Effects of irrigation with reclaimed water on leaf content and physiological aspects of carnation

Petousı I.[1*], Stavroulaki N.[1], Mpouki C.[1], Papadimitriou M.[1] And Manios T.[1]

[1]Department of Agriculture, Technological Educational Institute of Crete, Estavromenos, 71410 Heraklion, Greece

*corresponding author: Dr. Ioanna Petousi, Agronomist

e-mail: ioannapetousi@staff.teicrete.gr

Abstract

This study examined the feasibility of using reclaimed wastewater as irrigation water for the cultivation of carnation plants. The effect of three different qualities of treated wastewater on the growth of this important ornamental plant was investigated in comparison with tap water and tap water enriched with fertiliser. Growth characteristics in plants irrigated with reclaimed wastewaters were equal or higher than those of plants irrigated with tap water. Nitrogen and phosphorus content in leaves increased for plants irrigated with primary, secondary and tertiary treated wastewater in comparison with plants irrigated with tap water while Ca and Mg accumulation in leaves showed no significant difference. Micronutrient (Cu, Fe, Zn) content in leaves increased in plants irrigated with primary and secondary treated wastewaters. Maximum efficiency of PSII (Fv/Fm) and leaf SPAD measurements indicates the absence of photo-inhibition and of chlorophyll loss in plants irrigated with reclaimed wastewaters.

Keywords: *Dianthus caryophullus*, irrigation, water quality, carnations, domestic wastewater

1. Introduction

Water consumption is an important problem for the cultivation of ornamental plants both from environmental and economical point of view, especially in arid and semi-arid regions. For this reason, the utilization of alternative water sources for irrigation seems unavoidable for a continuing and increased floriculture production. The main alternative water sources available for irrigation are brackish groundwater, runoff water and treated wastewater. Each of these marginal-quality waters has individual characteristics which may have an effect on plant growth. In general, the effect of irrigation on ornamental plants has been investigated to a much lesser extent than the other crops because flowers are normally irrigated with high quality water. However during the last decade, there has been an increasing interest in marginal water use options for the irrigation of ornamental crops (Cassaniti *et al*., 2013). Salinity is the main parameter of marginal water that has been studied for it's possible negative effects on plants. Salt stress causes serious damage in ornamental shrubs according to Cassaniti *et al*. (2009). The overall conclusion was that salinity tolerance should be investigated for each plant species in order to find the most suitable for cultivation under saline conditions. Treated domestic wastewater is a significant part of marginal water in many countries. In addition, unlike other types of marginal water it could also contain nutrients that are essential for plants thereby improving plant growth and reducing fertilizer requirements (Gori *et al*., 2000). However, possible high concentrations of salts (Al-Hamaiedeh and Bino, 2010), heavy metals (Rusan *et al*., 2007) or pathogenic organisms (Mara *et al*., 2007) in wastewaters may have a negative effect on cultivations. Bernstein *et al*. (2006) examined the application of treated wastewater for cultivation of roses in soil-less culture. Results showed that all parameters examined (growth, quantity, size etc) were not affected by the irrigation treatments. In addition, Friedman *et al*. (2007) examined the effects of irrigation with secondary-treated domestic wastewater on the growth of sunflower and celosia plants for cut flower production. The observed reduction in sunflower and celosia characteristics will not pose any commercial problem for cut flowers. Recently, another study was conducted in order to examine the effect of diluted and undiluted wastewater on the growth, physiological aspects and visual quality of lantana and polygala plants (Banon *et al*., 2011). Results indicated that polygala could be irrigated with reused water without any significant problem while lantana was negatively affected by wastewater resulting in defoliation, leaf burn and chlorosis. In addition, Marinho *et al*. (2013) reported an increasing crop yield of 31.8% for rosebushes irrigated with reclaimed wastewater instead of traditional cultivation. Carnations (*Dianthus caryophullus*) are one of the most popular commercial cut flowers in the world ranked second in the commercial importance to roses. The irrigation quantities and the frequency of use with carnations varies with soil texture, photoperiod, air temperature and humidity, air movement, and the mass of the plants relative to loss of water by transpiration (Besemer, 1980). It was reported that yield and quality parameters were high in frequently irrigated carnations (Aydinsakir *et al*., 2009). The effect of saline stress on growth and ornamental characteristics has been reported in previous studies (Baas *et al*., 1995; Navarro *et al*., 2012). To the best of our knowledge very little is known regarding the effects of treated sewage water on carnation plants (Safi *et al*., 2005). In addition, preliminary results of this experiment about some growth characteristics (plant and flower height and weight) were already published

(Petousi *et al*., 2013). The aim of this study was to investigate the effect of irrigation with treated wastewater on leaf content and physiological aspects of carnation plants cultivated for producing cut flower crops.

2. Materials & Methods

2.1 Plant Cultivation

The experiment was conducted from June 2012 to September 2012 (112 days) in a greenhouse at the farm of the School of Agricultural Technology of Crete, Greece. Rooted cuttings of carnation viz. 'Dover' were established into pots filled with a mixture of perlite (33.3%), peat (33.3%) and potting soil (33.3%). The experiment had a random block design with 25 pots per treatment (total 125 pots). During the experiment plants were sprayed with a fungicide (Iprodione 75%, Rovral) and a miticide (milbemectin 0.93%, Milbemeknock) for fungi and mite control, respectively. Plants were irrigated 2-4 times per week, depending on the demand. The total amount of irrigation water applied to each pot for any treatment was 3.9 L corresponding to an average water addition of approximately 35 ml per day.

2.2 Irrigation treatments

The treatments were a) irrigation with primary treated wastewater (PTW), b) irrigation with secondary treated wastewater (STW), c) irrigation with tertiary treated wastewater (TTW), d) irrigation with tap water (TW) as the control treatment, and e) irrigation with tap water enriched with fertilizers (FTW). Primary treated wastewater was obtained from the sewage treatment plant of Heraklion (180,000 p.e.), Crete, Greece. This primary wastewater was further treated at the School of Agriculture using a compact packed bed filter (Advantex-AX20, Orenco) to produce the secondary treated wastewater. Tertiary wastewater was obtained by treating the effluent of the packed bed filter using a sand filter and a chlorination process. For FTW, 5 gr of a chemical fertilizer (12% N, 6% P_2O_5, and 30% K_2O) was added per 10 L of TW.

2.3 Growth monitoring

Leaf SPAD was measured at the midpoint of leaves with a chlorophyll meter SPAD-502 (Konica Minolta). In addition, chlorophyll fluorescence was measured using the chlorophyll fluorometer OS-30p (Opti-Sciences). At the end of the experiment the plants were harvested and their fresh weight, height, width and number of branches were measured. In addition, the number of open flowers and their fresh weight, height and diameter were also determined.

2.4 Chemical Analysis

Water samples were analyzed for chemical oxygen demand (COD) using the closed reflux colorimetric method, for total suspended solids (TSS) by the glass fibre method, while pH was measured with a pH-meter (WTW, 3110) and electrical conductivity (EC) with a conductimeter (Hanna, 8333) in accordance with Standard Methods (APHA, 1995). Total Nitrogen (TN) and Total Phosphorus (TP) were determined spectrophotometrically by use of standard test kits (Hach-Lange). The analysis of macro-elements (K, Mg, Ca) and micro-elements (B, Cu, Zn) was carried out by inductively coupled plasma mass spectroscopy (ICP-MS-Agilent 7500-CX).

2.5 Data Analysis

The data were analysed through one-way analysis of variance (ANOVA) to compare the effect of each irrigation type on plant growth characteristics. Differences between means were tested for significance ($p<0.05$) by Tukey's test.

3. Results

3.1 Irrigation water

The chemical composition of all sources of irrigation water is presented in Table 1. The pH value was high for all treated wastewaters as well as for tap water. The addition of nutrients to TW reduced the pH value in FTW. The salinity was about 2.2-2.3 mS/cm for all wastewaters, 1.0 mS/cm for FTW and 0.5 mS/cm for TW. The organic content as expressed by the COD values was quite different between the examined irrigation water sources. In particular, PTW had a COD value of about 300 mg/L, STW and TTW approximately 65 mg/L while FTW and TW less than 30 mg/L. For the wastewaters this was roughly in line with what we would expect from a properly performing wastewater treatment plant. Average potassium concentration in the FTW was 108 mg/L which was 2-3 times higher than in treated wastewaters (36-48 mg/L). Nitrogen content was about 75-78 mg/L for PTW and FTW while STW had a slightly lower concentration level (61 mg/L), and TTW had significantly lower value (24 mg/L). Similarly, phosphorus content was higher for PTW and FTW (15 mg/L and 13 mg/L, respectively) while STW and TTW had almost the half value (6 mg/L). The treated wastewaters contained higher levels of magnesium (75-80 mg/L), calcium (128-152 mg/L) and especially boron (285-319 mg/L) compared to TW and FTW (22-27 mg/L, 63-64 mg/L and 9-17 mg/L for Mg, Ca and B, respectively). Copper concentration was 70 μg/L for FTW while all other irrigation waters had values below 7μg/L. Tap water had a Zn concentration of 111.0 μg/L, which was about ten times higher than that measured for reclaimed wastewaters.

3.2 Growth and leaf content

Figure 1 shows the growth characteristics of carnations irrigated with five different water sources. Statistical analysis applied to growth parameters indicated that plants irrigated with treated wastewaters had no significant difference ($p<0.05$) compared with plants irrigated with tap water or fertilized tap water. Specifically, number of branches per plant was almost the same for all irrigation treatments ranging from 23.0 to 23.4. Assessment of treatments in terms of the number of open flowers showed the superiority of FTW over all other. In addition, the application of all treated wastewater had positive (for PTW and STW) or neutral (TTW) effect in comparison with the application of TW. Statistical analysis shown that all values were not significantly different for flower height. Similar no differences in plant and flower fresh weight and height was also observed (data presented in Petousi *et al*., 2013). Macroelement contents were affected by the treatments as presented in Table 2. Leaf nitrogen, phosphorus and potassium content increased in plants irrigated with wastewaters in comparison with TW.

Table 1. Chemical composition of water and treated wastewater used in the experiment

Parameter	**PTW**	**STW**	**TTW**	**FTW**	**TW**
pH	7.9 ± 0.2^{a}	8.5 ± 0.2^{b}	8.4 ± 0.2^{b}	7.8 ± 0.1^{a}	$8.3 \pm 0.1^{a,b}$
EC (mS/cm)	2.3 ± 0.1^{a}	2.2 ± 0.1^{a}	2.3 ± 0.1^{a}	1.0 ± 0.1^{b}	0.5 ± 0.1^{c}
COD (mg/L)	296 ± 28^{a}	65 ± 4^{b}	65 ± 6^{b}	29 ± 5^{b}	27 ± 3^{b}
BOD (mg/L)	142 ± 5^{a}	8 ± 1^{b}	6 ± 1^{b}	6 ± 1^{b}	6 ± 1^{b}
TSS (mg/l)	177 ± 23^{a}	28 ± 6^{b}	27 ± 5^{b}	1 ± 1	1 ± 1^{b}
TN (mg/L)	78 ± 3^{a}	61 ± 9^{a}	24 ± 3^{b}	76 ± 4^{a}	5 ± 1^{b}
TP (mg/L)	15 ± 2^{a}	6 ± 1^{b}	6 ± 1^{b}	13 ± 1^{a}	0.2 ± 0.1^{c}
K (mg/L)	36 ± 2^{a}	48 ± 4^{b}	47 ± 5^{b}	108 ± 2^{c}	$5 \pm {}^{d}2$
Mg (mg/L)	75 ± 3^{a}	75 ± 3^{a}	80 ± 4^{a}	27 ± 2^{b}	$22 \pm {}^{b}1$
Ca (mg/L)	128 ± 5^{a}	135 ± 6^{a}	152 ± 8^{b}	63 ± 1^{c}	$64 \pm {}^{c}1$
B (μg/L)	285 ± 10^{a}	304 ± 9^{a}	319 ± 10^{a}	17 ± 5^{b}	9 ± 5^{b}
Cu (μg/L)	7 ± 5^{a}	1 ± 1^{a}	5 ± 3^{a}	70 ± 8^{b}	5 ± 4^{a}
Zn (μg/L)	28 ± 9^{a}	7 ± 5^{a}	7 ± 5^{a}	149 ± 9^{b}	111 ± 23^{b}

a, b: In each raw, mean values followed by a different symbol are significantly different ($p < 0.05$)

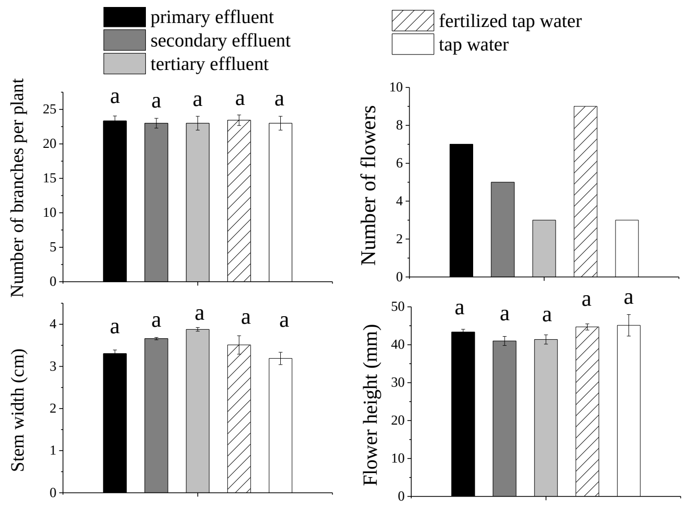

Figure 1. Effect of water treatments on carnation plants: a) number of branches, b) number of flowers, c) stem width and d) flower height. In each measurement, different small letters indicate significant differences ($p<0.05$) between treatments.

Furthermore, plants irrigated with PTW had nitrogen and phosphorus leaf content (23.3 g/kg and 7.7 g/kg, respectively) even higher than plants irrigated with FTW (21.1 g/kg and 5.8 g/kg, respectively). On the other hand, leaf calcium and magnesium concentrations were higher when the plants were irrigated with tap water (30.7 g/kg and 6.0 g/kg, respectively). However, these higher values for Ca and Mg were not statistically significantly different in comparison with the other treatments. Wastewater quality had no significant effect on the leaf boron concentration. Copper, Ferrous and Zinc content in carnation leaves increased in the order TTW<STW<PTW. Plants irrigated with PTW had microelement concentrations (8.1 mg/kg, 90.1 mg/kg and 180.5 mg/kg for Cu, Fe and Zn, respectively) higher than plants irrigated with FTW. On the other hand Cu, Fe and Zn leaf content in plants irrigated with TTW was 4.5 mg/kg, 25.8 mg/kg and 67.9 mg/kg, respectively, values almost equal with values observed in plants irrigated with TW. No effect in K, Ca and Mg content in plants irrigated with treated wastewaters was observed despite the higher levels found in these waters. On the other hand, results showed that the irrigation with treated wastewaters rich in N and P resulted in an increase of N and P content in the leaves of plants in comparison with tap water. In other words, irrigation with treated wastewater had a positive effect on

Table 2. Effect of irrigation in leaves of carnations

Parameter	**PTW**		**STW**		**TTW**		**FTW**		**TW**	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Macroelements										
N (g/kg)	23.3^{a}	1.7	22.6^{a}	2.3	16.2^{b}	2.0	21.1^{a}	0.2	12.0^{c}	0.6
P (g/kg)	7.7^{a}	0.6	4.6^{b}	0.2	5.9^{c}	0.4	5.8^{c}	0.5	4.0^{b}	0.5
K (g/kg)	43.5^{a}	3.7	46.4^{a}	1.7	42.6^{a}	4.4	80.2^{b}	7.7	40.3^{a}	6.6
Ca (g/kg)	27.9^{a}	2.5	25.1^{a}	4.7	23.8^{a}	4.0	29.0^{a}	5.2	30.7^{a}	7.4
Mg (g/kg)	6.5^{a}	0.7	5.8^{a}	1.0	5.2^{a}	1.3	5.7^{a}	1.5	6.0^{a}	1.0
Microelements										
B (mg/kg)	136.5^{a}	35.8	132.6^{a}	42.0	136.9^{a}	24.9	154.4^{a}	47.0	161.9^{a}	75.3
Cu (mg/kg)	8.1^{a}	2.1	$5.2^{a,b}$	0.5	4.5^{b}	0.9	$6.5^{a,b}$	1.3	4.6^{b}	0.8
Fe (mg/kg)	90.1^{a}	11.2	40.6^{b}	9.1	25.8^{b}	9.9	$63.0^{a,b}$	14.8	25.7^{b}	5.9
Zn (mg/kg)	180.5^{a}	19.8	$129.2^{b,c}$	11.8	67.9^{c}	12.2	135.0^{a}	29.5	78.4^{c}	15.5

a, b: In each raw, mean values followed by a different symbol are significantly different ($p < 0.05$)

plants similar to the positive effect of irrigation with fertilized tap water. Similar results were reported by Friedman *et al*. (2007) who examined the effects of irrigation with secondary treated wastewater on the growth of sunflower and celosia. They found that under irrigation with wastewater celosia accumulated significant higher levels of N and sunflower higher levels of P. In the same study the K content in leaves was similar for both species and irrigation treatments (39.5-42.0 g/kg) even if the concentration of K in wastewater was significant higher (35-50 mg/L) in comparison with potable water (0-5 mg/L). A previous study (Sonneveld and Woogt, 1986) on the supply and uptake of K, Ca and Mg of spray carnations found that a mole ratio of K:Ca:Mg of 55:35:10 in nutrient solution appeared to be optimal. Such ratios in addition led to ratios of 55:30:15 in the root environment. Green (1967) suggested that there were probably three systems operating in cation uptake of carnations: a) when potassium was in good supply, its presence suppressed the uptake of sodium rather effectively, b) when the potassium supply was deficient, the four ions K, Na, Ca, and Mg competed for uptake and c) magnesium and calcium may have been taken up by a separate system in which they competed equally for uptake. In this study, treated wastewaters contained about 300 μg/L of B, a value significantly higher than in the tap waters examined (9-17 μg/L). Nevertheless, that value is characterized as safe (<500 μg/L) even for boron-sensitive crops (Maas, 1996). The B content in leaves was found not to be statistically different for all examined irrigation treatments including tap waters and treated wastewaters with values between 132.6-161.9 mg/kg. A previous study reported that B becomes less available to plants with increasing soil pH. In addition B uptake by plants was reduced when the Ca content of the medium was increased (Gupta, 1993). So, increased values of pH and Ca in treated wastewaters may balance the uptake of B by plants.

3.3 SPAD and chlorophyll fluorescence

Leaf SPAD was significantly higher for all treated wastewaters in comparison with TW (Figure 2a). Specifically, SPAD values at the end of experiment were 79.4 , 76.4, 70.2 and 56.9 for PTW, STW, TTW and TW respectively. Similar results were observed for maximum photochemical efficiency of photosystem II as expressed by the ratio Fv/Fm (Figure 2b). Values between 0.81 to 0.83 were recorded for treated wastewaters while plants irrigated with TW had a mean value of 0.77 at the end of the experiment. In addition, comparing either SPAD or Fv/Fm ratio for FTW (75.6 and 0.82, respectively) with all wastewater treatments no significant difference was found. Leaf SPAD values and Fv/Fm recorded in this work indicated the absence of chlorophyll loss and of photo-inhibition in plants irrigated with treated wastewaters in comparison with tap waters. These two parameters are frequently used as indicators of photosynthetic stress of plants caused by salinity (Loreto *et al*., 2003), nutrient deficiency (Morales *et al*., 2000), and heavy metals (Mallicka and Mohnb, 2003). Banon *et al*. (2011), examined among others the effect of irrigation with reused water on leaf SPAD and Fv/Fm values for two ornamental plants. They found any change for polygala and significant reduced values for lantana.

4. Conclusions

Results show that reclaimed wastewater could be used without any significant problem as an alternative water source for the production of carnations in arid and semi-arid regions. The high salinity and boron concentrations levels in treated wastewater had no adverse effect on carnation cultivation as indicated by plant growth characteristics. Furthermore, treated wastewater contained significant amount of nutrients (nitrogen, phosphorus, potassium) and minerals (calcium, magnesium) which could reduce fertilizer requirements for carnation cultivation. In general the accumulation of nutrients in plant tissues was in accordance with the concentration levels in irrigation water used (primary treated>secondary treated>tertiary treated). Two other parameters not mentioned in this work should be investigated in the

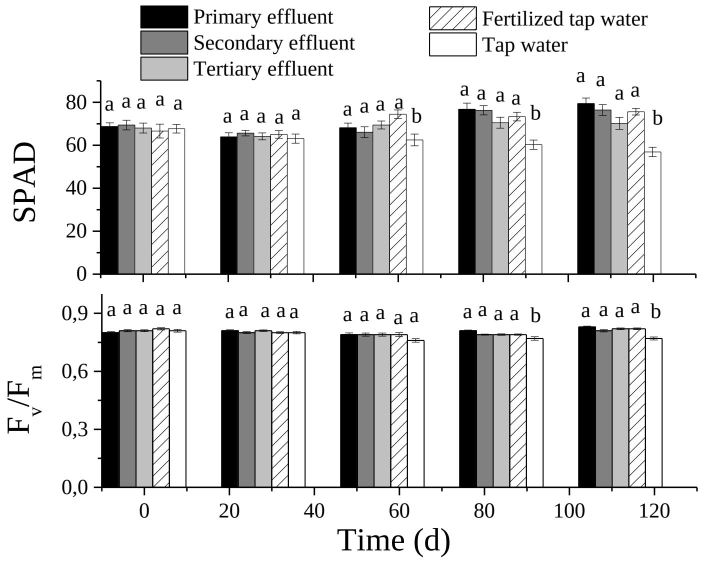

Figure 2. Effect of water treatments on a) leaf SPAD and b) maximum photochemical efficiency of photosystem II during cultivation period. Data are means (n=25) ± SE (vertical bars). In each measurement, different small letters indicate significant differences (p<0.05) between treatments.

future: a) the occurrence of pathogens in carnation cut flowers (even if, treated wastewater were chlorinated) and b) the long term effect of treated wastewater on cultivation medium.

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