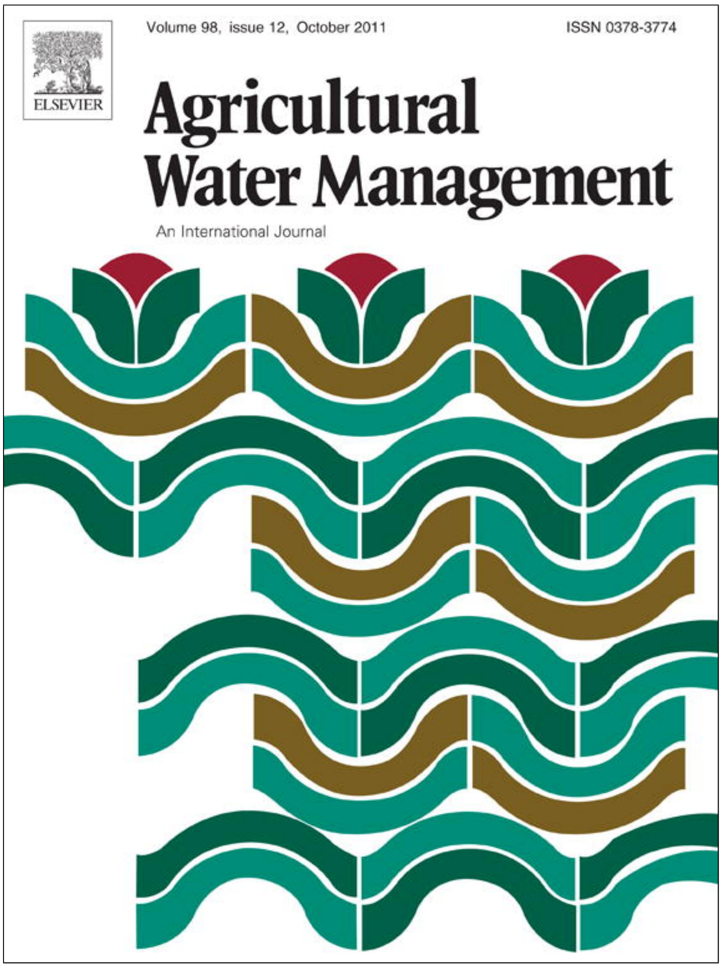
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# Reclaimed wastewater: Effects on citrus nutrition

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ABSTRACT

The effects of irrigation with reclaimed wastewater (RWW) were compared with well water (WW) on citrus (*Citrus paradisi* Macfad. X *Citrus aurantium* L.) nutrition. The deviation from the optimum percentage (DOP) index of macro- and micro-nutrients were used to evaluate the nutritional status: optimal (DOP = 0), deficiency (DOP < 0) or excess (DOP > 0). After 11 years of RWW irrigation the influence on nutrient concentration in plants decreased in the order: B > Zn > Mn = Ca > Cu > Mg > P > K. Reclaimed wastewater irritation positively affected citrus nutrition as it rendered the concentration of macro-nutrients, i.e. P, Ca, and K. closer to their optimum levels ($\Sigma\text{DOP}_{\text{macro}} = 7$). However micro-nutrients tended to be excessive in plants ($\Sigma\text{DOP}_{\text{micro}} = 753$) due to imbalanced supply of these elements in the RWW, particularly, for B and Cu. Citrus groves with long-term RWW irrigation may exercised caution in monitoring concentrations of B and Cu to avoid plant toxicity and soil quality degradation.

## 1. Introduction

Global warming and water scarcity are among the main concerns of the modern world. High temperature causes high vapor pressure deficit and evaporation reduces water availability for vegetation growth (Zhao and Running, 2010). Severe competition for water resources is predicted in the future as the population continues to grow, particularly in southern USA (O'Connor et al., 2008; Sun et al., 2008). In 2010, the fertilizers price index in USA was five folds higher than in 1960 (USDA, 2010). In this scenario, the use of reclaimed wastewater (RWW) could contemplate the water shortages as well as partial source of nutrients.

Compared with well water (WW) the RWW possess higher concentrations of macro-(N, P, K, S, Ca, Mg) (Barton et al., 2005; Fonseca et al., 2007; Jaiswal and Elliott, 2011; Sophocleous et al., 2009) and micro-nutrient (B, Cl, Cu, Co, Zn, Fe, Mn, Mo, and Ni) (Pedrero and Alarcón, 2009; Xu et al., 2010). Nutrients in RWW may not be in a balanced supply for plant nutrition, leading to a nutritional deficiency or excess in plants. Studies reported antagonistic and synergistic interactions of nutrients in plants irrigated with RWW (Kalavrouziotis et al., 2009). Morgan et al. (2008) reported higher concentrations of Mg and B in citrus after RWW irrigation corroborating with Pedrero and Alarcón (2009). Relationships were reported of RWW with excess or toxicity of some micro-nutrients (B, Cu, Ni and Zn) in citrus and *Brassica* (Aucejo et al., 1997; Kalavrouziotis et al., 2008; Khan et al., 2008; Maurer and Davies, 1993; Pedrero et al., 2010). Omran et al. (1988) previously found the following descending order of micro-nutrient concentration in citrus leaves after 60 years of irrigation with RWW: Fe > Zn > Mn > Cu.

Most of the studies have focused on the quality, management and methods of application, human health risk assessment, and environmental protection strategies. However, few studies have focused on the problem of nutrient disorder in plants as a result of continuous application of RWW (Fatta-Kassinos et al., 2010).

To avoid plant nutrient disorders, it is essential to know the tendency of excess or deficiency in nutrients and nonessential elements for the plants irrigated with RWW. The deviation from optimum percentage (DOP), is an alternative method to the traditional diagnosis and recommendation integrated system (DRIS), which is capable of accurately defining the quantity and quality of each nutrient in plants (Montañés et al., 1993). Besides, it provides the general nutritional status of all macro- and micro-nutrients through the sum of DOP indexes ($\Sigma\text{DOP}_{\text{macro}}$ and $\Sigma\text{DOP}_{\text{micro}}$).

The positive benefits and agronomical advantages of RWW in citrus crop as an alternative source of water for irrigation were demonstrated in previous studies located in Egypt, Greece, Israel, Italy, Jordan, Spain and USA (Ammary, 2007; Aucejo et al., 1997; Graber et al., 2006; Kalavrouziotis et al., 2009; Meli et al., 2002;

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Morgan et al., 2008; Omran et al., 1988; Pedrero and Alarcón, 2009, Pedrero et al., 2010). In Florida, USA, there are 440 RWW reuse systems irrigating more than 90,000 ha with about 6144 ha producing citrus (*Citrus* spp. L.) (Morgan et al., 2008). In Okeechobee county, FL, there is one of the few commercial growers of grapefruit (*Citrus paradisi* Macfad.) having been irrigating with RWW since 1997, while the others apply WW irrigation. Questions persist regarding the influences of long-term RWW irrigation on citrus nutrition. Therefore, the effects of RWW irrigation in comparison with WW on citrus nutritional status after 11 years of irrigation were investigated.

## 2. Materials and methods

### 2.1. Area description and irrigation system

This study was conducted in a commercial citrus grove in South Florida, USA. The soil is classified as loamy, siliceous, hyperthermic, Arenic Glossaqualfs (USDA, 1980) with physical and chemical characteristics (USDA, 1951) prior to the study presented in Table 1.

Two citrus blocks, 8.1 ha each, were selected because of similar combination of soil, rate of irrigation, citrus variety, and tree density. Both blocks were planted with grapefruit (*Citrus paradisi* Macfad.) grafted on sour orange (*Citrus aurantium* L.), 8 m × 5 m spaced. One block was irrigated with reclaimed wastewater (RWW) and the other with well water (WW) (control). The nursery trees of each block were transplanted in 1982 and 1974. In their respective blocks, RWW and WW have been applied since 1997 until 2008 with a micro-irrigation system. According to daily crop reference evapotranspiration (ETo) the irrigation rates were estimated by the Penman-Monteith equation (Allen et al., 1998) using a 360° micro-sprinklers (60.5 $\mathrm{L\,h^{-1}}$) at annual rate of 518 mm for RWW and 485 mm for WW. Granular fertilizer was applied at the rates of 450 $\mathrm{kg\,ha^{-1}}$ (14–0–18 + 3 Mg) in spring, 450 $\mathrm{kg\,ha^{-1}}$ (12–0–16 + 3 Mg) in early summer, 225 $\mathrm{kg\,ha^{-1}}$ (14–0–16 + 3 Mg) in autumn, and 2.2 $\mathrm{kg\,ha^{-1}}$ of spraying solution (0–29–26) twice a year.

### 2.2. Field sampling and laboratory analysis

Four grids (replications) were randomly distributed on each block (RWW or WW). Each grid was a 0.14 ha square with five points marked to collect 60 leaf sub-samples to produced one composite sample. The sampling points were located in the Google Earth$^{\mathrm{TM}}$ and their geographic coordinates were transferred to a Global Positioning System (GPS) instrument with an accuracy of ±3 m. Leaf samples were taken from three trees in each one of the five grid points in July 2008. For each tree four leaves of 4–6 month-old spring flush of non-fruiting twigs were collected (240 leaves/block) (4 grids × 15 trees × 4 leaves/tree). Leaves were rinsed in tap water to remove solid particles, then washed in a low concentration solution of detergent (phosphorus free), rinsed in deionized water, soaked in HCl solution (1%) for 1 min and, rinsed four times in deionized water. Leaves were then dried in a forced-air oven at 70 °C for 3 days and ground in a ball mill (4 canister, model 4200, Kleco-Garcia Machine, Visalia, CA, USA). A sample of 0.4 g oven dried leaf was accurately weighed and digested using 5 mL of concentrated nitric acid on a block digester (AIM 500-c, AI Scientific, Brisbane, Australia). The concentrations of P, K, Ca, Mg, B, Cu, Fe, Mn, Zn and Na in the digested solution were determined using inductively coupled plasma optical emission spectrometry (ICP-OES, Ultima II, JY Horiba Group, Edison, NJ, USA). Total S was determined by precipitation with $BaCl_2$ in 1 mL of acid solution (HCl 6N with 20 $\mathrm{mg\,L^{-1}}$ of S) and determined by turbidimetric method ($\lambda = 420$ nm) using a spectrophotometer (Hitachi U-3010, Tokyo, Japan). Total N and C contents of plant samples were determined by a CN analyzer (vario Max CN, Elemental Analysensystem GmbH, Hanau, Germany).

### 2.3. Yield data

Yield data were obtained from the harvest of 1997/1998 to 2007/2008 seasons. Fruit yield was recorded by harvesting all the fruit from the trees in each plot into a standard 10-box (1 box = 38.6 kg) bin. The total fruit weight in each 10-box bin was measured by using a scaled stick that was previously calibrated. The yield per plot was calculated by multiplying the number of bin by the fruit weight per bin.

### 2.4. Reclaimed wastewater and well water analysis

The domestic RWW was provided by a municipal Wastewater Treatment Plant (WTP) in south Florida. The primary treatment of wastewater removes heavy solids and floatable materials while the secondary treatment consists of extended aeration and chlorine disinfection. The WW used for irrigation was the water pumped from groundwater and stored in reservoir which also collected the rainwater. Reclaimed wastewater samples (90) were collected at the WTP from 2001 to 2008, whereas five WW samples were collected from the micro-sprinklers in the WW-irrigated block in 2008. The RWW and WW samples were analyzed according to the following references: electrical conductivity (EC) (APHA, 1992), pH, biochemical oxygen demand (BOD, 5-day at 20 °C), chemical oxygen demand (COD), total Kjeldahl N, total P, total suspended solids, $NO_3^-$–N, $NO_2^-$–N, $PO_4^{3-}$–P, $SO_4^{2-}$–S, B, Ca, Cu, Fe, K, Mn, Na, Zn, Cd, Cr, Mg, Ni, Pb and Se (USEPA, 1983) and Li (USEPA, 1986).

### 2.5. Data analyses

The nutritional status of citrus was evaluated for each plant nutrient using the DOP index which was defined as: $\mathrm{DOP} = [(C \times 100)/C_{\mathrm{ref}}] - 100$ where "$C$" is the measured concentration of a nutrient in the plant sample and "$C_{\mathrm{ref}}$" is the optimal nutrient concentration used as reference value (Montañés et al., 1993). The $C_{\mathrm{ref}}$ values of each nutrient were estimated according to the guidelines of interpretation for citrus nutrition (Obreza and Morgan, 2008; Reuter and Robinson, 1997). The DOP is an index and indicates the relative tendency of nutrient deficiency (DOP < 0), optimization (DOP = 0), or excess (DOP > 0) in plants. The sum of absolute DOP index value for macro ($\Sigma\mathrm{DOP_{macro}} = \mathrm{DOP_N} + \mathrm{DOP_P} + \mathrm{DOP_{Ca}} + \mathrm{DOP_{Mg}} + \mathrm{DOP_S}$) and micronutrients ($\Sigma\mathrm{DOP_{micro}} = \mathrm{DOP_B} + \mathrm{DOP_{Cu}} + \mathrm{DOP_{Fe}} + \mathrm{DOP_{Mn}} + \mathrm{DOP_{Zn}}$) was also calculated to evaluate the nutritional status of macro- and micro-nutrients. For the ΣDOP calculations only the nutrients with significant difference between RWW and WW irrigation were considered.

Means (Table 2) and standard errors (±SE) were used to compare statistical differences between RWW and WW. The Shapiro–Wilk test ($P > 0.05$) was used to evaluate the normality of the data of yield and DOP. Then, the *t*-test at $P < 0.05$ was used to compare the effect of RWW and WW on these two dependent variables (Table 3).

## 3. Results

### 3.1. Reclaimed wastewater

Generally, the RWW contained larger amounts of macro- ($NO_3^-$, $NH_4^+$, $PO_3^-$, $SO_4^{2-}$, K, Mg), micro-nutrients (B, Cl, Cu, Fe, Mn, Zn) and non-essential elements (Cd, Co, Cr, Li, Se) (Table 2) than WW. In the RWW, the macro-and micro-nutrients concentrations decreased in the following order: S > Ca > Mg > N > K > P > B > Zn = Fe > Mn = Cu (Table 2).

### 3.2. Effects of reclaimed wastewater on soil

In the same studied area after 11 years of RWW irrigation Pereira et al. (2011) reported increased concentration of KCl-extractable $NH_4^+$ (21%) and $NO_3^-$ (76%); Mehlich-3-extractable P (500%), Mg (56%), Fe (251%), Cu (66%), Mn (90%), Zn (109%), Ni (147%) and Co (345%); water-extractable $SO_4^{2-}$ (260%), hot-water-extractable B (21%) in soil when compared with soil irrigated with WW. Moreover, RWW irrigation acidified soil by 0.4–0.7 pH units and increased soil sodicity (SAR) and salinity (EC) about 2–3 times.

### 3.3. Deviation from optimum percentage range of citrus nutrients

#### 3.3.1. Macro-nutrients

The data of all studied nutrients in plant tissue was considered parametric according to the Shapiro–Wilk test ($P > 0.05$). The $DOP_{Ca}$ was negative and $DOP_P$ was positive regardless of water source. For these two macro-nutrients, plants irrigated with RWW tended to have a DOP value close to the optimum level (DOP = 0) and significantly different ($P < 0.05$) from that irrigated with WW (Table 3). $DOP_K$ was negative for plants irrigated with RWW and positive for plants with WW. Plants irrigated with RWW also displayed $DOP_K$ closer to the optimum status (Table 3). No effect of RWW was observed on $DOP_N$, $DOP_{Mg}$, and $DOP_S$ in citrus leaves (Table 3).

#### 3.3.2. Micro-nutrients

In general, the RWW increased leaf concentration of most studied micro-nutrients in citrus: Mn with 13%; Zn with 30%; Cu with 11%; and B with 87% (Table 3). The average $DOP_{Mn}$, $DOP_{Zn}$ and $DOP_{Cu}$ for plants irrigated with RWW and WW were positive, and higher values were observed in plants irrigated with RWW (Table 3). The $DOP_B$ was respectively positive and negative for plants irrigated with RWW and WW. Among the studied nutrients, the increase in $DOP_{Cu}$, $DOP_B$ and $DOP_{Zn}$ by RWW irrigation was the most pronounced. $DOP_{Fe}$ was similar for both RWW and WW irrigation.

#### 3.3.3. Macro- versus micro-nutrients

Compared with WW, the irrigation with RWW raised the concentrations of nutrients in the citrus plant in the following order: B > Zn > Mn = Ca > Cu > Mg > P > K (Table 3). Plants irrigated with RWW had a $\Sigma DOP_{macro}$ ($\Sigma DOP_{macro} = DOP_P + DOP_K + DOP_{Ca}$) of 7, as compared to 43 with WW. However, the $\Sigma DOP_{micro}$ ($\Sigma DOP_{micro} = DOP_B + DOP_{Cu} + DOP_{Mn} + DOP_{Zn}$) of plants irrigated with RWW was 753, as compared to 555 with WW.

### 3.4. Influence of RWW and WW on citrus yield

The normality of citrus yield data obtained from 1997/1998 to 2007/2008 was verified by the Shapiro–Wilk test for RWW ($P = 0.539$) and WW ($P = 0.510$). The average fruit yield was similar ($P = 0.704$) for RWW (228 ± 57 kg $tree^{-1}$) and WW irrigation (243 ± 115 kg $tree^{-1}$).

## 4. Discussion

### 4.1. Reclaimed wastewater

The RWW contained larger amounts of nutrients, and non-essential elements except for Ca and Al than the WW (Table 2). In general, RWW used in this study was adequate for irrigation in agriculture according to the observed properties. None of the measured properties including pH, SAR, alkalinity, biological oxygen demand (BOD), total soluble solids (TSS), N, P, K, Ca, Mg, S, B, Cl, Cu, Fe, Zn, non-essential elements (Cd, Co, Cr, Pb, Se, and Al), counts of pathogens (*Escherichia coli* and fecal coliforms) exceeded the reference limits established by USEPA (2004) or suggested by previous studies (Fatta-Kassinos et al., 2004; FAO, 1992; Feigin et al., 1991; James et al., 1982; Reboll et al., 2000) (Table 2).

The higher concentration of macro- than micro-nutrients (S > Ca > Mg > N > K > P > B > Zn = Fe > Mn = Cu) in RWW is compatible with the general nutritional requirements of plants, but did not follow the order of macro- and micro-nutrient concentrations in the Hoagland nutrition solution (Epstein and Bloom, 2005): N > K > Ca > P > S > Mg > Fe > B > Mn > Zn > Cu, implying that imbalanced supply of nutrients may occur in RWW.

### 4.2. Macro-nutrients and sodium

The DOP index is the relative concentration of assessed nutrients and used to indicate which element tend to be deficient (DOP < 0), optimal (DOP = 0), or in excess (DOP > 0) (Montañés et al., 1993). The most beneficial influence of RWW on the macro-nutrients was observed with $\Sigma DOP_{macro}$ closer to zero which is explained by the $DOP_P$, $DOP_K$ and $DOP_{Ca}$ being near to the optimum nutritional status ($DOP \approx 0$) (Table 3) as compared to WW.

The RWW irrigation decreased *P* concentration in plant tissue which is beneficial for the positive $DOP_P$ (Table 3) indicating the tendency of P excess in plants irrigated with WW. The results are consistent with those of Morgan et al. (2008) and Herpin et al. (2007) who reported reduced P concentration in citrus and coffee (*Coffea arabica* L.) with RWW irrigation. The P reduction in citrus leaves may be explained by the antagonistic interaction of Cu with P. Phosphorus concentration decreased 71% with increasing concentration of Cu in *Brassica oleracea* L. irrigated with RWW (Kalavrouziotis and Koukoulakis, 2010). We

**Table 1**
Basic physical and chemical properties of the studied soil (loamy, siliceous, hyperthermic, Arenic Glossaqualfs).

| Depth (cm) | Sand (%) | Silt (%) | Clay (%) | Bulk density ($g\,cm^{-3}$) | Extractable bases Ca ($mg\,kg^{-1}$) | Mg ($mg\,kg^{-1}$) | Na ($mg\,kg^{-1}$) | K ($mg\,kg^{-1}$) | Extractable acidity ($cmol_c\,kg^{-1}$) | CEC[a] (%) | BS[b] (%) | OC[c] (%) | EC[d] ($\mu S\,cm^{-1}$) | pH $CaCl_2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0–7 | 90.1 | 8.6 | 1.3 | 1.1 | 1894 | 86.3 | 69.0 | 27.4 | 4.0 | 14.5 | 73 | 3.4 | 260 | 6.3 |
| 7–15 | 96.8 | 2.5 | 0.7 | 1.4 | 482 | 19.4 | 25.3 | 3.9 | 0.5 | 3.2 | 84 | 0.5 | 180 | 7.3 |
| 15–30 | 97.5 | 1.3 | 1.2 | 1.5 | 476 | 14.6 | 25.3 | 3.9 | 0.4 | 3.0 | 86 | 0.4 | 150 | 7.5 |

[a] Cation exchange capacity.
[b] Base saturation.
[c] Organic carbon.
[d] Electrical conductivity.

also found 30% increased Zn concentration in plants irrigated with RWW.

The $DOP_{Ca}$ of plants irrigated with RWW and WW was −2 and −14, respectively (Table 3). Therefore, plants irrigated with RWW tended to be closer to the optimum Ca concentration and plants receiving WW slightly tended to deficiency. This beneficial influence of RWW irrigation on Ca concentration in citrus leaves was unexpected because RWW had less Ca ($28\,mg\,L^{-1}$) than WW (Table 3). Moreover, higher Ca concentration in soil irrigated with RWW was found only in the deepest layer of 90 cm (Pereira et al., 2011). Previous studies reported no influence of RWW on Ca concentration in citrus (Morgan et al., 2008; Pedrero and Alarcón, 2009) or lower Ca concentration (Maurer et al., 1995). The higher concentration of Ca in plants irrigated with RWW may be explained by the synergistic interaction observed between Ca and Mn (Kalavrouziotis et al., 2009), once we also observed higher Mn in plants receiving RWW (Table 3).

The negative $DOP_K$ indicated the tendency of K deficiency in plants irrigated with RWW (Table 3) although it was near the optimal level (DOP = 0), as compared with WW. Potassium reduction in plants irrigated with RWW was previously described in citrus and cabbage (*Brassica oleracea* L.) by Kalavrouziotis et al. (2008) and Reboll et al. (2000). This may be explained by: (i) decreased $K^+$ availability in the soil due to its leaching caused by higher $Na^+$ concentration in soil. Jalali et al. (2008) reported losses of $K^+$ in soil irrigated with RWW were due to leaching with rain water; (ii) high $Na^+$ concentration in soil solution inhibits the passive absorption of $K^+$ through the channel protein (Epstein and Bloom, 2005); (iii) the antagonistic effect of Ca, Mn, Ni, and Cd may also play an important role in K reduction in plants treated with RWW irrigation (Kalavrouziotis et al., 2009; Kalavrouziotis and Koukoulakis, 2010). Despite decreased K concentration in leaf tissue after long-term irrigation with RWW, the replacement of $K^+$ by $Na^+$ in a low proportion has no harmful effects on plant nutrition as $Na^+$ can partially substitute for $K^+$ in the non-specific functions of osmotic homeostasis (Epstein and Bloom, 2005). Moreover, K concentration in the plants was still considered to be within the optimal range ($12–17\,g\,kg^{-1}$) (Obreza and Morgan, 2008). Therefore, the negative effects of RWW irrigation on plant nutrition of K are minimal.

Plants irrigated with RWW had $0.4 \pm 0.08\,g\,Na\,kg^{-1}$, as compared with $0.1 \pm 0.01\,g\,Na\,kg^{-1}$ in plants with WW irrigation. The increase in plant Na concentration by RWW irrigation was previously discussed on different crops such as red cabbage (Kiziloglu et al., 2008), sugarcane (Leal et al., 2009), maize (Fonseca et al., 2005), coffee (Herpin et al., 2007) and citrus (Maurer et al., 1995; Maurer and Davies, 1993). The increased Na concentration in citrus plant merits attention since grapefruit trees are susceptible to salinity (Al-Yassin, 2004). Marschner (1995) stated that species with low salt tolerance (natrophobic) cannot prevent massive transport of $Na^+$ to the shoots due to the fine structure of the chloroplasts. However, even after long term RWW irrigation, the concentration of Na in citrus leaves ($0.4 \pm 0.1\,g\,Na\,kg^{-1}$) is still lower than the excessive range of beyond $2.5\,g\,Na\,kg^{-1}$ (Obreza and Morgan, 2008) and within the adequate range of $1.6\,g\,Na\,kg^{-1}$ (Reuter and Robinson, 1997).

### 4.3. Micro-nutrients

Despite $\Sigma DOP_{macro}$ was close to zero, the higher value of $\Sigma DOP_{micro}$ of RWW irrigated plants highlights the negative effect of RWW on citrus nutritional status due to the excess tendency of some micro-nutrients (DOP > 0). The $DOP_{Mn}$, $DOP_{Zn}$, $DOP_{Cu}$ and $DOP_B$ indexes of plants irrigated with RWW ranged from 41 to 608 (Table 3).

**Table 2**
Mean (±standard error) values of soil and elemental variables in the well water (WW) and reclaimed wastewater (RWW).

| Variables | Unit | WW | RWW |
|---|---|---|---|
| | | Mean ± SE | Mean ± SE |
| pH | – | 7.3 ± 0.16 | 8.1 ± 0.1 |
| SAR[a] | $mmol^{1/2}\,L^{-1/2}$ | 0.88 | 3.7 ± 0.1 |
| Na:Ca[b] | – | 0.26 | 2.0 |
| Alkalinity ($HCO_3^-$) | $mg\,L^{-1}$ | – | 150.6 ± 3.0 |
| BOD[c] | $mg\,L^{-1}$ | – | 13.9 ± 3.4 |
| COD[d] | $mg\,O_2/L$ | – | 64.4 ± 2.1 |
| EC[e] | $\mu S\,cm^{-1}$ | 522.0 ± 26.12 | 995.0 ± 15.2 |
| Turbidity | NTU | 1.1 ± 0.92 | 12.75 ± 0.92 |
| TSS[f] | $mg\,L^{-1}$ | – | 17.5 ± 1.1 |
| $NO_3^-$–N | $mg\,L^{-1}$ | 0.3 ± 0.20 | 5.7 ±± 0.3 |
| $NO_2^-$–N | $mg\,L^{-1}$ | <0.01 | 0.5 ± 0.2 |
| $NH_4^+$–N | $mg\,L^{-1}$ | 0.14 ± 0.04 | 1.47 ± 0.2 |
| $PO_4^{3-}$–P | $mg\,L^{-1}$ | <0.01 | 2.2 ± 0.2 |
| $SO_4^{2-}$–S | $mg\,L^{-1}$ | 2.7 ± 0.03 | 24.47 ± 5.7 |
| TKN[g] | $mg\,L^{-1}$ | – | 4.1 ± 0.4 |
| TP[h] | $mg\,L^{-1}$ | – | 2.9 ± 0.2 |
| Ba | $mg\,L^{-1}$ | – | 0.01 ± 0.0003 |
| B | $mg\,L^{-1}$ | 0.1 ± <0.01 | 0.31 ± 0.007 |
| Ca | $mg\,L^{-1}$ | 88.3 ± 2.8 | 60.6 ± 1.0 |
| Cl | $mg\,L^{-1}$ | 28.0 ± 8.08 | 232.56 ± 14.9 |
| Cu | $mg\,L^{-1}$ | <0.01 | 0.01 ± 0.001 |
| Fe | $mg\,L^{-1}$ | <0.01 | 0.05 ± 0.004 |
| K | $mg\,L^{-1}$ | 3.852 ± 0.085 | 17.1 ± 0.3 |
| Mn | $mg\,L^{-1}$ | 0.005 ± 0.002 | 0.01 ± 0.0004 |
| Na | $mg\,L^{-1}$ | 23.6 ± 0.1 | 122.1 ± 2.7 |
| Zn | $mg\,L^{-1}$ | 0.013 ± <0.01 | 0.06 ± 0.003 |
| Cd | $mg\,L^{-1}$ | <DL | 0.001 ± 0.0002 |
| Co | $mg\,L^{-1}$ | <0.01 | 0.01 ± 0.0005 |
| Cr | $mg\,L^{-1}$ | <DL | 0.016 ± 0.001 |
| Li | $mg\,L^{-1}$ | – | 0.007 ± 0.001 |
| Mg | $mg\,L^{-1}$ | 8.950 ± 0.242 | 11.1 ± 0.4 |
| Ni | $mg\,L^{-1}$ | <0.02 | 0.008 ± 0.001 |
| Pb | $mg\,L^{-1}$ | 0.018 ± 0.009 | 0.043 ± 0.003 |
| Se | $mg\,L^{-1}$ | – | 0.002 ± 0.0005 |
| Al | $mg\,L^{-1}$ | 1.191 ± 0.364 | 0.73 ± 0.1 |
| *E. coli* | $100\,mL^{-1}$ | <DL | <DL |
| Total coliforms | $100\,mL^{-1}$ | <DL | <DL |

<DL = below detection limit.

[a] Sodium adsorption ratio: $SAR = [Na^+]/([Ca^{2+}] + [Mg^{2+}])^{1/2}$.

[b] Ratio of sodium and calcium.

[c] Biochemical oxygen demand.

[d] Chemical oxygen demand.

[e] Electrical conductivity.

[f] Total soluble solids.

[g] Total Kjeldahl nitrogen.

[h] Total phosphorus.

**Table 3**
Deviation from the optimum level in percentage (DOP) of macro- and micro-nutrients in the leaf of citrus trees irrigated with reclaimed wastewater (RWW) or well water (WW) for 11 years. Average concentration and standard error of macro-nutrients ($g\,kg^{-1}$) and micro-nutrients ($mg\,kg^{-1}$) irrigated with RWW and WW.

| Variabes | DOP[*] | | Nutrients concentration in leaves | |
|---|---|---|---|---|
| | RWW | WW | RWW | WW |
| N | −7 A | −10 A | 24.2 ± 0.1 | 23.4 ± 0.4 |
| P | 15 A | 38 B | 1.6 ± 0.0 | 1.7 ± 0.0 |
| K | −5 A | 19 B | 13.7 ± 0.3 | 17.3 ± 0.6 |
| Ca | −2 A | −14 B | 38.6 ± 1.3 | 34.1 ± 0.9 |
| Mg | −12 A | −20 A | 3.4 ± 0.3 | 3.1 ± 0.1 |
| S | 63 A | 76 A | 5.0 ± 0.5 | 5.4 ± 0.1 |
| Na | – | – | 0.4 ± 0.1 | 0.1 ± 0.0 |
| Mn | 42 A | 25 B | 88.7 ± 3.0 | 78.4 ± 4.0 |
| Zn | 41 A | 9 B | 88.4 ± 2.8 | 68.0 ± 7.3 |
| Cu | 608 A | 535 B | 74.4 ± 4.0 | 66.7 ± 1.5 |
| Fe | −61 A | −68 A | 35.0 ± 6.5 | 28.8 ± 2.4 |
| B | 62 A | −14 B | 110.0 ± 6.2 | 58.7 ± 1.8 |

[*] Different letters mean significant difference at $P < 0.05$ according to the *t*-test.

Despite higher $DOP_{Mn}$ and $DOP_{Zn}$ in citrus leaf with RWW irrigation, their concentrations (Table 3) were far from excessive (>300 mg kg$^{-1}$) (Obreza and Morgan, 2008). The higher concentrations of Mn and Zn (Table 2) in the RWW, a slightly decreased soil pH and the higher concentration of extractable Mn and Zn in the soil, may have contributed to their increased availability to the plants. In addition, soil under RWW had respectively 90 and 109% higher concentration of extractable Mn and Zn than WW irrigated soil (Pereira et al., 2011). These authors also observed soil acidification under RWW irrigation. Higher concentration of metals (Cu, Zn, Mn, Ni, Cd, Pb, and Cr) was reported in plants irrigated with RWW than WW (Kiziloglu et al., 2008; Singh et al., 2009).

A concern remains regarding the high $DOP_{Cu}$ and $DOP_B$ of plants irrigated with RWW. Regardless of water source, excessive concentration of Cu (>20 mg kg$^{-1}$) was measured in the plant tissue, likely due to spray of Cu-containing chemicals which added 1–2 kg of Cu ha$^{-1}$ yr$^{-1}$ to the citrus grove, in Florida (He et al., 2005). However, Cu concentration in soil irrigated with RWW was 66% higher than in WW soil (Pereira et al., 2011). RWW irrigation increased Cu concentration in the citrus leaf with 11% as compared with WW irrigation. Therefore, a long-term RWW irrigation may potentiate Cu toxicity in citrus crop.

Of the micro-nutrients, B is frequently cited among the main problems of RWW irrigation (Aucejo et al., 1997; Pedrero and Alarcón, 2009). The RWW contained a concentration of B three folds higher than that in WW (Table 2), and consequently available B in soil was raised by 21% with RWW irrigated (Pereira et al., 2011). Besides, the synergistic interactions of B with Ca, Mn, Zn, and Ni (Kalavrouziotis et al., 2009) may have rendered B the most affected nutrient in plants with RWW irrigation. Boron concentration in the leaf tissue increased at a rate of 4.6 mg B kg$^{-1}$ yr$^{-1}$ ($110–58.7/11 = 4.6$), reaching the high range (101–200 mg kg$^{-1}$) in 11 years of irrigation with RWW. However, B toxicity symptoms were not observed in the fields. With the same scenario, using the estimated increasing rate, it would require about 30 years of RWW irrigation [$(200 - 110/4.6) + 11 = 30.5$] for plant B concentration to reach the excessive level of >200 mg of B kg$^{-1}$ yr$^{-1}$. Qian and Mecham (2005) confirmed the potential problems of B accumulation in the soil after 33 years of irrigation with RWW in golf course. Therefore, special attention needs to be paid to B toxicity if the RWW irrigation is extended beyond 30 years on citrus crops.

## 5. Conclusions

Although the concentration of macro-nutrients in the RWW was higher than micro-nutrients, the irrigation with RWW has a greater influence on the micro-nutrient than macro-nutrient concentration in plant leaves, following the order: $B > Zn > Mn = Ca > Cu > Mg > P > K$.

RWW positively influences citrus nutrition by rendering the concentration of macro-nutrients, i.e. P, Ca, and K closer to their optimum levels. Nevertheless imbalanced supply of micro-nutrient in RWW may cause nutritional excess of some micro-nutrients, including Mn, Zn, Cu and B in citrus plants.

The results from this study suggest that the irrigation with RWW can improve overall nutrition of citrus plants, particularly for macro-nutrients though no significant difference in fruit yield is observed. However, it is necessary to carefully monitor the concentrations of Cu and B in the plant if RWW irrigation is extended beyond 30 years to avoid toxicity to citrus plant.

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