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Original Article

Influence of Olive Mill Wastewater Incubated Organic Manures on Soil Chemical and Microbial Characteristics

Kemal Doğan[1], Necat Ağca[2], Nadir Doğru[3], Ali Sarıoğlu[4]✉

[1,2,3]Hatay Mustafa Kemal University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, Hatay/Serinyol, TÜRKİYE
[4]Harran University Faculty of Agriculture Department of Soil Science and Plant Nutrition, Haliliye/Şanlıurfa, TÜRKİYE

[1] https://orcid.org/0000-0002-5448-0009, [2] https://orcid.org/0000-0003-4864-844X, [3] https://orcid.org/0000-0003-2342-6509, [4] https://orcid.org/0000-0001-6269-4990

✉asarioglu@harran.edu.tr

ABSTRACT

In this research, it was aimed to increase the resistance of farm manure (FM) and vermicompost (VC) to microbial decomposition, to which olive mill wastewater (OMW) was added at different rates, and thus to extend the persistence of organic matter in the soil. The study was carried out in two stages. In the first stage, organic materials mixed with OMW at different rates were incubated for 30 days and some chemical and biological properties of the resulting organic mixtures were determined. In the second stage, the organic mixtures obtained as a result of incubation were applied to the soil at a rate of 10%, a pot trial was established and soybean plants were grown. The incubation results showed that OMW application increased pH by approximately 3–9% and electrical conductivity (EC) by 15–40%, without causing a significant reduction in organic matter content, indicating a slowdown in microbial decomposition. In the pot experiment, soil pH and EC increased by 4–12% and 20–45%, respectively. Macronutrient contents, particularly P_2O_5 and K_2O, increased by 15–60% in VC+OMW treatments, whereas FM+OMW combinations showed more variable responses depending on OMW dose. Micronutrient elements generally increased (up to 10–35%) under VC+OMW applications, while Mg exhibited slight decreases due to ionic competition with K^+. Soil biological indicators increased by 18–30% with FM and VC applications alone; however, high OMW doses caused reductions of approximately 10–25% in microbial respiration, dehydrogenase activity, and microbial biomass carbon. Overall, the results indicate that low to moderate OMW doses combined with organic fertilizers can improve nutrient availability and enhance organic matter stability while maintaining soil microbial functionality.

Key words: Olive mill wastewater, microbial decomposition of soil organic matter, increasing the durability of organic matter, vermicompost, farm manure

Zeytin Karasuyu ile İnkübe Edilmiş Organik Gübre uygulamalarının Bazı Kimyasal ve Mikrobiyal Toprak Özelliklerine Etkileri

ÖZ

Bu araştırmada, farklı oranlarda zeytin karasuyu (OMW) eklenen çiftlik gübresi (FM) ve solucan gübresinin (VC) mikrobiyal ayrışmaya karşı direncinin artırılması ve böylece organik maddenin topraktaki kalıcılık süresinin uzatılması amaçlanmıştır. Çalışma iki aşamalı olarak yürütülmüştür. İlk aşamada, farklı oranlarda OMW karıştırılmış organik materyaller 30 gün süreyle inkübasyona tabi tutulmuş ve elde edilen organik karışımların bazı kimyasal ve biyolojik özellikleri belirlenmiştir. İkinci aşamada ise, inkübasyon sonucu elde edilen organik karışımlar %10 oranında toprağa uygulanarak saksı denemesi kurulmuş ve soya bitkisi yetiştirilmiştir. İnkübasyon denemesi sonucunda, OMW uygulamasının organik madde içeriğinde önemli bir azalmaya neden olmadan pH'ı yaklaşık %3–9 ve elektriksel iletkenliği (EC) %15–40 artırdığını, yani mikrobiyal ayrışmanın yavaşladığını gösterdi. Saksı deneyinde toprağın pH'ı ve EC'si sırasıyla %4–12 ve %20–45 arttı. Makrobesin içerikleri, özellikle P_2O_5 ve K_2O, VC+OMW tedavilerinde %15-60 oranında artarken FM+OMW kombinasyonları, OMW dozuna bağlı olarak daha değişken tepkiler gösterdi. VC+OMW uygulamaları altında mikro besin elementleri genel olarak (%10-35'e kadar) artarken, Mg, K^+ ile iyonik rekabet nedeniyle hafif düşüşler sergiledi. Yalnızca FM ve VC uygulamalarıyla toprağın biyolojik göstergeleri %18–30 arttı; ancak yüksek OMW dozları mikrobiyal solunum, dehidrojenaz aktivitesi ve mikrobiyal biyokütle karbonunda yaklaşık %10-25 oranında azalmaya neden olmuştur. Genel olarak sonuçlar, organik gübrelerle birlikte düşük ila orta düzeyde zeytin yağı dozlarının, toprağın mikrobiyal işlevselliğini korurken besin kullanılabilirliğini artırabildiğini ve organik madde stabilitesini artırabildiğini göstermektedir.

Anahtar kelimeler: Zeytin karasuyu, toprak organik maddesinin microbiyal ayrışması, organik maddenin dayanıklılığının arttırılması, solucan gübresi, Çiftlik gübresi

INTRODUCTION

Olive oil production is of economic and cultural importance in many places, especially in countries in the Mediterranean climate zone. A significant amount of olive wastewater is generated during olive building production. Olive wastewater (OMW) is an environmental risk because it contains significant amounts of organic matter, phenolic compounds, acidity and salinity. When discharged directly onto the ground or into large bodies of water, it can increase oxygen demand, which can cause stress. (Montemurro et al., 2011).

Despite these environmental risks, OMW also represents a potentially valuable organic resource when applied to soil in a controlled and scientifically guided manner. Studies have reported that correct practices can increase soil organic matter, phosphorus (P), potassium (K), and nitrogen (N) content (Regni et al., 2021). Nevertheless, the agronomic benefits of OMW are closely related not only to the applied dose but also to its interaction with soil organic amendments. However, excessive and repeated application leads to negative effects such as increased salinity, phenolic toxicity and nutritional imbalance (Khalil et al., 2024).

Composting or stabilizing organic wastes to make them more resistant to microbial decomposition is a widely preferred practice to ensure longer persistence of these materials in the soil. Applying organic residues to soil can have positive effects on productivity by increasing carbon storage in the soil (Magdich et al., 2020). Long-term studies using materials composted with olive oil production waste have revealed that these practices lead to healing changes in both the chemical and biochemical properties of the soil (Regni et al., 2021).

There are various studies in Turkey examining the effects of OMW on soil health and focusing on its reflections on microbial activity, especially when applied together with VC and FM (Doğan et al., 2018). In these studies, it was reported that appropriate mixture ratios had significant effects on important biological indicators such as CO_2 production, dehydrogenase enzyme activity (DHA) and microbial biomass carbon (MBC) in the soil (Doğan et al., 2018). However, these studies mostly focus on short-term responses and direct soil applications. Information regarding the effects of pre-incubated OMW–organic fertilizer mixtures on organic matter stability and microbial decomposition remains limited.

Numerous studies conducted today show that the importance of organic matter for the soil ecosystem and the benefits it provides are now clearly demonstrated. However, it is emphasized in many studies that not only the amount of organic matter but also its permanence in the soil is at least as critical as its total content. Therefore, increasing the persistence of organic matter in soil is considered at least as important as increasing its total amount. While soil organic matter is an essential source of nutrients and energy for mesofauna and microflora, it can decompose quite rapidly under appropriate environmental conditions. Although the nutrients released during the decomposition process are beneficial for plant development and microbial activities, this rapid mineralization leads to a significant decrease in the organic matter level of the soil in the long term; As a result, the regulatory effects on soil structure, water retention capacity and productivity are weakened.

Soils in temperate and relatively humid regions, especially under the influence of the Mediterranean climate, have very favorable conditions in terms of microbial activity, causing the organic matter applied to the soil to decompose in a short time. Although it is not possible to directly control climatic conditions, it seems possible to increase the resistance of organic materials added to the soil against microbial decomposition. In this way, the capacity of organic matter to provide nutrients can be more balanced, while the permanence of its regulatory and healing effects on the physical and chemical properties of the soil can be increased. Under such conditions, strategies that slow down microbial decomposition are essential for sustaining soil organic matter levels.

In this study, OMW was combined with FM and VC in order to increase the resistance of these organic materials to microbial decomposition and thereby enhance their persistence in soil. OMW contains organic compounds that are difficult to manage and can be toxic, and its use with organic fertilizers can contribute to the stabilization of these materials. Organic mixtures prepared for this purpose were incubated for 30 days to ensure stabilization and then applied to the soil at a rate of 10%. Pot trials were set up under soybean vegetation to evaluate the effects on soil. Within the scope of these trials, various physical, chemical and biological soil properties were analyzed, especially considering the effects of OMW-modified organic materials on the basic microbial and chemical dynamics of the soil system.

MATERIALS AND METHODS

Materials

The study was conducted in the climate-controlled laboratory of the Department of Soil Science and Plant Nutrition, Faculty of Agriculture, Hatay Mustafa Kemal University, Turkey. Some initial properties of the soil used in the experiment were as follows: pH 7.75–7.85, electrical conductivity (salinity) 0.024–0.032%, lime content 25–27%, organic matter 0.86–1.74%, and texture classified as clay loam. Available potassium (K_2O) and phosphorus (P_2O_5) contents were 88–93 kg da^{-1} and 3.1–3.2 kg da^{-1}, respectively. Soil microbial parameters were also determined as CO_2 respiration 427 µg g^{-1} soil, dehydrogenase enzyme activity (DHA) 34.58 µg TPF g^{-1} soil, and microbial biomass carbon (MBC) 220 µg g^{-1} soil. Soil samples were air-dried, gently crushed, and passed through a 2mm sieve prior to analysis and experimental use.

As organic materials, VC and FM were used. The organic matter content of VC was 44%, with N–P–K contents of 2.6–0.18–0.25% and a pH of 7.6. The organic matter content of FM was 52%, with N–P–K contents of 1.7–0.16–0.71% and a pH of 8.2. OMW, a dark-colored liquid by-product of olive oil production characterized by high organic matter and phenolic compound contents, was mixed with VC and FM at different proportions. The OMW used in this study was obtained from a local olive oil factory located in the study area. The olive mill wastewater (OMW) consisted of approximately 90% water and 10% dry matter. On a dry matter basis, the solid fraction contained approximately 18% organic compounds, 2–5% sugars, 1–2% nitrogenous compounds, and 0.5–1.2% organic acids, along with minor amounts of oils, phenolic compounds, and pectins. Phenolic compounds were not individually characterized in this study and are referred to as total phenolic constituents based on general olive mill wastewater composition reported in the literature.

Methods

In the study, vermicompost (VC) and farmyard manure (FM) materials were mixed with OMW at three different ratios (1:0.5, 1:1, and 1:2). Each incubation unit consisted of 1 kg of organic material placed in plastic containers. The obtained mixtures were incubated at 28 °C for 30 days under field capacity moisture conditions, with daily weighing to maintain constant moisture levels. At the end of the incubation period, the mixtures were air-dried, prepared for analysis, and then mixed with soil at a 10% rate to establish pot experiments. Each pot contained 1 kg of air-dried soil, and the organic amendments were thoroughly mixed with soil on a dry weight basis.

Both incubation and pot trials were conducted in a randomized block design with 17 variants and three replications. The variants consisted of the control (S0), VC and FM alone (SVC, SFM), and their combinations with OMW (SVC-OMW1, SVC-OMW2, SVC-OMW3, SFM-OMW1, SFM-OMW2, SFM-OMW3). After adding soil and organic materials to the pots, soybean seeds were sown, and the experiments were terminated at the flowering stage. The experimental layout is presented in Table 1, where the first eight variants correspond to the incubation experiment and the remaining ones to the pot experiment. The experimental design included organic material type (VC and FM) and OMW dose as main factors. Each treatment was replicated three times, resulting in a total of 51 experimental units.

Before and after the experiments, soil chemical, physical, and biological properties were analyzed following standard procedures. Soil pH, electrical conductivity, organic matter, lime content, water-holding capacity, macro- and micronutrient contents, as well as soil respiration, dehydrogenase activity, and microbial biomass carbon were determined. Detailed analytical procedures are described below.

Table 1. Experimental treatments and composition of organic material–OMW mixtures used in incubation and pot experiments

App. No	Applıcatıon	Blackwater and Organic Fertilizer Mixtures	Composition Rates
1	VC	Vermicompost: Zero Blackwater	1 Kg VC
2	FM	Farmyard manure: Zero Blackwater	1 Kg FM
3	VC1 OMW1	Vermicompost: VC +1/2 OMW :OMW/2	1Kg VC + 0.5 Kg OMW
4	VC2 OMW2	Vermicompost: VC +1 OMW : 1 OMW	1Kg VC + 1 Kg OMW
5	VC3 OMW3	Vermicompost: VC +2 OMW :2 OMW	1Kg VC +2 Kg OMW
6	FM1 OMW1	Farmyard manure: FM + Blackwater :OMW/2	1Kg FM + 0.5 Kg OMW
7	FM2 OMW2	Farmyard manure: FM + Blackwater :OMW	1Kg FM + 1 Kg OMW
8	FM3 OMW3	Farmyard manure: FM + 2 Blackwater : 2OMW	1Kg FM +2 Kg OMW
		Soil and organic materials applied to soils	Composition Rates
9	S0	Soil: S- Zero organic and black water application	1 Kg S
10	S+VC	Soil + % 10 Vermicompost	1 Kg S + %10 VC
11	S+FM	Soil + % 10 Farmyard manure	1 Kg S + %10 FM
12	S+VC+OMW1	% 10 (Vermicompost: VC +1/2 Blackwater:OMW/2)	1Kg VC + 0.5 Kg OMW
13	S+VC+OMW2	% 10 (Vermicompost: VC +1 Blackwater : 1 OMW)	1Kg VC + 1 Kg OMW
14	S+VC+OMW3	% 10 (Vermicompost: VC +2 Blackwater :2 OMW)	1Kg VC +2 Kg OMW
15	S+FM+OMW1	% 10 (Farmyard manure: FM +Blackwater :OMW/2)	1Kg VC + 0.5 Kg OMW
16	S+FM+OMW2	% 10 (Farmyard manure: FM +Blackwater :OMW)	1Kg VC + 1 Kg OMW
17	S+FM+OMW3	% 10 (Farmyard manure: FM + 2 Blackwater : 2OMW)	1Kg VC +2 Kg OMW

VC: Vermicompost; FM: Farmyard manure; OMW: Olive mill wastewater. S+VC: Soil + 10% vermicompost; S+FM: Soil + 10% farmyard manure. S+VC+OMW1, S+VC+OMW2, S+VC+OMW3: Soil + 10% (VC + OMW mixture) with OMW additions at ratios of 1:0.5, 1:1, and 1:2, respectively. S+FM+OMW1, S+FM+OMW2, S+FM+OMW3: Soil + 10% (FM + OMW mixture) with OMW additions at ratios of 1:0.5, 1:1, and 1:2, respectively.

Soil texture: The soil texture was determined using the hydrometer method as described by Bouyoucos (1951).

Soil lime ($CaCO_3$): Soil lime ($CaCO_3$) content was determined using the Scheibler calcimeter method, based on volumetric measurement of CO_2 released after reaction with hydrochloric acid, as described by Nelson (1982).

Soil organic matter (SOM): Soil organic matter content was analyzed according to the modified Lichterfelder wet combustion method (Walkley & Black, 1934).

Macro and micronutrient contents: Available phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), manganese (Mn), and zinc (Zn) contents were determined following appropriate soil extraction procedures, and element concentrations were measured using an inductively coupled plasma optical emission spectrometer (ICP-OES) according to standard methods described by Page (1982) and Lindsay and Norvell (1978).

Water holding capacity (WHC): Water holding capacity was determined on saturated soil paste samples (Kacar, 2009).

Salinity: Total soluble salts were determined using the Wheatstone bridge method on saturated soil pastes (Allison et al., 1954).

Soil reaction (pH): Soil pH was measured in saturated paste using a Beckman glass electrode pH meter (Allison et al., 1954).

Soil respiration (CO_2 production): Soil respiration was determined according to the method described by (Isermeyer, 1952).

Dehydrogenase enzyme activity (DHA): Dehydrogenase enzyme activity was measured using the procedure described by Casida et al. (1964).

Microbial biomass carbon (MBC): Microbial biomass carbon was analyzed following the method proposed by Öhlinger (1993).

Statistical analysis

The data obtained for each variable were analyzed using the SPSS 21 software package. Analysis of variance (ANOVA) was performed to evaluate treatment effects, and treatment means were compared using Duncan's multiple range test at a 5% significance level ($P < 0.05$). Results are presented as mean ± standard error (SE).

RESULTS AND DISCUSSION

This study investigated the interactions between OMW, VC, and FM under both incubation and pot experiment conditions. The findings reveal that OMW application had distinct and predominantly positive effects on the physicochemical properties of the organic amendments and key soil quality parameters.

Soil pH and Electrical Conductivity (EC)

During the incubation experiment, the pH values of VC and FM materials remained mostly within the neutral range, yet the addition of OMW caused noticeable increases, particularly in the VC combinations (Figure 1A).

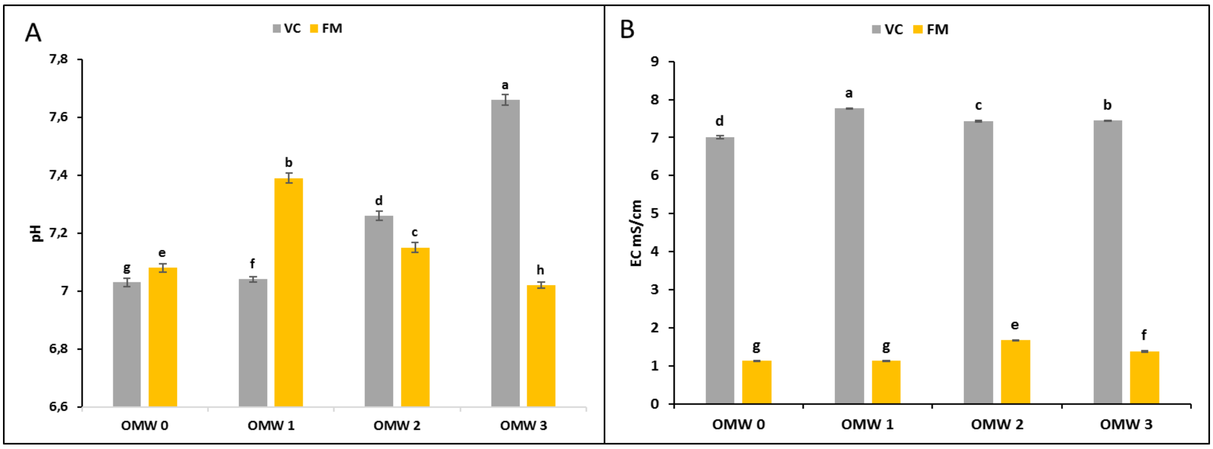

Figure 1. pH (A) and EC (B) pH values of different olive mill wastewater (OMW) and organic fertilizer mixtures after the incubation period (Mean ± S.E.). Different letters on the bars indicate statistically significant differences between means according to Duncan multiple range test (P < 0.05). VC: vermicompost; FM: farmyard manure; OMW: olive mill wastewater; VC OMW1: 1 kg VC + 0.5 kg OMW; VC OMW2: 1 kg VC + 1 kg OMW; VC OMW3: 1 kg VC + 2 kg OMW; FM OMW1: 1 kg FM + 0.5 kg OMW; FM OMW2: 1 kg FM + 1 kg OMW; FM OMW3: 1 kg FM + 2 kg OMW.

The rise ranged approximately between 3–9%, while FM showed only slight increases at low OMW doses and small decreases at higher levels. Such fluctuations may be related to interactions between organic acids and alkaline ions in the mixtures. In the pot experiment, soil pH values (Figure 2A) increased modestly under all OMW applications in both VC- and FM-amended soils. This pattern is generally consistent with studies reporting that the acidic nature of OMW can be buffered by soil carbonate systems, leading to mild upward shifts in pH (Sierra et al., 2001, Mekki et al., 2006).

Electrical conductivity showed a more distinct response. In the incubation stage (Figure 1B), EC increased clearly with OMW addition, with VC mixtures exhibiting stronger rises than FM, likely due to higher soluble salt accumulation. In the pot experiment (Figure 2B), OMW+VC mixtures again produced the highest EC values, whereas FM combinations showed a more limited increase, probably due to the strong ion-binding capacity of farmyard manure. Overall, these outcomes align well with the literature indicating that OMW increases EC in the short term due to its soluble salt content, but this effect may be moderated by organic amendments (Kotsou et al., 2004, Paredes et al., 1999).

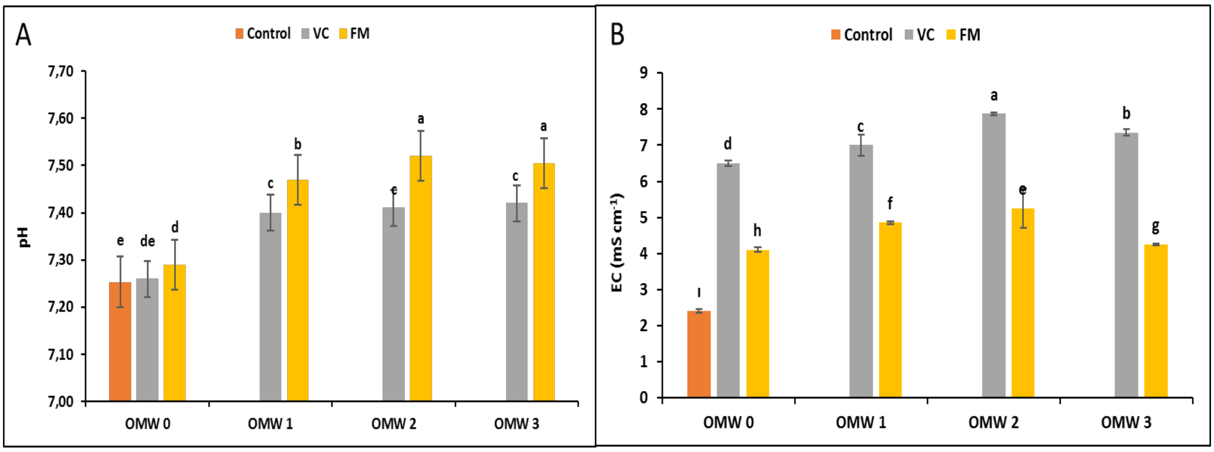

Figure 2. Soil pH (A) and EC (B) pH values of different olive mill wastewater (OMW) and organic fertilizer mixtures after the incubation period (Mean ± S.E.). Different letters on the bars indicate statistically significant differences between means according to Duncan multiple range test (P < 0.05). Control: non-application; VC: vermicompost; FM: farmyard manure; OMW: olive mill wastewater; VC OMW1: 1 kg VC + 0.5 kg OMW; VC OMW2: 1 kg VC + 1 kg OMW; VC OMW3: 1 kg VC + 2 kg OMW; FM OMW1: 1 kg FM + 0.5 kg OMW; FM OMW2: 1 kg FM + 1 kg OMW; FM OMW3: 1 kg FM + 2 kg OMW.

In our study, an upward trend in soil pH was observed following the application of OMW (olive mill wastewater). This finding is consistent with results reported in several modern studies. For instance, some researchers have indicated that the application of olive oil processing residues (OMW) may initially cause a temporary decrease in soil pH, but that the buffering capacity of soil carbonates can gradually normalize or even increase pH levels over time. Similarly, Enaime et al. (2024) noted that the application of OMW to soil generally produces “slight changes” in pH, since the acidic nature of OMW can be neutralized by the soil’s buffering capacity.

On the other hand, certain studies have reported a temporary reduction in pH following OMW addition. Regni et al. (2021) observed that this short-term acidification might result from the organic acids present in OMW, which can temporarily lower soil pH during incubation-type experiments. Conversely, Chaari et al. (2015) reported that in long-term applications, the soil’s buffering capacity restricted any major changes in pH, preventing significant acidification.

Overall, our findings suggest that despite the inherently acidic composition of OMW, its combination with organic materials and the soil’s buffering potential can lead to a slight alkaline shift in pH. This trend aligns well with the general direction reported in the literature, reinforcing the consistency of our results with previous studies.

In both the incubation and soil experiments, an increasing trend in electrical conductivity (EC) was observed, particularly in combinations that included OMW (olive mill wastewater). This finding aligns closely with current studies examining the effects of olive mill wastewater and organic fertilizers on soil salinity.

Regni et al. (2021) reported that the short-term rise in EC following the application of olive oil processing residues (OMW) is due to their high content of soluble salts, especially K+, Na+, and Cl- ions, while rainfall and microbial transformations later reduce salinity. Similarly, Barbera et al. (2013) observed that EC increases were most pronounced during the first weeks after OMW application in Mediterranean soils and that the magnitude of this effect was dose-dependent. Enaime et al. (2024) also highlighted that the high organic matter and mineral salt content of OMW elevates ionic concentration and EC, although the increase is typically temporary a trend consistent with the EC rise observed in our incubation phase.

In contrast, Kavvadias et al. (2015) found that in long-term applications, EC levels tend to stabilize over time, likely due to organic matter decomposition and ion adsorption by clay-humus complexes. The limited EC rise observed at higher OMW doses in our study supports this mechanism.

Additionally, Fernández-Hernández et al. (2014) studied vermicompost+OMW mixtures and noted that pure OMW can pose a risk of excessive salinity, while the addition of organic materials (such as vermicompost) partially offsets this increase by promoting ion immobilization. The moderate EC levels detected in our VC+OMW treatments can therefore be attributed to this buffering effect.

In conclusion, the present findings indicate that OMW tends to increase EC in the short term due to its high load of soluble salts; however, this effect can be mitigated by the ion binding capacity of organic materials such as vermicompost and farmyard manure. These results are largely consistent with previous studies reporting that the combined application of organic wastes can help reduce salinity-related risks (Enaime et al., 2024; Regni et al., 2021; Fernández-Hernández et al., 2014).

Organic Matter, Lime Content ($CaCO_3$) and Water Holding Capacity (WHC)

Organic matter levels during incubation (Figure 3A) remained relatively stable within VC and FM materials, with OMW causing only small fluctuations (1–7%). In the pot experiment (Figure 4A), both VC and FM significantly increased soil OM compared with the control. However, OMW slightly reduced OM in VC mixtures, while FM mixtures showed more irregular but generally modest changes. These results correspond with previous work indicating that OM decomposition patterns depend heavily on microbial activity and the chemical character of OMW (Mekersi et al., 2022, Matišić et al., 2024, Ahmed et al., 2025) .

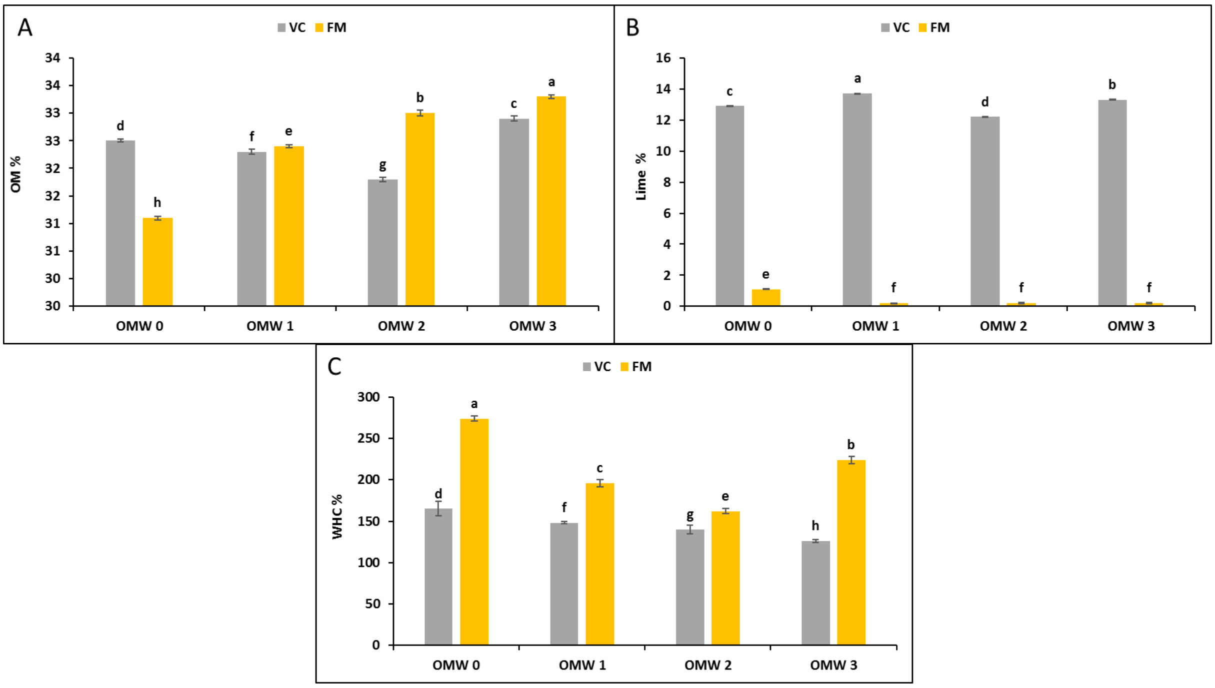

Figure 3. Organic matter (A), lime (B), and water holding capacity (C) values of different olive mill wastewater (OMW) and organic fertilizer mixtures after the incubation period (Mean ± S.E.). Different letters on the bars indicate statistically significant differences between means according to Duncan multiple range test ($P < 0.05$). VC: vermicompost; FM: farmyard manure; OMW: olive mill wastewater; VC OMW1: 1 kg VC + 0.5 kg OMW; VC OMW2: 1 kg VC + 1 kg OMW; VC OMW3: 1 kg VC + 2 kg OMW; FM OMW1: 1 kg FM + 0.5 kg OMW; FM OMW2: 1 kg FM + 1 kg OMW; FM OMW3: 1 kg FM + 2 kg OMW.

$CaCO_3$ contents during incubation (Figure 3B) increased by 3–6% in VC materials after OMW addition, while FM showed pronounced decreases. In the pot experiment (Figure 4B), OMW increased $CaCO_3$ in VC-amended soils but caused substantial reductions in FM mixtures. Such variability may reflect temporary $CaCO_3$ dissolution by OMW-derived organic acids, followed by reprecipitation depending on dose and soil chemistry.

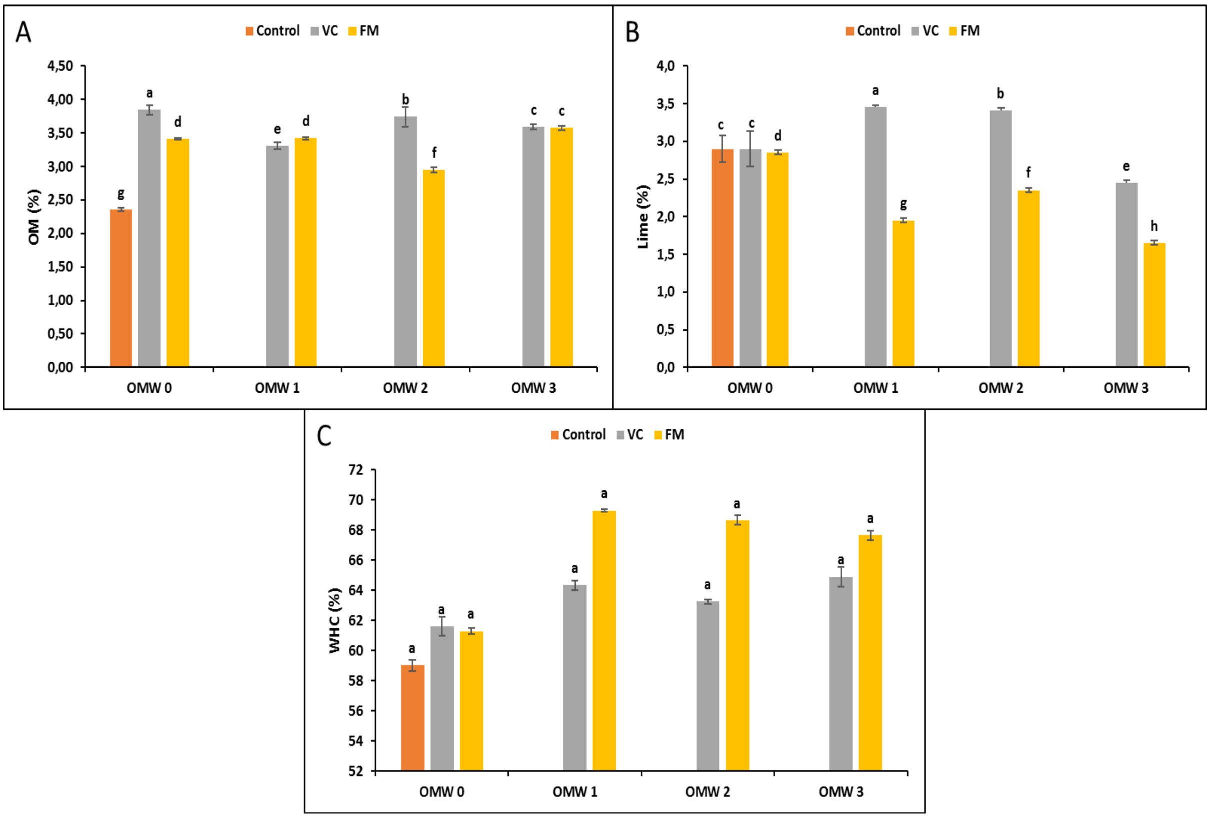

Figure 4. Soil Organic matter (A), lime (B), and water holding capacity (C) values of different olive mill wastewater (OMW) and organic fertilizer mixtures after the incubation period (Mean ± S.E.). Different letters on the bars indicate statistically significant differences between means according to Duncan multiple range test ($P < 0.05$). Control: non-application; VC: vermicompost; FM: farmyard manure; OMW: olive mill wastewater; VC OMW1: 1 kg VC + 0.5 kg OMW; VC OMW2: 1 kg VC + 1 kg OMW; VC OMW3: 1 kg VC + 2 kg OMW; FM OMW1: 1 kg FM + 0.5 kg OMW; FM OMW2: 1 kg FM + 1 kg OMW; FM OMW3: 1 kg FM + 2 kg OMW.

WHC values in the incubation stage (Figure 2C) decreased by 10–25% in VC materials, while FM showed reductions at low OMW levels and increases at the highest dose. In the pot experiment (Figure 6C), OMW consistently improved WHC in both VC and FM mixtures. This effect may be associated with altered organic matter structure and enhanced aggregation in OMW-amended soils.

The organic matter content exhibited variability in both short-term incubation and soil experiments, depending on environmental conditions, the chemical composition of the applied materials, and microbial activity levels. The outcomes of this study closely align with recent research investigating the effects of OMW and organic fertilizer combinations on soil organic matter dynamics. Enaime et al. (2024) reported that OMW application can increase organic carbon in the short term, but at higher doses, accelerated microbial oxidation may lead to partial carbon loss as CO2, which could explain the slight fluctuations in organic matter observed in our study.

Similarly, Regni et al. (2021) found that long-term OMW applications resulted in the accumulation of organic matter, particularly in surface soil layers, and that this effect became more stable when supported by the presence of organic amendments such as vermicompost or farmyard manure. This supports the view that the co-application of OMW with organic materials can have a synergistic effect on soil carbon sequestration and contribute to the long-term stabilization of soil organic matter

Fernández-Hernández et al. (2014) reported that while the application of OMW alone can lead to an increase in organic matter, excessive amounts may pose risks of phytotoxicity and microbial imbalance. However, when OMW is combined with organic materials, a more balanced accumulation of organic carbon is achieved. Similarly, Hassani et al. (2020), in a study conducted under Mediterranean conditions, found that OMW undergoes rapid humification in soil, leading to a short-term increase in organic matter, although this effect tends to be temporary under high-temperature conditions. This observation is consistent with the limited variations in organic matter detected during incubation in our study.

Kavvadias et al. (2015) also stated that the combined application of organic fertilizers and olive mill wastewater supports carbon stabilization processes and improves the long-term balance of soil organic matter.

Accordingly, the increase in organic matter may not only be an initial effect but can also create a sustainable impact over time.

In this context, the existing literature indicates that OMW serves as a direct source of organic matter input; however, its effect strongly depends on dose, duration, microbial activity, and the composition of the applied materials. The addition of vermicompost and farmyard manure plays a critical role in moderating carbon mineralization and maintaining organic matter stability.

Soil lime content (CaCO3) and stable soil carbon (SSC) dynamics are directly associated with the application of organic materials and serve as important indicators reflecting the effects of organic wastes like OMW, which are rich in soluble ions, on soil chemistry. In the literature, the responses of these two parameters to OMW and organic fertilizer combinations have shown variable trends depending on the duration and conditions of the experiments

Enaime et al. (2024) stated that the calcium, potassium, and organic acids present in OMW can alter the ionic balance in the soil solution, leading to fluctuations in soil pH and lime dynamics. They noted that organic acids may temporarily increase CaCO3 solubility, but over time, the re-association of Ca2+ ions with carbonates tends to stabilize lime levels.

Similarly, Regni et al. (2021) reported that in long-term OMW applications, lime content exhibited only minor fluctuations, which were linked to microbial carbonate dissolution and re-precipitation processes. This suggests that the limited variations in lime content observed in our experiments, especially in treatments combining OMW with organic fertilizers, may be attributed to biochemical buffering mechanisms.

Fernández-Hernández et al. (2014) found that during the humification process, Ca and Mg complexes in OMW can interact with organic carbon, promoting the accumulation of stable soil carbon (SSC). This effect becomes more persistent in applications supported by organic materials, where carbon tends to bind to mineral surfaces. Likewise, Hassani et al. (2020) demonstrated that although olive mill wastewater applications can increase carbon mineralization in the short term, they also enhance the SSC fraction over time through the accumulation of humic compounds.

Kavvadias et al. (2015), in their study on the effects of OMW in calcareous soils, emphasized that higher lime content can promote carbon stability because Ca2+ ions form complexes with humic acids, strengthening stable carbon bonds. This mechanism is consistent with the gradual increase in SSC observed in OMW + organic material combinations in our study

On the other hand, Chatzistathis and Koutsos (2017) reported that excessive applications of olive mill wastewater (OMW) may reduce carbon stability due to ion accumulation and permeability issues, emphasizing that application dosage plays a critical role. Overall, recent literature indicates that while OMW can influence lime dynamics in the short term because of its soluble calcium and organic acid content, it ultimately supports the accumulation of stable soil carbon (SSC) over the long term through Ca–C complex formation. The supplementation of organic materials such as vermicompost or farmyard manure acts as a stabilizing factor for both lime solubility and carbon stabilization processes.

Macronutrients: P_2O_5, K_2O, Ca and Fe

During incubation, P_2O_5 and K_2O concentrations (Figure 5A–B) increased markedly in VC+OMW combinations. In the pot experiment (Figure 6A–B), VC+OMW again showed strong increases in both nutrients, whereas FM+OMW treatments caused decreases in P_2O_5, particularly at high OMW doses. This agrees with earlier findings suggesting that OMW can enhance P solubility under certain combinations but may limit P mineralization when applied excessively (Gul et al., 2024, Mira-Urios et al., 2025).

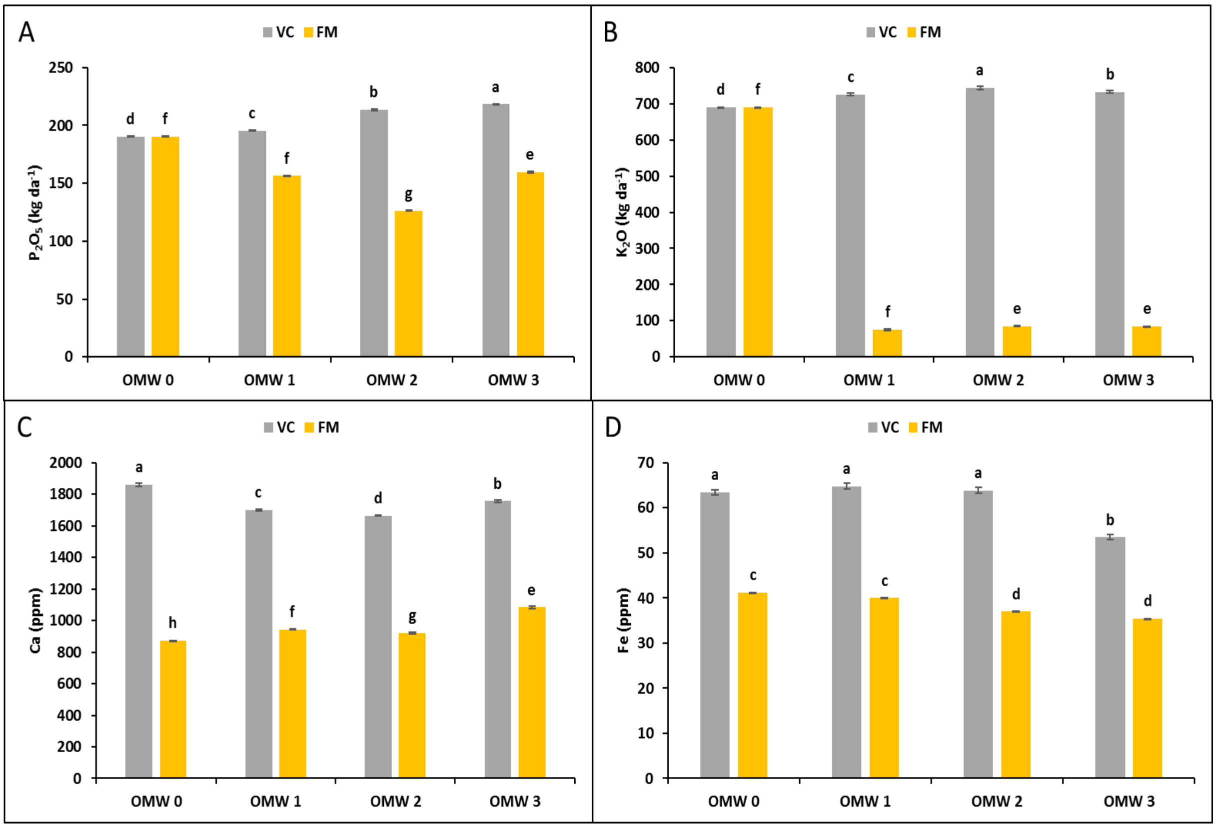

Figure 5. Phosphorus (P_2O_5) (A), potassium (K_2O) (B), calcium (Ca) (C) and iron (Fe) (D) values of different olive mill wastewater (OMW) and organic fertilizer mixtures after the incubation period (Mean ± s.e.). Different letters on the bars indicate statistically significant differences between means according to Duncan multiple range test ($P < 0.05$). VC: vermicompost; FM: farmyard manure; OMW: olive mill wastewater; VC OMW1: 1 kg VC + 0.5 kg OMW; VC OMW2: 1 kg VC + 1 kg OMW; VC OMW3: 1 kg VC + 2 kg OMW; FM OMW1: 1 kg FM + 0.5 kg OMW; FM OMW2: 1 kg FM + 1 kg OMW; FM OMW3: 1 kg FM + 2 kg OMW.

Calcium contents during incubation (Figure 5C) declined in VC materials and rose in FM materials with OMW addition. In the pot experiment (Figure 6C), Ca values showed dose-dependent increases and decreases in both VC and FM combinations. Fe contents followed a clearer pattern: incubation data (Figure 5D) showed consistent reductions in Fe after OMW addition, and pot results (Figure 6D) indicated especially strong decreases in FM+OMW mixtures. These shifts likely relate to redox changes and metal–organic complexation processes induced by OMW (Teixeira et al., 2024, Mira-Urios et al., 2025) .

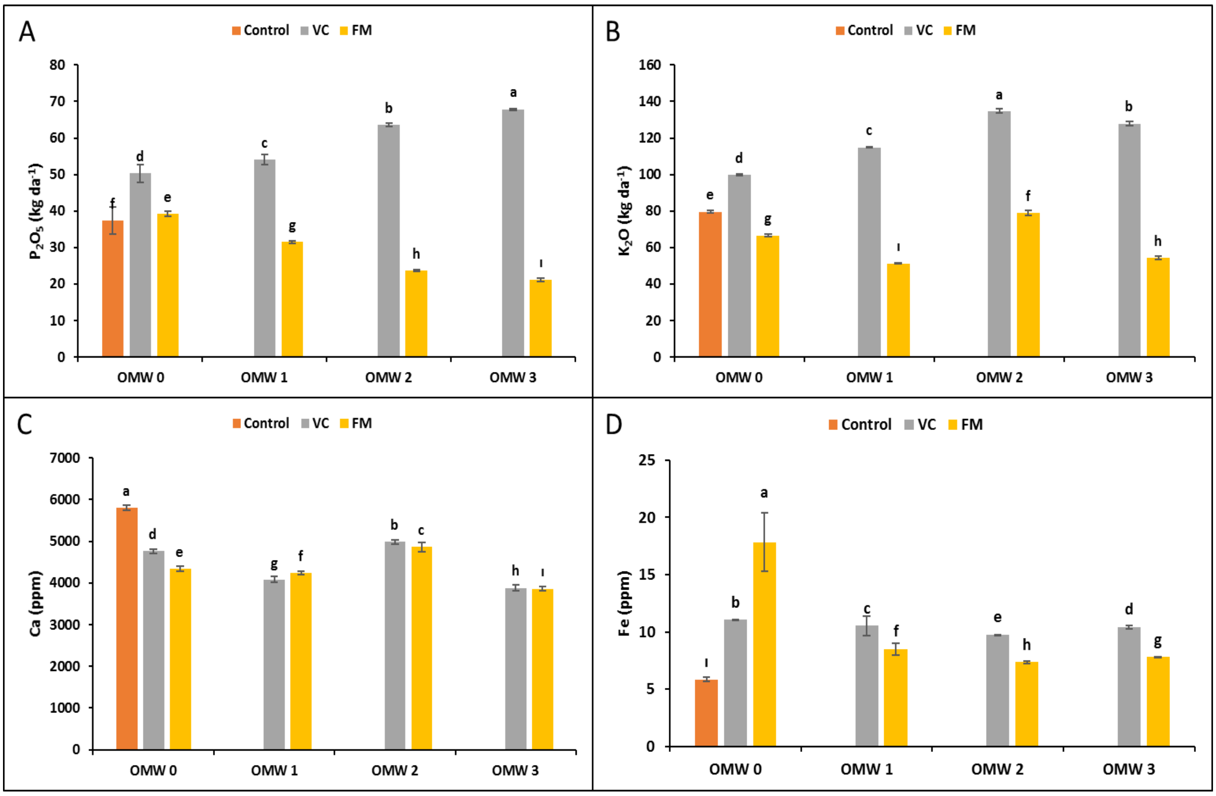

Figure 6. Soil Phosphorus (P_2O_5) (A), potassium (K_2O) (B), calcium (Ca) (C) and iron (Fe) (D) values of different OMW and organic fertilizer mixtures after the incubation period (Mean ± S.E.). Different letters on the bars indicate statistically significant differences between means according to Duncan multiple range test ($P < 0.05$). Control: non-application; VC: vermicompost; FM: farmyard manure; OMW: olive mill wastewater; VC OMW1: 1 kg VC + 0.5 kg OMW; VC OMW2: 1 kg VC + 1 kg OMW; VC OMW3: 1 kg VC + 2 kg OMW; FM OMW1: 1 kg FM + 0.5 kg OMW; FM OMW2: 1 kg FM + 1 kg OMW; FM OMW3: 1 kg FM + 2 kg OMW.

Combinations of OMW and organic fertilizers can directly affect the mobility of macronutrients, mineralization rates, and ion balance in soil. The findings of this study are largely consistent with trends reported in similar applications within the literature. Several studies have shown that the organic phosphorus compounds and soluble salts in OMW can increase soil P accumulation in the short term. Enaime et al. (2024) reported that phosphorus in the organic fraction of OMW becomes soluble through microbial mineralization, and that its availability increases particularly when OMW is co-applied with vermicompost.

Similarly, Regni et al. (2021) emphasized that the humic acid content of OMW can enhance the solubility of calcium-phosphate complexes, thereby increasing soil phosphorus levels. However, Kavvadias et al. (2015) noted that high OMW doses, due to the accumulation of organic acids, can suppress microbial phosphorus transformation, which may limit P accumulation over time.

The high potassium content of OMW is also a key factor contributing to K accumulation in soils. Hassani et al. (2020) demonstrated that OMW applications significantly increased K ion concentrations in the soil solution during the initial incubation stages, but this effect gradually balanced out as potassium became adsorbed by clay fractions

Similar results were also reported by Fernández-Hernández et al. (2014), who noted that OMW and vermicompost combinations increased potassium accumulation while maintaining ionic balance. In addition, Chatzistathis and Koutsos (2017) stated that such mixtures accelerate the transformation of K^+ ions into more plant-available forms, an effect particularly evident at lower OMW doses.

Calcium content in OMW-treated soils often shows fluctuating patterns. Regni et al. (2021) and Enaime et al. (2024) attributed this to the presence of organic acids in OMW, which can increase $CaCO_3$ solubility, leading to a short-term release of calcium followed by a decrease due to carbonate re-precipitation. Furthermore, Kavvadias et al. (2015) indicated that calcium fractions can bind to humic complexes, becoming more stable over time, and thus exhibit a tendency to accumulate near the soil surface under OMW + organic material treatments. The phenolic compounds in OMW and its potential to create reducing conditions can influence iron (Fe) bioavailability. Lamaizi et al. (2023) reported that OMW applications enhanced Fe solubility, particularly under conditions of high microbial activity, where the reduction of Fe^{3+} to Fe^{2+} was accelerated. However, Fernández-

Hernández et al. (2014) and Enaime et al. (2024) emphasized that during long-term applications, as organic acids are depleted, Fe can re-oxidize and become less soluble. This suggests that the observed fluctuations in Fe content between short- and long-term results may be related to chemical redox equilibrium dynamics.

Overall, the literature indicates that OMW and organic fertilizer combinations tend to enhance P and K accumulation, while the solubility dynamics of elements such as Ca and Fe vary depending on organic acid concentration, pH, and microbial activity. This highlights the decisive role of organic material type and application dosage in determining the forms and mobility of soil nutrients.

Micronutrients: Cu, Mn, Zn and Mg

Incubation data (Figure 7A–D) revealed increases in Cu and Mn, especially in VC+OMW mixtures, while Zn and Mg exhibited smaller variations. In the pot experiment (Figure 8A–D), Cu increased slightly in VC+OMW but decreased in FM mixtures. Mn rose substantially in all VC+OMW treatments and also increased at high OMW doses in FM mixtures. Zn levels showed minimal change in VC but increased in FM mixtures. Mg displayed a more irregular pattern, likely due to ion-exchange competition with abundant K^+ in OMW.

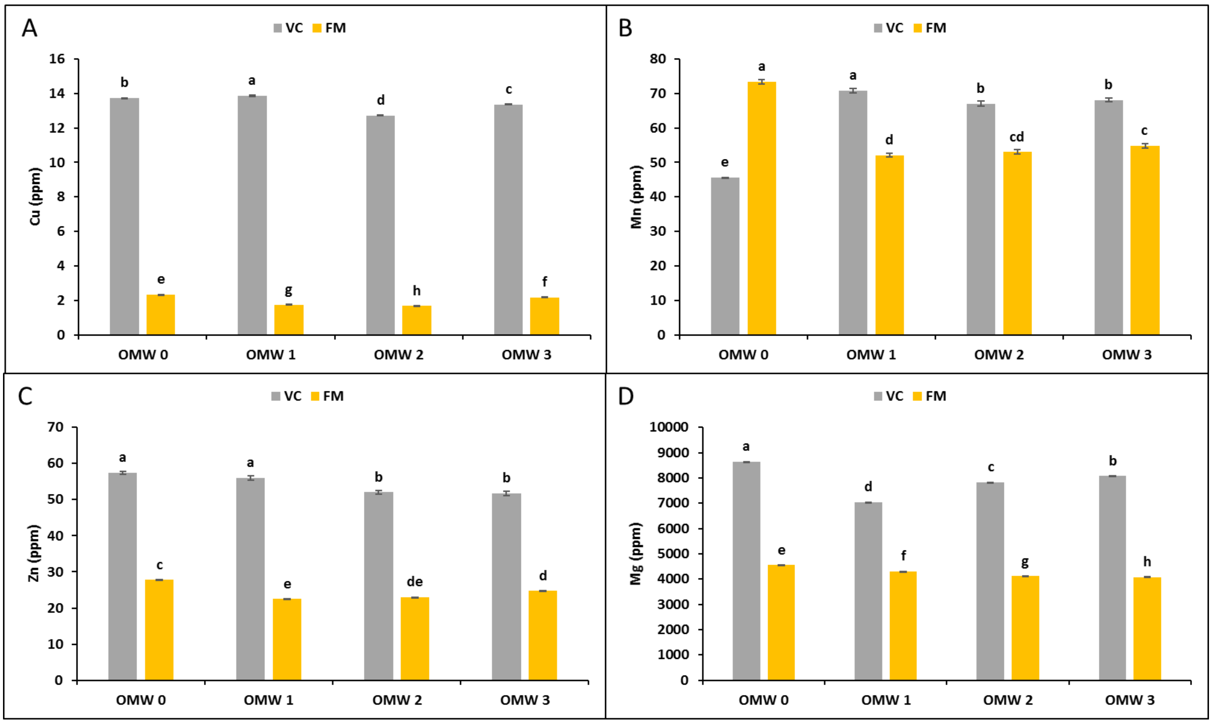

Figure 7. Copper (Cu)(A), manganese (Mn) (B), zinc (Zn) (C) and magnesium (Mg) (D) values of different olive mill wastewater (OMW) and organic fertilizer mixtures after the incubation period (Mean ± S.E.). Different letters on the bars indicate statistically significant differences between means according to Duncan multiple range test ($P < 0.05$). VC: vermicompost; FM: farmyard manure; OMW: olive mill wastewater; VC OMW1: 1 kg VC + 0.5 kg OMW; VC OMW2: 1 kg VC + 1 kg OMW; VC OMW3: 1 kg VC + 2 kg OMW; FM OMW1: 1 kg FM + 0.5 kg OMW; FM OMW2: 1 kg FM + 1 kg OMW; FM OMW3: 1 kg FM + 2 kg OMW.

These observations align with recent studies showing that OMW can modify the chemical forms and solubility of micronutrients through complexation, redox shifts, and competitive ion interactions (Khalil et al., 2024, Ahmed et al., 2025).

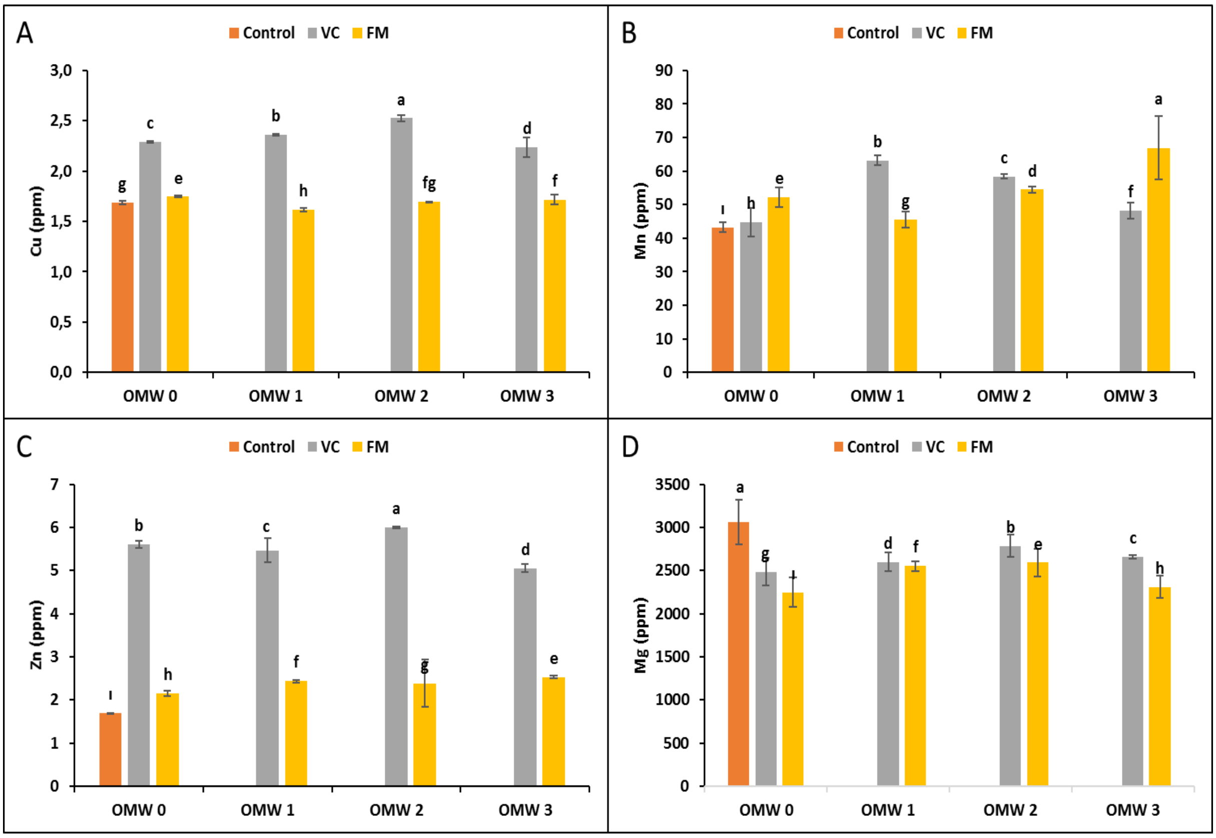

Figure 8. Copper (Cu)(A), manganese (Mn) (B), zinc (Zn) (C) and magnesium (Mg) (D) values of different olive mill wastewater (OMW) and organic fertilizer mixtures after the incubation period (Mean ± S.E.). Different letters on the bars indicate statistically significant differences between means according to Duncan multiple range test ($P < 0.05$). Control: non-application; VC: vermicompost; FM: farmyard manure; OMW: olive mill wastewater; VC OMW1: 1 kg VC + 0.5 kg OMW; VC OMW2: 1 kg VC + 1 kg OMW; VC OMW3: 1 kg VC + 2 kg OMW; FM OMW1: 1 kg FM + 0.5 kg OMW; FM OMW2: 1 kg FM + 1 kg OMW; FM OMW3: 1 kg FM + 2 kg OMW.

Applications of OMW and organic materials can significantly influence the availability and chemical forms of micronutrients in soil, particularly Cu, Mn, Zn, and Mg. In the literature, the responses of these elements to OMW and organic fertilizer combinations have been largely linked to soil pH, organic matter content, and redox conditions.

Several studies have shown that the phenolic compounds and organic acids in OMW can form metal complexes, thereby increasing the mobility of copper (Cu). Enaime et al. (2024) reported that in OMW treated soils, Cu initially increased due to its binding with organic complexes but later became stabilized within humic structures. Similarly, Regni et al. (2021) stated that the organic acid content of OMW enhances Cu solubility, although in the long term, this element tends to adsorb onto organic matter, reducing its bioavailability. Kavvadias et al. (2015) also found that organic fertilizers combined with OMW can form strong complexes with metal ions, which in turn limits Cu accumulation.

Manganese (Mn) generally tends to increase following OMW application. Lamaizi et al. (2023) observed that OMW creates oxygen-limited microzones in the soil, accelerating the reduction of Mn^{4+} to Mn^{2+} and thereby increasing Mn solubility. This mechanism helps explain the Mn increases observed in high-organic-matter treatments such as those containing vermicompost. In addition, Fernández-Hernández et al. (2014) reported that OMW application enhances microbial respiration activity, which can accelerate Mn redox transformations.

Zinc (Zn) dynamics typically follow a similar trend to those of Cu and Mn. Chatzistathis and Koutsos (2017) noted that in Mediterranean soils treated with OMW, the soluble form of Zn tends to increase, although at excessively high doses, Zn may temporarily form complexes with organic acids and subsequently precipitate. Abdennbi et al. (2025) emphasized that OMW enhances microbial activity, facilitating the release of Zn from the organic fraction and, particularly in the presence of vermicompost, promoting its transformation into a plant-available form. This finding suggests that OMW and organic fertilizer combinations may have a regulatory effect on Zn mobility in soils.

Magnesium (Mg) in OMW-treated soils generally exhibits a fluctuating behavior. Enaime et al. (2024) attributed this to ion-exchange processes driven by OMW's high potassium content, which competes with Mg^{2+}

ions. The displacement of Mg^{2+} by K^+ may lead to a short term decrease in Mg concentration within the soil solution. Conversely, Regni et al. (2021) highlighted that organic matter–rich materials can enhance Mg retention, thereby reducing its loss in the long term. Similarly, Kavvadias et al. (2015) noted that OMW + organic fertilizer combinations help stabilize Mg within clay humus complexes, keeping it in a more persistent form.

Overall, recent literature indicates that the response of micronutrients to OMW application depends on their chemical nature: Cu and Zn tend to show temporary increases through organic complexation; Mn becomes more soluble under reducing conditions; while Mg behaves more variably due to ion competition and adsorption processes. Organic fertilizers exert a moderating and buffering effect on these dynamics.

Soil Biological Activity: CO_2, DHA and MBC

Soil respiration decreased progressively with increasing OMW dose during incubation (Figure 9A), and a similar trend appeared in the pot experiment. DHA activity also declined with higher OMW levels (Figure 9B), suggesting that phenolic compounds and osmotic stress may inhibit enzymatic activity. MBC values in both incubation and pot experiments (Figure 9C) decreased as OMW levels increased, indicating a suppressive effect when OMW concentrations become excessive.

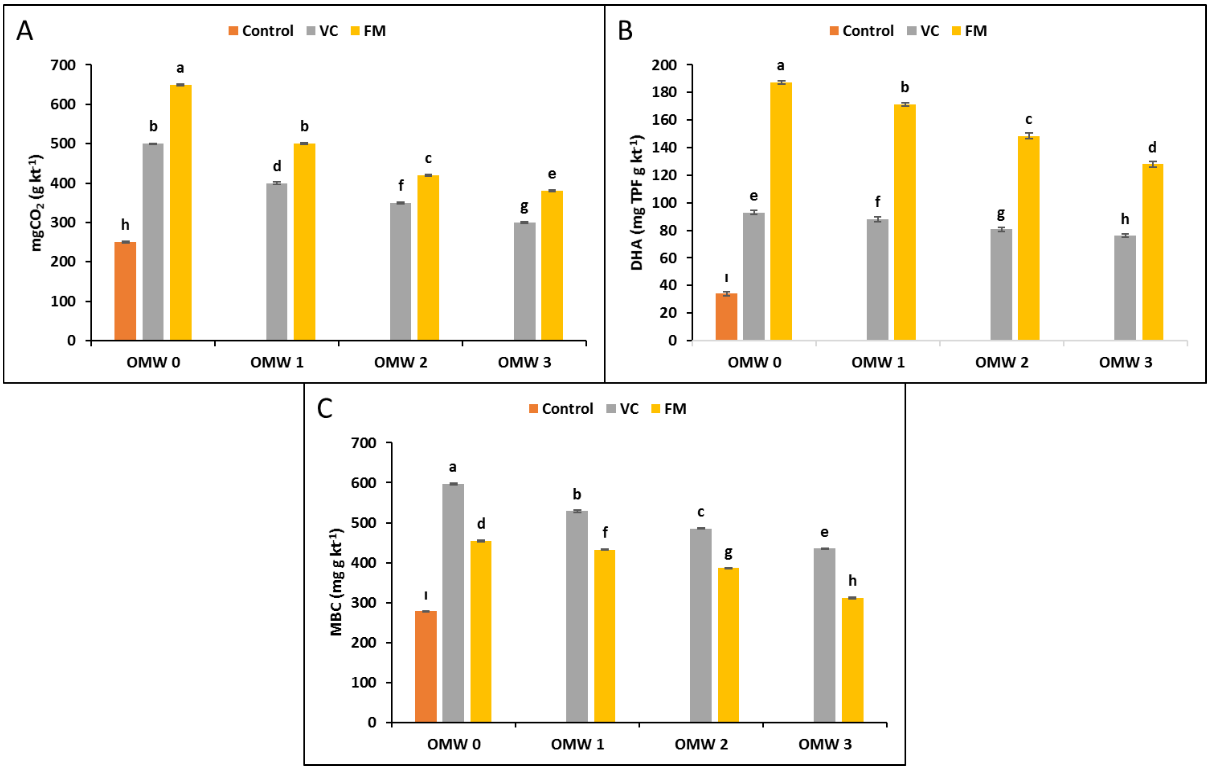

Figure 9. Soil carbondioxide (CO_2) (A), dehydrogenase enzyme (DHA) (B) and microbial biomass carbon (MBC) (C) values of different olive mill wastewater (OMW) and organic fertilizer mixtures after the incubation period (Mean ± S.E.). Different letters on the bars indicate statistically significant differences between means according to Duncan multiple range test ($P < 0.05$). Control: non-application; VC: vermicompost; FM: farmyard manure; OMW: olive mill wastewater; VC OMW1: 1 kg VC + 0.5 kg OMW; VC OMW2: 1 kg VC + 1 kg OMW; VC OMW3: 1 kg VC + 2 kg OMW; FM OMW1: 1 kg FM + 0.5 kg OMW; FM OMW2: 1 kg FM + 1 kg OMW; FM OMW3: 1 kg FM + 2 kg OMW.

These findings fit well with the broader literature: while OMW can stimulate microbial communities at low doses by providing easily decomposable carbon sources, high level may induce stress due to phenolic toxicity and elevated salinity (Yaakoubi et al., 2024, Ahmed and Khail, 2025) . Organic fertilizers such as VC and FM appear to buffer these stresses, supporting more stable microbial activity.

Soil microbial activity is closely related to the quality, amount, and degradable carbon content of the applied organic materials. The effects of OMW and organic fertilizer combinations on soil microbial respiration (CO_2), dehydrogenase activity (DHA), and microbial biomass carbon (MBC) have been reported in the literature with both positive and partially negative trends. Many studies have shown that OMW and organic manure applications accelerate soil carbon cycling, leading to increased CO_2 emissions, particularly in the short term when microbial respiration intensifies significantly. Enaime et al. (2024) explained that this rise results from the rapid microbial decomposition of OMW due to its high organic carbon and soluble phenolic content.

Dehydrogenase activity (DHA) serves as an important biochemical indicator reflecting microbial vitality and the rate of biological oxidation processes in soil. Kavvadias et al. (2015) found that low doses of OMW can stimulate microbial enzyme activity, whereas high doses tend to suppress enzymatic systems due to the presence of phenolic compounds. Fernández-Hernández et al. (2014) similarly observed that the organic carbon content of OMW can initially stimulate microbial populations and increase DHA, but over time, its high conductivity and phenolic load may limit this enhancement. Moreover, Tsiknia et al. (2014) demonstrated that OMW combined with organic fertilizers can improve the microbial redox balance, resulting in DHA activity that increases in parallel with organic matter content.

Microbial biomass carbon (MBC) reflects the size of the active microbial population and plays a crucial role in the sustainability of soil carbon cycling. Enaime et al. (2024) reported that OMW provides easily degradable carbon sources that support microbial biomass; however, at high application rates, microbial stress may increase, leading to declines in MBC. Similarly, Regni et al. (2021) noted that while OMW initially promotes microbial biomass growth, it can later decrease as respiration rates intensify.

Abdennbi et al. (2025) further showed that OMW applications, especially when combined with vermicompost, can enhance microbial biomass, as vermicompost contains humic substances and nitrogen compounds that promote microbial diversity.

Overall, recent literature indicates that OMW can act as an effective carbon source that stimulates soil microbial activity when applied at low to moderate rates (e.g., ≤10% v/w or equivalent field application rates), whereas higher application levels may exert inhibitory effects due to the accumulation of phenolic compounds and increased salinity (Masmoudi et al., 2024). The co-application of vermicompost (VC) and farmyard manure (FM) has been reported to mitigate these negative effects by enhancing soil enzyme activities and stabilizing microbial biomass, thereby promoting a more balanced and sustainable microbial activity profile (Yaakoubi et al., 2024).

CONCLUSION

This study revealed the effects of co-application of OMW with VC and FM on the physicochemical and biological properties of soils in both incubation and potting conditions. The results obtained showed that the observed responses varied greatly depending on the type of organic material used and the dose of OMW applied. OMW applications caused significant changes in soil pH and electrical conductivity (EC) values, especially when used with organic fertilizers. While pH values generally remained in the neutral to slightly alkaline range, significant increases in EC values were observed, especially in VC+OMW applications, due to the high soluble salt and potassium (K^+) content of OMW. On the other hand, lower EC levels in FM+OMW combinations suggest that farm manure has strong ion adsorption and buffering capacity. Overall, OMW applications increased soil EC by approximately 20–45% and enhanced soil organic matter content by up to 30% compared to the control, depending on the organic material type and application rate.

Soil organic matter (OM) content increased in all treatments compared to the control. The highest OM levels were determined in VC+OMW combinations, reflecting the synergistic interaction between the carbon-rich structure of OMW and the humic substance-rich composition of vermicompost. The changes observed in lime ($CaCO_3$) and total carbonate content indicate temporary dissolution and re-precipitation processes that most likely occur under the influence of organic acids in OMW.

When evaluated in terms of macronutrient elements, it was observed that P_2O_5 and K_2O concentrations increased significantly, especially when OMW was applied together with VC and FM. This reveals that olive mill wastewater has the potential to increase soil fertility thanks to its nutrient-rich structure. On the other hand, it was determined that Ca and Fe contents followed a more variable course. It is thought that this variability may be related to the effects of OMW on redox balance and metal complexation processes in the soil. While the levels of micronutrient elements Cu, Mn and Zn generally increased under VC + OMW applications, partial decreases occurred in Mg content due to ionic competition with K^+ ions.

Microbial respiration (CO_2), dehydrogenase activity (DHA) and microbial biomass carbon (MBC) values, which are the biological parameters of the soil, increased significantly, especially with VC and FM applications. These increases are associated with the fact that organic materials provide easily decomposable carbon sources for microorganisms. However, at higher OMW doses, decreases in these parameters were observed, most likely due to the toxic effects of phenolic compounds and increased salinity stress. This reveals that although OMW has a stimulating effect on microbial activity in the short term, it can create physiological stress on microorganisms in excessive applications.

In general, the use of olive mill wastewater with organic fertilizers stands out as an effective and sustainable practice in terms of increasing soil fertility, nutrient availability and microbial activity. However, the application dose of OMW remains a critical factor; While low and moderate applications support the biological functioning and nutrient cycle of the soil, high doses may cause negative effects such as salinity accumulation and enzyme inhibition. Based on the results of this study, OMW application rates of 1:0.5 and 1:1 combined with vermicompost or farmyard manure can be recommended for short-term soil improvement, whereas the 1:2 OMW ratio should be avoided or applied with caution due to its salinity and microbial stress potential.

It is important that future studies focus on long-term field trials in which parameters such as heavy metal accumulation, organic carbon stability and enzymatic resistance are evaluated. Such research will contribute to the creation of scientific guidelines for the controlled and balanced use of OMW and will enable the transformation of an environmentally problematic by-product into a valuable organic resource for sustainable agriculture.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Author contributions

Kemal DOĞAN: Responsible for the creation of the data set, design of the study, methodology development, evaluation and review of the manuscript.
Necat AĞCA**:** Design of the study, methodology development, evaluation and review of the manuscript.
Nadir DOĞRU**:** Conducted the literature review and integrated the relevant sources into the study.
Ali SARIOĞLU**:** Contributed to the analysis and interpretation of the analysis and the writing of the discussion and conclusion sections of the article.

ORCID

Kemal DOĞAN **https://orcid.org/0000-0002-5448-0009**
Necat AĞCA **https://orcid.org/0000-0003-4864-844X**
Nadir DOĞRU **https://orcid.org/0000-0003-2342-6509**
Ali SARIOĞLU **https://orcid.org/0000-0001-6269-4990**

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