

Influence of Biochar Derived from Olive Pomace–remediated Olive Mill Wastewater on Germination of Barley (*Hordeum vulgare*) and Fenugreek

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DOI: 10.15376/biores.21.4.11603-11621

GRAPHICAL ABSTRACT



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The disposal of olive mill wastewater (OMW) poses significant environmental challenges because of its high organic load and phytotoxic compounds. This study focused on treating OMW from a modern oil mill using a filtration-percolation technique and evaluated its impact on the germination of barley and fenugreek seeds. Filters were prepared using lime and charcoal derived from the mill's pomace. Four distinct treatments were applied: (1) no treatment (raw OMW) (T0), (2) a lime layer (1.0 cm) sandwiched between two layers of sand (T1), (3) an activated charcoal layer (1.0 cm) derived from pomace sandwiched between two layers of sand (T2), and (4) a combination of lime and activated charcoal derived from pomace, sandwiched between two layers of sand (T3). The physicochemical analyses included pH, electrical conductivity (EC), total suspended solids (TSS), moisture content (MC), dry matter (DM), total Kjeldahl nitrogen (TKN), biochemical oxygen demand (BOD₅), phosphorus (P), polyphenol (PP), and exchangeable cation content (Ca, Na, and K). The results revealed significant differences between treatments, with substantial reductions in polyphenols, EC, organic matter (OM), total organic carbon (TOC), and BOD₅. Treatments T2 and T3 were the most effective for depollution of OMW, and T3 exhibited the highest germination rate.

DOI: 10.15376/biores.21.4.11603-11621

Keywords: Olive mill wastewater; Treatment; Infiltration-percolation; Olive pomace; Seed germination

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INTRODUCTION

The production of olive oil is a vital economic and cultural sector in many Mediterranean countries. However, it presents significant environmental challenges, particularly due to the generation of olive mill wastewater (OMW) and olive mill pomace (OMP) (Abu-Dalo *et al.* 2023). The OMW, the primary waste product from olive oil extraction, is a highly polluted liquid whose composition varies depending on factors such

as olive characteristics, maturity, cultivation practices, and the oil extraction process (Carmona *et al.* 2023). Approximately 2.72 million tons of olive oil is produced annually worldwide, with the majority originating from the Mediterranean region (Hassen *et al.* 2023).

The OMW is comprised of water (83% to 92%), organic compounds (4% to 16%), inorganic compounds (1% to 2%), and contains high concentrations of suspended solids. It is characterized by elevated chemical oxygen demand (COD) and biochemical oxygen demand (BOD₅), and the presence of fats and phenolic compounds (Gueboudji *et al.* 2022). The color of the wastewater ranges from black to dark red (Mekersi *et al.* 2023), and its phenolic compounds can exert toxic effects on various microorganisms (Solomakou *et al.* 2023). Beyond its visual and odor-related impacts, OMW's high organic load depletes dissolved oxygen in water bodies, devastating aquatic fauna and flora. In agriculture, the direct application of untreated OMW has been shown to reduce crop yields (*e.g.*, tomatoes, beans, and soybeans) and inhibit seed germination in species such as pine, tomato, and atriplex seeds (Mekki *et al.* 2013; Belaqqiz *et al.* 2016; Salem *et al.* 2023; Shabir *et al.* 2023). The direct land application of untreated OMW negatively impacts water, soil, microorganisms, and plants (El Hassani *et al.* 2023; Hamli *et al.* 2024). Consequently, effective treatment of OMW is essential to mitigate its environmental consequences. Since the 1970s, scientific institutions, companies, and public organizations have focused on developing technologies to minimize OMW's environmental impacts, valorize its byproducts, or facilitate its safe disposal (Lombardo *et al.* 2022; Kounani *et al.* 2023).

Although OMW's low organic matter content limits its direct use as a value-added product (Goula and Gerasopoulos 2017; Lee *et al.* 2019), enrichment, mixing with other residues, concentration, drying, or purification can transform it into valuable materials. More than 20 processes or technologies for OMW treatment have been identified; these have been primarily tested at laboratory or pilot scales. They include thermal processes (*e.g.*, evaporation, incineration, distillation), physicochemical processes (*e.g.*, filtration, ultrafiltration, ozonation, coagulation), and biological processes (*e.g.*, anaerobic and aerobic treatments) (Benamar *et al.* 2020; Poerio *et al.* 2022; Saf *et al.* 2022; Cifuentes-Cabezas *et al.* 2023).

In Africa, OMW management is particularly challenging due to the widespread use of traditional production facilities that generate large quantities of untreated waste. This byproduct is often disposed of in evaporation ponds near olive cultivation areas. The OMW production is seasonal (November-February), necessitating annual temporary storage (Sáez *et al.* 2021; Bouhia *et al.* 2023). Recently, there has been growing interest in developing eco-friendly and cost-effective OMW treatment methods. One promising approach involves using biochar derived from olive pomace, a byproduct of olive oil extraction (Raupp *et al.* 2021). By converting olive pomace into biochar, this waste material can be repurposed as an effective absorbent for waste treatment, providing both environmental and economic benefits (Donner *et al.* 2022).

The objective of this study was to characterize OMW before and after its treatment using filtration through an biochar layer derived from olive pomace, with or without lime addition. The study further aimed to evaluate the effects of these treated effluents on the germination of barley (*Hordeum vulgare*, var. Tichdert) and fenugreek (*Trigonella foenum-graecum*) seeds. Additionally, this research sought to contribute valuable insights for researchers, practitioners, and policymakers involved in water and waste management, highlighting the potential of biochar from olive pomace for OMW treatment. By integrating this innovative approach into existing systems, this study envisions a transition towards a

more sustainable and environmentally conscious olive oil production sector, paving the way for greener and more efficient industry practices.

EXPERIMENTAL

Olive Mill Wastewater (OMW)

Olive mill wastewater was collected from the settling basin of a modern Italian oil mill located in Baghai, Khenchela Province, Eastern Algeria. Cold oil extraction was conducted by using a closed process on olives collected from the Khenchela region. The OMW, characterized by a red-brown color, was collected in November 2022 and transported to the laboratory in 5-L bottles. It was stored in a dark refrigerator at 4 °C to preserve its physicochemical characteristics until use.

Sand

Sand filtration is a widely used technique for treating effluents from small communities due to its low energy consumption, minimal maintenance requirements, and ease of integration. Sand filters are recognized for their effectiveness in removing carbon, Kjeldahl nitrogen (through nitrification), suspended solids (SS), and bacterial loads. The filter medium used in this study consisted of washed silica sand with a particle size ranging from 0.8 to 2.0 mm (effective size, d_{10} = 0.9 mm; uniformity coefficient = 1.8). The sand was predominantly composed of coarse particles, providing high permeability while ensuring effective filtration.

Lime

Calcium hydroxide ($\text{Ca}(\text{OH})_2$), commonly known as lime, is an economical, safe, and effective solution widely employed in wastewater and effluent treatment. Lime neutralizes the acidity of acidic effluents to environmentally acceptable levels and aids in removing dissolved metals and inorganic salts due to its highly alkaline nature.

Biochar (charcoal)

The biochar used in this study was prepared from olive pomace obtained from the same olive oil mill, following a modified method described by Heidarinejad *et al.* (2020). Briefly, the dried olive pomace was carbonized in a muffle furnace at 450 °C for 3 h. After cooling to room temperature, the resulting activated charcoal was rinsed three times with distilled water to remove ash and residual impurities. It was then immersed in freshly squeezed lemon juice (pH 2.0–2.5) for 12 h to enhance surface cleaning and improve porosity. Subsequently, the material was rinsed repeatedly with distilled water until a near-neutral pH was achieved and then oven-dried at 105 °C for 24 h before use.

The prepared activated charcoal exhibited a porous structure suitable for adsorption applications. Although physicochemical characterization, such as BET surface area, pore volume, adsorption capacity, and pH at the point of zero charge (pHPZC), was not performed in the present study, the material was prepared under standardized conditions and used consistently throughout all experiments.

Experimental Setup

The treatment system was based on the infiltration–percolation technique using laboratory-scale filtration columns. Each filter consisted of two sand layers enclosing a

reactive layer composed of lime, activated charcoal derived from olive pomace, or their mixture.

The experimental treatments were as follows:

T0: Raw olive mill wastewater (OMW; control).

T1: Sand–lime–sand filter, with a 1 cm lime layer.

T2: Sand–biochar–sand filter, with a 1 cm biochar layer.

T3: Sand–(lime + biochar)–sand filter, with a 1 cm mixed reactive layer.

The sand used in all filters had an average particle size of approximately 1 mm. The filtration materials were packed carefully to ensure uniform bed density and avoid preferential flow.

For each treatment, three independent filtration units ($n = 3$) were prepared to provide biological replicates. Each unit received the same volume of raw OMW (specify volume, *e.g.*, 500 mL or 1 L) under gravity-flow conditions. Filtration was carried out once per replicate (or specify the number of filtration cycles if multiple passes were used). The filtrate was collected immediately after percolation for physicochemical analyses. Fresh filtration media were used for each replicate to eliminate any effect of filter saturation or carry-over between treatments.

Physicochemical Analyses of OMW and Filtrates

pH

The pH was determined using an electronic pH meter (Bante instruments, China) with a 20 mL sample of OMW (Gueboudji and Kadi 2023).

Electrical conductivity

Electrical conductivity (EC) was measured using a Bante instruments conductivity meter, with results expressed in $\mu\text{S}/\text{cm}$ at 20 °C (Gueboudji and Kadi 2023).

Moisture content

The moisture content (MC) of OMW samples was determined by drying approximately 10 g of the sample in an oven at 103 °C for 24 hours. After cooling for 30 min in a desiccator, the samples were reweighed. Moisture loss was calculated as the weight difference between the wet and dry samples (Gueboudji and Kadi 2023).

Dry matter

The dry matter content (DM) was determined indirectly from the moisture content measurement following Gortzi *et al.* (2008). Moisture content was initially measured and expressed in g/L. To ensure consistency of units for calculation purposes, the moisture values were first converted into percentage form based on the appropriate experimental basis (w/w or w/v, clearly defined according to the sample type). Dry matter was then calculated using the following equation:

$$\text{DM (\%)} = 100 - \text{Moisture content (\%)} \quad (1)$$

All measurements were performed under identical conditions to ensure reproducibility, and results are reported as mean values.

Mineral matter and total organic carbon (TOC)

Ash content (mineral matter, MM) was determined by incinerating 1.0 g of the dried sample in a muffle furnace at 550 °C for 5 hours until constant weight was achieved. The ash content was then calculated using Eq. 2:

$$\text{Total ash (\%)} = (\text{Ash weight} \times 100) / (\text{Initial sample weight}) \quad (2)$$

The organic matter (OM) content was obtained by difference as follows:

$$\text{OM (\%)} = 100 - \text{Ash (\%)} \quad (3)$$

Total organic carbon (TOC) was subsequently estimated from the organic matter content using the empirical conversion factor of AFNOR (1975):

$$\text{TOC (\%)} = \text{OM (\%)} \times 1.725 \quad (4)$$

Total polyphenol content

Polyphenols (PP) were quantified using the Folin–Ciocalteu colorimetric method (Makkar *et al.* 1993). The phenolic extract was prepared prior to analysis and used for all subsequent determinations. The Folin–Ciocalteu reagent, a mixture of phosphotungstic acid ($\text{H}_3\text{PW}_{12}\text{O}_{40}$) and phosphomolybdic acid ($\text{H}_3\text{PMo}_{12}\text{O}_{40}$), is reduced by phenolic compounds, forming blue-colored tungsten (W_8O_{23}) and molybdenum (Mo_8O_{23}) complexes. The intensity of the developed blue color, measured at 725 nm (maximum absorption wavelength), is proportional to the total phenolic content of the sample.

Briefly, an aliquot of 50 μL of phenolic extract was mixed with 950 μL of distilled water, 500 μL of Folin–Ciocalteu reagent (1 N), and 2.5 mL of sodium carbonate solution (20% Na_2CO_3). The mixture was incubated in the dark at room temperature for 40 min, after which the absorbance was measured at 725 nm. A calibration curve was constructed using gallic acid standards at concentrations ranging from 0.02 to 0.06 mg/mL (Gueboudji *et al.* 2023).

Total Kjeldahl nitrogen (TKN) was determined according to the classical Kjeldahl method (Tianyun and Xiulai 2016). Organic nitrogen compounds were converted into ammonium sulfate by digestion with concentrated sulfuric acid in the presence of a catalyst. In a Kjeldahl digestion flask, a defined volume of OMW sample was mixed with concentrated sulfuric acid, selenium catalyst, and sodium sulfate to increase the boiling point. The mixture was heated until a clear solution was obtained, indicating complete digestion of organic matter.

After cooling, the digested sample was made alkaline using sodium hydroxide and subjected to steam distillation using a Büchi distillation unit. The liberated ammonia was distilled and captured in a receiving solution of standard sulfuric acid (N/10). The collected distillate was then titrated with standard sulfuric acid solution in the presence of Tashiro indicator until the endpoint was reached. A blank determination was performed under the same conditions. The same sample volume was used consistently throughout the procedure.

Total Kjeldahl nitrogen (TKN) was calculated using Eq. 5,

$$\% \text{TKN} = (V_1 \times 0.014 \times N \times 100) / V_0 \quad (5)$$

where N is the normality of the acid solution, V_0 is the volume of the sample (mL), V_1 is the volume of titrant used (mL), and 0.014 corresponds to the nitrogen equivalent factor. The extent of nitrogen reduction for each treatment was calculated based on the difference between nitrogen concentration in diluted OMW and the concentrations measured in each column (Gueboudji *et al.* 2022).

Phosphorus

Phosphorus content was determined according to the method described by Altieri and Esposito (2010) based on the formation of a blue phosphomolybdenum complex. In this method, orthophosphate reacts with ammonium molybdate in an acidic medium to form phosphomolybdic acid, which is subsequently reduced to a blue-colored complex (molybdenum blue), allowing spectrophotometric determination.

Briefly, a 10 mL aliquot of OMW was appropriately diluted with distilled water to obtain a phosphorus concentration in the range of approximately 2 to 5 mg/L. After addition of the color-developing reagents (ammonium molybdate and reducing agent under acidic conditions), the mixture was allowed to react until full color development.

The absorbance of the resulting solution was measured at 880 nm using a UV–Visible spectrophotometer (Spectrum SP-UV 2005). A calibration curve was prepared using standard phosphate solutions, and phosphorus concentration in the samples was expressed as mg/L (or ppm).

Calcium, sodium, and potassium

The determination of exchangeable cations (Ca, Na, and K) in treated and untreated OMW samples was carried out according to the method described by Vella *et al.* (2016). The concentrations of sodium (Na⁺), potassium (K⁺), and calcium (Ca²⁺) were quantified using flame photometry, (Model FP640, Shanghai Precision & Scientific Instrument Co., Ltd., China).

Prior to analysis, samples were appropriately diluted and filtered to remove any suspended particles. Calibration curves were prepared using certified standard solutions for each element, and all measurements were performed under optimized instrumental conditions. The results were expressed in mg/L.

Organic matter

The quantity of organic matter (OM) was determined using the method described by Chatzistathis and Koutsos (2017). Organic matter is calculated based on the amount of KMnO₄ used per liter of water, expressed in mg of KMnO₄. The formula is,

$$C_{\text{KMnO}_4} = [(V'_{\text{KMnO}_4} + V''_{\text{KMnO}_4}) \times N_{\text{KMnO}_4} - (V_{\text{H}_2\text{C}_2\text{O}_4} \times N_{\text{H}_2\text{C}_2\text{O}_4})] \times 8 \quad (5)$$

where V' is initial volume of KMnO₄ solution (10 mL); V'' is volume of KMnO₄ solution used for titration (3.5 mL); N_{KMnO_4} = 0.01 N; $V_{\text{H}_2\text{C}_2\text{O}_4}$ = 10 mL; $N_{\text{H}_2\text{C}_2\text{O}_4}$ = 0.01 N; and the volume of water analyzed = 50 mL.

Biochemical oxygen demand

Biochemical oxygen demand (BOD₅) was measured by the oxygen consumption at 20 °C in the dark over 5 days of incubation with a pre-seeded sample, representing the time required for the biological oxidation of carbonaceous organic matter. BOD₅ was determined using a respirometric method (Gueboudji 2022) with a BOD meter (BOD Sensor 6, VELP Scientifica). Results are expressed in mg of O₂/L.

Evaluation of Treated OMW Effect on Seed Germination

The phytotoxicity of untreated and treated olive mill wastewater (OMW) was evaluated through seed germination assays using a cereal crop, barley (*Hordeum vulgare* var. Tichdert), and a medicinal plant, fenugreek (*Trigonella foenum-graecum*). Barley seeds were obtained from the Cooperative of Cereals and Dry Legumes (CCLS) of

Khenchela (December 2022), while fenugreek seeds were purchased from a local herbalist in Khenchela.

The germination tests were conducted under laboratory conditions at a controlled temperature of 25 ± 2 °C. Healthy, uniform, and undamaged seeds were visually selected to ensure good viability before the experiment. The experimental design included five treatments: control (tap water), untreated OMW (T0), and the three filtered OMW treatments (T1, T2, and T3). Each treatment was performed in triplicate, with 10 seeds per replicate ($n = 30$ seeds per treatment for each plant species).

For each replicate, the seeds were placed in plastic cups lined with sterile cotton pads moistened with the corresponding treatment solution. The cotton was maintained moist throughout the germination period by adding the respective treatment solution as needed to prevent drying. The cups were kept on a laboratory bench under ambient light conditions throughout the experiment.

A seed was considered germinated when the radicle had visibly emerged (approximately 2 mm). The number of germinated seeds was recorded daily, and the final germination percentage was calculated according to Bhardwaj *et al.* (2012) using the following equation:

$$\text{Germination \%} = (\text{Number of germinated seeds} / \text{Total number of seeds}) \times 100 \quad (6)$$

Statistical Analysis

All experiments were conducted in triplicate, and the results are presented as the mean $\pm$ standard deviation (SD). Statistical analyses were performed using SAS software (version 9.3.1). Differences among treatments were evaluated by one-way analysis of variance (ANOVA).

When the ANOVA indicated significant differences, treatment means were compared using Fisher's least significant difference (LSD) post-hoc test at a significance level of $p < 0.05$. Principal component analysis (PCA) was performed using XLSTAT (Addinsoft, France) to investigate the relationships among the physicochemical parameters and to evaluate the clustering of the different treatment groups.

RESULTS AND DISCUSSION

Effect of Infiltration Treatment on Physicochemical Parameters of OMW

One-way ANOVA analysis of variance on physicochemical parameters of OMW

Based on the results of the one-factor ANOVA, the infiltration treatments had a highly significant effect on OMW parameters, including pH, electrical conductivity (EC), organic matter (OM), polyphenols (PP), total organic carbon (TOC), mineral matter (MM), total Kjeldahl nitrogen (TKN), dry matter (DM), calcium (Ca), sodium (Na), potassium (K), total suspended solids (TSS), biochemical oxygen demand (BOD₅), and phosphorus (P).

Significant differences were observed among the treatments involving infiltration through lime layers, biochar, and their mixtures, with a probability level of $P < 0.05$ (Table 1).

Table 1. Mean Squares for the Treatment Effect on the Physicochemical Parameters of OMW

Sources	Mean squares	Error
DF	3	8
pH	34.673***	0.033
EC	106.909***	3.35
MM	1.7572***	0.0066
TNK	0.0051***	0.0000081
TOC	50.287***	0.0340
OM	98.00***	1.00
BOD5	8611.561***	0.531
PP	0.0183***	0.0000907
Ca ⁺⁺	60.88***	6.5
Na ⁺	4.750***	0.5
K ⁺	60.750***	0.83
P	316.198***	0.123
TSS	53.306***	0.21
DM	7.716***	0.04

*** : $p < 0.001$ (very highly significant), DF: Degrees of freedom .EC, electrical conductivity; MM, mineral matter; TNK, total Kjeldahl nitrogen; TOC, total organic carbon; OM, organic matter; BOD₅, biochemical oxygen demand after 5 days; TSS, total suspended solids; DM, dry matter.

Effect of Infiltration on pH of OMW in Different Layers

The results presented in Table 2 indicate that the pH of untreated OMW was acidic (pH = 4.53), which was likely due to the presence of organic acids such as phenolic acids and fatty acids. This finding aligns with the results reported by Assas *et al.* (2002), Hassen *et al.* (2023), and Benamar *et al.* (2020), who observed pH values ranging from 4.2 to 5.9.

Table 2. Physicochemical Characteristics of OMW Based on the Treatments Using Infiltration

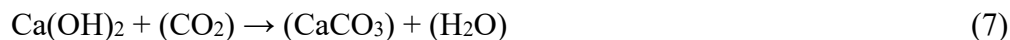
Treatments	T0	T1	T2	T3
pH*** (LSD= 0.342)	4.53 ^d	11.9 ^a	4.93 ^c	6.43 ^b
EC (μS/cm)*** (LSD=3.4483)	31.5 ^a	22.667 ^b	17 ^c	23.33 ^b
MM (%)*** (LSD= 0.53)	2.153 ^a	0.57 ^c	1.06 ^b	0.448 ^c
TKN (g/l)*** (LSD= 0.0054)	1.29 ^a	0.42 ^c	0.56 ^b	0.45 ^c
OM (g/l)*** (LSD= 1.882)	21 ^a	13 ^b	10 ^c	8 ^d
TOC (%)*** (LSD= 0.347)	43.7 ^a	31.66 ^b	21.6 ^c	33.6 ^d
BOD5 (gO ₂ /l)*** (LSD= 1.372)	174 ^a	135.8 ^b	67.33 ^c	65.03 ^d
PP (μg GAE / ml)*** (LSD= 0.0179)	189 ^a	32 ^b	39 ^b	28 ^b
Ca ⁺⁺ (ppm)*** (LSD= 4.8)	22.33 ^a	17.66 ^b	15 ^{bc}	11.661 ^c
Na ⁺ (ppm)*** (LSD= 1.331)	13 ^a	11.33 ^b	10.33 ^b	10.33 ^b
K ⁺ (ppm)*** (LSD= 1.71)	231 ^a	226 ^b	225 ^b	220 ^c
P (mg/l)*** (LSD= 0.660)	42.57 ^a	18.85 ^d	34.23 ^c	37.83 ^b
TSS (g/l)*** (LSD= 0.864)	37.83 ^a	28.17 ^d	35.65 ^b	35.6 ^b
DM (g/l)*** (LSD= 0.864)	119 ^a	89 ^c	98 ^b	82 ^d

a, b, c, d : Homogenous groups; LSD: Least Significant Difference, *** : $p < 0.001$ (very highly significant).

After applying the infiltration-percolation method using different filter layers, the pH of the treated OMW increased significantly, becoming 11.9 that are more alkaline. The pH rose to 4.93 in OMW filtered through a layer of biochar derived from olive pomace (1 cm) between two layers of sand (T2) and to 11.9 in OMW filtered through a lime layer (1

cm) between two layers of sand (T1). This increase is primarily attributed to the alkaline nature of lime, as reported by Khoufi *et al.* (2006).

Lime, being a strong base, reacts with carbonic acid in the OMW, resulting in the formation of calcium carbonate and water through the carbonation reaction:



This reaction effectively neutralizes the acidity, contributing to the observed increase in pH.

Effect of Infiltration-Percolation on Electrical Conductivity

The results in Table 2 show that the untreated OMW exhibited high electrical conductivity (EC) at 31.5 $\mu\text{S}/\text{cm}$. This value reflects the high mineral salt content in the OMW, particularly potassium, chlorides, calcium, and magnesium ions, as noted by Benyahia and Zein (2003) and Zaier *et al.* (2017). Subsequently after applying the infiltration-percolation methods through different filter layers, there was a significant reduction in EC compared to the untreated OMW. The recorded EC values were 22.7, 17, and 23.3 $\mu\text{S}/\text{cm}$ for treatments T1, T2, and T3, respectively. Electrical conductivity serves as an indicator of the overall degree of water mineralization and is influenced by the concentration of ions such as Cl^- , Mg^{2+} , Ca^{2+} , and K^+ , as well as by temperature (McDonald and Rogano 1986; Martínez 2018). The observed reductions in EC can be attributed to the adsorption of mineral salts onto the filtration layers, which included lime, biochar, and their mixture (Fuentes-López *et al.* 2018). Similar findings have been reported by Danish and Ahmad (2018) for filters composed of biochar from wood. These results also underscore the influence of the OMW extraction system and the degree of olive maturation during crushing on the mineralization of OMW, as reported by Gueboudji and Kadi (2023). These findings are consistent with those of Gargouri *et al.* (2022) and Al-Hmoud *et al.* (2020), who observed that soil salinity increases proportionally with the applied OMW dose. This further highlights the importance of effective filtration techniques to reduce the salinity and mineral content of OMW.

Effect of Infiltration-Percolation on Mineral Matter Content of OMW

The comparison of mean mineral matter (MM) contents (Table 2) revealed three statistically homogeneous groups (LSD = 0.53). Untreated olive mill wastewater (OMW) exhibited the highest MM content (2.15%), whereas all infiltration-percolation treatments significantly reduced the mineral load. The biochar filter (T2) reduced the MM content to 1.06%, corresponding to a 50.8% reduction compared with untreated OMW. Greater removal efficiencies were achieved with the lime filter (T1) and the combined lime-biochar filter (T3), yielding MM contents of 0.570% and 0.448%, which represent reductions of 73.5% and 79.2%, respectively.

The marked reduction in mineral matter can be attributed to the complementary removal mechanisms provided by the filtration media. The sand layers promoted the retention of suspended mineral particles through physical filtration, while the porous structure and high specific surface area of olive pomace-derived biochar enhanced the adsorption of dissolved inorganic constituents. In addition, lime increased the pH of the wastewater, promoting the precipitation of dissolved mineral salts and metal ions as insoluble hydroxides or carbonates, which were subsequently retained within the filter bed. The superior performance of the combined treatment (T3) suggests a synergistic effect

between lime and biochar, where precipitation induced by lime was complemented by adsorption onto the biochar surface, resulting in the highest mineral removal efficiency.

These findings are consistent with previous studies reporting that infiltration–percolation systems combining adsorption and chemical precipitation effectively reduce the mineral content of olive mill wastewater (Achak *et al.* 2008). The substantial reduction achieved by treatment T3 demonstrates that integrating lime with olive pomace-derived biochar significantly enhances the purification efficiency of the filtration system, thereby improving the suitability of the treated effluent for subsequent agricultural reuse or environmentally safe disposal.

Effect of Infiltration–Percolation on Total Kjeldahl Nitrogen (TKN) Content

The total Kjeldahl nitrogen (TKN) content in olive mill wastewater (OMW) originates primarily from nitrogen-containing organic compounds, including proteins, amino acids, and other organic residues derived from olive fruits and agricultural practices (Zenjari *et al.* 2006). The untreated OMW exhibited a TKN concentration of 1.20 g/L, which is comparable to the 1.60 g/L reported by Sáez *et al.* (2021) for OMW generated by a Spanish olive mill.

The infiltration–percolation treatments significantly reduced the TKN content, although the removal efficiency depended on the filter composition. Filtration through biochar reduced TKN to 0.56 g/L, while lime treatment decreased it to 0.42 g/L. The combined lime–biochar filter exhibited the highest removal efficiency, indicating that coupling adsorption and chemical precipitation enhances nitrogen removal. Biochar contributes through its porous structure, large specific surface area, and oxygen-containing functional groups, which adsorb dissolved organic nitrogen compounds. In contrast, lime increases the pH of the wastewater, promoting coagulation–flocculation and the precipitation of suspended organic particles that contain nitrogen. The simultaneous action of these mechanisms improves the retention of nitrogenous compounds within the filter bed.

The observed reduction in TKN is closely associated with the removal of suspended solids and organic matter during filtration. Since a substantial fraction of nitrogen in OMW is bound to particulate and colloidal organic materials, their removal directly decreases the TKN concentration. Similar mechanisms have been reported by Achak *et al.* (2008). Differences between the present study and previous reports may be attributed to variations in olive cultivar, extraction process (traditional, two-phase, or three-phase), seasonal conditions, initial wastewater composition, and the characteristics of the filtration materials.

Effect of Infiltration–Percolation on Organic Matter Content

Organic matter (OM) in OMW consists mainly of polysaccharides, lipids, proteins, and a wide range of aromatic and phenolic compounds (Bouaouine *et al.* 2020). The untreated OMW contained 21 g/L of OM, which was substantially reduced following infiltration–percolation. The OM concentration decreased to 13 g/L after lime filtration, 10 g/L after biochar filtration, and 8 g/L after treatment with the combined lime–biochar filter, demonstrating the superior efficiency of the combined treatment.

The reduction in organic matter results from complementary physicochemical mechanisms. Biochar removes dissolved organic compounds through adsorption within its porous structure and through interactions between its surface functional groups and organic molecules. Lime, on the other hand, promotes coagulation–flocculation and the

precipitation of colloidal organic matter by increasing the pH, thereby facilitating its retention within the sand filter. The superior performance of treatment T3 demonstrates the synergistic effect between adsorption and chemical precipitation, leading to more efficient removal of organic constituents than either material used individually.

Although the reduction observed in this study agrees with previous reports, differences in removal efficiencies among studies are expected because OMW characteristics vary considerably depending on olive variety, fruit maturity, climatic conditions, oil extraction technology, and treatment configuration. These factors strongly influence the initial organic load and, consequently, the effectiveness of the filtration process (Cassano 2016; achak *et al.* 2023).

Effect of Infiltration–Percolation on Total Organic Carbon (TOC)

The untreated OMW contained 43.7% total organic carbon (TOC), confirming its high organic load. Filtration significantly reduced the TOC content in all treatments, with the greatest reduction observed for the combined lime–biochar filter.

The decrease in TOC is mainly attributed to the adsorption of dissolved organic compounds and phenolic substances onto the biochar surface, together with the coagulation and precipitation processes induced by lime. These complementary mechanisms reduce both particulate and dissolved organic carbon fractions, thereby lowering the overall TOC content. Similar observations have been reported by Liu *et al.* (2023). Variations between the present results and those reported elsewhere are likely related to differences in biochar properties, lime dosage, wastewater composition, hydraulic loading, and operational conditions.

Effect of Infiltration–Percolation on Biochemical Oxygen Demand (BOD₅)

The untreated OMW exhibited a very high BOD₅ (174 g O₂/L), reflecting its elevated biodegradable organic content. Following filtration, BOD₅ progressively decreased, reaching its lowest value (65.0 g O₂/L) in treatment T3.

This reduction is primarily associated with the removal of biodegradable organic matter through adsorption onto biochar and coagulation–precipitation induced by lime. Consequently, fewer biodegradable substrates remain available for microbial degradation, resulting in lower oxygen demand (Shukla *et al.* 2014). The improved aeration within the porous filter media may also contribute to the reduction in BOD₅. The enhanced performance of the combined filter demonstrates the complementary action of adsorption and chemical precipitation, leading to more effective removal of biodegradable pollutants than either material alone.

Differences between the present findings and those reported in previous studies may reflect variations in olive processing technology, wastewater characteristics, climatic conditions, and filtration system design (Xiang *et al.* 2013).

Effect of Infiltration Treatment on Germination

The analysis of variance (Table 3) revealed a highly significant difference ($P < 0.0001$) between treatments and untreated OMW in terms of germination percentage for both tested species.

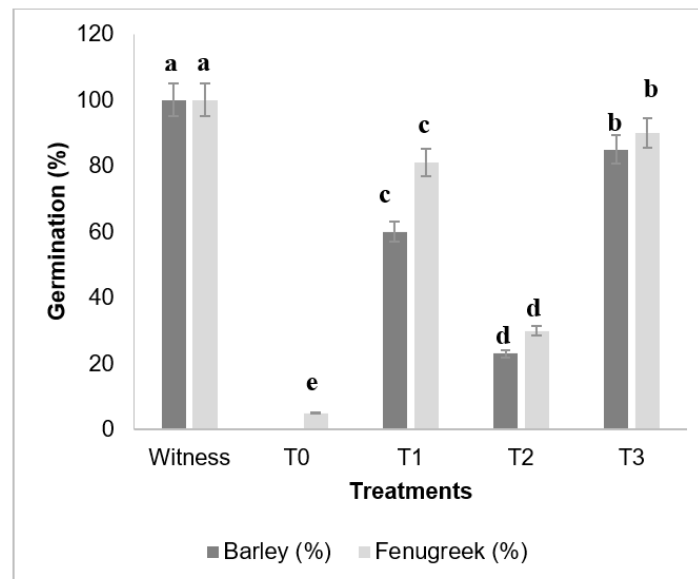
Table 3. Mean Squares of Variance Analysis for the Effect of Different Filter Treatments on the Germination of Two Different Seeds

Sources	DF	Barley	Fenugreek
Treatment	4	5226.66***	5158.566***
Error	10	15.86	10.26

*** : $p < 0.001$ (very highly significant); DF: Degrees of Freedom.

After three days of germination testing with raw and filtered OMW, it was observed that raw OMW inhibited germination entirely for barley (100% inhibition) and significantly for fenugreek (95% inhibition).

Barley exhibited the highest germination percentage (85%) when irrigated with filtrate obtained from the combined lime–biochar treatment (T3), whereas the lowest germination (23%) was observed with the biochar-only treatment (T2). Similarly, fenugreek showed germination percentages of 81% and 90% following irrigation with the lime (T1) and lime–biochar (T3) filtrates, respectively, while only 30% of the seeds germinated after treatment with the biochar filtrate (T2) (Fig. 1).

**Fig. 1.** Effect of OMW infiltration treatment on the germination percentage of two different seeds

These results indicate that seed germination was not governed solely by the electrical conductivity (EC) of the treated OMW. Although the filtrates from T1 and T3 exhibited relatively higher EC values, they also produced the highest germination percentages, suggesting that EC alone is not a reliable indicator of phytotoxicity. The improved germination observed after lime-containing treatments is more likely attributable to the decrease of toxic constituents, including phenolic compounds and excessive organic matter, together with the neutralization of the acidic nature of OMW. Lime promotes coagulation, flocculation, and precipitation of suspended and dissolved pollutants, while biochar adsorbs organic contaminants and phenolic compounds. The combination of these mechanisms substantially decreases the phytotoxicity of the wastewater despite the increase in dissolved mineral ions contributing to EC.

In contrast, the lower germination observed in T2 suggests that adsorption by biochar alone was insufficient to remove all phytotoxic compounds. Therefore, the

inhibition of seed germination should be attributed to the combined effects of residual phytotoxic substances, organic pollutants, and the overall chemical composition of the wastewater rather than to salinity alone (Bewley *et al.* 2013). These findings demonstrate that the decrease of phytotoxic compounds is more critical for seed germination than electrical conductivity by itself, emphasizing the importance of considering multiple physicochemical parameters when evaluating the suitability of treated OMW for agricultural reuse.

Principal Component Analysis (PCA)

Principal component analysis (PCA) was performed to investigate the relationships among the measured physicochemical characteristics of the substrates and the germination responses of barley and fenugreek. The analysis included 17 variables: pH, electrical conductivity (EC), organic matter (OM), polyphenols (PP), total organic carbon (TOC), mineral matter (MM), total Kjeldahl nitrogen (TKN), dry matter (DM), calcium (Ca^{2+}), sodium (Na^+), potassium (K^+), total suspended solids (TSS), biochemical oxygen demand (BOD_5), phosphorus, and the germination percentages of barley and fenugreek. The first two principal components accounted for 89.93% of the total variance, with F1 explaining 61.46% and F2 explaining 28.47% (Fig. 2).

The F1 axis was positively associated with TOC, OM, EC, Na^+ , and BOD_5 , indicating that this component mainly represented the organic load and salinity-related characteristics of the substrates. The F2 axis was primarily associated with pH, TSS, and the germination responses of barley and fenugreek. The relative positions of the germination variables and the physicochemical parameters on the factorial plane indicate the relationships between substrate characteristics and seed germination. Variables located in the same direction from the origin tend to be positively correlated, whereas variables located in opposite directions tend to show negative associations.

The PCA therefore revealed that the treatments modified the physicochemical characteristics of the wastewater/substrate system and, consequently, influenced seed germination. In particular, the distribution of the treatments on the F1–F2 factorial plane (Fig. 3) reflected differences in their organic, mineral, and saline characteristics and their corresponding germination responses.

According to Gairola *et al.* (2012) and Hmissi *et al.* (2023), successful seed germination under laboratory conditions depends on the physicochemical characteristics of the growing medium and its suitability for the tested species. In the present study, the PCA provides an integrated visualization of the relationships between these characteristics and germination performance. The predominance of F1, which explained 61.46% of the variance, indicates that organic and salinity-related parameters contributed substantially to the overall variability among the treatments, whereas F2 accounted for an additional 28.47% and helped discriminate the treatments according to other physicochemical characteristics and germination responses.

Overall, the PCA supports the effectiveness of the applied treatment strategies in modifying the physicochemical characteristics of the wastewater and improving the conditions associated with seed germination. However, the PCA describes associations among variables and should not be interpreted as demonstrating direct causal relationships between individual physicochemical parameters and germination.

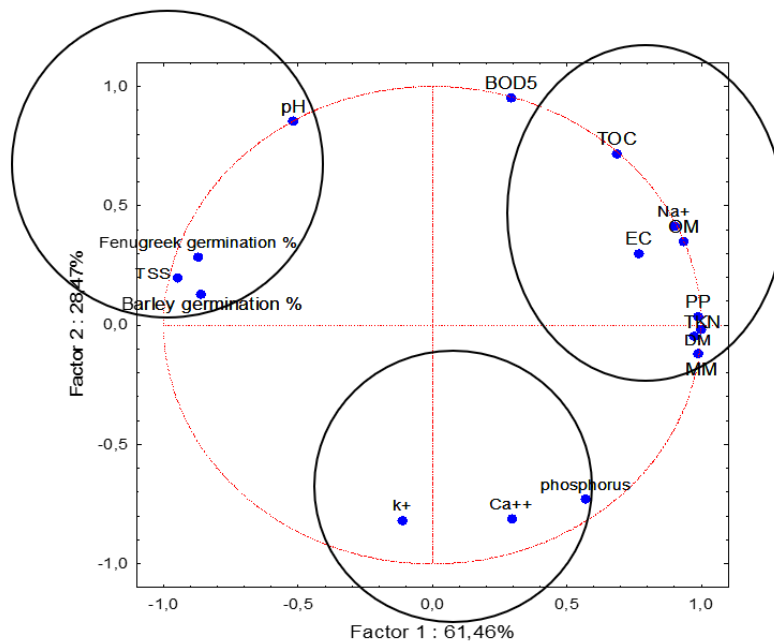


Fig. 2. Principal component analysis (PCA) of the measured parameters

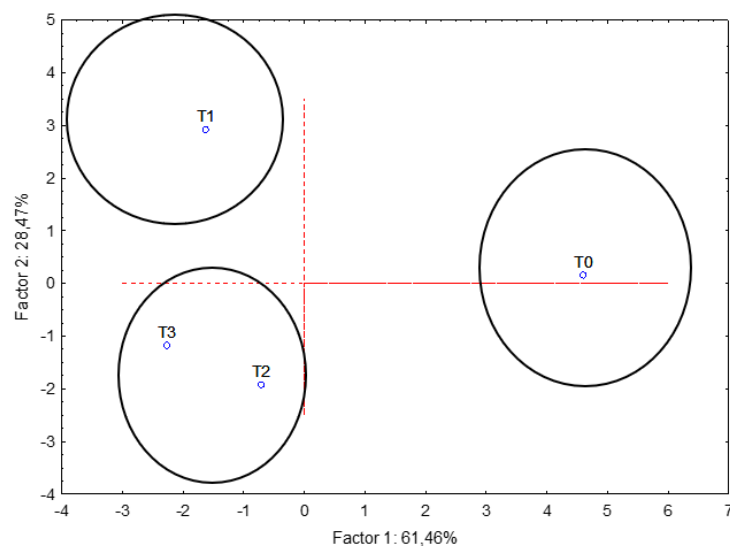


Fig. 3. Principal component analysis (PCA) of treatments

CONCLUSIONS

This study demonstrated the effectiveness of a sustainable approach for the treatment of olive mill wastewater (OMW) using olive pomace-derived biochar, highlighting its potential as an environmentally friendly and low-cost adsorbent for infiltrative wastewater remediation. Results from physicochemical analyses showed a clear improvement in OMW quality after treatment, with T2 (biochar) and T3 (lime and biochar) emerging as the most efficient conditions for pollutant removal. Among all treatments, T3 exhibited the best overall performance, particularly in terms of germination rate, indicating its potential suitability for agricultural reuse applications. These findings underline the dual benefit of the proposed approach, combining effective wastewater depollution with possible valorization in soil-plant systems.

ACKNOWLEDGEMENT

The authors extend their appreciation to Umm Al-Qura University, Saudi Arabia for funding this research work through grant number 26UQU4310078GSSR01.

Funding Statement

This research work was funded by Umm Al-Qura University, Saudi Arabia under grant number 26UQU4310078GSSR01.

Availability of Data and Materials

Data and materials are available upon request.

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Article submitted: February 21, 2026; Peer review completed: May 31, 2026; Revised version received: June 28, 2026; Accepted: August 15, 2026; Published: October 14, 2026.

DOI: 10.15376/biores.21.4.11603-11621

Erratum: October 22, 2026; A new caption for Figure 3 has been added, along with more analysis details on page 11615.