

EXPERIMENTAL METHODOLOGY FOR THE STUDY OF THE INTERACTION BETWEEN METAL – ORGANIC COMPLEXES AND MICROBIAL BIOMASS IN AQUATIC SYSTEMS

Research Team

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1. Introduction

Dissolved organic matter (DOM) represents a large and chemically diverse organic carbon pool in aquatic environments. DOM consists of a variety of macromolecules that may provide active sites which may bind a variety of contaminants and especially metal ions. Due to its complexation capacity DOM may influence the fate and transport of metals, potentially reducing their bioavailability and toxicity to marine and estuarine fauna (Haitzer, Hoss, Traunspurger, & Steinberg [1]). In fact, studies have shown that DOM reduces the bioavailability of various metals to aquatic organisms and alter their fate in the environment (Guo, Santschi and Ray [2]).

DOM could be classified into three categories concerning their ability to complex metals:

1. Strong organic ligands (Synthetic chelators of heavy metals, such as ethylenediamine tetra-acetic acid (EDTA) and nitrilotriacetic acid (NTA)).
2. Intermediate organic ligands (Humic Substances (HS)).
3. Weak organic ligands (Simple organic acids).

According to literature, the parameters that determine the formation and behavior of organic - metal complexes between strong organic ligands (EDTA, NTA) and metals, have been extensively studied (Alder, Siegrist, Gujer and Giger [3] and White and Knowles [4]). Moreover the study of the impact of weak organic ligands (simple organic acids) in the mobility and bioavailability of the metals is of secondary interest

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since they form weak and instable complexes. On the other hand, the understanding of the behavior of intermediate organic ligands (humic substances) is in an early state because of the complexation and heterogeneity of their nature, their isolation methods and the conventional methods used for its determination. For the above mentioned reasons the present project was focused on the study of the formed complexes between humic substances and metals in aquatic environment.

Humic substances consist 70 – 80 % of the organic matter in aquatic systems. Humic substances are naturally occurring, heterogeneous organic substances that are generally dark – colored (yellow to black), recalcitrant to biological degradation, and high in molecular weight (from hundreds to many thousands of Daltons). Humic substances are formed largely as a result of microbial activity on plant material, but further polymerization can occur abiotically. The resulting compounds are relatively resistant to further microbial degradation and tend to have low turnover rates in aquatic systems (Wetzel [5]).

The reactions between humic substances and metals are highly complex and depend on the metal, humic material and pH, according to Logan, Pulford, Cook and Mackenzie [6]. The understanding of the complex interactions of HS with metals requires a better knowledge of the structure and reactivity of HS. The lack of knowledge about the humic substances structure and the humic – metal interactions causes problems on the prediction of its chemical and biological effect in the environment.

A variety of analytical methods have been established in an attempt to understand the humic – metal complexing (Tipping [7]). In this study a method consisted of a high-performance liquid chromatography system (HPLC), equipped with a size exclusion column (SEC) (Rottmann and Heumann [8] and Vogl and Heumann [9]) and an ion selective electrode (ISE), was established in an attempt to investigate metal binding to humic substances.

Furthermore in the present research project, preliminary results on the interaction of humic – metal complexes with inactive as well as active microbial biomass.

In the present project the proposed research was mainly supported by the development of an accurate and precise analytical methodology in order to study the complexation mechanisms between metals and humic substances and of its interaction with microbial biomass.

2. Experimental

2.1 Sample preparation

Synthetic solutions of known initial concentration in organic substance and metal are prepared.

The organic substances which were used as organic ligands are:

1. Strong Organic Ligands:

- a) EDTA (Sigma – Aldrich)
- b) NTA (Sigma – Aldrich)

2. Intermediate Organic Ligands:

- a) Purified Suwannee River humic and fulvic acid isolated by the International Humic Substances Society (Humic acid (IHSS) and Fulvic acid (IHSS))¹.
- b) Humic acid (Aldrich).

2.2 Instrumentation

The organic substances determination is performed by a high-performance liquid chromatography system Metrohm.

Potentiometric measurements were performed with the aid of a pH-meter (Orion 420A). Copper selective electrode made possible the determination of the free copper ions.

The obtained chromatographs by the liquid chromatography system concerning humic substance solutions are given in figure 1(a), where it is obvious that the retention time for the standard Suwannee River humic acid is about 9.6 – 10.3 min.

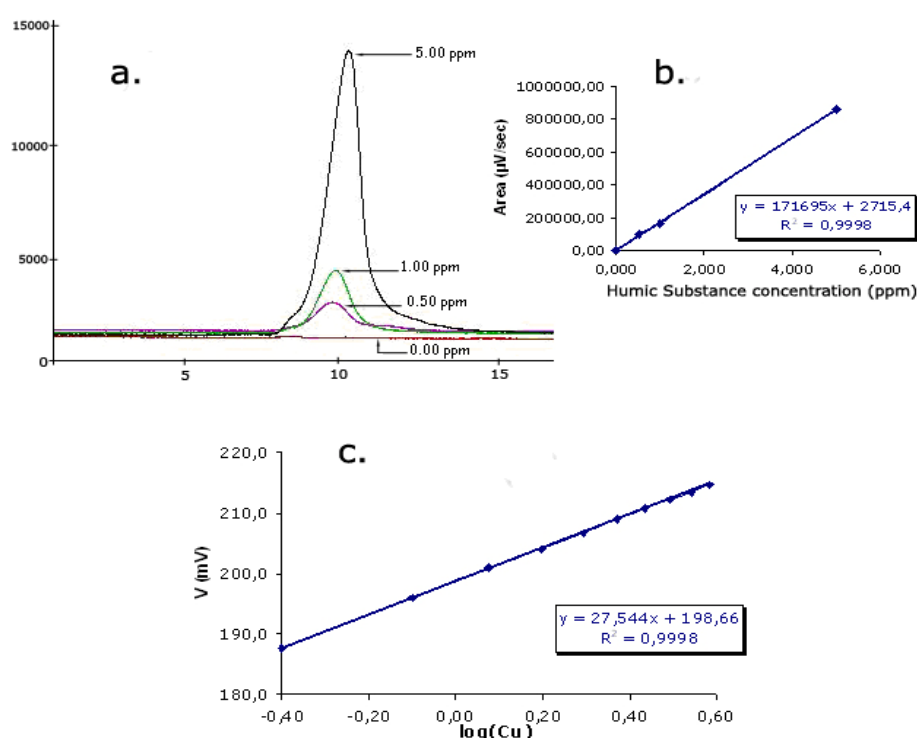


Figure 1. (a) Representative chromatographs of humic acid (HPLC – SEC), (b) Humic acid representative curve (HPLC – SEC), (c) Free copper ions representative reference curve (where $\log_{(Cu)}$: logarithm of the concentration of dissolved copper ions). (ISE– Cu)

A representative calibration curve for humic acid is shown in figure 1(b). The calculated detection and quantitation limit and the relative standard deviation of a sample containing 5 mg L^{-1} humic acid (Aldrich), are 0.30 mg L^{-1} , 0.35 mg L^{-1} and 0.0029, respectively. The good linearity of the calibration curves in combination with the small standard deviation of dissolved free humic acid concentrations determined, suggest that method developed is well suited for quantitation.

An example of calibration of the copper selective electrode is shown on figure 1(c).

¹ The isolation and purification method of humic/fulvic acid (IHSS) have been described by Aiken [10].

3. Results and Discussion

3.1 Strong Organic Ligands (EDTA – NTA)

The experimental results of the tested solutions determined by the ion selective electrode showed that EDTA/NTA bind the added copper with a mole proportion equal to 1:1, which is in accordance with the literature.

Furthermore, the HPLC system succeeded to determine qualitatively the formed complexes, but the system met difficulties in the quantitative determination of the formed complexes, as the HPLC system failed to distinguish the peaks of the free and complexed organic ligands.

3.2 Intermediate organic ligands (Humic Substances)

Knowing the initial concentrations of humic substance and copper of the synthetic solutions and by determining, the concentrations of the free humic substance (HPLC) and the free copper ions (ISE), it is possible to calculate the concentration of the complexed humic substance and the complexed copper from the equations (1) and (2).

$$HS_{total} = HS_{free} + HS_{complexed} \quad (1)$$

$$Cu^{++}_{total} = Cu^{++}_{free} + Cu^{++}_{complexed} \quad (2)$$

Figure 2 represents the decrease of free humic substance as a result of the increase in the concentration of the added dissolved Cu in the tested solutions.

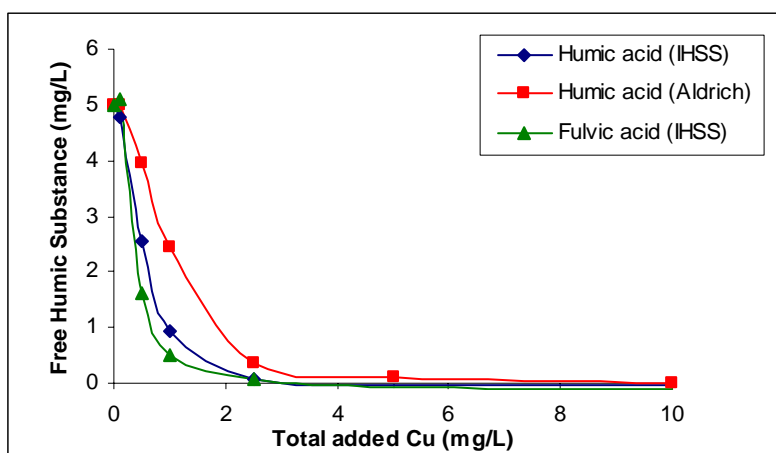


Figure 2. Influence of the concentration (mg L^{-1}) of the total added Cu to the concentration (mg L^{-1}) of free humic substance.

From figure 2 it is obvious that when the mass proportion of total metal Cu to total humic is equal to $\frac{1}{2}$, the free humic substance tends to zero. This behavior is similar to all the tested humic substances (Humic acid (IHSS), Fulvic acid (IHSS) and Humic acid (Aldrich)).

The potentiometric results of the tested solutions made possible the calculation of the complexed copper (equation (2)). Figure 3 provides information on the effect of the nature of humic substance on the concentration of complexed copper to humic substances.

From figure 3 it is obvious that the series of the complexation capacity of humic substances is:

$$\text{Fulvic Acid (IHSS)} > \text{Humic Acid (IHSS)} > \text{Humic Acid (Aldrich)}$$

In figure 3 someone can figure out that in the case of the humic acid (IHSS) and fulvic acid (IHSS), the complexed copper concentration is increased as the added copper is increased into the solution. The total humic substance in the solution is 5 mg L^{-1} and, as presented in figure 2, when the copper added to the solution exceeds 2.50 mg L^{-1} , the free humic substance tends to zero. However, the increase of the complexed copper above 2.50 mg L^{-1} of added copper shows that humic acid (IHSS) and fulvic acid (IHSS) have the ability to bind an extra amount of copper, despite the fact that there are no free humic/fulvic acid molecules into the solution. This humic/fulvic acid behavior is a result of the numerous active sites available by the molecule. Moreover, in figure 3, it is apparent that humic acid (Aldrich) is more rapidly saturated by copper, than the other two tested humic substances.

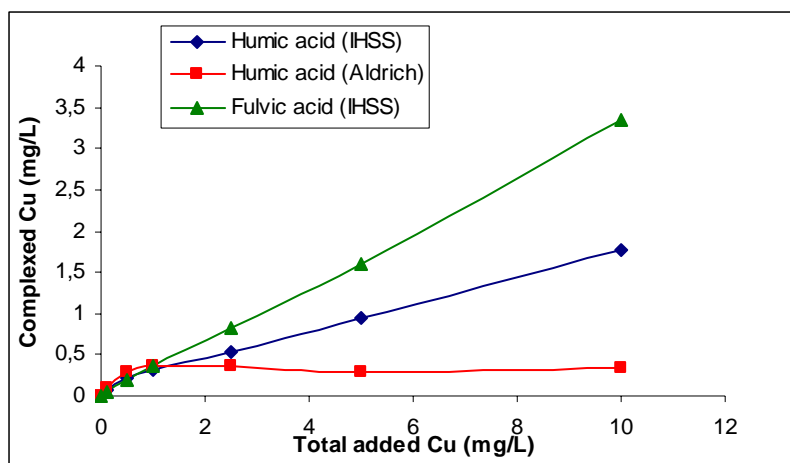


Figure 3. ◆ complexed Cu concentration (mg L^{-1}) to humic acid (IHSS) as a function of the total Cu added (mg L^{-1}), ■ complexed Cu concentration (mg L^{-1}) to humic acid (Aldrich) as a function of the total Cu added (mg L^{-1}), ▲ complexed Cu concentration (mg L^{-1}) to fulvic acid (IHSS) as a function of the total Cu added (mg L^{-1}), (Total Initial humic substance concentration : 5 mg L^{-1}).

3.3 Interaction of Humic -Metal Complexes with Microbial Biomass

During the preliminary experimental testing of the interaction of the humic - metal complexes with inactive and active microbial biomass, interferences have been observed during the measurement of the free humic substance with the aid of the HPLC system. Figure 4 shows a representative chromatograph of humic substance – copper solution after contact with microbial biomass, where the interferences in the free humic substance determination are obvious.

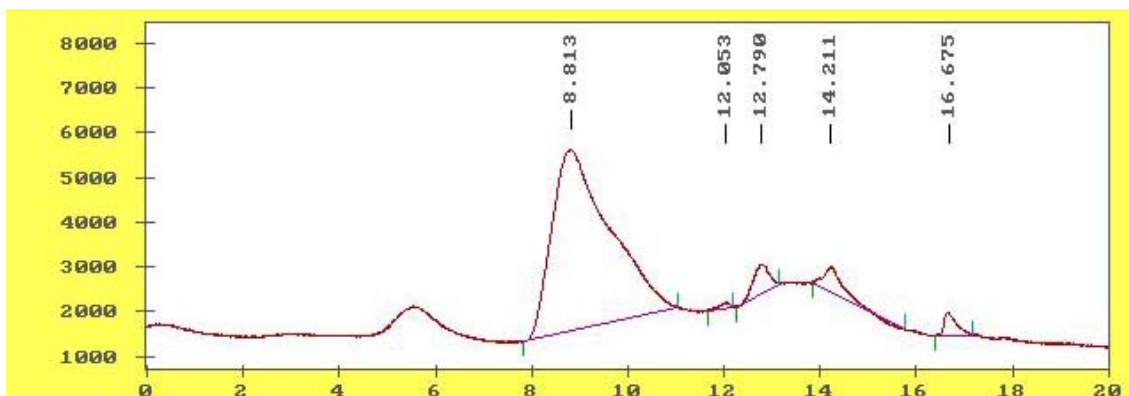


Figure 4. Representative chromatograph of humic substance – copper solution, after contact with microbial biomass.

4. Conclusions - Perspectives

The main result of the project is the development of an accurate and precise analytical methodology in order to approach the binding mechanisms of metals to humic substances.

The interaction of the formed complexes with microbial biomass in aquatic systems is also a main target of our research effort.

The experimental results mean that the phenomenon of Cu binding to humic substances molecules can be successfully studied by the developed analytical method.

Further work will focus to:

- Development of a new methodology for studying the interaction of humic substances – metal – microbial biomass.
- Identification of the active sites which are available from the humic substances for the binding of metals using spectroscopic methods (NMR).

Furthermore, two scientific articles will be published:

1. Copper Binding to Humic Acid Determined by Using a High-Performance Liquid Chromatography System and an Ion Selective Electrode (submitted to *Analytica Chimica Acta*)
2. Kinetic study of copper binding to various humic substances.

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