



## Hydrophilic olive cake extracts: Characterization by physicochemical properties and Cu(II) complexation

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### ABSTRACT

Disposed olive cake generates hydrophilic components that can be mobilized in the aquatic environment. This paper deals with the characterization of such components, isolated by alkaline extraction. It is shown that these substances possess properties very much resembling humic acid, including a substantial inventory of proton exchanging groups. Extraction and purification of the hydrophilic components from the disposed olive cake was performed by the standard approach for isolation of humic acids from solid sources, i.e. alternating alkaline dissolution and acid flocculation, leaving the purified extract in the protonated form. The purified sample was characterized by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), Fourier Transform Infra Red Spectroscopy (FTIR), UV–vis, Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS) and Asymmetrical Flow Field-Flow Fractionation (AF4). The complex formation properties were investigated by potentiometry using Cu(II) ion selective electrode under atmospheric conditions at  $I = 0.1 \text{ M NaClO}_4$  (aqueous solution) and pH 6. The formation constant for the CuHA complex is found to be  $\log \beta = 5.3 \pm 0.4$  which is close to the corresponding value ( $\log \beta = 5.2 \pm 0.4$ ) obtained from similar investigations with the commercially available Aldrich humic acid (this study) and corresponding published values for various humic acids. Both, structural properties and complex formation data show that the olive cake extract has considerable similarities with humic acids from different sources, pointing towards potential similarities in environmental behavior and impact.

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### 1. Introduction

Olive cake is a biomass by-product of olive oil production and is a very abundant agricultural waste in the Mediterranean area, where approx. 98% of the world's commercial olive trees are cultivated. Manufacturing of olive oil yields an aqueous (50%), an oily (30%) and a solid residue – the olive cake or pomace (20%). The olive cake generally remains stockpiled close to olive oil mills without further use [1]. Olive mills waste waters and leachates from olive cake deposits will potentially percolate into the groundwater. These solutes contain the hydrophilic organic compounds generated from the disposed olive cake [2]. Together with bounded heavy metals and metalloids they can contaminate groundwater, prob-

lematic especially in view of scarce drinking water resources in Mediterranean countries.

The distribution and fate of heavy metal pollutants in aqueous solutions is strongly influenced by the presence of natural organic material [3]. Humic acids (HA) and fulvic acids (FA) constitute the main fraction of dissolved organic matter (DOM). HA is soluble in aqueous solution with exception for the acidic range where it flocculates [4,5]. HAs are present in soils, natural waters, rivers, lakes and sea sediments, peat, brown and brown-black coals and other materials as a product of chemical and biological transformations of animal and plant residues [6]. Moreover, humic acids have considerable quantities of strong complexing groups (in the order of 5 meq/g carboxylic and phenolic groups), and thus they can form stable heavy metal ion complexes. Depending on their distribution between mobile and stationary phases, they can then cause either an enhancement or decrease in heavy metal mobility. They also affect the bioavailability of metals in soils, sediments and aquatic

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systems [7,8]. For the quantification of the different relevant processes, a basic knowledge of the nature of humic acids and their complexation behavior is necessary [9].

For the purpose of predicting the potential mobilization of heavy metals from olive cake deposits, the characteristic properties and the complex formation behavior of the hydrophilic components released by olive cake deposits needs to be known. A key objective thus is to compare the properties of the hydrophilic components with those from humic acids of frequently studied sources (e.g. Aldrich humic acid) and thus ensure applicability of that broad existing knowledge base.

The present paper reports results on hydrophilic components, which were extracted from olive cake that was disposed for about 1 year. Extraction, isolation and purification followed the standard scheme for isolation and purification of humic acid from solid sources, namely alternating alkaline dissolution and acid flocculation, leaving a purified material in the protonated form. The purified sample was characterized by various spectroscopic methods (e.g. Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), Fourier Transform Infra Red (FTIR), UV-vis, Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS)) and Asymmetrical Flow Field-Flow Fractionation (AFFFF). Furthermore, the complex formation properties were investigated by potentiometry, using a  $\text{Cu}^{2+}$  ion selective electrode. Potentiometry by means of ion selective electrodes has the advantage that measurements are performed in situ, i.e. there is no separation of the complexed from the free metal ion for their respective quantification. Consequently, the interaction parameters are determined without disturbing the equilibrium. Reference to the  $\text{Cu}^{2+}$  ion humic acid system has been chosen because of the stability of the  $\text{Cu}^{2+}$  ion selective electrode and the availability of extensive literature data on the complexation of Cu(II) with humic and fulvic acids [10–18].

Both, spectroscopic characterization and investigation of the complexation properties are performed in parallel (under similar conditions) for the olive cake extract and the commercially available Aldrich humic acid, in order to compare the two different types of hydrophilic organic matter. Similarities in the characteristic properties, including  $\text{Cu}^{2+}$  complexation, opens the possibility to use the broad experience available in the literature on humic acid for assessment of the environmental impact from disposal of residues from olive oil production.

## 2. Materials and methods

### 2.1. Sampling and analytical methods

The deposited olive cake material was supplied by a local (Cypriot) olive oil production plant. The biomass by-product (e.g. olive cake) had been disposed near to the plant for about 1 year. The material was pre-treated by means of hexane extraction and thus was dry and free of oil residues. Separation of hydrophilic components from the solid matrix was performed by alkaline extraction using 0.2 M NaOH. Isolation and purification of the material was carried out by duplicate cycles of flocculation with 1 M HCl and dissolution with 0.2 M NaOH. Finally, the protonated flocculate was washed with water and freeze-dried. Tris buffer was obtained from Fluka (93394) and  $\text{NH}_4\text{OH}$  solution (29.1 %) was obtained from Sigma (A-6899). All substances were used as delivered and all aqueous solutions were prepared with high purity water (Milli-QPLUS, Millipore).

Characterization included: (a) elemental analysis performed by Desert Analytics, (b) FTIR spectroscopy (FTIR spectrometer 8900, Shimadzu), (c) UV/vis spectroscopy (UV 2401 PC, Shimadzu,  $[\text{HA}] = 30 \text{ mg/L}$ , pH 6), (d) determination of inorganic impurities

by ICP-OES (7500 Shimadzu) after digestion in 60% nitric acid, (e) molecular mass distribution carried out by TOF-SIMS, (f) molecular size distribution by AFFFF, and (g) complexation with Cu(II).

#### 2.1.1. Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS)

The time-of-flight secondary ion mass spectrometer used in this work has been described elsewhere [19]. Solid samples for TOF-SIMS analysis were prepared by spray-deposition on cleaned silicon substrates with a hydrophilic character [20]. Solutions of 30 mg/L of extract were dissolved in a dilute aqueous solution of  $\text{NH}_4\text{OH}$  and typically 400  $\mu\text{L}$  of solution were sprayed. The total amount of sprayed extract was  $\sim 12 \mu\text{g}$ . This converts to area densities of roughly 5–6  $\mu\text{g}/\text{cm}^2$ . The recorded spectra were highly reproducible, both with respect to characteristic spectral features and in absolute terms. Usually the measured yields were the same to within 10% or better from different beam positions of the deposits.

#### 2.1.2. Asymmetrical Flow Field-Flow Fractionation (AFFFF)

Field-flow fractionation is a technique for size fractionation of macromolecules and colloids. The analytical technique and the types of equipments that available are symmetrical flow field-flow fractionation (FFFF) and the more recently developed AFFFF [21–23]. The AFFFF measurements were carried out at room temperature with a system delivered from Wyatt Technology using an asymmetrical fractionation channel from ConSensus (Germany) with a length of 286 mm and a spacer thickness of 0.54 mm. The regenerated cellulose membrane used has a cut-off of 1 kDa (related to globular proteins). As eluent, 5 mM Tris buffer with a pH of  $\sim 9.1$  at room temperature was used at a channel flow of 0.6 mL/min and a cross-flow of 3.0 mL/min. The absorbance of the effluent was recorded with an UV/vis detector (K-2500, Knauer, Germany) at 210 nm. The fractionated sample volume was 20  $\mu\text{L}$ . Humic acid and olive cake extract samples were dissolved in 5 mM Tris buffer with a concentration of 30 mg/L.

### 2.2. Complexation/potentiometric experiments

Hydrophilic olive-cake extract, Aldrich humic acid and copper sulphate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , Merck) stock solutions were prepared. Potentiometric measurements were performed using glass or Cu(II) ion selective electrodes (Inolap) attached to a pH meter (WTW). The systems were calibrated by means of buffer solutions (pH 2, 4, 7 and 10, Merck) and Cu(II) standard solutions, respectively, prior to and after each measurement.

Complexation experiments were carried out at room temperature ( $23 \pm 2^\circ\text{C}$ ) under atmospheric conditions in aqueous solutions at constant ionic strength (0.1 M  $\text{NaClO}_4$ ) and pH (pH 6, MES-Buffer,  $\text{C}_6\text{H}_{13}\text{NO}_4\text{S} \cdot \text{H}_2\text{O}$ , Merck). The test solutions were prepared by addition of defined amounts of Cu(II) stock solution ( $[\text{Cu(II)}] = 0.1 \text{ M}$ ) to 25 mL with 0.1  $\text{g L}^{-1}$  olive cake extract or humic acid solution in  $\text{NaClO}_4$ , that contained 1 mmol/L MES-Buffer (pH 6). After 2 days equilibration the concentration of the non-complexed Cu(II) was determined by potentiometry. Potentiometric titrations were also performed in blank samples of similar composition with the test solutions but without olive cake extract/humic acid (reference solutions). All experiments were performed in duplicate and the mean values have been used for data evaluation.

## 3. Results and discussion

### 3.1. Elemental analysis and spectroscopic characteristics

A yield of approx. 7% hydrophilic components was obtained by the alkaline extraction from the dry, oil free, olive cake. This value is

**Table 1**

Elemental composition of hydrophilic olive cake extract compared to literature values for humic acids

Element	Hydrophilic olive cake extract	Humic acid lit. values [4]
C	63.5	50–60
H	8.2	4–6
N	2.2	2–6
O	26.1	30–35

comparable to a yield of 9.8% hydrophilic components/humic acid extracted from wet olive cake [24]. The elemental composition (C, H, N and O) of the olive cake extract and the ranges of elemental compositions of soil humic acids is given in Table 1. The ranges of values represent humic acids from different sources and origin, including possible differences between different isolation procedures. The elemental composition of hydrophilic olive cake extract shows C and H values slightly above the typical range of humic acids and a correspondingly slightly lower O value. This is a first indication for the possible comparability between hydrophilic olive cake extracts and humic acids, however, with a tendency to a lower oxygen content, expected for a low degree of humification maturity.

Table 2 shows the content of inorganic constituents of the present sample and purified Aldrich humic acid [9]. Humic acids contain inorganic inventory that is not removed even by repeated acid base treatment, and, as of the purified Aldrich humic acid, also treatment with HF. As shown by the ratios at the bottom of Table 2, the Al, Fe and Se inventories in the hydrophilic olive cake extract is 1.4–5 times higher than that found in the Aldrich humic acid. With respect to the other inorganic constituents comparison data are missing or the inventory in olive cake extract is considerably higher. The significantly higher content of Hg and As in the olive cake extract can be attributed to the background lithology of the island (Cyprus), which is rich in pyrite minerals. In general, the data show an inventory of inorganic constituents that is not removed by purification, typical for humic acid.

The UV spectrum of humic acids shows an absorption continuum without recognizable peaks, increasing with decreasing wavelength [9]. The same spectrum is also obtained for hydrophilic olive cake extract. E4/E6 is a value defined as the ratio of absorption intensities at 465 and 665 nm and is used to characterize humic acid. It is generally considered that this ratio decreases with increasing molecular mass and delocalization of aromatic systems. The E4/E6 value of humic acids is usually <5, while for fulvic acids it ranges from 6.0 to 8.5 [9]. The E4/E6 value for the hydrophilic olive cake extract is found to be 2.6, falling in the range of humic acid. This may also be expected because of the extraction procedure where humic acid alike material is isolated by flocculation whereas the non-flocculating fulvic acid alike material is discarded.

The FTIR spectrum of the olive cake extract shows characteristic features of humic acids but also some differences compared to Aldrich humic acid (Fig. 1): OH-stretching at 3430 cm<sup>-1</sup> from numerous sources (including water) is very similar [25]; the bands at 2925 and 2850 cm<sup>-1</sup> from aliphatic C–H stretching (together with the band at 1450 cm<sup>-1</sup> from single bond vibrations) show for olive cake extract higher intensity and point to higher aliphatic moieties which is supported by the relatively high

**Table 2**

Inorganic constituents (in ppm) in hydrophilic olive cake extract and Aldrich humic acid [9]

	Al	Mn	Fe	Ni	Cu	Zn	As	Se	Hg
Hydrophilic olive cake extract	175	15	1195	10	215	95	50	3	45
Aldrich humic acid	35		360			2.6	0.16	2.2	1.8
Ratio	5.0		3.3			37	313	1.4	25

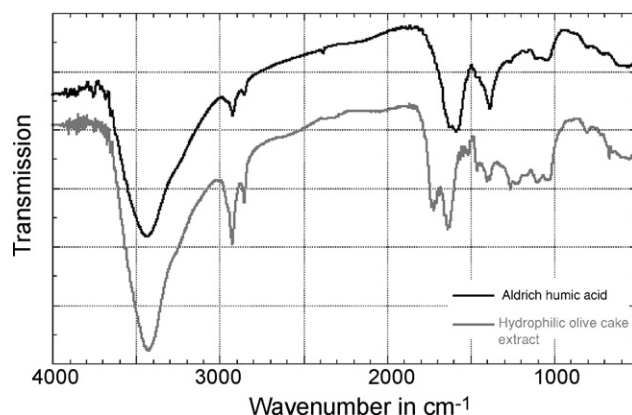


Fig. 1. FTIR spectra of hydrophilic olive cake extract and Aldrich humic acid.

H content (Table 1); olive cake extract shows a distinct band at 1725 cm<sup>-1</sup> from C=O stretching vibrations due to protonated carboxylic groups but on the other side relatively similar intensity of the 1625 cm<sup>-1</sup> band, which is attributed to C=C bonds conjugated with C=O and COO<sup>-</sup> groups; in the region 1400–1000 cm<sup>-1</sup> the hydrophilic olive cake extract shows smaller intensity of the peak at 1400 cm<sup>-1</sup> corresponding to COO<sup>-</sup> vibrations but in the entire region 1300–1000 cm<sup>-1</sup> much stronger intensity including the peak at 1260 cm<sup>-1</sup> corresponding to C–O vibrations probably caused by higher contributions of not so strongly humified plant material [26].

### 3.2. Molecular mass distribution

The negative TOF-SIMS spectrum of hydrophilic olive cake extract (Fig. 2) depicts a broad smooth distribution, characteristic for humic acids. Compared to Aldrich humic acid, the olive cake extract distribution is shifted to lower masses as a whole. The shift of the olive cake extract mass distribution is also clearly shown in Fig. 3, where the TOF-SIMS spectra are transformed to mass concentration vs. logarithmic mass (distribution maximum: hydrophilic olive cake extract 520 mass units, and Aldrich humic acid 800 mass units).

### 3.3. Size distribution by AFFFF

The fractograms obtained by AFFFF were transformed into size distributions (relative mass concentration related to the mass of the polycarboxylic acid (PCA) standards used) for a logarithmic mass scale. The molecular size distributions of the olive cake extract and of Aldrich humic acid are shown in Fig. 4. Hydrophilic olive cake extract falls in the typical range of size distributions found for humic acids. Detailed comparison with Aldrich humic acid shows the following differences: The size distribution of olive cake extract is broader and the maximum of the distribution is slightly shifted to a lower size. There is a shoulder in the size range between 2 and 6 kDa PCA.

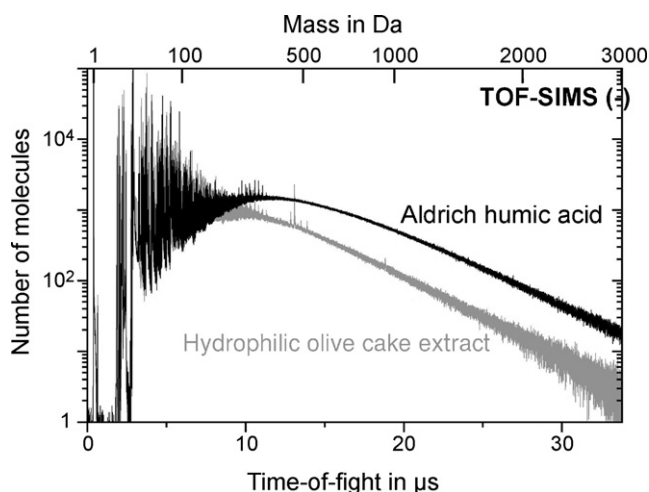


Fig. 2. Mass distributions of hydrophilic olive cake extract and Aldrich humic acid by TOF-SIMS in the negative ion mode.

### 3.4. Interaction between Cu(II) and olive cake extract

The interaction of hydrophilic olive cake extract with Cu<sup>2+</sup> was compared to that of Aldrich humic acid. Fig. 5 shows experimental data in the form of a  $\log[\text{Cu}^{2+}]_{\text{free}}/\log[\text{Cu}^{2+}]_{\text{tot}}$  diagram obtained from Aldrich and olive cake extract solutions and comparable reference solutions without olive cake extract/humic acid. The concentration of the free Cu<sup>2+</sup> ion in the olive cake extract/humic acid solutions is far lower than in the reference solutions, showing the impact of complexation with olive cake extract/humic acid. The results from the two different hydrophilic acids are strikingly similar. This visualizes their similar complexation behavior.

Hydrolysis of the Cu(II) ion at given pH (pH 6) is negligible. Experiments are conducted with a maximum concentration of non-complexed Cu(II) ions of about 10<sup>-4</sup> mol/L. Using thermodynamic data from [27], it is found that the Cu<sup>2+</sup> does not fall below 98.8%, i.e. the influence of hydrolysis, formation of carbonate and sulfate complexes is negligible. Thus, the interaction of the Cu(II) ion with humic acid can be written as (the same notation used in this paper

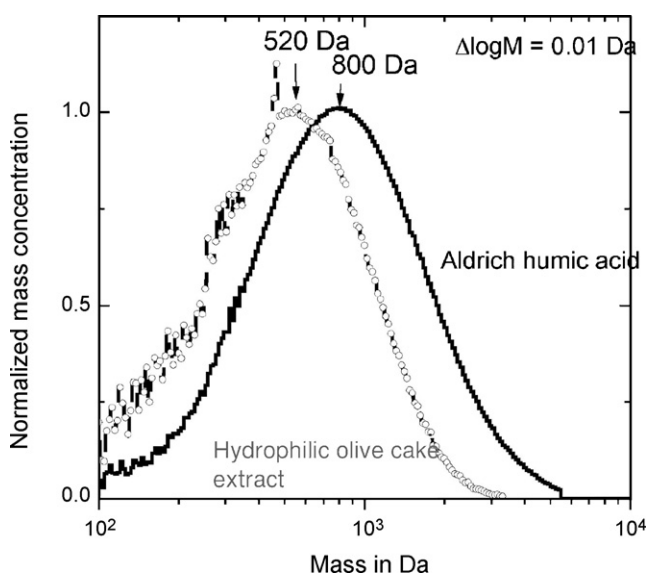


Fig. 3. Transformed TOF-SIMS spectra: Mass concentration vs. logarithmic mass (cf. Fig. 2).

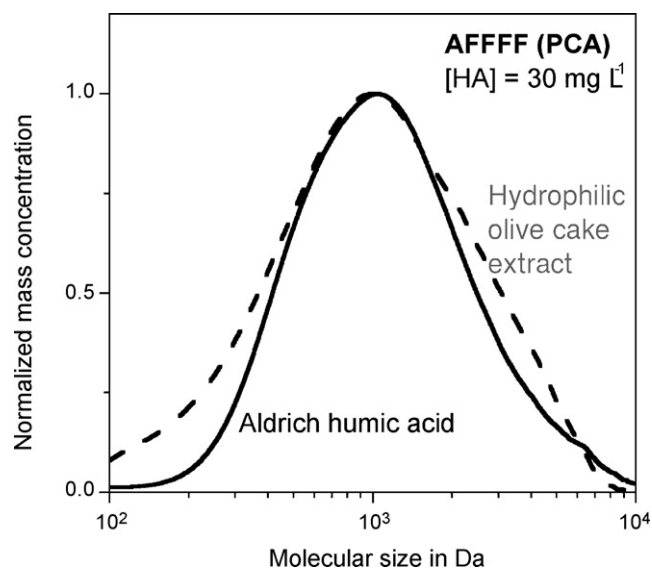


Fig. 4. Molecular size distribution of hydrophilic olive cake extract and Aldrich humic acid measured by AFFF, as the size of polycarboxylic acids (PCA) as a function of their mass.

for hydrophilic olive cake extract):



and the conditional stability constant for the Cu(II)–humate complex is given by

$$\beta = \frac{[\text{CuHA}^{(z-2)-}]}{[\text{Cu}^{2+}] \cdot [\text{HA}^{z-}]} \quad (2)$$

where  $[\text{CuHA}^{(z-2)-}]$  is the concentration of the complexed Cu(II) and is calculated as follows:

$$[\text{CuHA}^{(z-2)-}] = [\text{Cu}^{2+}]_{\text{tot}} - [\text{Cu}^{2+}]_{\text{aq}} \quad (3)$$

where  $[\text{Cu}^{2+}]_{\text{tot}}$  is the total Cu(II) ion concentration (initial Cu(II) ion concentration) and  $[\text{Cu}^{2+}]_{\text{aq}}$  the non-complexed Cu(II) ion concentration, which is determined by potentiometry. The  $[\text{HA}^{z-}]_{\text{tot}}$  is

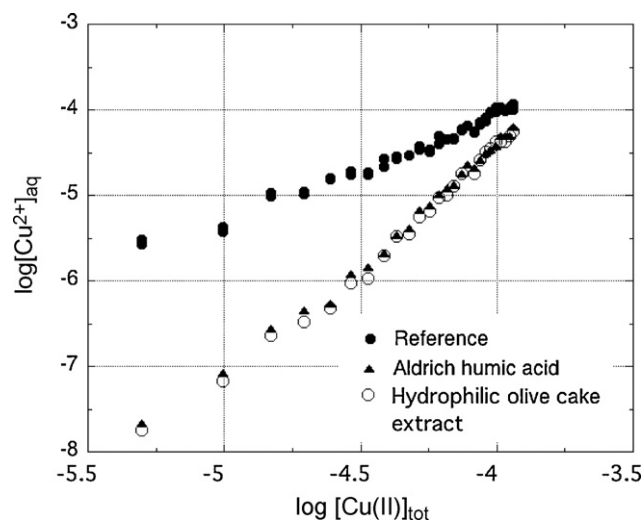


Fig. 5.  $\log[\text{Cu}^{2+}]_{\text{free}}$  as a function of  $\log[\text{Cu}^{2+}]_{\text{tot}}$  in solutions with and without olive cake extract/Aldrich humic acid. Experimental data are obtained from potentiometric measurements in aqueous solutions with 0.1 g L<sup>-1</sup> olive cake extract/humic acid, varying Cu(II) concentrations, 0.1 M NaClO<sub>4</sub> as background electrolyte and carried out at 23 ± 2 °C in contact with air.

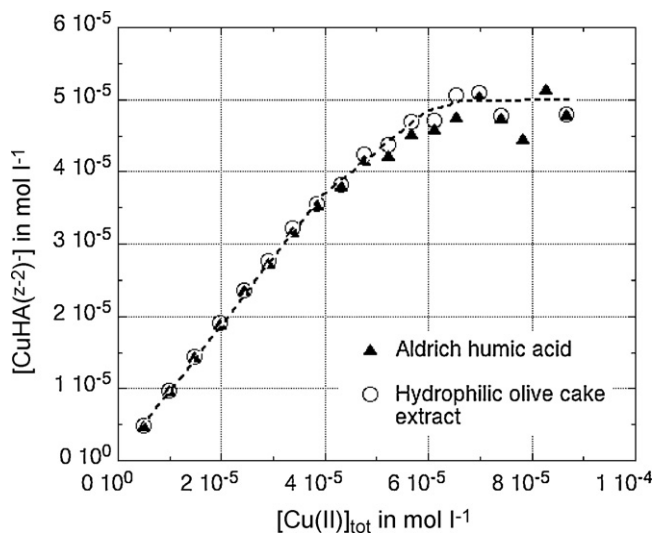


Fig. 6. Complexation capacity of hydrophilic olive cake extract (circles) and Aldrich humic acid (triangles) and for  $\text{Cu}^{2+}$  ions.

defined as the maximum concentration of complexes formed under given conditions. The free ligand concentration is calculated as the total ligand concentration minus the concentration of complexes:

$$[\text{HA}^{z-}] = [\text{HA}^{z-}]_{\text{tot}} - [\text{CuHA}^{(z-2)-}] \quad (4)$$

The value of  $[\text{HA}^{z-}]_{\text{tot}}$  is determined by gradual addition of  $\text{Cu}(\text{II})$  ions to humic acid ( $0.1 \text{ g L}^{-1}$ ) under the experimental conditions used (pH 6,  $I = 0.1 \text{ M}$  and atmospheric contact) until the saturation value is identified. It is found to be  $0.5 \text{ mmol Cu}(\text{II}) \text{ g}^{-1}$  both for Aldrich and the hydrophilic olive cake extract (Fig. 6). The similar value of the “complexation capacity” for both types of organic hydrophilic acids shows their similar complexation behavior.

The value of the formation constant for the  $\text{Cu}(\text{II})$ –humate complex ( $\beta$ ) can be calculated by linear regression analysis of the corresponding potentiometric data using Eq. (5) in its logarithmic form:

$$\log \frac{[\text{CuHA}^{(z-2)-}]}{[\text{HA}^{z-}]} = \log \beta + \log [\text{Cu}^{2+}] \quad (5)$$

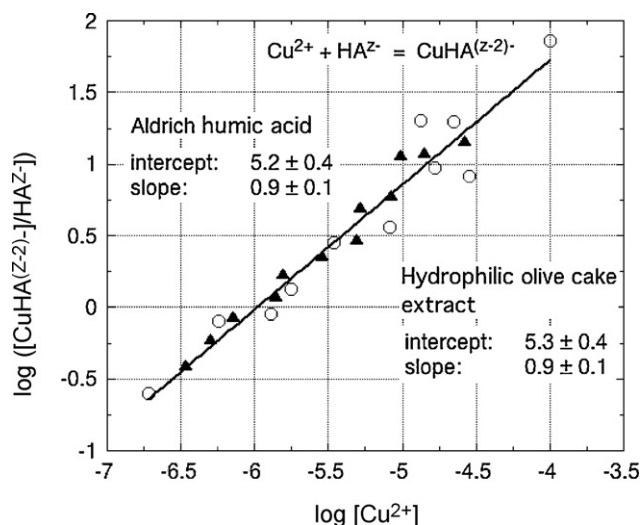


Fig. 7.  $\log([\text{CuHA}^{(z-2)-}]/[\text{HA}^{z-}])$  vs.  $\log[\text{Cu}^{2+}]$ . Data obtained from potentiometric measurements for the complexation of  $\text{Cu}(\text{II})$  with hydrophilic olive cake extract (circles) and Aldrich humic acid (triangles; both denoted as “HA”).

In Fig. 7, experimental data obtained from potentiometric measurements are presented in a  $\log([\text{CuHA}^{(z-2)-}]/[\text{HA}^{z-}])$  vs.  $\log[\text{Cu}^{2+}]$  diagram. The slope represents the stoichiometry of the reaction. With  $0.9 \pm 0.1$  it is close to unity. The stability constant is evaluated by linear regression with the slope set to unity. The resulting stability constant is given by the intercept with  $\log \beta = 5.3 \pm 0.4$ .

The slope of the curve (Fig. 7) is close to unity. Furthermore, the  $\log \beta$  value of the hydrophilic olive cake extract determined for the  $\text{Cu}(\text{II})$ –humate complex is close to  $\log \beta$  values (5.0–5.9) given in literature for the complexation of  $\text{Cu}(\text{II})$  with humic or fulvic acid [12–18] and similar to the  $\log \beta$  value ( $5.2 \pm 0.4$ ) evaluated from the experimental data corresponding to measurements with Aldrich humic acid (this work).

#### 4. Conclusions

The results obtained from this study lead to the general conclusion that hydrophilic olive cake extract possess the typical properties of frequently studied humic acids (e.g. Aldrich humic acid). In some more detail, according to this study:

- The C and H values of the elemental composition of the hydrophilic olive cake extract are slightly above the typical range of humic acids and are ascribed to the lower degree of humification.
- The higher content of Hg and As in the olive cake extract compared to Aldrich humic acid is attributed to the background lithology of the island (Cyprus), which is rich in pyrite minerals.
- The E4/E6 value and the peak positions in the FTIR spectrum of the hydrophilic olive cake extract are similar to the corresponding data of the Aldrich humic acid.
- TOF-SIMS and AFFF measurements show that the size distribution of the olive cake extract is broader and the maximum of the distribution is slightly shifted to a lower size, but in general, the particle size of the hydrophilic olive cake extract falls in the typical range of size distributions found for humic acids.
- Potentiometric studies have shown that hydrophilic olive cake extract and Aldrich humic acid possess similar “complexation capacity” and show identical chemical affinity for  $\text{Cu}(\text{II})$ .

Further laboratory experiments and field measurements are planned to understand the impact of the hydrophilic olive cake components on heavy metal mobility from olive cake deposits into the aquatic environment.

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