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THE OVERLOOKED ECOSYSTEM DRIVING FORCE IN NON-EUTROPHICATED FRESHWATER SYSTEMS: DISSOLVED HUMIC SUBSTANCES-A SHORT REVIEW AND OUTLOOK

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Abstract: This review starts with the description of the quantitative significance of dissolved organic material in general and dissolved humic substances (HS) in particular in various ecosystems. Despite their high quantities, the knowledge about the role of HS is still very low and full of old, but still recycled paradigms. HS are thought to be inert or at least refractory and too large to be taken up by aquatic organisms. Instead, I present evidence that dissolved HS that mainly derives from the terrestrial environment, are taken up and directly and/or indirectly interfere with freshwater organisms and, thus, structure biocenoses.

Relatively well known is in the meantime the fuelling function of allochthonous HS, which, upon irradiation, release fatty acids, which serve as substrates for microbial growth. This is an indirect effect of HS. Microbes, in turn, are food for mixotrophic algae and (heterotrophic) zooplankton. Thus, non-eutrophicated freshwaters are net-heterotrophic, meaning that respiration exceeds primary production. Furthermore, model calculations exemplify that only a very small portion of the terrestrial production is sufficient to cause net-heterotrophy in these freshwater bodies. But, recent papers show also that due to different stoichiometries the maximal plankton biomass production with algae or mixotrophs is higher than with bacteria.

Very recently, several direct effects of HS have been elucidated. Among them are: induction of chaperons (stress shock proteins), induction and modulation of biotransformation enzymes, modulation (mainly inhibition) of photosynthetic oxygen release of aquatic plants, production of an internal oxidative stress, modulation of the offspring numbers in the nematode *Caenorhabditis elegans*, feminization of fish and amphibs, interference within the thyroid system, and action as chemical attractant to *C. elegans*. We are still in the phase of identifying the various physiological, biochemical, and molecular-biological effects. Hence, the ecological and ecophysiological significance of these HS-mediated effects still remain somewhat obscure. Nevertheless, HS appear generally to have an impact on the individual as well as on the community and even ecosystem level comparable to that of, for instance, nutrients.

Key words: Humic substances; Modulation of sex ratio; Hormone-like effect; Chemical attractant; Biotransformation; Oxidative stress; Direct effects of humic substances; Indirect effects of humic substances; Net-heterotrophy; Forest stocking

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HS are complex organic molecules comprising the highest proportion (50%—80%) of dissolved organic matter (DOM) in all freshwater ecosystems. In any non-eutrophicated freshwater ecosystems and with concentrations between 1 and 100mg/L, [occasionally even more, for instance: Australian wetlands up to 300mg/L DOC[1], Brazilian coastal lagoons 160—200mg/L DOC[2], HS exceed the organic carbon of all living organisms by roughly one order of magnitude[3—6]. Jones (1998)[7] emphasizes that all freshwaters contain some HS of allochthonous origin. Therefore, humic-rich lakes with high water color must be seen as lying towards one end of a continuum between lakes with low concentrations of HS and lakes with high concentrations of HS.

Some textbook figures on the ratio of dead to living organic carbon are given in Tab. 1.

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Tab. 1 Estimates of carbon in organisms in relation to dead organic carbon in the ecosystems of the world (after various authors from Killops &Killops, 1993)[8]

	Ratios of carbon in living organisms to dead organic carbon	Dead organic carbon, % of total organic carbon
Terrestrial Systems	1∶3	75
Freshwater	1∶10	90
Oceans	1∶400	>99

In clear contrast to the quantitative significance of dead organic carbon in non-eutrophicated ecosystems, the knowledge on the qualitative significance (ecological function) is still minimal. Only the role of HS as direct or indirect external energy has intensely been studied in recent decades (see below). However, the most important objects of limnological studies are still living organisms, and their interactions with the ambient biotic and abiotic world. Organisms build the central dogma in ecology with the predominance of food webs on the one hand and nutrient and energy cycling on the other hand. Obviously, there is little space for dissolved organic carbon (including HS) in this perspective of the freshwater world. Hence, in many textbooks on freshwater ecology, HS are disregarded totally, or, if dealt with, are regarded refractory or even inert. However, recycling of outdated paradigms does not revive them or increase their actual validity.

In freshwater systems, the majority of HS derives from terrestrial plant debris, lignin building blocks (cumaryl alcohol, coniferyl alcohol, and sinapyl alcohol) being the main source. All of the mentioned alcohols are basically polyvalent alkylphenols-they are chemicals in the aquatic environment, natural environmental chemicals[9]. One of many potential structures, showing well the diverse structural components of soil HS, is displayed in Fig. 1. With their functional groups, they are able to react chemically with any chemical compound, be it a chemical compound in the freshwater body or in an organism. From studies of the electromobility of HS, Münster (1985)[10] was the first to postulate the ability of HS to interact with biomembranes; empirical evidence, however, was presented much later (see below).

This review will focus on three topics of HS ecology (1) uptake by organisms, (2) interaction with organisms, and (3) ecological significance. In all, it will draw to attention to an overlooked powerful ecosystem driving force in non-eutrophicated freshwater bodies.

1 Uptake of HS-like substances

The question as to whether or not HS are taken up by organisms has been argued intensively in the literature. To date, empirical evidence is increasing that HS are taken up. In a recent study, Wang et al. (1999)[11] showed that HS or at least parts thereof can be taken up by cell cultures and, at least, parts of these molecules can be found even in the DNA. Nardi et al. (2002)[12] showed that the physiological effects of HS on terrestrial plants depend on the source, concentration, and molecular mass of the HS. The authors presented evidence that HS< 3.5 kDa easily reach the plasmalemma of higher plant cells and, in part, are taken up.

Steinberg et al. (2003)[9] presented evidence that ^{14}C-labeled humic-like substances prepared by enzymatic oxidation of caffeic acid are taken up and bioconcentrated by aquatic organisms. The peak molecular mass of the caffeic acid oxidation product (KOP) detected from a non-radioactive sample was found to be 11.6kDa[13]. Fig.2 shows that, after 24 hours of exposure, a macrophyte (*Ceratophyllum demersum*), an invertebrate (*Gammarus pulex*), and a vertebrate (tadpoles of the moor frog *Rana arvalis*) are able to bioconcentrate ^{14}C in their bodies. The percentage uptake of the exposed [^{14}C] KOP lies between 7.3 and 11.7% of the exposed quantity. From kinetic experiments, we know that the uptake of [^{14}C] KOP in *Daphnia magna* is almost completed after 24h (Wiegand, Humboldt University at Berlin, pers. comm).

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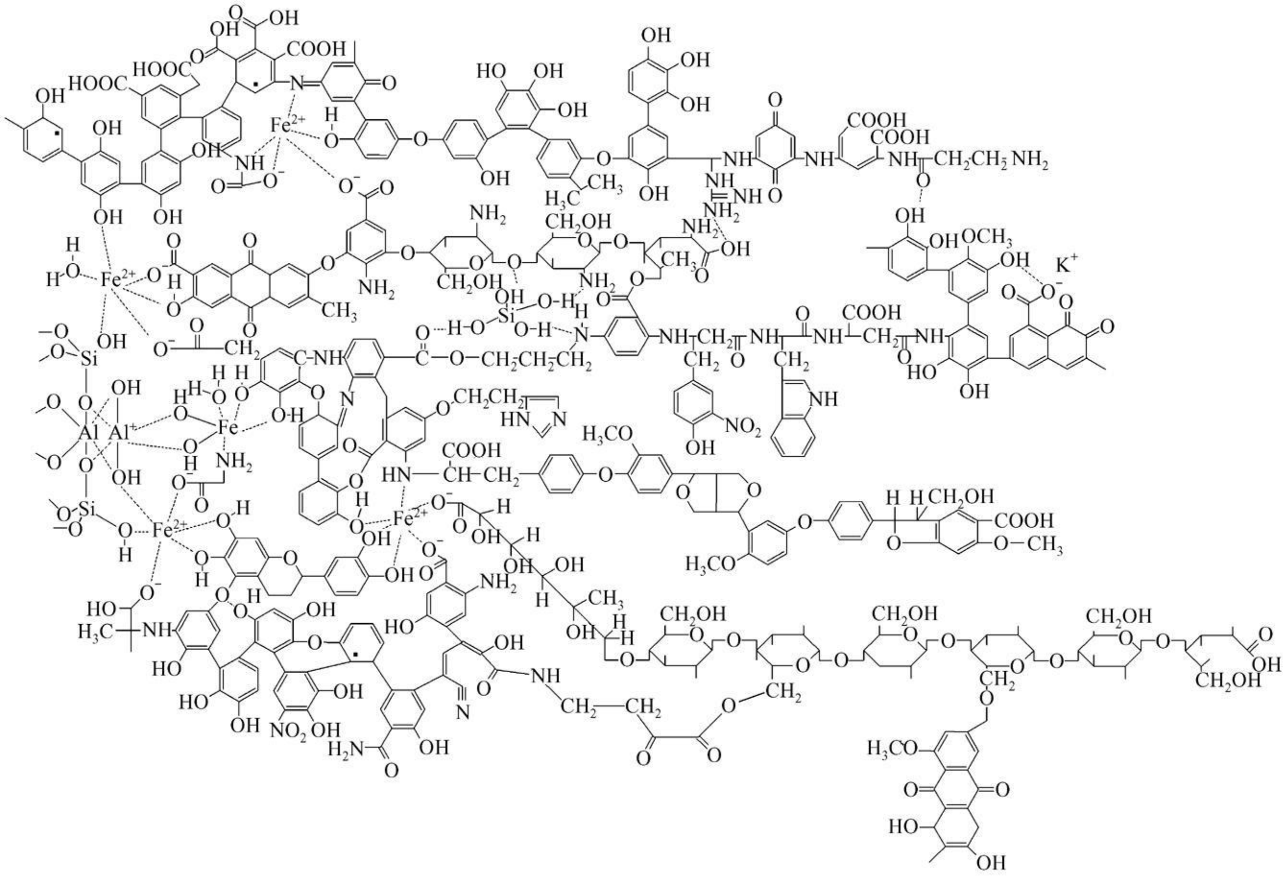

Fig. 1 Proposed structure of a soil humic substance (from Kleinhempel, 1970[14])

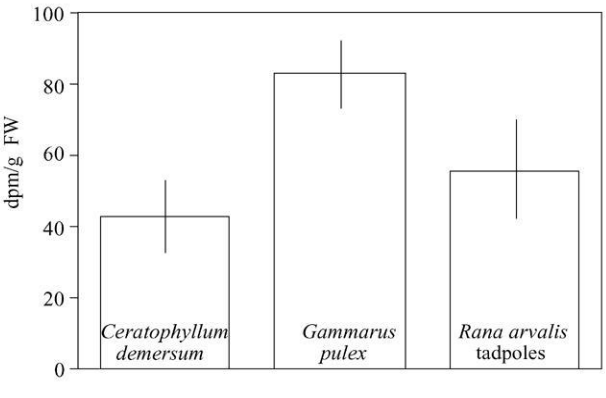

Fig. 2 Uptake of [^{14}C]-labeled caffeic acid oxidation product ([^{14}C] KOP) by three aquatic organisms within 24 hours (means + standard deviation) (from Steinberg et al., 2003[9])

It may still be argued that it is not the intact caffeic acid oxidation product, but also smaller photodegradation products, which cause the bioconcentrated ^{14}C. This argument does not refute our statement; it only means that at least low-molecular weight (photo-) products of the humic-like substances are taken up. Even if parts of the low-molecular weight products of the caffeic oxidation products are in the molecular mass range of < 1.0 kDa, they cover well the molecular masses of most fulvic acids (FA) in aquatic ecosystems[3]. Furthermore, very recent environmental-chemical studies support our statement: Reemtsma & These (2003)[15], Cooper et al. (2004)[16], and Hatcher et al. (2004)[17] show that water-soluble HS are relatively small by molecular weight and do not exceed ~0.5 kDa[1]. Hence, it is not surprising that aquatic organisms easily take up HS.

It also may be argued that HS accumulate at the membranes rather than penetrate into cell. Recent molecular biologiol studies show that the uptake may even be an active process, scince up on exposure to humic substances, several genes are activated in the nematode caenorhabditis elegans encoding for transporter proteins[6]. Futhermore, several indrect proofs also exist. If HS only accumulate at the membranes, how can

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[1] HS-associations with higher molecular weights that are often reported from freshwater studies are buit up by polyvalent cation bridges., 2004)[16,17]

one explain that cytosolic receptors, such as the estrogen receptor is induced as recently shown with the clawed frog after exposure to the synthetic HS 1500[18] Concluding, I am convinced that the paradigm that HS are not taken up must be considered outdated.

2 Interaction of HS with organisms

Once taken up, HS are able to migrate to effective organs or organelles. They act as ‘natural environmental chemicals’ or ‘natural xenobiotics’[1]. From recent environmental and microbial studies, it is evident that HS can act as redox catalysts. For instance, HS have the potential to act as electron acceptors for microbial respiration[19]. More recent work of Scott et al. (1998)[20] shows that it is the quinoide structures, which determine the properties as redox catalyst. In organisms or cells, HS must interfere within all electron transport reactions. To prove this hypothesis, we tested several of aquatic plants by exposing them to different HS and measuring photosynthetic oxygen release. Prior to photosynthesis measurement, the plants were transferred into HS-free media to exclude light quenching effects. With the coccal green alga *Scenedesmus armatus*, Fig. 3 confirms the validity of our hypothesis. Suwannee River natural organic matter (NOM), a forest soil leachate fulvic acid (FA), and a synthetic HS (HS 1500), all significantly reduce the photosynthetic oxygen release. By applying a so-called Quantitative-Structure-Activity-Relationship, Paul et al. (2004)[21] show that stable radicals, a substitute for quinone structures, may statistically count for up to 90% for the observed reduction of oxygen release.

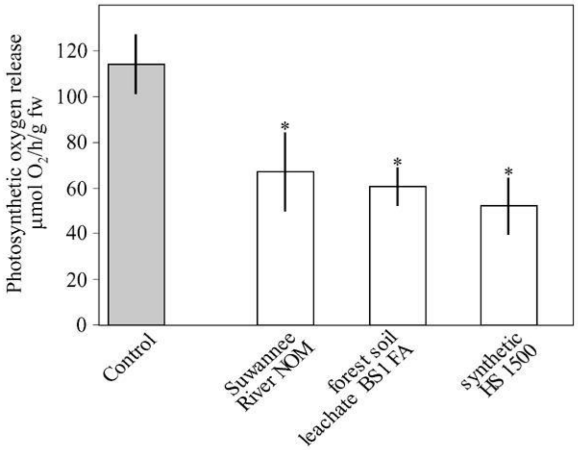

Fig. 3. Reduction of photosynthetic oxygen release in the coccal green alga *Scenedesmus armatus* after 18-h pre-exposure to 0.5mg/L DOC of three HS isolates. Data are means of three replicates ± standard deviation; * significantly different from control; fw = fresh weight (from Steinberg, 2003[6])

As any xenobiotic chemical or heavy metal, internal HS may produce an oxidative stress, caused by H_2O_2 and other reactive oxygen species (ROS) (see also Steinberg et al., 2004b[22]) that may also interfere with the photosynthesis. In an as yet unpublished study on another coccal green alga (*Monoraphidium convolutum*), Karasyova et al. (in prep.) elaborate that the reduction of the photosynthetic oxygen release actually can also be related to the cell-internal H_2O_2 concentration. Whichever mechanism applies, some ecological consequence might be derived from our findings: When comparing all three aquatic plants tested so far (Fig. 4), it is evident that there is no ‘most sensitive’ species. With Suwannee River NOM, the most sensitive species is the coccal green alga *S. armatus*; with the soil FA it is the angiosperm *Ceratophyllum demersum*; and with the synthetic HS 1500 it is the water moss *Vesicularia dubyana*. That means that a specific region, with specific terrestrial plant cover resulting in HS with specific chemical features, will, under non-eutrophicated conditions, produce a specific aquatic community of primary producers. Furthermore, anthropogenic or climate-induced changes in forest stocking most probably will result in subsequent changes of the aquatic biocenoses.

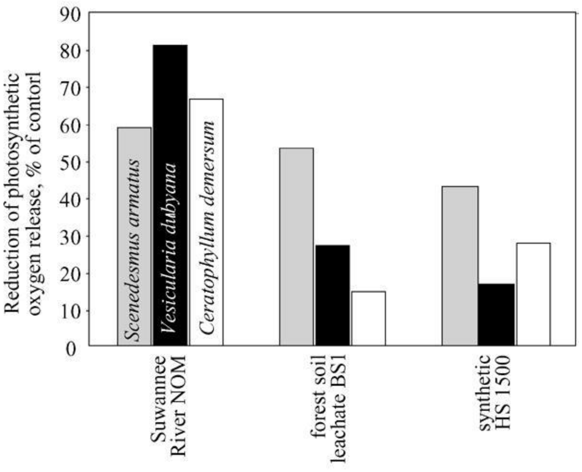

Fig. 4 Different susceptibilities of the three species of aquatic plants towards three different HS or NOM, as indicated by reduction of photosynthetic oxygen release (from Steinberg, 2003[6])

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1 These terms are contradictions by themselves; however, it appears to me that the term xenobiotic has to be re-defined.

With high plausibility, this conclusion might even be expanded to animals. There are several observations that vertebrates as well as invertebrates respond to exposure on HS by specific and non-specific defense mechanisms, such as expression of stress (heat) shock proteins, membrane (lipid) oxidation, activation of biotransformation enzymes, like cytochrome P-450 enzymes or glutathione *S* transferase[23—26] Even hormone-like effects have recently been observed with the nematode *Caenorhabditis elegan*[27—29], the ornamental fish *Xiphophorus helleri*[30], and the clawed frog *Xenopus laevis* (Fig. 5)[18, 29] With the clawed frog, the estrogenic mode (elevated levels of mER-RNA) of action has been identified so far. At least with the nematode, there is a very strong guess that alkylphenolic structures are responsible for the hormone-like effect[31] .

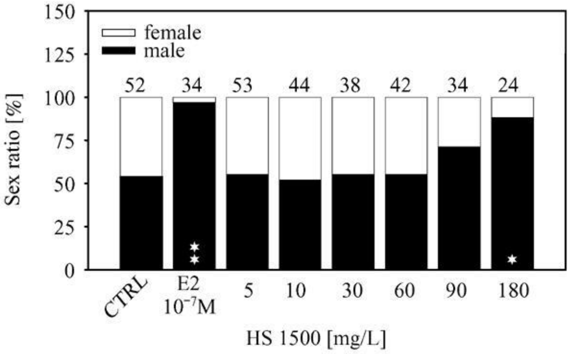

Fig. 5 Sexual differentiations of *Xenopus laevis* exposed to E2 10^{-7}M and the synthetic HS 1500. The estrogen 17β-estradiol (E2) serves as a positive control. * indicates significance at $P < 0.05$ level, * * significance at $p < 0.01$ level; one-way ANOVA correlation analysis (from Lutz et al., 2004[18])

Furthermore, HS have the potential to act as chemical cue, as recently discovered with the nematode *Caenorhabditis elegans*. With oligonucleotide-based whole genome microarrays of *C. elegans*, it can be indicated that synthetic as well as natural HS are obviously recognized as environmental signaling chemicals, because one or more genes coding for chemosensors, olfactory receptors, as well as enzymes of the biotransformation system (CYP, UDG, GST) are switched on (Menzel et. al 2005[69]). On a more phenomenological level, this molecular-biological result could be confirmed by a simple test. Petri dishes were prepared with six piles of food bacteria. Alternating, only three piles contained HS in addition to the bacteria. Adult and juvenile *C. elegans* were placed in the middle of the Petri dish. As a result, the adults, but not the juveniles preferred to move to the food bacteria with HS, as sketched in Fig. 6.

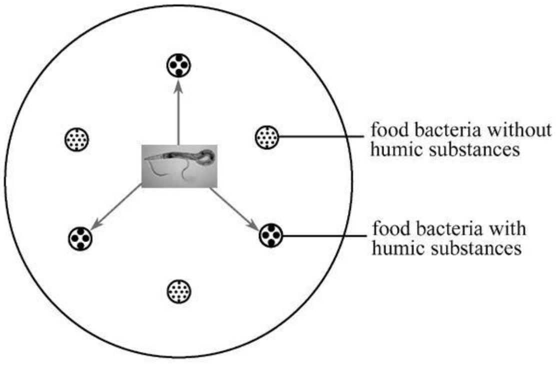

Fig. 6 Schematic presentation to prove the potential of HS to act as chemical cues towards *Caenorhabditis elegans*. Adult worms preferentially moved to the food bacteria with HS (Menzel, et al.)

At present, we are still in the phase of gathering and identification of direct effects of dissolved HS towards freshwater organisms. Essential ecological questions are still to be answered, such as:

1) Are the observed effects sustainable?

2) Or is there an acclimation or even adaptation?

3) If they are long-term effects, do they even affect the subsequent generation(s)?

4) In case of energy consumption, how may the energy loss be compensated? Or is the energy consumption in disfavor of katabolic or reproductive processes?

5) In case of feminization of lower vertebrates, is this only a bizarre phenomenon or is it of ecological relevance?

In general, the described effects remain still more or less on the individual level and are lacking the evidence of higher ecological levels. That means that studies on the ecosystem level, which relate biocoenotic community structures with chemical characteristics of allochthonous HS are not yet available; nevertheless, there exists some evidence from field studies that HS might have an controlling effect on aquatic biocenoses. For instance, Bjürk (2004)[32] reported that one of the European freshwater pearl mussels (*Magaritifera margaritifera*) significantly declined after the forest stocking has been changed from deciduous to coniferous trees. Similar observations are recorded from Upper Austria (D. Csar, University Salzburg, pers. comm.). The changes in stocking led to HS qualities with higher aromatic moieties that supported less microbial growth and very likely impacted aquatic organisms directly more than HS from deciduous or mixed forest. From a biochemical perspective, these field studies are substantially supported by the fact that in *M. margaritifera* specimen, biotransformation enzymes, such as glutathione-*S*-transferase, are significantly higher than the activity of any metabolic enzymes (Steinberg, Peuthert &Pflugmacher to be published). As a consequence, we assume that, due to the lower food quality of the HS from coniferous forest, the energy spent for their biotransformation could not be compensated. Some fifty and more years ago, when the changes in the forest stocking took place, sewage water simultaneously was diverted into streams and rivers. Hence, the impact of the less favorable humic material from coniferous forest on aquatic animals was overlooked or simply attributed to potentially adverse effects of sewage water.

3 Ecological significance of HS

Compared to the knowledge of direct effects of HS on aquatic organisms, much more is known about the indirect effects of HS. One of the most pronounced indirect effects is the input of biochemically fixed energy from allochthonous sources. Organic carbon from terrestrial sources clearly delivers energy and nutrients to aquatic bacteria. This energy is, in turn, transferred to zooplankton and to fish. The aquatic microorganisms therefore demonstrate the link between the terrestrial primary production and the aquatic secondary production[33]. The fixed C is made available to microorganisms in the aquatic system through the action of light on the chromophoric part of HS.

The first clear evidence of non-eutrophic lakes being net heterotrophic (respiration exceeds primary production by algae and macrophytes) is presented by Salonen et al. (1992)[34]. In nutrient-rich waters, the autochthonous production dominates. Additional evidence for net-heterotrophy is provided from Laurentian Great Lakes[35, 36], experimentally manipulated forest lakes[33, 37].

To test the applicability of the net heterotrophy hypothesis, Blomqvist et al. (2001)[38] add dissolved organic carbon, in the form of sucrose, to an oligotrophic humicpoor lake, initially characterized by a pronounced dominance of autotrophic phytoplankton. The authors assumed that it is organic carbon *per se* that determines the differences in structure of the planktonic ecosystem between humic-rich and humicpoor lakes. Actually, the additions of DOC result in a significant increase in bacterial biomass and a decrease in the biomass of autotrophic phytoplankton. The biomass of mixotrophic and heterotrophic flagellates increase significantly, whereas no effects are found to extend to higher trophic levels. As a result of the changes among biota, total planktonic biomass also decrease to a level typical of nearby humic lakes. Blomqvist et al. (2001)[38] suggest that it is the carbon component of HS, and its utilization by bacterioplankton that determines the structure and function of the pelagic food web in humic lakes.

It is striking that pigmented flagellates, mainly chrysophytes and cryptophytes, dominate the phytoplankton of humic lakes. Many of these algae are mixotrophic. Obviously, mixtrophy is an advantageous strategy when the availability of light and/or nutrients is low[39]. Jansson (1998)[40] assumes that it appears to be energetically more efficient for the phytoplankton to consume bacteria, which concentrate nutrients from the medium, than to activate and maintain a specific uptake mechanism. In addition, the bacteria can exploit the allochthonous detritus as a nutrient and energy source much more easily than can the phytoplankton. A frequent outcome for the mixotrophic phytoplankton is that as a consequence of the high P content of the consumed bacteria, the phytoplankton becomes N limited (Jansson, 1998[40]. The dominance of bacteria in humic lakes and the reaction of the various microplankton compartments (in terms of nutrient uptake) are summarized in Fig. 7. The enrichment of P and N alone, and in combination, supports the hypothesis above that the bacterial production is P limited, as bacterial biomass significantly increased only with P addition (Fig. 8). Not only in enclosures, but also in the lake itself, the biomass of mixotrophs is high. It is usually higher than the biomass of heterotrophic flagellates during summer. This dominance cannot be explained by higher grazing rates among mixotrophs. Instead, ratios between mixotrophic and heterotrophic biomass were positively related to light availability. Therefore, the authors suggest that photosynthesis can enable mixotrophic flagellates to outcompete heterotrophic flagellates[41].

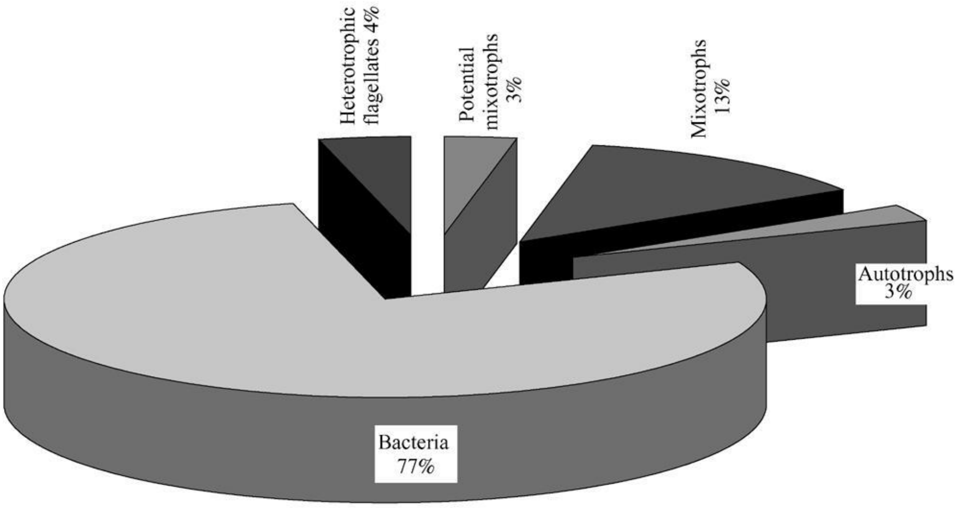

Fig.7 Increases in biomass (μg/L C) of bacterioplankton, autotrophic, and mixotrophic phytoplankton, and heterotrophic nanoflagellates in P enrichment experiments in the brownwater Lake Öträsket, northern Sweden (from Jansson et al., 1996[42])

The utilization of the energy in the dissolved carbon from terrestrial sources may be accomplished indirectly by photolysis and microbial uptake of the released short-chained fatty acids[43—49], or directly via attack of the carbon skeleton of the HS. Münster et al. (1998)[50] are the first limnological group to test an unspecific peroxidase in a humic lake and emphasize that enzymes, which directly attack the C skeleton of HS, rather than the side chains, evidently play a fundamental role also in aquatic systems.

Comparable results are reported from Lake Fuchskuhle (Fig.8). This lake has artificially been divided and one quarter (SW basin) receives seepage water from an oligotrophic bog. It is evident that the peroxidase activity is elevated in that basin. Interestingly, the highest activities are found during fall and winter months, which have the highest inflow of surface and seepage waters. Because the search for aquatic microorganisms responsible for the peroxidase activity has failed thus far, Burkert (Max-Planck-Institut of Marine Microbiology, Bremen pers. comm.) concludes that the peroxidase is probably washed in from the catchment during the high flow periods—as the HS are. If this assumption can be corroborated, it would mean that non-eutrophicated lakes and even their metabolism depend on their catchments in two ways: 1) C and energy is washed in as HS, and 2) the necessary tools to handle these substances are imported, too.

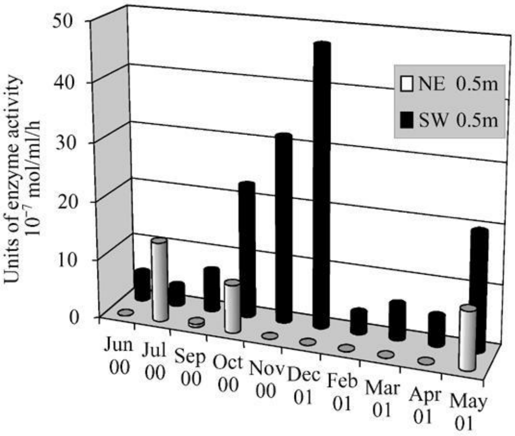

Fig.8 Direct utilization: peroxidase activity in the divided, differently humic Lake Fuchskuhle (Brandenburg, Germany), Summer 2000. SW basin: impacted by an ombotrophic oligotrophic bog; NE basin served as a control[28]

Pelagic food webs of non-eutrophicated freshwater systems are dominated by heterotrophic energy acquisition from allochthonous sources. Bacterial production exceeds primary production by far. In 21 major Scandinavian catchments, Algesten et al. (2003)[51] calculated the carbon loss for roughly 80,000 lakes and running water. Between 30% and 80% of the total organic carbon that entered the freshwater ecosystems was lost in lakes. Mineralisation in lakes and subsequent CO_2 emission to the atmosphere was by far the most important carbon loss process.

Now, the question rises, whether or not the alimentation does only apply to the basis of the foodweb or even to higher trophic levels. By use of two-compartment labeling, Hessen et al. (1990)[52] explain the relative roles of detritus, bacteria, and phytoplankton as C sources for various crustaceans (Fig. 9): approximately 50% or even more of the organic C in the bodies of zooplankton originates from detritus sources, 5%—10% from bacteria, and up to 40% from phytoplankton. Comparable data are also obtained by Jones et al. (1998)[53] in Loch Ness (Scotland), a relatively large, moderately humic lake. Here, around 50% of the body C comes directly, or indirectly, via bacteria from detritus sources. These findings clearly show that allochthonous energy sources can support the food web in lakes, independent of their size. Furthermore, a qualitatively similar tendency is found in the food web of subarctic lakes in northern Sweden[54].

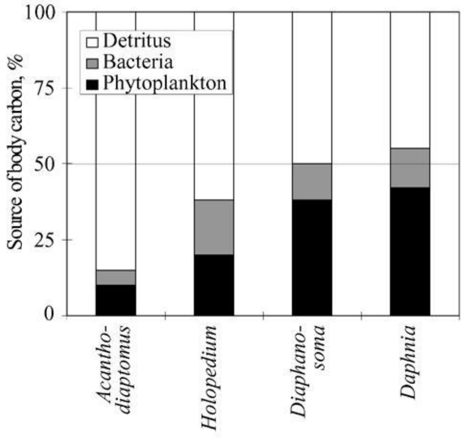

Fig. 9 Share of detritus, bacteria and phytoplankton in the diet of the dominating crustacean zooplankton in Lake Kjelsåsputten, based on separate labeling of food compartments. The shares are based on specific activity in seston fractions and animals at isotope equilibrium (after Hessen et al., 1990[52])

The acquisition of detritus carbon can be accomplished via bacteria and directly via detritus C uptake. Using a high-resolution X-ray microscope, Myneni et al. (1999)[55], for instance, describe the configuration of HS and FA build aggregates of various size and form, if the concentration is high (1g L^{-1}). Divalent cations cause thread-like structures, and trivalent cations cause globular as well as thread-like structures (Fig. 10). Provided that the above displayed structures also occur under conditions of natural DOC concentrations, one can easily see that aggregates > 1—2μm in diameter are formed in the presence of divalent metal cations hard-water lakes, and these can be taken up by zooplankton grazers as a major carbon source. A new picture of marine dissolved organic carbon showing that 10%—40% is in the form of colloids which can aggregate[56] indirectly supports the finding of Myneni et al.[55]. Hence, the aggregation is feasible and is an important initial step in the formation of particulate organic carbon, which can serve as C source for heterotrophic particle feeders.

Due to different stoichiometric compositions of phytoplankton (carbon-rich) and bacterioplankton (phosphorus-rich), biomass production in Lake Örträsket, however, is higher when the edible plankton is dominated by phytoplankton than by bacterioplankton[57]. As the productivity of bacterioplankton and phytoplankton is nutrient limited in Lake Örträsket, a probable explanation is that the cell C:N:P ratio permits phytoplankton to produce more biomass than bacteria per unit available N or P.

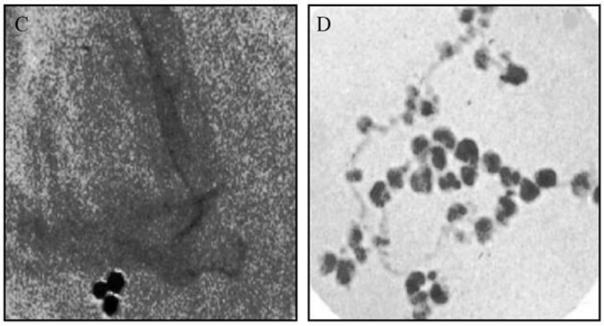

Fig. 10 HS studied by high resolution X-ray microscope, here effects of cations: C) threats, divalent cations; D) globular and threats, trivalent cations (scale bar = 500 nm). (Myneni et al., 1999[55], http://www-als.lbl.gov/als/science/sci-archive/humic.html.)

Concludingly, one may emphasize that a portion, and in boreal waters a large portion, of the organic C in the water body comes from the catchment area. To estimate the quantity of organic C necessary for net heterotrophy of aquatic ecosystems, Cole (1999)[33] set down a model calculation with the following assumptions: net heterotrophy is indicated by a CO_2 supersaturation for lakes with forested catchments when the supersaturation is around 1 matm[58]. The net efflux of C as CO_2 will be about 40g m^{-2} a^{-1} with 250 ice-free days per year. If the lake's catchment area is 10-fold larger than the lake surface, the forest needs to export only 4g m^{-2} a^{-1} of C to meet this demand. Given a typical forest net primary production of approximately 500g m^{-2} a^{-1}[59], then only 1% of this terrestrial production is required to sustain net heterotrophy in the lake. If this net heterotrophy respires 50% of the terrestrial DOC input to the lake[60], only 2% of terrestrial production is required for net heterotrophy. This is an almost negligible quantity of the terrestrial production.

4 Epilogue to evolution of HS and life on earth

From several discussions with ecotoxicologists, the question arose, why the biotransformation system in organisms is that conservative. It exists with very little variation in bacteria, algae, higher plants, invertebrates, and vertebrates, including man. Hence, it must be very old, very aboriginal. Phylogenetically, it must have been developed very early. Actually, it seems that one of the ancient' xenobiotic chemicals' are HS or humic-like substances. . For instance, Ziechmann (1996)[63] writes: '*According to new results, HS and related molecules are formed at a very early point in chemical evolution from* CH_4, H_2O, NH_3, *and* H_2 *as well as from their products. With this astonishing fact that HS can also be built from small inorganic building blocks and their derivatives, a corner stone of previous HS research has collapsed. Up to now, it is thought that HS are a typical component of active soils (and other media, e.g. or water)...*'

Humus or at least HS-like molecules become established in ecosystems independently from life and death events and play a definitive role in early evolution. Ziechmann (1994)[64] and Bada & Lazcano (2000)[65] point out that, in the pioneering studies of Stanley L. Miller (1955)[66] on the origin of life in the primitive reducing atmosphere 4× 10^9 years ago, colored tarry substances[1] were present, however, were not described or characterized in further detail since Miller focused on the amino acids to identify them as the missing biomolecules to prove the hypothesis that life on Earth has developed in a reducing atmosphere. Ziechmann (1994)[64] and his co-workers repeat Miller's experiment using more energy. Dark brown substances, HS, or at least HS-like materials, also form in this experiment, as shown in UV and IR spectra, and by precipitation with hydrochloric acid. According to their element composition, these materials are somewhere between humic acid precursors and humic acids themselves.

Ziechmann (1994)[64] sees the most important role of HS to be in chemical and biological evolution (Fig. 11): HS eliminate reactive radicals, which otherwise inhibit or prevent the synthesis of amino acids, carbohydrates, and nucleic acids. Thus, without HS serving as radical scavengers, in the primitive atmosphere, evolution would not be possible, since no biomolecules could otherwise form in the strong UV radiation.

The discovery of HS formation in the primitive atmosphere has further far-reaching consequences for the understanding of ecosystem functioning. The HS are to be granted the role of an independent ecosystem component, such as atmosphere, water, or light, since they come into being simultaneously with early life. This means that living organisms have to adapt to humus or HS-like materials with which they come in contact from the very time they evolve. Because HS are natural environmental chemicals, they exert a chemical stress on organisms in many ways, and thus determine ecological niche, and structure biocoenoses and guilds. The chemical stress applies not only to terrestrial systems, but also to freshwater systems in which the humus content is relatively low.

Once organisms have evolved, and through the formation of humus from dead biomass, the quality of HS becomes more diverse and its quantity greater. For organisms, the adaptive pressure rises. Already from this, the general statement follows that organisms can only develop with HS in their environment. Since HS interfere with cell-internal metabolism, biological systems have to defend themselves against their protector against UV radiation and chemical radicals-the HS. We hypothesize that this chemical stress exerted on bacteria, fungi, plants, and animals since the primitive Earth, is likely one major reason for the fact that the' defense' systems are very similar in all organisms. In fact, the biotransformation systems are very conservative, occurring in only slightly altered forms in organisms from bacteria to mammals. And all are responding to exposure to

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[1] In their congratulations to Miller on the occasion of his 70th birthday, Bada & Lazcano(2000)[65] write about the pioneering work of the laureate: "*He started the spark and began to heat the water flask gently. After two days, the water had turned pale yellow and a tarry residue coated the inside of the' atmosphere' flask around the electrodes. Anxious and unable to wait any longer, he stopped the experiment at that point and analyzed the water for amino acids.... Stanley repeated the experiment, this time sparking the apparatus for a week and boiling the water rather than heating it gently. At the end of the week, the inside of the sparking flask was coated with an oily scum and the water solution was yellow-brown. Now the glycine spot on the paper chromatogram was far more intense....*"

HS or humic-like substances as shown in several recent and previous papers[9, 22, 24-26, 67, 68].

Or in other words, although HS or humic-like substances obviously belong to the oldest organic molecules on Earth, the knowledge about them to date is only fragmentary and still full of wrong paradigms.

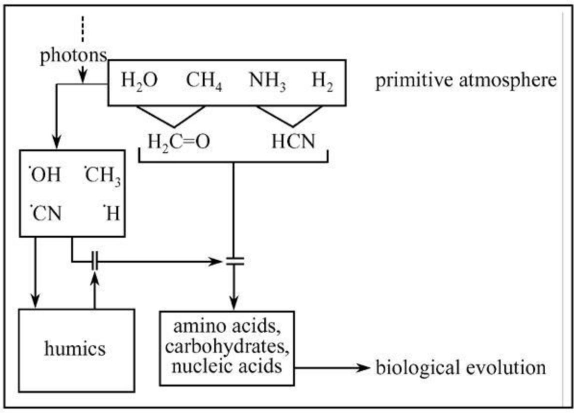

Fig. 11 The role of HS or HS-like materials in the ancient atmosphere as radical scavengers so that biomolecules such as amino acids, carbohydrates or nucleic acids can be formed (after Ziechmann 1994)[64]

5 Conclusion

As a kind of conclusion, I am going to relate structural features of dissolved HS with environmental, ecochemical, and ecophysiological effects in freshwater ecosystems as displayed in Tab. 2. It is obvious that dissolved HS have the potential to interfere within almost any biochemical and biogeochemical pathway in both aquatic organisms and freshwater systems. The major structural feature that is responsible for most of the reactions and effects of HS in freshwater ecosystems is the aromaticity. Aromaticity of HS originates from lignin of terrestrial plants. That means that freshwater ecosystem are mirrors of their terrestrial environments and the chemical cue is the humic material. In general, humic substances have to be considered an ecological controlling force as, for instance, nutrients, temperature, or even light.

Tab. 2 Structural features of dissolved HS and their potential effect in freshwater ecosystems and aquatic organisms respectively (after Steinberg, 2003[6], with reference to the original sources)

Structural feature of dissolved humic substances and precursors	Potential effects on various scales and targets
ionic structures⇒	water solubility of HS
ionic structures⇒	metal complexation at higher concentrations
aliphatic structures, such as carbohydrates, amino acids⇒	microbial (dark) production
lignin ⇒	***aromaticity of HS***
aromaticity⇒	UV absorption⇒shield against UV-B increase
aromaticity⇒	photomineralization⇒CO, CO_2 development
aromaticity⇒	photolytic release of organic (fatty acids) & inorganic nutrients (P, N)
aromaticity⇒	photo sensibilized degradation of xenobiotics and allelochemicals, such as cyanotoxins
alkylaromatics⇒	hormone-like effects
quinoide structures⇒	electron acceptors, redox catalysts

Acknowledgments

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