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Research article

Agricultural reuse of municipal wastewater through an integral water reclamation management

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ABSTRACT

The DESERT-prototype, a state-of-the-art compact combination of water treatment technologies based on filtration and solar-based renewable energy, was employed to reclaim water for agricultural irrigation. Water reclaimed through the DESERT-prototype (PW) from a secondary effluent of a wastewater treatment plant, as well as conventional irrigation water (CW) and the secondary effluent (SW) itself, were employed to cultivate baby romaine lettuces in a greenhouse in Murcia (Spain), by means of drip and sprinkler irrigation methods, thus establishing six treatments. Assessments of physicochemical and microbiological quality of irrigation water, as well as agronomic and microbiological quality of crops from all treatments, showed that results associated to PW complied in all cases with relevant standards and guidelines. In contrast, results linked to SW and CW presented certain non-compliance cases of water and crop microbiological quality. These assessments lead to conclude that the DESERT-prototype is an appropriate technology for safe water reclamation oriented to agricultural production, that can be complemented by a proper irrigation method in reaching safety targets.

1. Introduction

Water scarcity is a main issue currently affecting a large part of the global population (Eslamian, 2016). Along with this reality, agriculture stands out as the economic sector with the highest water demands, representing about 70% of global freshwater withdrawals worldwide (Eslamian, 2016; The World Bank Group, 2016). Moreover, 28% of the global cropland and 56% of the global irrigated cropland are located in areas under high (40–80%) or extremely high (>80%) water stress, based on the ratio of water withdrawal over available water (Gassert et al., 2013). In this sense, water reclaimed from municipal wastewater has become one of the major and less expensive non-conventional water sources for agriculture (Drechsel et al., 2015; Eslamian, 2016), which is, with roughly 20 out of 200 million Ha of irrigated land worldwide (Jaramillo and Restrepo, 2017), the largest reclaimed water consumer (Lazarova et al., 2013), and one of the economic sectors in which its use shows its real benefits (Younos and Parece, 2016).

Agricultural irrigation with reclaimed water brings several advantages: reduction of pressure over freshwater sources (Eslamian, 2016; Parsons et al., 2010), presence of nutrients that reduce the use of synthetic fertilizers (Lyu et al., 2016; Pedrero et al., 2013b; Vicente-Sánchez et al., 2014; Vivaldi et al., 2015), higher yields than freshwater-irrigated counterparts (Vergine et al., 2016; Vivaldi et al., 2015), amongst others. Contrariwise, water reclamation mismanagement may also arise negative impacts for both the environment and human health (Eslamian, 2016; Lazarova et al., 2013). Probably the most recognized and characterized concern is the presence of pathogens that may enter the food chain (Castro-Ibáñez et al., 2015; López-Gálvez et al., 2016b). Furthermore, crops and soils may be affected due to increasing salinity (Jiménez and Asano, 2008; Pedrero et al., 2010), phytotoxic elements can

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affect growth of crops reducing yields (Parsons et al., 2010; Pedrero, 2010), and the structure of soils may result damaged due to high sodicity levels (Jiménez and Asano, 2008; Pedrero, 2010).

There are several studies focused on the effects of different reclaimed water sources over tree crops (Nicolás et al., 2016; Pedrero et al., 2013a, 2013b; Pedrero and Alarcón, 2009; Vivaldi et al., 2015), as well as horticultural crops (Cirelli et al., 2012; Hoque et al., 2010; López-Gálvez et al., 2016b, 2014). From the latter, health risks associated to the intake of raw-eaten leafy greens irrigated with reclaimed water, such as lettuces, take particular attention (Castro-Ibáñez et al., 2015; Ceuppens et al., 2015; Makkaew et al., 2016; Sales-Ortells et al., 2015). In this respect, several studies highlight the relevance of irrigation methods in reducing health risks (López-Gálvez et al., 2016b; Qadir et al., 2010; Uyttendaele et al., 2015). Irrigation methods are one of the most relevant interventions within the World Health Organization (WHO) 'multiple-barrier approach', which offers, besides wastewater treatment, strategies at key points that aims to a safe agricultural production, avoiding recontamination and cross-contamination within the farm-to-fork cycle (Al-Baz et al., 2008; Drechsel et al., 2015, 2010; WHO et al., 2006).

Despite the wide amount of studies remarking its obvious advantages, reclaimed water is still a largely underused resource: barely 15% of the generated wastewater and 41% of the treated wastewater are destined worldwide for agricultural irrigation in around 20 million Ha (Jaramillo and Restrepo, 2017; Valipour and Singh, 2016). Besides, a major problem in many countries is the lack of appropriate criteria and realistic standards for using reclaimed water (Paranychianakis et al., 2015). In some cases, specific criteria and guidelines are adapted from other contexts, thus being not correctly oriented to local realities (Fulazzaky, 2010, 2009; Jeong et al., 2016; Norton-Brandão et al., 2013), whereas in other cases appropriateness of water for different uses still needs to be verified (Fulazzaky, 2013). A broad range of water reclamation technologies is available nowadays, being virtually able to achieve any required quality (Lazarova et al., 2013). However, the trend on agricultural-oriented water reclamation is the fit-to-purpose combination of technologies, mainly filters and membranes following a conventional (primary or secondary) treatment (Wang et al., 2011), whose aims are: regulation of salinity levels to the crops' needs, retention of valuable nutrients in the reclaimed water, and reduction of pathogenic loads to safe levels for irrigation (De La Cueva Bueno et al., 2016; Lazarova et al., 2013; Norton-Brandão et al., 2013). Low-cost compact technologies, suitable for rural croplands, can be obtained by coupling solar energy able to tackle with the costs that energy demand of high-pressure membranes implies (De La Cueva Bueno et al., 2016; Lazarova et al., 2013). Furthermore, incorporation of fertigation equipment to these compact, off-the-grid reclamation trains, would offer an ultimate solution to agricultural needs, such as the case of the DESERT-prototype (see supplementary information) of the DESERT project (Water JPI, 2016).

To foster and increase the practice of irrigating with reclaimed water while effectively coping with the associated risks, it is necessary to involve different factors beyond reclamation technologies: irrigation methods, quality of waters, type and quality of crops, risk assessments, amongst others (Cirelli et al., 2012; Valipour and Singh, 2016). However, there is a severe lack of literature addressing irrigation of reclaimed water for horticultural production from a holistic point of view, encompassing the aforementioned factors (Norton-Brandão et al., 2013). In this context, the goal of the present study is to evaluate, from an integral perspective and under the scope of current standards and guidelines, the effects that different (conventional and non-conventional) irrigation sources and methods may have over physical, chemical and microbiological qualities of soil-cultivated lettuces.

2. Materials and methods

2.1. Experimental set up

Baby romaine lettuces (*Lactuca sativa* var. *romana*) were grown between November 2016 and January 2017 (60 days), in a 680 m^2 greenhouse located in the *Roldán, Lo Ferro y Balsicas* municipal wastewater treatment plant (WWTP) facilities in Murcia, Spain (latitude 37° 47′ 48″ N, *longitude* 0° 57′ 36″ W). Inside the greenhouse, average temperature, relative air humidity, and daily transpiration were 15 °C, 67%, and 0.5 L m^{-2}, respectively. The crops were cultivated on silty clay loam with average pH and electrical conductivity (EC) values of 7.6 and 1.7 dS m^{-1}, respectively. Lettuce was the selected crop because its growth and nutritional composition is largely influenced by salinity stress (Kim et al., 2008). Furthermore, it is a highly representative horticultural crop for assessing safe agricultural production: it is the most common raw-eaten vegetable and its leafy configuration may protect pathogens from light and desiccation, thus promoting their persistence (Petterson et al., 2001).

2.2. Irrigation water sources and methods

Three types of water were used for irrigation: 1) water reclaimed through the DESERT-prototype (PW), 2) conventional irrigation water (CW), and 3) the secondary effluent (SW) from the WWTP. PW was reclaimed after feeding SW to the DESERT-prototype, whose reclamation train consists of two 130 μm disk filters, one 0.08 μm capillary ultrafiltration (UF) membrane module, one granular activated carbon filter, and four composite polyamide multi-pass reverse osmosis (RO) membrane elements, powered by eight 54.7 V monocrystalline-cell photovoltaic (PV) panels. CW was provided by an irrigation community, and is a mix of different conventional and non-conventional sources: Tajo-Segura water transfer (88.7%), Segura river basin (3.0%), reclaimed water from WWTPs (6.7%) and Mojón desalination plant (1.6%) (C.R.C.C., 2017). This water was mainly used for agronomic quality control due to its appropriate salinity levels. SW was obtained from the WWTP, after a treatment that consisted of pre-treatment (coarse screen, fine screen, sieving, degritter and degreaser), double-stage activated sludge with prolonged aeration, and secondary clarifier. SW was employed as a model of water with low microbiological quality. Irrigation waters were fertilized based on their initial concentration of nutrients. In terms of $N - P_2O_5 - K_2O$, the fertilization throughout the experiment was 60.4 − 20.0 − 75.5 kg ha^{-1} (balance 1 − 0.33 − 1.25), respectively.

The total irrigated water amount of 1163 m^3 ha^{-1} was applied through two irrigation methods: drip irrigation (DI) and sprinkler irrigation (SI), with a flow of 2 and 40 L h^{-1}, per dripper and micro-sprinkler, respectively. These were chosen because they are the most representative systems for growing vegetables (FAO, 2017), and due to their opposite ways of exposing crops to irrigation waters, leading to different consequences regarding microbiological risk (Jiménez and Asano, 2008; Uyttendaele et al., 2015). To ensure that irrigation demands of lettuces were fully covered, soil moisture was kept at field capacity. Moisture tension at 15 cm-depth (root zone) was daily monitored using ceramic cup tensiometers (Irrometer, USA), resulting in a range of 10.6–12.9 kPA.

Combining the three waters (PW, CW, SW) and the two irrigation systems (DI, SI), six treatments were set: PW-DI, PW-SI, CW-DI, CW-SI, SW-DI, SW-SI, with four replicates each (Figure 1 of supplementary data). A total of 144 lettuces were planted per each treatment plot (spacing of 12 plants m^{-2}), on ridges using a

randomized design, thus a total of 864 lettuces in the whole area.

2.3. Irrigation water analyses

For physicochemical analyses, the different types of irrigation water were grab-sampled biweekly (8 samples) during the experimental period, in clean, non-sterile bottles (not intended for microbiological analyses) that were first rinsed and filled with the corresponding water. Once transported to the lab, samples were stored at 5 °C before processing them. Macronutrients (NO_3^-, PO_4^{3-}, K, Ca, Mg), micronutrients (Fe, Mn, Zn, Cu), phytotoxic elements (B, Cl^-, Na) and metals were analyzed by mass spectrometry, using an Inductively Coupled Plasma spectrometer (ICP-ICAP 6500 DUO Thermo, England), and by ion chromatography, using a chromatograph (Metrohm, Switzerland). Samples for both spectrometry and chromatography were previously filtered using 45 μm filters, and stored in 10 mL test tubes. EC and pH were measured with a multiparameter equipment Eutech PC 2700 (Eutech instruments, Singapore). Turbidity was analyzed with a turbidity meter Dinko D-110 (Dinko Instruments S.A., Barcelona, Spain). Total Suspended Solids (TSS) were measured by filtering, drying and weighing water samples. Sodium Adsorption Ratio (SAR) was calculated based on the relation between soluble sodium and soluble calcium and magnesium divalent cations ($SAR = Na^{2+}/\sqrt{(Ca^{2+} + Mg^{2+})/2}$) (Jiménez and Asano, 2008). Sampling and analyses complied with the last edition of Standard Methods for the Examination of Water and Wastewater (American Public Health Association et al., 2012).

Regarding microbiological analyses, concentration of *E. coli* and presence of pathogenic bacteria (*Salmonella* spp. and Shiga-toxigenic *E. coli* (STEC)) were assessed in the different types of irrigation water. For *E. coli* analysis, three daily replicate samples of 2 L per irrigation water were taken in sterile containers, during the last three days of the lettuce growing cycle. Samples were refrigerated with ice using a fridge bag while on transit to the lab. Concentration of culturable *E. coli* was assessed by plating samples in Chromocult coliform agar (Merck, Darmstadt, Germany). After incubation for 24 h at 37 °C, colonies in dark blue-violet color were considered positives for *E. coli*. This microorganism was selected because it is considered the most precise indicator of fecal contamination (Tallon et al., 2005; WHO, 2011). For pathogenic bacteria analyses, two samples per treatment were taken each sampling day. Samples had a volume of 10 L, and were filtered through Modified Moore Swabs (MMS) prepared following the protocol of Sbodio et al. (2013). Water was pumped through the swabs, at the greenhouse, using sprayer pumps (Geolia, Lille, France). Swabs were then transported to the laboratory in refrigerated conditions. At the laboratory, MMS were placed in sterile stomacher bags in aseptic conditions. Buffered peptone water (BPW, 20 g L^{-1}) was added to the bags to cover the MMS, which was then massaged by hand for 1 min before incubating for 24 h at 37 °C. After the incubation period, 7 mL of enrichment were transferred to sterile tubes and mixed with glycerol (3 mL). Tubes were kept at −20 °C until DNA extraction was performed. For the DNA extraction, the Extraction Pack Food 1 (Pall Corporation, Port Washington, USA) was used. For the RT-PCR (detection of RNA expressions) analyses of water samples, GeneDisc Shiga Toxic *E. coli* & *Salmonella* spp. discs were used in a Genedisc Cycler (Pall Corporation, Port Washington, USA) following manufacturer instructions. When presumptive positive samples for *Salmonella* spp. were detected, confirmation by isolation in culture media from frozen samples was performed using the IBISA method (AES Chemunex, Bruz, France). Samples positive for STEC other than *E. coli* O157:H7 were confirmed by isolation in Chromagar STEC culture media (CHROMagar, Paris, France). Finally, samples positive for *E. coli* O157:H7 were confirmed by isolation in CHROMagar O157 (CHROMagar, Paris, France) and CT-SMAC (Scharlab, Barcelona, Spain) culture media, followed by further confirmation using the *E. coli* O157 Latex Test Kit (Oxoid, Basingstoke, UK).

2.4. Baby romaine lettuce analyses

For analyzing agronomic quality, sampling of lettuces was performed at their growth stage 49, according to the BBCH scale (Meier, 2001), when the produce reached commercial size. Each sample consisted of a whole lettuce head cut from its base, removing traces of soil. To analyze commercial weight, total N and C concentrations, macronutrients, micronutrients, and metals, three lettuces per treatment were randomly sampled during two consecutive days, thus 36 lettuces in total.

Fresh (whole lettuce) and commercial (cleaned lettuce, without outer leaves) weight were measured on-site, immediately after harvesting and drying the lettuces with paper towels, to avoid inaccuracies due to water losses in the plants and/or external moisture. For measuring dry weight, lettuces were dried for at least 2 day at 65 °C. Percentage of water content in lettuces was calculated based on fresh and dry weights values. To analyze C concentrations, macronutrients (total N, NO_3^-, PO_4^{3-}, K, Ca, Mg), micronutrients (Fe, Mn, Zn, Cu), phytotoxic elements (B, Cl^-, Na) and metals, lettuces leaves underwent a cleaning preparation process. This process consisted of detergent-washing (alconox 0.1%), rinsing with tap water, cleaning with 0.005% hydrogen chloride (HCl) solution, and rinsing with distilled water. Cleaned samples were then drained by leaving them on a filter paper. Later, they were oven dried at 65 °C for at least two days. Dried samples were blended and digested in nitric-perchloric acid (2:1) (Thompson and Erdman, 1982). Replicate samples of 25 g were also digested in Aqua Regia acid HCl/H NO_3. Total N and C concentrations were analyzed with an automatic micro-analyzer FlashEA 1112 Series (FlashEA, England) and Leco Truspec (Sant Joseph, USA). Elements and anions were assessed by mass spectrometry, using an Inductively Coupled Plasma spectrometer (ICP-ICAP 6500 DUO Thermo, England), and by ion chromatography, using a chromatograph (Metrohm, Switzerland). Samples for both spectrometry and chromatography were diluted using a standard leaf to distilled-water ratio of 1:2.5. Sampling and analyses complied with the standard methods contained in the Laboratory guide for conducting soil tests and plant analysis (Jones, 2001).

For microbial analyses, lettuces were sampled at their growth stage 49, according to the BBCH scale (Meier, 2001). Ten samples were taken per treatment per day, during three consecutive days, hence 180 lettuces in total. Each sample consisted of a whole lettuce head cut from its base. Traces of soil as well as outer leaves in bad shape were removed. Harvested lettuces were then aseptically stored inside individual food grade plastic bags and transported immediately to the lab for their analysis. Once in the lab, each lettuce head was chopped in an aseptic way and a homogeneous sample of 25 g was picked and stored in a Stomacher® bag without filter. The 25 g sample was then diluted 1:5 in buffered peptone water (BPW, 20 g L^{-1} concentration) and mixed in an automatic blender. For assessing culturable *E. coli*, the sample was then pour plated in Chromocult coliform agar (Merck, Darmstadt, Germany) directly from the bag. After incubating plates at 37 °C during 24 h, colonies in dark blue-violet color were considered positives for *E. coli*. After *E. coli* plating, 125 mL of BPW (20 g L^{-1}) were added to the stomacher bags, which were then incubated during 24 h at 37 °C for enrichment. After the incubation, 7 mL of enrichment were transferred to sterile tubes and mixed with glycerol (3 mL). Tubes were kept at −20 °C until DNA extraction was performed. DNA extraction, detection by RT-PCR and confirmation of the presence of pathogenic bacteria were performed as explained in

paragraph 2.3.

2.5. Statistical analysis

Data related to water and crops qualities were processed statistically through analysis of variance (ANOVA). Means where separated by Tukey's post hoc test, where $p < 0.05$ was considered statistically significant. Different lowercase letters (a-c) represent significant differences between treatments. All statistical analyses were performed with R version 3.3.3 (The R Foundation for Statistical Computing, Vienna, Austria).

3. Results and discussion

3.1. Irrigation water

To evaluate the quality of different types of irrigation water (PW, CW, SW), physicochemical and microbiological characteristics (presented in Table 1 of supplementary data) were compared with each other and against the Spanish legislation (Ministerio de la presidencia, 2007). Besides, EC was also compared against the threshold of lettuce tolerance to salinity (Shannon and Grieve, 1998; Tanji and Kielen, 2002), and phytotoxic elements against guidelines on crops tolerance to specific ions (Ayers and Westcot, 1985).

The water reclamation train of the DESERT-prototype obtained removal efficiencies of about 54%, 24%, 60%, and 62% of EC, SAR, turbidity, and TSS, respectively. Nutrients of agronomic interest, i.e. NO_3^-, PO_4^{3-}, and K, were removed from SW with an efficiency of 62%, −141%, and 58%, respectively. The negative removal efficiency, hence increase in PO_4^{3-} concentration, most likely responds to adding of phosphonic acid as antiscalant for RO membranes (Basile et al., 2015).

Furthermore, removal efficiency of *E. coli* was about 2.2 log units. This efficiency does not reflect the full removal capacity of the DESERT-prototype, due to its regulative design for meeting specific salinity levels (for this study EC in PW was kept at $\approx 0.8\ dS\ m^{-1}$), thus specific water irrigation qualities. This level of salinity was kept by mixing UF − RO permeates, which is considered an appropriate combination to safely reclaim water while preserving nutrients for crops irrigation (De La Cueva Bueno et al., 2016; Iglesias et al., 2017; Norton-Brandão et al., 2013).

Average values of EC, SAR, turbidity and TSS of all irrigation waters were under the correspondent Spanish legislation thresholds (Ministerio de la presidencia, 2007). In consequence, they theoretically do not pose any concerns in regard to salinity-stress for the crops, sodicity issues for the soil structure, and presence of particulate matter. However, PW is remarkably the only water complying with the recommended threshold of lettuce tolerance to salinity ($1.3\ dS\ m^{-1}$) (Shannon and Grieve, 1998; Tanji and Kielen, 2002), though Vicente-Sánchez et al. (2014) identify a maximum tolerable threshold of $1.7\ dS\ m^{-1}$. Furthermore, SAR of SW may theoretically reach critical levels that cannot be neglected, though these were not observed. Albeit the Spanish legislation (Ministerio de la presidencia, 2007) does not show any specific pH threshold, all irrigation waters complied with the tolerable pH range for crops eaten raw (min 6.0 − max 9.0) (Jiménez and Asano, 2008). Significant statistical differences between irrigation waters can be observed for EC, SAR and TSS (Table 1 of supplementary data).

All macronutrients and micronutrients presented significant differences between irrigation waters, and are in general lower in PW than in SW. This is consistent with the fact that RO membranes in the DESERT-prototype are able to remove even up to ionic particles. If compared to the applied nutrient solution, NPK contributions of PW were in general higher than CW, thereby reflecting the advantage of using reclaimed water rather than conventional irrigation sources. Although presence of nutrients in reclaimed water contributes in reducing the external input of fertilizers, their concentrations are normally considered too low to meet crops nutritional requirements (Parsons et al., 2010), hence needing external inputs. Furthermore, Fe concentrations were $<0.1\ mg\ L^{-1}$ in all the irrigation waters (data considered negligible thus not shown), hence disregarded. In respect of phytotoxic elements, B concentrations in all irrigation waters are below the tolerable phytotoxic range for irrigation of lettuces ($2.0–4.0\ mg\ L^{-1}$), which is considered a moderately tolerant crop (Ayers and Westcot, 1985); however, only CW complies with the respective Spanish legislation threshold (Ministerio de la presidencia, 2007). In contrast, PW is the only irrigation water whose Cl^- concentration is under the tolerable limits set by Ayers and Westcot (1985) for the type of produce ($140\ mg\ L^{-1}$). Concentrations of other metals and metalloids were under limit of detection, hence neglected for the case of non-accumulative effects in short-cycle cultivation. PW complied with Spanish standards and agronomic tolerance thresholds, that neither SW nor CW were able to fulfill; furthermore, and despite the membrane-nature removal capacities of the prototype, PW might imply the best long-term irrigation source, as reported by De La Cueva Bueno et al. (2016).

Presence of culturable *E. coli* showed significant differences amongst the different types of irrigation water. Concentration of *E. coli* in SW and CW was similar to that observed in a previous study performed in the same experimental setting (López-Gálvez et al., 2016a). *E. coli* concentrations of both PW (under limit of detection) and CW were within the threshold of the Spanish legislation (Ministerio de la presidencia, 2007), whereas SW slightly surpassed this threshold, indicating that the latter cannot be considered as a suitable irrigation water source unless other preventive measures are taken. The agricultural use of water mixtures obtained from conventional and non-conventional sources is a common practice in the Mediterranean basin (Pedrero et al., 2013b), as it is the case of the CW water source employed in this study. In this regard, storage and conveyance of CW through open air reservoirs and canals, respectively, render this water source prone to be contaminated before reaching the end user, thus explaining its reported *E. coli* concentrations. There were no positive samples for *Salmonella* spp. or *E. coli* O157:H7 in any of the water samples analyzed. On the other hand, for STEC (other than O157:H7), there were two and one confirmed positives in SW and CW, respectively. In a previous study performed in the same experimental setting in which a higher amount of water samples ($n = 104$) was analyzed for pathogen presence, no STEC or *Salmonella* spp. confirmed positives were found in CW and the tertiary treatment effluent from the same WWTP (López-Gálvez et al., 2014). In any case, the amount of water samples analyzed was too small to responsibly achieve conclusions about the presence of pathogens in these water types.

3.2. Baby romaine lettuce

To assess the quality of lettuces, physicochemical and microbiological characteristics (presented in Tables 2 and 3 of supplementary data, respectively) were compared against selected standards. Commercial weights of lettuces were compared against European commercial standards (OECD, 2002; UNECE, 2012), NO_3^- concentrations against the European Commission Regulation No 1881/2006 (European Commission, 2006), and macronutrients and micronutrients against optimum ranges and phytotoxic thresholds found in related literature (Hartz et al., 2007; Marschner, 2012).

Water content in lettuces amongst the six treatments were in the range of 93.0%–94.9% (data not shown), thus not presenting

significant differences. To prevent inaccuracies, irrigation of the different waters was evenly kept throughout the experiment for both DI and SI systems, according their technical specifications. Commercial weight showed significant differences with regard to the irrigation method, being higher in DI treatments than in SI ones. However, all treatments complied with the commercial minimum weight of 100 g for romaine lettuces (classes I and II) grown under protection (OECD, 2002; UNECE, 2012).

Total N concentrations presented significant differences in regard to the irrigation waters, and all of them are slightly over the optimum range (33–48 g kg^{-1}) for this type of crop (Hartz et al., 2007). Total C concentrations, in contrast, showed significant differences in respect of both irrigation waters (higher in SI) and irrigations methods. NO_3^- concentrations were evidently higher in SI treatments than in their DI counterparts; however, the standard deviation in DI-SW treatment is too large to arise it as a reliable data. Nonetheless, none of them reached the toxic threshold (5000 mg kg^{-1}) (European Commission, 2006) for being considered harmful for human health (Santamaria, 2006). P concentrations do not follow a specific pattern regarding the type of water nor the irrigation system; they are roughly within the optimum range (3.5–7.5 g kg^{-1}) (Hartz et al., 2007), but none of them reaches the detrimental threshold (10 g kg^{-1}) (Marschner, 2012). K, Ca and Mg concentrations were higher in DI treatments than in SI ones, and all of them are slightly above their optimum ranges (29–78, 6–11, and 2.5–4.5 g kg^{-1}, respectively) (Hartz et al., 2007).

Fe concentrations were higher in SI treatments than in DI ones, though some standard deviations are too large to become reliable data. However, it can be said that most of these are over the optimum range (115–257 mg kg^{-1}) (Hartz et al., 2007), but the detrimental threshold (500 mg kg^{-1}) (Marschner, 2012) is only surpassed by lettuces of SW-SI treatment. Considering that Fe concentrations in all irrigation waters was roughly <1 mg L^{-1} (data considered negligible thus not shown), it is assumed that such differences between lettuces of SI and DI treatments were brought by limited Fe solubility in soil, thus promoting a more efficient uptaking by direct contact through the leaves in sprinkler irrigation (Marschner, 2012).

Mn and B concentrations were higher in DI treatments than in SI ones, and almost all of them are over their respective optimum ranges (45–74 and 24–36 mg kg^{-1}, respectively) (Hartz et al., 2007); for the former, no detrimental threshold is defined in literature, whereas for the latter, none of the concentrations reached the strictest detrimental threshold (100 mg kg^{-1}) (Marschner, 2012). Cu concentrations of all treatments were over the optimum range (5.0–8.6 mg kg^{-1}) (Hartz et al., 2007), but none of them reaches the strictest toxicity threshold (20 mg kg^{-1}) (Marschner, 2012). Zn concentrations were higher in SI treatments than in DI ones; only SW-SI treatment slightly surpasses the optimum range (25–73 mg kg^{-1}) (Hartz et al., 2007), and none reaches the typical toxicity threshold (300 mg kg^{-1}) (Marschner, 2012). All macronutrients and micronutrients, with the exception of total N P, Fe and Cu, showed significant differences in respect of the irrigation method; and only total C and N, Fe, B and Zn in respect of the irrigation waters. Furthermore, Zn was the only element whose differences were significant regarding the two factors. Though Zn concentrations in irrigation waters were considered negligible (data not shown), lettuces of all treatments presented concentrations within normal ranges (14–200 mg kg^{-1}) (Tambasco et al., 2000). Concentrations of other elements not mentioned here were either under limit of detection or considered not relevant for the type of crop, thus neglected in this study.

Presence of *E. coli* was not detected in PW-irrigated lettuces (Table 3 of supplementary data), which is in agreement with the microbiological quality of the water source (PW) used for irrigation (Table 1 of supplementary data). Although SW showed higher *E. coli* concentration than CW, CW-irrigated plants presented the highest *E. coli* concentrations. According to Pachepsky et al. (2011), the concentration of microorganisms in the irrigation water is not necessarily the main factor in produce contamination. For example, Holvoet et al. (2014), in a study about contamination of lettuce in primary production, detected high prevalence of *E. coli* in irrigation water (26% of samples ≥100 cfu/100 mL), but not in lettuce (only 5% of samples ≥5 cfu/g). In a previous study performed in the same experimental setting, *E. coli* was detected in hydroponically cultivated peppers irrigated with CW and not in peppers irrigated with tertiary treatment effluent from the wastewater treatment plant (López-Gálvez et al., 2016a). Lonigro et al. (2016), observed similar *E. coli* contamination in lettuce irrigated with conventional irrigation water and in lettuce irrigated with SW. Li and Wen (2016) reported weak association between irrigation practices and presence of *E. coli* on lettuce leaves. Regarding the presence of pathogenic bacteria in lettuce samples, although there were some RT-PCR positives for *Salmonella* spp. and STEC (Table 4 of supplementary data), mainly in SW sprinkler irrigated samples, none of them could be supported using the confirmation protocols. Lonigro et al. (2016) reported absence of *Salmonella* in lettuce drip-irrigated with SW. Although SW irrigated lettuce samples showed more RT-PCR positive results for pathogenic bacteria than CW irrigated samples (Table 4 of supplementary data), the trend in the case of *E. coli* was the opposite (Table 3 of supplementary data). *E. coli* has been reported as a suitable indicator of the presence of pathogenic microorganisms in fresh produce (Ceuppens et al., 2015). However, correlation between indicator and pathogenic microorganisms is not always detected (Jongman and Korsten, 2017; Rangel-Vargas et al., 2015).

4. Conclusions

The reclamation train of the prototype employed in this experiment effectively coped with the main environmental/agricultural and public health concerns related to reclaimed water, namely salinity and pathogenicity, respectively. Altogether, data related to water quality, as well as agronomic and microbiological quality of crops lead to conclude that the DESERT-prototype stands out as an appropriate add-on tertiary technology for safe water reclamation oriented to agricultural production. Furthermore, irrigation method, as one of the post-harvest strategies of the multiple-barrier approach, is of utter importance in supporting the DESERT reclamation technology in reaching safety targets.

Notwithstanding the potential of the DESERT-prototype for producing very high quality reclaimed water for growing both horticultural and fruit tree crops, the aim must be an optimal, fit to purpose treatment performance. Tolerance ranges in the quality of irrigation waters regarding the type of crop, as well as in the agronomic and microbiological standards and guidelines, give place to fine tuning the prototype reclamation train according specific needs, thereby keeping the most out of valuable plant nutrients, while ensuring environmental compliance and a less risky agricultural production.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jenvman.2018.02.011.

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