The use of treated wastewater and fertigation in greenhouse pepper crop as affecting growth and fruit quality

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ABSTRACT

The performance and suitability of tertiary treated wastewater (TW) and/or fertigation (F) in pepper plants were studied over a 4-month period in greenhouse conditions. Four treatments were used consisting of (1) water, (2) water + F, (3) TW, and (4) TW + F. The F and/or TW application increased plant height and plant biomass compared with the control plants (irrigated with water) while no differences were observed in the number of leaves produced. Plants grown with TW + F were thicker than control plants. The addition of F increased fruit number in both water and TW, which resulted in increased plant yield and fruit marketability for the water application but decreased mean fruit weight for TW application. The application of TW increased fruit total soluble solids but decreased fruit firmness whereas adding F, these changes were normalized. The F and/or TW application reduced fruit total phenolics, fruit acidity, and fruit length but not fruit diameter. No differences were observed in fruit dry matter content, fruit color/lightness (a, b, and L value). Bacteria (total coliform and Escherichia coli) units on the fruits did not differ among the treatments. The results indicate that wastewater may act as an alternative means of irrigation if following strict safety aspects while the fertigation acted beneficially.

Key words | fertigation, fruit quality, pepper, wastewater, yield

INTRODUCTION

In recent years, the use of marginal-quality water for crop irrigation has gained importance in water-scarce regions (Qadir et al. 2010) as the demand for water is continuously increasing in arid and semi-arid countries. One of the major types of marginal-quality water is wastewater, which has been reused in agriculture for centuries; however, the amount of wastewater recycled has greatly increased in the last decade (Bhogal et al. 2003; Kalavrouziotis et al. 2008). Types of wastewater used for recycling include primary treated wastewater (PTW, Zabalaga et al. 2007), secondary treated wastewater (STW, Pedrozo & Alarcón 2009), and tertiary treated wastewater (TTW, Police et al. 2004). Several scientific studies have researched this area in order to determine the reuse of processed wastewater for the irrigation needs of crops following primary, secondary, and tertiary (chlorotic, UV, O₃, etc.) treatment aiming at the maximization of crop yields, not only quantitative, but also qualitative, without environmental risks (Manios et al. 2006; Petousi et al. 2013). Disinfection of wastewater is vital in addressing the potential health risks of urban water reuse and appropriate methods for disinfection as an essential purification step for safe urban reuse have been studied (Bischoff et al. 2013). The above methods of wastewater application will need to be accompanied by restrictions that ensure the protection of public health and the protection of air, aqueous, and soil sources. The use of wastewater affects the chemical properties of soil in the first 30 cm below ground, as well as the constitution of plants (Kiziloglu et al. 2008).

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In general, treated wastewater (TW) reuse has several advantages which include: (1) crop productivity improvement in water-constrained systems (Raschid-Sally et al. 2005); (2) reduced amount of fresh water used for irrigation; (3) reduced discharge of nutrients into surface waters; and (4) a decrease in the cost of wastewater treatment by eliminating the need for nutrient removal (Rosenqvist et al. 1997). Furthermore, irrigation with municipal wastewater is considered an environmentally friendly wastewater disposal practice that helps to minimize pollution of the ecosystem which is subjected to contamination by direct disposal of wastewater into surface or groundwater (Mohammad & Mazahreh 2003). In addition, wastewater is a valuable source for plant nutrients (exchangeable Na, K, Ca, Mg, and P) and organic matter needed for maintaining fertility and productivity of arid soils. However, the reuse of wastewater for irrigation may potentially create environmental problems if not properly treated and managed (Bahri & Brisaud 2011). Consequently, management of irrigation with wastewater should consider the nutrient content in relation to the specific crop requirements and the concentrations of plant nutrients in the soil, and other soil fertility parameters. Thus, wastewater irrigation in lettuce had a high influence on soil parameters: organic matter, N, P, Ca, Al, Fe, Pb, and Zn as well as pathogenic microorganisms (Manas et al. 2009). Several studies examined the effects of wastewater used in agriculture for irrigation needs as well as the impact of fertilizers in the crop-soil system, such as alfalfa, radish, and tomato (Albulbasher et al. 1998; Manios et al. 2006; Najafi et al. 2006; Aiello et al. 2007; Petousi et al. 2013), cucumber (Manios et al. 2006; Pilatakis et al. 2013), eggplant (Najafi et al. 2006; Cirelli et al. 2012), citrus (Morgan et al. 2008), ornamental (Lubello et al. 2004), cauliflower, and red cabbage (Kiziloglu et al. 2008).

At the market interface, only produce that corresponds to the expectations of the consumer can survive. Fruit quality encompasses many aspects, and includes not only flavor, color, nutritional aspects, and firmness, but also shelf life, processing attributes, and resistance to pathogens (Brumwell & Harpster 2003). Moreover, an increased interest in vegetables has been created by the fact that their consumption has been correlated with human health and the reduced risk of some types of cancer (Gerster 1997). The present study examined the impacts of mineral fertigation combined with different water quality (TTW) in soil, in plant growth, and yield and fruit quality related parameters, in greenhouse pepper production.

**MATERIAL AND METHODS**

**Plant material and tertiary treated wastewater source**

Pepper (*Capsicum annum* L. cv. Oregon) plants were grown under natural light from September to January (in 2011). PTW was obtained from the sewage treatment unit of Heraklion (180,000 p.e.), Crete, Greece. TW was obtained by treating the effluent of packed bed filter (Advantex-AX20, Oreno; used for STW) using a sand filter and a chlorination process. The physicochemical characteristics of the TTW used in the experiment were measured and presented in previous studies (Petousi et al. 2013). In detail, the physicochemical characteristics of TTW and of tap water (in parentheses) sources used in the experiment were: pH 7.5 (7.8); electrical conductivity – EC (mS/cm) 1.1 (0.7); chemical oxygen demand – COD (mg L⁻¹) 24 (16); total soluble solids – TSS (mg L⁻¹) 9.1 (2.6); total nitrogen – TN (mg L⁻¹) 20.0 (4.7); total phosphorus – TP (mg L⁻¹) 8.0 (2.0); boron – B (μg L⁻¹) 251.2 (16.4); magnesium – Mg (mg L⁻¹) 51.2 (19.1); calcium – Ca (mg L⁻¹) 123.0 (60.5); potassium – K (mg L⁻¹) 28.5 (not detected); and zinc – Zn (μg L⁻¹) 7.0 (not detected).

**Experimental design**

The experiment was carried out in an unheated plastic greenhouse with a north–south orientation at the Technological Educational Institute of Crete (TEI of Crete), Greece. Seedlings were produced in plastic seedling trays filled with expanded clay and were acquired from a local agriculture nursery. Fertigation (F) and/or TW were used to create four treatments (three replications/treatment; three plants/replication) which were (1) water, (2) water + F, (3) TW, and (4) TW + F.

Seedlings were transplanted in single pots (filled with substrate; 9 L capacity pot) and arranged in a single row with a completed randomized design for the replicates/
Row treatment. Rows were 1.0 m apart and plants were separated by 0.4 m. Drip irrigation emitters (one emitter/pot) were placed and irrigation took place twice (1 min/time) daily, via timer, by means of pressure pumps. Fertigation (EC: 2.5–3.0 mS/cm; 200 mL/plant twice a week) with commercial fertilizers took place manually. The drainage solution was collected in trays in each pot and was available for plant water needs through capillary suction. Plants were treated according to a twin lateral pruning system (two lateral stems growing vertically) on string.

Measurements

The nutritional status of soil was observed. Thus, we measured K and Na content (by a flame photometer), P (spectrophotometrically), total N (Kjeldahl method), organic matter content and organic carbon, pH, and EC.

Beginning the second week after transplanting, we studied the impact of fertigation and/or water quality on plant growth/development, yield and fruit quality in peppers. Every second week the plant height, main stem diameter, leaf number produced, flower and fruit number, and the leaf fluoresces (chlorophyll fluorometer, OS-30p, Opti-Sciences, UK) were measured. With the completion of the experiment, plant biomass (fresh weight and the % of dry matter) and plant yield were determined. Follow the completion of the experiment, plant biomass (fresh weight and the % of dry matter) and plant yield were determined.

Fruit fresh weight, dry matter content (%), and fruit size (length and diameter) were measured for each harvested fruit. Fruit marketability was observed by employing a 1–4 scale (1: extra quality; 2: good quality; 3: medium quality (i.e., small size, and decolorization); and 4: not marketable quality (i.e., malformation, wounds, and infection)). Fruit color measurements were taken around the fruit equator (two measurements per fruit) with a Minolta Chroma Meter CR300. Data are expressed in L x a x b units. Fruit firmness was measured at one point on the shoulder of the fruit, for each treatment, using a penetrometer FT 011 (TR Scientific Instruments, Forli, Italy). The amount of force (in Newtons – N) required to break the radial pericarp (i.e., surface) of each pepper was recorded at ambient (22–24 °C) temperature.

TSS concentration was determined on the fruit juice for each treatment with a digital refractometer 300017 (Sper Scientific Ltd, Scottsdale, AZ, USA) at 20 °C and results were expressed as the mean (%) of Brix. Sub-samples of homogenized fruit tissue were used to determine the pH and EC of fruit juice using a standard pH/EC meter (Orion 920A; Scientific Support, Hayward, CA, USA). Titratable acidity (TA) was determined by potentiometric titration, using fruit samples (5 g) diluted in 100 mL distilled water and titrated with 0.1 N NaOH, using phenolphthalein as pH indicator, and monitored up to 8.2 end point with a pH meter. The reported values were expressed in terms of citric acid percentage. Total phenolics was determined on blended fruit tissue (5 g) extracts following repeated (four-fold) addition of 2.5 mL of 50% (v/v) methanol, as reported previously (Tzortzakis et al. 2007). Results were expressed in terms of gallic acid equivalents (GAE; Sigma Aldrich, Athens, Greece) per 100 g fresh weight of tissue.

Fruits (n = 23) were assessed for bacteria (total coliform and Escherichia coli (E. coli)) units on the fruits as well as in the fruit, by employing ChromoCult® Coliform Agar (Merck KGaA), which is a selective and differential chromogenic culture medium. Harvested fruits were placed in sterile plastic bags (one fruit per bag), which were then filled with 225 mL sterile Butterfield’s phosphate buffered water (42.5 g/L KH₂PO₄, pH 7.2, Merck), and were gently shaken to rinse off most of the bacteria present. For isolation of coliform cultures, 1 mL of the solution was added to 0.45 μm membrane in vacuum under sterile contrition. Inoculated membrane was transferred into a Petri dish containing ChromoCult® Coliform Agar, and incubated at 35 °C for 24–48 h. Pink colonies resulting from salmon-galactoside cleavage by β-D-galactosidase were classified as TCC, whereas dark blue colonies resulting from salmon-galactoside and X-glucuronide cleavage by β-D-galactosidase and β-D-glucuronidase were classified as presumptive E. coli colonies (Byamukama et al. 2000). Similarly, soil samples (n = 5) were assessed for bacteria (total coliform and E. coli) units.

Statistical analysis

Data were tested for normality and then subjected to analysis of variance (ANOVA). Significant differences between mean values were determined using Duncan’s Multiple Range test (P < 0.05) following one-way ANOVA. Significant differences on percentage values (% dry weight) were
logarithmically transformed prior to analysis. Statistical analyses were performed using SPSS (SPSS Inc., Chicago, IL) and graphs produced using Prism v.2.0 (Graph Pad Inc., San Diego, CA).

RESULTS AND DISCUSSION

Soil properties

The nutritional status of soil used in the present study were 0.83% organic matter (0.48% organic C), C/N of 21.7, pH of 6.94, and EC 0.71 mS/cm as well as total N content of 0.14% and 21.73 mg P L⁻¹, 5.55 mg K L⁻¹ and 0.32 mg Na L⁻¹. The soil was of a white color, a calcareous clay loam with a CEC of 2.9.

Effect on plant growth

Examining the different F and/or TW application, plants grown in soil enriched with F and/or irrigated with TW were taller with significant changes observed after 45 days (average 40 cm) compared with the control plants (irrigated with tap water) while no differences were observed in leaf number produced (Figures 1(a) and 1(b)). The F-enrichment in both water and TW application affected positively plant height and was in agreement with previous studies in cucumber and tomato crops when irrigated with TW and/or fertigation (Manios et al. 2006). Plants grown with TW + F produced thicker stems than plants grown with tap water (control) and this is probably due to the nutrient lack of plants irrigated only with water. Plants irrigated with TW or water + F produced similar stem thickness highlighting the nutritive value that TW provided to the crop following 105 days plant growth (Figure 2(a)). No differences were observed in leaf fluorescence among treatments (data not presented).

The addition of F increased fruit number produced in both water and TW treatments and differed significantly with the fruit number obtained in plants irrigated only with water (Table 1 and Figure 2(b)). However, plants irrigated with water formed fruits 15 days earlier compared with other treatments (Figure 2(b)), which is of interest as fruit earliness is directly related with increased cost benefits of the producers. Fruit number produced followed a similar pattern as the number of the produced flowers (data not presented), implying that there was no flower absorption that was directly related to the applied treatments.

Effect on plant yield

Plants irrigated with water + F increased yield (averaged 327.61 g/plant) compared with plants irrigated with water or TW ± F (Table 1). Fruit fresh weight decreased in plants irrigated with TW + F compared with TW or water ± F, indicating that the increased nutrition (due to the combination of TW and F, as both are nutrient sources) affected negatively the fruit fresh weight. No differences were obtained in fruit dry matter content among treatments (Table 1).

*Figure 1 | Effects of fertigation (F) and/or TW into soil on plant height and leaf number produced of greenhouse pepper crop. Values represent mean (±SE) of measurements made on six plants per treatment.*
The increased yield obtained in plants irrigated with water + F is due to the increased fruit number (more fruits) rather than differences in fruit fresh weight. When municipal-TW was used in ornamental plants in nurseries, the nutrient content of the tertiary effluent was able to maintain as good plant growth as fertigated water for most of the tested species (Lubello et al. 2004), which is in accordance with the present study. Lettuce treated with wastewater increased yield compared with control as well as increased N, P, Pb, and Al content in plant tissues (Manas et al. 2013). Wastewater irrigation treatments also increased the yield as well as N, P, K, Ca, Mg, Na, Fe, Mn, Zn, Cu, Pb, Ni, and Cd contents of cauliflower and red cabbage plants (Kizilçöglu et al. 2008). Additionally, a part of the increased yield in tomato and eggplant irrigated with TW can be related to better soil moisture and increased available nitrogen in the root zone (Najafi et al. 2006; Cirelli et al. 2012), which is in line with the present study with the increased nutrient concentration, i.e., N, of TW compared with tap water.

The F and/or TW application increased plant biomass (fresh weight; fwt) compared with the control plants (irrigated with water) while TW + F reduced biomass dry matter content (Table 1).

**Effect on fruit quality**

Fruit size fluctuated among treatments and the effects were mainly in fruit length rather than fruit diameter (i.e., thicker fruits) (Table 2). In detail, the greatest fruit length was observed in the case of plants irrigated with water while plants irrigated with TW had a reduced (up to 34%) fruit length. Interestingly, the addition of fertilizers (TW + F) alleviated the reduction in fruit length. Fruit firmness reduced when plants irrigated with TW were compared with the control treatment (water) while the addition of F resulted in fruit firmness being maintained (see Table 2). Fruit firmness is an important quality attribute and is directly related to enhancing the storability potential and to inducing greater resistance to decay and mechanical damage (Barret & Gonzalez 1994).
Fruit marketability was maintained as good quality (marked as 2 out of the four values) for the water application but was enhanced when fertigation was applied and this is probably due to the better nutrition that plants achieved. No differences were observed when TW was used in combination with fertigation (Table 2). Fruit lightness (L) increased when TW was used while the addition of fertilizer reduced L value (Table 2). Fruit green color (Chroma a and b), fluctuated among treatments but did not differ significantly.

The application of TW increased fruit TAA and fruit sweetness (TSS/TA ratio) whereas upon the addition of F, these changes were normalized. The F and/or TW application reduced fruit total phenolics and fruit acidity (Table 3). Pepper fruits were less acid in plants irrigated with TW but no differences were observed in the pepper juice EC. In previous studies, irrigation with TW did not affect tomato fruit pH, increased their size up to 2 cm in diameter, and weight up to 78.7 g. Additionally, a decrease of 1.5% in the TSS, 0.59 kg in firmness, and 5.1% in weight loss of tomato fruit were recorded (Al-Lahham et al. 2003).

Bacteria (total coliform and E. coli) units on the fruits did not differ among the treatments (Table 4) being in agreement with previous studies in tomato and eggplant (Cirelli et al. 2012). In detail, the average total coliform units were 5.3 CFU/100 g fruit fwt while fertigation enhanced the presence of bacteria. The average number of E. coli was approximately 0.36 CFU/100 g fruit fwt and it was only present in the case of the application of fertilizer in soil (water + F and TW + F). When selected fruits were examined for the presence of bacteria inside the fruit, bacteria units were not detected (data not presented), implying that bacteria did not move through plant tissue.

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**Table 2** | Effects of fertigation (F) and/or TW into soil on fruit size (length and diameter in mm), fruit firmness (N) and fruit color (L, a, and b values), fruit marketability on 1–4 scale (1: extra quality, 2: good quality, 3: medium quality, and 4: not marketable quality) in greenhouse pepper crop

<table>
<thead>
<tr>
<th>Fruit size (mm)</th>
<th>Fruit color</th>
<th>Marketability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Length</td>
<td>Diameter</td>
</tr>
<tr>
<td>Water</td>
<td>7.31 a</td>
<td>6.77 a</td>
</tr>
<tr>
<td>Water + F</td>
<td>5.75 b</td>
<td>6.31 a</td>
</tr>
<tr>
<td>TW</td>
<td>4.84 c</td>
<td>6.53 a</td>
</tr>
<tr>
<td>TW + F</td>
<td>5.43 b</td>
<td>5.82 a</td>
</tr>
</tbody>
</table>

Values (n = 23) in columns followed by the same letter are not significantly different. P < 0.05.

**Table 3** | Effects of fertigation (F) and/or TW into soil on TSS (TSS: ‘Brix), titratable acidity (TA: % citric acid), sweetness (TSS/TA), pH, EC (mS/cm), and total phenols (gallic acid equivalent: GAE/100 g fwt) in greenhouse pepper crop

<table>
<thead>
<tr>
<th></th>
<th>TSS</th>
<th>TA</th>
<th>TSS/TA</th>
<th>pH</th>
<th>EC</th>
<th>Total phenols</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>3.26 b</td>
<td>4.68 a</td>
<td>0.79 c</td>
<td>5.30 a</td>
<td>1.78 a</td>
<td>296.91 a</td>
</tr>
<tr>
<td>Water + F</td>
<td>3.20 b</td>
<td>3.09 b</td>
<td>1.13 b</td>
<td>5.30 a</td>
<td>1.48 a</td>
<td>184.57 c</td>
</tr>
<tr>
<td>TW</td>
<td>3.76 a</td>
<td>3.10 b</td>
<td>1.28 a</td>
<td>5.11 b</td>
<td>1.62 a</td>
<td>263.15 b</td>
</tr>
<tr>
<td>TW + F</td>
<td>3.53 b</td>
<td>3.52 b</td>
<td>1.01 b</td>
<td>5.38 a</td>
<td>2.01 a</td>
<td>186.61 c</td>
</tr>
</tbody>
</table>

Values (n = 23) in columns followed by the same letter are not significantly different. P < 0.05.

**Table 4** | Effects of fertigation (F) and/or TW into bacteria (total coliform and E. coli) units (CFU/100 g fruit fwt) on the fruits as well as in the soil (CFU/100 g soil) in greenhouse pepper crop

<table>
<thead>
<tr>
<th>Fruits</th>
<th>Soil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total coliform</td>
</tr>
<tr>
<td>Water</td>
<td>3.81 a</td>
</tr>
<tr>
<td>Water + F</td>
<td>4.48 a</td>
</tr>
<tr>
<td>TW</td>
<td>5.82 a</td>
</tr>
<tr>
<td>TW + F</td>
<td>7.26 a</td>
</tr>
</tbody>
</table>

Values (n = 23) in columns followed by the same letter are not significantly different. P < 0.05.
However, this fact needs to be examined more precisely and in greater detail in the future, before final statements. It is worthwhile mentioning that the bacteria load into the soil was approximately $10^3$ times more than the one in fruits (which is in agreement with previous studies; Manios et al. 2006) for the equivalent treatments (see Table 4) while the wastewater application in tomato crops resulted in increased microbial contamination (E. coli and fecal Streptococci) on the soil surface (Aiello et al. 2007). In previous studies, the washing solution of tomato fruits grown with wastewater was analyzed for fecal coliforms. It appeared that the fruit skins were free of viable fecal coliforms 24 h after the wastewater application. Subsurface drainage analyses did not show any alarming levels of constituents irrespective of the source of the water: wastewater or freshwater (Albulbasher et al. 2012).

The use of TW in agriculture may act as an alternative method for crop irrigation considering the great shortage of water in the last years. Moreover, wastewater contains nutritive value (mainly N and P) that should be considered as additives in crop needs while the microbial load that wastewater may contain might act as a negative parameter for plant growth and fruit production. The use of TW for vegetable production, which are considered as edible fresh products, needs further exploration. Indeed, the negligible microbial contamination of tomato fruit and washing solution suggested that the TW can be used as a valid alternative for irrigation of tomatoes. However, the TW can be used as an alternative for irrigation practice in vegetables, i.e., tomatoes, eaten after cooking but not for those eaten raw provided that the effluent quality is continuously monitored to avoid contamination (Al-Lahham et al. 2005). In conclusion, the results indicate that wastewater may be used as an additional water resource and act as an alternative means of irrigation for pepper cultivation in water-scarce Mediterranean environments, following strict safety aspects while the fertigation acted beneficially.

ACKNOWLEDGEMENT

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REFERENCES


Kiziloglu, F. M., Turan, M., Sahin, U., Kuslu, Y. & Dursun, A. 2008 Effects of untreated and treated wastewater irrigation on some chemical properties of cauliflower (Brassica oleracea L. var. botrytis) and red cabbage (Brassica oleracea L. var. rubra) grown on calcareous soil in Turkey. Agri. Water Manage. 95, 716–724.


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