Dilution of olive mill wastewater eliminates its phytotoxicity and enhances plant growth and soil fertility

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Olive Mill Wastewater (OMW) is phytotoxic and all attempts to treat it are expensive and therefore alternative less expensive techniques should be developed to reduce its phytotoxicity. The objective of this study is to determine whether the dilution of OMW with water improves its suitability for soil application and enhances plant growth without polluting the environment. The following treatments were investigated in a randomized complete block design with four replications in a greenhouse pot experiment: 1) Potable water (W); 2) Undiluted OMW (100%OMW); 3) Diluted OMW at a ratio of 1_{water}:3_{omw} (75%OMW); 4) Diluted OMW at a ratio of 1_{water}:1_{omw} (50%OMW); and 5) Diluted OMW at a ratio of 3_{water}:1_{omw} (25%OMW). Pots filled with 5 kg air dry soil and seeded with hybrid maize were watered periodically according to the treatments applied. At the end of the growing period, the above ground biomass was harvested and plant and soil samples were taken for chemical analysis. The results indicated that undiluted OMW reduced plant growth and increased soil salinity. Dilution of OMW reduced its phytotoxic effect, increased soil organic matter, soil N, P, and K. However, even diluted OMW increased soil salinity thus should be taken into consideration with continuous OMW application. It was concluded that the dilution of OMW (25%OMW) eliminated the OMW phytotoxicity and enhanced plant growth. Such approach can be attractive to both farmers and owners of olive mill as this approach is a practical alternative to the expensive non-affordable by the owners of mills treatments techniques

Keywords: Olive mill wastewater; dilution; maize; soil properties

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Introduction:

The Mediterranean region is the largest olive oil producer in the Word accounting for about 97% of the world oil production [1]. Improving the olive oil processing in this region is of enormous importance for the whole region as well as for each individual country [2]. The process of oil extraction generates annually about 30 million cubic meter of olive mill wastewater (OMW) [3]. The disposal of untreated OMW imposes environmental and health hazards and is considered one of the most serious environmental problems facing most of the Mediterranean countries [4,5]. The major concerns associated with OMW disposal are the high level of the chemical oxygen demand (COD) and the high content of microbial growth-inhibiting compounds, such as phenolic compounds and tannins [6]. OMW has phytotoxic and inhibiting effect on plant growth and an antibacterial effect [5] and therefore, cannot be disposed neither directly to the environment nor to the sewage systems [7]. Consequently, OMW must be treated before reuse or before disposal to the environment. All physical, chemical and biological technologies tested and evaluated for OMW treatment have been proved to be technically effective but unfortunately economically not feasible [8]. In the absence of cost effective treatment technologies, many countries either discharge it directly into sewer systems and water streams or conduct a preliminary inefficient treatment through storing in the evaporation ponds where it degrades and releases greenhouse gas emissions [9]. Controlled soil application can constitute an inexpensive and reasonable option for OMW recycling by the farmers and the owners of the olive mills, in particular in the Mediterranean region where water resources are limited [8].

On the other hand, if OMW was properly treated and managed, it can be beneficial as a source of organic matter and nutrients essential to the plants and to the fertility of the soil [10]. Therefore, OMW can be a valuable source for water, organic matter and nutrients to the soil-plant system especially in the arid and semiarid region suffering from scarcity of water and low soil organic matter content [11-13]. Several researchers have reported that OMW is rich in nutrients essential for plant growth and contains high amount of beneficial organic compounds [10, 14-15]. These OMW characteristics can enhance the fertility and productivity of the soil of the Mediterranean region that are poor in soil organic matter and in fertility level [16]. The high content of organic carbon in the OMW can be used to restore the deficit in soil carbon and combat soil degradation which consequently enhances the sustainability of the Mediterranean agroecosystems [17-19].

Several researchers have shown that soil application of OMW increased crop productivity and enhanced soil fertility [8, 20-21]. Plant nutrients such as nitrogen, phosphorus and potassium increase with soil application of OMW [22]. On the other hand, OMW application tends to increase soil salinity [23], which can impose a negative impact on soil physical condition such as infiltration into soil, aggregate formation and water holding capacity [24]. In contrary, other researchers found that soil application of OMW increased soil water-holding capacity, total soil porosity and aggregate stability, while lowered bulk density which was attributed to the effect of the soil compounds provided to the soil with OMW application [19, 25].

In Jordan, the olive sector is one of the most important areas of agricultural production. The average annual production of olive fruits in Jordan is about 240,000 tons [26]. The process of oil extraction in Jordan annually produces 25,000 tons of olive oil and generates 200,000 M^3 of OMW [27]. OMW is prohibited to dispose into the environment to avoid contamination of the soil and water resources. Moreover, Jordanian regulations prohibit discharging OMW into municipal wastewater treatment plants, because OMW contains compound that are toxic to microorganisms [28]. So OMW in Jordan is not treated but instead it is disposed in dumping sites and sometimes disposed by the Owners of mills illegally into agricultural lands. Uncontrolled spreading of OMW into agricultural soil not only pollutes the environment and negatively affects the soil fertility and productivity but also is phytotoxic to the crops and prohibits seed germination [29].

Recently in Jordan, to avoid costly treatment of OMW, regulations have been issued to allow conditional and controlled direct land application of OMW. Other countries of the Mediterranean regions have issued somewhat similar conditional OMW land application. However, up to now there is no solid and scientifically-based protocol for proper management of OMW through direct land application. The objective of this study is to evaluate the impact of land application of OMW used as a raw and after dilution with potable water on plant growth and soil properties.

Materials and Methods

Soil:

A calcareous soil with a low organic matter content classified as fine-loamy, mixed, thermic, calcic Paleargid [30], was collected from the Research Center of Jordan University of Science and Technology and used in this study. The soil was air-dried and sieved through a 5mm screen. The soil was analyzed for general characteristics; texture was determined by hydrometer method [31]; soil pH was measured on 1:1 soil: water suspension [32]; soluble salts (EC) (electrical conductivity) were determined by measuring the electrical conductivity of 1:1 soil: water extracts [33]; organic matter content was determined by the Walkley-Black method [34]; cation exchange capacity (CEC) by [35]; total Kjeldahl nitrogen by [36] , available phosphorus by extraction with sodium bicarbonate [37]; exchangeable potassium by extraction with 1 M NH_4OAc [38]; $CaCO_3$ by acid neutralization method [39]; and heavy metals (Fe, Zn, Mn, Cu, Cd, Pb) by DTPA extractable microelements [40]; bulk density by [41]; The major characteristics of the soil are presented in Table 1.

Olive Mill Wastewater (OMW):

Olive mill wastewater (OMW) used in this experiment was collected from three phase olive oil Mill in Jordan. The OMW was not treated but was collected from the settled reservoirs. The OMW and potable water used as a source of irrigation water were analyzed for physical and chemical characteristics according to the standard methods described by the American Public Health Association (APHA) [42]. The major characteristics of OMW and potable water are illustrated in Table 2.

The settled OMW with and without dilution with potable water were applied to the soil as a source of irrigation water. The following treatments were investigated in a randomized complete block design with four replications in a greenhouse pot experiment: 1) potable water (W); 2) undiluted OMW (100%OMW); 3) diluted OMW at a ratio of $1_{water}:3_{omw}$ (75%OMW); 4) diluted OMW at a ratio of $1_{water}:1_{omw}$ (50%OMW); and 5) diluted OMW at a ratio of $3_{water}:1_{omw}$ (25%OMW).

Greenhouse Pot Experiment:
Each pot was filled with 5 kg air dry soil. Three maize seeds per pot were seeded. Pots were watered periodically with undiluted and diluted OMW according to the treatments to maintain approximate field capacity water content. After germination two similar plants were kept per pot. At the end of the growing period, the whole plants were harvested from each pot. The fresh weights were recorded. Then the plant samples were oven-dried at 70 ^{0}C for 48 hours, and then the oven dry weights were recorded. Plant parts were ground to a fine powder using a laboratory mill with 0.5 mm sieve. The milled plant samples were analyzed for the total nitrogen using a modified micro-Kjeldahl digestion procedure [43]. Total P, K, Fe, Mn, Zn, Cu, Pb and Cd were determined in the dry ash digestion. P was determined using Vanadate-Molybdate-Yellow method, K and Na by flame photometery and Fe, Mn, Zn, Cu, Pb, and Cd by atomic absorption spectroscopy [44]. At the end of the experiment, infiltration rate test was conducted for all treatments using cylinder infiltrometer [45]. Undisturbed soil (core) was taken to measure the soil Bulk density [41]. Representative soil sample was also taken from each pot after thoroughly mixing the soil. Soil samples were sieved through 2 mm sieve and analyzed for the same parameters mentioned above.

At the end of the experiment, analysis of variance (ANOVA) was used to determine the treatment effects. When F ratio was significant a multiple means comparison was performed using Fisher's Least Significance Test (0.05 probability level). Statistical analyses were performed with Systat statistical program [46].

Results and Discussion

Preliminary soil characteristics:
The analysis of the soil used in this study indicates that the soil is basic, alkaline and non saline, poor in organic matter, N, P and basic micronutrients. Available K is considered to be adequate for normal plant growth. The soil textured is silt clay loam with relatively high CEC. The major soil characteristics are summarized in Table 1.

OMW characteristics
The undiluted OMW is acidic and strongly saline. The total suspended solids (TSS) was relatively high (1236 mg L^{-1}), heavily loaded with organic material with chemical oxygen demand (COD) of 118 g L^{-1}. The levels of major plant nutrients (N, P, and K) were also relatively high which can be valuable sources for plant growth and soil fertility. The total bacterial count in undiluted OMW (100%OMW) was 2.13 X 10^{6} CFU/ml. OMW contains high phenolic content which is considered toxic to organisms [20]. The major characteristic of the undiluted OMW are presented in Table 2.

Infiltration rate into the soil:

Infiltration rate of diluted and undiluted OMW into the soil just after plant harvest are illustrated in Fig. 1. The undiluted OMW prevail the lowest infiltration rate followed by the 75% OMW (lowest dilution) during the first five minutes compared to other treatments that resulted in similar effect on infiltration rate. The highest infiltration rate was obtained by the 25%OMW treatment. The infiltration rate for all treatments decreased with time during the elapsed first 120 min of infiltration run. There were no significant differences among all treatments during the period from 30 minutes till 120 minutes. The observed decrease in the infiltration rate could be attributed to the abundant suspended matter in the OMW that might clog the soil pores [48-49]. Further, OMW contains oil that may increase soil hydrophobicity and expect to engage the macropores and cover soil aggregates which minimizing water film around these aggregates and decrease water retention and infiltration rate and thus reducing the movement of water through soil pores. [50]. The oily film around those aggregates increases the contact angle between soil solution and soil solid which eventually reduce infiltration rate [51]. In the long run and due to the positive effect of OMW on soil organic content, it is expected to improve the soil structure and eventually the soil porosity and infiltration rate [19].

Plant growth

Plant growth parameters as affected by the undiluted and diluted OMW are shown in Fig. 2. The plant dry weight was the highest with the soil application of the highest dilution of OMW with potable water (OMW at the water to OMW ratio of 3:1 coded as 25%OMW), followed by the control treatment where potable water alone was used (W). The undiluted OMW resulted in the lowest plant dry weight indicating the phytotoxic effect on the plant growth. The relative plant dry weight obtained by the 25%OMW was 23% more than that obtained by the control (W) and three times more than that obtained by the application of undiluted OMW (100%OMW) (Fig. 2) . There was a linear relationship between %OMW and plant dry weight. With each dilution unit (25%) investigated in this study the dry weight decreased by about 18% (Fig. 3).

These results indicate two findings. The first finding is that inhibiting and phytotoxic effect of OMW can be reduced with dilution of OMW with potable water and at the same time improves the plant growth. The best results obtained with the highest dilution, represented by the 25%OMW treatment, which approximately tripled the plant dry weight. The second finding is that the highest dilution (25%OMW) gave even higher plant dry weight compared to the control treatment (W). This indicates that by diluting the OMW at a ratio of water: OMW of 3:1 (25%OMW), one can eliminate the phytotoxicity of undiluted OMW and enhance plant growth. Such plant growth enhancement could be attributed to the beneficial organic substances and essential nutrients provided to the soil with OMW application. The decrease in OMW phytotoxicity following OMW dilution could be attributed to the reduction of the levels of the phenols and other phytotoxic compounds [52]. Several researchers have reported the positive response of plant growth to soil OMW application [28,53].

Plant nutrients contents

The plant contents of N, P and K were the highest for the 25%OMW treatment followed by the W (Table 3). The increase in the plant N, P and K with the 25%OMW compared with the control treatment (W) indicates the soil is deficient in these nutrients and that the OMW provided the soil with these nutrients or enhanced the original unavailable soil nutrients resulting in a an increase in their uptake by the plant.

The lowest plant contents of N, P and K was obtained by the application of the undiluted OMW and tended to increase with dilution of OMW. The higher the dilution was the higher the contents of plant nutrients. The decreasing trend in plant uptake of nutrients with dilution of OMW followed the trend of the effect of the same treatments on the plant dry weight. Obviously the lower the dilution of the OMW is the lower is the plant dry weight. Although the OMW contains considerable amounts of N, P and K which simultaneously would be added to the soil upon OMW application [54-55], their uptake by the plant irrigated with undiluted and diluted OMW remained low due to the low plant dry weight. The enhancement of plant uptake of N, P and K with OMW application have been reported by several researchers [10,14,22]. The uptake of micronutrients (Fe, Mn, Zn and Cu) and heavy metals (Cd and Pb) was not affected significantly by all the treatments investigated including the control (Table 4). This agrees with the results obtained by Rinaldi et al [56] who found that OMW application did not result on heavy metal accumulation in the soil.

Soil characteristics after plant harvest:

Soil pH at the end of the growing period was significantly lower in the soil where undiluted OMW was applied (Table 5). Other treatments of diluted OMW did not decrease the soil pH. The decrease in soil pH could be attributed to the acidic nature of olive mill wastewater [57]. It should be pointed out that the soil is calcareous with high buffer capacity. This could explain the small decrease in the soil pH with undiluted OMW while the diluted OMW was not affecting the soil pH. In addition, it is expected that the possible decrease in soil pH with diluted OMW could be temporal and will not persist longer due to the higher buffering capacity [58]. Such temporal decrease in the soil pH would enhance solubility, availability and the uptake of certain nutrients in calcareous soils such as phosphorus, Fe, Mn, Zn and Cu [58].

On the other hand, the soil salinity (EC) was increased drastically by the application of undiluted and diluted OMW (Table 5). The highest increase in soil EC was obtained by the undiluted OMW and then the EC decreased with decreasing the dilution of the OMW. The increase in EC with OMW application is obviously attributed to the high salt concentration in the OMW that would accumulate in the soil with continuous application. Chaari et al [59] has reported an increase in soil electrical conductivities following OMW application. The continuous buildup of salts in the soil surface with long-term application of OMW may adversely affect seed germination, seedling establishment and plant growth and may also deteriorate soil productivity. Therefore, this should be considered in managing soil application of OMW especially when OMW will be used for long term application [12]. Since these salts are water soluble, potential leaching beyond the rooting systems is possible [60].

Compared to the soil application of water with and without fertilizer, the soil contents of both organic matter and total polyphenols were the highest for the undiluted then by diluted OMW. Besides improving the soil fertility of the soil with OMW application [19], increasing the soil organic matter tends to enhance soil structure by enhancing the soil aggregation. This was also evidenced by enhancing the soil bulk density which was the lowest when undiluted OMW was applied. It has been documented that soil bulk density decreases and soil aggregation improved following the soil application of OMW and were attributed to the increased soil organic content [61]. In contrary, an increase in soil bulk density and decrease in soil hydraulic conductivity with soil application of OMW under coarse texture conditions has been reported [62]. Other researchers reported an increase in soil organic matter and in phenolic compounds with soil application of OMW [5,22,47,59].

Soil nutrients after plant harvest:
Soil N, P, K, Ca, Mg and Na drastically increased with undiluted and diluted OMW application in comparison with the control treatments where water was applied. The highest values for all these nutrients were obtained when undiluted OMW was applied (Table 6). The increase in soil N, P and K contents with OMW application can be attributed to their high content in the OMW used (Tables 2). Such enrichment of the soil with organic matter and macronutrients would improve the soil fertility and productivity levels. Positive effect of OMW on soil fertility level has been reported by other studies [5,61]. Increasing soil content of N, P and K with OMW application to the soil has been reported in other studies [5,22,53].

The soil contents of the soil DTPA-extractable Fe, Mn, Zn and Cu after crop harvest were not affected significantly by the application of neither of undiluted nor by diluted OMW (Table 7). This could be attributed to their very low concentrations in the OMW. In addition, fine textured soils have the capacity to treat OMW and retain considerable amount of micronutrients and heavy metals rendering them not bioavailable that commonly measured by DTPA extraction [58]. However, continuous application of OMW may lead to accumulation of certain nutrients in the soil to levels high enough to cause nutrient imbalance; therefore, one should take this into consideration before determining the rate of application [58].

Soil microorganisms:
The total bacterial count in the undiluted OMW was 2.13×10^6 CFU ml^{-1} . The total bacterial count in the soil irrigated with potable water was $4.52 \times 10^6 \pm 6.14 \times 10^5$ CFU ml^{-1}, while in the soil irrigated with the undiluted OMW (100% OMW) the total bacterial count was $2.74 \times 10^6 \pm 4.79 \times 10^5$ CFU ml^{-1} (Fig. 4). This indicates the toxic effect of OMW on soil microorganisms [5]. Sidari et al. [52] have reported that addition

of raw OMW to the soil reduced the numbers of bacteria and actinomycetes in the soil. Similar findings were obtained by other researchers [5, 63].

Conclusions

Based on the results obtained from this study it can be concluded that soil application of undiluted OMW had phytotoxic and prohibiting effect on plant growth and soil microorganisms. On the other hand, and due to high levels of organic matter, phenols and nutrients in the OMW, the soil fertility was improved following soil application of OMW. Dilution of OMW with potable water at water to OMW ratio of 3:1 (25%OMW) is recommended to before soil application to eliminate its phytotoxicty and to enhance plant growth. Such dilution can be adopted without any further treatment as an inexpensive technology before application. Finally, the enhancement of soil OM, N, P and K and improving soil fertility is of particular importance for the poor soils of the arid and semi-arid region. Thus, OMW in this region has the potential to be used as an organic soil amendment.

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Captions:

Table 1. Measured Soil Characteristics

Table 2. Characteristics of water and OMW used for irrigation

Fig. 1. Infiltration rate of diluted and undiluted OMW into the soil, mm hr^{-1}

Fig. 2. Plant dry weight as g plant-1 and relative to the control (W)

Fig. 3. Relationship between plant dry weight and %OMW in the omw:water mixture

Fig. 4. Total bacterial count, CFU ml^{-1}

Table 3. Plant uptake of macronutrients

Table 4. Plant uptake of micronutrients and heavy metals

Table 5. Soil characteristics after plants harvest

Table 6. Soil macronutrients and secondary nutrients after plants harvest

Table 7. Soil DTPA-extractable micronutrients after plants harvest

Table 1. Measured Soil Characteristics

pH (1:1 soil:water suspension)	8.18
EC (1:1 soil:water extract (dS m^{-1})	0.61
CEC (cmol kg^{-1})	34.32
O.M (%)	0.72
N (%)	0.01
P (mg kg^{-1})	7.11
K (mg kg^{-1})	452
$CaCO_3$ (%)	13.38
Fe (mg kg^{-1})	3.56
Mn (mg kg^{-1})	5.58
Zn (mg kg^{-1})	1.88
Cu (mg kg^{-1})	1.22
Pb (mg kg^{-1})	0.68
Cd (mg kg-1)	0.06
Bulk density (g cm^3)	1.38
Texture Class	Silty clay loam

Table 2. Characteristics of water and OMW used for irrigation

Parameters	W	OMW*
pH initial	7.8	4.7
EC dS m^{-1}	0.56	7.6
TSS mg 1^{-1}	10	1236.2
TP, mg l^{-1}	0.98	1666.7
COD, g l^{-1}	ND	118.8
N, mg l^{-1}	11.7	96.8
P_2O_5, mg l^{-1}	34.3	369.5
K_2O, mg l^{-1}	10.9	2441.8
Total bacterial count, CFU ml^{-1}	-	2.13 X 10^{-6}

OMW= Olive Mill Wastewater; EC= Electrical conductivity; TP= Total polyphenols; COD = Chemical oxygen demand; TSS = Total suspended solids; CFU = Community forming unit

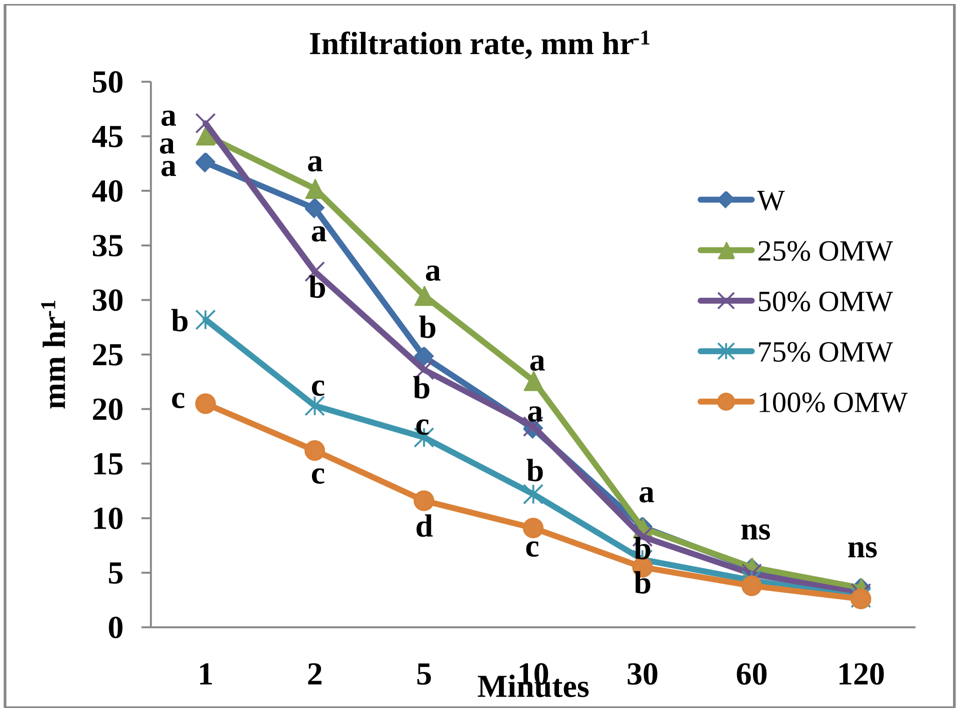

Fig. 1. Infiltration rate of diluted and undiluted OMW into the soil, mm hr^{-1}
Different letters in each column indicate significant difference at $p < 0.05$

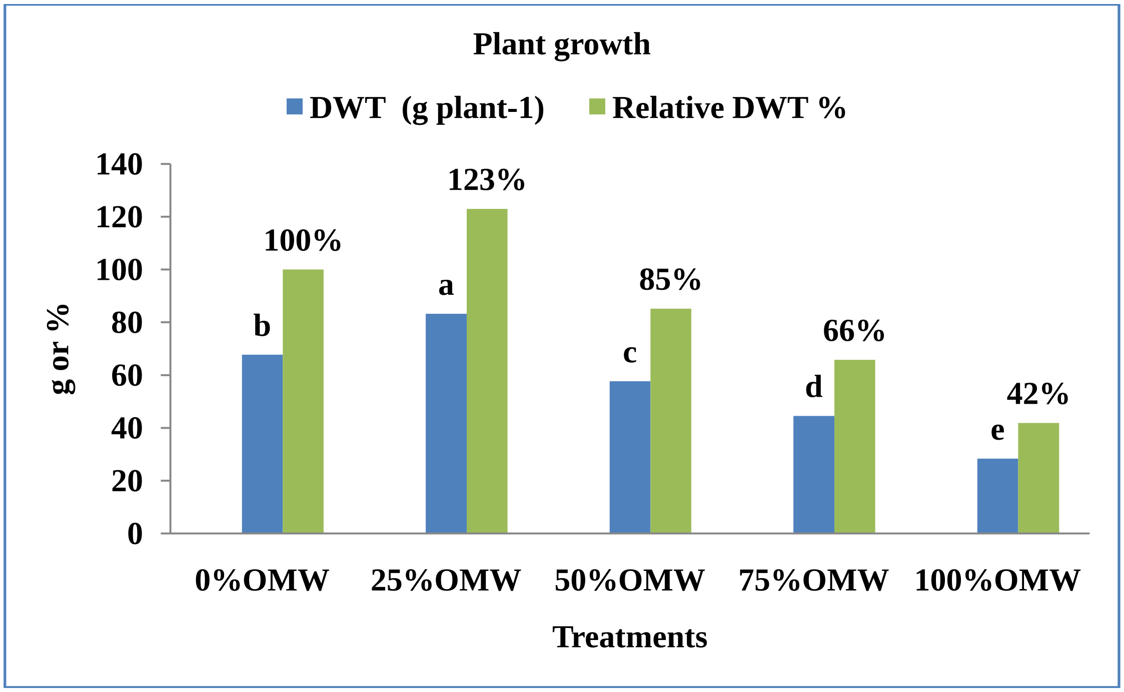

Fig. 2. Plant dry weight as g plant-1 and relative to the control (W)
Different letters in each column indicate significant difference at $p < 0.05$

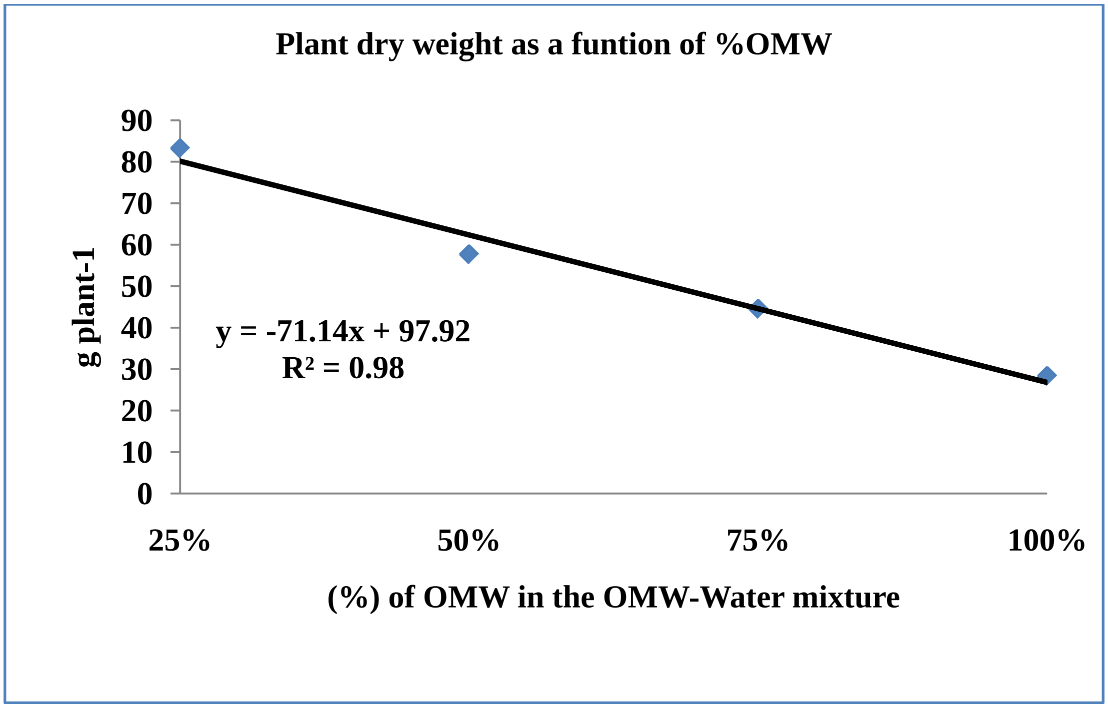

Fig. 3. Relationship between plant dry weight and %OMW in the omw:water mixture

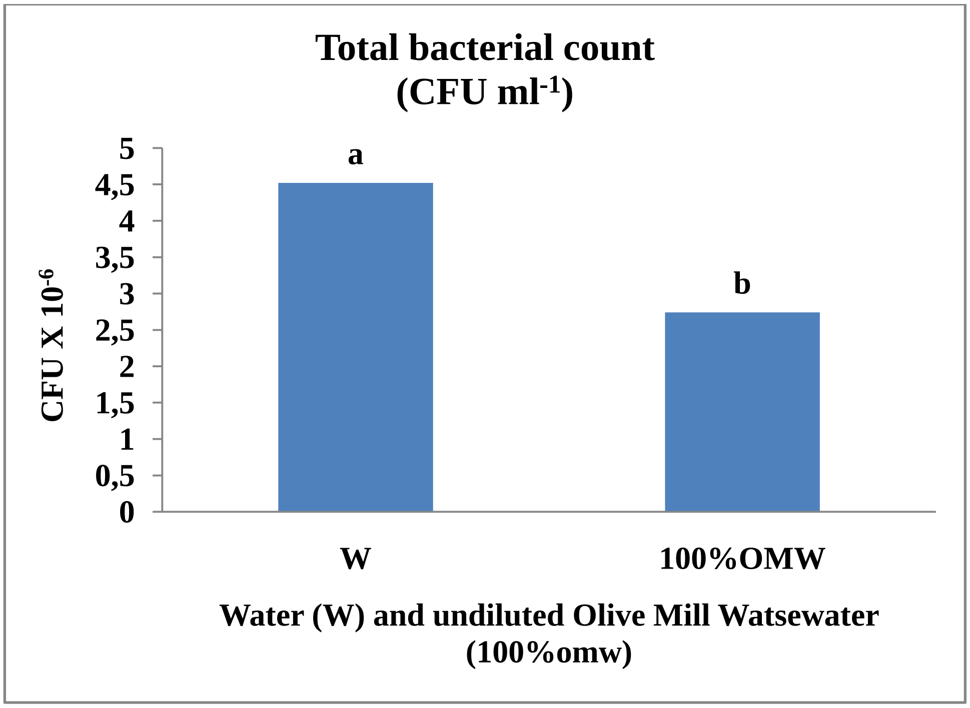

Fig. 4. Total bacterial count, CFU ml^{-1}
Different letters in each column indicate significant difference at $p < 0.05$

Table 3. Plant uptake of macronutrients

Treatments	N	P	K
	gm $Plant^{-1}$	gm $Plant^{-1}$	gm $Plant^{-1}$
W	0.99 b	0.17 b	1.38 b
$100\%_{OMW}$	0.48 d	0.06 d	0.44 d
$75\%_{OMW}$	0.66 c	0.08 d	1.04 c
$50\%_{OMW}$	0.98 b	0.11 c	1.48 b
$25\%_{OMW}$	1.74 a	0.26 a	2.14 a

Different letters in each column indicate significant difference at $p < 0.05$

Table 4. Plant uptake of micronutrients and heavy metals

Treatments	Fe	Mn	Zn	Cu	Cd	Pb
	mg $Plant^{-1}$	mg $Plant^{-1}$	mg $Plant^{-1}$	mg $Plant^{-1}$	mg $Plant^{-1}$	mg $Plant^{-1}$
W	3.68	1.86	0.39	0.02	0.05	0.17
$100\%_{OMW}$	1.74	1.61	0.27	0.03	0.02	0.08
$75\%_{OMW}$	1.72	1.64	0.21	0.03	0.07	0.08
$50\%_{OMW}$	1.95	1.72	0.22	0.02	0.14	0.09
$25\%_{OMW}$	1.81	1.92	0.25	0.02	0.16	0.11
$LSD_{.05}$	NS	NS	NS	NS	NS	NS

Different letters in each column indicate significant difference at $p < 0.05$

Table 5. Soil characteristics after plants harvest

Treatments	PH	EC	O.M	TP	BD
		dS/m	%	%	g/cm3
W	7.87 a	0.98 d	1.21 d	0.11 e	1.22 a
100%$_{OMW}$	7.70 b	5.88 a	2.10 a	20.67 a	1.09 b
75% $_{OMW}$	7.87 a	4.88 b	1.96 a	15.95 b	1.09 b
50% $_{OMW}$	7.90 a	3.22 c	1.83 ab	10.31 c	1.2 a
25% $_{OMW}$	7.87 a	2.83 c	1.65 bc	5.09 d	1.2 a

Different letters in each column indicate significant difference at $p < 0.05$

Table 6. Soil macronutrients and secondary nutrients after plants harvest

Treatments	N	Olsen-P	K	Ca	Mg	Na
	%	mg kg^{-1}	mg kg^{-1}	meq L^{-1}	meq L^{-1}	meq L^{-1}
W	0.09 b	8.83 d	631 d	3.47 e	3.13 e	2.96 d
100%$_{OMW}$	0.12 a	82.50 a	2926 a	23.73 a	22.70 a	8.10 a
75% $_{OMW}$	0.12 a	64.23 b	2558 b	17.70 b	16.73 b	7.59 a
50% $_{OMW}$	0.11 a	54.53 bc	2290 b	11.23 d	13.10 c	6.31 b
25% $_{OMW}$	0.12 a	28.23 c	1664 c	12.77 cd	8.27 d	5.03 c

Different letters in each column indicate significant difference at $p < 0.05$

Table 7. Soil DTPA-extractable micronutrients after plants harvest

Treatments	DTPA Fe	DTPA Mn	DTPA Zn	DTPA Cu
	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
W	1.50 b	3.10 d	0.88 d	1.49 a
100% $_{OMW}$	3.12 a	83.40 a	2.45 a	1.30 a
75% $_{OMW}$	3.69 a	65.27 b	2.40 a	2.10 a
50% $_{OMW}$	4.34 a	27.70 c	2.50 a	1.87 a
25% $_{OMW}$	2.86 a	22.77 c	1.93 b	1.56 a

Different letters in each column indicate significant difference at $p < 0.05$