

A study of olive mill wastewaters obtained from different treatment processes effects on chemical and microbial properties of a Typic Xerofluent soil and wheat yield

Nur OKUR¹, Hüseyin Hüsnü KAYIKÇIOĞLU^{1*}, Bülent OKUR¹,
Bülent YAĞMUR¹, Delia SPONZA TERESA², Recep Serdar KARA³

¹Department of Soil Science and Plant Nutrition, Faculty of Agriculture, Ege University, İzmir, Turkey

²Department of Environmental Engineering, Faculty of Engineering, Dokuz Eylül University, İzmir, Turkey

³Department of Water Resources, Faculty of Agrobiological, Food and Natural Resources,
Czech University of Life Sciences Prague, Czech Republic

Received: 22.02.2019 • Accepted/Published Online: 04.10.2019 • Final Version: 01.04.2020

Abstract: This study was carried out to investigate the potential for the usability of treated olive mill wastewater (OMW) as an organic amendment in agricultural soils under Mediterranean climate conditions. OMW was treated by two different treatment processes as economical (E-OMW) and advanced (A-OMW). The treated OMWs and raw OMW (R-OMW) were applied to a loamy soil at a rate of 100 m³ ha⁻¹ year⁻¹ for 2 years. Soils were sampled 15 days and about 5 months (at harvest) after OMW application for chemical and microbial analyses in each year. The total concentrations of N, P, Cu, Zn, and phenol of R-OMW decreased after both treatment processes while salinity (EC) and the total amounts of K, Na, and Ca increased. The applications of OMW caused changes in soil chemical (pH, EC, P_{ext}, K_{ext}) and microbial (microbial biomass-C (MB-C), microbial biomass-N (MB-N), basal soil respiration (BSR), N-mineralization (N-min)) characteristics (P < 0.05). In the second year of the experiment, initial samplings showed that the values of soil pH and EC increased significantly under all OMW applications compared to the control. High P_{ext} concentrations were determined in soils amended with R-OMW, while there were high K_{ext} concentrations in soils amended with the treated OMWs. The increases determined in MB-C and MB-N at all sampling times resulted in high MB-C/TOC and MB-N/TN ratios in soils amended with the treated OMWs. The wheat grain yield over the 2-year period showed that the application of the treated OMWs had a positive effect. It was determined that no negative effects occurred for either soil properties or wheat growth with the treated OMW applied at rates of up to 100 m³ ha⁻¹. The addition of treated OMW after removal of its phenolic components may be considered as a good option for evaluating this waste in countries where OMW causes serious environmental pollution.

Key words: Detoxification, soil conditioning, soil fertility, N-mineralization, phenolics, soil enzymes, wheat, grain yield

1. Introduction

The olive tree (*Olea europaea* L.) is one of the most important cultivated crops in Turkey, which gives the olive oil sector remarkable economic importance in West and Southwest Anatolia. About 178,000 t of olive oil is produced in Turkey, representing 5.8% of the world production (IOC, 2018). The countries producing olive oil have the problem of the disposal of the wastewater from olive mills where the olives are processed and oil is extracted (Erses Yay et al., 2012). The olive oil extraction process in Turkey annually generates about 923,000 m³ of olive mill wastewater (OMW) (Murat Hocaoglu, 2015). On the other hand, in today's world, where agricultural irrigation water quality and quantity problems are experienced, chemical fertilizer input costs have increased and ecosystem pollution is serious due to wastes produced in increasing

amounts from day to day. Therefore, investigation of the potential of OMW for use in agricultural soil has gained importance in Turkey, where there is no specific regulation regarding the discharge of OMW, as well as in other Mediterranean countries.

OMW is characterized by high organic load (BOD₅: 20–120 g L⁻¹; COD: 40–240 g L⁻¹), salinity, and phytotoxic levels of polyphenols, while it also consists of a high amount of organic compounds and mineral nutrients (Chatzistathis and Koutsos, 2017). One alternative and economical solution for OMW disposal is controlled land application (Erses Yay et al., 2012). In some Mediterranean countries, such as Italy, Portugal, and Spain, application rates of about 30 to 80 m³ ha⁻¹ year⁻¹ of OMW to agricultural lands are permitted (Sierra et al., 2007). In Turkey, there is no specific regulation or permitted rate regarding the

* Correspondence: husnu.kayikcioglu@ege.edu.tr

discharge of OMW, which contains only fruit constituents and water and not contains heavy metals, pathogenic microorganisms, or any synthetic matters. In this respect, OMW is considered as a natural fertilizer or a cheap water resource at proper application rates, especially in the Mediterranean region, characterized by a serious lack of quality and quantity of irrigation water and soil organic matter.

However, the agronomic use of OMW by directly spreading it on soils is limited by some constraints, such as acidity, salinity, N immobilization, lipids, organic acids, and accumulation of phenolic compounds (Sierra et al., 2007). These constraints, which may vary depending on the relative amounts of beneficial and toxic organic and inorganic compounds contained in OMW, include potential negative effects on the physical, chemical, and biological properties of the soil, potential phytotoxic effects on crops, and potential groundwater pollution (Barbera et al., 2013).

Continuous untreated application of OMW may cause surface sealing, affect soil properties, and contribute to eutrophication of freshwaters (S'habou et al., 2009; Chartzoulakis et al., 2010; Kavvadias et al., 2010; Lopez-Pineiro et al., 2011; Magdich et al., 2013, 2016). In studies investigating the use of treated OMW for irrigation/fertilization of agricultural soils, the cheapest and most easily implemented techniques were generally preferred. Based on the results of a 6-year study, Kayikcioglu and Sahin (2013) also suggested that dried OMW applications at a rate of 75 kg tree⁻¹ for every year and 100 kg tree⁻¹ every 2 years are the best amendment rates for degraded and poor Mediterranean soils in order to enhance soil microbial activity and plant yield. Mekki et al. (2006) reported that water holding capacity, salinity, and contents of total organic carbon, humus, total nitrogen, phosphate, and potassium increased when the spread amount of untreated and biologically treated OMWs increased. On the other hand, the authors also specified that a toxic effect of the untreated OMW appeared from 100 m³ ha⁻¹ and this toxicity was more significant at 200 m³ ha⁻¹, where microflora of total mesophiles, yeasts, molds, actinomycetes, and nitrifiers was seriously inhibited. The study of Brunetti et al. (2007) showed that the application of untreated OMW and catalytically digested OMW at two rates (300 and 600 m³ ha⁻¹) increased soil electrical conductivity and the contents of total organic C, total extractable C, humified and nonhumified C forms, and available P and K. Moraetis et al. (2011) applied lime-pretreated weathered-OMW mixed with fresh water at a ratio of 1:4 to a maize field and found that the soils received 6 times more N, 5 times more K, and 2 times less P than the recommended fertilization rates. Piotrowska et al. (2011) used dephenolized OMW (d-OMW) and crude OMW

(c-OMW) and concluded that the application of d-OMW resulted in a less inhibitory effect of some enzymatic activity (urease) and higher values of the respiratory quotient qCO₂ as compared to c-OMW amendment. According to these authors, the application of OMW, mainly after removal of its phenolic components, may be suggested as a good strategy for restoring soils in semiarid areas and soils that are poor in organic matter. Rusan et al. (2016) evaluated the potential use of OMW treated by 4 different technologies in irrigation of maize and indicated that the untreated OMW increased soil salinity and reduced plant growth, while the treated OMW improved plant growth and resulted in lower soil pH. OMW reduced phytotoxicity as an efficient treatment technique and it can be used to restore the deficit in soil carbon and improve soil fertility, consequently enhancing the sustainability of Mediterranean agroecosystems (Mohawesh et al., 2014).

Until now, little attention has been paid to the use of treated OMWs as an amendment for soils and additive organic fertilizer for crops in the Mediterranean countries producing olive oil. The objectives of this field study, which was prepared based on this idea, were to determine the properties of chemically detoxified OMW, to evaluate the potential use of treated OMW as a soil conditioner in a wheat field, and to determine the impacts on soil chemical and microbial parameters and plant growth. In addition, an attempt was made to evaluate the results of this study in terms of ecosystem health.

2. Materials and methods

2.1. Olive mill wastewater

Raw OMW (R-OMW) used in the field study was obtained from a three-phase extraction plant in Aydın Province in the Aegean region of Turkey). OMW was treated by two different treatment processes, one of which was economical and the other a novel advanced treatment process including the nanometal oxide composites. In the economical treatment process (E-OMW), Ca(OH)₂ was added to OMW at a rate of 40 g L⁻¹ and mixed quickly for 2 min and slowly 15 min. Then OMW was left to settle for 24 h. In the advanced treatment process (A-OMW), OMW was treated by photocatalytic oxidation by using 8 g L⁻¹ nano-Fe₃O₄/SiO₂ under UV. The OMWs were stored at 4 °C until application to the soil in closed plastic tanks.

2.2. Field experiment

The experiment was carried out between November 2014 and July 2016 on a Typic Xerofluvent type soil located in the Menemen region (İzmir, Aegean region of Turkey, 38°35'N, 27°02'E). The climate of this region is Mediterranean and semiarid, with an average rainfall of 543 mm year⁻¹ and an average annual temperature of 17 °C (Table 1). In 2016, temperatures of the winter months were higher and precipitation was less and more irregular

Table 1. Average air temperature and total rainfall during the experiment period.

	Year/Month	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Mean
Average temp. (°C)	2014–2015	18.0	13.2	11.3	7.8	7.4	11.2	14.1	20.7	23.6	27.7	28.3	24.5	17.3
	2015–2016	18.9	15.0	8.2	7.8	13.3	12.8	17.8	20.0	26.4	28.4	28.1	23.4	18.3
	Avg. of long years (55-year)	17.4	13.0	9.7	7.9	8.8	11.1	15.0	20.0	24.7	27.7	26.5	22.3	17.0
														Total
Average total rainfall (mm)	2014–2015	35.0	14.5	187.3	156.3	88.9	89.4	11.6	61.3	100.0	0.0	0.0	13.0	757.3
	2015–2016	102.1	61.2	0.0	169.7	55.1	118.8	17.8	17.8	4.8	0.0	0.0	0.0	547.3
	Avg. of long years (55-year)	37.7	74.6	108.0	91.3	73.5	62.1	42.5	27.4	8.6	2.4	2.7	12.5	543.3

than in 2015. In December 2015, there was 187.5 mm of precipitation, but in December 2016 there was no precipitation. Physicochemical characteristics of the soil studied were as follows: pH, 7.67; EC, 0.22 dS m⁻¹; sand, 47%; clay, 11%; CaCO₃, 4.85%; organic C, 0.83%; Kjeldahl N, 0.086%; Olsen P, 14.83 mg kg⁻¹; available K, 217.9 mg kg⁻¹. The experimental design consisted of 30 plots (1.5 × 4 = 6 m²) according to a randomized block design with three replicates. In November, winter wheat (*Triticum aestivum* L. var. Ceyhan-99) was sown in rows 15 cm wide at a seeding rate of 21 kg ha⁻¹ two months before OMW application. Fertilizers were applied to all the plots as 20:20:0 NPK 350 kg ha⁻¹ with the form of urea and di-ammonium phosphate, respectively. All the phosphorous and 1/3 of the nitrogen were applied at sowing and the remaining nitrogen was applied as a top-dress at the tillering (1/3) and jointing (1/3) stages of wheat as urea (1000 kg ha⁻¹) and ammonium nitrate (150 kg ha⁻¹). A single dose of R-OMW, E-OMW, and A-OMW was applied to the experimental plots at levels of 100 m³ ha⁻¹ year⁻¹. OMWs were spread annually in January using a 10-L manual sprayer with effort to apply the OMW homogeneously within the plot boundaries. The control plot was not amended with any water and served as the control in rainfed conditions. Soon after wheat physiological maturity was reached (mid-June), the above-ground wheat biomass was collected from each plot and growth parameters and grain yield were determined. The same practices were also repeated in the second year of the experiment (2016).

2.3. Soil sampling

Soils were sampled for chemical and microbiological analyses initially within 15 days of sowing and then 150 days later (after harvest). Soil samples for phytotoxicity tests were collected 1 day before and 1 day, 1 month, 2 months, and 3 months after OMW application. All sampling events included the collection of soil samples from random locations in each plot, from 0 to 20 cm, using

a metal core with internal diameter of 5 cm. Subsamples for microbiological analyses and phytotoxicity tests were kept at 4 °C and other subsamples were air-dried and used for chemical analyses.

2.4. OMW analysis

The pH and electrical conductivity of OMW samples were measured using a pH-meter (WTW 526) and conductivity meter (WTW 720), respectively. COD was measured by colorimetric method (5220 D) as explained in detail by the APHA (2012). Total phenols were measured using the Merck/WTW 14551 phenol reagent kits with a Photometer Nova 60/Spectroquant. Total N and P were analyzed by using WTW 14551 reactive kits (Merck). Total K, Ca, and Na were determined flame-photometrically, while Mg, Fe, Cu, Zn, and Mn were determined by atomic absorption spectrometry after wet digestion of OMW with HNO₃-HClO₄ (4:1) mixing solution (Kacar and Inal, 2010). HPLC (Alliance 2690, Waters) was employed to monitor the concentrations of the most common toxic phenolic compounds, e.g., tyrosol, hydroxytyrosol, and caffeic acid. Separation was achieved on an AC18-R reverse phase column (stainless steel, 250 × 4.6 mm, i.d. 5 µm), while detection was achieved through a diode array detector set at 320 nm. A mobile phase consisting of 0.02% TFA in water and 0.02% TFA in methanol was used. The flow rate was 0.5 mL min⁻¹. The column temperature was 25 °C and the injection volume was arranged to be 10 µL. For tyrosol and hydroxytyrosol, in the mobile phase 0.1% phosphoric acid and 70% acetonitrile were dissolved in water. The flow rate was adjusted to 0.5 mL min⁻¹ and it was measured at a wave length of 280 nm using a diode array detector with gradient conditions given for caffeic acid as mentioned above. The column temperature was 40 °C while the injection volume was 20 µL. The polyphenolic compounds were tentatively identified by matching their elution times from the column to those of standard stock solutions.

2.5. Physicochemical soil analysis

Soil texture analysis was performed by the hydrometer method (Bouyoucos, 1962). Total soluble salt, organic C (C_{org}), and pH (saturated) were determined according to Rhoades (1996), Nelson and Sommers (1982), and Thomas (1996), respectively. Total N and available P were determined by the Kjeldahl method (Bremner and Mulvaney, 1982) and Olsen's sodium bicarbonate method (Olsen and Sommers, 1982), respectively. Available K and Ca were measured by flame photometer after 1 mol L^{-1} $CH_3CO_2NH_4$ neutral extraction (Helmke and Sparks, 1996; Suarez, 1996). Available Fe, Mn, Cu, and Zn in DTPA extracts and Mg in 1 mol L^{-1} $CH_3CO_2NH_4$ extract were detected by atomic absorption spectroscopy (Lindsay and Norwell, 1978; Suarez, 1996).

2.6. Soil biological analysis

A fumigation-extraction method was used to estimate microbial biomass C (MB-C) and N (MB-N), with extractable C and N converted to microbial C and N using standard factors (Vance et al., 1987). Soil was fumigated with ethanol-free chloroform for 24 h. Fumigated and unfumigated soil samples were then extracted with 0.5 M K_2SO_4 .

After filtration, organic C in soil extracts was oxidized by dichromate digestion. The amount of dichromate left was back-titrated with an iron (II) ammonium sulfate complex solution (Kalembasa and Jenkinson, 1973). MB-C was calculated by the following formula:

$$MB - C = \frac{E_C}{k_{EC}}$$

where EC is the difference between the C extracted from the fumigated and nonfumigated samples and $k_{EC} = 0.45$ is the factor that converts the organic C flush to microbial C (Sparling, 1985). MB-N concentration was determined as the difference in total N content in the fumigated and nonfumigated extracts by the following formula:

$$MB - N = \frac{E_N}{k_{EN}}$$

where E_N is the difference between the N extracted from the fumigated and nonfumigated samples and $k_{EN} = 0.54$ is the fraction of biomass N-mineralized (Brookes et al., 1985). Total N in soil extracts was measured under strong acidic conditions by Kjeldahl digestion (Pruden et al., 1985). Basal soil respiration (BSR) was measured by the titration method (Isermeyer, 1952). Soil samples were incubated in a closed vessel at 25 °C for 24 h. The CO_2 produced was absorbed in NaOH and quantified by titration. N-mineralization (N-min) was assayed according to the method of Keeney (1982). This method involves the incubation of a sample under waterlogged conditions at 50 °C. At the end of 7 days, NH_4-N released from the soil was determined by modified Berthelot reaction.

Dehydrogenase (DHG) activity was assayed using the modified method of Thalmann (1968). Soil samples were suspended in a triphenyltetrazolium chloride solution and incubated for 16 h at 25 °C. The triphenylformazan (TPF) produced was extracted with acetone and measured photometrically at 546 nm. β -Glucosidase activity (GLU) was measured using the method of Hoffmann and Dedekam (1966). After the addition of β -glucosido-saligenin (salicin) as substrate, soil samples were incubated for 3 h at 37 °C and the saligenin released from the substrate was determined calorimetrically at 578 nm after coloring with 2,6-dibromchinon-4-chlorimide. Alkaline phosphatase (ALKP) activity was assayed using the method of Eivazi and Tabatabai (1977). After the addition of a buffered p-nitro phenyl phosphate solution (pH 11), soil samples were incubated for 1 h at 37 °C. The p-nitro phenol released by phosphomonoesterase activity was extracted and colored with sodium hydroxide and assigned photometrically at 400 nm. For the determination of urease activity (UR), the soil samples were incubated with a 79.9 mM urea solution for 2 h at 37 °C. Released ammonium was extracted with 2 M KCl solution and determined colorimetrically by a modified Berthelot reaction (Kandeler and Gerber, 1988). Nitrate reductase (NR) and arylsulfatase (ARS) were measured as described by Abdelmagid and Tabatabai (1987) and Kandeler (1996) using KNO_3 as the substrate and by Tabatabai and Bremner (1970) using p-nitrophenyl sulfate as the substrate, respectively.

2.7. Wheat grain and yield analyses

Wheat grain samples taken from each plot during harvest were ground to a fine powder using a microfine grinder with a screen-hole size of 0.25 mm. The milled plant samples were analyzed for total nitrogen using a modified micro-Kjeldahl digestion procedure (Bremner and Mulvaney, 1982). Total P was determined using the vanadate/molybdate method (yellow method) and K by flame photometry (Chapman and Pratt, 1961). Grain yield was first determined for each plot after harvest and then converted $kg ha^{-1}$.

2.8. Phytotoxicity analyses

Phytotoxicity of OMWs was evaluated in both OMW-treated soil and control soil, from which samples were taken from the experimental area on the dates specified in Section 2.3 by static-type germination assays using cress seeds (*Lepidium sativum* L.). Four milliliters of the respective soil saturation extract was placed on Whatman No. 1 filters and inserted into 90-mm glass petri dishes. Ten seeds were placed in each dish with three replicates. The petri dishes were wrapped with a polyethylene bag in order to prevent desiccation and passage of volatiles among treatments. The germination was conducted for 5 days in the dark at 25 °C. After incubation, the number of germinated seeds and their root lengths were used to

determine the germination index (GI) by the following formula:

$$GI = 100 \times \frac{Gs}{Gc} \times \frac{Ls}{Lc}$$

where Gs and Gc are germinated seeds in the sample and control, and Ls and Lc are mean root elongation in the sample and control, respectively (Piotrowska et al., 2011).

2.9. Statistical analyses

The data were analyzed by two-way multivariate analysis of variance (MANOVA), using OMW treatment and sampling time as factors in order to evaluate the effectiveness of these independent variables. In detail, sampling time was used as the fixed factor and OMW treatment as the random factor nested within time. In addition, the data were analyzed by one-way ANOVA using OMW treatment and time as the factors, and the means were compared by Duncan's test ($P < 0.05$) in order to evaluate the effect of independent variables on dependent variables. Principal component analysis of microbial parameters was performed using varimax rotation with Kaiser normalization in order to reduce the number of variables of a dataset while preserving as much information as possible. Initial components were extracted regarding their eigenvalues (≥ 1). All statistical analyses were performed with SPSS 16.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Chemical properties of OMWs

Chemical properties of R-OMW have changed depending on the treatment processes used (Table 2). The total concentrations of organic C, N, P, Cu, Zn, and phenol of R-OMW decreased after both treatment processes (advanced and economical), while the EC levels and the total amounts of K, Na, and Ca increased. The total concentrations of Mg, Fe, and Mn of R-OMW increased after the advanced treatment process while they decreased after the economical treatment process. The pH value of E-OMW increased to 10.5 due to the use of CaOH in the treatment process while the pH of A-OMW was more acidic (4.44) than that of R-OMW. Total phenols were removed by 86% and 60% after economical and advanced treatment processes, respectively. Polyphenolic compounds such as tyrosol, hydroxytyrosol, and caffeic acid, which are abundant in OMW, were considerably removed. While 67% of caffeic acid, 97% of hydroxytyrosol, and 30% of tyrosol was removed by economic treatment, these values were 43%, 51%, and 80% for caffeic acid, hydroxytyrosol, and tyrosol in the advanced treatment process, respectively (Table 2). Calcium hydroxide was used for chemical treatment of OMW and added by anionic polyelectrolytes for chemical coagulation/flocculation in the economical treatment process (Azbar et al., 2004). In the advanced treatment process, metal oxide photocatalysts, such as

Table 2. Main properties of OMW before and after economical and novel advanced treatment processes. Δ indicates the difference percentages between R-OMW and the treated OMWs.

Parameters	R-OMW	E-OMW		A-OMW		
		Δ (%)		Δ (%)		
pH	4.93	10.51	+53	4.44	-10	
EC (dS m ⁻¹)	7.26	10.49	+31	13.63	+47	
TOC (%)	2.70	1.83	-32	1.60	-41	
Total (mg L ⁻¹)	N	330	37	-89	50	-85
	P	630	19	-97	45	-93
	K	3520	5680	+61	5114	+45
	Na	190.0	258.6	+36	324.7	+70
	Ca	541.6	2860.0	+429	223.2	+59
	Mg	234.7	72.5	-69	321.6	+37
	Fe	9.76	0.33	-97	72.04	+638
	Cu	0.85	0.01	-99	0.17	-80
	Mn	2.93	0.28	-90	3.49	+19
	Zn	2.83	0.29	-90	2.22	-21
	Total phenols	660	93	-86	264	-60
	Caffeic acid	7.07	2.31	-67	4.00	-43
	Hydroxytyrosol	112.60	15.70	-97	55.20	-51
	Tyrosol	172.70	121.90	-30	34.80	-80

EC: Electrical conductivity, TOC: total organic carbon.

TiO₂ at semiconductor nanoscale (1–50 μ m), are effective in treating wastewater with UV light by forming a series of oxidation reactions that convert organic compounds into carbon dioxide and water (Comparelli et al., 2005; Fouad et al., 2006). As can be observed in Figures 1 and 2, the chromatograms show the disappearance of phenolic monomer peaks identified in R-OMW.

3.2. Chemical characteristics of OMW-treated soils

R-OMW and treated (E-OMW and A-OMW) OMW applications had different effects on the chemical properties of the experimental soils. In addition, these effects showed variation and changed over time (Table 3). Analysis of variance showed that the applications of R-OMW and treated OMWs did not affect the levels of pH and EC in the first year of the experimental soils. However, pH and EC values of the soils significantly increased compared to the control plot after 15 days of OMW applications in the second year (2016-1). Thereafter, for the 2016-2 sampling time, EC levels remained higher in the soils treated with OMWs while soil pH showed no significant differences between the control and treated soils. It was determined that EC levels of soils treated with OMWs

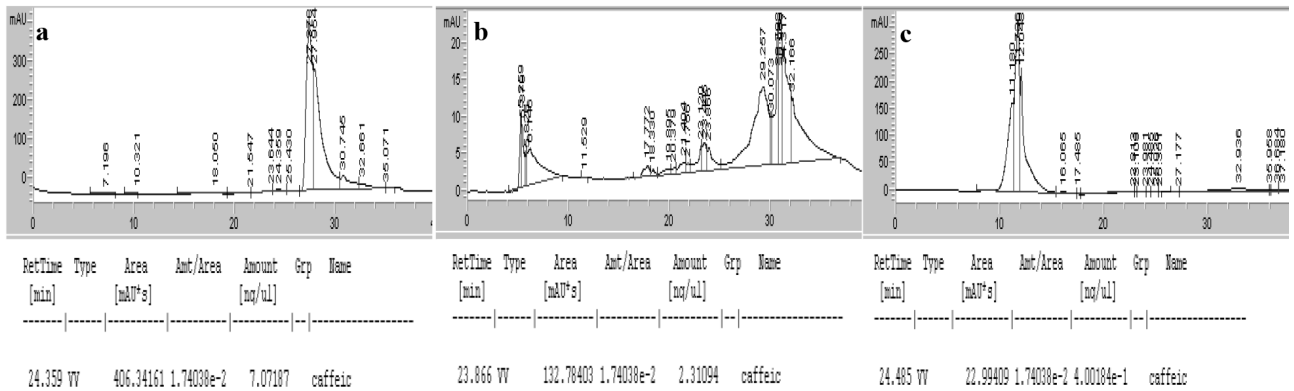


Figure 1. Chromatographic profile of (a) R-OMW, (b) E-OMW, and (c) A-OMW for caffeic acid.

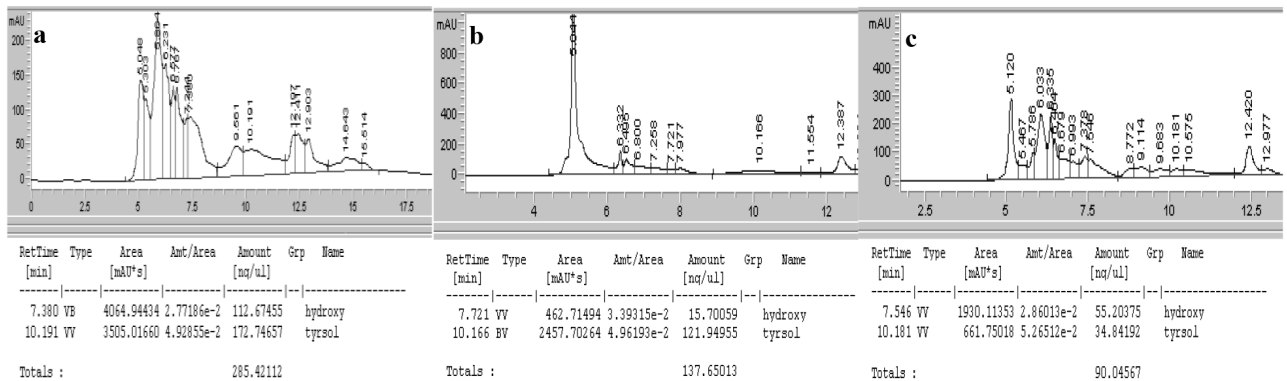


Figure 2. Chromatographic profile of (a) R-OMW, (b) E-OMW, and (c) A-OMW for hydroxytyrosol and tyrosol.

significantly increased during the entire experiment time. OMW applications did not have any effect on the contents of TOC and TN of soils in both years. Compared to the control, extractable P was greater in the soil with R-OMW applications at all sampling times. The most noticeable changes were the significant increases of extractable K after OMW applications due to the high potassium content. Of the other extractable ions, the amounts of Ca, Mg, and Na were not changed by OMW applications and time.

3.3. Microbial properties of soil amended with OMWs

Analysis of variance showed that OMW applications significantly increased the amounts of MB-C and MB-N compared to the control in all sampling periods (Table 4). As compared to the control and R-OMW applications, the addition of both E-OMW and A-OMW caused a significant increase in the ratio of MB-C to total organic C (MB-C/TOC) in the first year of the experiment. However, OMW-treated soils had higher MB-C/TOC ratios compared to the control plot in the last sampling period (2016-2). Similar to the increase in MB-C/TOC ratio, the ratio of MB-N to total N (MB-N/TN) was also increased in all sampling periods with OMW applications except for the 2015-2 sampling period. Higher amounts

of MB-C and the ratio of MB-C/TOC were determined in the first sampled soils (2015-1) while MB-N and the ratio of MB-N/TN were higher in the last sampled soils (2016-2). As compared to the control, OMW applications significantly increased CO₂ release from soils in the 2015-1, 2015-2, and 2016-1 sampling periods. However, in the last sampling period (2016-2), the differences among the treatments including the control disappeared. The application of OMWs caused a significant increase in N-min compared to the control in the first sampling periods of both years. The effect of OMW applications on N-min of the soils disappeared for the second sampling times (2015-2 and 2016-2)

3.4. Enzyme activities of soil amended with OMWs

DHG and NR, which are involved in redox soil reactions, had different behaviors in response to OMW applications (Table 5). Significant short- and long-term changes of DHG were observed in the amended soils with OMWs. Compared to the control, both R-OMW and the treated OMWs had significantly increased DHG at all sampling times while the NR activity in the soils was raised by only R-OMW application for the first sampling times (2015-1 and 2016-1). The analysis of variance did not show

Table 3. Some chemical properties of OMW-amended soils.

Parameter	Treatment	2015-1	2015-2	2016-1	2016-2
		15 days after OMW applications	5 months after OMW applications	15 days after OMW applications	5 months after OMW applications
pH	Control	7.69 a A**	7.52 a B	7.73 b A	7.73 a A
	R-OMW	7.67 a B	7.54 a C	7.82 a A	7.75 a AB
	E-OMW	7.76 a B	7.54 a C	7.91 a A	7.75 a B
	A-OMW	7.69 a B	7.54 a C	7.87 a A	7.72 a B
EC* ($\mu\text{S cm}^{-1}$)	Control	273 a A	274 a A	241 c A	280 b A
	R-OMW	258 a C	321 a AB	297 b BC	363 a A
	E-OMW	255 a C	297 a B	303 b B	345 a A
	A-OMW	288 a B	301 a AB	335 a AB	342 a A
TOC (g kg^{-1})	Control	8.21 a A	7.83 a A	6.77 a A	7.83 a A
	R-OMW	8.26 a A	7.93 a A	7.89 a A	8.43 a A
	E-OMW	7.45 a A	7.81 a A	6.90 a A	7.73 a A
	A-OMW	7.08 a B	8.02 a AB	7.56 a AB	8.47 a A
TN (g kg^{-1})	Control	0.86 a A	0.84 a A	0.86 a A	0.82 a A
	R-OMW	0.88 a A	0.84 a A	0.85 a A	0.86 a A
	E-OMW	0.86 a A	0.84 a A	0.77 a A	0.80 a A
	A-OMW	0.89 a A	0.86 a A	0.82 a A	0.85 a A
Extractable P (mg kg^{-1})	Control	17.87 b A	13.23 b A	13.04 ab A	14.87 b A
	R-OMW	22.54 a A	14.53 a C	16.28 a B	17.07 a B
	E-OMW	14.84 b A	12.12 b B	9.64 b C	10.03 c C
	A-OMW	15.80 b A	11.91 b B	12.84 ab B	14.01 b AB
Extractable K (mg kg^{-1})	Control	162.2 b B	237.7 b A	215.0 c A	226.5 b A
	R-OMW	262.7 a A	321.3 a A	307.5 b A	308.6 a A
	E-OMW	261.1 a B	324.6 a AB	336.2 ab A	313.5 a AB
	A-OMW	272.5 a AB	300.0 a BC	380.4 a A	343.0 a AB
Extractable Ca (mg kg^{-1})	Control	2037 a BC	2579 a AB	3080 a A	1771 a C
	R-OMW	2407 a B	2213 a BC	3333 a A	1683 a C
	E-OMW	1953 a C	2420 a B	3013 a A	1780 a C
	A-OMW	2273 a BC	2404 a B	3067 a A	1746 a C
Extractable Mg (mg kg^{-1})	Control	300 a A	471 a A	387 a A	307 a A
	R-OMW	292 a A	440 a A	362 ab A	222 a A
	E-OMW	303 a B	571 a A	334 b B	305 a B
	A-OMW	309 a A	352 a A	357 ab A	299 a A
Extractable Na (mg kg^{-1})	Control	24.7 a C	52.6 a B	36.8 a C	82.8 a A
	R-OMW	24.7 a C	44.1 a B	32.9 a BC	70.3 a A
	E-OMW	24.7 a B	48.7 a B	36.2 a B	81.3 a A
	A-OMW	32.5 a C	52.6 a B	36.1 a C	65.6 a A

*EC: Electrical conductivity, TOC: total organic C; TN: total N.

**: Lowercase letters in each vegetation period represent statistical differences between treatments; uppercase letters in each vegetation period represent the statistical difference between sampling periods ($P = 0.05$, Duncan's test).

Table 4. Some microbial properties of control and OMW-amended soils.

Parameter	Treatment	2015-1	2015-2	2016-1	2016-2
		15 days after OMW applications	5 months after OMW applications	15 days after OMW applications	5 months after OMW applications
MB-C* (mg kg ⁻¹)	Control	195.8 b A**	150.1 b AB	76.6 b B	123.3 b AB
	R-OMW	308.8 a A	257.3 a AB	117.0 a B	278.3 a AB
	E-OMW	384.4 a A	249.0 a AB	154.2 a B	234.7 a AB
	A-OMW	326.8 a A	222.7 a AB	118.1 a B	277.9 a AB
MB-N (mg kg ⁻¹)	Control	24.5 b C	53.3 b B	88.2 b A	52.1 b B
	R-OMW	51.0 a C	86.7 a B	88.2 a B	140.3 a A
	E-OMW	60.9 a C	84.3 a B	81.0 a B	124.3 a A
	A-OMW	65.5 a C	84.7 a B	95.8 a B	155.0 a A
MB-C (mg)/TOC (g)	Control	23.8 c A	19.2 b B	11.3 c C	15.7 b B
	R-OMW	37.4 b A	22.4 b C	14.8 b D	33.0 a B
	E-OMW	51.6 a A	31.9 a B	22.3 a C	30.4 a B
	A-OMW	46.2 a A	27.8 a C	15.6 b D	32.8 a B
MB-N (mg)/TN (g)	Control	28.5 b C	103.2 a A	79.3 b B	63.5 c B
	R-OMW	58.0 a C	78.9 b C	103.8 a B	163.1 b A
	E-OMW	70.8 a C	100.4 a B	105.2 a B	155.4 b A
	A-OMW	73.6 a C	98.5 a B	116.8 a B	182.4 a A
BSR (mg C 100 g ⁻¹ 24 h ⁻¹)	Control	9.9 b B	8.5 b B	12.0 b A	9.6 a B
	R-OMW	14.4 a A	14.7 a A	17.2 a A	9.9 a B
	E-OMW	13.8 a B	15.7 a A	16.6 a A	11.1 a B
	A-OMW	14.5 a A	15.6 a A	15.0 a A	12.4 a A
N-Mineralization ($\mu\text{g NH}_4\text{-N g}^{-1}\text{ day}^{-1}$)	Control	3.66 b A	2.79 a B	3.24 b A	2.32 a B
	R-OMW	4.42 ab A	2.96 a B	3.68 ab A	2.48 a B
	E-OMW	4.80 ab A	2.93 a B	4.44 a A	2.40 a B
	A-OMW	5.36 a A	3.46 a AB	3.93 a B	2.48 a B

*MB-C: Microbial biomass-C; MB-N: microbial biomass-N, TOC: total organic C; TN: total N; BSR: basal soil respiration. **: Lowercase letters in each vegetation period represent the statistical difference between treatments; uppercase letters in each vegetation period represent the statistical difference between sampling periods (P = 0.05, Duncan's test).

significant differences in hydrolytic enzymes (ALPK, GLU, UR, and ARS) between OMW-amended and control soils. The highest ALPK values were obtained in the last soil samples while the highest DHG values were seen in the first soil samples.

3.5. Phytotoxicity test

Compared to the control, the applications of OMW significantly decreased GI in soils taken 1 day after applications. The GI value of soil amended with R-OMW decreased almost 50%, while the other treatments (E-OMW and A-OMW) had higher GI values (Table 6). However, an increase of GI values was observed with increasing time, and after 1 month, the complete recovery of the soil germination occurred with all OMW applications. This situation also continued in subsequent times.

3.6. Wheat yield and leaf nutrient concentrations

The applications of OMWs significantly (P < 0.01) affected the wheat yield, but nonsignificant differences were obtained for N, P, and K concentrations of wheat (Table 7). Compared to the control, wheat yield increased by a 2-year average of 13% in soils amended with E-OMW and A-OMW. R-OMW application could not provide an increase over the control and had a similar effect as the control on yield. Yield values significantly decreased in 2017 depending on climatic conditions.

4. Discussion

4.1. Soil physicochemical parameters

The applications of OMW to soil caused significant changes in pH, EC, P_{ext}, and K_{ext} while there were no

Table 5. Enzyme activities of control and OMW-amended soils.

Parameter	Treatment	2015-1	2015-2	2016-1	2016-2
		15 days after OMW applications	5 months after OMW applications	15 days after OMW applications	5 months after OMW applications
DHG* ($\mu\text{g TPF g}^{-1}$)	Control	198.9 b A**	79.1 b B	101.5 b B	101.2 b B
	R-OMW	237.7 ab A	112.4 a B	123.3 a B	147.7 a B
	E-OMW	274.1 a A	109.9 a B	185.6 a AB	169.5 a AB
	A-OMW	250.8 ab A	124.9 a B	145.4 a B	182.2 a B
NR ($\mu\text{g N g}^{-1} 2 \text{ h}^{-1}$)	Control	0.25 b A	0.16 a A	0.19 b A	0.17 a A
	R-OMW	0.36 a A	0.22 a B	0.29 a AB	0.19 a B
	E-OMW	0.22 b A	0.17 a A	0.15 b A	0.16 a A
	A-OMW	0.18 b A	0.20 a A	0.12 b B	0.15 a A
GLU ($\mu\text{g saligenin g}^{-1} 3 \text{ h}^{-1}$)	Control	75.3 a BC	109.7 a A	58.9 b C	81.9 a B
	R-OMW	92.8 a AB	115.3 a A	86.2 a B	89.5 a B
	E-OMW	79.2 a B	109.7 a A	77.6 ab B	92.2 a AB
	A-OMW	78.2 a B	138.6 a A	78.2 ab B	89.4 a B
ALKP ($\mu\text{g pNP g}^{-1} \text{ h}^{-1}$)	Control	443.5 a A	479.2 a A	445.7 a A	468.0 a A
	R-OMW	378.5 a B	504.4 a A	503.7 a A	492.4 a A
	E-OMW	370.8 a B	479.8 a A	484.9 a A	513.1 a A
	A-OMW	392.4 a B	527.0 a A	505.5 a AB	502.8 a AB
UR ($\mu\text{g N g}^{-1} 2 \text{ h}^{-1}$)	Control	62.7 a AB	54.1 a B	53.1 b B	78.9 a A
	R-OMW	74.7 a B	60.1 a C	100.1 a A	80.1 a B
	E-OMW	67.1 a BC	63.3 a C	113.2 a A	89.9 a AB
	A-OMW	72.9 a B	58.4 a B	120.7 a A	93.3 a AB
ARS ($\mu\text{g p-n.phenol g}^{-1} \text{ h}^{-1}$)	Control	254.1 a A	167.8 a B	190.9 a B	217.3 a AB
	R-OMW	249.0 a A	170.2 a B	239.1 a A	250.4 a A
	E-OMW	217.8 a AB	150.9 a B	195.3 a AB	253.1 a A
	A-OMW	214.6 a A	167.7 a A	256.1 a A	241.6 a A

* DHG: Dehydrogenase activity; NR: nitrate reductase activity; GLU: β -glucosidase activity; ALKP: alkaline phosphatase activity; UR: urease activity; ARS: aryl sulfatase activity. **: Lowercase letters in each vegetation period represent the statistical difference between treatments; uppercase letters in each vegetation period represent the statistical difference between sampling periods ($P = 0.05$, Duncan's test).

Table 6. GI of control and R-OMW, E-OMW, and A-OMW treated soils.

Time*	Control	R-OMW	E-OMW	A-OMW	Analysis of variance
Before 1 day	100	92.8	98.7	99.1	ns
After 1 day	100	49.7	75.8	82.8	**
After 1 month	100	89.5	92.0	91.2	ns
After 2 months	100	99.2	95.4	95.9	ns
After 3 months	100	99.5	96.1	95.1	ns

* Time: Soil sampling time in relation to OMW applications given in detail in Section 2.3.

** Significant at $P > 0.01$ level; ns: not significant.

Table 7. Effects of the applications of OMWs on yield and N, P, and K concentrations of wheat.

Parameter	Year 2016				Year 2017				Analysis of variance		
	Control	R-OMW	E-OMW	A-OMW	Control	R-OMW	E-OMW	A-OMW	T ^a	S ^a	T × S
Yield (kg ha ⁻¹)	6691 b	6145 b	7534 a	7544 a	3886 d	3770 d	4634 c	4559 c	**	**	ns
Total N (%)	1.87	1.91	1.91	1.90	1.86	1.97	1.97	2.01	ns	**	ns
Total P (%)	0.35	0.36	0.36	0.37	0.20	0.23	0.23	0.23	ns	**	ns
Total K (%)	0.34	0.33	0.35	0.33	0.37	0.39	0.38	0.40	ns	**	ns

^a T: Treatment; S: sampling time; values with the same letter within a row are not significantly different at a P < 0.05 level of probability.

** Significant at P < 0.01 probability levels; ns: not significant.

significant changes in TOC, TN, Ca_{ext}, Mg_{ext}, and Na_{ext} among the studied chemical parameters (Table 3). The effects of the applications of R-OMW and A-OMW with acidic pH and E-OMW with alkaline pH on soil pH and EC were significant 15 days after application in the second year of the experiment. Compared to the control, soil pH was increased by an average of 0.13 units by the addition of OMWs, but thereafter this pH increase was neutralized by soil buffering capacity. OMW applications caused small pH variations in the treated soils (Mechri et al., 2008; Mekki et al., 2009). These small pH variations were temporary according to Piotrowska et al. (2011). Di Serio et al. (2008) applied OMW at 160 m³ ha⁻¹ year⁻¹ and determined a small increase in pH, similar to our results. The authors suggested that this increase was potentially caused by the production of ammonia from bacterial breakdown of OMW. Unlike pH, the enhancing effect of OMW applications on soil EC continued over the next 5 months. Generally, the increase in soil EC after applying OMW was attributed to the presence of high salt concentrations in the OMWs. High EC values after OMW applications were maintained in the soil for up to 6 or 16 weeks depending on the application rate (Chiesura et al., 2005). Sierra et al. (2007) and Mekki et al. (2009) found that soil EC values were proportional to the volume of supplied OMW.

After both economical and advanced treatment processes, the total phosphorus content of R-OMW significantly decreased while total potassium content increased (Table 2). As a result, high extractable P concentrations were determined only in soils amended with R-OMW while high extractable K concentrations were determined in soils amended with both R-OMW and treated OMWs (Table 3). Belaqziz et al. (2016) found that after spreading untreated OMW in soil, the amounts of nutrients increased by 66% for P and 88% for K, respectively. Some authors (Chartzoulakis et al., 2006; Ayoub et al., 2014) considered the increased soil K levels as the most positive effect of OMW application on soil chemical properties. At the end of the experiment, we also

determined higher soil K amounts compared to the control by 36%, 38%, and 51% in soils amended with R-OMW, E-OMW, and A-OMW, respectively. However, the amounts of TOC, TN, and extractable Ca, Mg, and Na in soils were not affected significantly by OMW applications at all sampling times (Table 3). Similar results were obtained by Gamba et al. (2005) and Rusan et al. (2016). However, positive effects of OMW applications were reported for TOC (Lopez-Pineiro et al., 2011; Magdich et al., 2013) and for TN (Moraetis et al., 2011; Belaqziz et al., 2016). The variation in these results probably resulted from the different chemical characteristics of the OMWs used, type of soil, and crop involved.

4.2. Soil microbial parameters

The applications of OMW caused significant changes in MB-C, MB-N, BSR, and N-min as microbial parameters (Table 4). Microbial biomass in natural and disturbed ecosystems acts as reservoir of important labile pools of C and mineral nutrients from which nutrients are liberated after the death of the microorganisms (Singh et al., 2010). For this reason, the size of microbial biomass can be considered as an index of soil fertility, which depends on the rate of nutrient fluxes. Higher amounts of MB-C and MB-N compared to the control were determined in soils amended with OMWs at all sampling times. Similar results were also found for BSR, except for the last sampling period. The high content of mineral nutrients in the OMWs could justify the significant increases of MB-C, MB-N, and BSR. Significant increases of MB-C and MB-N were also found in soils amended with dephenolized OMW and crude OMW by Piotrowska et al. (2011). Microbial biomass has a rapid rate of turnover (Jenkinson and Ladd, 1981). Therefore, its changes are detectable long before they are measurable in the total organic matter, thus providing an early indication of long-term trends in the total organic C of soils (Powlson et al., 1987). Increases in MB-C and MB-N resulted in high MB-C/TOC and MB-N/TN ratios, especially in soils amended with treated OMWs. These high ratios reflect organic matter inputs to

the soils, the efficiency of conversion to MB-C, and the stabilization of organic C by the soil fractions (Sparling, 1992) and suggest better conditions for the development of microorganisms as well as for the mineralization of organic matter. Usually, the decrease in microbial quotients could be caused by decreased microbial biomass and/or partially disabled function of its ability to mineralize organic matter (He et al., 2003). BSR is an indicator of the mineralization capacity of the soil's microbial biomass. OMW applications significantly increased BSR with respect to the control soil. Such a "priming effect" indicated by BSR can be correlated with the ability of microorganisms to decompose different organic substrates and seems to be induced by the labile part of the organic matter applied by OMW (Di Serio et al., 2008). However, as most of the organic substrates were probably decomposed and total precipitation was very low in 2016, the effect of OMW applications on BSR was not significant in the 2016-2 soil samples. N-min significantly increased in a short time after OMW applications, but then decreased, probably due to the mineralization, immobilization, and plant uptake. Higher total N content of R-OMW (Table 2) compared to the treated OMWs did not result in a higher N-min rate. This is probably due to the presence of highly inhibitory substances such as phenol in R-OMW (Table 2) (Piotrowska et al., 2006).

The applications of OMW caused significant changes in only DHG and NRA and no significant changes in GLU, ALKP, UR, or ARS from the tested enzyme activities (Table 5). DHG occurs intracellularly in all living microbial cells (Yuan and Yue, 2012) and can be used as an indicator of overall soil microbial activity (Salazar et al., 2011). Compared to the control, DHG activity increased by about 20%, 38%, and 26% for the 2015-1 sampling time and 21%, 83%, and 43% for the 2016-1 sampling time with R-OMW, E-OMW, and A-OMW amendments, respectively. This effect can be attributed to greater microbial biomass due to the addition of available organic substrates that can promote the growth of indigenous microorganisms (Benitez, 2000; Kayikcioglu, 2018). In the process of denitrification, dissimilatory nitrate reductase catalyzes the first step by reducing NO_3^- to NO_2^- under anaerobic conditions (Schinner et al., 1996). This activity was significantly increased in soils taken 15 days after only R-OMW amendment. The increase in NRA activity indicated that anaerobe conditions can occur in soils with R-OMW.

Phytotoxicity tests showed that phytotoxic effects of OMWs on seed germination disappeared 1 month after applications. The phenolics in the OMWs are the main compounds that inhibit germination (El Hadrami et al., 2004; Quarantino et al., 2007). Isidori et al. (2005) determined that the highest phytotoxic effects on germination of watermelon and sorghum were caused by

catechol and hydroxytyrosol. A day after application, the treated OMWs, having lower phenol (Table 2), showed a smaller phytotoxic effect on the soils as compared to the R-OMW amendment. One month after application, no difference between OMW-amended and control soils was observed (Table 6). This finding can be attributed to the degradation of polyphenols in a defined time depending on environmental conditions (Barbera et al., 2013).

4.3. Principal component analysis (PCA) of microbial parameters

The spread of OMWs causes drastic and immediate changes in the physical and chemical properties of the soils. This phenomenon primarily concerns the uppermost aerated layer of the soil in which the essential biological processes occur (Gargouri et al., 2014). These changes also induce a microbial habitat and affect it while organic degradation with various enzymatic activity is already occurring (Kotsou et al., 2004; Bustamante et al., 2010).

Indeed, focusing on microbial changes also requires a temporal perspective since it is a complex process with different degradation dynamics at different numbers of days after treatment (DAT). In this sense, two separate PCAs with varimax rotation were realized on the results from 15 DAT and harvest samples in order to elucidate major variation patterns. Furthermore, PCA was used to evaluate the effects of OMW on microbial parameters, to reveal the time-varying effects of OMW on soil microbial parameters, and to determine which microbiological parameter or parameters could explain the degradation of OMW in soil. This gave insight regarding which kinds of microbial activity trend are induced due to different OMW applications at certain periods after treatment. The microbiological PCAs for the first and second samplings in both vegetation periods were evaluated cumulatively, involving the results of each year.

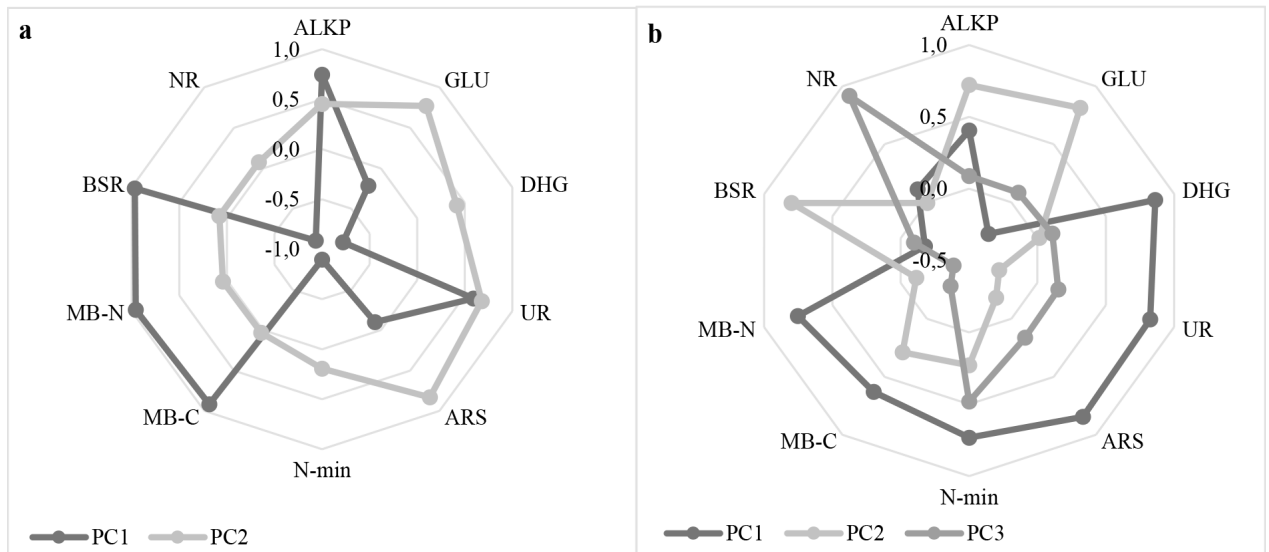
Two and three principal components were extracted for both sampling periods with <0.05 Bartlett significance and KMO values higher than 0.6 (Table 8). Over 70% of cumulative variance representation was achieved with those components.

PC1 of the early results (Figure 3a) was found to have the highest correlation with basal respiration (0.967), MB-N (0.959), and MB-C (-0.917), while PC2 had the highest correlation with GLU (0.772) and ARS activity (0.831). However, PC2, with the lower percentage of total variance, was found to present only these mentioned loading values significantly (>0.7). Interpreting harvest samples (Figure 3b), PC1 indicated the highest correlation with ARS (0.846), DHG (0.861), and UR activities (0.824). PC2 mainly represented the GLU (0.814) and ALKP activities (0.722) with BSR (0.798). PC3 was not found to have any significant (>0.7) loading values beside NR activity (0.916).

Table 8. The main principal components of soil microbial parameters.

Component	Rotation sums of squared loadings					
	Total 1st*	Total 2nd**	% of Variance 1st	% of Variance 2nd	Cumulative 1st	Cumulative 2nd
PC1	5.865	3.986	58.65	39.86	58.65	39.86
PC2	2.174	2.074	21.74	20.74	80.39	60.60
PC3	-	1.321	-	13.21	-	73.81

* Based on the results from soil samples taken at 15 DAT; **: Based on the results from soil samples taken at harvest.

**Figure 3.** Components in rotated space at (a) 15 DAT and (b) harvest.

After PCA, the highest portion of variance in 15 DAT results indicates a differentiation by stored microbiological supplies or relative quantitative properties (N-min, MB-C, DHG, MB-N, and BSR), which were ready to be easily transformed under agricultural practices. Conversely, the highest portion of variance in the harvest samples indicates that microbial dynamics of soils under OMW treatments were mainly differentiated by enzyme-specific microbial activity (and naturally with DHG). Briefly, at 15 DAT, treatments induced the variance in microbial parameters, which might explain the intensity of microbial activity due to different amounts of organic inputs. However, at the end of the experiment, more detailed observations became available due to induced variance in the type of microbial activity. This also supports the great temporal difference indicated in microbiological properties of soils (Tables 4 and 5), by using a concept of evaluation other than ANOVA.

It is known that especially treated OMWs may reduce the organic matter content of soils (Mekki et al., 2009; Moraetis et al., 2011) and the loss in TOC observed during

the early stages of incorporation. Moreover, important changes in microbial activity may be observed during/after the process following OMW treatment due to nutritional and physical supply sourced by organic decomposition (Bustamante et al., 2010). The wider-ranging changes in organic carbon content in the first (between 7.08 and 8.26 g kg⁻¹) period compared to the second period of sampling (between 7.81 and 8.02 g kg⁻¹) may also support this phenomenon (Table 3).

4.4. Grain yield of wheat

The wheat grain yield over the 2-year period showed that the application of the treated OMWs caused a significant increase in grain yield (Table 7). Although no significant difference in nutrient concentrations of wheat was measured between the control and the amended soils, total N and P concentrations of grain tended to increase with application of OMWs and were slightly higher in soils amended with A-OMW than the other amendments. Similar results were also found by Cereti et al. (2004) and Brunetti et al. (2007) and higher yield values were obtained in the soils amended with the treated OMWs compared to

those of the untreated OMW. The grain yield and quality characteristics of wheat can vary according to genetic capacity of the varieties (Krejčírová et al., 2007), cultivation conditions, agricultural applications in the period of grain filling (Branlard et al., 2001), nitrogen uptake capability (Baresel et al., 2008), and the competitiveness of weeds (Kaut et al., 2008). In 2016, the grain yield decreased at the same levels with all the amendments, including the control. It is thought that this low yield in 2016 resulted from higher temperatures in winter months and lower and irregular rainfall compared to 2015 (Table 1). Due to lack of precipitation in December, the newly germinated plants did not develop sufficiently. In addition, less rainfall in the spring may have affected the grain filling of the plants negatively. It has been reported that grain yield and yield characteristics are highly influenced by environmental conditions and may be different from year to year (Krejčírová et al., 2007).

4.5. Conclusions

Two-year application of raw and treated OMWs to an alluvial soil under wheat had different effects on the chemical and microbiological properties without negatively affecting wheat yield. Both R-OMW and the treated OMWs (E-OMW and A-OMW) had generally similar effects on soil chemical properties, except for available P content of the soil. However, the effect of the treated OMWs on microbiological properties of soil was more pronounced than the effect of R-OMW. The significant increases in the ratios of MB-C/TOC and MB-N/TN detected in the soils amended with the treated

OMWs suggest that the microorganisms use carbon and nitrogen in OMWs effectively and increase the microbial biomass. According to these results, this waste may not be toxic to soil microorganisms, at least at the application rate examined. Moreover, this positive effect was reflected by the yield values and higher wheat yields were obtained in soils amended with the treated OMWs compared to the control and R-OMW. Due to the fact that there was no significant difference between the two treatment methods in view of their effects on soil properties, it can be advised to use E-OMW since it is a more economical method. These results are important in terms of showing that toxic compounds and excessive organic load in R-OMW can be successfully removed by both treatment methods with no adverse effects on soil properties and wheat growth if the application rates are less than 100 m³ ha⁻¹. As a result, the use of treated OMW in agricultural soils was found to be a valuable alternative in the disposal of this waste. However, the results of this study are not sufficient for the emergence of legislation on the use of OMW in agricultural soils in Turkey. Studies with different perspectives are required for soils with different physical and chemical properties and different plants. Only in this way can recommendations on multifaceted effects of this waste on agricultural soils be developed.

Acknowledgment

This work was supported by the Scientific and Technological Research Council of Turkey (TÜBİTAK Grant Number 113O558).

References

- Abdelmagid HM, Tabatabai MA (1987). Nitrate reductase activity in soils. *Soil Biology and Biochemistry* 19: 943-946.
- APHA (2012). *Standard Methods for the Examination of Water and Wastewater*. 22nd ed. Washington, DC, USA: American Public Health Association.
- Azbar N, Bayram A, Filibeli A, Muezzinoglu A, Sengul F et al. (2004). A review of waste management options in olive oil production. *Critical Reviews in Environmental Science and Technology* 34: 209-247.
- Ayoub S, Al-Absi K, Al-Shdiefat S, Al-Majali D, Hijazeen D (2014). Effect of olive mill wastewater land-spreading on soil properties, olive tree performance and oil quality. *Scientia Horticulturae* 175: 160-166.
- Barbera AC, Maucieri C, Cavallaro V, Ioppolo A, Spagna G (2013). Effects of olive mill wastewater on soil properties and crops, a review. *Agricultural Water Management* 119: 43-10.
- Baresel JP, Zimmermann EG, Reents EHJ (2008). Effects of genotype and environment on N uptake and N partition in organically grown winter wheat (*Triticum aestivum* L.) in Germany. *Euphytica* 163 (3): 347-354.
- Belaqziz M, El-Abbassi A, Lakhal EK, Agrafioti E, Galanakis CM (2016). Agronomic application of olive mill wastewater: effects on maize production and soil properties. *Journal of Environmental Management* 171: 158-165.
- Benitez E, Melgar R, Sainz H, Gomez M, Nogales R (2000). Enzymes activities in rhizosphere of pepper (*Capsicum annuum* L.) grown with olive cake mulches. *Soil Biology and Biochemistry* 32: 1829-1835.
- Bouyoucos GJ (1962). A recalibration of the hydrometer method for making mechanical analysis of the soils. *Agronomy Journal* 54: 419-434.
- Branlard G, Dardevet M, Saccomano R, Lagoutte F, Gourdon J (2001). Genetic diversity of wheat storage proteins and bread wheat quality. In: Bedö Z, Láng L, editors. *Proceedings of the 6th International Wheat Conference, 5–9 June 2000; Budapest, Hungary. Wheat in a Global Environment: Developments in Plant Breeding*, Vol. 9. Dordrecht, the Netherlands: Springer, pp. 157-169.

- Bremner JM, Mulvaney CS (1982). Nitrogen-total. In: Page AL, Miller RH, Keeney DR (editors). *Methods of Soil Analysis: Part II. Chemical and Microbiological Properties*. Madison, WI, USA: American Society of Agronomy, pp. 595-641.
- Brookes PC, Landman A, Pruden G, Jenkinson DS (1985). Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method for measuring microbial biomass nitrogen in soil. *Soil Biology and Biochemistry* 17: 837-842.
- Brunetti G, Senesi N, Plaza C (2007). Effects of amendment with treated and untreated olive oil mill wastewaters on soil properties, soil humic substances and wheat yield. *Geoderma* 138: 144-152.
- Bustamante MA, Said-Pullicino D, Paredes C, Cecilia JA, Moral R (2010). Influences of winery-distillery waste compost stability and soil type on soil carbon dynamics in amended soils. *Waste Management* 30 (10): 1966-1975.
- Cereti CF, Rossini F, Federici F, Quarantino D, Vassilev N et al. (2004). Reuse of microbially treated olive mill wastewater as fertiliser for wheat (*Triticum durum* Desf.). *Bioresource Technology* 91: 135-140.
- Chapman HD, Pratt PF (1961). *Methods of Analysis for Soils, Plants and Waters*. Berkeley, CA, USA: University of California, Division of Agricultural Sciences.
- Chartzoulakis K, Psarras G, Moutsopoulou M, Stefanoudaki E (2006). Application of olive mill wastewater on olive orchards: effects on soil properties, plant performance and the environment. In: *Proceedings of Olivebioteq 2006, Vol. II: Second International Seminar Biotechnology and Quality of Olive Tree Products Around the Mediterranean Basin, 5-10 November 2006*. Mazara Del Vallo, Italy: Università degli Studi di Palermo, Regione Siciliana Assessorato Agricoltura E Foreste, pp. 37-44.
- Chartzoulakis K, Psarras G, Moutsopoulou M, Stefanoudaki E (2010). Application of olive mill wastewater to a Cretan olive orchard: Effects on soil properties, plant performance and the environment. *Agriculture, Ecosystems & Environment* 138: 293-298.
- Chatzistathis T, Koutsos T (2017). Olive mill wastewater as a source of organic matter, water and nutrients for restoration of degraded soils and for crops managed with sustainable systems. *Agricultural Water Management* 190: 55-64.
- Chiesura A, Marano V, De Francesco P, Maraglino A (2005). Verso la sostenibilita della filiera olivicola: trattamento, recupero e valorizzazione dei sottoprodotti oleari. Rome, Italy: UNASCO (in Italian).
- Comparelli R, Fanizza E, Curri ML, Cazzoli PD, Mascolo G et al. (2005). UV-induced photocatalytic degradation of azo dyes by organic-capped ZnO nanocrystals immobilized onto substrates. *Applied Catalysis B Environmental* 60 (1-2): 1-11.
- Di Serio MG, Lanza B, Mucciarella MR, Russi F, Iannucci E et al. (2008). Effects of olive mill wastewater spreading on the physico-chemical and microbiological characteristics of soil. *International Biodeterioration & Biodegradation* 62: 403-407.
- Eivazi F, Tabatabai MA (1977). Phosphatases in soils. *Soil Biology and Biochemistry* 9: 167-172.
- El Hadrami A, Belaqqiz M, El Hassni M, Hanifi S, Abbad A et al. (2004). Physico-chemical characterization and effects of olive oil mill wastewaters fertirrigation on the growth of some Mediterranean crops. *Journal of Agronomy* 3 (4): 247-254.
- Erses Yay AS, Oral HV, Onay TT, Yenigün O (2012). A study on olive mill wastewater management in Turkey: a questionnaire and experimental approach. *Resources, Conservation and Recycling* 60: 64-71.
- Fouad OA, Ismail AA, Mohammed RM (2006). Zinc oxide thin films prepared by thermal evaporation deposition and its photocatalytic activity. *Applied Catalysis B Environmental* 62 (1-2): 144-149.
- Gamba C, Piovaneli C, Rapini R, Pezzarossa B, Ceccarini L et al. (2005). Soil microbial characteristics and mineral nitrogen availability as affected by olive oil waste water applied to cultivated soil. *Communications in Soil Science and Plant Analysis* 36: 937-950.
- Gargouri K, Masmoudi M, Rhouma A (2014). Influence of olive mill wastewater (OMW) spread on carbon and nitrogen dynamics and biology of an arid sandy soil. *Communications in Soil Science and Plant Analysis* 45 (1): 1-14.
- He ZL, Yang XE, Baligar VC, Calvert DV (2003). Microbiological and biochemical indexing systems for assessing quality of acid soils. *Advances in Agronomy* 78: 89-136.
- Helmke PA, Sparks DL (1996). Lithium, sodium, potassium, rubidium, and cesium. In: Sparks DL, Page AL, Helmke PA, Loeppert RH, Soltanpour PN et al. (editors). *Chemical Methods: Methods of Soil Analysis, Part 3*. Madison, WI, USA: Soil Science Society of America and American Society of Agronomy, pp. 551-574.
- Hoffman G, Dedekam M (1966). Eine methode zur kolorimetrischen bestimmung der β -glucosidaseaktivitat in böden. *Zeitschrift für Pflanzenernahrung und Bodenkunde* 108: 195-201 (in German).
- IOC (2018). *World Olive Oil Figures (Production)*. Madrid, Spain: International Olive Council.
- Isermeyer H (1952). Eine einfache methode zur bestimmung der bodenatmung und der karbonate im boden. *Zeitschrift für Pflanzenernahrung und Bodenkunde* 56: 26-38 (in German).
- Isidori M, Lavorgna M, Nardelli A, Parella A (2005). Model study on the effects of 15 phenolic olive mill wastewater constituents on seed germination and *Vibrio fischeri* metabolism. *Journal of Agricultural and Food Chemistry* 53: 8414-8417.
- Jenkinson DS, Ladd JN (1981). Microbial biomass in soil: measurement and turnover. In: Paul EA, Ladd JN (editors). *Soil Biochemistry*. Vol. 5. New York, NY, USA: Marcel Dekker, pp. 415-471.
- Kacar B, İnal A (2010). *Bitki Analizleri*. 2nd ed. Ankara, Turkey: Nobel Yayın (in Turkish).
- Kalembasa SJ, Jenkinson DS (1973). A comparative study of titrimetric and gravimetric methods for the determination of organic carbon in soil. *Journal of the Science of Food and Agriculture* 24: 1085-1090.

- Kandeler E (1996). Nitrate reductase activity. In: Schinner F, Öhlinger R, Kandeler E, Margesin R (editors). *Methods in Soil Biology*. New York, NY, USA: Springer-Verlag, pp. 176-179.
- Kandeler E, Gerber H (1988). Short-term assay of soil urease activity using colorimetric determination of ammonium. *Biology and Fertility of Soils* 6: 68-72.
- Kaut AHE, Mason HE, Navabi A, Donovan JTO, Spaner D (2008). Organic and conventional management of mixtures of wheat and spring cereals. *Agronomy for Sustainable Development* 28 (3): 363-371.
- Kavvadias V, Doula M, Komnitsas K, Liakopoulou N (2010). Disposal of olive oil mill wastes in evaporation ponds: effects on soil properties. *Journal of Hazardous Materials* 182: 144-155.
- Kayikcioglu HH (2018). Can treated wastewater be used as an alternative water resource for agricultural irrigation? Changes in soil and plant health after three years of maize cultivation in western Anatolia, Turkey. *Applied Ecology and Environmental Research* 16 (6): 8131-8161.
- Kayikcioglu HH, Sahin B (2013). Long-term effects of olive mill wastewater sludge on soil biological properties and yield efficiency: a case study of a fig grove in southern Turkey. *Journal of Food, Agriculture and Environment* 11 (2): 950-958.
- Keeney DR (1982). Nitrogen availability indices. In: Page AL, Miller RH, Keeney DR (editors). *Methods of Soil Analysis: Part II. Chemical and Microbiological Properties*. Madison, WI, USA: American Society of Agronomy, pp. 711-733.
- Kotsou M, Mari I, Lasaridi K, Chaptzipavlidis I, Balis C et al. (2004). The effect of olive mill wastewater (OMW) on soil microbial communities and suppressiveness against *Rhizoctonia solani*. *Applied Soil Ecology* 26: 113-121.
- Krejcirová L, Capouchova I, Petr J, Bicanova E, Famera O (2007). The effect of organic and conventional growing systems on quality and storage protein composition of winter wheat. *Plant, Soil and Environment* 53 (11): 499-505.
- Lindsay WL, Norwell WA (1978). Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Science Society of America Journal* 42 (3): 421-428.
- Lopez-Pineiro A, Albarran A, Rato Nunes JM, Pena D, Cabrera D (2011). Long-term impacts of de-oiled two-phase olive mill waste on soil chemical properties, enzyme activities and productivity in an olive grove. *Soil and Tillage Research* 114: 175-182.
- Magdich S, Abid W, Boukhris M, Rouina BB, Ammar E (2016). Effects of long-term olive mill wastewater spreading on the physiological and biochemical responses of adult Chemlali olive trees (*Olea europaea* L.). *Ecological Engineering* 97: 122-129.
- Magdich S, Ben Ahmed C, Jarboui R (2013). Dose and frequency dependent effects of olive mill waste water treatment on the chemical and microbial properties of soil. *Chemosphere* 93: 1896-1903.
- Mechri B, Ben Mariem F, Baham M, Ben Elhadj S, Hammami M (2008). Change in soil properties and the soil microbiological community following land spreading of olive mill wastewater affects olive trees key physiological parameters and the abundance of arbuscular mycorrhizal fungi. *Soil Biology and Biochemistry* 40: 152-161.
- Mekki A, Dhoubi A, Sayadi S (2006). Changes in microbial and soil properties following amendment with treated and untreated olive mill wastewater. *Microbiological Research* 161: 93-101.
- Mekki A, Dhoubi A, Sayadi S (2009). Evolution of several soil properties following amendment with olive mill wastewater. *Progress in Natural Science Materials International* 19 (11): 1515-1521.
- Mohawesh O, Mahmoud M, Janssen M, Lennartz B (2014). Effect of irrigation with olive mill wastewater on soil hydraulic and solute transport properties. *International Journal of Environmental Science and Technology* 11 (4): 927-934.
- Moraetis D, Stamati FE, Nikolaidis NP, Kalogerakis N (2011). Olive mill wastewater irrigation of maize: impacts on soil and groundwater. *Agricultural Water Management* 98 (7): 1125-1132.
- Murat Hocaoglu S (2015). *Zeytin Sektörü Atıklarının Yönetimi Projesi*. Gebze, Turkey: TÜBİTAK Marmara Araştırma Merkezi Çevre ve Temiz Üretim Enstitüsü (in Turkish).
- Nelson DW, Sommers LE (1982). Total carbon, organic carbon and organic matter: In: Page AL, Miller RH, Keeney DR (editors). *Methods of Soil Analysis: Part II. Chemical and Microbiological Properties*. Madison, WI, USA: American Society of Agronomy, pp. 539-579.
- Olsen SS, Sommers LE (1982). Phosphorous. In: Page AL, Miller RH, Keeney DR (editors). *Methods of Soil Analysis: Part II. Chemical and Microbiological Properties*. Madison, WI, USA: American Society of Agronomy, pp. 403-430.
- Piotrowska A, Antonietta Rao M, Scotti R, Gianfreda L (2011). Changes in soil chemical and biochemical properties following amendment with crude and dephenolized olive mill waste water (OMW). *Geoderma* 161: 8-17.
- Piotrowska A, Iamarino G, Rao MA, Gianfreda L (2006). Short-term effects of olive mill waste water (OMW) on chemical and biochemical properties of a semiarid Mediterranean soil. *Soil Biology and Biochemistry* 38: 600-610.
- Powelson DS, Brooks PC, Christensen BT (1987). Measurement of soil microbial biomass provides an early indication of changes in total soil organic matter due to straw incorporation. *Soil Biology and Biochemistry* 19: 159-164.
- Pruden G, Kalembasa SJ, Jenkinson DS (1985). Reduction of nitrate prior to Kjeldahl digestion. *Journal of the Science of Food and Agriculture* 36: 71-73.
- Quarantino D, D'Annibale A, Federici F, Cereti CF, Rossini F et al. (2007). Enzyme and fungal treatments and a combination thereof reduce olive mill wastewater phytotoxicity on *Zea mays* L. seeds. *Chemosphere* 66: 1627-1633.
- Rhoades JD (1996). Salinity: electrical conductivity and total dissolved solids. In: Sparks DL, Page AL, Helmke PA, Loeppert RH, Soltanpour PN et al. (editors). *Chemical Methods: Methods of Soil Analysis, Part 3*. Madison, WI, USA: Soil Science Society of America and American Society of Agronomy, pp. 417-436.
- Rusan MJ, Albalasmeh AA, Malkawi HI (2016). Treated olive mill wastewater effects on soil properties and plant growth. *Water, Air, & Soil Pollution* 227: 135.

- Salazar S, Sanchez L, Alvarez J, Valverde A, Galindo P et al. (2011). Correlation among soil enzyme activities under different forest system management practices. *Ecological Engineering* 37: 1123-1131.
- Schinner F, Öhlinger R, Kandeler E, Margesin R (1996). Enzymes involved in nitrogen metabolism. In: Schinner F, Öhlinger R, Kandeler E, Margesin R, editors. *Methods in Soil Biology*. New York, NY, USA: Springer-Verlag, pp. 162-165.
- S'habou R, Zairi M, Kallel A, Aydi A, Dhia HB (2009). Assessing the effect of an olive mill wastewater evaporation pond in Sousse, Tunisia. *Environmental Geology* 58: 679-686.
- Sierra J, Martí E, Garau MA, Cruanas R (2007). Effects of the agronomic use of olive oil mill wastewater field experiment. *Science of the Total Environment* 378: 90-94.
- Singh JS, Singh DP, Kashyap AK (2010). Microbial biomass C, N and P in disturbed dry tropical forest soils, India. *Pedosphere* 20 (6): 780-788.
- Sparling GP (1985). The soil biomass. In: Vaughan D, Malcolm RE (editors). *Soil Organic Matter and Biological Activity*. Dordrecht, the Netherlands: Martinus Nijhoff/Dr W Junk, pp. 223.
- Sparling GP (1992). Ratio of microbial biomass C to soil organic C as a sensitive indicator of changes in soil organic matter. *Australian Journal of Soil Research* 30: 195-207.
- Suarez DL (1996). Beryllium, magnesium, calcium, strontium, and barium. In: Sparks DL, Page AL, Helmke PA, Loeppert RH, Soltanpour PN et al. (editors). *Chemical Methods: Methods of Soil Analysis, Part 3*. Madison, WI, USA: Soil Science Society of America and American Society of Agronomy, pp. 575-602.
- Tabatabai MA, Bremner JM (1970). Arylsulfatase activity of soils. *Soil Science Society of America Journal* 34: 225-229.
- Thalmann A (1968). Zur methodik der bestimmung der dehydrogenaseaktivitaet im boden mittels Triphenyltetrazoliumchlorid (TTC). *Landwirtschaftliche Forschung* 21: 249-258 (in German).
- Thomas GW (1996). Soil pH and soil acidity. In: Sparks DL, Page AL, Helmke PA, Loeppert RH, Soltanpour PN et al. (editors). *Chemical Methods: Methods of Soil Analysis, Part 3*. Madison, WI, USA: Soil Science Society of America and American Society of Agronomy, pp. 475-490.
- Vance ED, Brookes PC, Jenkinson DS (1987). An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry* 19: 703-707.
- Yuan BC, Yue DX (2012). Soil microbial and enzymatic activities across a chronosequence of Chinese pine plantation development on the loess plateau of China. *Pedosphere* 22: 1-12.