

# Applications of humic acids in the treatment of heavy metal contamination

S. Mazzuoli, A. Tognazzi, F. Bartolini, S. Loisel & C. Rossi  
*Department of Chemical and Biosystem Sciences, University of Siena, Italy*

## Abstract

Humic substances are macromolecules that naturally occur in all environments in which vegetation matter are present. The group of molecules that are considered humic substances vary greatly and are characterised by an extremely complex structure. Humic substances play a fundamental role in many ecosystems, since they interact with toxic metal ions present in the system, determining a decrease in the bio-availability of such ions. In the present paper, we report an investigation to quantify the ability of humic acids to complex the metal ions. We used Atomic Absorbance Spectroscopy to determine the heavy metal concentrations complexed in different concentrations and environmental conditions by humic acid. This method is based on the use of membranes of known molecular cut off, which were utilised to separate the metal-humic substances complex from the free metal. The results show that the humic acid preparation, utilised in this study, has a large affinity for several metal ions. The values of the calculated global constant indicate that at a pH of 4, the binding strength increases in the order  $Mn < Cd < As < Cu$ .

*Keywords: humic acid, heavy metals, interactions, Atomic Absorbance Spectroscopy, calculated global stability constant.*

## 1 Introduction

Humic substances are abundant, naturally occurring organic compounds which account for 60-70% of soil organic matter and 30-50% of organic matter present in surface waters [1]. They are produced from the degradation of organic matter and are highly polydisperse and chemically heterogeneous materials, with very broad molecular weight distribution (from 5000 to 100000 dalton).



These macromolecules are slightly acidic in solution. They have numerous functional groups such as carboxylic, hydroxyl and amino groups, but also have carbohydrate or amino acid moieties. Their structure determines the brownish colour that characterizes these molecules. Humic substances are divided into three classes based upon their solubility in water: humin is insoluble in aqueous systems at all pH values, humic acid is soluble at  $\text{pH} > 2$  and fulvic acid is soluble at all pH values [2]. This study concerns only the soluble humic acid fraction.

It is known that humic substances bind a large range of metals from “hard” alkali earth metals such as calcium and magnesium to very “soft” metals such as mercury and lead [2]. In general, these macromolecules bind a wide range of metals. With the variation of pH, ionic strength and redox potential conditions these macromolecules can be differently dissociated and different ligands can result. The interactions between cations and humic acid depend on the structural complexity of humic acid which is related to their origin (humic acids that come from a variety of sources, may have different ion-binding properties) and environmental conditions. The interaction also depends on the type of metal present. The interaction between metal ions and ligand is either mediated by water molecules or direct. In first case, it creates a condition of “outer sphere complex” and in the latter case of “inner sphere complex”. The direct interaction between ion and ligand is very strong and scarcely reversible. The environmental consequences of these interactions is related to the apply the humic acids to remove the heavy metals and in this manner to reduce their bio-availability.

Because of their heterogeneity, these macromolecules are very complicated to study. A single humic acid sample can be characterised by many molecules with a broad molecular weight distribution. Each molecule will display a range of binding sites with a different complexation capacity [3], and hence, may not described with a single equilibrium constant. Due to the many structural variations, functional groups in humic acids represent a vast collection of proton- or metal-binding sites that have a distribution of complex stability constants,  $K_i$ . Therefore, the ligands of humic acids can be considered as a distribution of binding sites, where the concentration of the type  $i$  ligand is  $C_i$  [4], [5]. Many method were applied to learn the metal-humic acid interaction and many models have been elaborated [4], [6], [7].

## 2 Model for metal-humic acid interactions

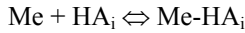
In this study, a simple model was elaborated to analyse the interaction between a standard humic acid (HA) and a set of cations. It is based on the assumption that the functional groups of humic acid can be treated as an ensemble of monoprotic acids or binding sites, even through polyprotic acids are undoubtedly present. For simplicity only 1:1 metal-ligand complexes are considered. A generic type or group of binding sites in humic macromolecules, that have the same complex stability constant to the metal  $\text{Me}$ , are indicated with subscript  $i$  ( $\text{HA}_i$ ). In changing the pH conditions, a change in the dissociation of  $\text{HA}_i$  occurs:



In thermodynamic equilibrium, the expression is

$$K_{\text{HA}_i} = \frac{[H^+] \cdot [A^-_i]}{[HA_i]} \quad (1)$$

As indicated above, the structure and ligand sites can be changed to vary the environmental conditions. At low pH conditions the protonated structure is going to increase and in the presence of a single metal Me type, the equilibrium is:



and the equilibrium constant

$$K_{\text{Me-HA}_i} = \frac{[\text{Me-HA}_i]}{[\text{Me}][\text{HA}_i]} \quad (2)$$

where concentrations in square brackets are in mol/L and  $K_{\text{Me-HA}_i}$  represents the formation or stability constant for every binding sites of type i.

The mass balance equations that need to be considered are

$$C_{\text{Me}} = [\text{Me}] + \Sigma[\text{Me-HA}_i] \quad (3)$$

$$C_{\text{HA}_i} = [\text{HA}_i] + [\text{Me-HA}_i] \quad (4)$$

where  $C_{\text{HA}_i}$  is the concentration of binding sites of type i.

For low analytical concentrations of the metal, it can be assumed that  $C_{\text{HA}_i} \cong [\text{HA}_i]$ , for which it

$$K'_{\text{Me-HA}_i} = K_{\text{Me-HA}_i} \cdot C_{\text{HA}_i} \cong \frac{[\text{Me-HA}_i]}{[\text{Me}]} \quad (5)$$

This binding model described above is widely used and it serves as the start point of more elaborate models [4], [8], [9]. The global stability constant can be examined considering all the binding site of humic macromolecules which can interact with the metal, even if with different “force” and in general;



where all concentrations in square brackets are in mol/L if it is possible to estimate the average molecular weight of the humic acid studied or

$$K_{\text{Me-HA}} = \frac{[\text{Me-HA}]}{[\text{Me}](\text{HA})} \quad (7)$$

where (HA) is the total concentration of humic acid in g/L. The [Me-HA] represents the amount of metal that is bound by the humic acid. If the analytical



concentration of the metal is low can be assume that  $C_{HA} \cong [HA]$  (where  $C_{HA}$  is the analytical concentration of humic acid), the Eq. 6 can be written;

$$K'_{Me-HA} = K_{Me-Ha} \cdot C_{HA} \cong \frac{[Me - HA]}{[Me]} \quad (8)$$

In this study, we have calculated a global stability constant (Eq.6), measuring the total concentration of the metal bound to humic acid.

### 3 Materials and methods

In this study, we have used a commercial humic acid (Humic Acid, Sigma-Aldrich, sodium salt). This material was subjected to a purification process to create a humic acid of know molecular weight and characteristics. To begin, 10 g of commercial solid humic acid were homogenised to obtain a powder, which was dissolved in 200 mL 0.1 M NaOH solution and stirred for 2 hours. The suspension was centrifuged at 4000g for 30 minutes to remove the humin (insoluble fraction). The supernatant was collected and then acidified at  $pH \cong 2$  with 1 M HCl solution. The solution was stored overnight at room temperature. The humic acid (precipitate) was centrifuged at 4000g for 30 minutes to remove the soluble fraction (fulvic acid). The humic acid precipitate was washed twice with 5M HCl-5%HF solution to remove any inorganic solid present in the sample. After the HCl/HF treatment, the solid was washed with MilliQ water. This humic acid was freeze-dried [10]. After this operation, the humic acid was dissolved using 20 mL of 0.1M NaOH solution and stirred for 2 hours. The suspension was centrifuged at 4000g for 20 minutes, filtered (0.45  $\mu m$ , Millipore filter) and freeze-dried. This material was utilised to determine the global stability constant in the metal interaction studies.

The standard solution of the Purified Commercial Humic Acid (PCHA) was analysed to identify its distribution of relative molecular weights. A set of centrifugal filters (membranes in cellulose regenerate) with a known cut-off (Centricon Plus-20, Amicon Bioseparations, Millipore Corp.) was used. The applied molecular weight cut-offs were by 100,000, 30,000 and 5,000 NMWL (Nominal Molecular Weight Limit). The determination of the HA concentration, in every molecular fraction, was made utilising UV/VIS spectroscopy [11], [12], [13]. The absorbance measurements were made on a Pharmacia Biotech Ultrospec® UV/VIS Spectrophotometer in 0.01 m path length quartz cuvettes. The filtered standard solution of PCHA was analysed by spectrophotometric methods and found to contain humic acid with a relative molecular weight between 30.000 and 100.000 NMWL, in agreement with literature data [14].

The absorbance of humic substances in the UV/VIS spectral region decreases with increasing wavelengths. This phenomenon is linked to the aromatic ring structure that constitutes a major part of the function groups of the humic substances formed from the degradation of lignin-cellulose materials. The responsible electronic transition is of the type  $\pi \rightarrow \pi^*$  and occurs in the wavelength region between about 270 and 280 nm for benzoic acids, aniline



derivates, polyenes and polycyclic aromatic hydrocarbons with two or more rings. A number of wavelengths have been used to measure DOM (Dissolved Organic Matter) and HA [15]. The wavelength 272 nm was selected as this wavelength permits a greater sensibility.

The reference heavy metal solutions used were 1 g/L of Mn ( $\text{Mn}(\text{NO}_3)_2$  in 0.5M  $\text{HNO}_3$ , CertiPUR® Merck), 1 g/L of Cu ( $\text{Cu}(\text{NO}_3)_2$  in 0.5M  $\text{HNO}_3$ , CertiPUR® Merck), 1 g/L of Cd (metallic Cd in 0.5M  $\text{HNO}_3$ , CertiPUR® Merck), 1 g/L ( $\text{H}_3\text{AsO}_4$  in 0.5M  $\text{HNO}_3$ , CertiPUR® Merck).

The method applied to study the metal-humic acid interactions is showed in Figure 1.

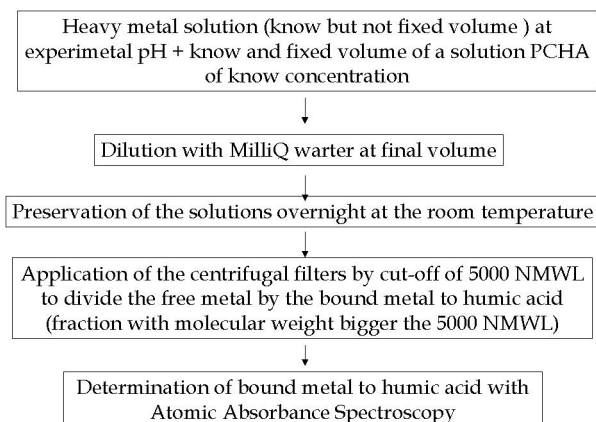


Figure 1: Methodological approach applied to study the metal-humic acid interactions.

Atomic Absorbance Spectroscopy was used to determine the concentration of metals bound by humic acid. The atomisation method that was used it was of electro-thermal type, that it has permitted to detect the concentrations of  $\mu\text{g/L}$  order. The concentrations of the solution of the purified humic acid analysed to the interaction study were 8.7 and 15.7 mg/L, the pH investigated were 4 and 7. The range of heavy metal concentrations was comprised between 0 and about  $2 \times 10^{-5}$  mol/L.

## 4 Results

The concentrations of complexed and free metal concentrations of Manganese-Humic Acid and Cadmium-Humic Acid are reported in Figure 2 and Figure 3 in low analytical concentrations of each heavy metal. The correlation between [Me-HA] and [Me] is good, in fact the  $R^2$  change between 0.94 and 0.98. The significant affinity of humic acid for the complexation with heavy metals and the resulting an high stability of the metal-humic acid complexes can be seen.



Figure 4 shows the relationship between the metal-humic acid interactions and the pH values. In general, the global stability constant is higher at a neutral pH respect to more acid conditions, due to a greater amount of the free binding sites not occupied by proton ions, and hence available to interact with metal ions.

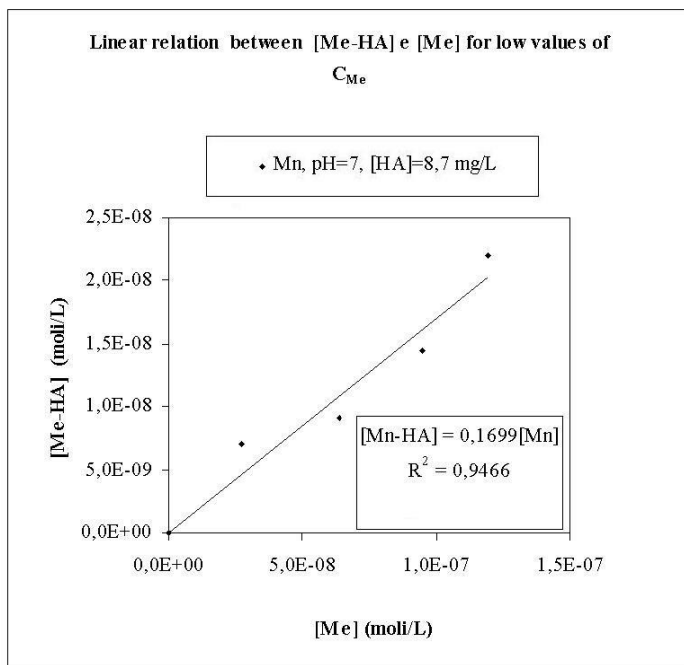


Figure 2: The relationship between Manganese bound in Humic Acid and original Manganese concentrations in solution, in conditions of low analytical concentrations of heavy metal.

Figure 4 shows the typical course of bound metal by humic acid when the analytical concentration of metal ions increase, and the binding sites upon the humic acid saturate.

Table 1: Minus Logarithm Calculated Global Stability Constants ( $pK_{Me-HA}$ 's) and data present in literature for some metals (Stevenson, 1982). The values written in bold style it referred at the calculated constants in this work.

pH	Cu	Cd	As	Mn
4	<b>6.8</b>	<b>6.4</b>	<b>6.6</b>	3.7
7	7.2	<b>6.6</b>	-	<b>5.7</b>

The minus log of calculated global stability constants ( $pK_{Me-HA}$ ) is reported in table 1 as well as those reported in the literature for some heavy metals [2], [6].



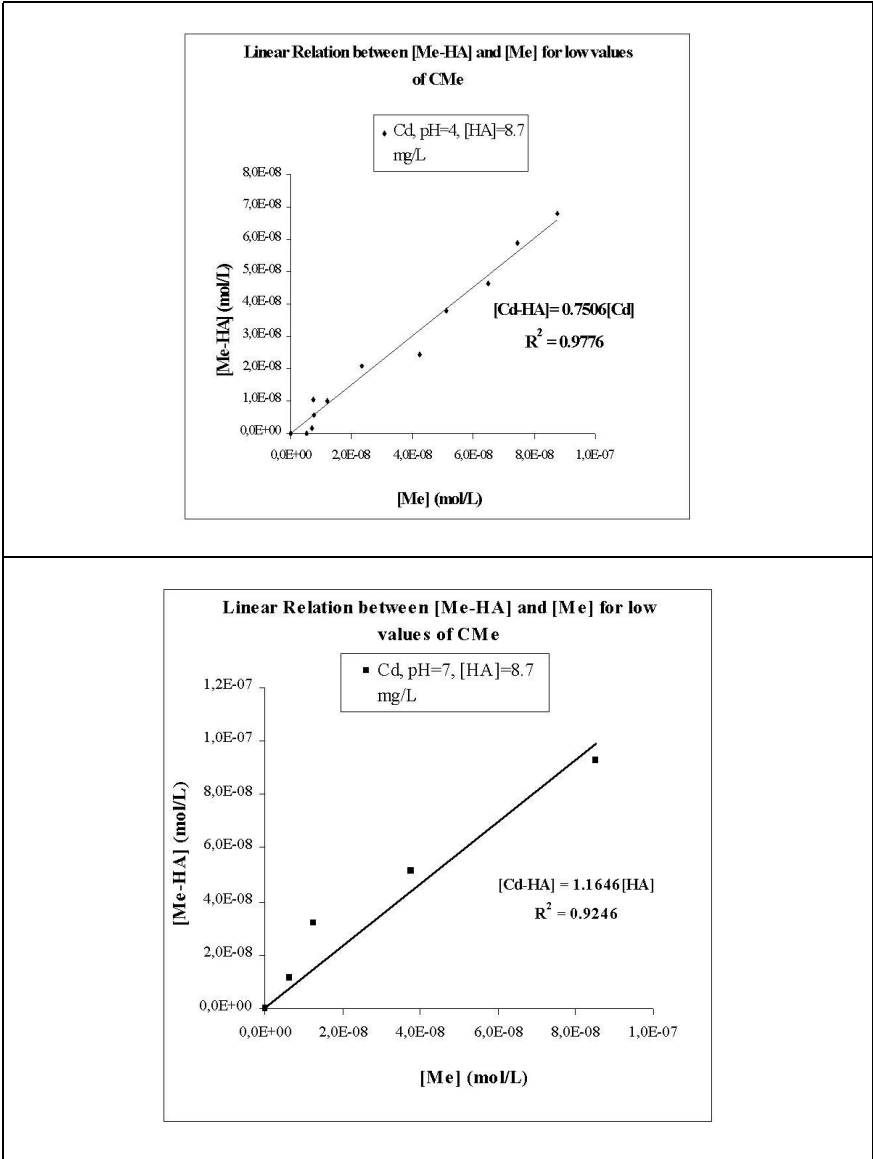


Figure 3: The relationship between Cadmium bound in Humic Acid and original Cadmium concentrations in solution, in conditions of low analytical concentrations of heavy metal.

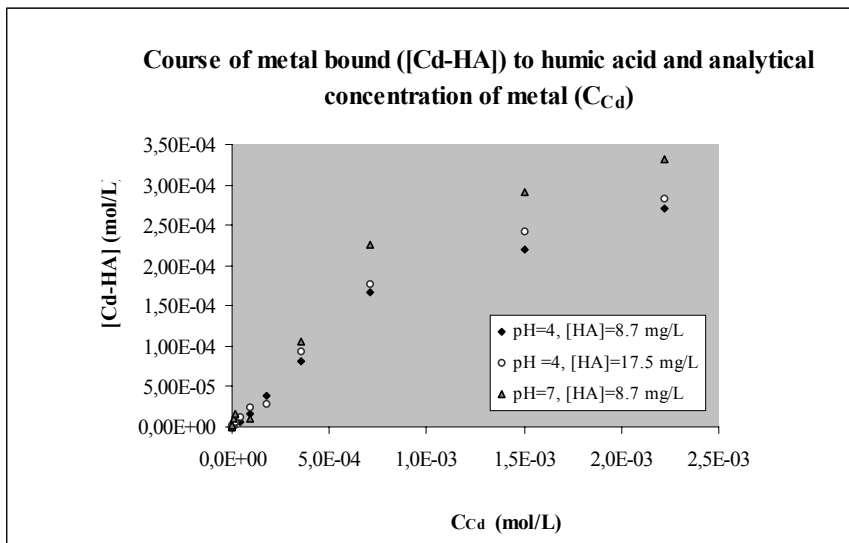


Figure 4: Saturation curve Cadmium interaction with humic acid at different experimental condition (pH).

## 5 Conclusion

The analytical method developed and applied here to determine the stability constants for the interaction between humic acid and heavy metal ions proved to be useful and efficient. Using this approach it was possible to quantify the capacity of humic acids, present in both soil and aquatic environments to complex low concentrations of heavy metals that are often available in contaminated sites. The calculated global stability constants and the pK values referred to the purified humic acid employed in this study and to the experimental conditions (pH) and contact time. However, the strong interaction is evident. In wetland ecosystems, where humic materials and water remain in constant contact, such complexation capabilities may provide a fundamental barrier to the release of contaminated waters and soils into non-contaminated areas. Further testing in such areas would provide needed information in the further understanding of the capacity of these macromolecules to mitigate the dispersion of point sources or extensive contamination.

## References

- [1] Thurman, E.M., Malcolm, R.,L., *Preparative Isolation of Aquatic Humic Substances*, Environ. Sci. Technol., 1981, 15, 4.
- [2] Stevenson, F., J., *Humic Chemistry: genesis, composition, reactions*, John Wiley & Sons, New York, 1982





- [3] Reuter, J.H., Perdue, E.M., *Importance of heavy metal-organic matter interactions in natural waters*, *Geochimica et Cosmochimica Acta*, 1977,41.
- [4] Susetyo, W., Dobbs, J.C., Carreira, L.A., Azarraga, L.V., Grimm, D.M., *Development of a Statistical Model for Metal-Humic Interactions*, *Anal. Chem.*, 1990, 62.
- [5] Susetyo, W., Carreira, L.A., Azarraga, L.V., Grimm, D.M, *Fluorescence techniques for metal-humic interactions*, *Fresenius J. Anal. Chem.*, 1991, 339.
- [6] Tipping, E., Hurley, M.A., *A unifying model of cation binding by humic substances*, *Geochimica et Cosmochimica Acta*, 1992, 56.
- [7] Bryan, N.D., Jones, D.M., Appleton, M., Livens, F.R., Jones, M.N., Warwick, P., King, S., Hall, A., *A physicochemical model of metal-humate interactions*, *Phys. Chem. Chem. Phys.*, 2000, 2.
- [8] Hummel, W., Glaus, M.A., Van Loon, L.R., *Trace metal-humate interactions. The „conservative roof” model and its application*, *Applied geochemistry*, 2000, 15.
- [9] Glaus, M.A., Hummel, W., Val Loon, L.R., *Trace metal-humate interaction. I. Experimental determination of conditional stability constants*, *Applied geochemistry*, 2000, 15.
- [10] Monteil-Rivera, F., Brouwer, E.B., Masset, S., Deslandes, Y., Dumonceau, J., *Combination of X-ray photoelectron and solid-state <sup>13</sup>C Nuclear Resonance Spectroscopy in the structural characterisation of humic acid*, *Analytica Chimica Acta*, 2000, 424
- [11] Traina, S.J., Novak, J., Smeek, N.J. *An ultraviolet absorbance method of estimating the percent aromatic carbon content of humic acids*. *J. Environ. Qual.*, 1990, 19
- [12] Peuravuori, J., Pihlaja, K. *Molecular size distribution and spectroscopic properties of aquatic humic substances*. *Anal. Chim. Acta*, 1997, 337.
- [13] Hautala, K., Peuravuori, J., Pihlaja, K. *Measurement of aquatic humus content by spectroscopic analyses*. *Wat. Res.*, 2000, 34.
- [14] Graso, D., Chin, Y.P., Weber, W.J., *Structural and behavioural characteristics of commercial humic acid and natural dissolved aquatic organic matter*, *Chemosphere*, 1990, 21, 10-11.
- [15] Kirk, J.T.O. *Light and Photosynthesis in Aquatic Ecosystems*. Cambridge Press, Cambridge 1994.

