

Introduction to Humic Substances: Compilation of data and Fundamental Concepts

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Abstract. Humic substances (HS) are still a challenge to understand and define even after centuries of study. HS are present everywhere, in soil or in natural waters, and play an important role in the environment. Even though it is not fully explained, scientists have observed some common features about the chemical and molecular composition and behavior under certain conditions for the HS, which allows the advancement of science and practical applications, such as in agriculture and in the treatment of places contaminated by heavy metals, organic or inorganic compounds. These uses are only possible because HS exhibit enormous variability in terms of functional groups, molecular size and behavior. A number of factors from the extraction method used to the mineralogy of the soil or characteristics of the water where the HS are sampled affects their features. Some general statements can be made about HS, which are broadly defined as structurally complex macromolecules resulting from the microbiological decomposition of organic matter, however details about its origin and structure are still unclear and are treated as a black box. In addition, it is still not possible to study HS *in situ* and some authors question whether samples obtained by classical extraction methods are able to represent faithfully the HS. In this context, this article compiles some information about molecular composition, molecular mass, colloid properties, interactions of HS with the environment, factors that affect its behavior or structure and some applications studied. This article aims to introduce the subject, explaining simple concepts and providing useful references.

Keywords. Humic substances, humification, soil, natural organic matter, colloidal particles

1. Introduction

Soil composition, specifically Natural Organic Matter (NOM), is influenced by several factors, such as mineralogy and microorganism ecosystems, which lead to biochemical transformations of matter through chemical reactions like polymerization. In this way, samples collected from the same soil, but at different depths, will have different characteristics, for example [1]. The evolution of NOM in the soil is better visualized in Figure 1, which summarizes the main factors and processes that influence its characteristics.

Therefore, the soil is classified as a complex and diverse matrix, which still presents aspects without consensus in the scientific community, as is the case of humic substances (HS), which constitute about 60-70% of the NOM [2]. In addition to soil, HS are also present in aquatic systems, whose research is more recent and has become frequent since HS can bind to different pollutants [3, 4].

To simplify the study of HS, generally they are classified into three different categories based on their solubility in water: humin is insoluble, humic acid (HA) is soluble only at pH greater than 2, and fulvic acid (FA) is soluble in all pH ranges [5].

The composition of HS is formed by carboxyl, phenolic hydroxyl, alcoholic hydroxyl, carbonyl, possibly quinone, methoxyl, ether, ester and ketone groups may be present [6, 7]. In general, HA and FA have similar concentrations of phenols, ketones and ethers, with FA being more alcoholic. Furthermore, FA structures are usually less aromatic than HA and have more carboxylic acid, phenolic, and ketonic groups [8]. For this reason, HA are less soluble at low pH (carboxylate groups are protonated) while FA are completely soluble [9]. In general, HS are characterized as structurally complex macromolecules present in soils and natural waters resulting from the microbiological decomposition of organic matter, such as plant or animal residues [10].

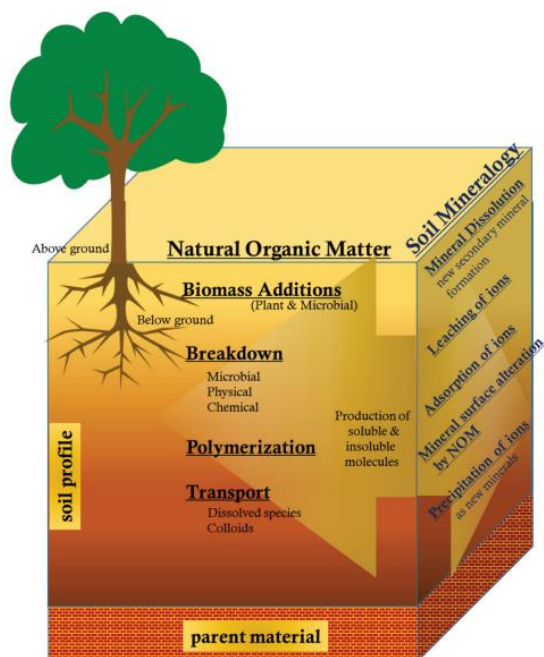


Fig. 1 - Synthesis of the main processes that affect NOM and soil mineralogy, showing how the accumulation of organic matter on the surface is linked to the rest of the soil layers. Source: [1]

Alkaline extraction is still the most used in HS research, a method first performed by Franz Karl Achard in 1786, which was adapted and modernized according to scientific advances. Some authors argue that this technique does not faithfully represent the HS in the soil [11]. In addition, there is also no consensus on the formation and origin of HS, nor on its composition and structure. Some authors argue that HS are linear macromolecular polyelectrolytes capable of aggregating under certain conditions, such as acidic pH and high ionic strength; others argue that HS are molecular aggregates of small molecules units by weak attractive forces, without macromolecular characteristics [12]. These are just some of the challenges involving HS.

The main difficulty in understanding and classifying HS is due to its great heterogeneity, mainly in relation to its structure and size [10]. In this context, the present article proposes to carry out a systematic review about the different colloid structures in a soil solution or in leachate, knowing that colloids are particles smaller than 10 μm that are usually suspended in water [13].

To achieve this goal, information from different articles was compiled on size distributions, elemental composition, behavior in relation to diffusion, formation of aggregates and interactions with the environment, also evaluating, when available, the methods of analysis and the type of soil.

2. Research Methods

2.1 Sampling Techniques

For the study of HS, it is common to apply alkaline extraction, which has been questioned by the

scientific community [11], as the extraction process can interfere with the results obtained. While others defend [14], since practical benefits were observed by the application of HS even without the certainty that the extraction is representative. In the present article, the data compiled come from alkaline extractions with the solvent sodium hydroxide (NaOH).

2.2 Molecular weight

There are different techniques to evaluate the HS molecular weight (MW), which are also used in the study of polymers, such as Ultracentrifugation, Field Flow Fractionation (FFF), Light Scattering by humic substances and Partial specific volumes of humic substances. With the advancement of instrumental analytical chemistry, new methods have been applied, mainly involving mass spectroscopy such as Fourier-transform ion cyclotron resonance and Fourier-transform infrared. [1, 14]. In this present article, the use of FFF and Multistage ultrafiltration (MST-UF) were observed.

2.3 Elemental composition

For elemental composition, studies there are also different types of methods. This paper presents: ultrafiltration and size exclusion chromatography with dynamic light scattering [12], elemental analysis of purified samples [15] and, together, elemental analysis, ^{13}C NMR spectroscopy and pyrolysis coupled to gas chromatography/gas spectrometry mass (Py-GC/MS) [16].

3. Results

3.1 Colloid characteristics of HS

As written by [7] "Dissolved' humic substances may be considered as true solutions of macro-ions or negatively charged hydrophilic colloids". In addition, colloids also provide sites for chemical reactions to occur, as they have a large surface area. [9]

The concentration of the HS, pH, and ionic strength of the system are the most important factors in controlling molecular conformation [17]. At high concentrations ($> 3.5 \text{ g}\cdot\text{L}^{-1}$), low pH (< 3.5), and high ionic strength ($> 0.05 \text{ M}$), humic materials are rigid, uncharged colloidal particles. In the opposite case, HA and FA exist as flexible linear polyelectrolytes, as occurs in fresh waters, where the concentration and ionic strength are generally low, and the pH is greater than 3.0 [9]. This other article [18], more recent, determines what the HA structure looks like under certain system conditions:

1. pH < 4 and sodium-form: HA exists in aqueous solutions as aggregates of approximately 30-120 nm.
2. pH > 4 : Structure of HA will depend on the concentration.
 - 2.1. Low concentrations: HA are individual molecules;

- 2.2. High concentrations: HA associate to form supramolecular structures at $\sim 5 \text{ mg/dm}^3$ and micelles at 8 g/dm^3 .

Furthermore, in [18] it was also observed that "supramolecular structures have increased adsorbability on clay minerals and promote the manifestation of solubilizing properties of humic acids in solutions even at 5 mg/dm^3 ."

3.2 Elemental composition of HS

In [15] composite samples (0-10 cm) of oxisol, LR, and mollisol, B, were collected in Brazil (RS). Two extraction methods were evaluated in each soil, one with HCl and NaOH, method 1, and another including pyrophosphate before the alkaline extraction step, method 2. It was concluded that for LR, method 1 generates higher yield, while for B the second is the most suitable. With these results, it was inferred that the HS in LR interact mainly through ligand exchange and hydrogen bonding reactions, while in B the interactions occur through the bridge of coordinated cations. Furthermore, this work showed the importance of soil mineralogy characterization in the evaluation of HS behavior. The chemical compositions of humic acids obtained by method 1 are summarized in Table 1.

In [16] humic acids were extracted with 0.5 mol.L^{-1} NaOH from the surface layer (0-20 cm) of a Brazilian ultisol and an oxisol. The objective was to evaluate the influence of the addition of residues on HA and, for that, the molecular composition was determined before and after the treatment. The data are synthesized in Table 1. An increase in carbohydrates was observed, indicating an increase in the labile structures of HA.

In [12] the objective was to investigate the presence of stable molecular aggregates in three different HS and their behavior (macromolecular and supramolecular). Samples were Aldrich HA, peat with HA and FA and compost of solid wastes of wineries with HA and FA. For this, the elemental analysis of each humic fraction was carried out and the results are summarized in Table 1, together with data from the other aforementioned articles.

Tab. 1 - Elemental composition of the HS extracted with NaOH from different samples.

Ref.	Sample	Classification	Elemental Composition of HS (%)			
			C	H	N	O
[15]	Oxisol (0-10 cm), NaOH, Brazil (RS)	Humate	51.4	4.6	3.9	40.1
	Mollisol (0-10 cm), NaOH, Brazil (RS)	Humate	52.6	4.4	3.6	39.5
[12]	Peat, NaOH	Humate	60.1	5.40	1.79	32.71
	Peat, NaOH	Fulvate	38.15	4.97	1.94	54.94
	Compost of solid wastes of wineries, NaOH	Humate	51.94	4.03	5.16	38.87
	Compost of solid wastes of wineries, NaOH	Fulvate	38.97	4.20	3.48	53.35
[16]	Oxisol (0-20 cm), NaOH, Brazil (RJ)	Humate	53.1	4.7	2.3	35.9
	Ultisol (0-20 cm), NaOH, Brazil (RJ)	Humate	57.9	5,00	2.3	34.7
	Oxisol (0-20 cm), NaOH, Brazil (RJ) with municipal compost	Humate	51.8	4,00	2.3	38.5
	Ultisol (0-20 cm), NaOH, Brazil (RJ) with municipal compost	Humate	55.8	5,00	2.9	32.3

3.3 Diffusion coefficient

In [5] the diffusion coefficient (D) of HA and HA of two samples, one of aqueous solution and the other of soil, was also determined. For this, the author used the FFF technique and two equations. It was observed that the highest values of D occurred for FA, which have the lowest MW. The results are compiled in Table 2.

Tab. 2 - Diffusion coefficients of the HS from two samples.

Ref.	Sample	Classification	Analysis technique	Diffusion Coef. ($10^6 \text{ cm}^2 \cdot \text{s}^{-1}$)
[5]	Suwannee (river)	Fulvate	FFF	4,1
		Humate	FFF	3,23
	Mattole (soil)	Fulvate	FFF	3,76
		Humate	FFF	3,01

3.4 Molecular weight of HS

With the same types of samples, the author D. P. Dick of [15] elaborated with another author a new publication concerning the kinetics of extraction, the size fractionation of the average molecular mass of the HS [19]. The results are compiled in Table 2. This time, three solvents were used, 0.1 mol.L^{-1} and 0.5 mol.L^{-1} NaOH and 0.15 mol.L^{-1} $\text{Na}_2\text{P}_2\text{O}_7$. It was observed that the HS extracted by method 2 had a higher molecular mass than the HS of method 1 in general. These results indicate that the solvent affects the solubility of different HS fractions.

In [5] a set of several samples was analyzed with FFF to have their molecular weight determined. The results are compiled in Table 2. It is interesting to note that even in this 1987 article some patterns can be observed such as the higher MW of AH in relation to FA and the lower MW of natural water samples than soil samples. These results are in agreement with other values in the literature [20].

3.5 HS interactions with the environment

HS can collaborate with the management of contaminated areas as they can interact with organic pollutants or metals due to their heterogeneous characteristics. In [21] HA and FA were applied to soils with metals. In general, it was observed that HA increases the solubility of metals and FA decrease it.

Tab. 3 - Molecular weight of the HS extracted with NaOH from different samples.

Ref.	Sample	Classification	Analysis technique	Mn ^a (Da)	Mw ^b (Da)	Polydispersion (Mw/Mn)
[5]	Suwannee river	Fulvate	Field flow fractionation (FFF)	1150	1910	1,66
	Suwannee river	Humate	Field flow fractionation (FFF)	1580	4990	3,16
	Mattole soil fulvate	Fulvate	Field flow fractionation (FFF)	1390	3900	2,81
	Mattole soil humate	Humate	Field flow fractionation (FFF)	1940	6140	3,16
	Florida sand humate	Humate	Field flow fractionation (FFF)	2250	7960	3,54
	Washington peat humate	Humate	Field flow fractionation (FFF)	3020	17800	5,89
	Leonardite coal humate	Humate	Field flow fractionation (FFF)	3730	18700	5,01
	Aldrich humate	Humate	Field flow fractionation (FFF)	3070	14500	4,72
[19]	Oxisol (0-10 cm), NaOH, Brazil (RS)	Humate	Multistage ultrafiltration (MST-UF)	-	≤ 100000	-
	Oxisol (0-10 cm), NaOH, Brazil (RS)	Fulvate	Multistage ultrafiltration (MST-UF)	-	≤ 1000	-
	Mollisol (0-10 cm), NaOH, Brazil (RS)	Humate	Multistage ultrafiltration (MST-UF)	-	≤ 100000	-
	Mollisol (0-10 cm), NaOH, Brazil (RS)	Fulvate	Multistage ultrafiltration (MST-UF)	-	≤ 5000	-

^a Mn is the number averaged molecular weight

^b Mw is the weight averaged molecular weight

This result shows that HS can act in soil treatment and agriculture, controlling the availability of substances, their retention and mobility. Several authors study how interactions between HS and various contaminants occur [14, 21, 22]. It is known that HS can act as surfactants, as they have the ability to bind hydrophobic and hydrophilic materials, being essential in transport of organic and inorganic substances in the environment [9].

In terms of metallic ions and their interaction with HS, [23] comments that in natural waters the interactions lead to the formation of complexes, usually chelates, and colloid structures, which would be generated by ion-exchange and surface absorption reactions.

In [9] the solubilizing properties of humic acids were studied through their binding to anionic surfactants (ASs) that do not involve electrostatic attraction. Furthermore, the sorption properties of HAs were studied by their adsorption on kaolinite, common in silt and colloidal fractions of surface water. HA was found to bind to ASs in a pH range of approximately 4 to 9, which indicates a relationship with pK dissociation values of aliphatic and aromatic carboxylic groups (4.2 and 8.1 respectively). It was also found that Ca²⁺ facilitates sorption more than Na⁺. [9] showed that the behavior of HA is strongly related to the concentration, pH and nature of the counter-ion.

4. Conclusion

With this research, it was possible to conclude that the HS present some global characteristics already established and well defined. The molecular composition found corresponded to the literature estimate made in 1989 [24]: 40-60% carbon, 30-50% oxygen, 4-5% hydrogen, 1-4% nitrogen, 1-2% sulfur, and 0-0.3% phosphorus. Therefore, with more modern and precise analysis techniques, it is possible to validate or not the results obtained a few decades ago, allowing the knowledge about HS to be increasingly solidified.

It is also possible to conclude that the interactions of HS with the environment are complex and are functions of different factors, as expected. Thus, applications of HS for decontamination of systems must be well studied and defined before they occur in practice, although with the results obtained, HS already have great potential for use.

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