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Original Research

Agricultural Valorization of Olive Mill Wastewater in Arid Regions of Tunisia: Short-Term Impact on Soil Biochemical Properties and Faba Bean Growth

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Abstract

Olive mill wastewater (OMW) is generated seasonally a large amount during the olive oil production in southern Tunisia and it is often discharged in the open environment. OMW has a high amount of phototoxic compounds, high salinity and acidity and therefore is challenging when disposed on soil. However, in southern Tunisia, the condition climatic is arid and semis-arid region. The soil sandy is in degradation and erosion processes.

New strategies have been developed to reduce these impacts in soil and to valorize a waste product as olive mill wastewater (OMW) loaded with minerals and organic matters as fertilizer in agronomy. The major aim of this study was to investigate the effects of OMW spraying onto on soil biochemical properties and Faba bean crop productivity was investigated.

The result showed that the irrigation of sandy soils by different OMW doses strongly influenced their chemical and microbiological characteristics. Indeed, spreading amounts from 15 m^3/ha to 45 m^3/ha for three consecutive years induced a considerable improvement of soil fertility. The pH and soil phosphorus content remain stable during the three years of study, while the soil salinity was increased for the 45 m^3/ha treatment where it exceeded to 6 dS/m.

In conclusions, the dose 15 m^3/ha is suitable for the vegetative development of the Faba bean tested plant according to the soil characteristics evolution.

Keywords: olive mill wastewater, agricultural valorization, sandy soil, Faba bean crop, impact

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Introduction

Olive mill wastewater (OMW) resulting from the extraction of olive oil is a major problem of the olive industry and is becoming a serious environmental challenge facing the Mediterranean countries such as Spain, Italy, Greece, Turkey, Morocco and Tunisia. These latter were produced more than 95% of the olive (Olea europaea L) oil in worldwide [1, 2]. Actually, Tunisia's olive-growing potential is estimated at nearly 90 million trees, occupying an area of 1.8 million hectares corresponding to about 79% of the total arboreal area.

Tunisia accounts for about 8 % of olive oil world production [3-5]. Despite, the economic dominance and the agricultural importance of olive growing, no one doubts that the olive-oil extraction process with the three-phase decanter generates considerable quantities of agro-industrial wastes. The annual OMW productions of Mediterranean olive are estimated to range from 7 million to over 30 million m^3 [6-8]. This enormous divergence of results can be explained by the fact that the production of olives varies from one year to another due to weather conditions and plagues that can affect the olive trees. The average total production amounts approximately to 10-12x10^6 m^3 per year and occurs over a brief period of the year (November-March) [1, 9, 10].

The OMW generate high values of chemical oxygen demand (COD, 40-200 g/ L) and biochemical oxygen demand (BOD5, 40-95 g/ L), phytotoxic properties and resistance to biodegradation due to the high polyphenol and the enormous supply organic matter (OM, 10 to 12 g/L) [11, 12]. Zenjeri et al. (2001) have reported that the addition of OMW sewage material would in soil cause a decrease pH value and induce an increase of the electric conductivity (EC) [13]. The OMW chemical composition is variable and depends on the olive varieties, the harvesting period and the extraction techniques. Moreover, it includes various simple and complex phenolic compounds, generating antimicrobial and phytotoxic effects [14, 15]. OMW characteristics is rich in water (83-96%), OM (3.5-15%) and in mineral nutrients (0.5 to 2%) such as nitrogen, phosphorous, potassium, iron and magnesium [16-20]. Consequently, increasing attention has been given to the spread of OMW on agricultural lands as organic fertilizer and to recycle both the OM and the nutritive elements in the soil crop [21-24]. The same researchers reported that the OMW caused negative changes in microbial soil properties, decreasing or inhibiting microflora growth [24-26]. Zenjeri et al.(2001) have established that the OMW spreading compromised soil fertility, altering physical and chemical soil properties [13]. In fact, the long-term applications of the OMW could increase the amount of polyphenol and salinity. They latter were influenced by soil structure and the substrate solubility. The negative effects of OMW on soil after 11 years of disposal of raw OMW were also [27, 28]. Mekki et al. (2006) have studied the effects of OMW on five species seeds germination tomato (Lycopersicon esculentum), chickpea (Cicer arietinum), bean (Vicia faba), wheat (Triticum durum) and barley (Hordeum vulgare) and have reported that raw OMW has strongly inhibited seeds [24].

Other studies reported that the long-term effect of OMW disposal on soil properties [28, 29] induced a positive effect of the effluent on physical, chemical and microbiological properties of soil. Chaari et al. (2014) have found that OMW spreading had beneficial effects on top soil such as nutrient availability for plant growth [29]. Furthermore, the results find by [30] showed that the application of the OMW for six successive years has improved olive productivity and oil quality. Recently, [31] have distinguished no negative effects of OMW on maize crop, despite of the amelioration in terms of fresh. In this perspective, several studies indicated beneficial effects of the OMW on soil fertility such as nutrient availability for plant growth [21, 22].

In Tunisia, several attempts to use the OMW as fertilizer have been made specifically towards the olive sector [2, 4, 5, 29]. They affirmed the utility of these effluents as a natural fertilizer for sandy soils and established that tree fruit shoots are long and bear more floral clusters and flowers. The tree is increased with the contribution of OMW, in trees receiving doses of 100 m^3/ha. However, at higher doses, these effluents caused a slight decrease in production. However, it remained higher than that given by trees with lower doses (50 m^3/ha), which was 49.8 kg per tree. The valorization of OMW as fertilizer in agriculture remains a potential initiative with both agronomic and environmental interests. It is in this perspective that we have proposed to carry out this research, which complements the previous work. In order to contribute the solutions allowing to circumvent the possible harmful effects of OMW and to value their contributions of organic compounds useful for the restoration of degraded lands and improved crop productivity in particular cereal crops in arid and semi-arid areas.

The aim of this work is to study of the effects of OMW application on soils in arid and semi-arid areas, focusing the effects of OMW application on specific physical, chemical and microbiological properties of the soils and studying the effects of the dose of the OMW on the final yield of productivity in the Faba bean crop.

Materials and Methods

Field Investigated

The field experiments were conducted at the Institute of arid and semi-arid regions, the governorate of Medenine in southern Tunisia (33°16'21"N, 10°19'30''E). The climatic conditions in this part of the country were typically Mediterranean with an average

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annual rainfall of 150 mm occurring mostly in autumn and spring. The mean annual air temperature was ranging from 18 to 20ºC. The field was divided into three-block design with three replicates per treatment. Four plots treatment was investigated among which three plots were annually dosed each December and May (from 2009 to 2012). The plots with 2 × 3 m^2 size were delimited mechanically ploughed and leveled to subsequently amend them with OMW (Olive Mill Wastewater). Over this period, the same raw OMW volume was achieved by spreading the waste on the soil surface, followed by arable level homogenization. After a period of 15 days of rest deemed necessary for drying the soil, the incorporation of OMW was subsequently carried out by manual tillage.

Each year, OMW application was done at rates equivalent to 15, 30 and 45 m^3/ha. The first was served as control soil. All the treatments were done in triplicate and the plots were distributed alternatively. The soil chosen was isohumic with the following characteristics: sandy texture (90 % sand, 3 % loam and 6 % clay), pH (7.11), EC (2.32 dS/m), % $CaCO_3$ (5.55) and % OM (0.98).

OMW Sampling and Characterization

The fresh OMW used in this study was taken from a three phase continuous extraction factory. The pH and EC were determined according to a standard method [32]. Dry matter and moisture contents were evaluated by drying a fresh sample of OMW at 105ºC. The total organic carbon (TOC) was made using Anne's method as described by [33]. The organic matter (OM) was oxidized by a mixture of bi-chromate of potassium and sulfuric acid. The excess of bi-chromate was titrated using the Mohr's salt. The OM was estimated as the difference between dry matter and the residue after calcinations at 550ºC for 4 h [1, 2]. The total nitrogen was assessed according to method of the Kjeldahl [34]. The chemical oxygen demand (COD) was evaluated according to [35] standard method. The biochemical oxygen demand (BOD_5) was determined by the manometric method with a respirometer. The exchangeable bases potassium and sodium (K and Na) were subjected to a treatment with a nitric acid in ash of dry matter after calcinations for 4 h in 550ºC, by using a flame photometer (model PFP7–JENWA). The total phenolic compounds were determined using the Folin-Ciocalteau method [36]. Table 1 summarizes the properties of raw OMW and all measurements were performed in triplicate.

Physicochemical and Microbiological Analysis of Soil

Soil samples were collected yearly from the different plots at 0-20 cm depths using a soil auger, air-dried for 10 days at room temperature. They were crushed with an agate mortar and sieved through a 2 mm mesh screen. The control and treated areas were collected in December and May for three years.

The pH and the EC were measured in a soil-water suspension (1:5 w/v extraction ratios). The TOC in soil was determined by dichromate oxidation [33] the total nitrogen (TN) by the Kjeldahl method [34] and the available phosphors with bicarbonate extraction according to the method of Olsen and Watanabe [37]. The phenolic compounds were extracted with sodium pyrophosphate 0.4 N and sodium hydroxide 0.1 N, and were quantified by Folin-Ciocalteau method [36]. Total Na and K were determined by spectrophotometer and chloride ions (Cl^-) was estimated using method of titration with chlorhydric acid.

The microbiological activity evolution of the amended soil such as C and N mineralization was determined. 50 grams (dry weight) of pre-incubated samples of soil were thoroughly mixed with OMW were placed in 1000 ml closed incubation vessels. Soil controls were run without any amendment. The CO_2 evolved was trapped in 10 ml of 0.1 M NaOH in small tubes, which were placed on top of the soil in the incubation vessels. Empty vessels were used as blanks. The trapped CO_2 was quantified by titration with 0.1 M HCl in an excess of $BaCl_2$. The incubation was carried out in a dark, temperature controlled incubator at 28ºC for 30 weeks. The amount of C evolved as carbon dioxide from the composting samples (extra C-CO_2) was calculated subtracting from the CO_2 evolved during the incubation of amended soil the CO_2 evolved during the incubation of the control. To study the mineralization of the organic-N, triplicate of 20 g of ground soil mixed with OMW and 3 ml of deionized water were placed in open polyethylene vessels of 100 ml. Control samples without the OMW were also prepared. Vessels were covered with Parafilm film to ensure O_2 and CO_2 exchange and minimize losses of water, and incubated for up to 30 weeks in the dark and at constant temperature (28ºC) and moisture content (15%). Moisture losses were controlled and corrected during the incubation. Samples were incubated for different periods of time and were analyzed to determine the exchangeable ammonium and the dissolved nitrate. Operational ranges of the methods were 0.5-20 mg/L NH_4-N and 0.5-50 mg/L NO_3-N. Total Kjeldahl-N in solid samples was determined at the start and at the end of the incubation period by the method described by [38]. Microbiological parameters (soil respiration performed as the C-CO_2, nitrate nitrogen and ammonium nitrogen) were studied in destructive samples.

Plant Material

A local variety (*Vicia Faba L*) of bean was adopted in amendment areas with OMW. This crop species is commonly cultivated in the Mediterranean area such as the Medenine governorate and this culture cycles coincide with the OMW production period. The intact seeds, and identical size and color were homogeneous.

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The free from wrinkles were chosen then sterilized with 10% Clorox for 10 min. A drop of (tween 20) per 100 ml was added to the solution, as a scattering material. Twenty seeds were grown in each plot. The number of plants per plot was decreased to 10, and only homogenous seedlings showing the strongest growth were selected for growth measurement parameters such as plant height (cm), leaf number, fresh and dry weight were determined.

The statistical analysis was performed either for each year or globally for the 3 years period using SAS (System for Windows version 9) and STATIGRAPHICS plus (version 5.1).

All statements of significance are based on a probability of $P<0.05$.

Chemical Characteristics of OMW

The chemical characteristic of the OMW is given in Table 1. The results showed that OMW is acidic liquid waste (4.8±0.2) with a high EC (10.0±0.52 dS/m). It can be explained by the high amount of salts added to conserve olive before extraction [12, 13]. The OMW was also characterized by the high amount of BOD_5 (66.0±2.4 g/L) and COD (98.0±2.1 g/L) [39, 40].

The phenolic amount (8.8±0.3 g/L) in OMW is in concordance with the result cited by [41] , which has been between 3g/L and 9 g/L and which has been significantly lower than the amounts of phenolic (1.74±0.02 g/L) compounds cited by [2]. Many reserchers showed that the high amounts of phenolic compounds constitute the main origin of its toxicity [10, 29].

The analysis of the mineral elements showed that the OMW is rich on the total nitrogen (NTK = 1.6±0.1 g/L), potassium (K = 6.1±0.2 g/L), phosphor (P = 0.35±0.02 g/L), sodium (Na = 1.57±0.01 g/L), chloride (Cl = 0.65±0.04g/L), calcium (Ca 1.1±0.1 g/L) and magnesium (Mg 0.42±0.01 g/L) (Table 1). Similar results were found by [2] in the OMW taken from a three phase continuous extraction factory located at Sfax, Tunisia. The variability of the characteristics of OMW depends on the various factors like the climatic conditions, the type of extraction process, the olive variety, and the ripening of olives [1, 2, 10, 42].

Table 1. Chemical characteristic of the used [4].

Parameters	Values ±SD
pH	4.8±0.2
EC (dS/m)	10±0.52
DCO (g/L)	98±2.1
DBO (g/L)	66±2.4
TOC (g/L)	26±2.4
Phenolic compounds (g/L)	8.8±0.3
Nitrogen ((g/L)	1.6±0.1
Ca (g/L)	1.1±0.1
Phosphore-Olsen (g/L)	0.35±0.02
Mg (g/L) K (g/L)	0.42±0.01 6.1±0.2
Na (g/L)	1.57±0.01
Cl (mg/L)	0.65±0.04

SD: Standard deviation ($P\leq0.05$).

Results and Discussion

Effect of OMW on Soil Composition

Effect of OMW on Soil Mineral Composition

Selected chemical properties of the soil are given in Table 2. The results show that the addition of the three doses of OMW and their variations with time has different effects on some of the physico-chemical properties of the soil. A slight decrease of pH has been observed for all amended soil with OMW of 15 m³/ha (T1), 30 m³/ha (T2) and 45 m³/ha (T3) compared to the control soil T. Thus, this parameter remains predominantly neutral for different treatment after three years. The slight modification of pH is due to the buffer capacity of the soil, which is relatively rich in limestone (general case of southern Tunisia soils). In this case, this buffering capacity can promote the activity of soil microflora by reducing the antimicrobial power of OMW phenolic compounds. Omar et al.(2019) showed that the carbonates present in the soil superficial horizon, by turning into bicarbonates have neutralized the acidic pH of the OMW [43].

EC makes it possible to estimate the overall content of the soluble salts in the soil. Based on the control soil EC, an increase in such parameter was observed as a function of OMW doses and time (Table 2).

Indeed, the EC has increased from 2.4 dS/m in the control to 6.72 dS/m in soil T3 after three years. Thus, the rise in the soil salinity reflects the amount of the main ionic species (Na, Cl, SO_4^{2-} and K) present in the soil. In instance, [10, 44] have noticed a gradual rise of soil salinity following the application of increasing quantities of OMW. The increase of the soil salinity involved the alteration of the cation exchange capacity (CEC) and could affect the soil fertility [45, 46]. These cumulative effects will have a negative impact on soil structure through deterioration of its permeability and inhibition of leaching possibilities, resulting in serious repercussions on the hydromineral balance of the plant and consequently on soil productivity.

The application of OMW did not have an immediate impact on the assimilable phosphorus content in the soil. The three successive applications seem have not an impact on the soil content of assimilable phosphorus. This parameter is often blocked by the adsorption

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Table 2. Effect of different doses of OMW on soil pH, EC, Sodium, Chlorides and potassium during three successive years.

Treatment	Year	December	May	December	May	December	May	December	May
		Ph		EC (dS/m)		Na (mg/kg)		Cl (mg/kg)	
T0 (*0 m³/ha*)	1	7.18±0.29 a	7.65±0.43 a	2.41±0.29 a	3.39±0.89 a	834±0.59 a	741±0.69 a	102±0.21 a	105±0.39 a
T1 (15 m³/ha)	1	7.35±0.21 b	7.66±0.51a	3.08±0.21 a	3.63±0.58 ab	1876±0.33 b	1297±0.83 b	162±0.78 a	135±0.53ab
T2 (30 m³/ha)	1	7.42±0.42 b	7.74±0.23 a	4.15±0.42 c	3.85±0.59 ab	2742±0.32 c	2188±0.55 c	264±0.66ab	187±0.55 b
T3 (45 m³/ha)	1	7.59±0.63 b	7.78±0.42 a	6.00±0.63 d	3.96±0.63 b	5134±0.74 d	4506 ±0.48 d	361±0.34 b	232±0.48 c
T0 (*0 m³/ha*)	2	7.12±0.29 a	7.62±0.21 a	2.86±0.29 a	2.69±0.79 a	856±0.33 a	916±0.61 a	144±0.46 a	137±0.61 a
T1 (15 m³/ha)	2	6.85±0.21 b	7.76±0.21 a	4.39±0.21 b	3.31±0.61 b	3253 ±0.74 b	2779±0.44 b	288±0.68 b	233±0.44 b
T2 (30 m³/ha)	2	6.78±0.42 b	7.85±0.42 a	5.53±0.42 c	4.24±0.28 c	4814±0.72 c	3979±0.27 c	477±0.32 c	277±0.27 c
T3 (45 m³/ha)	2	6.71±0.63 c	7.83±0.63 a	6.35±0.63 d	4.86±0.72 c	6529±0.63 d	4112 ±0.89 c	685±0.93 d	329±0.89 d
T0 (*0 m³/ha*)	3	7.14±0.29 a	6.84±0.24 a	2.79±0.93 a	3.24±0.44 a	1351±0.59 a	999 ±0.29 a	218±0.59 a	159±0.29 a
T1 (15 m³/ha)	3	7.02±0.21 a	6.72±0.55 a	4.61±0.38 b	3.74±0.68 b	4916±0.43 b	2556±0.21 b	397± 0.43 b	441±0.21 b
T2 (30 m³/ha)	3	7.03±0.42 a	6.75± 0.57 a	5.74±0.88 c	4.54±0.77 c	6825±0.92 c	4260±0.42 c	752±0.92 c	611±0.42 c
T3 (45 m³/ha)	3	7.16±0.63 a	6.68±0.81 a	6.72±0.51 d	5.13±0.53 d	9970±0.73 d	5910 ±0.63 d	933±0.73d	664±0.63 d
		SO_4^{2-} (mg/kg)		P (mg/kg)		K exch (mg/kg)			
T0 (*0 m³/ha*)	1	733±0.85 a	654±0. 92 a	1.33±0.52 a	2.46±0. 53 a	436.8 ±0.72 a	296.8±0.43 a		
T1 (15 m³/ha)	1	813±0.52 b	728±0.28 b	2.63±0.88 b	2.64±0.68 b	1566 ±0.18 b	843.5±0.31 a		
T2 (30 m³/ha)	1	958±0.66 c	831±0.69 c	3.08±0.34 c	2.96±0.77 c	1811 ±0.55 c	1107±0.78 b		
T3 (45 m³/ha)	1	1036±0.39d	982±0.42 d	3.63±0.57 c	4.78±0.88 d	2543±0.29 d	2107±0.92 c		
T0 (*0 m³/ha*)	2	736±0.86 a	623±0.84 a	2.12±0.45 a	2.15±0.66 a	527±0.39 a	421.87±0.53a		
T1 (15 m³/ha)	2	1033± 0.59 b	859±0.62 b	2.97± 0.67 b	3.49±0.57 b	1764±0.54 b	986.54±0.64b		
T2 (30 m³/ha)	2	1256± 0.77 c	1038±0.73c	3.15± 0.82 c	3.98±0.83 b	2128± 0.74 c	1385.86±0.77c		
T3 (45 m³/ha)	2	1385±0.67d	1128±0.92c	3.87±0.84 d	4.97±0.92 c	3195± 0.36 d	2122.65±0.63d		
T0 (*0 m³/ha*)	3	859±0.27 a	668±0.89 a	2.12±0.83 a	1.46±0.89 a	471,9±0.33 a	668.1±0.85 a		
T1 (15 m³/ha)	3	1236±0.69b	956±0.62 b	3.31±0.72 b	1.6±0.92 a	2040±0.47b	1480±0.92b		
T2 (30 m³/ha)	3	1369±0.44b	1089±0.86b	3.87±0.91 b	2.11±0.86 a	4269±0.89 c	2532±0.34 c		
T3 (45 m³/ha)	3	1568±0.63c	1203±0.57b	5.24±0.83 c	3.25±0.57 b	5634±0.52 d	3736.9±0.76c		

Different letters indicate significantly different values at $p<0.05$ (Duncan test). For each year of experimentation, values indicated by the same letter are not significantly different at $p < 0.05$

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mechanism or precipitation in the form of apatite phosphate in the soil when the pH is greater than 6. Compared to the control soil, a significant increase in exchangeable K level is in the relation to the added OMW quantities (Table 2). Indeed, this element was increased from 436.8 mg/kg in control soil to 2040 mg/kg, 4269 mg/kg and 5634 mg/kg in amended soil 15, 30 and 45m^3/ha, respectively. These results have been consistent to those obtained by [47] who have showed an increase in soil exchangeable K after application of gradual OMW quantities. Furthermore, [48] demonstrated that the K drastically increased with all OMW treatments compared with the control. We can conclude that OMW constitute a potential source of K showing a high fertilizing potential that can be valued for the rehabilitation of poor soil in this element. Here careful attention should be paid, since excessive potassium application through OMW may alter in both short and long-term the physico-chemical and biological equilibrium of the treated soils and consequently a plant nutritional disorder.

Effect of OMW on Soil Organic Composition

Rich in organic matter (26 g/L), the application of OMW has caused a positive effect on soil organic C content compared to the control soil during the three years of study experiment (Fig. 1). Comparatively to control soil (T0), a significant increase of organic matter has been showed relatively to the increase of OMW added doses. This is of the greatest importance in semiarid conditions where agricultural soils, poor in organic matter are dominant and are being subjected to intense processes of degradation [31, 49]. Moreover, these results are consistent with previous reports indicating that OMW application increases soil organic C content [50, 51]. In this context, [52] have reported also that OMW adding has increased the rate of organic matter with applied doses. Whereas, the total organic carbon content decreased significantly in the amended soil over time, from time 1 (December) to time 2 (May). This reduction has been observed for all OMW applied doses. Indeed, for the first year of study, we recorded a highly significant decrease to 27 %, 43% and 55% for T1 (15 m^3/ha), T2 (30 m^3/ha) and T3 (45 m^3/ha), respectively. These decreases were 34%, 45 % and 58% for the second year and were 50%, 58%, and 73% for the last year. The reduction is correlated with OMW applied dose. This could be explained by the fact that the OMW rich in OM has greatly stimulated the soil microbial activity, which has led consequently to a rapid degradation of the organic compounds supplied by these effluents [4]. On the other hand, this decrease in soil organic carbon content after harvest tends to increase over 3-years of experiment, which may be a sign of soil microflora proliferation in response to their adaptation to the chemical OMW properties [32]. At the end of the experiment, we can deduce that a relative stability of the total organic carbon content of soil has been maintained at an average 0.4% to 0.5%. These results are in agreement with those of [53], which showed that the annual contribution to a sandy soil initially containing 0.45% organic matter of 37 or 61 L/m^2 of OMW for three years resulting in a respective increase in organic matter of 1.62% and 1.98% respectively.

Despite the relatively weak OMW content in nitrogen (1.6 g/L), a significant improvement of this element compared to the soil control has been observed for soil irrigated by OMW. This improvement is in correlation with the dose supplied (Fig. 2). However, a slight decrease in total organic nitrogen has been observed over the time from time 1 (December) to time 2 (May) in the treated soils. This can be explained by the important growth of cultivated plants. Then, the plant root system has been well developed, so they can absorb more nitrogen essential in the form of nitrate ($N\text{-}NO_3$) [2].

On the other hand, OMW application induced a significant increase in the soil polyphenols content compared to the soil control with the increase of the dose applied. As well as from one year to the next following the cumulative effect of the successive OMW spreading (Fig. 3). However, a marked regression of polyphenol levels in comparison with those recorded immediately after OMW spreading was confirmed at the end of each year. This decrease can be attributed to the biodegradation process of phenolic compounds by soil microorganisms. The neutral pH caused by the buffering effect of calcareous soil is a factor favoring the activity of soil microorganisms. Furthemore, [54] demonstrated that at neutral or slightly alkaline pH (7.4 to 7.6), the phenolic compounds pass in the form of phenates and lose a great part of their antimicrobial power. Thus, microorganisms can use them as carbonaceous nutrients [55].

Effect of OMW on Soil Microbial Properties

The effects of OMW on the soil microbiological activities have been carried out through the follow-up of the respiratory activity of the different OMW amended soils according to the applied doses and the incubation time compared to the control soil [56].

The amended soils microbiological activity provides an overall picture of the capacity of the soil microflora to mineralize the added OM [10]. Consequently, the evaluation of the cumulative respiratory activity of the different soils has been assessed 15 days after the OMW application during 28 days of incubation for 3 years.

Fig. 4 shows the results of microbial properties and evolution of the amended soil. The analysis of the variance showed that OM mineralization kinetics (as cumulative $C\text{-}CO_2$) increased significantly with OMW applied doses in soil compared to the control. This considerable increase is especially recorded for the two highest rates T2 (30 m^3/ha) and T3 (45 m^3/ha). In this context, these results have been consistent with

previous research confirming that the application of OMW stimulated the respiration rate [32, 57]. This is can be attributed to the presence of high concentrations of labile organic-C in OMW [45]. However, the kinetics of cumulative respiration expressed as the cumulative C-CO_2 alone cannot provide a clear idea of the biological activity of the soil in relation to the OM added [58, 49]. This is why we must always think in terms of specific respiration ratio in relation with total organic carbon added (C-CO_2/TOC) in order to assess the capacity of the soil microflora to biodegrade the OM and there degree of biodegradability [43].

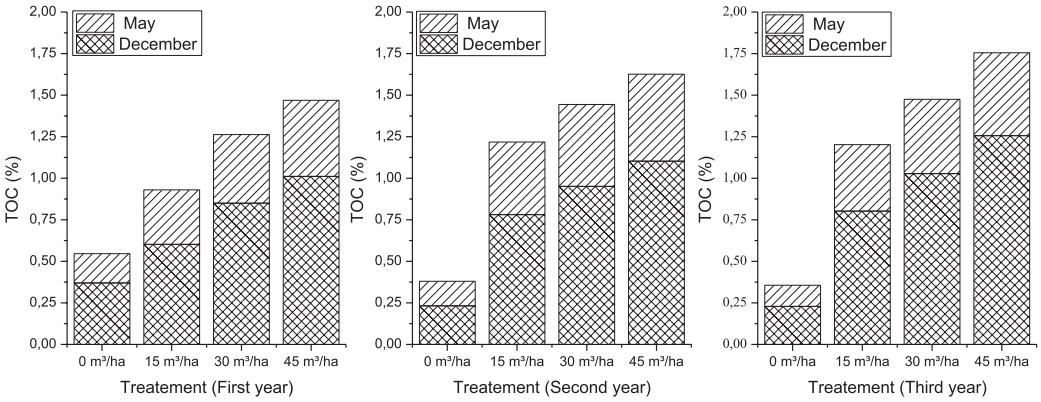

Fig. 1. Evolution of soil total organic carbon (TOC) content (%) after the first, tow and third OMW spreading of different doses: control: 0 m^3/ha, 15 m^3/ha, 30 m^3/ha and 45 m^3/ha in the layers 0-20 cm.

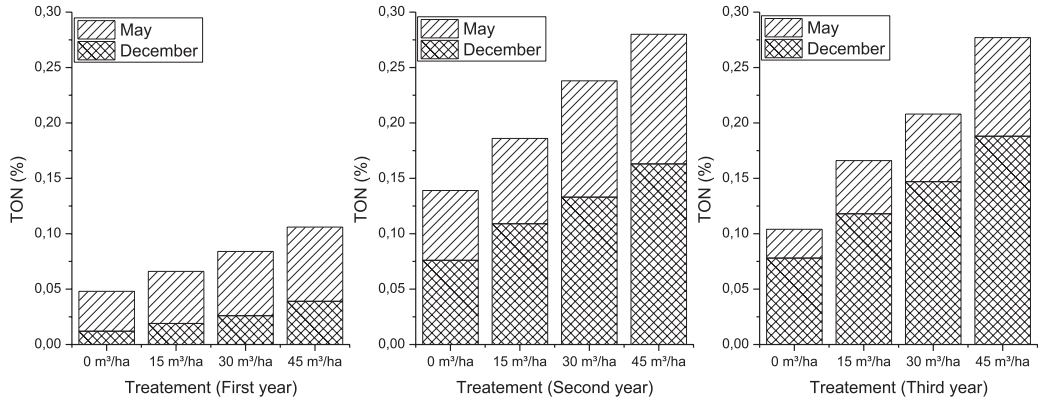

Fig. 2. Evolution of soil total organic nitrogen (TON) content (%) after the first, tow and third OMW spreading of different doses: control: 0 m^3/ha, 15 m^3/ha, 30 m^3/ha and 45 m^3/ha in the layers 0-20 cm.

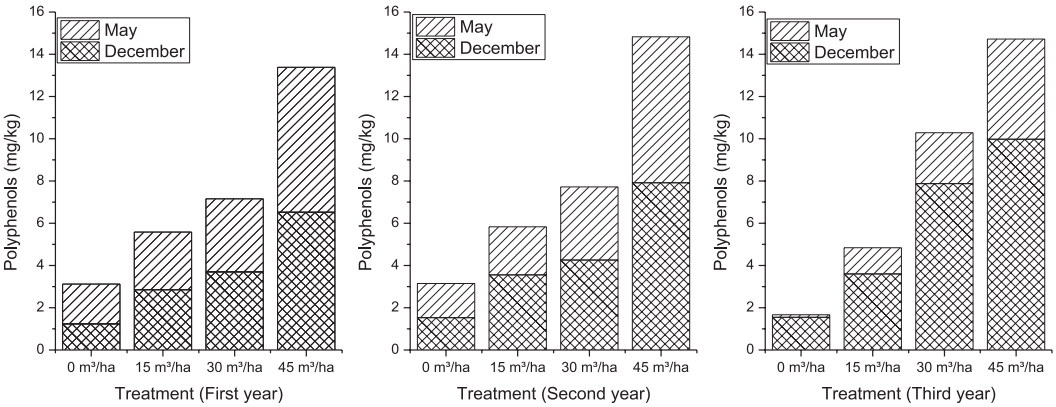

Fig. 3. Evolution of soil polyphenol (mg/kg) content (mg/ kg) after the first, tow and third OMW spreadings of different doses: control: 0 m^3/ha, 15 m^3/ha, 30 m^3/ha and 45 m^3/ha in the layers 0-20 cm.

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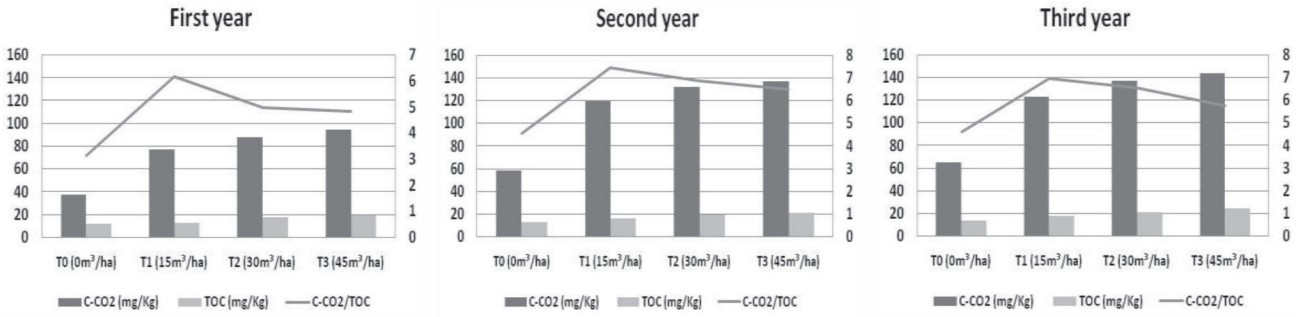

Fig. 4. Evolution of the respirometric activity C-CO_2/TOC in functions of OMW doses during three years.

Fig. 4 shows that the dose 45 m^3/ ha gives more important organic supplement in terms of the TOC in the soil, while the dose 15 m^3/ha which has given higher specific respiration values in terms of C-CO_2/TOC. In fact, the optimum respirometric activity was found for 15 m^3/ha and this value decreased with increasing the dose of OMW, 30 m^3/ha and 45 m^3/ha. Such findings have confirmed the previous results regarding microflora count evolution in different soils studied and were in accordance with many previous studies [2, 59, 60]. This decrease can be explained by the fact that the phenolic compounds may inhibit the soil respiration, especially in the high OMW doses, and thus neutralize the favorable influence of its higher nutrient contents [57, 61, 32]. Thus, the inhibition of soil respiration could be caused by the fact that the big amount of carbon added to the soil was unavailable to the microflora under the effect of its strong adsorption or its reaction with the components of the soil [24]. On the other hand, this biological activity dose-response increasing is observed also regarding soil N mineralization (Fig. 5).

Thus, soil mineral N content continued to increase in OMW-treated pots until the first year incubation, the point in time that approached its highest values. Then, soil mineral N content decreased sharply during the second year and they maintained more or less approximately constant until the end of third year when a slight, but statistically significant, increase took place in all the treatments especially that treated with the highest load of OMW rates T2 (30 m^3/ha) and T3 (45 m^3/ha) (Fig. 5). This regression can be attributed, on the one hand to the use and depletion, by the microbial biomass of the soil of the more labile organic compounds. It also, because the microorganisms themselves cannot provides sufficient exo enzymes necessary for the degradation of organic compounds.

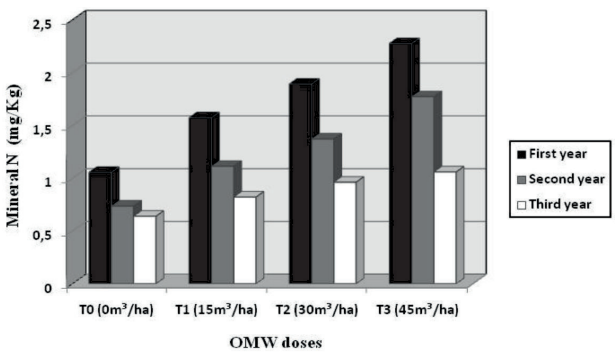

Fig. 5. Effect of OMW spreading on mineral N amount during three consecutive years.

The fresh input with OMW of easily degradable C and N substrates resulted in high levels of extractable C and N and led consequently to an increase of microbial biomass stimulation. This suggests that the addition of available organic substrates promoted the growth of indigenous microorganisms and possibly resulted in a high synthesis of enzymes activities such as dehydrogenase, ureas [4, 62, 63] However, the most available OM was decomposed during the first two-week's incubation and activity of enzymes involved in both C and N mineralization including dehydrogenase, urease, β-glucosidase and phenol oxidase activity rate stabilized or decreased after 14 days [57]. We can conclude that the degradation rate of many organic compounds is limited the capacity of the soil microflora to biodegrade the added OM and the degree of biodegradability of OM. At a certain threshold, it manifests deficiencies in the mineralization process correlated with the increase of OMW applied dose. This is can be attributed to the performance limits of microorganisms in a medium rich in this sludge (pH, salinity and polyphenols).

Indeed, phenolic compounds, such as p-coumaric acid and ferulic acid, can also affect the physiology of both prokaryotic and eukaryotic organisms [64]. Moreover, other OMW components, for example, lipids, cannot be excluded. In addition, the low pH and the osmotic stress caused by the presence of high Na+ and Cl− concentrations may play a role in OMW acute toxicity [64].

Effect of OMW on Plant Growth

Effect of OMW on Plant Growth

The effect of OMW compost on Faba bean for three successive years has been achieved by monitoring several agro-physiological parameters such as plant

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Table 3. Some growth characters of Faba bean affected by OMW application.

Treatment (OMW)	Years					
	1	2	3	1	2	3
	Plant height (cm)			Leaf number		
T0 (0m³/ha)	50.33±0.51 a	51.73±0.88 a	50.02±0.62 a	23.33±0.92 a	22.66±0.57 a	21.33±0.57 a
T1 (15m³/ha)	58.33±0.66 b	55.01±0.84 b	51.66±0.96 a	29.66±0.23 b	27.33±0.78 b	23.66±0.84 b
T2 (30m³/ha)	43.66±0.38 c	38.77±0.69 c	31.53±0.52 c	21.65±0.69 c	17.57±0.63 c	11.33±0.33 c
T3 (45m³/ha)	38.36±0.58 c	32.67±0.33 d	22.33±0.55 d	17.33±0.88 d	11.33±0.41 d	6.66±0.79 d
	Fresh weight (g)			Dry weight (g)		
T0 (0m³/ha)	25.33±0.5 1 a	23.53±0.56 a	22.66±0.67 a	10.33±0.72 a	10.53±0.51 a	11.66±0.28 a
T1 (15m³/ha)	30.33±0.66 b	27.33±0.83 b	25.05±0.66 b	14.03±0.69 b	13.38±0.66 b	13.63±0.66 b
T2 (30m³/ha)	18.66±0.38 c	15.66±0.75 c	11.27±0.38 c	2.66±0.53 c	1.76±0.458c	0.89±0.44 c
T3 (45m³/ha)	14.36±0.58 c	10.36±0.77 d	7.66±0.26 d	2.03±0.78 c	1.12±0.93 c	0.59±0.89 c

Values are mean±standard error. Mean values with different letters at the same raw differ significantly at P = 0.05

height, leaves number, plant productivity (as fresh weight and dry weight). Table 3 shows the increase of the growth parameters compared to the control soil. The maximum growth of plants was observed for the soil amended with 15 m³/ha of OMW. The minimum growth was observed for the soil amended with 45 m³/ha of OMW. Such a reduction in growth could be attributed to the influence of high osmotic stress and ion toxicity [65] or due to altered cell wall structure induced by stress [66]. Further, salinity stress might inhibit cell division, cell enlargement and expansion as mentioned by Radi et al. (2013) [67].

Similar observations were found for the leaves number. Other works supported this finding results, are those on moth bean (*Vigna aconitifolia* L.) by [68], on radish plant, (*Raphanus sativus* L.) by [69], on cowpea (*Vigna unguiculata* L.) by [70] and on (*Vigna mungo* L.) by [71]. This can be due to the phytotoxicity caused by higher OMW doses applied [30, 72]. We can be also explained by the high load of OM and mineral elements brought by the higher OMW doses, which in turn mainly exerts a saline stress affecting leaves plant growth. .

In this regard, [73] reported that the growth of tomato plants was seriously affected by the high level of OMW acidity and lowering of uptake of elements was observed for the plants cultivated on soil amended by OMW. At pH less than 4-5, the solubility of nutrients such as iron (Fe), aluminum (Al) and manganese (Mn) rises into toxic level that toxifies plant in different levels.

As regards the effects of OMW on the Faba plants biomass productivity in terms of fresh weight, and dry weight, the results showed that plants grown in greater doses are a gradual decrease in their productivities compared to control plants has been distinguished. Thus, the lowest productivity is recorded for plants grown under 45 m³/ha. This can be explained by the high salinity of the OMW dose applied, which can exert phytotoxic and nutritional disturbances for the soil plant interactions [28, 59]. They found that increasing of the concentrations of NaCl developed a decline in the lengths of the plants. On the other hand, the noticed decrease in the length of the stem, also due to treatment with NaCl solution, could be due to the negative effect of this salt on the rate of photosynthesis, the changes in enzyme activity (that subsequently affects protein synthesis), and the decrease in the level of carbohydrates and growth hormones. The decrease in the level of carbohydrates and growth hormones can lead to the inhibition of the growth plants [64, 74-76].

Conclusions

The present study investigated the quality of untreated OMW and their effects on the chemical and microbiological properties of the soils. The effects of OMW on the final yield of productivity in the Faba bean crop were also studied.

The results showed that the spreading of OMW on cultivated agricultural sandy soil by the dose 15 m³/ha have strongly enhanced its physicochemical and microbiological characteristics. The height organic matter and a rise in the concentrations of the different mineral elements have been established. Therefore, the same dose has significantly improved its biological activity correlated with the enrichment of the soil with OM and microflora, thereby positively affecting plant growth. Although, the high doses of OMW (30 m³/ha and 45 m³/ha) constituted an inhibitory dose for the soil microflora and caused as a consequence a significant decrease in all yield components of plots. This reduction, although observed in the first year,

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has become more and more accentuated over the years under the cumulative effect of chemical substances in the soil after three years of OMW application. The best vegetative development and productivity of Faba been was observed in the dose 15 m^3/ha of OMW.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical Approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Informed Consent

Informed consent was obtained from all individual participants included in the study.

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