# UPTAKE

Agricultural reuse of wastewater and sewage sludge: uptake and distribution of emerging organic contaminants in plants

WP 1 - Project management

## (BIO)ANALYTICS
Contaminants of Emerging Concern (CECs)

- Compound selection
- Analytical methods
- Sample analysis
- Microbiological evaluation
- Sensors supporting measurements

WP 2

## EXPERIMENTS

Sludge fertiliser

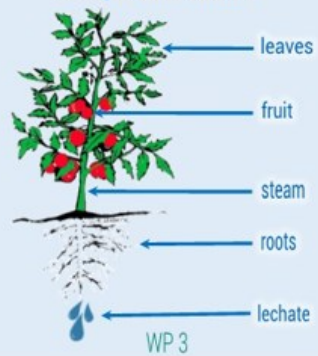
Pot experiment

Irrigation with wastewater

Hydroponic experiments

Lysimeter experiments

Sample collection




WP 3

## MODELLING
plant uptake

WP 4

## EFFECTS ON FRUIT QUALITY
effects of wastewater and sludge on fruit quality attributes

WP 5

## RISK ASSESSMENT
health and environment

WP 6

## IMPACTS

| Agriculture | Recyling | Policy |
|---|---|---|
| Holistic approach for wastewater and sludge reuse | Circular economy | Socio-economic acceptance |
| Soil fertilisation and stimulation of plant growth | Water shortage management | Development of EU regulation on wastewater and treated sludge reuse |
| Health saftey and environmental safety | | |
| Alternative water source | Treated sludge management | Environmental resilience |

WP 7 - Dissemination and knowledge brokerage

(agriculture, water-managmnet organisation, waste water treatment plants, technology providers, reserch centres, public administration, policy makers, CAP RDP measures, The European Green Deal)

UPTAKE

Agricultural reuse of wastewater and sewage sludge: uptake and distribution of emerging organic contaminants in plants

**_UPTAKE_ research objectives are to:**

- Develop analytical workflows (target, suspect and non-target analysis) for the determination of CEC residues in different compartments (soil, plant, aq. phase)
- Evaluate the distribution of CEC and their residues between plant tissues, soil and water in the presence of simulated and real TWW and TS
- Include micro/nanoplastics and faecal markers among the parameters investigated
- Model plant uptake under different experimental conditions
- Evaluate the effects of TWW and TS on fruit quality attributes
- Assess the risks to human health of eating plants irrigated with treated TWW or when grown in TS amended soil
- Disseminate results to relevant stakeholders, scientific community and public

UPTAKE

Agricultural reuse of wastewater and sewage sludge: uptake and distribution of emerging organic contaminants in plants

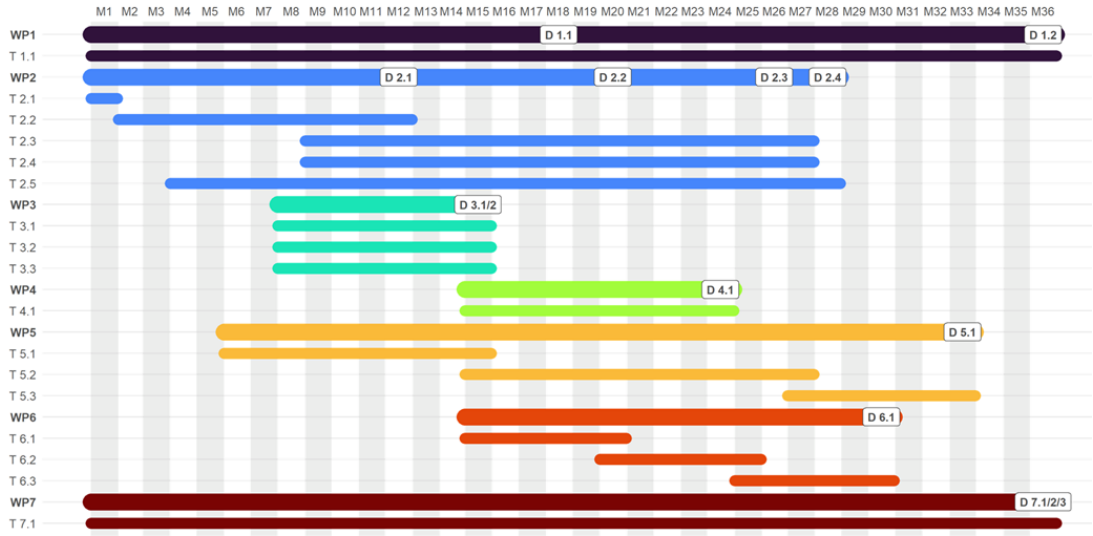
| RESEARCH PROJECT PARTNER |
| --- |
| **JSI-O2** (Jožef Stefan Institute-JSI, Department of Environmental Sciences) |
| **JSI-E2** (JSI, Department of Systems and Control) |
| **UL-BF** (Biotechnical Faculty, University of Ljubljana) |
| **UL-ZF** (Faculty for Health Sciences, University of Ljubljana) |
| **UL-FGG** (Faculty of Civil Engineering, University of Ljubljana) |
| **NIHP** (National Institute of Public Health) |

| |
| --- |
| Department of Civil and Environmental Engineering, University of Cyprus, Nicosia, Cyprus (Prof. Dr Despo Fatta, **UC**) |
| Department of Soil Science, Agricultural Research Institute Cyprus, Nicosia, Cyprus (Dr Anastasis Christou, **ARIC**) |
| La Sapienza University, Faculty of Mathematical, Physical and Natural Sciences, Department of Chemistry, Rome, Italy (Prof Dr Alessandra Gentili, **UR**) |
| National Institute for Agricultural and Food Research and Technology, Madrid, Spain (Dr Dolores Hernando, CSIC) |
| The Aristotle University of Thessaloniki, Department of Chemistry, Environmental Pollution Control Laboratory, Thessaloniki, Greece (Dr Dimitra Lambropoulou, **AUTH**) |
| The University of Antwerpen, Department of Pharmaceutical Sciences, Antwerpen, Belgium (Prof Dr Adrian Covaci, **UAnt**) |
| University of Ankara, Faculty of Pharmacy, Department of Analytical Chemistry (Prof Dr Sibel A. Ozkan, **UAnk**) |
| The Hebrew University of Jerusalem, Rehovot, Israel (Prof Dr Benny Chefetz, **HUJI**) |

| CO-FINANCING PROJECT PARTNER/*stakeholder |
| --- |
| Centralna čistilna naprava Domžale Kamnik, d.o.o. (Dr Marjeta Stražar, director, **WRRF DK**) |
| Komunalno stanovanjska družba d. o. o. Ajdovščina (Nataša Uršič Praček, **WRRF Ajd**) |
| Komunala Novo mesto d.o.o. (Bojan Kekec, director, **WRRF NM**) |
| Komunalno podjetje Velenje d.o.o. (Nataša Uranjek Ževart, **WRRF VE**) |
| Komunala Javno podjetje d.o.o., Murska Sobota (Tomislav Zrinski, director, **WRRF MS**) |
| Municipality of Krško (Mayor Miran Stanko, Rafael Jurečič, MOK) |
| Komunala Kranj, JP, Kranj (Lucija Janeš, **WRRF KR**) |
| Chamber of Commerce and Industry in Slovenia (Sebastjan Rozman, **CCIS**) |
| Association of Municipality and Towns of Slovenia, (Jasmina Vidmar, **AMTS**) |

**The project is divided into seven thematic parts (work packages, WP):**

- **WP 1:** PROJECT MANAGEMENT
- **WP 2:** (BIO)ANALYTICAL ISSUES
- **WP 3:** EXPERIMENTAL ISSUES
- **WP 4:** EFFECTS OF WASTEWATER/TREATED SLUDGE ON FRUIT QUALITY ATTRIBUTES
- **WP 5:** MODELLING CEC PLANT UPTAKE
- **WP 6:** RISK ASSESSMENT
- **WP 7:** DISSEMINATION



## WP 1 PROJECT MANAGEMENT (PM 1-36)

- T (Task) 1.1 Project management (PM 1-36)

## WP 2 (BIO)ANALYTICAL ISSUES (PM 1-28)

T 2.1 Compound selection (PM 1)

T 2.2 Development of analytical methods for determining compounds of concern in different matrices (PM 2-12)

T 2.3 Sample analysis: compounds of interest in different matrices (PM 9-27)

T 2.4 Microbiological evaluation of WW, sludge, tomato fruits (PM 9-27)

T 2.5 Quantification of plastics in soil (MP) and plant (NP) (PM 4-28)

# CECs

| Emerging contaminants | CAS No | Molecular formula |
|---|---|---|
| **PPCPs** | | |
| ***Antibiotics*** | | |
| Amoxicillin | 26787-78-0 | $C_{16}H_{19}N_3O_5S$ |
| Azithromycin | 83905-01-5 | $C_{38}H_{72}N_2O_{12}$ |
| Ciprofloxacin | 85721-33-1 | $C_{17}H_{18}FN_3O_3$ |
| Clarithromycin | 81103-11-9 | $C_{38}H_{69}NO_{13}$ |
| Erythromycin | 114-07-8 | $C_{37}H_{67}NO_{13}$ |
| ***Pharmaceuticals*** | | |
| Diclofenac | 15307-86-5 | $C_{14}H_{11}Cl_2NO_2$ |
| Naproxen | 22204-53-1 | $C_{14}H_{14}O_3$ |
| Carbamazepine | 298-46-4 | $C_{15}H_{12}N_2O$ |
| Ibuprofen | 15687-27-1 | $C_{13}H_{18}O_2$ |
| ***EDCs*** | | |
| ß-estradiol | 17916-67-5 | $C_{18}H_{24}O_2$ |
| 17α-ethinylestadiol | 57-63-6 | $C_{20}H_{24}O_2$ |
| Estrone | 53-16-7 | $C_{18}H_{22}O_2$ |
| Progesterone | 57-83-0 | $C_{21}H_{30}O_2$ |
| Testosterone | 58-22-0 | $C_{19}H_{28}O_2$ |
| ***Industrial Chemicals*** | | |
| BPA | 80-05-7 | $C_{15}H_{16}O_2$ |
| BPS | 80-09-1 | $C_{12}H_{10}O_4S$ |
| BPAF | 1478-61-1 | $C_{15}H_{10}F_6O_2$ |
| 4,4-BPF | 620-92-8 | $C_{13}H_{12}O_2$ |
| ***Stimulants*** | | |
| Caffeine | 58-08-2 | $C_8H_{10}N_4O_2$ |
| Nicotine | 54-11-5 | $C_{10}H_{14}N_2$ |
| ***Antimicrobials*** | | |
| Triclosan | 3380-34-5 | $C_{12}H_7Cl_3O_2$ |
| Triclocarban | 101-20-2 | $C_{13}H_9Cl_3N_2O$ |
| ***Pesticides*** | | |
| Acetamiprid | 160430-64-8 | $C_{10}H_{11}ClN_4$ |
| Dimethomorph | 113210-97-2 | $C_{21}H_{22}ClNO_4$ |
| ***UVA/UVB filters*** | | |
| Benzophenone | 119-61-9 | $C_{13}H_{10}O$ |
| ***Parabens*** | | |
| Methylparaben | 99-76-3 | $C_8H_8O_3$ |
| Propylparaben | 94-13-3 | $C_{10}H_{12}O_3$ |
| ***Musks*** | | |
| Tonalide | 21145-77-7 | $C_{18}H_{26}O$ |

Final CEC conc.:

Aq. samples: 1mg/L

Soil samples: 12mg/kg

# WP 3 EXPERIMENTAL ISSUES (PM 8-15)

T 3.1 Pot experiments using activated sludge as fertiliser (EXP-1: PM 8-15):

T 3.2 Hydroponic experiments using wastewater (EXP-2: PM 8-15)

T 3.3 Lysimeter experiments using wastewater (EXP-3: PM 8-15)

*Table 1. Review of experiments in the UPTAKE project, with treatment remarks in brackets (* plant nutrients deriving from sludge and TWW will be taken into account in the total amount of fertiliser).*

| Type of experiment | | K | A | B | C |
|---|---|---|---|---|---|
| EXP-1 | **Pot** (irrigated with potable water) | Peat substrate fertilised with water-soluble fertiliser (WSF) (K1) | Peat substrate spiked with CEC and a fertiliser with WSF (A1) | Peat substrate fertilised with treated sludge* (B1) | Peat substrate fertilised with reated sludge spiked with CEC* (C1) |
| EXP-2 | **Hydroponic** | Nutrient solution by potable water (K2) | Nutrient soultion from potable water spiked with CEC (A2) | Nutrient solution prepared by TWW* (B2) | A nutrient solution prepared by TWW spiked with CEC* (C2) |
| EXP-3 | **Lysimeter** | Soil, irrigated with potable water (K3) | Soil, irrigated with potable water spiked with CEC (A3) | Soil, irrigated with TWW* (B3) | Soil, irrigated with TWW spiked with CEC* (C3) |

# WP 4 EFFECTS OF WASTEWATER/TREATED SLUDGE ON FRUIT QUALITY ATTRIBUTES (PM 15-24)

## T 4.1 Evaluation of CEC effects on plant fruits (PM 15-24)

Besides assessing the exposure, i.e. occurrence and concentration of the CEC and the consumed quantity of tomato fruit (T 6.2), fruit quality attributes including fruit mass and colour, the content of sugars, organic acids, vitamin C and carotenoids, antioxidant activity, amino acids, lipids, and proteins will be determined in tomatoes (Table 1, A, B, C). These parameters will be compared to the control group from the same experiments (Table 1, K). Parameters such as lipids, amino acids and proteins represent the primary inputs to the plant uptake model (WP 5).

**WP 5 MODELLING CEC PLANT UPTAKE (PM 6-33)**

T 5.1: Plant-uptake model design (PM 6-15)

T 5.2: Model parameterisation and calibration (PM 15-27)

T 5.3: Predictions with plant-uptake model (PM 27-33)

**WP 6 RISK ASSESSMENT (PM 15-30)**

T 6.1. Hazard assessment (PM 15-20)

T 6.2. Exposure assessment (PM 20-25)

T 6.3. Risk characterisation and uncertainty analysis (PM 25-30)

# WP 7 DISSEMINATION (PM 1-36

## T 7.1 Project dissemination activities

The goal is to:

i) Raise awareness among the relevant stakeholders and policymakers necessary to exploit the project results related to WW reuse and CEC distribution and uptake in plants.

ii) Share the project results with the academic and research community interested in the topic and stimulate further research.

iii) Disseminate information on planned and project activities and relevant results to the public and stakeholders, including policy makers through various media, e.g., open access scientific articles and presentations at conferences and presentations for wider public.

# Pot Experiments – Sludge Amendment

- Started: 19.05.2022, Finalized: 14.09.2022

- 8 different treatments with 5 replicates (C=peat substrate)
  - C, C+S1, C+S2, C+CEC1, C+CEC2, C+S1+CEC1, C+S2+CEC2
  - and C+S (1:1) (three replicates)

- SLUDGE AMENDMENT
  - S1: low sludge amount: N requirements
  - S2: covering K requirement

- CEC SPIKING:
  - Spiking performed either to sludge or peat substrate, by mixing stock solution with ethanol, at final conc of 12 mg/kg in sludge

- HARVESTING
  - First harvest: 8/7
  - After sample collection weekly

# Pot Experiments

Intermediate issues:

1. Ca deficiency by the end of June (25/06) causing tomatoes turning black
   - All plants treated with Ca foliar additive every 10 days, starting from 8/7
   - Additive was changed after 25/7/2022 to Calboron (30g/ 10L)

2. Infection of plants on 20/7/2022 (grey mole)
   - starting from C+CEC1/III and IV, followed by C/IV, C+SI/II, C+S2+CEC/V, C+S(1:1)/III on 9/8/2022

3. On 21/7/2022 additional fertilizing
   - added to all treatments without sludge and also to parallels I and III of treatments including sludge (theoretically: sludge should serve as a fertilizer)

4. Sampling from ZF (Microbiology)
   - on 21/7/2022 from C/II, C/IV,C/V, C+S1/II, C+S1/III, C+S1/V, C+S2/I, C+S2/III, C+S2/IV, C+S(1:1)/I, C+S(1:1)/II, C+S(1:1)/III

# Pot Experiments

| Treatment | Total | July | August | September |
|---|---|---|---|---|
| Controls (C) | 44 | 12 | 19 | 13 |
| Low addition of CEC (C + CEC1) | 45 | 12 | 21 | 12 |
| High addition of CEC (C + CEC2) | 69 | 10 | 35 | 24 |
| Low sludge amount (C + S1) | 30 | 6 | 12 | 12 |
| High sludge amount (C + S2) | 43 | 16 | 19 | 8 |
| Low sludge amount + CEC (C + S1 + CEC) | 31 | 12 | 11 | 8 |
| High sludge amount + CEC (C + S2 + CEC) | 41 | 14 | 22 | 5 |
| Mix of sludge and peat (C:S1:1) | 17 | 4 | 10 | 3 |

∑Tomatos= 320

3 harvesting time groups of tomatoes to analyse?

(in 2020 no diff in uptake, but in 2022 diff conditions)

# Pot exp: Set Up

# Pot exp – 2nd week



C + S (1:1)/II

# Pot exp – 4 week


C + S2/III


C + CEC1/V

# Pot exp – 6 & 7 week



# Pot exp – 8 week


C + S1 + CEC /IV


C + S1 + CEC /II

# Pot exp – 9 week

# Pot exp – 10 & 12 week




# Pot exp – 14 & 16 week

# Pot exp: Final

# Hydroponic Experiments – PW & WW

- EXP
  - Started: 17.05.2022
  - Finalized: 26.06.2022 & 15.07.2022
- 9 different treatments with triplicates (I, II and III; 4 plants in each tub, addition of nutrient solution) with:
  - Potable Water
  - Wastewater
  - Potable Water + CEC (1mg/L)
  - Wastewater + CEC (1 mg/L)
- pH and conductivity measured weekly
- pH adjustment in the tubs (BF)
- Water samples for chem analyses collected before and after each tub refill

# Hydroponic Experiments – PW & WW

Intermediate issues:

1. After 2 weeks (2/6/2022) noticed HR+CEC not growing

(yellow leaves and poor root system)

1. 9/06/2022: 3 more tubs added with HR+CEC spiked at a lower level (0.1 mg/L as in 2020), but no significant difference in growth

2. Potable waters tubs refilled twice a week after 15/6/2022 (due to fast growth)

3. Started finalizing on 26/06/2022 because some plants were already dead/dried (except two plants in each control – PW and lower concentration tubs planted on 9/6)

4. ZF (microbiological parametres) not sampled during finalizing the exp (JSI mistake and tomatos not ripe at harvest)

# Hydroponics

| Treatment | Total | Large | Medium | Small | Mini |
|---|---|---|---|---|---|
| Potable Water | 74 | 26 | 28 | 11 | 9 |
| Potable Water + CEC (1 mg/L) | 7 | - | - | 3 | 4 |
| Potable Water + CEC (0.1 mg/L) | 9 | - | - | 3 | 5 |
| Wastewater | 15 | 2 | 3 | 1 | 9 |
| Wastewater + CEC (1 mg/L) | 8 | - | 2 | 1 | 5 |

∑Samples (green) = 113

3 harvesting time groups of tomatoes not possible here…..

# Hydroponics - Water Samples

## 0.5 and 1L: CSIC, AUTH, JSI+NPK

| | 18/05/2022 | 31/05/2022 | 07/06/2022 | 26/06/2022 | 15/07/2022 |
|---|---|---|---|---|---|
| Potable Water | 4 | 1 | 1 | 3 | 4 |
| Potable Water + CEC | 5 | 1 | 1 | 3 | - |
| Wastewater | 5 | 1 | 1 | 3 | - |
| Wastewater + CEC | 5 | 1 | 1 | 3 | - |
| Potable Water + CEC Low | - | - | 3 | - | 4 |
| Total: 45 | | | | | |

Additional samples of 10 ml from tubs, before and after refill: 65

Additional samples of 500 ml from tubs during finalizing: 18

# Hydroponics – Set Up

# Hydroponics – 2nd Week

HR + CEC / I


HR + CEC / I

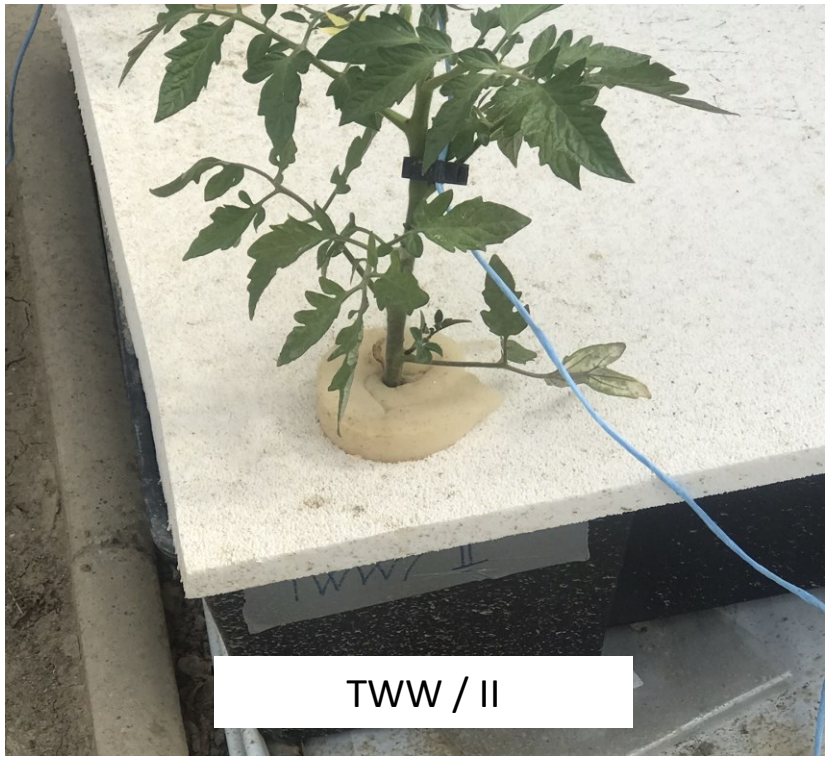
HR + CEC / II

# Hydroponics – 3rd Week



# Hydroponics – 4 & 5 Week

HR + CEC / II



TWW / II

HR + CEC - Low / I

Addition of 3 more tubs with HR+CEC spiked at a lower level (0.1 mg/L as 2020)

# Hydroponics – 6 & 7 Week

HR + CEC / I

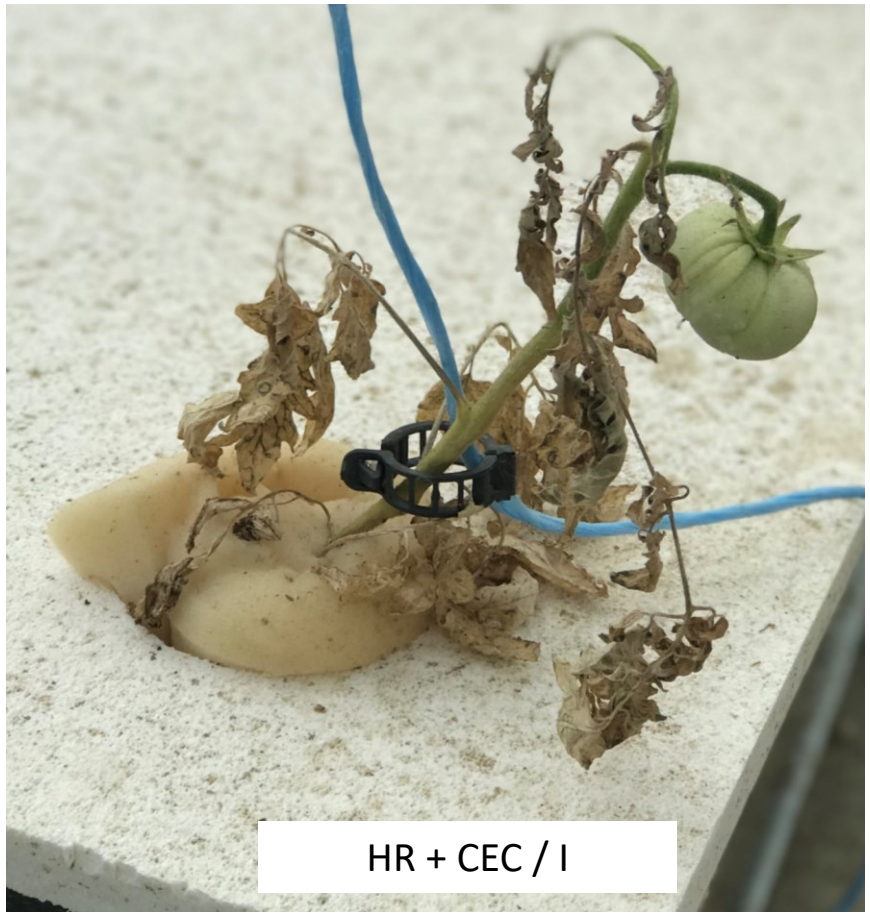
HR + CEC Low / I

TWW/ I

# Hydroponics – 6 & 7 Week

HR + CEC / III

TWW + CEC / III



HR + CEC / I

# Hydroponics – 8 Week


HR + CEC Low / III


HR + CEC Low / II


PW/ III

# Hydroponics – Final

# Lysimeter Experiments – PW & WW

- Started: 02.06.2022 - Finalized: 12.09.2022

- 4 different treatments with 7 replicates (except PW including 9 lysimeters), fertiliser added to soil day 0 and 14/7 (not dissolved at end of exp!!!):
  - Potable Water
  - Wastewater
  - Potable Water + CEC
  - Wastewater + CEC

- Sampling of water from cannisters every Friday (14 x 4x  10 mL samples)

# Lysimeters - Water Levels and Refills

| Report day | 2/6/2022 | 19/6/2022 | 8/7/2022 | 3 weeks refill | 12/8/2022 |
|---|---|---|---|---|---|
| Potable Water | 100% | 100% (18/6) | 100 (8/07??) | Drained, Refilled 100% | Refilled 100% (12/8) |
| Potable Water + CEC | 100% | 30% left Refilled 70% | 30% left, Refilled 70% | Drained, Refilled 50% | 20% left, Drained and refilled 50% |
| Wastewater | 100% | 25% left, Refilled 75% | 50% left, Refilled 50% | Drained, Refilled 50% | ??% left, Drained and refilled 50% |
| Wastewater + CEC | 100% | 60% left, Refilled 40% | 50% left, Refilled 50% | Drained, Refilled 50% | 20 % left, Frained and refilled to 50% |
| Refill | | 22/6/2022 | 14/7/2022 | 04/8/2022 | 15/8/2022 |

For 4/8 and 15/8 cannisters were emptied whole, washed and refilled again
14/7: extra fertilizer added to soil

# Lysimeters - Water Samples

## 0.5 and 1L: CSIC, AUTH, JSI

| | 02/06/2022 | 22/06/2022 | 14/07/2022 | 24/07/2022 | 04/08/2022 | 15/08/2022 | 12/09/2022 |
|---|---|---|---|---|---|---|---|
| Potable Water | 3 | 5 | 5 | 5 | 5 + 5 | 5 + 3 | 5 |
| Potable Water + CEC | 4 | 4 | 4 | 5 | 5 + 5 | 5 + 3 | 4 |
| Wastewater | 4 | 5 | 5 | 5 | 5 + 5 | 5 + 3 | 5 |
| Wastewater + CEC | 4 | 4 | 4 | 5 | 5 + 5 | 5 + 3 | 4 |
| Total: 161 | | | | | Before + After | Before + After | |

Additional samples of 10 ml from cannisters every week: 56

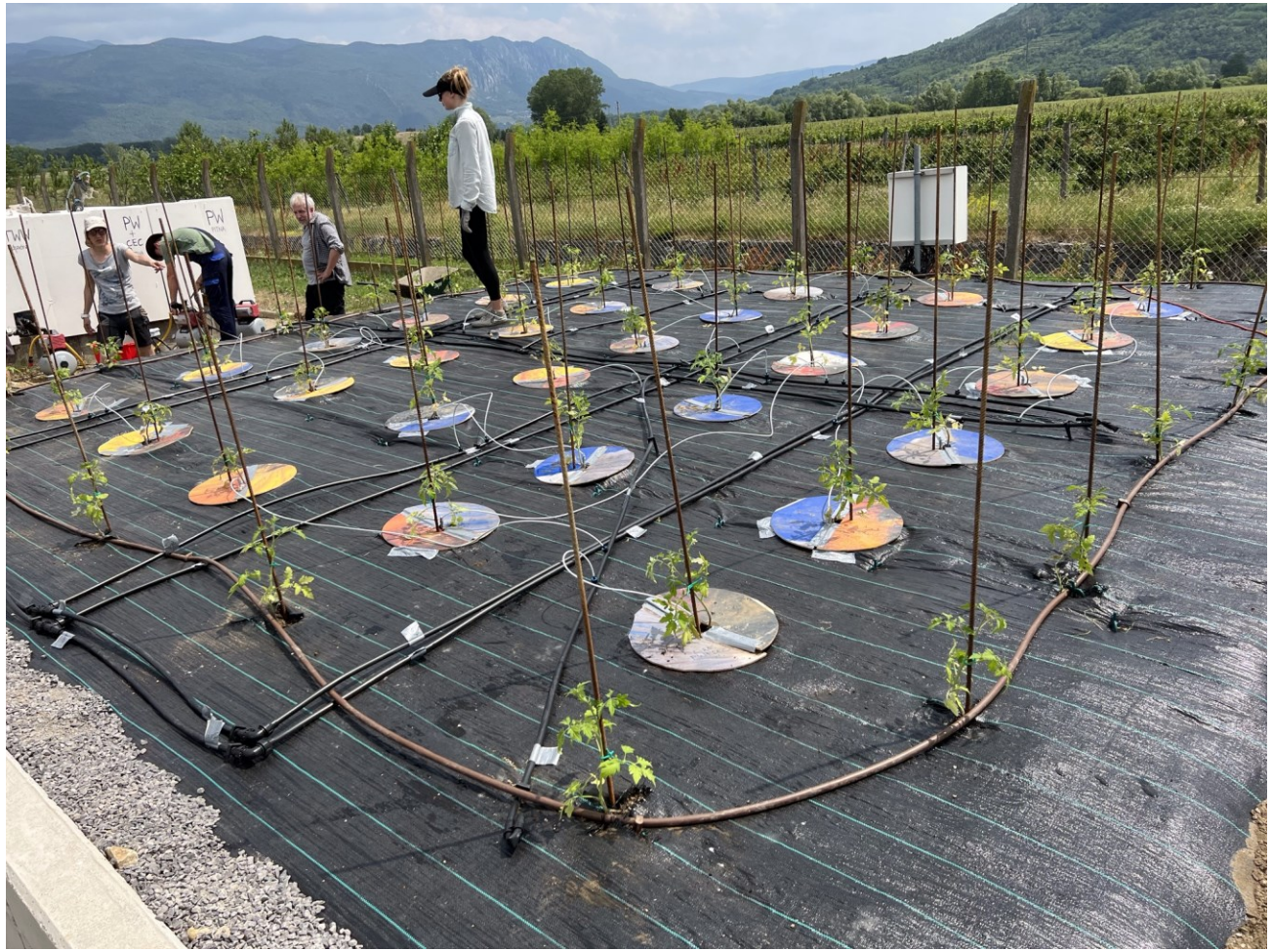
# Lysimeters

| Treatment | Total | First Sampling (14-29/7) | Intermediate Sampling (1-15/8 – 4) | Final Sampling (24.8 – 12.9 – 3) |
|---|---|---|---|---|
| Potable Water | 67 | 10 | 28 | 29 |
| Potable Water + CEC (1 mg/L) | 48 | 8 | 19 | 21 |
| Wastewater | 55 | 10 | 22 | 23 |
| Wastewater + CEC (1 mg/L) | 55 | 12 | 30 | 13 |
| Total | 225 | | | |

$\sum$ Samples = 225

3 harvesting time groups of tomatoes to analyse?

# Lysimeters – Set Up



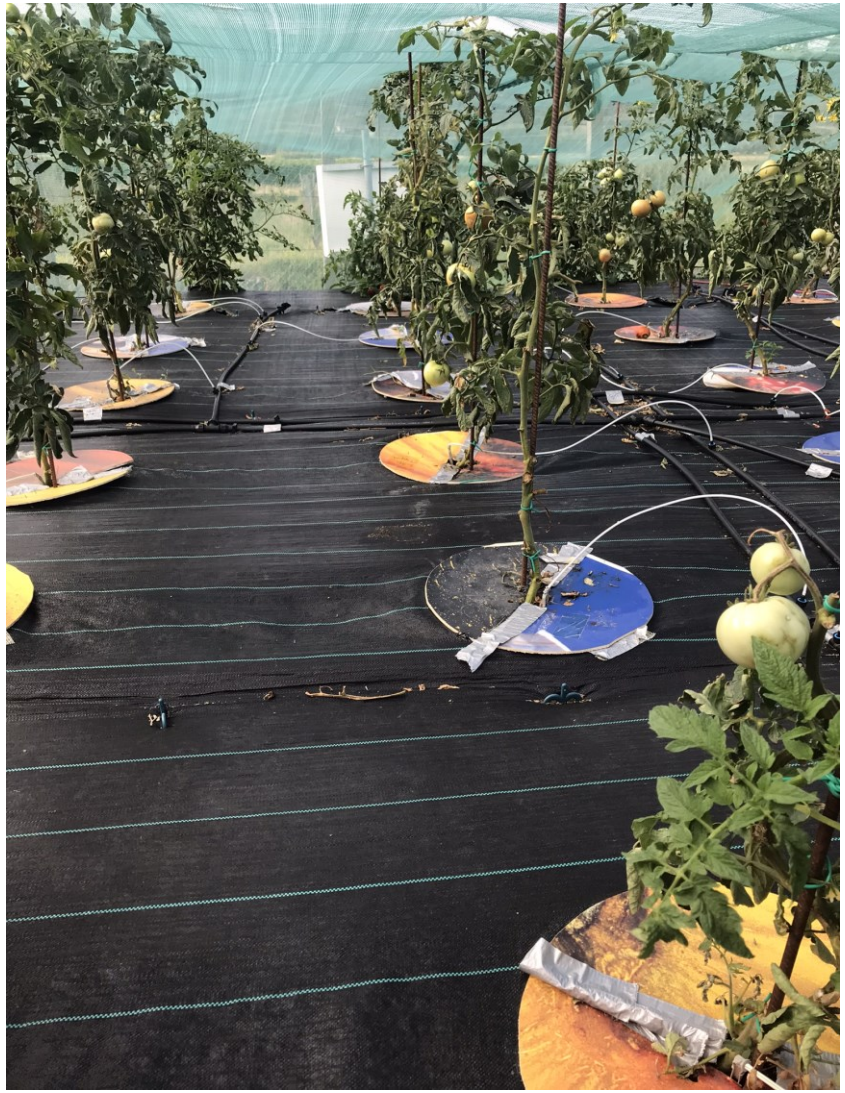
# Lysimeters - 3 week


# Lysimeters – 8 week

TWW + CEC

# Lysimeters – 9 week


# Lysimeters – 12 week



# Lysimeters - Final

# To discuss .....

- JSI:
- all matrices: CEC, elements
- Tomato fruit: amino acids, lipids, fatty acids, polyphenols, aroma (JSI)

Besides assessing the exposure, i.e. occurrence and concentration of the CEC and the consumed quantity of tomato fruit (T 6.2), fruit quality attributes including fruit mass and colour, the content of sugars, organic acids, vitamin C and carotenoids, antioxidant activity, amino acids, lipids, and proteins will be determined in tomatoes (Table 1, A, B, C). These parameters will be compared to the control group from the same experiments (Table 1, K). Parameters such as lipids, amino acids and proteins represent the primary inputs to the plant uptake model (WP 5).

- BF:
- Sludge/soil analysis (pedology group?)
- Quality of tomato fruit- parameters?
- Toxicity tests?

- ZF
- microbiological parameters in soil/sludge/tomatoes
- MP in sludge/soil before and after exp+after 1 year

- NPK in water samples? (Mg, Ca…?)
  - BF or Hmeljarski Institut?

TOMATOS

- How to divide tomato samples – in 3 picking up periods like in 2020?
- Amounts of tomato samples/parameter (JSI, BF (ZF sampled themselves)
- How to prepare tomato samples – fresh, liofilised for each partner?

PLANT PARTS and SOIL

- JSI: CEC and elem.
- BF?