Interreg
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IR2MA
European Regional Development Fund

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University of Ioannina
HELLENIC REPUBLIC REGION OF EPIRUS
ISPA ISTITUTO DI SCIENZE DELLE PRODUZIONI ALIMENTARI
CIHEAM IAM BARI
CONSORZIO PER LA BONIFICA DELLA CAPITANATA
Regione Puglia

WP5

Deliverable 5.3.2

Experiments regarding the use of treated wastewater for cultivations in greenhouse by using floating hydroponic system

Interreg V- A Greece-Italy Programme 2014 2020

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IR2MA
Large Scale Irrigation Management Tools for Sustainable Water Management in Rural Areas and Protection of Receiving Aquatic Ecosystems

Subsidy Contract No: I1/2.3/27

Project co-funded by
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Partners

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PB2 REGION of EPIRUS (ROE) http://www.php.gov.gr/

PB2 ISTITUTO SCIENZE DELLE PRODUZIONI ALIMENTARI (ISPA/CNR) http://www.ispacnr.it/

PB4 CIHEAM - ISTITUTO AGRONOMICO MEDITERRANEO – BARI (IAMB) http://www.iamb.it/

PB5 CONSORZIO PER LA BONIFICA DELLA CAPITANATA (CBC) http://consorzio.fg.it/

Associated partners

REGION OF PUGLIA (ROP) http://www.regione.puglia.it/

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Notes

Deliverable 5.3.2 - Experiments regarding the use of treated wastewater for cultivations in greenhouse by using floating hydroponic system

The IR2MA case-study: application of different treated wastewater for production of baby leaf vegetables in floating hydroponic system

Involved partners:

Institute of Sciences of Food Production (ISPA) National Council of Research (CNR).

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Project Summary

The main objectives of the InterregIR2MA project are the implementation of advanced smart decision support systems (DSS) for irrigation management, the definition of optimal strategies for the reuse of treated wastewaters for the production of safe and high-quality horticultural products, as well as the dissemination of technological solutions suitable for optimizing water resource uses.
The project involves for the Italian side the Institute of Sciences of Food Production, National Council of Research (CNR-ISPA), Consorzio di Bonifica della Capitanata (CBC), the Mediterranean Agronomic Institute of Bari (CIHEAMB) and Puglia Region as Associate partner, while on the Greek side the University of Ioannina and the Region of Epirus.
Among the several ways to use water, irrigation represents the largest quantity (even up to 70% of the total used volume). Therefore, considering the problem of the climate changes and their effects on the future scenarios in agriculture, it is essential to put in place suitable strategies for optimizing the irrigation practice, avoiding water reservoir pollution and contamination and, where possible, taking advantage of unconventional water usage, for example by reusing wastewater.

Foreword

This report contains a description of the activities carried out by CNR-ISPA in the framework of WP5 - Specialized research actions, Deliverable 5.3.2 - Experiments regarding the use of treated wastewater for cultivations in greenhouse by using floating hydroponic system.

The activities focused on producing different leafy vegetables by using wastewater, with the aim to test treated wastewater for preparing nutrient solution for growing vegetables in floating hydroponic system. We implemented both laboratory and on-greenhouse activities, focusing on three different baby leaf vegetables as the most important ready to eat vegetables crops in horticulture field.

Beside an introductive examination of the state of the art and of the concepts at the base of the carried out activities, a description of experiments and the results is included in this report. We outline that the full dataset from the experiments is currently under consideration for publication in scientific international journals, with the aim to present the outcomes of the IR2MA project to the wide scientific community. The present document contains an exhaustive overview of the findings of the project activities. All the publications that will arise from the activities carried out in the project will clearly refer to the IR2MA project as source of funding for the conducted research.

Introduction

The increase in world population suggests that higher food demand will be expected in the future, in fact, over the past few years, the major challenge of agriculture was to produce “more with less” in order to obtain crops with high nutritional values and with low environmental impact. In addition, the transition to sustainable food systems also represents a huge economic opportunity for farmers (European Commission 2020). However, the increasing population causes intensification of resources used to produce foods, such as soil and water. At the heart of the Green Deal Farm to Fork strategy points to a new system of production, in general more sustainable (European Commission 2020).

Water is a renewable resource, but its availability is limited and variable in relation to countries. Furthermore, in most regions of the world, over 70% of freshwater is used for agriculture. By 2050, feeding a planet of 9 billion people will require an estimated 50% increase in agricultural production and a 15% increase in water withdrawals (FAO, 2017).

There are different approaches that should be combined to properly tackle the current water scarcity, such as: i) improve the water use efficiency; ii) adoption of alternative water resources and iii) improvement of resource management practices by balancing demand and supply (GWP, 2012).

Wastewater is a general term, describing discharge of effluent from wastewater treatment which receives wastewater from households, commercial establishments, and industries. Combined sewer/separate storm overflows are included in this category (European Environment agency, European Directive91/271/cee).

The municipal wastewater is comprised of water together with variable concentrations of organic and different mineral elements deriving from domestic and industrial processes. In different countries wastewater reuse is an important source of water, in fact, in the more arid areas of USA and Australia wastewater is used in agriculture, releasing high quality water supplies for potable use.

A variety of wastewater treatment technologies are available to achieve recycled water of a quality (Bixio et al., 2005). The Membrane BioReactor (MBR) is a wastewater treatment technology resulting from the integration of membrane filtration into the activated sludge process, and it is one of the most important innovations developed in this field (Judd, 2011). However, long term operation of ultrafiltration-based MBRs has shown that the fouling of polymeric membranes causes gradual reduction of their productivity, which can only be contrasted through periodic chemical cleanings (Krzeminski et al., 2017).

An innovative approach that aims to avoid these drawbacks is represented by the Self-Forming Dynamic Membrane BioReactor (SFD MBR), based on the replacement of polymeric ultrafiltration membranes with relatively coarse filtering supports (pore size range 10–100 μm) made of low-cost materials. During the filtration process, the accumulation of mixed liquor-activated sludge on the backing support due to external forces (suction and gravity filtration) results in the growth of a biological cake layer, which is the dynamic membrane. The pores within the dynamic membrane are much smaller compared to those of the filtering support, and therefore the filtration through the dynamic membrane can lead to a treated wastewater of good quality, with values of turbidity close to 1 nephelometric turbidity unit (Hu et al., 2016; Xiong et al., 2016; Vergine et al., 2018). The system’s productivity can be stably maintained through simple mechanical cleaning procedures (Vergine et al., 2018; 2021). The low-cost materials, the low energy needs, and the simple care make the SFD MBR a good substitute for conventional MBR.

In agriculture, treated wastewater represents a resource of strategic importance. In fact, it could satisfy different exigency, such as water and nutrients availability for plant growth (Mastrorilli et al., 2018; Campi et al., 2014). The macro and micronutrients in treated wastewater can help to meet the nutritional needs of crops reducing synthetic fertilizer uses.

The possibility to obtain alternative water source from wastewater treatment is a valid mean in Mediterranean Basin. In fact, in this region, the combination of environmental and geographic conditions, such as absence of rivers or natural lakes, the progressive groundwater salinization and dry weather conditions requires the adoption of alternative and/or non-conventional resources. Different studies reported the possibility to use treated wastewaters as water sources for different typologies of fruit and vegetables production in open filed (Al-Lahham et al., 2007; Perrulli et al., 2021), however, less information are available about the possibility to use the treated wastewater resources in soilless system and their effects on quality of edible products. In these systems the plant roots grow in the nutrient solution with or without growing media. Soilless culture has been used successfully to grow a variety of crops such as aromatic herbs, fruit, flowers and many types of leafy and fruit vegetables. In addition, these system can be used an important tool for produce vegetables with high nutritional profile and low environmental impact (Montesano et al., 2016). The main advantages over conventional production systems are faster growth, higher productivity, easier handling, greater water efficiency (Montesano et al., 2016) and lesser use of fertilizers (Massa et al., 2020). In these systems, macro and micronutrients concentration can be accurately controlled making easier to improve the nutritional quality of edible products (Montesano et al., 2016; Gonnella et al., 2019; D'Imperio et al., 2020) and to observe the symptoms of nutrient deficiency or toxicity in plants (Adrover et al., 2013). Soilless systems can use different sources of water (tap, well and rain water); in general, respect to open field the soilless system require less quantity of water for vegetables production.

Floating system is a common soilless system and it is known as one of the simplest hydroponic systems and is particularly adapt to leafy vegetables. Among cropping systems floating is gaining interest for these kind of productions because it allows to overcome agronomic problems such soil-born disease, it is dynamic and flexible allowing to increase number of cycles and, thus, production. Furthermore, also quality can be enhanced because of the better hygienic-sanitary characteristics and the possibility to modulate plant nutrition so that nitrate continent is reduced (Zanin et al., 2009, Fontana et al., 2004).

However, an alternative source of water could be the treated wastewater. One of the main economic and environmental challenges of the century is to profit from waste management.

The IR2MA case-study: application of different treated wastewater for production of baby leaf vegetables in floating hydroponic system

The objective of this study was to test a sustainable way to produce baby leaf vegetable in floating system based on the use of treated wastewater as water source for preparing nutrient solution. Specifically, we focused on investigating the effects of use wastewater use on the mineral and preliminary organic profile (chlorophylls, carotenoids and polyphneols) of three different species of baby leaf vegetables, mizuna (*Brassica rapa* L.), rocket (*Diplotaxistenuifolia*) and lettuce (*Lactuca sativa* L. lollo group). For each sample, the edible epiphytic part was analyzed, in order to enumerate viable cell density of Enterobacteriaceae spp., Pseudomonas spp. and Escherichia coli microbial populations.
The hypothesis of this study was to use wastewater for preparing nutrient solution for growing vegetables in hydroponic floating system.

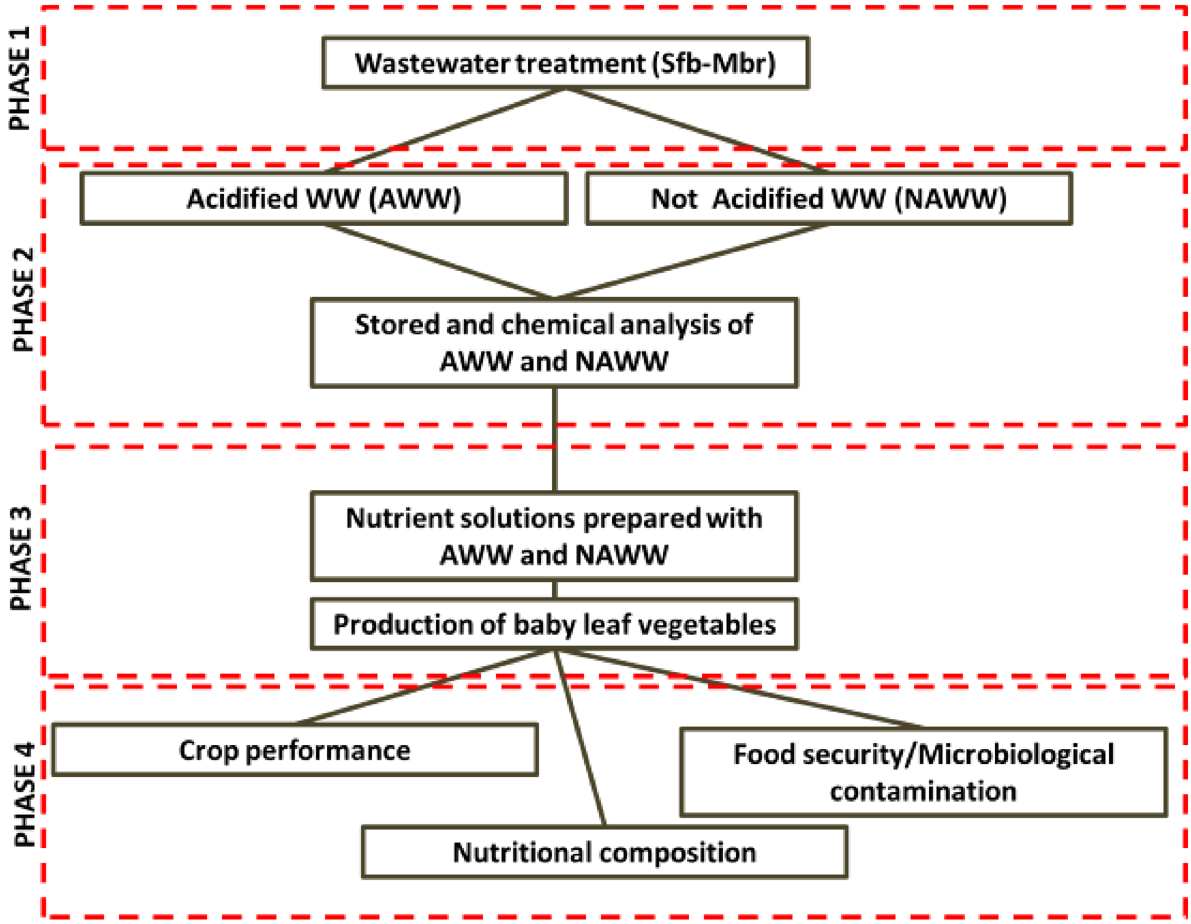

Figure 1. Workflow IR2MA case-study: Application of treated wastewaters for production of baby leaf vegetables in floating hydroponic system.

PHASE 1: Wastewater treatment (in collaboration with IRSA-CNR)

A specific agreement was established between ISPA-CNR (PB3 of IR2MA project) and IRSA-CNR (Institute for Research on Water resources) in the framework of IR2MA activities (Bari, 27/03/2019) with the aim to test treated wastewater obtained with an innovative process studied by IRSA-CNR. Treated wastewater used in the experiments described in this document were implemented using different types of treated wastewater provided by IRSA.

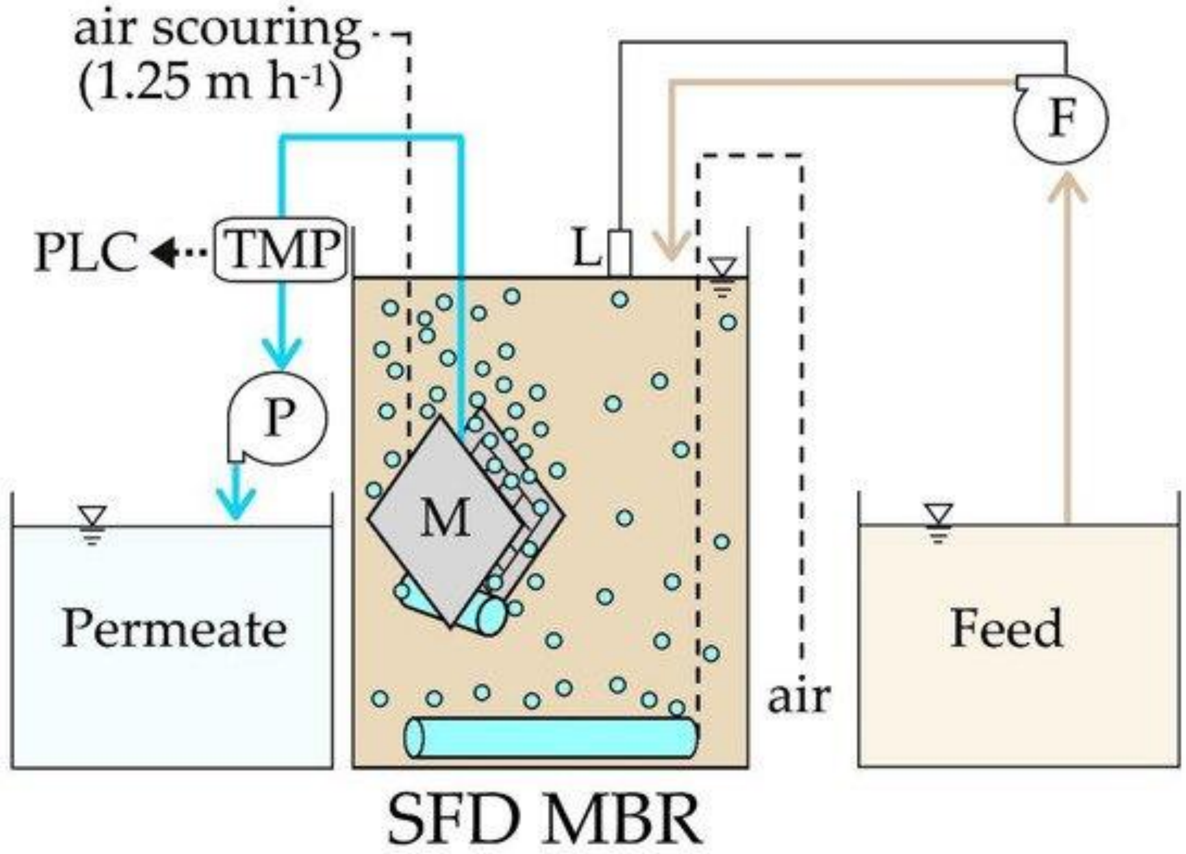

Figure 2. Plant scheme of the Self-Forming Dynamic Membrane BioReactor (SFD MBR). P, permeate pump; TMP, manometer with transmembrane pressure transducer connected to a PLC; M, filtration module; F, feed pump; L, level control connected to F (Vergine et al., 2021).

PHASE 2: Water storing, handling and analysis

Two different treated wastewaters (acidified and not acidified, AWW and NAWW respectively) were stored in two distinct PVC containers (1000 L capacity) in order to obtain enough volume of water for the vegetable growing cycle. The storage period lasted six months. At the end of this period, AWW,NAWW and rain water were analyzed by using ion exchange chromatography for F, Cl, NO_2, NO_3, PO_4 and SO_4, and Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES; 5100 VDV) for Al, B, Ba, Cd, Cr, Fe, Pb, Cu, Mn, Ca, K, Mg, Na and Zn determinations (see paragraph 2.4 for more details). The pH and EC parameters were measured by using pH (WTW InoLab) and conductivity meter (WTW InoLab).

Figure 3. Inductively coupled plasma - optical emission spectrometer (ICP-OES) and ion exchange chromatography used for chemical analysis of treated wastewater.

PHASE 3: Plant Materials and Experimental Conditions

Two experiments were conducted in a plastic greenhouse located at the experimental farm "La Noria of the Institute of Sciences of Food Production (ISPA-CNR) located in Mola di Bari (BA), Southern Italy (41°03′ N, 17°04′ E; 24 m a.s.l.).

Figure 4. Experimental station "La Noria", CNR – ISPA (Mola di Bari, BA, Italy).

The first experiment was carried out from 23 September to 17 October, 2019, while the second experiment was carried out from 28 October to 26 November, 2019. Mean air temperature, relative humidity, and photosynthetically active radiation (PAR) inside the greenhouse during the experiments were: 23 °C, 69.3%, and 200 µmol/m2/sec and 18 °C, 75.2%, and 135 µmol/m^2/sec during second experiment. In both experiments mizuna *(Brassica rapa* L.), rocket (Diplotaxistenuifolia) and lettuce (*Lactuca sativa* L. group lollo) were growth. Major information about species were reported in table 1.

Table 1. Vegetable scientific and common name, sowing and harvest dates, cycle length, of the experiment.

Scientific name	Common name	Sowing	Harvest	Cycle length (days)
I experiment				
Brassica rapa L.	Mizuna	23/09/19	17/10/19	25
Diplotaxis tenuifolia	Rocket			
*Lactuca sativa*L	Lettuce, Lollo green			
II experiment				
Brassica rapa L.	Mizuna	28/10/19	26/11/19	29
Diplotaxis tenuifolia	Rocket			
*Lactuca sativa*L	Lettuce, Lollo Red			

The seeds were sown in cell pots containing peat. When seedlings reached the two-true leaf stage (after seven and six days from sowing, respectively, for the first and second experiment) the cell pots were moved on a floating hydroponic system, where plants were grown up to the end of the experiments.

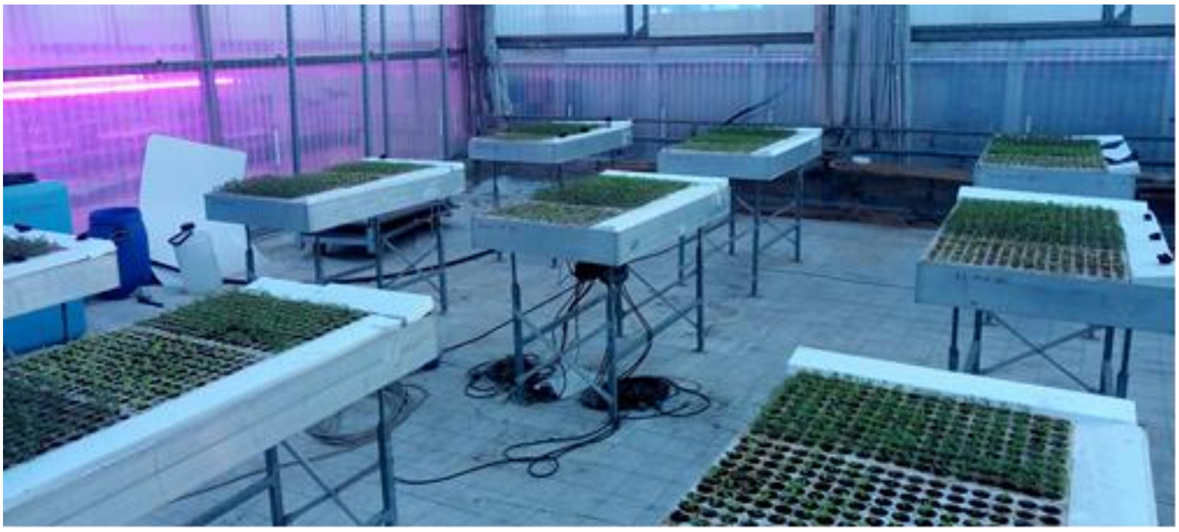

Figure 5. Mizuna (*Brassica rapa* L.), rocket (Diplotaxistenuifolia) and lettuce (*Lactuca sativa* L. group lollo) growing in floating hydroponic system.

The vegetables were grown with nutrient solutions (NS) prepared with rain water, NAWW and AWW. The NS were adjusted to contain similar levels of macronutrients for both irrigation treatments. The compositions of rain water, and treated waters (NAWW and AWW) were reported in table 1 .The NS pH was measured every three days and it was adjusted to 5.5–6.0 using 1 M H_2SO_4. An air pump was used in order to promote oxygenation of the NS and avoid possible root anoxia issues.

Table 2. Mineral elements detected in the treated and rain waters used for preparing nutrient solution.

Macro-nutrients								
	pH	**EC**	**N_NO3**	**P tot**	**Ca**	**K**	**Mg**	**Na**
		(µS/cm)			mg/L			
NAWW	7.6	1876	53.25	8.10	86.15	30.70	43.44	233.561
AWW	4.3	1819	49.15	10.10	88.00	29.29	41.60	220.725
RW	7.0	60.8	n.d.	n.d.	4.68	5.03	0.69	4.575

Micro-nutrients					
	Fe	**Mn**	**B**	**Cu**	**Zn**
			mg/L		
NAWW	0.037	0.025	0.104	n.d.	0.539
AWW	0.023	0.036	0.054	n.d.	0.992
RW	0.010	0.047	0.003	n.d.	1.443

*not detected.

Figure 6. Mizuna (*Brassica rapa* L.), rocket (Diplotaxistenuifolia) and lettuce (*Lactuca sativa* L. group lollo) harvest at phenological stage of fourth or fifth true leaf.

A split-plot design, with nutrient solutions in the main plot and vegetable species in the sub-plot, with three replications was used. Each replication was constituted of 160 plants.

CTR	**Mizuna Rocket Lettuce**	**NAWW**	**Mizuna Rocket Lettuce**	**AWW**	**Rocket Mizuna Lettuce**

NAWW	**Lettuce Rocket Mizuna**	**CTR**	**Rocket Mizuna Lettuce**	**NAWW**	**Rocket Lettuce Mizuna**

AWW	**Lettuce Mizuna Rocket**	**AWW**	**Rocket Mizuna Lettuce**	**CTR**	**Lettuce Mizuna Rocket**

Figure 7. Split-plot design.

PHASE 4: yield and chemicals characterization of baby leaf vegetables

At the harvest, 25 days after sowing in the 1st experiment and 29 day in the 2nd experiment, yield [expressed as kg of fresh weight (FW) m^{-2}] was evaluated. Fresh baby leaf samples were maintained in a forced draft oven at 65 °C until constant weight for the measurement of dry weight (DW). For each replication, baby leaf vegetable samples were freeze-dried by Freeze Dry System (LABCONCO 7754030, Kansas City, MI, USA). The freeze-dried samples were ground at 500 µm by using a laboratory mill (Retsch, Torre Boldone, BG, Italy) to obtain a homogeneous powder samples.

The total phenol (TP) content was determined according to the Folin–Ciocalteu method by using the extraction methods reported by D'Imperio et al., (2020). Briefly, 200 mg of lyophilized sample were mixed with 10 mL of solvent mixture ($MeOH:H_2O:CH_3COOH$, 79:20:1% v/v/v). The vials were then placed in a sonicator bath at ambient temperature for 30 min, followed by 1 h in a magnetic stirrer. The mixture was centrifuged at 10,000 x g at 4 °C for 10 min and the supernatant was transferred into a volumetric tube. The residue was resuspended in 10 mL of $MeOH:H_2O:CH_3COOH$ (79:20:1% v/v/v), gently mixed manually, and sonicated for an additional 30 min, followed by stirring (1 h) and centrifugation (10,000 x g at 4 °C 10 min). The TP content was determined using gallic acid (R2 = 0.9999) as a calibration standard by using a Perkin–Elmer Lambda 25 spectrophotometer (Boston, MA, USA).

Chlorophylls (CHLa, CHLb and CHLTOT) and total carotenoid contents were determined spectrophotometrically, using the extraction procedure reported by Montesano et al., (2018). Briefly, lyophilized samples were homogenized in a fresh solution of 80% acetone ($C_3H_6O:H_2O$, v/v) and stirred for 24 h at room temperature. After extraction, the samples were diluted and filtered by using 0.45 µm (RC) and the absorbance of the extracts were measured at 662, 645 and 470 nm, using a UV-1800 spectrophotometer (Perkin–Elmer Lambda 25 spectrophotometer, Boston, MA, USA).

PHASE 4: minerals and Anions Analysis baby leaf samples

For NO_3, SO_4 and Cl, determinations, ion exchange chromatography (Dionex DX120, Dionex Corporation, Sunnyvale, CA, USA) with a conductivity detector was used as re-ported by D'Imperio et al., (2020). Briefly, the anions were extracted from DW samples with 3.5 mM (Na2CO3) and 1 mM (NaHCO3) for 30 min. After extraction, the samples were diluted and filtered by using 0.45 µm followed by Dionex OnGuard IIP (ThermoScientific). The resulting solutions were analyzed by ion chromatography (IC-Dionex DX120, Dionex Corporation) with a conductivity detector by using an IonPac AG14 pre-column and an IonPac AS14 separation column (Dionex Corporation). For Al, B, Ba, Fe, Mn, Ca, K, Mg, Na, Mo, Sr, Co, Cu and Zn determination, 0.3 g of dried sample were mineralized with 10 mL of 65% HNO_3 (Pure grade, Carlo Erba) in a closed-vessel microwave assisted digestion system (MARS 6, CEM Corporation, Matthews, NC, USA). The digestion procedure was performed in two steps: 15 min to reach 200 °C and 10 min maintained at 200 °C (power set at 900–1050 W; 800 psi). The digested solutions were cooled at room temperature. The mineralized samples were diluted with ultrapure H_2O (Milli-Q Millipore 18 M Ω/cm) and filtered using a 0.45 µm filter. Samples were analyzed with a Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES; 5100 VDV, Agilent Technologies, Santa Clara, CA, USA) to measure Ca, K, Mg, and Na in radial mode and Al, B, Ba, Fe, Mn, Mo, Sr, Co, Cu and Zn in axial mode. In addition, accuracy and precision of chemical analysis (NO_3, Ca, K, Mg, Na, Al, B, Cr, Mn, Zn and Fe) were evaluated by using two different certified reference materials (CRM): NIST_1573a —tomato leaves and SPIN-1_spinach. The certified and experimental value of CRM are provided in supplementary material (Table 3). The limits of detection (LOD) and the limit of quantification (LOQ) of the methods were calculated with standard deviation (sd) of the blank (n=10), LOD (sd x 10) and LOQ (sd x 10).

Table 3. Concentrations obtained for minerals and NO_3 determination in NIST-1573a and NRC/SPIN-1(certified reference material).

	LOD	LOQ	Certified value and uncertainty	Experimental value and uncertainty
	µg/l		mg/kg of DW	
NIST-1573a				
Al	0.03289	0.0996	598±7.10	575±19.6
B	0.59262	1.7958	33.1±0.42	32±6.1
Ca	0.20223	0.6128	50,450±550	50051±2049
Cr	0.20801	0.6302	1.988±0.034	2.075±0.2913
Fe	0.81112	2.4579	367±4.37	355±27
K	15.9942	46.467	26,760±480	25,611±1100
Mg	0.34969	1.0596	12000	12,758±2949
Na	0.07113	0.2155	136±3.70	140±19
Mn	0.74266	2.2504	246±7.11	231±1.6
Zn	1.45585	4.4116	30.9±0.55	29.9±4.9
NRC-SPIN-1			mg/g of DM	
NO_3			22.5±0.43	22.9±0.50

Mg: Non-Certified Value. Insufficient information is available to assess the uncertainty associated with the value, and therefore no uncertainty is provided (NIST).

PHASE 4: microbiological analyses

Microorganisms naturally contaminating the edible epiphytic vegetable parts were enumerated immediately after the harvest. For each vegetable, mizuna (*Brassica rapa* L.), rocket (*Diplotaxis tenuifolia*) and lettuce (*Lactuca sativa* L. group lollo), similar amount of each baby leaf were recovered, under microbiological safety cabinet, from 3 replications in order to obtain a single pooled sample of about 100 g. This sample was manually cut with a sterile blade, and after a gentle hand mixing, about 15 g were aseptically transferred into a stomacher bag containing 9 parts (w/w) of sterile saline solution (0.9% NaCl) and homogenised for 2 min using a stomacher (Bag Mixer, Interscience, Saint-Nom, France). The saline solution containing microorganisms removed from the epiphytic part of each sample was decimally diluted and plated in triplicate on different selective agar media for the enumeration of Pseudomonas spp, *Enterobacteriaceae* spp. and Escherichia coli viable cells as previously reported (Pinto et al., 2015; ISO 16649-1:2018).

Results and considerations

Experiment 1

The wastewater used did not modified the crop performance parameters evaluated in this study, in fact, no differences were found in the first experiment as regard yield and dry matter. Similar trend result was founded for leaf area, leaf number and roots FW and dry matter. The average values of leaf area, leaf number and roots fresh weight dry matter were 3.59 g/plant, 81.5 cm^2/plant, 11.5 n/plant, 0.34 g/plant and 5.52 g/100 g of FW, respectively. In addition, in all treatments no phytotoxicity effects on plants were observed. On the contrary, huge differences in relation to the species were found. Respect to the rocket the baby leaf vegetables of mizuna and lettuce showed high value of yield, leaf area and roots FW; while, rocket had a higher number of leaves compared with mizuna and lettuce. As regard DM, the major value was found in mizuna (6.35 g/100 g of FW) followed by rocket (6.09 g/100 g of FW) and lettuce (4.11 g/100 g of FW). The baby leaf vegetables are known to be rich sources of bioactive compounds, such as polyphenols, glucosinolates, ascorbic acid, carotenoids, and tocopherols, which have human-health effects reportedly involved in preventing cardiovascular diseases and some types of cancers. In our study the use of NAWW and AWW, for prepare NS, allowed to improve the TP content in edible parts of all baby leaf vegetables present in this current study, respect to control treatment, with an increase to 51% and 39%, respectively for WW and AWW. The species with high content was mizuna, followed by rocket and lettuce. The increase of TP in edible parts of vegetables might be related with Na content in both wastewater. In general, high increase of TP were founded when the plants were growing in stress (biotic and/or abiotic) conditions as reported (Lattanzio 2013; Nedbal et al., 2000). The NO3 content in vegetables is an important nutritional and consumer health parameter to be considered for quality of fresh leafy vegetable products because this anion is listed as anti-nutritional factor in vegetables (Santamaria, 2006). In fact, as suggested by European Food Safety Authority (EFSA, 2008) the current acceptable daily intake for this element is 3.7 mg/kg of body weight. The use of AWW and NAWW did not induce an increase of NO_3 content in edible part of baby leaf vegetables. In addition, in our study, the NO3 contents in mizuna, rocket and lettuce plant tissues were, in general, lower respect to limit imposed by Commission Regulation (EU) No 1258/2011 for other leafy vegetables, such as lettuce (3–5 g/kg of FW) and rocket (6–7 g/kg of FW).The Na content in baby leaf vegetables produced by using WW, was higher respect to our CTR treatment. This result is certainly due to the presence of high concentrations of Na in WW. In addition, in all species the contents of Cd, Cr, Li, V and Ni was below the limit of quantification.

Experiment 2

In the second trial experiment the species with high yield value was mizuna (2.79 g/plant), followed by lettuce (1.93 g/plant) and rocket (1.33 g/plant). In mizuna the use of NAWW allowed to obtain an increase of yield, on the contrary, using AWW did not induce in mizuna and rocket statistical differences about this parameter. However, in all species the use of both WW did not induce phytotoxicity effects on plants. In the rocket the NAWW and AWW, respect to control treatment, allowed to obtain a slight increase of production, while in the lettuce both WW did not induce reduction of yield. Regarding leaf area, the major value was found in mizuna produced by using NAWW (73.5 cm^2/plant), in this species no differences were observed between control treatment and AWW treatment. Also in the rocket, the treatments, in this case with both wastewaters allowed to obtain a slight increase of this parameter, major with NAWW treatment. On the contrary, in lettuce the use of both wastewater reduced leaf area, with a major reduction in lettuce grown by using NAWW.

The kind of water used for NS did not modify leaf number and DM. The average value of leaf number and DM were, respectively, 14.1 and 5.07 g/100 g of FW. The use NAWW highly increased root FW respect to the control and AWW treatments. Others differences were found in relation to species, such as leaf number and DM. As for yield and leaf area parameters, also TP showed statistical interaction ($p \leq 0.001$) between water and species. In mizuna and rocket plants the use of NAWW and AWW allowed to increase TP, greater in rocket (+51% and 98% respectively for NAWW and AWW). As regard lettuce, no differences were observed among treatments. In general, the water used for preparing NS can modify the ions content. The use of both wastewater did not modify the NO3 content, while differences in relation to species were found ($p<0.001$). The rocket was the species with the highst NO_3 content, followed by mizuna and lettuce. In all species the control treatment showed low content of Na, moreover, the use of both wastewaters allowed to increase more this mineral element in edible part, with major increase in mizuna produced by using AWW (13.81 mg/kg of DW). In addition, in all species the contents of Cd, Cr, Li, V and Ni was below the limit of quantification.

As regards the microbial analyses of edible baby leaf vegetables, no samples showed viable E. coli cells, whereas viable populations of *Enterobacteriaceae* and *Pseudomonas* spp. ranged from 2.5-6.5 logufc/g fresh weight depending on vegetable. The use of waste water resulted in a marked reduction (up to 100 times) in the population of *Pseudomonas* spp. in rocket and (up to 10 times) of Enterobacteriaceae in lettuce. In all other cases, the values found were not significantly different between the two types of water used, and in line with those of vegetables for fresh consumption.

Conclusions

The use of non-conventional water resources can help to mitigate water stress and might support the agricultural sector. Treated municipal wastewater is one of the most readily available alternative water resources, and its use in agriculture should be adopted to reduce fresh water usage in several countries, under their respective water quality regulations. In our experiments the use of two different waste waters (acidified and not) did not modified the crop performance and nutritional quality of three different baby leaf vegetables.

The results of microbiolgical analyses of samples of this research activity indicate a good level of hygiene of the vegetables when harvested, which can be used as fresh-cut products.

Synopsis in English language

The objective of this study was to test a sustainable way to produce baby leaf vegetable in floating system based on the use of treated wastewater as water source for preparing nutrient solution. Specifically, we focused on investigating the effects of wastewater use on mineral and preliminary organic profile (chlorophylls, carotenoids and polyphenols) of three different species of baby leaf vegetables, mizuna (*Brassica rapa* L.), rocket (*Diplotaxis tenuifolia*) and lettuce (*Lactuca sativa* L. lollo group). For each sample, the edible epiphytic part was analyzed, in order to enumerate viable cell density of Enterobacteriaceae spp., Pseudomonas spp. and Escherichia coli microbial populations.

In our experiments the use of waste water (acidified and not) did not modified the crop performance and nutritional quality of three different baby leaf vegetables. The results of microbiological analyses of samples of this research activity indicate a good level of hygiene of the vegetables when harvested, which can be used as fresh-cut products.

Σύνοψη στην ελληνική γλώσσα

?? Synopsis in Greek language – not obligatory

Sinossi in lingua italiana

Tra le fonti irrigue alternative, le acque reflue depurate possono garantire un approvvigionamento idrico costante nel tempo e indipendente dalla variabilità del clima. La presenza, in questo tipo di acque, di macro e microelementi utili per le piante consentirebbe, inoltre, di ridurre la quantità di fertilizzanti di sintesi utilizzata.

In Italia, un limite all'utilizzo irriguo di questa tipologia di acque è rappresentato dagli stringenti vincoli relativi ai parametri chimico-fisici e microbiologici imposti dalla vigente legislazione nazionale e regionale. Al contrario, le acque reflue depurate possono rappresentare una risorsa di non trascurabile entità, soprattutto quando utilizzate con sistemi di coltivazione sostenibili che garantiscano la salvaguardia ambientale e produzioni orticole di qualità, come nel caso dei sistemi fuori suolo a ciclo chiuso.

Per verificare la possibilità di utilizzare le acque reflue depurate per la preparazione della soluzione nutritiva nella coltivazione di specie da foglia per il consumo fresco, sono state allestite prove sperimentali in serra presso l'azienda sperimentale La Noria di Cnr-ISPA.

L'acqua reflua depurata, necessaria per la formulazione della soluzione nutritiva, è stata fornita dall'IRSA-CNR ed è stata ottenuta mediante sistema sperimentale Sfd-Mbr. Questa tecnologia unisce i vantaggi dei processi biologici di depurazione a quelli della separazione solido/liquido mediante filtrazione di superficie.

La prova sperimentale è stata condotta in *floating system*. La semina di rucola, lattuga e mizuna è avvenuta in pannelli alveolati riempiti con torba e posti su bancali. Le piante sono state inizialmente irrigate con sola acqua piovana; a partire dalla fase fenologica "cotiledoni completamente distesi", è stata utilizzata soluzione nutritiva tipo Hoagland preparata con acqua di pioggia o con acqua reflua depurata, acidificata e non acidificata, precedentemente analizzata. La formulazione della soluzione nutritiva è stata effettuata in funzione della dotazione di elementi nutritivi rilevati.

Al momento della maturazione commerciale sono stati registrati la produzione per unità di superficie, il contenuto di nitrati e di sodio nelle foglie. Sulle parti eduli è stata determinata anche la presenza di *Enterobacteriaceae* spp., *Pseudomonas* spp. ed Escherichia coli.

Dai risultati della prova risulta che l'uso di acque reflue depurate per la preparazione della soluzione nutritiva può consentire di ottenere risultati sovrapponibili, in termini quantitativi e qualitativi, a quelli ottenuti con acqua piovana. Ciò permette, al tempo stesso, di ridurre l'utilizzazione di acqua di buona qualità e di fertilizzanti. Se da un lato è auspicabile che il legislatore consenta l'utilizzazione di acque reflue depurate con un grado di affinamento finalizzato al tipo di riutilizzo, d'altro canto va posta particolare attenzione alla possibile contaminazione biologica o chimica dei prodotti. In particolar modo quando sono destinati al consumo fresco. A tale proposito, l'implementazione di un sistema di controllo qualità e la scelta di un sistema di coltivazione che crei una barriera fisica tra parte edule e soluzione nutritiva può garantire l'ottenimento di un prodotto sicuro per il consumatore.

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