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ORIGINAL RESEARCH

Agronomic assessment of solar dried recycled olive mill sludge on Maize agrophysiological traits and soil fertility

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Abstract

Purpose Olive mill waste sludge (OMWS) is a solid by-product resulting from olive oil extraction, OMWS is usually left decanting in landfills causing environmental pollution and a significant loss of recyclable organic resources. This study aims to evaluate the feasibility of producing an organic amendment through treating OMWS with solar drying (SDy), which is a low-cost method, highly adapted to semi-arid and low-income countries.

Method We investigated the effect of 90 days SDy on OMWS physico-chemical properties, then the agronomic efficiency of the resulting product was assessed under greenhouse conditions, using *Zea mays* as a crop model.

Results The SDy treatment significantly reduced OMWS initial moisture and the C/N ratio, while the nutrient content of the final product was improved. Pot trial under greenhouse conditions revealed that the application of SDy-OMWS improved the soil physico-chemical properties. The initial application decreased soil pH from 8.19 to 7.06, and soil phosphorus (P), potassium (K), iron (Fe) and zinc (Zn) increased by 209%, 162.4%, 290% and 270% respectively compared to the unamended control. SDy-OMWS application initially induced a delayed seed germination and plant growth at early stages, which was followed by a significant improvement of plant above and below ground traits, including photosynthetic activity, stomatal conductance, and root parameters (RL, RSA and RV).

Conclusion Overall, SDy significantly reduced the C/N ratio, moisture, and improved the nutrient content of OMWS. Despite improving soil fertility, SDy-OMWS application negatively affected the plant development at early stages. However, such effect was completely alleviated at the end of the experiment.

Keywords Olive mill waste sludge, Organic amendment, Soil toxicity, Plant development, Microorganisms

Introduction

Olive oil production is an important socio-economic driver of several countries of the Mediterranean basin. The worldwide production of olive oil is dominated by Spain, Italy, Greece, Tunisia and Morocco (FAO 2015). Whether it is for modern or rudimentary production units, olive processing generates several wastes, notably, a complex matrix known as olive mill wastewater (OMWW), which constitute an environmental issue due to its resistance to biodegradation and its richness in phytotoxic compounds. It is estimated that more than 30 million m^3 of OMWW is generated on an annual basis (Souilem et al. 2017). More specifically, Paraskeva and Diamadopoulos (2006) reported that for 1000 kg of olives, the resulting OMWW may range from 0.5 to 1.5 m^3. Various methods have been proposed for the treatment of OMWW, and while physico-chemical processes are expensive and unsustainable for developing countries. While, their biological counterparts demonstrated promising results.

Integrating waste recycling in agriculture production systems is essential for sustainable development. For example, in Morocco, soils are globally poor with a low total organic carbon (TOC) < 1.5%, a nitrogen (N) content of about 0.1 to 0.5%, and an available phospho-

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rus (AP) varying between 10 and 30 ppm (Achuba et al. 2020; Dahan et al. 2012; Khodrani et al. 2017). Consequently, classical mineral fertilization approaches are often not enough to attain the required yield, and novel practices and products should be exploited to ensure the improvement of soil overall properties by combining organic matter rich matrices and chemical inputs. Considering its rich composition, OMWW could constitute a suitable candidate to achieve this objective, given that an efficient valorisation strategy is implemented.

OMWS is an oleaginous solid black substrate with a highly variable chemical properties, that depend on the olive characteristics. Worth mentioning, OMWS and OMWW properties are completely different. For example, OMWS pH and electrical conductivity can range from 4.9 to 9.69 and from 3.72 dS/m to 9.69 dS/m respectively (Bouhia et al. 2020; Hachicha et al. 2009, 2012; Mekki et al. 2013). The plausible fertilization benefits of OMWS are mostly attributed to its high moisture and TOC% content (50 to 66%), as well as its richness in K, calcium (Ca) and P (Bouhia et al. 2020; Hachicha et al. 2009; Hytiris et al. 2004; Rigane et al. 2015). Moreover, OMWS contains other high value-added compounds such as sugars, tannins, Lipids, phenols, polysaccharides, pectin and proteins (Chtourou et al. 2004; Jarboui et al. 2010). However, its composition is highly dominated by phytotoxic compounds, antioxidants (13 to 16 g/kg) and fatty acids which represent more than 20% of OMWS total weight (Bouhia et al. 2020; Filippi et al. 2013; Linares et al. 2003). According to several research investigations, OMWS treatment via composting and anaerobic digestion could significantly reduce the phenol and lipid loads, thus generating products, which could improve soil fertility, with a positive impact on soil microflora (Bouhia et al. 2020; Moreno et al. 2013; Pierantozzi et al. 2013).

Using such product as a soil amendment to improve soil fertility and structure may be beneficial for semi-arid regions like Morocco, which suffer from serious water and carbon content deficiencies (Boulmane et al. 2017; Francaviglia et al. 2017). However, other less complex methods for OMWS valorisation should be studied, such as treatment via SDy. In fact, the use of solar radiation for drying purposes is one of the oldest applications of solar energy that have been proposed as a treatment for sewage sludge (Bennamoun et al. 2013; Lima et al. 2012; Phiri et al. 2014). Given the climatic traits of Morocco (3000 h/year of sunshine), SDy is theoretically suitable as it could adjust the pollution level in OMWS, given that the large quantity of OMWS is generated during the summer period where solar radiation is at its maximum. To the best of our knowledge, the great majority of research investigations mainly focuses on OMWW application (directly or prior to treatments). However, studies on OMWS which is chemically and physically different are still lacking. Consequently, in this work, we propose to study the effect of SDy on OMWS physico-chemical properties and to assess the agronomic efficiency of the resulting product using *Zea mays* as a crop model.

Material and methods

OMWS treatment and characterization

OMWS was collected from the evaporation basins of a semi-modern unit of olive oil production in Chichaoua city (Marrakech-Safi region, Morocco) (31° 38’ N, 7° 59’ O). OMWS was recovered and solar dried for 3 months, from August to the end of October. Briefly, OMWS was spread out below a layer of cement (20 cm) of 2 m^3 in a windrow (1.5m width X 0.5m height) in order to avoid any leakage and soil contamination, and thus optimizing the effect of heat on the substrate. Electrical Conductivity (EC) and pH were measured on an aqueous extract at ambient temperature (1 g/10 ml of sludge and distilled water) (AFNOR NF T90- 008). Moisture content was determined by drying 100 g of OMWS at 105 °C for 48 h (AFNOR 2000). Total organic carbon (TOC) was calculated after calcination in a muffle furnace at 600 °C for 6 h according to AFNOR, European standard EN 1085. Total Kjeldahl nitrogen (TKN) was assayed in 0.5 g samples by using the classical Kjeldahl procedure according to AFNOR (2004).

Trial set-up and experimental design

Agronomic trials were conducted at the greenhouse of Mohammed VI polytechnic University experimental farm for 90 days (from April to July 2019). *Zea mays (MACHA* variety*)* was grown in pots filled with 3.5 kg of alkaline soil. The used soil was collected from 0-30cm depth (pH_{water} = 8.29; Electrical conductivity (EC) = 0.43 dS/m; TOC (%) = 1.31; $P_2O_{5(available)}$ = 19.07 Mg kg^{-1}; for exchangeable cations K = 0.75 Cmol. kg^{-1}; Ca^{2+} = 44.4 Cmol. kg^{-1}; Mg^{2+} = 10.71 Cmol kg^{-1}). Two treatments with five replicates were assessed: 1: soil amended with SDy-OMWS [1:20 (v/v)], 2: unamended soil as a control. For all the treatments, soil was solely fertilized with Nitrogen (180 kg N ha^{-1}) through adding urea. Before planting, seeds were surface sterilized with 1/5 diluted sodium hypochlorite, followed by successive washing with sterile distilled water, and then transferred to pots. Pots were daily irrigated with distilled water to maintain a moisture content of 50-60%.

Soil physico-chemical properties and nutrients analysis

At the end of the experiment soil samples were oven dried at 30 °C for 72h. Then, sieved at 2-mm prior to analysis. The pH and electrical conductivity (EC) were assayed on an aqueous extract according to ISO standards (10390, 1994). (%TOC) was evaluated, following the oxidation of organic matter by $K_2Cr_2O_7$ according to Walkley and Black (1934). Total Kjeldahl nitrogen (%TKN) was assayed in 0.5g of dried samples by using Kjeldahl reactive according to AFNOR T90-1110 standard. The soil AP concentration was evaluated using Olsen and Sommers (1982) methods. The determination of exchangeable cations (K^+, Ca^{2+} and Mg^{2+}) was carried out by a direct extraction from the soil using ammonium acetate (1:20 (m/v)) at pH=7, then values were determined using flame spectrometry following NF X 31-108 standard. Soil content of iron (Fe), zinc (Zn), manganese (Mn), boron (B) and copper (Cu) was evaluated after acid treatment using aqua regia mixture (1/3 of nitric acid and 2/3 of hydrochloric acid), using ICP-OES (Agilent 5110, Santa Clara, California, USA).

Morphological root traits and plant biomass

Plants were carefully harvested and separated into shoots and roots at different growing periods, namely, germination, 15 days, 1 month, 2 months and 3 months. The root part was kept in a good state using moistened cellulose paper after soil recovery and directly analysed using the automated image software Winrhizo LA2400 (3^{RD} Gen.). Each root sample was separately submerged in a distilled water in a transparent glass of polymethyl methacrylate, and an image was recorded at a resolution of 300 dpi using the Epson expression 836 L), (Regent Instruments Canada Inc, scanner and winseedle). The analysed parameters were root length (RL), average root diameter (RD), root surface area (RSA), and root volume (RV). Afterwards, plant weight was determined by over drying shoot and root biomass at 70 °C for 72h prior to recording their dry weight. Then, shoot biomass was ground to a fine powder for plant elementary analysis, using a multi-elemental trace analysis of previously digested 1 g dried samples (plants and soil) and carried out using ICP-OES (Agilent 5110, Santa Clara, California, USA). The analysed elements were Iron (Fe), Zink (Zn), Manganese (Mn), Boron (B) and Copper (Cu).

Chlorophyll fluorescence measurements

The plant chlorophyll fluorescence was carried out in quintuplicate using a modulated fluorometer instrument ($OS30P^+$ opti-science, USA). The Fluorescence measurement was recorded at midday (3500 µmol m^{-2} s^{-1}). Five pulse Modulated Tests were used for measurement of fluorescence levels, (F0) was the minimal value measured at (<0.05 µmol m^{-2} s^{-1} for about 1.8 µs). The maximal fluorescence values (Fm) was measured after applying a saturating red-light actinic of 6000 µmol m^{-2} s^{-1} for 0.7 s. Those measurements were made to record the maximal quantum efficiency (MQF) of PSII according to the following the equations:

$$MQF = Fv/Fm=(Fm-F0)/Fm$$

Where: F0: Was the minimal value measured at (<0.05µmol m^{-2} s^{-1} for about 1.8µs)
Fm: The maximal fluorescence values was measured after applying a saturating red-light actinic, and
MQF: the maximal quantum efficiency

Quantitative analysis of polyphenols

Polyphenols extraction was carried out during different period following the procedure of Waterman (1994). Soils were carefully recovered from the pots by slowly stirring without damaging roots at 15 days, 1 month, 2 months and 3 months. The measurement was mainly based on the reduction of the acid complexes, namely, phosphomolybdic and phosphotungstic (Folin-ciocalteu reagent) by phenols fractions. The purified phenolic extract (50 µL) was mixed with distilled water (1.25 ml) and Folin-Ciocalteu reagent (200 µL). After 3 min of reaction, the stabilization of the phenolic oxidation was performed by adding 20% sodium carbonate (1 ml). After 30 min of incubation at 40° C, resulting in the formation of a blue complex, the concentrations were determined Spectrophotometrically at 760 nm with respect to a standard solution of gallic acid (Vasquez et al. 1974).

Extraction of the lipid fractions

Lipids were extracted at 15 days, 1 month, 2 months and 3 months. Briefly, 10 g of each soil sample was Soxhlet-extracted at ambient temperature during 20 hours with 150ml of ethyl ether (EE) for the easily biodegradable lipid fraction and then with 150ml of chloroform ($CHCl_3$) for the bio-resistant lipid fraction following method of Filippi et al. (2013). Afterward, the extract was dried on a rotatory evaporator at 45 °C to remove the solvent, then kept at ambient temperature for five days, and weighed.

Enumeration of cultivable microbial community (Actinobacteria, Bacteria and Fungi)

The enumeration of soil cultivable actinobacteria, bacteria and fungi were assayed in triplicate on SDy OMWS amended soil and the control, at different periods of the agronomic trial (2 weeks, 1 month, 2 and 3 months). After uniform homogenization, 1g of soil was suspended in 10 ml of sterilized physiological water (9g Nacl/L distilled water), then, vortexed several times to disperse the mixture particles. Finally, the suspensions were serially diluted up to 10^{-9} and transferred to sterile petri dishes containing specific growth media.

For Actinobacteria, Actinomycete Isolation Agar (AIA) was supplemented with 50 µg/ml of cycloheximide and 10 µg/ml of nalidixic acid to inhibit fungi and Gram-negative bacteria without affecting the growth of Actinobacteria. For bacteria, a standard medium (GN) supplemented with 50 µg/ml of cycloheximide was used, and for fungi, Potato Dextrose Agar (PDA) supplemented with 5 ug/ml of chloramphenicol was used. Microbial enumeration was done according to ISO 2718 (Rajhi et al. 2018), and results were expressed as number of colonies forming units (CFU) per gram of soil.

Statistical analyses

Results are means of five replicates for growth parameters (shoot and root dry weight, number of leaves, and physico-chemical analysis). Data was collected and analysed by one-way ANOVA using SPSS 20. Statistically significant differences between means were determined by the SNK test (Student, Newman, Keuls) at $p < 0.05$. Soil chemical parameters, nutrient content of plants and their correlation with treatments were subject to Pearson component analysis using SPSS 20.

Results and discussion

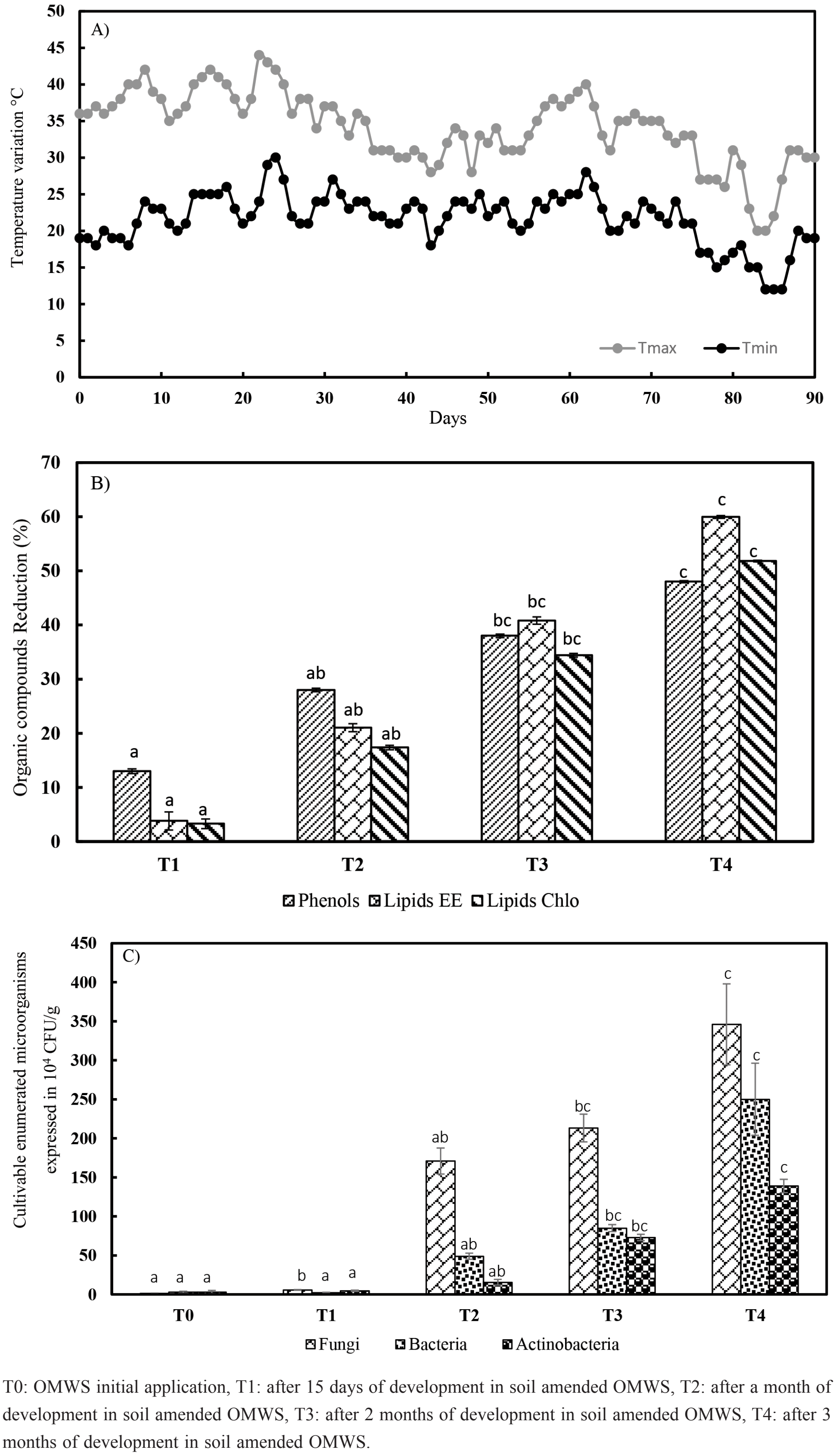
Effects of solar drying on OMWS physico-chemical proprieties

Our study revealed that SDy affected most of OMWS physico-chemical properties. For example, the high moisture content initially found in OMWS was significantly reduced by 37% (Table 1).

The increase in ambient temperature generated from sunlight in summer period (Fig. 1A) increased the waste temperature and evaporation and reduced its water content.

The reduction of moisture content could be also attributed to microbial activity during the mineralisation process (Aikaite-Stanaitiene et al. 2010; Bouraoui and Faycxal 2020). More importantly, a lower moisture allows a better heat transfer, which can improve the degradation of some easily biodegradable organic pollutants such as lipids and phenols (Liu et al. 2014). Such observation was corroborated by our findings (Table 1), as the SDy treatment induced a significant reduction of the easily biodegradable and bio-resistant lipid fraction by 15% and 9% respectively, and similarly phenol content was reduced by 7.5%. After 3 months of SDy, the pH value of OMWS was reduced by 0.8 units, and %TOC slightly decreased by 2.4%. Furthermore, SDy did not affect OMWS EC value, total P, Fe and B, while it significantly improved TKN, K, manganese (Mg), Mn and Zn by 6%, 20%, 21%, 38% and 23.86%, respectively. The decrease in phenol content under elevated temperature (between 30 and 50°C) was reported by many authors (Ait Baddi et al. 2004; Hafidi et al. 2005; Rigane et al. 2015). According to Galliou et al. (2018), treatment of OMWW by solar drying under greenhouse conditions decreased the concentration of total phenols to almost 3.7 g/kg after 6 months of experiment (February to August) with a maximal variation of temperature between 30°C and 33 °C.

Similarly, other authors suggested that in much warmer conditions (up to 50 °C) the content of some specific phenolic compounds such as Hydroxytyrosol could be significantly reduced via oxidation (Abu-lafi et al. 2017; Sa et al. 2019; Sklavos et al. 2015). Regarding the lipid fraction, many authors reported that lipids with short fatty acid chains such as, oleic, palmitic and linoleic acid, represent more than 90% of total lipid content of olive mill wastes (OMW), which may explain the progressive reduction dynamic during com-



T0: OMWS initial application, T1: after 15 days of development in soil amended OMWS, T2: after a month of development in soil amended OMWS, T3: after 2 months of development in soil amended OMWS, T4: after 3 months of development in soil amended OMWS.

Fig. 1 A: Variation of air temperature during OMWS drying process, B: the evolution of organic compounds in soil amended SDy-OMWS, C: the evolution of soil cultivable microorganisms (Actinobacteria, Bacteria and Fungi) in the SDy-OMWS treatment

Table 1 Physico-chemical properties and organic compounds composition of OMWS and SDy-OMWS

Parameters	OMWS	OMWS- SD
Moisture[1] (%)	72.36 (3.78) [a]	36.11 (2.3) [b]
pH[2]	4.81 (0.12) [a]	5.6 (0.01) [a]
EC[2] (dS/m)	2.83 (0.25) [a]	2.67 (0.47) [a]
TOC[1]%	51.95 (0.5) [a]	50.72 (0.1) [b]
TKN%	1.49 (0.08) [a]	1.58 (0.08) [b]
C/N	34	32
Phenols (g/kg) [1]	18.5 (0.2) [a]	17.1 (0.5) [a]
EE-extracted lipids [1]	261.07 (1.4) [a]	239.5 (1.8) [b]
Chloro- extracted lipids [1]	43.7 (0.7) [a]	37.14 (1.3) [b]
Total phosphorus (%)[1]	0.14 (0.01) [a]	0.16 (0.01) [a]
K[1]%	1.5 (0.05) [a]	1.8 (0.05) [b]
Ca[1]%	0.58 (0.05) [a]	0.60 (0.04) [a]
Mg[1] (ppm)	18.27 (1.2) [a]	22.08 (0.9) [b]
Mn[1] (ppm)	25.8 (3.4) [a]	35.7 (0.4) [b]
Fe[1] (ppm)	1152.25 (124.02) [a]	1380.5 (119.01) [a]
Zn[1] (ppm)	46.98 (1.3) [a]	58.19 (4.9) [b]
Bore [1] (ppm)	77.3 (4.9) [a]	82.3 (7.9) [a]

Data are mean values ± SD of 3 replicates. Numbers in the same column noted by a different letter are significantly different at $P < 0.05$. [1]: dry samples, [2]: fresh samples.

posting (Estaun and Calvet 1985; Filippi et al. 2013). According to Rodis et al. (2002), only 2% of the polyphenols present in the olives will be found in the olive oil, and more than 53% in OMWS, and the rest in the pomace. Moreover, the climatic conditions could play a critical role in improving microbial activity as higher temperature (case of compost thermophilic phase) are often correlated with improved microbial degradation of phenols and lipids (El Hajjouji et al. 2014; Ntougias et al. 2013; Rubio et al. 2019). The SDy treatment affected positively the OMWS pH values by a significant increase from 4.81 to 5.6, which is directly related to the reduction of lipids and phenols proportion, especially that presenting about thirty percent of OMWS composition (Table 1).

The effect of OMWS-SD on soil physico-chemical parameters and nutrient status

The effect of SDy-OMWS application on soil properties was studied directly after homogenizing with soil and at the harvest time. The main results are represented in (Table 2). Overall soil EC, pH, TOC, AP and available potassium (AK) were positively altered by SDy-OMWS treatments (Table 2A, B).

The % TOC initially increased by 2.47%, then decreased by 22% after 3 months compared to the control treatment, which could be related to the microbial degradation of easily degradable organic compounds (Magdich et al. 2020; Mohawesh and Al-hamaiedeh 2019). Soil AP and exchangeable K content significantly increased at the beginning of the experiment by 300% and 260%, respectively, and further increased at harvesting by 330% and 317%, respectively, compared to the control (Table 2). Soil exchangeable Ca was initially reduced by 43.3% and then increased by 14% after 90 days. Regarding micronutrients, amending the soil with SDy-OMWS improved total Mn, Fe and Zn by 298%, 470% and 660%, respectively after harvesting (Table 2b). Our results are in line with several studies investigating the effect of OMWW on soil fertility (Belaqziz et al. 2016, Chalkia et al. 2020; Rigane et al. 2015).

Soil pH decreased initially from 8.19 to 7.06 after application compared to the control treatments, and slightly increased by 0.3 units after 3 months. pH reduction may be attributed to the acidic functions of fatty acids and phenols. Some studies showed that pH values usually decrease following soil application of OMW; however, such effect is often temporary, and pH usually returns to its initial value after a prolonged

Table 2 Effect of SDy-OMWS applications on soil physico-chemical properties at the beginning of the experiment (A), and after harvesting (B)

A) Amendment	pH	EC (dS/m)	TOC (%)	P assimilable mg/Kg	Exch K^+ mg/kg	Exch Ca^{2+} g/kg	Exch Mg^{2+} mg/kg	Tot Mn mg/kg	Tot Fe mg/kg	Tot Zn mg/kg
Unamended (T=0 days)	8.19 (0.01) ^{a}	0.41 (0.05) ^{a}	1.28 (0.07) ^{a}	22.33 (0.9) ^{a}	300.5 (13.8) ^{a}	8.01 (0.8) ^{a}	1128 (47.9) ^{a}	8.89 (0.1) ^{a}	6.56 (0.11) ^{a}	0.71 (0.04) ^{a}
SDy-OMWS Amendement T=0 days)	7.06 (0.03) ^{b}	0.95 (0.1) ^{b}	3.75 (0.2) ^{b}	69 (2.6) ^{b}	788.6 (56.6) ^{b}	4,54 (0.10) ^{b}	620.3 (8.9) ^{a}	122.1 (10.5) ^{b}	25.57 (0.6) ^{b}	2.62 (2.62) ^{b}
Signifiance	***	**	***	***	***	***	NS	***	**	***
B) Amendment										
Unamended (T=3 months)	8.18 (0.03) ^{a}	0.43 (0.06) ^{a}	1.06 (0.02) ^{a}	19.33 (1.1) ^{a}	224.12 (16) ^{a}	5.62 (0.16) ^{a}	668.32 (38.1) ^{a}	4.11 (0.1) ^{a}	7.28 (0.29)a	0.42 (0.07) ^{a}
SDy-OMWS Amendement T= 3 months)	7.36 (0.03) ^{b}	0.79 (0.1) ^{a}	2.98 (0.2) ^{b}	65 (5.33) ^{b}	710.9 (53) ^{b}	4,023 (0.2) ^{b}	689.51 (35.7) ^{a}	122.8 (22) ^{b}	34.89 (4.3) ^{b}	2.79 (0.3) ^{b}
Signifiance	***	NS	***	***	***	***	***	***	***	***

Data are mean values ± SD of 3 replicates. Numbers in the same column denoted by a different letter are significantly different according to the student, Newman-Keuls test at *P<0.05, **P<0.01, ***P<0.001

time (López-Piñeiro et al. 2011; Magdich et al. 2013). In our experiment the pH reduction was partially maintained even after harvesting, which can be related to the persistence of organic compounds in soil (Barbera et al. 2013).

Soil EC increased initially by 0.54 dS/m compared to the control and significantly decreased by 0.2 dS/m after 3 months in the treated soil. Similar results were reported by (Kavvadias et al. 2010), who attributed the increase of EC values to the high content of nutrients in soil after OMW application. Moreover, EC increase may be related to the richness of OMW in alkaline metals such as K^+, Ca^{2+} and Mg^{2+} (Tsai and Chang 2019; Bouhia et al. 2021) as well as heavy metals accumulation (Moraetis et al. 2011).

The effect of SDy-OMWS on plants agro-physiological parameters and nutrient status

Despite the initial soil improvement induced by SDy-OMWS application, the studied plant agro-physiological parameters were negatively affected compared to the control, which was plausibly due to the phytotoxic effect of the amendment (Fig. 2A). Soil amendments significantly reduced ($p<= 0.05$) shoot and root dry weight by 57% and by 55% respectively.

Likewise, a negative impact on germination of maize seeds were initially recorded compared to the control, thus, slowing down the overall plant development. Notably, root morphological traits were negatively affected by the SDy-OMWS treatment except for RD where no difference was recorded compared to the control (Fig. 3).

Soil application of SDy-OMWS reduced significantly RL (96%), RSA (89.5%) and RV (71.5%) of the 15 days old seedlings (Table 3). However, the toxicity was gradually alleviated after 1, 2 and 3 months of the experiment, with 83.9%, 78.6% and 72% for (RL), 68.5%, 84.3% and 61.68% for (RSA) and 71.5%, 33% and 59.15% for (RV) respectively.

The fluorescence and stomatal conductance measurement showed a lesser significant value in the amended soil during the whole experiment compared to the control (Fig. 4). For Fv/Fm ratio (Fig. 4A) at the first month of the experiment, the amended soil presented a lesser ratio of 0.55 compared to 0.63 recorded in control plants. This value increased after 2 months to almost 0.6 and 0.72 for soil amended SDy-OMWS and the control, respectively.

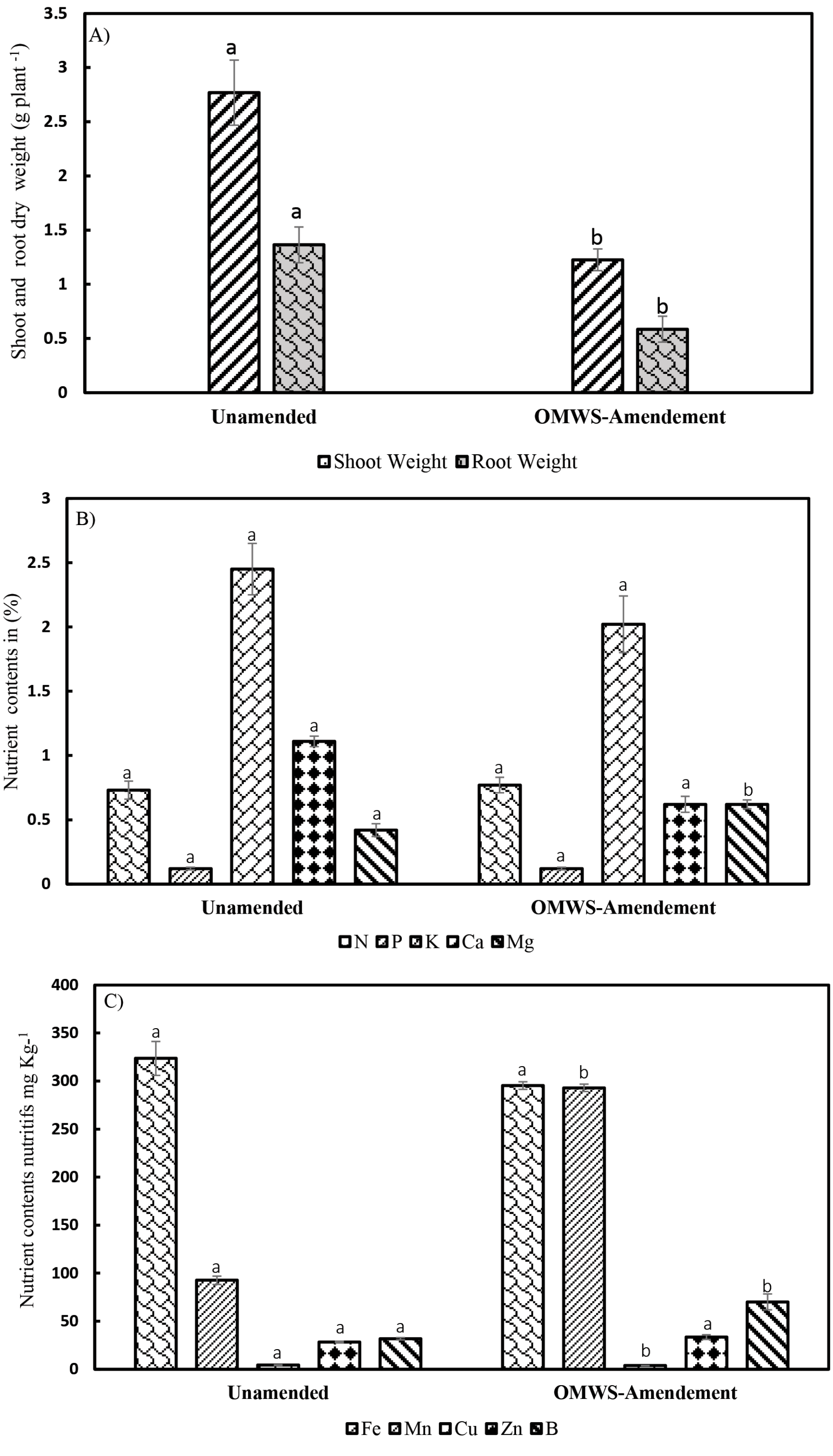


Fig. 2 A: The effect of SDy-OMWS on plant biomass, B: plant majors' nutrients uptake (N, P, K, Ca and Mg), and, C: micronutrients Fe, Mn, Cu, Zn and B

Table 3 Variation in morphological root traits at 2 weeks, 1 months, 2 months and 3 months old of *Zea mays* plants in response to SDy-OMWS amendment compared to the control

	Days	Morphological root traits parameters Root Length (RL) (cm)	Root Diameter (RD) (mm)	Root Surface Area (RSA) (cm^2)	Root Volume (RV)cm^3
SDy-OM-WS-Amended soil	15-day old seedlings	41.3 (17.3) [a]	0.35 (0.02) [a]	12.86 (3.3) [a]	0.33 (0.03) [a]
	1 months old plants	250.23 (101.1) [a]	0.385 (0.04) [a]	61.15 (18) [a]	1.21 (0.2) [a]
	2 months old plants	1142.28 (189.05) [a]	0.85 (0.1) [b]	100.35 (13.3) [a]	1.15 (0.3) [a]
	3 months old plants	4713.4 (112.5) [b]	1.08 (0.1) [b]	656.5 (19.5) [b]	5.31 (0.9) [b]
Unamended soil	15-day old seedlings	1032.81 (212.6) [a]	0.374 (0.01) [a]	122.69 (30.3) [a]	1.16 (0.3) [a]
	1 months old plants	1557.5 (236.4) [a]	0.38 (0.01) [a]	184.8 (38.4) [a]	1.82 (0.4) [a]
	2 months old plants	5353.9 (488.5) [b]	0.37 (0.05) [a]	637.73 (146) [a]	6.16 (2.2) [b]
	3 months old plants	16884 (1258.4) [c]	1 (0.14) [b]	1695 (142.8) [b]	13 (1.4) [c]

Data are mean values ± SD of 5 replicates. Numbers in the same column noted by a different letter are significantly different at $P < 0.05$.

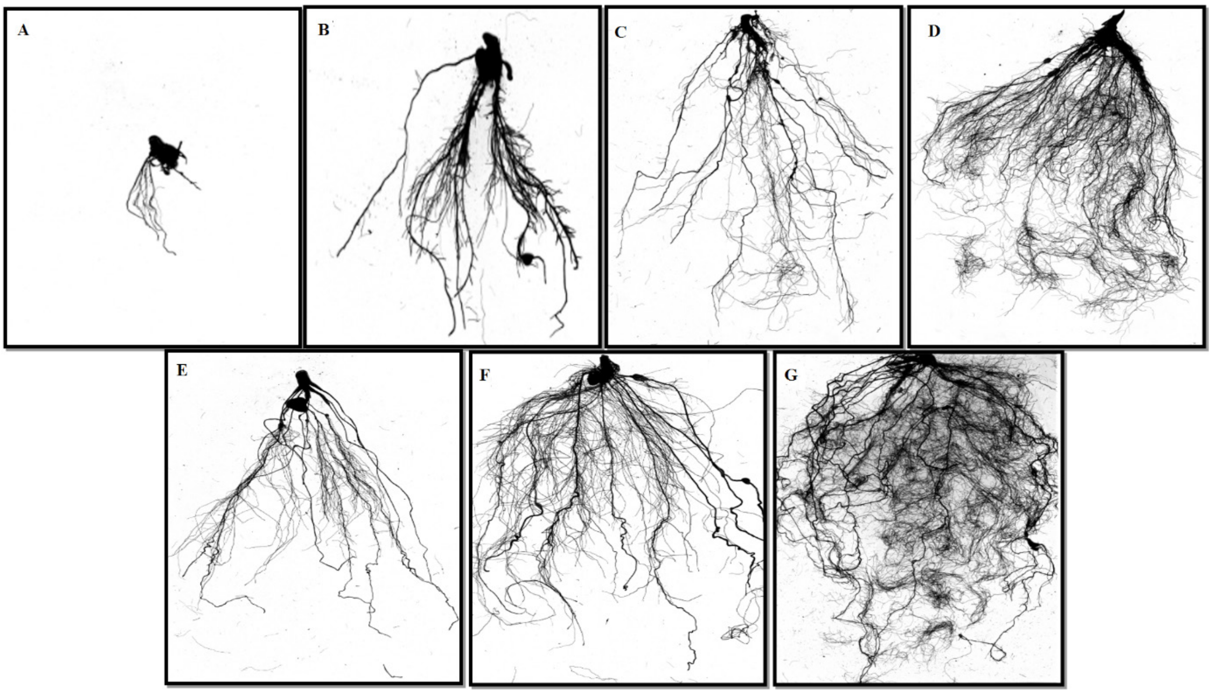


Fig. 3 A: Root morphology after two weeks of plant development in soil amended SDy-OMWS and E: the control, B: after one month of plant development in soil amended SDy-OMWS and F: the control, C: after two months of plant development in soil amended SDy-OMWS and G: the control, and D; after 3 months of plant development in soil amended SDy-OMWS

After 3 months of experiment, the values of Fv/Fm ratio decreased in the control treatment and kept increasing in the amended treatments. Regarding the stomatal conductance our results (Fig. 4B) showed a reduction of plant respiration after the first and second month in the amended soil compared to the control with value ranging from 312 to 220 gs umol m^{-2}. s^{-1} and 340 to 255 gs umol m^{-2}. s^{-1}. Similarly, after 3 months of experiment, plants were always stressed, which was reflected by a reduction of about 51% of stomatal respiration efficiency compared to the control. Moreover, regarding the plant's nutrient assimilation, no significant difference between the treated and unamended soil was recorded concerning plant NPK (Fig. 2B). However, plant Mg in the amended treatment (Fig. 2C) increased significantly by 48% compared to the control. Inversely, a reduction of 55.8% was recorded in the plant Ca in the amended treatment compared to the control, although not statistically significant.

Plant Mn, and B significantly increased by 216.3% and 121% respectively, and plant Cu decreased by 13% compared to the control; however, the value of plant Fe and Zn remained insignificant between treated plants and the control. Many works using OMW in agriculture reported a negative impact on seed germination due to the occurrence of phytotoxic organic compounds, such as phenols (Filippi et al. 2013; Linares et al. 2003). For example (Cavallaro et al. 2014; Magdich et al. 2013; Piotrowska et al. 2006) revealed that OMW could significantly decrease tomato seed germination. Those authors showed that germination parameters depend on the plant species which could have differential genotypic sensitivity to phenol toxicity. The variable composition of OMWW has a direct influence on the final recovered sludge regarding its physico-chemical properties and organic compounds load, especially on polyphenols, total lipids and tannins, which are not easily degraded even with a variety of methods (aerobic, anaerobic degradation and biological treatments), which directly influence the initial pH values and its properties (Alonso-fariñas et al. 2020; Bouhia et al. 2020). Our results are in agreement with many authors, which supported that the phytotoxic effect on crop development and growth is due to the higher polyphenols and lipid contents of OMW (Ait Baddi et al. 2009; Hachicha et al. 2009; Rigane et al. 2015).

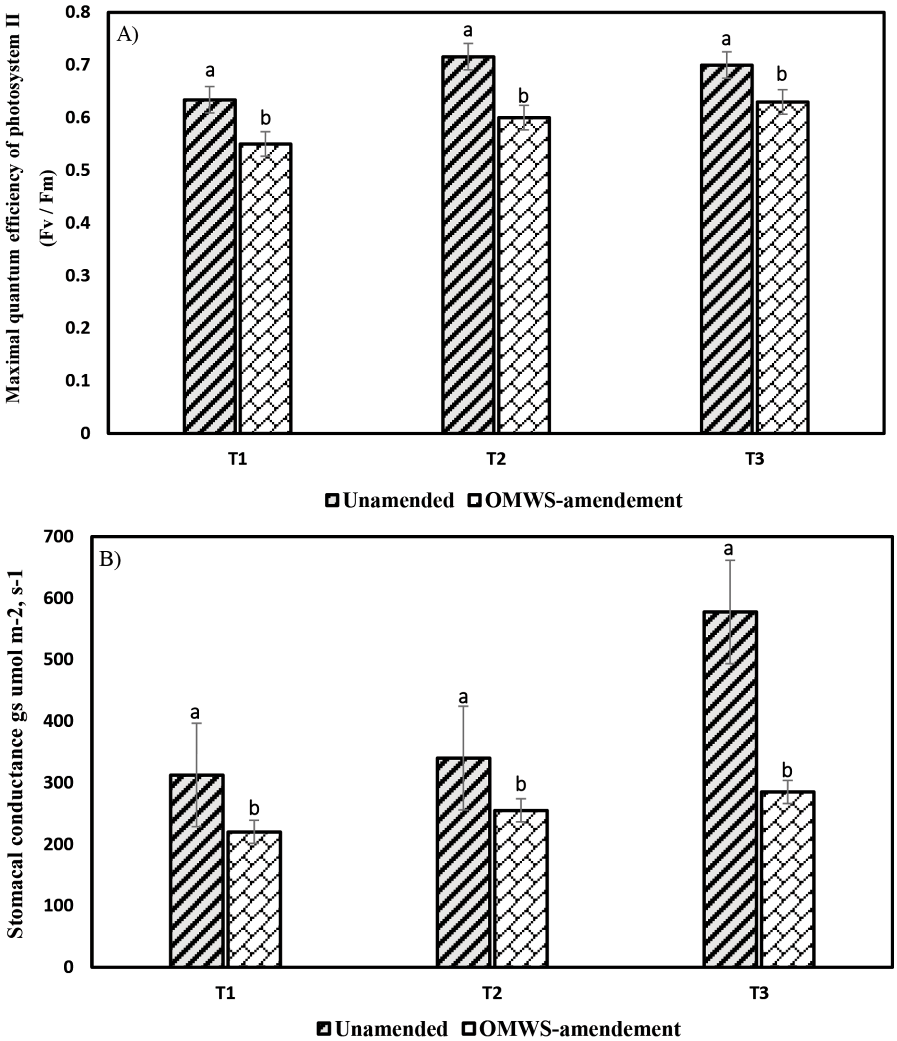
The reduction of the photosynthetic rates and the photochemical efficiency of PSII due to phenol toxicity, could be a result of suboptimal nutrient uptake by the plant (Chehab et al. 2019; Tajini and Ouerghui 2020). According to Mechri et al. (2011), who studied the effect of OMWW application rates on olive tree physiology and nutritional quality, the application of a high rate of OMWW significantly reduced the photosynthetic rate, as well as the plant yield and photochemical efficiency of PS II. Similar results were reported on tomato grown in sandy soil (Ouzounidou et al. 2008). Interestingly, those authors noted that roots were more sensitive to organic content in OMW than shoots. On a different note, the effect of toxicity induced by OMW application at the early growth stage of the plant has been well documented by (Kapellakis et al. 2015). Indeed, following a long-term agronomic trial, those authors reported a significant increase of *Eucalyptus camaldulensis* tree biomass and that an application rate of 180m^3 ha^{-1} allowed to avoid the toxicity linked to organic compounds and soil salinity.

Similarly, (Magdich et al. 2013) studied the effect of OMW on olive tree yield and change in soil microbial activity linked to phenols and found a positive correlation between crop improvement and OMW applied at 200 m^3 ha^{-1}. Other studies demonstrated the beneficial role of OMW as soil amendments on plant growth and final yield, which was attributed to the improvement of soil fertility (Mechri et al. 2011, Belaqziz et al. 2016; Nair et al. 2014). Inversely, Chartzoulakis et al. (2010) reported a reduction in plant leaf and fruit nutrient concentration of Olive trees using OMW compared to the control. Notably, the N and P leaf contents were reduced, and a significant increase in leaf Mg was recorded. In our study, the low nutrient content in maize plants could be a result of many factors, including salinity and the concentration of organic compounds persisting into the soil (Chehab et al. 2019), as well as the delayed growth of plants due to the state of stress during germination (Fig 3).

The variation of organic compounds on SDy-OMWS amended soil

Olive wastes are rich in organic compounds that could showcase toxicity depending on their concentration. For example, phytotoxic effect during all plant development stages was reported to be directly correlated with the phenols and lipids content (Baddi et al. 2004; Hachicha et al. 2012). Our study showed similar results as the application of SDy-OMWS increased phenols and lipid soil content. Thus, affecting plant growth at several development phases Fig. 3.

Phenols and lipid content decreased progressively during the 3 months of experimenting. For instance, after 15 days, phenols, easily biodegradable lipids and bio-resistant lipid decreased by 13%, 3.82% and 3.31% respectively. These compounds content was further reduced by 23%, 21% and 17.37%, and by 38%, 40.82% and 34.4% after 1 and 2 months, respectively. By the end of the experiment, the content was highly reduced by 48%, 60% and 52% for total phenols and easily biodegradable and bio-resistant's lipid, respectively. Diminution of phenols and lipid soil content could be attributed to soil microbial activity, as they can both be



Different letters above the bars indicate significant differences between mean values at each sampling occasion (P < 0.05).

Fig. 4 Effect of SDy-OMWS amended soil on plant physiological parameters. A: maximum quantum efficiency of PSII (Fv/Fm), B: Stomatal conductance gu umol m^{-2}. s^{-1}

used as a source of carbon through degrading phenolic compounds by oxidation of benzene ring and lipids by using the carbon chain (Hachicha et al. 2009; Khayer et al. 2013). Additionally, phenols could be used as precursor during the humification process, which could also explain such reduction (Baddi et al. 2004). Furthermore, the richness of OMWS in lignin compounds can generate more phenol compounds under the microbial biodegradation actions. Similarly, for both easy and bio-resistant lipid fractions, the progressive degradation of lipid fraction was previously evaluated by (Barje et al. 2012; Bouhia et al. 2020; Dinel et al. 2013; Filippi et al. 2013; Madejon et al. 1998). Based on the ratio of both easy and bio-resistant lipid fraction who shifted from 6 to 1.9 after composting treatment (Filippi et al. 2013). The high decrease of lipid fraction could be explained by the microbial biodegradation through using the less resistant lipid forms such as fatty acid compounds and glycerides as a carbon source. Also, the long- chain fatty acids could be degraded via the enzymatic activity of β-oxidation of the soil microbial community (Barje et al. 2012).

Effect of SDy-OMWS applications on soil cultivable microorganisms

SDy-OMWS application negatively affected soil cultivable microorganisms, namely Fungi (1.4 x 10^4

CFU/g), Bacteria (3 x 10^4 CFU/g) and Actinobacteria (3.2 x 10^4 CFU/g). The high microbial inhibition was maintained after 2 weeks of the experiment, except for fungi which slightly increased (6 x 10^4 CFU/g). After 1- and 2-months of experiment, soil microbial load significantly increased for fungi (17 x 10^5), bacteria (49 x 10^4 CFU/g) and actinobacteria (16 x 10^4 CFU/g), then further increased at the end of the experiment to reach 35 x 10^5, 25 x 10^5 and 13 x 10^5 CFU/g for Fungi, Bacteria and Actinobacteria, respectively. In the short-term, the application of SDy-OMWS affected the main chemical and biological parameters of soil which was linked to the inhibition of microbial activity. Our results were in accordance with those reported by (Pierantozzi et al. 2013; Rajhi et al. 2018), except for soil fungal biomass as it was not affected by SDy-OMWS application. According to Piotrowska et al. (2006), OMW application can induce changes in soil enzymatic activity. These authors showed that OMW application leaded to a decrease of soil phosphatase, β-glucosidase, nitrate reductase and diphenol oxidase activities, and inversely an improvement of urease and dehydrogenase activities and soil respiration, which was attributed to the richness of OMW in easily degradable C and N substrates. On a related note, several authors reported that the negative effect of OMW is not limited to bacteria and non-biotrophic fungi but could as well concern functionalities of arbuscular mycorrhizal fungi (AMF) and even some soil invertebrates (Di et al. 2012, Ipsilantis et al. 2009, Cayuela et al. 2008; D'addabb et al. 1997; Thligene et al. 2019). Conversely beneficial effect of OMW on soil microbiome has been also reported. For example, (Casacchia et al. 2012; El Hassani et al. 2010) demonstrated that the application of OMW significantly increased the abundance of actinomycetes and cellulolytic soil bacteria. Furthermore, (Barbera et al. 2013) evidenced the role of OMW in enhancing soil carbon and microbial activity and the inhibition of plant pathogens. Interestingly, changes induced by OMW in microbial diversity and functionalities could also be due to competition between microbial community for the mineral N in phenolic compounds as well as for others nutrient including AP and AK (Ipsilantis et al. 2009; Karpouzas et al. 2010).

Conclusion

Treatment of OMWS by SDy induced an important modification of its physico-chemical properties, namely a significant reduction of its moisture and C/N ratio and an increase of its nutrient concentrations. Furthermore, application of SDy-OMWS reduced soil pH and improved soil AP, exchangeable K, total Fe and Zn. However, it negatively affected plant development at early stages, suggesting that the phytotoxic effect partially remained, although such effect was completely alleviated by the end of the experiment. Likewise, Enumeration of soil cultivable microorganisms (actinobacteria, bacteria, and fungi), revealed that OMWS-SDy negatively affected the initial soil microbial load at the beginning, followed by lesser inhibition effect after 2 and 3 months of plant growth. The observed dynamic with regards to plant growth and soil cultivable microbes was strongly correlated with the evolution of the concentration of phytotoxic organic compounds in the soil, as those latter were reduced by 48%, 60% and 52% for phenols, easily and bio-resistant's lipid fractions, respectively. Overall, treatment of OMWS by SDy generated a product with interesting fertilization properties, however the discrepancies of the observed effect on soil and plant parameters suggest that an agronomic evaluation over more than one crop cycle may be required as to assess the whole potential of using such approach for OMWS valorisation.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest associated with this study.

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