PKP
PUBLIC KNOWLEDGE PROJECT

Journal of Biological Studies
ISSN 2209-2560

JBS
An Open Access Peer Reviewed Journal

Physicochemical and microbiological characterization of olive oil mill wastewater (OMWW) from Algerian Sahara, region of Ghardaïa

Meriem Meziani[1*], Rabah Arhab [2], Imane Lamraoui[3], Ilham Kaddour[3]

[1] *Laboratory of Applied Biochemistry, Department of Microbiology, Faculty of Nature and Life Sciences, University of Constantine 1, Algeria.*

[2] *Laboratory of natural substances, biomolecules and biotechnological applications, Faculty of Nature and Life Sciences, Oum El Boouaghi University, Algeria.*

[3] *Faculty of Nature and Life Sciences, Oum El Boouaghi University, Algeria.*

Received: December 27, 2022; **Accepted:** February 10, 2023; **Published online:** March 29, 2023.

Abstract: The aims of this study were to determine the physical-chemical and microbiological characteristics of olive oil mill wastewater (OMWW) resulting from the extraction of olive oil in Algerian Sahara in Ghardaïa region, and to evaluate the antibacterial activity of the OMWW extracts manifested by their phenolic compounds on the identified bacteria. This is the first study of physicochemical and microbiological characterization of the olive mill wastewater resulting from the modern industrial unit of trituration of olives by three-phases centrifugation in Ghardaïa region in Algerian Sahara. The results showed that this effluent is too rich in organic matter that is expressed by BOD5 and COD. It is also characterized by an acidic pH (4.8) and a high level of phenolic compounds. The microbiological study of the olive oil mill wastewater showed the isolation of three GRAM positive bacterial strains (*Staphylococcus aureus*, *Staphylococcus epidermidis* and *Bacillus subtilis*) and five fungal strains (*Penicillium* sp, *Aspergillus ochraceus*, *Aspergillus fumigatus*, *Penicillium chrysogenum* and *Aspergillus niger*), and one strain of yeast *Saccharomyces cerevisiae*. The results of the antibacterial activity showed that strains which were isolated from the olive oil mill wastewater were resistant to the phenolic compounds whereas clinical strains were sensitive.

Keywords: Olive oil mill wastewater; physicochemical and microbiological characterization; phenolic extracts; antibacterial effect; Algerian Sahara.

*e-mail: Meziani_meri25@yahoo.fr

1 Introduction

The olive industry is an important economic activity, located mainly in the Mediterranean countries which hold about 95 % of world production, including 1 % for Algerian production in 2001. The olive industry, in addition to its main production of oil (virgin olive oil and pomace oil), leads to two residue generations: a liquid (olive mill wastewater) and the other solid (olive pomace) (Nefzaoui, 1991).

Olive oil mill wastewater are considered to be one of the most harmful effluents produced by the agro-food industries (Cardinali et al., 2010), because of their polluting load and their toxicity for the ecosystem (plants, microorganisms and aquatic and aerial organisms) as a consequence of their acid pH, and their richness in organic matter, in particular in polyphenols (Abbassi et al., 2012; Dermeche et al., 2013). The discharge of these effluents into rivers and sewers without any prior treatment poses serious problems for aquatic ecosystems (Bouranis et al., 1995; Cabrera et al., 1996; Sayadi et al., 2000). These considerations have led to several studies in order to choose the way of treatment and recovery of the olive oil mill wastewater to limit their pollution (Gharsallah et al., 1999; Garcia Garcia et al., 2000; Leger et al., 2000; Kissi et al., 2001). To overcome this problem, various processes have been developed but remain until today very limited and very expensive (Garrido Hoyos et al., 2002; Pozo et al., 2002; Fenice et al., 2003; Hamdi, 1993). However, several microorganisms have been shown that they are able to grow in the olive oil mill wastewater and use them as a carbon source, in particular yeasts and fungi (Vazequez, 1978; Aissam et al., 2002). In this context, researches are very rare.

This study relates to a physico-chemical and microbiological characterization of the microflora of the waste water of the oil mills resulting from the modern industrial unit of trituration of the olives by three-phase centrifugation in the Algerian Sahara precisely the region of Ghardaïa, and to an evaluation of the antibacterial activity of OMWW extracts manifested by its phenolic compounds.

2 Materials and Methods

2.1. Sampling:

The olive oil mill wastewater analyzed in this study was resulting from a modern industrial unit for trituration of olives by three-phase centrifugation, located in Ghardaïa region in southern Algeria. The samples were taken from the olive oil mill wastewater storage basin and transported in sterile vials of 2 L, then were stored in the dark at 4 °C for physicochemical and microbiological characterization.

2.2. Physico-chemical analysis:

2.2.1. Determination of the pH:

The pH is determined from the quantity of free hydrogen ions contained in the OMWW. The pH is determined by a pH meter with electronic display previously calibrated at pH 4 and 7 (AFNOR, 1992).

2.2.2. Determination of the conductivity:

Electrical conductivity was measured using a conductivity meter (Rodier, 1996).

2.2.3. Turbidity:

Turbidity was measured using a multi-parameter HACH-lange model sension TM + MM150 and expressed in g/l.

2.2.4. Determination of biological oxygen demand (BOD):

The biochemical oxygen demand represents the quantity of oxygen necessary for the degradation of organic matter by environmental microorganisms. The BOD5 in this study was measured by a respirometric method (Rodier, 2009; Michaud, 2012).

2.2.5. Determination of chemical oxygen demand (COD):

COD is the measure of the amount of oxygen required for chemical degradation of all organic matter in the sample. COD was determined using the small-scale closed tube method (Rodier, 2009; Michaud, 2012).

2.3. Biochemical analysis:

2.3.1. Determination of mois:ture content and dry matter:

The moisture and dry matter were determined by the method adapted by Tsagariki et al. (2007), in order to calculate the difference between the weight of the wet sample and that of the dried sample.

2.3.2. Organic and mineral matter:

The ashes are determined by incineration of 1 g of dry sample in a muffle furnace at 550 °C for 5 hrs. The organic matter corresponds to the difference between the test sample and the resulting ash (Tsioulpas et al., 2002).

2.4. Determination of total polyphenols:

The extraction of the total polyphenols is carried out using ethyl acetate which is a suitable solvent for the recovery of the phenolic compounds contained in the olive mill wastewaters. The polyphenols are determined using the colorimetric technique of Folin Ciocalteu described by Vazequez (1978). Folin Ciocalteu's reagent consists of an acidic yellow solution. It oxidizes phenols to phenolate ions in an alkaline medium and partially reduces these heteropolyacids, hence the formation of a blue complex. The blue color obtained is proportional to the amount of phenols present. The revelation of the blue color is obtained after incubation in the dark and at room temperature for 40 min.The absorbance is measured at 725 nm. The concentration of phenolic compounds in the extract is determined from the calibration curve of gallic acid used as standard (Figure 1).

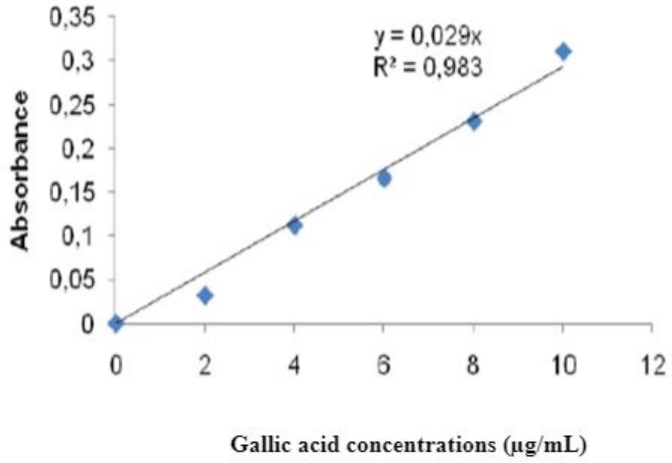

Figure 1: Standard curve corresponding to the determination of total phenols.

2.5. Microbiological analysis of the olive mill wastewaters:

The olive oil mill wastewaters that were the subject of our work underwent pre-treatment operations, in order to eliminate suspended matter and grease (Zahari et al., 2014). The isolation of all microbial flora (bacteria, yeasts, etc.) from the OMWW, and the identification of the microbial strains were carried out using conventional techniques based on morphological and biochemical studies (Esmail et al., 2014). The morphological study was carried out on the basis of two parts: macroscopic and microscopic which is based on the preparation of the smear and the Gram staining for the various bacterial germs or a simple staining for the yeasts. The biochemical study is based on the realization of various biochemical tests.

2.6. Evaluation of the antibacterial activity of phenolic compounds:

The isolated bacterial strains from the OMWW samples that have undergone antibacterial activity are: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Saccharomyces cerevisiae*, and *Bacillus subtilis*. Three clinical strains were also tested and are listed in table 1. Antibacterial activity was assessed using the Mueller-Hinton agar disk diffusion method; Whatman paper discs soaked in 10 μl of phenolic compound extract (Rožman and Jeršek, 2009).

Table 1. Clinical isolates tested for antibacterial activity of olive oil mill wastewaters.

Bacterial strains (ATCC)	Code	Origin
Staphylococcus aureus	43900	Public Hospital Establishments (EPH)Constantine
Escherichia coli	25925	EPH Constantine
Pseudomonas aeruginosa	27853	EPH Constantine

3 Results and Discussions

3.1. Physicochemical and biochemical characterization of the olive mill wastewaters:

The table 2 recapitulates the physicochemical and biochemical characteristics of the OMWW resulting from the traditional 3 phase's system. The olive oil mill wastewaters have highly polluting discharges in residual liquid form whose composition is variable. This variability depends on the type of olives, the degree of maturation, the cultivation systems, the practice of salting for the preservation of the olives, the climatic conditions and the process used for the extraction of the olive oil (De Marco et al., 2007). The pH measurements of OMWW gave a value of (4.8), this result can be explained that the olive oil mill wastewaters (OMWW) are therefore acidic effluents, due to the presence of organic acids (phenolic acids)., this result is consistent with that of Djeziri et al. (2022). The value recorded in our study is at the lower limit of the range reported in the literature (4.5 to 6) by Zaier et al. (2017) and Mohamed et al. (2020). This variation depends on the factors mentioned above.

Table 2. Physicochemical and biochemical characteristics of the olive oil mill wastewaters.

Parameters	Values
pH	4,8
Conductivity	1711 μs/cm
Turbidity	666,66 NTU
BOD5	1200 mg O2/L
COD	1300 mg O2/L
% of dry matter	10,5 %
% of organic matter	9,5 %
% of mineral matter	1 %
polyphenols	2,2 μg/mL

The olive oil mill wastewaters studied have a low electrical conductivity (1711 us/cm) compared to those found by Sbai and Loukli (2015). The low conductivity of the olive mill wastewaters analyzed could be explained by their high content of organic compounds compared to salts. Indeed, the dissolved mineral salts in solution are the good conductors whereas the organic compounds are the poor conductors. This result is corroborated by the richness of the olive oil mill wastewaters analyzed in our study in organic matter expressed in terms of BOD5 and COD, this result is mentioned in previous works by Zaier et al. (2017) and Djeziri et al. (2022).

These discharges are also characterized by the predominance of toxic substances, in particular phenolic compounds (2,2 μg/mL) which give them an antimicrobial power. This result is confirmed by the results of other authors (Zaier et al., 2017; Djeziri et al., 2022). The olive oil mill wastewaters analyzed have a turbidity of around 666,66 NTU due to the high content of organic substances.

The rate of total dry matter (in g/l) was determined as (109.3 g/l). The value recorded in our study is closer to that found by EL Abbassi (2012) having found that their OMWW sample contained 90 g/l. These variations can be due to climatic and geological parameters, botanical variations, the stage of maturation of the olive and the oil extraction process.

3.2. Microbiological characterization:

The results of the germ count presented in the table 3 confirmed the complete absence of pathogenic microorganisms in the OMWW analyzed. These results are similar to those reported by Ranalli (1991). Consequently, the total absence of these germs in the OMWW could only be

explained by its richness content in phenolic compounds inhibiting the microbial growth (Ranalli, 1991). The total microbial load was evaluated by counting the total aerobic mesophilic flora (TAMF). It was found relatively low (6.35 10^4 CFU. ml^{-1}) compared to that recorded in previous studies; that recorded in slaughterhouse effluents (1.23 10^6 CFU. ml^{-1})(Aissam et al., 2002). That is linked to the physicochemical characteristics of the OMWW, particularly the presence of antimicrobial substances (phenolic compounds) which inhibits microorganism growth (Aissam et al., 2002; Zaier et al., 2017).

Table 3. Microbiological characterization of the olive oil mill wastewaters studied.

Microbial flora	CFU. ml^{-1}
Total aerobic mesophilic flora TAMF	6.35 10^4
Fungi	1.08 105
Yeasts	1.7 104
Faecal coliforms	0
Total coliforms	0
Faecal streptococci	0

Yeasts and fungi represent the major flora of the OMWW. They were found in the range of 1.08 10^5 CFU. ml^{-1} and 1.7 10^4 CFU. ml^{-1} respectively. These results are consistent with those reported by other authors who showed that in the olive oil mill wastewaters, yeasts and fungi are able to develop better than bacteria (Aissam et al., 2002; Zaier et al., 2017).

In addition, analyzes of faecal contamination indicator germs showed a total absence of total coliforms (CT), faecal coliforms (CF) and faecal streptococci (SF); these results are in agreement with those reported by Mouncif et al. (1993). The microbiological study of the olive oil mill wastewaters showed the isolation of three GRAM positive bacterial strains (*Staphylococcus aureus*, *Staphylococcus epidermidis* and *Bacillus subtilis*) and five fungal strains (*Penicillium* sp, *Aspergillus niger*, *Aspergillus ochraceus*, *Aspergillus fumigatus* and *Penicillium chrysogenum*), and one yeast strain (*Saccharomyces cerevisiae*).

The results of the evaluation of antimicrobial activity against strains isolated from the olive oil mill wastewaters (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Saccharomyces cerevisiae* and *Bacillus subtilis*) (Figure 2) showed that there is no effect of these phenolic compounds on the isolated strains and therefore these strains are resistant to phenolic compounds, while a very effective effect is observed against the clinical bacterial strains tested (*Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*) therefore, these strains are sensitive to phenolic compounds (Table 4) and (Figure 3).

Table 4. Diameter of the zones of inhibition in (mm) showing the antibacterial activity of the phenolic compounds on the clinical strains tested.

Microbial group	Clinical strains tested	Inhibition diameters in (mm)
$GRAM^+$	*Staphylococcus aureus*	26
$GRAM^-$	*Escherichia coli*	14
-	*Pseudomonas aerugino*	22

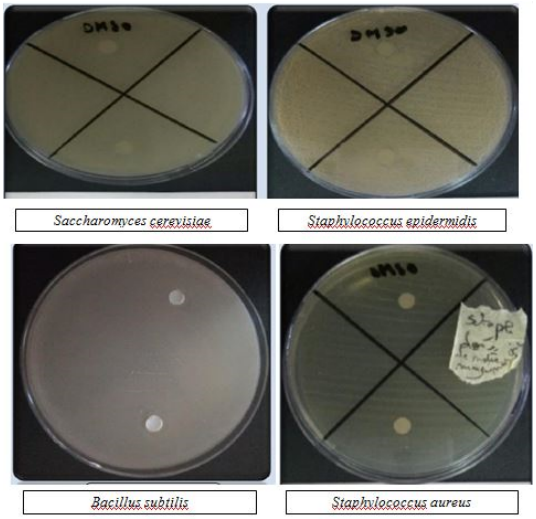

Figure 2: Antibacterial activity of phenolic compounds on strains isolated from the olive oil mill wastewaters.

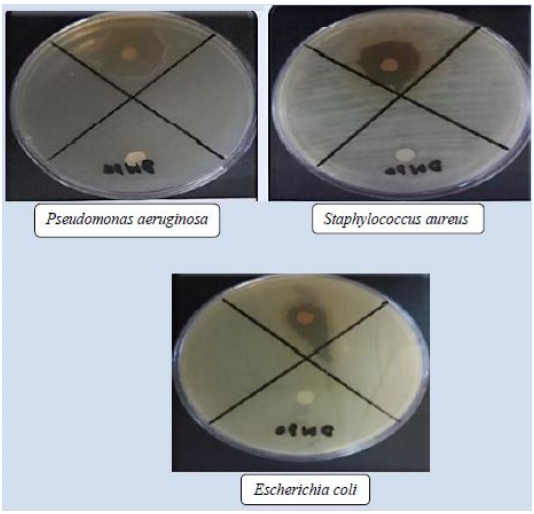

Figure 3: Antibacterial activity of phenolic compounds on clinical bacterial strains tested.

Few works have been carried out on the characterization of the microflora of the olive oil mill wastewaters in Ghardaïa region in the Algerian Sahara. This work constitutes the first study of physico-chemical and microbiological characterization of the OMWW from the modern industrial unit of trituration of olives by centrifugation in three phases, in the region of Ghardaïa.

4 Conclusions

The physicochemical characterization of the olive oil mill wastewaters analyzed in this study showed a high organic pollution indicated particularly by the presence of phenolic compounds. The OMWW are also characterized by a high acidity with a lower value of electrical conductivity and a high pollution load as determined by COD and BOD5. The resistance profile to phenolic compounds revealed that the microbial strains isolated from The OMWW are resistant while the clinical strains tested are sensitive to the phenolic compounds. To remediate problems of pollution caused by the production of the OMWW, scientific research must be devoted to propose alternatives to the direct discharge of these effluents into the natural environment.

5 Acknowledgements

We would like to thank the reviewers for their valuable comments and suggestions to improve paper quality.

Conflict of interests

The authors have no conflict of interest to declare.

References

Abbassi, A., Kiai, H., & Hafidi, A. (2012). Phenolics profile and antioxidant activities of olive mill wastwater. Food chemistry, 132, 406–412.

Aissam, H., Errachidi, F., & Merzouki M. (2002). Identification des levures isolées des margines et étude de leur activité catalase. Cahiers de l'Association Scientifique Européenne pour l'Eau et la Santé, 7, 23–30.

Bouranis, L., Vlyssides, G., Drosopoulos, B., & Karvouni, G. (1995). Some characteristics of new organic soil conditioner from the co-composting of olive oil processing wastewater and solid residue. Soil science and plant analysis, 26, 2461–2472.

Cabrera, F., Lopez, R., Martinez-Bourdiu, E., Dupuy de Lome, E., & Murillo, J.M. (1996). Land treatment of olive oil mill wastewater. International Biodegradation and Biodeterioration, 38, 215–225.

Cardinali, A., Cicco, N., & Linsalata, V. (2010). Compounds in the phenolic fraction of olive oil. Clinical Chemistry, 46, 976-988.

De Marco, E., Savarese, M., Paduano, A., & Sacchi, R. (2007). Characterization and fractionation of phenolic compounds extracted from olive oil mill wastewaters. Food Chemistry, 104, 858–867.

Dermeche, S., Nadour, M., Larroche, C., Moulti-Mati, F., & Michaud, P. (2013). Olive mill wastes: Biochemical characterizations and valorization strategies. Process Biochemistry, 48, 1532–1552.

Djeziri, S., Taleb, Z., & Djellouli, M. (2022). Physicochemical and microbiological characterisation of Olive Oil Mill Wastewater (OMW) from the region of Sidi Bel Abbes (Western Algeria). Moroccan Journal of Chemistry. [In press].

El-Abbassi, A., Kiai, H., & Hafidi A. (2012). Phenolic profile and antioxidant activities of olive mill wastewater. Food Chemistry, 132(1), 406–12.

Esmail, A., Abed, H., Firdaous, M., & Chahboun, N. (2014). Étude physico-chimique et microbiologique des margines de trois régions du Maroc. (Ouazzane, Fès Boulman et Béni Mellal). Journal of Materials and Environmental Science, 5, 121–126.

Fenice, M., Giovannozzi Sermanni, G., Federici, F., & D'Annibale, A. (2003). Submerged and solid-state production of laccase and Mn-peroxidase by Panus tigrinus on olive mill waste water-based media. Journal of Biotechnology, 100 (1), 77–85.

Garcia Garcia, I., Jimenez Pena, P.R., Bonilla Venceslada, J.L., Martın Martın, A., Martın Santos, M.A., & Ramos Gómez, E. (2000). Removal of phenol compounds from olive mill wastewater using Phanerochaete chrysosporium, Aspergillus niger, Aspergillus terreus and Geotrichum candidum. Process Biochemistry, 35(8), 751–758.

Garrido Hoyos, E., Martinez Nieto, L., Camacho Rubio, F., & Ramos, C.A. (2002). Kinetics of aerobic treatment of olive-mill wastewater (OMW) with Aspergillus terreus. Process. Biochemistry, 37, 1169–1176.

Gharsallah, N., Labat, M., Aloui, F., & Sayadi S. (1999). The effect of Phanerochaete chrysosporium pretreatment of olive mill wastewaters on anaerobic digestion. Ressources Conservation and recycling, 27, 187–192.

Hamdi, M. (1993). Valorisation et épuration des effluents des huileries d'olives: l'utilité de la microbiologie industrielle. Olivae, 46, 20–24.

Kissi, M., Mountadar, M., Assobhei, O., Gargiulo, E., Palmieri, G., Giardina, P., & Sannia, G. (2001). Roles of two white-rot basidiomycete fungi in decolorisation and detoxification of olive mill waste water. Applied Microbiology Biotechnology, 57(1-2), 221–226.

Leger, L., Kadiri-Hassani, N., & Descomps B. (2000). Decreased superoxide anion production in cultured human promonocyte cells (THP-1) due to polyphenol mixtures from olive oil processing wastewaters. Journal of Agricultural and Food Chemistry, 5061-5067. doi: 10.1021/jf991349c. PMID: 11052778.

Michaud, P. (2012). Olive mill wastes: Biochemical characterizations and valorization strategies. Process Biochemistry, 48, 1532–1552.

El Yamani, M., Sakar, E.L, Boussakouran, A., Ghabbour, N., & Rharrabti, Y. (2020). Physicochemical and microbiological characterization of olive mill wastewater (OMW) from different regions of northern Morocco. Environmental Technology, 41(23), 3081–3093.

Mouncif, M., Tamoh, S., Faid, M., & Achkari-Begdouri, A. (1993). A study of chemical and microbiological characteristics of olive mill waste water in Morocco. Grasas y Aceites, 44, 335–338. DOI: https://doi.org/10.3989/gya.1993.v44.i6.1059.

Nefzaoui, A. (1991). Valorisation des sous-produits de l'olivier. Options Méditerranéennes. Série A. Séminaires Méditerranéens, 16, 101–108.

Pozo, C., Martinez-Toledo, V., Rodelas, B., & González-López, J . (2002). Effects of culture conditions on the production of polyhydroxyalkanoates by *Azotobacter chroococcum* H23 in media containing a high concentration of alpechin (wastewater from olive oil mills) as primary carbon source. Journal of Biotechnology, 97, 125–131.

Ranalli, A. (1991). The effluent from olive mills: Proposals for re-use and purification with reference to Italian legislation. Olivae, 37, 30–39.

Rodier, J. (1996). L'analyse de l'eau : eaux naturelles, eaux résiduaires, eaux de mer. Huitième édition DUNOD Paris. 1384 pp.

Rodier, J. (2009). L'analyse de l'eau, 9e édition. DUNOD (éditeur), Paris, France, 1579 pp.

Rožman, T., & Jeršek, B. (2009). Antimicrobial activity of rosemary extracts (Rosmarinusofficinalis L) against different species of Listeria. Acta agriculturae Slovenica, 93(1), 51–58.

Sayadi, S., Allouche, N., Jaoua, M., & Aloui, F. (2000). Detrimental effects of high molecularmass polyphenols on olive mill wastewater biotreatment. Process Biochemistry, 35(7), 725-735.

Sbai, G., & Loukili, M. (2015). Traitement des margines par un procède couplant la coagulation floculation et la voie électrochimique. European Scientific Journal, 9, 1857–7881.

Tsagaraki, E., Lazarides, H.N, & Petrotos, B.K. (2007). Olive mill waste water treatment. Sprigerlink, 133–157.

Tsioulpas, A., Dimou, D., Iconomou, D., & Aggelis G. (2002). Phenolic removal in olive mill wastewater by strains of Pleurotus spp. In respect to their phenol Oxidase (laccase) activity. Bioresource Technology, 84, 251–257.

Vazequez, A. (1978). Les polyphénols de l'huile d'olive et leur influence sur les caractéristiques de l'huile, Revue française des corps gras, 25(1), 21–26.

Zahari, A., Tazi, A., & Azzi, M. (2014). Optimization of treatment conditions of Olive Oil Mill Wastewater by superoxidant K3FexMnyO8. Journal of Materials and Environmental Science, 5 (2), 484–489.

Zaier, H., Chmingui, W., Rajhi, H., & Bouzidi, D. (2017). Physico-chemical and microbiological characterization of olive mill wastewater (OMW) of different regions of Tunisia (North, Sahel, South). Journal of new sciences, Agriculture and Biotechnology, 48(2), 2888-2897.