

## Article

# Pomological and Olive Oil Quality Characteristics Evaluation under Short Time Irrigation of Olive Trees cv. Chemlali with Untreated Industrial Poultry Wastewater

Amira Oueslati <sup>1</sup>, Samia Dabbou <sup>2</sup>, Nosra Methneni <sup>1</sup>, Giuseppe Monteverchi <sup>3</sup>, Vincenzo Nava <sup>4</sup>,  
Rossana Rando <sup>4</sup>, Giovanni Bartolomeo <sup>4</sup>, Andrea Antonelli <sup>3</sup>, Giuseppa Di Bella <sup>4</sup> and Hedi Ben Mansour <sup>1,\*</sup>

<sup>1</sup> Research Unit of Analysis and Process Applied on the Environment—APAE UR17ES32—Higher Institute of Applied Sciences and Technology of Mahdia, University of Monastir, Mahdia 5100, Tunisia

<sup>2</sup> Unit of Bioactive and Natural Substances and Biotechnology UR17ES49, Dentistry Faculty, University of Monastir, Avicenne Street, Monastir 5019, Tunisia

<sup>3</sup> Department of Life Sciences (Agro-Food Science Area), BIOGEST-SITEIA Interdepartmental Centre, University of Modena and Reggio Emilia, Piazzale Europa 1, 42124 Reggio Emilia, Italy

<sup>4</sup> BioMorf Department, University of Messina, Viale Annunziata, Polo Universitario, 98168 Messina, Italy

\* Correspondence: hdbenmansour@gmail.com

**Abstract:** The aim of this work was the investigation of the effect of wastewater generated from the poultry meat industry on the irrigation of olive trees, during a short time period, in order to evaluate its impact on pomological criteria and olive oil quality. Olive trees were subjected to irrigation with different water qualities: (i) poultry wastewater (PWW), (ii) poultry wastewater diluted with tap water 50:50 (*v/v*) (PWTWW), (iii) rain-fed cultivation system (control). The results showed that PWTWW contains the optimal mineral proportions, leading to improved pomological criteria. However, the highest significant pulp oil content was obtained using poultry wastewater irrigation (69.51%), while this was 66.71% using diluted poultry wastewater, and 58.03 % for the control. Poultry wastewater irrigation yielded the best results in oil standard quality indices. In addition, an enrichment in oil total polyphenols content was achieved. The oil fatty acid profile was not affected following irrigation with poultry wastewater. Nevertheless, there was a significant increase in the contents of oleic acid and alcohols, accompanied by a decrease in total sterols. However, heavy metals accumulation was observed in both fruits and olive oil. In conclusion, our results suggest that among the three water qualities, poultry wastewater is the best alternative to improve olive oil quality.

**Keywords:** *Olea europaea*; fruits; agro-industrial wastewater reuse; water shortage



**Citation:** Oueslati, A.; Dabbou, S.; Methneni, N.; Monteverchi, G.; Nava, V.; Rando, R.; Bartolomeo, G.; Antonelli, A.; Di Bella, G.; Ben Mansour, H. Pomological and Olive Oil Quality Characteristics Evaluation under Short Time Irrigation of Olive Trees cv. Chemlali with Untreated Industrial Poultry Wastewater. *Sustainability* **2023**, *15*, 4198. <https://doi.org/10.3390/su15054198>

Academic Editors: Amélia Martins Delgado and Pasqualina Laganà

Received: 15 January 2023  
Revised: 18 February 2023  
Accepted: 19 February 2023  
Published: 25 February 2023



**Copyright:** © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

Statistical estimations suggest that, given the current demographic trends, about 60% of the worldwide population may suffer water scarcity by the year 2025 [1]. This dramatic situation is due to climate change as a result of greenhouse gas emissions from industrial activities [2]. It has been predicted that increasing temperatures will contribute to about 20% of the global expansion in water scarcity [3], which would affect the development and functioning of social and economic communities.

In arid and semi-arid countries such as Tunisia, this problem is accentuated, since they have limited freshwater resources [4]. Agriculture is by far the largest consumer sector of freshwater, accounting for 69% of annual global water withdrawals, followed by industry (19%) and households (12%) [5]. In order to manage water demand well, current governmental strategies are geared towards the reuse of wastewater in crop irrigation. In fact, wastewater has been considered as a source of nutrients for crops, leading to a reduction in chemical fertilizer use [6,7]. Furthermore, it has been mentioned that the cost of agricultural production could be reduced by 10–20% [8].

In Tunisia, olive trees (*Olea europaea* L.) are considered the major evergreen culture, by participating in olive oil production [9]. Chemlali cultivar (cv.) is grown in the warm coastal zone and in the lower steppe. It represents nearly 85% of olive plantations and contributes to more than 80% of the national production of olive oil [10,11]. The management of olive trees is essentially rain-fed, however, previous studies have shown that irrigation can increase fruit tree productivity and oil content [12,13]. Irrigation is considered a determining factor of oil quality. Indeed, high-quality olive oil cannot be obtained from fruits that have suffered from severe water stress [14]. Bibliographic research showed that the poultry meat industry generates a large amount of wastewater, as a result of various production activities [15,16]. Furthermore, mineral characterization revealed this wastewater's richness in essential elements such as nitrogen and magnesium [17,18]. According to [19] the response of olive crops to irrigation depends on the variety, which can react differently, where the quality of the oil can be improved or altered. Previous studies have demonstrated that fertilization with high levels of N and P is positively correlated with an increase in linolenic acid content, and free fatty acids, accompanied by a reduction in oleic acid content [20–22].

Irrigation of olive trees cv. Chemlali aged 160 years, using dairy wastewater characterized by a moderate salinity, was shown to preserve the commercial value of the fruits by increasing their fresh weight and improving their nutritional value in  $Mg^{2+}$  and  $K^+$ . Moreover, no significant difference in oil phenolic compounds was observed, compared to olive trees cultivated via rain-feeding [23]. On the contrary, irrigation conducted for 3 years in Greece, with poor-quality wastewater, recovered from municipal treatment systems, was shown to induce an increase in the polyphenol content in the oil and a reduction in the percentage of fatty acids [24,25].

The hypothesis underlying this study was that the composition of the poultry wastewater could improve the pomological quality of the olives, as well as the oil obtained from them. In fact, the poultry wastewater (PWW) used in the current experience has been evaluated for its richness in essential mineral elements such as total nitrogen and potassium. Short-term irrigation with PWW led to the promotion of the vegetative growth of young olive trees, cv. Chemlali, and their physiological metabolism [18]. Moreover, the bacteriological characterization of PWW used in this experiment revealed the occurrence of *Enterobacteriaceae* members (40%) [26]. According to bibliographic data, the identified *Enterobacteriaceae* genus are described as part of the intestinal microbial flora of healthy animals [27]. For these reasons, the present study aimed to evaluate the effect of poultry wastewater reuse, for a short period, on the final pomological fruit and olive oil quality. In particular, rain-fed olive trees (control) were compared with olive trees irrigated with poultry wastewater (PWW), and olive trees irrigated with poultry wastewater diluted with tap water 50:50 (v/v) (PWTWW).

## 2. Material and Methods

### 2.1. Water Sampling

Poultry wastewater (PWW) was collected from the outlet of a poultry industry specializing in the production of poultry meat and its derivatives. PWW is the result of different production chain activities such as, (i) scalding for feather removal, (ii) bird washing before and after the evisceration process, (iii) chilling, (iv) cleaning and sanitizing equipment and facilities, and (v) cooling of mechanical equipment. Tap water (TW) was borehole water which was sampled from a tap located in the industry. Samples were transported to the laboratory in plastic bottles and immediately analyzed for their physicochemical parameters and their mineral concentrations. The physicochemical parameters, except for the mineral characterization were described by [18]. The samples were stored at  $-20\text{ }^{\circ}\text{C}$  for further analysis.

### 2.2. Plant Material

The study was conducted during the 2018/2019 crop year, in the governorate of Mahdia, in the east of Tunisia (latitude  $35^{\circ}28'11''$  N and longitude  $10^{\circ}57'23''$  E), characterized

by a Mediterranean climate. Olive trees (*Olea europaea* L. cv. Chemlali), aged 150 years old, were cultivated at 11 m × 16 m in clay loam soil, and usually irrigated through a basin irrigation system. The experimental field was divided into three lots of four olive trees. The first lot was irrigated with poultry wastewater (PWW), the second lot with PWW diluted with tap water 50:50 (v/v) (PWTWW), and only rain-feeding was used for the control trees. Each tree was irrigated with 1000 L of water. The irrigation was applied twice per month from June to October, and once per month for the rest of the year. It is important to highlight that the irrigation frequency depended also on the rainfall.

Fruit samples were collected in January 2020. Harvesting was performed manually using rakes. Olives were randomly plucked, at the height of a man, and around the perimeter of each olive tree, when they were fully mature. Only healthy olive fruits, free of physical damage or infection, were selected. The olive oil was extracted within 24 h of harvesting, using laboratory scale equipment, then, through a mechanical extraction unit, the oil was transferred to a sterile dark glass bottle, and stored at +4 °C until analysis.

### 2.3. Determination of Maturity Index and Pomological Parameters

The maturity index (MI) was determined based on the appreciation of the color of 100 olives, randomly selected from a 1 kg gross sample. These olives were divided into eight classes, ranging from olives with a dark green epidermis to olives with a black epidermis and entirely dark pulp [28].

The pomological characteristics of the randomly collected olive samples were determined according to the procedures described by the International Olive Council. The measure of each descriptor was obtained from the average of 100 fresh fruits.

The average fresh weight (AFW) of the fruits was determined based on the weight of 100 fresh fruits. The diameter was measured from the center of the fruit using a caliper, with an accuracy of  $10^{-3}$  mm, then the olives were pitted. The stones (S) and the pulp (P) were also weighed. Then, the ratio of pulp to stone (P/S), and the percentage of pulp, were determined.

Pitted and dried olive samples were crushed, and 10 g of the paste was put in a Soxhlet cartridge. In a previously weighed round-bottom flask, 150 mL of hexane was added, and the extraction of olive oil was carried out at 70 °C for 6 h. Finally, the hexane was evaporated using a rotary evaporator and the flask was reweighed after putting it in the oven overnight, and then for 30 min in the desiccator [29]. The oil content was determined as a percentage.

### 2.4. Quality Indices

Free fatty acids (FFA), peroxide value (PV) and specific UV absorbance at 232 and 270 nm (K232 and K270, respectively) were carried out following the analytical methods described in the Commission Regulations EEC 2568/91 and EEC 1429/92 of the European Union [30], to attribute the oil samples a commercial class.

### 2.5. Fatty Acid Methyl Esters (FAMES) Profile Analysis

The fatty acid composition of the olive oil samples was carried out by GC-FID after derivatization to their methyl esters (FAMES) [31,32]. A gas chromatograph (GC) (Dani Instrument, Master GC1000, Milan, Italy), equipped with a split/splitless injector, a flame ionization detector (FID), and a capillary column (SLB-IL100, Supelco, Merck Sigma Aldrich, USA), 60 m × 0.25 mm ID × 0.20 μm thickness film, was used for the GC analysis.

The following chromatographic conditions were used: column temperature of 165 °C to 210 °C (held for 10 min) at 2 °C min<sup>-1</sup>; injector and detector temperatures of 250 °C; a constant linear velocity of ultrapure helium (carrier gas) at 30 cm sec<sup>-1</sup>. The injection volume was 1 μL, with a split ratio of 1:100. The identification was carried out by comparing the retention times of the compounds identified in the oil to the retention times of a reference FAME mixture. The percentage of individual FAMES was calculated relative to the total area of the chromatograms.

### 2.6. Dosage of Total Phenolic Content (TPC)

The TPC was determined using the Folin–Ciocâlteu assay [33]. A triple-extraction, with water–methanol (80:20 *v/v*), was carried out from fruit and oil samples, according to the method described by [34] and [32], respectively. TPCs were measured at 760 nm via a UV-spectrophotometer (UV-2401 PC, Shimadzu, Japan). The results were expressed as mg gallic acid equivalent (GAE) kg<sup>−1</sup>.

### 2.7. Determination of Mineral Elements and Heavy Metals

Sample preparation was carried out according to the method described by [35], with minor modifications. In short, 0.5 g from each sample (fruits and oils) was used. The digestion was made with 8 mL of HNO<sub>3</sub> (65%) and 2 mL of H<sub>2</sub>O<sub>2</sub> (30%). The mixtures obtained were placed in a microwave digester (Milestone ETHOS) and the mineralization was carried out at 180 °C, with a power of 1000 W, for 10 min, as described in the microwave operation manual for fruits and oils. The determination of the heavy metal content was performed using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS), using an iCAP Q (Thermo Scientific, Waltham, MA, USA) spectrometer equipped with an autosampler ASX520 (Cetac Technologies Inc., Omaha, NE, USA). The quantification of Hg levels was performed using a Direct Mercury Analyzer (DMA-80, Milestone, CT, USA). The operating conditions of the ICP-MS and DMA-80 used are described by [36], and the results were given as mg kg<sup>−1</sup>.

### 2.8. Statistical Analysis

Univariate statistical analysis was performed using the SPSS v22 software (SPSS, Chicago, IL, USA). Differences between means were assessed using the one-way ANOVA test, after checking the normal distribution of the dataset. Comparative analysis was performed by Tukey's HSD test. Values of  $p \leq 0.05$  were considered significant. Multivariate statistical analysis (principal component analysis, PCA) was performed using the Statistica v8.0 software (former Stat Soft Inc., now TIBCO Software Inc., Palo Alto, CA, USA).

## 3. Results

### 3.1. Effects of Poultry Wastewater Irrigation on Pomological Parameters of 'Chemlali' Olives

The results of the visual assessment based on the pulp color of 100 randomly selected fruits, revealed that the reuse of industrial effluent PWW and PWTWW in the irrigation of olive trees delayed the maturation of olive fruits, in comparison to those collected from olive trees cultivated in rain-fed conditions, where the maturity index (MI) values were 5.30, 5.36, and 6.12, respectively (Table 1). The one-way ANOVA showed that different pomological parameters: fruit average weight and diameter, weight and percentage of pulp, stone weight and diameter, and pulp/stone recorded in the lot irrigated with PWTWW, were significantly higher ( $p \leq 0.05$ ) than those recorded in the control lot, grown under rain-fed conditions, and the lot irrigated with PWW. No significant difference was detected between the latter two lots.

The fruit water content did not present statistically significant inter-lots differences, where the values recorded were in the range from 46% to 48%. However, the highest pulp oil content (69.51%) was recorded in the lot of olives harvested from the lot irrigated with PWW.

### 3.2. Effect of Wastewater Irrigation on Olive Oil Quality

Table 2 shows the results of quality indices of the olive oil samples obtained using wastewater irrigation. All values are within the limits established by the International Olive Council [35] for extra virgin olive oils (EVOOs).

**Table 1.** Effect of wastewater irrigation on the pomological criteria of fruit from the *Olea europaea* L. cv. Chemlali.

	Control	PWW	PWTWW
Maturity index (MI)	6.12	5.30	5.36
Average fruit fresh weight (g)	0.86 <sup>a</sup> ± 0.13	0.88 <sup>a</sup> ± 0.11	0.97 <sup>b</sup> ± 0.14
Water fruit content (%)	46.00 <sup>a</sup> ± 0.00	46.00 <sup>a</sup> ± 1.00	48.00 <sup>a</sup> ± 1.00
Fruit diameter (mm)	0.84 <sup>a</sup> ± 0.08	0.85 <sup>a</sup> ± 0.06	0.88 <sup>b</sup> ± 0.06
Average pulp fresh weight (g)	65.00 <sup>a</sup> ± 0.10	0.67 <sup>a</sup> ± 0.09	0.74 <sup>b</sup> ± 0.11
Pulp percentage (%)	75.49 <sup>a</sup> ± 1.58	76.00 <sup>a</sup> ± 1.73	77.00 <sup>b</sup> ± 1.65
Pulp oil content (%)	58.03 <sup>a</sup> ± 0.02	69.51 <sup>c</sup> ± 0.01	66.71 <sup>b</sup> ± 0.01
Average stone fresh weight (g)	0.21 <sup>a</sup> ± 0.03	0.21 <sup>a</sup> ± 0.03	0.22 <sup>b</sup> ± 0.03
Stone diameter (mm)	0.38 <sup>a</sup> ± 0.03	0.38 <sup>a</sup> ± 0.04	0.39 <sup>b</sup> ± 0.03
Pulp/stone	3.11 <sup>a</sup> ± 0.26	3.20 <sup>a</sup> ± 0.30	3.38 <sup>b</sup> ± 0.31

Control: olive trees grown under rain-fed conditions; PWW: olive trees irrigated with poultry wastewater; PWTWW: olive trees irrigated with poultry wastewater diluted with tap water (50:50) (v/v). ± standard deviations. Same superscript letters indicate not significant differences at  $p \leq 0.05$ , according to Tukey's HSD test.

**Table 2.** Effect of wastewater irrigation on the quality of olive oils from cv. Chemlali.

	Control	PWW	PWTWW	* Standards	
				Extra-Virgin	Virgin
Free fatty acids (% Oleic acid)	0.2	0.2	0.2	≤0.80	≤2.0
Peroxide value (mEqO <sub>2</sub> kg <sup>-1</sup> )	13	13	16	≤0.20	≤0.20
K232	2.48	2.42	2.59	≤2.50	≤2.60
K270	0.08	0.13	0.08	≤0.22	≤0.25
ΔK	−0.005	0	−0.005	≤0.001	≤0.001

Control: olive trees grown under rain-fed conditions; PWW: olive trees irrigated with poultry wastewater; PWTWW: olive trees irrigated with poultry wastewater diluted with tap water (50:50) (v/v); \* Commercial standards applicable to olive oils [37].

The results of the peroxide value revealed that the samples of oil produced from trees that were irrigated with PWTWW had the highest value (16 mEq O<sub>2</sub> kg<sup>-1</sup>), whereas the other oil samples showed similar values (13 mEq O<sub>2</sub> kg<sup>-1</sup>).

The olive oil extracted from trees irrigated with PWTWW presented the highest spectrophotometric absorbance value (2.59) at 232 nm, while the lowest value was recorded in the PWW samples (2.42), while the control group displayed a value in between (2.48). The oil sample from the group receiving PWW irrigation had a high concentration of oxidized secondary products, with a value of 0.13, according to the data obtained for the absorbance at 270 nm. However, oil samples obtained with the PWTWW and control groups showed lower secondary oxidation levels (0.08).

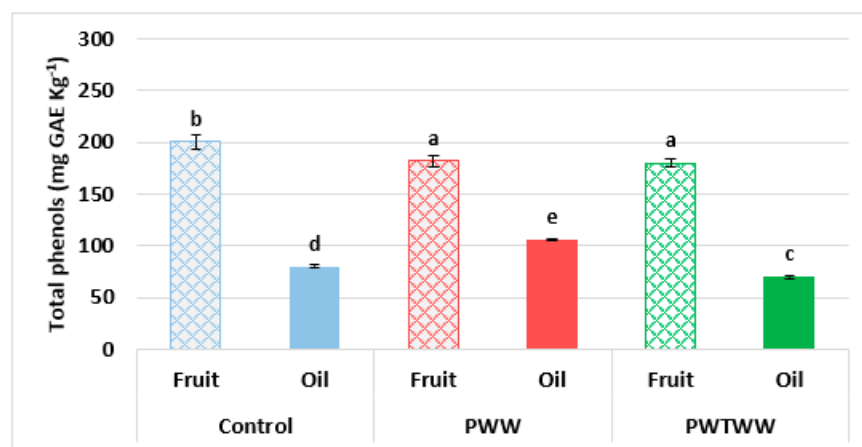
All Δk values recorded were lower than 0.001, which shows that the reuse of wastewater did not affect the purity of the oils obtained. On the basis of these classification criteria, established according to the commercial standard of the International Olive Council [37], the olive oils extracted from the lot grown under rain-fed conditions, and the lot irrigated with PWW, belong to the extra-virgin olive oil category, while the oil obtained from the PWTWW irrigated group belongs to the virgin olive oil category, this is due to the K232 extinction value, which was in the range 2.50–2.60.

### 3.3. Effect of Wastewater Reuse on Total Phenolic Content

Figure 1 shows the total phenolic contents determined in the samples. Olive oil samples showed a high statistical intragroup variability ( $F = 314.190$ ), compared to fruit samples ( $F = 7.688$ ). The reuse of PWW and PWTWW in olive trees' irrigation caused a decrease in TPC concentrations of drupes, i.e.,  $182 \pm 5$  and  $180 \pm 4$  mg GAE kg<sup>-1</sup>,



respectively. However, olive oil samples showed a significant increase in TPC using PWW ( $106 \pm 1 \text{ mg GAE kg}^{-1}$ ).



**Figure 1.** Influence of wastewater irrigation on olive fruit and oil total phenolic contents ( $\text{mg GAE kg}^{-1}$ ). Control: olive trees grown under rain-fed conditions; PWW: olive trees irrigated with poultry wastewater; PWTWW: olive trees irrigated with poultry wastewater diluted with tap water (50:50) (*v/v*). Same superscript letters indicate not significant differences at  $p \leq 0.05$ , according to Tukey's HSD test.

### 3.4. Effects of Wastewater Reuse on Mineral and Heavy Metal Accumulation on Fruits and Olive Oil Samples

Table 3 shows the results of mineral and heavy metal determination on the fruit and olive oil samples. Olive fruits harvested from PWW-irrigated trees were rich in Mg, B, and Cu, with values of about  $27.490$ ,  $3.660$ , and  $0.240 \text{ mg kg}^{-1}$ , respectively. In addition, a very marked Fe fruit enrichment was noticed in the lot irrigated with PWTWW ( $2.590 \text{ mg kg}^{-1}$ ), compared with the PWW ( $1.480 \text{ mg kg}^{-1}$ ) and control ( $1.060 \text{ mg kg}^{-1}$ ) lots. No significant difference, at  $p \leq 0.05$ , was registered in the concentrations of the nutrients Mn, Zn, and Co.

Statistical analysis showed that the reuse of wastewater had a significant effect, at  $p \leq 0.05$ , on the rate of accumulation of heavy metals in the olive pulp (Table 3). Fruits harvested from trees irrigated with PWTWW showed a higher accumulation rate of Cr, Al, Ti, V, Ni, Sr, and Ba than those irrigated with PWW or rain-fed. Except for Li and Ti, the lowest heavy metal concentrations were detected in the control lot.

Regarding the olive oil samples, the reuse of PWW induced a very significant accumulation of mineral elements such as Mg, Fe, and Mn, with concentrations of  $12.090$ ,  $8.360$ , and  $0.250 \text{ mg kg}^{-1}$ , respectively (Table 3). According to standards established in [37], Fe concentrations exceeded the set limits. The lowest amounts of these minerals were found in olive oil derived from the control olive trees, while the highest concentrations were found in the PWTWW-irrigated olive trees.

The evaluation of heavy metals accumulation showed the absence of some metals, such as Be, Se, and Ag, in the three olive oil samples, as well as the absence of a significant difference, at  $p \leq 0.05$ , in the Hg, Tl, and Bi content. Olive oil extracted from olive trees irrigated with PWTWW revealed very high concentrations of Cr and Ni, on the order of  $0.100 \text{ mg kg}^{-1}$  (Table 3). However, the concentrations recorded in the sample of oil extracted from the trees irrigated with PWW had higher values than the other two samples, especially in the contents of Li, Al, Ti, V, Sr, and Ba, where the concentrations were  $0.010$ ,  $13.610$ ,  $1.180$ ,  $0.030$ ,  $0.100$ ,  $0.050 \text{ mg kg}^{-1}$ , respectively. In addition, Cd and Sb were not detected in the PWW olive oil sample (Table 3).

**Table 3.** Determination of minerals (mg kg<sup>-1</sup>) in fruits and olive oil samples.

	Control		PWW		PWTWW		* Standard for Olive Oil
	Fruit	Olive Oil	Fruit	Olive Oil	Fruit	Olive Oil	
Mg	23.460 <sup>a</sup> ± 0.090	2.070 <sup>a</sup> ± 0.005	27.490 <sup>b</sup> ± 0.400	12.090 <sup>c</sup> ± 0.067	26.510 <sup>b</sup> ± 0.290	3.100 <sup>b</sup> ± 0.001	-
B	2.020 <sup>a</sup> ± 0.010	nd	3.660 <sup>b</sup> ± 0.050	nd	2.070 <sup>a</sup> ± 0.030	nd	-
Cu	0.240 <sup>b</sup> ± 0.000	nd	0.240 <sup>b</sup> ± 0.000	nd	0.200 <sup>a</sup> ± 0.010	nd	<0.1
Fe	1.060 <sup>a</sup> ± 0.040	0.020 <sup>a</sup> ± 0.001	1.480 <sup>b</sup> ± 0.010	8.360 <sup>c</sup> ± 0.004	2.950 <sup>c</sup> ± 0.040	0.710 <sup>b</sup> ± 0.004	<0.3
Mn	0.250 <sup>a</sup> ± 0.030	0.001 <sup>a</sup> ± 0.000	0.230 <sup>a</sup> ± 0.020	0.250 <sup>b</sup> ± 0.003	0.290 <sup>a</sup> ± 0.030	0.010 <sup>a</sup> ± 0.003	-
Mo	nd	nd	nd	nd	nd	0.004 <sup>a</sup> ± 0.001	-
Zn	0.100 <sup>a</sup> ± 0.000	0.100 <sup>a</sup> ± 0.002	0.120 <sup>a</sup> ± 0.010	0.100 <sup>a</sup> ± 0.000	0.100 <sup>a</sup> ± 0.000	0.100 <sup>a</sup> ± 0.000	-
Co	0.002 <sup>a</sup> ± 0.000	nd	0.002 <sup>a</sup> ± 0.000	0.010 <sup>b</sup> ± 0.001	0.002 <sup>a</sup> ± 0.000	0.001 <sup>a</sup> ± 0.000	-
Hg	0.004 <sup>a</sup> ± 0.001	0.002 <sup>a</sup> ± 0.001	0.005 <sup>a</sup> ± 0.001	0.001 <sup>a</sup> ± 0.001	0.003 <sup>a</sup> ± 0.001	0.001 <sup>a</sup> ± 0.001	-
Cr	nd	nd	nd	0.020 <sup>a</sup> ± 0.002	0.002 <sup>a</sup> ± 0.001	0.100 <sup>b</sup> ± 0.001	-
Li	0.050 <sup>b</sup> ± 0.001	nd	0.070 <sup>c</sup> ± 0.001	0.010 <sup>a</sup> ± 0.001	0.040 <sup>a</sup> ± 0.001	nd	-
Be	nd	nd	nd	nd	nd	nd	-
Al	1.320 <sup>a</sup> ± 0.004	0.100 <sup>a</sup> ± 0.005	1.840 <sup>b</sup> ± 0.002	13.610 <sup>b</sup> ± 0.003	3.531 <sup>c</sup> ± 0.001	0.100 <sup>a</sup> ± 0.002	-
Ti	0.240 <sup>b</sup> ± 0.001	0.030 <sup>a</sup> ± 0.002	0.210 <sup>a</sup> ± 0.001	1.180 <sup>b</sup> ± 0.005	0.500 <sup>c</sup> ± 0.001	0.020 <sup>a</sup> ± 0.001	-
V	0.003 <sup>a</sup> ± 0.001	0.001 <sup>a</sup> ± 0.000	0.004 <sup>a</sup> ± 0.001	0.030 <sup>b</sup> ± 0.001	0.010 <sup>b</sup> ± 0.001	0.002 <sup>a</sup> ± 0.001	-
Ni	0.010 <sup>a</sup> ± 0.001	nd	0.010 <sup>a</sup> ± 0.001	0.010 <sup>a</sup> ± 0.001	0.030 <sup>b</sup> ± 0.001	0.100 <sup>b</sup> ± 0.007	-
As	0.001 <sup>a</sup> ± 0.000	nd	0.001 <sup>a</sup> ± 0.000	0.003 <sup>b</sup> ± 0.001	0.001 <sup>a</sup> ± 0.000	nd	-
Se	0.001 <sup>a</sup> ± 0.000	nd	0.001 <sup>a</sup> ± 0.000	nd	0.001 <sup>a</sup> ± 0.000	nd	-
Sr	0.700 <sup>a</sup> ± 0.010	0.010 <sup>a</sup> ± 0.001	0.760 <sup>b</sup> ± 0.001	0.100 <sup>c</sup> ± 0.002	1.550 <sup>c</sup> ± 0.004	0.020 <sup>b</sup> ± 0.001	-
Ag	nd	nd	nd	nd	nd	nd	-
Cd	0.010 <sup>a</sup> ± 0.000	0.002 <sup>a</sup> ± 0.001	0.010 <sup>a</sup> ± 0.000	nd	0.010 <sup>a</sup> ± 0.000	0.001 <sup>a</sup> ± 0.000	-
Sb	0.002 <sup>a</sup> ± 0.000	0.003 <sup>a</sup> ± 0.001	0.003 <sup>a</sup> ± 0.001	nd	0.002 <sup>a</sup> ± 0.001	0.003 <sup>a</sup> ± 0.001	-
Ba	0.040 <sup>a</sup> ± 0.000	0.002 <sup>a</sup> ± 0.001	0.040 <sup>a</sup> ± 0.000	0.050 <sup>b</sup> ± 0.001	0.100 <sup>b</sup> ± 0.007	nd	-
Tl	0.001 <sup>a</sup> ± 0.000	0.001 <sup>a</sup> ± 0.000	0.001 <sup>a</sup> ± 0.000	0.001 <sup>a</sup> ± 0.000	0.001 <sup>a</sup> ± 0.000	0.001 <sup>a</sup> ± 0.000	-
Pb	0.002 <sup>a</sup> ± 0.000	0.001 <sup>a</sup> ± 0.000	0.002 <sup>a</sup> ± 0.000	0.003 <sup>b</sup> ± 0.000	0.002 <sup>a</sup> ± 0.000	nd	-
Bi	0.003 <sup>a</sup> ± 0.000	0.004 <sup>a</sup> ± 0.001	0.003 <sup>a</sup> ± 0.000	0.004 <sup>a</sup> ± 0.001	0.003 <sup>a</sup> ± 0.000	0.003 <sup>a</sup> ± 0.001	-

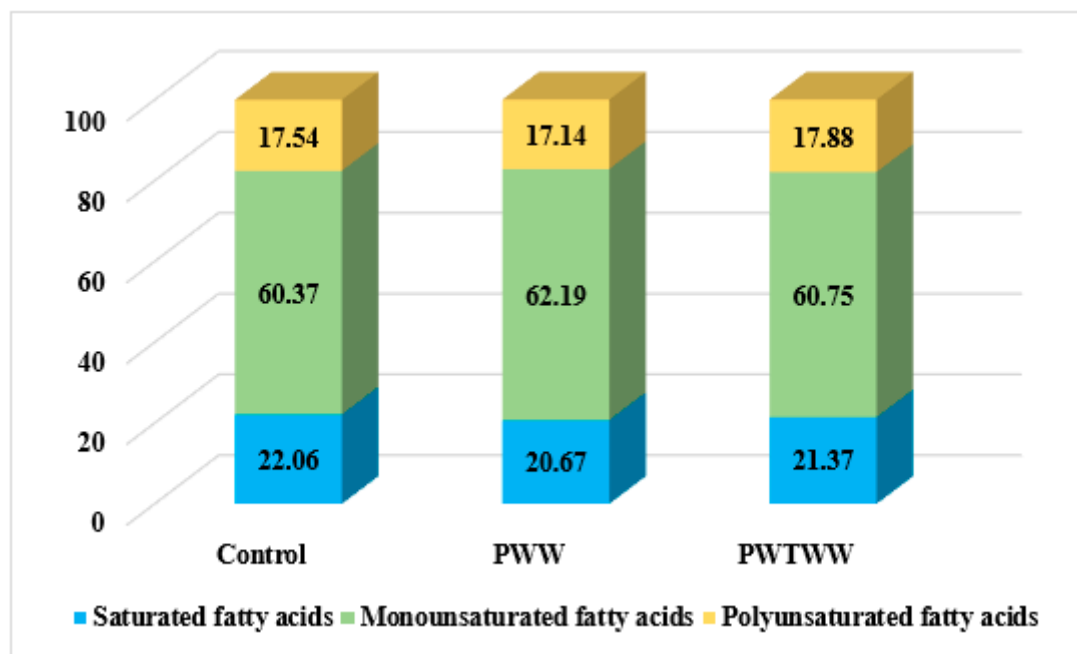
Control: olive trees grown under rain-fed conditions; PWW: olive trees irrigated with poultry wastewater; PWTWW: olive trees irrigated with poultry wastewater diluted with tap water (50:50) (v/v). \* Commercial standards applicable to olive oils [37]. ± standard deviations. Same superscript letters indicate not significant differences at  $p \leq 0.05$ , according to Tukey's HSD test. nd: not detected (concentration < 0.001).

### 3.5. Effect of Wastewater Irrigation on Olive Oil Fatty Acid Profile

Figure 2 shows the results of the determination of fatty acids in the olive oil samples. A relatively higher concentration of monounsaturated fatty acids was found (62.19%) in olive oil extracted from the PWW-irrigated lot versus the control (60.37%) and PWTWW (60.75%) lots, respectively. This abundance was accompanied by a decrease in the relative percentage of saturated fatty acids.

As shown in Table 4, all fatty acid contents quantified in the olive oil samples were within the range set by the IOC for the classification of olive oil as extra-virgin (2021). The characterization of these fatty acids showed that oleic acid was the major representative compound (57.62–59.71%), followed by palmitic acid (17.41–18.82%), and linoleic acid (16.51–17.25%). According to the obtained results, statistical differences were only observed in the PWW irrigated lot, which presented the highest percentages of oleic acid and eicosenoic acid. The reuse of industrial wastewater had no effect on the content of the remaining fatty acids.

In rain-fed conditions, a significant increase in the percentage of total sterols was detected, where apparent  $\beta$ -sitosterols were the major representative. The analysis of cholesterol content did not show a difference between the two rain-fed and PWW groups, with values of 0.20% and 0.15%, respectively, while the PWTWW lot presented the lowest content, with a percentage of 0.13%.



**Figure 2.** Effect of irrigation with poultry wastewater on olive oil fatty acid concentrations (%). Control: olive trees grown under rain-fed conditions; PWW: olive trees irrigated with poultry wastewater; PWTWW: olive trees irrigated with poultry wastewater diluted with tap water (50:50) (v/v).

**Table 4.** Fatty acid (%), sterol (%), and alcohol (%) contents in olive oils from cv. Chemlali, obtained after wastewater irrigation.

Fatty Acids	Common Name	Control	PWW	PWTWW	* Standards
Fatty acids (%)					
C14:0	Myristic acid	0.01 <sup>a</sup> ± 0.00	0.02 <sup>a</sup> ± 0.00	0.01 <sup>a</sup> ± 0.00	≤0.03
C16:0	Palmitic acid	18.82 <sup>a</sup> ± 0.55	17.41 <sup>a</sup> ± 0.26	18.11 <sup>a</sup> ± 0.61	7.00–20.00
C16:1	Palmitoleic acid	2.55 <sup>a</sup> ± 0.19	2.21 <sup>a</sup> ± 0.29	2.26 <sup>a</sup> ± 0.38	0.30–3.50
C17:0	Heptadecanoic acid	0.05 <sup>a</sup> ± 0.01	0.04 <sup>a</sup> ± 0.02	0.08 <sup>a</sup> ± 0.02	≤0.40
C17:1	Heptadecenoic acid	0.05 <sup>a</sup> ± 0.02	0.03 <sup>a</sup> ± 0.01	0.06 <sup>a</sup> ± 0.02	≤0.60
C18:0	Stearic acid	2.72 <sup>a</sup> ± 0.06	2.68 <sup>a</sup> ± 0.03	2.66 <sup>a</sup> ± 0.03	0.50–5.00
C18:1	Oleic acid	57.62 <sup>a</sup> ± 0.11	59.71 <sup>b</sup> ± 0.39	58.25 <sup>a</sup> ± 0.13	55.00–85.00
C18:2	Linoleic acid	16.89 <sup>a</sup> ± 0.36	16.51 <sup>a</sup> ± 0.31	17.25 <sup>a</sup> ± 0.05	2.50–21.00
C18:3	Linolenic acid	0.65 <sup>a</sup> ± 0.01	0.63 <sup>a</sup> ± 0.01	0.63 <sup>a</sup> ± 0.01	≤1.00
C20:0	Arachidic acid	0.44 <sup>a</sup> ± 0.01	0.46 <sup>a</sup> ± 0.02	0.44 <sup>a</sup> ± 0.01	≤0.60
C20:1	Eicosenoic acid	0.15 <sup>a</sup> ± 0.01	0.24 <sup>b</sup> ± 0.01	0.18 <sup>a</sup> ± 0.01	≤0.50
C22:0	Behenic acid	0.03 <sup>a</sup> ± 0.01	0.03 <sup>a</sup> ± 0.01	0.04 <sup>a</sup> ± 0.01	≤0.20
C23:0	Tricosylic acid	0.02 <sup>a</sup> ± 0.00	0.03 <sup>a</sup> ± 0.00	0.03 <sup>a</sup> ± 0.00	≤0.20
Sterols (%)					
	Cholesterol	0.20 <sup>b</sup> ± 0.02	0.15 <sup>a,b</sup> ± 0.01	0.13 <sup>a</sup> ± 0.02	-
	Brassicasterol	0.02 <sup>a</sup> ± 0.00	0.03 <sup>b</sup> ± 0.00	0.05 <sup>c</sup> ± 0.00	-
	Campesterol	2.86 <sup>a</sup> ± 0.13	3.21 <sup>b</sup> ± 0.03	3.04 <sup>a,b</sup> ± 0.05	-
	Stigmasterol	0.60 <sup>a</sup> ± 0.14	1.04 <sup>b</sup> ± 0.04	0.95 <sup>b</sup> ± 0.03	-
**	Apparent β-sitosterols	95.40 <sup>c</sup> ± 0.15	94.80 <sup>b</sup> ± 0.05	94.22 <sup>a</sup> ± 0.12	-
	Δ7-stigmasterol	0.29 <sup>a,b</sup> ± 0.00	0.25 <sup>a</sup> ± 0.01	0.31 <sup>b</sup> ± 0.02	-
	Total sterols (%)	0.23 <sup>b</sup> ± 0.00	0.19 <sup>a</sup> ± 0.01	0.20 <sup>a</sup> ± 0.00	-
Alcohols (%)					
	Erythrodiol and uvaol	2.58 <sup>a</sup> ± 0.12	3.15 <sup>b</sup> ± 0.09	2.76 <sup>a,b</sup> ± 0.11	<4.50

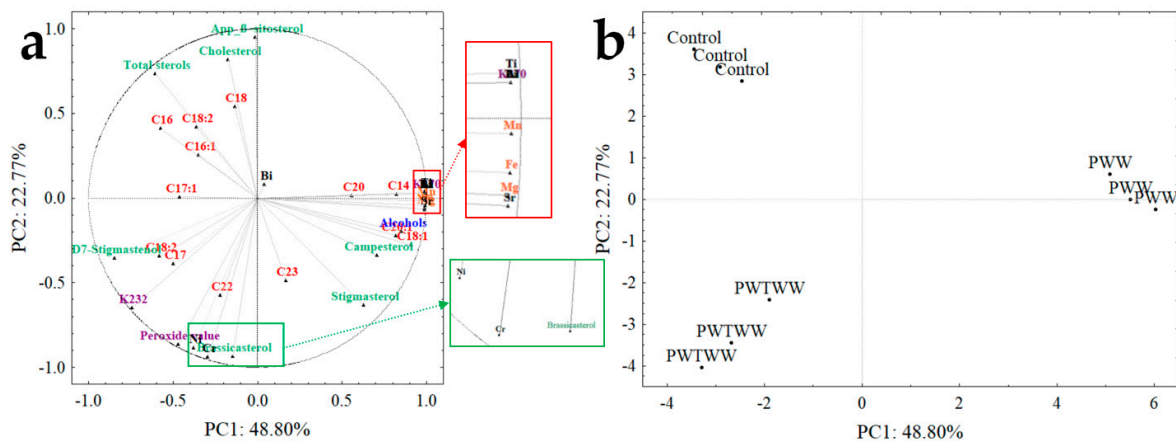
Control: olive trees grown under rain-fed conditions; PWW: olive trees irrigated with poultry wastewater; PWTWW: olive trees irrigated with poultry wastewater diluted with tap water (50:50) (v/v); \* Commercial standards applicable to olive oils [37]; \*\* Apparent β-sitosterols: Δ-5.23-stigmastadienol + cholesterol + β-sitosterol + sitostanol + Δ-5-avenasterol + Δ-5.24-stigmastadienol. ± standard deviations. Same superscript letters indicate not significant differences, at  $p \leq 0.05$ , according to Tukey's HSD test. nd: not detected (concentration < 0.001).

Erythrodiol and uvaol, determined as a sum, were the only alcohols detected with a higher percentage in the PWW lot, with a value of 3.15%, compared to 2.58% in the control



lot. Erythrodiol and uvaol levels did not exceed 4.5%, the acceptable COI limit for edible virgin olives [37].

Principal component analysis (PCA) was performed on the autoscaled data obtained for the olive oil samples, in order to reduce such a large dataset without losing information (Figure 3a,b). The first principal component (PC1) and the second principal component (PC2) explained 48.80% and 22.77% of the total variance, respectively.



**Figure 3.** Principal component analysis of the elemental analysis of oils derived from different irrigation lots. Control: olive trees grown under rain-fed conditions; PWW: olive trees irrigated with poultry wastewater; PWTWW: olive trees irrigated with poultry wastewater diluted with tap water (50:50) (v/v).

Oleic acid, myristic acid, arachidic acid, eicosenoic acid, K270, the sum of the two alcohols, and campesterol weighed on PC1 with a positive sign and were highly correlated with the mineral elements (Mg, Mn, and Fe), but also with Li, Al, Ti, V, Sr, and Ba (Figure 3a). PC2 was mainly characterized by apparent  $\beta$ -sitosterols, cholesterol, and stearic acid with a positive sign, while brassicasterol, peroxide value, Ni, and Cr weighed on PC2 with a negative sign. Figure 3b shows the scatterplot of PC1 vs. PC2. The samples are perfectly separated in three different clusters. PWW is located on the positive quadrant of PC1, while PWTWW is set in the negative quadrant of PC1 and PC2, and finally, the controls are placed in the negative quadrant of PC1 and the positive quadrant of PC2.

PC3 represents 13.26% of the total variance. However, this PC is mainly characterized by minor fatty acids, which reflects in the more scattered positions of the samples in the score plot. However, PWW samples are clearly separated by the remaining samples, which are instead more mixed.

#### 4. Discussion

Wastewater reuse is becoming a worldwide agricultural practice, especially for olive tree irrigation. In fact, wastewater plays a primary role in supplementing irrigation water requirements [19,38]. However, maintaining the commercial value of the olives, such as their size, is still critical. In fact, their commercial value increases with the increase in pulp to stone ratio [39].

Several previous works have shown that the reuse of treated wastewater has improved the pomological criteria of Chemlali olives [23,40]. In order to determine the optimal harvesting moment, the calculation of the maturity index (MI) value is a very common method to assess the degree of ripeness of olives. MI is very useful for farmers, because it allows for an improvement in the quantitative and qualitative characteristics of olive oil [41]. Indeed, it has been proven that the pomological characteristics of fruits, and the quality of olive oil, depend on several factors, including the stage of maturation [42,43].

After 12 months of irrigation, the results showed that using PWW and PWTWW delayed the maturity of olives compared with those harvested from olive trees grown under rain-fed conditions. The nutritional state of the tree can influence the maturity of the fruits. In particular, fertilization with nitrogen reduces the biosynthesis of anthocyanins (phenolic compounds responsible for changes in the color of the fruits) and, consequently, delays the olive maturity [44,45].

Another study has found that under conditions of high nutrient availability, phenylalanine is preferentially directed towards protein synthesis rather than phenylpropanoid synthesis, via phenylalanine ammonia-lyase (PAL), the main pathway for flavonoid biosynthesis [46]. Those findings agree with our results, since PWW and PWTWW induced a decrease in fruits' total polyphenols content.

According to [47] the stage of maturity of Chemlali fruits is the most important factor determining the fat content of olives, in relation to the fresh weight. In addition, it has been shown that the irrigation of olive trees is a promoting factor for increasing fruit productivity, by increasing both size, number, and oil content [12,48,49].

In the present work, the quality of water irrigation influences the pomological characteristics of fruits. Aside from for the pulp oil content, the best criteria were recorded in the olives harvested from the PWTWW-irrigated trees that showed the highest pulp to stone ratio, which allows the improvement of their commercial value [39]. These findings suggest that the nutrient contents of PWTWW represent the ideal nutritional ratios that allow for the improvement of the fruits' pomological features [18]. According to [50] the application of appropriate dilutions of olive oil mill wastewater has stimulated the productivity of olive trees. In fact, magnesium and potassium, at suitable concentrations, act as pomological stimulator factors (fruit weight, fruit dimensions, pulp weight, and pulp to stone ratio), by enhancing the plant physiological processes [51].

A previous study has shown that foliar application of potassium improved fruit quality by promoting the synthesis and transfer of carbohydrates from the shoot to the storage organs (fruits) [52]. Magnesium, which also affects the biosynthesis of proteins, chlorophylls, and carbohydrates in plant anabolism, is responsible for the improvement in fruit quality. By increasing the plant capacity for photosynthetic energy, magnesium makes assimilates for fruit growth more readily available [53]. Fertilization with magnesium and potassium, which function as controllers of the enzyme activity involved in oil production, results in an enhanced assimilation translocation to the storage organs [51,54]. In addition, it has been proven that the accumulation of oil in the olive is a process that depends on the amount of carbohydrates from the fruits and leaves [55], which is consistent with our earlier findings about the carbohydrate content in olive tree leaves [18]. Regarding the health status of fruits, [56] did not mention any contamination of fruits harvested directly from the canopy.

According to the classification proposed by [37], the PWW preserved the oil quality as extra-virgin. However, the oil obtained following the PWTWW irrigation was classified as virgin oil, due to the slight increase in the K232 extinction value, which could be due to its oxidation during storage. The UV spectrophotometric absorbance test is a complementary analysis to determine oil purity degree, by evaluating the oxidation state of unsaturated fatty acids and the formation of oxidized products. Absorbance at 232 nm allowed the measurement of the concentration of linoleic hydroperoxides, which act as oxidation initiators, giving rise to volatile and non-volatile compounds [57]. The oxidation of PWTWW oil samples can be attributed to the higher fruit water content, in comparison with the remaining samples. In fact, lipases naturally present in olive pulp or seeds, are active in the aqueous phase [58].

The absorbance at 270 nm is a tool to measure secondary products of oxidation such as diketones and unsaturated ketones [59]. The determination of the  $\Delta k$  value is fundamental in order to assess the degree of purity of the oil [60]. The peroxide value corresponds to the number of peroxides present in the oil, which is used to assess the state of conservation of fat during storage and its degree of primary oxidation [61].

Previous works have mentioned that the reuse of treated wastewater from different origins (domestic, industrial, the dairy industry) did not affect the standard indices (free acidity, specific absorbance at ultraviolet K232 and K270) used for evaluating the quality of Chemlali olive oil [56,62,63].

The fatty acids profiles of the olive oils showed that PWW and PWTWW preserved the olive oil quality as extra-virgin, according to the commercial standard applicable to olive oils [35]. Furthermore, higher percentages of oleic and eicosenoic acids were observed in the lots irrigated with PWW. These findings contradict those made by [62,64], who claimed that the reuse of treated wastewater in the watering of Chemlali olive trees was the cause of the increase in the contents of linoleic, stearic, palmitic, palmitoleic, and linolenic acids.

Free fatty acids are a very important parameter for determining olive oil quality. This parameter is expressed as the percentage of oleic acid present in 100 g of oil. In fact, it has been mentioned that the stage of maturity of the olives is one of the major factors that can influence the oil quality, where the high oleic acid content is positively correlated with less mature fruits. In addition, a high concentration of oleic acid, accompanied by low concentrations of palmitic, stearic, linoleic, and linolenic acids, can improve the commercial value of olive oil [65].

According to [66], the fatty acid profile of olive oil is affected by the irrigation water quality. Furthermore, previous research has revealed that fertilization slightly affects the contents of the main fatty acids [67,68]. In addition, it is recommended to carefully control fertilization, especially nitrogen levels, in order to guarantee the final quality of the olive oil [69]. Indeed, oleic acid is one of the most important fatty acids in olive oils, due to its important nutritional value and its role in maintaining oxidative stability [70], which is in agreement with our results relating to the peroxide values and extinction coefficients.

Sterols are among the most important minor compounds that influence the final virgin olive oil quality, by contributing to its resistance to oxidative deterioration [71]. In addition, they constitute part of the non-saponifiable lipid fraction, which possesses several beneficial health properties [72]. In our study, the sterols content recorded, following irrigation with different water qualities, varied between 0.19% and 0.23%, which is consistent with the range of values reported in the literature (0.13–0.29%) [73–75].

The results showed that olive trees cultivated under rain-fed conditions produced an oil richer in sterol compounds, compared to those irrigated with PWW and PWTWW. However, the control lot recorded the lowest stigmaterol percentages. The authors of [76] considered that stigmaterol is the main sterol that causes alterations in virgin olive oil quality. In fact, its high content negatively affects the oil's sensory quality. In addition, previous studies have mentioned that a high stigmaterol content is positively correlated with higher acidity [77,78].

In the present study, the decrease in the concentration of total sterols could be due to the fact that steroid compounds are formed during the first phases of maturation, during which the oil content normally increases. This increase is explained by the dilution of sterols [79]. The fruits collected from the PWW and PWTWW lots were less mature, with higher oil contents than those collected from the control batch. According to [71], the reuse of treated wastewater for 13 years in the irrigation of Chemlali olive trees, induced an increase in the content of total sterols, campesterol, stigmaterol, and cholesterol, with a decrease in  $\beta$ -sistosterol. These results agree with ours only in terms of total sterols and cholesterol content. The authors of [67] found that foliar fertilization based on boron, manganese, magnesium, and sulfur decreased cholesterol levels in Picholine olive oil, which is consistent with our results, given the richness of PWW in these nutrients. Contrary to our findings, the combination of these elements with nitrogen does not seem to have an impact on the amounts of brassicasterol, campesterol, and stigmaterol.

Erythrodiol and uvaol are triterpenic dialcohols [80]. The increase in erythrodiol and uvaol levels observed in the oil sample extracted from the PWW lot can be explained by the fact that these triterpene diols are mostly located in fruit epicarp. During maturation, the epicarp becomes more fragile as the fruit ripens, and better breaks during milling [81].

According to the high correlation shown by PCA among mineral elements (Mn, Mg, and Fe), major fatty acids (oleic and myristic acid), and alcohols, this increase may be attributed to PWW's richness in these elements. As far as we know, no literature has established a link between oils' mineral and fatty acid profiles.

The content of phenolic compounds is responsible for major olive oil properties, such as oxidative stability [82]. It is widely known that the highest quality of olive oil is obtained during the initial ripeness stage, due to its composition of phenolic compounds [83,84]. In fact, less mature harvested fruits produce oil with a high polar phenol content, which contributes to the sensations of bitterness and pungency, and to better oil stability [82]. These assertions agree with the results obtained in the present study, since the olives harvested from the PWW lot remained less ripe than the control lot and consequently had a higher total phenolic content. Nevertheless, previous studies have mentioned that the increases in olive oil polyphenols can be a response to salinity stress [85–87]. In a previous study, physicochemical characterization of PWW showed a high level of electric conductivity, which resulted in a high salinity [18].

Besides the stage of fruit maturity and salinity, a higher olive water content could explain the differences in the oil phenolic content between the PWW and PWTWW groups. In fact, phenolic compounds are soluble in both water and oil. Fruits harvested from the PWTWW group showed the highest water content, even though it was not significant, meaning significant amounts of total phenol were carried away from the oily phase [88].

The nutritional fruit profile showed a high concentration of the minerals Mg, B, and Cu in the lot irrigated with PWW. The authors of [89] have hypothesized that the transfer of N and K from the leaves, for the synthesis of amino acids, proteins, and secondary metabolites could be the cause of the enrichment of fruits in these minerals. The same work mentioned an accumulation of Zn and Mn as well. An increase in the Mg content of fruits has also been described in a study on the Tunisian cultivar Chemlali, treated using wastewater from a dairy company [23]. However, the concentrations of the mineral elements Mn, Fe, and Zn, as well as the levels of heavy metals (Pb and Hg), decreased [23]. Conversely, in the present study an increase in the concentrations of Fe and several heavy metals, such as Al, Ba, Sr, Ni, and Ti, was observed.

The accumulation of heavy metals in the soil, and their translocation to different plant organs, is one of the main negative consequences of the reuse of wastewater in agriculture. The results obtained in the present study suggest that PWW and PWTWW contributed to the enrichment of olive oil in the minerals Mg, Fe, Mn, and Co. However, in parallel, there was a considerable immobilization of heavy metals along the transition from fruit to olive oil. In contrast to the findings mentioned by [90], the same observations were made by [91] and [63]. In our case, before the beginning of the experience, high concentrations of heavy metals were detected in the soil. The reuse of PWW and PWTWW induced a significant decrease in their levels, accompanied by an increase in the electrical conductivity. Relevant decreases recorded in the soil after irrigation could be correlated to the effect of NPK fertilization. Indeed, it has been proven that NPK fertilization promotes the absorption of elements (in particular zinc) by plants, as well as heavy metals [92,93]. Furthermore, this result can be explained by the soil electrical conductivity increase, which is considered as a determining factor of the bioavailability of heavy metals, where its increase leads to a greater bioavailability [94,95].

## 5. Conclusions

Reusing poultry wastewater for the irrigation of olive trees, revealed that its 50% dilution (PWTWW) improved the pulp to stone ratio, which is a relevant parameter of olive tree productivity. Furthermore, two considerable phenomena were observed using poultry wastewater to irrigate of olive trees, a delay in the fruit ripening process (for both PWW and PWTWW), and a higher pulp oil content (PWW).

The reuse of PWW and PWTWW resulted in the production of extra-virgin and virgin olive oil qualities, respectively, according to the classification proposed by the [35].

Regarding the fatty acid profile of the olive oil, it was unaffected following irrigation with PWW. Nevertheless, PWW induced a significant increase in the content of oleic acid, triterpenic alcohols (erythrodiol and uvaol), and total phenolic content, and a decrease in total sterols, in comparison with the other samples. Heavy metals analysis revealed that PWW irrigation induced heavy metal bioaccumulation in olive oil translocated from already contaminated soil.

The reuse of PWW can be a good alternative to minimize the reuse of fresh water and improve the quality of olive oil, provided that it is ensured in advance that the irrigated soil is free of heavy metals.

**Author Contributions:** Conceptualization, A.O. and H.B.M.; data curation, A.O.; formal analysis, G.M.; funding acquisition, H.B.M. and G.D.B.; investigation, G.B., V.N. and R.R.; methodology, A.O. and N.M.; project administration, H.B.M. and G.D.B.; resources, A.A., G.D.B. and H.B.M.; supervision, H.B.M. and S.D.; validation, G.M.; visualization, A.O., N.M. and G.M.; writing—original draft, A.O.; writing—review and editing, H.B.M. and S.D. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Data and Materials are available.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Cosgrove, W.J.; Rijsberman, F. *World Water Vision: Making Water Everybody's Business*; World Water Council, World Water Vision, and Earthscan; Routledge: London, UK, 2000.
2. Ungureanu, N.; Vladut, V.; Voicu, G. Water scarcity and wastewater reuse in crop irrigation. *Sustainability* **2020**, *12*, 9055. [CrossRef]
3. Food and Agriculture Organization of the United Nations (FAO). *Coping with Water Scarcity—Challenge of the Twenty-First Century, World Water Day*; FAO: Rome, Italy, 2007. Available online: <http://www.fao.org/3/a-aq444e.pdf> (accessed on 16 September 2020).
4. World Bank. World Bank eAtlas of Global Development The World Bank, Washington, USA. 2012. Available online: <http://www.worldbank.org> (accessed on 14 January 2023).
5. AQUASTAT—FAO's Global Information System on Water and Agriculture. Water Use. 2021. Available online: [www.fao.org/nr/water/aquastat/water\\_use/index.stm](http://www.fao.org/nr/water/aquastat/water_use/index.stm) (accessed on 14 January 2023).
6. Aghtape, A.A.; Ghanbari, A.; Sirousmehr, A.; Siahshar, B. Effect of irrigation with wastewater and foliar fertilizer application on some forage characteristics of foxtail millet (*Setaria italica*). *Plant. Physiol. Biochem.* **2011**, *3*, 34–42. Available online: [https://jcesc.um.ac.ir/article\\_36040.html?lang=en](https://jcesc.um.ac.ir/article_36040.html?lang=en) (accessed on 14 January 2023).
7. Sioud, O.; Beltifa, A.; Ayeb, N.; Mansour, H.B. Characterization of industrial dairy wastewater and contribution to reuse in cereals culture: Study of phytotoxic effect. *Austin J. Environ. Toxicol.* **2016**, *2*, 1013. Available online: <https://austinpublishinggroup.com/environmental-toxicology/fulltext/ajet-v2-id1013.php> (accessed on 14 January 2023).
8. Khalid, S.; Shahid, M.; Natasha, B.I.; Sarwar, T.; Haidar, S.; Ali, N.N.; Khan, N.N. A review of environmental contamination and health risk assessment of wastewater use for crop irrigation with a focus on low and high-income countries. *Int. J. Environ. Res. Public Health.* **2018**, *15*, 895. [CrossRef] [PubMed]
9. Hannachi, H.; Msallem, M.; Ben Elhadj, S.; El Gazzah, M. Influence du site biologique sur les potentialités agronomiques et technologiques de l'olivier (*Olea europea L.*) en Tunisie. *C. R. Biol.* **2007**, *330*, 135–142. [CrossRef] [PubMed]
10. IOC. *General Overview of Olive Growing in Tunisia*; IOC: Paris, France, 2012; p. 10.
11. Zlaoui, M.; Dhraief, M.Z.; Jebali, O.; Benyoussef, S. Assessment of the Tunisian olive oil value chain in the international markets: Constraints and Opportunities. *FARA Res. Result.* **2019**, *4*, 36. Available online: <http://www.onagri.nat.tn/uploads/Etudes/etude-ho.pdf> (accessed on 14 January 2023).
12. Moriana, A.; Orgaz, F.; Fereres, E.; Pastor, M. Yield responses of mature olive orchard to water deficits. *J. Am. Soc. Hortic. Sci.* **2003**, *128*, 425–431. [CrossRef]
13. Grattan, S.R.; Berenguer, M.J.; Connell, J.H.; Polito, U.S.; Vossen, P.M. Olive oil production as influenced by different quantities of applied water. *Agric. Water. Manag.* **2006**, *85*, 133–140. [CrossRef]
14. Gomez-Rico, A.; Salvador, M.D.; Moriana, A.; Perez, D.; Olmedilla, N.; Ribas, F.; Fregapane, G. Influence of different irrigation strategies in a traditional Cornicabra cv. olive orchard on virgin olive oil composition and quality. *Food. Chem.* **2007**, *100*, 568–578. [CrossRef]



15. Mittal, G.S. Characterization of the effluent wastewater from abattoirs for land application. *Food. Rev. Int.* **2004**, *20*, 229–256. [CrossRef]
16. Kosseva, M.R.; Webb, C. Food industry wastes: Assessment and recuperation of commodities. *Acad. Press* **2020**, *516*, 67–85.
17. Amorim, A.K.B.; De Nardi, I.R.; Del Nery, V. Water conservation and effluent minimization: Case study of a poultry slaughterhouse. *Resour. Conserv. Recycl.* **2007**, *51*, 93–100. [CrossRef]
18. Oueslati, A.; Montevecchi, G.; Antonelli, A.; Ben Mansour, H. Short-time irrigation on young olive tree (*Olea europaea* L. cv. Chemlali) with untreated industrial poultry wastewater: Investigation of growth parameters and leaves chemical composition. *Environ. Sci. Pollut. Res.* **2021**, *28*, 50420–50429. [CrossRef] [PubMed]
19. Pedrero, F.; Grattan, S.R.; Ben-Gal, A.; Vivaldi, G.A. Opportunities for expanding the use of wastewaters for irrigation of olives. *Agr. Water. Manag.* **2020**, *241*, 106333. [CrossRef]
20. Morales-Sillero, A.; Jiménez, R.; Fernández, J.E.; Troncoso, A.; Beltrán, G. Influence of fertigation in ‘Manzanilla de Sevilla’ olive oil quality. *Hort. Science.* **2007**, *42*, 1157–1162. [CrossRef]
21. Morales-Sillero, A.; Fernández, J.E.; Troncoso, A. Response of mature ‘Manzanilla’ olive trees to different doses of NPK fertilizer applied by fertigation. *Acta. Hortic.* **2008**, *791*, 345–349. [CrossRef]
22. Dag, A.; Ben-David, E.; Kerem, Z.; Ben-Gal, A.; Erel, R.; Basheer, L.; Yermiyahu, U. Olive oil composition as a function of nitrogen, phosphorus and potassium plant nutrition. *J. Sci. Food. Agric.* **2009**, *89*, 1871–1878. [CrossRef]
23. Sdiri, W.; Dabbou, S.; Nava, V.; Di Bella, G.; Ben Mansour, H. Pomological descriptors, phenolic compounds, and chemical monitoring in olive fruits irrigated with dairy treated wastewater. *Chemosensors* **2021**, *9*, 130. [CrossRef]
24. Petousi, I.; Fountoulakis, M.S.; Saru, M.L.; Nikolaidis, N.; Fletcher, L.; Stentiford, E.I.; Manios, T. Effects of reclaimed wastewater irrigation on olive (*Olea europaea* L. Cv. ‘Koroneiki’) trees. *Agr. Water. Manag.* **2015**, *160*, 33–40. [CrossRef]
25. Bourazanis, G.; Roussos, P.A.; Argyrokastritis, I.; Kosmas, C.; Kerkides, P. Evaluation of the use of treated municipal wastewater 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]
26. Oueslati, A.; Hassen, W.; Ellafi, A.; Alibi, S.; Jaziri, A.; Bachkouel, S.; Oueslati, I.; Snoussi, M.; Adnan, M.; Patel, M.; et al. Assessment of Bacterial Diversity of Industrial Poultry Wastewater by Denaturing Gradient Gel Electrophoresis (DGGE) and the Cultivation Method in Order to Inform Its Reuse in Agriculture. *BioMed. Res. Int.* **2022**, *2022*, 1–12. [CrossRef] [PubMed]
27. Homeier-Bachmann, T.; Heiden, S.E.; Lübcke, P.K.; Bachmann, L.; Bohnert, J.A.; Zimmermann, D.; Schaufler, K. Antibiotic-Resistant Enterobacteriaceae in Wastewater of Abattoirs. *Antibiotics* **2021**, *10*, 568. [CrossRef] [PubMed]
28. Garcia, J.M.; Gutierrez, F.; Barrera, M.J.; Albi, M.A. Storage of mill olives on an industrial scale. *J. Agric. Food. Chem.* **1996**, *44*, 590–593. [CrossRef]
29. Agar, I.T.; Hess-Pierce, B.; Sourour, M.M.; Kader, A.A. Quality of fruit and oil of black-ripe olives is influenced by cultivar and storage period. *J. Agric. Food. Chem.* **1998**, *46*, 3415–3421. [CrossRef]
30. European Union (EU). Commission Delegated Regulation (EU). Amending regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. *Off. J. Eur. Union L.* **2016**, *326*, 1–6.
31. Dabbou, S.; Rjiba, I.; Nakbi, A.; Gazzah, N.; Issaoui, M.; Hammami, M. Compositional quality of virgin olive oils from cultivars introduced in Tunisian arid zones in comparison to Chemlali cultivars. *Sci. Hortic.* **2010**, *124*, 122–127. [CrossRef]
32. IOC: International Olive Council. Coi/t.20/doc. No 33/rev.1. Determination of Fatty Acid Methyl Esters by Gas Chromatography. 2017. Available online: <https://www.internationaloliveoil.org/wp-content/uploads/2019/11/COI-T.20-Doc.-No-33-Rev-1-2017.pdf> (accessed on 14 January 2023).
33. Singleton, V.L.; Orthofer, R.; Lamuela-Raventós, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Method. Enzymol.* **1999**, *299*, 152–178. [CrossRef]
34. Vinha, A.F.; Silva, B.M.; Andrade, P.B.; Seabra, R.M.; Pereira, J.A.; Oliveira, M.B. Development and evaluation of an HPLC/DAD method for the analysis of phenolic compounds from olive fruits. *J. Liq. Chromatogr. Relat.* **2002**, *25*, 151–160. [CrossRef]
35. Benincasa, C.; Lewis, J.; Perri, E.; Sindona, G.; Tagarelli, A. Determination of trace element in Italian virgin olive oils and their characterization according to geographical origin by statistical analysis. *Anal. Chim. Acta.* **2007**, *585*, 366–370. [CrossRef]
36. Di Bella, G.; Naccari, C.; Bua, G.D.; Rastrelli, L.; Lo Turco, V.; Potortì, A.G.; Dugo, G. Mineral composition of some varieties of beans from Mediterranean and tropical areas. *Int. J. Food Sci. Nut.* **2016**, *67*, 239–248. [CrossRef]
37. IOC: International Olive Council. *Trade Standard on Olive Oils and Olive-Pomace Oils*; IOC: Paris, France, 2021; pp. 1–16.
38. Perry, D.M.; Praskievicz, S.J. A New Era of Big Infrastructure? (Re) developing Water Storage in the U.S. West in the Context of Climate Change and Environmental Regulation. *Water. Altern.* **2017**, *10*, 437–454.
39. Mele, M.A.; Mohammad, Z.I.; Ho-Min, K.; Angelo Maria, G. Pre-and post-harvest factors and their impact on oil composition and quality of olive fruit. *Emir. J. Food. Agric.* **2018**, *30*, 592–603. [CrossRef]
40. Gharsallaoui, M.; Zaanouni, N.; Gabsi, S. Study of variations in pomological characteristics of olive fruits following irrigation by treated wastewater (EUT). *J. New. Sci.* **2018**, *52*, 3479–3487. Available online: [https://file:///C:/Users/amira/Downloads/JNS\\_AgriBiotech\\_Vol\\_52\\_01%20.pdf](https://file:///C:/Users/amira/Downloads/JNS_AgriBiotech_Vol_52_01%20.pdf) (accessed on 14 January 2023).
41. Guzmán, E.; Baeten, V.; Pierna, J.A.F.; García-Mesa, J.A. Determination of the olive maturity index of intact fruits using image analysis. *J. Food. Sci. Technol.* **2015**, *52*, 1462–1470. [CrossRef] [PubMed]
42. Ben Temime, S.; Taamalli, W.; Baccouri, B.; Abaza, L.; Daoud, D.; Zarrouk, M. Changes in olive oil quality of Chétoui variety according to origin of plantation. *J. Food. Lipids.* **2006**, *13*, 88–99. [CrossRef]



43. Garcia, J.M.; Seller, S.; Perez-Camino, M.C. Influence of fruit ripening on olive oil quality. *J. Agric. Food. Chem.* **1996**, *44*, 3516–3520. [[CrossRef](#)]
44. Fernández-Escobar, R.; Braz Frade, R.; Lopez Campayo, M.; Beltrán Maza, G. Effect of nitrogen fertilization on fruit maturation of olive trees. *Acta. Hort.* **2014**, *1057*, 101–105. [[CrossRef](#)]
45. Ângelo Rodrigues, M.; Coelho, V.; Arrobas, M.; Gouveia, E.; Raimundo, S.; Correia, C.M.; Bento, A. The effect of nitrogen fertilization on the incidence of olive fruit fly, olive leaf spot and olive anthracnose in two olive cultivars grown in rainfed conditions. *Sci. Hort.* **2019**, *256*, 108658. [[CrossRef](#)]
46. Tovar, M.J.; Romero, M.P.; Alegre, S.; Girona, J.; Motilva, M.J. Composition and organoleptic characteristics of oil from Arbequina olive (*Olea europaea* L.) trees under deficit irrigation. *J. Sci. Food Agric.* **2002**, *82*, 1755–1763. [[CrossRef](#)]
47. Lazzez, A. Etude des Caractéristiques Pomologiques des Olives et Physico-Chimiques de l'huile de la Variété Chemlali: Impact de la Maturité, du Site d'Implantation et de la Campagne Oléicole. Master's Thesis, University of Sfax, Sfax, Tunisia, 2009; p. 189.
48. Inglese, P.; Barone, E.; Gullo, G. The effect of complementary irrigation on fruit growth ripening pattern and oil characteristics of olive (*Olea europaea* L.). *Carolea. J. Hort. Sci.* **1996**, *71*, 257–263. [[CrossRef](#)]
49. Dag, A.; Ben-Gal, A.; Yermiyahu, U.; Basheer, L.; Nir, Y.; Kerem, Z. The effect of irrigation level and harvest mechanization on virgin olive oil quality in a traditional rain-fed 'Souri' olive orchard converted to irrigation. *J. Sci. Food. Agric.* **2008**, *88*, 1524–1528. [[CrossRef](#)]
50. Ayoub, S.; Al-Absi, K.; Al-Shdiefat, S.; Al-Majali, D.; Hijazeen, D. Effect of olive mill wastewater land spreading on soil properties, olive tree performance and oil quality. *Sci. Hort.* **2014**, *175*, 160–166. [[CrossRef](#)]
51. Thanaa, S.M.; Mahmoud, E.S.; Mohamed, A.; El-Sharony, T.F. Influence of Foliar Application with Potassium and Magnesium on Growth, Yield and Oil Quality of "Koroneiki" Olive Trees. *Am. J. Food Technol.* **2017**, *12*, 209–220. Available online: <https://scialert.net/abstract/?doi=ajft.2017.209.220> (accessed on 14 January 2023).
52. Ramezani, S.; Shekafandeh, A. Influence of Zn and K sprays on fruit and pulp growth in olive (*Olea europaea* L. cv. 'Amygdalifolia'). *Iran. Agric. Res.* **2011**, *30*, 1–10. Available online: [https://iar.shirazu.ac.ir/article\\_489\\_a91ca482ca232a11158bc9e22520ee6d.pdf](https://iar.shirazu.ac.ir/article_489_a91ca482ca232a11158bc9e22520ee6d.pdf) (accessed on 14 January 2023).
53. Gerendas, J.; Fuhrs, H. The significance of magnesium for crop quality. *Plant. Soil.* **2013**, *368*, 101–128. [[CrossRef](#)]
54. Mahmoud, T.S.; Kassim, N.E.; Abou Rayya, M.S. Effect of foliar application with dry yeast extract and benzyladenine on growth and yield of manzanillo olive trees. *Res. J. Pharmaceut. Biol. Chem. Sci.* **2015**, *6*, 1573–1583. Available online: [https://www.rjpbcs.com/pdf/2015\\_6/\[233\].pdf](https://www.rjpbcs.com/pdf/2015_6/[233].pdf) (accessed on 14 January 2023).
55. Conde, C.S.; Delrot, G.H. Physiological, biochemical and molecular changes occurring during olive development and ripening. *J. Plant. Physiol.* **2008**, *165*, 1545–1562. [[CrossRef](#)] [[PubMed](#)]
56. Palese, A.M.; Pasquale, V.; Celano, G.; Figliuolo, G.; Masi, S.; Xiloyannis, C. Irrigation of olive groves in Southern Italy with treated municipal wastewater: Effects on microbiological quality of soil and fruits. *Agric. Ecosyst. Environ.* **2009**, *129*, 43–51. [[CrossRef](#)]
57. Rossignol-Castera, A. et Qualité des Aliments Oxydation et Rancissement des Matières Grasses; Conséquences sur la Qualité Nutritionnelle et la Durée de vie des Produits Alimentaires, ITERG- Expertise Corps Gras. 2006, p. 6. Available online: <https://docplayer.fr/12261075-Contexte-technologique-des-matieres-grasses-alimentaires-anne-rossignol-castera-directrice-developpement-iterg.html> (accessed on 14 January 2023).
58. Jimenez-Lopez, C.; Carpena, M.; Lourenço-Lopes, C.; Gallardo-Gomez, M.; Lorenzo, J.M.; Barba, F.J.; Prieto, M.A.; Simal-Gandara, J. Bioactive compounds and quality of extra virgin olive oil. *Foods.* **2020**, *9*, 1014. [[CrossRef](#)]
59. Tanouti, K.; Elamrani, A.; Serghini-Caid, H.; Khalid, A.; Bahetta, Y.; Benali, A.; Harkous, M.; Khiar, M. Caractérisation d'huiles d'olive produites dans des coopératives pilotes (lakrarma et kentine) au niveau du Maroc oriental. *Les Technol. De Lab.* **2010**, *5*, 18–26.
60. Houshia, O.J.; Zaid, O.; Shqair, H.; Zaid, M.; Fashafsheh, N.; Bzoor, R. Effect of olive oil adulteration on peroxide value, delta-k and on the acidity nabali-baladi olive oil quality. *Adv. Life. Sci.* **2014**, *4*, 235–244. Available online: <http://article.sapub.org/10.5923.j.als.20140405.04.html> (accessed on 14 January 2023).
61. Kouba, M.; Mourot, J. A review of nutritional effects on fat composition of animal products with special emphasis on n-3 poly-unsaturated fatty acids. *Biochimie* **2011**, *93*, 13–17. [[CrossRef](#)] [[PubMed](#)]
62. Bedbabis, S.; Clodoveo, M.L.; Ben Rouina, B.; Boukhris, M. Influence of irrigation with moderate saline water on Chemlali extra virgin olive oil composition and quality. *J. Food. Q.* **2010**, *33*, 228–247. [[CrossRef](#)]
63. Sdiri, W.; Dabbou, S.; Hechmi, C.; Selvaggini, R.; Servili, M.; Di Bella, G.; Ben Mansour, H. Quality characteristics and chemical evaluation of Chemlali olive oil produced under dairy wastewater irrigation. *Agric. Water. Manag.* **2020**, *236*, 106–124. [[CrossRef](#)]
64. Bedbabis, S.; Palese, A.M.; Ben Rouina, B.; Boukhris, M.; Rhouma, A.; Gargouri, K. The effect of irrigation by treated wastewater on 'Chemlali' olive oil quality. *J. Food. Qual.* **2009**, *32*, 141–157. [[CrossRef](#)]
65. Rousseaux, M.C.; Cherbiy-Hoffmann, S.U.; Hall, A.J.; Searles, P.S. Fatty acid composition of olive oil in response to fruit canopy position and artificial shading. *Sci. Hort.* **2020**, *271*, 109477. [[CrossRef](#)]
66. Romero-Trigueros, C.; Vivaldi, G.A.; Nicolás, E.; Paduano, A.; Pedrero, S.F.; Camposeo, S. Ripening indices, olive yield and oil quality in response to irrigation with saline reclaimed water and deficit strategies. *Front. Plant. Sci.* **2019**, *10*, 1–16. [[CrossRef](#)]
67. Tekaya, M.; Mechri, B.; Bchir, A.; Attia, F.; Cheheb, H.; Daassa, M.; Hammami, M. Effect of nutrient-based fertilizers of olive trees on olive oil quality. *J. Sci. Food. Agric.* **2013**, *93*, 2045–2052. [[CrossRef](#)]

68. Basheer, L.; Dag, A.; Yermiyahu, U.; Ben-Gal, A.; Zipori, I.; Kerem, Z. Effects of reclaimed wastewater irrigation and fertigation level on olive oil composition and quality. *J. Sci. Food. Agric.* **2019**, *99*, 6342–6349. [[CrossRef](#)]
69. Dag, A.; Erel, R.; Kerem, Z.; Ben-Gal, A.; Stern, N.; Bustan, A.; Zipori, I.; Yermiyahu, U. Effect of nitrogen availability on olive oil quality. *Acta. Hortic.* **2018**, *1199*, 465–470. [[CrossRef](#)]
70. Kesen, S.; Amanpour, A.; Sonmezdag, A.; Kelebek, H.; Selli, S. Effects of cultivar, maturity index and growing region on fatty acid composition of olive oils. *Euras. J. Food. Sci. Technol.* **2017**, *1*, 17–27. Available online: <https://dergipark.org.tr/en/download/article-file/483410> (accessed on 14 January 2023).
71. Bedbabis, S.; Rouina, B.B.; Mazzeo, A.; Ferrara, G. Irrigation with treated wastewater affected the minor components of virgin olive oil from cv. Chemlali in Tunisia. *Eur. Food. Res. Technol.* **2017**, *243*, 1887–1894. [[CrossRef](#)]
72. Plat, J.; Mensink, R.P. Plant stanol and sterol esters in the control of blood cholesterol levels: Mechanism and safety aspects. *Am. J. Cardiol.* **2005**, *96*, 15–22. [[CrossRef](#)]
73. Matos, L.C.; Cunha, S.C.; Amaral, J.S.; Pereira, J.A.; Andrade, P.B.; Seabra, R.M.; Oliveira, B.P.P. Chemometric characterization of three varietal olive oils (Cvs. *Cobrançosa*, *Madural* and *Verdeal Transmontana*) extracted from olives with different maturation indices. *Food. Chem.* **2007**, *102*, 406–414. [[CrossRef](#)]
74. Giuffrè, A.M.; Louadj, L. Influence of crop season and cultivar on sterol composition of monovarietal olive oils in Reggio Calabria (Italy). *Czech. J. Food. Sci.* **2013**, *31*, 256–263. [[CrossRef](#)]
75. Rebedo-Rodríguez, P.; González-Barreiro, C.; Cancho-Grande, B.; Valli, E.; Bendini, A.; Gallina Toschi, T.; Simal-Gandara, J. Characterization of virgin olive oils produced with autochthonous Galician varieties. *Food. Chem.* **2016**, *212*, 162–171. [[CrossRef](#)]
76. Konuskan, D.B.; Mungan, B. Effects of variety, maturation and growing region on chemical properties, fatty acid and sterol compositions of virgin olive oils. *J. Am. Oil. Chem. Soc.* **2016**, *93*, 1499–1508. [[CrossRef](#)]
77. Gutierrez, F.; Varona, I.; Albi, M.A. Relation of acidity and sensory quality with sterol content of olive oil from stored fruit. *J. Agric. Food. Chem.* **2000**, *48*, 1106–1110. [[CrossRef](#)]
78. Gracia-Gómez, M.S. Composicion quimica de distintas calidades de aceites de oliva virgen de la variedad ‘Empetre’ en el bajo Aragon. *Grasas. Aceites.* **2001**, *52*, 52–58. Available online: <https://fr.scribd.com/document/538821081/396-Article-Text-399-1-10-20071108> (accessed on 14 January 2023).
79. Sodeif, A.D.; Paresh, C.D. *Chapter 27-Phytosterol Classes in Olive Oils and their Analysis by Common Chromatographic Methods*; Victor, R., Ed.; Preedy, Ronald Ross Watson, olives and olive oil in health and disease prevention; Academic Press: Cambridge, MA, USA, 2010; pp. 249–257.
80. Gunstone, F. *vegetable Oils in Food Technology: Composition, Properties and Uses*, 2nd ed.; John Wiley and Sons: West Sussex, UK, 2011; pp. 243–272.
81. Sánchez Casas, J.; Osorio Bueno, E.; Montaña García, A.M.; Martínez Cano, M. Sterol and erythrodiol + uvaol content of virgin olive oils from cultivars of Extremadura (Spain). *Food. Chem.* **2004**, *87*, 225–230. [[CrossRef](#)]
82. Nick, K.; Andriana, C.K. *Effect of Fruit Maturity on Olive OIL phenolic Composition and Antioxidant Capacity*; Dimitrios, B., Ed.; Olive and olive oil bioactive constituents; Aocs Press: Urbana, IL, USA, 2015; pp. 123–145.
83. Rotondi, A.; Bendini, A.; Cerretani, L.; Mari, M.; Lercker, G.; Gallina Toschi, T. Effect of olive ripening degree on the oxidative stability and organoleptic properties of Cv. Nostrana di Brisighella extra virgin olive oil. *J. Agric. Food. Chem.* **2004**, *52*, 3649–3654. [[CrossRef](#)] [[PubMed](#)]
84. Gallina Toschi, T.; Cerretani, L.; Bendini, A.; Bonoli Carbognin, M.; Lercker, G. Oxidative stability and phenolic content of virgin olive oil: An analytical approach by traditional and high resolution techniques. *J. Sep. Sci.* **2005**, *28*, 859–870. [[CrossRef](#)]
85. Artajo, L.S.; Romero, M.P.; Tovar, M.J.; Motilva, M.J. Effect irrigation applied to olive trees (*Olea europaea* L.) on phenolic compound transfer during olive oil extraction. *Eur. J. Lipid. Sci. Technol.* **2006**, *108*, 19–27. [[CrossRef](#)]
86. Ben Ahmed, C.; Magdich, S.; Ben Rouina, B.; Boukhris, M.; Ben Abdul-lah, F. Saline water irrigation effects on soil salinity distribution and some physiological responses of field grown Chemlali olive. *J. Environ. Manage.* **2012**, *113*, 538–544. [[CrossRef](#)]
87. Tietel, Z.; Dag, A.; Yermiyahu, U.; Zipori, I.; Beiersdorf, I.; Krispin, S.; Ben-Gal, A. Irrigation-induced salinity affects olive oil quality and health-promoting properties. *J. Sci. Food. Agric.* **2018**, *99*, 1180–1189. Available online: <https://pubmed.ncbi.nlm.nih.gov/30047164/> (accessed on 14 January 2023). [[CrossRef](#)]
88. Rodis, P.S.; Karathanos, V.J.; Mantzavinou, A. Partitioning of olive oil antioxidants between oil and water phases. *J. Agric. Food. Chem.* **2002**, *50*, 596–601. [[CrossRef](#)] [[PubMed](#)]
89. 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](#)]
90. Odeh, L.H.; Musameh, S.; Abdelraziq, I.R. Influence of wastewater used in irrigation on the physical properties of olive oil in Palestine. *J. Material. Sci. Eng.* **2015**, *5*, 209–215. Available online: <https://www.hilarispublisher.com/open-access/influence-of-waste-water-used-in-irrigation-on-the-physical-properties-of-olive-oil-in-palestine-2169-0022-1000209.pdf> (accessed on 14 January 2023).
91. Zaanouni, N.; Gharssallaoui, M.; Eloussaief, M.; Gabsi, S. Heavy metals transfer in the olive tree and assessment of food contamination risk. *Environ. Sci. Pollut. Res.* **2018**, *25*, 18320–18331. [[CrossRef](#)]

92. Pogrzeba, M.; Rusinowski, S.; Sitko, K.; Krzyżak, J.; Skalska, A.; Małkowski, E.; Ciszek, D.; Werle, S.; McCalmont, J.P.; Mos, M.; et al. Relationships between soil parameters and physiological status of *Miscanthus × giganteus* cultivated on soil contaminated with trace elements under NPK fertilisation vs. microbial inoculation. *Environ. Pollut.* **2017**, *225*, 163–174. Available online: <https://pubmed.ncbi.nlm.nih.gov/28365513/> (accessed on 14 January 2023). [[CrossRef](#)]
93. Tang, G.; Zhang, X.; Qi, L.; Li, L.; Guo, J.; Zhong, H.; Liu, J.; Huang, J. Nitrogen and phosphorus fertilization increases the uptake of soil heavy metal pollutants by plant community. *Bull. Environ. Contam. Toxicol.* **2022**, *109*, 1059–1066. [[CrossRef](#)] [[PubMed](#)]
94. Singh, A.; Sharma, R.K.; Agrawal, M.; Marshall, F. Effects of wastewater irrigation on physicochemical properties of soil and availability of heavy metals in soil and vegetables. *Commun. Soil Sci. Plant Anal.* **2009**, *40*, 3469–3490. [[CrossRef](#)]
95. Chaoua, S.; Boussaa, S.; El Gharmali, A.; Boumezzough, A. Impact of irrigation with wastewater on accumulation of heavy metals in soil and crops in the region of Marrakech in morocco. *J. Saudi Soc. Agric. Sci.* **2018**, *18*, 429–436. [[CrossRef](#)]

**Disclaimer/Publisher’s Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.