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Increasing expertise in micro- and nanoplastics analysis through twinning action

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D 3.2 Knowledge exchange on protocol for generation of ‘model’ microplastics from mulching films using accelerated weathering

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Abstract:	The Deliverable 3.2 Knowledge exchange on protocol for generation of 'model' microplastics from mulching films using accelerated weathering summarizes the InPlasTwin activities carried out under Task 3.2: Generation of 'model' microplastics for subsequent experiments. The primary objective of this task was to establish a reliable and reproducible protocol for producing 'model' microplastics from biodegradable mulching films, achieved through hands-on training of two researchers from JSI and AUA at DTU Sustain. This also included the identification of key characterization requirements for the generated 'model' microplastics and the performance of selected quantitative and chemical analyses.

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List of Abbreviations and Acronyms	
AUA	Agricultural University of Athens
BST	Black standard temperature
C-H	Carbon-hydrogen
C-O	Carbon-oxygen
DSC	Differential scanning calorimetry
DTU	Technical University of Denmark
DTU Sustain	Department of Environmental and Resource Engineering
FTIR	Fourier transform infrared
JSI	Jožef Stefan Institute
LDIR	Laser direct infrared
LDPE	Low-density polyethylene
MNP	Micro- and nanoplastic
MP	Microplastic
NP	Nanoplastic
PBAT	Polybutylene adipate terephthalate
PVGIS	Photovoltaic Geographical Information System
QCL-μIR	Quantum cascade laser micro-infrared
RUC	Roskilde University
SEM	Scanning electron microscopy
TGA	Thermogravimetric analysis
UV	Ultraviolet
WP	Work package
WP3	Work Package 3
WP8	Work Package 8

1. Introduction

Plastics play an important role in agriculture, with mulching films accounting for approximately 50 % of the total mass of agricultural plastics (Hofmann et al., 2023). Their use in food production offers several benefits, including increased soil temperature, reduced weed growth, improved moisture retention, and higher crop yields (Kasirajan & Ngouajio, 2012). Traditionally, mulching films have been produced from conventional plastic, most commonly low-density polyethylene (LDPE) (Hofmann et al., 2023). However, as LDPE and other conventional plastics are non-biodegradable, they must be collected and disposed of after harvest – a process that is both costly and labor-intensive (Hayes et al., 2019). Furthermore, incomplete recovery after use will lead to the accumulation of persistent plastic residues in soils, with potential negative impacts on ecosystems (Hofmann et al., 2023). To address these issues, biodegradable plastic mulching films have been developed (Hayes et al., 2019). Unlike conventional plastics, these biodegradable films can be left on the soil after use, where they are expected to undergo biodegradation, offering a less labor-intensive and more environmentally sustainable alternative (Hayes et al., 2019; Khalid et al., 2023). However, if not fully degraded, these biodegradable plastic mulching films can generate micro- and nanoplastics (MNPs). Such plastic particles can contribute to soil contamination and may be transferred through food chains, raising concerns for both ecosystems and human health (Khalid et al., 2023).

Addressing these challenges requires reliable methodologies to study how biodegradable mulching films generate microplastics (MPs) under realistic conditions, along with the development of suitable laboratory test materials that mimic naturally aged MPs. Laboratory-scale production of true-to-life MPs can be achieved through mechanical fragmentation of ultraviolet (UV)-weathered mulching films, where UV exposure accelerates polymer aging. The resulting particles closely resemble those found in natural environments in terms of size, morphology, and chemical composition, thereby enhancing the environmental relevance of the test materials (Ducoli et al., 2025). This approach enables a more accurate and realistic assessment of the environmental fate and behavior of MPs in laboratory exposure experiments, ultimately contributing to improved environmental risk evaluation and mitigation strategies (Ducoli et al., 2025).

1.1. Aim

In this context, the main aim of Work Package 3 (WP3) is to strengthen the capacity of researchers at Jožef Stefan Institute (JSI) and the Agricultural University of Athens (AUA) to investigate the generation of microplastics (MPs) from biodegradable mulching films under environmentally relevant degradation conditions. Task 3.2 specifically focuses on generating ‘model’ MPs for subsequent experiments. It includes training of scientists from JSI and AUA to establish a reliable and reproducible protocol for producing such MPs from biodegradable mulching films. The training also covers defining key characterization requirements for the generated MPs and performing selected quantitative and chemical analysis using visual inspection and Fourier transform infrared (FTIR) spectroscopy. Finally, the produced ‘model’ MPs will be supplied to other work packages (WPs) for further experimental work and analysis. Thus, the outcome of this task is both capacity building and the establishment of a protocol for producing and characterizing ‘model’ MPs, thereby supporting collaborative research across subsequent WPs.

2. Methodology

The above-mentioned aims of the training activities under Task 3.2. were focused on selected topics as listed in Table 1. All activities were hands-on oriented.

Table 1: Topics and training activities under Task 3.2.

Training activity	Purpose
Generation of 'model' microplastics (MPs) (accelerated weathering and cryo-milling)	Providing participants with practical experience in accelerated weathering of plastics under simulated sunlight exposure, as well as in cryo-milling techniques to produce 'model' MPs from UV-weathered mulching films.
Defining characterization requirements for 'model' MPs	Enhancing participants' understanding of key characterization approaches for 'model' MPs, including quantification, size distribution, and chemical analysis

The training activities took place at the Technical University of Denmark (DTU), Department of Environmental and Resource Engineering (DTU Sustain) during the period 01/09-2025 to 02/10-2025.

DTU Sustain contributes leading expertise in the field of MNP research. This includes research on their environmental impacts, development of testing guidelines, and investigation of their role as carriers of co-pollutants. This knowledge was shared with partner institutions in two Widening countries – JSI (Slovenia) and AUA (Greece) – to strengthen their research capacity, particularly in areas where they had limited expertise in analytical techniques for MNP analysis and in studying MP formation from agricultural mulching films.

The training thus involved DTU (host) and JSI and AUA (visitors). Dr. Majda Nikezić, a postdoctoral researcher from JSI, participated in the training from 01/09-2025 to 26/09-2025. Postdoctoral researcher Dr. Konstantina Argyropoulou from AUA participated in the training from 01/09-2025 to 09/09-2025, and Prof. Dr. Dimitrios Savvas from AUA participated in the training from 15/09-2025 to 02/10-2025.

3. Trainings

3.1 Generation of ‘model’ microplastics (accelerated weathering and cryo-milling)

Method: Hands-on training and discussions

Description:
This activity provided participants with practical experience in the accelerated weathering of plastics, i.e., biodegradable mulching film, under simulated sunlight exposure, as well as in cryo-milling techniques for producing ‘model’ MPs from UV-weathered biodegradable mulching film.

Given that the degradation experiments on mulching films conducted under controlled, environmentally relevant conditions in Task 3.1 (comprising six days of accelerated weathering followed by 180 days of incubation in agricultural soil) resulted in limited film breakdown and insufficient formation of MPs for further project activities, a more efficient approach was adopted. This approach involves 9 days of accelerated weathering of the mulching films, followed by cryo-milling to generate the desired ‘model’ MPs.

The biodegradable mulching film used in this task was supplied by PLASTIKA KRITIS, a Greek manufacturer of agricultural films, which confirmed that this is the only biodegradable mulching film currently produced by the company. In strawberry production in the Western Peloponnese – and generally across Greece and other Mediterranean regions – growers exclusively use conventional polyethylene mulching films due to their robustness and ability to be reused for 4-5 successive years within the same greenhouse. To ensure relevance to actual strawberry cultivation practices in Greece and alignment with the strawberry experiment conducted within Work Package 8 (WP8), this single biodegradable film was therefore selected for testing in Task 3.2. In parallel, two conventional polyethylene films, representing the materials most widely used by Greek strawberry growers, were included in other project activities (Task 3.1 and WP8) to provide a realistic reference for current agricultural practices, rather than sourcing additional biodegradable alternatives from other manufacturers.

According to FTIR analysis, the selected material is composed of a blend of two biodegradable polymers: polybutylene adipate terephthalate (PBAT) and corn starch. The biodegradable mulching film showed a high spectral quality match index (0.95) with a previously analyzed sample - Mater-Bi Biobag (Figure 1) - known to consist of PBAT and corn starch (Afshar et al., 2025). Based on this similarity, the biodegradable mulching film is therefore assumed to be made of comparable material.

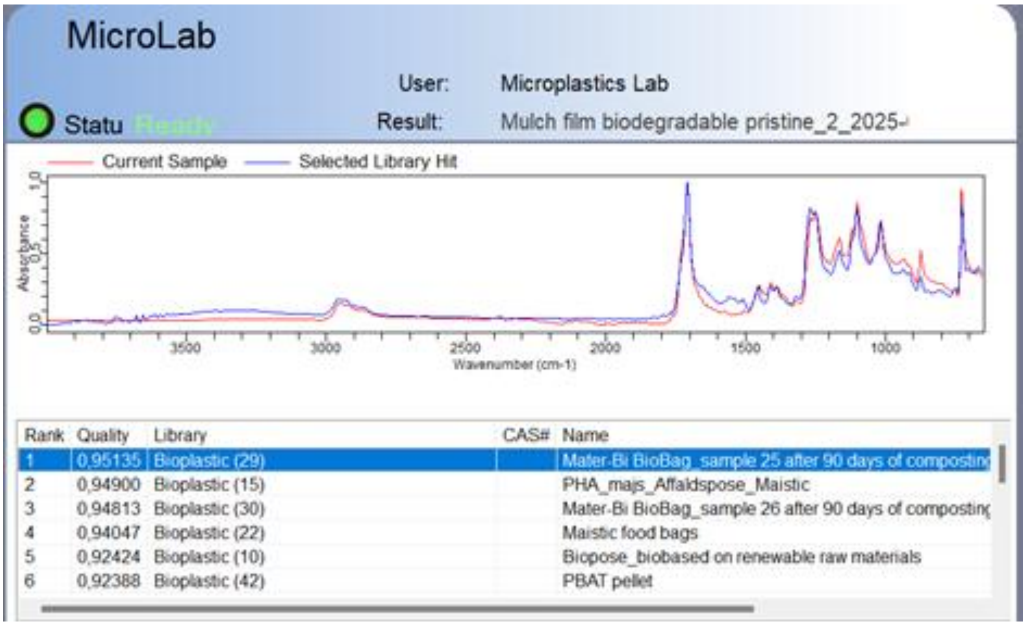

Figure 1: FTIR spectra of pristine biodegradable mulching film, including spectral quality match index with plastics in the reference library.

The training included a demonstration of accelerated weathering using simulated solar radiation. An Atlas SunTest XLS+ chamber was used to expose biodegradable mulching films to controlled UV radiation and temperature conditions. Participants were guided through the setup and operation of the equipment and subsequently conducted accelerated weathering of the mulching films themselves (Figure 2). The UV-weathered films were later characterized by FTIR spectroscopy, building on analytical skills introduced during the previous training (Task 3.1).

The generation of 'model' MPs includes the following steps:

1. Accelerated weathering
2. Cryo-milling
3. Sieving

After sieving, MPs < 63 µm are collected and classified as the 'model' MPs. Particles > 63 µm can be processed through additional cryo-milling to obtain larger amounts of smaller size fractions.

3.1.1. Accelerated weathering

The Atlas SunTest XLS+ chamber is equipped with a metal sample plate measuring 40 cm x 30 cm. A 5 cm x 5 cm area on the plate contains a black standard temperature (BST) sensor, which monitors and regulates the sample temperature during exposure. This area was left uncovered to ensure accurate temperature measurement. Samples of biodegradable mulching film (3.73 g) were cut to cover the remaining surface area of the plate around the sensor.

The samples were exposed to accelerated weathering under the following conditions:

- **Standard:** ISO 4892-2:2013 (dry conditions, no water spray)
- **UV radiation:** 300-400 nm wavelength range
- **BST:** 65 °C
- **Total exposure time:** 211 h 49 min (≈ 9 days)
- **UV dosage:** 38122 kJ/m² (equivalent to ≈ 121 days of natural exposure under Greek conditions)

To estimate the equivalence between accelerated and natural exposure, data from the Photovoltaic Geographical Information System (PVGIS) were used. The annual solar energy intercepted by uncovered ground in the Ilia region (Western Peloponnese) ranges from 1,700 to 1,850 kWh m^{-2} $year^{-1}$, corresponding to approximately 6,400,000 kJ m^{-2} $year^{-1}$ on average (*JRC Photovoltaic Geographical Information System (PVGIS) - European Commission*, n.d.). Dividing this by 365 days yields an average daily solar energy of 17,534 kJ m^{-2} day^{-1}.

Considering that UV radiation in the range of 280-400 nm accounts for 6 % of total solar energy at the Earth's surface (Daxini & Wu, 2024), the mean UV energy reaching the surface in Western Peloponnese is approximately 1,052 kJ m^{-2} day^{-1}. Standard films used to cover greenhouse tunnels used for strawberry production in Western Peloponnese typically transmit about 30 % of UV radiation (information from PLASTIKA KRITIS), resulting in an estimated 316 kJ m^{-2} day^{-1} of UV irradiance inside a standard greenhouse tunnel. This estimate assumes direct and continuous sunlight exposure without shading by soil or vegetation, potentially underestimating the calculated natural UV exposure period.

Given that the accelerated weathering test delivered a total UV dosage of 38,122 kJ m^{-2}, this exposure corresponds to approximately:

$$\frac{38{,}122}{316} = 121\ days\ of\ natural\ UV\ exposure$$

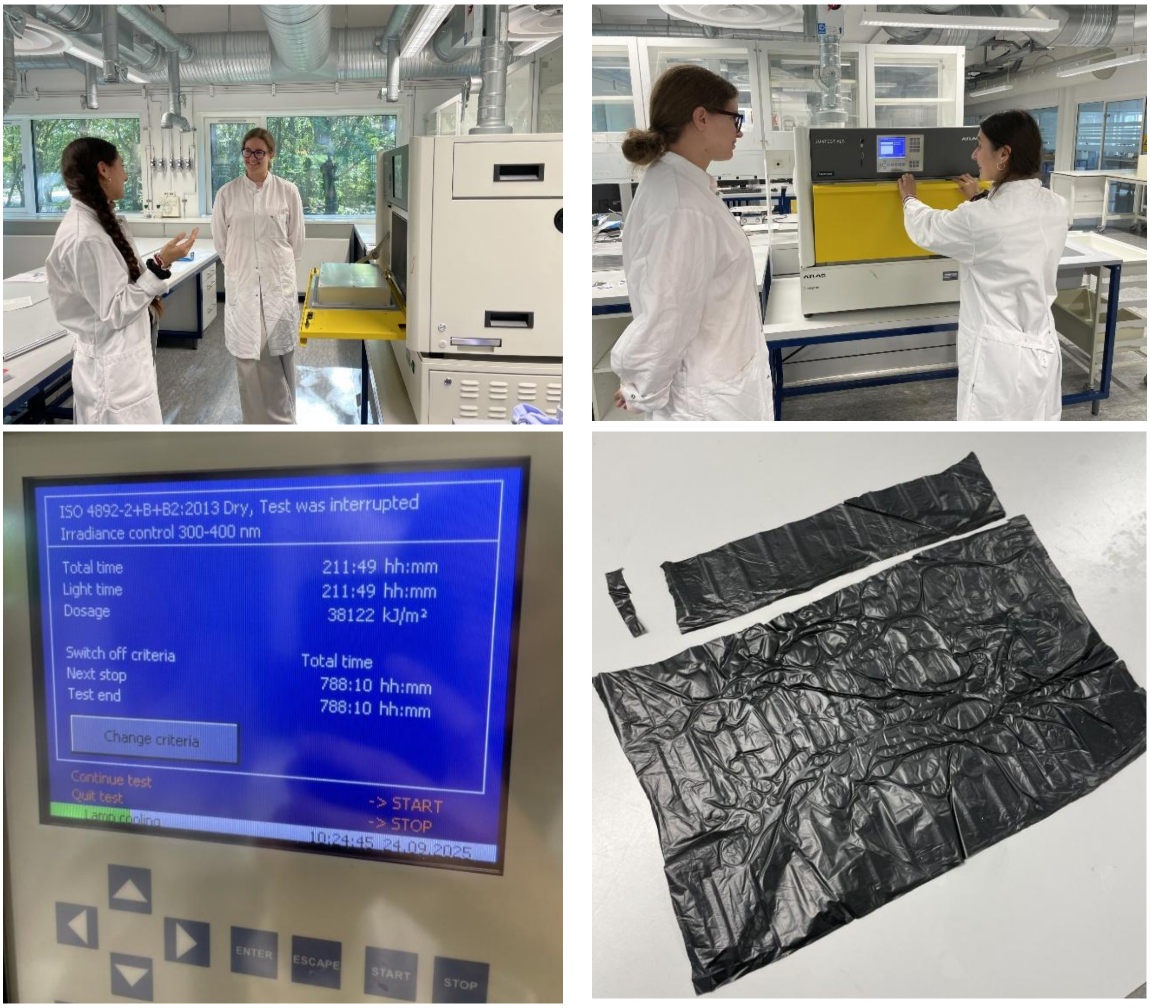

Figure 2: Accelerated weathering of biodegradable mulching film using the Atlas SunTest XLS+ chamber.

3.1.2. Cryo-milling

The cryo-milling procedure was based on McColley et al. (2023) with modifications done by collaborators at Roskilde University (RUC) (Ugwu et al., under preparation). Briefly, the protocol (Figure 3) consisted of the following steps:

Sample preparation:

- Cut UV-weathered plastic samples into smaller pieces to facilitate cryo-milling.
- Smaller initial pieces generally result in finer particles after cryo-milling.

Cryo-milling procedure:

- Add up to 5 g of the cut plastic pieces (≈ 70-80 % of the 50 mL stainless steel milling chamber volume) along with a 25 mm stainless steel ball.
- Use a Retsch CryoMill and ensure a continuous supply of liquid nitrogen throughout the process to maintain low temperatures, ensuring that the plastics become brittle for efficient fracturing and are protected from heat-induced degradation.
- Pre-cool the chamber for 4 minutes at 5 Hz.
- Perform six milling cycles: 5 minutes at 30 Hz followed by 1 min of cooling at 5 Hz.

Pristine cryo-milled plastics were included to evaluate the performance and feasibility of the cryo-milling process prior to processing UV-weathered materials. Milling of pristine biodegradable mulching films demonstrated that the method could produce MP particles in sufficient quantity for subsequent use. The results demonstrated that the cryo-milling process was effective, providing confidence in its suitability for generating representative ‘model’ MPs from UV-weathered plastics for subsequent experimental applications.

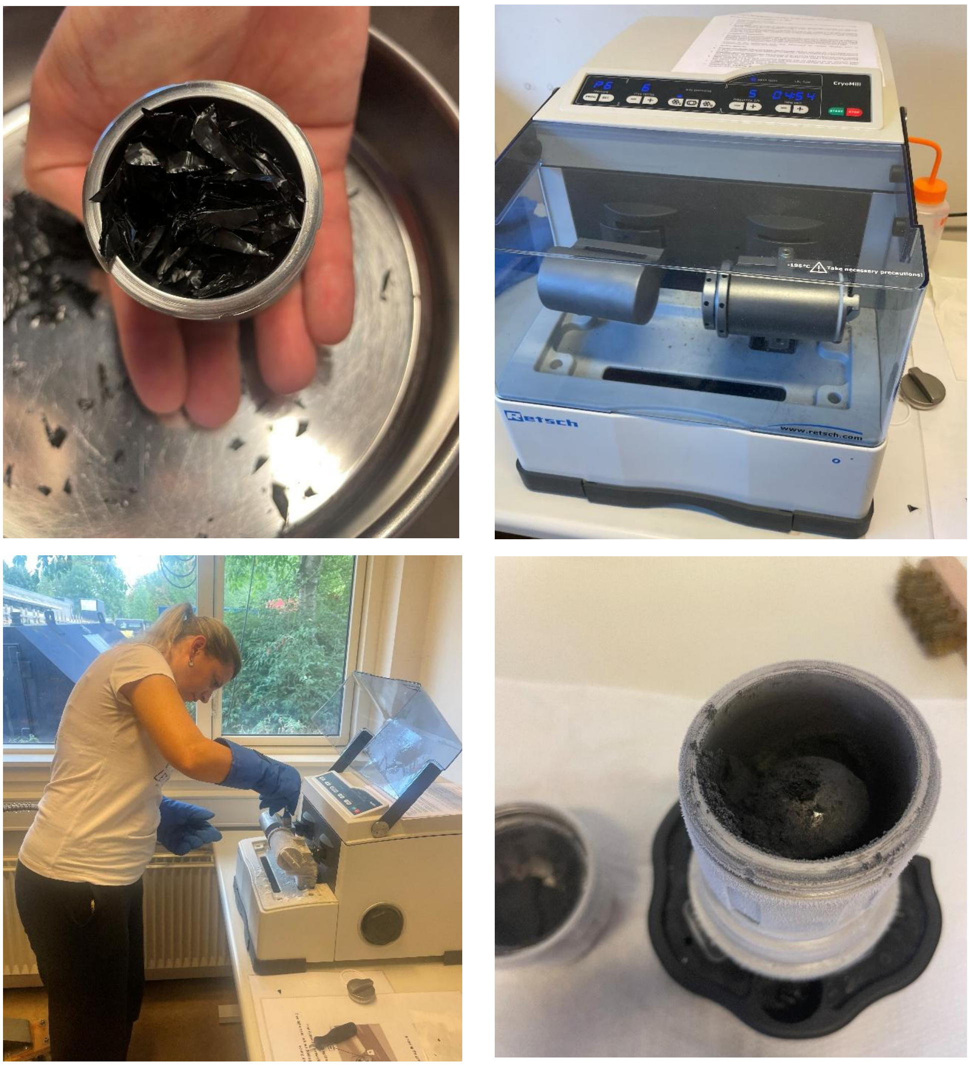

Figure 3: Cryo-milling of UV-weathered biodegradable mulching films using Retsch CryoMill.

3.1.3. Sieving

Separation and collection of ‘model’ MPs by sieving:

- Resulting ‘model’ MPs are stored in amber glass vials and kept away from direct sunlight to minimize the effects of UV photo-oxidation.
- Following the cryo-milling, the bulk cryo-mill material is fractionated using stainless-steel sieves (125 µm and 63 µm) in a vibratory sieve shaker to obtain three different size fractions: > 125 µm, 63-125 µm, and < 63 µm (Figure 4).

The size fraction < 63 µm, comprising MPs and potentially a fraction of nanoplastics (NPs), is classified as the 'model' MPs and will be used in other work packages (WPs) for subsequent experimental work and analysis.

Figure 4: Sieving of cryo-milled UV-weathered biodegradable mulching film.

3.2. Characterization requirements for 'model' microplastics

Method: Hands-on training and discussions

Description:
This activity aimed to enhance participants' understanding of key characterization techniques for 'model' MPs, focusing on mass quantification, size distribution, and chemical composition analysis. The training emphasized how to select appropriate analytical approaches depending on sample type, size range, and material composition.

The following characterizations are recommended for the produced 'model' MPs:

Mass quantification:

- Weigh all collected size fractions after sieving.
- Determine the total mass of 'model' MPs (< 63 µm) generated per cryo-milling cycle.
- Calculate the production yield of 'model' MPs relative to the input material loaded into the cryo-mill.

Chemical characterization:

- Perform FTIR analysis before and after accelerated weathering and cryo-milling to verify polymer identity and detect potential chemical alterations.
- Compare spectra to reference materials to confirm polymer type and assess the extent of degradation.

Size distribution:

- Assess the particle size distribution across fractions using sieving, Coulter counter, or imaging methods, such as laser direct infrared (LDIR) analysis, µ-Raman spectroscopy, and quantum cascade laser micro-infrared (QCL-µIR) spectroscopy.

Morphological characterization:

- Examine particle shape, surface features, and fragmentation patterns using optical microscopy or scanning electron microscopy (SEM).

Thermal analysis:

- Conduct differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) before and after accelerated weathering and cryo-milling to evaluate changes in thermal stability, crystallinity, and decomposition profiles resulting from accelerated weathering or mechanical processing.

Together, these characterization steps will provide a comprehensive understanding of the physical, chemical, and surface properties of the produced 'model' MPs, thereby supporting their application in subsequent project activities, including capacity building activities and studies on their uptake by hydroponically grown strawberries.

3.2.1. Characterization results

During the training, participants discussed key characterization requirements for 'model' MPs and performed selected analyses. The remaining analyses listed in Section 3.2 will be implemented within the corresponding project activities where relevant. Quantitative and chemical characterization results obtained during this training are summarized below.

Mass quantification

Loading 3.73 g of UV-weathered biodegradable mulching film material into the cryo-mill yielded a total of 1.93 g across all particle size fractions, corresponding to an overall recovery of 51.7 % (Table 2). Only particles in the < 63 µm fraction, encompassing both MPs and possibly NPs, were designated as 'model' MPs. Based on Table 2, a single cryo-milling procedure of 3.73 g UV-weathered biodegradable mulching film yields a recovery of 2.88 % for particles < 63 µm. Larger fractions can be re-milled to generate additional particles in the < 63 µm range if needed.

Table 2: Distribution of particle size fractions (g) obtained from 3.73 g of UV-weathered biodegradable mulching film loaded into the cryo-mill.

Particle size	Weight (g)
< 63 µm	0.108
63-125 µm	1.44
> 125 µm	0.378
Total weight (g)	1.93

Chemical characterization

Before and after cryo-milling, the plastic was analyzed using FTIR in the wavenumber range from 4000 cm^{-1}to 400 cm^{-1} (Figure 5). Data was collected at a spectral resolution of 4 cm^{-1} and processed and visualized using the Spectragryph software.

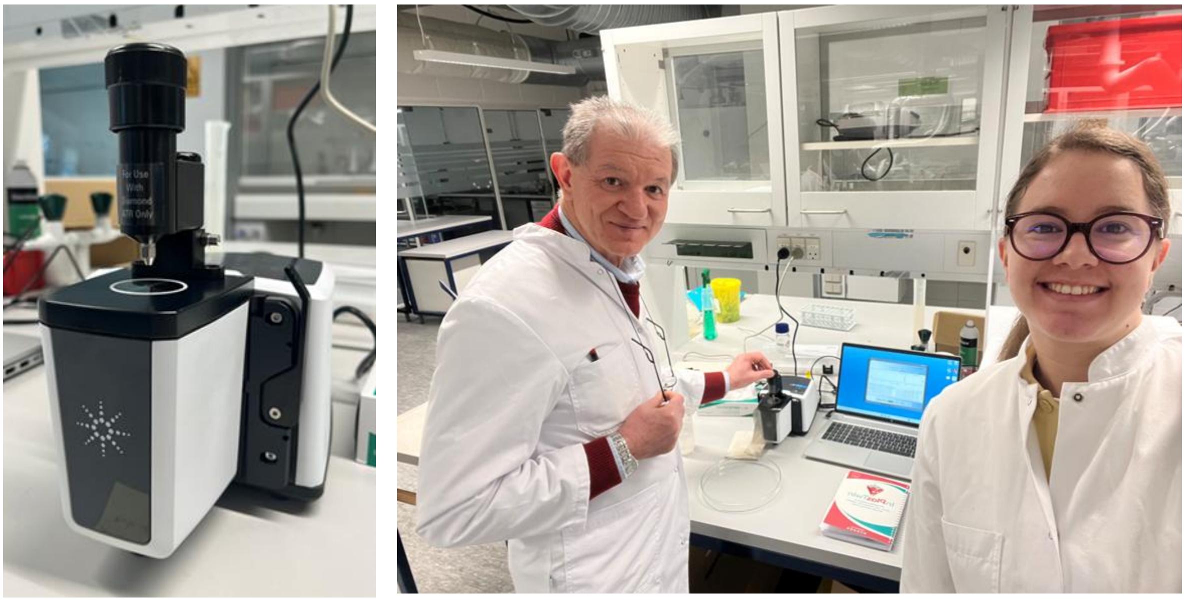

Figure 5: FTIR instrument (left) and FTIR analysis of plastic mulching films in progress (right).

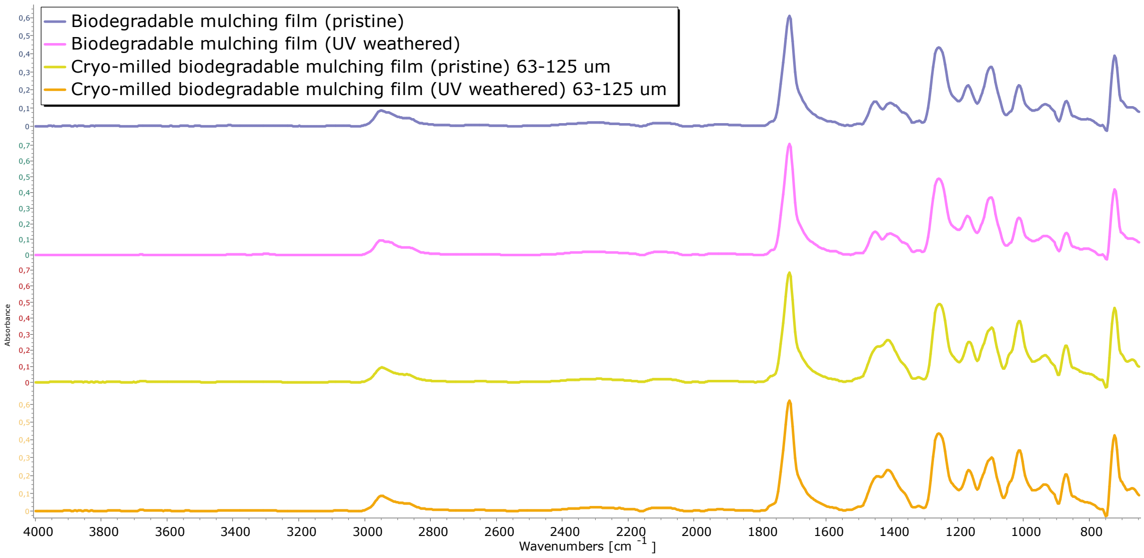

Figure 6: FTIR spectra of biodegradable mulching film, from top to bottom: pristine film, UV-weathered film, cryo-milled film (pristine) 63-125 µm, cryo-milled film (UV-weathered) 63-125 µm.

Figure 6 shows that cryo-milling of biodegradable mulching films slightly modifies the shape and intensity of the FTIR bands at around 1410 and 1450 cm^{-1}, corresponding to carbon-hydrogen (C-H) bending, and increases the intensity of the band at around 1010 cm^{-1}, associated with carbon-oxygen (C-O) stretching, which may indicate photo-oxidation. The observed spectral differences between the cryo-milled and non-cryo-milled films could also result from the inherent heterogeneity of the pristine test material, which is a blend of two polymers (Figure 1). Fragmentation during cryo-milling likely produced particles originating from both the surface and interior of the film, which may differ in chemical composition. Furthermore, Figure 6 shows that accelerated weathering of the material, in both forms – the film and the cryo-milled 63-125 µm fraction – results in minimal or no detectable changes in the FTIR spectra.

To further examine minor spectral differences and confirm the overall consistency across material forms – beyond what can be observed through visual interpretation of FTIR spectra – the spectral quality match index was obtained directly from the FTIR analysis by comparing the UV-weathered and cryo-milled biodegradable mulching films in the two size fractions (63-125 µm; Table 3 and > 125 µm; Table 4) with reference polymers from the spectral library.

Table 3: FTIR results showing the spectral quality match index of the UV-weathered and cryo-milled biodegradable mulching film (63–125 µm) with reference plastics in the spectral library.

Rank	Quality index	Name
1	0.99954	Pristine cryo-milled biodegradable mulching film (63-125 µm)
2	0.99944	UV-weathered + cryo-milled biodegradable mulching film (> 125 um)
3	0.99333	Pristine cryo-milled biodegradable mulching film (< 63 µm)
4	0.98091	Pristine biodegradable mulching film
5	0.97811	UV-weathered biodegradable mulching film

Table 4: FTIR results showing the spectral quality match index of the UV-weathered and cryo-milled biodegradable mulching film (> 125 µm) with reference plastics in the spectral library.

Rank	Quality index	Name
1	0.99962	UV-weathered cryo-milled biodegradable mulching film (63-125 µm)
2	0.99905	Pristine cryo-milled biodegradable mulching film (63-125 µm)
3	0.99446	Pristine cryo-milled biodegradable mulching film (< 63 µm)
4	0.98070	Pristine biodegradable mulching film
5	0.97698	UV-weathered biodegradable mulching film

Table 3 and Table 4 show that both size fractions of the UV-weathered and cryo-milled biodegradable mulching film (63–125 µm and > 125 µm) exhibit a high spectral match quality index (> 0.97) with reference spectra of the same material under different conditions, including pristine, UV-weathered, and cryo-milled forms. The consistently strong spectral similarity (quality index > 0.97) across all comparisons indicates that the chemical structure of the biodegradable mulching film remains largely unchanged after both UV-weathering and cryo-milling. Notably, the non-cryo-milled films exhibited the lowest spectral match percentages when compared with the UV-weathered and cryo-milled samples, further confirming that some structural modifications of the polymers occurred as a result of the cryo-milling process.

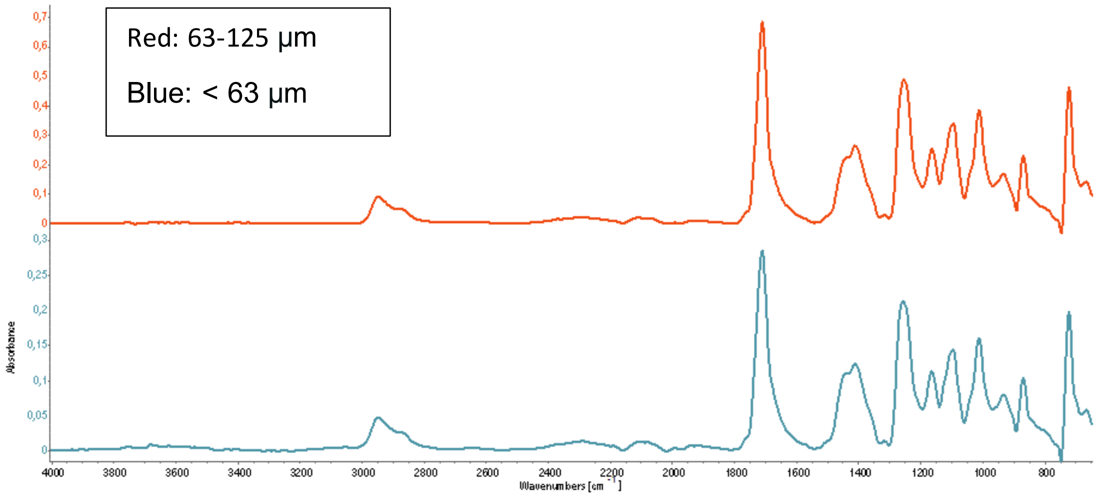

Figure 7: FTIR spectra of cryo-milled biodegradable mulching film (pristine).

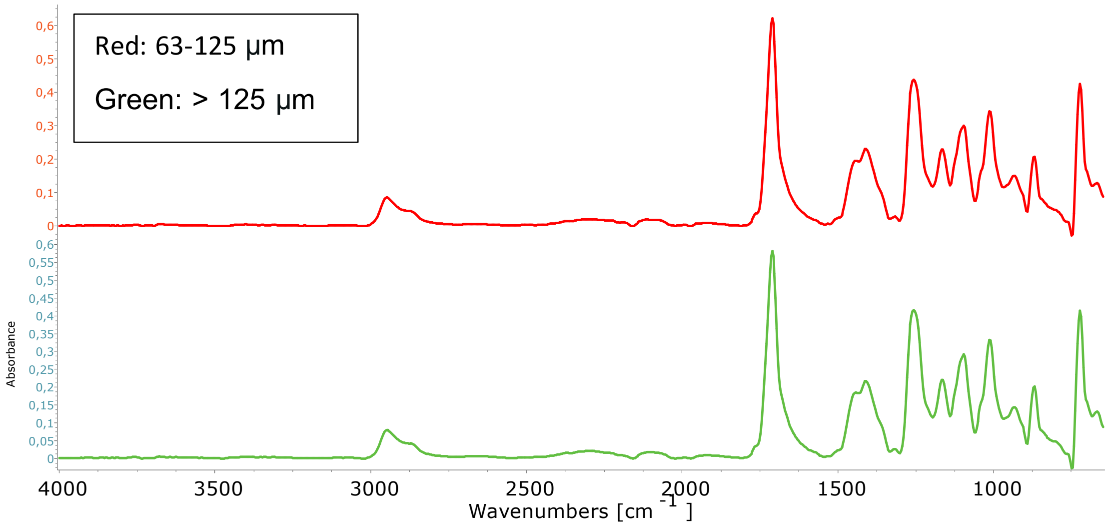

Figure 8: FTIR spectra of cryo-milled biodegradable mulching film (UV-weathered).

The FTIR spectra of the cryo-milled pristine biodegradable mulching film (Figure 7) showed no significant differences between the 63–125 µm and <63 µm fractions. This indicates that cryo-milling reduced the particle size without altering the chemical structure of the polymer, confirming that the process preserved the material's molecular integrity. Similarly, the FTIR spectra of the UV-weathered cryo-milled biodegradable mulching film (Figure 8) showed consistent band positions and intensities for the 63-125 µm and > 125 µm fractions, suggesting that accelerated weathering did not result in additional polymer degradation or oxidation among the different size fractions.

The strong consistency between the two analyzed size fractions further suggests that the polymer chemistry is uniform across particle sizes. Accordingly, the < 63 µm UV-weathered and cryo-milled fraction – which has not yet been analyzed by FTIR – is also expected to show comparable spectral characteristics.

4. Training outcomes

The training produced several key outcomes:

- **Competence in plastic accelerated plastic weathering and MP generation:**
  Participants acquired practical skills in performing accelerated weathering and conducting cryo-milling of UV-weathered biodegradable plastics using Retsch CryoMill. Participants also gained proficiency in sieving techniques for particle size fractionation and handling of MP fractions.

- **Establishment of a reproducible protocol for “model” MP generation:**
  A standardized workflow was established for producing “model” MPs, including accelerated weathering, cryo-milling, sieving, and storage procedures. The workflow ensures reproducibility of particle size fractions and supports consistent preparation of materials needed for subsequent WPs.

- **Strengthened analytical capacity in MP characterization:**
  Participants received training in the use of analytical instruments for chemical characterization of MPs by using FTIR analysis.

- **Qualitative data interpretation skills:**
  The training enhanced participants’ abilities to interpret spectral data by comparing FTIR spectra before and after UV-weathering and cryo-milling. They were also trained to use the Spectragryph software for FTIR data processing and visualization.

- **Capacity building and knowledge transfer:**
  The training enabled direct knowledge exchange between the host and visiting researchers, strengthening the participants’ competencies in MP generation and analysis. The acquired skills enhance the long-term research capacity of their home institutions and reinforce ongoing collaboration within the InPlasTwin project.

- **Strengthened overall researcher well-being and networking:**
  Beyond technical skills, the training fostered inclusion and collaboration. The integration of participants into the Plastics research group created a supportive and motivating work environment for professional development. Informal interactions and joint activities with DTU Sustain researchers further expanded networking opportunities, facilitating exchange of ideas and future research discussions within and beyond the InPlasTwin consortium.

5. Conclusions

Biodegradable mulching films that are not fully degraded can generate MNPs, which may contribute to soil contamination and enter food chains, raising concerns for ecosystems and human health. Realistic assessment of their environmental fate and behavior requires standardized laboratory methodologies capable of producing environmentally relevant MNPs that closely resemble naturally aged particles.

The activities carried out under Task 3.2 successfully met their objectives, combining capacity building with the development of a robust protocol for generating 'model' MPs from biodegradable mulching films. Practical training on accelerated UV-weathering and cryo-milling provided researchers from JSI and AUA with hands-on experience in producing environmentally relevant MPs and performing their quantitative and chemical characterization using visual inspection and FTIR spectroscopy. Participants also gained an understanding of key characterization approaches for 'model' MPs, including particle quantification, size distribution, and chemical analysis.

The outcome of Task 3.2 is the establishment of a standardized and reproducible protocol for producing 'model' MPs of different size fractions, including accelerated weathering, cryo-milling, sieving, and storage procedures. FTIR analysis confirmed that this approach does not significantly alter the chemical structure of the original material, ensuring that the resulting 'model' MPs are representative of naturally aged particles. These materials can be reliably applied in subsequent project activities, including capacity building activities and investigations of MNP uptake and effects in hydroponically grown strawberries.

Overall, this work provides a solid methodological foundation for studying the generation of environmentally relevant biodegradable MPs, supplying a test material suitable for plant exposure studies. It also supports the InPlasTwin project's broader objectives of strengthening the research capacity of the consortium, advancing analytical expertise, and improving the scientific basis for environmental risk assessment and mitigation strategies related to plastic pollution in agriculture.

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