

## Article

# Chemical Characteristics of Two-Phase Olive-Mill Waste and Evaluation of Their Direct Soil Application in Humid Mediterranean Regions

Maja Podgornik <sup>1,\*</sup>, Milena Bučar-Miklavčič <sup>1</sup>, Alenka Levart <sup>2</sup>, Janez Salobir <sup>2</sup>, Vida Rezar <sup>2</sup> and Bojan Butinar <sup>1</sup> 

<sup>1</sup> Institute for Oliveculture, Science and Research Centre Koper, Garibaldijeva 1, 6000 Koper, Slovenia; milena.bucarmiklavcic@zrs-kp.si (M.B.-M.); bojan.butinar@zrs-kp.si (B.B.)

<sup>2</sup> Department of Animal Science, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana, Slovenia; alenka.levart@bf.uni-lj.si (A.L.); janez.salobir@bf.uni-lj.si (J.S.); vida.rezar@bf.uni-lj.si (V.R.),

\* Correspondence: maja.podgornik@zrs-kp.si

**Abstract:** Over the last decade, the two-phase centrifugation system for olive-oil extraction has become dominant in Slovenia. There are many suggestions for the exploitation of two-phase OMW, but among the suggested methods, direct spreading on agricultural land appears to be operationally simple and economically feasible for Slovenia. As there is little information available about two-phase OMW produced in the northern Mediterranean regions, the aim was to determine its composition and evaluate its use as a soil amendment in olive groves. This study shows that the characteristics of two-phase olive-mill waste produced in northern Mediterranean regions are similar to those of other countries. In addition, the calcareous characteristics of the Mediterranean soil can reduce its phytotoxic effects and might thus represent a natural system for olive-mill waste treatment. Phenolic compounds in the two-phase olive-mill waste are rapidly decomposed, and the soil has a high buffering capacity. Furthermore, the results of the soil analysis also showed some effects on the soil properties, such as a significant increase in K<sub>2</sub>O and soil organic carbon. The combined application of two-phase olive-mill waste and mineral fertilizer to olive groves on eutric cambisols has positive effects on the physical, chemical and biochemical properties of the soil.

**Keywords:** two-phase olive-mill waste; wet pomace; soil amendment; organic matter; phenolic compounds



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## 1. Introduction

Olive-oil production and the associated industry are very important in southern European countries and in the Near East and North Africa, where olive (*Olea europaea* L.) cultivation is a centuries-old tradition [1]. Due to climate change and new climatic conditions, northern Mediterranean countries can now become important oil producers. Slovenia is one of the most northern parts of the Mediterranean basin where olives can still be grown [2].

Over the last decade, the two-phase centrifugation system for olive-oil extraction has become dominant in Slovenia, where approximately 80% of olive mills use this technology. This system was developed during the early 1990s to minimize olive-mill wastewater (OMWW), which comprises the olive vegetation water plus water added in the different steps of olive production, and it also serves to reduce phenol leaching [3]. This technology is also known as an 'ecological system' because it greatly reduces the quantities of OMWW produced and the consequent land contamination. Indeed, OMWW has for many years been the most polluting and troublesome waste product of olive mills throughout the Mediterranean countries [1,4].

This two-phase olive-oil extraction technology generates a liquid phase (i.e., the olive oil) and an organic slurry (i.e., the two-phase OMW) [5]. Two-phase OMW has very different characteristics compared to olive pomace from the traditional olive presses and the three-phase systems [1]. Two-phase OMW is also known as “alperujo”, olive wet husk, olive wet pomace, and olive wet cake, and it is a semi-solid waste with a strong odor and a doughy texture, which makes its management and transport difficult. The olive vegetation water (i.e., an OMW of the three-phase system) is here included in the two-phase OMW, which represents the greatest problem for its exploitation because of its high moisture content (65–70%). Thus, this residue has become a serious problem for olive mills because its management requires specific facilities (e.g., storage tanks with special valves, mass pumps, and tank trucks) [4]. Furthermore, the high water content of two-phase OMW makes the extraction of olive pomace oil more expensive [6].

There have been many suggestions for disposal or exploitation of OMW, but there are also many factors that need to be considered when selecting the best method; for instance, the total amount of waste, the investment required for any treatment, the available land, the industrial and/or agronomic environment, and the local laws. To date, there remains no unique method; indeed, any method used will depend on the specific needs of the local area [4].

Among the suggested methods for exploitation and recycling of two-phase OMW that have been proposed (e.g., drying and solvent extraction of residual oil, biogas production, composting) reviewed by [4], direct spreading on agricultural land appears to be operationally simple and economically feasible for Slovenia. Two-phase OMW has a high organic matter content (i.e., 90% of the dry matter), and therefore it has been proposed as a way to improve the characteristics of Mediterranean soil, which has low levels of organic matter and is exposed to progressive degradation processes [1,4]. In the Mediterranean zone, soils are usually much shallower than in the humid tropics and in the temperate zone, where pedogenesis is faster and erosion less ancient. Shallow soils with low nutrients and water storage capacity are a major constraint to natural vegetation and crop cover, which in turn affords weak protection to soils from water and wind erosion [7]. Also, for organic farming systems, the application of two-phase OMW to soil represents an interesting option that also ‘closes’ the cycle of residue resources [4]. In addition, two-phase OMW is practically free of heavy metals and pathogenic microorganisms, in contrast to other organic residues, such as sewage sludge [5]. However, although several studies have shown that two-phase OMW is less phytotoxic than OMWW, [1,8] reported that its application to the soil might have negative agronomic and environmental impacts. This arises from its acid pH and high content of compounds that are potentially phytotoxic (e.g., inhibition of plant growth) and antimicrobial (e.g., effects on soil bacteria), such as phenols, tannins, and fatty acids.

This is in contrast to a multi-year study by [6], who demonstrated that two-phase OMW as a soil amendment might be of great agronomic interest as it can lead to increased vegetative and productive olive trees. No long-term consequences were reported for the chemical and microbiological characteristics of the soil. Indeed, in some European countries such as Italy and Portugal, national legislation allows the spreading of specified amounts of two-phase OMW on the soil surface. However, the chemical composition of two-phase OMW is very variable. It depends upon the variety of the olive trees, the fruit ripening at harvest, and above all, the processing method, which strongly influences its water content [9]. Moreover, soils from different origins can have different intrinsic buffering capacities, and therefore they will respond differently to such applied perturbants [10].

Considering that there is little information available about two-phase OMW produced in northern Mediterranean regions, the aim of the present study was to determine the composition of samples from different olive mills in Slovenia and to correlate their characteristics with the main chemical characteristics that have been reported previously. In addition, the potential use of two-phase OMW as a soil amendment in north Mediterranean olive groves was also investigated. On the basis of the two-phase OMW characteristics

and its effects on the soil, new legislation for the olive-oil extraction industry needs to be implemented.

## 2. Materials and Methods

### 2.1. Sampling of the Two-Phase Olive-Mill Waste

To characterize two-phase OMW, 14 samples were taken from 7 two-phase olive mills in Slovenian Istria during the olive harvest in 2016 and 2017. Fresh samples were collected immediately after the extraction of the olive oil and transported directly to the laboratory. Each of these was divided into two sub-samples.

The first half of these two-phase OMW samples were air-dried and homogenized. They were then used to determine their main characteristics based on the Official Methods of analysis of AOAC International [11]. These included the dry matter (934.01), total nitrogen and crude protein (984.13), fat (920.39), and the levels of the mineral nutrients phosphorous (965.17), potassium (985.35), calcium, iron, and copper (968.08). The numbers in the parentheses indicate the method number from [10].

The second half of these two-phase OMW samples were lyophilized (Alpha 1-2/LD; Martin Christ, Osterode am Harz, Germany) for 3 days at  $-60\text{ }^{\circ}\text{C}$  using a chamber pressure of 0.110 mbar. The lyophilized samples were kept deep-frozen ( $-80\text{ }^{\circ}\text{C}$ ) until later HPLC analysis for the phenols. Before the HPLC analysis, extraction solvent was added (80:20, v v-1; methanol: water). The supernatants from these extracted samples were concentrated using a reduced pressure rotary evaporator and analyzed for their phenolic compounds. The modified International Olive Council (IOC) method [12] with diode array (G4212B; 190–600 nm) and triple quadrupole electrospray ionization detection was used for the HPLC analysis (Infinity 1260; Agilent Technologies, Waldbronn, Germany). The HPLC system included the binary pump (G1312B), degasser (G4225), autosampler (G1329B), thermostat (G1330B), and thermostated column compartment (G1316A) and was interfaced with a triple-quadrupole mass spectrometer (Triple Quad G6420A LC-MS; Agilent Technologies Singapore International, Singapore). The chromatographic column used (Synergi 4  $\mu\text{m}$  Hydro-RP 80 A;  $250 \times 4.6\text{ mm}$ ) was protected with a guard column (Security Guard Cartridge AQC;  $184 \times 3.0\text{ mm}$ ; both from Phenomenex, Torrance, CA, USA). The gradient was as proposed by the IOC, with phosphoric acid substituted with 0.1% formic acid as the aqueous phase. The mass spectrometry was operated in negative ion mode, as follows: gas temperature,  $300\text{ }^{\circ}\text{C}$ ; gas flow,  $10\text{ L min}^{-1}$ ; nebulizer pressure, 50 psi; and capillary voltage, 3 kV.

### 2.2. Experimental Site and Soil Amendments

This study was carried out across four different nonirrigated production olive groves on Cambisol (Eutric) in Slovenian Istria, with an overall plantation density of 300 plants  $\text{ha}^{-1}$  (trees spaced at  $6\text{ m} \times 5\text{ m}$ ). The soil texture at the sampling sites was loam with about 22–25% clay, 45–47% silt, and 32–35% sand. The pruning and pest-management practices carried out in these experimental olive groves were the same. All of the agronomic treatments were carried out according to the standard agricultural practices of the area.

Southwestern Slovenia, where Slovenian Istria is located, has a sub-Mediterranean climate. This region is characterized by sunny days (up to  $2350\text{ h year}^{-1}$ ), with daily mean temperatures between  $4.1\text{ }^{\circ}\text{C}$  in winter (January) and  $22.5\text{ }^{\circ}\text{C}$  in summer (July). The temperature is  $>30\text{ }^{\circ}\text{C}$  for a mean of  $29.3\text{ days year}^{-1}$  [13]. The mean annual reference evapotranspiration is 1035 mm, which represents the loss of water to the atmosphere by evaporation and transpiration from a reference crop, usually given as well-watered grass. The precipitation regime is sub-Mediterranean (annual 20-year mean, 1991–2010: 953 mm) [14,15]. Each grove represented one replication with two treatments. Therefore, 8 plots with 10 olive trees each and the vegetative and productive characteristics representative of the entire area were selected; 4 were amended with  $80\text{ m}^3\text{ ha}^{-1}$  of two-phase OMW, while the other 4 served as the control.

The two-phase OMW was obtained from the two-phase oil extraction systems in November and then directly spread on the soil surface (in November 2016, November 2017). The soil surface was covered with a natural green cover, and thus the two-phase OMW was not incorporated into the soil. Each year, 4 months after the amendment, 300 kg ha<sup>-1</sup> mineral fertilizer (NPK 15:15:15) was spread on the control (not two-phase OMW treated) and treated plots.

### 2.3. Soil Analysis

To determine the differences between the soil of the control and amended (experimental) plots, fresh soil samples were collected three times (21 March 2017, 30 November 2017, and 30 March 2018) after the two-phase OMW application was performed. Each soil sample was collected as sub-samples (10 sub-samples) that were randomly collected from the topsoil (10–20 cm deep) from each plot after removing the superficial vegetation and any amendment. These randomly collected sub-samples were mixed to form one sample per plot. Before analysis, the soil samples were air-dried.

For each soil sample from each plot, measurements were taken for pH, K (K<sub>2</sub>O), total organic carbon (TOC), and total nitrogen (TN), and the C/N ratios were calculated. Soil pH was measured for soil suspensions in 0.01 M KCl [16]. The K<sub>2</sub>O was determined using the ammonium-lactate (AL) extraction method of [17] and the molybdenum blue method of [18]. TOC was determined using sulfochromic oxidation without external heating and using titration with 0.5 M FeSO<sub>4</sub>, following the Walkley-Black method [19]. TN was determined using the method from [20,21].

### 2.4. Statistical Analysis

All of the statistical analyses were performed using the statistical software package R, which is a free software environment for statistical computing and graphics. One-way ANOVA was used for comparisons of the mean effects on soil properties between the control and treated plots. When ANOVA showed statistically significant differences between treatments, Duncan's multiple comparison tests were used to examine the differences between treatments' means.

## 3. Results and Discussion

### 3.1. Main Characteristics of the Two-Phase Olive-Mill Waste

The two-phase OMW had a moisture content of 68.7% (Table 1), which was higher than that of two-phase OMW from different provinces in Spain (64.0%) [1] and particularly lower than that of two-phase OMW collected from new generation two-phase centrifugation olive oil mills in Italy (79%) [8].

**Table 1.** Main characteristics and components of the two-phase olive-mill waste.

Parameter	Units	Mean	Range	Coefficient of Variation (%)
Moisture	% FW	68.7	52.1–77.3	11.80
Total nitrogen	g kg <sup>-1</sup> DW	10.1	7.5–14.4	23.02
P	g kg <sup>-1</sup> DW	1.4	0.4–2.0	37.14
K	g kg <sup>-1</sup> DW	19.4	4.8–23.7	37.90
Ca	g kg <sup>-1</sup> DW	1.9	1.4–2.7	18.72
Fe	mg kg <sup>-1</sup> DW	104.6	60.3–163.1	28.94
Cu	mg kg <sup>-1</sup> DW	14.6	8.2–21.4	22.94
Protein	g kg <sup>-1</sup> DW	63.0	47.0–90.2	20.89
Hemicellulose	g kg <sup>-1</sup> DW	141.5	109.1–173.8	18.74
Fat	g kg <sup>-1</sup> DW	158.1	105.5–229.1	24.54

FW, fresh weight; DW, dry weight.

Some small differences were also seen for the two-phase OMW K content of  $19.4 \text{ g kg}^{-1}$  dry weight (DW) here ( $19.8 \text{ g kg}^{-1}$  DW, [1];  $17.0 \text{ g kg}^{-1}$  DW, [8]). However, while these two-phase OMW samples were rich in K, they were poor in P ( $1.4 \text{ g kg}^{-1}$  DW), which is a common characteristic of two-phase OMW from different countries [1,8]. The Fe content of  $104.6 \text{ mg kg}^{-1}$  DW here was low compared to other studies where it showed mean values between 526 and  $769 \text{ mg kg}^{-1}$  DW [1,22,23]. These other studies also showed that the main micronutrient in two-phase OMW was Fe, while Cu, Mn, and Zn showed similar mean contents that were lower than for Fe. Similarly low levels of Cu were seen in the present study ( $8.20\text{--}21.39 \text{ mg kg}^{-1}$  DW). The TN was calculated according to the Kjeldahl method, and these data agree with those of [1,8,22,23], where the TN content ranged from  $10.0$  to  $16.0 \text{ g kg}^{-1}$  DW.

As well as the high moisture content (55.6–74.5%), according to [1], the organic fraction of two-phase OMW contained lignin ( $323.0\text{--}556.5 \text{ g kg}^{-1}$  DW), hemicellulose ( $273.0\text{--}415.8 \text{ g kg}^{-1}$  DW), cellulose ( $140.2\text{--}249.0 \text{ g kg}^{-1}$  DW), carbohydrates soluble in water ( $12.9\text{--}164.0 \text{ g kg}^{-1}$  DW), fat ( $77.5\text{--}194.6 \text{ g kg}^{-1}$  DW), protein ( $43.8\text{--}115.0 \text{ g kg}^{-1}$  DW), and phenols ( $6.2\text{--}23.9 \text{ g kg}^{-1}$  DW). In the current study, the hemicellulose, fat, protein, and phenols were analyzed. These data are given in Tables 1 and 2.

**Table 2.** Main phenolic compounds in the two-phase olive-mill waste and the olive grove soil. All data are expressed using the response factor for tyrosol at 280 nm. Phenolic compounds are assigned based on mass spectrometry (MS) and/or chemical standards (CS).

Phenolic Group	Phenolic Compound	Assigned by	Two-Phase Olive-Mill Waste			Soil		
			Mean ( $\text{mg kg}^{-1}$ )	Range ( $\text{mg kg}^{-1}$ )	CV (%)	Mean ( $\text{mg kg}^{-1}$ )	Range ( $\text{mg kg}^{-1}$ )	CV (%)
Phenyl alcohols	Hydroxytyrosol glucoside	MS	438	135–973	0.72	0.00	0.00	0.00
	Hydroxytyrosol	CS, MS	3203	148–8505	0.87	1.25	0.00–4.00	1.51
	Tyrosol glucoside	MS	314	205–466	0.36	0.75	0.00–2.00	1.28
	Tyrosol	CS, MS	532	176–963	0.59	0.25	0.00–1.00	2.00
Phenylethanoid glycoside	Verbascoside	CS, MS	2434	84–8370	1.16	0.25	0.00–1.00	2.00
Flavonoids	Luteolin-7-O-glucoside	CS, MS	338	93–901	0.73	0.00	0.00	0.00
	Luteolin	CS, MS	710	310–1368	0.48	0.00	0.00	0.00
	Apigenin	CS, MS	107	55–184	0.36	3.25	3.00–4.00	0.15
Total assigned phenolic compounds		CS, MS	6769	1388–12,399	0.68	5.50	3.00–8.00	0.43
Total unassigned phenolic compounds		–	12,523	3327–25,393	0.52	404	148–760	0.66
Total phenolic compounds		–	17,600	5409–36,073	0.61	459	156–767	0.66

DW, dry weight; CV, coefficient of variation.

### 3.2. Phenolic Composition of the Two-Phase Olive-Mill Waste

The phenolic composition of the two-phase OMW depends on several factors, including the olive variety and origin, climate conditions, fruit maturation, cultivation practices, and the olive-oil extraction process [24]. The present data show that hydroxytyrosol was the major phenolic compound identified in this two-phase OMW ( $148\text{--}8505 \text{ mg kg}^{-1}$  DW). Apart from hydroxytyrosol, there were also relevant levels of verbascoside ( $84\text{--}8370 \text{ mg kg}^{-1}$  DW) (Table 2). The other phenolics identified and quantified at lower concentrations were hydroxytyrosol glucoside, tyrosol glucoside, luteolin-7-O-glucoside, luteolin, and apigenin (Table 2). These data are in partial agreement with the data of [25,26], who reported that hydroxytyrosol is the main phenolic compound in olive pomace, with concentrations between  $1624$  and  $2872 \text{ mg kg}^{-1}$  DW. The differences in verbascoside levels in two-phase OMW between the present study and other studies might be attributed to the ripening stage of the olives and to the different amounts of original vegetation water in the two-phase OMW. A

study by [27] investigated the phenolic composition in olives in terms of the ripening time, and they detected verbascoside only in the earlier maturation stages, and [28] reported that species that show high levels of oleuropein have minimum levels of verbascoside, although its concentration during fruit maturation increases steadily [29]. The present study also indicated that there was verbascoside in all parts of the olive trees (i.e., olives, leaves, stems) and in the vegetation water (our unpublished data).

Considering that the main changes in the soil chemistry and microbiology are likely to occur during the first weeks after the application of organic amendments [30], the phenol levels in the soils where the two-phase OMW was applied were also determined after 1 week. The content of total phenols in the two-phase OMW (5409–36,073 mg kg<sup>-1</sup> DW) was considerably higher than that of the 1-week-amended soil (156–767 mg kg<sup>-1</sup> DW). Thus, differences were seen in the phenolic composition of the two-phase OMW and the amended soils (Table 2). Among the assigned phenolic compounds, hydroxytyrosol and verbascoside were the major constituents in the two-phase OMW, while apigenin and hydroxytyrosol were the major constituents in the amended soils. The differences in the phenolic compositions and contents between the two-phase OMW and the amended soils might be attributed to the soil's environmental conditions. Environmental factors, such as soil pH, temperature, oxygen, and substrate, can affect the degradation of phenols [10,31]. A study by [31] reported that there were positive relationships between the phenolic degradation enzyme activities and soil pH across their ecosystem. Fungi (e.g., Basidiomycetes, Ascomycetes) and bacteria (e.g., Pseudomonas) released extracellular enzymes into the soil that broke down the phenolic compounds. These enzymes can cause nonspecific oxidation of phenolic compounds, consuming oxygen and hydrogen peroxide as the electron acceptors, respectively [31].

Furthermore, the amended soil was a eutric cambisol, which is characterized by high saturation of alkaline cations (e.g., K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>). A study by [32] reported that the addition of Ca(OH)<sub>2</sub> to two-phase OMW, which had a pH of 4–6, resulted in the formation of alkaline secondary waste (i.e., alkaline two-phase OMW). These pH changes favor alkali tolerant and alkaliphilic bacteria that have some degree of halophilicity. The majority of halotolerant alkaliphile and alkali tolerant and/or halotolerant bacteria isolated from alkaline two-phase OMW effectively used the phenolic compounds as their sole carbon source [32]. On this basis, and also considering that climatic conditions such as temperature, warming, and drought might affect degradation in soils, the direct application of raw two-phase OMW in humid Mediterranean regions should be carried out according to the characteristics of the soils and the environmental conditions. However, more investigations of the relationships between the application of two-phase OMW and the soil bacteria need to be carried out to further estimate the impact of such two-phase OMW applications on the soil bacteria and phenolics content.

### 3.3. Effects of Two-Phase Olive-Mill Waste Application on the Soil Properties

Previous studies have indicated that the low N content in two-phase OMW can cause a great nutrition imbalance in soils. This has been reflected in a high C/N ratio [33] and results in strong competition between plants and microorganisms for the available N. Therefore, [34] recommended the use of two-phase OMW soil amendment together with an N fertilizer. In the present study, the effects of the combined application of two-phase OMW and a mineral fertilizer (NPK 15:15:15) (two-phase OMW+NPK) were evaluated to explore the potential benefits of such combined applications.

Based on the results of the soil analysis of the three soil samples taken during the application of two-phase OMW+NPK (21 March 2017, 9 November 2017, 30 March 2018), some effects on the soil properties were observed (Table 3). After the second season of application of two-phase OMW+NPK to the soil (30 March 2018), significant beneficial changes were seen going from the control plots (NPK [15:15:15] mineral fertilized only) to those with two-phase OMW+NPK, particularly for TOC (2.5% vs. 3.6%;  $p < 0.05$ ) and the C/N ratio (10.2 vs. 13.0;  $p < 0.05$ ) (Table 3). These data are consistent with previous

studies that indicated that two-phase OMW and de-oiled two-phase OMW applications significantly increased soil organic carbon [5]. The increase in soil organic carbon (i.e., organic matter) as a result of the application of increasing amounts of wet pomace was shown to have effects on the soil structural stability and to improve its water-holding at field capacity and wilting point [35].

**Table 3.** Short-term effects on soil properties after application with the two-phase olive-mill waste.

Parameter	Date of Measure	Control	Two-Phase Olive-Mill Waste
Total organic carbon (%)	21 March 2017	2.0 ± 0.42 c	3.1 ± 0.84 ab
	9 November 2017	2.3 ± 0.39 bc	2.4 ± 0.42 bc
	30 March 2018	2.5 ± 0.26 bc	3.6 ± 1.02 a
Total nitrogen (%)	21 March 2017	0.19 ± 0.04 a	0.29 ± 0.12 a
	9 November 2017	0.21 ± 0.04 a	0.23 ± 0.04 a
	30 March 2018	0.23 ± 0.02 a	0.28 ± 0.09 a
Carbon/ nitrogen ratio	21 March 2017	11.0 ± 0.82 b	11.5 ± 1.73 ab
	9 November 2017	10.7 ± 0.96 b	10.7 ± 0.50 b
	30 March 2018	10.2 ± 1.26 b	13.0 ± 0.82 a
pH	21 March 2017	7.4 ± 0.05 a	7.3 ± 0.11 a
	9 November 2017	7.3 ± 0.06 ab	7.1 ± 0.19 b
	30 March 2018	7.2 ± 0.13 b	7.1 ± 0.07 b
K <sub>2</sub> O (mg [100 g] <sup>-1</sup> )	21 March 2017	30.1 ± 5.78 b	61.0 ± 25.06 a
	9 November 2017	26.8 ± 3.33 b	49.1 ± 24.11 ab
	30 March 2018	33.5 ± 13.92 b	72.9 ± 23.96 a

Data are means ± standard deviation ( $n = 4$ ; Duncan's multiple comparison tests). Data with different letters within a soil parameter are significantly different ( $p < 0.05$ ).

The higher TOC in the soils treated with two-phase OMW+NPK indicated that decomposition rates of the organic carbon were low and remained lower than the rates of the annual applications. As a result of these low decomposition rates, the C/N ratio increased overall from 11.5 to 13.0 through all of these applications here (Table 3). However, it is commonly assumed that materials with a C/N ratio >15 lead to initial N immobilization, whereas N mineralization should be conserved with a C/N ratio <15, providing greater mineralization for the lowest C/N ratios [36]. Furthermore, compared to the control plots, there were no significant differences for the TN where two-phase OMW+NPK were applied (0.23% vs. 0.28%;  $p > 0.05$ ) (Table 3). This reflects the relatively low content of N in the two-phase OMW here (10.1 g kg<sup>-1</sup> DW) (Table 1). Different effects on soils were seen by [5], who reported that two-phase OMW and de-oiled two-phase OMW applications led to significant linear increases in total N in the soil after 2 years of continued applications. A study by [37] showed that organic N in olive-mill pomace was particularly resistant to mineralization and was therefore retained in the soil. Two-phase OMW is rich in phenolic compounds that can disturb the N mineralization processes by direct harmful effects on the soil microbial biomass or indirectly due to their binding capacity [38].

A significant increase in K<sub>2</sub>O was seen for two-phase OMW addition (Table 3). Throughout the whole study, some significant differences were seen for K<sub>2</sub>O between the control and treated plots. Similar effects of two-phase OMW on the soil were reported by [5,6] who investigated solid OMW application to soils and the effects of two-phase OMW application on olive grove production and soil properties, respectively. Both of these studies showed that the available P and K increased in the soils treated with solid OMW and two-phase OMW.

However, further studies in deeper layers (20–50 cm) with many samples and over several years under these growing conditions are needed to evaluate more accurately the direct soil application of two-phase OMW to soils in humid Mediterranean regions.

Although the soil pH showed small but significant decreases during this study, this was not significantly affected by the addition of the two-phase OMW. The decreased pH values might be explained in terms of organic matter decomposition and the formation of organic acids in the initial stages of decomposition [39]. This could be beneficial in the upper alkaline horizons of soils on soft carbonate rocks (flysch, marl, limestone), where pH can also exceed 7.2, which negatively affects the uptake of certain plant nutrients.

#### 4. Conclusions

This study initially showed that the characteristics of the two-phase OMW produced in northern Mediterranean regions are similar to those in other countries. This two-phase OMW was rich in K and poor in P and micronutrients and contained intermediate levels of N. It had a high moisture content and a slightly acidic pH. The contents of the total phenols were a little higher than those reported in other studies.

We have also shown here that the calcareous characteristics of the soil can reduce any phytotoxic effects, but only if the direct application of two-phase OMW is limited to a maximum of  $80 \text{ m}^3 \text{ ha}^{-1}$ . Applications of larger amounts of two-phase OMW (more than  $80 \text{ m}^3 \text{ ha}^{-1}$ ) to the soil could have negative agronomic and environmental impacts. This arises from its acid pH and high content of compounds that are potentially phytotoxic (e.g., inhibition of plant growth) and antimicrobial (e.g., effects on soil bacteria), such as phenols, tannins, and fatty acids.

The calcareous characteristics of the soil thus represent a natural system for treatment of this two-phase OMW, as the phenolic compounds are rapidly decomposed, and the soil has a high buffering capacity. Therefore, direct land spreading of two-phase OMW can be considered an environment-friendly practice to exploit and recycle such organic materials, even though two-phase OMW is considered a serious environmental issue due to the heavy load of phenolic compounds and organic acids. However, we also recommend that after direct land spreading of two-phase OMW, the soil characteristics and contents of total phenolics are regularly monitored.

These data also indicated that the combined application of two-phase OMW and a mineral fertilizer (here as NPK 15:15:15) to olive groves on eutric cambisols has positive effects on the physical, chemical, and biochemical properties of the soil, due to the high organic matter content. Further studies are necessary to define the best application rates of such two-phase OMW and mineral fertilizers to avoid excess accumulation of K in the soil and to estimate the continued application of two-phase OMW to prevent potential environmental pollution.

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