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Physicochemical and microbiological characterisation of Olive Oil Mill Wastewater (OMW) from the region of Sidi Bel Abbes (Western Algeria)

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Abstract: The aim of the present work is to evaluate the physicochemical and microbiological quality of the Olive oil mill wastewater (OMW) from the region of Sidi Bel Abbes (Western Algeria). The results of the physicochemical analysis showed that the Olive oil mill wastewater of this region has an acid pH. The average value was 4.52. OMW is rich in organic matter. This last parameter was obtained by BOD_5 and COD. The BOD_5 was 29 g/L and the COD was 90.5 g/L. Acidity, polyphenols and fatty acids content were also determined. Fourier Transformed Infrared Spectroscopy and UV-Visible analysis confirm the presence of polyphenols in OMW. Moreover, the microbial load of this Olive oil mill wastewater was evaluated by the determination of the total aerobic mesophilic flora FMAT, which take the value of 7.9 x 10^3 CFU/mL, yeasts and moulds were 0.95 x 10^3 CFU/mL and lactic bacteria was 6.8 x 10^3 CFU/mL. These analyses reveal that OMW effluents is not within the permitted values and require treatment before reuse or any direct discharge into the environment.

Keywords: Olive oil mill wastewater, Polyphenols, physicochemical parameters, microbiological parameters, FTIR, UV-Vis.

1. Introduction

Olive oil is one of the most important components of the Mediterranean diet because of its nutritional and biological characteristics (Botitsia *et al.*, 2004). The olive oil industry is an important economic activity, concentrated mainly in the Mediterranean countries, which account for about 97% of world production (Izghri *et al.*, 2019). Spain is the main world producer (1,790,309 tons) followed by Greece (327,718 tons), Tunisia (278,300 tons), Italy (277,713 tons), Morocco (174,400 tons) and Turkey (154,326 tons) (FAOSTAT, 2022). On a global scale, the olive production in 2018 exceeded 3,574,336 tons. In Algeria, production of olive oil passed from 19,124 tons in 1961 to 96,632 tons in 2018 (FAOSTAT, 2022).

Extraction of olive oil requires large quantities of water; therefore this industry generates large quantities of liquid effluents: the Olive oil mill wastewater (Gharby *et al.*, 2014; Bouknana *et al.*, 2021) and solids (i.e. stone and pomace). The solid waste obtained after the production of olive oil does present an environmental problem and cannot be used directly in any agricultural application, thus, a treatment is needed. Compost (S'Habou *et al.*, 2010), although probably the most common process is not the only one, other treatments as Anaerobic Digestion, combustion, etc., are well studied and in use in several countries.

Olive oil mill wastewater or vegetation water has a very high polluting power highly loaded with organic matter (phenolic compounds, lipids) (Gharby *et al.*, 2014). They are characterized by a blackish brown color and a strong odor and acid pH (Esmail *et al.*, 2014) and affect particularly the quality of water in which they are discharged. The OMW toxicity is principally due to the polyphenol component and is classified as a highly polluting effluent in the agro-food sector (Noukeu *et al.*, 2016; Elabdouni *et al.*, 2022). Spread on the soil, the Olive oil mill wastewater is undergoing a very severe degradation of the environment quality (Ouabou *et al.*, 2014; El Hassani *et al.*, 2023). The quality and quantity of the Olive oil mill wastewater depends on the olive oil extraction operation. They are also influenced by the varieties of olive, harvesting season, ripening rate fruits and climatic conditions (Achak *et al.*, 2009). Moreover, their high organic loads require a high consumption of oxygen (Ouabou *et al.*, 2014). They contain high concentrations of suspended solids. Olive oil mill wastewater (OMW) is also characterized by high COD and BOD levels then fatty and phenolic compounds. Phenolic compounds can cause toxic effects to many microorganisms. In addition to the visual inconvenience and bad odors, the high organic load of the Olive oil mill wastewater destroys the fauna and the flora by absorption of the oxygen dissolved in the water (Ouabou *et al.*, 2014).

In the present work, a complete characterization of the Olive oil mill wastewater (OMW) in the region of Sidi Bel Abbes (Algeria) was to carry out: physicochemical and microbiological parameters were studied of such as acidity, turbidity, conductivity, etc. This assessment will make it possible to choose the best appropriate treatment system. Further, OMW was analyzed by FTIR and UV-Vis to confirm its composition.

2. Methodology

2.1 Sampling

Olive oil mill wastewater sampling was carried out from factory in the region of Sidi Bel Abbes in the west of Algeria (**Figure 1**) during the olive campaign in November 2020. The used system was extraction with three-phase (**Figure 2**). The samples were taken and homogenized from the storage tank and then transported in bottles and were kept in the dark at 4°C for later use.

2.2 FTIR and UV-Vis analysis

The FTIR spectrum was obtained using a Perkin–Elmer Model Frontier/Multi scope spectrophotometer in the 4000-400 cm^{-1} wavenumber range and 2 cm^{-1} resolution while UV-Vis spectrum was carried out using Perkin–Elmer Lambda 45 UV-Visible spectrophotometer between 250 to 320 nm.

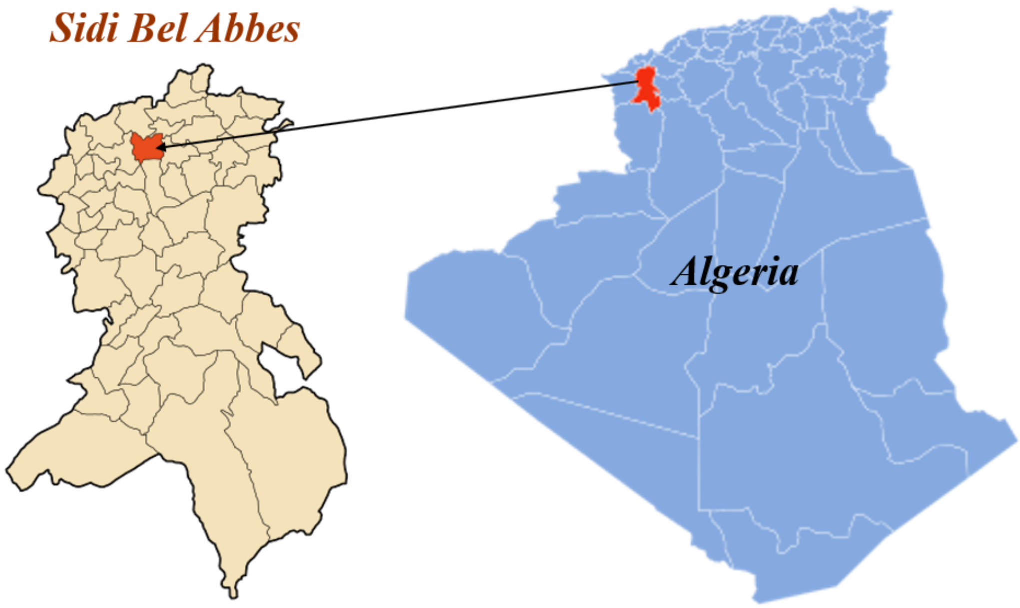

Figure 1. Location of the olive oil factory in the region of Sidi Bel Abbes (Algeria)

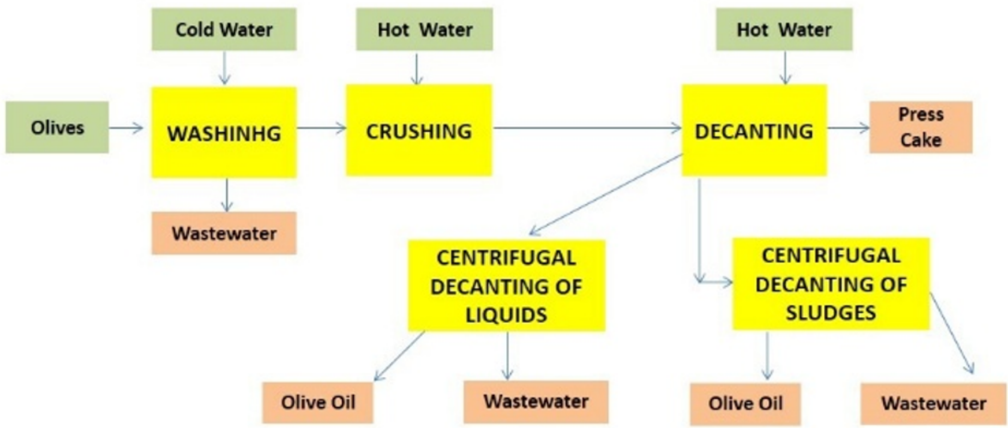

Figure 2. Process flow chart of three-phase olive oil extraction method

2.3 Physicochemical parameters

The physicochemical parameters determined in this study were according to the Standard Methods and other reference sources (APHA, 1995; Ma *et al.*, 2020): pH, Acidity, Electrical conductivity, Suspended Solids (SS), Turbidity, Total nitrogen matter, Biological oxygen demand (BOD_5), Chemical oxygen demand (COD), Chlorides and mineral composition (sodium, potassium and calcium). Dry matter content, Ash content, Volatile matter content, Organic matter, Fatty acids and polyphenols content were also measured:

Hydrogen Potential pH: was measured by a pH meter (multi parameter) type INOLAV 7110.

Acidity*:* Acidity or percentage of oleic acid is the quantitative capacity of a water to neutralize an alkali: 10 mL of Olive oil mill wastewater was transferred into three 100 mL beakers. Titration was carried out with a NaOH solution (0.1N). The test was repeated three times.

***Electrical conductivity**:* was measured by an INOLAV 7110 multi-parameter conductivity meter expressed in $ms.cm^{-1}$.

Total suspended solids: was determined by the difference in weight of the filter simple through a 0.45 µm pore diameter membrane filters and drying residue at 105°C for 4 h.

Turbidity: was estimated in nephalometric turbidity units (NTU) using a HACH DRB 890 optical turbidimeter.

Total Nitrogen Matter: The total nitrogen content in Olive oil mill wastewater is done through the Kjeldahl method which consists of a sample mineralization at high temperature with concentrated sulfuric acid and the catalyst (Cu) (6.25% in $CuSO_4.5H_2O$). After addition of sodium for solution neutralization, a mineralization product release nitrogen as ammonia (NH_3). This last was vaporised and trapped in boric acid then titrated with hydrochloric acid.

Chemical Oxygen Demand (COD): The determination of COD was performed by potassium dichromate. The principle based of this method is a boiling oxidation (150°C during 2 hours) of the reducing materials in acid medium (H_2SO_4) by an excess of potassium dichromate in the presence of silver sulfate as catalyst and mercury sulfate as a complex agent of chlorides. The optical density of the sample was obtained by spectrophotometry at a wavelength of 620 nm.

Biochemical oxygen demand (BOD_5): BOD_5 was determined for 5 days by the respirometric method in a thermostatic chamber at 20°C in the dark. The Olive oil mill wastewater samples was diluted, mixed with urban wastewater and its pH was adjusted to neutral range.

Biodegradability Index I_b: Ratio COD/BOD_5 gives idea on the biodegradability of OMW.

BOD_5/COD: This ratio determines the level of pollution presented by the liquid effluents.

Total oxidable Matter (TOM): It is an important parameter to determine the biodegradable part of the organic pollution released. It was calculated by the formula: **$(COD + 2\ BOD_5)/3$**

Chlorides: Chlorides were determined by the titrimetric method of Mohr with silver nitrate and potassium chromates.

Mineral composition: Concentrations of sodium, potassium and calcium in the Olive oil mill wastewater were determined using a flame spectrophotometer type BWB technologies calibrated with NaCl, KCl and $CaCl_2$ standard solutions.

***Dry Matter content*:** consists of organic and inorganic substances contained in solution or in suspension of Olive oil mill wastewater. The dry matter content was determined by weighing a sample of Olive oil mill wastewater before and after 24 hours evaporation time at 105°C (Aggoun *et al.*, 2016). It is expressed in g/100 g fresh weight.

Ash content (mineral matter): Obtained by incineration of the dry Olive oil mill wastewater during 6 hours in a Nebertherm muffle furnace at 550°C (Aggoun *et al.*, 2016).

Organic matter: The organic matter is the weight difference between the dry and the resulting ash content. The weight loss observed during calcination corresponds to organic matter and the residue to ash (Iboukhoulef *et al.*, 2014).

***Volatile matter**:* The volatile matter was determined by making the difference between the dry matter and the ash calcined residue at 550°C for 2 hours (Iboukhoulef *et al.*, 2014). It is expressed in g/L.

Fatty Acids content: The chloroform / methanol method was used in order to quantify the fatty acids in OMW (Esmail *et al.*, 2014).

Total polyphenols concentration: In the aim to determinate the total polyphenols concentration, Olive oil mill wastewater was mixed with ethyl acetate for polyphenols extraction. The polyphenolic compounds and ethyl acetate were recovered after centrifugation for 10 minutes at the rotation speed of 3200 rpm. Then the ethyl acetate was evaporated at 37°C. Polyphenols were then recovered in methanol (Zahari *et al.*, 2014). Then, the total polyphenols concentration was performed by the colorimetric method with Folin Ciocalteu. The optical density was reading at 760 nm by the spectrophotometrer (Mouzaoui *et al.*, 2014; Singleton *et al.*, 1999).

2.4 Microbiological parameters

The microbiological analyses of the Olive oil mill wastewater involved the enumeration of total aerobic mesophilic flora (FMAT), yeasts and moulds (Y and M) and lactic acid bacteria (LAB). After homogenization of the OMW samples, a series of dilutions in sterile saline solution (0.9% NaCl) were performed. The dilutions were obtained in sterile bottles from 1 mL of solution and 9 mL of saline solution. 1 mL of each dilution was placed in five Petri dishes and then poured in the choice agar medium. All the microbiological results were reported as CFU/mL (Colony Forming Units per milliliter).

Total Aerobic Mesophilic Flora: provides information on the overall bacterial load and was estimated on Plate Count Agar (PCA) incubated at 37°C for 48 h (Esmail *et al.*, 2014).

Lactic acid bacteria: The best-known medium for counting is MRS (Man Rogosa Sharpe, Difco, Detroit, USA). To prevent from the growth of yeast and moulds, cycloheximide was added to the basal medium and colonies were counted after 24 h of incubation at 30°C (Esmail *et al.*, 2014).

***Yeasts and moulds**:* The evaluation of the abundance of yeasts and moulds was done on Sabouraud chloramphenicol Agar medium after incubation at 30°C during 48 h for yeasts and 5-7 days for moulds (Zaier *et al.*, 2017).

3. Results and Discussion

3.1 Physicochemical characterisation

The collected olive oil mill wastewater studied in our research has a strong smell that reminds one of olive oil. They have a cloudy appearance with a dark brown color to reddish-brown, which becomes darker during storage. In order to interpret physicochemical parameters of this olive OMW, results were regrouped and compared with effluents regulation recommended in interministerial

Algerian decrees (**Table 1**). In all results mentioned in Table 1, it appears that OMW do not meet Algerians norms. For more understanding and interpreting these parameters excess, it would be better to compare results with OMW physicochemical parameters obtained previously by authors in different countries (see **Table 2**).

Table 1. Physicochemical parameters of OMW compared with effluents regulation recommended in interministerial Algerian decrees

Parameters	Units	Raw (OMW)	Effluent regulation in Algeria (JORADP, 2006; 2012)
pH	/	4.52	6.5 - 8.5
Acidity	%	1.35	/
Conductivity	mS/cm	12.67	3
Dry matter content	g/L	12	/
Ash content	g/L	3.45	/
Volatile matter content	g/L	6.9	/
Fatty acids content	%	1.25	0.20
Suspended Solids	g/L	18.5	0.03 - 0.035
Total Nitrogen Matter	g/L	0.095	0.03
Turbidity	NTU	1780	/
Organic matter	g/L	68.9	/
COD	g/L	90.5	0.03 – 0.12
BOD_5	g/L	29	0.035 - 0.09
I_b	/	3.12	/
BOD_5/COD	/	0.32	/
Total oxidable Matter	/	49.5	/
Polyphenols	mg/L	450	0.002
Chloride	g/L	8.16	0.354
Sodium	g/L	2.875	/
Potassium	g/L	6.75	/
Calcium	g/L	21.125	/

Table 2. Physicochemical parameters of Olive oil mill wastewater compared with OMW obtained in different countries

Parameters	Literature values								
	Algeria (Our Study)	**Egypt** (Benamar *et al.*, 2020)	**Portugal** (Lourenço *et al.*, 2017; Davies *et al.*, 2004)	**Greece** (Galanakis, 2017 ; Roulia *et al.*, 2021)	**Tunisia** (Zaier *et al.*, 2017)	**Spain** (Gassan *et al.*, 2019; Pulido and Férez, 2017)	**Morocco** (Esmail *et al.*, 2014; Achak *et al.*, 2009)	**Jordan** (Al Bsoul *et al.*, 2019; Khdair *et al.*, 2019)	**Italy** (Vuppala *et al.*, 2021; Stoller *et al.*, 2016)
pH	4.52	4.81	4.7-6.85	5.48	4.7-5.1	5.35	4.65 - 5.16	4.6 -4.9	4.2 - 4.65
Acidity (%)	1.35		/	/	/	/	1.287 - 1.755	/	/
Conductivity (mS/cm)	12.67	16.79	/	11	11.1-12.3	0.7-1.8	6.85– 19.09	23.5-25.1	/
Dry matter content (g/L)	12	/	/	/	80.7-90.5		/	/	/
Ash content (g/L)	3.45	/	/	19.8	12.72-13.57	1.2	/	/	/
Volatile matter content (g/L)	6.9	/	7.1 – 94.3	/	71.8-79.4		1.51	/	/
Fatty acids content (%)	1.25	/	/	0.03-1.1			1.5 - 2.5	/	/
Suspended Solids (g/L)	18.5	29.25	5.99 - 106	3.2-30	16.42-17.06	0.0036	0.5-2.07	23 - 71.4	/
Total Nitrogen Matter (g/L)	0.095	/	/	0.58- 2	/	0.039	0.03-1.96	/	/

Turbidity (NTU)	1780	/	3440	/	/	2056	/	/	3200
Organic matter (g/L)	68.9	/	/	80.2	57.2-58.06	20	/	/	/
COD (g/L)	90,5	227.33	9.08 - 134.0	/	267.65-286.3	0.73 - 6.621	70.22 - 104.31	50 - 105	0.024 – 21.83
BOD_5 (g/L)	29	46.40	4.75 – 42.0	/	80.35-86.71	/	16.74 - 35	0.06 – 41	/
I_b	3.12	/	/	/	3.301- 3.331	/	/	/	/
BOD_5/COD	0.32	/	/	/	/	/	/	/	/
Total oxidable Matter	49.5	/	/	/	/	/	/	/	/
Polyphenols (mg/L)	450	11410	30-6160	/	5.63-10.093	118	0.39 - 0.42	$7.8e^{-4}$ - 3100	260
Chloride (g/L)	8.16	/	/	/	/	0.503	5 - 5.8	/	/
Sodium (g/L)	2.875	/	/	0.04-0.48	/	0.0127 10^{-3}	/	/	/
Potassium (g/L)	6.75	/	/	0.3-0.9	/	/	/	/	/
Calcium (g/L)	21.125	/	/	0.32-0.53	/	/	/	/	/

The results shows that the OMW effluent is acidic with pH = 4.52. This measured value is not allowed in effluent regulation in Algeria (6.5 < pH > 8.5) as mentioned in Table 1 but it is at the limit of those observed in the literature for vegetable waters (**Table 2**). This acid pH is due to the richness of the Olive oil mill wastewater in organic acids (phenolic acids, fatty acids, etc.) (Belaid *et al.*, 2002). The low pH of our Olive oil mill wastewater is confirmed by an acidity value of 1.35 %.

The electrical conductivity (EC) is one of the most important parameter for the wastewater quality control. The obtained high value of EC (12.67 mS/cm) is probably related to the salting (addition of salt in large quantities to preserve the olives) practiced before crushing olives, in addition to the natural richness of vegetable waters in dissolved mineral salts (Gharby *et al.*, 2014). To confirm this hypothesis, the contents of potassium, sodium and calcium ions are measured and are respectively of the order of 6.750 g/L, 2.875 g/L and 21.125 g/L. For the same reasons probably, the chloride rate greatly exceeds the values allowed in Algerian standards. The measured quantity of chloride is 8.160 g/L while the values allowed in wastewater must not be more than 0.354 g/L. The total nitrogen content is about 0.095 g/L. The result obtained in our study is in the interval of several studies, such as between 0.0476 and 0.0756 g/L during 2012 (Esmail *et al.*, 2014) and between 0.442 and 0.487 g/L found in Morocco during 2014 and 2016 (El yamani *et al.*, 2020).

The dry matter content is relatively low (12 g/L) compared to the maximum value of Tunisian Olive oil mill wastewater (80.7 - 90.5 g/L) reported by Zaier *et al.* (2017). However, the water content obtained in the OMW is important (96%). it can be explained by three elements: i/ Water presents in the olives; ii/ Water added during the olive oil extraction process; and iii/ Washing water. The ash content, which represents the mineral fraction of vegetable water, is in the order of 3.45 g/L. This value is relatively low compared to the organic matter 68.9 g/L, which shows the low mineral constitution of these vegetable waters. These values remain within the range found previously in the literature: Mineral matter between 12.72 - 13.57 g/L and volatile matter was 64 g/L (Zaier *et al.*, 2017).

On the other hand, volatile matter content represents 6.9 g/L of dry matter. It is higher than the ash content but the two values remain far from the quantity of organic matter (68.9 g/L) which confirms the organic nature of vegetable waters. This shows the high oxygen demand for the complete oxidation of the organic matter contained in these effluents, which reflects their very high polluting power (Zaier *et al.*, 2017). The content of residual fatty acids present in the Olive oil mill wastewater depends on the extraction system of olive oil (with three-phase in our case). The content obtained in our experiment is 1.25%. Compared with content fatty detected previously by other researchers in Morocco as one of the most olive oil producing countries in the Mediterranean, Esmail A. *et al.* (2014) found 1, 1.5 and 2.5% when they studied physicochemical quality of OMW from three different regions: Ouazzane, Fes Boulman and Beni Mellal, respectively.

Suspended solids and turbidity are determined for particles in Olive oil mill wastewater. Turbidity of the studied Olive oil mill wastewater is about 1780 NTU and suspended solids measured is 18.5 g/L. High turbidity levels can complicate the disinfection processes (US EPA, 2012) and high suspended solids in effluent can lead to clogging of infrastructure and soil, sludge deposition and anaerobic conditions. It can may be associated with higher microbial contamination. The SS result exceeds the permitted values in Algerian wastewater effluent (0.030 - 0.035 mg/L).

The pollutant content expressed in terms of Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD_5) is about 90.5 g/L and 29 g/L, respectively. These values exceed the standards allowed in Algeria (**Table 1**). Moreover, the biodegradability index higher than 3 (= 3.12) prove that OMW is rich in non or partially biodegradable organic matter. In addition, the ratio BOD_5/COD is 0.32

pouves that OMW can be treated by selected microbial strains (Gueboudjia *et al.*, 2021). In the other hand, total oxidisable matter is 49.5 g/L. Oxidizable matters represent a significant pollutant load in urban effluents and industrial establishments. It helps to connect the industrial facility to a municipal sewer system. In our case, any connection of the units' trituration olives can lead to dysfunctional treatment plant wastewater (Bouknana *et al.*, 2014).

The phenolic compounds can be either polyphenols which result from polymerisation of the simple phenols or flavonoids. The content of phenolic compounds is about 0.45 g/L. Phenolic composition of vegetable waters depends on the variety and maturity of the fruit and on climatic conditions, as well as on the process for separating the aqueous phase (vegetable water) from the oily phase (Esmail *et al.*, 2014). However, these compounds are strong inhibitors of flora hence the high toxicity of these rejects who make the treatment of OMW imperative (Belaid *et al.*, 2002). The color of vegetable waters reflects the state of degradation of the phenolic compounds and olives from which they are derived.

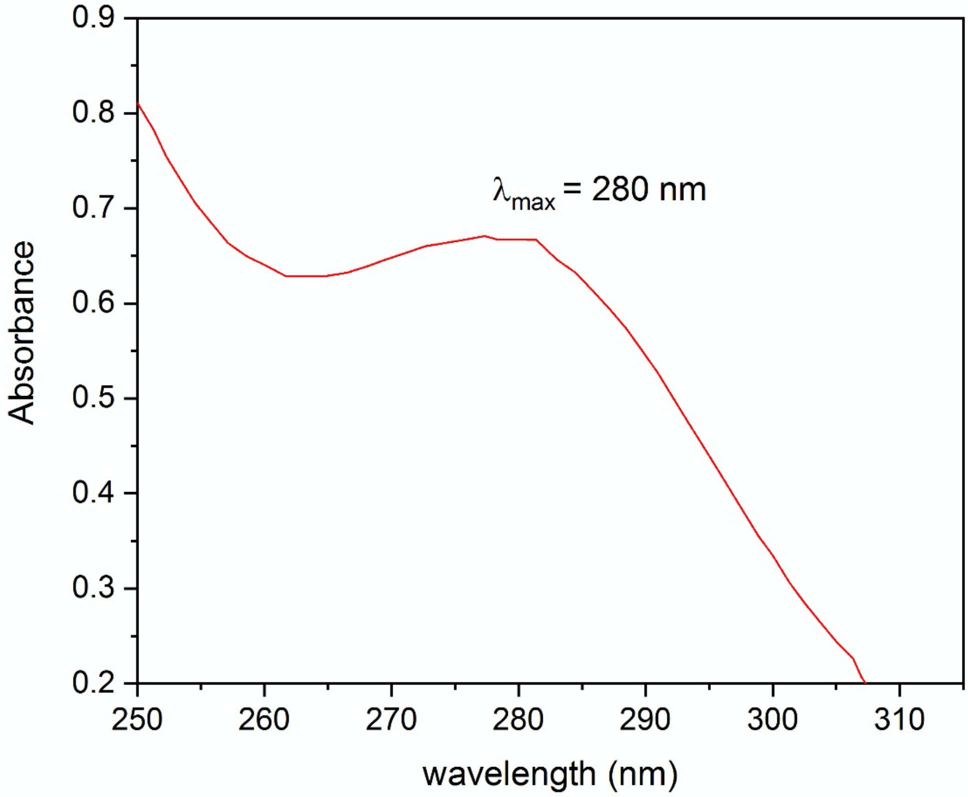

Figure 3. UV-Vis spectrum of Olive oil mill wastewater

UV-Vis (**Figure 3**) and FTIR (**Figure 4**) Spectra of olive mill wastewater are used in this work to confirm our obtained analytical results. UV-Vis spectrum recorded shows an absorption band with λ_{max}= 280 nm attributed to the chromophore OH of phenols (Aleixandre-Tudo and Wessel du Toit, 2018; Elayadi *et al.*, 2019). The primary role of Fourier Transformed Infrared Spectroscopy (FTIR) is to identify organic compounds of this black wastewater. The spectrum reveals a centered band at 3237 cm^{-1} correspond to the OH vibration of carboxylic groups, alcohols and phenols (Hafidi *et al.*, 2005; Ricca and Severini, 1993); In addition, band situated at 1566 cm^{-1} due to the double bonds C=C and at 1405 cm^{-1} attributed to the OH vibration of the aromatic. Bands at 2923 and 2854 cm^{-1} attributed to C-H, CH_2 and CH_3 groups in aliphatic compounds (waxes, fatty acids, etc.) (Socrates, 1994; Fransisco, 2007). A C=O stretching vibrations band at 1719 cm^{-1} related to ketone groups, esters and carboxyl COOH. The last bands at 1082 cm^{-1} and 1042 cm^{-1} can be attributed to deformation vibration C-O of phenols and acids or to monosaccharides and polysaccharides, present also in OMW (Fransisco, 2007; El hassani *et al.*, 2020).

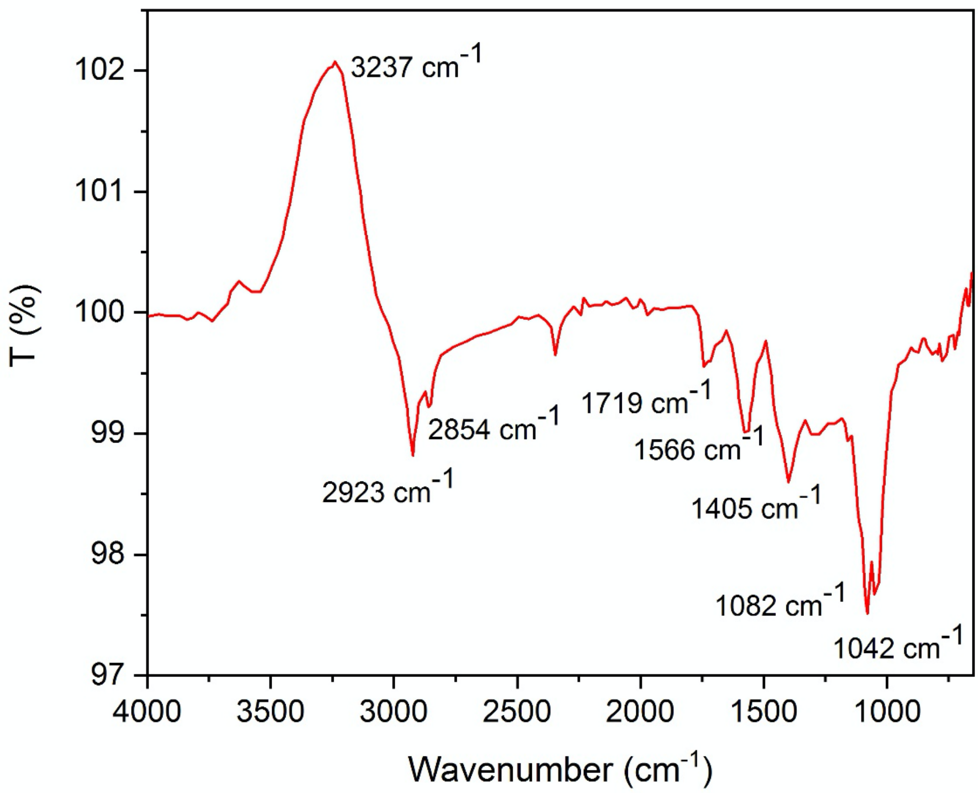

Figure 4. FTIR spectrum of Olive oil mill wastewater

In order to study the effect of flocculant on particles size, several samples were prepared with phosphate particles at different solid concentration. The flocculant used in this study is the polyacrylamide [$-CH_2-CH(-CONH_2)-$]n, with a concentration of 5g/L. Final samples and their characteristics are represented below:

The result is coherent with previous studies: It is well known that olives are among fruits that containing phenolic compounds (Macheix *et al.*, 1990) and therefore their derivatives such as vegetable water are also. Akretche H. et al. (Akretche *et al.*, 2019) identified polyphenols in OMW from Olea europaea oil extraction in the region of Tizi Ouzou (Algeria) using the Folin Ciocalteu method, with gallic acid as the standard. Polyphenols were quantified using the Shimadzu UV-1700 spectrophotometer at 750 nm as 0.20 ± 0.05 %. In the same study and with HPLC analysis, authors detected some phenolic compounds: caffeic acid 0.015 ± 0.004 g/L, hydroxytyrosol 0.215 ± 0.009 g/L, tyrosol 0.092 ± 0.003 g/L and coumaric acid 0.052 ± 0.005 g/L. The percentage of sugars determined by phenol-sulfuric acid colorimetric method and glucose was 0.31 ± 0.06 % and water was 98.15 ± 0.10 %. In another study (Cassano *et al.*, 2016), in addition to the phenolic compounds cited in the preceding paragraph, other phenols are mentioned: secoiridoid aglycons and lignans, such as elenolic acid, oleuropein, vanellic acid, p-cumaric acid, catechol, verbascoside and rutin.

3.2 *Microbiological characterisation*

The microbiological analyses of the Olive oil mill wastewater was carried out on the indicator germs of pollution, which include heterotrophic aerobic mesophilic bacteria such as Total Aerobic Mesophilic Flora (TAMF), yeasts and moulds and also lactic acid bacteria (**Table 3**).

Table 3. Olive oil mill wastewater (OMW) Microbiological parameters

Total Aerobic Mesophilic Flora (CFU/mL)	Lactic acid Bacteria (CFU/mL)	Yeasts and moulds (CFU/mL)
7.9×10^3	6.8×10^3	0.95×10^3

The total microbial load of the olive oil wastewater in this region was assessed by counting the total aerobic mesophilic flora which is around 7.9×10^3 CFU/mL. In another study, the TAMF of olive oil wastewater in different Tunisian regions was evaluated by Zaier *et al*. (2017). The results indicated that TAMF ranged from 12×10^3 CFU/mL to 413×10^3 CFU/mL. However, this total microbial load may be related mainly to the extraction process, operating conditions adopted from the region and also the storage conditions of Olive oil mill wastewater and physicochemical characteristics that hinder the growth of microorganisms including the presence of antimicrobial substances (pH, phenolic compounds, mineral salts, heavy metals, tannins, fatty acids ...). Moreover, lactic acid bacteria are reported for the olive oil mill wastewater and the found value was 6.8×10^3 CFU/mL. Their presence is a witness of their resistance in acidic environments. Indeed, LAB can grow even at pH below 4. This resistance decreases with time, and therefore their disappearance becomes normal with time. The factor of disappearance is probably related to the presence of phenolic compounds (Esmail *et al*., 2014).

In olive oil mill wastewater, only a few microorganisms manage to develop. These are mainly yeasts and moulds (0.95×10^3 CFU/mL). In most cases, pathogenic microorganisms are absent and do not cause any health problems. The antimicrobial power of Olive oil mill wastewater is mainly related to the action of monomeric phenols and brown catechol-melaninic pigments. These effluents act on bacteria by denaturing cellular proteins and altering membranes. They can also inhibit the activity of symbiotic nitrogen-fixing bacteria in the digestive tract of ruminants by inhibiting their enzymatic activity (Jeddi *et al*., 2016).

Conclusion

The discharge of Olive oil mill wastewater from olive oil producing industries is a major problem especially in Mediterranean basin countries due to the content of an important organic fraction and cause several types of pollution.

The physicochemical study carried out on this Olive oil mill wastewater showed that it was acidic (pH = 4.52), suspended solids (18.5 g/L) and conductivity (12.67 mS /Cm). It was characterised by a high organic pollution evaluated in terms of COD (90.5 g/L) and BOD_5 (29 g/L), this shows that this effluent cannot be purified by natural biodegradation. The content of phenolic compounds was high 0.45 g/L. Dry matter, fatty acids, mineral matter, volatile matter and mineral composition were also determined. The microbiological results showed that the values obtained for our Olive oil mill wastewater are high, too. These values constitute strong inhibitors of the flora hence the high toxicity of this discharge.

The excess in the physicochemical and microbiological parameters of the Olive oil mill wastewater is due mainly to the quality and the method used in the process of extraction of olive oil, the practice of salting for the preservation of olives, the type of olives, the degree of their ripening and the method of storage in covered basins or pools. The data presented in this study further reinforces the idea that the treatment of these effluents is necessary before any discharge.

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