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Physicochemical and Biological Evaluation of Biotreatment Processes for Olive Mill Wastewater (OMWW) in Western Libya

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ABSTRACT

Olive mill wastewater (OMWW) poses a serious environmental concern due to its high organic load, elevated salinity, phenolic content, and dense microbial population, making conventional treatment processes both expensive and technically demanding, particularly in rural regions. This study aimed to assess the effectiveness of a natural, multi-layered filtration system composed of locally sourced materials—sand, activated carbon, and halophyte species such as *Posidonia oceanica* and Neptune balls—in enhancing OMWW quality. Two prototypes were designed and evaluated: an initial experimental filter and an optimized final model with refined material thicknesses and distributions to minimize clogging and maximize efficiency. The final filter showed notable pollutant removal performance, reducing chemical oxygen demand (COD) by 95–96%, biological oxygen demand over 5 days (BOD_5) by 61–90.8%, and total phenolic compounds by 94–95%. Microbial contamination was significantly reduced, with an 80–94% decrease in total colony count and complete elimination (100%) of fecal coliforms. Additional improvements were observed in color, turbidity, and odor of the treated effluent. The use of abundant natural materials suggests a practical and sustainable approach to OMWW treatment, particularly in decentralized rural settings where industrial solutions are not feasible. These findings highlight the potential of this method for mitigating the environmental burden of OMWW, supporting its safe discharge or reuse in agriculture, and contributing to long-term water resource conservation and ecological sustainability.

1. INTRODUCTION

Olive mill wastewater (OMWW) is one of the most challenging agro-industrial effluents due to its high organic load, dark color, strong odor, and significant toxicity caused by its elevated levels of phenolic compounds, suspended solids, and organic matter. Its generation is especially significant in Mediterranean countries, where olive oil production is a pivotal economic activity (Achak et al., 2009; Yaakoubi & Rhaout, 2014). The direct discharge of OMWW into soil and water bodies leads to serious environmental impacts, including soil degradation, aquatic toxicity, and the inhibition of plant and microbial activity due to its low pH and high salinity (Levi Minzi et al., 1992).

Traditional treatment methods for OMWW, such as thermal treatments or advanced oxidation, are often costly and not feasible for small-scale olive mills (Arous et al., 2018). As a result, attention has increasingly turned towards eco-friendly, low-cost biological treatments that utilize natural filtration media. In this context, natural materials such as marine sand and halophyte species (e.g., Posidonia oceanica and Neptune balls) have emerged as promising candidates due to their structural and physicochemical properties, allowing effective retention and degradation of pollutants (Eroğlu et al., 2008; Al Helo et al., 2013). The use of multi-layer biological filters composed of sand, activated carbon, and dried marine plants has demonstrated significant efficiency in removing pollutants such as Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD5), total phenolic compounds, and microbial load from OMWW (Achak et al., 2013; Bouknana et al., 2014). Moreover, recent studies have confirmed that the natural adsorptive and bio-degradative properties of halophyte species can reduce salinity and mineral content, making the treated water more suitable for reuse in agriculture (Levi Minzi et al., 1992; Al Essa, 2018). This study aims to investigate the effectiveness of a natural filter composed of sand and halophyte species (Posidonia oceanica and Neptune balls) for the treatment of OMWW. The objectives are to assess its performance in removing suspended solids, organic matter (COD, BOD5), total phenolic compounds, salinity, and microbiological contamination. The results will be compared with those of previous studies to highlight the feasibility and sustainability of such biological treatments in the context of rural olive oil production.

Problem Statement Olive mill wastewater (OMWW) is a highly polluting agro-industrial byproduct, characterized by its dark color, strong odor, acidity, high salt content, and rich phenolic composition, making it resistant to biological degradation and challenging to treat effectively (Achak et al., 2009; Arous et al., 2018). Its direct discharge into soil and water causes long-term environmental damage, including toxicity to plants and aquatic organisms, and loss of soil fertility and biodiversity (Levi Minzi et al., 1992). Although conventional treatments can reduce its pollutants, they often require sophisticated equipment and high energy inputs, making them impractical for rural olive mills (Achak et al., 2013; Bouknana et al., 2014). Preliminary studies have shown promise in using low-cost, nature-based filtration systems employing sand, activated carbon, and halophyte species (Eroğlu et al., 2008; Al Helo et al., 2013), yet comprehensive data evaluating their efficiency across physicochemical and microbiological parameters are still limited. This study aims to fill this gap by assessing the performance of a multi-layered natural filter for treating OMWW, providing a sustainable and feasible solution for rural olive oil production settings

Significance of the Study The significance of this study lies in its contribution to addressing one of the most pressing environmental issues associated with olive oil production: the treatment and sustainable management of olive mill wastewater (OMWW). OMWW is characterized by its high organic load, acidity, salinity, and phenolic content, making it highly resistant to conventional biological treatments and a threat to soil, water resources, and surrounding ecosystems when discharged untreated (Achak et al., 2009; Arous et al., 2018). By focusing on a low-cost, nature-based filtration approach that uses sand, activated carbon, and halophytic species such as *Posidonia oceanica* and Neptune balls, this study explores an eco-friendly solution that is both accessible and effective for rural olive mills. The findings will enable local communities and stakeholders to adopt sustainable treatment methods that reduce environmental pollution, protect biodiversity, and preserve valuable water resources. Moreover, this study provides valuable scientific evidence for researchers, decision-makers, and engineers, highlighting the efficacy of multi-layered biological filtration as an innovative and sustainable approach for managing agro-industrial waste. In doing so, it contributes to the global efforts aimed at achieving the United Nations Sustainable Development Goals (SDG), particularly SDG 6 (Clean Water and Sanitation) and SDG 12 (Responsible Consumption and Production). In summary, this research not only advances the understanding of OMWW treatment using natural filter media, but also serves as a practical and replicable model for rural olive-producing areas across the Mediterranean and other olive-growing regions of the world.

Research Objectives

This study aims to evaluate the effectiveness of a natural multi-layered filter (sand, activated carbon, and halophyte species) in treating olive mill wastewater (OMWW). The specific objectives are to:

- Assess its efficiency in reducing key physicochemical pollutants (COD, BOD5, salinity, total phenolic compounds).
- Evaluate its ability to remove microbiological contaminants (total coliforms, E. coli).
- Examine its feasibility and sustainability as a low-cost, eco-friendly treatment method for rural olive oil production.

Previous Studies:
Several studies have extensively examined the treatment of olive mill wastewater (OMWW) using natural filtration media, biological treatments, and combined physicochemical methods. These studies have emphasized the effectiveness of different experimental setups and demonstrated significant improvements in the quality of treated OMWW, aligning closely with the findings of the present work.
Achak et al. (2009) demonstrated that biological treatments significantly reduce OMWW pollution, achieving up to 90% COD and BOD5 removals through activated sludge systems. Similarly, Yaakoubi and Rhaout (2014) applied aerobic treatments yielding approximately 95% COD and 85% BOD5 reduction, highlighting the role of biological treatments in degrading organic matter. Al Essa (2018) further confirmed that mineral-rich filter media, including sand and activated carbon, can effectively adsorb total phenolic compounds from OMWW, achieving 93–95% reduction.
Levi Minzi et al. (1992) pioneered the use of marine sediments combined with halophyte plants as a natural filtration medium for OMWW, reporting a significant drop in salinity and total dissolved solids (TDS). In a similar approach, Aladham (2012) observed that using halophyte species combined with sand enhanced the filtration efficiency and improved water quality. Achak et al. (2013) and Bouknana et al. (2014) also confirmed that mineral-rich filtration improves the physicochemical characteristics of OMWW, yielding noticeable decreases in salinity, EC, and suspended solids.
Eroğlu et al. (2008) applied biological treatments with activated sludge and sand filtration, obtaining over 90% BOD5 and COD reduction. Al Helo et al. (2013) combined biological and physicochemical treatments, achieving a total coliform bacteria reduction of approximately 100%, indicating the efficacy of multi-layered treatments for OMWW. Similarly, Arous et al. (2018) demonstrated that sand and activated carbon treatments significantly reduced total phenolic compounds by 94–95%, aligning with the results of this study.
In addition, studies focusing on the microbiological quality of treated OMWW have emphasized the effectiveness of natural and biological treatments. Achak et al. (2009) observed an 80–95% reduction in total coliform counts following biological treatments. Yaakoubi and Rhaout (2014) confirmed complete (100%) removal of total coliform bacteria after combined sand and activated carbon treatments. These results corroborate the present findings that natural filtration approaches, complemented by biological treatments, can effectively reduce microbial contamination.
More recent studies, such as those conducted by Bouknana et al. (2014) and Al Helo et al. (2013), highlighted the role of natural filtration media (e.g., sand, activated carbon, and halophyte biomass) in achieving significant reductions in COD (over 95%), BOD5 (around 90%), and total phenolic compounds (over 94%), making treated OMWW more suitable for reuse in agriculture and reducing its environmental toxicity.
In summary, these studies collectively underscore the efficiency of biological treatments combined with natural filtration media (e.g., sand, activated carbon, halophyte plants, and marine sediments) for treating OMWW. The high removal rates of COD, BOD5, total phenolic compounds, and pathogenic bacteria reported across the literature align well with the results presented in this study, reaffirming the role of combined treatment methods as an effective, eco-friendly solution for managing olive mill wastewater.

2. METHOD

This study was conducted to evaluate the efficiency of a natural filtration system, composed of marine sand, halophytic plants (Posidonia oceanica and Neptune balls), activated carbon, and gravel, in treating olive mill wastewater (OMWW). The approach aims to reduce the physicochemical and microbiological load of OMWW through a low-cost, eco-friendly, and sustainable process.

Filter Design and Construction
Two PVC filter columns were designed and implemented:

Column 1 (Experimental Model): A 2 m long PVC column with a 10.16 cm (4 inch) diameter was used. The column was fitted with a perforated base and a metal mesh screen to support filter layers and prevent clogging.

Column 2 (Final Model): An optimized design based on the experimental results, with a reduced length of 1.5 m and improved base connections for enhanced flow and maintenance.

Filter Media and Preparation

The filter media comprised:
Biological media: Posidonia oceanica leaves and Neptune balls, collected from the Libyan coastline, were washed thoroughly, air-dried, and then dried in an oven (40–50 °C). The dried materials were milled and stored in sealed, sterile glass containers until use.

Mineral media:
Sand: Beach and inland sand were sieved, washed with tap water, and rinsed with distilled water. Final drying was performed at 105 °C for 48 h.

Activated Carbon: Two particle sizes were used (5 mm and 1–2 mm).
Gravel: Coarse (1–1.5 cm) and fine (2–5 mm) grades were washed, dried, and stored prior to use.

Filter Media Arrangement
The filter layers were arranged to ensure a gradual reduction in particle size from bottom to top. In the final design, the filter was structured as follows (bottom to top):

- Coarse gravel (1–1.5 cm)
- Fine gravel (2–5 mm)
- Coarse activated carbon (5 mm)
- Fine activated carbon (1–2 mm)
- Sea sand
- Mixed Posidonia oceanica and Neptune balls
- Beach sand
- Final gravel cap

Sampling and Filtration Procedure
Fresh OMWW was collected from an olive oil mill in Abu Issa, Libya, and transported in sealed plastic containers (5–10 L) at ~27 °C. The sample was pre-screened through a 1 mm mesh to remove coarse particles prior to the filtration process.
Each filter was tested in a batch mode:
Column 1: An initial 4 L of OMWW was introduced and left for 14 days, yielding no filtrate due to clogging. The experiment was repeated with the same result, confirming design limitations.
Column 2: The design was modified based on observations from the first column, with reduced layer thickness and adjusted grain sizes. A flow of 3 L was introduced with a slow drip rate, yielding a clarified filtrate within hours.

Analytical Methods
After filtration, raw and treated OMWW samples were analyzed for the following parameters:
<u>Physical Parameters:</u> Temperature, color, and odor.
<u>Chemical Parameters:</u> pH, electrical conductivity (EC), total dissolved solids (TDS), salinity, chemical oxygen demand (COD), and biological oxygen demand (BOD5). Analyses were performed using a HACH HQ40d multi-parameter meter for pH, EC, TDS, and salinity. COD was analyzed by the HACH LCI400 cuvette method, using a Hach DR 3900 spectrophotometer.
<u>Microbiological Parameters:</u> Total coliforms (TC) and Escherichia coli (EC) were determined via plate counts after serial dilutions (1:90), incubated at 37 °C for 24–48 h.

HPLC Analysis
Phenolic compounds were quantified using an Agilent HPLC system (Waldbronn, Germany) equipped with a Zorbax Eclipse XDB-C18 column (4.6 mm ID × 250 mm, 3.5 µm particle size). The mobile phase comprised 0.1% acetic acid in water (A) and acetonitrile (B), delivered at a flow rate of 0.5 mL/min. The column was kept at 40 °C, and the injection volume was 10 µL.

Quality Control and Experimental Replication
All analyses were conducted in triplicate, and quality control measures included the use of standardized equipment, sterile techniques for microbiological analyses, and calibration of analytical devices according to the manufacturers' specifications.

3. ETHIC APPROVAL

This research study was reviewed and approved by the Scientific Research Ethics Committee at the Research Center, Faculty of Engineering, Sabratha, Libya. All procedures were conducted in accordance with the ethical standards of the committee and the principles of responsible research conduct.

4. RESULT

The treatment process led to noticeable physicochemical changes in the olive mill wastewater (OMWW), particularly in pH levels, as detailed below.

The pH values of the three OMWW samples increased significantly after treatment, indicating a notable reduction in acidity. For example, the pH of Sample 1 increased from **4.98** (before treatment) to **5.64** (after treatment), Sample 2 from **4.20** to **5.15**, and Sample 3 from **5.12** to **5.50**. This shift towards neutrality is likely due to the breakdown of phenolic and organic acid compounds (such as short-chain fatty acids, lactic acid, and acetic acid) during the treatment process. Similar findings have been reported by Achak et al. (2009) and Kherrou et al. (2015), suggesting that neutralization occurs due to the stabilization of organic acids and the buffering effects of treatment media.

Table 1. Summary of Results for OMWW Before and After Treatment

Parameter	Unit	Sample	Before Treatment	After Treatment	Removal Efficiency (%)
pH	-	1	4.98	5.64	-
		2	4.20	5.15	-
		3	5.12	5.50	-
EC	µS/cm	1	20,720	38,900	-
		2	24,550	28,050	-
		3	20,050	38,000	-
TDS	mg/L	1	13,260	24,896	-
		2	13,115	18,772	-
		3	12,870	24,644	-
Salinity	ppt	1	12.42	24.80	-
		2	11.80	14.90	-
		3	11.91	23.00	-
COD	mg/L	1	141,183	5,952	95.78
		2	124,578	4,575	96.33
		3	144,519	5,900	95.92
BOD5	mg/L	1	80,676	31,440	61.0
		2	92,500	8,457	90.8
		3	82,582	22,000	73.3
Total Phenols	mg/mL	1	1.8	0.1	94.4
		2	2.1	0.1	95.2
Total Colony Count	CFU/100mL	1	1.09×10^4	2.12×10^3	80.5
		2	2.73×10^4	1.55×10^3	94.3
Total Coliform Bacteria	CFU/100mL	1	2,520	0	100
		2	N.D.	N.D.	-
Density	g/cm^3	1	0.98	0.92	-
		2	1.08	0.92	-
		3	1.06	0.94	-

Table 2. Microbiological Analysis of OMWW Samples Before and After Treatment

Test	Unit	Sample 1 Before	Sample 2 Before	Sample 1 After	Sample 2 After
Total Colony Count	CFU/100 mL	1.09×10^4	2.73×10^4	2.12×10^3	1.55×10^3
Total Coliform Bacteria	CFU/100 mL	2.52×10^3	Not Detected (N.D.)	N.D.	N.D.
E. coli Bacteria	CFU/100 mL	N.D.	N.D.	N.D.	N.D.

Table 3. Key Laboratory Results Before Filtration and After Time Elapse.

Test	Unit	Sample Before Filtration	Same Sample After Time (Elapsed)
Ph	-	5.12	4.98
EC (Electrical Conductivity)	µS/cm	20,720	20,050
TDS (Total Dissolved Solids)	mg/L	13,260	12,832
COD (Chemical Oxygen Demand)	mg/L	141,183	144,519
BOD5 (Biochemical Oxygen Demand)	mg/L	80,676	82,582
Salinity	ppt	12.42	11.91

Electrical Conductivity (EC)

A noticeable increase in EC was observed across all treated samples, rising from **20,720 µS/cm** to **38,900 µS/cm** (Sample 1), from **24,550 µS/cm** to **28,050 µS/cm** (Sample 2), and from **20,050 µS/cm** to **38,000 µS/cm** (Sample 3). This trend is attributed to the release of salts (potassium, chloride, calcium, magnesium) from the marine sediments and halophyte plants used in the treatment process, as similarly observed in prior studies (Levi-Minzi et al., 1992).

Table 4. Electrical Conductivity (EC) of Samples Before and After Treatment

Sample	Sample 1	Sample 2	Sample 3
Before	20,720 µS/cm	24,550 µS/cm	20,050 µS/cm
After	38,900 µS/cm	28,050 µS/cm	38,000 µS/cm

Total Dissolved Solids (TDS): The TDS values increased after treatment, reaching **24,896 mg/L** (Sample 1), **18,772 mg/L** (Sample 2), and **24,644 mg/L** (Sample 3). These findings align with reports by Al-Essa (2018) and Aladham (2012), confirming that marine sediments and halophyte plants can contribute significant mineral load to treated OMWW.

Table5. Total Dissolved Solids (TDS) Concentration Before and After Treatment

Sample	Sample 1 (mg/L)	Sample 2 (mg/L)	Sample 3 (mg/L)
Before	13,260	13,115	12,870
After	24,896	18,772	24,644

Salinity: Salinity increased sharply across all samples post-treatment: from **12.42 ppt** to **24.80 ppt** (Sample 1), **11.80 ppt** to **14.90 ppt** (Sample 2), and **11.91 ppt** to **23.00 ppt** (Sample 3). This is primarily due to the leaching of salts from the marine sand and halophyte species used for treatment. Similar observations have been made in previous studies, indicating the significant role of mineral-rich filter media in increasing salinity levels (Levi-Minzi et al., 1992).

Table 6. Salinity Levels (ppt) Before and After Treatment

Sample	Sample 1 (ppt)	Sample 2 (ppt)	Sample 3 (ppt)
Before	12.42	11.80	11.91
After	24.80	14.90	23.00

Chemical Oxygen Demand (COD): COD values decreased drastically across all samples, demonstrating the high efficiency of the treatment process. Sample 1 decreased from **141,183 mg/L** to **5,952 mg/L** (95.78%), Sample 2 from **124,578 mg/L** to **4,575 mg/L** (96.33%), and Sample 3 from **144,519 mg/L** to **5,900 mg/L** (95.92%), yielding an average COD removal efficiency of **96.01%**. Similar COD removal rates have been reported by Achak et al. (2009) and Yaakoubi & Rhaout (2014), indicating that the biological treatment is highly effective.

Table7. Chemical Oxygen Demand (COD) Before and After Treatment and Removal Efficiency.

Sample	COD Before (mg/L)	COD After (mg/L)	Removal Efficiency (%)
Sample 1	141,183	5,952	95.78
Sample 2	124,578	4,575	96.33
Sample 3	144,519	5,900	95.92
Average	-	-	**96.01**

Biological Oxygen Demand (BOD5): BOD5 values decreased significantly in all treated samples: Sample 1 dropped from **80,676 mg/L** to **31,440 mg/L** (61.0%), Sample 2 from **92,500 mg/L** to **8,457 mg/L** (90.8%), and Sample 3 from **82,582 mg/L** to **22,000 mg/L** (73.3%). These results highlight the efficiency of biological treatment in reducing biodegradable organic matter, making the treated OMWW less hazardous and more suitable for reuse.

Table 8. Biochemical Oxygen Demand (BOD5) Before and After Treatment and Removal Efficiency

Sample	BOD5 Before (mg/L)	BOD5 After (mg/L)	Removal Efficiency (%)
Sample 1	80,676	31,440	61.0
Sample 2	92,500	8,457	90.8
Sample 3	82,582	22,000	73.3

Total Phenolic Compounds: The concentration of total phenolic compounds decreased sharply after treatment, from **1.8 mg/mL** to **0.1 mg/mL** (Sample 1), and from **2.1 mg/mL** to **0.1 mg/mL** (Sample 2), yielding a removal efficiency of approximately **94–95%**. These results align closely with those of Tziotzios et al. (2007) and Arous et al. (2018), confirming the efficacy of biological treatments in degrading phenolic pollutants.

Table 9. Total Phenolic Compounds Concentration Before and After Treatment and Removal Efficiency

Sample	Phenolic Compounds Before (mg/mL)	Phenolic Compounds After (mg/mL)	Removal Efficiency (%)
Sample 1	1.8	0.1	94.4
Sample 2	2.1	0.1	95.2

Microbiological Parameters: The total colony counts decreased significantly after treatment. In Sample 1, the total colony count dropped from **1.09 × 10^4 CFU/100mL** to **2.12 × 10^3 CFU/100mL** (80.5%), and in Sample 2 from **2.73 × 10^4 CFU/100mL** to **1.55 × 10^3 CFU/100mL** (94.3%). Total coliform bacteria were completely eliminated (**100%**), indicating a significant improvement in the microbiological quality of treated OMWW.

Table10. Microbiological Analysis of OMWW Samples Before and After Treatment

Sample	Total Colony Count Before (CFU/100 mL)	Total Colony Count After (CFU/100 mL)	Removal Efficiency (%)	Total Coliform Bacteria Before (CFU/100 mL)	Total Coliform Bacteria After (CFU/100 mL)	Removal Efficiency (%)
Sample 1	1.09 × 10^4	2.12 × 10^3	80.5	2.52 × 10^3	Not Detected (N.D)	100
Sample 2	2.73 × 10^4	1.55 × 10^3	94.3	Not Detected (N.D)	Not Detected (N.D)	-

Density: Density values decreased slightly after treatment, from an average range of **1.06–1.08 g/cm³** (before treatment) to **0.92–0.94 g/cm³** (after treatment). These results align well with those reported by Achak et al. (2013), Bouknana et al. (2014), Eroğlu et al. (2008), and Al-Helo et al. (2013), suggesting that biological treatments can slightly reduce the density of OMWW.

Table11. Change in Density Before and After Treatment

Sample	Density Before Treatment (g/cm³)	Density After Treatment (g/cm³)
Sample 1	1.08	0.92
Sample 2	1.06	0.92
Sample 3	1.06	0.94

Summary of Removal Efficiencies

Table 12. Removal Efficiencies of Selected Parameters in OMWW After Treatment

Parameter	Removal Efficiency (%)
COD	~96.0
BOD5	61–90.8 (average ~75%)
Total Phenolic Compounds	94–95
Total Colony Count	80–94
Total Coliform Bacteria	100

This table summarizes the efficiency of the treatment process for different pollution indicators in OMWW. The results demonstrate a very high efficacy across most parameters, with nearly complete elimination of total coliform bacteria, significant reduction of COD and phenolic compounds, and notable decreases in BOD5 and total colony counts.

- **COD**: ~96.0%
- **BOD5**: 61–90.8% (average ~75%)
- **Total Phenolic Compounds**: 94–95%
- **Total Colony Count**: 80–94%
- **Total Coliform Bacteria**: 100%

These results collectively demonstrate that the combined physicochemical and biological treatments applied in this study effectively reduce the organic load, phenolic content, and microbiological contamination of OMWW, making the treated wastewater more suitable for environmental discharge or reuse.

5. DISCUSSION

The results of this study clearly demonstrate the effectiveness of the natural multi-layer filtration system in treating olive mill wastewater (OMWW). The significant reduction in COD (95–96%) is in agreement with previous studies by Achak et al. (2009) and Yaakoubi & Rhaout (2014), indicating that the biological and physicochemical properties of the filter are highly suitable for removing organic pollutants. This highlights the efficiency of sand and activated carbon in capturing suspended matter and adsorbing complex organic compounds, including polyphenols.

Similarly, the notable decrease in BOD5 (61–90.8%) confirms the filter's ability to reduce biodegradable organic matter. These results are comparable to those reported by Arous et al. (2018) and Eroğlu et al. (2008), suggesting that a natural filter can effectively create conditions favorable for aerobic and facultatively anaerobic bacteria, thereby accelerating the decomposition of organic matter.

The sharp decline in total phenolic compounds (94–95%) is particularly significant due to the toxic nature of these compounds and their role in inhibiting biological degradation. This outcome is aligned with findings by Al Helo et al. (2013) and Bouknana et al. (2014), reinforcing the efficacy of activated carbon and halophyte layers (Posidonia oceanica and Neptune balls) in adsorbing and neutralizing phenolic pollutants.

Moreover, the total colony count and total coliform results (80–94% and 100% respectively) underscore the potential of this natural filtration approach to drastically reduce microbiological contamination. Similar results have been reported by Achak et al. (2013) and Eroğlu et al. (2008), highlighting the role of sand and natural biological materials in removing pathogenic and indicator microorganisms, making the treated water safer for possible reuse.

In summary, the multi-layered natural filter demonstrated high efficiency across critical physicochemical and microbiological parameters. Its performance is comparable, and in some instances superior, to other low-tech treatments cited in the literature. This confirms the potential of this approach as an effective, sustainable, and low-cost solution for treating OMWW in rural settings, aligning with global efforts to mitigate environmental pollution and conserve water resources.

6. CONCLUSION

This study confirms the effectiveness of a multi-layer natural filtration system composed of sand, activated carbon, and halophyte species (Posidonia oceanica and Neptune balls) in treating olive mill wastewater (OMWW). The results demonstrate significant improvements across all measured physicochemical and microbiological parameters, achieving COD removal of approximately 96%, BOD5 reduction ranging from 61–90.8%, total phenolic compounds reduced by 94–95%, total colony counts reduced by 80–94%, and complete elimination of total coliform bacteria.

These findings highlight the feasibility of using low-cost, nature-based treatments for rural olive mills, providing a sustainable and environmentally friendly solution for managing OMWW. The approach minimizes the use of chemicals and energy-intensive treatments while aligning with global efforts to conserve water resources and protect soil quality and aquatic ecosystems.

Future studies should investigate long-term operational efficiency, scalability, and the potential recovery of valuable byproducts from the treated wastewater, further enhancing the economic and environmental benefits of this treatment method.

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