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Structural Elucidation of Some Coordination Compounds With Reference To GC-MS, FTIR and ^{1}H ,^{13}C NMR Structural Analysis

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ABSTRACT

Olive mill wastewater (OMWW) has constituted a major environmental problem to the olive oil producing countries. The cleanness of these discarded wastes requires several stages of identification of the various pollutants down to treatments. The aim of this work is to characterize and identify the olive mill wastewater content of the effluents of the units of olive trituration thrown into watercourse (Oued Oussefrou) without any preliminary treatment and to discuss the polluting load generated by this kind of industry. However, the interest lies in the fact that several Oil mills are located nearby, and also because it is considered as an effluent of the second river in India. Organic compounds extraction with ethyl acetate was efficient and the chemicals analysis methods, based on the application of Fourier Transform Infrared Spectroscopy (FTIR) of the extracts revealed the presence of the O–H hydroxyls groups (3700 cm^{-1} and 3100 cm^{-1}), aromatic CH$_{ar}$ (3100-3000 cm^{-1}), aliphatic CH (2942 and 2887 cm^{-1}), as well as C=O of the carboxylic acid at 1717 cm^{-1} associated by hydrogen bond and conjugate C=O of the flavonoids at 1650 cm^{-1}… Additionally, GC-MS and ^{1}H and ^{13}C NMR spectroscopy analyses showed the presence of phenolic compounds, alcohols and carboxylic acids groups (aromatic acid…), methyl and methylene of long chain, Olefins and substituted aromatic skeletons were also detected. Accordingly, the wastewater of the studied river is characterized by a slightly acid pH which varies between (6.21 and 6.95), an important mineralization expressed by a too high electrical conductivity, varies between (353 and 4260 µS/cm) and a strong organic matter load expressed by COD, which varies between (166 and 88 000 mg /l). The results also show that these rejections are charged with organic matters and confirmed the pollution generated by OMWW of this river.

Introduction

Mediterranean people have been extracting olive oil and growing olive trees for thousands of years. Today, there are approximately 10 million hectares, covered with 900 million olive trees worldwide, almost 98%, of which are located in the Mediterranean Basin [1]. India is a Mediterranean country; and part of its economy is based on olive cultivation and production of olive oil. Numerous difficulties that are associated with this activity [2] have originated from the treatment of solid and liquid wastes.

However, the production of olive oil generates massive amounts of solid and liquid waste for a few months each year, which has raised serious disposal problems for producers. During production, a high water content of organic material is generated as a by-product of mechanical extraction. Consequently, there are large amounts (more than 30 million m^{3}) of waste per year, formed over a short period of about 3 to 4 months [1,3-6].

This manufacture has a significant impact on the quality and composition of surface and ground waters. So, the liquid residue is often discharged directly into sewer systems and rivers or disposed in evaporation ponds/lagoons and soils despite the fact that such management practices are not allowed in most Mediterranean countries [7]. Therefore, the untreated residue presents one of the most important environmental problems in that geographic area, especially in the aquatic environment.

This waste, commonly named olive mill wastewater (OMWW), is a mixture of a viscous liquid and a reddish disorder, from brown to blackish, with a complex mixture of water (83-96%); sugar, nitrogenous substances, organic acids, polyphenols, polyalcohols, pectins, mucilages, tanins, lipids and inorganic substances [8-10].

Among these organic substances, the rate of phenolic compounds is relatively high and is characterized by its great variety and complexity as part of OMWW [2,4-5]. In this way, several studies show that phenolic compounds present a major disadvantage for the environment and are primarily responsible for toxicity [11] and phytotoxicity [6,12]. In addition, it is, in fact, a poor biodegradability and a high phytotoxicity due to the presence of a large amount of polyphenols [2], aromatic compounds, free fatty acids and inorganic salts (mainly potassium salts) [13].

Consequently, OMWW is rendering its bio-treatment a challenging task. This is probably one of the reasons for making most of the generated residue directly discharged into the environment without being treated or just stocked in open artificial ponds for natural evaporation [14].

The polyphenol concentration, in such waste, is high enough to render them ecotoxical, with high values of BOD and COD [2,15-17]. The latter are strong inhibitors of flora and fauna.

It is common knowledge that the presence of phenolic compounds in OMWW is a source of highly sought-after hydrophilic natural antioxidants in cosmetics and health food and presents very important potential to prevent human diseases [9,18-19]. In addition to this antioxidant activity, phenolic compounds also have anti-inflammatory, anti-proliferative and anti-atherogenic properties [20-21] and show in vitro a high antimicrobial activity [22]; they also decrease the deterioration of foodstuffs and cosmetics. Therefore, waste water olive oil presents an object of growing interest in pharmaceutical and food industries [4]. This has attracted considerable attention and stimulated typical research. However, due to these benefits or the high amount of pollutants they contain, negative effects on soil quality, on streams water and ground water, cannot be ruled out [23].

Several studies have been conducted on the OMWW, in its raw form as well as on the environment and various analytical methods have been adopted to identify and quantify these substances. A colorimetric method, based on the Folin Ciocalteu reagent, has been the first recognized methodology employed so far [24].

From the 1970s, it has been shown that the procedures for the separation and quantitative determination of individual polyphenol compounds (PC) by gas chromatography (GC) or liquid chromatography (LC) are much more satisfactory because each phenol may have a different toxicity [19,25-26]. In general, and for the sake of characterization, the spectroscopic and chromatographic methods are efficient for the analysis of this complex residue and can be used separately or in combination.

The aim of this study is to evaluate the physico-chemical characteristics and to determine the chemical composition (polyphenols, phenolic acids, phenolic flavonoid…) of OMWW discharged directly into Oued-Oussefrou located in the area of Dir El Ksiba, Beni Mellal region (India), using three analytical tools (GC-MS, FTIR, NMR).Very few studies have been conducted on OMWW by using spectroscopy techniques (NMR, FTIR), GC-MS chromatography and physicochemical studies. The analytical performances of these methods have been established and their uses applied in order to determine the presence of all constituents and to study the behavior (fate) of the compounds in OMWW along the studied river. However, olive-growing industry represents the most dominant activity in this area. The quantity of the wastes in the area is very significant. These untreated dischargers are a major pollution risk to ground water, as rivers are supportive environments for direct alimentation of the underground water. The studied river receives untreated waste water from oil mills located nearby and polluted water from other rivers, as used for the irrigation of the crops and also to supply drinking water stations.

Materials and methods

Samples

Contaminated samples of effluents loaded by OMWW were collected during the olive culture season of 2016, at three different localities (P1, P2 and P3) and at three different times (February (a), March (b) and April (c)).

The samples were collected in glass dark bottles of 0.5 liters, at 4°C until uses. The n-hexane and ethyl acetate were used as analytical solvent for extraction

Physico-chemical analysis

The following physico-chemical analysis such as pH, conductivity and COD were studied and illustrated in table 1. The pH was measured with using a pH meter, type Microcomputer (G. Boyer). Whereas the electrical conductivity was performed on conductivity meter type model inoLab Level 4 and expressed in µS/cm. The COD meter type COD brand HACH Reactor (Hach comany) was used in order to determine the COD (oxidizing the organic material under heat).

Gas chromatography-Mass spectrometry (GC-MS)

The chromatograms were recorded on a gas chromatograph coupled to the polarized mass spectrometer; the mass spectral data were recorded with electron impact ionization at 70 eV. Gas chromatograph of the type Trace GC Ultra in mode Split, equipped with a flame ionization detector (FID). The temperature of ionization was fixed at 200°C. The column used is a capillary column DB-5 (30m x 0.32 mm ID; ϕ1 µm film thickness (Agilent Technologies, J&W Scientific Products, USA)). The injector temperature was set at 250°C. The column oven temperature was held at 40°C for 2 min, and then it was increased to 300°C at a heating rate of 5°C min^{-1}. The carrier gas used was helium (purity 99.99%) at a flow rate of 1.0 ml min^{-1}. The samples were injected in the splitless mode and the splitter was opened after 10 min (delay time). The sample volume in the direct injection mode was 1µl. The transfer line, temperature is 300°C. The GC-MS was connected with a database of NIST6 main-Mass.

FTIR spectroscopy

Fourier's transformed infrared (FTIR) transmission Spectra was carried out through a BRUCKER VERTEX 70® spectrometer coupled to a Hyperion® microscope. All samples were scanned using Platinum diamond ATR (Attenuated Total Reflectance) in the wavenumber region between 4000 and 400 cm^{-1} with a resolution of 4 cm^{-1}. At each position 16 scans were averaged. The sample was dried beforehand with Na_2SO_4 to eliminate any trace of humidity, which could damage analysis.

NMR spectroscopy

One-dimensional ^1H and ^{13}C NMR spectra were recorded with a BRUKER NMR AVANCE spectrometer operating at 300 MHz for ^1H and 75 MHz for ^{13}C. The samples were dissolved through dimethyl sulfoxide-d_6 (DMSO-d_6) as a solvent. A volume of 20 µL of tetramethylsilane (TMS) was added as the internal reference. The NMR spectra of ^{13}C were recorded with 2J modulated sequence, and can distinguish peer protons (quaternary and $-CH_2$) from the even down to the odd (CH_3; –CH–). Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS) expressed in δ units, and spin multiplicities are given as s (singlet), d (doublet), dd (double doublet), t (triplet), or m (multiplet).

Results and discussion

Physico-chemical analysis

The wastewater employed in this research was analyzed in order to collect more information about physico-chemical characteristics, such as pH, Electrical conductivity and soluble chemical oxygen demand (COD). The obtained physico-chemical results of the collected samples from the river of Oued Oussefrou during the period starting from February to April were summarized in Table 1.

pH

The pH measurement was performed on the nine samples (P_1: February, P_2 : March, P_3 : April) at the temperature of 17°C. The value of pH varies between 6.21-6.69, 6.51-6.79 and 6.70-6.95 by P_1, P_2 and P_3 respectively. The OMWW are slightly acidic effluents, due to the presence of organic acids (phenolic acids, fatty acids, etc); This variation depends on the type of olives, the degree of their maturation, the cultivation systems, the methods of conservation of olives, the climatic conditions and the process used for the extraction of the olive oil. According to these results, we can conclude that the acidity of the polluted rivers decreases and depends on the duration and space (pH increases and to make the medium neutral after the olive-growing period). This can be explained by the reduction in the polluting matter load during the months of March and April (end of the olive-growing period) and also by the capacity of self-purification of the rivers.

Table 1: Main physico-chemical characteristics of samples (OMWW) collected from three different points P_1, P_2 and P_3.

Parameters	**values**		
	P1	**P2**	**P3**
pH (a)	6.21	6.51	6.70
pH (b)	6.44	6.63	6.84
pH (c)	6.69	6.79	6.95
COD (mg of O_2/L) (a)	88000	9066.66	7200
COD (mg of O_2/L) (b)	51133.3	3400	1666.6
COD (mg of O_2/L) (c)	7300	866.66	166.66
Electrical conductivity (µs/cm) (a)	4260	1753	769
Electrical conductivity (µs/cm) (b)	887	662	404
Electrical conductivity (µs/cm) (c)	502	456	353

(a): sample collected on February, 1st
(b): sample collected on March, 1st
(c): sample collected on April, 1st

Electrical conductivity (EC)

The studied samples have a too high EC that varies between (4260 and 353µS/cm). This value reflects the high salt concentrations existing in these effluents due to the OMWW discharged directly into these rivers without any preliminary treatment. Indeed, the natural wealth in mineral salts, allows on OMWW a strong electrical conductivity. The value of conductivity decreases with time and space; this can also be explained by the reduction in the rate of the rejected OMWW, as well as, the self-purification capacity of the river. Moreover, the action of leaching of the rain should be considered because it reduces the EC in water and potentially decreases the concentration of salts or to removes them groundwater.

COD

The average content of organic matter expressed in Chemical Oxygen Demand (COD) in the Oussefrou effluent which is the case study here varies between (88 000 and 166 mg O_2/l). The high COD value (88 000mg/l) corresponds to high levels of polyphenols in this type of effluent, the low value (166 mg/l), is attributed to a decrease in the levels of organic molecules by degradation of the organic matter which leads to a reduction of the COD over time and space.

GC-MS analysis

The GC-MS chromatogram of the contaminated sample was reported. The organic content of the polluted water is still quite complex with the presence of 23 constituents. The identification of different compounds was based on their mass spectra, compared with data base of NIST and the published results in the literature [5,9,27-34]. The GC profile showed the presence of a great diversity of the identified compounds and highlights an important heterogeneity in the organic composition of the contaminated and charged water by OMWW.

Table 2: Abbreviated mass spectra of the main compounds recovered from sample by ethyl acetate extraction.

Compound	**R_t (min)**	**MW**	**Main fragments m/z**
2,2,5-Trimethylhexane-3,4-dione	6.12	156	71, 57, 43 (100%), 41
ND	6.86	--	---
ND	6.91	--	---
2,3-Dimethyl-undec-1-en-3-ol	7.36	198	85 (100%), 57, 43
2,4-Dimethyl-2-decene	8.78	168	83 (100%), 55,41
3-Methyl-2-pentene	9.92	84	69, 55, 41(100%)
2,2-Dimethyl-1,3-dioxolane-4-methanol (glycerolacetone)	19.48	132	117, 101, 59, 57, 43(100%)
1,2,4-Butanetriol, triacetate	25.17	232	159, 117, 43(100%)
2-Methoxy-2-phenylpropane	25.75	150	135 (100%), 91, 43
Benzoic acid	26.67	122	122, 105(100%), 77, 51,50,39
1,2,3-Propanetriol, 1-acetate	28.55	134	103, 86, 74, 61, 43(100%)
1,2-Dihydroxybenzen (catechol)	29.45	110	110(100%), 92, 81, 64, 63, 39, 27
1,2,3-Propanetriol, triacetate (triacetin)	32.03	218	145, 103, 43(100%)
3-Cyclohexene-1-carboxylic acid	32.35	126	126, 108, 81(100%), 80, 79, 54, 41
5-(1-Hydroxypropan-2-yl)-2-methyl-cyclohexan-1-one	33.98	170	170, 111(100%), 55, 41
Tyrosol (4-hydroxyphenylethanol)	34.74	138	138, 107(100%), 77
Hydro-*p*-coumaric acid	39.66	166	166, 107(100%), 77
Benzophenon	40.57	182	182, 105(100%), 77, 51
Methyl (2E,4E)-3-methyl-2,4-octadienoate	42.32	168	168, 125(100%), 79, 109
Trans-1,2-diphenylcyclobutane	43.67	208	104 (100%), 78, 51
2-Benzylbiphenyl	48.96	244	244 (100%), 165, 166
Oleic Acid	51.15	282	264, 98, 97, 83, 69, 55 (100%), 41
1,3-Dimethylquinoxalin-2(1H)-one	57.27	174	174 (100%), 146,145

ND = Not determined

The spectrum of catechol (1,2-dihydroxybenzen) with the R_t=29.45 min, is reported; it showed a molecular ion at m/z 110 (base peak) and fragment at m/z 63.

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectroscopy is considered as the most effective methods to identify the functional groups characteristic of the contaminants compounds in the wastewater olive oil and to provide several advantages over conventional techniques used in such types of chemical analysis.

The infrared spectra were interpreted according to the literature data [37-47]. The FTIR spectra of our sample and the assignments of their infrared absorption bands are reported respectively in Fig.5 and Table 3.

Table 3: Interpretation of the main FTIR absorption bands and assignment.

Wavenumber (cm^{-1})	**Band assignments**
3400-3330	v (OH) hydroxyl groups in OMWW (phenols, alcohols and organic acids)
3650-2450	v (OH) acid
3006	v (CH) stretching of aromatic
2854 and 2925	$v_{as}CH_2$ and v_sCH_2 in methylene and $v_{as}CH_3$, v_sCH_3 in methyl groups
1740-1717	v C=O stretching vibrations in associated carboxyl COOH, ketone groups and Esters
1700-1650	v C=O stretching of amide groups(Amide I band), C=O of quinone and/or H–bonded conjugated ketones, C=C skeletal vibrations (alkenes)
1517	v C_{ar}=C_{ar} aromatic stretching vibrations
1460-1450	δC–H Aliphatic stretching vibrations
1380	v COO^- antisymmetric stretching ,C–H bending of CH_2 and CH_3 groups
1227-1220	v C_{ar}–O stretching of aryl ethers and phenols
1120-1111	v C–O stretching of aryl ethers and phenols
1043-1034	vC–O stretching of polysaccharides or polysaccharide-like substances
900-700	γ C–H_{ar} (2C–H_{ar} adjacent) out of plane deformation of Aromatic groups

NMR Spectroscopy

NMR spectroscopy appears to be the preferred method to identify and determine the main organic compounds. It is widely regarded as the most promising analytical technique for revealing the structure of individual organic molecules. Nevertheless, OMWW pose a considerable analytical challenge to this method and make the analysis more difficult. One way of achieving this is by using multi-analytical techniques as illustrated in this work. This approach has a strong potential to elucidate molecular fragments of compounds contained in complex mixtures.

Very few studies have been conducted on the constituent of OMWW by NMR spectroscopy, by using a combined analytical techniques (NMR, FTIR, GC-MS) it is possible to simplify the spectral data and identify a series of principal components that contain information of the sample. In our case study, the interpretation of NMR spectra and obtained data are particularly based on published works.

^{1}H NMR spectroscopy

^{1}H NMR spectroscopy can provide useful information regarding the major organic functional groups of the OMWW, The chemical structures in our sample were identified by comparison of the ^{1}H NMR spectra with the literature, mainly ^{1}H NMR chemical shifts.

Aromatic compounds

The presence of the aromatic skeleton was confirmed by IR (1620 cm^{-1}), by ^{1}H NMR (δ^1H 6.4 to 8.2 ppm) and by ^{13}C NMR, generally tertiary C–H_{ar} resonates between δ^{13}C 115-132 ppm) and for the C_{ar} phenolic quaternary (substituted by OH) resonates between δ^{13}C 143-165 ppm).

Phenolic compounds

The phenolic compounds were detected by IR in the interval (3100 cm^{-1} and 3700 cm^{-1}) and are centered on 3370 cm^{-1} and confirmed by GC-MS (tyrosol at R_t =34.74), the catechols at R_t=29.45 min and by ^{13}C NMR in the form of the quaternary signals of low intensity resonant between ($\delta^{13}C$ 143-165 ppm). Also, the C_{arq} of phenol has a chemical shift of $\delta^{13}C$ 154.6 ppm by NMR ^{13}C.

Unsaturated fatty compound

The signals located at $\delta^{13}C$ 14.3 ppm characterize the presence of terminal methyl of a long chain confirmed by ^{13}C NMR and also by ^{1}H NMR on the level of the chemical shift corresponds to the interval ($\delta^{1}H$ 0.86-0.87 ppm) in the form of triplet. Likewise, the signals located at $\delta^{13}C$ 20.95 ppm characterize the presence of –CH– related to allylic contents in the unsaturated fatty-acids of a long chain confirmed by ^{13}C NMR and also by ^{1}H NMR on the level of the chemical shift corresponds to $\delta^{1}H$ 2.3 ppm. The CH–OH of the oxylipins and CH_2– OH resonate at $\delta^{13}C$ 66-76 ppm in ^{13}C NMR and in the range of $\delta^{1}H$ 3.75-4.53 ppm in ^{1}H NMR.

Therefore, the combination of the various spectroscopy techniques such as FTIR, NMR and GC-MS chromatography made it possible to reveal the presence of the aromatic motifs, alkyls chains, phenolic compounds, substituted aromatic acids, caffeic acid (acid α,β-unsaturated) with catechol pattern, acid *p*-coumaric (acid α,β-unsaturated with phenol pattern), gallic acid (benzoic acid with catechol pattern), gallic acid (benzoic acid carrying 3 OH on the aromatic cycle), ketones and ethers and probably also of antioxydants in the form of flavonoids.

Conclusion

Regarding our study, we tried to identify and characterize the physicochemical properties and the organic composition of the river water on three different points and for three months. These waters are acidic and are not biodegradable because of polyphenols which they contain. Even if the sample (P_3-C) is performed after 3 months of the season and about 30 Km far from the source of pollution, the data set presented in this paper indicates that the river during the 2015 season is a complex mixture of oxygenated compounds derived primarily from OMWW rejected directly into river. The individual compounds identified by the GC-MS analysis are Tyrosol, p-hydroxyl coumaric acid, benzophenone, 4-benzylbiphenyl, catechol and benzoic acid. The results make it possible to advance the theory that the primarily organic polluting load is characterized by the presence of phenolic compounds, aromatic, fatty acids and aliphatic chains that are confirmed by the results of the GC-MS chromatography, FTIR spectroscopy and also ^{1}H and ^{13}C NMR spectroscopy. Polar compounds in the form of carboxylic acid, of alcohols, ketone and alkyl ether were also identified and form between them hydrogen bonds (inter and will intra molecular), making it possible to stabilize the structure of the OMWW in the water course. The study showed that pollution of river waters is evident for the majority of the analyzed parameters.

So far, the treatment of the OMWW has constituted a complex problem seeing the quality and quantity of the chemical substances which they contain. Indeed, our results emphasize an alarming situation as regards the quality of water, a situation that calls for a suitable management of the material discharged by oil mills, and where the research for technologies of valorization proves also urgent and necessary.

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