Asian Journal of Water, Environment and Pollution. Vol. 23, No. 1 (2026), pp. 293-301.
doi: 10.36922/ajwep.6618

ORIGINAL RESEARCH ARTICLE

Valorization of olive mill wastewater through irrigation trials of *Triticum durum* seeds using the dilution approach

Omar Elrhaouat[1]*, Mohamed Hadji[1], Sakina Belhamidi[2], and Mohammed Ouhssine[1]

[1]Department of Biology, Faculty of Sciences, Ibn Tofail University, Kenitra, Kenitra Province, Morocco
[2]Department of Chemistry, Higher School of Technical Sciences, Ibn Tofail University, Kenitra, Kenitra, Kenitra Province, Morocco
***Corresponding author:** Omar Elrhaouat (omar.elrhaouat@uit.ac.ma)

Received: November 27, 2024; Revised: January 29, 2025; Accepted: February 5, 2025; Published Online: November 26, 2025

Abstract: Olive mill wastewater (OMW) generated by industrial extraction units in olive oil mills is a significant source of pollution for the ecosystem. This pollution manifests through inhibited soil microbial activity and reduced plant germination rates due to high acidity, excessive salinity, and the presence of heavy metals. This study aimed to enhance the value of these effluents by restoring soil microbial activity and promoting plant germination through a series of increasing dilutions to determine the optimal dose. This dilution process effectively neutralized the pH and rebalanced the microbial load, facilitating the reappearance of various bacterial species, such as fecal coliforms, total coliforms, *Escherichia coli*, and *Staphylococcus*. This resurgence significantly contributed to improved soil fertility. Furthermore, at a 10/100 dilution ratio, wheat seed germination reached 98%, with seedling growth reaching 18 cm – observations comparable to the control batch. These results demonstrate the effectiveness of this dilution strategy in mitigating the negative effects of OMW on agricultural soils.

Keywords: Germination; Dilution; Soil; Microbes; Heavy metals; Olive mill wastewater

1. Introduction

Morocco, like other Mediterranean countries, has emerged as a leading olive oil producer. However, this success is accompanied by a significant environmental challenge – generating huge quantities of wastewater from olive oil extraction units. If not properly treated beforehand, this wastewater poses a considerable risk, resulting in environmental pollution. Disposal of untreated olive mill wastewater (OMW) onto the soil can have devastating effects, including sterilization and defertilization. This is primarily due to its high content of heavy metals, minerals, and organic compounds – particularly phenols – along with various physicochemical elements, such as a highly acidic pH and low dissolved oxygen levels. Moreover, OMW also contains a specific microbial load that tolerates the environment's acidity. Hence, sustainable management and recovery strategies are essential for mitigating these negative impacts.

The direct application of OMW to agricultural soils has several adverse effects, such as compromising soil microbial life and reducing durum wheat (*Triticum durum*) germination rates. These negative effects are attributed to factors including high acidity levels, the presence of phenols and salts, and the proliferation of *Lactobacillus* bacteria that exacerbate environmental acidification. Furthermore, a lack of oxygen asphyxiates plant roots, while heavy metals disrupt bacterial metabolism, compromising soil fertility. Under normal conditions, microorganisms are essential in maintaining soil fertility.[1]

A study used an approach based on progressive dilution of OMW effluents applied to durum wheat seeds to determine the optimum dose for restoring soil biological functions. The results showed that at a dilution of 10/100, soil microorganisms were preserved and fulfilled their metabolic role within the ecosystem.[2] Conversely, uncontrolled dispersion of these effluents leads to soil biotoxicity, damaging microbial flora.[3,4] The persistent, non-biodegradable presence of heavy metals in the soil adversely affects the biotope, biocenosis, and human health.[5-7]

The wastewater was diluted across increasing concentrations to determine the optimal dose for durum wheat germination and growth. Results indicated that at a 10/100 dilution ratio, seedling size increased significantly compared to those grown in water-irrigated control soil. This dilution also facilitated normal activation and development of soil microorganisms due to the gradual neutralization of pH and the reduction in heavy metal concentrations, thereby reducing their inhibitory effect on microbial metabolism.

These results highlight the importance of carefully managing OMW to preserve soil biodiversity and promote a sustainable ecological balance. By adopting appropriate strategies, such as optimized dilution, it is possible to reduce the negative impact on ecosystems while maintaining their biological fertility. This approach aligns well with broader sustainable development goals aimed at reconciling agricultural needs alongside environmental protection measures.

2. Materials and methods

The study was conducted in Sidi Kacem (Figure 1), located in central Morocco, where olive oil extraction plants produce OMW. The production of OMW is associated with adverse effects on the region's ecosystem. Effluent samples were collected 5 times a month during the olive harvesting and extraction season (from October to November) across 12 olive oil extraction plants (Figure 1). These plants were divided into three extraction categories: Traditional discontinuous system (TDS), modern discontinuous system (MDS), and modern continuous system (MCS).

Table 1 below presents the descriptive statistics for the specific microbial load and key physicochemical parameters of the OMW.

2.1. Microbiological analysis and culture

For microbiological analysis purposes, the samples were filled into sterile 1,000 mL bottles and stored in a cooler at a temperature of 4°C. Twenty-five mL of wastewater from the mill was added to 100 mL of buffered peptone water. This mixture was homogenized and then subjected to a series of cascade dilutions in sterile distilled water. Subsequently, a 0.1 mL aliquot from the stock solution was inoculated onto selective agar to facilitate accurate enumeration during incubation at specific temperatures for each species studied. Microbial cultures revealed the presence of *Lactobacillus*, yeasts, and total aerobic mesophilic flora (TAMF), while species such as total coliforms

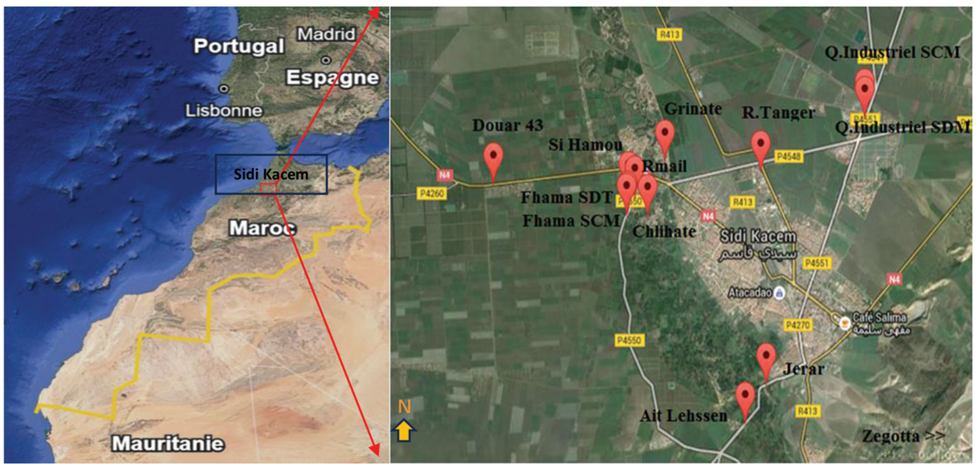

Figure 1. Location of the study areas in Sidi Kacem city

Table 1. Descriptive statistics for the microbial load and physicochemical parameters of olive mill wastewater samples by type of extraction system

Variable	Mean	SD	CV	Minimum	Q1	Median	Q3	Maximum
TAMF ($\times 10^4$ CFU)	1.055	0.768	72.8	0.28	0.47	0.865	1.365	3
Lactobacillus ($\times 10^4$ CFU)	0.796	0.382	47.95	0.34	0.45	0.685	1.185	1.4
Yeast ($\times 10^4$ CFU)	0.94	0.409	43.53	0.36	0.558	0.92	1.35	1.63
Anaerobic SR ($\times 10^4$ CFU)	3.083	1.346	43.64	1.5	1.625	3	4.625	5
Fecal coliforms ($\times 10^4$ CFU)	0	0	-	0	0	0	0	0
Total coliforms ($\times 10^4$ CFU)	0	0	-	0	0	0	0	0
Staphylococcus ($\times 10^4$ CFU)	0	0	-	0	0	0	0	0
pH	4.902	0.407	8.3	4.25	4.545	4.95	5.143	5.59
Salinity (mg/L)	12.129	2.63	21.68	7.65	9.92	12.635	13.818	17.11
Dissolved oxygen (mg/L)	0.5347	0.1452	27.16	0.24	0.4825	0.565	0.6145	0.77
BOD_5 (mg/L)	23.582	10.086	42.77	12.140	17.184	21.382	28.473	50.104
Electrical conductivity (µS/cm)	19.430	6.158	31.7	10.752	13.250	18.820	25.175	29.080

Abbreviations: BOD: Biochemical oxygen demand; CFU: Colony forming unit; CV: Coefficient of variation; Q: Quartile; SD: Standard deviation; SR: Side-stream reactors; TAMF: Total aerobic mesophilic flora.

(TC), fecal coliforms (FC), *Escherichia coli*, and *Staphylococcus* were absent.

Durum wheat cultivation was conducted in homogeneous soils, with each batch containing 50 seeds – a control group included for comparison. These batches were irrigated with increasing concentrations of OMW diluted with decreasing volumes of water. After one week post-irrigation, the growth of durum wheat in each batch was evaluated, and soil microbiological analyses were conducted to assess changes before and after irrigation with OMW. Soils irrigated with increasing concentrations of inversely diluted effluents D1 to D5 with a control soil were subjected to microbiological analyses on various culture media: deMan, Regosa, and Sharpe agar for *Lactobacillus* incubated at 30°C, Sabourand agar for yeasts incubated at 30°C over 48 – 72 h, supplemented with chloramphenicol (0.25 g/L) to inhibit bacterial growth, Violet Red Bile Agar for total coliforms and fecal coliforms incubated at 37°C and 44°C, respectively, for 24 h, and Baird Parker agar for *Staphylococcus* incubated 35 – 37°C for 24 – 48 h according to the protocols provided by BioMérieux.

2.2. AAS spectroscopic analysis

The samples were analyzed using flame atomic absorption spectroscopy to determine the concentration of each metallic element such as arsenic (As), chromium (Cr), mercury (Hg), nickel (Ni), cadmium (Cd), lead (Pb), selenium (Se), zinc (Zn), copper (Cu), and iron (Fe). This analysis was performed using NOVAA 350 (Analytik Jena, Germany) with acetylene/air flame (standard 50 mm slit burner) and a pneumatic annular slit nebulizer (platinum or rhodium with a graphite cannula) with a flow rate of 4 – 6 mL/min. The NOVAA 350 concentrations of the specific standard for each metal to be analyzed were prepared (Standards are available at 1,000 ppm in 120 – 500 mL vials for flame analysis).

Sampling was conducted during the olive oil crushing period at olive extraction plants. Wastewater samples were placed in 100 mL borosilicate flasks and subjected to wet mineralization through acid attack with concentrated nitric acid (HNO_3) and perchloric acid ($HClO_4$). This process involved several steps:

(i) Sample preparation: The samples were filtered through a 0.45 µm filter to remove suspended solids, followed by homogenization. The pH of each sample was adjusted to less than two to stabilize the metal ions[8] by adding concentrated HNO_3 at a recommended dosage of 1% by volume, corresponding to adding 1 mL of concentrated HNO_3 per 100 mL of sample. The final volume was achieved by adding ultrapure or demineralized water, followed by homogenization of the sample with gentle agitation.

(ii) Wet mineralization procedure: Fifty mL of homogenized OMW was collected for analysis. A mixture of acids consisting of HNO_3 and $HClO_4$ with a ratio of 3 to 1 was prepared. The mixture was heated to 180 – 200°C until near complete evaporation, resulting in a clear residue formation after 2 – 3 h. Following thermal digestion, 25 mL of ultrapure water was added to the residue, followed by gentle shaking to dissolve any remaining residue. Another filtration step using a 0.45 μm membrane was performed to remove insoluble particles. The final dilution to make up the final volume of 50 mL was performed with ultrapure water while maintaining acidity by adding 1% of HNO_3 (add 0.5 mL HNO_3 for 50 mL sample). Samples were stored at 4°C before analysis.

(iii) Standard calibration solutions: Calibration standards for heavy metals were prepared from certified standard solutions (1000 mg/L) diluted to final concentrations of 0.5, 1, 2, 5, and 10 mg/L. One percent HNO_3 was used to stabilize the standard solutions.

2.3. Statistical analysis

Statistical analyses were conducted using various software, such as Origin (version 9) and Statistical Analysis System JMP Pro (version 17). Appropriate statistical tests, including regression analysis, principal component analysis, and analysis of variance (ANOVA) mean comparison, were applied across three distinct groups of olive oil extraction processes.

3. Results

This work employed physicochemical analyses, notably the quantification of heavy metals, and microbiological analyses to reveal significant correlations between certain heavy metals while others exhibited weaker relationships (Figure 2). In addition, the effect of progressive dilution of OMW on wheat germination and growth was evaluated, revealing significant variations as a function of applied concentrations (Figure 3).

The application of a dilution process enabled the identification of the optimal doses for seed germination and ecosystem preservation, opening promising prospects for valorizing these effluents. Furthermore, a comparative analysis was conducted on the three olive extraction processes – MCS, MDS, and TDS – using ANOVA followed by Tukey's *post hoc* test, highlighting significant differences between these processes.

Figure 2 represents the correlation matrix of various metallic elements, with high correlation coefficients observed between As-Cd (0.97), Se-Zn (0.96), Cr-Pb (0.94), As-Pb (0.86), As-Cr (0.85), Cd-Cr (0.83), and Cd-Pb (0.82). These correlations represent the interactions affecting the microbial metabolism as well as carbon and phosphorus limitations, such as Zn's relationship with Cd, Cr, Pb, and As, with values of 0.74, 0.72, 0.71, and 0.7, respectively. An ANOVA test followed by a *post hoc* analysis was applied to explore the differences between the three systems studied.

The ANOVA test revealed a significant difference with probability $P = 0.00$, which is less than the significance value set at $P < 0.05$. For a good fit of the ANOVA model, the R-squared and root mean square of

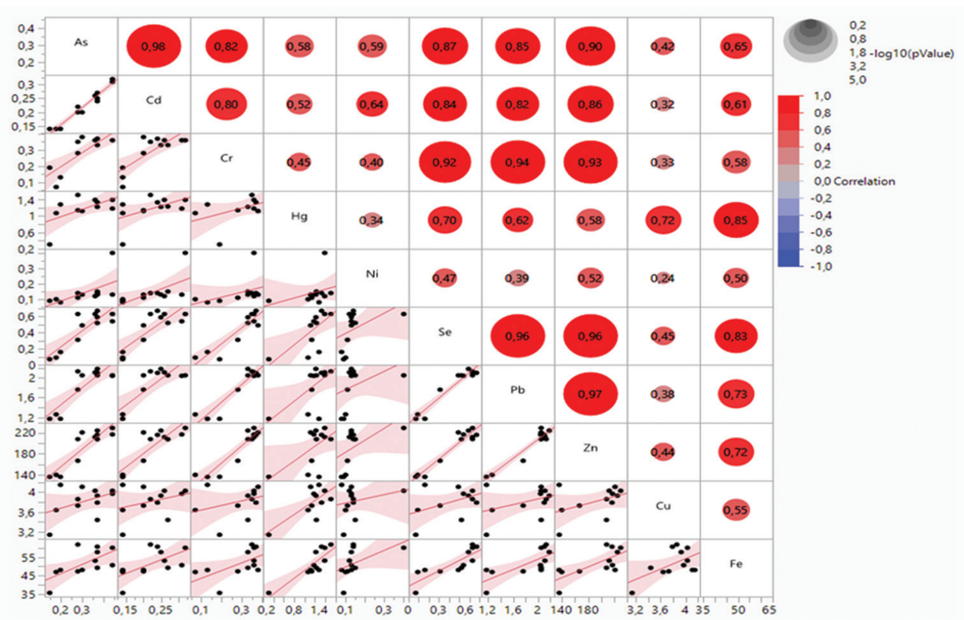

Figure 2. A scatterplot matrix showing the regression and correlation analyses of heavy metals

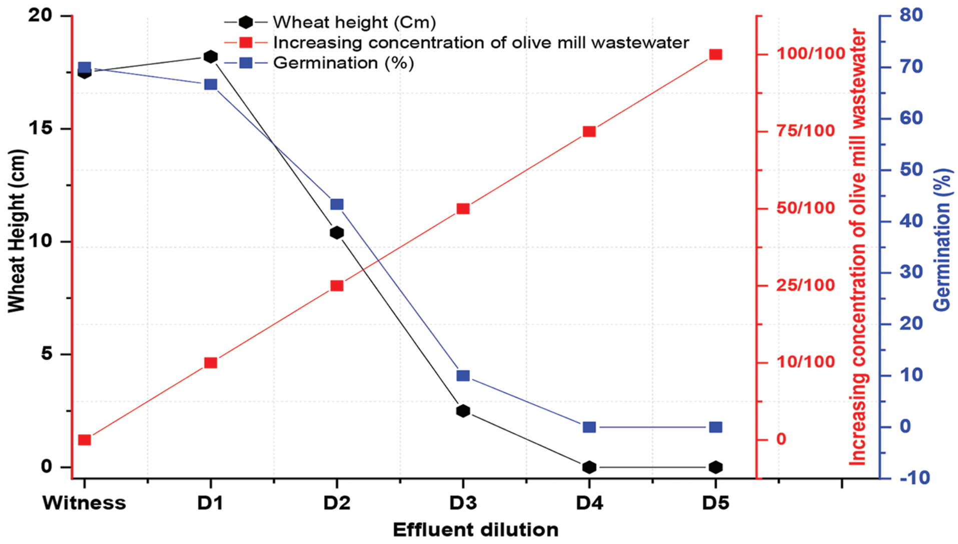

Figure 3. Variation in plant size and germination with increasing dilution of oil mill wastewater from D1 to D5

Table 2. Analysis of variance for the three different olive extraction systems

Overall ANOVA						Errors adjustment
Source	**DF**	**Sum of squares**	**Mean square**	**F value**	**Pro > F**	**R^2, root MSE**
Model	2	0.08786	0.04393	17.46792	7.97×10^{-4}	
Error	9	0.02263	0.00251			0.79, 0.050
Total	11	0.1105				

Abbreviations: ANOVA: Analysis of variance; DF: Degrees of freedom; MSE: Mean square of the error.

Table 3. Multiple comparisons by pairs of groups using Tukey's *post hoc* test

Pairs of groups	Mean diff	SEM	q value	Probability	Alpha	Sig.	LCL	UCL
MCS-MDS	-0.18333	0.03546	7.31149	0.00152	0.05	1	-0.28234	-0.08433
TDS-MDS	0.03467	0.03546	1.38254	0.6082	0.05	0	-0.06434	0.13367
TDS-MCS	0.218	0.04095	7.52924	0.00124	0.05	1	0.10368	0.33232

Abbreviations: LCL: Lower control limit; MCS: Modern continuous system; MDS: Modern discontinuous system; Sig: Significance; TDS: Traditional discontinuous system; UCL: Upper control limit

the error coefficient values were significant, at 0.79 and 0.05, respectively (Table 2).

Tables 3 and 4 show the comparisons of the means using Tukey's *post hoc* test with values 0 or 1 corresponding to the significance of the test. A value of 0 indicates no significant difference ($P > 0.05$) as it falls within the interval (−0.06434, 0.13367), while a value of 1 indicates a significant difference between the MCS and MDS/TDS systems with a probability of $P < 0.05$ and a confidence interval far from zero (Table 3).

Following the *post hoc* analysis, a Tukey test was used to determine the differences between the three extraction systems. Results in Table 4 indicate that the MCS system differs significantly from the TDS and MDS systems. TDS and MDS systems did not show a difference in means, as indicated by the *P*-value below the error risk threshold of 0.05 (Table 4). This suggests

Table 4. The similarity of the extraction systems characterized by letters A and B

System	Mean	Groups	Groups
TDS	0.39533	A	
MDS	0.36067	A	
MCS	0.17733		B

Abbreviations: MCS: Modern continuous system; MDS: Modern discontinuous system; TDS: Traditional discontinuous system.

that the two discontinuous pressure systems cause more pollution to the ecosystem than the MCS.

Table 4 presents the groups characterized by the letters A and B, where the letter A reflects the similarity of the TDS and MDS systems, and the letter B indicates a difference in the MCS system compared to the others. This classification aligns with the results from the comparison of means (Table 3) from Tukey's test following the *post hoc* analysis.

According to Figure 3, this study demonstrates that as the dilution of the OMW progresses from sample D1 (10/100 dilution) to sample D5 (100% undiluted), the germination rate of wheat seeds gradually decreases until it is completely inhibited. Concurrently, as the concentration of these effluents increases, the size of the wheat seedlings decreases due to the adverse effects associated with higher concentrations.

Seed germination is negatively impacted by several factors, such as heavy metals, salinity-causing obstructions, organic matter, and acidic pH, which collectively render agriculture challenging due to soil infertility. In Figure 3, it is illustrated that as the dilution of OMW increases, some chemical parameters decrease, thereby maintaining normal conditions for microbial communities and various microorganisms in the soil. This leads to the reappearance of species such as *E. coli*, TAMF, TC, and FC, contributing to improved soil fertility and plant germination.

In a series of batches with increasing concentrations of irrigated OMW alongside inversely increasing dilutions, the bacterial load of *Lactobacillus* increased compared to TC and FC, which abruptly decreased until they disappeared. This disappearance is mainly due to the acidity of *Lactobacillus* within this ecosystem. The impact of increased dilution causes these microorganisms to reappear in the soil at a diluted concentration, specifically at the 10/100 ratio, which is suitable for wheat seed germination (Figure 4).

Adequate dilution was achieved at a ratio of 10/100, corresponding to a high and balanced microbial load; however, this gradually decreased as effluent concentration increased across successive batches. As a result, FC, TC, *E. coli*, and *Staphylococcus* species showed decreasing loads until they disappeared as effluent concentration approached the raw state. In contrast, despite an acidic environment, *Lactobacillus*, TAMF, and yeasts persisted when undiluted effluents were used (Figure 5).

4. Discussion

The discharge of industrial effluents into the environment, especially into the soil, poses significant environmental challenges, due to their high content of heavy metals, such as Hg, Cr, Zn, Cd, As, Ni, Cu, and Pb. These contaminants disrupt microbial metabolism within soils because they are characterized by high acidity levels that some microbial species tolerate better than others. Consequently, this intrusion compromises the soil's stability, maintained typically through organic matter's role in preserving water and nutrient retention

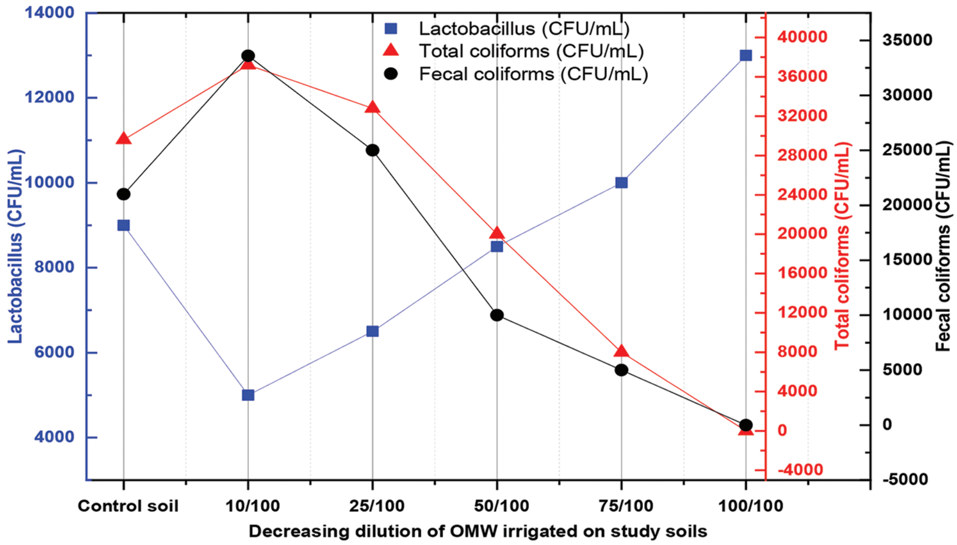

Figure 4. Variation in *Lactobacillus*, total coliforms, and fecal coliform concentrations with increasing dilution of irrigated OMW in soil
Abbreviations: CFU: Colony forming unit; OMW: Olive mill wastewater.

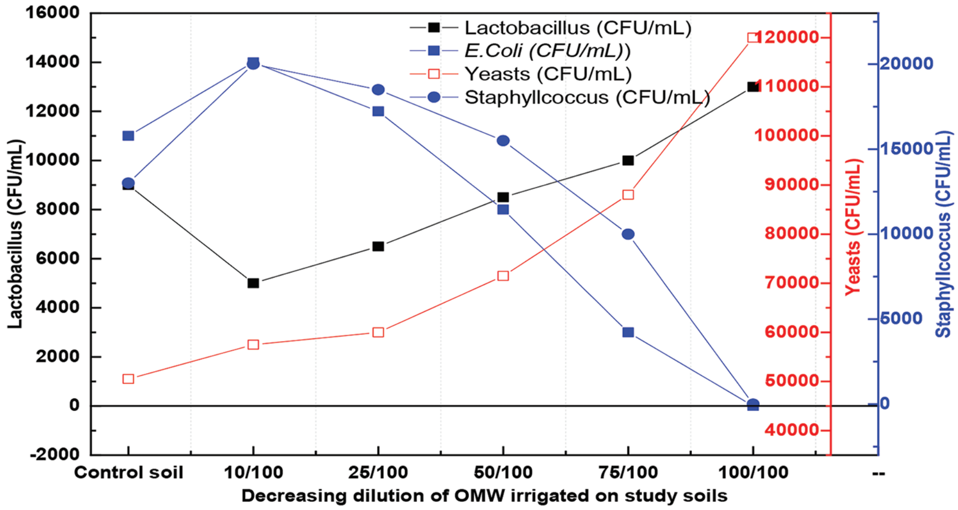

Figure 5. Variation in microbial loads in soils irrigated with increasing concentrations of OMW/dilution Abbreviations: CFU: Colony forming unit; OMW: Olive mill wastewater.

capabilities.[9] Soil is a balanced ecosystem but becomes unbalanced when intrusive matter like heavy metals settles in it. Heavy metal contamination leads to a decline in soil fertility by inducing toxicity and causing damage to soil microorganisms.[10] Previous research indicates that pollution by these heavy metals would cause changes in microbial diversity and structure.[11] Moreover, these heavy metals accumulate in plant and animal food chains, causing problems at environmental and public health levels.[12] Soils deteriorated by factors such as salinization and desertification are subjected to conditions affecting their normal function and productivity.[13] Nitrogen present in these effluents represents a very important fertilizing factor for the soil, contributing to plant growth in the form of nitrate.[14] In general, when the pH level decreases, such as in this study, where the pH is 5.48, heavy metals become highly soluble with improved bioavailability.[15]

Excessive nickel can be toxic to plants by decreasing root growth, leading to leaf chlorosis,[16] while arsenic impairs seed germination, severely reducing root development[17] due to membrane dysfunction, including fluidization effects.[18] Consequently, an excess of arsenic can cause the death of vegetation.[19] However, some bacterial species metabolize certain heavy metals for energy sources[20], and through dilution, soil bacteria are activated by heavy metals, which also helps maintain their revitalization potential.[21]

Soil bacterial activities are influenced by various factors, such as heavy metal concentrations, bacterial species, soil properties, and the coexistence of metals and bacteria.[22,23] The accumulation of these metals can have serious consequences on soil ecosystems, inhibiting bacterial activity and growth.[24] Our study, along with previous research,[25,26] demonstrates that bacterial revitalization and the restoration of their activities can be triggered by the dilution of OMW in soil plots irrigated with increasing concentrations. The effectiveness of dilution is associated with the bacterial species in the soil and their ability to adapt to less toxic conditions,[27] reducing the inhibition effects of heavy metals on bacterial growth.[28] The presence of heavy metals induces biotoxicity, altering soil microbial activities.[1] Moreover, an increase in heavy metals also limits carbon production by soil microbes[29] by increasing the energetic metabolism to resist heavy metal stress, which results in greater carbon release.[30] These pollutants affect the soil's nutrient cycle, limiting microbial metabolism and decreasing the availability of essential nutrients, thereby reducing soil fertility.[31]

5. Conclusion

This study showed that applying OMW to agricultural soils hinders microbial life and inhibits durum wheat germination. The negative effects are attributed to several factors, including high acidity, the presence of phenolic compounds, salinity, and the activity of *Lactobacillus* bacteria that exacerbates environmental acidification.

To identify an appropriate solution, a series of irrigation trials were conducted using different dilutions of effluent on multiple batches of wheat seeds. This approach made it possible to determine the optimal dilution ratio for wheat germination and growth, comparable to that observed in the control batch. In addition, adequate dilution preserved soil biodiversity by restoring the microbiological and physicochemical balance of the ecosystem.

Thus, the adoption of an optimized dilution process, with a ratio of 10/100, proved effective in revitalizing soil microbial activity, ensuring optimal germination, and promoting satisfactory plant growth. These results offer promising prospects for sustainable effluent management in the agri-food sector while preserving soil fertility and biodiversity.

Acknowledgments

None.

Funding

None.

Conflicts of interests

The authors confirm that there were no conflicts of interest.

Author contributions

Conceptualization: Omar Elrhaouat
Investigation: Sakina Belhamidi
Methodology: Mohamed Hadji
Writing – original draft: Omar Elrhaouat, Mohamed Hadji
Writing – review & editing: Mohammed Ouhssine

Availability of data

Data of this study are available from the corresponding author on reasonable request.

References

1. Xu M, Cui Y, Beiyuan J, Wang X, Duan C, Fang L. Heavy metal pollution increases soil microbial carbon limitation: Evidence from ecological enzyme stoichiometry. *Soil Ecol Lett*. 2021;3(3):230-241. doi: 10.1007/s42832-021-0094-2
2. Stuczynski TI, McCarty GW, Siebielec G. Response of soil microbiological activities to cadmium, lead, and zinc salt amendments. *J Environ Qual*. 2003;32(4):1346-1355. doi: 10.2134/jeq2003.1346
3. Xiao XY, Wang MW, Zhu HW, Guo ZH, Han XQ, Zeng P. Response of soil microbial activities and microbial community structure to vanadium stress. *Ecotoxic Environ Safe*. 2017;142:200-206. doi: 10.1016/j.ecoenv.2017.03.047
4. Tang J, Zhang J, Ren L, *et al*. Diagnosis of soil contamination using microbiological indices: A review on heavy metal pollution. *J Environ Manag*. 2019;242:121-130. doi: 10.1016/j.jenvman.2019.04.061
5. Yang J, Yang F, Yang Y, *et al*. A proposal of a bioindicator of central enzyme in areas of long-term Pb-Zn contamination based on the analysis of surface soil properties. *Environ Pollut*. 2016;213:760-769. doi: 10.1016/j.envpol.2016.03.030
6. Mierzwa-Hersztek M, Gondek K, Klimkowicz-Pawlas A, Baran A, Bajda T. Sewage sludge biochars management-ecotoxicity, mobility of heavy metals, and soil microbial biomass. *Environ Toxicol Chem*. 2018;37(4):1197-1207. doi: 10.1002/etc.4045
7. Lin Y, Ye Y, Hu Y, Shi H. The variation in microbial community structure under different heavy metal contamination levels in paddy soilsils. *Ecotoxic Environ Safe*. 2019;180:557-564. doi: 10.1016/j.ecoenv.2019.05.057
8. Rodier J, Legube B, Merlet N, *et al*. *Water Analysis, Natural Waters, Waste Waters, Sea Water, Chemistry, Physical Chemistry, Microbiology, Biology, Interpretation of Results*. 9th ed. Paris: Dunod; 2009.
9. Annabi M, Bissonnais Y, Villio-Poitrenaud M, Houot S. Improvement of soil aggregate stability by repeated applications of organic amendments to a cultivated silty loam soil. *Agri Ecosyst Environ*. 2011;144(1):382-389. doi: 10.1016/j.agee.2011.07.005
10. El-Naggar A, Lee SS, Rinklebe J, *et al*. Biochar application to low fertility soils: A review of current status, and future prospects. *Geoderma*. 2019;337:536-554. doi: 10.1016/j.geoderma.2018.09.034
11. Oliveira A, Pampulha ME. Effects of long-term heavy metal contamination on soil microbial characteristics. *J Biosci Bioeng*. 2006;102(3):157-161. doi: 10.1263/jbb.102.157
12. Nabulo G, Young SD, Black CR. Assessing risk to human health from tropical leafy vegetables grown on contaminated urban soils. *Sci Total Environ*. 2010;408(22):5338-5351. doi: 10.1016/j.scitotenv.2010.06.034
13. Antoniadis V, Levizou E, Shaheen SM, *et al*. Trace elements in the soil-plant interface: Phytoavailability, translocation, and phytoremediation-a review. *Earth Sci Rev*. 2017;171:621-645. doi: 10.1016/j.earscirev.2017.06.005
14. Beiyuan J, Fang L, Chen H, Li M, Liu D, Wang Y. Nitrogen of EDDS enhanced removal of potentially toxic elements and attenuated their oxidative stress in a phytoextraction process. *Environ Pollut*. 2021;268(Part A):115-719.

doi: 10.1016/j.envpol.2020.115719

15. Schachtman DP, Kumar R, Schroeder JI, Marsh EL. Molecular and functional characterization of a novel low-affinity cation transporter (LCT1) in higher plants. *Proc Natl Acad Sci U S A*. 1997;94(20):11079-11084. doi: 10.1073/pnas.94.20.11079
16. Himmelbauer ML, Puschenreiter M, Schnepf A, Loiskandl W, Wenzel WW. Root morphology of Thlaspi goesingense Hálácsy grown on a serpentine soil. *J Plant Nutr Soil Sci*. 2005;168(1):138-144. doi: 10.1002/jpln.200420434
17. Garg N, Singla P. Arsenic toxicity in cultivated plants: Physiological effects and tolerance mechanisms. *Environ Chem Lett.* 2011;9:303-321. doi: 10.1007/s10311-011-0313-7
18. Smith SE, Christophersen HM, Pope S. Arsenic uptake and toxicity in plants: Integrating mycorrhizal influences. *Plan Soil.* 2010;327:1-21. doi: 10.1007/s11104-009-0089-8
19. Tripathi RD, Srivastava S, Mishra S, *et al*. Arsenic Hazards: Strategies for tolerance and remediation by plants. *Trends Biotechnol*. 2007;25(4):158-165. doi: 10.1016/j.tibtech.2007.02.003
20. Bielińska EJ, Mocek-Płóciniak A. Biochemical and chemical indices of soil transformations on goose farms in the years 1996-2011. *Arch Environ Prot*. 2015;41(1):80-85. doi: 10.1515/aep-2015-0010
21. Yasmin R, Zafar MS, Tahir IM, Asif R, Asghar S, Raza SK. Biosorptive potential of *Pseudomonas* species RY12 toward zinc heavy metal in agriculture soil irrigated with contaminated waste water. *Dose Response*. 2022;20(3):15593258221117352. doi: 10.1177/15593258221117352
22. Arora PK. Bacilli-mediated degradation of xenobiotic compounds and heavy metals. *Front Bioeng Biotechnol*. 2020;8:570307. doi: 10.3389/fbioe.2020.570307
23. Uridia RZ, Kavtaradze, NA, Kochiashvili KN, *et al*. Bioremediation of soils contaminated with heavy metals. *Inter Acad J Web Sch*. 2021;2(52). doi: 10.31435/rsglobal_wos/30062021/7618
24. Gong S, Wang H, Lou F, Qin R, Fu T. Calcareous materials effectively reduce the accumulation of cd in potatoes in acidic cadmium-contaminated farmland soils in mining areas. *Inter J Environ Res Public Health*. 2022;19(18):11736. doi: 10.3390/ijerph191811736
25. Oladele EO, John T, Odeigah P, Taiwo IA. The influence of Pb and Zn contaminated soil on the germination and growth of Bambara Nut (*Vigna subterranea*). *J Appl Sci Environ Manag*. 2017;21(4):761-768. doi: 10.4314/jasem.v21i4.17
26. Wang L, Zhou F, Zhou J, *et al*. Genomic analysis of *Pseudomonas asiatica* JP233: An efficient phosphate-solubilizing bacterium. *Genes* (*Basel*). 2022;13(12):22-90. doi: 10.3390/genes13122290
27. Patel JK, Agrawal R, Sidhdhapara R. Bacterial endophytes associated with the roots of Poaceae plants: Identification, characterization, and promotion of plant growth. *J Microb Biotechnol Food Sci*. 2020;10(3):478-483. doi: 10.15414/jmbfs.2020.10.3.478-483
28. Kalu CM, Ogola HJO, Selvarajan R, Tekere M, Ntushelo K. Fungal diversity and metabolome of the rhizosphere and endosphere of *Phragmites australis* in an environment contaminated by AMD. *Heliyon*. 2021;7(3):E06399. doi: 10.1016/j.heliyon.2021.e06399
29. Wang X, Cui Y, Zhang X, *et al*. A novel extracellular enzyme stoichiometry method to evaluate soil heavy metal contamination: Evidence derived from microbial metabolic limitation. *Sci Total Environ*. 2020;738:139709. doi: 10.1016/j.scitotenv.2020.139709
30. Li C, Zhou K, Qin W, *et al*. A review on heavy metals contamination in soil: Effects, sources, and remediation techniques. *Soil Sediment Contam Int J*. 2019;28(4):380-394. doi: 10.1080/15320383.2019.1592108
31. Seraj F, Rahman T. Heavy metals, metalloids, their toxic effect and living systems. *Amer J Plant Sci*. 2018;9(13):2626-2643. doi: 10.4236/ajps.2018.913191