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## Training in tools to develop quantitative risk assessment of fresh produce using water reuse systems in Mediterranean production

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### Abstract

Water resources are increasingly coming under pressure specially around the Mediterranean area, leading to water scarcity and a deterioration in water quality. The use of treated wastewater represents an alternative source to enhance the demand for irrigation water. Water reuse in combination with the promotion of the use of water-efficient technologies in industry and water-saving irrigation techniques could lead to good qualitative and quantitative water status for surface and ground water bodies. Nevertheless, food-borne outbreaks linked to fresh produce irrigated with partially or untreated wastewater caused by bacteria, parasites and enteropathogenic viruses have been widely reported. In the absence of solid scientific understanding of the actual risks involved, consumers are likely less receptive to buy leafy greens irrigated with treated wastewater, also known as reclaimed water. In this study, we aimed to assess the microbiological risks of leafy green vegetables irrigated with treated wastewater in Spain using Norovirus as a model organism to facilitate the development of risk management strategies. A conceptual exposure model was designed to describe the virus fate and transport from the Wastewater treatment plant (WWTP) secondary effluent to the consumers' fork. This study is an example of the use of reclaimed water for irrigation of commercial fields producing leafy greens in the south-east of Spain and tries to assess potential microbiological risks to the consumers by establishing their safety.

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## Table of contents

Abstract.....	1
1. Introduction.....	4
2. Description of work programme .....	5
2.1. Aims.....	5
2.2. Activities/Methods .....	6
2.2.1. Practical work, research project on QMRA of leafy greens irrigated with reclaimed water. ....	6
2.2.2. Training in risk assessment.....	6
2.3. Secondary scientific activities during fellowship.....	6
2.3.1. Participating in various conferences/webinars/meetings.....	7
2.3.2. Oral presentation .....	7
3. Results .....	7
4. Conclusions from the participation in the EU-FORA programme.....	9
References.....	9
Abbreviations.....	10
Annex A – Oral presentation at First Workshop of EU+ Sustainability Lab June 2021.....	11

## 1. Introduction

The present work, dedicated to the training in tools to develop quantitative risk assessment of fresh produce using water reuse systems in Mediterranean production, was conducted in the context of the EFSA EU-FORA fellowship programme. This programme aims at early to mid-career professionals from European Union (EU) and European Free Trade Association (EFTA) countries, offering candidates the chance to widen their knowledge and hands-on experience of food safety risk assessment. EU-FORA fellowship programme provides competency based training and practical experience using the 'learning by doing' approach in acknowledged training sites across EU and European Economic Area (EEA) Member States.

Theofilos Papadopoulos is a Veterinary surgeon who has been working in the Directorate of Veterinary Centre of Thessaloniki. He graduated from the School of Veterinary Medicine, Aristoteles' University in Greece in 2003 and he received a Master of Science's degree in Aquatic Animal Health in 2008, and a Master of Science's degree in Public Health in 2011. The fellow completed his PhD in Molecular Microbiology in 2015, and he is EBVS European Veterinary Specialist in Veterinary Microbiology and a *de facto* member of the European College of the Veterinary Microbiology. Before EUFORA, he worked for nearly 10 years in the field of Surveillance of Zoonoses and Food Safety in Greece and in the field of Epidemiology in SCIENSANO - Belgium. In January 2021, he started the fellowship at the Polytechnic University of Cartagena (UPCT), joining the research group of Food Safety and Preservation in the Agronomic Engineering Department under the supervision of Professor Pablo Fernández Escámez. The fellowship was developed jointly by the UPCT and CEBAS-CSIC (Spain).

Water is a critical input for agricultural production and agricultural water has been identified as one of the main risk factors of microbial contamination for fresh produce. Agriculture consumes a significant amount of water resources in Europe, accounting for around 30% of total water use. In Europe, the main water sources are surface waters (rivers, lakes), reservoirs supplied by well or rain water, well water and potable quality water particularly in the case of hydroponics (EFSA BIOHAZ Panel, 2014). However, water is a limited resource, particularly for at least nine Mediterranean countries which are currently considered as water-stressed countries, related to pressure on the quantity and quality of water resources. This water stress situation is expected to be aggravated by the expected increasing water demand during the coming years and climate change (Mancuso et al., 2020). For this reason, alternative water sources have started to be used in an attempt to reduce the water stress. The use of reclaimed wastewater is a promising alternative water resource, particularly for agriculture, which is currently the main user of renewable water resources.

The use of reclaimed wastewater mainly allows to preserve the freshwater stock. However, it also represents a source of nutrients, namely nitrogen, phosphorus, and other salts, which are necessary for the physiological growth of crops (Mancuso et al., 2020). The wastewater treatment process usually includes primary (sedimentation) and secondary treatments (biological oxidation), as well as more advanced tertiary treatments such as chemical coagulation, filtration and/or chemical disinfection (EFSA BIOHAZ Panel, 2014). Wastewater cannot be used for irrigation, and reclaimed water needs to be of a particular quality (European Union, 2020). The use of reclaimed water for irrigation is beneficial in agriculture but if wastewater treatments are not well implemented, it may be associated with potential human health risks. One of the important challenges when using reclaimed water in agriculture is ensuring the safety of food products considering that if the water reuse systems are not properly implemented, human pathogens may be still present.

The consumption of leafy salads has increased internationally in recent years as promotion of healthier lifestyles. These foods are generally consumed fresh and are not subject to further processing to eliminate pathogenic microorganisms such as viruses (Callejón et al., 2015). Since leafy greens eaten raw as salads do not include any processing steps or control points which will ensure removal or inactivation of biological hazards, it is particularly important to consider risk factors and control options at the point of production (EFSA BIOHAZ Panel, 2014). Food-borne outbreaks linked to fresh produce irrigated with partially or untreated wastewater have been reported (Lynch et al., 2009; Berger et al., 2010; Gelting and Baloch, 2013), while contamination of crops with enteric viruses, faecal coliforms, and bacterial pathogens and parasites has also been evidenced (Hamilton et al., 2006; Sales-Ortells et al., 2015; Adegoke et al., 2018).

Food-borne outbreaks may be caused by bacteria, parasites and enteropathogenic viruses including rotaviruses, astroviruses, adenoviruses, noroviruses and caliciviruses (Domínguez et al., 2009). During 2019, norovirus was associated with 457 food-borne outbreaks and, most importantly, with 11,125 related illnesses (22.5% of total cases) meaning one in five of all outbreak-related illnesses in the EU.

During the same period, 51 outbreaks were associated with the consumption of food of non-animal origin (leafy green vegetables, olives, tomatoes, cucumbers and radish sprouts) with leading causes norovirus (14 outbreaks) and *Salmonella* (12 outbreaks) (EFSA and ECDC, 2021). Norovirus caused most of the outbreaks associated with the consumption of leafy vegetables in USA during 2014–2018 (CDC, 2021). The microbiological risks derived from irrigation of fresh produce with reclaimed water have been previously reported for enteric viruses (Hamilton et al., 2006; Sales-Ortells et al., 2015; López-Gálvez et al., 2016; Adegoke et al., 2018; Summerlin et al., 2021). Some studies have also focused on norovirus risks/presence in fresh produce (Mara and Sleigh, 2009; Barker, 2014; Sales-Ortells et al., 2015; Eregno et al., 2017; Torok et al., 2019; Emilse et al., 2021). Studies on the prevalence and infectivity of Norovirus are limited, and quantitative data on viral load are scarce making establishment of microbiological criteria for Norovirus on leafy greens difficult (EFSA BIOHAZ Panel, 2014).

Quantitative microbiological risk assessment (QMRA) is a methodology used to organise and analyse scientific information to estimate both the probability and severity of an adverse event as well as prioritise efforts to reduce the risk of food-borne pathogens (Habib et al., 2020). The development of QMRA involves a four-phase process to estimate the human health risk associated with exposure to the target pathogen. The microbial risk assessment process consists of four distinct steps: (i) the hazard identification; (ii) the hazard characterisation; (iii) the exposure assessment; and (iv) the risk characterisation (Lammerding and Fazil, 2000; Koutsoumanis and Aspridou, 2016). QMRA is based on a quantitative description of the microbial response to the different conditions encountered during each step of the field-to-fork chain of the product based on mathematical models.

The results of the quantitative model of pathogens associated to fresh produce using water reuse systems in Mediterranean production will provide an estimation of the burden of pathogenic microorganisms present in the plant products that are irrigated with reclaimed water considering the different scenarios and production practices. These data, combined with the existing data in the literature on the consumption of leafy vegetables and dose-response curves will provide an estimate of the possible risks associated with this practice.

## 2. Description of work programme

### 2.1. Aims

The work programme will focus on the QMRA of pathogens associated with fresh produce using water reuse systems in Mediterranean production. It will cover hazard identification, exposure assessment, hazard characterisation and risk characterisation, applying a robust statistical method. It will be developed in collaboration with CEBAS-CSIC, the partner that was included in the application. Main activities related to the Work Programme where the fellow is going to be involved in:

- Training of the fellow person on methodologies related to risk assessment routinely used by the supervisors and co-supervisors at UPCT and CEBAS-CSIC. This will include databases, different software available for predictive microbiology and risk assessment, statistical analysis and programming in R.
- Data selection to characterise the microbial response required for QMRA. Data available in both institutions will be used, as well as data from literature. If there are data gaps, they will be filled performing experimental work.
- Development of mathematical models to describe microbial behaviour along the food change (prevalence, growth, inactivation, acclimation, contamination, etc.), based on data gathered from the groups and from literature. The models will be validated so that they can be applied for a QMRA. Dose–response models will be selected from those already published. When possible, R statistical software (open access) and f.eg. Shiny app will be used.
- Estimation of the risk based on different scenarios. Health risks will be established on the basis of conditions included in the study using web-based tools (such as MicroHibro, FDA-iRisk, @Risk) and the data and models developed. This will allow the establishment of a risk ranking and the interpretation of the impact of variability and uncertainty on a QMRA.

## 2.2. Activities/Methods

### 2.2.1. Practical work, research project on QMRA of leafy greens irrigated with reclaimed water

As part of the fellowship, the priority of the hosting site was to provide the fellow with the basic theoretical background required to perform a QMRA. The fellow joined a working team based in the UPCT in collaboration with CEBAS-CSIC and with proved expertise in the use of risk assessment tools.

The fellow performed a comprehensive literature mining to collect published information and identify potential data gaps in performing QMRA. This also included training in handling of available databases (EFSA, FAO and ECDC). The fellow was also trained in growth and inactivation modelling (such as Combase or Bioinactivation FE, the latter developed in the group).

Together with his supervisors, the fellow agreed to work in a study project in QMRA of norovirus gastroenteritis associated with the consumption of leafy greens from commercial fields irrigated with reclaimed water. The QMRA application covered all the steps from the secondary effluent of a wastewater treatment plant to the consumer's fork.

A total of 570 samples (water and crop) were analysed across the water reuse system used to irrigate leafy greens in commercial growing fields. Reclaimed water originated from two WWTPs in Murcia region (Spain) using different wastewater treatment processes. Samples were tested for the presence of several microbiological hazards during 2017–2019. Sampling was performed in WWTP inlet (N = 100), WWTP outlet (N = 100), WWTP reservoir (N = 100), grower reservoir (N = 75), irrigation point ((N = 100) and crop (N = 95). A database, provided by the supervisors, was analysed including the prevalence data of several Norovirus I, Norovirus II, hepatitis virus and bacteriophages in leafy green primary production. After analysing the data, some laboratory work was planned to cover potential gaps for the QMRA application. However, due to the uncertainty of COVID-19 situation, and the lockdown at the university performing laboratory work was not feasible. Therefore, a more theoretical approach for the project had to be adapted.

The fellow received training in implementation of statistical analysis using Monte Carlo and Bayesian methods and risk ranking methodologies. He was trained in using various software tools specific for risk assessment (e.g. MicroHibro, @Risk, FDA-iRISK). He also gained experience in the separation between variability and uncertainty, the quantification of these terms and the incorporation in predictions from the point of view of experimental design and statistical analysis.

### 2.2.2. Training in risk assessment

During the fellowship, the fellow obtained general information on Risk assessment activities. Due to the COVID-19 pandemic, all courses were held as online modules. Initially, the fellow followed the 3-week induction training in microbiological and chemical risk assessment by EFSA (11–29 January 2021). He attended an additional 1-week training module focusing on risk communication and crisis response, organised by the BfR (22–26 March 2021) and the 6-day training module focusing on emerging risks, nanotechnology, omics, new concepts and tools in toxicology, risk ranking organised by the Hellenic Food Authority (7–14 June 2021). He also attended the 4-day training module on data collection and reporting (4-7 October 2021) and finally the 1-week in module 'module 4 training of the European Food Risk Assessment Fellowship Programme' (22–26 November 2021), both organised by EFSA.

The fellow joined a 2-day visit in the headquarters of the Spanish Agency for Food Safety and Nutrition (AESAN) on October 28, 2021, in Madrid and on 29 in Centro Nacional de Alimentación in Majadahonda. During this visit, he had the opportunity to meet in person all the other fellows hosted in Spanish Institutes and to receive information and in-hand training in risk assessment regarding biological and chemical risks, risk management, official control in foods and alerts, food contact materials, residues of veterinary drugs, microbiology, and antimicrobial resistance.

Moreover, the fellow presented and discussed the results of his project at the First Workshop of EUt+ (European University of Technology) Sustainability Lab on 14 June, 2021.

## 2.3. Secondary scientific activities during fellowship

Along with the scheduled activities, additional training and other opportunities were provided by the hosting and other organisations face to face or online. This helped the fellow further improve his general knowledge on risk assessment.

### 2.3.1. Participating in various conferences/webinars/meetings

- 1) 'Elicitation and practical use of disability weights for quantifying years lived with disability'. Webinar provided by Burden of Disease Network, January 2021 (online).
- 2) 'Basic Concepts in Epidemiology and Surveillance'. Training school provided by Animal Health Ireland, 1-2 February 2021 (online).
- 3) 'Learning R'. LinkedIn learning online course, March 2021 (online).
- 4) 'Risk communication' EPIET Alumni Network webinar, March 2021 (online).
- 5) 'Activities and achievements of the Italian Global Burden of Disease Initiative'. Webinar provided by Burden of Disease Network, April 2021 (online).
- 6) 'The ESCMID study group in Public Health Microbiology Virtual Meeting', May 2021, (online).
- 7) 'Burden of foodborne diseases: how can we estimate it and why do we need it?' Webinar provided by World Health Organization, June 2021, (online).
- 8) 'Forecasting the Global Burden of Disease Study'. Webinar provided by SCIENSANO, June 2021, (online).
- 9) 'First Workshop of EUt+ Sustainability Lab' June 2021, (online).
- 10) 'Basic pharmacokinetics and pharmacodynamics – focus on antibiotics'. Training School, School of Veterinary Medicine, University of Padova, Italy, August 2021
- 11) 'European Network for Optimization of Veterinary Antimicrobial Treatment working group meeting'. University of Padova, Italy, August 2021
- 12) 'Epidemiology of Methicillin Resistant *Staphylococcus aureus* in abattoirs and slaughtered ruminants', (25 days) Short Term Scientific Mission by Risk-based meat inspection and integrated meat safety assurance CA18105, Thessalonikí, Greece, August 2021.
- 13) 'Animal Health economics'. Webinar provided by Standardizing output-based surveillance to control non-regulated Diseases of cattle in the EU (SOUND) COST action, September 2021 (online).
- 14) 'Innovative Dairy Science education material development, focused on Products, Processes, Quality, Safety & Entrepreneurship, using Information and Communication Technologies (ICTs) and Open Educational Resources (OER)' final meeting, Larissa, Greece, September 2021.
- 15) 'AGM meeting of the European College of Veterinary Microbiology, October 2021 (online).
- 16) 'Novel technologies for surveillance and characterization of Extended-spectrum  $\beta$ -lactamase and Carbapenemase producing Enterobacteriaceae, in humans and animals (CARBATECH)' seminar, Ioannina, Greece, November 2021
- 17) 'Virtual training module 4' Training module provided by the European Food Risk Assessment Fellowship Programme, 22–26 November 2021 (online).

### 2.3.2. Oral presentation

**'Water reuse in agricultural irrigation of commercial fields producing leafy greens in South-east Spain'**. Papadopoulos T., Allende A., Egea J.A., Gomez A.P., Fernandez P.S. Oral presentation at First Workshop of EUt+ Sustainability Lab June 2021, Cartagena, Spain (online) (**Annex**).

## 3. Results

Leafy greens, eaten raw as salads, represent a minimally processed, ready-to-eat food. In order to provide an analysis of the risks associated with the consumption of this food item we considered all the production processes of leafy greens starting from the field, growing, harvesting, distribution, retail and handling in domestic environments. On top of this, we considered the irrigation of the leafy greens with reclaimed water and the risks associated with this practice using norovirus as the model organism (Figure 1).

One of the main pathways for the contamination at primary production is due to the microbial quality of the reclaimed water used for irrigation. Water treatment usually includes primary (sedimentation) and secondary (biological oxidation) treatments, as well as more advanced tertiary treatments such as chemical coagulation, filtration and/or chemical disinfection. Nevertheless, they can vary even if there are standards established at EU level for treatment of municipal wastewater to be used for irrigation (European Union, 2020). However, the water reuse system includes not only the water treatment process but also the distribution and storage of the water before the reclaimed water

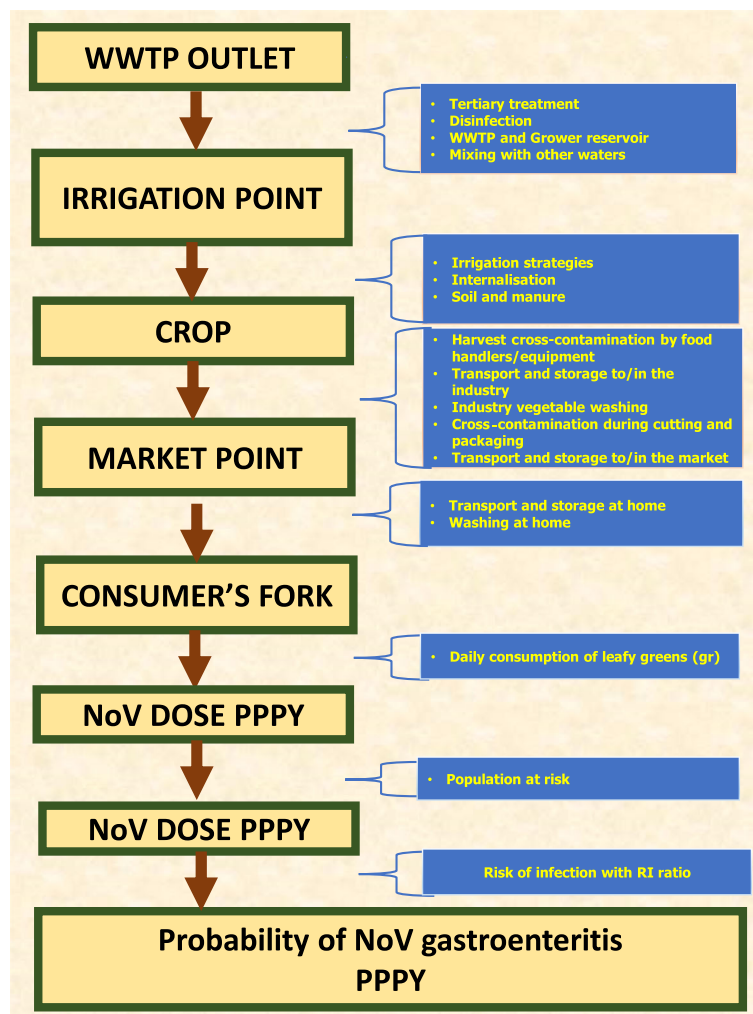
is used in the delivery point at the grower field. Therefore, the microbiological quality of the reclaimed water not only depends on the good implementation of the water treatments but also on the management of all the steps of the water reuse system.

Contamination risk depends also on the irrigation strategies (mixing with other waters, irrigation system) and the use of manure as fertiliser and Norovirus (NoV) internalisation from irrigation or the soil. Irrigation with untreated or partially treated wastewater together with spraying prior to harvest and overhead irrigation that leads to wetting of edible parts are the main risk factors regarding this stage of production.

We considered, as basic scenario, leafy greens consumed after harvesting without any processing from the industry (washing, cutting, packaging, etc.) but washing by the consumer. As an alternative scenario we considered all these processes made by industry, leafy greens sold packed and eaten raw without any other process from the consumer.

The main factors for contamination of leafy greens during primary production is the use of untreated wastewater for irrigation and the irrigation system. During processing, the main factors are contamination or cross-contamination via equipment, water or by food handlers. Finally, at distribution in domestic and commercial environments, cross contamination by food handlers or equipment are very important factors, while at home and according to the basic scenario, washing practices before consumption play the most important role.

Figure represents a flow chart for the QMRA while table includes all the inputs used for QMRA application (Figure 1).



**Figure 1:** Flow chart of QMRA for predicting the probability of developing NoV acute gastroenteritis for an individual after consuming raw leafy greens



#### 4. Conclusions from the participation in the EU-FORA programme

Participation in the EFSA EU-FORA work programme was a valuable opportunity for the fellow to obtain experience in tools used in quantitative microbial risk assessment. This was also a perfect chance to expand his knowledge and skills in food safety, particularly in the field of QMRA, by working in a professional environment according to European guidelines and standards. It also provided the hosting institutions, Universidad Politécnica de Cartagena and CEBAS-CSIC, an excellent opportunity to interact with the EU-FORA Programme through a motivated and highly capable fellow to improve our training capacity in QMRA. A multidisciplinary biological risk assessment was developed.

The fellow learned many new skills through the modules, and had opportunities to broaden his understanding of the risk assessment methodology in a wider range of hazards including infectious diseases. Besides all the learning-by-doing, he had the chance to further develop his network of professionals by attending the EU-FORA training modules, conferences, and meetings.

The EU-FORA programme provided a great environment to build a strong professional and personal network that will be used for future collaborations between the sending and both the hosting institutions. Finally, fellow's training in risk assessment methodology was a great added value also for the sending institute and will help both fellow and the Institute to apply risk assessment methodology in practice.

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## Abbreviations

EEA	European Economic Area
EFTA	European Free Trade Association
QMRA	quantitative microbiological risk assessment
UPCT	Polytechnic University of Cartagena
WWTP	Wastewater treatment plant

## Annex A – Oral presentation at First Workshop of EUT+ Sustainability Lab June 2021

### Water reuse in agricultural irrigation of commercial fields producing leafy greens in South-east Spain

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- Water scarcity and deterioration in water quality in the Mediterranean area due to climate change
- Use of reclaimed water is alternative water source for irrigation water.
  - Promote sustainability of the water in surface and ground bodies
  - Way of restoring nutrients to the ground
  - Water transfers and desalination have significant costs

#### Study objectives:

- Give an example of the use of reclaimed water for irrigation of leafy green fields in the S. Spain
- Monitor indicator and pathogenic microorganisms across leafy green production
- Identify potential microbiological risks to the consumers
- Perform microbiological risk assessment- quantify this risk to the consumers

**Agricultural products irrigated with reclaimed water should ensure safety across EU Ms.**

EU legislation=Wastewater treatment plants operators have the main responsibility

**BUT**

End-users have responsibility for the microbiological quality of the water to produce safe food

*E. coli* for irrigation water is 2 log CFU/100 ml

### 2017-2019, growing cycles of leafy greens in Murcia-Spain (570 samples)

**2 WWTPs with tertiary treatments for water disinfection**

**Different sampling points:** WWTP inlet, WWTP outlet, WWTP reservoirs, grower reservoirs, irrigation (3 types) and leafy greens

**Microorganisms:** *E. coli*, Norovirus I and II, Bacteriophages, HVA, non-O157:H7 Shiga-toxicogenic *E. coli* (STEC), *E. coli* O157:H7 and *Salmonella* spp.

**Modelling the microbial behaviour from WWTP to the crop:** concentration in effluent, removal by tertiary treatment, inactivation in the field and during storage and transport, transfer through irrigation.

**Estimating the health risks to the consumers for different scenarios:** washing removal, leafy greens consumption, dose, food poisoning in different populations, burden of disease.

**What is the risk for the consumers of leafy greens irrigated with reclaimed water?**

Workshop of the EUT+ Sustainability Lab 14 & 15 June, 2021