Analysis of Metal Cations and Inorganic Anions in Olive Oil Mill Waste Waters by Atomic Absorption Spectroscopy and Ion Chromatography. Detection of Metals Bound Mainly to the Organic Polymeric Fraction

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Metal cations were quantitatively detected by atomic absorption spectrometry in samples of olive oil mill waste waters obtained by a pressure process (omww$_1$) (K, 17.1; Mg, 2.72; Ca, 2.24; Na, 0.40; Fe, 0.123; Zn, 0.0630; Mn, 0.0147; Cu, 0.00860 g L^{-1}) and a centrifugation process (omww$_2$) (K, 9.80; Mg, 1.65; Ca, 1.35; Na, 0.162; Fe, 0.0330; Zn, 0.0301; Mn, 0.00910; Cu, 0.00980 g L^{-1}). The inorganic anions, determined in the same samples by ion chromatography, proved to be Cl$^-$, H$_2$PO$_4^-$, F$^-$, SO$_4^{2-}$, and NO$_3^-$ (1.61, 1.05, 0.66, 0.52, and 0.023 g L^{-1}, respectively, in omww$_1$ and 0.61, 0.40, 0.25, 0.20, and 0.0090 g L^{-1}, respectively, in omww$_2$). Most of the metal cations were revealed to be bound to the omww organic polymeric fraction (opf), composed of polysaccharides, phenol polymers, and proteins. Opf relative molecular weight was substantially estimated in the range between 1000 and 30000 Da for ~75% and in the range from 30000 to 100000 Da for ~25%. The free residual cations pool proved to be neutralized by the inorganic counteranions. Finally, the possible exploitation of this material in agriculture and in environmental biotechnology processes is also discussed in the light of its chemical and biochemical oxygen demand parameters.

INTRODUCTION

Olive oil mill waste waters (omww) are an agricultural waste material produced in high quantities mainly in the Mediterranean basin and also in many other countries throughout the world where olive oil is produced (Demicheli and Bontoux, 1997).

Omww possesses highly polluting properties for which many legal restrictions against its discharge into sewage plants exist; however, it can be spread on soil, according to the laws of the various producer countries.

Its highly polluting capacity is documented by high chemical oxygen demand (COD) and biochemical oxygen demand (BOD) values (Pacifico, 1989) and is mainly based on its polyphenol content and synergic effect with other inorganic and organic compounds (Capasso et al., 1992, 1995).

Omww is rich in inorganic and organic compounds (Pacifico, 1989) and may also be regarded as an inexpensive source of products to be recovered because of its potential economic interest and/or ability to be transformed into products for use in agriculture and industry.

In this connection, several processes for its utilization have been proposed (Sanjut et al., 1991; Garcia-Barriounevo et al., 1992; Sayadi and Ellouz, 1992), and the main polyhenols have been isolated from omww and chemically and biologically characterized, together with some of their synthetic derivatives (Capasso, 1997;

Capasso et al., 1992, 1995). Among these polyphenols, hydroxytyrosol (Capasso et al., 1999) has been revealed to be the most interesting because of its remarkable pharmacological and antioxidant properties (Chimatsu et al., 1996).

We are currently investigating omww polymeric and inorganic fractions with the aim to chemically characterize and possibly recycle them totally in agriculture.

This study analyzes the content, composition, and physicochemical status of metal cations and inorganic anions in raw omww processed by pressure (omww$_1$) as well as centrifuge (omww$_2$).

Determinations were also performed on the nonpermeated and permeated pools of omww obtained by precipitation of the organic polymeric fraction (opf) by cold methanol and subsequent dialysis through a 3500 Da cutoff membrane. The cations were quantitatively determined by atomic absorption spectroscopy (AAS), whereas the anions were detected by ion chromatography (IC).

The chemical nature of the opf binding the metal cations was analyzed and its sizes in terms of relative molecular weight (rmw) were also investigated by combining the UV−vis spectroscopic analysis with ultrafiltration experiments.

Finally, the COD/BOD values of the obtained fractions were also determined to verify whether the proposed treatment, that is, cold methanol precipitation, reduced the organic load of the initial raw material and its related environmental risks and to evaluate the

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potential for use of these fractions as a soil amendment and in environmental biotechnology processes.

EXPERIMENTAL PROCEDURES

Materials. Two samples of omww were respectively supplied by a pressure (omww$_1$) and centrifuge (omww$_2$) processing plant located in Monteroduni (Isernia, Italy). Samples were kept refrigerated at -20 °C in tightly closed PVC vessels. Solvents were of HPLC grade and reactive of analytical grade. High-quality water (hqw), obtained using a MilliQ system (Millipore), was used throughout.

Safety and General Experimental Conditions for the Handling of Omww Samples. The handling of the omww samples was only carried out using disposable gloves. The dialysis procedure was performed in the freezeroom at 10 °C mainly in the dark.

AAS. Metal cations were determined by a Perkin-Elmer model 3030 B atomic absorption spectrometer equipped with deuterium-arc background correction. Either air/acetylene or nitrous oxide/acetylene flames were used as an atomization source. Measurements were performed using hollow cathode lamps (Perkin-Elmer) operated at 765.5 nm, with a current of 12 mA and a bandwith of 0.7 nm for potassium; 285.2 nm, 25 mA, and 0.7 nm, respectively, for magnesium; 422.7 nm, 25 mA, and 0.7 nmm respectively, for calcium; 589 nm, 12 mA, and 0.2 nm, respectively, for sodium; 248.3 nm, 25 mA, and 0.2 nm, respectively, for iron; 213.9 nm, 20 mA, and 0.7 nm, respectively, for zinc; 279.5 nm, 30 mA, and 0.2 nm, respectively, for manganese; and 324.8 nm, 10 mA, and 0.7 nm, respectively, for copper. The spectrometer was controlled by AA Winlabtm software (Perkin-Elmer, Norwalk, CT). Background correction (AA-BG) was used for lower wavelength elements (Zn, Mn, Fe, and Cu), which could be more susceptible to interferences from molecular absorbance. The average and standard deviation of three absorption measurements were recorded for each sample.

All glassware, plasticware, and storage bottles used were previously immersed for several hours in a solution containing 10% v/v concentrated hydrochloric acid and 20% w/v concentrated nitric acid (Carlo Erba, Milan, Italy) to avoid any kind of contamination and air-dried before use. Stock standard solution of cations (1 g L^{-1}) was obtained from BDH Reagents (Poole, U.K.) and diluted in water containing the same amount of acids as the samples to obtain working standards.

IC. Cl$^-$, PO$_4^{2-}$, F$^-$, SO$_4^{2-}$, and NO$_3^-$ were determined by a Dionex model 2000i/SP ion chromatograph, equipped with an Ion Pac AS4A column (4 $\times$ 250 mm) and an Ion Pac AG4A (4 $\times$ 50 mm) guard column and detected by suppressed conductivity. Eluent was 1.8 mM Na$_2$CO$_3$/1.7 mM NaHCO$_3$ at a flow rate of 2 mL min^{-1} for a pressure of 69 bar.

A multianion stock solution from Dionex (Sunnyvale, CA) containing 1 g L^{-1} of each anion was used as reference standard.

UV Spectroscopy. A Perkin-Elmer Lambda 3B UV–vis spectrophotometer was used to analyze the total polyphenol, protein, and carbohydrate contents in omww.

Metal Determination by AAS. A fraction of 250 mL of omww was concentrated to 100 mL at 50 °C under reduced pressure and then lyophilized. Samples were digested with 42 mL of nitric acid (65% w/v) and 8 mL of perchloric acid (72% w/v) on a heating sand bath at 80 °C. Once dried, a 5% HCl solution was added to them, and the samples were filtered through No. 40 Whatman paper filter, $\varnothing$ 12.5 cm, and raised to a final volume of 50 mL with 5% HCl solution.

To evaluate whether the cations were completely free in solution or bound to organic polymers, another set of concentrated omww samples of 100 mL were treated with 350 mL of cold methanol, agitated on an orbital shaker for 20 min, and centrifuged at 8000 rpm in 500 mL Nalgene tubes in a Sorvall RC 5 C centrifuge. The methanol-treated sample appeared to be divided into two main fractions: a solid aliquot precipitating at the bottom of the centrifuge vessel (P-omww) and a liquid fraction represented by the overlying supernatant (S-omww).

P-omww$_1$ (the precipitated fraction regarding omww$_1$) was oven-dried at 50 °C and then redissolved in 100 mL of hqw, and S-omww$_1$ (the supernatant fraction regarding omww$_1$) was concentrated to 100 mL under vacuum. Both aliquots were dialyzed using 3500 Da molecular weight cutoff membranes (Spectrum Medical Industry, Houston, TX) against four sequential volumes of 500 mL of hqw per time of 2 h intervals. Dialysis was stopped when no further phenol UV absorption was recorded in the permeated fraction at 270 nm. The permeated and nonpermeated dialyzed samples were made up to a final volume of 100 mL under vacuum and then digested.

Inorganic Anions by IC and Metal Cations by AAS Determination. To detect the anions by IC and avoid the contemporary coelution of phenols, 25 mL of omww$_1$ and 25 mL omww$_2$ were treated with ethyl acetate (6 $\times$ 25 mL) to remove phenols. The combined exhausts were evaporated under reduced pressure, redissolved in 25 mL of hqw, and analyzed by TLC silica gel plates (20 $\times$ 20 cm, Merck Kieselgel 60 f$_{254}$, 0.25 mm), using as eluent petroleum ether/acetone (1: 1). After elution, the plates were observed by exposure to UV light selected at 254 nm and then sprayed with 10% sulfuric acid in methanol and next with 3% phosphomolybdic acid in EtOH before being heated for 5 min at 105 °C. Spots were compared with hydroxytyrosol and catechol standards. After phenol extractions, the exhausted omww$_1$ and omww$_2$ of 25 mL were applied on an RP-18 column (5 $\times$ 1 cm, Merck, Darmstadt, Germany), which were eluted with H$_2$O at low pressure (3 bar). The resulting colorless solutions were divided into two aliquots, which were analyzed by IC and AAS according to the procedure described above.

pH and Electrical Conductivity Determination. pH was determined using an Orion expandable ion analyzer EA 940, and electrical conductivity was estimated by means of an HI 8733 Hanna instrument.

Organic Substance and Organic Carbon Determination. The organic carbon contents of omww raw samples R-omww, P-omww, and S-omww were determined by treating 1 g of lyophilized sample with 1 N potassium dichromate solution in the presence of concentrated sulfuric acid (U.S. Department of Agriculture, 1996).

BOD Determination. BOD$_5$ of R-omww, P-omww, and S-omww was determined on 50 mL of sample added of 50 mL of hqw in 1 L bottles with glass stoppers and incubated at 20 $\pm$ 1° according to the methods reported by the AOAC (1979) using domestic sewage as seeding material.

COD Determination. COD was determined by potassium dichromate in sulfuric acid (1 + 1) at reflux temperature with silver sulfate as catalyst and mercuric sulfate to remove Cl$^-$ interference. Excess dichromate was titrated with Fe^{2+} using phenanthroline as indicator (AOAC, 1979).

Protein Content Determination. Concentration was estimated according to the Bradford (1976) method using bovine serum albumin as standard.

Carbohydrate Content Determination. Carbohydrates were analyzed by means of an anthrone reagent (Fung et al., 1953) using D-mannose and D-galactose as standards.

Polyphenol Content Determination. Total phenol/polyphenols were determined according to the Folin–Ciocalteu method (Jennings, 1981) using gallic acid as reference standard and reading the absorbance at 760 nm.

Relative Molecular Weight Determination of the Opf. To estimate the relative molecular weight (rmw) of the opf of omww, a 200 mL stirred cell for ultrafiltration (Amicon), equipped with a disk membrane of 1000 Da cutoff (AmiconYM1, $\varnothing$ 6.2 cm), was filled with omww$_1$ of 150 mL previously centrifuged at 8000 rpm for 30 min at 4 °C. The cell was placed under nitrogen flow and connected to an hqw reservoir. The operating pressure inside the cell was regulated at 3.5 bar.

Just before the start of the ultrafiltration experiment (at 0 time), a sample of dark omww$_1$ showed the following UV–vis absorption peaks: $A_{270} = 27.6$ and $A_{400} = 6.55$. The ultrafiltration of omww$_1$ terminated when the permeated solution did not reveal any further UV–vis absorption in the range 200–470 nm and any residual substance, as monitored by drying 10 mL of the control fraction. The remaining 50 mL of

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Table 1. Concentrations of Cations (C_{c1} and C_{c2})[a] and Anions (C_{a1} and C_{a2})[a] in Olive Waste Water by Pressure (Omww₁) and Centrifuge (Omww₂), Respectively

	cations, g L^{-1}			anions, g L^{-1}	
cation	omww₁ (C_{c1})	omww₂ (C_{c2})	anion	omww₁ (C_{a1})	omww₂ (C_{a2})
K^+	17.10 ± 0.31	9.80 ± 0.12	Cl^-	1.63 ± 0.06	1.3 ± 0.02
Mg^{2+}	2.72 ± 0.13	1.65 ± 0.11	$H_2PO_4^-$	1.07 ± 0.06	0.85 ± 0.04
Ca^{2+}	2.24 ± 0.14	1.35 ± 0.10	F^-	0.57 ± 0.01	0.530 ± 0.01
Na^+	0.40 ± 0.17	0.162 ± 0.08	SO_4^-	0.53 ± 0.05	0.420 ± 0.02
Fe^{2+}	0.129 ± 0.05	0.033 ± 0.01	NO_3^-	0.023 ± 0.01	0.0109 ± 0.008
Zn^{2+}	0.0630 ± 0.001	0.0301 ± 0.001			
Mn^{2+}	0.0147 ± 0.001	0.0091 ± 0.001			
Cu^{2+}	0.0086 ± 0.001	0.0098 ± 0.001			

[a] Standard deviation values were calculated on the basis of three detererminations.

Table 2. Metal-Cation Concentrations Determined by AAS in Raw Omww₁ ($C_{c1} - C_{cm1}$, Respectively) and in an Aliquot of the Same Omww₁ Used for the Determination of the Anions ($C_{c1'}$ and $C_{cm1'}$)[a]

	cations					inorganic anions		
	total cations		cations determined in an aliquot of omww₁ used also for anion determination					
cation	conc (C_{c1}), g L^{-1}	concn (C_{cm1}), mequiv L^{-1}	concn ($C_{c1'}$), g L^{-1}	concn ($C_{cm1'}$), mequiv L^{-1}	anion	concn[a] (C_{a1}), g L^{-1}	concn[a] (C_{am1}), mequiv L^{-1}
K^+	17.10 ± 0.31	437.2	3.68 ± 0.15	94.11	Cl^-	1.63 ± 0.05	45.9
Mg^{2+}	2.72 ± 0.13	223.8	0.027 ± 0.01	2.1	$H_3PO_4^-$	1.07 ± 0.05	11.0
Ca^{2+}	$2.24 \pm 0,14$	111.8	$0.031 \pm 0,01$	1.5	F^-	0.57 ± 0.01	30.0
Na^+	0.40 ± 0.17	17.4	0.0030 ± 0.001	0.13	SO_4^{2-}	0.53 ± 0.05	10.8
Fe^{2+}	0.129 ± 0.05	4.6	0.0023 ± 0.001	0.082	NO_3^-	0.023 ± 0.01	0.37
Zn^{2+}	0.063 ± 0.01	1.9	0.0002 ± 0.0001	0.006			
Mn^{2+}	0.0147 ± 0.001	0.5	0.0002 ± 0.0002	0.007			
Cu^{2+}	0.0086 ± 0.001	0.2	0.00054 ± 0.0002	0.017			
		$\sum C_{cm1} = 797.4$		$\sum C_{cm1'} = 97.95$			$\sum C_{am1} = 98.07$

[a] Anion concentrations (C_{ag} and C_{am}) were determined by IC. Anion concentrations refer to that present in raw omww₁. The sum of the mequiv L^{-1} of these anions ($\sum C_{am1} = 98.07$ mequiv/L) is smaller than the sum of the mequiv of cations determined in raw omww₁ ($\sum C_{cm1} = 797.4$ mequiv/L). This means that only a small fraction of the cations ($\sum C_{cm1'} = 97.95$ mequiv/L) has the inorganic anions as counterions, whereas most of the cations have the charged groups of the polymeric organic fraction of the water as counterions. This was also shown by the difference between the sum of the mequiv L^{-1} of the total cations and the sum of the mequiv L^{-1} of the counterions in the solution of the anions: ($\sum C_{fp1} = 797.4 - 97.95 = 699.45$ mequiv/L). The solution of the anions was obtained by a purification procedure involving the elimination of the dark polymers of omww₁. In this solution the residual cations were determined by AAS. Standard deviation values were determined on the basis of three determinations ($n = 3$).

nonpermeated omww₁ dark solution (opf₁) was removed from the cell and diluted to 150 mL with hqw. The relative UV–vis absorption peaks appeared to be greatly reduced: $A_{270} = 19$ and $A_{400} = 5.55$. This dark solution was placed again into the cell equipped with a 30000 Da cutoff membrane (YM30, Amicon), undergoing the same ultrafiltration procedure as described above. The resulting nonpermeated brown solution (opf₃₀) showed a further reduced UV–vis absorption: $A_{270} = 4.5$ and $A_{400} = 1.4$. The same procedure was repeated using a 100000 Da cutoff disk membrane (YM 100, Amicon). The nonpermeated colorless solution (opf₁₀₀) thus obtained, after dilution to 150 mL, did not show any UV–vis absorption. A very small residue (0.1 mg) was detected upon evaporattion under reduced pressure, and it was positive at the carbohydrate test (Fung et al., 1992).

RESULTS AND DISCUSSION

Table 1 shows the concentration values of the cations (C_{c1} and C_{c2}) and inorganic anions (C_{a1} and C_{a2}) in omww₁ and omww₂, respectively. In the case of omww₁, K was the predominant metal (17.10 g L^{-1}), followed in decreasing order by Mg (2.72 g L^{-1}), Ca (2.24 g L^{-1}) Na (0.40 g L^{-1}), Fe (0.129 g L^{-1}), Zn (0.063 g L^{-1}), Mn (0.0147 g L^{-1}), and Cu (0.0086 g L^{-1}).

Lower concentration levels of cations were detected in omww₂ (third column of Table 1) due to the dilution of the water during the centrifugation processing of the olive oil.

With regard to the anions (Table 1), the prevailing anion proved to be Cl^- ($C_{a1} = 1.63$ g L^{-1} and $C_{a2} = 1.3$ g L^{-1}), followed by the biacid phosphate, $H_2PO_4^-$ (C_{a1} = 1.07 g L^{-1} and $C_{a2} = 0.85$ g L^{-1}), which was in this form as a consequence of the acid pH value of the waters (pH 5.1, see Table 6). In omww₁, the anions F^- and SO_4^- presented very similar concentrations (0.57 and 0.53 g L^{-1}), whereas in omww₂ the concentrations of the same anions were slightly different (0.53 and 0.42 g L^{-1}, respectively). With respect to the other anions, NO_3^- ions were present at very low concentrations in both kinds of waste waters ($C_{a1} = 0.023$ g L^{-1} and $C_{a2} = 0.0109$ g L^{-1}).

The sum of the milliequivalents of the cations in omww₁, $\sum C_{cm1} = 797.4$ mequiv (third column of Table 2) is ~10 times higher than the sum of the milliequivalents of the anions in the same omww₁ sample ($\sum C_{am1} = 98.07$ mequiv, eighth column of Table 2). These data suggest that only a small part of the overall cation pool had these anions as countering ions and that a large fraction of the cations could be bound to the organic fraction of the waste waters.

To confirm this hypothesis, the cation content was analyzed (a) in an aliquot of omww₁ in which the anions were quantitatively detected and (b) in the omww₁ fractions (P-omww₁ and S-omww₁) resulting from the processes of precipitation and dialysis through 3500 Da cutoff membranes.

With regard to the first point (a), the sum of the milliequivalents of the cations, $\sum C_{cm1} = 97.95$ mequiv, was close to the above-mentioned sum of the anions, $\sum C_{am1} = 98.07$ mequiv (fifth and eighth columns in Table 2, respectively).

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Table 3. Concentrations of Metals Bound to the Opf Expressed in Grams per Liter (C_{cfp1}), in Milliequivalents per Liter (C_{cfpm1}), and in Percent of Bound Ions over the Total (C_{cfp1}) in Omww$_1$, of Free Metals in Omww$_1$ Expressed in Grams per Liter (C_{cls1}, C_{clsm1} C_{clsp1}), and of Anions in Omww$_1$ Expressed in Milliequivalents per Liter (C_{am1})

	cations						anions	
	cations bound to ofp of omww$_1$[a]			free cations in solution (ls) of omww$_1$[b]			anions in omww$_1$	
	concn (C_{cfp1}), g L^{-1}	concn (C_{cfpm1}), mequiv L^{-1}	concn of ions bound to polymeric fraction (C_{cpfp1}), %	concn (C_{cls1}), g L^{-1}	concn (C_{clsm1}), mequiv L^{-1}	concn (C_{clsp1}), % free ions		concn (C_{am}), mequiv L^{-1}
K$^+$	13.2 ± 0.6	337.5	78.0	3.70 ± 0.16	94.87	22.0	Cl$^-$	45.9
Mg^{2+}	2.51 ± 0.16	206.4	98.8	0.029 ± 0.01	2.4	1.2	H$_2$PO$_4^-$	11.0
Ca^{2+}	2.11 ± 0.12	105.3	98.5	0.032 ± 0.01	1.5	1.5	F$^-$	30.0
Na$^+$	0.373 ± 0.03	16.2	99.1	0.0031 ± 0.001	0.13	0.9	SO$_4^-$	10.8
Fe^{2+}	0.105 ± 0.01	3.8	97.7	0.0024 ± 0.001	0.08	2.3	NO$_3^-$	0.37
Zn^{2+}	0.059 ± 0.01	1.8	99.6	0.0002 ± 0.0001	0.006	0.4		
Mn^{2+}	0.0113 ± 0.01	0.4	98.3	0.00019 ± 0.0001	0.007	1.7		
Cu^{2+}	0.0058 ± 0.001	0.2	91.2	0.00056 ± 0.0002	0.017	8.8		
		$\sum C_{cfpml} = 671.6$			$\sum C_{lsml} = 99.0$			$\sum C_{am1} = 98.07$

[a] The fraction of ions bound to polymeric fraction was determined by summing the cation concentration of the nonpermeated fraction of the precipitate and supernatant (dialysis performed at a cutoff = 3500 Da) of omww$_1$. This sum, $\sum C_{cfpm1} = 671.6$ mequiv L^{-1}, was very close to $\sum C_{fp1} = 699.45$ mequiv L^{-1}, deriving from the difference between the sum of mequivalents of the total ions and the sum of the mequivalents of the anions. This attested to the fact that these ions were mainly bound to the polymeric fraction of omww$_1$. [b] The concentration of free ions was determined in the permeated fraction of supernatant: $\sum C_{lsm1} = 99.0$ mequiv L^{-1}. This value was close to the sum $\sum C_{am1} = 98.07$ mequiv L^{-1} of the free anions in solution and to the sum $\sum C_{cpm1} = 97.95$ of the cations determined in an aliquot of the same ommww$_1$ sample used to determine the anions. These data confirm the status of the occurrence of residual free ions in solution of omww$_1$ and in all likelihood of omww$_2$.

With regard to the second point (b), the concentration values of the cations C_{cfp1} (second column of Table 3) were obtained by summing the values determined on the nonpermeated fraction of the dialyzed precipitated, P-omww$_1$, and that on the nonpermeated supernatant, S-omww$_1$. This sum calculated in terms of milliequivalents was $\sum C_{cfpm1} = 671.7$ mequiv (third column of Table 3) and was very close to $\sum C_{cfp1} = 699.45$ mequiv, which was obtained by the difference between the sum of the total cations determined in omww$_1$, $\sum C_{cm1} = 797.4$ mequiv (third column of Table 2) and the sum of the cations, $\sum C_{cm1} = 97.95$ mequiv (fifth column of Table 2), balanced by the inorganic anions ($\sum C_{am1} = 98.07$ mequiv, eighth column of Table 2).

The analysis of the permeated fraction of the precipitate P-omww$_1$ did not reveal the presence of any cation, whereas C_{cls1} was present in the permeated fraction of supernatant, S-omww$_1$ (fifth column of Table 3). The sum of the milliequivalents of the cations, $\sum C_{clsm1} = 99.00$ mequiv (sixth column of Table 3), was close to the sum of the milliequivalents of the cations, $\sum C_{cm1} = 97.95$ mequiv, determined in an aliquot of the same omww$_1$ solution in which the anions were determined, and close to that of the anions, $\sum C_{am1} = 98.07$ mequiv.

All of these data indicate that most of the metal cations are bound to the opf of the water, whereas only a small fraction is free, that is, in equilibrium with the bound cations and has the inorganic anions as counterions.

These data seem to be consistent with those shown by various authors (Darnall et al., 1986; Gardea-Torresdey et al., 1990; Drake et al., 1996; Tiemann et al., 1999), who revealed that nonliving biomass materials have a high potential in binding metals. Metal ion uptake by biomaterials is believed to occur through interactions with functional groups such as carboxyl, amino, sulfhydryl, phenolic, or hydroxyl moieties. These proved to be native to the proteins, lipids, and carbohydrates that make up the cell walls.

As shown in the fourth and seventh columns of Table 3, all of the metal cations, except for K, are >90% bound to opf. In the case of K, 78% of the cation was bound to opf and 22% was free in solution.

Table 4. Maximum Absorbance at $\lambda = 270$ and 400 nm and Rmw Values of the Opf Obtained in the Ultrafiltration Experiments

nonpermeated opf	A_{270}	A_{400}	rmw of opf, Da
raw omww$_1$	27.6	6.55	
opf$_1$[a]	19	5.5	>1000
opf$_{30}$[b]	4.5	1.4	>30000
opf$_{100}$[c]	ND[d]	ND	

[a] Opf$_1$ = nonpermeated organic polymeric fraction through 1000 Da cutoff disk membrane. [b] Opf$_{30}$ = nonpermeated organic polymeric fraction through 30000 Da cutoff disk membrane. [c] Opf$_{100}$ = no detected opf. This fraction left only a colorless solid residue of 0.1 mg 150 mL^{-1} which resulted positive at the carbohydrate assay (Fung et al., 1953). [d] Not detected.

This trend was consistent with the chemical nature of the metals analyzed. In fact, except for K and Na, all of the remaining cations are bivalent and possess strong chelating properties. The relative abundance of negative charged sites of the opf could explain the consistent binding of K to the opf. In the case of sodium, 99.1% of the metal was bound to opf, thus competing with K, which has a smaller ionic ray, 0.95 and 1.53 Å, respectively. This difference should make the Na more reactive toward the negative sites of opf than K.

Regarding the chemical composition of the opf, the analysis of P-omww$_1$ plus S-omww$_1$ and P-omww$_2$ plus S-omww$_2$ revealed carbohydrate contents of 7.0 and 2.8 g L^{-1}, polymeric phenol contents of 4.2 and 3.4 g L^{-1}, and protein contents of 3.8 and 2.0 g L^{-1}, respectively. These results indicate the complex nature of the opf, constituted by polysaccharides, polymeric polyphenols, and proteins to which K and Na are essentially bound by single electrostatic bonding, whereas all other ions are more strongly bound, even under chelated form, by means of anionic functional groups and/or having Lewis base properties natively bound to opf considered.

As shown in Tables 4 and 5, the molecular sizes in terms of the relative molecular weight of such fractions were relatively estimated for ~75% ranging substantially in the interval 1000−30000 Da and for ~25% in the interval 30000−100000 Da, by combining UV−vis spectroscopic data with those obtained by ultrafiltration

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Table 5. Pecentage Values of the Opf Included in the Range of Rmw of 1000−30000 and 30000−100000 Da

opf	A_{270}	A_{400}	% of opf included in rmw range of 1000−30000 Da relative to A_{270}	% of opf included in rmw range of 1000−30000 Da relative to A_{400}	% opf included in rmw range of 30000−100000 Da relative to A_{270}	% of opf included in rmw range of 30000−100000 Da relative to A_{400}
opf_1	19	5.5				
opf_{30}	4.5	1.4				
$opf_{1,30}{}^a$	19 − 4.5 = 14.5	5.5 − 1.4 = 1.4	(14.5/19) × 100 = 76.3	(4.1/5.5) × 100 = 74.5		
$opf_{30,100}{}^b$	4.5	1.4			(4.5/19) × 100 = 23.7	(1.4/5.5) × 100 = 25.4
opf_{100}	ND^c	ND				

^{a} $Opf_{1,30}$ = opf included in the range 1000−30000 Da. ^{b} $Opf_{30,100}$ = opf included in the range 30000−100000 Da. ^{c} Not detected.

Table 6. Physicochemical Characteristics of Olive Oil Mill Waste Waters by Pressure (Omww₁) and Centrifuge (Omww₂)

parameter	R-omww₁^a	S-omww₁	P-omww₁	R-omww₂	S-omww₂	P-omww₂
pH	5.1	5.3	6.5	5.4	5.4	6.3
electrical conductivity (ms cm^{-1})	15.1	10.0	4.0	8.2	5.7	1.1
COD (g L^{-1})	180	100	95	80	40	24
BOD (g L^{-1})	18.5	9.2	5.2	17.5	10.2	8.5
OM (g %)	11.8	7.4	2.6	3.2	2.4	0.8
C (g L^{-1})	65	43	15.2	23.1	13.6	4.6

^{a} R = raw; S = supernatant; P = precipitate.

experiments performed on omww₁ (see Experimental Procedures).

The data of Table 4 indicate that the relative molecular weight of the overall opf is included in the range 1000−100000, because the nonpermeated opf through the 1000 Da cutoff membrane (opf₁) showed high UV−vis absorption values $A_{270} = 19$ and $A_{400} = 5.5$, whereas no absorption peaks were detected by the nonpermeated opf through the membrane having a cutoff of 100000 Da (opf₁₀₀). This fraction left a colorless solid residue of polysaccaridic nature, the amount (0.1 mg) of which was not significant enough to determine the molecular weight of opf₁₀₀.

By elaborating the data reported in Table 4 according to the procedure shown in Table 5, the percentages of opf included in the ranges 1000−30000 and 30000−100000 Da can be calculated. In fact, considering that the UV−vis absorbance values of opf₁ are $A_{270} = 19$ and $A_{400} = 5.5$ and the UV−vis absorbance values of opf₃₀ (the organic polymeric fraction nonpermeated through the membrane having a cutoff of 30000 Da) are $A_{270} = 5.5$ and $A_{400} = 1.4$ and the UV−vis absorbance values of opf₁₀₀ are not detected, then the UV−vis absorbance values of the opf included in the range 1000−30000 Da (opf₁,₃₀) are $A_{270} = 19 − 5.5 = 14.5$ and $A_{400} = 5.5 − 1.4 = 4.1$; therefore, the percentages of opf having a molecular weight included in the range 1000−30000 Da (opf₁,₃₀) are (14.5/19) × 100 = 76.3% (relative to A_{270}) and (14.1/5.5) × 100 = 74.5% (relative to A_{400}), that is, ∼75%. Similarly, the percentages of opf having a molecular weight included in the range 30000−100000 Da (opf₃₀,₁₀₀) are (4.5/19) × 100 = 23.7% (relative to A_{270}) and (1.4./5.5) × 100 = 25.4 (relative to A_{400}), that is, ∼25%.

In all likelihood, all of the data reported above relating to the percentages of the metal binding and molecular weight sizes, which were determined for omww₁ opf, may also be extended to omww₂ opf, the qualitative organic and inorganic contents of which remain unchanged.

Table 6 shows that the values of COD, BOD, and the organic substance of the precipitate fractions (P-omww), which contains only the polymeric fraction, are remarkably lower than those of the raw omww (R-omww). The considerable presence of macroelements (K, Ca, and Mg) and microelements (Fe, Zn, Mn, and Cu) bound to the polymeric fraction of raw omww (R-omww), which is also confirmed by the highly significant values of electrical conductivity (see Table 6) as well as the consistent abatement of the COD and BOD values of the polymeric material obtained by cold methanol precipitation with respect to the raw waste, suggests the use of this byproduct as a soil amendment in future experimentation trials.

The high metal binding capacity of opf represents a potential industrial tool for the extraction of toxic metal ions from waste waters and mining effluents. The single-step chemical omww treatment can in fact offer an opportunity to mitigate waste disposal problems, to reduce the costs of chemical byprocess, and to aid in the development of a filtration system to remove and recover metal ions from contaminated waters. The development of the technical and quantitative aspects of these applications is the object of our current research.

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