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Soil activity behaviors after farming techniques application in The Chammak olive tree field

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Article info

Abstract

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The olive tree arranges no more water reserve that can be exploited during extremely dry periods. A failure of the olive yields observed during the last two decades. The annual production decreased from 150 000 tons to 50 000 tons of olives in the south of Tunisia. This degradation results, in fact from an obvious reduction in the biological activity of the ground. A new Strategy was employed to improve the organic status and restore the biological activity of the soil ground of the long-term. We have evaluated the effect of the of different plot treatment. A plot of olive tree speeded with 200 m³/ha dose of OMW during 10 years, P4; a plot of olive tree treated with tillage accompanied by 50 m³/ha dose OMW spreading lasting only a one year, P5 and a plot of olive tree cultivated with the introduction of the fig tree in parallel, P3. Untouched ground was used as control plot, P0. A comparison of these plots with a degraded ground of olive tree implanted since 1900, P2 and other ground degraded during 10 years, P1. The plot treated with OMW showed an OM value close to the value founded with PO (Control Plot). The Phytotoxcity measured via germination index GI (percentage) was determined; an important increment ranged from 200% to 230% was obtained in the P3 and P5, respectively. Correlation analyses, among physiochemical parameters of soil and microbial biomass indicated several positive significant trends. The highest significant correlation was found between OM and TAMF (r=0.999, p<0.001). These results corroborate the notion that the microbial community structure is a good indicator of soil quality and the effects of different management practices, because the microorganisms respond against changes in soil management more rapidly than chemical or physical soil properties. However, in this work we found a negative correlation between respiration soil activity (Resp) and with OM (r=-0.533, p<0.05). In fact, CO₂ sequestration took place. Indeed, increasing soil OM enhances the sequestering carbon dioxide (CO2) to mitigate anthropogenic greenhouse gas (GHG) emissions.

1. INTRODUCTION

The even warmer climatic conditions and higher vulnerability of the dry lands due to wind erosion and excess cultivation led a progressive soil degradation in many places. The case of the drought observed in the South of Tunisia. The olive tree occupies a social and economic crucial place in the life. However, the olive tree arranges no more water reserve that can be exploited during extremely dry periods (Nefzaoui, 1991). This besides the senescence of a big part of the olive grove to Medenine contributes to explain the failure of the yields observed during the last two decades. The annual production decreased from 150 000 tons to 50 000 tons of olives (Abichou, 2011). This degradation results, in fact from an obvious reduction in the biological activity of the ground. This activity is maintained by the contribution of organic matters and by the

presence of diverse living beings such as fauna, the microorganisms, the roots of plants, etc. One of the important components of the biological activity of the ground is the one that refers to the microbial activity, given the role of catalyst that play microorganisms in the fertility of the ground. It is known that soil microbial activity is related to ecosystem stability and fertility, and that soil fertility is a great problem in dry lands. (Hannachi et *al.*, 2014). Indeed, the slowing down of the microbial activity was closely linked to a progressive impoverishment of the ground in terms of carbon, nitrogen, phosphor and some mineral salts. Thus, it is necessary to find or develop techniques for appropriate management and protection of soil fertility. Moreover. agricultural irrigation with wastewater effluents became a common practice in arid and semiarid regions, where it was used as a readily available and inexpensive option to fresh water (Angelakis et al., 1999; Mekki et al., 2006). Despite the critical problem of the olive mill wastewater (OMW), especially in the Mediterranean area. Generally, OMW has high values of biological oxygen demand (BOD) and chemical oxygen demand (COD), high contents of organic matter, suspended solids (Di Giovacchino et al., 1988; Dhouib et al., 2005; Dermeche et al., 2013; Aggoun et al., 2016). Some characteristics of this material are favourable for agriculture since this effluent is rich in organic matter, nitrogen (N), phosphorous (P), potassium (K) and magnesium (Mg). For these reasons, the controlled spreading of OMW increased the fertility of the soil, offering the opportunity to recycle the various compounds. Because of the high amounts of organic matter and micronutrients, OMW could be considered as a useful, low cost amendment and fertilizer (Mekki et al., 2006; Di Serio et al., 2008). The effect of the OMW on the physical and chemical characteristics of the soil are well documented (Paredes et al., 1999; Sierra et al., 2001; Mekki et al., 2006, Sahraoui & Mellouli, 2010; Toscano et al., 2013; Morugán-Coronado et al., 2015). In addition, the quantities of disposing OMWare considered as a low cost method with fertilizing properties and no harmful effects to crops and soil (Chartzoulakis et al., 2010; Saadi et al.,2007; Buchman et al., 2015). The objective of this work is to improve the organic status of the ground and the biological activity to restore the physical and chemical properties of the long-term ground. To reach this goal two working stages will be undertaken: the first one consists in the study of the evolution of the activity of the ground to know the physicochemical proprieties

and the microbiology of the ground, given the role of catalyst, which play microorganisms in the fertility of the ground. The second consists in proposing solutions through the implementation of new experimental plots of land.We have evaluated the effect of the of different plot treatment. A plot of olive tree speeded with 200 m³/ha dose of OMWduring 10 years, P4; a plot of olive tree treated with tillage accompanied by 50 m³/ha dose OMW spreading lasting only a one year, P5 and a plot of olive tree cultivated with the introduction of the fig tree in parallel, P3. Untouched ground was used as control plot, Po. A comparison of these plots with a degraded ground of olive tree implanted since 1900, P2 and other ground degraded during 10 years, P1.

2. MATERIALS AND METHODS

2.1. **Experimental plot**

Soil samples were taken throughout soil at a depth of 10-20 cm. Each sample was composed of soil cores collected from four different points. The soil was brought back to the laboratory on ice, stored at 4 °C and sieved (2 mm pore size mesh) about 24 h after sampling. The treated soil was located in the region of Chammakh-Zarzis located in southern Tunisia, in an environment with an arid Mediterranean climate with a mean annual rainfall of 180 mm, as a long-term average since 1923. The soil is moderately deep with a sandy texture. The soil of this area is moderately with a slight texture. The characteristics of the soil texture were finding Sand 85.9%, Clay 7.7%, coarse sand 5.1% and Silt 0.7%.

2.2. Preparation of the ground sample

We have evaluated the effect of the of different plot treatment. A plot of olive tree speeded with 200 m3/ha dose of OMW during 10 years, P4; a plot of olive tree treated with tillage accompanied by 50 m³/ha dose of OMW spreading lasting only a one year, P5 and a plot of olive tree cultivated with the introduction of the fig tree in parallel, P3. Untouched ground was used as control plot, P0. A comparison of these plots with a degraded ground of olive tree implanted since 1900, P2 and other ground degraded during 10 years, P1.

For the plot treated with OMW:the OMW is pumped from a pit cistern in a tank and brought by a tractor to the field. Then it was sprayed homogeneously on the sandy soil surface, previously tilled to a 10-15 cm depth. OMW was sprayed yearly since 2005 during the winter period (December-January) using at doses of 200

m3/ha in the plot P4. OMW was sprayed only during one year just before the sampling, in the plot 5.The tillage as well the introduction of the fig tree in parallel could change the soil chemical and physical properties of long-term.

2.3. **OMW origin and Characteristics**

The fresh OMW was taken from a three-phase discontinuous extraction factory located in the region of Chemmakh-Zarzis (Southern Tunisia, 33° 36'N, 11° 02'E). The chemical characteristic composition of OMW was in (g/l-1): 105 DCO, 55DBO, 107 Organic matter, 11.4, Reducing 3.9 Glucose. 5.8 Phenols, Greasinessmatter,13.7 Mineral matter, 1.4 0.32 Phosphates, 7.5 Nitrogen. Potassium, 0.65Magnesium, 1.31 Sodium, 0.71 Calcium. 0.56 Chlorures. Its humidity was 87.9 %, pH 5.5 and electrical conductivity was 18.6 (Ms/cm).

2.4. **VPhysic-chemical Soil determinations**

The pH and the Soil and Electrical Conductivity (EC) of every plot were determined. The pH was measured using a glass electrode at water/soil ratio 2.5:1 v/w. The water content was determined after drying at 105 °C. Electric conductivity (EC) of the soil aqueous extract (1:2.5) was measured with a conductimeter, wtw model inolabcond 730. Total organic carbon (C) was determined following the Walkley-Black method and organic matter (OM) was calculated by multiplying the total carbon by 1.724.

2.5. Respirometric test

Biological activity in the soil was achieved by measuring CO2 evolution in the aerobic condition (Ohlinger, 1995). The soil sample was humidified to 50% of its water holding capacity, and then was incubated at 30 °C in the dark. The CO2 evolved was trapped in NaOH solution and titrated with HCl.

2.6. **Phytotoxicity**

The Phytotoxicity was studied according to the method proposed by (Zucconi et al.,1981). This technique consists in making germinate, in petri dish, 10 seeds of tomatoes with the different samples studied. Petri dish incubated during a one week in the darkness at 25°C. The Control ground used as positive control. After incubation, the reading of the result is determined by counting of the number of the germinated seeds. The length of the roots of seeds having germinated measured in mm. The germination index 'GI' is calculated according to the following formula:

GI (%) = [Nbre of seed germinated* root lenght]/[Control Nbre of seed germinated* control root length | 1 *100

2.7. **Microbial estimation**

Ten grams of the soil sample were suspended in an Erlenmeyer flask containing 90 ml of a sterile solution (0.2% of sodium polyphosphate (NaPO3) in distilled water, pH 7.0) and 10 g of sterile glass beads (1.5mm diameter). 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 Sabouraud containing chloramphenicol at 25 °C for yeasts, on Desoxycholate (0.1%) lactose agar at 37 °C for total Coliforms.

2.8. Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS, Version 20.0). Data are presented as means \pm SD. Values were obtained from triplicate determinations and the differences were examined using one-way analysis of variance (ANOVA) followed by the Fischer's LSD (Least Significant Difference) post hoc test. Statistical significances of the correlations between data sets were calculated using Pearson's R-values. At least three replicates were performed for each laboratory measurement.

3. RESULTS AND DISCUSSION

3.1. Chemical and physic Soil characteristics

The comparison of physical and chemical soil properties between the different study samples shows an important significant difference in pH and EC (p=0.01). However, any difference was registered in OM parameters (Fig. 1).

A comparative physic and chemical study of parameters of the ground between different plots was realized. The studied Soils are generally alkaline where their pH ranged from 8.31 to 8.74.In addition, the electric conductivity showed values between of 0.539 and 1.744ms / cm. As can be seen, both pH and EC registered higher values in the all studied plots. As can be seen, the pH of plot treated with OMW remained higher, close to 8.64. This increase of the salinity in the soil could result of ground composition in the South Tunisian, with high content of the buffer, could explain this unlikely behavior. The ground of the Tunisian South is essentially rich in limestone (Sahrawi et al., 2012). Moreover, in

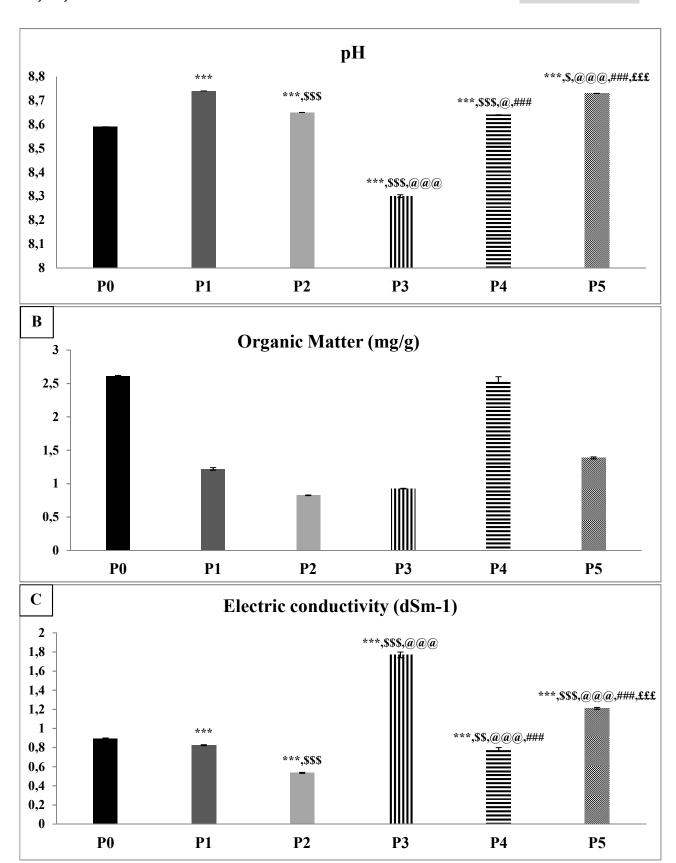


Fig. 1. Physico-chemical analysis of the studied soils.pH(A), Organic Matter(B), Electric conductivity(C). Data are presented as means ± SD. Values were obtained from triplicate determinations and statistical significance was examined by one-way analysis of variance (ANOVA) followed by the Fischer's LSD (Least Significant Difference) post hoc test.

***p<0.001 as compared to **P0**; \$ p<0.05 as compared to **P1**; \$\$ p<0.01 as compared to **P1**; compared to P1; @ p<0.05 as compared to P2; @@@p<0.001 as compared to P2; ### p<0.001 as compared to **P3**; *EEE* p<0.001 as compared to **P4**.

long-term applications, replacement of the soil calcium by the cations of Na, K and Mg could lead to the degradation of the soil structure. Previous studies suggested the formation of saline soils as earlier (Sierra et al., 2007; Zenjari & Nejmeddine, 2001).

The OM contents show different behavior in the different studied plots. Indeed, this contents in organic matter deprived with the plot degraded from 10 years (P1) with a value close 1.241 mg/ g, this content registered a failure to 0.827 mg/ Cultivated with the soil since 1901(P2). However, these values increase gradually with the P3. The P5 and P4 recorded an

Table 1. Soil Respiration activity in the different studied plot.

Parameters	Respiration				
P0	0.26±0.04				
P1	0.74±0.01***				
P2	0.52±0.02***,\$\$\$				
Р3	0.275±0.005\$\$\$,@@@				
P4	0.265±0.045\$\$\$,@@@				
P5	0.69±0.01***, \$, @@@, ###, £££				

P2 and P0, P1and P3, P2and P3, P4and P1, P4and P2. However, any difference was registered between P3andP4, P3 and P0. Well, the plot 5 shows an important difference with the different plots (Table 2). A respirometric test was determined on soils sampled. CO2 production increased in the most studied plots compared with the Control plot. Indeed, the scrum of the fresh organic residues in the top layer of the ground what has for consequence that the major part of the ground is converted in atmospheric CO2. In Contrast, in the plot OMW treatment P4, the specific respiration remained very low, eventhough the carbon content was very important approximately 2.6 mg g1 very close to

Table 2. Phytotoxicity soil in the different studied plot.

Parameters	Phytotoxicity				
P0					
P1	21.04±0.04				
P2	141.395±0.095 ^{\$\$\$}				
Р3	218.82±0.22\$\$\$,@@@				
P4	60.975±0.025\$\$\$,@@@,###				
P5	206.9±0.1***, \$\$\$, @@@, ###, £££				

Data are presented as means \pm SD. Values were obtained from triplicate determinations and statistical significance was examined by one-way analysis of variance (ANOVA) followed by the Fischer's LSD (Least Significant Difference) post hoc test. ***p<0.001 as compared to **P0**; \$ p<0.05 as compared to **P1**; \$\$\$\$ p<0.001 as compared to **P1**; @@@ p<0.001 as compared to P2;### p<0.001 as compared to P3; £££ p<0.001 as compared to P4.

important increase to 1.37 mg/g and 2.62mg/g, respectively. As can be seen, the plot treated with OMW showed an OM value close to the value founded with P0 (Control Plot). Indeed, several works indicate that the contribution of the olive mill wastewater entails the enrichment of the ground and the improvement of the yield on the culture (Garcia-Gomez & Bernal, 2003; Mekkkiet al., 2013; Piotrowskaet al., 2006; Dakhli et al., 2013; Morugán-Coronado et al., 2015). Most studies have concluded that OMW application on soil could have positive effects on soil structure. However, negative environmental impacts of OMW spreading have also been reported (Zenjari & Nejmeddine, 2001), although the results of these studies are sometimes contradictory and depending on soil parameters and application rate.

3.2. **Soil Respiration**

The comparison of respiration soil activity in the different plots shows different behaviors (Table1). A significant difference (p=0.01) between the flowing plots: Pland P0, Pland P2, control plot. This 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 favourable influence of its higher nutrient contents as was demonstrated by (Paredes et al., 1987; Ribo, 1997; Sierra et al.,2001; Zenjari & Nejmeddine 2001; Alloucheet al., 2004; Obied et al.,2005). In simple terms, the inhibition of soil respiration could be caused by the fact that the biggest amount of carbon added to the soil was unavailable to the microflora under the effect of its strong adsorption or its reaction to the components of the soil. However, organic matter increasing was the soil accompanied by a failure of respiration soil activity (Table2/Fig. 1). Several studies suggested that increasing soil OM is also a strategy for sequestering carbon dioxide (CO2) to mitigate anthropogenic greenhouse gas (GHG) emissions (Luo et al., 2010; Liu et al., 2014; Barton et al., 2016). Increasing soil OM can enhance nitrous oxide (N20) emissions, a potent GHG, by increasing the availability of nitrogen (N) and C to soil microorganisms (Stehfest & Bouwman, 2006). Crop residues are subject to N mineralization, and in turn nitrification and denitrification; microbial processes that lead to N20 production (Butterbach-Bahlet al., 2013). For example, nitrifying microbes convert soil ammonium (NH4+) to nitrate (NO3) under aerobic conditions, which may result in N20 as a by-product of the N transformation. Likewise, anaerobic denitrifies sequentially reduce N oxides (e.g. NO3) to nitric oxide, N2O and finally N2; with N2O emissions resulting from an incomplete conversion. Soil methane (CH4) uptake could also be inhibited, should increase soil OM result in increased soil N concentrations (Le Mer & Roger, 2001). While soil C sequestration would be expected to reach a maximum threshold (Ingram & Fernandes, 2001), the effects on soil GHGs emissions could continue if soil OM concentrations were maintained (Barton et al., 2016).

3.3. **Phytotoxcity**

Table 3 shows a significant difference (p<0.001) in the different compared plots. The Phytotoxcity determined germination via index (percentage). The GI is relatively low, 20 and 60 % for P2 and P4 respectively, increases gradually with P 2(145), then P3 (200) and maximal for the P5 (230). We notice that P4 (Plot treated with high dose OM Walong-time) registered a lower value of GI compared with other treatments. Further, assuming that phenolic substances are less easily degradable, they should remain longer times in soil compared to other organic substances from OMW, resulting in a high toxic level at the beginning of the incubation experiment (Mekkiet al., 2007; Deebet al., 2012; Buchmannet al., 2015). If our assumptions were right, the subsequent degradation of those phenolic substances should come along with a decrease of phytotoxicity in OMW treated soil and its extracts over time. However, the higher values of GI founded with another treatment such as in P3 as well as P5. The tillage as well the introduction of the fig tree in parallel could change the soil chemical and physical properties of long-term. Furthermore, it is well-known that higher dose of phenolic compounds can lead to an enhanced phytotoxicity in the case of the P4, plot of olive tree speeded with 200 m3/ha dose of OMW during 10 years compared to P5, plot treated with tillage accompanied by 50 m3/ha dose OMW spreading lasting only a one year.

3.4. **Microbial Activity**

Any significant difference was noted in the microbial biomass between the different studied plots (Table 3).

The biological soil activity is characterized by an important microbial activity. An increase in the total aerobic mesophilic flora TAMF count was observed in P4. However, at P1, P2and P3 the total bacterial counts remained much higher compared to the control soil, but at lower counts were observed in P5 compared to the other plots. In comparison with the control soil, an overall high CFU of yeast in all plots was found (Table). In all dates of sampling, the total coliform CFU number was not detected in all plot compared with the control soil except in P3, which had ahigher CFU than the control. However, Soil OMW treated with (P4) created some modifications in the average values for the total number of microorganisms and their repartition. Results showed an initial increase in the numbers of CFU in most microflora groups after the OMW amendment. In line with this finding (Mekkiet al., 2013) reported also an increase in the total viable counts in the soil treated with OMW. (Mekkiet al., 2006). The bacterial analyses of the soil showed a very spectacular diversity with the treated olive mill wastewater. Bacteria play an important role

Table 3. Microbial analysis

Parameters	Total aerobic mesophilicflora (10 ⁴ g ⁻¹) UFC	Yeast (10 ⁴ g ⁻¹) UFC	CT (10 ⁴)	Coliform bacteria (10 ⁴ g ⁻¹) UFC	Streptococcus (10 ⁴ g ⁻¹) UFC
OMW	100475±25a	1127±27a		0±0	0.67±0.15
P0	188.33±97b	50005±5b	5±0.36	0±0	0±0
P1	1307.67±135.6b	99995±5¢	0±0	0±0	0±0
P2	263.67±204.74b	30015±15d	0±0	0±0	0±0
Р3	172±57.37b	8950±50e	3.7±0.51	0±0	0±0
P4	1120.33±581.69b	210005±5f	0.33±0.08	0±0	0±0
P5	71±25.36b	7950±50g	0±0	0±0	0±0

Means in the same column with different letters are significantly different ($p \le 0.05$) according to Fischer's LSD (Least Significant Difference) post hoc test. Data expressed as mean \pm SD of triplicate determinations.

in the cycling of OM on the ground, indeed, thanks to their activity metabolism and proteolytic bacteria are capable of degrading very complex organic molecules. In addition, the olive mill wastewater establishes a good substratum usable spoil by microorganisms, which are capable of degrading the rather complex phenolic compounds (Mekkiet al., 2006; Dhouha Saidana et al., 2014).

3.5. **Correlation parameters**

Correlation analyses, among physiochemical parameters of soil and microbial biomass indicated several positive significant trends. The highest significant correlation was found between OM and TAMF (r=0.999, p<0.001) (Table 4). Our results was agreed with previous pH, which in its turn was negatively correlated with EC and OM. The negative correlation was found for pH and EC (r=-0.996; P<0.01) flowed by pH and OM (r=-0.993; P<0.01). This result can be explained that mesopilic bacterial led to further as by-products which can decrease the pH ground.

However, in this work we found a negative correlation between respiration soil activity (Resp)and with OM. This result was in contrast with previous work which shown a strongly positive effect with these both soil parameters (Brant et al., 2006; Hoorman&Rafig 2010). In fact, CO2 sequestration took place. Thus OM increase CO2 sequestration, which inhibiting CO2 liberation by respiration phenomena.

Table 4. Correlation matrix (Pearson's r values) between the different parameters determined in soils subjected to the different treatments. OM (Organic Matter), EC (Electric Conductivity), TAMF (Total Aerobic Mesophilic Flora), CT (Total Coliform Bacteria), Strep (Streptococcus), Resp(Respiration), **Phytox**(Phytotoxicity).**p*<0.05 ***p*<0.01

Parameters	ОМ	EC	pН	TAMF	Yeast	CT	Strep	Resp	Phytox
OM	1								
EC	0.991**	1							
pН	-0.993**	-0.996**	1						
TAMF	0.999**	0.991**	-0.993**	1					
Yeast	-0.323	-0.385	0.363	-0.325	1				
СТ	0.228	0.309	-0.445	-0.201	-0.195	1			
Strep	-0.084	-0.099	0.087	-0.094	-0.027	0.428	1		
Resp	-0.533*	-0.219	0.677**	0.143	-0.234	-0.450	-0.283	1	
Phytox	-0.443	0.673**	-0.541*	-0.570*	-0.783**	0.312		-0.192	1

researches concluded that increasing in soil organic matter enhance the bacterial mesohiplic activity, (Mekkiet al., 2013). In addition, correlation studies showed significant positive correlations (r=0.991, P<0.01) between the EC and TAMF. These results corroborate the notion that the microbial community structure is a good indicator of soil quality, perturbations and the effects of different management practices (Di Serio et al., 2008, Morugán-Coronado et al., 2015), because the microorganisms respond against changes in soil management more rapidly than chemical or physical soil properties. However, OM increase can lead an accumulation of salts soil, which further a higher rate of EC. This fact is further supported by the existence of a positive correlation (r= 0.991P < 0.01) between OM and EC (Table5). In general, TAMF was positively correlated with the soil properties, except for the The higher Phytotoxicity (Phytox) rate decreases the pH and inhibit the microflora such as TAMF and yeast. As can be seen a negative correlation of -0.541, -0.570 and-0.783, was observed with Phytox and pH, Phytox and TAMF, Phytox and Yeast, respectively.

However, the positive relation of phytotoxicity with EC explained the accumulation of salts and reduced water infiltration flowing the soil phytotoxcity. This could be confirmed by the high correlation (R2 = 0.673, P < 0.01) betweenPhytox EC. Indeed. and negative effects on physiochemical soil properties such as accumulation of salts (Di Serio et al., 2008; Mahmoud et al., 2010), potential groundwater pollution (Buchmannet al., 2015), slow degradation and biological mineralization for some of its substance (Pierantozzi et al., 2013), are limiting for the application of OMW on

4. CONCLUSION

The Soils are generally alkaline, with different treatment; this behaviorcould result of ground composition in the South Tunisian, with high content of the buffer. However, the plot treated with OMW showed an important OM value (2.62mg / g) close to the value founded withuntouched plot. Thus, OMW application on soil could have positive effects on soil structure. In contrast, we note that P4 (Plot treated with high dose OMW for a long-time) registered a lower value of GI compared with other treatments. It is well known that higher dose of phenolic compounds can lead to an enhanced phytotoxicity in the case of the P4 compared to P5 and P3. Indeed, it is necessarily required an additional treatment to higher doses of OMW Correlation analyses, among physiochemical parameters of soil and microbial biomass indicated several positive significant trends. The highest significant correlation was found between OM and TAMF (r=0.999, p<0.001). These results corroborate the notion that the microbial community structure is a good indicator of soil quality, perturbations and the effects of different management practices, because the microorganisms respond against changes in soil management more rapidly than chemical or physical soil properties. The finding in this study was the negative correlation between respiration soil activity (Resp) and with OM (r=-0.533, p<0.05). Indeed, increasing soil OM enhances the sequestering carbon dioxide (CO2) to mitigate anthropogenic greenhouse gas (GHG) emissions.

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