Desalination and Water Treatment
www.deswater.com

1944-3994/1944-3986
doi: 10.1080/19443994.2012.747654

51 (2013) 2488–2496
February

Taylor & Francis
Taylor & Francis Group

Influence of irrigation with saline reclaimed water on young grapefruits

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Received 23 December 2011; Accepted 3 April 2012

ABSTRACT

The use of non conventional water resources, as strategy to an efficient water management, is receiving greater attention. We have designed an experiment, during four year under field conditions in a commercial grapefruit orchard located in Campotejar (Murcia, Spain). The main objective was to evaluate the effects of irrigation with saline reclaimed water compared with traditional irrigation water (Tajo-Segura water transfer) on growth, leaf mineral content, plant and soil water status, yield, and fruit quality. Na, B and Cl concentrations exceeded the recommended level in reclaimed water, for this reason, soil salts accumulation and infiltration problems were observed during last season in this treatment. Leaf B concentration was over the phytotoxic limit in reclaimed water plants, although no visual toxicity symptoms were observed. No differences were observed concerning to leaf Cl and Na concentration. The canopy volume, the number of fruits per trees, and the total yield were reduced by the effect of reclaimed water; however a tendency of higher fruit weight was observed in plants irrigated with this type of water. Salinity and boron accumulation were the main problems associated with the use of reclaimed water because although leaf toxicity levels were not observed, it could pose a risk for grapefruit production at medium and long term. The microbiological water quality was always below the threshold; therefore, the reclaimed irrigation water for grapefruit trees did not represent a microbial risk.

Keywords: Wastewater; Irrigation; Salinization; Citrus

1. Introduction

The social, economic, and environmental impacts of historic water resources development practices and the inevitable prospects of water scarcity are driving a new approach in water resources management, incorporating the principles of sustainability, environmental ethics, and public participation [1]. Sustainable water resources management seeks to design integrated and adaptable systems, increasing efficiency of water use, and making continuous efforts toward protecting ecosystems.

Water withdrawals for agricultural uses were doubled between 1950 and 1995 [2], that is, because irrigated agriculture is the primary user of diverted water globally, reaching a proportion that exceeds 70–80% of the total in the arid and semi-arid zones. To develop strategies to meet water needs and extend freshwater availability is important in these regions [3]. These strategies in the agronomic sector have been focused in two big blocks, the use of efficient water management strategies and the use of nonconven-

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tional water resources, as reclaimed water (RW). Wastewater as a water resource is currently receiving greater attention because of the global water crisis [4]. The reuse of this nonconventional water resource represents a viable method of reducing the competition between urban and agricultural water demands [5]. The use of wastewater is practiced all over the world although not always in the correct way. The benefits, potential health risks, and environmental impacts resulting from wastewater use for irrigation have been well documented [6], and it has been reported that successful wastewater reuse in agriculture requires selection of salt-tolerant crops, appropriate irrigation systems, water management practices, site/soil suitability, and salinity management strategies [7–9]. Irrigation with poor-quality wastewater may create undesirable effects on soils and plants or pose a potential health threat to the consumer [10]. Studies in RW use in agriculture have shown that an integrated planning approach that takes into account the technological aspects of the irrigation system, as well as the production and contamination issues is essential for the safe irrigation of vegetables crops [11].

One of the problems frequently associated with the RW use in the agriculture is the salinization, in fact, secondary salinization from irrigation water is a growing worldwide problem as more agricultural land has become saline [12]. Murcia, as a semi-arid Mediterranean agronomic region, uses $100\,Hm^3$ of RW per year in their fields. However, the 93% of this volume of water has an electrical conductivity (EC) higher than $2\,dS\,m^{-1}$ and 37% has EC values higher than $3\,dS\,m^{-1}$ [13].

Although reuse of saline water for different irrigating crops has been studied [14–17], little is known of the long-term effects of the use of saline reclaimed irrigation on trees in arid and semi-arid agricultural areas with severe water shortage. Also, few studies have focused on the problem of nutrient disorder in plants as a result of continuous application of RW [18]. The report written by Ayers and Westcot [19], are still the basic reference applicable to irrigation with treated municipal wastewater [20]. For this reason, the main objective was to evaluate the effects of irrigation with saline RW compared with traditional irrigation water (Tajo-Segura water transfer) on growth, leaf mineral content, plant and soil water status, yield, and fruit quality.

2. Materials and methods

2.1. Experimental conditions

The experiment was conducted during four years (2007–2010), at a commercial orchard located in Campotejar (Murcia) Spain (38°07′18′′N; 1°13′15′′W). The experimental plot of 0.5 ha was cultivated with 5-year young 'Star Ruby' grapefruit trees (Citrus Paradisi Macf) grafted on Macrophylla rootstock [*Citrus Macrophylla*]. The plant spacing was 6×4 m. The water is supplied by drip irrigation with three compensated pressure drips per tree, each with a flow rate of $4\,L\,h^{-1}$ and spaced 0.9 m. The soil had at 30–90 cm depth a loam texture (43% sand, 24% clay, and 33% loam) with an average bulk density of $1.41\,g\,cm^{-3}$. The irrigation doses were scheduled on the basis of weekly crop evapotranspiration estimated as reference evapotranspiration (ETo), calculated with the Penman–Monteith equation [21], and a monthly crop factor [22].

The irrigation head was equipped and supplied with two water sources; the first TW was pumped from the Tajo-Segura water transfer (EC $\approx$ 1 $dS\,m^{-1}$) and the second water sources RW was pumped from the ''Molina de Segura'' wastewater treatment plant (WWTP) (EC $\approx$ 3–4 $dS\ m^{-1}$). The treatment in the WWTP is a conventional activated sludge with ultraviolet tertiary treatment. The irrigation head was equipped with two types of filters, first sand filters and then disc filters, to avoid emitters clogging. Therefore, a total of two irrigation treatments with two replicates each one were distributed using a completely randomized design. Each replicate consisted of 6×4 trees with the four central trees being used for periodic sampling. During the four seasons, the annual reference ETo was 1,299, 1,332, 1,385, and 1,262 mm in 2007, 2008, 2009, and 2010 respectively. The irrigation treatments were applied daily from January 2007 until December 2010. Total amounts of water applied were measured with inline water meters, placed on the four replicates of each treatment. Fertilizers were applied through the drip irrigation system with N_2–P_2O_5–K_2O ($kg\,ha^{-1}$) at rates of 450,325,225, 1,158,530,481, 1,350,560,255, and 1,350,560,255 for 2007, 2008, 2009 and 2010 respectively.

2.2. Water analysis

Three water samples from each irrigation water source were collected monthly between 2007 and 2010 to characterize irrigation water quality. For this purpose, the water sample was collected in glass bottles, transported in an ice chest to the lab and stored at 5°C before being processed for chemical analyses. The concentration of macronutrients (Na, K, Ca, Mg), micronutrients (Fe, B, Mn) and heavy metals (Ni, Cd, Cr, Cu, Pb, Zn) were determined by inductively coupled plasma (ICP-ICAP 6500 DUO Thermo, England); anions (chloride, nitrate, phosphate, and sulfate) were analysed by ion chromatography with a Chromatograph (Metrohm, Switzerland), pH was measured with a pH-meter Cryson-507 (Crisom Instruments S.A., Barcelona, Spain) and EC total dissolved solids (TDS) were determined using the multi-range equipment Cryson-HI8734 (Crisom Instruments, S.A., Barcelona, Spain), and turbidity was measured with a turbidity-meter Dinko-D-110 (Dinko Instruments S.A., Barcelona, Spain).

The microbiological quality of irrigation water was assessed by determining the number of fecal coliforms and *E. coli* by a membrane filtration procedure [23]. Samples were filtered using a vacuum system through a sterile 0.45 μm-pore-size membrane filters (Millipore, Billerica, USA). Colony formation was measured after incubation on top of Chromocult agar plates (Merck, Darmstadt, Germany) for 24 h. Incubation temperatures were 37˚C for *E. coli*, and 44.5˚C for fecal coliforms. Microbial counts were expressed as CFU $100\,ml^{-1}$. The helminth eggs were measured following the Bailenger´s method [24].

2.3. Soil analysis

Gravimetric soil samples were collected every three months during 2008, 2009, and 2010 from 0.2 and 0.6 m depths at 0.3 m away from the irrigation emitter. Soluble-salt contents of soils were determined by the saturation-extract method as described by Rhoades et al. [25]. The EC of the saturated paste extract (ECe) were measured with a multi-range equipment Cryson-HI8734 (Crison Instruments, S.A., Barcelona, Spain). Soluble Ca and Mg were measured using the EDTA tritation method and Na was measured using a frame photometer [26].

The soil water content (SWC) was measured biweekly. The SWC was measured at 0.2 m away from the first emitter and at right angle to the irrigation lateral, using the time-domain reflectometry (TDR) probes (model 1502C, Tektronix Inc., OR, USA), for the top 0.1 m and the neutron probe Troxler 4300 (Troxler, Raleigh, NC, USA) from 0.2 down to 1 m depth, following a 0.1 m step. The neutron and the TDR probes were installed in one tree per each replicate (2 trees per treatment).

2.4. Plant measurements

For mineral analysis, spring flush leaves from non-fruiting branches were sampled every three months during 2008, 2009 and 2010. Twenty leaves per tree were sampled in the two central trees of each replicate per treatment. Leaves were washed with a detergent (alconox 0.1%), rinsed with tap water, cleaned with a dilute solution of 0.005% HCl and finally rinsed with distilled water and left to drain on a filter paper before being oven-dried for at least 2 days at 65˚C. Dried leaves were ground and a nitric-perchloric acid (2:1) digestion [27] was executed. Replicate samples (0.25 g) were digested by Aqua Regia acid HCl/HNO_3. The concentration of macroelements, microelements, and heavy metals were determined by inductively coupled plasma (ICP-ICAP 6500 DUO Thermo, England). Anions were analysed by ion chromatography with a Chromatograph Metrohm (Switzerland) after using a standard leaf-to-distilled-water ratio of 1:2.5 (w:w).

The midday stem water potential (Ψ_{stem}) was measured biweekly, throughout the season. Two mature, fully expanded leaves from the canopy close to the trunk, were taken from the two inner trees of each replicate per treatment. The leaves were enclosed within foil-covered aluminum envelopes, at least 1 h before the midday measurement [28]. The midday stem water potential was measured at noon (12:00 h GMT), using a pressure chamber (model 3000; Soil Moisture Equipment Corp., Santa Barbara, CA, USA) and following the recommendations of Turner [29].

The tree canopy height and perimeter were measured at the beginning and at the end of each season during the experimental period in all the trees of the orchard. The canopy volume was estimated from height and diameter of the tree, measured with ranging rods in two perpendicular directions. The formula is that proposed by Hutchinson [30], considering that the tree is shaped like a pyramid.

2.5. Fruit set, yield and fruit quality

Fruit set and fruit load were determined from flower and fruit counts from four secondary branches in the two inner trees per each replicate (eight trees per treatment), from bloom to harvest. These branches were facing in the four directions, and their basal diameter was between 2 and 3 cm. Fruit set was calculated as the percentage of the total initial flowers and those flowers that continued growing actively.

Yield was entered in eight trees per treatment. In each tree, measurements were made: number of fruits per tree, total kg, and the distribution in commercial diameters (<90, <95, <100, <105, <120, <140 mm and extra (>140 mm) [31].

Fruit quality was measured in 100 fruits per treatment in each year randomly collected during harvesting. The parameters included the peel thickness (PT), fruit weight (g/fruit), and juice volume (JV). A sample of 50 mL per fruit was used to assess internal fruit quality, including titratable acidity (TA) and soluble solid content (SSC). TA was determined by titration of 10 mL of juice with 0.1 mol L^{-1} NaOH to pH 8.1 [30] and refractible SSC with a handheld refractometer (Atago N1, Tokyo, Japan).

2.6. Statistical design and analysis

A total of 144 trees were used in this study. The experimental design of each treatment was four standard experimental plots distributed randomly in blocks. The standard plot was made up of 24 trees, organized in three adjacent rows. The two central trees of the middle row were used for measurements. Statistical analysis was performed by weighted analysis of variance (ANOVA) using a linear model for SPSS software (version 17.0, SPSS Inc., Chicago, USA).

3. Results

3.1. Irrigation water quality

In both sources of irrigation water, TW and RW, the water quality differed. RW had the highest levels in salinity and sodicity risk, with EC values close to 3 dS m^{-1} and sodium adsorption ratio (SAR) around 6 (Fig. 1), while for the Tajo-Segura TW, the EC and SAR values were lower (close to 1 dS m^{-1} and 2, respectively) (Fig. 1). The high level of salinity observed in the RW treatment was mainly due to the high concentration of Cl (> 350 mg l^{-1}) and Na (> 200 mg l^{-1}) (Fig. 1), although Ca, Mg, and SO_4 were also more concentrated in this treatment (data not shown). In RW, there was also a higher concentration of N, P and K compared with the Tajo-Segura TW treatment (Fig. 1). RW covered the 24% and 15% of N and P fertilization needs, respectively, and completely satisfied K requirements. No differences in the concentration of heavy metals were found between the different irrigation water sources (data not shown).

In relation to the microbiological parameters measured, at the beginning of the experiment, the RW reached values always higher than Tajo-Segura TW, between 400–600 *E. coli* and 1,000–1,500 fecal coliforms CFU 100 mL^{-1}, but in the last period, the Tajo-Segura TW reached the maximum values, around 400 *E. coli* and 3,000 fecal coliforms CFU 100 mL^{-1} (Fig. 2).

3.2. Soil measurements

Soil analysis showed higher EC_e in the RW during 2009 and 2010 (near to 4 dS m^{-1}), while for the

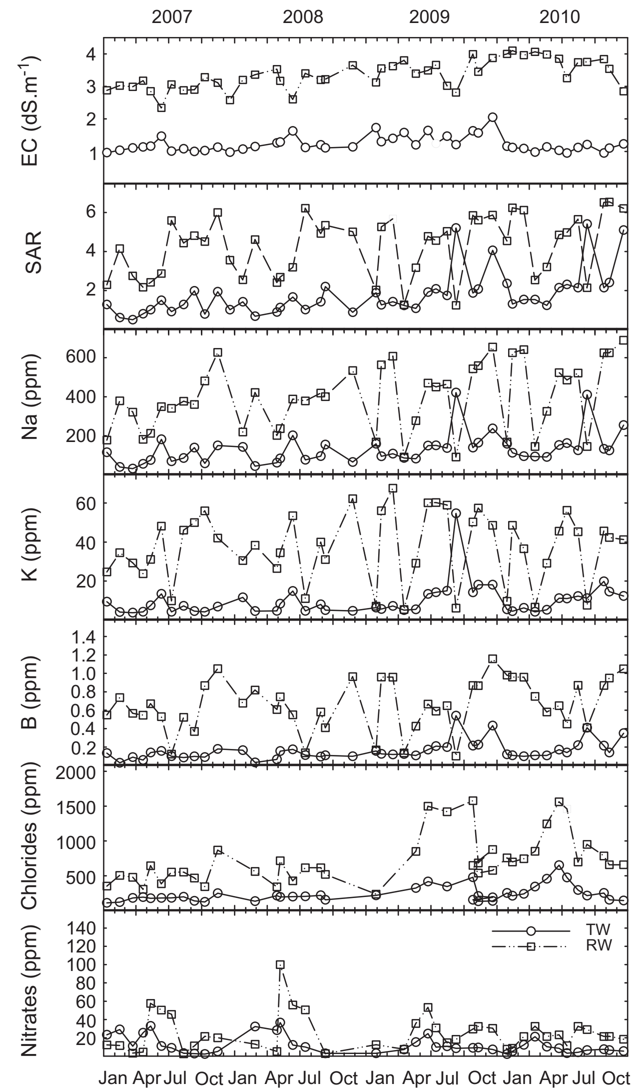

Fig. 1. Evolution of water EC (dS m^{-1}), SAR, and chemical compositions ([Na], [B], [Cl], [K] and [NO_3], ppm) in the two irrigation treatments (TW, open circles and RW, open squares) measured monthly during three years (2007, 2008, 2009, and 2010). Each value is the mean of 4 values.

Tajo-Segura TW, EC_e values were almost the half (2 dS m^{-1}) (Fig. 3). Considering the soil SAR values, only in 2009 the RW treatment differed significantly with Tajo-Segura TW treatment, reaching values up to 8 (Fig. 3). Soil water store was maintained to field capacity (around 250 mm m^{-1}) in both irrigation water treatments during entire experiment (Fig. 4).

3.3. Plant–water relations and leaf mineral concentration

The Ψ_{stem} was observed in the same range in both treatments, with values close to 0.4–0.8 MPa during the experiment (Fig. 4).

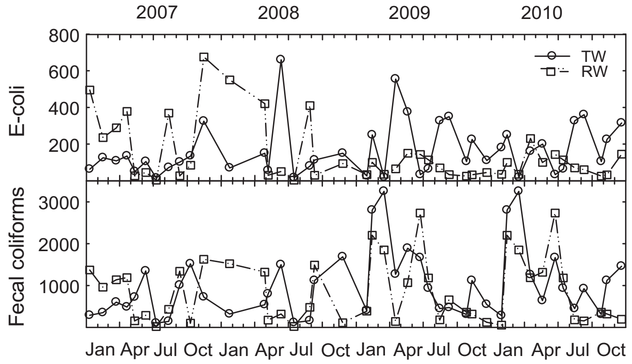

Fig. 2. Evolution of microbiological water quality, *E. coli* and Fecal coliforms (colonies formation units, CFU, $100\,ml^{-1}$), in the two irrigation treatments (TW, open circles and RW, open squares) measured monthly during three years (2007, 2008, 2009, and 2010). Each value is the mean of 4 values.

The leaf Na and Cl concentrations were maintained in a nontoxic range (0.3–0.4%) of accumulation for both treatments during all seasons. The foliar B concentration was increasing in RW treatment, reaching values between 140 and 160 ppm in 2009 and 2010, while in TW treatment, this concentration was around 110 ppm in the last two seasons.

3.4. Vegetative growth, yield and fruit quality

In the beginning of the assay, in 2007 the trees had similar volumes of canopy. However, after three years applying different sources of irrigation water, there were significant differences between the canopies of the RW trees and the TW trees, and thus, the canopy of RW trees was about $13.4\,m^3$ while the canopy of TW trees was close to $15.9\,m^3$ (Table 1).

Only during the season 2009, it was possible to report significant differences between the yield observed in the RW treatment ($83\,kg\ tree^{-1}$) in respect to the crop production measured in the Tajo-Segura TW treatment ($98\,kg\ tree^{-1}$). This difference was mainly due to a decrease in fruit set (52.3% in RW compared with 62.5% in TW) (Table 1).

In general, fruits quality parameters such as PT, JV, SSC, TA, and maturity index (MI) were not affected by the irrigation water quality treatments considered in the experiment (Table 2).

4. Discussion

Besides of high concentrations of Cl and Na observed in RW, the most remarkable data was the high level of B that was always above phytotoxic range for sensitive crops ($>0.7\,mg\,kg^{-1}$) [19] (Fig. 1).

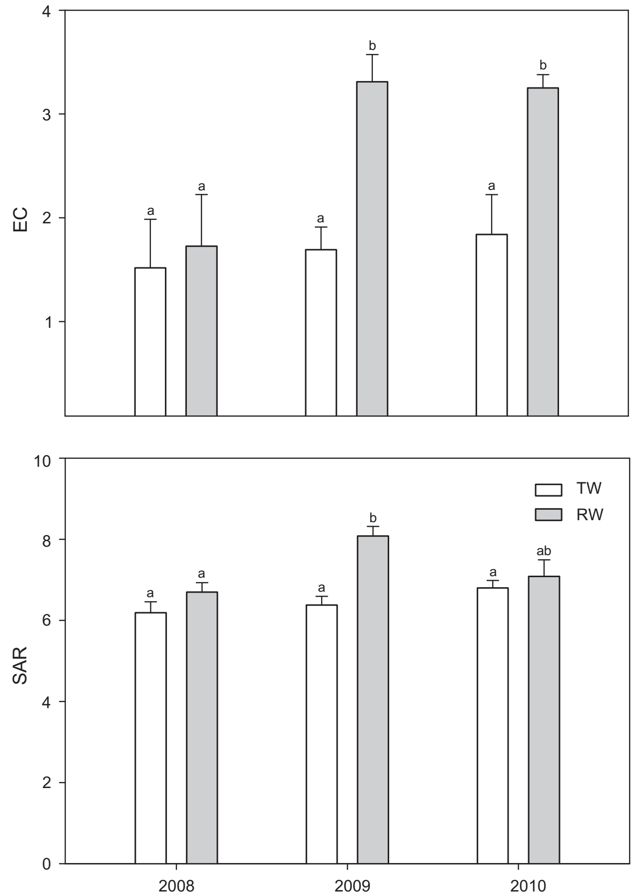

Fig. 3. ECe ($dS\,m^{-1}$) and SAR measured in soil gravimetric samples during 2008, 2009, and 2010 in the two irrigation treatments (TW, white histogram and RW, gray histogram). Each column is the annual average of 12 measurements (4 samples per irrigation treatment and three times per year). Different letters above each column indicate significant differences between means according to Tukey's test ($p < 0.05$).

This high value can be explained because of the use of cleaning agents, such us detergents, may also elevate B concentrations in RW [32]. Considering the water analysis, it was also remarkable that the microbiological load in the different irrigation water sources was highly variable. In several samples during the experiment, the TW microbial load was higher than RW (Fig. 2), these data suggest that open channel water distribution networks may have a higher microbiological risk than tertiary treated wastewater. These episodic contaminations emphasize the need for periodic microbiological analysis of the irrigation water supplies, independently of the water source considered, to minimize negative public health impacts [33]. The intestinal nematode eggs (data not shown) and *E. coli* were always below the threshold

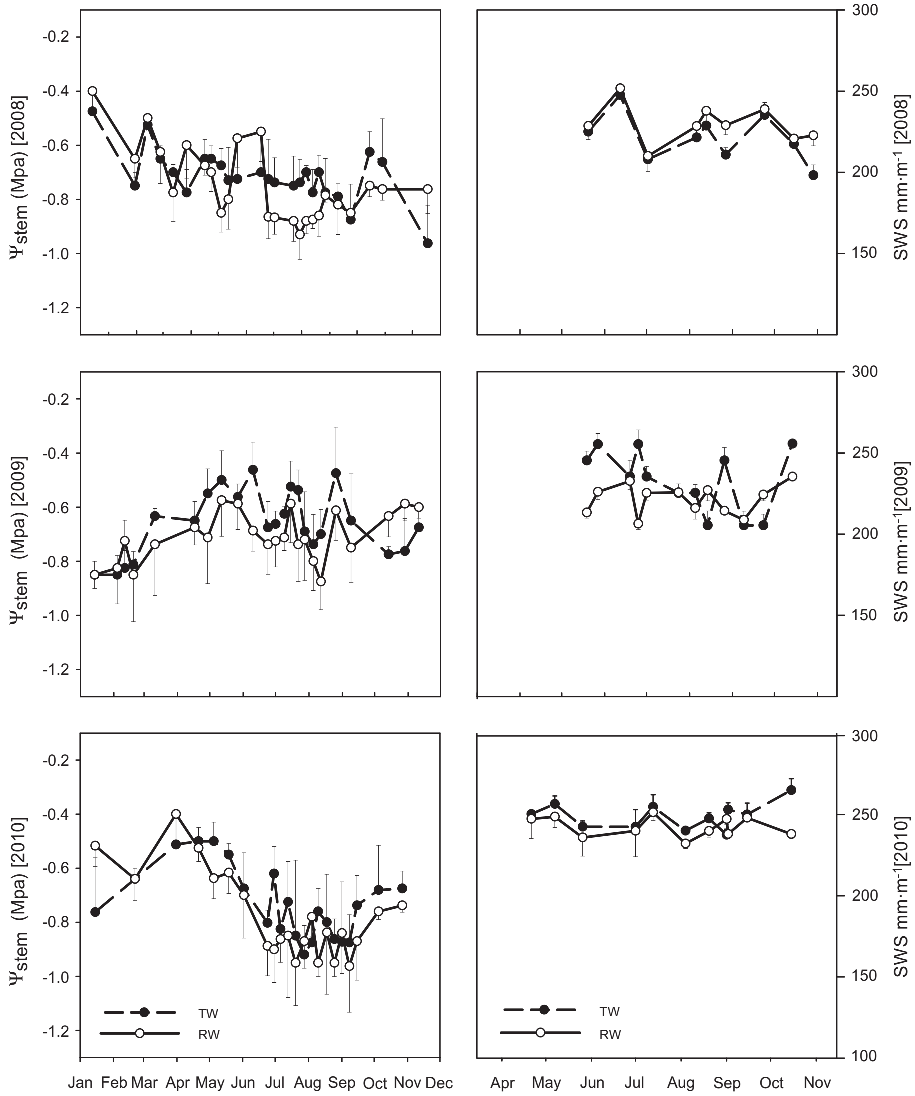

Fig. 4. Evolution of soil water store (SWS, mm m^{-1}) and midday stem water potential (Ψ_{stem}) during 2008, 2009, and 2010 in the two irrigation treatments (TW, black circles and RW, open circles). Each value of SWS and Ψ_{stem} stem is the mean of four and sixteen measurements ± SE, respectively.

Table 1
Tree canopy (m^3), fruit set (%), crop load (fruit tree^{-1}), fruit weight (g), and yield (kg tree^{-1}) in the two irrigation treatments (TW and RW)

Year	Treatment	Tree canopy	Fruit set	Crop load	Fruit weight	Yield
2008	TW	11.1 ± 0.2 a	18.6 ± 2.5 a	289 ± 10 a	107 ± 3 a	31 ± 3 a
	RW	9.6 ± 0.3 a	20.3 ± 3.9 a	326 ± 14 a	107 ± 2 a	35 ± 8 a
2009	TW	14.7 ± 0.9 a	62.5 ± 6.5 a	998 ± 65 a	98 ± 3 a	98 ± 9 a
	RW	10.1 ± 0.5 b	52.3 ± 7.3 a	743 ± 23 b	112 ± 3 b	83 ± 8 b
2010	TW	15.9 ± 0.6 a	33.7 ± 4.5 a	618 ± 24 a	104 ± 3 a	64 ± 4 a
	RW	13.4 ± 0.5 b	34.3 ± 6.2 a	504 ± 24 b	115 ± 3 b	58 ± 5 b

Values are the mean of 48 trees ± SE in tree canopy and eight trees ± SE in the other parameters. Letters in each column for each year indicate significant differences between means according to Tukey's test ($p < 0.05$).

Table 2
Fruit quality parameters: PT (mm), JV (%), SSC (°Brix), TA (%), and MI (SSC/TA ratio) in the two irrigation treatments (TW and RW)

Year	Treatment	PT	JV	SSC	TA	MI
2008	TW	8.8 ± 0.6 a	136.0 ± 53.5 a	9.9 ± 0.1 a	1.9 ± 0.2 a	5.0 ± 0.8 a
	RW	9.6 ± 1.5 a	158.6 ± 41.8 a	9.2 ± 0.5 a	1.7 ± 0.1 a	4.8 ± 0.7 a
2009	TW	9.3 ± 1.2 a	159.0 ± 10.8 a	8.1 ± 0.9 a	2.0 ± 0.2 a	4.1 ± 0.9 a
	RW	9.0 ± 1.4 a	162.6 ± 16.8 a	9.4 ± 1.6 a	2.2 ± 0.3 a	3.8 ± 0.5 a
2010	TW	8.5 ± 1.0 a	158.2 ± 9.5 a	9.8 ± 0.6 a	2.4 ± 0.1 a	4.0 ± 0.2 a
	RW	9.2 ± 0.5 a	162.2 ± 14.3 a	9.4 ± 0.5 a	2.4 ± 0.1 a	3.8 ± 0.5 a

Values are the mean of 100 fruits ± SE. Letters in each column for each year indicate significant differences between means according to Tukey's test ($p < 0.05$).

(< 10 egg 10 L^{-1} and 10,000 fcu 100 mL^{-1}, respectively) imposed by Royal Decree-Law 1620/2007 [34], which regulates the use of RW in Spain. This result is in accordance with the previous studies carried out in Murcia [35], which concluded that in 43 WWTP effluent samples analyzed, nematode eggs were absent in 79% of the samples water treated with a secondary treatment, and completely absent in the WWTP effluents that had undergone tertiary treatment. In general, considering the microbiological load in both irrigation water sources, non microbiological risks are expected in this assay. These results are similar to those reported by Pedrero et al. [10] working with lemon trees in Murcia Region.

A tendency was identified in terms of salts accumulation in the soil, during the last two seasons in the RW treatment (Fig. 3). This result is in accordance with the long-term study developed by Pereira et al. [36], which soil salinity increased about 2–3 times after 11 years applying RW in citrus. According to the data reported by Ayers and Westcot [19], our soil has also a moderate risk of sodification problems in the long term if RW is used during ten or more years. According to Ganjecunte et al. [3], to prevent these problems, periodic flushing of salts with freshwater and intensive soil status monitoring is needed to avoid the salt accumulation and a reduction in the physical soil properties.

Applying the same quantity of water in both treatments, the SWC in both treatments was maintained at the same level of field capacity during all seasons in the experiment (Fig. 4). These results were not according to some studies that showed that orchards irrigated with RW had higher SWC [37]. In recent years, the use of plant-based water status indicators have become very popular to study plant–water relations and for planning citrus irrigation programs [38,39]. In this sense, although plant–water relations parameters should be affected by both water availability and quality, in this experiment Ψ_{stem} was similar in both treatments (Fig. 4). These results are corroborated by previous studies that demonstrated no effect of RW on midday stem water potential [40,41].

Citrus is considered sensitive to B [42], and B toxicity is a concern in arid environments where salinity problems exist [43]. Although no toxicity symptoms were observed in our experiment, if we consider the accumulation of B in the leaf tissue after four years of treatment (Fig. 5), it is possible to assume that if RW is applied during more seasons, the B leaf concentration could reach toxic levels, as it was observed by Pedrero and Alarcon [44] using less saline RW than in our experiment. These results are according to our previous articles studying the use of RW on mandarin and lemon trees in which we reported that saline RW use can induce some problems in the long term because of the B accumulation on plant and soil [45,46].

Although different authors found significantly higher sodium and chlorine concentrations in citrus leaf samples [37] and ornamental shrubs [46] irrigated with RW, in our case not increment of leaf Na and Cl during the experiment was observed (Fig. 5). The explanation could be because in citrus grown under saline conditions, calcium was found to be effective in reducing the transport of both sodium and chloride from roots to leaves, thereby alleviating foliar injury and/or defoliation [37,47–50].

The use of RW irrigation on different crops has improved fruit quality parameters such as size, pH, and TA [51,52]. A higher fruit size related to the tendency to reduce the number of fruits in the trees irrigated with RW (Table 1) was observed in the experiment in the last two seasons (2009–2010). This result was similar than observed by Pedrero [45] on mandarin trees irrigated with saline RW. The rest of the quality parameters were unaffected by the effect of different treatments (Table 2). Although some

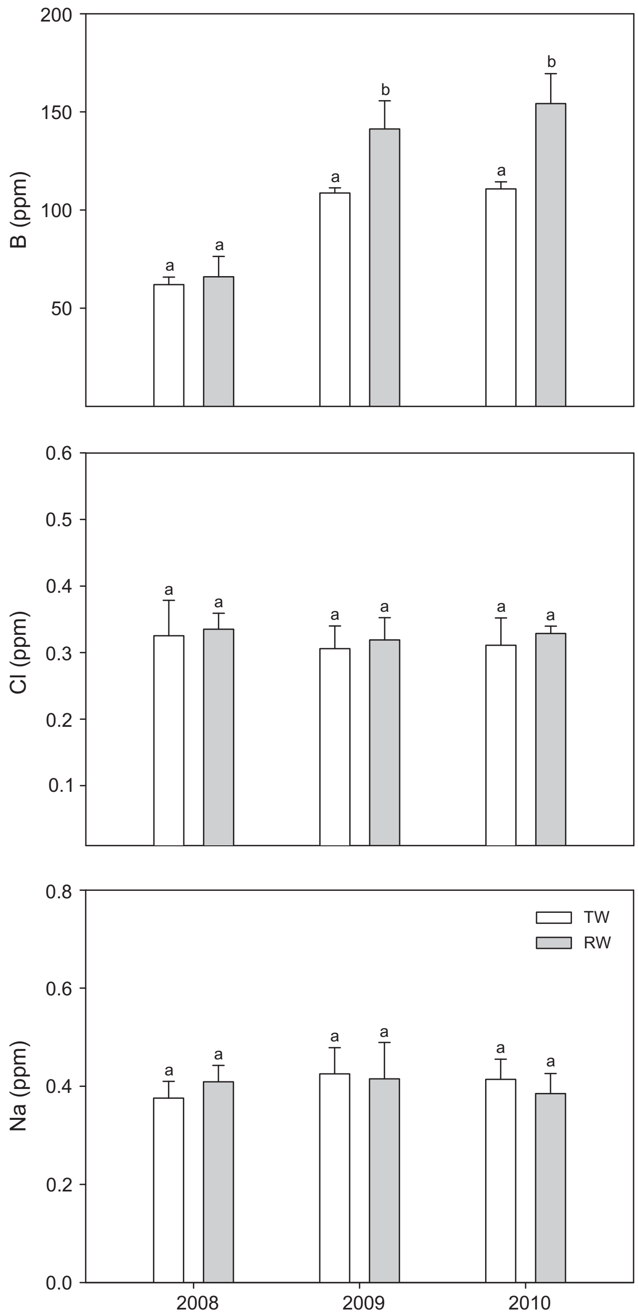

Fig. 5. Annual average of leaf mineral concentration of Cl (%), Na (%) and B (ppm) measured during 2008, 2009, and 2010 in the two irrigation treatments (TW, white histogram and RW, gray histogram). Each column is the average of 4 measurements ± SE (160 leaf samples per irrigation treatment and four times per year, $n = 1,280$). Different letters above each column indicate significant differences between means according to Tukey's test ($p < 0.05$).

studies claim that irrigation with RW increases the yield and canopy volume in citrus trees [37,44]; in this experiment, the tree canopy and yield were slightly lower in the RW treatment compared with the Tajo-Segura TW treatment, this reduction was significant during 2009 and 2010 (Table 1).

In conclusion, there were not any important effects on plant water status, crop production and fruit quality. Nonmicrobiological risks were observed by the use of RW for grapefruit production. However, salinity and boron concentrations were the main problems associated with RW use in this experiment, and although leaf toxicity levels were not observed, these problems can suppose a risk for grapefruit production with this type of water at medium and long term.

Acknowledgments

This study was supported by four projects granted to the authors, SIRRIMED (FP7-KBBE-2009-3-245159), CONSOLIDER-INGENIO 2010 (MEC CSD2006-0067), SENECA (11872/PI/09), and CICYT (AGL2010-17553).

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