Completed chemical analysis from field plot experiments on plant growth

Milestone no 16.

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Field plot experiment

The PAPILLONS field trial was carried out in three different countries, representing different vegetation and climatic zones in Europe: Finland, Germany and Spain. At each site, 25 study plots of 3.5 m x 4.5 m were established in an area of 51 m x 51 m.

Micronised pellets of recycled mulching film were used as test materials: conventional polyethylene (PE-MP; P3) and biodegradable PBAT-BD starch blend (PBAT-BD-MP; P4). The test materials were applied to the study plots at two estimated concentrations, both representing environmentally relevant concentrations in agricultural soils: approximately 0.005% and 0.05% dry weight. Each treatment was replicated five times. After being applied to the soil surface, the MP test materials were mixed into the top soil using a rotavator and malt barley was sown in the plots (Figure 1).

Figure 1. Field plot experiment with barley (Hordeum vulgare) in Jokionen, Finland. Photos: Sylwia Adamczyk

The aim of WP4 is to elucidate the effects of micro- and nanoplastics (MNPs) on plant seed germination and development, plant traits and environmental factors on plant growth and performance in representative European agro-ecological zones. The field experiment will validate MNP effects on plant production in the laboratory under field conditions and elucidate the potential effects of conventional and biodegradable plastics on the physiological and biochemical properties of barley under different climatic conditions (boreal, temperate and Mediterranean). The aim of the experiment was to provide a comprehensive picture of the effects and fate of microplastics and plastic additives in real, varying environmental conditions across Europe.

Table 1. Activities and progress of WP4 Field plot experiment analysis of plant-soil MNP interaction

Research question	MNP	Methods
Do the conventional (PE) and biodegradable (PBAT) plastics affect physiological properties of barley in the natural conditions?	PE, PBAT	**Physiological analyses:** biomass, specific leaf area, necrotic tissue, harvest index
Is there an effect of plastics on plant processes and is plastics induce oxidative stress reactions chain?	PE, PBAT	**Biochemical analyses:** - **from grains and leaves:** nitrogen (N) and carbon (C) content in the grains and leaves, - **from the leaves:** chlorophyll content, lipid peroxidation, total phenolic content, salicylic acid, sugars, proteins.
Can we observe plastic additives in grains?	PE, PBAT	**Plastic additives:** quantitative analysis of various plastic additives in grains

Plant samples and physiological analyses

During the growing season, barley was sampled at various stages of growth and the leaf area and necrotic tissue were measured (Figure 2).

Figure 2. Leaves of barley prepared for specific leaf and necrotic tissue analyses in different growing stage (GS) (left - GS32, middle - GS38, right - GS58). Photo: Sylwia Adamczyk

Biochemical indicators and plastic additives

In the end of the growing season plant material was sampled during daylight. The second-highest leaf (Figures 24-25) from 10 plants growing in a row, were selected randomly by sampling every second plant from that row. Altogether 20 leaves/plot were collected. The sampling was done in the same way from all 25 plots. The fresh weight of 20 leaves per plot was measured (g/plot). The lengths and widths of the leaves were measured in mm (the picture of all leaves with white paper and scale were taken). The dry mass of all 20 leaves were determined by drying them at 60 °C for 48 h in a paper bag (g/plot). The leaves samples were milled and C and N content were measured with an elemental CN analyzer.

After the last plant sampling, barley was harvested, and grain samples were collected to analyse yield, quality and plastic additives.

Samples for biochemical and toxicological analyses (lipid peroxidation, total phenolic content, and stress hormones; SA) and for chlorophyll analyses were collected at flag leaf stage (Figure 3).

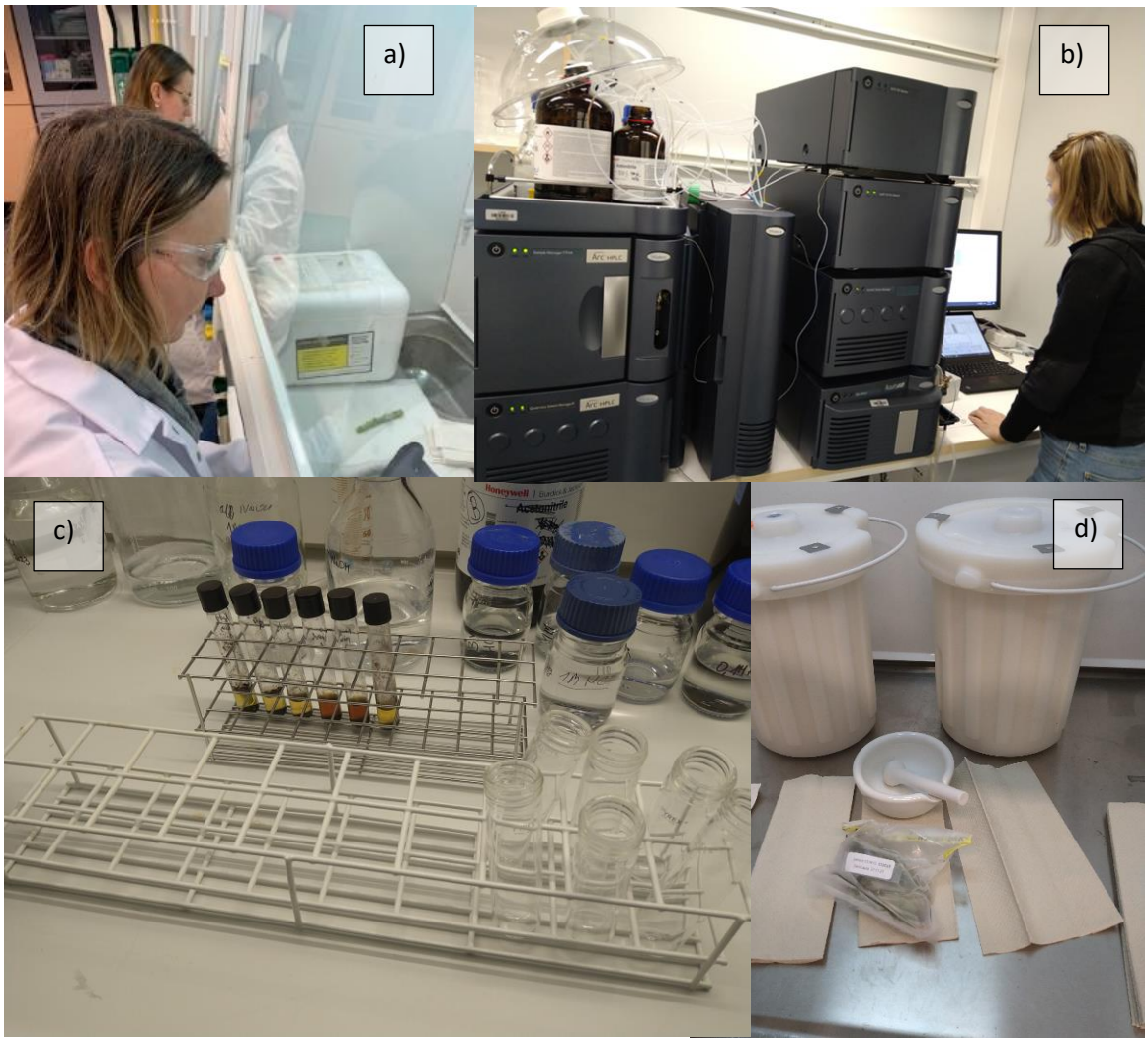

Figure 3. Analysing of samples from field experiment in Luke's laboratory, Helsinki. Preparing samples by grinding in liquid nitrogen, (a) in the front Sylwia Adamczyk. Photos: Sannakajsa Velmala and Sylwia Adamczyk

A team of researchers from UCT Prague has developed and validated an analytical method for determining plastic additives in barley grains. The method enables detection of 13 different plastic additives at very low concentrations, ranging from 1 to 10 ng/g. The analytical procedure involves a straightforward sample preparation using only small amount of sample (2.5 g) and employs advanced sensitive analytical instruments for measurement.

Despite some analytical challenges due to the complex nature of barley matrix, the method demonstrates reliable results with very good precision (repeatability < 20%) (Figure 4). Special attention needs to be paid during sample handling to prevent any background contamination from laboratory environment, e.g., use only plastic materials that do not contain any of the analytes. Overall, this analytical development represents an important contribution to food safety monitoring and environmental contamination assessment, allowing for reliable tracking of plastic additives in grain samples within field plot experiment.

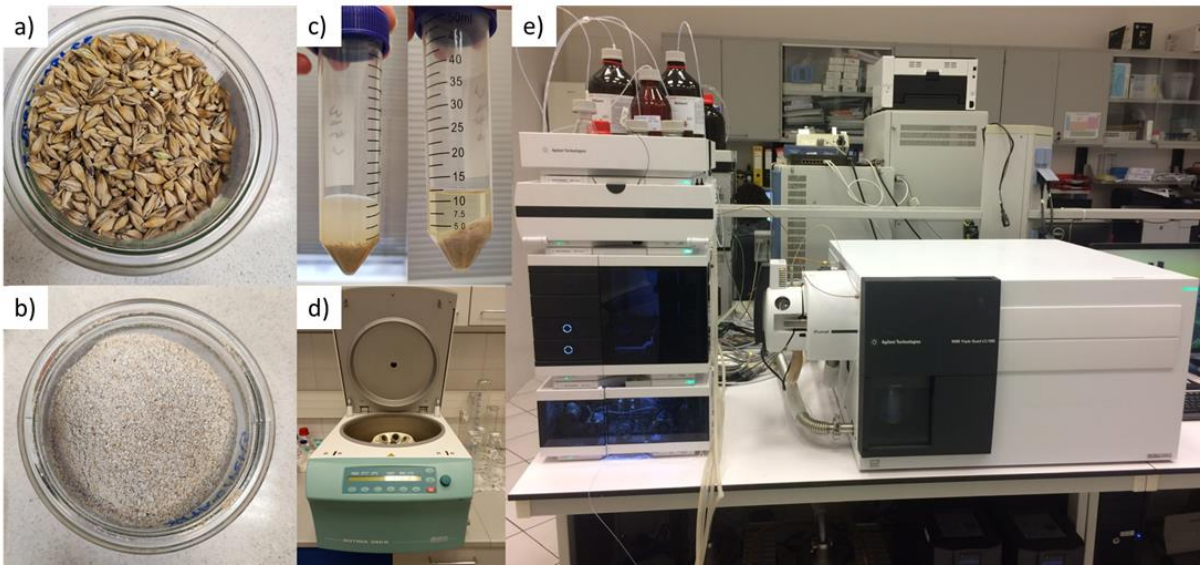

Figure 4. Analysing of grains from field experiment at the Department of Food Analysis and Nutrition, UCT Prague. a) barley grain delivered in laboratory, b) barley grain after homogenization, c) organic extract of sample after 30 min of ultrasound assisted extraction before and after centrifugation, d) refrigerated centrifuge, e) ultra-high performance liquid chromatograph with mass detector. Photos: Darina Dvorakova