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Growth Development, Physiological Status and Water Footprint Assessment of Nursery Young Olive Trees (*Olea europaea* L. 'Konservolea') Irrigated with Urban Treated Wastewater

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Abstract: Application of urban treated wastewater (TWW) has been practiced globally as an alternative irrigation water source in areas where access to safe and abundant freshwater is limited. Water footprint (WF) has been employed over the last decades as a tool for the assessment of the sustainable management of water resources. In the present study, the suitability of TWW for the irrigation of nursery young olive trees (*Olea europaea* L. 'Konservolea'), one of the main table olive cultivars in Greece, the second global table olive exporter, was tested and compared to tap water irrigation and application of zeolite on soil. Plant growth and physiological parameters and stress indicators were measured. Additionally, a WF assessment was performed, distinguishing TWW from freshwater (blue water) resources in order to examine the possibility of minimizing the environmental impact through the limitation of freshwater use. Plants irrigated with TWW performed better in most of the growth and physiological parameters measured compared to the other treatments. Stress indicators revealed that TWW did not induce any additional stress. TWW could be used as an irrigation water source for young olive trees for at least a short period during their growth as a safe and sustainable alternate of blue water resources. Additionally, the WF assessment showed that the application of TWW could be a significant blue water saving measure.

Keywords: blue water; table olives; total phenols; proline

1. Introduction

1.1. Pressure on Global Freshwater Resources

During the last 50 years, the amount of total available renewable freshwater has decreased by almost 50% when at the same period, freshwater withdrawal, which serves not only the fundamental living standards for the growing population but also for the compliance to modern consumption and production patterns, has almost doubled (114%) [1,2]. Comparing the average rate of global freshwater withdrawal (4000×10^9 m^3 per year) to the total renewable freshwater resources ($54{,}730 \times 10^9$ m^3 per year) [2], an almost 9% global water stress is calculated, but, when these figures are expressed at the country level, withdrawals reach an average global water stress equal to 57.82% [2]. With this pace and taking into consideration the effects of climate change on the global water reservoir, it is obvious that pressure on water resources will be intensified the following years. To this pressure, degradation of the quality of fresh water resources due to the increasing loads of pollutants that human activities release to receiving aquifers should also be added.

Agriculture, as a basic food providing activity, is globally the largest freshwater consumer (72%), followed by industrial activities (16%) and households (12%). In Europe, which consumes 7% of global freshwater, industry is the largest consumer (44.9%), while agriculture and household use follow at 29.4% and 25.7%, respectively. The Mediterranean region and Western Europe withdraw almost equal quantities of water (83.7×10^9 m^3 per year and 82.7×10^9 m^3 per year, respectively) but in the Mediterranean, the largest portion is allocated to agriculture while in Western Europe, it is allocated to the industrial sector. Among the Mediterranean countries that are the first line of the European continent exposed to climate change effects on precipitation rates and most expected to face serious water scarcity issues in the future, Greece consumes more water per unit of cultivated area (ratio: 2.5), compared to Italy (ratio: 1.8) and Spain (ratio: 1.2) [2].

Intersectoral countermeasures for the halting of the proceeding intensity of global water scarcity include rational use of water resources, decrease in pollution, and the employment of alternative water resources. Specifically in agriculture, rationalization of irrigation, application of alternative water resources, and an increase in fresh water productivity consist of key elements of any strategy drawn for the preservation of water resources.

1.2. Application of Urban Treated Wastewater

Application of treated wastewater (TWW) for irrigation as an alternative irrigation water resource in arid areas dates back in antiquity [3,4] while the first relevant organized project of the modern era was established in California in the early 1900s [5]. Since then, significant progress has been made in many levels of treated wastewater reuse such as legal = issues, sanitation, treatment procedures, transport, etc. Currently, 6.8×10^9 m^3 per year of treated wastewater are globally applied to irrigate 4276.7×10^3 ha of cultivated land [2].

Application of treated wastewater for irrigation purposes has strong supporters among policy makers and scientists globally since it cannot only replace freshwater resources safely when treated adequately, but in many cases, it enhances crop development and yield. Numerous studies have shown that TWW contains important quantities of macro- and micronutrients, which through irrigation, are added to the soil to be uptaken by the plants [6,7]. Due to its composition, wastewater is not only an important source of nitrogen, phosphorous, potassium, and other nutrients such as zinc, iron, manganese, and copper, but also facilitates the nutrients' uptake by plants [8–10]. Application of TWW to crops has fertilizing effects, decreases the need for synthetic fertilizer application, and thus limits water pollution. TWW has a high content of organic matter, which leads to a general enhancement of soil quality through the improvement in nutrient availability and recycling, soil stability, and reduction in soil erodibility [6,11]. Microbial population and diversity in soil can also be positively affected by TWW [12]. Considering the role of microbial communities in soil and hence plant growth, application of TWW can affect soil fertility and enhance plant development [13]. Furthermore, many microbes can act as remediating agents against contaminants such as pesticides, heavy metals, and emerging contaminants (antibiotics etc.) [14]. Skepticism on TWW application is mainly related to the impacts of TWW reuse on public health, the soil–plant system, and the environment, since it may contain pathogens, heavy metals, and other toxic substances that accumulate not only in soil, but also in the edible parts of plants when wastewater treatment is inadequate [12–16]. To the direction of minimizing the risks of TWW reuse, relative legislative framework and guidelines have been developed at both national and international levels. Recently, the European Committee has developed the 2020/741 Directive on Minimum Quality Requirements for Treated Wastewater Reuse, which will be in force in 2023, setting the standards for TWW reuse among the European countries. Salinization of soil and the effect on its hydraulic properties as a result of high cation (sodium, magnesium, iron, calcium) or anion (chloride) concentration in TWW is considered to be the main threat to plant development and health [6,9,15–17]. Additionally, the presence of nutrients in TWW, although favorable up to a certain level, above a threshold concentration, they can induce plant toxicity [7]. The severity of the above-mentioned impact of TWW on the soil–plant nexus is directly related to the TWW composition, the irrigation frequency and volume, soil properties, and of course, the plant's characteristics and salt tolerance [6]. This negative impact could be moderated or even eliminated through a customized application scheme that involves the tolerable species choice, the TWW dilution, the frequent "rinsing" (irrigation at several intervals with freshwater), and the adjustment of applied quantities. Economic feasibility of TWW reuse projects is often questioned since large investments regarding treatment and transportation are required [18], but even in this case, the reuse of TWW can relieve the costs of pumping the effluent from the treatment site to the receiving water body if sites of reuse are closer [19] and generally, it can be accepted that the environmental benefits coming from TWW reuse may recover the investment and operation costs, especially when more and more countries are under the threat of serious water stress. The most important point is that application of TWW for irrigation reduces fresh water abstraction and preserves fresh water resources, partially relieving the global freshwater pressure. There are many cases of arid or semi-arid countries that have compensated for the effect of prolonged drought periods by employing TWW in irrigation schemes and the adoption of such an alternative irrigation water resource has constantly gained ground among European countries, but the substantial increase in its reuse globally also consists of one of the UN's SGD6 indicators [20]. Considering that 380×10^9 m^3 of TWW is produced annually and 80% of it is discarded, the potential of TWW reuse to address water scarcity is significant [21].

1.3. Application of Water Footprint Assessment for Rational Irrigation

Among the many competent tools that can be incorporated in a rational irrigation scheme, most of which are focusing on soil moisture deficit management, water footprint (WF) is largely used as an indicator of water appropriation and pollution during cultivation practices. WF is a measure of a procedure's effect on the quantity and the quality of water resources. It is computed as the sum of three components: the green and blue water footprints that respectively refer to the rain consumed or the surface or underground water resources used during a production process or incorporated in the end product and the grey water footprint, which is the theoretical quantification of pollution that the production process causes to the receiving water bodies [22]. In a cultivation process, the WF links the water consumption only to the amount that covers the crop's water needs, which is expressed through the crop's evapotranspiration and not the total water applied since it suggests that excess water cannot be considered as "lost", as long as it returns back to the water basin and is not transferred to another or to the sea.

The water footprint assessment includes (a) the determination of the scope of the WF analysis, (b) the WF computation, (c) the WF sustainability assessment, and (d) the WF response formulation [22]. Applying the WF approach, the hot spots of water use and pollution during a process are revealed and the action toward the minimization of WF can be the base of a rational water management strategy drawn in many levels (individuals, company, regional, national, global, crop, water basin, etc.). As the WF of many products or services has been computed by researchers during the last decades and global datasets of crops and end products have become available, one should look beyond the absolute numbers presented and focus on the WF's sustainability, taking into account the available renewable water resources. There are many studies that point out the unsustainable fresh water use (either groundwater or surface water) in major crops and countries [23]. The WF considers as blue water all the water resources and does not diversify the alternative ones. Fridman et al. [24] proposed the extension of blue water footprint to include alternative water sources as different modules so that the actual blue water used (surface and groundwater) is not overestimated.

1.4. Olive Cultivation

Since antiquity, olive cultivation has been a typical characteristic crop of the Mediterranean basin landscape and culture. In the last decades, it has rapidly expanded in the area, but also in other countries with a similar climate (California, Chile, Saudi Arabia, etc.). Globally, 12,763,498 ha of olive crops [25] produce 23,642,927 tons of olives annually [25]. The predominant countries in olive production are traditionally Spain, Italy, and Greece, which together account for more than half of global production (55.5%), but countries such as Turkey and Tunisia as well as new entries in the last 15 years such as Albania, Chile, Australia, Saudi Arabia have steadily increased their production, claiming their position in the global market. Having been established globally in the conscience of consumers for its healthy attributes, the Mediterranean diet has introduced olive oil and relevant olive products in many culinary activities, hence significantly increasing the demand. The global production of olive oil has reached 3.1 million tons, which has almost doubled in the last thirty years (1990: 1.45 million tons) [26]. The global consumption, which has reached 3.2 million tons, has increased 1.5-fold since 1990 [26]. In the case of table olives, the increase in global production and consumption (which in 2020 reached 2.8 and 2.7 million tons, respectively) has tripled over the past thirty years [26]. The figures make clear the dynamics and perspectives of olive products globally, but also the increasing competition of traditional countries and new producers for their share in the global olive market.

1.5. Table Olives

In Greece, which ranks third in global olive production, olive groves cover a total area of almost one million ha and more than 2.8 million tons of olives were produced in 2020 [25]. Regarding table olives, Greece is the second worldwide producer (230,000 tons in 2020) [26] and exporter (80,000 tons in 2021) [26] following Spain, and the prospectives are very optimistic. One of the three most important, in terms of production and export activity, table olive cultivars in Greece is the 'Konservolea' (*Olea europaea* L.). It is predominant in the central zone of Greece and in the plain of Arta (northwestern Greece), where it accounts for almost 8% of the total table olive production in Greece. Traditionally, the 'Konservolea' olive groves in Arta are rainfed, as are the majority of olive groves in the Mediterranean basin. Although the olive tree is a rather drought tolerant species, favorable cultivation practices such as irrigation enhance its development and final fruit yield and quality. In the area, irrigation is applied during the summer months when rainfall is scarce. Olive seedlings budded with the 'Konservolea' cultivar (as this cultivar is impossible to propagate commercially with cuttings) are propagated mainly in open or covered nurseries where they are kept for at least one year before they are transplanted in the field. Irrigation in nurseries on some occasions and mainly in Arta is performed by a water consuming alternative of the "ebb-and-flow" method where large quantities of water flood the basin of the nursery and then the excess water is disposed to the environment.

1.6. Scope of the Experiment

The objective of the experiment was to evaluate the growth, development, and physiological status of nursery 'Konservolea' olive young trees irrigated with urban TWW. Additionally, the WF of nursery 'Konservolea' olive young trees irrigated using TWW was computed and compared to the respective ones of those irrigated using fresh (tap) water (TaW), differentiating the blue water footprint according to the water resource. A preliminary attempt was also made to assess the WF of young olive trees in local nurseries irrigated based on the local practice.

2. Materials and Methods

2.1. Experimental SITE

The experiment was carried out in a twin-span glass-covered greenhouse, W–E oriented, located at the premises of University of Ioannina, Department of Agriculture in the Kostakii Campus (latitude 39°0.7′ N, longitude 20°56′ E, altitude 5 m) near Arta, at the northwestern part of Greece. The climate in the area is typical Mediterranean with rainy cold winters and hot and dry summers. The mean annual average temperature is 17.2 °C and the average annual precipitation reaches 1084 mm, concentrated mainly during the winter months. A preliminary experiment was conducted in the period from May to November 2019 in order to assess the primary growth and tolerance characteristics and the main experiment was conducted from May to November 2020.

2.2. Plant Material, Experimental Conditions, Treatments

Fifty (50) uniform one-year-old young olive plants of the 'Konservolea' cultivar (*Olea europaea* L.) were supplied by a local nursery accompanied with their phytosanitary certificate. Single stem young olive trees had an average height of 70 cm. The rootstock was a wild olive seedling budded with 'Konservolea' buds. The plants were transplanted to three liter pots filled with sandy loam soil. Irrigation was performed by a micro irrigation system with pressure compensating emitters (4 Lh^{-1}). The full fertilizer application following the local practice was application of 1.5 g of 20–20–20 fertilizer to each pot. The pots were kept under ambient conditions in shade for at least one and a half months. During the acclimatization period, all plants were irrigated with fresh (tap) water. Then, they were transferred to the greenhouse in order to prevent rain from affecting the experiment. The mean minimum temperature in the greenhouse during the experimental period ranged between 15 and 22.7 °C and the mean maximum temperature ranged between 35.6 and 44.6 °C. The relative humidity ranged from 29% to 78% and the average incoming daily solar radiation ranged from 4.7 to 56 W m^{-2}. After establishment in the greenhouse, the plants were subjected to the following treatments:

1. Irrigation with municipal treated wastewater and application of full quantity of fertilizer (TWW);
2. Irrigation with municipal treated wastewater and application of half quantity of fertilizer (TWW1/2F);
3. Irrigation with fresh (tap) water and application of full quantity of fertilizer (TaW);
4. Irrigation with 75% quantity of tap water and application of full quantity of fertilizer (TaW75%); and
5. Irrigation with fresh (tap) water, with 10% zeolite substrate (v/v) and application of full quantity of fertilizer (Zeolite). Zeolites are hydrated alluminosilicates, which, due to their structure, have high adsorption capacity. In agriculture, they are applied on the soil, improving its physical, chemical, and biological properties. They have been highly recommended for soil application during the last years due to their ability to store water and also capture, store, and slowly release nutrients to soil solution [27].

2.3. Water Resources

TaW was provided by Arta's Municipal Water Supply and Sewerage Company. TWW was provided by the Arta's Municipal Wastewater Treatment Plant. The recycled water had undergone tertiary treatment. TWW was collected the same day that it was used. The chemical properties of tap water and TWW are summarized in Table 1.

2.4. Water Needs and Irrigation Schedule

Over the experimental period, all plants were irrigated according to their actual water needs based on measurements of the evapotranspiration. Irrigation volume was calculated twice a week as the difference of the weight of the system pot–plant early in the morning and the weight of the system pot–plant at the same time the next morning. A balance model 60000 G SCS (Persica, Dietikon, Switzerland) was used for pot weighing. TWW treatments were irrigated once a month with tap water in order to avoid the risk of salt concentration. Table 2 summarizes the monthly amount of water applied to each plant during the experimental period.

Table 1. Chemical characteristics of the experimental water resources, fresh water (TaW), and treated wastewater (TWW).

Parameter	TaW	TWW
pH	7.63	7.53
EC (dS m^{-1})	0.58	1.02
NO_3^- (mg l^{-1})	0.00	12.30
NH_4^+ (mg l^{-1})	0.00	0.21
K^+ (mg L^{-1})	0.95	20.7
PO_4^{-3} (mg L^{-1})	0.00	8.99
Ca^{2+} (mg L^{-1})	76	80.78
Mg^{2+} (mg L^{-1})	15.6	11.6
Na+ (mg L^{-1})	18.4	86
HCO_3^- (mg L^{-1})	218	269
Cl^- (mg L^{-1})	28	68
SO_4^{2-} (mg L^{-1})	76	98.9

Table 2. Amount of water applied to each plant during each month of the experimental period.

	June	July	August	September	October	November
Water per plant (mL)	820	3700	4800	3500	2450	700
75% water per plant (mL)	615	2775	3600	2625	1838	525

2.5. Plant Development (Height, Number of Leaves and Stem Diameter, Biomass, and Leaf Area)

Plant development was measured monthly. The length of the central stem was measured from the height of 10 cm from the budding point to the top of the plant. The number of lateral shoots developed was counted and the length of lateral shoots was measured from the basal point to the top of each lateral shoot each month. The increase in height of each plant was computed as the increase rate between the sum of the length of the central stem and the lateral shoots at the beginning and the end of the experiment. Additionally, the number of leaves of both the central and lateral shoots were counted and computed as the increase rate between the sum of leaves at the beginning and at the end of the experiment. Increase in stem diameter represents the change in stem diameter 10 cm over the budding point at the beginning and end of the experiment.

Plant biomass was measured at the end of the experiment (9 November 2020). Leaves, stems, and roots of the ten plants (replications) of each treatment were harvested, washed with distilled water, and dried at 70 °C for 48 h to obtain the dry weight of each plant's tissues. Leaf area of harvested plants was measured by a leaf area meter AM 300 (ADC Bioscientific Ltd., Holdsden, UK).

2.6. Total Chlorophyll and Carotenoids Content

Chlorophyll a, chlorophyll b, and carotenoid content were measured twice during the experiment at midterm (27 August 2020) and at the end of the experiment (9 November 2020), according to [28]. A quantity of 0.10 g of fresh olive leaves was homogenized in 10 mL of pure acetone and centrifuged at 3000 rpm for 5 min in a centrifuge (Biofuge primo R, Heraeus). The absorbance of the extract was measured using a V-630 UV Visible spectrophotometer (Jasco, Tokyo, Japan) at 661.6, 644.8, and 470 nm.

2.7. Proline Content

Proline content was measured twice during the experimental period, at midterm (27 August 2020) and at the end of the experiment (9 November 2020) according to a modification to the protocol in [29]. Ten mg of dried olive leaves were homogenized with 4 mL 80:20 ethanol:water, and then centrifuged at 4000 rpm for 10 min. One mL of the supernatant was mixed with 2 mL of ninydrin, vortexed for 15 s, and then placed in a water bath at 90 °C for 25 min. The extracts were again centrifuged at 4000 rpm for 5 min and the absorbance was measured in the supernatant at 520 nm.

2.8. Total Phenolic Content

Total phenolics were measured twice during the experiment, at midterm (27 August 2020) and at the end of the experiment (9 November 2020), according to the Wissam et al. method [30]. Twenty mg of dried olive leaves were homogenized with 10 mL 80:20 ethanol:water and the extract was centrifuged at 5000 rpm for 5 min. A total of 250 µL of the supernatant was diluted in 9.75 mL of distilled water. One mL of the diluted extraction was then mixed with 500 µL of Folin–Ciocalteau (2N) reagent, 4.5 mL of distilled water, and left to stand at room temperature for 3 min. Then, 4 mL of 7.5% Na_2CO_3 was added and then samples were placed in a water bath for 30 min at 40 °C. The absorbance was measured at 734 nm and the results were expressed as g of gallic acid equivalents per 100 g of dry matter (DM).

2.9. Statistical Analysis

Statistical analysis was performed with SPSS software (SPSS 20.0, IBM Corp., Armonk, NY, USA). The Univariate Analysis of Variance (ANOVA) was applied to compare the significant differences between the values of all measured parameters. Post hoc test was performed with the Tukey HSD test ($p \leq 0.05$).

2.10. Water Footprint Computation

The WF was computed for the TaW and TWW treatment plants based on the water footprint network approach [22] as the sum of green water footprint (WF_{green}), blue water footprint (WF_{blue}), and grey water footprint (WF_{grey}) according to the equation:

$$WF_{total} = WF_{green} + WF_{blue} + WF_{grey} \tag{1}$$

which equals Equation (2):

$$WF_{total} = CWU_{green}/Yield + CWU_{blue}/Yield + (a \times AR/(c_{max} - c_{nat}))/Yield \tag{2}$$

where a is the leaching factor; AR is the application rate of pollutant (nitrogen—N); and c_{nat} and c_{max} are the natural concentration and the maximum acceptable concentration of the pollutant in the free flowing surface water bodies, respectively. Since the plants were kept in a nursery protected from rain, the WF_{green} was considered to be zero. The WF_{blue} was diversified to the actual blue water footprint referring to the TaW applied or the TWW applied. Since all plants were irrigated according to their actual water needs, the amount of TaW water and TWW applied were considered to be the actual crop water used (CWU). Additionally, since all plants received no excess water regarding their water needs, no leaching took place so factor a and WF_{grey} were considered to be zero. When the agricultural product is countable, the water footprint is expressed as water volume per piece [22], so in the present study, yield was considered to be each item of young olive tree.

2.11. Local Nursery of 'Konservolea' Nursery Plants Water Footprint Computation

The WF for young olive tree plants propagated in local nurseries was computed based on data provided by local nursery owners (interviews). Irrigation in local nurseries was realized by an alternative ebb-and-flow method. The floor of the nursery was filled with water up to a height of 15 cm. At the end of each irrigation event, the excess water was discarded outdoors. Plants were kept in the nursery for at least one year before they were transplanted to the field.

3. Results and Discussion

3.1. Plant Development and Physiological Status

Performance of plants irrigated with TWW was equivalent, if not better in some cases, to that of the tap water (TaW) and zeolite treatment plants for most of the growth parameters measured (Table 3). Plant height increase did not differ significantly between TWW, tap water, and zeolite treatments. Stem diameter increase in TWW and TWW1/2F plants was higher by 21 and 39%, respectively, compared to that of the tap water plants, but zeolite treatment plants exhibited a similar stem diameter increase to that of the TWW plants. Tap water and zeolite promoted the development of new leaves, since plants of both treatments exhibited the highest leaf number increase rate. On the other hand, irrigation with TWW may have promoted the final size of the leaves as the plants' leaf area was 6 and 8%, higher compared to tap water and zeolite treatments, respectively. Above and below ground biomass did not differ significantly between tap water, TWW, and zeolite treatments (Table 3). Plants irrigated with half quantity of tap water and plants irrigated with TWW but accepted half quantity of fertilizer exhibited the weakest performance. Application of TWW in various crops increased vegetative growth, which was mainly attributed to the increased nutrient content of TWW as well as the TWW facilitation of nutrient uptake by plants [6,7]. Studies in olive trees have shown a positive correlation between irrigation with TWW and growth development [31–33], although there have been reported cases where growth reduction was observed mainly due to elevated EC content of TWW [34]. Although soil acidity was found not to effect the olive trees' vegetation and final yield [35], the soil EC content is a determining factor in olive tree development. Erel et al. [36] found that olive tree growth was affected only after eight years of TWW application. In the present study, there was no detrimental effect observed on the growth and development of young olive trees. In contrast, TWW treatment plants exhibited a similar height increase and biomass and higher leaf area and stem diameter increase when compared to the tap water irrigated plants and zeolite treated plants. The fertilizer effect of TWW is influenced by the TWW composition and irrigation volumes applied [9]. Since olive trees do not require large amounts of water, the TWW effect on their growth, especially during a short application period, is expressed to a lesser extent.

Table 3. Growth parameters of plants measured for each treatment at the end of the experiment.

Treatment	Shoot Length Increase (%)	Stem Diameter Increase (%)	Leaves Increase (%)	Leaf Area (cm^2)	Plant Biomass (g)
TaW	35.9 a	16.4 c	69.2 a	583.8 b	28.6 a
TaW75%	27.7 b	10.7 d	46.7 b	519.6 d	25.9 b
TWW	35.8 a	19.9 ab	43.0 b	618.7 a	28.6 a
TWW1/2F	27.0 b	22.8 a	47.0 b	556.6 c	26.9 ab
Zeolite	39.3 a	19.5 b	68.3 a	570.0 bc	27.9 ab
Significance	***	***	***	***	***

Different letters indicate significant differences between treatments. *** = $p < 0.001$.

Irrigation with TWW increased chlorophyll a, b, total chlorophyll, and carotenoid content in the leaves of young olive trees by 23, 28, 25, and 26%, respectively, compared to the TaW plants. It should be mentioned that TWW1/2F treatment also exhibited a significant increase in those parameters. The addition of zeolite in soil increased the total chlorophyll and carotenoid content in young olive trees by 8% and 6%, respectively, in comparison to the TaW plants (Table 4).

Photosynthetic activity is the food/energy providing mechanism to plants and chlorophyll, which plays a key role in this reaction. Chlorophyll, in a large proportion, consists of nitrogen and its deficiency in plants causes a reduction in leaf chlorophyll, limiting the plant's development and physiological status [6,7,37]. TWW is rich in nitrogen and other micronutrients that are involved in the synthesis of chlorophyll, thus, the application of TWW increases the chlorophyll content in plants and enhances their physiological activity [38]. In the present study, the application of TWW increased chlorophyll a, chlorophyll b, and total chlorophyll content, which is in agreement with the findings of other studies [31,38,39].

Table 4. Chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid content in the leaves of young olive trees at the end of the experiment.

Treatment	Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Total Chlorophyll (mg g^{-1})	Carotenoids (mg g^{-1})
TaW	1.16 b	0.41 b	1.57 c	0.31 c
TaW75%	1.23 b	0.45 b	1.68 bc	0.35 b
TWW	1.43 a	0.52 a	1.95 a	0.39 a
TWW1/2F	1.42 a	0.51 a	1.92 a	0.38 a
Zeolite	1.24 b	0.45 b	1.70 b	0.33 bc
Significance	***	***	***	***

Different letters indicate significant differences between treatments. *** = $p < 0.001$.

3.2. Stress Indicators

Irrigation with TWW reduced the total phenolic content in plants compared to the TaW ones (Table 5). It should be noted that TWW treatment plants exhibited similar levels of phenolic compounds compared to the TaW plants during the midterm sampling at 27/8, when total phenolic content was slightly higher in these treatments and much higher in the TWW1/2F and zeolite, probably as a result of the stress plants experienced during that period due to elevated temperatures.

Table 5. Concentration of total phenolics in the leaves of young olive trees at the end of the experiment and at midterm sampling.

Treatment	Total Phenolics End (mg g^{-1})	Total Phenolics Midterm (mg g^{-1})
TaW	39.5 a	36.3 c
TaW75%	32.7 b	35.7 c
TWW	33.1 b	35.1 c
TWW1/2F	32.6 b	49.5 a
Zeolite	33.9 b	43.5 b
Significance	***	***

Different letters indicate significant differences between treatments. *** = $p < 0.001$.

Phenols are plant secondary metabolites that are expressed in elevated concentration when the plant experiences biotic or abiotic environmental stress [40–43]. Irrigation with TWW may result in soil salinization, which induces osmotic stress in plants, increasing as a defense mechanism the content of stress metabolites [44,45]. In the present study, irrigation with TWW reduced total phenolic content in the plant leaves, which implies that its application did not induce abiotic stress on plants. This is in line with the findings by Tekaya et al. [41], who also observed a decrease in olive leaf total phenol content when irrigated with TWW compared to treatments that induced water stress.

Proline is an amino acid, which, like phenols, can be perceived as a stress indicator. This is implicated in osmoregulation in drought conditions, acting as an osmolyte [46]. At the end of the experiment, proline content did not differ significantly between the tap water, TWW, and zeolite treatments. TWW1/2F exhibited the highest proline content in the leaves of young olive trees (Table 6). During the midterm sampling (27/8), the lowest proline content value was measured in the leaves of TaW plants and the highest in the leaves of TWW1/2F treatment plants (Table 6).

Table 6. Concentration of proline in the leaves of young olive trees at the end of the experiment and at midterm sampling.

Treatment	Proline End (mg/100 g Fresh Leaf)	Proline Midterm (mg/100 g Fresh Leaf)
TaW	4.8 ab	4.6 c
TaW75%	4.5 b	5.0 bc
TWW	5.0 ab	5.3 ab
TWW1/2F	5.5 a	5.9 a
Zeolite	5.2 ab	4.8 bc
Significance	***	***

Different letters indicate significant differences between treatments. *** = $p < 0.001$.

Proline accumulation has been reported in young olive trees under water stress conditions [40,42,45]. In the present study, proline content in irrigated with TWW young olive trees leaves did not differ from the TaW leaves, indicating that irrigation with TWW did not pose any water stress on young olive trees.

3.3. Water Footprint

The WF was computed at the end of the experiment as the sum of the amount of water applied to each plant (L per plant). The blue water was distinguished according to the water source into blue water for the tap water (TaW) and black water for the TWW. Results are presented in Tables 7 and 8.

Table 7. Water footprint of young olive trees irrigated with tap water (TaW) and treated wastewater (TWW).

Treatment	CWU_{blue} (L)	CWU_{black} (L)	CWU_{total} (L)	WF_{blue} (L plant^{-1})	WF_{black} (L Plant^{-1})	WF_{total} (L Plant^{-1})	WF_{total} (m^3 Plant^{-1})
Tap	15.97	0	15,97	15.97	0	15.97	0.01597
TWW	1.50	14.47	15.97	1.5	14.47	15.97	0.01597

Table 8. Crop water use of a single young olive tree in a local nursery.

	Volume of Water in Each Irrigation Event (m^3)	Irrigation Events per Year	Total Water Volume per Period (m^3)	Number of Plants Irrigated	Volume per Plant per Period (m^3) (CWU)	WF_{blue} (m^3/plant/year)
Full year irrigation	27	72	1944	8000	0.243	0.243
5 month irrigation (summer)	27	40	1080	8000	0.135	0.135

The WF was estimated for the young olive trees of the experiment irrigated with TWW and TaW (Figure 1). Since rain was prevented from reaching the plants as they were kept in the greenhouse and irrigation volume was based on the actual water needs, no N leaching took place and only the WF_{blue} was computed. The blue WF was distinguished in two components based on the water source, thus WF_{blue} and WF_{black} were computed for the TaW and TWW treatments, respectively. Fridman et al. [24] proposed the extension of WF_{blue} to include alternative water sources. If we consider the fact that treated wastewater is discarded in the receiving water bodies, hence being wasted, then its reuse in agriculture appears as a great opportunity for the reduction in fresh water resource abstraction for irrigation.

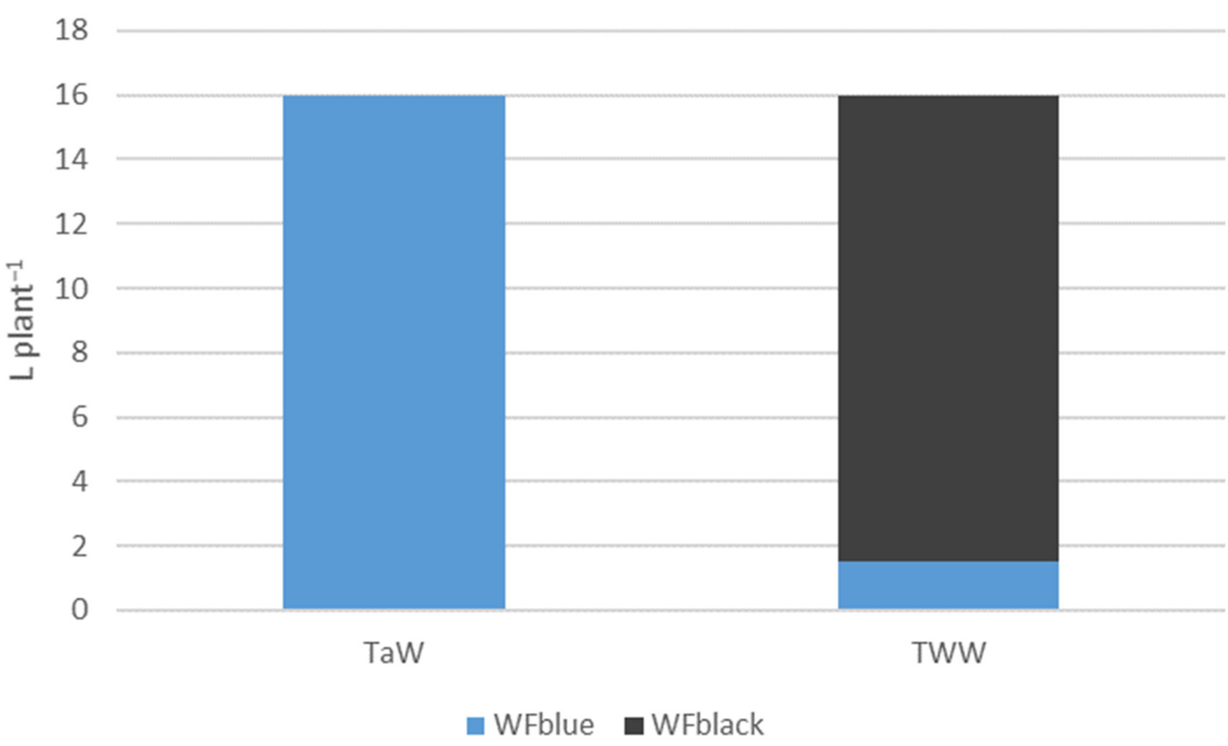

Figure 1. Water footprint of young olive trees irrigated with tap water (TaW) and treated wastewater (TWW) and the water source components (WF_{blue} for fresh water and WF_{black} for treated wastewater).

3.4. Local Nursery Water Footprint

The CWU and WF_{blue} for a single plant in local nurseries for 'Konservolea' are presented in Table 8. The WF_{grey} is presented in Table 9. The WF_{total} for a single plant in a local nursery was computed as $WF_{total} = WF_{blue} + WF_{grey} = 0.24$ m^3 plant^{-1}. Both CWU and WF_{blue} was additionally computed for the corresponding experimental period so values could be comparable.

Table 9. WF_{grey} for a single young olive plant in a local nursery.

Amount of N per Plant (kg)	WF_{grey} (m^3/Plant)
0.000144	0.001309

Since there are no similar data available in the literature, the main purpose of this computation was to underline the magnitude of water savings that could be achieved from the application of TWW in local olive nurseries, especially when the rapid expansion of 'Konservolea' olive groves in the area has increased the demand of olive nursery plants for the establishment of new olive orchards. As observed, the WF_{total} of local nurseries, even when calculated for the same period in the present study, was 10* fold higher than the corresponding one of the experimental young olive trees. This, of course, is mainly attributed to the irrigation method applied in the area, which was not based on the actual plants' water needs and is not realized with precise and water saving methods such as micro irrigation. The ebb-and-flow method applied in the local nurseries for irrigation is not an effective method in terms of sustainable water management as large quantities of water are withdrawn for irrigation and discarded to the environment. This not only causes huge freshwater losses, but also increases the pollutants' dispersion since fertilizers are carried away with water. Micro-irrigation using pressure compensating emitters achieves precise and uniform water application, minimizes drainage, and optimizes water and mineral uptake, providing a highly efficient irrigation method [47].

4. Conclusions

The present study showed that application of treated wastewater for irrigation for a short period of 'Konservolea' young nursery grown olive trees is an effective and safe alternative to fresh water. Plants irrigated with TWW performed better in most of the growth and physiological parameters measured compared to tap watered plants or plants growing in a substrate enriched with zeolite. Stress indicators such as total phenols and proline revealed that TWW did not induce any additional stress. Furthermore, no detrimental effects were observed in plants irrigated with TWW. The water footprint calculated for the young olive trees irrigated with TWW was compared to plants irrigated with tap water, introducing the water source based blue water assessment approach and set a baseline for the water consumption needs of nursery grown young olive trees in the area. Taking into account the high water volume consuming irrigation methods used in the local 'Konservolea' nurseries, we propose at least the partial replacement of fresh water resources for irrigation with municipal treated wastewater combined with micro-irrigation methods.

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References

1. Ritchie, H.; Roser, M. Water Use and Stress. Available online: Ourworldindata.org/water-use-stress (accessed on 31 January 2022).
2. FAO. AQUASTAT Core Database. Available online: https://www.fao.org/aquastat/statistics/query/index.html?lang=en (accessed on 31 January 2022).
3. Angelakis, A.N.; Asano, T.; Bahri, A.; Jimenez, B.E.; Tchobanoglous, G. Water reuse: From ancient to modern times and the future. *Front. Environ. Sci.* **2018**, *6*, 26. [CrossRef]
4. Singh, A. A review of wastewater irrigation: Environmental implications. *Resour. Conserv. Recycl.* **2021**, *168*, 105454. [CrossRef]
5. Olivieri, A.W.; Pecson, B.; Crook, J.; Hultquist, R. California water reuse—Past, present and future perspectives. *Adv. Chem. Pollution, Environ. Manag. Prot.* **2020**, *5*, 65–111. [CrossRef]
6. Ofori, S.; Puškáčová, A.; Růžičková, I.; Wanner, J. Treated wastewater reuse for irrigation: Pros and cons. *Sci. Total Environ.* **2021**, *760*, 144026. [CrossRef] [PubMed]
7. Hashem, M.S.; Qi, X. Bin Treated wastewater irrigation—A review. *Water* **2021**, *13*, 1527. [CrossRef]
8. Seleiman, M.F.; Al-Suhaibani, N.; El-Hendawy, S.; Abdella, K.; Alotaibi, M.; Alderfasi, A. Impacts of long-and short-term of irrigation with treated wastewater and synthetic fertilizers on the growth, biomass, heavy metal content, and energy traits of three potential bioenergy crops in arid regions. *Energies* **2021**, *14*, 3037. [CrossRef]
9. Vergine, P.; Salerno, C.; Libutti, A.; Beneduce, L.; Gatta, G.; Berardi, G.; Pollice, A. Closing the water cycle in the agro-industrial sector by reusing treated wastewater for irrigation. *J. Clean. Prod.* **2017**, *164*, 587–596. [CrossRef]
10. Shtull-Trauring, E.; Cohen, A.; Ben-Hur, M.; Israeli, M.; Bernstein, N. NPK in treated wastewater irrigation: Regional scale indices to minimize environmental pollution and optimize crop nutritional supply. *Sci. Total Environ.* **2022**, *806*, 150387. [CrossRef]
11. Bedbabis, S.; Rouina, B.B.; Boukhris, M.; Ferrara, G. Effect of irrigation with treated wastewater on soil chemical properties and infiltration rate. *J. Environ. Manage.* **2014**, *133*, 45–50. [CrossRef]
12. Farhadkhani, M.; Nikaeen, M.; Yadegarfar, G.; Hatamzadeh, M.; Pourmohammadbagher, H.; Sahbaei, Z.; Rahmani, H.R. Effects of irrigation with secondary treated wastewater on physicochemical and microbial properties of soil and produce safety in a semi-arid area. *Water Res.* **2018**, *144*, 356–364. [CrossRef]

13. Dang, Q.; Tan, W.; Zhao, X.; Li, D.; Li, Y.; Yang, T.; Li, R.; Zu, G.; Xi, B. Linking the response of soil microbial community structure in soils to long-term wastewater irrigation and soil depth. *Sci. Total Environ.* **2019**, *688*, 26–36. [CrossRef] [PubMed]
14. Becerra-Castro, C.; Lopes, A.R.; Vaz-Moreira, I.; Silva, E.F.; Manaia, C.M.; Nunes, O.C. Wastewater reuse in irrigation: A microbiological perspective on implications in soil fertility and human and environmental health. *Environ. Int.* **2015**, *75*, 117–135. [CrossRef] [PubMed]
15. Bedbabis, S.; Ben Rouina, B.; Boukhris, M.; Ferrara, G. Effects of irrigation with treated wastewater on root and fruit mineral elements of Chemlali olive cultivar. *Sci. World J.* **2014**, *2014*, 973638. [CrossRef] [PubMed]
16. Tunc, T.; Sahin, U. The changes in the physical and hydraulic properties of a loamy soil under irrigation with simpler-reclaimed wastewaters. *Agric. Water Manag.* **2015**, *158*, 213–224. [CrossRef]
17. Elfanssi, S.; Ouazzani, N.; Mandi, L. Soil properties and agro-physiological responses of alfalfa *(Medicago sativa* L.) irrigated by treated domestic wastewater. *Agric. Water Manag.* **2018**, *202*, 231–240. [CrossRef]
18. Giannoccaro, G.; Arborea, S.; de Gennaro, B.C.; Iacobellis, V.; Piccinni, A.F. Assessing reclaimed urban wastewater for reuse in agriculture: Technical and economic concerns for Mediterranean regions. *Water* **2019**, *11*, 1511. [CrossRef]
19. Verlicchi, P.; Al Aukidy, M.; Galletti, A.; Zambello, E.; Zanni, G.; Masotti, L. A project of reuse of reclaimed wastewater in the Po Valley, Italy: Polishing sequence and cost benefit analysis. *J. Hydrol.* **2012**, *432–433*, 127–136. [CrossRef]
20. *UN-Water Summary Progress Update 2021: SDG 6—Water and Sanitation for All*; UN Water Publications: Geneva, Switzerland, 2021; pp. 1–58.
21. Qadir, M.; Drechsel, P.; Jiménez Cisneros, B.; Kim, Y.; Pramanik, A.; Mehta, P.; Olaniyan, O. Global and regional potential of wastewater as a water, nutrient and energy source. *Nat. Resour. Forum* **2020**, *44*, 40–51. [CrossRef]
22. Hoekstra, A.Y.; Chapagain, A.K.; Aldaya, M.M.; Mekonnen, M.M. *The Water Footprint Assessment Manual*; Earthscan: London, UK, 2011; ISBN 9781849712798.
23. Mekonnen, M.M.; Hoekstra, A.Y. Sustainability of the blue water footprint of crops. *Adv. Water Resour.* **2020**, *143*, 103679. [CrossRef]
24. Fridman, D.; Biran, N.; Kissinger, M. Beyond blue: An extended framework of blue water footprint accounting. *Sci. Total Environ.* **2021**, *777*, 146010. [CrossRef]
25. FAO. Food and Agriculture Data. Available online: http://fenix.fao.org/faostat/internal/en/#data/QCL (accessed on 31 January 2022).
26. IOC. World Olive Oil and Table Olive Figures. Available online: https://www.internationaloliveoil.org/what-we-do/economic-affairs-promotion-unit/#figures (accessed on 31 January 2022).
27. Jarosz, R.; Szerement, J.; Gondek, K.; Mierzwa-Hersztek, M. The use of zeolites as an addition to fertilisers—A review. *Catena* **2022**, *213*, 106125. [CrossRef]
28. Lichtenthaler, H.K.; Buschmann, C. Chlorophylls and Carotenoids: Measurement and Characterization by UV-VIS Spectroscopy. *Handb. Food Anal. Chem.* **2005**, *2*, 171–178. [CrossRef]
29. Bates, L.S.; Waldren, R.P.; Teare, I.D. Rapid determination of free proline for water-stress studies. *Plant Soil Vol.* **1973**, *39*, 205–207. [CrossRef]
30. Wissam, Z.; Ali, A.; Rama, H. Optimization of extraction conditions for the recovery of phenolic compounds and antioxidants from Syrian olive leaves. *J. Pharmacogn. Phytochem.* **2016**, *5*, 390–394.
31. Ben Hassena, A.; Zouari, M.; Trabelsi, L.; Khabou, W.; Zouari, N. Physiological improvements of young olive tree (*Olea europaea* L. cv. Chetoui) under short term irrigation with treated wastewater. *Agric. Water Manag.* **2018**, *207*, 53–58. [CrossRef]
32. Bourazanis, G.; Roussos, P.A.; Argyrokastritis, I.; Kosmas, C.; Kerkides, P. Evaluation of the use of treated municipal waste water on the yield, oil quality, free fatty acids' profile and nutrient levels in olive trees cv Koroneiki, in Greece. *Agric. Water Manag.* **2016**, *163*, 1–8. [CrossRef]
33. Bedbabis, S.; Ferrara, G. Effects of long term irrigation with treated wastewater on leaf mineral element contents and oil quality in Olive cv. Chemlali. *J. Hortic. Sci. Biotechnol.* **2018**, *93*, 216–223. [CrossRef]
34. Bedbabis, S.; Ferrara, G.; Ben Rouina, B.; Boukhris, M. Effects of irrigation with treated wastewater on olive tree growth, yield and leaf mineral elements at short term. *Sci. Hortic.* **2010**, *126*, 345–350. [CrossRef]
35. Barouchas, P.E.; Vatista, P.; Kalantzis, E.; Moustakas, N.K. Seasonal changes of macronutrients concentration in olive trees grown in acid and in alkaline soils. *Not. Bot. Horti Agrobot. Cluj-Napoca* **2021**, *49*, 12498. [CrossRef]
36. Erel, R.; Eppel, A.; Yermiyahu, U.; Ben-Gal, A.; Levy, G.; Zipori, I.; Schaumann, G.E.; Mayer, O.; Dag, A. Long-term irrigation with reclaimed wastewater: Implications on nutrient management, soil chemistry and olive (*Olea europaea* L.) performance. *Agric. Water Manag.* **2019**, *213*, 324–335. [CrossRef]
37. Singh, P.K.; Deshbhratar, P.B.; Ramteke, D.S. Effects of sewage wastewater irrigation on soil properties, crop yield and environment. *Agric. Water Manag.* **2012**, *103*, 100–104. [CrossRef]
38. Alvarez-Holguin, A.; Sosa-Perez, G.; Ponce-Garcia, O.C.; Lara-Macias, C.R.; Villarreal-Guerrero, F.; Monzon-Burgos, C.G.; Ochoa-Rivero, J.M. The Impact of Treated Wastewater Irrigation on the Metabolism of Barley Grown in Arid and Semi-Arid Regions. *Int. J. Environ. Res. Public Health* **2022**, *19*, 2345. [CrossRef] [PubMed]
39. Al-Suhaibani, N.; Seleiman, M.F.; El-Hendawy, S.; Abdella, K.; Alotaibi, M.; Alderfasi, A. Integrative effects of treated wastewater and synthetic fertilizers on productivity, energy characteristics, and elements uptake of potential energy crops in an arid agro-ecosystem. *Agronomy* **2021**, *11*, 2250. [CrossRef]

40. Arji, I.; Ahmadipour, S.; Ebadi, A.; Abdosi, V. Biochemical changes of some olive (*Olea europaea* L.) cultivars under water-deficit stress. *Acta Hortic.* **2021**, *1315*, 383–390. [CrossRef]
41. Tekaya, M.; Mechri, B.; Dabbaghi, O.; Mahjoub, Z.; Laamari, S.; Chihaoui, B.; Boujnah, D.; Hammami, M.; Chehab, H. Changes in key photosynthetic parameters of olive trees following soil tillage and wastewater irrigation, modified olive oil quality. *Agric. Water Manag.* **2016**, *178*, 180–188. [CrossRef]
42. Petridis, A.; Therios, I.; Samouris, G.; Koundouras, S.; Giannakoula, A. Effect of water deficit on leaf phenolic composition, gas exchange, oxidative damage and antioxidant activity of four Greek olive (*Olea europaea* L.) cultivars. *Plant Physiol. Biochem.* **2012**, *60*, 1–11. [CrossRef]
43. Varela, M.C.; Arslan, I.; Reginato, M.A.; Cenzano, A.M.; Luna, M.V. Phenolic compounds as indicators of drought resistance in shrubs from Patagonian shrublands (Argentina). *Plant Physiol. Biochem.* **2016**, *104*, 81–91. [CrossRef]
44. Mechri, B.; Tekaya, M.; Hammami, M.; Chehab, H. Effects of drought stress on phenolic accumulation in greenhouse-grown olive trees (*Olea europaea*). *Biochem. Syst. Ecol.* **2020**, *92*, 104112. [CrossRef]
45. Ahmadipor, S.; Arji, I.; Ebadi, A.; Abdossi, V. Physiological and biochemical responses of some olive cultivars (*Olea europaea* L.) to water stress. *Cell. Mol. Biol.* **2018**, *64*, 20–29. [CrossRef]
46. Hayat, S.; Hayat, Q.; Alyemeni, M.N.; Wani, A.S.; Pichtel, J.; Ahmad, A. Role of proline under changing environments: A review. *Plant Signal. Behav.* **2012**, *7*, 1456–1466. [CrossRef]
47. Dong, S.; Wang, G.; Kang, Y.; Ma, Q.; Wan, S. Soil water and salinity dynamics under the improved drip-irrigation scheduling for ecological restoration in the saline area of Yellow River basin. *Agric. Water Manag.* **2022**, *264*, 107255. [CrossRef]