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Effect of Olive Mill Vegetable Water Spreading on Soil Microbial Communities and Soil Properties

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Abstract: Olive Mill Vegetable Water (OMVW) discharge is one of the most challenging environmental problems in Mediterranean countries. To the various treatment process proposed, controlled land spreading of untreated OMVW has been suggested as an alternative solution. The Effect of OMVW spreading on soil microbial communities and soil physicochemical characteristics was investigated in this study. A field study was conducted in the Saïs Valley in Morocco, between April 2004 and October 2004. The experiment included two application levels of OMVW (8 and 16 l m^{-2}). Results showed that the abundances of soil total microflora, fungi, yeasts and actinomycetes enhanced after OMVW spreading with levels of 8 and 16 l m^{-2}. This enhancement occurs after a latency phase that is longer when the amount spread is more important. Temporary decrease in soil pH, increased conductivity and elevated phenols concentrations were also observed. No correlation was found between profiles of soil phenolics and soil microflora after OMVW spreading with 8 and 16 l m^{-2}. OMVW spreading caused an enhancement of soil humification that could de beneficial for soil fertility.

Key words: Olive mill wastewater · spreading · phenolic compounds · soil · microflora · Morocco

INTRODUCTION

The disposal of Olive Mill Vegetable Water (OMVW) is a critical and still increasing problem in Mediterranean countries that produced the whole world production of olive oil [1]. The olive oil industry worldwide and especially in the Mediterranean and Middle East countries is constantly growing, with an average annual growth of ca. 5% in world production over the last 15 years [2]. Moroccan industrial olive oil produces huge amounts of OMVW corresponding to nearly 250000 m^3/year. Some characteristics of this material are favourable for agriculture since this effluent is rich in organic matter, nitrogen (N), phosphorous (P), potassium (K) and magnesium (Mg), substantial amounts of plant nutrientss [2-4]. However, OMVW contains compounds with recognized toxicity towards plants and soil living microorganisms [5-7]. Phenolic compounds are the OMW fraction which is considered as the principal cause of toxicity [7-10].

The effect of OMW spreading on soil physicochemical characteristics was reviewed by several researchers [4, 11-13] but less interest was given to the effect of OMVW spreading on soil microflora [14]. Soil microflora is so interesting to prospect because it contributes to organic matter modification and stable humus formation.

In north Mediterranean countries, some laws have been established about soil capability to endure the OMVW spreading, particularly in Italy (Law N°574, 1996) [15]. In Morocco, up to now, investigations undertaken were interested to biological and/or physico-chemical treatment of OMVW [16]. Little interest was given to the valorisation of OMVW in agricultural field. OMVW agronomic valorisation is interesting in south Mediterranean countries that produce huge quantities of OMVW and when water everlastingness and soils poverty are essential limits for development. The purpose of this work was to contribute to the knowledge of the effects of untreated OMVW spreading on a Moroccan

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soil and to evaluate the effects on soil microflora and soil characteristics.

MATERIAL AND METHODS

OMW origin and collection: The fresh OMVW was taken from a press extraction factory located in Fès city, Morocco. and stored in closed plastic tanks at 4°C before use. OMVW physico-chemical characterization is presented in Table 1.

Study site: The study area consisted in a field of the botanic garden of the Faculty of Sciences and Techniques (FST), Saïs, Fès in Morocco. The weather typical Mediterranean, semiarid to arid, with an average rainfall of 400 mm/year and an average annual temperature of 18-20 °C. The field was divided in three plots of 5m^2 that were seeded with maize. Plots C, P1 and P2 were amended in April 2004 with 0, 8 and 16 l m^{-2} of fresh untreated OMVW respectively. Plots did not receive any OMVW before. The soil is constituted by 13.42% of sand (Ø>0.63mm) and 86.58% clay-silt (Ø<0.63mm) (in the 10 cm upper layer). Soil samples were collected from different parts of each plot in the top 10 cm of soil layer which is the most relevant to rhizosphere and microflora activity. Samples were taken from four random locations in each plot.

For each plot, soil analysis was followed-up for a period of six months just after OMVW spreading except for the plot (8 l m^{-2}) for which there was no sampling in April. All soil samples, taken from each plot were mixed, air-dried, sieved with a mesh size of 2 mm and stored at 4°C prior to use.

Physicochemical determination: OMVW was analyzed for COD according to standard method (COD meter, HACH). BOD was determined by the manometric method with a respirometer (OXI TOP IS6). pH and conductivity of soil were determined in a soil/distilled water mixture agitated for two hours: 1/2.5 (V/V) for pH and 3/2 (V/V) for conductivity. Soil humus was estimated by Tiurin method that consists in humus acidification with $K_2Cr_2O_7$ 0.4N. There was no humus measure for April (16 l m^{-2}) because of the high soil organic matter load limiting for the Tiurin method. OMVW total phenols were determined using the Folin-Ciocalteu colorimetric method [17] using acid tannic as standard. Soil phenolic compounds were determined in a 1:25 (W/V) aqueous extract, which was shaken for 12h, then phenolic compounds were quantified in the extract by means of the Folin-Ciocalteu method [18]. OMVW total phosphorus and total nitrogen were determined according to Rodier [19]. Dry weight and moisture content were determined by weighing samples before and after drying at 105°C.

Table 1: OMVW characterization

Parameter	Value
pH (25°C)	4.70
Electric conductivity (25°C) (mS cm^{-1})	12.01
COD (g O_2 l^{-1})	120.00
BOD_5 (g O_2 l^{-1})	67.50
Total P (g l^{-1})	0.079
Total N (g l^{-1})	0.303
Total phenols (g l^{-1})	7.30
Fats	2.6%

Microbiological determination: Microbial counts (Colony forming units; CFU) were conducted using the spread plate method. Soil inocula were prepared by suspending 5 g of the soil in 100 ml of sterile physiologic water (0.9% NaCl in bidistilled water, pH 7.0) containing 100 μl of tween 20, then shaking with a rotary shaker (150 rpm for 30 min). Serial 10-fold dilutions of the samples were plated in triplicate in a 0.9% NaCl solution on different medium: Tryptone-Soja-Agar (Biokar Diagnostics, France) for total microflora, Yeast Extract Peptone Glucose (YPG) containing ampicillin and chloramphenicol for yeasts, Malt extract (Biokar Diagnostics, France) for fungi and Starch-Casein-Agar for actinomycetes. Plates were incubated at 28 °C for 48 h for total microflora and yeasts and up to 7 days for fungi. For actinomycetes, plates were incubated for 7 to 15 days. For actinomycetes, soil samples were pre-treated by incubation at 50°C for 10 min before samples microbial analyses. All these counts were expressed as Colony Forming Units (CFU) per gram of dried soil.

Statistical analysis: All physico-chemical and microbiological analyses were followed-up in triplicate. Statistical analyses were conducted using prism pad, version 4. Analyses of variance (one way) were used to test the significant differences among data. The correlation (pearson-test) between soil phenols and soil microflora groups was calculated and the statistical significance was tested. All tests were performed at 0.05 significance level.

RESULTS AND DISCUSSION

Characterisation of the effluent: The physico-chemical characterization of the effluent is reported in Table 1. As

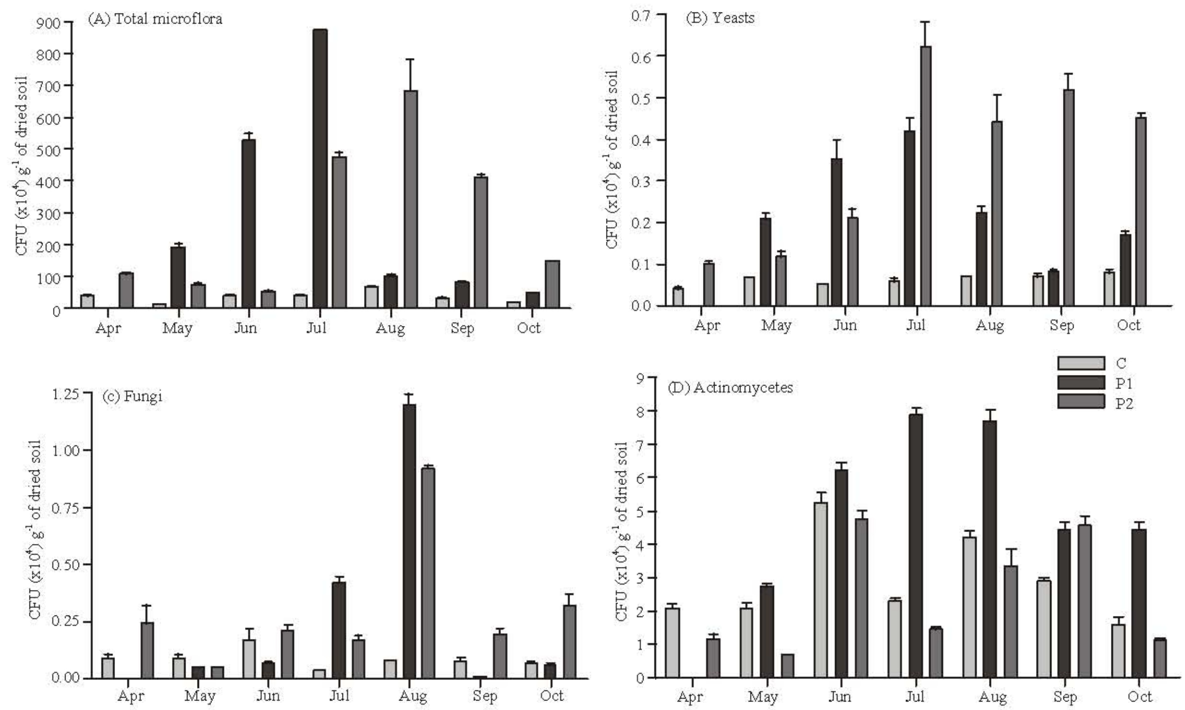

Fig. 1: Effects of OMVW on the profile of soil microflora. C: control plot, P1: OMVW spread (8 l m^{-2}), P2: OMVW spread (16 l m^{-2})

shown, OMVW is an acidic effluent highly charged with organic matter (COD = 120 gO_2 l^{-1}, BOD5 = 67.5 gO_2 l^{-1}, COD /BOD5 = 1.77). OMVW is also characterized by a high load of phenolic compounds (7.3 g l^{-1}) and salts (conductivity = 12.01 ms cm^{-1}). OMVW is so a by-product that we should not discharge in aquatic ecosystem because it will affect the water quality. Otherwise, OMVW high charge with organic matter and his appreciable charge of nitrogen (0.30 g l^{-1}) and phosphorus (0.07 g l^{-1}) are interesting for its agricultural use.

Microbial soil evolution: Microbial counts of total microflora, yeasts, fungi and actinomycetes were followed-up during 6 months. Analyses were performed on soil samples collected from 0 to 10 cm depth. Addition of OMVW to the soil created an enhancement in soil communities prospected that are total microflora, fungi, yeasts and actinomycetes (Fig. 1). This is in agreement with previous findings [7, 20, 21].

For total microflora, when the dose spread is 8 l m^{-2}, the abundances increase progressively to reach 8.7 10^6 cfu g^{-1} compared with the control assay (4. 10^5 cfu g^{-1}) after 3 months of the spreading (Fig. 1a). With an amount of spreading of 16 l m^{-2}, the abundances of total microflora increased but less than for 8 l m^{-2}. The increase was also observed for yeasts, fungi and actinomycetes and occurs latter when the amount spread is higher (Fig. 1a, 1b, 1c). The latency phase can be explained by the fact that soil microflora needs a phase of adaptation to the high load of OMVW to start using the organic matter [11]. Also, it could be explained by the fact that OMVW organic matter added to the soil on a high load was unavailable to the microflora under the effect of adsorption or reaction with soil compounds [14].

After spreading, the increase in abundances of the microbial groups studied seems to be temporary (Fig. 1) that follows the Monod theory: $\mu max = S/Ks+S$ (μ: growth rate; μmax: maximum growth rate; S: limiting substrate concentration (OMVW available organic matter); Ks: concentration in substrate for which the growth rate is half-maximum. The abundances increase to reach a maximum level because of the substrate availability [7] and then start decreasing. The top is generally retarded on time when the dose spread is 16 l m^{-2} in comparison with the dose 8 l m^{-2} because of the need of a latency phase when the dose spread is higher.

The increase of actinomycetes number is more abundant when OMVW is spread as 8 l m^{-2} than when it is as 16l m^{-2} during all the period of study (6 months) (Fig. 1d). This result is in agreement with previous studies [14] where enhancement of soil actinomycetes abundances is more power full when OMVW is spread at the rate of 5 l m^{-2}, 10 l m^{-2} than with 20 l m^{-2}. These results could be explained by the fact that increasing OMVW concentration may become toxic for the microbial group [6, 8]. Some compounds in OMVW may inhibit soil respiration especially in the high doses and neutralize the favourable influence of its higher nutrients contents [18, 14]. The decrease of actinomycetes number for the dose 16 l m^{-2} in comparison with the dose 8 l m^{-2} does not necessarily mean an elimination of these microorganisms. It could be a state of viability without cultivability appearance in response to the stress caused by OMVW concentration. At the same time, OMVW could be considered as a temporary enrichment of the soil with available carbon source and as a product containing inhibiting components to some microorganisms. OMVW can cause a decrease in abundances of some soil groups like nitrifiers [14] but not prolonged and may [11] not be appeared if the spread doses are not high.

For yeast and fungi, abundance become more pronounced when the dose is 16 l m^{-2} than when the dose spread is 8 l m^{-2}, after spreading by three and five months respectively (Fig. 1b, 1c). Fungi and yeasts are groups recognized for their capability to degrade OMVW. Amhajji *et al.* [22] reported the ability of some mould strains to degrade polyphenols with a rate that reached up to 95%. Yeast are a group recognized by its capability to survive in a high osmotic pressure ambiance increase earlier than fungi that prefer a low soil water activity that seems to decrease in August (Fig. 1c). At high loads of OMVW spread, yeasts and fungi were more effective on OMVW degradation than actinomycetes as its abundances in 16 l m^{-2} never exceed 8 l m^{-2} during 6 months from the OMVW spreading (Fig. 1).

Changes in soil communities should be linked to changes in the diversity of the microbial community structure. Indeed, Tardioli *et al.* [23] showed changes in soil fungal biodiversity after OMVW application, with substantial decrease in *Scopulariopsis brevicaulis* and increase in *Penicillium cyclopium*. Kotsou *et al.* [7] found a shift toward copiotrophic bacteria (r-selected species) that are first colonizers of a newly added organic matter after OMVW application. Changes that OMVW causes to soil microflora should not be considered as harmful for soil. Soil organic additives like composts or manures can also cause such changes in soil microflora in a reversible way [24].

physico-chemical changes: Spreading of OMVW decreased soil pH decrease from 7.82 to 6.24 just after spreading (Fig. 2a), because of the low OMVW pH value (4.7) (Table 1). However, only one month after OMVW spreading, soil pH increased to reach 8.15 when OMVW was spread at a rate of 16 l m^{-2} while control has a pH of 7.95. Already one month after OMVW spreading, soil receiving OMVW established its pH because of its buffering capacity. The acidity of the OMVW is compensated by the soil carbonate alkalinity that became bicarbonates [18].

Table 2: Correlation between soil phenols and soil microflora groups

		Total microflora	Yeasts	Fungi	Actinomycetes
P1*	Pearson r	-0.1459	-0.3153	-0.1207	-0.2182
	Is the correlation significant? (α=0.05)	No	No	No	No
P2µ**	Pearson r	-0.3510	-0.4275	0.0475	-0.5886
	Is the correlation significant? (α=0.05)	No	No	No	No

* P1: plot 1 (OMVW spread with 8l m^{-2}); ** P2: plot 2 (OMVW spread with 16l m^{-2})

OMVW spreading causes an immediate increase in soil electric conductivity (Fig. 2b) that could be justified by OMVW high electric conductivity (12.01 ms cm^{-1}) due to the effect of ions especially sodium and chlorides. Already one month after spreading, mainly adsorption of OMVW ions in soil colloids causes a deep decrease in soil electric conductivity (Fig. 2b). Mekki *et al.* [14] reported that OMVW high conductivity is not limiting for soil microflora development. Long-term OMVW application can cause a replacement of soil calcium with Na, K and Mg and so a salinisation and decrease of soil quality could be appeared [18, 25].

OMVW spreading causes an immediate increase in soil phenolic compounds that pass from 0.19 mg g^{-1} for control to 2.68 mg g^{-1} when OMVW is spread with 16 l m^{-2} (Fig. 2c). Already one month after OMVW spreading, Phenolic compounds decreased significantly in the aqueous extract of the upper layer soil (10 cm) and pass from 2.68 mg g^{-1} to 0.24 mg g^{-1} this could be due to that soil phenolic compounds are nearly totally fixed by the soil colloids and became not ready in the soil aqueous extract. That should be linked to several phenomena such as adsorption with the clay-humic substances particles of the soil (clay-silt = 86.58% in that soil), degradation by soil organisms and infiltration by irrigation water. Mekki *et al.* [26] reported that in a sandy Tunisian soil, phenolic compounds migrated according to their molecular mass while polyphenols are adsorbed in the soil upper layers, the monomers migrated in depth. After decrease of soil phenolics, an increase is registered in

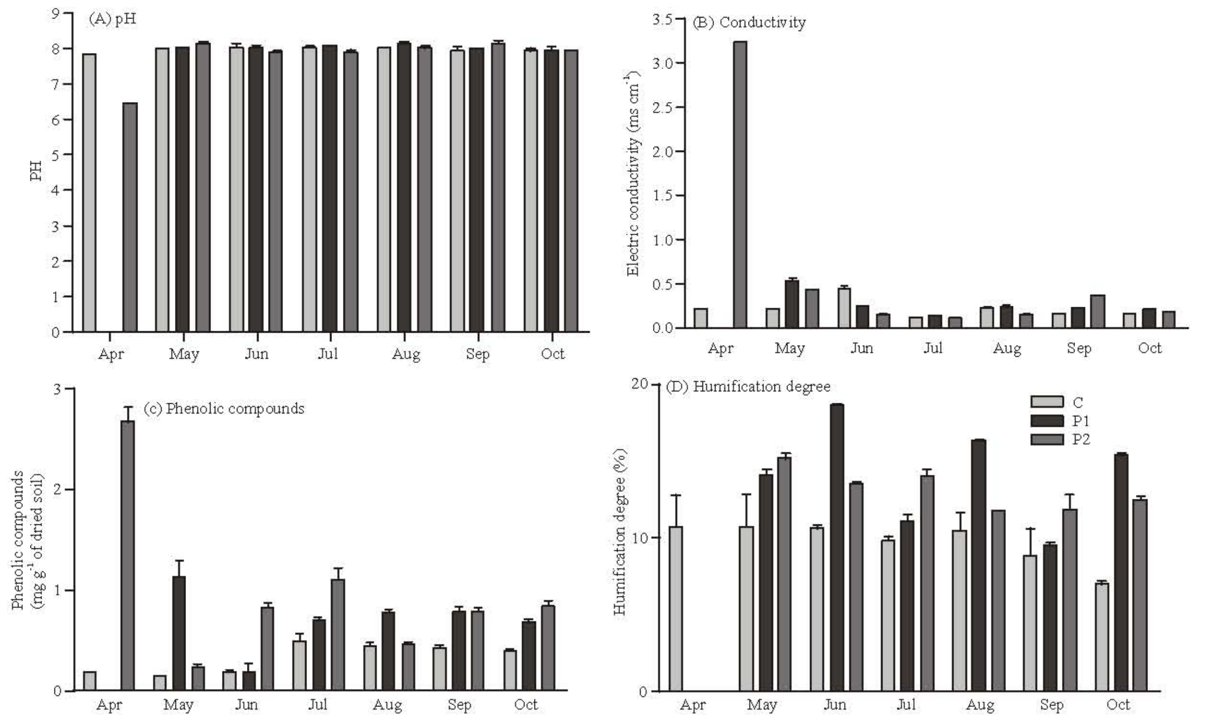

Fig. 2: Effects of OMVW on the profile of soil pH (a), Electric conductivity (b), Phenolic compounds(c) and humus (d). C: control plot, P1: OMVW spread as 8l m^{-2}, P2: OMVW spread as 16l m^{-2}

June samples that correspond to an increase from 0.24 to 0.84 mg g^{-1}. Soil phenolic compounds could be undergo adsorptions and desorpstions according to the soil conditions like soil granulometric, pH and salts concentration which in turn affect soil microbial activity [27].

Phenolic compounds are considered as the principal cause of OMVW toxicity against microorganisms [6, 8]. Theses compounds are considered as harmful to the aerobic processes of the soil microflora, particularly to the nitrification processes [26]. Correlation between soil phenolic coumpounds and soil microflora was evaluated and no reverse correlation was detected (Table 2). Other OMVW compounds should be responsible for the toxicity like fats [5]. Results could be explained by the soil physical role in protecting soil microorganisms against OMVW phenols.

OMVW spreading causes an increase in soil humification degree even for the dose 8 l m^{-2} or the dose 16 l m^{-2} (Fig. 2d) that is in agreement with several investigations [4, 26]. In soil, OMVW organic matter constituted basically by carbohydrates, proteins, fats, polyalcools and polyphenols [13] is transformed to highly polymerized polyphenolic compounds such as humic acid-like substances [27] by microorganisms, insects, larvae and earthworms. Humus forms with clay a colloidal complex which retains water and the biogenic salts and progressively redistributes them to plants and soil organisms. Soil enrichment with humus is an important factor for soil structure and fertility [4, 15, 21].

CONCLUSION

OMVW spreading with high amounts of 8 l m^{-2} and 16 l m^{-2} didn't cause suppression of none of the soil microbial groups studied (total microflora, fungi, yeasts and actinomycetes). OMVW spreading caused an increase in abundances of soil microbial groups and soil humification degree that is so interesting for soil fertility. This work could help for setting a Moroccan regulations concerning OMVW spreading on soil. According the results, we can consider that controlled OMVW spreading could be an eco-compatible practice for Moroccan agriculture field.

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