

REVIEW

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Review: Effects of olive mill wastewater application on soil properties and plants growth

Ali Mekki*, Abdelhafidh Dhouib and Sami Sayadi

Abstract

Comparative effects of untreated olive mill wastewater (UOMW), treated olive mill wastewater (TOMW) and bioaugmented olive mill wastewater (BOMW) on soil properties, on seeds germination and on plants growth were investigated.

The water holding capacity, the salinity, the organic carbon content, humus, total nitrogen, phosphate and potassium increased when the spread amounts of UOMW (50, 100 and 200 m³ ha⁻¹.year⁻¹) or TOMW increased. TOMW increased the total mesophylic number while the number of fungi and nitrifiers decreased. Actinomycetes and spore-forming bacteria were neither sensitive to TOMW nor to UOMW. The total coliforms number increased with higher doses of TOMW and UOMW.

Hazard assessments of toxicity were conducted for UOMW, untreated olive mill wastewater organic extract (UOE), TOMW, treated olive mill wastewater organic extract (TOE) and extracts of soils amended with UOMW (S_{UOMW}) and with TOMW (S_{TOMW}).

Results showed an increase in the germination index when seeds species were cultivated with TOMW. Plants irrigated by TOMW showed an improvement in biomass, spike number, plants growth and a similar or even better dry productivity than plants irrigated with water.

Keywords: Microbial communities; Olive mill wastewater; Phenolic compounds; Soil fertility; Toxicity

Review

Highlights

Olive mill wastewater (OMW) is the liquid by-product generated during olive oil production. The annual production of OMW in Mediterranean countries reached 30 million cubic meters and 700 000 cubic meters in Tunisia alone. Olive mill wastewater is a critical problem; this waste contains an enormous supply of water, organic and inorganic matters. For these reasons, increasing attention has been given to find the best methods to spread OMW on agricultural lands and to recycle both the organic matter and the nutritive elements in the soil crops system. Moreover, agricultural irrigation with wastewater effluents became a common practice in arid and semiarid regions, where it was used as a readily available and inexpensive option to fresh water. Latest studies have investigated the effects of untreated OMW on soil characteristics and microbial activities and the application of OMW for soil

irrigation represents now a controversy discussion and a debate of actuality between those that are for and those that are against this strategy. For this purpose, this work built on previous studies in the same Laboratory (Sayadi and Ellouz 1995; Sayadi et al. 2000; Dhouib et al. 2005; Mekki et al. 2006a; Mekki et al. 2006b; Mekki et al. 2007; Mekki et al. 2008; Mekki et al. 2009; Mekki et al. 2012) attempted to assess the benefits of reusing treated OMW in ferti-irrigation. A comparison of their effects with those of the application of untreated OMW on seeds germination, plants growth and soil fertility were undertaken.

Introduction

Olive mill wastewater (OMW) is the liquid by-product generated during olive oil production (Mekki et al. 2009). The OMW annual production in Mediterranean countries reached 30 million cubic meters and 700 000 cubic meters in Tunisia alone (Dhouib et al. 2006; Kapellakis et al. 2006). OMW is a critical problem; this waste contains an enormous supply of organic matter, COD between 40 and 210 g. m⁻³ and BOD₅ between 10

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and 150 g. m⁻³ (Saadi et al. 2007). Some OMW characteristics are favourable for agriculture since this effluent is rich in water, organic matter, nitrogen, phosphorous, potassium and magnesium (Lesage-Meessen et al. 2001). Furthermore, agricultural irrigation with wastewater effluents became a common practice in arid and semiarid regions, where it was used as a readily available and inexpensive option to fresh water (Oved et al. 2001). The most frequently used method nowadays to solve the problems associated with this wastewater is the direct application to agricultural soils as organic fertilizers (Komilis et al. 2005; Mekki et al. 2006a). In this perspective, several studies found positive effects of OMW on soil fertility and crops growth (Casa et al. 2003; Cereti et al. 2004; Paredes et al. 2005). Feria (2000) and Rinaldi et al. (2003) indicated that OMW spreading does not result in heavy metal accumulation in the soil. However, recent studies found that the addition of unprocessed OMW causes significant shifts in the structure and function of microbial communities which in turn influences the soil fertility (Sierra et al. 2001; Mekki et al. 2006b, 2007). Moreno et al. (1987) warned that untreated OMW application causes serious environmental problems due to its antibacterial effects and its phytotoxicity. According to Levi-Menzi et al. (1992) the high COD value and the presence of phytotoxic and antibacterial polyphenols in OMW can be a serious pollution risk for superficial and underground waters. Moreover, the presence of phenolic compounds in OMW makes them highly toxic and ecologically noxious (Capasso et al. 1992; Aggelis et al. 2003).

To solve the problems associated with OMW, different treatments methods such as aerobic treatment, anaerobic digestion and composting process have been proposed (Sayadi and Ellouz 1995; Ehaliotis et al. 1999; Peredes et al. 2000; Kissi et al. 2001; Marques 2001; D'Annibale et al. 2004; Abid and Sayadi 2006).

An integrated approach using a pre-treatment of the UOMW with the white-rot fungus *Phanerochaete chrysosporium* followed by an anaerobic digestion was developed in our Laboratory (Laboratory of Bioprocesses, Center of Biotechnology of Sfax, Tunisia) in order to reuse the effluent in agriculture (Sayadi et al. 2000; Dhouib et al. 2005; Khoufi et al. 2006). Thus, the aim of our work was to investigate the effects of untreated (UOMW), treated (TOMW) and bioaugmented (BOMW) olive mill wastewater on soil physicochemical and biological properties, on microbial communities' respiration, on seeds germination and on plants growth.

This review is a synthesis of previous works in the same Laboratory (Sayadi and Ellouz 1995; Sayadi et al. 2000; Dhouib et al. 2005, 2006; Mekki et al. 2006a, b, 2007, 2008, 2009, 2012) attempted to assess the benefits of reusing TOMW in ferti-irrigation. A comparison of their effects

with those of the application of UOMW on seeds germination, plants growth and soil fertility were undertaken.

Olive mill wastewater characterization

OMW contain an enormous supply of organic matter very rich in phenolic compounds, which are toxic. Untreated olive mill wastewater (UOMW) is an acidic effluent with a high nutrient content that can be used to fertilize the soil. However, UOMW had an elevated chemical oxygen demand and a high phenolic content, which have toxic properties. UOMW totally inhibited *V. fischeri*. This toxicity was essentially due to its high content of phenolics compounds. Its C/N ratio was unfavourable for the biodegradation and humification processes (Mekki et al. 2006b).

However, treated olive mill wastewater (TOMW) is a slightly alkaline effluent, rich in inorganic loads such as potassium, calcium, magnesium and iron. Its phenolic compounds content was lower than 1 g L⁻¹, reflecting a significant reduction of its toxicity from 99% B_I in UOMW to only 30% B_I. This high content of non-toxic organic compounds, macro-elements and micro-elements indicated a significant fertilizing potential of the TOMW that could be used advantageously in agronomy (Table 1).

Table 1 Physicochemical characteristics of untreated olive mill wastewater (UOMW) and treated olive mill wastewater (TOMW) (Mekki et al. 2006b)

Characteristics	UOMW	TOMW
pH (25°C)	5 ± 0.2	8.1 ± 0.2
Electrical conductivity (25°C) (dS m ⁻¹)	8.1 ± 0.1	14.2 ± 0.1
Chemical oxygen demand (g L ⁻¹)	53.3 ± 1.8	4.5 ± 0.41
Biochemical oxygen demand (g L ⁻¹)	13.42 ± 0.8	1.8 ± 0.16
COD/BOD ₅	4 ± 0.72	2.5 ± 0.45
Water content (g L ⁻¹)	960.6 ± 19	984 ± 19
Total solids (g L ⁻¹)	39.4 ± 1.8	16 ± 0.8
Mineral matter (g L ⁻¹)	6.5 ± 0.3	10.15 ± 0.5
Volatile solids (g L ⁻¹)	33 ± 1.5	4.8 ± 0.2
Total organic carbon (g L ⁻¹)	17.6 ± 0.88	3.2 ± 0.16
Phenolic compounds (g L ⁻¹)	8.6 ± 0.5	0.77 ± 0.08
Total nitrogen (g L ⁻¹)	0.5 ± 0.05	0.25 ± 0.03
Carbon/nitrogen	35.2 ± 7.04	12.8 ± 2.56
Toxicity by LUMISTox (B _I (%))	99 ± 2	30 ± 0.7
Phosphore (P) (mg L ⁻¹)	36 ± 3.6	15 ± 1.5
Sodium (Na) (g L ⁻¹)	0.8 ± 0.08	0.86 ± 0.09
Chlorures (Cl) (g L ⁻¹)	1.45 ± 0.15	1.3 ± 0.13
Potassium (K) (g L ⁻¹)	8.6 ± 0.8	5.34 ± 0.5
Calcium (Ca) (g L ⁻¹)	0.9 ± 0.09	3.2 ± 0.3
Iron (Fe) (mg L ⁻¹)	23.4 ± 2.3	38.3 ± 3.8
Magnesium (Mg) (mg L ⁻¹)	186.9 ± 18.7	281 ± 28.1

OMW impacts on the physicochemical soil properties

The soil of the study area had an important content of active calcareous (0.6% w/w) at the surface, and was composed of sand (89.82% w/w), clay (7.44% w/w) and silt (2.74% w/w). It had an alkaline pH and a weak electrical conductivity (Mekki et al. 2006a). The soil was very poor in nitrogen (0.5 g kg⁻¹ dry soil) and in organic matter (0.16% w/w). The levels of potassium and phosphorus were 0.014 (% w/w) and 0.002 (% w/w), respectively. The soil water content was very low and it varied between 0.8% and 1.15 (% w/w) (Table 2).

The addition of treated or untreated OMW without or after C/N ratio correction did not show any effect on the initial soil pH (Mekki et al. 2009). Indeed, in spite of the initial UOMW acidity, the follow-up of this parameter during 6 months showed that these OMW provoked no significant reduction in the soil pH (0.2 U), whereas the addition of TOMW provoked a weak augmentation. Similarly, OMW application increased soil electrical conductivity (EC), and this increase was proportional to the added OMW quantity.

The studied soil was initially poor in organic matter (OM). OMW improved the soil organic and mineral matter's contents. The follow-up of the biodegradation kinetics of this OM brought by OMW revealed that for the same quantity of added OM, soil receiving the TOMW exhibited a potential of biodegradation three times bigger than that of soil receiving UOMW (Mekki et al. 2009).

Soil irrigated with TOMW presented more important total nitrogen content in comparison with soil receiving UOMW. The weak reduction in the total nitrogen or even its constancy as a function of amended OMW and time could be explained by the retraining of the different soil nitrogen shapes.

OMW phenolic compounds dynamics

UOMW application increased the total phenolic compounds content in all soil layers. Besides that the majority of phenolic compounds are kept in the soil upper layers. The phenolic concentration decreased rapidly from 0 to 25 cm then continued to decrease weakly with depth but remained even detectable at 120 cm. Comparison of phenolic compounds spectra shows that especially the high molecular-mass compounds decreased with depth while the low molecular-mass polyphenols remained more abundant (Mekki et al. 2007).

According to the HPLC principle, polymers were eluted with low retention time, while monomers needed higher retention time. HPLC analyses show that phenolic compounds with low retention time were detected in upper layers (0–25 cm), while higher retention time phenolic compounds infiltrated in deeper layers of the soil (50–120 cm).

OMW impacts on the biological soil properties

The aerobic heterotrophic bacteria counted on the studied soil were relatively weak (10⁵–10⁶ CFU g⁻¹ of dry soil). OMW addition induced an elevation of the total heterotrophic bacteria count of the soil microflora (Table 2). The control soil was very poor in organic nitrogen; so, the number of nitrifiers was feeble. The OMW addition enlarged, in a meaningful manner, their number. This increase was more remarkable in soils receiving TOMW. As it was the case for the nitrifying bacteria, the OMW addition enhanced the denitrifier's subsistence, whose number increased correlatively with the added OMW quantity.

The soil's urease and ammonium oxidases activities were stimulated distinctly in soils irrigated with TOMW,

Table 2 Physicochemical and biochemical characteristics of unamended soil (C), soil amended with untreated olive mill wastewater (S_{UOMW}) and soil amended with treated olive mill wastewater (S_{TOMW}) (Mekki et al. 2006b)

Soil characteristics	C	S _{UOMW}	S _{TOMW}
pH (21°C)	8.4 ± 0.2	8.26 ± 0.2	8.9 ± 0.2
Electrical conductivity (dS m ⁻¹)	0.2 ± 0.1	0.5 ± 0.1	0.7 ± 0.1
Water content (mg kg ⁻¹)	11 ± 0.35	18.1 ± 0.36	28.3 ± 0.57
Organic matter (%) (w/w)	1.8 ± 0.18	4.5 ± 0.45	2.9 ± 0.29
Total nitrogen (%) (w/w)	0.1 ± 0.01	0.12 ± 0.01	0.16 ± 0.015
Carbon/nitrogen	10.4 ± 2.1	21.75 ± 4.35	10.37 ± 2.1
Aerobic bacteria counts (10 ⁶ CFU g ⁻¹)	58 ± 6	60 ± 6	71 ± 7
Nitrifiers counts (10 ² MPN g ⁻¹)	1.4 ± 0.14	0.8 ± 0.08	22 ± 2.2
Urease (µg NH ₄ -N g ⁻¹ 2 h ⁻¹)	42 ± 0.42	38 ± 0.38	112 ± 11.2
Nitrate reductase (µg NO ₂ -N g ⁻¹ 24 h ⁻¹)	0.53 ± 0.05	1.42 ± 0.14	1.18 ± 0.12
Ammonium oxydase (µg NO ₂ -N g ⁻¹ 24 h ⁻¹)	0.24 ± 0.02	0.17 ± 0.02	0.71 ± 0.07
Xylanase (µgGE g ⁻¹ 24 h ⁻¹)	34.6 ± 3.5	106.5 ± 10.7	124.5 ± 12.5
Cellulase (µgGE g ⁻¹ 24 h ⁻¹)	21.7 ± 2.2	26.8 ± 2.7	58.4 ± 5.8

CFU: colony-formant unit; GE: glucose equivalent; MPN: most probable number; NH₄-N: ammonium nitrogen; NO₂-N: nitrate nitrogen; w/w: weight/weight.

whereas the UOMW addition inhibited these two enzymatic activities (Mekki et al. 2006b).

UOMW inhibited the soil respirometric activity, while TOMW exhibited significantly higher respiration levels compared to the unamended and the UOMW amended soils. The ratio of $C-CO_2/C_{tot}$ decreased from 6.7 in the unamended soil to 6.34, to 2.74 and to 1.6 in soils amended consecutively with 50, 100 and 200 $m^3 ha^{-1}$ year⁻¹ of UOMW. Based on these results, the UOMW dose of 50 $m^3 ha^{-1}$ showed the elevated $C-CO_2/C_{tot}$ ratio in comparison with the two other doses (100 and 200 $m^3 ha^{-1}$) so that it was chosen to test the effects of its bioaugmentation with *P. chrysosporium* on the soil biodegradation activities. The soil amended with 50 $m^3 ha^{-1}$ of bioaugmented olive mill wastewater (BOMW) showed higher $C-CO_2/C_{tot}$ ratio in comparison with control soil (unamended) and with soil amended with 50 $m^3 ha^{-1}$ of UOMW. The $C-CO_2/C_{tot}$ ratio increased from 6.34 in the soil amended by 50 $m^3 ha^{-1}$ of UOMW and 6.7 in the control soil to 27 (nearly 4 fold) in soil amended by BOMW (Mekki et al. 2012).

Hazard assessments of toxicity were conducted for UOMW, untreated olive mill wastewater organic extract (UOE), TOMW, treated olive mill wastewater organic extract (TOE) and extracts of soils amended with UOMW (S_{UOMW}) and with TOMW (S_{TOMW}). Measures of toxicity were achieved by the determination of the bioluminescence inhibition (B_I (%)) of *Vibrio fischeri* and by the growth inhibition (GI) of *Bacillus megaterium*, *Pseudomonas fluorescens* and *Escherichia coli*. A B_I of *V. fischeri* of 100%, 100%, 65%, 47%, 46% and 30% were obtained with UOMW, UOE, TOMW, TOE, S_{UOMW} and S_{TOMW} respectively. Indeed, even diluted 24 times, a significant B_I of 96% was obtained by UOMW. However, only 30% B_I was obtained by 24 times diluted TOMW. Whereas, 24 times diluted, S_{UOMW} and S_{TOMW} did not show a significant B_I . The GI of *B. megaterium*, *P. fluorescens* and *E. coli* were, respectively, 93%, 72% and 100% by UOMW; 100%, 80% and 100% by UOE; 70%, 60% and 89% by TOMW; 63%, 54% and 68% by TOE; 39%, 27% and 43% by S_{UOMW} and 23%, 0% and 34% by S_{TOMW} . The incubation of UOMW or TOMW in the soil during four months reduced their toxicity by 54% and 35%, respectively (Mekki et al. 2008).

OMW impacts on seeds germination and crops growth

Seeds germination was conducted both on UOMW and on TOMW. The results showed that seeds germination was strongly inhibited for all the studied species when UOMW dilution was lower than 1/10 (UOMW/Water). For the TOMW diluted to 1/10 (TOMW/water), positive effects on all seeds germination were observed and the germination ratio was higher than in the control. TOMW did not show any inhibitory effect on seeds germination

and all crops presented a high germination ratio (>50% in all species used) (Mekki et al. 2006b).

In order to evaluate UOMW and TOMW on plants growth, some agronomic tests were performed in field experiments. The same species used for the germination tests were also used for this study. TOMW application did not show any morphological or physiological inhibition effect on any of the species used. Indeed, the maximum height of the treated plants was better than that of the control ones, especially for *Vicia faba* and *Cicer arietinum*. The average protein content, productivity, grain weight and the number of spikes per plant were the most sensitive yield components to the treatments and the most important for seed yield. The positive effects of the TOMW ferti-irrigation seemed evident, allowing optimal ripening and kernel filling. The chlorophyll a/chlorophyll b ratio and the root/shoot ratio were similar to control species values. The amounts of organic nitrogen and proteins in plants irrigated with TOMW were comparable with the control species or sometimes better, as for *Hordeum vulgare* and *Cicer arietinum* (Mekki et al. 2006b).

Discussion

Several studies have been devoted to develop efficient treatment technologies for OMW through various kinds of physicochemical and biological pretreatments (Mantzavinos and Kalogerakis 2005). Yet, such systems are in many cases not economical, considering the short olive oil season, the typical biennial olive harvest cycle, and the fact that many olive mills are small and isolated (Azbar et al. 2004). Results presented here showed that several chemical and biochemical properties of the investigated soils changed in response to UOMW, TOMW and BOMW application.

The UOMW acidity was compensated by the soil carbonate alkalinity as given away by Sierra et al. (2001). The raise in the soil salinity could result from the main ionic species (Na, Cl and SO_2), which came from UOMW (Zenjari and Nejmeddine 2001). Achak et al. (2009) reported that the OMW acidity was due to the presence of phenolic and fatty acids, subsequently the application of this effluent to soils could accumulate salts and phytotoxic compounds, change pH and leach nutrients that could contaminate the ground water source.

The use of *P. chrysosporium* for the practical treatment of OMW was investigated because this fungus could significantly reduce the color of this effluent and degrade the high and low molecular-mass aromatics compounds (Sayadi et al. 2000). Wang et al. (2008) and Taccari et al. (2009) reported that white rot fungi (WRF) had a good ability to degrade and metabolize polymeric lignin and a broad range of organopollutants.

Addition of the untreated or the treated OMW to the soil created some modifications in the average values for total number of microorganisms and their repartition. Our results showed an initial increase in the numbers of CFU in most microflora groups after the OMW amendment, excepted for nitrifiers which decreased. In line with this finding, Peredes et al. (2000) reported also an increase in the total viable counts in the soil polluted with OMW. The chemolithotrophic ammonia-oxidizing bacteria (AOB) are responsible for the first rate limiting step in nitrification in which ammonia (NH_3) is transformed to nitrate (NO_3^-) via nitrite (NO_2^-). The AOB play a critical role in the natural nitrogen cycle (Oved et al. 2001). This microflora could be affected by a variety of chemical conditions including aromatic compounds and salts (Mendum and Hirsch 2002). Some authors reported that higher pH is not favourable for some phylogenetic groups of nitrifying bacteria (Kowalchuk et al. 2000). Moreover, some residual polyphenolic compounds present in TOMW may be toxic for this sensitive category of microorganisms (Paredes et al. 1987). Actinomycetes and spore-forming bacteria play a significant role in the organic matter cycle in nature, by virtue of their considerable powers and ability to break down complex organic molecules. Actinomycetes counts were strongly enhanced by treated and untreated OMW amendment. The introduction of organic pollutants, which can potentially act as toxic substances and nutrient sources, was shown to preferentially stimulate specific populations (Atlas et al. 1991). The increase of the CFU count of spore-forming bacteria was in accordance with the earlier investigations of Paredes et al. (1987).

Fungi populations are known by their considerable depolymerising enzymes and their resistance to recalcitrant substances. The OMW enhanced fungi, the most important organisms decomposing lignin and polyphenols (Borken et al. 2002). Consequently, this population was favoured in soil amended by UOMW, where pH and C/N ratio were also more favourable compared to the control. This observation confirms previous findings by Perkiomaki and Fritze (2002).

The requirements for determining the activities of a large number of enzymes were emphasized to provide information on soil microbial activities (Sukul 2006). Urease and ammonium oxidases constituted two major enzymes of nitrogen metabolism. Urease played a key role in the transformation of the organic nitrogen in ammoniacal and assimilated nitrogen (N-NH_4). Ammonium oxidases assured the transformation of the product of the ammonification in plants' assimilated nitrogen (Tscherko et al. 2003). These two enzymatic activities were stimulated distinctly in soils irrigated with OMW. In this context, Deni and Penninckx (1999) mentioned that the addition of hydrocarbon to an uncontaminated

soil stimulated immobilization of nitrogen and reduced nitrification and soil urease activity. In contrast, Piotrowska et al. (2006) announced a rapid increase in soil respiration, deshydrogenase and urease activities and the microbial biomass of OMW amended soils.

UOMW increased the soil carbon content while the specific respiration remained very low. However, the amendment with TOMW positively affected the soil-specific respiration. Indeed, Piperidou et al. (2000) proclaimed that the wealth of UOMW organic matters in toxic phenolic compounds made its biodegradation difficult. Zheng and Obbard (2002) and Dzul-Puc et al. (2005) reported that the lignin-degrading WRF *P. chrysosporium* had the ability to degrade a wide variety of organopollutants such as polycyclic aromatic hydrocarbons due to its non-specific extracellular enzymes. These investigations were aligned with our results viewing that the bioaugmentation of $50 \text{ m}^3 \text{ ha}^{-1}$ by *P. chrysosporium* was the very beneficial for the stimulation of the respirometric and consequently the biodegradation activities of soil autochthonous microflora.

The study of phenolic compounds dynamics showed that compounds of low molecular-mass migrated more in depth than those of high molecular-mass. This is in line with previous findings of a correlation between places of olive oil mill waste spreading and wells with high phenolic concentrations (Spandre and Dellomonaco 1996).

The acute toxicity of UOMW, UOE, T, TOE, S_{UOMW} and S_{TOMW} , was assessed on the marine bacterium *V. fischeri* and on representing soil and aquatic bacteria as *B. megaterium*, *P. fluorescens* and *E. coli*. Toxicity assays based on bioluminescence in *V. fischeri* can provide a rapid assessment of chemical toxicity (Ribo 1997). They are widely used for routine screening of waste effluents or as part of more elaborate environmental assessments that involve several forms of bioassay and employ a range of different organisms (Jennings et al. 2001).

UOMW totally inhibited the bioluminescence of *V. fischeri*. This toxicity was essentially due to its high content of phenolic compounds and more precisely to phenolic monomers as hydroxytyrosol and tyrosol. Indeed, similar toxicity was obtained with the UOE which is essentially composed by hydroxytyrosol and tyrosol. These findings are in line with previous findings of Dhouib et al. (2006) who put in evidence the toxicity exercised by the main phenolic monomers of the OMW on the microbial flora implied in the treatment of this waste. Fiorentino et al. (2003) reported that the most toxic fraction to the test organisms *Pseudokirchneriella subcapitata* (alga), *Brachionus calyciflorus* (rotifer) and the two crustaceans *Daphnia magna* and *Thamnocephalus platyurus* was the low molecular weight (350 Da) and especially catechol and hydroxytyrosol, the most abundant components of

this fraction. Allouche et al. (2004) and Obied et al. (2005) reported that compounds found in OMW that exhibited antibacterial activity were tyrosol, hydroxytyrosol, oleuropein, 3–4 dihydroxyphenyl acetic acid, and 4-hydroxybenzoic acid. Our results showed that the treatment of the OMW reduced considerably its phenolic content and eliminate essentially phenolic monomers. In line with this, Sierra et al. (2001) showed the fast degradation of these monomers by the biologic activities of soil or their infiltration in the deep layers of soil.

The monitoring of the growth of bacteria representing the soil or the aquatic microflora as *B. megaterium*, *P. fluorescens* and *E. coli* cultivated in the presence of OMW is very instructive and permits to predict their behaviour, the day where they will be in contact with this waste or submitted to its toxicity in the nature. The bacterial responses regarding various substrates UOMW, UOE, TOMW, TOE, SU or ST were different. The GI values for *B. megaterium*, *P. fluorescens* and *E. coli* allow visualizations of the fact that the *E. coli* response is the most sensitive to the toxic effect of monomers present in UOMW and UOE. *P. fluorescens* showed the high resistance to OMW toxicity. This is quite normal because this bacterium is known by its powerful capacity to degrade the recalcitrant compounds and its ubiquitous distribution in soil and water environments. This bacterium has often been found during biodegradation studies of petroleum hydrocarbons contaminated samples (Bugg et al. 2000; Abbondanzi et al. 2003; Evans et al. 2004). On the other hand, Ramos-Cormenzana et al. (1996) noted that antibacterial activity of OMW phenolic compounds was higher on Gram positive than on Gram negative bacteria.

Conclusions

Olive mill wastewater constitutes a serious environmental problem. Several physico-chemical and biological processes to reduce their contaminant impacts have been proposed. Many researchers have established that this wastewater have a high fertiliser value when applied to the soil.

Soils in semi-arid and arid areas are known to have low organic matter levels, a low fertility and a high exposure to degradation, desertification and pollution. Currently, organic wastes of various origins and nature are widely used as amendments to increase soil organic matter and crop productivity.

Treated olive mill wastewater still contains relatively high organic matter amounts in an important volume of water and could be used as a potential fertilizer, especially for soils and crops.

Abbreviations

OMW: Olive mill wastewater; UOMW: Untreated olive mill wastewater; TOMW: Treated olive mill wastewater; BOMW: Bioaugmented olive mill wastewater; UOE: Untreated olive mill wastewater organic extract; TOE: Treated olive mill wastewater organic extract; S_{UOMW} : Soil amended

with UOMW; S_{TOMW} : Soil amended with TOMW; COD: Oxygen chemical demand; BOD_5 : Biochemical oxygen demand; EC: Electrical conductivity; OM: Organic matter; *P. chrysosporium*: *Phanerochaete chrysosporium*; *V. fischeri*: *Vibrio fischeri*; *B. megaterium*: *Bacillus megaterium*; *P. fluorescens*: *Pseudomonas fluorescens*; *E. coli*: *Escherichia coli*; Bi: Bioluminescence inhibition; Gi: Growth inhibition; CFU: Colony formant unity.

Competing interests

The author declares that they have no competing interests.

Authors' contributions

This work built on previous studies in the same Laboratory. Abdelhafidh Dhouib and Sami Sayadi contribute by development of an integrated approach using a pre-treatment of the UOMW with the white-rot fungus *Phanerochaete chrysosporium* followed by an anaerobic digestion. Ali MEKKI as Corresponding author contributes by the most part of this work as the studies of effects of various OMWs on soil biochemical properties and on crops growth. All authors read and approved the final manuscript.

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