Journal of Scientific Research & Reports
2(2): 559-570, 2013; Article no. JSRR.2013.007

SCIENCEDOMAIN *international*
www.sciencedomain.org

Changes Induced by Olive Mill Wastewaters Used as Organic Fertilizer on Polyphenol Content, pH and Salinity of an Alkaline Soil

Rachid Razouk[1*], Kaltoum Oubella[2] and Abdelaziz Chergaoui[1]

[1]Regional Agricultural Research Center, P.O. Box 578, Meknès, Morocco.
[2]Department of Biology, Moulay Ismail University, P.O. Box 4010, Meknès, Morocco.

Authors' contributions

This work was carried out in collaboration between all authors. Authors RR and AC designed the study and wrote the protocol. Author RR performed the experiment and statistical analysis and wrote the first draft of the manuscript. Author KO assisted in the realization of measurements. All authors read and approved the final manuscript.

Research Article

Received 21st June 2013
Accepted 4th July 2013
Published 16th August 2013

ABSTRACT

Aims: The main objective of this paper is to determine the recommended amounts of olive mill wastewaters (OMW) that should be applied as a fertilizer in alkaline soil, based on evolution of its pH, salinity and polyphenol content.
Study Design: Randomized complete block design with three replications where the variable factor was amount of OMW.
Place and Duration of Study: The trial was carried in an open glass greenhouse in the Regional Agricultural Research Center of Meknes - Morocco, between February and March 2012.
Methodology: Four amounts of OMW (50, 75, 100 and 150 m^3/ha) were tested as organic fertilizer on an alkaline soil in comparison with water treatment based on measurement of soil pH, electrical conductivity and polyphenol content using fenugreek as an indicator plant. The Indicators of stress measured on plants were leaf chlorophyll content, stomatal conductance and leaf temperature.
Results and Discussion: The application of the four amounts of OMW induced a decrease of soil pH and an increase of its electrical conductivity whereas soil polyphenol

**Corresponding author: E-mail: razouk01@yahoo.fr;*

content following significant regression models. Soil pH and electrical conductivity changed slightly under all treatments with a maximal values under the treatment 150 m^3/ha. However, soil polyphenol content increased widely under different treatments of OMW. The physiological parameters of the fenugreek showed a certain stress under 150 m^3/ha.

Conclusion: OMW may be used as organic fertilizer up to 100 m^3/ha on alkaline soil without adversely affecting its quality in the arable layer. Beyond this amount, a particular attention should be paid, to soil polyphenols as far as their phytotoxicity is concerned, since a high concentration of polyphenols may exert an allelochemical effect.

Keywords: Olive mill wastewaters; alkaline soil; Trigonella foenum-graecum; soil pH,;soil salinity; soil polyphenol content.

1. INTRODUCTION

The development of olive oil industry imposed environmental challenges residing mainly in pollution caused by olive mill wastewaters (OMW) that are liquid residues formed by 40 to 50% of vegetable water naturally contained in olives and those added for extracting oil [1]. It is generally estimated that trituration of 1 kg of olives generates 1 to 1.5 liters of OMW [2], depending on the extraction system used. The volume of OMW is steadily increasing because of the extension of olive plantation. In Morocco, for an annual olives production of 700.000 tons on an area of 790.000 ha [3], olive oil industry produces more than 250.000 m^3 of OMW [4].

Purification of OMW is an expensive operation and difficult to undertake because of their high richness in organic matter and minerals [5,6]. In Morocco, because of absence of effective purification systems, OMW are dumped in nature without any treatment prior, either directly or across the public sewerage system [7]. This practice poses serious pollution problems, especially on surface water and groundwater, and adverse effects on soils and crops [8]. Pollutant power of OMW is limited to three main factors that are: the acidity, the salinity [9-11] and the phenolic compounds which are phytotoxic by allelochemical effect [12].

In parallel with attempts to reduce the pollution load of OMW in several countries, particularly Mediterranean including Morocco, by natural evaporation in the storage basins, several trials are engaged to identify the economically feasible solutions for their valorization in agriculture and industry. In agriculture, these trials consist essentially to use OMW as an additive in animal feed [13] to extract phenolic and aromatic products [14], natural antioxidants and their use as herbicidal product [15].

The trials regarding use OMW as organic fertilizer, gross or composted, carried out since 1970 in Spain, Italy and Greece, have shown encouraging results especially for olive, vine, maize and rice productions without significant negative effects on soil fertility. The retained amounts vary between 50 and 150 m^3/ha depending on crops, soil type and climatic conditions [16,17]. In Morocco, Algeria and Tunisia, the transfer of this technology has been started recently, especially on olive, vine, tomato and date palm. Results of these trials were similar to those found in the northern countries of the Mediterranean and portend the possibility of using OMW as fertilizer with condition to not exceed a certain amount. However, the decision-making of this technique still confronted to some adverse effects reported by other authors, especially related to the acidity, salinity and polyphenol content [18,19]. It is within this mind that the present experiment has been carried to identify the temporal evolution of the main factors that could adversely affect soil quality and fertility due to OMW, that are salinity, acidity and polyphenol content by taking fenugreek as an indicator plant.

2. MATERIALS AND METHODS

2.1 Olive Mill Wastewaters Used

OMW used were taken approximately after one month of their production (end of January) from an accumulation basin of a modern oil mill in Meknes region, in the North of Morocco, which does not add salt in extraction process. The chemical characteristics of OMW are presented in Table 1, showing that the used OMW are characterized by an acid pH, a high electrical conductivity (EC) and a high organic matter content, expressed by values of biological and chemical oxygen demand.

Furthermore, it is noted that the used OMW far exceed specific limits established by Moroccan Secretariat of the Environment regarding pH, electrical conductivity, biological oxygen demand (BOD) and polyphenol content.

Table 1. Chemical characteristics of OMW used

Parameters	Values
pH	4.56
EC (mS.cm^{-1} at 20°C)	6.8
Total phenolic compounds (g l^{-1})	3.6
Chemical oxygen demand COD (g l^{-1})	78
Biological oxygen demand at 5 days BOD_5 (g l^{-1})	49.1
Solid matter in suspension (mg l^{-1})	710

2.2 Soil Type and Indicator Plant

The soil used is of calci-magnesic type which is the dominant type in Meknes region [20]. The samples of soil were taken from a field previously cultivated by wheat. The soil is silty clay, moderately rich in organic matter, with an average of 1.40% in the top soil surface layer (0-30 cm). The soil pH is basic (7.98) and moderately saline with an average of electrical conductivity around 1.04 mS/cm.

The indicator plant used was fenugreek (*Trigonella foenum-graecum*) cultivated largely in association with olive trees in several olive-growing regions of Morocco [21]. This herb has been chosen for its sensitivity to salt stress especially during the vegetative growth phases [22,23].

2.3 Treatments, Experimental Design and Measurements

The trial was carried between February and March 2012 in 10 liters pots (30 cm height and 21 cm diameter) placed into an open glass greenhouse in the Regional Agricultural

Research Center of Meknes located in the Saïs plain, in North center of Morocco (33° 56’ E, 5° 13’ N; 500 m).

The pots were filled with the same amount of soil, homogenized prior, on which fenugreek seeds were sown at the rate of five per pot. After sowing, different amounts of OMW were applied on pots uniformly. However, in order to apply the same irrigation, amounts of water were added to OMW (Table 2). During the two months of the experiment, the pots were weekly irrigated at maximal evapotranspiration, with an average of 7 mm/week.

Table 2. Amounts of OMW tested as a fertilizer of fenugreek

Treatments	OMW (ml pot^{-1})	Water added (ml pot^{-1})	Equivalent of OMW per hectare (m^3 ha^{-1})
T0	0	675	0
T1	225	450	50
T2	340	335	75
T3	450	225	100
T4	675	0	150

The experimental design was a randomized complete block, with three replications. Each of the three block consisted of five pots, each of which corresponds to an OMW treatment. Measurements consisted at weekly follow of pH, electrical conductivity and polyphenol content on soil and of physiological indicators of stress on fenugreek crop, that are chlorophyll content, stomatal conductance and leaf temperature.

Soil analysis was realized on samples taken by a plastic tube with 1 cm of diameter and 20 cm of length. Soil pH and electrical conductivity were measured on soil previously dried under ambient temperature and sieved at 2 mm. Polyphenol content was measured on lyophilized fine soil by spectrophotometry, following the method of Singleton and Rossi [24].

Chlorophyll content, stomatal conductance and leaf temperature of fenugreek plants were measured using a SPAD chlorophyll-meter, a leaf porometer and an infrared thermometer. The measurements were realized in the morning at 11h on the second and the third leaves from shoot apex and averages were taken.

3. RESULTS AND DISCUSSION

3.1 Effect of OMW on Soil pH, Salinity and Polyphenol Content

A high linear correlation was found between values of soil pH and amount of OMW applied (Fig. 1). This result was also found by other authors but with different significance level depending on soil type [25]. The linear relationship remained significant even after one month of OMW application, maintaining the same determination coefficient. However, the decrease of soil pH, influenced by the OMW, was attenuated after this period because fort probably of soil elasticity effect. This is related to presence of calcium bicarbonate in alkaline soils, which reacts with carbon dioxide and water in the soil to produce bicarbonate (HCO^{3-})' which is able to take the ions H_3O^+ and Al^{3+}, thereby raising the soil pH [26,27].

Practically, decrease of soil pH, observed under all treatments, is not able to adversely affect plants growth, especially in alkaline soils as the case of the tested soil type. Indeed, the higher dose of OMW (150 m^3/ha) decreased soil pH just by 0.29 points which has reduced to 0.17 points (Table 3) after one month because of soil elasticity and drainage of ions H_3O^+ to deeper layers. The extrapolations of this result on neutral soils (pH 7) that are prevalent in Morocco suggests the possibility of application of OMW on this soil type until an amount of 150 m^3/ha without make it acid. The slight acidification of this soil type may greatly favors plant nutrients availability. In fact, the majority of macronutrients and micronutrients (N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, Ni, and Zn) are most available within a pH range of 6 to 7. Outside of these optimal ranges, nutrients are available to plants at lesser amounts [28].

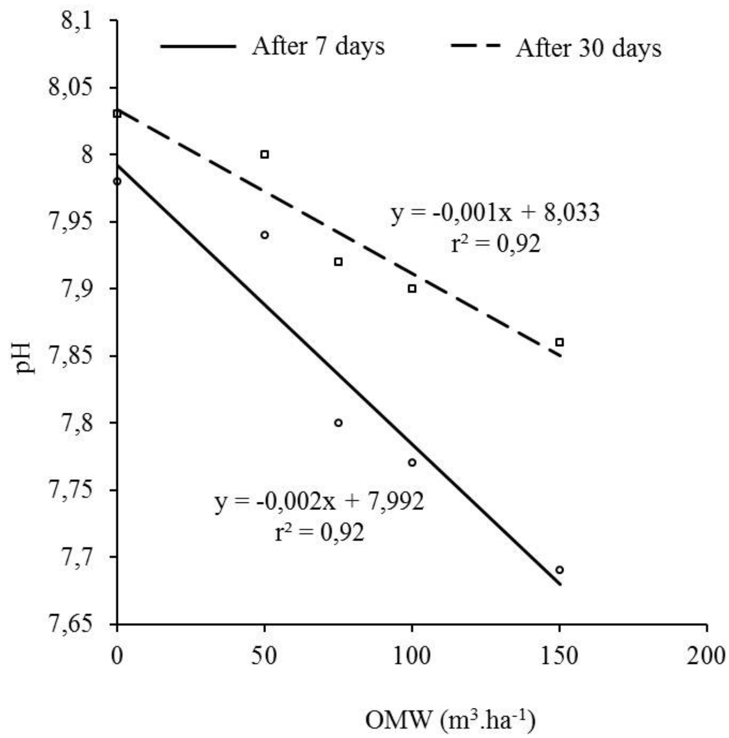

Fig. 1. Relationship between soil pH and amount of OMW applied

As for the soil salinity, it shown that all amounts tested of OMW induced a slight increase of soil electrical conductivity (EC), but with significant levels even after one month of their application (Table 3). Indeed, the amounts 50, 75 and 100 m^3/ha of OMW increased respectively soil EC by 1.5%, 2.5% and 3.2% compared to the control treatment without OMW application. Under 150 m^3/ha of OMW, soil EC was increased by 50 μS/cm which is equivalent to 5%. This last result is in disagreement with those of Levi-Minzi et al. [29] who found that the increase of the soil EC does not exceed 3.5% even with application of high amounts of OMW up to 300 m^3/ha. By against, Hanifi and El Hadrami [30] found that soil EC did not change after application of 150 m^3/ha of OMW over three consecutive years. The contrary results may be related to drainage capacity of the soil type used which was sandy in the last trial. In fact, more soil texture is sandy more the mineral salts are drained to the deeper layers, thereby decreasing the soil EC in the arable layer [31].

Variation of soil EC was significantly correlated in polynomial relationship with the amount applied of OMW (Fig. 2). The relationship indicates that the amount of OMW required for increase soil EC by 0.1 mS/cm is about 260 m^3/ha, which is equivalent to 10400 m3/ha to increase it to 4 mS/cm which constitute the limit value beyond which the soil is considered saline [32,33]. However, these OMW effects concern only the soil arable layer (0-30 cm) and remain to study their impact on mineral salts accumulation in deeper layers.

Table 3. Weekly evolution of soil pH, electrical conductivity and polyphenol content under different amounts of OMW

	Week	Control	50 m^3 ha^{-1}	75 m^3 ha^{-1}	100 m^3 ha^{-1}	150 m^3 ha^{-1}
Soil pH	1	7.98	7.94	7.80	7.77	7.69
	2	8.00	7.97	7.82	7.80	7.74
	3	8.02	7.98	7.86	7.84	7.79
	4	8.03	8.00	7.92	7.90	7.86
Soil EC ($mS.cm^{-1}$)	1	1.04	1.06	1.07	1.07	1.09
	2	1.04	1.05	1.06	1.07	1.09
	3	1.04	1.04	1.05	1.06	1.09
	4	1.04	1.04	1.05	1.06	1.08
Soil polyphenol content ($mg.gDW^{-1}$)	1	0,17	0,20	0,24	0,35	0,52
	2	0,12	0,19	0,21	0,27	0,42
	3	0,07	0,12	0,15	0,17	0,35
	4	0,02	0,05	0,08	0,10	0,17

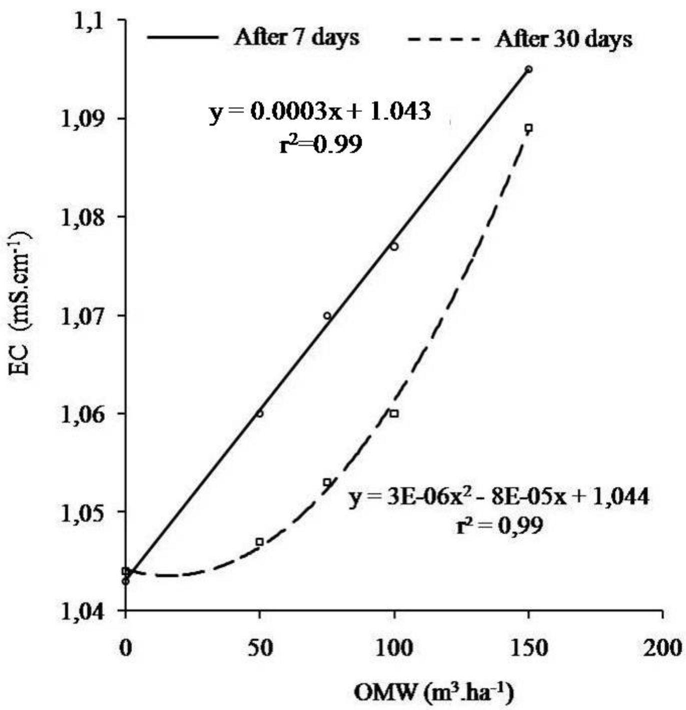

Fig. 2. Relationship between soil electrical conductivity and amount of OMW applied

Soil polyphenol content (PC) was amply increased under effect of OMW. After one week of application, the initial soil PC (0.175 mg/gDW) doubled under the treatment 100 m^3/ha to reach three fold value under 150 m^3/ha (Table 3). The moderate treatments 50 and 75 m^3/ha increased soil PC, respectively by 17 and 40%. The relationship between the soil PC and the amount of OMW was significant following a polynomial equation (Fig. 3) which indicates that application of $1m^3$/ha of OMW increased soil PC by an average of 1.40 µg/gDW.

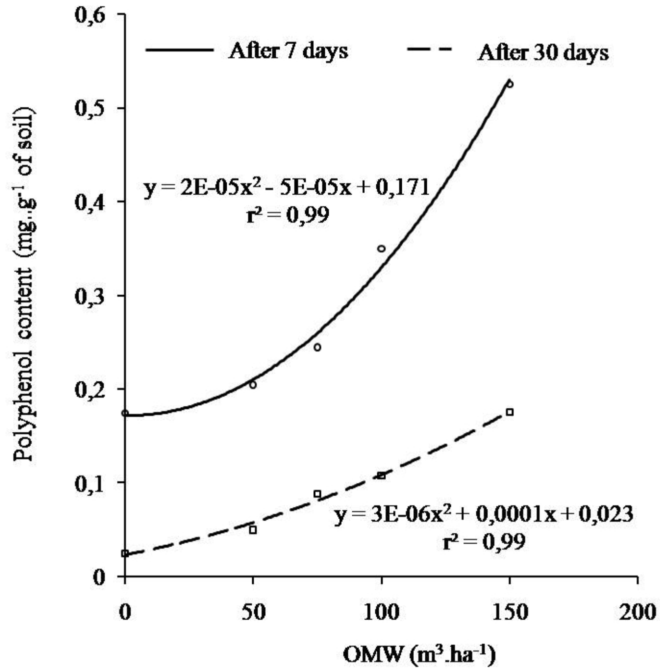

Fig. 3. Relationship between soil polyphenol content and amount of OMW applied

However, after one month of OMW application, soil PC decreased under all treatments by an average of 72%. This decrease was due to their deep infiltration and biodegradation in the soil. In fact, the phenolic compounds of OMW may be degraded by microorganisms [34,35] or by microbial enzymes [36,37]. However, the duration necessary for their complete degradation may be considerable. Indeed, Hanifi and El Hadrami [30] found that soil PC was undetectable in soil surface layer after three years of application of 150 m^3/ha of OMW.

3.2 Effect of OMW on Physiological Behavior of Fenugreek

The physiological response of fenugreek, taken as indicator plant, varied depending to the amounts applied of OMW based on weekly evolution of chlorophyll content index, stomatal conductance and leaf temperature values (Fig. 4).

As for chlorophyll, an improvement of its concentration was observed under the treatments 50 and 75 m^3/ha especially from the third week of OMW application and from the fourth week under the treatment 100 m^3/ha. This positive effect was more related to nitrogen and organic acids content of OMW. However, the treatment 150 m^3/ha induced a spectacular decrease of chlorophyll content more pronounced from the second week of its application. This depressive effect was more related to the allelochemical effect of polyphenols whose concentration in the soil tripled under treatment 150 m^3/ha to reach 0.52 mg/g. Secondary, this effect may be also due to the soil salinity which was relatively elevated under this treatment compared to the control treatment. This is in agreement with the findings of Tewari and Singh [38] and Sivtsev [39] who observed that a slight increase of soil salinity induced a significant decrease of chlorophyll content in tomato and lentil.

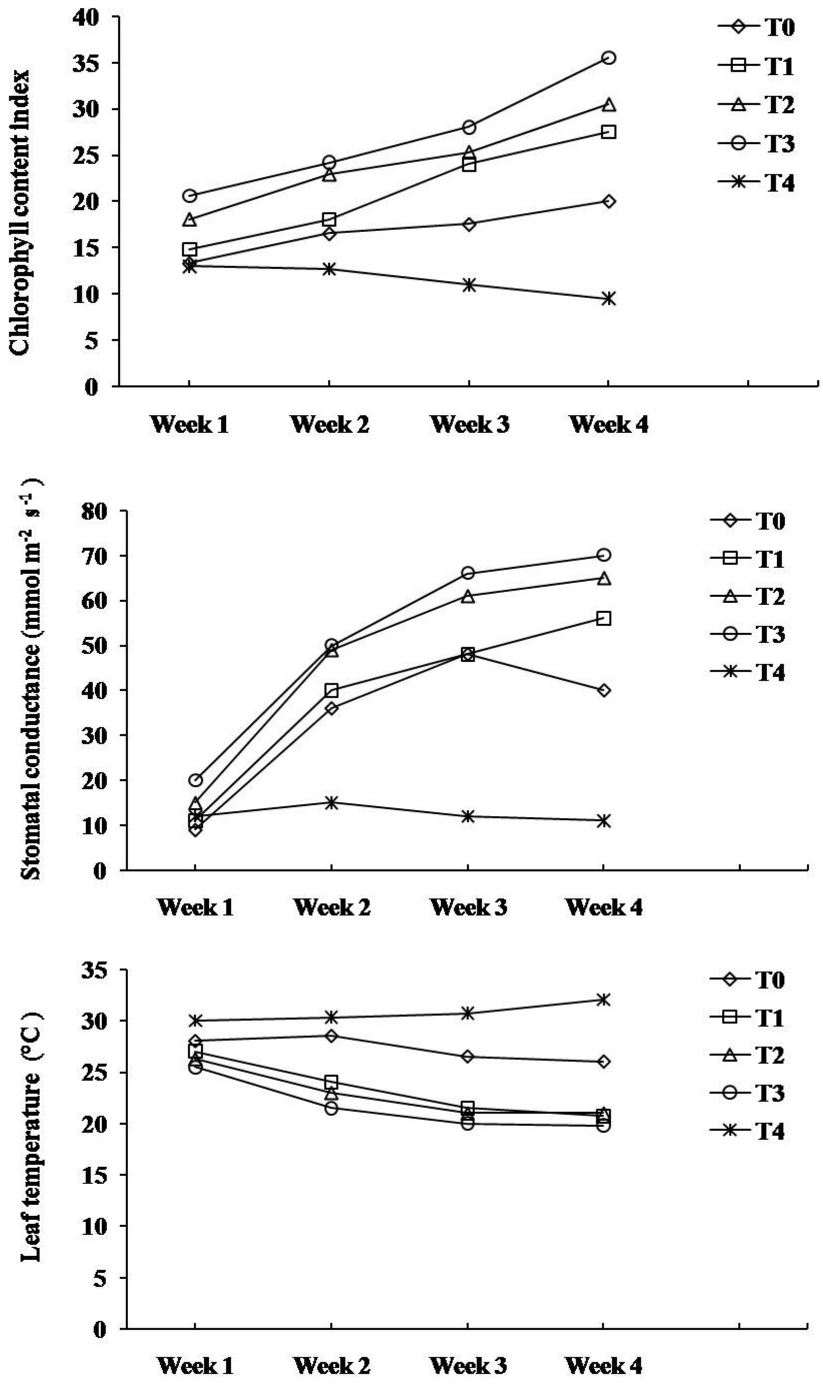

Fig. 4. Evolution of some physiological parameters of fenugreek fertilized by different amounts of OMW

The effect of treatment 150 m^3/ha on chlorophyll content of fenugreek was also explained by an induction of water stress by raising soil polyphenol content and soil salinity. Water stress may reduce chlorophyll content by decreasing assimilation and translocation of nitrogen [40]. Indeed, water deficit induced a nitrogen deficit which comes mainly from reductions in nitrogen flow at the roots, and secondarily from capacity reductions of root absorption and reduction of transport between leaves and roots due to transpiration feebleness [41]. This explanation was based on values of stomatal conductance and leaf temperature which indicate that fenugreek was submitted to water stress under the treatment 150 m^3/ha.

Indeed, under treatment 150m^3/ha, transpiration flow of the fenugreek was reduced from the first week of OMW application, marked by a significant decrease of stomatal conductance and an increase of leaf temperature. The changes of these physiological parameters are explained by the fact that the transpiration mitigation induces stomata closure and a decrease of their density, thereby decreasing the stomatal conductance. Consequently, it reduces the layer of steam water on leaf surface and makes accordingly leaf temperature more controlled by air temperature changes. However, fenugreek water status improved under the three treatments 50, 75 and 100 m^3/ha based on stomatal conductance and leaf temperature values. The positive effect of these treatments on fenugreek water status comes in large part from nitrogen and organic acids contained in OMW that increase rate of CO_2 assimilation, thereby improving the stomata functioning [42,43].

4. CONCLUSION

Soil application of OMW as an organic fertilizer of fenugreek has differently influences the alkaline soil quality and physiological behavior of fenugreek depending on the amounts applied. The amounts 50, 75 and 100 m^3/ha of OMW induced slight changes of soil quality without significant effect on the physiological behavior of fenugreek accordingly to values of chlorophyll content, stomatal conductance and leaf temperature. By against, application of 150 m^3/ha seemed detrimental to soil quality by increasing significantly soil electrical conductivity and amply soil polyphenol content that are phytotoxic by allelochemical effect. These changes in soil quality induced water stress, detected through measurement of stomatal conductance and leaf temperature on fenugreek, as well as nutritional stress revealed through measurement of leaf chlorophyll content.

ACKNOWLEDGEMENTS

Our thanks go to Dr. Mohammed El Asri, Head of the Regional Agricultural Research Center of Meknes – Morocco, and to Dr. Abdellah Kajji, coordinator of the Research Unit of Agronomy and Plant Physiology, for their logistical support.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ranalli G, Alfano G, Bahammi M, Belli C, Lustrato G. Gestion des sous-produits de la filière oléicole au Maroc: Identification des solutions éco-compatibles et économiquement soutenables pour la valorisation des résidus de l'huile d'olive et la réduction de l'impact environnemental dans les régions de Fès, Meknès et Marrakech. Rabat, Morocco: United Nations Organization for Industrial Development; 2003. French.

2. Achak M, Ouazzani N, Mandi L. Traitement des margines d'une huilerie moderne par infiltration-percolation sur un filtre à sable. Revue des Sciences de l'Eau. 2009;22(3):421-33.
3. Anonymous. Les aides financières de l'état pour l'encouragement des investissements agricoles. Rabat, Morocco: Ministry of Agriculture and Marine Fisheries; 2011. French.
4. Anonymous. L'Industrie Oléicole au Maroc et son impact sur l'Environnement, propositions d'actions de lutte contre la pollution générée par les Huileries d'Olives: cas de la Province de Taounate. Rabat, Morocco: Secretariat of Environment; 2004. French.
5. Fiestas JA, Borja R. Use and treatment of olive mill wastewater: current situation and prospects in Spain. Grasas y Acietes. 1992;43(2):101-06.
6. Rosario L, Ana R, Bernardo M, Gerardo AC, Antonio G. Biodegradation of olive mill wastewaters (OMW) by strains of scopulariopsis spp. Isolated from OMW contaminated habitats. Toxicology and Environmental Chemistry. 1999;72:127-34.
7. Gartet A, El Fengour M, Gartet J. Dégradation de la qualité des eaux du barrage Sahla: traitement et gestion des risques de pollution. Papeles de Geografía. 2009;49:41-54. French.
8. Benyahia N, Zein K. Analyse des problèmes de l'industrie de l'huile d'olive et solutions récemment développées. Lausanne, Switzerland: second international conference of Swiss Environmental Solutions for Emerging Countries (SESEC II); 2003. French.
9. Zenjari B, Nejmeddine A. Impact of spreading olive mill wastewater on soil characteristics: laboratory experiments. *Agronomy*. 2001;21:749-755.
10. Fakharedine N, El Hajjouji H, Ait Baddi G, Revel JC, Hafidi M. Chemical and spectroscopic analysis of organic matter transformations during aerobic digestions of olive-mill waste-waters. Process Biochemistry. 2006;41:398-04.
11. Mekki A, Dhouib A, Sayadi S. Polyphenols dynamics and phytotoxicity in a soil amended by olive mill wastewaters. Journal of Environmental Management. 2007;84:134-140.
12. Wang Q, Li ZH, Ruan X, Pan CD, Jiang DA. Phenolics and plant allelopathy. Molecules. 2010;15:8933-52.
13. Nefzaoui A. Valorisation des sous produits de l'olivier. Options Méditerranéennes – Série A. 1991;16:101-08. French
14. Camurati F, Rizzolo A, Fedeli E. Chemical components of the anatomical parts of the olea Europea fruit - Alcoholic extracts. Rivista Italiana Delle Sostanze Grasse. 1981;58(11):541-47.
15. Kaddour S, Kharrat M, Souissi T, Bouya D, Bouhache M. Chemical control of orobanche spp In Faba Bean (*Vicia Faba*) In the Maghreb. El-Beida, Libya: Proceeding of the eighth arab congress of plant Protection; 2003.
16. Alfano G. Valorisation des sous-produits de l'olivier issus de système de trituration 2 et 3 phases: Expérience Italienne. Meknes, Morocco: Proceeding of the fourth edition of the Mediterranean olive days; 2009. French.
17. Nieto LM. Valorisation des sous-produits de l'olivier et des effluents liquides de l'industrie d'huile d'olive: biomasse et production d'énergie. Meknes, Morocco: Proceeding of the fourth edition of the Mediterranean olive days; 2009. French.
18. Moreno E, Perez J, Ramos-Cormenzana A, Martinez J. Antimicrobial effect of waste water from olive oil extraction plants selecting soil bacteria after incubation with diluted waste. Microbios. 1987;51(208):169-74.
19. Yesilada O, Sik S, Sam M. Biodegradation of olive oil mill wastewater by Coriolus versicolor and Funalia trogii: effects of agitation, initial COD concentration, inoculum size and immobilization. World Journal of Microbiology and Biotechnology. 1998;14:37-42.

20. El Idrissi R.M. Différentiation des sols du plateau de Meknès. Paris, France: Paris VI University; 1992. French.
21. Razouk R, Daoui K. Croissance et production de l'olivier sous les conditions pluviales en relation avec l'occupation temporelle des cultures intercalaires. Rabat, Morocco: Revue Annuelle du Projet Arboriculture Fruitière (PAF – MCA). 2012;1:173-79. French.
22. Al-Saady NA., Khan AJ., Rajesh L, Esechie HA. Effect of salt stress on germination, proline metabolism and chlorophyll content of Fenugreek (*Trigonella foenum-graecum*). Journal of Plant Sciences. 2002;7(5):176-85.
23. Pour AP, Farahbakhsh H, Saffari M, Keramat B. Response of fenugreek plants to short-term salinity stress in relation to photosynthetic pigments and antioxidant activity. International Journal of Agriculture: Research and Review. 2013;3(1):80-86.
24. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic – phosphotungstic acid reagents. American Journal of Enology and Viticulture. 1965;16:148-58.
25. Bonari E, Macchia M, Angelini LG, Ceccarini L. The waste waters from olive oil extraction: their influence on the germinative characteristics of some cultivated and weed species. Agricoltura Mediterranea. 1993;123:273-80.
26. Tisdale SL, Nelson WL, Beaton JD, Havlin JL. Soil fertility and fertilizers. 5th ed. New York: Macmillan Publishers; 1993.
27. Brady NC, Weil RR. The nature and properties of soils. 12^{th} Edition. New Jersey: Prentice Hall; 1999.
28. McCauley A, Jones C, Jacobsen J. Soil pH and organic matter. Nutrient Management module. 2009;8:1-12.
29. Levi-Menzi R, Saviozzi A, Riffaldi R, Falzo L. Lo smaltimento in campo delle acque di vegetazione: effetti sulleproprieta del terreno. Olivae. 1992;40:20-25. Spanish.
30. Hanifi S, El Hadrami I. Olive mill wastewaters fractioned soil-application for safe agronomic reuse in date palm (*Phoenix dactylifera L.*) fertilisation. Journal of Agronomy. 2008;7(1):63-69.
31. Bouarfa C, Slama F, Bahri A, Chaumont S, Bouhlila R. Pratiques d'irrigation et rôle du drainage pour le contrôle de la salinité: Cas du périmètre irrigué de Kalaat Landelous en Tunisie. Rabat, Morocco: Proceeding of the Irrigated agriculture modernization seminar; 2004. French.
32. Horneck DA, Ellsworth JW, Hopkins BG, Sullivan DM, Stevens RG. Managing salt - affected soil for crop production. Corvallis, USA: Oregon State University; 2007.
33. Rhoades JD, Chanduvi F, Lesch S. Soil salinity assessment: methods and interpretation of electrical conductivity measurements. Rome, Italy: Food and Agriculture Organization; 1999.
34. Tsioulpas A, Dimou D, Iconomou D, Aggelis G. Phenolic removal in olive mill wastewater by strains of Pleurotus spp. In respect to their phenol oxidase (laccase) activity. Bioresource Technology. 2002;84:251-57.
35. Fountoulakis MS, Dokianakis SN, Kornaros ME, Aggelis GG, Lyberatos G. Removal of phenolics in olive mill wastewaters using the white-rot fungus Pleurotus ostreatus. Water Research. 2002;36(19):4735-44.
36. Robles A, Lucas R, De Cienfuegos GA, Galvez A. Phenol-oxidase (laccase) activity in strains of the hyphomycete Chalara paradoxa isolated from olive mill wastewater disposal ponds. Enzyme and Microbial Technology. 2000;26(7):484-90.

37. Casa R, D'Annibale A, Pieruccetti F, Stazi SR, Giovannozzi Sermanni G, Lo Cascio B. Reduction of the phenolic components in olive-mill wastewater by an enzymatic treatment and its impact on durum wheat (*Triticum durum Desf.*) germinability. Chemosphere. 2003;50(8):959-66.
38. Tewari TN, Singh BB. Stress studies in lentil (*Lens esculenta Moench*). II. Sodicity-induced changes in chlorophyll, nitrate, nitrite reductase, nucleic acids, proline, yield and yield components in lentil. Plant and Soil. 1991;136(2):225-30.
39. Sivtsev MV, Ponomareva SA, Kuznetsova EA. Effects of Salinization and herbicide on chlorophyllase activity in tomato leaves. Russian Journal of Plant Physiology. 1973;20(1):62-65.
40. Bojovié B, Markovié A. Correlation between Nitrogen and Chlorophyll Content in Wheat (*Triticum aestivum L.*). Kragujevac Journal of Science. 2009;31:69-74.
41. Tardieu F, Cruiziat P, Durand JL, Triboi E, Zivy M. Perception de la sécheresse par la plante, conséquences sur la productivité et sur la qualité des produits récoltés. In Anonymous, editors. Sécheresse et agriculture, Paris, France: National Agricultural Research Institute; 2006. French.
42. Weng JH, Hsu FH. Gas exchange and epidermal characteristics of Miscanthus populations in Taiwan varying with habitats and nitrogen application. Photosynthetica. 2001;39(1):35-41.
43. Tazoe Y, Noguchi K, Terashima I. Effects of growth light and nitrogen nutrition on the organization of the photosynthetic apparatus in leaves of a C_4 plant, *Amaranthus cruentus*. Plant Cell and Environment. 2006;29(4):691-700.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=250&id=22&aid=1885