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Influence of geographical origin, harvesting time and processing system on the characteristics of olive-mill wastewater: A step toward reducing the environmental impact of the olive oil sector

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ABSTRACT

The olive oil production sector generates considerable quantities of liquid by-products (olive-mill wastewater, OMWW) which, if left untreated, can cause detrimental environmental impacts. This study aimed to investigate the influence of the Lebanese geographical origin, harvesting time and processing system and their interactions on the chemical and biological characteristics of these by-products. The results showed that the interaction of all the three studied factors on the chemical composition of OMWW, were highly significant ($p < 0.001$). Furthermore, all the OMWW could be considered as a rich source of natural phenolic and antioxidant compounds. The average Total Phenolic Content in OMWW samples was 807.56 GAE/ kg with significantly higher values for OMWW obtained from the North (1027.7 GAE/ kg) at early harvest (1024.00 GAE/ kg) and using press system (1036.13 GAE/ kg) as compared to the other samples from the South, intermediate and late harvesting time using 3-phases and Sinolea ($p < 0.05$). The overall results of this study shed light on the need to establish a sustainable means of treating the liquid effluents of olive mills. The results can help prioritize the regions in most need for a treatment system based on the values reported, while redirecting our attention to the possibility of valorizing the phenolic contents as potent raw material rather than pollutants, in an aim to enhance sustainable agriculture and help achieve (sustainable development goal) SDG6 for improved water quality by 2030.

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1. Introduction

Several studies have supported the hypothesis that the Mediterranean diet is as an efficient way to boost the immunity and prevent several non-communicable diseases as well as a potent factor to combat the severe cases of COVID-19 (Issa

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and Doumit, 2023; Finicelli et al., 2022; Serra et al., 2022; Perez-Araluce et al., 2022). Consequently, the consumption of olive oil, the main type of fats in the Mediterranean diet, has increased steadily, and the production has grown by approximately 40% in the last decade (Dermeche et al., 2013). This increase is not only based on the attributed health benefits and nutritional properties, but also on its unique and distinct organoleptic characteristics (El Riachy et al., 2011). However, olive oil production results in significant amounts of polluting by-products, in the olive mill wastewater (OMWW) released, which their management is both an environmental and an economic challenge (Christoforou and Fokaides, 2016). Although Lebanon is rich in its natural water resources, however, sustainable management of this resource is a pressing matter in order to achieve SDG 6 (sustainable development goal 6) by 2030 which aims to reduce water pollution and improve its quality. Additionally, the OMWW generated is characterized by its high content of phenolic compounds. While these compounds may pose environmental concerns if left untreated, they also possess potent antioxidant properties that can be exploited through various extraction and valorization methods. The generated by-products amount, and composition differ according to the olive oil processing system applied. Olive oil processing includes four main steps: the washing and defoliation step followed by crushing and malaxation, then separation of the oil from the solid pomace, and finally separating the oil from the liquid portion. The first two steps and the last one are common among all processing systems. However, the third step, which is separation of the oil from the solid pomace, differs between the traditional press, the modern horizontal centrifugation system that could be either 2-phase or 3-phase systems, percolation or the supercritical fluid extraction (Sciancalepore et al., 2000; Souilem et al., 2017). The press and the 3-phases systems generate a dry solid (pomace) and liquid by-product (olive-mill wastewater, OMWW) (Kapellakis et al., 2008). The 2-phases system produces a pomace with high moisture content, sometimes known as 'margion' (Berbel and Posadillo, 2018). The pomace is composed of skins, pulp residues, fragments of olive stones and fractions of the oil called secondary oil; it constitutes on average a third of the weight of the fresh olives crushed (M'Sadak et al., 2016). The OMWW is a viscous liquid effluent having a blackish brown color and a foul odor. It is the combination of the water present in the olive cells, the washing waters and those used in the treatment process (Elhag et al., 2017). The processing of one ton of olives produces about 1.5 tons of OMWW with slight variations depending on the specific processing technique employed (Gargouri et al., 2013).

The polluting effect of these by-products varied based on the chemical composition, including the phenolic compounds, its high chemical (COD) and biochemical oxygen (BOD) demand (Capasso et al., 1992). The management of OMWW has varied over time and across regions, with some olive oil producers resorting to environmentally harmful practices such as releasing it into evaporation ponds or directly into streams or sewage systems. However, these methods are widely recognized as polluting and are prohibited in many countries due to the negative impacts they cause (Sobhi et al., 2007). OMWW release into water bodies can lead to an increase in the phosphorous content and a reduction in the clarity of the water due to its dark color (Sobhi et al., 2007; Elhag et al., 2017). Moreover, its release into open stagnant water bodies also increased the rate of anaerobic fermentation of its organic compounds which produces methane and other harmful products associated with a bad smell (Arvaniti et al., 2012). Some farmers resorted to using untreated OMWW as an organic fertilizer. However, this method also showed a negative impact on the soil with undesirable abscission and seed germination inhibition (Arvaniti et al., 2012) since it is high in mineral content, has a low pH and a phytotoxic effect that is mainly linked to its high phenolic compounds content (Roig et al., 2006; Hanafi et al., 2013).

Consequently, different methods, including various physical, physicochemical and biological techniques, have been used to treat the OMWW before their release into the environment, in order to prevent environmental degradation (Bettazzi et al., 2006; Hashwa and Mhanna, 2008; Arvaniti et al., 2012). Choosing one waste management approach over another for OMWW can be challenging due to the significant variability in its chemical composition. Factors such as olive fruit maturity (Reboredo-Rodríguez et al., 2020; Diarte et al., 2021), processing system employed (Torrecilla, 2010; Souilem et al., 2017), and the type of solvent used for oil extraction, if applicable, as well as the geographical origin (Leouifoudi et al., 2014) can all influence the composition of OMWW. As a result, determining the most suitable waste management method for OMWW becomes complex, considering the multifaceted nature of these parameters (Kalderis and Diamadopoulos, 2010).

Accordingly, the physico-chemical characterization of olive oil by-products based on various processing variables is an important task for understanding, predicting, and evaluating effective, justifiable, and sustainable waste management strategies. This work aimed at studying the effects of geographical origin, harvesting time and processing system and their interaction on the chemical composition of the olive-mill wastewater released in different regions of Lebanon, with a special focus on the phenolic content of OMWW and its antioxidant capacity in order to suggest effective methods for their safe release in the environment, based on multifactorial assessment.

2. Materials and methods

2.1. Materials

Boric acid, sodium hydroxide, ammonium vanadate, Folin-Ciocalteu reagent (2 M), powdered 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid (3,4,5-trihydroxybenzoic acid), powdered sodium carbonate (Na_2CO_3), *n*-hexane (HPLC grade), ethyl acetate, and methanol were purchased from Sigma Chemical Co. (St. Louis, MO, USA); sulfuric acid (H_2SO_4), hydrochloric acid (HCl), phosphoric acid (H_3PO_4), ammonium molybdate and lanthanum chloride from Analar (VWR

Table 1
Repartition of the samples according to their origin and to the processing systems.

Region	Rainfall	Altitude	Latitude	Longitude	Processing system	OMWW
Hasbaya	650 mm	650–1050 m	33.3833°	35.6833°	Press	9
					Sinolea ^a	9
					3-phases	9
					Press	9
Akkar	850 mm	300–700 m	34.5506°	36.0781°	3-phases	9
Total number of samples						45

^a Sinolea coupled to 3-phases decanter.

international, Leuven, Belgium); diphenylamine and potassium and calcium standards from Merck (Darmstadt, Germany); potassium dichromate from Acros organic (New Jersey, USA); and, Mohr salt from Uni-Chem, (Belgrade, Serbia). All used chemicals were analytical grade and deionized water was prepared in the lab using Labconco carbon water Pro-PS (Kansas, USA).

2.2. Samples collection

The by-products were collected from Akkar in North Lebanon and Hasbaya in the South following the described repartition in [Table 1](#).

These two regions are well-known for their high olive and olive oil production capacity where local farmers depend on the olive sector as one of the main sources of income. In each region, three samples of OMWW were collected from three olive lots at three harvesting times: early harvest (late October), intermediate harvest (mid-November) and late harvest (early December); and from the most widespread processing systems found in each region: From Hasbaya, samples were collected from press, 3-phases decanter and Sinolea coupled to 3-phases decanter systems which are the mostly used in the area. Whereas, samples from Akkar were collected from press, 3-phases decanter and 2-phases decanter systems.

2.3. Physico-chemical analyses

2.3.1. pH, electrical conductivity (EC), dry matter (DM), total nitrogen (N), potassium content and phosphorus content

In order to measure the pH and the electrical conductivity (EC) a volume of 100 mL of the sample was used. The measurements were performed using a Mettler Toledo MP200 pH meter (Mettler Toledo, Ohio, USA) and a conductivity meter conductimeter 522 (Crison Instruments, S.A., Barcelona, Spain). Dry matter (DM) was assessed following the official method ([AOAC 930.15, 1998](#)), using oven drying at 65 °C. Total nitrogen (N) was determined using the Kjeldahl method ([AOAC 955.04, 1998](#)). The percentage of potassium was determined as potassium oxide (K₂O), using a flame photometer M410 (Corning, Medfield, MA, USA) ([AOAC 955.06, 1998](#)). The percentage of phosphorus was determined as phosphorus pentoxide (P₂O₅) following the [AOAC 958.01 \(1998\)](#) official method.

2.3.2. Oil content

The oil content (OC) in OMWW was assessed using the method described by [Aggoun et al. \(2016\)](#). An aliquot of 4 g (approximately 4 mL) was mixed with 200 µl HCl (2 N) and approximately 16 mL of hexane as solvent (4 times the volume of OMWW). Then, the solution underwent a first centrifugation at 1000 rpm for 15 min and a first extraction. The centrifugation and extraction were repeated 2 more times by adding the same volume of hexane each time. The 3 extracts were collected together, and the resulting solution was placed in a rotary evaporator at 80 °C for 10 to 15 min depending on the oil dissolved quantity. Finally, the remaining solution was evaporated under a fume hood until the total disappearance of the solvent. the oil content in OMWW (OCOMWW) was determined as follows:

$$\text{OCOMWW (g/kg)} = \frac{(W_2 - W_1)}{W_0} \times 1000 \quad (1)$$

where, W₀ is the weight of the dried sample in g; W₁ is the weight of empty extraction beaker with boiling chips in g; W₂ is the weight in g of empty extraction beaker with the oil fraction after drying.

2.3.3. Potassium content

Potassium content in OMWW was determined by dissolving 1.5 g of the sample with approximately 75 mL of distilled water to obtain a homogeneous solution. The mixture was placed in a water bath for 30 min to obtain a homogeneous solution. The dissolved mixture was then introduced into a volumetric flask of 100 mL and the volume was completed to the mark with distilled water. Then, the solution was diluted 25 times with distilled water. For each solution, standard and sample values were measured.

The % of K₂O was calculated using the following two formulas:

$$K (\%) = \frac{C \times D}{W \times 10000} \quad (2)$$

and

$$K_2O (\%) = K (\%) \times 1.205 \quad (3)$$

where, D is the dilution factor of the solution and is equal to 100 × 50; C is the concentration of the solution obtained through the calibration curve; and W is the weight of the sample in g.

2.3.4. Calcium content (Ca)

The calcium content (Ca) in OMWW was calculated using atomic absorption spectroscopy (AAS, Shimadzu AA 6800, Kyoto, Japan) (AOAC 965.09, 1998). 1.5 g of samples were mixed with 20 mL of concentrated HCl and heated for 30 min. The volume was adjusted to 100 mL with distilled water. Then, 0.25 mL of the dissolved sample was diluted with 1% lanthanum chloride up to a final volume of 50 mL. Standard solutions were also prepared. The calculation was done as follows:

$$Ca (\%) = \frac{C \times D}{W \times 10000} \quad (4)$$

where, D is the dilution factor of the solution and is equal to 100 × 50; C is the concentration of the solution obtained through the calibration curve; and, W is the weight of the sample in g.

2.3.5. Biochemical oxygen demand (BOD) and chemical oxygen demand (COD)

To determine the BOD of the OMWW, the BOD 5 method was used APHA (2005). This method measures the quantity of oxygen consumed, for 5 days, by the microorganisms involved in natural purification mechanisms. For this, 100 mL of samples were introduced into flasks and six pellets of sodium hydroxide were introduced into each inner plug. The flasks were hermetically sealed and kept in the dark in an incubator at 20 °C. Oxygen consumption was monitored daily, as a function of time, for 5 days.

The determination of the COD was accomplished using HACH kits (USEPA, 2014). In this process, 2 mL of OMWW were carefully added to the mixture in the commercial kit (83% H₂SO₄, mercury sulfate and chromium trioxide), and thoroughly mixed. Then, the kit was placed in a HACK DRB200 reactor (HACK, Germany) for 2 h at 150 °C. The kits were then placed to cool at room temperature and the color variation was measured using a HACK spectrophotometer DR 2800 (HACK, Germany). The results of BOD and COD were expressed in mg of O₂/L.

2.3.6. Organic matter (OM)

The percentage of organic matter (OM) was determined using Walkley and Black method (Walkley and Black, 1934). In a 400 mL beaker, concentrated samples of 0.1 g each (or 1.5 g if diluted) were weighed and mixed with 10 mL of potassium dichromate and 20 mL of concentrated H₂SO₄. This mixture was allowed to stand for 30 min before mixing with 170 mL of distilled water and 10 mL of 85% H₃PO₄ solution. Then, the mixture was allowed to cool before titration. The titration was performed with Mohr salt and using 0.5 mL of diphenylamine as indicator. The end of the titration was marked by the change of color from black to green. The percentage of OM was calculated as follows:

$$OM (\%) = \frac{((B - V) \times 0.39 \times C \times 1.72)}{(W)} \quad (5)$$

where, B is the volume in mL of Mohr salt used for blank titration; V is the volume in mL of Mohr salt used for sample titration; C is the Mohr salt concentration; and, W is the weight of the sample in g.

2.3.7. Total phenols and antioxidant capacity

The phenolic extraction was performed following the method described by Aggoun et al. (2016). In brief, 4 g of OMWW were acidified with 200 µL of 2 N HCl to pH 2 and washed 3 times with 4 volumes of *n*-hexane. The solution was mixed for 1 min using a vortex (Mini vortex VWR Scientific Product state, USA) and centrifuged (1000 g) for 15 min at room temperature using a Thermo Electron Corporation (USA) centrifuge.

The lipid-free sample was treated, 3 times, with 2 volumes of ethyl acetate, vortexed for 1 min, and agitated using a shaker (Shaker Bath SBS 30: Stuart Scientific) for 5 min. The sample was then centrifuged at 1000 g for 15 min at room temperature. The ethyl acetate phases containing the phenolic compounds were then pooled and transferred to a rotavap (Stuart Scientific) for drying at 35 °C. The final drying stages were performed under nitrogen flow. The extracts were then reconstituted in 1 mL of methanol:water (60:40, v/v) before the quantification step.

For the quantification of the TPC, the Folin-Ciocalteu method proposed by Favati et al. (1995) was followed. First a solution of 20 g Na₂CO₃ in 80 mL of distilled water and a solution of 100 mg gallic acid in 100 mL methanol/water (60/40 w/v) were prepared. In a 1 mL pipette, solutions of different concentrations were prepared including a blank, 25, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, and 1000 µg/L. Samples were tested at three different dilutions. To each 100 µL phenolic extract, 675 µL distilled water were added, followed by 25 µL of Folin-Ciocalteu reagent and 200 µL of Na₂CO₃.

Table 2

Multivariate analysis (MANOVA) of OMWW total phenolic content and the antiradical scavenging activity.

Interactions/Factors	TPC and DPPH in OMWW						
	F	Partial η^2	Power	Wilk's Λ	F	Partial η^2	Power
Origin * System * Harvest	9.73***	0.82	1.00	0.76	3.44	0.24	0.58
Origin * System	4.23**	0.67	0.96	0.94	0.67	0.06	0.15
Origin * Harvest	65.21***	0.97	1.00	0.58	3.40*	0.24	0.81
System * Harvest	10.19***	0.81	1.00	0.57	1.78	0.25	0.69
Origin	71.67***	0.97	1.00	0.62	6.80**	0.38	0.88
System	33.28***	0.94	1.00	0.74	1.81	0.14	0.51
Harvest	45.52***	0.96	1.00	0.68	2.34	0.18	0.63

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

The mixture was stirred and allowed to stand in the dark for 90 min. The absorbance was then assessed at 765 nm with a slit 0.5 nm using spectrophotometer (Analytic Jena Specord 250 Plus double beam, Germany) and using the software Winaspect Plus (Version 4.2). Results were expressed as mg GAE (Gallic acid equivalents)/Kg of OMWW.

2.3.8. DPPH radical scavenging assay

The free radical scavenging activity was determined using the DPPH method described by [Del Monaco et al. \(2015\)](#). A stock solution of 250 μmol DPPH in methanol was prepared. The stock solution (500 μL) was diluted with 500 μL of methanol and read using a spectrophotometer at 515 nm to give the absorbance value of 1.2. The samples were then tested, in triplicates, by mixing 10 μL of the extract with 490 μL of methanol and 500 μL of DPPH in methanol solution, then by incubating them in the dark for 15 min at room temperature. Appropriate dilutions were conducted to obtain quantifiable measures. The reduction of DPPH absorbance was measured at 515 nm against methanol blank, using UV/visible light spectrophotometer (Analytic Jena Specord 250 Plus double beam, Germany) and using the software Winaspect Plus (Version 4.2). Tocopherol was used as a standard for preparing the calibration curve. Results were expressed as Tocopherol Equivalent (TE).

2.3.9. Statistical analysis

All analyses were done in duplicate. The statistical software IBM-SPSS (version 22.0, IBM, USA) was used to analyze the collected data. A multivariate analysis of the variance (MANOVA) and a One-Way ANOVA were used for this purpose. However, to ensure the validity of the results, all assumptions required for the mentioned tests were checked before running them. A result was considered statistically significant for a p -value less than 0.05 and the p -value levels were adjusted using Bonferroni corrections for multiple comparisons.

3. Results and discussion

3.1. Physico-chemical and biological characteristics of the Olive-mill Waste Water_(OMWW)

3.1.1. Physico-chemical parameters

[Table 2](#) showed that the mean values of OC, COD and BOD in the collected OMWW were 10.50 g/kg, 89.55 g O₂/L and 5.77 g O₂/L, respectively. The average values were 4.89 for pH, 4.49 mS/cm for EC, 7.20% for OM and 0.06% for N. These values were comparable to those reported in the literature ([Toscano and Montemurro, 2012](#)). [Sierra et al. \(2001\)](#) also reported values of OC between 0.3 and 23 g/L. [Moreno et al. \(1987\)](#) reported values of COD ranging between 39.9 and 210 g of O₂/L. However, BOD values (5.77 g O₂/L) were lower than those obtained by [Moreno et al. \(1987\)](#) (9.6 to 150 g of O₂/L).

The effects of the processing system, geographical origin and olive harvesting time on the chemical composition of OMWW, were investigated ([Table 2](#)). The results showed a very highly significant effect ($p < 0.001$) of the interaction of the three considered factors, geographical origin*processing system*harvesting time, on the set of all OMWW analyzed parameters (OCOMWW, BOD, COD, pH, EC, N, OM, P₂O₅ and K₂O). In addition, each of the considered factors alone showed a very highly significant effect ($p < 0.001$).

These results were in close agreement with several previous studies reporting that all the factors including processing systems, harvesting time and geographical origin presented significant effect on the physico-chemical characteristics of OMWW ([De Felice et al., 1997](#); [Mennane et al., 2010](#); [Anastasiou et al., 2012](#)). In addition, it was notable that the coefficient of variation (CV) was high, mainly in the case of OC, BOD, COD, EC, N and OM. This reflected the great variation of these parameters between regions, harvesting times and processing systems. Therefore, the combined effect of the three mentioned factors was of primary importance to the composition of the OMWW and consequently on their degree of pollution as well as their safe disposal.

Furthermore, the results showed that the OMWW originated from the North recorded significantly higher OC (21.02 g/kg), BOD (6.28 O₂/kg), COD (122.60 g O₂/kg), EC (6.00 mS/cm²), N (0.11%), OM (10.74%), P₂O₅ (1.07%) and K₂O (0.32%) as compared to those obtained in the South. Since processing systems were different among various regions, the processing

conditions can also vary. The crushing temperature, crushing size and malaxation duration can also vary between different olive mills as well as different olive varieties and soil qualities. Gomez-Rico et al. (2009) reported that the malaxation temperature affected the partition coefficient between oil and water phases in the olive paste as well as the release of organic compounds from the olive tissues. Furthermore, it had an impact on various enzymes including β -glucosidases and esterases. The high oxygen demand for the complete oxidation of the OM contained in OMWW originated from the North, exerted a great polluting capacity that destroys all life in the rivers where it is released. The polluting effect was traced to several of its components, including the phytotoxic action of the polyphenolic contents. Chaari et al. (2015) reported that in controlled soils the levels of phenolic contents could range between 2835 and 3010 ppm at a depth from 40 to 80 cm respectively. However, in OMWW these levels can reach around 4200 ppm or even higher. Boulimi et al. (2019) reported that the phenolic compounds are very toxic to plant seedlings, and demonstrated the high levels of some of the phenols in OMWW such as Tyrosol (11.8%), Hydroxytyrosol (45.3%), p-Coumaric acid (28.58%) and Gallic acid (12.62%) as compared to treated OMWW. In parallel, the OMWW effluents have a relatively high EC, which reflected their high content of salts as well as high contents of OM, Ca and N (Halalah, 2003). Accordingly, it is imperative to establish efficient and effective OMWW treatment systems in the North region to mitigate the detrimental environmental impact caused by the discharge of OMWW. In addition, direct discharge of untreated OMWW into the sewage that flows into the Mediterranean Sea poses an alarming risk of pollution. It is also suggested that these OMWW could be valorized for their high content in TPC and antioxidant capacity.

Regarding the harvesting time, the oil loss with the OMWW was significantly higher in the early harvesting (22.92 ± 30.74) in comparison with intermediate (6.44 ± 7.39) and late harvesting (4.62 ± 4.17) ($p < 0.05$), where no significant differences were recorded. On the other hand, early and late harvesting time led to OMWW with higher BOD (8.37 ± 1.35 , and 5.93 ± 3.51 respectively) and COD (97.34 ± 51.72 and 106.28 ± 35.49 respectively) and lower pH (4.76 ± 0.19 and 4.78 ± 0.30 respectively) than intermediate harvesting time with BOD (3.18 ± 3.32), COD (65.54 ± 35.87) and pH (5.14 ± 0.86). However, these harvesting times led to OMWW with higher EC, N, OM, P_2O_5 and K_2O (Table 2). Accordingly, early and late harvesting time led to OMWW with higher polluting capacity but higher fertilization value than intermediate harvesting. In contrast, intermediate harvesting time led to OMWW with lower polluting effect but also lower fertilization value. These results are partially in agreement with those obtained by Ntougias et al. (2013) who reported an increase in N, K_2O and P_2O_5 concentrations during ripening of olives although the differences were not significant. The same authors reported no significant effect of harvesting period on N, total organic carbon and total solids.

As per the processing system, the press and the 3-phases systems recorded significantly higher OCOMWW than Sinolea (Table 2). In addition, the press and the 3-phases systems showed significantly higher polluting effect as they recorded significantly higher BOD, COD and EC and a lower pH than Sinolea system. Nevertheless, the same systems showed higher contents of total N, OM, P_2O_5 and K_2O . While comparing the press to the 3-phases system, it could be noticed that the oil loss, the BOD and pH in the press system were higher than in the 3-phases system. These results were completely in agreement with those obtained by Azbar et al. (2004). However, the obtained N in the present study was higher in the 3-phases than in the press system.

Despite the high polluting effect observed for the OMWW originated from the North, from the early and late harvesting periods and from both the press and the 3-phases systems, their richness in OM has a positive impact on the life of the microorganisms in the soil. Moreover, their elevated levels of N, OM, P_2O_5 and K_2O allows the valorization of these by-products either by their composting or by applying them as fertilizers to agricultural lands, which if applied in proper doses and with correct plowing, will result in the improvement of the soil fertility (Buldini et al., 2000; Capasso et al., 2002; Halalah, 2003). For instance, Chaari et al. (2015) applied regularly three doses of OMWW (50, 100 and 200 m³/ha) for nine successive years and noticed a significant increase of soil EC, OM, N, P_2O_5 and K_2O with the increase of OMWW application dose. The phenolic contents remained high as compared with the control especially in the top centimeters of the soil rendering it more fertile if applied in the right doses.

On the other hand, the overall results suggested that the OMWW obtained from the Sinolea system in the South region and at intermediate harvest were considerably less polluting than the other samples. In that perspective, olive oil producers should be encouraging the use of the Sinolea systems as the least polluting system and more sustainable process.

3.1.2. Total phenolic content (TPC) and antiradical scavenging activity (AC) and their correlation

The results (Table 3) showed that the average TPC in OMWW was 807.56 GAE/kg with significantly higher values for OMWW obtained from the North (1027.7 GAE/kg) at early harvest (1024.00 GAE/kg) and using press system (1036.13 GAE/kg) as compared to the other samples from the South, intermediate and late harvesting time using 3-phases and Sinolea ($p < 0.05$).

The multivariate analysis (MANOVA) (Table 2) of the combined effect of the three factors considered on the TPC in OMWW showed a p -value equal to 0.05. Thus, the geographical origin of the olive fruits, their harvesting time and the oil processing system are factors of primary importance on the TPC of OMWW. In addition, the two-way interaction geographical origin*harvesting time presented a significant effect ($p < 0.05$) (Table 2). El Abbassi et al. (2011) reported that OMWW obtained from semi-modern extraction processes contained higher phenolic content as compared to those obtained from modern 3-phases with values of 9.8 g/L and 6.1 g/L, respectively. Aggoun et al. (2016) also reported that

Table 3

Quantitative chemical characterization of the olive mill waste water (OMWW) obtained from different geographical origin, processing system and at different harvesting time. Different letters in the same row indicate significant differences (Duncan, $p < 0.05$).

Factor		OC ^a (%)	BOD ^b (g O ₂ /L)	COD ^c (g O ₂ /L)	pH	EC ^d (mS/cm)	N ^e (%)	OM ^f (%)	P ₂ O ₅ (%)	K ₂ O (%)	TPC ^g (GAE/kg of oil)	DPPH ^h (TE)	
Geographical origin	South (n = 27)	4.65 ± 3.26	5.45 ± 3.22	68.73 ± 38.62	5.04 ± 0.65	3.54 ± 1.86	0.04 ± 0.03	4.96 ± 3.46	0.96 ± 0.28	0.25 ± 0.08	637.45 ± 471.07 ^b	682.05 ± 642.09 ^a	
	North (n = 18)	21.02 ± 28.24	6.28 ± 4.11	122.60 ± 30.67	4.67 ± 0.24	6.00 ± 1.67	0.11 ± 0.04	10.75 ± 3.09	1.07 ± 0.07	0.32 ± 0.05	1027.70 ± 697.32 ^a	1880.07 ± 1091.49 ^b	
Harvesting time	Early (n = 15)	22.92 ± 30.74 ^a	8.37 ± 1.35 ^a	97.34 ± 51.72 ^a	4.76 ± 0.19 ^b	5.11 ± 2.62 ^a	0.07 ± 0.05 ^b	8.71 ± 4.89 ^b	1.06 ± 0.07 ^a	0.30 ± 0.07 ^a	1024.00 ± 780.27 ^a	1426.87 ± 1269.38	
	Intermediate (n = 15)	6.44 ± 7.39 ^b	3.18 ± 3.32 ^c	65.54 ± 35.87 ^b	5.14 ± 0.86 ^a	3.69 ± 1.82 ^b	0.05 ± 0.04 ^b	4.14 ± 2.89 ^c	0.90 ± 0.37 ^b	0.25 ± 0.11 ^b	644.59 ± 478.53 ^b	903.65 ± 863.26	
	Late (n = 15)	4.62 ± 4.17 ^b	5.93 ± 3.51 ^b	106.28 ± 35.49 ^a	4.78 ± 0.30 ^b	4.70 ± 1.82 ^a	0.08 ± 0.05 ^a	8.85 ± 3.50 ^a	1.04 ± 0.03 ^a	0.29 ± 0.04 ^a	699.98 ± 392.89 ^b	1207.32 ± 907.69	
Processing system	Press (n = 18)	13.07 ± 20.94 ^a	7.05 ± 3.04 ^a	104.11 ± 32.53 ^a	4.80 ± 0.16 ^b	5.27 ± 1.79 ^a	0.06 ± 0.03 ^b	8.42 ± 3.67 ^a	1.06 ± 0.06 ^a	0.32 ± 0.05 ^a	1036.13 ± 652.54 ^a	1498.63 ± 1287.30	
	3-Phases (n = 18)	10.71 ± 20.84 ^a	5.75 ± 3.92 ^b	105.03 ± 31.46 ^a	4.64 ± 0.25 ^c	5.11 ± 1.42 ^a	0.09 ± 0.05 ^a	8.79 ± 3.82 ^a	1.05 ± 0.04 ^a	0.30 ± 0.04 ^a	880.64 ± 562.72 ^b	1352.52 ± 840.12	
	Sinolea (n = 9)	5.27 ± 4.04 ^b	3.24 ± 2.62 ^c	31.16 ± 38.25 ^b	5.56 ± 0.92 ^a	1.74 ± 1.78 ^b	0.01 ± 0.02 ^c	1.76 ± 1.39 ^b	0.80 ± 0.45 ^b	0.17 ± 0.10 ^b	271.69 ± 218.83 ^c	275.59 ± 269.05	
Total	(n = 45)	Mean ± SD Min Max	10.50 ± 18.49 0.17 87.68	5.77 ± 3.56 0.06 9.73	89.55 ± 44.23 5.35 149.35	4.89 ± 0.56 4.25 6.85	4.49 ± 2.15 0.62 8.51	0.06 ± 0.05 0.00 0.16	7.20 ± 4.35 0.62 14.32	1.00 ± 0.23 0.00 1.28	0.28 ± 0.08 0.00 0.43	807.56 ± 604.82 64.31 2221.97	1200.11 ± 1042.83 14.62 3945.12

^a OC is the oil content on humid matter.

^b BOD stands for biochemical oxygen demand.

^c COD stands for chemical oxygen demand.

^d EC stands for electrical conductivity.

^e N stands for Nitrogen.

^f OM stands for organic matter.

^g TPC stands for total phenolic content.

^h DPPH is the antiradical scavenging activity expressed per Tocopherol Equivalent (TE).

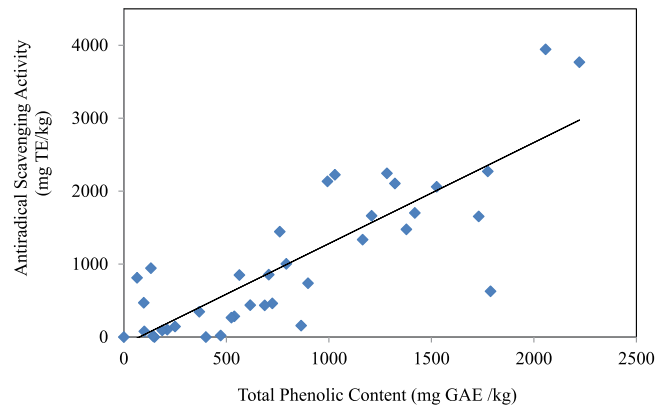


Fig. 1. Correlation between total phenolic content (TPC) and antiradical scavenging capacity (AC) of the olive mill wastewater (OMWW) obtained using different processing systems at different harvesting time and from different regions.

the content of specific phenolic compounds varied significantly according to the olive variety and the processing system. [Ntougias et al. \(2013\)](#) showed that the total phenolics were significantly higher at the end of the harvest season (4.66 g/L) as compared to the early harvest ones (3.88 g/L). The differences in the phenolic content could also be attributed to several other factors not considered in this study including the farming practices, the olive variety, the olive storage prior processing and the phenolic solubility ([Obied et al., 2005](#)). The average value of DPPH was $1200.11 \text{ TE kg}^{-1}$. The results indicated that only the geographical origin of the samples had a significant impact on the DPPH antioxidant activity ($p < 0.01$), with significantly higher DPPH values observed in the North ($1880.07 \text{ TE kg}^{-1}$) compared to the South ($682.05 \text{ TE kg}^{-1}$). Several studies reported that antioxidant activity depends on the olive variety, on the climatic conditions, storage time, malaxing time and process of milling ([Obied et al., 2005](#)). For instance, [Leouifoudi et al. \(2015\)](#) reported that extracts originating from OMWW of mountainous regions had higher antioxidant scavenging activity ($\text{EC}_{50} = 12.1 \pm 5.6 \mu\text{g mL}^{-1}$) than those produced in plain areas ($\text{EC}_{50} = 157.7 \pm 34.9 \mu\text{g mL}^{-1}$). The authors attributed this difference to varying factors such as precipitation, humidity, and soil composition in the respective regions. Moreover, [El Abbassi et al. \(2011\)](#) reported that the processing time had significant effect on the antioxidant capacity of the OMWW, where the highest values were obtained using semi-modern processing system as compared to the 3-phases system. The antioxidant activity of OMWW was believed to be influenced by its phenolic composition, and the variation in antioxidant capacity can be attributed to the differences in phenolic compounds released during different processing techniques ([Aggoun et al., 2016](#)). Moreover, it has been reported that different phenolic compounds possess varying antioxidant capacities ([Bedouhene et al., 2014](#)). In a study by [Bengana et al. \(2013\)](#), it was found that the antioxidant capacity of OMWW was attributed to its phenolic contents as well as α -tocopherol. Interestingly, the level of α -tocopherol was observed to be the highest during the early harvesting stages and gradually decreased, reaching the lowest level during the third harvesting stage. However, this difference was not statistically significant. On the contrary, [Bouaziz et al. \(2004\)](#) showed that the total antioxidant capacity of the TPC increased with fruit maturation, and attributed this increase to the possible surge of the TPC level associated with the fruit ripening. In the present study, the relation between the TPC and antiradical scavenging capacity obtained was also assessed. The results ([Fig. 1](#)) presented a strong positive correlation between the TPC and antiradical scavenging capacity with a Pearson correlation coefficient, r , of value equal to 0.784 ($r^2 = 0.6147$). Statistical analysis showed that an increase in GAE by 1 causes an increase in the TE by 1.326 times. The coefficient for GAE was significantly different from 0 ($p = 0.000 < 0.05$). [Leouifoudi et al. \(2015\)](#) showed a strong correlation ($r = 0.977$) between the TPC and the antioxidant capacity. The antioxidant effect of the OMWW has been attributed to its phenolic contents ([Abu-Lafi et al., 2017](#)). This again confirmed that the phenolic content of the OMWW could be a good determinant of the antioxidant capacity of the OMWW product ([Azaizeh et al., 2012](#)).

3.1.3. Valorization of OMWW

Although OMWW has been long regarded as a hazardous waste with negative impacts on the environment and an economic burden on the olive oil industry, this view should be redirected since OMWW could be considered as a low-cost starting material rich in bioactive compounds, particularly phenolics, which can be extracted and used as natural antioxidants in various applications in the food, pharmaceutical and cosmetic industries. Pharmaceutically relevant characteristics such as antioxidant, anti-allergic, anti-inflammatory, anti-tumor, and anti-hypertensive actions were demonstrated by polyphenolic compounds recovered as by-products ([Russo et al., 2022](#)). Moreover, these substances exhibit antibacterial properties that could be used in agriculture to combat phytopathogens and in the search for novel therapeutic medicines that are effective against human antibiotic-resistant diseases ([Russo et al., 2022](#)). OMWW is a potent source for ingredients for applications in the food industry and food formulation as well as some polyphenols that can be used as natural food additives of antimicrobial benefits ([Zahi et al., 2022](#); [Caporaso et al., 2018](#)). Additionally,

the recovered polyphenols from OMWW can be used to produce functional foods. Extra virgin olive oil with moderate amounts polyphenols, exhibited extra health benefits to consumers, when enriched with phenolic compounds extracted from OMWW (Valls et al., 2015). The addition of the extracted phenols to fruit juice, is a useful way of producing functional foods with natural antioxidants as a “clean label”. Foti et al. (2022) reported that the fortified juice with hydroxytyrosol still complied with the EFSA daily intake levels even after 60 days of refrigeration. As for the extraction methods, various valorization processes can be used such as solvent extraction, adsorption onto resins, supercritical fluid extraction, and membrane separation (Fleyfel et al., 2022). The extraction of polyphenols offers a dual advantage by yielding valuable compounds while simultaneously reducing the toxicity and oxygen demand (BOD and COD) of OMWW (Bouslimi et al., 2019). This enhanced quality makes OMWW particularly well-suited for application as fertilizers, showcasing a more environmentally beneficial and economically viable option. Bouhia et al. (2023) reviewed several possibilities to valorize the sludge settling from OMWW. All this would have an important socio-economic effect on low-income areas, especially in the Mediterranean region (Bouhia et al., 2023). Furthermore, composting the OMWW sludge prior to its direct application to the soil can have a significant enhanced fertility by increasing cation exchange capacity as well as phosphorus and exchangeable K, Ca, and Mg levels (Rigane et al., 2015). Interestingly, the soil treated with the composted OMWW sludge showed a corrected organic C and organic N by 260% and 100% compared to those of the unaltered soil. This amendment was evident by the increase in the harvested potato yield, which improved by 9.4% compared to that of the unaltered soil without chemical fertilizer (Rigane et al., 2015). The proper use of organic and inorganic matter from OMMW can help attain the food security and sustainability goals by promoting nutrient use efficiency (Bouhia et al., 2022). As a result, the aforementioned proposed treatments consider tailor-made valorization of OMWW, taking into account its chemical and biological characteristics.

4. Conclusion

The results of this study indicated that OMWW composition was affected by all the assessed factors. Those obtained from the South region, at the intermediate harvest period using Sinolea appeared to be the most environmentally friendly. The OMWW obtained from the North, at early harvesting period using press systems could be considered rich sources of natural phenolic and antioxidant compounds. Thus, sustainable OMWW valorization pathways should be thought in agreement with all the factors assessed in this study. Moreover, processing should take into consideration valorization of its contents that could be considered a potential source of beneficial functional ingredients, rather than polluting compounds. Further studies could be carried out to validate the applicability of the suggested pathways and to assess the individual phenolic contents and their relative antioxidant capacities.

Perspective

This study shed light on the double-edged sword of the OMWW effect on the Environment and water quality in Lebanon and its potential valorization. Further studies are needed with a larger number of samples to establish a statistical significance between the three studied factors and the TPC of OMWW. Moreover, further investigation must be conducted to address the benefits of OMWW rich in phenolic compounds and endowed with higher antioxidant capacity, as well as the individual phenolic composition in order to further clarify this possible association.

CRedit authorship contribution statement

Aline Issa: Data curation, Formal analysis, Methodology, Project administration, Visualization, Writing – original draft, Writing – review & editing. **Milad El Riachy:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Validation, Writing – original draft, Writing – review & editing. **Christelle Bou-Mitri:** Conceptualization, Formal analysis, Funding acquisition, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing. **Jacqueline Doumit:** Conceptualization, Formal analysis, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing. **Wadiah Skaff:** Conceptualization, Formal analysis, Funding acquisition, Methodology, Validation, Writing – original draft, Writing – review & editing. **Layal Karam:** Formal analysis, Funding acquisition, Methodology, Validation, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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