STUDY OF THE EFFECT OF OLIVE MILL WASTE WATER SLUDGE ON SOIL CHEMICAL PROPERTIES AND ON AUTOCHTHONOUS PEAR MILLET ECOTYPE (*PENNISETUM GLAUCUM* (L) R.BR) BEHAVIOR IN SOUTHERN TUNISIAN

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Abstract

The extraction of the oil produces olive mill waste water "OMWW" which has a very strong polluting power resulting in high levels of COD (chemical oxygen demand), high salinity and a strong phenolic compounds causing environmental pollution. 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 (margines), 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. The search for new technologies or processes for recovery of the effluent is necessary. The spreading of OMWW in the sandy soils in arid conditions of southern Tunisia is a potential alternative for this purpose. In this study, spreading of 20, 40 and 60 m³/ha was tested in the presence of one autochthonous pear millet ecotype *Pennisetum glaucum* (L) R.Br) collected from southern Tunisia in order to assess the impact of the incorporation of this effluent on soil Chemical properties.The results of the study showed that at rate of 20 m³/ha, margines do not present risks regarding salinity, high concentrations of phenolic substances, high potassium content and pH. On the contrary, they induce an improvement of some chemical properties of the soil (organic matter content and potassium) without improving the productivity of pear millet. Application doses of Margines greater than 20 m³/ha generates a very significant decrease of yield of pear millet with disruptions of phenological stages as a result of the accumulation of phenolic substances and the excessive increase of the levels of sodium, chlorides and consequently higher levels of soil salinity in the short and long term.

Key words: *olive mill wastewater, soil, pear millet, yield.*

Introduction

Olive oil production is highly important for the economy, ecology and social life of many Mediterranean countries such as Spain, Italy, Greece and especially Tunisia, which accounts for about 8 % of olive oil world production (Doula et al., 2012;Dakhli, 2013a, b).

The industrial olive oil sector generates large quantities of wastes, including solid and liquid wastes, which have to be suitably managed in order to avoid the

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associated environmental impacts. (Sierra., 2001). The OMWW annual production in Mediterranean countries reached 30 million cubic meters and 700 000 cubic meters in Tunisia alone (Dhouib et al., 2006 ; Kapellakis et al., 2006). OMWW is a critical problem. This sludge presents high salinity (16ds/m), high values of chemical oxygen demand COD between 40 and 200 g/l (Namara et al, 2008 ; Mahmoud et al., 2012 ; Dakhli, 2015).), phytotoxic properties and resistance to biodegradation due to the enormous supply of polyphenol and organic substance (10 to 12 g) (Mekki et al. 2007). Some OMWW characteristics are favourable since this effluent is rich in water, organic substance, and in mineral nutrients, such as nitrogen, phosphorous, potassium, iron and magnesium (Taamallah, 2007).

Consequently, increasing attention has been given to the spread of OMWW on agricultural lands as organic fertilizer and to recycle both the organic substance and the nutritive elements in the soil crop system (Mekki et al. 2006a, Belghith, 2008).

Some studies indicated a positive effect of OMWW on physical, chemical and microbiological properties of soil. Pagliai et al. (2001) reported that OMWW spreading had beneficial effects on topsoil.

Conversely, Mekki et al. (2006) found that OMWW caused negative changes in microbial soil properties, decreasing or inhibiting microflora growth. Zenjari and Nejmeddine (2001) established that OMWW spreading compromised soil fertility, altering physical and chemical soil properties.

Recent studies have evaluated the long-term effect of OMWW disposal on soil properties. Chaari et al. (2015); Kavvadias et al. (2010) observed a positive soil effect due to the supply of organic matter (OM) and macronutrients, but when a high dose (200 $m^3.ha^{-1}.year^{-1}$) was provided for nine successive years, a cumulative effect of soil salinization became evident. Current research assesses the chemical and biological properties of soils after treatment with raw OMWW.A study was performed on two different soils, located in Avellino province (Italy), that have been irrigated for 11 years with a low volume of OMWW (30 $m^3.ha^{-1}.year^{-1}$), corresponding to the annual OMWW production of the factory mill and soils were analyzed after the last spreading performed in October 2013. In this context, several works have been carried out on the valorisation of these effluents in agriculture as fertilizer. Indeed, Albi and Ros de Ursinos (1960); demonstrate the richness in inorganic loads such as this high content of non-toxic organic compounds, macro-elements and micro-elements that indicated a significant fertilizing potential of the OMWW that could be used advantageously in agronomy.In Tunisia, several attempts to use OMWW as fertilizer have been made and directed specifically towards the olive sector. Ammar and Ben Rouina (1999); Ben Rouina and Taâmallah (2000) affirmed the utility of these effluents as a natural fertilizer for sandy soils.The same authors in (2000) and Ben Rouina et al, in (2001) worked on the olive tree and demonstrated that tree fruit shoots are longer and bear more floral clusters and flowers, The tree is increased with the contribution of OMWW, 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 present study was aimed at evaluating under field experiment, the short and medium term effects of different application rates of olive mill wastewater on several chemical soil properties and the evolution of phenolic compounds. Also, the assessment of the effects on the final yield of pear millet crop will be addressed.

Materials and Methods

Experimental site

Experimental work was carried out in field experiment in the CFRA farmer located at the IRA (Institute of Arid Areas) in southern Tunisia (governorate of Medenine). North latitude: 33° 16′ 21″, East longitude: 10° 19′ 30″ (Figure 1).

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.

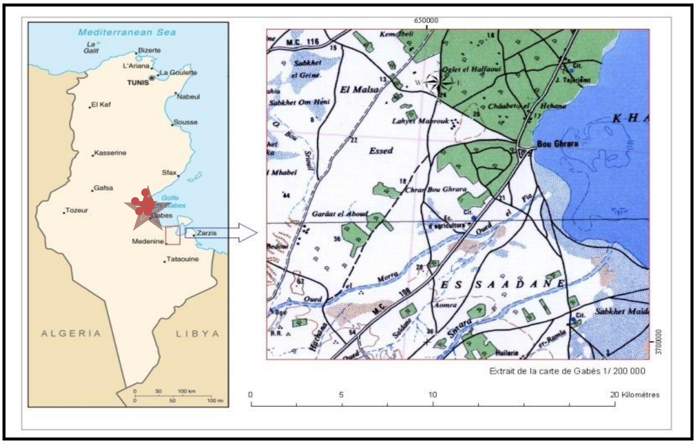

Figure 1
Study area location (Remote sensing Unit-Institute of Arid Areas Medenine).

Soil

It is an isohumic soil, mollic epipedon (Soil Taxonomy - Soil Survey Staff, 2014), typical soil for the arid southern Tunisia with the following characteristics: sandy texture (90% sand, 3% loam, and 6% clay), pH (7.11), % $CaCO_3$ (5.55) and % OM (0.98). The soil is also characterized by a high level of the electrical conductivity which increases with the depth. Indeed, this parameter increased from 5 dS/m in surface layer to more than 8 dS/m in depth (75 cm).

OMWW

The fresh OMWW used in the current experiment was collected from a local three-phase olive oil producing unit located in Saadane-Tunisia southern Tunisia. It was characterized as acidic (pH 4.8) with high electrical conductivity (10 dS m^{-1}), high

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concentration of total solids (42 g l^{-1}) and high organic (chemical oxygen demand 98 g l^{-1}; total organic carbon 26 g l^{-1}; phenolics 8.8 g l^{-1}) and inorganic (total N 1.6 g l^{-1}; K 6.1 g l^{-1}; Ca 1.1 g l^{-1}; PO_2 0.35 g l^{-1}; Mg 0.42 g l^{-1}; Na 1.57 g l^{-1}).
The experimental design consisted of thirty six plots according to a complete randomized block design with three replicates per treatment. Plots with 2 × 3 m^2 size, were delimited, mechanically ploughed and leveled to subsequently amend them with OMWW (olive mill waste water). Annually, since 2009, OMWW spreading application was done in one application on the same plot for two consecutive years: 2009 (First year), 2010 (Second year). The two contributions were made on the same site and with the same experimental design. Amendments were applied annually in December (from 2009 to 2011), 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 OMWW was subsequently carried out by manual tillage. Each year, OMWW application was done at rates equivalent to 20, 40, and 60 $m^3 \cdot ha^{-1}$. Also T0 plots were not amended and served as control: soil where OMWW was never spreading. All the treatments were done in triplicate, distributing the plots alternatively. For each year, four soil subsamples from each plot were taken randomly at 0- 20 cm and 0- 40 cm depth at first time in December (1 day after each amendment) and at second time (after: six months: After pear millet harvest date). Samples were air dried and ground, and the fraction that passed through a 2-mm sieve was used for chemical analyses. A local variety of pear millet (*Pennisetum glaucum* (L) R.Br was adopted in irrigated areas.
Pennisetum glaucum (L) R.Br (Poaceae family): is a potential crop in southernTunisia, with an ecotype collected in Zarzis (Gouvernorate of Medenine) characterized by the length of tillers, thin candles with a loose structure, a relatively long vegetative cycle and a higher Productivity (L.Radhouane et al., 2003).
Pennisetum glaucum (L) R.Br a very common crop in the experimental area, was used in the 2 experimental years.

Soil sampling and parameters analysis

In order to evaluate the length and the range of OMWW spreading effects, we choose two sampling times: short-term and highest expected effects (time1): 1day after spreading; long-term and residual/lowest expected effects (time 2): 6 months after spreading. Soil samples were collected at two layers such as: 0 – 20 cm and 20-40 cm for each plot. The field-moist soil samples were sieved (< 2 mm), delivered and then stored at 4˚C prior to analysis.

Chemical soil analysis

The following parameters were monitored in the soil layer (0 – 40 cm): pH and EC 25 (1:2.5 aqueous extracts), TOC (Total Organic C) was determined by Dichromate oxidation, total N by the Kjeldahl method (1883), Na+ and total K+ contents were determined by fire Spectrophotometer by Chapman and Pratt (1961) method; Chlorides ions was estimated using method of titration with Chlorhydric acid by the Mohr method (Skoog et al., 2015). PC (phenolic compounds) (water soluble

and extracted with sodium pyrophosphate 0.4 N/sodium hydroxide 0.1 N, 1:1) quantified by Folin-Ciocalteau method (Box, 1983).
All determinations were made in three replications. In order to investigate the influence of Olive mill waste water application on crop production, panicle number/m^2 was estimated. At harvest, grain and straw yield and thousand-kernel weights were recorded. The data regarding panicle number and thousand-kernel weights were recorded on 50 plants at time of maturity.

Statistical Analysis

All results were subjected to analysis of variance. Data were analyzed using the ANOVA procedure. A probability level of $\alpha = 0.05$ 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).

Results and discussions

Effect of OMWW spreading on soil organic composition

The addition of the 4 doses of OMWW had different effects on the chemical properties of the soil and their variation with time. In detail, after disposal, results show that OMWW treatment significantly affected total organic C, producing a strong impact with respect to the control, during the three years of the experiment (Table 1).
The rate of organic total carbon increases with the doses administrated. This rate was proportional to the increasing of OMWW doses application. Because of it's binding and hydrophobic effects, the application of OMWW resulted in a more stable soil and created mulch reducing the losses of water evaporation.
Many researchers have established that these wastes have a high fertilizer value when applied to the soil; OMWW is known to increase soil organic matter and the concentrations of essential inorganic elements for plant growth resulting in enhanced soil fertility (Bonari et al., 1993; Cabrera et al., 1996; Paredes et al., 1999).
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 (Sierra., 2007 ; A. López et al., 2008). Moreover these results are consistent with previous reports indicating that olive mill waste application increases soil organic C (E. Madejón et al., 2007; López et al, 2007 and Taamallah., 2007). This increase tends to evolve positively from one year to the next due to the cumulative quantities of OMWW spreading during two years of study (Table 1). Whereas, the total organic carbon content decreased significantly in the amended soils over time. This reduction was observed for the three doses of OMWW applied and for the two years of study. Indeed, for the first year of study, we recorded for the soil top layer a highly significant decrease by 18 % for T1 (20 m^3/ha), 39 % for T2 (40 m^3/ha) and 44 % for T3 (60 m^3/ha). This decrease is respectively by 42 %, 44 % and 47 % for the second year. Reduction is correlated with OMWW applied dose.

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Table 1. *Effect of different doses of OMWW on soil organic composition during two successive years of studies. *For each year different letters indicate a significant difference among the means at LSD test (P > 0:001).*

Treatment	Year	After OMWW spreading				After Harvest			
		Layer 0-20 cm		Layer 20-40 cm		Layer 0-20 cm		Layer 20-40 cm	
		Total organic carbon (TOC) Average*							
T0 (0 m^3/ha)	1	0.230 ±0.31	A	0.180 ±0.63	A	0.146 ±0.05	A	0.116 ±0.05	A
T1 (20 m^3/ha)	1	0.459 ±0.23	B	0.264 ±0.51	B	0.328 ±0.14	B	0.268 ±0.14	B
T2 (40 m^3/ha)	1	0.633 ±0.85	C	0.419 ±0.23	C	0.513 ±0.18	C	0.313 ±0.18	C
T3 (60 m^3/ha)	1	1.018 ±0.18	D	0.562 ±0.86	D	0.568 ±0.07	C	0.358 ±0.07	C
T0 (0 m^3/ha)	2	0.232 ±0.22	A	0.132 ±0.22	A	0.146 ±0.05	A	0.086 ±0.08	A
T1 (20 m^3/ha)	2	0.584 ±0.33	B	0.344 ±0.33	B	0.328 ±0.14	B	0.237 ±0.85	B
T2 (40 m^3/ha)	2	0.962 ±0.52	C	0.462 ±0.52	C	0.513 ±0.18	C	0.369 ±0.64	C
T3 (60 m^3/ha)	2	1.141 ±0.77	D	0.541 ±0.77	D	0.568 ±0.07	C	0.392 ±0.95	C
		Total organic Nitrogen (TON) Average*							
T0 (0 m^3/ha)	1	0.012 ±0.015	A	0.008 ±0.031	A	0.072 ±0.028	A	0.032 ±0.059	A
T1 (20 m^3/ha)	1	0.019 ±0.019	B	0.042 ±0.026	B	0.088 ±0.036	B	0.058 ±0.085	B
T2 (40 m^3/ha)	1	0.067 ±0.012	C	0.067 ±0.032	C	0.103 ±0.052	C	0.093 ±0.042	C
T3 (60 m^3/ha)	1	0.095 ±0.023	D	0.088 ±0.056	D	0.147 ±0.035	D	0.107 ±0.077	D
T0 (0 m^3/ha)	2	0.076 ±0.023	A	0.035 ±0.082	A	0,063 ±0.026	A	0,023 ±0.033	A
T1 (20 m^3/ha)	2	0.119 ±0.049	B	0.076 ±0.053	B	0.077 ±0.032	B	0.045 ±0.084	B
T2 (40 m^3/ha)	2	0.168 ±0.054	C	0.109 ±0.047	C	0.105 ±0.037	C	0.088 ±0.069	C
T3 (60 m^3/ha)	2	0.199 ±0.088	D	0.116 ±0.093	D	0.117 ±0.062	D	0.093 ±0.023	D
		Phenolic compounds Average *							
T0 (0 m^3/ha)	1	1.237 ±0.41	A	0.832 ±0.41	A	0.569 ±0.67	A	0.269 ±0.51	A
T1 (20 m^3/ha)	1	2.263 ±0.88	B	1.423 ±0.88	B	1.657 ±0.39	B	1.022 ±0.47	B
T2 (40 m^3/ha)	1	3.366 ±0.59	C	1.695 ±0.59	C	2.881 ±0.62	C	1.697 ±0.88	C
T3 (60 m^3/ha)	1	4.352 ±0.95	D	3.839 ±0.95	D	3.863 ±0.45	D	2.408 ±0.39	D
T0 (0 m^3/ha)	2	1.369 ±0.16	A	1.422 ±0.88	A	1.619 ±0.69	A	1.125 ±0.52	A
T1 (20 m^3/ha)	2	3.778 ±0.26	B	2.652 ±0.52	B	2.274 ±0.83	B	1.882 ±0.59	B
T2 (40 m^3/ha)	2	4.169 ±0.77	C	3.853 ±0.67	C	3.460 ±0.77	C	2.587 ±0.57	C
T3 (60 m^3/ha)	2	6.637 ±0.69	D	4.967 ±0.23	D	4.910 ±0.97	D	3.740 ±0.88	D

This could be explained by the fact that OMWW rich in organic matter have greatly stimulated the soil microbial activity, which has led consequently to a rapid degradation of the organic compounds supplied by these effluents. On the other hand, this decrease in soil organic carbon content after harvest tends to increase over 2-years of experiment, which may be a sign of soil microflora proliferation in response to their adaptation to the Chemical OMWW properties. This hypothesis is consistent with the results obtained by Taamallah (2007) who was demonstrated that the OMWW incorporation in soil stimulates its microbial activity. At the end of the second year, we were able to deduce that each new contribution of OMWW was followed by a restoration of the stock of soil total organic carbon of the previous year, with a margin of gain. The proportion missing after mineralization process and other factors is restored by the next OMWW application.

From one year to another, there was a tendency towards the disappearance of differences in effect between doses. Only the highly significant improvement over the control remains unchanged. At the end of experiment, we can deduce that a relative stability of the total organic carbon content of soil has been maintened at an average 0.4 % to 0.5 %. In addition to OMWW, a small proportion of the increase in soil organic carbon content can be attributed to the previous crop. Indeed, Kaur et al. (2000) have shown that root exudation contributes substantially to the increase of the organic carbon content.

These results are in agreement with those of Cabrera et al (1996), which showed that the annual contribution to a sandy soil initially containing 0.45 % organic matter of 37 or 61 l/m^2 of OMWW for three years Resulting in a respective increase in organic matter of 1.62 % and 1.98 % respectivly. Abichou (2011) showed that after the first application, the level of soil organic matter initially very low is markedly improved for the different doses of OMWW.

Despite the relatively low nitrogen content of OMWW (1.6 g/l), a significant improvement of this element compared to the control was observed for the different doses of OMWW. This improvement is in correlation with the quantity supplied (Table 1).

The organic nitrogen levels recorded during the first year are relatively low immediately after OMWW application. However, they improved markedly after the first harvest. This increase is attributed, on the one hand, to the contribution of pear millet root fraction and irrigation water, on the other hand, to the low rate of mineralization process.

From the second year, OMWW spreading resulted in a marked increase in the organic nitrogen content in the soil. Indeed, this rate was multiplied by ten for the three doses of OMWW applied in comparison with those recorded after the first spreading operation. This considerable increase can be attributed to the effect of cumulative quantities of OMWW after two successive years and especially to the passage of the activity of microorganisms already adapted to the environment at the cruising speed. The same trend towards the increase is observed during the third year. However, we have seen a marked decrease in total organic nitrogen at the end of the crop cycle compared to that at the beginning of the experiment.

The incorporation of OMWW has made it possible to establish a balance between the supply of organic nitrogen and the export of mineral nitrogen and the conservation of a relatively acceptable residue in this element. After each OMWW application, there was a significant increase in the soil content of polyphenols compared to the controls. This increase was amplified from one year to the next following the cumulative effect of the three successive OMWW spreading..

The regression of polyphenol levels in comparison with those recorded immediately after OMWW spreading was confirmed at the end of each crop year.

This decrease is observable at the level of the different doses allocated. However, the increase in soil content of this element as a function of the incorporated dose is always retained and the difference between treatments applied is highly significant between them and with respect to the control without OMWW. This decrease is attributed to the biodegradation process of phenolic compounds accelerated after the second application following the adaptation process initiated by the first OMWW application. The progressive neutralization of the pH due to the buffering effect of the calcareous soil is a factor favoring the activity of soil microorganisms. Indeed, Medeci et al. (1985) 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

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carbonaceous nutrients (Borja et al., 1995). On the other hand, according to the sandy composition and the porous property of the studied soil, we observed an increase both of TOC and total N in 2 layers of soil with the increase of the dose of OMWW used in the irrigation. This suggests an important leaching of OMWW from the surface to the soil deeper layers and therefore of phenolic compounds.

Effect of OMWW application on soil chemical properties

Figure 2 shows the evolution (mean of three replicates) of the pH and electrical conductivity for two soil profiles (0-20 cm) and (0-40 cm) after OMWW spreading during 2 years. Results presented here show that several chemical propertiesof the investigated soil changed in response to OMWW application.

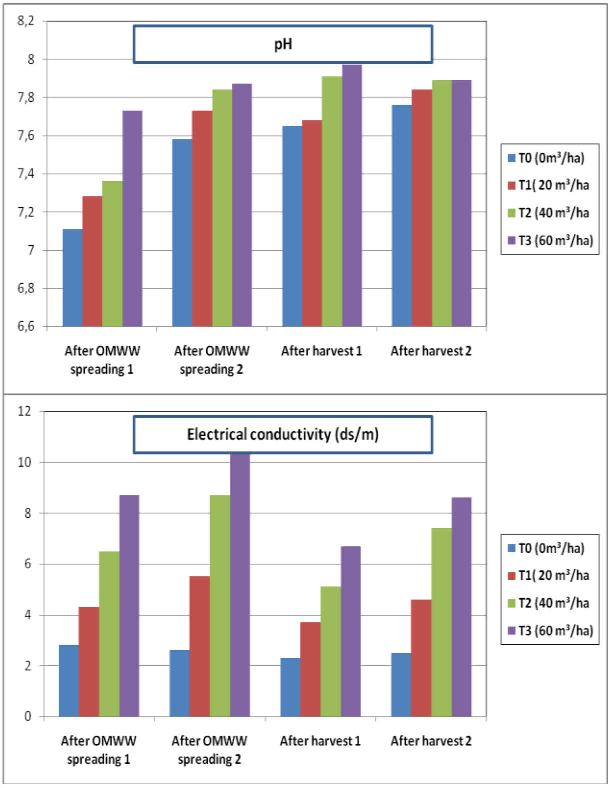

Figure 2
Effect of different doses of OMWW on soil pH and Electrical Conductivity value during two successive years of studies.

Taking into consideration pH deviation, in first time sampling day, this parameter indicated no significant difference with the application of increasing OMWW doses. Indeed, the pH variation (7–7.8) for treated soil showed no difference compared to the soil taken as reference (Figure 2). The soil pH remains unchangeable despite the pH acidic of OMWW (4.8) mainly due to the presence of organic acids (Mekki et al., 2009). Indeed, in spite of the initial OMWW acidity, the time evolution of this parameter show a light increment in pH values (first year of experiment) that were maintained to approximately the 2th year of observation but without being statistically significant. It is probably due to the substantial ability of the $CaCO_3$ soil compound to neutralize such OMWW as given away by (Sierra et al., 2001; Dakhli et al., 2013; Zenjari et al., 2009). These results are also consistent with those of Levi-Minzi et al. (1992), which demonstrated that, despite their high acidity, OMWW do not modify the pH 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 OMWW phenolic compounds (Dakhli., 2015). Besides, the variability in pH values can be attributed to the transformation of the substrate form reduced to oxidized material during anaerobic mineralization and to the intense liberation of CO_2 predominantly during aerobic mineralization. It can be said also that this parameter does not vary as a function of depth. Similarly, OMWW application increased soil EC and this increase was proportional to the added OMWW quantity (Figure 2). After first OMWW spreading, the EC value for the untreated soil registered was 2.21 dS m^{-1}. For the treated soil, these values were 4, 6 and 8 ds·m^{-1} for each doses applied respectively for 20, 40 and 60 m^3/ha. (Sierra et al, 2001) showed the raise of soil EC with increasing OMWW rates and the highest OMWW dose applied almost duplicate the control salinity. The increase in soil EC was related to the high salts concentration in the OMWW which presented an EC value about 10 dS m^{-1}. The three applications of OMWW resulted in a significant increase in soil salinity compared to the control. This increase recorded from the first application. It increased from one year to the next, particularly in the presence of the higher doses 40 m^3/ha and 60 m^3/ha thereafter to cumulative effects on the successive annual application of OMWW. The increase of the soil salinity involved the alteration of the CEC (Cation Exchange Capacity) and could affect the soil fertility. Regarding the deviation of the soil salinity determination, Chlorides and Sodium concentrations increases also with OMWW rates application (Figure 3). All this parameters increased as a function of depths compared to the control soil which suggest the leaching of OMWW from the surface to the soil deeper layers. The increase of the soil salinity involved the alteration of the CEC (cation exchange capacity) and could affect the soil fertility. So as a consequence, this application might affect negatively the most sensitive crops. 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 the growth and productivity of the soil. Indeed, the sodium ion replaces the calcium and magnesium ions adsorbed on the clay particles, resulting in a hard, compact soil with an unstable structure that becomes excessively impermeable to water. The quantities of salts brought by this waste are greater and concentrated in the superficial layers of the soil.

Indeed, Berndt et al. (1996) have demonstrated that the viscous and greasy character of OMWW leads to the formation of an oily deposit on the soil and provokes its waterproofing thus generating an accumulation of the salts.

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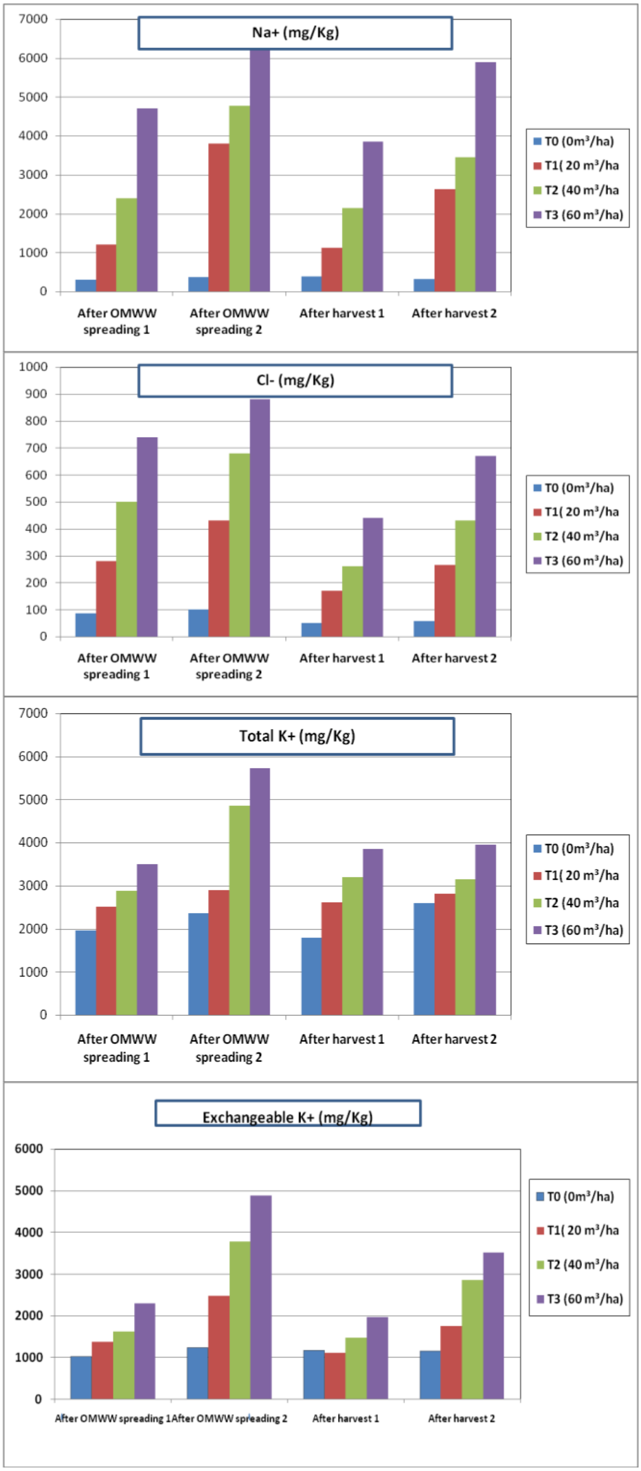

Figure 3
Effect of different doses of OMWW on soil Sodium, Chlorides and Total and Exchangeable Potassium concentrations during two successive years of studies.

The raise in the soil salinity could result from the main ionic species (Na, Cl and SO_2), which came from OMWW. Dakhli et al (2013) reported that the OMWW acidity was due to the presence of phenolic and fatty acids, subsequently the application of this effluent to soils could accumulate salts and phytotoxic compounds, change pH and leach nutrients that could contaminate the ground water source (Zenjari et al, 2001). However, this soluble salts can be effectively mobilized, transported and eliminates from the soil both by infiltrating rain water accumulated during the experimental period (150 mm) and the quantities of water deliberated by irrigation, as can be seen by the decrease of the soil salinity through with depth the experimental period after OMWW spreading for all the parameters following as: EC, Chlorides, Sodium and total K concentrations.

For the two years of trial and after each application, OMWW produced a significant increase in the soil total potassium content compared to the controls. This increase is only significantly observed for 40 and 60 m^3 /ha doses.

During the cropping cycle, and over two years, of experiment there was a gradual overall decrease Irrespective of dose in total soil potassium concentration.

The cumulative effect of successive OMWW applications in the soil total potassium content begins to be observed from the first crop year to increase markedly during the second.

The contribution of OMWW to soil total potassium content is certainly positive. However, the accumulation of excessive amounts recorded represents a potential source of toxicity that can threaten the activity of soil microorganisms and the mineral nutrition of pear millet.

The level of potassium exchangeable in the soil increased in a highly significant way as the dose of OMWW is increased. However, a drop in the exchangeable potassium concentration was observed at the end of each crop cycle. In addition to the consumption of the crop, leaching may also be responsible for the decrease in post-harvest soil potassium content, especially since in our case the limited cation exchange capacity of the soil does not Permits long-term storage of high quantities of exchangeable potassium. However, the stock in this element is restored after the renewal of each spraying operation. (Taamallah., 2007), showed that a drop in the concentration of exchangeable potassium was recorded following cessation of spreading operations. The stock in this element was restored almost completely after a single application. Besides, Chartzoulakis et al. (2010) has shown that soil application of OMWW increased available soil K.

Furthemore, Rusan et al (2016) demonstarded that K drastically increased with all OMWW treatments compared with the control, where the highest value for soil K was 2926 mg Kg^{-1} for the untreated OMWW. The increase in soil K contents with OMWW application can be attributed to their high content in the OMWW used in the irrigation (2441.8 mg l^{-1}). As an indication, previous studies carried out by (Morisot, 1979) have shown that the application of 100 m^3/ha of OMWW corresponds to a very high potassic fertilization of 350 to 1100 Kg in the form of K2O. From the results obtained, we can conclude that OMWW constitute a

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potential source of potassium showing a high fertilizing potential that can be valued for the rehabilitation of poor soil in this element, taking into account that higher concentrations In this element could cause an imbalance at the cation exchange complex and consequently a plant nutritional disorders.

Effect of different doses of OMWW on pera millet yield components.

Panicle number/m². For the two years of study, there was a significant reduction of the number of panicle/m² compared to the controls for 40 m³/ha and 60 m³/ha doses. This reduction, although observed in the first year, is becoming more and more over the two years of trial. This amplification of damage can be attributed to the cumulative effect of successive OMWW applications.In the second year of experiment, this reduction was by 12% for T1 (20 m³/ha) and by 20% and 43% respectively for T2 (40 m³/ha) and T3 (60 m³/ha). However, for the first year, a significant increase of the number of panicle/m² with regard to the control was recorded for the less dose 20 m³/ha (Table 2).

Straw yield. For the first year of study, The application of T1 dose (20 m³/ha) of OMWW induced a significant increase in straw yield with respect to the control with an improvement of 25%. The T2 dose (40 m³/ha) did not induce a difference with regard to the control. However, the T3 dose (60 m³/ha) of OMWW induced a significant reduction of straw yield compared to the control. This reduction is of 12%. This result is consistent with an earlier study conducted by (Dakhli et al., 2009). Which showed that application of OMWW at a dose of 50m³/ha resulted in a Significant reduction of barley straw yield.

Pear millet straw yield over the 2-years period decreased from the first to the second years in request to the control whatever the dose applied. This reduction is correlated with the assigned dose. At the end of the second year, this reduction is by 38% and 40% respectively for T2 (40 m³/ha) and T3 (60 m³/ha) doses.

Treatment	First year		Second year	
	Panicle number/m²		**Panicle number/m²**	
T0 (0 m³/ha)	28.0 ± 0.13	A	33.0 ± 0.51	A
T1 (20 m³/ha)	**32.6 ± 0.88**	**B**	27.9 ± 0.63	B
T2 (40 m³/ha)	25.0 ± 0.47	C	20.0 ± 0.93	C
T3 (60 m³/ha)	22.2 ± 0.82	D	12.6 ± 0.23	D
	Straw yield (T/ha)		**Straw yield (T/ha)**	
T0 (0 m³/ha)	3.375 ± 0.53	A	3.843 ± 0.36	A
T1 (20 m³/ha)	**4.533 ± 0.69**	**B**	2.874 ± 0.59	B
T2 (40 m³/ha)	3.279 ± 0.57	A	2.028 ± 0.64	C
T3 (60 m³/ha)	2.841 ± 0.91	D	1.696 ± 0.78	D
	Grain yield (T/ha)		**Grain yield (T/ha)**	
T0 (0 m³/ha)	3.038 ± 0.87	A	3.258 ± 0.94	A
T1 (20 m³/ha)	**3.364 ± 0.98**	**B**	2.694 ± 0.85	B
T2 (40 m³/ha)	2.879 ± 0.53	C	1.856 ± 0.77	C
T3 (60 m³/ha)	2.133 ± 0.59	D	1.239 ± 0.59	D

Table 2
Effect of different doses of OMWW on on pera millet yield components during two successive years of studies.

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

Grain yield. For the first year, a slight increase in grain yield by 9% compared to the control was recorded for the T1 dose (20 m³/ha). However, a highly significant decline was recorded for T2 (40 m³/ha) and T3 (60 m³/ha) and is by 14% and 37% respectively. Concerning the second year of trial, this reduction is much more important. Indeed, it is by 17% and 43% relative to the control respectively for the doses T1 and T2 and by 62% for the higher dose T3.

In conclusion, all pear millet yield components were negatively affected by OMWW, in particular the yields of the plots receiving the highest doses of 40 m^3/ha and 60 m^3/ha. The straw yield and of course the grain yield are very affected with relatively variable degrees depending on the dose applied but also the cumulative effect of the successive OMWW spreadings during two years of study.

In this context Dakhli et al (2009,2013) have shown that at a dose more than 50 m^3/ha of OMWW a significant reduction in dry matter and grain yield will be recorded. The yield is necessarily dependent on the ecotype of pear millet used, but mainly on the availability of adequate mineral nutrition. These negative results can only be attributed to an exogenous nutritional stress caused by the OMWW imput.

Indeed, the richness of these effluents in salts, polyphenols and other more or less toxic compounds is at the origin of the physiological disturbances which have translated negatively at the level of the various phenological stages of pear millet such as germination, Emergence, tillering, run-up and heading, thus yielding the catastrophic results obtained in terms of grain yield.

The excesses of the salts brought by OMWW are therefore among the major causes of the reduction of the growth and the productivity of the pear millet. The reduction in crop aerial biomass (straw yield) in proportion to the applied dose increase of OMWW observed over the two years of study, particularly at doses of 40 m^3/ha and 60 m^3/ha, constitutes an index Relevance of guilt of the salts relative to the contributions of the different doses of OMWW.

It is the excess concentrations of salts especially Na^+ and Cl^- brought by OMWW that are the cause of a temporary change in the water-plant relationship resulting in the increase in the concentration of apoplasmic ions generating a reduction in osmotic potential and water. This can be at the origin of the ionic perturbations greatly limiting the supply of the plants with major mineral elements necessary for their growth. Essentially the negative effect of this effluent on the permeability of the soil and consequently on the leaching process, which leads to an increase in the concentration of salts at the level of superficial layers of soil. As a result, this excessive salinity causes a reduction in leaf area, a physiological imbalance and a slowdown in growth resulting in a reduction in plant biomass and grain production of pear millet. (Radhouane, L., 2008) also showed that six millet ecotypes presented the same similarities to salinity. Indeed, they retain the same stature on the productive Performance component was penalized under the influence of salinity. Research by (Mansour, 1990) studies of barley and (Bounaquba, 1996) in wheat, triticale and barley demonstrated an increasing reduction in leaves due to an increase in salt concentration . This reduction in foliar area, according to the same authors, is attributed to the slowing down of cell divisions or to a decrease in cellular expansion. These results are in agreement with those obtained by Calu. (2006), which demonstrated that extreme salt stress leads to dwarfism and inhibition of root growth. The leaves become sclerotized even before they have

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finished growing and the entire organism runs the risk of dying quickly enough. Indeed, other research has shown that in the presence of high concentrations of NaCl, the reduction in barley growth is accompanied by an increase in the accumulation of $Na^{+}$and Cl^{-} in the tissues and a Decrease in K^{+} and Ca^{2+}. On the other hand, in the isolated walls of barley roots, Na^{+} and Ca^{2+} compete for the same adsorption sites, while K^{+} is fixed at other sites (Stassart, 1981). This leads to an alteration in the absorption K^{+}/Na^{+} selectivity and loss of tissue K^{+} (The Hague, 1969) (Kent., 1985) and (Hajji., 1985). K^{+} and Ca^{2+} become limiting factors for growth when the medium is enriched in Nacl. (Greenway, 1962a, b) and (Munns., 1982). These results coincide with those obtained by (Soltani, 1990) which showed that Nacl exerts its depressive effect on growth by limiting the supply of the plant to Ca^{2+}. Moreover, as has been mentioned by other authors, the reduction in plant growth and productivity may also result from direct salt inhibitory effects on the biochemical reactions of photosynthesis (Ziska, 1990) and (Jeschke, 1992) or stomatic restrictions on CO_2 induction by the induction of stomatal closure (Kingsbury, 1984) (Kyparassis, 1995) and (Wang., 1997).

In general, as with all plants, the response to stresses results in a precipitation of the phenological stages and a shortening of the vegetative cycle in order to arrive as quickly as possible in the reproduction of the seeds and to ensure the perenniality of the species. This rather genetic behavior does this despite the productivity and quality of the seeds.The use of OMWW as a fertilizer with a dose up to 20 m^3/ha has therefore not yielded encouraging results with respect to straw and grain yields of barley. On the contrary, it has been a factor of stress and metabolic disturbance.

Conclusion

Containing many nutritive elements leading to an improvement of agricultural production, OMWW can be considered as a fertilizer for pear millet cultivation. Indeed, used with amounts less than 20 m^3/ha, OMWW do not present any risks for the soil salinity, the phenolic substances concentration, the potassium content and acidity. In addition, they generate an improvement of some physical and chemical soil properties and an increase in the pear millet yields whereas a spreading with strong amounts of this effluent causes an ionic imbalance and an increase in the soil salinity. It was concluded that OMWW applied with high doses (40 m^3/ha and 60 m^3/ha) reduce the production whereas the low doses (less than 20 m^3/ha) improve the soil characteristics. It provides an economically acceptable production alternative for pear millet cultivation and can reduce the problems caused by this sludge. Although some elements of answers to the agronomic valorization of OMWW have been made, several aspects deserve further study: a comparative study of several ecotypes of millet in order to be able to affirm their tolerance to OMWW, a study of the physiological mechanisms, Biochemical (oxidative stress) and metabolic factors responsible for the stimulation of germination of pear millet.

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ETUDE DE L'EFFET DE MARGINES SUR LES PROPRIETIES CHIMIQUES DU SOL ET SUR LE COMPORTEMENT D'UN ÉCOTYPE DE MIL (*PENNISETUM GLAUCUM* (L) R.BR) AUTOCHTONE DE SUD TUNISIEN

Resumé

Les Margines, eaux résiduaire produites lors de la trituration des olives ont un pouvoir polluant très élevé se traduisant par une valeur assez élevée de DCO (demande chimique en oxygène), une salinité élevée et une forte charge en composés phénoliques responsable de la pollution de l'environnement. La valorisation de ces déchets en agriculture sans traitement préalable est très limitée vu leur toxicité pour les sols et les plantes. La recherche de nouvelles technologies ou des procédés de valorisation de cet effluent s'avère nécessaire. L'épandage des margines dans les sols sableux en conditions arides du sud tunisien constitue une alternative potentielle à cette fin. En effet, au cours de notre étude, des apports de 20, 40 et 60 m³ à l'hectare ont été testé en présence d'un écotype de mil (*Pennisetum Glaucum* (L) R.Br) autochtone de sud Tunisien en irriguée afin d'évaluer l'impact de l'incorporation de ces effluents sur les caractéristiques chimiques du sol et sur le comportement de la plante. Les résultats de l'étude, ont montré qu'à une dose de 20 m³/ha, les margines ne présentent pas de risques considérables en ce qui concerne la salinité, les fortes concentrations en substances phénoliques, la forte teneur en potassium et le pH. Au contraire, elles engendrent une amélioration de quelques propriétés physiques et chimiques du sol à savoir la teneur en matière organique et en potassium sans toutefois améliorer la productivité de mil. L'épandage des doses de margines supérieures à 20 m³/ha génère une diminution très significative du rendement de de mil avec des perturbations des stades phénologiques à la suite de l'accumulation des substances phénoliques et de l'augmentation excessive des teneurs en sodium et chlorures et par conséquent une hausse des degrés de salinité du sol à court et à long terme.

Mots clés: *Margines, Sol, mil, Rendement*.