Minutes – Uptake "kick-off" meeting – 19/10/2022

Present:

JSI: Ester Heath (EH), David Heath (DH), Eirini Andreasidou (EA), Anja Vehar (AV), Ana Kovačič (AK)

BF: Nina Kacjan-Maršić (NKM), Špela Železnikar (ŠŽ), Jure Ferlin (JF)

ZF: Tjaša Griessler Bulc (TGB), Darja Istenič (DI)

NIHP: Urška Blaznik (UB)

-Round table presentation

-Presentation of objectives of the project

-Presentation of research partners and supporting research partners

-Presentation of co-financing partners (all associated with wastewater treatment plants). Municipality of Krsko required involvement of their WWTP and include their wastewater and surface waters used for irrigation for analysis (suitable for irrigation of not)

-Presentation of tasks for each WP

WP2: JSI except microbiology and microplastics (ZF)

-Presentation of CEC included in exp part

WP3: Experimental issues, performed already (BF)

WP4: Quality attributes, to be decided between JSI and BF later

WP5: Modelling (use the measurements from experiments to relate uptake with different parameters and characteristics of compounds)

WP6: Risk assessment (taking place at the end of the project); Hazard assessment should be performed earlier as it might be hard to find data for all the compounds. Compounds are already selected and available from WP2, thus they can already be provided to UB to proceed with HA. Risk assessment and exposure assessment based both on Slovenian and EU data.

WP7: Project Dissemination – Additional presentation to the public of Municipality of Krško

-Presentation of experiments

<u>Pot experiments</u>: 4 months of growing, presentation of different treatments included, explanation of how sludge was treated before application, presentation of problems regarding pots, presentation of available samples (tomatoes and plant parts, soil, water). Intermediate on the level of healthy plants and problems presented. Addition of fertilizer also in sludge pots but only to I and III so we do not interrupt all of them.

<u>Hydroponics experiments</u>: Experiments finalized after first month. Explanation of why plants were decaying (increased CEC spiking level and extreme heat) and what was the problems and steps. Presentation of samples (tomatoes and plant parts). available and water samples that were collected (before and after due to evaporation and plant uptake). Explanation of later addition of 3 extra tubs

with lower concentration. Microbiological parameters were not sampled and all harvested fruits were green.

<u>Lysimeters experiments</u>: Explanation of replicates, short explanation of stability and refill every 3 weeks, issue with fertilizing: not dissolved when we sampled due to the fact they were at the surface while we used drip irrigation. However, plants generally remained healthy but we should take this in account for future experiments (e.g., add it manually).

-Questions after the presentation: No

-Miscellaneous

-Microplastics experiments (pots): grown in peat substrate nex to CEC pots: Franja who was in charge of the experiment was not able to finish the tests for microplastics in sludge and peat but TGB mentioned that a pH student (PhD on microplastics) will take over the experiment and process the samples. Environmental samples might be a challenge to analyze as ZF was working with spiked samples up to this point.

-Mention that a separate hydroponic experiment was performed by JSI-TES (Group for Trace element Speciation) with elemental tracers and nanoplastics, harvested even before us.

-JSI: Analysis of CEC (EA) and elements as they can come from WW (AV)

-Quality attributes: explanation of what was promised in the project application

NKM: In 2020 determination of: content of sugars and organic acids, content of ascorbic acid and vitamin C and carotenoids.

JSI: Attempt to determine: amino acids, lipids, fatty acids, aromas and polyphenols.

NKM: They can also analyze polyphenols, because JSI group was planning to send the samples to external partners from other projects due to the fact that they don't have an in-house method.

BF: Polyphenols as they have a method (to be confirmed), carotenoids, sugars, organic acids, ascorbic acid and vitamin C

-Soil analysis - Sludge: To be analyzed by Pedology Group (BF), samples are already collected. Discussed about the parameters determined in soil analysis: e.g. inclusion of heavy metals (SŽ) but they will be analyzed from JSI as soil samples were already collected for that purpose (multielement analysis including heavy metals).

-Soil samples were collected before mixing with new soil so we can possibly check also the old soil samples (2020) and compare also with data from cultivation of pumpkin (2019) to see if there is any trend to the accumulation of elements/ CEC/ microbiology to the soil after 3 seasons. Microbiology is planning to sample next spring (2023) before the start of season, include also CEC and elements! Lysimeters are covered now to prevent weed growing and should be opened and exposed to weathering (ŠŽ will organize this). During previous years they were also not covered. SŽ updated us after the meeting that lids will be removed in order to allow weathering of soil in lysimeters.

-NPK: NPK analysis in water from Hop Institute (cost 35e only for NPK, additional cost for Ca, Mg..) – Pedology performs NPK in soil, SŽ will check if this is available for water, otherwise Hmeljarski Institit will analyze and JSI will cover the costs. NHM also suggest to include analysis of Ca, Mg in S. ŠŽ updated us after the meeting that these analysies cannot bne done at BF.

-Separation of samples: Separation in 3 harvesting periods during 2020 did not show any statistically significant difference but this year we have different conditions. The separation for dividing won't have significant effect to the final number of samples so we agree to, again, divide the samples in 3 harvesting periods (except hydroponics where exp lasted only for a month).

-How are we going to present the problems that we faced: SZ: we should consider conditions we experienced as "normal" conditions in agricultural experiments.

-Repeat of hydroponics next year: different conditions for example bury the tubs-baths in soil, there is no greenhouse in BF with controlled temperature, CEC influenced obviously but even control plants were not as healthy as 2020. New greenhouse in use next year by NHM, still not controlled atmosphere, but tubs will be buried in ground (reduce water heating during summer)

Consider repeating hydroponics exp in 2023. NHM: let BF know before the end of 2022 so they can plan ahead.

-Toxicity tests to be performed: NKM – check of literature, if 2 pesticides are tested it's important to determine if you are looking at the germination stage or plants. We go with plants as we performed exposure to CEC after germination. Suggested - EH: exposure of plants separately to each compound and to mixture of compounds at concentration of 1 mg/L (as exp) and if possible at 0,1 mg/L (2020). Experiment to be implemented as a part of MSc student from BF (to be discussed further, planned to begin in March, 2023, but needed to be designed earlier)

-Sample analysis (tomato) for CEC: go ahead with pots and lysimeters, when comes to hydroponics we can create a composite sample and analyze it to see if any CECs are accumulated in tomatoes. Then decide how to proceed with this exp (repeat in Spring, 2023?).

-Discussion to use rockwool in hydroponics (NKM, TGB): it was suggested but there might be a problem of CEC adsorption so a separate test should be performed first before we perform the experiments. Suggestion of Nutrient Film Technique Hydroponics (NFT) use but need to be taken into account that tomatoes have long and strong roots.

-Regarding stems and leaves suggestion to first develop a method and then proceed with analysis to check if we need to take any measures for future experiments (plants were left drying for a long period and exposed to UV, next year freezing samples?)

-Samples to be divided between JSI and BF; 2020 samples were divided in half, JSI will make a plan as we need samples for CEC (TA & NTA), Elements, Quality Attributes and Aromas (non liofilised). Both BF and JSI have freeze dryers available. Check again if ZF needs additional tomato plants -NO. BF does not need plant parts (dried) for any analysis – only fruits. JSI will evaluate how much samples they need and then propose to BF the amounts that can be dedicated for their analysis. Frozen samples will be given to BF and every institution (BF and JSI) will prepare (lyophilize) samples for themselves.