academicJournals

Vol. 8(12), pp. 684-690, December, 2014
DOI: 10.5897/AJEST2008.105
Article Number: D1E8B7749138
ISSN 1996-0786
Copyright © 2014
Author(s) retain the copyright of this article
http://www.academicjournals.org/AJEST

African Journal of Environmental Science and Technology

Full Length Research Paper

Changes in microbial and soil organic matter following amendment with olive mill wastewaters

Ali Mekki*, Abdelhafidh Dhouib and Sami Sayadi

Laboratoire des bioprocédés, Pôle d'Excellence Régionale AUF (PER-LBP), Centre de Biotechnologie de Sfax, BP: « K », 3038 Sfax, Tunisie.

Received 21 July, 2008; Accepted 2 September, 2008

In this work, the occurrence of the untreated and treated olive mill wastewaters (OMW) amendment on the soil organic matter recycling and on its microbial biomass evolution were investigated. Compared to the control, soils amended with untreated and treated OMW showed high levels of organic and mineral matters. Soil amended with untreated OMW presents low levels of total and inorganic nitrogen (0.38 ± 0.03 and 0.08 ± 0.02 mg g^{-1} dry soil). Treated OMW had a little content of pollutants (COD = 4 g l^{-1}± 0.4; phenolic compounds = 0.6 g l^{-1}± 0.04) and organic matter brought by these residues was rapidly mineralized in the soil. The number of heterotrophic bacteria was increased (from 54 ± 5 .10^5 CFU g^{-1} dry soil in control soil to 123 ± 11. 10^5 CFU g^{-1} dry soil) in response of the OMW amendment, mainly following C/N ratio correction. The amendment of the soil with untreated OMW improved the soil carbon content (2.18 times higher) while the specific respiration remained very low. However, the amendment with treated OMW positively affects the soil specific respiration that increases from 6.1 in control soil to 9.75 in soil amended with treated OMW. This phenomenon was accompanied by an enhancement (from 12 ± 2 .10^4 CFU g^{-1} dry soil in control soil to 83 ± 5. 10^4 CFU g^{-1} dry soil in soil amended with OMW) of nitrifiers number , urease and ammonium oxidases activities.

Key words: Olive mill wastewaters, soil, organic matter, nitrifiers, mineralization.

INTRODUCTION

Olive oil industries are of fundamental economic importance for many Mediterranean countries that account for approximately 95% of the worldwide olive oil production (Dhouib et al., 2006a). Olive mill wastewaters (OMW) are the liquid by-product generated during olive oil production (Kapellakis et al., 2006). They are characterized by the following chemical properties: a very high content of organic matter (COD between 60 and 185 g l^{-1}; BOD_5 between 14 and 75 g l^{-1}), a low pH, and high polyphenols, potassium and phosphorus contents (Rinaldi et al., 2003). Extremely high organic load and the toxic nature of olive mill wastewaters (OMW) prevent their direct discharge into domestic wastewaters treatment systems. To solve the problems associated with these wastewaters, different disposal methods have been proposed (Sayadi and Ellouz, 1995; Paredes et al., 2000; Casa et al., 2003; Dhouib et al., 2006b). However, the most frequently used method nowadays is the direct application to agricultural

*Corresponding author. E-mail: jayanthybiochem85@gmail.com. Tel: 98947 07275. Fax: 0422-2575622.

Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

Table 1. Physico-chemical characteristics of untreated and treated olive mill wastewaters.

Characteristics	Untreated OMW	Treated OMW
pH (25°C)	5.1 ± 0.2	8.2 ± 0.2
Electrical conductivity (25°C) (dS m^{-1})	8.9 ± 0.1	14.1 ± 0.1
Chemical oxygen demand (g l^{-1})	72 ± 2.8	4 ± 0.4
Biochemical oxygen demand (g l^{-1})	13 ± 0.9	1.8 ± 0.14
COD/BOD_5	5.53 ± 0.72	2.2 ± 0.32
Salinity (g l^{-1})	6.75 ± 0.66	12.1 ± 1.1
Water content (g l^{-1})	948 ± 17.2	984 ± 19.5
Total solids (g l^{-1})	52 ± 2.98	15.9 ± 0.7
Ash (g l^{-1})	8 ± 0.43	10.15 ± 0.5
Volatile solids (g l^{-1})	44 ± 1.85	4.8 ± 0.22
Total organic carbon (g l^{-1})	25.52 ± 1.18	3.2 ± 0.12
Total nitrogen Kjeldahl (g l^{-1})	0.6 ± 0.06	0.21 ± 0.03
Carbon/Nitrogen	43 ± 1.8	15 ± 0.65
P (mg l^{-1})	36 ± 3.6	15 ± 1.1
Na (g l^{-1})	0.94 ± 0.09	0.86 ± 0.08
Cl (g l^{-1})	1.6 ± 0.15	1.3 ± 0.11
K (g l^{-1})	8.8 ± 0.8	5.34 ± 0.6
Ca (g l^{-1})	1.2 ± 0.11	3.2 ± 0.2
Fe (mg l^{-1})	32 ± 2.9	38.3 ± 3.3
Mg (mg l^{-1})	187 ± 18.8	281 ± 27.1
Ortho-diphenols (g l^{-1})	9.2 ± 1.8	0.6 ± 0.04
Toxicity by LUMIStox (% I_B)	99 ± 9	30 ± 2.9

soils as organic fertilizers (Fiestas and Borja, 1992; Nieto and Garrido, 1994; Mekki et al., 2006b; Mekki et al., 2007a). OMW physico-chemical composition and characteristics are well documented (Fiorentino et al., 2003; Allouche et al., 2004; Obied et al., 2005). Little is known, however, of the impact of OMW on the chemical, biochemical and biological properties of soil (Aggelis et al., 2003; Mekki et al., 2007b). Indeed, the effect of OMW addition on soil organic matter cycles is one of topics that need to be addressed from an agricultural point of view.

The present work was aimed at evaluating the occurrence of untreated and treated OMW amendment on the soil organic matter recycling and then on its microbial biomass evolution. The main goal is to know how much the OMW treatment application and its incubation in soil can remove its toxicity and their effects on the soil matter recycling.

MATERIALS AND METHODS

Untreated OMW origin

Untreated olive mill wastewater was taken from a three-phase discontinuous extraction factory located in Sfax, Tunisia.

Treatment of untreated OMW

The treated OMW was obtained with an integrated process based on aerobic fungal pre-treatment using *Phanerochaete chrysosporium* DSMZ 6909 followed by a decantation step then anaerobic digestion (Sayadi and Ellouz, 1995). The characteristics of the treated and untreated OMW are given in Table 1.

Physico-chemical analyses

The pH and the electrical conductivity were determined according to Sierra et al. (2001) standard method. Organic matter (OM) was determined by combustion of the samples in a furnace at 550°C for 4 h. Total organic carbon was determined by dry combustion (TOC Analyser multi N/C 1000). Total nitrogen was determined by Kjeldahl method (1883). Chemical oxygen demand (COD) was determined according to Knechtel method (1978). Five-day biochemical oxygen demand (BOD_5) was determined by the manometric method with a respirometer (BSB-Controller Model 620 T WTW) and phenolic compounds (ortho-diphenols) were quantified by means of Folin-Ciocalteu colorimetric method using caffeic acid as standard (Box, 1983). The absorbance was determined at l = 765 nm.

Study sites and sampling

The soil of the study area was described in a previous study (Mekki et al., 2006 a). The field was divided in 5 plots. S_1 and S_2 were amended with 100 m^3 ha^{-1} of untreated OMW. The amendment is achieved without (S_1) or after (S_2) correction of the C/N/P ratio to 100/10/1. S_3 and S_4 were amended with 400 m^3 ha^{-1} of treated OMW. The amendment is achieved without (S_3) or after (S_4) correction of the C/N/P ratio to 100/10/1. The fifth plot, plot S_c, was not amended and served as a control.

Amendment was realized in February 2006. Soil samples (S_1, S_2,

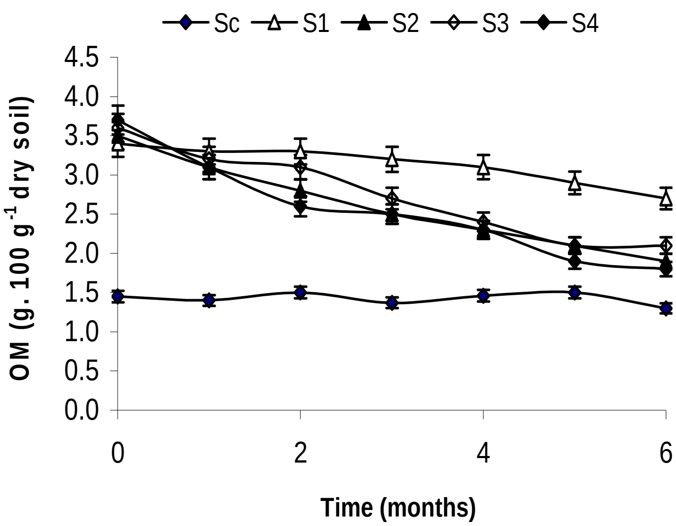

Figure 1. Soil organic matter evolution as a function of amended OMW and time.

S_3, S_4 and S_c) were collected monthly (during 6 months) from different parts of each plot from 0 to 20 cm deep, using a soil auger. All soil samples, taken from each plot were then mixed, air-dried, sieved with a mesh size of 450 µm and stored at 4°C prior to use. Water content was immediately determined before air-drying the sample.

Physico-chemical soils analyses

pH and electrical conductivity were determined according to Sierra et al. (2001) standard method. Organic matter was determined after furnacing samples at 550°C for 4 h. Total nitrogen was determined by the Kjeldahl method (1883). Inorganic nitrogen was determined by the Kandeler method (1995). Organic nitrogen is determined by difference between total and inorganic nitrogen.

Biological soil analyses

10 g of each soil sample was suspended in an erlenmeyer flask containing 90 ml of a sterile solution (0.2% 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 PCA at 30°C for total bacterial counts. Nitrifiers and denitrifiers microorganisms were enumerated by the most probable number (MPN) procedure (Trolldenier, 1995). The soil biological activity was achieved by measuring respirometric evolution in the aerobic condition (Öhlinger, 1995). Urease and ammonium oxidases activities were determined according to Kandeler et al. (1999).

Statistical analysis

For physico-chemical analyses, three replications were used for each parameter. For biological soils analyses, each soil sample was analysed in duplicate and the dilution series were plated in triplicate for each medium. Data were analysed using the ANOVA procedure. Variance and standard deviation were determined using Genstat 5 (second edition for windows).

RESULTS AND DISCUSSION

Treated and untreated OMW characteristics

The physico-chemical characteristics of untreated and treated OMW are summarized in Table 1. The high pollutant load of untreated OMW and its acidity could be observed. However, treated OMW was a slightly alkaline effluent, rich in inorganic loads such as potassium, calcium, magnesium and iron. Its content of phenolic compounds was lower than 1 g l^{-1}, reflecting a significant reduction of its toxicity from 99%I_B before treatment to only 30%I_B after treatment application (Table 1).

OMW impact on the physico-chemical soil parameters

Untreated and treated OMW effects on some soil physico-chemical parameters such as pH, electrical conductivity (EC), organic matter (OM), soil nitrogen and soil C/N ratio have been studied. Results showed that several chemical and biochemical properties of the investigated soil changed in response to the application of OMW. The addition of treated or untreated OMW with or without C/N ratio correction didn't show a significant effect on the initial soil pH. Indeed in spite of the initial untreated OMW acidity, the follow-up of this parameter during 6 months showed a weak reduction of the soil pH (0.2 units). Then, the acidity of the untreated OMW was compensated by the soil carbonate alkalinity as was shown by Sierra et al. (2001), whereas the addition of treated OMW caused a weak increase. Indeed, the alkalinity of this waste was not buffered by the soil components.

In the same way, the count of OMW induced an increase of the soil electric conductivity. This increase is proportional to the added OMW quantity. The increase of the soil Salinity could result from the main ionic species, sodium chloride and sulphate, coming from the treated or untreated OMW (Zanjari and Nejmeddine, 2001).

The studied soil is initially poor in organic matter. OMW addition improves the soil organic and mineral matters amounts. The organic matter supplement brought by 400 m^3 ha^{-1} of treated OMW is comparable to the one brought by 100 m^3 ha^{-1} of untreated OMW (Figure 1). The follow-up of the biodegradation kinetics of this organic matter brought by OMW shows that for the same quantity of added organic matter, soil receiving the treated OMW (S_3) presents a potential of biodegradation 3 times higher than the one of soil receiving the untreated OMW (S_1). The correction of the C/N ratio accelerates this biodegradation for soil irrigated with untreated OMW (S_2) and show a low effect in the case of soil receiving the treated OMW (S_4) (Figure 1).

The increase of organic and mineral nutrient contents may have a beneficial effect on the soil fertility. Nevertheless, the difficulty of untreated OMW organic matter biodegradation can be explained by its richness in phenolic compounds which are toxic for the soil biological

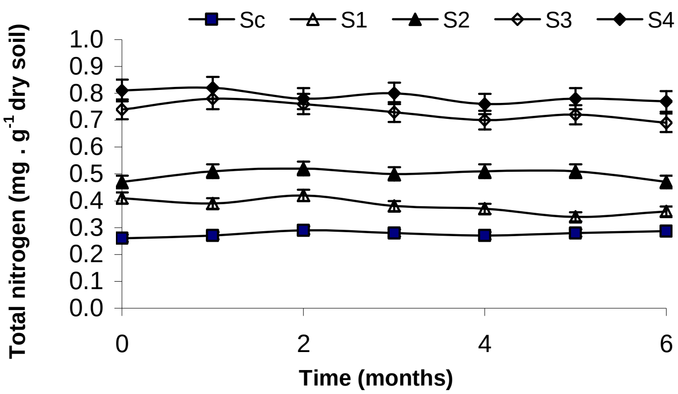

Figure 2. Soil total nitrogen evolution as a function of amended OMW and time.

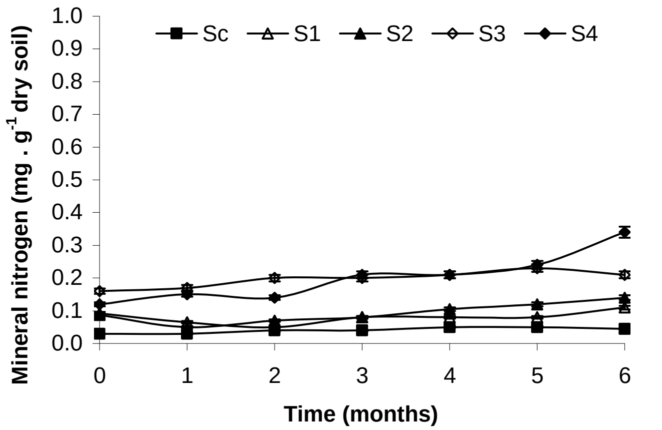

Figure 4. Soil inorganic nitrogen evolution as a function of amended OMW and time.

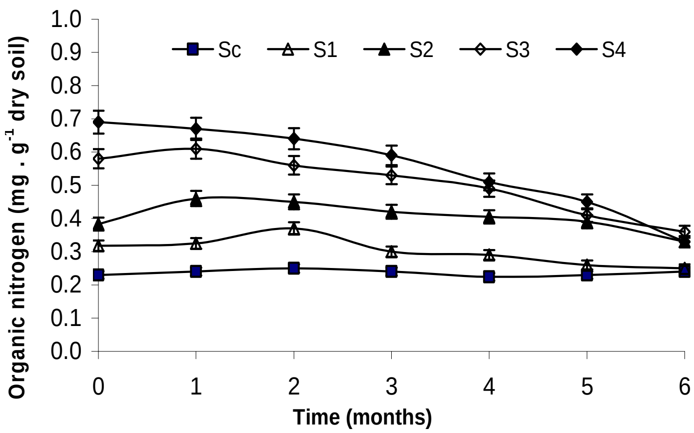

Figure 3. Soil organic nitrogen evolution as a function of amended OMW and time.

activity (Cox et al., 1998).

The OMW nitrogen content remained negligible compared to the other elements as the carbon and the potassium (Table 1). Control soil (S_C) is initially poor in nitrogen. Soil irrigated with the treated OMW presents more important total nitrogen content in that of soil receiving untreated OMW (Figure 2). The weak reduction of the total nitrogen or even its constancy during the time can be explained by the retraining of the different soil nitrogen shapes.

The organic nitrogen brought by the untreated OMW presents a slow mineralization. Indeed the affluence in suspended matters of untreated OMW enhances the immobilization of soil nitrogen. However, the organic nitrogen brought by the treated OMW turns quickly into inorganic nitrogen, what explains the important reduction of the content in organic nitrogen of soil irrigated with treated OMW (Figures 3 and 4).

The slow mineralization of the untreated OMW organic nitrogen is due to the composition of this material that facilitates its adsorption to soil particles since the untreated OMW is very rich in suspended matters. Negative effects of raw OMW on soil properties have also been recorded, including the immobilization of available nitrogen (Kissi et al., 2001).

OMW impacts on the biological soil parameters

OMW effects on the soil aerobic heterotrophic bacteria, nitrifiers and denitrifiers microorganisms and on the soil enzymatic and respirometric activities have been studied.

The aerobic heterotrophic bacteria counted on the studied soil are relatively weak (10^5 to 10^6 CFU $g^{-1}$of dry soil). Results showed an initial increase in total number of heterotrophic bacteria after the OMW amendment, mainly following C/N ratio correction. In line with this finding, Paredes et al. (1987) reported also an increase in the total viable counts in the soil polluted with OMW (Table 2).

The control soil was very poor in organic matter and particularly in nitrogen, so the number of nitrifiers microorganisms is very weak. The OMW addition increases in a meaningful manner their number. This increase is more remarkable in the case of soil receiving treated OMW (Figure 5). However, from the 4^{th} month after OMW addition, a reduction of these microorganisms number in all tested samples was observed. This decrease could be explained by the reduction of the soil organic nitrogen content. As it is the case for the nitrifiers, the OMW addition improves the presence of denitrifiers whose number increases correlatively with the OMW quantity (data not shown).

Indeed, nitrifiers and denitrifiers microorganisms play a critical role in the natural nitrogen cycle (Oved et al., 2001; Mendum and Hirsch, 2002). This microflora could be affected by a variety of chemical conditions including aromatic compounds and salts. The number of nitrifiers shifted from the CFU g^{-1} number ranging from 11.2 to 12.4 x 10^4 in the control soil to CFU g^{-1} number ranging

Table 2. Aerobic heterotrophic bacteria count in the different plots and time (CFU 10^5 g^{-1}).

Time (month)	March	April	May	Jun	Jul	August
Sc	54±5	49±5	52±5	49±5	50±5	51±5
S_1	75±7	78±7	72±7	70±6	63±6	64±6
S_2	88±8	103±9	98±8	93±9	96±9	94±8
S_3	117±10	114±10	108±10	106±1	105±10	98±9
S_4	123±11	126±11	117±11	109±10	111±10	107±1

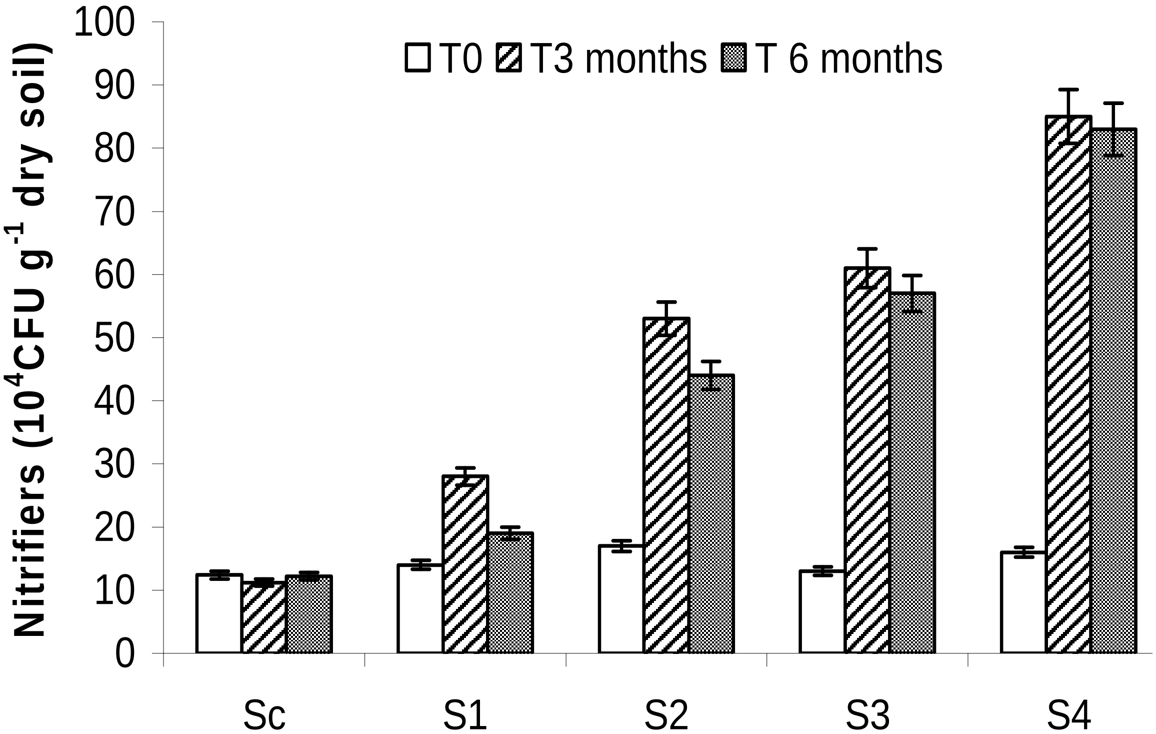

Figure 5. Nitrifiers numbers evolution as a function of amended OMW and time.

from 13 to 61 10^4 in the soil amended with treated OMW. Additionally, the decomposition of OMW by soil microbes could have induced oxygen depletion in the surface soil, thereby inhibiting aerobic microbial activity (Kowalchuk et al., 2000; Schloter et al., 2003; Gianfreda et al., 2006). Besides, the OMW addition improves the presence of denitrifiers whose number increases correlatively with the OMW quantity. Indeed, these microorganisms appear more resistant to the poisonous compounds that the nitrifying bacteria.

The need to measure the activities of a large number of enzymes has been emphasized to provide information on soil microbial activity (Sukul, 2006). Urease and ammonium oxidases constitute the two major enzymes of nitrogen metabolism. Urease plays a key role in the nitrogen cycle; it contributes to the transformation of the organic nitrogen in ammoniacal assimilated nitrogen (N-NH_4). Ammonium oxydases assures the transformation of the product of the ammonification in assimilated nitrogen by plants (N-NO_3) (Tscherko et al., 2003). Soils urease and ammonium oxidases activities are stimulated distinctly in soils irrigated with treated OMW, whereas the untreated OMW addition inhibits these two enzymatic activities (Table 3).

In this context, Deni and Penninckx (1999) reported that addition of hydrocarbon to an uncontaminated soil stimulated immobilization of nitrogen and reduced nitrification and soil urease activity.

The soil respirometric activity constitutes an important parameter to understand its biologic activity (Javorekova et al., 2001). A respirometric test was achieved on soils during 49 days. The specific respiration rate expressed as the ratio of C-CO_2/Ctot for the different soil samples is shown in Figure 6. The amendment of the soil with untreated OMW increased the carbon content while the specific

Table 3. Urease and ammonium oxidases activities detected in soils 6 months after OMW application.

Enzymatic activities	Urease (µg NH_4 g^{-1} 2 h^{-1})	Ammonium oxidases (µg NO_2 g^{-1} 24 h^{-1})
Sc	30±2.8	0.22±0.02
S_1	18±2	0.17±0.01
S_2	29±2.5	0.21±0.02
S_3	84±7	0.69±0.06
S_4	98±8.2	0.89±0.07

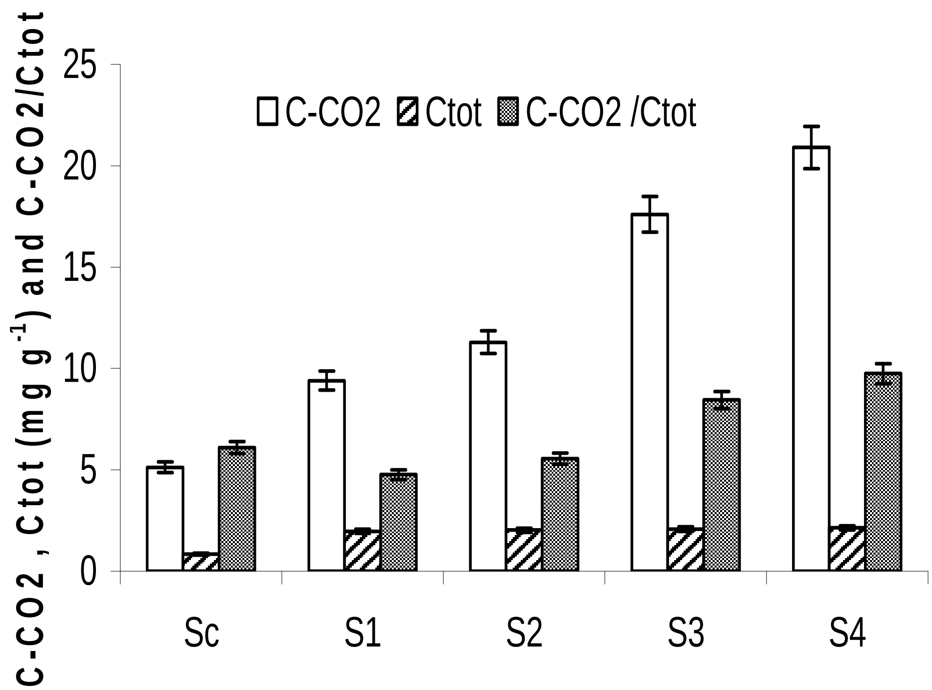

Figure 6. Specific respiration C-CO_2/C_{tot}, cumulative C-CO_2, and total carbon C_{tot} of the soil samples studied.

respiration remained very low. Indeed, Rinaldi et al. (2003) reported that the affluence of the untreated OMW organic matter in toxic phenolic compounds makes difficult its biodegradation in the nature. However, the amendment of the soil with treated OMW positively affects its specific respiration. The initial correction of the C/N ratio enhances the specific respiration rate (Figure 6).

Conclusion

Olive mill wastewaters constitute a serious environmental problem. Our results seem to confirm that the impact of OMW on soil properties was the result of opposite effects, depending on the relative amounts of beneficial and toxic organic and inorganic compounds present.

Soil organic matter recycling is assured by its autochthonous microflora. A contaminated soil constitutes an environment limiting for the microbial normal development, and therefore an unbalance of the soil matter cycles. OMW treatment before their application on soil is therefore necessary to limit negative impact on the soil biological activity.

Treated olive mill wastewaters with white-rot fungi followed by anaerobic digestion contains again relatively high amounts of dissolved and suspended organic matter in a large volume of water and are potential candidates for use as liquid organic amendment especially for soils and crops. The use of these waters for soil amendment improves its organic pool, bring assimilated nitrogen and stimulate the organic matter mineralization.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGMENTS

This research was funded by E.C program “Medusa water” contract ICA-CT-1999-00010 and contracts programme (MESRS, Tunisia).

REFERENCES

Aggelis G, Iconomou D, Christou M, Bokas D, Kotzailias S, Christou G, Tsagou V, Papanikolaou S (2003). Phenolic removal in a model olive oil mill wastewater using *Pleurotus ostreatus* in bioreactor cultures and biological evaluation of the process, Water Res. 37:3897-3904.

Allouche N, Fki I, Sayadi S (2004). Toward a high yield recovery of antioxidants and purified hydroxytyrosol from olive mill wastewaters, J. Agri. Food Chem. 52:267-273.

Box JD (1983). Investigation of the Folin-Ciocalteau phenol reagent for the determination of polyphenolic substances in natural waters, Water Res. 17:511-522.

Casa R, D’Annibale A, Pieruccetti F, Stazi SR (2003). Reduction of the phenolic components in olive-mill wastewater by enzymatic treatment and its impact on durum wheat (*Triticum durum Desf*.) germinability, Chemosphere. 50:959-966.

Cox L, Celis R, Hermosin MC, Beker A, Cornejo J (1998). Porosity and herbicide leaching in soils amended with olive-mill wastewater, Agr. Ecosyst. Environ. 65:151-161.

Deni J, Penninckx MJ (1999). Nitrification and autotrophic nitrifying bacteria in a hydrocarbon-polluted soil, Appl. Environ. Microbiol. 56 (9):4008-4013.

Dhouib A, Aloui F, Hamad N, Sayadi S (2006 a). Pilot-plant treatment of olive mill wastewaters by *Phanerochaete chrysosporium* coupled to anaerobic digestion and ultrafiltration, Process Biochem. 41:159-167.

Dhouib A, Ellouz M, Aloui F, Sayadi S (2006 b). Effect of bioaugmentation of activated sludge with white-rot fungi on olive mill wastewater detoxification, Lett. Appl. Microbiol. 42:405-411.

Fiestas JA, Borja R (1992). Use and treatment of olive mill waste-water: current situation and prospects in Spain, Grasas y Aceites. 43:101-106.

Fiorentino A, Gentili A, Isidori M, Monaco P, Nardelli A, Panella A, Fabio T (2003). Environmental effects caused by olive mill waste waters: Toxicity comparison of low-molecular–weight phenol components, J. Agri. Food chem. 51:1005-1009.

Gianfreda L, Piotrowska A, Iamarino G, Antonietta Rao M (2006). Short-term effects of olive mill waste water (OMW) on chemical and biochemical properties of a semiarid Mediterranean soil, Soil Biol. Biochem. 38:600-610.

Javorekova S, Stevlikova T, Labuda R, Ondrisik P (2001). Influence of xenobiotic on the biological soil activity. J. Central Eur. Agric. 2:3-4.

Kandeler E (1995). Total nitrogen. In: Schinner F., Ohlinger R., Kandeler E., Margesin R. (Eds.). Methods in Soil Biology, Springer. Berlin, pp. 406-408.

Kandeler E, Stemmer M, Klimanek EM (1999). Response of soil microbial biomass, urease and xylanase within particle size fractions to long-term soil management, Soil Biol. Biochem. 31, 261-273.

Kapellakis IE, Tsagarakis KP, Avramaki C, Angelakis AN (2006). Olive mill wastewater management in river basins: A case study in Greece, Agri.Water Manag. 82:354370.

Kissi M, Mountadar M, Assobhei O, Gargiulo E (2001). Roles of two white-rot basidiomycete fungi in decolourization and detoxification of olive mill waste water, Appl. Microbiol. Biotechnol. 57:221-226.

Kjeldahl J (1883). A new method for the determination of nitrogen in organic matter, Anal. Chem. 22:366.

Knechtel RJ (1978). A more economical method for the determination of chemical oxygen demand, Water Pollut. Control. (May/June) pp.25-29.

Kowalchuk GA, Stienstra AW, Heilig GH, Stephen JR, Woldendorp JW (2000). Molecular analysis of ammonia-oxidizing bacteria in soil of successional grasslands of the Drentsche A (The Netherlands), FEMS Microbiol Ecol. 31:207-215.

Nieto Martinez L, Garrido H (1994). El Alpechin: Un problema medioambiental en vias de solución (1), Quibal. 41:755-765.

Mekki A, Dhouib A, Sayadi S (2006 a). Changes in microbial and soil properties following amendment with treated and untreated olive mill wastewater, Microbiol. Res. 161:93-101.

Mekki A, Dhouib A, Aloui F, Sayadi S (2006 b). Olive wastewater as an ecological fertiliser, Agron. Sustain. Dev. 26:61-67. DOI: 10.1051/agro: 2005061.

Mekki A, Dhouib A, Sayadi S (2007 a). Polyphenols dynamics and phytotoxicity in a soil amended by olive mill wastewaters, J. Environ. Manage. 84:134140.

Mekki A, Dhouib A, Fki F, Sayadi S (2007 b). Assessment of toxicity of the untreated and treated olive mill wastewaters and soil irrigated by using microbiotests, Ecotoxicol. Environ. Saf. doi:10.1016/j.ecoenv.2007.04.008.

Mendum TA, Hirsch PR (2002). Changes in the population structure of b-group autotrophic ammonia oxidizing bacteria in arable soils in response to agricultural practice, Soil Biol. Biochem. 34: 1479-1485.

Obied HK, Allen MS, Bedgood DR, Prenzler PD, Robards K, Stockmann R (2005). Bioactivity and analysis of biophenols recovered from olive mill waste, J. Agri. Food chem. 53:823-837.

Öhlinger R (1995). Soil respiration by titration. In: Schinner F., Öhlinger R., Kandeler E., Margesin R. (Eds.). Methods in soil biology. Springer, Berlin, pp. 95-98.

Oved T, Shaviv A, Goldrath T, Mandelbaun RT, Minz D (2001). Influence of effluent irrigation on community composition and function of ammonia-oxidizing bacteria in soil, Appl. Environ. Microbiol. 67: 3426-3433.

Paredes MJ, Moreno E, Ramos-Cormenzana A, Martinez J (1987). Characteristics of soil after pollution with waste waters from oil extraction plants, Chemosphere. 16:1557-1564.

Paredes C, Roig A, Bernal MP, Sanchez-Monedero MA, Cegarra J (2000). Evolution of organic matter and nitrogen during co-composting of olive mill wastewater with solid organic wastes, Biol. Fert. Soils. 32:222-227.

Rinaldi M, Rana G, Introna M (2003). Olive-mill wastewater spreading in southern Italy: effects on a durum wheat crop, Field Crops Res. 84: 319-326.

Sayadi S, Ellouz R (1995). Roles of lignin peroxidase and manganese peroxidase from *Phanerochaete chrysosporium* in the decolourization of olive mill wastewaters, Appl. Environ. Microbiol. 61: 1098-1103.

Schloter M, Dilly O, Munch JC (2003). Indicators for evaluating soil quality, Agr. Ecosyst. Environ. 98:255-262.

Sierra J, Marti E, Montserrat G, Cruanas R, Garau MA (2001). Characterization and evolution of a soil affected by olive oil mill wastewater disposal, Sci. Total Environ. 279:207-214.

Sukul P (2006). Enzymatic activities and microbial biomass in soil as influenced by metalaxyl residues, Soil Boil. Biochem. 38:320-326.

Trolldenier G (1995). Nitrifiers by MPN method. In: Schinner F., Öhlinger R., Kandeler E., Margesin R. (Eds.). Methods in soil biology. Springer, Berlin, pp. 32-36.

Tscherko D, Bölter M, Beyer L, Chen J, Elser J, Kandeler E, Kuhn D, Blume H-P (2003). Biomass and enzyme activity of two soils transect at King George Island, Maritime Antarctica; Arctic, Antarctic, and Alpine Research. 35:34-47.

Zanjari B, Nejmeddine A (2001). Impact of spreading olive mill waste waters on soil characteristics: Laboratory experiments, Agronomie. 21:749-755.