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Evaluating the quality of marine composts by using humification and spectroscopy

A summary of workshop presentations and learning outcomes from the MARIGREEN_TECB project

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SAMMENDRAG:

Denne rapporten oppsummerer presentasjonene og aktivitetene gjort under en workshop arrangert i regi av prosjektet MARIGREEN_TECB (Training, Exchange, and Capacity Building), som var finansiert av den tredje tilleggsutlysningen til ERA-NET Cofund on Blue Bioeconomy (BlueBio)-programmet (tilskuddsnummer 346849).

MARIGREEN_TECB var en utvidelse av MARIGREEN (bærekraftig utnyttelse av MARINe-ressurser for å fremme GREEN planteproduksjon i Europa), et prosjekt finansiert av den første tilleggsutlysningen til ERA-NET BlueBio-programmet (EUs Horizon 2020-tilskuddsavtale 817992). MARIGREEN har som mål å verdigjøre dårlig utnyttede restmaterialer fra blå sektor ved å behandle

dem med passende teknologier som ekstraksjon, kompostering og biokullimpregnering etter utvinning, og anvende dem i økologisk landbruk. Prosjektet arbeider med tilgjengelig restmateriale fra fiskefangst, brunalgeindustrien, blåskjellindustrien og økologisk havbruk.

Hovedmålet til MARIGREEN_TECB var å forbedre forståelsen av humifisering under kompostering av marine råvarer og hvordan man kan forbedre kvaliteten på "blå" kompost og for å få kompetanse i FT-IR-analyse og spektratolkning for humusstoffer.

MARIGREEN_TECB var et samarbeid mellom NORSØK og USAMV i Bucuresti. Workshopen ble arrangert av Research Center for Studies of Food Quality and Agricultural Products ved USAMVs campus i Bucuresti og ble holdt fra 23. til 26. oktober 2023. Temaene for workshopen ble valgt fordi de er direkte relevante for MARIGREEN-hovedprosjektet og flere av arbeidspakkene. Imidlertid har de også global relevans. Mens fokuset var på marinbasert kompost, er metodene dekket under workshopen relevante for alle typer komposter, organisk-basert gjødsel og jordforbedringsmidler. Metoder for å forbedre kompostkvaliteten og forbedre humifiseringen kan brukes på kompost laget med alle råvarer. Det samme gjelder for evaluering av kompostkvalitet ved mer tradisjonelle metoder og ved FT-IR-spektroskopi.

COUNTRY:	Norway
COUNTY:	Møre og Romsdal
MUNICIPALITY:	Tingvoll

APPROVED	PROJECT LEADER
Cecilie Løkken	Joshua F. Cabell
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Preface

MARIGREEN_TECB (Training, Exchange, and Capacity Building) was a project funded by the 3rd Additional Call of the ERA-NET Cofund on Blue Bioeconomy (BlueBio) program (grant number 346849). This call was open to all projects currently being funded by previous calls of the BlueBio program and are receiving funding in Croatia, Iceland, Ireland, Malta, and Norway. The goal of this call was to "amplify project reach and knowledge impact" for the main BlueBio Cofund projects.

MARIGREEN_TECB was an extension of MARIGREEN (*Sustainable utilization of MARIne resources to foster GREEN plant production in Europe*), a project funded by the 1st Additional Call of the ERA-NET BlueBio program (EU's Horizon 2020 grant agreement 817992). MARIGREEN aims to valorize poorly utilized residual materials from the blue sector by treating them with appropriate technologies such as extraction, composting, and biochar impregnation after extraction, and applying them in organic agriculture. The project works with available residual materials from fish capture, the brown algae industry, the mussel industry, and organic aquaculture. MARIGREEN is coordinated by the National University of Science and Technology POLITEHNICA Bucharest (UNSTPB), with research consortium partners from the University of Agronomic Sciences and Veterinary Medicine of Bucharest (USAMV), the Norwegian Center for Organic Agriculture (NORSØK), the Aristotle University of Thessaloniki (AUTh), Norwegian Research Center (NORCE), the Technical University of Denmark (DTU Aqua), and the University of Copenhagen (UCPH). Industry partners are Alumichem A/S (Denmark), Fjordlaks AS (Norway), Algea AS (Norway/Italy), Sigurd Folland AS (Norway), and Norgeskjell AS (Norway). The project period is from November 1st, 2021, to October 31st, 2024. For more information about the project and its activities, visit http://www.marigreen-project.eu/.

MARIGREEN_TECB was a collaboration between NORSØK and USAMV. The main activity in the project was a workshop hosted by the Research Center for Studies of Food Quality and Agricultural Products at USAMV's campus in Bucharest, held from October 23rd to 26th, 2023. The workshop topics were the use of infrared (FT-IR) spectroscopy for evaluating humification in marine-based composts, optimizing the composting process for increased quality and maximum humification, and learning methods for extracting humic and fulvic acids from compost. These topics were chosen because they are directly relevant to the main MARIGREEN project and several of the work packages. However, they also have global relevance. While the focus was on marine-based composts, the methods covered during the workshop are relevant for all types of composts, organic-based fertilizers, and soil improvers. Methods for improving compost quality and enhancing humification can be applied to composts made with all feedstocks. The same is true for evaluating compost quality by more traditional methods and by FT-IR spectroscopy.

Thank you to all the staff at USAMV for the hard work they put into making it a successful workshop.

Tingvoll, 24.04.24

Joshua Fenton Cabell

Project leader for MARIGREEN_TECB

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1 Introduction

1.1 Workshop objectives

The primary objective of MARIGREEN_TECB was to improve the understanding of humification during the composting of marine feedstocks and how to improve the quality of "blue" compost. Secondary objectives were:

1. To gain competence in FT-IR analysis and spectra interpretation for humic substances.

2. Facilitate an exchange of knowledge and experience between the invited experts and the PhD candidates associated with the MARIGREEN project.

3. Help the PhD candidates in MARIGREEN build their professional network.

4. Further promote the MARIGREEN project and the BlueBio Cofund program.

5. Disseminate the outcomes to relevant audiences through recorded lectures, social media, and a published report.

A total of ten PhD and Postdoc candidates participated in the workshop, along with various other faculty members, researchers, and guests from USAMV and UNSTPB. Two of the participants were from Norway, two from Greece, and six from Romania (from both UNSTPB and USAMV). Two external experts were invited to give lectures and lead the participants in the practical work, in addition to researchers from USAMV. The two external instructors were Dr. Jean Robertson and Erwin Binner. Dr. Robertson is the head of the spectroscopy group at the James Hutton Institute in Aberdeen, Scotland. She taught us about using FT-IR (MIR) and interpreting the results. Mr. Binner is a research engineer at the Institute for Waste Management and Circularity at the BOKU University of Natural Resources and Life Sciences Vienna in Austria. He taught us about composting, composting quality, humification during composting, and using FT-IR (NIR) to evaluate compost quality. From USAMV, researcher Dr. Violeta Alexandra Ion taught us a method for extracting humic and fulvic acids from compost, as well as various methods of chemical and elemental analysis. She was also one of the co-organizers. Other contributors from USAMV included Professor Liliana Bădulescu, Dr. Oana Crina Bujor-Nenita, Andrei Mot, Ailin Moloşag, and Cristian Nedelcu; from UNSTPB, Professor Oana Pârvulescu; and Marios Maroulis from AUTh/Modern Analytics.

In addition to the workshop, the other activities in MARIGREEN_TECB included planning and coordinating the workshop and disseminating the project outcomes. Dissemination includes the current report documenting the learning outcomes and video recordings of the lectures that will be published and shared with relevant audiences.

1.2 Workshop program

The workshop consisted of four days of lectures, visits to field trials, tours of the facilities, hands-on learning in the lab, and social and cultural activities in the evenings. Four PhD candidates and one instructor travelled to Bucharest from Norway, Greece, and Scotland. The rest of the participants and instructors live in Bucharest and study or work at UNSTPB or USAMV. One of the Instructors, Erwin Binner, was unable to attend physically and therefore gave his lectures on composting live on Zoom.

The first day of the workshop began with a welcome from Professor Gina Fîntîneru, Vice-Rector of Scientific Research at USAMV. This was followed by a presentation of a selection of current research activities in MARIGREEN. Joshua Cabell (NORSØK/Norwegian University of Life Sciences [NMBU]) described the experiments with composting marine residues; Marios Maroulis (Modern Analytics/AUTh) presented his work on extracting biostimulants from marine residues; Violeta Alexandra Ion (USAMV) and Oana Pârvulescu (UNSTPB) presented their work on impregnating biochar with compost extracts; and finally, Ailin Moloşag (USAMV) gave a tour of her field trial testing marine-based composts and extracts in strawberry production. There was a tour of the facilities, including the new greenhouses, following the presentations.

The second day began with a tour of the laboratory facilities at the Research Center for the Study of Food Quality and Agronomic Sciences, led by the head of the research center Professor Liliana Bădulescu. Following the tour was the first session on extracting humic substances in the lab. The participants were divided into three groups, with each group given a different marine-based compost to practice with over the next three days. In between steps in the extraction process, participants learned about other analysis methods such as combustion elemental analysis (CHNS). The final lecture of the day was given by Erwin Binner on Zoom. The topics of this day's lecture were the fundamentals of composting and the state of the art of composting in Austria.

Day three began in the lab with a resumption of the extraction process and more discussions about the various analysis techniques that are relevant to compost. Jean Robertson gave her first lecture, titled "FT_IR Spectroscopic Analysis: a powerful tool for chemical characterization". This was an introduction to the instruments, methods, applications, and the interpretation of results for spectroscopy in the mid-infrared range (MIR). Day three concluded with Erwin Binner's second lecture in which he covered the topics of compost quality, methods for evaluating the humification of compost, and how to enhance humification.

The final day began in the FT-IR lab where Jean Robertson taught participants how to use an FT-IR-spectrometer with an ATR sampling accessory and how to interpret IR spectra. The same samples of marine compost that were used for extraction were used for MIR analysis. The discussion about interpretation continued in the meeting room where other examples of spectra from the MARIGREEN project were reviewed. The final day ended with a viewing of the movie "The Need to Grow", which is about finding practical solutions to creating a more sustainable food system.

In addition to the scientific program, there were excursions and social activities throughout the week. There was a tour of the Parliament building, a dinner at a traditional restaurant in the old city, a tour of Bucharest's largest urban park, and time for socializing during lunches and breaks during the workshop.

A copy of the full program is included as an appendix to this report.

2 Learning outcomes

2.1 Marine composts

Aerobic composting is a popular and effective method for stabilizing organic residues and converting them into a resource for improving soil and plant health. Composting can be done on a range of scales, from the backyard to the municipality, and with a wide range of feedstocks. Almost any material of biological origin can be composted. One source of feedstocks that is less common is the ocean. There are several reasons why, such as the high salt content of marine residues and the fact that they are mostly available near coastlines, far from where major agricultural areas are located. However, marine residues contain valuable minerals and nutrients such as nitrogen, phosphorus, and potassium (NPK) in addition to micronutrients and plant biostimulants (e.g., phytohormones). In this session, Joshua Cabell from NORSØK/NMBU presented composting experiments he has conducted with marine residues, including two types of algae fiber (low and high nitrogen concentrations) from rockweed (*A. nodosum*), dried rockweed, fish meal, ground fish bones, mussels, kelp, and various farmyard wastes. These experiments were conducted at three scales: 2-liter Dewar flasks, in a 270-liter rotating composting drum, and an outdoor windrow.

Dewar flasks are extremely well-insulated "thermoses" that were originally designed by James Dewar to store and transport cryogenically frozen samples. However, they have also become a common tool for measuring compost maturity and stability in a standardized method developed in Germany in the late 1980s and known as the "Dewar self-heating" or "Rottegrad" test (see Brinton et al., 1995). It has also been used for screening the potential degradability of feedstocks and combinations of feedstocks (Miloştean & Flori, 2021). For both tests, Dewar flasks of a standard dimension are placed in a room set at 20 °C and filled with either compost or raw feedstocks with the optimal moisture content and bulk density. The temperature in the flasks is monitored and the level of maturity (or degradability) is then determined by the maximum temperature reached before returning to room temperature. If no heat is generated, the compost is mature and ready to use. If, on the other hand, the compost gets warm it means that it is not mature nor stable and therefore needs more time to cure. There are five (alternatively three) levels of maturity based on the range of temperature in the flasks (see Brinton et al., 1995 and an update from 2009). The higher the temperature in the flask, the less mature the compost. In the case of testing for degradability, higher temperatures indicate that the feedstocks (or combination of feedstocks) have a greater potential for making compost at a larger scale.

NORSØK had never used the Dewar self-heating test before the MARIGREEN project and conducted some pilot experiments with horse manure, straw, and wood chips to become familiar with the equipment and the method. These feedstocks are well-known for making compost. NORSØK also added some of the marine residues to see if there would still be heat generation and tested the composting of fresh kelp that was rinsed (to remove salt) and unrinsed. These experiences laid the foundation for further experiments with the marine residues that are part of the MARIGREEN project. The results from these trials were published in the report "Blue biomass composting technology" (Cabell & Løes, 2023).

2.2 Biostimulants based on marine residues

Marios Maroulis from AUTh and Modern Analytics presented his work with extracting, identifying, and analyzing potential biostimulants based on some of the same marine residues discussed in the previous section in addition to others like fertilizer pellets made with algae fiber and fish meal, dried sludge from aquaculture, a commercial blue mussel compost, and other types of fish meal that were not used in the compost experiments. He described how these materials were analyzed for micro- and macronutrients and contaminants via ICP-OES and ICP-MS, how he determined total and organic carbon and nitrogen, extracted fatty matter, conducted fatty acid methyl ester (F.A.M.E.) analysis, and finally did solvent extractions of organic compounds and GC-MS screenings. Results are presented in Maroulis et al. (2023).
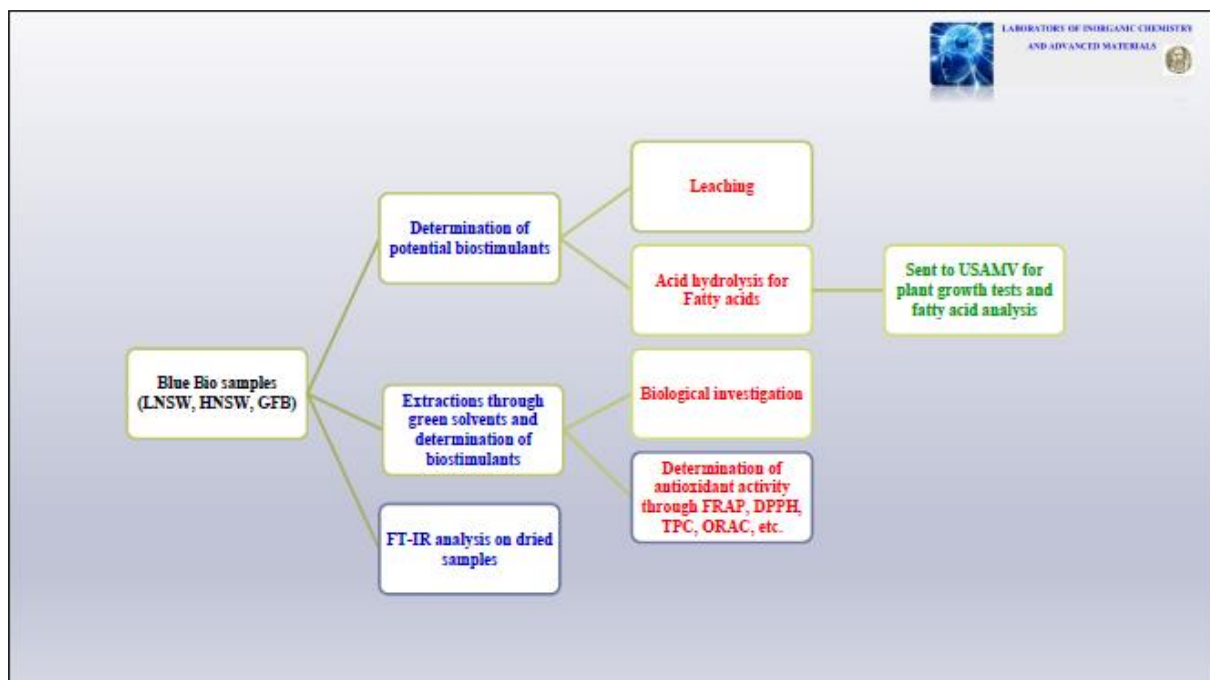

Figure 1. Workflow of pre-treatment, extractions, and analysis of marine biowastes.

2.3 Biochar impregnation with compost tea

This presentation began with an overview of mass transfer and adsorption as these are the foundation for biochar impregnation. Adsorption is a surface process that leads to the transfer of a molecule from a fluid bulk to a solid surface. There is both physical and chemical adsorption. Physical adsorption is caused mainly by Van der Waals forces and electrostatic forces between adsorbate molecules and electrostatic forces between adsorbate molecules and the atoms that compose the adsorbate surface. Chemical adsorption (chemosorption) is characterized by strong interactions that have a characteristic of covalent bonding. Adsorption materials include alumina, silica gel, zeolite, metal-organic frameworks (MOF), cellulose, and activated carbon.

The impregnation trials were based on previous experiments with volatile organic compounds coupled with mathematical modeling to establish the effect of processing parameters on performance. The adsorbates used were i-propanol (IPA), n-hexane (HEX), and acetone. The

adsorbents were bacterial cellulose (BC), BC impregnated with magnetite (M/BC), granular activated carbon (GAC), GAC impregnated with copper (CU/GAC), and granular silica gel (GSC). The independent variables used in the trial were air superficial velocity (0.7-1.7 cm/s), temperature (25-40 °C), magnetite concentration in bacterial cellulose (0%, 16%), copper concentration in granular activated carbon (0%, 13.8%), and particle size of granular silica gel (0.21, 0.54 cm). The dependent variables were adsorption capacity, saturation adsorption capacity, and average adsorption rate.

The conclusions from these initial experiments were that the maximum values of the adsorption capacity, i.e., $q_{i,sat}$=0.70-0.81 g/g =0.70-0.81 g/g, were obtained for HEX and IPA on BC and M/BC (1.7 cm/s, 40 °C), and they are larger than the ones obtained in the literature (<0.5 g/g). BC membranes present high chemical, mechanical, and thermic stability. BC and M/BC can be successfully used as adsorbents for removing VOC from the air stream. The obtained results can be a base for projecting, scaling up, and optimization of fixed bed columns used for air purification.

The next experiment involved the impregnation of biochar with a compost tea (fermented compost extract) obtained from two composts: one made in a reactor that used exclusively algae fiber, and another from an outdoor windrow that was made with algae fiber, cattle manure, wood chips, and acidified fish bones. A liquid digestate obtained from the anaerobic fermentation of aquaculture sludge (trout) was also tested for impregnation. For impregnation with compost tea, the extractions were first made by mixing water and compost in different ratios and stored at 25 °C in the dark. The mixing ratios and storage time were determined by using a central composite design (CCD) model. The next step in the impregnation trial will be to mix compost tea with biochar. Again, CCD is used to determine process parameters, which in this case are the liquid/solid ratios and temperature for impregnating over 24 hours. The impregnated biochar will be used in greenhouse and field trials to evaluate its effectiveness as a biostimulant and/or fertilizer. This step of the trial had not begun yet at the time of the workshop. Results from these trials will be published in a forthcoming article.

The experimental design for impregnating biochar with liquid sludge digestate from fish was similar. CCD was used to identify which ratios of liquid to solid (5.1-24.9 g/g) and temperatures (23.9-45.1 °C) to test. Results showed a recovery rate of up to 46% for nitrogen (NH4+) and 63% for phosphorus (PO43-). Maximum adsorption capacities for these two nutrients were 10.5 and 2.5 mg/g, respectively.

2.4 Effect of marine compost on strawberry

Ailin Moloşag and Violeta Alexandra Ion presented results from a growth trial with strawberries given eight treatments: soil amended with one of the two types of composts described earlier (reactor and windrow) in two applications (20% and 40% of soil volume), biochar, biostimulant from AUTh, a positive control (commercial fertilizer), and a negative control (no amendment/fertilizer). The treatments were divided into five blocks with five replicates of each treatment placed randomly in one of each block (experimental block design). The strawberry plants were planted in raised, irrigated beds outdoors. Weekly monitoring of the plants was done for leaf number, plant height, inflorescence number, and fruit number. Weekly monitoring of the substrate included temperature, conductivity (EC), and pH. Monthly monitoring for plants consisted of leaf area (Winfolia), dry matter content, the ratio between leaf area/dry matter, and mineral content.

The experiment was ongoing and only preliminary results were available at the time of the workshop. Results from a previous experiment are published in Moloșag et al. (2023).

2.5 What are humic substances?

This presentation gave a general overview of what humification and humic substances are (or are believed to be). Humification is the conversion (or synthesis) of a fraction of the soil organic matter (SOM) to recalcitrant humic substances through various biochemical pathways (Hayes & Swift, 2020; Tan, 2014; Weil & Brady, 2017). There are many definitions of humic substances, though two were chosen for the workshop. First was "Highly complex, large polymers made up of aromatic rings that are formed during humification" (Weil & Brady, 2017). Next was the definition from the International Humic Substances Society: "Complex and heterogeneous mixtures of polydispersed materials formed in soils, sediments, and natural waters by biochemical and chemical reactions during the decay and transformation of plant and microbial remains (a process called humification)"(International Humic Substances Society (IHSS), 2023).
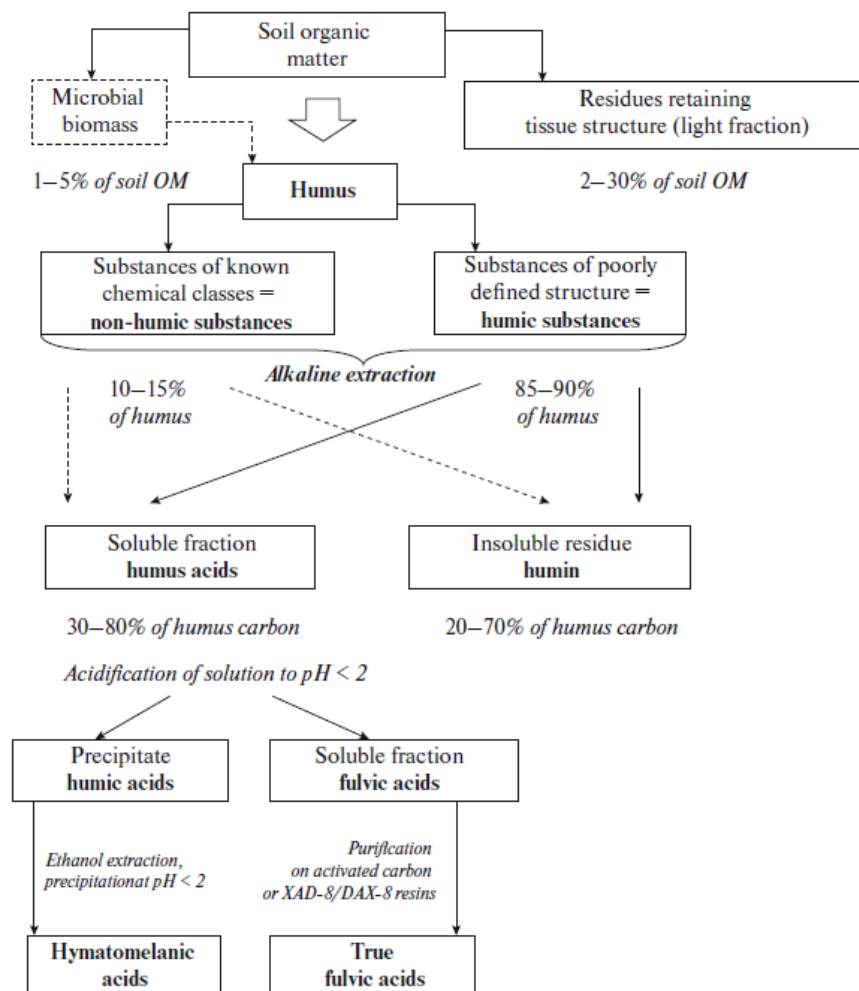

Figure 1. Humification model from Zavarzina et al. (2021)

Humic substances can be divided into three main fractions based on their solubility in acids and bases. The first fraction is fulvic acid, which is soluble in both alkaline and acidic pH and highly soluble in water. It has the lowest molecular weight (1-10 kDa). Humic acid is soluble in alkaline pH,

precipitates in acid, is partly soluble in water, and has a molecular weight of 10-100 kDa. Humin is the third fraction and is not soluble in either alkaline or acid pH, nor in water. It is the most recalcitrant fraction. It has the heaviest molecular weight at >1000 kDa.

The path from plant and animal remains to humic substances is disputed. At least seven different models have been proposed (Figure 3), including sugar-amine, polyphenol, lignin, polyphenol originating from lignin, cell autolysis, microbial synthesis, etc.

The humification model is not universally accepted within the soil science community. Other models include the Soil Continuum Model, Selective Preservation, Progressive Decomposition, and others. Nor is the existence of humic substances universally accepted. Some argue that they are an artifact of the extraction process and are not naturally found. However, products based on humic substances have many uses such as in water purification, soil improvement, and as biostimulants in plant production and horticulture. Numerous studies have confirmed their effectiveness in improving yields and plant health.
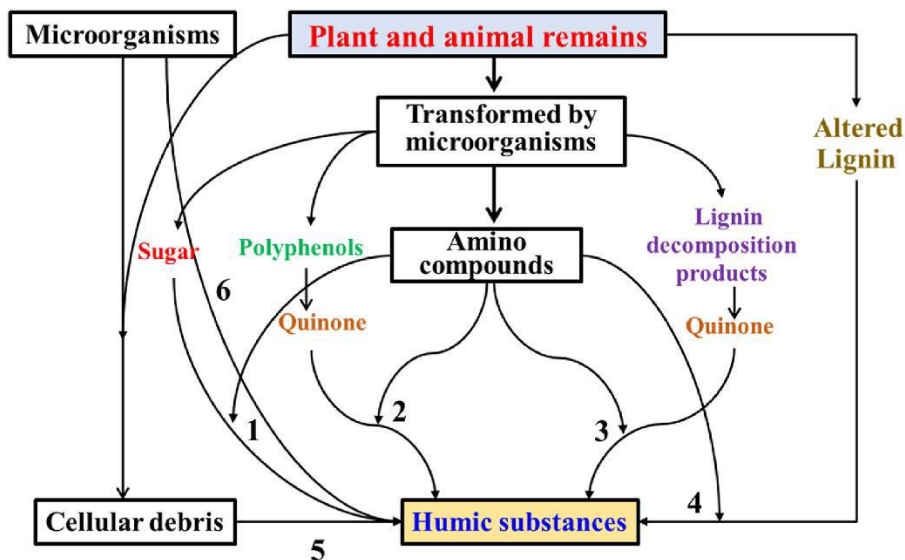

Figure 2. Pathways of humification. 1: Sugar-amine condensation; 2: Polyphenol; 3: Lignin; 4: Polyphenol originating from lignin; 5: Cell autolysis; 6: Microbial synthesis; 7: +++. Figure from Wei et al. (2022)

2.6 Extraction of humic and fulvic acid

Dr. Violeta Alexandra Ion taught participants how to extract humic substances (humic acid, fulvic acid, humin) from a soil/compost matrix based on the method described in Lamar et al. (2014). It involved the following steps:

Step 1	Dry the sample (air dry or in the oven below 50 °C).	
Step 2	Remove large objects and sieve the dried sample through a 2.0 mm sieve.	
Step 3	Weigh 3 g of the prepared sample in a 50 mL centrifuge tube.	
Step 4	Add enough 1 M HCl solution to the sample to reach a pH of 1-2, but not more than a ratio of 1 g sample/10 mL solution.	
Step 5	Adjust to the required volume of 30 mL with 0.1 M HCl.	
Step 6	Shake the suspension for 1 hour.	Day 1
Step 7	Separate the supernatant from the residue by centrifugation (5 min, 5000 rpm). Label this fraction FA-1.	
Step 8	Neutralize the residue with 1 M NaOH solution to reach a pH of 7.0.	
Step 9	Add 0.1 M NaOH solution to reach 30 mL (again, 1 g sample/10 mL solution).	
Step 10	Sake the suspension for 4 hrs.	
Step 11	Allow the solution to settle overnight.	
Step 12	Separate the supernatant from the residue by centrifugation (5 min, 5000 rpm). Collect supernatant. This contains both humic and fulvic acid.	Day 2
Step 13	Acidify the supernatant with 6 M HCL under constant stirring to a pH = 1.0 and then allow the suspension to stand for 12-16 hrs.	
Step14	Centrifuge (5 min, 5000 rpm) to separate the HA (precipitate) and FA (supernatant – FA-2) fractions.	Day 3
Step 15	Separate the fractions and purify or analyze separately.	

2.7 Extraction of humic and fulvic acid

*Analytical chemistry of humic substances*

Drs. Oana Crina Bujor-Nenita and Violeta Alexandra Ion presented the theoretical background of analytical chemistry of humic substances. They began with a detailed description of the chemistry of humic acids (HAs) as these are often the most relevant for soil environments. Some of the key characteristics of HAs include:

• It is a natural, acidic organic polymer extracted from humus found in soil, sediment, or aquatic environments.
• HAs are mainly composed of quinones, phenolic, carboxylic, enolic, and ether functional groups, but may also include carbohydrates and peptides (Stevenson, 1994).
• HAs are insoluble in strong acid (pH = 1).
• A 1:1 hydrogen-to-carbon ratio indicates a significant degree of aromatic character (i.e., the presence of benzene rings in the structure).
• A low oxygen-to-carbon ratio indicates fewer acidic functional groups than occur in fulvic acid.
• Transition and heavy metals—for example, $Fe3+$ or $Pb2+$—as well as other compounds having aromatic or hydrophobic (water-insoluble) chemical structures (i.e., organic pesticides or anthropogenic hydrocarbons), react strongly with humic acid, which makes it an effective agent in sequestering many of the pollutants in terrestrial and aquatic environments.

Figure 3. Humic (bottom) and fulvic (upper) acids extract after centrifugation.

Figure 4. HA possible structure & chemical formula $C_{187}H_{186}O_{89}N_9S_1$
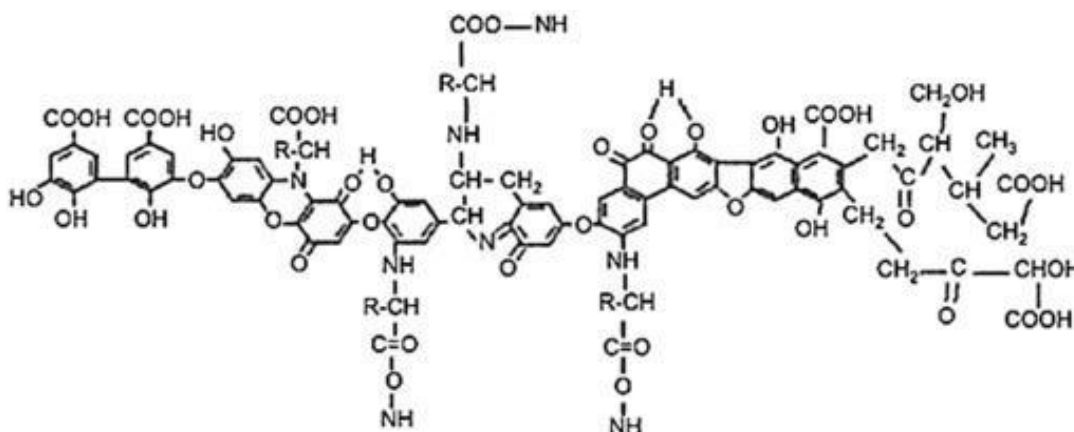
Approaches for testing HAs fall into five categories: Precipitation, extraction, and purification (standard approach); Spectroscopic methods such as UV-Vis, Fourier transform infrared (FT-IR), and 13C NMR (rapid, less accurate methods); Ash content measurement (typically for measuring only

total humic content); Thermogravimetry; and Ion chromatography. The methods used during the workshop were extraction and spectroscopy. The extraction method used during the workshop was based on the standard described by Lamar et al. (2014). The selection of both the extractant and the method used for the extraction process should be made with an understanding of the chemical and physical characteristics of the fraction(s) of humic substances required to be separated from the soil (or compost) matrix.

Drs. Bujor-Nenita and Ion described a selection of methods that are commonly used. First was **UV-Vis**. Ultraviolet (UV) and visible radiation are small parts of the electromagnetic spectrum, which includes other forms of radiation such as radio, infrared (IR), cosmic, and X-rays. UV-Vis spectrophotometers use a light source to illuminate a sample with light across the UV to the visible wavelength range (typically 190 to 900 nm). From the spectrum obtained it is possible to determine the chemical or physical properties of the sample, identify molecules in a solid or liquid sample, determine the concentration of a particular molecule in solution, characterize the absorbance or transmittance through a liquid or solid (over a range of wavelengths), characterize the reflectance properties of a surface or measuring the color of a material, and study chemical reactions or biological processes.

UV-Vis is used to obtain the E4/E6 ratio (the ratio between absorbances at 465 and 665 nm) which indicates the degree of humification (decomposition of organic matter). Progressive humification is indicated by a decrease in the E4/E6 ratio. Lower E4/E6 ratios are associated with molecular condensation and a high level of hydrophobicity, which in turn indicate higher degrees of humification.

Fourier Transform Infrared (FT-IR) is a method of spectroscopic analysis and measures the vibration of molecules. Each functional group has its own discrete vibrational energy which can be used to identify a molecule through the combination of all the functional groups that are identified in a sample. This makes FT-IR spectroscopy ideal for sample ID, multilayer film characterization, and particle analysis. Compared to classic FT-IR, using transmission, the ATR (Attenuated total reflection) method enables samples to be examined directly in the solid or liquid state without further preparation. This minimizes the time for sample preparation, is a nondestructive measurement, and is easy to handle.

FT-IR analysis can be performed using an FT-IR spectrometer in conjunction with a range of different sampling systems. The classic approach has been to use transmission but using an ATR accessory has become widely used in recent years. For the classic approach, a known amount of humic acid (2 mg) is mixed with 400 mg potassium bromide (KBr) and pressed under a vacuum to make pellets. Both samples and KBr can be dehydrated at 105 °C to remove moisture before production of the pellets. IR spectra of the pellets should be recorded at room temperature, after pressing. They should then be heated overnight at $105^{o}C$ and repressed before re-recording the spectra. For the ATR method, use the dried extracted HAs directly. The bands can be measured in the range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}. More information about interpreting FT-IR spectra will be presented in a later section.

2.8 Fundamentals of composting and composting quality

Mr. Erwin Binner from The BOKU University of Natural Resources and Life Sciences Vienna gave two lectures about composting. The first was about the fundamentals of composting and state-of-the-art in Austria (with relevant examples from other countries). The second focused on humic acids as a quality criterion, methods for evaluating humification, and methods for enhancing humification.

Composting is defined by the Austrian Compost Ordinance as a "controlled, aerobic, exothermic biological transformation of biodegradable organic materials (including a thermophilic phase) into a product rich in stable humic compounds with minimum 20 % (m/m) of organic fraction". Compost has many benefits. It adds stable organic substances to the soil, is a source of slow-release nutrients, promotes growth and increased yields of plants under sub-optimal conditions, promotes aggregate stability, improves soil structure, and increases filter and buffer capacity.

The heat generated during composting is a result of the metabolic activities of the microorganisms, and the threshold for reaching the thermophilic phase is 55 °C. The goal of composting is to convert organic compounds into stable humic substances through a fast (but not too fast, low-loss degradation with minimal mineralization of CO_2, H_2O, and loss of N (runoff or off-gassing). Compost should have a high content of nutrients and minimal presence of pollutants, pathogens, and other impurities (all of which are regulated and have limits).

Quality compost begins with a separate source collection of biogenic waste, with low concentrations of pollutants and contaminants. It is also dependent on the proper mix of feedstocks (i.e., browns and greens, fine and coarse, etc.) and an optimized process. Composting is an aerobic process, meaning that the microorganisms doing the work need oxygen. They also need a balanced diet of carbon and nitrogen and access to water. Carbon is the energy source, and nitrogen is used to build cell structures. Oxygen is consumed and CO_2 is released. Organic matter is converted to metabolic products such as fatty acids (carbon acids), ammonium (NH_4), ammonia (NH_3), and humic substances.

Structure of the mixed feedstocks is essential for allowing an aerobic process. This is dependent on a large enough pore space between the aggregates. As the substrate heats up, convection from the heat rising draws fresh air in through the bottom and supplies the microorganisms with oxygen. A poorly structured or overly wet substrate will turn anaerobic, leading to odors, and degradation slows down. A well-balanced and properly aerated feedstock mixture will heat up rapidly and reach the thermophilic phase within a day or two of mixing. Once the easily degradable carbon is consumed the temperature will drop to the mesophilic range again until it is turned, at which time it may become thermophilic again. After several rounds of turning, all the easily degradable carbon will be used up and the microbial population shifts to become more dominated by fungi, which are better at degrading the more recalcitrant carbon compounds like cellulose and lignin. The composting process can be sped up by pre-treating the feedstocks, supplying oxygen, maintaining optimal moisture levels, and even regulating the temperature, though these interventions are not always necessary.

Once the active phase of composting is finished compost must be cured before it can be used. This phase of composting lasts 8-12 weeks (ideally longer) and cannot be sped up. It is dependent on soil organisms, both micro and macro such as earthworms. During this phase, phytotoxic compounds are broken down, carbon and nitrogen are mineralized, and stable humic compounds develop.

Suitable wastes for composting include institutional and commercial food wastes (catering, commercial kitchens, restaurants, hotels), wastes from processing industries and agriculture (abattoir trimmings, food wastes), manure, road trimmings, garden waste, household kitchen wastes, and many others. These feedstocks should be collected separately, often, and mixed in amounts that give optimal C/N ratios, pore space (free air space), and moisture content. The compost should be a well-balanced mix of scarcely to easily degradable feedstocks (e.g., kitchen + yard wastes) and turned frequently during all phases to promote aeration, homogenization, and contact between microorganisms and nutrients.

Commercial and municipal compost quality is regulated by ordinances that set certain requirements for the sourcing of raw materials and process parameters, and set limits to the content of pollutants, physical and chemical contaminants, heavy metals, and pathogens. These ordinances are determined by government regulators and vary from country to country. Compost quality in Austria is regulated by the Austrian Compost Ordinance (2001).

2.9 Methods for evaluating humification and how to enhance humification during composting

The next lecture was about humic substances as an indicator of compost quality, humification during composting, and how to enhance humification. Evaluating compost quality and maturity can be done in several ways. The more common tests include organoleptic analysis (odor, crumb structure) and degradation parameters (ignition loss, carbon content, nutrient content, ammonium/nitrate ratio, C/N ratio). For measuring the level of maturity and stability, the simplest tests are Dewar "self-heating" (described previously), respiration activity test (CO_2 evolution or oxygen uptake like e.g., AT_4), and germination tests. Germination tests measure whether phytotoxins and other compounds harmful to plants have been broken down. An immature compost will often suppress germination, as will composts that contain herbicides.

The presence of humic acids is a very good parameter for quality and in the opinion of Mr. Binner possibly the best definition, but they are time-consuming to measure directly (see previous section about extraction and purification methods). However, they can also be evaluated indirectly by using FT-IR. The development of humic substances begins during the intensive, thermophilic phase and concentrations can get up to 45% of organic solids in finished compost. They are composed of approximately 50-60% C and 2-8% N. They originate from reactive metabolic compounds produced during the degradation of lignin, polysaccharides, protein, and others by the biological activity of microorganisms.

The determination of compost quality with FT-IR focuses on two aspects: material composition and parameter prediction. Material composition is determined by analyzing spectra at specific frequencies, such as the CH stretching groups around 2900 cm^{-1}, amide (1650), amide II (1550), and carboxylate (1400), and in some cases the fingerprint areas below 1500 where aliphatic methylene bands inorganic compounds (silicates/carbonates) can be found, though these are difficult to interpret. These bands identify compounds and functional groups that are indicative of the presence of humic substances. The other aspect of FT-IR determination – parameter prediction – requires collecting a range of data from a large data pool and running statistical models to connect

parameters to FT-IR spectra. Mr. Binner was part of a team that developed a model using Partial Least-Squares Regression (PLS-R) to "predict" extractable humic acids, loss on ignition, total organic carbon, total nitrogen, and O_2 uptake (AT_4) with FT-IR based on results from chemical and physical analyses.

The final topic was the enhancement of humification. Enhanced humification begins with the right feedstock. Biowaste (kitchen and food waste) combined with yard waste resulted in the highest concentrations of HA. Compost containing sewage sludge or made from pure yard waste had the lowest. An initial anaerobic processing of some or all the feedstocks, e.g., in a biogas facility, can also help. Humification takes time. While the development of humic substances begins during the initial thermophilic phase, they need time to fully develop. Treatment of more than 20 weeks resulted in significantly higher concentrations of HA than treatment of compost in under 8 weeks. Finally, respiration should be moderate and degradation unhurried. Too much oxygen and rapid degradation can lead to a high level of mineralization and losses of carbon and nitrogen that could form the basis of HAs.

2.10 FT-IR spectroscopic analysis – a powerful tool for chemical characterization

Dr. Jean Robertson began her lecture with a presentation of The James Hutton Institute and their work with marine biomass research, for example, processing into alginates, biostimulants, and food. She then continued with a presentation about FT-IR in the mid-infrared (MIR) range, the applications of FT-IR analysis, how to approach the interpretation of spectra (sampling method, sample preparation, and spectral quality), spectral features of organic and inorganic components, and gave a summary of steps for interpretation of spectra.

Infrared radiation provides a level of energy that corresponds to that of vibrational energy transitions in molecules that are absorbed by molecular inter-atomic vibrations. There are three ranges of wavelength (nm/μm) and wavenumber (cm^{-1}) within the IR region: Near (780-2500 nm or 12820-4000 cm^{-1}), Mid (2.5-25 μm or 4000-400 cm^{-1}), and Far (30-300 μm or 400-33 cm^{-1}). Molecules interact with the electric vector of IR radiation resulting in absorption at different frequencies. Compounds are required to have covalent bonds and exhibit a change in dipole moment during chemical bond vibrations to be IR-active. Different functional groups absorb at several frequencies involving different types of chemical bond vibrations e.g., stretching and bending.

Infrared radiation is absorbed by a sample at specific frequencies depending on the chemical composition of the sample. It allows us to "see" what something is made of by producing a chemical "fingerprint" or chemical profile (IR spectrum). Importantly, IR spectra can provide information about both the organic and inorganic components of a sample.
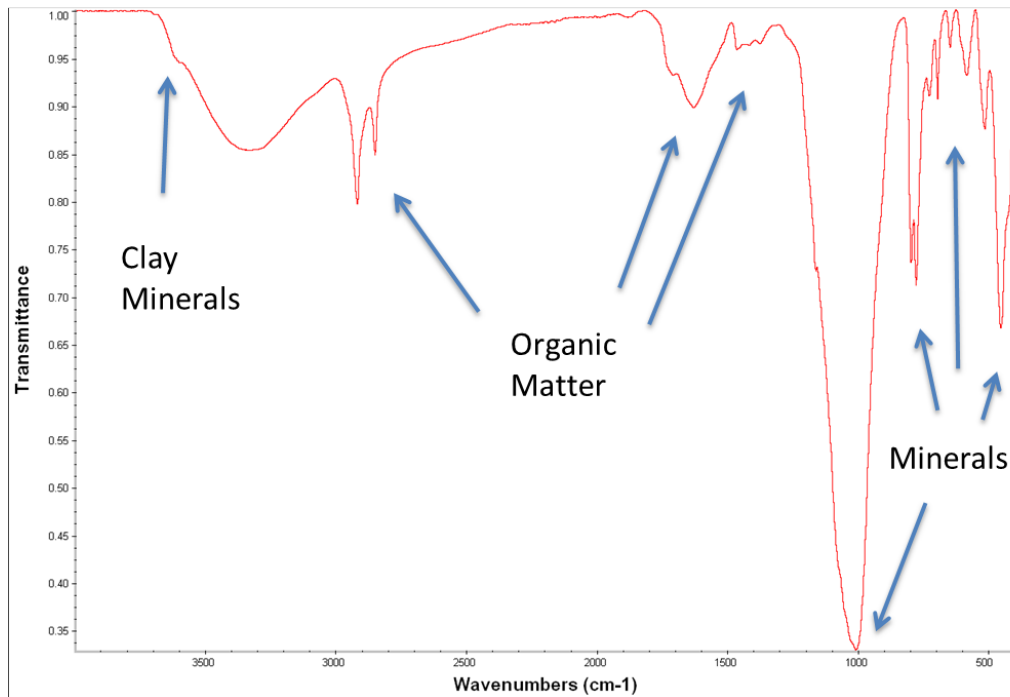
Figure 5. IR spectrum of a soil sample.

Different regions of the IR spectrum give different information. The most common to use are the mid-infrared (MIR) or near-infrared (NIR) regions. Qualitative interpretation in the MIR region gives "insight". Quantitative prediction of properties (NIR or MIR) can be used as a tool instead of "wet chemistry". Multiple parameters can be predicted at once using statistical models (chemometrics, i.e., the method that Mr. Binner and his team used). For MIR (wavenumber 4000-400 cm^{-1}), fundamental vibrations of bonds within molecules absorb IR light. Spectra provide "fingerprints" of substances that are more readily interpreted and give a more complete profile of inorganic and organic components. NIR (wavelength 1100-2500 nm) gives combination bands and overtones of fundamental bands found in the MIR, mainly for vibrations of the lighter elements. The NIR does provide complementary information to the MIR but generally it is harder to interpret and provides more limited spectral information than the MIR region does. More energetic radiation in NIR allows for deeper penetration into samples.
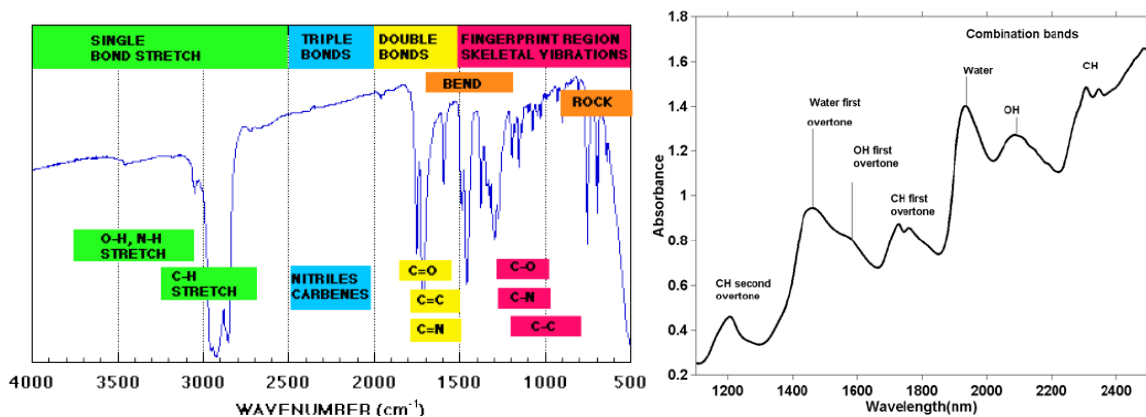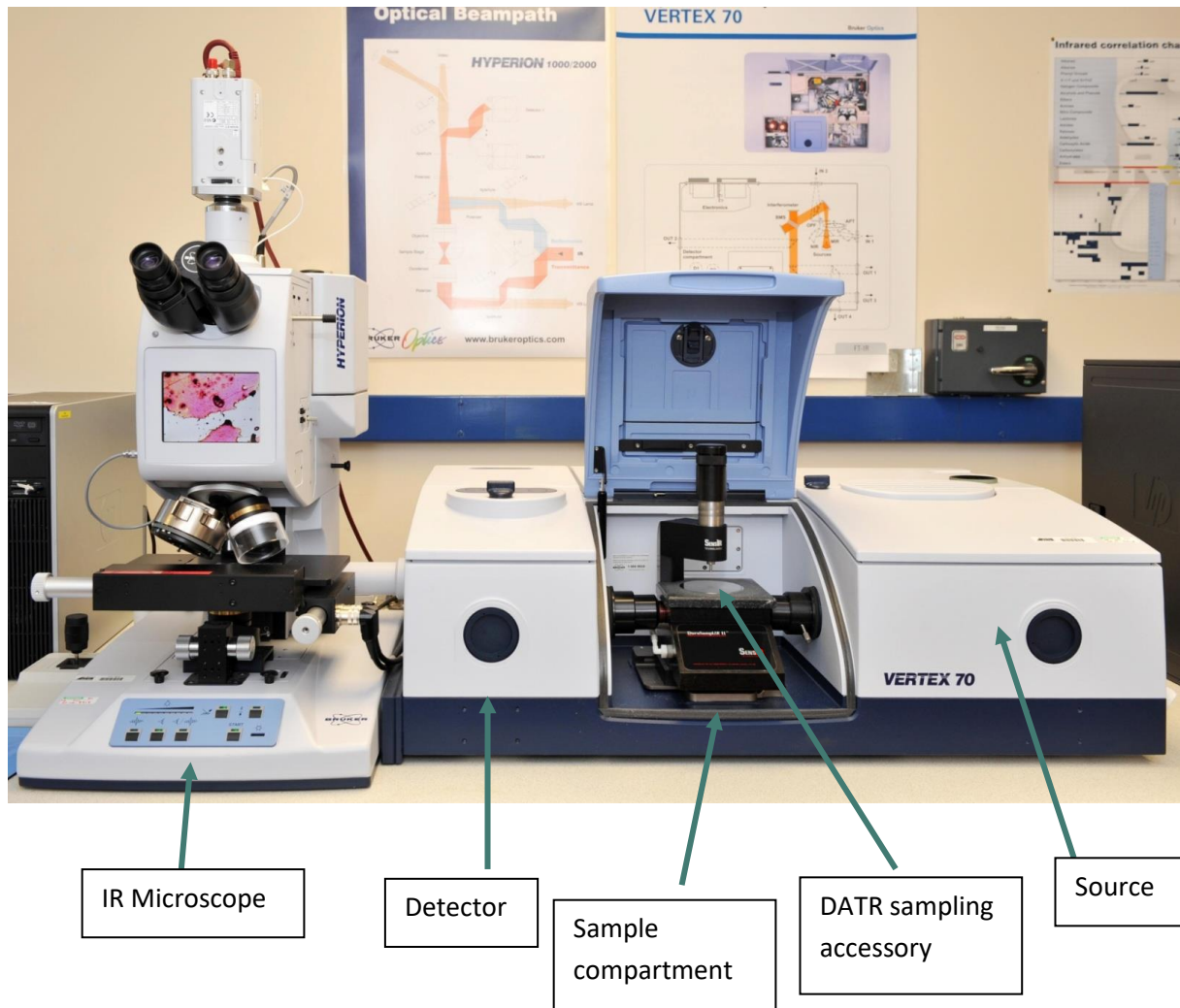

Figure 6. Examples of MIR (left) and NIR (right) spectra and the regions of major functional groups.

Recording FT-IR spectra is more straightforward than interpreting the spectra, which can be highly complex, particularly for natural materials. However, ensuring that you have recorded good-quality spectra that are properly representative of your samples, is essential for any study using FT-IR analysis. Therefore, make sure that you use good sampling techniques, and high-quality spectral data with a good signal/noise ratio and without water vapor spikes or CO_2 interference (though these can be subtracted with software). The most intense band in your spectrum should also be less than 2 units in absorbance.
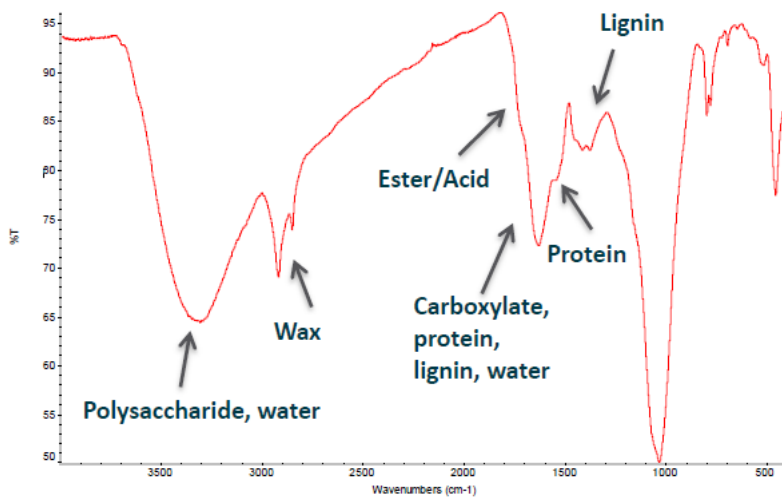
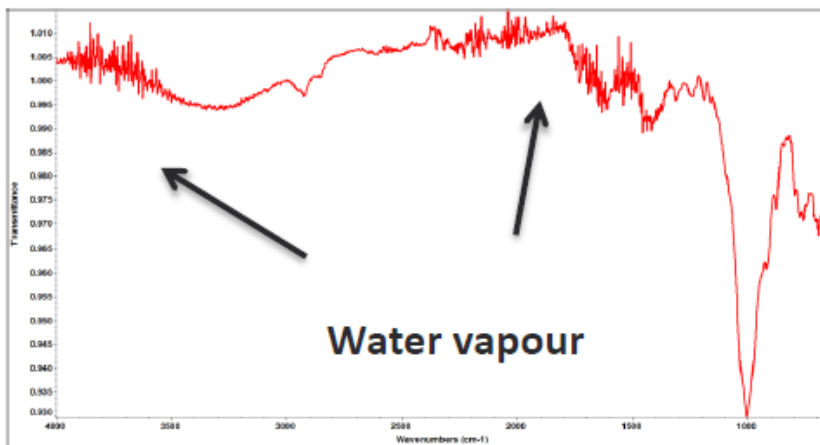*Figure 7. Example of a spectrum from un-decomposed vegetation.*

Figure 8. Example of a poor-quality spectrum.

As mentioned above, FT-IR spectra can be recorded using a range of different sampling methods including Transmission, Diffuse Reflectance (DRIFTS) and Attenuated Total Reflectance (ATR). Both DRIFTS and ATR spectra can also be recorded in situ. Be aware that the appearance of the two types of spectra can be very different, especially if DRIFTS is recorded of a "neat" sample i.e. one which is undiluted by KBr (Nguyen et al., 1991). The DATR accessory has several advantages. The sample can be placed, without processing, on the window. It is non-destructive so the sample can be recovered and requires < 1mg of sample. Diamond crystal allows analysis of hard particles e.g. quartz. IR beam only penetrates the sample by a few μm independent of sample thickness so there are no absorbance issues. Spectra are "Transmission-like" and relatively easy to interpret. Optics are all internal so there is no issue with lighting conditions.

Spectra must be representative and reproducible. For the highest quality FT-IR spectra of solids, samples should be dry and milled to < 2μm. A sample preparation protocol, which produces samples with the optimal particle size, while avoiding problems of contamination or degradation, is required. Once a quality spectrum is obtained, to start the interpretation process, look over it, moving from left to right, and noting the frequency (cm^{-1}) of the intense absorption bands. Bearing in mind your

knowledge of the sample (e.g., is it largely inorganic or organic) assign the most intense bands first using correlation tables or a reliable paper. Track down any secondary bands for the functional groups if possible – e.g., for protein/amide, there should be amide I and amide II. Then assign other bands as needed. Finally, write down the organic and/or inorganic functional groups you think exist in the sample. Get help from spectral libraries or interpretation software as natural material can be very complex mixtures.
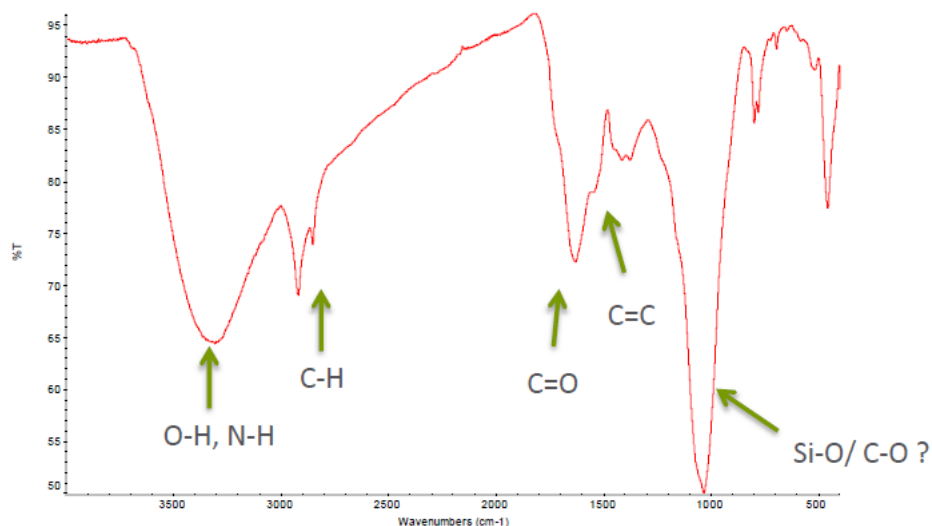

Figure 9. Some of the important functional groups and their wavenumbers.

Dr. Robertson presented some examples of interpreting spectra from a variety of organic materials, beginning with organic soils. Often referred to as peat deposits, organic soils represent accumulations of partly or completely decomposed plant residues formed under anaerobic conditions. Their spectra are related to that of the un-decomposed vegetation, and peat spectra will differ according to plant population. The peat spectra will also differ from the vegetation depending on the extent of decomposition. Cellulose and lignin are major components of plant material. Key frequencies from their spectra can be identified in the spectra of vegetation and peat samples.

In addition to cellulose (polysaccharide – 1030 cm^{-1} and 3330 cm^{-1}) and lignin (aromatic – 1600, 1510 cm^{-1}) organic soils that are largely un-decomposed (often at the top of the peat) are likely to have other polysaccharides (C-O 1100-900 cm^{-1}), ester (C=O ~1730 cm^{-1}), and protein (amide I ~1650 cm^{-1}, amide II ~1550cm^{-1}) functional groups identifiable in the spectra. The CH stretching region (3000-2800 cm^{-1}) of these soils will show no distinct peaks as the absorption is derived largely from the polysaccharides. Organic soils that are more decomposed (often deeper in the peat) are likely to have reduced polysaccharides (C-O 1100-900 cm^{-1}). Although some ester may remain, there is likely to be predominantly carboxylic acid present (C=O ~1710 cm^{-1}). The CH stretching region (3000-2800 cm^{-1}) of these soils will also show evidence of long chain or waxy compounds with sharp distinct peaks at 2920 cm^{-1} and 2850 cm^{-1}, which are derived from CH$_2$ stretching vibrations. In addition, there is a small but sharp CH$_2$ "wagging" vibration which appears at 720 cm^{-1}.

The IR spectrum of a soil sample can rapidly provide an insight into the proportion and nature of soil organic matter (SOM) present and the level of humification. In the IR spectra of the top horizons, the SOM often still has functional groups that are identifiable. In lower horizons, humification can lead to a smoother profile in the IR spectrum with no distinct bands arising from discernible functional

groups. Broad bands arising from carboxylates can arise in lower horizons. Examples of IR spectra of humic and fulvic substances are given below, in figs 11 and 12.
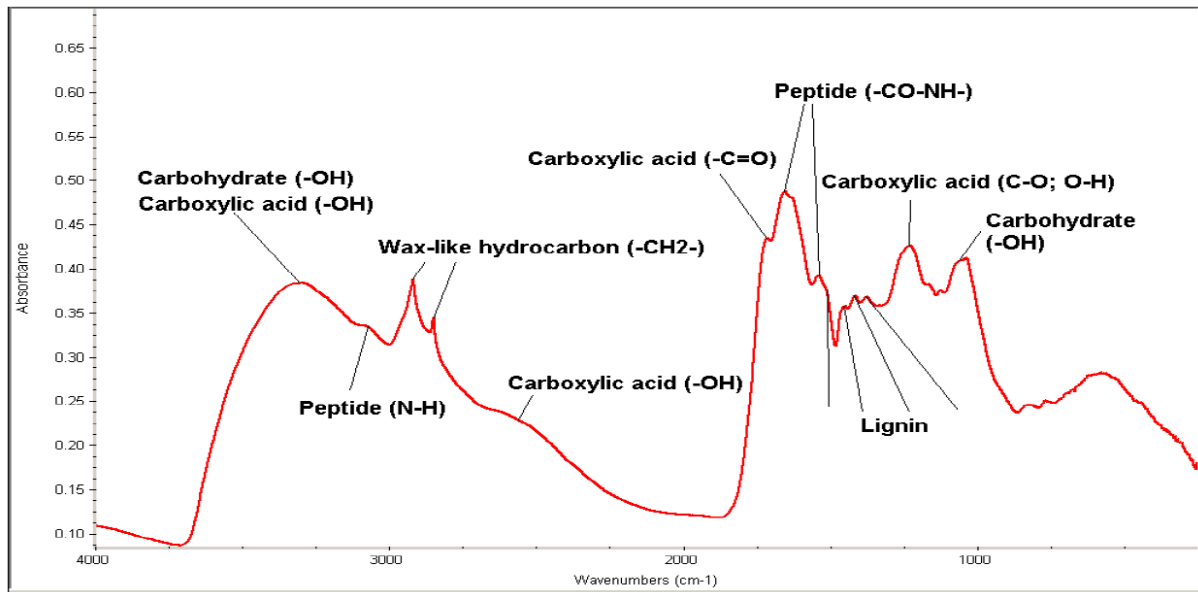

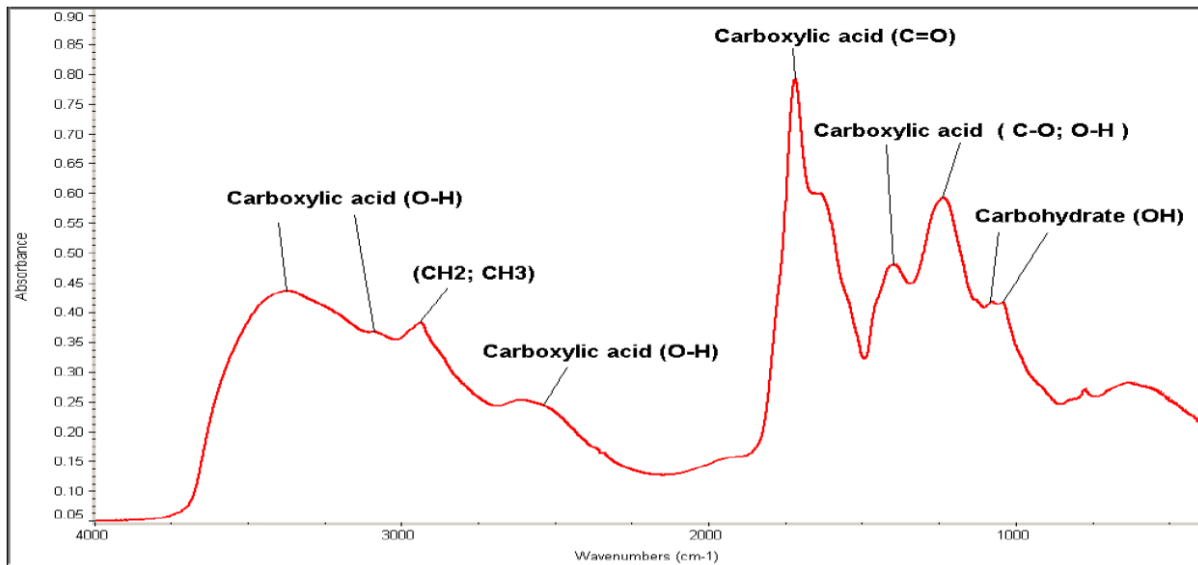*Figure 10. IR spectrum of mineral soil humic acid.*

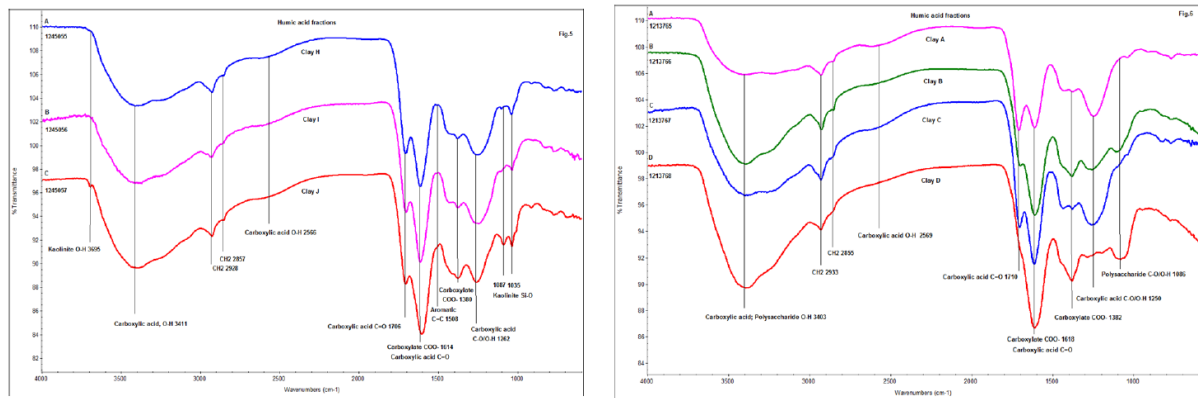*Figure 11. IR spectrum of mineral soil fulvic acid.*

Figure 12. Spectra from HA fractions extracted from seven different clays. Samples H, I, and J are on the left; A, B, C, and D are on the right.

Spectra were presented (see Fig 13) for a study of HA fractions extracted from a set of clay samples. The HA fractions of samples H, I, and J (Figure 13) have very similar chemical compositions to the HA fractions of samples A, B, C, and D, with the dominant functional groups being carboxylic acid/carboxylate in varying relative proportions. Traces of lignin-type material and kaolinite components were detected in HA fractions H, I, and J but not in HA fractions A, B, C, and D. Polysaccharide, which was detectable in HA fractions B and D, does not appear to be present in HA fractions A, C, H, and I but may be detectable in HA fraction J.
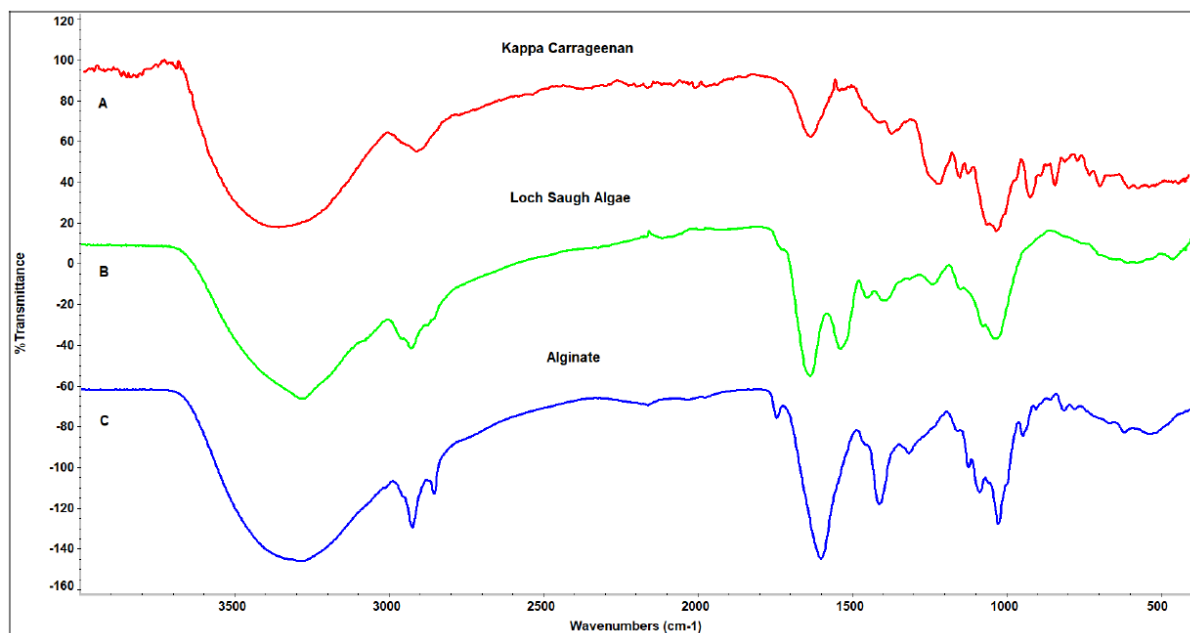

Figure 13. FT-IR spectra of some marine products.

In summary, when recording FT-IR spectra, care must be taken to avoid interference by water vapor and CO_2. Spectra should also have a good signal to noise ratio. Sample preparation methods will affect the spectra produced and a protocol to ensure representative and reproducible spectra should be used. It is important to be aware that different sampling methods (e.g., DRIFTS and ATR) can cause large differences in the appearance of spectra, which needs to be taken into account when interpreting them. Always ensure that you are using high-quality data, e.g. good signal/noise ratio,

with no water vapor spikes or carbon dioxide interference. The most intense band should be less than 2 in absorbance.

When interpreting the IR spectra start by looking over the spectrum from left to right, noting the frequency (cm $^{-1}$) of the intense absorption bands. Taking into account your knowledge of the sample, assign the most intense bands first (using correlation tables). Track down the secondary bands for the functional groups you have already assigned, if possible. Assign other bands as needed. Write down the organic functional groups and/or minerals you think exist in the sample. Get help from spectral libraries or interpretation software as natural materials are very complex mixtures.

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Appendices

Appendix 1: Workshop program

<u>Monday, 23rd October</u>

8:30-9:30	Registration and coffee at Research Center for Food Quality and Agronomic Sciences
9:30-9:40	Welcome to USAMV (*Prof. Gina Fîntîneru, Vice-rector Scientific Research, USAMV*)
9:40-10:15	Presentation of the workshop schedule, practical information, introductions of attendees and guests, and expectations for the workshop (*Workshop coordinators Joshua Cabell and Violeta Ion*)
10:15-10:45	MARIGREEN activities/experiments related to marine composts (*Joshua Cabell, NORSØK/NMBU*)
10:45-11:00	Break
11:00-11:30	MARIGREEN: Biostimulants based on aquatic residues (*Marios Mariupolis, AUTh/Modern Analytics*)
11:30-12:00	MARIGREEN: Growth trials/biochar impregnation (*Violeta Alexandra Ion, USAMV; Oana Cristina Pârvulescu, UNSTPB*)
12:00-12:30	Effect of compost on strawberry trials at USAMV (*Ailin Molosag, USAMV*)
12:30-13:30	Lunch
13:30-14:30	Tour of facilities, visit field trials
14:30-15:00	Coffee break
15:00-	Visit Parliament

<u>Tuesday, 24th October</u>

8:30-9:00	Registration and coffee
9:00-10:00	Introduction to and tour of the Research Center for Studies of Food Quality and Agricultural Products (*Professor Liliana Badulescu, director of the research center*)
10:00-11:00	Extraction of humic substances – sample preparation (I) – lab work (*Violeta Ion, Oana Bujor-Nenita, Andrei Mot, Cristian Nedelcu, USAMV*)
11:00-11:15	Coffee break
11:15-12:30	Extraction of humic substances – sample preparation (II) – lab work (*Violeta Ion, Oana Bujor-Nenita, Andrei Mot, Cristian Nedelcu, USAMV*)
12:30-13:30	Lunch
13:30-14:30	Analytical chemistry of humic substances (*Violeta Ion, Oana Bujor-Nenita, USAMV*)

14:30-14:45   Coffee break

14:45-16:00   Fundamentals and state-of-the-art of composting in Austria – via Zoom (*Erwin Binner, BOKU*)

Wednesday, 25th October

8:30-9:00   Registration and coffee

9:00-11:00   Extraction of humic substances (I) – lab work (*Violeta Ion, Oana Bujor-Nenita, Andrei Mot, Cristian Nedelcu, USAMV*)

11:00-11:15   Break

11:15-12:30   Extraction of humic substances (II) – lab work (*Violeta Ion, Oana Bujor-Nenita, Andrei Mot, Cristian Nedelcu, USAMV*)

12:30-13:30   Lunch

13:30-14:45   FT-IR spectroscopic analysis – a powerful tool for chemical characterization (*Jean Robertson, James Hutton Institute*)

14:45-15:00   Break

15:00-16:30   Compost quality, humic acids, humification – via Zoom (*Erwin Binner, BOKU*)

19:00   Dinner celebration

Thursday, 26th October

8:30-9:00   Registration and coffee

9:00-11:00   FT-IR analysis of humic acids, comparing and interpreting spectra (*Jean Robertson, James Hutton Institute; Violeta Ion, Oana Bujor-Nenita, Cristian Nedelcu, Andrei Mot, USAMV*)

11:00-11:15   Coffee break

11:15-12:30   UV-VIS analysis of humic acids, comparing and interpreting spectra (*Jean Robertson, James Hutton Institute; Violeta Ion, Oana Bujor-Nenita, Cristian Nedelcu, Andrei Mot, USAMV*)

12:30-13:30   Lunch

13:30-14:45   Open discussion of results and workshop, wrap-up, final impressions

14:45-15:00   Coffee break

15:00-17:00   Viewing of documentary "The Need to Grow", directed by Rosario Dawson (*Event provided by the Hort4EUGreen project "Enhancing skills of horticultural specialists to better address the demands of the European Green Deal", project coordinator Roxana Ciceoi, USAMV*)

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