

*humic substances
in the Environment*

M. Schnitzer and S. U. Khan

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PREFACE

After many years of indifference, interest is rapidly increasing in humic substances, the principal organic components of soils and waters. While formerly only soil scientists and agronomists were concerned with the subject, now oceanographers, water scientists, geochemists, environmentalists, biologists and chemists are increasingly coming to realize that humic substances participate in, and often control, many reactions which occur in soils and waters. The availability of such sophisticated and powerful analytical tools as the gas chromatographic-mass spectrometric system, Nuclear Magnetic Resonance and Electron Spin Resonance Spectrometers has made possible significant advances in our knowledge of the chemical structure and reactions of these complex materials. Thus, important and exciting developments are occurring. Therefore, we believe that the time has arrived to write an account of the present state of knowledge in this field. Since this is not a history of the subject, we have chosen to refer to and to discuss only those publications that in our opinion have significance and relevance at this time.

This book is directed to both students and advanced researchers. We hope that it will assist new and older investigators and stimulate further research in this field.

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Chapter 1

INTRODUCTION TO HUMIC SUBSTANCES

I. HISTORY

Humic substances are probably the most widely distributed natural products on the earth's surface, occurring in soils, lakes, rivers, and the sea. In spite of their extensive distribution, much remains to be learned about their origins, synthesis, chemical structure, and reactions.

For a comprehensive historical review of chemical investigations on humic substances the reader is referred to the book by Kononova (1). Especially noteworthy are observations recorded in the 1760's by Wallerius, who pointed out the capacity of humic substances to adsorb water and plant nutrients, and by Lemonosov, who suggested that soils with a high humic content originated from prolonged rotting of animal and plant bodies (1). In the 1830's Berzelius attempted to classify humic substances into three fractions. These were: (a) humic acid (HA), which was soluble in solutions of alkalis; (b) humin, which was supposedly inert; and (c) crenic and apocrenic acids, which had the ability of forming salts and complexes with di and trivalent metal ions (1). Berzelius' classification scheme was extended by Mulder between 1840 and 1860. Mulder subdivided humic substances on the basis of color and solubility in water and solutions of alkalis into the following groups: (a) materials which were

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insoluble in alkali were referred to as ulmin and humin; (b) those soluble in alkali were classified as ulmic acid when brown and as HA when black; and (c) materials soluble in water were referred to as crenic and apocrenic acids (1). Most early workers in the field regarded the different humic fractions as chemically distinct compounds with definite properties, but failed to see the molecular complexities of these materials. Of particular significance are the contributions of Odén between 1912 and 1919, who proposed that crenic and apocrenic acids be referred to as fulvic acid (FA), and who also drew attention to the colloid chemical characteristics of humic substances (1). Major contributions were also made in the early 1920's by Shmuck, who considered the different humic fractions as a group of compounds with similar structural features (1). He showed that humic substances had characteristics that were typical of materials in the colloidal state. For example, they could be precipitated by electrolytes, and exhibited adsorption properties, swelling, etc. He also demonstrated the occurrence of carboxyl and phenolic hydroxyl groups in humic substances (1).

II. CLASSIFICATION

The organic matter of soils and waters consists of a mixture of plant and animal products in various stages of decomposition, of substances synthesized biologically and/or chemically from the breakdown products and of microorganisms and small animals and their decomposing remains. To simplify this very complex system, organic matter is usually divided into two groups: (a) nonhumic substances, and (b) humic substances.

Nonhumic substances include compounds that exhibit still recognizable chemical characteristics. To this

class of compounds belong carbohydrates, proteins, peptides, amino acids, fats, waxes, resins, pigments, and other low-molecular-weight organic substances. In general, these compounds are relatively easily attacked by micro-organisms in the soil and have a relatively short survival rate.

The bulk of the organic matter in most soils and waters consists of humic substances. These are amorphous, brown or black, hydrophilic, acidic, polydisperse substances of molecular weights ranging from several hundreds to tens of thousands. Based on their solubility in alkali and acid, humic substances are usually divided into three main fractions: (a) humic acid (HA), which is soluble in dilute alkaline solution but is precipitated by acidification of the alkaline extract; (b) fulvic acid (FA), which is that humic fraction which remains in the aqueous acidified solution, i.e., it is soluble in both acid and base; and (c) the humic fraction that cannot be extracted by dilute base and acid, which is referred to as humin. There is increasing evidence that the chemical structure and properties of the humin fraction are similar to those of HA, and that its insolubility arises from the firmness with which it combines with inorganic soil and water constituents. Data available at this time suggest that structurally the three humic fractions are similar to each other, but that they differ in molecular weight, ultimate analysis, and functional group content, with the FA fraction having a lower molecular weight but higher content of oxygen-containing functional groups per unit weight than HA and the humin fraction. While the fractionation scheme is arbitrary, the fractions are still molecularly heterogeneous, it has nonetheless been widely accepted. The fractions are generally more suitable starting materials for further investigations than

unfractionated humic substances.

Important characteristics exhibited by all humic fractions are resistance to microbial degradation, and ability to form stable water-soluble and water-insoluble salts and complexes with metal ions and hydrous oxides and to interact with clay minerals and organic chemicals often added by man, which may be toxic pollutants. Thus, reactions of humic substances in soils and waters with inorganic and organic compounds and the properties of the products so formed should be of considerable interest to those concerned with environmental problems.

III. DISTRIBUTION

Swanson and Palacas (2) have observed accumulation of humic substances in surface and subsurface soil layers, in and beneath marsh deposits, in shore and beach sands of bayous and bays, commonly near the mouths of tea-colored streams and near ground-water seepages, and as a type of organic sediment in bodies of brackish and saline waters. They believe that the humic materials are leached from decaying plant materials or humus on the land surface and transported by surface and subsurface waters in the soluble or colloidally dispersed form to subsurface sand environments or to brackish or saline water bodies where flocculation or precipitation of the humic substances is triggered by various physical-chemical mechanisms. While the geochemical role of humic substances is complex and not yet well understood, it is likely that humic substances are important constituents of coal, black shales, and other carbonaceous sedimentary rocks, particularly those deposited in coastal environments (2).

IV. SYNTHESIS

The mode of formation of humic substances has been the subject of much speculation. Felbeck (3) lists four hypotheses for their synthesis: (a) The plant alteration hypothesis; (b) the chemical polymerization hypothesis; (c) the cell autolysis hypothesis; and (d) the microbial synthesis hypothesis.

The plant alteration hypothesis implies that fractions of plant tissue that are resistant to microbial attack, especially lignified tissues, are altered only superficially in the soil to form humic substances. The nature of the original plant material strongly influences the nature of the humic substance formed. The higher-molecular-weight HA's and humin fractions represent the first stages of humification. These materials are degraded by microbes into FA and ultimately to CO_2 and H_2O .

According to the chemical polymerization hypothesis, plant materials are degraded microbially to small molecules which are used by microbes as carbon and energy sources. The microbes then synthesize products such as phenols and amino acids which are excreted into the surrounding environment where chemical oxidation and polymerization to humic substances take place. In this instance the nature of the original plant material has no effect on the kind of humic substance formed.

The cell autolysis hypothesis assumes that humic substances are products of the autolysis plant and microbial cells after their death. The resulting materials are heterogeneous, formed by the random condensation and free radical polymerization of cellular debris (such as sugars, amino acids, phenols, and other aromatic compounds). The free radicals are formed with the aid of autolytic enzymes.

1. INTRODUCTION TO HUMIC SUBSTANCES

The microbial synthesis hypothesis states that microbes use plant tissue as carbon and energy sources but synthesize high-molecular-weight humic-like substances intracellularly; these substances are released in the soil after the microbes die and their cells are lysed. Thus, the high-molecular-weight compounds represent the first stages of humification, followed by extracellular microbial degradation to HA, FA; and finally to CO_2 and H_2O .

It is difficult to state at this time which of the four hypotheses is the more valid one. All four refer to processes that may take place simultaneously and lead to the formation of humic substances.

V. USES

Finally, let us consider possible uses for humic substances. It has been known for a long time that humic substances enhance the fertility of soils by improving their physical properties such as crumb structure, aeration, drainage, and movement of water and nutrients (4), thus creating a more favorable environment for plant growth and facilitating the transport and availability of nutrient elements, especially trace metals. For these reasons humic substances are used as soil conditioners, stabilizers, and fertilizers. Humic substances also exert favorable physiological effects in the areas of cell division and cell elongation (5), and have been shown to act as denitrifiers in soils (6). Industrially they are used in drilling muds for oil well rigs, and as boiler-scale removers, pigment extenders, and emulsifiers (6). It is most likely that in the future greater attempts will be made to utilize the remarkable adsorption

properties of humic substances as well as their capacity to form stable complexes with metals.

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Chapter 2

EXTRACTION, FRACTIONATION, AND PURIFICATION OF HUMIC SUBSTANCES

I. INTRODUCTION

Extraction of humic substances from soils and sediments is often the first task that confronts the investigator. The ideal extractant should remove practically all of the humic material without altering its physical and chemical properties. The search for suitable extractants has been and still is a matter of high priority. Following extraction, it is usual to fractionate and purify the humic materials. It is in this area that a number of interesting and useful procedures have been developed during the past ten years.

II. EXTRACTION

Of the large number of extractants that have been tested, dilute aqueous NaOH solution remains the most commonly used and quantitatively the most effective reagent for extracting humic substances from soils or sediments. The use of alkaline solutions has been criticized on the ground that the material extracted is modified (1-8). There is some evidence that under alkaline conditions, autoxidation of humic constituents in contact with air may occur. Increases in uptake of oxygen and release of carbon dioxide as the alkalinity of the solution increases have been reported (2,9). The

use of stannous chloride has been suggested for mitigating autoxidation reactions during the extraction of humic materials (4,10): If the extraction is carried out in air-tight flasks, stannous chloride absorbs oxygen contained in the extractant and in the air space over the suspension. The principal objections to the use of stannous chloride are that it may be difficult to remove from the extracted humic material and that it may reduce quinone groups in HA's and FA's. Alternatively, oxygen can be displaced from the soil-alkali system by bubbling an inert gas such as nitrogen into it; the container is filled with nitrogen and made air-tight. In recent years the latter procedure has been adopted in several laboratories for the extraction of humic substances from soils and sediments.

Several workers have presented evidence to show that alkali extraction does not change the nature of humic materials. Thus, a HA extracted with 0.5% NaOH solution did not differ in light absorption characteristics from that extracted with 1% NaF solution (11,12). Similarly, Rydalevskaya and Skorokhod (13) found no essential differences in the elementary composition and carboxyl group content between HA's extracted with 1% NaF and with 0.4% NaOH solutions from different soils and peats. Smith and Lorimer (14) noted that HA extracted with dilute $\text{Na}_4\text{P}_2\text{O}_7$ solution was in all respects similar to that extracted with dilute NaOH solution from peat soil. Forsyth (15) found that FA extracted with dilute NaOH solution had identical properties to FA extracted with water. Schnitzer and Skinner (16) examined FA extracted with 0.5N NaOH solution under nitrogen and with 0.1N HCl from a Podzol Bh horizon. Following purification, each preparation was characterized by ultimate and functional groups analysis, by ir spectrophotometry, and by gel

filtration. It was found that the elementary composition of the two materials was very similar and that the oxygen-containing functional groups were also of the same order of magnitude. Furthermore, the ir spectra of both preparations and their fractionation behavior on Sephadex gels were almost identical. From this study they concluded that the damaging effects ascribed by some workers to alkali extraction of soil humic compounds might have been exaggerated.

The yield of humic materials extracted is affected by the concentration of the NaOH solution. Thus, Ponomareva and Plotnikova (17) found 0.1N NaOH solution most effective for the extraction of humic substances from several soils. Lévesque and Schnitzer (18) noted that the highest proportions of carbon and nitrogen were extracted from a Podzol Bh horizon by using 0.1N and 0.15N NaOH solutions. However, the material thus obtained contained the highest percent ash. These workers observed that the most suitable extractant for obtaining humic materials low in ash was 0.4N or 0.5N NaOH solution.

Soils with a high content of exchangeable Ca and other bases or CaCO_3 need to be decalcified with dilute mineral acids prior to extraction. This pretreatment brings about a more complete extraction of humic materials from soils by alkaline solutions. Caution should be exercised in pretreating soils with dilute mineral acids, as considerable amounts of humic material may thereby be removed. Thus, treatment of a Russian Podzol Bh horizon with cold dilute HCl solution resulted in the dissolution of large quantities of FA (19). On the other hand very little material was extracted by treating a Chernozem or a Forest soil with cold dilute HCl solution. Schnitzer and Wright (20) used 0.5% aqueous (v/v) HCl, HF and a HCl-HF mixture for the extraction of humic materials from