

Trace metal metabolism in plants



Training school for working with metalloproteins



České Budějovice, 17-19 July 2023

Day 1

8:30 am (theory, all together in IPMB boardroom)

- Welcome (Hendrik, Filis, Marie-Theres)
- Photosynthesis: role of metalloproteins, theory of Chl fluorescence kinetics (Filis)
- Safety measures and good laboratory practice in the PPB lab and overall introduction to lab facilities during centrifugation (Hendrik)

10.30 am (lab, 2 parallel groups)

- Group I extraction of proteins for analytical HPLC-ICP/MS and spectrophotometric analyses (Bojan, Marie-Theres) –includes 1 centrifugation step of 2h.
- Group II Photosynthesis: Measurements of Chl fluorescence kinetics (Filis); micro-X-ray Fluorescence (μ XRF) method for element visualization: sample mounting and start of measurement (Hendrik)

Approx. 12.30 am: Lunch in the IPMB canteen

1.30 pm (lab / office , 2 parallel groups)

- Group I: starting the analyses of soluble proteins (Nadeem) and spectrophotometric measurements (Marie-Theres)
- Group II Analyses of photosynthesis imaging measurements

3 pm coffee break in the IPMB boardroom

3.30 pm IPM boardroom (lab, 2 parallel groups))

- Group I: theory: Work with metalloproteins: expression, isolation, purification and characterization by biochemical assays and metalloproteomics (Hendrik)
- Group II PME assay: Start of incubation (Marie-Theres)

approx... 17.30 end of work day

Day 2

8:30 (Theory, all together in IPMB boardroom)

- Basics of metalloprotein biochemistry (Hendrik)
- Metal-protein prediction programs and (Marie-Theres)

10:30 am (lab, 2 parallel groups)

- Group II extraction of proteins for analytical HPLC-ICP/MS (Bojan) and spectrophotometric analyses (Marie-Theres) – includes 1 centrifugation step of 2h.) + few minutes for destaining the PME assay
- Group I: Photosynthesis: measurements of Chl fluorescence kinetics (Filis) and microXRF method for element visualization- sample mounting and start of measurement (Hendrik)

ca 12.00 am: Lunch in the IPMB canteen

1.00 pm (lab)

- Group I Photosynthesis measurements (Filis): Analyses of photosynthesis imaging measurements
- Group II: starting the analyses of soluble proteins (Nadeem) and spectrophotometric measurements

3 pm coffee break, Discussion in the IPMB boardroom

3.30 pm (lab, 2 parallel groups)

- Group I: PME assay: Start of incubation (Marie-Theres)
- Group II: theory: Work with metalloproteins: expression, isolation, purification and characterisation by biochemical assays and metalloproteomics (Hendrik)

Ca 18.30 Joined dinner in town (place to be selected)

Day 3

8:30 (Theory, all together in IPMB boardroom)

- Theory of X-ray emission spectroscopy (EDX, PIXE, μ XRF) for analysing element distribution in biological samples and X-ray absorption spectroscopy (XANES, EXAFS) for analysing biological metal complexes (Hendrik)

10 am (office/lab, 2 parallel groups)

- Group I: Analysis of metalloproteomics results (Filis) and μ XRF maps (Hendrik) + few minutes for destaining the PME assay
- Group II Analysis of Spectrophotometric assays (Marie-Theres) + analysis of the PME assay

ca 12.00 am: Lunch in the BC canteen

1.00 pm (Office, 2 parallel groups)

- Group I: Analyses of Spectrophotometric and PME assays (Marie-Theres)
- Group (II) Analysis of metalloproteomics results (Filis) and micro-XRF images (Hendrik)

3 pm coffee break and discussion in IPMB boardroom

3:30 – 17:00: Talks (all together, in IPMB boardroom)

- Mechanisms of metal deficiency & toxicity (Hendrik),
- Metal sensing of the cell wall (Marie-Theres)

End of the school

