Ljubljana, 7.12.2023

**UPTAKE project**

**MINUTES**

The 2nd annual meeting of the UPTAKE project (Agricultural reuse of wastewater and sewage sludge: Uptake and distribution of contaminants of emerging concern in tomato plant as a model) took place on 23rd November 2023, at 1 p.m. in “Sejna soba” at Jožef Stefan Institute (JSI), Jamova cesta 39, Ljubljana, Slovenia.

Present research partners:

JSI: Eirini Andreasidou, Ester Heath, David Heath, Nadja Hvala, Nives Ogrinc, Janez Ščančar, Anja Vehar, Janja Vidmar in Taja Verovšek

BF: Nina Kacjan Maršič in Špela Železnikar (BF)

ZF: Karmen Godič Torkar in Darja Istenič

FGG: Nataša Atanasova

Excused absent research partners:

JSI: Ana Kovačič (JSI), Juš Kocijan

ZF: Tjaša Griessler Bulc

NIJZ: Urška Blaznik

Project manager Prof. Dr. Ester Heath welcomed everyone, announced previously excused project members and briefly recaptured project work packages and objectives.

Each research partner then introduced themselves.

The following agenda was proposed and adopted:

1. Presentation and discussion of the results of the analysis of chemicals of emerging concern (CEC) by Eirini Andreasidou
2. Presentation and discussion by Anja Vehar of the results of food quality analysis performed by the Jožef Stefan Institute
3. Presentation and discussion by Špela Železnikar of the effects of contaminants on plant growth performed by the Biotechnical Faculty
4. Presentation and discussion by Karmen Torkar on microbiological analysis performed by the Faculty of Health Sciences
5. Presentation and discussion by Darja Istenič on analysis of microplastics performed by the Faculty of Health Sciences
6. Presentation and discussion by Janja Vidmar on analysis of elements and nanoplastics by Jožef Stefan Institute
7. A.O.B

**Ad1)**

Eirini Andreasidou (JSI) presented the experimental design for growing tomato plants (2020 and 2022 experiments), sampling procedures, analytical methods and results (stability data, CEC analysis of water for tomato irrigation, tomato plants and fruits). It has been shown that most CEC of interest are stable under tomato growing conditions, selected CEC enter tomato plants and fruits from irrigation water spiked with CEC, and that CEC at measured levels in tomato fruits do not pose a risk to human health. The data presented was related to the 2020 growing season. Future work was also highlighted, namely the development of analytical methods for determining CEC in soil and tomato roots and implementing a risk assessment for the 2022 experiment.

Following, toxicity experiments performed in 2023 were discussed. The problem of growing tomatoes was exposed and explained by the exposure of tomato plants to ethanol – a solvent used to prepare CEC standards, which were added to the irrigation water. Although this resolved during the last repetition of the experiment, there was a noticeable difference between the growing progress of plant replicates. As a result, the last experiment consisted of high standard deviations. Given these results, it was suggested that the toxicity test (hydroponics) should be repeated. Accordingly, a date for a separate meeting between JSI and BF project partners was set in January 2024.

**Ad2):**

Anja Vehar (JSI) presented analytical methods applied and results obtained for the assessment of fruit quality attributes for tomatoes grown using hydroponics and lysimeters in 2020. The analysis included elements, amino acids, polyphenols, carotenoids, fatty acids, volatile organic compounds (VOC), and stable isotopes. It has been shown that the levels of elements present in tomato fruits do not pose a risk to human health growing conditions (including media), and the addition of CEC in irrigation water affects the quality of tomato fruits in terms content of amino acids, polyphenols, carotenoids, fatty acids. In contrast, VOC content is only affected by growing conditions.

Future work was highlighted, namely data processing (machine learning, experiment 2020), analysis of 2022 samples. In addition, results on quality attributes parameters obtained at JSI and BF will be compared on separate meeting.

Three scientific publications are predicted to be published from this segment of research (CEC and 2022 experiment, quality attributes from 2020 and 2022 experiments).

**Ad3):**

Špela Železnikar (BF) supplement Eirini Andreasidou’s presentation (under point 1) with a detailed explanation of growing media and conditions (hydroponics, lysimeters, and pots) for the cultivation of tomato plants in 2020 and 2022. Additionally, toxicological experiment (2023), including different treatment of tomato plants, i.e., the addition of nutrient solution with different CEC concentrations, treated wastewater and treated wastewater with the addition of nutrients, was presented along with methods used to evaluate tomato plant health. It has been shown that CEC content (0.1 mg/L) promotes root growth (possibly due to the antimicrobial effect of individual CEC, e.g., antibiotics) even higher than the control replicate, while higher CEC concentration (1 mg/L) inhibits root growth.

The problem with the high variability of tomato plants was exposed, and repetition of the experiment was suggested for February/March 2024 (to avoid high summer temperatures). More replicates will be considered (at least five considering statistical requirements), but fewer concentrations are suggested to be tested (e.g. 0.1 and 1 mg/L). A new experiment with hydroponics during the summer of 2024 will be omitted for technical reasons. The JSI and BF project partners will organise a separate meeting in January 2024 to design the experiment (see point 1).

One scientific publication (BF, ZF and JSI) is predicted to be published on toxicity and one on tomato quality attributed from 2020 experiment (BF).

**Ad4):**

Karmen Godič Torkar (BF) presented results on the presence of microorganisms in potable and wastewater (alone and with added CEC and nutrients) used for irrigation in the 2022 and 2023 toxicity experiments and the associated tomato fruits; soil, sludge and tomato fruits in pot experiment and lysimeter (Ajdovščina) soil. It has been shown that the content of coliforms, *Salmonella* and *E. Colli* decreases in hydroponic experiments with increased addition of CEC, dry sludge has a lower concentration of microorganisms (coliforms and *E. Colli*) compared to wet sludge (due to thermal treatment), the concentration of microorganisms in soil was constant throughout the pot experiment, all obtained concentrations of microorganisms in tomato fruit samples were within EU directives and guidelines, indicating good tomato quality in terms of microbiology regardless of wastewater/sludge irrigation/fertilisation.

As future work, analysis of the presence of microorganisms will be included in the toxicological study performed in February/March 2024 (described under point 3), and the results will be published in a joint scientific publication with BF. In January 2024, a separate meeting between project partners IJS and BF will be organised to set up the experiment (see points 1 and 3), where ZF will also be present.

**Ad5):**

Darja Istenič (ZF) presented an analytical method (oil extraction) for the determination of microplastics in plant substrate (soil), sludge (untreated, treated) and fertilisers and the obtained results. Samples were collected from a parallel pot experiment. It has been shown that treated sludge had the highest number and diversity of microplastic particles, soil contained the lowest number of microplastic particles, and fertilisers were an additional source of microplastic in the experiment.

It was concluded that the analysis of tomato fruits for microplastics would not be done since microplastics are too large to enter the tomato plants/fruits.

One scientific publication is predicted from microplastic experiment in pots.

**Ad6):**

Janja Vidmar (JSI) presented experimental design (exposure of growing tomato plants to elements and polystyrene nanoplastics), analytical methods used to determine uptake and distribution of selected elements (Cr, Pb, Zn and Cd) and europium-doped polystyrene nanoplastics in tomato plants and fruits. It has been shown that the highest concentration of selected elements and nanoplastics was determined in tomato roots and the lowest in fruits. Zn and Cd were taken up more efficiently by the tomato plants than Cr and Pb, which could be explained by the different forms of the elements present in the nutrient solution. At a pH around 7, Cd and Zn are mainly present in the nutrient solution as relatively small positively charged Cd²⁺ and Zn²⁺ ions, while Cr and Pb are mainly in the form of larger neutral molecules Cr(OH)₃⁰ and PbCO₃⁰. The concentrations of Cd and Pb in tomato fruits do not pose a risk to human health, the nanoplastic reduced the uptake of Cr and Pb by tomato plants, most likely due to steric effects and/or promoted adsorption of Cr and Pb on the nanoplastics. However, the interpretation of the results must consider the state of tomato fruits (not fully mature).

Future work was highlighted: confirming the presence of nanoplastic by SEM analysis and performing modelling to predict maximum levels of selected elements in wastewater used for irrigation of tomato plants so that tomato fruits would still be safe to eat (in connection with Nadja Hvala - JSI and Urška Blaznik – NIJZ). Additionally, some suggestions were made for determining the content of elements and nanoplastics considering levels (e.g., separate analysis of leaves, growing at the bottom of the plant and growing on the top) and analysing soil samples.

Two scientific publications are predicted, one on europium-doped polystyrene nanoplastics and one on labelled element uptake.

**Ad7):**

It was concluded that Nadja Hvala (JSI) and Urška Blaznik (NIJZ) will present their results at the next meeting.

Meeting minutes and presentations represented at the 2nd annual UPTAKE meeting will be loaded on a common drive.

Present research partners agreed that good progress has been made within the UPTAKE project so far and that at least xx scientific publications are to be expected from common work.

Next meeting will take place in the Autumn 2024, when stakeholders will also be invited.

The meeting ended at 4 p.m.

 Project Coordinator

 Prof. Dr. Ester Heath