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# Irrigation of *Myrtus communis* plants with reclaimed water: morphological and physiological responses to different levels of salinity

By J. R. ACOSTA-MOTOS[1], S. ÁLVAREZ[1], J. A. HERNÁNDEZ[2] and M. J. SÁNCHEZ-BLANCO[1,3]*

[1]Departamento de Riego, Centro de Edafología y Biología Aplicada del Segura (CSIC), P.O. Box 164, Espinardo E-30100, Murcia, Spain
[2]Grupo de Biotecnología de Frutales, Departamento de Mejora Vegetal, Centro de Edafología y Biología Aplicada del Segura (CSIC), P.O. Box 164, Espinardo E-30100, Murcia, Spain
[3]Horticultura Sostenible en Zonas Áridas, Unidad Asociada al CSIC-CEBAS, 52 Paseo Alfonso XIII Cartagena 30203, Spain
(e-mail: quechu@cebas.csic.es)

SUMMARY

The influence of irrigation with different sources of reclaimed water on physiological and morphological changes in *Myrtus communis* plants was investigated to evaluate their adaptability to such conditions. *M. communis* plants, growing in a growth chamber, were subjected to four irrigation treatments over 4 months (120 d): a control [tap water (0.8 dS m$^{-1}$), leaching 10% (v/v) of the applied water] and three reclaimed water irrigation treatments, namely 1.5 dS m$^{-1}$ leaching 25% (v/v) of the applied water (RW1), 4.0 dS m$^{-1}$ leaching 40% (v/v) of the applied water (RW2), and 8.0 dS m$^{-1}$ leaching 55% (v/v) of the applied water (RW3). After treatment, all plants were irrigated with tap water, as for the control plants, for a further 2 months (60 d). At the end of the first period (4 months), none of the myrtle plants showed any adverse change in biomass and the average total dry weight (DW) increased by 53% in treatment RW2. However, at the end of the treatment and recovery period (180 d), accumulations of Cl$^-$ ions, and especially Na$^+$ ions, negatively affected the growth of all RW3 plants. Plants irrigated with all three reclaimed water samples had increased difficulty in taking-up water from the substrate (i.e., they had lower leaf water potential and relative water content values). RW2 plants showed a better response in their gas exchange parameters. The use of reclaimed water decreased leaf K$^+$/Na$^+$ and Ca$^{2+}$/Na$^+$ ratios, but no chlorosis or necrosis were observed. The three reclaimed water samples had different effects on the myrtle plants depending on the specific chemical properties of the water. Leaching was found to be important to minimise the negative effects of salinity in the irrigation water.

**R**eclaimed water is water that has previously been used, suffered a loss in quality, but has been treated to a point where it is suitable for additional use. Use of this water in agriculture is a common practice in many areas of the World, especially in arid and semi-arid environments where access to water is a limiting factor (Yermiyahu *et al.*, 2008). Several studies have reported environmental and agronomic interest in using waste water for irrigation in different crops (Parson *et al.*, 2001; Pedrero and Alarcón, 2009; Pedrero *et al.*, 2010). Treated municipal waste water can be regarded as an alternative source of water and as fertilisation for the production of landscape plants, since it contains nutrients which can reduce the application of fertiliser, thus reducing costs and risks of environmental pollution (Gori *et al.*, 2000; Gomez-Bellot *et al.*, 2013). In spite of these potential benefits, reclaimed waste water is usually of poor quality compared to fresh water. Depending on its source and treatment, reclaimed waste water may contain high concentrations of salts, heavy metals, and/or pathogenic organisms. Nevertheless, the potential physical, chemical, or biological problems associated with the application of

waste water to irrigate crops are of less concern for landscape plant production (Gori *et al.*, 2000).

A high concentration of salts in the irrigation water causes water stress due to a decrease in the water potential of the rooting medium (an osmotic effect). In addition, specific ions such as Na$^+$ and Cl$^-$ can accumulate in plants, where they can reach toxic levels (ion toxicity) and induce nutritional imbalances with those mineral elements that are essential for the correct functioning of the plant. In some cases, reclaimed water also contains high concentrations of boron (B; Feigin *et al.*, 1991) and significant quantities of toxic heavy metals (Barar *et al.*, 2000; Yadav *et al.*, 2002).

Salinity affects the establishment, growth, and development of plants, leading to significant losses in productivity (Giri *et al.*, 2003; Katerji *et al.*, 2003; Mathur *et al.*, 2007; Álvarez *et al.*, 2012), and may also affect the ornamental quality of both cultivated and wild species (Morales *et al.*, 2001). In the case of landscape plants, maximum growth is not always essential and visual quality may or may not be related to biomass production and/or photosynthetic responses (Zollinger *et al.*, 2007; Álvarez *et al.*, 2011). Another way to determine the effect of salinity would be to study plant responses during a

*Author for correspondence.

recovery period after salinity stress. Recovery from water stress is generally characterised by an increase in leaf water potential, followed by a recovery of stomatal conductance (Chaves *et al.*, 2011). However, the physiological mechanisms involved in the recovery of plants subjected to high salinity are still poorly understood.

To minimise crop losses, it is necessary to identify new irrigation management strategies such as increased leaching to maintain a high and constant substrate humidity (Bañón *et al.*, 2011), or to use salt-tolerant plants, or to develop salt-tolerant crops through breeding programmes (Wu and Dodge, 2005).

*Myrtus communis* L. is a sclerophyllus evergreen shrub (Mendes *et al.*, 2001) of interest for ornamental use in re-vegetation projects in semi-arid and degraded land, and in landscaping (Romani *et al.*, 2004). Although *M. communis* is a typical Mediterranean species, with good adaptability to environmental stresses, it may, under natural conditions, suffer from abiotic stresses (Navarro *et al.*, 2009). Nevertheless, little is known about the growth and physiological responses of *M. communis* to irrigation with recycled water of different quality. Many research studies have been conducted on the effects of waste water on the physiology of ornamental species, with contradictory results, probably due to the different cultivation techniques, environments, and species used (Parnell *et al.*, 1998; Gori *et al.*, 2000; Schuch, 2005; Bañón *et al.*, 2011).

The objective of this work was to study the negative and positive impacts that reclaimed water of different origins and composition could have on the development and quality of myrtle plants. The aim was to evaluate whether reclaimed water with a high level of salinity could be used as an alternative source of water and nutrients for the production of *M. communis* plants. The responses of several physiological parameters related to water status, photosynthetic efficiency, and nutrient content were also considered. The present study was conducted under controlled environment conditions to avoid other possible effects due to climatic variables. The information generated by this study would be valuable for both landscape and nursery irrigation management.

## MATERIALS AND METHODS
### Plant material and experimental conditions

Single rooted cuttings (120) of native myrtle (*Myrtus communis* L.) were transplanted into 14 cm $\times$ 12 cm pots (1.2 l) filled with an 8:7:1 (v/v/v) mixture of coconut fibre, sphagnum peat, and perlite and amended at 2.0 g l$^{-1}$ substrate with Osmocote Plus (Scotts Australia Pty. Ltd.,

The Hills Shire New South Wales, Australia; 14:13:13 N, P, K, plus micro-elements). The experiment was conducted in a controlled environment growth chamber set to simulate natural conditions. The temperature in the canopy was 23ºC during the 16 h photoperiod, and 18ºC during darkness. Relative humidity (RH) ranged from 55 – 70%. A mean photosynthetically active radiation (PAR) level of 350 µmol m$^{-2}$ s$^{-1}$ at canopy height was supplied from 08.00 – 00.00 h. Although the level of radiation in the growth chamber was lower than in the open field, we assumed that the PAR level used was of secondary importance compared with the different irrigation treatments.

### Treatments

*M. communis* plants (n = 30 per treatment) were exposed to four irrigation treatments for 4 months (120 d; Period I) using water from different sources. The irrigation treatments consisted of a control, where the electrical conductivity (EC) of the tap water was 0.8 dS m$^{-1}$ (indicating no use-restrictions or only slight restrictions according to FAO classifications; FAO, 2003), and three reclaimed water treatments. The latter used water from three sewage treatment plants located in the Province of Murcia (Spain), namely: RW1 (EC 1.7 dS m$^{-1}$) from Jumilla, RW2 (4.0 dS m$^{-1}$) from Campotejar, and RW3 (8.0 dS m$^{-1}$) from Mazarrón. FAO classifications indicated severe restrictions on the use of the latter two types of water. All three waste water treatment plants applied a conventional activated-sludge process, followed by ultraviolet radiation as the tertiary treatment. At the start of the experimental period the concentrations of Na$^+$, Cl$^-$, and B$^{3+}$ ions in each irrigation water were analysed. The results are shown in Table I.

After 4 months (120 d; Period I), all plants were exposed to a 2-month (60 d) recovery period (Period II) in which the plants were irrigated with the same tap water used for the control plants. Throughout the 6 months (180 d) of the experiment, all plants were irrigated twice a week to above-container capacity. To determine the maximum water-holding capacity of the substrate, the medium was uniformly mixed and packed to a bulk density of 0.165 g cm$^{-3}$ in all pots. Each substrate surface was covered with aluminium foil to prevent water evaporation and the lower part of each pot was submerged to half its height in a water bath, then left to equilibrate overnight. The next day, the pots were removed and left to drain freely until the drainage became negligible. The fresh weight was then recorded and calculated for each individual pot and considered as the weight at field capacity (WFC). The volume of irrigation water to be applied was determined for each treatment as the point at which the leaching fraction reached 10% (v/v) of the water applied in the control treatment, 25% in RW1, 40% in RW2, or 55% of the applied water in RW3. Each plant was weighed before each irrigation event and the volume of irrigation water required to refill the pot to its threshold level (i.e., its WFC plus its pre-determined level of leaching, depending on treatment) was calculated and added to each plant.

### Growth and colour measurements

At the ends of Period I and Period II, the substrate

TABLE I
*Chemical analyses of the water samples used for the different irrigation treatments*

| Ion (concentration) | Control[‡] | RW1[§] | RW2[§] | RW3[§] |
|---|---|---|---|---|
| Na$^+$ (mg l$^{-1}$) | 140 | 260 | 362 | 1,492 |
| Cl$^-$ (mg l$^{-1}$) | 184 | 720 | 862 | 1,557 |
| B$^{3+}$ (mg l$^{-1}$) | 0.13 | 0.18 | 0.55 | 1.26 |

[§]RW1, reclaimed water irrigation treatment at 1.5 dS m$^{-1}$, leaching 25% (v/v) of the applied water; RW2, reclaimed water irrigation treatment at 4.0 dS m$^{-1}$, leaching 40% (v/v) of the applied water; RW3, reclaimed water irrigation treatment at 8.0 dS m$^{-1}$, leaching 55% (v/v) of the applied water.
[‡]Control, tap water (0.8 dS m$^{-1}$).

was gently washed from the roots of eight plants per treatment and each plant was divided into leaves, stems, and roots. These were oven-dried at 80ºC until they reached a constant weight to measure their respective dry weights (DW). Leaf numbers and leaf areas ($cm^2$) were determined for the same plants before drying using a leaf area meter (AM 200; ADC BioScientific Ltd., Hoddesdon, UK). The root:shoot DW ratio was determined for each plant by dividing the root DW by the leaf DW.

At the ends of Period I and Period II, plant heights were measured for 20 plants per treatment and leaf colour and relative chlorophyll concentration (RCC) were measured at the mid-point of a mature leaf using three leaves from each plant and six plants per treatment. Plant height was taken as the vertical distance from the surface of the substrate to the node of the highest leaf. Leaf colour was measured using a CR-10 colorimeter (Konica Minolta Sensing Inc., Osaka, Japan), which provided values for the colour co-ordinates lightness (L*), chroma (C*), and hue angle (hº; McGuire, 1992). RCC was estimated using a SPAD-502 chlorophyll meter (Konica Minolta Sensing Inc.).

### Plant water relations and gas exchange

At the ends of Period I and Period II, changes in leaf water potential ($\Psi_l$), relative water content (RWC), stomatal conductance ($g_s$) and the net rate of photosynthesis ($P_n$) were determined in six plants per treatment midway through the photoperiod. $\Psi_l$ was estimated according to Scholander *et al.* (1965), using a pressure chamber (Model 3000; Soil Moisture Equipment Co., Santa Barbara, CA, USA). Each leaf was placed in the chamber within 20 s of collection and pressurised at a rate of 0.02 MPa $s^{-1}$ (Turner, 1988). The RWC of leaves was measured according to Barrs (1968). $g_s$ and $P_n$ were determined in attached leaves using a gas exchange system (LI-6400; LI-COR Inc., Lincoln, NE, USA).

### Mineral concentrations and water and substrate analyses

At the end of the salinity treatment and recovery periods (Period I and Period II), eight plants per treatment were separated into leaves, stems, and roots, washed with distilled water, dried at 70ºC, and stored at room temperature for inorganic solute analyses. The concentrations of $Cl^-$ ions were measured using a chloride analyser (Model 926; Sherwood Scientific Ltd., Cambridge, UK) in aqueous extracts obtained by mixing 100 mg DW of each tissue sample with 40 ml of water, shaking for 30 min, and filtering. The concentrations of $Na^+$, $B^{3+}$, $K^+$, and $Ca^{2+}$ ions were determined by digesting 100 mg DW of tissue powder with 50 ml of a 2:1 (v/v) mix of 14 M $HNO_3$:12 M $HClO_4$ and using an inductively-coupled plasma-optical emission spectrometer (ICP-OES; IRIS Intrepid II XDL; Thermo-Fisher Scientific Inc., Loughborough, UK).

The inorganic solute concentrations and EC values of each irrigation water sample were measured at the start of the experiment by collecting 100 ml in glass bottles and storing them at 5ºC until being processed for chemical analyses. EC values were measured using a multirange, Cryson-HI8734 conductivity meter (Crisom Instruments S.A., Barcelona, Spain). $Na^+$ and $B^{3+}$ ion

concentrations were determined using an ICP-OES (IRIS Intrepid II XDL; Thermo Fisher Scientific Inc.) and $Cl^-$ ion concentrations were measured using a Metrhom Chromatograph (Metrohm Ltd., Herisau, Switzerland).

Eight samples of the substrate were collected per treatment and sent for analysis to an external laboratory (Antonio Abellán Caravaca S.L., Murcia, Spain) at the ends of Period I and Period II. The substrate was dried at room temperature for 1 week. $Na^+$, $Ca^{2+}$, and $Mg^{2+}$ ion concentrations were then determined using an ICP-OES (IRIS Intrepid II XDL; Thermo Fisher Scientific Inc.) in a saturated soil extract. $Cl^-$ ion concentrations were measured by chromatography. EC values were measured in a saturated soil paste using a Cryson-HI8735 conductivity meter (Crisom Instruments S. A.).

### Statistical analysis of the data

Thirty plants were attributed at random to each of the four treatments. The data were analysed by one-way ANOVA using Statgraphics Plus for Windows 5.1 software (Manugistics Ltd., Rockville, MD, USA). Root:shoot ratio data were subjected to arcsine square-root transformation before statistical analysis to ensure homogeneity of variance. Treatment means were separated using Duncan's Multiple Range Test at $P \leq 0.05$.

## RESULTS

The EC of the substrate at the end of Period I increased in line with the increase in the EC of the irrigation water applied due to the accumulation of $Cl^-$ and $Na^+$ ions, although no significant differences were observed between RW2 and RW3 (Table II). The latter two treatments also gave the highest concentrations of $Ca^{2+}$ and $Mg^{2+}$ ions, especially RW2. After irrigating with reclaimed water, all plants were irrigated with low conductivity (0.8 dS $m^{-1}$) tap water in Period II. At the end of Period II, although the EC of the substrate was similar in all treatments, substrate $Na^+$ ion concentrations were higher in RW2 and RW3. In general, at the end of Period II a greater accumulation of salts was observed in the substrate of the control treatment than at the end of Period I. However, $Na^+$ and $Cl^-$ ion concentrations decreased in the substrate of the RW2 and RW3 treatments compared to the values recorded after Period I.

At the end of Period I, the growth of all 120 myrtle plants showed no adverse changes after the four irrigation treatments (Table III). Surprisingly, total DWs were higher in plants subjected to RW2 than in control plants. This was due to an increase in the biomass of all parts of the plant, up to 38% in leaves, 56% in stems, and 69% in roots. Leaf areas and the numbers of leaves per plant were also significantly higher in RW2 plants compared to the other treatments. Growth parameters of the aerial parts of plants irrigated with RW3 showed no significant changes compared to control plants, although root DWs increased. Root:shoot DW ratios were higher in RW3 plants, which were shorter, than in plants from the control treatment (Table III). At the end of Period II, when all plants had been watered with the same water as was used for the control plants (Table III), RW2 plants

TABLE II

*Influence of four irrigation treatments on the physico-chemical properties of the substrate collected from* M. communis *plants at the end of Period I and Period II[‡]*

| Period | Parameter | Control | RW1[§] | RW2[§] | RW3[§] |
|---|---|---|---|---|---|
| I (4 months; 120 d) | EC (dS m$^{-1}$) | 1.83 ± 0.22a[†] | 3.30 ± 0.33b | 8.57 ± 0.38c | 8.33 ± 0.23c |
| | Cl$^-$ (mmol kg$^{-1}$ DW) | 7.53 ± 1.11a | 18.11 ± 1.84b | 55.65 ± 2.92c | 63.44 ± 2.17d |
| | Ca$^{2+}$ (mmol kg$^{-1}$ DW) | 1.03 ± 0.09a | 1.50 ± 0.13a | 6.26 ± 0.40c | 3.90 ± 0.28b |
| | Mg$^{2+}$ (mmol kg$^{-1}$ DW) | 0.83 ± 0.11a | 1.34 ± 0.16a | 8.99 ± 0.43c | 6.59 ± 0.23b |
| | Na$^+$ (mmol kg$^{-1}$ DW) | 9.83 ± 1.21a | 21.61 ± 2.12b | 59.13 ± 2.76c | 63.57 ± 1.75c |
| II (4 + 2 months; 180 d) | EC (dS m$^{-1}$) | 3.21 ± 0.28a | 4.08 ± 0.34a | 4.32 ± 0.37a | 4.16 ± 0.24a |
| | Cl$^-$ (mmol kg$^{-1}$ DW) | 11.95 ± 1.41a | 16.31 ± 1.62ab | 19.50 ± 2.80b | 20.38 ± 1.37b |
| | Ca$^{2+}$ (mmol kg$^{-1}$ DW) | 1.61 ± 0.19a | 1.96 ± 0.16a | 2.51 ± 0.24b | 1.84 ± 0.12a |
| | Mg$^{2+}$ (mmol kg$^{-1}$ DW) | 1.41 ± 0.18a | 1.60 ± 0.15a | 3.03 ± 0.32b | 2.51 ± 0.24b |
| | Na$^+$ (mmol kg$^{-1}$ DW) | 20.87 ± 1.73a | 27.85 ± 2.32b | 31.46 ± 2.36bc | 34.84 ± 2.05c |

[‡]Period I, a 4-month (120 d) period with control or waste water irrigation; Period II, a 2-month (60 d) period of recovery with control (low EC) tap water irrigation after Period I.
[§]RW1, reclaimed water irrigation treatment at 1.5 dS m$^{-1}$, leaching 25% (v/v) of the applied water; RW2, reclaimed water irrigation treatment at 4.0 dS m$^{-1}$, leaching 40% (v/v) of the applied water; RW3, reclaimed water irrigation treatment at 8.0 dS m$^{-1}$, leaching 55% (v/v) of the applied water.
[†]Mean values (n = 8) ± SD in each row followed by different lower-case letters are significantly different according to Duncan's Multiple Range Test at $P \leq 0.05$.

had the highest values for all growth parameters studied, although the differences in leaf and stem DWs, leaf numbers and leaf areas compared with the controls were not significant. After the recovery period (Period II), plants that had been irrigated at the highest salinity level (RW3) had lower shoot DWs and lower leaf areas than control plants, and again had the highest root:shoot ratios. As regards plant height, the differences between control and RW3 plants observed after Period I were maintained at the end of Period II (180 d; Table III).

At the end of Period I, leaf water potential values ($\Psi_l$) became more negative as the level of salinity increased. Thus, RW3 plants had the lowest values (–1.0 MPa) and control plants had the highest values (–0.6 MPa), with intermediate values for plants irrigated with RW1 or RW2 (Figure 1A). Relative water content (RWC) values showed a similar behaviour to that observed for $\Psi_l$, with RW3 plants having the lowest values (82%; Figure1B). However, the corresponding values for plants irrigated with RW2 were slightly higher than those shown by plants treated with RW1. At the end of Period II, plants from the most saline treatment (RW3) did not reach the RWC values recorded for the other treatments (Figure 1B). At the end of Period I, lower stomatal conductance ($g_s$) and net photosynthesis ($P_n$) values were observed in all 90 plants irrigated with waste water compared to the 30 control plants (Figure 1C, D). When RW1 and RW2 plants were compared, gas exchange values ($g_s$ and $P_n$) were higher after the RW2 treatment. At the end of Period II, myrtle plants from the RW2 treatment had similar $P_n$ values to control plants (Figure1D).

Relative chlorophyll content (RCC) values did not change at any point during the experiment in any of the treatments studied (Figure 2A). In contrast, leaf colour parameters (L*, C*, and h°) were affected by the different irrigation treatments (Figure 2B-D). At the end of the recovery period (Period II), RW2 and RW3 plants had similar L* and C* values, which were lower than those in control and RW1 plants, while their h° values were higher (Figure 2B, C). The higher h° and lower L* and C* values recorded for the leaves of RW2 and RW3 plants confirmed the visibly darker and less-vivid green colour of their foliage compared to the control plants.

At the end of Period I, control and RW1 plants had similar Cl$^-$, Na$^+$, B$^{3+}$, K$^+$, and Ca$^{2+}$ ion concentrations in their leaves, stems, and roots (Table IV). Cl$^-$ ion

TABLE III

*Influence of four irrigation treatments on the growth of* M. communis *plants at the end of Period I and Period II[‡]*

| Period | Parameter (units) | Control | RW1[§] | RW2[§] | RW3[§] |
|---|---|---|---|---|---|
| I (4 months; 120 d) | Leaf DW (g plant$^{-1}$) | 6.00 ± 0.63a[†] | 5.53 ± 0.31a | 8.27 ± 0.34b | 5.84 ± 0.56a |
| | Stem DW (g plant$^{-1}$) | 5.19 ± 0.55a | 5.47 ± 0.46a | 8.08 ± 0.57b | 5.00 ± 0.42a |
| | Aerial DW ( plant$^{-1}$) | 11.20 ± 1.15a | 11.00 ± 0.73a | 16.35 ± 0.74b | 10.83 ± 0.90a |
| | Root DW (g plant$^{-1}$) | 5.45 ± 0.69a | 5.14 ± 0.63a | 9.19 ± 0.21c | 7.22 ± 0.19b |
| | Total DW (g plant$^{-1}$) | 16.64 ± 1.74a | 16.14 ± 1.31a | 25.54 ± 0.56b | 18.06 ± 0.89a |
| | Root:shoot DW ratio | 0.91 ± 0.07a | 0.92 ± 0.08a | 1.13 ± 0.07b | 1.32 ± 0.15b |
| | Leaf number | 685 ± 36a | 691 ± 31a | 918 ± 34b | 557 ± 50a |
| | Leaf area (cm$^2$) | 1109 ± 98.54a | 1119 ± 87.67a | 1640 ± 55.76b | 975 ± 52.54a |
| | Plant height (cm) | 36.8 ± 1.0b | 34.4 ± 0.7ab | 35.4 ± 1.1ab | 32.9 ± 1.0a |
| II (4 + 2 months; 180 d) | Leaf DW (g plant$^{-1}$) | 5.89 ± 0.36ab | 5.43 ± 0.51a | 7.21 ± 0.72b | 5.19 ± 0.46a |
| | Stem DW (g plant$^{-1}$) | 7.47 ± 0.64b | 6.70 ± 0.60b | 7.26 ± 0.64b | 4.41 ± 0.56a |
| | Aerial DW ( plant$^{-1}$) | 13.36 ± 0.82b | 12.14 ± 1.05ab | 14.48 ± 1.24b | 9.60 ± 0.89a |
| | Root DW (g plant$^{-1}$) | 8.65 ± 0.71a | 8.26 ± 0.43a | 11.65 ± 1.04b | 8.91 ± 1.04a |
| | Total DW (g plant$^{-1}$) | 22.02 ± 1.28ab | 20.40 ± 1.19a | 26.13 ± 2.25b | 18.51 ± 1.85a |
| | Root:shoot DW ratio | 1.50 ± 0.14a | 1.63 ± 0.15a | 1.66 ± 0.11a | 1.76 ± 0.16b |
| | Leaf number | 629 ± 35ab | 610 ± 48a | 759 ± 60b | 616 ± 40a |
| | Leaf area (cm$^2$) | 2,127 ± 135b | 2,042 ± 159b | 2,431 ± 191b | 1,561 ± 114a |
| | Plant height (cm) | 38.2 ± 1.3b | 36.2 ± 0.9ab | 36.8 ± 1.4ab | 33.8 ± 1.2a |

[‡]Period I, a 4-month (120 d) period with control or waste water irrigation; Period II, a 2-month (60 d) period of recovery with control (low EC) tap water irrigation after Period I.
[§]RW1, reclaimed water irrigation treatment at 1.5 dS m$^{-1}$, leaching 25% (v/v) of the applied water; RW2, reclaimed water irrigation treatment at 4.0 dS m$^{-1}$, leaching 40% (v/v) of the applied water; RW3, reclaimed water irrigation treatment at 8.0 dS m$^{-1}$, leaching 55% (v/v) of the applied water.
[†]Mean values (n = 8; except for plant height, when n = 30) ± SD in each row followed by different lower-case letters are significantly different according to Duncan's Multiple Range Test at $P \leq 0.05$.
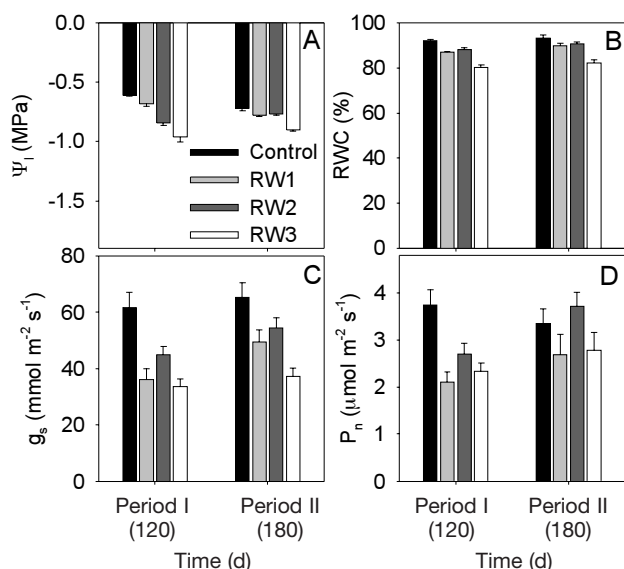
FIG. 1

Leaf water potential ($\Psi_l$; Panel A), relative water content (RWC; Panel B), stomatal conductance ($g_s$; Panel C), and rate of net photosynthesis ($P_n$; Panel D), at the end of Period I [a 4-month (120 d) period with control or waste water irrigation] and at the end of Period II [a 2-month (60 d) period of recovery after Period I (120 d) with control tap water irrigation] in *M. communis* plants under four different irrigation treatments: control, RW1, RW2, or RW3 (see Materials and Methods). Values are means (n = 6) and vertical bars indicate ± SE.
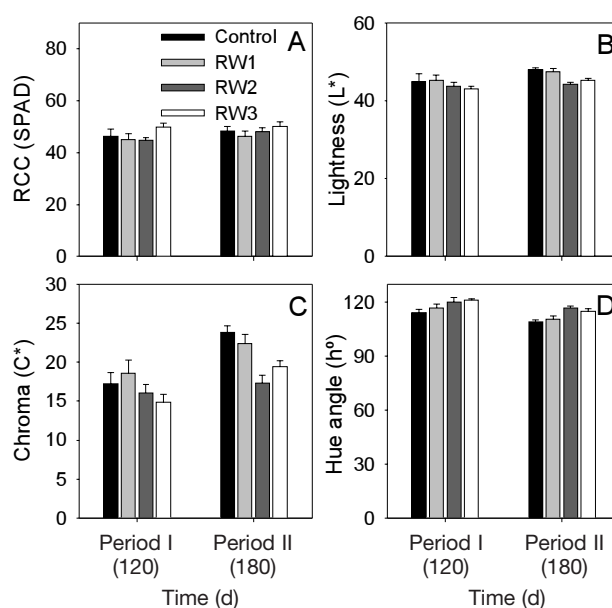


FIG. 2

Relative chlorophyll concentration (RCC in SPAD meter values; Panel A), lightness (L*; Panel B), chroma (C*; Panel C), and hue angle (h°; Panel D) at the ends of Period I [a 4-month (120 d) period with control or waste water irrigation] and Period II [a 2-month (60 d) period of recovery after Period I (120 d) with control tap water irrigation] in *M. communis* plants under four different irrigation treatments: control, RW1, RW2, or RW3 (see Materials and Methods). Values are means (n = 6) and vertical bars indicate ± SE.

concentrations at the end of Period I in all parts of the plant were similar for RW2 and RW3 and were significantly higher in the RW3 treatment than in the controls. Na$^+$ ion concentrations in leaves were higher in

RW2 plants than in control plants, while the highest Na$^+$ concentrations in all parts of the plant were found using RW3. Boron accumulation in leaves was higher in plants

TABLE IV

*Influence of four irrigation treatments on Na$^+$, Cl$^-$, B$^{3+}$, K$^+$, and Ca$^{2+}$ ion concentrations (in mmol kg$^{-1}$ DW tissue) in* M. communis *plants at the end of Period I and Period II*[‡]

| Period | Ion | Tissue | Control | RW1[§] | RW2[§] | RW3[§] |
|---|---|---|---|---|---|---|
| I (4 months; 120 d) | Cl$^-$ | Leaf | 74.37 ± 10.96a[†] | 94.84 ± 12.42ab | 104.41 ± 7.84ab | 120.19 ± 12.41b |
| | | Stem | 66.85 ± 4.99ab | 57.84 ± 7.40a | 91.27 ± 9.97bc | 99.34 ± 13.71c |
| | | Root | 64.60 ± 1.90a | 88.64 ± 12.24ab | 121.50 ± 13.52b | 123.19 ± 17.48b |
| | Na$^+$ | Leaf | 25.38 ± 1.69a | 36.80 ± 3.38ab | 59.35 ± 6.41b | 128.10 ± 17.40c |
| | | Stem | 60.38 ± 6.25a | 94.26 ± 19.85a | 86.72 ± 7.63a | 167.85 ± 18.69b |
| | | Root | 162.13 ± 16.53a | 188.51 ± 12.74a | 184.88 ± 18.67a | 355.83 ± 19.43b |
| | B$^{3+}$ | Leaf | 8.02 ± 0.16a | 9.28 ± 0.27ab | 10.46 ± 1.36b | 10.58 ± 0.34b |
| | | Stem | 5.63 ± 0.13a | 6.21 ± 0.28a | 8.79 ± 0.84b | 6.13 ± 0.13a |
| | | Root | 6.95 ± 0.40a | 7.29 ± 0.47ab | 6.80 ± 0.55a | 8.53 ± 0.29b |
| | K$^+$ | Leaf | 449.15 ± 15.75b | 456.52 ± 26.24b | 558.21 ± 19.69c | 333.28 ± 8.20a |
| | | Stem | 305.28 ± 30.59a | 311.52 ± 51.30a | 331.45 ± 23.99b | 232.69 ± 16.82a |
| | | Root | 176.21 ± 17.13ab | 135.72 ± 19.12a | 202.93 ± 11.15b | 134.19 ± 5.97a |
| | Ca$^{2+}$ | Leaf | 108.73 ± 6.90a | 112.22 ± 5.14a | 161.32 ± 15.59b | 159.64 ± 2.27b |
| | | Stem | 113.04 ± 12.21a | 120.43 ± 17.88a | 120.06 ± 16.54b | 230.85 ± 5.28b |
| | | Root | 119.60 ± 11.56 | 101.30 ± 17.86 | 111.66 ± 7.70 | 99.09 ± 0.11 |
| II (4 + 2 months; 180 d) | Cl$^-$ | Leaf | 69.86 ± 14.89 | 75.72 ± 12.11 | 84.73 ± 15.55 | 96.00 ± 14.52 |
| | | Stem | 71.89 ± 16.28 | 82.70 ± 12.05 | 83.38 ± 13.58 | 77.97 ± 9.24 |
| | | Root | 75.72 ± 14.06 | 58.82 ± 5.67 | 59.61 ± 7.03 | 68.96 ± 7.81 |
| | Na$^+$ | Leaf | 31.62 ± 4.57a | 94.85 ± 15.66b | 39.92 ± 8.52a | 75.47 ± 13.10b |
| | | Stem | 98.80 ± 17.59a | 124.75 ± 9.78ab | 109.43 ± 21.10a | 167.60 ± 16.13b |
| | | Root | 190.09 ± 15.37a | 282.83 ± 13.48b | 209.44 ± 14.52a | 274.95 ± 22.27b |
| | B$^{3+}$ | Leaf | 10.26 ± 0.37a | 12.96 ± 0.55c | 11.54 ± 0.39b | 14.11 ± 0.41c |
| | | Stem | 6.18 ± 0.14a | 7.67 ± 0.19c | 7.08 ± 0.18b | 7.79 ± 0.19c |
| | | Root | 6.55 ± 0.19a | 9.09 ± 0.22d | 7.75 ± 0.19b | 8.45 ± 0.15c |
| | K$^+$ | Leaf | 401.14 ± 6.85b | 314.63 ± 13.52a | 419.56 ± 17.44b | 310.82 ± 10.49a |
| | | Stem | 298.96 ± 16.77c | 226.44 ± 11.67b | 203.87 ± 16.06ab | 181.85 ± 5.86a |
| | | Root | 122.65 ± 8.89b | 99.13 ± 7.13a | 98.91 ± 8.01a | 87.58 ± 7.40a |
| | Ca$^{2+}$ | Leaf | 116.24 ± 4.89ab | 131.76 ± 6.89bc | 105.24 ± 5.62a | 135.96 ± 4.76c |
| | | Stem | 139.74 ± 13.46b | 136.11 ± 10.84b | 95.38 ± 8.25a | 139.14 ± 11.49b |
| | | Root | 152.87 ± 9.57a | 202.81 ± 17.07b | 154.73 ± 9.82a | 164.18 ± 13.10a |

[‡]Period I, a 4-month period (120 d) with control or waste water irrigation; Period II, a 2-month period (60 d) of recovery with control (low EC) tap water irrigation after Period I.

[§]RW1, reclaimed water irrigation treatment at 1.5 dS m$^{-1}$, leaching 25% (v/v) of the applied water; RW2, reclaimed water irrigation treatment at 4.0 dS m$^{-1}$, leaching 40% (v/v) of the applied water; RW3, reclaimed water irrigation treatment at 8.0 dS m$^{-1}$, leaching 55% (v/v) of the applied water.

[†]Mean values (n = 8) ± SD in each row followed by different lower-case letters are significantly different according to Duncan's Multiple Range Test at $P \leq 0.05$.

TABLE V
*Influence of four irrigation treatments on $K^+$/$Na^+$ and $Ca^{2+}$/$Na^+$ ion ratios in leaves of* M. communis *plants at the end of Period I and Period II*[‡]

| Period | Ratio | Control | RW1[§] | RW2[§] | RW3[§] |
|---|---|---|---|---|---|
| I (4 months) | $K^+$/$Na^+$ | $18.19 \pm 1.63$c[†] | $12.99 \pm 1.42$b | $10.14 \pm 1.45$b | $2.84 \pm 0.36$a |
| | $Ca^{2+}$/$Na^+$ | $4.18 \pm 0.36$c | $3.23 \pm 0.26$b | $3.01 \pm 0.33$b | $1.23 \pm 0.23$a |
| II (6 months) | $K^+$/$Na^+$ | $14.20 \pm 1.28$b | $4.14 \pm 0.70$a | $14.25 \pm 2.44$b | $5.05 \pm 0.70$a |
| | $Ca^{2+}$/$Na^+$ | $4.21 \pm 0.48$b | $1.72 \pm 0.26$a | $3.83 \pm 0.76$b | $2.19 \pm 0.28$a |

[‡]Period I, a4-month period (120 d) with control or waste water irrigation; Period II, a 2-month period (60 d) of recovery with control (low EC) tap water irrigation after Period I.
[§]RW1, reclaimed water irrigation treatment at 1.5 dS m^{−1}, leaching 25% (v/v) of the applied water; RW2, reclaimed water irrigation treatment at 4.0 dS m^{−1}, leaching 40% (v/v) of the applied water; RW3, reclaimed water irrigation treatment at 8.0 dS m^{−1}, leaching 55% (v/v) of the applied water.
[†]Mean values (n = 8) ± SD in each row followed by different lower-case letters are significantly different according to Duncan's Multiple Range Test at $P \leq 0.05$.

irrigated with all three reclaimed water samples. Similarly, $B^{3+}$ ions accumulated in the stems of RW2 plants and in the roots of RW3 plants. RW2 plants had the highest $K^+$ ion concentrations and RW3 plants had the lowest values. However, the highest $Ca^{2+}$ ion concentrations were found in the leaves and stems of plants irrigated with RW2 or RW3.

At the end of Period II, plants from all treatments had similar $Cl^-$ ion concentrations in all parts (leaves, stems, and roots). RW1 and RW3 plants had higher $Na^+$ ion concentrations in their leaves and roots than in the controls and RW2 plants. $B^{3+}$ ion concentrations were higher than in the controls in the three reclaimed water irrigation treatments, especially RW1 and RW3. In general, $K^+$ ion concentrations were lower in plants watered with reclaimed water than in control plants. RW3 plants had the lowest $K^+$ ion concentrations.

Leaf $K^+$/$Na^+$ and $Ca^{2+}$/$Na^+$ ion ratios at the end of Period I were lower in plants irrigated with all three reclaimed water samples, especially RW3, which produced the lowest values (Table V). At the end of Period II, the highest $K^+$/$Na^+$ and $Ca^{2+}$/$Na^+$ ion ratios in leaves were found in RW1 and RW3, while the values measured in RW2 plants did not differ from those in the controls.

## DISCUSSION

Treated waste waters have a variable salt content that depends on their origin, which makes their use problematic when irrigation strategies are unsuitable. One important aspect when using low quality reclaimed water is the technique used for plant culture, which affects the development and agronomic performance of the crop (Bañón *et al*., 2012). For example, regulating the drainage is considered to be a valid tool to reduce the problems associated with salinity. The lower the quality of the water, the higher the drainage necessary to prevent the accumulation of salts in the substrate (Evans, 2004). In this experiment, adjusting the amount of drainage according to the EC of the irrigation water applied reduced the toxic negative effects of the salts. Even after 4 months (120 d) of applying reclaimed waste water with EC values of 1.5 – 8.0 dS m^{−1}, no reduction in growth parameters was observed in the myrtle plants. Moreover, using RW2 resulted in higher shoot and root DWs than the other three treatments.

One possible advantage of using reclaimed waste water can be the composition of the water, which often has higher organic matter and nutrient contents than fresh water (Janssen *et al*., 2005). However, it is important to know the concentrations of solutes in the irrigation water, since high concentrations of $Na^+$ and $Cl^-$ ions may be offset by the beneficial effects of other solutes such as $Mg^{2+}$, $K^+$, $PO_4^{3-}$, and $Ca^{2+}$ ions. Analysis of the treated waste waters used here identified high levels of these elements, meaning that their concentrations in the myrtle plants were not diminished by the effect of NaCl, and were even increased, as in the case of P (data not shown; Gómez-Bellot *et al*., 2013). The highest levels of $Ca^{2+}$ and $Mg^{2+}$ ions were found in the substrate of the RW2 treatment (Table II).

Some differences in plant growth parameters were observed between the different treatments after the recovery period (day-180). For example, plants irrigated with RW3 had lower biomass and leaf areas, suggesting that, although they were irrigated with good quality water during the recovery Period II, the accumulation of toxic ions such as $Na^+$ and $B^{3+}$ had had a negative effect on plant growth. This did not occur in RW2-irrigated plants, which generally had a higher biomass than RW1 plants.

The low $\Psi_l$ and RWC values of plants irrigated with RW3 (the highest salinity) reflect the increased difficulty for plants to take-up water from the substrate due to the high accumulation of salts (Álvarez *et al*., 2012). Despite the availability of water in the substrate, the osmotic effect of the salts in the root zone limit the absorption of water (Hardikar and Pandey, 2008), as reflected in the water status of the plants (Figure 1). This behaviour has been observed in other ornamental species grown under similar conditions (Navarro *et al*, 2007; Miralles *et al*., 2011). However, the most significant response was the decrease in $g_s$ values in all plants treated with reclaimed water, which acted as a mechanism to prevent excessive loss of water by transpiration (Muns and Tester, 2008; Figure 1C). $P_n$ values were also affected. The highest $P_n$ values among the reclaimed water treatments were observed in RW2 plants, which correlated with their higher DW, increased leaf area, and greater numbers of leaves (Table III). Although recovery after a period of salinity was characterised by an increase in leaf water parameter values (Chaves *et al*., 2009), this was not observed in plants irrigated with RW3.

In many studies, the effects of salinity on $P_n$ and $g_s$ have been shown to depend on species, salinity level, and the duration of the saline stress imposed (Tattini *et al*., 2002; Álvarez and Sánchez-Blanco, 2014). Another parameter used to detect differences in the salt-tolerance of different species used for landscaping is RCC. In some species, it has been observed that reductions in leaf RCC values due to high salt levels reflect a low degree of stress tolerance (Cabrera, 2003). However, under our

conditions, RCC values did not change significantly in the four different treatments applied.

Aesthetic value is an important trait in ornamental plants and an absence of visible leaf damage such as chlorosis, necrosis, or premature leaf drop is critical to the evaluation of plant quality. None of these symptoms were observed in our experiments. Controlled environment conditions (i.e., light, temperature and humidity) and irrigation practices can affect plant responses (Fox *et al*., 2005). The inhibition of photosynthesis observed at the end of Period II (day-180) led to a reduction in photo-assimilates and less dry matter production in RW3 plants (i.e., the lowest total DW, stem DW, and height). This could be related to higher concentrations of toxic ions, especially $Na^+$ and $B^{3+}$, in the leaves of RW3 plants compared with the other treatments (Álvarez *et al*., 2012). Plants in the RW2 treatment had similar $Cl^-$ ion concentrations to those in the controls and were the least affected. Thus, $Cl^-$ ion concentrations were similar in all treatments, whereas $Na^+$ and $B^{3+}$ ions accumulated more in RW3 plants compared to the controls.

High concentrations of $B^{3+}$ ions are another problem associated with the use of reclaimed water, and high levels of $B^{3+}$ ions were observed in leaves and roots, especially in RW3 plants. However, no $B^{3+}$-related toxicity symptoms were observed, perhaps because the higher concentrations of $Na^+$ ions interfered with the absorption of $B^{3+}$ ions (El-Motaium *et al*, 1994; Edelstein *et al*., 2005). Moreover, symptoms associated with the accumulation of $Na^+$ and $Cl^-$ ions may mitigate the damage typically caused by excess $B^{3+}$ ions (Bañón *et al*., 2012). While salinity has been shown to aggravate the symptoms of B toxicity in wheat (Wimmer *et al*., 2003), it has also been reported that the addition of B to the nutrient solution can prevent the reduction in NaCl-induced plant growth in pea plants (El-Handaui *et al*., 2003).

High levels of salinity reduce the absorption of $K^+$ and $Ca^{2+}$ ions in many species (Niu *et al*., 1995; Chaparzadeh *et al*., 2003). In our study, $K^+/Na^+$ and $Ca^{2+}/Na^+$ ion ratios decreased in the leaves of all plants irrigated with all reclaimed waters, but to the greatest extent with RW3.

RW2 plants showed similar ion ratios to the controls after 60 d of irrigation with low EC, tap water (the recovery phase). Many species exhibit some degree of tolerance to salinity (Heuer and Ravina, 2004), which appears to be related to a higher selective uptake of $K^+$ ions than $Na^+$ ions (Heuer and Ravina, 2004; Colmer *et al*., 2006). The severe reduction in growth, even at relatively low salt levels (2.0 – 3.0 dS $m^{-1}$), has been attributed to increases in $Na^+$ and $Cl^-$ ions, accompanied by a major reduction in $Ca^{2+}$ and $K^+$ ion concentrations in plant tissues (Valdez-Aguilar *et al*., 2009). However, these effects did not occur in our study. In addition, plants irrigated with reclaimed water showed relatively high $K^+/Na$ and $Ca^{2+}/Na^+$ ion ratios, especially in leaves, which correlated with their response to salinity. In this sense, $K^+$ and $Ca^{2+}$ ions not only play important roles in plant growth and development, but are also vital for osmotic adjustment and the maintenance of cell turgor (Osakabe *et al*., 2014).

In conclusion, the three reclaimed water samples had different effects on *M. communis* plants, depending on their chemical properties. This was most evident in the ability of RW-treated plants to recover from salinity. Reclaimed water of moderate conductivity (EC = 4.0 dS $m^{-1}$; RW2) was able to maintain the quality of the ornamental plants and could be regarded as safe for a nutrient management strategy. None of the problems associated with reclaimed water, such as salinity, were seen in the RW2 treatment. However, *M. communis* plants irrigated with reclaimed water of high EC (RW3; 8.0 dS $m^{-1}$) were stunted and showed reductions in their gas exchange parameters, which did not recover after a 60 d period of irrigating with low EC, tap water. EC values, the different salts present in the irrigation water, and the extent of the leaching fraction, must all be considered when using reclaimed water for irrigation purposes.

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