



Assessment and valorization of treated and non-treated olive mill wastewater (OMW) in the dry region

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Abstract

Purpose The quantity of Olive by-products does not stop increasing and a water shortage that threatens the olive tree culture; require a serious valuation of these by-products. A comparative and valorization study of two kinds of OMW; Fresh OMW (FOMW), directly issue from three-phase continuous extraction factory and Disposal Evaporation Ponds OMW (DOMW) were done.

Methods Physico-chemical and biological parameters of OMWs and soil irrigated with OMWs, respectively, were determined. An antibacterial activity test of FOMW against Clinic Standard Bacteria was determined. A statistical analysis was performed for all defined parameters.

Results A significant increase of pH value of 6 and a lower failure of the EC in 8.94 (mS/cm⁻¹) were registered after OMW disposal in evaporation ponds. We registered a fall of BOD₅ and COD from 61.05–116.37 (g/L) to 55.67–103.82 (g/L), respectively. A significant increment of phenol compound removal was observed after OMW disposal. However, a switch of fatty acids distribution and content was observed, which several fermentation pathways could explain took place. This result suggested by a clear shift in biomass composition. An important soil fertility after DOMW soil irrigated was traduced by an important value of the germination index (170.55%) and efficient organic matter increment of 2.3%. A CMI rate of 32.76 (µg mL⁻¹) was determined by FOMW against different clinic standard bacteria.

Conclusion A spectacular soil fertility effect was obtained from DOMW soil spreading, that efficiently evaluate the OMW biological treatment. In addition, the FOMW was valorized as its powerful antibacterial.

Keywords Olive mill water valorization · Ponds evaporation · OMW compounds · Soil treatment · Soil fertility · OMW antibacterial activity

Introduction

Every year, olive oil production industry release large quantities of olive mill wastewater (OMW). They are estimated approximately 30 million m³ in the world and 700 million m³ in Tunisia (Mekki et al. 2013). They are obtained from olive tree after discontinuous press or from the continuous centrifugation (Niaounakis and Halvadakis 2006; Aggoun

et al. 2016). The chemical characterization of OMW demonstrates that are highly rich of phenolic compounds, lipids and organic acids with a poor biodegradability that limits their treatment (Dermeche et al. 2013; Aggoun et al. 2016). Generally, different factors influence the characteristics of OMW such as the method of extraction, the technological process separation, the climatic conditions and the variety and the fruit maturity of olive tree (Dermeche et al. 2013; Aggoun et al. 2016). In fact, after oil extraction, OMW contained 98% of the total phenolic content of the olive fruits with concentration varying from 0.5 to 24 g/l (Paraskeva and Diamadopoulou 2006; Alaoui et al. 2016; Leouifoudi et al. 2014).

Different phenolic compounds were detected essentially for simple phenols and flavonoids and polyphenols resulting from polymerisation of the simple phenols (Comandini et al. 2014; Leouifoudi et al. 2014). They are phytotoxic and can

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inhibit plant growth limiting the use of OMW for irrigation. In addition, their direct release onto soil may affect soil's physical and chemical properties including soil porosity and pH (Niaounakis and Halvadakis 2006; Mekki et al. 2013). Moreover, release of OMW in the environment can discolour streams and rivers due to the high concentration of darkly coloured polyphenols. Indeed, discharge of the OMW in the environment has a serious environmental drawback (Paraskeva and Diamadopoulos 2006).

In addition to phenolic compounds, the highly reduced sugars content of OMW can stimulate microbial respiration, lowering therefore, dissolved oxygen concentrations (McNamara et al. 2008). Besides, the high phosphorus content can lead to eutrophication (McNamara et al. 2008). Likewise, the large-volume OMW and the seasonal nature of olive oil production limit their storage operation and their displacement (McNamara et al. 2008). Regardless, olive oil production remains a high polluting industry due to the utilization of large amounts of water and the production of large amounts of waste water and sludge.

To resolve these problems, pretreatment of OMW can improve the quality of the wastewater and remove some of its toxicity. Different strategies to know, physical–chemical, and biological process were applied to treat OMW. Dilution, evaporation, sedimentation, filtration and centrifugation were used largely as a physical process to treat OMW (Paraskeva and Diamadopoulos 2006; Villegas et al. 2016). Moreover, several biotechnological methods have been used to reduce the polluting load of OMW including decantation with lime and clay, coagulation–flocculation, electrocoagulation, natural evaporation and thermal concentration (Paraskeva and Diamadopoulos 2006; Villegas et al. 2016). Also, evaporation ponds and artificial ponds with very large surface areas that are designed to efficiently evaporate water by sunlight and exposure to the ambient temperatures can be used for OMW treatment (Barbera et al. 2013). Besides, different biological treatment technologies of OMW were reported as efficient strategies to clean OMW, including microbial degradation, aerobic and anaerobic process (codigestion with other effluents and composting) (Paraskeva and Diamadopoulos 2006; Villegas et al. 2016). It presents many advantages over other techniques including simplicity; less expensive for the reduction of pollutants that perfectly meets many sustainable social systems and ecological advantages (Barbera et al. 2013). Note in this regard that the arid southern Tunisia is a wide field of biochemical exploration and has many characteristics associated with the continuous presence of light and sunshine throughout the year.

Moreover, soil serves as a medium for organic waste disposal, both solid and liquid. OMW was known to have a fertilizing effect, assessing a possible valorization of these effluents in agronomy (Dakhli and Maalej 2017). Spreading represents an interesting solution for OMW treatment and

disposal. In fact, the higher content of micronutrients, especially potassium and organic matter induce plant growth (Di Serio et al. 2008; Dakhli and Maalej 2017). However, the high organic load of OMW due mainly to the presence of polyphenols as well as short and long chain fatty acids may cause phytotoxic and antimicrobial effects of these effluents (Dakhli and Maalej 2017). The inhibition of the growth of microorganisms, especially bacteria, may reduce the mineralization process in the soil (Dakhli and Maalej 2017). For these reasons, the controlled spreading of OMW can increase the fertility of the soil and offer the opportunity to recycle various compounds.

Indeed, the goal of this work is constituted of two parts; the first one is to characterize different types of OMW. A comparison between Fresh OMW directly issue from three-phase continuous extraction factory and disposal evaporation ponds OMW in the south of Tunisia (Chammakh-Zarzis) was done. The second goal of this work was the investigation of the effects of different types of OMW spreading on the soil characteristics. The soil treated fertility was evaluated to evaluate the OMW spreading phytotoxicity potential. In addition, we tried to evaluate the antibacterial activities of the fresh OMW against different clinic bacteria, making the possibility of further production of antibiotic with high industrial and biotechnological values.

Materials and methods

Study zone

The study zone is located at Chammakh-Zarzis in the south-eastern Tunisia. The climate of this region is an arid Mediterranean climate with an annual average rainfall of 180 mm. The soil of this area is moderately deep with a slight texture, very filterable and relatively poor in organic matter (Abichou et al. 2009).

Meteorological parameters

Average monthly values of different meteorological factors of evaporation ponds are detailed in Fig. 1 including Month temperature (°C), Evaporation rate (mm per days), Relative humidity (%), Wind speed (m per second) and Dominant wind direction. The annual temperature and precipitation was 21 °C and 15.16 mm per year, respectively. The evaporation extended from 1 mm to 5.6 mm. In fact, the OMW evaporation rate can be considered around 4.03 mm per year. The maximal air humidity and wind speed was around 74% and 1.6 m per second, respectively, followed by (230°) as a maximal value of a wind direction Dig. The meteorological conditions described above can facilitate an efficient natural evaporation of OMW.

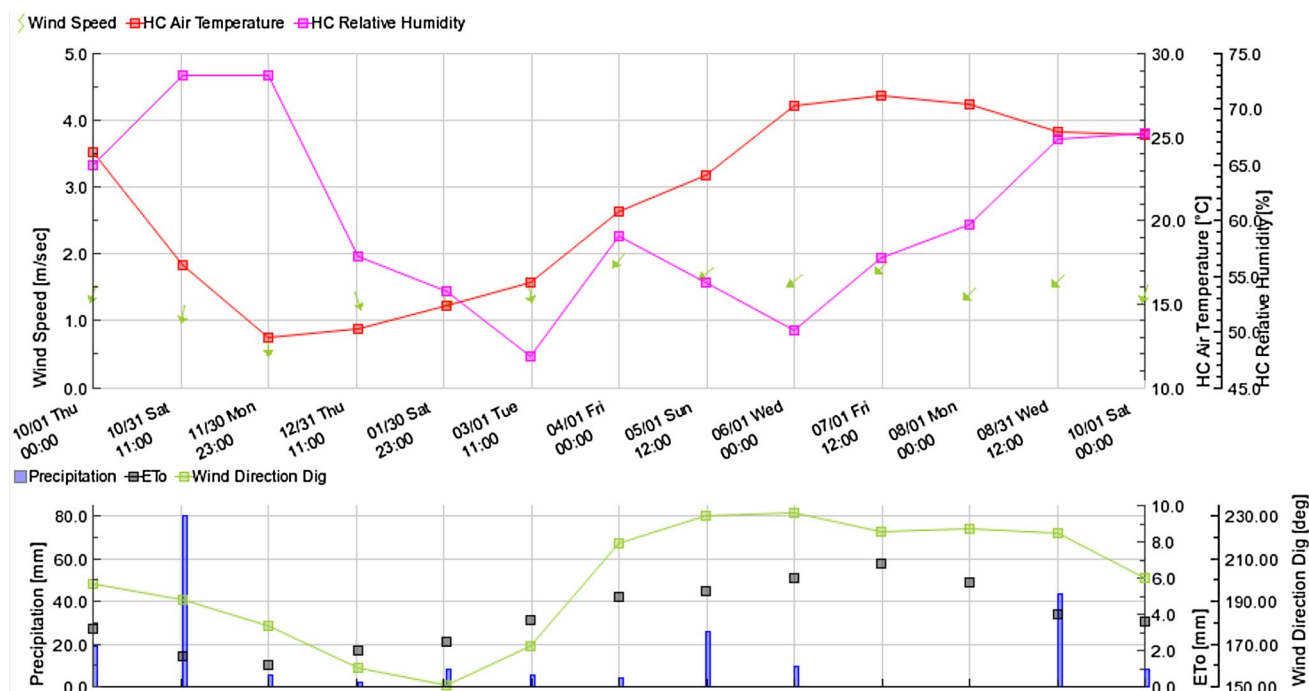


Fig. 1 The meteorological factors of study Zone; Month temperature ($^{\circ}\text{C}$) Evaporation (mm per days); Relative humidity (%), Wind speed (m per second) and Dominant wind direction

Sampling

FOMW was taken from a three-phase continuous extraction factory located in the region of Chemmakh-Zarzis (Southern Tunisia, $33^{\circ}36'N$, $11^{\circ}02'E$). DOMW Samples were collected from the evaporation ponds after one year of fresh OMW disposal. The characteristics of ponds were in m: 70 of Length, 40 of Width, 4 of Height. The volume of ponds was 11200 m^3 . The OMW was sprayed homogeneously on the sandy soil surface, previously tilled to a 20 cm of depth. DOMW and FOMW were sprayed in the soil olive tree culture plot during 5 years in the winter period (December–January) using at doses of $50\text{ m}^3/\text{ha}^{-1}$. The dimension of soil plot was 1 ha. The soil sample was collected between two olive trees using the Hand auger tube *H-4268*. The soil sampling was taken in March. Each type of OMW was used in each different site. Non-OMW spreading soil (Untouched soil) was used as a control.

Physico-chemical analysis

Different physico-chemical parameters including organic matter, mineral content, biological oxygen demand (BOD), chemical oxygen demand (COD), electrical conductivity (EC) and pH were performed according to the Standard Methods for the Examination of Water and Wastewater, 20th Edition 1998. The pH value of soil was determined with pH meter XP with a pH 50 lab model. Electrical Conductivity

(EC) of each site was determined Conductivity *inoLab WTW 7110 model*. Total Organic Carbon (TOC) was determined following the Walkley–Black method (Walkley 1947) and organic matter (OM) was calculated by multiplying the total carbon by 1.724.

Sugar and phenolic compounds analysis

High-performance liquid chromatography (HPLC 2010 PLUS, SHIMADZU) was used to quantify Sugar and Phenolic compounds. For sugar compounds identification, stationary phase consisted of NH_2 column (AQUASILC C18 with length and inner diameter of 150 and 30 mm, respectively) and mobile phase consisted of acetonitrile with a flow rate of (1 ml/min). For phenolic compound quantification, the mobile phase was an equal mixture of (A) 0.1% of formic acid in water and (B) 0.1% of formic acid in methanol with a flow of 0.4 ml/min. The column TSK (TSK gel with length and inner diameter of 30 cm and 7.8 mm, respectively) was used (Pérez et al. 1990; Kemal 1994). The extraction of soil polyphenols was realized according to FOLIN-DENIS method by the ethanol (Ranalli 1997).

Fatty acid analysis

Free fatty acids were analyzed with a Shimadzu gas chromatograph system, (GC–MS GP2010ULTRA) adapted for capillary columns. For the analysis, a fused silica capillary



column, 30 m×0.25 mm×0.25 µm film thickness, was used. The injector and detector temperatures were set at 200 °C with interface of 220. The column temperature was set at 50 °C, then raised to 250 °C with flow of 50 °C/minute, then increased from 200 to 230 °C at the rate of 50 °C/min. Peak heights were determined by integration software (D'Annibale et al. 1998).

Microbial enumeration

Microbial enumeration was done according to ISO 2718 (Technical Committee 1996). Results were expressed as the total number of colonies forming units (CFU). For the enumeration of total aerobic mesophilic bacteria, PCA plate were inoculated and incubated at 37 °C for 24 h. While, Sabouraud medium containing a bacterial inhibitor (chloramphenicol) was used to enumerate yeasts after incubation at 25 °C for 5 days. The microbial results were expressed by calculating the ratios of decimal logarithmic value of each flora (bacteria and yeasts). Triplicates plated were used in each test.

For soil Microbial estimation, a soil suspension was prepared as follows; 10 g of the soil sample were suspended in an Erlenmeyer flask containing 90 ml of a sterile solution (0.2% of sodium polyphosphate (NaPO₃) in distilled water, pH 7.0) and 10 g of sterile glass beads (1.5 mm diameter). The flask was shaken at 200 rpm for 2 h. Serial tenfold dilutions of the samples in a 0.85% NaCl solution were plated in triplicate on PCA at 37 °C and Sabouraud containing chloramphenicol at 25 °C for total bacterial counts and for yeasts counting, respectively.

Antibacterial activity study

Antibacterial activities of different OMWF concentration were studied in triplicate using glass tubes as described by (Aziz et al. 1998). The antimicrobial activity of the different test was tested against Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Klebsiella pneumonia*, *Salmonella typhimurium* and *Enterobacter cloacae*) to determine the minimal inhibitor concentration (MIC). The tubes were incubated at 37 °C for 18 h. Results of bacterial growth inhibition were expressed by a visual examination: the inhibition was considered positive when there was no microbial growth in all the three tubes of the triplicate. The MIC was determined as the lowest concentration caused complete growth inhibition in the triplicate tubes of each treatment.

A Stock solution containing 10 mL of Mueller–Hinton medium and 1 mL of culture was prepared. A 1.8 mL of stock solution was mixed with 0.8 mL of the filtrate of FOMW extracted. A solution of 100, 50 and 25% dilution of OMW extracted range was prepared. Pure water was used as a control. The same protocol described above was repeated in triplicate for each strain.

Respirometric tests

Biological activity in the soil was achieved by measuring CO₂ evolution in the aerobic condition. Respirometric analysis was done according to the method of Ohlinger (1995). The soil sample was humidified to 50% of its water holding capacity, and then was incubated at 25 °C in the dark. Released CO₂ was trapped in NaOH solution and titrated with HCl.

Germination index evaluation

The phytotoxicity of the treated soils was evaluated by the measurement of the germination index according to the method proposed by (Zuconni et al. 1981). This technique consists of making germinate, in petri dish, 10 seeds of tomatoes with the different samples studied. Petri dish incubated during a 1 week in the darkness at 25 °C. The untouched soil was used as positive control. After incubation, the reading of the result is determined by counting the number of germinated seeds. The length of the roots of seeds having germinated measured in mm. The germination index “GI” is calculated according to the following formula:

$$GI (\%) = \frac{[\text{Number of seed germinated} \times \text{root length}]}{[\text{Control Number of seed germinated} \times \text{Control root length}]} \times 100$$

Statistical analysis

The Statistical Package for the Social Sciences (SPSS, Version 20.0) was used to perform the statistical analysis. The Phenol, fatty acids and sugar compounds of both OMW Samples (fresh and disposed in the evaporation ponds) values were compared using Student test at the “5% ($P=0.05$)” significance level. Data are presented as mean ± standard deviation. Values were obtained from triplicate determinations and the differences were examined using one-way analysis of variance (ANOVA) followed by the Fischer’s LSD (Least Significant Difference) post hoc test. Statistical significances of the correlations between datasets were calculated using Pearson’s R values. At least three replicates were performed for each laboratory measurement.

Results and discussion

The physico-chemical properties

The physico-chemical properties of FOMW and DOMW are reported in Table 1. A significant increase of pH value of 6 and a lower failure of electrical conductivity of 8.94 (mS/cm⁻¹) were registered after OMW disposal in evaporation

Table 1 Physico-chemical properties of FOMW and DOMW

Samples	pH	EC (mS/cm ¹)	BOD ₅ (g/l)	COD (g/l)	OM (g/l)	MM (g/l)
FOMW	5.045 ^a ±0.007	9.200 ^a ±0.28	61.050 ^a ±1.47	116.370 ^a ±2.81	89.590 ^a ±0.3	11.480 ^a ±0.2
DOMW	6.020 ^b ±0.02	8.940 ^b ±0.19	55.670 ^b ±0.89	103.820 ^b ±1.66	41.260 ^b ±0.02	5.300 ^b ±0.6

Test Student was determined with FOMW and DOMW ($p=0.01$, $*p<0.05$). Mean scores in the same column with different letter (“a” and “b”) are significantly different (for $p<0.05$) (Results are compared for the same parameters and the two different OMW)

Table 2 Phenolic composition of FOMW and DOMW; reduction of single phenolic components was represented in removal percent

Phenolic compound	FOMW (mg/l ⁻¹)	DOMW (mg/l ⁻¹)	Removal (%)
Quinic acid	0.556 ^a	0.508 ^b	8.630
Protocatechuic acid	7.863 ^a	0.000 ^b	100
Caffeic acid	39.907 ^a	0.000 ^b	100
p-coumaric acid	38.617 ^a	0.000 ^b	100
Naringin	0.912 ^a	0.000 ^b	100
Hyperoside (quercetin-3-o-galactoside)	1.600 ^a	0.313 ^b	80.430
Rutin	0.649 ^a	0.000 ^b	100
Salviolinic acid	5.064 ^a	0.000 ^b	100
Naringenin	0.309 ^a	0.122 ^b	60.510
Luteolin	14.924 ^a	1.708 ^b	88.550
Cirsiliol	5.658 ^a	2.570 ^b	54.570
Apegenin	0.551 ^a	0.244 ^b	55.710
Acacetin	14.447 ^a	14.774 ^b	–
Syringic acid	0.000 ^a	39.193 ^b	–

Test Student was determined with FOMW and DOMW ($p=0.01$, $*p<0.05$). Mean scores in the same line with different letter (“a” and “b”) are significantly different (for $p<0.05$) (Results are compared for the same parameters and the two different OMW)

ponds. The OMW are rich in organic matter expressed in terms of BOD₅ and COD. However, a fall of BOD₅ and COD from 61.05 and 116.37 (g/L) to 55.67 and 103.82 (g/L), respectively, after the disposal of OMW more than 1 year in evaporation ponds. These results suggest the occurrence of oxidative phenomena, which led to further removal of the acidic compounds in OMW (Paraskeva and Diamadopoulos 2006).

The phenol compounds

Table 2 summarizes the phenol compound concentration founded in OMWF and DOMW. Comparative study of phenol compounds between fresh and disposal OMW shows a significant difference in the concentration values ($p=0.023$).

Regarding previous studies, it is not easy to compare phenol content in OMW in different experiments because

phenol compounds are influenced by many factors, including the impact of geographical and climatic conditions.

OMW composition was studied in various studies (Bouk-nana et al. 2014; Leouifoudi et al. 2014; Alaoui et al. 2016). It was characterized by its complexity and it was found to be rich in hydroxytyrosol and secoiridoids derivatives (Peixoto et al. 2008; Leouifoudi et al. 2014). In this work, we tried to analyze phenolic compounds in OMW before and after ponds evaporation. An important failure of phenol compound concentration rate as well as the efficient removal of a large number of phenolic compounds was observed. Significant increment, ranging from 54 to 100% of phenol compound removal of the most compound after disposal of OMW in the evaporation ponds, was observed.

Phenol compounds such as protocatechuic acid, caffeic acid, p-coumaric acid, Naringin, Rutin and Salviolinic acid, were completely removed. The decrease and removal of phenol compounds may be due to their oxidation and their possible further transformations (Belaid et al. 2002). In fact, the complete oxidation of organic compounds to CO₂ and H₂O results from the split of the various aromatic compounds during the aerobic catabolism permitting their use in the cycle of the Krebs (Anderson and Dagley 1980). Regardless, a higher concentration rate of 39.193 of syringic acid compounds in DOMW was detected in this study. A previous study suggested that syringic acid is the least toxic phenol compound (Rahouti et al. 1997). These results showing the significant decrease of phenolic compounds along with their toxic effect are of great interest. However, previous studies showed that degradation of phenolic compounds is considered as an obstacle in the biological OMW treatment, including their natural breakdown is not easy (Tsioulpas et al. 2002; Martins et al. 2008).

The fatty acid content

The comparison of fatty acids between the fresh and disposal OMW shows a significant difference in the fatty acid concentration ($p=0.01$). Figure 2 and Table 3 shows a significant change of the fatty acid distribution.

Several acids are completely removed such as ethylbenzene, styrene; ethyl 9-hexadecenoate, cyclobutane, 3-diphenyl-, trans-, cyclopropyl phenyl methane, benzene, 1,1'-(1,2-Cyclobutanedilyl) bis-, trans-, octadecanoic acid,



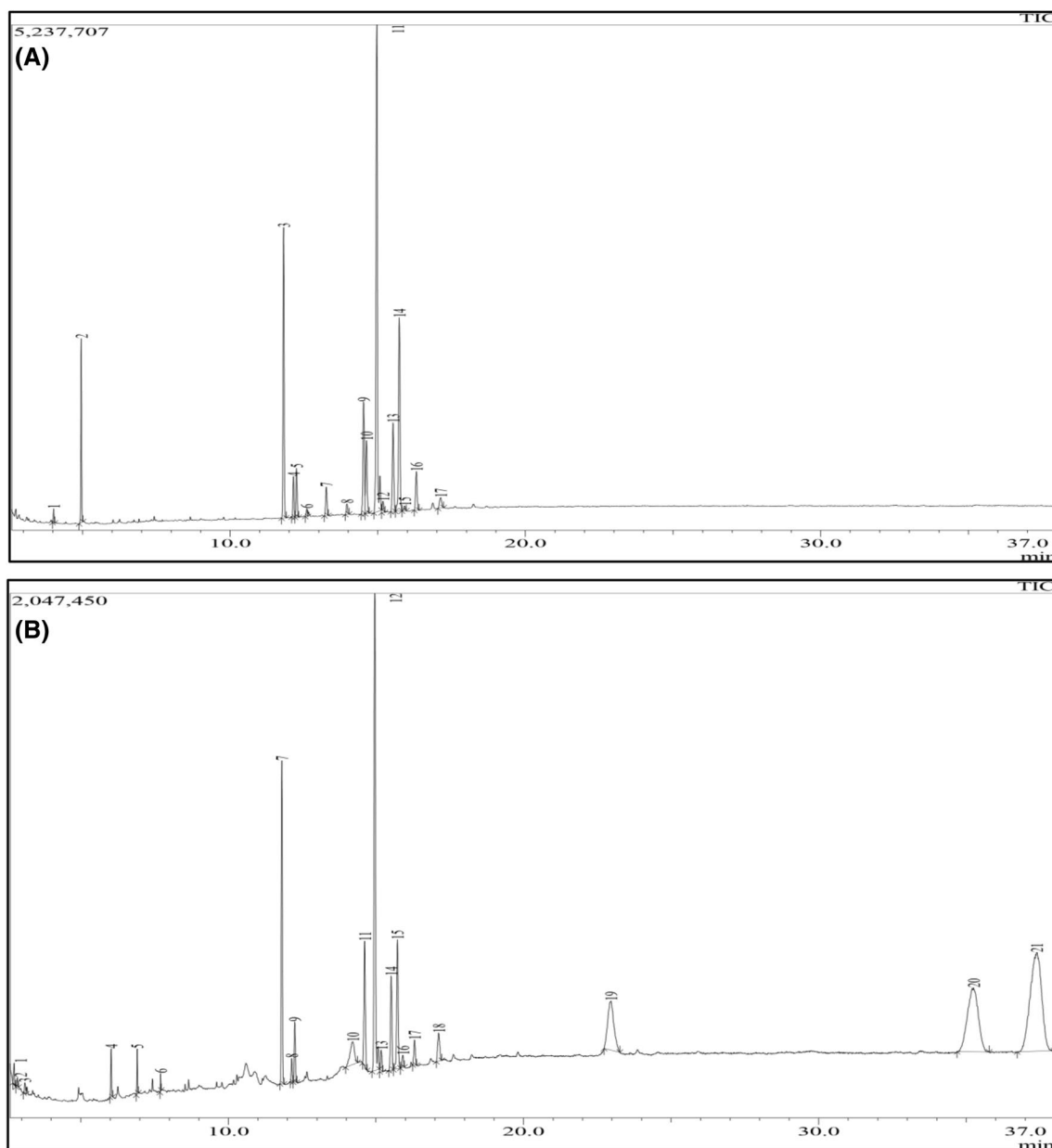


Fig. 2 Representative GC/MS chromatograms of the acid compounds of FOMW (a) and DOMW (b). The order and identification pic was detailed in Table 3

methyl ester, Octadecanoic acid, ethyl ester and 1-Eicosanol. Most of these acids have a little content that is quickly degraded. In addition, we note a remarkable failure for other acids such as, Hexadecanoic acid, methyl ester, 9-Hexadecenoic acid, methyl ester, (*Z*)-;hexadecanoic acid, ethyl ester; 9-Octadecenoic acid, methyl ester, (*E*)-; ethyl oleate 9,12-Octadecadienoic acid (*Z,Z*), methyl ester (*E*) and ethyl (9*Z*,12*Z*)-9,12-Octadecadienoate. However, an appearance of the other acids reduced to know Propionic acid, ethyl ester, propyl ester and acetic acid. Generally, these acids are a direct product of the main fermentation

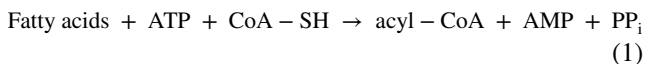
pathway, such as a propionic or acetic fermentation (Staples et al. 2001; Antonopoulou et al. 2010). OMW can produce glycerol and long chain fatty acids (LCFA). According to Schönfeld et al. (2004), long chain free fatty acids (C16–C48) are naturally occurring fats in olive fruits, which exhibit toxic effects towards microorganisms. Indeed, the fermentation of glycerol gives 1,2-propanediol and ethanol, which can be further oxidized to acetate and propionate (Rajhi et al. 2015). Moreover, LCFA can be degraded by β -oxidation releasing, therefore, one molecule of acetate per cycle (Rajhi et al. 2015). To know, a

Table 3 Fatty acids concentration rate in FOMW and DOMW

Fatty acids	FOMW (%)	DOMW (%)
Ethylbenzene	5.893 ^a	0.000 ^b
Styrene	5.893 ^a	0.000 ^b
Hexadecanoic acid, methyl ester	14.681 ^a	8.972 ^b
9-Hexadecanoic acid, methyl ester, (Z)-	2.113 ^a	0.627 ^b
Hexadecanoic acid, ethyl ester	2.651 ^a	1.672 ^b
Ethyl 9-hexadecenoate	0.433 ^a	0.000 ^b
Cyclobutane, 1,3-diphenyl-, trans-	1.800 ^a	0.000 ^b
Cyclopropylphenylmethane(C ₁₀ H ₁₂)	0.676 ^a	0.000 ^b
Benzene, 1,1'-(1,2-Cyclobutanedilyl) Bis-, Trans-	7.704 ^a	0.000 ^b
Octadecanoic acid, methyl ester	4.786 ^a	16.789 ^b
9-Octadecenoic acid, methyl ester, (E)-	34.252 ^a	16.789 ^b
Octadecanoic acid, ethyl ester	0.750 ^a	0.722 ^b
Ethyl Oleate	5.998 ^a	3.286 ^b
9,12-Octadecadienoic acid (Z,Z)-, Methyl ester	13.634 ^a	4.805 ^b
Acetic acid <i>n</i> -octadecyl ester	0.362 ^a	0.474 ^b
Ethyl (9Z,12Z)-9,12-Octadecadienoate #	2.729 ^a	0.902 ^b
1-Eicosanol	1.059 ^a	0.000 ^b
Propanoic acid, ethyl ester	0.000 ^a	0.500 ^b
Acetic acid, Propyl ester	0.000 ^a	0.274 ^b
3,4-Dihydrothienyl-[3,4,B]-5-carboxythiol	0.000 ^a	0.324 ^b
Cycloheptasiloxane, Tetradecamethyl-	0.000 ^a	0.952 ^b
Cyclooctasiloxane, Hexadecamethyl-	0.000 ^a	0.629 ^b
Cyclononasiloxane, Octadecamethyl-	0.000 ^a	0.227 ^b
1-Propen, 3-(2-Cyclopentenyl)-2-methyl-1,1-diphenyl-	0.000 ^a	2.471 ^b
Octacosanol	0.000 ^a	1.391 ^b
Benzene, 1,1',1'',1'''-(1,3,4,6-hexanetetrayl)tetrakis-, (R*,R*)	0.000 ^a	7.330 ^b
(E)-3,3-Diphenyl-4-hexenoic acid	0.000 ^a	16.831 ^b
Methyl (Z)-3,3-diphenyl-4-hexenoate	0.000 ^a	27.460 ^b

Test Student was determined with FOMW and DOMW ($p=0.01$, $*p<0.05$). Mean scores in the same line with different letter (“a” and “b”) are significantly different (for $p<0.05$) (Results are compared for the same parameters and the two different OMW)

possible triglyceride hydrolysis, β -oxidation presented in Eq. 1 can take place (Singh 1997).



Meanwhile, the increase of pH close to 6 led further to an eventual enhancement of the anaerobic digestion process of OMW, during their disposal in the evaporation ponds leading to the degradation of long chain fatty acids by methanogen communities (Speece 1996). Generally, long Chain Fatty Acids (LCFA), in anaerobic condition and at neutral pH can be ionized (Alves et al. 2009). However, studies have shown that LCFA inhibited anaerobic digestion (Neves et al. 2009; Palatsi et al. 2009). These inhibiting potential can be due to the complexity of lipids molecules in addition to their carbon chain length and their saturation (Kuang et al. 2006).

Sugar analysis

Carbohydrates are primarily represented by the parietal components; in particular cellulose and pectin; where they account for approximately 0.6% of the weight of the fresh pulp (Dermeche et al. 2013). Generally, they play an important role in texture olives. Previous studies have shown that the OMW is very rich in simple sugars (Dermeche et al. 2013). Analysis of these OMW shows the predominance of fructose, glucose, sucrose, palatinose and maltose. Sugars generated from different OMW samples are shown in Table 4. The comparison with Fresh and disposal OMW shows any significant difference ($p=0.14$) for the different sugar fractions. As can be seen, an accumulation of sucrose concentration is observed in DOMW. Despite, the sucrose rate was completely removed in DOMW.

Table 4 Sugar concentration rate in FOMW and DOMW; Test Student was determined with FOMW and DOMW ($p=0.14$, $p>0.05$)

Sugar compounds (g/l)	FOMW	DOMW
Fructose (C ₆ H ₁₂ O ₆)	0.689 ^a	3.074 ^b
Glucose (C ₆ H ₁₂ O ₆)	0.689 ^a	1.489 ^b
Sucrose (C ₁₂ H ₂₂ O ₁₁)	0.053 ^a	0.000 ^b
Palatinose (C ₁₂ H ₂₂ O ₁₁)	0.201 ^a	0.462 ^b
Maltose (C ₁₂ H ₂₂ O ₁₁)	0.000 ^a	0.149 ^b

Test Student was determined with FOMW and DOMW ($p=0.14$, $p>0.05$). Mean scores in the same line with different letter (“a” and “b”) are significantly different (for $p<0.05$) (Results are compared for the same parameters and the two different OMW)

With regard to DOMW, we noted an accumulation of fructose and glucose. They can be by-products of the oxidation of intermediates released by the complex chain carbon such as phenol or from long chain fatty acid degradation. Indeed, the OMW disposal in evaporation ponds led a further oxidation of the fermented product that enhances the COD degradation. The complexity of the results requires a more detailed analysis and a further treatment.

Microbial community

It is known that OMW contained all essential elements for microbial growth. However, they may contain several growth inhibitors, such as organic acids and phenolic compounds (Borja et al. 1992). As observed in Table 5, microbial community analysis before and after pond evaporation showed a clear shift in biomass composition. Bacterial aerobic mesophilic community present in the FOMW disappeared completely after disposal in pond evaporation, even though there was available substrate. Since, competing species or new species can be appeared (data not shown). Above all, the Archaea community defined as the act of anaerobic digestion. In fact, the pH increment has been previously reported (Section: The physico-chemical properties) would be enhancing the development of some Methanomicrobiales. It is well known that methanogens are more sensitive to low pH than other anaerobic microorganisms (Speece 1996). In addition, we showed a failure of yeast number. This can be explained by the reduction of the soluble oxygen in the pond evaporation.

Table 5 Microbial diversity in FOMW and DOMW

Samples	MB (CFU/mL)	Yeats (CFU/mL)
OMW F	1005×10^2	$38,100 \times 10^2$
OMW T	0	1632

Antibacterial activity

To valorize the FOMW as an antibacterial agent, MIC against different clinic bacterial strains (Gram-positive and Gram-negative bacteria) were determined. The antibacterial activity shows a MIC value of 32.76 ($\mu\text{g mL}^{-1}$) for the different studied strains (Table 6). A significant important MIC value was registered compared with others works. The MIC was established against bacterial isolates responsible for human intestine and respiratory tract infections such as *Staphylococcus aureus* ($50.00 \mu\text{g mL}^{-1}$) and *Klebsiella pneumoniae* ($50.00 \mu\text{g mL}^{-1}$) (Tafesh et al. 2011). The MIC defined in this study was the result of a combined effect of several phenolic compounds present in the FOMW extract. Indeed, previous studies suggested that bioactivity of the single phenolic component against clinic bacterial strains was found to be very low and required high concentration exceeding $1000 \mu\text{g mL}^{-1}$ to inhibit the growth of the these strains (Obied et al. 2007).

The observed antibacterial activities against Gram-negative and Gram-positive bacteria were similar. In contrast, previous study explains that there is a relationship between antibacterial activity and cell wall composition (Khoufi et al. 2008; Yangui et al. 2008). However, the comparison of antimicrobial data of plant extracts is very difficult, depends on the different factors, such bacterial strains, growth media, inoculum size, methods and the relative purity (Leouifoudi et al. 2015).

Soil chemical and biological and features, behaviour after FOMW and DOMW spreading

All OMW compounds are involved in several chemical and biological soil transformations, which influence their mobility and biodegradation. Indeed, just after spreading, the OMWs modify the soil. Table 7 summarizes the physico-chemical properties and biological quality of two soils treated with OMWF and OMWD, respectively. During the

Table 6 Antibacterial activity of different OMW F concentration ($\mu\text{g mL}^{-1}$) against the flowing bacteria: Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Klebsiella pneumonias*, *Salmonella typhimurium* and *Enterobacter cloacae*)

Culture Studies	Different OMW F concentration ($\mu\text{g mL}^{-1}$)			
	Control	32.76	65.52	131.04
<i>Staphylococcus aureus</i>	+	–	–	–
<i>Klebsiella pneumoniae</i>	+	–	–	–
<i>Enterobacter cloacae</i>	+	–	–	–
<i>Salmonella typhimurium</i>	+	–	–	–

(+) Bacterial growth observed and (–) No bacterial growth observed

Table 7 Physicochemical and microbial soil characteristics of two sites treated with OMWF and OMWD

Parameters	Non-treated soil	Soil treated FOMW	Soil treated DOMW
OM (%)	2.6±0.2	0.87±0.12	2.3±0.26
pH	8.7±0.16	8.64±0.01	7.66±0.01
EC (mS/cm ⁻¹)	0.86±0.05	1.76±0.15	0.48±0.02
Soil respiration (mg CO ₂ g ⁻¹ dry soil)	0.22±0.02	0.66±0.02	0.3±0.01
GI (%)	100	61.65±3.03	170.55±1.88
Mesophilic bacterial (CFU g ⁻¹)	18.93×10 ⁶ ±1	29×10 ⁶ ±1	50×10 ⁶ ±2
Fungi (CFU g ⁻¹)	51×10 ⁶ ±3.6	6×10 ⁶ ±1	12×10 ⁶ ±2
Polyphenols contain (ppm)	–	1850±50	10±800

Non-treated soil was used as a control

– not detected

experimented period, the soil treated with DOMW showed a significant failure of pH and EC values, compared to the soil FOMW spreading. As can be seen, the soil treated with DOMW showed an OM value close to the value found with control soil, which emphasizes the efficiency and the interest of the OMW treatment before use in soil irrigation. Results are similar to those published by Mahmoud et al. (2010) and Munir et al. (2016) showing an increase of soil organic matter after OMW treatments.

Moreover, this OM increase is accompanied by an important rate of soil fertility represented by GI and microflora biomass by 170.55% and 50 × 10⁶ CFU g⁻¹, respectively. Indeed, OMW treatment entails the soil enrichment and the improvement of the yield on the culture. Mekki et al. (2013) showed that microbial growth could increase by soil fertility. The lowest polyphenols soil rate (10 ppm) was recorded when irrigated with DOMW. These results can be explained by the high decrease and removal of phenolic compounds for DOMW (see section *The Phenol Compounds*). In fact, phenolic compounds are non-degradable and can inhibit seed germination and decrease soil fertility. In addition, we note that low-molar-mass polyphenols remained in the DOMW did not affect the bacterial growth (50 × 10⁶ CFU g⁻¹). This result corroborates with previous studies that showed a

lower toxicity of Low-molar-mass polyphenols for bacteria (Fiorentino et al. 2003; Isidori et al. 2005).

However, higher concentrations of salts and polyphenols, found in the FOMW explained the highest rate of EC (1.76 mS/cm⁻¹) found in FOMW spreading soil. Results are similar to those presented by Rinaldi et al. (2003) explaining the increase of soil electrical conductivity. Generally, the increase of EC after direct OMW spreading was attributed to the highest contain of salt concentrations in the non-treated OMW (Di Serio et al. 2008).

Correlation analyses, among soil parameters of the studied sites indicated different significant trends (Table 8). A significant positive correlation between Polyphenols and EC ($r=0.732$) was established. In fact, the soil salinity increases with the content of polyphenols. These results agree with previous results showing a higher rate of soil EC due to the accumulation of polyphenols compounds (Di Serio et al. 2008). However, a positive correlation founded between Bacteria and the index of germination of ($r=0.775$). This result confirmed the increase of soil fertility by the bacterial growth. In addition, a positive correlation with soil organic matter and fungi biomass ($r=0.716$) confirmed that soil organic additives, (such as glucose or/and lipid OMW contain), can cause changes in soil microflora (Stenström

Table 8 Correlation matrix (Pearson's r values) between the different parameters determined in soils subjected to the two different treatments

	pH	EC	OM	Resp	IG	Poly	Bac	Fungi
pH								
EC	0.683*							
OM	-0.310*	-0.873**						
Resp	0.292	0.877**	-0.957**					
GI	-0.908**	-0.908**	0.638	-0.651				
Poly	0.29	0.732*	-0.939**	0.953**	-0.418			
Bac	-0.948**	-0.462	0.034	-0.038	0.775*	0.242		
Fungi	0.418	-0.340	0.716*	-0.710*	-0.045	-0.883**	-0.658	

pH, EC (Electrical Conductivity) OM (Organic Matter), Resp (Respiration), GI (Germination index), Poly (Polyphenol compounds), Bac (Bacterial mesophilic), Fungi (fungi)

* $p < 0.05$; ** $p < 0.01$



et al. 2001). In fact, sugars and lipids can increase bacterial growth. Indeed, previous studies suggested that OMW enriched the soil in organic substrates affecting the nutritional status of the soil; influencing, therefore, the structure of the soil microbial communities (Barbera et al. 2013).

The significant negative correlation founded between polyphenols compound and fungi biomass ($r = -0.883$) could be explained by polyphenols phytopathogenic soil agents (Yangui et al. 2010; Debo et al. 2011). Of it made, the polyphenols content was negatively correlated with OM ($r = -0.939$), the result was in agreement with previous works (Cardinali et al. 2010). Having a phytotoxic effect and antimicrobial activities, polyphenols are the main limiting factor for spreading OMW. In fact, polyphenols are difficult to decompose. Indeed, polyphenols present a microbial toxicity suppressing soil microorganisms and inhibiting their growth (Obied et al. 2007). In addition, the high level of polyphenols in OMW can also pollute surface and groundwater resources (Obied et al. 2007).

However, the negative correlation between Respiration values and OM content ($r = -0.957$) seems to be strange. Indeed, this result was in contrast to previous works which showed a strongly positive effect with these between both soil parameters (Di Serio et al. 2008; Hoorman and Rafiq 2010). The negative correlation between the organic matter and soil respiration in our case could corroborate with previous studies which demonstrated that increasing soil OM is a strategy for sequestering carbon dioxide (CO₂) a greenhouse gas (Luo et al. 2010; Liu et al. 2014; Barton et al. 2016).

Conclusion

The natural biological treatment of OMW, which is obvious in the disposal of OMW in an evaporation pond promoted an important phenol compounds and long chain fatty acid degradation. Ponds disposal OMW also affected the microbial biomass composition. Both shift in microbial and LCFA composition was a consequence of an anaerobic digestion, which promoted further oxidation of organic by-products, thus generating more reduced product and led to disappearance of total aerobic mesophilic bacteria, which in turn prevented the growth of anaerobic bacteria. The profitable effect of DOMW was successfully presented after the DOMW soil spreading. Indeed, we note an important development of the soil microorganism communities accompanied by a very spectacular soil fertility without forgetting that FOMW can be also valorized as soon in the axis of powerful antibacterial activity against certain standard clinical species.

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