Participation of cadmium (II) and copper (II) ions in intermolecular forces of humic acids in solutions

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Abstract. Using membrane ultrafiltration and size exclusion chromatography (SEC), the effect of Cd (II) and Cu (II) ions on the molecular weight distribution of humic acids (HA) in solution was investigated. It was shown that Cu (II) ions, unlike Cd (II), can take part in the formation of supramolecular HA associates (> 10 kDa).

1. Introduction
Humic acids (HA) have a major role in the transport elements in soils and natural waters. Acting as macroligands, HA molecules not only change the migration parameters of metal ions in the environment, but also affect the mechanisms of ion transport into living cells.

Initially, special attention was paid to the study of the formation of strong complexes of metal ions with HA with the participation of donor-acceptor and ionic interactions [1-3]. Afterwards, the attention of researchers focused on weak intermolecular interactions between humic acid molecules in solutions, leading to the formation of supramolecular associates (complexes, supramolecules) [4-6]. Metal ions can take part in the formation of such associates, built at the expense of weak interactions (hydrogen bonds, hydrophobic interactions), but this phenomenon remains poorly understood.

The aim of the work was to study the role of Cd (II) and Cu (II) ions in the molecular and supramolecular organization of HA in solution.

2. Objects and methods
We used Aldrich HA (cas. No. 1415-93-6), dissolved in 0.01 M NaOH and adjusted to pH 6 with 1 M HCl. CdCl2 or CuCl2 solutions to a concentration of 50 μM were added to the HA solution (100 mg/l) and incubated in the dark at 20 °C for 12 hours.

The separation of the HA into fractions was performed by 2 methods: ultrafiltration and size exclusion chromatography. It was used membrane filter 10 kDa (Millipore) and stirred cell (Amicon) for HA’s ultrafiltration. Exclusion chromatography (SEC) was performed by HPLC (Agilent 1100 with a diode array detector; Ultropac G2000SW column, LKB; eluent: 0.1 M Na-phosphate buffer (pH 7) with 0.1% SDS).

Determination of Cd and Cu content in samples was carried out on an Agilent 5110 ICP-OES inductively coupled plasma optical emission spectrometer.
Organic C in the samples was determined on a liquid elemental analyzer (LiquiTOC trace, Elementar). To estimate the proportion of metal ions associated with HA, we used data on the content of COOH groups in HAs, taken from the literature [7].

3. Results and discussion
The introduction of metal ions into the HA solution does not cause, according to SEC HPLC a noticeable change in the molecular weight distribution of HA (table 1, figure 1). Simultaneously, the results of ultrafiltration (table 2) show that the introduction of Cu (but not Cd) ions into the solution containing HAs causes a noticeable increase in the fraction fraction> 10 kDa.

| Variant  | Initial HA (kDa) | HA >10 kDa (kDa) | HA <10 kDa (kDa) |
|----------|------------------|------------------|------------------|
| HA       | 29300            | 30400            | 500              |
| HA+Cd    | 29700            | 31900            | 650              |
| HA+Cu    | 30200            | 31600            | 730              |

Table 2. Concentration of total organic carbon, carboxyl groups and metals before and after fractionation.

| Variant | C (mg/l) | Metal (µM) | COOH-group (µM) | Metal / COOH |
|---------|----------|------------|-----------------|--------------|
| HA      | 37       | -          | 320             | -            |
| HA+Cu   | 24       | -          | 210             | -            |
| HA+Cu >10 kDa | 12 | - | 100 | - |
| HA+Cu < 10 kDa | 37 | 50 | 320 | - |
| HA+Cd   | 26       | 38         | 230             | 0.17         |
| HA+Cd >10 kDa | 9 | 7 | 80 | 0.09 |
| HA+Cd < 10 kDa | 37 | 50 | 320 | - |
| HA+Cd < 10 kDa | 10 | 19 | 90 | 0.21 |

When using the method of ultrafiltration separation occurs at the level of individual molecules HA and supramolecular associates. In SEC method we used an eluent based on phosphate buffer (possible competitive binding of Cd and Cu ions in solution) and surfactant (SDS), which blocks hydrogen bonds and hydrophobic interactions, therefore even if solid binary Cu-HA complexes (and to a lesser extent Cd-HA) are formed in solution, SEC HPLC will not reveal a significant increase in the molecular masses of HA. A small part of the carboxyl groups associated with metal ions, also reduces the probability of significant changes in the molecular weight distribution of HA (table 2).

Simultaneously, the existence of supramolecular structures formed due to weak interactions (for example, HA·Cu·HA) can be registered by ultrafiltration, but not by the SEC method using “aggressive” eluent. The formation of supramolecular complexes with the predominant binding of high molecular weight HA fractions was studied with the introduction of iron ions [8] and cobalt into solutions of HAs (Aldrich) [9].
Figure 1. Chromatogram of unfractionated HA (1), fraction >10 kDa (2) and fraction <10 kDa (3). A – initial HA, B – HA+Cd, C – HA+Cu.

In early studies [2,3], the formation of supramolecular associates of the HA·M·HA type was recorded by gel filtration using “soft” eluents (water, diluted alkali), but the separation of these associates by the strength of ion binding metal in these conditions is impossible to hold. Simultaneously, a combination of ultrafiltration and SEC HPLC methods allows not only to detect supramolecular structures formed by weak intermolecular interactions in systems containing metal ions and humic acids, but also to separate them from more durable metal ion complexes with HA.

It can be assumed that the initial stage of the formation of labile supramolecular complexes is the formation of binary complexes of the type M-HA, in which the carboxyl groups of the macroligand participate. After partial compensation of a negative charge on a macroligand, the probability of its interaction with another macroligand increases, which leads to the formation of structures like M-HA·HA or M-HA·HA-M. A similar situation occurs when joining metal to a solution of supramolecular structures HA·HA. Figure 2 shows a diagram of this interaction.

The structure identified as a standard of fulvic acid IHSS was used as a macroligand [10]. The pK value for two adjacent COOH-groups is less than 2, therefore, they will be deprotonated at pH values characteristic of soils and natural waters. Another aliphatic COOH-group has a pK of 4.8–5.0 and, therefore, will be partially protonated at pH below 6. At the first stage, the metal ion is bound to form a bidentate 5-membered cycle. The probability of this reaction is proportional to the stability of the chelate complex formed, as a rule, complexes involving Cu (II) are stronger than with Cd (II) [2]. Further interaction proceeds with the participation of the protonated COOH-group, which forms a dimer with a similar functional group of another macroligand. Other types of weak interactions (hydrophobic, with the participation of H2O bridges, etc.) can also occur [10]. HA benzene rings can also form an additional bond between two molecules by pi stacking (figure 3).
Figure 2. The formation of intermolecular complexes of humic acids with metals by hydrogen bonds.

Figure 3. The formation of intermolecular complexes of humic acids with metals by hydrogen bonds and pi stacking.

These supramolecular structures formed by organic molecules and metal ions are more sensitive to the physico-chemical parameters of the environment compared to the complexes of M-HA or HA-M-HA, formed by donor-acceptor bonds or ionic interactions. The study of the entire spectrum of particles formed in solutions containing HA and metal ions is necessary both in the study of metal migration in ecosystems and in assessing the bioavailability and toxicity of metals for living organisms in soils and surface waters.

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