



## WP4: Sustainable Water Re-use with Innovative Purification and Sensing system for the agri-food supply chain

Presenter Rim WERHENI AMMERI

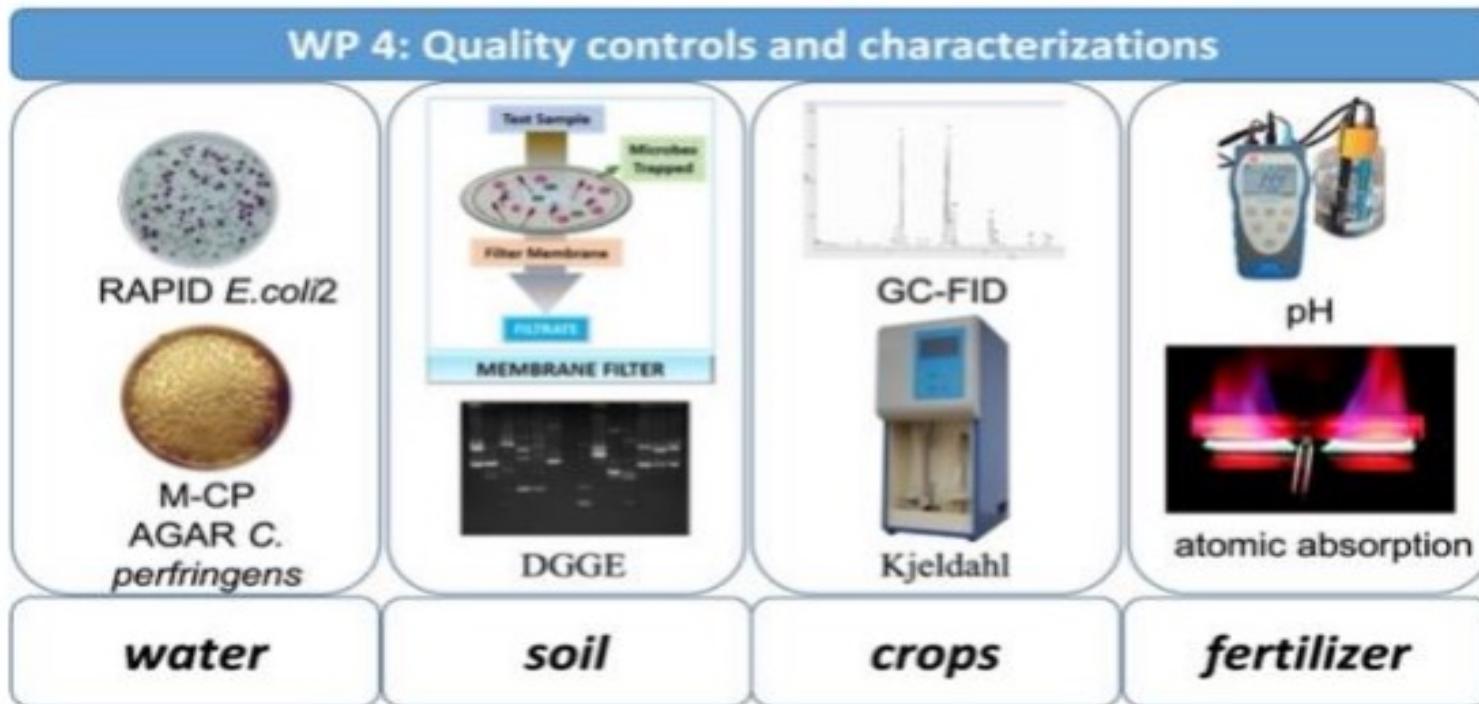
Second SWRIPS General Meeting

Palermo 07-08 October 2024



## WP4 aims

- ❑ Analyses of the products of the whole chain: water, sludge soil, crops to measure the effects produced by the system (WP4) in terms of environmental impacts (water and soil) and agriculture and human health impacts (crops).

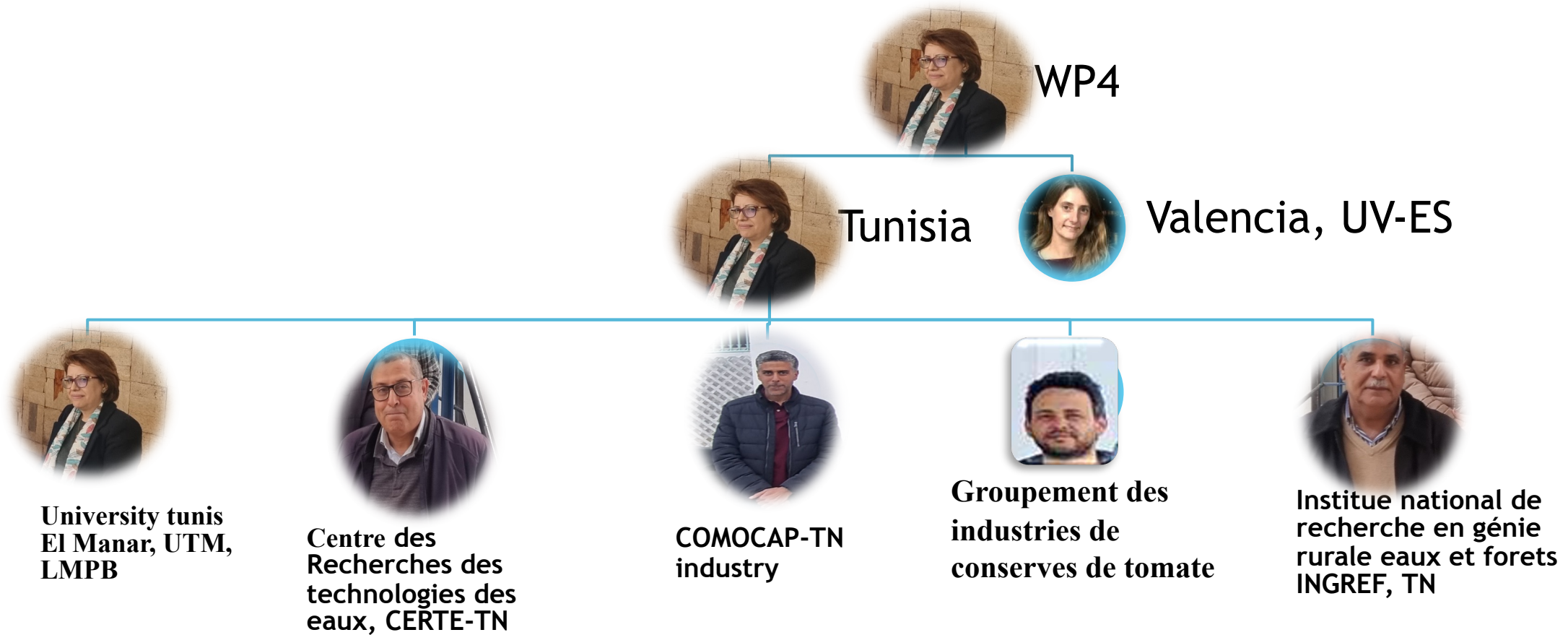


# WP4 Tasks

- ▶ Task 4.1 Microbiological water quality test (UNITU-TN, CERTE-TN, UV-ES) M16-M24
- ▶ Task 4.2 Soil Quality test (UV-ES, ENSA-DZ, UNITU-TN, INRGREF-TN, GICA-TN, ENSCR-FR) M16-M32
- ▶ Task 4.3 Crop Quality test (UV-ES, ENSA-DZ, UNITU-TN, GICA-TN, COMOCAP-TN, AGRUCOR-IT) M18-M36
- ▶ Task 4.4 Water characterization as Fertilizer (ENSA-DZ, UV-ES, UNIPA-IT, AGRUCOR-IT, INRGREF-TN, GICA-TN, ENSCR-FR) M18-M36
- ▶ Task 4.5 Sludge characterization and re-use as Fertilizer (UNIPA-IT, ENSA-DZ, UNITU-TN)- M16-M36

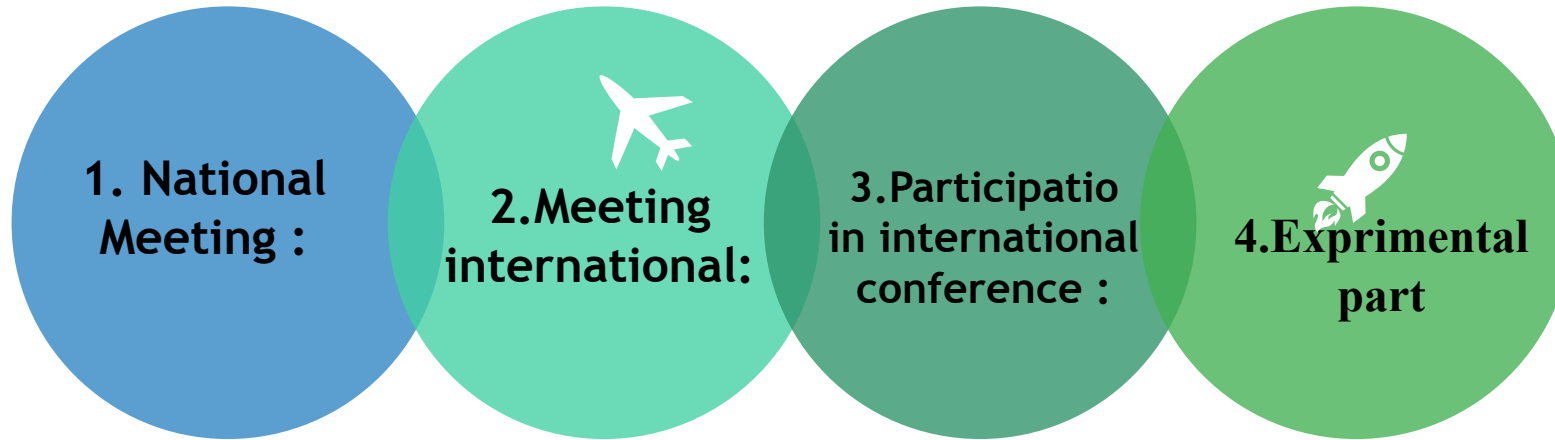


# WP 4 Participation





# WP4 Activity during the first year



01

National Meeting :

Ingref  
Certe  
UTM  
COMOCAP

02

Meeting international:

Catania (December 2023)

03

Participatio in international conference :

SIDISA 2024

04

Exprimental part



# Meeting

**CERTE**



**COMOCAP**



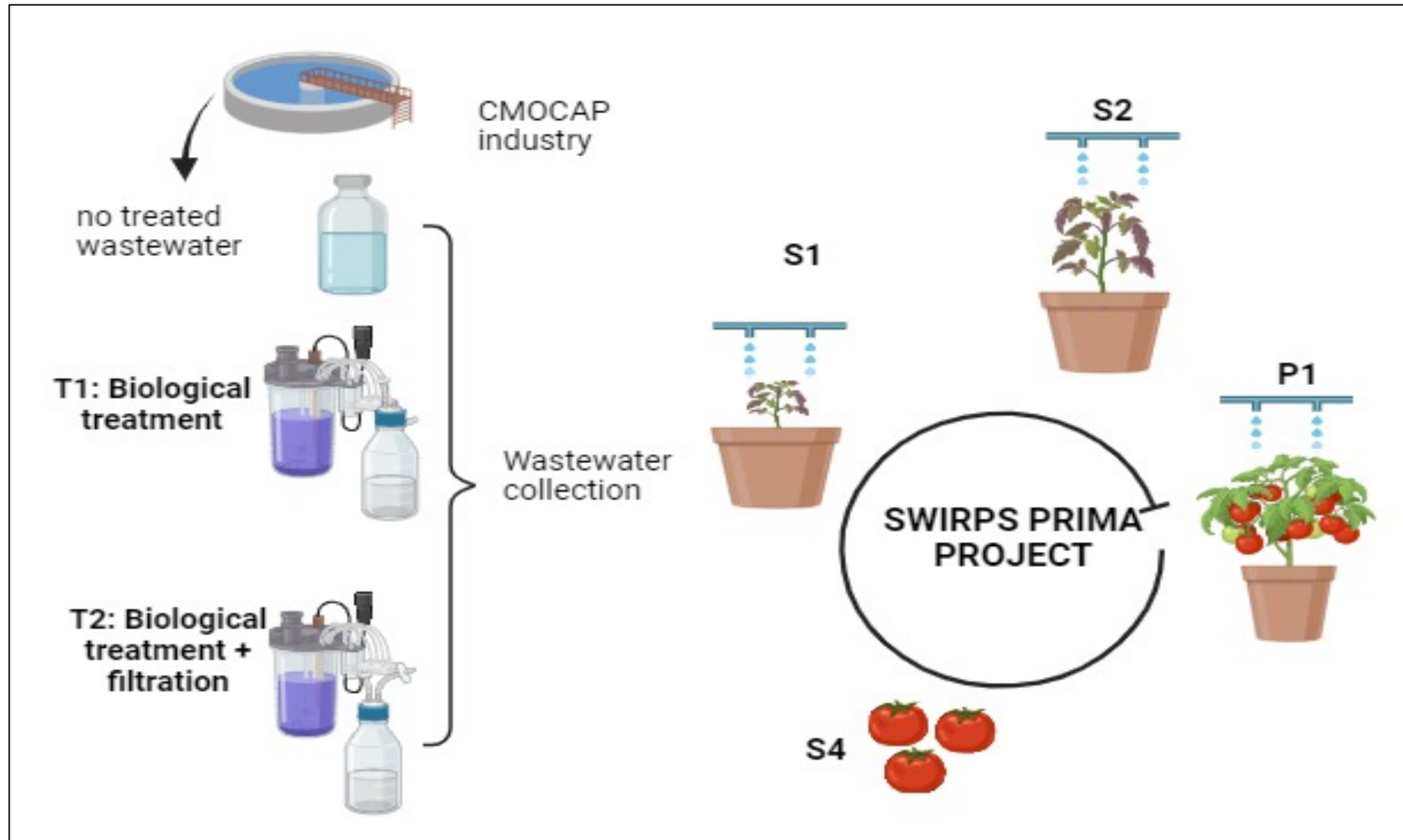
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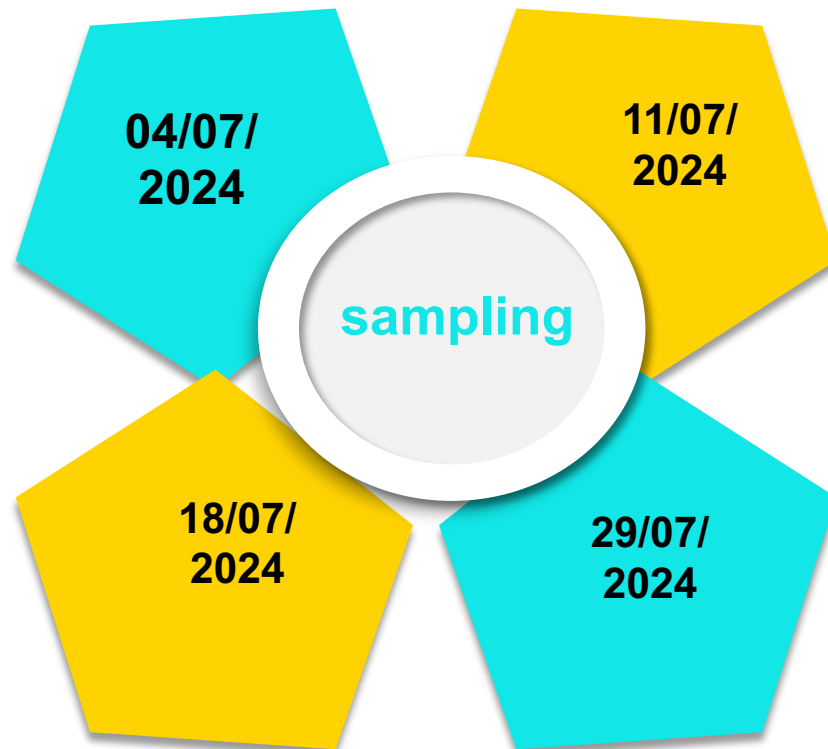
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# WP4 aims



## CERTE Participation

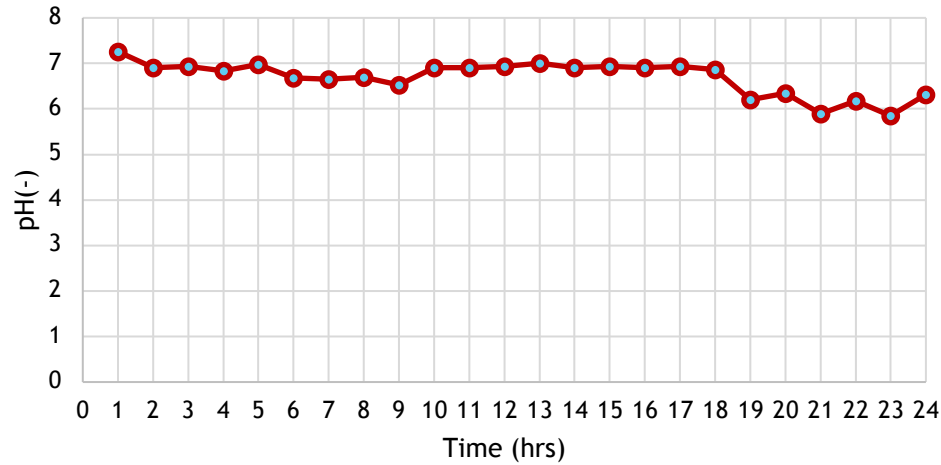


## Automatic sampling raw comocap wastewater

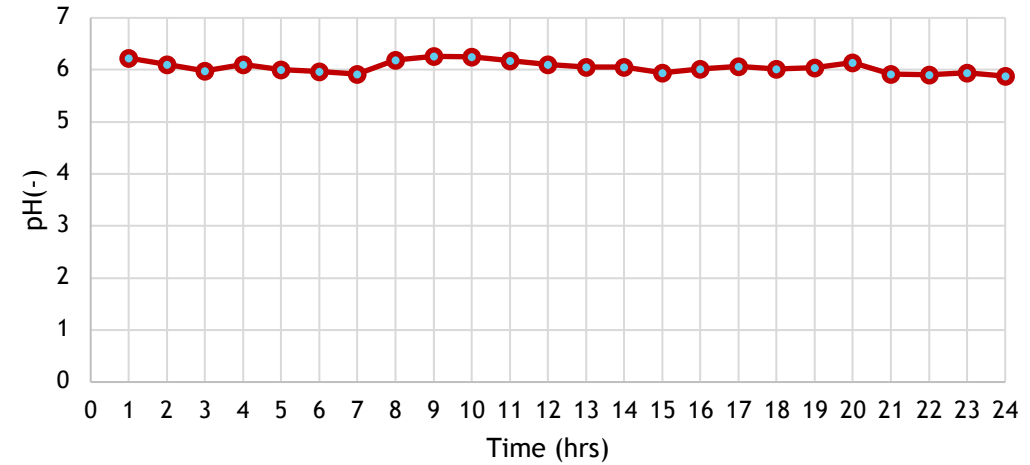


## □ Variation of comocap's raw wastewater within 24hours (four samples were taken)

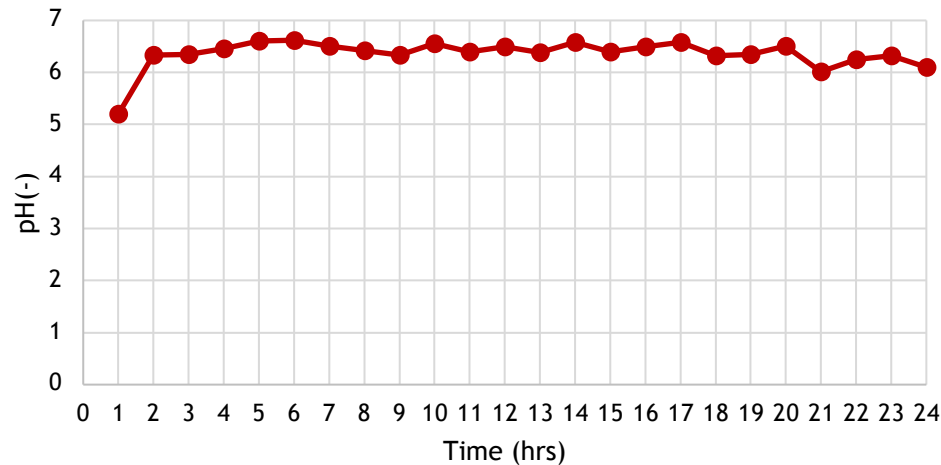
04/07/2024



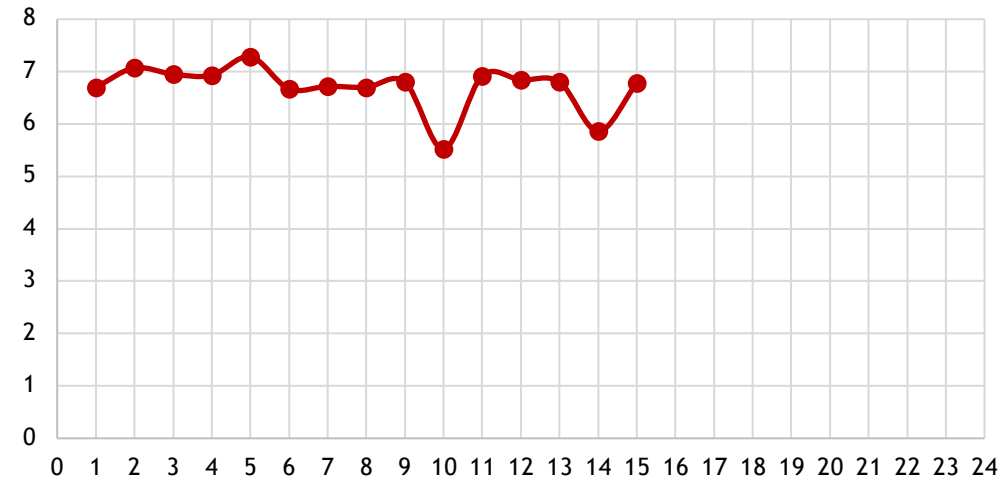
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18/07/2024



29/07/2024



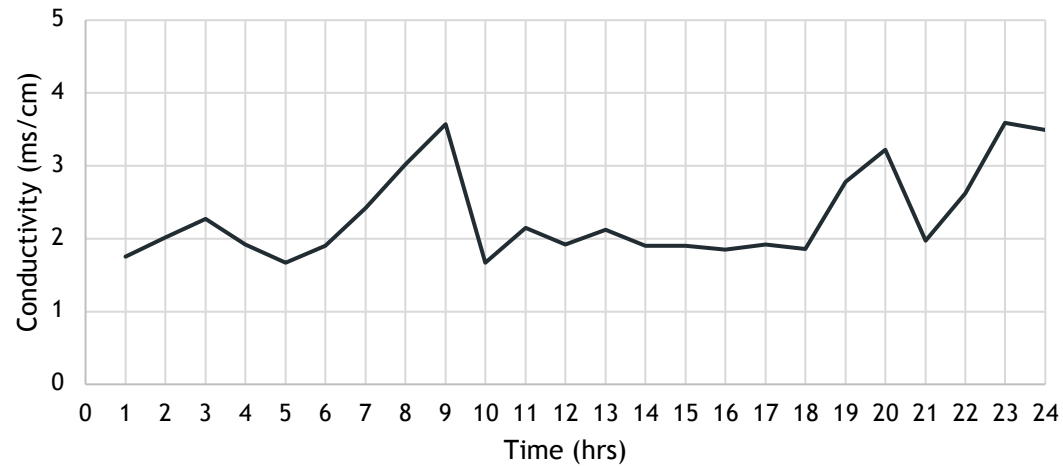
pH varied from 5,2 to 7,3



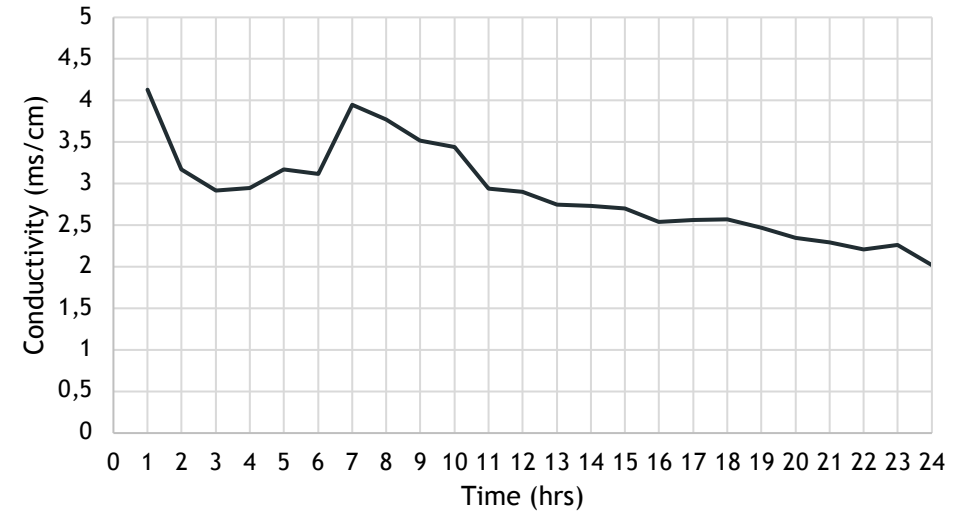


# □ conductivity variations of comocap's raw wastewater within 24hours (four samples were taken)

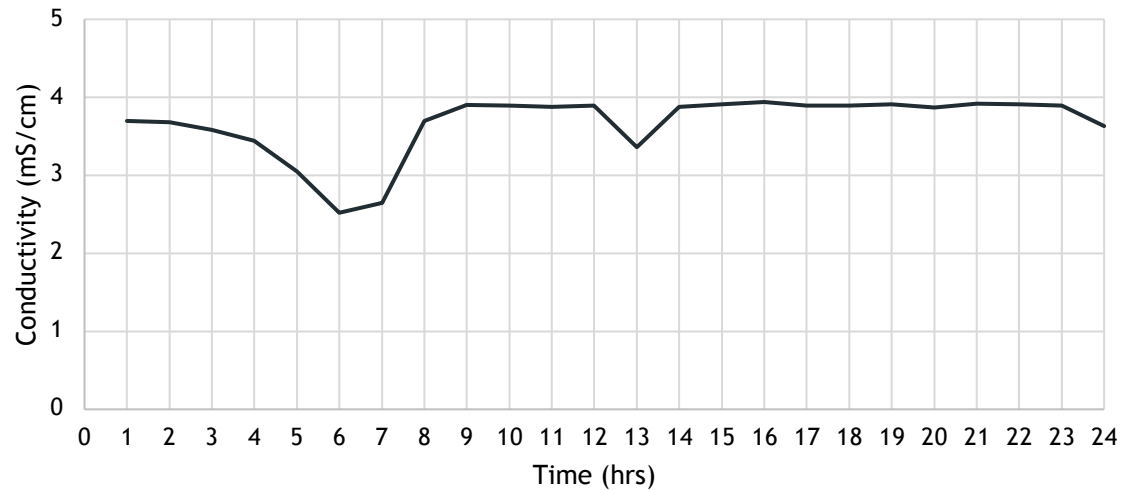
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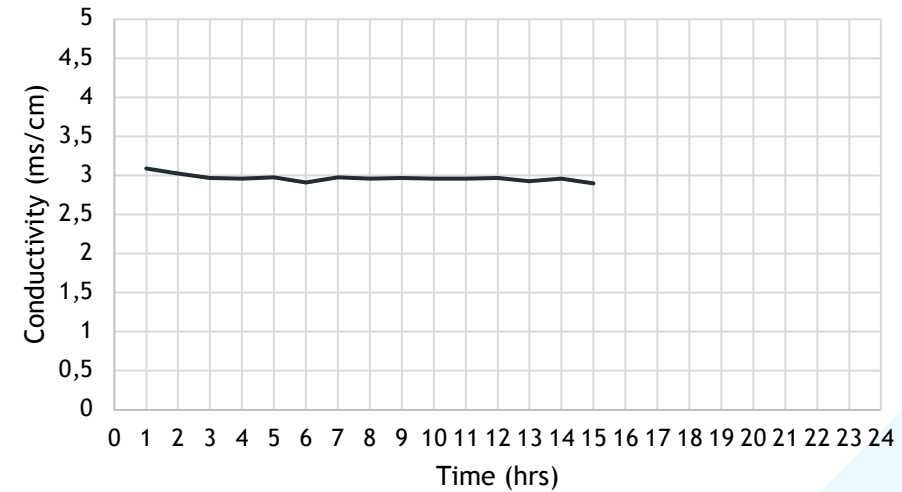
11/07/2024



18/07/2024



29/07/2024





## CERTE Participation



## ❑ Caractéristiques of Comocap 's wastewater

Parameters	Range
pH(-)	5,2 - 7,3
Conductivity (mS/cm)	2,5 - 4,2
Suspended solid (mg/L)	1100 - 2500
COD (mg/L)	<b>9600-28800</b>
BOD <sub>5</sub> (mg/L)	3200- 9600
TKN (mg N/L)	138-148
TP (mg P/L)	70-110



## CERTE participation

### *Granular Activated carbon*

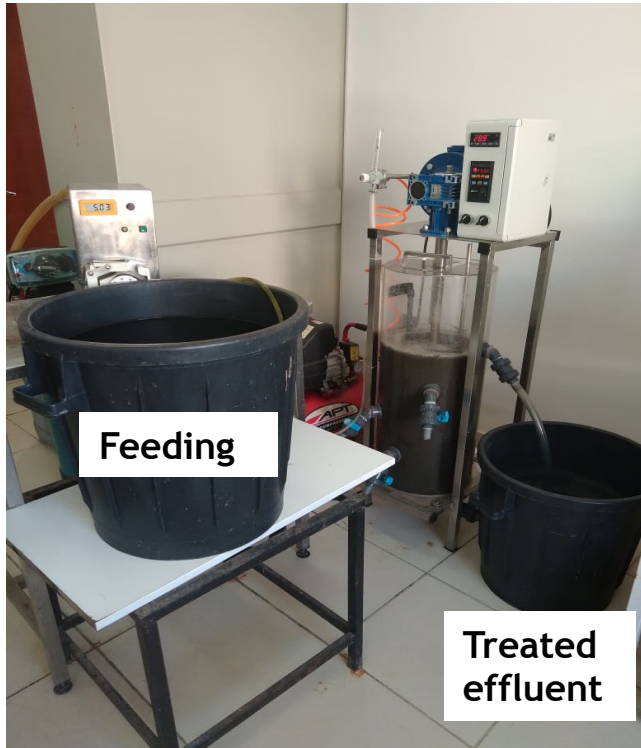
Start : 17/07/2024

Height (cm)	74,5
Diameter (cm)	28,5
Air flowrate (L/min)	10
Mixing (rpm)	0-200
Sludge volume	1/6 Volume of reactor
pH	7-9 no adjustment of pH
Temperature (°C)	Ambient temperature

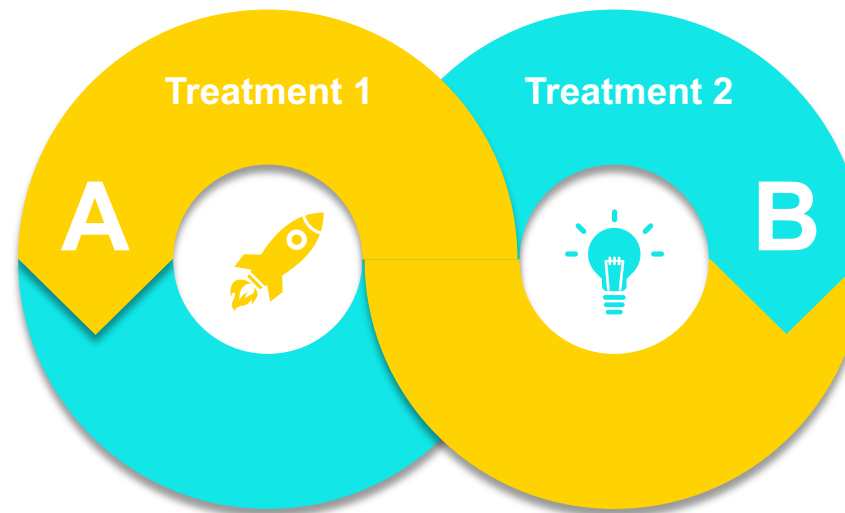
Feeding 20L of raw wastewater/ day



# CERTE participation

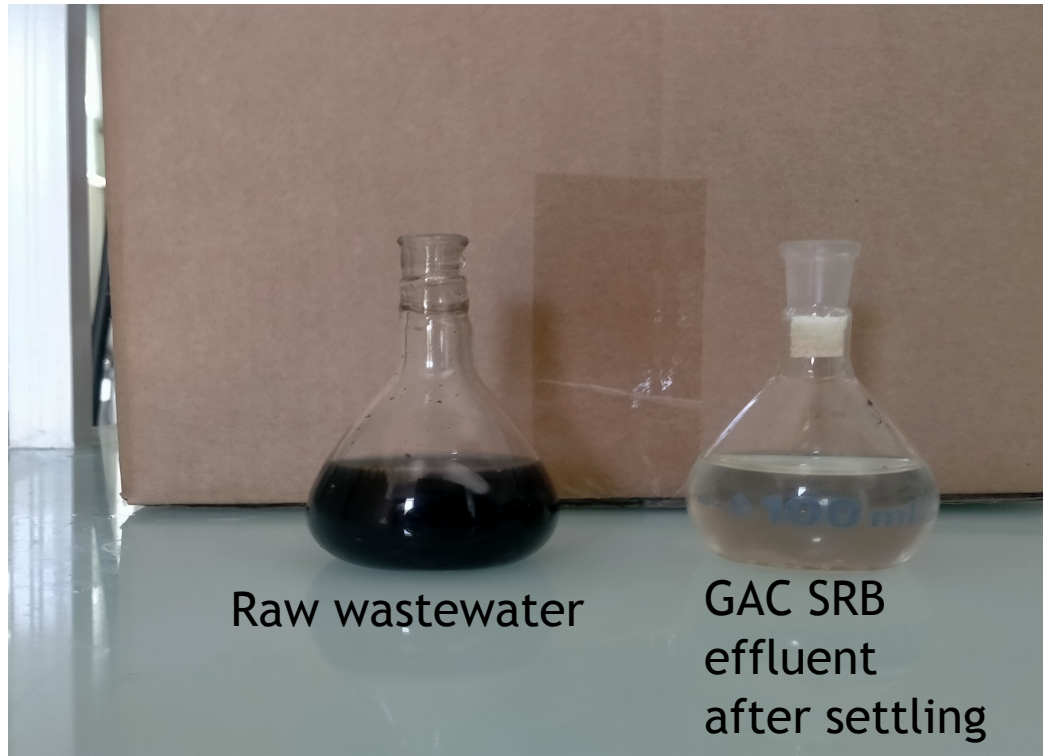


➤ GAC SRB at CERTE

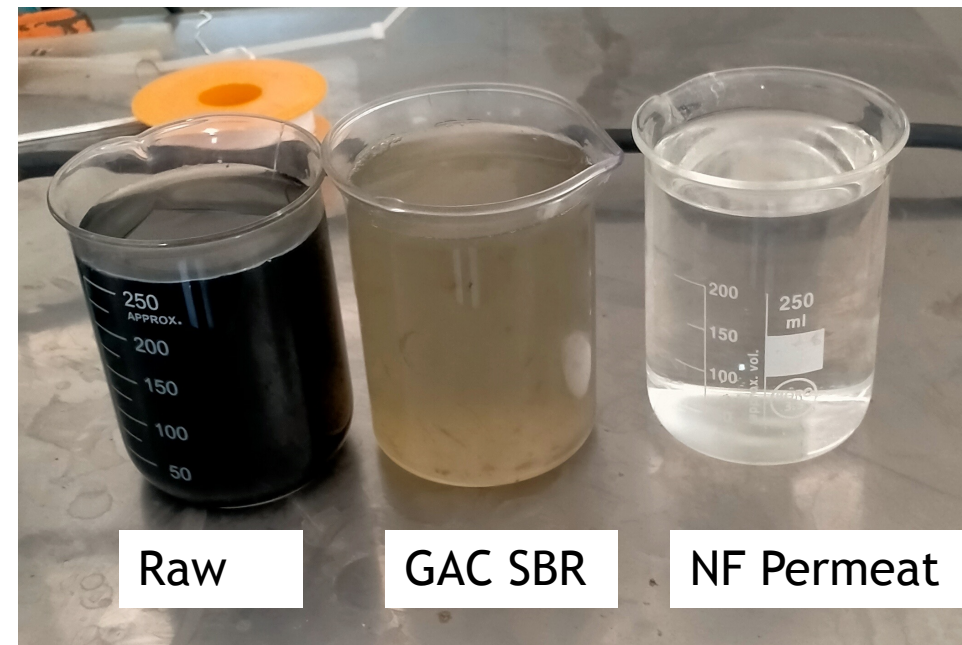


➤ Nanofiltration Unit (Filmtech NF40-40)





## Wastewater treatment results





# INRGREF Work Packages



## ❖ WP1: Technical analysis of the water/land/agro-ecosystem degradation

- ▶ Task 1.1 Characterization of water/soil/agri-food product

## ❖ WP2: Development of Innovate Integrated Water Purification System

- ▶ Task 2.1 Development of innovative agri-food wastewater first stage of treatment based on AGSB

## ❖ WP4: Quality Control on Water, Soil and Crop

- ▶ Task 4.2 Soil Quality Test
- ▶ Task 4.5 Fertilizer characterization



Co-funded by  
the European Union





# WP1: Technical analysis of the water/land/agro-ecosystem degradation



- ▶ **Task 1.1 Characterization of soil**
- Physico-chemical analysis of the soil used for to produce COMOCAP tomato plants (Physical and chemical characterization)



Co-funded by  
the European Union



## WP2: implementation of an experimental tomato cultivation protocol and study of the effect of irrigation by treated waste water

- ▶ implementation of an experimental tomato cultivation protocol and study the effect of treated wastewater
- ▶ Three classes of waters (T1:raw waste water, T2: waste water treated by mini-bioreactor and T3:bioreactor water treated by Nano filter) are used for to grow tomato plants in soil took from COMOCAB exploitation



**WP2:** implementation of an experimental tomato cultivation protocol and study of the effect of irrigation by treated waste water

## Morphologicals , Physiologicals and biochemicals variables determined

Morphological variables :

- growth in height
- number of leaves
- number of flowers
- leaf area
- leaf size
- biomass of aerial and root parts



# WP2: implementation of an experimental tomato cultivation protocol and study of the effect of irrigation by treated waste water

## Physiological variables:

- ▶ relative water content
- ▶ Fluorescence
- ▶ Electrolyte leakage
- ▶ chlorophyll content.3.3

## Biochemical variables:

- ▶ MDA, proline
- ▶ soluble sugars
- ▶ phenolic compounds (polyphenols, flavonoids and tannins).





## ❖ preliminary results

- Plants treated with bio water have the largest leaf areas
- Plants treated with nano water show the smallest leaf area
- Plants treated with raw water developed roots better than the other treatments
- Plants treated with nano water showed the least root development,.



## Effect of Water classes on Tomato growth

### Interpretation:

- ✓ **Treated water:** Globally favorable for stem growth and leaf production, but performs less well in terms of flowering.
- ✓ **Raw water:** It may seem less refined, raw water showed excellent results for stem, leaf production, especially for flowering.
- ✓ **Nano water:** Nano water showed decent initial growth, it did not promote flowering or a noticeable increase in the number of stems.





# WP4 Participation

## ▶ Microbiological analysis of Water:

### □ Enumeration in solide medium:

- ✓ Total Coliform
- ✓ Mesophll
- ✓ Clostridium sp,

### □ Molecular analysis

- ✓ 16 S sequencing
- ✓ ERIC PCR and RTpcr

## ▶ Microbiological analysis of soil:

### □ Enumeration in solide medium:

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- ✓ Clostridium sp,

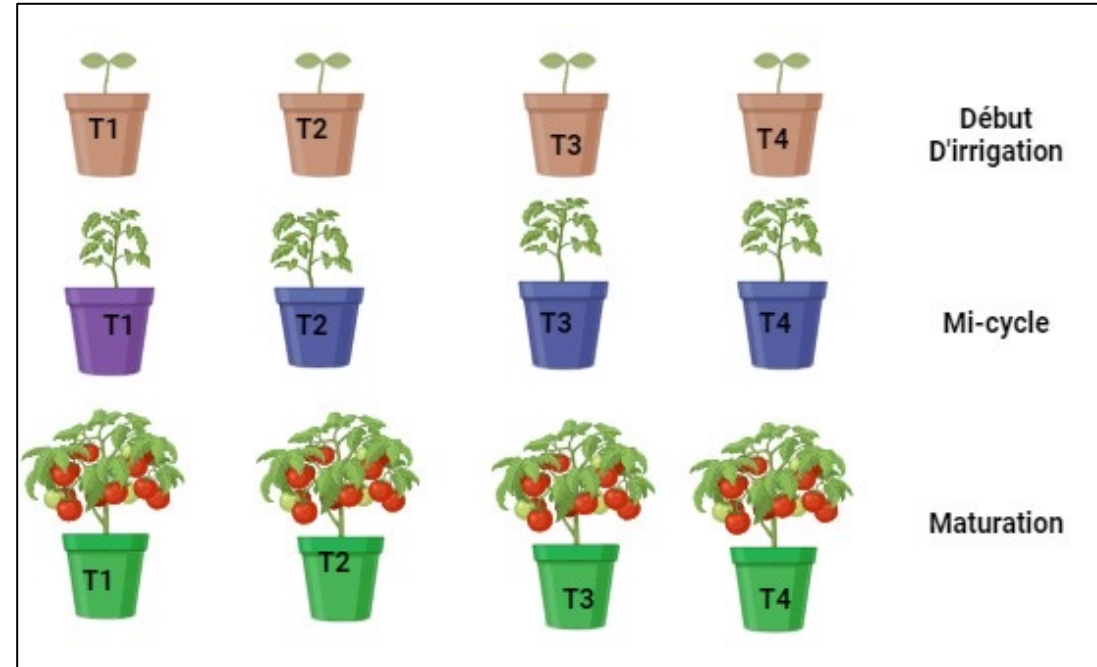
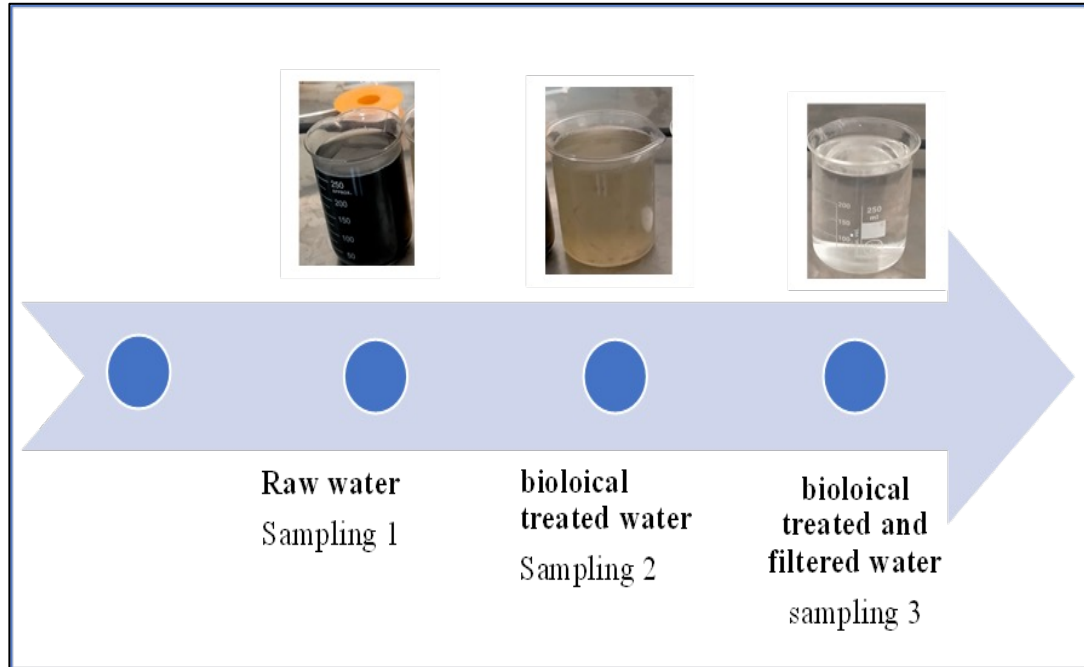
### □ Molecular analysis

- ✓ 16 S sequencing
- ✓ ERIC PCR and RTpcr

### □ Carbon and nitrogen Biomass



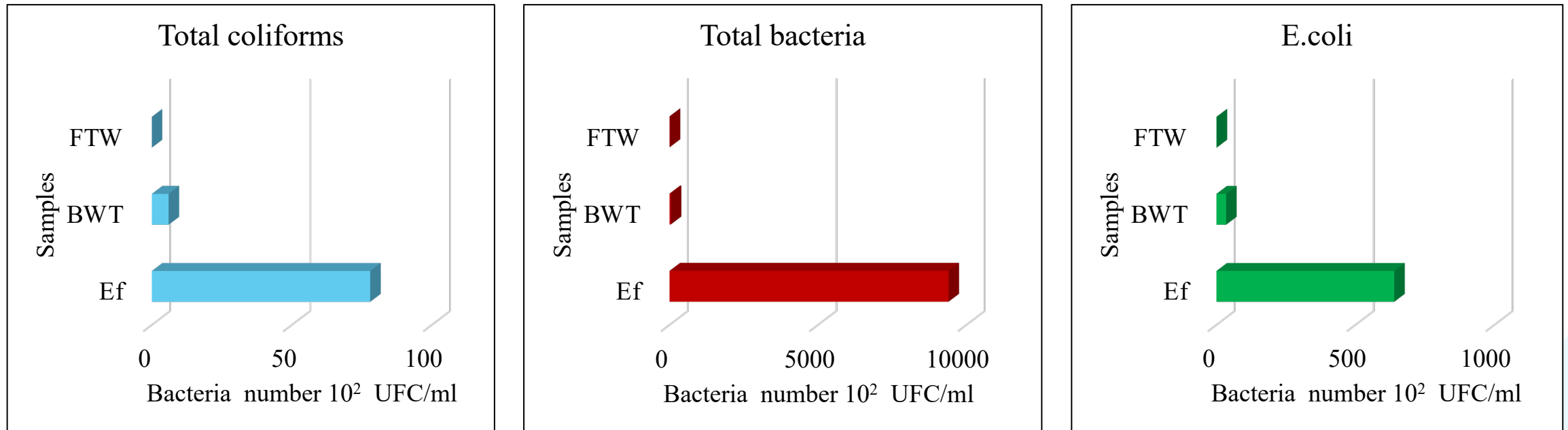
# WP Implementation



# WP4 Results

## Wastewater analysis:

- ❑ Microbiological analyses of raw effluents revealed inadequate sanitary quality.
- ❑ The combined treatment of biological process and nanofiltration proves to be the most effective in achieving the desired objectives.



**Figure 3.** Results of bacterial wastewater analysis

# WP4 Results

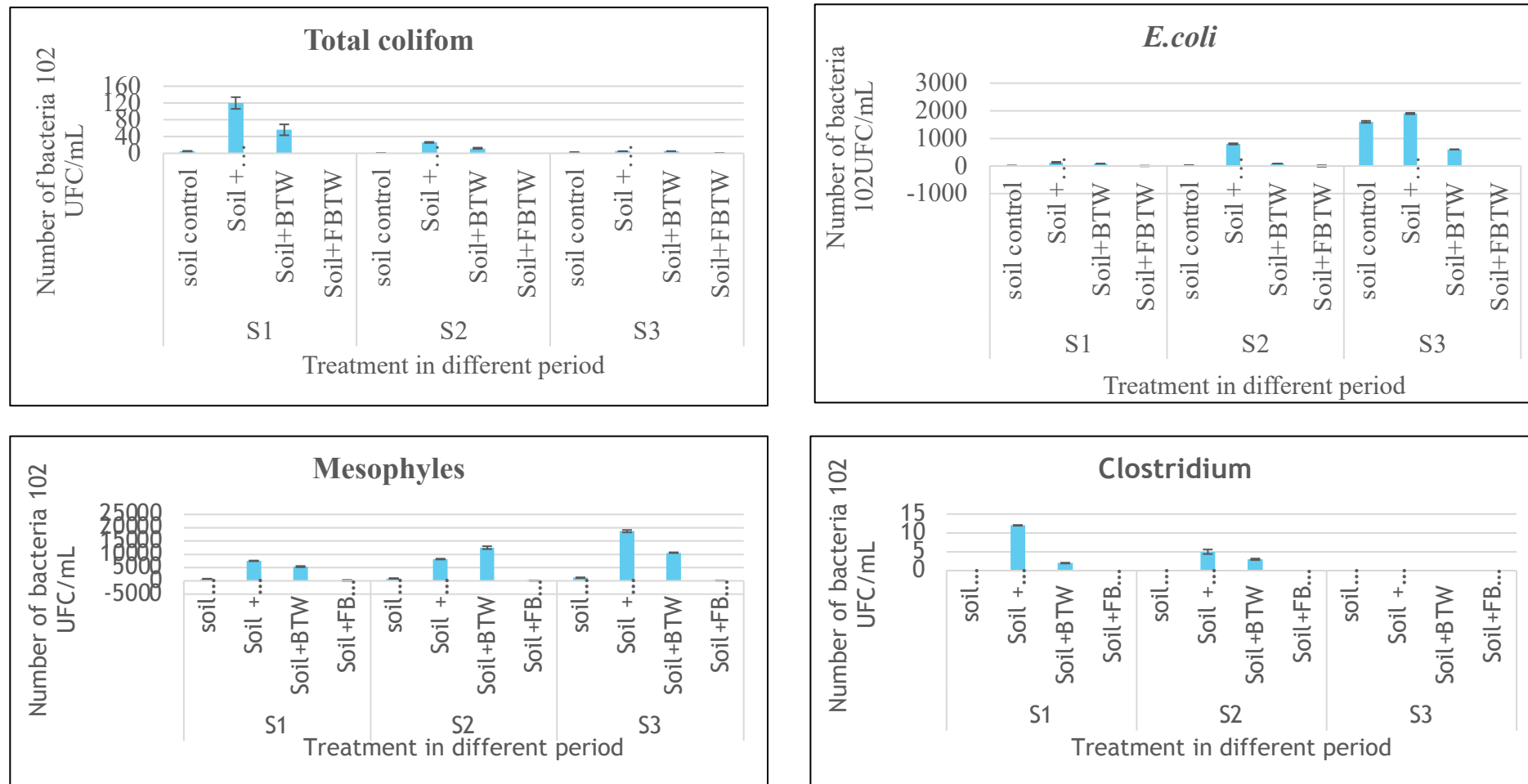
## Soil microbial analysis:

- ❑ The bacterial load in the soil increases during the growth cycle of the tomato plant, especially with irrigation using raw effluent.
- ❑ Therefore, irrigation with non-conventional water increases the load of total coliforms and consequently *E. coli*.

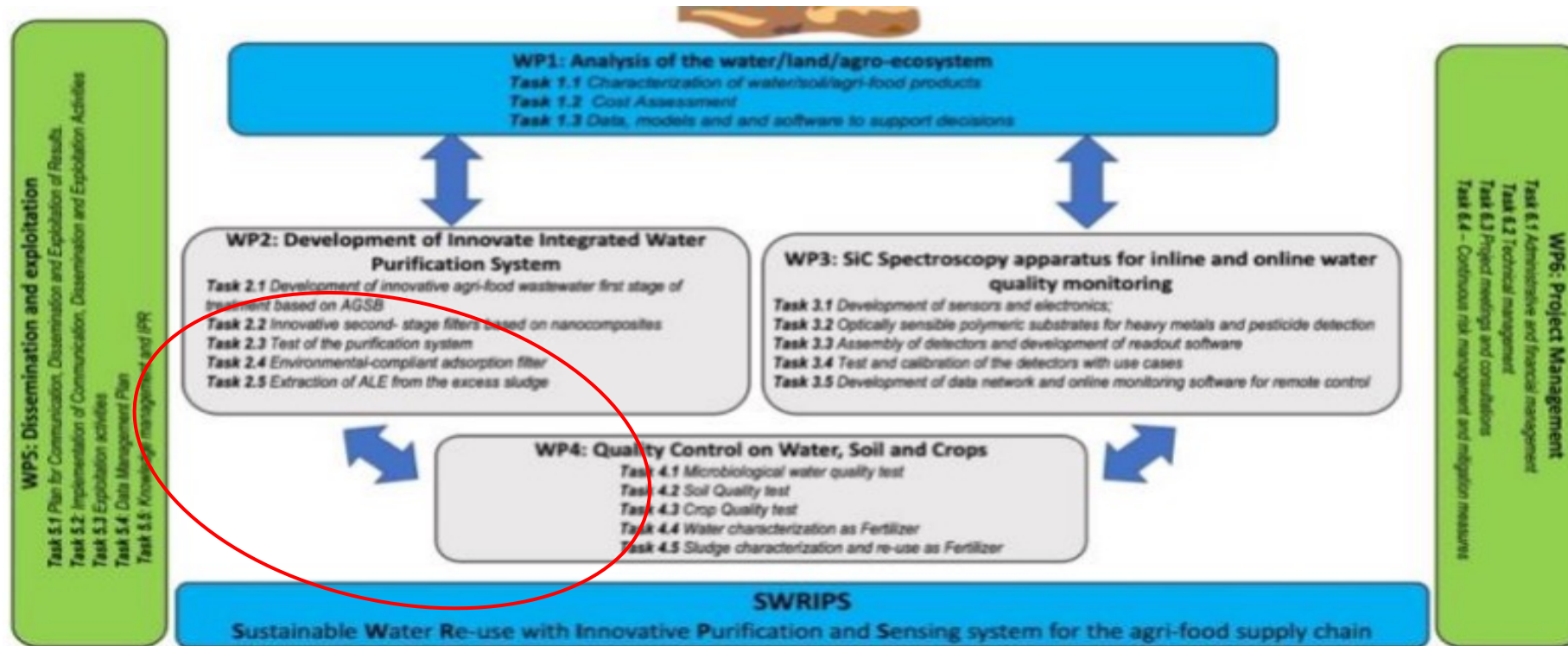


# WP4 Results

**Figure 4.** Results of bacteriological analyses ( $10^2$  UFC/mL): total coliforms (A), *E. coli* (B), mesophiles (C), *Clostridium* (D).





# WP interaction with other WPs





# WP4 UTM Participation

## ► Protocol Water

PRIMA Full Proposal Template	
 	
Title	Sustainable Water Re-use with Innovative Purification and Sensing system for the agri-food supply chain
Acronym:	SWRIPS
Task 4.1 Microbiological water quality test (UNITU-TN)	

### 1. Description of work

This task is aimed to ensure that the microbiological property of the purified water is compliant with the EU and International standards.

### 2. Experimental part

#### 2.1. Water sampling

Microbiological analyses were conducted to assess the sanitary quality of the COMOCAP water. Samples were collected from raw effluents, water treated by a biological reactor, and water that underwent biological treatment followed by nanofiltration. Each sample was collected in sterile glass bottles and stored at a temperature of 4°C during transport.

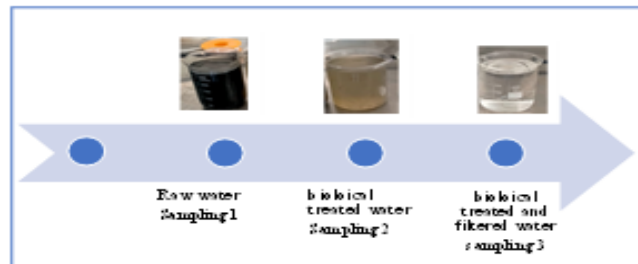




Figure. Representation of different step of water sampling

## ► Protocol soil

PRIMA Full Proposal Template	
 	
Title	Sustainable Water Re-use with Innovative Purification and Sensing system for the agri-food supply chain
Acronym:	SWRIPS

### Specific objectives of WP4 are:

microbiological water control; soil quality control; crop quality control; water characterization for irrigation purpose; sludge characterization for agriculture purpose.]

### soil quality control;

#### I. Soil Sampling:

As part of the SWIREPES project, we will collect soil samples throughout the tomato growing season. Samples will be taken from both control plots (not irrigated with treated wastewater) and plots irrigated with treated wastewater. We will collect samples at three key points: the beginning, middle, and end of the cultivation period. To collect representative soil samples from agricultural fields irrigated with treated wastewater at two depths (0-20 cm and 20-40 cm) for both microbiological and molecular analysis.

Different samples immediately place the sample bags on ice within a cooler to maintain a cool temperature (ideally 4°C). This helps preserve microbial communities. The various samples will be taken in triplicate.

#### II. Microbial enumeration in soil



##### 1. Enumeration of sulfate-reducing clostridium

The enumeration of sulfate-reducing clostridium is done by culture on agar meat-liver agar, Diagnostic Pasteur (meat-liver base 30g, glucose 2g, starch 2g, agar 11g, distilled water 100 ml), a standard medium for the detection of these germs (Marshall et al., 1987). Once



# WP4 Participation

## ► Water and soil Sampling:

Title:	Sustainable Water Re-use with Innovative Purification and Sensing system for the agri-food supply chain
Acronym:	SWRIPS
PRIMA Full Proposal Template  	

### Sampling plan for microbiological analysis

#### Experimental design (5 repetitions):

##### Water sampling:

Type	Sampling date
T <sub>0</sub> Raw water	05 August 2024
T <sub>1</sub> Water treated by the biological reactor	
T <sub>2</sub> <del>Raw water</del> <del>external water</del>	

##### Soil sampling:

Type	Sampling date
T <sub>0</sub> <del>0 days of irrigation</del>	05 August 2024
T <sub>1</sub> 15 <del>days of irrigation</del>	17 August 2024
T <sub>2</sub> 40 <del>days of irrigation</del>	02 <del>September</del>
T <sub>3</sub> 60 <del>days of irrigation</del>	<del>02 September</del>

##### Plant sampling:

Type	Sampling date
T Start of cycle to maturity	05 August 2024
P <sub>0</sub> Start of irrigation	17 August 2024
P <sub>1</sub> <del>cycle</del>	02 <del>September</del>
P <sub>2</sub> End of cycle to maturity	<del>02 September</del>



# Poster participation :3 October 2024

**XII International Symposium on Engineering Technology**  
1-4 October 2024

**LMPF IOE FST**

**Tide: Microbial Quality Control on Soil irrigated by treated secondary wastewater: case tomato plant**

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**Somrat TRAELEP, Nijala SAKUP-SOMLOUP**

\* Laboratory of Biology, Pathology and Veterinary Faculty at Khon Kaen University, Khon Kaen Province, Thailand; \*\*Faculty of Science, Khon Kaen University, Khon Kaen Province, Thailand; \*\*\*Department of Statistics at Khon Kaen University, Khon Kaen Province, Thailand; \*\*\*\*Department of Health Sciences at Khon Kaen University, Khon Kaen Province, Thailand; \*\*\*\*\*Department of Health Sciences at Khon Kaen University, Khon Kaen Province, Thailand.

### Introduction:

The tomato supply chain, a cornerstone of the Mediterranean agricultural industry, provides a complex food matrix. With water scarcity, water footprint, especially in the growing process (Santos, 2023), the tomato sector offers opportunities for sustainable waste management. Agricultural waste management of wastewater can offer environmental, economic and social benefits, including improved crop yields, reduced energy consumption, and avoided nutrient pollution (Nieto et al., 2023).

### Background

This study was conducted within the framework of the IOWSP project 2023/2024, which aims to assess the impact of treating unconventional water from the agri-food industry.

**Figure 1.** Experimental design for the biotechnological study within the IOWSP project.

### Methodology

Samples of CORDEX® soil were collected from one of the tomato fields, water treated by a biological reactor (PTRC), and water that under wet biological treatment followed by nano-filtration (PTNC).  
Samples of soil will be taken from four plots types irrigated with spring water (control), PT, PTRC and with PTNC.

**Figure 2.** Sampling scheme for water (A) and soil (B) according to the different treatments.

**Bacterial analysis:** Microbiological analysis samples using culture media specific to total coliforms (methyl red), total coliforms, *E. coli* (TC) and *Campylobacter* (CC).

**Fungal identification:** Fungi were identified using phenotypic, molecular, and molecular characterization.

### Results

**Bacterial analysis:** Microbiological analyses of soils from tomato revealed inadequate sanitary quality.  
The results of treatment of biological processes and nanofiltration process for the reuse of water in achieving the microbiological criteria.

**Figure 3.** Results of bacterial analysis.

**Fungal analysis:** The fungal load in the soil increases during the growth cycle of the tomato plant, especially with irrigation using raw effluent.

**Figure 4.** Results of biotechnological analysis (TP-LPC-CC); total coliforms (AC), *E. coli* (BC), campylobacter (CC), *Campylobacter* (DC).

### Conclusion

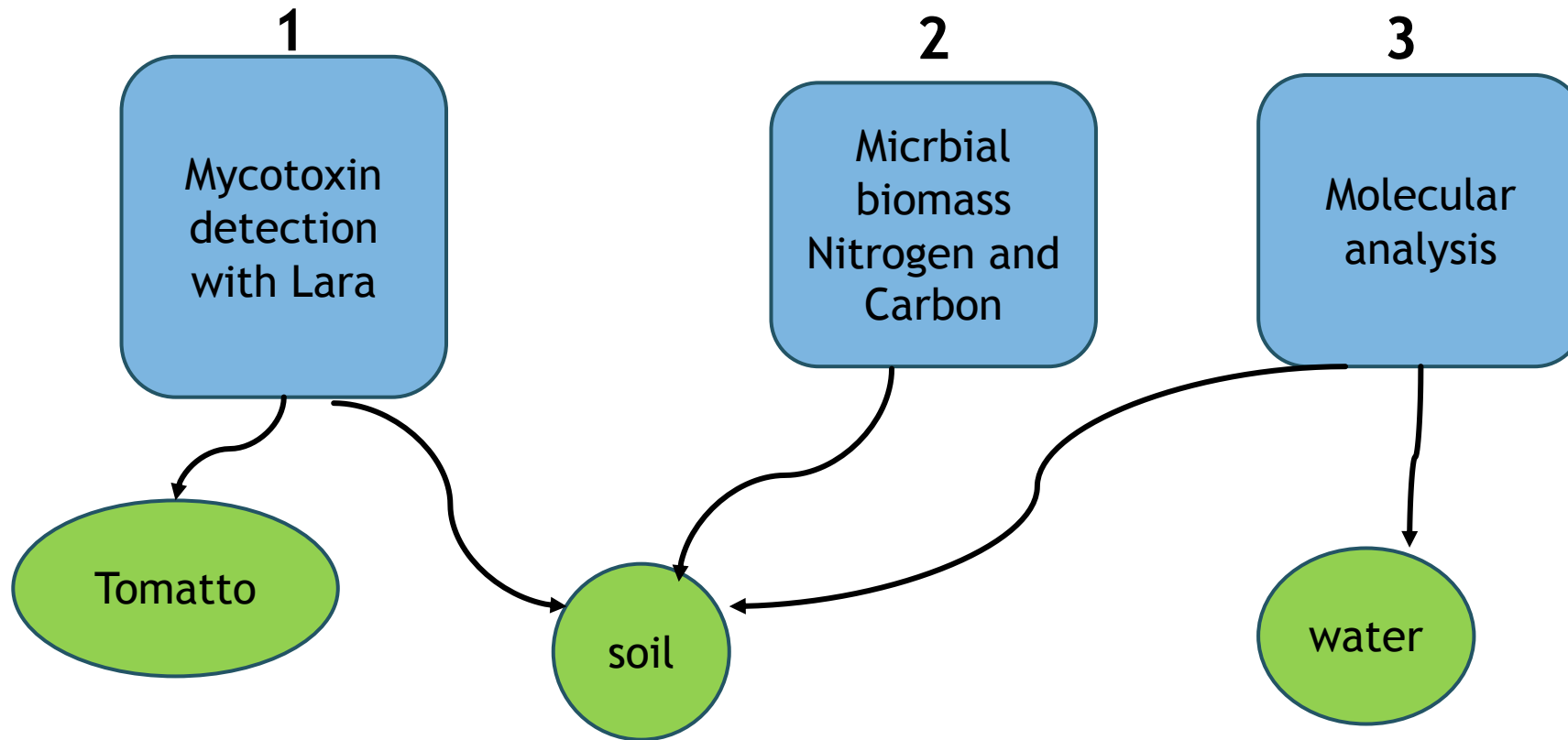
With reference to climate change and the environment, IOWSP has the additional long-term ambition to exploit the innovation of this technology not only to purify the wastewater but also to contribute to agro biodecontamination. Higher availability of nutrient can contribute to the reduction of farmers' expenditure for synthetic fertilizers which, as a result of climatic stress, tend to increase the phenomenon of nitrate leaching.

PRIMA Full Program Symposium

**PRIMA**



## WP4 going on



# On going activities

- ❑ In-person GICA meeting
- ❑ Dissemination through national and international conferences
- ❑ Preparation of the first scientific article
- ❑ Phd student with prof Lara



# Risk and contingencies

Experimental risk	Other risk
<p><b>Sample Collection:</b></p> <ul style="list-style-type: none"><li><input type="checkbox"/> <b>Container:</b> Sterile flasks</li><li><input type="checkbox"/> <b>Storage:</b> 4° C for short-term storage</li><li><input type="checkbox"/> <b>Sampling times:</b> Before and after treatment</li><li><input type="checkbox"/> <b>Minimum volume:</b> 50-100 mL</li></ul>	<ul style="list-style-type: none"><li><input type="checkbox"/> The exchange of protocols</li><li><input type="checkbox"/> The similarity of types of analysis</li></ul>
<ul style="list-style-type: none"><li><input type="checkbox"/> Soil sample collected in a sterile plastic bag or container.</li><li><input type="checkbox"/> Sampling schedule: at the beginning, middle, and end (harvest time) of the irrigation period using the tested water.</li></ul>	





# WP Deliverables

Deliverables (brief description and month of delivery)

- ▶ D4.1 Report on the presence of microorganisms in water (M30)
- ▶ D4.2 Report on the presence of *E. coli*, and *Clostridium* spp. in water (M30)
- ▶ D4.3 Report on soil and crop quality (M34)
- ▶ D4.4 Report on ALE characterization as fertilizer (M36)



# THANK YOU!

LMPB  
FST

