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Treatment of olive mill wastewaters using infiltration/percolation system: effect on microbial communities in the soil filter

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ABSTRACT

Infiltration/percolation is a low-technology process that has low energy and maintenance requirements and could be used to completely oxidize and decontaminate primary or secondary wastewaters. The objective of this work is to determine the performance of a soil filter implemented in Aït Ourir (Marrakech region – Morocco) for the treatment of olive mill wastewater (OMW). The experimental pilot consists of three basins of 12 m × 8 m each (length × width) built using reinforced concrete. A storage basin followed by a soil filter filled with 60 cm height of soil and 90 cm of gravel at the bottom. The 3rd basin was used to collect the treated OMW. The alimentation of the filter was continuous from the storage basin starting on November 15th until the end of January. The OMW was very acidic with a pH of 4.3, an electrical conductivity of 19.33 mS/cm, a total phenolic content of 2.78 g/L, and a total organic carbon of 3.12 g/L. The percolation of the OMW through the soil filter with gravel causes a reduction of 68% of suspended solids, 52% of fat contents, 69% of the total organic carbon, and 55% of the phenolic content. An analysis of microbial communities of soil filter was undertaken in comparison to control soil. The obtained results show the potentiality of an infiltration/percolation system (soil filter) to treat OMW before its use as fertilizer. Further investigations are needed to assess the toxicity of the treated effluents and their suitability for irrigation since some of their physico-chemical characteristics did not meet the Moroccan standards for wastewater reuse in irrigation.

Keywords: Olive mill wastewater; Infiltration/percolation; Soil filter; Phenolic compounds; Microbial communities

1. Introduction

Olive oil production generates huge volumes of wastewaters known as olive mill wastewaters (OMW) which is a major environmental concern in many Mediterranean countries [1]. Several methods have been suggested to treat these polluting effluents including membrane processing [2], surfactant-mediated processes [3–5], advanced oxidation [6], and biological treatment [7] among other techniques. The main limitation of the wide application of such processes at a large scale is their high costs. For this reason, an eco-friendly and economically viable solution should be developed. Infiltration/percolation is a low-technology process that could be used to completely oxidize and decontaminate primary or secondary wastewaters.

Owing to its low energy and maintenance requirements, infiltration/percolation systems have been increasingly used for the treatment of wastewater effluents [8,9]. The infiltrated effluent percolates through an unsaturated porous medium. The treated wastewater is then collected by

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a drainage system. Soil and gravel filters behave as aerobic fixed biomass reactors. wastewater is firstly filtered, and a great part of the organic load is retained in the first basin. When percolating through the filter, the filtered wastewater is treated by an aerobic biological process resulting in the mineralization of organic matter and the oxidation of nitrogen compounds [9]. A recent study evaluated the potential application of infiltration/percolation before a biological treatment of OMW [10]. The reported results indicated that the use of this system in columns as pretreatment of raw olive mill wastewater could be realized before biological treatment to obtain a satisfactory reduction of pollutants. More recently, a low-cost process called biobed was proposed to treat OMW [11]. The study aimed at the determination of the appropriate mixture of filling material (a mixture of compost and soil) and the corresponding bed layer thickness for the optimum removal of pollutants. However, the effect of continuous application of OMW on soil in the infiltration/percolation system was rarely studied. In one of the few studies where bacterial communities of soil filter were characterized and their evolution over time investigated, Karpouzas et al. [12] investigated the effect of continuous olive mill wastewater applications on the structure of rhizosphere–soil fungal communities in a loamy sand and a sandy loam for a period of 3 months.

In this work, we investigated the efficiency of an infiltration/percolation system to treat OMW. Most of the previous studies on treating agro-industrial wastewaters using an infiltration/percolation system focus on studying the changes in the chemical composition of soil and effluents. In the present study, we are investigating, also, the changes in the soil microstructure and microbial communities.

The objective is to develop an easy-to-handle low-cost method suitable for the treatment of such effluents to reuse them in ferti-irrigation. The efficiency of the system was assessed by comparing the physico-chemical characteristics of soils and OMW before and after treatment. Microbiological analysis was also carried out and soil texture was studied using scanning electron microscopy.

2. Experimental

2.1. Infiltration/percolation system

The experimental pilot system is located in Ait Ourir (Haouz Region, Morocco). The climate of the experimental area is characterized by weak and variable rains with an annual average of about 240 mm, for 40 d of rain approximately, a high average temperature (18.3°C in January to 36.7°C in July) with daily and monthly important variations, and a weak hygroscopy which varies in the monthly average from 40% (August) to 70% (January). The experimental area is characterized also by a very strong evaporation rate of approximately 2,300 mm/y.

The experimental pilot consists of a system of three basins of 12 m × 8 m each (length × width) built using reinforced concrete. The basin in the middle was designed as a filter filled from the bottom with 90 cm of gravel of decreasing diameters (2–18 cm) followed by 60 cm of soil collected from the region (Fig. 1). The sand layer blocks solid particles from the wastewater and gravel in the bottom provides a more open area for a smoother flow of effluent after it has passed through the sand. The gravel sizes were chosen to avoid clogging the infiltration system. The alimentation of the filter was continuous (10–25 m^3/d) from the storage basin starting on November 15th until the end of January. Outside this period the feeding was intermittent from the storage basin. The hydraulic loading rate (HLR) was between 100 and 260 L/d/m^2. The effluents have an average organic load of 92 g/L and pass through the filter by gravity flow. Samples of the treated effluent were collected and analyzed for various characteristics and biotests.

2.2. Olive mill wastewater sampling and characterization

The fresh OMW used in this experiment was taken from an olive mill equipped with a three-phase centrifugal decanter located in Aït Ourir (Haouz Region, Morocco) close to the location of the infiltration/percolation pilot system. The storage basin of the system was filled continuously by OMW coming from the mill. Treated OMW was compared to non-treated samples in terms of physico-chemical and microbiological characteristics. Analyses of various OMW characteristics were performed shortly after sampling and were carried out as described by standard methods. All analyses were done in triplicate.

2.3. Soil sampling and physico-chemical analysis

Filter soil was sampled for chemical, microbial counts, and activity analysis, before and after OMW application.

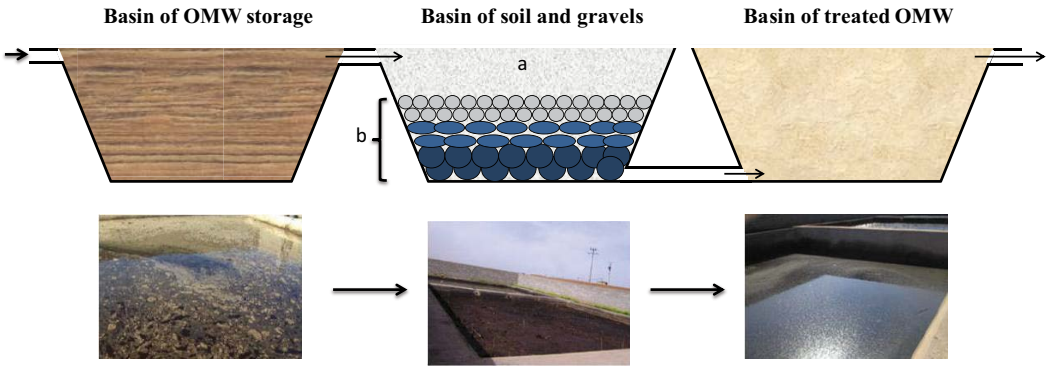

Fig. 1. Pilot plant of the infiltration/percolation system: (a) soil collected from the region and (b) gravels of increasing diameters.

All sampling events included the collection of soil samples from 4 random locations, from 0 to 5 and 5 to 10 cm, using a 5 cm internal diameter metalcore. Fresh samples were used for microbiological analysis. Samples of soil were brought back to the laboratory and sieved (2 mm pore size mesh). All analyses were done immediately after sampling, otherwise, if necessary, samples were stored at 4°C about 24 after sampling. The basic characteristics of the soil texture were: 35.7% clay, 25.3% fine silt, 10% coarse silt, 16.7% fine sand, 12.4% coarse sand, and 8.5% $CaCO_3$. The parameters analyzed are pH value, electrical conductivity (EC) using a conductivity meter (WTW F318), soluble contents of potassium using a flame photometer (JenWay PEP7), and available phosphorus (P) analysis measured according to the method of Olsen et al. [13]. Organic C analysis was carried out using Walkley–Black method [14], total nitrogen by Kjeldahl digestion [15], and the soluble phenolics using the method described by El Hadrami et al. [16].

2.4. Microbiological analysis of soil

2.4.1. Microbiological enumerations

10 g of the soil sample was suspended in an Erlenmeyer flask containing 90 mL of a sterile solution (0.3% of sodium polyphosphate $(NaPO_3)_n$ in distilled water, pH 7.0). The flask was shaken at 200 rpm for 2 h. Serial 10-fold dilutions of the samples in a 0.85% NaCl solution were plated in triplicate on Tryptic Soy Agar Medium TSA (40 g/L; pH 7) at 30°C for total bacterial counts, on Potato Dextrose Agar PDA (39 g/L; pH 5.6) at 25°C for fungi and on AIA agar (22 g/L, pH 8) for *Actinomycetes*. Each soil sample was analyzed in triplicate for each medium. All these counts were expressed as CFU (colony forming units)/g of dried soil (for 24 h at 105°C).

2.4.2. Respirometric test

Biological activity in the soil was assessed by measuring CO_2 evolution in the aerobic condition and closed system. 50 g of soil was moistened with sterilized tap water up to 55% of its water-holding capacity to enable the biodegradation process to proceed. Soil respiration was determined by placing soil samples in hermetically sealed bottles equipped with a CO_2 trap (a bottle containing 25 mL of NaOH 0.05 M). Incubation was carried out in the dark at 30°C and the amount of CO_2 generated by soil microflora was daily monitored colorimetrically using HCl (0.05 M) in presence of a few drops of phenolphthalein indicator. To avoid the fixation of atmospheric CO_2 on NaOH, 10 mL of a solution of barium chloride ($BaCl_2$, 0.5 M) was added. The amount of NaOH available to titrate is inversely proportional to the amount of trapped CO_2. The rate of respiration was expressed in mg of CO_2/g of dry soil/d.

2.4.3. Amylolytic, proteolytic and cellulolytic activity

Three microbial activities were highlighted in this study: amylolytic, cellulolytic, and proteolytic activity. The evaluation of the microbial activities was carried out by counting the number of positive tubes throughout the dilutions to calculate the 'dilution index' as a function of time according to the following formula:

Dilution index = the number of positive tubes/the number of tubes inoculated by dilution.

Dilutions were then aseptically seeded in different media specific to each physiological group. These environments make it possible to highlight a specific character in each group. Three repetitions per dilution were performed.

2.4.3.1. Cellulolysis

Cellulolysis is the breakdown of cellulose into oligosaccharides and soluble sugars. These carbohydrates are used by these germs as a source of carbon and energy. The culture medium used in this test contains a sheet of filter paper as the only carbon source. The reading is made after incubation at 28°C as a function of time for 15 d. The positive tube is characterized by the appearance of pigmented spots on the paper or even cutting of the latter after shaking.

2.4.3.2. Amylolysis

Amylolytic germs hydrolyze starch into simple sugars. The culture medium used contains starch as the only carbon source. The reading is made after 15 d of monitoring and incubation at 28°C using the appropriate reagent. The positive tube is colored slightly yellow.

2.4.3.3. Proteolysis

It is the degradation of many protein molecules into polypeptides, amino acids, and then amides. In hemolysis tubes containing 2 mL of saline medium, with gelatin added as the sole source of carbon and nitrogen, 0.5 mL of suspensions-dilutions of soil was inoculated. Tubes were then incubated at 28°C and monitored for 15 d. Results' reading was performed at the end of incubation by cooling the tubes at +2°C for 90 min. Liquefaction of the medium indicates that proteolysis has occurred.

2.5. Acute toxicity LUMIStox bioassay

The microtoxicity test consisted of the inhibition of the bioluminescence of *Vibrio fischeri* LCK480 according to ISO 11348-2 [17] using the LUMIStox system (Dr. Lange GmbH, Duesseldorf, Germany) was investigated in Sfax Biotechnology Center (Tunisia). The inhibition percentage of the bioluminescence was determined by mixing 0.5 mL of OMW and 0.5 mL of the luminescent bacterial suspension. After 15 min exposure at 15°C, the decrease in light emission was measured. The OMW toxicity was expressed as the percentage of inhibition of the bioluminescence (% IB) compared to a non-contaminated reference. A positive control (7.5% NaCl) was included for each test.

2.6. Scanning electron microscopy

The microstructure of samples was examined by scanning electron microscopy (SEM) using JEOL JSM 5500 SEM equipped with an EDAX Flacon EDS analyzer. Specifications of the scanning electron microscope JEOL JSM-5500 are

Max resolution 3.5 Nm; Growth X 18 to 300 000; Tension of acceleration 0.5 to 30 kV; Secondary detectors of electrons (topographic contrast); Automatic adjustment of X-ray, contrast, brightness, and stigmatism. The SEM is coupled with a powerful system of microanalysis X: Detector If (Li) with the dispersion of energy with beryllium (Be) super-ultra-thin window; Detection of the elements starting from Boron; Quantitative analysis with or without witnesses (ZAF); Resolution: 127 eV with the MnKα line for a time-constant of 100 μs.

3. Results and discussions

3.1. OMW physico-chemical characterization

OMW samples were dark-colored aqueous wastes, foul-smelling and turbid. First, the physico-chemical characterization of OMW samples collected in Aït Ourir (Marrakech region) shows that this effluent is slightly acidic (pH = 4.3). The acidity of OMW is due to the presence of carboxylic organic molecules such as fatty acids and phenolic compounds. The OMW electrical conductivity was relatively high and reached 19.33 ± 2.43 mS/cm (Table 1). The salinity of the OMW can be explained by the traditional use of salt (NaCl) to conserve olives before processing. The total phenolic content (2.78 g/L) is relatively low compared to the values reported in our previous studies (9.37 ± 1.05 g/L) [18,19]. This could be attributed to the use of a huge volume of water during olives processing resulting in a dilution of the generated effluents. The total organic carbon was found to be 3.12 g/L. The percolation of the OMW through the soil filter with gravel does not cause a significant increase in the pH (4.3 to 4.9), however, it causes a reduction of 68% of suspended solids, 52% of fat contents, 69% of the total organic carbon, and 55% of the phenolic content (Table 1). However, many parameters such as pH, chlorine, and nitrate concentrations are beyond the limits sited by Moroccan standards [20]. In Morocco, there is no specific legislation for the reuse of olive mill wastewater in irrigation, thus farmers can reuse these effluents as fertilizer.

3.2. Soil microbiological analyzes

3.2.1. Respirometric test

The basal parameters of the quality of the soil in terms of its biological activity are the respiration activity (RA) and the capacity of the microflora (number and species of micro-organisms) to degrade the substrate. These are the main indicators of the mineralization capacity of microbial biomass. The evaluation of the respirometry is made by measuring the quantity of dioxide carbon released with mineralization by the soil filter microorganisms. In Fig. 2 the soil filter activities after OMW spreading are reported. OMW treatment increased the RA in the untreated soil, especially in the upper soil layer (10–20 cm). The soil respiration activity is highly correlated with the decomposition of organic matter in the soil. Higher RA indicates the ability of the micro-organisms to utilize and decompose variable organic substrates introduced with OMW. Indeed, a maximum production of 54.76 and 38.02 CO_2/g was measured for the OMW amended soil filter at t_0 and t_f (after 7 d), respectively (Fig. 2).

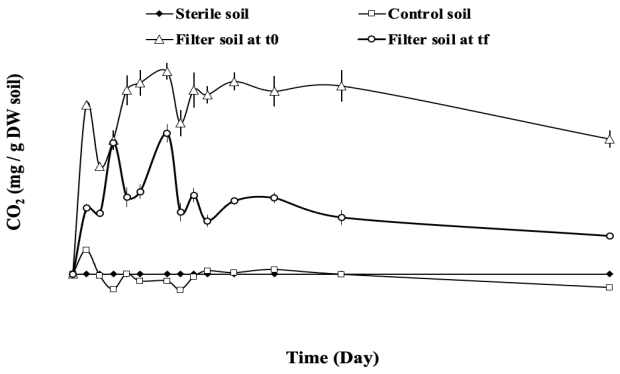

Fig. 2. Respirometric activity of the soil filter at t_0 and t_f compared to the control soil.

Table 1
Physico-chemical composition of the raw and treated OMW

Parameters	Unit	Crude OMW (untreated)	Treated OMW	Moroccan standards*
pH (25°C)	–	4.3 ± 0.1	4.9 ± 0.52	6.5–8.4
Electrical conductivity (20°C)	mS/cm	19.33 ± 2.43	9.51 ± 2.76	<12
Total suspended solids (TSS)	g/L	52.25 ± 2.31	16.61 ± 0.51	<0.2
Salinity	g/L	16.2 ± 1.36	7.16 ± 0.65	<7.68
Fat matter	g/L	0.79 ± 0.02	0.38 ± 0.01	–
Total organic carbon	g/L	3.12 ± 0.1	0.96 ± 0.05	–
Total soluble phenols (g/L)	g/L	2.78 ± 0.12	1.26 ± 0.06	–
Nitrate	mg/L	123 ± 32	36.2 ± 4.6	<30
Chemical oxygen demand	g/L	57.3 ± 6.1	21.6 ± 3.7	–
Biochemical oxygen demand	g/L	35 ± 4.9	14.8 ± 2.9	–
Cl	mg/L	1,398 ± 121	636 ± 29	<350
Na	mg/L	123 ± 9.5	64.85 ± 7.8	<69

*For wastewater reuse in irrigation according to Article 51 of the Decree No. 2-97-787 of February 4, 1998 of the water law which defines the quality standards that water must meet according to the use that will be made of it.

The observed increase in soil respiration during the entire period of OMW addition could be attributed to the high organic load of OMW, and a part of this organic load has been removed in the form of CO_2 during the aerobic condition. Soil microorganisms responded immediately to OMW addition, as demonstrated by the direct increase in soil respiration, following the first OMW addition. Concerning the control soil, the microbial activity is much less compared to the other samples with a weak CO_2 release which does not exceed 6.45 CO_2/g. Released CO_2 is due to the biodegradation of the substrates to be tested and the organic matter presents naturally in the soil. For the sterile soil sample that does not comprise any substrate, the microbial activity remains absent considering the absence of micro-organisms and substrate (Fig. 2). During the biological degradation process of OMW, the total assimilation of the carbon substrate results from the synergy of several species which cohabit in the soil. The results of these experiments confirm the economic and environmental validity of the OMW spreading on cultivated soil since it does not induce toxic effects on the soil, and helps to reduce, or avoid, the chemical fertilizer with macronutrients.

3.2.2. Respirometric test confirmation: OMW biodegradability

The results representing the metabolic activity of the micro-organisms are illustrated in Fig. 3. The observed production of CO_2 confirms on the one hand that the organic matter of the OMW is mineralized in CO_2 and on the other hand, that the activity obtained by the first respirometric test has a biological origin. The control is conducted in the same conditions as the biodegradation test. The quantity of released CO_2 is only the result of the endogenous activity of the inoculum.

3.2.3. Amylolytic, proteolytic and cellulolytic activities

Results of the amylolytic test (Fig. 4) show well the presence in the three samples of the soil of amylolytic germs with different activity ratios which vary from 1 in the soil filter at t_0 to 1.33 for the soil filter at t_f. However, the activity ratio is 2 in the control soil. Concerning the control soil, these amylolytic activities are better than that of the OMW treated soil due especially to the natural attack of the starch in the soil. This weak activity of the micro-organisms at the beginning is due to the additional starch contribution by the OMW.

The evolution of the proteolytic activity of the control soil and soil filter at t_0 and t_f (Fig. 5) shows a similar activity in the control soil and filter soil at t_f after 3 d.

In the same way, the cellulolysis test (Fig. 6) shows well the presence of cellulolytic germs in the various samples of the soil with more important activity ratios in the OMW treated soil compared to the control one. Thus, the tested soil presents indices of dilution respectively of 3.2, 3.21, and 1.68 for the samples of soil filter at t_0, t_f, and control soil.

3.3. Scanning electron microscopy and microbial activity

This study attempted to demonstrate that soil amended with OMW showed modification of its structure, texture, and microbial communities (Fig. 7). OMW application causes a change in the soil's biological [21], chemical, and physical properties [22], and irrigation with wastewater, in

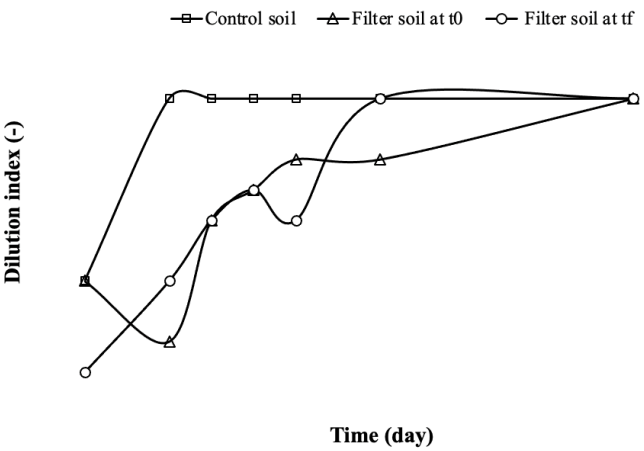

Fig. 4. Amylolytic activity soil filter at t_0 and t_f compared to control soil.

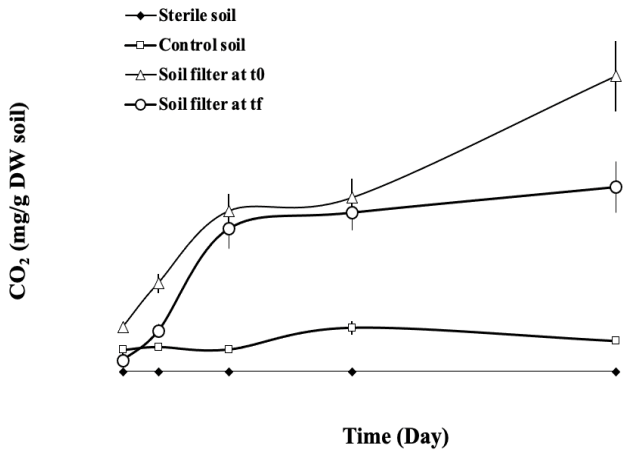

Fig. 3. Confirmation of the respirometric activity of the soil filter at t_0 and t_f compared to the control soil.

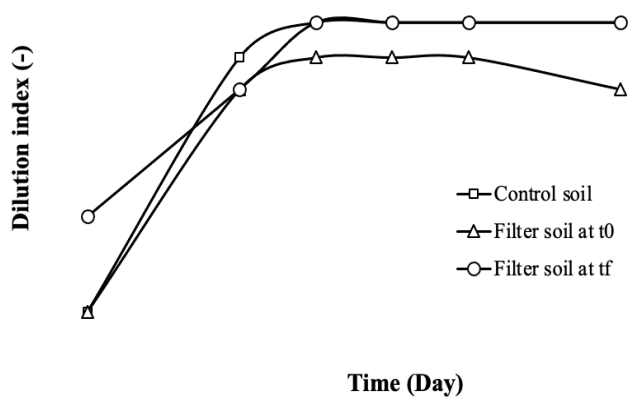

Fig. 5. Proteolytic activity of soil filter at t_0 and t_f compared to control soil.

general, has been shown to reduce the saturated hydraulic conductivity [23]. This decrease can be explained by two processes: (i) partial blockage of soil pores by suspended solids and organic matter [24] or clogging up of small soil pores by swelling of dispersed clay particles resulting in limited water movement in the soil [25], and (ii) redeployment of soil porosity by an increase of narrow pores and decrease of large pores [26]. Rasa et al. [27] reported that generally, the water-repellency increases at the soil surface after the accumulation of organic matter. The increase of water-repellency may be caused by the generation of hydrophobic components resulting from the decomposition of organic matter; these products of decomposition and the oil residues are wax-like substances that could form a coating on soil particles [28]. Tarchitzky et al. [25] reported that soils irrigated with freshwater were hydrophilic, while soil hydrophobicity increased in those irrigated with wastewater. The effects of the OMW on the physico-chemical characteristics of the soil were well documented [29–31]. However, a few studies are treating the impacts of this effluent on the microbial communities of soil [32–35].

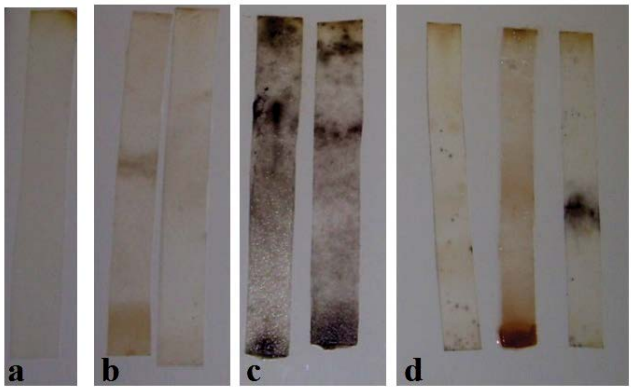

Fig. 6. Cellulolytic activity of soil samples: (a) sterile soil, (b) control soil, (c) soil filter after the OMW application, and (d) soil filter at t_f.

Knowledge about the structure of soil microbial communities is very important in view to predict the fluxes of nutrients and understand the impacts of xenobiotics. The addition of OMW to the soil filter creates some modifications in the average values of the total number of microorganisms and their repartition. Generally, OMW enhanced the soil capacity of holding water and consequently increased soil humidity. The total microflora increases with the increase of soil humidity. The results showed, for most of the studied microflora, an increase in the number (in UFC/g of dry soil) of germs after OMW spreading. The impact of OMW on soil microflora may be understood from two perspectives: (1) the temporal enrichment of the soil with readily available carbon source; (2) the presence of antimicrobial components in the amended wastewater. The results of this study demonstrate that OMW potentially stimulates the microbial activity of the soil at the applied levels and is generally not toxic to the soil microflora. Such

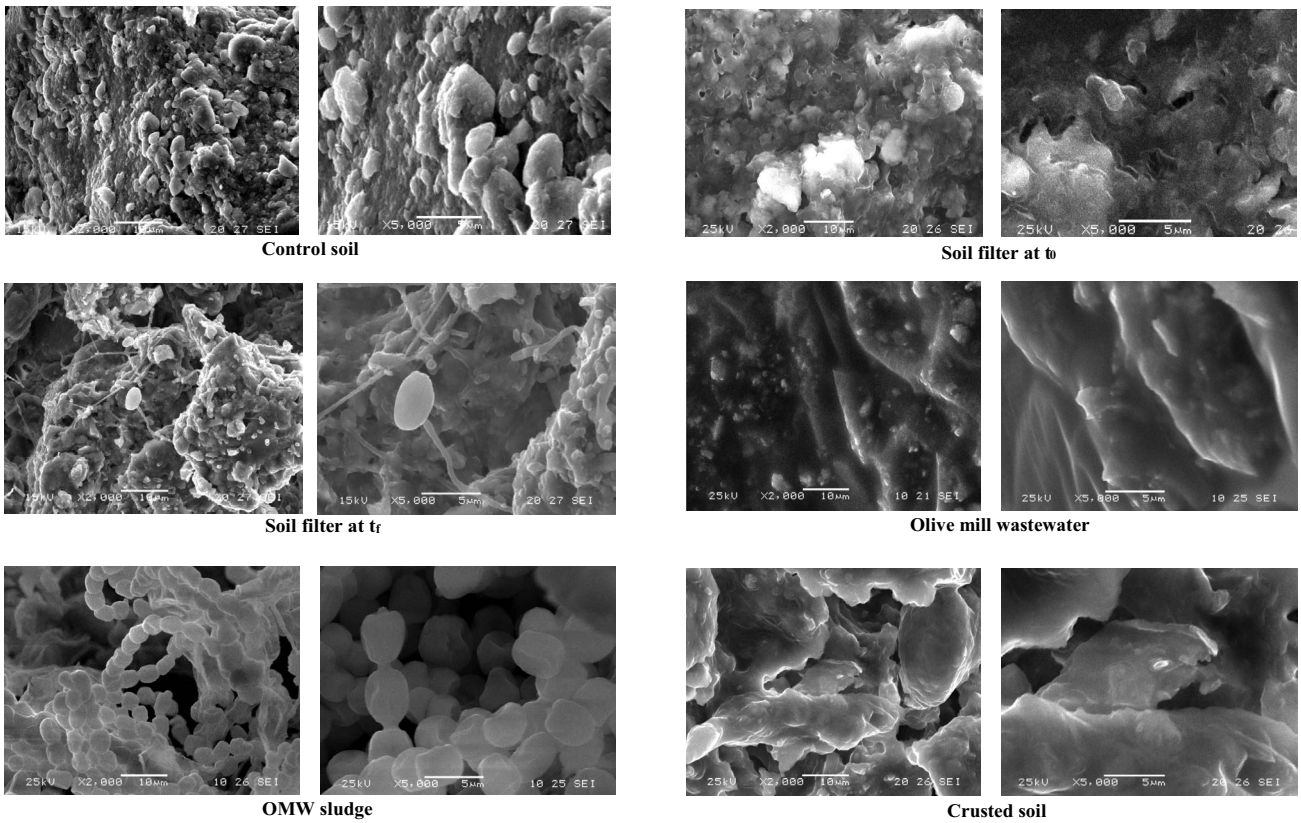

Fig. 7. Microstructure of soil and OMW samples captured by scanning electron microscopy (SEM) (Magnification ×2,000 and ×5,000).

Table 2
Physico-chemical characteristics of control soil

Parameters	Mean ± SD
pH (25°C)	8.30 ± 0.20
Electrical conductivity (mmhos/cm at 25°C)	0.24 ± 0.006
Salinity (g/L)	0.76 ± 0.01
Total organic carbon (%)	0.59 ± 0.11
Organic matter (%)	1.01 ± 0.009
Total nitrogen (%)	0.10 ± 0.006
C/N	6.08 ± 0.31
K_2O (mg/kg)	566.49 ± 29.10
Olsen-P (mg/kg)	60.80 ± 0.53

Table 3
Microbiological enumeration of control soil and the filter soil at t_0 and t_f

	Bacteria	*Actinomycetes*	Fungi
Control soil	3.21×10^5	0.13×10^5	0.44×10^5
Filter soil at t_0	10.6×10^5	3.34×10^5	69.8×10^5
Filter soil at t_f	10.6×10^5	0.31×10^5	8.79×10^5

temporal stimulation of soil microflora is most likely associated with soil enrichment with readily available carbon sources from these wastewaters. The low pH (4–5) of OMW (Table 1) may affect the pH of the soil (Table 2) and makes it less alkaline, thus improving soil microbial growth. Increased microbial activity, counts, and biomass, after OMW application, were also reported in previous studies [36,37]. Yet, they did not exclude possible changes in the structure of the microbial community in treated soils. Only limited information is available in the literature concerning such changes. Tardioli et al. found changes in the fungal composition of soil after OMW application, with an increase in *Penicillium cyclopium* and an important decrease in *Scopulariopsis brevicaulis*. The fungus *P. cyclopium* was found to be abundant in OMW [36]. The observed changes in soil bacterial and fungal communities could be beneficial to soil fertility. Such changes that were shown to be reversible [38], do not necessarily reflect the effect of inhibiting components from OMW but may reveal community adjustments in response to the high amount of readily available carbon source supplied with OMW. As shown by Kaiser and Esterby [39], the addition of OMW resulted in a shift toward copiotrophic bacteria (r-selected) species, which comprise the first colonizers of the newly added organic load. Such changes are likely to occur with many types of soil amendments like composts and manures [38]. The rate of OMW mineralization was faster during the first days, most probably due to the rapid decomposition of the OMW labile organic fraction. In the various samples of the soil the microbial density of the bacteria is much more important compared to the *Actinomycetes* and fungus this is can be explained by their fast multiplication, except for the soil filter at t_0 which presents a more fungi population of 6.98 10^6 UFC/g PS compared to the bacteria with a rate of 1.06 10^6 UFC/g PS (Table 3). Sayadi et al. [40] reported that the biodegradation of a high concentration OMW with low-weight molecular phenolic compounds can be done by new isolated or genetically modified bacteria in anaerobic treatment. On the other hand, phenolic compounds of high molecular weight are particularly degraded by fungi. According to the obtained results, the fungi are more important in the OMW amended soil filter compared to the control soil (Table 3).

The basidiomycetes growth on the fresh OMW metabolizing sugars and other simple compounds allow the reduction of the chemical oxygen demand (COD) and color [41]. Fungi such as *Aspergillus Niger* and *Phanerochaete chrysosporium* [42], are known for their enzymes of depolymerization and their high resistance to toxic compounds. The spread of OMW on the soil filter stimulated such micro-organisms that have activities of degradation of lignin and polyphenols [43,44]. Consequently, this microbial population was favored in the soil amended by the OMW where the pH and C/N ratio were more favorable than in the control soil (Table 2). This observation confirms the observations founds in previous works [45]. Concerning the *Actinomycetes* group, they exist with a low microbial density compared to the bacteria (Table 3). The *Actinomycetes* and the sporulating bacteria play a considerable role in organic matter recycling in nature. This is due to their considerable capacities to mobilize complex organic molecules. The number of *Actinomycetes* was improved by the addition of OMW. The degradation of the fresh organic matter was carried out gradually; the first stages correspond to the action of the bacteria and fungus; the last one corresponds to the action of the *Actinomycetes*. Thus, the *Actinomycetes* cannot grow in the first stages because of their ineptitude to the competition; on the other hand, they can grow on partially degraded organic matter and inapt to carry a fungus and bacterial microflora. *Actinomycetes* could participate in the humification processes by producing compounds whose chemical structure is like humic acids. Thus, *Streptomyces globisporus* can synthesize aromatic polymeric molecules of a high cations exchange capacity like humic substances that constitute the precursors of humic and fulvic acids. The fungi seem to take part in the organization of nitrogen than in its mineralization. Indeed, their crucial role is probably in the mineralization of organic matter. The aptitude to degrade great quantities of organic matter with small quantities of nitrogen can be a consequence of their accelerated recycling reserves and can be also explained by their capacity to mobilize poor or resistant resources of nitrogen. This explains their preponderance in poor soils, in the remains of fresh plants, and especially in the remains of old plants where the C/N can reach considerable values.

4. Conclusion

The comprehension of the mechanisms of biotransformation of the phenolic compounds of the OMW by microorganisms of the soil will allow optimization and the control of the whole process of treatment and valorization of olive mill wastewater using an infiltration/percolation system. Moreover, this detoxified agricultural water would be used in agriculture as water resources and fertilizer for poor soils. However, since the obtained treated effluent did not meet all the Moroccan standards for its reuse in irrigation,

an assessment of its toxicity on crops should be undertaken before its agronomic application as fertilizer.

During this work, several bacterial strains, fungi, and *Actinomycetes* were isolated and purified (data not shown) presenting varied morphologies and potentialities of degradation. It will be also preceded by the physiological characterization and the determination of the phylogenetic position of all the isolated stocks. Studies of the direct involvement of these isolated stocks in the processes of degradation and especially the transformation of the aromatic compounds contained in these OMW effluents will be also considered.

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