Algerian Journal of Natural Products 5:3 **(2017)** 548-561

OPEN ACCESS

Online ISSN: 2353-0391

Algerian Journal of Natural Products

www.univ-bejaia.dz/ajnp

Type of the Paper (Article)

Influence Of Olive Mill Waste Water Spreading On soil Microbial Activity. Short Term Effect On Carbon And Nitrogen Mineralization Process

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Received: 060/06/2017 /Accepted: 26/12/2017 https://doi.org/10.5281/zenodo.1133241

Abstract: Mineralization is the core of the symbiotic relation between soil - microorganism and plant. It is the generator of mineral elements essential to the plants nutrition. Microorganisms are the main biotic actors in this process. The product of the mineralization depends, on the one hand, on the biomass of the soil and on the other hand on the quantity, nature and characteristics of the organic matter.
The monitoring of the mineralization is therefore essential after any input of organic matter with a view to its valorisation which effects are unpredictable.
It is in this context that we propose to study the impact of the three successive annual intakes of OMWW on the process of mineralization of organic matter and the soil content of carbon and mineral nitrogen.

Keywords: *mineralization, mineral carbon, mineral nitrogen.*

I. Introduction

Olive oil extraction produces large amounts of waste water, known as olive mill waste water (OMWW). This effluent has a high chemical oxygen demand, contains high level of phenolic compounds, and is therefore a cause of environmental pollution and public health. The exploitation of this waste without preliminary treatment is very limited considering its toxicity for soils and plants. In Tunisia, 700,000 tons of OMWW, produced annually, are generating many types of pollution. They are dried in special basins and then put in heap to be used as compost while an important fraction of the product is poured directly in the natural channel beds (wadi). Several methods for OMW treatment have been employed in recent years such as evaporation, electrocoagulation, oxidation by ozone and using fenton reagent, aerobic and anaerobic biological treatments as well as reuse by spreading onto agricultural soil as an organic fertilizer (Eus´ebio et al. 2007; Mekki et al. 2013).
However, the richness of that sludge in mineral and organic compound raised to investigate other techniques to valorise this residue in agronomy.
Indeed, OMWW contains high amount (80–150 kg m^{-3}) of beneficial organic compounds and plant mineral nutrients for the soil–plant system that can sustain fertility and productivity of the soils (Bene et al. 2013; Weber et al. 1996). Soils of the Mediterranean region are characterized by lack in soil organic matter rate with low level of fertility and productivity (Brunetti et al. 2007). To compensate for

the negative balance with respect to soil carbon, external sources of organic matter should be periodically added to the soil. Currently, organic wastes of various origins and nature are widely used as amendments to increase soil organic matter.
OMWW which is rich in organic compounds can be used to restore the deficit in soil carbon and nitrogen, combat soil degradation, and improve soil fertility which consequently enhancing sustainability of the Mediterranean agroecosystems (Burgos et al. 2002; Madejo´n et al. 2003 ; Mohammad and Mazahreh 2003; Mohawesh et al. 2014).
This work fits into this context and aims to study the impact of three consecutive spreading of OMWW on soil carbon and nitrogen mineralization.

II. Materials and Methods
II.1. Incubation experiment

For this study surface layer samples (0–20 cm) of soil were collected at the IRA (Institute of Arid Regions) in southern Tunisia (governorate of Medenine). North latitude: 33° 16′ 21″, East longitude: 10° 19′ 30″. The climate of the region is typical Mediterranean, semiarid to arid, with an average rainfall of 150 mm and average annual temperature of 18-20 °C.
The soil chosen was an isohumic with the following characteristics: sandy texture (90% sand, 3% loam, and 6% clay), pH (7.11), EC (2.32 $dS \cdot m^{-1}$), % $CaCO_3$ (5.55) and % OM (0.98).
The fresh OMWW was taken from a three-phase continuous extraction factory located in Saadane-Tunisia southern Tunisia. The physicochemical characteristics of crude OMWW are shown in Table 1 and correspond to the mean values of 3 analyses.
Soil samples were air-dried, sieved to 2 mm and then placed in 500 mL polyethylene pots at a rate of 2 kg per pot.

Table 1. *Physicochemical characteristics of olive mill waste water used in ferti-irrigation (values after represent ± standard deviation).*

Characteristics	Data
pH	4.8 ± 0.2
Electrical conductivity (Ds/m)	10.0 ± 0.52
COD (g/L)	98.0 ± 2.1
BOD (g/L)	66.0 ± 2.4
Total organic carbon (g/L)	26.0 ± 2.4
Total nitrogen Kjeldahl (g/L)	1.6 ± 0.1
Carbon/Nitrogen	16.25 ± 0.48
Phenolic compounds (g/L)	8.8 ± 0.3
Potassium (g/L)	6.1 ± 0.2
Calcium (g/L)	1.1 ± 0.1
Phosphorus-Olsen (g/L)	0.35 ± 0.02
Magnesium (g/L)	0.42 ± 0.01
Sodium (g/L)	1.57 ± 0.01
Chlorides (mg/L)	0.65 ± 0.4

OMWW intended to be used were also homogenized and then incorporated into the soil. The equivalent to the doses was adopted, T1: 15 m^3/ha, T2: 30 m^3/ha, T3: 45 m^3/ha. Since the soil area at the pots is 0.018 m^2, the doses of OMWW applied per pot correspond to 90 ml for the dose 15 m^3/ha, 180 ml for the dose 30 m^3/ha and 270 ml for the dose 45 m^3/ha.
The experimental design consists of 12 pots divided into three treatments; one control was run without any amendment and three replications.
The same doses of OMWW were applied three successive times spaced five months apart.
Ten days after the first application, a first sample of 12 soil samples of 250 g was taken. A second sampling at two months and a third sampling at four months of the day of OMWW spreading.
The same sampling operations were carried out with the same chronology after the second and third OMWW spreading.

Soil controls were run without any amendment. Deionised water was added to the soil–waste mixtures and the soil samples in order to bring their moisture content to 60% of their water-holding capacity.

II.2. Analytical methods

50 grams (dry weight) of pre-incubated samples of soil were thoroughly mixed with OMWW 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 28 C 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 OMWW and 3 ml of deionized water were placed in open polyethylene vessels of 100 ml. Control samples without the OMWW 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 ($28_{–}^{C}$) and moisture content (15%).
Moisture losses were controlled and corrected during the incubation (Keeney and Bremner 1967).
Samples incubated for different periods of time, were analysed for the determination of exchangeable ammonium and dissolved nitrate. Exchangeable ammonium was extracted with 2 M KCl and dissolved nitrate with deionized water. Both extractions were carried out at a rate of 2:5 (w/v) soil:solution, shaking for 30 min at room temperature. Ammonium and nitrate in solution were analysed using an ionselective electrode (Banwart et al. 1972; Davies et al. 1972). Operational ranges of the methods were 0.5–20 mg $l^{–1}$ NH_4–N and 0.5–50 mg $l^{–1}$ 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 Hesse (1971).
Microbiological parameters (soil respiration performed as the C-CO_2, nitrate nitrogen and ammonium nitrogen) were studied in destructive samples at 0, 7, 14, 21, 35, 63, 70, 77, 84, 98, 105, 112, 119, 126, 133, 140, 147, 154, 161, 168, 175, 182, 189, 196, 203, 210 and 214 days of incubation.

2.3 Statistical Analysis

All results were subjected to analysis of variance. Data were analyzed using the ANOVA procedure.
A probability level of α = 0.05 was chosen to establish the statistical significance among treated and control samples. Variance and standard deviation were determined using SAS 9.3 (version for windows).

III. Results and discussions
III.1. Effect of OMWW spreading in C an N mineralization
III.1.1. Carbon mineralization

Whatever the dose, the intake of OMWW into the soil induced a greater release of C-CO_2 than that produced by a soil without any input. Application of OMWW stimulated respiration consistent with earlier studies (Piotrowska et al. 2006; Sierra et al. 2007).
Indeed, at the end of the seventh day of incubation we recorded cumulations of the order of 16.61 mg/kg, 18.57 mg/kg and 24.01 mg/kg respectively for the samples having received the three doses of OMWW adopted such as T1 (15 m³/ha), T2 (30 m³/ha) and T3 (45 m³/ha) (table 2).

Table 2. *C-CO_2 average (mg/kg) rate after three consecutive OMWW spreading.*

Each year different letters indicate a significant difference among the means at LSD test (P > 0:001).

	First OMWW spreading			Second OMWW spreading		Third OMWW spreading	
Treatment (m³/ha)	**Since (7day's)**	**Since (63 day's)**	**Since (7 day's)**	**Since (7 day)**	**Since (35 day's)**	**Since (7 day's)**	**Since (35 day's)**
T0 (0 m³/ha)	6,5 A	39,5 A	50,66 A	54,3 A	56,88 A	63 A	65,22 A
T1 (15 m³/ha)	16,61 B	81,31 B	106,66 B	115,33 B	120,6 B	127,38 B	122,33 B
T2 (30 m³/ha)	18,57 B	94,18 BC	115,66 C	127,92 C	132,85 C	141,38 C	136,33 C
T3 (45 m³/ha)	24,01 C	102,48 C	119,66 D	132,88 C	137,9 D	147,33 D	143,11 D

The differences are statistically significant compared to the control without OMWW in which we recorded an accumulation of the order of 6.5 mg/kg. However, we did not observe significant differences between the T1 and T2 doses.
This can be explained by the fact that at this stage and at a certain threshold it appears that the amount of organic matter contributed is not critical in the productivity of the mineralization process.
The increase in mineralization potential cannot be positively correlated with the amount applied beyond the T2 dose.
However, the situation of the incubation with respect to the date of the OMWW intake proves to be influential on the activity of the microorganisms of the soil (Figure 1).
Indeed, during this first phase (first week), it is the acceleration of the mineralization process which is most remarkable. It is observed independently of the incorporated OMWW dose (Figure 1).

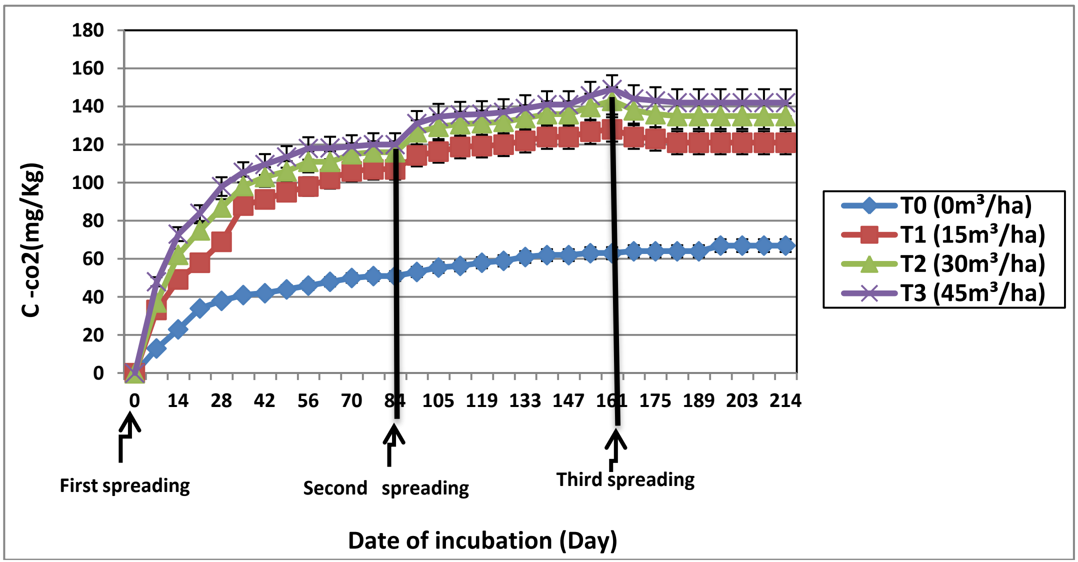

***Figure 1**. Effect of three consecutive OMWW spreading on (C-CO_2) amount (mg/Kg).*

Moreover, whatever the quantity of organic matter contributed by these effluents, this mineralization flush is on the same incubation date (seventh day) for the three applied doses of OMWW.
This process, which is also called the "priming effect" (Fontaine et al., 2004), is related to an acceleration of the mineralization of native organic matter following the contribution of compounds rich in energy potentially exploitable by microorganisms.
Following the application of organic amendments to the soil, respiration rates were among the highest reported in the literature (Busby et al. 2007; Khalil et al. 2005), an effect attributed to the presence of high concentrations of labile organic-C in OMWW (Niaounakis and Halvadakis 2006). Although the

increase in respiration rate was not proportional to hydraulic loading (Figure 1), this response does not imply a toxic effect, since it is a typical response to the increasing rate of substrate (Huang and Chen 2009; Sierra et al. 2007). Issues related to the accessibility of organic compounds by microorganisms, soil properties, and/or the diffusion of O_2 have been suggested to contribute to this effect (Busby et al. 2007; Khalil et al. 2005). Indeed, Meli et al (2003) demonstrated that it is not the amount of organic carbon that would be involved at the beginning of incubation but rather the diversity of sources of organic carbon.

It is for this reason that during this phase of decomposition, the input of organic matter is followed by an increase in the microbial populations "strategy r", which are not specific to a type of substrate. They have the ability to use the readily degradable resources available and are responsible for the mineralization peak that occurs immediately after the incorporation of organic matter (Grossbelet, 2008). According to Swift et al (1979), in this first phase of decomposition, bacteria would be the first actors because of their affinity to labile carbonaceous substrates, while fungi dominate in the later stages of decomposition (Decomposition of recalcitrant compounds predominate).

In addition, work by Boer et al (2005); Denef et al. (2009) have shown interactions between certain groups of fungi or bacteria within microbial domains to optimize the decomposition of certain compounds.

Besides, previous work has provided evidence for an increase in the fungi/bacteria ratio with OMWW application that was linked to the biodegradation of recalcitrant compounds found in OMWW (Mekki et al. 2006).

A stimulation of fungi growth, especially of basidiomycetes, which contain multiple laccase gene copies (Theuerl and Buscot 2010) may be responsible for this increase.

A second phase appeared on the eighth day of incubation. During this period, a slowing down of the mineralization process from the first period was observed. This decrease in the rate of decomposition resulted in an asymptotic curve.

This phase was much more spread over time (56 days). Indeed, it continued until the 63^{th} day of incubation.

At the end of this period and in comparison with the first phase, mineralization amount is relatively proportional to the increase of the OMWW attributed dose. This can be explained by the change of strategy of the microorganism under the influence of the availability of the labile fraction of the organic matter over time.

We found cumulations of 81.31 mg/kg, 94.18 mg/kg and 102.48 mg/kg of $C\text{-}CO_2$ respectively for the T1 (15 m^3/ha), T2 (30 m^3/ha) and T3 (45 m^3/ha) with a statistically significant difference between them and highly significant difference compared to the control where we recorded a cumulative of 39.5 mg/kg (Table 2).

This decrease in the rate of decomposition of organic matter can be attributed, on the one hand, to the exhaustion of a large part of the labile fraction of the organic matter and, on the other hand, to the richness of OMWW of substances with difficulty Biodegradable materials, in particular lignin and phenolic compounds; As a result, microorganisms more specialized in the degradation of these more complex polymers develop slowly during this second phase. From Leij et al. 1993 described this maneuver as a strategy (k).

Decomposition processes are very complex with microbial dynamics that are difficult to generalize (Hu et al., 1999). Indeed, for the same substrate, the degradation of organic matter involves successive populations that interact according to their enzymatic equipment (Schlegel, 1993; Swift et al., 1979). Some organisms can adapt to environmental conditions: this is the case of fungi whose nitrogen content in the mycelium can vary depending on the amount of nitrogen in the environment (Swift et al., 1979).

The more organic residues are rich in lignin, the more difficult their biodegradation is due to the recalcitrant effect of their plant polymers (Parnaudeau, 2005, Bertrand et al., 2003, Hammel, 1997).

Numerous studies on plant residues also show the depressive effect of lignin on global decomposition, because its macromolecular structure including multiple types of bonds is difficult to degrade (Bertrand et al., 2003; Hammel, 1997).

Moreover, (Houot et al., 2004) have shown that the toxicity of certain compounds may limit the decomposition of organic matter. In this case, the concentration of toxic phenolic compounds initially bound to other molecules.

(Benzarti., 2003) attributes this slowing of biodegradation to the time required to adapt and select populations of soil microorganisms specific to the new medium rich in complex organic matter.
From the 70th day of incubation, we witnessed a third phase which lasted only two weeks. This phase is characterized by a regression of the mineralization potential which has resulted in a decrease in productivity compared to the second phase.
We recorded C-CO_2 accumulations in the order of 106.66 mg/kg, 115.66 mg/kg and 119.66 mg/kg respectively for samples receiving 15 m³/ha, 30 m³/ha and 45 m³/ha; with a significant difference between them and compared to the control which accumulated an amount of 50.66 mg/kg (Table 2).
The values for each applied dose were maintained at a practically constant level up to the 84th day of incubation.
We can deduce that at this stage the mineralization process tends to stabilize with a low and constant production of C-CO_2. This would indicate a regression of microbial activity. 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 (Torri et al., 2003).
On the other hand, because the microorganisms themselves can not provide sufficient exo enzymes necessary for the degradation of organic compounds at this stage.
The kinetics of carbon mineralization after the second intake of OMWW is characterized by two phases (Figure 1).
During the first phase, as with the first application, a mineralization flush was observed.
This flush also lasted one week but the flows of C-CO_2 emitted were small, not exceeding 7,10 and 11 mg/kg respectively for T1 (15 m³/ha), T2 (30 m³/ha) and T3 (45 m³/ha) compared with those reached after the first application.
Indeed, with the highest dose T3 (45 m³/ha), the amounts of easily biodegradable compounds increase. However, the C-CO_2 flux seems to be limited even for significant intakes.
The mineralized carbon content is inversely proportional to the increase in the amount of organic matter contributed by the dose. The statistically significant difference disappears as we increase the dose, especially between the two highest doses T2 and T3 (Table 2).
This seems to limit the microbial activity that will be confirmed during the second phase.
The second phase is characterized by a slowing of the mineralization rate which resulted in a plateau of constancy that lasted five weeks indicating the stabilization of microbial respiration.
The quantities of C-CO_2 emitted are maintained at rates equivalent to 120.6 mg /kg, 132.85 mg/kg and 137.9 mg/kg respectively for T1 doses (15 m³/ha), T2 (30 m³/ha) and T3 (45 m³/ha) compared to a flux of 56.88 mg/kg for the control without OMWW. The difference between them is highly significant (Table 2).
This slowdown can be attributed as demonstrated at the end of the first application, not only to the exhaustion of the microbial communities, but also to the fact that the successive application of OMWW brings more compounds that are difficult to mineralize in proportion to the doses adopted, which accelerated the decline of microbial activity.
Moreover, the kinetics of carbon mineralization in soil-compost mixed (Annabi., 2005) showed that the carbon mineralization observed corresponded to the maximum potential of activity of the microflora present, therefore mineralization activity would increase proportionally to the dose of available carbon without a limiting factor appearing. This limiting factor is observable only in the presence of the highest dose.
This would indicate that it is the nature of the material supplied that defines the rate of mineralization.
The mineralization curves recorded after the last spreading operation have two phases (Figure 1):
Beyond the 147th day of incubation, a new "priming effect" appeared during which there was a slight increase in the C-CO_2 flux which did not exceed an average of 7 mg/kg, 9 mg/kg and 10 mg/kg respectively for T1 doses (15 m³/ha), T2 (30 m³/ha) and T3 (45 m³/ha) (Table 2).
The effect of the new labile fraction of the organic matter brought by the third spreading of OMWW is at the origin of this increase which also lasted only one week.
Indeed, Young and Crawford (2004) have shown that some environmental changes favorable to the local increase of the microbial activity of the soil could be at the origin of a reorganization of the sol-microorganisms complexes resulting in the appearance of new bacterial communities that have been able to adapt to environmental conditions.

In comparison with the first and second spreading, the "priming effect" observed after the third injection is less important. This can be attributed to the cumulative effect of phenolic compounds on the mineralization potential of microorganisms.
The second phase is characterized by a drop in mineralization potential compared to that recorded during the first phase regardless of the amount of organic matter supplied. Indeed, the rate of mineralization gradually decreases with time until the end of the experiment to stabilize at the enivrons of 122 mg/kg, 136 mg/kg and 143 mg/kg respectively for the T1 doses (15 m³/ha), T2 (30 m³/ha) and T3 (45 m³/ha) compared to a control flux of 65 mg/kg (Table 2).
It should be noted that the slight increase in flow of (C-CO_2) released during the entire incubation period of the control soil is related to the mineralization of the small amount of initial organic matter of the soil by the various microorganisms.
Considering that this fall in microbial respiration can be attributed to the depletion of labile fractions of soil carbon during the incubation period as demonstrated by Torri et al in 2003, which shows that Rate of mineralization is related to the amount of easily mineralizable carbon, and that this fraction is effectively fully mineralized. The rate of mineralization should be expected to be constant regardless of its amount. However, the results showed that the rate of mineralization decreases according to the amount of organic matter supplied whatever the type of organic input. We are therefore experiencing a mineralization deficit as the amount of organic matter added increases.
During this stage and at a certain threshold the microbial activity forms a limiting factor of the mineralization in spite of the increase of the quantity of the organic matter.
This regression of the mineralization potential observed after the "priming effect" of the third spreading approves the results of other studies carried out by (Marschner, 2003, Saison et al., 2009, Lejon et al., 2007, 2008) which showed that organic amendments like compost altered both the composition of bacterial communities, their size and activity.
Pascual et al. (1997) also explained the phenomenon of the fall of bacterial respiration in the soil by total depletion of biodegradable organic carbon. The shortage of this metabolizable fraction represents a limiting factor to bacterial proliferation.
In addition, mineralization of organic matter is a process that involves microorganisms that consume organic resources. The latter provide them with energy (carbon bond) and essential mineral elements for their proliferations (especially nitrogen).
Indeed, increasing the amount of organic matter brought in requires increasing nitrogen requirements, which if not in sufficient quantities would limit microbial growth and thus inhibit the mineralization of organic carbon (Recous et al., 1995). Given that a fraction of mineral nitrogen is assimilated by microorganisms for their growth. This is the organization of mineral nitrogen, also called immobilization (Anglo-Saxon term) (Parnadeau, 2004).
The research carried out by Busby et al (2006) showed similar results in the case of non-composted household waste associated with a soil rich in carbon and organic nitrogen, compared to organic waste composted in a soil low in native organic matter.
Moreover, the dough-like texture and high moisture of these wastes cause the physical compaction of the material, limiting free aeration through the composting piles and slowing down the process and accelerated the decline of microbial activity. Indeed,, Berndt (1996), Abichou (2003) Taamallah (2007) and (Dakhli et al., 2009; Dakhli, 2015) have shown that OMWW contain oily emulsion residues which tend to disperse as small vesicles and then form crusts on the surface aggregates causing waterproofing in the first stage and subsequently promoting anaerobic conditions (asphyxia) (Ros de Ursinos et al., 1996)
In addition, the chemical composition of the wastes is mainly based on lignocellulosic materials (32.6–56.0% lignin; 27.3–41.6% hemicellulose and 14.0–24.9% cellulose; Darwis., 1993 and Derenne and Largeau., 2001; Alburquerque et al., 2006; Cayuela et al., 2006) that are slowly degraded by microorganisms. Finally, the antimicrobial properties of these wastes, mainly due to the presence of phenols, are normally one of the main reasons for their low degradation rate, the antimicrobial properties of these wastes, mainly due to the presence of phenols, are normally one of the main reasons for their low degradation rate, at least during the earlier stages of the composting process. Whereas low molecular weight phenols are well known to have antimicrobial and phytotoxic properties (Paredes et al., 1999; Capasso et al., 1992; RamosCormenzana et al., 1996; Della Greca et al., 2001), high molecular weight phenols have also been shown to inhibit the effect of lignolitic

enzymes such as lignin peroxidase in fungi and bacteria (Sayadi et al., 2000). All these characteristics cause a very low degradation rate of these wastes (Alburquerque et al., 2006; Cayuela et al., 2006). The intrinsic characteristics of exogenous organic matter are therefore one of the main determinants of their mineralization (Heal et al., 1997).
These effects, linked to the biochemical properties of this sludge can negatively affect the degradation process, in particular by modifying the access of the microorganisms to the substrate (Angers et al., 1997, Vanlauwe et al., 1997). For example the availability of nitrogen (Corbeels et al., 2003) and the inhibition of the assimilation of O_2 and other nutrients essential for the growth of microorganisms.
Environmental factors also affect the process of mineralization of organic matter (Parnadeau, 2004). These include temperature, humidity, O2 availability and soil pH (Kirschbaum, 1995, Franzluebbers et al., 2001; Cookson et al., 2002, Papatheodorou et al., 2004, Annabi, 2005 and Grossbelet, 2008).
Indeed, and starting from the fact that the microorganisms in micro-habitats are distributed inside the pores of the soil mainly according to their sizes. Nannipieri (2003) demonstrated that the rate of decomposition of organic compounds varies with the porosity gradient.

III.1.2. Nitrogen Mineralization

The kinetics of the nitrogen mineralization as a function of the different rates of OMWW is illustrated in figure (2). The soil incubation lasted 214 days after three successive spraying operations.
The three curves illustrating the kinetics of the nitrogen mineralization relative to each dose show a concomitance of the evolution of the respective quantity of mineralized nitrogen throughout the incubation period.
As with carbon, the application of OMWW generated a potential for mineralization throughout the incubation period that was higher than that recorded for the control without OMWW.
We note that for the three doses, the mineral nitrogen varies in a similar way over time and the curves of evolution of this parameter present the same shape of kinetics.
Regardless of the dose, the first application of this sludge resulted in a rapid acceleration of the nitrogen mineralization potential in the soil (Figure 2).

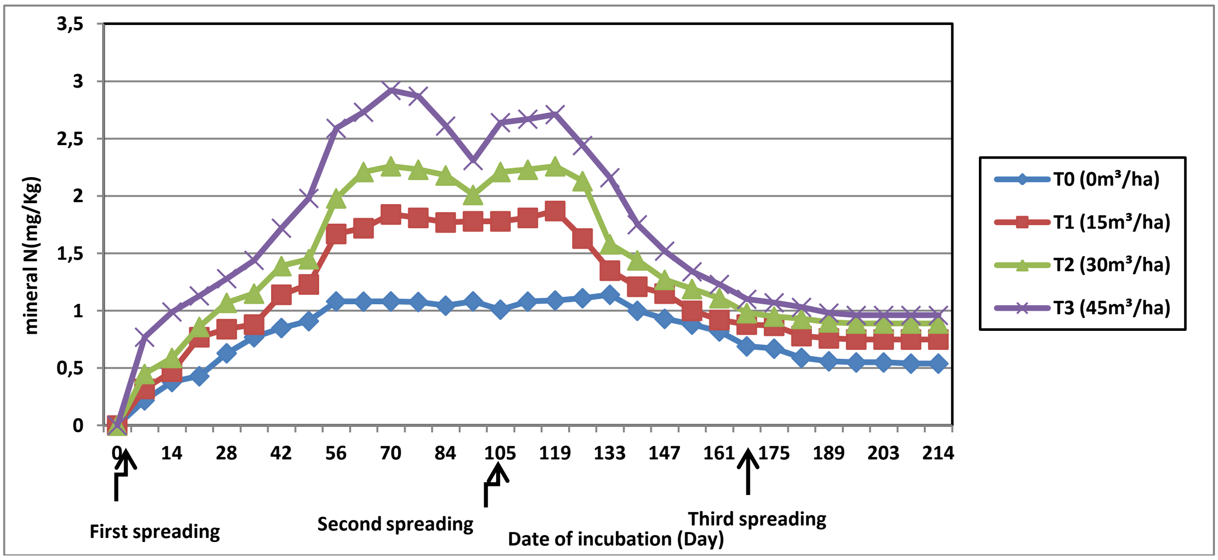

Figure 2. *Effect of three consecutive OMWW spreading on mineral N amount (mg/Kg).*

This stimulation lasted seven days at the end of which a cumulative mineral nitrogen of the order of 0.32 mg/kg, 0.45 mg/kg and 0.77 mg/kg was recorded for the T1 doses (15 m³/ha), T2 (30 m³/ha) and T3 (45 m³/ha) compared to 0.22 mg/kg for the control without OMWW (Table 3).
The mineralization potential, although statistically insignificant, nevertheless recorded differences that were positively proportional to the applied dose (Table 3, Figure 2).

Table 3*: Mineral N average (mg/kg) rate after three consecutive OMWW spreading.*

	First OMWW spreading			Second OMWW spreading		Third OMWW spreading		
Traitement (m³/ha)	**(Since 7days)**	**(Since 49days)**	**(Since 56days)**	**(Since 7days)**	**(Since 119 days)**	**(Since 7days)**	**(Since 14days)**	**(After 214days)**
T0 (0 m³/ha)	0,11 A	0,81 A	1,04 A	1,01 A	56,88 A	0,93 A	0,79 A	0,57 A
T1 (15 m³/ha)	0,32 A	1,17 AB	1,77 B	1,78 B	120,6 B	1,15 AB	0,93 AB	0,77 B
T2 (30 m³/ha)	0,45 A	1,44 BC	2,18 C	2,21 BC	132,85 C	1,27 BC	1,09 BC	0,9 C
T3 (45 m³/ha)	0,77 A	1,86 C	2,81 D	2,64 C	137,9 D	1,52 C	1,22 C	0,98 D

Each year different letters indicate a significant difference among the means at LSD test (P > 0:001).

Indeed, (Elherradi et al., 2003) demonstrated that this stimulation is more pronounced in mixed soils and increases with the addition of the highest dose of OMWW.
Similar results were observed after one week (Hadas and Portnoy, 1994) and two weeks (Jedidi et al., 1995) incubation in single or amended manure compost soils.
On the other hand, (Jenkinson, 1966) and (Bottner, 1985) demonstrated that the marked increase in mineral nitrogen production during the first two weeks of incubation, called "priming effect" was induced by the addition of a highly labile organic material capable of being rapidly mineralized and the microbial biomass destroyed in part by drying.
This phenomenon is thus caused in this case by the mineralization of the labile fraction of the organic matter brought by OMWW.
However, after this peak observed seven days after incubation, we are witnessing a slowing of the rate of mineralization of nitrogen. This rhythm lasted about two months. The evolution of the mineralization potential remains proportional to the incorporated marginal dose.
This phase is characterized by an increasing microbial activity as a function of the dose of the margins attributed, resulting in a consistent yield of mineral nitrogen. There were cumulations of 1.17 mg/kg, 1.44 mg/kg and 1.86 mg/kg of mineral nitrogen respectively for T1 doses (15 m³/ha), T2 (30 m³/ha) And T3 (45 m³/ha) versus cumulative 0.81 mg/kg for the control without OMWW with a significant difference compared to the control and between the three doses applied (Table 3).
The microorganisms of the soil have gradually adapted and have been arranged either by the development of colonies or by the diversification of species, to the quantity of organic matter brought in. This may explain the slow rate of mineralization and the increase in productivity.
A first highly labile pool where the easily biodegradable labile fraction generates a mineralization flush and a second recalcitrant pool responsible for a slowing rate of mineralization following the accumulation of polymers that are difficult to biodegrade, such as the case of lignin and other phenolic compounds Brought by OMWW.
This observation confirms the inferences of (Elherradi et al., 2003) that soil nitrogen and soil-marginal nitrogen consist of two pools that do not have the same mineralization ability.
It has also been demonstrated by (Bremner & Mc Carty., 1993), (Handayanto et al., 1994), that phenolic compounds could inhibit microbial activity and growth, thus delay the mineralization process of nitrogen.
We can say that the mineralization of nitrogen depends on the chemical nature and quality of the organic products brought.
Application of organic residues normally results in an increase in the nitrogen mineralization potential (N0) of soils – that is, an increase in the content of easily mineralizable nitrogen (Benı´tez et al. 2003; Nira and Nishimune 1993). However, the quantity of residual N available to plants is influenced by residue characteristics, rate, timing and method of application. Therefore, knowledge of the N-supplying capacity of an organic waste is important to achieve optimum crop yields while avoiding possible contamination of groundwater by nitrate leaching (Griffin and Laine 1983; Douglas and Magdoff 1991).
During this same phase and beyond the 49^{th} day, we witnessed a new peak of concomitant mineralization for the various treatments that lasted a week to fade thereafter. This acceleration may be due to the proliferation of new populations of microorganisms specific to the decomposition of molecules with a more advanced degree of biodegradability.
The increase in mineralization potential was subsequently continued at a slower pace until the 70^{th} day of incubation where we recorded cumulative mineral nitrogen of the order of 1.84 mg/kg, 2.26 mg/kg, 2.92 mg/Kg respectively for the doses T1, T2, and T3. The difference between treatments has nevertheless continued.

Beyond this date, we have witnessed a gradual regression of the mineralization process, which tends towards constant productivity in the vicinity of 1.77 mg/kg, 2.18 mg/kg and 2.81 mg/kg with a highly significant difference compared to Control and between the three doses (Table 3). This period lasted two weeks.

This regression is detectable regardless of the dose applied to OMWW. This stage represents the maximum productivity threshold of the mineralization process for each dose.

The regression of observed mineral nitrogen levels can be attributed to the critical threshold of performance of soil microbial biomass and/or depletion of the nitrogen-generating fraction. The chronological concomitance of this phase allows us to privilege the first hypothesis.

Compared with the first application, the degree of primary stimulation after the second application was not the same for all treatments.

Indeed, this "priming effect" can be detected only for the highest dose T3 (45 m^3/ha) and to a lesser extent for the T2 dose (30 m^3/ha). However, the lowest dose did not produce any "priming effect.

This flush of nitrogen mineralization lasted one week at the end of which we recorded cumulation of the order of 2.21 mg/kg and 2.64 mg/kg respectively for T2 and T3 with a significant difference between them and a highly significant with respect to the control and to the T1 dose (Table 3).

Beyond the 105th day of incubation, the mineralization potential showed a slow progression which lasted two weeks (Table 3).

From the 119th day of incubation, we witnessed a considerable drop in mineralization potential. This decrease simultaneously affected all samples treated independently of the dose applied.

This one-month regression continued until the respective levels of mineral nitrogen were stabilized.

In this phase, the difference between treatments tends to decrease with incubation chronology to stabilize without fading (Table 28).

The drop in the mineralization potential recorded during the first spreading was amplified during the second OMWW intake except for the low "priming effect".

This fall can be attributed to a reorganization and/or a deficit of microbial activity following a probable inhibiting effect relative to the impact of the successive intakes of the margins (cumulative effects).

The targets of this inhibition are obviously the communities of key microorganisms which regulate the relative availability of ammonium and nitrate in a soil, ie the total heterotrophic community, ensuring the biodegradation of organic matter in the soil, in this case the ammonia .The mineralization of nitrogen and the nitrifying community, essentially autotrophic for carbon, but whose substrate, ammonium, is produced by the mineralization of organic matter or brought by these effluents.

In the same context, organic modification modifies the composition, the size and the activities of bacterial communities, as shown by (Marschner, 2003, Saison et al., 2009, Lejon et al., 2007, 2008).

Moreover, the porosity of the soil as indicated by (Cox et al., 1997) will be reduced by the combined effect of the accumulation of suspended solids and the very high COD Oxygen) formed mainly by one of the polymerized polyphenolic compounds, such as acidic and humic substances.

As a result, the soil has become impermeable which has led to an inhibition of microbial activity in soil aerobic conditions such as fungi and actinomycetes following oxygen depletion at the soil surface.

This slowing down of the metabolic activity of soil microorganisms therefore causes a reduction in the absorption of the mineral elements essential for the growth and development of the plant.

The drop in the nitrogen mineralization potential observed during the second marginal spraying operation and the regression of the soil content in this element continued and even increased after the third addition. However, the concomitance of the kinetics of mineralization was preserved independently of the quantity of organic matter brought in (figure 2).

This time, even the posting effect of margines "the priming effect" has completely faded.

This observation leaves no doubt about the implication of the negative effects of the successive contributions of OMWW. Indeed, at a rate of 15 m^3/ha per intake, the third successive spreading of OMWW resulted in the disappearance of the spreading stimulation flush and the aggressive fall of the mineralization potential.

This fall is very abrupt during the first two weeks following the spreading of OMWW (Table 3).

The rate and degree of regression of the mineralization potential subsequently begins to decrease gradually and subsequently to stabilize at the lowest of the levels in comparison with the second and particularly the first spread for all the doses adopted.

Cumulative mineral nitrogen levels of 0.77 mg/kg, 0.9 mg/kg and 0.98 mg/kg were recorded respectively for T1 (15 m^3/ha), T2 (30 m^3/ha) and T3 (45 m^3/ha) (Table 3).

IV. Conclusion

Whatever the dose, OMWW induced an emission of C-CO_2 higher than that recorded for a soil without any contribution.
Indeed, during the first incubation phase, a mineralization peak was recorded independently of the allocated marginal dose. This peak, which lasted a week, was observed, also after each operation of OMWW spreading.
However, the C-CO_2 flux recorded at the end of each mineralization flush tends to decrease gradually from one spreading to another. This can be attributed to the cumulative effect of the phenolic compounds contributed by the successive intakes of the different doses of the margins during three years of study.
From the eighth day of incubation, a slowing down of mineralization processes was observed in comparison with that recorded during the first phase. This is confirmed during the same period after each spraying operation.
This slowing can be attributed to the depletion of microbial communities.
Whatever the dose, the first application of OMWW produced a rapid acceleration of the mineralization of nitrogen in the soil.
The mineralization potential that lasted one week, although statistically insignificant, nevertheless recorded differences that were positively proportional to the applied dose.
However, in comparison with the first application, the degree of primary stimulation begins to decrease gradually from one spreading to another. After a second intake, this mineralization flush was only detectable for the highest T3 (45 m^3/ha) and to a lesser extent for the T2 dose (30 m^3/ha). The lowest dose, on the other hand, produced no stimulation. However, the third application of margines did not record any mineralization flush.
The regression of the mineralization potential observed at the second application to widen further after the third intake of the margins can be attributed to an inhibitory effect of the activity of the soil microorganisms relative to the acid pH and essentially to the cumulations of the phenolic compounds relative to the Successive annual intakes of OMWW.
The rate and degree of regression of the mineralization potential subsequently begins to decrease gradually and subsequently to stabilize at the lowest of the levels in comparison with the second and particularly the first spread for all the doses adopted.

5. Acknowledgements

This work was carried out in the Institute of Arid Areas Medenine-Tunisia.The facilities and services of the Institute are gratefully acknowledged. It was supported by the Ministry of Agriculture and Water Resources and the Ministry of Higher Education and Scientific Research of Tunisia.

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Please cite this Article as:

Raja Dakhli, Houcine Khatteli, Influence Of Olive Mill Waste Water Spreading On soil Microbial Activity. Short Term Effect On Carbon And Nitrogen Mineralization Process*, Algerian J. Nat. Products,* 5:3 **(2017)** 548-561

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