Twinning excellence on organic soil amendments effect on nutrient and contaminant dynamics in the subsurface

TwinSubDyn

Deliverable D1.2 1st Summary report on mobility

Written by the TwinSubDyn project consortium

N°	Short	Beneficiary	Role
1	UNSPMF	University of Novi Sad Faculty of Sciences	CO
2	UNIVIE	University of Vienna	BEN
3	FZJ	Forschungszentrum Jülich	BEN
4	MLU	Martin-Luther-Universität Halle-Wittenberg	BEN
5	CSIC	Spanish National Research Council	BEN

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1.0	05.02.2024.	Deliverable	Marijana Kragulj Isakovski	Drafting deliverable
2.0	21.03.2024.	Deliverable	Jens Kruse, Snežana Maletić	Technical corrections

Contents

EXECUTIVE SUMMARY

Deliverable 1.2 encompasses all completed, ongoing, and upcoming training activities within the TwinSubDyn project outlined in WP1 (tasks 1.1, 1.2 and 1.3). In November 2022, we delivered a comprehensive Mobility and Training Plan, addressing the specific needs of UNSPMF researchers and staff. The primary objectives were to foster networking excellence and facilitate knowledge transfer with our esteemed partners UNIVIE, FZJ, MLU, and CSIC.

The plan incorporated short-term scientific exchanges (STSE) between EU institutions and UNSPMF, featuring two-way exchanges of qualified (QR) or recognized researchers (RR) and one-way exchanges of early-stage researchers (ESR). An overview of the planned and realized training activities includes:

Short visits by UNSPMF-qualified researchers to EU partnering institutions (1 week): Successfully implemented 4 visits out of 9.

Short-term staff exchanges (STSE) for the training of UNSPMF experienced researchers, post-doctoral, and early-stage researchers at EU-partner organization facilities at EU partnering institutions (1-6 months): Realized 15 out of 21.

Hosting at least 10 scientists to deliver seminars, conduct short courses, and train researchers in experimental techniques at UNSPMF (1-2 weeks): visit 10 top scientists from partnering institutions to UNSPMF.

Seminars/training of UNSPMF staff by EU partner institutions: Organized 5 out of 7.

The Steering Community carefully monitored the Short-Term Scientific Exchange (STSE) program, ensuring both accuracy and timeliness in reporting. Researchers were required to generate comprehensive reports following their visits. Training materials and videos from events hosted by UNSPMF are now readily available on the project website and the TwinSubDyn knowledge hub, enhancing the dissemination of project outcomes.

INTRODUCTION

TwinSubDyn's specific objective is to facilitate knowledge transfer and best practices exchange between UNSPMF and EU partners (UNIVIE, FZJ, MLU, and CSIC) in understanding the impact of OSA on SOM, contaminants, and nutrient dynamics in the soil subsurface and its implications for groundwater quality. The project entails planned training and mobility for UNSPMF researchers over the PM30. Short-term staff exchanges (STSE) with EU partners address research gaps and needs, aligning with UNSPMF's strategic partners' expertise. Training is closely connected with partner expertise:

- UNIVIE: Chromatography-mass spectrometry methods, natural colloids, nanoparticles, hydrogeological modeling, and phthalates analysis.
- FZJ: Isotope signatures as tracers, Lysimeters experiments, and large-scale field experiments on OSA impact.
- MLU: SOM functional analysis, large-scale field experiments, and meta-analysis on organic amendments.
- CSIC: Solid-state NMR spectroscopy, analytical pyrolysis, isotope-based approaches, and respirometry for assessing OSA impact on SOM dynamics.

Knowledge transfer occurs through various activities:

- Short visits by qualified researchers from UNSPMF to EU institutions for up to 1 week (Task 1.1).
- Short-term staff exchanges (STSE) available for all UNSPMF researchers (ESR, RR, QR) (Task 1.2).
- Training sessions conducted by senior EU researchers at UNSPMF last 1-2 weeks (Task 1.3).
- Seminars and training sessions for UNSPMF staff organized by EU partners (Task 1.4).

In Table 1, a comprehensive summary of all successfully completed STSEs and visits conducted throughout the project is presented, along with a comparison against the predetermined targets set for each mobility type.

Table 1: General overview of planned and realized mobilities within WP1.

Activity	No planned	No realized	Means of verification
Short visits of UNSPMF qualified researchers to EU partnering institutions (1 week) (PM1-34) (Task 1.1)	9	4	Photos, attendance list, Agendas
Short-term staff exchanges (STSE) for training of UNSPMF experienced researchers, post-doctoral and early-stage researchers at EU-partner organization facilities at EU partnering institutions (1-6 months) (PM1-30) (Task 1.2)	21	15	Photos, mobility reports, obtained training materials and research data
Hosting a minimum of 10 scientists to deliver seminars, conduct short courses, train researchers in experimental techniques at UNSPMF (1-2 weeks) (PM1-PM33) (Task 1.3)	10	10	Photos, training materials, agenda, attendance list, open accessibility on TwinSubDyn knowledge platform

It's important to note that an overview of all training activities related to task 1.4 and corresponding materials will be included in Deliverable D1.5 Training Material for Boosting Research Capacity and EU Grant Acquisition scheduled for submission by PM34.

REPORTS ON TRAINING ACTIVITIES

Task 1.1 (leader UNSPMF) Short exchange visits (up to 1 week) between EU research institutions and UNSPMF for 9 QR/RR from UNSPMF.

Throughout the duration of the project, there are plans for nine one-week exchange visits, which involve researchers from both UNSPMF and EU research institutions. These visits are designated for Consortium (CM) and Steering Committee Meetings (SCM), as well as for executing project activities. These one-week exchanges play a pivotal role in facilitating visits to each partner and their respective laboratories, fostering the exchange of ideas, and nurturing potential future collaborations. Details of the completed short exchanges are presented in Table 2.

Table 2: Completed one-week exchange visits.

Month /Year	Host	Paricipants from EU or UNSPMF	Actions
20-21 September 2022	UNSPMF	Gabriel Sigmund, Martin Stockhausen, Melissa Torres, Roland Bol, Lutz Weihermuller, Bruno Glaser, Arthur Gross, Heicke Knicker	Kick off
19-20 April, 2023	CSIC	Snežana Maletić, Srđan Rončević, Marijana Kragulj Isakovski, Tamara Apostolović	Consortium and SC meetings
28-29 September 2023	UNSPMF	Bruno Glaser, Arthur Gross, Lutz Weihermuller, Heicke Knicker, Gabriel Sigmund (online), Thorsten Huffer (Online), Roland Bol (online)	Consortium and SC meetings

The Kick-off meeting at UNSPMF in Serbia took place on September 20-21, 2022. Detailed discussions during the event are documented in D5.1. The UNSPMF visit included a guided tour of the Department of Chemistry, Biochemistry, and Environmental Protection. At the kick-off meeting, 8 project members from EU partnering institutions were hosted at UNSPMF.

Following this, the Consortium Meeting (CM) and Steering Committee Meeting (SCM) were held at IRNAS in Sevilla, Spain, from April 19th to 20th, 2023. During this session, partners explored various IRNAS laboratories and engaged in discussions on pyGC/MS, TGA, ultra-high Resolution Analytical Pyrolysis (Py-GC-Q-TOF), and Pyrolysis compound-specific isotopic analysis (Py-CSIA). During CM and SCM at IRNAS, four UNSPMF researchers (Snežana Maletić, Srđan Rončević, Marijana Kragulj Isakovski, and Tamara Apostolović) presented realized project activities.

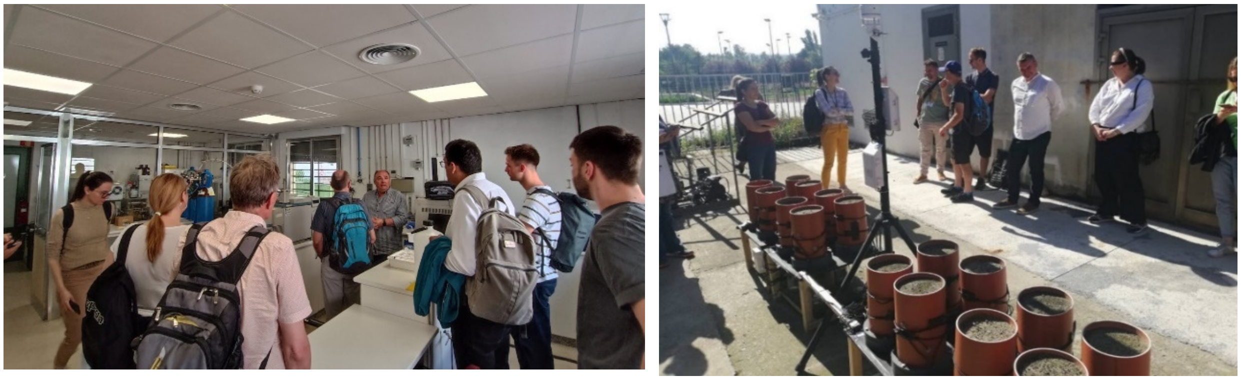


Figure 1: The TwinSubDyn consortium tours and engages in discussions during a visit to the IRNAS laboratoties (left) and Lysimeters experiment at UNSPMF (right) during SC and SCM meetining

The third Consortium Meeting (CM) and Steering Committee Meeting (SCM) took place in a hybrid format on September 28-29, 2023, featuring workshops held at UNSPMF. During this workshop, four project members, Bruno Glaser, Arthur Gross, Lutz Weihermuller, and Heicke Knicker, attended in person at UNSPMF, while three project participants, Gabriel Sigmund, Thorsten Huffer, and Roland Bol, participated remotely online.

The forthcoming one-week visit is scheduled for the Consortium Meeting (CM) and Steering Committee Meeting (SCM) at UNIVIE, coinciding with the EGU conferences in April 2024. Concurrently, project members will actively contribute to the EGU2024 conference as oral speakers in the dedicated session focusing on the TwinSubDyn topic. For this meeting, five UNSPMF researchers are planned to be present both at EGU and at the CM.

Task 1.2 Short-term scientific visits and stays in EU research institutions for researchers (leader CSIC).

The outline for short-term scientific visits and stays in EU research institutions was detailed in Deliverable D1.1, the Mobility and Training Plan, submitted on 30.11.2022. To adhere to the activities outlined in the Plan, a series of UNSPMF researchers' mobilities and training sessions, crucial for the project's 34 months, were realized. Dates, locations, and specific actions within the training program were adapted and detailed as described in D1.1. In some instances, adjustments were made to fulfill project requirements, ensuring scientific validation and benefits for all involved researchers and institutions. A summary of the scheduled and completed 6-month STSE and visits lasting up to 1 month within specific subtasks is presented in Table 3 and Annex I.

Table 3: Overview of all 6-month STSE and up to 1-month training sessions at EU institutions

No	Place/subtask	Researcher	Time	Status	Mean of verification
			6-month STSE		
1.	CSIC/1.2.1	T. Apostolović	15 March to 14 September 2023.	DONE	Photos, ppt, Mobility report (Annex I)
2.	MLU/1.2.3	M. Solić	16 February to 31 August 2023.	DONE	Photos, ppt, Mobility report (Annex I)
3.	UNIVIE/1.2.6	S. Vasiljević	1 February to 31 July 2023	DONE	Photos, ppt, Mobility report (Annex I)
4.	UNIVIE/1.2.6	I. Jevrosimov	1 March to 28 August 2023	DONE	Photos, ppt, Mobility report (Annex I)
5.	FZJ/1.2.4	N. Đukanović	5 June to 30 November 2023.	DONE	Photos, ppt, Mobility report (Annex I)
6.	UNIVIE/1.2.7	T. Apostolović	November 2023 – April 2024	Ongoing	In preparation
7.	CSIC/1.2.2	M. Šolić	March 2024-September 2024	In preparation	-
8.	FZJ/1.2.5	S. Tenodi	October 2024-March 2025	In preparation	-
			1-month visits		
1.	MLU/1.2.3	T. Apostolović	16 February to 14 March 2023	DONE	Photos, ppt, Mobility report (Annex I)
2.	FZJ/1.2.4	J. Beljin	5 June to 23 June 2023.	DONE	Photos, ppt, Mobility report (Annex I)
3.	UNIVIE/1.2.7	A. Tubić	25 June to 23 July 2023	DONE	Photos, Mobility report (Annex I)
4.	CSIC/1.2.1	S. Maletić	1 July to 31 July 2023	DONE	Photos, ppt, Mobility report (Annex I)
5.	CSIC/1.2.1	M. Kragulj Isakovski	1 July to 31 July 2023	DONE	Photos, ppt, Mobility report (Annex I)
6.	UNIVIE//1.2.6	I. Jevrosimov	30 October to 28 November 2023	DONE	Photos, ppt, Mobility report (Annex I)
7.	UNIVIE//1.2.6	S. Vasiljević	30 October to 28 November 2023	DONE	Photos, ppt, Mobility report (Annex I)
8.	FZJ/1.2.4	S. Maletić	2024	In preparation	-
9.	FZJ/1.2.5	S. Rončević	2024	In preparation	-
10.	MLU/1.2.3	S. Maletić	2024	In preparation	-
11.	UNIVIE/1.2.7	M. Kragulj Isakovski	2024	In preparation	-
			2-week training		
1.	UNIVIE/1.2.8	S. Rončević	25 to 30 June 2023.	DONE	Photos, Mobility report

			3 to 8 September 2023		(Annex I)
2.	UNIVIE/1.2.8	A. Tubić	25 to 30 June 2023. 3 to 8 September 2023	DONE	Photos, Mobility report (Annex I)

Two additional 1-month visits related to task 1.2.6 have been approved for Sanja Vasiljević and Irina Jevrosimov. These supplementary months have proven to be pivotal for consolidating the training they received earlier during the 6-month STSE at UNIVIE.

The training materials, including researchers' presentations at partnering institutions, utilized relevant methods were hosted on the Knowledge Platform (https://knowledge-hub.pmf.uns.ac.rs/) for the benefit of the academic and scientific communities.

Additionally, Mobility Reports for each researcher detailing completed training activities, accompanied by photos and comprehensive descriptions of the techniques learned and skills acquired, are provided in Annex I. It should be noted that Tamara APOSTOLOVIĆ is currently on a 6-month STSE at UNIVIE (subtask 1.2.7), which will be fully realized in April 2024. Therefore, her Mobility Report is not included in the content of this Deliverable.

Furthermore, presentations delivered by researchers from UNSPMF during their stay at collaborating institutions, focusing on either their scientific expertise or TwinSubDyn outcomes, are complemented by corresponding Mobility Reports found in Annex I. Moreover, the research outcomes resulting from these visits are integrated into Deliverable D3.1, the Midterm Research Progress Report, and are slated for use in forthcoming scientific publications.

Please be advised that Marko ŠOLIĆ is presently preparing for a 6-month STSE at CSIC, supervised by Heike KNICKER, scheduled from March 2024 to September 2024, with a focus on subtask 1.2.2. Furthermore, all remaining visits linked to subtasks 1.2.3, 1.2.4, 1.2.5, and 1.2.7 are scheduled to occur in 2024, following the timetable provided in Table 3.

Task 1.3 Hosting at least 10 high-level experts/scientists from abroad in research themes of interest to UNSPMF (leader UNSPMF).

Ten scientists visited UNSPMF, delivering seminars, providing training in experimental techniques, and engaging in discussions about setting up research experiments. Information about all trainings, including agendas, timeframes, and links for online participants, was made available in advance on the project website's "News and Events" subsection (https://twinsubdyn.pmf.uns.ac.rs/index.html).

Training materials, such as PowerPoint presentations and video recordings, are hosted on the TwinSubDyn website under the "HUB/Education Resources" section: https://knowledge-hub.pmf.uns.ac.rs/course/view.php?id=3. Additionally, numerous video recordings of training sessions can be accessed on the TwinSubDyn YouTube channel at https://www.youtube.com/@twinsubdynproject/videos.

The analysis of the audience for all organized training sessions at UNSPMF is presented in Table 4 and Figure 2, while all agendas and corresponding attendance records are provided in Annex II.

Table 4: Summary of Training Activities Conducted at UNSPMF and Participant Types

No	Institution Subtask	Speaker	Time	No of participants per day	Audience (estimated)
1.	UNIVIE 1.3.1	Thorsten HÜFFER, Charlotte HENKEL	18-19th January 2023.	28+8	UNSPMF: PhD: 7 Researchers: 10 Prof: 11
2.	UNIVIE 1.3.2	Michael ZUMSTEIN, Katharina SODNIKAR	3rd-5th April 2023.	10+20+7	UNSPMF: PhD: 7 Researchers: 4 Prof: 9
3.	MLU 1.3.3	Bruno GLASER, Arthur GROSS	Online and on-site 20th-21st September 2023.	15+13	UNSPMF PhD: 2 Researchers: 6 Prof: 7
4.	MLU 1.3.5	Bruno GLASER, Arthur GROSS	Online and on-site 22nd and 25th September 2023.	13+13	UNSPMF, UNSFT: PhD: 2 Researchers: 5 Prof: 6

5.	FZJ 1.3.5	Lutz WEIHERMÜLLER, Jens KRUSE	27th-31st March 2023.	10	UNSPMF MSc: 1 PhD: 3 Researchers: 3 Prof: 3
6.	CSIC 1.3.6	Heike KNICKER, José A. GONZÁLEZ PÉREZ	6th-9th February 2023	16+15+22+9	UNSPMF, UNSFT PhD: 6 Researchers: 9 Prof: 7

Across all completed training sessions, the number of onsite participants ranged from 8 to 28, varying depending on the type of training. In total, there were 108 participants across all training sessions. Most participants were from the University of Novi Sad, primarily from the Faculty of Sciences, with a smaller number coming from the Faculty of Technology (UNSFT). It should be noted that all training sessions were organized as hybrid events. However, due to limitations in the data obtained from online attendance lists, it was not possible to determine the specific types of participants or their affiliations. Therefore, this category of participants was not considered for the analysis.

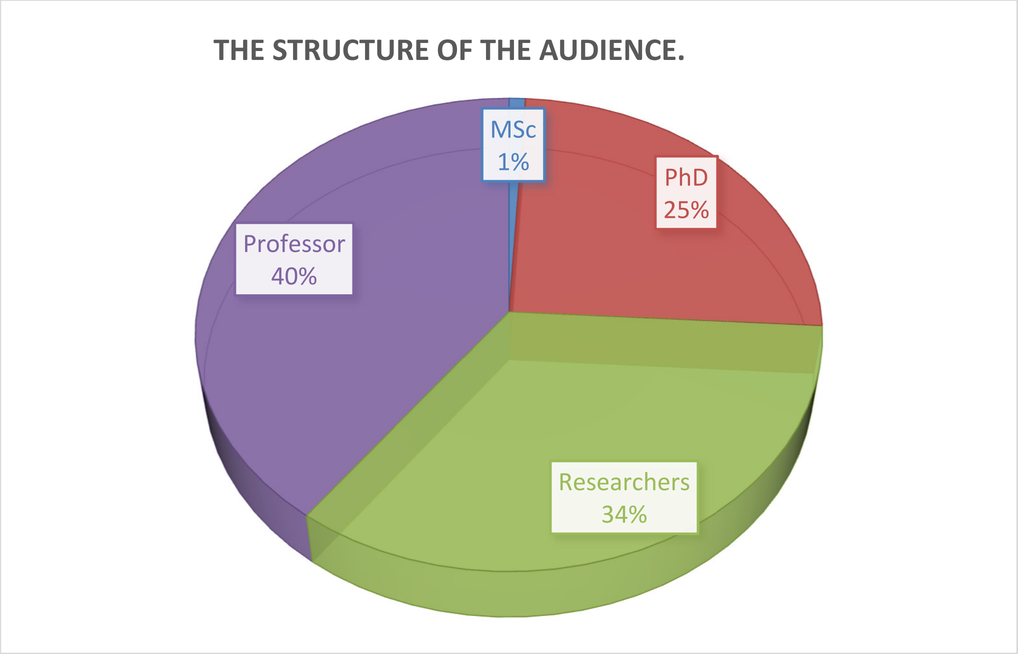


Figure 2: The structure of the audience for all training seasons.

Based on the participant data gathered from the attendance lists, the composition of the audience indicates that professors (40%) and researchers (34%) were the most dominant groups in attendance. This aligns with the goal of the training, which is to transfer knowledge and enhance the capacity of scientists at UNSPMF. The training sessions were primarily designed for academia, and the distribution of attendees reflects this objective. The following text provides a overview of these training activities, including their respective topics. Agendas and attedence list for all training are given in the Annex II.

Subtask 1.3.1 (leader UNIVIE) 1 visit of QR/RR from UNVIE to train on-site UNSPMF researchers and laboratory personnel in the optimization and improvement of experimental designs.

Thorsten HÜFFER and **Charlotte Henkel from UNIVIE** visited UNSPMF on January 18th-19th, 2023. They realized training titled “On-site training in sample preparation and analysis of contaminants and selected transformation products”. The focus was on analyzing microplastics and their additives. After introductions and a lab tour, discussions covered sample preparation techniques at UNSPMF. **Thorsten HÜFFER** delivered lecture titled "(Micro-)plastics in the environment - lessons learned and current challenges," followed by in-depth discussions on analytical methods and experimental approaches for additive leaching from microplastics.

The lecture slides and video recordings are accessible on the TwinSubDyn Knowledge Hub to enhance accessibility and impact: https://knowledge-hub.pmf.uns.ac.rs/course/view.php?id=3, https://knowledge-hub.pmf.uns.ac.rs/mod/resource/view.php?id=12&forceview=1. A combined total of 36 individuals participated in the two-day training session. According to the provided data, the most common participant category consisted of 7 PhD students, 10 researchers, and 11 professors from UNSPMF. The agenda and attendance lists for each day of the microplastic training can be found in Annex II.

Figure 3: Keynote lecture by Thorsten HÜFFER (left) and in-depth discussions between UNIVIE and UNSPMF researchers (right).

During the visit, Snežana MALETIĆ provided an overview of the Department of Chemistry, Biochemistry, and Environmental Protection at the Faculty of Sciences, University of Novi Sad. Partners from UNIVIE were acquainted with the department's main sections, study programs, and the Environmental Protection research group, including their primary research areas and accomplishments. Following this, the UNSPMF TwinSubDyn research group members (Jasmina AGBABA, Srđan RONČEVIĆ, Aleksandra TUBIĆ, Marijana KRAGULJ ISAKOVSKI, Jelena BELJIN, Tamara APOSTOLOVIĆ, Marko ŠOLIĆ, Irina JEVROSIMOV, Sanja VASILJEVIĆ, and Nina ĐUKANOVIĆ) were introduced. The research group members elaborated on their respective research fields and the project tasks they will undertake throughout the project duration. Additionally, Thorsten HÜFFER and Charlotte HENKEL presented the experimental and analytical methods utilized for plastic additives analysis at UNIVIE.

Subtask 1.3.2 (leader UNIVIE) 1 visit of QR/RR from UNVIE to train on-site UNSPMF researchers in sample preparation and analysis of contaminants (PM6)

Michael ZUMSTEIN and Katharina SODNIKAR from UNIVIE visited UNSPMF from April 3rd to 5th, 2023, focusing on the analysis of biotransformation and biodegradation of organic contaminants and their transformation products. They offered “On-site training in the application of LC-MS(/MS) for Organic Contaminants in soil science”. The visit included introductions and a lab tour, followed by one-on-one meetings between the visitors and UNSPMF researchers to explore collaboration opportunities. Keynote lectures were delivered by Michael ZUMSTEIN on "Environmental Biodegradation and Biotransformation of Organic Chemicals: Applying LC-MS/MS and Related Analytical Approaches," and by Katharina SODNIKAR on "Fate of DNA & dsRNA in Soils: Interaction with Minerals and Biodegradation."

Figure 4: Keynote lecture by M. ZUMSTEIN (left) and discussions during the complementary workshop with UNIVIE and UNSPMF researchers (middle) and Katharina SODNIKAR presentation

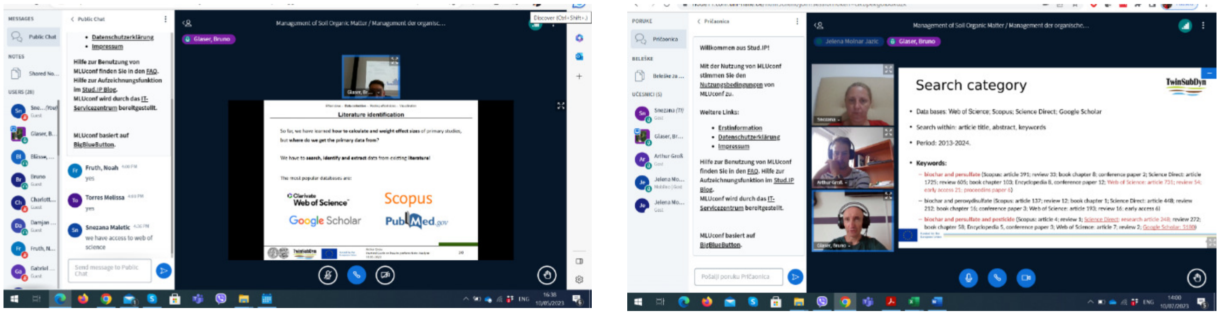
The lectures and supporting materials are openly accessible via the TwinSubDyn knowledge hub to maximize their impact and usability (https://knowledge-hub.pmf.uns.ac.rs/course/view.php?id=3). Video records is available on knowledge hub https://knowledge-hub.pmf.uns.ac.rs/mod/resource/view.php?id=40&forceview=1.

The overall attendance numbered 27 individuals, comprising 7 PhD students, 4 researchers, and 9 professors (Table 4).

Subtask 1.3.3 (leader MLU) 2-person visits of QR/RR from MLU to deliver seminars focused on the meta-analysis

The course on meta-analysis, with a focus on organic soil amendments, was led by Bruno GLASER and Arthur GROSS, both from MLU. The course comprised a series of online lectures and seminars (part 1) followed by on-site training at UNSPMF (part 2).

Online courses (part 1) and relevant materials on meta-analysis are accessible on the TwinSubDyn website under the section "HUB/subsection Education Resources"



Literature identification

So far, we have learned **how to calculate and weight effect sizes** of primary studies, but **where do we get the primary data from?**

We have to **search, identify and extract** data from existing **literature!**

The most popular databases are:

Search category

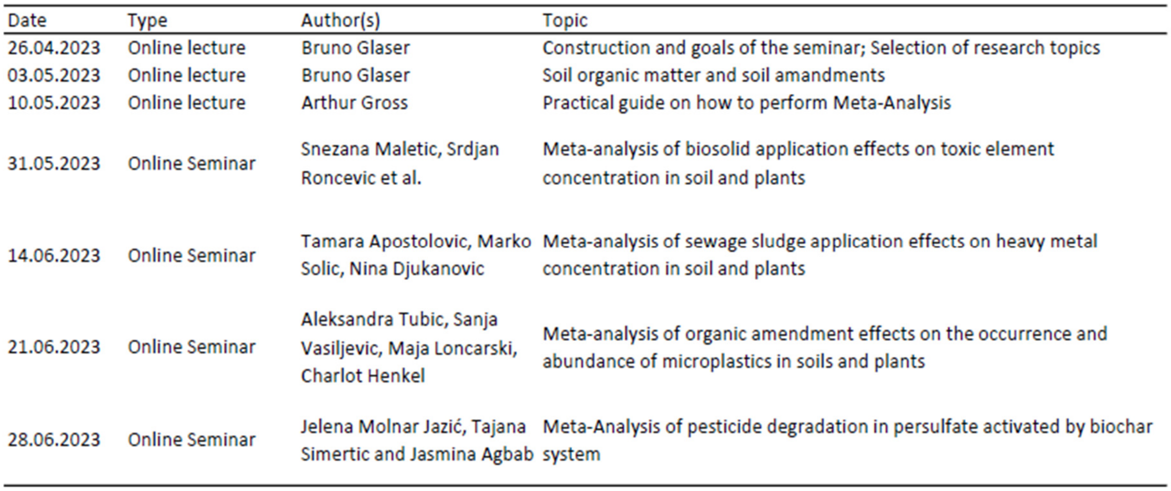
- Data bases: Web of Science; Scopus; Science Direct; Google Scholar
- Search within: article title, abstract, keywords
- Period: 2013-2024.
- **Keywords:**
  - biochar and persulfate (Scopus: article 391; review 33; book chapter 8; conference paper 2; Science Direct: article 1725; review 605; book chapter 103; Encyclopedia 8, conference paper 12; Web of Science: article 731; review 54; early access 21; proceedins paper 6)
  - biochar and peroxydisulfate (Scopus: article 137; review 12; book chapter 1; Science Direct: article 448; review 212; book chapter 16; conference paper 3; Web of Science: article 193; review 16; early access 6)
  - biochar and persulfate and pesticide (Scopus: article 4; review 1; Science Direct: research article 248; review 272; book chapter 58; Encyclopedia 5, conference paper 3; Web of Science: article 7; review 2; Google Scholar: 5180)

Figure 5: Screenshot of the online lecture given by Arthur GROSS on 10.05.2023 (left). Screenshot of one of the unscheduled online meetings (right).

In the initial lecture, attendees of the seminar were divided into four groups, with each group assigned a project topic for meta-analysis, as outlined in Table 5. The objective of each project was to collaborate on a distinct meta-analysis topic. Project titles were selected under the condition that none of the topics had previously been covered in an existing meta-analysis, ensuring the potential for successful publication of project outcomes in the future.

During the second lecture, Bruno GLASER discussed the impacts of organic amendments on soil organic matter (SOM) and carbon storage. Subsequently, the third lecture, delivered by Arthur GROSS, focused on introducing the methodology of meta-analysis. These lectures aimed to establish a shared knowledge base among all seminar participants for their individual meta-analysis projects. Over the following weeks, the groups were tasked with defining appropriate meta-analysis search criteria and determining inclusion and exclusion parameters for primary studies, as well as gathering pertinent literature data and conducting initial analyses. In collaborative online seminars held in June and July 2023, the groups presented their progress and preliminary findings. Following each presentation, project groups had the opportunity to address challenges and issues with Bruno GLASER, Arthur GROSS, and fellow seminar participants.

Table 5: Timetable of the online lectures and seminar



Date	Type	Author(s)	Topic
26.04.2023	Online lecture	Bruno Glaser	Construction and goals of the seminar; Selection of research topics
03.05.2023	Online lecture	Bruno Glaser	Soil organic matter and soil amandments
10.05.2023	Online lecture	Arthur Gross	Practical guide on how to perform Meta-Analysis
31.05.2023	Online Seminar	Snezana Maletic, Srdjan Roncevic et al.	Meta-analysis of biosolid application effects on toxic element concentration in soil and plants
14.06.2023	Online Seminar	Tamara Apostolovic, Marko Solic, Nina Djukanovic	Meta-analysis of sewage sludge application effects on heavy metal concentration in soil and plants
21.06.2023	Online Seminar	Aleksandra Tubic, Sanja Vasiljevic, Maja Loncarski, Charlot Henkel	Meta-analysis of organic amendment effects on the occurrence and abundance of microplastics in soils and plants
28.06.2023	Online Seminar	Jelena Molnar Jazić, Tajana Simertic and Jasmina Agbab	Meta-Analysis of pesticide degradation in persulfate activated by biochar system

Unscheduled online meetings were held between the online lectures and seminars and the on-site training in September 2023. These meetings were necessary to ensure the successful continuation of the project work.

The on-site training in meta-analysis, conducted on September 20th and 21st, 2023, commenced with a session led by Arthur GROSS. He provided instruction on calculating and visualizing meta-analyses using Excel and R, covering both theoretical concepts and practical demonstrations with the software. Following this, the four groups presented their ongoing progress and interim findings. The presentations prepared by the four groups for the onsite training are available in Annex III, and the agenda along with the corresponding attendance list can be found in Annex II. The training session was attended by a total of 28 participants, consisting of 2 PhD students, 6 researchers, and 6 professors, as detailed in Table 4.

Additionally, all course materials from both the online sessions and the on-site training are available on the TwinSubDyn website under the "HUB/subsection Education Resources" section https://knowledge-hub.pmf.uns.ac.rs/course/view.php?id=3. Following the training activities on meta-analysis, a peer-reviewed scientific paper has been submitted to a high-impact journal

Biochar (IF=13) by authors Jelena Molnar Jazić, Arthur Gross, Bruno Glasser, Jasmina Agbaba, Tajana Simetić, Jasmina Nikić, and Snežana Maletić.

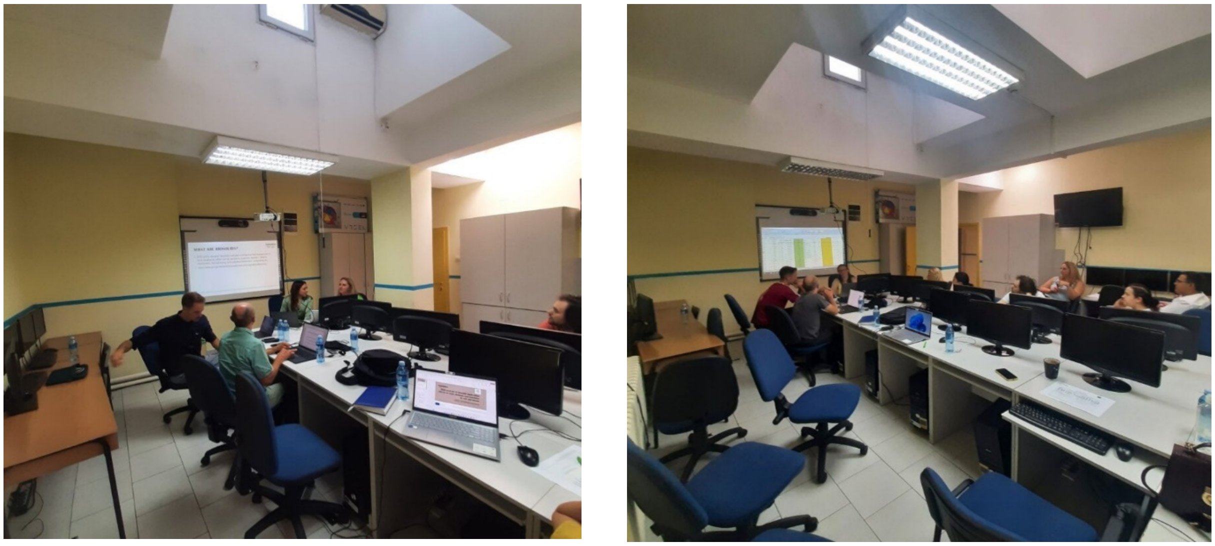


Figure 6: Onsite training on meta-analysis at UNSPMF

Subtask 1.3.4 (leader FZJ) 2 person Two-week visits (PM29) of QR/RR from FZJ to deliver training on modelling of nutrient and element cycling, colloid and nanoparticle transport in the unsaturated zone.

Training in nutrient and element cycling will be conducted in two parts. The first part is tentatively planned for March 2024 and will be organized online to introduce participants to the theoretical aspects of modeling. The second part of the training is scheduled to take place onsite at the University of Novi Sad from September 9th to 13th, 2024.

Subtask 1.3.5 (leader MLU) 3 person visits of QR/RR from FZJ and MLU to deliver training on site for the large-scale field experiments.

MLU and FZJ delivered training on large-scale field experiments at UNSPMF. On-site training, conducted by FZJ, took place from March 27th to 31st, 2023, coinciding with the setup of lysimeter experiments at UNSPMF. FZJ researchers collaborated with UNSPMF staff to plan and execute lysimeter experiments, with installation completed in March 2023. The training highlighted the significance of soil parameter measurements and optimal sampling strategies for nutrient cycling and pollutant fate. FZJ is currently processing the data to ensure its quality and promptly identify any system breakdowns. A total of 10 researchers (1 MSc, 3 PhD, 3 reserchers and 3 professoers) participated in setting up the lysimeter experiments as part of the field training.

Figure 7: The lysimeter setup at UNSPMF in March 2023 with staff from UNSPMF and FZJ

Figure 8: Field training on sampling large-scale field experiments at MLU (left); On-site training at UNSPMF on field experiment provided by Bruno GLASER and Arthur GROSS (right)

MLU provided training in two parts. The first training was conducted during the stay of Marko ŠOLIĆ and Tamara APOSTOLOVIĆ at MLU to sample large-scale field experiments in Bayreuth, Germany. The hosts of the training were Bruno GLASER and Arthur GROSS, accompanied by the two UNSPMF researchers Tamara Apostolović and Marko Šolić.

The field trial was established in 2010 to explore the impact of different organic soil amendments on soil characteristics and crop productivity. The tested organic soil amendments included biochar, compost, and two combinations of these materials: a direct blend of biochar and compost, and a mixture of biochar and raw plant material subsequently co-composted. Each treatment was administered in two different doses, resulting in a total of 8 treatments alongside an untreated control soil. Soil sampling was conducted as follows (Figure 8): Treatment plots were arranged in the experimental field following a Latin rectangle pattern in a row-column layout (5-10), with each treatment replicated five times. Magnets were buried along the field edges and located using a metal detector, with stakes marking the division point of each plot. Subsequently, the plots were identified, and soil samples were collected from each plot using a hand auger (6 augers per plot) from a depth of 0-30 cm. All soils from the same treatment were combined to create one composite sample per treatment.

On-site lectures were conducted at UNSPMF on large-scale field experiments. The second segment of the training for large-scale field experiments occurred on 22 September at UNSPMF, provided by Bruno GLASER and Arthur GROSS. This training consisted of two lectures. During the initial presentation, Bruno GLASER discussed the evolution of large-scale field experiments on soil biogeochemistry at MLU (Figure 8). He provided a comprehensive overview of their characteristics and the evolutionary changes in their design based on accumulated experience over the years. Additionally, he highlighted the most significant findings obtained thus far.

Arthur GROSS delivered the second lecture on the statistical analysis of large-scale field trials. After covering statistical fundamentals, he introduced an iterative approach to the statistical workflow for analyzing agronomic field trials. Subsequently, participants engaged in discussions about their experiences with statistics and encountered statistical challenges. In certain instances, the course instructors offered immediate assistance in resolving statistical issues.

A virtual visit to the UNSPMF field experiment in Serbia, originally planned for September 25th, 2023, was canceled due to heavy rainfall that lasted the whole day. The excursion was replaced by a presentation by Snežana MALETIĆ, who conducted a "virtual visit" to the site.

The two-day training session on large field experiments had 26 participants, comprising 2 PhD students, 5 researchers, and 6 professors, as specified in Table 4 and Annex II.

Subtask 1.3.6: 2 visits (PM6) of QR/RR from CSIC to train UNSPMF researchers in the application of solid-state NMR in soil science and the interpretation of NMR data (leader CSIC).

Heike KNICKER and José A. GONZÁLEZ PÉREZ from IRNAS-CSIC conducted one-week training sessions from February 6th to 9th at UNSPMF. They provided on-site training focusing on the application of solid-state NMR and analytical pyrolysis techniques in soil science.

Heike KNICKER delivered training lectures encompassing an introduction to NMR theory, interactions during NMR experiments, solid-state NMR, cross-polarization magic angle spinning experiments, interpretation of solid-state NMR data, and examples of their application in soil science and biochar research. José A. GONZÁLEZ PÉREZ provided lectures on analytical pyrolysis techniques in soil science, covering an introduction to analytical pyrolysis, Py-GC/MS, Py-GC-Q-TOF, and examples of their application in biomass and soil science. He also provided training on stable isotope analysis and Py-CSIA, along with examples in soil science. Following the training sessions, in-depth discussions took place between researchers from IRNAS and UNSPMF.

Training materials and lectures are accessible on the TwinSubDyn Knowledge Hub, while video recordings of completed training sessions are hosted at https://knowledge-hub.pmf.uns.ac.rs/mod/resource/view.php?id=33&forceview=1.

The agenda and timeline for the training were available in advance on the TwinSubDyn website (https://twinsubdyn.pmf.uns.ac.rs/news.html#news_events). The agenda and attendance records for each day are included in Annex II. The training was conducted in a hybrid format, with a total of 62 participants attending on-site, including 9 researchers, 7 professors, and 6 PhD students from the Faculty of Sciences and Faculty of Technology at the University of Novi Sad (Table 4).

Furthermore, while at UNSPMF, the Serbian national broadcasting network attended the training session and created a television segment covering the TwinSubDyn project.

Figure 9: Heike KNICKER teaches solid-state NMR (left), José A. GONZÁLEZ PÉREZ teaches Py-GC/MS (in the middle); Heike Knicker's interview for the national broadcast network (right).

The training materials aimed at enhancing research capacity and acquiring EU grants, including PowerPoint presentations, PDF documents, guidelines, and other relevant materials obtained during the WP1 activities, will be compiled, and submitted in Deliverable D1.5: "Training Material for Boosting Research Capacity and EU Grant Acquisition," by the deadline of PM34.

CONCLUSION AND OUTLOOK

Currently, five out of 8 six-month STSEs have been successfully completed, enabling UNSPMF researchers to acquire knowledge and skills concerning the impacts of organic soil amendments on carbon, nutrient, and pollutant dynamics in soils and the subsurface. Additionally, one six-month STSME is ongoing at UNIVIE. The researchers have gained familiarity with state-of-the-art techniques and experimental setups utilized for soil and organic soil amendment analysis. Two more STSEs are scheduled to be realized in 2024 and at the beginning of 2025. Furthermore, four additional one-month visits (two at FZJ, MLU, and UNIVIE) for QR are in the preparation phase and will be carried out in 2024 and early 2025. UNSPMF hosted 10 top scientists from EU partnering institutions who provided training on relevant topics within the TwinSubDyn project. One more training session, provided by FZJ on nutrient and element modeling, will conclude in September 2024. All research findings and acquired knowledge will be disseminated through scientific papers to meet the required publication criteria.

ACKNOWLEDGEMENTS

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List of Annexes:

ANNEX I: Mobility Reports for all UNSPMF researchers, along with the corresponding PowerPoint presentations delivered during their visits.

ANNEX II: Agendas and corresponding attendance list for all completed training sessions provided by experts from EU partner institutions at UNSPMF.

ANNEX III: Prepared PowerPoint presentations by researchers from UNSPMF for META analysis training.

ANNEX I

Subtask 1.2.1: 6 months secondment for an ESR/RR and two 1 month’s STSM for a QR/RR at CSIC focused on impact of soil organic amendment on soil organic matter characterization and dynamics by GC/MS, FTIR and solid-state NMR (leader CSIC)

MOBILITY REPORT

Researcher: Dr. Tamara Apostolović, UNSPMF

Assigned supervisor: Prof. Dr. Heike Knicker

Duration of the visit: 15.03.2023. - 07.09.2023.

Executive Summary

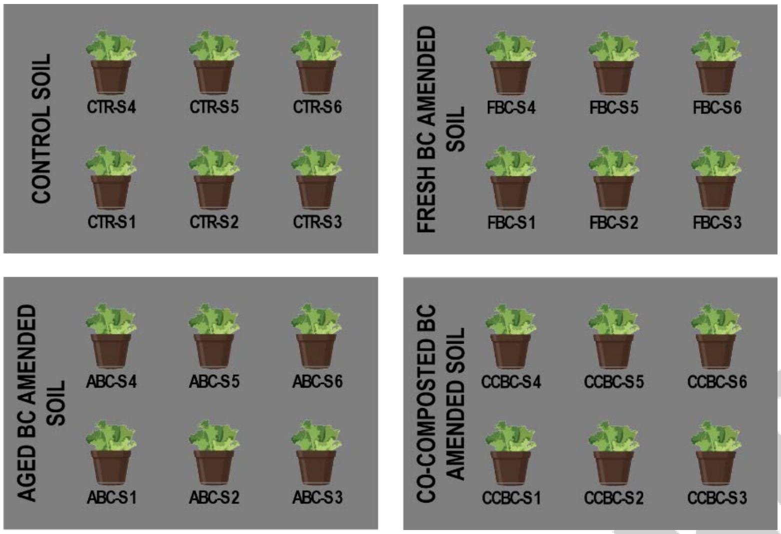
The 6 months visit to the Spanish National Research Council (Consejo Superior de Investigaciones Científicas, CSIC) and the Institute for Natural Resources and Agrobiology (Instituto de Recursos Naturales y Agrobiología de Sevilla, IRNAS) had two main focuses: the training of UNSPMF researcher in techniques for soil organic matter characterization and a joint research investigating the effect of aging of biochar treated soil on soil properties and plant growth. The training part of the visit included skill-building for the utilization of techniques and instruments such as solid state nuclear magnetic resonance (NMR), pyrolysis coupled with gas chromatography / mass spectroscopy (Py/GCMS), thermogravimetric analysis (TGA) and Respicond. In addition, the use of softwares for data analysis as well as data interpretation were also part of the training. The greenhouse pot experiment designed to investigate how different soil treatments (biochar, co-composted biochar), and aging (soil treated with biochar in 2010 and soil treated with biochar just before the experiment) affected the quality of soil and the performance of plants growing in said soils. Soil samples were analysed in terms pH, electrical conductivity, water holding capacity, water repellence, organic matter and carbon content and characterization, total and available nutrients content, microbial activity. In addition, plant material collected after 60 days was also analyzed in terms of biomass growth, nutrient content, plant performance (chlorophille production, photosynthesis efficiency).

Introduction

Background

Soil samples: The pot experiments are carried out in soils from an experimental field near Bayreuth, Germany established in 2010. Composite samples of three treatment variants from the field were used: Control (untreated soil used as a reference soil), K_31.5 (soil treated with 31.5 t/ha biochar) and BGK31.5/70 (soil treated with co-composted biochar in the ratio 31.5 t/ha biochar and 70 t/ha compost material). In addition, for the pot experiment, a fourth variant of soil (Fresh_BC) was prepared by treating control soil with fresh biochar (the same biochar and dose used for biochar treatment in 2010).

Experimental setup: After taking samples for soil characterization, the composite soil samples were divided into six pots per treatment (24 pots in total). Lettuce seeds were sown, and grown for 60 days, maintaining moderate irrigation conditions by keeping the water content at around 60% of water holding capacity.



Scope of the secondment

Part of the scope of the secondment was knowledge transfer on techniques for soil organic matter characterization, including solid state nuclear magnetic resonance (NMR), pyrolysis coupled with gas chromatography / mass spectroscopy (Py/GCMS), thermogravimetric analysis (TGA) and soil microbial activity measured by Respicond. This included laboratory training in soil sample preparation and analysis of as well as training in data analysis and interpretation.

Furthermore, the secondment included research into the long-term effects of biochar amendment on soil properties and its soil organic matter and the effect of aging of biochar on plant performance. The research consisted of the setup of a greenhouse pot experiment with lettuce which lasted for 60 days. In order to investigate the effect of both different biochar treatments (pure biochar / co-composted biochar amended soil) and the aging of biochar (freshly treated soil / aged biochar treated soil), characterization and analysis of the soils (at the beginning and the end of the pot experiment) and the characterization of the biomass were carried out.

Content

1. Methods

pH and electrical conductivity (EC)

Materials: 1 centrifuge tube per sample (30 ml), 1 test tube per sample (Ø≈16 mm, small), rotator, filter-blotting paper, funnels, clock-timer, notebook, pen and marker.

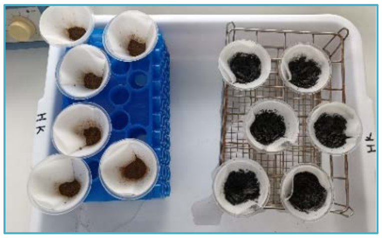
Procedure:

1. Label the tubes and measure 1 g of biochar (soil).
2. Add 10 ml of mili-Q/elix water (tipe II distilled water).
3. Shake in the rotator 2 h.
4. Let set for 30 min.
5. Measure the pH (clean with distilled water and dry the electrode between each measurement).
6. After the pH measurement, filter the supernatant (into a test tube, because part of the water remaines retained in the sample+filtee, the volume of the filtrate obtained is small, and the electrode must be well immersed).
7. Directily measure EC in the filtrate.
8. The biochar (soil) colected on the filter can be dried (< 40ºC) and reused for other analysis.

NOTE: Before starting the protocol, verify the necessary conditions for the pH-meter (pre-ignite to stabilize, calibrate, verify the pH of the water to be used…).

Water Holding Capacity (WHC)

Materials: 1 set “flask + funnel + filter (Whatman 2)” and 1 beaker per sample, distilled water, notebook, pen and marker.



Procedure:

1. Mark/label and weigh each set (“flask + funnel + filter”), with a dry filter and with a humid filter.
2. Weigh 2 g of sample and record the weight (“flask + funnel + humid filter + dry sample”)

3. Add distilled water in excess to saturate the sample (~10 ml).
4. Leave for 2 hours (with the “filter+funnel+sample” on the beaker).
5. After 2 hours, weigh “flask + funnel + humid filter + humid sample”.
6. Calculate the wet weight of the sample considering the weight of the funnel and filter paper. The difference in weight of the wet sample after 2 hours and the dry sample represents the weight of the water that the sample can retain (WHC). WHC is expressed as:

$$WHC\ (\%) = \frac{weight\ of\ water\ retained\ by\ sample}{initial\ weight\ of\ the\ sample}\ x\ 100$$

__Determination of organic matter, total organic carbon and total nitrogen content in soil__

<u>Organic matter content by loss-on-ignition method:</u> This method estimates soil organic matter based on gravimetric weight change associated with high temperature oxidation of organic matter. After initial oven drying at 105ºC overnight, the samples are ignited in a muffle furnace for 6 hours at 550ºC. The percent weight loss during the ignition step is reported as OM-LOI (% wt. loss). Organic carbon content can be estimated from the obtained organic matter content by assuming that organic matter contains 58% carbon.

<u>Total organic carbon (TOC) and total nitrogen (TN)</u> content in soil and plants was determined using a TOC analyzer. Determining total carbon (C) and total nitrogen (N) content in soil using a Total Organic Carbon (TOC) analyzer is a modern and efficient method. TOC analyzers are widely used in environmental and soil science laboratories for these measurements. The TOC analyzer introduces the dry and grinded sample into a combustion chamber where it is heated to a high temperature (usually around 680-720°C). During combustion, all the carbon in the sample is converted to carbon dioxide (CO2). The released CO2 is then measured using an infrared detector. In a TOC analyzer equipped with a TN module, the same combustion process used for

TOC analysis can be adapted to measure total nitrogen. The nitrogen in the sample is converted to nitrogen oxides (NOx) during combustion. The released NOx is then chemically reduced to produce nitric oxide (NO), which is measured using a chemiluminescence detector.

Determination of micro- and macronutrients in soil

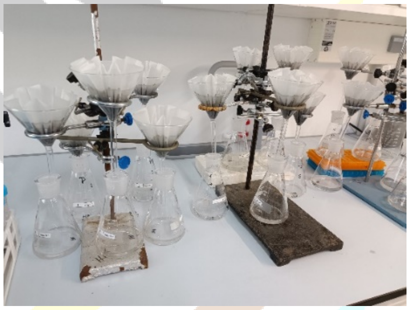
Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) is a powerful analytical technique used to determine both micro and macronutrients in soil samples. It's widely employed in soil and environmental analysis due to its accuracy, precision, and ability to detect a wide range of elements. In order to analyze soil by ICP-OES, samples must be dried and grinded to fine powder to ensure homogeneity. Depending on the elements of interest and the nature of the soil, you may need to perform a digestion step to solubilize the nutrients. Common digestion methods include using strong acids (e.g., nitric acid, perchloric acid, hydrochloric acid) or a combination of acids to break down the soil matrix and release the elements of interest into solution.

Extraction of soluble phosphorus in sodium bicarbonate

The available phosphorus is extracted from the soil with 0.5 M sodium bicarbonate ($NaHCO_3$) as an extraction solution, at a pH of approximately 8.5. In limestone, alkaline, or neutral soils containing calcium phosphate, the concentration of phosphorus in the extracting solution increases by precipitation of calcium as $CaCO_3$. In acid soils containing iron and aluminum phosphates, the concentration of P in the solution will increase as the pH is raised.

Phosphorus forms insoluble compounds with many cations, so the amount of phosphorus in the soil solution at any given time is very small. Plants apparently absorb phosphorus only from the soil solution and a simple calculation shows that it must be renewed several times a day in order to nourish the plant. It is this factor that can be limiting and is carried out by the processes of dissolving and diffusion. The factors involved in this renewal are: the amount of phosphorus that can be solubilized, the degree of solubility and the speed of diffusion from the solid surface to the root of the plant. A phosphorus extraction can roughly measure the first two factors but never the third, so a subsequent field calibration will always be necessary for the analysis to be useful. The types of phosphorus compounds present and other chemical properties of the soil will determine the characteristics of the extractant used.

Materials and equipment:



- Analytical balance with a minimum sensitivity of 0.001 g,
- Bottle shaker
- Falcon tubes, 50 ml
- Test tubes, 50 ml
- 1 L beaker
- 1 L volumetric flask
- Erlenmeyer flasks, 250 ml
- Large funnels for first filtration
- Small funnels for second filtration
- General purpose phased filters
- ALBERT fitlers Whatman number 2
- glass bottles, 100 ml
- pH meter
- AA3 BRAN+LUEBBE segmented flow autoanalyzer

Reagents:

- Commercial activated carbon for analysis
- Sodium hydroxide 2 M: Dissolve 80 g of NaOH in 1 L of distilled water (ELIX). It is the same concentration as the NaOH that is used for the determination of carbonates in soil. I it is not available, prepare about 100 ml weighing 8 g of NaOH, as it is only used to adjust the pH.
- Sodium bicarbonate solution 5 M (buffer solution pH 8.5 or Olsen extractor solution): Weigh 42 g of sodium bicarbonate ($NaHCO_3$). Add approximately 90 ml of ELIX water into a 1 L beaker. Adjust the pH of the solution to 8.5 with 2 N NaOH. Make up to 1 L with ELIX water. Check the pH if the solution is going to be used another day.

Procedure:

1. Weigh 2 g of soil, previously dried, ground and passed through a 2 mm sieve, in 50 ml falcon tubes labeled with the name of the sample.

2. Add 0.12 g of activated carbon to the falcon tube. (It can also be weighed in reverse order, first the activated carbon and then the sample, it does not influence the analysis).
3. Add 40 ml of the sodium bicarbonate extractor solution.
4. Make a blank: in a falcon tube, add 0.12 g of activated carbon and 40 ml of extracting solution.
5. Shake for 30 min in a bottle shaker. Beforehand, it is convenient to shake the falcon tubes manually with the stopper on.
6. Filter the first time through folded filters (general) and collect in 250 ml Erlenmeyer flask.
7. Filter a second time through Whatman No. 2 filters and collect in previously labeled glass container jars.
8. Measure by spectroscopy in an AA3 BRAN+LUEBBE autoanalyzer. See protocol *PNT_SU Olsen phosphorus determination in AA3 BRAN+LUEBBE autoanalyzer*.

Observations: Never wash labware (extraction bottles, flasks, funnels, etc.) for phosphorus analysis with soap. All the material should be washed with ELIX water or diluted HCl.

Determination of ammonium and nitrate in soils and plants

The largest part of nitrogen in soil is in organic form, which is unavailable for plants. The inorganic (mineral) forms of nitrogen available for plant uptake are ions of nitrates and amonia, which can be extracted with a KCl solution, and analized by a spectrophotometer.

a. KCl extraction

materials

- 50 ml falcon tubes
- shaker
- funnels
- Whatman 2V filter paper

reagents

- 1 M KCl solution: dissolve 74.56 g KCl in 600 mL of distilled water and make up to 1000 mL.

procedure

- Weigh 1 g soil (dry) into the 50 mL falcon tubes and add 10 mL of the KCl solution.
- Shake for 1 hour at 180 rpm
- Centrifuge the samples for 5 min at 4000 rpm, then filter through Whatman 2
- Measure immediately or store in the refrigerator (-18ºC)

b. Measurement of ammonium (based on DIN 38406/5)

For the determination of NH_4-N content in soil, we used the Nessler method.

reagents

- Stock solution: dissolve 4.719 g dry ammonium sulfate ($(NH_4)_2SO_4$) in 400 mL of distilled H_2O in a 1 L flask and make up to the mark with water to 1000 mL (= 1000 mg/L NH_4-N stock solution). *Maximum conservation time: 1 week.*

- indicator (indicator solution): dissolve 4 g sodium salicylate ($C_7H_5O_3Na$), and 0.1 g of sodium nitroprusside ($Na_2[Fe(CN)_5NO]\cdot 2H_2O$) in 60 mL of Elix water and make up to 100 mL. *Save in dark. Maximum conservation time: 1 week.*

- buffer solution: dissolve 1.48 g of sodium hydroxide (NaOH), 4.98 g of monohidrogen phosphate and 5 mL of hypochlorite solution (5% available chloride) in Elix water, and make up to 1000 mL with distilled water. *Exothermic reaction. Must be prepared on the day of use.*

- EDTA 6%: dissolve 3 g of Disodium diacid ethylenediaminetetraacetate in 50 ml Elix water. *Maximum conservation time: 1 month in fridge.*

calibration

- from the stock solution, prepare work solutions (1, 10 and 100 mg/l)

- pipette an appropriate amount of work solutions into the micro plate to prepare calibration standards with concentrations from 0.0 to 2.0 ppm NH_4-N. Make up the volume with Elix water. *Standards should be prepared each measurement day immediately before use.*

procedure

1. In a micro-plate, measure 50 µl of soil extracts, add 10 µl of EDTA solution, 60 µl of indicator, 100 µl of Elix water and 30 µl of buffer solution.
2. Shake for 1 min, then incubate for 30 min at 37°C
3. Wait 5 min
4. Measure at 667 nm wavelength
5. For the blank, measure 50 µl of extraction solution (1 M KCl) instead of sample

6. For calibration points: measure 50 µl of 1 M KCl and then an appropriate volume of work standards into the microplate and make up to a 100 µl with Elix water.

c. Measurement of nitrates

reagents

- Copper sulphate solution (Sol. 1): dissolve 0.5 g of $CuSO_4 * 5 H_2O$ in 40 ml Elix water and make it up to 50 ml.

- Zinc sulphate solution (Sol. 2): dissolve 0.5 g of $ZnSO_4 * 7 H_2O$ in 40 ml Elix water and make it up to 50 ml.

- Catalyst solution: dissolve 0.5 g of NaOH (lentils) in 40 ml of Elix water + 0.4 ml of 50% orthophosphoric acid and make up to 50 mL with Elix water.

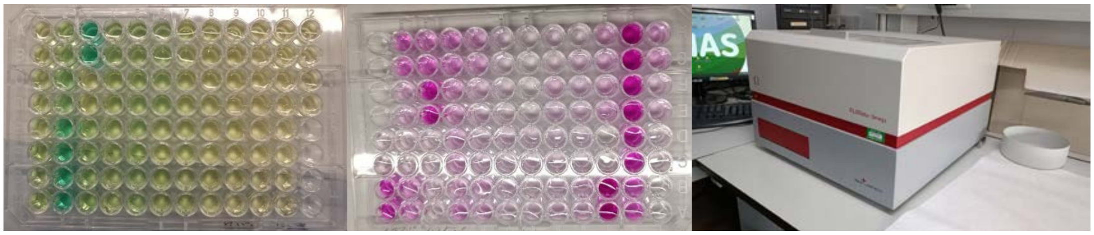
- Hydrazine sulphate solution: add 0.5 ml of Sol. 1, 0.5 ml of Sol. 2 and 0.1 g of hydrazine sulphate in 40 ml of Elix water, make up to 50 ml.

- SNEDD colouring solution: dissolve 0.5 g of sulphanilamide in 40 ml of Elix water (heat if necessary), add 0.025 g of N-(1-naphtyl) ethylenediamine and 10 ml of 50% orthophosphoric acid. Make up to 50 ml.

- Stock solution, 1000 ppm N-NO_3: dissolve 1.0804 g of KNO_3 in Elix water and make it up to 250 ml.

procedure

- in a microplate, measure 30 µl of samples (extracts) or 40 µl 1 M KCl.
- only for blanks and calibration standards, add 20 µl of KCl
- add 60 µ of the catalyst solution, agitate
- add 20 µl of hydrazine sulphate solution, agitate
- cover the plate with parafilm and agitate for 30 seconds
- put in incubator at 37°C for 20 min
- add 60 µl of SNEDD solution, agitate
- add 80 µl of Elix water to the samples or 50 µl to the calibration standards and blanks.
- measure N-NO_3 content on spectrophotometer at 540 nm.



Biomass parameters

The SPAD index, also known as the Soil-Plant Analysis Development index, is a non-destructive and quick method for measuring the chlorophyll content in plant leaves. It's commonly used to assess the health and nutrient status of plants. Measuring the quantum yield in plants, specifically the quantum yield of photosystem II (PSII), is crucial for assessing the efficiency of photosynthesis and the health of plants. The quantum yield of PSII is a measure of how effectively plants convert absorbed light energy into chemical energy through photosynthesis. The most common method to measure the quantum yield of PSII is through a technique called pulse amplitude modulation (PAM) fluorometry.

Furthermore, the fresh and dry weight of shoots and roots was measured and root/shoot ratio was determined, as well as the leaf area of the plants using an open-source image processing software Fiji.

Solid state nuclear magnetic resonance (NMR)

In order to analyze the soil and biochar samples by NMR, the samples were subjected to demineralization with hydrofluoric acid (HF) as follows:

a. Objectives

→ Eliminate the mineral fraction and paramagnetic elements (Fe, Al) to minimize interfirences in the NMR.

→ Concentrate the organic matter.

b. Reagents

→ Hydrochloric acid 1M (HCl):

- for 1 L of HCl solution: measure 84 ml of HCl (37% purity) and make up to 1 L with water.

→ Hydrofluoric acid 10% (HF):

- for 1 L of HF solution: measure 208 ml of HF (48% purity) and make up to 1 L with distilled water. If the HF is 40% purity, measure 250 ml and make up to 1 L.

c. Procedure

→ **Removal of particulate organic matter (POM):** in case of high content.

- Weigh:
    - Write down the weight of the bottle with the lid (sample may remain on the lid)
    - Tare and record the weight of simple (in g): 10-20 g (or less, 5 g, depending on C content).
- Add 40 ml (20 ml) of distilled water and shake
- Centrifuge the cans (previously tared) at 3000 rpm for 10 minutes.
- Collect the organic matter in suspensión into a previously tared beaker, by filtration (make sure not to drag simple from the bottom of the beaker).

→ **Treat with HCl, 1 M:** in case the simple contains carbonates ($CaCO_3$) to avoid the formation of CaF_2 from HF, which implies an increase in the weight of the sample due to the presence of white precipitates

- Add 40 ml (20 ml) od 1 M HCl (do not cover the cans completely, gases are generated).
- Shake slightly and leave to react/settle.
- Centrifuge the cans at 3000 rpm for 10 minutes.

- Remove the supernatant (re-add HCl as many times as necessary).
- Wash with distilled water several times to remove excess HCl (≈ neutral pH).

→ **Treat with 10% HF:** use the specific protection material and proceed with extreme care

- Add 40 ml (20 ml) of 10% HF to the sample left in the bottle.
- Shake the samples for 2 h at 250 rpm.
- Centrifuge at 3000 rpm for 10 minutes.

Repeat 4 times

- Carefully remove the supernatant from the bottles
- Add 50 ml (20 ml) of distilled water, shake, tare, centrifuge and carefually remove the supernatant (wash until pH reaches 5-6).
- Freeze samples and lyophilize.
- After lyophilizing, weigh the jar+sample (with a lid, and the remains of the sample that have remained in it), to determine the content of organic matter that has resisted the acid attack.

Notes

- Before starting work: place the no-entry sign on the laboratory door. Put the gluconate ointment (stored in the fridge) and the emergency kit material (HF safety sheet, emergency sheet, powders, red gloves, spare face shield) on the entrance table. And notify the rest of the group that you are going to start working with HF.
- Always wear a lab coat, gloves specific for working with HF over laboratory gloves (never latex) and a face protection mask.
- Do not touch anything outside the marked work area with the gloves, in case they are stained with HF.
- Do not use glass containers, HF attacks the silica.
- The values in parentheses are for a quantity of 5 g of sample.

- Take into account the purity of the reagents, to make the calculations of the necessary reagent.
- Check that the material to be used is in perfect condition.
- When you finish working, clean all the material (gloves, inside of centrifuge...) and the surfaces (table, scale, cabinet covers...) and put away the emergency kit material.

Liofilization - Operating instructions

NOTES: Always wear a face shield while operating the machine.

The air-conditioning must be ON when the machine is in operating mode (22°C).

If preparation takes too long, place the samples back in freezer.

Switching on the machine

Check the level of oil in the vacuum pump.

Put the plug in the drain valve

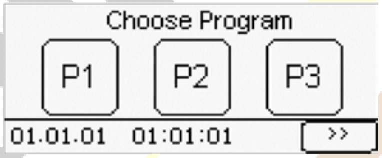
Switch-on the device by means of turning the main switch to “ON”.

Now starts the Refrigeration machine and cools down the condenser.

Place the frozen samples on the holder.

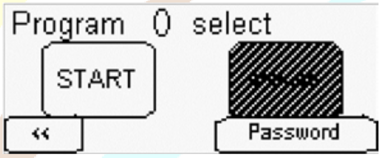
Place the cylinder and the top with the valves in their place.

The display appears after the startup of the controller as follows:


Choose Program

Starting a program

Select the program button for the appropriate program (P1).

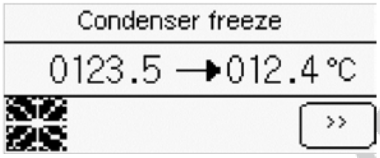

Program 0 select

Press the START button.


All hand valves closed ?

Before starting the program, check the cleanliness and the correct fitting of all gaskets. You also check the drain valve on right closure, the position of the rubber valves and the oil of the vacuum pump.

If you closed all hand valves, you can press the START button. The following image will now appear:



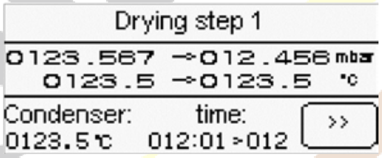
Condenser freeze

0123.5 →012.4 °C

Might be necessary to apply light pressure to the cylinder or between the cylinder and the top for the pressure to start dropping.

When the pressure is down to between 0.5 and 5 mbar, turn the switch on the vacuum pump from position II to position 0.

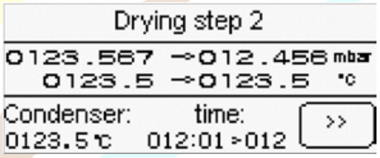
If the condenser has reached the set value, starts the first drying stage. The device then cools further down to the lowest possible temperature.



Drying step 1

0123.567 →012.456 mbar
0123.5 →0123.5 °C
Condenser: time:
0123.5 °C 012:01 >012

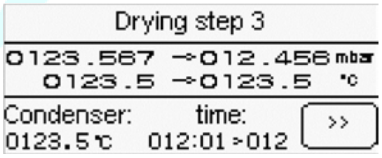
When the drying start, the pressure and the temperature will reach the set points. If the set time is reached, continue with the second drying stage.



Drying step 2

0123.567 →012.456 mbar
0123.5 →0123.5 °C
Condenser: time:
0123.5 °C 012:01 >012

When the drying start, the pressure and the temperature will reach the set points. If the set time is reached, continue with the third drying stage.

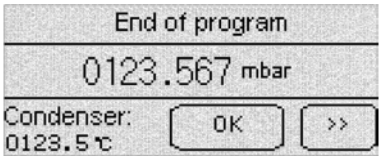


Drying step 3

0123.567 →012.456 mbar
0123.5 →0123.5 °C
Condenser: time:
0123.5 °C 012:01 >012

When the drying start, the pressure and the temperature will reach the set points.

If the set time is reached, the following picture appears:


End of program
0123.567 mbar
Condenser:
0123.5 ℃

Switching off the machine

The chamber pressure and the temperature will continue to be regulated to the last set value. When you press the "OK" button, the program is finished and turned off the vacuum pump.

Turn off the machine by pressing the OFF button.

You can ventilate the chamber now by partially opening one of the valves (preferably one facing away from you).

When the pressure is equalized, remove the top and the cylinder.

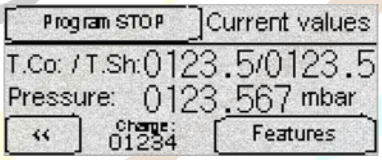
Retrieve your samples.

Clean any excess water from the top, place cylinder and valve top back on the machine.

Remove plug from the drain valve.

Turn the switch on the vacuum pump back to position II.

If you want to quit the program in between, press the right arrow key and then press the
"STOP" button.



Current values
T.Co: / T.Sh: 0123.5/0123.5
Pressure: 0123.567 mbar
Charge:
01234

→ Sample analysis

- The rotor is filled with ground sample using the support and the stick to push. Only the hole for the plug should remain free. A black half stripe is painted below with a permanent marker.

- When removing the previous sample, the speed must be progressively decreased.
- The sample is always inserted with the stopper facing up.
- With new sample you do not have to adjust all the parameters, instead you can open another previous sample and drag the spectrum. → "ased" → To control the parameters (it is only done in the first)
- edc → To create a new one. Change the name and title. SR: that of the last revision/change of probe.
- wobb → tuning
- Insert
- Go→ C: 0 a 4000Hz→8000Hz→12000Hz→14000Hz. N: 0 a 2000Hz→4000Hz→6000Hz
- Adjust Tunning and Matching (first blue screw and then the yellow one [you can press NEXT on the screen on the left on the ground])
- zg → start measurement. Sometimes extra signals come out (especially at 14000Hz). Sometimes there are echoes with the same frequency as the speed of rotation (the spinning size bands). ro: It has to match the speed you're on
- ft → transformation of Fourier (go from time to frequency). With this type of experiment there can be no negative signals → We adjust to 0
- lb →Line broadening: We can see that the noise/background is reduced, but the bands are widened.
- efp → to transform Acqu to Spectrum.
- tr → save provisionally (to see if the measurement is ok)→ efp
- To end: halt (no acquisition running)→ drop to 4000hz → Halt (in action)→ Eject



<u>Pyrolysis coupled with gas chromatography / mass spectroscopy (Py/GCMS)</u>

Analyzing soil organic matter by pyrolysis coupled with gas chromatography/mass spectrometry (Py/GCMS) allows for the characterization of complex organic compounds present in soil. This method involves two main steps: pyrolysis, where organic matter is thermally degraded into volatile compounds, and GC/MS, which separates and identifies these compounds.

A weighed portion of the dry soil/biochar sample was placed into a sample container (crucibel) suitable for pyrolysis. The sample size depends on the specific instrument and method used. The pyrolysis process, which involves heating the sample rapidly to high temperatures (typically between 400°C to 800°C) in an inert atmosphere (usually helium or nitrogen), causes the organic matter in the soil/biochar to break down into volatile compounds. These compounds, released during pyrolysis, are carried into the gas chromatograph for further analysis. The resulting data provides information about the types and relative abundance of organic compounds present in the soil/biochar. Py/GCMS is a technique which helps in understanding the composition of organic materials in soil, and can have significant implications for agriculture, environmental science, and soil management. It provides detailed information about the complex mix of organic compounds present, enabling deeper understanding of soil quality and behavior.

Thermogravimetric analysis (TGA)

Thermogravimetric analysis (TGA) is an analytical technique used to determine the composition and thermal properties of materials, including soil. TGA measures the weight change of a sample as it is heated or cooled over a specified temperature range. In the case of soil, TGA can provide information about its organic matter content, moisture content, and thermal stability.

Procedure:

Sample Preparation - Grind the dried soil samples into a fine powder to ensure uniformity and increase the surface area for analysis. Weigh a known amount of the powdered soil sample (typically 5-10 mg) accurately. Record the initial mass.

Sample Loading - Place the weighed soil sample into a sample crucible. Make sure it is evenly distributed across the bottom of the crucible.

Instrument Setup - Install the sample crucible containing the soil sample onto the balance in the TGA instrument.

TGA Analysis - Set the desired temperature range and heating rate for your analysis. The temperature range and heating rate will depend on your research objectives. Start the TGA instrument, and it will begin to heat the sample at the specified rate while continuously measuring the weight of the sample. Monitor the weight change of the sample as it undergoes thermal decomposition or phase transitions.

Data Interpretation - The TGA instrument will generate a thermogram, which is a plot of weight loss (or gain) as a function of temperature. Analyze the thermogram to identify key weight loss events. In the case of soil, typical weight loss events may include:

- Loss of moisture content (dehydration)
- Degradation of organic matter (oxidation or decomposition)
- Mineral decomposition or phase transitions

Calculate and Interpret Results - Calculate the percentage of weight loss for each significant event based on the initial mass of the soil sample. Interpret the results to determine the composition and thermal properties of the soil, including the moisture content, organic matter content, and thermal stability.

Respicon - microbial respiration

Aim - Study the rate of degradation/decomposition of organic material, through respiration of microorganisms, CO_2 emission from microbial activity.

Reagents

- Inoculum (solution of microorganisms): take a small amount of soil (e.g. pot). Add water, stir and leave to rest. Centrifugate, filter and collect the filtrate. Store in the respicond chamber to acclimatize.
- 0,6 M KOH (PM = 56.11 g/mol; 85% purity). For 1 L solution → 39.61 g KOH. CAUTION: corrosive reaction! Prepare the solution under a hood and with caution, EXOTHERMIC. Allow to cool before making up to the mark.

Protocol

- Calculate the water holding capacity of the samples (WHC).
- Prepare the sample:
  - Weigh 10 g of sample (sieved, Ø<2mm) into small glass beakers (note the weight of the beaker). Prepare replicates (≈ 4) of each sample. If the sample has less than 4 % organic carbon, C-org, weigh 20 g.

  - Add the amount of distilled water (previously acclimatized in the respicond chamber) necessary to achieve 60% of the WHC, deducting the volume of the inoculum to be added.
  - Add 0,5 - 1 mL of the microorganisms solution (inoculum) to promote initiation of microbial activity.
- Installation of the Respicond experiment/test:
  - Add 10 mL of KOH into the electrode containers located in the lid of each cup.
  - Insert the beakers with the prepared samples into the plastic cups of the respicond (vessel), mark very clearly beaker+vessel/channel (it is possible that during the experiment, some sample beakers have to be relocated, so the samples must be clearly identifiable).
  - Configure the program in the Respicond:
    - Select the program: WinRrj.ewe-shortent.
    - File → open new: WRITE SAMPLE NAME → save.
    - Edit → description → close.
    - Configuration → run option → scan interval → close.
    - Start time (first run a “manual scan” to verify that the used channels are working properly).
- Change the KOH solution in each beaker when the conductivity decreases below 60 mS or the curve stabilizes.
- At the end of the experiment, the data will be exported, treated with sigmaplot to see the degradation curves. The samples are dried (< 40 ºC) and weighed in the same beakers.

<u>Notes</u>

- Store the KOH solution and distilled water in the Respicond chamber to acclimatize (25ºC).
- The respicond bath always has to have the water level touching the metal plate where the beakers are placed. Fill with acclimatized distilled water.
- Each time the experiment is started, all the solutions in all the beakers must be changed and the electrodes must be checked to ensure that they are 1 cm apart.
- Put a small amount of bleach or borate in the bath on a regular basis and turn on the recirculation pump to prevent the growth of microorganisms.
- After 1000 data points, the device no longer saves data, a new folder should be created.

- Make an Excel sheet for each respicond experiment, (where it is located, the weight of the sample, the number of the beaker where it is places...) → see Excel sheets from the computer and notebook.
- Cleaning of equipment:
    - Remove the water from the water bath.
    - Clean the cables of the vessels with 70% ethanol.
    - Pour clean distilled water in the bath, add borate or bleach.



2. Results

2.1 Soil characterization

pH and electrical conductivity (EC)

Soil and biochar samples were prepared in triplicated for pH and EC measuring. Results obtained for triplicate measurments are given in Tables 1 and 2 and Figure 1.

Table 1. Results of pH measurement

			pH					
Sample	**Repetition**	**m (g)**	**1**	**2**	**3**	**Avg.**	**SD**	**RSD**
Control soil	**CTR 1**	1.014	6.95	6.78	6.74	6.82	0.112	1.63
	CTR 2	1.016	7.31	7.09	7.05	7.15	0.140	1.96
	CTR 3	1.008	7.10	7.01	6.96	7.02	0.071	1.01
					Average:	**7.00**	**0.172**	**2.46**
FBC-soil	**FBC-s 1**	1.016	7.22	7.35	7.42	7.33	0.101	1.38
	FBC-s 2	1.013	7.41	7.43	7.47	7.44	0.031	0.41
	FBC-s 3	1.014	7.52	7.53	7.51	7.52	0.010	0.13
					Average:	**7.43**	**0.098**	**1.32**
ABC-soil	**ABC-s 1**	1.004	6.87	6.83	6.84	6.85	0.021	0.30
	ABC-s 2	1.001	6.81	6.82	6.81	6.81	0.006	0.08
	ABC-s 3	1.001	6.82	6.83	6.80	6.82	0.015	0.22
					Average:	**6.83**	**0.021**	**0.30**
CCBC-soil	**CCBC-s 1**	1.000	6.79	6.83	6.81	6.81	0.020	0.29
	CCBC-s 2	1.006	6.85	6.87	6.85	6.86	0.012	0.17
	CCBC-s 3	1.010	6.90	6.88	6.83	6.87	0.036	0.52
					Average:	**6.85**	**0.035**	**0.51**
Biochar	**BC 1**	0.999	9.25	8.93	8.79	8.99	0.236	2.62
	BC 2	1.000	8.85	8.87	8.77	8.83	0.053	0.60
	BC 3	1.005	8.95	8.94	9.01	8.97	0.038	0.42
					Average:	**8.93**	**0.143**	**1.61**

Table 2. Results of EC measurements

			EC (μS/cm)					
Sample	**Repetition**	**m (g)**	**1**	**2**	**3**	**Avg.**	**SD**	**RSD**
Control soil	**CTR 1**	1.013	107.2	107.6	105.7	106.8	1.002	0.94
	CTR 2	1.028	104.0	103.3	105.1	104.1	0.9	0.9
	CTR 3	1.031	90.3	90.2	91.2	90.6	0.55	0.61
					Average:	**100.5**	**7.6**	**7.5**
FBC-soil	**FBC-s 1**	1.014	221.0	219.0	225.0	221.7	3.06	1.38
	FBC-s 2	1.023	222.0	215.0	221.0	219.3	3.79	1.73
	FBC-s 3	1.013	205.0	212.0	216.0	211.0	5.57	2.64
					Average:	**217.3**	**6.10**	**2.81**
ABC-soil	**ABC-s 1**	1.042	79.7	79.3	80.6	79.9	0.666	0.83
	ABC-s 2	1.002	85.8	85.3	86.8	86.0	0.76	0.89
	ABC-s 3	1.001	77.2	77.0	78.3	77.5	0.70	0.90
					Average:	**81.1**	**3.83**	**4.73**

Sample	Repetition	m (g)	EC (µS/cm)					
			1	2	3	Avg.	SD	RSD
CCBC-soil	**CCBC-s 1**	1.000	88.6	87.6	89.3	88.5	0.85	0.97
	CCBC-s 2	1.019	81.7	81.8	81.4	81.6	0.21	0.26
	CCBC-s 3	1.002	94.3	92.9	90.9	92.7	1.71	1.84
					Average:	**87.6**	**4.93**	**5.63**
Biochar	**BC 1**	1.030	403.0	386.0	386.0	391.7	9.81	2.51
	BC 2	1.012	428.0	422.0	429.0	426.3	3.79	0.89
	BC 3	1.007	366.0	358.0	364.0	362.7	4.16	1.15
					Average:	**393.6**	**28.18**	**7.16**

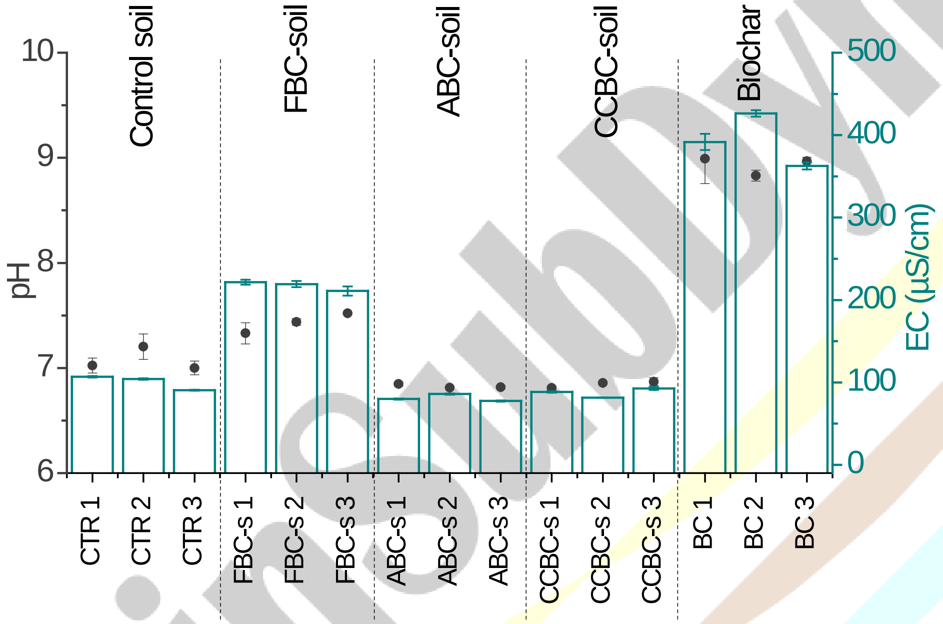


Figure 1. pH and electrical conductivity of soils and biochar

The obtained results show that the biochar has a significantly higher pH (8.93) and EC (394 µS/cm) compared to the control soil (pH 7 and EC 100.5 µS/cm). The freshly treated soil (fresh BC) showed an increase in both parameters, and had a pH 7.43 and an EC 217 µS/cm. On the other hand, both soils treated in 2010 had pH and EC very similar to the control soil. The pH of the soil treated with biochar (aged BC) and the soil treated with co*composted biochar (co-comp. BC) was 6.83 and 6.85, and the EC was 81.1 and 87.6, respectively. These results indicate that, whereas immediately after biochar treatment soil pH and EC increase, these parameters decrease with time, subsequently resulting in soil returning to its initial properties.

Water Holding Capacity (WHC)

WHC was determined for the four variants of soil and the biochar in 6 replicates (Figure 2).

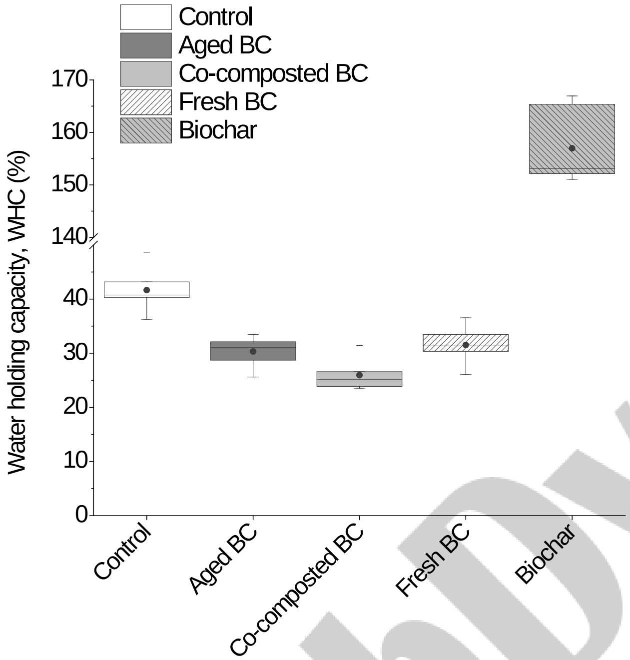


Figure 2. Water holding capacity for soils and biochar

Reults indicate that the applied biochar has about 4 times higher WHC in comparison to the control soil. This suggests that, in theory, application of biochar should increase the WHC of the treated soil. In general, biochar is often added to improve water retention in soil due to its porous structure and ability to hold moisture. However, WHC of all treated soils (fresh BC, aged BC and co-composte BC) all showed slightly lower WHC than the original, untreated soil. The impact of biochar on WHC can indeed vary widely depending on specific factors, including the type of biochar, the application rate, and the initial properties of the soil. Specifically, biochar can alter the physical properties of soil by increasing pore space. This can lead to an increase in macropores, which allow water to drain more readily and can, subsequently reduce the soil's WHC. Further, biochar's porous structure can reduce the capillary rise of water in soil. Capillary action is the ability of soil to draw water upwards against gravity. By reducing the capillary action, biochar can make it more challenging for the soil to hold onto water. The size of the biochar particles can also influence water retention. Finer biochar particles can have a larger surface area, which can help retain water, whereas larger particles, such as the biochar used in this study, may not have the same effect. In addition, the quantity of biochar added to the soil can also affect WHC. Higher application rates of biochar can lead to more significant changes in soil structure and may reduce WHC, which could be the reason for the decrease in water retention of the treated soils.

Micro- and macronutrient content in soil

Micro- and macronutrient content in all four variants of soil were very similar (Table 3). In general, the content of almost all measured elements were slightly higher in the control soil than in the treated soils, however, there was no significant difference observed between the different treatments.

Table 3. Micro- and macronutrients in soils and biochar

Sample	**Al**	**As**	**B**	**Ba**	**Ca**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**K**
						mg/kg					
Control	14536	14.7	4.99	48.8	2230	< 0,10	5.15	53.9	11.0	11294	1880
Aged BC	12912	11.3	3.85	44.6	2131	< 0,10	5.07	31.2	9.15	9573	1674
Co-comp. BC	12344	12.6	3.77	44.1	2245	< 0,10	4.63	48.9	10.0	10167	1612
Fresh BC	13931	11.2	4.82	57.0	2933	< 0,10	3.95	42.6	9.76	9957	1907
BC	336	< 0,10	6.96	101	10028	< 0,10	0.358	5.41	5.08	2576	1860
Sample	**Li**	**Mg**	**Mn**	**Na**	**Ni**	**P**	**Pb**	**S**	**Sr**	**V**	**Zn**
						mg/kg					
Control	14.0	1401	526	44.4	8.96	955	23.2	352	19.8	43.0	38.8
Aged BC	11.9	1209	550	40.1	7.35	851	19.3	318	17.8	36.7	34.4
Co-comp. BC	11.0	1188	497	35.3	10.0	786	20.7	340	18.3	35.6	33.6
Fresh BC	11.8	1223	510	48.4	8.30	779	25.5	299	20.8	36.9	34.7
BC	0.602	903	821	27.7	0.734	387	4.07	189	21.9	0.465	12.4

Organic matter content

Organic matte content was determined according to the loss-on-ignition method in triplicates. Result are shown in Figure 3.

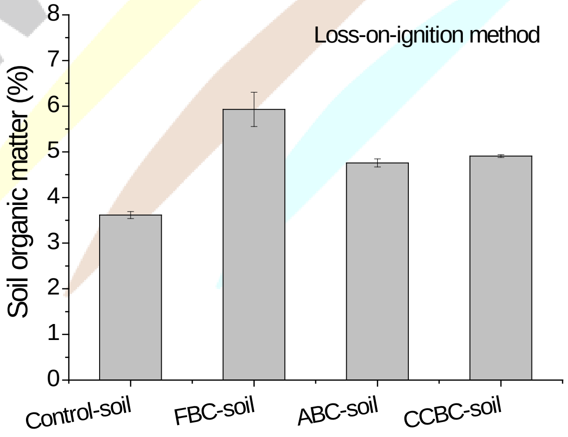


Figure 3. Loss-on-ignition organic matter content

Biochar addition to the soil had a positive effect on the organic carbon content, which increased from 3.62% in control soil to 5.93%, 4.76% and 4.91% in fresh BC, aged BC and co-composted BC treated soils, respectively. With aging, the organic matter content decreased, however, after 13 years is still higher than the control soil.

Total carbon (TOC) and total nitrogen (TN) content

TOC and TN measured in soil samples are shown in Figure 4.

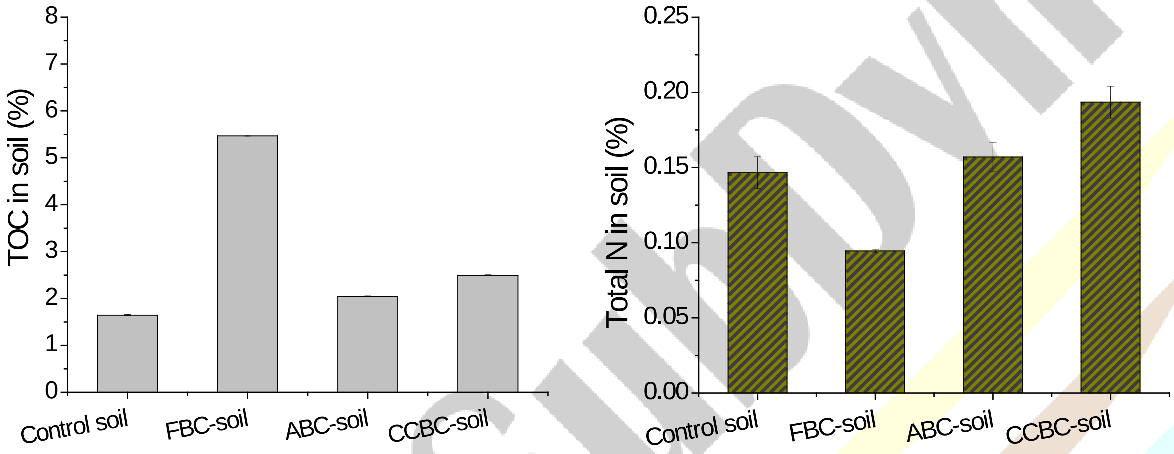


Figure 4. Total organic carbon content (left) and total nitrogen (right) in untreated and treated soils

As presented in the figure, TOC content was significantly higher in the soil freshly amended with biochar. The increase in TOC due to biochar addition can have several positive effects on soil quality and fertility. With aging, a significant decrease in TOC can be observed. When compared to the TOC estimated from the loss-on-ignition method, it can be observed that the measured TOC is about 60% higher than the estimated TOC. When biochar is incorporated into soil, several mechanisms can contribute to an increase in TOC such as carbon sequestration, microbial activity, adsorption of organic compounds, improvement of soil structure, reduced decomposition of existing soil organic matter. Addition of biochar decreased the relative TN content. With aging, those values returned to the initial level in the untreated soil (Figure 4). The soil treated with co-composted biochar contained the highest amount of nitrogen which can be related to the addition of a significant amount of nitrogen with the green waste during the co-composting process the biochar (70 t/ha).

Solid state nuclear magnetic resonance (NMR)

Results of the organic matter characterization by solid state nuclear magnetic resonance are presented in Figures 5 and 6. The biochars are characterized by their high aromaticity (chemical shift region between 160 and 90 ppm). This high content of aromatic structures leads to an increase of the aromaticity of the SOM in the soil amended with fresh biochar relative to the unamended soil. Note that in the spectra of the soils, the intensity of the anomeric C of carbohydrates is contribution to the chemical shift region between 140 and 90 ppm. With aging of the biochar, the contribution of aromatic C is decreasing (ABC-soil) which may indicate either a leaching of biochar to deeper soils or biochar degradation. The latter may be indicated by a relative increase of carboxyl C which may be formed by ongoing oxidation of the ring structures. In general, the obtained NMR data showed significant increase in aromaticity of the soil amended with fresh biochar which can be directly linked with the high content of aromatic structures in the biochar. With aging, NMR spectra showed decrease in more libel and easily decomposable organic compounds (carbonyl and carboxyl fractions, as well as aromatic compounds) and an increase in more complex and stable organic fractions (O-alkyl and alkyl), indicating a stabilization effect of biochar aging on SOM.

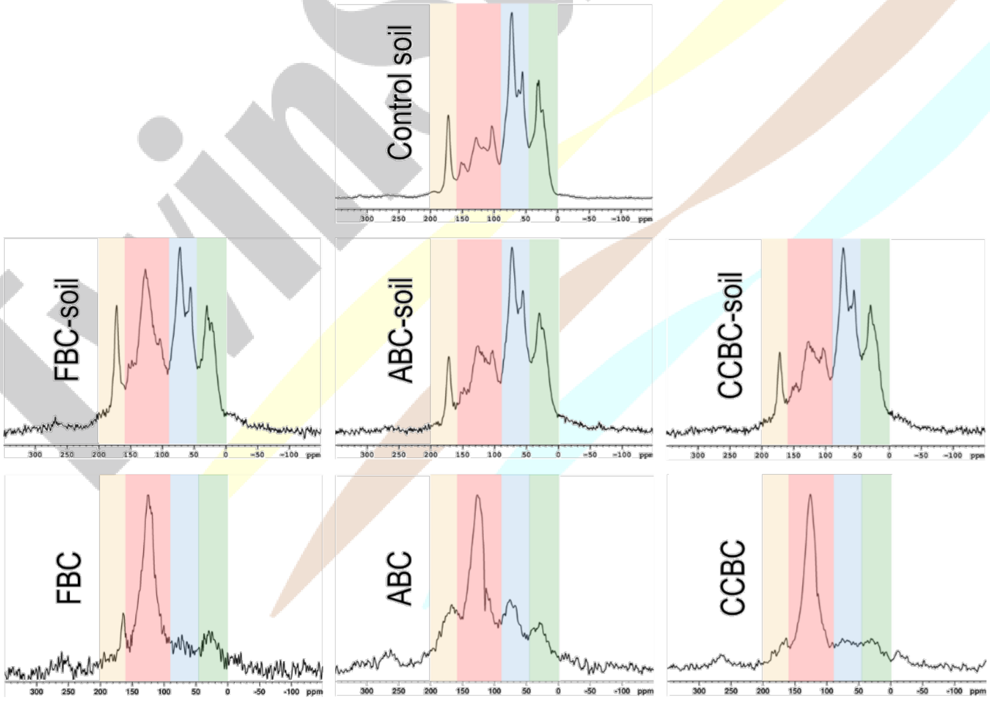
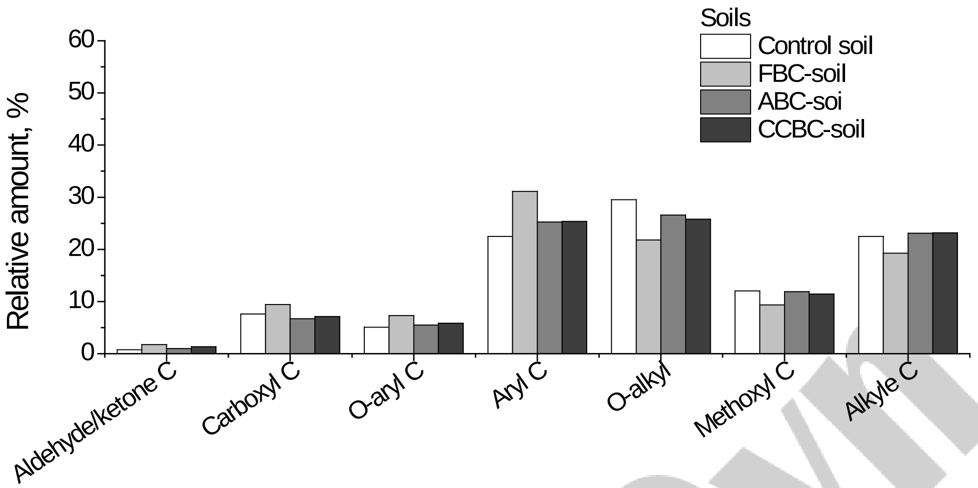


Figure 5. NMR spectra of the untreated and treated soils (up) and biochars isolated from the treated soils (down)



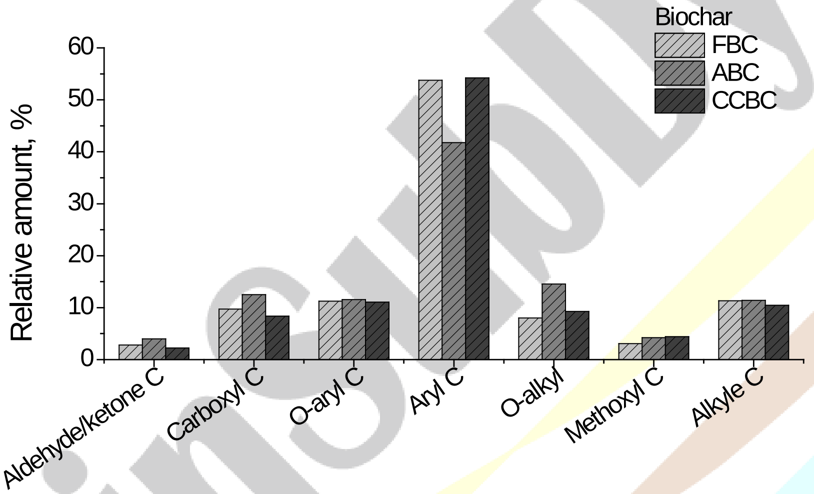


Figure 6. Relative amounts of various carbon forms in untreated and treated soils (up) and biochars isolated from the treated soils (down)

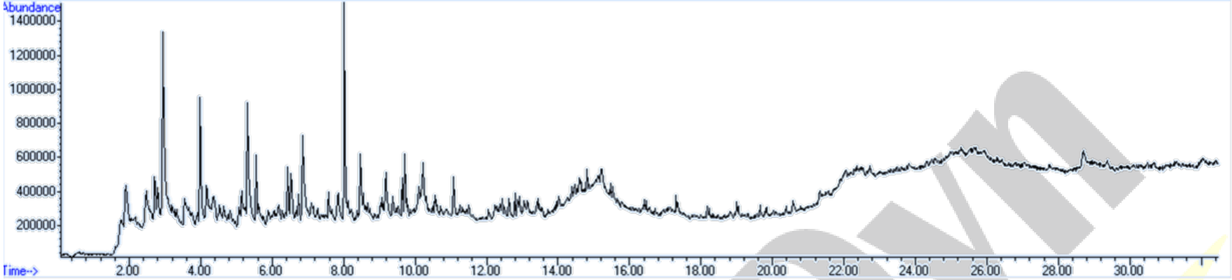
<u>Pyrolysis coupled with gas chromatography / mass spectroscopy (Py/GCMS)</u>

The used soil and the biochar were analyzes by Py/GCMS. Soil samples were pyrolyzed at 400°C, whereas biochar was pyrolyzed initially at 400°C, and then also at 600°C and 800°C. The pyrograms of the untreated and treated soils are given in Figure 7(a-d), and the pyrograms of the fresh biochar obtained after pyrolysis at 400°C, 600°C and 800°C are shown in Figure 8(a-c).

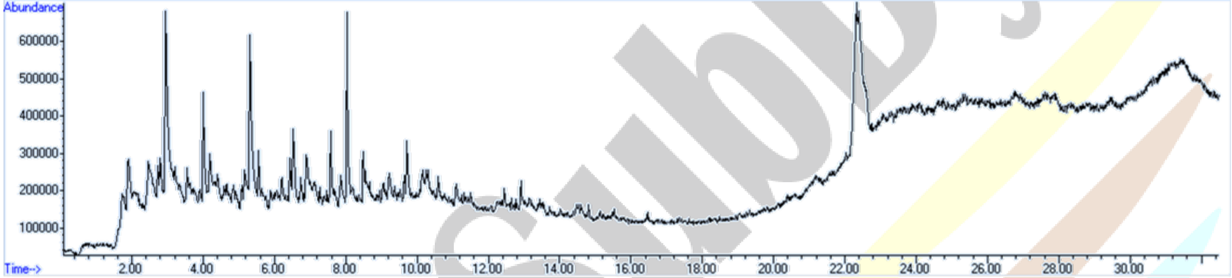
As can be observed from Figure 8, the pyrograms obtained from pyrolysis of the biochar did not show any peaks that could give further insight into the composition of this soil amendment. The lack of detected compounds, even at 800°C indicate during the production temperatures of about

800°C must have been reached, since all compounds that would have been pyrolyzable were absent. Thus, even though the production process was intended to be a low temperature pyrolysis, short periodes with high temperatures must have occurred during the long residence time of of 36 h.

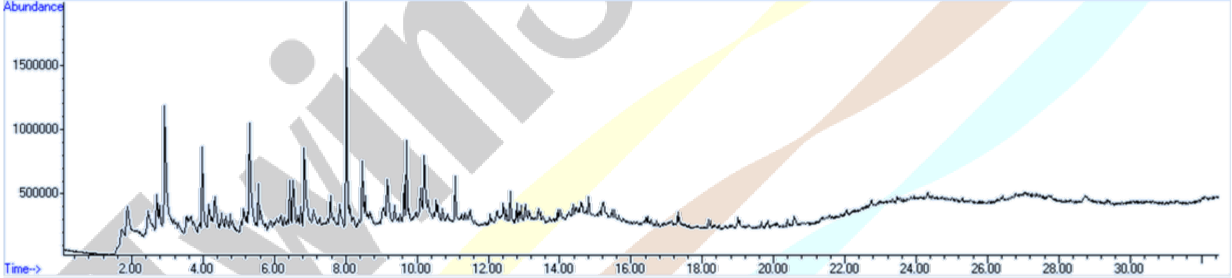
a) Control soil



b) Fresh BC amended soil



c) Aged BC amended soil



d) Co-composted BC amended soil

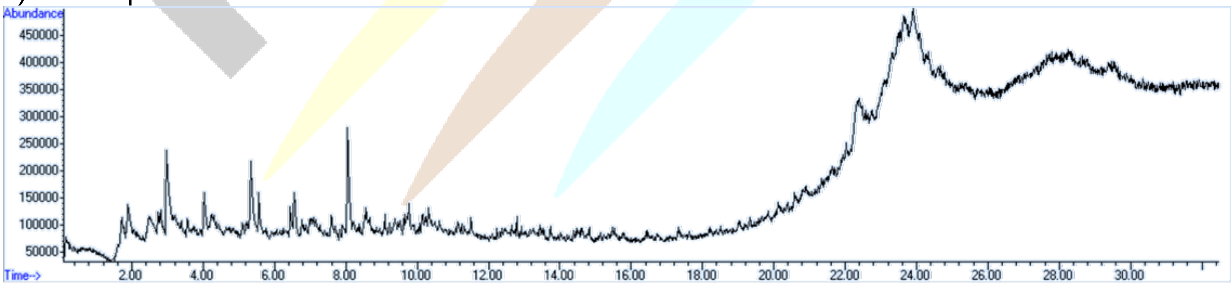
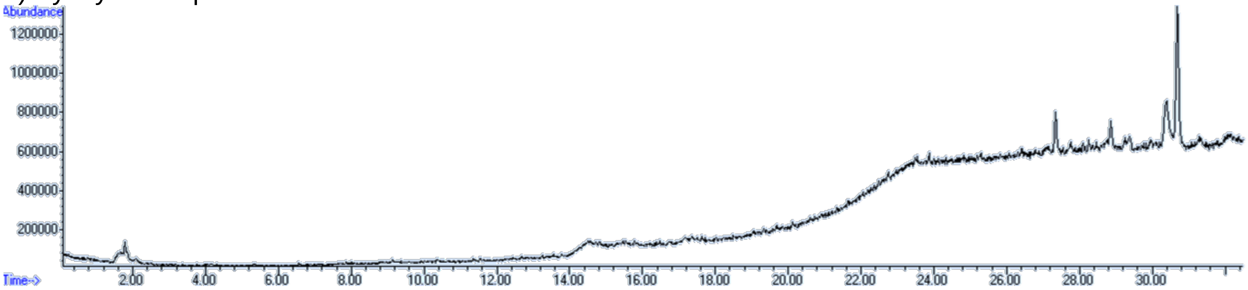
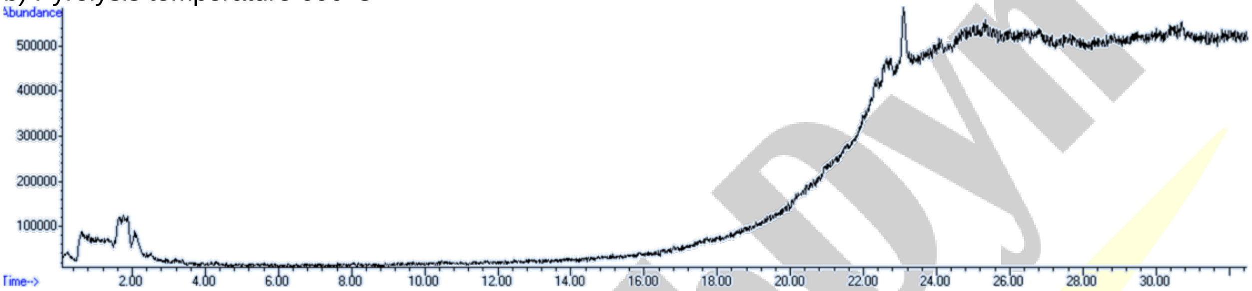


Figure 7. Pyrograms of untreated (7a) and treated soils (7b-7d) obtained at pyrolysis temperatue of 400°C

a) Pyrolysis temperature 400°C



b) Pyrolysis temperature 600°C



c) Pyrolysis temperature 800°C

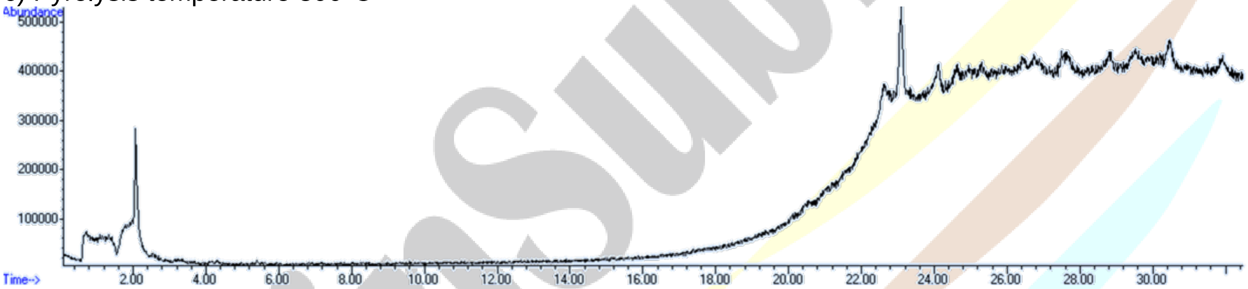


Figure 8. Pyrograms of the fresh biochar obtained at a) 400°C, b) 600°C and c) 800°C pyrolysis temperature

Figure 9 (up) shows fragments of the overlapped pyrograms with marked peaks assigned to compounds that are typically found in soil organic matter. Furfurals are formed during the thermal decomposition of carbohydrates. In soils they are mostly derived from f hemicellulose. Guaiacol is typically produced during the breakdown of lignin, a complex polymer found in plant cell walls. Lignin contributes with about 20% to the plant biomass. Creosol can be produced in soil through several processes, including the decomposition of lignin but also aromatic amino acids. Vanillin represents a further component of lignin. Figure 9 (down) presents peaks detected in the pyrogram originating from long-chain hydrocarbons (both saturated and unsaturated), typical for plant waxes. These results indicate that the soil organic matter primarly originate from plant biomass, likely from waste plant residues left after the removal of crops.

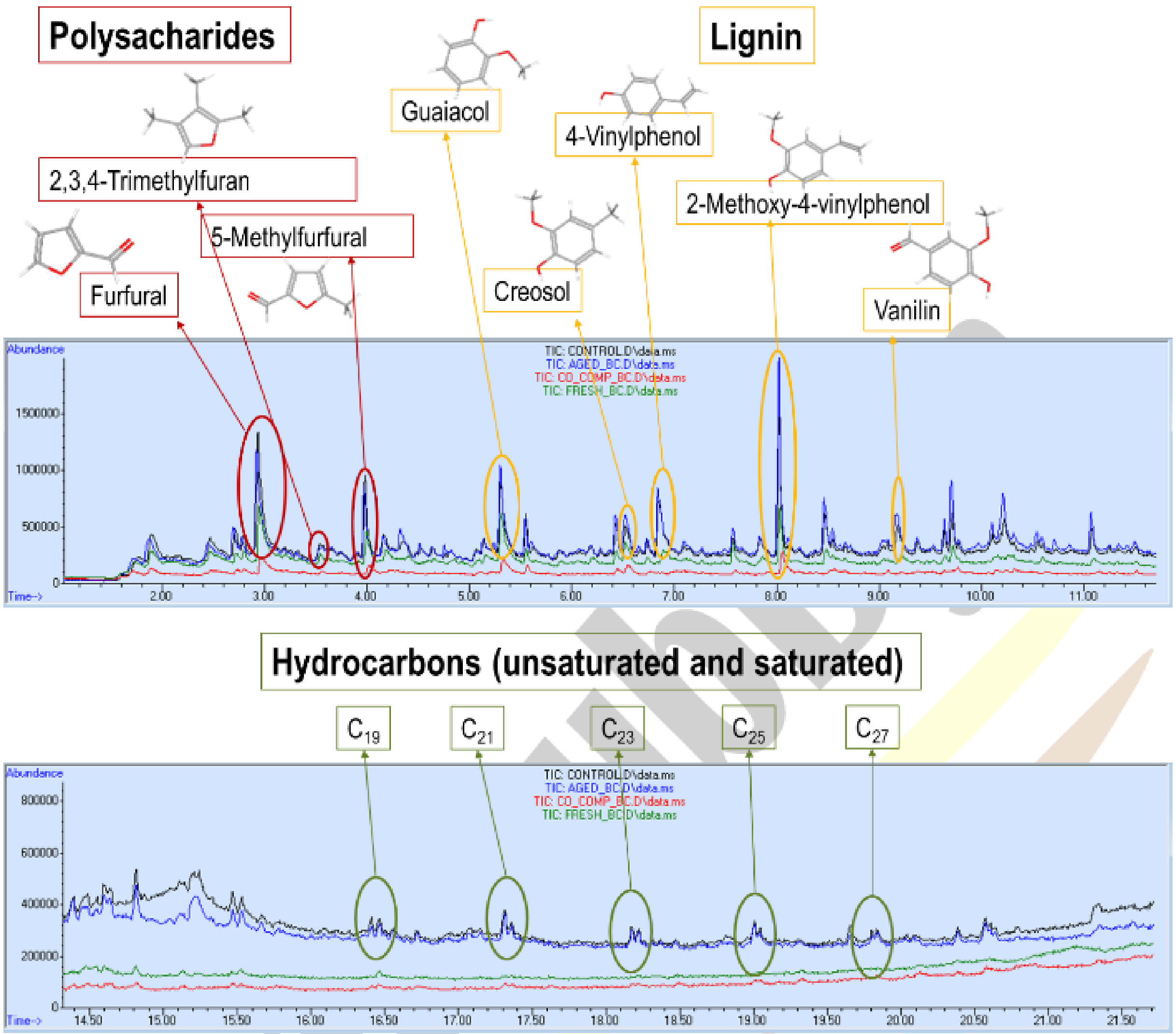


Figure 9. Pyrograms of the untreated and treated soils

Thermogravimetric analysis (TGA)

Thermogravimetric analysis (TGA) is an analytical technique used to determine the composition and thermal properties of materials, including soil. The used soil samples showed similar TGA curves (Figure 10), except for the fresh biochar amended soil, where an additional peak in the recalcitrant range occurs, which can be assigned to the added biochar. With aging, this peak disappears. Also, in the soil treated with co-composted biochar, the recalcitrant fraction is less abundant which is best explained with the co-addition of compost being less thermally stable than biochar. For the biochar, a significant decrease in the recalcitrant fraction can be observed, which might indicate that during the aging process, biochar turns less stable, whereas the labile fraction increases. The total mass loss is nearly the same for the control soil and the freshly amended soil and decreases with aging (Figure 11). This same trend can be observed in the biochar samples as well, where the fresh biochar lost almost 50% of its mass, whereas the aged biochar and co-composted biochar (both added to soil in 2010) showed less than 30% weight loss.

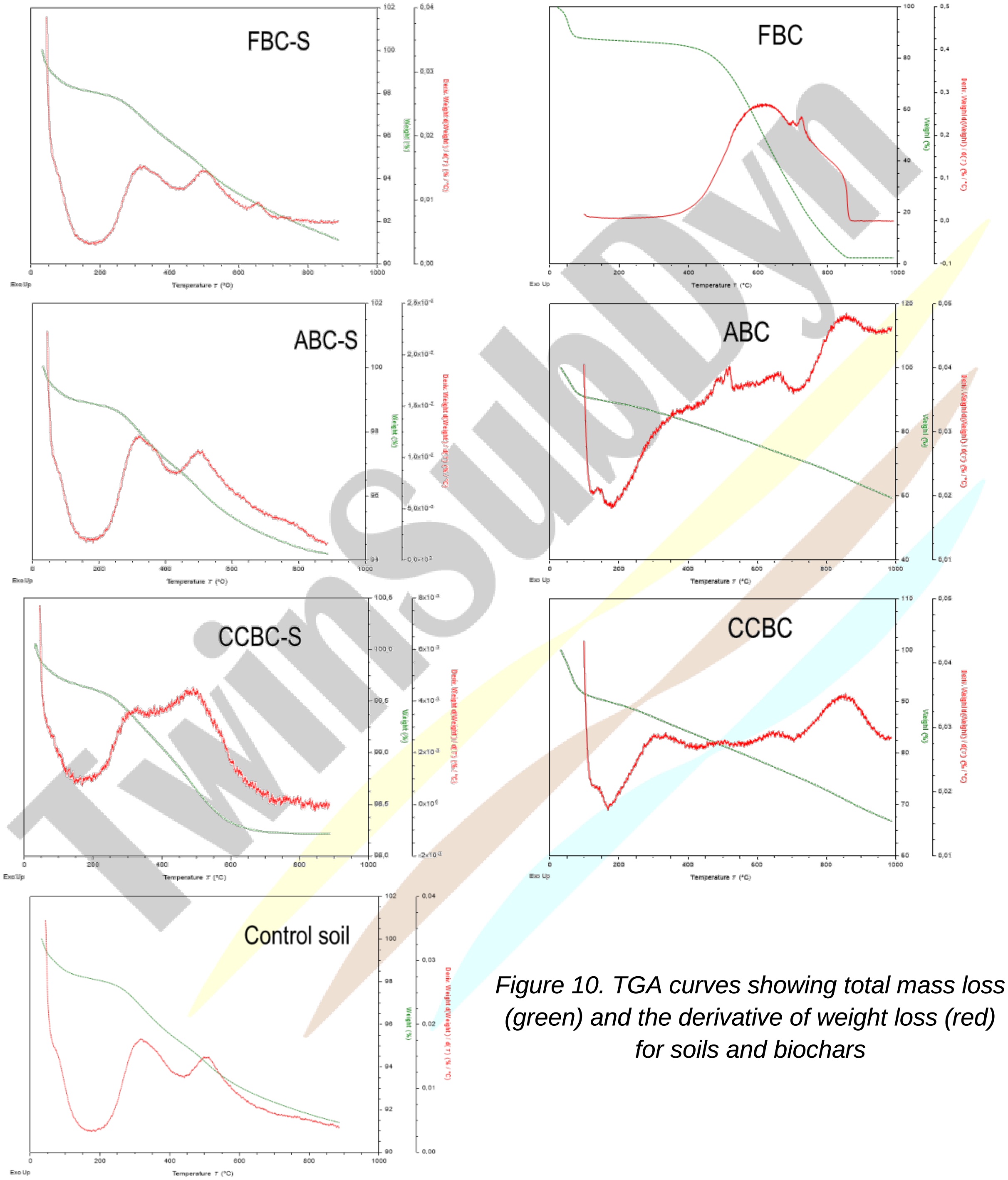


Figure 10. TGA curves showing total mass loss (green) and the derivative of weight loss (red) for soils and biochars

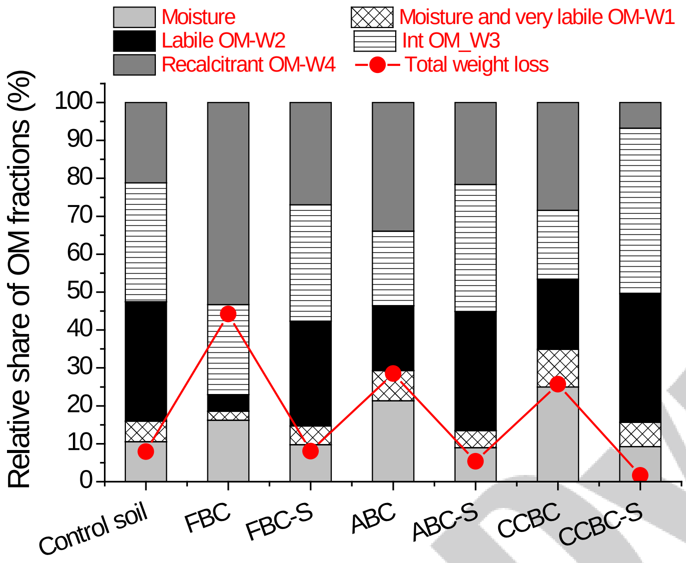


Figure 11. The relative share of different fractions of organic matter and the total mass loss in the soils and biochars

Microbial respiration

Measuring microbial respiration in soil helps in assessing the metabolic activity of microorganisms and can be used to estimate the rate at which microorganisms in soil are breaking down organic matter and releasing CO_2. Soil samples in triplicates of the four treatment variants were prepared for the respiration experiment. After weighing the soil into glass beakers of the Respicond, the soil is watered (to 60% of WHC) and inoculum is added to promote initiation of microbial activity, and the production of CO_2 is tracked over an extended period of time. Data processing is in progress.

2.2 Greenhouse incubation experiments

In order to investigate the impact of aging of amended biochar on plant growth, pot experiments were conducted with the selected soils and cultivating lettuce. The pot experiment was carried out for 60 days after sowing (DAS). After this period, samples of both soils and biomass were collected for further analysis and characterization.

Total organic carbon and total nitrogen in soil, 60 DAS

Results of TOC and TN in soils 60 DAS are given in Figure 12. TOC in control soil, aged biochar and co-composted biochar treated soils did not change significantly during pot experiment. However, the soil treated with fresh biochar showed significant decrease in TOC from 0 DAS (5.47%) to 60 DAS (3.38%), indicating higher degradability of the fresh biochar, which is in line with NMR and TGA results showing increase in stable fractions of soil organic matter with aging of biochar. Total N did not change significantly after the pot experiment in any of the soils.

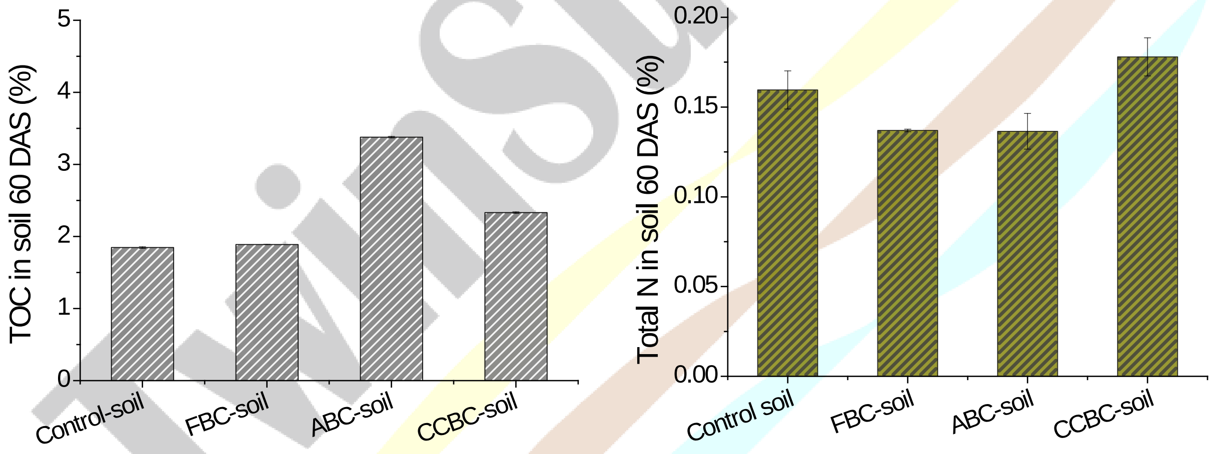


Figure 12. TOC and TN in soils 60 DAS

Micro- and macronutrients in soil, 60 DAS

In gerenal, for most micro- and macronutrients, a slight decrease in values was observed in soils 60 DAS, compared to the content of these components in soils analyzed prior to the pot experiment (0 DAS). This could indicate an uptake of nutrients by the plants or their leaching from the soil. Result are shown in Table 4.

Table 4. Micro- and macronutrients in soils 60 DAS

	Al	As	B	Ba	Ca	Cd	Co	Cr	Cu	Fe	K
Sample						mg/kg					
Control soil	11718	13.7	4.65	55.9	1784	0.300	3.77	87.3	7.56	10387	2075
ABC-soil	10543	9.89	3.61	50.5	1765	0.384	3.97	39.7	6.58	9192	1834
CCBC-soil	10953	11.6	3.92	52.2	2060	0.297	3.50	53.5	7.39	9454	1985
FBC-soil	11041	11.3	4.25	59.7	2389	0.344	3.77	45.0	6.66	9296	2062
	Li	**Mg**	**Mn**	**Na**	**Ni**	**P**	**Pb**	**S**	**Sr**	**V**	**Zn**
Sample						mg/kg					
Control soil	10.6	1135	497	43.3	7.54	826	22.8	261	14.7	55.6	36.1
ABC-soil	9.43	1035	452	31.7	5.63	751	19.4	248	13.6	48.5	33.3
CCBC-soil	9.60	1095	431	39.5	6.40	813	18.5	288	14.3	49.0	36.4
FBC-soil	9.55	1082	467	42.2	6.64	732	18.4	242	14.9	48.7	32.3

Available nitrogen in soil

The largest part of nitrogen in soil is in organic form, which is unavailable for plants. The inorganic (mineral) forms of nitrogen available for plant uptake are ions of nitrates and ammonia. The concentration of these two forms of available nitrogen in soil are similar, which can be significant for plant growth and nutrient availability, as different plants have varying preferences for nitrogen forms. In soils after the experiment, an increase in N-NO_3 and a slight decrease in N-NH_4 can be observed in comparision with soils before the experiment (Figure 13).

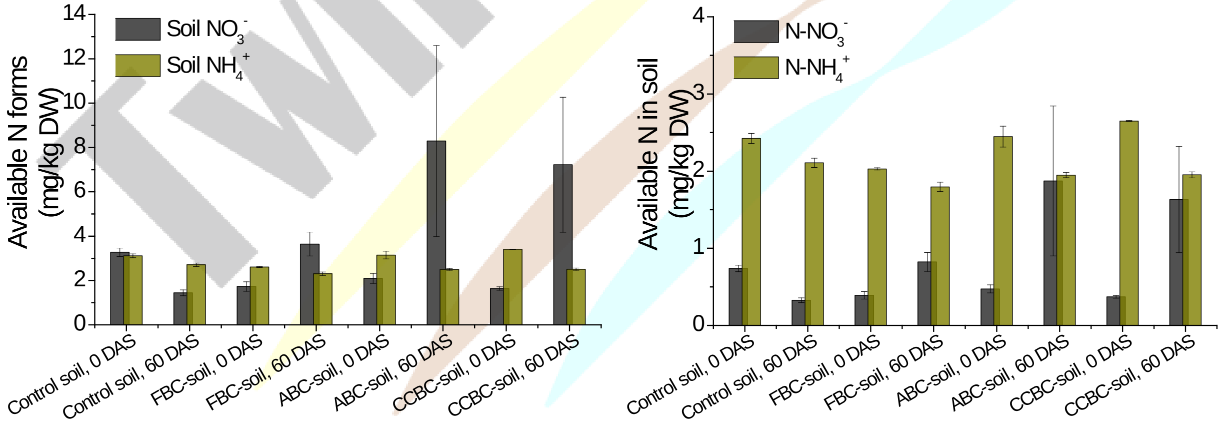


Figure 13. Available nitrogen forms in soil

The primary reason for the increase in nitrate nitrogen is nitrification, which is a microbial process in which ammonia nitrogen is oxidized into nitrate nitrogen by nitrifying bacteria. This process occurs in well-aerated soils and is favored by near-neutral pH conditions. Nitrification is responsible for the conversion of ammonia nitrogen into nitrate, leading to an increase in N-NO_3 levels.

Available phosphorus in soil

Available phosphorus content in soil varied between around 45 and 60 mg/kg (Figure 14).

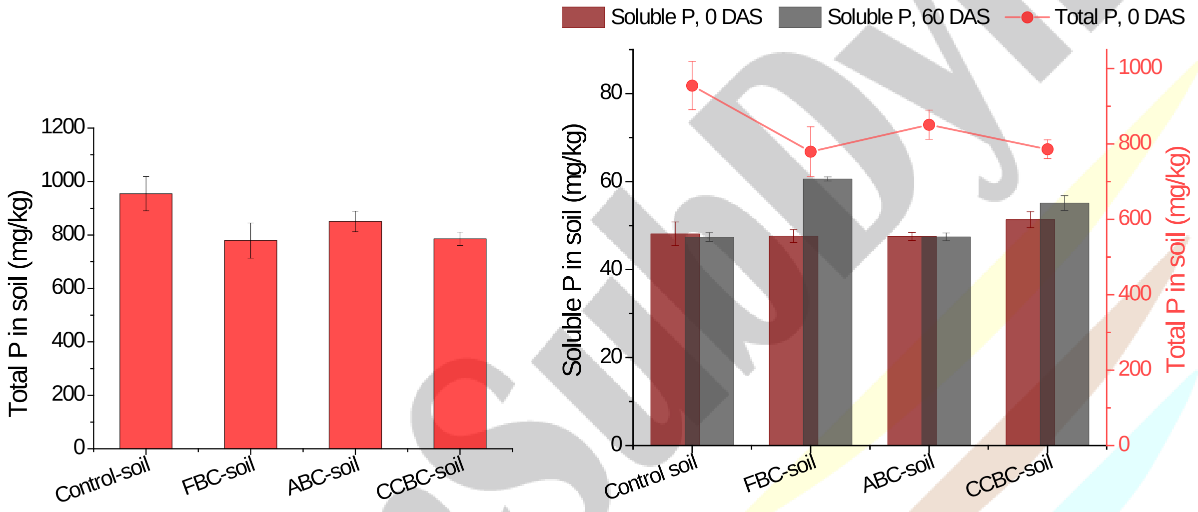


Figure 14. Content of total phosphorus obtained by ICP-OES (left) and available phosphorus (right) in untreated and treated soils

Comparing soils at the beginning (0 DAS) and at the end of the pot experiment (60 DAS), significant difference was only observed for the soil treated with fresh biochar. The other soil variants did not show change in available phosphorus content during the greenhouse pot experiment. Compared to the total phosphorus content, the available phosphorus makes up 4.98-7.78%. The highest percentage of available phosphorus in relation to total phosphorus was found in fresh biochar treated soil and was 7.07% and 7.78% for soil before and after the pot experiment, respectively.

Biomass parameters

Germination varied slightly depending on the treatment. Compared to the germination in the untreated soil (67%), the germination in the soil amended with fresh biochar decreased (56%)

whereas, the two soils treated with aged biochar and co-composted biochar yielded higher germination rates of 72% and 78%, respectively. This experiment included the analysis of plant growth (fresh and dry weight of shoots and roots) as well as plant stress parameters. The latter included the SPAD index, quantum yield in plants, specifically the quantum yield of photosystem II (PSII) which are parameters describing the photosynthetic activity (Figure 15).

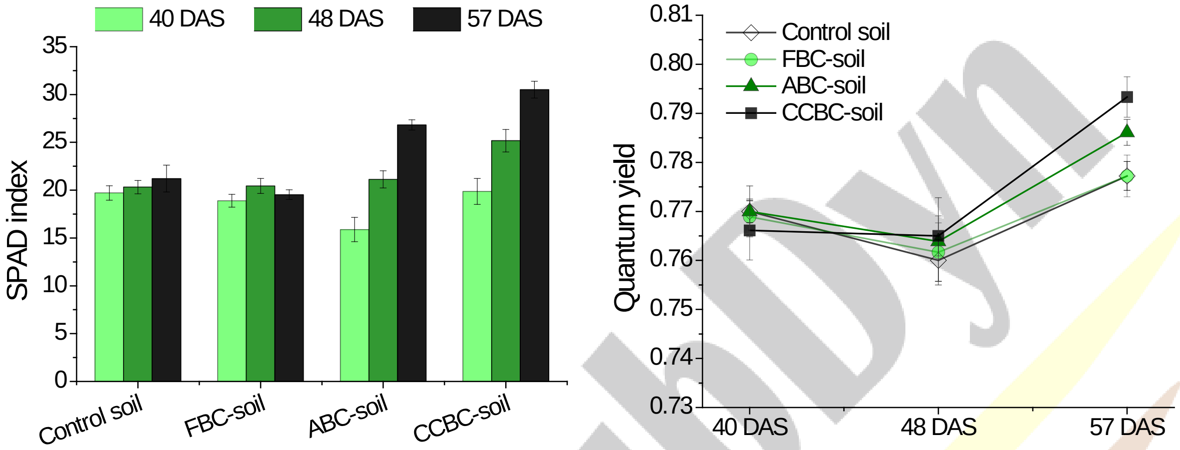


Figure 15. SPAD index and Quantum yield measured in plants 40, 48 and 57 DAS

For all samples, a slight decrease in quantum yield in the 7th week, and a significant increase in the 8th week was observed. For the SPAD index, control soil and freshly amended soil did not show any change during the three weeks that this parameter was monitored. On the other hand, the SPAD index of the aged biochar and the co-composted biochar treated soils was increasing during the same period. An increase in both the SPAD index and quantum yield during plant growth is generally indicative of healthy and vigorous plant development. A rising SPAD index during plant growth typically indicates an increase in chlorophyll content. This is a positive sign, as it suggests that the plant is producing more chlorophyll to capture more light energy for photosynthesis. An increase in quantum yield indicates improved photosynthetic efficiency. As plants grow and develop, they often become better at utilizing available light energy for photosynthesis.

The aged and co-composted biochar treated soils showed the highest yield for shoot, whereas the control soil, and the fresh biochar treated soil showed highest root yield (Figure 16).

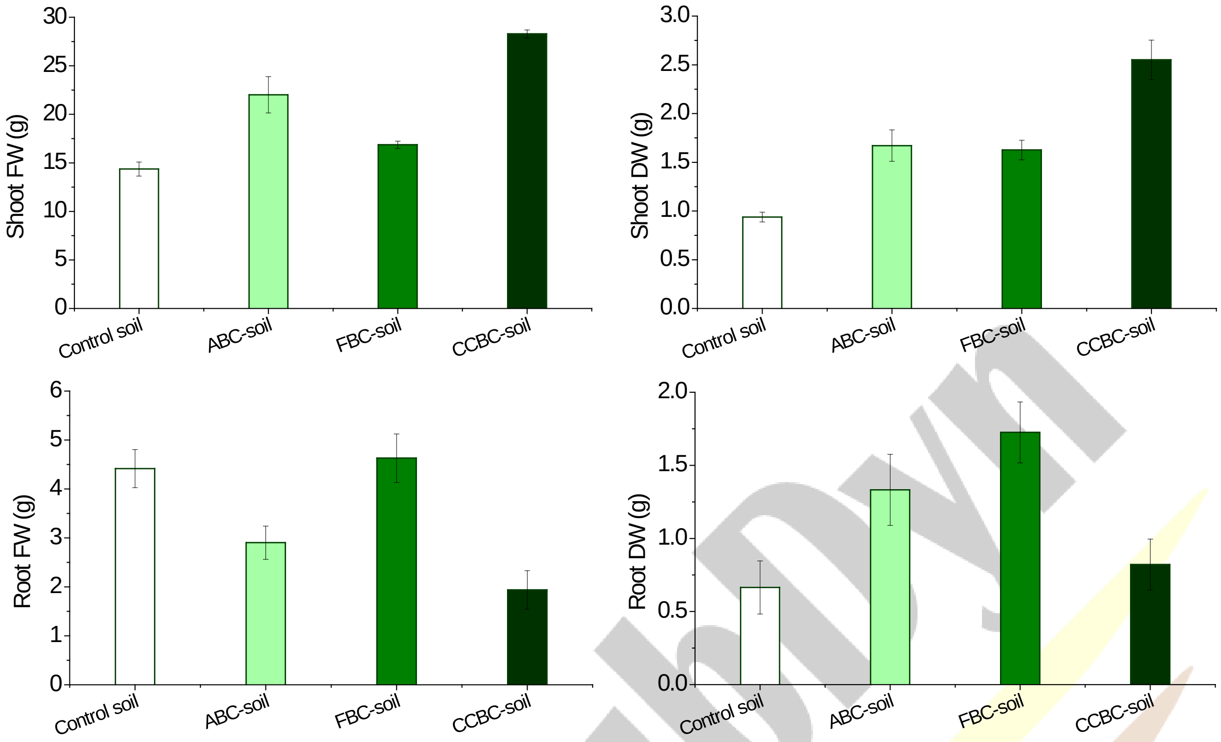


Figure 16. Yields of lettuce plants cultivated on untreated and treated soil

This reflects in the root/shoot ratio (Figure 17) which was the highest for fresh biochar amended soil. The root-to-shoot ratio is a valuable indicator in plant physiology and ecology that can provide insights into the response of plants to various environmental stressors, such as drought, nutrient deficiency, or other adverse conditions.

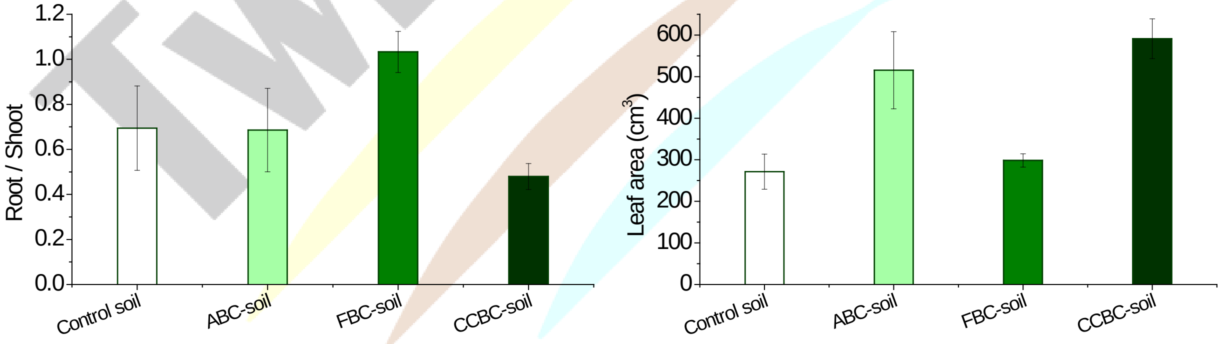


Figure 17. Growth parameters of lettuce plants cultivated on untreated and treated soil

Thus, the results of root-to-shoot ratio (Figure 17) show that plants grown in soil amended with fresh biochar have a higher root-to-shoot ratio (~ 1) than the plants grown in the untreated soil (~0.7), indicating that fresh the frech biochar amendment might be causing plant stress symptoms. On the other hand, plants grown in soil amended with aged biochar have approximately the same root-to-shoot ratio (~ 0.7), and plant grown in soil amended with co-composted biochar have slightly lower (~ 0.5) root-to-shoot ratios than plants grown in control soil, which indicated a positive effect of both aging and co-composting of biochar on plant health.

Micro- and macronutrients in plants

The content of nutrients in plant materials was determined for roots and shoots of the lettuce plants, and result are given in Table 5.

Table 5. Micro- and macronutrients in different parts of the plants

Sample		Micro- and macronutrient content										
Treatment	**Plant part**	**Al** mg/kg	**As** mg/kg	**B** mg/kg	**Ba** mg/kg	**Ca** %	**Cd** mg/kg	**Co** mg/kg	**Cr** mg/kg	**Cu** mg/kg	**Fe** mg/kg	**K** %
Control soil	**Shoot**	94.5	< 0.10	26.1	6.54	0.895	0.301	0.449	0.458	4.98	136	8.20
	Root	8136	< 0.10	14.0	42.7	0.243	0.220	0.175	22.5	10.8	7291	1.67
FBC-soil	**Shoot**	89.3	< 0.10	25.0	4.77	0.729	0.103	0.174	0.865	1.99	140	7.56
	Root	6618	< 0.10	9.40	45.3	0.382	< 0.10	0.141	19.3	7.75	7233	2.25
ABC-soil	**Shoot**	187	< 0.10	19.2	4.18	0.790	< 0.10	0.207	0.747	4.61	228	7.38
	Root	7817	< 0.10	14.0	45.8	0.366	0.146	0.234	22.5	9.03	7789	1.51
CCBC-soil	**Shoot**	136	< 0.10	25.2	3.47	0.792	0.154	< 0.10	0.646	5.15	179	5.51
	Root	7194	< 0.10	11.4	39.9	0.382	0.113	0.394	22.1	8.53	7272	1.28
Treatment	**Plant part**	**Al** mg/kg	**As** mg/kg	**B** mg/kg	**Ba** mg/kg	**Ca** %	**Cd** mg/kg	**Co** mg/kg	**Cr** mg/kg	**Cu** mg/kg	**Fe** mg/kg	**K** %
Control soil	**Shoot**	0.021	0.239	100	0.025	1.27	0.427	< 1.0	0.182	15.7	1.33	44.56
	Root	11.5	0.161	338	0.031	3.76	0.384	11.6	0.117	19.5	32.9	38.68
FBC-soil	**Shoot**	0.110	0.178	85.2	0.027	1.26	0.330	1.07	0.138	11.5	0.950	22.27
	Root	10.8	0.174	373	0.033	2.91	0.294	11.6	0.152	20.3	31.7	34.03
ABC-soil	**Shoot**	0.201	0.255	70.0	0.046	1.77	0.435	< 1.0	0.206	13.3	1.24	32.77
	Root	12.0	0.217	395	0.148	3.47	0.192	15.5	0.118	21.2	35.9	49.93
CCBC-soil	**Shoot**	0.198	0.268	69.3	0.047	1.27	0.418	< 1.0	0.196	13.2	1.24	34.42
	Root	10.3	0.224	338	0.133	3.80	0.164	11.6	0.103	20.6	37.7	45.26

Total organic carbon and total nitrogen in plants

Roots and shoots were analyzed for TOC and TN content (Figure 18). In the shoots, there was no difference in TOC content between different treatments, whereas in the roots, slightly higher TOC was observed for the fresh biochar treated soil. Total nitrogen in shoots increased with biochar application, and further increased with aging. This is in line with results for soils, which also showed an increase in available nitrogen forms with the aging process.

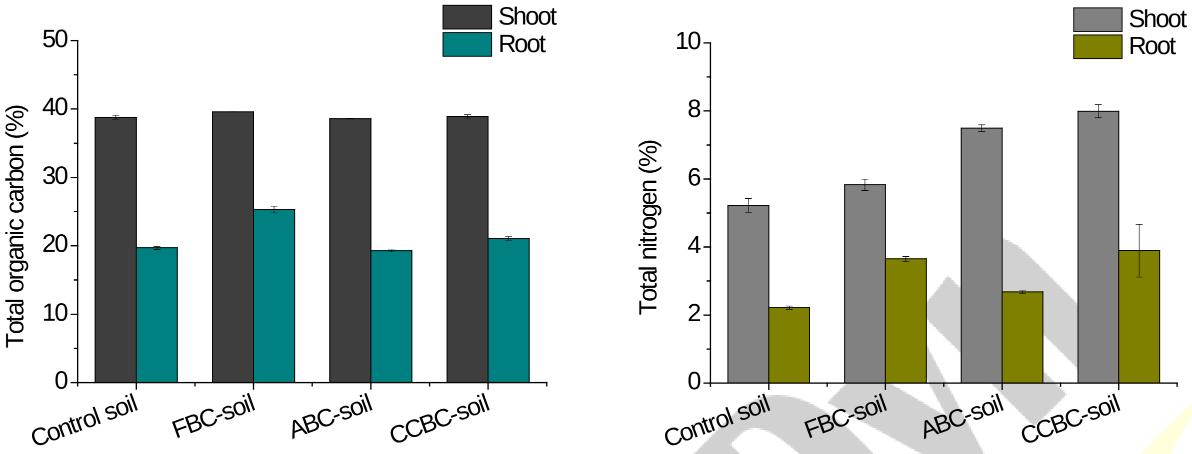


Figure 18. TOC and TN in biomass

Impact on your project

The training in soil organic matter characterization was an excellent opportunity for knowledge transfer between the CSIC/IRNAS and UNSPMF project groups. The techniques (NMR, TGA, Py/GCMA, Respicond) are partially novel to the Novi Sad group as similar but simpler methods for soil characterization are already used in our labs. However, the Serbian partner lacks experience on the instrumental techniques included in the training of the UNSPMF researchers, thus the secondment provided a great opportunity for them to gain knowledge and experience that can be implemented in laboratory work, but also to further transferred the gained knowledge and skills to both TwinSubDyn participants as well as other researchers and stakeholders. This will subsequently broaden the possibilities for further research in the field of soils science. In addition, the results obtained by analyzing samples through this training will be included in the publications planned as the output of the research portion of the 6 months secondment. The investigation carried out was focused on both analysis of the soils, but also the plants grown in the greenhouse pot experiment. The working hypothesis for the carried out experiment is that the aging of biochar changes biochar properties. These properties enhance its efficacy as a soil organic amendment, improving soil properties and promoting plant growth. Thus two publications are planned, focusing on: (I) the long-term effects of biochar amendment on soil properties and its soil organic matter, and (II) the effect of the aging of biochar in soils on plant performance. Also, the methodology carried out for this research is somewhat new for the UNSPMF researchers, and can be transferred and implemented in in our laboratories as practical learning tools and to complement future projects and investigations.

MOBILITY REPORT

Researchers: Snežana MALETIĆ and **Marijana KRAGULJ ISAKOVSKI**

Assigned supervisor: Prof. Dr. Heike KNICKER

Duration of the visit: 1 July to 31 July 2023

Executive Summary

The one month's visit for two researchers from UNSPMF to the Spanish National Research Council (Consejo Superior de Investigaciones Científicas, CSIC) and the Institute for Natural Resources and Agrobiology (Instituto de Recursos Naturales y Agrobiología de Sevilla, IRNAS) was realized during July 2023. The focus was training of UNSPMF researchers in techniques for soil organic matter characterization. The training included skill-building for the utilization of techniques and instruments such as solid state nuclear magnetic resonance (NMR), pyrolysis coupled with gas chromatography/mass spectroscopy (Py/GCMS), thermogravimetric analysis (TGA) and Respicond. Additionally, the application of software for data processing and interpretation was included as part of the training. A total of fifteen samples of Organic Soil Amendments (OSAs) and soils were selected for the training session.

Introduction

Scope of training

Fifteen samples were carefully chosen for training purposes, and their details, including their place of origin, are presented in Table 1. These samples were used to conduct training activities. The training sessions concentrated on imparting knowledge regarding techniques for characterizing soil organic matter, such as solid-state nuclear magnetic resonance (NMR), pyrolysis combined with gas chromatography/mass spectrometry (Py/GCMS), thermogravimetric analysis (TGA), and assessing soil microbial activity using Respicond. This comprehensive training encompassed hands-on experience in soil sample preparation, laboratory analysis, and the skills required for data analysis and interpretation.

Table 1. OSA and soil samples used for training activities

Samples	Type of samples	Origin
HTC_M_180	Hydrochair obtained from *Miscanthus×giganteus* during HTC at 180°C	Harvested during 2017 from the experimental field of Institute 106 INEP, Serbia

HTC_M_200	Hydrochair obtained from *Miscanthus×giganteus* during HTC at 200°C	
HTC_M_220	Hydrochair obtained from *Miscanthus×giganteus* during HTC at 220°C	
B_M	Biochar obtained from *Miscanthus×giganteus* during slow pyrolysis at 400°C	
HTC_S_180	Hydrochair obtained from *Beta vulgaris* during HTC at 180°C	Arising from the sugar manufacturing process in Serbia
HTC_S_200	Hydrochair obtained from *Beta vulgaris* during HTC at 200°C	
HTC_S_220	Hydrochair obtained from *Beta vulgaris* during HTC at 220°C	
B_S	Biochar obtained from *Beta vulgaris* during slow pyrolysis at 400°C	
AD	Anaerobic digestate	Obtained during biogas production in Serbia
BC	Biochar obtained from *Miscanthus×giganteus* during pyrolysis at 700°C	Produced in Julich and delivered in Serbia, for the Lysimeter experiment
Compost NS	Obtained from green waste from Novi Sad	Produced in Novi Sad, Serbia
Compost SU	Obtained from green waste from Subotica	Produced in Subotica, Serbia
Sediment	Sediment from Itebej	Serbia
Sludge	Sludge from wastewater treatment plant from Subotica	Serbia
Sandy soil	Danube alluvial sandy soil used in column and Lysimeter experiment	Serbia

The following sections will provide an overview of the content covered in the training activities and a detailed description of the analytical and instrumental methods employed. Subsequently, the key findings and results will be presented. Tamara Apostolović participated in these training activities during her six-month secondment.

Content

1. Methods

Solid state nuclear magnetic resonance (NMR)

To analyze the soil and sediment samples by NMR, the samples were subjected to demineralization with hydrofluoric acid (HF) as follows:

a. **Objectives**

→ Eliminate the mineral fraction and paramagnetic elements (Fe, Al) to minimize interfirences in the NMR.

→ Concentrate the organic matter.

b. **Reagents**

→ Hydrochloric acid 1M (HCl):

- for 1 L of HCl solution: measure 84 ml of HCl (37% purity) and make up to 1 L with water.

→ Hydrofluoric acid 10% (HF):

- for 1 L of HF solution: measure 208 ml of HF (48% purity) and make up to 1 L with distilled water. If the HF is 40% purity, measure 250 ml and make up to 1 L.

c. **Procedure for sample preparation**

→ **Removal of particulate organic matter (POM):** in case of high content.

- Weigh:
- Write down the weight of the bottle with the lid (sample may remain on the lid)
- Tare and record the weight of simple (in g): 10-20 g (or less, 5 g, depending on C content).
- Add 40 ml (20 ml) of distilled water and shake
- Centrifuge the cans (previously tared) at 3000 rpm for 10 minutes.
- Collect the organic matter in suspensión into a previously tared beaker, by filtration (make sure not to drag simple from the bottom of the beaker).

→ **Treat with HCl, 1 M:** in case the simple contains carbonates ($CaCO_3$) to avoid the formation of CaF_2 from HF, which implies an increase in the weight of the sample due to the presence of white precipitates

- Add 40 ml (20 ml) of 1 M HCl (do not cover the cans completely, gases are generated).
- Shake slightly and leave to react/settle.
- Centrifuge the cans at 3000 rpm for 10 minutes.

- Remove the supernatant (re-add HCl as many times as necessary).
- Wash with distilled water several times to remove excess HCl (≈ neutral pH).

→ **Treat with 10% HF:** use the specific protection material and proceed with extreme care

- Add 40 ml (20 ml) of 10% HF to the sample left in the bottle.
- Shake the samples for 2 h at 250 rpm.
- Centrifuge at 3000 rpm for 10 minutes.

Repeat 4 times

- Carefully remove the supernatant from the bottles
- Add 50 ml (20 ml) of distilled water, shake, tare, centrifuge and carefually remove the supernatant (wash until pH reaches 5-6).
- Freeze samples and lyophilize.
- After lyophilizing, weigh the jar+sample (with a lid, and the remains of the sample that have remained in it), to determine the content of organic matter that has resisted the acid attack.

Liofilization - Operating instructions

- NOTES: Always wear a face shield while operating the machine.
- The air conditioning must be ON when the machine is in operating mode (22°C).
- If preparation takes too long, place the samples back in freezer.
- **Switching on the machine**
- Check the level of oil in the vacuum pump.
- Put the plug in the drain valve
- Switch-on the device by means of turning the main switch to “ON”.
- Now starts the Refrigeration machine and cools down the condenser.
- Place the frozen samples on the holder.
- Place the cylinder and the top with the valves in their place.
- The display appears after the startup of the controller as follows:

Starting a program

- Select the program button for the appropriate program (P1).
- Press the START button.
- Before starting the program, check the cleanliness and the correct fitting of all gaskets. You also check the drain valve on right closure, the position of the rubber valves and the oil of the vacuum pump.
- If you close all hand valves, you could press the START button. The following image will now appear:

- It might be necessary to apply light pressure to the cylinder or between the cylinder and the top for the pressure to start dropping.
- When the pressure is down to between 0.5 and 5 mbar, turn the switch on the vacuum pump from position II to position 0.
- If the condenser has reached the set value, the first drying stage starts. The device then cools further down to the lowest possible temperature.
- When the drying starts, the pressure and the temperature will reach the set points.
- If the set time is reached, continue with the second drying stage.
- When the drying starts, the pressure and the temperature will reach the set points.
- If the set time is reached, continue with the third drying stage.

Switching off the machine: The chamber pressure and the temperature will continue to be regulated to the last set value. When you press the "OK" button, the program is finished and turned off the vacuum pump. Turn off the machine by pressing the OFF button.

Figure 1: Snežana MALETIĆ, Marijana KRAGULJ ISAKOVSKI and Tamara APOSTOLOVIĆ conduct HF treatment of soil (upper left); Tamara APOSTOLOVIĆ performs lyophilization (upper right); UNSPMF researchers, including Heike KNICKER and Marinos THEODORAKOPOULOS, work in the NMR lab (middle left); Snežana MALETIĆ, Marijana KRAGULJ ISAKOVSKI, and Heike KNICKER engage in discussions during NMR analysis (middle right); Snežana MALTIĆ and Heike KNICKER prepare the samples for NMR analysis (right bottom) and NMR (right bottom).

Pyrolysis coupled with gas chromatography/mass spectroscopy (Py/GCMS)

Analyzing soil organic matter by pyrolysis coupled with gas chromatography/mass spectrometry (Py/GCMS) allows for the characterization of complex organic compounds present in soil and OSA samples. This method involves two main steps: pyrolysis, where organic matter is thermally degraded into volatile compounds, and GC/MS, which separates and identifies these compounds.

A weighed portion of the dry soil/biochar sample was placed into a sample container (crucibel) suitable for pyrolysis. The sample size depends on the specific instrument and method used. The pyrolysis process, which involves heating the sample rapidly to high temperatures (typically between 400°C to 800°C) in an inert atmosphere (usually helium or nitrogen), causes the organic matter in the soil/biochar to break down into volatile compounds. These compounds, released during pyrolysis, are carried into the gas chromatograph for further analysis. The resulting data provides information about the types and relative abundance of organic compounds present in the soil/biochar. Py/GCMS is a technique which helps understand the composition of organic materials in soil and can have significant implications for agriculture, environmental science, and soil management. It provides detailed information about the complex mix of organic compounds present, enabling deeper understanding of soil quality and behavior.

Figure 2. Marijana KRAGULJ ISAKOVSKI performs PyGC/MS analysis (left) and Tamara APOSTOLOVIĆ and Marijana KRAGULJ ISAKOVSKI durining PyGC/MS analysis

Thermogravimetric analysis (TGA)

Thermogravimetric analysis (TGA) is an analytical technique used to determine the composition and thermal properties of materials, including soil and OSA. TGA measures the weight change of a sample as it is heated or cooled over a specified temperature range. In the case of soil, TGA can provide information about its organic matter content, moisture content, and thermal stability.

Procedure:

Sample Preparation - Grind the dried soil and OSA samples into a fine powder to ensure uniformity and increase the surface area for analysis. Weigh a known amount of the powdered soil sample (typically 5-10 mg) accurately. Record the initial mass.

Sample Loading - Place the weighed sample into a sample crucible. Make sure it is evenly distributed across the bottom of the crucible.

Instrument Setup - Install the sample crucible containing the soil sample onto the balance in the TGA instrument.

TGA Analysis - Set the desired temperature range and heating rate for your analysis. The temperature range and heating rate will depend on your research objectives. Start the TGA instrument and heat the sample at the specified rate while continuously measuring its weight. Monitor the weight change of the sample as it undergoes thermal decomposition or phase transitions.

Data Interpretation - The TGA instrument will generate a thermogram, which is a plot of weight loss (or gain) as a function of temperature. Analyze the thermogram to identify key weight loss events. In the case of soil, typical weight loss events may include:

- Loss of moisture content (dehydration)
- Degradation of organic matter (oxidation or decomposition)

- Mineral decomposition or phase transitions

Calculate and Interpret Results - Calculate the percentage of weight loss for each significant event based on the initial mass of the soil sample. Interpret the results to determine the composition and thermal properties of the soil, including the moisture content, organic matter content, and thermal stability.

Figure 3. Snežana MALETIĆ prepares the samples for TGA analysis

Respicon - microbial respiration

This experiment was conducted while Tamara Apostolović was on six secondments at IRNAS, with Snezana and Marijana joining the experiment and receiving corresponding training.

The objective was to investigate the rate of organic material degradation and decomposition through the respiration of microorganisms, specifically by measuring CO_2 emissions resulting from microbial activity. A short procedure of the Respicon usage is given below.

Reagents

- Inoculum (solution of microorganisms): take a small amount of soil (e.g. pot). Add water, stir and leave to rest. Centrifugate, filter and collect the filtrate. Store in the respicond chamber to acclimatize.
- 0,6 M KOH (PM = 56.11 g/mol; 85% purity). For 1 L solution à 39.61 g KOH. CAUTION: corrosive reaction! Prepare the solution under a hood and with caution, EXOTHERMIC. Allow to cool before making up to the mark.

Protocol

- Calculate the water holding capacity of the samples (WHC).
- Prepare the sample:

  - Weigh 10 g of sample (sieved, Ø<2mm) into small glass beakers (note the weight of the beaker). Prepare replicates (≈ 4) of each sample. If the sample has less than 4 % organic carbon, C-org, weigh 20 g.
  - Add the amount of distilled water (previously acclimatized in the respicond chamber) necessary to achieve 60% of the WHC, deducting the volume of the inoculum to be added.
  - Add 0,5 - 1 mL of the microorganisms solution (inoculum) to promote initiation of microbial activity.
- Installation of the Respicond experiment/test:
  - Add 10 mL of KOH into the electrode containers located in the lid of each cup.
  - Insert the beakers with the prepared samples into the plastic cups of the respicond (vessel), mark very clearly beaker+vessel/channel (it is possible that during the experiment, some sample beakers have to be relocated, so the samples must be clearly identifiable).
  - Configure the program in the Respicond:
    - Select the program: WinRrj.ewe-shortent.
    - File → open new: WRITE SAMPLE NAME → save.
    - Edit → description → close.
    - Configuration → run option → scan interval → close.
    - Start time (first run a “manual scan” to verify that the used channels are working properly).
- Change the KOH solution in each beaker when the conductivity decreases below 60 mS or the curve stabilizes.
- At the end of the experiment, the data will be exported, treated with sigmaplot to see the degradation curves. The samples are dried (< 40 ºC) and weighed in the same beakers.

Figure 4. Tamara APOSTOLOVIĆ perform Respicon experiments

2. Results

Solid state nuclear magnetic resonance (NMR)

To facilitate NMR analysis of the soil and OSA samples, they were first dried and finely ground. In the case of soil and sandy materials, a demineralization process was performed using hydrofluoric acid (HF). Obtained results are shown in Tables 2 and 3 and Fig. 5.

Table 2. Relative amount of organic groups obtained by solid state NMR for OSAs used in column experiments

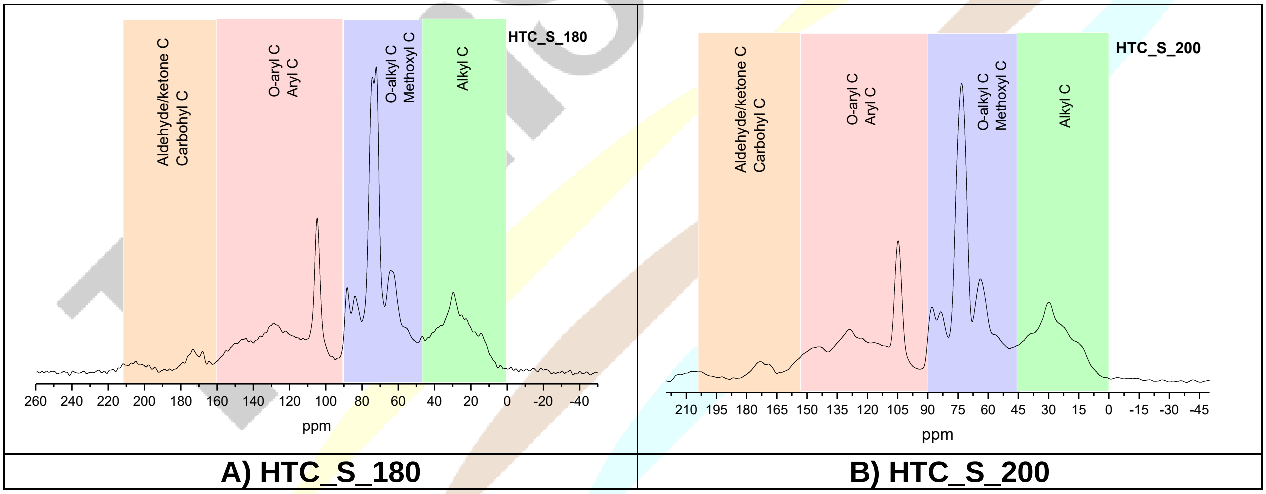
Range (ppm)	Organic groups	Relative amount (%)								
		HTC_S_180	HTC_S_200	HTC_S_220	B_S	HTC_M_180	HTC_M_200	HTC_M_220	B_M	Soil
0-25	Terminal methyl groups	7.92	8.12	7.73	6.83	6.67	3.75	4.10	3.05	14.06
25-45	Methylene groups in aliphatic rings and chains	11.99	12.81	14.33	4.38	10.37	7.65	7.94	3.12	21.08
45-60	Methoxyl C	6.54	6.88	6.52	2.90	10.93	9.14	8.65	1.59	11.25
60-90	O-alkyl C	39.82	38.05	37.63	5.97	27.47	41.62	35.94	2.71	18.99
90-110	Anomeric C in the pure cellulose	10.58	10.38	10.60	6.39	10.26	12.51	11.57	2.72	8.93
110-140	Aryl C-H carbons	13.21	13.82	14.29	50.92	15.93	15.48	18.62	60.97	14.05
140-160	O-aryl C	5.87	6.10	5.70	12.90	9.21	7.80	10.11	16.45	5.78
160-185	Carboxyl C	3.42	3.22	2.51	6.59	8.23	1.62	2.25	6.57	4.93
185-240	Aldehyde/ketone C	0.65	0.64	0.70	3.13	0.94	0.44	0.83	2.83	0.93

Spinning side bands, constituting approximately 5.96%, were observed in the biochar derived from sugar beet shred (B_S). This phenomenon is attributed to incomplete removal of spin-spin interactions and contributes to the overall intensity of the parent signal.

Table 3. Relative amount of organic groups obtained by solid state NMR for OSAs used in column experiments Lysimeter experiments.

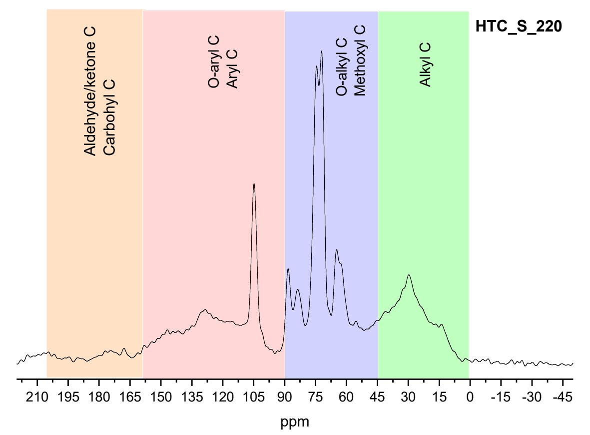
Range (ppm)	Organic groups	Relative amount (%)					
		AD	Biochar	Compost NS	Compost SU	Sediment	Sludge SU
0-25	Terminal methyl groups	0.17	2.06	10.02	6.67	10.93	18.40
25-45	Methylene groups in aliphatic rings and chains	5.40	2.20	14.16	10.37	16.88	20.38
45-60	Methoxyl C	6.65	1.18	10.25	10.93	10.09	10.48
60-90	O-alkyl C	12.11	2.27	20.67	27.47	19.82	18.45
90-110	Anomeric C in the pure cellulose	12.70	3.57	8.31	10.26	7.07	5.53
110-140	Aryl C-H carbons	42.94	72.29	14.52	15.93	15.52	7.88
140-160	O-aryl C	9.89	13.72	8.20	9.21	6.91	3.23
160-185	Carboxyl C	5.81	2.34	10.21	8.23	11.48	15.43
185-240	Aldehyde/ketone C	4.31	0.37	3.66	0.94	1.31	0.32

In all samples the highest amount Aryl C-H carbons show presence of aromatic structure. After that, a significant amount of anomeric C in the pure cellulose was observed. The NMR spectrum of pure cellulose typically reveals information about the carbon atoms in the cellulose structure, including the anomeric carbon. In the 13C NMR spectrum of cellulose, the anomeric carbon typically appears as a distinctive peak.

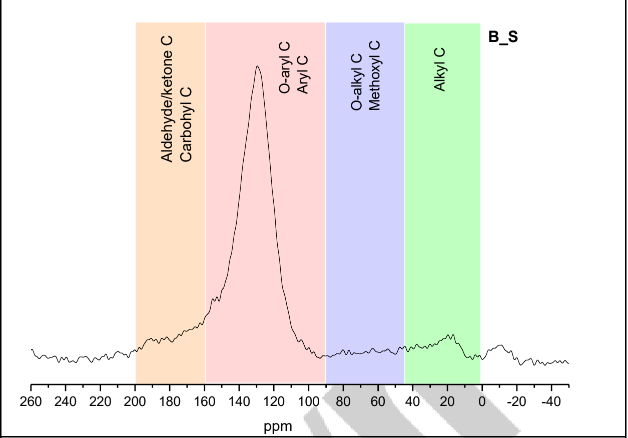


A) HTC_S_180

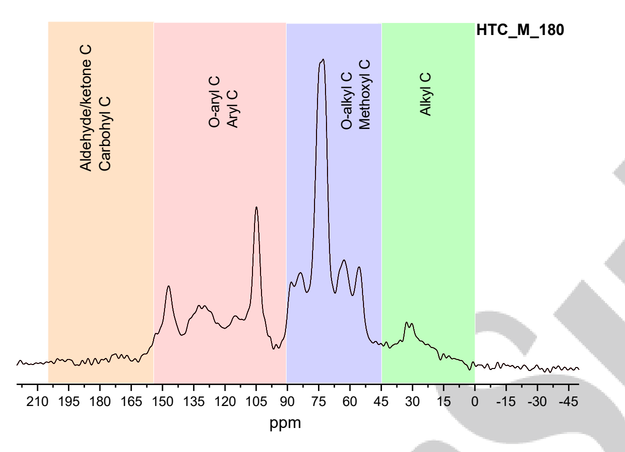
B) HTC_S_200



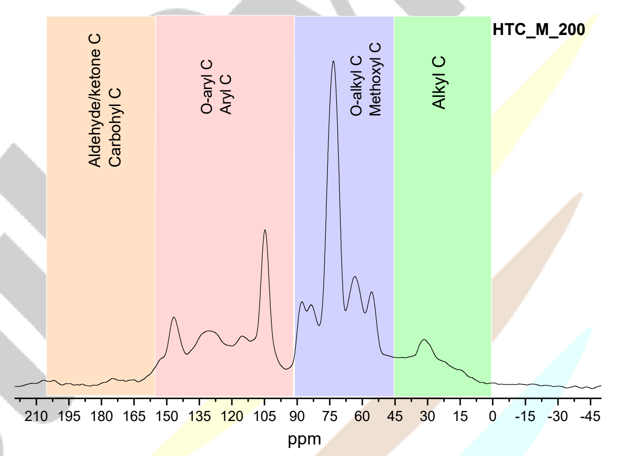
C) HTC_S_220



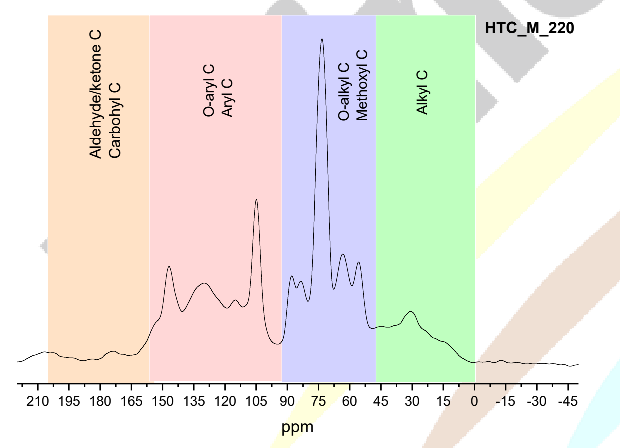
D) B_S



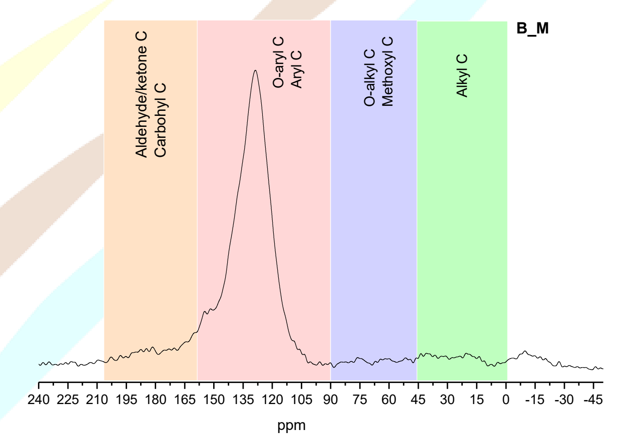
F) HTC_M_180



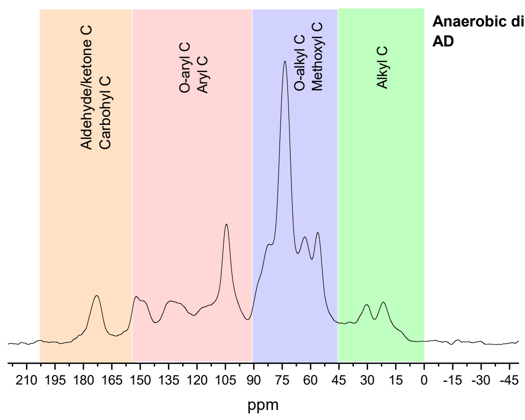
F) HTC_M_200



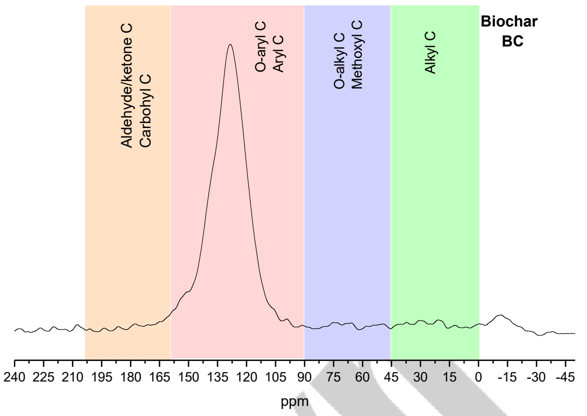
G) HTC_M_220



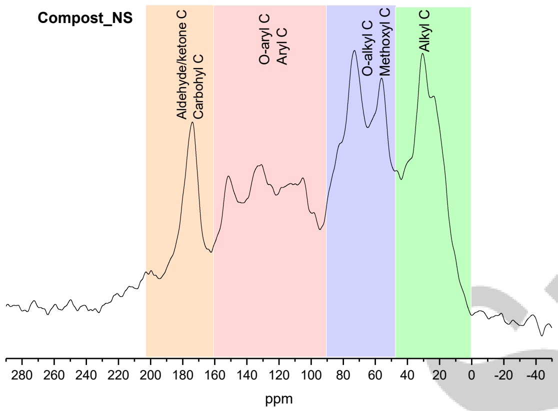
H) B_M



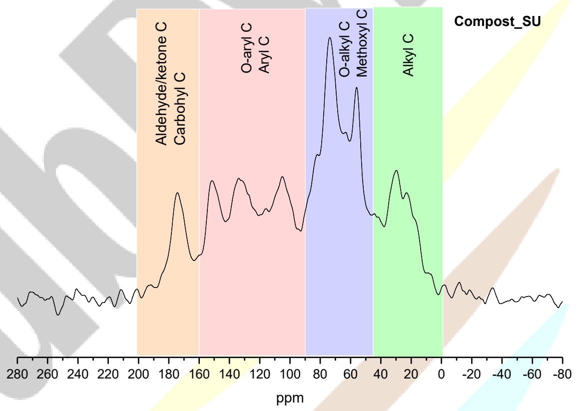
I) AD



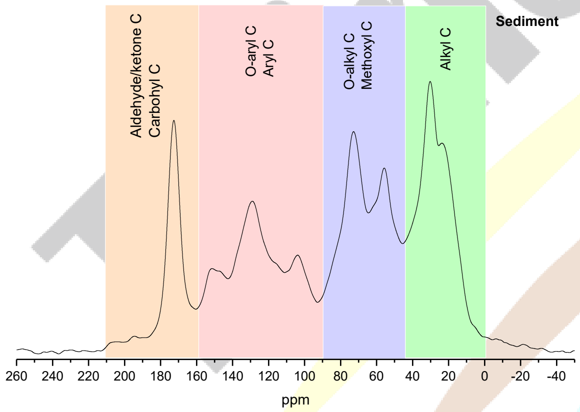
J) Biochar, BC



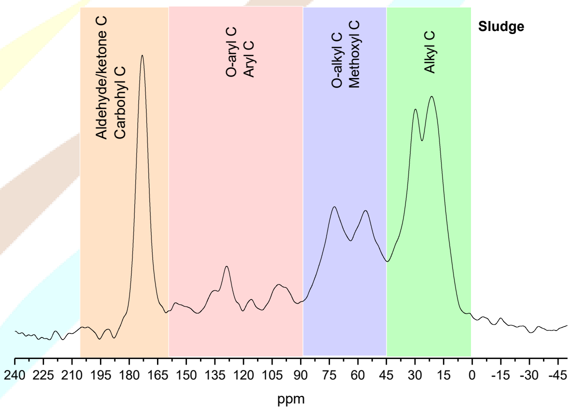
K) Compost NS



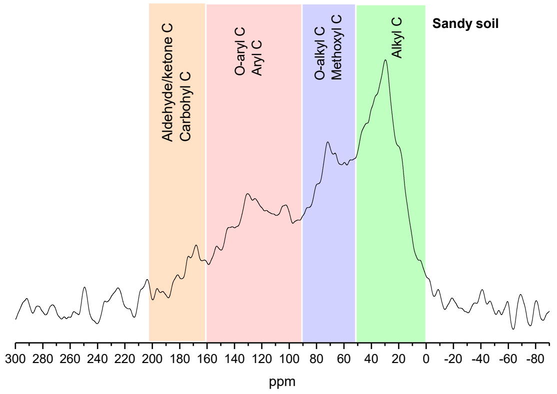
L) Compost SU



M) Sediment



N) Sludge SU



O) Sandy soil

Figure 5. NMR spectra of soil, sediment and OSA used in research activities

Pyrolysis coupled with gas chromatography / mass spectroscopy (Py/GCMS)

The analysis of organic matter by pyrolysis coupled with gas chromatography/mass spectrometry (Py-GC/MS) allows for the characterization of complex organic compounds present in soil and OSA. This method involves two main steps: pyrolysis and GC/MS identification of these compounds. For this analysis the samples of anaerobic digestate, biochar from Julich, Compost from Novi Sad and Subotica, sewage sludge, sediment were selected. Those samples are used for Lysimeter experiment.

A weighed portion of the dry soil and OSA sample was placed into a sample container (crucibel) suitable for pyrolysis. The sample size depends on the specific instrument and method used. The pyrolysis process, which involves heating the sample rapidly to high temperatures (typically between 400°C to 800°C) in an inert atmosphere (usually helium or nitrogen), causes the organic matter in the soil/biochar to break down into volatile compounds. Py/GCMS analysis of all soil and OSA samples were conducted during our stayed in IRNAS, Spain. The obtained pyrograms are presneted in Table 4.

Table 4. Identified compounds by PyrGC/MS analysis.

OSA	Detected compounds	Origin
AD, Sediment, BC, CompostSU, CompostNS	2,3-dimethylfuran	Polysaharides
AD	2-Furanmethanol	Polysaharides
AD, CompostSU, CompostNS	2,3-dihydro-furan	Polysaharides
Sediment, CompostSU, CompostNS	2,4-Dimethylfuran	Polysaharides
Sediment, CompostSU, CompostNS	2-Furanmethanamine	Polysaharides
Sediment, BC	5-methyl-2-Furancarboxaldehyde	Polysaharides
AD, BC, CompostSU, CompostNS	5-Ethyl-2-furaldehyde	Polysaharides
AD, BC, CompostSU, CompostNS	2-Acetyl-5-methylfuran	Polysaharides
AD, BC, CompostNS	5-Ethyl-2-furaldehyde	Polysaharides
AD	3,4-dihydro-2H-1-benzopyran-3-ol	Polysaharides
AD, BC, CompostSU, CompostNS	Furfural	Polysaharides
BC, CompostSU, CompostNS	2-Vinylfuran	
AD	m-Guaiacol	Lignin
AD, BC, CompostSU, CompostNS	Creosol and other its derivates	Lignin
AD, CompostSU, CompostNS	4-Vinylphenol	Lignin
AD, BC, CompostSU, CompostNS	2-Methoxy-4-vinylphenol	Lignin
AD, BC, CompostSU, CompostNS	2,6-dimethoxy- Phenol	Lignin
AD, BC, CompostSU, CompostNS	3-hydroxy-4-methoxy- Benzaldehyde	Lignin
AD, BC, CompostSU, CompostNS	4-Methyl-2,5-dimethoxybenzaldehyde	Lignin

AD, BC, CompostSU, CompostNS	2,4,6-Trimethoxytoluene and toluene derivatives	Lignin
AD, BC, CompostSU, CompostNS	3,4-Dimethoxy-6-amino toluene	Lignin
AD, BC	3-cyclopropylmethoxy-toluene	Lignin
CompostSU, CompostNS	Eugenol	Lignin
AD, Sediment, BC, CompostSU, CompostNS	Benzene and its derivatives	Lignin
Sediment	PAHs	Lignin

Applying Py-GC/MS analysis, we investigated the thermal decomposition of anaerobic digestate - AD, Compost SU, Compost NS, Sediment and Sludge SU (Table 13). The pyrolysis process generated a diverse array of volatile compounds, and the mass spectra provided valuable insights into the chemical composition of the sample. The Py-GC/MS analysis revealed the presence of several dominant compounds, including furan, furfural, vanillin, phenols, creosol, Eugenol, which are indicative of polysaharide and lignin origin. All these pyrolytic products are aromatic compounds which are identified in almost all analyzed OSAs samples. Numerous phenolic derivatives, such as (2,6-dimethoxy-phenol, 4-Vinylphenol, Eugenol) were detected. These compounds are associated with lignin. In the sediment and sludge identified is higher number of PAHs and other benzene derivatives. It's important to note that the presence of polysaccharides and lignin can vary among OSAs depending on the origin of native biomass and process used for OSA production.

Thermogravimetric analysis (TGA)

Thermogravimetric Analysis (TGA) was performed using thermal analyzer SDT Q600, TA Instruments, USA. Thermogravimetric analysis determines the amounts of moisture, labile, intermediate, and recalcitrant organic matter. The results of TGA analysis are presented in Table 5 and Fig. 6

Table 5. Type of OM obtained by TGA analysis of soil and OSA used in column.

	B-M	B-S	HTC_M_180	HTC_M_200	HTC_M_220	HTC_S_180	HTC_S_200	HTC_S_220	Soil
	Relative Weight Loss (%)								
Moisture	14	13	3	2	2	3	3	3	2
Moisture and very labile OM	4	8	1	1	1	2	2	3	1
Labile OM	10	11	78	76	72	66	66	63	3
Int OM	29	26	12	14	18	19	19	21	26
Recalcitrant OM	43	41	7	6	8	10	10	11	68

In general, the predominant portion of labile organic matter (OM) was found in all types of hydrochar, ranging from 63% to 78% (Table 5, Figure 6). However, this fraction was significantly lower for both biochars, accounting for only 11%. The second notable stage, characterized by a mass loss of 12% to 21%, in hydrochars was associated with intermediate organic matter. A similar proportion was also observed for both biochars in this stage. The substantial mass loss observed during the labile organic matter stage in hydrochars can be attributed to the relatively high content of sugars present in these materials. In contrast, the biochar's higher mass loss during the intermediate organic matter stage indicates that a considerable amount of aromatic structures with low condensation degree are present. Soil showed high recalcitrance, most likely this is due to the high mineral content of the sample.

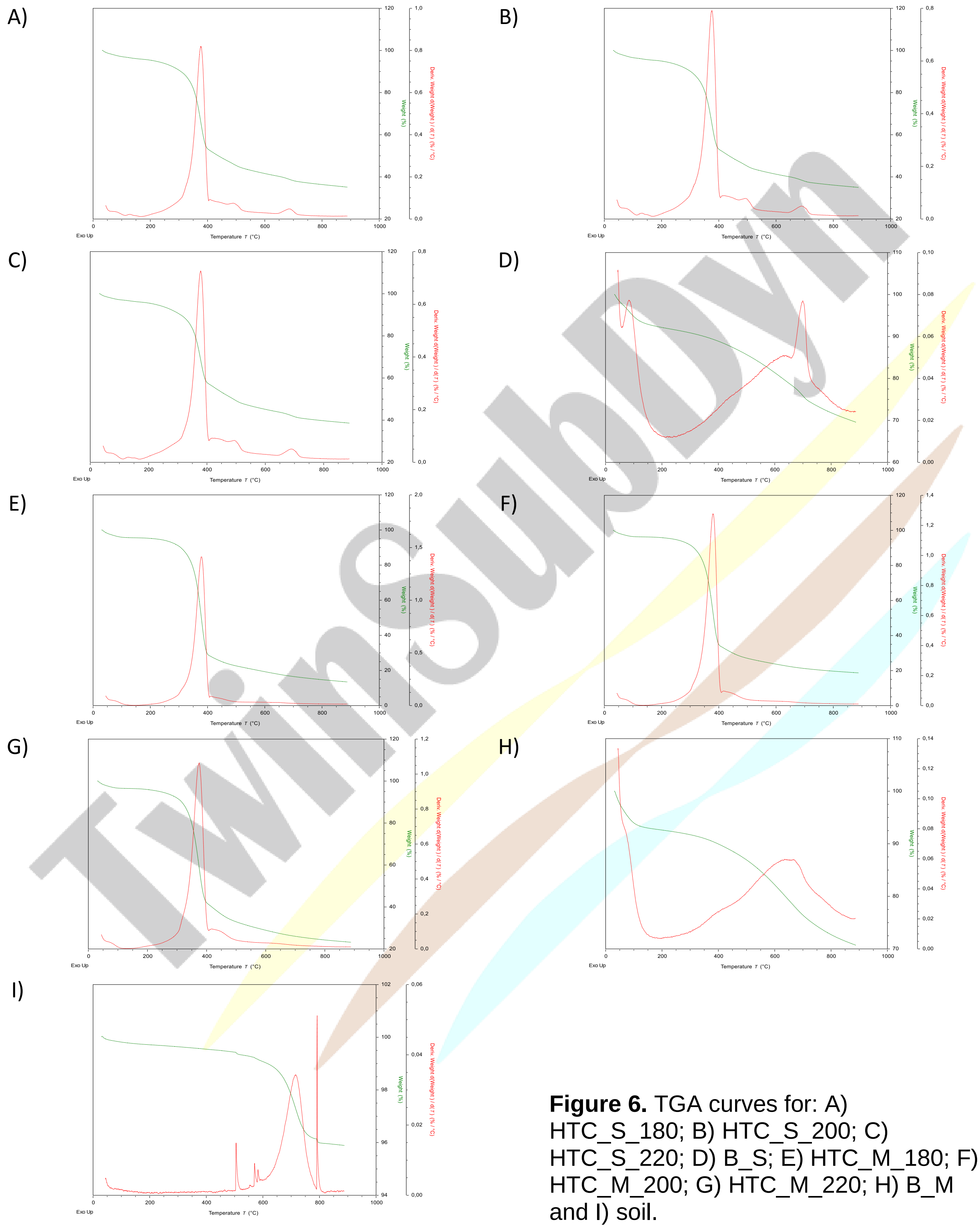


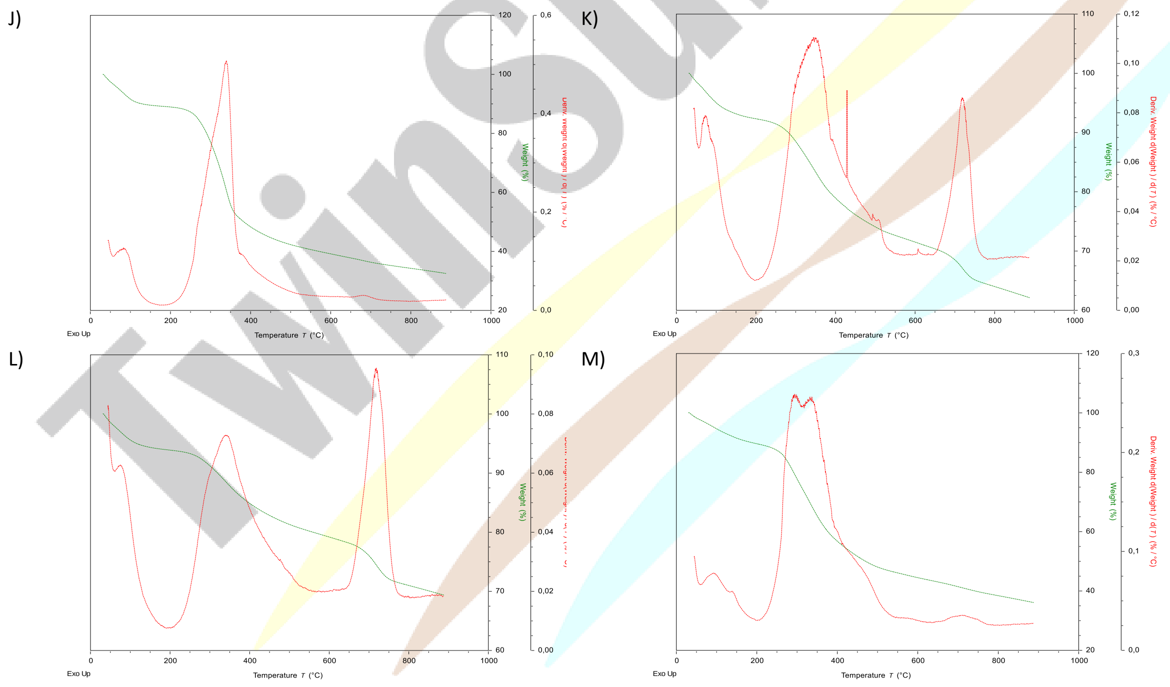
Figure 6. TGA curves for: A) HTC_S_180; B) HTC_S_200; C) HTC_S_220; D) B_S; E) HTC_M_180; F) HTC_M_200; G) HTC_M_220; H) B_M and I) soil.

Composted sewage sludge from Subotica, city compost from Novi Sad, anaerobic digestate-AD, sediment, sewage sludge from Subotica, biochar used in Lysimeter experiment are also analyzed during our team stay in CSIC. These data are presented in Table 7 and Figure 7.

Table 7. TGA analysis of other OSA used in Lysimeter experiments.

Fraction	Tem. range	AD	Biochar	Compost NS	Compost SU	Sediment	Sludge SU
		Relative Weight Loss (%)					
Moisture	50-105 ºC	10	10	11	10	16	6
Moisture and very labile OM-W1	105-200 ºC	3	6	5	7	9	7
Labile OM-W2	200-400 ºC	63	11	31	37	20	53
Int OM_W3	400-600 ºC	14	19	20	20	28	20
Recalcitrant OM-W4	600-750 ºC	10	55	34	26	28	13

In general, the largest proportion of easily decomposable organic matter (labile OM) was found in anaerobic digestate, compost, and sludge from Subotica, ranging from 37% to 63%. On the other hand, biochar exhibited the highest mass loss of 55% for at temperatures between 600 and 750°C. Commonly this fraction is assigned to be also biochemically recalcitrant.



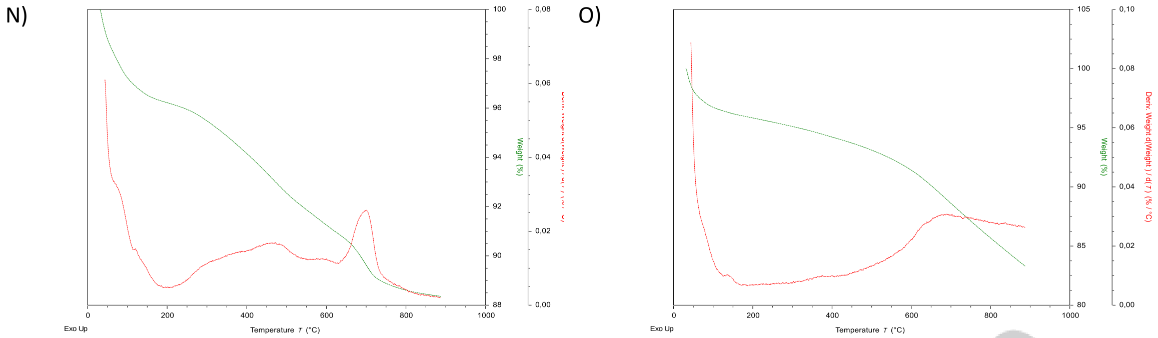
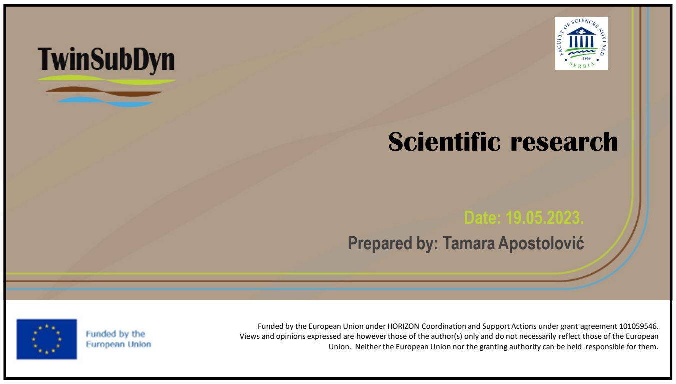


Figure 7. TGA curves of the OSAs used in lysimeter experiments: J) anaerobic digestate, AD; K) Compost from Subotica (SU); L) Compost Novi Sad (NS); M) sludge from Subotica (SU); N) Sediment; O) Biochar.

Impact on your project

The training on soil organic matter characterization presented a valuable opportunity for knowledge exchange between the CSIC/IRNAS and UNSPMF project groups. While the techniques employed (NMR, TGA, Py/GCMA, Respicond) were somewhat new to the UNSPMF group, which had previously utilized simpler methods for soil characterization in their laboratories, the Serbian team lacked experience with the advanced instrumental techniques incorporated in the UNSPMF researchers' training. Consequently, the secondment offered an excellent chance for UNSPMF team to acquire knowledge and hands-on experience that can be applied in laboratory work. Moreover, it facilitated the transfer of this acquired knowledge and skill set to both TwinSubDyn participants and other researchers and stakeholders, thereby expanding the scope for future research in soil science. The results obtained from analyzing samples during this training will be integrated into the planned publications that constitute the research output of the six-month secondment.



TwinSubDyn

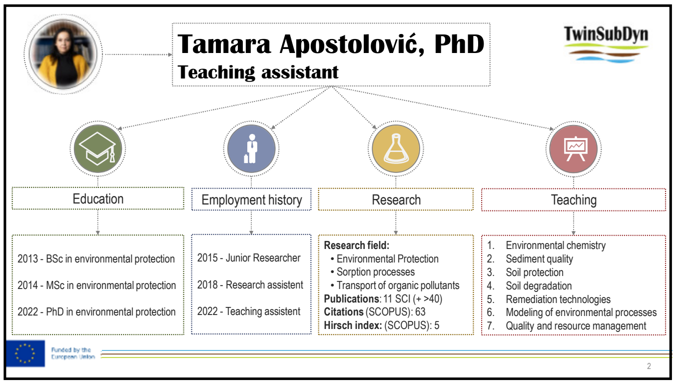
Scientific research

Date: 19.05.2023.

Prepared by: Tamara Apostolović

Funded by the European Union

Funded by the European Union under HORIZON Coordination and Support Actions under grant agreement 101059546.



Tamara Apostolović, PhD

Teaching assistant

TwinSubDyn

Education

- 2013 - BSc in environmental protection
- 2014 - MSc in environmental protection
- 2022 - PhD in environmental protection

Employment history

- 2015 - Junior Researcher
- 2018 - Research assistent
- 2022 - Teaching assistent

Research

Research field:

- Environmental Protection
- Sorption processes
- Transport of organic pollutants

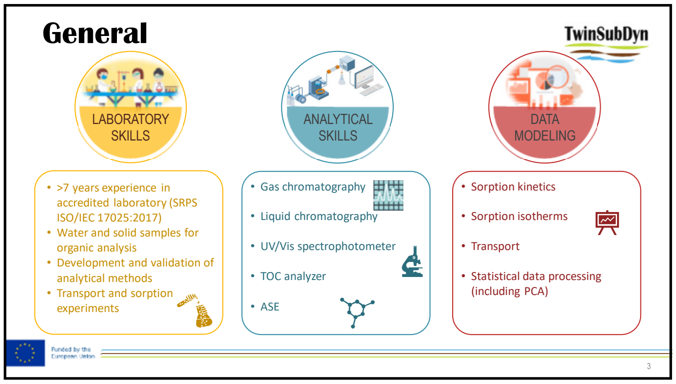
Publications: 11 SCI (+ >40)

Citations (SCOPUS): 63

Hirsch index: (SCOPUS): 5

Teaching

1. Environmental chemistry
2. Sediment quality
3. Soil protection
4. Soil degradation
5. Remediation technologies
6. Modeling of environmental processes
7. Quality and resource management



General

TwinSubDyn

LABORATORY SKILLS

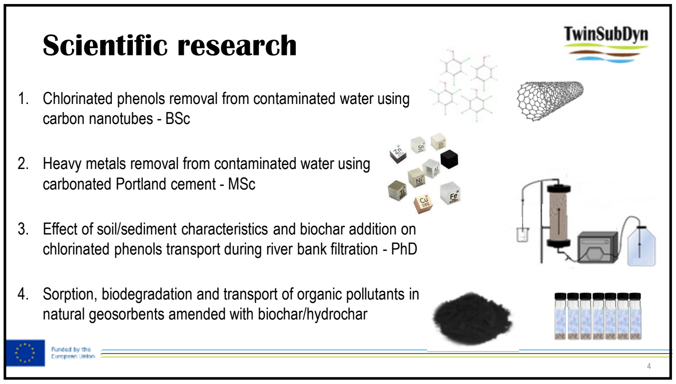
- >7 years experience in accredited laboratory (SRPS ISO/IEC 17025:2017)
- Water and solid samples for organic analysis
- Development and validation of analytical methods
- Transport and sorption experiments

ANALYTICAL SKILLS

- Gas chromatography
- Liquid chromatography
- UV/Vis spectrophotometer
- TOC analyzer
- ASE

DATA MODELING

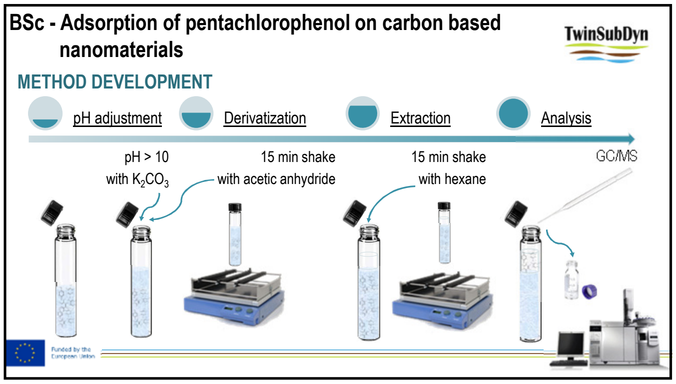
- Sorption kinetics
- Sorption isotherms
- Transport
- Statistical data processing (including PCA)



Scientific research

TwinSubDyn

1. Chlorinated phenols removal from contaminated water using carbon nanotubes - BSc
2. Heavy metals removal from contaminated water using carbonated Portland cement - MSc
3. Effect of soil/sediment characteristics and biochar addition on chlorinated phenols transport during river bank filtration - PhD
4. Sorption, biodegradation and transport of organic pollutants in natural geosorbents amended with biochar/hydrochar



BSc - Adsorption of pentachlorophenol on carbon based nanomaterials

TwinSubDyn

METHOD DEVELOPMENT

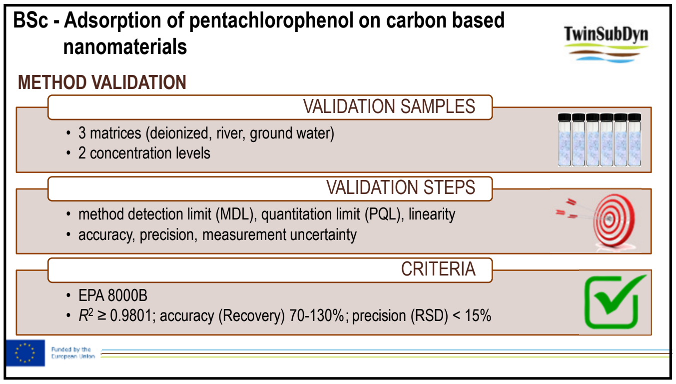
pH adjustment → Derivatization → Extraction → Analysis

pH > 10 with K_2CO_3

15 min shake with acetic anhydride

15 min shake with hexane

GC/MS



BSc - Adsorption of pentachlorophenol on carbon based nanomaterials

TwinSubDyn

METHOD VALIDATION

VALIDATION SAMPLES

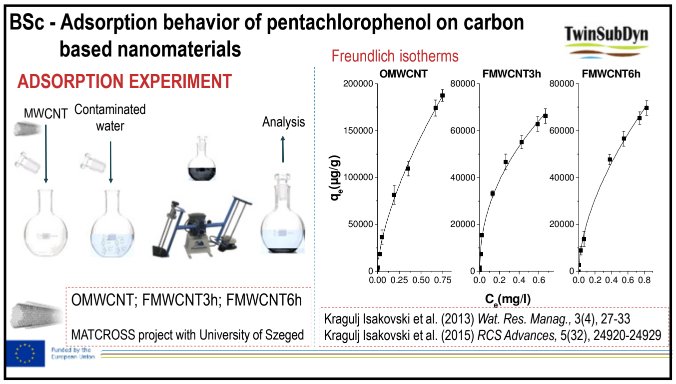
- 3 matrices (deionized, river, ground water)
- 2 concentration levels

VALIDATION STEPS

- method detection limit (MDL), quantitation limit (PQL), linearity
- accuracy, precision, measurement uncertainty

CRITERIA

- EPA 8000B
- $R^2 \geq 0.9801$; accuracy (Recovery) 70-130%; precision (RSD) < 15%



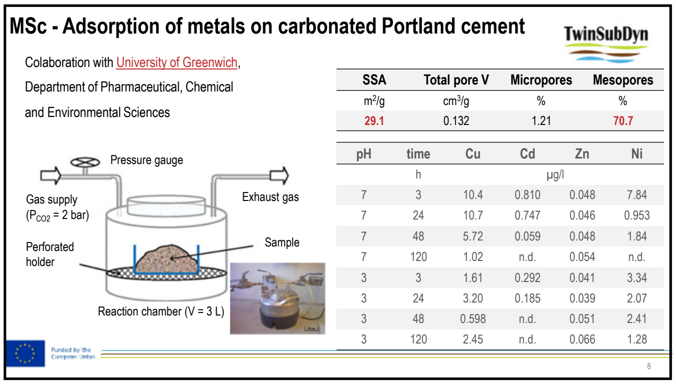
BSc - Adsorption behavior of pentachlorophenol on carbon based nanomaterials

ADSORPTION EXPERIMENT

OMWCNT; FMWCNT3h; FMWCNT6h

MATCROSS project with University of Szeged

Kragulj Isakovski et al. (2013) *Wat. Res. Manag.*, 3(4), 27-33
Kragulj Isakovski et al. (2015) *RCS Advances*, 5(32), 24920-24929

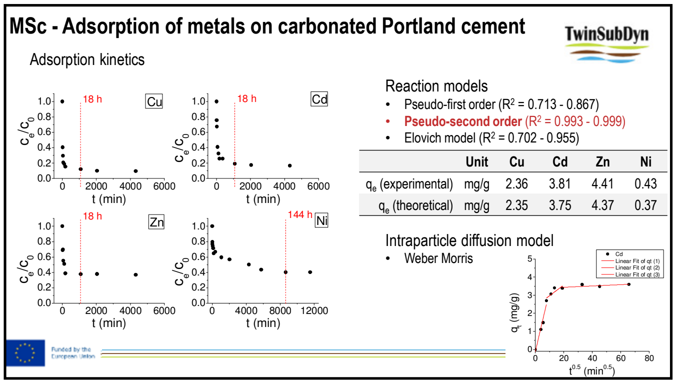


MSc - Adsorption of metals on carbonated Portland cement

Colaboration with University of Greenwich,
Department of Pharmaceutical, Chemical
and Environmental Sciences

SSA	Total pore V	Micropores	Mesopores
m^2/g	cm^3/g	%	%
29.1	0.132	1.21	70.7

pH	time	Cu	Cd	Zn	Ni
	h		µg/l		
7	3	10.4	0.810	0.048	7.84
7	24	10.7	0.747	0.046	0.953
7	48	5.72	0.059	0.048	1.84
7	120	1.02	n.d.	0.054	n.d.
3	3	1.61	0.292	0.041	3.34
3	24	3.20	0.185	0.039	2.07
3	48	0.598	n.d.	0.051	2.41
3	120	2.45	n.d.	0.066	1.28



MSc - Adsorption of metals on carbonated Portland cement

Adsorption kinetics

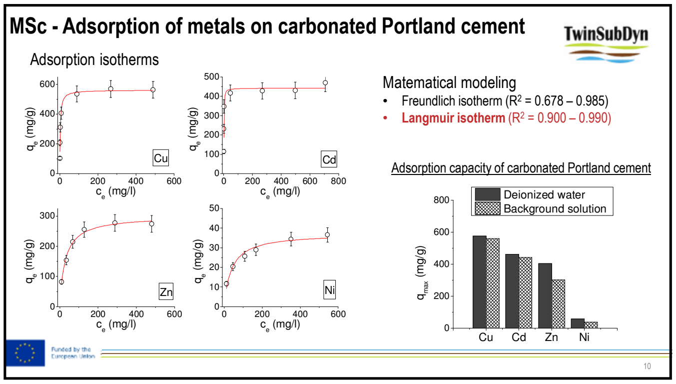
Reaction models

- Pseudo-first order (R^2 = 0.713 - 0.867)
- **Pseudo-second order** (R^2 = 0.993 - 0.999)
- Elovich model (R^2 = 0.702 - 0.955)

	Unit	Cu	Cd	Zn	Ni
q_e (experimental)	mg/g	2.36	3.81	4.41	0.43
q_e (theoretical)	mg/g	2.35	3.75	4.37	0.37

Intraparticle diffusion model

- Weber Morris



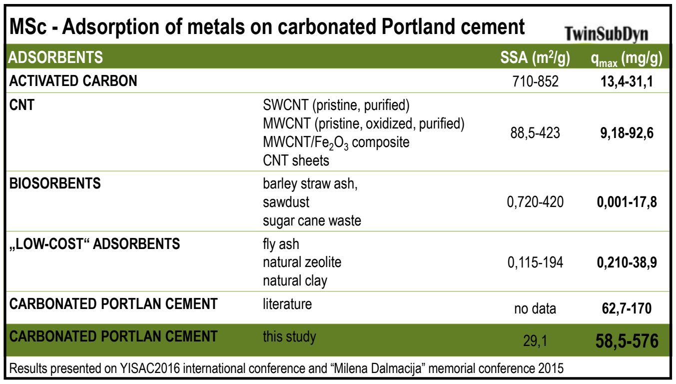
MSc - Adsorption of metals on carbonated Portland cement

Adsorption isotherms

Matematical modeling

- Freundlich isotherm (R^2 = 0.678 – 0.985)
- **Langmuir isotherm** (R^2 = 0.900 – 0.990)

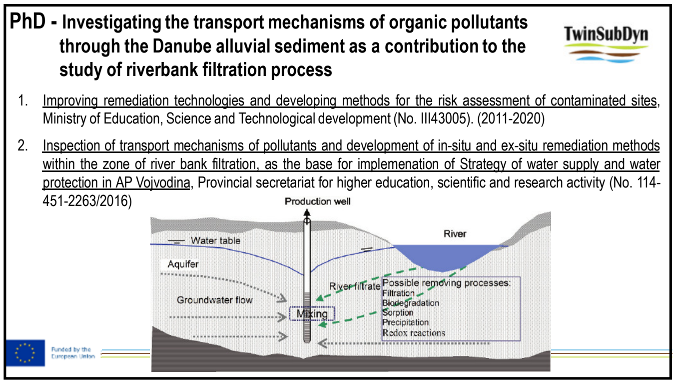
Adsorption capacity of carbonated Portland cement



MSc - Adsorption of metals on carbonated Portland cement

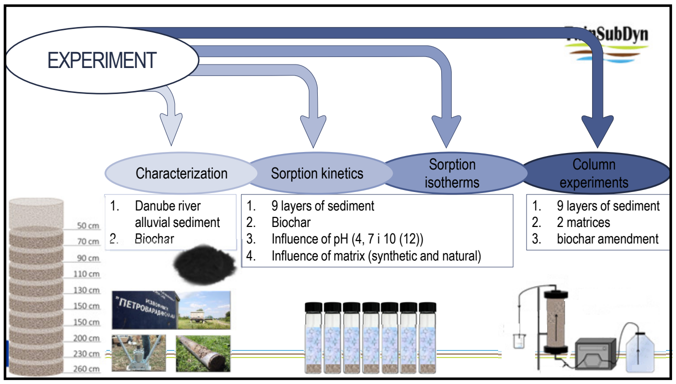
ADSORBENTS		SSA (m^2/g)	q_{max} (mg/g)
ACTIVATED CARBON		710-852	**13,4-31,1**
CNT	SWCNT (pristine, purified) MWCNT (pristine, oxidized, purified) MWCNT/Fe_2O_3 composite CNT sheets	88,5-423	**9,18-92,6**
BIOSORBENTS	barley straw ash, sawdust sugar cane waste	0,720-420	**0,001-17,8**
„LOW-COST“ ADSORBENTS	fly ash natural zeolite natural clay	0,115-194	**0,210-38,9**
CARBONATED PORTLAN CEMENT	literature	no data	**62,7-170**
CARBONATED PORTLAN CEMENT	this study	29,1	**58,5-576**

Results presented on YISAC2016 international conference and “Milena Dalmacija” memorial conference 2015



PhD - Investigating the transport mechanisms of organic pollutants through the Danube alluvial sediment as a contribution to the study of riverbank filtration process

1. Improving remediation technologies and developing methods for the risk assessment of contaminated sites, Ministry of Education, Science and Technological development (No. III43005). (2011-2020)
2. Inspection of transport mechanisms of pollutants and development of in-situ and ex-situ remediation methods within the zone of river bank filtration, as the base for implemenation of Strategy of water supply and water protection in AP Vojvodina, Provincial secretariat for higher education, scientific and research activity (No. 114-451-2263/2016)



EXPERIMENT

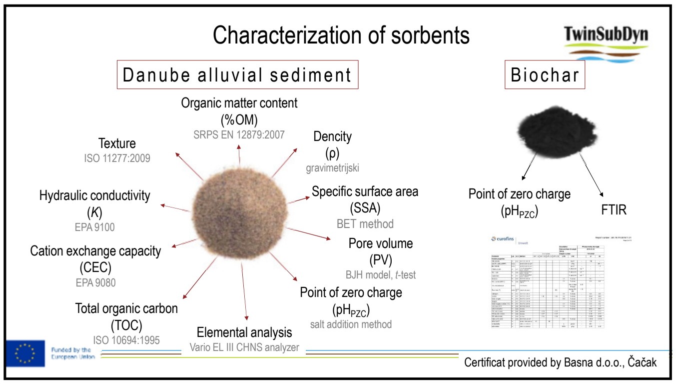
Characterization
1. Danube river alluvial sediment
2. Biochar

Sorption kinetics
1. 9 layers of sediment
2. Biochar
3. Influence of pH (4, 7 i 10 (12))
4. Influence of matrix (synthetic and natural)

Sorption isotherms

Column experiments
1. 9 layers of sediment
2. 2 matrices
3. biochar amendment

13



Characterization of sorbents

Danube alluvial sediment

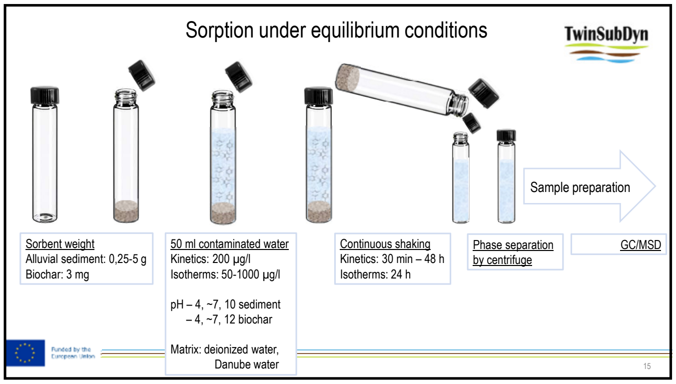
Organic matter content (%OM) SRPS EN 12879:2007
Dencity (ρ) gravimetrijski
Texture ISO 11277:2009
Hydraulic conductivity (*K*) EPA 9100
Specific surface area (SSA) BET method
Cation exchange capacity (CEC) EPA 9080
Pore volume (PV) BJH model, *t*-test
Total organic carbon (TOC) ISO 10694:1995
Point of zero charge (pH_{PZC}) salt addition method
Elemental analysis Vario EL III CHNS analyzer

Biochar

Point of zero charge (pH_{PZC})
FTIR

Certificat provided by Basna d.o.o., Čačak

14



Sorption under equilibrium conditions

Sample preparation

Sorbent weight
Alluvial sediment: 0,25-5 g
Biochar: 3 mg

50 ml contaminated water
Kinetics: 200 µg/l
Isotherms: 50-1000 µg/l

pH – 4, ~7, 10 sediment
– 4, ~7, 12 biochar

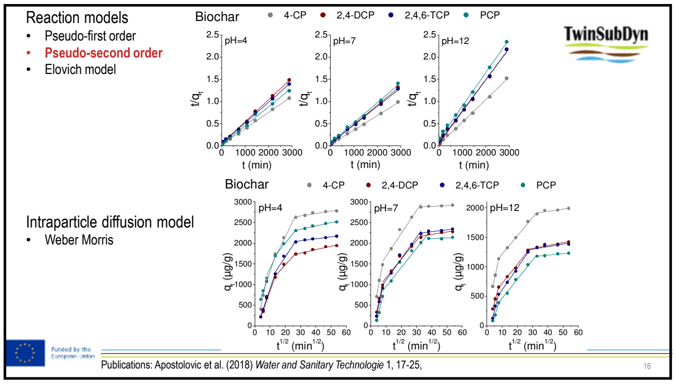
Matrix: deionized water, Danube water

Continuous shaking
Kinetics: 30 min – 48 h
Isotherms: 24 h

Phase separation by centrifuge

GC/MSD

15

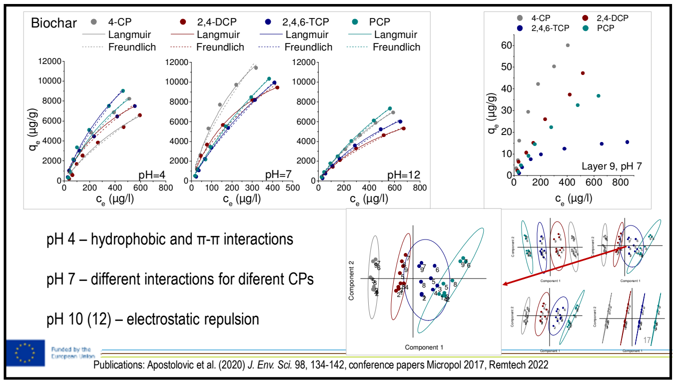


Reaction models
- Pseudo-first order
- **Pseudo-second order**
- Elovich model

Intraparticle diffusion model
- Weber Morris

Publications: Apostolovic et al. (2018) *Water and Sanitary Technologie* 1, 17-25,

16



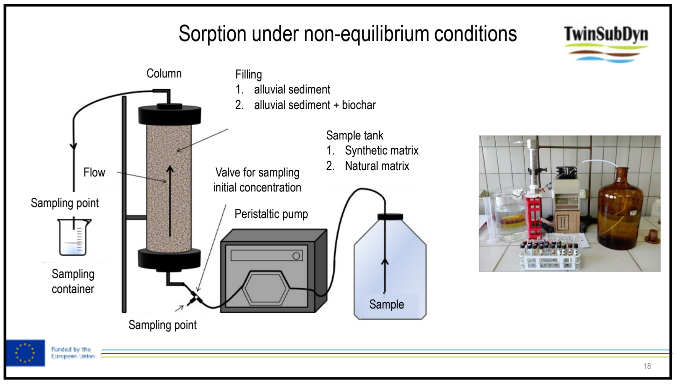
pH 4 – hydrophobic and π-π interactions

pH 7 – different interactions for diferent CPs

pH 10 (12) – electrostatic repulsion

Publications: Apostolovic et al. (2020) *J. Env. Sci.* 98, 134-142, conference papers Micropol 2017, Remtech 2022

17

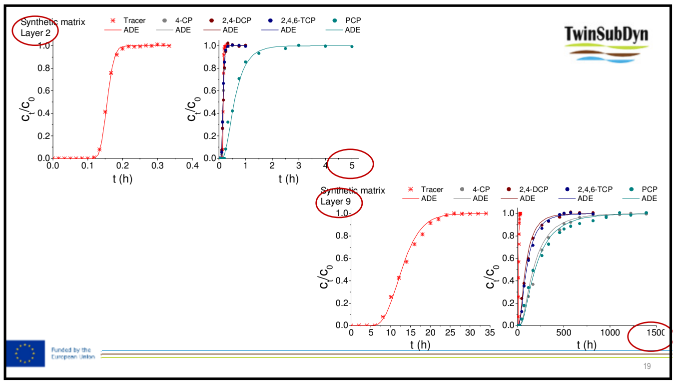


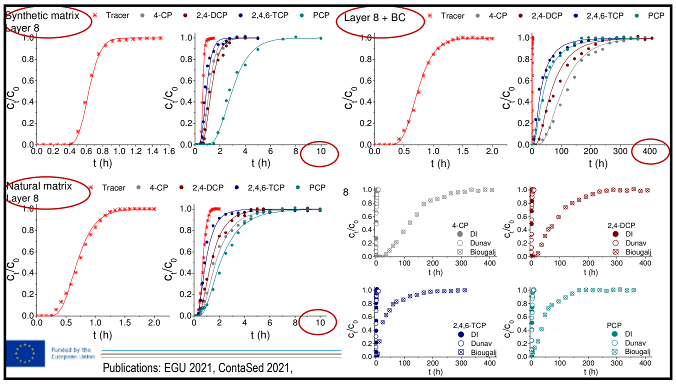
Sorption under non-equilibrium conditions

Filling
1. alluvial sediment
2. alluvial sediment + biochar

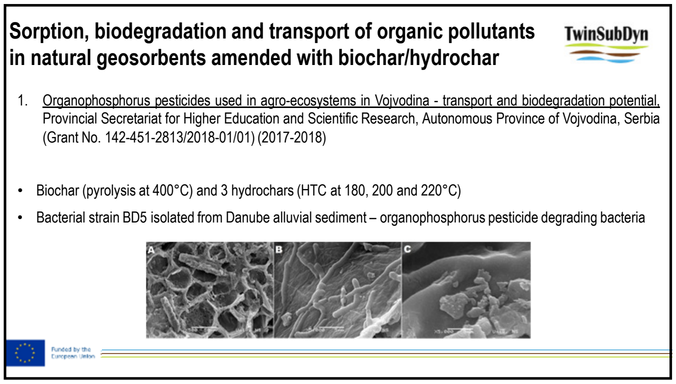
Sample tank
1. Synthetic matrix
2. Natural matrix

18





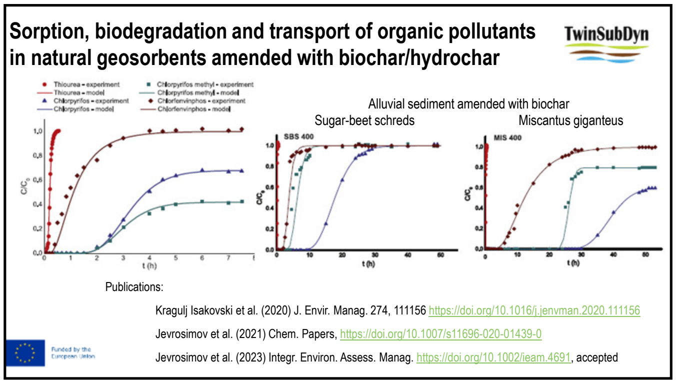
Publications: EGU 2021, ContaSed 2021,



Sorption, biodegradation and transport of organic pollutants in natural geosorbents amended with biochar/hydrochar

1. Organophosphorus pesticides used in agro-ecosystems in Vojvodina - transport and biodegradation potential, Provincial Secretariat for Higher Education and Scientific Research, Autonomous Province of Vojvodina, Serbia (Grant No. 142-451-2813/2018-01/01) (2017-2018)

- Biochar (pyrolysis at 400°C) and 3 hydrochars (HTC at 180, 200 and 220°C)
- Bacterial strain BD5 isolated from Danube alluvial sediment – organophosphorus pesticide degrading bacteria



Sorption, biodegradation and transport of organic pollutants in natural geosorbents amended with biochar/hydrochar

Alluvial sediment amended with biochar

Sugar-beet schreds

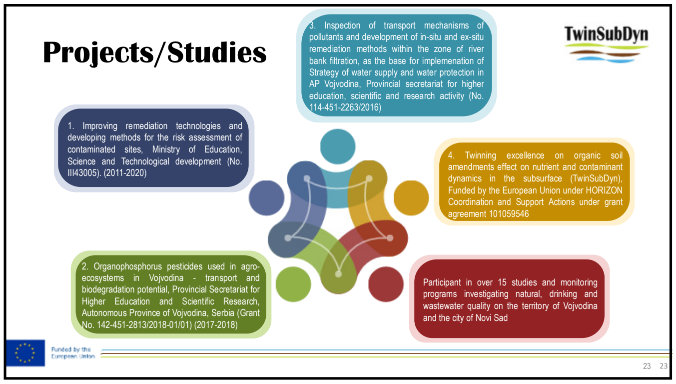
Miscantus giganteus

Publications:

Kragulj Isakovski et al. (2020) J. Envir. Manag. 274, 111156 https://doi.org/10.1016/j.jenvman.2020.111156

Jevrosimov et al. (2021) Chem. Papers, https://doi.org/10.1007/s11696-020-01439-0

Jevrosimov et al. (2023) Integr. Environ. Assess. Manag. https://doi.org/10.1002/ieam.4691, accepted



Projects/Studies

1. Improving remediation technologies and developing methods for the risk assessment of contaminated sites, Ministry of Education, Science and Technological development (No. III43005). (2011-2020)

2. Organophosphorus pesticides used in agro-ecosystems in Vojvodina - transport and biodegradation potential, Provincial Secretariat for Higher Education and Scientific Research, Autonomous Province of Vojvodina, Serbia (Grant No. 142-451-2813/2018-01/01) (2017-2018)

3. Inspection of transport mechanisms of pollutants and development of in-situ and ex-situ remediation methods within the zone of river bank filtration, as the base for implemenation of Strategy of water supply and water protection in AP Vojvodina, Provincial secretariat for higher education, scientific and research activity (No. 114-451-2263/2016)

4. Twinning excellence on organic soil amendments effect on nutrient and contaminant dynamics in the subsurface (TwinSubDyn), Funded by the European Union under HORIZON Coordination and Support Actions under grant agreement 101059546

Participant in over 15 studies and monitoring programs investigating natural, drinking and wastewater quality on the territory of Vojvodina and the city of Novi Sad

Thank you!

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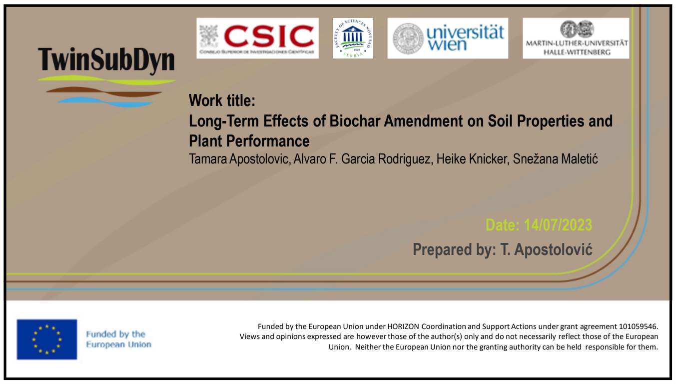
Funded by the European Union under HORIZON Coordination and Support Actions under grant agreement 101059546.



TwinSubDyn

Work title:

Long-Term Effects of Biochar Amendment on Soil Properties and Plant Performance

Tamara Apostolovic, Alvaro F. Garcia Rodriguez, Heike Knicker, Snežana Maletić

Date: 14/07/2023

Prepared by: T. Apostolović

Funded by the European Union

Funded by the European Union under HORIZON Coordination and Support Actions under grant agreement 101059546. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union. Neither the European Union nor the granting authority can be held responsible for them.

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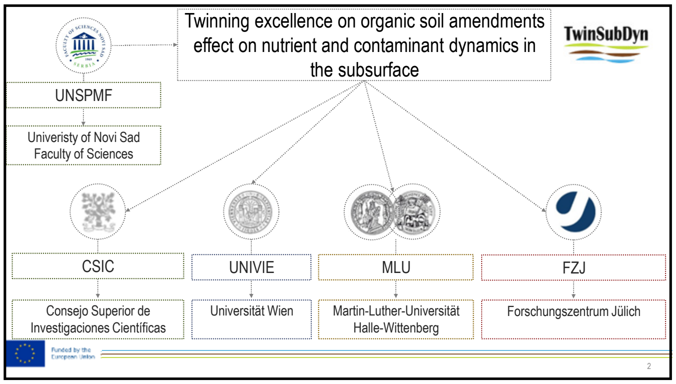


Twinning excellence on organic soil amendments effect on nutrient and contaminant dynamics in the subsurface

TwinSubDyn

UNSPMF

Univeristy of Novi Sad Faculty of Sciences

CSIC

Consejo Superior de Investigaciones Científicas

UNIVIE

Universität Wien

MLU

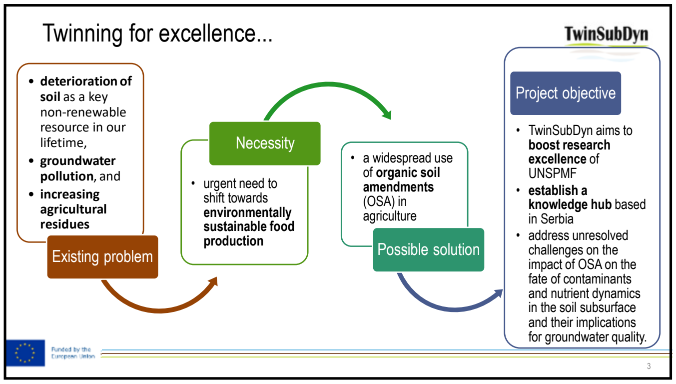
Martin-Luther-Universität Halle-Wittenberg

FZJ

Forschungszentrum Jülich

Funded by the European Union

2



Twinning for excellence...

TwinSubDyn

- **deterioration of soil** as a key non-renewable resource in our lifetime,
- **groundwater pollution**, and
- **increasing agricultural residues**

Existing problem

Necessity

- urgent need to shift towards **environmentally sustainable food production**

- a widespread use of **organic soil amendments** (OSA) in agriculture

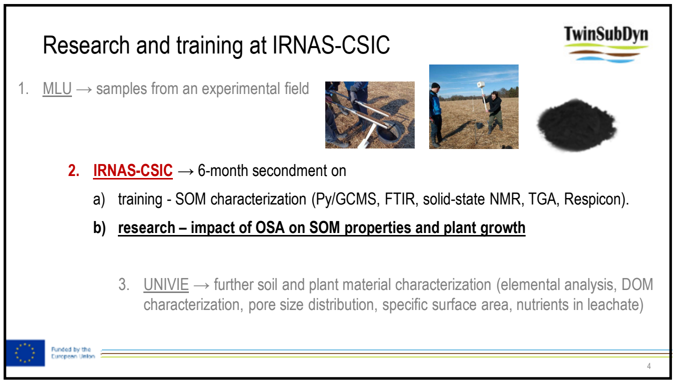
Possible solution

Project objective

- TwinSubDyn aims to **boost research excellence** of UNSPMF
- **establish a knowledge hub** based in Serbia
- address unresolved challenges on the impact of OSA on the fate of contaminants and nutrient dynamics in the soil subsurface and their implications for groundwater quality.

Funded by the European Union

3



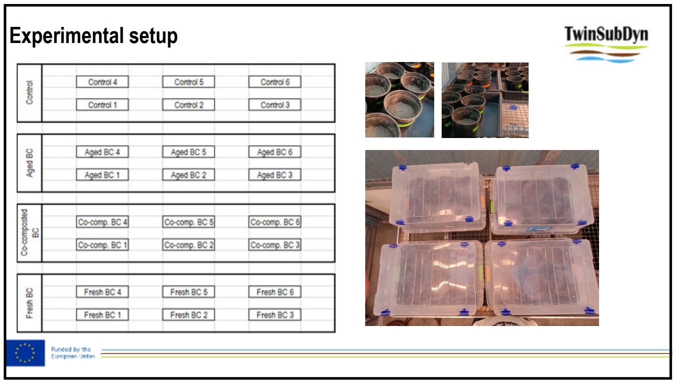
Research and training at IRNAS-CSIC

TwinSubDyn

1. MLU → samples from an experimental field
2. IRNAS-CSIC → 6-month secondment on
   a) training - SOM characterization (Py/GCMS, FTIR, solid-state NMR, TGA, Respicon).
   b) **research – impact of OSA on SOM properties and plant growth**
3. UNIVIE → further soil and plant material characterization (elemental analysis, DOM characterization, pore size distribution, specific surface area, nutrients in leachate)

Funded by the European Union

4



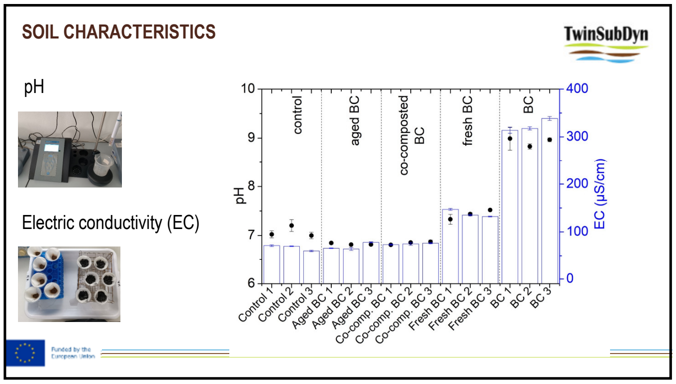
Experimental setup

TwinSubDyn

Control	Control 4	Control 5	Control 6
	Control 1	Control 2	Control 3
Aged BC	Aged BC 4	Aged BC 5	Aged BC 6
	Aged BC 1	Aged BC 2	Aged BC 3
Co-composted BC	Co-comp. BC 4	Co-comp. BC 5	Co-comp. BC 6
	Co-comp. BC 1	Co-comp. BC 2	Co-comp. BC 3
Fresh BC	Fresh BC 4	Fresh BC 5	Fresh BC 6
	Fresh BC 1	Fresh BC 2	Fresh BC 3

Funded by the European Union

5



SOIL CHARACTERISTICS

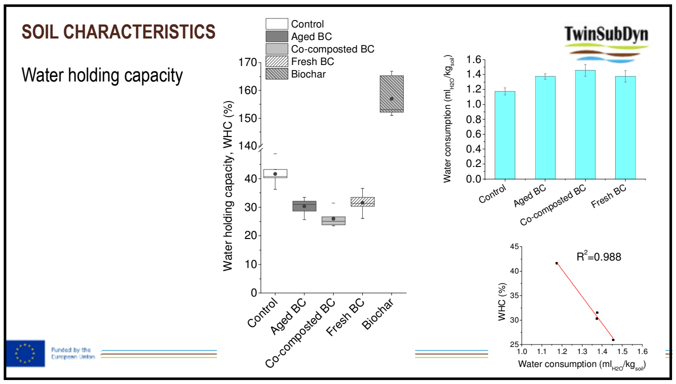
TwinSubDyn

pH

Electric conductivity (EC)

Funded by the European Union

6



SOIL CHARACTERISTICS

Water holding capacity

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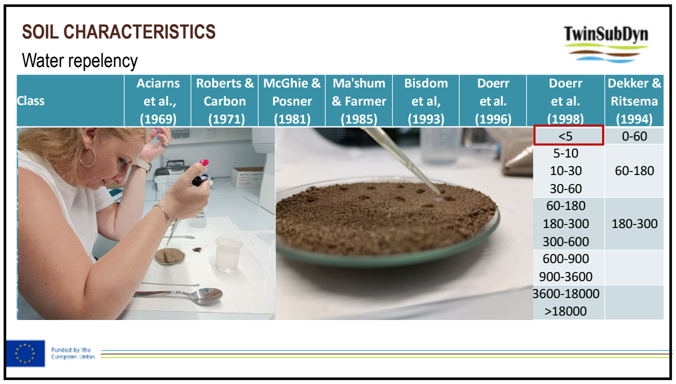
7



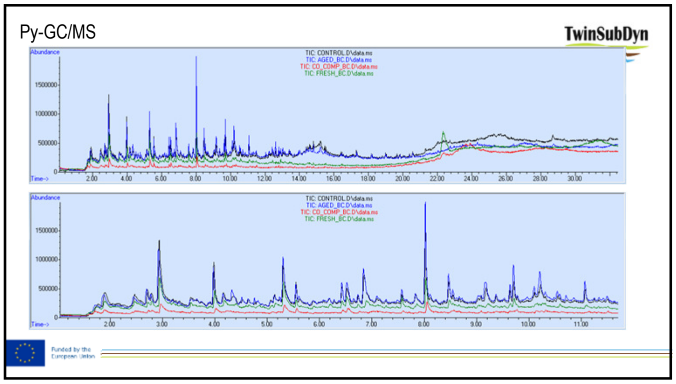
SOIL CHARACTERISTICS

Water repelency

Class	Aciarns et al., (1969)	Roberts & Carbon (1971)	McGhie & Posner (1981)	Ma'shum & Farmer (1985)	Bisdom et al, (1993)	Doerr et al. (1996)	Doerr et al. (1998)	Dekker & Ritsema (1994)
							<5	0-60
							5-10	60-180
							10-30	
							30-60	
							60-180	180-300
							180-300	
							300-600	
							600-900	
							900-3600	
							3600-18000	
							>18000	

Funded by the European Union

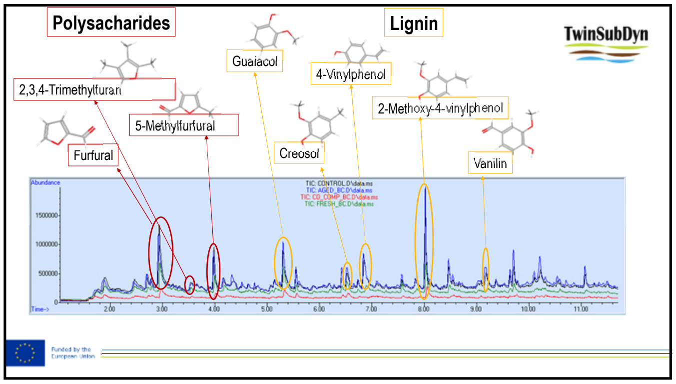
8



Py-GC/MS

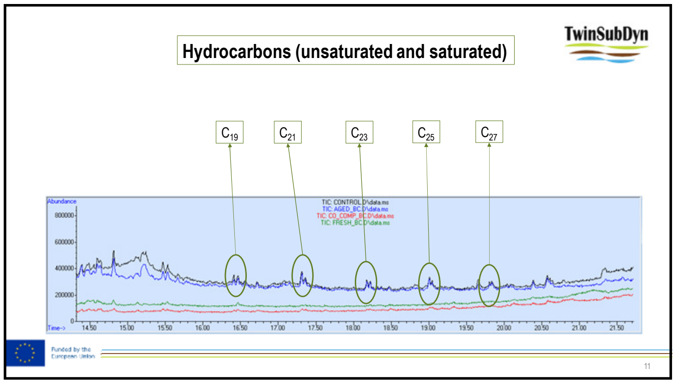
Funded by the European Union

9



Funded by the European Union

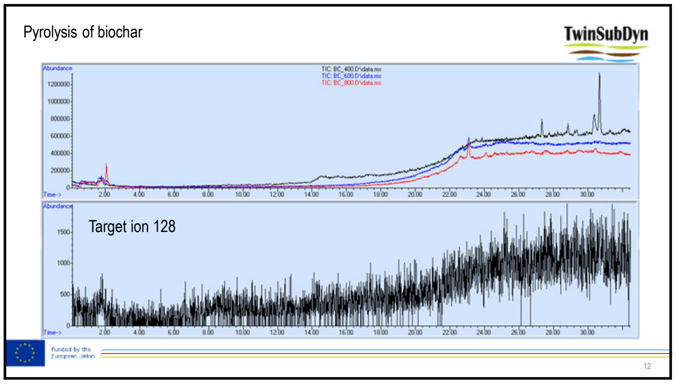
10



Hydrocarbons (unsaturated and saturated)

Funded by the European Union

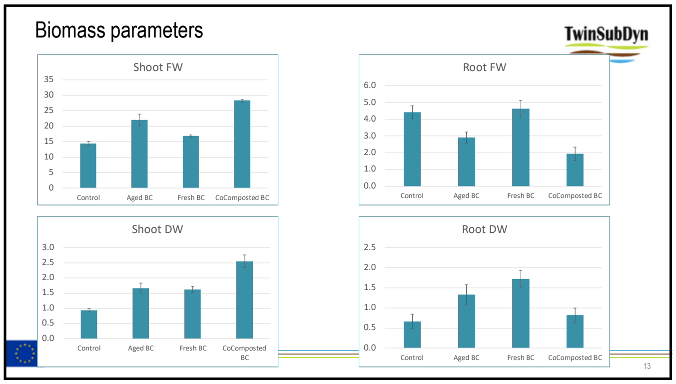
11



Pyrolysis of biochar

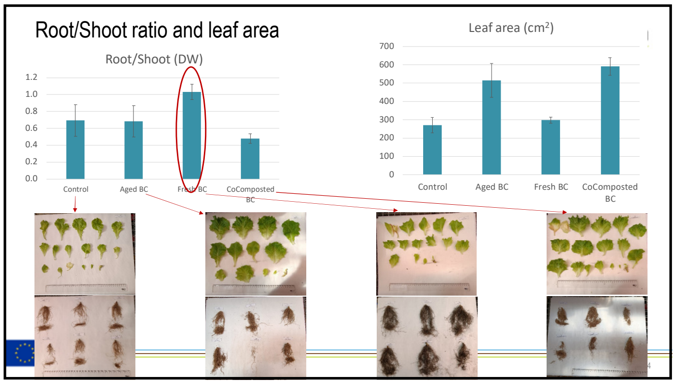
Funded by the European Union

12



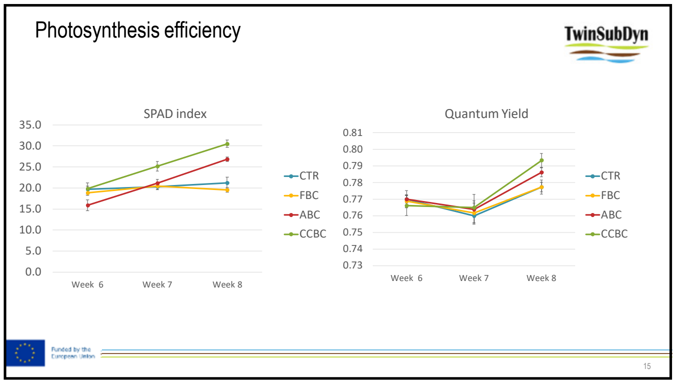
Biomass parameters

13



Root/Shoot ratio and leaf area

14



Photosynthesis efficiency

15

Thank you to the members of MOSS group
Rocio Reinoso Limones
Laura Gismero Rodriguez
Jose Maria Garcia de Castro
Francisco Moreno Racero

Thank you!

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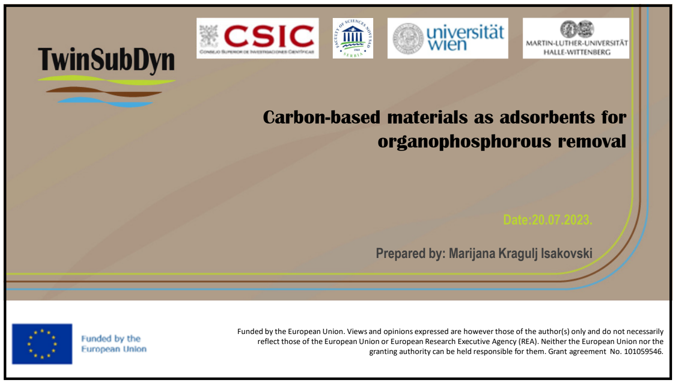

TwinSubDyn

Carbon-based materials as adsorbents for organophosphorous removal

Date:20.07.2023.

Prepared by: Marijana Kragulj Isakovski

Funded by the European Union

Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency (REA). Neither the European Union nor the granting authority can be held responsible for them. Grant agreement No. 101059546.

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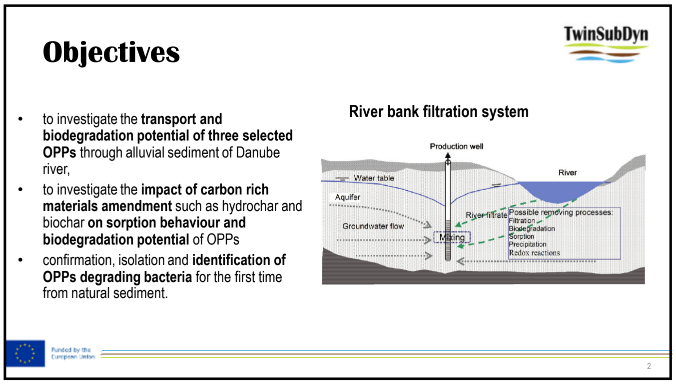
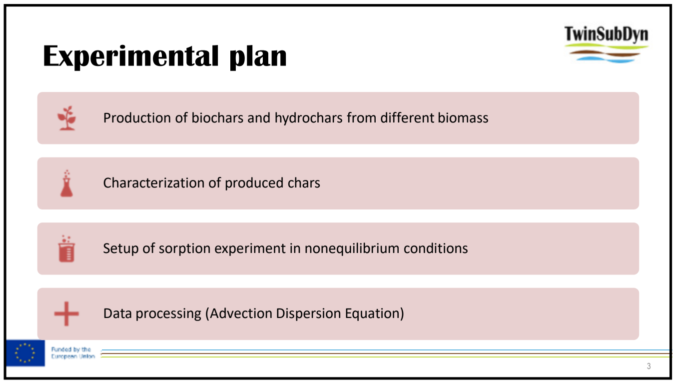


Objectives

- to investigate the **transport and biodegradation potential of three selected OPPs** through alluvial sediment of Danube river,
- to investigate the **impact of carbon rich materials amendment** such as hydrochar and biochar **on sorption behaviour and biodegradation potential** of OPPs
- confirmation, isolation and **identification of OPPs degrading bacteria** for the first time from natural sediment.

River bank filtration system

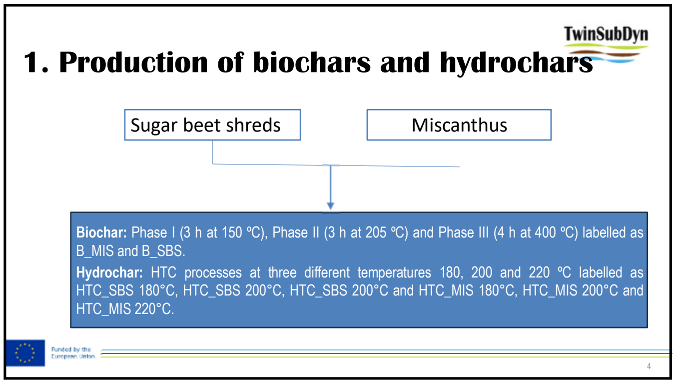
2



Experimental plan

- Production of biochars and hydrochars from different biomass
- Characterization of produced chars
- Setup of sorption experiment in nonequilibrium conditions
- Data processing (Advection Dispersion Equation)

3



1. Production of biochars and hydrochars

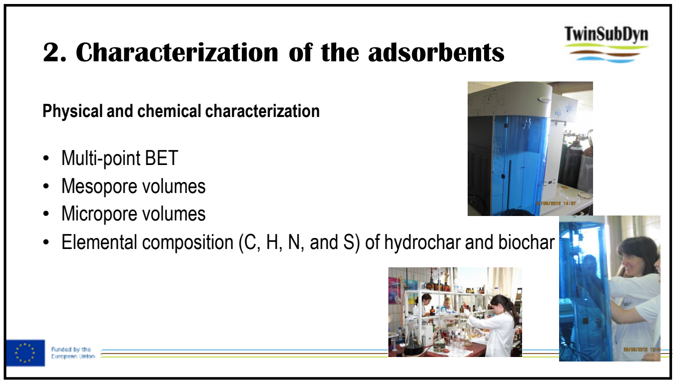
Sugar beet shreds

Miscanthus

Biochar: Phase I (3 h at 150 °C), Phase II (3 h at 205 °C) and Phase III (4 h at 400 °C) labelled as B_MIS and B_SBS.

Hydrochar: HTC processes at three different temperatures 180, 200 and 220 °C labelled as HTC_SBS 180°C, HTC_SBS 200°C, HTC_SBS 200°C and HTC_MIS 180°C, HTC_MIS 200°C and HTC_MIS 220°C.

4

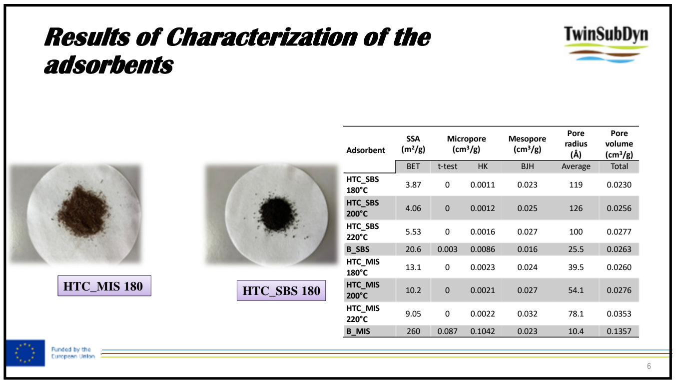


2. Characterization of the adsorbents

Physical and chemical characterization

- Multi-point BET
- Mesopore volumes
- Micropore volumes
- Elemental composition (C, H, N, and S) of hydrochar and biochar

5



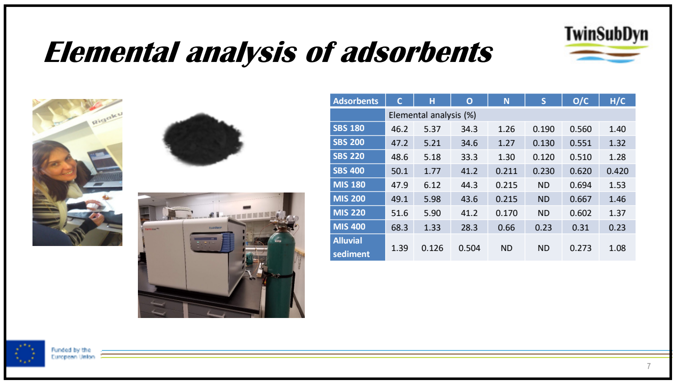
Results of Characterization of the adsorbents

HTC_MIS 180

HTC_SBS 180

Adsorbent	SSA (m²/g)	Micropore (cm³/g)		Mesopore (cm³/g)	Pore radius (Å)	Pore volume (cm³/g)
	BET	t-test	HK	BJH	Average	Total
HTC_SBS 180°C	3.87	0	0.0011	0.023	119	0.0230
HTC_SBS 200°C	4.06	0	0.0012	0.025	126	0.0256
HTC_SBS 220°C	5.53	0	0.0016	0.027	100	0.0277
B_SBS	20.6	0.003	0.0086	0.016	25.5	0.0263
HTC_MIS 180°C	13.1	0	0.0023	0.024	39.5	0.0260
HTC_MIS 200°C	10.2	0	0.0021	0.027	54.1	0.0276
HTC_MIS 220°C	9.05	0	0.0022	0.032	78.1	0.0353
B_MIS	260	0.087	0.1042	0.023	10.4	0.1357

6



Elemental analysis of adsorbents

Adsorbents	C	H	O	N	S	O/C	H/C
	Elemental analysis (%)						
SBS 180	46.2	5.37	34.3	1.26	0.190	0.560	1.40
SBS 200	47.2	5.21	34.6	1.27	0.130	0.551	1.32
SBS 220	48.6	5.18	33.3	1.30	0.120	0.510	1.28
SBS 400	50.1	1.77	41.2	0.211	0.230	0.620	0.420
MIS 180	47.9	6.12	44.3	0.215	ND	0.694	1.53
MIS 200	49.1	5.98	43.6	0.215	ND	0.667	1.46
MIS 220	51.6	5.90	41.2	0.170	ND	0.602	1.37
MIS 400	68.3	1.33	28.3	0.66	0.23	0.31	0.23
Alluvial sediment	1.39	0.126	0.504	ND	ND	0.273	1.08

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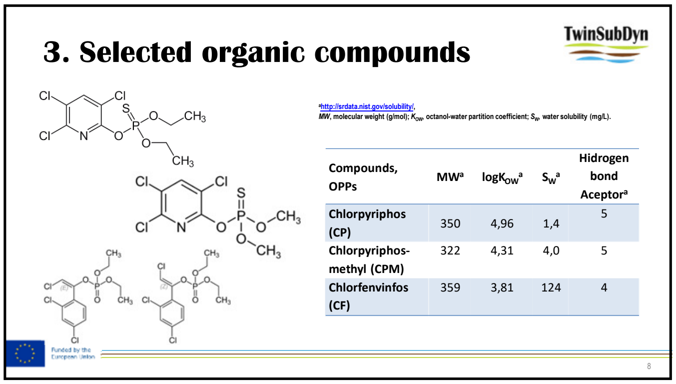

3. Selected organic compounds

[a]http://srdata.nist.gov/solubility/,
MW, molecular weight (g/mol); K_{OW}, octanol-water partition coefficient; S_W, water solubility (mg/L).

Compounds, OPPs	MW[a]	logK_{OW}[a]	S_W[a]	Hidrogen bond Aceptor[a]
Chlorpyriphos (CP)	350	4,96	1,4	5
Chlorpyriphos-methyl (CPM)	322	4,31	4,0	5
Chlorfenvinfos (CF)	359	3,81	124	4

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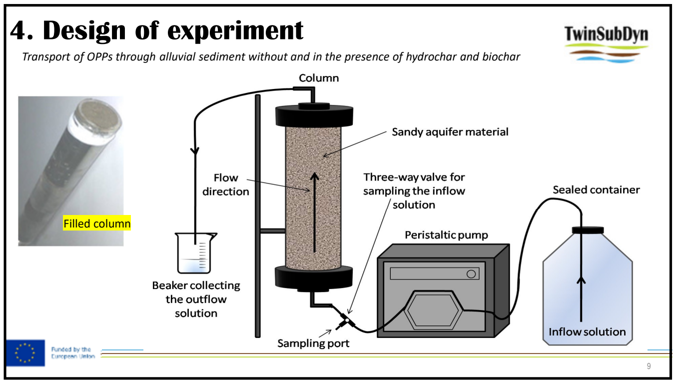

4. Design of experiment

Transport of OPPs through alluvial sediment without and in the presence of hydrochar and biochar

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4. Data analysis

$$c(z, t) = \frac{c_0}{2} exp\left(\frac{v_c z}{2D^*}\right)\left\{exp\left[-\frac{z\,F}{2\,D^*}\right] ercf\left[\frac{z - F\,t}{2\sqrt{D^*\,t}}\right] + exp\left[\frac{z\,F}{2\,D^*}\right] ercf\left[\frac{z + F\,t}{2\sqrt{D^*\,t}}\right]\right\} \tag{1}$$

$$F = \sqrt{v_c^2 + 4\,\frac{\lambda}{R_d}\,D^*} \tag{2}$$

$$v_c = \frac{v_w}{R_d} \tag{3}$$

$$D^* = \frac{D_{ax}}{R_d} \tag{4}$$

c(*z,t*)–concetration at time *t* and at position *z*; v_c–velocity of the transported substance; *z*–position; *t*–time; *λ*–biodegradation rate; R_d–retardation factor; v_w–pore water velocity; D_{ax}–dispersion coefficient in axial direction (=longitudinal dispersion coefficient).

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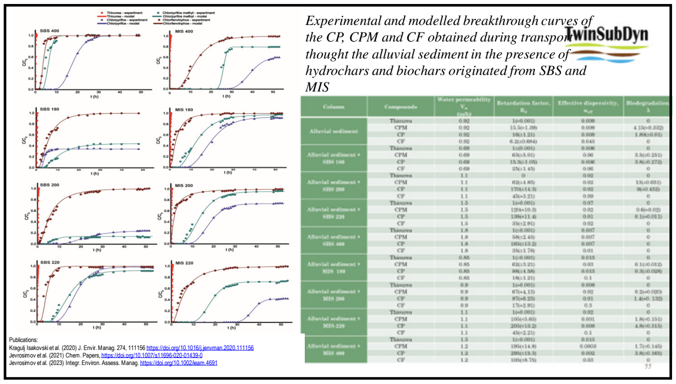
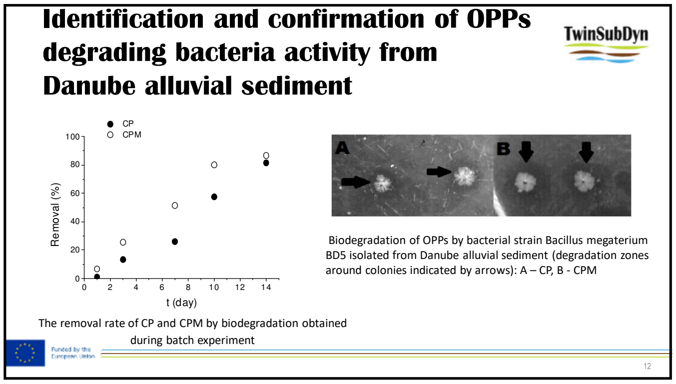


Experimental and modelled breakthrough curves of the CP, CPM and CF obtained during transport thought the alluvial sediment in the presence of hydrochars and biochars originated from SBS and MIS

Column	Compounds	Water permeability V_w (m/h)	Retardation factor, R_d	Effective dispersivity, α_{eff}	Biodegradation λ
Alluvial sediment	Thiourea	0.92	1(±0.001)	0.009	0
	CPM	0.92	15.5(±1.39)	0.009	4.15(±0.332)
	CP	0.92	16(±1.21)	0.009	1.80(±0.01)
	CF	0.92	6.2(±0.684)	0.045	0
Alluvial sediment + SBS 180	Thiourea	0.69	1(±0.001)	0.006	0
	CPM	0.69	63(±5.01)	0.06	3.5(±0.251)
	CP	0.69	15.5(±1.05)	0.006	3.8(±0.272)
	CF	0.69	25(±1.45)	0.06	0
Alluvial sediment + SBS 200	Thiourea	1.1	0	0.02	0
	CPM	1.1	62(±4.85)	0.02	13(±0.651)
	CP	1.1	170(±14.3)	0.02	9(±0.452)
	CF	1.1	45(±3.21)	0.09	0
Alluvial sediment + SBS 220	Thiourea	1.5	1(±0.001)	0.07	0
	CPM	1.5	120(±10.3)	0.02	0.6(±0.02)
	CP	1.5	138(±11.4)	0.01	0.1(±0.011)
	CF	1.5	35(±2.91)	0.02	0
Alluvial sediment + SBS 400	Thiourea	1.8	1(±0.001)	0.007	0
	CPM	1.8	58(±2.45)	0.007	0
	CP	1.8	165(±13.2)	0.007	0
	CF	1.8	35(±1.78)	0.01	0
Alluvial sediment + MIS 180	Thiourea	0.85	1(±0.001)	0.015	0
	CPM	0.85	62(±3.21)	0.03	0.1(±0.012)
	CP	0.85	88(±4.58)	0.015	0.3(±0.028)
	CF	0.85	18(±1.21)	0.1	0
Alluvial sediment + MIS 200	Thiourea	0.9	1(±0.001)	0.008	0
	CPM	0.9	67(±4.15)	0.02	0.2(±0.020)
	CP	0.9	87(±6.25)	0.01	1.4(±0.132)
	CF	0.9	17(±2.91)	0.3	0
Alluvial sediment + MIS-220	Thiourea	1.1	1(±0.001)	0.02	0
	CPM	1.1	105(±5.65)	0.001	1.8(±0.151)
	CP	1.1	205(±13.2)	0.008	4.8(±0.315)
	CF	1.1	45(±2.21)	0.1	0
Alluvial sediment + MIS 400	Thiourea	1.5	1(±0.001)	0.015	0
	CPM	1.2	195(±14.8)	0.0003	1.7(±0.145)
	CP	1.2	295(±15.3)	0.002	3.8(±0.165)
	CF	1.2	105(±8.75)	0.03	0

Publications:
Kragulj Isakovski et al. (2020) J. Envir. Manag. 274, 111156 https://doi.org/10.1016/j.jenvman.2020.111156
Jevrosimov et al. (2021) Chem. Papers, https://doi.org/10.1007/s11696-020-01439-0
Jevrosimov et al. (2023) Integr. Environ. Assess. Manag. https://doi.org/10.1002/ieam.4691



Identification and confirmation of OPPs degrading bacteria activity from Danube alluvial sediment

Biodegradation of OPPs by bacterial strain Bacillus megaterium BD5 isolated from Danube alluvial sediment (degradation zones around colonies indicated by arrows): A – CP, B - CPM

The removal rate of CP and CPM by biodegradation obtained during batch experiment

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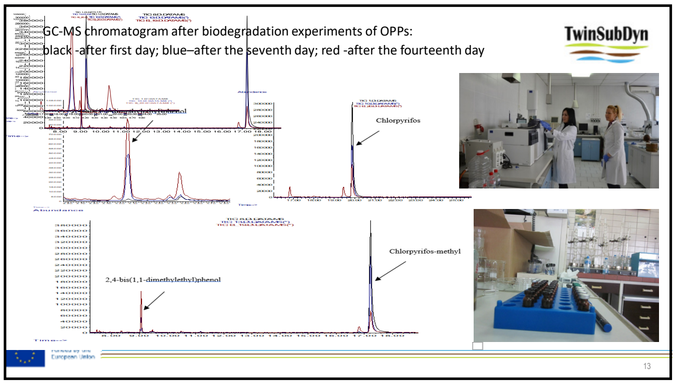

GC-MS chromatogram after biodegradation experiments of OPPs:
black -after first day; blue–after the seventh day; red -after the fourteenth day

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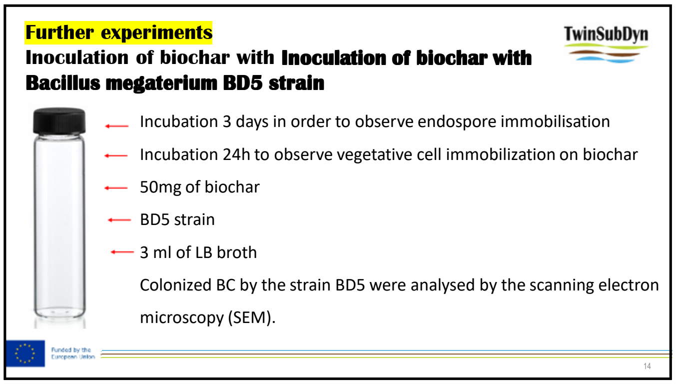

Further experiments

Inoculation of biochar with Inoculation of biochar with Bacillus megaterium BD5 strain

- Incubation 3 days in order to observe endospore immobilisation
- Incubation 24h to observe vegetative cell immobilization on biochar
- 50mg of biochar
- BD5 strain
- 3 ml of LB broth

Colonized BC by the strain BD5 were analysed by the scanning electron microscopy (SEM).

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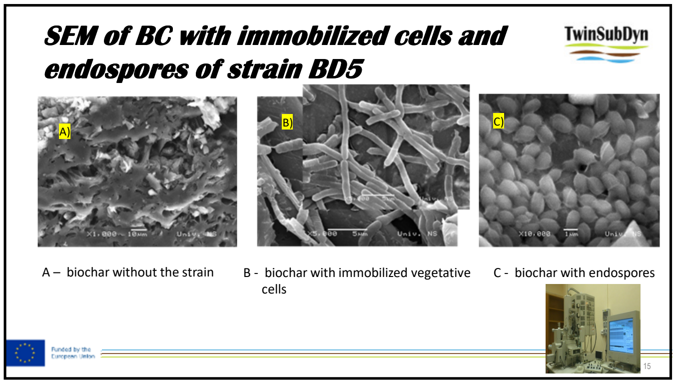

SEM of BC with immobilized cells and endospores of strain BD5

A – biochar without the strain

B - biochar with immobilized vegetative cells

C - biochar with endospores

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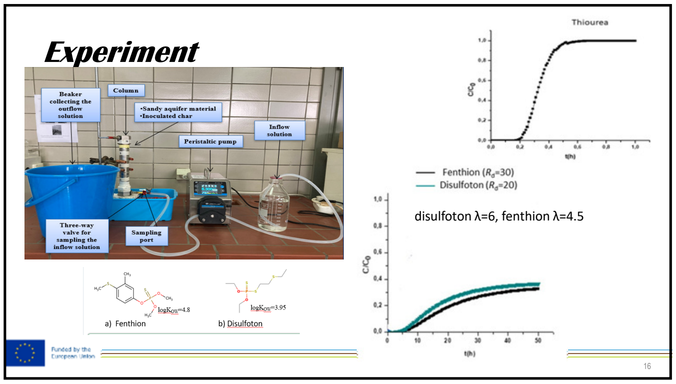

Experiment

disulfoton λ=6, fenthion λ=4.5

a) Fenthion

b) Disulfoton

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Thank you!

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Subtask 1.2.3 6-months secondment for ESR/RR, and two 1-months secondments for a QR/RR at MLU focused on SOM functional analysis using molecular markers and stabile isotope techniques (leader MLU).

MOBILITY REPORT

Researcher: MSc Marko Šolić, UNSPMF

Assigned supervisor: Prof. Dr. Bruno Glaser, MLU

Duration of the visit: 17.02.2023. - 17.08.2023.

Executive Summary

The main reasons for visiting Martin-Luther University Halle-Wittenberg included the following:

1. Participation in the course *Soil Biogeochemical Analysis*. The general topic of the course was *The molecular memory of the soils: Identification of fire and human molecular markers in the Bronze Age tell Toboliu (Western Romania)*. The objective of the course was to learn and apply sophisticated soil biogeochemical methodology for the determination of molecular markers: black carbon (fire-derived organic matter; BC) and steroids and bile acids (human and animal intestine), as well as their analysis by gas chromatography (GC) with mass spectrometry (MSD) and flame ionization detection (FID). Samples from two sites and five depths were analyzed, an anthrosol sampled near the tell, and a phaeozem that was considered as a control. Based on the results a few conclusions were be drawn: (I) BC content was higher in anthrosol than in phaeozem, with shares of benzene penta- and hexacarboxylic acids indicating intensive burning events at high temperatures; (II) both steroids and bile acids content was significantly higher in the anthrosol samples, indicating that the focus of both human and animal activity might have been at/near the tell; (III) based on the calculated biomarker ratios, dominant input by pigs in all anthrosol and most phaeozem can be suspected.
2. Learning about the long-term field experiments with organic soil amendments. For this purpose, an experimental field near Bayreuth, Germany was visited and sampled. Samples of the control soil without treatment, as well as soils treated with biochar and co-

composted biochar were sampled and prepared (dried and packed) for further analysis and experiments at the University of Novi Sad, University of Vienna and IRNAS, Sevilla.

3. Learning and adapting the methodology for the analysis of free benzene polycarboxylic acids (BPCAs). The objective of this activity was to learn and apply sophisticated soil biogeochemical methodology for the determination of free BPCAs using ion exchange chromatography (IC) with variable wavelength detection (VWD), as well as to adapt this methodology for the purpose of conducting free BPCAs analyses through GC-FID technique. For this purpose, samples collected from an experimental field near Bayreuth were used. The samples were gathered from two treatment variants, over three years of sampling and at two different depths. Based on the results obtained so far, the following conclusions can be drawn: (I) the methodology for determining free BPCAs using GC-VWD has been successfully adapted for potential application with the GC-FID technique, as demonstrated by the high recovery values obtained; (II) the levels of free BPCAs, if they were identified at all, in the analyzed soil samples were very low. This suggests either the high stability of the applied biochar or the possibility of certain dissipation of free BPCAs from the soil. Both of the above stated conclusions must be further verified through the original methodology, which involves the use of the GC-VWD technique. This verification has not been conducted yet due to the instrument's failure.
4. Obtaining fundamental skills in utilizing stable isotope ratio mass spectrometry techniques (IR-MS). For bulk sample measurements, this activity included a theoretical introduction to the instrument's operation. In the case of compound-specific measurements, the training encompassed not only the theoretical background but also the development of a method for quantifying ^{13}C in lignin. This process primarily involved setting up a system for conducting the analysis itself.

Introduction

Background

Specific molecules or compounds whose presence indicates an influence of particular processes, organisms, or environmental conditions in soil ecosystems are commonly referred to as molecular biomarkers. Detected in soil, these biomarkers can provide valuable information about soil health, microbial activity, contaminant presence, nutrient cycling, anthropogenic influence and overall ecosystem functioning. Black carbon (BC) refers to the solid, carbonaceous material that remains after incomplete combustion of organic matter. BC in soil can serve as an indicator of human activity, particularly related to the combustion of fossil fuels and biomass. The composition and characteristics of BC can vary depending on the source and combustion conditions, and its detection in soil can help identify the contributions of human activities and assess their impact on soil quality. Furthermore, due to the persistence of BC in soil, it can potentially provide a historical record of human activities. The distribution and characteristics of BC across soil profiles or sediment layers can help reconstruct past human impacts, such as the use of fire by indigenous communities or changes in land use practices over time. Steroids, including various classes such as hormones, phytosterols, and sterols, can also serve as biomarkers in soil. Phytosterols, such as β-sitosterol and stigmasterol, can be used as biomarkers to assess the input of plant-derived organic matter and track the decomposition processes in soil. Animal feces, including those from livestock and wildlife, contain sterols that can be released into the soil during the deposition and decomposition of organic matter. Sterols, such as cholesterol and coprostanol, can serve as indicators of animal presence or fecal contamination in soil. Bile acids are derived from cholesterol metabolism in animals. Their presence in soil can indicate inputs from animal sources or the activity of specific microbial communities, whereas their ratio can point toward the influence of a specific animal group. BPCAs, which are generated when condensed polycyclic aromatic structures within various types of black carbon (BC) or coal are exposed to strong laboratory oxidants, also occur naturally within soil through intensive BC oxidation, leading to the formation of what are referred to as free BPCAs. These compounds are widely distributed in all soil types, with the potential exception of consistently oxygen-deprived soils. As a result, free BPCAs can be valuable indicators for tracking the degradation of black carbon, offering insights into the processes and mechanisms involved in black carbon degradation within the environment. However, it's important to note that concentrations of BPCAs cannot be relied upon as a precise measure of BC degradation due to their significant susceptibility to leaching.

Scope of the secondment

The scope of the secondment included the following:

1. Knowledge transfer involving techniques for analyzing molecular markers in soils, encompassing soil sample preparation, method adaptation, and the analysis of various biomarkers: BC derived BPCAs, Δ^5-sterols, stanols, stanones, bile acids, and free BPCAs.

2. Training in the design and sampling of long-term field experiments involving soil organic amendments.
3. Acquisition of fundamental knowledge and skills in utilizing IR-MS techniques.

In the following chapters, a detailed description of the methodologies applied within this secondment, along with the results obtained through their application, will be provided.

Content

1. Methods

Determination of black carbon derived benzene polycarboxylic acids by GC-FID

<u>Principle</u>: Due to the highly aromatic structure of black carbon (BC), specific determination in soil is carried out by nitric acid degradation which yields a suite of aromatic degradation products. The obtained benzene polycarboxylic acids (BPCAs) are indicators of BC. After conversion into trimethylsilyl derivatives, BPCAs can be separated by capillary gas chromatography and detected with flame ionization detection. An overview of the method is given in Figure 1.

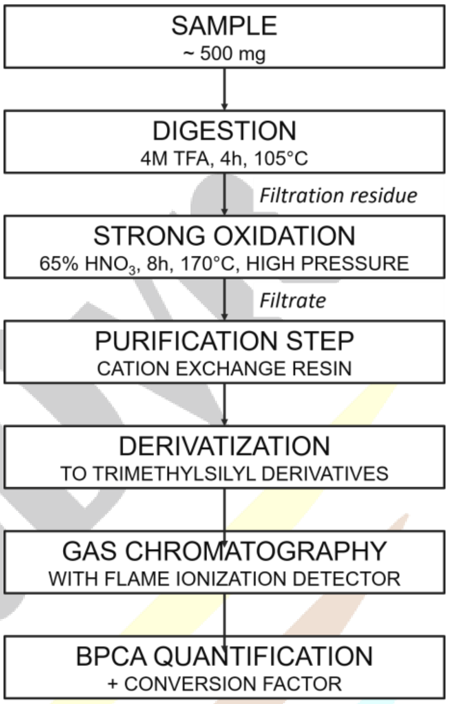


Figure 1. Scheme of BPCAs analysis method

<u>Hydrolysis</u>: Approximately 500 mg of soil sample is weighed and placed into a 25 ml hydrolysis flask (if high BC concentrations are expected, weight is reduced to equal about 5 mg of organic carbon). In order to remove polyvalent metal cations, 10 ml of 4 M trifluoroacetic acid (TFA, prepared by diluting 153 ml of TFA to 500 ml with deionized water) is added, the flask is capped and placed into a furnace for 4 h at 105 °C for hydrolysis (Photo 1). The hydrolysate and residue are transferred onto a glass fiber filter (placed in a Buchner funnel and fitted onto a vacuum flask), rinsed with 5 × 5 ml of deionized water to remove polyvalent cations (particularly Fe^{3+} and Al^{3+}) and the filtrate is discarded (Photo 2). The filter with the hydrolysis residue is transferred onto a watch glass and dried at 40 °C in a drying cabinet (about 2 - 4 hours).

Photo 1. Sample preparation for hydrolysis

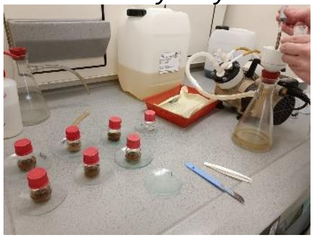


Photo 2. Filtration of the hydrolysate and residue

Photo 3. Oxidation

Oxidation: After it is dry, the hydrolysis residue is quantitatively transferred from the glass fiber filter into a digestion tube and 2 ml of 65% nitric acid is added (Photo 3). The digestion tube is closed and placed in a high-pressure digestion apparatus for 8 hours at 170 °C. The digestion solution is filtered into a 10 ml volumetric flask. The residue is rinsed several times with deionized water, and the flask is filled to the mark.

Photo 4. Conditioning of Dowex 50W×8 resin

Photo 5. Sample purification

Purification: In order to purify the solution from any residual polyvalent cations and impurities, the sample is passed through a conditioned Dowex 50W×8 resin column. Preparation and conditioning of Dowex 50W×8 resin column is done as follows (Photo 4): a 20 cm glass column is fitted with about half a centimeter of glass wool at the base to hold the resin in place, and then filled with 4 g of Dowex extensively prerinsed with, and then slurred in deionized water. The column is rinsed by eluting one column with 2 M NaOH and then washing extensively with 2 columns of deionized water. The column is then activated by eluting one column with 2 M HCl and then rinsing the column with 2 columns of deionized water. The pH of the eluate should be about 6 to 7, and the column must not be allowed to dry at any time. After the column is prepared, a 2 ml aliquot of the solution is transferred to a 10 ml test tube, adding 4 ml of deionized water and 100 µl of internal standard 1 (Internal standard 1 is 1000 ppm phthalic acid solution prepared by dissolving 25 mg of phthalic acid in 25 ml of deionized water, adding 2 or 3 drops of 2 M NaOH for an easier dissolving; prepared freshly on the day of use). A pear shaped quick fit flask (100 ml) is placed under the conditioned Dowex 50W×8 resin column and the sample solution is slowly passed through (Photo 5). The test tube is rinsed with 3 × 1 ml of deionized water, and the column is rinsed with 5 × 10 ml of deionized water, letting each volume eluate first before adding the next. The flask containing the combined eluates is sealed with parafilm, placed in freezer at -18 °C overnight, and freeze dried.


Photo 6. Derivatization

Derivatization: The freeze-dried residue is re-dissolved in 3 × 1 ml of methanol and the solution is transferred into a 5 ml reaction vial (after adding the first 1 ml of methanol, the flask is placed in an ultrasonic bath before transferring the solution, and then rinsed two more times with 1 ml of methanol to recover the entire sample). The solution is evaporated until dry using a nitrogen stream with heat application. In the vial, 100 µl of internal standard 2 is added (internal standard 2 is 1000 ppm biphenylen-2,2-dicarboxylic acid solution prepared by dissolving 25 mg biphenylen-2,2-dicarboxylic acid in 25 ml of methanol), and then evaporated until dry using a nitrogen stream with heat application. After the solution is evaporated, 98 µl of N,O-bis(trimethylsilyl)-trifluoroacetamide (BSTFA), 2 µl of N-trimethylsilylimidazole (TSIM) and 50 µl of Toluol are added, the vial is closed with a Teflon seal and vortexed. The vial is heated for 2 hours at 90 °C, then after 15 min vortexed again, left to cool down to room temperature and finally the solution is transferred into a GC vial with a glass insert (Photo 6).

External standards preparation: An external standard solution (100 ppm) is prepared by dissolving 25 mg of each standard (phthalic acid, hemimellitic acid, trimellitic acid, trimesic acid, pyromellitic acid, benzene pentacarboxylic acid, and mellitic acid) in 25 ml of methanol. In six 5 ml reaction vials, 10, 25, 50, 100, 250, and 500 µl of external standard solution is transferred, and evaporated until dry using a nitrogen stream with heat application. In each vial, internal standard 1 and internal standard 2 is added (100 µl each), the content is evaporated until dry using a nitrogen stream with heat application, and a derivatization step is carried out following the same procedure as with the samples.


Photo 7. GC-FID analysis

Analysis: The prepared sample is analyzed by capillary gas chromatography with flame ionization detection (GC-FID) (Photo 7). The gas chromatographic conditions applied for the analysis are as follows: *GC* - Shimadzu GC-2100; *Column* - Supelco SPB-5 or Agilent HP-5 (30 m, 0.25 mm inner diameter, 0.25 µm film); *Injector* - Temp.: 300 °C; Carrier: He; Flow control mode: const. flow (79.3 ml/min); Split ratio: 1:50; Purge: 3.0 ml/min; Injection volume: 1 µl (or 2 µl); *Detector* - Flame ionization detector (FID); Temp.: 330 °C; Make-up: (He) 30.0 ml/min; H_2 flow: 40.0 ml/min; Air flow: 400.0 ml/min; Sampling rate: 40 msec;

Temperature Program - 100 °C for 2 min, then up to 240 °C at 20 °C/min; 240 °C for 7 min, then up to 300 °C at 30 °C/min; 300 °C for 10 min.

Calculation and interpretation: Individual compounds are identified by comparing retention times with those of standard compounds (Figure 2); validation may be supported by GC-MS and mass spectral library searches. For routine analysis, only GC-FID is used. Phthalic acid and biphenylen-2,2-dicarboxylic acid are used as internal standards due to their similar structures to BPCAs. They occur rarely in hydrolysates of natural soil samples. Sum of BPCAs indicates total BC in sample; estimation of BC can be done by applying a conversion factor of 2.27 to BPCAs-C, which corrects for the loss of CO_2 during the partial oxidation of BC structures.

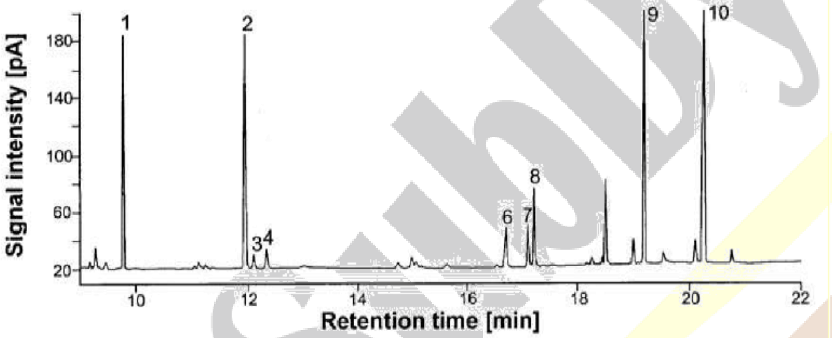


Figure. Chromatogramm of BPCAs of a Haplic Chernozem (1) phthalic acid (IS 1); (2) biphenyl-2,2'-dicarboxylic acid (IS2); (3) hemimellitic acid; (4) trimellitic acid; (5) trimesic acid; (6) pyromellitic acid; (7) mellophanic acid; (8) prehnitic acid; (9) benzenepentacarboxylic acid; (10) mellitic acid.

Figure 2. An example of a chromatogram for BPCAs analysis

Method for extraction and purification of Δ^5-sterols, stanols, stanones and bile acids (+alkanes) in soil and sediment samples

Principle: Stanols, stanones, Δ^5-steroles and bile acids are extracted from soil samples by a single Soxhlet extraction yielding a total lipid extract (TLE). The TLE is saponified with KOH in methanol. Sequential liquid–liquid extraction is applied to recover the biomarkers from the saponified extract and to separate the bile acids from the neutral stanoles, stanones and Δ^5-steroles. The neutral fraction is directly purified using solid phase extraction (SPE) columns packed with 5% deactivated silica gel. The bile acids are methylated in dry HCl in methanol and purified on SPE columns packed with activated silica gel. A mixture of hexamethyldisilazane (HMDS), trimethylchlorosilane (TMCS) and pyridine is used to silylate the hydroxyl-hydroxyl groups of the stanols and Δ^5-sterols avoiding a silylation of the keto groups of the stanones in their enol-form.

Silylation of the bile acids is carried out with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing N-trimethylsilylimidazole (TSIM). Standard addition experiments showed mean recoveries of individual substances ≥85%. The recoveries of biomarkers within each biomarker group did not differ significantly. Precisions were ≤0.22 (RSD) and quantification limits were between 1.3 and 10 ng/g soil. Figure 3 shows a schematic diagram of the procedure.

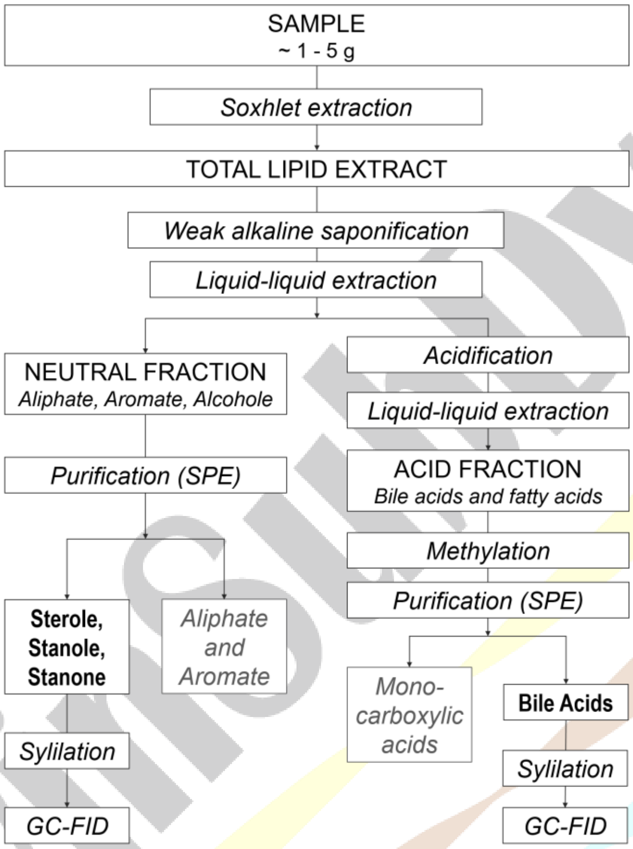


Figure 3. Schematic diagram of stanols, stanones, Δ^5-steroles and bile acids analysis method

<u>Soxhlet extraction</u>: Approximately 5 g of dried and ground soil is weighed into a fiberglass thimble (up to 10 g for soil with low C content). Quartz sand (~1-2 cm) is added over the sample to avoid encrustation, along with 5-10 boiling chips. The sample is extracted (Photo 8) for approximately 40 h with 210 ml dichloromethane (DCM)/methanol (MeOH) mixture (v/v=2:1). Heat is adjusted to 3-5 drops per second. Internal standard 1 solution is added to the extract, and extract is transferred with DCM/MeOH (v/v=2:1) into a 100 ml pear

Photo 8.Soxhlet extraction

shaped flask (wash 3 times during transfer). The volume is reduced using a rotary evaporator (40 °C, 800 → 200 mbar; 800 mbar for 3 min, then to 250 or 200 mbar in 50 mbar steps). The residue is transferred into a 5 ml reaction vial with DCM/MeOH (v/v=2:1) (wash 3 times during transfer).

<u>“Mild” saponification</u>: The reaction vial is placed under a gentle stream of nitrogen to evaporate the extract until dry. For saponification, 3.5 ml of 0.7 M KOH in MeOH is added and then shaken to dissolve (using an ultrasonic bath or vortex). The vial is left at room temperature for 10-14 h (overnight) to allow the reaction. The sample is transferred into a separation funnel, and the vial is washed 3 times with MeOH during the transfer. Then, 10 ml of distilled water is added.

<u>Liquid-liquid extraction</u>: For the extraction, 15 ml of chloroform is added into the separation funnel (Photo 9), the sample is shaken for approximately 1 min, and the chloroform layer (lower layer) is collected in a 50 ml pear shaped flask. The extraction step is repeated two more times. The collected extract is labeled as the “AAA-fraction” and contains aliphates, aromates and alcohols including stanols, stanones and Δ^5-steroles. The remaining aquatic fraction in the funnel is acidified with 6 M HCl to achieve a pH of ≤ 2. The extraction step (3 extractions with 15 ml of chloroform) is repeated and the extracts are collected in a new 50 ml pear shaped flask. This extract is labeled as the “BAFA-fraction” and contains bile acids and fatty acids. The aquatic fraction is discarded. The volume of the AAA-fraction is reduced in a rotary evaporator (Photo 10) at 600-400 mbar, and if necessary, a gentle stream of nitrogen can be used at the end. The residue is dissolved in a small amount of hexane using an ultrasonic bath and then transferred into a 5 ml reaction vial. The volume of the BAFA-fraction is reduced to ~200 µl in a rotary evaporator at 600-400 mbar. The remaining sample is transferred into a 5 ml reaction vial using 5% MeOH in chloroform (it may be necessary to use ultrasonic bath for washing to ensure quantitative transfer).

Photo 9. Liquid-liquid extraction

Photo 10. Rotary evaporator

Photo 11. Purification/SPE

SPE of “AAA-fraction” (stanols, stanones and ∆5-steroles): A clean-up step is carried out by solid-phase extraction with 5% deactivated silica gel (silica gel is activated in a dry cabinet at 120 °C overnight and then allowed to cool slightly in an exicator; the still warm silica gel is deactivated by adding 5% (m/m) distilled water and shaking until the reaction is complete (silica gel cools down); after deactivation, it can be stored in hexane). The SPE column (Photo 11) is filled with hexane, then with the 5% deactivated silica gel, until about 0.5 cm of empty space remains at the top (the empty space is filled with hexane). During filling, some hexane is eluting; this amount of hexane is sufficient for the conditioning of the silica gel. An aliquot of the sample (the volume should be marked) is transferred onto the column, and eluted with 5 ml of hexane (aliphatics and aromatics, AA fraction) in two steps of 2.5 ml each. This fraction is discarded, unless AA fraction is required for analysis, in which case, it is eluted with 4 ml of hexane in to steps of 2 ml each and collected in a reaction vial. Sterols, stanols and stanones (SSS fraction) are eluted with 3 ml of DCM (in two steps of 1.5 ml each) and 2 ml of DCM/acetone (2:1 v/v) (in two steps of 1 ml each). The eluates are collected in a reaction vial or in small centrifuge tubes, and evaporated under a gentle nitrogen stream until dry.

Photo 12. Vortexer

Silylation of hydroxy groups of stanols and Δ^5-sterols: Methanol is added to the reaction vial and the sample is dissolved in the ultrasonic bath and by shaking (vortexer, Photo 12). An aliquot of the sample is then transferred into a GC-V-vial. External standards need to be silylated together with the samples (preparation of external standards: 5, 10, 25, 50, 100, 250, and 500 µl of a methanol work solution containing ~10 µg/ml of each sterol is pipetted into GC-V-vials). The sample and external standards are dried under a gentle stream of nitrogen. In each vial, 50 µl of a BSTFA/TMCS + pyridine mixture (3:1, v/v) is added (the mixture is prepared by adding 3 parts of N,O-bis(trimethylsilyl)trifluoroacetamide (BSFTA)/trimethylchlorosilane (TMCS) mixture (99:1 v/v) and one part of dry pyridine in a reaction vial just before addition to the samples/external standards). Samples and external standards are heated at 90 °C for 1 h, and then dried under a gentle stream of nitrogen. After drying, internal standard 2 (α-Cholestan in dry toluene) is added to all vials (samples and external standards), and more dry toluene is added to achieve a final volume of 150 µl. Samples and external standards are transferred to GC vials with inlet (transfer is done with Pasteur pipette, GC-V-vials should not be washed as all internal standards have been added at this point, and measurement of concentration will be done by GC/MS/MS).

Methylation of carboxy groups of bile acids: External bile acid standards need to be methylated together with the samples in this step of the analysis (preparation: 5, 10, 25, 50, 100, and 250 µl of methanol work solution containing ~10 µg/ml of each bile acid is transferred into reaction vials for methylation). The solvents of the BAFA-fraction (if an aliquot is used, the exact volume must be noted) and external standards are evaporated under a gentle stream of nitrogen. Into the reaction vials 1 ml of HCl in MeOH is added, and the vials are tightly closed with cup, metal disc and PTFE-septum (the flask containing HCl in MeOH should be closed under a gentle stream of nitrogen or argon, in order to push out air/humidity above the solution). The vials are heated at 80 °C for 2 h in a heating block to allow reaction. After cooling to room temperature, 1 ml distilled water (millipore) and 1 ml of hexane are added, and vials are shaken for 1 min or vortexed. The hexane (upper) layer is collected and transferred into a 5 ml reaction vial. The separation is repeated additional 2 times with 1 ml of hexane each, and the hexane layer is collected into the reaction vial (overall, 3 step separation with hexane). The reaction vial with the collected hexane layers is closed with cup and PTFE-septum. The aqueous layer is discarded of.

SPE of bile acids: A clean-up step is carried out by solid-phase extraction using activated silica gel (silica gel is activated in a dry cabinet at 120 °C overnight and stored in an exicator; it can be stored in hexane after activation). The SPE column is filled with hexane, then with the activated silica gel, until about 0.5 cm of empty space remains at the top (the empty space is filled with hexane). During filling, some hexane is eluting; this amount of hexane is sufficient for the conditioning of the silica gel. An aliquot or the whole sample in DCM/hexane (2:1, v/v, dissolved in ultrasonic bath and washed 3 times with DCM/hexane) is transferred onto the column, and eluted with 4 ml DCM/hexane (2:1, v/v) (fatty acid methyl esters fraction) in two steps of 2 ml each. This fraction is discarded. Bile acids (BA fraction) are eluted with 5 ml DCM/MeOH (2:1, v/v) in two steps of 2.5 ml each. The eluates are collected in a reaction vials and dried under a gentle stream of nitrogen (Photo 13).

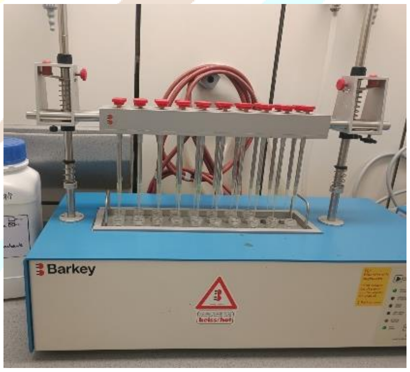


Photo 13. Evaporation in N_2 stream

Silylation of hydroxy groups of bile acids: The dried samples and standards are diluted in 20 µl of toluene, and 98 µl of BSTFA and 2 µl of TSIM (100 µl mixture) are added (the mixture of 98 parts BSTFA and 2 parts TSIM in a reaction vial is prepared just before addition to the samples and standards). Samples and external standards are heated for 1 h at 180 °C, and internal standard 2 (α-Cholestan in dry toluene) is added. If necessary, dry toluene is added to archive a final volume of dry toluene addition of 50 µl (standard solution + maybe additional dry toluene) to samples and external standards. Samples and external standards are transferred to GC vials with inlet (transfer is done with Pasteur pipette, GC-V-vials should not be washed as all internal standards have been added at this point, and measurement of concentration will be done by GC/MS/MS).

Determination of free benzene polycarboxylic acids by IC-VWD

Principle: Free BPCAs naturally occurring in soil are extracted using a NaOH solution. Following the extraction and removal of humic acids, the free BPCA extracts undergo a series of steps, including purification, freeze-drying, redissolution, and filtration. The isolated free BPCAs are further separated using ion exchange chromatography and subsequently detected by variable wavelength detector. Analyzing free BPCAs, which primarily result from black carbon degradation, can enhance understanding of black carbon's fate and degradation mechanisms in the environment. An overview of the method is given in Figure 4.

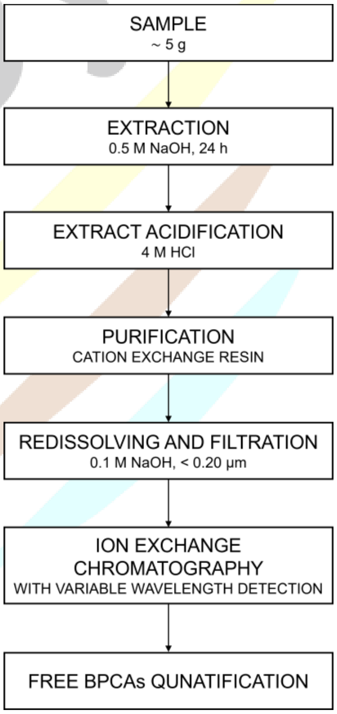


Figure 4. Scheme of free BPCAs analysis method

Extraction: Approximately 5 g of air-dried, crushed, and sieved soil sample (< 2 mm) is directly weighed into a 50 ml centrifuge tube. Subsequently, 100 µl of internal standard 1 (Internal standard 1 is a 1000 ppm phthalic acid solution, freshly prepared by dissolving 25 mg of phthalic acid in 25 ml of deionized water and adding 2 or 3 drops of 2 M NaOH for easier dissolving) and 25 ml of 0.5 M NaOH are added to the weighed soil sample. In the following step, the centrifuge tube containing the sample is placed on a horizontal shaker and agitated at 250 rpm for 24 hours.

Following this agitation period, the sample is then subjected to centrifugation at 3700 g for 15 min, leading to the separation of the liquid phase, which is subsequently carefully transferred to a fresh centrifuge tube. To eliminate humic acids present within the extracted solution, 4 ml of a 4 M HCl is introduced into the centrifuge tube containing the extract. After subjecting it to centrifugation at 3700 g for 15 minutes, the resulting extract, now free from humic acids (Photo 14), is carefully transferred to a clean glass vial for further analysis or storage (Photo 15).

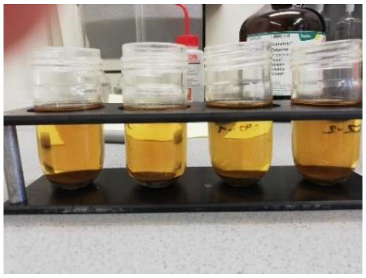


Photo 14. Precipitation of humic acids


Photo 15. Free BPCAs extract

<u>Purification</u>: In order to purify the extract from any residual polyvalent cations and impurities, the sample is passed through a conditioned Dowex 50W×8 resin column. The process of preparing and conditioning a Dowex 50W×8 resin column is carried out as outlined below: a glass column, measuring 35 cm in length and having an inner diameter of 2.6 cm, is fitted with approximately 0.5 cm of glass wool at the bottom to secure the resin in place. This column is then filled to a 10 cm level (equivalent to a volume of 53 cm^3) with Dowex resin that has been rigorously pre-rinsed and then suspended in deionized water. Following this initial step, the column is rinsed by passing one column volume of 2 M NaOH through it, followed by washing with two column volumes of deionized water. Subsequently, the column is activated by eluting with a single column volume of 2 M HCl, followed by rinsing with two column volumes of deionized water. The pH of the eluate should fall within the range of 6 to 7, and it is imperative to ensure that the column remains consistently moist, never allowing it to dry out. A pear-shaped quick-fit flask (250 ml) is positioned beneath the conditioned Dowex 50W×8 resin column, and a 20 ml aliquot of the extract is passed through (Photo 16). Following the complete elution of the aliquot, the column is washed twice with 55 ml of deionized water, allowing the first eluate volume to completely pass through before introducing the next one. The flask containing the combined eluates is sealed with parafilm, then placed in a freezer set to -18°C overnight, and subsequently subjected to freeze-drying (Photo 17).


Photo 16. Sample purification


Photo 17. Sample freeze-drying

<u>Redissolving and filtration</u>: The freeze-dried residue (Photo 18) is redissolved in one step by adding 2 ml of a 0.1 M NaOH solution already containing internal standard 2 (internal standard 2 is 500 ppm biphenylen-2,2-dicarboxylic acid solution, freshly prepared by dissolving 12.5 mg of biphenylen-2,2-dicarboxylic acid in 250 ml of a 0.1 M NaOH solution). To ensure complete dissolution of the residue within these 2 ml, the flask is firstly immersed in an ultrasonic bath, and its inner walls are subsequently flushed several times with the solution it contains. Prior to analysis, it is essential to pass the sample through a filter with a pore size of less than 0.2 µm.

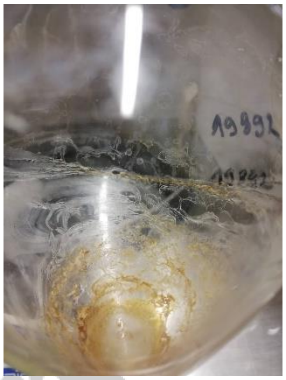


Photo 18. Freeze-dried residue

<u>External standards preparation</u>: An external standard solution (1000 ppm) is prepared by dissolving 25 mg of each individual standard compound, including phthalic acid, hemimellitic acid, trimellitic acid, trimesic acid, pyromellitic acid, benzene pentacarboxylic acid, and mellitic acid, in the same 25 ml of methanol. Subsequently, aliquots of 10, 25, 50, 100, 250, and 500 µl from this external standard solution are transferred to six 5 ml reaction vials and dried to completion using a nitrogen stream with heat application. Following the evaporation process, 2 ml of a 0.1 M NaOH solution, already containing internal standard 2, is added to each of the six vials. The vials are sealed with Teflon caps and thoroughly vortexed. The contents of six vials are transferred into clean vials, and just before analysis, they are filtered into IC vials using filters with a diameter of less than 0.2 µm.

<u>Analysis</u>: The prepared sample is analyzed by ion exchange chromatography with a variable wavelength detector (IC-VWD) (Photo 19). The ion exchange chromatographic conditions applied for the analysis are as follows: *IC* - Dionex ICS 5000 (Thermo Fisher, Bremen, Germany); *Guard column* - AG11-HC; *Column* - Dionex IonPac AS11-HC (2 × 250 mm); *Injection volume* - 5 µl; *Flow rate* - 350 µl/min; *Mobile phase* - Sodium hydroxide (0.1 M, eluent A) and deionized water (eluent B); *Gradient elution* - 0–1 min 30–35%, 1–5 min 35–40%, 5–20 min 40–80%, 20–30 min 80–100%, 30–35 min 100%, 35–40 min 100–30%, 40–50 min 30% (% of eluent A); *Detector* - Variable wavelength detector (VWD); *Detection wavelength* - 254 nm; *Total runtime* - 50 min. Before analyzing each batch, the system is rinsed with 200 mM NaOH for 30 minutes, followed by a 1-hour washing cycle with deionized water to equilibrate the system.

Photo 19. IC-VWD analysis

Calculation and interpretation: Individual free BPCAs are identified by comparing their retention times with those of standard compounds (Figure 5). Phthalic acid and biphenylen-2,2-dicarboxylic acid are used as internal standards due to their structural similarities with BPCAs. These compounds are infrequently found in the hydrolysates of natural soil samples.

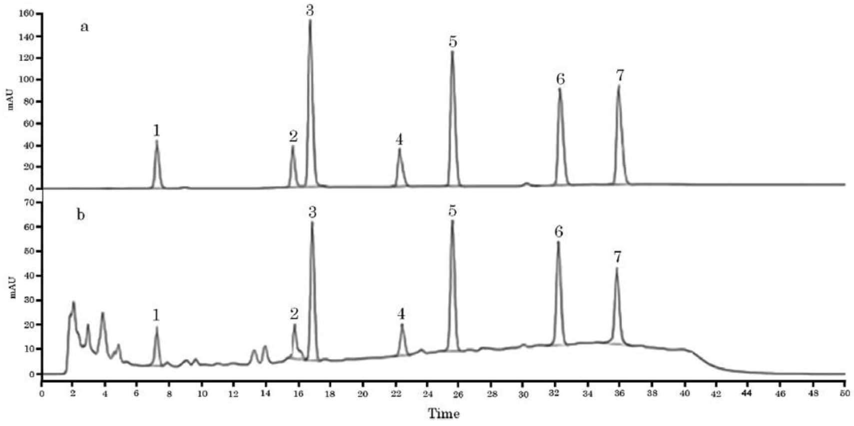


Figure 2: Ion exchange chromatogram of a benzenepolycarboxylic acids standard (a) and a Chernozem sample (b). (1) Phthalic acid (internal standard); (2) hemimellitic acid; (3) trimellitic acid; (4) trimesic acid; (5) pyromellitic acid; (6) benzene pentacarboxylic acid; (7) mellitic acid.

Figure 5. An example of a chromatograms for free BPCAs analysis

Determination of free benzene polycarboxylic acids by GC-FID

Principle: Free BPCAs naturally occurring in soil are extracted using a NaOH solution. Following the extraction and removal of humic acids, the free BPCA extracts undergo a series of steps, with the most important ones being purification and conversion into trimethylsilyl derivatives. The isolated free BPCAs are further separated using gas chromatography and subsequently detected by flame ionization detector. Analyzing free BPCAs, which primarily result from black carbon degradation, can enhance understanding of black carbon's fate and degradation mechanisms in the environment. An overview of the method is given in Figure 6.

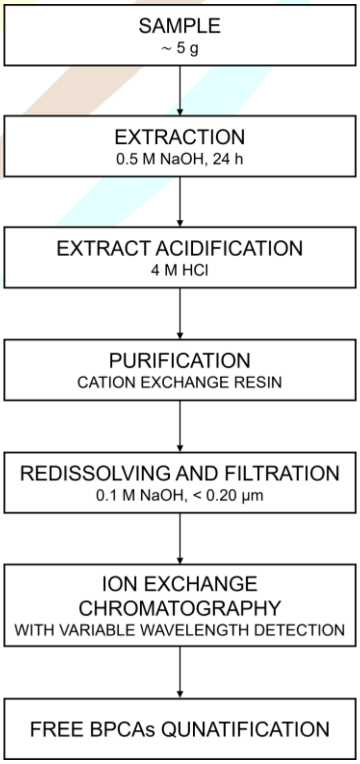


Figure 6. Scheme of free BPCAs analysis method

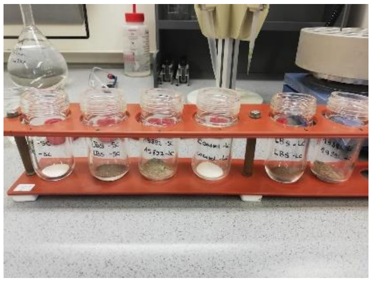


Photo 20. Soil and sand samples

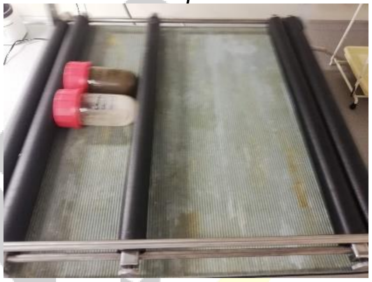


Photo 21. Free BPCAs extraction

Extraction: Approximately 5 g of air-dried, crushed, and sieved soil sample (< 2 mm) is directly weighed into a 50 ml centrifuge tube, (Photo 20). Subsequently, 100 µl of internal standard 1 (Internal standard 1 is a 1000 ppm phthalic acid solution, freshly prepared by dissolving 25 mg of phthalic acid in 25 ml of deionized water and adding 2 or 3 drops of 2 M NaOH for easier dissolving) and 25 ml of 0.5 M NaOH are added to the weighed soil sample. In the following step, the centrifuge tube containing the sample is placed on a horizontal shaker and agitated at 250 rpm for 24 hours (Photo 21). Following this agitation period, the sample is then subjected to centrifugation at 3700 g for 15 min, leading to the separation of the liquid phase, which is subsequently carefully transferred to a fresh centrifuge tube. To eliminate humic acids present within the extracted solution, 4 ml of a 4 M HCl is introduced into the centrifuge tube containing the extract. After subjecting it to centrifugation at 3700 g for 15 minutes, the resulting extract, now free from humic acids, is carefully transferred to a clean glass vial for further analysis or storage.

Photo 22. Pear-shaped flasks with pre-purified samples

Photo 23. Pear-shaped flasks with purified samples

Purification: In order to purify the extract from any residual polyvalent cations and impurities, the sample is passed through a conditioned Dowex 50W×8 resin column. The process of preparing and conditioning a Dowex 50W×8 resin column is carried out as outlined below: a glass column, measuring 20 cm in length and having an inner diameter of 1 cm, is fitted with approximately 0.5 cm of glass wool at the bottom to secure the resin in place. This column is then filled with 4 g of Dowex resin that has been rigorously pre-rinsed and then suspended in deionized water. Following this initial step, the column is rinsed by passing one column volume of 2 M NaOH through it, followed by washing with two column volumes of deionized water. Subsequently, the column is activated by eluting with a single column volume of 2 M HCl, followed by rinsing with two column volumes of deionized water. The pH of the eluate should fall within the range of 6 to 7, and it is imperative to ensure that the column remains consistently moist, never allowing it to dry out. Once the column is prepared, a 2 ml aliquot of the extract is transferred to a pear-shaped quick-fit flask (50 ml), and 4 ml of deionized water is added (Photo 22). A 100 ml pear-shaped quick-fit flask is placed beneath the conditioned Dowex 50W×8 resin column, and the solution from a 50 ml pear-shaped quick-fit flask is passed through (Photo 23). A 50 ml pear-shaped quick-fit flask is rinsed three times with 1 ml of deionized water, and after that the column is rinsed five times with 10 ml of deionized water, allowing each volume to elute completely before introducing the next. The flask containing the combined eluates is sealed with parafilm, then placed in a freezer set to -18°C overnight, and subsequently subjected to freeze-drying.

<u>Derivatization</u>: The freeze-dried residue is redissolved by adding three separate 1 ml portions of methanol, and the resulting solution is then transferred into a 5 ml reaction vial (after adding the first 1 ml of methanol, the flask is placed in an ultrasonic bath before transferring the solution, and subsequently rinsed twice with 1 ml of methanol to ensure the complete recovery of the entire sample). The solution from the vial is then evaporated using a nitrogen stream with heat application. Thereafter, 100 µl of internal standard 2 is introduced into the vial (internal standard 2 is 1000 ppm biphenylen-2,2-dicarboxylic acid solution, freshly prepared by dissolving 25 mg of biphenylen-2,2-dicarboxylic acid in 25 ml of methanol) and evaporated using a nitrogen stream with heat application. Following the evaporation process, 98 µl of N,O-bis(trimethylsilyl)-trifluoroacetamide (BSTFA), 2 µl of N-trimethylsilyl(imidazole) (TSIM) and 50 µl of toluol are added, the vial is closed with a Teflon seal and vortexed. The vial is heated at 90°C for 2 hours, vortexed again after the initial 15 minutes of heating, allowed to cool down to room temperature (Photo 24), and finally the solution is transferred into a GC vial fitted with a glass insert (Photo 25).


Photo 24. Derivatized samples


Photo 25. GC vials with glass inserts

<u>External standards preparation</u>: An external standard solution (1000 ppm) is prepared by dissolving 25 mg of each individual standard compound, including phthalic acid, hemimellitic acid, trimellitic acid, trimesic acid, pyromellitic acid, benzene pentacarboxylic acid, and mellitic acid, in the same 25 ml of methanol (Photo 26). Subsequently, aliquots of 10, 25, 50, 100, 250, and 500 µl from this external standard solution are transferred to six 5 ml reaction vials and


Photo 26. External standard solution

dried to completion using a nitrogen stream with heat application. Following the evaporation process, 100 µl of internal standard 2 is added to each vial, and the vial contents are further evaporated using a nitrogen stream with heat application. The derivatization step follows the same procedure as with the samples.

Analysis: The prepared sample is analyzed by capillary gas chromatography with a flame ionization detector (GC-FID). The gas chromatographic conditions applied for the analysis are as follows: *GC* - Shimadzu GC-2100; *Column* - Supelco SPB-5 or Agilent HP-5 (30 m, 0.25 mm inner diameter, 0.25 µm film); *Injector* - Temp.: 300 °C; Carrier: He; Flow control mode: const. flow (79.3 ml/min); Split ratio: 1:50; Purge: 3.0 ml/min; Injection volume: 1 µl (or 2 µl); *Detector* - Flame ionization detector (FID); Temp.: 330 °C; Make-up: (He) 30.0 ml/min; H_2 flow: 40.0 ml/min; Air flow: 400.0 ml/min; Sampling rate: 40 msec; *Temperature Program* - 100 °C for 2 min, then up to 240 °C at 20 °C/min; 240 °C for 7 min, then up to 300 °C at 30 °C/min; 300 °C for 10 min.

Calculation and interpretation: Individual free BPCAs are identified by comparing their retention times with those of standard compounds. Validation can be further substantiated through the utilization of GC-MS analysis and mass spectral library searches (for routine analysis, only GC-FID is used). Phthalic acid and biphenylen-2,2-dicarboxylic acid are used as internal standards due to their structural similarities with BPCAs. These compounds are infrequently found in the hydrolysates of natural soil samples.

2. Locations and sampling

Analyses of black carbon, stanols, stanones, Δ^5-steroles and bile acids were conducted on samples collected from the Bronze Age tell settlement of Toboliu - a complex site consisting of a central mound, two concentric enclosing ditches, and an impressive contemporary outer settlement, situated in Bihor County, Western Romania (this samples were analyzed as part of the *Soil Biogeochemical Analysis* course) (Figure 7).

Figure 7. Toboliu tell - sampling locations

Soil from two locations within the site was sampled (Figure 7): a phaeozem from the outer settlement that was considered as control soil, and an anthrosol near the tell suspected to show anthropogenic influence of burning activities. Soil from both locations was sampled at five depths, as shown in Table 1.

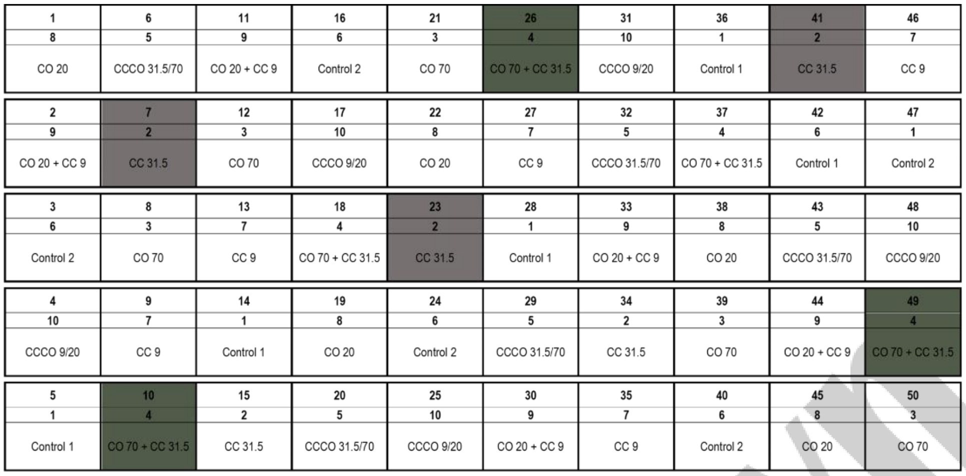
Table 1. Sampling locations and depths of the analyzed soils from Toboliu tell

Soil/Sample	Horizon/Depth		
Phaeozem	cm		
35089	20 - 25		
35090	37 - 40		
35091	45 - 50		
35092	70 - 75		
35093	90 - 95		Site 5, core 5
Anthrosol	cm		
35094	40 - 43		Site 9, core 6
35095	48 - 52		
35096	65 - 75		
35097	70 - 80		Site 9, core 8
35098	58 - 70 (pit)		

Free BPCAs analyses were performed on soil samples collected from a long-term field experiment in Donndorf/Eckersdorf, near Bayreuth, Germany, in northeast Bavaria. The samples were obtained from two experimental variants, one with the individual application of a high dose of biochar (31.5 t/h) and the other combining biochar (31.5 t/h) with composted material (70 t/h). Sampling was conducted in 2009, prior to the experiment's establishment, and subsequently at two-year intervals in 2013 and 2021. In 2009 and 2013, samples were taken at depths of 0-10 cm and 10-30 cm, while in 2021, samples were collected at a depth of 0-30 cm. In all cases, both variants were sampled in triplicate. The location of the experimental field is depicted in Figure 8, while the samples used are shown in Photo 27. The design of the experiment is shown in Figure 9.

Figure 8. Bayreuth field experiment - location

Photo 27. Analyzed samples



1	6	11	16	21	26	31	36	41	46
8	5	9	6	3	4	10	1	2	7
CO 20	CCCO 31.5/70	CO 20 + CC 9	Control 2	CO 70	CO 70 + CC 31.5	CCCO 9/20	Control 1	CC 31.5	CC 9
2	7	12	17	22	27	32	37	42	47
9	2	3	10	8	7	5	4	6	1
CO 20 + CC 9	CC 31.5	CO 70	CCCO 9/20	CO 20	CC 9	CCCO 31.5/70	CO 70 + CC 31.5	Control 1	Control 2
3	8	13	18	23	28	33	38	43	48
6	3	7	4	2	1	9	8	5	10
Control 2	CO 70	CC 9	CO 70 + CC 31.5	CC 31.5	Control 1	CO 20 + CC 9	CO 20	CCCO 31.5/70	CCCO 9/20
4	9	14	19	24	29	34	39	44	49
10	7	1	8	6	5	2	3	9	4
CCCO 9/20	CC 9	Control 1	CO 20	Control 2	CCCO 31.5/70	CC 31.5	CO 70	CO 20 + CC 9	CO 70 + CC 31.5
5	10	15	20	25	30	35	40	45	50
1	4	2	5	10	9	7	6	8	3
Control 1	CO 70 + CC 31.5	CC 31.5	CCCO 31.5/70	CCCO 9/20	CO 20 + CC 9	CC 9	Control 2	CO 20	CO 70

Figure 9. Design of the experiment. The analyzed variants are marked in gray and green (CC - biochar; CO - compost)

3. Results

Black carbon

The results obtained by the analysis of BC derived BPCAs by GC-FID are given in Figure 10. In general, concentrations in both phaeozem and anthrosol were lower than in the laboratory control sample (LBS) which is a black chernozem with typically high BC content used in the study as a reference soil.

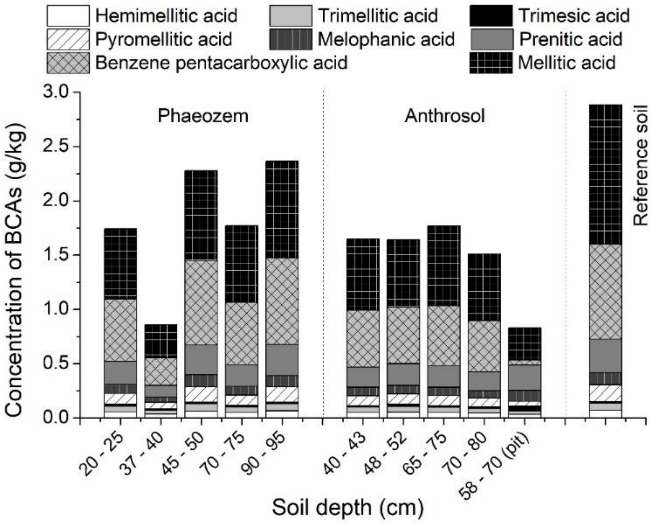


Figure 10. BPCAs content in the phaeozem and anthrosol samples

The content of BC, BC_{ABS}, in the samples was calculated by multiplying the sum concentration of the analyzed BPCAs with the factor of 2.27. Results are shown in Figure 11. The BC_{ABS} content in most of the anthrosol samples was higher than the content of BC_{ABS} in the phaeozem samples from similar depths. The higher values of absolute BC content in the anthrosol may be a potential indicator of higher anthropogenic influence in the soil closer to the tell. For both investigated soils, the BC_{ABS} values were lower compared to the reference soil. This could be the consequence of several factors and processes, such as erosion, weather effects and possible decay.

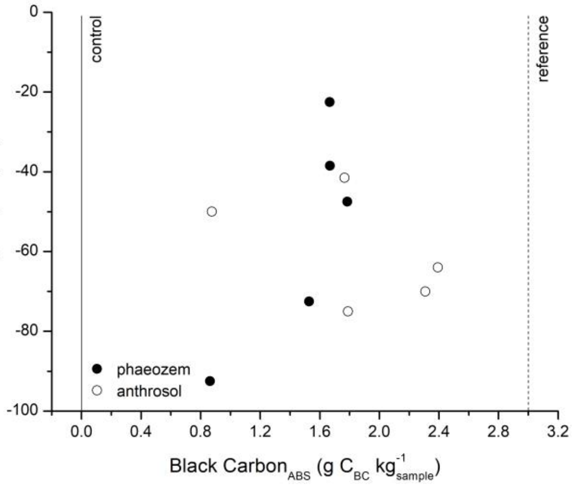


Figure 11. Black carbon content in phaeozem and anthrosol

The organic carbon content (C_{ORG}) was in the range 6.8-13.1% and 11.5-21.2% for phaeozem and anthrosol samples, respectively (Figure 12). All anthrosol samples have higher C_{ORG} than phaeozem samples from similar depths, which is in consistency with the higher BC levels in this soil. Samples from both soil types have in general lower organic carbon content compared to the reference soil which is a chernozem with typically high total organic carbon (TOC_{REF}=1.94%). The only exception is the anthrosol taken from 48-52 cm depth, which had a slightly higher organic carbon content than the reference soil (2.12%). A trend of decreasing organic carbon content was observed with the increasing depth of the samples for both analyzed soils.

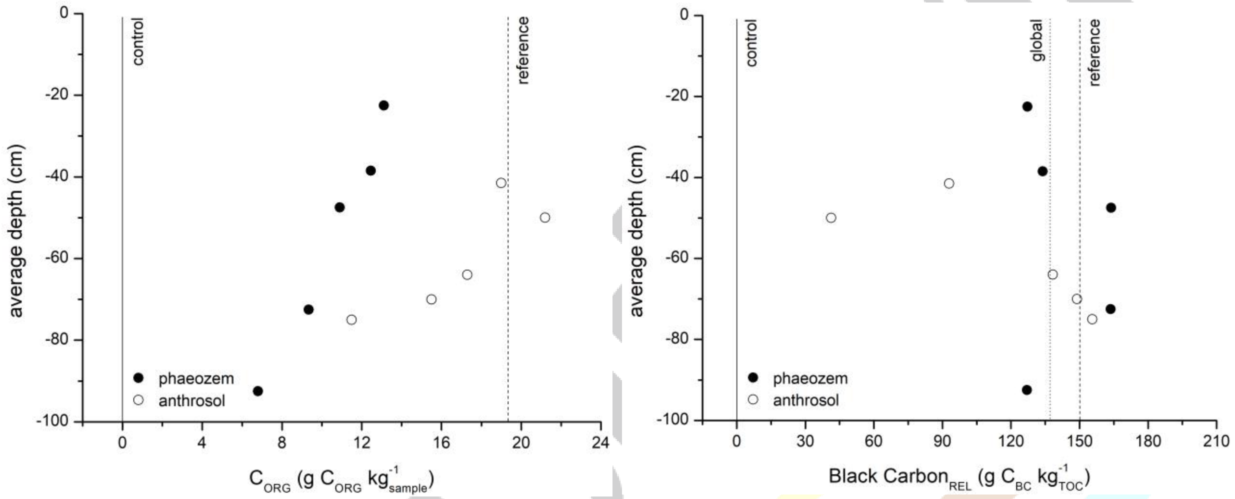


Figure 12. Organic carbon content (left) and relative black carbon content (right) in phaeozem and anthrosol samples

The relative black carbon content normalized to the TOC (BC_{REL}) was lower in the anthrosol samples compared to the phaeozem samples from similar depths, which is likely the consequence of higher TOC values in anthrosol (Figure 12). According to literature, BC_{REL} values of 4.5%, 9.2% and 24.4% in soils coincide with low, medium and high fire frequencies, respectively. The average BC_{REL} values obtained for the analyzed samples were 14.3% for phaeozem and 11.5% for anthrosol, whereas the reference soil had a BC_{REL} of 15.0%. These results indicate medium frequency burning activities at the study site.

The share of BPCAs with 3 (B3CA), 4(B4CA), 5 (B5CA) and 6 (B6CA) carboxylic groups was calculated and is presented in Figure 13. The B5CA and B6CA shares in total BPCAs were 38.1% (67.1%) to 70.6% for phaeozem, 62.2% to 70.0% for anthrosol and 72.6% for the reference soil. High levels of BPCAs with 5 and 6 carboxylic groups indicate intensive burning events at high temperatures. Furthermore, the results do not indicate any significant occurrence of decay. Also, the BPCAs patterns of the investigated anthrosol and phaeozem are comparable to BPCAs distribution that can be found in literature.

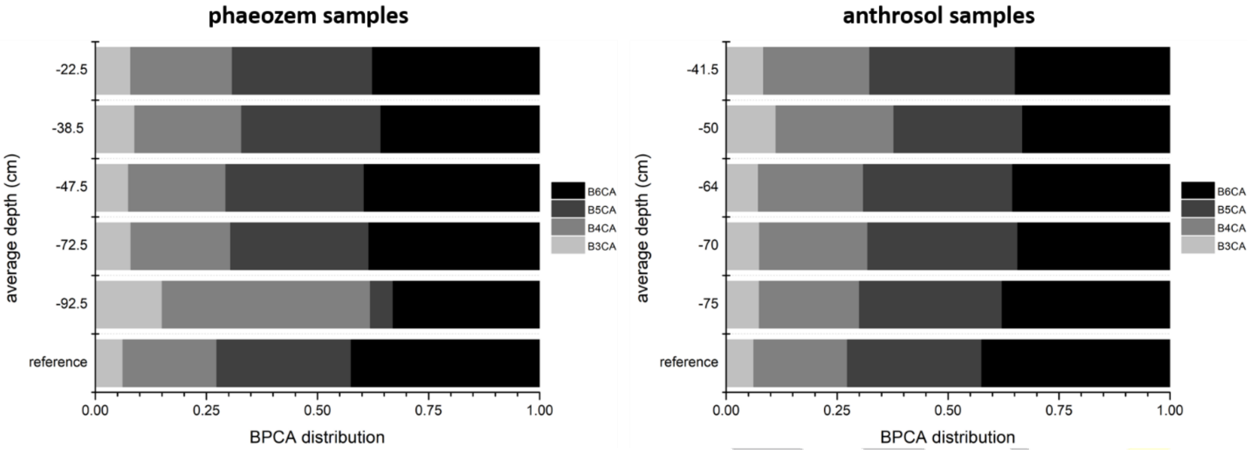


Figure 13. Distribution of BCAs with different number of carboxylic groups

Stanols, stanones, Δ^5-steroles and bile acids

The sum concentration of steroids in the analyzed soils (Figure 14, left) varied in the range 1410-2560 µg/kg for anthrosol samples and 583-1490 µg/kg for phaeozem samples. In the reference soil the sterols were detected in a total concentration of 1830 µg/kg.

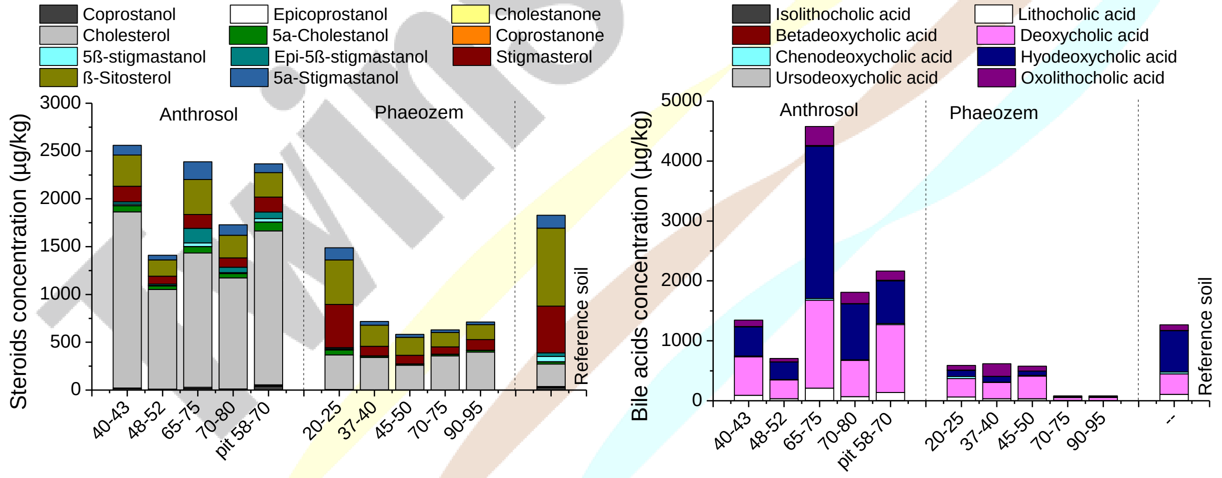


Figure 14. Sum concentrations of steroids (left) and bile acids (right) in anthrosol and phaeozem samples and reference soil

Cholesterol was detected in the highest concentration, followed by β-sitosterol and stigmasterol. This is to be expected as cholesterol is a component in nearly all eukaryotic cells, and can be found in animals, plants, root exudates and several fungal species, whereas both stigmasterol and β-sitosterol are typical Δ^5-sterols for plant biomass. The same trend was observed when concentrations were normalized to the TOC content in the soil samples (Figure 15).

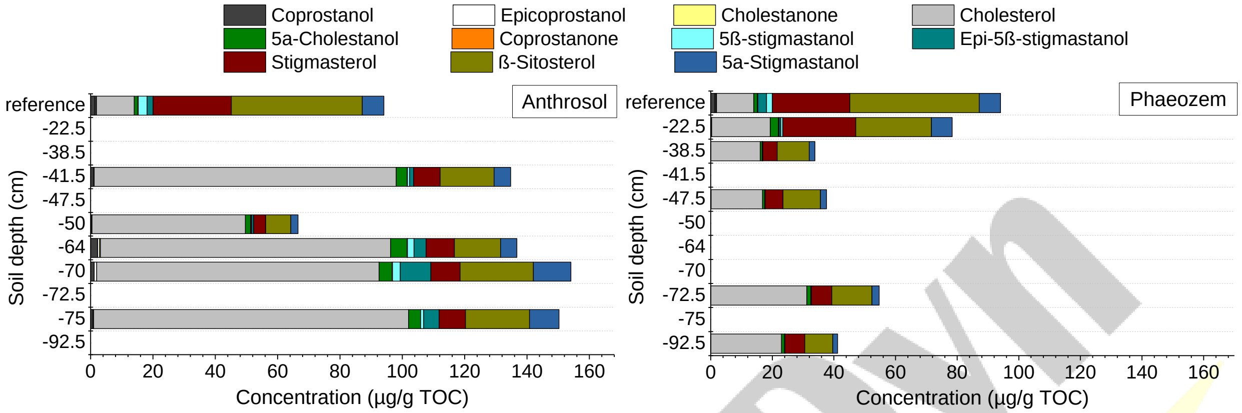


Figure 15. Content of stanols, stanones and Δ^5-steroles in phaeozem and anthrosol samples normalized to TOC content

Both the absolute (Figure 14, right) and the relative to TOC (Figure 16) concentrations showed the same general trend of bile acids content. Hyeodeoxycholic acid (HDCA) and deoxycholic acid (DCA) were found in the highest concentrations nearly all samples, followed by oxolithocholic acid (OLCA) and lithocholic acid (LCA). This trend was also observed in the reference soil.

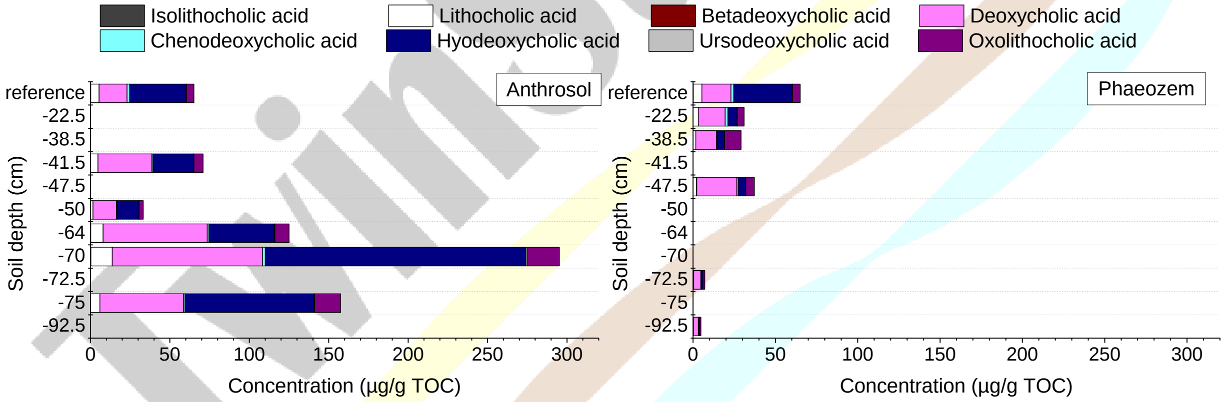


Figure 16. Content of bile acids in phaeozem and anthrosol samples normalized to TOC content

Figure 17 shows the comparison of steroids content in the two analyzed soils. For both groups of compounds, higher content was observed in the anthrosol compared to the phaeozem samples. These results indicate higher animal input in the soil closer to the tell. Moreover, it can be observed that the concentration of the biomarkers increases with depth of anthrosol, especially for bile acids, whereas, both groups of compounds show no change or only a slight decrease in concentration with depth of phaeozem.

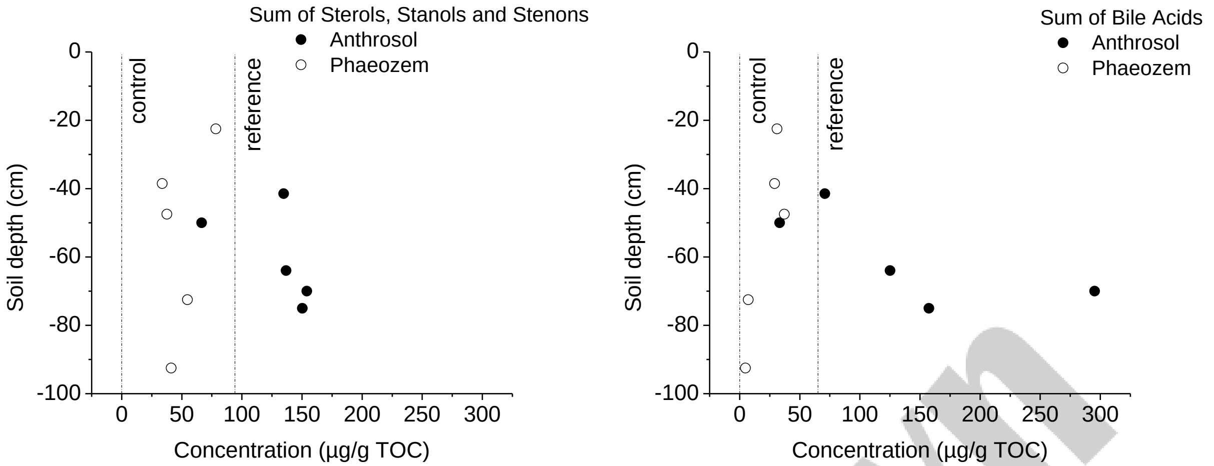


Figure 17. Comparison of biomarkers content in phaeozem and anthrosol

Table 2. presents the calculated typical ratios of sterols and bile acids indicative of inputs from specific groups of animals.

Table 2. Calculated ratios of sterols and bile acids

Sample	Depth (cm)	Sterols		Bile Acids					Input
		A*	B**	DCA/LCA	DCA/CDCA	CDCA/LCA	HDCA/LCA	HDCA/DCA	
Anthrosol	40-43	57.4		7.27	46.4	0.156	5.48	0.755	pig
Anthrosol	48-52	55.6		9.48	39.2	0.242	8.81	0.929	pig
Anthrosol	65-75	31.5		7.05	56.5	0.125	12.2	1.73	pig
Anthrosol	70-80	41.9		8.95	62.4	0.143	13.8	1.54	pig
Anthrosol	pit 58-70	52.3		8.44	63.1	0.134	5.30	0.627	pig
Phaeozem	20-25	**26.3**	**1.56**	5.06	9.71	0.521	1.71	0.339	horse
Phaeozem	37-40	44.1		7.57	49.1	0.154	2.72	0.360	pig
Phaeozem	45-50	**67.7**		11.4	29.7	0.385	2.11	0.185	human
Phaeozem	70-75	41.7		10.1	12.5	0.809	2.29	0.227	pig
Phaeozem	90-95	48.3		13.0	15.6	0.834	2.02	0.156	pig

*A = [coprostanol/(coprostanol + 5β-stigmastanol)]*100%
**B = (epi-5β-stigmastanol/5β-stigmastanol)+(epicoprostanol/coprostanol)
CDCA = Chenodeoxycholic acid; HDCA = Hyodeoxycholic acid; LCA = Lithocholic acid; DCA = Deoxycholic acid

Sterol ratios A and B were calculated as indicated in Table 2. In most samples, the ratio A was between 29 and 65% which suggests pig input. Phaeozem soil from 20-25 cm depth had an A ratio lower than 29%. This could indicate input from herbivores rather than pig or human. For this sample, ratio B was also calculated, which was higher than 1.2 (1.56) and leads to the assumption that the herbivore influence might be from horse input. Lastly, the A ration calculated for phaeozem soil from 45-50 cm depth was slightly higher than 65% (67.7%), and this could suggest input from humans. However, whereas ratios A and B indicate certain inputs, literature suggests additional criteria such as the presence or absence of specific bile acids to confirm these inputs, which in most samples from this research were not met.

Soil sampling

In addition to the laboratory training in soil sample preparation for biomarkers analysis, the secondment also included on-site training in soil sampling. Soil samples were collected from an experimental field located Donndorf/Eckersdorf (near Bayreuth, Germany) in northeast Bavaria. The field experiment was established in 2010 for investigating the influence of different organic soil amendments (OSA) on soil properties and crop yield. The OSA used in the research include biochar, compost and two mixtures of these materials: direct mixture of biochar and compost, and a mixture of biochar and raw plant material which was then co-composted. All treatments were applied in two doses, which resulted in a total of 8 treatments and an untreated control soil. All treatment variants, as well as the variants that were sampled as part of the on-site training are listed in Table 3.

Table 3. Treatment variants included in the field experiment

Variant	Treatment	Dose (t/h)		Sampled for TSD*	Depth sampled	Amount sampled for TSD*
		biochar	compost			
control	no treatment	/	/	✓	0-30 cm	24 kg
K9	biochar	9	/			
K31.5	biochar	31.5	/	✓	0-30 cm	6 kg
GK20	compost	/	20			
GK70	compost	/	70			
GK20+K9	biochar+compost mix.	9	20			
GK70+K31.5	biochar+compost mix.	31.5	70			
BGK9/20	co-composted biochar	9	20			
BGK31.5/70	co-composted biochar	31.5	70	✓	0-30 cm	6 kg

*TSD - TwinSubDyn

The sampling was carried out as follows: the treatment plots were arranged within the experimental field according to a Latin rectangle in row-column (5-10) design, and each treatment was replicated 5 times. Magnets buried along the edges of the field were located with a metal detector, and pols were placed to mark the dividing point of each plot. Once the field was divided, the plots of interest were located and soils from each plot were sampled with a manual corer (6 corers/plot) from 0-30 cm depth (Photos 28 and 29). All soils of the same variant were collected together to make a composite sample. For the TwinSubDyn project, three variants of the soil were sampled: the control soil without any application of OSA, soil K31.5 which was treated with biochar (31.5 t/h) and soil BGK31.5/70 which was treated with co-composted biochar (31.5 t/h biochar and 70 t/h compost). The soils were homogenized, dried at 40°C (Photo 30), packed and labeled. Control, K35.1 and BGK31.5/70 soils (approximately 6 kg each) were sent to Sevilla, Spain to IRNAS for characterization and pot experiments. An additional 6 kg of control soil was sent to IRNAS to be mixed with biochar in order to investigate the impact of biochar aging on soil, as well as approximately 0.5 kg of all three sampled variants for phospholipid fatty acids analysis, which were previously freeze dried. Control soil was also sent to project partners at University of Vienna and University of Novi Sad for additional research.


Photo 28. Soil sampling


Photo 29. Corer


Photo 30. Drying

Free benzene polycarboxylic acids

The results obtained from the analysis of soil samples for free BPCAs using an adapted methodology that incorporates the GC-FID technique are presented in Table 4.

As indicated by the data in Table 4, free BPCAs were not detected in most of the analyzed soil samples. In the remaining cases, free BPCAs were identified, at both investigated soil depths, but only in concentrations that can be considered relatively low, ranging from 3.96×10^{-5} to 5.51×10^{-2} g/kg. For comparison, Simeone et al. (2018) reported concentrations for the sum of free BPCAs in different soil types, which fell within the range of 0 to 0.24 g/kg. In this study, the sum of free BPCAs ranged from 0 to 5.51×10^{-2} g/kg. These findings suggest two possible explanations: either the applied biochar is highly stable, indicating minimal degradation over time, or alternatively, these outcomes may be attributed to other soil-related phenomena. According to Haumaier (2010), the latter primarily involves the possibility of leaching or further oxidation, resulting in the loss of free BPCAs from the soil. However, considering the specific characteristics of the investigated soil, with a pH ranging from 5.4 to 5.8 and a clay content of 26%, this outcome should not occur. Hence, the loss of biochar, identified in a study beyond the scope of this secondment, likely results from other processes, such as vertical or lateral transport. Finally, it is important to emphasize that the conclusions presented here require further validation through the analysis of the tested samples using a methodology that incorporates the LC-VWD technique. Unfortunately, this validation is currently not possible due to instrument failure.

Table 4. Free BPCAs amounts detected in analyzed soil samples by adapted GC-FID methodology

Variant	Sampling year	Sampling depth	Recovery	Hemimellitic	Trimellitic	Trimesic	Pyromellitic	Melophanic	Prenitic	B5CA	Mellitic
		cm	%	g/kg							
CC31.5	2009	0-10	81.34	n.d.	4.76×10^{-4}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			89.00	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	5.14×10^{-3}
			84.17	2.64×10^{-3}	3.96×10^{-5}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		10-30	75.76	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			68.73	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			72.11	n.d.	1.67×10^{-3}	n.d.	n.d.	n.d.	n.d.	1.24×10^{-4}	n.d.
	2013	0-10	85.54	n.d.	n.d.	n.d.	n.d.	1.48×10^{-3}	n.d.	n.d.	n.d.
			90.03	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			81.96	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		10-30	79.49	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			108.42	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.75×10^{-2}
			84.74	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	8.92×10^{-4}	n.d.
	2021	0-30	130.33	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			89.27	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			111.80	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
CO70 + CC31.5	2009	0-10	131.11	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			101.47	n.d.	n.d.	5.28×10^{-2}	n.d.	n.d.	n.d.	n.d.	n.d.
			116.76	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		10-30	71.23	n.d.	1.13×10^{-2}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			101.84	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			81.61	n.d.	6.72×10^{-4}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	2013	0-10	112.35	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			119.23	n.d.	n.d.	4.51×10^{-2}	n.d.	n.d.	n.d.	n.d.	n.d.
			108.98	n.d.	n.d.	4.93×10^{-2}	n.d.	n.d.	n.d.	n.d.	n.d.

Continuation of table 4. Free BPCAs amounts detected in analyzed soil samples by adapted GC-FID methodology

Variant	Sampling year	Sampling depth	Recovery	Hemimellitic	Trimellitic	Trimesic	Pyromellitic	Melophanic	Prenitic	B5CA	Mellitic
		cm	%	g/kg							
CO70 + CC31.5	2013	10-30	102.95	n.d.	n.d.	5.22×10^{-2}	n.d.	n.d.	n.d.	n.d.	n.d.
			97.32	n.d.	n.d.	5.51×10^{-2}	n.d.	n.d.	n.d.	n.d.	n.d.
			112.19	n.d.	n.d.	4.79×10^{-2}	n.d.	n.d.	n.d.	n.d.	n.d.
	2021	0-30	118.96	n.d.	n.d.	4.65×10^{-2}	n.d.	n.d.	n.d.	n.d.	n.d.
			108.72	n.d.	n.d.	4.93×10^{-2}	n.d.	n.d.	n.d.	n.d.	n.d.
			113.66	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Impact on your project

The activities carried out during this secondment provided an excellent opportunity for knowledge exchange between the MLU and UNSPMF working groups. The sample preparation methodologies for biomarker analysis, which include BC, steroids, bile acids and free BPCAs, are relatively new to UNSPMF members. Certain steps within these methods are not part of our regular practices. The incorporation of these methodologies into UNSPMF laboratories, facilitated by the available infrastructure, will undoubtedly enhance and diversify our research perspectives in the field of soil science. The trained UNSPMF researcher will have the opportunity to transfer the gained knowledge and skills to both TwinSubDyn participants as well as other researchers and stakeholders.

Through active participation in the long-term field experiment, the UNSPMF researcher has developed essential skills in designing, monitoring, and sampling for this type of experiments. The insights gained can be efficiently shared with fellow UNSPMF team members, other researchers and stakeholders. The importance of acquired knowledge becomes particularly evident when considering the intended transition of experiments from the laboratory to the field level within the UNSPMF team. Furthermore, the field training offered the chance to collect soil samples subjected to treatment in 2010. This opportunity allows for extended research on how the aging of SOA treated soils affects both soil and SOA properties. These studies will be conducted at IRNAS, UNIVIE, and UNSPMF over the course of the TwinSubDyn project.

MOBILITY REPORT

Researcher: Dr. Tamara Apostolović, UNSPMF

Assigned supervisor: Prof. Dr. Bruno Glaser, MLU

Duration of the visit: 17.02.2023. - 14.03.2023.

Executive Summary

The main purpose of the visit to Martin-Luther University Halle-Wittenberg was participation in the course *Soil Biogeochemical Analysis*. The general topic of the course was *The molecular memory of the soils: Identification of fire and human molecular markers in the Bronze Age tell Toboliu (Western Romania)*. The objective of the course was to learn and apply sophisticated soil biogeochemical methodology for the determination of molecular markers: steroids and bile acids (human and animal intestine) and black carbon (fire-derived organic matter), as well as their analysis by gas chromatography (GC) with mass spectrometry (MSD) and flame ionization detection (FID). Samples from two sites and five depths were analyzed, an anthrosol sampled near the tell, and a phaeozem that was considered as a control. Based on the results a few conclusions were be drawn: (I) black carbon content was higher in anthrosol than in phaeozem, with shares of benzene penta- and hexacarboxylic acids indicating intensive burning events at high temperatures; (II) both steroids and bile acids content was significantly higher in the anthrosol samples, indicating that the focus of both human and animal activity might have been at/near the tell; (III) based on the calculated biomarker ratios, dominant input by pigs in all anthrosol and most phaeozem can be suspected.

Furthermore, the visit included learning about the long-term field experiment with organic soil amendments at the experimental field near Bayreuth, Germany. Samples of the control soil without treatment, as well as soils treated with biochar and co-composted biochar were sampled and prepared (dried and packed) for further analysis and experiments at the University of Novi Sad, University of Vienna and IRNAS, Sevilla.

Introduction

Background

Specific molecules or compounds whose presence indicates an influence of particular processes, organisms, or environmental conditions in soil ecosystems are commonly referred to as molecular biomarkers. Detected in soil, these biomarkers can provide valuable information about soil health, microbial activity, contaminant presence, nutrient cycling, anthropogenic influence and overall ecosystem functioning. Black carbon (BC) refers to the solid, carbonaceous material that remains after incomplete combustion of organic matter. BC in soil can serve as an indicator of human activity, particularly related to the combustion of fossil fuels and biomass. The composition and characteristics of BC can vary depending on the source and combustion conditions, and its detection in soil can help identify the contributions of human activities and assess their impact on soil quality. Furthermore, due to the persistence of BC in soil, it can potentially provide a historical record of human activities. The distribution and characteristics of BC across soil profiles or sediment layers can help reconstruct past human impacts, such as the use of fire by indigenous communities or changes in land use practices over time. Steroids, including various classes such as hormones, phytosterols, and sterols, can also serve as biomarkers in soil. Phytosterols, such as β-sitosterol and stigmasterol, can be used as biomarkers to assess the input of plant-derived organic matter and track the decomposition processes in soil. Animal feces, including those from livestock and wildlife, contain sterols that can be released into the soil during the deposition and decomposition of organic matter. Sterols, such as cholesterol and coprostanol, can serve as indicators of animal presence or fecal contamination in soil. Bile acids are derived from cholesterol metabolism in animals. Their presence in soil can indicate inputs from animal sources or the activity of specific microbial communities, whereas their ratio can point toward the influence of a specific animal group.

Scope of the secondment

The scope of the secondment was knowledge transfer on techniques for molecular markers analysis in soils. This included laboratory training in soil sample preparation and analysis of several biomarkers: black carbon derived benzene carboxylic acids, Δ^5-sterols, stanols and stanones and bile acids. The detailed methodology for the determination of molecular markers in soil and the results obtained throughout the course will be given in the following chapters.

Content

1. Methods

Determination of black carbon derived benzene carboxylic acids by GC-FID

<u>Principle:</u> Due to the highly aromatic structure of black carbon (BC), specific determination in soil is carried out by nitric acid degradation which yields a suite of aromatic degradation products. The obtained benzene carboxylic acids (BCAs) are indicators of BC. After conversion into trimethylsilyl derivatives, BCAs can be separated by capillary gas chromatography and detected with flame ionization detection. An overview of the method is given in Figure 1.

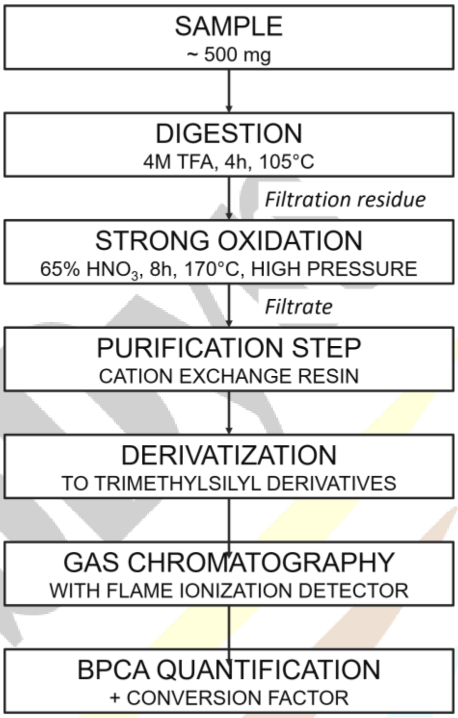


Figure 1. Scheme of BCAs analysis method

<u>Hydrolysis:</u> Approximately 500 mg of soil sample is weighed and placed into a 25 ml hydrolysis flask (in case of high BC concentrations expected, weight is reduced to equal about 5 mg organic carbon). In order to remove polyvalent metal cations, 10 ml of 4 M trifluoroacetic acid (TFA, prepared by diluting 153 ml of TFA to 500 ml with deionized water) is added, the flask is capped and placed into a furnace for 4 h at 105 °C for hydrolysis (Photo 1). The hydrolysate and residue are transferred onto a glass fiber filter (placed in a Buchner funnel and fitted onto a vacuum flask), rinsed with 5 x 5 ml of deionized water to remove polyvalent cations (especially Fe^{3+} and Al^{3+}) and the filtrate is discarded (Photo 2). The filter with the hydrolysis residue is transferred onto a watch glass and dried at 40 °C in a drying cabinet (about 2 - 4 hours).

Photo 1. Sample preparation for hydrolysis

Photo 2. Filtration of the hydrolysate and residue

Photo 3. Oxidacion

Oxidation: After it is dry, the hydrolysis residue is quantitatively transferred form the glass fiber filter into a digestion tube and 2 ml of 65% nitric acid is added (Photo 3). The digestion tube is closed and placed in a high pressure digestion apparatus for 8 hours at 170 °C. The digestion solution is filtered into a 10 ml volumentric flask. The residue is rinsed several times with deionized water, and the flask is filled to the mark.

Photo 4. Conditioning of Dowex 50W×8 resin

Photo 5. Sample purification

Purification: In order to purify the solution from any residual polyvalent cations and impurities, the sample is passed through a conditioned Dowex 50W×8 resin column. Preparation and conditioning of Dowex 50W×8 resin column is done as follows (Photo 4): a 20 cm glass column is fitted with about half a centimeter of glass wool at the base to hold the resin in place and filed with 4 g of Dowex extensively pre-rinsed with, and then slurred in deionized water. The column is rinsed by eluting one column with 2 M NaOH and then washing extensively with 2 columns of deionized water. The column is then activated by eluting one column with 2 M HCl and then rinsing the column with 2 columns of deionized water. The pH of the eluate should be about 6–7, and the column must not be allowed to dry at any time. After the column is prepared, a 2 ml aliquot of the solution is transferred to a 10 ml test tube, adding 4 ml of deionized water and 100 µl of internal standard 1 (Internal standard 1 is 1000 ppm phthalic acid solution prepared by dissolving 25 mg of phthalic acid in 25 ml of deionized water, adding 2 or 3 drops of 2 M NaOH for an easier dissolving; prepared freshly on the day of use). A pear shaped quick fit flask (100 ml) is placed under the conditioned Dowex 50W×8 resin column and the sample solution is slowly passed through (Photo 5). The test tube is rinsed with 3 × 1 ml of deionized water, and the column is rinsed with 5 x 10 ml of deionized water, letting each volume eluate first before adding the next. The flask containing the combined eluates is sealed with parafilm, placed in freezer at -18 °C overnight, and freeze dried.


Photo 6. Derivatizacion

Derivatization: The freeze dried residue is re-dissolved in 3 x 1 ml of methanol and the solution is transferred into a 5 ml reaction vial (after adding the first 1 ml of methanol, the flask is placed in ultrasonic bath before transferring the solution, and then rinsed two more time with 1 ml of methanol to recover the entire sample). The solution is evaporated under air stream until dry. In the vial, 100 µl of internal standard 2 is added (internal standard 2 is 1000 ppm biphenylen-2,2-dicarboxylic acid solution prepared by dissolving 25 mg biphenylen-2,2-dicarboxylic acid in 25 ml of methanol), and then evaporated under air stream until dry. After the solution is evaporated, 98 µl of N,O-bis(trimethylsilyl)-trifluoroacetamide (BSTFA), 2 µl of N-trimethylsilylimidazole (TSIM) and 50 µl of Toluol are added, the vial is closed with a Teflon seal and vortexed. The vial is heated for 2 hours at 90 °C, then after 15 min vortexed again, left to cool down to room temperature and the solution transferred into a GC vial with a glass insert (Photo 6).

External standards preparation: External standard solution (100 ppm) was prepared by dissolving 25 mg of each standard (phthalic acid, hemimellitic acid (benzene-1,2,3-tricarboxylic acid), trimellitic acid (benzene-1,2,4-tricarboxylic acid), trimesic acid (benzene-1,3,5-tricarboxylic acid), pyromellitic acid (benzene-1,2,4,5-tetracarboxylic acid), benzolpentacarboxylic acid (benzene-1,2,3,4,5-tetracarboxylic acid), mellitic acid (benzene-1,2,3,4,5,6-hexacarboxylic acid)) in 25 ml of methanol. In six 5 ml reaction vials, 10, 25, 50, 100, 250 and 500 µl of external standard solution is transferred, and evaporated under air stream until dry. In each vial, internal standard 1 and internal standard 2 is added (100 µl each), the content is evaporated under air stream until dry, and a derivatization step is carried out following the same procedure as with the samples.


Photo 7. GC-FID analysis

Analysis: The prepared sample is analyzed by capillary gas chromatography with flame ionization detection (GC-FID) (Photo 7). The gas-chromatographic conditions applied for the analysis are as follows: *GC* - Shimadzu GC-2100, equipped with flame ionization detector (FID); *Column* - Supelco SPB-5 or Agilent HP-5 (30 m, 0.25 mm inner diameter, 0.25 µm film); *Injector* - Temp. = 300 °C; Carrier: He; Flow control mode: const. flow (79.3 ml/min); Split ratio 1:50; Purge 3.0 ml/min; Injection volume = 1 µl (or 2 µl); *Detector* - Flame ionization detector (FID); Temp. = 330 ˚C; Make-up: (He) 30.0 ml/min;

H_2 flow = 40.0 ml/min; Air flow = 400.0 ml/min; Sampling rate = 40 msec; *Temperature Program* - 100 °C for 2 min, then up to 240 °C at 20 °C/min; 240 °C for 7 min; then up to 300 °C at 30 °C/min 10 min.

Calculation and interpretation: Individual compounds are identified by comparing retention times with those of standard compounds (Figure 2); validation may be supported by GC-MS and mass spectral library searches. For routine analysis, only GC-FID is used. Phthalic acid and biphenylen-2,2-dicarboxylic acid are used as internal standards because of featuring similar structures as BCAs. They occur rarely in hydrolysates of natural soil samples. Sum of BCAs indicates total BC in sample; estimation of BC can be done by applying a conversion factor of 2.27 to BCA-C, which corrects for the loss of CO_2 during the partial oxidation BC structures.

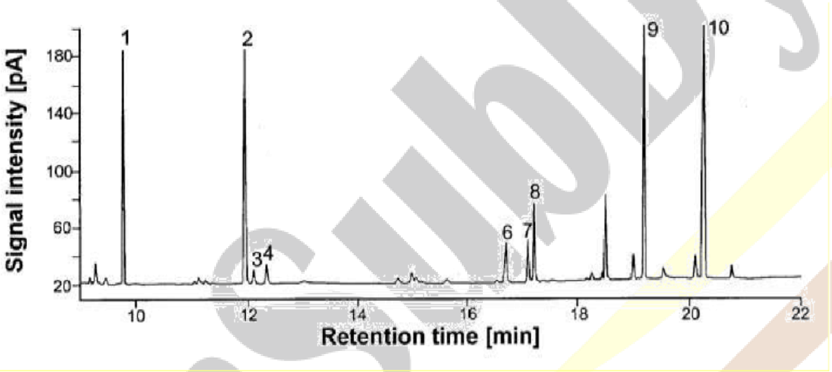


Figure. Chromatogramm of BPCAs of a Haplic Chernozem (1) phthalic acid (IS 1); (2) biphenyl-2,2'-dicarboxylic acid (IS2); (3) hemimellitic acid; (4) trimellitic acid; (5) trimesic acid; (6) pyromellitic acid; (7) mellophanic acid; (8) prehnitic acid; (9) benzenepentacarboxylic acid; (10) mellitic acid.

Figure 2. An example of a chromatogram for BCAs analysis

Method for Extraction and Purification of Δ^5-Sterols, Stanols, Stanones and Bile Acids (+Alkanes) in Soil and Sediment Samples

Principle: Stanols, stanones, Δ^5-steroles and bile acids are extracted from soil samples by a single Soxhlet extraction yielding a total lipid extract (TLE). The TLE is saponified with KOH in methanol. Sequential liquid–liquid extraction is applied to recover the biomarkers from the saponified extract and to separate the bile acids from the neutral stanoles, stanones and Δ^5-steroles. The neutral fraction is directly purified using solid phase extraction (SPE) columns packed with 5% deactivated silica gel. The bile acids are methylated in dry HCl in methanol and purified on SPE columns packed with activated silica gel. A mixture of hexamethyldisilazane (HMDS), trimethylchlorosilane (TMCS) and pyridine is used to silylate the hydroxyl-hydroxyl groups of the stanols and Δ^5-sterols avoiding a silylation of the keto groups of the stanones in their enol-form. Silylation of the bile acids is carried out with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing N-trimethylsilylimidazole (TSIM). Standard addition experiments showed mean recoveries of individual substances ≥85%. The recoveries of biomarkers within each biomarker group did not differ significantly. Precisions were ≤0.22 (RSD) and quantification limits were between 1.3 and 10 ng/g soil. Figure 3 shows a schematic diagram of the procedure.

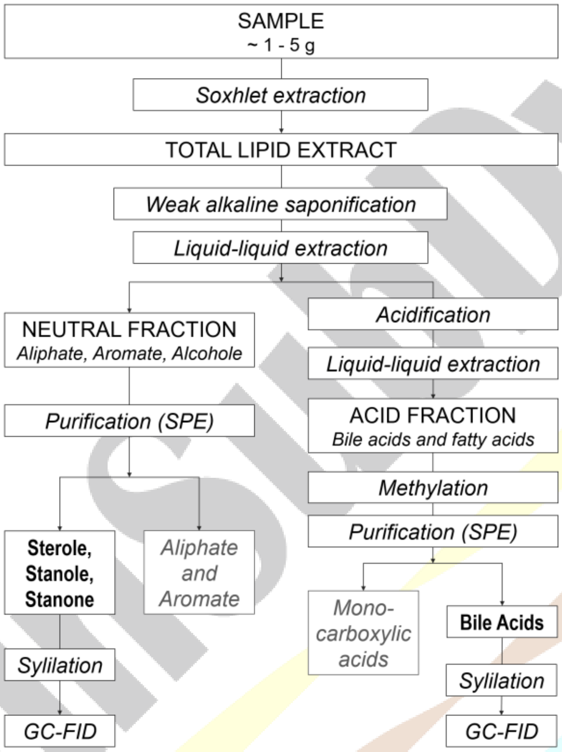


Figure 3. Schematic diagram of stanols, stanones, Δ^5-steroles and bile acids analysis method

<u>Soxhlet extraction:</u> Approximately 5 g of dried and ground soil is weighed into a fiberglass thimble (up to 10 g for soil with low C content), quartz sand (~1-2 cm) is added over the sample to avoid encrustation and 5-10 boiling chips. The sample is extracted (Photo 8) for approximately 40 h with 210 ml dichloromethane (DCM)/methanol (MeOH) mixture (v/v=2:1). Heat is adjusted to 3-5 drops per second. Internal standard 1 solution is added to the

Photo 8. Soxhlet extraction

extract, and extract is transferred with DCM/MeoH (v/v=2:1) into a 100 ml pear shaped flask (wash 3 times during transfer). The volume is reduced using a rotary evaporator (40 °C, 800 → 200 mbar; 800 mbar for 3 min, then to 250 or 200 mbar in 50 mbar steps). The residue is transferred into a 5 ml reaction vial with DCM/MeOH (v/v=2:1) (wash 3 times during transfer).

<u>“Mild” saponification:</u> The reaction vial is placed under gentle stream of nitrogen to evaporate the extract until dry. For saponification, 3.5 ml of 0.7 M KOH in MeOH is added, then shaken to dissolve (ultrasonic bath or vortex). The vial is left at room temperature for 10-14 h (overnight) to allow reaction. The sample is transferred into a separation funnel, the vial is washed 3 times with MeOH during transfer and 10 ml of distilled water is added.

<u>Liquid-liquid extraction:</u> For the extraction, 15 ml of chloroform is added into the separation funnel (Photo 9), the sample is shaken for approximately 1 min, and the chloroform layer (lower layer) is collected in a 50 ml pear shaped flask. The extraction step is repeated two more times. The collected extract is labeled as the “AAA-fraction” and contains aliphates, aromates and alkohols including stanols, stanones and Δ^5-steroles. The remaining aquatic fraction in the funnel is acidified with 6 M HCl to achieve pH ≤ 2. The extraction step (3 extractions with 15 ml of chloroform) is repeated and the extracts are collected in a new 50 ml pear shaped flask. This extract is labeled as the “BAFA-fraction” and contains bile acids and fatty acids. The aquatic fraction is discarded. The volume of the AAA-fraction is reduced in a rotary evaporator (Photo 10) at 600-400 mbar, and if necessary, a gentle stream of nitrogen can be used at the end. The residue is dissolved in a small amount of hexane using an ultrasonic bath and transferred for transfer into a 5 ml reaction vial. The volume of the BAFA-fraction is reduced to ~200 µl in a rotary evaporator at 600-400 mbar. The remaining sample is transferred into a 5 ml reaction vial with 5% MeOH in chloroform (might be necessary to use ultrasonic bath for washing to ensure quantitative transfer).

Photo 9. Liquid-liquid extraction

Photo 10. Rotary evaporator

Photo 11. Purification / SPE

<u>SPE of "AAA-fraction" (stanols, stanones and Δ^5-steroles):</u> A clean-up step is carried out by solid-phase extraction through 5% deactivated silica gel (silica gel is activated in a dry cabinet at 120 °C overnight and let to cool a little in an exicator; the still warm silica gel is deactivated by adding 5% (m/m) distilled water and shaking until the reaction is complete (silica gel cools down); can be stored in hexane after deactivation). The SPE column (Photo 11) is filled with hexane, then with the 5% deactivated silica gel, until about 0.5 cm of empty space remains at the top (the empty space is filled with hexane). During filling, some hexane is eluting; this amount of hexane is enough for the conditioning of the silica gel. An aliquot of the sample (volume should be marked) is transferred onto the column, and eluted with 5 ml hexane (aliphatics and aromatics, AA fraction) in two steps of 2.5 ml each. This fraction is discarded, unless AA fraction is required for analysis, in which case, it is eluted with 4 ml of hexane in to steps of 2 ml each and collected in a reaction vial. Sterols, stanols and stanones (SSS fraction) are eluted with 3 ml DCM (in two steps of 1.5 ml each) and 2 ml DCM/acetone (2:1 v/v) (two steps of 1 ml each). The eluates are collected in a reaction vial or in small centrifuge tubes, and evaporated under gentle nitrogen stream until dry.

Photo 12. Vortexer

<u>Silylation of hydroxy groups of stanols and Δ^5-sterols:</u> Methanol is added to the reaction vial and the sample is dissolved in ultrasonic bath and by shaking (vortexer, Photo 12). An aliquot of the sample is transferred into a GC-V-vial. External standards need to be silylated together with the samples (preparation of external standards: 5, 10, 25, 50, 100, 250 and 500 µl of a methanol work solution containing ~10 µg/ml of each sterol is pipetted into GC-V-vials). The sample and external standards are dried under a gentle stream of nitrogen. In each vial, 50 µl of a BSTFA/TMCS + pyridine mixture (3:1, v/v) is added (the mixture is prepared by adding 3 parts N,O-Bis(trimethylsilyl)trifluoroacetamide (BSFTA) / trimethylchlorosilane (TMCS) mixture (99:1 v/v) and one part dry pyridine in a reaction vial just before addition to the samples / external standards). Samples and external standards are heated at 90 °C for 1 h, and then dried under a gentle stream of nitrogen. After drying, IS2 in dry toluene is added to all vials (samples and external standards), and more dry toluene is added to achieve

a final volume of 150 µl. Samples and external standards are transferred to GC/vials with inlet (transfer is done with Pasteur pipette, GC-V-vials should not be washed as all internal standards have been added at this point, and measurement of concentration will be done by GC/MS/MS).

Methylation of carboxy groups of bile acids: External bile acid standards need to be methylated together with the samples in this step of the analysis (preparation: 5, 10, 25, 50, 100 and 250 µl of methanol work solution containing ~10 µg/ml of each bile acid is transferred into reaction vials for methylation). The solvents of the BAFA-fraction (if an aliquot is used, the exact volume must be noted) and external standards are evaporated under a gentle stream of nitrogen. Into the reaction vials 1 ml of HCl in MeOH is added, and the vials are tightly closed with cup, metal disc and PTFE-septum (the flask containing HCl in MeOH should be closed under a gentle stream of nitrogen or argon, in order to push out air/humidity above the solution). The vials are heated at 80 °C for 2 h in a heating block to allow reaction. After cooling to room temperature, 1 ml distilled water (millipore) and 1 ml of hexane are added, and vials are shaken for 1 min or vortexed. The hexane (upper) layer is collected and transferred into a 5 ml reaction vial. The separation is repeated additional 2 times with 1 ml of hexane each, and the hexane layer is collected into the reaction vial (overall, 3 step separation with hexane). The reaction vial with the collected hexane layers is closed with cup and PTFE-septum. The aqueous layer is discarded of.

SPE of bile acids: A clean-up step is carried out by solid-phase extraction through activated silica gel (silica gel is activated in a dry cabinet at 120 °C overnight and stored in an exicator; can be stored in hexane after activation). The SPE column is filled with hexane, then with the activated silica gel, until about 0.5 cm of empty space remains at the top (the empty space is filled with hexane). During filling, some hexane is eluting; this amount of hexane is enough for the conditioning of the silica gel. An aliquot or the whole sample in DCM/hexane (2:1, v/v, dissolved in ultrasonic bath and washed 3 times with DCM/hexane) is transferred onto the column, and eluted with 4 ml DCM/hexane (2:1, v/v) (fatty acid methyl esters fraction) in two steps of 2 ml each. This fraction is discarded. Bile acids (BA fraction) are eluted with 5 ml DCM/MeOH (2:1, v/v) in two steps of 2.5 ml each. The eluates are collected in a reaction vials and dried under a gentle stream of nitrogen (Photo 13).

Photo 13. Evaporation in N_2 stream

<u>Silylation of hydroxy groups of bile acids:</u> The dried samples and standards are diluted in 20 µl of toluene, and 98 µl of BSTFA and 2 µl of TSIM (100 µl mixture) are added (the mixture of 98 parts BSTFA and 2 parts TSIM in a reaction vial is prepared just before addition to the samples and standards). Samples and external standards are heated for 1 h at 180 °C, and IS2 (α-Cholestan) in dry toluene is added. If necessary, dry toluene is added to archive a final volume of dry toluene addition of 50 µl (standard solution + maybe additional dry toluene) to samples and external standards. Samples and external standards are transferred to GC/vials with inlet (transfer is done with Pasteur pipette, GC-V-vials should not be washed as all internal standards have been added at this point, and measurement of concentration will be done by GC/MS/MS).

2. Location and sampling

The samples analyzed through the course *Soil Biogeochemical Analysis* were taken from the Bronze Age tell settlement of Toboliu - a complex site consisting of a central mound, two concentric enclosing ditches, and an impressive contemporary outer settlement, situated in Bihor County, Western Romania (Figure 4).

Figure 4. Toboliu tell - sampling locations

Soil from two locations within the site was sampled (Figure 4): a phaeozem from the outer settlement that was considered as control soil, and an anthrosol near the tell suspected to show anthropogenic influence of burning activities. Soil from both locations was sampled at five depths shown in Table 1.

Table 1. Sampling locations and depths of the analyzed soils

Soil/Sample	Horizon/Depth	
Phaeozem	cm	
35089	20 - 25	
35090	37 - 40	
35091	45 - 50	
35092	70 - 75	
35093	90 - 95	Site 5, core 5
Anthrosol	cm	
35094	40 - 43	
35095	48 - 52	Site 9, core 6
35096	65 - 75	
35097	70 - 80	
35098	58 - 70 (pit)	Site 9, core 8

3. Results

Black carbon

The results obtained by the analysis of black carbon derived BCAs by GC-FID are given in Figure 5. In general, concentrations in both phaeozem and anthrosol were lower than in the laboratory control sample (LBS) which is a black chernozem with typically high BC content used in the study as a reference soil.

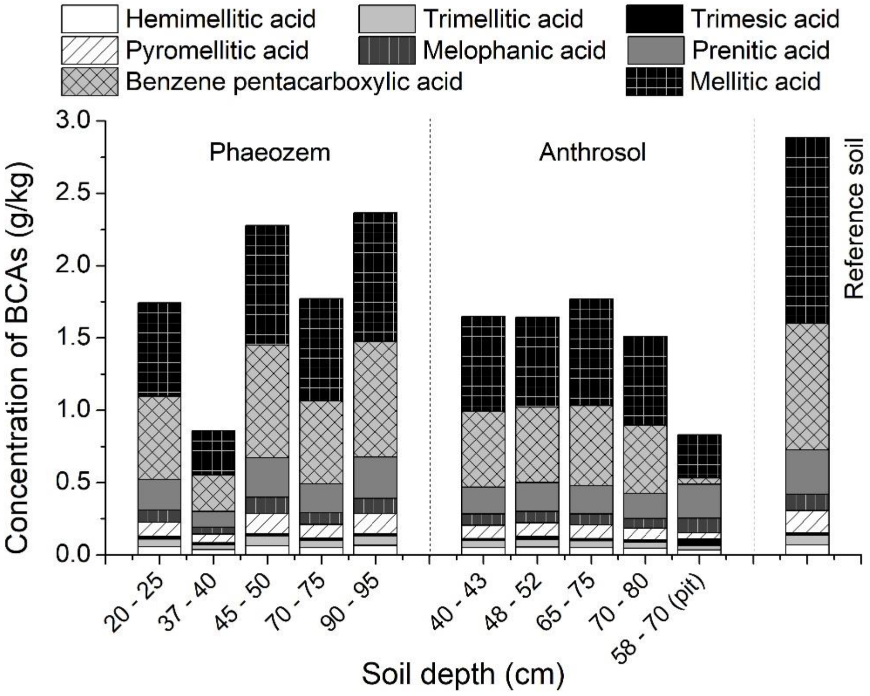


Figure 5. BCAs content in the phaeozem and anthrosol samples

The content of black carbon, BC_{ABS}, in the samples was calculated by multiplying the sum concentration of the analyzed BCAs with the factor of 2.27. Results are shown in Figure 6. The BC_{ABS} content in most of the anthrosol samples was higher than the content of BC_{ABS} in the phaeozem samples from similar depths. The higher values of absolute BC content in the anthrosol may be a potential indicator of higher anthropogenic influence in the soil closer to the tell. For both investigated soils, the BC_{ABS} values were lower compared to the reference soil. This could be the consequence of several factors and processes, such as erosion, weather effects and possible decay.

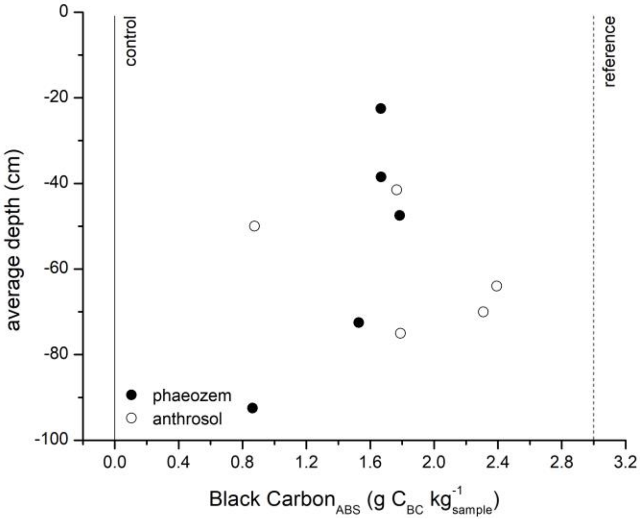


Figure 6. Black carbon content in phaeozem and anthrosol

The organic carbon content (C_{ORG}) was in the range 6.8-13.1% and 11.5-21.2% for phaeozem and anthrosol samples, respectively (Figure 7). All anthrosol samples have higher C_{ORG} than phaeozem samples from similar depths, which is in consistency with the higher BC levels in this soil. Samples from both soil types have in general lower organic carbon content compared to the reference soil which is a chernozem with typically high total organic carbon (TOC_{REF}=1.94%). The only exception is the anthrosol taken from 48-52 cm depth, which had a slightly higher organic carbon content than the reference soil (2.12%). A trend of decreasing organic carbon content was observed with the increasing depth of the samples for both analyzed soils.

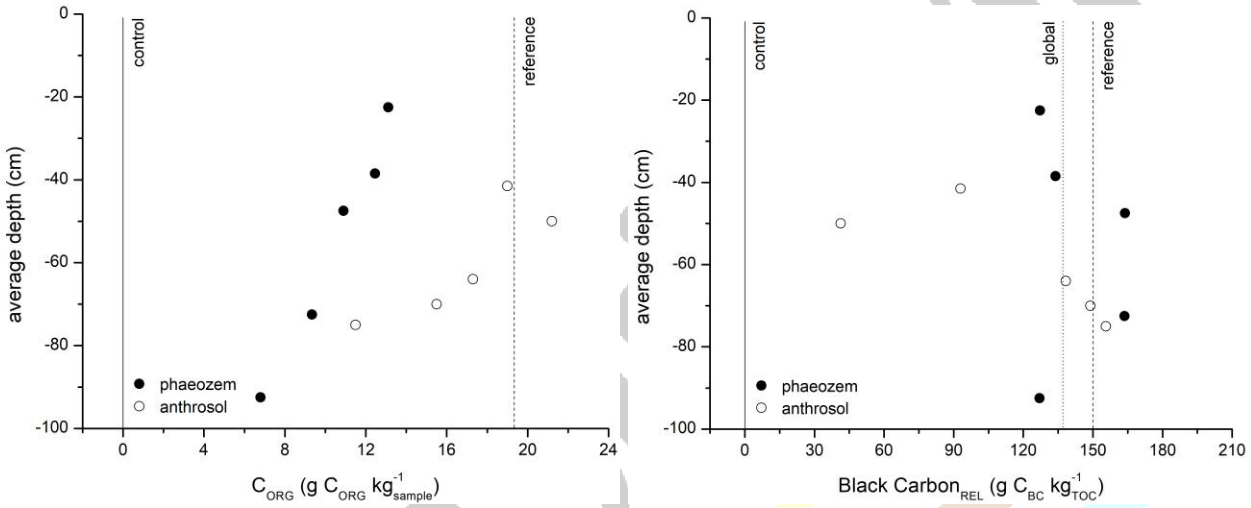


Figure7. Organic carbon content (left) and relative black carbon content (right) in phaeozem and anthrosol samples

The relative black carbon content normalized to the TOC (BC_{REL}) was lower in the anthrosol samples compared to the phaeozem samples from similar depths, which is likely the consequence of higher TOC values in anthrosol (Figure 7). According to literature, BC_{REL} values of 4.5%, 9.2% and 24.4% in soils coincide with low, medium and high fire frequencies, respectively. The average BC_{REL} values obtained for the analyzed samples were 14.3% for phaeozem and 11.5% for anthrosol, whereas the reference soil had a BC_{REL} of 15.0%. These results indicate medium frequency burning activities at the study site.

The share of BCAs with 3 (B3CA), 4(B4CA), 5 (B5CA) and 6 (B6CA) carboxylic groups was calculated and is presented in Figure 8.

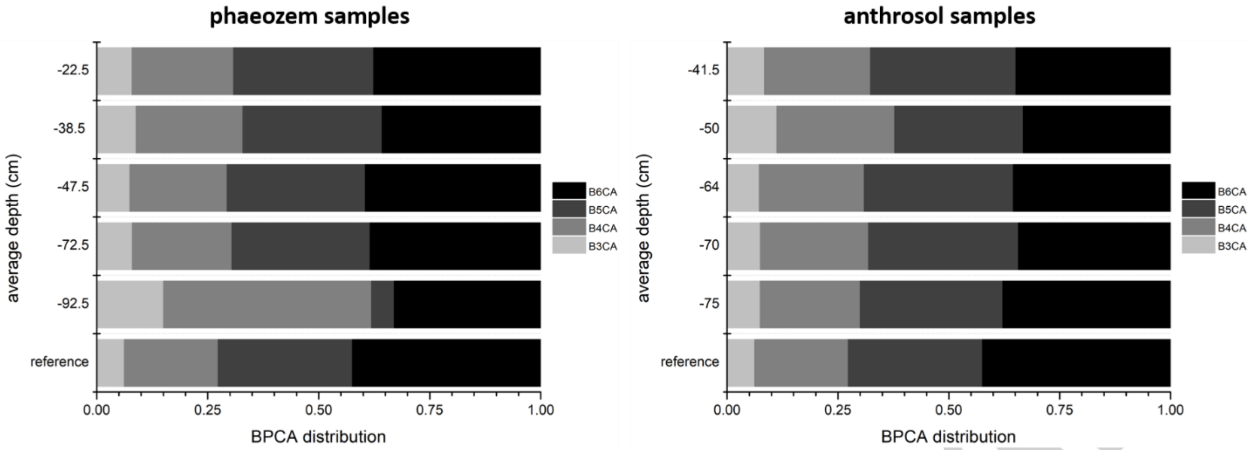


Figure 8. Distribution of BCAs with different number of carboxylic groups

The B5CA and B6CA shares in total BCAs were 38.1% (67.1%) to 70.6% for phaeozem, 62.2% to 70.0% for anthrosol and 72.6% for the reference soil. High levels of BCAs with 5 and 6 carboxylic groups indicate intensive burning events at high temperatures. Furthermore, the results do not indicate any significant occurrence of decay. Also, the BCAs patterns of the investigated anthrosol and phaeozem are comparable to BCAs distribution that can be found in literature.

<u>Stanols, stanones, Δ^5-steroles and bile acids:</u>

The sum concentration of steroids in the analyzed soils (Figure 9, left) varied in the range 1410-2560 µg/kg for anthrosol samples and 583-1490 µg/kg for phaeozem samples. In the reference soil the sterols were detected in a total concentration of 1830 µg/kg.

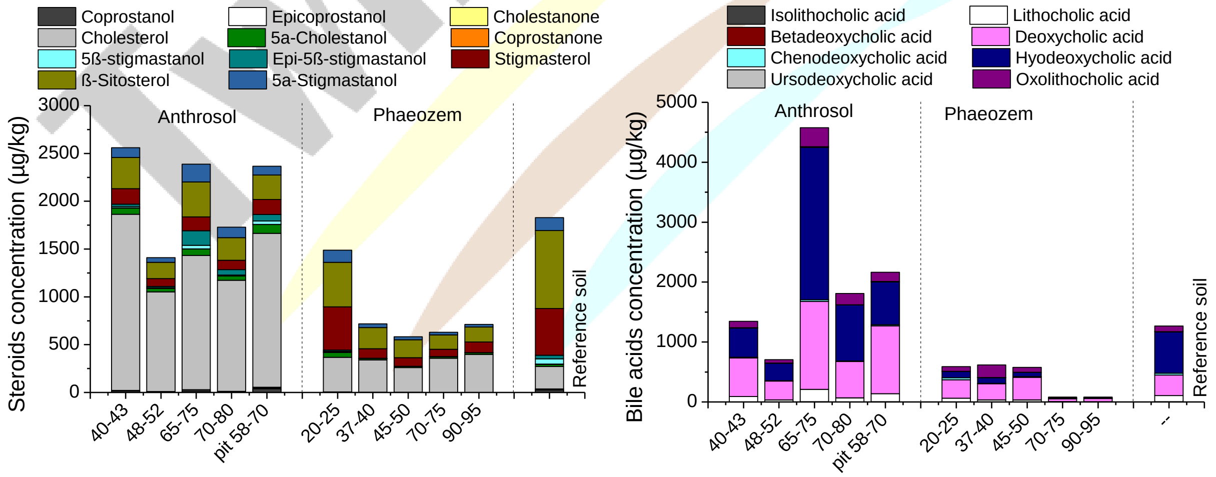


Figure 9. Sum concentrations of steroids (left) and bile acids (right) in anthrosol and pheaozem samples and reference soil

Cholesterol was detected in the highest concentration, followed by β-sitosterol and stigmasterol. This is to be expected as cholesterol is a component in nearly all eukaryotic cells, and can be found in animals, plants, root exudates and several fungal species, whereas both stigmasterol and β-sitosterol are typical Δ^5-sterols for plant biomass. The same trend was observed when concentrations were normalized to the TOC content in the soil samples (Figure 10).

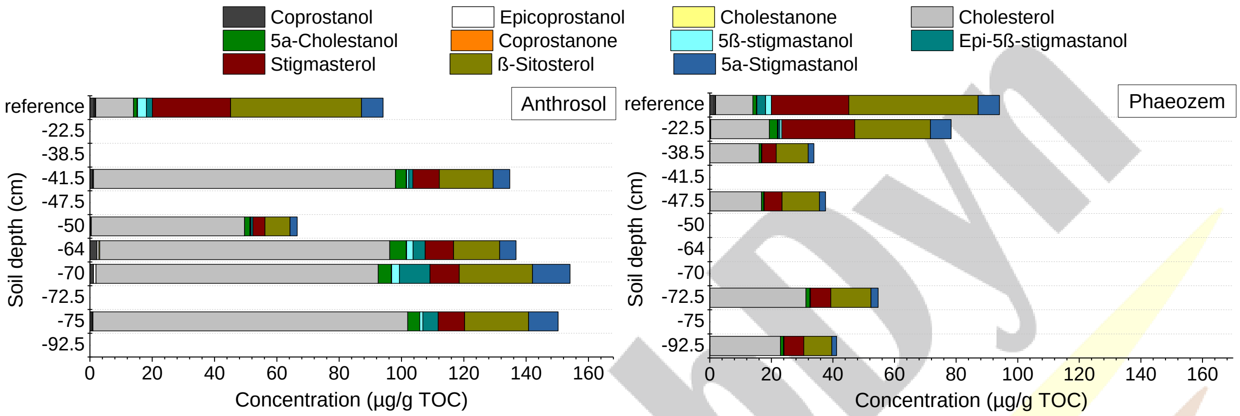


Figure 10. Content of stanols, stanones and Δ^5-steroles in phaeozem and anthrosol samples normalized to TOC content

Both the absolute (Figure 9, right) and the relative to TOC (Figure 11) concentrations showed the same general trend of bile acids content. Hyeodeoxycholic acid (HDCA) and deoxycholic acid (DCA) were found in the highest concentrations nearly all samples, followed by oxolithocholic acid (OLCA) and lithocholic acid (LCA). This trend was also observed in the reference soil.

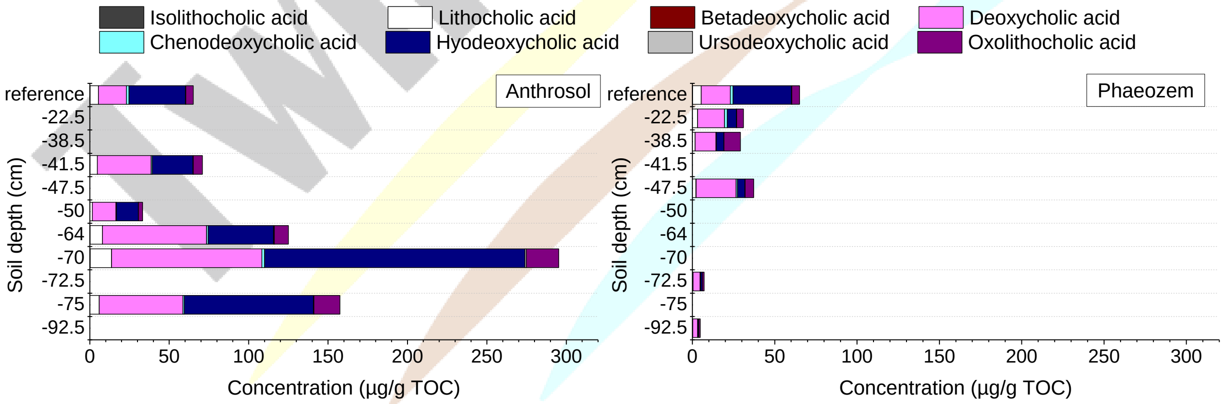


Figure 11. Content of bile acids in phaeozem and anthrosol samples normalized to TOC content

Figure 12 shows the comparison of steroids content in the two analyzed soils. For both groups of compounds, higher content was observed in the anthrosol compared to the phaeozem samples.

These results indicate higher animal input in the soil closer to the tell. Moreover, it can be observed that the concentration of the biomarkers increases with depth of anthrosol, especially for bile acids, whereas, both groups of compounds show no change or only a slight decrease in concentration with depth of phaeozem.

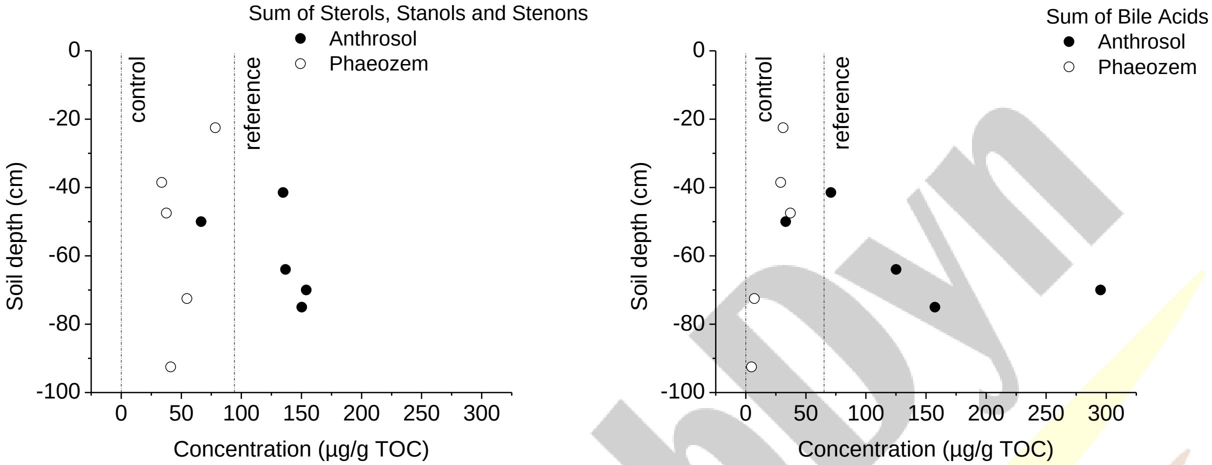


Figure 12. Comparison of biomarkers content in phaeozem and anthrosol

Table 1. presents the calculated typical ratios of sterols and bile acids indicative of inputs from specific groups of animals.

Table 1. Calculated ratios of sterols and bile acids

Sample	Depth (cm)	Sterols		Bile Acids					Input
		A*	B**	DCA/LCA	DCA/CDCA	CDCA/LCA	HDCA/LCA	HDCA/DCA	
Anthrosol	40-43	57.4		7.27	46.4	0.156	5.48	0.755	pig
Anthrosol	48-52	55.6		9.48	39.2	0.242	8.81	0.929	pig
Anthrosol	65-75	31.5		7.05	56.5	0.125	12.2	1.73	pig
Anthrosol	70-80	41.9		8.95	62.4	0.143	13.8	1.54	pig
Anthrosol	pit 58-70	52.3		8.44	63.1	0.134	5.30	0.627	pig
Phaeozem	20-25	**26.3**	**1.56**	5.06	9.71	0.521	1.71	0.339	horse
Phaeozem	37-40	44.1		7.57	49.1	0.154	2.72	0.360	pig
Phaeozem	45-50	**67.7**		11.4	29.7	0.385	2.11	0.185	human
Phaeozem	70-75	41.7		10.1	12.5	0.809	2.29	0.227	pig
Phaeozem	90-95	48.3		13.0	15.6	0.834	2.02	0.156	pig

*A = [coprostanol/(coprostanol + 5β-stigmastanol)]*100%
**B = (epi-5β-stigmastanol/5β-stigmastanol)+(epicoprostanol/coprostanol)
CDCA = Chenodeoxycholic acid; HDCA = Hyodeoxycholic acid; LCA = Lithocholic acid; DCA = Deoxycholic acid

Sterol ratios A and B were calculated as indicated in Table 1. In most samples, the ratio A was between 29 and 65% which suggests pig input. Phaeozem soil from 20-25 cm depth had an A ratio lower than 29%. This could indicate input from herbivores rather than pig or human. For this sample, ratio B was also calculated, which was higher than 1.2 (1.56) and leads to the assumption that the herbivore influence might be from horse input. Lastly, the A ration calculated for phaeozem soil from 45-50 cm depth was slightly higher than 65% (67.7%), and this could suggest input from humans. However, whereas ratios A and B indicate certain inputs, literature suggests additional criteria such as the presence or absence of specific bile acids to confirm these inputs, which in most samples from this research were not met.

Soil sampling

In addition to the laboratory training in soil sample preparation for biomarkers analysis, the secondment also included on-site training in soil sampling. Soil samples were collected from an experimental field located Donndorf / Eckersdorf (near Bayreuth, Germany) in northeast Bavaria. The field experiment was established in 2010 for investigating the influence of different organic soil amendments (OSA) on soil properties and crop yield. The OSA used in the research include biochar, compost and two mixtures of these materials: direct mixture of biochar and compost, and a mixture of biochar and raw plant material which was then co-composted. All treatments were applied in two doses, which resulted in a total of 8 treatments and an untreated control soil. All treatment variants, as well as the variants that were sampled as part of the on-site training are listed in Table 2.

Table 2. Treatment variants included in the field experiment

Variant	Treatment	Dose (t/h)		Sampled for TwinSubDyn	Depth sampled	Amount sampled for TwinSubDyn
		biochar	compost			
control	no treatment	/	/	✓	0-30 cm	24 kg
K9	biochar	9	/			
K31.5	biochar	31.5	/	✓	0-30 cm	6 kg
GK20	compost	/	20			
GK70	compost	/	70			
GK20+K9	biochar+compost mixture	9	20			
GK70+K31.5	biochar+compost mixture	31.5	70			
BGK9/20	co-composted biochar	9	20			
BGK31.5/70	co-composted biochar	31.5	70	✓	0-30 cm	6 kg

The sampling was carried out as follows: The treatment plots were arranged within the experimental field according to a Latin rectangle in row-column (5-10) design, and each treatment was replicated 5 times. Magnets buried along the edges of the field were located with a metal detector, and pols were placed to mark the dividing point of each plot. Once the field was divided, the plots of interest were located and soils from each plot were sampled with a manual corer (6 corers/plot) from 0-30 cm depth (Photos 14 and 15). All soils of the same variant were collected together to make a composite sample. For the TwinSubDyn project, three variants of the soil were sampled: the control soil without any application of OSA, soil K31.5 which was treated with biochar (31.5 t/h) and soil BGK31.5/70 which was treated with co-composted biochar (31.5 t/h biochar and 70 t/h compost). The soils were homogenized, dried at 40°C (Photo 16), packed and labeled. Control, K35.1 and BGK31.5/70 soils (approximately 6 kg each) were sent to Sevilla, Spain to IRNAS for characterization and pot experiments. An additional 6 kg of control soil was sent to IRNAS to be mixed with biochar in order to investigate the impact of biochar aging on soil, as well as approximately 0.5 kg of all three sampled variants for phospholipid fatty acids analysis, which were previously freeze dried. Control soil was also sent to project partners at University of Vienna and University of Novi Sad for additional research.

Photo 14. Soil sampling

Photo 15. Corer

Photo 16. Drying

Impact on your project

The training in molecular biomarkers analysis was an excellent opportunity for knowledge transfer between the MLU and UNSPMF project groups. The sample preparation methodology is partially novel to the Novi Sad group as techniques such as hydrolysis and oxidation are not routinely used in solid samples preparation for organic analysis. However, the methods carried out throughout the course can be transferred and adopted to be used in the UNSPMF labs, which will subsequently broaden the possibilities for further research in the field of soils science. The trained UNSPMF researchers will have the opportunity to transfer the gained knowledge and skills to both TwinSubDyn participants as well as other researchers and stakeholders. In addition, the acquired training in biomarkers analysis will be further utilized through a 6 month secondement of an early stage researcher from UNSPMF at the MLU. They will continue the research in analysis of biomarkers optimizing sample preparation methodology for the detection of available benzene carboxylic acids. The main focus will be on investigating natural degradation processes of BC over an extended period of time.

The field training carried out during the short visit gave insight into the design and methodology of field experiments which can be transferred to other groups within the project, as well as other stakeholders. The expertise of the MLU group in experimental design and field research could be an excellent starting point for future filed investigations. In addition, the field training provided the opportunity to sample soils that underwent treatment in 2010, thus enabling further research into the impact of aging of SOA treated soils on both soil and SOA properties. These investigations will be carried out at IRNAS, UNIVIE and UNSPMF throughout the duration of the TwinSubDyn project.

Black Carbon analysis of phaeozem and anthrosol at the Bronze age tell Toboliu (Western Romania)

Soil Biogeochemical Analysis - AGE.06353.01

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02.03.2023
MARTIN-LUTHER-UNIVERSITÄT
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Study site

- **Toboliu** - Bronze age (2200 - 800 BC) tell in **Western Romania**

map location

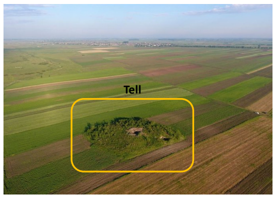
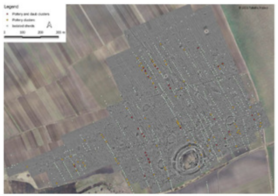


photo of the tell

Study site

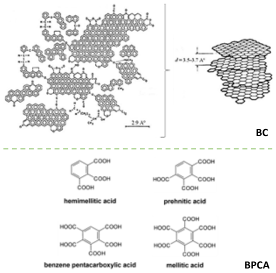


geophysical measurements
and surface survey

Black Carbon analysis

Black Carbon (BC)

- Most commonly **fire derived**
- Molecular marker:
  benzene polycarboxylic acids (**BPCA**)
- BPCA: **3 - 6 carboxyl groups** with specific isomers
- No. of –COOH: **temperature** and **time** of **combustion**

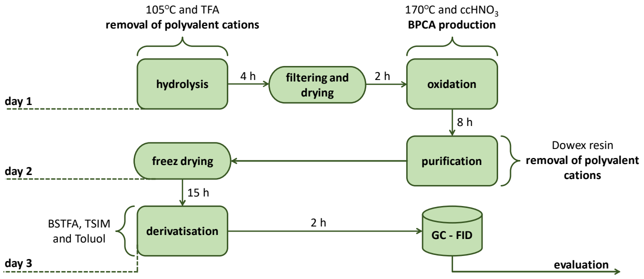


Hypothesis

H1: Due to **anthropogenic impact**, the **Black Carbon content is higher in anthrosol** samples **than in phaeozem** samples

H2: **Black Carbon content** of the **anthrosol** at **study site** is **comparable** to Black Carbon content of **anthrosols at different locations**

Methodology



105°C and TFA
removal of polyvalent cations

170°C and $ccHNO_3$
BPCA production

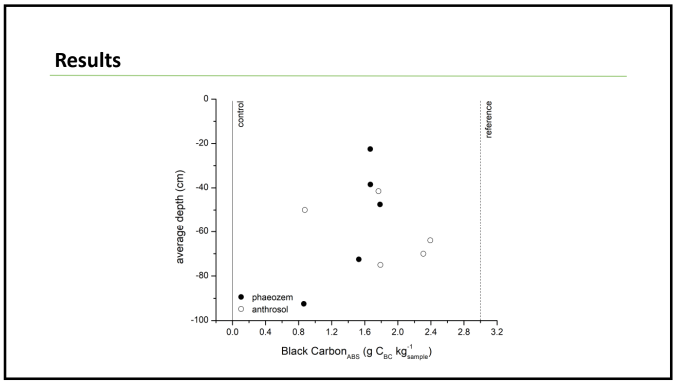
day 1: **hydrolysis** → 4 h → **filtering and drying** → 2 h → **oxidation** → 8 h → **purification**

Dowex resin
removal of polyvalent cations

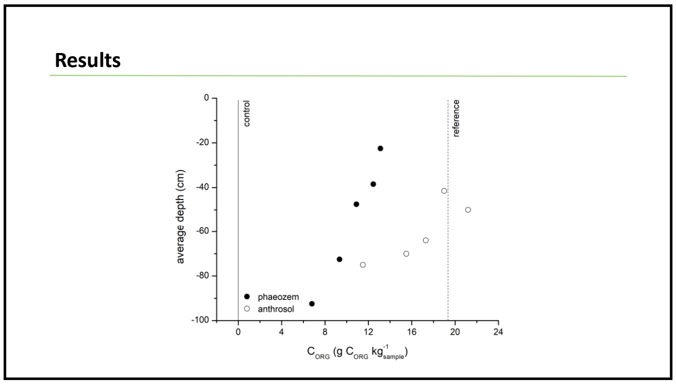
day 2: **purification** → **freez drying** → 15 h → **derivatisation**

BSTFA, TSIM
and Toluol

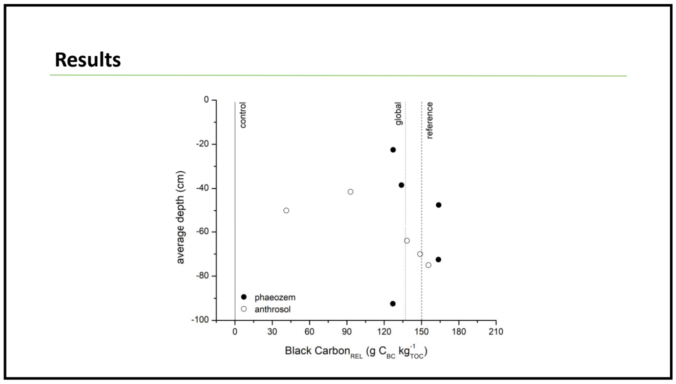
day 3: **derivatisation** → 2 h → **GC - FID** → **evaluation**



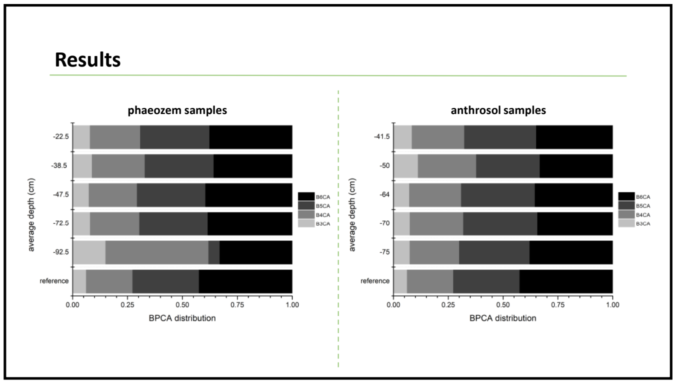
Results



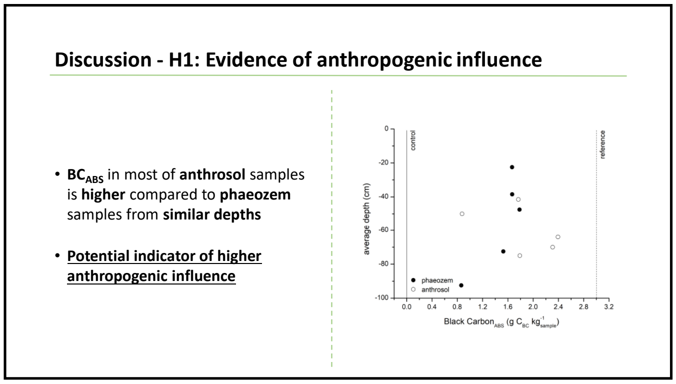
Results



Results

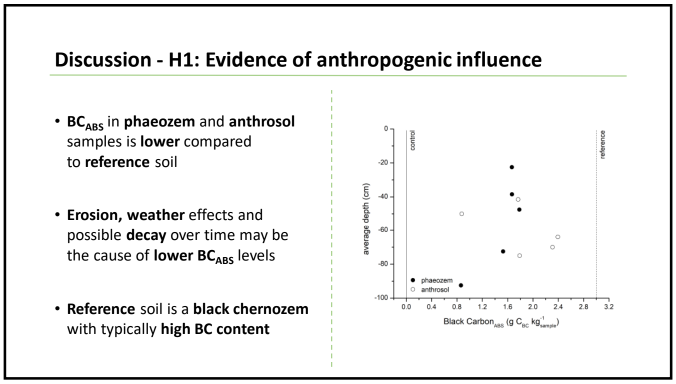


Results



Discussion - H1: Evidence of anthropogenic influence

- **BC$_{ABS}$** in most of **anthrosol** samples is **higher** compared to **phaeozem** samples from **similar depths**
- **Potential indicator of higher anthropogenic influence**

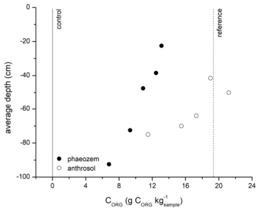


Discussion - H1: Evidence of anthropogenic influence

- **BC$_{ABS}$** in **phaeozem** and **anthrosol** samples is **lower** compared to **reference** soil
- **Erosion, weather** effects and possible **decay** over time may be the cause of **lower BC$_{ABS}$** levels
- **Reference** soil is a **black chernozem** with typically **high BC content**

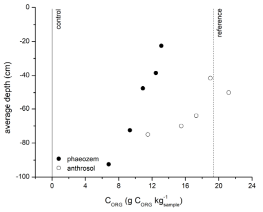
Discussion - H1: Evidence of anthropogenic influence

- **TOC** in **anthrosol** samples is **higher** compared to **phaeozem** samples from **similar depths**
- **<u>Potentially a partial result of higher BC levels</u>**



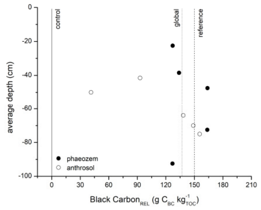
Discussion - H1: Evidence of anthropogenic influence

- **TOC** in **phaeozem** and **anthrosol** samples is mostly **lower or similar** compared to **reference** soil
- **TOC** in **phaeozem** and **anthrosol** samples **decreases with depth**
- **Reference** soil is a **black chernozem** with typically **high TOC**



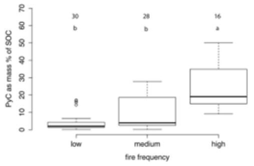
Discussion - H1: Evidence of anthropogenic influence

- **BC_{REL}** in all **anthrosol** samples is **lower** compared to **phaeozem** samples from **similar depths**
- Consequence of **higher TOC** values for **anthrosol** samples



Discussion - H1: Evidence of anthropogenic influence

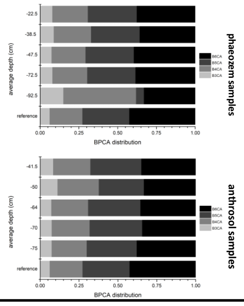
- **BC_{REL} for:**
  Low fire frequencies - 4.5%
  Medium fire frequencies - 9.2%
  High fire frequencies - 24.4%
- **Average BC_{REL} for:**
  phaeozem samples - 14.3%
  anthrosol samples - 11.5 (13.4)%
  reference soil - 15.0%
- **<u>Indication of medium frequency burning activities</u>**



(Reisser et al. 2016)

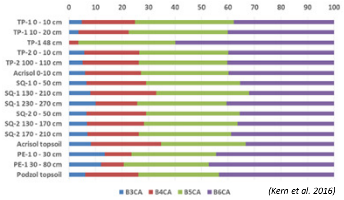
Discussion - H1: Evidence of anthropogenic influence

- **B5CA and B6CA shares in total BPCA:**
  phaeozem samples - 38.1 (67.1) to 70.6%
  anthrosol samples - 62.2 to 70.0%
  reference soil - 72.6%
- **<u>Indication of intensive burning events at high temperatures</u>**
- **<u>The results do not indicate the occurrence of decay</u>**

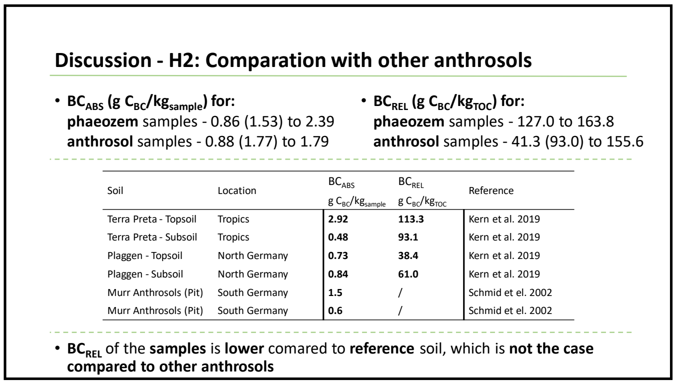


Discussion - H2: Comparation with other anthrosols

- The **BPCA distribution** in the **presented samples** is **very similar** to the **BPCA pattern** of **anthrosol** and **phaeozem** samples from the **study site**



(Kern et al. 2016)

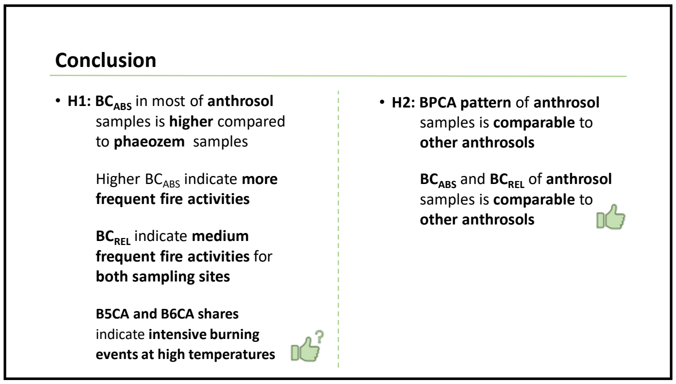


Discussion - H2: Comparation with other anthrosols

- **BC_{ABS} (g C_{BC}/kg_{sample}) for:**
  phaeozem samples - 0.86 (1.53) to 2.39
  anthrosol samples - 0.88 (1.77) to 1.79
- **BC_{REL} (g C_{BC}/kg_{TOC}) for:**
  phaeozem samples - 127.0 to 163.8
  anthrosol samples - 41.3 (93.0) to 155.6

Soil	Location	BC_{ABS} g C_{BC}/kg_{sample}	BC_{REL} g C_{BC}/kg_{TOC}	Reference
Terra Preta - Topsoil	Tropics	**2.92**	**113.3**	Kern et al. 2019
Terra Preta - Subsoil	Tropics	**0.48**	**93.1**	Kern et al. 2019
Plaggen - Topsoil	North Germany	**0.73**	**38.4**	Kern et al. 2019
Plaggen - Subsoil	North Germany	**0.84**	**61.0**	Kern et al. 2019
Murr Anthrosols (Pit)	South Germany	**1.5**	/	Schmid et el. 2002
Murr Anthrosols (Pit)	South Germany	**0.6**	/	Schmid et el. 2002

- **BC_{REL}** of the **samples** is **lower** comared to **reference** soil, which is **not the case compared to other anthrosols**



Conclusion

- **H1: BC_{ABS}** in most of **anthrosol** samples is **higher** compared to **phaeozem** samples

  Higher BC_{ABS} indicate **more frequent fire activities**

  BC_{REL} indicate **medium frequent fire activities** for **both sampling sites**

  B5CA and B6CA shares indicate **intensive burning events at high temperatures**

- **H2: BPCA pattern** of **anthrosol** samples is **comparable** to **other anthrosols**

  BC_{ABS} and **BC_{REL}** of **anthrosol** samples is **comparable** to **other anthrosols**

Determination of human and animal biomarkers in the soil from the Bronze Age tell Toboliu, Western Romania

Soil Biogeochemical Analysis

TAMARA APOSTOLOVIĆ

Halle, 02.03.2023.

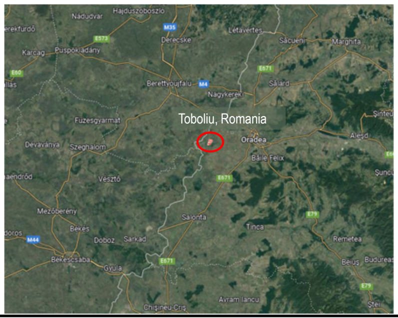
MARTIN-LUTHER-UNIVERSITÄT
HALLE-WITTENBERG

INTRODUCTION

SAMPLING SITE

Toboliu tell, Romania

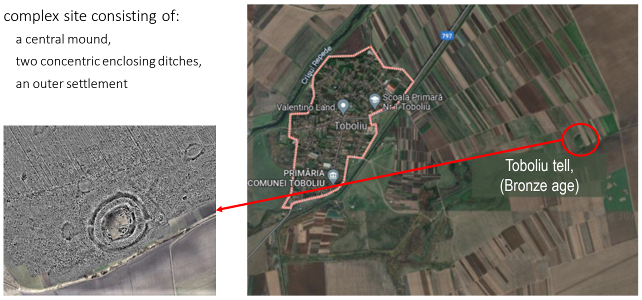
- A Bronze Age tell settlement of Toboliu
- Situated in Bihor County, Western Romania



TOBOLIU TELL, ROMANIA – BRONZE AGE

A complex site consisting of:

- a central mound,
- two concentric enclosing ditches,
- an outer settlement

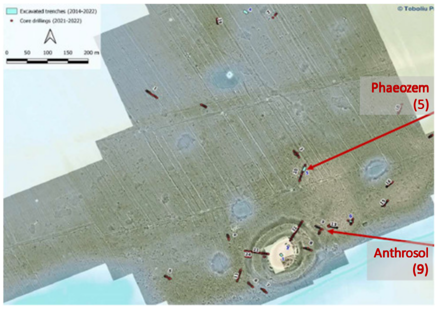


https://ufg.phil-fak.uni-koeln.de/forschung/forschungsprojekte/bronze-eisenzeit/toboliu-project

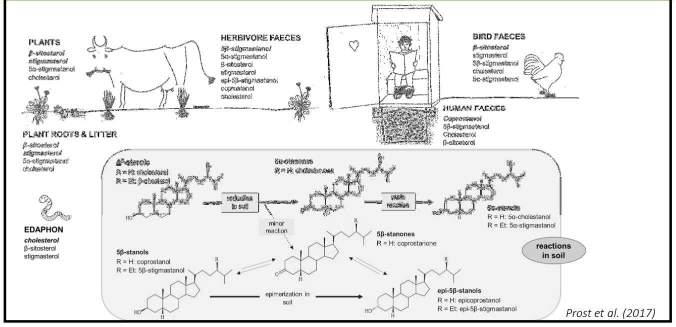
TOBOLIU TELL, ROMANIA – BRONZE AGE

Samples

- Site 9 – anthrosol
  - 40-43 cm
  - 48-52 cm
  - 65-75 cm
  - 70-80 cm
  - pit 58-70 cm
- Site 5 – phaeozem
  - 20-25 cm
  - 37-40 cm
  - 45-50 cm
  - 70-75 cm
  - 90-95 cm

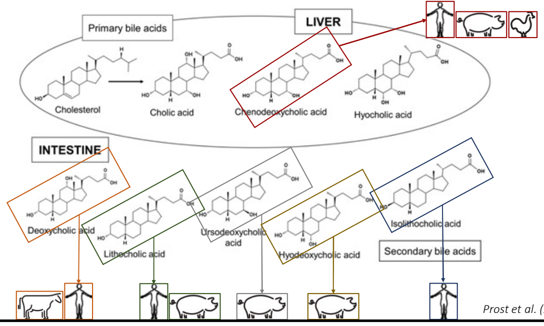


STEROLS, STANOLS, STANONES



Prost et al. (2017)

BILE ACIDS



Prost et al. (2017)

OBJECTIVE AND HYPOTHESES

- OBJECTIVE – identifying human and/or animal presence at the Toboliu tell, Romania by determining the content of biomarkers (sterols, stanols, stanones, bile acids) in soil samples.

- HYPOTHESES

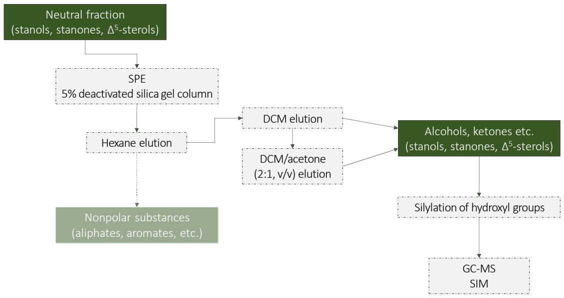
H.1 Anthrosol have a higher content of the target biomarkers than phaeozem.

H.2 Closer to the tell, biomarkers indicating human presence are found in higher content than those indicating animal presence.

METHODOLOGY

METHODOLOGY

METHODOLOGY



Neutral fraction
(stanols, stanones, Δ^5-sterols)

SPE
5% deactivated silica gel column

Hexane elution

DCM elution

DCM/acetone
(2:1, v/v) elution

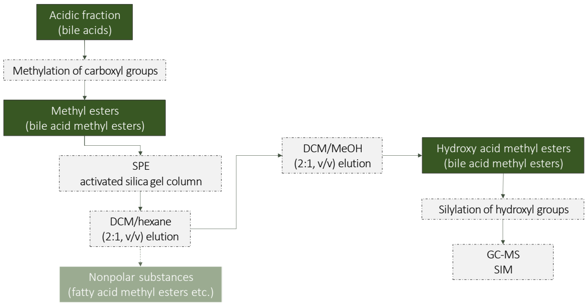
Alcohols, ketones etc.
(stanols, stanones, Δ^5-sterols)

Silylation of hydroxyl groups

GC-MS
SIM

Nonpolar substances
(aliphates, aromates, etc.)

METHODOLOGY



Acidic fraction
(bile acids)

Methylation of carboxyl groups

Methyl esters
(bile acid methyl esters)

SPE
activated silica gel column

DCM/hexane
(2:1, v/v) elution

Nonpolar substances
(fatty acid methyl esters etc.)

DCM/MeOH
(2:1, v/v) elution

Hydroxy acid methyl esters
(bile acid methyl esters)

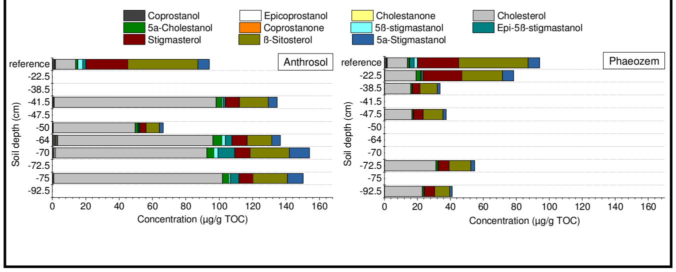
Silylation of hydroxyl groups

GC-MS
SIM

RESULTS

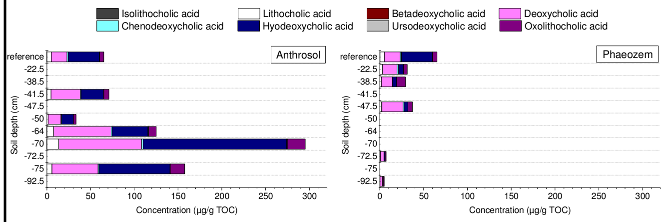
RESULTS

Content and distribution of sterols, stanols and stanones in soil samples



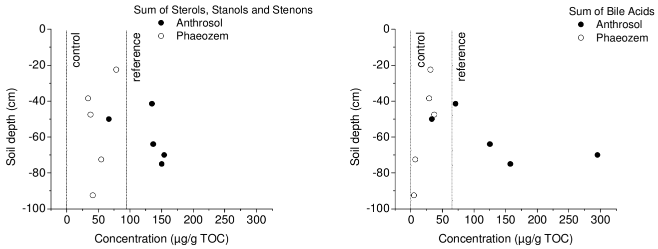
RESULTS

Content and distribution of bile acids in soil samples



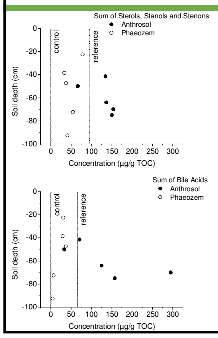
RESULTS

Comparation of biomarkers content in anthrosol and phaeozem



DISCUSSION

DISCUSSION – H.1 ✓

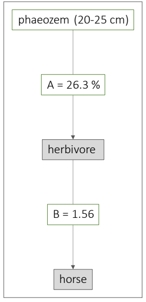


- H.1 Anthrosol have a higher content of the target biomarkers than phaeozem
- Anthrosol have higher content of the target biomarkers than the reference chernozem
- Bile acids content increase with soil depth in anthrosol

DISCUSSION – H.2

Sample	Depth (cm)	Sterols		Bile Acids				
		A	B	DCA/LCA	DCA/CDCA	CDCA/LCA	HDCA/LCA	HDCA/DCA
Anthrosol	40-43	57.4		7.27	46.4	0.156	5.48	0.755
Anthrosol	48-52	55.6		9.48	39.2	0.242	8.81	0.929
Anthrosol	65-75	31.5		7.05	56.5	0.125	12.2	1.73
Anthrosol	70-80	41.9		8.95	62.4	0.143	13.8	1.54
Anthrosol	pit 58-70	52.3		8.44	63.1	0.134	5.30	0.627
Phaeozem	20-25	**26.3**	**1.56**	5.06	9.71	0.521	1.71	0.339
Phaeozem	37-40	44.1		7.57	49.1	0.154	2.72	0.360
Phaeozem	45-50	**67.7**		11.4	29.7	0.385	2.11	0.185
Phaeozem	70-75	41.7		10.1	12.5	0.809	2.29	0.227
Phaeozem	90-95	48.3		13.0	15.6	0.834	2.02	0.156

DISCUSSION – H.2 ✗

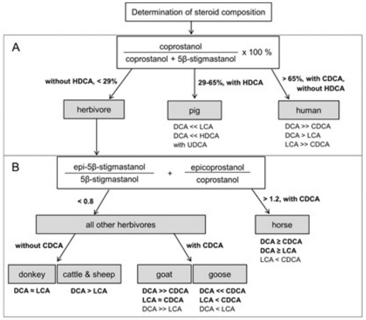

phaeozem (20-25 cm)

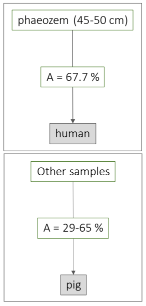
A = 26.3 %

herbivore

B = 1.56

horse




phaeozem (45-50 cm)

A = 67.7 %

human

Other samples

A = 29-65 %

pig

Prost et al. (2017)

DISCUSSION – H.2

Sample	Depth (cm)	Ratio		Bile Acids		
		A	B	CDCA	**HDCA**	
Anthrosol	40-43	57.4		0.730	**25.6**	🐖
Anthrosol	48-52	55.6		0.377	**13.8**	🐖
Anthrosol	65-75	31.5		1.68	**164**	🐖
Anthrosol	70-80	41.9		0.845	**81.3**	🐖
Anthrosol	pit 58-70	52.3		1.04	**41.1**	🐖
Phaeozem	20-25	**26.3**	**1.56**	1.68	**5.53**	🐎
Phaeozem	37-40	44.1		0.257	**4.55**	🐖
Phaeozem	45-50	**67.7**		0.819	**4.49**	🧍
Phaeozem	70-75	41.7		0.343	**0.971**	🐖
Phaeozem	90-95	48.3		0.192	**0.464**	🐖

CONCLUSION

CONCLUSIONS

✓ H.1 Anthrosol have a higher content of the target biomarkers than phaeozem.

- The result show a clear distinction between the analyzed anthrosol and phaeozem.
- Biomarkers content was significantly higher in anthrosol indicating that the focus of both human and animal activity might have been at/near the tell.

✗ H.2 Closer to the tell, biomarkers indicating human presence are found in higher content than those indicating animal presence.

- Based on the calculated biomarker ratios, in all anthrosol and most phaeozem samples the dominant compounds are those linked to pigs
- The presence of human and horse input was also observed in phaeozem (one sample each).

Subtask 1.2.4 (leader FZJ) 6-months secondment for ESR/RR two 1-months secondments for QR/RR at FZJ focused on methods to characterize biogeochemical processes, nutrient cycling in different soil layers using state-of-the-art physical-chemical and analytical methods.

MOBILITY REPORT

Researcher: MSc Nina Đukanović, UNSPMF

Assigned supervisor: Prof. Dr. Roland Bol and Dr. Jens Kruse, FZJ

Duration of the visit: 05.06.2023. - 30.11.2023.

Executive Summary

The main purpose of the visit to Forschungszentrum Jülich was to acquire new knowledge and skills related to soil chemistry and sophisticated techniques used for soil characterization and soil organic matter dynamics and element cycles. The first part of the training was specifically focused on the development of methods for the characterization of biogeochemical processes and nutrient cycling in different soil layers using the most up-to-date physicochemical and analytical methods. These methods included the use of advanced instruments such as isotope ratio mass spectrometry (IRMS), inductively coupled plasma mass spectrometry (ICP-MS) and multi-collector inductively coupled plasma mass spectrometry (MC-ICP-MS), as well as techniques such as are such as flow field fractionation (AF4, SPLITT), microscopy (SEM/TEM, fluorescence) and scattering (light, X-ray). The second part of the training was focused on a medium-scale lysimeter experiment with soil contaminated with heavy metals, to investigate the effect of organic amendments on the uptake of heavy metals by plants and the risk of leaching of colloidally bound heavy metals. The last part of the training was focused on the preparation and analysis of soil, plant and leachate samples, collected during and after the experiment in lysimeters, as well as characterisation of raw-materials from this experiment.

Introduction

Background

Soil organic matter (SOM) dynamics refer to the processes by which organic materials are added, decomposed, and transformed in the soil. SOM plays a crucial role in soil fertility, nutrient cycling, and carbon sequestration. Microorganisms in the soil, such as bacteria and fungi, break down the organic compounds that are added to the soil. Nitrogen, phosphorus and sulfur are among the nutrients that are released during this decomposition process and become available to plants. Research objectives related to SOM and nutrient cycling are: (i) Obtain information on the origin, residence time, cycling and priming of organic matter and elements (N, P, K, Ca, Si, Fe etc.) in the soil-plant system and from there into surface - and groundwater. (ii) Improve the understanding of the complex carbon and nutrient biogeochemistry processes and interactions that occur at the field and laboratory scale. (iii) Research involves the use of innovative modern technologies for examining the complex effects of natural factors and factors caused by human activities on biogeochemical cycles. (iv) Research involves the collection of field and laboratory scientific data, in order to enable sustainable management of the system. This is achieved by integrating the findings within a comprehensive systems biogeochemistry framework. A significant aspect of the research is the utilization of isotope signatures as tracers for carbon, other elements, and sediments in the environment. This involves employing approaches such as IRMS, ICP-MS, and MC-ICP-MS, as well as techniques like field flow fractionation (AF4, SPLITT), microscopy (SEM/TEM, fluorescence), and scattering (light, X-ray).

Scope of the secondment

The scope of the first part of the secondment was knowledge transfer on techniques for soil characterizations, organic matter dynamics and nutrient cycle. This included laboratory training in soil sample preparation and analysis. The scope of the next part of the secondment was to gain knowledge about setting up medium-scale lysimeter experiments in the glasshouse (including mixing soil with amendments, the addition of macro and micronutrients, labeling, irrigation, etc.) , as well as collecting and analyzing samples before (soil and amendments samples - raw materials and mixtures), during (leachate samples) and after (plants and soil samples) experiments. The detailed methodology for the preparation and determination of colloids in soil and the results obtained throughout the first part of the course will be given in the following chapters, as well as an explanation of the sample collection and analysis, and preparation of lysimeter experiments.

Content

FIELD FLOW FRACTIONATION (FFF) – (AF4, Postnova Analytics, Landsberg, Germany)

Colloidal organo−mineral associations contribute to soil organic matter (OM) preservation and mainly occur in two forms: (i) as water-dispersible colloids that are potentially mobile (free colloids) and (ii) as building units of soil microaggregates that are occluded inside them (occluded colloids). The obtained free and occluded colloids can be size-separated using asymmetric flow field-flow fractionation. Field-flow fractionation coupled online to various detectors, including inductively coupled plasma mass spectrometry (ICP-MS), an organic carbon detector (OCD), an ultra-violet (UV) detector and dynamic light scattering (DLS), was used for the size separation of natural colloidal particles.

Separation in the AF4 is achieved according to molar mass or particle size as dictated by the principles of asymmetrical flow field-flow fractionation (asymmetrical flow FFF). This separation is carried out in a thin ribbon-like channel in which the channel is constructed by clamping a thin spacer between porous and nonporous plates as shown in Figure 1.

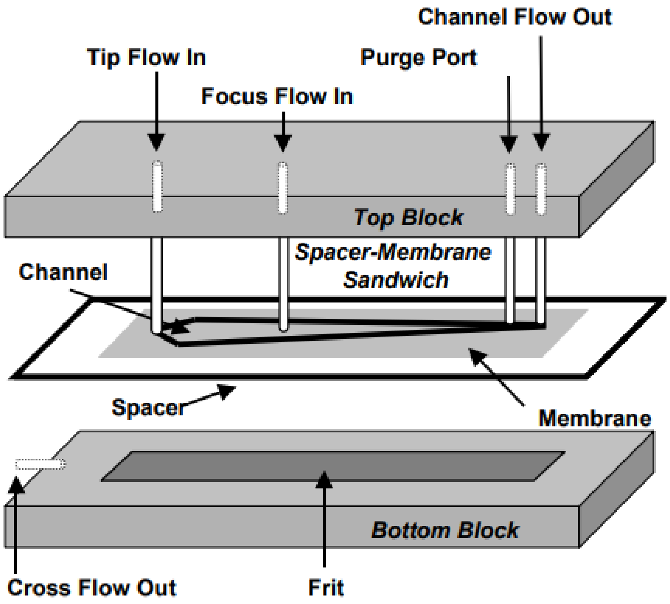


Figure 1. Schematic of the asymmetrical flow FFF channel

Carrier liquid entering at the tip of the channel makes up both the longitudinal fluid flow (termed channel flow) and the horizontal fluid flow (termed crossflow). The channel flow is parabolic (flow velocity is highest in the center of the channel and zero at the walls) and this flow carries sample down the channel. The crossflow is created because the porous wall (a membrane supported by a frit) allows the carrier liquid to exit through the bottom channel wall. Thus carrier flow entering the channel divides between flow down the channel and flow across the channel. The crossflow forms fluid lines which are mostly perpendicular to the channel flow. The crossflow causes a viscous force that drags the sample material to the membrane surface (termed accumulation wall). Once the sample is positioned at the accumulation wall, the individual species equilibrate into zones of a specific thickness. Diffusion and/or size properties of the sample species and the magnitude of crossflow field determine the thickness of the zone. Components which are compressed tightly against the accumulation wall are carried slowly by flow because they are immersed in streamlines of low velocity adjacent to the wall. Sample components with higher diffusivities for more expanded distributions which travel more rapidly and elute earlier. The result is differential elution of sample components. A mechanism of the asymmetric flow FFF separation is illustrated in Figure 2.

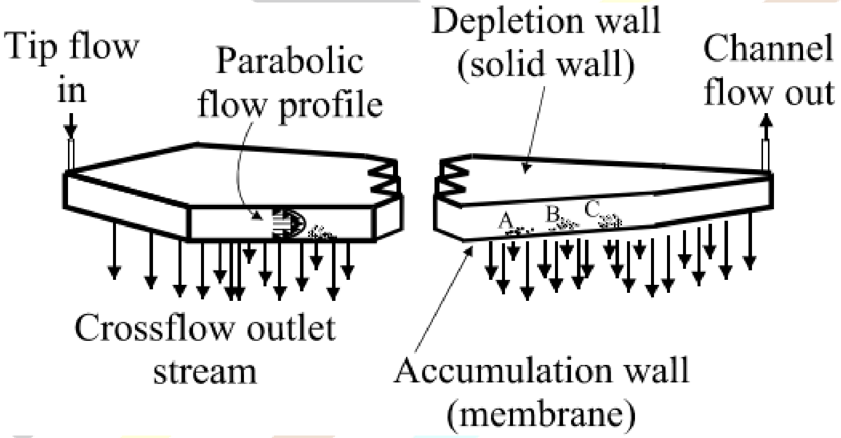


Figure 2. Separation mechanism of asymmetrical flow FFF. Three components, shown here A, B, and C are compressed to different levels against the accumulation wall by the crossflow. Component A lies closest to the wall where fluid flow approaches zero. Component B, protruding into faster flow streams, travels more rapidly downstream. Component C, least compacted by the crossflow, is displaced most rapidly by the carrier liquid.

General things to prepare

- Prepare fresh FFF eluent (25 |M NaCl; 1.461 g NaCl/L) and if necessary, OCD solution (K2S208 2.5 g/5 L; concentrated H3PO4: 20 mL/5 L; do not change/refill during measurement) using MiliQ water
- Clean channel and use new membrane
  Change all filters (check eluent flow direction)
- Correct 'Loop' for your method installed?
- Reboot the computer before a new measuring campaign (help to prevent software bugs)

Detectors

UV

- Switch on lamp at least 30 min before measurement o
- func. -> enter -> func. -> func. -> lamp: 1

OCD

- Switch on about 3-4 h before measurement -> baseline should be stable
- Make sure that there is enough solution for acidification of the OCD (do not refill during measurement)
- Connect to FFF onlv shortly before measurement
- Please always disconnect FFF from OCD after measurement and insert plug

Figure 3. FFF-UV-OCD

Software

New sequence

Open 'Basic Run' -> Load 'Method' that was previously created

- Determine number of samples -> Set 'End Position' to the number of samples
- Then press 'Create

(For single samples if you just want to use an existing sequence as a basis):

- File -> new -> sequence
- In the 'Sequence' window -> click on empty 'run' field - 'Open run' -> load run file
- Press 'Show run' -> Check 'Auto'
- then change the name of the sample -> if you press 'next' (bottom left) you get to the next sample and can change the name here
  When you are finished press 'Save/Exit'
- Then save the entire sequence

- To change the autosampler position of the sample press 'Vial'
- Before starting the measurements, the settings of the flows of the used method should be pre-set -› 'Run' -> show run -> In the 'Method' window -> 'preset flows' (center left
- If you want to change the method of all samples again you should create a new method and reload it under 'basic Run'

  - If you only want to change something for single samples you can click on Method in the list and then change the method for single samples

- Before each measurement the settings of the autosampler should be checked again (e.g., does the used tray match the settings etc.).
- You can cool the autosampler -> useful for long measurements

Troubleshooting:

1. back pressure is too low: can be increased by the length of the red capillary - the longer the higher the pressure (pressure becomes even higher after coupling with detectors, e.g., ICP-MS or OCD)
2. reset the pumps: Press the button at the bottom right and then turn the wheel at the bottom right to the left until it stops, then press the button at the top. You also must reset in the software -› Tools -› Interface -> K hloen Pumps -> Reset
3. strange signs on the pump display: everything must be restarted
   a. Turn off ALL devices at the back of the switch
   b. Close software
   c. Switch on the devices at the back of the switch
   d. Restarting the software
4. pressure is unstable -> check valves of pump heads (tip and focus pump) in ultrasonic bath (approx. 5 min in 2 % EtOH) -> see FFF manual

Preparation of separation channel/filter/loop

Channel (figure 4):

- Unscrew channel from AF4 - › Pump flow all to 0 mL/min
- Loosen screws: Loosen the screws from the outside to the inside
- Tighten screws: Tighten the screws from inside to outside (with a torque wrench)
- Cut the membrane (to size (29.5 x 3.4 cm) -> Cut slightly larger than the frit, but must not protrude over the seal
- Place the membrane in Milli-Q overnight or rinse the instrument for 2-3 hours (at 0.5, 0.5, 0.5)
- Assembly: aluminium top plate with screws - top plate - spacer - membrane (shiny side up) - frit
- bottom plate with sealing ring - metal plate with screw thread
- Rinse everything with Milli-Q and then screw it back together again -› Caution Frit may fall out

-Vent the channel: 1. screw in Out port for detector, 2. screw in Focus Port and purge with Focus pump (press buttons ^ and < simultaneously) until there is no more air in the channel, 3. turn Purge Port, 4. fill the channel with Focus "Purge" until there is no more air in the channel and then turn Tip Port and Cross Flow Port in

Figure 4. Nina Đukanović and Jay Carl Cacerez during preparation of the membrane

Filter (figure 5):

- Open with 23" wrench - Pay attention to the direction of the running medium
- Structure: screw - metal nut with "-›" - membrane - frit – screw
- Both screws should be in the middle of the metal nut (do not tighten one first and then the other, but both at the same time and then tighten)
- To purge the two filters press "Purge" without entering the system (or simply rinse the system 2-3 hours before measurement at 0.5, 0.5, 0.5)
- Filter holders should be cleaned in an ultrasonic bath from time to time -> 2 % EtOH for 5 min

Figure 5. Preparation of the filter

Loop:

- Pay attention to correct loop
- Purge new loop by purging the tip pump -> Flow rate of the pump must be 0 mL/min (or simply run the system for 2-3 hours at 0.5, 0.5, 0.5)
- Test if the loop is vented: Tools -> Interface -> Autosampler -> 'manual washing' until the loop is filled - test if there is still air in the loop by switching 'injection valve' between 'inject and 'load' -> if the pressure drop is > 1 bar then there is still air in the loop

Leakage test:

- Tip, Focus, & Cross: 0.5 ml/L -> run for 10 min -> the purge valve must be closed back pressure should be between 6-9 bar
- Then test method pressures -› Preset flows

Rinse system -> Open purge valve

Once the system is set up, we can put our samples to analysis and then get the results as in Figure 6.

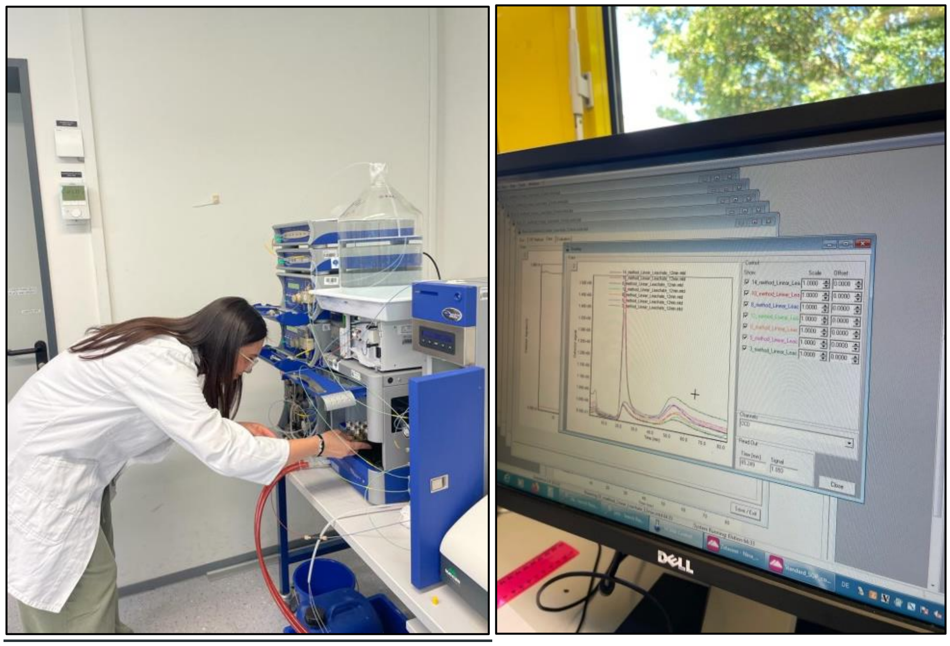


Figure 6. Nina Đukanović starts of the analysis and gained results

FFF-DLS

Dynamic Light Scattering (DLS, figure 7) detectors provides detailed, quantitative size distributions and structural information, sampled over large ensembles, for good statistical robustness. It is based on the Brownian motion of particles - this states that smaller particles move faster, while larger ones move slower in a liquid. The light scattered by particles contains information on the diffusion speed and thus on the size distribution. Dynamic light scattering enables the analysis of particles in a size range from 0.3 nm to 10000 nm.

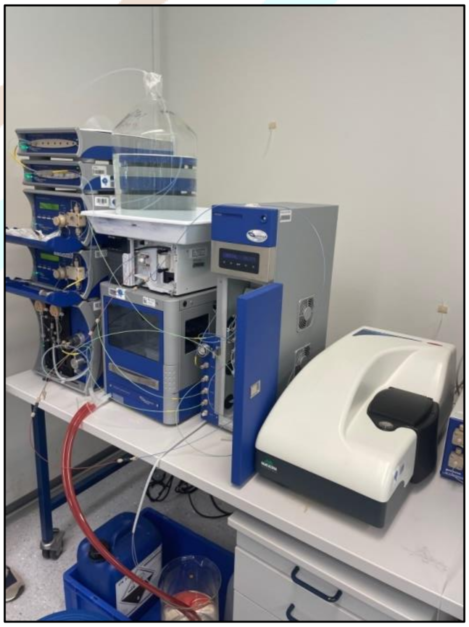


Figure 7. FFF-DLS

Standard Operation Procedure FFF-DLS coupling

- Use the flow cuvettes
    - Connect the flow cuvette with the tube coming from the UV-detector
    - Be careful with the inlet and outlet - inlet right side, outlet left side o 1 tube into waste (the long tube)
    - insert the cuvette into the DLS (arrow on cuvette should be located right hand side on the front) - the cuvette tubing should exit at the side
- start the UV detector
- start the DLS
- Only 1 sample at the time can be measured > label the sample manually within the DLS software, as there is no communication between the DLS and the FFF software
- Prepare FFF and us the method that was also used for normal FFF measurements
    - ➢ only 1 sample in the sequence
- Open Zetasizer software
    - Select -> flow cuvette

      Browse for SOP (use existing one or create new one)
    - Example SOP for small natural colloids:
    - Measurement type: flow
    - Material: clay_smaller_022
    - Temperature: 19 °C
    - Cell: flow cell
    - " Measurement: change time - must be if the time of method
    - Safe SOP
- Browse for SOP -> open SOP
- Measurement -> start online window
- In the FFF software start the run

  When the FFF injection starts start DLS measurement in the Zetasizer software

Water dispersible colloids (WDC) extraction (Figure 8)

- 10g (fresh samples, <2mm) (figure 5) + 20 ml distilled water. Literature: usually soil:water ratio is 1:2 (w/v). Suggestion: flasks of 100 ml;

- Shaking 6h, 150 pm;
- Add 60 ml distilled water. Homogenize gently;
- Sedimentation based on Stoke's Law. The time for sedimentation is calculated based on the particle diameter and the bottle size (water table height into the tube), so it is possible to collect the volume of extract needed (for further analyses) free of particles with a predefined diameter (for example 0.02mm, common in literature).
- After waiting for the sedimentation time. Collect the extract using a pipette (the pipette volume is defined in the previous step. Transfer the collected extract to a centrifuge tube;
- Centrifugation. The centrifugation time and force are calculated based on centrifuge settings/characteristics and particle diameter according to the formula of Hathaway et al. (1956) in Harry et al. (1984). Here for example if the target diameter (to be collected) is 500 nm, the r (radius) in the formula would be equivalent to 250 nm.
- Use an aliquot of the centrifuged sample to test the average particle size obtained. This can be done with a quick DSL test;
- As the sedimentation and centrifugation steps are theoretical, it is possible that the obtained average particle size differs from that initially desired. In this case, batches of fixed centrifugation force and increasing time, with DSL test of each test sample, can be performed to adjust the procedure.

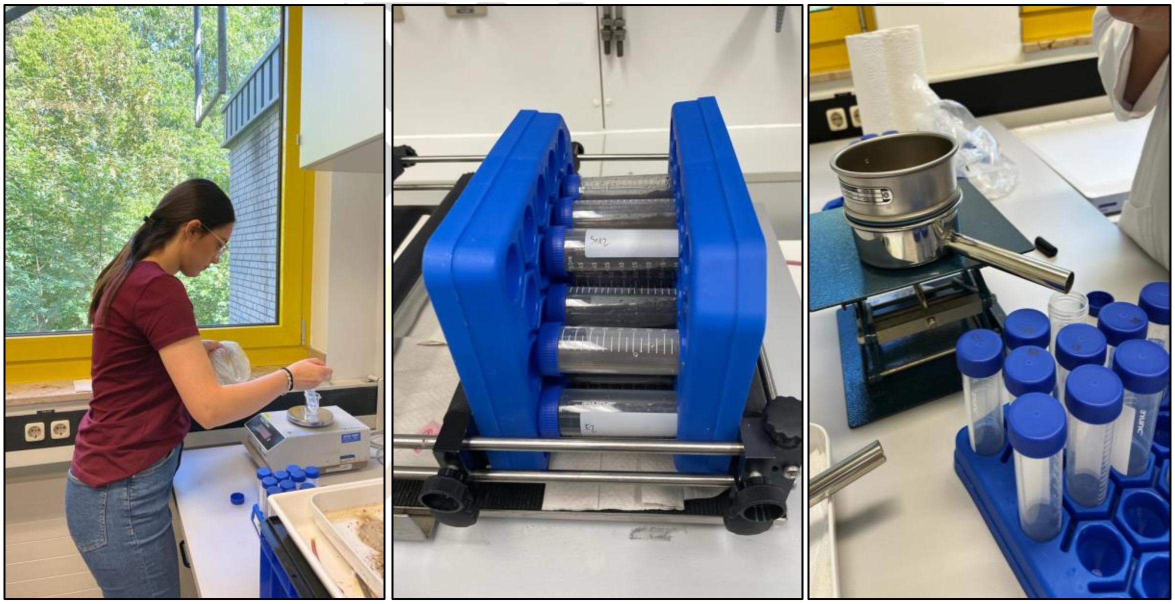


Figure 8. Water dispersible colloids (WDC) extraction

ICP-MS

ICP-MS (inductively coupled plasma-mass-spectrometry, figure 9) is a technique to determine low-concentrations (range: ppb = parts per billion = µg/l) and ultra-low-concentrations of elements (range: ptt = parts per trillion = ng/l). Atomic elements are lead through a plasma source where they become ionized. Then, these ions are sorted on account of their mass. The advantages of the ICP-MS technique above AAS (Atomic Absorption Spectroscopy) or ICP-OES (inductively coupled plasma optical emission spectrometry) are: (i) Extremely low detection limits; (ii) A large linear range and (iii) Possibilities to detect isotope composition of elements.

Figure 9. ICP-MS

Particle Size analyzer LA960, Horiba

The principle of laser diffraction is the relationship that exists between light scattering (its angle and intensity) and particle size (figure 10). The larger the particle, the smaller the angle and the higher the intensity of the scattering. The device does not directly measure the size of the particle but the angle and intensity of light scattering from the particles. This data is entered into an algorithm that uses the Mie scattering theory to yield information about the particle size from the data on light scattering. Size measurement can only be reliable if the data on light scattering is good. The analyzer is therefore designed to acquire the best data from what is available, which is then sent to the algorithm that makes use of it to produce a particle size distribution.

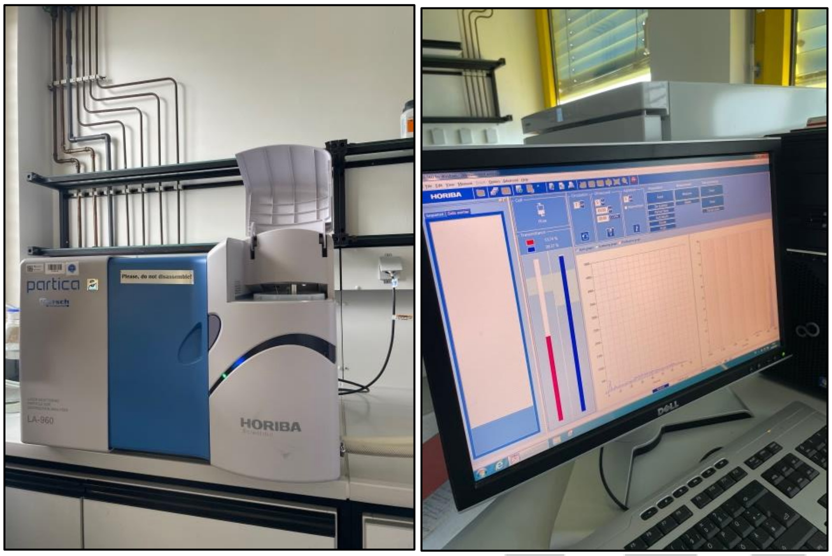


Figure 10. Particle size analyzer LA960, Horiba

FFF-ICP-MS

Field flow fractionation (FFF) with inductively coupled plasma mass spectrometry (ICP-MS) is a powerful technique for identification and quantification of particle bound metals, but has not been applied for quantitative determination of particulate carbon. This technique combines the separation capabilities of FFF with the elemental analysis capabilities of ICP-MS. It allows for the separation and quantification of nanoparticles or colloids based on their size and elemental composition. FFF-ICP-MS is particularly useful for studying nanoparticles in environmental samples, such as water or soil, as well as in various industrial applications. While both FFF-OCD and FFF-ICP-MS are separation techniques, FFF-OCD focuses on size and shape separation with online detection, while FFF-ICP-MS combines size separation with elemental analysis using mass spectrometry.

In case of FFF-ICP-MS (figure 11) the injection volume was 5 mL for water samples with a focusing time of 30 min at 0.2 mL min1 tip flow and for the soil extracts injection volume was 200 mL with focusing time of 12 min at 0.3 mL/min tip flow. The isotopes were monitored in helium collision cell mode (Agilent 7500).

In case of FFF-OCD injection volume was 1 mL with a focus time of 10 min for the water samples using the same flow rates; injection conditions for the soil extracts were the same as for FFF-ICP-MS.

Figure 11. FFF-ICP-MS

IRMS

Isotopes are variants of an element that have the same number of protons but different numbers of neutrons. Isotope Ratio Mass Spectrometry (IRMS, figure 12) provides analysis of isotopic ratios of the light stable isotopes C, N, O, S and H. By analyzing the isotopic composition of a substance, IRMS can provide valuable information about its origin, authenticity, and various biological, geological, and environmental processes. IRMS is a powerful analytical technique that allows scientists to determine the relative abundance of isotopes in a sample.

It involves three main steps: sample preparation, ionization, and mass analysis. During sample preparation, the substance of interest is extracted and purified to remove any impurities that could interfere with the analysis. This step ensures accurate and precise measurements.

Next, the purified sample is introduced into the mass spectrometer, where it is ionized. This process involves converting the atoms or molecules in the sample into charged particles (ions) by bombarding them with high-energy electrons or by using other ionization techniques. Once ionized, the ions are accelerated and separated based on their mass-to-charge ratio using magnetic fields or electric fields within the mass spectrometer. The different isotopes present in the sample will have slightly different masses, allowing them to be distinguished and measured.

The final step is the mass analysis, where the separated ions are detected and their abundance ratios are determined. This information is then used to calculate the isotopic composition of the sample.

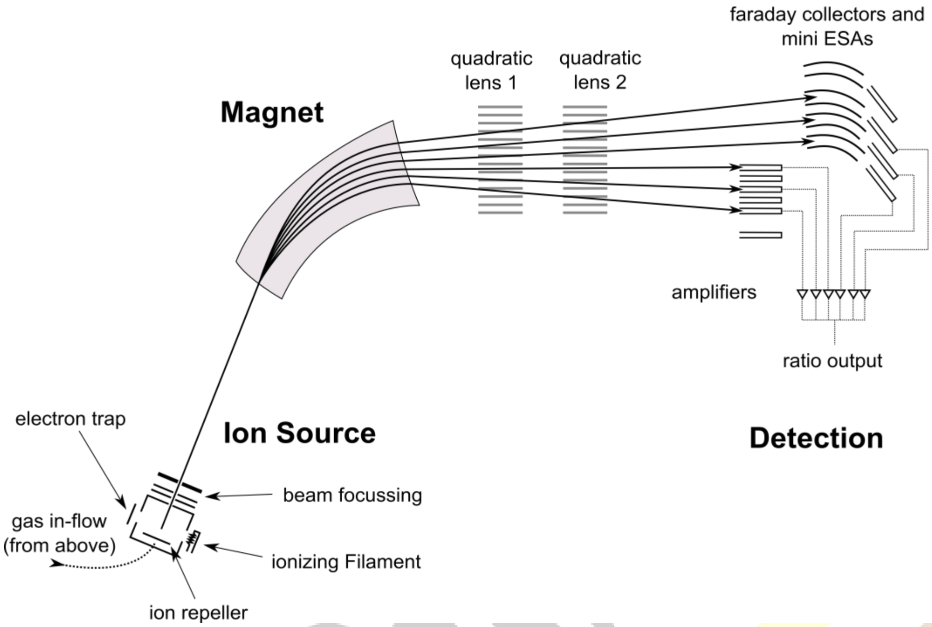


Figure 12. IRMS

MC-ICP-MS

Multicollector Inductively Coupled Plasma Mass Spectrometry (MC-ICP-MS, figure 13) is a highly advanced analytical technique used for precise and accurate isotopic analysis of a wide range of elements. MC-ICP-MS combines two primary components: inductively coupled plasma (ICP) as the ionization source and a mass spectrometer with multiple detectors (multicollector) to measure the isotope ratios of elements.

The sample, typically in liquid form, is introduced into the ICP, which generates a high-temperature, ionized plasma. In the ICP, the sample is vaporized and ionized, forming positively charged ions. The positively charged ions generated in the plasma are then passed into the mass spectrometer. Inside the mass spectrometer, these ions are separated based on their mass-to-charge ratio (m/z). Unlike conventional ICP-MS, which typically uses a single detector, MC-ICP-MS utilizes multiple detectors. These detectors are capable of measuring the different isotopes of an element simultaneously. This allows for precise measurement of isotope ratios, making it especially useful in geochronology, geochemistry, and environmental science.

The primary advantage of the MC-ICPMS is its ability to analyze a broader range of elements, including those with high ionization potential that are difficult to analyze by TIMS. The ICP source also allows flexibility in how samples are introduced to the mass spectrometer and allows the analysis of samples introduced either as an aspirated solution or as an aerosol produced by laser ablation.

Fundamental Principles of Multicollector-Inductively Coupled Plasma Mass Spectrometer (MC-ICPMS). As a hybrid mass spectrometer, MC-ICPMS combines an inductively coupled plasma (ICP) plasma source, an energy filter, a magnetic sector analyzer, and multiple collectors for the measurement of ions. The ions are produced by introducing the sample into an inductively coupled plasma which strips off electrons thereby creating positively charged ions. These ions are accelerated across an electrical potential gradient (up to 10 KV) and focused into a beam via a series of slits and electrostatically charged plates. This ion beam then passes through an energy filter, which results in a consistent energy spectrum in the ion beam and then through a magnetic field where the ions are separated based on their mass to charge ratio. These mass-resolved beams are then directed into collectors where the ions reaching the collectors are converted into voltage. Isotope ratios are calculated by comparing voltages from the different collectors.

The electronics of these instruments must operate to very close tolerances to produce isotope ratios that are precise to 0.01-0.001%. In addition, a high vacuum needs to be maintained along the path of the ion beam to avoid scattering of the ions due to interaction with air molecules.

Data Collection, Results and Presentation. Measured isotope ratios must be properly corrected for all instrumental biases, including mass fractionation. Once corrected, these ratios are suitable for plotting in any diagrams requiring atomic ratios (e.g., isochron, concordia, etc.).

Figure 13. MS-ICP-MS

Fluorescence microscopy

For the evaluation of biological material, fluorescence microscopy (figure 14), which enables the selective and precise detection of molecules at low concentrations, has become an essential microscopy technique. In fluorescence microscopy, specific molecules or structures of interest are labeled with fluorescent dyes or fluorophores. These dyes can bind to specific targets and emit fluorescent light when excited by a particular wavelength of light. A fluorescence microscope uses a light source to generate a narrow and specific wavelength of light (excitation light). This excitation light is typically of a higher energy (shorter wavelength) than the emitted fluorescence. An excitation filter is placed in the optical path to allow only the desired excitation wavelength to pass through and reach the specimen. This light excites the fluorophores attached to the target molecules. When the fluorophores are excited, they absorb the energy from the excitation light and then re-emit this energy as fluorescent light at a longer wavelength. The emitted light is typically of a different color than the excitation light and can be specific to the type of fluorophore used. An emission filter is placed in the optical path to block the excitation light and only allow the emitted fluorescence to pass through to the detector.

The detector converts the emitted light into an electronic signal, which is then used to generate an image of the specimen. Fluorescence microscopy produces images that show the distribution and intensity of the labeled molecules throughout the object, giving precise details on their localization and concentration. To learn a lot about molecular localization, a correlative research combining fluorescence and electron microscopy is typically used. The scanning electron microscope's (SEM) technology has evolved significantly in recent years, and its image resolution is now comparable to that of the transmission electron microscope (TEM).

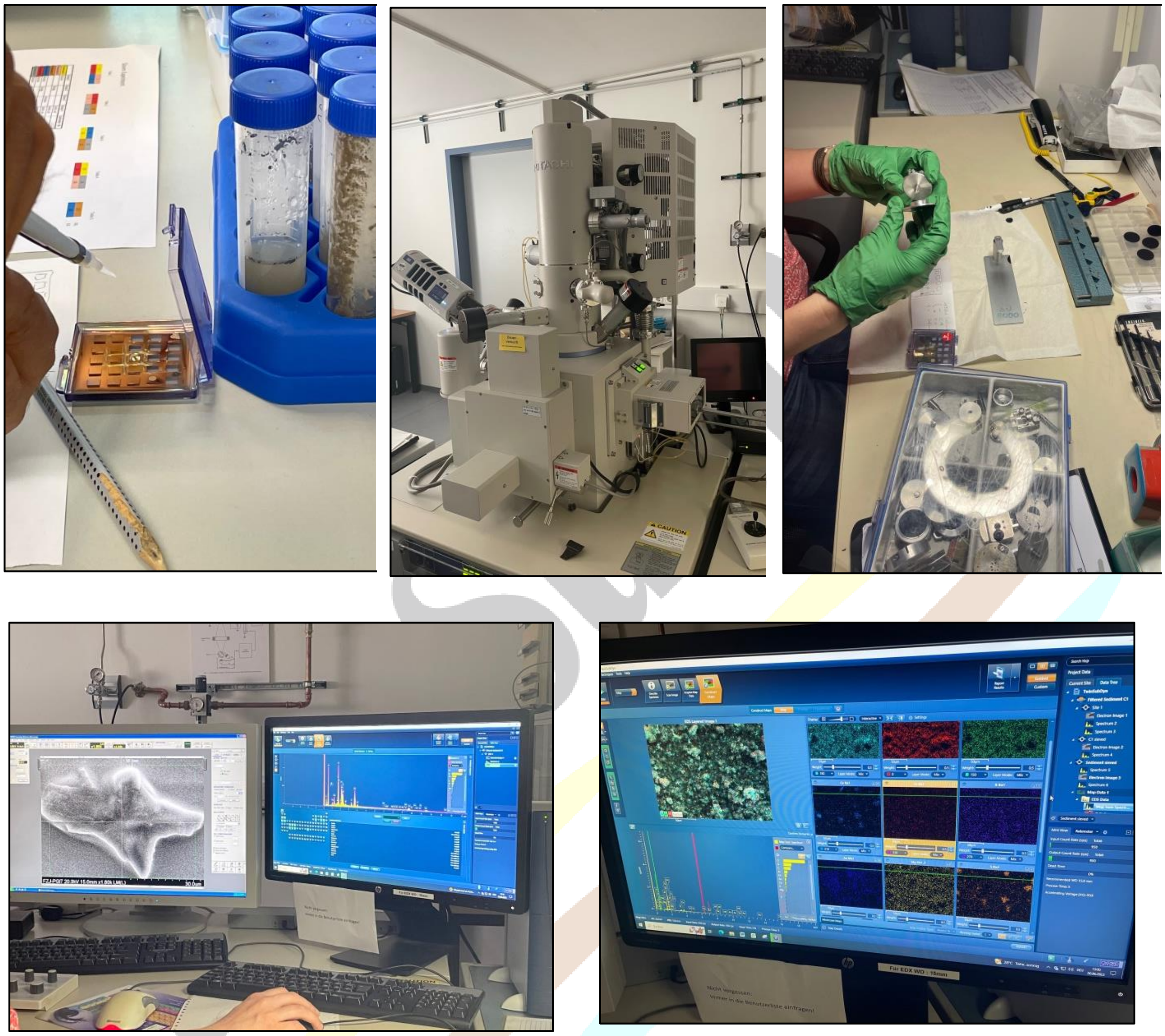


Figure 14. Jelena Beljin and Nina Đukanovic during microscopic analysis

RESULTS

The carbon composition of non-discharge normalized leachates, as revealed by FFF measurements using an OCD detector, shows that the majority of carbon is released in the form of truly dissolved carbon in all treatments (table 1). The treatment with 1% Biochar + Sandy soil had the smallest losses of carbon, while treatments with compost had the highest losses. This is likely due to differences in application rates.

Furthermore, there were noticeable differences among treatments in the size distribution of particulate leached carbon. The data suggest that in the initial stages of the experiment compost seam to be a significant source of carbon-containing particles of small size was observed (up to 70% in fraction 1). In contrast, larger particles (up to 70% in fraction 3) were leached from the Biochar treatment.

Graph averaged fractorgams of leachates and are given in figure 15. In figure 16 are given size resolved leachates OCD RAW, while table 2 has data about chemical composition biochar.

Table 1. Results from FFF measurements using an OCD detector

Lysimeter Number	Treatment	Total carbon	Truly dissolved carbon	Proportion of truly dissolved on total carbon	Total particulate carbon	Proportion of truly dissolved on total carbon	Size faction					
							Fraktion 1		Fraktion 2		Fraktion 3	
		mg/L	*mg/L*	*%*	*mg/L*	*%*	*mg/L*	*%*	*mg/L*	*%*	*mg/L*	*%*
1	**1% Biochar + Sandy Soil**	6.58	6.1	92.8	0.5	7.23	0.104	22	0.04	8.6	0.33	70
11	**1% Biochar + Sandy Soil**	5.57	5.2	93.6	0.4	6.36	0.069	19	0.02	6.3	0.26	74
13	**1% Biochar + Sandy Soil**	7.48	7.0	93.8	0.5	6.24	0.127	27	0.03	6.4	0.31	66
6	**5% Compost + Sandy Soil**	35.45	32.9	92.9	2.5	7.10	1.706	68	0.09	3.6	0.72	29
12	**5% Compost + Sandy Soil**	34.36	31.8	92.6	2.5	7.35	1.872	74	0.12	4.7	0.53	21
17	**5% Compost + Sandy Soil**	36.30	33.3	91.8	3.0	8.19	2.041	69	0.15	5.2	0.78	26
2	**20% Sediment + Sandy Soil**	23.76	22.9	96.6	0.8	3.40	0.205	25	0.07	8.9	0.53	66
7	**20% Sediment + Sandy Soil**	24.22	23.4	96.7	0.8	3.35	0.235	29	0.08	9.6	0.50	61
16	**20% Sediment + Sandy Soil**	24.30	23.4	96.1	0.9	3.88	0.240	25	0.08	8.5	0.62	66
3	**1% Biochar + 5% Compost + Sandy soil**	39.18	36.4	92.8	2.8	7.19	1.464	52	0.10	3.5	1.25	45
5	**1% Biochar + 5% Compost + Sandy soil**	38.23	35.2	92.1	3.0	7.86	1.871	62	0.13	4.4	1.00	33
15	**1% Biochar + 5% Compost + Sandy soil**	38.60	35.5	91.9	3.1	8.12	2.008	64	0.17	5.3	0.96	31
4	**1% Biochar + 20% Sediment + Sandy soil**	27.50	26.7	97.0	0.8	2.99	0.227	28	0.08	9.6	0.52	63
9	**1% Biochar + 20% Sediment + Sandy soil**	26.70	25.8	96.6	0.9	3.40	0.249	27	0.10	11.3	0.56	61
18	**1% Biochar + 20% Sediment + Sandy soil**	26.09	25.4	97.2	0.7	2.83	0.227	31	0.11	14.3	0.41	55
8	**1% Biochar + 5% Compost + 20% Sediment + Sandy soil**	33.23	31.5	94.9	1.7	5.10	0.522	31	0.22	12.9	0.95	56
10	**1% Biochar + 5% Compost + 20% Sediment + Sandy soil**	32.80	31.8	97.0	1.0	3.00	0.349	36	0.06	6.1	0.57	58
14	**1% Biochar + 5% Compost + 20% Sediment + Sandy soil**	32.06	31.1	97.0	1.0	3.04	0.371	38	0.09	9.5	0.51	52

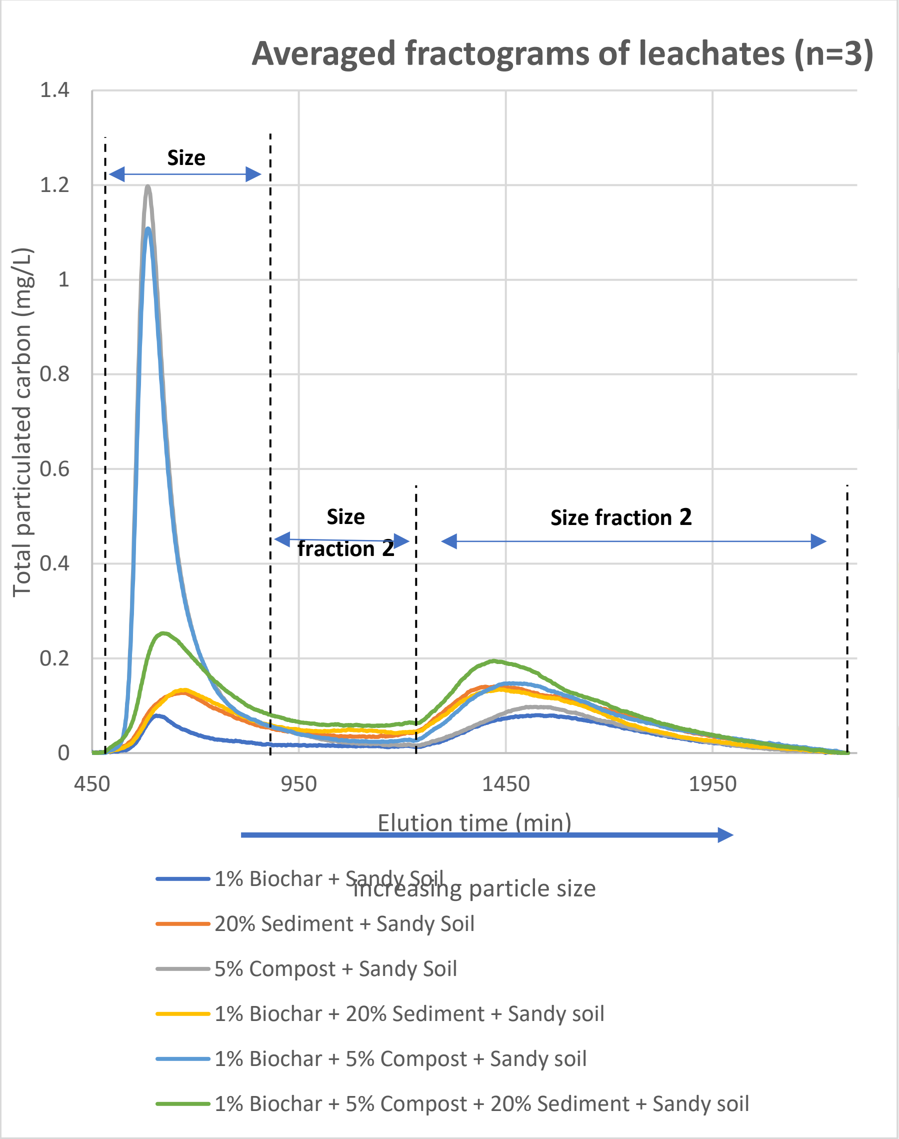


Figure 15. Graph averaged fractorgams of leachates

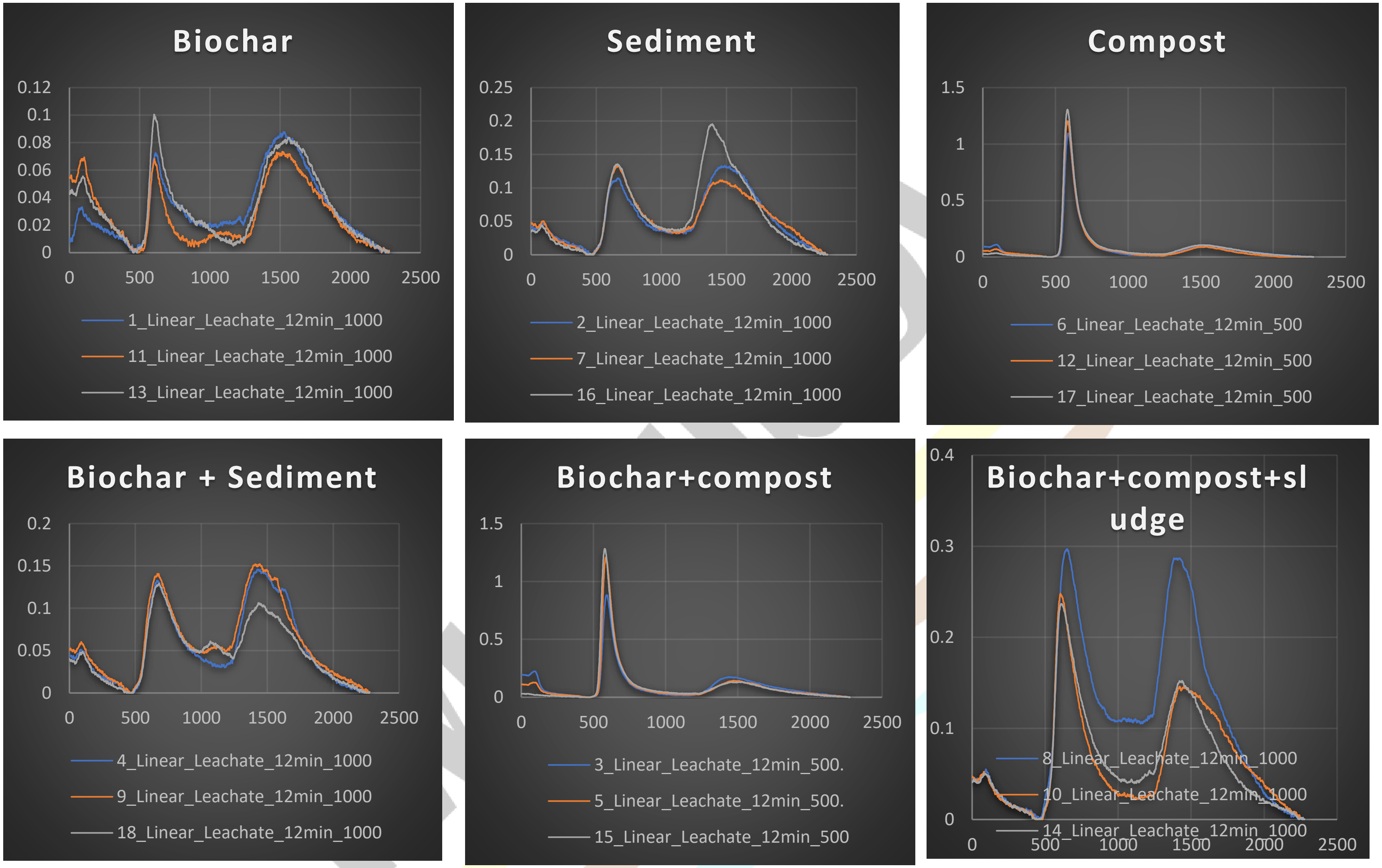


Figure 16. Size resolved leachates OCD RAW

Table 2. Total elemental concentration biochar (Miscanthus)

Mean			Mean		
C	(g/kg)	772,5±12500	Cu	(mg/kg)	4,6±0,5
Si	(g/kg)	23,7±2873	B	(mg/kg)	4,2±0,5
H	(g/kg)	25,5±1500	Cr	(mg/kg)	2,3±0,4
K	(g/kg)	15,1±990	Ni	(mg/kg)	1,3±0,3
P	(mg/kg)	3014,7±323	Pb	(mg/kg)	1±0,2
Ca	(mg/kg)	2391,7±83	Co	(mg/kg)	0,5±0,1
N	(mg/kg)	2000±0	Mo	(mg/kg)	0,3±0
Mg	(mg/kg)	1033,3±24	V	(mg/kg)	0,2±0,1
Mn	(mg/kg)	295,9±15	Cd	(mg/kg)	0±0
Fe	(mg/kg)	228,7±117	Se	(mg/kg)	<0,2
Al	(mg/kg)	214,7±97	As	(mg/kg)	<0,1
Na	(mg/kg)	184,3±34	Hg	(mg/kg)	<0,1
Zn	(mg/kg)	70,7±29	Tl	(mg/kg)	<0,1
Ba	(mg/kg)	14,5±0,3			

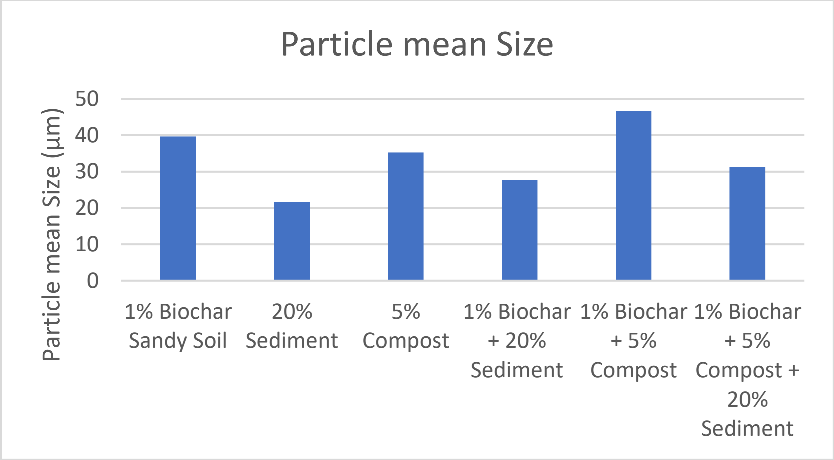
Results obtained from particle analyzer measurements to characterize the size distribution of the raw materials employed in the experiment and the mixtures of various treatments before the start of the experiment. Pure biochar could not be analyzed due to a high risk of clogging in the particle analyzer. There is variation in both median and mean particle sizes among the different treatments. Treatments with 1% Biochar tend to have larger median and mean sizes compared to other treatments.The addition of 5% Compost to Sandy Soil generally results in larger particle sizes compared to Sandy Soil alone, with increased median and mean sizes and higher D10, D50, and D90 values. When Biochar and Compost are

combined with Sandy Soil and Sediment, the particle size trends are influenced by the individual characteristics of Biochar and Compost. For example, the combination of 1% Biochar and 5%

Compost results in larger particle sizes compared to Sandy Soil alone, as seen in the increased median and mean sizes and higher D10, D50, and D90 values (Table 3, figure 17).

Table 3. Particle analyzer

Treatment sandy soil with	**Median Size**	**Mean size**	**D10**	**D50**	**D90**
	(µm)				
1% Biochar + Sandy Soil	10.5	21.6	3.4	10.5	39.7
20% Sediment + Sandy Soil	9.9	12.7	3.5	9.9	21.6
5% Compost + Sandy Soil	11.8	20.7	4.3	11.8	35.3
1% Biochar + 20% Sediment + Sandy Soil	11.6	14.9	3.8	11.6	27.7
1% Biochar + 5% Compost + Sandy Soil	13.3	26.9	5.5	13.3	46.7
1% Biochar + 5% Compost + 20% Sediment + Sandy Soil	13.3	20.2	4.8	13.3	31.3
Raw Compost	11.9	16.7	4.0	11.9	31.3
Raw Sand soil	9.9	14.8	3.2	9.9	28.7
Raw Sediment	9.8	12.0	3.8	9.8	19.3
	Standard deviation				
Treatment	Median Size	Mean size	D10	D50	D90
1% Biochar + Sandy Soil	**2.24**	**2.90**	**0.38**	**2.24**	**8.56**
20% Sediment + Sandy Soil	**0.60**	**2.29**	**0.02**	**0.60**	**2.12**
5% Compost + Sandy Soil	**0.49**	**5.75**	**0.28**	**0.49**	**5.78**
1% Biochar + 20% Sediment + Sandy Soil	**1.65**	**2.83**	**0.26**	**1.65**	**5.40**
1% Biochar + 5% Compost + Sandy Soil	**0.25**	**10.10**	**0.15**	**0.25**	**10.02**
1% Biochar + 5% Compost + 20% Sediment + Sandy Soil	**1.38**	**7.11**	**0.25**	**1.38**	**5.65**
Raw Compost	**0.27**	**2.07**	**0.04**	**0.27**	**1.55**
Raw Sand soil	**1.49**	**5.17**	**0.30**	**1.49**	**9.94**
Raw Sediment	**0.46**	**1.66**	**0.28**	**0.46**	**0.20**



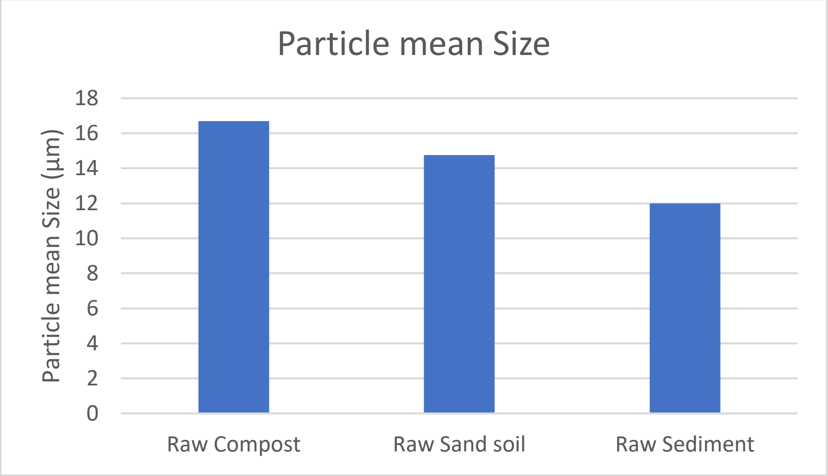


Figure 17. Particle analyzer

II part: Medium scale lysimeter experiment with heavy metal contaminated soil

Preparation for the experiment

The sampling of the soil contaminated with heavy metal was the beginning step of the experiment. The samples were collected from the immediate vicinity of the town Goslar, a historic town in Lower Saxony, Germany. About 200 kg of soil contaminated with heavy metals, especially zinc and cadmium, was collected, and the contamination originates from the smelter that was previously located on the given site. The samples were collected from the first 20 cm of the surface (figure 18).

Figure 18. Sampling (Nina Đukanović and Jens Kruse)

After the samples were brought and stored in the FZJ, they were dried in the open air and mechanically ground. In the meantime, we were preparing tables, lysimeters and other equipment (figure 19).

Figure 19. Nina Đukanović prepare samples and equipment

A small amount of soil samples was taken and subjected to analysis. In addition to the basic chemical parameters, the samples were analyzed for the content of heavy metals, as well as the content of bioavailable heavy metals. The results are shown in table 4.

Table 4. Chemical parameters and heavy metal content in soil.

Parameter	Unit	Value
pH value in CaCl2		5,8
Cu (total)	mg/kg	178
Zn (total)	mg/kg	4381
As (total)	mg/kg	56
Pb (total)	mg/kg	2144
Cd (total)	mg/kg	90,3
Ni (total)	mg/kg	23
Cr (total)	mg/kg	26
Hg (total)	mg/kg	19,6
Cu (available)	mg/kg	54
Zn (available)	mg/kg	1815
Mn (available)	mg/kg	12
B (available)	mg/kg	0,48
N (total)	%	0,185
N (soluble)	mg/100g	0,7

The plan of the experiment was to mix the polluted soil with two organic amendments, biochar and manure, and to set up control without organic amendments. All three treatments were set in 6 repetitions, with the fact that in 3 of each, the energy plant rapeseed was sown (figure 20).

Biochar was transported to FZJ from Novi Sad, and in table 5 are shown total elemental concentrations in biochar.

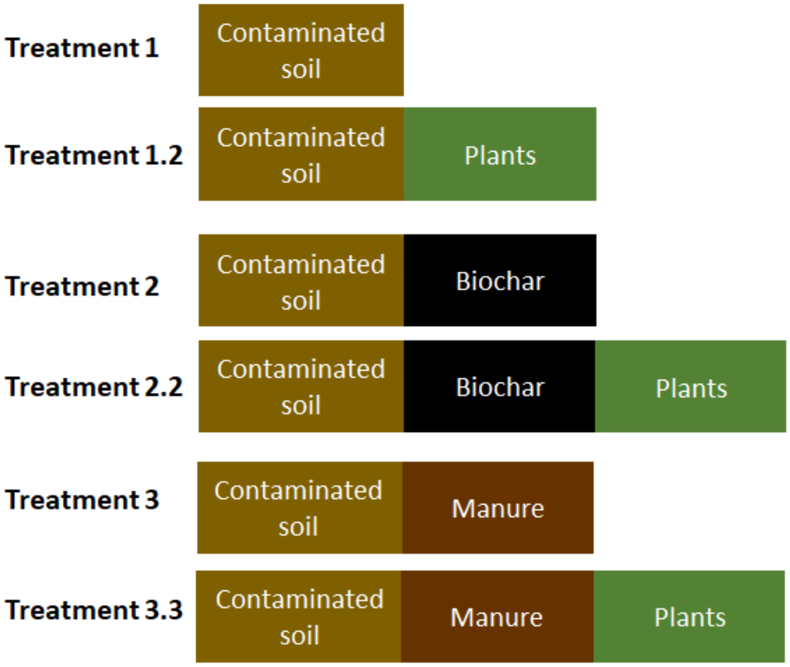


Figure 20. Experiment set up

Table 5. Total elemental concentrations in biochar.

Parameter	Unit	Value	Parameter	Unit	Value
C	g/kg	772,5±13	Cd	mg/kg	0±0
Si	g/kg	23,7±3	Se	mg/kg	<0,2
H	g/kg	25,5±2	As	mg/kg	<0,1
K	g/kg	15,1±1	Hg	mg/kg	<0,1
P	mg/kg	3014,7±323	Tl	mg/kg	<0,1
Ca	mg/kg	2391,7±83	Cu	mg/kg	4,6±0,5
N	mg/kg	2000±0	B	mg/kg	4,2±0,5
Mg	mg/kg	1033,3±24	Cr	mg/kg	2,3±0,4
Mn	mg/kg	295,9±15	Ni	mg/kg	1,3±0,3
Fe	mg/kg	228,7±117	Pb	mg/kg	1±0,2
Al	mg/kg	214,7±97	Co	mg/kg	0,5±0,1
Na	mg/kg	184,3±34	Mo	mg/kg	0,3±0
Zn	mg/kg	70,7±29	V	mg/kg	0,2±0,1
Ba	mg/kg	14,5±0,3			

Manure was collected in the bulls farm in Dorsten, North Rhine-Westphalia, Germany (figure 21). The manure was subjected to the analysis and the results are shown in table 6.

Figure 21. Manure collection (Nina Đukanović and Jens Kruse)

Table 6. Chemical parameters and heavy metal content in manure.

Parameter	Unit	Value (in fresh substance)	Value (in dry matter)
pH		7,4	
dry matter content	%	8,8	
Organic matter	%	6,96	79,16
Total N	%	0,428	4,87
NH4-N	%	0,21	
P2O5 (total)	%	0,2	2,24
K2O (total)	%	0,4	
MgO (total)	%	0,145	
CaO (total)	%	0,21	
Cu (total)	%	0,00073	<0,010
Na (total)	%	0,086	
S (total)	%	0,051	0,58
Zn (total)	%	0,00333	<0,050
Pb (total)	mg/kg	< 0,440	<5,00
Cd (total)	mg/kg	< 0,0352	<0,400
Cr (total)	mg/kg	< 0,440	<5,00
Ni (total)	mg/kg	< 0,440	<5,00
C/N ratio		9	
As (total)	mg/kg	< 0,088	<1,00
B (total)	%	0,000132	0,0015

Lysimeter experiment set up

The lysimeter experiment was performed in a glasshouse, under controlled conditions (figure 22). The surface of the lysimeter is 288 cm^2, and the height is 35 cm. We filled the lysimeters by placing a filter on the bottom, and then filled 1 cm of sand and 1 cm of gravel. Calculating based on the area of the lysimeters and the height to which we wanted to fill them (2 cm to leave an empty space at the top), as well as the target density in the column, we arrived at a soil mass of 11,1 kg which fits into the lysimeter.

We filled 6 lysimeters designated as controls with 11,1 kg of soil.

6 lysimeters with added biochar, we filled by mixing 11,1 kg of soil with 1% biochar (about 0,111 g), which corresponds to the application of 38.8 t/ha.

Lysimeters filled with a mixture of contaminated soil and manure required somewhat more complex calculations. The goal was that the amount of manure corresponds to 40 m^3/ha, and based on that data and the surface of the lysimeter, it was calculated that 115,3 ml of manure should be added per lysimeter. Since the pH value of the fresh manure was 7,4, we decided to lower the pH value to increase metal mobility. We added 50% water to the mentioned quantity of manure, in order to adjust the pH more easily and dose the manure in a more liquid form. Before adjusting the pH, we added N^{15}, in organic and inorganic form, as urea and NH_4-Cl in a 1:1 ratio, to the manure. Under laboratory conditions, we added different volumes of cc H_2SO_4, until we reached a suitable pH value of about 4,5. Based on that experiment, we determined the volume of acid that we should add to the mixture of manure, water, NH_4-Cl and urea, in order to achieve the desired pH value. We then filled each lysimeter by mixing 11,1 kg of soil with 175 ml of labeling manure.

Before we planted the plants, we added macro and micro nutrients to the lysimeters. We added micronutrients (B, Cu, Mn, Mo) to all lysimeters, and macronutrients (P, N, S, K, Mg) to control and biochar lysimeters. The reason for this was that we wanted to have the same starting conditions in all treatments, so we calculated the amount of micronutrients we would add to the control and biochar based on the amount of nutrients we had in the manure lysimeters. The volume of micronutrients dissolved in the water added to the control and biochar lysimeter is about 153 ml, which corresponds to the fertilizer volume of 175 ml, given that the proportion of dry matter in the fertilizer is only 8%. With this, we achieved that at the beginning we have approximately the same water content in all lysimeters.

Figure 22. Lysimeter experiment set up

Before we sowed the rapeseed, we left the seeds overnight in water and then on wet paper to germinate (figure 23). After 7 days, we transplanted 4 small plants into 9 lysimeters (3 control, 3 biochar and 3 manure lysimeters).

Figure 23. Rapeseed germination process

We installed an irrigation system and added about 100-150 ml of water every day. During the next two weeks, the plants did not make much progress, nor did we collect water in the bottles placed under the lysimeters. After two weeks, we noticed spots on the leaves of the plants, which began to dry and soon died. When we took the plants out of the lysimeters, we saw that their roots were stunted and underdeveloped (figure 24).

Figure 24. Appearance of the plant after 2 weeks

We assumed that the plants probably died due to the poor hydraulic conductivity of the soil and the high sensitivity of rapeseeds to high concentrations of Zn (Zn-toxicity).

We decided to repeat the experiment with a plant species that is more resistant to elevated zinc concentration, and oats were chosen for this purpose. In order to avoid the poor hydraulic conductivity that we observed in the first experiment setup, since we did not collect leachates in glass bottles under the lysimeters for 2 weeks, we decided to mix the lysimeter mixtures with 50% sand. This required that we carefully remove the mixture from each lysimeter, so as not to disturb the layer of sand and gravel at the bottom. We mixed the contents of each lysimeter with 5,55 kg of sand. After that, we filled the lysimeters again with about 11,1 kg of new mixture. However, in the treatments with biochar, we made dilutions with additional sand, so we no longer had 1% biochar but about 0,5%. Based on all the data, we calculated that by mixing soil with sand, we achieved a reduction of 41%, and based on that, we calculated that we should add another 33 g of biochar to the mixture with biochar, in order to have about 1% biochar again.

We had the same problem due to the reduction of soil mixing with sand in treatments with manure, as well as with N^{15} added to the manure. We prepared the manure again, according to the same recipe as the previous time (with water, cc H_2SO_4, urea and NH_4-Cl), only this time we added a smaller volume (75 ml per lysimeter), in order to compensate for the 41% lost by diluting with sand. We also added macro and micro nutrients again, 41% of the amount added last time.

After that, we sowed the winter oat variety, 15 seeds per lysimeter, to a depth of 3 cm. After 5-7 days, the plants sprouted (figure 25), and after 17 days, the first leachate sampling was carried out.

Figure 25. Oat seeds and plants

After taking the leachate samples, we treated the plants with KNO_3 equivalent to 30 kg N/ha, because we noticed that the leaves were turning yellow. 7 days later, the yellow color became even more intense, so we also applied iron, because yellow leaves can also indicate a lack of iron.

The situation with the color and behavior of the leaves was the same after iron (figure 26) and we decided to harvest before the plants die.

Figure 26. Oat plants before harvest

After watering, we harvested with a spoon and a small spatula and recorded the number of plants from each lysimeter. We cleaned the roots from the soil, separated the roots and shoots and left the plants in the laboratory to dry (figure 27). When the plants are dried, we will grind and homogenize them and make an analysis.

Figure 26. Oat plants after harvest

After harvesting, we continue watering in lysimeters because we want to have more leachate samples. We will decide at what point it is time to stop the experiment.

In a meantime, we analyzed heavy metal content and carbon content in a leachate samples from Novi Sad (the scheme of the experiment is in figure 27). We also analyzed isotope ratio in a lysimeter mixtures from Novi Sad which contains biochar (because of C_{13}) – treatment A, D, E and F.

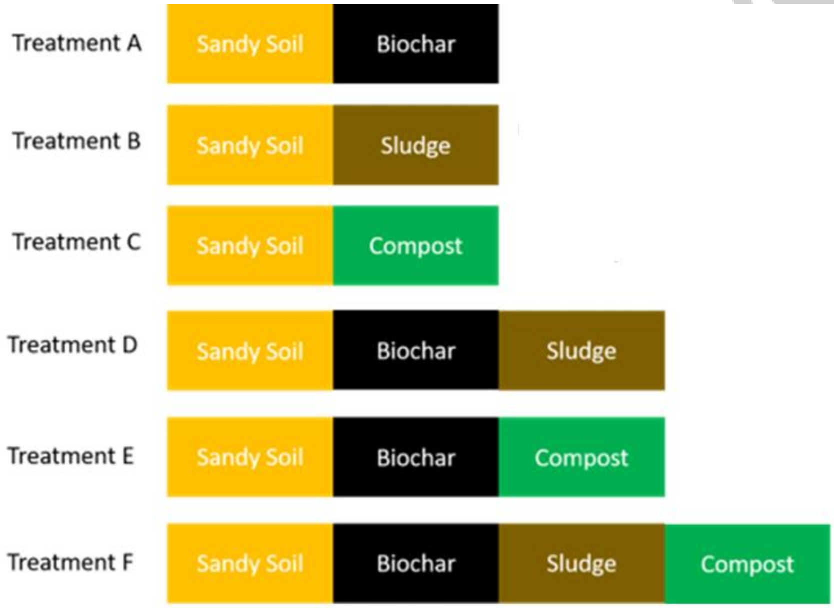


Figure 27. Scheme of the experiment in Novi Sad

We had 6 sampling date (6 set of samples), from April to September, and our task was to analyze heavy metal content on FFF-ICP-MS and carbon content on FFF-OCD. When the data processing is complete, the result of the FFF analysis will be shown.

C_{13} and N_{15} content was analyzed on an Elemental analyzer (Figure 28), but solid samples were prepared by wet sieving, freeze drying and homogenization (Figure 29).

Figure 28. Elemental analyzer

Wet sieving was done for approximately 100 g of sample, through sieves of 2 mm, 250 µm and 20 µm. 4 fractions per sample were obtained (>2 mm, >250 µm, 250-20 µm and <20 µm) and each was transferred to a small glass bottle. The samples were frozen in a freezer and then placed in a freeze dryer. After about 10 days, all samples were dry and ready for homogenization and measurement. The mass of samples (each fraction) after freeze drying were recorded too.

Figure 29. Sample preparation for isotope measurement

To determine carbon content, samples are weighed into silver capsules, then acidified with cc HCl and the opened capsules are left in a desiccator overnight to release inorganic carbon. After that, they are packed in larger capsules, the capsules are closed and arranged in the auto-sampler of the analyzer. To determine the nitrogen content, the samples are weighed into tin capsules, the capsules are immediately sealed and distributed into the analyzer's auto-sampler. Determination is made on the basis of calibration (standards are prepared in the same way as samples) (Figure 30).

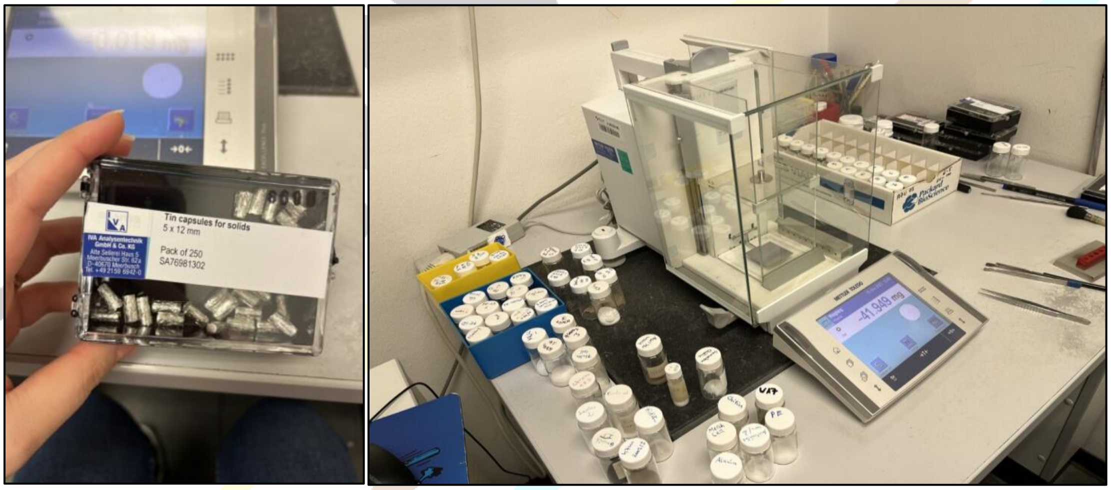


Figure 30. Weighing samples for nitrogen and carbon analysis

The results of the analysis are given in Table 7. In addition to samples from Novi Sad, we also analyzed fresh and labeled manure as well as biochar.

Table 7. Carbon and nitrogen content and ratio.

SAMPLE	C (%)	N (%)	C/N
Treatment A (250-20 µm)	0,1	0,01	13
Treatment F (250-20 µm)	0,3	0,02	13
Treatment E (250-20 µm)	1,5	0,10	16
Treatment D (250-20 µm)	0,4	0,04	11
Treatment A (>250 µm)	0,3	0,01	36
Treatment D (>250 µm)	0,6	0,04	16
Labeled manure	30,2	3,58	8
Treatment F (>250 µm)	1,5	0,09	16
Treatment E (>250 µm)	0,7	0,05	16
Treatment E (>2mm)	42,4	0,94	45
Biochar	72,4	0,23	318
Raw manure	37,5	2,12	18
Treatment D (>2mm)	37,8	0,30	125
Treatment A (>2mm)	61,4	0,29	211
Treatment F (>2mm)	15,6	0,45	35

MOBILITY REPORT

Researcher: Dr. Jelena Beljin, UNSPMF

Assigned supervisor: Prof. Dr. Roland Bol and Dr Jens Kruse, FZJ

Duration of the visit: 05.06.2023. - 23.06.2023.

Executive Summary

The main purpose of the visit to Forschungszentrum Jülich was to acquire new knowledge and skills related to soil chemistry and sophisticated techniques utilized for soil characterization and soil organic matter dynamics and elemental cycles. The training was specifically focused on developing methods to characterize biogeochemical processes and nutrient cycling in different soil layers using state-of-the-art physical-chemical and analytical methods. These methods included the utilization of advanced instruments such as the isotope-ratio mass spectrometer (IRMS), Inductively coupled plasma mass spectrometry (ICP-MS), and Multicollector-Inductively Coupled Plasma Mass Spectrometer (MC-ICP-MS), as well as techniques like field flow fractionation (AF4, SPLITT), microscopy (SEM/TEM, fluorescence), and scattering (light, X-ray).

Introduction

Background

The current research in natural and managed systems is driven by both “Soil organic matter dynamics and elemental cycles” personal and group objectives. These objectives encompass several key areas: (i) Firstly, the aim is to gather valuable information pertaining to the origin, residence time, cycling, and priming of organic matter and essential elements (such as nitrogen, phosphorus, potassium, calcium, silicon, iron, etc.) within the soil-plant system and their subsequent movement into surface and groundwater. (ii) Secondly, there is a focus on enhancing the understanding of the intricate carbon and nutrient biogeochemical processes and interactions occurring at both field and catchment scales. This improved understanding contributes to a broader comprehension of ecosystem functioning.

(iii) Thirdly, the research involves employing innovative and reliable high-resolution tools and technologies to examine the diverse and multi-dimensional impacts of natural factors and human-induced environmental perturbations on biogeochemical cycles. This investigation sheds light on the complexities of ecosystem responses to changing environmental conditions. (iv) Lastly, the research entails the collection of field and laboratory-based scientific data, with the overarching goal of providing sustainable management options for natural and managed systems. This is achieved by integrating the findings within a comprehensive systems biogeochemistry framework. A significant aspect of the research is the utilization of isotope signatures as tracers for carbon, other elements, and sediments in the environment. This involves employing approaches such as IRMS, ICP-MS, and MC-ICP-MS, as well as techniques like field flow fractionation (AF4, SPLITT), microscopy (SEM/TEM, fluorescence), and scattering (light, X-ray).

Scope of the secondment

The scope of the secondment was knowledge transfer on techniques for soil characterizations, organic matter dynamics and nutrient cycle. This included laboratory training in soil sample preparation and analysis. The detailed methodology for the preparation and determination of colloids in soil and the results obtained throughout the course will be given in the following chapters.

Content

Standard Operation Procedure FFF-UV-OCD (figure 1)

General things to prepare

- Prepare fresh FFF eluent (25 |M NaCl; 1.461 g NaCl/L) and if necessary, OCD solution (K2S208 2.5 g/5 L; concentrated H3PO4: 20 mL/5 L; do not change/refill during measurement) using MiliQ water
- Clean channel and use new membrane
  Change all filters (check eluent flow direction)
- Correct 'Loop' for your method installed?
- Reboot the computer before a new measuring campaign (help to prevent software bugs)

Detectors

UV

- Switch on lamp at least 30 min before measurement o
- func. -> enter -> func. -> func. -> lamp: 1

OCD

- Switch on about 3-4 h before measurement -> baseline should be stable
- Make sure that there is enough solution for acidification of the OCD (do not refill during measurement)
- Connect to FFF only shortly before measurement
- Please always disconnect FFF from OCD after measurement and insert plug

Figure 1. FFF-UV-OCD

<u>Software</u>

New sequence

Open 'Basic Run' -> Load 'Method' that was previously created

- Determine number of samples -> Set 'End Position' to the number of samples
- Then press 'Create

(For single samples if you just want to use an existing sequence as a basis):

- File -> new -> sequence
- In the 'Sequence' window -> click on empty 'run' field - 'Open run' -> load run file
- Press 'Show run' -> Check 'Auto'
- then change the name of the sample -> if you press 'next' (bottom left) you get to the next sample and can change the name here
  When you are finished press 'Save/Exit'
- Then save the entire sequence

- To change the autosampler position of the sample press 'Vial'
- Before starting the measurements, the settings of the flows of the used method should be pre-set -› 'Run' -> show run -> In the 'Method' window -> 'preset flows' (center left
- If you want to change the method of all samples again you should create a new method and reload it under 'basic Run'
- If you only want to change something for single samples you can click on Method in the list and then change the method for single samples

- Before each measurement the settings of the autosampler should be checked again (e.g., does the used tray match the settings etc.).
- You can cool the autosampler -> useful for long measurements

<u>Troubleshooting:</u>

1. back pressure is too low: can be increased by the length of the red capillary - the longer the higher the pressure (pressure becomes even higher after coupling with detectors, e.g., ICP-MS or OCD)

2. reset the pumps: Press the button at the bottom right and then turn the wheel at the bottom right to the left until it stops, then press the button at the top. You also must reset in the software -› Tools -› Interface -> K hloen Pumps -> Reset
3. strange signs on the pump display: everything must be restarted
    a. Turn off ALL devices at the back of the switch
    b. Close software
    c. Switch on the devices at the back of the switch
    d. Restarting the software
4. pressure is unstable -> check valves of pump heads (tip and focus pump) in ultrasonic bath (approx. 5 min in 2 % EtOH) -> see FFF manual

<u>Preparation of separation channel/filter/loop</u>

Channel:

- Unscrew channel from AF4 - › Pump flow all to 0 mL/min
- Loosen screws: Loosen the screws from the outside to the inside
- Tighten screws: Tighten the screws from inside to outside (with a torque wrench)
- Cut the membrane (figure 2) (to size (29.5 x 3.4 cm) -> Cut slightly larger than the frit, but must not protrude over the seal
- Place the membrane in Milli-Q overnight or rinse the instrument for 2-3 hours (at 0.5, 0.5, 0.5)
- Assembly: aluminium top plate with screws - top plate - spacer - membrane (shiny side up) - frit - bottom plate with sealing ring - metal plate with screw thread
- Rinse everything with Milli-Q and then screw it back together again -› Caution Frit may fall out

Figure 2. Preparation of the membrane

- Vent the channel: 1. screw in Out port for detector, 2. screw in Focus Port and purge with Focus pump (press buttons ^ and < simultaneously) until there is no more air in the channel, 3. turn Purge Port, 4. fill the channel with Focus "Purge" until there is no more air in the channel and then turn Tip Port and Cross Flow Port in

Filter:

- ○ Open with 23" wrench - Pay attention to the direction of the running medium
- ○ Structure: screw - metal nut with "-›" - membrane - frit – screw
- ○ Both screws should be in the middle of the metal nut (do not tighten one first and then the other, but both at the same time and then tighten)
- ○ To purge the two filters press "Purge" without entering the system (or simply rinse the system 2-3 hours before measurement at 0.5, 0.5, 0.5)
- ○ Filter holders should be cleaned in an ultrasonic bath from time to time -> 2 % EtOH for 5 min

Loop:

- ○ Pay attention to correct loop
- ○ Purge new loop by purging the tip pump -> Flow rate of the pump must be 0 mL/min (or simply run the system for 2-3 hours at 0.5, 0.5, 0.5)
- ○ Test if the loop is vented: Tools -> Interface -> Autosampler -> 'manual washing' until the loop is filled - test if there is still air in the loop by switching 'injection valve' between 'inject and 'load' -> if the pressure drop is > 1 bar then there is still air in the loop

  Leakage test:

  - ○ Tip, Focus, & Cross: 0.5 ml/L -> run for 10 min -> the purge valve must be closed

    back pressure should be between 6-9 bar
  - ○ Then test method pressures -› Preset flows

  Rinse system -> Open purge valve

  Once the system is set up we can put our samples for analysis (figure 3).

Figure 3. Jelena Beljin starts of the analysis

Standards for OCD

- The concentration range of the calibration solution is dependent on the Cors -› must first measure samples and then prepare standards for the OCD depending on the OC concentration of the sample

- Stock solution: 100 L of the TOC standard in 9.9 mL Mili-Q (10 mg/L) (Table 1)

Table 1. Preparation of standards

Conc. (µg/L)	*TOC (µL)*	*Mili-Q (µL)*
Cal 0	0	1000
Cal 100	100	9900
Cal 1000	1000	9000
Cal 2000	2000	8000
Cal 5000	5000	5000

Standards for ICP-MS

Stocksolutions (Cal_Stock) (10 mL)

- P, Mn, Ca, Mg, Cu, Zn -> 100 ML of the 1 g/L Std -> 0.01 g/L
- Fe, Al, Si, S, K -> 1000 M L of the 1 g/L Std -> 0.1 g/L
- Acid_IS -> add to obtain 10 mL including volumes of standards

Internal Standard (Acid_IS)

- Rh, Y -> 50 pL of 1 g/L Std
- 30% HCl (suprapur) -> 8 mL
- DI-Wasser -> 472 mL

Calibration standards - 10 times dilution of the stock solution.

Gained results are given in a figure 4.

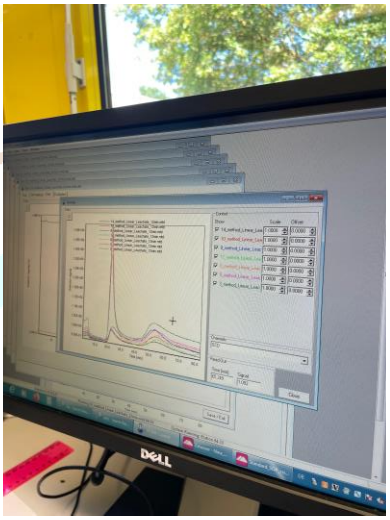


Figure 4. FFF-UV-OCD data processing

Standard Operation Procedure SOP FFF-DLS coupling

- Use the flow cuvettes
  - Connect the flow cuvette with the tube coming from the UV-detector
  - Be careful with the inlet and outlet - inlet right side, outlet left side o 1 tube into waste (the long tube)
  - insert the cuvette into the DLS (arrow on cuvette should be located right hand side on the front) - the cuvette tubing should exit at the side
- start the UV detector
- start the DLS
- Only 1 sample at the time can be measured > label the sample manually within the DLS software, as there is no communication between the DLS and the FFF software
- Prepare FFF and us the method that was also used for normal FFF measurements
  - ➢ only 1 sample in the sequence
- Open Zetasizer software
  - Select -> flow cuvette

    Browse for SOP (use existing one or create new one)
  - Example SOP for small natural colloids:
  - Measurement type: flow
  - Material: clay_smaller_022
  - Temperature: 19 °C
  - Cell: flow cell
  - " Measurement: change time - must be if the time of method
  - Safe SOP
- Browse for SOP -> open SOP
- Measurement -> start online window
- In the FFF software start the run

  When the FFF injection starts start DLS measurement in the Zetasizer software

Water dispersible colloids (WDC) extraction

- 10g (fresh samples, <2mm) (figure 5) + 20 ml distilled water. Literature: usually soil:water ratio is 1:2 (w/v). Suggestion: flasks of 100 ml;
- Shaking 6h, 150 pm;
- Add 60 ml distilled water. Homogenize gently;

- Sedimentation based on Stoke's Law. The time for sedimentation is calculated based on the particle diameter and the bottle size (water table height into the tube), so it is possible to collect the volume of extract needed (for further analyses) free of particles with a predefined diameter (for example 0.02mm, common in literature).
- After waiting for the sedimentation time. Collect the extract using a pipette (the pipette volume is defined in the previous step. Transfer the collected extract to a centrifuge tube;

Figure 5. Jelena Beljin during measuring the sediment sample

- ○ Centrifugation. The centrifugation time and force are calculated based on centrifuge settings/characteristics and particle diameter according to the formula of Hathaway et al. (1956) in Harry et al. (1984). Here for example if the target diameter (to be collected) is 500 nm, the r (radius) in the formula would be equivalent to 250 nm.
- ○ Use an aliquot of the centrifuged sample to test the average particle size obtained. This can be done with a quick DSL test;
- ○ As the sedimentation and centrifugation steps are theoretical, it is possible that the obtained average particle size differs from that initially desired. In this case, batches of fixed centrifugation force and increasing time, with DSL test of each test sample, can be performed to adjust the procedure.

ICP MS

ICP-MS (inductively coupled plasma-mass-spectrometry, figure 6) is a technique to determine low-concentrations (range: ppb = parts per billion = µg/l) and ultra-low-concentrations of elements (range: ptt = parts per trillion = ng/l). Atomic elements are lead through a plasma source where they become ionized. Then, these ions are sorted on account of their mass. The advantages of the ICP-MS technique above AAS (Atomic Absorption Spectroscopy) or ICP-OES (inductively coupled plasma optical emission spectrometry) are: (i) Extremely low detection limits; (ii) A large linear range and (iii) Possibilities to detect isotope composition of elements.

Figure 6. ICP-MS

FFF AF2000

Asymmetric flow field flow fractionation was performed using an AF2000 system (figure 7) (Postnova, Landsberg, Germany).

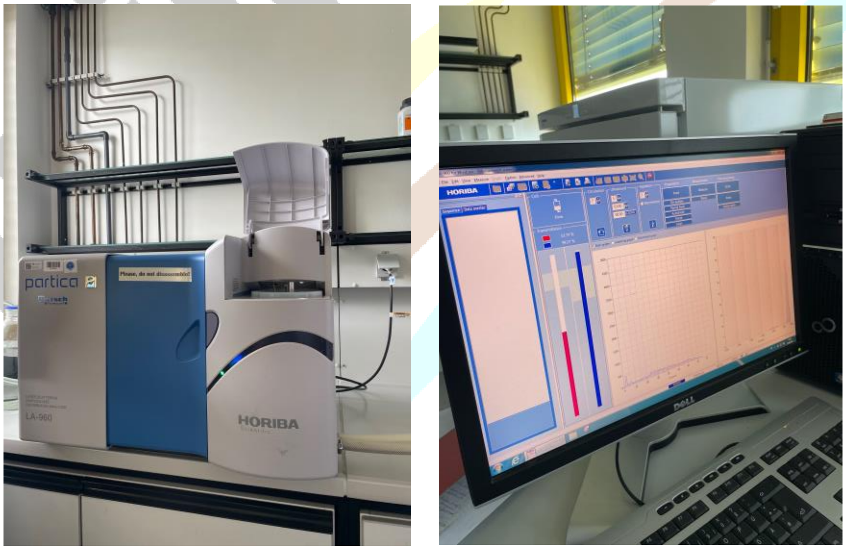


Figure 7. AF2000 system

FFF-ICP-MS

In case of FFF-ICP-MS (figure 8) the injection volume was 5 mL for water samples with a focusing time of 30 min at 0.2 mL min1 tip flow and for the soil extracts injection volume was 200 mL with focusing time of 12 min at 0.3 mL/min tip flow. The isotopes were monitored in helium collision cell mode (Agilent 7500).

Figure 8. FFF-ICP-MS

In case of FFF-OCD injection volume was 1 mL with a focus time of 10 min for the water samples using the same flow rates; injection conditions for the soil extracts were the same as for FFF-ICP-MS.

IRMS

IRMS (figure 9) provides analysis of isotopic ratios of the light stable isotopes C, N, O, S and H. Samples are introduced to the ion source as pure gas (dual inlet - DI) or carried on a flow of helium carrier gas (continuous flow - CF). A range of preparation systems are available to convert solid, liquid and gas samples containing stable isotopes into a gas that can be analyzed, while preserving the isotopic signature of the original sample. The light elements, their stable isotopes and the gases used for analysis are listed in the table 2.

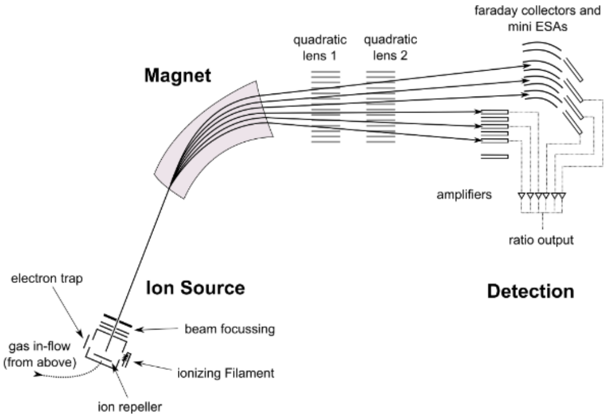


Figure 9. IRMS

Table 2. Elements, their stable isotopes and the gases

ELEMENT	STABLE ISOTOPE RATIO	GAS ANALYSED
Carbon	$^{13}C/^{12}C$	CO_2, CO
Nitrogen	$^{15}N/^{14}N$	N_2, N_2O
Oxygen	$^{18}O/^{16}O$	CO_2, CO, O_2
Sulphur	$^{34}S/^{32}S$	SO_2, SO
Hydrogen	$^{2}H/^{1}H$	H_2

MC-ICP-MS

A MC-ICPMS (figure 10) is a hybrid mass spectrometer that combines the advantages of superior ionization of an inductively coupled plasma source and the precise measurements of a magnetic sector multicollector mass spectrometer. The primary advantage of the MC-ICPMS is its ability to analyze a broader range of elements, including those with high ionization potential that are difficult to analyze by TIMS. The ICP source also allows flexibility in how samples are introduced to the mass spectrometer and allows the analysis of samples introduced either as an aspirated solution or as an aerosol produced by laser ablation.

Fundamental Principles of Multicollector-Inductively Coupled Plasma Mass Spectrometer (MC-ICPMS). As a hybrid mass spectrometer, MC-ICPMS combines an inductively coupled plasma (ICP) plasma source, an energy filter, a magnetic sector analyzer, and multiple collectors for the measurement of ions. The ions are produced by introducing the sample into an inductively coupled plasma which strips off electrons thereby creating positively charged ions. These ions are accelerated across an electrical potential gradient (up to 10 KV) and focused into a beam via a series of slits and electrostatically charged plates. This ion beam then passes through an energy filter, which results in a consistent energy spectrum in the ion beam and then through a magnetic field where the ions are separated based on their mass to charge ratio. These mass-resolved beams are then directed into collectors where the ions reaching the collectors are converted into voltage. Isotope ratios are calculated by comparing voltages from the different collectors.

The electronics of these instruments must operate to very close tolerances to produce isotope ratios that are precise to 0.01-0.001%. In addition, a high vacuum needs to be maintained along the path of the ion beam to avoid scattering of the ions due to interaction with air molecules.

Data Collection, Results and Presentation. Measured isotope ratios must be properly corrected for all instrumental biases, including mass fractionation. Once corrected, these ratios are suitable for plotting in any diagrams requiring atomic ratios (e.g., isochron, concordia, etc.).

Figure 10. MS-ICP-MS

Fluorescence microscopy

Fluorescence microscopy (figure 11) has become an indispensable microscopy technique for the examination of biological specimens, because it allows selective and specific detection of molecules at small concentrations with a good signal-to-background ratio. It even allows one to work with intact samples, including living cells, and to see samples with the naked eye; these advantages are not available with other methods, such as electron microscopy. Furthermore, recent developments in fluorescence imaging techniques have enabled the well-known Abbe barrier of about 200 nm lateral resolution to be crossed, that is, the diffraction limit for an optical microscope has approached the level of 100 nm as with 3D structural illumination microscopy (3D-SIM)4 or even less than 100 nm, as with other techniques such as 4Pi, simulated emission depletion (STED) and photoactivated localization (PALM In order to obtain a stable image with a higher resolution, however, an electron microscope is still required. Correlative study using both fluorescence and electron microscopy is usually employed to obtain substantial information on molecular localization. The technology of the scanning electron microscope (SEM) is now well advanced and the image resolution of the SEM approaches that of the transmission electron microscope (TEM). For most biological samples, it is easier and less time-consuming to prepare samples for scanning electron microscopy than for transmission electron microscopy. Furthermore, the structure of the SEM makes it easier and more cost-effective to incorporate other devices into it.

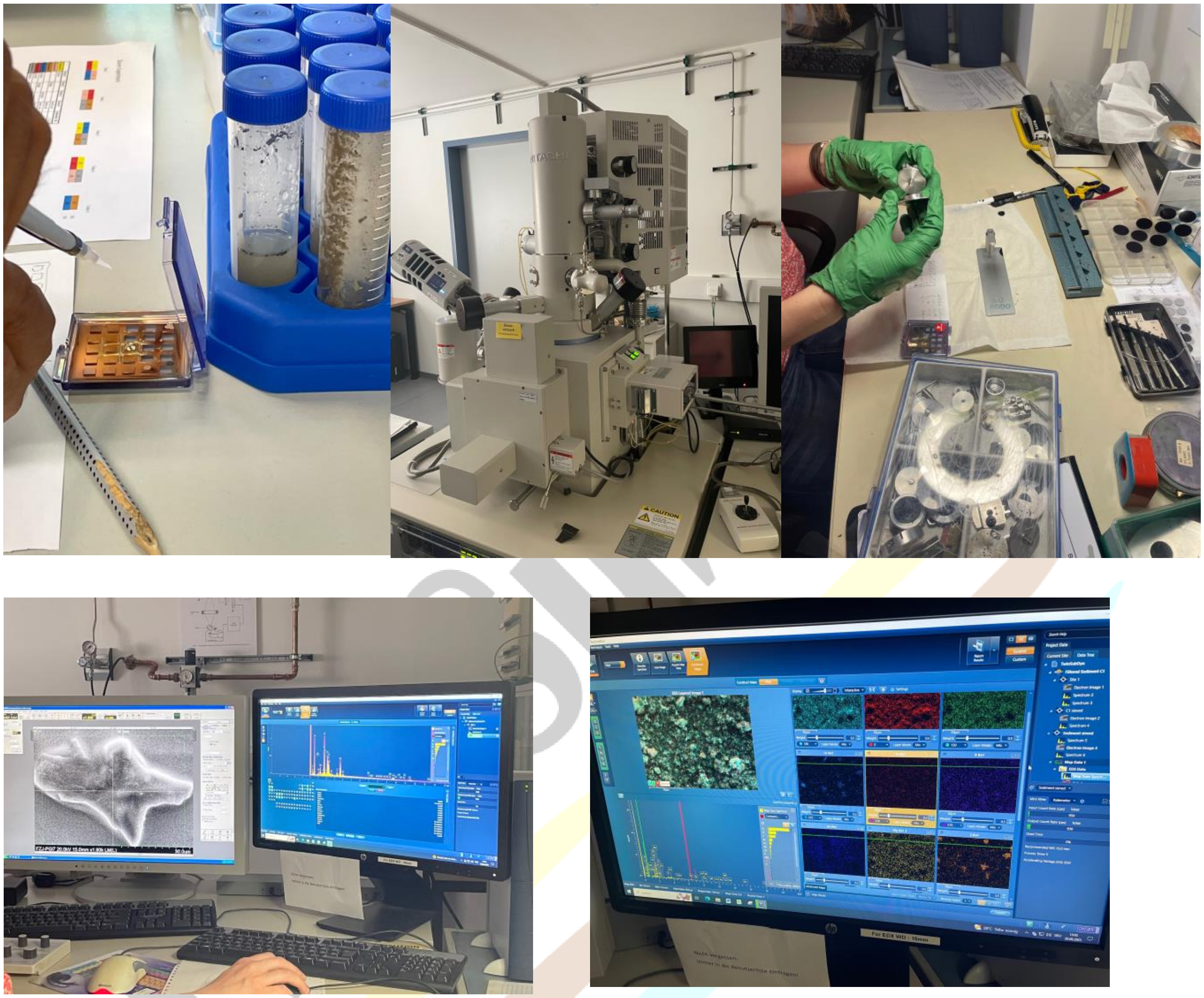


Figure 11. Jelena Beljin and Nina Đukanović during microscopic

Results:

The carbon composition of non-discharge normalized leachates, as revealed by FFF measurements using an OCD detector, shows that the majority of carbon is released in the form of truly dissolved carbon in all treatments (table 3). The treatment with 1% Biochar + Sandy soil had the smallest losses of carbon, while treatments with compost had the highest losses. This is likely due to differences in application rates.

Furthermore, there were noticeable differences among treatments in the size distribution of particulate leached carbon. The data suggest that in the initial stages of the experiment compost seam to be a significant source of carbon-containing particles of small size was observed (up to 70% in fraction 1). In contrast, larger particles (up to 70% in fraction 3) were leached from the Biochar treatment.

Graph averaged fractorgams of leachates and are given in figure 12. In figure 13 are given size resolved leachates OCD RAW, while table 4 has data about chemical composition biochar.

. Table 3. Results from FFF measurements using an OCD detector

Lysimeter Number	Treatment	Total carbon	Truly dissolved carbon	Proportion of truly dissolved on total carbon	Total particulate carbon	Proportion of truly dissolved on total carbon	Size faction					
							Fraktion 1		Fraktion 2		Fraktion 3	
		mg/L	*mg/L*	*%*	*mg/L*	*%*	*mg/L*	*%*	*mg/L*	*%*	*mg/L*	*%*
1	**1% Biochar + Sandy Soil**	6.58	6.1	92.8	0.5	7.23	0.104	22	0.04	8.6	0.33	70
11	**1% Biochar + Sandy Soil**	5.57	5.2	93.6	0.4	6.36	0.069	19	0.02	6.3	0.26	74
13	**1% Biochar + Sandy Soil**	7.48	7.0	93.8	0.5	6.24	0.127	27	0.03	6.4	0.31	66
6	**5% Compost + Sandy Soil**	35.45	32.9	92.9	2.5	7.10	1.706	68	0.09	3.6	0.72	29
12	**5% Compost + Sandy Soil**	34.36	31.8	92.6	2.5	7.35	1.872	74	0.12	4.7	0.53	21
17	**5% Compost + Sandy Soil**	36.30	33.3	91.8	3.0	8.19	2.041	69	0.15	5.2	0.78	26
2	**20% Sediment + Sandy Soil**	23.76	22.9	96.6	0.8	3.40	0.205	25	0.07	8.9	0.53	66
7	**20% Sediment + Sandy Soil**	24.22	23.4	96.7	0.8	3.35	0.235	29	0.08	9.6	0.50	61
16	**20% Sediment + Sandy Soil**	24.30	23.4	96.1	0.9	3.88	0.240	25	0.08	8.5	0.62	66
3	**1% Biochar + 5% Compost + Sandy soil**	39.18	36.4	92.8	2.8	7.19	1.464	52	0.10	3.5	1.25	45
5	**1% Biochar + 5% Compost + Sandy soil**	38.23	35.2	92.1	3.0	7.86	1.871	62	0.13	4.4	1.00	33
15	**1% Biochar + 5% Compost + Sandy soil**	38.60	35.5	91.9	3.1	8.12	2.008	64	0.17	5.3	0.96	31
4	**1% Biochar + 20% Sediment + Sandy soil**	27.50	26.7	97.0	0.8	2.99	0.227	28	0.08	9.6	0.52	63
9	**1% Biochar + 20% Sediment + Sandy soil**	26.70	25.8	96.6	0.9	3.40	0.249	27	0.10	11.3	0.56	61
18	**1% Biochar + 20% Sediment + Sandy soil**	26.09	25.4	97.2	0.7	2.83	0.227	31	0.11	14.3	0.41	55
8	**1% Biochar + 5% Compost + 20% Sediment + Sandy soil**	33.23	31.5	94.9	1.7	5.10	0.522	31	0.22	12.9	0.95	56
10	**1% Biochar + 5% Compost + 20% Sediment + Sandy soil**	32.80	31.8	97.0	1.0	3.00	0.349	36	0.06	6.1	0.57	58
14	**1% Biochar + 5% Compost + 20% Sediment + Sandy soil**	32.06	31.1	97.0	1.0	3.04	0.371	38	0.09	9.5	0.51	52

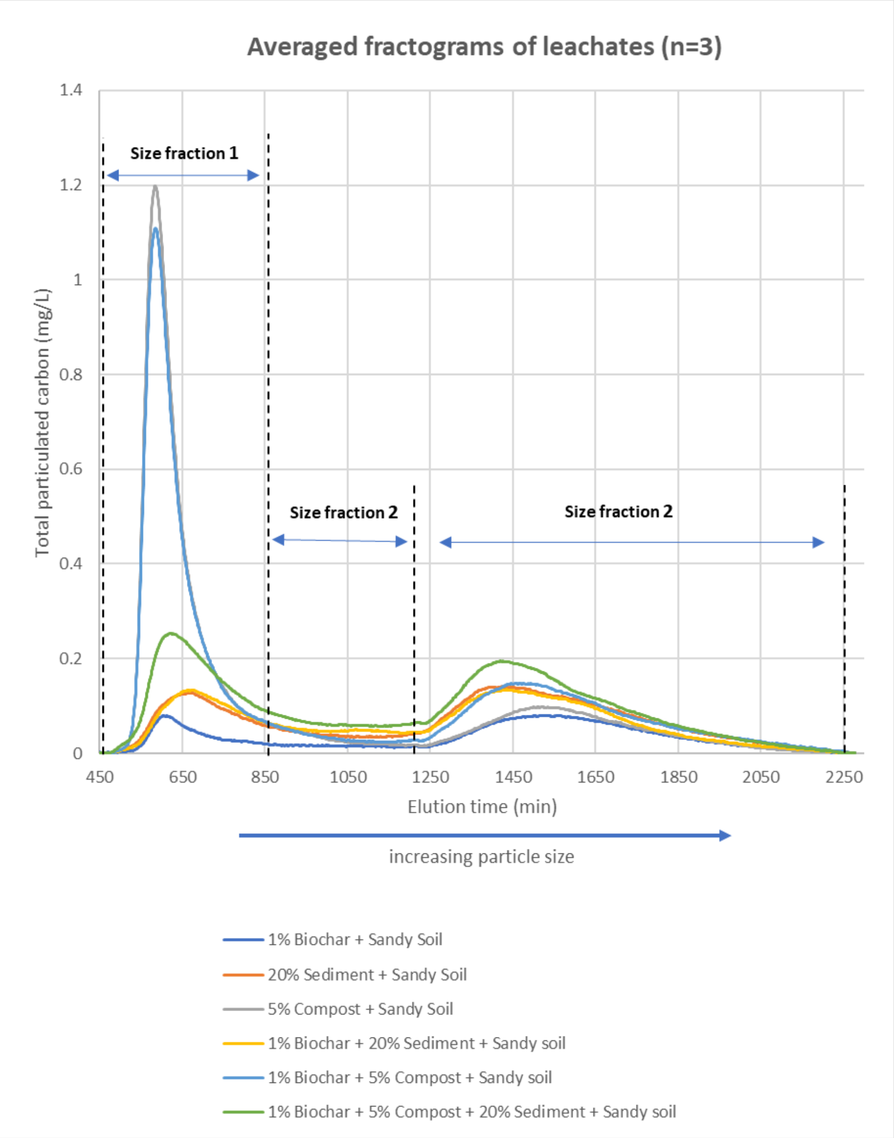
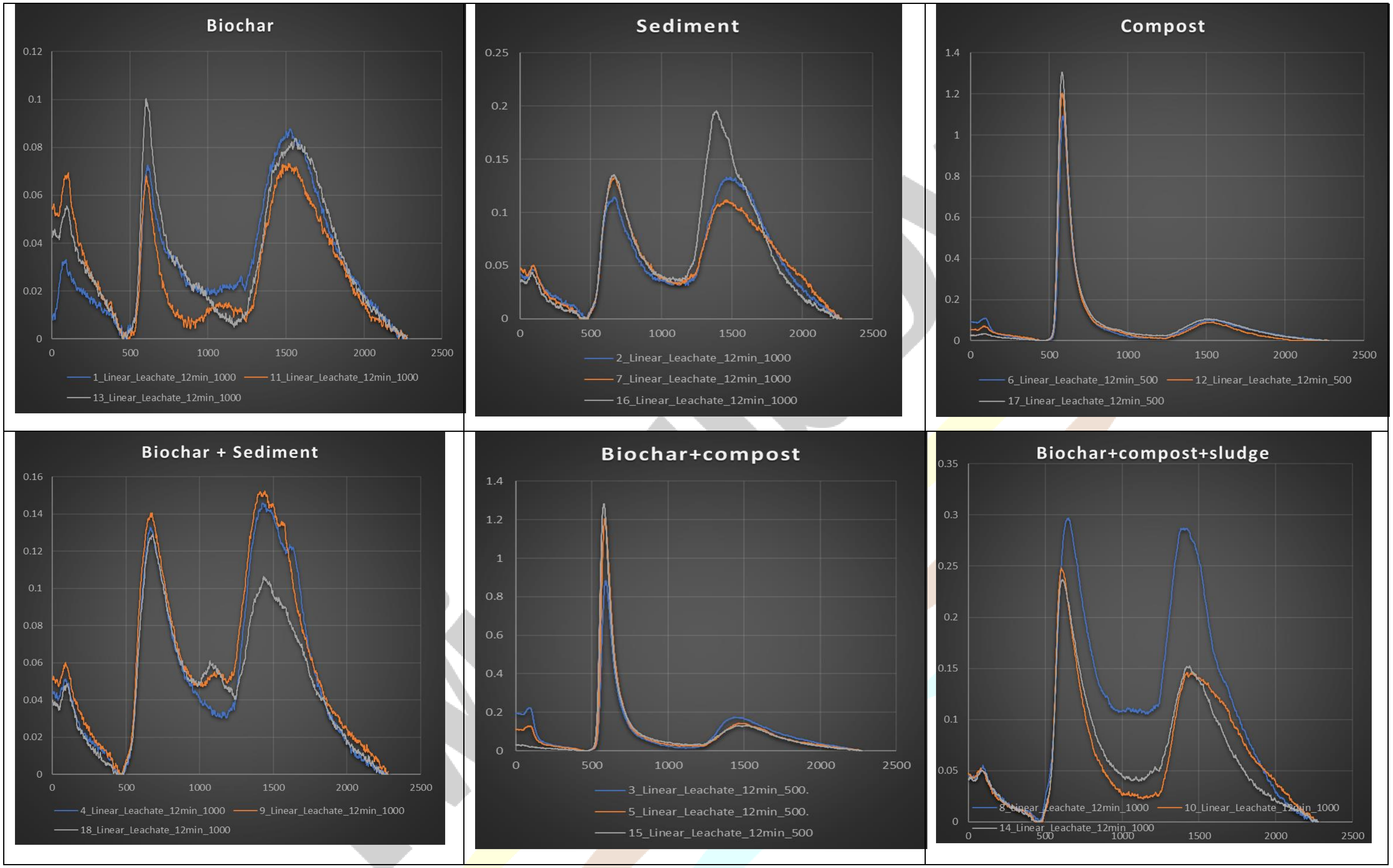


Figure 12. Graph averaged fractorgams of leachates


Biochar

Sediment

Compost

Biochar + Sediment

Biochar+compost

Biochar+compost+sludge

Figure 13. Size resolved leachates OCD RAW

Table 4. Total elemental concentration biochar (Miscanthus)

Mean			Mean		
C	(g/kg)	772,5±12500	Cu	(mg/kg)	4,6±0,5
Si	(g/kg)	23,7±2873	B	(mg/kg)	4,2±0,5
H	(g/kg)	25,5±1500	Cr	(mg/kg)	2,3±0,4
K	(g/kg)	15,1±990	Ni	(mg/kg)	1,3±0,3
P	(mg/kg)	3014,7±323	Pb	(mg/kg)	1±0,2
Ca	(mg/kg)	2391,7±83	Co	(mg/kg)	0,5±0,1
N	(mg/kg)	2000±0	Mo	(mg/kg)	0,3±0
Mg	(mg/kg)	1033,3±24	V	(mg/kg)	0,2±0,1
Mn	(mg/kg)	295,9±15	Cd	(mg/kg)	0±0
Fe	(mg/kg)	228,7±117	Se	(mg/kg)	<0,2
Al	(mg/kg)	214,7±97	As	(mg/kg)	<0,1
Na	(mg/kg)	184,3±34	Hg	(mg/kg)	<0,1
Zn	(mg/kg)	70,7±29	Tl	(mg/kg)	<0,1
Ba	(mg/kg)	14,5±0,3			

Results obtained from particle analyzer measurements to characterize the size distribution of the raw materials employed in the experiment and the mixtures of various treatments before the start of the experiment. Pure biochar could not be analyzed due to a high risk of clogging in the particle analyzer. There is variation in both median and mean particle sizes among the different treatments. Treatments with 1% Biochar tend to have larger median and mean sizes compared to other treatments.The addition of 5% Compost to Sandy Soil generally results in larger particle sizes compared to Sandy Soil alone, with increased median and mean sizes and higher D10, D50, and D90 values. When Biochar and Compost are combined with Sandy Soil and Sediment, the particle size trends are influenced by the individual characteristics of Biochar and Compost. For example, the combination of 1% Biochar and 5% Compost results in larger particle sizes compared to Sandy Soil alone, as seen in the increased median and mean sizes and higher D10, D50, and D90 values (Table 5, figure 14).

Table 5. Particle analyzer

Treatment sandy soil with	**Median Size**	**Mean size**	**D10**	**D50**	**D90**
	(µm)				
1% Biochar + Sandy Soil	10.5	21.6	3.4	10.5	39.7
20% Sediment + Sandy Soil	9.9	12.7	3.5	9.9	21.6
5% Compost + Sandy Soil	11.8	20.7	4.3	11.8	35.3
1% Biochar + 20% Sediment + Sandy Soil	11.6	14.9	3.8	11.6	27.7
1% Biochar + 5% Compost + Sandy Soil	13.3	26.9	5.5	13.3	46.7
1% Biochar + 5% Compost + 20% Sediment + Sandy Soil	13.3	20.2	4.8	13.3	31.3
Raw Compost	11.9	16.7	4.0	11.9	31.3
Raw Sand soil	9.9	14.8	3.2	9.9	28.7
Raw Sediment	9.8	12.0	3.8	9.8	19.3

	Standard deviation				
Treatment	Median Size	Mean size	D10	D50	D90
1% Biochar + Sandy Soil	**2.24**	**2.90**	**0.38**	**2.24**	**8.56**
20% Sediment + Sandy Soil	**0.60**	**2.29**	**0.02**	**0.60**	**2.12**
5% Compost + Sandy Soil	**0.49**	**5.75**	**0.28**	**0.49**	**5.78**
1% Biochar + 20% Sediment + Sandy Soil	**1.65**	**2.83**	**0.26**	**1.65**	**5.40**
1% Biochar + 5% Compost + Sandy Soil	**0.25**	**10.10**	**0.15**	**0.25**	**10.02**
1% Biochar + 5% Compost + 20% Sediment + Sandy Soil	**1.38**	**7.11**	**0.25**	**1.38**	**5.65**
Raw Compost	**0.27**	**2.07**	**0.04**	**0.27**	**1.55**
Raw Sand soil	**1.49**	**5.17**	**0.30**	**1.49**	**9.94**
Raw Sediment	**0.46**	**1.66**	**0.28**	**0.46**	**0.20**

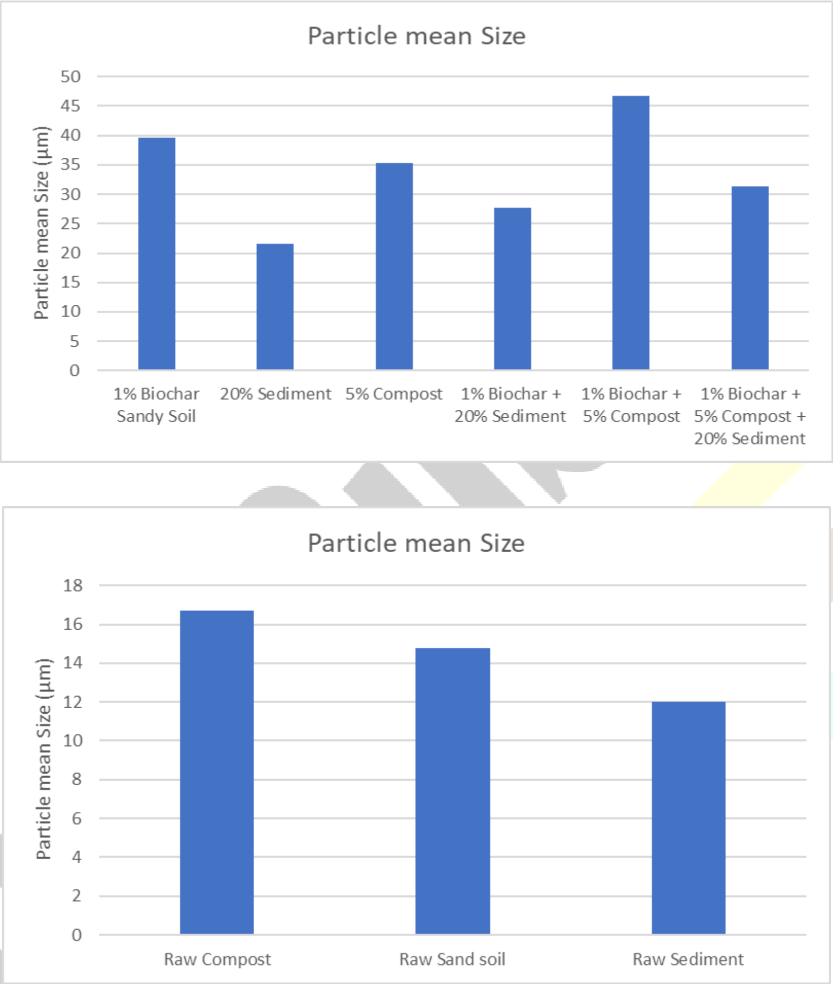
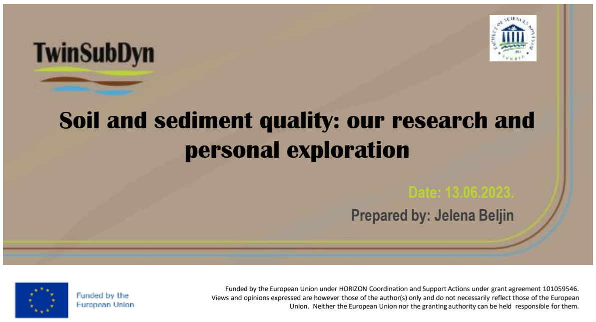


Figure 14. Particle analyzer

Impact on your project

The training in soil characterizations, organic matter dynamics and nutrient cycle analysis was an excellent opportunity for knowledge transfer between the FZJ and UNSPMF project groups. The sample preparation methodology is partially novel to the Novi Sad group and the methods carried out throughout the training can be transferred and adopted to be used in the UNSPMF labs, which will subsequently broaden the possibilities for further research in the field of soils science. The trained UNSPMF researchers will have the opportunity to transfer the gained knowledge and skills to both TwinSubDyn participants as well as other researchers and stakeholders. In addition, the acquired training in this subject will be further utilized through a 6-month secondment of an early-stage researcher from UNSPMF at the FZJ. They will continue the research in analysis of used organic amendments on soil characteristics and nutrient cycle. The expertise of the FZJ group in experimental design and laboratory research could be an excellent starting point for future experiments and lab investigations.



TwinSubDyn

Soil and sediment quality: our research and personal exploration

Date: 13.06.2023.

Prepared by: Jelena Beljin

Funded by the European Union

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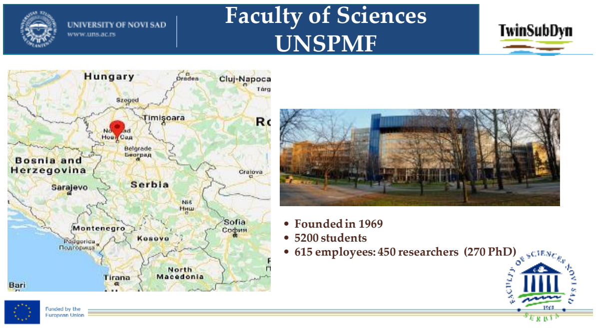


UNIVERSITY OF NOVI SAD
www.uns.ac.rs

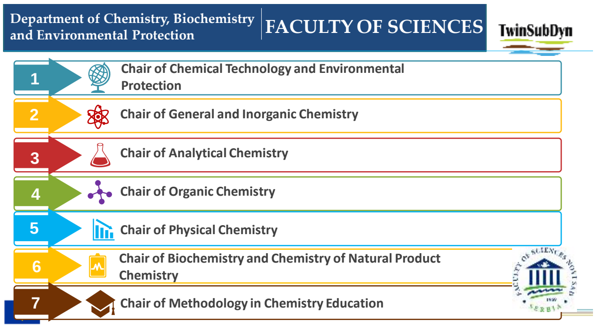
Faculty of Sciences UNSPMF

TwinSubDyn

- Founded in 1969
- 5200 students
- 615 employees: 450 researchers (270 PhD)

Funded by the European Union

2



Department of Chemistry, Biochemistry and Environmental Protection

FACULTY OF SCIENCES

TwinSubDyn

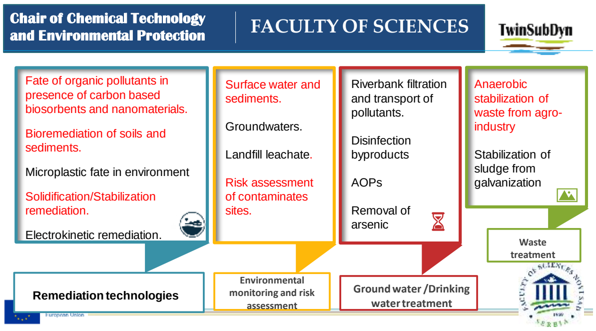
1. Chair of Chemical Technology and Environmental Protection
2. Chair of General and Inorganic Chemistry
3. Chair of Analytical Chemistry
4. Chair of Organic Chemistry
5. Chair of Physical Chemistry
6. Chair of Biochemistry and Chemistry of Natural Product Chemistry
7. Chair of Methodology in Chemistry Education

3

Chair of Chemical Technology and Environmental Protection

7 full professors, 7 associate professors, 2 assistant professors, 4 assistenat and 19 PhD researchers

4



Chair of Chemical Technology and Environmental Protection

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TwinSubDyn

Fate of organic pollutants in presence of carbon based biosorbents and nanomaterials.

Bioremediation of soils and sediments.

Microplastic fate in environment

Solidification/Stabilization remediation.

Electrokinetic remediation.

Remediation technologies

Surface water and sediments.

Groundwaters.

Landfill leachate.

Risk assessment of contaminates sites.

Environmental monitoring and risk assessment

Riverbank filtration and transport of pollutants.

Disinfection byproducts

AOPs

Removal of arsenic

Ground water /Drinking water treatment

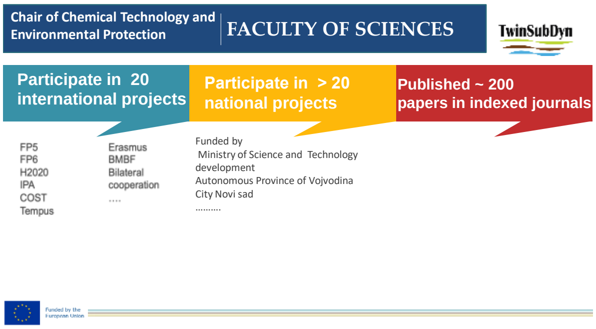
Anaerobic stabilization of waste from agro-industry

Stabilization of sludge from galvanization

Waste treatment

European Union

5



Chair of Chemical Technology and Environmental Protection

FACULTY OF SCIENCES

TwinSubDyn

Participate in 20 international projects

FP5
FP6
H2020
IPA
COST
Tempus

Erasmus
BMBF
Bilateral cooperation
....

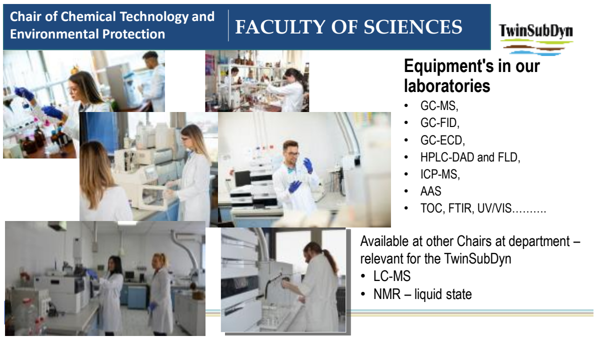
Participate in > 20 national projects

Funded by
Ministry of Science and Technology development
Autonomous Province of Vojvodina
City Novi sad
……….

Published ~ 200 papers in indexed journals

Funded by the European Union

6



Chair of Chemical Technology and Environmental Protection

FACULTY OF SCIENCES

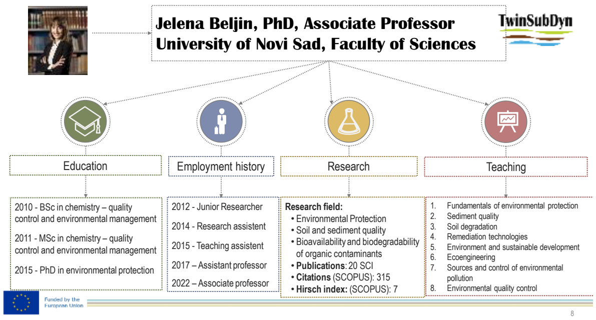
TwinSubDyn

Equipment's in our laboratories

- GC-MS,
- GC-FID,
- GC-ECD,
- HPLC-DAD and FLD,
- ICP-MS,
- AAS
- TOC, FTIR, UV/VIS……….

Available at other Chairs at department – relevant for the TwinSubDyn
- LC-MS
- NMR – liquid state

7



Jelena Beljin, PhD, Associate Professor
University of Novi Sad, Faculty of Sciences

TwinSubDyn

Education
- 2010 - BSc in chemistry – quality control and environmental management
- 2011 - MSc in chemistry – quality control and environmental management
- 2015 - PhD in environmental protection

Employment history
- 2012 - Junior Researcher
- 2014 - Research assistent
- 2015 - Teaching assistent
- 2017 – Assistant professor
- 2022 – Associate professor

Research

Research field:
- Environmental Protection
- Soil and sediment quality
- Bioavailability and biodegradability of organic contaminants
- **Publications**: 20 SCI
- **Citations** (SCOPUS): 315
- **Hirsch index:** (SCOPUS): 7

Teaching
1. Fundamentals of environmental protection
2. Sediment quality
3. Soil degradation
4. Remediation technologies
5. Environment and sustainable development
6. Ecoengineering
7. Sources and control of environmental pollution
8. Environmental quality control

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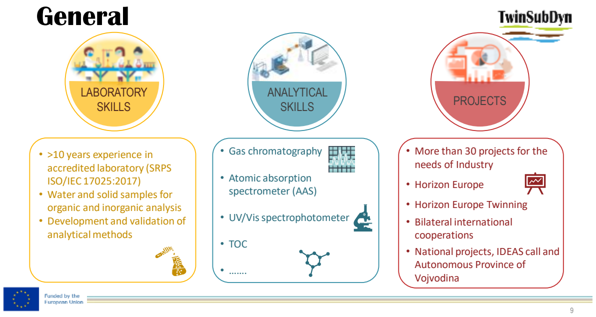

General

TwinSubDyn

LABORATORY SKILLS
- >10 years experience in accredited laboratory (SRPS ISO/IEC 17025:2017)
- Water and solid samples for organic and inorganic analysis
- Development and validation of analytical methods

ANALYTICAL SKILLS
- Gas chromatography
- Atomic absorption spectrometer (AAS)
- UV/Vis spectrophotometer
- TOC
- …….

PROJECTS
- More than 30 projects for the needs of Industry
- Horizon Europe
- Horizon Europe Twinning
- Bilateral international cooperations
- National projects, IDEAS call and Autonomous Province of Vojvodina

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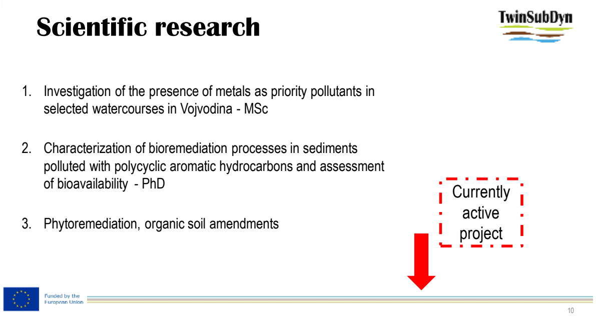
9



Scientific research

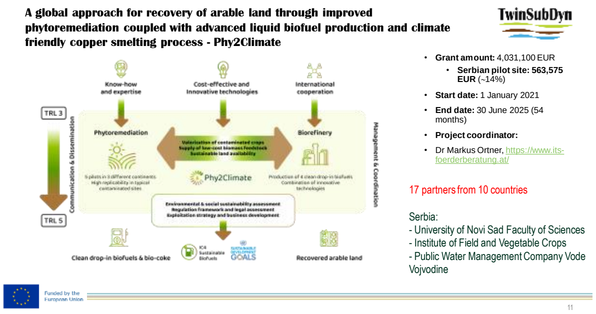
TwinSubDyn

1. Investigation of the presence of metals as priority pollutants in selected watercourses in Vojvodina - MSc
2. Characterization of bioremediation processes in sediments polluted with polycyclic aromatic hydrocarbons and assessment of bioavailability - PhD
3. Phytoremediation, organic soil amendments

Currently active project

Funded by the European Union

10



A global approach for recovery of arable land through improved phytoremediation coupled with advanced liquid biofuel production and climate friendly copper smelting process - Phy2Climate

TwinSubDyn

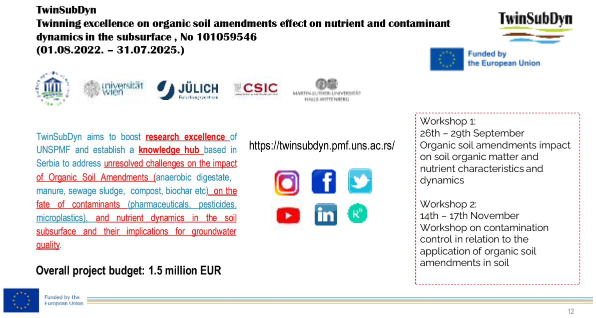
- **Grant amount:** 4,031,100 EUR
  - **Serbian pilot site: 563,575 EUR** (~14%)
- **Start date:** 1 January 2021
- **End date:** 30 June 2025 (54 months)
- **Project coordinator:**
- Dr Markus Ortner, https://www.its-foerderberatung.at/

17 partners from 10 countries

Serbia:
- University of Novi Sad Faculty of Sciences
- Institute of Field and Vegetable Crops
- Public Water Management Company Vode Vojvodine

Funded by the European Union

11



TwinSubDyn
Twinning excellence on organic soil amendments effect on nutrient and contaminant dynamics in the subsurface , No 101059546
(01.08.2022. – 31.07.2025.)

TwinSubDyn

Funded by the European Union

TwinSubDyn aims to boost **research excellence** of UNSPMF and establish a **knowledge hub** based in Serbia to address unresolved challenges on the impact of Organic Soil Amendments (anaerobic digestate, manure, sewage sludge, compost, biochar etc) on the fate of contaminants (pharmaceuticals, pesticides, microplastics), and nutrient dynamics in the soil subsurface and their implications for groundwater quality.

Overall project budget: 1.5 million EUR

https://twinsubdyn.pmf.uns.ac.rs/

Workshop 1:
26th – 29th September
Organic soil amendments impact on soil organic matter and nutrient characteristics and dynamics

Workshop 2:
14th – 17th November
Workshop on contamination control in relation to the application of organic soil amendments in soil

Funded by the European Union

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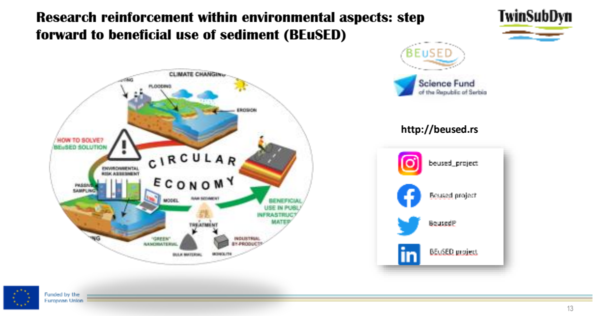

Research reinforcement within environmental aspects: step forward to beneficial use of sediment (BEuSED)

TwinSubDyn

BEuSED

Science Fund of the Republic of Serbia

http://beused.rs

beused_project

Beused project

BeusedP

BEuSED project

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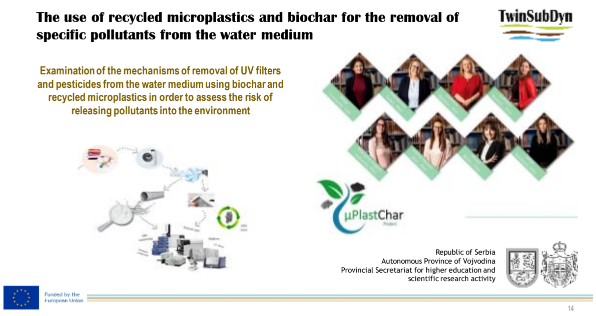

The use of recycled microplastics and biochar for the removal of specific pollutants from the water medium

TwinSubDyn

Examination of the mechanisms of removal of UV filters and pesticides from the water medium using biochar and recycled microplastics in order to assess the risk of releasing pollutants into the environment

µPlastChar Project

Republic of Serbia
Autonomous Province of Vojvodina
Provincial Secretariat for higher education and scientific research activity

Funded by the European Union

Bilateral cooperations

TwinSubDyn

- Republic Serbia and Republic of Hungary: Development of new nanocomposites based on biochar for the remediation of polluted sediment (2021-2023)
- Republic Serbia and Republic of Belarus: Designing and environmental application of nano-architectures materials based on magnesium titanates (DINANTIAN) (2022-2024)

COST actions

- CA19123 - Protection, Resilience, Rehabilitation of damaged environment (PHOENIX) – COST ACTION

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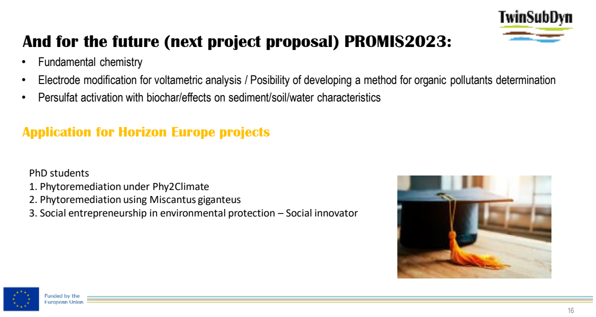

And for the future (next project proposal) PROMIS2023:

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- Fundamental chemistry
- Electrode modification for voltametric analysis / Posibility of developing a method for organic pollutants determination
- Persulfat activation with biochar/effects on sediment/soil/water characteristics

Application for Horizon Europe projects

PhD students
1. Phytoremediation under Phy2Climate
2. Phytoremediation using Miscantus giganteus
3. Social entrepreneurship in environmental protection – Social innovator

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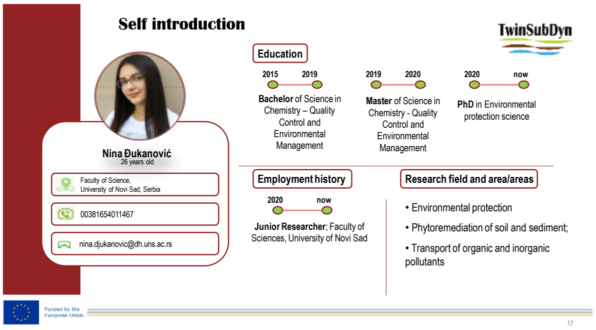

Self introduction

TwinSubDyn

Nina Đukanović
26 years old

Faculty of Science,
University of Novi Sad, Serbia

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nina.djukanovic@dh.uns.ac.rs

Education

2015 – 2019
Bachelor of Science in Chemistry – Quality Control and Environmental Management

2019 – 2020
Master of Science in Chemistry - Quality Control and Environmental Management

2020 – now
PhD in Environmental protection science

Employment history

2020 – now
Junior Researcher; Faculty of Sciences, University of Novi Sad

Research field and area/areas

- Environmental protection
- Phytoremediation of soil and sediment;
- Transport of organic and inorganic pollutants

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Skills

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LABORATORY	• Working in regular laboratory equipment • 2 years experience in accredited laboratory (SRPS ISO/IEC 17025:2017)
SAMPLE PREPARATION	• Preparation of solid and liquid samples for analysis of organic pollutants (extraction, purification, fractionation). ✓ **ASE** (accelerated solvent extraction). • Preparation of solid and liquid samples for heavy metal analysis (**microwave digestion**). • Preparation of solid and liquid samples for organic carbon analysis (**TOC**).
INSTRUMENTAL	• **FAAS** (flame AAS), **GFAAS** (furnace AAS), **FIAS-AAS** (flow injection analyses system). • **ICP-MS.** • **GC-MS, GC-ECD.** • **UV-VIS spectrophotometer, pH meter, turbidimeter, conductometer.**

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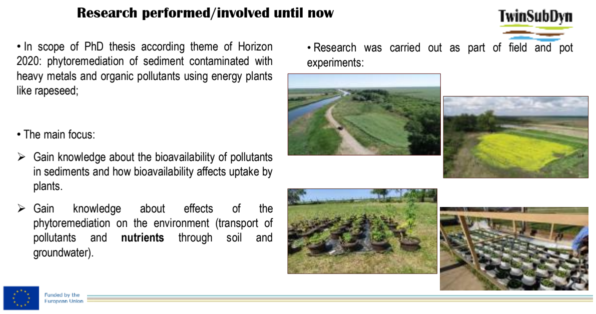

Research performed/involved until now

- In scope of PhD thesis according theme of Horizon 2020: phytoremediation of sediment contaminated with heavy metals and organic pollutants using energy plants like rapeseed;

- The main focus:
  - Gain knowledge about the bioavailability of pollutants in sediments and how bioavailability affects uptake by plants.
  - Gain knowledge about effects of the phytoremediation on the environment (transport of pollutants and **nutrients** through soil and groundwater).

- Research was carried out as part of field and pot experiments:

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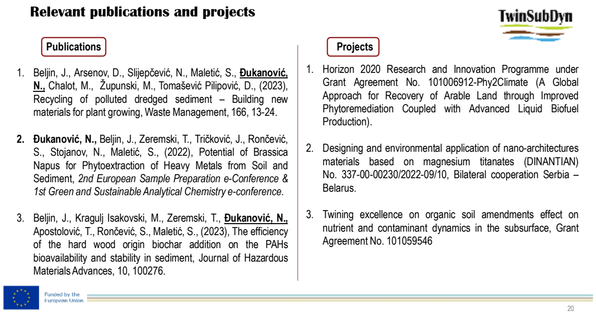

Relevant publications and projects

Publications

1. Beljin, J., Arsenov, D., Slijepčević, N., Maletić, S., **Đukanović, N.,** Chalot, M., Županski, M., Tomašević Pilipović, D., (2023), Recycling of polluted dredged sediment – Building new materials for plant growing, Waste Management, 166, 13-24.
2. **Đukanović, N.,** Beljin, J., Zeremski, T., Tričković, J., Rončević, S., Stojanov, N., Maletić, S., (2022), Potential of Brassica Napus for Phytoextraction of Heavy Metals from Soil and Sediment, *2nd European Sample Preparation e-Conference & 1st Green and Sustainable Analytical Chemistry e-conference.*
3. Beljin, J., Kragulj Isakovski, M., Zeremski, T., **Đukanović, N.,** Apostolović, T., Rončević, S., Maletić, S., (2023), The efficiency of the hard wood origin biochar addition on the PAHs bioavailability and stability in sediment, Journal of Hazardous Materials Advances, 10, 100276.

Projects

1. Horizon 2020 Research and Innovation Programme under Grant Agreement No. 101006912-Phy2Climate (A Global Approach for Recovery of Arable Land through Improved Phytoremediation Coupled with Advanced Liquid Biofuel Production).
2. Designing and environmental application of nano-architectures materials based on magnesium titanates (DINANTIAN) No. 337-00-00230/2022-09/10, Bilateral cooperation Serbia – Belarus.
3. Twining excellence on organic soil amendments effect on nutrient and contaminant dynamics in the subsurface, Grant Agreement No. 101059546

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Thank you!

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Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union. Neither the European Union nor the granting authority can be held responsible for them.

Subtask 1.2.7 6-months secondment for ESR/RR and two 1-months secondments for QR/RR at UNVIE focused on methodologies for the characterization and analysis of particulate contaminants (leader UNIVIE).

MOBILITY REPORT

Researcher: MSc Sanja Vasiljević, UNSPMF

Assigned supervisor: Thorsten Hüffer, UNIVIE

Duration of the visit: 01.02.2023-31.07.2023.

Executive Summary

The main goal of this visit was working on leaching and long-term dynamics of plastic-derived additives, with a special focus on PVC-derived phthalates. For this, time dependent leaching curves of DEHP from PVC microplastics will be determined over a period of up to 60 days using methods established at UNIVIE. To account for leaching processes occurring under environmentally relevant conditions, experiments were done in OSA matrices. DEHP and its transformation products leached from PVC will be quantified using a gas chromatograph coupled to a triple quadrupole mass spectrometer in next one month visit at UNIVIE. The experimental data will allow for an interpretation of the governing mass transfer process for leaching using models for intraparticle diffusion and aqueous boundary layer diffusion.

The main knowledge transfer was focused on:

- Leaching procedure: Preparation of carbon sinks, experiment set up, analysis procedures
- ASE and GC/MS/MS technique for phthalate analysis

1. Characterization

Elementar Analyzer (CHNS) – Quick guide

CHNS measuring principle Thevario MACRO in CHNS mode analyzes the C, H, N, and S content of a dry sample. Sample (in mg range) is mixed with a catalyst (WO_3) in tin foil sample boats, folded, weighted and placed on a rotating carousel. The samples are transferred to the furnace area using a ball valve that rotates to receive a sample pushed towards it by the carousel. Any outside air is removed using helium and the sample is then transported to the combustion tube. This tube contains an ash crucible with WO_3 granulate at the bottom, where the sample drops into. The combustion tube also contains WO_3 granulate that sits below the ash crucible (separated from it by corundum balls). The combustion tube is maintained at a temperature of 1100°C, and oxygen is dosed for a set time for the following gaseous reaction products to form from the elements of interest: CO_2, H_2O, N_2, NO_x, SO_2, SO_3.

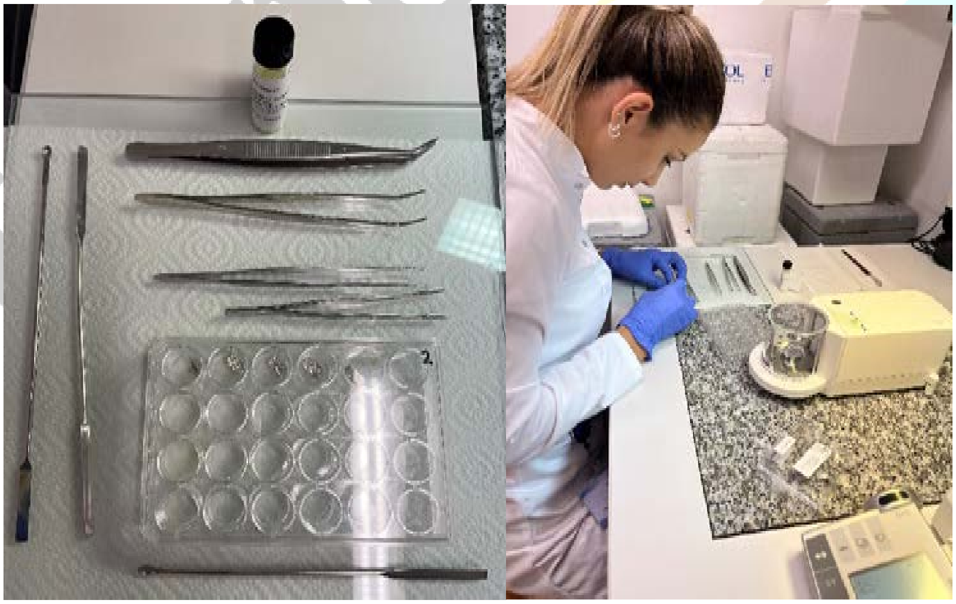


Figure 1. Sample preparation for CHNS analysis

Following the analysis of this final element, a cooling fan reduces the temperatures of the adsorption columns so that they are ready for the next sample. It takes approximately 11 minutes to analyze each sample. Samples need to be perfectly dry.

Carbon analyzer (LECO) – Quick guide

The TOC analyzer RC-612 from LECO is used to determine the total carbon content (TC), the total inorganic carbon content (TIC), the total organic carbon content (TOC), and the water content of an unknown sample. The sample is burnt at 550°C in a quartz tube to determine TOC or at 1000°C to determine TC. For an analysis of an unknown sample, a temperature program with a slow heating curve can be chosen. Increasing the temperature step-wise causes different carbon forms to oxidize at a respective temperature. By adding oxygen, the released carbon is oxidized to CO_2. For complete oxidation, the use of CuO as a catalyst and an afterburner is needed. For detection, an infrared (IR) detector is used. The present carbon of the converted gaseous sample absorbs the radiation of the IR source while passing through the measurement cell. Quantification is done by measuring extinction by the Lambert-Beer law. The measurement of the water vapour is performed analogue. The amount of the available CO_2 and H_2O can be detected continuously and simultaneously. Two differently long CO_2 measurement cells can be installed in the RC-612 (LECO), which have to be separately calibrated depending on the expected amounts of the respective samples.

TOC-L (Shimadzu) – Quick guide

The TOC-L instrument measures the amount of total carbon (TC), inorganic carbon (IC) and total organic carbon (TOC) in a liquid sample. If the water sample is filtered through a 0.45 µm or 0.20 µm filter membrane, the dissolved organic carbon (DOC) content is measured. TOC or DOC concentrations are measured by direct measurement, which is called non-purgeable organic carbon (NPOC) method. By using the NPOC method, firstly, the sample will be acidified to pH<2 with hydrochloric acid (HCl). This can be done automatically by the system or also manually during sample preparation. Then, the sample will be automatically sparged by the carrier gas (synthetic air) for 150 seconds. The sparging causes the inorganic carbon (IC) to transform into CO2, which is then released from the sample. Sparging can take place in a 5 mL syringe installed in the inside of the instrument or by an external sparging option. The remaining organic fraction (NPOC) will be detected by catalytic combustion oxidation with an NDIR-detector. There is also the possibility to use the TC-IC method to determine the TC, IC and TOC concentration of a respective sample. It measures the amount of IC evolved from an acidified aliquot of a sample and also the amount of TC present in the sample. TOC is calculated by subtraction of the IC value from the TC in the sample. This method is not installed at the moment.

Figure 2. Sample preparation for DOC measurments

UV-vis (PerkinElmer) – Quick guide

The Lambda 35 is a versatile spectrometer operating in the ultraviolet (UV) and visible (Vis) spectral ranges. The spectrometer features a double-beam and an all-reflecting system. UVvis spectrometry is used to obtain the absorbance spectra of a compound in solution. What is being observed spectroscopically is the absorbance of light energy or electromagnetic radiation, which excites electrons from the ground state to the first singlet excited state of the compound or material. The UV-vis region relates to a wavelength range of 800 nm to 200 nm. The Beer-Lambert Law is the principle behind absorbance spectroscopy. For a single wavelength, A is absorbance (unitless), is the molar absorptivity of the compound or molecule in solution (M-1 cm^{-1}), b is the path length of the cuvette or sample holder (usually 1 cm), and c is the concentration of the solution: A = bc UV-vis spectroscopic data can give qualitative and quantitative information of a given compound or molecule. Irrespective of whether quantitative or qualitative information is required it is important to use a reference cell to zero the instrument for the solvent the compound is in. For quantitative information on the compound, calibrating the instrument using known concentrations of the compound in question in a solution with the same solvent as the unknown sample would be required.

Characterization results:

Table 1. Characterization of materials (mean value of three measurements)

	pH	UV-Vis	DOC (mgC/l)	SUVA	LECO (%C)
Compost UNSPMF	7.20	0.3856	329.75	0.1169	24.57
Sludge UNSPMF	6.05	0.2567	236.5	0.1085	37.32
Cow manure	7.16	1.9267	326	0.5910	42.59
Urban compost (Vienna)	7.57	0.4700	284.15	0.1654	31.53
Rural compost (Vienna)	7.43	0.9156	327.55	0.2795	12.58
Danube sediment	7.87	0.151	4.78	3.1590	0.78
Bayreuth soil	6.13	1.926	42.19	4.5651	0.92
Compost UNSPMF new	6.80	1.908	182.4	1.0461	20.65

Table 2. CHNS results

	C	H	N	S
Compost UNSPMF	16.94	1.46	1.44	1.17
Sludge UNSPMF	32.97	4.19	5.71	2.63
Cow manure	44.81	5.19	3.18	0.86
Urban compost (Vienna)	24.50	2.61	25.45	0.54
Rural compost (Vienna)	34.68	3.43	95.14	0.31
Danube sediment	0.94	0.01	0.04	0.03
Bayreuth soil	1.74	0.12	0.18	0.09
Compost UNSPMF new	17.54	2.20	1.25	0.26

For Danube sediment, Bayreuth soil and Compost UNSPMF (new): No or almost no HNS in the samples. Therefore, the measurements here are below the detection limit.

ASE extraction of materials

The aim of the experiment was to determine the presence of phthalates in different soils and organic amendments, to select materials that will be further used for leaching experiment. Materials which was used are:

1. *Sludge UNSPMF*
2. *Compost UNSPMF*
3. *Compost Vienna*
4. *Compost Thorsten*
5. *Cow manure*
6. *Bayreuth soil*

Before the experiment itself, a recovery test was performed.

1. ***Procedure for ASE recovery test***

- ➔ Preparation of dishes and ASE cilindres (all glassware were cleaned with ultra-pure water, dried and rinsed with acetone, and after that were heated at 550ºC for six hours in a muffle oven); ASE cilindres were cleaned with soap and water, ultra-pure water, dried in oven 3 hours and rinsed with acetone in ultrasonic bath);
- ➔ 3 blanks (each of them has 15 g of sand and 1ug/ml DEHP-d4)
- ➔ 3 samples (15 g of sand + 1ug/ml DEHP-d4 + 5ug DEHP)
- ➔ Samples are collected in 60 ml vials
- ➔ After ASE measurment samples are concetrated to 1 ml (in 20 ml vials))
- ➔ Measuring on GC-MS/MS

Result: 85%

Figure 3. ASE coloumn prearation

After a successful recovery test of 85%, soil extraction was performed in triplicate.

2. ***Procedure for extraction of OSA using ASE***

A defined amount of each organic soil amendment is weight into an ASE extraction cell. For the quantification of DEHP, 1 µg of DEHP-d4 was added to each OSA.

Depending on the weight of the materials, different masses were added. Table of how much of each OSA was used is in Table 1:

Table 1: Masses of materials used in ASE extraction experiment

Materials	*Mass*
1. Compost UNSPMF	4 g
2. Sludge UNSPMF	6 g
3. Compost UNIVIE	3 g
4. Compost from Thorsten	5 g
5. Cow manure	2 g
6. Soil Bayreuth	10

Extraction using ASE following EPA protocol. After ASE extraction samples are collected in 60 ml vials and it was found that sample purification is necessary (clean-up experiment).

The soil samples were dirty and, as such, could not be subjected to further analysis. The sample purification procedure described below was applied.

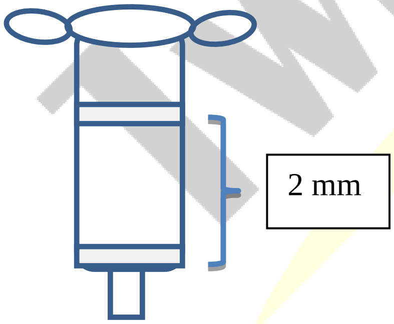
Figure 4. Soil extracts after ASE extraction

Clean-up procedure was done in syringes, so the recovery test of the syringes was done first.

3. ***Procedure for recovery test for clean-up experiment (ASE extracts)***

✓ Preparation of glass cartridge (=syringe):

- ➔ Glass cartridges are cleaned with MQ water and muffled 5h at 550 °C
- ➔ Cartridges are packed with a 1:1 mixture of silica gel (also muffled 5h at 550 °C) and Na_2SO_4



✓ <u>Recovery test: conducted in triplicates-**RT1**</u>

- ➔ Blanks: 1 ug DEHP-d4 spiked to 20 mL of n-hexane in a 60 mLvials

- ➔ Sample: 1 ug DEHP-d4 and 5 µg of DEHPare spiked to 20 mL of n-hexane in a 60 mL vials
- ➔ Blanks and samples are poured in a glass cartridge and the extracts are collected in 20 mL vials. Afterwards, extracts are concentrated to 1 mL. Samples are stored in the fridge until measurement.
- ➔ DEHP is quantified using GC-MS/MS

Results: we cannot indetify DEHP

- ✓ <u>Repeat recovery test with **reduce amount of silica gel** and sodium sulfate and **adding preconditioning with hexane**: conducted in triplicates-**RT2**</u>
  - ➔ Blanks: 1 ug DEHP-d4 spiked to 20 mL of n-hexane in a 60 mL vials
  - ➔ Sample: 1 ug DEHP-d4 and 5 µg of DEHP are spiked to 20 mL of n-hexane in a 60 mL vials
  - ➔ Hexane is used for preconditioning (10 mL) and collected in separate vials. After that blanks and samples are poured in a glass cartridge and the extracts are collected in 20 mL vials. Afterward, extracts are concentrated to 1mL. Samples are stored in the fridge until measurement.
  - ➔ DEHP is quantified using GC-MS/MS

Results: we still cannot identify DEHP

Figure 5. Clean-up procedure for ASE soil extracts

- ✓ Repeat the recovery test with **reduce amount of silica gel** and sodium sulfate an**d using instead of filters**: conducted in triplicates-**RT3**
  - ➔ Blanks: 1 ug DEHP-d4 spiked to 20 mL of n-hexane in a 60 mL vials
  - ➔ Sample: 1 ug DEHP-d4 and 5 µg of DEHP are spiked to 20 mL of n-hexane in a 60 mL vials
  - ➔ Hexane is used for preconditioning (10 mL) and collected in separate vial. After that blanks and samples are poured in a glass cartridge and the extracts are collected in 20 mL vials. Afterwards, extracts are concentrated to 1 mL. Samples are stored in the fridge until measurement.
  - ➔ Cotton wool is used instead of filters
  - ➔ DEHP is quantified using GC-MS/MS

Results: we cannot identify DEHP

- ✓ Repeat recovery test with **reduced amount of silica gel** and sodium sulfate and **using cotton wool instead of filters**: conducted in triplicates and **add postconditioning with hexane-RT4**
  - ➔ Blanks: 1 ug DEHP-d4 spiked to 20 mL of n-hexane in a 60 mL vials
  - ➔ Sample: 1 ug DEHP-d4 and 5 µg of DEHP are spiked to 20 mL of n-hexane in a 60 mL vials
  - ➔ Hexane is used for preconditioning (10 mL) and collected in separate vial. After that blanks and samples are poured in a glass cartridge. Postconditioning was done with 10 mL hexane and the extracts are collected in 20 mL vials. Afterwards, extracts are concentrated to 1 mL. Samples are stored in the fridge until measurement.
  - ➔ Watte is used instead of filters
  - ➔ DEHP is quantified using GC-MS/MS

Results: we can not identify DEHP

After conducting these measurements, it was determined that the identification of DEHP was not possible. Due to the limited time available during the visit, there was no opportunity to develop a new procedure for sample extraction. As a result, we decided to proceed with the experiment without further delay.

Organic matter extraction from OSA

Next step was organic matter extraction from OSA for PVC leaching experiments. First, a recovery test was performed.

1. **Procedure for recovery test- plastic tubes, background test**

➔ -blue (17000 g) and green tubes test (20000g)
➔ -rinsed 3 times with isopropanol and 1 time with MQ water
➔ -filled with MQ water (not completely) and left on the shaker for the weekend
➔ -air dried 1 day
➔ -cleaned with hexane (10 ml) and shaked 1 hour
➔ -transfered to 20 ml vial + 1ug DEHP-d4 and concentrated to 1 ml
➔ -measured on GC-MS/MS

2. **OSA extraction-procedure**

Sludge UNSPMF, compost UNIVIE and cow manure are used. Also one blank with MQ water

➔ Crush samples (30 Hz-20s)

➔ Concetration of samples was 50 g/l in MQ water (leave headspace in tubes for shaking)
➔ 24h shaking
➔ Centrifuge samples 2h in 17 000g and filtered them (0,22µm filters)
➔ Left samples on dialyzing (with a regenerated cellulose membrane <1kD)- cuted membranes, left in MQ water 30 min and rinsed inside with MQ water because of presence of sodium-azide
➔ After that put clip on one side and samples were added with glass pipets. Other clip was added and left sample in water on magnetic stirrer (about 300 RPM)
➔ Water was changed twice in day till conductivity is not <1µS/cm
➔ Measured in TOC (Shimadzu)-DOC

Materials to be used in leaching were selected based on their characteristics: ***sludge UNSPMF, compost UNIVIE and cow manure*** and one blank sample (MQ water).

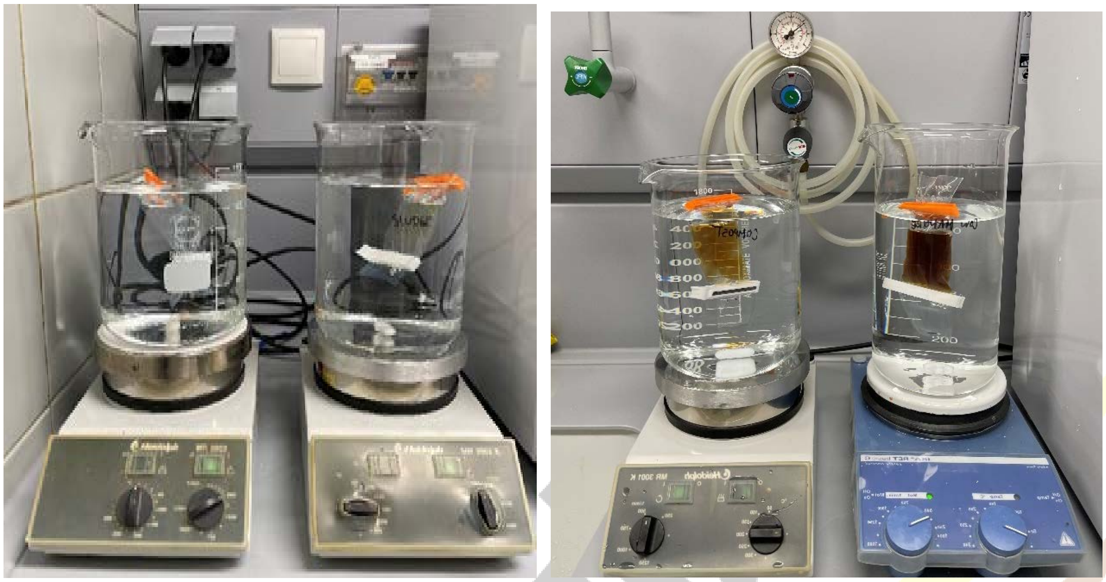


Figure 6. Dialyzing procedure

Concetration of DOC (mg C/l) after dyalizing	
Blank sample	3.815
Sludge UNSPMF	390
Compost UNIVIE	303
Cow manure	496

Leaching experiment

1. Preparation of infinite sinks

Today, PVC is one of the most commonly used polymers in the production of plastics, and it contains a high amount of additives, i.e. phthalates. Therefore, it is necessary to monitor the kinetics of potential leaching of additives from plastics, especially in aqueous environments. Taking into account everything mentioned, in this experiment, the leaching of additives from PVC in aqueous solutions of organic soil amendments was monitored.

To investigate the leaching of phthalates from PVC into the aqueous solutions of organic soil amendments, infinite sink method was used. This method was developed at UNIVIE.

First, all glassware were cleaned with ultra-pure water, dried and rinsed with acetone, and after that were heated at 550ºC for six hours in a muffle oven. Everything was closed with caps equipped with teflon septa. The infinite sink consisted of 10 mg activated carbon powder packed in a 4 cm x 4 cm piece of Grade 50 filter paper. A copper wire kept the rectangular infinite sink in shape and increased the mechanical stability.

2. Leaching experiment- set up

Based on results DOC, 4 solutions were prepared for leaching test:

A-KCl
B-sludge UNSPMF
C-compost UNSPMF
D-cow manure

Leaching points: 1, 5, 8, 15, 45 and 60 days (in triplicates)

The infinite sink was added to a 60 mL glass vial filled with 40 mL of the 1 mM KCl solution as a control and also in solutions of organic soil amendments (sludge UNSPMF, compost Vienna and cow manure). In order to achieve a equilibration between sinks and solutions, the vials with the infinite sinks were placed on a horizontal shaker (in dark to prevent photodegradation) at room temperature and 125 rpm for a minimum of 12h. An infinite sink should take up phthalates from the aqueous phase and keep their concentration well below the solubility.

A spiking experiment provided the information on the capacity and velocity of the infinite sink to remove phthalates from the aqueous phase. 20 µL of a 500 µg mL $^{-1}$ DEHP standard were spiked into the KCl and soil amendments solutions containing the infinite sink. For the leaching experiment, three PVC pellets (about 85 mg) were added to each vial. The pellets should have a similar size,weight and shape and thus asimilar surface area. The vials with the pellets were horizontally shaken at 125 rpm and room temperature until sampling. To determine the method blank value, samples containing only the background solution and the infinite sink were prepared.

3. *Sampling:*

- ➔ The pellets and infinite sinks are removed from the water phase with tweezers
- ➔ For the quantification of phthalates in the infinite sinks and the water phase were spiked 20µl of deuterated 50 µg mL $^{-1}$ internal standard DEHP-d4
- ➔ Sinks are dried overnight in oven at 40ºC. After that dried sinks are transferred from oven to desiccator and stored until ASE extraction

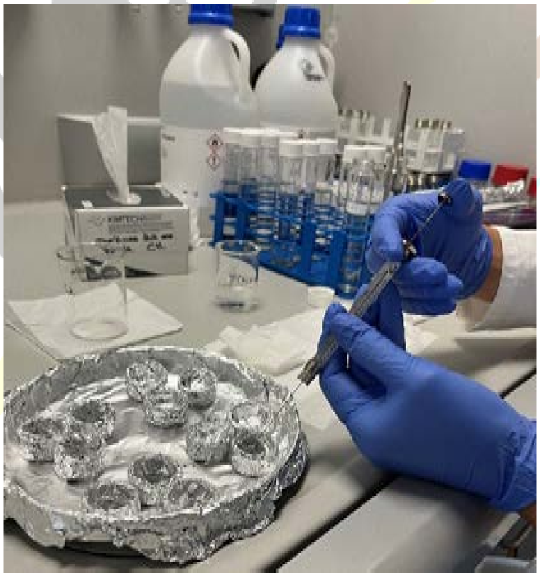


Figure 7. Preparation of samples for quantification of phthalates in the infinite sinks

4. ***Extraction***

- Solid phase extraction

The infinite sinks were extracted using Accelerated Solvent Extraction (ASE 200, Thermo Fisher, Waltham, US). The extraction was conducted in two cycles at 120ºC and 40 bar for seven minutes in 11 mL cylinder bodies using n-hexane as a solvent. The solid phase extraction for each sample resulted in about 30 mL of n-hexane extract, which was transferred in portions of 15 mL into a 20 mL glass vial and concentrated before the refill.

- Liquid-liquid extraction

To extract DEHP from the aqueous phase 5 mL of n-hexane were added. The vial containing both phases was vigorously shaken by hand for 2 min and placed in a vial rack. After the water and n-hexane phase separated, the n-hexane phase was transferred into a 20 mL glass vial. The extraction was repeated three times and the n-hexane extracts were pooled in the same 20 mL vial. The n-hexane extracts were concentrated to 1 mL using a laboratory evaporator (Barkey vapotherm basic mobil I, Leopoldshöhe, Germany) at 40ºC and nitrogen aeration. The concentrated extracts were transferred into 1.5 mL brown glass measurement vials and stored at 4 ºC until measurement.

Instruments measuring

GC-MS/MS (Agilent)- *Quick guide*

Principle of Gas chromatography is a separation technique for volatile compounds, favoured for non-polar compounds. Samples analyzed by GC-MS/MS are separated in a gaseous state. The sample is either a gas or a liquid that is vaporized in the injection port. The mobile phase for gas chromatography is a carrier gas, typically helium because of its low molecular weight and chemical inertness (He 5.0 in our system). The pressure is applied and the mobile phase moves the analyte through the column. The separation is based on the various physical and chemical properties of analytes and their interaction with the analytical column's stationary phase.

Principle of Mass Spectrometry Mass Spectrometry (MS) is an analytical technique that identifies the amount and type of chemicals by measuring the mass-to-charge ratio and abundance of gas-phase ions. • A sample is converted to rapidly moving positive ions by electron bombardment and charged particles are separated according to their mass-to-charge ratio.

Principle of Tandem Mass Spectrometry (MS/MS) • Tandem Mass Spectrometry is a detection technique that ionizes and fragments molecules. • Upon exiting the analytical column, the analytes enter the tandem mass spectrometer (MS/MS) which consists of two scanning mass analyzers separated by a collision cell. • Fragments selected in the first analyzer(= first quadrupole) (precursor ions) react with an inert gas in the collision cell, resulting in further fragmentation (daughter/product ions). • These daughter/product ions are then resolved in the third quadrupole for analysis. • Both precursor and product ions are measured which increases the specificity and selectivity of this detection technique.

ASE (Thermo Scientific Dionex) – Quick guide

Accelerated solvent extraction (ASE) is an automated extraction technique that uses elevated temperatures and pressures to extract samples in a very short time, using fewer solvents than traditional extraction techniques (Soxhlet, microwave extraction, or sonification). Higher temperature increases the capacity of solvents to solubilize analytes as well as analyte diffusion rates. Higher pressure allows the use of temperatures above the solvent’s boiling point and ensures a better contact of the solvent with analytes. To perform an extraction, (homogenized and dry) solid sample is loaded into a sample cell and the end caps (each containing a frit) are tightened by hand onto the cells. The filled sample cells are loaded onto a cell tray and collection vessels onto a collection tray. A robotic arm transfers each cell separately into the oven for extraction.

Sample preparation Samples must be dry, finely grounded and homogenized before extraction. This may not be valid for some samples, such as POM, microplastic pellets etc. This is valid e.g., soils and sediments, biochar, SPM and similar (the latter is already fine-grained and needs to be just dried and homogenized).

Selection of solvents: The solvent must be able to solubilize the target analytes while leaving the sample matrix intact. The polarity of the extraction solvent should closely match that of the target compounds. Generally, the more polar the solvent or solvent mixture, the less selective it will be.

What should be finish during one-month visit:

- ASE extraction: B, C and D solvent for each point and A solvent for 45 and 60 days (about 70 samples)
- Concetration of samples after ASE and samples after LL extraction (about 140 samples)
- Measuring everything on GC-MS/MS
- 1:10 experiment with Irina

Researcher: MSc Sanja Vasiljević, UNSPMF

Assigned supervisor: Thorsten Hüffer, UNIVIE

Duration of the visit: 30.10.2023-24.11.2023.

Executive Summary

The main goal of this visit was to finish measurements on leaching dynamics of plastic-derived additives, with a special focus on PVC-derived phthalates. Measurements were performed on the ASE. DEHP and its transformation products leaching from PVC were quantified using a gas chromatograph coupled to a triple quadrupole mass spectrometer (GC-MS/MS). The experimental data will allow for an interpretation of the impact of OSA extract on the governing mass transfer process for leaching using models for intraparticle diffusion and aqueous boundary layer diffusion.

The main knowledge transfer was focused on:

- Finishing data point (30 days) on leaching and long-term dynamics of plastic-derived additives, with a special focus on PVC-derived phthalates
- ASE extraction
- Measurements on GC-MS/MS
- Data analysis

1. *Leaching experiment*

(Completion of the final leaching point from the 30-days period)

Based on the results from DOC analysis, 4 solutions were prepared for the leaching test:

A-KCl
B-sludge UNSPMF
C-compost UNIVIE
D-cow manure

Table 1. Samples used in experiment and DOC values

Solutions	DOC (mg C/L)
Blank sample	3.815
Sludge UNSPMF	390
Compost UNIVIE	303
Cow manure	496

The infinite sink was added to a 60 mL glass vial filled with 40 mL of either the 1 mM KCl solution as a control or the background solutions from the organic soil amendments (sludge UNSPMF, compost UNIVIE and cow manure). To achieve an equilibration of infinite sinks in solutions, the vials with the infinite sinks were placed on a horizontal shaker (in dark to prevent photodegradation) at room temperature and 125 rpm for a minimum of 12 h. An infinite sink should take up phthalates from the aqueous phase and keep their concentration well below solubility.

A spiking experiment provided information on the capacity and velocity of the infinite sink to remove phthalates from the aqueous phase. 20 µL of a 500 µg mL $^{-1}$ DEHP standard were spiked into the KCl and soil amendments solutions containing the infinite sink. For the leaching experiment, three PVC pellets (about 85 mg) were added to each vial. The pellets should have a similar size, weight, and shape and thus a similar surface area. The vials with the pellets were horizontally shaken at 125 rpm and room temperature until sampling. To determine the method blank value, samples containing only the background solution and the infinite sink were prepared.

1. ***Sampling:***

- ➔ The pellets and infinite sinks were removed from water phase with tweezers.
- ➔ For the quantification of phthalates in the infinite sinks and the water phase were spiked 20 µL of deuterated 50 µg mL^{-1} internal standard DEHP-d4.

Figure 1. Sampling of pellets and infinite sinks from water

- ➔ Sinks were dried overnight in the oven at 40ºC. Dried sinks were transferred from the oven to an desiccator and stored until ASE extraction

Figure 2. Infinite sinks after sampling

2. *Extraction*

➢ Solid phase extraction

The infinite sinks were extracted using Accelerated Solvent Extraction (ASE 200, Thermo Fisher, Waltham, US). The extraction was conducted in two cycles at 120ºC and 40 bar for seven minutes in 11 mL cylinder bodies using n-hexane as a solvent. The solid phase extraction for each sample resulted in about 30 mL of n-hexane extract, which was transferred in portions of 15 mL into a 20 mL glass vial and concentrated before the refill.

Figure 3. ASE extraction

➢ Liquid-liquid extraction

To extract DEHP from the aqueous phase 5 mL of n-hexane were added. The vial containing both phases was vigorously shaken by hand for 2 min and placed in a vial rack. After the water and n-hexane phases separated, the n-hexane phase was transferred into a 20 mL glass vial. The extraction was repeated three times and the n-hexane extracts were pooled in the same 20 mL vial. The n-hexane extracts were concentrated to 1 mL using a laboratory evaporator (Barkey vapotherm basic mobil I, Leopoldshöhe, Germany) at 40ºC and nitrogen aeration. The concentrated extracts were transferred into 1.5 mL brown glass measurement vials and stored at 4 ºC until measurement.

2. *ASE extraction*

ASE (Thermo Scientific Dionex) – Quick guide

Accelerated solvent extraction (ASE) is an automated extraction technique that uses elevated temperatures and pressures to extract samples in a very short time, using fewer solvents than traditional extraction techniques (Soxhlet, microwave extraction, or sonification). Higher temperature increases the capacity of solvents to solubilize analytes as well as analyte diffusion rates. Higher pressure allows the use of temperatures above the solvent's boiling point and ensures a better contact of the solvent with analytes. To perform an extraction, (homogenized and dry) solid sample is loaded into a sample cell and the end caps (each containing a frit) are tightened by hand onto the cells. The filled sample cells are loaded onto a cell tray and collection vessels onto a collection tray. A robotic arm transfers each cell separately into the oven for extraction.

Sample preparation Samples must be dry, finely grounded and homogenized before extraction. This may not be valid for some samples, such as POM, microplastic pellets etc. This is valid e.g., soils and sediments, biochar, SPM and similar (the latter is already fine-grained and needs to be just dried and homogenized).

Selection of solvents: The solvent must be able to solubilize the target analytes while leaving the sample matrix intact. The polarity of the extraction solvent should closely match that of the target compounds. Generally, the more polar the solvent or solvent mixture, the less selective it will be.

1. *Procedure for ASE*

- ➔ All glassware were cleaned with ultra-pure water, dried and rinsed with acetone, and after that heated at 550ºC for six hours in a muffle oven); ASE cylinders were cleaned with soap and water, ultra-pure water, dried in an oven for 3 hours, and rinsed with acetone in ultrasonic bath).

Figure 4. Preparation of ASE cylindres

- ➔ Dried infinite sinks were placed into ASE cylinders
- ➔ Samples were collected in 60 ml vials
- ➔ After ASE measurement samples were concetrated to 1 mL (in 20 mL vials)
- ➔ Measurements were performed on GC-MS/MS

3. GC-MS/MS measurements

GC-MS/MS (Agilent)- *Quick guide*

Principle of gas chromatography is a separation technique for (semi-)volatile compounds, favored for non-polar compounds. Samples analyzed by GC-MS/MS are separated in a gaseous state. The sample is either a gas or a liquid that is vaporized in the injection port. The mobile phase for gas chromatography is a carrier gas, typically helium because of its low molecular weight and chemical inertness (He 5.0 in our system). The pressure is applied and the mobile phase moves the analyte through the column. The separation is based on the various physical and chemical properties of analytes and their interaction with the analytical column's stationary phase.

Figure 5. GC-MS/MS measurements

Principle of Mass Spectrometry Mass Spectrometry (MS) is an analytical technique that identifies the amount and type of chemicals by measuring the mass-to-charge ratio and abundance of gas-phase ions. A sample is converted to rapidly moving positive ions by electron bombardment and charged particles are separated according to their mass-to-charge ratio.

Principle of Tandem Mass Spectrometry (MS/MS) Tandem Mass Spectrometry is a detection technique that ionizes and fragments molecules. Upon exiting the analytical column, the analytes enter the tandem mass spectrometer (MS/MS) which consists of two scanning mass analyzers separated by a collision cell. Fragments selected in the first analyzer (= first quadrupole) (precursor ions) react with an inert gas in the collision cell, resulting in further fragmentation (daughter/product ions). These daughter/product ions are then resolved in the third quadrupole for analysis. Both precursor and product ions are measured which increases the specificity and selectivity of this detection technique.

4. Data analysis

1. Quantification of DEHP

DEHP was quantified using a GC 8890 coupled to a triple quadrupole MS 7000 (both Agilent Technologies, Santa Clara, U.S.) and a PAL 3 (RSI 120) autosampler (CTC Analytics AG, Zwingen, Switzerland). The injection volume was 1 µL. The MM Inlet mode was split (15:1) with an initial temperature of 70 °C followed by a ramp of 900 °C min^{-1} to 300 °C. Two HP-5ms ultra inert columns (15 m x 250 µm x 0.25 µm, Agilent Technologies, Santa Clara, U.S.) were used for the chromatographic separation and Helium 5.0 (Linde Gas GmbH, Stadl-Paura, Austria) served as carrier gas. The column flow was 1.4 mL min^{-1} for the first column and 1.5 mL min^{-1} for the second column. The oven temperature was 40 °C held for 2 min, followed by a ramp of 50 °C min^{-1} to 300 °C held for 4 min. The MS/MS parameters were as follows: the EI-source temperature was 230 °C, the electron energy was 70 eV. All transitions are given in **Table 2**.

Table 2. MS parameters for the quantification of DEHP. The transitions used as quantifiers are indicated by an asterisk.

Compound	**Precursor ion (m/z)**	**Product ion (m/z)**	**Collision energy (V)**	**Retention time (min)**
DEHP	279	149	16	8.6
DEHP	149	121	18	8.6
DEHP	149	93	21	8.6
DEHP*	149	65	30	8.6
DEHP-d4	283	153	20	8.6
DEHP-d4	153	125	17	8.6
DEHP-d4	153	97	23	8.6
DEHP-d4*	153	69	29	8.6

2. ***Data analysis:***

Conducting the leaching experiments resulted in the mass of phthalates in the infinite sink M_{sink} (µg) and in water M_w (µg) for each sampling time. The total mass of DEHP leached is therefore calculated as the sum of both: $M_{leach}=M_{sink} + M_w$ and will be calculated for each OSA solution. Time-dependent leaching curves will be obtained by plotting M_{leach} (y-axis) versus the respective sampling time (x-axis). Since the leaching of DEHP from PVC microplastic is slow, we expect that leaching curve scan be described by linear regression lines; the slope of the leaching curves gives the leaching rate in µg d^{-1}.

To identify the limiting mass transfer process, time-dependent leaching curves for DEHP into different OSA solutions will be evaluated using models for internal and external mass transfer, that is, intraparticle diffusion (IPD) and aqueous boundary layer diffusion (ABLD). The leaching of DEHP into 1 mM KCl is limited by ABLD. Adding 5 mg L^{-1} DOC (using extracts from different OSA) to the system will most likely not change the limiting diffusion process: we expect ABLD to remain the limiting mass transfer process for the leaching of DEHP from PVC microplastics. For fitting the ABLD model, an aqueous diffusion coefficient of $4.45*10^{-10}$ m^2s^{-1} and an aqueous boundary layer thickness of 38.4 µm, determined in an earlier study will be used. $K_{PVC/W}$ will be used as a fitting parameter. Fitted $K_{PVC/W}$ and the experimentally determined SUVA data for each OSA will be used to identify the influence of each OSA on leaching. Details on each step of the data analysis are described in Henkel et al., 2022.

References

Henkel C., Hüffer T., Hofmann T. (2022) Polyvinyl Chloride Microplastics Leach Phthalates into the Aquatic Environment over Decades, Environ. Sci. Technol. 2022, 56, 20, 14507–14516.

MOBILITY REPORT

Researcher: Dr. Irina Jevrosimov, UNSPMF

Assigned supervisor: Dr. Gabriel Sigmund, UNIVIE

Duration of the visit: 02.03.2023. - 25.08.2023.

Executive Summary

The main purpose of the visit to University of Vienna was to learned and researched targeted extraction and analysis of OSA-derived organic contaminants and assessing their dynamics in OSA and OSA-soil mixtures. Also, one of the puposes was to investigated mobility of selected pollutants (pesticides, pharmaceuticals) through soil within sorption. The goal was to learn development and application of tailored extraction procedures and targeted analysis via LC-MS/MS. Mobilizable fractions from aqueous elution experiments will be cleaned up and prepared for subsequent measurement via high-performance liquid chromatography – mass spectrometry (HPLC-MS) aiming to identify selected organic contaminants. In addition to the pure OSAs, both control soils used consistently throughout TwinSubDyn will also be intermixed with organic soil amendments (OSAs) to investigate how the easily leachable contaminant fraction from OSA is affected by soils. These investigations was done using the same OSA amendment rate in both soils (e.g., 2%). Control experiments for elution from unamended soils was complement the measurements for better interpretability of the results. During of the visit at University of Vienna Irina was performing experiments on the sorption and mobility of OSA derived organic contaminants. These investigations had a particular focus on ionizable organic contaminants which are of special interest because of their complex sorption behavior and often higher mobility compared to neutral compounds of similar size and structure. Sorption parameters of compounds were determined at different environmentally relevant conditions (pH=7 , ionic strength 1.5 mmol/L and pH=3, ionic strength 89.2 mmol/L) using sorbent (soil + OSA) packed HPLC columns. Additionaly, the enzymatic transformation experiments of selected organic contaminants was investigated.

Introduction

Background

The increasing use of pharmaceuticals and pesticides causes great concern because of their behavior and fate in the environment, and also because of the possibility of their biodegradation by-products. A large number of these compounds can be found in the soil, so it would be very interesting to investigate the effect of the addition of organic amendments to the soil on the fate and behavior of these compounds.

Generally, organic soil amendments (organic wastes) are considered the main source of plant nutrients, especially nitrogen (N). In addition, organic soil amendments (OSA) provide macro- and micronutrients, including carbon for the restoration of soil physical and chemical properties. Certain organic soil amendments contain a large number of pollutants that can be harmful to human health. A large number of organic soil amendments include compost, sewage sludge, manure from animals, etc. All the mentioned OSA are natural sources of carbon (C) for microorganisms, where certain species can be pathogenic for humans. The main reason for applying organic soil amendments is to improve the physical and chemical properties of soil and decreasing concentrations of many pollutants which are present in the soil.

The main objectives of this investigation was:

- ✓ Characterization of soils and organic soil amendments
- ✓ Biotransformation of selected organic pollutants in enzyme extracts
- ✓ Investigation of the mobility of selected organic compounds using HPLC column tests with and without the addition of organic amendments to the soil

Scope of the secondment

The scope of the secondment was transfer knowledge from UNIVIE to UNSPMF in the case novel approach to determine sorption will be a key skill and method. In addition, also learning biotransformation experiments of organic pollutants in enzyme extracts. Biotransformation experiments enabled an estimation of biological degradability of OSA derived organic pollutants.

Content

Methods

Characterization of selected materials (soils and organic soil amendments)

Elementar Analyzer (CHNS)

The vario MACRO in CHNS mode analyzes the C, H, N, and S content of a dry sample. Sample (in mg range) is mixed with a catalyst (WO_3) in tin foil sample boats, folded, weighted and placed on a rotating carousel. The samples are transferred to the furnace area using a ball valve that rotates to receive a sample pushed towards it by the carousel. Any outside air is removed using helium and the sample is then transported to the combustion tube. This tube contains an ash crucible with WO_3 granulate at the bottom, where the sample drops into. The combustion tube also contains WO_3 granulate that sits below the ash crucible (separated from it by corundum balls). The combustion tube is maintained at a temperature of 1100°C, and oxygen is dosed for a set time for the following gaseous reaction products to form from the elements of interest: CO_2, H_2O, N_2, NOx, SO_2, SO_3. Following the analysis of this final element, a cooling fan reduces the temperatures of the adsorption columns so that they are ready for the next sample. It takes approximately 11 minutes to analyze each sample. Samples need to be perfectly dry.

Carbon analyzer (LECO)

The TOC analyzer RC-612 from LECO is used to determine the total carbon content (TC), the total inorganic carbon content (TIC), the total organic carbon content (TOC), and the water content of an unknown sample. The sample is burnt at 550°C in a quartz tube to determine TOC or at 1000°C to determine TC. For an analysis of an unknown sample, a temperature program with a slow heating curve can be chosen. Increasing the temperature step-wise causes different carbon forms to oxidize at a respective temperature. By adding oxygen, the released carbon is oxidized to CO_2. For complete oxidation, the use of CuO as a catalyst and an afterburner is needed. For detection, an infrared (IR) detector is used. The present carbon of the converted gaseous sample absorbs the radiation of the IR source while passing through the measurement cell. Quantification is done by measuring extinction by the Lambert-Beer law. The measurement of the water vapour is performed analogue. The amount of the available CO_2 and H_2O can be detected continuously and simultaneously. Two differently long CO_2 measurement cells can be installed in the RC-612 (LECO), which have to be separately calibrated depending on the expected amounts of the respective samples.

Figure 1. *Measurements on LECO analyzer*

TOC-L (Shimadzu)

The TOC-L instrument measures the amount of total carbon (TC), inorganic carbon (IC) and total organic carbon (TOC) in a liquid sample. If the water sample is filtered through a 0.45 µm or 0.20 µm filter membrane, the dissolved organic carbon (DOC) content is measured. TOC or DOC concentrations are measured by direct measurement, which is called non-purgeable organic carbon (NPOC) method. By using the NPOC method, firstly, the sample will be acidified to pH<2 with hydrochloric acid (HCl). This can be done automatically by the system or also manually during sample preparation. Then, the sample will be automatically sparged by the carrier gas (synthetic air) for 150 seconds. The sparging causes the inorganic carbon (IC) to transform into CO_2, which is then released from the sample. Sparging can take place in a 5 mL syringe installed in the inside of the instrument or by an external sparging option. The remaining organic fraction (NPOC) will be detected by catalytic combustion oxidation with an NDIR-detector. There is also the possibility to use the TC-IC method to determine the TC, IC and TOC concentration of a respective sample. It measures the amount of IC evolved from an acidified aliquot of a sample and also the amount of TC present in the sample. TOC is calculated by subtraction of the IC value from the TC in the sample. This method is not installed at the moment.

UV-vis (PerkinElmer)

The Lambda 35 is a versatile spectrometer operating in the ultraviolet (UV) and visible (Vis) spectral ranges. The spectrometer features a double-beam and an all-reflecting system. UV-vis spectrometry is used to obtain the absorbance spectra of a compound in solution. What is being observed spectroscopically is the absorbance of light energy or electromagnetic radiation, which excites electrons from the ground state to the first singlet excited state of the compound or material. The UV-vis region relates to a wavelength range of 800 nm to 200 nm. The Beer-Lambert Law is the principle behind absorbance spectroscopy. For a single wavelength, A is absorbance (unitless), ε is the molar absorptivity of the compound or molecule in solution (M-1 cm^{-1}), b is the path length of the cuvette or sample holder (usually 1 cm), and c is the concentration of the solution: $A = \varepsilon bc$ UV-vis spectroscopic data can give qualitative and quantitative information of a given compound or molecule. Irrespective of whether quantitative or qualitative information is required it is important to use a reference cell to zero the instrument for the solvent the compound is in. For quantitative information on the compound, calibrating the instrument using known concentrations of the compound in question in a solution with the same solvent as the unknown sample would be required.

pH meter

A pH meter is an instrument used to measure hydrogen ion activity in solutions (this instrument measures acidity/alkalinity of a solution). The degree of hydrogen ion activity is ultimately expressed as pH level, which generally ranges from 1 to 14.

Biotransformation experiments

Within these experiments the investigation of biotransformation of selected organic pollutants (melamine, 2,4 DB, citalopram and carbamazepine) in enzyme extracts of two soils (Bayreuth soil, Danube sediment) and two organic soil amendments (sludge UNSPMF and cow manure) was done.

Enzyme extraction

Protocol:

Water extraction:

Weigh 5g (oven dry basis) of soil into a 50 mL tube and fill up to 50mL with distilled water, close the bottle and place the bottle on a rotational shaker for 1h.

Sample preparation:

2. Take the sample off the shaker and sonicate for 300s at P 30W (pulse on 4s, pulse off 2s).

3. Centrifuge the sample at 7000g for 10min at RT.

4. Vacuum filtrate the sample (Whatman GF/F, glass microfiber filters, diameter: 47 mm) and transfer the cleaned up supernatant into fresh 50 mL tubes (roughly 45 mL) or split into amounts of your choosing.

Autoclavation:

5. Take the sample designated for autoclavation and place them without a cap into the autoclave.

6. Autoclave for 2h at 121°C.

pH measurements:

7. Measure the pH of all samples.

8. Store the samples in the freezer until further use.

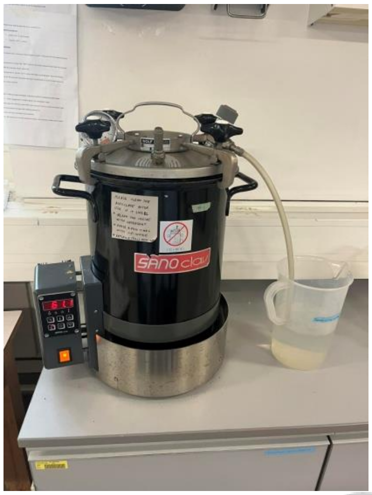


Figure 2. *Autoclave*

Biotransformation of selected organic pollutants in enzyme extracts

Protocol:

Solutions preparation :

1) Prepare a 1% formic acid solution in acetonitrile. Add 1 mL of formic acid (100%) to 99 mL of acetonitrile (LC-MS grade).

Sample preparation for the shaker:

2) Prepare samples for the shaker: Add 10 uL (10000 ug/L) of the mixture of selected compounds into 2 mL Eppendorf tubes.

3) Afterwards, add 1 mL of the enzyme extract to the dried mixture of selected compounds.

4) Place the tubes on the horizontal shaker: 300 rpm, leave on infinite time

Sample preparation for the LC-MS/MS analysis:

5) Prepare 1.5 mL collecting tubes. Add 450 uL of 1% FA/ACN in the collecting tubes and add 150 uL of the samples from the shaker after the desired time periods (T0, T1, T2,…).

6) Place the collecting tubes in a centrifuge at 16000 rpm, for 1 min at RT.

7) Prepare additional 1.5 mL Eppendorf tubes to collect the supernatant. Transfer 550 uL of the supernatant into the fresh tubes.

8) Place the tubes in the centrifugal evaporator (45°C, AL) for around 1-3h till the liquid evaporates completely.

Figure 3. *Centrifugal evaporator*

9) Resuspend the formed pellet in 150 uL of (MeOH+0.1%FA) and vortex until the pellet dissolves.

10) Transfer the sample into the brown LC-MS/MS vials with inlets. Keep in the fridge until measurement.

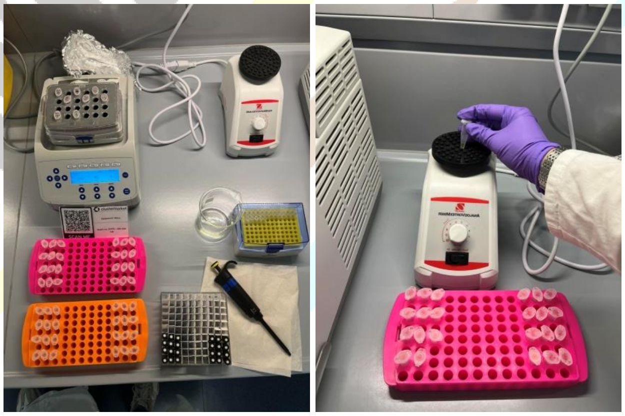


Figure 4. *Sample preparation for analysis on LC-MS/MS (biotransformation experiments)*

LC-MS/MS

High Performance Liquid Chromatography (HPLC) HPLC is used to separate (in a liquid state), identify, and quantify analytes within a sample. The method relies on pumps to pass a pressurized liquid solvent (or a solvent mixture), called “mobile phase” through a column (called “stationary phase”). The sample is added to the mobile phase and flows through the column. The separation is based on the various physical and chemical properties of analytes and their interaction with the column’s stationary phase. The time for an analyte to elute from the column is called retention time, and is an identifying characteristic of a given analyte. In normal-phase chromatography a non-polar mobile phase is used in conjunction with a polar stationary phase. Reversed-phase chromatography (RP-HPLC), which is currently applied within our system, refers the use of a polar mobile phase and a non-polar (hydrophobic) stationary phase. In the RP-HPLC the common mobile phases are acetonitrile and methanol but any combination of water with organic solvents is often used, depending on the sample properties. Acetonitrile is often used because of its low UV cutoff, lower viscosity (methanol forms highly viscous mixtures with water at certain concentrations), and higher boiling point. Buffers/acids (such as formic acid, acetate) can be added to the aqueous phase. Usually 0.1% formic acid is added (pH ≈ 2.7) in order to provide a suitable environment for the ionization of the analytes in the MS when measuring in positive mode, and to control pH, which results in reproducible retention times, and thus better separation of analytes in the sample.

The composition of the mobile phase can stay constant during the analysis (isocratic elution mode) or can be changed (gradient elution mode). Gradient elution is used for samples that contain analytes with similar affinities for the column’s stationary phase, while isocratic elution is used for analytes with highly varying physiochemical properties. When using isocratic elution make sure that your analyte completely elutes from the system (i.e., carefully optimize the eluent composition depending on your analyte and verify complete elution by running blank measurements with gradient elution). When using gradient elution make sure that the last composition of the mobile phase matches that of the starting composition and that these changes are gradual and not abrupt. This is important for maintaining column performance. You can specify this either within the method gradient, or you can use the “stop time” option (in the method editor) by editing the time during which the mobile phase composition goes to the starting one.

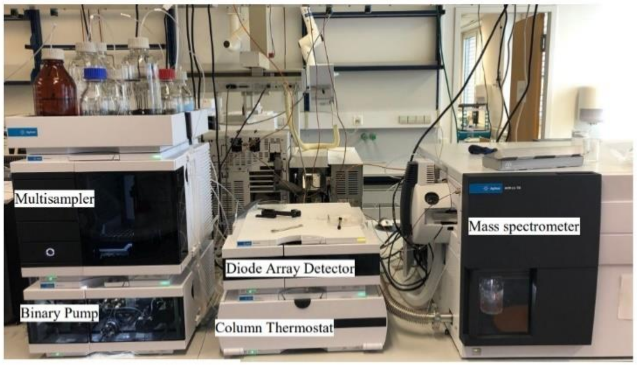


Figure 5. *Modules of the LC-MS/MS*

Column experiments on HPLC

As part of the HPLC column experiments, the transport of selected organic compounds such as carbamazepine, citalopram, 2,4 DB and melamine was investigated through a column filled only with quartz, then a column filled with a mix of quartz and Bayreuth soil, also column filled with a mix of quartz, Bayreuth soil and biochar, as well as a column filled with a mix of quartz, Bayreuth soil and with the addition of UNSPMF sludge. Column experiments were performed in different conditions, the first one at pH 7 and 1.5 mmol/L ionic strength of the solution, and the second at pH 3 and 89.2 mmol/L ionic strength of the solution.

Figure 6. *Irina Jevrosimov performs column experiments.*

Column packing procedure for Reverse Liquid Chromatography

(and pratical considerations)

Cleaning the column

Before use, the column must be cleaned with methanol or isopropanol, then rinsed with MilliQ water and left to air dry. In the case of cleaning the column after use itself, it is necessary to remove all filters (metal and fiber glass), as well as the O-ring, then throw out all the material with which the column was filled and then rinse with the above-mentioned solvents and MiliQ water and leave to dry in the air. Use carefully the sterile needle for remove all material (sorbent) from column so as not to damage the walls of the column inside.

Materials

(× 1) *stainless steel column (5.3 x 0.3 cm)*

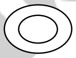
(× 2) *stainless steel nozzles (inserts)*

(× 2) *stainless steel plugs*

(× 4 +2 *pre-column*) *stainless steel sieves (5.4 mm OD; 3 µm)*

(× 6) *glass fiber filters (5.4 mm OD; 0.5 µm)*

(× 2 +1 *pre-column*) *O-rings (sealing gaskets; PTFE)*



(× 1) *stainless steel pre-column*

(× 1) *stainless steel connector*

General lab materials

(× 1) *HPLC pump*

(× 1) *Balance*

(× 1) *Long capillary*

(× 1) *Small capillary*

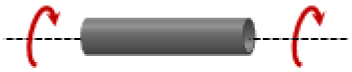
(× 1) *Beacker*

(× 1) *Spatula*

(× 1) *Stem*

Column preparation

- Grind previously dried sorbent to obtain a homogeneous particle size e.g. by using the swing mill. Column homogeneity prevents preferential flow of the mobile phase that would disturb measurements.
- Determine an appropriate weight ratio of sorbent and inert material (such as quartz powder or glass beads). Inert material needs to be added to sorbent to avoid clogging of the column.
- A total mass of 3g is convenient to pack stainless steel column with a 5.3 cm length and a 0.3 cm diameter purchased from Bischoff Chromatography.
- After weighing to the desired ratios, mix the two materials together.
- Mix the two materials by rolling the tube to avoid particle size fractionation and keep it as homogeneous as possible.



Prepare and weigh the following column components:

- 4 metallic sieves
- 6 fiberglass filters
- 2 O-rings.

Weigh the other column components :

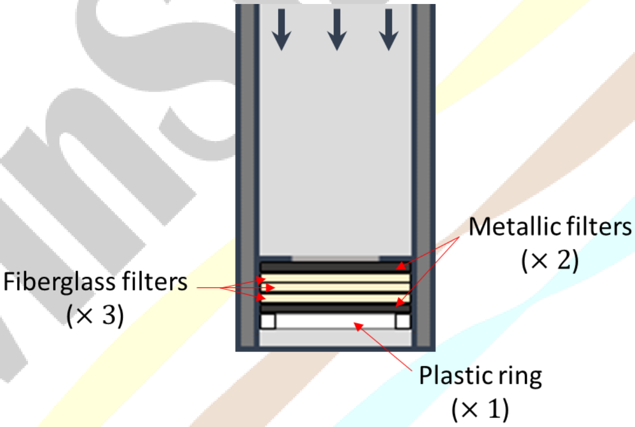
- ✓ 1 column
- ✓ 2 plugs
- ✓ 2 nozzles

Prepare for pre-column inlet:

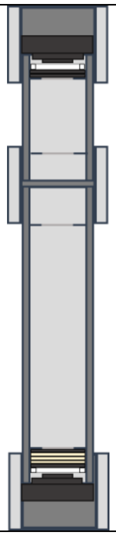
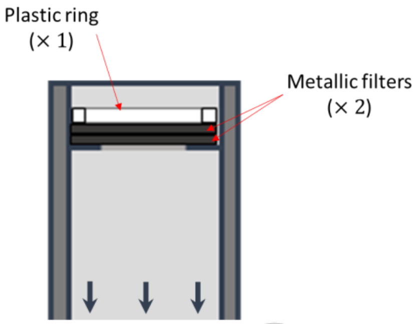
- 2 metallic sieves
- 1 O-ring

No need to weigh pre-column components

- Install half of the filters and O-rings on column outlet as described on the scheme (1 metal; 3 fiberglass ; 1 metal ; 1 O-ring) and insert and plug for later connection with capillary.



Scheme 1 ***: Column outlet***

	
Scheme 2* :** ***General packing setup	***Scheme 3* :** ***Pre-column inlet***

Screw a small capillary to column outlet.

Screw the connector on column inlet and install empty pre-column on top (Scheme 2)

Use wrenches to strongly screw these different components

Instal 2 non-weighed metallic filters and 1 O-ring on pre-column inlet (Scheme 3).

Instal plugs and nozzles on top of pre-column as shown in Scheme 2.

Filling the column

- Place a funnel with filter paper on top of the column and slowly fill the column with the appropriate material.
- Install the column **vertically** on a stem. This position prevents heterogeneity and the creation of preferential flow.
- Carefully fill the column with sorbent up to pre-column top.

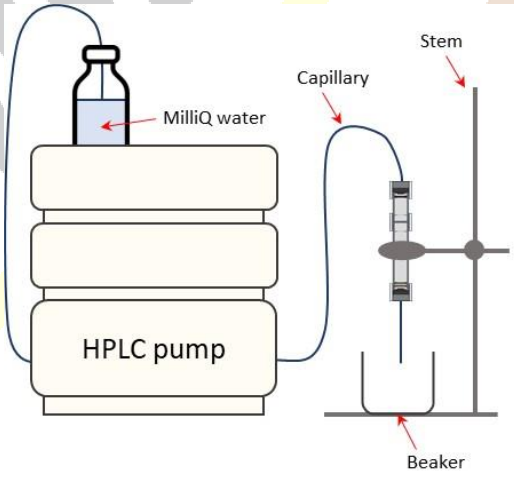
Do not **agitate or hit the** column (e.g., with spatula) to prevent fractionation. If you hit something, roll the tube again before continuing to fill column.

If your spatula is too full, put it all back instead of hitting it on an edge to reduce the amount of sorbent.

- Only when the column is full, gently hit the column with a stick to compact sorbent.
- Add sorbent material to fill the new empty space in column.
- Repeat two last steps until column is completely full and compacted.
- Install 2 metallic sieves and 1 O-ring on pre-column top (new set, not the components that were weighed earlier) and close it with nozzle and plug.

Packing the column

- Purge supply circuit of HPLC system with MilliQ water (purge valve open).
- Detach the capillary after the pump and purge it with MilliQ water (purge valve closed).
- Stop pump flow and attach capillary to pre-column top.
- Use wrenches to strongly screw capillary to plug
- Set a $0.01\ ml/min$ flow in column and keep an eye on back-pressure evolution over time until the first drop comes out of the column. Note the time and pressure associated with the first drop.
- The first drop will appear approximately after 40 *min*
- Every 15 min, gradually raise the flow following the procedure in Table 1 (next page) while continuing to monitor back-pressure.
- Verify that back pressure never goes above 400 *bar* (HPLC limit)



<u>Scheme 4</u> : HPLC Packing setup

Table 1. *Pressuring procedure*

Duration	Until 1st drop	15 min	15 min	15 min	15 min	15 min	15 min	15 min
Flow $(mL/\min)$	0.01	0.05	0.1	0.2	0.3	0.45	0.7	1

Duration	15 min	15 min	15 min	15 min	15 min	15 min	15 min	15 min
Flow $(mL/\min)$	1.5	2	2.5	2.7	2.9	3.1	3.3	3.4

- After that, set up flow 3.5 ml/min and wait 3.5 h to be stable pressure
- When packing is completed, remove capillaries and detach pre-column from column.
- Clean column inlet from sorbent excess carefully and place remaining rings and filters on it following the same order as earlier and screw plug and nozzle to close column.
- Install column in HPLC and connect the capillaries, making sure that flow follows the same direction as when packing.
- Before any measurement, pre-equilibrate column with eluent for a prolonged time (typically 12 hours).

A steady flow in column of *0.1 mL/min* is often suitable for soil sorption experiments.

Reverse Liquid Chromatography calculations

Reverse liquid chromatography can be used to measure contaminants K_D. It´s determination is directly based on retention time measurements with Equations (1) and (2):

$$R = \frac{t_r - t_0}{t_d - t_0} \tag{1}$$

$$R = 1 + \frac{\rho_b}{\theta} K_D \tag{2}$$

Where ρ_b (g/mL) and θ $(-)$ represent column bulk density and porosity respectively and are determined via Equation (3) and (4) :

$$\rho_b = \frac{\Delta m_{column} - \Delta m_{water}}{\pi r_c^2 l_c} \tag{3}$$

(4)

$$\theta = \frac{t_{r_{tracer}}^{column} - t_{r_{tracer}}^{no\ column}}{\pi r_c^2 l_c}$$

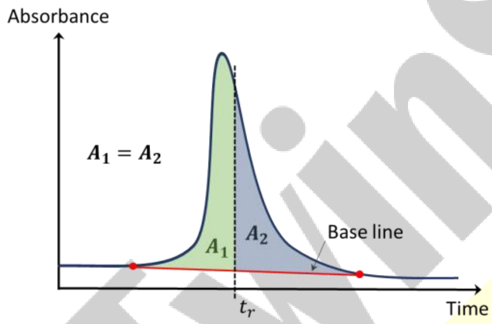
- $\Delta m_{column} = m_{column}^{packed} - m_{column}^{empty}\ (g)$: Mass difference between packed and empty column
- $m_{water}\ (g)$: Mass of water in the column (deduced from time and flow until first drop out of column)
- $t_{r_{tracer}}^{column}\ (\mathrm{min})$: Tracer retention time measured when column is installed
- $t_{r_{tracer}}^{no\ column}\ (\mathrm{min})$: Tracer retention time measured when there is no column in HPLC system

$r_c\ (cm)$: Column radius

$l_c\ (cm)$: Column length

Peaks integration

Chromatograms analysis requires peak integration to determine retention times. This can be done via an Excel Macro programmed in VBA by Antoine Raoult, and available at EDGE.



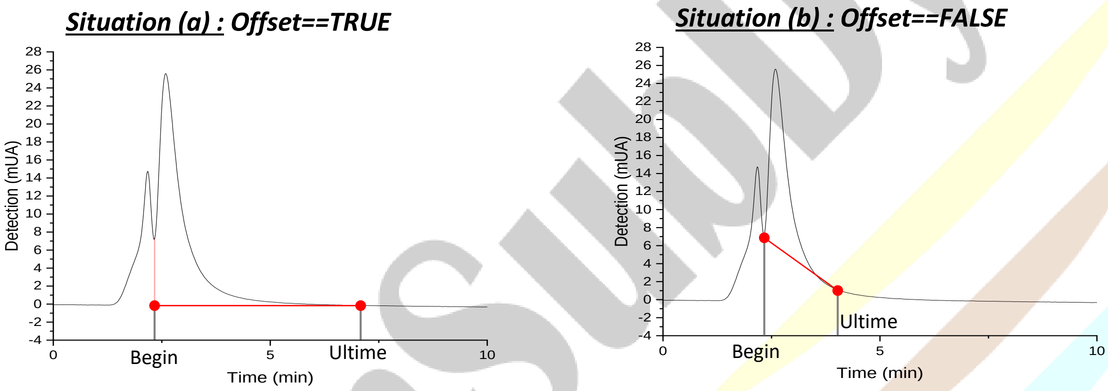
***Scheme 5* : Half mass method schematisation**

The half mass method was preferred over other numerical solutions for retention times determination because it has been shown to be quite insensitive to peak shape. Therein, the retention time corresponds to the time when the peak areas on both sides are equal (Scheme 5).

To maximize flexibility, two different function were programmed (@peakArea and @HalfMass) determining respectively total peak area and retention time by half mass method. They are using the following variables as input:

Table 2. *Parameters required for peak integration*

Parameters list	***Explanation***	***Determination***
T_begin (Double)	Line number of selection beginning	=LINE(Cell1)
T_end (Double)	Line number of selection ending	=LINE(Cell1)+NBVAL(Selection)
ColumnTime (Double)	Column number of Time in selection	=COLONNE(CellTime)
ColumnData (Double)	Column number of Data in selection	=COLONNE(CellData)
Begin (Double)	Wanted time for integration inferior bound	
Ultime (Double)	Wanted time for integration final bound	
NameSheet (String)	Name of the Sheet were selection is located	Exact same name as Sheet
Offset (Boolean)	If TRUE ➔ situation (a) If FALSE ➔ situation (b) (Scheme 6)	


Situation (a) : ***Offset==TRUE***

Situation (b) : ***Offset==FALSE***

HPLC 1260 Infinity II (Agilent)

The high-performance liquid chromatography (HPLC) is a technique for separation, identification and quantification of single compounds in a complex mixture. The separation is based on the distribution of the analyte between the mobile phase (eluent) and the stationary phase (packing material of the column). Each component in the sample reacts slightly different from the adsorbent material, depending on its chemical structure. This interaction leads to different retention times of single compounds. The HPLC 1260 Infinity II from Agilent is equipped with a Diode Array Detector (DAD) and a Refraction Index (RI) Detector.

Results

Characterization of materials

Table 3. *Results of characterization of selected soils and organic soil amendments (OSA)*

Materials	pH	UV-Vis	DOC (mgC/l)	SUVA	LECO (%C)
Compost UNSPMF	7.20	0.3856	329.75	0.1169	24.57
Sludge UNSPMF	6.05	0.2567	236.5	0.1085	37.32
Cow manure	7.16	1.9267	326	0.5910	42.59
Compost Vienna	7.57	0.4700	284.15	0.1654	31.53
Compost Thorsten	7.43	0.9156	327.55	0.2795	12.58
Danube sediment	7.87	0.151	4.78	3.1590	0.78
Bayreuth soil	6.13	1.926	42.19	4.5651	0.92
Compost UNSPMF new	6.80	1.908	182.4	1.0461	20.65

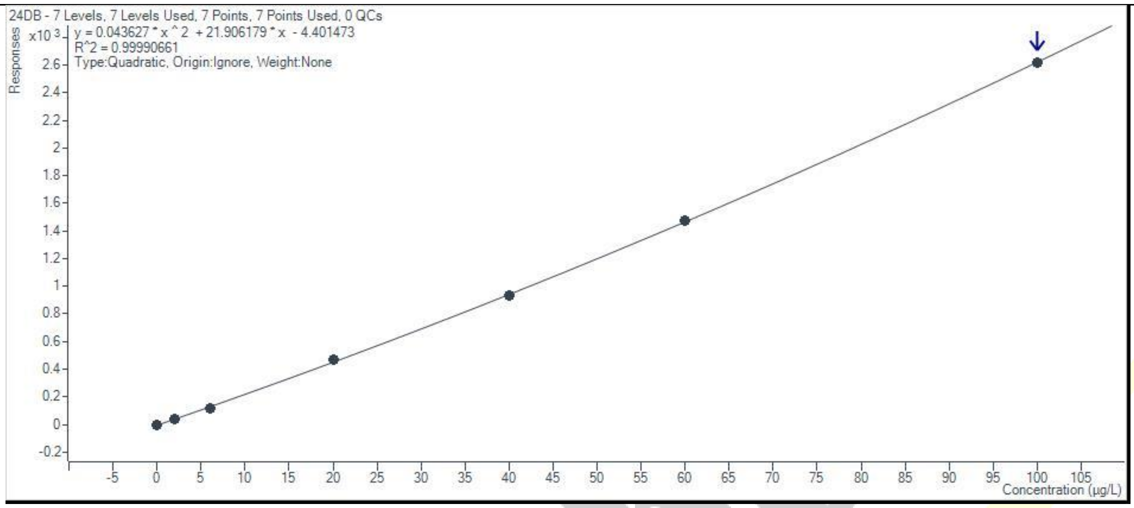
Table 3 presents the characterization of the investigated materials. Based on the performed characterization of the samples, it can be concluded that the highest DOC value (mgC/L) was observed for UNSPMF compost. Also, highest percent of carbon was observed for cow manure. pH values was in range from 6.05-7.87 and values for UV-Vis was in range from 0.151-1.9267.

Table 4. *Elemental analysis of selected materials*

Materials	C	H	N	S
Compost UNSPMF	16.94	1.46	1.44	1.17
Sludge UNSPMF	32.97	4.19	5.71	2.63
Cow manure	44.81	5.19	3.18	0.86
Compost Vienna	24.50	2.61	25.45	0.54
Compost Thorsten	34.68	3.43	95.14	0.31
Danube sediment	0.94	0.01	0.04	0.03
Bayreuth soil	1.74	0.12	0.18	0.09
Compost UNSPMF new	17.54	2.20	1.25	0.26

Elemental analysis (Table 4) also confirmed the highest carbon content in cow manure compared to other materials, which is consistent with the carbon content analysis on the LECO instrument. Also, for Danube sediment, Bayreuth soil and Compost UNSPMF (new): No or almost no HNS in the samples. Therefore, the measurements here are below the detection limit.

Biotransformation experiments

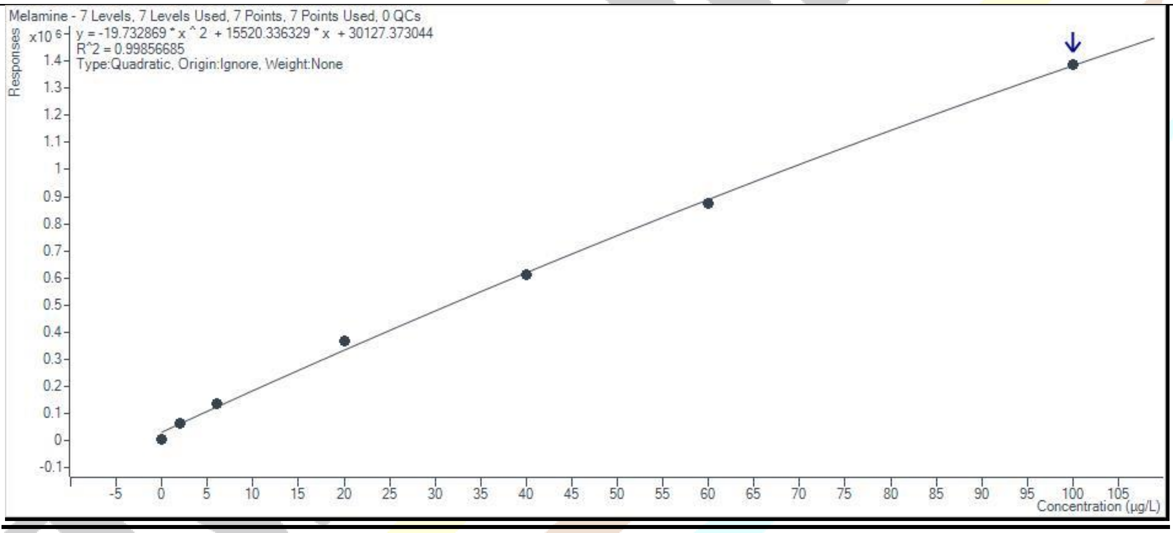


24DB - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 0 QCs

y = 0.043627 * x ^ 2 + 21.906179 * x - 4.401473

R^2 = 0.99990661

Type:Quadratic, Origin:Ignore, Weight:None



Melamine - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 0 QCs

y = -19.732869 * x ^ 2 + 15520.336329 * x + 30127.373044

R^2 = 0.99856685

Type:Quadratic, Origin:Ignore, Weight:None

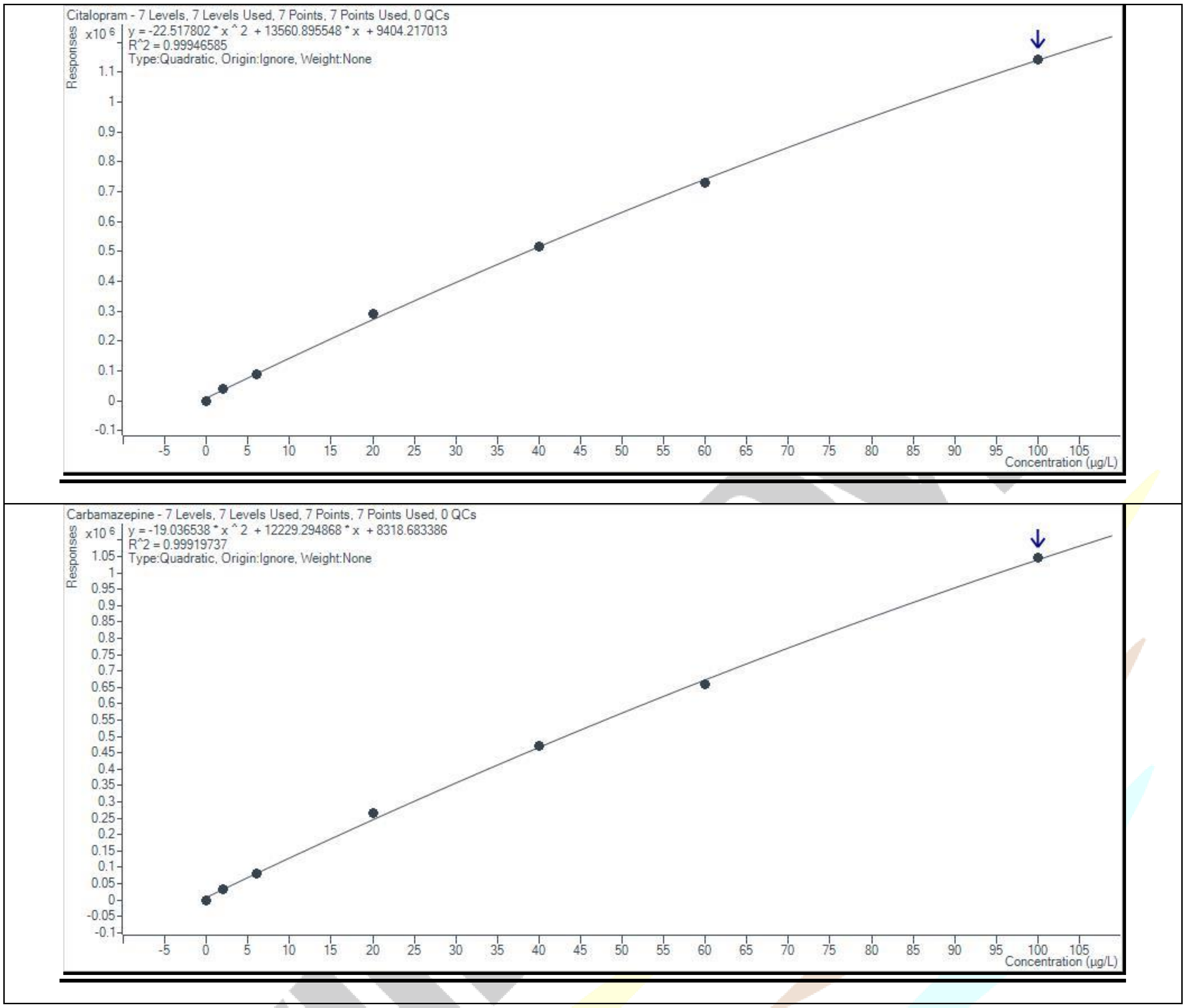


Figure 7. *Calibration curves for all compounds (24DB, Melamine, Citalopram, Carbamazepine)*

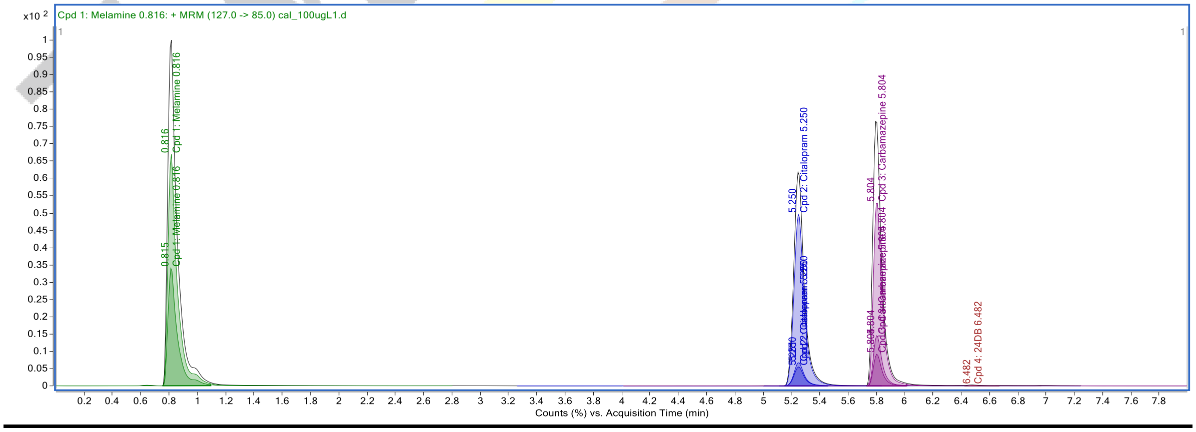


Figure 8. *MRM spectra of all compounds*

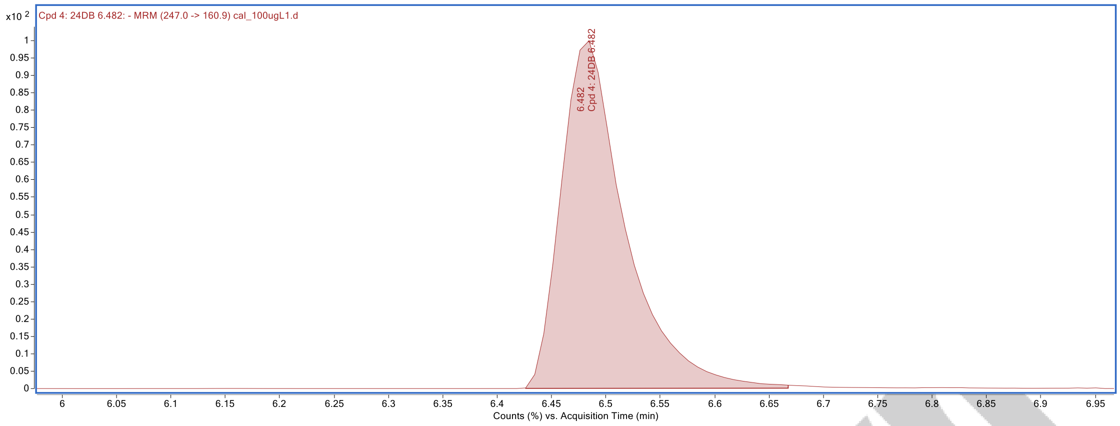


Figure 9. MRM spectra of 24DB

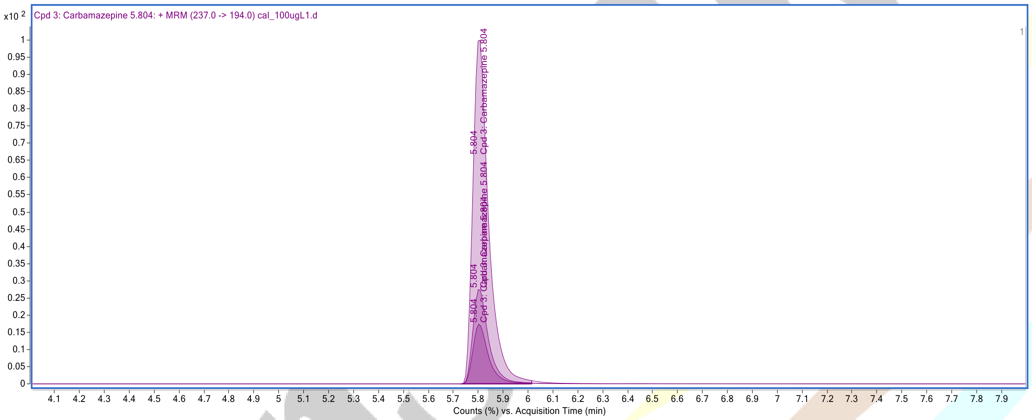


Figure 10. MRM spectra of Carbamazepine

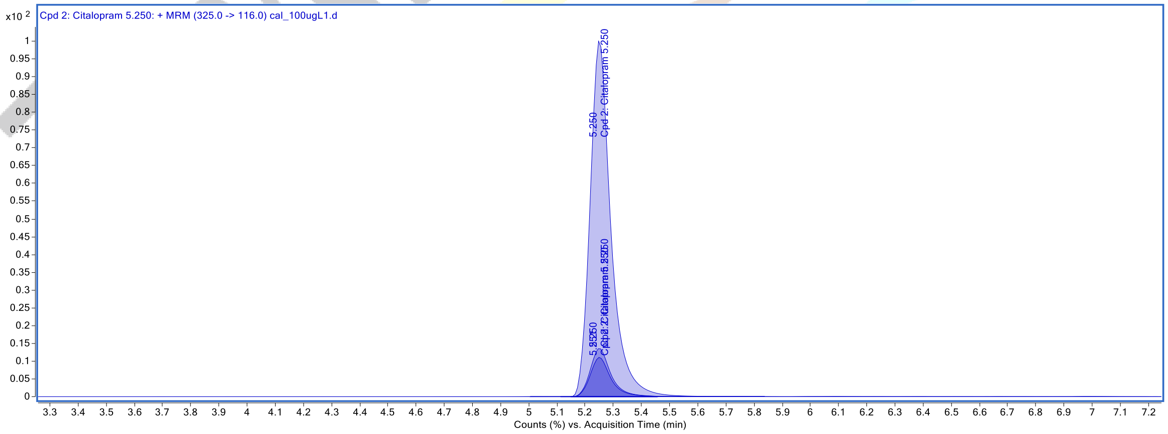


Figure 11. MRM spectra of Citalopram

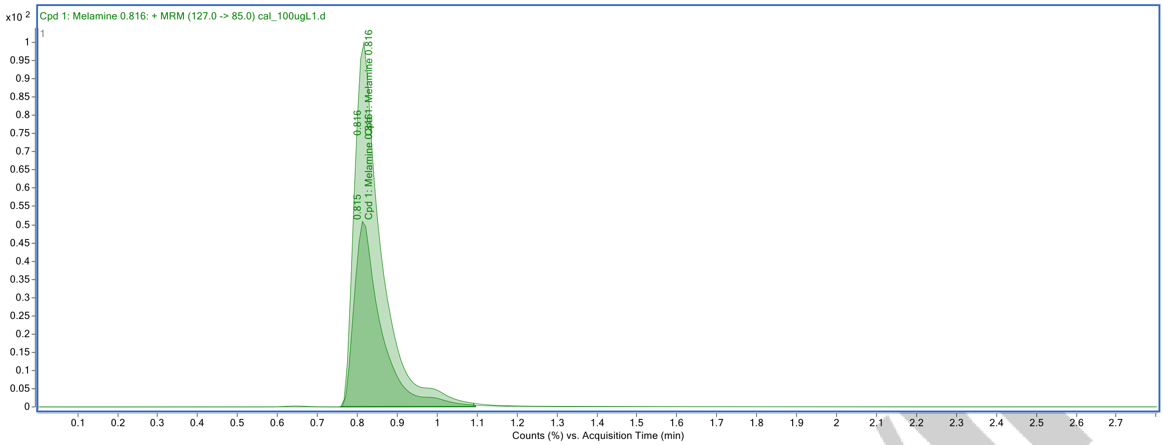
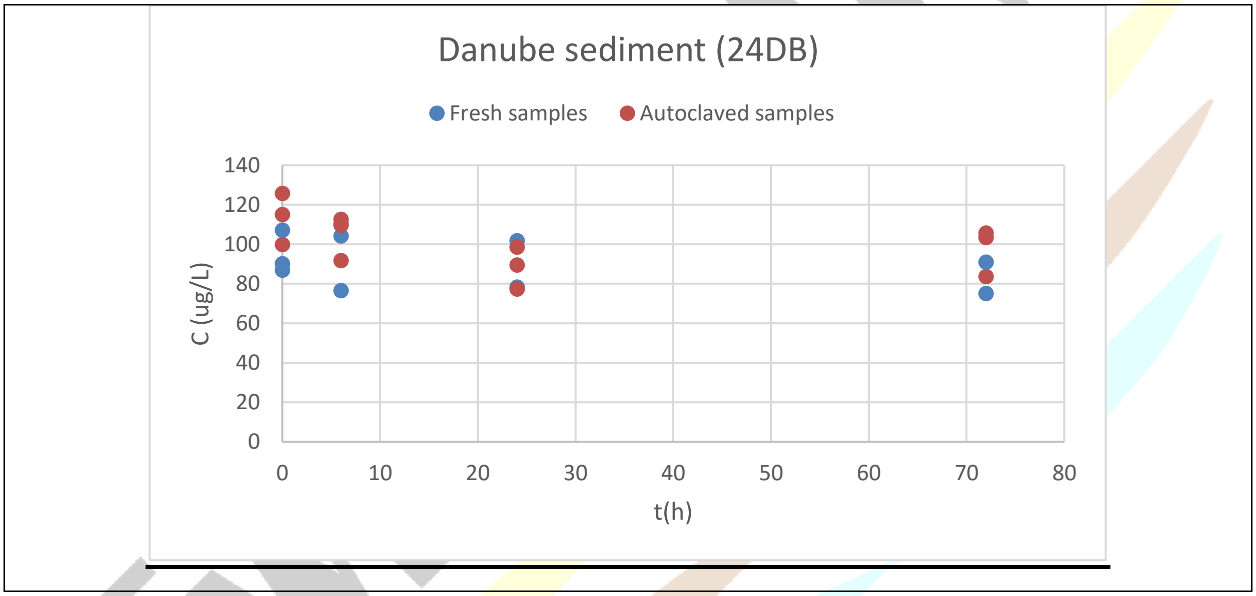
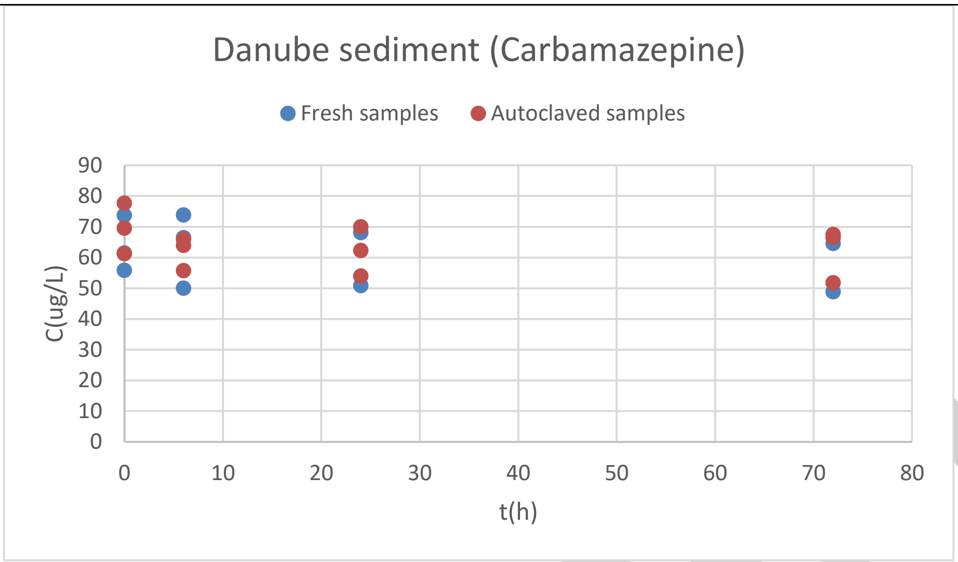
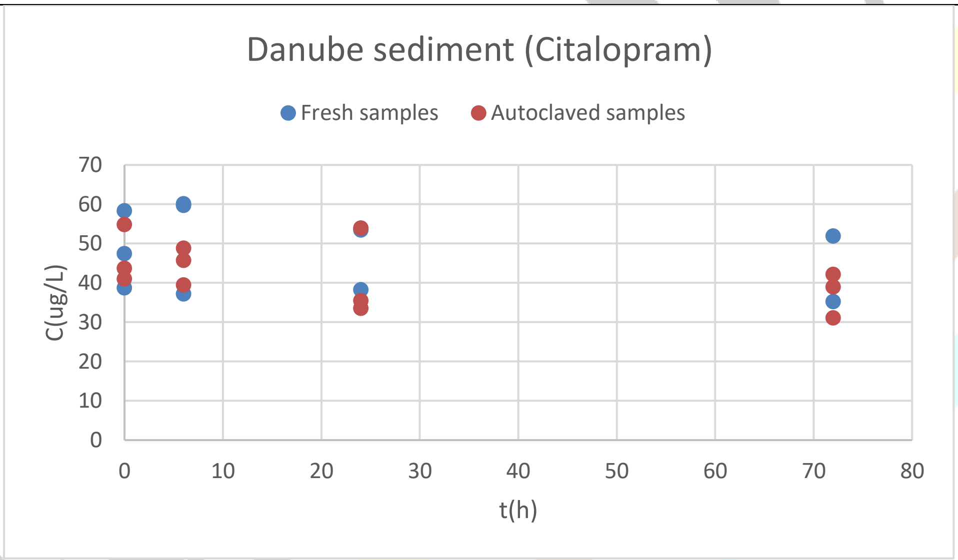


Figure 12. *MRM spectra of Melamine*




Danube sediment (Carbamazepine)
● Fresh samples
● Autoclaved samples
C(ug/L)
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
80
t(h)


Danube sediment (Citalopram)
● Fresh samples
● Autoclaved samples
C(ug/L)
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
80
t(h)

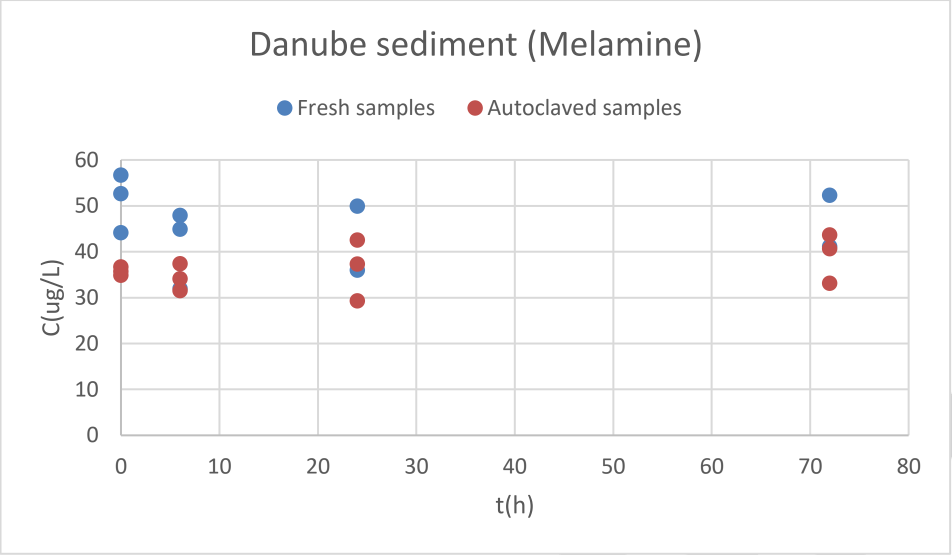
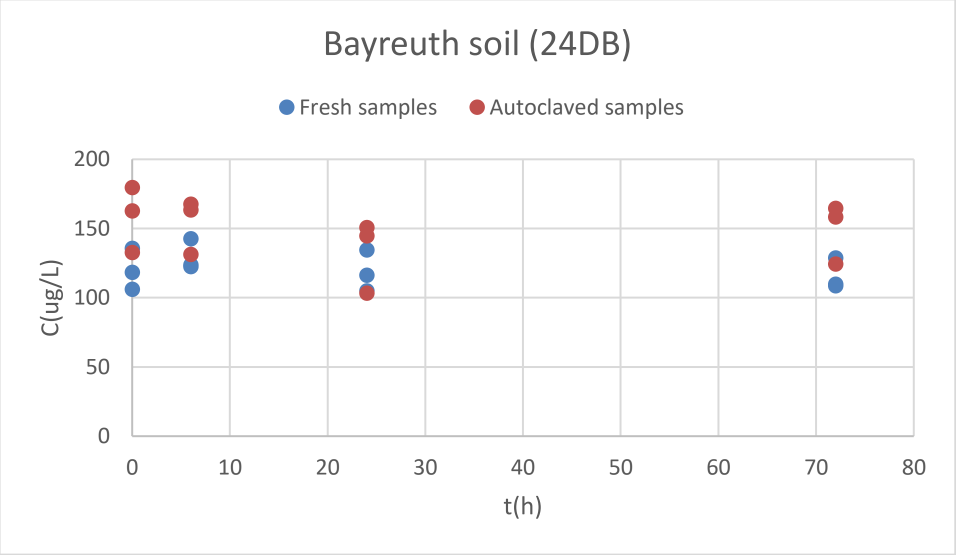
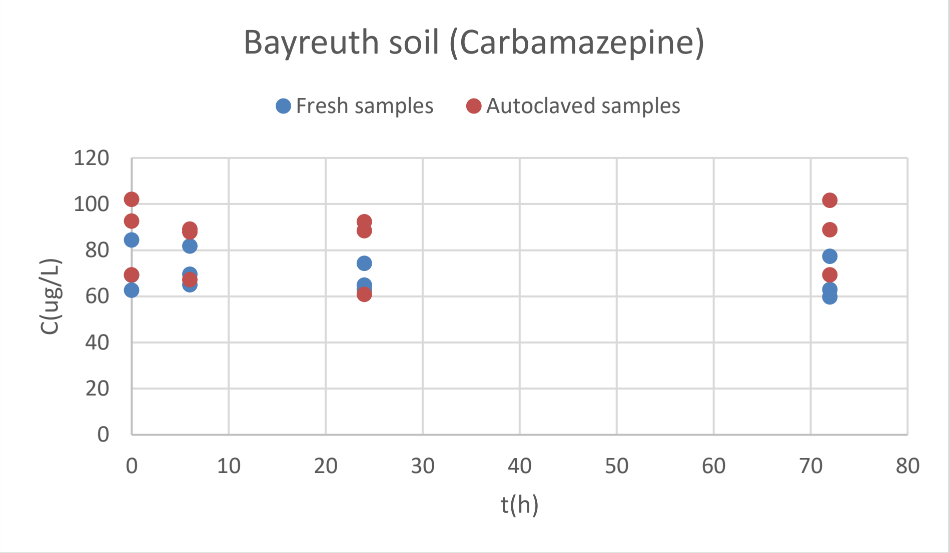
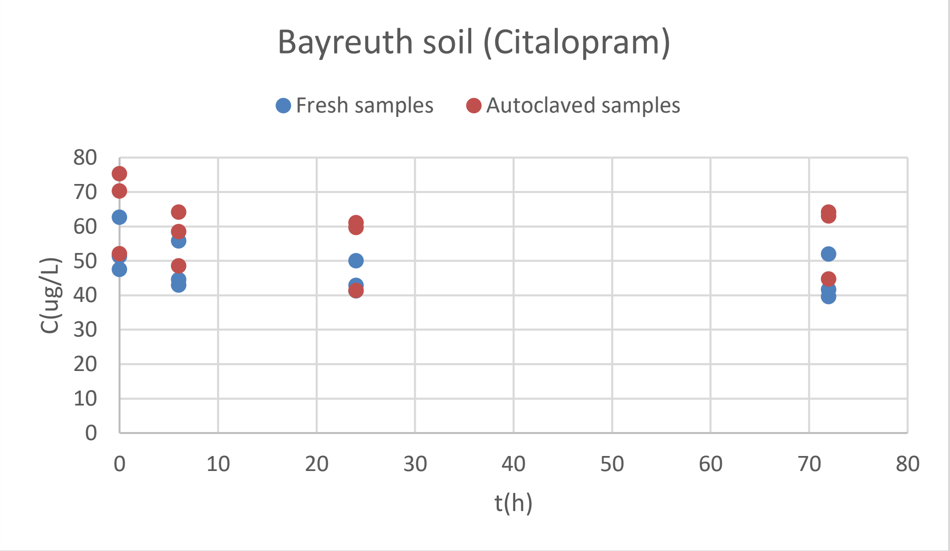


Figure 13. *Biotransformation of selected compounds in Danube sediment extracts (fresh samples and autoclaved)*




Bayreuth soil (Carbamazepine)
● Fresh samples
● Autoclaved samples
C(ug/L)
120
100
80
60
40
20
0
0
10
20
30
40
50
60
70
80
t(h)


Bayreuth soil (Citalopram)
● Fresh samples
● Autoclaved samples
C(ug/L)
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
80
t(h)

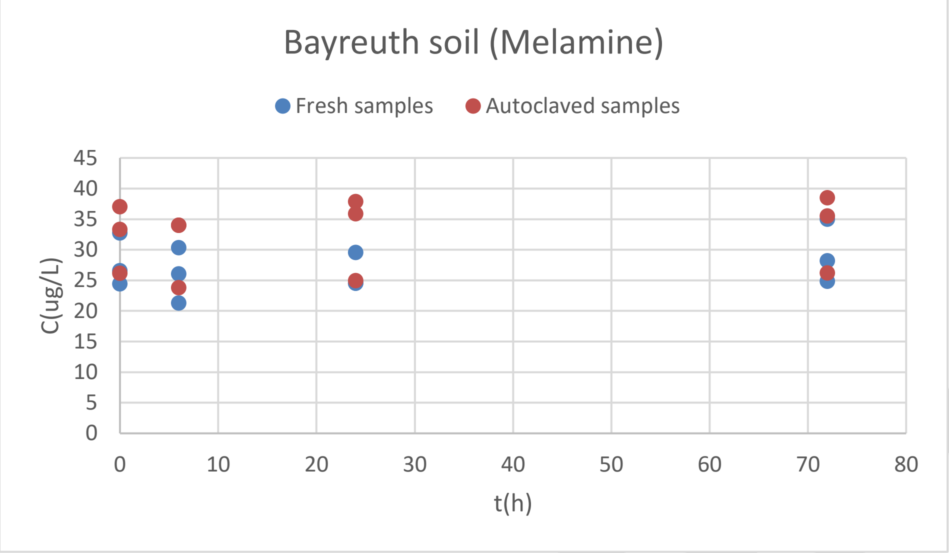
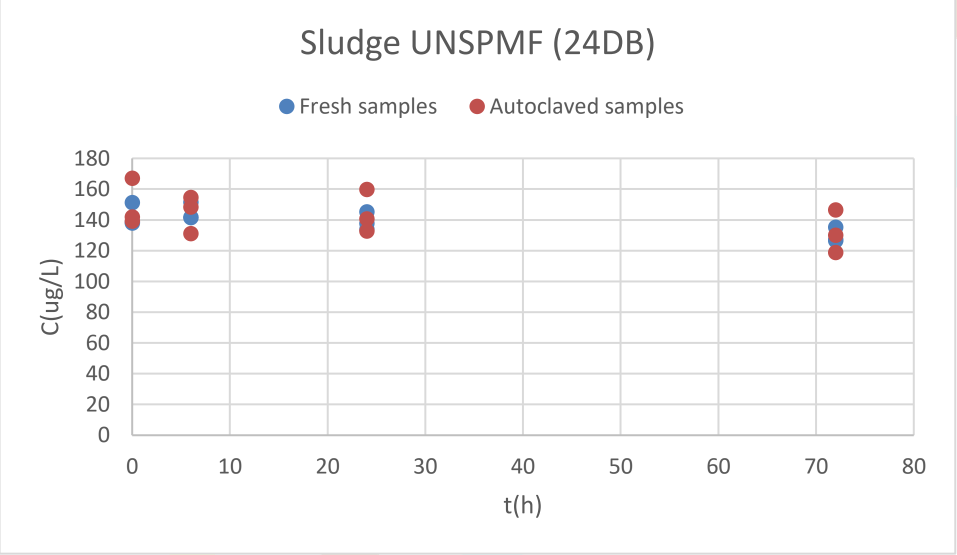
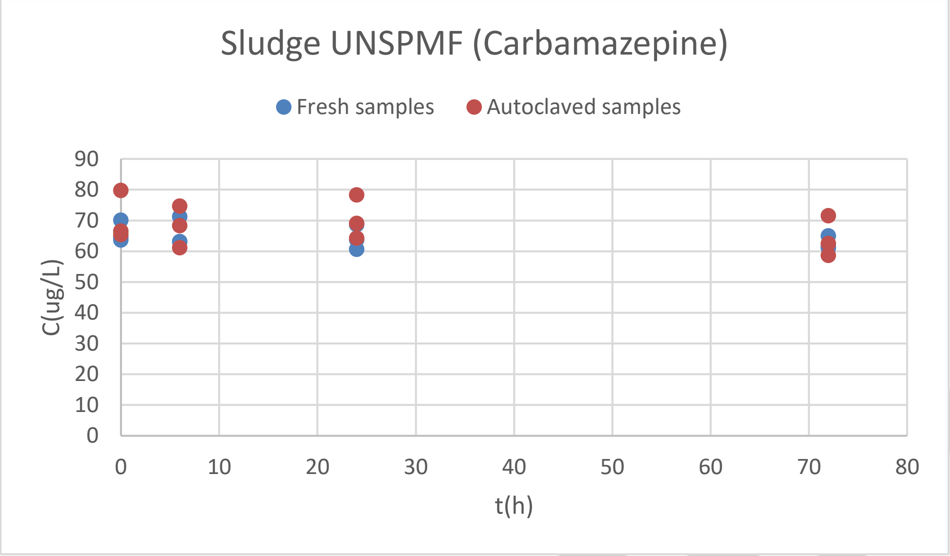
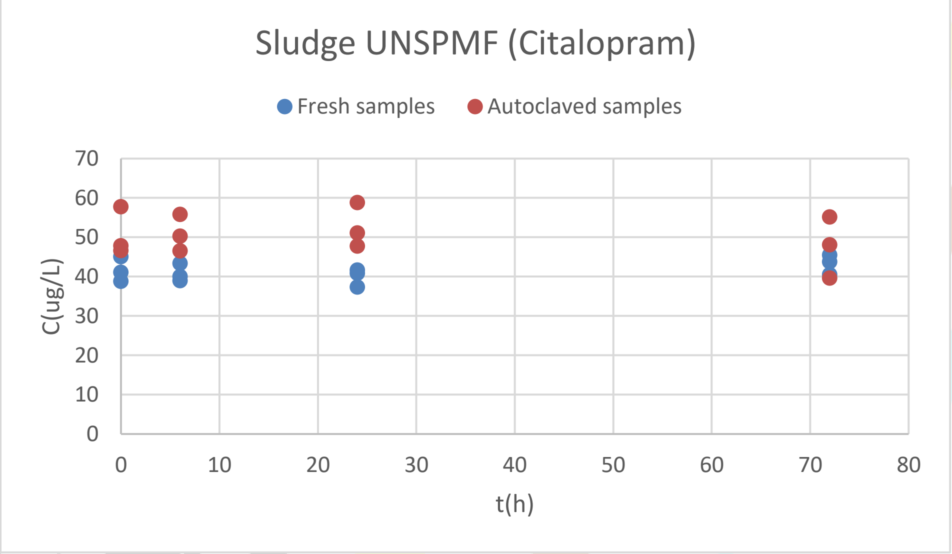


Figure 14. *Biotransformation of selected compounds in Bayreuth soil extracts (fresh samples and autoclaved)*




Sludge UNSPMF (Carbamazepine)
● Fresh samples
● Autoclaved samples
C(ug/L)
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
80
t(h)


Sludge UNSPMF (Citalopram)
● Fresh samples
● Autoclaved samples
C(ug/L)
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
80
t(h)

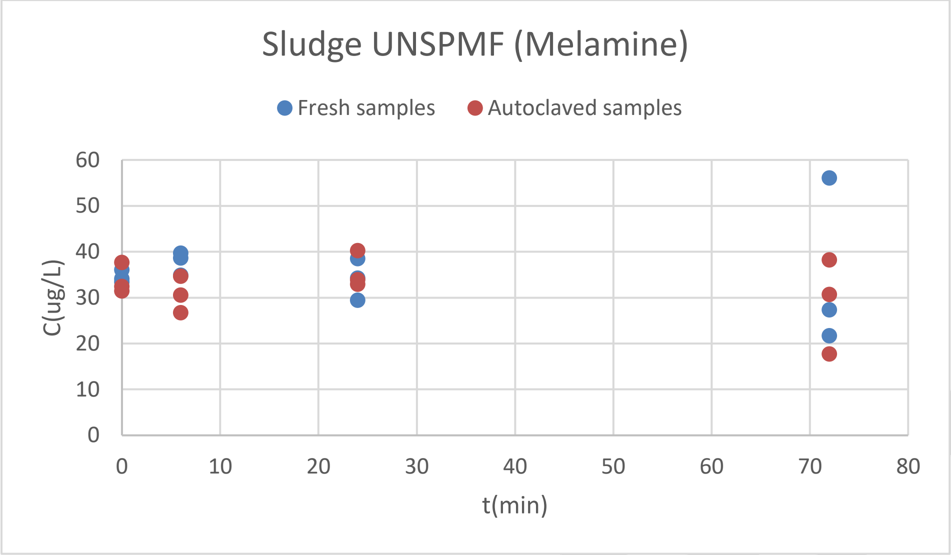
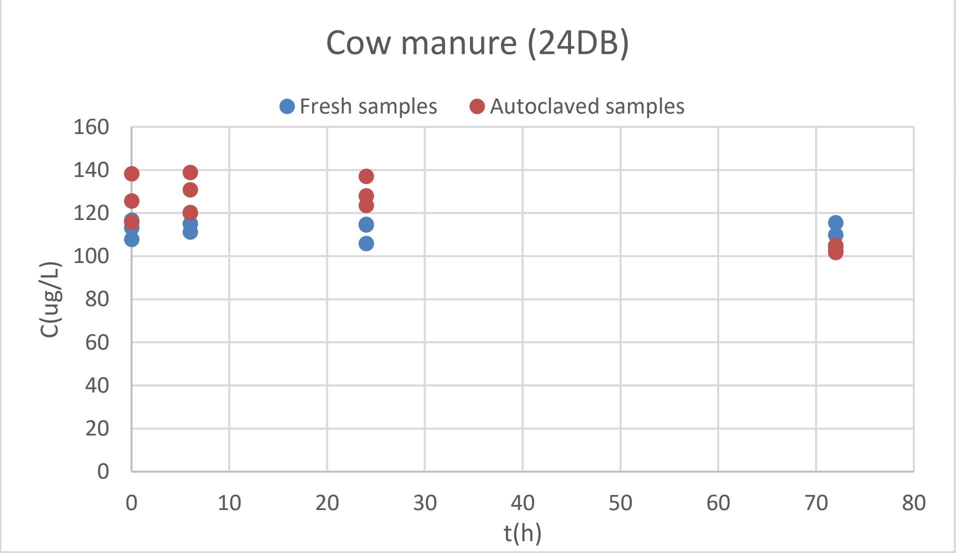
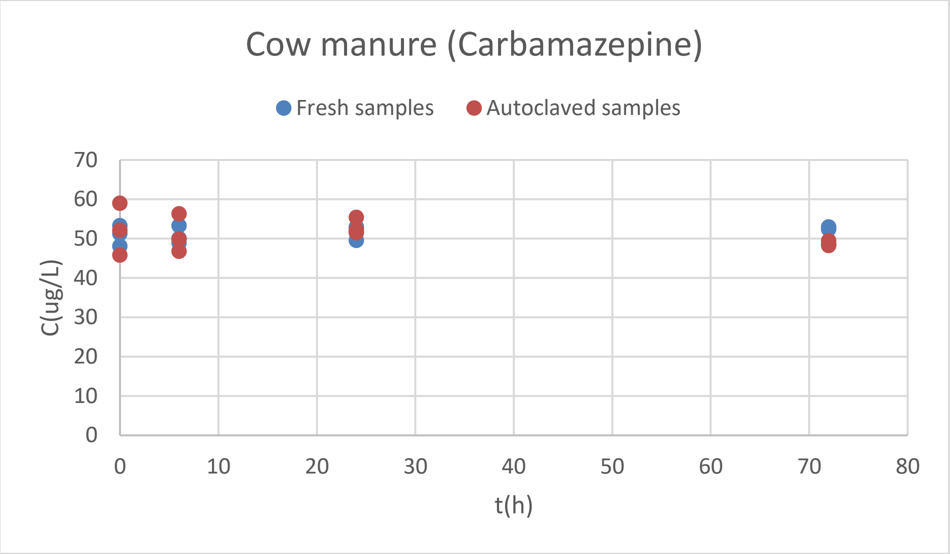
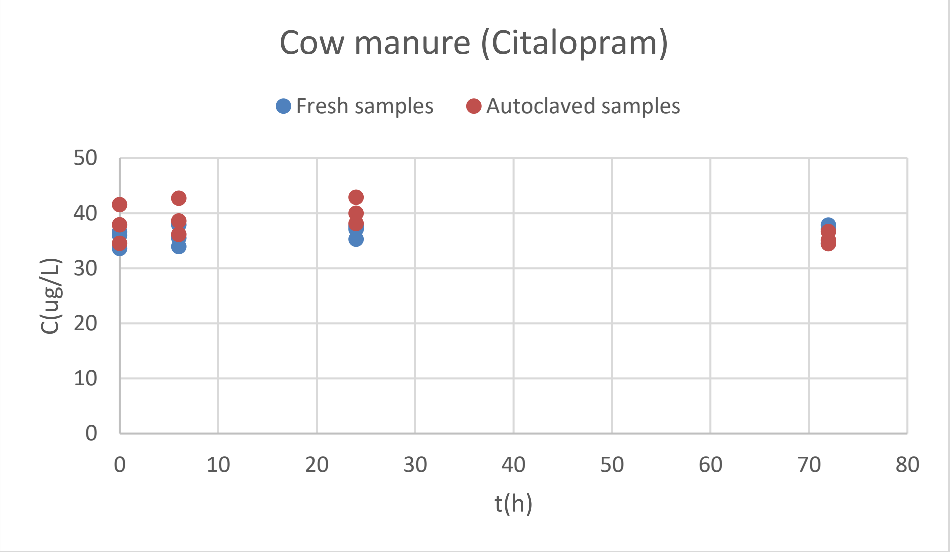


Figure 15. *Biotransformation of selected compounds in Sludge UNSPMF extracts (fresh samples and autoclaved)*




Cow manure (Carbamazepine)
Fresh samples
Autoclaved samples
70
60
50
40
30
20
10
0
C(ug/L)
0
10
20
30
40
50
60
70
80
t(h)


Cow manure (Citalopram)
Fresh samples
Autoclaved samples
50
40
30
20
10
0
C(ug/L)
0
10
20
30
40
50
60
70
80
t(h)

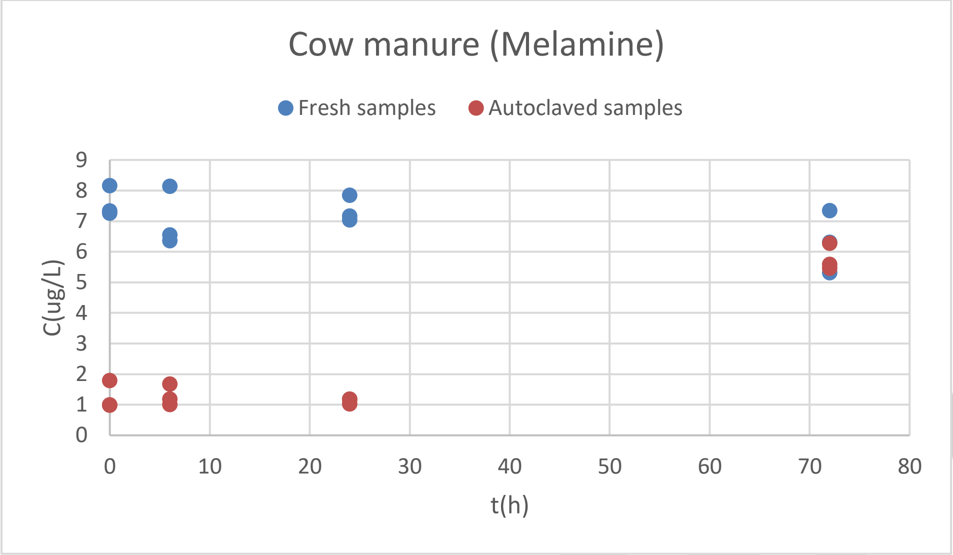


Figure 16. *Biotransformation of selected compounds in Cow manure extracts (fresh samples and autoclaved)*

<u>Conclusions for biotransformation experiments:</u>

- Generally, biotransformation of all compounds <u>was not observed</u>.
- Melamine potentially could interact with organic matter of investigated materials (pKa=5).
- For moisture content we don't see differences in mass of materials after 48 h of drying the materials.
- For repeated measurments of moisture content in sludge (3g of sludge was measured in triplicates)-losses 0.42; 0.4; 0.44 g of sample after 48 h drying.
- <u>In the future:</u>

✓ Investigate impact of matrix effect on biotransformation of selected pollutants

✓ Enzyme assay with sludge UNSPMF and cow manure

✓ For Bayreuth soil and cow manure-spike 100 ug/L of mix of all compounds in old extracted samples (repeat).

- ✓ <u>1:10 experiments :</u> Will be finished in November 2023 on one month visit on the University of Vienna.

Impact on your project

Trainings on UNIVIE was very nice experience and new knowledge for our members on the project TwinSubDyn. The main trainings was to learn targeted extraction and analysis of OSA-derived organic contaminants and assessing their dynamics in OSA and OSA-soil mixtures and transfer this knowledge from UNIVIE to UNSPMF.

MOBILITY REPORT

Researcher: Dr. Irina Jevrosimov, UNSPMF

Assigned supervisor: Prof. Dr. Gabriel Sigmund, UNIVIE

Duration of the visit: 30.10.2023. - 24.11.2023.

Executive Summary

As part of a one-month visit to the University of Vienna the main goal was to learn and to do methodologies of leaching and extraction experiments of selected organic compounds (tire additives and phthalates) from organic soil amendments (OSA) and learn development and application of tailored extraction procedures and targeted analysis via LC-MS/MS.

Introduction

Background

Tire additives represent the largest sources of microplastics in the urban environment that ensure the leaching of chemical additives into the environment. In addition to the previously mentioned compounds, phthalates can also be found in the organic soil amendments. Second group of plastic micropollutants - phthalates represent plasticizers and can be found in plastic products and they are also a problem for the environment. A large number of these compounds can be found in the organic soil amendments, so it would be very interesting to investigate fate and behavior of these compounds. Organic soil amendments represent organic waste and have been the main source of plant nutrients, especially nitrogen (N). Organic soil amendments ensure macro- and micronutrients, including carbon (C) for the restoration of soil physical and chemical properties.

The main objective of this investigation was:

- ✓ OSA elution experiments (batch-based water extraction) in a 1:10 solid to liquid ratio

  These experiments included recovery test, leaching and extraction experiments of selected compounds from organic soil amendments (OSA).

Scope of the secondment

The scope of the secondment was to learn and transfer knowledge from UNIVIE to UNSPMF toward to learn development and application of tailored extraction procedures and targeted analysis via LC-MS/MS. As part of this visit, leaching and extraction experiments were performed of selected organic pollutants from organic soil amendments (OSA).

Content

Methods

OSA elution experiments (batch-based water extraction) in a 1:10 solid to liquid ratio

Within these experiments were performed recovery test, leaching and extraction experiments of selected organic micropollutants such as tire additives and phtalates from 6 organic soil amendments (sludge from UNSPMF, cow manure, compost UNSPMF, mix of compost and sludge from UNSPMF, compost from Vienna, compost from Pixendorf).

Leaching and extraction of plastic additives (tire derived compounds, phthalates (in separate LC-MSMS method)) from OSA

1. ***Recovery test included next steps:*** Measure 20 mL of MQ water and transferred in glass centrifuge tubes (50 mL) in triplicates (3 samples and 3 blanks). After that, spiked all selected compounds (in MiliQ water) and placed glass centrifuge tubes on an overhead shaker for 24 hours. Internal standards will be added to the aqueous phases before SPE.

SPE extraction: After that, transfer the aqueous phases into the cartridges in portions; flow rate through cartridge 1 drop/s. SPE cartridges are then extracted using 3 x 5 mL of methanol; flow rate 1 drop/s, methanol extracts are collected in 20 mL vials (Figure 1).

Figure 1. SPE extraction of samples

Concentration: Methanol extracts are concentrated to 500 µL using N_2 at 40 °C and after that measuring on LC-MS/MS and doing data analysis.

Calculations for recovery test:

$$Recovery\ (\%) = \frac{experimental\ yield}{theoretical\ yield} * 100$$

2. ***Leaching experiment (1:10) included next steps:***

Measure selected organic soil amendments in triplicates in glass centrifuge tubes (50 mL) and add 20 mL of MQ water. After that, glass centrifuge tubes are placed on an overhead shaker for 24 hours and glass centrifuge tubes are centrifuged for 30 minutes at 20 °C, 1000 G. With glass pipettes transfer supernatant in 40 mL glass vial. If we see particles after centrifugation, samples need to be filtered using 0.45 µm nylon syringe filters before SPE. Internal standards will be added to the aqueous phases before SPE. SPE extraction: After that, transfer the aqueous phases into the cartridges in portions; flow rate through cartridge 1 drop/s. SPE cartridges are then extracted using 3 x 5 mL of methanol; flow rate 1 drop/s, methanol extracts are collected in 20 mL vials.

Concentration: Methanol extracts are concentrated to 500 µL using N_2 at 40 °C and after that measuring on LC-MS/MS.

3. ***<u>Extraction experiments included next steps:</u>***

Measure 0.2 g dried organic soil amendments (in triplicates) and placed in 50 mL centrifuge tubes with Teflon-line screw caps; Add 10 mL of DCM: acetone mix (80:20) and placed vials in ultra-sonication bath for 1 h. After that, centrifuge extracts 30 min (20 °C, 1000 G) and after centrifugation transfer supernatant in 40 mL glass vials (repeat that two times with extraction with DCM:acetone mix). After that, add internal standards and vortex the samples. Extracts are filtered using 20 mL glass syringe and 0.2 µm Nylon filters into a fresh 40 ml vials. Extracts are transferred to 20 mL vials and concentrated to 0.2 mL and after that add 0.8 mL of methanol, vortex the sample and measure it on LC-MS/MS (Figure 2 and 3).

Figure 2. Concentration of samples

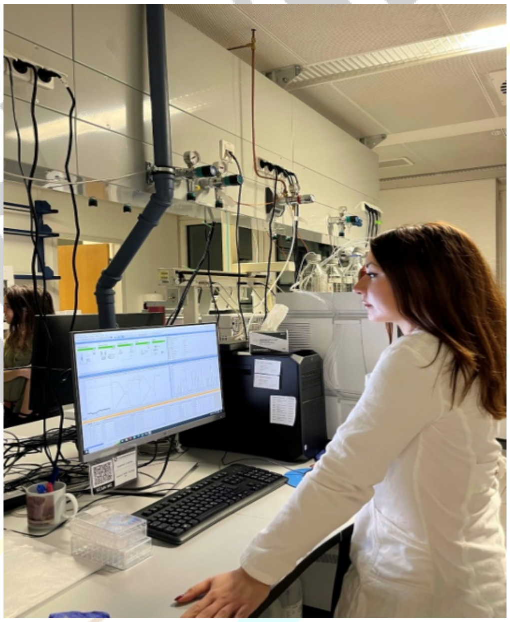


Figure 3. Measuring on LC-MS/MS

LC-MS/MS

High Performance Liquid Chromatography (HPLC) HPLC is used to separate (in a liquid state), identify, and quantify analytes within a sample. The method relies on pumps to pass a pressurized liquid solvent (or a solvent mixture), called "mobile phase" through a column (called "stationary phase"). The sample is added to the mobile phase and flows through the column. The separation is based on the various physical and chemical properties of analytes and their interaction with the column's stationary phase. The time for an analyte to elute from the column is called retention time and is an identifying characteristic of a given analyte. In normal-phase chromatography a non-polar mobile phase is used in conjunction with a polar stationary phase. Reversed-phase chromatography (RP-HPLC), which is currently applied within our system, refers to the use of a polar mobile phase and a non-polar (hydrophobic) stationary phase. In the RP-HPLC the common mobile phases are acetonitrile and methanol but any combination of water with organic solvents is often used, depending on the sample properties. Acetonitrile is often used because of its low UV cutoff, lower viscosity (methanol forms highly viscous mixtures with water at certain concentrations), and higher boiling point. Buffers/acids (such as formic acid, acetate) can be added to the aqueous phase. Usually 0.1% formic acid is added (pH ≈ 2.7) in order to provide a suitable environment for the ionization of the analytes in the MS when measuring in positive mode, and to control pH, which results in reproducible retention times, and thus better separation of analytes in the sample.

The composition of the mobile phase can stay constant during the analysis (isocratic elution mode) or can be changed (gradient elution mode). Gradient elution is used for samples that contain analytes with similar affinities for the column's stationary phase, while isocratic elution is used for analytes with highly varying physiochemical properties. When using isocratic elution make sure that your analyte completely elutes from the system (i.e., carefully optimize the eluent composition depending on your analyte and verify complete elution by running blank measurements with gradient elution). When using gradient elution make sure that the last composition of the mobile phase matches that of the starting composition and that these changes are gradual and not abrupt. This is important for maintaining column performance. You can specify this either within the method gradient, or you can use the "stop time" option (in the method editor) by editing the time during which the mobile phase composition goes to the starting one. Modules of the LC-MS/MS were shown in Figure 4.

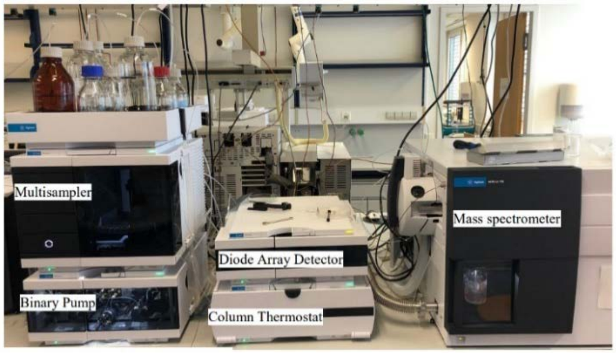


Figure 4. *Modules of the LC-MS/MS*

Calculations for extraction experiments:

$$Total\ extraction\ (ng/g) = \frac{Concentration\ of\ compound * final\ volume\ of\ extract}{Amount\ of selected\ material}$$

Calculations for leaching experiments:

$$Mass\ of\ compound = \frac{Concentration\ of\ compound\ *\ final\ volume\ of\ extract}{Mass\ of selected\ \ material}$$

$$Leachate\ fraction\ (\%) = \frac{Mass\ of\ compound}{Mass\ of\ selected\ material} * 100$$

Impact on your project

The training on UNIVIE was very useful and significant for the members of the TwinSubDyn project. The main training included learning methodologies of leaching and extraction experiments of selected organic compounds (tire additives and phthalates) from organic soil amendments (OSA) and learning development and application of tailored extraction procedures and targeted analysis via LC-MS/MS and also transferring this knowledge from UNIVIE to UNSPMF.

TwinSubDyn

Removal of textile fibers from water by coagulation and flocculation

Date: 19.06.2023.

Prepared by: Sanja Vasiljević

Funded by the European Union

1

Introduction

TwinSubDyn

The aim

- The aim was to investigate the **potential of coagulation/flocculation treatment in the removal of a complex mixture of textile fibers** from natural and synthetic materials.

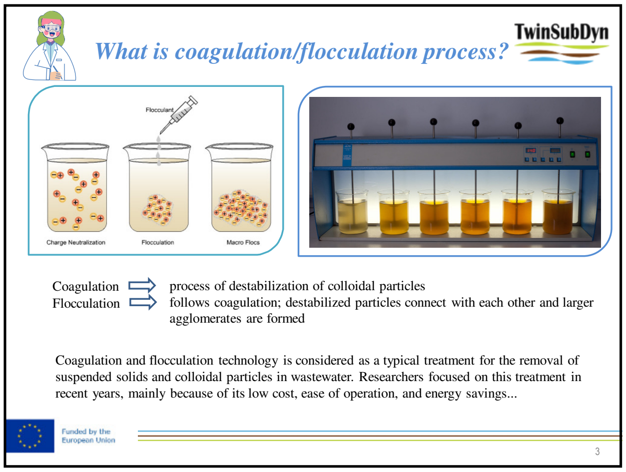
Coagulants

- Two common coagulants:
- **ferric chloride (FeCl3) and**
- **polyaluminum chloride PaCl (Aln(OH)mCl3(n-m))**
- the potential of their combination.

Matrices

- **Wastewater from textile washing and surface water (Danube river)** were selected as representatives of real water matrices in comparison with **synthetic matrix** (distilled water enriched with Na, Ca, and Mg salts.

2



What is coagulation/flocculation process?

TwinSubDyn

Coagulation ⇨ process of destabilization of colloidal particles

Flocculation ⇨ follows coagulation; destabilized particles connect with each other and larger agglomerates are formed

Coagulation and flocculation technology is considered as a typical treatment for the removal of suspended solids and colloidal particles in wastewater. Researchers focused on this treatment in recent years, mainly because of its low cost, ease of operation, and energy savings...

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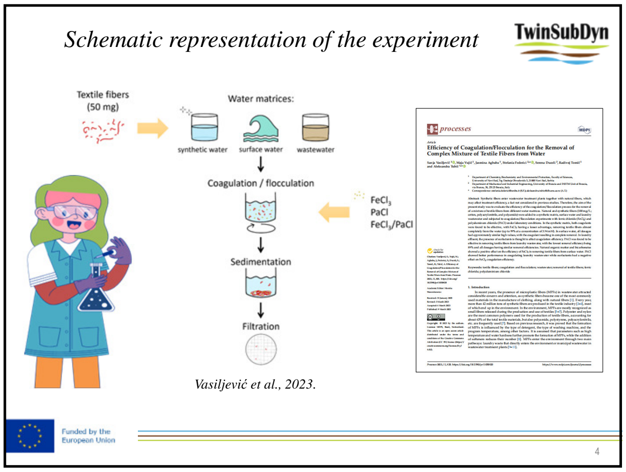

Schematic representation of the experiment

TwinSubDyn

Vasiljević et al., 2023.

Article

Efficiency of Coagulation/Flocculation for the Removal of Complex Mixture of Textile Fibers from Water

Sanja Vasiljević, Maja Vujić, Jasmina Agbaba, Stefania Federici, Serena Ducoli, Radivoj Tomić and Aleksandra Tubić

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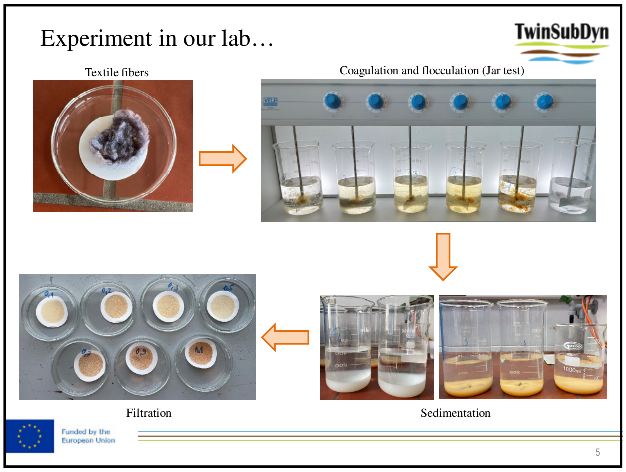


Experiment in our lab…

TwinSubDyn

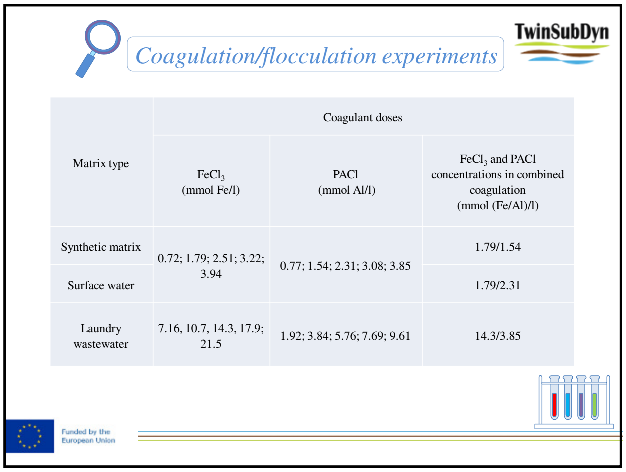
Textile fibers

Coagulation and flocculation (Jar test)

Filtration

Sedimentation

5



Coagulation/flocculation experiments

TwinSubDyn

Matrix type	Coagulant doses		
	$FeCl_3$ (mmol Fe/l)	PACl (mmol Al/l)	$FeCl_3$ and PACl concentrations in combined coagulation (mmol (Fe/Al)/l)
Synthetic matrix	0.72; 1.79; 2.51; 3.22; 3.94	0.77; 1.54; 2.31; 3.08; 3.85	1.79/1.54
Surface water			1.79/2.31
Laundry wastewater	7.16, 10.7, 14.3, 17.9; 21.5	1.92; 3.84; 5.76; 7.69; 9.61	14.3/3.85

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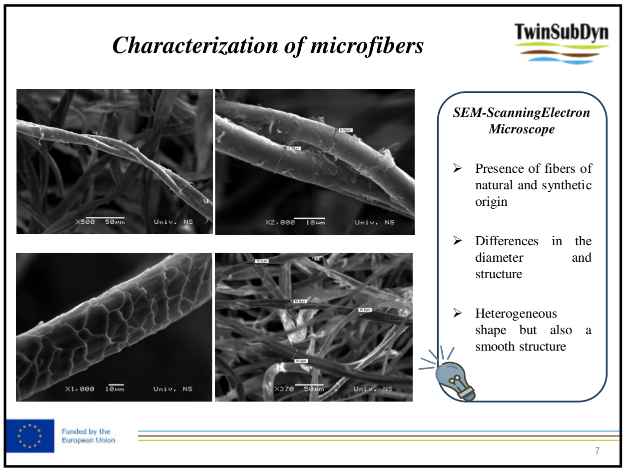
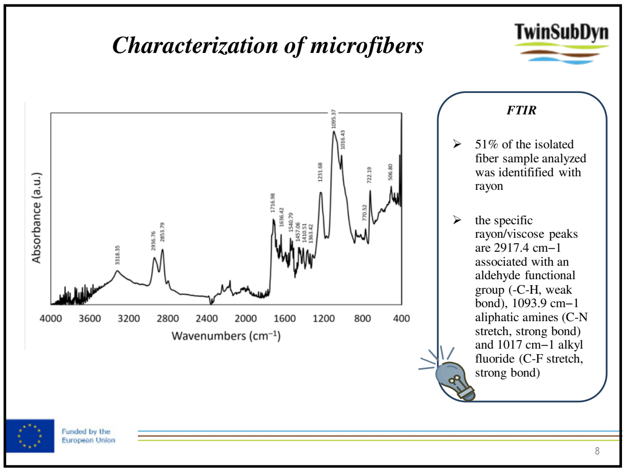


Characterization of microfibers

SEM-ScanningElectron Microscope

- Presence of fibers of natural and synthetic origin
- Differences in the diameter and structure
- Heterogeneous shape but also a smooth structure

7



Characterization of microfibers

FTIR

- 51% of the isolated fiber sample analyzed was identifified with rayon
- the specific rayon/viscose peaks are 2917.4 cm−1 associated with an aldehyde functional group (-C-H, weak bond), 1093.9 cm−1 aliphatic amines (C-N stretch, strong bond) and 1017 cm−1 alkyl fluoride (C-F stretch, strong bond)

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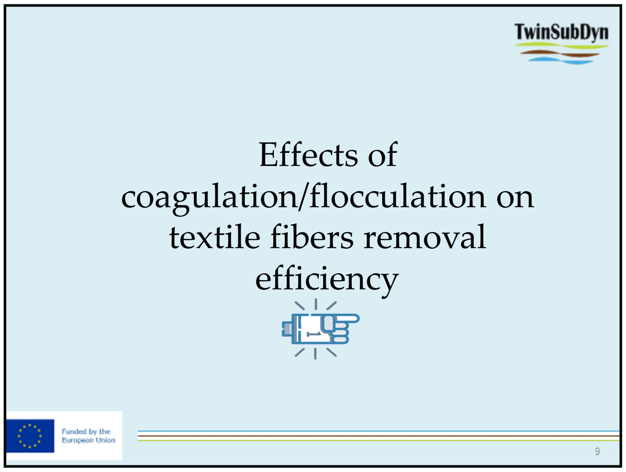

Effects of coagulation/flocculation on textile fibers removal efficiency

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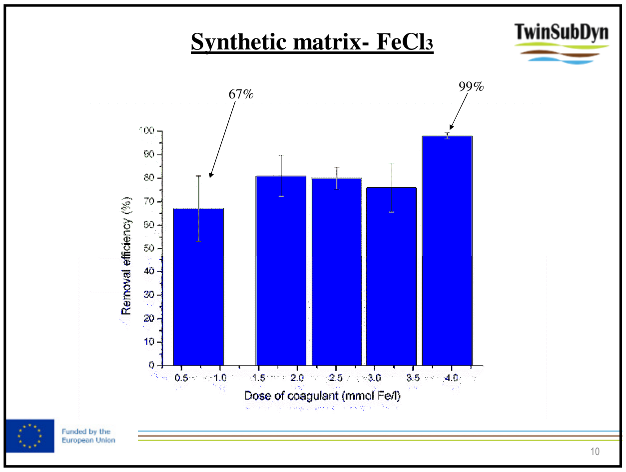

Synthetic matrix- FeCl3

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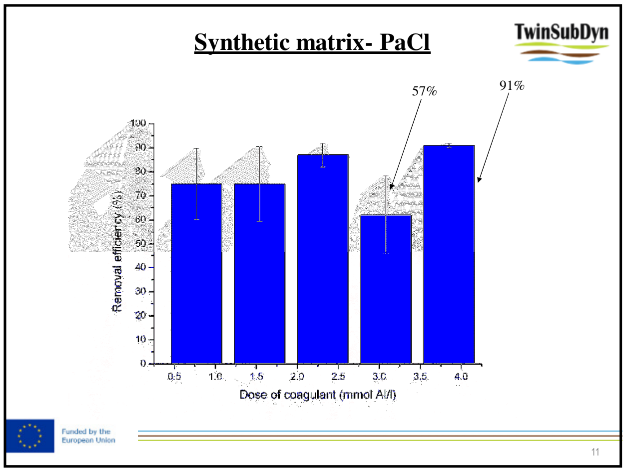
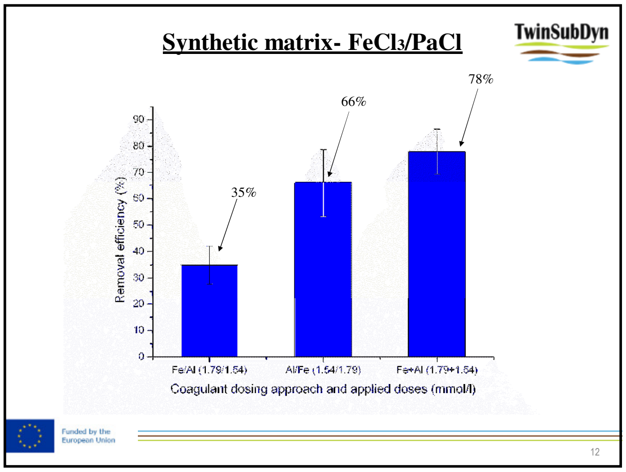


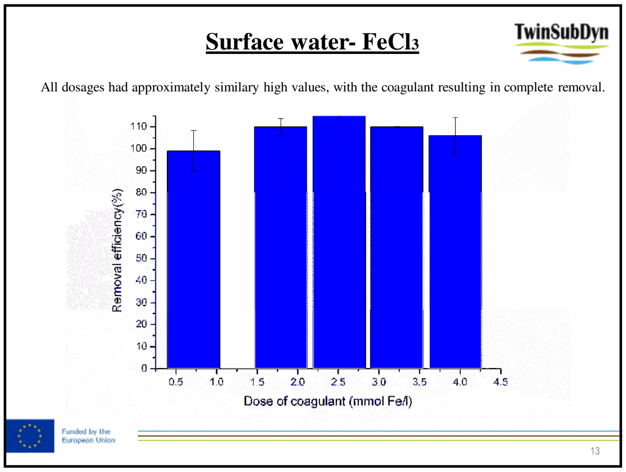
Synthetic matrix- PaCl

11



Synthetic matrix- FeCl3/PaCl

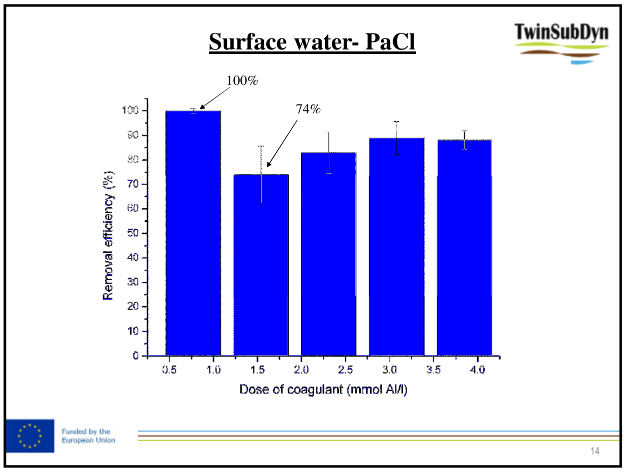
12



Surface water- FeCl3

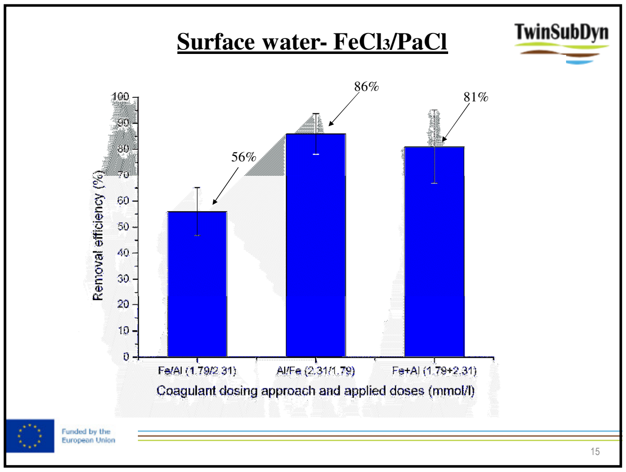
All dosages had approximately similary high values, with the coagulant resulting in complete removal.

13



Surface water- PaCl

14



Surface water- FeCl3/PaCl

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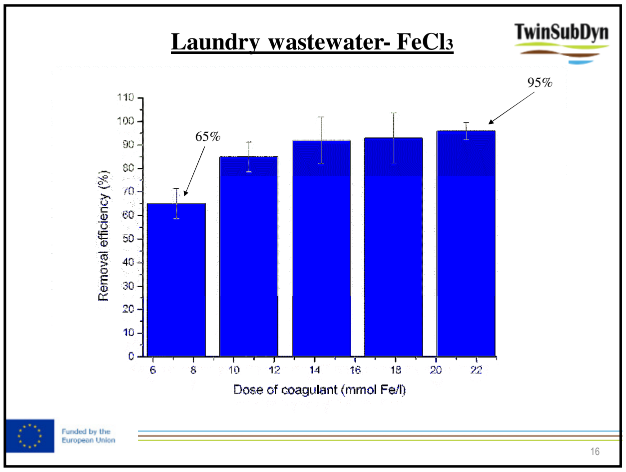
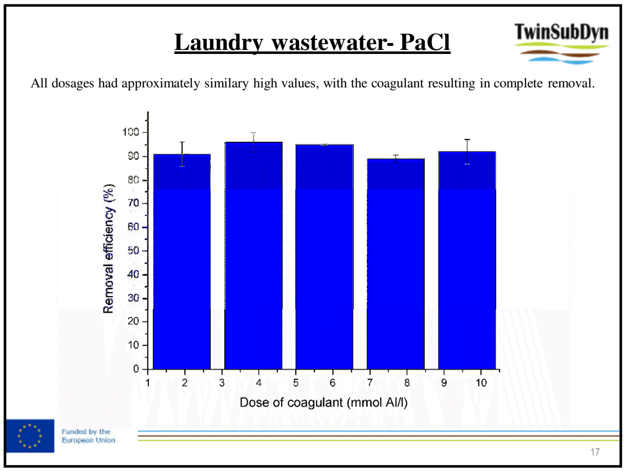


Laundry wastewater- FeCl3

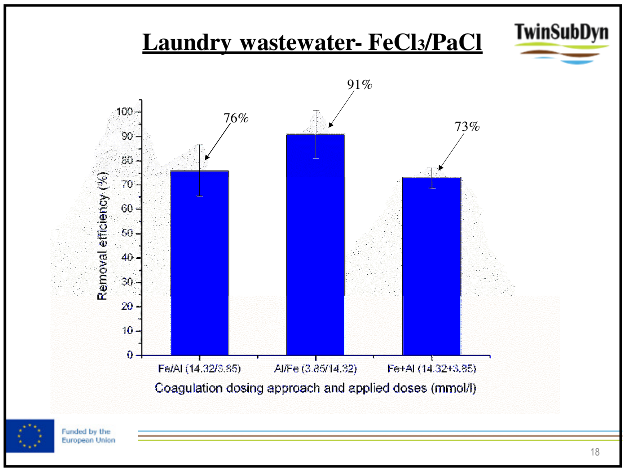
16



Laundry wastewater- PaCl

All dosages had approximately similary high values, with the coagulant resulting in complete removal.

17



Laundry wastewater- FeCl3/PaCl

18

Conclusion

TwinSubDyn

According to the literature data, the removal effificiency depends on the type of material to be removed.

The effectiveness of the coagulant also depends on the characteristics of the water matrix, which was also confifirmed by this work, as much higher coagulant dosages were required to effectively remove textile fibers from laundry wastewater compared to surface water and synthetic matrix.

The type and dosage of coagulant is another factor affecting the treatment effificiency, where PACl was more effective on laundry wastewater, while Fe-based coagulants were more effective at removing textile fifibers from synthetic matrix and surface water.

Future studies should focus on optimizing the coagulation–flocculation treatment (e.g., by adding activated carbon) to reduce the coagulant dose required for effificient treatment and better removal of textile fifibers from water.

Funded by the European Union

TwinSubDyn

Thank you for your attention!

Questions and comments?

Funded by the European Union

<u>Subtask 1.2.7 6-months secondment for ESR/RR and two 1-months secondments for QR/RR at UNVIE focused on methodologies for the characterization and analysis of particulate contaminants (leader UNIVIE).</u>

MOBILITY REPORT

Researcher: Prof. Dr. Aleksandra Tubić, UNSPMF

Assigned supervisor: Dr. Thorsten Hüffer, UNIVIE

Duration of the visit: 25.06.2023. - 23.07.2023.

Executive Summary

The main purpose of the visit Aleksandra Tubić (senior UNSPMF team member) to University of Vienna, Department of Environmental Geosciences, was participation in training related to impact of microplastics on organic soil amendments quality under the supervision of Dr. Thorsten Hüffer and with active participation of Charlotte Henkel. The activities included familiarization with the equipment for the analysis of different types of compounds in UNIVIE laboratories, training for leaching experiments, sample preparation and analysis of phthalates, as leachable compounds from microplastics, discussions with members of the UNIVIE team regarding the experiments and processing of results, as well as joint publications and future joint projects.

Introduction

Background

One of the specific objectives of the TwinSubDyn is to provide knowledge transfer and exchange of best practice between UNSPMF and EU leading partners UNIVIE, FZJ, MLU and CSIC in the field on the impact of OSA on SOM, contaminant and nutrient dynamics in the soil subsurface and their implications for groundwater quality. In order to achieve this objective, a certain number of UNSPMF researchers' mobilities and training are required. Short-term staff exchange with EU partners will allow UNSPMF researchers to acquire new knowledge and bridge the identified research gaps and needs. Training activities will be realized in cooperation with UNSPMF's strategic partners and in accordance with their field of expertise.

Scope of the secondment

The scope of the training exchange was knowledge transfer on methodologies for the characterization and analysis of colloids and microplastics. This was realized through one month participation in the experiments and analysis related to microplastic impact on organic soil amendments.

Content

WP1 Networking for excellence and knowledge transfer road map

Subtask 1.2.6 Two 1-mounth secondment for two QRs focused on methodologies for the characterization and analysis of colloids and microplastics.

The training focused on methodologies for the characterization and analysis of colloids and microplastics. The training was realized in the period of 25 June to 23 July 2023.

Location: Department of Environmental Geosciences, UNIVIE

Description of activities:

Training started with the discussion with UNIVIE researchers Dr. Thorsten Hüffer, Charlotte Henkel and Sanja Vasiljević on leaching and long-term dynamics of plastic-derived additives, with a special focus on PVC-derived phthalates, TOC analysis results of OSA needed for the evaluation of the OSA quality etc. The tour through the laboratories and safety measures instructions were made by lab manager Martin Stockhausen. The experiments included special preparation of the labware for the phthalate analysis, leaching and long-term dynamics experiment set-up, extraction and analysis method for PVC-derived phthalates from the water and activated carbon sinks, as well as GC-MS analysis. Additionally, three more meetings were held with the discussion of the obtained results in leaching tests, as well as three meetings with Gabriel Sigmund, Sanja Vasiljević, Irina Jevrosimov, Katharina Sodnikar, Charlotte Henkel discussing the experiments performed by UNSPMF team members in UNIVIE.

Apart from the training, Aleksandra Tubić was actively involved in the weekly meetings of the UNIVIE research team discussing potential future projects and other current topics in the UNIVIE team. Prof. Dr. Thilo Hoffman and Aleksandra Tubić agreed to jointly participate in the project proposal preparation in frame of HORIZON-MISS-2023-OCEAN-01-02 (Danube River basin lighthouse – Demonstration of effective and sustainable management of sediments in the Danube river-Black sea system) call, leaded by Dunarea de Jos University of Galati from Romania. Additionally, she participated in the interview of new PhD candidates, which was new very useful experience, since this is not practice in UNSPMF.

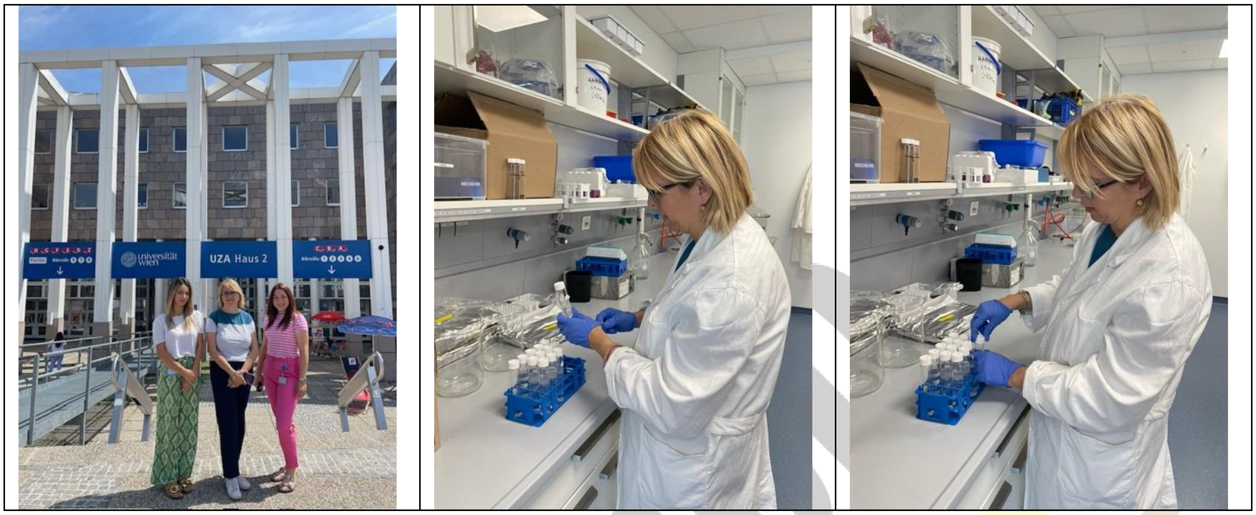


Figure 1. Sanja VASILJEVIĆ, Irina JEVROSIMOV and Aleksandra TUBIĆ at UNIVIE (left), Aleksandra TUBIĆ prepare the sample for phthalate analysis (right)

Impact on your project

The training gave researchers from UNSPMF improved knowledge on methodologies for the characterization and analysis of colloids and microplastics in OSA. The overall experience strengthens the connections with UNIVIE team, it enabled the exchange of ideas and knowledge, improved knowledge of UNSPMF researcher in project and research management, and added new elements to the cooperation of the two teams.

Subtask 1.2.8: 2-person two-weeks visits of QR/RR at UNVIE for intensive knowledge transfer focused on hydrogeological groundwater modelling and particle transport (leader UNIVIE).

MOBILITY REPORT

Researcher: Prof. Dr. Srđan Rončević, Prof Dr Aleksandra Tubić, UNSPMF

Assigned supervisor: Prof. Dr. Thilo Hofmann, UNIVIE

Duration of the visit: 26.06.2023. - 30.06.2023.

Executive Summary

The main purpose of the visit two senior faculty members from UNSPMF (Aleksandra Tubić and Srđan Rončević) to University of Vienna, Department of Environmental Geosciences, was participation in the course *Groundwater Systems - Module Groundwater Modeling* held by Prof. Thilo Hofmann and Dr. Andreas Darsow. This course covered distribution, measurement and analysis of the most important hydrogeological parameters, e.g. rainfall, discharge, groundwater recharge. Introducing analytical and numerical methods to solve flow and transport problems, the participants know how to process, analyze and interpret pumping test data and derive transmissivity and storage coefficients and can build a simplified numerical 3D groundwater model, understand boundary conditions, numerical discretization, calibration and validation of groundwater flow models.

Introduction

Background

One of the specific objectives of the TwinSubDyn is to provide knowledge transfer and exchange of best practices between UNSPMF and EU leading partners UNIVIE, FZJ, MLU and CSIC in the field on the impact of OSA on SOM, contaminant and nutrient dynamics in the soil subsurface and their implications for groundwater quality. To achieve the vision above, a certain number of UNSPMF researchers' mobilities and training are required. Short-term staff exchange with EU partners will allow UNSPMF researchers to acquire new knowledge and bridge the identified research gaps and needs. These STSE and training will be realized in cooperation with UNSPMF’s strategic partners and in accordance with their field of expertise.

Scope of the secondment

The scope of the short-term staff exchange was knowledge transfer on hydrogeological groundwater modeling for subsurface contaminant and particle transport. This was realized through participation in the course Groundwater Systems - Module Groundwater Modeling, which included lectures (introduction + theory), practical exercises, and presentations of real projects.

Content

WP1 Networking for excellence and knowledge transfer road map

Task 1.2 Short-term scientific visits and stays in EU research institutions for researchers from UNSPMF

Subtask 1.2.8 Two persons two-week visits of QR/RR at UNVIE for intensive knowledge transfer

The training focused on hydrogeological groundwater modeling for subsurface contaminant and particle transport, as well as particle transformation in the subsurface. The training is divided into two parts, the first held in June, and the second will be organized in September.

Course Title: Groundwater Systems - Module Groundwater Modeling

Teachers: Prof. Thilo Hofmann and Dr. Andreas Darsow

Time: 26-30/June/2023, 09:00-17:00

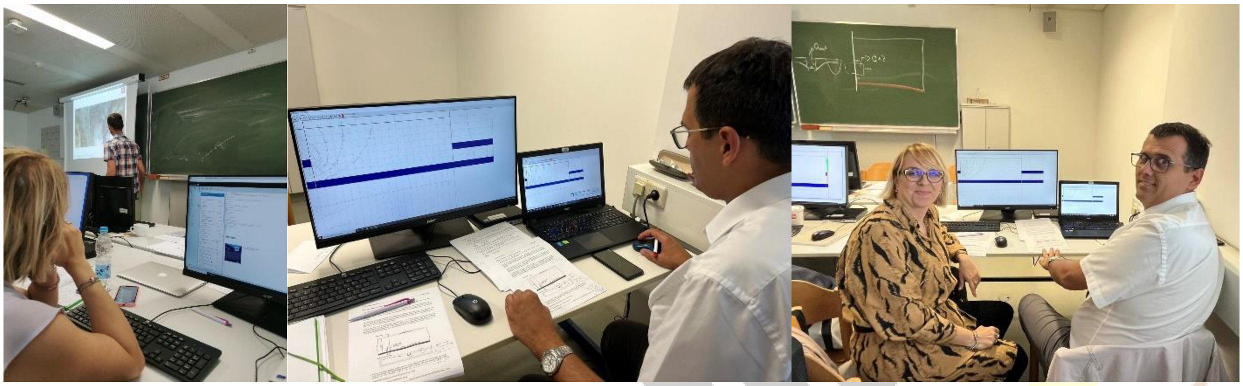
Location: EDV-Raum 4 2C502 5.OG UZA II, Department of Environmental Geosciences, UNIVIE

The course consists of lectures (introduction + theory), practical exercises, and presentations of real projects.

Software: Processing MODFLOW 8

LECTURES IN GROUNDWATER FLOW and TRANSPORT: from nature to model / model construction: discretization, flow velocities, Analog model, introduction PMWIN / MODFLOW, basics of numerical flow modeling, Darcy / flow equation, initial and boundary conditions, steady-state & transient flow, horizontal/vertical (2D/3D), isotropic/anisotropic, calibration/inverse modeling, aquifer parameters - determination of parameters in practice, basics solute transport

PRACTICAL EXERCISES: Model with three zones of different hydraulic conductivity; Well close to a river; Flow net and seepage under a weir; Isolation of a contaminated area; Pumping test; Isolation of a contaminated area II; Inflow of water into an excavation pit; Parameter estimation.



Furthermore, on June 26, 2023, we met with Giovanni Formentin and Victor Nogay in preparation for the second part of training in September. We discussed preparation for building a model that simulates the Strand source in Novi Sad. We will focus on vinyl chloride, to locate the sources of vinyl chloride in groundwater. We will analyse the data: location of wells and monitoring wells (table with X, Y, Z), the position of screened intervals of wells and monitoring wells, stratigraphic logs of drillings and geological/hydrogeological cross-sections, results from pumping tests performed in wells and monitoring wells, measurements of groundwater levels, concentrations of chlorinated compounds (PCE, TCE, DCA, vinyl chloride...), records of rainfall and Danube water levels.

Impact on your project

The training gave researchers from UNSPMF basic knowledge about groundwater flow modeling and the basic use of the software Modflow 8. This will help us to understand the limits of the use of modeling and to provide a better interpretation of data on subsurface contaminant dynamics.

The advancements and progress in Subtask 3.6.2 will be coordinated with ongoing efforts in Subtask 3.6.1 with FZJ to optimize progress and outcomes by utilizing synergies between the two tasks.

MOBILITY REPORT

Researcher: Prof. Dr. Srđan Rončević and Prof. Dr Aleksandra Tubić, UNSPMF

Assigned supervisor: Prof. Dr. Thilo Hofmann / Giovanni Formentin, UNIVIE

Duration of the visit: 04.09.2023. - 08.09.2023.

Executive Summary

The main purpose of the visit two senior faculty members from UNSPMF (Aleksandra Tubić and Srđan Rončević) to University of Vienna, Department of Environmental Geosciences, was training focused on hydrogeological groundwater modeling for subsurface contaminant and particle transport. Using real data, a model was created simulating the Štrand source using QGIS and ModelMuse software. Contamination leakage has been demonstrated, but further modeling work with real contamination data is needed.

Introduction

Background

One of the specific objectives of the TwinSubDyn is to provide knowledge transfer and exchange of best practices between UNSPMF and EU leading partners UNIVIE, FZJ, MLU and CSIC in the field on the impact of OSA on SOM, contaminant and nutrient dynamics in the soil subsurface and their implications for groundwater quality. To achieve the vision above, a certain number of UNSPMF researchers' mobilities and training are required. Short-term staff exchange with EU partners will allow UNSPMF researchers to acquire new knowledge and bridge the identified research gaps and needs. These STSE and training will be realized in cooperation with UNSPMF’s strategic partners and in accordance with their field of expertise.

Scope of the secondment

The scope of the short-term staff exchange was knowledge transfer on hydrogeological groundwater modeling for subsurface contaminant and particle transport. This was realized through training in the practical use of QGIS and ModelMuse software and building models on real data.

Content

WP1 Networking for excellence and knowledge transfer road map

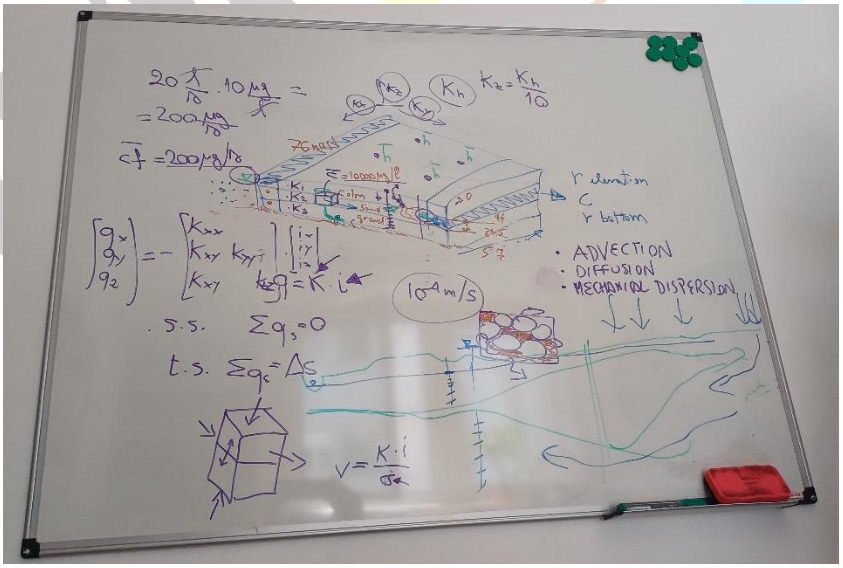
Task 1.2 Short-term scientific visits and stays in EU research institutions for researchers from UNSPMF

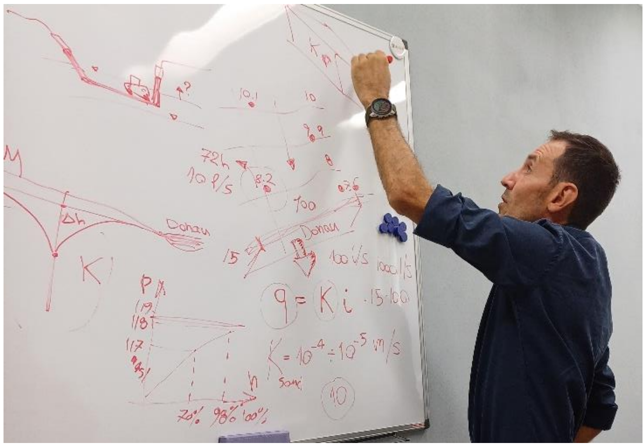
Subtask 1.2.8 Two persons two-week visits of QR/RR at UNVIE for intensive knowledge transfer

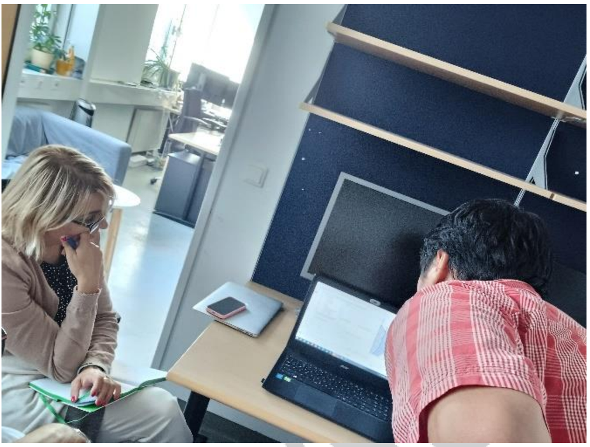
The training focused on hydrogeological groundwater modeling for subsurface contaminant and particle transport, as well as particle transformation in the subsurface. The training is divided into two parts, the first held in June, and the second was organized in September.

Softwares: QGIS and ModelMuse

From September 4 to 8, prof. Thilo Hofmann, Giovanni Formentin, and Victor Nogay taught us how to use QGIS and ModelMuse software. We worked on real data from the Štrand source in Novi Sad: location of wells and control wells, measurements of groundwater levels, stratigraphic records of wells and geological/hydrogeological sections, water levels of the Danube, etc. We created a model that simulates the Štrand source. Finally, we have demonstrated contamination leakage, but we need further modeling work with real contamination data.







Impact on your project

The training gave researchers from UNSPMF basic knowledge about groundwater flow modeling and the basic use of the QGIS and ModelMuse software. This will help us understand the limits of modeling use, provide a better interpretation of data on subsurface contaminant dynamics, and better design groundwater monitoring and data collection.

The advancements and progress in Subtask 3.6.2 will be coordinated with ongoing efforts in Subtask 3.6.1 with FZJ to optimize progress and outcomes by utilizing synergies between the two tasks.

ANNEX II

AGENDA

On-site training in sample preparation and analysis of contaminants and selected transformation products

Twinning excellence on organic soil amendments effect on nutrient and contaminant dynamics in the subsurface – TwinSubDyn

DAY 1.	**18.01.2023.**	
09:00-09:15	Meet up & agenda overview	UNSPMF (dr Snežana Maletić)
9:15 – 10:45	Lab tour at UNSPMF	Dr Thorsten HÜFFER, MSc Charlotte HENKEL Dr Snežana Maletić All participant
10:45-11:00	Coffee break	
11:00-12:00	"(Micro-)plastics in the environment - lessons learnt and current challenges "	Dr Thorsten HÜFFER
12:00-13:00	Lunch break	
13:00-14:00	Sample preparation techniques for phthalates analysis	MSc Charlotte HENKEL
14:00-15:00	A brief round of introduction of UNSPMF researchers	Project members
15:00-17:00	Discussion on phthalate analysis	All participant
DAY 2.	**19.01.2023.**	
09:00 – 09:15	Meet up & agenda overview	UNSPMF (dr Snežana Maletić)
09:15 -13:00	Experiment planning and discussion related to microplastics and their associated additives analysis	UNSPMF and UNIVIE team (dr Thorsten HÜFFER; MSc Charlotte HENKEL)

Link for online participation (MS Teams):

Day 1. https://teams.microsoft.com/l/meetup-join/19%3ameeting_ODEyZDA1NzktNzBiMC00YjMyLTk2NWYtMGU5ZWVmYWY0ZDM0%40thread.v2/0?context=%7b%22Tid%22%3a%22e7a31644-4c56-46ed-af60-8b0906c738c0%22%2c%22Oid%22%3a%225c753ce1-8943-4c10-9e28-cb51e72c6d55%22%7d

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ATTENDANCE LIST

On-site training in sample preparation and analysis of contaminants and selected transformation products

Day 1 18. Jan. 2023.

Twinning excellence on organic soil amendments effect on nutrient and contaminant dynamics in the subsurface – TwinSubDyn (Grant agreement No 101059546)

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ATTENDANCE LIST

On-site training in sample preparation and analysis of contaminants and selected transformation products

Day 2 19. Jan. 2023.

Twinning excellence on organic soil amendments effect on nutrient and contaminant dynamics in the subsurface – TwinSubDyn (Grant agreement No 101059546)

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AGENDA

On-site training in the application of LC-MS(/MS) for Organic Contaminants in soil science

Twinning excellence on organic soil amendments effect on nutrient and contaminant dynamics in the subsurface – TwinSubDyn (Grant agreement no 101059546)

DAY 1.	**03.04.2023.**	
14:00-14:15	Meet up & agenda overview	UNSPMF (dr Snežana Maletić)
14:15 – 15:00	Tour through the facilities and labs	Key members
15:00-17:00	Individual meetings UNIVIE with UNSPMF researchers. UNSPMF researchers prepare a few slides to introduce their research as a basis for discussions	All participants
DAY 2.	**04.04.2023.**	
09:00 – 09:15	Agenda overview	UNSPMF (dr Snežana Maletić)
9:15 – 10:00	Biodegradation and Biotransformation of Organic Contaminants in Receiving Environment – Applying LC-MS/MS and Related Approaches	Asst. Prof. Michael Zumstein
10:00-10:15	Coffee break	
11:15-12:00	Fate of DNA&RNA in Soils: Interaction with Minerals and Biodegradation	Dr. Katharina Sodnikar
12:00-13:00	Lunch break	
13:00-13:45	Predicting Biotransformation Products of Organic Pollutants and Hunting them with LC-HRMS/MS	Asst. Prof. Michael Zumstein
13:45-14:30	General Principles working with LC-MS(/MS) and Method Development (including Sample Preparation) for Selected Examples of Organic Contaminants.	Dr. Katharina Sodnikar
14:30-14:45	Coffee break	
14:45-17:00	Discussion between UNIVIE and UNSPMF researchers	UNIVIE and UNSPMF
DAY 3.	**05.04.2023.**	
09:00 – 09:15	Agenda overview	UNSPMF (dr Snežana Maletić)
9:15 – 12:00	Discussion on identified topics during Mon/Tues	All participants

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Link for online participation (MS Teams):

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ATTENDANCE LIST

On-site training in the application of LC-MS(/MS) for Organic Contaminants analysis and related Biodegradation/Biotransformation products in soil science

Day 1 3 April 2023.

Twinning excellence on organic soil amendments effect on nutrient and contaminant dynamics in the subsurface – TwinSubDyn (Grant agreement No 101059546)

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ATTENDANCE LIST

On-site training in the application of LC-MS(/MS) for Organic Contaminants analysis and related Biodegradation/Biotransformation products in soil science

Day 2 4 April 2023.

Twinning excellence on organic soil amendments effect on nutrient and contaminant dynamics in the subsurface – TwinSubDyn (Grant agreement No 101059546)

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ATTENDANCE LIST

On-site training in the application of LC-MS(/MS) for Organic Contaminants analysis and related Biodegradation/Biotransformation products in soil science

Day 3 5 April 2023.

Twinning excellence on organic soil amendments effect on nutrient and contaminant dynamics in the subsurface – TwinSubDyn (Grant agreement No 101059546)

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AGENDA

On-site training in Meta-analysis

Twinning excellence on organic soil amendments effect on nutrient and contaminant dynamics in the subsurface – TwinSubDyn (Grant agreement no 101059546)

DAY 1.	**20.09.2023.**	
08:45 – 09:00	Agenda overview	UNSPMF (dr Snežana Maletić)
9:00 – 10:00	Seminar block 1 "Introduction; Plan of the upcoming days"	Prof. Dr. Bruno Glaser
10:00-10:30	Coffee break	
10:30-12:00	Seminar block 2 "Meta-analysis calculation in R"	Arthur Groß
12:00-13:00	Lunch break	
13:00-15:00	Seminar block 3 "Interim presentation Group 1 and 2"	Group and 5
15:00-16:00	Q&A Meta-analysis	All participants
DAY 2.	**21.09.2023.**	
09:00 – 09:15	Agenda overview	UNSPMF (dr Snežana Maletić)
9:15-11:00	Seminar block 4 "Interim presentation Group 3 and 4"	Group 3 and 4
11:00-12:00	Q&A Meta-analysis	All participants
12:00-13:00	Lunch break	
13:00-15:00	Seminar block 5 "Interim presentation Group 5"	Group 5
15:00-16:00	Q&A Meta-analysis	All participants

Link for online participants: https://teams.microsoft.com/l/meetup-join/19%3ameeting_MDM3NzAyZDItNjkxYy00ODk0LWExZTQtZjgzMTRkNmJhZWRh%40thread.v2/0?context=%7b%22Tid%22%3a%22e7a31644-4c56-46ed-af60-8b0906c738c0%22%2c%22Oid%22%3a%225c753ce1-8943-4c10-9e28-cb51e72c6d55%22%7d

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ATTENDANCE LIST
On-site training in Meta analysis
Day 1, 20. September 2023.
Twinning excellence on organic soil amendments effect on nutrient and contaminant dynamics in the subsurface – TwinSubDyn (Grant agreement No 101059546)

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ATTENDANCE LIST
On-site training in Meta analysis
Day 2, 21. September 2023.
Twinning excellence on organic soil amendments effect on nutrient and contaminant dynamics in the subsurface – TwinSubDyn (Grant agreement No 101059546)

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AGENDA

On-site training on large-scale field experiments for the purpose of the assessment of the impact of the organic amendment's application on the soil subsurface and groundwater quality – II part

Twinning excellence on organic soil amendments effect on nutrient and contaminant dynamics in the subsurface – TwinSubDyn
(Grant agreement no 101059546)

DAY 1.	**22.09.2023.**	
09:00 – 09:15	Agenda overview	UNSPMF (dr Snežana Maletić)
9:15 – 11:15	Part 1: Introduction, Overview four BC-LTEs in Germany, Planning of a new field trial	Prof. Dr. Bruno Glaser
11:15-11:30	Coffee break	
11:30-12:30	Part 2: Statistics	Arthur Groß
12:30-13:30	Lunch break	
13:30-16:00	Discussion between MLU and UNSPMF researchers	MLU and UNSPMF
DAY2.	**25.09.2023.**	
09:00-16:00	Sampling soil on Itebej field trial, Serbia	All participants

Link for online participation (MS Teams): https://teams.microsoft.com/l/meetup-join/19%3ameeting_YWRiNmEzZjAtMTVmOC00NGE1LWJkYzEtMTYzZjIwNzYyNmE3%40thread.v2/0?context=%7b%22Tid%22%3a%22e7a31644-4c56-46ed-af60-8b0906c738c0%22%2c%22Oid%22%3a%225c753ce1-8943-4c10-9e28-cb51e72c6d55%22%7d

ATTENDANCE LIST

On-site training in large-scale field experiments for the purpose of the assessment of the impact of the organic amendment's application on the soil subsurface and ground water quality – II part

22 September 2023.

Twinning excellence on organic soil amendments effect on nutrient and contaminant dynamics in the subsurface – TwinSubDyn (Grant agreement No 101059546)

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ATTENDANCE LIST

On-site training in large-scale field experiments for the purpose of the assessment of the impact of the organic amendment's application on the soil subsurface and ground water quality – II part

25 September 2023.

Twinning excellence on organic soil amendments effect on nutrient and contaminant dynamics in the subsurface – TwinSubDyn (Grant agreement No 101059546)

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ATTENDANCE LIST
On-site training in large-scale field experiments for the purpose of the assessment of the impact of the organic amendment's application on the soil subsurface and ground water quality
27-31 April 2023.
Twinning excellence on organic soil amendments effect on nutrient and contaminant dynamics in the subsurface – TwinSubDyn (Grant agreement No 101059546)

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AGENDA

On-site training in the application of solid-state NMR and analytical pyrolysis techniques in soil science

Twinning excellence on organic soil amendments effect on nutrient and contaminant dynamics in the subsurface – TwinSubDyn

(Grant agreement no 101059546)

DAY 1.	**06.02.2023.**	
09:00-09:15	Meet up & agenda overview	UNSPMF (dr Snežana Maletić)
9:15 – 11:00	Introduction into the theory of NMR	Dr Heike Knicker
11:00-11:15	Coffee break	
11:15-13:00	Introduction into the theory of NMR	Dr Heike Knicker
13:00-14:00	Lunch break	
14:00-15:00	Introduction to analytical pyrolysis and hyphenated techniques	Dr. José A. González Pérez
15:00-15:15	Coffee break	
15:15-17:00	Introduction to analytical pyrolysis and hyphenated techniques	Dr. José A. González Pérez
DAY 2.	**07.02.2023.**	
09:00 – 09:15	Agenda overview	UNSPMF (dr Snežana Maletić)
9:15 – 11:00	Interactions during an NMR experiment	Dr Heike Knicker
11:00-11:15	Coffee break	
11:15-13:00	Interactions during an NMR experiment	Dr Heike Knicker
13:00-14:00	Lunch break	
14:00-15:00	Conventional analytical pyrolysis (Py-GC/MS) and High resolution analytical pyrolysis (Py-GC-Q-TOF). Examples of application to biomass and soil science	Dr. José A. González Pérez
15:00-15:15	Coffee break	
15:15-17:00	Conventional analytical pyrolysis (Py-GC/MS) and High resolution analytical pyrolysis (Py-GC-Q-TOF). Examples of application to biomass and soil science	Dr. José A. González Pérez
DAY 3.	**08.02.2023.**	
09:00 – 09:15	Agenda overview	UNSPMF (dr Snežana Maletić)

9:15 – 11:00	Basic of solid-state NMR - The cross polarization magic angle spinning experiment -Interpretation of solid-state NMR data	Dr Heike Knicker
11:00-11:15	Coffee break	
11:15-13:00	Basic of solid-state NMR - The cross polarization magic angle spinning experiment -Interpretation of solid-state NMR data	Dr Heike Knicker
13:00-14:00	Lunch break	
14:00-15:00	Short Introduction to stable isotope analysis and Pyrolyis compound specific isotope analysis (Py-CSIA). Examples of application to biomass and soil science.	Dr. José A. González Pérez
15:00-15:15	Coffee break	
15:15-17:00	Short Introduction to stable isotope analysis and Pyrolyis compound specific isotope analysis (Py-CSIA). Examples of application to biomass and soil science.	Dr. José A. González Pérez
DAY 4.	**09.02.2023.**	
09:00 – 09:15	Agenda overview	UNSPMF (dr Snežana Maletić)
9:15 – 11:00	Examples of the application of solid-state NMR spectroscopy in soil science and biochar research	Dr Heike Knicker
11:00-11:15	Coffee break	
11:15-13:00	Examples of the application of solid-state NMR spectroscopy in soil science and biochar research	Dr Heike Knicker
13:00-14:00	Final discussion	All participant

Link for online participation (MS Teams):

https://teams.microsoft.com/l/meetup-join/19%3ameeting_YWUzMGFjMjctZjg2MC00MWFmLThhYzktZWM1YjE0Nzg4MjFl%40thread.v2/0?context=%7b%22Tid%22%3a%22e7a31644-4c56-46ed-af60
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ATTENDANCE LIST

On-site training in the application of solid-state NMR and analytical pyrolysis techniques in soil science

Day 1 6. Feb. 2023.

Twinning excellence on organic soil amendments effect on nutrient and contaminant dynamics in the subsurface – TwinSubDyn (Grant agreement No 101059546)

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ATTENDANCE LIST

On-site training in the application of solid-state NMR and analytical pyrolysis techniques in soil science

Day 3 8. Feb. 2023.

Twinning excellence on organic soil amendments effect on nutrient and contaminant dynamics in the subsurface – TwinSubDyn (Grant agreement No 101059546)

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ATTENDANCE LIST

On-site training in the application of solid-state NMR and analytical pyrolysis techniques in soil science

Day 2 7. Feb. 2023.

Twinning excellence on organic soil amendments effect on nutrient and contaminant dynamics in the subsurface – TwinSubDyn (Grant agreement No 101059546)

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ATTENDANCE LIST

On-site training in the application of solid-state NMR and analytical pyrolysis techniques in soil science

Day 4 9. Feb. 2023.

Twinning excellence on organic soil amendments effect on nutrient and contaminant dynamics in the subsurface – TwinSubDyn (Grant agreement No 101059546)

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ANNEX III

TwinSubDyn

Meta-analysis of biosolid application effects on toxic element concentration in soil and plants

Date: 20.09.2023.

Prepared by: Snežana Maletić, Srđan Rončević, Irina Jevrosimov, Jelena Beljin, Marijana Kragulj Isakovski

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WHAT ARE BIOSOLIDS?

- EPA (2019), the term “biosolids” indicates a sludge that had received one or more treatments, which can be: aerobic or anaerobic digestion, alkaline stabilization, thermal drying, acid oxidation/disinfection, composting, etc.
- https://www.epa.gov/biosolids/biosolids-laws-and-regulations#process

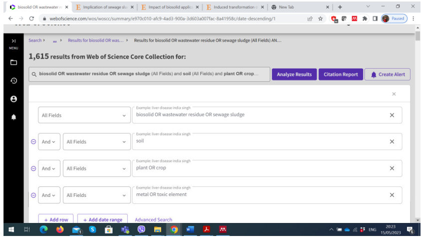
---

1. Research question:

- Weather application of biosolid increase toxic elements concentration in soil and plants?

Search category

- Data bases: Web of Science
- Key words - search term: →
- NO of hints 1615



1,615 results from Web of Science Core Collection for:

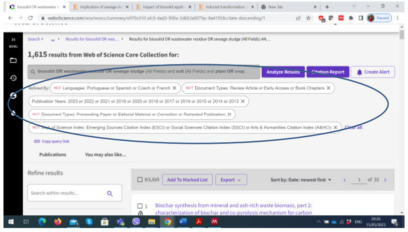
biosolid OR wastewater residue OR sewage sludge (All Fields) and soil (All Fields) and plant OR crop...

	All Fields	biosolid OR wastewater residue OR sewage sludge
And	All Fields	soil
And	All Fields	plant OR crop
And	All Fields	metal OR toxic element

https://www.webofscience.com/wos/woscc/summary/e970c010-afc9-4ad3-900a-3d603a007fac-8a41958c/relevance/1

Search category

- Last 10 years 2013-2023.
- Excluding review
- Paper
- ….
- NO of hints 1615



1,615 results from Web of Science Core Collection for:

biosolid OR wastewater residue OR sewage sludge (All Fields) and soil (All Fields) and plant OR crop...

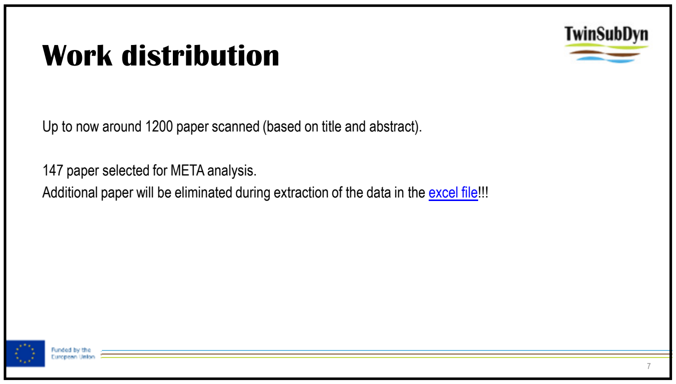
Refined By: NOT Languages: Portuguese or Spanish or Czech or French
NOT Document Types: Review Article or Early Access or Book Chapters
Publication Years: 2023 or 2022 or 2021 or 2019 or 2020 or 2018 or 2017 or 2016 or 2015 or 2014 or 2013
NOT Document Types: Proceeding Paper or Editorial Material or Correction or Retracted Publication
NOT Web of Science Index: Emerging Sources Citation Index (ESCI) or Social Sciences Citation Index (SSCI) or Arts & Humanities Citation Index (A&HCI)

1 Biochar synthesis from mineral and ash-rich waste biomass, part 2: characterization of biochar and co-pyrolysis mechanism for carbon

https://www.webofscience.com/wos/woscc/summary/e970c010-afc9-4ad3-900a-3d603a007fac-8a41958c/relevance/1

Elimination criteria

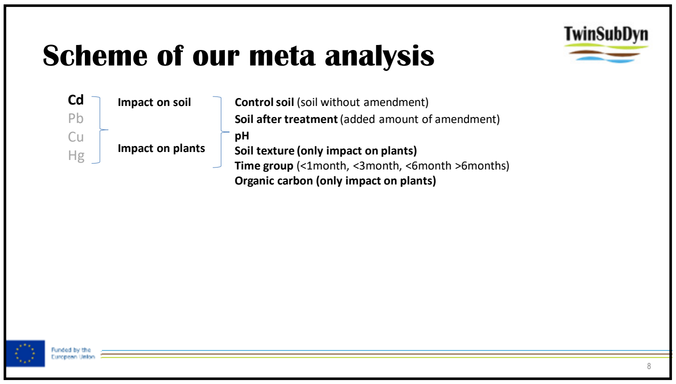
- Completely irrelevant (production biochar from BS, …………….)
- Term biosolids are not in line with the EPA (2019) definition.
- Lack of treatment free control.
- Agroforestry systems (parklands, forests, home gardens)
- Include only intensive crop farming - Exclude all except experiments related to cultivation of crops: corn, cereal, and oil plants
- Mixing biosolids with other amendments.
- Not dealing with toxic elements (e.g dealing with nutrients..)
- Exclude organic pollutants
- Application of biosolids on soil without including crops



Work distribution

Up to now around 1200 paper scanned (based on title and abstract).

147 paper selected for META analysis.
Additional paper will be eliminated during extraction of the data in the excel file!!!

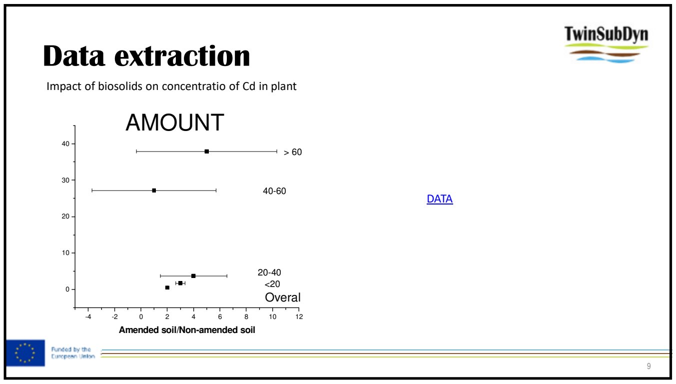


Scheme of our meta analysis

Cd
Pb
Cu
Hg

Impact on soil
Impact on plants

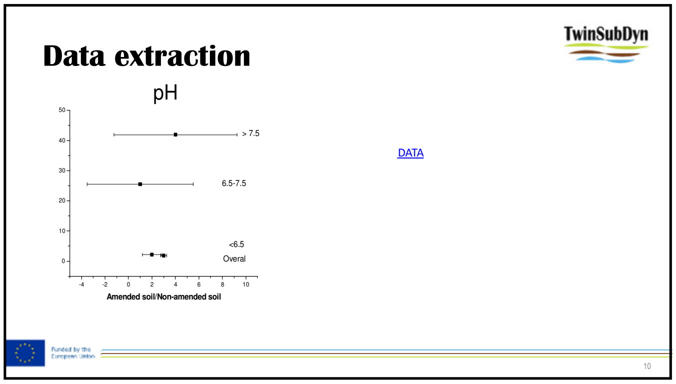
Control soil (soil without amendment)
Soil after treatment (added amount of amendment)
pH
Soil texture (only impact on plants)
Time group (<1month, <3month, <6month >6months)
Organic carbon (only impact on plants)



Data extraction

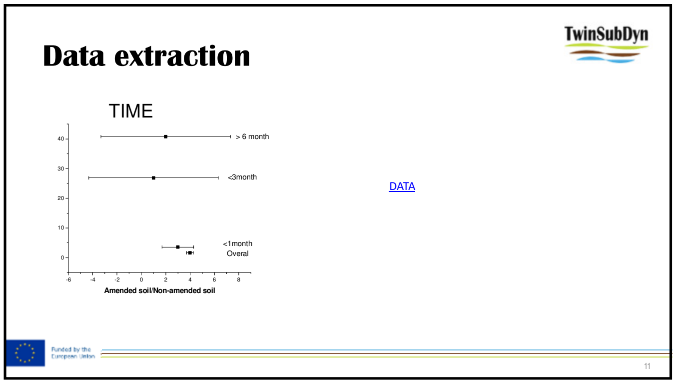
Impact of biosolids on concentratio of Cd in plant

DATA



Data extraction

DATA



Data extraction

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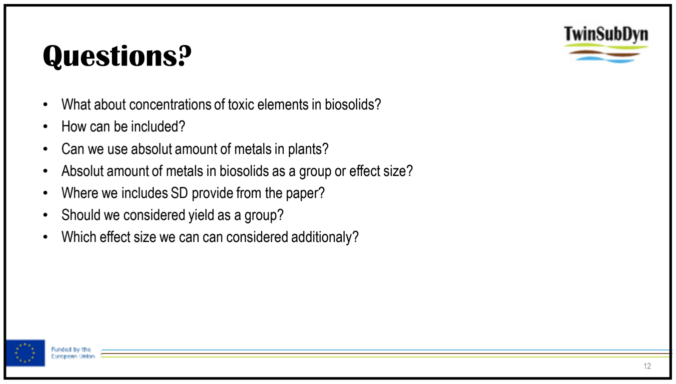

Questions?

- What about concentrations of toxic elements in biosolids?
- How can be included?
- Can we use absolut amount of metals in plants?
- Absolut amount of metals in biosolids as a group or effect size?
- Where we includes SD provide from the paper?
- Should we considered yield as a group?
- Which effect size we can can considered additionaly?

TwinSubDyn

Thank you!

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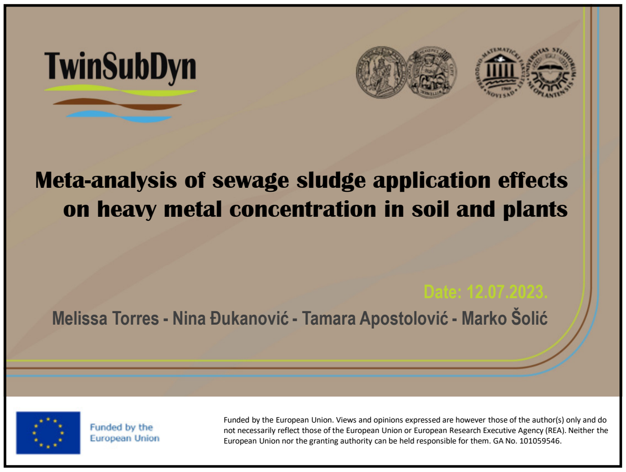

TwinSubDyn

Meta-analysis of sewage sludge application effects on heavy metal concentration in soil and plants

Date: 12.07.2023.

Melissa Torres - Nina Đukanović - Tamara Apostolović - Marko Šolić

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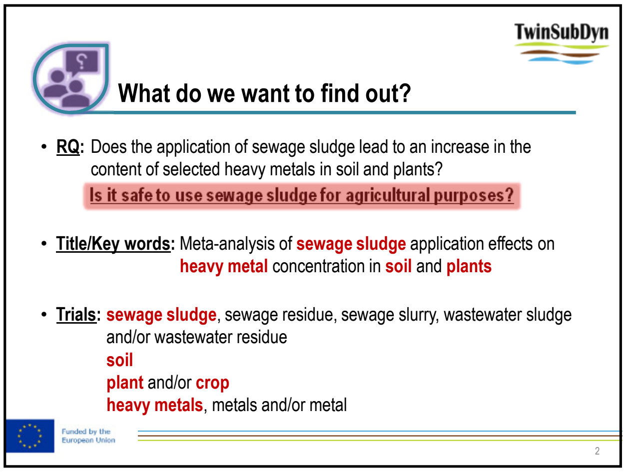

TwinSubDyn

What do we want to find out?

- **RQ**: Does the application of sewage sludge lead to an increase in the content of selected heavy metals in soil and plants?
  Is it safe to use sewage sludge for agricultural purposes?
- **Title/Key words**: Meta-analysis of **sewage sludge** application effects on **heavy metal** concentration in **soil** and **plants**
- **Trials**: **sewage sludge**, sewage residue, sewage slurry, wastewater sludge and/or wastewater residue
  soil
  plant and/or **crop**
  heavy metals, metals and/or metal

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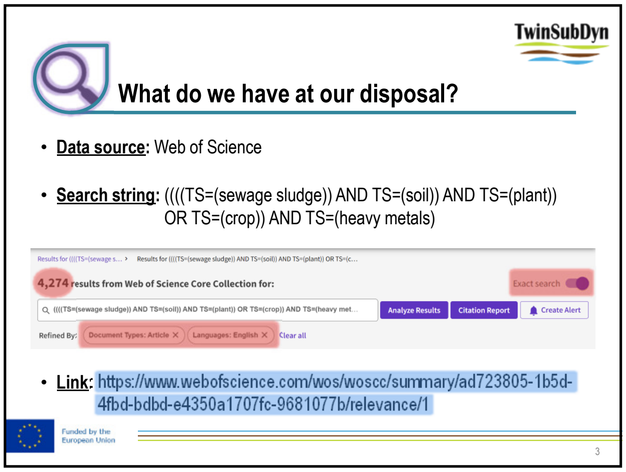

TwinSubDyn

What do we have at our disposal?

- **Data source**: Web of Science
- **Search string**: ((((TS=(sewage sludge)) AND TS=(soil)) AND TS=(plant)) OR TS=(crop)) AND TS=(heavy metals)

Results for ((((TS=(sewage s… > Results for ((((TS=(sewage sludge)) AND TS=(soil)) AND TS=(plant)) OR TS=(c…

4,274 results from Web of Science Core Collection for:

((((TS=(sewage sludge)) AND TS=(soil)) AND TS=(plant)) OR TS=(crop)) AND TS=(heavy met…

Refined By: Document Types: Article ✕ Languages: English ✕ Clear all

- **Link**: https://www.webofscience.com/wos/woscc/summary/ad723805-1b5d-4fbd-bdbd-e4350a1707fc-9681077b/relevance/1

Funded by the European Union

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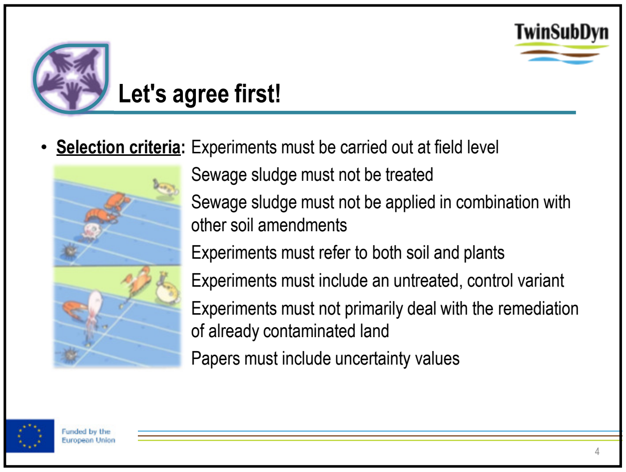

TwinSubDyn

Let's agree first!

- **Selection criteria**: Experiments must be carried out at field level
  Sewage sludge must not be treated
  Sewage sludge must not be applied in combination with other soil amendments
  Experiments must refer to both soil and plants
  Experiments must include an untreated, control variant
  Experiments must not primarily deal with the remediation of already contaminated land
  Papers must include uncertainty values

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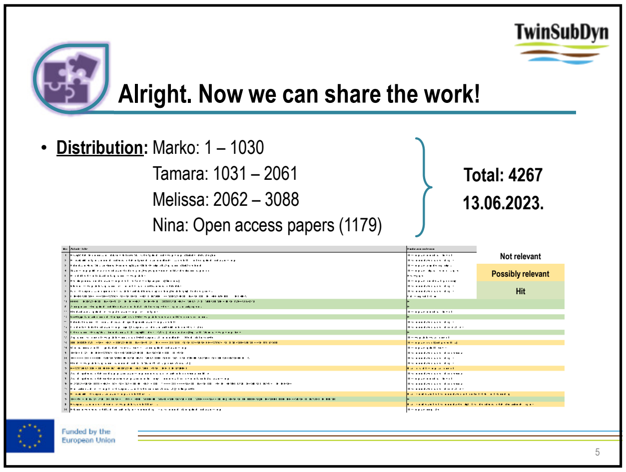

TwinSubDyn

Alright. Now we can share the work!

- **Distribution**: Marko: 1 – 1030
  Tamara: 1031 – 2061
  Melissa: 2062 – 3088
  Nina: Open access papers (1179)

Total: 4267
13.06.2023.

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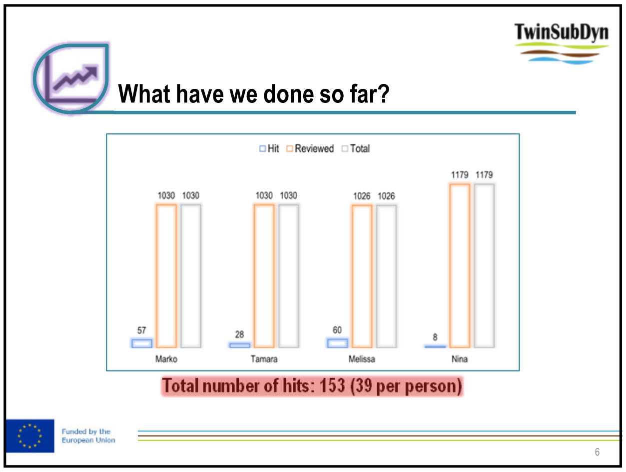

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What have we done so far?

Total number of hits: 153 (39 per person)

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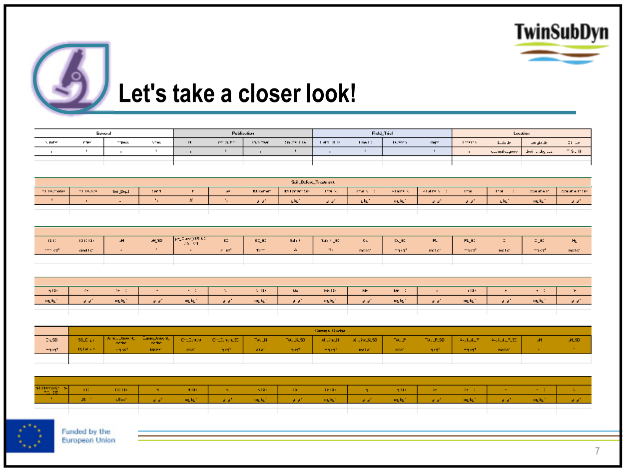

Let's take a closer look!

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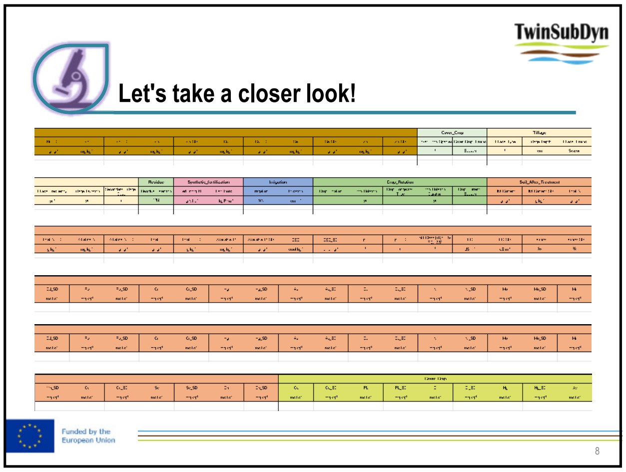

Let's take a closer look!

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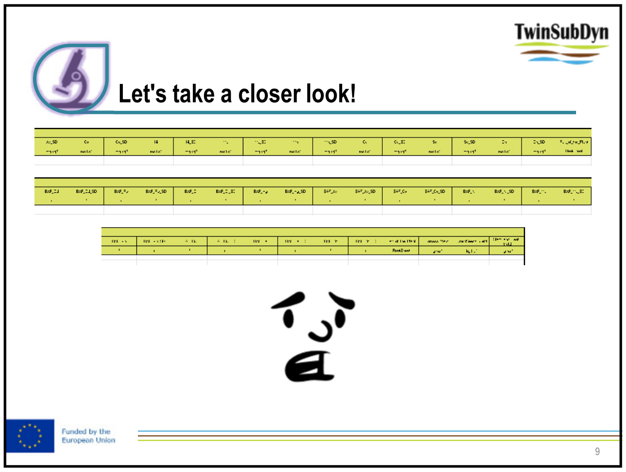

Let's take a closer look!

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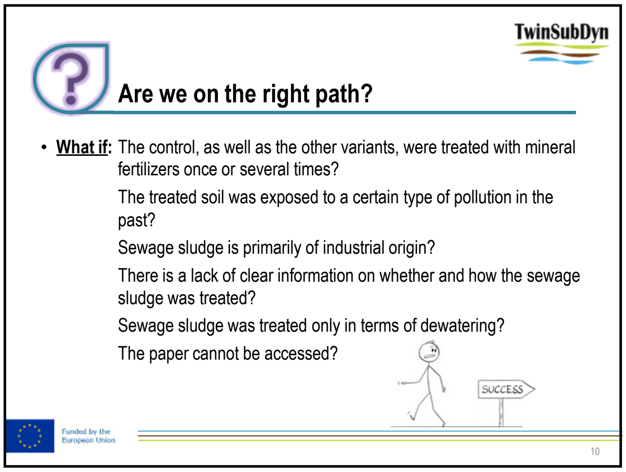

Are we on the right path?

- **What if**: The control, as well as the other variants, were treated with mineral fertilizers once or several times?

  The treated soil was exposed to a certain type of pollution in the past?

  Sewage sludge is primarily of industrial origin?

  There is a lack of clear information on whether and how the sewage sludge was treated?

  Sewage sludge was treated only in terms of dewatering?

  The paper cannot be accessed?

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Thank you!

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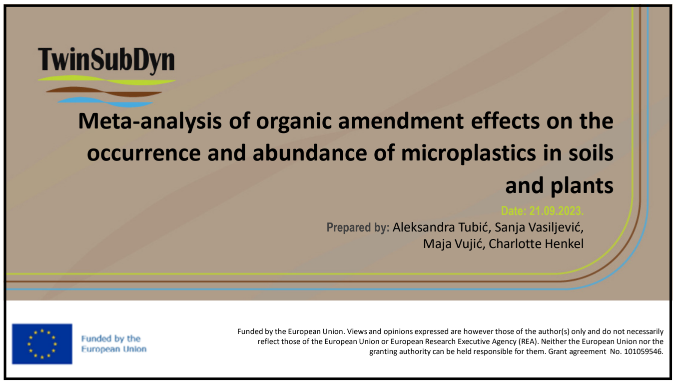

TwinSubDyn

Meta-analysis of organic amendment effects on the occurrence and abundance of microplastics in soils and plants

Date: 21.09.2023.

Prepared by: Aleksandra Tubić, Sanja Vasiljević,
Maja Vujić, Charlotte Henkel

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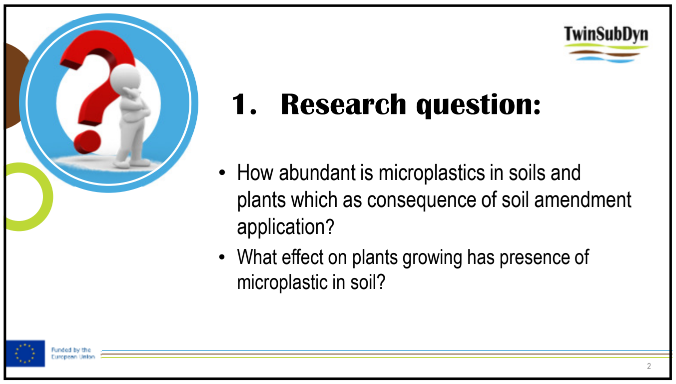
1



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1. Research question:

- How abundant is microplastics in soils and plants which as consequence of soil amendment application?
- What effect on plants growing has presence of microplastic in soil?

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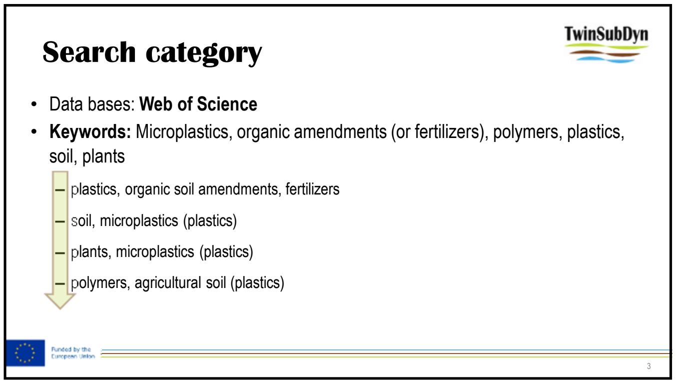

Search category

TwinSubDyn

- Data bases: **Web of Science**
- **Keywords:** Microplastics, organic amendments (or fertilizers), polymers, plastics, soil, plants
  - plastics, organic soil amendments, fertilizers
  - soil, microplastics (plastics)
  - plants, microplastics (plastics)
  - polymers, agricultural soil (plastics)

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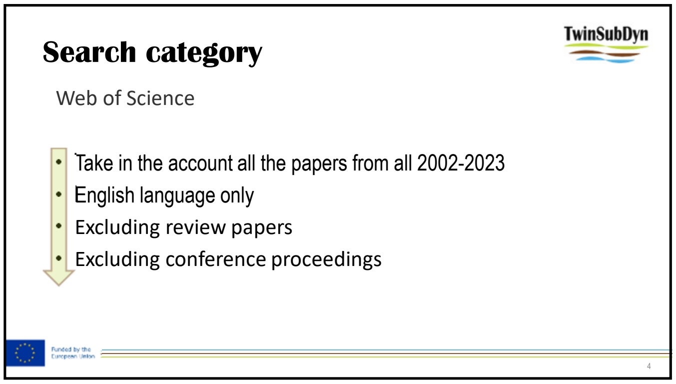

Search category

TwinSubDyn

Web of Science

- Take in the account all the papers from all 2002-2023
- English language only
- Excluding review papers
- Excluding conference proceedings

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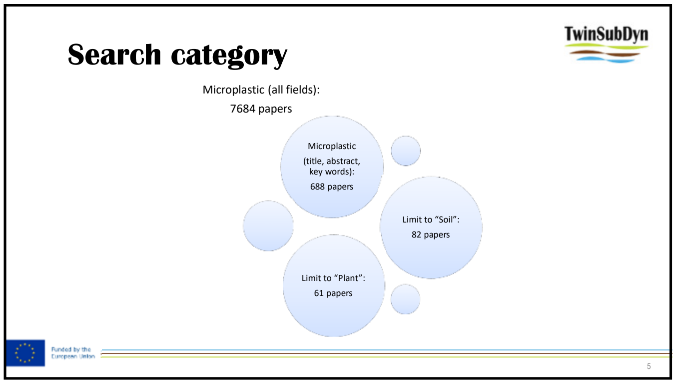

Search category

TwinSubDyn

Microplastic (all fields):
7684 papers

Microplastic
(title, abstract, key words):
688 papers

Limit to "Soil":
82 papers

Limit to "Plant":
61 papers

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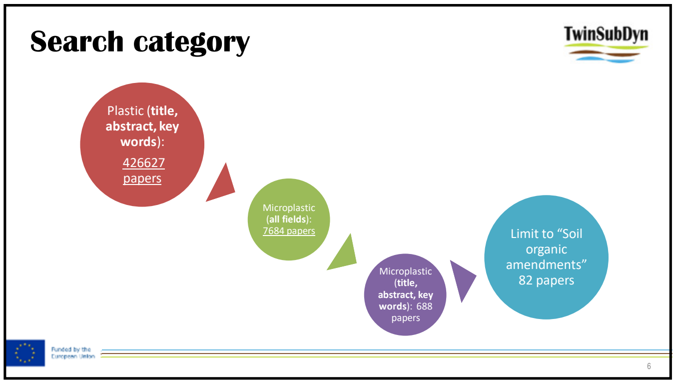

Search category

TwinSubDyn

Plastic (**title, abstract, key words**):
426627 papers

Microplastic (**all fields**):
7684 papers

Microplastic (**title, abstract, key words**): 688 papers

Limit to "Soil organic amendments"
82 papers

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- All the papers scanned based on title, abstract and key words
- **Elimination criteria**
  - Completely irrelevant (plastic pipes for the plant growth, fruit, water treatment, wastewater treatment plants, inorganic pollutants, etc.)
- 118 papers selected for META analysis.
- Additional paper selection will be during the extraction of the data in the **Excel file**!!!

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Data extraction will be done in Excel

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Microplastic characteristics

Plant characteristics

Soil characteristics

Soil characterization before experiment

Soil after experiment (measured parameters)

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Questions?

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- Data available in papers are mostly in range (eg. Size of PE ranged 0.2-3 mm), how to extract those kind of data?
- How to create groups?
- How should we add reported errors/standard deviations from papers?

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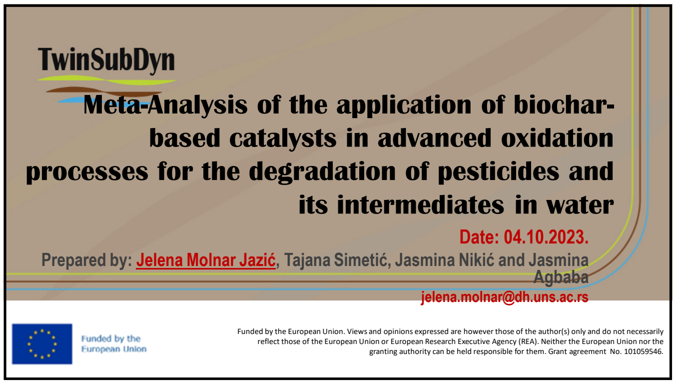

TwinSubDyn

Meta-Analysis of the application of biochar-based catalysts in advanced oxidation processes for the degradation of pesticides and its intermediates in water

Date: 04.10.2023.

Prepared by: Jelena Molnar Jazić, Tajana Simetić, Jasmina Nikić and Jasmina Agbaba
jelena.molnar@dh.uns.ac.rs

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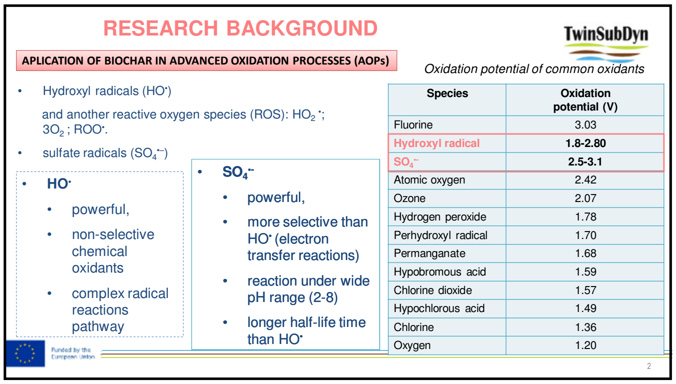

RESEARCH BACKGROUND

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APLICATION OF BIOCHAR IN ADVANCED OXIDATION PROCESSES (AOPs)

- Hydroxyl radicals (HO$^•$)

  and another reactive oxygen species (ROS): $HO_2{}^•$; $^3O_2$; ROO$^•$.
- sulfate radicals ($SO_4{}^{•-}$)

- **HO$^•$**
  - powerful,
  - non-selective chemical oxidants
  - complex radical reactions pathway

- **$SO_4{}^{•-}$**
  - powerful,
  - more selective than HO$^•$ (electron transfer reactions)
  - reaction under wide pH range (2-8)
  - longer half-life time than HO$^•$

Oxidation potential of common oxidants

Species	Oxidation potential (V)
Fluorine	3.03
Hydroxyl radical	**1.8-2.80**
$SO_4{}^{•-}$	**2.5-3.1**
Atomic oxygen	2.42
Ozone	2.07
Hydrogen peroxide	1.78
Perhydroxyl radical	1.70
Permanganate	1.68
Hypobromous acid	1.59
Chlorine dioxide	1.57
Hypochlorous acid	1.49
Chlorine	1.36
Oxygen	1.20

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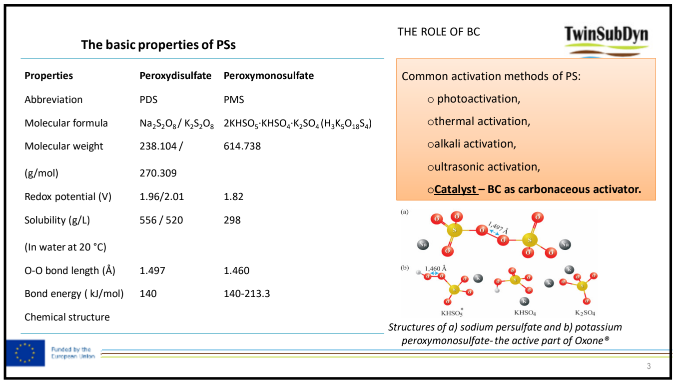

The basic properties of PSs

Properties	Peroxydisulfate	Peroxymonosulfate
Abbreviation	PDS	PMS
Molecular formula	$Na_2S_2O_8$ / $K_2S_2O_8$	$2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$ ($H_3K_5O_{18}S_4$)
Molecular weight	238.104 /	614.738
(g/mol)	270.309	
Redox potential (V)	1.96/2.01	1.82
Solubility (g/L)	556 / 520	298
(In water at 20 °C)		
O-O bond length (Å)	1.497	1.460
Bond energy (kJ/mol)	140	140-213.3
Chemical structure		

THE ROLE OF BC

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Common activation methods of PS:
- ○ photoactivation,
- ○thermal activation,
- ○alkali activation,
- ○ultrasonic activation,
- ○**Catalyst – BC as carbonaceous activator.**

Structures of a) sodium persulfate and b) potassium peroxymonosulfate- the active part of Oxone®

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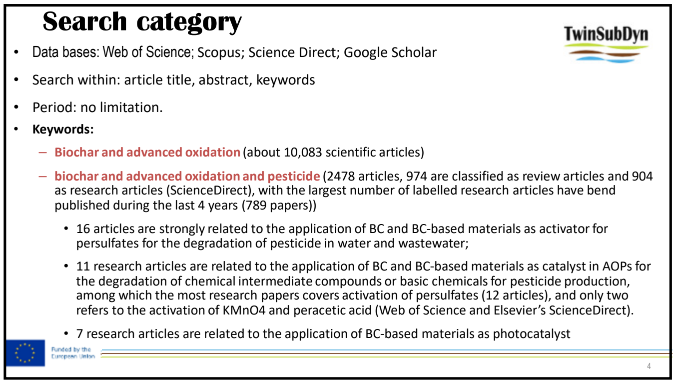

Search category

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- Data bases: Web of Science; Scopus; Science Direct; Google Scholar
- Search within: article title, abstract, keywords
- Period: no limitation.
- **Keywords:**
  - **Biochar and advanced oxidation** (about 10,083 scientific articles)
  - **biochar and advanced oxidation and pesticide** (2478 articles, 974 are classified as review articles and 904 as research articles (ScienceDirect), with the largest number of labelled research articles have bend published during the last 4 years (789 papers))
    - 16 articles are strongly related to the application of BC and BC-based materials as activator for persulfates for the degradation of pesticide in water and wastewater;
    - 11 research articles are related to the application of BC and BC-based materials as catalyst in AOPs for the degradation of chemical intermediate compounds or basic chemicals for pesticide production, among which the most research papers covers activation of persulfates (12 articles), and only two refers to the activation of KMnO4 and peracetic acid (Web of Science and Elsevier's ScienceDirect).
    - 7 research articles are related to the application of BC-based materials as photocatalyst

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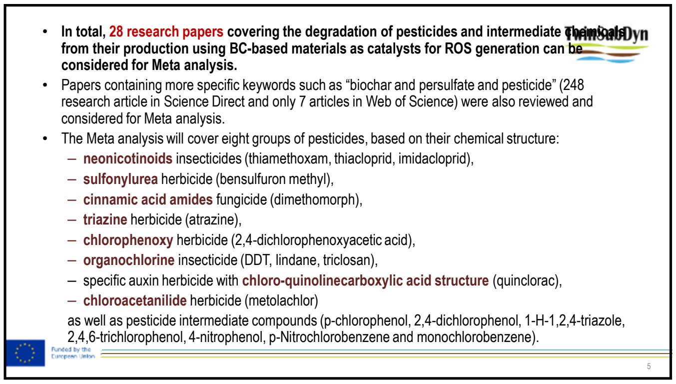

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- **In total, 28 research papers covering the degradation of pesticides and intermediate chemicals from their production using BC-based materials as catalysts for ROS generation can be considered for Meta analysis.**
- Papers containing more specific keywords such as "biochar and persulfate and pesticide" (248 research article in Science Direct and only 7 articles in Web of Science) were also reviewed and considered for Meta analysis.
- The Meta analysis will cover eight groups of pesticides, based on their chemical structure:
  - **neonicotinoids** insecticides (thiamethoxam, thiacloprid, imidacloprid),
  - **sulfonylurea** herbicide (bensulfuron methyl),
  - **cinnamic acid amides** fungicide (dimethomorph),
  - **triazine** herbicide (atrazine),
  - **chlorophenoxy** herbicide (2,4-dichlorophenoxyacetic acid),
  - **organochlorine** insecticide (DDT, lindane, triclosan),
  - specific auxin herbicide with **chloro-quinolinecarboxylic acid structure** (quinclorac),
  - **chloroacetanilide** herbicide (metolachlor)

  as well as pesticide intermediate compounds (p-chlorophenol, 2,4-dichlorophenol, 1-H-1,2,4-triazole, 2,4,6-trichlorophenol, 4-nitrophenol, p-Nitrochlorobenzene and monochlorobenzene).

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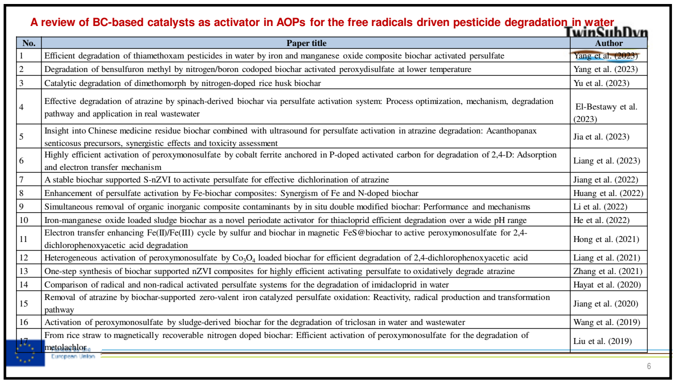

A review of BC-based catalysts as activator in AOPs for the free radicals driven pesticide degradation in water

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No.	Paper title	Author
1	Efficient degradation of thiamethoxam pesticides in water by iron and manganese oxide composite biochar activated persulfate	Yang et al. (2023)
2	Degradation of bensulfuron methyl by nitrogen/boron codoped biochar activated peroxydisulfate at lower temperature	Yang et al. (2023)
3	Catalytic degradation of dimethomorph by nitrogen-doped rice husk biochar	Yu et al. (2023)
4	Effective degradation of atrazine by spinach-derived biochar via persulfate activation system: Process optimization, mechanism, degradation pathway and application in real wastewater	El-Bestawy et al. (2023)
5	Insight into Chinese medicine residue biochar combined with ultrasound for persulfate activation in atrazine degradation: Acanthopanax senticosus precursors, synergistic effects and toxicity assessment	Jia et al. (2023)
6	Highly efficient activation of peroxymonosulfate by cobalt ferrite anchored in P-doped activated carbon for degradation of 2,4-D: Adsorption and electron transfer mechanism	Liang et al. (2023)
7	A stable biochar supported S-nZVI to activate persulfate for effective dichlorination of atrazine	Jiang et al. (2022)
8	Enhancement of persulfate activation by Fe-biochar composites: Synergism of Fe and N-doped biochar	Huang et al. (2022)
9	Simultaneous removal of organic inorganic composite contaminants by in situ double modified biochar: Performance and mechanisms	Li et al. (2022)
10	Iron-manganese oxide loaded sludge biochar as a novel periodate activator for thiacloprid efficient degradation over a wide pH range	He et al. (2022)
11	Electron transfer enhancing Fe(II)/Fe(III) cycle by sulfur and biochar in magnetic FeS@biochar to active peroxymonosulfate for 2,4-dichlorophenoxyacetic acid degradation	Hong et al. (2021)
12	Heterogeneous activation of peroxymonosulfate by Co_3O_4 loaded biochar for efficient degradation of 2,4-dichlorophenoxyacetic acid	Liang et al. (2021)
13	One-step synthesis of biochar supported nZVI composites for highly efficient activating persulfate to oxidatively degrade atrazine	Zhang et al. (2021)
14	Comparison of radical and non-radical activated persulfate systems for the degradation of imidacloprid in water	Hayat et al. (2020)
15	Removal of atrazine by biochar-supported zero-valent iron catalyzed persulfate oxidation: Reactivity, radical production and transformation pathway	Jiang et al. (2020)
16	Activation of peroxymonosulfate by sludge-derived biochar for the degradation of triclosan in water and wastewater	Wang et al. (2019)
17	From rice straw to magnetically recoverable nitrogen doped biochar: Efficient activation of peroxymonosulfate for the degradation of metolachlor	Liu et al. (2019)

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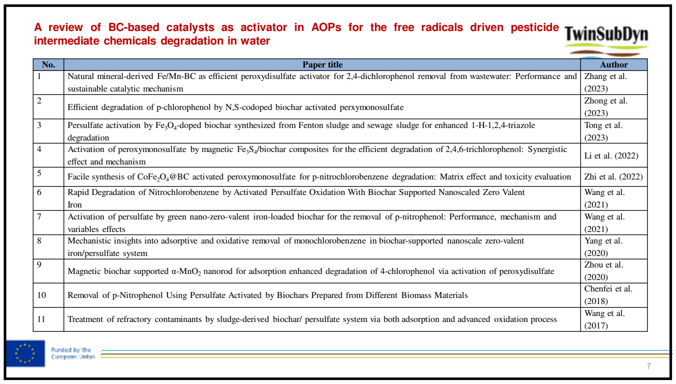

A review of BC-based catalysts as activator in AOPs for the free radicals driven pesticide intermediate chemicals degradation in water

No.	Paper title	Author
1	Natural mineral-derived Fe/Mn-BC as efficient peroxydisulfate activator for 2,4-dichlorophenol removal from wastewater: Performance and sustainable catalytic mechanism	Zhang et al. (2023)
2	Efficient degradation of p-chlorophenol by N,S-codoped biochar activated perxymonosulfate	Zhong et al. (2023)
3	Persulfate activation by Fe_3O_4-doped biochar synthesized from Fenton sludge and sewage sludge for enhanced 1-H-1,2,4-triazole degradation	Tong et al. (2023)
4	Activation of peroxymonosulfate by magnetic Fe_3S_4/biochar composites for the efficient degradation of 2,4,6-trichlorophenol: Synergistic effect and mechanism	Li et al. (2022)
5	Facile synthesis of $CoFe_2O_4$@BC activated peroxymonosulfate for p-nitrochlorobenzene degradation: Matrix effect and toxicity evaluation	Zhi et al. (2022)
6	Rapid Degradation of Nitrochlorobenzene by Activated Persulfate Oxidation With Biochar Supported Nanoscaled Zero Valent Iron	Wang et al. (2021)
7	Activation of persulfate by green nano-zero-valent iron-loaded biochar for the removal of p-nitrophenol: Performance, mechanism and variables effects	Wang et al. (2021)
8	Mechanistic insights into adsorptive and oxidative removal of monochlorobenzene in biochar-supported nanoscale zero-valent iron/persulfate system	Yang et al. (2020)
9	Magnetic biochar supported α-MnO_2 nanorod for adsorption enhanced degradation of 4-chlorophenol via activation of peroxydisulfate	Zhou et al. (2020)
10	Removal of p-Nitrophenol Using Persulfate Activated by Biochars Prepared from Different Biomass Materials	Chenfei et al. (2018)
11	Treatment of refractory contaminants by sludge-derived biochar/ persulfate system via both adsorption and advanced oxidation process	Wang et al. (2017)

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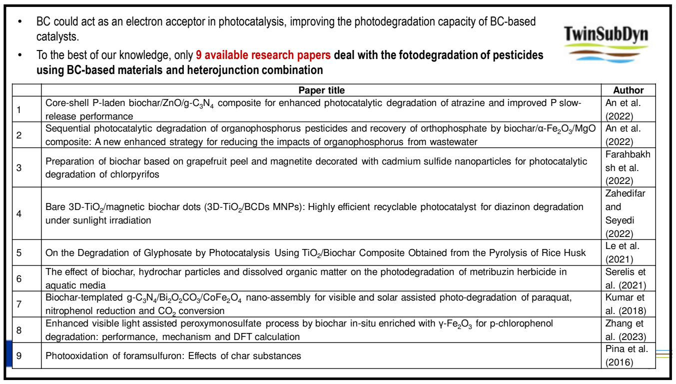
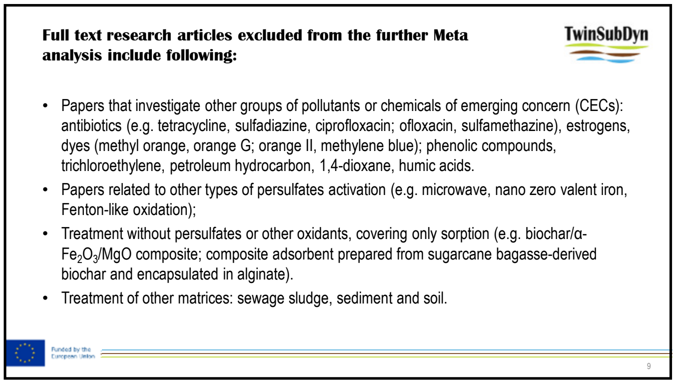


- BC could act as an electron acceptor in photocatalysis, improving the photodegradation capacity of BC-based catalysts.
- To the best of our knowledge, only **9 available research papers deal with the fotodegradation of pesticides using BC-based materials and heterojunction combination**

	Paper title	Author
1	Core-shell P-laden biochar/ZnO/g-C_3N_4 composite for enhanced photocatalytic degradation of atrazine and improved P slow-release performance	An et al. (2022)
2	Sequential photocatalytic degradation of organophosphorus pesticides and recovery of orthophosphate by biochar/α-Fe_2O_3/MgO composite: A new enhanced strategy for reducing the impacts of organophosphorus from wastewater	An et al. (2022)
3	Preparation of biochar based on grapefruit peel and magnetite decorated with cadmium sulfide nanoparticles for photocatalytic degradation of chlorpyrifos	Farahbakhsh et al. (2022)
4	Bare 3D-TiO_2/magnetic biochar dots (3D-TiO_2/BCDs MNPs): Highly efficient recyclable photocatalyst for diazinon degradation under sunlight irradiation	Zahedifar and Seyedi (2022)
5	On the Degradation of Glyphosate by Photocatalysis Using TiO_2/Biochar Composite Obtained from the Pyrolysis of Rice Husk	Le et al. (2021)
6	The effect of biochar, hydrochar particles and dissolved organic matter on the photodegradation of metribuzin herbicide in aquatic media	Serelis et al. (2021)
7	Biochar-templated g-C_3N_4/$Bi_2O_2CO_3$/$CoFe_2O_4$ nano-assembly for visible and solar assisted photo-degradation of paraquat, nitrophenol reduction and CO_2 conversion	Kumar et al. (2018)
8	Enhanced visible light assisted peroxymonosulfate process by biochar in-situ enriched with γ-Fe_2O_3 for p-chlorophenol degradation: performance, mechanism and DFT calculation	Zhang et al. (2023)
9	Photooxidation of foramsulfuron: Effects of char substances	Pina et al. (2016)



Full text research articles excluded from the further Meta analysis include following:

- Papers that investigate other groups of pollutants or chemicals of emerging concern (CECs): antibiotics (e.g. tetracycline, sulfadiazine, ciprofloxacin; ofloxacin, sulfamethazine), estrogens, dyes (methyl orange, orange G; orange II, methylene blue); phenolic compounds, trichloroethylene, petroleum hydrocarbon, 1,4-dioxane, humic acids.
- Papers related to other types of persulfates activation (e.g. microwave, nano zero valent iron, Fenton-like oxidation);
- Treatment without persulfates or other oxidants, covering only sorption (e.g. biochar/α-Fe_2O_3/MgO composite; composite adsorbent prepared from sugarcane bagasse-derived biochar and encapsulated in alginate).
- Treatment of other matrices: sewage sludge, sediment and soil.

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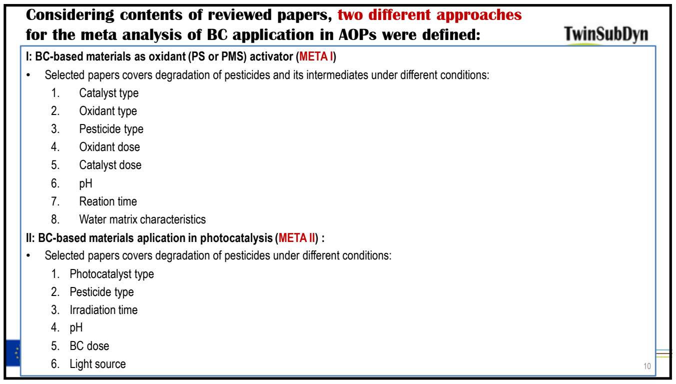


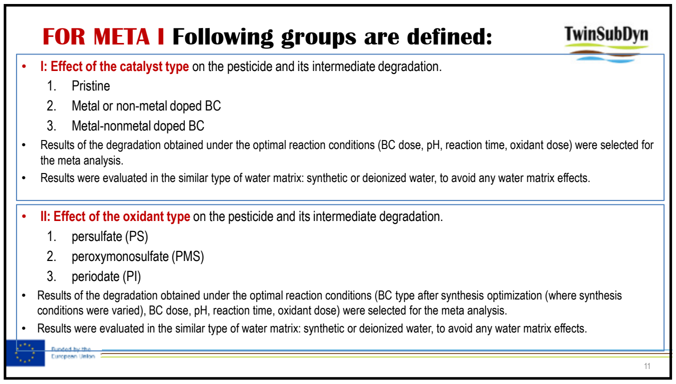
Considering contents of reviewed papers, two different approaches for the meta analysis of BC application in AOPs were defined:

I: BC-based materials as oxidant (PS or PMS) activator (META I)

- Selected papers covers degradation of pesticides and its intermediates under different conditions:
  1. Catalyst type
  2. Oxidant type
  3. Pesticide type
  4. Oxidant dose
  5. Catalyst dose
  6. pH
  7. Reation time
  8. Water matrix characteristics

II: BC-based materials aplication in photocatalysis (META II) :

- Selected papers covers degradation of pesticides under different conditions:
  1. Photocatalyst type
  2. Pesticide type
  3. Irradiation time
  4. pH
  5. BC dose
  6. Light source



FOR META I Following groups are defined:

- **I: Effect of the catalyst type** on the pesticide and its intermediate degradation.
  1. Pristine
  2. Metal or non-metal doped BC
  3. Metal-nonmetal doped BC
- Results of the degradation obtained under the optimal reaction conditions (BC dose, pH, reaction time, oxidant dose) were selected for the meta analysis.
- Results were evaluated in the similar type of water matrix: synthetic or deionized water, to avoid any water matrix effects.

- **II: Effect of the oxidant type** on the pesticide and its intermediate degradation.
  1. persulfate (PS)
  2. peroxymonosulfate (PMS)
  3. periodate (PI)
- Results of the degradation obtained under the optimal reaction conditions (BC type after synthesis optimization (where synthesis conditions were varied), BC dose, pH, reaction time, oxidant dose) were selected for the meta analysis.
- Results were evaluated in the similar type of water matrix: synthetic or deionized water, to avoid any water matrix effects.

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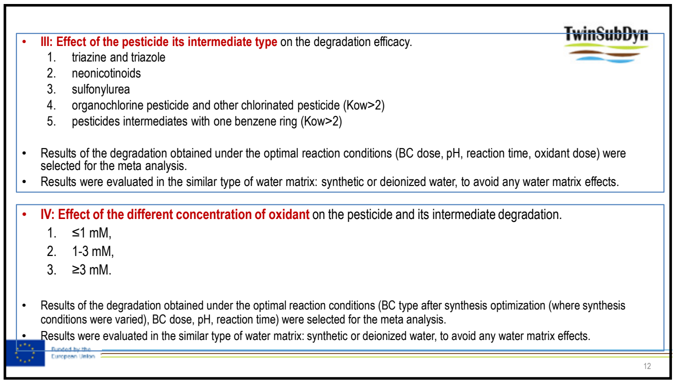

- **III: Effect of the pesticide its intermediate type** on the degradation efficacy.
  1. triazine and triazole
  2. neonicotinoids
  3. sulfonylurea
  4. organochlorine pesticide and other chlorinated pesticide (Kow>2)
  5. pesticides intermediates with one benzene ring (Kow>2)
- Results of the degradation obtained under the optimal reaction conditions (BC dose, pH, reaction time, oxidant dose) were selected for the meta analysis.
- Results were evaluated in the similar type of water matrix: synthetic or deionized water, to avoid any water matrix effects.

- **IV: Effect of the different concentration of oxidant** on the pesticide and its intermediate degradation.
  1. ≤1 mM,
  2. 1-3 mM,
  3. ≥3 mM.
- Results of the degradation obtained under the optimal reaction conditions (BC type after synthesis optimization (where synthesis conditions were varied), BC dose, pH, reaction time) were selected for the meta analysis.
- Results were evaluated in the similar type of water matrix: synthetic or deionized water, to avoid any water matrix effects.

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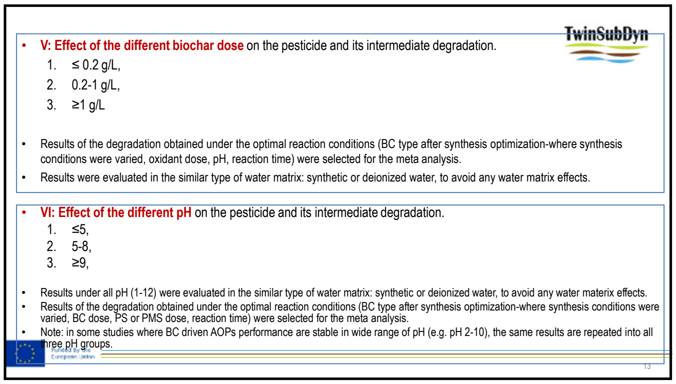
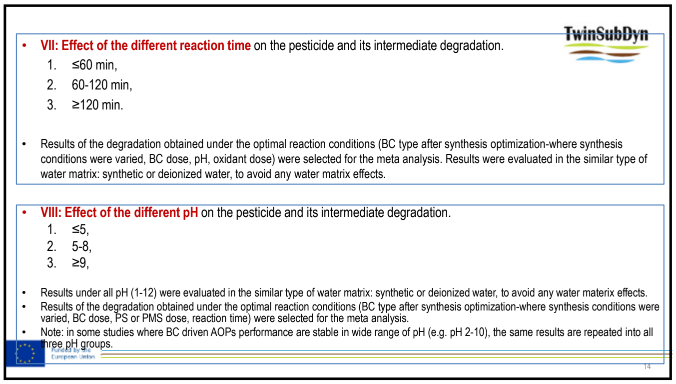


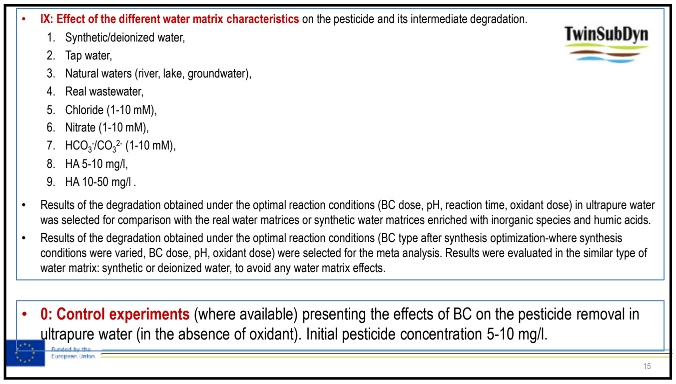
- **V: Effect of the different biochar dose** on the pesticide and its intermediate degradation.
  1. ≤ 0.2 g/L,
  2. 0.2-1 g/L,
  3. ≥1 g/L
- Results of the degradation obtained under the optimal reaction conditions (BC type after synthesis optimization-where synthesis conditions were varied, oxidant dose, pH, reaction time) were selected for the meta analysis.
- Results were evaluated in the similar type of water matrix: synthetic or deionized water, to avoid any water matrix effects.

- **VI: Effect of the different pH** on the pesticide and its intermediate degradation.
  1. ≤5,
  2. 5-8,
  3. ≥9,
- Results under all pH (1-12) were evaluated in the similar type of water matrix: synthetic or deionized water, to avoid any water materix effects.
- Results of the degradation obtained under the optimal reaction conditions (BC type after synthesis optimization-where synthesis conditions were varied, BC dose, PS or PMS dose, reaction time) were selected for the meta analysis.
- Note: in some studies where BC driven AOPs performance are stable in wide range of pH (e.g. pH 2-10), the same results are repeated into all three pH groups.



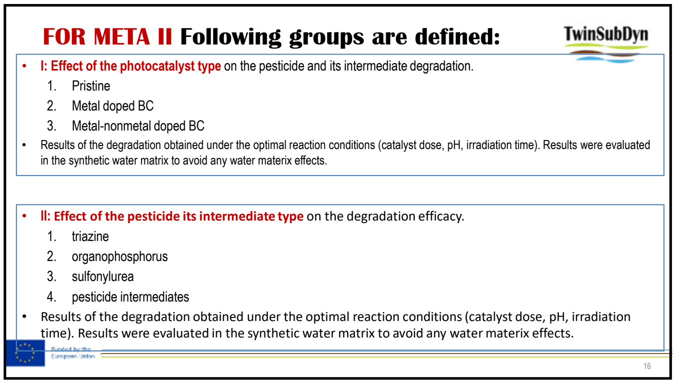
- **VII: Effect of the different reaction time** on the pesticide and its intermediate degradation.
  1. ≤60 min,
  2. 60-120 min,
  3. ≥120 min.
- Results of the degradation obtained under the optimal reaction conditions (BC type after synthesis optimization-where synthesis conditions were varied, BC dose, pH, oxidant dose) were selected for the meta analysis. Results were evaluated in the similar type of water matrix: synthetic or deionized water, to avoid any water matrix effects.

- **VIII: Effect of the different pH** on the pesticide and its intermediate degradation.
  1. ≤5,
  2. 5-8,
  3. ≥9,
- Results under all pH (1-12) were evaluated in the similar type of water matrix: synthetic or deionized water, to avoid any water materix effects.
- Results of the degradation obtained under the optimal reaction conditions (BC type after synthesis optimization-where synthesis conditions were varied, BC dose, PS or PMS dose, reaction time) were selected for the meta analysis.
- Note: in some studies where BC driven AOPs performance are stable in wide range of pH (e.g. pH 2-10), the same results are repeated into all three pH groups.



- **IX: Effect of the different water matrix characteristics** on the pesticide and its intermediate degradation.
  1. Synthetic/deionized water,
  2. Tap water,
  3. Natural waters (river, lake, groundwater),
  4. Real wastewater,
  5. Chloride (1-10 mM),
  6. Nitrate (1-10 mM),
  7. HCO_3^-/CO_3^{2-} (1-10 mM),
  8. HA 5-10 mg/l,
  9. HA 10-50 mg/l .
- Results of the degradation obtained under the optimal reaction conditions (BC dose, pH, reaction time, oxidant dose) in ultrapure water was selected for comparison with the real water matrices or synthetic water matrices enriched with inorganic species and humic acids.
- Results of the degradation obtained under the optimal reaction conditions (BC type after synthesis optimization-where synthesis conditions were varied, BC dose, pH, oxidant dose) were selected for the meta analysis. Results were evaluated in the similar type of water matrix: synthetic or deionized water, to avoid any water matrix effects.

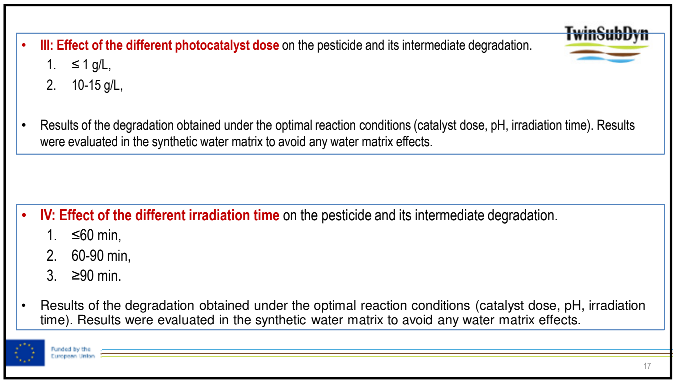
- **0: Control experiments** (where available) presenting the effects of BC on the pesticide removal in ultrapure water (in the absence of oxidant). Initial pesticide concentration 5-10 mg/l.



FOR META II Following groups are defined:

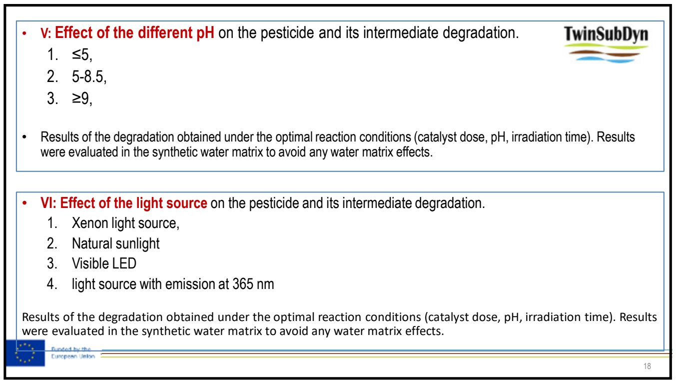
- **I: Effect of the photocatalyst type** on the pesticide and its intermediate degradation.
  1. Pristine
  2. Metal doped BC
  3. Metal-nonmetal doped BC
- Results of the degradation obtained under the optimal reaction conditions (catalyst dose, pH, irradiation time). Results were evaluated in the synthetic water matrix to avoid any water materix effects.

- **II: Effect of the pesticide its intermediate type** on the degradation efficacy.
  1. triazine
  2. organophosphorus
  3. sulfonylurea
  4. pesticide intermediates
- Results of the degradation obtained under the optimal reaction conditions (catalyst dose, pH, irradiation time). Results were evaluated in the synthetic water matrix to avoid any water materix effects.



- **III: Effect of the different photocatalyst dose** on the pesticide and its intermediate degradation.
  1. ≤ 1 g/L,
  2. 10-15 g/L,
- Results of the degradation obtained under the optimal reaction conditions (catalyst dose, pH, irradiation time). Results were evaluated in the synthetic water matrix to avoid any water matrix effects.

- **IV: Effect of the different irradiation time** on the pesticide and its intermediate degradation.
  1. ≤60 min,
  2. 60-90 min,
  3. ≥90 min.
- Results of the degradation obtained under the optimal reaction conditions (catalyst dose, pH, irradiation time). Results were evaluated in the synthetic water matrix to avoid any water matrix effects.



- **V: Effect of the different pH** on the pesticide and its intermediate degradation.
  1. ≤5,
  2. 5-8.5,
  3. ≥9,
- Results of the degradation obtained under the optimal reaction conditions (catalyst dose, pH, irradiation time). Results were evaluated in the synthetic water matrix to avoid any water matrix effects.

- **VI: Effect of the light source** on the pesticide and its intermediate degradation.
  1. Xenon light source,
  2. Natural sunlight
  3. Visible LED
  4. light source with emission at 365 nm

Results of the degradation obtained under the optimal reaction conditions (catalyst dose, pH, irradiation time). Results were evaluated in the synthetic water matrix to avoid any water matrix effects.

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