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Humic Substances in Aquatic Ecosystems: A Review

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ABSTRACT: Humic substances, including humic acid, fulvic acid and humin, represent a class of naturally occurring complex compounds found in soil, water and sediments that are formed by the transformation of biomolecules that originate from dead plants and animals, and microbial activity, a process that is going on along with the natural decay processes. In aquatic ecosystems, humic substances exhibit an important influence on the properties and functions of natural water systems as they play a direct role in the formation of physical and chemical environment in a water body that ultimately determines its biological community. This review summarises information regarding the composition, structure, formation and role in aquatic ecosystems, also pointing to their application in other fields like environment, agriculture and biomedicine.

KEYWORDS: Humic, Fulvic, Humin, Formation, Aquatic ecosystems, Organisms, Pesticides.

I. INTRODUCTION

Among the various organic substances occurring naturally, humic substances are the most widespread [56], being present in soil, water, geological organic deposits such as lake sediments, peat, brown coal and shales, and representing about 25% of the total organic carbon on earth and comprise 50-75% of the dissolved organic carbon in water [42, 18]. Humic substances are high molecular weight compounds that are produced by partial degradation of complex biomolecules and recombination of these with simple biomolecules and their breakdown products [66]. This ‘humification’ process is an important biogeochemical process, but this has remained as good as unstudied, especially in aquatic systems possibly because of their low concentration, lack of suitable analytical tools and presumably the missing relevance of the problem [54, 15].

Over the years, various attempts have been made to define these complex compounds. Waksman defined humus as “a complex aggregate of brown to dark colored amorphous substances, which have originated during the decomposition of plant and animal residues by micro-organisms under aerobic and anaerobic conditions, usually in soils, composts, peat bogs and water basins” [30].

According to International Humic Substance Society (IHSS), Humic substances are complex and heterogeneous mixtures of polydispersed materials formed by biochemical and chemical reactions during decay and transformation of plant and microbial remains (a process called humification).

Humic substances can be divided into 3 main fractions –

- Humic acid –the fraction of HS that is not soluble in water under acidic conditions ($\text{pH} < 2$), but is soluble at higher pH values
- Fulvic acid –the fraction of HS that is soluble in water under all pH values
- Humin -the fraction of HS that is not soluble in water at any pH value [22].

Humic substances can be easily differentiated from the non-humic substances that include organic compounds (such as amino acids, sugars, fats and organic acids like citric acid) that are easily degraded and utilized by micro-organisms and hence they are present in low concentrations in aquatic ecosystems [30].

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The remarkable properties of humic substances have attracted the attention of many investigators worldwide. Extensive research on the humic substances for the last two centuries has shown these compounds to have anti-viral, anti-inflammatory, hormonal system stimulant, profibrinolytic and heavy metal binding capabilities [51] that has credited them with so many practical applications in industry, agriculture, environment and biomedicine [45, 39]. Now a days, they are used as feed and food additives, in dyes and cosmetics. The aim of this article is to have more insight into humic substances, their structure, composition and role in aquatic ecosystems.

II. RELATED WORK

Study of humic substances as an essential component of ecosystem began with the work of German chemist Achard in 1786 and his German School. They developed isolation and classification schemes for the first time and also used the term ‘Huminstoffe’ (humic substances) that comes from the Latin word ‘Humus’ which means soil or earth, and that was subsequently used by Saussure to describe dark coloured organic materials in soils. In 19th century, Berzelliuss made an important contribution in study of aquatic humic substances when he isolated them from a spring in Sweden. Later, Aschan and Oden made important contributions regarding the role of humic substances in aquatic ecosystems and Oden developed the division of humic substances into humins, humic acids and fulvic acids, which is still valid today [54].

III. COMPOSITION

Extensive research over the years has not been able to propose a single structural formula for humic substances as they consist of a heterogeneous mixture of compounds [30], however, the chemical composition and functional groups are well characterized. The main constituent elements of humic substances are carbon, hydrogen, oxygen, nitrogen and sulfur and these elements are always present in these compounds regardless of their origin and country or continent [45].

The most important functional groups in humic substances are carboxylic, phenolic, alcoholic, carbonyl, amino, and sulphydryl (SH) groups and the predominance of the carboxyl groups attributes acidic character to humic substances [66].

Along with these functional groups, humic substances incorporate a variety of biomolecules in their structures, including sugars, fatty acids, polypeptides and amino-acids, carbohydrates accounting for about 1% of carbon in dissolved humic substances. Amino acids are present in humic substances in smaller amounts with glycine, aspartic acid, glutamic acid and alanine being most abundant [66, 30].

Table 1. Elemental composition (weight %) and functional group content (mequ/g) in humic acid and fulvic acid [after 48].

ELEMENT	HUMIC ACID	FULVIC ACID
C	53.6-58.7	40.7-50.6
H	3.2-6.2	3.8-7.0
N	0.8-5.5	0.9-3.3
O	32.8-38.3	39.7-49.8
S	0.1-0.5	0.1-3.6

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FUNCTIONAL GROUPS	HUMIC ACID	FULVIC ACID
Acid groups, total	5.6-8.9	6.4-14.2
Carboxylic acids	1.5-5.7	5.2-11.2
Phenolic OH	2.1-5.7	0.3-5.7
Alcoholic OH	0.2-4.9	2.6-9.5
Quinoid/keto C=O	0.1-5.6	0.3-3.1
Methoxy OCH ₃	0.3-0.8	0.3-1.2

However, there is a difference in elemental composition of humic and fulvic acids, with humic acids poorer in hydrogen and oxygen and richer in nitrogen than fulvic acids and also in the functional group composition with fulvic acids having a predominance of carboxylic acid groups while humic acid being rich in phenolic groups [66]. HS are known to be of high molecular mass with Humic acids 1500 – 3000 A size or >3,500 Da molecular weight, Fulvic acids 800 A and <3,500 Da [3, 37, 30].

IV. STRUCTURE:

The scientific understanding of the structure of humic substances is indispensable in order to understand their role in aquatic ecosystems. Earlier view regarding structure of HS was that these compounds exist in the form of polymeric branched or linear chains, formed by condensation and oxidation processes of simple but a heterogenous assemblage of monomeric units to form a high molecular weight compound. These polymers structures could assume different configurational forms that makes the compound inert and refractory. Alvarez Puebla et al. (2005) was a supporter of this model [1].

However, now accepted view regarding their structure is that HS are a supra-molecular association of small heterogenous molecules derived from the decomposition of organic matter and the forces stabilizing this association predominantly are weak dispersive forces like hydrophobic (van der Waals, $\pi-\pi$, CH- π) and hydrogen bonds instead of covalent linkages. Moreover, microspaces are present that act like molecular sieves responsible for the sorption of low molecular compounds such as organic xenobiotics, herbicides, pesticides, metal ions, etc. this model was supported by Piccolo (2001, 2002), Simpson et al. (2002), and Schaumann (2006) [41, 40, 50, 46].

V. FORMATION

According to Stevenson (1982), “the biochemistry of the formation of humic substances is one of the least understood aspects of humus chemistry and one of the most intriguing”.

Several theories have been proposed to explain the origin of humic substances since very long time.

The earliest theory was the **sugar amine condensation theory** which states that humus is produced as a result of condensation reaction between carbohydrates and amino acids, without the involvement of micro-organisms. This theory was supported by Malguti (1835), Mulder (1839), Maillard (1912), Eller (1921) and Beckley (1921) [32, 36, 29, 10, 4].

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Lignin theory was the most accepted theory for a very long time and was accepted by many researchers. According to this theory, incomplete utilization and chemical modification of lignin by micro-organisms leads to formation of unknown compounds that after further changes yield humic acids and then fulvic acids.

The lignin degradation theory was supported by Wehmer (1915), Fischer and Schrader (1921), Waksman (1936) and in 1950s, this was the most dominant theory [62, 13, 61].

Waksman(1936) in his book ‘Humus’ has strongly supported the microbial alteration of lignins as the way in which humus is formed. He has provided evidences in favour of this theory as

1. Decomposing wood and peat increase in lignin content with time.
2. Lignin, as opposed to cellulose, gives rise to aromatic transformation products. Humic acids are found to contain aromatic moieties as well.
3. Both lignin and humic acids contain methoxyl groups; the methoxy content decreases with proceeding decomposition.
4. Both lignin and humic acids are acidic in nature; both can react with bases and both are characterized by their capacity of cation exchange, all be it to different degrees.
5. Both lignin and humic acids are insoluble in cold concentrated acids, the degree of insolubility increasing with proceeding decomposition.
6. When lignins are heated in aqueous alkaline solutions, they are transformed into methoxyl-containing humic acids, which does not hold for cellulose.
7. On oxidation under pressure, lignin gives rise to humic acids and finally to aromatic carboxylic acids, but cellulose is changed to other products.
8. Oxidation of brown coal under pressure gives benzenecarboxylic acids and no furancarboxylic acids.
9. Both lignin and humic acids are oxidized by oxidizing agents, such as permanganate and hydrogen peroxide.
10. Both lignin and humic acids are soluble in alkalis and precipitated by acids.
11. Both lignin and humic acids are partly soluble in alcohol and pyridine, depending also on the method of preparation; some lignin and some humus preparations are completely soluble in alcohol.
12. Both lignin and humic acids are decomposed with great difficulty or not at all by the great majority of fungi and bacteria [61].

Presently, the **polyphenol theory** supported by Stevenson (1982) has become the most accepted one.

Two pathways are proposed regarding the polyphenol model. According to one, microbial degradation of lignin produces phenolic aldehydes and acids that undergo enzymatic reactions to from quinines, which may polymerize to form humic like macromolecules in the presence or absence of amino compounds. The other pathway suggests the synthesis of polyphenols from non-lignin source (e.g., cellulose) by micro-organisms. These polyphenols are then enzymatically oxidized to quinines and later converted to humic substances [56].

This theory is also supported by Flaig(1964) and Kononova(1966) [14, 24]. Kononova(1966) has emphasized the role of micro-organisms as the source of polyphenols.

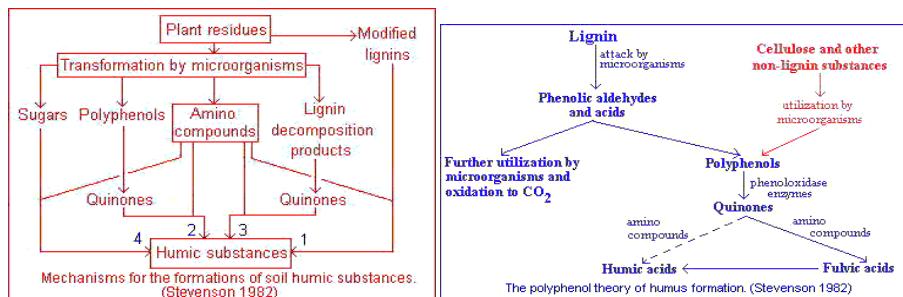


Fig. Formation pathways

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The origin of aquatic humic substances is not well understood. Humic matter in water bodies may be attributed to two main sources :

- i. Allochthonous substances i.e., having a terrestrial origin from plants and soil
- ii. Autochthonous substances i.e., having source within the water body itself [66, 15].

Humic substances whose origin is from allochthonous sources may be formed by the possible 4 possible mechanisms mentioned, but humic substances originating from autochthonous sources may have other mechanisms of formation, including originating from the degradation of aquatic organisms and bottom sediments. Therefore, a composite hypothesis was proposed by Stevenson in 1982 for humic substances in water.

Composite hypothesis-

It is proposed that aquatic humic substances are the result of several processes that are related to the aquatic environment, including;

1. Leaching of plant organic matter directly into the water,
2. Leaching of plant organic matter through the soil profile with subsequent alteration both chemical and biochemical within the soil,
3. Leaching of soil fulvic and humic acid into water,
4. Lysis of algal remains and bacterial action on phytoplankton,
5. Ultraviolet oxidation of surface active organic matter in the microlayer of streams, lakes and seawater, which is followed by polymerization reactions,
6. Polymerization reactions among phenolic, amine and aldehyde functional groups from biological products in natural waters. Natural waters that are concentrated in DOC are most likely sources for these reactions. They include dystrophic and eutrophic lakes, soil waters, and interstitial waters of bottom sediments.

VI. HUMIC SUBSTANCES IN AQUATIC ECOSYSTEMS

Humic substances constitute a major component (50-80%) of organic matter pool in freshwater ecosystems [57, 65] and due to their ubiquity and the variety of functional groups, HS have the potential to affect almost any biochemical and biogeochemical pathway in both freshwater organisms and ecosystems. Humic substances play a direct role in the formation of physical and chemical environment in a water body that ultimately determines its biological community.

Table 2 shows typical ranges for dissolved organic carbon concentration and humic substance concentration in selected water bodies [15].

SOURCE	DOC (mgL^{-1})	HS (mgL^{-1})	REFERENCE
Sea water	0.2-2.0	0.06-0.6	Thurman,1985
Ground water	0.1-0.2	0.03-0.6	Thurman,1985
River	1-10	0.5-4.0	Thurman,1985
Lake	1-50	0.5-40	Thurman,1985
Waste water effluent	8.6	1.7	Frimmel and Abbt-Braun,1999

- Humic substances have been shown to have a major effect on the optical properties of water. It causes light attenuation in aquatic ecosystems that results in the reduction of photic zone. This ultimately lowers the levels of photosynthetically available light in turn restricting photosynthesis. At the same time, humic substances protect biota from direct radiation damage as they act as an important screen against UV radiations that are harmful to biota.
- Some other features of humic substances such as acidity, buffering properties and high calorific value have a considerable impact on the formation of physical and chemical features of water. Because of the presence of acid functional groups, humic substances act as a pH buffer in aquatic systems, especially between pH 4 and 5 [25, 44]. Hedin et al. (1990) and Roila et al. (1994) estimated that organic anions on humic compounds provide 1.8 to 2.0 meq

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acid-neutralizing capacity per mg C of DOC [17, 44]. The acid-base properties of DOC also mean that it is a natural background source of acidity, particularly in humus-rich waters, and that recovery of pH will be slowed by DOC buffering [11, 25, 44].

- Humic substances also have the ability to bind heavy metals and aromatic hydrocarbons, and thus, they decrease their negative effect on aquatic organisms. As already mentioned, the supra molecular structure of humic substances with microspaces act as sieves for sorption of these compounds. Numerous studies have shown that the humic substances along with other compounds that comprise dissolved organic carbon in water complex with various metals like cadmium, aluminum, silver, zinc, lead and copper, thus reducing the toxicity of these metals [55, 49, 23, 52, 68, 33, 6, 21, 64, 7]. Similar relation between the humic substances and the bioavailability of organics such as PAHs and PCBs, chlorothalonil and cationic surfactants has also shown [8, 67, 26].

Thus, humic substances have direct effect on the physical and chemical properties of water bodies. This means that all sections of organisms inhabiting water body are affected by its quality and quantity.

- As revealed by several studies conducted, it has been noted that in lakes having high humic content, the classical food chain starting with algae or macrophytes is replaced with the detritus food chain having increased bacterial productivity and supporting denitrification [19, 63]. This has been proved by the fact that bacterial secondary production in humic lakes far exceed the phytoplankton primary production, even in the upper layers that are exposed to photosynthetically available light [59]. For example, Hessen (1985) observed that the mean annual biomass of bacteria in holarctic oligotrophic lakes was 7.8-12.1 µg C/L in clear lakes, 10.5 µg C/L in slightly coloured lakes and 16.2-44.1 µg C/L in highly coloured humic lakes [19].

- Humic substances are also associated with reduced algal production. Bacterial and zooplankton biomass were high relative to algal biomass. This can be demonstrated from the fact that eutropic humic rich lakes do not support the cyanobacterial blooms characteristic of eutrophic but humic poor lakes.

- Humic substances form particulates that serve as a food source for filter and deposit feeders. These are used as a food source by zooplankton as observed by Naumann. Also, a direct link between humic substances and heterotrophic bacteria has been demonstrated by Hessen (1985) and Tranvik (1988) [59, 58].

- Humic substances are a major component of bottom organic pool of a water body that is the chief source of nutrients for aquatic plants and benthic organisms. Hence, their quality and quantity also determines the bottom communities of these aquatic systems. Allotrophic aquatic systems receiving large inputs of particulate organic carbon tend to have macroinvertebrate communities with high proportions of shredders and detritivores [2, 5, 9].

- These bottom sediments are also used as a habitat for benthic organisms. It has also been shown that humic substances have the potential to act as environmental signals in aquatic ecosystems. The adult worms of *Caenorhabditis elegans* have been shown to migrate towards humic substances. This finding points to the ability of organisms to select their habitat on the basis of its humic substance content and probably its quality [35].

- Humic substances also have an effect on the physiology of organisms. Organisms living in humic rich water bodies are exposed to continuous stress conditions that helps in development of stress resistance in these organisms, thereby helping them in survival.

Experimentally, it has been shown that exposure of young swordtails (*Xiphophorus helleri*) to synthetic humic substances significantly enhanced their growth as compared with the controls [34].

- Recently, hormone like effects have been shown in nematode *C. elegans*, the ornamental fish *Xiphophorus helleri* (Heckel), the clawed frog *Xenopus laevis* [20, 53, 34, 28]. The ecological implication of this effect, however, still remains obscure.

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VII. APPLICATION OF HUMIC SUBSTANCES IN OTHER FIELDS

Apart from that, humic substances play an important role in various fields such as agriculture, industry, environment and biomedicine.

Agriculture	Role in improving the quality and productivity of soil by improving aeration, moisture retention and soil structure Reduce diseases in plants and for control of weeds, even more efficiently than commercial fertilizers and herbicides [47, 60, 16]
Environmental chemistry	remove toxic metals, anthropogenic organic chemicals and other pollutants such as herbicides, fungicides, insecticides, nematicides, dioxins and also some pharmaceutical products like estrogenic compounds from water by forming water soluble complexes with them [27, 12, 38].
Biomedicine	direct correlation between natural soil substances (humus, HA, FA) and the absence of tuberculosis in humans, as humic extracts contain a magnificently powerful spectrum of natural micronutrients, phytochemicals, antiviral and antibiotic like agents that directly inhibit and destroy disease pathogens, while fortifying and regulating the immune system[43].

VIII. CONCLUSION

The article provided a detailed review regarding some components of humic chemistry, their structure, formation and role in aquatic ecosystems and also about their potential application in agriculture, medicine and environmental research. The research around the world is going on to elucidate more information regarding their structure and complexation properties, but since it is a major component of aquatic ecosystems, more research about the role of humic substances in natural ecosystems should be encouraged as they are known to influence the environment in a water body and also affect its organisms directly and indirectly.

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