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Water relations, nutrient content and developmental responses of ormanental *Euonymus* plants irrigated with water of different degrees of salinity and quality

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Abstract

For twenty weeks, the physiological responses of *Euonymus japonica* plants to different irrigation sources were studied. Four irrigation treatments were applied at 100% water holding capacity: Control (electrical conductivity (EC) < 0.9 dS m^{-1}); irrigation water normally used in the area, (irrigator's water) IW (EC: 1.7 dS m^{-1}); NaCl solution, NaCl (EC: 4 dS m^{-1}); and wastewater, WW (EC: 4 dS m^{-1}). This was followed by a recovery period of thirteen weeks, when all the plants were rewatered with the same amount of irrigation water as the control plants. Despite the differences in the chemical properties of the water used, the plants irrigated with NaCl and WW showed similar alterations in growth and size compared with the control even at the end of the recovery period. Leaf number was affected even when the EC of the irrigation water was of 1.7 dS m^{-1} (IW), indicating the salt sensitivity of this parameter. Stomatal conductance and photosynthesis, as well as stem water potential, were most affected in plants irrigated with the most saline waters (NaCl and WW). At the end of the experiment the above parameters recovered, while IW plants showed similar values to the control. The higher Na^+ and Cl^+ uptake by NaCl and WW plants led them to show osmotic adjustment throughout the experiment. The highest amount of boron found in WW plants did not affect root growth. Wastewater can be used as a water management strategy for ornamental plant production, as long as the water quality is not too saline, since the negative effect of salt on the aesthetic value of plants need to be taken into consideration.

Keywords: Gas exchange; NaCl; Reclaimed wastewater; Quality plants; Water relations.

Abbreviattions

EC, electrical conductivity; DW, dry weight; g_s, stomatal conductance; J, absorption rate of ions by roots; NTU, Nephelometrical Turbidity Units; P, significance level; PAC, percentage of plants in acceptable conditions; PDB, percentage of plants with dry branches; PIC, percentage of plants in ideal conditions; DP, percentage of dry plants;

P_n, net photosynthesis; RH, relative humidity; TDS, total dissolved solids; VPD, vapour pressure deficit; Ψ_{stem}, stem water potential; Ψ_{100s}, osmotic potential at full turgor.

Introduction

In the Iberian Peninsula, particularly in Mediterranean areas, the increasing shortage of water resources and their low quality mean that greater effort is needed for their management to satisfy the needs of crops (Jordan et al. 2009; Ruiz-Sánchez et al. 2010). Indeed, the use of low quality water, in many species is becoming an alternative, in order to guarantee irrigation. Several studies have shown the environmental and agronomical interest of using wastewater for irrigation in different crops (Parsons et al. 2001; Aiello et al. 2007). Nevertheless, in ornamental plant production the high concentration of toxic ions that this kind of water contains can decrease the quality and landscape value of these plants (Wu and Dodge 2005). Depending on their source and treatment, such waters may have a high salt content, especially sodium and chloride and significant quantities of toxic metals (Barar et al. 2000; Yadav et al. 2002). Their long-term use therefore may result in the toxic accumulation of heavy metals with unfavourable effects on plant growth (Rattan et al. 2005). Phosporus concentration levels may also be very high, contributing to surface water eutrophication (Kalavrouziotis et al. 2005) and high levels of boron can cause phytotoxic problems in crops. For example, numerous articles have demonstrated that B^+ reduces citrus tree growth and productivity and contributes to defoliation and yellow leaves (Chapman HD 1968, Aucejo et al. 1997; Barar et al. 2000). This kind of water may also contain high

K^+ and S^- levels that can induce magnesium and phosphorus deficiency, respectively (Rattan et al. 2005) reducing the growth and development of plants. Nevertheless, potential irrigation problems associated with low water quality will depend on the duration of its application (Bansal et al. 1992; Palaniswami and Sree Ramulu 1994) and the different characteristics of the plant species. One alternative that has produced goods results and avoided many of the problems associated with wastewater use in agriculture was the blending reclaimed water with well water (Pedrero 2010; Bañón et al. 2011).

Salinity can cause imbalances in the uptake of mineral nutrients as well as a gradual accumulation of electrolytes (mainly Na^+ and Cl^-) in the aerial parts, causing damage to the plant metabolism, when no compartmentation of saline ions in the vacuole takes place. One of the main mechanisms that plants use to adapt to osmotic stress is osmotic adjustment which maintains the positive turgor required for stomata opening and cell enlargement (Torrecillas et al. 2003; Navarro et al. 2007; Alvarez et al. 2012). When osmotic adjustment occurs, the osmotic potential decreases and, as a consequence, the water potential falls, diminishing the availability of water to the root, affecting growth and vegetal development (Greenway and Munns 1980; Tanji 1990; Neumann 1997).

If poor quality water is to be used, it is important to select plants that have mechanisms which help tolerate osmotic and saline damage without damaging their development.

Euonymus japonica is a popular compact shrub, which is usually cultivated as a hedge to adorn gardens. It is well adapted to coastal zones where high concentrations of salt accumulate in the soil. The objectives of this work were: 1) to study the effect of different irrigation water sources on plant growth and quality, water relations, gas exchange and nutrient content in *Euonymus japonica*, and 2) to evaluate whether reclaimed water with a high salinity level can be used as an alternative source of water and nutrients for Euonymus plant production. The results will increase the little information that exists on the impact of low quality water of different chemical properties on shrub species of ornamental and landscape interest in the Mediterranean region.

Material and methods

Plant material and growth conditions

Euonymus japonica plants (n= 160) with an initial height of 15 cm, were transplanted on 23 March 2010 into 2.5 L polyethylene pots (diameter 17 cm, height 14 cm) containing a substrate of coconut fibre, black and blond peat, and perlite, (8:7:1) amended with 2 g L^{-1} of Osmocote Plus (14:13:13 N, P, K plus microelements). The pots were placed in a plastic greenhouse in the CEBAS experimental farm located in Santomera (Murcia, Spain), equipped with a cooling system. The micro-climatic conditions, recorded with a Hoboware Lite Data Logger (Escort Data Loggers, Inc., Buchanan, Virginia, USA), showed maximum/minimum average temperatures of 20/17 ºC and maximum/minimum average RH of 70/50 %. During the experimental period, the average values of the air temperature, RH and vapour pressure deficit (VPD) were around 22 ºC, 64% and 1.3 KPa, respectively.

Irrigation with saline water began on 29 April 2010, five weeks after transplanting. For twenty weeks (saline phase) plants of different irrigation sources were studied. Four irrigation treatments were applied at 100% water holding capacity: Control (EC < 0.9 dS m^{-1}, leaching 10-15% of the applied water); irrigation water normally used in the area (irrigator's water, reclaimed wastewater blended 50% with well water), IW (EC: 1.2-1.8 dS m^{-1}, leaching 20-25%); NaCl solution, NaCl (EC: 4 dS m^{-1}, leaching 30-40%); and reclaimed wastewater, WW (EC: 4 dS m^{-1}, leaching 30-40%) from a sewage treatment plant located in Campotejar (Murcia, Spain). The wastewater treatment plant applies a conventional activated sludge process followed by ultraviolet application for tertiary treatment. The saline period ended on 15 September 2010.

After the saline period, the plants of the IW, NaCl and WW treatments were rewatered maintaining the same conditions as the control plants for a further thirteen weeks (recovery period). The experiment finished on 16 December 2010, thirty eight weeks after transplanting.

One drip nozzle, delivering 2 l h^{-1} per pot, was connected to two spaghetti tubes, one on each side of every pot. Plants were irrigated daily and the duration of each irrigation episode depended on the season, climatic conditions and plant development. Water consumption was measured gravimetrically throughout the experimental period and was determined from the difference in weights (weight after irrigation, when drainage stopped, and before irrigating again).

Water and substrate analyses

The inorganic solute content, pH and EC of the irrigation waters were assessed at the beginning of the experiment. The samples were collected in glass bottles and stored at

5ºC before being processed for chemical analyses. pH was measured with a Cryson-507 pH-meter (Crisom Instruments S.A. Barcelona, Spain); EC and total dissolved solids (TDS) were determinated using the multirange equipment, Cryson-HI8734 (Crisom Instruments S.A. Barcelona, Spain); turbidity was measured with a Dinko-D-110 turbidity meter (Dinko Instruments S.A., Barcelona, Spain); the concentrations of macronutrients (Na^+, K^+, P^+, Ca^{2+} and Mg^{2+}) and micronutrients (B^+ and S^-) were determined by inductively coupled plasma optical emission spectrometer (ICP-ICAP 6500 DUO Thermo, England) and anions (chloride, nitrate, phosphate and sulphate) were analysed by ion chromatography with a Metrhom Chromatograph (Switzerland);

Five substrate samples per treatment were collected and sent to an external analysis laboratory (Antonio Abellán Caravaca S.L. (Fitosoil)) at the end of the saline period and at the end of the recovery period. The substrate was dried at room temperature for a week. Na^+, Ca^{2+} and Mg^{2+} were determined by Inductively Coupled Plasma ICP-AES in a saturated soil extract and Cl^- was determined by ion chromatography. EC was determined on saturated soil paste.

Measurements of growth, ornamental characters and mineral content

At the end of the saline and recovery periods, the substrate was gently washed from the roots of five plants per treatment. The plants were divided into shoots (leaves and stems) and roots. These were then oven-dried at 80 ºC until they reached a constant weight to measure the respective dry weights (DW). Leaf number was counted directly, while succulence was calculated as the shoot fresh weight/ shoot dry weight ratio, and leaf area (cm^2) was determined in the same plants, using a leaf area meter (Delta-T;

Devices Ltd., Cambridge, UK). Root length was analyzed by a root system analyzer (Winrhizo LA 1600 Regent Inc., USA).

At the end of the saline and recovery periods all the plants were visually evaluated as follows: (1) PIC, percentage of plants in ideal condition; (2) PAC, percentage of plants in acceptable condition; (3) PDB, percentage of plants with dry branches; and (4) DP, percentage of dry plants.

The inorganic solute content of leaves and roots was determined from the dry mass in five plants per treatment at the end of the saline period. The concentration of Cl^- was analysed by a chloride analyzer (Chloride Analyser Model 926, Sherwood Scientific Ltd.) in the aqueous extracts obtained by mixing 100 mg of dry vegetable powder with 40 ml of water before shaking for 30 min and filtering. The concentrations of Na^+, K^+, Ca^{2+}, Mg^{2+} and B^+ were determined in a digestion extract obtained by mixing 1 g of dry plant material with 10 mL of concentrated HNO_3 and 5 mL of concentrated $HClO_4$. After digestion, they were filtered through Whatman filter paper and a volume of 100 mL was obtained by adding distilled water (Rashid 1986). Na^+, K^+, Ca^{2+}, Mg^{2+} and B^+ were analized by Inductively Coupled Plasma optical emission spectrometer (ICP-OES IRIS INTREPID II XDL).

The absorption rate of Na^+, Cl^- and B^+ ions by the root system (J) was calculated considering the total salt content of five plants per treatment at harvest, expressed as mmol Na^+, Cl^- and B^+ and the mean root weight, using the formula described by Pitman (1975):

$$J = (M_2 - M_1) / (WR * t)$$

where M_1 and M_2 correspond to concentration in mmol of Na^+, Cl^- or B^+ in the total plant at the beginning and at the end of saline period, respectively, t corresponds to time in days and WR is the logarithmic mean root biomass, calculated as (WR_2-WR_1)/Ln (WR_2/WR_1), with WR_1 and WR_2 being the dry weight of roots at the beginning and at the end of saline period respectively.

Water relations

Seasonal changes in leaf stomatal conductance (g_s), net photosynthesis (P_n), stem water potential (Ψ_{stem}) and leaf osmotic potential at full turgor (Ψ_{100s}), were determined at midday in six plants per treatment periodically during the assay.

Leaf stomatal conductance and net photosynthesis were determined in sunny leaves using a gas exchange system (LI-6400; LI-COR Inc., Lincoln, NE, USA) in greenhouse conditions of temperature, light irradiation, CO_2 concentration and relative humidity.

Stem water potential was estimated immediately in the same leaves as g_s and P_n were measured, according to Scholander et al. (1965), using a pressure chamber (Model 3000; Soil Moisture Equipment Co., Santa Barbara, CA, USA) in which leaves were placed in the chamber within 20s of collection and pressurised at a rate of 0.02 MPa s^{-1} (Turner 1988). Leaves for Ψ_{stem} were taken from the north facing side and were covered with aluminium foil for at least 2 h before measurements. Leaves from plants were excised with their petioles and placed in distilled water overnight to reach full saturation before being frozen in liquid nitrogen (-196 °C) and stored at -30 °C. After thawing, the osmotic potential at full turgor (Ψ_{100s}) was measured in the extracted sap using a WESCOR 5520 vapour pressure osmometer (Wescor Inc., Logan, UT, USA), according to Gucci et al. (1991).

Statistics

For the experiment, 40 plants were randomly attributed to each treatment. The data were analysed by one-way ANOVA using Statgraphics Plus for Windows 5.1 software. Ratio and percentage data were subjected to an arcsine square-root transformation before statistical analysis to ensure homogeneity of variance. Treatment means were separated with Duncan's Multiple Range Test ($P \leq 0.05$). Pearson's correlation analysis was used to test for any relationship between leaf ion concentrations and leaf dry weight.

Results

Chemical characteristics of water and substrate

At the beginning of the experiment, the physicochemical properties of the irrigation waters were analyzed (Table 1). The NaCl and WW waters had similar pH, EC, and TDS values. The highest EC value of both waters compared with the control water was due to the higher salt content. The sodium and the chloride content of the NaCl water was about 15 and 18-times higher, respectively, than the corresponding values of the control water, meaning that Nephelometrical Turbidity Units (NTU) were up to 4 times higher than in the control treatment. The highest boron, calcium, potassium, magnesium, phosphorus, sulphur and sulphate values were observed in the WW water. The IW water showed intermediate values between the control and the highest saline waters (NaCl and WW) for most ions.

At the end of the saline period irrigation with NaCl and WW treatment caused an accumulation of Na^+ and Cl^- in the substrate, especially of Cl^- in the NaCl treatment (Table 2). There were no significant differences in Ca^{2+} content between the three saline treatments, while the Mg^{2+} content was only higher than the control in the WW treatment. The salt content increased the EC values of the substrate in the NaCl and WW treatments to around 10 dS m^{-1}. At the end of the experiment, after irrigation with control water, Na^+ and Cl^- concentrations were similar in all treatments. Only the NaCl treatment showed lower Ca^{2+} and Mg^{2+} levels than the other three treatments.

Growth, ornamental characteristics and mineral content

At the end of saline period, the aerial biomass of *euonymus* plants irrigated with NaCl and WW water was statistically lower than in the control plants due to the lower in leaf DW (11.02±0.69 g $plant^{-1}$ and 14.35±2.03 g $plant^{-1}$ in NaCl and WW treatments, respectively), as well as a decrease in leaf number (36% and 25% lower than the control treatment in NaCl and WW treatments, respectively) and total leaf area (43% and 36% lower than the control treatment in NaCl and WW treatments, respectively). An increase in succulence (40% and 21% higher than the control treatment in NaCl and WW treatments, respectively) was observed in these treatments (Table 3). As regards the root/shoot ratio, this parameter only increased in plants irrigated with NaCl due to the marked reduction in leaf DW in this treatment. In contrast, root DW did not show significant differences between all the treatments, although a significant decrease in total root length was observed in NaCl and WW plants (Table 3), more specifically in thin (Ø≤0.5 mm) and medium thickness (0.5<Ø≤2.0 mm) roots (data not shown). No growth parameters in the IW treatment were significantly altered during this time, except for leaf number (Table 3).

At the end of recovery period, leaf DW in the two most saline treatments (NaCl and WW) continued to be significantly lower than in the control treatment, and the values were even lower than at the end of the saline period (Table 3). The same behaviour was observed for leaf number and total leaf area.

As regards root growth, neither the dry weight nor the total length of the roots of NaCl and WW plants recovered (Table 3) by the end of the experiment although the root/shoot ratio did not show significant differences between any of the treatments.

As regards the visual characteristics, both at the end of the saline and the recovery periods, a high percentage of PIC were observed in the control treatment (around 94%), followed by plants irrigated by IW (around 81%) (Fig. 1A, B). In contrast, the NaCl treatment produced 68 % of PAC at the end of saline phase (Fig. 1A), a percentage that decreased by the end of the assay, when the number of DP or PDB had increased. A similar response was seen in the plants from the WW treatment, where the quantity of DP increased from 6.45 % in the saline phase to 23.33 % at the end of the recovery period (Fig. 1A, B).

Cl- and Na^+ accumulated in the leaves of the plants subjected to the two most saline treatments, especially in the NaCl treatment. No differences between the control and IW were observed at the end of the saline period in this respect (Table 4). Sodium concentrations were higher in leaves than in roots in the NaCl treatment, while the Cl^- concentration was similar in both leaves and roots.. The highest K^+ values in roots were found in IW plants. B^+ only accumulated in WW plants (in both leaves and roots). In general, K^+ and Ca^{2+} accumulation was greater in the leaves than in the roots in all plants. While the contrary was observed for Mg^{2+}.

The highest rates of Na^+ and Cl^- absorption by roots were observed in plants of the NaCl and WW treatments (Fig. 2A, B), especially the NaCl treatment. As regards B^+ absorption by roots, WW had the highest values, although no statistically significant differences were found with respect to the control (Fig. 2C). The lowest rates of B^+ absorption were found for the NaCl and IW treatments.

Plant water relations and gas exchange

During the saline period, midday Ψ_{stem} in the control plants ranged between -0.6 and -1.0 MPa, but was significantly lower for the most experimental period in NaCl and WW plants, reaching values of -0.87 to -1.15 MPa for NaCl and of -0.8 to -1.20 MPa for WW (Fig. 3A). The Ψ_{stem} values of IW plants were close to those of control plants.

Significant differences in Ψ_{100s} values were found between the two highest saline treatments and the control along almost throughout the assay (Fig. 3B), pointing to the plants osmotic adjustment which induced higher pressure potential values (data not shown). No differences were observed in the Ψ_{100s} values between the control and IW treatment.

When control water was applied to all the plants (recovery phase), neither the Ψ_{stem} nor the Ψ_{100s} values of the NaCl and WW treatments recovered (Fig. 3A, B).

As regards gas exchange, the control plants had the highest stomatal conductance and photosynthesis values during the saline period (Fig. 3C, D). Both parameters were significantly lower in the other treatments, particularly in NaCl and

WW, which showed similar values. Both g_s and P_n tended to recover at the end of the experiment in NaCl and WW plants (Fig. 3C, D). Statistical differences in the gs values of IW plants compared with control disappeared, while the P_n was higher at the end of the experiment (Fig. 3C, D).

Discussion

As seen in many other ornamental species submitted to saline conditions (Gori et al. 2000), the toxic effect of the salt accumulated as a result of using NaCl and WW delayed the growth and development of *Euonymus* plants. There are few references to the effect of using reused water for irrigation on the physiological response of ornamental plants, although Bañon et al. (2011) observed that the growth of lantana and polygala plants was reduced when saline reused water (5.11dS m^{-1}) was applied, but differently in each species. In our case, growth reductions were very similar in the plants of both treatments (NaCl and WW), despite the different results obtained for the chemical properties of the waters (Table 1) and the differences in salt accumulation in the substrate, especially in the case of Cl^- (Table 2). As regards the development parameters the root/shoot ratio was higher in the plants irrigated with NaCl than with WW. Similar behaviour was observed in herbaceous perennials under saline irrigation (Niu and Rodriguez 2006; Navarro et al. 2008). Salinity affects leaf number and, in our case, this occurred even when the EC of the irrigation water was around 1.7 dS m^{-1}(IW). One of the first symptoms of plants exposed to high salinity is a restriction in leaf expansion with a subsequent decrease in leaf area (Navarro et al. 2007). It can be explained by a decrease in leaf turgor, changes in cell wall properties or decreased photosynthesis rates (Rodriguez et al. 2005). It has been demonstrated that a sudden increase in soil salinity causes cells to lose water, although the loss of cell volume and turgor is transient, as was observed in our conditions. With time, the cells regain their original volume and turgor owing to osmotic adjustment, but despite this, cell elongation rates are reduced (Passioura and Munns 2000). Reductions in cell elongation and cell division lead to leaves appearing more slowly and to a smaller final size. Cell dimensions change, with greater reductions in area than depth, so leaves are smaller and thicker. Most of these biomass related responses (reductions in leaf DW and leaf number) were especially marked during the recovery period (Table 3), indicating that the observed effects were not reversible, even though leaching was efficient, as seen from Cl^- and Na^+ accumulation in the substrate during the recovery phase (Table 2).

At the end of the saline period root dry weight was the only biomass parameter to remain unaltered in treated plants, indicating that shoots and roots responded differently to salinity (Álvarez et al. 2012). In polygala, shoot growth was more sensitive to saline reused water than root growth (Bañon et al. 2011). This indicates that the changes that take place in the cell wall properties of roots differ from those in leaves, although the mechanism is unknown. With time, the initiation of new seminal or lateral roots is probably reduced, as seen in other ornamental species submitted different saline levels (Fornes et al. 2007). In our experiment, total root length was reduced at the end of the saline period, especially in thin roots which take up water and nutrients for the plant. This reduction can be considered a mechanism to avoid the entry of toxic ions and heavy metals into the plant, although in our experiment the amounts of heavy metals were small, with no significant differences between treatments, implying no risk to plants. Nonetheless, an increase in toxic ions, mainly sodium and chloride in irrigation water was evident. Ouzounidou et al. (1995) suggested that the inhibitory action of toxic ions and heavy metals on root length, shoot height and leaf area seems principally to be due to chromosomal aberrations and abnormal cell division. This could also be correlated with the metal-induced inhibition of photosynthesis and respiration in the shoot and protein synthesis in the root (Iannelli et al. 2002, Maria and Tadeusz 2005). However, after the two month recovery period, the effect of the salt continued, since these parameters (including root size) in WW and, especially, in NaCl treated plants were lower than in the control plants, meaning that the toxic effects of salts were not reversible during the time studied (Rodríguez et al. 2005).

Injury symptoms, like chlorosis and senescence, were a consequence of Na^+ and Cl^- accumulation in the leaves of NaCl and WW plants, causing the death of 20% by the end of experiment (Fig.1). Chloride has been described as being more toxic than Na^+ when it accumulates in excess in the leaves (Bennet, 1993). At cellular level, high amounts of Na^+ and Cl^- in leaves can be tolerated through anatomical adaptations, such as increased succulence due to increases in vacuole volume, which was observed in our experiment (Table 3), although it did not mitigate the damage caused by these ions.

Frequently, the presence of high concentrations of Na^+ and Cl^- in the medium leads to a decrease in the uptake of K^+ and Ca^{2+}, given the competition that exists with Na^+ for membrane transporters. It is not clear whether this occurred in our plants since Ca^{2+} was not displaced but accumulated in the leaves of NaCl plants, and K^+ concentrations were similar in the leaves of the plants of all treatments. The same results were obtained by Fornes et al. (2007) in Petunia, Calendula and Calceolaria plants submitted to saline irrigation.

The differences found in other elements in the water of the NaCl and WW treatments, such as boron, magnesium and sulphur (Table 1), did not differently affect the growth and quality of the plants of these treatments (Table 3 and Fig. 1). According to Wu and Dodge (2005), these elements and other heavy metals are rarely found in reclaimed water at levels that are damaging to landscape plants. In the specific case of boron, its high rate of absorption by roots (Fig. 2) and its accumulation in the leaves and roots of the plants irrigated with WW did not inhibit a leaf and root growth (Cervilla et al. 2009) compared with NaCl plants. Bañon et al. (in press) showed that plant tolerance to B^+ differs widely among species and cultivars. In species with no B^+ toxicity symptoms, the B^+ concentrations ranged from 100 to 400 mg kg^{-1}, which is similar to the values observed in our assay (Table 3).

Plants responded to salinity by decreasing stomatal aperture (Fig. 3C), and showing a lower Ψ_{stem}. However, in salt- treated plants the osmotic adjustment observed throughout the experiment was able to maintain leaf cell turgor. Salinity affects stomatal conductance immediately and transiently owing to perturbed water relations (Muns and Tester 2008). The reduction in P_n in NaCl and WW plants was related with a lower g_s, which could be related to the high concentration of Cl^- and Na^+ accumulated in the leaves. In extremely saline situations photosynthesis is depressed due to reductions in stomatal and mesophyll conductance to CO_2 (Flexas et al. 2004). The inhibition of photosynthesis observed in the saline treatments was more marked at the end of the experiment, as reflected in the inhibition of photo-assimilation and dry matter production (Table 3) even through leaf turgor was maintained.

In conclusion, our results indicate that, regardless of irrigation sources, no differences in the aesthetic and growth response were found between WW and NaCl plants due to the high salt content in the irrigation water of both treatments. Considering our results and the fact that after a two month recovery period 20% of the plants had died, *Euonymus* should not be irrigated with EC values exceeding to 4 dS m^{-1}, specially for long periods. The use of wastewater with a moderate EC (IW treatment) could be regarded as a safe water management strategy, since the problems that are associated with this water are of little importance in landscape plants.

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Legends of figures

Fig.1 (print in B/W). Percentage of plants according to the visual characteristics of *Euonymus japonica* plants irrigated with water from different sources and quality at the end of the saline (A) and recovery period (B). PAC, percentage of plants in acceptable conditions; PDB, percentage of plants with dry branches; PIC, percentage of plants in ideal conditions and DP, percentage of dry plants.

Fig.2 (print in B/W). Absorption rate (J) of Na^+(A), Cl^- (B) and B^+ (C) ions by roots in *Euonymus japonica* plants irrigated with water from different sources and of different quality at the end of the saline period. Values are mean of five plants. Different lower case letters indicate significant differences between treatments according to $Duncan_{0.05}$ test.

Fig.3 (print in B/W). Evolution of stem water potential (Ψ_{stem}) (A) osmotic potential at full turgor (Ψ_{100s}) (B), stomatal conductance (g_s)(C) and net photosynthetic rate (P_n) (D) at midday in *Euonymus japonica* plants irrigated with water from different sources and of different quality at the end of the saline period. Values are means of six plants per treatment.

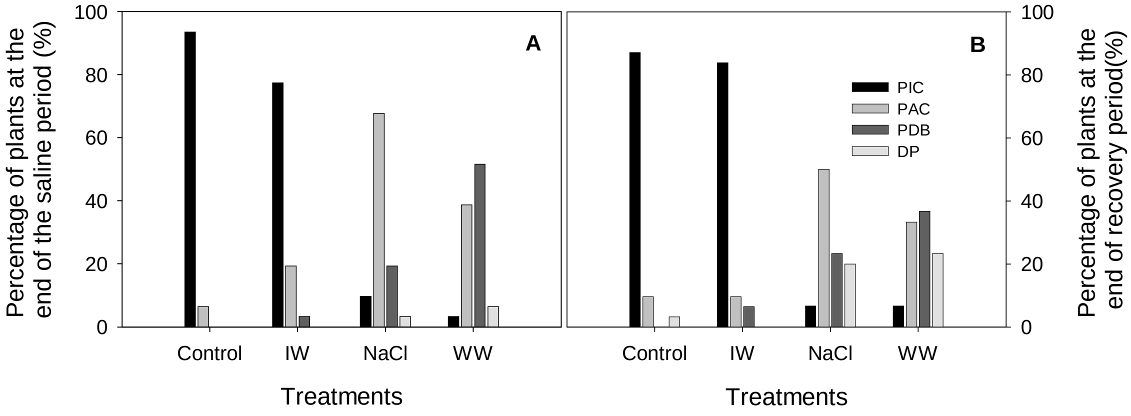
A
B
Percentage of plants at the end of the saline period (%)
Percentage of plants at the end of recovery period(%)
PIC
PAC
PDB
DP
0
20
40
60
80
100
Control
IW
NaCl
WW
Treatments

Fig.1

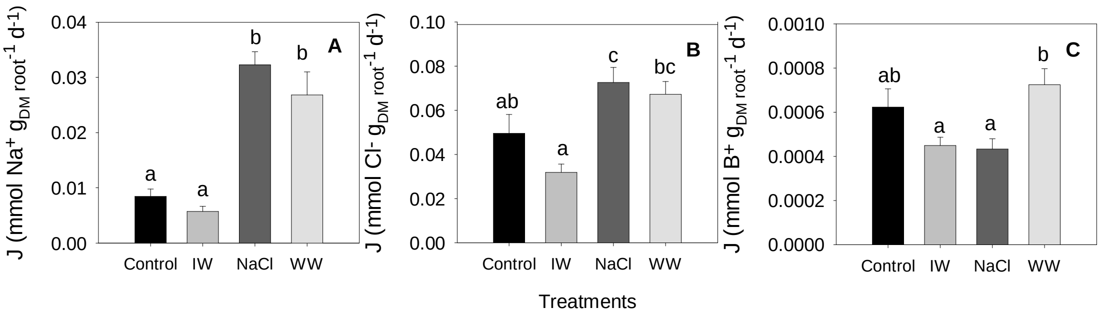
A
B
C
J (mmol Na+ gDM root-1 d-1)
J (mmol Cl- gDM root-1 d-1)
J (mmol B+ gDM root-1 d-1)
0.04
0.03
0.02
0.01
0.00
0.10
0.08
0.06
0.04
0.02
0.00
0.0010
0.0008
0.0006
0.0004
0.0002
0.0000
Control
IW
NaCl
WW
Treatments
a
b
ab
c
bc

Fig.2

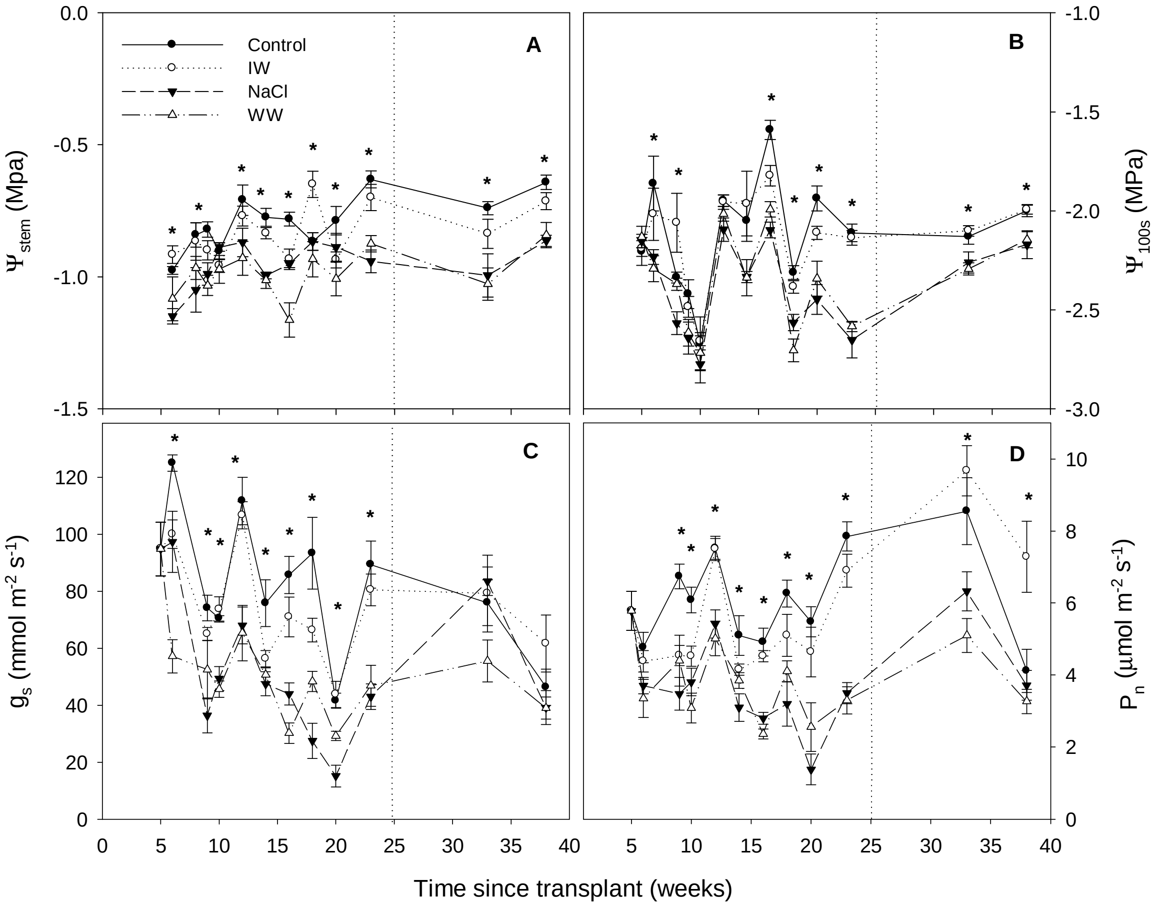

Fig.3

Asterisks indicate statistically significant differences by $Duncan_{0.05}$ test.

Tables

Table 1. Physicochemical analyses for the irrigation treatments. Data are values from samples collected at the beginning of saline period.

Physicochemical Analyses	Treatments			
	Control	**IW**	**NaCl**	**WW**
pH	7.97	8.17	7.78	7.67
EC (dS m^{-1})	0.87	1.71	4.24	4.19
TDS (mg L^{-1})	383	720	1626	1785
Turbidity (NTU)	2.10	2.88	8.16	3.2
B (mg L^{-1})	0.09	0.20	0.06	1.08
Ca (mg L^{-1})	94.21	92.45	82.54	186.35
K (mg L^{-1})	3.39	10.51	4.17	48.27
Mg (mg L^{-1})	41.87	54.14	37.79	148.80
Na (mg L^{-1})	52.07	187.90	801.3	662.30
P (mg L^{-1})	0.22	0.7	<0.1	1.62
S (mg L^{-1})	85.6	112.90	64.81	310.30
Chlorides (mg L^{-1})	69.50	600.70	1295.90	816.80
Sulphates (mg L^{-1})	220.12	750.20	157.87	1044,03

Table 2. Physicochemical analyses of substrate collected from plants irrigated with water from different sources and of different quality at the end of the saline (S) and recovery (R) periods. Values are means ± SEM (n=5 plants).

Measured parameters		**Treatments**				**P**
		Control	**IW**	**NaCl**	**WW**	
Na^{+} (mg kg^{-1} DW)	**S**	213.89 ± 37.62 a	375.70 ± 55.64 a	2073.06 ± 260.19 b	1523.56 ± 333.82 b	***
	R	330.66 ± 71.28	412.37 ± 113.14	254.68 ± 21.02	577.09 ± 103.74	ns
Cl^{-} (mg kg^{-1} DW)	**S**	334.82 ± 69.12 a	625.78 ± 73.85 a	3936.68 ± 558.18 c	2390.78 ± 495.44 b	***
	R	460.58 ± 99.50	563.62 ± 166.00	355.96 ± 26.93	807.80 ± 197.41	ns
Ca^{2+} (mg kg^{-1} DW)	**S**	306.31 ± 73.37	396.57 ± 36.73	327.05 ± 48.48	381.11 ± 43.26	ns
	R	450.68 ± 42.31 b	423.03 ± 80.00 b	92.05 ± 22.95 a	457.98 ± 38.78 b	***
Mg^{2+} (mg kg^{-1} DW)	**S**	153.27 ± 40.74 a	199.38 ± 19.88 a	181.10 ± 34.98 a	350.02 ± 73.30 b	*
	R	291.11 ± 29.05 b	221.04 ± 49.92 b	88.65 ± 58.94 a	253.32 ± 27.28 b	*
EC (ds m^{-1})	**S**	2.97 ± 0.58 a	4.35 ± 0.40 a	10.43 ± 2.05 b	10.25 ± 1.75 b	**
	R	2.64 ± 0.50 ab	4.61 ± 0.99 ab	2.44 ± 0.35 a	6.13 ± 0.83 b	*

Means within a row without a common letter are significantly different by $Duncan_{0.05}$ test.

P, probability level; ns, not significant; * P≤ 0.05; ** P≤ 0.01;***P≤ 0.001.

Table 3. Growth and development parameters in *Euonymus japonica* plants irrigated with water from different sources and of different quality at the end of saline (S) and recovery (R) periods. Values are means ± SEM (n=5 plants)..

Measured parameters		**Treatments**				**P**
		Control	**IW**	**NaCl**	**WW**	
Leaf DW (g plant^{-1})	**S**	24.38 ± 2.90 b	18.12 ± 3.34 ab	11.02 ± 0.69 aB	14.35 ± 2.03 aB	**
	R	25.21 ± 2.46 b	26.69 ± 4.39 b	5.37 ± 1.06 aA	8.80 ± 2.30 aA	***
Leaf number	**S**	344 ± 33 b	226 ± 23 aA	220 ± 12 aB	258 ± 19 aB	**
	R	407 ± 31 b	474 ± 69 bB	121 ± 15 aA	173 ± 38 aA	***
Total leaf area (dm^2)	**S**	17.82 ± 2.20 b	13.03 ± 1.59 ab	10.11 ± 1.24 aB	11.41 ± 1.82 aB	*
	R	17.28 ± 2.87 b	22.21 ± 3.22 b	4.29 ± 0.63 aA	7.60 ± 1.77 aA	***
Root DW (g plant^{-1})	**S**	9.07 ± 1.54 A	7.95 ± 1.31 A	5.80 ± 0.34	5.72 ± 0.71	ns
	R	17.18 ± 1.38 bB	15.60 ± 2.59 bB	5.36 ± 1.29 a	7.92 ± 1.77 a	***
Root/Shoot ratio	**S**	0.26 ± 0.04 aA	0.32 ± 0.02 ab	0.38 ± 0.04 b	0.27 ± 0.02 aA	*
	R	0.45 ± 0.03 B	0.22 ± 0.09	0.52 ± 0.05	0.48 ± 0.04 B	ns
Total root length (cm)	**S**	5008 ± 514 cA	4214 ± 603 bcA	3124 ± 291 ab	2161 ± 508 aA	**
	R	12611 ± 425 cB	7989 ± 661 bB	3117 ± 783 a	5544 ± 1305 abB	***
Suculence	**S**	2.40 ± 0.10 aA	2.39 ± 0.13 a	3.37 ± 0.23 b	2.92 ± 0.16 bA	**
	R	2.62 ± 0.04 aB	2.80 ± 0.12 a	3.67 ± 0.21 b	3.76 ± 0.18 bB	***

Means within a row without a common lowercase letter are significantly different by $Duncan_{0.05}$ test. Means within a column without a common capital letter are significantly different by $Duncan_{0.05}$ test.
P, probability level; ns, not significant; * P≤ 0.05; ** P≤ 0.01;***P≤ 0.001.

Table 4. Leaf and root Na^+, Cl^-, B^+, Ca^{2+},K^+ and Mg^{2+} concentration in *Euonymus japonica plants* irrigated with water from different sources and of different quality at the end of saline period. Values are means ± SEM (n=5 plants)...

Solutes (mg kg^{-1} DW)		Treatments: Control	IW	NaCl	WW	P
Na^+	**Leaf**	1943 ± 445 aA	1565 ± 320 aA	21385 ± 3098 cA	12538 ± 2403 b	***
	Root	4002 ± 204 aB	4163 ± 209 aB	10907 ± 1102 cB	8606 ± 948 b	***
Cl^-	**Leaf**	10560 ± 1434 a	8560 ± 627 a	27200 ± 1374 c	23040 ± 1366 bB	***
	Root	12960 ± 3113 a	10960 ± 3360 a	31680 ± 6641 b	15680 ± 1562 aA	*
B^+	**Leaf**	92.64 ± 4.37 a	87.87 ± 2.92 a	103.87 ± 8.57 a	127.69 ± 6.70 b	**
	Root	84.85 ± 1.52 a	82.78 ± 1.34 a	100.62 ± 7.54 a	158.5 ± 14.63 b	***
Ca^{2+}	**Leaf**	8671 ± 497 aB	8740 ± 613 aB	11539 ± 644 bB	9432 ± 681 a	*
	Root	5816 ± 402 aA	5714 ± 208 aA	7049 ± 314 aA	8969 ± 703 b	***
K^+	**Leaf**	10568 ± 236 B	10756 ± 486 B	11305 ± 772 B	11698 ± 597 B	ns
	Root	5046 ± 346 aA	6485 ± 456 bA	4195 ± 372 aA	5280 ± 351 aA	**
Mg^{2+}	**Leaf**	1296 ± 105 aA	1296 ± 235 aA	1695 ± 80 abA	1867 ± 124 bA	*
	Root	5470 ± 365 aB	4964 ± 265 aB	4578 ± 207 aB	6495 ± 305 bB	**

Means within a row without a common lowercase letter are significantly different by $Duncan_{0.05}$ test. Means within a column without a common capital letter are significantly different by $Duncan_{0.05}$ test.

P, probability level; ns, not significant;* P≤ 0.05; ** P≤ 0.01; ***P≤ 0.001.