

Review Series 'Humic Substances': Preface

Humic Substances in the Environment with an Emphasis on Freshwater Systems

Christian E.W. Steinberg (Editor)

Humboldt University at Berlin, Institute of Biology, Freshwater and Stress Ecology; postal address: Arboretum, Späthstr. 80/81, 12437 Berlin, Germany (christian_ew_steinberg@web.de)

DOI: <http://dx.doi.org/10.1065/espr2007.12.458>

Despite swimming in brown-water ponds originating from peat cutting with their very steep and inconvenient thermal gradient in my early days, I first came across humic substances (HS) in freshwater systems as a graduate student during my dissertation some few years ago. From my laboratory work, I got the impression that these substances must be very complicated and nasty, hard to describe by chemical means, and that ecologists had better stay away from this brown stuff. My first impression was that of annoying substances which puzzled the chemical analytics of dissolved amino acids, peptides and proteins, and reduced the recovery rate of the substances to be analyzed. The behavior of HS in freshwaters can by no means be predicted. Indeed, some hundred years ago, analytical chemists stopped dealing with HS more or less abruptly. Disappointingly, the chemists discovered that HS did not comprise a specific class of compounds, such as proteins, carbohydrates, and lipids, and they consequently terminated these studies. Later, after that decrease in HS research, an important impetus had been provided by soil science and soil chemistry in the 20th century. Aquatic HS were considered as seepage from soils and wetlands. During this period, substantial contributions on humification theory were made by Russian, German and Canadian authors. After the Second World War, HS research was dominated by Russian laboratories, but this work, due to the language barrier, is not known in western countries. Only recently, they were made accessible by a polyglot friend from Riga, Dr. Maris Klavinš (Klavinš 1997). Some enlightening examples: Skopintsev (1934 ff), and not an American or German scholar, was the first to emphasize the importance of aquatic HS in the cycling of organic matter. He analyzed HS in oceans, groundwater, and freshwater and describes their formation. Skopintsev stated that about 90% of marine HS have a planktonic origin, which means that they are of autochthonous origin – a very insightful statement. A special structural feature of these substances is the predominance of aliphatic compounds. A further visionary opinion of Skopintsev has been that aquatic HS are not only a reservoir for nutrients, but are also a source of energy. This aspect has been independently proven much later by the pioneering works of Geller (1985) using laboratory cultures and Salonen et al. (1992) in enclosures within a Finnish lake.

The success of research on HS is closely linked to the development of isolation procedures. The present phase of HS

research is linked to the introduction of adsorption methods for isolation, purification and fractionation. Improved isolation methods provide low-ash content samples for further study. The introduction of gel-filtration (Gjessing 1965) is an important step towards the quantitative determination of aquatic HS, as well as for the determination of their properties and ecological role. Malcolm (1985) describes the introduction of gel-filtration as the beginning of the 'Sephadex period'.

Furthermore, the success of HS research is connected to developments in (protein) biochemistry. However, the conventional methods which were developed for biopolymers need modification for HS. Some specific methods for the isolation, fractionation and separation of these very complex molecules were necessary. The best examples of such methods were the improvements by a research group of the US Geological Survey at that time in Denver, Colorado (which I amicably called the 'Denver-Clan' according to the soap series shown in German TV), particularly involving the isolation of aquatic HS as suggested by Thurman & Malcolm (1981) and the fractionation according to Leenheer (1981). At present, development and refinement of specific methods is still an important subject of HS research. For instance, the introduction of high resolution mass spectrometry with soft ionization revealed some unexpected results (Reemtsma & These 2005, Cooper et al. 2004, Hatcher et al. 2004), which strongly overrule embosomed, but still recycled, paradigms: Water soluble and ionizable HS are very regularly structured, their building blocks are low-molecular(!) weighted. On this basis, several features of the ecochemical behavior of HS can be predicted, for instance, the so-called natural attenuation of organic chemicals or the production of reactive oxygen species.

Nevertheless, freshwater ecology students continue to learn that HS are inert and useful only upon irradiation, to fuel the microbial heterotrophic compounds in a lake ecosystem and to control the underwater light climate. The students continue to learn that HS do not directly interact with organisms. If there are effects to be observed, these effects are results of more or less indirect interactions. Keeping in mind the mentioned structural features, including the various functional groups of HS, however, one can easily deduce that they are soft natural environmental chemicals and that there must be interactions, comparable to those of weak, man-

made chemicals. From studies on the electromobility of HS, Münster (1985), interestingly a chemist by training, was the first to postulate the ability of HS to interact with biomembranes. Empirical evidence, however, was presented much later (Timofeyev et al. 2006). During our study of invertebrates and their struggle with or against HS, we came across a very strange behavior: the nematode *Caenorhabditis elegans* was actively looking for the stressful environment (Menzel et al. 2005) – does it benefit from the oxidative stress it has to suffer?

In summary, these interactions are supposed to structure ecological guilds by suppressing sensitive species and leaving less sensitive species unaffected or even promoted behind. To my mind, dissolved HS have to be considered abiotic ecological controlling factors as, for instance, nutrients, temperature or even light (Steinberg et al. 2006). However, we are just at the beginning of understanding the complete functions of HS in the environment.

The time seems to be right to review the present knowledge of ecological and ecochemical features of HS, for two reasons: (1) there is still a gap between eco-chemists and ecologists dealing with HS; and I, in concert with potent co-authors, shall try to bridge this gap, and (2), since the book on the *Ecology of Humic Substances in Freshwater* (Steinberg 2003) has been published, the scientific progress on HS has been overwhelmingly large and deserves to be reviewed in detail in several mini-reviews. The series will start with a description of interesting results of HS application in aquaculture, and the subsequent papers will try to explain the phenomena displayed in the first review in more detail and conclude with a broader perspective on HS in the framework of global climate change. In particular, the seven mini-reviews will cover

- 1 'Dissolved humic substances in aquaculture and ornamental fish breeding' by Meinelt et al. (2008)
- 2 'Interactions with organisms' by Steinberg et al. (2008)
- 3 'Reviewing DOC removal paradigms in highly humic aquatic ecosystems' by Farjalla et al. (in press)
- 4 'Sorption of hydrophobic organic contaminants' by Pan et al. (in press a)
- 5 'Sorption of pharmaceutical and personal care products' by Pan et al. (in press b)
- 6 'New findings on the chemical building blocks' by William T. Cooper, Florida State University (in preparation)
- 7 'Humic substances as geochemical determinants and subject of global climate change', by Peter Dillon, Trent University, Ontario (in preparation)
- 8 'Humic substances as precursors of potential toxic compounds in drinking water' by David Reckhow, University of Massachusetts, Amherst (in preparation)

References

Cooper WT et al. (2004): Ultrahigh resolution mass spectrometry of aquatic humic substances: Recurring molecular themes and polymeric character. In: Martin-Neto L et al. (eds), Humic Sub-

- stances and Soil and Water Environment. Embrapa, Brazil, pp 257–260
- Farjalla VF, Amado AM, Suhett AL (2008): Humic Substances. Part 3: Reviewing DOC removal paradigms in highly humic aquatic ecosystems. *Env Sci Pollut Res* (in press)
- Geller A (1985): Light-induced conversion of refractory, high molecular weight lake water constituents. *Schweiz Z Hydrol* 47, 21–26
- Gjessing ET (1965): Use of Sephadex gels for the estimation of molecular weight of humic substances in natural waters. *Nature* 208, 1091–1092
- Hatcher P et al. (2004): Intercomparisons of some new approaches for investigating the molecular weight distribution of dissolved organic matter. In: Martin-Neto L et al. (eds), Humic Substances and Soil and Water Environment. Embrapa, Brazil. pp 241–243
- Klavinš M (1997): Aquatic humic substances: Characterization, structure and genesis. Riga University Press, Riga
- Leenheer JA (1981): Comprehensive approach to preparative isolation and fractionation of dissolved organic carbon from natural waters and wastewaters. *Environ Sci Technol* 15, 558–587
- Malcolm RL (1985): Geochemistry of stream fulvic and humic substances. In: Aiken GR et al. (eds), Humic substances in soil, sediment, and water. Wiley, New York, pp 181–210
- Meinelt T, Schreckenbach K, Pietrock M, Heidrich S, Steinberg CWB (2008): Humic Substances. Part 1: Dissolved humic substances (HS) in aquaculture and ornamental fish breeding. *Env Sci Pollut Res* 15 (1) 17–22
- Menzel R et al. (2005): Humic material induces behavioral and global transcriptional responses in the nematode *Caenorhabditis elegans*. *Environ Sci Technol* 39, 8324–8332
- Münster U (1985): Investigation about structure, distribution and dynamics of different organic substrates in the DOM of lake Plusssee. *Arch Hydrobiol Suppl* 70, 429–480
- Pan B, Ning P, Xing BS (2008a): Humic Substances. Part 4: Sorption of hydrophobic organic contaminants. *Env Sci Pollut Res* (in press)
- Pan B, Ning P, Xing BS (2008b): Humic Substances. Part 5: Sorption of pharmaceuticals and personal care products. *Env Sci Pollut Res* (in press)
- Reemtsma T, These A (2005): Comparative investigation of low-molecular-weight fulvic acids of different origin by SEC-Q-TOF-MS: New insights into structure and formation. *Environ Sci Technol* 39, 3507–3512
- Salonen K et al. (1992): Planktonic food chains of a highly humic lake. II. A mesocosm experiment in summer during dominance of heterotrophic processes. *Hydrobiologia* 229, 143–157
- Skopintsev BA (1934): Analysis of organic matter in waters with high content of chlorides. *Zh Prikl Him* 7, 376–382 (in Russian)
- Steinberg CEW (2003): Ecology of Humic Substances in Freshwaters. Springer, Berlin, 440 pp
- Steinberg CEW et al. (2006): Dissolved humic substances – Ecological driving forces from the individual to the ecosystem level? *Freshwat Biol* 51, 1189–1210
- Steinberg CEW, Meinelt T, Timofeyev MA, Bittner M, Menzel R. (2008): Humic Substances. Part 2: Interactions with organisms. *Env Sci Pollut Res*, DOI: <http://dx.doi.org/10.1065/espr2007.07.434>
- Timofeyev MA et al. (2006): Natural organic matter (NOM) induces oxidative stress in freshwater amphipods *Gammarus lacustris* Sars and *Gammarus tigrinus* Sexton. *Sci Total Environ* 366, 673–681
- Thurman EM, Malcolm RL (1981): Preparative isolation of aquatic humic substances. *Environ Sci Technol* 15, 463–466