Chapter

Chemical Characteristics of Humic Substances in Nature

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Abstract

Humic substances are the main constituents of natural organic matter, found in both aquatic and terrestrial environments. Humic substances are a complex, dispersed, and heterogeneous mixture of various organic compounds synthesized from organic matter residues, decomposed by microorganisms. Most scientists indicate that humic substances are as a supramolecular association of small heterogeneous molecules stabilized by weak intermolecular bonds. When these substances are presented in water intended for drinking or industrial use, it can have a significant impact on the treatability of this water and on the success of chemical disinfection processes, due to possible formation of organic compounds harmful to human health. Moreover, the humic substances can be used of several ways such as fertilizer to help in the development of plants, to improve soil erosion and to removal of organic compounds and metals from soils and waters. In addition, humic substances suggest an important role in mitigating areas degraded by the phytoremediation technique. The purpose of chapter is to provide an overview of humic substances and to discuss their concepts, chemical characteristics, ecological effects and technological applications for soils and aquatic systems.

Keywords: Humic substances, Humic acid, Fluvic acid, Humin, Organic matter, Soils, Aquatic system

1. Introduction

Humic substances are the main constituents of natural organic matter, found in both aquatic and terrestrial environments. Natural organic matter can be classified into aquagenic organic matter and paedogenic organic matter. Aquagenic organic matter occurring in ocean waters is formed by the excretion and decomposition of plankton and aquatic bacteria. Paedogenic organic matter is produced by the decomposition of land plants and microorganisms, including leached material from the soil anchored in the aquatic system. This type of organic matter is formed by the degradation of lignin, polysaccharides and proteins, which lead to the formation of organic compounds of carboxylic, phenolic, benzoic, and aliphatic compounds. In addition, humic substances are the main carbon reservoir in the biosphere and account for approximately 70 to 90% of soil organic matter [1].

According to the International Humic Substances Society (IHSS), humic substances are complex heterogeneous mixtures of polydisperse materials formed by a humification process, in which chemical and biochemical reactions occur during the decomposition of plant and microbial residues [2]. In [3] was also noted this new understanding, where humic substances are heterogeneous and relatively small molecular components of soil organic matter in supramolecular associations with a variety of organic compounds of biological origin and synthesized by abiotic and biotic reactions in soil. The first publications on humic substances were reported in 1786 with the extraction of humic acids from peat by Achard in Germany and by Vauquelin in 1797 with the extraction of plant residues. Between 1829 and 1837, studies to understand the origin and composition of humic substances began with the research conducted by Sprengel, reporting that more alkaline soils exhibited greater abundance of humic acids, making them more fertile [4]. In the 19th and 20th centuries, humic substances were often examined through the acid–base theory. In the mid-20th century studies were initiated in order to classify humic fractions. The author in [5] created the nomenclatures of fulvic acids, humic acids and humins. This classification is based on their solubility in aqueous medium. Then, fulvic acids are soluble at acidic or alkaline pH, humic acids are soluble at alkaline pH, and humins are insoluble at any pH [4, 6, 7]. Researchers have been discussing, in recent years, models in order to explain the chemical structure of humic substances. Currently, there are three widely discussed models - the macromolecular, the micellar, and the supramolecular structure models. The macromolecular model assumes that humic substances are a soil polymer and that humification is the process by which organic compounds from plants and animals are not fully oxidized to CO_2 and H_2O and accumulate in the soil as humus [8–10].

The micellar model states that humic substances consist of macro-structures of high molecular mass and that the macromolecular properties result from associations of small molecular species in micellar structures [11]. The author in [12] defines humic substances as a supramolecular association of small heterogeneous molecules stabilized by weak intermolecular bonds, which can be broken by small amounts of organic acids. Among the three main structural models (micellar, macromolecular and supramolecular) of humic substances, currently, the supramolecular model is the most widely accepted by researchers in the IHSS [2].

However, the scientific community has been discussing the macromolecular and supramolecular models, due to the undefined chemical structure of humic substances.

2. Concept and characterization of humic substances

Researchers in this area has been discussing the concept, origin, and composition of humic substances for the last decades, and these questions have not yet been clarified. According to [13, 14] humic substances are a complex, dispersed, and heterogeneous mixture of various organic compounds synthesized from organic matter residues, decomposed by microorganisms.

Humic substances exhibit a wide variety of structures and chemical compositions. As an example, a considerable part of these substances present the benzene ring (phenols and quinones), as shown in **Figure 1**. In addition, several functional groups are present in humic substances such as carboxylic, hydroxyl (phenolic and alcoholic), carbonyl, and amino groups [15]. The authors [16] list the substances identified in humic extracts, that include mono-, di- and trihydroxy acids, fatty acids, dicarboxylic acids, linear alcohols, phenolic acids, terpenoids, steroid compounds, carbohydrates and amino acids.

In [9], the author reports that the functional groups that contribute most to the surface charge and reactivity of humic substances are phenolic and carboxylic groups. Humic acids behave as mixtures of dibasic acids, with pK_a value around 4

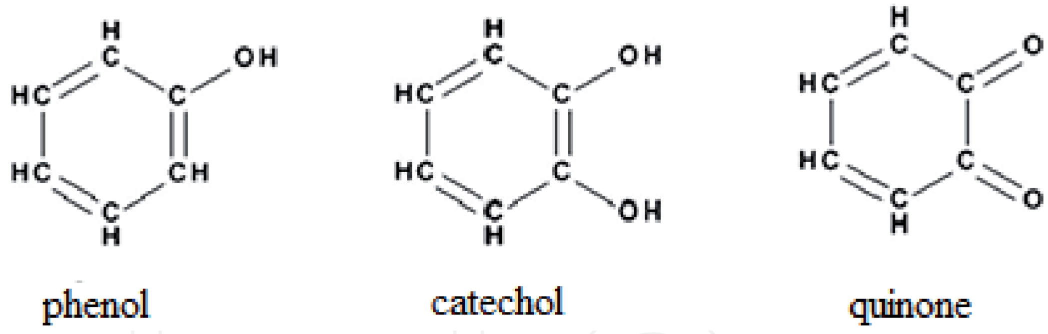

Figure 1.
Molecular building blocks that form humic substances containing the benzene ring.

for protonation of carboxylate groups and around 8 for protonation of phenolate groups. There is considerable overall similarity between the individual humic acids [17].

For this reason, the pK_a values measured for a given sample are mean values relative to the constituent species. The other important characteristic is charge density. Fulvic acids are defined as associations of small hydrophilic molecules which several acidic functional groups that form the fulvic clusters dispersed in solution at any pH. Humic acids are made by associations mainly of hydrophobic compounds which it are stabilized at neutral pH by hydrophobic dispersive forces such Van der Waals forces, π-π bonds, CH-π bonds. Their conformations grow progressively in size when intermolecular hydrogen bondings are increasingly, flocculate at lower pH [11]. The presence of carboxylate and phenolate groups gives humic acids the ability to form complexes with ions such as Mg^{2+}, Ca^{2+}, Fe^{2+} and Fe^{3+}. Many humic acids have two or more of these groups arranged in such a way that allows the formation of chelate complexes [18]. The development of complexes (chelates) is an important aspect of the biological role of humic acids in regulating the bioavailability of metallic ions [17].

According to [19] the elemental composition of humic substances can be divided and vary as follows: fulvic acids (35.1 to 75.7% carbon, 16.9 to 55.8% oxygen, 0.4 to 7.9% hydrogen, 0.5 to 8.2% nitrogen and 0.1 to 3.6% sulfur), humic acids (37.2 to 75.8% carbon, 7.9 to 56.6% oxygen, 1.6 to 11.7% hydrogen, 0.5 to 10.5% nitrogen and 0.1 to 8.3% sulfur) and humin (48.3 to 61.6% carbon, 28.8 to 45.1% oxygen, 7.3 to 14.2% hydrogen, 2.9 to 6.0% nitrogen and 0.1 to 0.9% sulfur).

The presence and relative abundance of fulvic acids, humic acids, and humin is inferred by laboratory extraction, a process that modifies their original state. Humic and fulvic acids are extracted as a colloidal solution from the soil to other solid phase sources in a strongly basic aqueous solution of sodium or potassium hydroxide. Humic acids are precipitated into the solution by adjusting the pH to 1 with hydrochloric acid, keeping fulvic acids in solution. This is the operational difference between humic and fulvic acids. Humin is insoluble in diluted alkali. The alcohol-soluble portion of the humic fraction is generally referred to as humic acid. The named "gray humic acids" (GHA), according to [20], are soluble in alkaline media of low ionic strength; "brown humic acids" (BHA) are soluble under alkaline conditions regardless of ionic strength; and fulvic acids (FA) are soluble regardless of pH and ionic strength. Humic acid, as traditionally produced in the laboratory, is not a single acid; instead, it is a complex mixture of many different acids containing carboxylic and phenolic groups so that the mixture behaves functionally as a dibasic acid or, occasionally, as a tribasic acid. Humic acid used for soil correction is manufactured using the same well-established procedures. Humic acids can form complexes with ions commonly found in the environment creating humic colloids. Humic acids are insoluble in water at acid pH, while fulvic acids are also derived from humic substances, but are water soluble throughout the pH range [21]. Humic and fulvic acids are often used as a soil supplement in agriculture and, less commonly, as a human nutritional supplement. As a nutritional supplement, fulvic acid can be found in liquid form as a component of mineral colloids. Fulvic acids are polyelectrolytes and are unique colloids that diffuse easily through membranes, whereas all other colloids do not [22]. A sequential chemical fractionation called Humeomics can be used to isolate more homogeneous humic fractions and determine their molecular structures by advanced spectroscopic and chromatographic methods [23].

3. Determination of humic substances in water samples

The water naturally found in springs has organic matter and microorganisms that can be pathogenic. The organic matter originates from the remains of dead plants and animals and is carried to the springs by surface runoff after rainfall. Domestic and industrial effluents discharged directly into water bodies also contribute to an increase in the amount of organic matter in the springs. The presence of humic substances in water intended for drinking or industrial use can have a significant impact on the treatability of this water and on the success of chemical disinfection processes. For example, according to [24, 25] humic and fulvic acids can react with the chemicals used in the chlorination process, forming disinfection by-products, such as dichlorocetonitriles, which are toxic to humans. In addition, the color occurring in natural waters is largely due to the presence of humic substances. When found in water distributed for human consumption, they impart a dark color and taste to the water. Moreover, they can form by-products during pre-oxidation and disinfection and can complex with metals and organic micropollutants. Such by-products, if originating from the reaction with chlorine, are potentially carcinogenic chlorinated organic compounds, known as trihalomethanes (THM) [26, 27]. Therefore, accurate methods for establishing humic acid concentrations are essential for maintaining water supplies, especially from watersheds. Since many different bio-organic molecules, in many diverse physical associations, are mixed together in natural environments, it is complicated to measure their precise concentrations in the humic superstructure. For this reason, humic acid concentrations are traditionally estimated from organic matter concentrations (usually from concentrations of total organic carbon or dissolved organic carbon). The organic carbon dissolved in waters accounts for 1/3 to 1/2 of the total in natural waters [28]. Humic substances, mainly humic acid, represent the largest fraction of dissolved organic carbon present in surface water and groundwater [29]. Humic extracts are composed of a large number of different bio-organic molecules that have not yet been fully separated and identified. However, unique classes of residual biomolecules have been identified by selective extractions and chemical fractionation and are represented by alkanoic and hydroxy-alkanoic acids, resins, waxes, lignin residues, sugars, and peptides. **Table 1** shows several methods that are used in the extraction of humic substances, such as lyophilization, co-precipitation, ultrafiltration, solvent extraction and resins [30, 31]. The separation of humic substance in water by adsorption chromatography has been widely employed [14, 32, 33].

The authors in [34] developed a methodology based on resin adsorption and carbon concentrations. The method is quantitative for determining the levels of humic substances in water. According to [35], this method was evaluated as the most appropriate and it produces more accurate results due to the use of spectrophotometry to evaluate only part of the humic substances present in a given sample.

Method	Benefit	Limitation
Lyophilization	Soft method and high concentration factor	Solutes are concentrated, less the volatile
Co-precipitation	Good cost-effective and effective for waters with a high content of dissolved organic carbon.	Low efficiency for large sample volumes.
Ultrafiltration	Fractionation of solutes by molecular weight and indicated for large sample volumes	Interaction with the membrane and clogging may occur
Solvent extraction	Exclusion of inorganic salts	Slow method and irreversible interactions occur in the sample/solvent
Resins	Suitable for large sample volumes, high concentration factor and easy regeneration of adsorbent	Changes in the sample and contamination from the resin

Table 1.
Benefits and limitations of the applied methods for extracting humic substances in aquatic environment.

Another technique used to determine the concentration of humic substances in water is using visible and ultraviolet spectroscopy. For this purpose, a standard absorbance vs. concentration line is constructed and thus it is possible to determine the concentration of humic substances based on the absorbance readings of the samples (aqueous solution of humic substances). The authors in [31] performed the isolation of humic substances from water and used this technique to determine the content of aromatic compounds and the color intensity of humic substances as well as the analysis of dissolved organic carbon for determine the total concentration of humic substances. Others authors, as cited in [36, 37], also used these techniques in their studies of humic substances.

4. Ecological effect

The additives of soil organic matter have been known by farmers to be beneficial to plant growth for longer than recorded history. However, the chemistry and function of organic matter have been the subject of controversy since humans began to postulate about it in the 18th century. Until Liebig's time, it was assumed that humus was used directly by plants but after Liebig showed that plant growth depends on inorganic compounds, many soil scientists argued that organic matter was useful for fertility only when it was decomposed with the release of its constituent nutrient elements in inorganic forms. Currently, soil scientists take a more holistic view and have recognized that humus influences soil fertility through its effect on water holding capacity of soil. Moreover, since it has been shown that plants absorb and translocate the complex organic molecules of systemic insecticides, they can no longer discredit the idea that plants may be able to absorb the soluble forms of humus.

The positive effects of humic substances on plant growth may be related to indirect effects, such as increased efficiency in fertilization and reduced soil compaction, or to direct effects, such as improvements in plant biomass [38, 39]. Such substances induce growth and increase the absorption of nitrogen, phosphorus and potassium [40]. Nitrogen is responsible for the formation of amino acids, proteins, enzymes and nucleotides, which ensures better growth and functioning of plants. Humic substances can also improve plant metabolism and absorption. In summary, the different fractions of humic substances can affect plant growth and development, leading to structural and physiological improvements. In addition, it favors the development of rhizoids (specialized epidermal cells whose function is water absorption and anchoring) and lateral roots [41]. The authors in [42] reported that humic substances induce the presence of nitric oxide in the lateral roots, which promotes their development. Root exudates also benefit from humic substances, which are responsible for the solubilization and mobility of nutrients. In addition, the use of humic substances in the soil is possible to observe the length of the rhizoid, the density and cell proliferation in the root [41]. Another important factor of these substances in the soil is the ability to improve the soil's retention capacity, decreasing erosion processes and thus, favoring a good soil structure which influences its workability, nutrient availability and development and growth of agricultural crops [9, 11].

Finally, in aquatic environments, humic substances are naturally present in dissolved, particulate or suspended forms and their importance is related to the availability of organic and inorganic nutrients for bacteria, fungi, phytoplankton and aquatic macrophytes. Another relevant factor is the relationship between humic substances with the complexation, absorption and immobilization of organic contaminants and heavy metals, which consequently increases the bioavailability and availability for organisms [1, 43].

5. Technological applications

Scientific research has shown different technological applications related to humic substances to improve the quality of water and soil.

In the aquatic environment, the humic acid binding skills of heavy metals have been exploited to develop remediation technologies for the removal of heavy metals from wastewater. For this, the authors in [44] used nano magnetic particles coated with humic acids. After capturing the lead ions, the nanoparticles can be captured using a magnet. On the other hand, to guarantee the potability of water for human consumption, it is necessary to remove humic substances due to the presence of organic compounds harmful to human health. Then, in the last few decades, several methods to improve water quality can be applied such as treatment methods, including coagulation, filtration and advanced oxidation processes [45]. The use of heterogeneous photocatalysis using titanium dioxide was analyzed by [46] to degrade the organic matter in water for human consumption. The results showed the complete removal of humic substances from the clarifier was after 220 minutes of irradiation.

Another important issue is the landfill leachate which is rich in humic substances, such as humic acid and fulvic acid, and these substances are important constituents of organic fertilizers used for plant growth. A study that evaluated the removal of humic substances from leachate for use as fertilizer was presented by [47]. The authors concluded that when using 100 ppm of the concentrate of humic substances extracted from the leachate, the green bean seedlings had an increase of 54.7% in height and 121.4% in weight, verifying that there was no phytotoxicity of the extracted.

Regarding the application of substances to the soil, the authors in [48] studied the effects of humic acid on plant growth, observing that "humic acids accelerate the growth of plants" and there are "relatively large responses to low application speeds". In [49] the authors showed that the addition of humate to the soil significantly increases the mass of the roots in creeping grass of folded grass. In the field of soil mechanics and geology, the term humate refers to geological materials, such as coal beds, mudrock or pore material in sandstones, which are rich in humic acids. The authors in [50] report that humate has been extracted from the Fruitland Formation of New Mexico for use as a soil corrector since 1970, with almost 60,000 metric tons produced in 2016. According to [51] humate deposits may also play an important role in the genesis of uranium ore bodies. Another contribution of humic substances refers to their potential use in reducing soil erodibility [52–54]. The authors in [55] evaluated the incorporation of humic substances extracted from the coal tailings in the material of the gneiss mining. The authors concluded that the use of these substances favored the reduction of soil erodibility in mining areas. This way of applying humic substances allows an alternative use to the tailings to be implanted and still providing the fight against erosion of degraded areas.

In the case of heavy metals, the authors in [56] evaluated the ability to simultaneously remove a soil artificially contaminated with copper, lead, zinc, cadmium and chromium through the use of humic substances extracted from the compost of livestock manure. The results showed that washing the soil with the studied compound allowed a greater capacity to remove metals from the soil due to the variety of structures found in the material used. In addition, the authors concluded that the metals have become more bioavailable for plants. Therefore, the use of humic substances combined with the phytoremediation technique is an excellent strategy to mitigate areas contaminated with heavy metals [57]. This technique uses plants to reduce the levels of contaminants to levels safe and compatible with the preservation of human health, preventing or hindering the spread of substances harmful to the environment [58]. Studies report that different species of plants are highly efficient for mitigating metals in soils when these elements are bioavailable [59]. Therefore, the use of humic substances in soils is desirable as it improves development and metals are more bioavailable for plants in the phytoremediation process.

6. Final considerations

Humic substances have made great strides in understanding the supramolecular structure and it have been widely accepted by several researchers. However, its structure is not yet fully defined and several issues remain under discussion by the scientific community. The fact is that these substances can be used to improve the quality and structure of the soil. Humic substances have unique chemical properties that benefit the soil in different ways, for example, it acts to combat soil erosion, improves the development of plants and removes pollutants from the soil. These characteristics indicate that humic substances play an important role in mitigating areas degraded by the phytoremediation technique. As for the aquatic system, humic substances can be used in the remediation of wastewater. In the treatment of leachate in landfills, humic substances can act as organic fertilizers for agricultural soils. On the other hand, these substances are a problem in drinking water for human consumption and must be treated efficiently to avoid damage to human health.

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