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Effects of treated wastewater irrigation on the establishment of young grapevines

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Abstract

Irrigation with treated wastewater could produce excessive accumulations within the plant and soil, negatively affecting the yield and production quality. In addition, the presence of biological and chemical contaminants could harm the agricultural environment, as well as the health of farmers and consumers. During this work, the suitability of secondary and tertiary treated wastewater for use in young grapevines was evaluated by studying the effect of the wastewater irrigation on the soil-plant system, crop yield, fruit quality and the presence of inorganic chemical contamination (salts, elements and heavy metals), organic chemical contamination (polycyclic aromatic hydrocarbons) and microbial contamination (E. coli, total coliforms). The results show that tertiary treated wastewater had positive impact on plant growth and yield while secondary treated wastewater had negative impact on fruit safety in comparison with tap water. Sodium levels in soils irrigated with treated wastewater increased at the end of the irrigation period while decreased during the wet season. The total polycyclic aromatic hydrocarbon concentrations in the soils ranged from 363 μg/kg to 374 μg/kg at the end of the experiment for all irrigation treatments applied. The use of tertiary treated wastewater was recommended for the irrigation of young grapevines as an alternative water source secured protection of environment, plant health and fruit quality.

1. Introduction

Today many countries face substantial problems of water scarcity and deterioration of its quality. One of the primary reasons for the observed water scarcity is that the fraction of water available for human consumption, in rivers and streams, lakes, reservoirs and groundwater aquifers, is not distributed uniformly around the world

(Shiklomanov, 1993). Simultaneously, the increasing need for water resources is a consequence of demographic growth, economic development and improvement of living standards, climate change and pollution (FAO, 2012). In this context, the reuse of treated wastewater represents a valid option, in some cases urged by the absence of viable alternatives (Niemczynowicz, 1999 and WHO, 2006). The 2030 Agenda for Sustainable Development defines 17 Sustainable Development Goals (SDGs) to end poverty, protect the planet and ensure that all people enjoy peace and prosperity (U.N. General Assembly, 2015). Wastewater treatment and reuse is recommended in target 6.3 "improve water quality by …halving the proportion of untreated wastewater and substantially increasing recycling and safe reuse globally" and target 3.9 "reduce the number of deaths and illnesses from … water and soil pollution and contamination". Moreover, treated wastewater reuse in agriculture could contribute to achieve targets of SDG 2 about zero hunger as this practice "increase agricultural productivity and strengthen capacity for adaptation to climate change and drought"

Irrigation with treated wastewater has already been implemented in several countries, such as Israel (Hamilton et al., 2007), Australia (Muyen et al., 2011), USA (USEPA, 2012), Spain (Iglesias and Ortega, 2008) Cyprus (Fatta and Anayiotou, 2007) and Jordan (Ammary, 2007) for cultivation of olive trees, grapevines and vegetables. The use of treated wastewater in agriculture continues due to the benefits it offers. These include: a solution to irrigation water scarcity; the availability of large amounts throughout the year; the possibility of reserving better-quality water for human consumption; the reduction of fertilizers needed due to the nutrients contained in this type of water; protection of the environment; the reduction of effluent waters in the surrounding area; avoiding marine intrusion in coastal areas and overexploitation.

While there is a wide scope for reuse potential for using wastewater for irrigation, there are reasons for concern when it is not carefully managed. Treated wastewater contains nutrients, heavy metals, salts and harmful chemicals. Environmental issues are associated with each of these components and their fate cannot be ignored. This might include irrigation induced runoff and rainfall runoff from the wastewater irrigation area resulting in eutrophication of surface water (Kontas et al., 2004). Moreover, wastewater irrigation entails some potential environmental risks for soils and groundwater (Wu et al., 2009). Many recent studies have focused on the impacts of wastewater irrigation on salinity and heavy metals in soils and groundwater (Khan et al., 2008; Leal et al., 2009; Pereira et al., 2012; Travis et al., 2010). In recent years, the impact of the migration of persistent organic pollutants such as polycyclic aromatic hydrocarbons, organochlorine pesticides and nonylphenols into soils and groundwater has raised broad concerns (Calderón-Preciado et al., 2011, Chung et al., 2008 and Zhou et al., 2013).

In addition, soluble constituents could be present in the treated wastewater at levels that may be toxic to plants, and they can also be stored in the soil profiles. Salinity is a very important issue for many horticultural reuse schemes (Muyen et al., 2011). Salts can affect plants either through causing osmotic stress or via direct toxicity. Sodicity induces changes in the soil's physical properties, the most notable effect being the dispersion of soil aggregates. Dispersion, in combination with other processes such as swelling and slacking, can affect plants through decreasing the permeability of water and air through the soil, water-logging, and impeding root penetration (Warrington et al., 2007).

Wastewater irrigation poses a number of potential risks to human health via the consumption of or exposure to pathogenic microorganisms, heavy metals and harmful organic chemicals. A wide variety of pathogenic microorganisms is found in wastewater, including bacteria, viruses, protozoans and parasitic worms. The symptoms and diseases associated with such infections are also diverse, including typhoid, dysentery, gastroenteritis, diarrhoea, vomiting and malabsorption. Heavy metals are of great concern due to their uptake in plants and their accumulation in vegetal tissue, resulting in a health hazard associated with the consumption of these heavy metal-contaminated vegetables over a long period of time (Kalavrouziotis et al., 2008; Gupta et al., 2010). In addition, the occurrence of harmful organic chemicals in treated wastewater may have adverse effects on human health if they accumulate in the edible parts of plants (Mapanda et al., 2005). Previous works have proved the accumulation of high concentrations of polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides and phthalic esters in plants cultivated on contaminated soils (Zohair et al., 2006; Khan et al. 2008; Cai et al., 2008; Petousi et al., 2014).

In Europe, only 2.4% of treated wastewater (700 Mm^3/year) is reused, mostly in Spain, while irrigation represents 75% of water reuse (Hochstrat et al., 2006). This is clearly not enough if the need to develop alternative water supplies is to be met in a context of growing water scarcity and increasing climate change impacts. So far, no specific regulation on reclaimed wastewater use exists at the European level, which may explain the low uptake of water reuse practices across Europe. The only references to reclaimed wastewater use are Article 12 of the European Wastewater Directive (91/271/EEC) (EC, 1991), the Water Framework Directive 2000/60/EC (EC, 2000) and, specifically, EU Directive 2008/105/EC on Environmental Quality

Standards (EC, 2008). According to Directive 91/271/EEC - Article 12, treated wastewater must be reused whenever appropriate and disposal routes must minimize any adverse effects on the environment. Directive 91/271/EEC focuses on conventional wastewater treatment quality parameters with the aim of avoiding eutrophication and oxygen depletion. Quality requirements for pathogenic contamination and microorganic pollution are not set in this directive. The levels of priority pollutants, which include pesticides, PAHs, phenolic compounds and volatile organic compounds, are currently regulated through the European Water Framework Directives (EC, 2000; EC, 2008).

Several factors, including soil-plant-water interactions (irrigation water quality, plant sensitivity and tolerance, soil characteristics, irrigation management practices, and drainage) are important in crop production. Plant sensitivity is generally a function of a plant’s tolerance to constituents encountered in the root zone or deposited on the foliage, and reclaimed water tends to have higher concentrations of some of these constituents than the groundwater or surface water sources from which the water supply is drawn. Determining the suitability of a given reclaimed water supply for use as a supply of agricultural irrigation is, in part, site-specific, and agronomic investigations are recommended before implementing an agricultural reuse program (US EPA, 2012).

In many grape-growing regions, shortages in water suitable for irrigating grapevines have led to an increased use of poorer-quality waters such as municipal wastewater. However, studies on the effect of treated wastewater for vineyard irrigation are limited, focussing mainly on salinity, nutrient status and plant growth

(Paranychianakis et al, 2004; Paranychianakis et al, 2006; Hepaksoy et al., 2006; Paranychianakis and Angelakis, 2008; Mendoza-Espinoza et al., 2008; Laurenson et al., 2011; Mosse et al., 2013; Netzer et al., 2014). Field measures show that irrigating vineyards with municipal wastewater can increase soil salinity, alter vine nutrient uptake and reduce subsequent wine quality.

According to Paranychianakis et al. (2006), irrigation of potted “Soultanina” grapevines growing in sandy-loam soil with municipal effluent can meet the needs of vines for P, K, Mg and Fe and eliminate the need to apply commercial fertilizers. Enhanced levels of Zn and Cu were also found in the leaves of vines irrigated with recycled water, but their content remained within the acceptable range reported for grapevines. In terms of N, additional fertilizer should generally be applied, in particular during the early development of grapevines when their water requirements are too low to meet the increased N-needs. In the same experimental grapevines, Paranychianakis and Angelakis (2008) reported that the presence of salts in recycled water occurs at levels which may damage the irrigated crops. Furthermore, they suggested that deficit irrigation should not be practiced when irrigating with water with elevated salt concentrations.

Mendoza-Espinosa et al. (2008) in Mexico found that irrigating Cabernet Sauvignon and Merlot vineyards with treated wastewater can cause earlier growth and extension of the growing period compared to well water irrigation. This may be associated with the higher concentration of total nitrogen (nitrate and ammonia) and phosphates in the treated wastewater. Measurements of sugar content in the grapes, pH and titratable solids showed that the biochemical characteristics are not modified by wastewater irrigation. The quality of the products is also not modified by applying treated wastewater.

Another study on silt loam soil in California, USA (Mosse et al., 2013) shows that irrigation of Syrah grapevines with simulated winery wastewater resulted in increased soil salt concentrations. Attributes related to berry and juice quality differed among irrigation treatments, although the majority of these were slight and therefore unlikely to have significant impact on wine quality. In clay loam soil in Israel, Netzer et al. (2014) reported that the yield of Superior table grapes was not affected by the water quality treatments. Nevertheless, the observed trends of Na accumulation in the vines exposed to tertiary treated wastewater may pose a potential risk in subsequent years.

The aim of this study was to assess for the first time the effect of treated domestic wastewater on young Crimson Seedless grapevines with regard to soil characteristics, plant growth and fruit quality. Crimson Seedless is a particularly vigorous, late-season red seedless table grape extensively cultivated in California (Goldspink and Cameron, 2004), Australia (Jayasena and Cameron 2008), Spain (Conesa et al., 2015), Italy (Ferrara et al., 2015), Chile (Lutz et al., 2011) and other parts of the world with a similar climate.

2. Materials & Methods

2.1 Experimental vineyard

The experiment was conducted at the farm of the Technological Educational Institute of Crete in Southern Greece (N 35^o, 19”; E 25^o, 10”). Crimson Seedless grapevines on

1103P rootstock were planted in March 2010 and monitored for three irrigation periods (spring 2010 - autumn 2012). In total, 33 vines were grown using drip irrigation with three different qualities of irrigation water. The experimental plot was divided into three experimental rows of 11 vine stocks each, and was irrigated using different qualities of irrigation water. The first row was irrigated with secondary treated wastewater (STW), the second with tertiary treated wastewater (TTW) and the third with tap water (TW). Each row (treatment) was isolated from the next by a plastic film (1.5 m in depth) to ensure that no wastewater treatment would interfere with the neighbouring ones. After planting, the irrigation line system was laid out and irrigation with different treatments started. After the first germination in May the shoots were underpinned and pruned, leaving two lateral shoots pinned to the first line of coated wire at a height of 80 cm. During the first irrigation period, the shoots were pruned again to produce a linear bilateral shape. In January 2011 the woody shoots were cut. The grapevines were pruned in February in order to develop a bilateral cordon. The trellis system consisted of 1.6 m stakes and three cross-arms (30 cm, 40 cm and 50 cm wide). Sprouting of the new vegetation for the second year of irrigation application was at the beginning of June. The same cultivation methods were applied in the third application period. The vineyard experiment ended at the end of August 2012 after harvesting.

Climate data for the experimental site (rainfall, air temperature and humidity) were obtained from a meteorological station located about 200 m from the vineyard. Average temperature and rainfall values were presented in a previous work (Petousi et al., 2015). The highest monthly mean temperature was 28.4 ^{o}C (July 2012) and the lowest 10.5 ^{o}C (January 2012). Average annual precipitation was 442 mm, 457 mm and 563 mm for 2010, 2011 and 2012 respectively, falling mainly between October to April. Average annual relative humidity (RH) ranged from 53% to 73%.

2.2 Irrigation treatments

The treatments were a) irrigation with STW, b) irrigation with TTW, and c) irrigation with TW. Details about the origin and the quality of the examined water sources are described in a previous article (Petousi et al., 2015). Briefly, STW and TTW contained higher concentrations of organic matter, nutrients, minerals, PAHs and pathogens (BOD: 6-8 mg/l, TN: 61 mg/l for STW and 24 mg/l for TTW, TP: 6 mg/l, K: 47 mg/l, Mg: 75-80 mg/l, Ca: 135-150 mg/l, B: 0.3 mg/l, Σ10PAHs: 0.8 μg/l, total coliforms: 4090 Most Probable Number (MPN)/100ml for STW and 85 MPN/100ml for TTW, E. coli: 3580 MPN/100ml for STW and 23 MPN/100ml for TTW) in comparison with tap water (BOD: 6 mg/l, TN: 5 mg/l, TP: 0.2 mg/l, K: 5 mg/l, Mg: 22 mg/l, Ca: 64 mg/l, B: 0.01 mg/l, Σ10PAHs: not detected, total coliforms: <1 MPN/100ml, E. coli: <1 MPN/100ml).

The water was channelled through a pipeline (25mm diameter) to the grapevines using a drip irrigation system. One drip emitter was used per vine, placed at 0.10 m from the trunk discharging 12 l/h. Grapevines were irrigated twice per week and the volume of water applied was 110 l/vine/week. Irrigation was applied for three months (Jul-Sep) for 2010 and four months (Jun-Sep) for 2011 and 2012, corresponding to a total amount of irrigation water of 660 mm for the first irrigation season and 880 mm for the next two.

2.3 Samplings

Soil and leaves were sampled in May before the beginning of the irrigation period (S1) and at the end of August (S2). Soil samples were collected near the drip emitters from a depth of 0-30 cm, dried before the stones were removed, and sieved through a 2mm screen prior to analysis. Leaf samples (3 per vine) were collected from the 11th - 13th node. Leaves were washed once with tap water and twice with distilled water before being dried at 75 °C and ground before analysis.

2.4 Chemical Analyses

2.4.1 Soil

Air-dried soil samples were sieved through a 2 mm screen before analysis. The values of pH and EC were determined for saturated paste solution using a pH-meter (Crison, GLP 21) and EC-meter (Crison, 525) respectively. The organic matter in soil was determined according to the Walkley-Black acid dichromate digestion method (Walkley, 1946), and total Kjeldahl N using the Kjeldahl digestion method. The sand, silt, and clay content of the soil samples were determined using the Bouyoucos method (Bouyoucos, 1962). Available P was extracted with sodium carbonate and measured by spectrophotometry (Olsen et al. 1954). For the determination of K and Na, soil samples were extracted with ammonium acetate and the extracts analysed by flame photometer (Model 410, Sherwood). The extraction of macro-elements (Mg, Ca) and micro-elements (B, Cu, Zn, Cr, Ni) prior to ICP-MS was performed by acid digestion using a microwave digestor (Multiwave 3000 by Anton Paar) according to the standard EPA 3051a operating procedure. The extraction of PAHs for soil samples was performed according to a modified USEPA method 3541 (USEPA, 1994). Dried samples were transferred into pre-cleaned cellulose extraction thimbles and extracted with 50 ml of acetone-hexane (1:1) by a Soxhlet system (SER148, Velp Scientific) for 2h. Chysene-d_{12} was used as internal standard solution.

2.4.2 Leaves

Leaf samples were collected, washed with distilled water, oven-dried, ground, homogenized and stored. Phosphorus in leaves was determined by the vanado-molybdate yellow method (Allen, 1976). K and Na analysis was carried out by flame photometer (Model 410, Sherwood) after dry-ashing at 550 ^{o}C in an oven and digestion of the ashes with HCl. For nitrogen, macro-elements and heavy metals analysis the same methodology was used as for soils.

2.5 Microbial analysis

Total coliforms and Escherichia coli were determined in irrigation waters, leaf and fruit samples using the IDEXX Quanti-Tray® enumeration procedure with Colilert-18® reagent (APHA, 2005). For leaves and grapes, ten grams of sample were extracted with 100 ml of sterilized Ringer's solution. The extracts were incubated for 18 h at 37 ^{o}C, after which the MPN of total coliforms and E. coli were determined. For soil samples the membrane filtration technique (APHA, 2005) was used to enumerate the same bacterial indicators. Ten grams of soil were added to 95ml of Ringer's solution. The m-Endo LES Agar and HiCrome Coliform Agar were used as culture media for total coliforms and E. coli respectively, while the incubation conditions were 36 ^{o}C for 21h for total coliforms and 37 ^{o}C for 24h for E. coli.

2.6 Growth monitoring

Trunk diameter (20 cm above ground level) and plant height of each vine were measured at the beginning (August 2010) and end of the experiment (Aug 2012). Leaf chlorophyll fluorescence was measured annually (in August) using an OS-30p chlorophyll fluorometer (Opti-Science) after 30 min dark adaptation period. Measurements were taken in the morning (09:00-10:00 local time) at ambient conditions. The ratio between variable and maximal fluorescence (F_v/F_m) was calculated. Leaf soil plant analysis development (SPAD) was also measured during the last irrigation period at the midpoint of leaves with a SPAD-502chlorophyll meter (Konica Minolta). Mature leaves without visible injury symptoms were selected for both analyses.

2.7 Yield and fruit quality

On 5 November 2012 all vines were harvested individually and the fruit production was measured. Total soluble solids content (^{o}Brix) was determined with PAL-1 pocket refractometer (Atago) in a subsample of 20 berries per treatment. Grape juice was used for the measurement of titratable acidity expressed as tartaric acid per liter. Color parameters were measured in a subsample of 150 berries per treatment using a CR-300 colorimeter (Konica Minolta). This technique is widely used for measuring grape color (Faci et al., 2014). The colorimeter uses three parameters: L*, a* and b*. In addition, two derived functions were computed from the recorded L*, a* and b* values as follows:

$$\text{Chroma:} \quad C = \left[(a)^2 + (b)^2\right]^{1/2}$$

$$\text{Hue angle:} \quad H = \tan^{-1}(b/a)$$

Finally, Color Index of Red Grapes (CIRG) was calculated according to Carreno et al. (1995) as follows:

$$\text{CIRG}=\frac{180-H}{C+L}$$

2.8 Data Analysis

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

3. Results & Discussion

3.1 Plant growth

Vines were healthy without any visible symptoms during the experimental period for all irrigation treatments applied. Vine growth was found not significantly different ($p>0.05$) for plants irrigated with STW and TTW compared to plants irrigated with TW. Specifically, trunk diameter increased from 18-19 mm in 2010 to 33-35mm in 2012 for all treatments. Similarly, pruning weight per vine was found 0.06-0.09 kg in 2010 and 0.9-1.2 kg in 2012 for all treatments. There is no data from previous studies on the effect of treated wastewater on trunk diameter and pruning weight. Weekly shoot elongation of Cabernet Sauvignon and Merlot grapevines was determined by Mendoza-Espinosa et al. (2008). In contrast to the present study, they found that plants irrigated with treated wastewater grew faster and for a longer period than plants irrigated with groundwater.

Leaf SPAD values are presented in Figure 1. Vines irrigated with treated wastewater had significantly higher SPAD values compared to vines irrigated with TW. Leaf SPAD value varied between 30-33 % during August 2012 for plants irrigated with STW and TTW, while leaf SPAD value for plants irrigated with TW was 24%. Similar leaf SPAD values ranging between 36-42% were reported by Ferrara and Brunetti (2010) for the Italia table grape. Highly significant correlations between SPAD values and nitrogen content in the leaves were also reported by Ferrara and Brunetti (2008). The absence of nutrients and minerals in TW may result in a decrease in leaf SPAD values.

Maximum quantum yield (Fv/Fm) of vines was about 0.81 after three irrigation periods for all irrigation treatments. Pech et al. (2013) reported a mean decrease of Fv/Fm ratio for Shiraz, 140 Ruggeri and K51-32 Lider vine genotypes from 0.80 (control) to 0.71 and 0.76 for irrigation water containing boron (7.2 mg/l) and boron plus salinity (4.8 mS/cm) respectively. However, during this study boron and salinity levels in treated wastewater (~0.3 mg/l and ~2.2 mS/cm respectively) were well below these values.

3.2 Soil

Soil of the experimental site was classified as clay loam (40.0% sand, 34.1 % silt and 25.9% clay) with 19.2 g/kg organic matter and a pH of 7.6. The chemical composition of the soil at the beginning and end of the experiment is presented in Table 1. Irrigation with treated wastewater had no effect on soil concentrations. Heavy metal and PAH concentrations in soil are similar for all irrigation treatments. Σ10PAH concentrations ranged from 363 μg/kg to 374 μg/kg at the end of the experiment, while almost the same concentrations (359-389 μg/kg) were observed at the beginning of the experiment. Phenanthrene, Fluoranthene and Pyrene (23%, 24% and 34% of total PAHs, respectively) were the most abundant PAHs. These three compounds accounted for 81% of total examined PAHs. The vineyard was in the Heraklion urban area (population 230,000) near a major road junction on the island of Crete. As a result, atmospheric deposition of PAHs was the major source of soil contamination. A similar finding was reported in the area from a previous study on the effect of wastewater irrigation in olive trees (Petousi et al. 2015). In addition, there are many reports worldwide on the occurrence of PAHs in soils. For example, Wang et al. (2015) found mean total concentrations of 16 PAHs (Σ16PAHs) ranging from 1,060 μg/kg (rural areas) to 3,300 μg/kg (urban areas) in Nanjing, China. Surface natural soils in Piedmont, Italy had a Σ16PAHs mean value of 160 μg/kg and a very wide range of concentrations, from 80 to 601 μg/kg (Fabietti et al. 2010). Morillo et al. (2008) reported Σ15PAHs concentrations ranging from 89 μg/kg to 4004 μg/kg in urban soils in Sevilla, Spain.

The wastewater used in the experiment came from the wastewater treatment plant of Heraklion, one of the largest facilities in Greece, receiving mainly domestic sewage from a combined system with limited industrial input. Results show that PAH concentrations in domestic wastewater in Greece (with limited industrial activity) are expected to be far below limits suggested by EU Directive 2008/105/EC (EC 2008) and Greek Law (JMD 145116/20110). So the risk of soil pollution by reclaimed water is significantly lower than that by airborne PAHs.

No significant difference was observed in Cu concentration in vineyard soil after the implementation of irrigation for all examined treatments, as expected (nonappearance of Cu in irrigation waters). Mean values ranged between 29-41 mg/kg, significantly lower than the legal limit (140 mg/kg) set by EU Council Directive 86/278/EC (EC 1986) for Cu concentrations in agricultural soils. It is known that the use of Cu-fungicides over the years increases the Cu content of many vineyard soils, with Cu amounts ranging from 100 up to 1500 mg/kg (Chaignon et al. 2003, Mirlean et al. 2007, Chopin et al. 2008, Fernandez-Calvino et al. 2008). However, in this work a newly established vineyard was used with no long-term impact from Cu-fungicides. Increased concentrations of Zn in soils were observed after the implementation of three year irrigation, especially with the use of treated wastewater. Zn content of agricultural soils increased mainly due to the addition of commercial fertilizers, liming materials or manures (Senesi et al., 1999). Moreover, pesticides and fungicides containing Zn also contribute to its presence in vineyard soils (Weingerl and Kerin 2000). During the present study, no fertilizers or manures were added but pesticides and fungicides were applied in order to control pests (no tilling applied) and fungi (downy mildew and powdery mildew), respectively. In addition, the occurrence of Zn at a concentration of about 7 μg/l in STW and TTW resulted in the increase of Zn in vineyard soil irrigated with STW and TTW.

Among the metals analyzed, nickel presented the highest content. Ni concentration was about 105 mg/kg in the vineyard. Similar high mean concentrations of Ni were observed in the Thriassio Plain (103 mg/kg), Greece by Gasparatos et al. (2015) and the Argolida Basin (147 mg/kg), Greece by Kelepertzis (2014). Irrigation resulted in a decrease of Ni concentration to levels of about 70-80 mg/kg. Ni was absent from all irrigation waters. So irrigation may result in the mobilization of Ni in the deepest soil layers. Agnieszka and Barbara (2012) reported that the mobility of Ni in soils was higher than that of Cr and V. Mean calcium concentrations were not significantly different between irrigation treatments but increased at the end of the experiment. Sodium levels in soils irrigated with wastewater increased at the end of the irrigation period (August) and decreased until the following season (Figure 2). Rainfall between autumn and spring was probably the main cause of the movement of sodium into lower soil layers. Netzer et al. (2014) examined the effect of wastewater irrigation on table grape vineyards, focussing on sodium accumulation in soil and plant. Their results showed that no differences were yet apparent after the first irrigation season. In the same study, significant or nonsignificant differences were established over the next years, depending on the quantity of irrigation water applied (increased differences) and the quantity of rainfall (decreased differences).

3.3 Leaf content

The leaf nutrient status of vines irrigated with three different qualities of irrigation water presented no significant differences (Table 2). A slight increase of Mg, Ca, Cu, and Zn concentration was observed in leaves of grapevines irrigated with STW and TTW compared to grapevines irrigated with TW. It is difficult to obtain reliable references for grapevines due to the wide range of varieties, genetics, rootstocks, growing techniques, water regime or simply the variation across different climates and soils (Failla et al. 1997). Nicolas et al. (2014) examined the nutrient status of Crimson Seedless in a vineyard in Murcia, Spain. Comparable results were found, with slightly higher values for nitrogen, potassium and copper (2.9%, 1.2% and 39 mg/kg respectively) and slightly lower values for magnesium and zinc (0.4% and 12 mg/kg respectively). Hirzel et al. (2017) reported that leafs from vines (cv. Cabernet

Sauvignon and cv. Sauvignon blanc) irrigated with winery wastewater contained more Na and Mg in comparison with leafs from vines irrigated with well water. However, the values recorded were still far below the toxicity level.

3.4 Pathogens

The concentrations of total coliforms and E. coli in soil, leaves and fruits of the experimental vineyard are shown in Table 3. Since table grapes are eaten raw or as a component of fresh ready-to-eat fruit salads, contaminated grapes might pose a health problem to the consumer. Results show that soil irrigated with STW was highly contaminated by total coliforms and E. coli. According to Oron et al. (2001), the organic matter content in the soil is very important factor affecting pathogen survival. When the organic matter content is above 8.5 g/kg a significant concentration of pathogens may be observed. However, the examined pathogens were not detected at all in grapes irrigated with TTW and TW. Faecal and total coliforms were also not present in the Cabernet Sauvignon and Merlot grapevines irrigated with reclaimed wastewater in a previous study in Mexico (Mendoza-Espinoza 2008).

3.5 Fruit quality

Grape yield and colour characteristics are presented in Table 4. Production of grapes per vine fluctuated for every irrigation treatment as the vineyard was still too young (not in full production). Higher grape production was observed for vines irrigated with TTW and lower for vines irrigated with TW. However, no statistically significant differences were observed ($P<0.05$).

In contrast, significant differences were observed in colour characteristics. Grapes from vines irrigated with STW were less red than the grapes irrigated with TTW and TW according to CIRG values. It is known that Crimson Seedless grapes may fail to achieve the desired level of red colour, in part due to high temperatures which inhibit the accumulation of anthocyanins (Spayd et al. 2002), the class of pigments that impart red colour to grape berries (Peppi et al. 2006). According to this study the application of low-quality treated wastewater, such as STW, also seems to inhibit the accumulation of anthocyanins. However, long-term irrigation should be examined in the future to have more clear results about the effect on colour characteristics.

Quality characteristics of grape juice are presented in Table 5. A correlation between grape production rate and ^{o}Brix values was observed (a higher production rate resulted in a lower ^{o}Brix). High °Brix has been associated with high consumer acceptance in different fruits, such as cherries (Crisosto et al. 2003), peaches (Robertson et al. 1988) and grape cultivars (Sonego et al. 2002). Jayasena and Cameron (2008) found that consumer acceptance of Crimson Seedless in Australia increased from 55 to 84% with the increase in °Brix from 16 to 20, whereas berries with °Brix values higher than 20 could not achieve better consumer acceptance. In the same study, they stated that ^{o}Brix/TA ratio is a better indicator (than °Brix or TA) of consumer acceptability, suggesting that the best time to harvest Crimson Seedless is when the ^{o}Brix/TA ratio is 35-40.

The application of STW and TTW to vines had no significant effect on the mineral concentration in grape juice compared to vines irrigated with TW (Table 5). Peuke (2009) examined the nutrient composition of leaves and grape juice (cv Riesling) as affected by soil and nitrogen fertilization. He found N, P, K, Ca, Mg and B concentrations in grape juice of about 0.7 g/l, 0.3 g/l, 1.0 g/l, 0.2 g/l, 0.1 g/l and 3.6 mg/l respectively. Mineral analysis of soils, leaves and juice revealed no consistent relationships. PAHs were not detected in grape juice for any examined treatments, indicating that there is no risk to human health. In general, food crops take up carcinogenic PAHs through roots from the soil and accumulate them in their tissues (Jian et al. 2004). This food chain route is considered to be one of the most serious concerns and accounts for 90% of ingestion of contaminants into the human body (Martorell et al. 2011). A number of previous works show accumulation of PAHs in crops irrigated with wastewater, such as lettuce (Khan et al. 2008), radish (Petousi et al. 2014) and spinach (Khan and Cao 2014). On the other hand, there are no reports on the fate of PAHs in vineyards irrigated with treated wastewater.

4. Conclusions

Many grape-producing countries around the world have significant levels of water scarcity, making it imperative to exploit wastewater as an alternative irrigation source. However, the use of treated wastewater may affect the agricultural environment, as well as the health of farmers and consumers. For this reason, further studies on the effect of treated wastewater in grapevines are required in order to guarantee safe reuse for the environment and public health. During this study, the effect of secondary and tertiary treated domestic wastewater on soil characteristics, plant growth and fruit quality of Crimson Seedless young grapevines was examined. The application of TTW did not appear to have any negative effect on the grapevines, while in some cases it improved growth parameters of the vines including leaf chlorophyll concentration (SPAD value) and yield. In addition, the results show that secondary treated wastewater had also no negative impact on vine growth. Heavy metals and PAHs occurred in wastewater but did not appear to have an adverse effect on soil quality. The application of both types of treated wastewater had no significant effect on the soil and leaf content with the exception of pathogens. Specifically, soil irrigated with STW was found to be highly contaminated by total coliforms and E. coli. Overall, it is concluded that high-quality treated wastewater such as tertiary treated wastewater can be used for the establishment of grapevines from planting to year 3. The risk of microbial and chemical contamination of the vineyard (soil-plant-fruit system) is limited, guaranteeing safe reuse for the environment and public health.

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Table 1. Chemical composition of soil in the vineyard at the beginning of the experiment (April 2010) and at the end of the experiment (August 2012)

Parameter	**2010**			**2012**		
	STW	**TTW**	**TW**	**STW**	**TTW**	**TW**
pH	7.4 ± 0.2	7.7 ± 0.3	7.3 ± 0.4	7.5 ± 0.1	7.4 ± 0.1	7.4 ± 0.1
N (g/kg)	0.5 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.8 ± 0.2	0.6 ± 0.1	0.5 ± 0.1
P (mg/kg)	7.1 ± 0.5	7.3 ± 0.2	7.5 ± 0.3	6.7 ± 0.2	7.1 ± 0.5	7.0 ± 0.4
K (mg/kg)	2.3 ± 0.3	2.4 ± 0.3	2.1 ± 0.3	2.7 ± 0.3	2.2 ± 0.2	2.3 ± 0.5
Mg (g/kg)	7.4 ± 0.9	7.0 ± 0.6	7.5 ± 0.8	10.2 ± 0.9	8.4 ± 0.6	9.7 ± 0.5
Ca (g/kg)	132 ± 21	148 ± 29	141 ± 25	308 ± 41	272 ± 35	311 ± 18
B (mg/kg)	n.d	n.d	n.d	12 ± 5	6 ± 2	7 ± 1
Cu (mg/kg)	35 ± 5	29 ± 8	32 ± 6	41 ± 1	30 ± 1	29 ± 1
Ni (mg/kg)	105 ± 12	104 ± 11	107 ± 10	81 ± 2	73 ± 2	76 ± 1
Zn (mg/kg)	36 ± 8	27 ± 5	25 ± 9	54 ± 2	54 ± 14	46 ± 7
Cr (mg/kg)	57 ± 9	45 ± 14	49 ± 10	52 ± 3	45 ± 4	50 ± 5
Σ10PAHs (μg/kg)	361 ± 54	389 ± 63	359 ± 62	374 ± 29	368 ± 72	363 ± 59

n.d: not detected

Table 2. Chemical composition of leaves at the time of grape veraison for the 3rd irrigation period.

	STW	**TTW**	**TW**
N (%)	1.99 ± 0.08	1.96 ± 0.05	1.97 ± 0.08
P (%)	0.11 ± 0.02	0.11 ± 0.02	0.13 ± 0.03
K (%)	0.78 ± 0.18	0.74 ± 0.21	0.80 ± 0.22
Na (%)	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.01
Mg (%)	0.90 ± 0.04	0.71 ± 0.02	0.66 ± 0.02
Ca (%)	4.38 ± 0.06	4.66 ± 0.02	3.95 ± 0.02
B (mg/kg)	53.5 ± 7.2	66.2 ± 3.3	53.5 ± 9.6
Cu (mg/kg)	8.2 ± 6.1	6.8 ± 2.6	6.2 ± 0.2
Ni (mg/kg)	3.6 ± 2.3	3.6 ± 1.4	3.4 ± 0.6
Zn (mg/kg)	21.7 ± 5.2	20.5 ± 17.0	17.8 ± 8.3

Table 3. Total coliforms and E.coli on soil, leaves and fruit in the vineyard

Irrigation treatment	**Total Coliforms**			**E.coli**		
	Soil[a]	Leaves	Fruit	Soil[a]	Leaves	Fruit
STW	3,900-12,000	400-3,000	0-200	10-122	0	0
TTW	3.500-10,000	0-132	0	0-40	0	0
TW	2.000-4.300	0-485	0	0	0	0

[a] CFU/g dry weight for soil and MPN/g fresh weight for leaves and fruit.

Table 4. Grape yield and colour characteristics at the end of experiment

	STW	**TTW**	**TW**
Production (kg/vine)	0.44 ± 0.14	0.91 ± 0.94	0.38 ± 0.26
Color			
L^*	36.0 ± 6.6[a]	33.5 ± 5.0[b]	33.9 ± 5.8[b]
a^*	10.0 ± 5.7[a]	9.8 ± 3.2[a]	8.8 ± 3.7[a]
b^*	3.7 ± 5.2[a]	0.8 ± 2.9[b]	1.1 ± 4.3[b]
CIRG	3.9[a]	4.2[b]	4.2[b]

a, b: In each row, mean values followed by a different letter are significantly different ($p<0.05$).

Table 5. Quality characteristics of grape juice

	STW	TTW	TW
^{o}Brix	20.4 ± 2.0	19.4 ± 3.2	21.8 ± 1.4
TA (%)	0.6 ± 0.1	0.8 ± 0.3	0.5 ± 0.1
^{o}Brix/TA ratio	33 ± 3	25 ± 4	46 ± 2
N (g/l)	0.7 ± 0.2	0.7 ± 0.1	0.6 ± 0.2
P (g/l)	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
K (g/l)	1.6 ± 0.1	1.5 ± 0.2	1.5 ± 0.2
Mg (mg/l)	186 ± 10	144 ± 7	147 ± 17
Ca (mg/l)	96 ± 14	92 ± 8	90 ± 1
B (mg/l)	8.2 ± 0.1	10.9 ± 4.8	11.9 ± 0.6
Cu (mg/l)	0.4 ± 0.1	0.3 ± 0.1	0.2 ± 0.1
Zn (mg/l)	1.1± 0.2	1.6 ± 0.2	1.6 ± 0.4
Ni (μg/l)	4.6 ± 2.1	4.8 ± 1.3	4.8 ± 2.2
Cr (μg/l)	n.d	n.d	n.d
Σ10PAHs (μg/l)	n.d	n.d	n.d

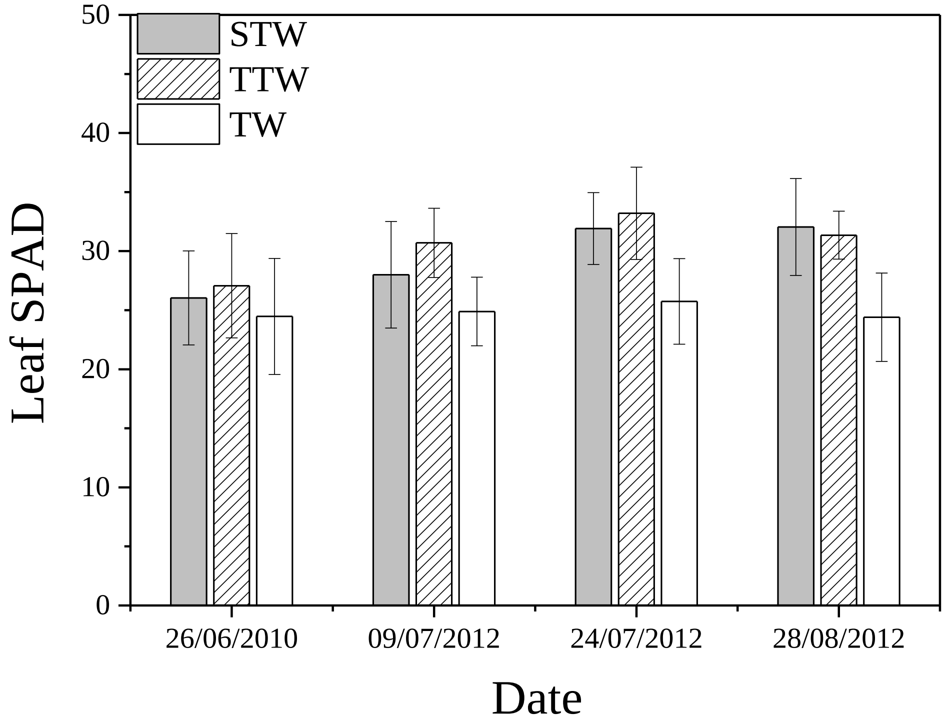

Figure 1. Effect of water irrigation treatments on the leaf SPAD of grapevines. Data are means (n=11) ± SD (vertical bars).

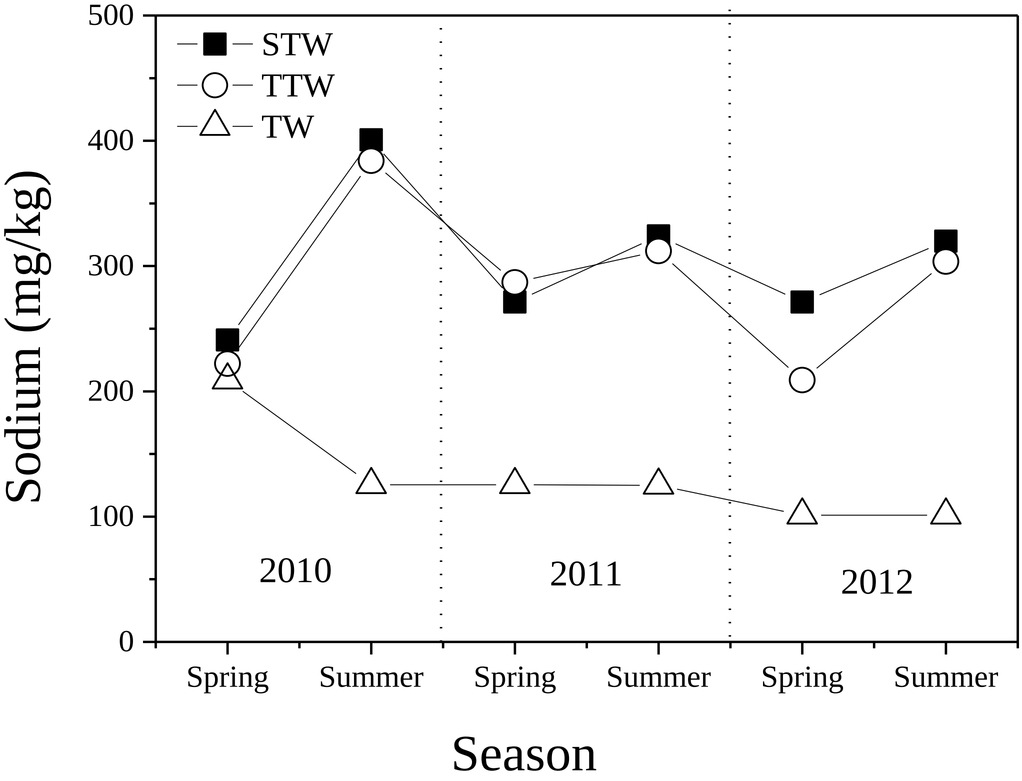

Figure 2. Sodium seasonal fluctuations in the soil in the vineyard during the experimental period (Spring 2010 –Autumn 2012).