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A MULTI METHOD APPROACH TO STUDY HUMIC COMPOUNDS AND METAL SPECIATION IN MARINE SEDIMENT SAMPLES

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A multi-method experiment was carried out to study humic compounds and metal speciation in a marine sediment sample. The humic and fulvic acid structures were investigated by infrared spectrometry, ^{13}C NMR, thermogravimetry, and elemental analyses. Trace metals (Cu, Pb, Cd, Zn, Fe, Cr, Ni, Co, Mn and Al) were determined in digests of the sample and in solutions obtained by selective extraction procedures. The distribution of metals associated with humic substances has also been investigated by high pressure liquid chromatography using inductively coupled plasma atomic emission spectrometer as detector.

The results show that manganese, iron and aluminum are present in inorganic form, whereas 37% of nickel and 8.8% of copper are present in the metal-humic compound form.

KEY WORDS: Sediments, metals, speciation, humic compounds.

INTRODUCTION

Speciation is an important aspect of environmental analytical chemistry for the characterization of natural samples. During the last years the interest in the chemistry of humic compounds, that represent one of the largest sources of natural organic carbon on earth, is increased owing to the important role of humic materials in the environment. Because humic compounds are able to form both water-soluble and water-insoluble complexes with metals and to react with organic compounds as pesticides and hydrocarbons, they play a crucial

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role in the speciation, transport and deposition of a variety of compounds ranging from metal ions to lipophilic compounds. The functional groups present in humic compounds, responsible for their reactivity, complexation ability and solubility, are carboxyls, alcohols and phenols.

In the marine ecosystem, sediments are the "reservoir" in which trace metals can accumulate. From the sediment, metals can move into different biogeochemical cycles, through several ways such as formation of suspended particulate matter, solubilization in seawater, and uptake by benthonic organisms.

Humic substances are a major component of organic matter contained in marine sediments¹, consequently in speciation studies, the knowledge of structural composition of humic acids is also important.

We propose in the present study an experimental multi-method approach in which the leaching technique with different solutions, that generally is taken into consideration to study metal speciation in marine sediments, is supported by the spectroscopic characterization of the humic fraction present.

EXPERIMENTAL

Apparatus

Varian (Mount Waverley, Victoria, Australia) Zeeman AA 300 spectrophotometer equipped with AA 300 Graphite Atomizer, with Zeeman background corrector. The instrument was interfaced to an IBM 30 computer.

Jobin Yvon (Longjumeau-Paris, France), JY 24 inductively coupled plasma atomic emission spectrometer, equipped with a Reyton 2300 RF plasma torch fitted with Scott glass spray chamber and a Meinhard concentric glass nebulizer led by a Gilson IIA peristaltic pump.

Varian HPLC Model 5000, equipped with a Rheodyne injector with a 200 μ l loop. Column BIO-GEL TSK 10.

Varian Star 9065 Polychrom diode array detector, interfaced to a Compaq computer.

Perkin-Elmer TGA 7 thermogravimetric analyzer

Philips PV 9800 FTIR spectrometer

Varian XL 300 NMR spectrometer and model 240-B elemental analysis apparatus

Reagents

Standard solutions (Spectrosol Grade) were obtained from BDH Chemicals Ltd (Poole, England). Water nanopure grade was produced with a Milly-Q Water System (Millipore). Hydrofluoric, perchloric, nitric acids and sodium hydroxide were of analytical grade.

Sample preparation

A Van Veen grab was utilized to collect the superficial part of the marine sediment samples to a depth of 2 cm. Five samples were collected during the "G.R.O.-G" Oceanographic Cruise in the eastern Ligurian Sea at a station in front of Chiavari (44°18'N, 09°16'E). These samples were mixed and homogenized to form a representative sample.

This sediment sample was dried to 45°C for humic substance extraction and trace metal determination, after selective solubilization, and to 110°C for the total metal determination.

Procedure

Humic and fulvic acids were extracted and separated as proposed by several authors²⁻⁶.

The sediment sample (600 g) was shaken for 24 hours by an automatic device with 0.1 M hydrochloric acid (solution 1); the residue of this step was treated with 0.5 M sodium hydroxide for 24 hours. The basic extract, acidified to pH 1, yielded a solution (solution 2, fulvic acids) and a precipitate (humic acids). Solutions 1 and 2 were dried under an IR lamp (60 °C), then the residues were dialyzed (membrane size 100) against distilled water to absence of chloride ions and again dried. Humic acids were redissolved in sodium hydroxide and the precipitation/dissolution cycle was repeated two times. Final purification was carried out with 0.1 M hydrochloric and 0.3 M hydrofluoric acid solution (1:1) for 48 hours. The residue was dialyzed (membrane size 1000) and dried under an IR lamp.

The three aliquots (fulvic acids, humic acids and compounds soluble in hydrochloric acid) were analyzed by IR and ¹³C-NMR spectrophotometry, thermogravimetry, combined IR and thermogravimetry⁷ and elemental analysis. Total acidity, carboxyl and carbonyl groups were determined⁸⁻¹³. Phenolic groups were obtained by difference between total and carboxylic acidity.

Determination of metals bound to humic substances

Different aliquots of the solutions containing humic substances, only humics and only fulvic acids, were analyzed for metals with ICP-AES and by gel filtration (HPLC), using a DEAE column (eluting phase: phosphate buffer, pH 7.50, flow rate 0.7 ml/min).

The chromatographic fractions were detected with the diode array detector, which provides the desired ultraviolet (254 or 220 nm) chromatograms directly and stores the spectra at all the other wavelengths. After ultraviolet detection, the eluate pass into an ICP-AES detector which provides the chromatograms of the metal-organic compounds.

Total metal solubilization

0.5 g of dried (110 °C) and finely powdered sediment were transferred in a Teflon bomb (Perkin-Elmer, Italy) and digested adding 5 ml of HF, 1 ml HCl-HNO₃ (3:1) and 5 ml of

Milli-Q water. 5 ml of saturated H_3BO_3 solution were then added to the teflon bomb previously heated to 180 °C for an hour and a half and cooled to room temperature.

The solution, after dilution to 50 ml, was analyzed by ICP-AES. (Operating conditions: sample rate 0.8 ml/min, Ar torch 18 l/min, Ar nebulizer 0.6 l/min, Ar coating 0.4 l/min, power 1/ kW, monochromator scan step 0.002 n).

To avoid the interferences from the major elements, cadmium was determined after an ion-exchange separation^{14,15}.

For testing precision and accuracy of the total solubilization method, we have analyzed two international standard sediments (MAG-1, U.S. Geological Survey and BCSS-1, National Research Council of Canada). The results obtained were in good agreement with certified values.

Selective metal solubilization

According to the selective dissolution procedure¹⁶⁻¹⁸, 5 g of finely powdered sediment were shaken overnight with 90 ml of 1 M ammonium acetate buffered at pH = 5 in a rotating action agitator (GLS, 40 rotations/min). After centrifugation (solution "A") the remaining residue was treated overnight with 90 ml of 1 M hydroxylammonium chloride plus 25% acetic acid in a rotating vessel (solution "B"); finally the residue was digested with 25 ml nitric acid (3 hours at 80°C) (solution "C"). After dilution at 100 ml the metals were determined by ICP-AES in the solutions A, B and C respectively, in order to detect the concentrations of metals present in the sediment in ionic exchanging form and bound to the carbonate (solution A), in the reductive phase bound to manganese-iron oxides (solution B) and bound to sulphide and organic phase (solution C).

¹³C-NMR spectra

Samples for ¹³C-NMR spectra were prepared by dissolving the dried residue in 1 ml of HCl 1 M or NaOH 0.5 M.

After dispersion in an ultrasonic bath, 0.5 ml of solution were placed in a 5 mm NMR tube to which 0.5 ml of D₂O was also added. NMR spectra were run at 75.0 MHz using a waltz-16 decoupling, a 45° pulse, an acquisition time of 0.1 sec and a delay time of 0.5 sec. Between 450,000 and 600,000 scans were accumulated.

RESULTS AND DISCUSSION

In Table 1 are reported the data concerning elemental analysis, H/C and N/C ratios, ash at 1000°C and the values of most important oxygenated groups of humic acids, fulvic acids and compounds soluble in hydrochloric acid. IR and ¹³C-NMR spectra and thermogravimetric trends are shown in Figures 1,2,3 and 4.

In 0.1 M HCl are solubilized aliphatic structures (0–50 ppm range), partly constituted by

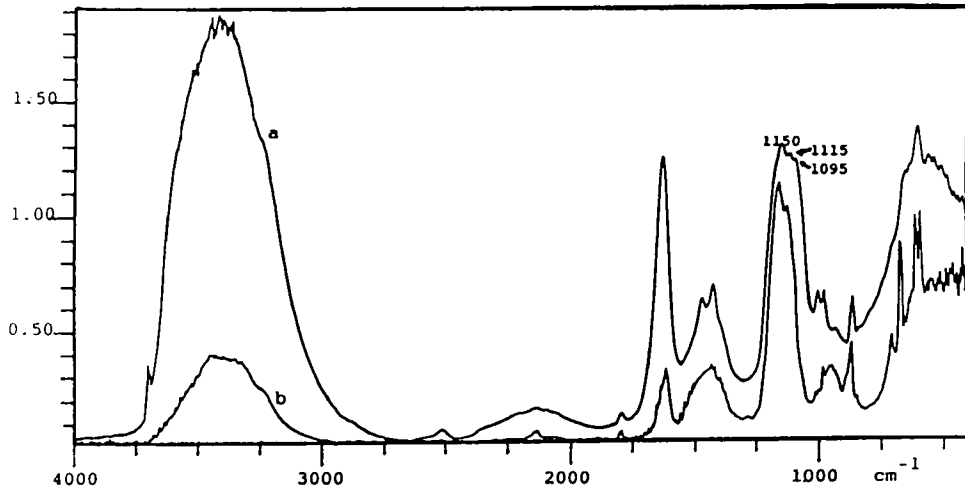


Figure 1 IR spectra of residue in HCl (a) and of residue after heating at 560°C (b).

branched chains (0–20 ppm range), aromatic compounds (120–150 ppm) and carbohydrates (60–80 ppm range and 105 ppm peak of ^{13}C -NMR spectrum; 3000–3600 cm^{-1} range and 1020–1150 cm^{-1} peaks of IR spectra). A thermogravimetric loss in the range 150–300 °C was observed for this fraction, as reported by Sheppard and Forgeron¹⁹. Aminoacids and nitrogenous compounds are present in low concentrations, as shown by the values of elemental analysis.

To obtain additional information regarding the assignment of specific absorption bands, particularly in the range 1000–1200 cm^{-1} (Figure 1a), we have performed a thermogravimetric analysis in the range 0–560 °C and then we have carried out an infrared spectrophotometric analysis on the residue.

The IR spectrum (Figure 1b) shows a strong decrease of the peak at 3000–3600 cm^{-1} (alcoholic OH) and the disappearance of the peak at 1095 cm^{-1} which confirmed the presence of carbohydrates. In fact, the absorption bands due to carbohydrates, silica and some other inorganic salts as sulphates and phosphates cover the range 1150–1030 cm^{-1} , but thermal destruction only of carbohydrates occurs below 560°C²⁰. The presence of silica and inorganic salts, outlined by the high ash value (Table 1) and thermogravimetric analysis (Figure 4a), is evidenced. In this fraction the concentration of carboxyl groups is not significant (Table 1).

The results obtained suggest that the compounds soluble in 0.1 M hydrochloric acid are not able to act as complexing agents towards metals.

The ^{13}C -NMR spectrum of fulvic acids shows absence of carbohydrates (60–80 ppm) and presence of structures like branched aliphatic chains (0–50 ppm), protonated aromatic rings (115–130 ppm), substituted aromatic rings (130–147 ppm), carboxyl groups (170–180 ppm); the last confirmed by the data of Table 1 and IR spectra (Figure 2a). The presence of

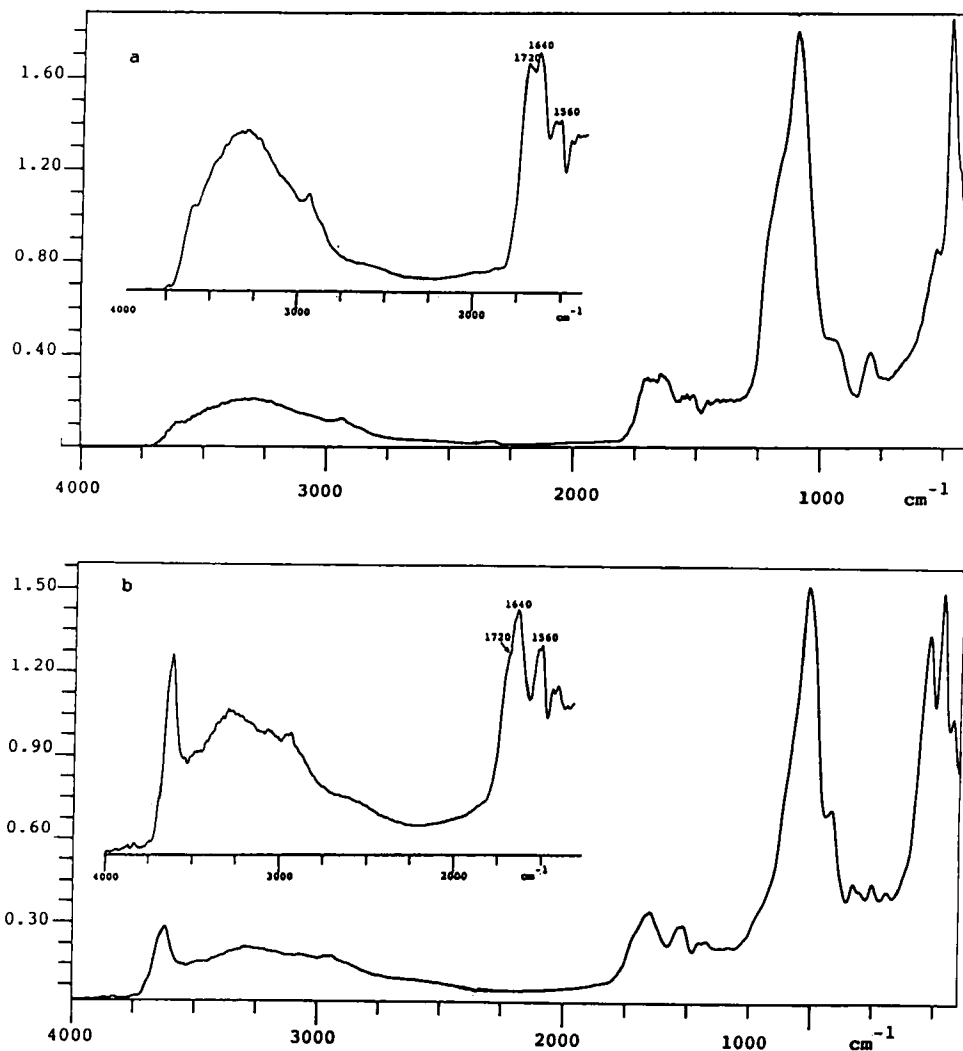


Figure 2 IR spectra of fulvic (a) and humic (b) acids.

protein-like substances (peaks at 1640 and 1560 cm^{-1} of IR spectrum due to amide I $\text{C}=\text{O}$ stretching and amide II NH deformation - CN stretching) is confirmed by elemental analysis. The very high amount of oxygen is probably due to the presence of inorganic salts. In fact, the high loss of weight (65%) beyond 750°C (thermogram of Figure 4b) is due to thermal destruction of inorganic salts, other than silicates (relatively little value of ash).

In the humic acid structures it is possible to identify carbohydrates (80–100 ppm region and peak at 105 ppm), protonated aromatic rings (120–130 ppm), substituted aromatic rings

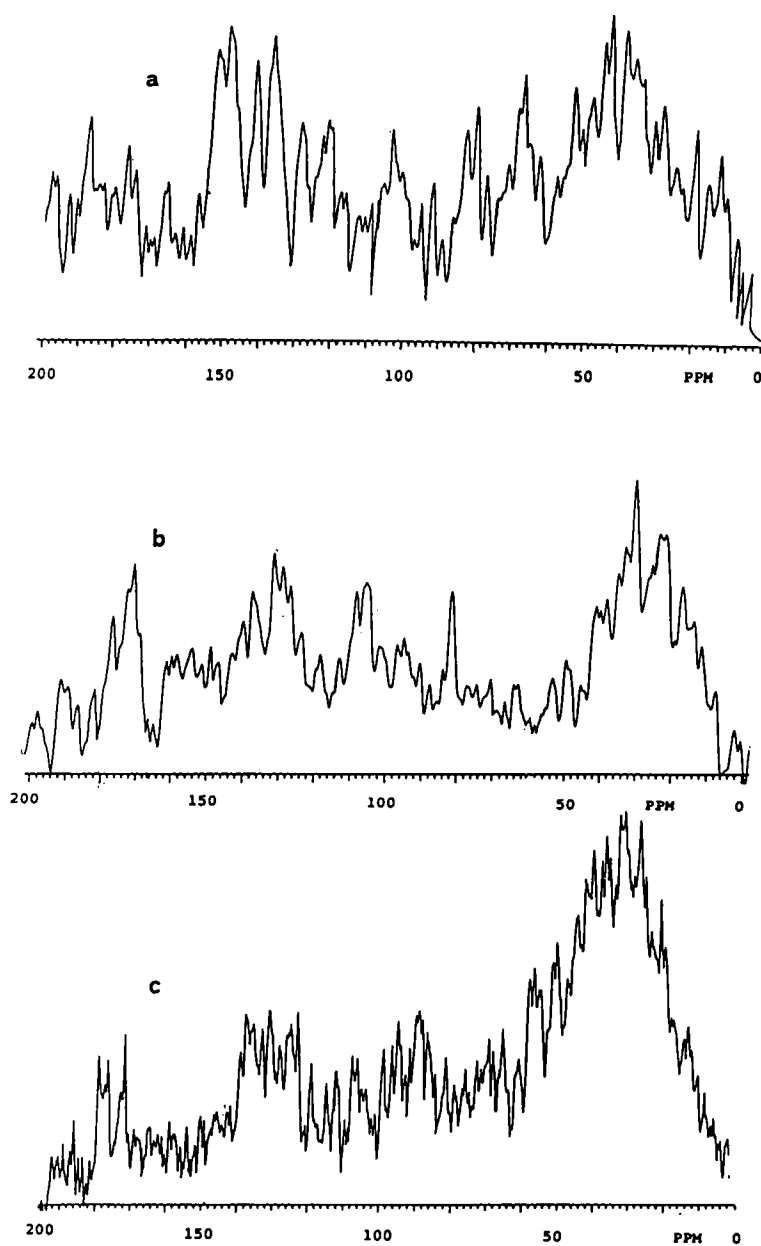


Figure 3 ^{13}C -NMR spectra of residue in HCl (a), fulvic (b) and humic (c) acids.

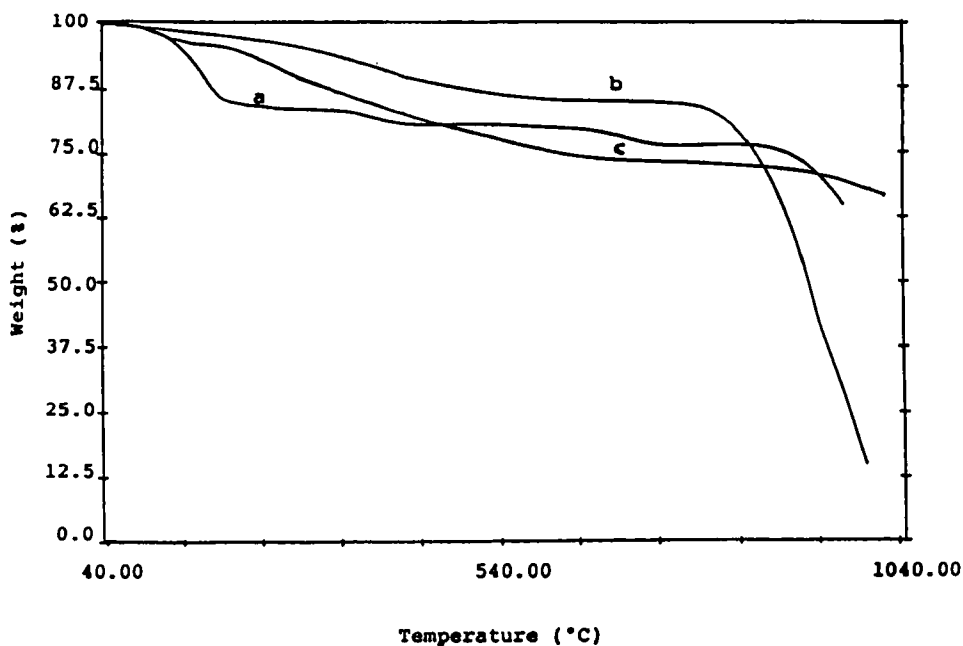


Figure 4 Thermograms of residue in HCl (a), fulvic (b) and humic (c) acids.

(130–140 ppm), carboxyl groups (peaks at 180 ppm and 1720 cm^{-1}) together with aliphatic structures (these last ones are less branched than those present in fulvic acid fraction), aminoacids (peaks at 1640 and 1560 cm^{-1}) and silica (peaks at 1150 – 1030 cm^{-1} and high ash content), bound to the humic acid structures as clay-humic complexes. The very high value of total acidity, and consequently that of phenolic groups, may derive from the silica bound to humic structures²¹.

Putting together the results reported above, free aminoacids or salts of weak organic acids with low molecular weight are not present in the analyzed sediment. Aminoacids take part only in the structures of humic and fulvic acids, probably through hydrogen bonding²². Carbohydrates are present partly free or adsorbed on the humic structures and partly bound to the humic acid structures through an ester linkage²³.

Humic compounds and particularly humic acids, owing to the high contents of amino and carboxylic groups are able to bind large quantities of metals both as salts and complexes or chelates.

The results of chemical analyses in the three solutions derived from selective treatments and the total content are shown in Table 2 for Cu, Pb, Cd, Zn, Fe, Cr, Ni, Co, Mn and Al. The average imprecision at the 95% confidence level, calculated from five replicate determinations is $\pm 5\%$ for total attack ($\pm 3\%$ for Cu and $\pm 4\%$ for Ni), $\pm 8\%$, $\pm 6\%$ and $\pm 10\%$ for the A, B and C solutions respectively.

The extraction efficiency of the three solutions increase from "A" to "C" for all metals

Table 1 Elemental analysis percentage (ash free), atomic ratios and concentration (meq/g) of major oxygen containing groups from HCl residue, fulvic (HF) and humic acids (HU).

| | <i>HCl residue</i> | <i>HF</i> | <i>HU</i> |
|---------------|--------------------|-----------|-----------|
| C | 5.66 | 15.76 | 45.37 |
| N | — | 1.11 | 6.48 |
| H | 6.06 | 2.07 | 6.93 |
| O* | 88.28 | 81.06 | 41.22 |
| H/C | — | 1.57 | 1.83 |
| N/C | — | 0.06 | 0.12 |
| Ash(1000°C) | 64.77 | 15.80 | 64.00 |
| Total acidity | 2.10 | 10.09 | 30.69 |
| -COOH | — | 2.06 | 3.18 |
| Phenolic -OH | 2.10 | 8.03 | 27.68 |
| -CO | — | — | 15.06 |

*obtained by difference

—not significant

except for copper and nickel that show for reagent "B" values higher than for "C". For all metals except lead the sum of the values of the amounts of metals solubilized in the three selective extractions is much smaller than the total contents reported in "T".

Lead is the only metal that shows high concentration values in all the three selective solutions equally distributed. Similar values were formerly found in this sampling site, that is evidently very polluted²⁴. For iron, aluminum and manganese the major part of the metal is present in the residual matrix. Regarding the macroelements, the aluminum content is higher than the iron content, as also reported by Hieke Merlin *et al.*²⁵.

Table 3 shows the results of trace metal analysis performed on humic substances and on humic and fulvic acid fractions.

Analysing the reported data, we must keep in mind that the sample was shaken with 0.1 M HCl before the isolation of humic compounds with basic solution. In this step also a fraction of metals bound at the humic substances may be solubilized for acid-base effect.

Table 2 Concentrations of metals (in µg/g and ng/g for Cd) in the solutions derived from selective extractions (A,B,C) and after total solubilization (T).

| <i>Element</i> | <i>A</i> | <i>B</i> | <i>C</i> | <i>T</i> |
|----------------|----------|----------------------|----------------------|----------------------|
| Cu | 4.3 | 16.7 | 12.1 | 45.6 |
| Pb | 50.2 | 55.6 | 61.7 | 197 |
| Zn | 0.5 | 3.7 | 20.2 | 54.7 |
| Cd | 0.45 | 1.2 | 1.6 | 7.2 |
| Fe | 210 | 4.50×10 ³ | 4.10×10 ⁴ | |
| Cr | - | 6.8 | 8.0 | 91.5 |
| Ni | 4.3 | 16.1 | 8.6 | 108 |
| Co | - | 0.06 | 0.34 | 1.00 |
| Mn | - | 1.2 | 4.5 | 219 |
| Al | 400 | 3.32×10 ³ | 1.27×10 ⁴ | 9.00×10 ⁴ |

Table 3 Concentrations of metals (in $\mu\text{g/g}$ and ng/g for Cd) associated with humic substances, humic and fulvic acids.

| <i>Element</i> | <i>humic substances</i> | <i>humic acids</i> | <i>fulvic acids</i> |
|----------------|-------------------------|--------------------|---------------------|
| Cu | 1.06 | 0.73 | 0.48 |
| Pb | 2.01 | 1.51 | 0.80 |
| Zn | 2.39 | 1.50 | 0.95 |
| Cd | 0.08 | 0.05 | 0.03 |
| Fe | 428 | 219 | 200 |
| Cr | 0.90 | 0.46 | 0.28 |
| Ni | 3.20 | 1.79 | 1.40 |
| Co | 0.04 | 0.02 | 0.015 |
| Mn | 0.15 | 0.12 | 0.07 |
| Al | 668 | 353 | 322 |

Comparing the data reported in Table 3 with that of column "C" (Table 2), we can conclude that the amount of nickel bound to humic substances is high (37% of total content in the organic and sulphide phases). These metal-humic compounds seem not to be affected by the acid-base reaction that occurs in the HCl solution, according with the stability order of humic complexes obtained from different authors^{4,26}. For copper we have 45.6 $\mu\text{g/g}$ as total content, 36.6% bound to Mn-Fe oxides and 26.3% to the sulphide and organic phases, but only 8.8% of the latter is found as metal-humic complexes (Table 3). Whereas copper-humic compounds are affected only in small quantity (1–20% as reported by Pickering²⁷) by the effect of acid-base reactions that occur during the 0.1 M HCl treatment, only low concentrations of this metal are present in solution "C". 37% of total zinc and lead are present in the organic and sulphide phases, but it is not possible to distinguish between these two forms.

The first experiments on HPLC coupled with ICP-AES showed good results for copper, zinc and manganese. Their distributions between high molecular weight (HMW) and low molecular weight (LMW) are summarized in Table 4. The three metals are preferentially bound at the high molecular weight fraction (humic acids), as already hypothesized on the base of functional group content and according to literature data²⁸.

Table 4 Distribution (%) of copper, zinc and manganese in the organic fraction soluble in NaOH.

| <i>Element</i> | <i>HMW 20000</i> | <i>LMW 5000</i> |
|----------------|----------------------|---------------------|
| Cu | 73.4 | 26.6 |
| Zn | 76.7 | 23.3 |
| Mn | 68.0 | 32.0 |

CONCLUSION

From the analysis of all results obtained we can conclude that manganese is present only in inorganic form, as are most of iron and aluminum. The fraction of metals bound to organic compounds is exclusively associated with humic substances and particularly with humic acids.

Among the considered metals, nickel is the metal most strongly bound to humic substances with 37% in the metal-humic compound form. On the other hand, 30–35% of zinc, lead and cobalt are bound to humic compounds.

Moreover, it may be hypothesized that the metal fraction not solubilized in the considered solutions is partly present in the sediment as insoluble inorganic salts and partly bound both at humic acids and silica.

In conclusion, the multi-method analysis proposed in this work seem to be able to provide a good spectrum of informations about the speciation of the metals. The knowledge of the structures of humic and fulvic acids provides then information on the potential of organic matter to participate in biogeochemical cycles and to act as complexing agents towards metals.

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