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Characterization of Two Olive Mill Wastewater and Its Effect on Fenugreek (*Trigonella foenum-graecum*) Germination and Seedling Growth

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ABSTRACT

In Morocco, the olive industry produces a large quantity of olive mill wastewater (OMW) every year for a short period (November to February). The physicochemical properties and quantities of these effluents depend largely on the extraction system used. The aim of this study is to characterize these effluents for the purpose of evaluating their impact on Fenugreek (*Trigonella foenum-graecum*) germination and seedling growth. For this purpose, a germination experiment was conducted, wherein 25 Fenugreek seeds were positioned on filter paper within Petri dishes. Subsequently, these seeds were subjected to varying concentrations of water sourced from two olive crushing processes: the Continuous System (OMW-CS) and the Semi-Modern System (OMW-SM) (0%, 1%, 2%, 5%, 7%, 10%, 25%, 50%, 75%, and 100%). The Petri dishes were then placed in an incubator set at 25 °C for a duration of 8 days. The results of the physicochemical analysis showed that both types of water had an acid pH and were rich in organic matter. The 5-day biological oxygen demand (BOD_5) values were similar, while the chemical oxygen demand (COD) values were quite different. Microbiological analysis revealed that yeasts and fungi were the main constituents of the flora of these wastewaters, with no significant difference between the two types of OMW. However, the total number of aerobic mesophilic flora was higher for OMW-CS than for OMW-SM. Analysis of variance revealed a highly significant difference between OMW-CS concentrations ($F = 2998.667$; $p < 0.000$) and OMW-SM ($F = 2839.778$; $p < 0.000$). A minimal germination rate (30%) was recorded for OMW-CS at a concentration of 10%. For OMW-CS, total inhibition of germination was observed at concentrations of 25%, 50%, 75%, and 100%, while for OMW-SM, this inhibition was recorded above a concentration of 2%.

Keywords: olive mill wastewater, continuous system, semi-modern, physicochemical parameters, microbiological parameters, principal component analysis, germination, Fenugreek.

INTRODUCTION

The olive tree is a traditional crop in the Mediterranean region. Currently, more than 750 million hectares are cultivated with olive trees, with 95% concentrated in the Mediterranean basin. Olive cultivation in Morocco covers almost the entire national territory due to the adaptability of the olive tree to different bioclimatic zones. The olive oil extraction systems used are as follows: the traditional system (Maâsra), discontinuous press crushing, three-phase continuous systems (CS), and two-phase or semi-modern systems (SM).

In Morocco, the olive industry faces significant environmental challenges in waste management (pomace and olive mill wastewater), with an annual production of approximately 685,000 m^3 of olive mill wastewater (OMW) during the harvest season (November-February) [Lakhtar et al., 2010; Massadeh et al., 2022]. The OMW consists mainly of an oil emulsion, fruit pulp, crushed seeds, and a substantial amount of water [Shabir et al., 2022]. They have a dark color, high acidity, significant organic matter content, and exhibit strong resistance to biodegradation due to their high levels of phenolic compounds [Alaoui et al., 2022; Enaime et al., 2020]. Several factors influence the physicochemical and microbiological properties of OMW, including olive variety, cultivation conditions (climate, fertilization, irrigation technique, etc.), olive ripeness at the time of harvest, storage conditions and duration before crushing, as well as the extraction method used (continuous or semi-modern system) [De Marco et al., 2007; Zahra El Hassani et al., 2023].

This study focuses on the evaluation by principal component analysis (PCA) of physicochemical and microbiological parameters of two types of olive mill wastewater (CS and SM) and Its Effect on the germination of fenugreek (*Trigonella foenum-graecum*) and Seedlings Growth.

MATERIALS AND METHODS

Sampling

The OMWs subjected to this study were sampled from the storage basin of two olive crushing units, using the Semi-modern system and the continuous three-phase system, located in the city of Tifelt, Khémisset province, in the Rabat-Salé-Kénitra region of Morocco. The effluents were collected and transported in sterile containers.

Physicochemical characterization

The physicochemical parameters measured are: pH was measured using an ORION STAR A111 pH meter. Electrical conductivity (EC) was measured directly in the fresh OMW using a CON 700 conductimeter (mS/cm). Suspended solids (SS) were determined by filtration through membranes with 0.45µm pore diameter. The SS content was determined by weighing the filter before and after filtration and drying in an oven at 105 °C for 2 hours. Organic matter (OM) was determined after calcination of dried OMW in a muffle furnace at 525 °C for 5 hours [Zaier et al., 2017]. Chemical oxygen demand (COD) was determined by excess potassium dichromate titration in an acidic medium and in the presence of silver sulphate [Rodier et al., 2009]. The biochemical oxygen demand (BOD_5) expresses the amount of oxygen in (mgO_2/L) required by bacteria to oxidize organic molecules in aerobic conditions. BOD_5 was determined using the respirometric method in the dark for 5 days [Rodier et al., 2009]. The ratio between COD and BOD_5 allows the determination of Ib (Biodégradabilité Index), which is an indicator of the importance of non- or poorly biodegradable pollutants [Rodier et al., 2009]. If: Ib > 6, it is hardly biodegradable; 3 <Ib> 6, it is partially biodegradable; Ib < 3, it is highly biodegradable.

The total phenolic content was determined using the Folin-Ciocalteu colorimetric method, with gallic acid as the standard, and the absorbance was measured at λ = 760 nm [Box, 1983].

Before starting the analysis, the samples were homogenized, and each test was performed in triplicate. The analysis of heavy metals present in OMW was conducted at the National Center for Scientific and Technical Research (CNRST), Division: Unit of Technical Support for Scientific Research (UATRS), in Rabat.

Microbiological characterization:

Microbiological analyses were performed on Total Aerobic Mesophilic Flora (TAMF) using the colony counting method on agar plates. The culture medium used was PCA and incubation was performed at 37 °C for 24 hours. The abundance of yeasts and molds (YM) was evaluated using Sabouraud medium, with incubation at 30 °C for 48 hours for yeasts and 5–7 days for molds (Zaier et al., 2017). All results are expressed in colony formation units (CFU).

Germination test:

The phytotoxicity of OMW on the germination of Fenugreek seeds (*Trigonella foenum-graecum*) was investigated. Twenty-five seeds were positioned on filter papers within glass Petri dishes and subsequently subjected to various concentrations of OMW (0%, 1%, 2%, 5%, 7%, 10%, 25%, 50%, 75% and 100%).

The evaluated metrics encompassed Germination Percentage (GP) and Seedling Vigor Index (SVI), both quantified using the provided equations.

$$GP = (S_G/S_T) \times 100 \quad (1)$$

where: S_G – the number of seeds germinated,
S_T – the total number of seeds.

$$SVI = GP \times (L_R+L_E) \quad (2)$$

where: L_R – average root length (cm),
L_E – average epicotyl length (cm).

Statistical analysis

The results were analyzed by a 5% ANOVA (Analysis of Variance), with a concentration of OMW as the factor. When a statistically significant difference was observed, a multiple means comparison was performed using the Tukey test. The statistical analysis of the physicochemical and microbiological parameters was carried out using Principal Component Analysis (PCA).

RESULTS

Physicochemical characterization

The two types of OMW (CS and SM) are characterized by a blackish-red color with a strong odor of olive oil (Table 1). In fact, the variance analysis shows a significant difference ($p < 0.05$) between the average contents of the two OMWs for all physicochemical parameters, except for the orthophosphate content ($F = 0.11$; $p < 0.761$). However, the pH of both types of OMW is acidic, with OMW-CS having a pH of 4.09 and OMW-SM having a pH of 5.07. Regarding electrical conductivity, it is 17.467 mS/cm for OMW-SM and 9.283 mS/cm for OMW-CS, exceeding the phytotoxic discharge standard (high conductivity >7 mS/cm). Furthermore, the turbidity in OMW-SM and OMW-CS is 403.33NTU and 223.33NTU, respectively. However, suspended solids are 12.581 g/l for OMW-CS and 16.80 g/l for OMW-SM, representing the total of mineral particles (11.42 g/l for OMW-CS and 47.93 g/l for OMW-SM) and organic matter (41.77 g/l for OMW-CS and 112.47 g/l for OMW-SM).

Regarding the total dry matter content, it is 53.10 g/l for OMW-CS and 160.33 g/l for OMW-SM. The pollutant content expressed in terms of COD and BOD_5 is approximately 10560 mgO_2/l and 410.33 mgO_2/l for OMW-CS, and 27370 mgO_2/l and 559.83 mgO_2/l for OMW-SM, respectively. However, the biodegradability index in both types of OMW exceeds the threshold of 6, confirming that our samples are partially or nonbiodegradable. Furthermore, the nitrate content is 464.97 mg/l and 509.40 mg/l, and the sulphate content is 581.50 mg/l for OMW-CS. In terms of the orthophosphate content in both OMWs, it is 29 mg/l. However, the polyphenol content is relatively low, with 0.321 g/l for OMW-CS and 0.26 g/l for OMW-SM.

Metallic trace elements

Compared with OMW-CS, OMW-SM has a lower proportion of metal components, Table 4 shows descriptive results for the mean heavy metal content of OMW-CS and OMW-SM (Fisher's test showed significant differences between the means). In fact, the minimum observed in OMW-CS was (0.2338 mg/l); Ni (0.2937 mg/l) and Cd (0.0076 mg/l), and the maximum was Fe (18.315 mg/l).

Microbiological characterization

The results of the microbiological analysis show that yeasts and molds (YM) represent the

Table 1. Physicochemical characteristics of OMW-CS and OMW-SM

ParameterS	Units	OMW-CS	OMW-SM	p value	Discharge limit values
pH		4. 09 ± 0.100	5.05±0.153	0.001**	5.5–9.5
EC	mS/cm	9.28 ±0.104	17.47±0.814	0.000***	2700 µs/cm
Turbidity	NTU	223.33 ±0.577	403.33±1.528	0.000***	-
SS	g/L	12.58 ±0.401	16.80±0.954	0.002**	100 mg/l
ST	g/L	53.10±0.557	160.33±1.528	0.000***	-
MM	g/L	11.42±0.100	47.93±0.551	0.000***	-
OM	g/L	41.77±0.473	112.47±1.501	0.000***	-
BOD_5	mgO_2/L	410.33±1.528	559.83±9.018	0.000***	100
COD	mgO_2/L	10560±0.700	27360±0.808	0.000***	500
Nitrate	mg/L	509.40±0.656	464.97±0.586	0.000***	-
Orthophosphate (mg/l)	mg/L	29.92±0.852	29.70±0.800	0.761^{ns}	15
Sulfate	mg/L	581.50±0.500	3313.50±1.000	0.000***	600
Phenol	g/L	0.321±0.025	0.162±0.015	0.001**	0.5

Table 2. Heavy metal content in the two types of OMW

Metallic trace (mg/L) elements	OMW-CS (mg/l)	OMW-SM (mg/l)	p value	Discharge limit values
Cr	2.421	0.0092	0.000***	2
Zn	2.371	0.0113	0.000***	5
Pb	0.2338	0.0689	0.000***	1
Ni	0.2937	0.0279	0.000***	5
Cd	0.0076	0.0019	0.000***	0.25
Mn	0.5238	0.0143	0.000***	2
Fe	18.315	0.4625	0.000***	5
Cu	0.0216	0.003	0.000***	2

Table 3. Correlations between the physicochemical and microbiological characteristics of OMW-CS and OMW-SM

Parameter	pH	EC	TURB	SS	ST	MM	OM	BOD	COD	COT	Nitrate	Orthophosphate	Sulfate	Phenol	TAMF	YM
pH	1	.982**	.998**	.953**	.997**	.997**	.996**	0.803	.998**	.999**	-.998**	-0.180	.998**	-.984**	.998**	.998**
EC		1	.980**	.936**	.979**	.979**	.978**	.835*	.983**	.983**	-.976**	-0.256	.980**	-.937**	.980**	.980**
TURB			1	.964**	1.000**	1.000**	1.000**	0.788	1.000**	1.000**	-1.000**	-0.156	1.000**	-.977**	1.000**	1.000**
SS				1	.967**	.967**	.966**	.823*	.962**	.961**	-.958**	0.015	.962**	-.932**	.962**	.962**
ST					1	1.000**	1.000**	0.789	1.000**	1.000**	-.999**	-0.149	1.000**	-.977**	1.000**	1.000**
MM						1	1.000**	0.791	1.000**	1.000**	-.999**	-0.149	1.000**	-.977**	1.000**	1.000**
OM							1	0.779	.999**	.999**	-.999**	-0.155	1.000**	-.975**	1.000**	1.000**
BOD								1	0.799	0.792	-0.777	0.079	0.786	-0.790	0.786	0.786
COD									1	1.000**	-.999**	-0.155	1.000**	-.977**	1.000**	1.000**
COT										1	-.999**	-0.173	1.000**	-.978**	1.000**	1.000**
Nitrate											1	0.158	-1.000**	.980**	-1.000**	-1.000**
Orthophosphate												1	-0.161	0.097	-0.161	-0.161
Sulfate													1	-.978**	1.000**	1.000**
Phenol														1	-.978**	-.978**
TAMF															1	1.000**
YM																1

Note: EC – electrical conductivity; TURB – turbidity; SS – suspended solids; ST – total solids; MM – mineral matter; OM – organic matter; BOD – biochemical oxygen demand; COD – chemical oxygen demand; COT – total organic carbon; TAMF – total aerobic mesophilic flora; YM – yeasts and molds.

main microbial flora in the olive mill wastewaters, with $16.5 \cdot 10^4$ CFU for OMW-CS and $24 \cdot 10^4$ CFU for OMW-SM. While the total microbial load was assessed by counting Total Aerobic Mesophilic Flora (TAMF), the highest value ($9 \cdot 10^4$ CFU) is observed for OMW-SM, while the lowest value ($2.77 \cdot 10^4$ CFU) is recorded for OMW-CS.

Correlations between the physicochemical and microbiological characteristics

The correlations were calculated on the average values of physicochemical and microbiological parameters (Table 3). Significant correlations were found among most of the parameters studied. pH showed strong positive correlations with EC (r = 0.982) and turbidity (r = 0.998). as well as with organic load indicators: SS (r = 0.953), ST (r = 0.997), MM (r = 0.997), OM (r = 0.996), COD (r = 0.998), COT (r = 0.999), and Sulphate (r = 0.998). Except for orthophosphate (r = -0.180^{ns}).

Phenol and nitrate were negatively correlated with all parameters. Strong correlations (r = 1) were recorded between turbidity and ST, MM, OM, COD, COT, and sulphate, as well as between sulfate and ST, MM, OM, COD, and COT.

Highly significant positive correlations were observed between physicochemical and microbiological parameters. The growth of TAMF and YM was strongly stimulated by pH as well as by all the parameters (SS, ST, MM, OM, BOD, COD, COT, etc.). On the other hand, growth was greatly and negatively affected by the presence of nitrate (r = -1) and phenol (r = -0.978).

PCA analysis

Principal component analysis (PCA) allows grouping the physicochemical and microbiological parameters into new factorial variables. The two main components (PC1 and PC2) alone absorb 99.470% of the total information: 90.236% for PC1 and 7.234% for PC2 (Figure 1).

The projection of physicochemical and microbiological parameters onto the factorial plane (PC1 and PC2) revealed 3 groups:

The first group, on the positive side of component1 includes: pH, EC, turbidity, ST, MM, OM, COD, BOD, and SS which form a new variable describing organic pollution, these parameters are positively correlated with each other.

The second group, located on the negative side of PC1, includes nitrate and phenol, these parameters are correlated with each other (r = 0.980).

The third group, located on the positive side of PC2, consists of orthophosphates. Groups 1 and 2 evolve in opposite directions and are independent of group 3.

The projection of the sample means according to the olive crushing system in the space delimited by PC1 and PC2 showed that PC2 distinctly separates the two olive mill wastewaters: OMW-SM characterized by high levels of organic pollutants (MES, BOD, etc.) on the positive side of PC1, and OMW-CS characterized by high levels of nitrates and phenol on the negative side.

Germination test

Germination percentage Analysis of variance for the germination percentage of Fenugreek seeds watered with different concentrations of OMW revealed a highly significant difference between the OMW-CS concentrations (F = 2998.667; p <0.000) and the OMW-SM concentrations (F = 2839.778; p <0.000) (Figure 2). Tukey's test was conducted, which identified three distinct and nonoverlapping groups for OMW-CS, the first group is composed of the control 1%, 2%, and 5%, the second group has the concentration 7%, while the final group is composed of the concentration 10%, which exhibited a minimum germination percentage of 30%. No germination was observed beyond this concentration. For OMW-SM, Tukey's test grouped concentrations of 0%, 1%, and 2% into a single category. Furthermore, no germination was recorded for concentrations exceeding 2%.

The independent sample 'student' t-test showed no significant differences between the final germination percentage for the two types of OMW, at concentrations 1 and 2% (t = 1; p <0.423).

Seedling vigor index

The Fisher test revealed a significant difference between the concentrations for both types of OMW-CS (F = 105.47; p <0.000) and OMW-SM (F = 42.916; p <0.000). For OMW-CS, an increase in SVI was observed up to the concentration of 5% (637.6±84.85%), which exceeded the values obtained by the control (634±22.64 %). The lowest ratio was observed at a concentration of 10% (6.928±1.29%). For OMW-SM, the highest SVI

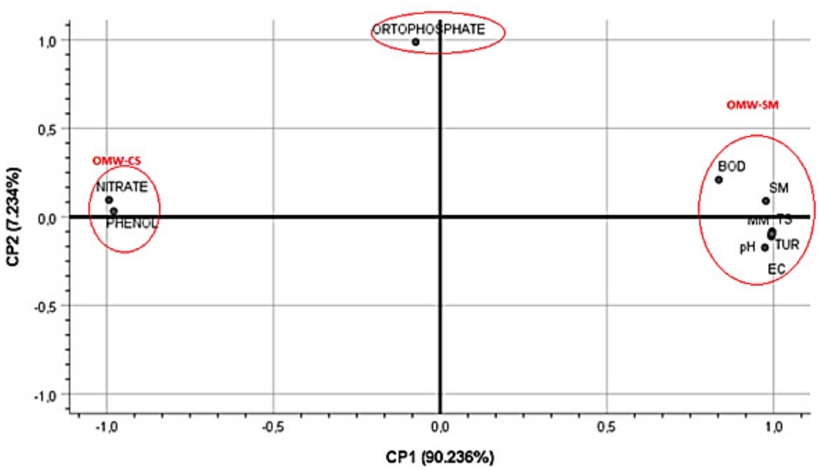

Figure 1. Projection of variables in the factorial plane (PC1 × PC2 : 99.047%)

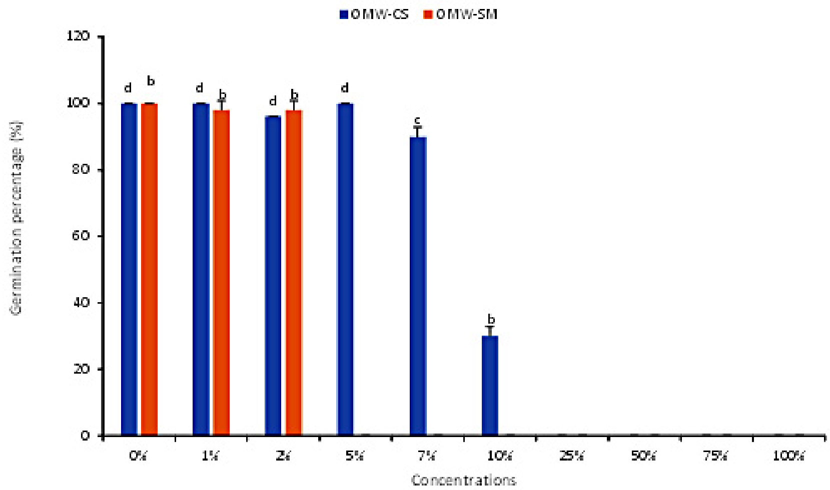

Figure 2. Effects of OMW-CS and OMW-SM at different concentrations on Fenugreek seed germination

(461.8±81.74%) was obtained at a concentration of 0%. The variance analysis for OMW-CS did not reveal significant differences between concentrations of 1%, 2%, and 5%, including the control, and between the concentrations of 1%, 2%, and the control for OMW-SM. Independent samples t-test revealed a significant difference between the two types of OMW for concentrations of 5%, 7%, and 10% ($t = 10.627$; $p < 0.009$), ($t = 49.6$; $p < 0.000$), ($t = 7.596$; $p < 0.017$) respectively, and non-significant for 1% and 2% ($t = 3.057$; $p < 0.092$), ($t = 1.506$; $p < 0.271$) respectively.

DISCUSSION

The differences observed in the physico-chemical characterizations between the two types of olive mill wastewater (OMW-CS and OMW-SM) depending on the variety, maturity and the origin of the olives. It could also be due to climatic conditions, farming methods, and oil extraction technology [El Yamani et al., 2020; Sassi et al., 2006]. Other previous studies have also highlighted that variation in the composition of the wastewaters is influenced by the extraction system used [Aissam et al., 2002; Bouknana et al., 2014; El Ghadraoui et al., 2021; Sassi et al., 2006]. However, both types of OMW are acidic, with pH values within the range described in the literature (3 to 6) [Shabir et al., 2022]. Furthermore, this high acidity could be explained by the presence of organic acids, namely phenolic and fatty acids [Achak et al., 2009; Elabdouni et al., 2020]. The high conductivity of olive mill wastewater is mainly due to the salting practice used for olive preservation and the natural richness of dissolved mineral salts [Achak et al., 2009; Alaoui et al., 2022; Bouknana et al., 2014; Elabdouni et al., 2020]. Conductivity reaches a high level, exceeding the limit of phytotoxic discharge (high conductivity >7 ms/cm). However, it remains significantly lower than that reported by Ghadraoui et al., (2021) (28.23 ms/cm) [El Ghadraoui et al., 2021]. Furthermore. the turbidity values are relatively low compared to those found by Meziani et al. (2023) and Djeziri et al., (2023). which were 666.66 NTU and 1780 NTU, respectively [Djeziri et al., 2023; Meziani et al., 2023]. This turbidity is attributable to the presence of suspended particles, especially colloidal organic matter. However, suspended matter represents the totality of mineral and organic particles. Our measured values (MM: 11.42 g/l for OMW-CS and 47.93 g/l for OMW-SM. OM: 41.77 g/l for OMW-CS and 112.47 g/l for OMW-SM) are close to those reported by Djeziri et al., (2023) and higher than those of El Yamani et al., (2020) and Rajhi et al. (2018) [El Yamani et al., 2020; Rajhi et al., 2018]. The levels of organic and mineral matter in both types of olive mill wastewaters are relatively high. which may be related to agronomic practices, irrigation management, and the degree of olive ripeness [El Yamani et al., 2020; Fernández-Escobar et al., 2006; Rharrabti and EI Yamani, 2019]. The quantity of organic matter in the effluents of each type of OMW is influenced by the olive oil extraction method, the OMW exhibit a high content of organic matter as indicated by the values of COD and BOD_5. However, these values are relatively low compared to those recorded in several previous studies, such

Table 4. Effects of different concentrations of OMW-CS and SM on Seedling vigor index

Concentration	OMW-CS	OMW-SM	t	pvalue
0%	634±22.64^{c}	461.8±81.74^{b}	2.871	0.103
1%	561.656±78.55^{c}	391.848±0.78^{b}	3.057	0.092
2%	595.2±57.02^{c}	454.68±118.96^{b}	1.506	0.271
5%	637.6±84.85^{c}	0±0.00^{a}	10.627	0.009
7%	261.888±7.47^{b}	0±0.00^{a}	49.6	0.000
10%	6.928±1.2^{9}a	0±0.00^{a}	7.596	0.017
25%	0±0.00^{a}	0±0.00^{a}	-	-
50%	0±0.00^{a}	0±0.00^{a}	-	-
75%	0±0.00^{a}	0±0.00^{a}	-	-
100%	0±0.00^{a}	0±0.00^{a}	-	-
Fisher	**105.47**	**42.916**		
p value	**0.000**	**0.000**		

Note: Values in the same column followed by the same letter are not statistically different at 5%.

as those conducted by other authors [Djeziri et al., 2023; Rajhi et al., 2018; Sassi et al., 2006; Yamani et al., 2019]. These findings suggest that the organic matter present in these effluents undergoes almost complete oxidation, indicating a high pollution potential. Nevertheless, despite this significant oxidation, the biodegradability index for both types of OMW was found to exceed the threshold of 6, confirming that our samples are partially or nonbiodegradable. Nitrate concentrations vary depending on the olive oil extraction system. In our study. these concentrations range between 509.40 mg/l for OMW-CS and 464.97 mg/l for OMW-SM. These values are lower than those reported by [Rajib et al., 2015] (798 mg/l) and higher than those of [Bouknana et al., 2014] (0.25 to 1.34mg/l for OMW-SM and 0.06 to 0.95 mg/l for OMW-CS). The rise in nitrate levels might be attributed to the utilization of elevated doses of fertilizers, causing their initial buildup in olive fruits and subsequently finding their way into wastewater streams [Ntougias et al., 2013]. The orthophosphate concentration in both OMW types (29 mg/l) surpasses the values reported by [Bouknana et al., 2014] (ranging from 3.83 to 5.75 mg/l) and remains below the levels described by Ena et al., (2009) and Rajib et al., (2015) for OMW-CS, which were 926.3mg/l and 54mg/l respectively [Bouknana et al., 2014; Ena et al., 2009; Rajib et al., 2015]. It depends on climatic conditions and whether fertilizers are used or not [Hanafi et al., 2009]. However, polyphenol levels remain relatively low compared to those reported in the literature (0.1 to 17.5 g/l) [Benaddi et al., 2022] and below discharge standards. Toxic substances such as phenols present in olive mill wastewaters can bind to soils and inhibit microbial activity. This inhibition may be related to olive ripeness, extraction technology, climatic conditions, and the duration of storage of OMW [Esmail et al., 2015; Sassi et al., 2006; Yaakoubi and Aghanchich, 2021; Zaier et al., 2017].

Regarding the microbial load on OMW, our results indicate differences between OMW-CS and OMW-SM. However, our values for both types of OMW are consistent with those found by El Yamani and al., (2020) for Moroccan OMW with TAMF ($22.64 \cdot 10^{4}$ CFU/ml) and YM ($17.95 \cdot 10^{4}$ CFU/ml), but they exceed those reported by [Djeziri et al., 2023] for OMW from the Sidi Bel Abbès region in western Algeria, produced from a three-phase extraction unit, with TAMF ($7.9 \cdot 10^{3}$ CFU/ml) and YM ($0.95 \cdot 10^{3}$ CFU/ml) [Djeziri et al., 2023; El Yamani et al., 2020]. For Meziani and al., (2023), TAMF $6.35 \cdot 10^{4}$ CFU/ml and $12.15 \cdot 10^{4}$ CFU/ml for YM in the case of OMW-CS from the Ghardaa region in southern Algeria. However, these values remain lower than those cited in previous studies [Amaral et al., 2008; Sassi et al., 2006]. The OMW microbial results show differences in microbial load between OMW-CS and OMW-SM. These differences may be related to the physicochemical characteristics, particularly pH and phenols. Previous studies suggest that the bacterial composition can be influenced by various factors, such as the environment (soil and freshwater), olive variety, cultivation and the harvesting methods, and extraction system [Amaral et al., 2008; El Yamani et al., 2020; Tsiamis et al., 2012].

The observed differences in some physicochemical and microbiological parameters compared to previous studies may be explained by the nature of the OMW. In fact, our samples were collected from open storage tanks. According to El Yamani and al. (2019), changes in microorganisms in OMW are closely related to their physicochemical properties, including pH, total phenols, mineral salts, heavy metals, fatty acids, among others [Djeziri et al., 2023; Elayadi and Naman, 2019]. The results of the analysis of variance revealed that the variation between the two types of OMW was mainly due to the influence of the olive milling system, which is consistent with several previous studies [El Hadrami et al., 2004; El Yamani et al., 2020; Saouini et al., 2023; Sassi et al., 2006].

Regarding the metal content compared to their general limits for discharge into surface waters. only the amount of Cr (2.421mg/l) in OMW-CS exceeds the discharge limit value. These levels remain lower than those found by Bouknana et al. (2014) for OMW-SM (19.4–22.6 mg/l) and OMW-CS (31.7–39.2 mg/l). The metal composition of the OMW depends on the quality of crushed olives, especially for Zn, Cu, and Fe. According to Zbakh and El Abbassi (2012), the contamination of the OMW with metals can occur during the handling and processing of olive fruits [Zbakh and El Abbassi, 2012].

The correlation between nitrate and phenol could be attributed to fertilizer use. Alizadeh et al. (2010) observed an increase in total phenolic content in "Satureja hortensis" after fertilizer use [Alizadeh et al., 2010]. Similarly, Tovar and al. (2001) reported that the production of phenolic compounds in olives was induced by stress conditions related to water scarcity and that the phenol content decreased with increasing irrigation levels [Greven et al., 2009; Tovar et al., 2001].

In a study conducted in the "Bni Frassen" region. El Yamani et al. (2020) also found that the total phenol content was higher at lower pH values. Various factors influence phenolic content, including olive maturity, olive crushing system, and OMW storage conditions, as highlighted in several previous studies [Belaid et al., 2002]. To study the relationships between the physicochemical and microbiological characteristics of the two types of OMW, we used two statistical methods: Principal Component Analysis (PCA) and Correlation Studies.

The PCA results confirmed the conclusions of the ANOVA by demonstrating that the composition of the OMW depends mainly on the extraction system. The first major component (PC1) was identified as the predominant factor in distinguishing microbiological characteristics such as SM, ST, MM, OM, BOD, COD, and COT explaining 90.236% of variability. The second main component (PC2) discriminated phenol and nitrates, which explained 7.234% of the variability. Previous studies have also used PCA to describe associations between physicochemical and microbiological variables. For example, El Rhaouat et al., (2014) found that PC1 and PC2 accounted for 88% of the total variability, with characteristics such as TS, ST, COD, Cl, and CE being on the positive side of PC1, while the pH was in the opposite direction on the PC2 axis [Rhaouat et al., 2014]. Similarly, Rajib et al., (2015) reported that the first two axes explained 76% of the observed variability, with 45% for PC1 and 31% for PC2 [Rajib et al., 2015]. El Yamani and al., (2020), showed that PC1 associated with microbiological characteristics as well as COD, sugars, OM, MM, ST, pH, and explained 58%, while the phenol was placed on the left side. PC2 is associated with the Cl, EC, and C/N ratio on the positive side 16% [El Yamani et al., 2020]. in the study conducted by Saouini and al., (2023), the findings from the PCA reveal that PC1, PC2, and PC3 account for a combined 79.8% of the overall variability. PC1 accounts for 47.3% of the variance and demonstrates a contrast between pH and EC versus dissolved oxygen. PC2, representing 19.8% of the total variance, distinguishes the different OMW samples based on their lipid content and BOD. While the third axis PC3 explains 11.8% of the total variance and illustrates the relationship between the extraction system and COD [Saouini et al., 2023]. These results show that PCA is a powerful tool to highlight the main associations between the physicochemical and microbiological characteristics of olive mill wastewaters, confirming the importance of the extraction system in determining the composition of these effluents.

The results of the germination of Funegrec seeds treated with OMW-CS and OMW-SM at different concentrations show that the phytotoxicity of OMW increases with increasing concentration. The seed vigor index (SVI) indicates an increase for OMW-CS between 1% and 5%. while concentrations above 2% for OMW-SM result in inhibition of seed germination. These effects can be attributed to the high conductivity of OMW and the presence of dissolved salts, organic acids, and polyphenols that directly affect seeds and limit their ability to absorb the water necessary for germination [Elayadi and Naman, 2019; Shabir et al., 2022; Yaakoubi and Aghanchich, 2021]. The percentage of germination of Funegrec seeds in OMW-CS is 30% at a concentration of 10%, same results are found by [EL KAFZ et al., 2023] in case of Tomato seeds. This percentage is relatively low compared to that recorded by Muscolo and al., (2010), which was greater than 80% for the four studied species (durum wheat, chicory, chamomile and fava bean) at the same concentration of OMW from a continuous extraction system in Italy (pH = 5.50; Total phenol = 11.3 mg/l) [Muscolo et al., 2010]. Regarding OMW-SM, inhibition of Funegrec seed germination at concentrations above 2% could be explained by its high conductivity (17.47 mS/cm). A high content of dissolved salts such as sodium, potassium, calcium, magnesium, and other minerals in OMW-SM can create an osmotically stressful environment for seeds, limiting their ability to absorb the water needed for germination. Previous studies have also shown inhibition of lettuce seed germination with high concentrations of OMW-CS [Elayadi and Naman, 2019]. The toxic effects of the salts, organic acids, and polyphenols contained in OMW can directly act on the embryo and affect seed germination [Yaakoubi and Aghanchich, 2021]. The seed vigor index (SVI) showed an increase for OMW-CS between 1% and 5%. which can be explained by an increase in the percentage of germination and the length of the seed radicle of Fenugreek seeds. However, studies have shown that the vigor index can be reduced by the effect of salinity [Shabir et al., 2022].

CONCLUSION

The management of OMW is an environmental challenge for the Moroccan olive industry. The physicochemical and microbiological parameters and the metal elements of the two OMW depended mainly on the olive oil extraction system, the olive salting practices, olives type and maturity, and the OMW preservation method. All the results of these analyzes underline the need to treat these effluents before they are discharged into the receiving environment. Several studies have examined the treatment processes for these often very costly effluents (biological, physicochemical, thermal, etc.). A comparison of the effects of OMW-CS and SM on the germination of Fenugreek seeds showed that OMW-SM had a major inhibitory effect on seed germination above 2%. On the contrary, OMW-CS showed an increase in GP of more than 90% for all concentrations below 10%.

Due to its abundance in organic materials and beneficial soil nutrients, along with the positive outcomes observed with OMW-CS, we propose the utilization of this effluent as an economical and eco-friendly soil fertilizer, as well as a potential water source for irrigation in Mediterranean nations grappling with water scarcity and soil deterioration.

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