Reuse of treated domestic wastewater on Mediterranean agricultural crops

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Abstract

Wastewater is considered as a new and unconventional source of water for agricultural production in many arid and semi-arid countries worldwide. As a result, careful monitoring of soil and plants for a range of parameters including salts, nutrients, micro-elements, heavy metals, toxic pollutants and pathogens is required.

During this study, the application of three different qualities of treated domestic wastewater on four agricultural crops was examined in Crete, Greece: a typical Mediterranean semi-arid area. Primary treated (low quality), secondary treated (medium quality) and tertiary treated (high quality) wastewater were applied to a) olive trees, b) grapevines, c) radishes and d) carnations. Tap water and fertilized tap water (controls) were also applied in all the above agricultural crops for comparison with treated wastewaters.

In general, increased concentrations of sodium, phosphorus, potassium and nitrogen in soils could be observed after wastewater irrigation. High salinity and boron concentrations in treated wastewater had no adverse effect on the examined cultivations. Low quality treated wastewater should not be used for irrigation mainly due to high levels of pathogens. In addition, they were found to a) to inhibit grapevine growth b) to degrade grape quality characteristics, and c) to accumulate polycyclic aromatic hydrocarbons (PAHs) in soil and radish roots.

On the other hand, high quality treated wastewater had no negative effect on soil, plant growth, health safety and fruit quality of all the examined agricultural crops. Furthermore, the application of tertiary treated wastewater a) improved leaf chlorophyll concentration and yield of grapevines, b) improved yield and fruit quality characteristics of radishes and c) improved plant growth of carnations. Finally, olive trees were found to be less sensitive to irrigation water quality suggesting that even medium-quality wastewater could be safely applied.

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Preface

This thesis was produced as part of the requirements for a degree of Doctor of Philosophy (PhD) at the University of Leeds.

The thesis it is divided into five different chapters, where chapter 1 and chapter 2 contain a general introduction and a literature review with regard to wastewater treatment and reuse. Chapter 3 contains Material and Methods used for the monitoring of agricultural crops, Chapter 4 deals with the results obtained from the application of wastewater on agricultural crops and the discussion about it. Chapter 5 contains the overall conclusions from the study and recommendations for further research.

The experiments were conducted on the island of Crete in Greece. Three different qualities of treated wastewater were applied in a broad-spectrum of Mediterranean crops including a) olive trees, b) grapevines, c) vegetables (radishes) and d) flowers (carnations) and compared with the application of tap water and fertilized tap water.

The effect of wastewater on a) plants, b) soil, c) health and safety (pathogens) and d) fruit quality was examined in comparison with tap water and fertilized tap water.

Parts of the work presented in this thesis have previously been published in or submitted to international scientific journals. A list of the relevant references in chronological order is given below.

1. **Petousi, I.,** Stavroulaki, N., Fountoulakis, M.S., Papadimitriou, M., Stentiford, E.I., Manios, T. (2013) Application of treated wastewater for cultivations of carnations. *Water Practice Technology* **8**, 457-460.

2. **Petousi I.,** Fountoulakis M.S, Tzortzakis N., Dokianakis, S., Stentiford, I., Manios T. (2014) Occurrence of micro-pollutants in a soil-radish system irrigated with several types of treated domestic wastewater. *Water, Air & Soil Pollution* **225**, 1791-1798.

3. **Petousi I.,** Fountoulakis M.S, Saru M.L., Nikolaidis N., Fletcher, L., Stentiford, I., Manios T. (2015) Effects of reclaimed wastewater irrigation on olive (*Olea europaea* L. cv. 'Koroneiki') trees. *Agric. Water Manage.* **160**, 33-40.

4. **Petousi I.,** Daskalakis G., Fountoulakis M.S, Lydakis D., Fletcher, L., Stentiford, I., Manios T. (2014) Application of reclaimed wastewater for cultivation of 'Crimson seedless' grapevines. *Agric. Water Manage.* In preparation

Chapter 1

Introduction

1.1 Introduction

Recycling treated wastewater is becoming ever more important for two reasons. The first is that the discharge of effluent into surface waters is now becoming more difficult, as treatment standards become more stringent in order to protect aquatic organisms and water users downstream of discharge sites. The second reason is that wastewater discharges are an important water source that can be used for various beneficial purposes, particularly in areas with scarce water resources.

Typical treated wastewater applications include: a) irrigation (of crops and green belt areas), b) industrial use, c) aquifer enrichment, d) recreational and environmental applications, e) aquaculture and f) indirect potable water (Paranychianakis *et al.*, 2009).

Given the farming practices applied to irrigated fields, and the potential risks of environmental pollution due to the reuse of treated urban wastewater in crop cultivation, this study investigated the effect of irrigation with treated domestic wastewater of three different qualities on olive, vine, carnation and radish cultivation.

1.2 Reclaimed water for irrigation purposes

Although crop irrigation has been known for millennia, the quality of irrigation water, as a basic parameter of agricultural development, was only recognised during the last century. Designing an effective irrigation project utilising treated wastewater depends primarily on the basic aim of the project, i.e. whether this is to provide crops with water, or for further treatment of the wastewater (Tzanakakis *et al.*, 2003). Over recent decades, there has been increased interest in urban wastewater reuse in agriculture due to increased demand for irrigation water. Population growth, increased water use per capita, and the demands of industry and agriculture, all put pressure on us to find immediate solutions in the water resources sector. Wastewater treatment provides water of satisfactory quality which can be used beneficially (Asano, 1998).

Irrigation of agricultural and other land is the largest mass use of water, particularly in arid and semi-arid areas. For instance, in the US in 1985, 190 billion m^3 of water were used to irrigate 23 million hectares and irrigation represents 34% of the total water use. Nine US water regions, led by California, consumed 91% of the total irrigation water used in 1980 and 1985 (Solley *et al*., 1988). Wastewater reuse for irrigation purposes across a wide range of crops was successful, and has reportedly resulted in a 10-30% increase in yields (Asano, 1998).

In California, over 70% of treated urban wastewater is used for irrigation (Special Secretariat for Water, 2013). Crop irrigation is the oldest and commonest category of treated wastewater reuse (Agelakis and Koutsoyiannis, 2003; US EPA, 2004). In Israel, over 80% of treated urban wastewater is applied in agriculture, while approximately 70% of the water used in agriculture is reclaimed wastewater. The main types of crop irrigated using treated wastewater are trees, pastureland, arable and fibre crops, which is due to the relatively low public health risk. Irrigation with wastewater discharges can also be applied to food crops that are consumed raw, as long as the discharges have been subjected to advanced treatment and meet the necessary standards (Asano *et al*., 2006).

The main benefits include (Paranychianakis et al., 2009):

a) Control of surface water pollution.

b) Preservation of natural water sources for future use.

c) Increased soil productivity, as wastewater discharges not only supply water to the soil but also contain the basic (nitrogen, phosphorus and potassium) nutrients required for crop development.

d) Financial savings for producers, since they use less commercial fertiliser.

e) Improvement of the physical soil characteristics through the addition of organic material. Retaining soil humus also prevents erosion

In Israel, of the total water consumption (2.115 billion m^3) in 1987, 73.1% represents agricultural use. In Greece, agricultural use is estimated at 83.7% of total water consumption, which was approximately 5.037 billion m^3 in 1981, rising by 40% in 1995 (Agelakis et al., 1999). In many arid and semi-arid areas water is considered a rare natural resource, therefore scientists attempt to utilise every available source of water which can be considered financially viable and effective for development. Although the use of high-quality water is rare, low-quality water can be used in agriculture. In Japan, as opposed to other countries in arid and semi-arid regions, the main categories of urban wastewater reuse are environmental upgrade, cleaning toilets, industrial use and snowmaking.

Wastewater irrigation is often considered to be a wastewater treatment method known as soil treatment. From this point of view, wastewater treatment or discharge in the soil is a method of controlled soil application, achieving a significant degree of treatment via the physical, chemical and biological processes inherent in the plant-soil-water system.

Currently, water quality criteria established for crop irrigation with clean water are the best available criteria for wastewater reuse in irrigation. However, since wastewater contains additional substances not usually found, or only in insignificant amounts, in natural water sources, special standards for wastewater reuse must be set out.

1.3 Environmental risk

While the reuse potential for wastewater in irrigation is huge, 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 the fate of them 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 that irrigation with wastewater has on salinity and heavy metals in soils and groundwater (Khan et al., 2008, Leal et al., 2009, Pereira et al., 2012 and 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 concern (Calderón-Preciado et al., 2011, Chung et al., 2008 and Zhou et al., 2013).

In addition, soluble constituents present in the treated wastewater could be at levels that possibly can 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 (Moyen 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).

1.4 Health risk

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 tissue vegetal body parts; implicating 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 part of plants (Mapanda et al., 2005). Previous works approved 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).

1.5 Drivers for wastewater reuse

Nowadays, many countries face significant problems of water scarcity and quality deterioration. One of the first reasons for the observed water scarcity is that the fraction of water available for the human consumption, in rivers and streams, lakes, reservoirs and groundwater aquifers, is not distributed uniformly around the world (Shiklomanov, 1993). Simultaneously, the increasing need of water resources is a consequence of the demographic growth, the economic development and the 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, 2006a). Besides the reduction in the use and abstraction of freshwater, wastewater reuse will also contribute to reduce the discharge of effluents into freshwater ecosystems (Bixio et al., 2006 and Toze, 2006). This scenario makes wastewater an increasingly valuable resource rather than a waste product. Indeed, irrigation with treated wastewater is already implemented, mainly for agriculture and landscaping, in countries such as France, Italy, Spain, Cyprus, Malta, Israel, Jordan or the USA (Aquarec Project, 2006, EMWIS, 2007, EPA, 2012, Kalavrouziotis et al., 2013, Ndour et al., 2008 and Pedrero et al., 2010).

According to the World Health Organisation, international statutory framework for wastewater reuse, protection measures come into four main categories: a) wastewater treatment, b) limiting irrigated crop types, c) selection of irrigation method, and d) control of human exposure to pathogenic microorganisms. Some countries have extra regulations in place to protect human health. For example in the USA, the California state Code of Regulations (EPA, 2012) attempts to minimise the theoretical risks of wastewater reuse, based on the possibility of human exposure to the reused wastewater. Wastewater reuse is divided into two categories, restricted and unrestricted, depending upon strict criteria necessitating tertiary treatment. The US Environmental Protection Agency (EPA, 2012) guidelines for the protection of the environment cover basic aspects of wastewater reuse, and include proposed treatment processes, quality limits of reclaimed water, control frequency, safety distances and other control measures.

Europe lacks integrated legislation as there is currently no single European directive on wastewater reuse. Efforts are, however, being made to enact strict criteria and limits along the lines of the California regulations. In France, regulations have been drawn up based on the EPA criteria, supplemented by strict regulations on the protection of ground and surface waters. In Italy there is a strict legislative framework regarding the presence of pathogenic microorganisms, varying from region to region. Spain has a combination of strict national legislation and more relaxed local criteria. In Cyprus, quality criteria have been implemented in combination with the EPA Guidelines and the California Code of Regulations, adapted to the particular conditions of the island.

In Greece, there have been improvements to the legislative framework in recent years, in an attempt to cover the gaps caused by the lack of an integrated European legislation, to promote the exploitation of treated wastewater, and to safeguard public health by the implementation of appropriate rules and criteria. The Greek legislative framework (JMD 145116/2011) divides wastewater reuse for irrigation into restricted and unrestricted irrigation. Restricted irrigation is applied to crops that are consumed after processing (e.g. after thermal treatment), that are not intended for consumption, or that do not come into contact with the soil. Wastewater treatment should be at least secondary with disinfection, with the following concentration limits: *E. coli* <200 EC/100ml, BOD <25mg/l, SS <35 mg/l. In unrestricted irrigation, all types of crops can be irrigated using various methods, including sprinkling and there are no irrigation restrictions. Permissible *E. coli* concentration limits are up to 5 EC/100ml for 80% of samples, with BOD up to 10ml for 80% of samples, SS up to 10mg/l for 80% of samples, and turbidity <2 ntu, with the application of secondary and tertiary treatment and disinfection.

In addition for both types of irrigation maximum permitted concentrations was defined for 19 heavy metals (Cu, Ni, Cr, Zn, Hg etc) and elements (B, Al, Fe etc) as well as 41 emerging organic pollutants (fluoranthene, benzo(a)pyerene, nonylphenol, dichloromethane, diuron, Aldrin, isoproturon etc). The maximum permitted concentrations according to Greek regulation and several international guidelines for both types of irrigation are set out in Table 1.1.

Table 1.1 Maximum permitted concentrations of several parameters for treated wastewater used for irrigation, according to different international guidelines.

	US EPA (2012)[1]		WHO (2006)[2]		Italy (2003)[3]	Spain (2007)[4]		France (2010)[5]		Greece (2011)[6]	
	UR	R	UR	R	ND	UR	R	UR	R	UR	R
pH	6-9	6-9	-	-	6-9.5	-	-	-	-	-	-
NTU	2	-	-	-	-	10	-	-	-	2	-
SS (mg/l)	30	-	-	-	10	20	35	15	-	10	35
BOD (mg/l)	10	10	-	-	20	-	-	-	-	10	25
TN (mg/l)	-	-	-	-	15	-	-	-	-	15	45
Faecal Coliforms (CFU/100ml)	0	200	-	-	-	-	-	4	2-3	-	-
E. coli (CFU/100ml)	-	-	10^3	-	100	100	10^3	250	10^4	5	200
Nematode eggs (no./l)	-	-	1	1	-	-	-	-	-	-	-
Copper (mg/l)	0.2	0.2	0.2	0.2	1.0	0.2	0.2	-	-	0.2	0.2
Nickel (mg/l)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	-	-	0.2	0.2
Zinc (mg/l)	2.0	2.0	2.0	2.0	0.5	-	-	-	-	2.0	2.0
Boron (mg/l)	0.75	0.75	-	-	1.0	0.5	0.5	-	-	2.0	2.0
Fluoranthene (μg/l)	-	-	-	-	-	-	-	-	-	1.0	1.0
Benzo(a)pyrene (μg/l)	-	-	-	-	0,01	-	-	-	-	0.1	0.1

UR, unrestricted irrigation; R, restricted irrigation; NTU, Nephelometric Turbidity Unit; SS, suspended solids; BOD, biological oxygen demand; TN, total nitrogen; ND. no determination. [1]US EPA, 2012, [2]WHO, 2006, [3]Decreto Ministeriale 15/2003, [4]Real Decreto 1620/2007, [5]NOR-SASP1013629A, 2010, [6]JMD 145116/2011

Furthermore, Greek law (JMD 145116/2011) contains guidelines for interpretation of water quality for irrigation (Table 1.2). These guidelines were adapted from US Envrionmental Protection Agency (US EPA, 2012) and Food and Agriculture Organization (FAO, 1984). 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.

Table 1.2 Guidelines for interpretation of water quality for irrigation (Greek law 354/2011)

Potential irrigation problem	Unit	Degree of restriction for irrigation		
		None	Slight to Moderate	Severe
Salinity (affects crop water availability)				
EC_w[(1)]	dS/m	<0,7	0,7-3,0	>3,0
or				
TDS	mg/l	<450	450-2000	>2000
Infiltration affects infiltration rate of water into the soil; evaluate using ECw and SAR together				
SAR[(2)]=0-3 and EC_W=		>0,7	0,7-0,2	<0,2
3-6		>1,2	1,2-0,3	<0,3
6-12		>1,9	1,9-0,5	<0,5
12-20		>2,9	2,9-1,3	<1,3
20-40		>5,0	5,0-2,9	<2,9
Specific Ion Toxicity				
Sodium (Na)				
Surface irrigation	SAR	<3	3-9	>9
Sprinkler irrigation	mg/l	≤70	>70	
Chloride (Cl)				
Surface irrigation	mg/l	<140	140-350	>350
Sprinkler irrigation	mg/l	≤100	>100	
Miscellaneous Effects (affects susceptible crops)				
Nitrate (NO_3-N)	mg/l	<5	5-30	>30
Bicarbonate HCO_3	mg/l	<90	90-500	>500
pH	Normal Range 6.5-8.5			

[1]EC:Electrical Conductivity, [2]SAR: Sodium adsorption ratio

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).

1.6 Aims and Objectives

The aim of this work was to assess the effect of treated domestic wastewater in four Mediterranean crops (olives, grapes, radishes and carnations) on soil characteristics, plant growth and fruit quality. In order to achieve this overall aim a number of study objectives were developed as follows:

- To determine the effect of treated wastewaters irrigation on soils chemical properties including salts, elements, heavy metals and polycyclic aromatic hydrocarbons
- To determine the effect of treated wastewaters irrigation on plants growth, leaf content, photosynthesis and chlorophyll fluorescence
- To determine the effect of treated wastewaters irrigation on yield and quality of agricultural products including size, colour and content of fruit
- To determine the fate of pathogens (total coliforms and *E.coli*) on soil-plants systems irrigated with treated wastewaters

Chapter 2

Literature Review

2.1 Wastewater quality parameters with agronomic significance

Important agricultural water quality parameters include a number of specific properties of water that are relevant in relation to the yield and quality of crops, maintenance of soil productivity, and protection of the environment (Lazarova, 2005). The water quality of treated wastewater depends to a great extent on the quality of the municipal water supply, the nature of the wastes added during use, and the degree of treatment the wastewater has received. In contrast, the water characteristics of importance in agricultural and landscape irrigation are specific chemical elements and compounds that affect plant growth or soil permeability. Not all these characteristics are measured or reported by wastewater treatment agencies as part of their routine water quality monitoring programme (Pedrero *et al*., 2010). When obtaining data to evaluate a treated wastewater irrigation system, it is often necessary to sample and analyze the wastewater for those constituents that define the suitability of the water for agricultural and landscape irrigation (Pettygrove and Asano, 1985).

2.1.1 Organic content and total suspended solids

According to Asano (1998), total suspended solids (TSS) can lead to sludge deposits and anaerobic conditions. Excessive amounts cause clogging of irrigation systems. The presence of solids in wastewater can be related to microbial contamination and turbidity, and interfere with the effectiveness of disinfection.

Wastewater quality data routinely measured and reported at the wastewater treatment plant are mostly for treated effluent disposal or discharge requirements in terms of gross pollution parameters (e.g. biochemical oxygen demand (BOD), chemical oxygen demand (COD), and TSS) that are of interest in water pollution control. (Pedrero *et al*., 2010).

The organic indicators total organic carbon (TOC) and degradable organics (COD, BOD) allow organic carbon to be measured. Their biological decomposition can lead to oxygen depletion. Only excessive amounts cause problems for irrigation. Low to moderate concentrations are beneficial.

2.1.2 Nutrients

The nutrients in treated municipal wastewater provide fertilizer value to crop or landscape production (Westcot and Ayers, 1985), because they reduce the need to add nutrients in chemical fertilizer. However, in some cases excessive nutrients in municipal wastewater can cause problems for some crops. The general principle is to carry out regular checks to evaluate wastewater nutrients, to calculate the quantities provided to the soil and, of course, the crops through irrigation.

The most important nutrients for crops are nitrogen, phosphorus, potassium, zinc, boron, and sulphur. Usually, recycled water contains enough of these elements to supply a large portion of a crop's needs. The nutrients N, P, and K in surface waters lead to eutrophication. In irrigation they are a beneficial source of nutrients. Nitrates in large concentrations can cause groundwater pollution.

The most beneficial nutrient for plants is nitrogen. Both the concentration and forms of nitrogen (nitrate and ammonium) need to be considered in irrigation water. Nitrogen is a macronutrient for plants that is applied on a regular basis. Nevertheless, at very high concentrations, it can over-stimulate plant growth, causing problems such as lodging and excessive foliar growth, and also delay maturity or result in poor crop quality. Nitrogen sensitivity varies with the development stage of the crops. It may be beneficial during growth stages, but causes yield losses during flowering/fruiting stages. The long-term effects of excess nitrogen include weak stalks, stems, and/or branches unable to support the weight of the vegetation under windy or rainy conditions (Lazarova and Bahri, 2005).

Pollution of groundwater from the percolation of nitrogen presents a health concern. This usually results from excessive application of nutrients in areas having permeable soils. When nitrogen is washed from soils and reaches streams, lakes, canals, and drainage ditches, it stimulates algae growth, which can result in plugged filters, valves, pipelines, and sprinklers (Lazarova and Bahri, 2005). In addition, excessive nitrogen application to pastures may be hazardous to livestock that consume the vegetation. Potassium in recycled water has little effect on crops.

The phosphorus content in recycled water is too low to meet crop needs. Over time, phosphorus can build up in the soil and reduce the need for supplementation. Although excessive phosphorus does not appear to cause serious immediate problems to crops, it may affect future land use because some plant species are sensitive to high phosphorus concentrations (Silber et al., 2002; Webb and Loneragan, 1998)). On the other hand, soil properties such as pH, organic matter, clay content and mineralogy are factors determining P dynamics. Moreover, in calcareous soil phosphorous solubility may be readily controlled by geochemical processes such as the solid phase dicalcium phosphate, by chemisorption of P on calcite, and by the formation of secondary $CaCO_3$ (Pizzeghello et al., 2011). As a result of these highly efficient retention processes most arable alkaline Mediterranean soils generally show extremely low P availability increasing the risk of P losses to aquatic ecosystems (Heredia and Cirilli, 2007). Phosphorus can also be a problem in surface water runoff as a limiting factor.

For sprinkler irrigation, excessive residual chlorine in recycled water causes plant damage if high residual chlorine exists at the time of irrigation. As free chlorine (Cl_2) is highly reactive and unstable in water, a high level of residual chlorine rapidly dissipates if the treated water is stored in reservoirs for more than a few hours. Residual free chlorine concentrations below 1 mg/L are not likely to affect plant foliage. Some damage may occur in very sensitive species at relatively low levels of about 0.5 mg/L (Lazarova and Bahri, 2005). Severe plant damage of a burning nature can occur in the presence of excessive free chlorine. Most reuse strategies will not face this problem if an intermediate storage facility is used, but care is needed during any period where the storage facility is bypassed for direct irrigation from the treatment plant.

2.1.3 The effects of pH

pH affects the solubility of metals, the alkalinity of soils, soil structure, and plant development. Soil pH has been shown to have a significant effect on plant uptake of trace elements in biosolids, much more consistently than other soil variables such as organic matter content, cation exchange capacity, and soil texture (Bibak *et al*., 1999). Many wastewaters contain high concentrations of bicarbonate (Kumar and Christen 2009), and application to soils with irrigation can increase soil pH (Suarez *et al*. 2006). At pH greater than 8, the formation of carbonate precipitates has been shown to occur in soils irrigated with waters of high bicarbonate content

(Eshel *et al*. 2007). Divalent cations, Mg^{2+} and Ca^{2+}, in solution and on exchange sites can act as conjugate cations in this precipitation.

The normal pH range for irrigation water is from 6.5 to 8.4. pH values outside this range provide an indication that the water is abnormal in quality. In this case, irrigation water may cause a nutritional imbalance affecting plant growth and health. Moreover, abnormal pH can be very corrosive to such appurtenances as pipelines, sprinklers, and control valves. Normally, pH is a routine measurement in irrigation water quality assessment as it may be an indication of the presence of toxic ions.

Trace element toxicities to plants are more common in acid soils. Other soil components such as clay, organic matter, hydrous iron and hydrous manganese oxides, organic acids, amino acids, and humic and fulvic acids can also react to prevent trace element movement. pH is an indicator of the acidity or alkalinity of water but is seldom a problem by itself (Lazarova and Bahri, 2005). Accumulation of heavy metals by the plants when irrigated with solution is affected mainly by pH. Low pH allows easier absorbance of heavy metals by plants (Mclaren and Crawford, 1973, Mitchell and Karathanasis, 1995, Salt et al., 1995).

2.1.4 The effects of salinity

The quality of irrigation water has been determined by the quantity and kind of salt present in these water supplies. Although crops vary considerably in their ability to tolerate saline conditions (Maas and Grattan, 1999), in general, as salinity increases in the treated wastewater used for irrigation, the probability for certain soil, water, and cropping problems increases. Tolerances for many common field, vegetable, forage and tree crops are given in Table 1.2. Yield Potential is a measure of the yield under different conditions, for example in Table 2.1 the zero yield potential (0%) indicates the theoretical salinity at which crop growth ceases.

Table 2.1 Crop tolerance and yield potential (%) of selected crops influenced by irrigation water salinity (mS/cm) (FAO, 2008)

	Yield Potential				
	100%	**90%**	**75%**	**50%**	**0%**
Barley *(Hordeum vulgare)*	5.3	6.7	8.7	12	19
Wheat *(Triticum aestivum)*	4.0	4.9	6.3	8.7	13
Rice (paddy) *(Oriza sativa)*	2.0	2.6	3.4	4.8	7.6
Corn (maize) *(Zea mays)*	1.1	1.7	2.5	3.9	6.7
Tomato *(Lycopersicon esculentum)*	1.7	2.3	3.4	5.0	8.4
Potato *(Solanum tuberosum)*	1.1	1.7	2.5	3.9	6.7
Pepper *(Capsicum annuum)*	1.0	1.5	2.2	3.4	5.8
Lettuce *(Lactuca sativa)*	0.9	1.4	2.1	3.4	6.0
Radish *(Raphanus sativus)*	0.8	1.3	2.1	3.4	5.9
Carrot *(Daucus carota)*	0.7	1.1	1.9	3.0	5.4
Corn (forage) (maize) *(Zea mays)*	1.2	2.1	3.5	5.7	10
Date palm *(phoenix dactylifera)*	2.7	4.5	7.3	12	21
Orange *(Citrus sinensis)*	1.1	1.6	2.2	3.2	5.3
Apricot *(Prunus armeniaca)*	1.1	1.3	1.8	2.5	3.8
Grape *(Vitus sp.)*	1.0	1.7	2.7	4.5	7.9

Compared to many other irrigation waters, recycled water generally has a low to medium salinity with electrical conductivity of 0.6 to 1.7 dS/m. Some dissolved mineral salts are identified as nutrients and are beneficial for plant growth, while others may be phytotoxic or may become so at high concentrations (Lazarova, 2005). Establishing a net downward flux of water and salt through the root zone is the only practical way to manage a salinity problem (Westcot and Ayers, 1985).

Where grapevines have been irrigated with wastewater, a decline in the nutrient status of vines has been reported by Nielsen et al. (1989) and McCarthy (2010). McCarthy (1981) and Paranychianakis et al. (2006) also report vine nutrient deficiencies due to a progressive increase in soil salinity over several consecutive years of irrigation. Vines grown in high-salinity soils may have reduced shoot and root growth, bunch number and berry weight, and adverse fruit acidity that results from a decline in soil structure, lower photosynthesis activity, poor nutrient utilization and greater osmotic stress.

The major salinity sources in recycled water are drinking water (especially hardness and naturally occurring salts), salts added by urban or industrial water use, infiltration of brackish water into sewers, and agricultural irrigation (impact on ground water salinity). As a rule, residential use of water typically adds about 300 + 100 mg/L of dissolved salts (Lazarova, 2005).

Good drainage is essential in order to allow a continuous movement of water and salt below the root zone. Long-term use of reclaimed wastewater for irrigation is not generally possible without adequate drainage. Where drainage water salinity exceeds crop threshold levels, the water can be blended with freshwater. Blending, which can be done before or during irrigation, enables farmers to extend the volume of water available (Rhoades, 1999; Oster and Grattan, 2002).

Salinity in the soil is related to, and often determined by, the salinity of irrigation water. The rate at which salts accumulate to undesirable levels in soils depends on the following factors:

- The concentration in the irrigation water
- The amount of water applied annually
- Annual precipitation
- Evapotranspiration
- Soil characteristics, both physical and chemical

The importance of applying excess water beyond evaporative demand is recognized by irrigators as a means of reducing the salt concentration in the vine root zone (Russo et al. 2009). The quality of irrigation water is of particular importance in arid zones, where extremes of temperature and low relative humidity result in high rates of evaporation with consequent deposition of salt, which tends to accumulate in the soil profile. The physical and mechanical properties of the soil, such as soil structure (stability of aggregates) and permeability, are very sensitive to the type of exchangeable ions present in irrigation water. Thus, when water reuse is being planned, several factors related to soil properties must be taken into consideration (Lazarova, 2005).

Dissolved salts increase the osmotic pressure of soil water and consequently lead to an increase in the energy plants must expend to take up water from the soil. As a result, respiration is increased and the growth and yield of most plants decline progressively as osmotic pressure increases (Lazarova, 2005).

Sodium salts in irrigation water, apart from their immediate negative effects on plants, can also affect soil structure, reducing both the rate at which the water infiltrates the soil and the aeration of the soil. If leaching is drastically reduced, it may become impossible to apply the necessary amount of water for good plant development. Consequences of soil structure degradation are surface pooling of water, crust formation, excessive weed growth and insufficient aeration of the soil. Irrigation with treated urban wastewater is often applied to already degraded soils, exacerbating the problem (Mahmoud, 2006).

Leaching problems generally concern surface soil to a shallow depth and are mainly associated with high sodium content or very low calcium content in this zone or in the applied water. Calcium deficiency problems are caused by irrigation with very low salinity water, which dissolves and washes away the calcium in the soil, or with water with very high sodium levels, which causes a high concentration of sodium in the soil compared to calcium. High-salinity water increases leaching and partially offsets (Rhoades, 1977) the problems caused by high sodium adsorption ratio (SAR). SAR is a measure of the suitability of water for use in agricultural irrigation, as determined by the concentrations of solids dissolved in the water. It is also a measure of the sodicity of soil, as determined from analysis of water extracted from the soil. The formula for calculating SAR is:

$$S.A.R = \frac{Na^{+}}{\sqrt{\frac{1}{2}(Ca^{+2} + Mg^{+2})}}$$

Where, sodium, calcium, and magnesium are in milliequivalents/litre.

If irrigation water with a high SAR is applied to a soil for years, the sodium in the water can displace the calcium and magnesium in the soil. This will cause a decrease in the ability of the soil to form stable aggregates and a loss of soil structure and tilth. This will also lead to a decrease in infiltration and permeability of the soil to water. The potential for water infiltration and/or soil dispersion problems can only be adequately addressed when the salinity and SAR indexes are considered together.

At a given SAR, the infiltration rate increases as salinity increases or decreases as salinity decreases. The SAR and electrical conductivity

(EC) of the water should therefore be taken into account for the evaluation and treatment of leaching problems (Mahmoud, 2006).

Sodium is a unique cation because of its effect on soil. When present in the soil in exchangeable form, sodium causes adverse physical-chemical changes, particularly to soil structure, which results in dispersion of particles and, consequently, reduced infiltration rates of water and air into the soil. As a rule, recycled water could be a source of excess Na in the soil compared to other cations (Ca, K, Mg), and for this reason it should be monitored (Halliwell et al. 2001).

As reported by Lieffering and McLay (1996), the high alkalinity and Na^+ content of some wastewaters can dissolve organic carbon. Sparling et al. (2001), for instance, reported a considerable increase in unsaturated hydraulic conductivity where dairy factory effluent had been used for irrigating for more than 2 years because, in part, of the dissolution of carbon compounds that may otherwise clog soil pores. The availability of nutrients for plant uptake is also strongly influenced by soil pH, and alkaline soil conditions can limit the supply of nutrients to vines (Bolan and Hedley 2003, Holzapfel *et al*. 2009).

2.1.5 Pathogens

Microbial pathogens which can be potentially present in wastewater can be divided into three separate groups. These groups are the viruses, bacteria and the pathogenic protozoan/helminths. The majority of these pathogens are enteric in origin, that is, they are excreted in faecal matter, contaminate the environment and then gain access to new hosts through ingestion infection rate from these viruses. These population groups are also particularly at risk of developing the more rare forms of disease caused by these viruses (Table 2.2).

Viruses are among the most important, and potentially most hazardous of the pathogens found in wastewater. Untreated wastewater can contain a range of viruses which are pathogenic to humans. Viral numbers have been detected in concentrations in excess of 10^3-10^4 viral particles/litre of wastewater. Viruses are generally more resistant to treatment processes, are more infectious, and require smaller doses to cause infection than most of the other pathogen types. Viruses are also generally more difficult to detect in environmental samples such as wastewater.

Bacteria are the most common of the microbial pathogens found in wastewater. There are a wide range of bacterial pathogens and opportunistic pathogens which can be detected in wastewaters. Many of the bacterial pathogens are enteric in origin, however, bacterial pathogens which cause non-enteric illnesses have also been detected in wastewaters (Neuman et al. 1997).

Table 2.2 Examples of diseases caused by pathogens present in wastewater (Lowe *et al*., 2007)

Pathogen	Disease caused
Viruses	
Enteriviruses	Gastroenteritis, heart anomalies, meningitis
Hepatitis A virus	Infectious hepatitis
Rotavirus	Gastroenteritis
Bacteria	
Escherichia Coli	Gastroenteritis
Salmonella typhi	Typhoid fever
Leptospira	Leptospirosis
Protozoa	
Giardia Lamblia	Giardiasis
Entamoeba histolytica	Amebiasis
Balantidium coli	Balantidiasis

Pathogenic protozoa are detected more regularly in wastewater than in other environmental sources. There are a number of protozoan pathogens which have been isolated from wastewater sources. The most commonly detected are *Entamoeba histolytica, Giardia intestinalis*, *Cryptosporidium parvum, E. histolytica, G. intestinalis*, and C*. parvum*. These are all common enteric pathogens and have been frequently detected in wastewater which has been contaminated with faecal material (Ferguson *et al*. 1996, Wallis *et al*. 1996).

The detection, isolation and identification of the many different types of microbial pathogens known to contaminate groundwater would be a difficult, time consuming and hugely expensive undertaking if attempted on a regular basis. To avoid the necessity of undertaking such huge ventures, indicator microorganisms are used to determine the relative risk of the possible presence of pathogenic microorganisms in a sample. To function effectively as indicators for the presence of these pathogens, indicator microorganisms should be present in equivalent or higher numbers and be as, or more resistant to environmental factors and treatment processes than the pathogenic microorganisms.

As most of the microbial pathogens present in waters and wastewaters are faecal in origin, the detection of faecal contamination of water has been the main aim of water testing authorities. Historically, the faecal coliforms, in particular *E. coli*, have been used as indicators of faecal contamination of water sources (APHA 1989).

E. coli is used as its growth characteristics and behaviour in the environment are relatively well known. Faecal coliforms which have been excreted by warm blooded animals can be grown on selective media at 44.5°C. This ability to be cultured at elevated temperatures has lead them to be know as the thermotolerant coliforms and they have become the mainstay indicator for the water industry.

2.1.6 Specific ion toxicity

Toxicity due to a specific ion occurs when that ion is taken up by the plant and accumulates in the plant in amounts that result in damage or reduced yield. Toxicity normally results in impaired growth, reduced yield, changes in the morphology of the plant, and even its death. The degree of damage depends on the crop, its stage of growth, concentration of the toxic ion or ions, its relationships, climate, and soil conditions. The most common phytotoxic ions that may be present in municipal effluents in concentrations high enough to cause toxicity are boron (B), chloride (Cl), and sodium (Na). Each can cause damage individually or in combination (Lazarova and Bahri, 2005).

Sodium and chloride

Sodium and chloride are usually absorbed by the roots but can also enter directly into the plant through the leaves when moistened during sprinkler irrigation. This typically occurs during periods of high temperature and low humidity. Leaf absorption speeds up the rate of accumulation of a toxic ion and may be a primary source of toxicity. The concentration of these ions should be determined on an individual case basis to assess the suitability of wastewater quality for agricultural or landscape irrigation, although concentration changes are usually not relevant for short and medium periods of time (Lazarova, 2005). Excessive sodium concentration can cause leaching problems. Chloride and sodium also increase during domestic usage, especially where water softeners are used. For sensitive crops, toxicity is difficult to correct without changing the crop or the water supply. The problem is usually accentuated by severe (hot) climatic conditions (Westcot and Ayers, 1985).

Boron

The source of boron (B) is usually household detergents or discharges from industrial plants (Westcot and Ayers, 1985). Boron can become toxic at levels only slightly greater than those required by plants for good growth. The predominant source of anthropogenic boron is domestic effluents, due to the use of perborate as a bleaching agent. As a result, boron can be found in urban wastewater at concentration levels as high as 5 mg/L (dry countries and concentrated sewage), with an average level around 1 mg/L. It should be noted that boron at concentrations of less than 1 mg/L is essential for plant development, but higher levels can cause problems in sensitive plants. Most plants exhibit toxicity problems when the concentration of boron exceeds 2 mg/L (Lazarova, 2005).

2.1.7 The effect of heavy metals

Heavy metals (Li, Zn, Ni, etc.) can accumulate in the soil and cause plant toxicity. Surveys of irrigation with recycled water have shown that more than 85% of the applied trace elements are likely to accumulate in the soil, most at or near the surface, and may be leached to groundwater.

Trace elements are not normally included in the routine analysis of regular irrigation water, but attention should be paid to them when using treated municipal effluents, particularly if contamination with industrial wastewater discharge is suspected. These elements include aluminum (Al), beryllium (Be), cobalt (Co), fluoride (F), iron (Fe), lithium (Li), manganese (Mn), molybdenum (Mo), selenium (Se), tin (Sn), titanium (Ti), tungsten (W), and vanadium (V). Heavy metals include a special group of trace elements that have been shown to create definite health hazards when taken up by plants. In this group are included arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), and zinc (Zn) (Gupta et al., 2010).

The pH of the soil system is a very important parameter, directly influencing sorption/desorption, precipitation/ dissolution, complex formation, and oxidation-reduction reactions of metals in soils. The soil's ability to immobilize heavy metals increases with rising pH and peaks under mildly alkaline conditions. Heavy metals mobility is related to their immobilization in the solid phase (Fuller,1977). He considered that in acid soils (pH 4.2-6.6) the elements Cd, Ni, and Zn are highly mobile. Cr is moderately mobile; and Cu and Pb practically immobile and in neutral to alkaline (pH 6.7-7.8), Cr is highly mobile whereas Cd and Zn; and Ni are moderately mobile and immobile respectively. Certain management practices will not only remove the heavy metal contaminants, but will also help to immobilize them in the soil and reduce the potential of adverse effects from the metals. Cationic metals are more soluble at lower pH level, therefore, increasing the pH in soil makes them less available to plants and therefore less likely to be incorporated in their tissues and ingested by humans. Raising pH has the opposite effect on anionic elements like As, Mo and Se (Hartley et al., 2004).

The concentration of heavy metals in the wastewater and the effect on the absorption of heavy metals by the plants are directly correlated. The higher the concentration of heavy metals in the solution, the higher the concentration in the plants (Gadallah, 1994, Mitchell and Karathanasis, 1995, Mungur et al., 1995, Scholes et al., 1999).

Several long-term field experiments have been conducted in different countries on the impact of land application of recycled water on soils, micro-organisms, and plants. Tejon et al., (2010) examined the presence of four metals (Cd, Ni, Hg, Pb) at the Llobregat delta, south of Barcelona (Spain). In the area, reclaimed water is destined to satisfy environmental uses, irrigation and the construction of a hydraulic barrier against seawater intrusion in the deep aquifer of the delta. Except for Hg, all compounds have been found in groundwater samples with mean concentration values between 0.45 and 12.2 µg/L. In another study (, the effect of reclaimed wastewater irrigation on the alteration of soil properties and accumulation of trace metals in soil profiles was investigated by monitoring different plots from Palmdale, California that had been irrigated with effluents for various lengths of time (3, 8, and 20 years, respectively). They concluded that heavy metals in the upper horizons may be accumulated, which may eventually lead to deterioration of soil and groundwater quality and affect the sustainability of land-based disposal of effluent.

Pollution of the biosphere with toxic heavy metals has accelerated dramatically since the beginning of the industrial revolution (Salt et al., 1995). The primary sources of this pollution are the burning of fossil fuels, mining and smelting of metalliferous ores, municipal wastes, fertilizers, pesticides and sewage. As a result, toxic metal contamination of soil, aqueous wastes, waste streams and groundwater poses a major potential environmental and human health problem which is still in need of an effective and affordable technological solution (Salt *et al*., 1995).

The metal removal ability of plants

All plants have the ability to accumulate, from soil and water, heavy metals that are essential for their growth and development. These metals include Fe, Mn, Zn, Cu, Mg, Mo and possibly Ni. Certain plants also have the ability to accumulate heavy metals which have no known biological function; these include Cd, Cr, Pb, Co, Ag, Se and Hg (Wallace and Wallace, 1994, Salt *et al*. 1995). However, excessive accumulation of heavy metals can be toxic to humans (Wallace and Wallace, 1994).

Heavy metal concentration in soil

Heavy metals attached to the organic matter in the soil are relatively unavailable to plants, especially in the short term (Mitchell and Karathanasis, 1995). The ability of organic matter to retain elements necessary for plant growth for a long period of time and release them as needed is one of the most important benefits derived from the presence of organic matter in soils (Garcia *et al*., 1995). However, accumulation of heavy metals by plants is directly proportional to the total concentration of metals in the substrate, including the organically bound fraction (Garcia *et al*., 1995).

Plant response to heavy metals

The response of plants in an environment containing heavy metals in high concentrations varies. It is quite difficult to find a common pattern of reaction for all plant species. Plants can absorb heavy metals which are either in exchangeable form or in the soil solution (Wallace and Wallace, 1994, Garcia *et al*., 1995).

The metals in wastewater could be regarded as existing in the form of soil solution. However, part of those metals will be absorbed by the substrate, and mostly by the organic matter, which will immobilize them. For a plant to accumulate these metals they must first be released into the soil solution (Fernandes and Henriques, 1991, Salt *et al*., 1995).

Metal-chelating molecules can be secreted into the rhizosphere to chelate and solubilize soil-bound metals. Roots can reduce soil-bound metal ions by using specific plasma membrane-bound metal reductases. Plant roots can solubilize heavy metals by acidifying their soil environment with protons produced by the roots (Fernandes and Henriques, 1991, Salt *et al*., 1995). The mechanisms of metals removal by plant roots are not necessarily similar for all metals and all plants.

Copper has a transfer coefficient between soil and plants up to 13 times lower than that determined for Zn, Cd and Ni, whether the metals were added as inorganic salts to a sandy soil or incorporated in sewage sludge.

This was due to the increased selectivity of Cu by soil organic and inorganic colloids (Fernandes and Henriques, 1991). The dominant method of metal uptake seems to be the sorption of metals by the root. Surface sorption is a combination of chemical and physical processes such as chelation, ion exchange and specific absorption. This component does not require biological activity and will take place in dead roots.

Once metal ions have entered the root they can be either stored or exported to the shoot. Metal transfer to the shoot probably takes place through the xylem. However, metals may redistribute in the shoot via the phloem. For metal ions to enter the xylem vessels they must first cross the Casparian strip, which divides the endodermis from the epidermis. To cross this strip of water and the impermeable cell wall, metals ions must move symplastically, as apoplastic motion within the endodermis is a rate limiting step. Xylem cell walls have a high CEC, which would be expected to severely retard the movement of metal cations (Salt *et al*., 1995).

For a plant to resist the toxic effects of heavy metals, it must either limit cellular uptake (avoidance), detoxify heavy metals once they enter the cells, or develop heavy metal resistant meristem metabolisms (Salt *et al*., 1995). The evidence for the avoidance of heavy metal toxicity, by reduced cellular uptake, is very limited. Nevertheless, avoidance may be a viable strategy for certain sensitive tissues like the root-tip meristem (Salt *et al*., 1995).

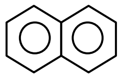
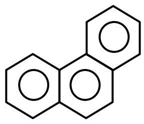
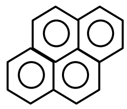
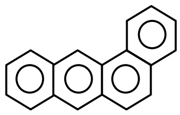
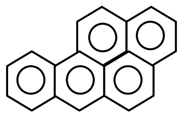
Some plant ecotypes endemic to heavy metal polluted soils have been shown to contain heavy metal resistant enzymes, for example cell wall acid phosphatases. However, it is unlikely that the development of heavy metal resistant biochemical processes could be a viable heavy metal resistant mechanism (Baker, 1981, Fernandes and Henriques, 1991, Salt *et al*., 1995). Once heavy metals accumulate within cells they will need to be detoxified. This can occur in a number of ways depending on the metal, whether through chelation, compartmentalization or precipitation (Fernandes and Henriques, 1991, Salt *et al*., 1995). Fernandes and Henriques (1991) suggested a number of possible mechanisms which explain how the tolerance of plants under metallic stress can be achieved.

2.1.8 Polycyclic aromatic hydrocarbons (PAHs)

PAHs are a group of compounds polymerized by two or more benzene rings in different ways (Table 2.3). Due to the increasing reuse of wastewater, there is an increasing concern regarding the fate of PAHs in the treated wastewater (Song *et al*. 2006; Fatta-Kassinos *et al*. 2011).

PAHs are of particular interest because: (a) they are persistent pollutants in the environment; (b) they have the lowest metabolism and degradability by most living organisms; and (c) due to their low water solubility and high hydrophobicity, PAHs are adsorbed onto solid particles. The main sources for PAHs in sewers are municipal and industrial wastes, rainfall and storm runoff waters, particularly from road surfaces (Mangas *et al*. 1998).

Table 2.3 Structure and physicochemical characteristics of some PAHs

PAH	Structure[1]	Water solubility (mg/l)	log K_p	Vapor pressure (torr) 20 °C
Naphthalene		30	3.36	0.082
Phenanthrene		1.29	4.46	6.8×10^{-4}
Pyrene		0.14	5.32	6.8×10^{-7}
Benzo(a)anthracene		0.014	5.61	5.0×10^{-9}
Benzo(a)pyrene		0.0038	6.04	5.0×10^{-7}

[1]benzene rings

Several PAHs have been shown to be potentially highly carcinogenic and mutagenic (Blanchard *et al*. 1999) and, as a result, have been listed by regulatory agencies such as the US EPA as top priority pollutants. These pollutants accumulate in the soil through wastewater irrigation and aerial deposition and then crop uptakes these pollutants from the soil through roots (Khan *et al*., 2008) and atmospheric aerial deposition on plants which affect the food quality (Wei et al., 2014). Air and root uptakes are considered as the main pathways of PAH accumulation in vegetable but their entry mostly depends upon the variety of vegetable, locality and nature of PAH compounds (Wang *et al*., 2011).

In 2002, the Scientific Committee on Food (SCF) concluded that 15 PAHs may potentially be genotoxic (damaging to DNA) and carcinogenic (cancer causing) to humans and should be prioritised when looking at the dietary intake of PAHs. The SCF concluded that it was not possible to establish a threshold level below which risk would be insignificant and therefore a Tolerable Daily Intake (the amount of a substance that can be ingested daily over a lifetime without appreciable health risk) could not be set. Consequently, it recommended that exposures to PAHs from food should be as low as reasonably achievable.

In April 2005, the European Commission introduced regulatory limits for BaP in a range of foods, including smoked meat and smoked fish in Commission Regulation (EC) 208/2005. New and revised regulatory limits for BaP and new limits for PAH4 (BaP, CHR, BaA and BbFl) were published in Commission Regulation (EU) No 835/2011 on 20/08/2011. On the other hand, there are no regulatory limits for PAHs in cereals and vegetables.

2.2 Wastewater treatment technology

Effective wastewater treatment to meet water reuse quality standards, and efforts to protect public health, are the basic preconditions for wastewater reuse systems. Urban wastewater treatment consists of a combination of physical, chemical and biological processes for the removal of solids, organic substances, pathogens, metals and sometimes nutrients from wastewater. The general terms used to describe the different treatment stages are primary, secondary, tertiary and/or advanced treatment. Disinfection to control pathogen populations is the final treatment stage and takes place shortly before the storage or distribution of the treated wastewater.

While reuse projects in Europe typically have very high standards for wastewater treatment, in lower-income countries, raw sewage is often used directly. It is estimated that 20 million hectares (10% of all irrigated land) are irrigated with raw, partially treated, or fully treated wastewater (United Nations, 2003). The criteria of wastewater treatment in guidelines related to the use of treated wastewater effluent for irrigation purposes as shown in Table 2.4. When wastewater is treated with the intention of using the effluent for agricultural irrigation and not disposal in receiving waters, the important quality criteria are those relevant to human health rather than environmental criteria and those related to the health of fish in receiving waters.

Table 2.4 Wastewater treatment criteria for irrigation of reclaimed water according to several guidelines.

Country	Irrigation of	Treatment required
Cyprus	All crops	Secondary + tertiary + disinfection
	Crops for human consumption - Amenity areas of limited public access	Secondary + storage >1 week and disinfection
	Industrial crops	Secondary + storage >30 days
Spain	Irrigation of crops for human consumption not avoiding direct contact of regenerated water with edible parts	Filtration + disinfection
	Localized irrigation of ligneous crops impeding contact of regenerated water with food for human consumption. Irrigation of ornamental flowers, greenhouses and nurseries with no direct contact of regenerated water with crops	Filtration + disinfection
Greece	All crops	Secondary + tertiary + disinfection
	Fodder, industrial crops, pastures, seed crops, crops that produce products which are processed before consumption.	Secondary + disinfection
Mediterannean[1]	All crops	Secondary, filtration and disinfection
	Cereals, fruit trees, plant nurseries, ornamental nurseries	Secondary + few days storage
	Cereals, fruit trees, plant nurseries, ornamental nurseries using trickle irrigation systems	Primary treatment
US EPA	Food crops	Secondary + tertiary + disinfection
	Processed food crops and Non Food crops	Secondary + disinfection
WHO	A. Vegetable and salad crops eaten uncooked, sports fields, public parks	stabilization ponds, sequential batch-fed wastewater storage and treatment reservoirs
	B.Cereal crops, industrial crops, fodder crops, pasture and trees	Retention in stabilization ponds for 8-10 days
	C.Localised irrigation of crops in category B if exposure of workers and the public does not occur	Pre-treatment as required by irrigation technology, but not less than primary treatment

[1] Recommended guideline for water reuse in the Mediterranean Region (Bahri and Brissaud, 2002)

2.2.1 Primary treatment

The term primary treatment refers to the initial treatment of wastewater to remove specific substances. In conventional treatment systems, primary treatment consists of screening, de-sanding and the removal of large particles. Conventional wastewater treatment is effective in removing solids over 50 μm. Generally, 50% of suspended solids and 25-50% of BOD_5 are removed during primary treatment (Metcalf and Eddy, 1991). Nutrients, hydrophobic particles, metals and microorganisms associated with the removed particles can also be removed by primary treatment. Approximately 10-20% of organic nitrogen and 10% of phosphorus is also removed by conventional primary treatment. For most wastewater reuse systems, primary treatment is not sufficient to achieve the required quality of treated wastewater.

Despite this, a large number of wastewater treatment plants, especially in the Asian and African part of Mediterranean comprise solely primary treatment. Currently it is considered as the absolute minimum level of treatment before water is discharged. Some countries (Palestine, Syria, Libya) are still struggling to achieve this minimal level of wastewater treatment (Kellis et al., 2013).

2.2.2 Secondary treatment

Secondary treatment systems consist of a series of biological processes combined with the separation of the liquid and solid phase.

The biological processes are designed to provide effective microbiological metabolism of the dissolved or suspended organic substrate present in the wastewater.

Conventional treatment systems include an aerobic biological reactor combined with secondary sedimentation for the dissolved or suspended organic substrate produced by the treatment of the wastewater components. Conventional treatment systems result in suspended solids and BOD_5 levels ranging both from 10 to 30 mg/l. Depending on the process, 10-50% of the organic nitrogen is removed during conventional secondary treatment and phosphorus is converted into phosphoric ions (PO_4^{-3}). The resulting solids are treated using aerobic or anaerobic digestion, composting or other types of treatment technology. There is only partial removal of pathogens, trace elements and pathogens combined with biological filtration and physical separation.

For many wastewater treatment and reuse systems, secondary treatment results in the satisfactory removal of organic substances from wastewater. Secondary treatment is often combined with filtration for further removal of particles and disinfection.

2.2.3 Tertiary and/or advanced treatment

Tertiary and/or advanced wastewater treatment is applied when specific wastewater components must be removed but this cannot be achieved by secondary treatment. Advanced treatment refers to the removal of specific substances such as ammonia or nitrates, using nitrification/denitrification processes, ion exchange or removal of the total dissolved solids by reverse osmosis. Tertiary and/or advanced wastewater treatment usually follows the other biological treatment processes.

2.3 Wastewater reuse for irrigation

Water supply and water quality degradation are global concerns that will intensify with increasing water demand, the unexpected impacts of extreme events, and climate change. For this reason, worldwide, marginal-quality water will become an increasingly important component of agricultural water supplies, particularly in water-scarce countries (Qadir *et al*., 2007).

One of the major types of marginal-quality water is the wastewater from urban and peri-urban areas. Wastewater has been recycled in agriculture for centuries as a means of disposal in cities such as Berlin, London, Milan and Paris (AATSE, 2004). However, in recent years wastewater has gained importance in water-scarce regions. In Pakistan 26% of national vegetable production is irrigated with wastewater (Ensink *et al*., 2004). In Hanoi 80% of vegetable production is from urban and peri-urban areas (Lai, 2000). In

Ghana, informal irrigation involving diluted wastewater from rivers and streams occurs on an estimated 11,500 ha, an area larger than the reported extent of formal irrigation in the country (Keraita and Drechsel, 2004). In Mexico about 260,000 ha are irrigated with wastewater, mostly untreated (Mexico CAN, 2004). In most of these cases, farmers irrigate with diluted, untreated, or partly treated wastewater. The failure to properly treat and manage wastewater generates adverse health effects. Farmers and their families using untreated wastewater are exposed to health risks from parasitic worms, viruses and bacteria.

The potential health risks and environmental impacts resulting from wastewater use for irrigation have been well documented (Angelakis *et al*., 2003). Health and environmental aspects are particularly sensitive issues and important prerequisites, since wastewater effluent must not be used and/or be accepted to replace conventional or possibly other non-conventional water sources for irrigation, unless it is adequately treated and safely applied (Salgot *et al*., 2003).

The overarching goals of water reuse in agriculture are to provide an adequate supply of high-quality water for growers and to ensure food safety (Dobrowolski et al., 2008). Therefore, in developed countries, public institutions usually determine water quality objectives by considering health risks and requiring wastewater treatment to achieve these goals. In these developed countries there are integrated programmes for planned reuse of wastewater. These programmes are developed by public institutions and include policies to improve the management of wastewater in agriculture that can be implemented before wastewater is generated, while it is being used, and after crops have been irrigated and products are prepared for sale and consumption.

The State of California pioneered efforts to promote water reclamation and reuse. The first reuse regulations were promulgated in 1918 (Asano and Levine, 1996). Currently, in the United States municipal water reuse accounts for 1.5% of water withdrawals, and California residents reuse 656 million cubic meters of municipal wastewater annually. Following rules and directives of this type, the use of reclaimed wastewater in agriculture is a growing practice that may help ensure safe and sustainable food crops.

Most articles on “treated municipal wastewater” relate to the development and description of methodologies and techniques in order to improve the water quality of the effluents proceeding from water recycling plants. The results show that disinfection strategies that included both UV and chlorine produced reclaimed water of a better and more reliable quality from the point of view of public health protection, as compared with the results obtained with only a disinfectant agent, even if applied at higher doses (Montemayor *et al*., 2008).

2.3.1 Wastewater reuse for irrigation in EU countries

With climate change, population growth and water scarcity, there is a growing need to manage water resources in a sustainable manner. Almost all Mediterranean countries in the EU regularly experience severe water supply and demand imbalances, particularly in the summer months. This is due to the simultaneous occurrence of low precipitation, high evaporation and increased demands for irrigation and tourism. However, this situation have been extended to other regions water, when periods of drought are becoming more frequent and long lasting as a result of global climate change. France, Bulgaria, Malta, Belgium, and the UK have suffered the negative impact of successive droughts over the last twenty years (EU, 2013).

In Europe, only 2.4% of treated waste water (700 Mm3/year) is reused, mostly in Spain. Irrigation represents 75% of water reuse. This is clearly not enough if the need to develop alternative water supplies is to be met in a context of growing water scarcity (structural unbalance) and increasing climate change impacts (modified rain patterns).

So far, no specific regulation on reclaimed wastewater use exists at European level that may explain the little 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). Regulations and guidelines adopted by EU countries about wastewater reuse are shown in Table 2.5

Table 2.5 Sectors in which reclaimed water is currently applied on by EU country (European Commission, 2013).

	Agriculture	Groundwater recharge	Industrial	Environment	Regulations /Guidelines
Austria			x		No
Belgium	x	x	x		Under prep.
Bulgaria			x		Under prep.
Cyprus	x	x	x	x	D 296/ 03.06.2005
Czech					No
Denmark			x		No
Finland			x		No
France	x	x	x		D 94/463.3.1994 DGS/SD1.D.91 Guidelines 1996
Germany	x	x	x	x	Under prep.
Greece	x	x	x		JMD 145116/11 GG B' 192/97
Italy	x	x	x		D152/2006
Ireland					No
Malta	x		x		Under prep.
Poland					Under prep.
Portugal	x	x	x		RecIRAR 2/2007 ERDAR Guideline
Slovakia					No
Spain	x	x	x		RD 1620/2007 Guidelines from the Regional Health Authorities
Sweden	x	x	x		No
UK		x	x	x	Under prep.

According to Directive 91/271/EEC - Article 12, treated wastewater must be reused whenever appropriate and disposal routes must minimise any adverse effects on the environment. Therefore, before disposal of treated wastewater into water bodies, the treated wastewater from municipal wastewater treatment plants must meet the water quality parameters shown in Table 2.6 (EC, 1991).

Table 2.6 Requirements for discharge from urban wastewater treatment plants (Directive 91/271/EEC).

Parameter	Concentration (mg/l)	Reduction (%)
Biochemical Oxygen Demand	25	70-90
Chemical Oxygen Demand	125	75
Total Suspended Solids	35-60[1]	70-90[2]
Total Nitrogen[3]	2	80
Total Phosphorus[3]	15	70-80

[1]Depending on population. 35 mg/L for more than 10 000 person equivalents (PE) and 60 mg/L for 2000-1000 PE, [2]Depending on population. 90% for more than 10 000 PE and 70% for 2000-1000 PE. [3]These parameters are required in sensitive areas

As can be seen from Table 2.6, 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/determined in this directive.

The levels of priority pollutants, which include pesticides, polycyclic aromatic hydrocarbons (PAH), phenolic compounds and volatile organic compounds, is currently regulated through the European Water Framework Directives (EC, 2000; EC, 2008). The environmental quality standards (EQS) are presented in Table 2.7.

Table 2.7 Environmental Quality Standards (EQS) for priority pollutants. Table adapted as is from the source (EC, 2008).

Name of substance	Annual average-EQS		Maximum allowable Concentration-EQS	
	Inland surface waters	Other surface waters	Inland surface waters	Other surface waters
Alachlor	0.3	0.3	0.7	0.7
Anthracene	0.1	0.1	0.4	0.4
Atrazine	0.6	0.6	2.0	2.0
Benzene	10	8	50	50
Brominated diphenylether	0.0005	0.0002	n.a	n.a
Cadmium and its compounds	0.08-0.25[1]	0.2	0.45-1.5[1]	0.45-1.5[1]
Carbon- tetrachloride	12	12	n.a	n.a
C10-C13 Chloroalkanes	0.4	0.4	1.4	1.4
Chlorfenviphos	0.1	0.1	0.3	0.3
Chlorpyrifos	0.03	0.03	0.1	0.1
Cyclodiene pesticides[2]	Σ=0,01	Σ=0,005	n.a	n.a
DDT total	0.025	0.025	n.a	n.a
Para-para-DDT	0.01	0.01	n.a	n.a
1,2-Dichloroethane	10	10	n.a	n.a
Dichloromethane	20	20	n.a	n.a
DEHP	1.3	1.3	n.a	n.a
Diuron	0.2	0.2	1.8	1.8
Endosulfan	0.005	0.0005	0.01	0.004
Fluoranthene	0.1	0.1	1	1
Hexachloro-benzene	0.01	0.01	0.05	0.05
Hexachloro-butadiene	0.1	0.1	0.6	0.6
Hexachloro-cycloexane	0.02	0.002	0.04	0.02
Isoproturon	0.3	0.3	1.0	1.0
Lead and its compounds	7.2	7.2	n.a	n.a
Mercury and its compounds	0.05	0.05	0.07	0.07
Naphthalene	2.4	1.2	n.a	n.a
Nickel and its compounds	20	20	n.a	n.a
Nonylphenol	0.3	0.3	2.0	2.0
Octylphenol	0.1	0.01	n.a	n.a
Pentachloro-benzene	0.007	0.0007	n.a	n.a
Pentachloro-phenol	0.4	0.4	1	1
PAHs (10)[3]	n.a	n.a	n.a	n.a
Benzo(a)pyrene	0.05	0.05	0.1	0.1
Benzo(b)fluor-anthene	Σ=0,03	Σ=0,03	n.a	n.a
Benzo(k)fluor-anthene				
Benzo(g,h,i)-perylene	Σ=0,002	Σ=0,002	n.a	n.a
Indeno(1,2,3,-cd)-pyrene				
Simazine	1	1	4	4
Tetrachloro-ethylene	10	10	n.a	n.a
Trichloro-ethylene	10	10	n.a	n.a
Tributyltin compounds	0.0002	0.0002	0.0015	0.0015
Trichloro-benzenes	0.4	0.4	n.a	n.a
Trichloro-methane	2.5	2.5	n.a	n.a
Trifluralin	0.03	0.03	n.a	n.a

n.a: not applicable, [1]Depending on water hardness, [2] Aldrin, Deldrin, Endrin, Isodrin, [3]For the group of priority substances of polyaromatic hydrocarbons (PAH), each individual EQS is applicable, i.e. the EQS for Benzo(a)pyrene, the EQS for the sum of Benzo(b)fluoranthene and Benzo(k)fluoranthene and the EQS for the sum of Benzo(g,h,i)perylene and Indeno(1,2,3-cd)pyrene must be met.

2.3.1.1 Wastewater reuse in Greece

Greece suffers seriously from lack of water every 40–45 years, and from periodical cycles of water shortages every 5–7 years due to drought. The water demand per year in Greece is estimated at 8243hm^3, of which 83% is used for crop irrigation. Approximately 40% of the total land area of Greece is under irrigation. The volume of available natural water resources in Greece is 14,340 hm^3. On an annual basis, the total water demand for cultivation is 6833hm^3. According to Bixio *et al*. (2006), 10% of total water use goes to urban use, 80% goes to agriculture, 5% goes to industry and the remaining 5% goes to cooling and other uses.

Almost 65% of the Greek population is connected to over 350 centralised wastewater treatment plants with a total capacity of over 1.45 Mm^3/d (Tsagarakis *et al*., 2001). An analysis of data concerning the water balance of the areas of the wastewater treatment plants (WWTP) demonstrated that more than 83% of the treated wastewater is produced in regions with a deficient water balance (Tchobanoglous and Angelakis, 1996).

Despite adequate average precipitation, a water imbalance is often observed, due to temporal and regional variations in precipitation, increased water demand during the summer months, and the difficulty of transporting water due to the mountainous terrain. Moreover, in many areas of southeast Greece there is severe pressure to discover additional freshwater sources, due to the especially high demand for water for tourism and irrigation. The integration of wastewater treatment into water resource management projects is a particularly important issue.

Tsagarakis *et al*. (2001) concluded that wastewater reclamation by existing WWTP, particularly for irrigation purposes, could be increased to 242 Mm^3/yr, increasing current water use by 3.2%. Moreover, several small-scale wastewater treatment and reclamation projects are currently underway, and wastewater reuse guidelines and criteria have been adopted in the Thessaloniki area.

In east Crete, two research projects are underway, based on wastewater treatment by constructed wetland systems for reuse in vine irrigation. The main aims of these projects are the investigation of existing plant species, and the examination of urban and olive mill wastewater treatment processes. The behaviour of vines irrigated with treated wastewater under monitoring conditions is also being studied.

Another pilot study is under way in Crete, with the primary aim of developing new wastewater treatment and reuse technologies in small settlements, villages and towns, mainly based on septic tanks and hygroscopic systems. In the area of Hersonissos, Crete, one of the largest tourist resorts in the country, olive trees have been watered with secondary treated wastewater from 2004 to the present, with positive results on olive tree growth and production. A research project was carried out from 2010 to 2014 with the aim of upgrading the unit, providing citizen and farmer awareness, and monitoring effects on soil and olive cultivation. The WWTP of Heraklion, Crete, has obtained a permit to provide secondary treated wastewater for the irrigation of a large vine-cultivation zone near Heraklion (Plate 2.1) while this application is also supported by a research project on wastewater reuse and its effects on nature and people.

Plate 2.1 Grapevines and olive trees irrigated with reclaimed wastewater in Heraklion, Crete.

In Chalkida, the wastewater reclamation and reuse research project includes water improvement by filtration and disinfection during secondary treatment of approximately 7,500 m^3 of effluent per day, and landscape irrigation of the residential area around the city. Reclamation and reuse in the Argos-Nafplio area includes water improvement by filtration and disinfection during secondary treatment of approximately 17,000 m^3 of effluent per day, and irrigation of approximately 900 ha of agricultural land.

In Thessaloniki there is an urban wastewater treatment plant, built in 1982-1992, in the Sindos area, between the new and the old bridge over the River Gallikos. The treatment system used is stabilization tanks and activated sludge, while in recent years the wastewater has been subject to higher-level treatment with active sludge and a denitrification system. The plant commenced operation in February 1992, with a wastewater flow equivalent to 40,000 m^3/d. Subsequently, following improvements in efficiency, flow increased to 60,000 m^3/d, corresponding to 30-40% of the total wastewater load for Thessaloniki. From November 2000, wastewater volume increased by 40,000 m^3/d, while today it stands at 150,000-160,000 m^3/d. A significant proportion of this total wastewater volume is used for irrigation. Several research projects have been carried out on the farm of the Land Reclamation Institute of Thessaloniki, to study the possibility of reusing treated urban wastewater for irrigation, instead of disposing of it in the Bay of Thessaloniki.

Antonopoulos and Diamantidis (1995) investigated the effect of environmental factors on nitrogen transformations in soil irrigated using treated wastewater. Their results showed that the effect of water content and temperature on nitrification and denitrification is particularly significant for models simulating nitrogen dynamics under changing field and environment conditions.

Panoras *et al*. (2000) examined the possibility of reusing wastewater treated using activated sludge or stabilization tanks, for drip and channel irrigation of beet. The results showed that there was no health risk, as no pathogens were found in the treated wastewater. In one of the studies carried out by Panoras *et al*. (2001a), they investigated the effect of wastewater treated with activated sludge or stabilization tanks on cotton yield and natural soil properties using drip and channel irrigation. They concluded that wastewater treated with activated e sludge posed no risk to health. There may be a risk from wastewater treated using stabilization tanks. However, the use of treated wastewater causes increased soil salinity in both cases.

According to the JMD 145116/11 (GG B' 354/2011) the wastewater reuse shall apply on urban liquid waste and industrial wastewater as defined in the JMD 5673/400/1997 (GG B' 192/1997). The reuse may lead to production of drinking water, usually through mixing of the elaborated water with clean underground aqueous systems, and production of irrigating water allowed for agricultural use. Further the Joint Ministerial Decision determines the measures, procedures and processes for the reuse of treated wastewater.

2.3.1.2 Wastewater reuse in Spain

A new National Hydrological Plan has recently been published, which is favourable to the reuse of effluent for irrigation. In any case, the reuse of treated wastewater is already a reality in several Spanish regions for four main applications: golf course irrigation, agricultural irrigation, groundwater recharge (to stop saltwater intrusion in coastal aquifers) and river flow augmentation. Commercial interest exists and some private water companies invest in Research and Development activities, in collaboration with the Universities. Multiple projects have been implemented treating brackish wastewater for irrigation and seawater desalination for irrigation in water short regions. Since 1989, Consorci of Costa Brava in Girona, have operated an increasing number of water reclamation and reuse projects for non-potable uses. The total flow of reclaimed water produced by the 14 water reclamation plants (WRP) during 2010 was 6,400,000m^3, which represents 19% of the secondary effluent produced.

In Spain, the water reuse is being regulated by the Royal Decree 1620/2007, 7th of December, which establishes the legal regime for the reuse of treated wastewater. The main aim of this RD is to maintain a balance between the protection of health and the environment, providing a scarce and necessary resource as water, with a high level of quality. The RD 1620/2007 defines the reuse of the wastewater as the application, before its return to the public hydraulic domain, for a new use once having received the necessary treatments as to accomplish the water quality parameter values set.

In order to reuse the treated wastewater, it is necessary to possess reuse authorization and it will granted by the Basin Organization. The state Governments, regional or local, in order to encourage the reuse of the water and the efficient use of water resources carry out plans and programs for wastewater reuse. On the other hand, on the touristy Mediterranean coastline, the development of wastewater reuse is especially promising for golf course irrigation. In spite of the lack of progress at national level for the moment, various initiatives have been taken at regional level. Andalusia, Catalonia and Balearic islands have issued comprehensive wastewater reuse Guidelines essentially following the WHO Guidelines and are encouraging the practice.

Reclaimed wastewater use in cultivation is about 346 Mm3 /year (Pedrero et al., 2010). Future reclaimed wastewater use in Spain is expected to focus on coastal areas of the Mediterranean, the South Atlantic arc and the Balearic and Canary Islands (Iglesias et al., 2010) Barcelona Metropolitan Area covers 600 km2 and includes more than 30 municipalities, with a total population close to 3.5 million people, which is about 50% of the total population of Catalonia (currently estimated at 7.4 million) (Mujeriego et al., 2008). The treatment plant for wastewater from Barcelona metropolitan region was upgraded in 2002 to include biological secondary treatment using activated sludge and tertiary treatment of coagulation-flocculation, filtration, UV disinfection, post-disinfection and oxygen saturation for a volume of 14 400 m3 wastewater per hour (Cazurra, 2008). The plant produces wastewater with a quality suitable for environmental flow injection; ≤10mg

BOD5/L, < 10 CFU/100mL of faecal coliforms, 0.6 mg/L residual chlorine and ≥7.5 mg/L dissolved oxygen (Cazurra, 2008).

In another study examining the presence of pathogens, bacteria and protozoa in treated wastewater, Mosteo et al. (2013) surveyed effluent wastewater from five treatment plants in the region of Navarra in Spain. All treatment plants disposed of their effluent to the river Ebro, from which water is reused. The study revealed that the physical and chemical parameters of the treated wastewater are more or less in compliance with Royal Decree 1620/2007 and Directive 91/271/EEC, with some exceptions for turbidity and solids. However, the pathogen content of the effluent of all these treatment plants places restrictions on its use. With the current pathogen content, reuse of the effluent is limited to applications where there is no contact with humans or crops eaten raw. Beside the conventional pollutants usually found in wastewater (degradable organics solids and bacteria), chemical input from households and industrial activities to the sewerage system result in wastewater pollution with persistent organic compounds, also referred to as priority pollutants (Martí et al., 2011). These include compounds belonging to pesticides, polycyclic aromatic hydrocarbons (PAH), phenolic compounds, volatile organic compounds (VOD) and pharmaceuticals and personal care products (PPCPs). Barco-Bonilla et al. (2013) concluded that PAHs are the most predominant organic pollutants.

2.3.1.3 Wastewater reuse in Italy

The use of untreated wastewater has been practiced in Italy at least since the beginning of this century, especially in small towns and near Milan. Nowadays, treated wastewater is mainly used for agricultural irrigation covering over 4,000 ha. One of the largest applications is in Emilia Romagna, where over 450,000 m^3/yr of treated effluent is used to irrigate over 250 ha.

The use of wastewater for irrigation in Italy was regulated, since 1977 and till 2003, in the frame of the 1976 Water Protection Act (Annex 5, CITAI, 1977), being considered an extensive treatment process. The approach was in some respects quite stringent, no standards were set for toxic or bio-accumulative substances and a specific evaluation of the volume of wastewater which can be yearly applied, depending on soil and crops, was required. Following the frame of Law-decree n. 152, a new legislative set of rules was promulgated on June 12th, 2003 (Ministry Decree, D.M. no 185/03) applicable for agriculture, non-potable urban and industrial. The proposed standards seemed to follow a quite restrictive approach, especially for some chemical compounds: in many cases the quality standards for reclaimed wastewater were the same as drinking water. This approach surely led to some difficulties in promoting wastewater reuse, when the compliance with some very strict standards asked for advanced treatments, with all the related consequences on the economics of the reclamation. Finally, Italian National Standards for reclaimed wastewater are exposed in

Ministerial Decree nr 152 of May 2006, in order to regulate the use of wastewater.

2.3.1.4 Wastewater reuse in Cyprus

In Cyprus, the wastewater from the main cities is approximately 25 Mm^3/yr. It is planned to collect and use this wastewater for irrigation following tertiary treatment. According to this plan, agricultural irrigation will expand by 8-10%, while an equivalent amount of water will be used in other sectors (Papadopoulos, 1995).

Treated wastewater (3 Mm^3/yr) produced at Limassol WWTP (Plate 2.2) is used, directly or after storage in a reservoir, for irrigation of crops, green areas in hotels and for industrial use (cement factory). The main crops cultivated are fodder, olive trees, and fruit trees

Plate 2.2 Wastewater treatment plant of Limassol, Cyprus

Since 1990, there are provisional standards for treated wastewater reuse with quality criteria for irrigation. These standards are stricter than the WHO Guidelines and take the specific conditions of Cyprus into account. Since 2005, these standards went from provisional to definitive (Decree no 296/03.06.05). These criteria are followed by a code of practice to ensure the best possible application of the water for irrigation

2.3.1.5 Wastewater reuse in France

In France, crops have been irrigated with wastewater for many years (almost a century). Interest in wastewater reuse was revived in the early 1990s for two main reasons:

a) the development of intensive irrigated farming (such as maize); and

b) the fall of water tables following several recent severe droughts.

Due to this new interest in wastewater reuse, the Health Authorities issued in 1991 guidelines on the reuse of wastewater for crop and green spaces irrigation, after treatment. These guidelines essentially follow the WHO guidelines. In France, 20 to 30 wastewater treatment plants for water reuse cover over 3,000 ha of irrigated land. Today, one of the largest studies in progress in Europe is the recycling scheme for irrigating over 700 ha of maize.

2.3.1.4 Wastewater reuse in Malta

Malta is facing the most severe natural wastewater scarcity, compared to the rest of Mediterranean countries and has been characterized as an area of drought by the European Union. The country does not have any permanent streams, lakes or rivers. Wastewater reuse practices in Malta are governed by legislation number 340 of 2001 and came into force in 2004 (Matla EPA, 2004). The water supply problem has always been high up in the priority list of the authorities governing these islands. The Sant' Antnin Sewage Treatment Plant produce 12,000 m^3/day of reclaimed water (Gauci, 1993).

2.3.2 Wastewater reuse for irrigation in non-EU Mediterannean countries

Many non-EU Mediterranean countries face specific challenges, but the state of economic development and the availability of water resources mainly determine the extent of wastewater reuse. Lebanon for example has a high per capita gross domestic product (GDP) but hardly has any functioning sewer system, let alone wastewater treatment plants or a reuse scheme. Due to the available water resources and alternative income opportunities, reuse has no high priority in the governmental action plans. In contrast, Jordan has only half of the per capita GDP but a reuse rate of more than 90 % of its treated wastewater. Reasons are the severe water stress and political dependency on agriculture in the country (ACWUA, 2010). There is lack of sanitation strategies in most Arab Countries. Only Egypt and Jordan have more than 90 % sanitation coverage. Several countries have water management strategies (Egypt, Jordan, Tunisia) consider wastewater as an important alternative water resource and also implemented measures accordingly (Table 2.8). Other countries have set standards for WWTP effluents and irrigation water however, do not enforce or monitor them.

Table 2.8 Strategies and laws about reclaimed water applied on by non-EU Mediterranean countries (ACWUA, 2010).

Country	Strategies and laws
Egypt	Reuse is a basic element in agricultural irrigation due to the Nile river. Egypt has implemented a Code of Reuse of Treated Wastewater in Agriculture (2001/ 2005) it regulates quality criteria for reuse in agriculture, requirements for irrigation techniques, requirements for health protection, enforcements, monitoring, inspection and corrective measures. According to the code no edible crops or export crops can be cultivated and irrigated on wastewater – regardless of the treatment level.
Jordan	Jordan considers wastewater as a crucial water resource and promotes reuse in irrigation. The laws regulate monitoring duties and responsibilities, which are partly overlapping. Standards are set for WWTP effluents and sludge quality. Further guidelines exist for wastewater reuse. These guidelines are currently under revision and planned to become standards as well. Despite the existing regulations, no clear coordination among authorities exist which defines cooperation, data exchange and evaluation among these organisations. No institution signs responsible for overall coordination and guidance in case the public health is threatened by bad practices of reclaimed water use
Lebanon	Wastewater reuse is not considered in the national water policy. Laws, standards and regulations for water management are outdated due to the political situation. Minimum standards exist to assure the quality of drinking water and environmental limit values for regulating the discharge of wastewater. Standards for the water used or reused for irrigation do not exist yet..
Morocco	Wastewater reuse was just recently acknowledged as a strategy to combat the ever increasing water shortage. It will become part of an IWRM strategy. Laws and quality standards are sufficiently set with regard to wastewater reuse, however they are only partly enforced.
Syria	The Water Law of 2005 and other regulations are considering Water Demand Management, including Reuse of Wastewater. Strict quality standards exist but are hardly enforced and met by the plants.
Tunisia	Tunisia Government gives high priority to wastewater reuse as it is an important measure to safe and protect freshwater resources for drinking purposes. The legal framework (Water law) provides a good basis for wastewater reuse, but requires further definitions and amendments. Existing quality standards are not enforced due to a lack of treatment capacity.

2.3.2.1 Wastewater reuse in Israel

In Israel, integrated programs for wastewater reuse have led to wastewater accounting for 20% of water resources used in agriculture. In Europe, municipal wastewater treatment is required by Directive 91/271/EEC, and the degree of pre-application treatment is an important factor in the planning, design, and management of wastewater irrigation systems (Pedrero *et al*., 2010). Israel has been a pioneer in the reuse sector, quickly followed by Tunisia, Cyprus, and Jordan. Egypt, Palestine, Morocco and Syria belong to the group of countries in which the development of water reuse practices is vital. These practices, however, must be feasible in view of current socioeconomic conditions, i.e. lack of capital and limited experience, in both the construction and the operation of complex management systems, and also the unsuitable infrastructure, including sewers and wastewater treatment plants.

Strict reuse criteria, such as those proposed in California, by the US EPA (1992), and by industrialized countries, cannot be easily applied to these countries, due to economic, technological and industrial conditions. In Israel, approximately 92% of wastewater is collected by municipal sewers. Of that 92%, 72% is used for irrigation (42% of the total wastewater generated) or groundwater recharge (30% of the total wastewater generated). Effluent used for irrigation must meet water quality criteria set out by the Ministry of Health.

Plate 2.3 shows the WWTP of Tel-Aviv, the biggest in Israel. Tertiary treated effluent (125,000,000 m^3/yr) is produced which is then used to replenish the groundwater table via seven infiltration basins (Soil Aquifer Treatment). Water from the aquifer is then pumped southward about 100 km and stored in reservoirs for irrigation of more than 4000 private farms (mostly market gardening for export). The project is part of a national policy on production of non-conventional water sources.

Plate 2.3 Wastewater treatment plant of Tel-Aviv in Israel

2.3.2.2 Wastewater reuse in Tunisia

In Tunisia, wastewater is used (or treated) in about 45 wastewater treatment plants, with a total design capacity of 130 Mm^3 per year. Municipal wastewater is mainly domestic in origin (approximately 82% domestic effluent, 12% from industry and 6% from tourism) and is subjected to secondary biological treatment. No further treatment is provided due to cost. In 1992-1996, the annual volume of reclaimed wastewater was 147 Mm^3/yr, potentially allowing the irrigation of a further 18,000 ha. In 2001, treated effluent reached 152 Mm^3/ yr. The annual volume of reclaimed water is expected to reach 290 Mm3 in the year 2020. The expected amount of reclaimed water will then be approximately equal to 18% of the available groundwater resources and could be used to replace groundwater currently being used for irrigation in areas where excessive groundwater mining is causing salt-water intrusion in coastal aquifers. Plate 2.4 shows the WWTP of Chotrana having a capacity of 78,000 m^3/day.

Plate 2.4 Wastewater treatment plant of Chotrana in Tunisia

Salinity and the microorganisms content are the two major constraints related to secondary effluent quality. Reclaimed water is often salt-affected due to sea- or groundwater seepage into the sewerage network, to the plant location, and to industrial activities. This salt load limits the range of crops to irrigate and the benefits related to water reuse, and may affect the soil chemical and chemical properties (Bahri, 2007).

Tunisia Government gives high priority to wastewater reuse as it is an important measure to safe and protect freshwater resources for drinking purposes. The legal framework (Water law) provides a good basis for wastewater reuse, but requires further definitions and amendments. Existing quality standards are not enforced due to a lack of treatment capacity (ACWUA, 2010).

2.3.2.3 Wastewater reuse in Morocco

Most Moroccan towns are equipped with sewage networks that also collect industrial effluent. The annual volumes of wastewater discharges have risen sharply over the past three decades. They went from 48 million to 600 million m^3 between 1960 and 2005 to reach 700 million by the year 2010 (Salama et al., 2014). Of the 60 largest Moroccan towns, however, only 7 have treatment plants, and their design and operation are considered insufficient (US AID, 2009).

The Moroccan application Decree (No 2-97-875, 1998) related to the use of wastewater stipulates that no wastewater can be used if it has not been recognized as treated wastewater; however, most of the wastewater produced by inland towns is reused, mainly as raw or insufficiently treated wastewater, to irrigate about 8,000 hectares. The irrigated crops are mainly fodder crops, fruit, cereals, and produce. The growing and selling of vegetables to be eaten raw is prohibited if they have been irrigated with wastewater (US AID, 2009)

The country does not yet have any specific wastewater reuse regulations and usually defers to the WHO recommendations. The lack of wastewater treatment before reuse in inland cities has resulted in adverse health impacts, and Morocco experiences a high incidence of waterborne diseases. Amahmid and Bouhoum (2000) detected that the incidence of parasitic diseases in consumers of sewage irrigated crops was higher than that of the control population. In addition, Hajjami et al. (2013) found that 50% of crops from a farmland irrigated by the treated wastewater were contaminated by helminth eggs.

Improvement in wastewater reuse methods and in the quality of reuse water for irrigation is recognized as essential. The application Decree (No 2-97-875, dated February 4, 1998), acting as Water Law 10-95 related to the use of wastewaters, stipulates that no wastewater can be used if it has not been recognized as treated wastewater. The use of raw wastewaters is thus prohibited and banished.

2.3.2.4 Wastewater reuse in Jordan

Water resources in Jordan depend on variable rainfall and therefore are characterized by scarcity, variability, and uncertainty. The per capita share of renewable water resources is 145 m3 /capita/year and Jordan is therefore ranked fourth poorest country with regard to water resources worldwide.

The utilization of recycled water within Jordan has been made possible by the development and evolution of a sound legislative and legal foundation. There are several sets of standards that have paved the way. These include the first law regarding the operation of municipal sewer systems, which was first established in 1955, and the original public health standards first enacted in 1971. Today there are several sets of standards and guidelines for wastewater, sludge, soil and crops that were derived from the work of the Water Authority of Jordan and the Ministry of Water and Irrigation. However, also other organizations are involved. The existing standards and laws that directly apply to wastewater reuse are

- the Water Authority of Jordan Law No.18/1988 and its amendments,
- the Jordan Standard No. 202/2007 for Industrial Wastewater Discharges,
- Jordanian Standard 893/2006 for Discharge of Treated Domestic Wastewater, and
- Jordanian Standard No. 1145/2006 regarding the use of sludge.

The 2006 Standard 893 includes the following categories of wastewater reuse standards depending on the fate of domestic wastewater after it is released from the wastewater treatment facility:

- Recycling of water for irrigation of vegetables that are normally cooked,
- Recycling of water used for tree crops, forestry and industrial processes,
- Discharges to receiving water such as wadis and catchments areas,
- Use in artificial recharge to aquifers not used for drinking purposes,
- Discharge to public parks or recreational areas,
- Use in irrigation of animal fodder.
- Use of reclaimed water for cut flowers

The sewer networks in Jordan drain its load into 22 existing central Waste Water Treatment Plants (WWTP). The effluent is used for agriculture purposes inside the premises of WWTP and in their vicinities. Three out of 22 WWTPs (Khirbet Al Samra, Jerash, Baq'a) drain the biggest share of the total effluents to King Talal Reservoir where it is diluted by the annual rainfalls (Plate 2.4). Farmers in the middle Jordan Valley totally depend on this resource as they don't receive any surface water. Therefore, this dam is considered as a vital necessity for agriculture in Jordan Valley.

Wastewater in Jordan can be characterized as very strong with high salinity and insignificant heavy metals and toxic organic compounds. The effluent of Al-Samra wastewater treatment plant had an electrical conductivity of 2.65 mS/cm, a Na concentration of 355 mg/l and a chloride concentration of 350 mg/l that may be detrimental to certain trees and vines (Matouq, 2008). Yield potential of most crops grown in the Jordan Valley lie between 50–80% if effluent alone is used for irrigation (Ammary, 2007).

Plate 2.4 King Talal Reservoir in Jordan

2.3.2.5 Wastewater reuse in Turkey

Although the Turkish legislation on wastewater reuse in agriculture has already been established in 1991, there is no major improvement in its application since that time. In Turkey, only few wastewater reuse applications exist in small communities, where wastewater of domestic nature is used for irrigation of forest areas, gardens and parks (UEST, 2010). For instance, treated effluent originating from Ankara Metropolitan Sewage Treatment Works is used for irrigation of several crops. Currently there are planning efforts in Konya province, which is particularly known as the “grain cellar” and is the largest agricultural area of the country, to use secondary (biologically) treated urban wastewater for the irrigation of cereals. A comprehensive, regional project known as the Southern Anatolia Project also features wastewater treatment and reuse for agricultural irrigation purposes.

On the other hand, it has been reported that the indirect use of domestic wastewater as irrigation water is eventually illegally practiced in Turkey. The above mentioned and recently completed MEDA-Water project demonstrated that in most cases the quality of even secondary treated urban wastewaters sampled from different Turkish urban wastewater treatment plants is not suitable for agricultural use, mainly because these effluents do not meet most of the irrigation water quality criteria, such as total Coliform, sodium absorption ratio (SAR), conductivity, and salinity values given by the National Water Pollution Control Regulation Technical Aspects Bulletin (Arslan-Alaton et al. 2011).

2.4. Case studies on the effect on soils and plants of irrigation with treated municipal wastewater

Several research and pilot projects dealing with wastewater recycling and reuse have been carried out in Greece (Angelakis *et al*., 1999), and relevant research work continues on the plains of Agrinion (Kalavrouziotis *et al*., 2005b) and Patras (Kalavrouziotis *et al*., 2006), as well as in the vine-growing area of Metamorphosis in the Region of Attica (Sakellariou-Makrantonaki *et al*., 2006) and in Macedonia.

In recent years, experimental applications of wastewater in agricultural irrigation have also been carried out on *Agrostis* crops in the Thessaloniki area and on vines and olives in Heraklion and Hersonissos, Crete. Further research is necessary on the effect of treated wastewater on plant yields, natural soil properties and groundwater pollution.

2.4.1 Evaluation of wastewater on soil

According to Laurenson *et al*. (2011), elevated levels of exchangeable Na^+ and, to a lesser extent, K^+ can cause clay swelling and dispersion. Soils with a high exchangeable potassium percentage, however, are unlikely to disperse to the same extent as those with a high exchangeable sodium percentage and will require lower soil electrical conductivity concentrations in order to maintain flocculation. Monovalent ions applied with wastewater may have confounding effects on soils beyond that imposed by salinity alone. The divalent cations calcium (Ca^{2+}) and, to a lesser extent, magnesium (Mg^{2+}) contribute to the structural stability of soils. When the concentration of monovalent cations in the soil solution is high, however, divalent cations are readily displaced from the soil surface, resulting in a reduction in soil stability (Rengasamy and Marchuk 2011).

Both Na^+ and Cl^- are typically the most abundant salts in water used for irrigation; however, bicarbonate and K^+ are also abundant in many wastewaters (Stevens *et al.* 2004). With excess application of irrigation, accumulated Cl^- is readily mobilised towards the edge of the dripper zone and downward through the soil profile (Russo *et al*. 2009). Exchangeable monovalent cations (Na^+ and K^+) are less mobile in soils, and high concentrations can often be problematic (Dudley *et al*. 2008). Wienhold and Trooien (1995), for instance, reported considerable Cl leaching and subsequent lowering of the soil salinity during winter precipitation. A decline in soil macro porosity associated with an accumulation of monovalent cations can reduce the drainage capacity of soils, which in turn limits the percolation of water and subsequently the ability of growers to leach salts (Prior *et al*. 1992). Stevens *et al*. (2003) reported a reduction in hydraulic conductivity when irrigating with municipal wastewater. This was due to an increase of exchangeable Na^+ within B horizon soils that lead to a decline in salt leaching and a progressive increase in soil salinity of the A horizon.

The EU funded project with the title "Sustainable use of irrigation water in the Mediterranean Region/SIRRIMED) examine the use of treated wastewater for deficit irrigation of Mandarin trees in a commercial farm located in Campotejar-Murcia Spain. The accumulation of salts within different soil layers and at a different distance for the emitter was evaluated by measuring the electrical conductivity of a saturated paste extract. First year results shown that the electrical conductivity only increased with depth at 10 cm from the emitter in both treated wastewater and tap water treatments, with a steeper gradient under the treated wastewater treatment (www.sirrimed.org).

The EU funded project with the title “Sustainable orchard irrigation for improving fruit quality and safety/IRRIQUAL” deals with the valuation of new irrigation practices (including water doses implementation, water quality use and fertigation management). Applying treated wastewater to mandarin trees, grapefruit trees and lemon trees they concluded that the effects of different types of water were not evident on the seasonal evolution of soil water content. In soil salinity, the use of regulated deficit irrigation and reclaimed wastewater produced a salt accumulation more marked from 30 cm to the emitter (www.irriqual.eu)

2.4.2. Wastewater irrigation impact on tree crops

The use of TMWW on *Eucalyptus* sp., *Forsythia* sp., *Medicago arborea*, *Buddleia variabilis* and *N. oleander*, according to Mavrogianopoulos and Kyritsis (1995), significantly favoured plant growth, perhaps owing to the beneficial effect of the nutrients present in the treated wastewater.

Aucejo *et al*. (1997) reported boron toxicity in a citrus plantation in Villarreal (Valencia) irrigated with a mix of surface water, groundwater and treated wastewater. However, Reboll *et al*. (2000), after studying the effect of treated wastewater in Navelina orange trees for three years, observed that both growth and fruit quality parameters were unaffected by the high levels of sodium, chloride and boron in wastewater. It was observed that chloride, sodium and boron foliar concentrations did not exceed toxicity levels.

Similar results were obtained by Pedrero and Alarcon (2009), evaluating the effects of applying treated wastewater on citrus trees. Some negative effects were observed in the plant canopy due to salinity from the application of treated wastewater. It was concluded that the possibility of using reclaimed wastewater mixed with well water is a good solution for improving the agronomic quality of treated wastewater, that high salinity and boron concentrations are the main problems associated with treated wastewater use in the Region of Murcia, and that treated municipal wastewater seems to be an alternative water resource for citrus tree irrigation with correct salt management.

According to Pedrero *et al*. (2013), irrigation with reclaimed water could increase soil salinity and leaf boron concentration. The nutritional contribution of RWW could provide 24% and 15% respectively of the annual nitrogen and phosphorus (N and P_2O_5) fertilizer requirement for mandarin oranges, and RWW treatment could also satisfy the entire potassium requirement (K_2O). They observed that the quality parameters of mandarins were not affected by the use of RWW. Salinity on the other hand can be a major problem for mandarin trees irrigated with RWW. Sodium, B and Cl concentrations in RWW may exceed phytotoxic levels. The use of reclaimed water can cause some problems in the long term due to the accumulation of salts, sodium and B in the soil. Intensive monitoring is needed to avoid the degradation of agro-physical soil properties when reclaimed water is used for irrigation. It is apparent therefore that the use of RWW in agriculture requires appropriate crop selection and good irrigation and soil management practices.

According to the results obtained during the implementation of IRRIQUAL project: The microbial quality of the irrigation water did not influence the microbial quality of lemon fruits. Thus, the use of reclaimed wastewater as irrigation water for lemon trees did not represent a microbial risk for lemon fruits. In addition, irrigation with water from a tertiary wastewater treatment plant influenced the sensory quality of mandarins because reduced the overall visual qualityas well as the juiciness and flavour while promote the presence of white membrane. On the other hand, grapefruits treated with water from a tertiary wastewater treatment plant showed higher weight and size and also higher juice production. This could be probably due to the high content in organic material of this type of water

2.4.3. Treated municipal wastewater irrigation impact on olive trees (*Olea europaea L.)*

One of the plants selected for treated wastewater application in the present study was the olive, specifically the Koroneiki variety, which is best suited to olive oil production on the island of Crete. The olive is a vitally important tree in the Mediterranean area, due to the high economic importance of olive oil production. Greece is a major olive oil producing country with significant exports.

Although the olive is a perennial tree crop resistant to salinity and suitable even for barren, semi-arid regions, its yield is increased through correct irrigation and fertilization, and large quantities of water are used to irrigate olive groves each year. It is worth noting that the olive tree is alternate bearing, with a high yield every other year. Thus treated wastewater application in olive grove irrigation is extremely interesting and forms the subject of several studies.

According to Mufeed *et al*. (2011), investigating the impact of irrigation with reused treated municipal wastewater on soil and olive leaves, the heavy metal uptake by the olive plants (leaves and fruits) was not always related to the corresponding concentration of the wastewater, suggesting a selective absorption. Generally, smaller quantities of heavy metals compared to essential elements accumulated in olive fruits and leaves. Higher levels of Fe, Mn, Ca, and Mg accumulated in olive fruits than in leaves. The soil of the olive grove studied seems to be polluted with Mn. However, more work is needed in this respect to accurately quantify the seriousness and severity of the pollution. The trend of heavy metal transfer from soil to plant was similar for both olive fruits and leaves (fruits: Cu>Zn>Mn>Fe>Ni.>Pb>Cd, and leaves: Fe>Zn>Mn>Cu>Ni>Pb>Cd), suggesting a consistency in metal transfer from soil to plant.

Palese *et al*. (2009), investigating the effects of irrigation with municipal wastewater on the microbiological quality of the soil and the fruits of olive groves, concluded that even using wastewater with *E. coli* populations over mandatory limits, the correct management can ensure soil and fruit quality is not adversely affected. Irrigation with treated effluent can affect the soil hygienic features especially in the top 10 cm during the irrigation season, but there is soil quality recovery in winter.

Compared to other bacteria, *Clostridium* shows a slightly different behavior, especially in its distribution through the soil profile because of its resistance to the environment and the reversible adsorption mechanism of its spores in the soil. Therefore it may not be appropriate to use indicator species such as faecal coliforms to predict the behavior of species such as *Clostridium* that are spore formers. This suggests that long-term safe reuse of low-quality wastewater for irrigation of olive trees (and also other fruit crops) should be supported by guidelines which take into account more suitable indicators for the assessment and monitoring of microbiological quality of wastewater, soil and products.

According to Melgar *et al*. (2009), irrigation with treated wastewater (TWW) provides higher yields in olive trees than in those irrigated with well water (WW), due to the nutrition from elements such as N, P and K present in the wastewater. The irrigation treatment worked as fertigation. According to Bedbabis *et al*. (2010), irrigation with TWW caused a significant increase of leaf N, P and K in low and higher yield periods due to the amounts of N, P, and K supplied by TWW compared to WW. Olive trees generally exhibit significant variations in the seasonal levels of leaf nutrients. Phosphorus (P) concentration in the leaves during peak olive yield reaches high levels in winter and falls in summer. This decrease could have been caused by a high demand of P from the fruit (sink) and by the absence of irrigation, whereas the increase was associated with a slight demand of P for lipid biosynthesis and limited vegetative growth.

Based on the data provided by Pescod (1992), TWW can be used as complementary N and P fertilization sources and partially of K. According to Bedbabis *et al*. (2010), irrigation with TWW could cause limited vegetative growth retardation but a highly significant increase of the yield. The TWW could work as fertigation supplying N, P and K in large amounts. The application of TWW could cause an increase of Mn and Zn in soil and leaves but within the usual range detected in plants. Salt tolerance in olive trees can be based on the ability to limit ion (mainly Na and Cl) uptake by the roots and/or ion transport from the roots to the shoots (Chartzoulakis *et al*., 2002).

According to Segal et al. (2011), the transition to reclaimed wastewater (RWW) could not have an effect on olive tree growth and productivity, but could have environmental repercussions due to the transport of salts below the root zone. The utilization of nutrients in reclaimed wastewaters RWW allows the reduction of applied fertilizer and facilitates the minimization of nutrient transport below the root zone during the rainy season. Optimization between the reduced nutrients and increased salt transport requires continued long-term evaluation of crop production and environmental aspects of irrigation with RWW.

2.4.4. Wastewater irrigation impact on vines

One of the plants selected for the application of treated wastewater irrigation in this study was the vine (*V. vinifera* L.), specifically the Crimson seedless variety. This is a particularly vigorous, late-season red seedless table grape that adapts well to soils with limited fertility, does not benefit from excessive watering and N fertilization, and is a very good alternative for Crete, where the main cultivar for standardized production is the white Thompson Seedless. According to Goldspink and Cameron (2004), Crimson Seedless has been cultivated in California since 1989; in 2004 planting reached 8,000 ha, of which 6,300 were for commercial production. According to Younger (1996), the vine was one of the first irrigated plant crops.

Vine water deficits are mainly due to irregular temporal and spatial distribution of water. This phenomenon is more obvious in vines in arid and semiarid areas like Crete, growing in conditions of low soil moisture for the greater part of the germination cycle. Vine irrigation has a positive effect on the photosynthesis rhythm, yield, germination parameters and quality of the end product. The study of wastewater application for vineyard cultivation is thus extremely interesting.

In many grape-growing regions, shortages in water suitable for irrigating grapevines has led to an increased use of poorer-quality waters such as municipal wastewater. According to studies on the effect of treated wastewater on vineyard irrigation, field measures show that irrigating vineyards with municipal wastewater can increase soil salinity, alter vine nutrient uptake and reduce subsequent wine quality.

A high chloride (Cl^-) concentration in the leaf lamina can also decrease photosynthetic activity. Downton (1977), for instance, reported a 50% reduction in photosynthetic activity in Thomson Seedless grapes when laminae Cl^- concentrations exceeded 2%. Prior *et al*. (1992) demonstrated a similar correlation between Cl^- concentration in the leaf and a reduced rate of photosynthesis. According to Paranychianakis *et al*. (2008), the presence of salts in recycled water occurs at levels which may damage the irrigated crops. There is no particular leaf salt content above which leaf injury occurs, but it appears to depend on the prevailing climatic conditions. Irrigation of grapevines at sub-optimum levels exacerbated the impact of salinity on vine performance; suggesting that deficit irrigation should not be practiced when irrigating with water with elevated salt concentrations.

According to Paranychianakis *et al*. (2006), irrigation with municipal effluent can meet the needs of vines for P_2O_3, K_2O, MgO and Fe_2O_3 and eliminate the applied rates of commercial fertilizers. P^{3+} and K^+ occur in excess in recycled effluent, and attention should be paid to limit the potential impacts on the environment and on grape vine performance. In terms of N, generally additional fertilizer should be applied, in particular during the early development of grapevines when their water requirements are too low to meet the increased N-needs. Trace elements do not appear to represent a risk for vine performance or human health. They reported that soil water content had a significant effect on K^+ and Mg^{2+} uptake, implying that their availability may be managed with irrigation in order to alleviate potential impacts on the qualitative and quantitative components of yield in grapevines. The use of suitable rootstock appears to be an efficient practice to manage the availability of nutrients.

According to Mendoza-Espinosa *et al*. (2008), irrigating vineyards with treated wastewater can cause earlier growth and extension of the growing period compared to well water irrigation. This could 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.

2.4.5. Wastewater irrigation impact on ornamental crops

Kalavrouziotis and Drakatos (2002) studied the capacity of three Mediterranean forest plants: *Myoporum* sp. (Myoporum), *Nerium oleander* (Oleander) and *Geranium* sp. (Geranium) to absorb heavy metals from reused wastewater from sewage treatment plants. The results obtained showed that M*yoporum* sp. (Myoporum) and *Geranium* sp. (Geranium) tolerated the highest concentrations of Zn in the leaves, without displaying any signs of toxicity. Using as a reference point the plant heavy metal concentration data reported by Kabata – Pendias and Pendias (1992), according to which the toxic levels of Cu, Mn, and Zn in plants are 100, 500 and 400 mg/kg respectively. It may be concluded that *Myoporum* sp. (Myoporum) and *Geranium* sp. (Geranium) are accumulators of Zn, and therefore very tolerant to high concentrations of this heavy metal. *Geranium* sp. also showed a high tolerance for extremely high concentration of Cu in the roots (2015 mg/kg); however, *Nerium Oleander* (Oleander), though it accumulated high levels of Cu, showed signs of leaf toxicity (Kalavrouziotis and Drakatos, 2002).

The results showed low concentrations of Mn in the leaves and roots of *Nerium oleander* (Oleander), *Geranium* sp. (Geranium) and *Myoporum* sp. (Myoporum). It was concluded that the tolerance of forest for wastewater heavy metals varies according to plant species, and that this variable response must be taken into account when irrigating these and eventually other plant species with treated wastewater, to avoid toxicities.

<u>Wastewater irrigation impact on *Dianthus caryophullus* (carnation) cultivation</u>

Carnations have long been grown as a cut flower in many parts of the world, while their presentation as a pot plant is more recent and follows the development of dwarf species. *Dianthus caryophullus* (carnation) is one of the most popular commercial cut flowers in the world, ranked second only to roses in commercial importance. It is an herbaceous perennial plant growing to 80 cm tall. The leaves are glaucous greyish green to blue-green, slender, up to 15 cm long. The flowers are produced singly or up to five together in a cyme; they are 3–5 cm diameter, and sweetly scented; the original natural flower color is bright pinkish-purple, but cultivars of other colors, including red, white, yellow and green, have been developed (Huxley, 1992). Carnation is a plant that is relatively resistant to poor-quality water. To produce good-quality cut flowers, however, the elements contained in the irrigation water must be within the limits set out in Table 2.9.

Table 2.9 Maximum ion concentrations in irrigation water, above which carnation growth is adversely affected. (Kokas, 1991)

Ion	Concentration
pH	6.0-7.5
K^+	240 mg/L
Na^+	180 mg/L
Ca^+	200 mg/L
Mg^{++}	160 mg/L
Fe^{++}	200 mg/L
NH_4^+	40 mg/L
HCO_3^-	360 mg/L
Cl^-	140 mg/L
SO_4^{--}	800 mg/L
$H_2PO_4^-$	100 mg/L
NO_3^-	800 mg/L

*Dianthus caryophullus*_(carnation) cultivation is fertilizer demanding. At higher salt concentrations, the plants suffer even though they are hardier than other varieties. High EC levels cause a fall in carnation production and cut flower quality, leading to tough plants, narrow leaves, short stalks, grey plants and wilted flowers. Carnation cultivation is highly demanding in nitrogen (N). 1,000 m^2 of carnations require 80-100 kg of pure N per year. Nitrogen fertilization must always be applied in small doses throughout the year. This prevents the whole of the nitrogen fertilizer being absorbed into the soil; only part of it is absorbed, since it raises the total soil salts. The best application method is fertigation. The best soils for carnation cultivation are those with neutral to alkaline pH. Soil pH for carnations is also associated with diseases such as Fusarium wilt. When the soil pH is over 8, the plants suffer from various deficiencies such as iron deficiency.

Each 1,000 m^2 of planted carnations requires 15 - 20 kg of pure phosphorus per year. However, because phosphorus is less soluble in an alkaline environment, larger quantities are always necessary, approximately 40-50 kg per 1,000 m^2/yr. The potassium requirement of carnations is approximately 180 - 240 kg per 1,000 m^2. The ideal N-P-K ratio is 3.5:1:2 in the summer months and 2:1:3.5 in the winter. Carnations are also very demanding in Ca and Mg. According to Safi *et al*. (2014), irrigating carnations with treated wastewater can cause, after two years, intermediate values of Ca, high concentrations of Mg, Na, Fe and K, low concentrations of P and no accumulation of Mn, Cu and Zn in soil (Table 1.5).

There are limited publications on treated wastewater reuse for irrigating carnation crops. For the reasons stated above, the production of carnations in the floriculture industry using treated wastewater seems a very promising practice, especially in areas with water scarcity. This is why carnations are the floriculture plant selected for study in this dissertation.

2.4.6. Wastewater irrigation impact on vegetables

The effects of irrigating various vegetables with treated wastewater have been extensively studied. Kalavrouziotis *et al*. (2005), studying the elemental accumulation in *Alium cepa* (onion) and *L. sativa* (lettuce), concluded that higher levels of heavy metals accumulated in lettuce than in onion plant dry matter. In a similar study of *Brassica oleracea* var *italica* (Broccoli) and *Brassica oleracea* var *gemminifera* (Brussels sprouts), Kalavrouziotis *et al*. (2008), observed that irrigation with treated wastewater increased levels of P, Zn, Cd and pH in the soil of both crops, although within the necessary limits. They also noted that irrigation with treated wastewater reduced the concentration of P and Zn in leaf dry matter of Broccoli and increased the concentration of Ni, while it reduced Pb and increased Ni levels in the roots. It also increased the concentration of Cd, Co, Ni, and Pb in the heads of Broccoli and in Brussels sprouts and leaves. Irrigation with treated wastewater also increased levels of Cd, Co and Ni, while reducing Pb values in the root system of Brussels sprouts.

Kabata – Pendias and Pendias (1992) found that Zn, Cd and Pb accumulated in soil after irrigation with treated wastewater, although within the critical limits for normal plants. In a corresponding study on leafy vegetables, particularly *L. Sativa* L., Sardan Khan (2008) concluded that vegetables grown in wastewater-irrigated soils were contaminated with Cd, Cr, Pb and Ni exceeding the permissible limits for vegetables. The transfer factor for HMs was found to be in the order of Ni >Cd >Cu > Pb >Cr.

Maize irrigation with wastewater treated using activated sludge or stabilisation tanks was studied by Panoras et al. (2011). They concluded that there was no health risk from wastewater treated using activated sludge. Moreover, irrigation using channels closed at the end and drip irrigation satisfactorily protects farmers from coming into contact with the water. Trace element concentrations in the soil and plant tissues were quite low, in accordance with international criteria. However, the use of treated wastewater increased soil salinity.

Polycyclic aromatic hydrocarbons in vegetables

Vegetables grown in soils irrigated with wastewater may take up polycyclic aromatic hydrocarbons (PAHs) in sufficient quantities to cause negative effects on consumers. The build-up of PAHs in plants depends on soil concentrations, plant species, and microbial population (Kapusta 2004). Previous work reported the accumulation of high concentrations of PAHs in plants cultivated on PAH-contaminated soils (Zohair *et al.* 2006; Khan et al. 2008; Cai *et al*. 2008). Different mechanisms may be responsible for the transfer of organic contaminants from soil to plants, including sorption, uptake through transpiration or volatilization and subsequent deposition on leaves (Wild *et al*. 2004). PAHs are known to be recalcitrant and mutagenic/carcinogenic pollutants, and there is serious concern about their presence in the environment, especially their tendency for bioaccumulation in food chains (Jian et al., 2004). The accumulation of PAHs and HMs in the soil environment is of increasing concern because of their impacts on soil health, food safety and potential health risks. Food chain contamination is one of the important pathways for the entry of these toxic pollutants into the human body.

Vegetables cultivated on wastewater-contaminated soils may take up these pollutants in sufficient quantities to cause consumer health problems. Plant uptake of PAHs varies significantly, and is affected by several factors including initial soil concentrations, plant species and soil microbial population (Kapusta *et al*., 2004). Numerous studies have demonstrated that vegetables accumulate high concentrations of PAHs when grown in PAH-contaminated soils (Samsoe-Petersen *et al*., 2002). According to Fryer and Collins (2003) and Wild *et al*. (2004), several mechanisms, including uptake through transpiration stream, volatilization and subsequent re-deposition on leaves and sorption from direct contact with soil particles, are responsible for the transfer of organic pollutants from soil to plant tissues. In recent years, a number of articles have addressed the sources, accumulation and transfer of HMs of wastewater contaminated soils (Rattan *et al*., 2005; Liu *et al*., 2005). However, information regarding the combined uptake, translocation and accumulation of PAHs and HMs present in wastewater-contaminated soil is still under study.

According to Sardan Khan *et al*. (2008), leafy vegetables, particularly *L. satuva* L., grown on wastewater-contaminated soils contain PAHs and HMs in shoots and roots in elevated concentrations. The concentration of PAHs in roots and shoots is related to their solubility. The soil-to-plant transfer is one of the major pathways of PAH transport into shoot and root of plants grown in wastewater-contaminated soils. LMW-PAHs (R^2 between 0.51 and 0.92) such as Naphthalene (Na), Acenapthlene (Ace), Acenaphthylene (Acy), Fluorene (Fl), Phenanthrene (Ph), and Anthracene (An) can dominate in shoots and roots due to their high solubility, thus resulting in greater uptake and translocation of PAHs into plants. The concentrations of LMW-PAHs in the roots can be two to three times lower than the soil concentrations, while LMW-PAH concentrations in shoots can be four to five times lower than the respective soil concentrations. Shoot and root concentrations are positively related to soil concentrations. Similarly, the HMW-PAHs (R^2 0.02 and 0.60) such as Fluoranthene (Flu), Pyrene (Pyr), Benzo(a)anthracene (BaA), Crycene (Chr), Benzo(b)fluoranthene (BbF), Benzo(k)Fluoranthene (BkF), Benzo(a)pyrene (BaP), Dibenzo(a,h)antracene (DBA), Indeno(1.2.3-cd)pyrene (InP), and Benzo(ghi)perylene (BghiP) can be concentrated in the root samples at values two to three times lower, while shoot concentrations can be 10–16 times lower than the respective soil concentrations.

2.5. Summary of literature review

Each country around the Mediterranean Basin has a different character as far as wastewater reuse for municipal and industrial applications is concerned. Among the Mediterranean countries, Israel, Cyprus and Spain are leading in water reclamation and reuse technologies and applications. All countries are explored separately in terms of their reuse profile. Adoption of wastewater reuse practices does not seem to be independent of the adoption of legislature in each country, although the stage of development of the country itself does play a role in the adoption of wastewater reuse practices. In European Union countries, because of the financial incentives associated with EU guidelines established in member states, a push for development can be observed. In the non-EU countries the stage of adoption is linked to the state of economic development of the country.

In any case, the use of treated wastewater for agricultural usage continues to expand in Mediterranean countries due to the benefits it offers such as: a solution to irrigation water scarcity; the availability of large amounts throughout the year; the possibility to reserve 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.

However, inadequate handling of irrigation with treated wastewater could produce excessive accumulations within the plant and soil, negatively affecting the yield and production quality. The main problems caused by the use of wastewater result from the presence of biological and chemical contaminants, most importantly those that have not been treated. These could harm the agricultural environment, as well as the health of farmers and consumers as they could cause a build-up of chemical contaminants in the soil, cause the mobilisation of contaminants from the soil to the crop due to cultivation, lead to soil salinization and cause diseases for both the farmers, who are in direct contact with the water, and for consumers if the crops have been colonised by pathogenic micro-organisms.

Inorganic chemical contamination is basically due to heavy metals, As and Na. The concern over these elements is due to the fact that they are not biodegradable. They are absorbed by the crops and they can easily accumulate in different parts of the human body, even if they are present in low concentrations, as the body has no effective elimination mechanism. Organic contaminants that appear in urban wastewater are from diverse origins. The majority are found in the remnants of soaps, detergents, general cleaning products, pesticide residues and organic material in the stages of decomposition. There are certain groups of contaminants that, due to their chemical properties, are not very soluble in water, and as a result they appear in wastewater in very low concentrations. This is the case with PAHs, which are important contaminants because they are highly toxic, and have mutagenic, teratogenic and carcinogenic properties.

The aim of this thesis, was to evaluate the suitability of treated wastewater for irrigation in four Mediterranean crops (olives, grapes, radishes and carnations) by studying the effect of irrigation waters on the soil-plant system, the crop yield, fruit quality and the presence of inorganic chemical contamination (salts, elements and heavy metals), organic chemical contamination (PAHs) and microbial contamination (E.Coli, total coliforms) as crop food safety parameters.

Chapter 3

Material and Methods

3.1. Introduction

Four crop experiments were conducted using: a) olive trees, b) grapevines, c) radishes and d) carnations. The experiments were carried out in open fields for the first two crops and in a greenhouse for the other two as farmers in Crete grown these crops with the same type of cultivation (open fields for olives and grapes and greenhouses for vegetables and ornamental plants). All experimental fields were located on the farm of the Technological Educational Institute of Crete (TEIC), Greece (N 35^{o}, 19”; E 25^{o}, 10”).

All experiments were based on the implementation of five different treatments in terms of the quality of the irrigation water: 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 enriched with fertilizers (FTW) and e) irrigation with tap water (TW) as the control treatment.

3.2. Irrigation treatments

Primary treated wastewater was obtained from the wastewater treatment plant of Heraklion (180.000 p.e.), Crete, Greece. The wastewater treatment plant of Heraklion (WWTPH) is one of the largest facilities in Greece and was designed to treat the sewage of municipalities of Heraklion, Nea Alikarnassos and Gazi receiving mainly domestic sewages from a combined system with limited industrial input. The total capacity is 30,500 m^3/day, however the plant currently receives approximately 20,000 m^3/day. The facilities include four ellipsoidal aeration tanks, which form two parallel subsystems and accomplish full nitrification and denitrification (Plate 3.1).

Plate 3.1 Municipal wastewater treatment plant of Heraklion, Greece.

A small amount of primary treated wastewater was pumped from the WWTPH to the experimental wastewater treatment plant of TEIC (Plate 3.2), where it was further treated using a free water surface constructed wetland and a compact packed bed filter (Advantex-AX20, Orenco) arranged in series 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.

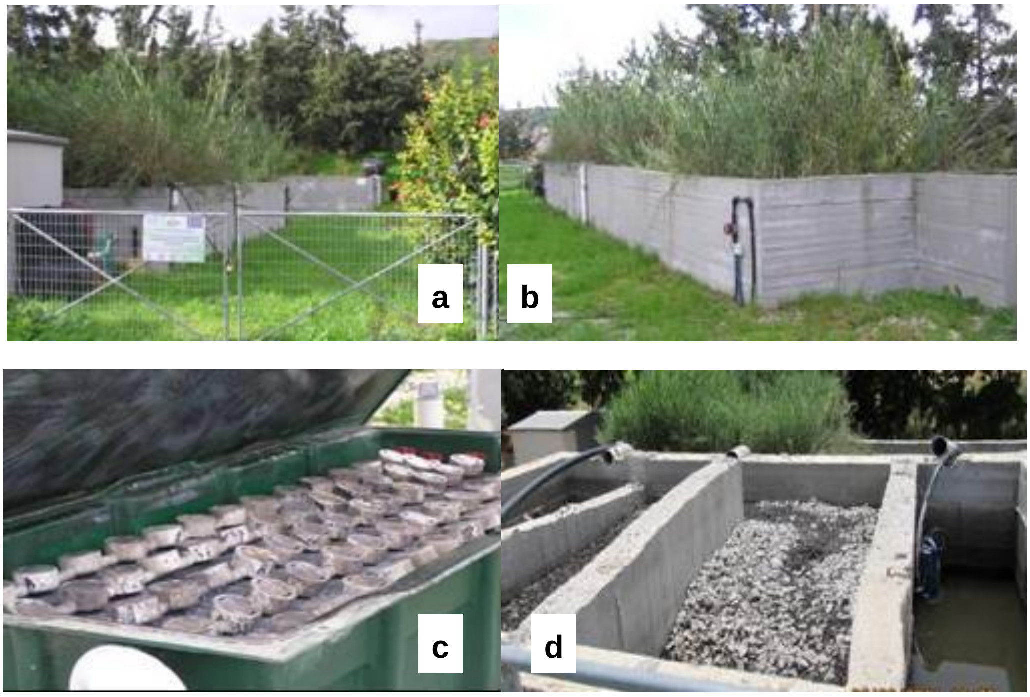


Plate 3.2 Experimental wastewater treatment plant at TEIC, a: general view, b: constructed wetland, c: packed bed filter and d: sand filter.

For the treatment using tap water enriched with fertilizer the appropriate fertilizer was added to each crop according to the schedule presented below:

- Olive trees: Fertilizer (400g/plant) was added to the soil 20cm around of the tree base, by the end of December each year. A compound fertilizer with the brand name Nitrophoska was used, consisting of 14% N, 7% P_2O_5, 17% K_2O, 2% MgO, 9% SO_3 and microelements (0,02% B, 0.01% Zn)
- Grapevines : Fertilizer (400 g/plant) was added to the soil, under vine foliage, by the end of December each year. The type of fertilizer was the same as that for olive trees.

- Radishes : A hydroponic solution was used, consisting of calcium nitrate, ammonium nitrate, Fe-chelate, potassium nitrate, magnesium sulphate, magnesium nitrate, potassium sulphate, phosphoric acid, manganese sulphate, zinc sulphate, copper sulphate, boric acid, ammonium molybdate and nitric acid. The final concentration of nutrients in the irrigation water was: NO3-N =17.0, NH_4-N=0.6, K=8.0, PO_4-P=1.0, Ca=5.5, Mg=2.25, SO_4-S=1.56, Na=1.3 mmol/L and B=40.52, Fe=2-.39, Mn=12.02, Cu=1.1, Zn=5.02, Mo-0.52 µmol/L with a pH value of 5.6 and EC value of 2.41 mS/cm.
- Carnations : 5 g of chemical fertilizer (12% N, 6% P_2O_5, and 30% K_2O) was added per 10 litres of tap water.

3.3. Climate

Climatic data for the experimental site (rain, temperature and humidity) were obtained from a meteorological station at a distance of approximately 0.2km from the olive grove and the vineyard. Average temperature and rainfall values are presented in Figure 3.1. The highest monthly mean temperature was 28.4°C (July 2012) and the lowest 10.5°C (January 2012). Mean annual precipitation was 442 mm, 457 mm and 563 mm for 2010, 2011 and 2012 respectively, falling mainly between October and April. The average annual humidity ranged from 53% to 73%.

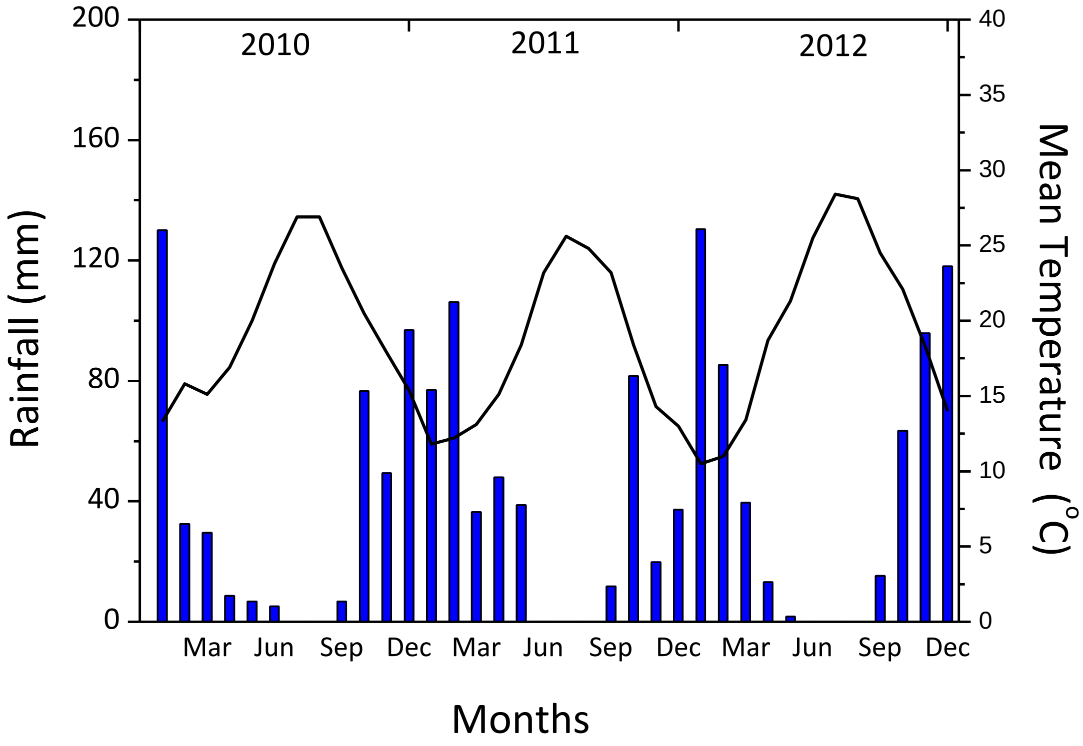


Figure 3.1 Monthly rainfall (blue bars) and monthly mean temperature (black line) recorded at the experimental site in 2010, 2011 and 2012.

Climate could be characterized as typical “Mediterranean” as the experimental site had relatively mild winters and very warm summers receiving almost all of their precipitation during their winter seasons while the summers are dry.

3.4. Experimental sites, plant material and design

3.4.1. Olive grove

3.4.1.1 General

The olive experiment was conducted over 3 years (2010-2012) in an experimental olive grove located on the farm of TEIC. Three-year-old olive trees (*Olea europea L.,cv. 'Koroneiki'*), approximately 1m in height, were tested. In total, fifty olive trees were grown outdoors using drip irrigation with five different qualities of irrigation water. The experimental plot was divided into five experimental rows, each row consisting of ten olive trees and irrigated by different qualities of irrigation water. The first row was irrigated with PTW, the second row with STW, the third with TTW, the fourth with FTW and the fifth with TW (Figure 3.2). Each row (treatment) was isolated from the next by a plastic film (1.5 m in depth), to ensure that no irrigation water treatment would interfere with the neighbouring ones.

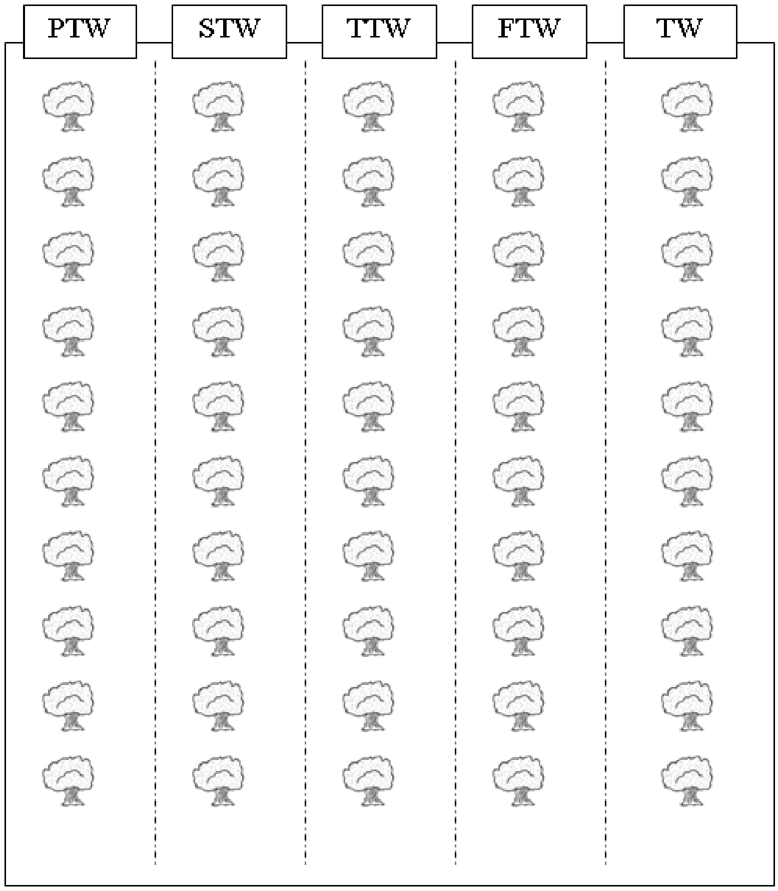


Figure 3.2 Layout of the experimental olive grove: five irrigation treatments x ten replicates. Dotted lines represent the plastic film used to separate adjacent rows (1.5m depth).

3.4.1.1. Application of wastewater irrigation in olive grove

In the first year, the application period of wastewater ran from July to September 2010, through an irrigation system with drip emitters. A 200L tank was placed in front of each treatment. The first tank was filled with PTW, the second with STW, the third with TTW, the forth with FTW and the fifth with TW. The water from each tank was supplied through a line (diameter 32mm) to the olive trees using drip irrigation (Plate 3.3). Irrigation was supplied by one drip emitter per tree using one line for each tank. The line was spaced 0.2m from the tree trunks. The emitters had a discharge rate of 12 L/h, to meet the irrigation requirements. Irrigation frequency was once per week and the amount of water was 40 L per irrigation per tree, corresponding to an annual amount of irrigation water of 0.48 m^3 per tree.

Plate 3.3 Olive grove irrigation lines, 2010.

In order to irrigate olive trees faster and easier, the irrigation system changed at the start of the second year irrigation period. The tanks were abolished and hydrometers were placed at the start of each line in order to measure the amount of irrigation water. The irrigation frequency was again once per week while the amount of water was 75L per irrigation per tree. The total amount of water used for irrigation was 1.2 m^3 per tree. The second and third irrigation period was from June to September. The olive grove experiment was completed in December 2012 when the olives were harvested.

3.4.1.2. Sampling procedure

Soil sampling was conducted four times per year, in spring (May), summer (Aug), autumn (Oct) and winter (Feb). The spring, summer and autumn soil samples were collected before, in the middle and at the end of the irrigation period respectively, while winter samples were collected in the middle of the rainy period. Surface soil samples (0-30cm) were collected near the drip emitters in each irrigated row and air dried. Stones were removed and the samples were sieved through a screen before analysis (Sparks, 1996).

Leaf samples were collected simultaneously with the soil samples. The fourth and the fifth pair of leaves from the new germination of annual growth from each tree were collected in order to gather fifth- and sixth-month leaves and prepare three samples of 200 leaves from each treatment. The leaves were washed once with tap water and twice with distilled water before being dried at 75^{0}C and ground before analysis (Jones *et al*., 1991).

3.4.1.3. Growth monitoring and yield

For the olive tree experiment, the trunk diameter (20 cm above ground level) and the height of each tree was measured at the start (May 2010), in the middle (Oct 2011) and at the end of the experiment (Aug 2012). Leaf chlorophyll fluorescence was measured annually (in August) using an OS-30p chlorophyll fluorometer (Opti-Science) after a 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 then calculated. Leaf chlorophyll content as expressed by SPAD value (Special Products Analysis Division) was also measured during the last irrigation period (every 20 days from June to August 2012) at the midpoint of leaves with a SPAD-502 chlorophyll meter (Konica Minolta). Mature leaves without visible injury symptoms were selected for both analyses. In order to measure the yield, 200 olive fruits from each treatment were collected to measure diameter and length and they were also weighed. The stone was then separated from the fruit and they were weighed again and and oven-dried at 75^0C to measure the dry weight. Tree height was also measured.

3.4.2. Vineyard

3.4.2.1 General

The grapevine (*Crimson Seedless*) experiments took place simultaneously with the olive tree experiments. The experimental plot was exactly the same as the olive tree experimental concept. Each row consisted of eleven vine stocks and was irrigated with the five different qualities of irrigation water (Figure 3.3).

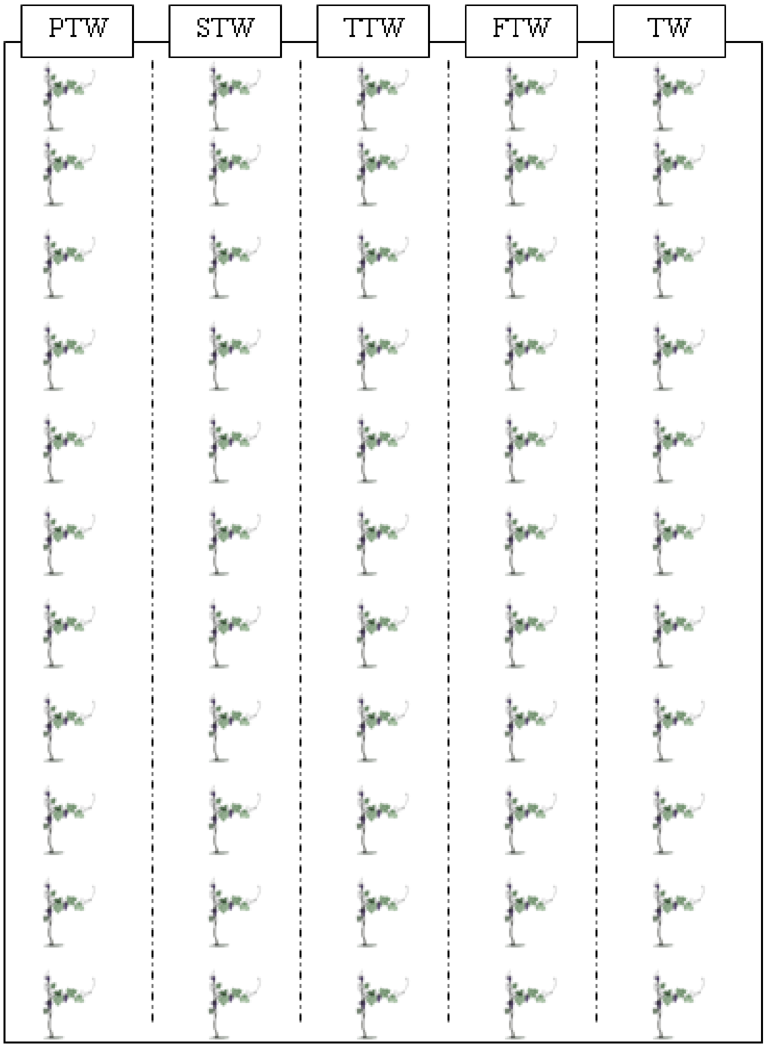


Figure 3.3 Layout of the experimental vineyard: five irrigation treatments x eleven replicates. Doted line represents the plastic film used to separate adjacent rows (1.5 m depth).

3.4.2.2. Vineyard planting

The vineyard was set up on the farm of TEIC next to the olive grove. By the end of March 2010 fifty-five vine shoots of *Crimson Seedless* grapevines on 1103P rootstock were planted (Plate 3.4), 2m apart, in five rows. Each row (treatment) was isolated from the next by a plastic film (1.5 m in depth) (Plate 3.5), to ensure that no wastewater treatment would interfere with the neighbouring ones. The vineyard experiment ended at the end of August 2012 after harvesting.

Plate 3.4 Vineyard planting, spring 2010

Plate 3.5 Isolation of each row before planting using plastic film.

3.4.2.3. Application of wastewater irrigation in vineyard

In the first year the application period for the vineyard was again from July to September, through an irrigation system with drip emitters. Irrigation was supplied by one drip emitter per plant using one line. The line was spaced

0.2m from the trunk. The emitters had a discharge rate of 12 L/h to meet irrigation requirements. Irrigation frequency was two times per week. In the second year, the irrigation system was changed. The tanks were abolished and hydrometers were placed in order to control the amount of water used for irrigation. The total amount of water used for irrigation was 1.8 m^3 per vine per year.

3.4.2.4. Cultivation techniques

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 80cm. During the first irrigation period the shoots were pruned again in order to give a linear bilateral shape. In January 2011 the woody shoots were cut. The vine plants were pruned in February in order to develop a bilateral cordon (Plate 3.6). The trellis system consisted of 1.6m stakes and three cross-arms (30 cm, 40cm and 50 cm wide respectively). Sprouting of the new vegetation for the second year of implementation was at the beginning of June. The same cultivation methods were applied in the third application period.

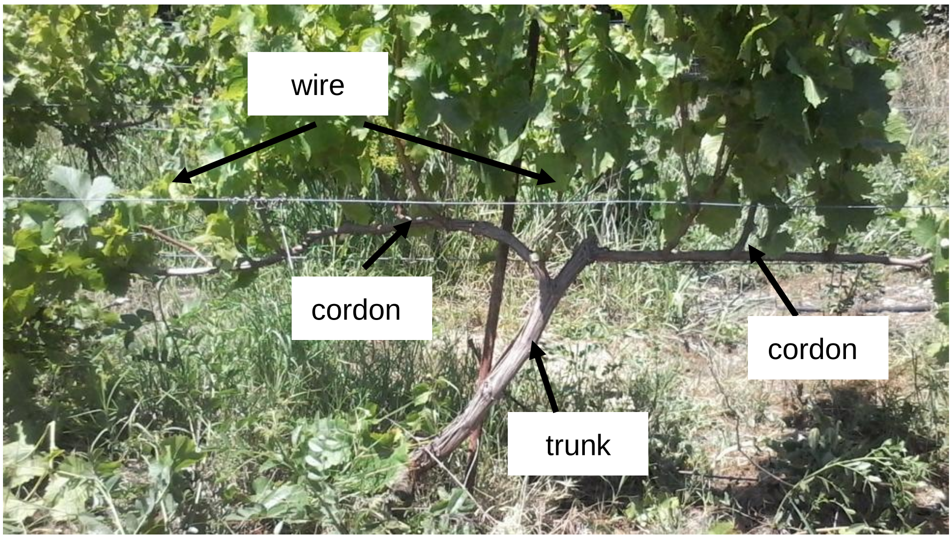


Plate 3.6 Bilateral cordon as training system for the experimental vineyard.

3.4.2.5. Sampling procedure

Soil and leaf sampling was conducted 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, were dried before the stones were removed, and sieved through a 2mm screen before analysis. Leaf samples (3 per vine) were collected from the 11^{th} to the 13^{th} node. Leaves were washed once with tap water and twice with distilled water before being dried at 75°C and ground before analysis.

3.4.2.6. Growth monitoring

Trunk diameter (20 cm above ground level) and plant height of each vine were measured at the beginning (August 2010) and at the 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 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.

3.4.2.7 Yield and fruit quality

On the 5th November 2012 all vines were individually harvested and the fruit production was measured. Total soluble solids content (^{o}Brix) was determined with a 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 litre. 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).

3.4.3. Radish

3.4.3.1 General

The radish (*Rapanus sativus*) pot experiment began in November 2010 and lasted 67 days (November 8th to January 14th. Four earth banks within a greenhouse were shaped and covered with plastic in order to place the radish pots. The radish pots were 17 cm deep, with a total capacity of 20L and holes for the proper runoff of the irrigation water. The plant material used was seeds of the “Large Red” variety. The soil was taken from an open field of TEIC, mixed thoroughly with sand in a 2:1 ratio and passed through a 5-mm sieve. Twenty radish seeds were sown in each pot (Plate 3.7), at 1.5cm depth. After germination, five days after sowing, the seedlings were thinned to 4 uniform plants per pot

Plate 3.7 Radish planting

3.4.3.1. Application of wastewater irrigation in the radish experiment

The irrigation treatments were with the same five water types as those used in the olive tree and grapevine projects. Eight pots containing four plants each were used for each treatment (Figure 3.4). Watering was performed manually using a can and a fabric at first, in order to avoid seed translocation (Waters gently newly planted seeds without washing the soil away). The frequency and amount of irrigation was 1L per two days at first and then twice a week with 0.5 L. In total, the amount of the medium used for irrigation was 14 L/pot during the entire irrigation period. The 40 pots were placed in such a way that no irrigation treatment could interfere with the next (Plate 3.7).

Figure 3.4 Layout of the experimental radishes: five irrigation treatments x eight pots per treatment x four plants.

3.4.3.2. Plant cultivation

Pots were weeded manually every week. On the 5th January 2011 foliar spraying with copper compound (Redomil) was carried out in order to prevent fungal diseases. No other cultivation treatments took place.

3.4.3.3. Sampling procedure

Before the first watering, a soil sample was taken from every pot to make a total sample of 1 kg. From then on, soil samples from 0 to 10 cm depth were taken every 10 days. The radishes were harvested on the 14^{th} January 2011. Root and shoot samples were separated for further analysis using the same methods as the olive tree and grapevine experiments.

3.4.3.4. Growth monitoring, yield and fruit quality

In the radish experiment, leaf chlorophyll fluorescence and leaf SPAD were measured, as well as root colour, in the same way as for the grapevine experiment. In January 2011 red root length, root thickness, shoot thickness, number of leaves, and fresh and dry weight of roots and leaves were measured. Cracking and market quality were also assessed. In the case of cracking, the two measurements used were: a) percentage of radishes with crack and b) the grade of crack per radish. The grade of crack was assigned on the basis of the depth of radial crack on each radish and shown in Table 3.1.

Table 3.1 Grade of radial cracking for radish

Grade	Crack
Grade 1	Very Shallow, 0-15mm length
Grade 2	Shallow, 0-25mm length
Grade 3	Deep, whole radial
Grade 4	Open fruit

For fruit marketability, four customers were asked to grade each radish on a scale of 1 to 5 where 1 is not of a marketable quality; 2 is of a low quality, 3 is medium quality, 4 is good quality and 5 is extra quality. Root color was measured at the end of the experiment using a CR-300 colorimeter (Konica Minolta).

3.4.4. Carnations

3.4.4.1 General

The experiment was conducted from July 2012 to September 2012 in a greenhouse (Plate 3.8) at the farm of the School of Agricultural Technology of Crete, Greece. Rooted cuttings of carnations viz. ‘Dover’ were established into 650ml plastic pots, one plant per 10.5cm pot, filled with a mixture of perlite (33.3%), peat (33.3%) and potting soil (33.3%) by weight. The experiment had a random block design with 25 pots per treatment (total 125 pots: Figure 3.5).

Plate 3.8 Carnation growth during the experiment

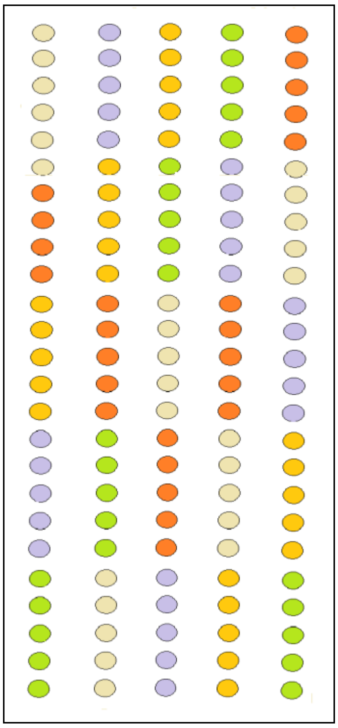


Figure 3.5 Layout of the experimental carnations. Each treatment is indicated by a different color (green: PTW, purple: STW, yellow: TTW, orange: FTW, white: TW).

3.4.4.2. Application of irrigation with carnations

Plants were irrigated 2 to 4 times per week, depending on 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.

3.4.4.3. Cultivation techniques

All plants were pinched 15 days after planting, leaving two or three nodes. During the experiment plants were sprayed with a fungicide (Iprodione 75%, Rovral) and a miticide (milbemectin 0.93%, Milbemeknock) for fungal and mite control respectively.

3.4.4.3. Sampling procedure

Leaf samples were collected at the end of the experiment, washed once with tap water and twice with distilled water, dried at 75°C and ground before analysis.

3.4.4.4. Growth monitoring and yield

Leaf SPAD was measured at the midpoint of leaves with a SPAD-502 chlorophyll meter (Konica Minolta). Chlorophyll fluorescence was measured using an OS-30p chlorophyll fluorometer (Opti-Sciences). When two flowers per plant were opened (at the end of the cultivation cycle) the following measurements were made: fresh weight, plant height, width and number of branches. The number of open flowers and their fresh weight, height and diameter were also determined.

3.5. Measurements

3.5.1. Water/wastewater analysis

Water and wastewater samples were analysed for total suspended solids (TSS) by the glass fibre method in accordance with Standard Methods (APHA, 2005). The pH was measured with a WTW, 3110 pH-meter and electrical conductivity (EC) with a Hanna, 8333 conductivity meter. Chemical oxygen demand (COD), total nitrogen (TN) and total phosphorus (TP) were determined spectrophotometrically using standard test kits (Hach-Lange).

The analysis of macro-elements (K, Mg, Ca) and micro-elements (B, Cu, Zn, Cr, Ni) was carried out using inductively coupled plasma mass spectroscopy (ICP-MS-Agilent 7500-CX) according to the EPA 6020A method. The ICP-MS operating conditions that were used can be seen in Table 3.2

Table 3.2 ICP-MS operating conditions

Parameter	Conditions
RF power (W)	1500
Plasma gas flow (L/min)	0.82
Auxiliary gas flow (L/min)	0.26
Sampling/Skimmer cone	Ni
Nebulizer type	MicroMist
S/C temperature	2°C
Replicates	3
Collision gas	He
Collision gas flow (mL/min)	4.5
Reaction gas	H_2
Reaction gas flow (mL/min)	3.5
Solution uptake (uL/min)	160
Integration time (sec per mass)	0.3

PAHs were recovered from water/wastewater samples using liquid-liquid extraction with hexane as an extraction solvent, as described by Manoli and Samara (1996). All extracts were filtered through 0.45-μm PTFE membrane filters and analyzed using High Pressure Liquid Chromatography (HPLC-Agilent 1200 Series) with programmable Fluorescence Detection by injecting 20 μL into the system. In total, the PAHs determined were: fluorene (Fl), phenanthrene (Phe), fluoranthene (Flu), anthracene (Ant), pyrene (Pyr), chrysene (Chr), benzo(a)anthracene (Baa), benzo(a)pyrene (Bap), benzo(b)fluoranthene (Bbf) and benzo(k)fluoranthene (Bkf). The chromatographic separation was done using a reversed-phase Hypersil Green PAH analytical column of 150mm x 4.6mm and 5μm particle diameters from Thermo Scientific. The mobile phase started with a 5 min isocratic step at 55% acetonitrile and 45% water, followed by a linear gradient to 100% acetonitrile at 22 min. The time program of the fluorescence detector (FLD) is presented in Table 3.3. The mobile phase flow rate was 1.2 ml/min and the column temperature was 25°C.

Table 3.3 Time programme of FLD for PAHs analysis

Time (min)	0	14
Excitation wavelength (nm)	280	260
Emission wavelength (nm)	324	420

3.5.2. Soil and plant analysis

Air-dried soil samples were sieved through a 2mm 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-Blank 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 spectrophotometrically (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, Sherwwod). The extraction of macro-elements (Mg, Ca) and heavy metals (B, Cu, Zn, Cr, Ni) prior to ICP-MS was performed by Microwave extraction (Microwave 3000, Anton Paar). For microwave extraction a modified EPA 3051A was performed. An appropriate amount of sample (0.25-0.5 g) was digested with 9 ml of 69% HNO_3 according to the program shown in the Table 3.4.

Table 3.4 Microwave extraction parameters for heavy metals

Stage	Power	Ramp in	Hold	Comment
1	600 W	6 min		At 800W:
2	800 W		14min	Max Temperature: 200^{o}C Max Pressure: 40bar
3	0 W		15min	Reach room temperature

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. The extracts were centrifuged and filtered (0.45μm) prior to ICP-MS analysis.

Leaf samples were collected, washed with distilled water, oven-dried, ground, homogenized and stored. P in leaves was determined by the vanado-molybdate yellow method (Allen, 1976). K and Na analysis was carried out using a flame photometer (Model 410, Sherwood) after dryashing at 550°C in an oven and digestion of the ashes with HCl. For N, PAHs and heavy metals analysis the same methodology was used as for soils.

3.5.3. Microbiological 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 olives, 10g of sample were extracted with 100 ml of sterilized Ringer's solution. The extract was added to the tray. Sealed trays were incubated for 18 h at 37°C, after which the MPN of total coliforms and *E. coli* were determined. (Plate 3.9).

Plate 3.9 Determination of pathogens with IDEXX Quanti-Tray®

For soil samples the membrane filtration technique (APHA, 2005) was used to enumerate the same bacterial indicators (Plate 3.10). 10g of soil was added to 95ml of Ringer's solution. 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°C for 21h for total coliforms and 37°C for 24h for *E. coli***.**

Plate 3.10 Determination of pathogens with membrane filtration technique.

3.5.4. Colour analysis

Colour in both grapes (Plate 3.11) and radishes was measured using a CR-300 colorimeter (Konica Minolta).

Plate 3.11 Measuring color of grapes with CR-300 colorimeter

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:

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

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

The L*a*b* colour space (also referred to as CIELAB) is presently one of the most popular colour spaces for measuring object colour and is widely used in virtually all fields. In this colour space, L* indicates lightness and a* and b* are the chromaticity coordinates. Plate 3.12 shows the a*, b* chromaticity diagram. In this diagram, the a* and b* indicate color directions: +a* is the red direction, -a* is the green direction, +b* is the yellow direction, and -b* is the blue direction. The centre is achromatic; as the a* and b* values increase and the point moves out from the center, the saturation of the color increases (Konica Minolta).

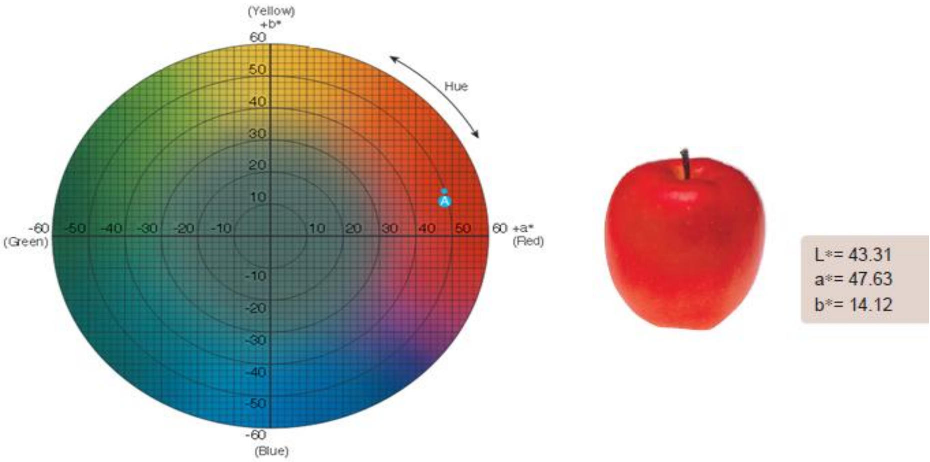


Plate 3.12 A representation of the colour space at a constant L* value.

The L*C*h colour space uses the same diagram as the L*a*b* colour space, but uses cylindrical coordinates instead of rectangular coordinates. In this colour space, L*indicates lightness and is the same as the L* of the L*a*b* colour space,C* is chroma, and h is the hue angle. The value of chroma C* is 0 at the centre and increases according to the distance from the centre. Hue angle h is defined as starting at the +a* axis and is expressed in degrees; 0° would be +a* (red), 90° would be +b *(yellow), 180° would be -a* (green), and 270° would be b* (blue). If the colour of an apple for example is measured using the L*C*h colour space, typical results are shown in Plate 3.13.

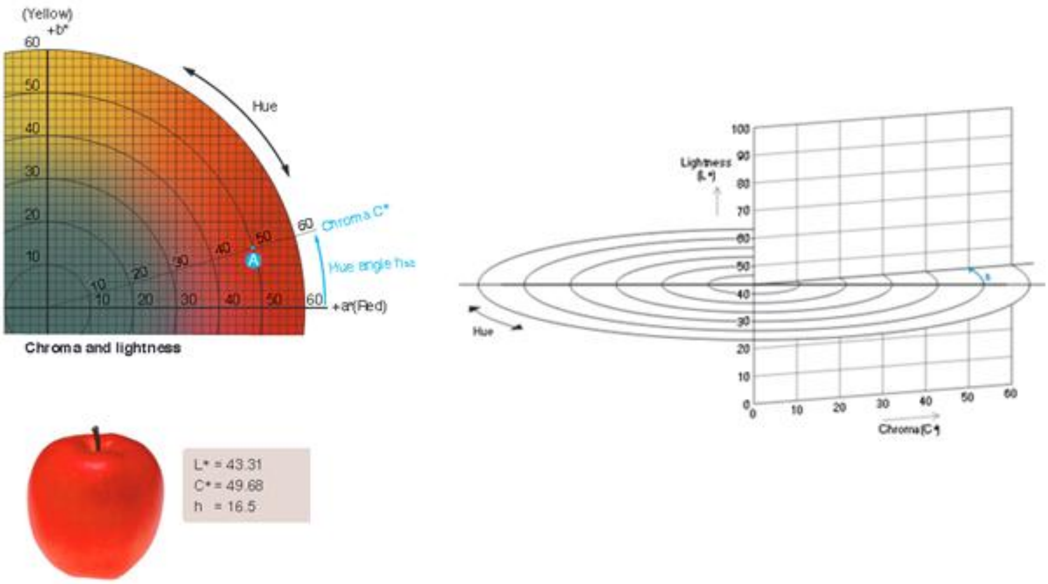


Plate 3.13 A representation of the L*C*h colour space (Konica-Minolta).

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

$$CIRG = \frac{180 - h}{C^* + L^*}$$

3.6 Data Analysis

Each sample (water, soil, leaf) was analysed in triplicate. If one of the replicates substantially disagreed with the other two, it was discarded and the average and standard deviation of the remaining two were used to calculate concentration. For irrigation water measurements, there were 18 samples during 3-year monitoring for each irrigation treatment (PTW, STW, TTW and TW) with the exception of FTW in radishes and carnations (7 samples).

Mean values and standard deviations were defined for all examined parameters using the MicroCal Origin 7.0 (Origin Lab). For microbial contamination (total coliforms and *E.coli*) median instead of mean concentration was reported as suggested by standards of Greek law (354/2011) about wastewater reuse.

For olive trees and grape vines 10 and 11 plants (replicates) were used respectively. The reason for this was the use of an existing olive orchard and the limited available land for the plantation of grapevine. For radish 8 replicates were used. Each replicate consists of 4 plants. For carnations 5 replicates were used. Each replicate consists of 5 plants. In all cases 5 treatments (irrigation water) were applied. Statistical analyses were conducted using the MicroCal Origin 7.0 (Origin Lab). The data were analysed through one-way analysis of variance (ANOVA) to compare the effect of each irrigation water source on specific parameter. Differences between means were tested for significance ($p<0.05$) using Tukey's test.

The following assumptions were met: a) response variable residuals are normally distributed, b) samples are independent, c) variances of populations are equal and d) responses for a given group are independent and identically distributed normal random variables.

Data for each parameter was fulfilled in columns (one column per treatment) as shown in Plate 3.14.

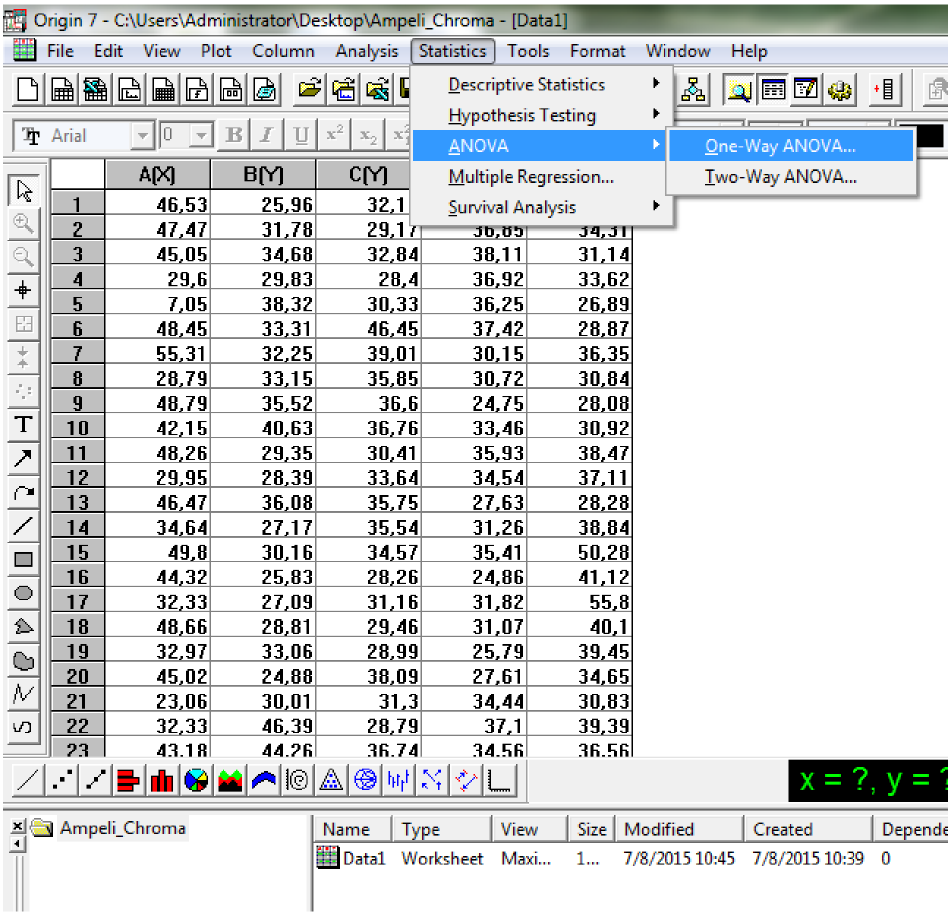


Plate 3.14 Data inserted in Origin 7 program

In the example (Plate 3.14), data are for parameter “L” (color in grapes). Each column represents values of “L” in grapes irrigated with different irrigation treatment (A: Primary treated wastewater, B: secondary treated wastewater, C: tertiary treated wastewater, D: fertilized tap water and E: tap water).

Then one-way ANOVA analysis was chosen defined significance level at 0.05 and using Tukey test for means comparison (Plate 3.15). Results obtained in a separate sheet including mean, standard deviation, standard error, df, mean square, f value, p value and others (Plate 3.16).

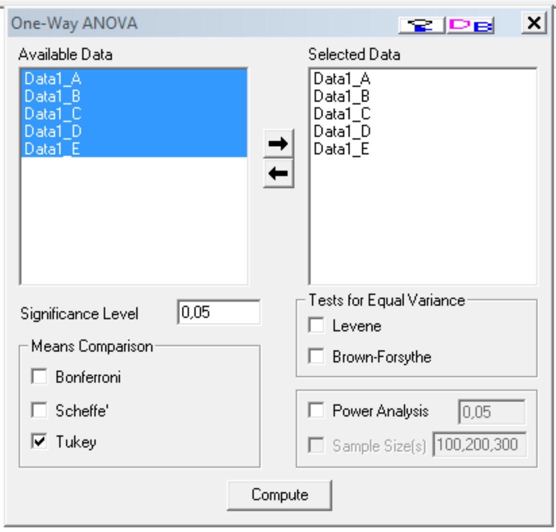


Plate 3.15 Selection of significance level and Tukey test

One-Way ANOVA

Summary Statistics

Dataset	N	Mean	SD	SE
Data1_A	171	38,96766	10,21452	0,78112
Data1_B	192	36,00781	6,55127	0,4728
Data1_C	126	33,49405	5,04331	0,44929
Data1_D	125	36,05176	8,43181	0,75416
Data1_E	60	33,919	5,84164	0,75415

Null Hypothesis: The means of all selected datasets are equal
Alternative Hypothesis: The means of one or more selected datasets are different

ANOVA

Source	DoF	Sum of Squares	Mean Square	F Value	P Value
Model	4	2549,12566	637,281415	10,67366	2,16966E-8
Error	669	39943,3011	59,7059808		

At the 0,05 level,
the population means are significantly different.

Means Comparison using Tukey Test

Dataset	Mean	Difference between Means	Simultaneous Confidence Intervals Lower Limit	Upper Limit	Significant at 0,05 Level
Data1_A	38,96766				
Data1_B	36,00781	2,95985	0,73752	5,18218	Yes
Data1_C	33,49405	5,47361	2,9922	7,95502	Yes
Data1_D	36,05176	2,9159	0,42878	5,40302	Yes
Data1_E	33,919	5,04866	1,87737	8,21995	Yes
Data1_B	36,00781				
Data1_C	33,49405	2,51376	0,09061	4,93692	Yes
Data1_D	36,05176	-0,04395	-2,47295	2,38505	No
Data1_E	33,919	2,08881	-1,03711	5,21473	No
Data1_C	33,49405				
Data1_D	36,05176	-2,55771	-5,2258	0,11038	No
Data1_E	33,919	-0,42495	-3,74007	2,89017	No
Data1_D	36,05176				
Data1_E	33,919	2,13276	-1,18664	5,45216	No

Plate 3.16 Results obtained from statistical analysis of parameter "L" (color in grapes) using Microcal Origin 7 program.

Chapter 4

Results and Discussion

4.1 Irrigation Water

The chemical composition of all sources of irrigation water is presented in Table 4.1 and Table 4.2. There are three different qualities of FTW: a) One for olive trees and vineyard added to soil, b) one for radishes added to TW and c) one for carnations added to TW. The pH value was high (7.9-8.5) for all treated wastewaters as well as for tap water. In addition, pH value in wastewater slightly increased after treatment (from 7.9 to 8.5). Treated wastewaters had significant higher salinity as EC values were 2.2-2.3 mS/cm for PTW, STW and TTW while TW had an EC value of 0.5 mS/cm.

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 TW less than 30 mg/l. Treated wastewaters contain significant quantities of nutrients as well as essential elements compared to tap water. For example, STW contains nitrogen and potassium at a concentration of 61.2 mg/l and 47.6 mg/l while TW had quantities lower than 5mg/l for both compounds. Similar PTW, STW and TTW have more than double concentrations of Mg and Ca in comparison with TW. Boron an important mineral for crop production (Saadati et al., 2013) was found in treated wastewaters at a concentration of about 0.3 mg/l.

Table 4.1 Chemical characteristics of water and treated wastewater used in the experiment

Parameter	PTW	STW	TTW	TW
pH	7.9 ± 0.2	8.5 ± 0.2	8.4 ± 0.2	8.3 ± 0.1
EC (mS/cm)	2.3 ± 0.1	2.2 ± 0.1	2.3 ± 0.1	0.5 ± 0.1
COD (mg/l)	296 ± 28	65 ± 4	65 ± 6	27 ± 3
BOD (mg/l)	142 ± 5	8 ± 1	6 ± 1	6 ± 1
TSS (mg/l)	177 ± 23	28 ± 6	27 ± 5	1 ± 1
TN (mg/l)	78 ± 3	61 ± 9	24 ± 3	5 ± 1
TP (mg/l)	14.9 ± 1.5	6.4 ± 0.7	6.1 ± 1.4	0.2 ± 0.1
K (mg/l)	36 ± 2	48 ± 4	47 ± 5	5 ± 2
Na (mg/l)	339 ± 23	328 ± 35	328 ± 43	14 ± 7
Mg (mg/l)	75 ± 3	75 ± 3	80 ± 4	22 ± 1
Ca (mg/l)	128 ± 5	135 ± 6	152 ± 8	64 ± 1
B (mg/l)	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	<0.1
Cu (μg/l)	n.d	n.d	n.d	n.d
Ni (μg/l)	n.d	n.d	n.d	n.d
Zn (μg/l)	7.2 ± 5.3	7.2 ± 5.3	7.0 ± 6.1	n.d
Σ10PAHs (μg/l)	2.1 ± 1.7	0.8 ± 0.7	0.8 ± 0.4	n.d
Total coliforms (MPN/100ml)	$4{,}0 \times 10^6$	$4{,}1 \times 10^3$	85 ± 27	<1
E. coli (MPN/100ml)	$1{,}3 \times 10^6$	$3{,}6 \times 10^3$	23 ± 18	<1

n.d: not detected, values expressed as mean values except for total coliforms and E. coli where median values are provided, PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

Table 4.2 Chemical characteristics of fertilized tap water (FTW) used in the experiments with radishes and carnations

Parameter	FTW (for radishes)	FTW (for carnations)
pH	5.6 ± 0.3	7.8 ± 0.1
EC (mS/cm)	2.4 ± 0.2	1.0 ± 0.1
COD (mg/l)	30 ± 5	29 ± 5
BOD (mg/l)	5.6 ± 0.7	5.8 ± 0.6
TSS (mg/l)	1.2 ± 0.4	1.3 ± 0.3
TN (mg/l)	251 ± 10	76 ± 4
TP (mg/l)	15 ± 1	13 ± 1
K (mg/l)	144 ± 13	108 ± 2
Na (mg/l)	28 ± 7	22 ± 7
Mg (mg/l)	49 ± 2	27 ± 1
Ca (mg/l)	172 ± 6	63 ± 1
B (mg/l)	0.21 ± 0.01	0.02 ± 0.01
Cu (μg/l)	32 ± 4	70 ± 8
Ni (μg/l)	2 ± 1	28 ± 4
Zn (μg/l)	153 ± 7	149 ± 9
Σ10PAHs (μg/l)	n.d	n.d
Total coliforms (MPN/100ml)	<1	<1
E. coli (MPN/100ml)	<1	<1

n.d: not detected, values expressed as mean values except for total coliforms and E. coli where median values are provided, PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

The occurrence of PAHs and a greater number of microorganisms was observed in wastewaters in comparison with tap water. PAHs were found in the wastewater effluents at a concentration of approximately 2.1 μg/l for PTW and 0.8 μg/l for STW and TTW while total *coliforms* in PTW, STW and

TTW were $4.0x10^6$ MPN/100ml, 4.1 $x10^3$ MPN/100ml and 85 MPN/100ml, respectively.

Furthermore *E. coli* was found in PTW and STW at a median concentration of $1.3x10^6$ MPN/100ml and $3.6x10^6$ MPN/100ml, respectively. Chromium, Copper and Nickel concentrations were not detected in the wastewaters analysed while Zinc concentration was quite low (approximately 7.0 μg/l). In Greece, and especially in Crete, industrial activities are very limited and this is reflected in the total amount of heavy metals found in wastewater. In general results were roughly in line with what we would expect from a properly performing wastewater treatment plant.

Comparing the data from Table 4.1 and Table 4.2 we can say that FTW in the experiments with radishes and carnations had higher concentrations of basic nutrients (N, P, and K) and some micro-elements (Cu, Ni, Zn) than that contained in treated wastewaters.

As already mentioned, fertilization in the experiment with olive groves and grape vines was added directly to soil. The total amount of nutrients added into the soils per year for an olive grove and a vineyard for every irrigation treatment are presented in Table 4.3. The total amount of irrigation water added to each plant annually was 1.2 m^3 for olive tree and 1.8 ^{3} for grapevine. As a result the amount of nutrients added to the olive trees was lower than that added to the grapevines.

Table 4.3 Total amount of nutrients added in soil for olive trees and grapevines

Parameter	Olive trees					grapevines				
(g/plant/yr)	PTW	STW	TTW	FTW	TW	PTW	STW	TTW	FTW	TW
N	94	73	28	62	6	141	110	42	64	8
P	18	8	7	8	0	27	12	11	8	0
K	44	57	57	53	5	65	86	85	56	8
Na	406	393	394	17	17	610	590	591	25	25
Mg	89	90	60	29	26	134	135	90	42	39

PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

According to Table 4.3 nutrients added in soil-plant systems irrigated with PTW and STW was higher than soil-plants systems irrigated with FTW.

4.2 Olive grove

4.2.1 Plant growth

Olive trees were healthy without any visible symptoms during the experimental period for all irrigation treatments applied (Plate 4.1). Mentioned that, at the beginning of the experiment the experimental field was tilled so the undergrowth was less.

Plate 4.1 Olive tree's growth at the beginning (2010) and at the end of the experiment (2012)

Trunk diameter and plant height of the examined olive trees are presented in Table 4.4. Olive tree growth was similar both for plants irrigated with treated wastewaters as well as for plants irrigated with FTW and TW. Segal et al. (2011) examined the effect or reclaimed water on tree growth of two olive cultivars ("Barnea" and "Leccino") in Israel in comparison with fresh water. For both cultivars in each year, no significant differences were found between treatments.

Table 4.4 Trunk number and plant height of olive trees during the experiment

Year			Trunk diameter (cm)		
	PTW	STW	TTW	FTW	TW
2010	2.4 ± 0.5	1.7 ± 0.7	1.9 ± 0.8	1.9 ± 0.9	2.3 ± 0.8
2011	7.0 ± 1.0	6.1 ± 0.7	6.5 ± 0.7	6.5 ± 1.4	6.8 ± 1.7
2012	7.7 ± 1.7	7.5 ± 1.3	7.9 ± 1.1	7.2 ± 1.6	8.4 ± 1.1
			Plant Height (m)		
2010	1.8 ± 0.3	1.7 ± 0.3	1.6 ± 0.4	1.6 ± 0.4	1.9 ± 0.3
2011	2.9 ± 0.3	2.8 ± 0.4	2.8 ± 0.3	2.7± 0.4	3.1 ± 0.3
2012	n.m	n.m	n.m	n.m	n.m

Values are the mean of ten trees ± standard deviation. For each year means are not significantly different according to Tukey's test ($P<0.05$), n.m: not measured, PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

Moreover, Leaf SPAD (Figure 4.1) values and the F_v/F_m ratio (Figure 4.2) 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. Leaf SPAD value varied between 74-85 % and F_v/F_m ratio was about 0.82 after three irrigation periods for all irrigation treatments.

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), heavy metals (Mallicka and Mohnb, 2003) and the application of olive mill wastewaters (Mechri *et al*. 2011).

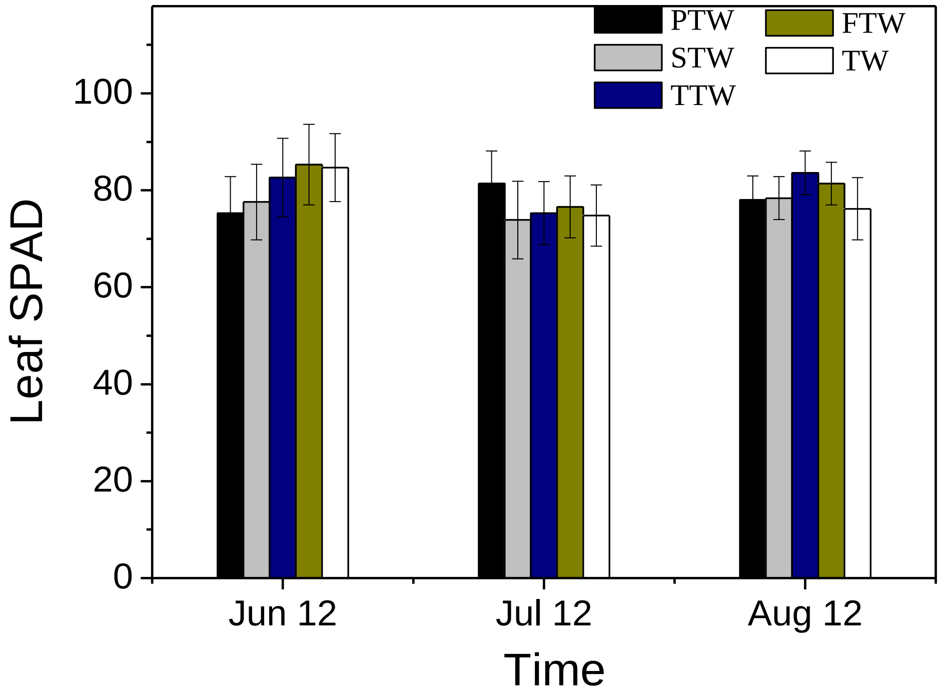


Figure 4.1 Effect of water irrigation treatments on leaf SPAD of the olive grove.

Data are means (n=10) ± standard deviations (vertical bars). PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

The results are in accordance with previous works about chlorophyll content of leaves for "Koroneiki" cultivar. In Tunisia, a nitrogen stress experiment was conducted with olive trees subjected to four nitrogen supply regimes (Boussadia *et al*. 2011). Results show SPAD values of the "Koroneiki" cultivar range from 75 to 90%. Khaou *et al*. (2013), examined the photosynthetic response of five olive cultivars to salinity. They found that F_v/F_m ratio of "Koroneiki" cultivar was stable between 0.74-0.78 at salinity values from 0.5 to 200 mM NaCl.

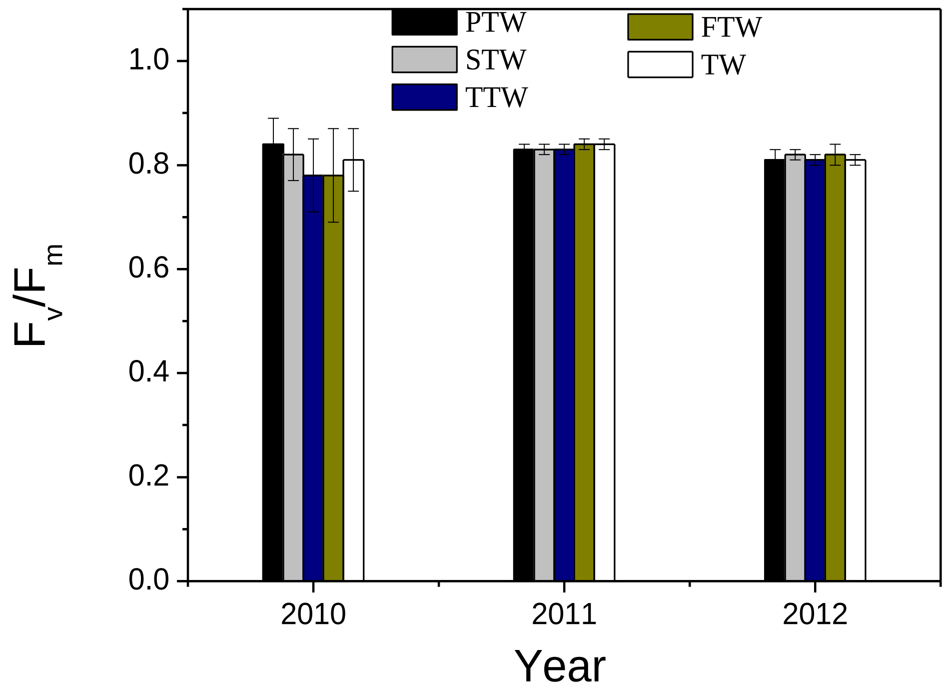


Figure 4.2 Effect of water irrigation treatments on the maximum photochemical efficiency of photosystem II during the cultivation period of olive grove.

Data are means (n=10) ± standard deviations (vertical bars). PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

4.2.2 Soil

The soil of the experimental site was classified as loam (42.6% sand, 34.4 % silt and 23% clay) with 20.7 g/kg organic matter and a pH of 7.5. Nitrogen and potassium concentrations in soil are reported in Figures 4.3 and 4.4, respectively. No significant effect on N and K in soil was observed for the first two years of wastewater application. On the other hand, after three years of irrigation a small difference was recorded.

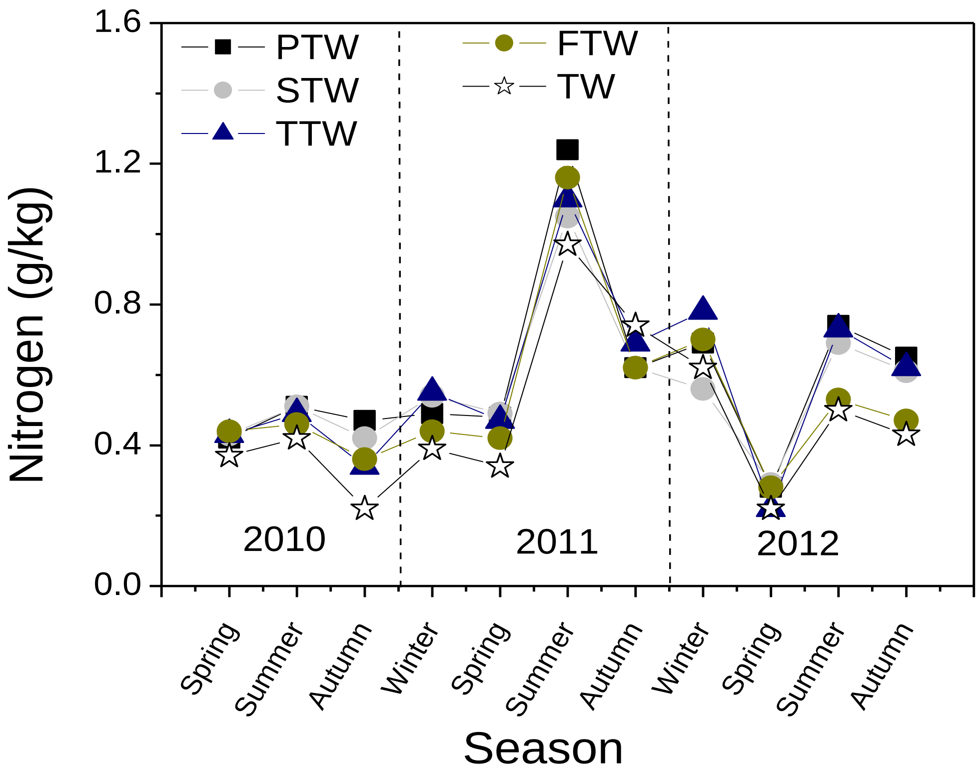


Figure 4.3 Nitrogen seasonal fluctuations in soil of olive grove during experimental periodPTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

Results were in contrast with those previous reported by Bedbabis *et al.* (2010) who found that the increase of N and K was highly significant from the first year of irrigation with wastewater. Plant uptake or movement of N and K from the examined layer may be the reasons (Heidarpour *et al*., 2007) for no significant accumulation of N and K in the soil.

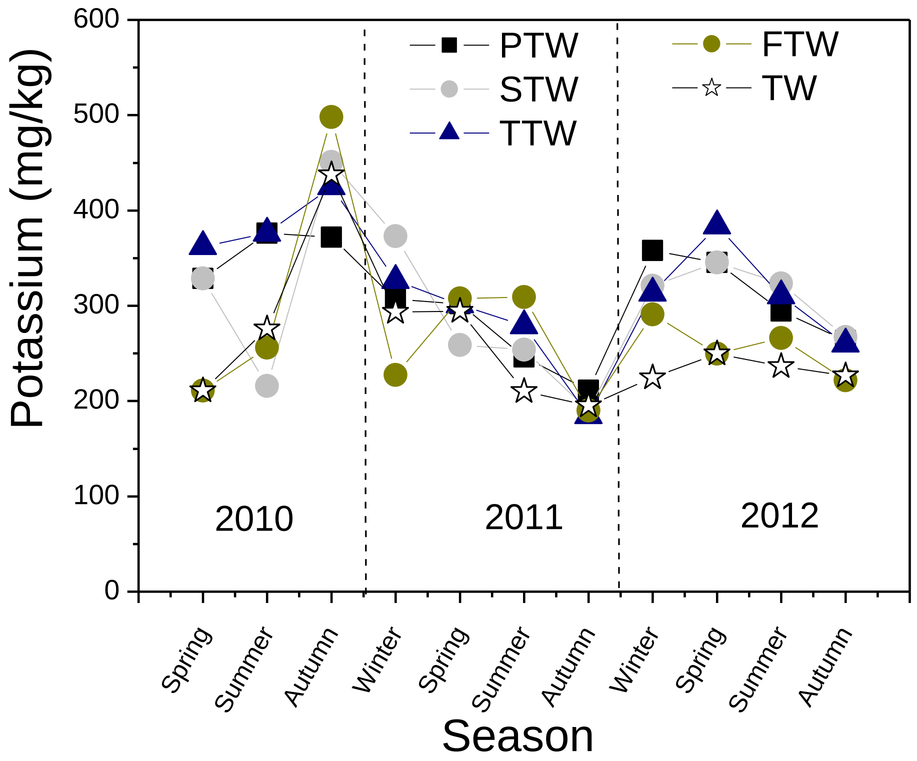


Figure 4.4 Potassium seasonal fluctuations in olive grove soil during the experimental period

PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

Phosphorus and sodium concentrations in the soil are reported in Figures 4.5 and 4.6, respectively. A clear difference in concentrations of these compounds seems to be established between the soils irrigated with treated wastewaters and the soil irrigated with TW.

In particular, phosphorus concentration in soils irrigated with TW and FTW was about 2 mg/kg and 3 mg/kg respectively during 2012 while in soils irrigated with PTW, STW and TTW was 4-8 mg/kg. Similar, sodium concentration in soils irrigated with TW and FTW was about 25 mg/kg during 2012 while in soils irrigated with PTW, STW and TTW was 50-413 mg/kg.

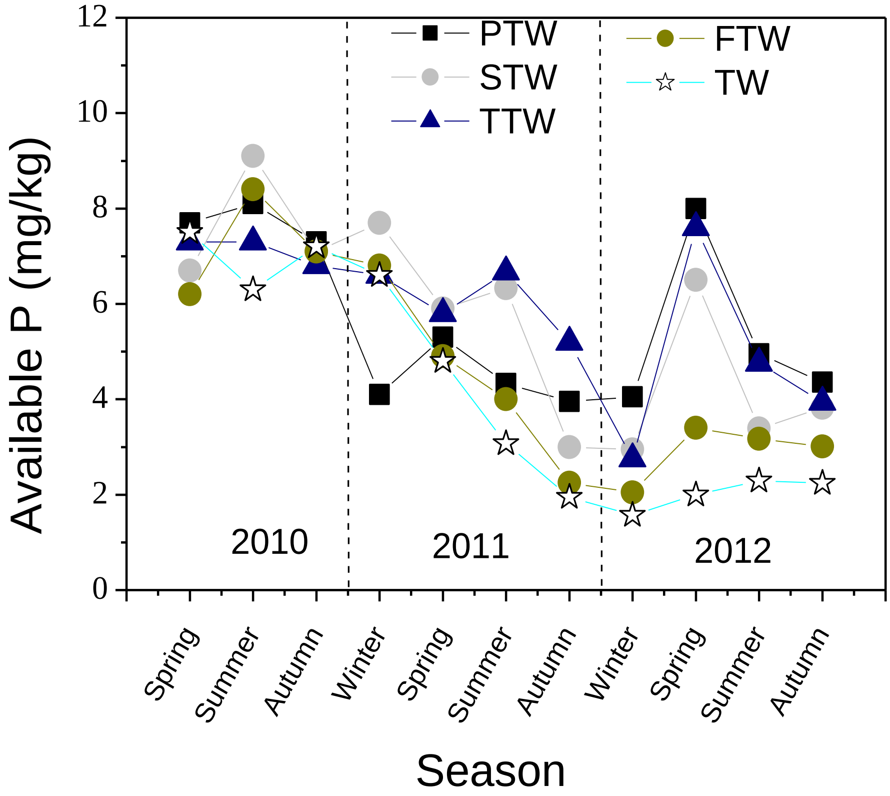


Figure 4.5 Phosphorus seasonal fluctuations in olive grove soil during experimental period

PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

Maximum values of P and Na were recorded during summer period when the treated wastewaters were applied. On the other hand, rainfalls between autumn and spring had as a result the movement of these compounds in lower soil layers. Bedbabis *et al.* (2010) found a similar increase of P and Na in the soil of olive groves irrigated with treated wastewaters (containing 10.3 mg/l P and 470 mg/l Na) after a period of 19 months (Feb 2003-Nov 2004) from 66.3 mg/kg and 105.3 mg/kg to 76.6 mg/kg and 182.5 mg/kg, respectively while the same period P and Na concentration in soil irrigated with well water was increased to 69.6 mg/kg and 150.8 mg/kg, respectively.

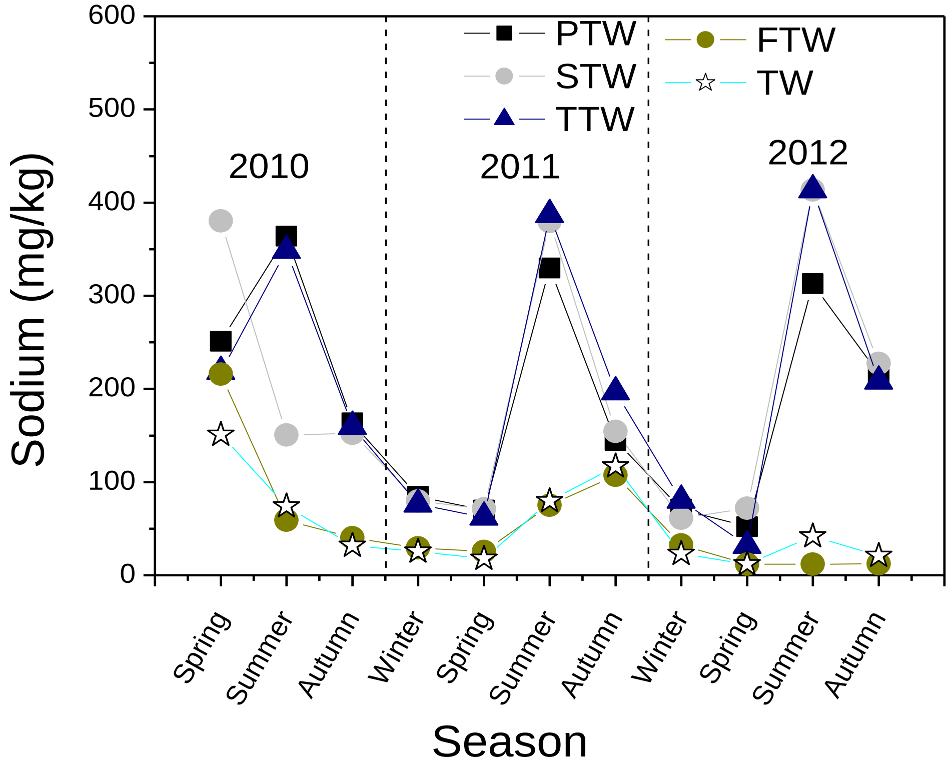


Figure 4.6 Sodium seasonal fluctuations in olive grove soil during the experimental period

PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

Heavy metals, minerals and PAHs concentration in soil during the three years of the study are presented in Table 4.5. Irrigation with treated wastewaters had no effect on soil concentrations. Heavy metals concentrations in soil were similar for all irrigation treatments. As already mentioned PTW, STW, TTW as well as TW do not contained significant quantities of any heavy metal. In addition, other parameters such as salinity or organic matter of wastewater did not affect the concentration of heavy metals in soil.

Magnesium, calcium and boron concentration in treated wastewaters was higher than TW. However no effects on soil concentrations were reported.

Table 4.5 Chemical composition of soil in the olive grove during the experimental period

Parameter	**PTW**	**STW**	**TTW**	**TTW**	**TW**
			2010		
Mg (g/kg)	6.2 ± 0.9	8.1 ± 0.4	6.9 ± 0.6	5.9 ± 0.6	7.1 ± 0.4
Ca (g/kg)	124.1± 31.7	146.7 ± 28.3	135.7 ± 53.4	127.3 ± 48.2	136.2 ± 21.9
B (mg/kg)	n.m	n.m	n.m	n.m	n.m
Cu (mg/kg)	24.8 ± 5.3	23.8 ± 4.3	25.7 ± 9.7	20.6. ± 4.7	21.1 ± 6.6
Ni (mg/kg)	88.9 ± 11.5	111.1 ± 10.2	91.7 ± 12.3	89.8 ± 10.6	79.5 ± 6.9
Zn (mg/kg)	39.1 ± 6.4	33.5 ± 3.1	31.8 ± 5.2	32.0 ± 4.8	27.4 ± 13.7
Σ10PAHs[1]	394.8 ± 87.6	378.9 ± 34.6	395.4 ± 80.7	390.2 ± 62.1	390.0 ± 58.1
			2011		
Mg (g/kg)	8.9 ± 0.3	9.0 ± 0.2	8.9 ± 0.8	9.7 ± 0.3	9.2 ± 0.3
Ca (g/kg)	181.5 ± 35.1	180.5 ± 32.2	174.5 ± 30.8	189.1 ± 40.1	182.3 ± 38.0
B (mg/kg)	9.6 ± 1.7	10.6 ± 1.5	10.2 ± 0.6	13.3 ± 4.5	9.1 ± 1.1
Cu (mg/kg)	28.1 ± 2.4	25.5 ± 1.3	24.8 ± 3.0	30.2 ± 3.8	26.2 ± 2.8
Ni (mg/kg)	95.3 ± 5.7	94.4 ± 9.8	93.0 ± 12.8	101.1 ± 11.4	98.2 ± 11.0
Zn (mg/kg)	44.4 ± 5.7	42.5 ± 5.3	40.5 ± 4.6	47.0 ± 5.1	43.4 ± 4.3
Σ10PAHs	351.9 ± 81.1	349.0 ± 76.5	366.3 ± 72.9	341.7 ± 61.8	322.0 ± 88.6

			2012		
Mg (g/kg)	10.7 ± 2.9	9.5 ± 0.3	9.8 ± 0.1	9.4 ± 0.2	9.8 ± 0.1
Ca (g/kg)	325.8 ± 26.7	306.2 ± 11.7	311.4 ± 14.8	303.0 ± 4.4	306.1 ± 3.0
B (mg/kg)	12.1 ± 5.8	9.5 ± 2.9	12.2 ± 5.3	9.9 ± 0.9	9.5 ± 3.3
Cu (mg/kg)	29.4 ± 6.3	23.5 ± 1.9	23.7 ± 0.7	25.9 ± 4.3	24.9 ± 0.3
Ni (mg/kg)	79.4 ± 3.9	73.1 ± 4.2	74.6 ± 0.3	73.5 ± 1.8	77.2 ± 0.6
Zn (mg/kg)	48.3 ± 2.9	47.2 ± 1.2	56.5 ± 5.9	54.8 ± 10.2	44.4 ± 0.7
Σ10PAHs	375.6 ± 73.4	369.0 ± 93.2	360.7 ± 52.2	371.1 ± 68.8	371.4 ± 61.1

Values are the mean of three different samplings (spring, summer, autumn) per year ± standard deviations. For each year means are not significantly different according to Tukey's test (P<0.05), [1](μg/kg), PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

The Mg and B mean concentrations were between 6.9-10.7 mg/kg and 9.1-12.2 mg/kg respectively for all irrigation treatments and all sampling periods. Ca mean concentrations were not significantly different between irrigation treatments but increased every year. No significant differences for Ca and Mg concentration in olive grove's soils irrigated with well water and treated wastewater for a period of 4 years were reported in a previous study in Tunisia (Bedbabis *et al*., 2014). In the same study Ca accumulation over the years was also observed in irrigated soils, indicating that the absorption of this element on the exchangeable complex was higher than the plant root's uptake.

The occurrence of PAHs was detected at concentrations between 349.0-395.4 μg/kg not only in soils irrigated with treated wastewaters but also in soils irrigated with TW. It was concluded that PAHs enter the soils mainly through atmospheric deposition and not with irrigation water. It is known that soils could be polluted by PAHs which can be transported over large distances (Nam et al., 2008). A previous study found that mean concentration of Σ16PAHs in arable soils in Poland (216 samples) was 435 μg/kg (Maliszewska-Kordybach *et al*., 2009). In addition, a mean concentration of 640 μg/kg and 150 μg/kg in UK and Norwegian soils respectively were reported by Nam *et al*. (2008).

During this study, Phe (22% of total PAHs) was the most abundant low-molecular PAHs (2-3 rings) while Flu (30%) and Pyr (32%) were the most abundant high-molecular PAHs (4-6 rings). These three compounds accounted for 84% of the total PAHs analysed. Comparing the results of the present work with other studies into PAH distributions in soils, it is apparent that the trends are very similar. Flu, Pyr and Phe were the most dominated hydrocarbons in the soils of Australia (Nguyen, et al., 2014), Poland (Maliszewska-Kordybach *et al.,* 2009) and Spain (Nadal *et al*., 2004).

4.2.3 Leaf content

Concentrations of macro-nutrients and heavy metals in the leaves evaluated throughout the experiment are presented in Table 4.6. No significant differences between irrigation treatments for each mineral were observed indicating no effect of wastewater toxicity. No significant differences in olive (Barnea and Leccino cultivars) leaves content between reclaimed wastewater and fresh water were also observed by Segal *et al.* (2011).

Nitrogen and potassium content in leaves ranged between 1.1-1.5% and 0.3-0.7%, respectively considered as slightly deficient (N deficient <1.4%, K deficient <0.4%). Even if, N and K concentrations in leaves were not the optimum, not visible symptoms were observed. On the other hand, the measured concentrations of P, Mg, Ca, B, Cu and Zn were within a range considered adequate for the olive trees in all treatments (International Olive Council, 2007).

Na content in leaves showed a tendency to increase in trees irrigated with treated wastewaters as the value after three years was 0.16% for all treated wastewaters while the relative value for TW was 0.12%. However, this difference was not statistically significant ($p<0.05$). Even if Na content was below the toxicity limit (>0.2%) this tendency to increase may affect plant growth in a long-term application. Segal *et al*. (2011) found no difference in Na content of olive trees after four years of irrigation using reclaimed water with an EC of 1.65 mS/cm. On the other hand Bedbabis *et al*. (2010) observed a significant increase of Na concentration after two years of irrigation with treated wastewaters with an EC of 6.30 mS/cm. In accordance with previous results, during this study the EC value of treated wastewaters was approximately 2.2 mS/cm and no significant difference was observed after three years.

Table 4.6 Chemical composition of leaves in the olive grove during the experimental period

	PTW	STW	TTW	FTW	TW
			2010		
N (%)	1.49 ±0.16	1.25 ±0.12	1.26 ± 0.39	1.38 ±0.33	1.27 ± 0.39
P (%)	0.10 ± 0.05	0.09 ± 0.05	0.09 ± 0.03	0.10 ± 0.02	0.10 ± 0.02
K (%)	0.33 ± 0.31	0.35 ± 0.31	0.36 ± 0.32	0.31 ± 0.27	0.33 ± 0.29
Na (%)	0.12 ± 0.02	0.11 ± 0.01	0.10 ± 0.02	0.09 ± 0.01	0.09 ± 0.01
Mg (%)	0.18 ± 0.02	0.16 ± 0.02	0.18 ± 0.04	0.17 ± 0.03	0.17 ± 0.05
Ca (%)	2.04 ± 0.24	2.04 ± 0.31	2.38 ± 0.26	2.04 ± 0.59	1.93 ± 0.84
B (mg/kg)	19.1 ± 7.1	17.1 ± 4.8	17.9 ± 5.4	16.9 ± 6.9	19.2 ± 3.9
Cu (mg/kg)	7.9 ± 4.2	10.6 ± 2.8	6.4 ± 2.2	7.6 ± 2.8	7.3 ± 2.0
Ni (mg/kg)	4.3 ± 1.5	2.7 ± 0.2	3.0 ± 0.5	3.9 ± 1.8	2.2 ± 0.8
Zn (mg/kg)	28.4 ± 8.4	20.9 ± 3.4	17.9 ± 5.9	31.4 ± 7.1	29.7 ± 2.7
			2011		
N (%)	1.35 ± 0.49	1.44 ± 0.54	1.45 ± 0.54	1.41 ± 0.49	1.27 ± 0.42
P (%)	0.12 ± 0.02	0.11 ± 0.03	0.12 ± 0.02	0.12 ± 0.02	0.13 ± 0.01
K (%)	0.67 ± 0.11	0.67 ± 0.12	0.66 ± 0.10	0.75 ± 0.07	0.65 ± 0.22
Na (%)	0.15 ± 0.02	0.15 ± 0.03	0.15 ± 0.01	0.11 ± 0.03	0.12 ± 0.01

Mg (%)	0.18 ± 0.01	0.15 ± 0.01	0.19 ± 0.01	0.18 ± 0.01	0.18 ± 0.01
Ca (%)	2.48 ± 0.08	2.17 ± 0.31	2.40 ± 0.18	2.61 ± 0.08	2.15 ± 0.07
B (mg/kg)	17.6 ± 1.9	20.7 ± 8.0	14.6 ± 2.2	17.8 ± 3.7	15.9 ± 3.2
Cu (mg/kg)	8.7 ± 0.7	7.4 ± 2.5	7.3 ± 2.0	6.9 ± 1.6	7.5 ± 2.6
Ni (mg/kg)	6.8 ± 0.3	5.6 ± 0.4	6.3 ± 0.4	5.7 ± 0.3	4.8 ± 0.3
Zn (mg/kg)	36.7 ± 12.4	31.9 ± 16.4	19.7 ± 7.7	29.0 ± 11.6	27.1 ± 1.1
			2012		
N (%)	1.30 ± 0.49	1.13 ± 1.07	1.04 ± 1.00	1.08 ± 1.02	1.14 ± 0.35
P (%)	0.15 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.16 ± 0.01	0.15 ± 0.01
K (%)	0.49 ± 0.03	0.55 ± 0.05	0.60 ± 0.07	0.59 ± 0.06	0.62 ± 0.08
Na (%)	0.16 ± 0.04	0.16 ± 0.02	0.16 ± 0.07	0.12 ± 0.07	0.12 ± 0.03
Mg (%)	0.19 ± 0.02	0.21 ± 0.02	0.18 ± 0.02	0.18 ± 0.02	0.17 ± 0.02
Ca (%)	4.32 ± 0.16	4.94 ± 0.21	5.76 ± 0.38	4.06 ± 0.23	3.85 ± 0.14
B (mg/kg)	25.0 ± 5.8	27.7 ± 5.3	31.9 ± 4.8	29.0 ± 3.6	29.0 ± 1.9
Cu (mg/kg)	7.8 ± 3.5	6.2 ± 2.4	7.4 ± 1.9	7.7 ± 3.1	12.0 ± 3.2
Ni (mg/kg)	4.8 ± 0.8	4.9 ± 0.5	4.2 ± 0.4	4.4 ± 0.6	4.6 ± 0.7
Zn (mg/kg)	27.0 ± 9.8	26.5 ± 11.9	19.1 ± 12.9	28.4 ± 10.1	29.6 ± 14.1

Values are the mean of three different samplings (spring, summer, autumn) per year ± standard deviations. For each year means are not significantly different according to Tukey's test ($P<0.05$), PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

4.2.4 Pathogens

The concentrations of *total coliforms* and *E.Coli* on soil, leaves and fruits of the experimental olive grove are shown in Table 4.7. As expected the soils of the olive trees irrigated with the PTW were more contaminated by total *coliforms* and *E. coli* with values ranging from 3,000-60,000 CFU/g and 50-1270 CFU/g respectively. Significant microbial pollution was also detected for STW with values ranging from 3,000-32,000 CFU/g and 10-84 CFU/g. Total coliforms were also detected in leaves and fruits of trees irrigated with TTW, FTW or TW although with different contamination levels. This result does not indicate a significant health risk as it is known that total coliforms are ubiquitous in agricultural environments (Materon, 2003).

Table 4.7 Total coliforms and *E.Coli* on soil, leaves and fruits in olive grove

Irrigation treatment	**Total Coliforms**			***E.Coli***		
	Soil[a]	Leaves	Fruit	Soil[a]	Leaves	Fruit
PTW	5,000-60,000	500-4,000	20-120	50-1,270	0	0
STW	3,000-32,000	400-5,000	20-160	10-84	0	0
TTW	500-19,000	200-4,500	0-84	0	0	0
FTW	200-3,000	200-1,000	0-84	0	0	0
TW	0-113	0-485	0-43	0	0	0

[a]CFU/g dry weight for soil and MPN/g fresh weight for leaves and fruit. PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

On the other hand, *E.Coli* contamination was not detected in leaves and fruits of olive trees for all the examined irrigation treatments. Results are in accordance with what reported by Vivaldi *et al*. (2013) for nectarines irrigated with treated wastewater. Moreover, Palese *et al.* (2009) found that although the *E. coli* content of treated wastewater applied for irrigation of olive groves in Italy was often over the limits the hygienic quality of soil and fruits was preserved.

The implementation of drip irrigation reduces the risk for leaves and fruit contamination. According to WHO guideline 2000, grove wastewater irrigation should be stopped two weeks before harvest and no fruit should be picked up off the ground. For olive groves the harvesting period in Greece begins at the end of November while irrigation stops in September. So at least there is a period of six weeks before harvest reducing the risk for contamination.

4.2.5 Fruit quality

Characteristics of olives are presented in Table 4.8. Olive trees irrigated with TTW had larger olive fruits. However, fruit size was generally negatively correlated to fruit number (Segal *et al*., 2011). Comparing water content of olives irrigated with different qualities of irrigation water no significant difference was found. Olive trees have an irregular crop load from year to year (biennial bearing cycle). In an "on" year too much fruit is set, leading to small fruit size and the subsequent year will be an "off" year (too little fruit). During the measurements was an “off” year for almost all trees. So there was not enough data to conclude about the effect of treated wastewaters on fruit quality.

Table 4.8 Olive quality characteristics at the end of the experiment

Irrigation treatment	Length (mm)	Diameter (mm)	Weight (g)	Kernel weight (g)	Water content (g/g)
PTW	16.0 ± 2.6[a]	10.9 ± 0.7[a]	1.14 ± 0.10[a]	0.39 ± 0.08[a]	0.57± 0.08[a]
STW	16.6 ± 2.9[a]	10.7 ± 0.9[a]	1.14 ± 0.10[a]	0.39 ± 0.08[a]	0.59± 0.06[a]
TTW	18.2 ± 2.2[b]	11.7 ± 0.8[b]	1.35 ± 0.14[b]	0.57 ± 0.11[b]	0.57± 0.08[a]
FTW	17.3 ± 2.6[c]	11.1 ± 0.9[c]	1.21 ± 0.07[c]	0.52 ± 0.07[c]	0.54± 0.04[a]
TW	17.2 ± 2.7[c]	11.4 ± 0.9[d]	1.31 ± 0.08[d]	0.47 ± 0.07[d]	0.53± 0.08[a]

mean values and standard deviations, a, b, c : In each row means values followed by a different symbol are significantly different to one another ($p<0.05$), PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

4.3 Vineyard

4.3.1 Plant growth

Vines were healthy without any visible symptoms during the experimental period for all irrigation treatments applied (Plate 4.2). Before the installation of the vineyard tilling was carried out therefore the undergrowth that was visible in the summer (top photograph) was less than that visible in the photograph taken at the end of the experimental period (bottom).

Plate 4.2 Vines at the beginning (2010, top) and at the end of the experiment (2012, bottom)

Trunk diameter and pruning weight during the experimental period are presented in Table 4.9. Vines growth was not significantly different for plants irrigated with STW and TTW in comparison with plants irrigated with FTW and TW. On the other hand trunk diameter as well pruning weight of plants irrigated with PTW was lower in comparison with all other plants.

Table 4.9 Trunk diameter and pruning weight of vines during experimental period.

Year	Trunk diameter (mm)				
	PTW	STW	TTW	FTW	TW
2010	17.6 ± 2.3	18.5 ± 2.5	19.4 ± 2.3	17.6 ± 2.9	18.9 ± 4.4
2012	28.3 ± 8.1[a]	32.7 ± 10.5[a,b]	35.2 ± 12.6 [a,b]	40.4 ± 9.8[b]	33.9 ± 8.3[a,b]
	Pruning weight (kg/vine)				
2010	0.08 ± 0.06	0.09 ± 0.04	0.08 ± 0.04	0.07 ± 0.04	0.06 ± 0.04
2012	0.66 ± 0.33	0.86 ± 0.34	1.20 ± 0.59	0.97 ± 0.40	1.02 ± 0.46

Values are the mean of eleven vines ± standard deviations. In each raw, mean values followed by a different symbol are significantly different ($p<0.05$), PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

Leaf SPAD values are presented in Figure 4.7. According to the results vines irrigated with treated wastewaters had significantly higher SPAD values in comparison with vines irrigated with TW and similar SPAD values in comparison with FTW. Leaf SPAD value varied between 30-33 % during August 2012 for plants irrigated with PTW, STW, TTW and FTW 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 table grape “Italia”. In addition, in another work (Ferrara and Bruneti, 2008) they found highly significant correlations between SPAD values and nitrogen content in the leaves. The absence of nutrients and minerals in TW may result in a decrease in leaf SPAD values.

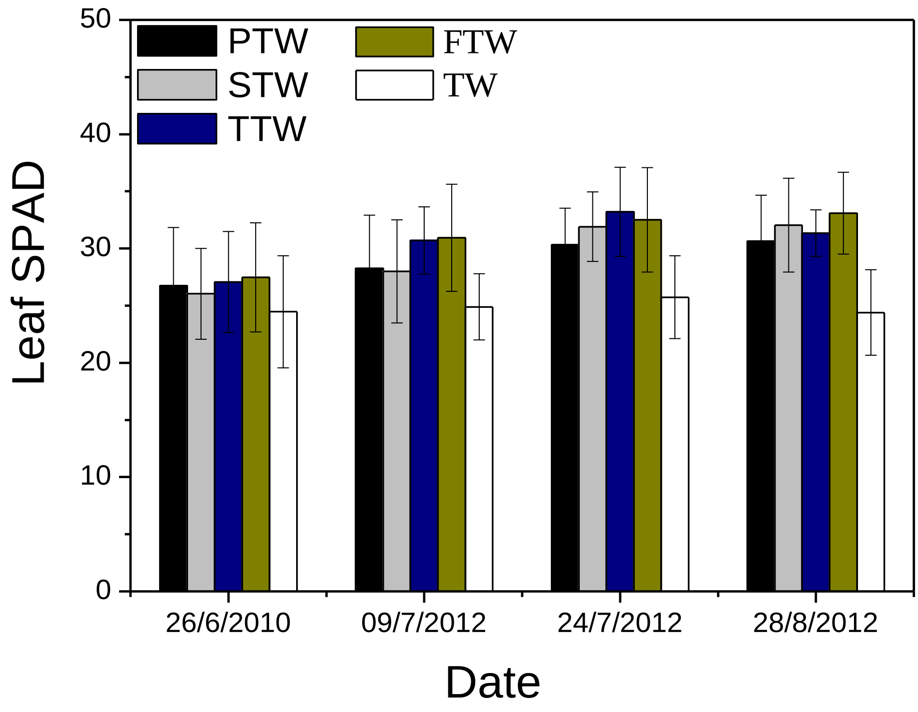


Figure 4.7 Effect of water irrigation treatments on the leaf SPAD of grapevines.

Data are means (n=11) ± standard deviations (vertical bars). PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

Maximum quantum yield (F_v/F_m) of vines was about 0.81 after three irrigation periods for all irrigation treatments (Figure 4.8). Pech *et al.* (2013) reported a decrease of Fv/F_m ratio from 0.80 (control) to 0.71 and 0.76 for irrigation water which contained 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 were well below these values, ~0.3 mg/l and ~2.2 mS/cm, respectively.

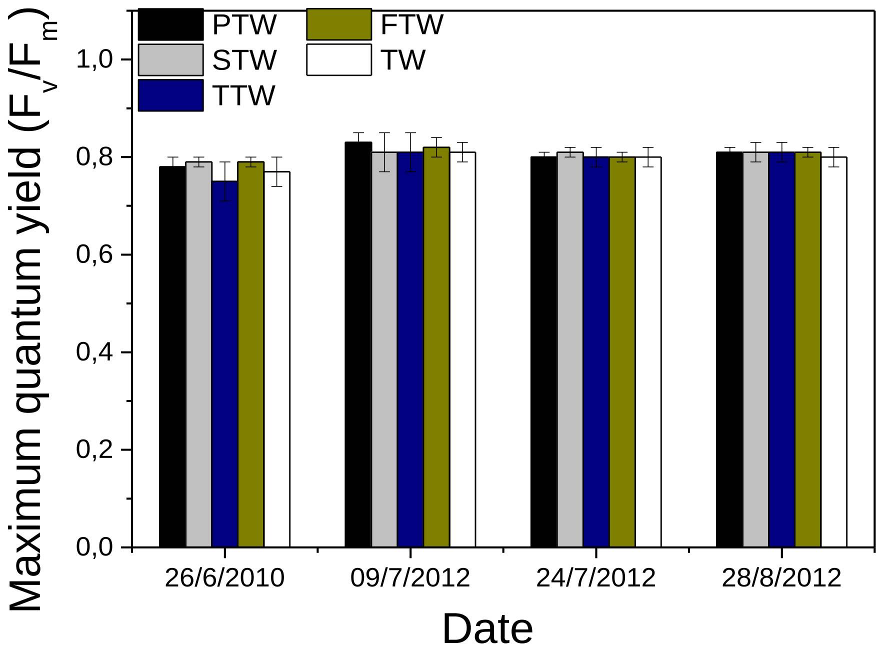


Figure 4.8 Effect of water irrigation treatments on the maximum photochemical efficiency of photosystem II of grapevines during the cultivation period.

Data are means (n=110) ± standard deviations (vertical bars). PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

4.3.2 Soil

The 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. A chemical composition of soil at the beginning of the experiment is presented in Table 4.10 while the composition of soil at the end of the experiment presented in Table 4.11. Irrigation with treated wastewaters had no effect on soil concentrations. Heavy metals and PAHs concentrations in soil are similar for all irrigation treatments. Ca mean concentrations were not significantly different between irrigation treatments but increased at the end of the experiment similarly to the experiment with olive trees.

Table 4.10 Chemical composition of soil in the vineyard at the beginning of the experiment (April 2010)

Parameter	PTW	STW	TTW	FTW	TW
pH	7.6 ± 0.3	7.4 ± 0.2	7.7 ± 0.3	7.6 ± 0.5	7.3 ± 0.4
N (g/kg)	0.6 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.5 ± 0.2
P (mg/kg)	7.4 ± 0.4	7.1 ± 0.5	7.3 ± 0.2	6.8 ± 0.5	7.5 ± 0.3
K (mg/kg)	2.2 ± 0.2	2.3 ± 0.3	2.4 ± 0.3	2.1 ± 0.4	2.1 ± 0.3
Mg (g/kg)	7.9 ± 0.7	7.4 ± 0.9	7.0 ± 0.6	7.4 ± 0.6	7.5 ± 0.8
Ca (g/kg)	144 ± 25	132 ± 21	148 ± 29	142 ± 23	141 ± 25
B (mg/kg)	n.m	n.m	n.m	n.m	n.m
Cu (mg/kg)	33 ± 4	35 ± 5	29 ± 8	34± 5	32 ± 6
Ni (mg/kg)	111. ± 10	105 ± 12	104 ± 11	106 ± 15	107 ± 10
Zn (mg/kg)	30 ± 8	36 ± 8	27 ± 5	31 ± 6	25 ± 9
Σ10PAHs (μg/kg)	325 ± 45	361 ± 54	389 ± 63	386 ± 70	359 ± 62

Mean values and standard deviations, n.m: not measured, PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

Table 4.11 Chemical composition of soil in the vineyard at the end of the experiment (August 2012)

Parameter	PTW	STW	TTW	FTW	TW
pH	7.4 ± 0.2	7.5 ± 0.1	7.4 ± 0.1	7.2 ± 0.3	7.4 ± 0.1
N (g/kg)	0.7 ± 0.1	0.8 ± 0.2	0.6 ± 0.1	0.7 ± 0.1	0.5 ± 0.1
P (mg/kg)	7.2 ± 0.4	6.7 ± 0.2	7.1 ± 0.5	7.1 ± 0.6	7.0 ± 0.4
K (mg/kg)	2.7 ± 0.2	2.7 ± 0.3	2.2 ± 0.2	2.5 ± 0.2	2.3 ± 0.5
Mg (g/kg)	10.2 ± 0.9	10.2 ± 0.9	8.4 ± 0.6	9.5 ± 0.5	9.7 ± 0.5
Ca (g/kg)	310.9 ± 32.6	308.2 ± 40.6	271.8 ± 35.2	290.5 ± 13.9	310.7 ± 17.5
B (mg/kg)	12.8 ± 1.4	12.3 ± 4.6	6.2 ± 1.7	8.0 ± 1.2	7.4 ± 1.3
Cu (mg/kg)	35.7 ± 1.6	41.3 ± 0.9	30.2 ± 0.2	41.6 ± 7.1	29.4 ± 0.2
Ni (mg/kg)	76.0 ± 10.0	80.8 ± 2.2	72.6 ± 2.4	79.9 ± 2.6	75.5 ± 0.8
Zn (mg/kg)	47.1 ± 4.0	53.5 ± 1.8	53.9 ± 13.6	53.8 ± 4.9	45.7 ± 7.1
Σ10PAHs (μg/kg)	352.2 ± 57.8	374.1 ± 28.6	368.4 ± 72.1	368.8 ± 51.7	363.1 ± 58.5

Mean values and standard deviations, PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

Sodium levels in soils irrigated with wastewater increased at the end of irrigation period (August) and decreased until the following season (Figure 4.9). Rainfall between autumn and spring was likely to be the main cause of the movement of sodium into lower soil layers. Netzer *et al.*, (2014) examined the effect of irrigation using wastewater on table grape vineyards focused on sodium accumulation in soil and plant. Results showed that after the first irrigation season no differences were yet established. In the same study, significant or not significant differences were established in the next years, depended on the quantity of irrigation water applied (increase differences) and the quantity of rainfall (decrease differences).

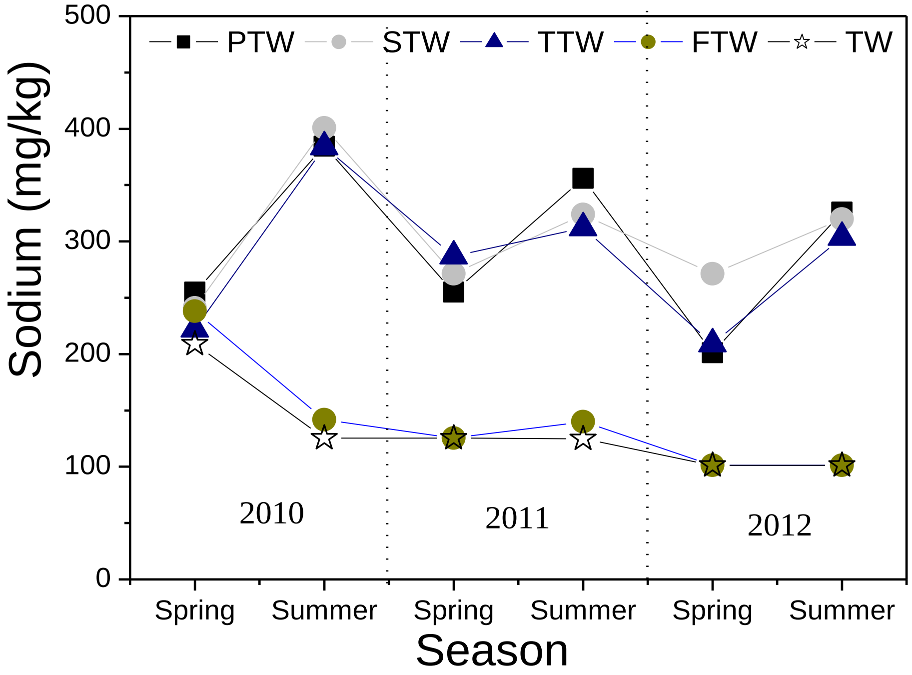


Figure 4.9 Sodium seasonal fluctuations in the soil in the vineyard during the experimental period (Spring 2010 – Autumn 2012).

PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

4.3.3 Leaf contents

Leaf nutrient status of vines irrigated with five different qualities of irrigation water had no significant differences (Table 4.12). For, grapevine, it is difficult to obtain reliable references 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 little higher values for Nitrogen and Potassium and Copper (2.9%, 1.2% and 39mg/kg, respectively) and little lower values for Magnesium and Zinc (0.4% and 12 mg/kg, respectively).

Table 4.12 Chemical composition of leaves at the time of grape veraison for the 3rd irrigation period.

	PTW	STW	TTW	FTW	TW
N (%)	1.93 ± 0.05	1.99 ± 0.08	1.96 ± 0.05	1.95 ± 0.05	1.97 ± 0.08
P (%)	0.11 ± 0.03	0.11 ± 0.02	0.11 ± 0.02	0.10 ± 0.03	0.13 ± 0.03
K (%)	0.80 ± 0.26	0.78 ± 0.18	0.74 ± 0.21	0.85 ± 0.15	0.80 ± 0.22
Na (%)	0.06 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.03	0.04 ± 0.01
Mg (%)	0.85 ± 0.08	0.90 ± 0.04	0.71 ± 0.02	0.78 ± 0.02	0.66 ± 0.02
Ca (%)	4.31 ± 0.16	4.38 ± 0.06	4.66 ± 0.02	4.40 ± 0.47	3.95 ± 0.02
B (mg/kg)	68.3 ± 4.6	53.5 ± 7.2	66.2 ± 3.3	58.5 ± 3.5	53.5 ± 9.6
Cu (mg/kg)	7.9 ± 1.3	8.2 ± 6.1	6.8 ± 2.6	7.4 ± 4.3	6.2 ± 0.2
Ni (mg/kg)	3.8 ± 2.5	3.6 ± 2.3	3.6 ± 1.4	5.1 ± 3.2	3.4 ± 0.6
Zn (mg/kg)	20.9 ± 6.9	21.7 ± 5.2	20.5 ± 17.0	26.1 ± 9.4	17.8 ± 8.3

mean values and standard deviations, PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

4.3.4 Pathogens

The concentrations of *total coliforms* and *E.Coli* on soil, leaves and fruits of the experimental vineyard are shown in Table 4.13. 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.

Table 4.13 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
PTW	3,700-20,000	100-2,500	0-250	50-1,270	0	0
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
FTW	2.000-3,000	0-500	0	0	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, PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

Results show that soil irrigated with the PTW and 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 could be observed.

On the other hand, the examined pathogens were not detected at all in grapes irrigated with TTW, FTW 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).

4.3.5 Fruit quality

Grape yield and colour characteristics are presented in Table 4.14. Production of grapes per vine fluctuated for any irrigation treatment as the vineyard was still too young (not in full production). Higher grape production was observed for vines irrigated with PTW and lower for vines irrigated with TW. However, no significant differences were observed according to statistics ($P<0.05$).

On the hand, significant differences on colour characteristics were observed. Grapes from vines irrigated with PTW and STW was less red than the grapes irrigated with TTW, FTW and TW according to RGCI values. This colour difference was clear during harvesting as presented in Plate 4.3.

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 it could be stated that the application of low quality treated wastewaters, such as PTW, also inhibit the accumulation of anthocyanins.

Table 4.14 Grape yield and colour characteristics at the end of experiment

	PTW	**STW**	**TTW**	**FTW**	**TW**
Production (kg/ vine)	1.00 ± 0.72	0.44 ± 0.14	0.91 ± 0.94	0.59 ± 0.42	0.38 ± 0.26
Color					
L	39.0 ± 10.2^{a}	36.0 ± 6.6^{b}	33.5 ± 5.0^{c}	$36.1 \pm 8.4^{b,c}$	33.9 ± 5.8^{c}
a	$7.9 \pm 8.5^{a,c}$	10.0 ± 5.7^{b}	9.8 ± 3.2^{b}	6.6 ± 7.0^{c}	$8.8 \pm 3.7^{b,c}$
b	9.0 ± 7.1^{a}	3.7 ± 5.2^{b}	0.8 ± 2.9^{c}	4.5 ± 9.6^{b}	1.1 ± 4.3^{c}
RGCI	3.5	3.9	4.2	4.1	4.2

mean values and standard deviations, a, b, c : In each row means values followed by a different symbol are significantly different to one another ($p < 0.05$), PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

Plate 4.3 The colour of Crimson seedless grapes in relation to the irrigation treatment (from left to right: PTW, STW, TTW, FTW, TW).

Quality characteristics of grape juice are presented in Table 4.15. The application of PTW caused a decrease in ^{o}Brix values. A correlation between grape production rate and ^{o}Brix values was observed (a higher production rate resulted in a lower ^{o}Brix).

Table 4.15 Quality characteristics of grape juice

	PTW	STW	TTW	FTW	TW
°Brix	15.8 ± 2.6[a]	20.4 ± 2.0[b,c]	19.4 ± 3.2[b]	18.6 ± 1.9[b]	21.8 ± 1.4[c]
TA (%)	0.61 ± 0.11[a]	0.61 ± 0.13[a]	0.79 ± 0.27[a]	0.63 ± 0.15[a]	0.47 ± 0.14[a]
°Brix/TA ratio	25.9 ± 3.5	33.4 ± 2.7	24.6 ± 4.1	29.5 ± 2.4	46.4 ± 2.1
N (g/l)	0.74 ± 0.05[a]	0.74 ± 0.16[a]	0.65 ± 0.14[a]	0.71 ± 0.19[a]	0.60 ± 0.15[a]
P (g/l)	0.22 ± 0.02[a]	0.25 ± 0.04[a]	0.26 ± 0.03[a]	0.29 ± 0.04[a]	0.25 ± 0.02[a]
K (g/l)	1.09 ± 0.08[a]	1.58 ± 0.12[b]	1.50 ± 0.23[b]	1.31 ± 0.20[b]	1.54 ± 0.15[b]
Ca (g/l)	0.10 ± 0.02[a]	0.10 ± 0.01[a]	0.09 ± 0.01[a]	0.09 ± 0.01[a]	0.09 ± 0.01[a]
Mg (g/l)	0.14 ± 0.01[a]	0.16 ± 0.01[a]	0.14 ± 0.01[a]	0.13 ± 0.01[a]	0.15 ± 0.01[a]
B (mg/l)	6.3 ± 0.7[a]	8.2 ± 0.1[a,b]	10.9 ± 4.8[b]	9.9 ± 4.1[a,b]	11.9 ± 0.6[b]

mean values and standard deviations, a, b, c : In each row means values followed by a different symbol are significantly different to one another ($p < 0.05$), TA: Titratable acid, PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water, PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

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 grapes 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 get a better consumer acceptance. In the same study, they stated that °Brix/TA ratio is a better indicator (than °Brix or TA) of consumer acceptability suggested as the best time to harvest Crimson seedless when °Brix/TA ratio is 35-40.

The application of STW and TTW on vines had no significant effect on minerals concentration of grape juice in comparison with vines irrigated with FTW and TW (Table 4.15). On the other hand the application of PTW produced the lowest values in potassium and boron concentration in grape juice. 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 approximately 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 soil, leaves and juice revealed no consistent relationships.

4.4 Radish

4.4.1 Plant growth

Plate 4.4 shows the radish plants growing during the experiment.

Plate 4.4 Radishes in pots during the experimental period.

The plants with all the treatments were healthy without any visible problem. In addition a clear difference in plant size was observed according to this series: FTW > PTW, STW = TTW >TW.

Figure 4.10 shows the dry mass of radish roots and shoots for the five treatments at the end of the experiment. Radishes irrigated with tap water had a mean dry mass production of 3.2 ± 0.8 g for roots. All types of wastewater used showed a similar effect on dry mass production of roots, with values of 4.7 ± 0.8, 4.2 ± 0.7, and 4.4 ± 1.0 g for primary, secondary and tertiary treated wastewater respectively. On the other hand, plants irrigated with FTW had the higher dry mass production of 5.8 ± 1.3 g. The irrigation with treated wastewater seems to increase the dry mass production compared with tap water, however one-way ANOVA Analysis showed no significant difference ($p<0.05$). Similar results were also observed for shoots of radishes (FTW > PTW, STW = TTW > TW).

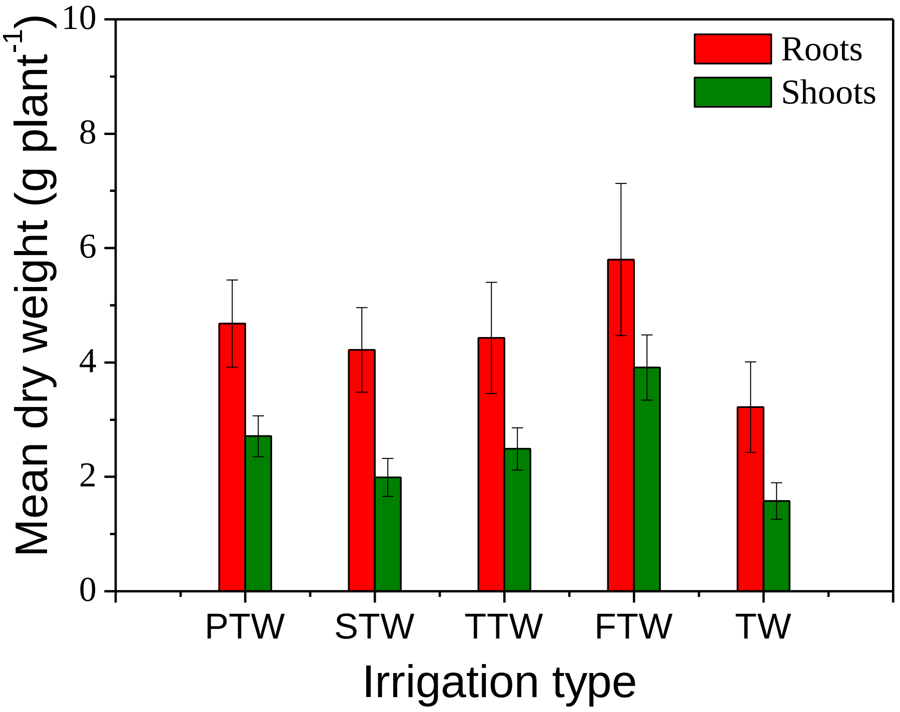


Figure 4.10 Plant biomass (dry mass of roots and shoots) of *Raphanus sativus* at the end of experiment.

PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

The maximum photochemical efficiency of photosystem II is expressed by the F_v/F_m ratio. The values recorded in this work (Figure 4.11) indicated the absence of photo-inhibition in plants irrigated with treated wastewaters in comparison with tap water. The F_v/F_m ratio was about 0.80 at the end of the experiment for all irrigation treatments. Similar values of F_v/F_m ratio were observed by Guo *et al*. (2005) who examined the photosynthetic responses of radish (*Raphanus sativus var.longipinnatus*) plants to infection by turnip mosaic virus. Healthy plants as well as infected plants had an F_v/F_m ratio from 0.81 to 0.84.

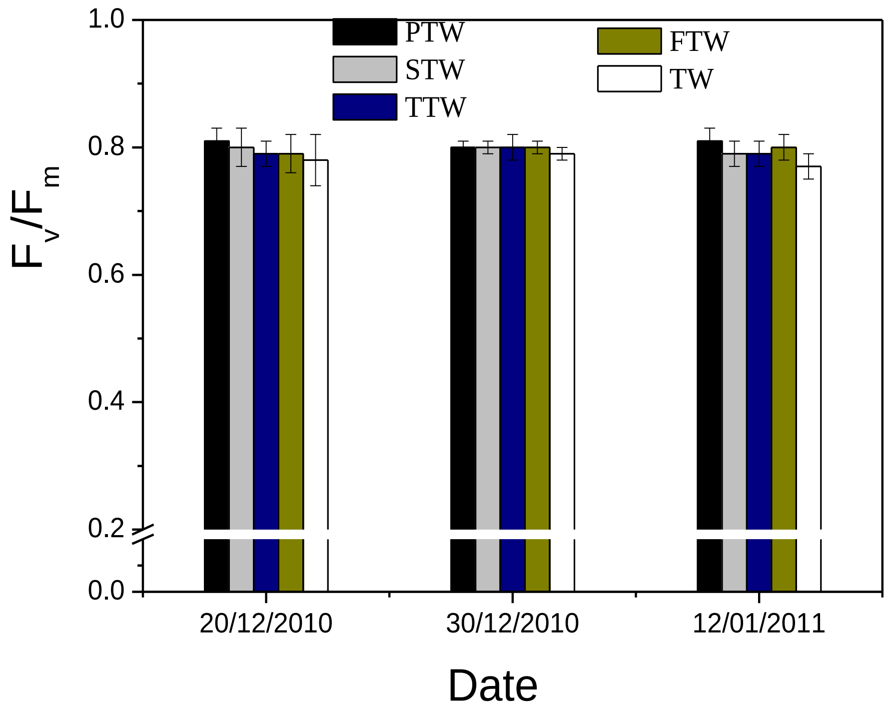


Figure 4.11 The effect of water irrigation treatments on the maximum photochemical efficiency of photosystem II during the cultivation period of radishes.

Data are means (n=9) ± standard deviations (vertical bars). PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

4.4.2 Soil

The soil mixture used in the experiment was classified as sandy loam (71% sand, 18 % silt and 11% clay) with 12.6 ± 3.3 g/kg of organic matter, a pH value of 7.6 ± 0.1 and an electrical conductivity of 3.0 ± 0.2 mS/cm. The variation of pH and EC of soil during the experimental period are shown in Figure 4.12. No effect on pH value was observed for all the examined irrigation treatments. On the other hand the salinity of soil was found to be higher in pots irrigated with treated wastewaters (3-4 mS/cm) in comparison with pots irrigated with tap water with or without fertilizer (1-2 mS/cm).

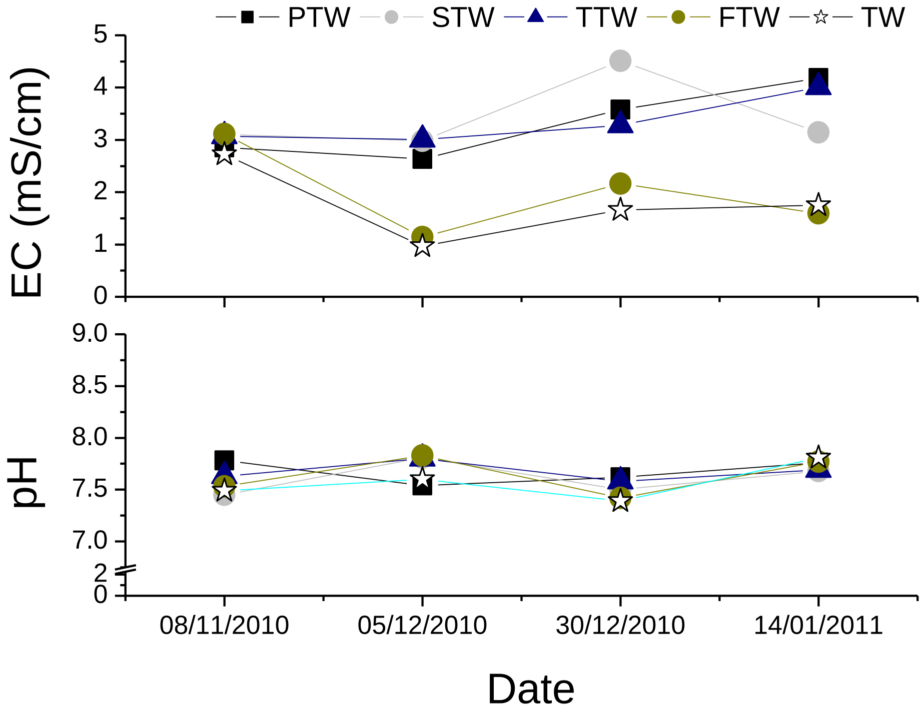


Figure 4.12 The variation of EC and pH in the soil planted with radishes during the experimental period.

PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

Macronutrients and heavy metal content in soils irrigated with the different qualities of irrigation water are shown in Table 4.16. In general, no significant differences were found in soils irrigated with treated wastewaters in comparison with soil irrigated with tap water. On the other hand, the use of FTW resulted in a higher concentration of phosphorus and potassium in the soil.

The Ca and Mg concentrations in the different wastewaters was approximately double (120 mg/l and 50 mg/l respectively) that of tap water (60.5 mg/l and 19.1 mg/l respectively) while no differences were found in the concentrations in the soil. Kalavrouziotis *et al*. (2008) found that Ca concentration decreased and Mg concentration increased in soil irrigated with treated wastewater in comparison with soil irrigated with tap water. In that case, the wastewater:water concentration ratio was slightly lower for Ca (90mg L^{-1} in wastewater and 49mg/l in tap water) and significant higher (21mg/l in wastewater and 4.2 mg/l in tap water) for Mg. Heavy metal content was not significantly different in all cases.

Table 4.16 Macro-nutrients and heavy metal concentrations in the soil of radishes at the end of experiment

Parameter	PTW	STW	TTW	FTW	TW
K (g/kg)	1.6 ± 0.6	1.6 ± 0.3	1.6 ± 0.4	1.8 ± 0.2	1.5 ± 0.1
Mg (g/kg)	11.8 ± 2.9	11.3 ± 1.2	10.8 ± 2.1	11.1 ± 0.8	11.7 ± 1.0
Ca (g/kg)	146.6 ± 4.9	143.1 ± 13.6	141.6 ± 12.1	147.8 ± 8.6	142.7 ± 13.6
P (mg/kg)	28.5 ± 6.4^{a}	14.8 ± 2.8^{b}	14.7 ± 0.5^{b}	37.0 ± 0.9^{c}	16.7 ± 0.8^{b}
Na (mg/kg)	2.0 ± 0.5^{a}	2.2 ± 0.5^{a}	2.3 ± 0.5^{a}	0.5 ± 0.1^{b}	0.5 ± 0.1^{b}
B (mg/kg)	7.6 ± 1.6	6.3 ± 1.5	5.8 ± 1.6	7.0 ± 1.3	5.1 ± 1.5
Cu (mg/kg)	25.9 ± 9.3	27.0 ± 9.6	24.8 ± 3.1	26.7 ± 4.3	26.7 ± 2.3
Ni (mg/kg)	71.2 ± 5.6	74.8 ± 7.4	75.7 ± 9.4	79.0 ± 7.6	74.7 ± 7.0
Cr (mg/kg)	84.1 ± 1.9	76.5 ± 7.4	80.1 ± 7.2	83.5 ± 15.9	80.4 ± 7.3
Zn (mg/kg)	31.6 ± 8.3	26.3 ± 3.0	35.4 ± 8.2	32.3 ± 5.1	32.3 ± 5.2

*mean values and standard deviations, a, b: In each row means values followed by a different symbol are significantly different to one another ($p < 0.05$) PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

The treated wastewaters used for the experiment were not containing heavy metals with the exception of Zn. The results show that the other characteristics of water and wastewater (pH, EC, organic content) did not affect the heavy metal concentrations in the irrigated soils. Boron concentration in soil irrigated with primary treated wastewater was 7.7 ± 1.6 mg/kg, while the soil irrigated with tap water had a boron concentration of 5.1 ± 1.5 mg/kg.

Table 4.17 shown the individual PAH concentrations in the soils irrigated with the different types of water and wastewater at the end of the experiment. The soil used for the experiment was found to be contaminated with remarkable PAH concentrations compared to those found by previous authors.

Table 4.17 Concentrations (µg/kg) of individual PAH and ΣPAHs in the soil of radishes at the end of experiment

Parameter	PTW	STW	TTW	FTW	TW
Fl	1.2 ± 0.1	0.4 ± 0.3	0.4 ± 0.5	0.4 ± 0.2	0.4 ± 0.3
Phe	20.4 ± 5.6	16.6 ± 1.1	15.6 ± 1.0	12.6 ± 2.2	12.5 ± 1.0
Ant	0.3 ± 0.3	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.3	0.1 ± 0.1
Flu	15.6 ± 2.6	14.3 ± 0.6	14.4 ± 0.4	14.1 ± 2.1	14.2 ± 0.6
Pyr	32.4 ± 0.1	31.0 ± 2.7	30.8 ± 2.3	30.9 ± 3.5	30.6 ± 2.4
Baa	1.0 ± 0.8	0.2 ± 0.1	0.2 ± 0.2	0.2 ± 0.4	0.2 ± 0.1
Chr	0.9 ± 0.4	0.5 ± 0.3	0.6 ± 0.4	0.5 ± 0.2	0.4 ± 0.1
Bbf	1.8 ± 1.3	0.5 ± 0.2	0.4 ± 0.2	0.5 ± 1.1	0.4 ± 0.2
Bkf	0.2 ± 0.1	n.d	n.d	n.d	n.d
Bap	3.9 ± 1.6	2.3 ± 0.6	2.5 ± 1.0	2.2 ± 0.7	2.2 ± 0.4
Σ10PAHs	77.7 ± 13.3	65.9 ± 6.0	64.9 ± 6.0	61.3 ± 12.2	61.0 ± 5.2

mean values and standard deviations, *n.d.: not detected,* PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

Notable PAH levels have been previously reported in soils from urban sites mainly due to traffic (Papageorgopoulou *et al*., 1999). In addition, Nadal *et al.* (2004) found a median concentration of 37 ± 27 μg/kg in unpolluted sites from Tarragona County, Spain. The most abundant PAHs investigated were phenanthrene, fluoranthene and pyrene. These three compounds accounted for 93% of total examined PAHs. Comparing the results of the present work with other studies into PAH distributions in anthropogenically contaminated soils, it is apparent that the trends are very similar. Generally, the medium-molecular-weight PAHs (e.g. fluoranthene and pyrene) are present in the greatest quantities (Nadal et al., 2004; Morillo et al., 2007). ΣPAH concentrations were found to be slightly higher for soils irrigated with secondary and tertiary wastewater compared with the control soil. However, significantly higher concentrations of PAHs were observed in the soil irrigated with primary wastewater. As expected, soil contamination (Table 4.17) was correlated with the PAH levels in the irrigation media (Table 4.1).

4.4.3 Root content

The data presented in Table 4.18 shows the comparative macro-nutrients and heavy metal composition of roots irrigated with five different qualities of irrigation water. There are no significant differences in the inorganic composition of radishes irrigated with treated wastewaters in comparison with radishes irrigated with tap water. On the other hand, the use of FTW had as a result increased concentration of minerals in radish roots.

The root concentration factor (RCFs) expressed as the ratio of heavy metals concentration in the mass root (dry weight) to the residual concentration in the soil was similar for all treated wastewaters applied. Higher RCFs were observed for Cu (0.95) and Zn (0.58), while Ni and Cr did not transfer from soil to roots. The ratio of metals between soil and roots (RCFs) may be affected by several factors, such as the type of heavy metal, soil, temperature, pH, organic matter and plant species (Antoniadis and Alloway, 2001; Kachenko and Singh, 2006; Kalavrouziotis *et al*., 2012)

Table 4.18 Macro-nutrients and heavy metals content in root dry matter of radishes at the end of experiment

Parameter	PTW	STW	TTW	FTW	TW
K (g/kg)	26.9 ± 1.4	26.4 ± 2.2	31.7 ± 1.2	41.6 ± 7.7	28.3 ± 8.2
Mg (g/kg)	1.4 ± 0.3	1.6 ± 0.3	1.8 ± 0.2	2.1 ± 0.6	1.5 ± 0.3
Ca (g/kg)	5.4 ± 0.8	6.5 ± 1.0	7.4 ± 2.2	7.7 ± 1.1	5.8 ± 1.4
B (mg/kg)	26.2 ± 11.4	20.7 ± 5.2	22.8 ± 1.8	26.9 ± 10.8	23.8 ± 7.7
Cu (mg/kg)	23.5 ± 5.6	26.8 ± 4.6	23.9 ± 4.2	32.7 ± 7.0	25.1 ± 8.7
Ni (mg/kg)	0.3 ± 0.2	0.4 ± 0.3	0.4 ± 0.3	0.5 ± 0.2	0.3 ± 0.2
Cr (mg/kg)	n.d	n.d	n.d	n.d	n.d
Zn (mg/kg)	18.7 ± 7.5	17.4 ± 5.0	17.3 ± 9.2	20.4 ± 6.3	19.2 ± 8.3

*mean values and standard deviations, PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

Examining the concentrations of PAHs in radish roots at the end of the experiment there was a substantial variation in the values, from non-detectable (Chr, Bbf and Bkf) to 53.04 μg/kg for Pyr. Radish roots were enriched with low and medium molecular weight PAHs such as Fl, Phe, Flu and Pyr (Figure 4.13). Slightly higher values of ΣPAHs were observed in the radish roots irrigated with primary treated wastewater. Furthermore, the results indicated that the PAH concentrations in the roots were correlated with the soil concentrations (high PAH concentration in the soil (Table 4.17) resulting in high PAH concentration in the root (Figure 4.13).

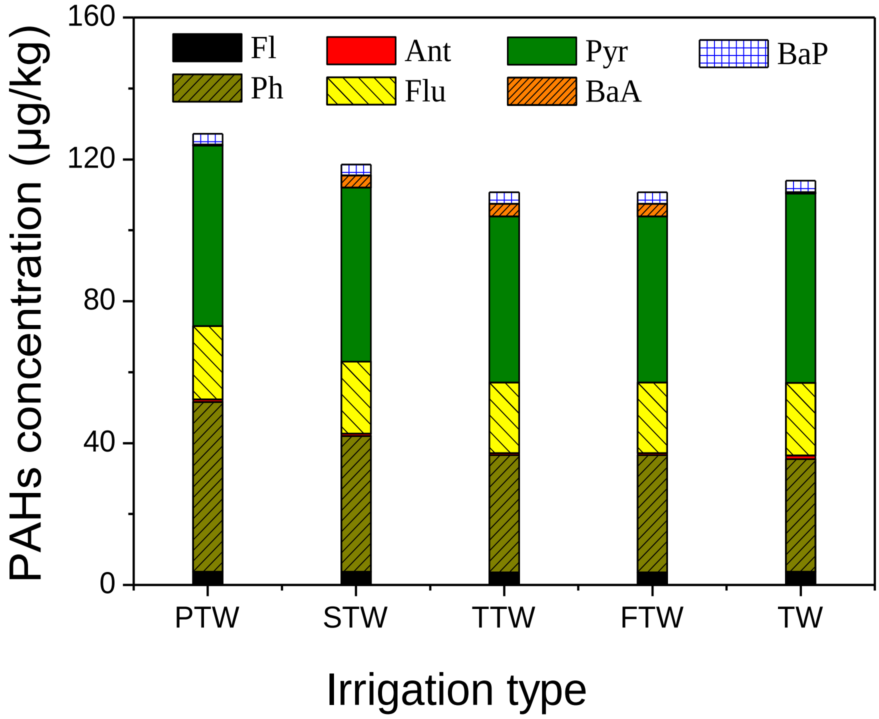


Figure 4.13 The mean concentrations of PAHs detected in radish roots on a dry weight basis.

PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

Cai et al. (2008) examined the accumulation of PAHs in *R. sativus* after sewage sludge and compost application. They found that at a concentration of 107 µg/kg of ΣPAHs in sewage sludge amended soil, the accumulation of ΣPAHs in radish roots was 104 µg/kg. The results observed in this experiment show a slightly higher accumulation of PAHs in radish roots

(107-124 μg/kg) compared with the concentration of PAHs in the soil (61-77 μg/kg). The maximum limits for PAHs in foods vary from country to country, while many countries have not established the tolerable limits in both soil and vegetables. Current EU legislation (2005) sets maximum allowed concentrations for BaP in various food products (not including vegetables) in the 1-10 μg/kg wet weight range.

The root concentration factors (RCFs) expressed as the ratio of PAH concentration in the mass root (dry weight) to the residual concentration in the soil are presented in Table 4.19. These factors are often used to determine contaminant concentrations in plants because soil-to-plant transfer is one of the major pathways for pollutants to enter the food chain (Khan and Cao, 2011).

It was found that the accumulations in radish roots were higher for low-molecular weight PAHs. Wang et al. (2011) examined PAH concentrations in roots and shoots of six vegetables from wastewater irrigated areas in China, and found the highest PAH concentration in radish roots and higher RCFs for acenaphtene, fluorene and phenanthrene. In general, the RCFs depend on the type of vegetable, PAH concentration in soil and PAH solubility, as well as the physicochemical properties of the soil. The RCF values found in this study were higher than those previously reported (Cai *et al*., 2008; Khan and Cao, 2011; Wang *et al*., 2011). In the pots the soil was watered to almost 100% water holding capacity, and as a result the mass transfer (bioavailabilty) issues that would be present in open field soils were partly reduced. It is reported that hydroponic systems remove mass transfer limitations, resulting in higher heavy metal accumulation in plants, and a similar process could have occurred in the pots with fully wetted soil (January *et al*., 2008).

Table 4.19 Root Concentration factors of PAHs in the examined radishes

PAHs	Irrigation type				
	PTW	STW	TTW	FTW	TW
Fl	3.2	8.3	9.8	8.2	9.3
Phe	2.3	2.3	2.1	2.6	2.6
Ant	2.6	17.1	16.1	17.5	24.4
Flu	1.3	1.4	1.4	1.6	1.4
Pyr	1.6	1.6	1.5	1.6	1.7
Baa	0.2	15.7	22.7	2.4	1.5
Chr	<0.1	<0.1	<0.1	<0.1	<0.1
Bbf	<0.1	<0.1	<0.1	<0.1	<0.1
Bkf	<0.1	-	-	-	-
Bap	0.8	1.3	1.3	1.8	1.5
Σ10PAHs	1.6	1.8	1.7	1.8	1.9

PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

4.4.4 Pathogens

The concentrations of *total coliforms* and *E. coli* on soil and fruit of the experimental olive grove are shown in Table 4.20. The application of PTW and STW resulted in an increased microbial contamination of soil and fruit. Results indicate a clear relationship between the degree of contamination with bacteria and the irrigation treatment. A similar relationship was found also by Al-Lahham *et al*. (2003) who examined the impact of treated wastewater irrigation on the contamination of tomato fruit. Armon *et al*. (1994) found that vegetables (including radish) irrigated with highly polluted effluents, displayed elevated numbers of indicator microorganisms.

Table 4.20 Range of total coliforms and *E.Coli* on soils and radishes

Irrigation treatment	**Total coliforms (CFU/g)**		**E. coli (CFU/g)**	
	Soil	Fruit	Soil	Fruit
PTW	154-258	5-40	20-195	0.4-0.5
STW	92-247	3-7	19-80	0.2-0.3
TTW	55-72	3-6	0-20	n.d
FTW	58-78	1-3	0-19	n.d
TW	68-94	1-3	0-19	n.d

[a]CFU/g dry weight. n.d: not detected, PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

High levels of organic matter in treated wastewaters can also enhance re-growth of bacteria (Shatanawi, 1994). Furthermore, the increased soil moisture occurred during this study may have prolonged bacterial survival or even allowed for bacterial re-growth (Bastos and Mara, 1995).

4.4.5 Radish quality

Plate 4.5 shown radishes after harvesting. Colour parameters, cracking, root fresh weight and fruit marketability of the radishes were determined.

Plate 4.5 Radishes after harvesting

Colour parameters of radish skin just after cutting were presented in Table 4.21. According to the results radishes irrigated with PTW had the highest lightness (L* value) while radishes irrigated with TTW had the lowest lightness. The chroma for radishes irrigated with STW and TTW were significant different in comparison with radishes irrigated with PTW, FTW and TW. On the other hand, Hue angle was not statistically different for all irrigation treatments.

Table 4.21 Color characteristics of radishes at the end of experiment

Parameters	PTW	STW	TTW	FTW	TW
L*	42.1 ± 4.3^{a}	36.5 ± 3.9^{c}	35.6 ± 5.7^{c}	40.4 ± 4.7^{b}	40.6 ± 3.8^{b}
a*	30.1 ± 5.9^{a,b}	41.3 ± 3.7^{c}	34.4 ± 5.5^{a}	27.3 ± 5.3^{b}	30.1 ± 4.7^{a,b}
b*	9.3 ± 1.9^{a,b}	14.3 ± 2.4^{c}	11.1 ± 2.5^{a}	8.1 ± 1.5^{b}	10.5 ± 2.3^{a}
Chroma	31.5 ± 6.0^{a,b}	43.7 ± 4.4^{c}	36.1 ± 6.0^{a}	28.5 ± 5.3^{b}	31.9 ± 5.0^{b}
Hue angle (deg)	17.3 ± 2.5^{a}	19.0 ± 1.5^{a}	17.7 ± 1.7^{a}	17.0 ± 3.4^{a}	19.3 ± 2.7^{a}

mean values and standard deviations , a, b, c: In each row means values followed by a different symbol are significantly different to one another ($p < 0.05$), L* indicates lightness and a* and b* are the chromaticity coordinates. +a* is the red direction, -a* is the green direction, +b* is the yellow direction, and -b* is the blue direction, PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

Schreiner et al. (2002) examined the seasonal climate effect on root colour of the red radish ‘Nevadar’ in Germany. From November until March the radishes were cultivated in a greenhouse; from April until October they were field grown. Monthly mean temperature varied from 2^{o}C to 15^{o}C during experimental period. Hue angle values ranged from 17.5 to 22.5 and chroma ranged from 34.3-48.8. The highest values were observed during the summer period while they were lowest during winter. According to Berger-Schunn (1994) a difference of 1 to 2 units in L*, a* or b* is noticeable to most observers. A consumer acceptance test in Germany demonstrated that consumers preferred bright-reddish radishes with hue angle values above 23^{o} and chroma values above 35 to assure high consumer acceptance (Schreiner et al., 1999). So it would be stated that the use of treated wastewater improved the colour characteristics (chroma) of radishes.

The cracking rate in radishes is shown in table 4.22. According to the results radishes irrigated with TW had the highest cracking rate (24.2%). When a cracking phenomenon occurred the grade of cracking was generally observed to be Grade 4 for all examined irrigation waters. The use of treated wastewater as well as FTW tended to reduce the incidence of cracking.

Table 4.22 Cracking rate in radishes

Irrigation	**Cracking rate (%)**				
Treatment	Grade 1	Grade 2	Grade 3	Grade 4	Overall
PTW	1.5	0	1.5	4.5	7.6
STW	1.4	1.4	2.9	7.1	12.9
TTW	1.5	1.5	3.1	4.6	10.8
FTW	2.0	0	2.0	6.1	10.2
TW	0	3.2	6.5	14.5	24.2

*1: very shallow (0-15 mm); 2: shallow (15-25mm); 3 deep, (whole radial); 4: open fruit, PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

Kang and Wan, (2005) found a cracking rate between 1.4-18.9% of radishes at different soil water potential. In general, cracking is related to plant nutrients, climatic conditions, water stress as well as physiological and morphological changes of fruit (Odemis *et al*., 2014). In this study, low levels of nutrients and minerals in TW in comparison with treated wastewaters and FTW may have as a result caused an increasing cracking rate.

Fruit marketability according to four independent customers (selected members of staff from TEI Crete) as well as root fresh weight are shown in Table 4.23. An improvement of quality of radishes irrigated with treated wastewaters was observed in comparison with radishes irrigated with TW.

Table 4.23 Root fresh weight and fruit marketability if radishes

	Root fresh weight	Fruit marketability* (% of the radishes)				
		Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
PTW	68 ±30	16 ± 7	16 ± 7	30 ± 11	28 ± 2	10 ± 6
STW	61 ± 26	17 ± 7	12 ± 4	33 ± 5	27 ± 6	11 ± 4
TTW	58 ± 21	20 ± 6	10 ± 6	30 ± 9	29 ± 5	11 ± 5
FTW	97 ± 45	17 ± 4	11 ± 6	34 ± 10	27 ± 4	11 ± 5
TW	44 ± 20	30 ± 8	20 ± 3	18 ± 10	25 ± 3	7 ± 3

mean values and standard deviations, *1: not marketable quality; 2: low quality 3: medium quality; 4: good quality; 5 extra quality, PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

As already mentioned the cracking rate of radishes irrigated with TW was higher than the other treatments resulting to lower fruit marketability. In addition, radishes irrigated with TW were smaller further decreasing their market quality (Plate 4.6).

Plate 4.6 Radishes just after harvesting (from left to right: TW, FTW, TTW, STW, PTW)

In general, in fruit markets, there are different qualities of agricultural products (fruit, vegetables, nuts etc) depending on the size. Even if the weight of a vegetable bought by customers is the same bigger vegetables are preferable. Moreover, in many cases there are different prices depending on the fruit size.

4.5 Carnations

4.5.1 Plant Growth

Plate 4.7 shows the carnations after a cultivation period of about 2 months. Plants were healthy without any visible symptoms of distress.

Plate 4.7 Carnation's growth at the end of the experiment

Growth characteristics of carnations irrigated with the five different water sources were presented in Figures 4.14, 4.15 and 4.16. 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.

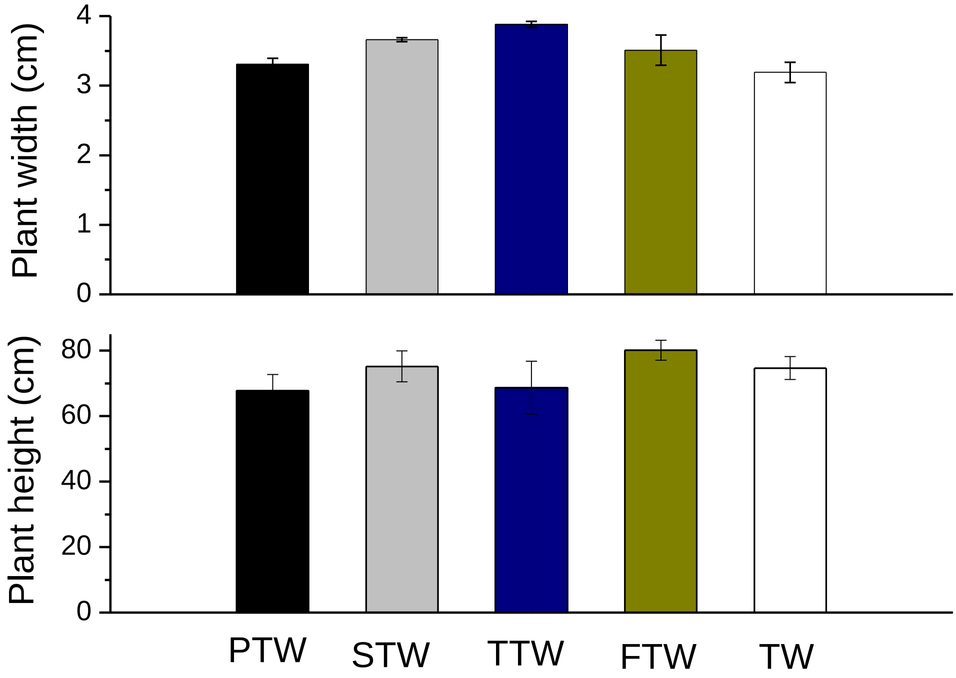


Figure 4.14 Height and width of carnations irrigated with different irrigation treatments.

PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

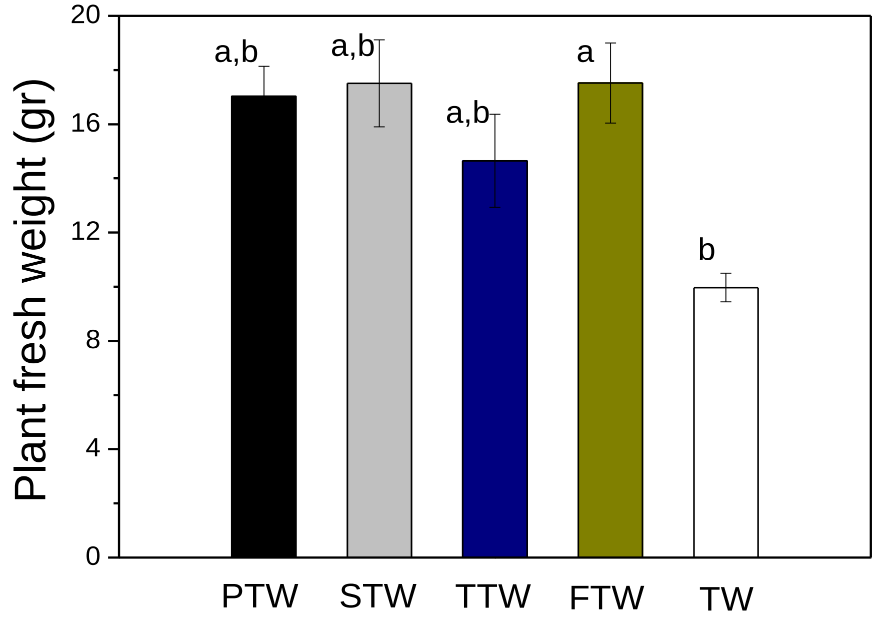


Figure 4.15 Fresh weight of carnations with different irrigation treatments.

In each measurement, different small letters indicate significant differences (p<0.05) between treatments. PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

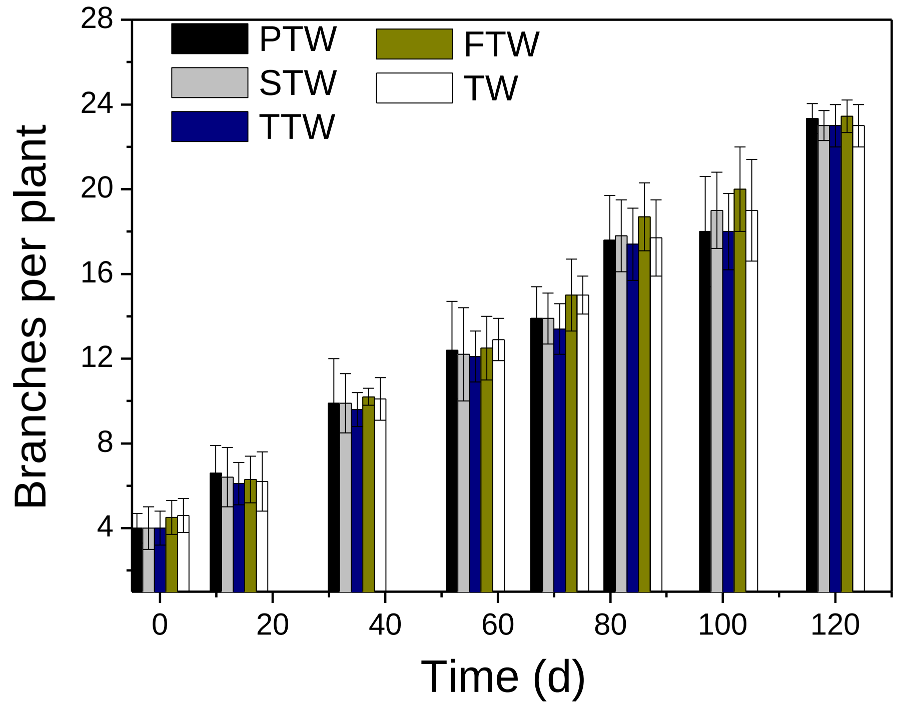


Figure 4.16 Number of branches per plant during the experiment

PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

Average plant height was 67.7 cm, 75.2 and 68.7 for PTW, STW and TTW respectively. These values were not statistically significantly different from the average plant height found for FTW and TW. Similar, plant width ranged from 3.2 cm (for TW) to 3.9 cm (for TTW) without any statistical difference between them. In addition, plant fresh weight was almost the same for PTW, STW and FTW. Lower but not statistically different fresh weight (with the exception of FTW vs TW) was found for plants irrigated with TTW and TW. The number of branches per plant was almost the same for all irrigation treatments ranging from 23.0 to 23.4.

Leaf SPAD was significantly higher for all treated wastewaters in comparison with TW (Figure 4.17). 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 4.18).

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.

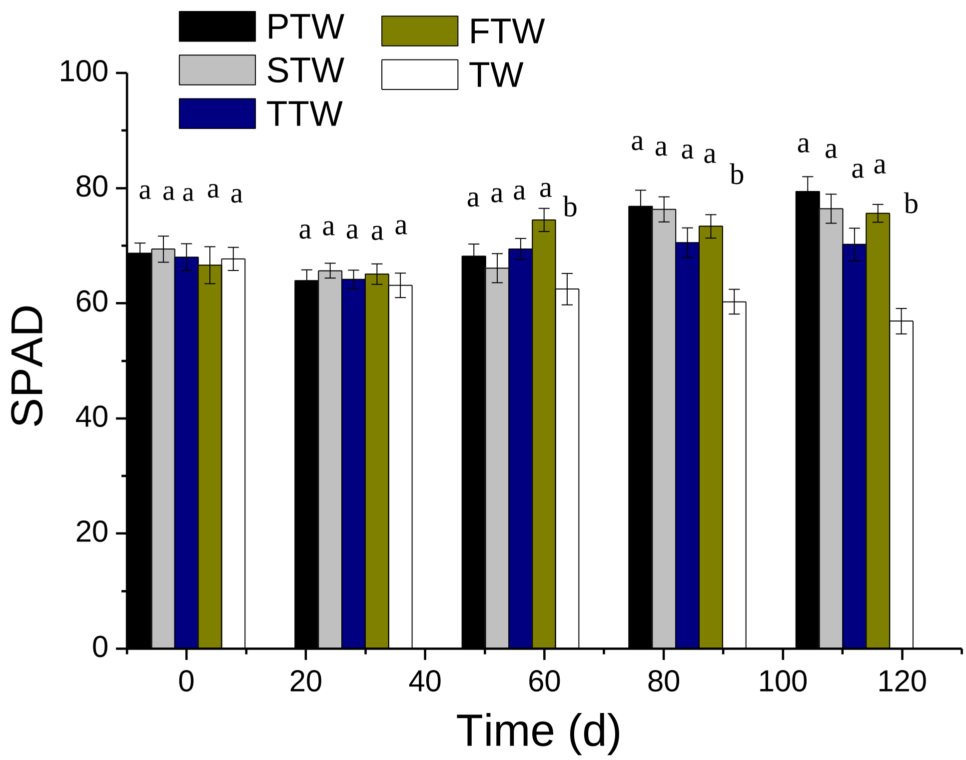


Figure 4.17 The effect of water treatments on the leaf SPAD of carnations. In each measurement, different small letters indicate significant differences ($p<0.05$) between treatments, PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

Leaf SPAD values and F_v/F_m 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 F_v/F_m values for two ornamental plants. They did not find any change for polygala and significant reduced values for lantana.

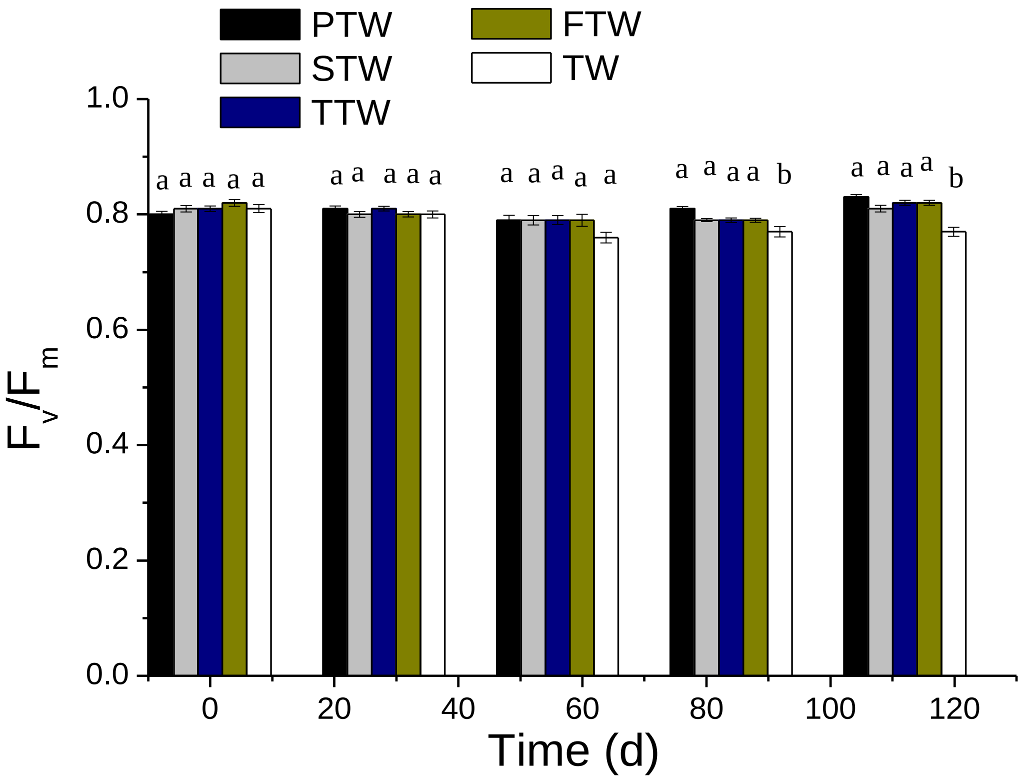


Figure 4.18 The effect of water treatments on maximum photochemical efficiency of photosystem II of carnations.

In each measurement, different small letters indicate significant differences ($p<0.05$) between treatments. PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

4.5.2 Leaf content

Macroelement contents were affected by the treatments as presented in Table 4.24. Leaf nitrogen, phosphorus and potassium content increased in plants irrigated with wastewaters in comparison with TW. 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.

Table 4.24 The effect of irrigation on the leaves of carnations

Parameter	PTW	STW	TTW	FTW	TW
N (g/kg)	23 ± 2 [a]	23 ± 2[a]	16 ± 2[b]	21 ± 1[a]	12 ± 1[c]
P (g/kg)	8 ± 1[a]	5 ± 1[b]	6 ± 1[c]	6 ± 1[c]	4 ± 1[b]
K (g/kg)	44 ± 4[a]	46 ± 2[a]	43 ± 4[a]	80 ± 8[b]	40 ± 7[a]
Ca (g/kg)	28 ± 3[a]	25 ± 5[a]	24 ± 4[a]	29 ± 5[a]	31 ± 7[a]
Mg (g/kg)	7 ± 1[a]	6 ± 1[a]	5 ± 1[a]	6 ± 2[a]	6 ± 1[a]
B (mg/kg)	137 ± 36[a]	133 ± 42[a]	137 ± 24[a]	154 ± 47[a]	162 ± 75[a]
Cu (mg/kg)	8 ±2 [a]	5 ± 1[a,b]	5 ± 1[b]	7 ± 1[a,b]	5 ± 1[b]
Fe (mg/kg)	90 ± 1[a]	41 ± 9[b]	26 ± 10[b]	63 ± 15[a,b]	26 ± 6[b]
Zn (mg/kg)	181 ± 20[a]	129 ± 12[b,c]	68 ± 12[c]	135 ± 30[a]	78 ± 16[c]

mean values and standard deviations, a, b: In each row, mean values followed by a different symbol are significantly different ($p < 0.05$), PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

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 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 (Gupta, 1993). 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.

4.5.3 Flower quality

The variety "Dover" used in the experiment produced tall white flowers (Plate 4.8). Assessment of treatments in terms of the number of open flowers showed the superiority of FTW over all other irrigation waters (Figure 4.19). In addition, the application of all treated wastewater had a positive (for PTW and STW) or a neutral (TTW) effect in comparison with the application of TW. The mean diameter of flowers (Figure 4.20a) was 46.0 mm for PTW, 42.8 mm for STW, 49.3 for TTW, 52.0 mm for FTW and 44.2 mm for TW. Flower height (Figure 4.20b) ranged from 41.0 mm (STW) to 45.1 mm (TW) for all treatments. Statistical analysis shows that all values were not significantly different both for flower diameter and flower height.

Plate 4.8 Cut flowers of carnation at the end of experiment (from left to right: primary treated wastewater, fertilized tap water and secondary treated wastewater)

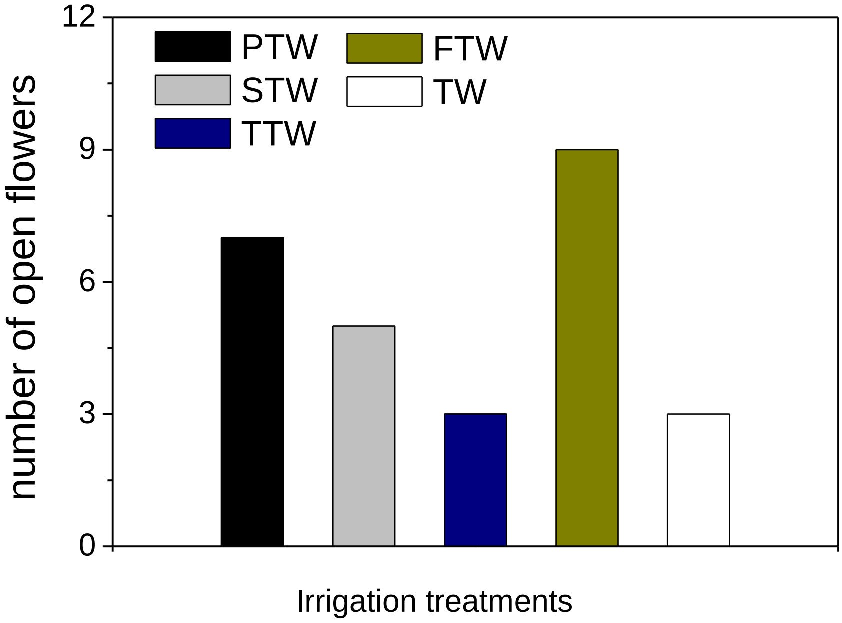


Figure 4.19 The number of carnation flowers for the five irrigation treatments

PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

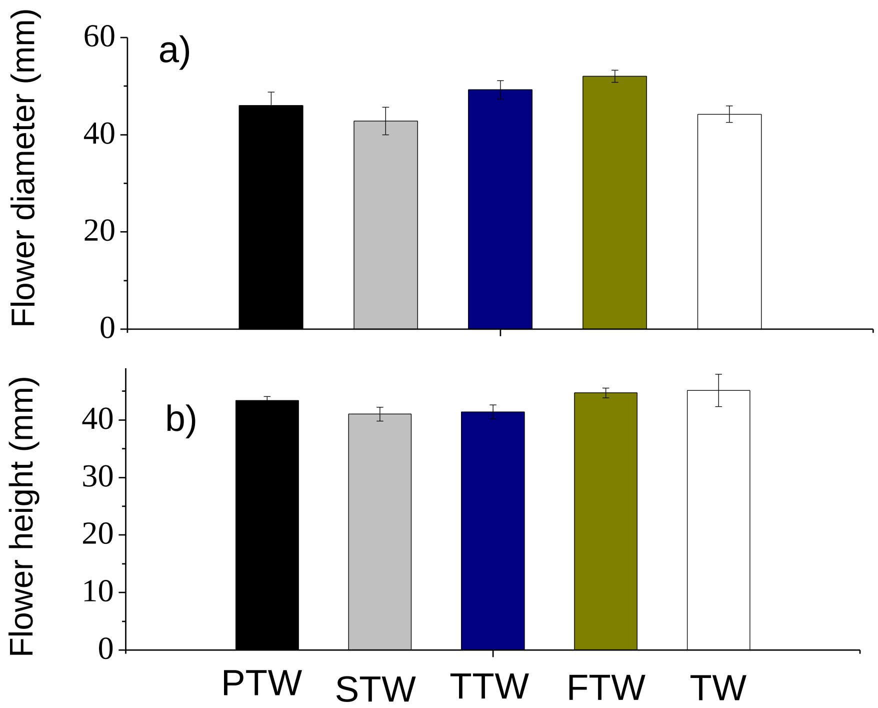


Figure 4.20 This shows in a) the diameter and b) the height of carnation flowers at the end of the experiment.

PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

Flower fresh weight (Figure 4.21) was found to be higher for all treated wastewaters (4.6 g, 4.9 g, 4.5 g for PTW, STW and TTW respectively) in comparison with TW (4.2 g). The use of fertilizer in the water resulted in an increase of flower fresh weight (5.6 g).

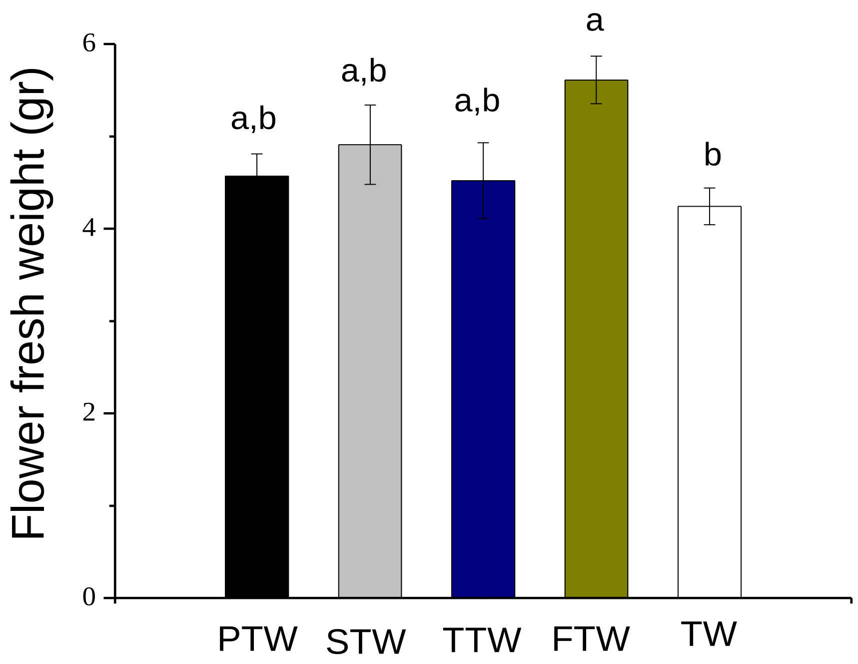


Figure 4.21 Fresh weight of carnation flowers at the end of the experiment.

In each measurement, different small letters indicate significant differences ($p<0.05$) between treatments. PTW: Primary treated wastewater, STW: Secondary treated wastewater, FTW: Fertilized tap water, TW: Tap water

Chapter 5

Conclusions and Recommendations for further research

5.1 Conclusions

The countries around the Mediterranean have significant levels of water scarcity make it imperative to exploit wastewater as an alternative irrigation source of water. Tertiary treated wastewater is used for irrigation in the European part of the Mediterranean as well as in Israel. On the other hand, in many cases in the Middle East and North Africa untreated or partially treated wastewater is also used for irrigation. In any case, 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. For this reason further studies are need to better clarify the acceptable level of contamination in treated wastewaters, specific for each crop, in such a way as to encourage low cost treatment and at the same time to guarantee safe reuse for the environment and the public health.

During this thesis, the suitability of treated wastewater for use in four Mediterranean crops (olives, grapes, radishes and carnations) was evaluated by studying the water's effect on the soil-plant system, the crop yield, fruit quality and the presence of inorganic chemical contamination (salts, elements and heavy metals), organic chemical contamination (PAHs) and microbial contamination (E.Coli, total coliforms) as crop food safety parameters. The novelty of this work lies on the fact that: a) the effect of treated wastewater on “Koroneiki” olive variety and “Crimson seedless” grape variety was examined for the first time, b) previous relative works on radishes and carnations are very limited and not in Mediterranean conditions, c) the monitoring of PAHs (priority pollutants according European Water Framework Directive) in soil-plant systems irrigated with treated wastewater was examined for the first time and d) monitoring of fruit quality and plant growth using parameters such as fruit colour, leaf SPAD and chlorophyll fluorescence were not used before in crops irrigated with treated wastewaters.

According to the results it is suggested that in comparison with tap water, tertiary treated wastewater could be applied safely in all the examined crops having more or less a positive effect on plant growth and fruit quality and no significant negative effect on soil. Secondary treated wastewater improved crop growth but due to the presence of pathogens could be applied safely only in olive trees Primary treated wastewater improved crop growth but could not be applied in all the examined crops not only due to the presence of pathogens but also because they accumulate PAHs in radishes and decreased fruit quality characteristics of radishes and grapes. Specifically:

For the olive trees, no significant differences were found between treated wastewater and tap water from an agronomic point of view as indicated by trunk diameter, plant height, the concentration of minerals in the leaves and photosynthetic activity. On the other hand, increased concentrations of sodium, phosphorus, potassium and nitrogen in soils irrigated with treated wastewaters were also observed. Even if the Na content was below the toxicity limit the observed tendency for it to increase may affect plant growth after long-term implementation of wastewater irrigation.

Heavy metals and PAHs occurred in wastewater but did not appear to have an adverse effect on soil quality. The pollution of soils by airborne PAHs is likely to be the major contributor to PAHs in the soil rather than the PAHs contained in the treated wastewater.

This study has shown the successful use of tertiary treated wastewater for the healthy and safe irrigation of olive groves while the results for secondary treated wastewater suggest that even medium-quality wastewater could be safely applied.

For grapevines the results of this study have shown that the application of treated wastewaters had no significant effect on the soil and leaf quality. However, low quality treated wastewaters (PTW) had a negative impact on vine growth and grape quality. Trunk diameter and pruning weight was found to be lower in vines irrigated with PTW in comparison with all the other irrigation treatments. Fruit quality characteristics such as colour, total soluble solids (^{o}Brix) and titratable acidity were also adversely affected by PTW. On the other hand, the application of TTW did not appear to have any negative effect on the grapevines while in some cases it improved growth the parameters of the vines including leaf chlorophyll concentration (SPAD value) and yield.

For radishes, no significant differences in the macro and micro- nutrient status were found in the soils as well as in radish roots irrigated with treated wastewaters in comparison with soil irrigated with tap water. On the other hand, the use of FTW resulted in higher concentrations of some compounds such as phosphorus and potassium. PAHs were taken up by radish from soils and therefore the application of primary treated wastewater could lead to the accumulation of PAHs in soil and radish roots. Even though there are no regulatory limits in the EU for PAHs in vegetables it is recommended that exposures to PAHs from food should be as low as reasonably achievable.

The concentration of ΣPAHs in the roots was found to be positively correlated with the concentration of PAHs in the soil. The most abundant PAHs were observed to be phenanthrene, fluoranthene and pyrene, both in the soil and in radish roots. Comparing the calculated bioaccumulation factors with those estimated in the past for other vegetables, it was concluded that radish had a higher potential (higher health risk) as a result of contamination by PAHs.

Significant differences between treatments were found in selected fruit quality characteristics such as colour, cracking and fruit marketability of radishes. The application of all the different types of treated wastewaters appeared to improve all these parameters.

The presence of pathogens was found in the edible part of the fruit for plants irrigated with PTW and STW. Radishes are often eaten raw, or with rich dressings which may result in re-growth of some pathogenic bacteria and may therefore threaten the public health.

For carnations, 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 were found to have no adverse effect on carnation cultivation as indicated by plant growth characteristics.

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 the irrigation water used (primary treated>secondary treated>tertiary treated).

5.2 Recommendations for future work

- Effects of long term irrigation (>10 years) with treated wastewater on soil status, plant growth and fruit quality should be further examined. Results from this study have shown that the Na concentration in treated wastewater was higher than in tap water. Therefore long term irrigation may result in the accumulation of Na in soils at levels which could affect plant growth and fruit quality.
- Further investigations on the effect of treated wastewater on fruit quality should be conducted as the results shown that several parameters as color, cracking and fruit content of radishes and grapes affected by treated wastewater.
- The results of this study indicate the potential health risk of consuming radishes from wastewater irrigated areas due to the presence of PAHs. Mechanisms regarding the transfer of PAHs in radishes and other vegetables should be examined
- Other types of irrigation techniques such as sub-surface irrigation and sprinkler irrigation should be tested since they may have an impact on the microbiological quality of soil and fruits. In addition, the cultivation of vegetables and ornamental plants in hydroponics using treated wastewaters is a very interesting option.

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List of Abbreviations

Ant	Anthracene
Baa	Benzo(a)anthracene
Bap	Benzo(a)pyrene
Bbf	Benzo(b)fluoranthene
Bkf	Benzo(k)fluoranthene
Chr	Chrysene
BOD	Biochemical oxygen demand
CFU	Colony Forming Units
CIRG	Color Index of Red Grapes
COD	Chemical oxygen demand
EC	Electrical Conductivity
Fl	Fluorene
FLD	Fluorescence Detector
Flu	Fluoranthene
FTW	Fertilzed tap water
F_v/F_m	Variable fluorescence (F_v) / maximal fluorescence (F_m) ratio
HPLC	High Performance Liquid Chromatography
MPN	Most Probable Number

PAHs	Polycyclic aromatic hydrocarbons
Phe	Phenanthrene
PTW	Primary treated wastewater
Pyr	Pyrene
RCF	Root Concentration Factor
SCF	Scientific Committee on Food
SPAD	Special Products Analysis Division
STW	Secondary treated wastewater
TA	Titratable acidity
TEIC	Technological Educational Institute
TN	Totan Nitrogen
TP	Total Phosphorus
TSS	Total Suspended Solids
TTW	Tertiary treated wastewater
TW	Tap water
USEPA	United States Environmental Protection Agency
WHO	World Health Organization
ΣPAHs	Sum of 10 PAHs