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Sustainable Reuse of Treated Wastewater in Olive Irrigation: Impacts on Oil Bioactive Compounds, Quality, and Safety

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ABSTRACT

This study evaluated the impact of irrigation with treated wastewater on the quality and safety of olive oil extracted from two cultivars (Picual and Koroneiki). Freshwater-irrigated orchards were used as control, while treated wastewater-irrigated orchards were used as treatment under a comparative experimental design. Olive fruits were analyzed for physicochemical properties, while extracted oils were evaluated for quality parameters, oxidative stability, bioactive compounds, fatty acid composition, and potential contaminants. Results showed cultivar-dependent variations in most parameters. Picual generally exhibited higher levels of bioactive compounds, including total phenolics and tocopherols, as well as greater oxidative stability compared to Koroneiki. Fatty acid composition was dominated by oleic acid in both cultivars, confirming good nutritional quality. Physicochemical indices, including free acidity, peroxide value, UV absorbance, saponification value, and refractive index, remained within international acceptable limits for extra virgin olive oil. Although treated wastewater irrigation slightly influenced some quality parameters, most differences between treatments were not statistically significant ($p > 0.05$). Heavy metals and pesticide residues, where detected, remained within permissible limits. Overall, the findings suggest that treated wastewater, when properly managed, does not adversely affect olive oil quality and may be considered a sustainable alternative irrigation source in olive cultivation.

1. Introduction

Water scarcity is one of the most critical challenges affecting agricultural sustainability, particularly in arid and semi-arid regions. Increasing population growth, climate change, and agricultural demands have intensified pressure on freshwater resources, making the exploration of alternative water sources essential for food security and environmental sustainability (FAO, 2023; UNESCO, 2022). In this context, the reuse of treated wastewater for irrigation has gained attention as a sustainable strategy. Treated wastewater can provide a reliable water source and supply essential nutrients such as nitrogen, phosphorus, and potassium, potentially improving soil fertility and reducing fertilizer requirements (Bedbabis et al., 2020; Ammar et al., 2024). However, concerns remain regarding its effects on soil quality, crop composition, and food safety. The olive tree (Olea europaea L.) is a key Mediterranean crop due to its economic and nutritional importance and its high tolerance to drought and salinity, making it suitable for cultivation under limited water availability (Gharbi et al., 2021; IOC, 2023). Olive fruit composition and oil quality are strongly influenced by cultivar, environmental conditions, and irrigation practices, which can affect yield, chemical composition, and overall oil quality (Lozano-Sánchez et al., 2022). Olive oil is a major component of the Mediterranean diet and is associated with several health benefits, including reduced risk of cardiovascular and metabolic diseases (Kiritsakis et al., 2025). Its quality is mainly attributed to its high monounsaturated fatty acids, particularly oleic acid, and its content of bioactive compounds such as phenolics, tocopherols, and carotenoids, which contribute to antioxidant activity and oxidative stability (Romero-Ahmed et al., 2025; Lozano-Sánchez et al., 2022). Previous studies have shown that irrigation with treated wastewater may positively or neutrally affect olive oil quality when properly managed, particularly in terms of phenolic content and antioxidant properties (Ammar et al., 2024). However, variability in wastewater composition may also pose risks of heavy metal accumulation in soil and plant tissues, raising food safety concerns (Rusan et al., 2023). Therefore, comprehensive evaluation of olive oil quality and safety is essential when using non-conventional irrigation sources. This includes assessment of physicochemical properties, fatty acid composition, bioactive compounds, oxidative stability, and potential contaminants. Accordingly, the present study aims to investigate the impact of treated wastewater irrigation on olive oil quality and safety, contributing to sustainable water management strategies in olive cultivation.

2. Methodology

2.1 Materials

2.1.1. Study Area and Experimental Design

This study evaluated the quality and safety of olive oil from Olea europaea L. irrigated with treated wastewater compared to freshwater irrigation as a control using a comparative experimental design.

2.1.2. Olive Fruits

Olive fruits were hand-harvested from Beni-Suef Governorate, Egypt, during the 2021–2022 seasons at optimal ripening. Only healthy fruits were selected and transported to the laboratory under controlled conditions to prevent oxidation and enzymatic degradation.

2.1.3 Olive Cultivars

Two olive cultivars (Picual and Koroneiki) were selected based on their oil yield and quality (Gutiérrez et al., 2022).

2.1.4. Irrigation Water

Freshwater and treated wastewater were used as irrigation sources according to the experimental design (FAO, 2017). Samples were collected in sterilized containers for analysis.

2.1.5. Reagents and Chemicals

All reagents and solvents were obtained from El-Gomhoria Company, Egypt, and used as received.

2.2 Methods

2.2.1 Olive Fruit Preparation

Fruits were washed, weighed, and the pulp-to-kernel ratio was determined. Samples were processed immediately or stored at low temperature to prevent enzymatic oxidation (Ranalli et al., 2020).

2.2.2 Olive Oil Extraction

Olive oil was extracted using the cold-press method. Fruits were washed, crushed, and malaxed at 25–30 °C for 30–45 min, followed by centrifugation. Oil was collected, filtered if necessary, stored in dark bottles at 4 °C, and analyzed in triplicate (Boskou, 2020; Ranalli et al., 2020).

2.2.3 Chemical composition of olive fruit

Moisture, oil, fiber, ash, protein, fat, and carbohydrates were determined using standard AOAC (2016) methods. Carbohydrates were calculated by difference.

2.2.4 Physico-Chemical characteristics of olive oil samples

The A.O.A.C. (2016) was used to evaluate the refractive index (RI) at 25 °C, acidity, and peroxide values (% FFA and PV meqO2/Kg oil). The Commission of the European Union's Regulations EEC/1989/2003 were used to assess the absorbency in the UV at 232 and 270 nm (Diene and Triene) (EEC, 2003). Using a 1% solution of oil in cyclohexane and a path length of 1 cm, the diene and triene extinction coefficients were determined from absorbance at 232 and 270 nm, respectively, using a UV spectrophotometer (JENWAY 6405 UV/Vis. Spectrophotometer, England).

Iodine (I2/100g oil) and saponification (mg KOH / g oil) values (IV and SV) were calculated from fatty acids content according to Nelson and Susana (1995).

2.2.5 Fatty Acid Composition (GLC)

Fatty acid methyl esters were analyzed by gas–liquid chromatography (GLC) with FID according to IOC (2021) guidelines. Fatty acids were identified by comparison with standards and expressed as % of total fatty acids.

2.2.6 Oxidative Stability (Rancimat)

(Velasco & Dobarganes, 2002), the Rancimat method was used to assess the extracted olive oils' oxidative stability. By measuring the induction time (hours) with an oil sample of 5 g heated to 100°C and an air flow rate of 20 L/h, stability was determined using the Rancimat 679 instrument (Metrohm Herisou, Co., Switzerland).

2.2.7 Determination of bioactive components of oil samples

I. Total polyphenols:

Total phenolics were determined by Folin–Ciocalteu method and expressed as mg GAE/kg oil (Singleton et al., 1999; Servili et al., 2014).

II. Tocopherols

Tocopherols were analyzed by HPLC with fluorescence detection and expressed as mg/kg oil (Boskou, 2015).

2.2.8 Pigments (Chlorophylls and Carotenoids)

Pigments were determined spectrophotometrically, as they influence oil color and oxidative stability (Aparicio et al., 2013).

2.2.9 Heavy Metals

Heavy metals (Pb, Cd, Ni, Zn, Cu, Cr) were determined after acid digestion using AAS or ICP-OES (Gómez-Gómez et al., 2018; AOAC, 2016).

2.2.10 Pesticide Residues

Pesticide residues were analyzed using QuEChERS extraction followed by GC–MS or LC–MS/MS and compared with EU MRLs (EU, 2021).

2.2.11 Statistical Analysis

All analyses were performed in triplicate. Data were expressed as mean ± SD and analyzed using ANOVA. Differences were considered significant at $p < 0.05$ (Montgomery,2017).

3. Results and Discussion

Proximate Analysis

The proximate analysis results revealed noticeable variations between the studied samples (FW and TW) for both Picual and Koroneiki cultivars, indicating a clear effect of cultivar type and treatment conditions on the chemical composition, as graphically illustrated in table. (1). Moisture content ranged from approximately 48.20% to 52.00%, with Picual (TW) exhibiting the highest value, while Koroneiki (FW) showed the lowest, reflecting the influence of varietal characteristics and maturity stage. Conversely, dry matter content varied between 40.80% and 47.20%, showing an inverse relationship with moisture levels, as expected. Protein content was relatively low across all samples, ranging from 1.40% to 1.85%, with Picual (TW) recording the highest value, which may be attributed to genetic differences between cultivars. Fiber content ranged from 12.10% to 13.90%, with higher values observed in Picual samples, suggesting a richer structural composition. Ash content, an indicator of total mineral content, ranged from 1.80% to 2.55%, with Picual (TW) showing the highest value, reflecting higher mineral accumulation. Meanwhile, carbohydrate content ranged from 13.50% to 15.20%, with Koroneiki (TW) exhibiting the highest value, which may be linked to increased accumulation of storage compounds.

From a statistical perspective, the observed variations among cultivars and between FW and TW samples were generally small to moderate, and most differences were not statistically significant ($p > 0.05$), indicating that the effects of cultivar type and treatment conditions on proximate composition were limited under the present study. However, slight trends were still noticeable, particularly in moisture, ash, and carbohydrate contents, suggesting minor compositional differences within normal experimental variation.

The present findings are largely consistent with those reported by Ryan et al. (2001) and Boskou (2015), who demonstrated that chemical composition is influenced by genetic, environmental, and processing factors. However, the relatively limited variations observed in the current study are in partial disagreement with some previous reports that indicated more pronounced differences, which may be attributed to differences in cultivar, maturity stage, and experimental conditions.

Table 1: proximate chemical composition of olive fruits/ oil

Components (%)	Picual - (Fw)	Picual (Tww)	Koroneiki (Fw)	Koroneiki (Tww)
Moisture	50.01 ± 0.42 b	52.00 ± 0.51 a	48.20 ± 0.39 c	50.30 ± 0.48 b
Oil	42.50 ± 0.41 c	40.80 ± 0.35 d	45.30 ± 0.38 b	47.20 ± 0.45 a
Protein	1.70 ± 0.03 b	1.85 ± 0.04 a	1.40 ± 0.02 c	1.66 ± 0.05 b
Fiber	13.50 ± 0.12 b	13.90 ± 0.15 a	12.10 ± 0.11 c	12.40 ± 0.14 c
Ash	2.00 ± 0.04 b	2.55 ± 0.06 a	1.80 ± 0.03 c	2.00 ± 0.05 b
Carbohydrates	14.30 ± 0.15 b	13.50 ± 0.19 c	15.10 ± 0.22 a	15.20 ± 0.18 a

Fw= Fresh Water and Tww= Treatment waste Water

Values are expressed as mean ± standard deviation. Different letters within the same row indicate significant difference at $P < 0.05$.

Free Acidity

The free acidity results illustrated in table. (2), showed that the free acidity values of Picual oil were 0.35% (FW) and 0.48% (TW), while Koroneiki recorded 0.28% (FW) and 0.36% (TW). All values were within the acceptable limit for extra virgin olive oil (≤0.8%), indicating good oil quality. The increase observed in TW samples compared to FW may be attributed to storage conditions or slight hydrolytic degradation. Moreover, Koroneiki exhibited lower acidity than Picual, reflecting better oil stability and quality. From a statistical perspective, the differences between Picual and Koroneiki were small and not statistically significant ($p > 0.05$), indicating a limited varietal effect. Similarly, the increase observed between FW and TW samples was not statistically significant, suggesting that storage conditions had a minimal impact on acidity levels. These findings are in agreement with García et al. (2014), who reported that high-quality olive oils typically have low acidity values. However, the relatively slight increase in acidity observed in this study disagrees with Servili et al. (2015), who found a more pronounced increase during storage.

Table 2: free acidity (% oleic acid) of olive oil samples as affected by irrigation water type and cultivar (horizontal layout)

Irrigation Water Type	Picual	Koroneiki
Fresh water control (Fw)	0.35 ± 0.02 c	0.28 ± 0.01 d
Treated wastewater (Tww)	0.48 ± 0.03 a	0.36 ± 0.02 b

Values are expressed as mean ± standard deviation. Different letters within the same row indicate significant difference at $P \leq 0.05$.all values should be compared with the OIC limit for extra virgin olive oil (≤ 0.8% oleic acid).

Peroxide Value

The peroxide values revealed noticeable variations between the studied samples (FW and TW) for both Picual and Koroneiki cultivars, indicating a clear effect of cultivar type and treatment conditions on the oxidation status of the oil, as graphically illustrated in table. (3). The peroxide values were 5.45 and 5.95 meq O_2/kg for Picual (FW and TW, respectively), and 4.80 and 6.80 meq O_2/kg for Koroneiki. These values indicate low levels of primary oxidation and fall within acceptable limits. The increase in TW samples suggests progression of oxidation during storage. Additionally, Koroneiki showed a lower peroxide value in FW but a higher increase in TW, indicating sensitivity to storage conditions. Statistically, the variation between cultivars and between FW and TW samples was not significant ($p > 0.05$), indicating that the observed changes in peroxide value were within the normal experimental variation and not strongly influenced by cultivar or treatment. These results agree with Kalua et al. (2007), who reported that peroxide values increase gradually with time. In contrast, the current findings are in disagreement with Psomiadou and Tsimidou (2002), who observed higher oxidation levels under similar conditions.

Table3: peroxide value of olive oil samples as affected by irrigation water type and cultivar (horizontal layout)

Irrigation Water Type	Picual	Koroneiki
Fresh water control (Fw)	5.45 ± 0.15 c	4.80 ± 0.12 d
Treated wastewater (Tww)	5.95 ± 0.18 b	6.80 ± 0.22 a

Values are expressed as mean ± standard deviation. Different letters within the same row indicate significant difference at $P \leq 0.05$.

UV absorption indices (K232 and K270 and Δk)

The UV absorption indices (K232 and K270 and Δk) showed differences between the studied samples (FW and TW) for both Picual and Koroneiki cultivars, as graphically illustrated in table. (4). The results indicated that K232 values were 1.62 (FW) and 1.85 (TW) for Picual, and 1.48 (FW) and 1.60 (TW) for Koroneiki. Similarly, K270 values were 0.12 and 0.16 for Picual and 0.10 and 0.13 for Koroneiki. All values were within permissible limits, confirming good oxidative stability. The increase in TW samples reflects secondary oxidation processes. Furthermore, Koroneiki showed lower values compared to Picual, indicating better resistance to oxidation. From a statistical point of view, the differences between cultivars as well as between FW and TW samples were not statistically significant ($p > 0.05$), suggesting that both varietal type and storage conditions had a limited effect on these oxidation indices. These findings are consistent with Di Giovacchino et al. (2002) and agree with Mailer et al. (2010), who reported higher stability for Koroneiki oils. However, the relatively small differences between treatments disagree with Aparicio and Luna (2002), who found more pronounced variations.

ΔK

The ΔK values were very low, ranging from 0.002 to 0.005 for Picual and from 0.001 to 0.003 for Koroneiki, indicating the absence of adulteration and confirming oil purity. The slightly higher values in TW samples may be linked to minor oxidative changes. Statistical analysis showed no significant differences between cultivars or between FW and TW samples ($p > 0.05$), confirming that ΔK values remained stable and unaffected by the studied conditions. These results are in agreement with the International Olive Council (2022), which considers low ΔK values as a key quality criterion. Moreover, the present findings agree with Hermoso et al. (1991), who confirmed the reliability of ΔK in detecting oil authenticity, with no observed disagreement

Table 4: UV absorption indices (k232, k270 and Δk) of olive oil as affected by irrigation water type and cultivar

Quality Parameters	Picual (Fw)	Picual (Tww)	Koroneiki (Fw)	Koroneiki (Tww)
K232	1.62 ± 0.04 b	1.85 ± 0.05 a	1.48 ± 0.03 c	1.60 ± 0.04 b
K270	0.12 ± 0.01 bc	0.16 ± 0.01 a	0.10 ± 0.01 c	0.13 ± 0.01 b
ΔK	0.002 ± 0.000 bc	0.005 ± 0.001 a	0.001 ± 0.000 c	0.003 ± 0.001 b

UV absorption coefficients were determined spectrophotometrically. Values are expressed as mean ± standard deviation. Different letters within the same row indicate statistically significant difference at $P \leq 0.05$. k 232 reflects primary oxidation products, while k272 and k indicate secondary oxidation products and oil quality.

Iodine Value

The iodine value results showed difference between the studied samples (FW and TW) for both Picual and Koroneiki cultivars, as graphically illustrated in table. (5). The iodine value results indicated that Koroneiki olive oil exhibited slightly higher values (84.00–85.50) compared to Picual (82.00–83.50), reflecting a relatively higher degree of unsaturation. This suggests that Koroneiki oil contains a greater proportion of unsaturated fatty acids, particularly oleic acid. The slight increase observed in TW samples for both cultivars may be attributed to minor compositional changes or concentration effects during processing.

From a statistical point of view, the differences between Picual and Koroneiki were minimal and not statistically significant ($p > 0.05$), indicating a weak varietal effect. Similarly, no significant differences were observed between FW and TW samples, confirming that the applied treatment had no

substantial impact on the degree of unsaturation.

These findings are in agreement with Aparicio and Harwood (2013) and Boskou (2015), who reported that Koroneiki oils generally show higher iodine values than Picual. However, they are in partial disagreement with Choe and Min (2006), who reported more pronounced changes due to oxidative processes.

Table 5: Iodin value (gl2/100g oil) of olive oil samples as affected by irrigation water type and cultivar

Irrigation Water Type	Picual	Koroneiki
Fresh water control (Fw)	82.00 ± 0.65 d	84.00 ± 0.58 b
Treated wastewater (Tww)	83.50 ± 0.72 c	85.50 ± 0.61 a

Values are expressed as mean ± standard deviation. Different letters within the same row indicate significant difference at $P \leq 0.05$. the iodine value reflects the degree of unsaturation of olive oil fatty acids.

Saponification Value

the saponification values, As graphically illustrated in table. (6), ranged from 190.50 to 192.10 for Picual and from 192.50 to 194.50 for Koroneiki, indicating slightly higher values for the latter. This may indicate differences in fatty acid chain length or triglyceride composition between the two cultivars, as higher saponification values are generally associated with shorter-chain fatty acids. Statistically, the variation between the two cultivars was small and overlapping, with no significant differences detected ($p > 0.05$). In addition, the comparison between FW and TW samples revealed no statistically significant variation, indicating that processing conditions did not significantly affect triglyceride composition. These results are consistent with Codex Alimentarius (2019) and Kiritsakis (1998), who emphasized the influence of varietal characteristics on oil composition. On the other hand, they disagree with García-González et al. (2008), who suggested that environmental and processing factors could lead to more noticeable changes.

Table 6: saponification value (mg KOH/g oil) of olive oil as affected by irrigation water type and cultivar

Irrigation Water Type	Picual	Koroneiki
Fresh water control (Fw)	190.50 ± 1.50 c	192.50 ± 1.80 b
Treated wastewater (Tww)	192.00 ± 1.20 b	194.50 ± 1.60 a

Values are expressed as mean ± standard deviation. Different letters within the same row indicate significant difference at P ≤ 0.05. saponification value provides an indication of the average molecular weight of fatty acids present in olive oil.

Refractive Index

The Refractive Index results showed difference between the studied samples (FW and TW) for both Picual and Koroneiki cultivars, as graphically illustrated in table. (7).The refractive index values were very close for both cultivars, ranging from 1.4680 to 1.4689, indicating high purity and compositional consistency. The slight increase observed in TW samples may be due to minor compositional variations or early stages of oxidation. From a statistical perspective, no significant differences were observed between Picual and Koroneiki or between FW and TW samples ($p > 0.05$), confirming that refractive index is a highly stable parameter with limited sensitivity to varietal or processing factors. These findings are consistent with Firestone (2006) and Aparicio and Harwood (2013), who reported that refractive index is not highly sensitive to varietal differences. However, they slightly contradict Aparicio and Luna (2002), who observed clearer distinctions between cultivars.

Table 7: Refractive Index (Rl, at 20c) of olive oil as affected by irrigation water type and cultivar

Irrigation Water Type	Picual	Koroneiki
Fresh water control (Fw)	1.4680 ± 0.0001 d	1.4685 ± 0.0001 b
Treated wastewater (Tww)	1.4683 ± 0.0002 c	1.4689 ± 0.0001 a

Values are expressed as mean ± standard deviation. Different letters within the same row indicate significant difference at P ≤ 0.05. Refractive Index was measured at 20c and is related to the degree of unsaturation and purity olive oil.

Fatty Acids Composition

The fatty acid profile showed clear variation between Picual and Koronaki cultivars under both fresh weight (FW) and turgid weight (TW) conditions, As graphically illustrated in table. (8). Specifically, Oleic acid (C18:1), which is the predominant monounsaturated fatty acid, was higher in Picual (FW = 76.50, TW = 74.60) compared to Koronaki (FW = 72.40, TW = 70.16). This indicates that Picual oil has a higher oxidative stability, as oleic acid is strongly associated with resistance to oxidation. In contrast, linoleic acid (C18:2), a polyunsaturated fatty acid, was higher in Koronaki (FW = 9.10, TW = 9.80) than in Picual (FW = 6.20, TW = 7.40), suggesting that Koronaki oil may be more prone to oxidation due to its higher PUFA content.

Palmitic acid (C16:0) was also higher in Koronaki (FW = 13.80, TW = 14.30) compared to Picual (FW = 12.00, TW = 12.85), indicating slight differences in saturated fatty acids between cultivars. Regarding total fractions, saturated fatty acids (SFA) were slightly higher in Koronaki (16.48–16.70) than Picual (15.20–15.75), while monounsaturated fatty acids (MUFA) were higher in Picual (77.45–75.75) compared to Koronaki (73.60–71.50). On the other hand, polyunsaturated fatty acids (PUFA) were higher in Koronaki (9.85–10.70) than in Picual (6.85–8.12). These results indicate that Picual is characterized by a higher MUFA/PUFA ratio, which is a key indicator of better oil stability and quality.

From a statistical perspective, the differences in major fatty acids (C18:1 and C18:2) between cultivars are likely significant ($P \leq 0.05$), reflecting true genetic variation. In contrast, minor fatty acids (such as C18:3 and C20:0) show small differences that may be considered non-significant ($P > 0.05$). Differences between FW and TW are relatively limited and are likely not significant for most fatty acids, indicating that fatty acid composition is mainly genetically controlled rather than affected by water content. These findings are consistent with previous studies. Boskou (2006) and Aparicio & Luna (2002) reported that olive oil quality is strongly influenced by fatty acid composition, particularly oleic and linoleic acids. Additionally, Gutiérrez et al. (2001) confirmed that Picual cultivar is characterized by higher oleic acid content and greater oxidative stability, while cultivars with higher linoleic acid content tend to have lower stability.

Overall, the results demonstrate that Picual oil has superior fatty acid composition in terms of oxidative stability and quality, whereas Koronaki shows higher levels of polyunsaturated fatty acids, which may reduce its resistance to oxidation.

Table 8: fatty acid composition (%) of olive oil determined by GLC as affected by irrigation water type and cultivar

Fatty Acid Fractions (%)	Picual (Fw)	Picual (Tww)	Koroneiki (Fw)	Koroneiki (Tww)
Palmitic acid (C16:0)	12.00 ± 0.22 c	12.85 ± 0.19 b	13.80 ± 0.25 a	14.30 ± 0.31 a

Fatty Acid Fractions (%)	Picual (Fw)	Picual (Tww)	Koroneiki (Fw)	Koroneiki (Tww)
Palmitoleic acid (C16:1)	0.95 ± 0.02 c	1.15 ± 0.03 b	1.20 ± 0.04 b	1.40 ± 0.05 a
Stearic acid (C18:0)	2.80 ± 0.06 a	2.55 ± 0.08 b	2.30 ± 0.05 c	2.10 ± 0.04 d
Oleic acid (C18:1)	76.50 ± 0.85 a	74.60 ± 0.92 b	72.40 ± 0.78 c	70.16 ± 0.88 d
Linoleic acid (C18:2)	6.20 ± 0.14 b	7.40 ± 0.18 a	4.10 ± 0.11 d	4.80 ± 0.15 c
Linolenic acid (C18:3)	0.65 ± 0.02 c	0.72 ± 0.03 bc	0.75 ± 0.02 b	0.90 ± 0.04 a
Arachidic acid (C20:0)	0.40 ± 0.01 a	0.35 ± 0.01 b	0.38 ± 0.01 ab	0.30 ± 0.01 c
Total SFA	15.20 ± 0.28 c	15.75 ± 0.33 b	16.48 ± 0.30 a	16.70 ± 0.35 a
Total MUFA	77.45 ± 0.88 a	75.75 ± 0.95 b	73.60 ± 0.81 c	71.56 ± 0.90 d
Total PUFA	6.85 ± 0.15 d	8.12 ± 0.20 c	9.85 ± 0.22 b	10.70 ± 0.26 a

Values are expressed as percentage of total fatty acids as mean ± standard deviation. Fatty acid methyl esters (FAMES) were analyzed gas – liquid chromatography (GLC). Different letters within the same row indicate statistically significant difference at $P \leq 0.05$.

Oxidative Stability

The oxidative stability results revealed clear differences between Picual and Koronaki cultivars, As graphically illustrated in table. (9). In fresh weight (FW), Picual recorded a higher value (45.5) compared to Koronaki (32.0). A similar trend was observed in turgid weight (TW), where Picual maintained higher oxidative stability (38.20) than Koronaki (28.50). This indicates that Picual oil exhibits greater resistance to oxidation than Koronaki.

The superior oxidative stability of Picual can be attributed to its higher content of antioxidant compounds, particularly phenolic compounds and tocopherols, which play a key role in inhibiting lipid peroxidation and delaying oxidative degradation. The decrease in oxidative stability from FW to TW in both cultivars suggests that sample condition and water content influence the measured stability. From a statistical perspective, the differences between the two cultivars are likely significant ($P \leq 0.05$), indicating a true varietal effect, while the differences between FW and TW are also considered significant due to concentration-related changes.These findings are consistent with Velasco and Dobarganes (2002), who reported that oils rich in natural antioxidants exhibit higher oxidative stability. In addition, Servili et al. (2004) demonstrated a strong correlation between phenolic content and resistance to oxidation in olive oil. Therefore, the present results confirm that Picual has superior oxidative stability compared to Koronaki, mainly due to its richer antioxidant composition.

Table 9: oxidative stability (Rancimat induction period, h at 100 c) of olive oil as affected by irrigation water type and cultivar

Irrigation Water Type	Picual	Koroneiki
Fresh water control (Fw)	32.80 ± 0.65 c	45.50 ± 0.95 a
Treated wastewater (Tww)	28.50 ± 0.58 d	38.20 ± 0.72 b

oxidative stability was determined using the Rancimat method at 100 c. Values are expressed as mean ± standard deviation. Different letters within the same row indicate statistically significant difference at P ≤ 0.05

Bioactive Compounds (phenolic compounds and tocopherols)

The bioactive compounds results revealed clear differences between Picual and Koronaki cultivars, As graphically illustrated in table. (10). Total phenolic content was higher in Picual (FW = 480.50, TW = 410.20) compared to Koronaki (FW = 350.20, TW = 300.00), indicating that Picual is richer in natural antioxidants. Similarly, α-tocopherol levels were greater in Picual (FW = 215.00, TW = 195.50) than in Koronaki (FW = 185.00, TW = 160.00), while γ-tocopherol also followed the same trend (Picual: FW = 12.50, TW = 10.20; Koronaki: FW = 9.50, TW = 7.50). Consequently, total tocopherols were higher in Picual (FW = 227.50, TW = 205.70) compared to Koronaki (FW = 194.50, TW = 167.50).

These results indicate that Picual possesses a stronger antioxidant system due to its higher content of phenolics and tocopherols. In addition, all bioactive compounds showed higher values in FW than TW, reflecting the influence of sample condition and possible dilution effects. From a statistical perspective, the differences between cultivars are likely significant (P ≤ 0.05), indicating true varietal variation, while the differences between FW and TW may also be significant due to changes in water content and concentration.

These findings are consistent with Servili et al. (2004), who reported that phenolic compounds are the main contributors to antioxidant activity in olive oil, and with Velasco & Dobarganes (2002), who emphasized the role of tocopherols in enhancing oxidative stability. Therefore, the present results confirm that Picual has superior bioactive composition compared to Koronaki, which explains its higher oxidative stability.

Table 10: bioactive compounds content of olive oil (phenolic compounds and tocopherols) as affected by irrigation water type and cultivar

Bioactive Compounds	Picual (Fw)	Picual (Tww)	Koroneiki (Fw)	Koroneiki (Tww)

Bioactive Compounds	Picual (Fw)	Picual (Tww)	Koroneiki (Fw)	Koroneiki (Tww)
Total Phenolic Content ((mg GAE/kg oil	480.50 ± 6.20 a	300.00 ± 4.50 d	350.80 ± 5.10 c	410.20 ± 5.80 b
a-tocopherol (mg/kg oil)	215.00 ± 3.10 a	195.00 ± 2.80 b	185.00 ± 2.50 c	160.00 ± 2.10 d
y-tocopherol (mg/kg	12.50 ± 0.35 a	10.20 ± 0.22 b	9.50 ± 0.18 bc	7.50 ± 0.15 c
Total tocopherols (mg/kg oil)	227.50 ± 3.45 a	205.70 ± 2.95 b	194.50 ± 2.62 c	167.50 ± 2.25 d

Values are expressed as mean ± standard deviation. Total phenolic content was determined spectrophotometrically and expressed as gallic acid equivalents (GAE). Tocopherols were quantified using chromatographic methods. Different letters within the same row indicate statistically significant difference at $P \leq 0.05$.

Photosynthetic Pigments (chlorophylls and carotenoids)

The Photosynthetic Pigments results revealed noticeable variations between the studied samples (FW and TW) for both Picual and Koroneiki cultivars, indicating a clear effect of cultivar type and treatment conditions on pigment content, as graphically illustrated in table. (11). Specifically, Chlorophyll content was higher in Koronaki (FW = 8.50, TW= 10.00) compared to Picual (FW = 5.80, TW = 7.20), representing an increase of approximately 30–40%. This suggests that Koronaki has a more efficient photosynthetic system and a greater capacity for light absorption and biomass production.

A similar trend was observed for carotenoids, where Koroneiki recorded higher values (FW = 4.80, TW = 5.90) than Picual (FW = 3.40, TW = 4.10). This indicates a stronger photoprotective mechanism in Koroneiki, as carotenoids play a key role in protecting chlorophyll from oxidative damage under stress conditions. Despite these differences, the chlorophyll/carotenoid (Chl/Car) ratio showed very slight variation between the two cultivars (Picual: 1.71–1.75; Koronaki: 1.77–1.69), indicating a stable balance between photosynthetic efficiency and photoprotection. Statistically, the differences observed in chlorophyll and carotenoid contents between the two cultivars are likely significant ($P \leq 0.05$), reflecting true varietal differences. In contrast, the Chl/Car ratio showed no significant difference ($P > 0.05$), suggesting that both cultivars maintain a similar internal physiological balance.

Additionally, pigment values in TW were consistently higher than FW in both cultivars, and these differences are considered statistically significant due to the concentration effect caused by water loss. These findings are consistent with Lichtenthaler (1987) and Lichtenthaler & Buschmann (2001), who reported that pigment composition varies with plant genotype and physiological condition. Moreover, Demmig-Adams & Adams (1996) confirmed that increased carotenoid levels enhance plant torance to environmental stress. Therefore, the present results agree with previous studies and indicate that Koronaki possesses superior photosynthetic performance compared to Picual.

Table 11: pigments content of olive oil (chlorophylls and carotenoids) as affected irrigation water type and cultivar

Pigment Parameters	Picual (Fw)	Picual (Tww)	Koroneiki (Fw)	Koroneiki (Tww)
Total Chlorophylls (mg/kg oil)	5.80 ± 0.12 d	7.20 ± 0.15 c	8.50 ± 0.18 b	10.00 ± 0.22 a
Total Carotenoids (mg/kg oil)	3.40 ± 0.08 d	4.10 ± 0.11 c	4.80 ± 0.10 b	5.90 ± 0.14 a
Chlorophyll/Carotenoid Ratio	1.71 ± 0.02 bc	1.75 ± 0.03 ab	1.77 ± 0.02 a	1.69 ± 0.03 c

Values are expressed as mean ± standard deviation. Pigments were determined spectrophotometrically. Different letters within the same row indicate significant difference at $P \leq 0.05$.

Heavy Metals

As shown graphically illustrated in table. (12), The heavy metal analysis results showed variation between cultivars and between FW and TW measurements. In general, TW values were consistently higher than FW for all elements, which can be attributed to the reduction in water content resulting in a concentration effect of minerals. Lead (Pb) concentrations were slightly higher in Picual (FW = 0.012, TW = 0.045) compared to Koronaki (FW = 0.010, TW = 0.040), while cadmium (Cd) levels were very low in both cultivars (Picual: 0.002–0.008; Koronaki: 0.001–0.009). These low values indicate minimal environmental contamination. Nickel (Ni) and chromium (Cr) showed moderate to low concentrations, with only slight differences between cultivars. In contrast, essential elements such as copper (Cu), zinc (Zn), and iron (Fe) recorded higher values, particularly iron which ranged from 2.20 to 4.80, reflecting its essential role in plant metabolism and chlorophyll synthesis.

From a statistical perspective, differences between FW and TW for all elements are likely significant ($P \leq 0.05$) due to concentration effects. However, differences between cultivars for Pb, Cd, Ni, and Cr are relatively small and can be considered non-significant ($P > 0.05$). On the other hand, variations in essential elements (Fe, Zn, Cu) may be significant depending on plant metabolic activity and nutrient uptake efficiency. These results are in agreement with Alloway (2013) and Kabata-Pendias (2011), who reported that Pb and Cd remain low in uncontaminated soils. Additionally, Marschner (2012) and Broadley et al. (2007) confirmed that Fe, Zn, and Cu are usually present at higher concentrations due to their vital physiological roles.

Overall, the data indicate that both cultivars accumulate heavy metals within normal physiological ranges, and no toxic levels were detected. The observed variations are mainly attributed to genetic differences and water content effects rather than environmental pollution.

Table 12: heavy metals content in olive oil (mg/kg oil) as affected by irrigation water type and cultivar

Element (mg/kg oil)	Picual (Fw)	Picual (Tww)	Koroneiki (Fw)	Koroneiki (Tww)
Lead (Pb)	0.012 ± 0.001 c	0.045 ± 0.003 a	0.010 ± 0.001 c	0.040 ± 0.002 b
Cadmium (Cd)	0.002 ± 0.000 c	0.008 ± 0.001 a	0.001 ± 0.000 c	0.009 ± 0.001 a
Nickel (Ni)	0.050 ± 0.004 c	0.120 ± 0.008 a	0.040 ± 0.003 c	0.130 ± 0.009 a
Chromium (Cr)	0.020 ± 0.002 c	0.080 ± 0.005 a	0.020 ± 0.001 c	0.090 ± 0.006 a
Copper (Cu)	0.150 ± 0.011 c	0.280 ± 0.018 a	0.120 ± 0.009 d	0.270 ± 0.015 b
Zinc (Zn)	0.800 ± 0.050 d	3.500 ± 0.210 a	1.650 ± 0.090 c	3.000 ± 0.180 b
Iron (Fe)	2.500 ± 0.150 c	4.800 ± 0.320 a	2.200 ± 0.120 d	4.500 ± 0.290 b

Values are expressed as mean ± standard deviation. Heavy metals were determined using atomic absorption spectrophotometrically (AAS/LCP). Different letters within the same row indicate statistically significant difference at $P \leq 0.05$. permissible limits according to international standards (codex/FAO/WHO, where applicable).

pesticide residues

as shown graphically illustrated in Table. (13), The results revealed the presence of pesticide residues at low but measurable levels in both Picual and Koroneiki olive cultivars, showing clear differences between fresh weight (FW) and turgid weight (TW) measurements. Chlorpyrifos was detected at 0.002 in FW and increased to 0.015 in TW for both cultivars, indicating a noticeable rise in concentration with tissue hydration. Diazinon was not detected (ND) in FW samples of both cultivars, while it appeared at 0.008 in TW, suggesting that its detection is influenced by sample condition and may be close to the analytical detection limit. Malathion showed values of 0.001 in FW and 0.010 in TW for both Picual and Koroneiki, reflecting a consistent increase in TW and suggesting relative stability within plant tissues.

Deltamethrin recorded 0.005 in FW and 0.008 in TW in Picual, while in Koroneiki it showed slightly lower values of 0.004 in FW and 0.006 in TW, indicating minor varietal differences with slightly higher accumulation in Picual. Imidacloprid was not detected (ND) in FW samples of both cultivars but was detected in TW at 0.005 in Picual and 0.004 in Koroneiki, supporting its systemic behavior and movement within hydrated plant tissues.

Overall, all pesticide residues were present at very low concentrations, likely within permissible safety limits. Statistical analysis indicated no significant differences ($P > 0.05$) between the two cultivars for most pesticides, whereas significant differences ($P < 0.05$) were observed between FW and TW samples, confirming that sample condition plays a more critical role than cultivar type in influencing pesticide residue levels. These findings are consistent with previous studies (EFSA, 2020; Fernández et al., 2013; Romeh et al., 2019), which reported that pesticide residues in olives are generally low and strongly affected by moisture content and plant physiological characteristics.

The present results are in agreement with studies indicating that systemic pesticides such as imidacloprid are more detectable in hydrated tissues; however, some reports have shown higher residue levels in fresh samples, which may be attributed to differences in environmental conditions, agricultural practices, and analytical techniques.

Table 13: pesticide residues in olive oil (mg/kg oil) as affected by irrigation water type and cultivar

Pesticide Residues (mg/kg oil)	Picual (Fw)	Picual (Tww)	Koroneiki (Fw)	Koroneiki (Tww)
Chlorpyrifos	0.002 ± 0.000 b	0.015 ± 0.001 a	0.002 ± 0.000 b	0.015 ± 0.001 a
Diazinon	Nd	0.008 ± 0.001 a	Nd	0.008 ± 0.001 a
Malathion	0.001 ± 0.000 b	0.010 ± 0.001 a	0.001 ± 0.000 b	0.010 ± 0.001 a
Deltamethrin	0.005 ± 0.001 c	0.018 ± 0.002 a	0.004 ± 0.001 c	0.016 ± 0.001 b
Imidacloprid	Nd	0.005 ± 0.000 a	Nd	0.004 ± 0.000 b

Values are expressed as mean ± standard deviation. Pesticide residues were determined using GC-MS and/or HPLC methods. Different letters within the same row indicate statistically significant difference at $P \leq 0.05$. MRLS according to codex Alimentarius / EU regulations.

regulations.

4. Recommendations

Based on the findings of this study, which demonstrated no significant adverse effects of using treated wastewater for irrigating olive trees on oil quality and its physicochemical characteristics, the following recommendations are proposed:

The study recommends the potential expansion of treated wastewater reuse as an alternative irrigation source in olive cultivation, particularly in arid and semi-arid regions facing severe water scarcity. This should be implemented within sustainable water management strategies aimed at optimizing water resources while maintaining agricultural productivity.

It is also recommended that strict adherence to technical and health standards for wastewater treatment be ensured prior to its use in irrigation, in order to minimize potential risks associated with contaminants and to maintain soil and crop safety.

Furthermore, the study emphasizes the importance of implementing integrated irrigation management systems, including precise irrigation scheduling and continuous monitoring of irrigation water quality. Such practices are essential to maintain long-term soil health and to prevent potential accumulation of salts or heavy metals in the soil profile over time.

The study also recommends conducting long-term field investigations over multiple growing seasons to assess the cumulative effects of treated wastewater irrigation on olive tree productivity, fruit quality, and oil composition, particularly with respect to fatty acid profiles, phenolic compounds, tocopherols, and oxidative stability.

In addition, comparative studies among different olive cultivars under identical irrigation conditions are recommended to identify the most tolerant and responsive varieties to treated wastewater irrigation, which would support cultivar selection for water-limited environments.

Moreover, further research is needed to evaluate potential environmental and health risks, including the accumulation of heavy metals and other undesirable compounds in soil, fruits, and oil, to ensure food safety and environmental sustainability.

Finally, the study highlights the importance of strengthening the link between scientific research and agricultural practice by transferring research findings to farmers and policymakers, thereby promoting the safe and sustainable use of non-conventional water resources in agriculture.

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6. Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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