Subject Area 2.1: Behavior of chemicals in water and their interactions with organisms

Review Series

Humic Substances

Part 2: Interactions with Organisms*

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Abstract

Goal, Scope and Background. Freshwater bodies which chemistry is dominated by dissolved humic substances (HS) seem to be the major type on Earth, due to huge non-calcareous geological formations in the Northern Hemisphere and in the tropics. Based on the paradigm of the inertness of being organic, direct interactions of dissolved HS with freshwater organisms are mostly neglected. However, dissolved organic carbon, the majority of which being HS, are natural environmental chemicals and should therefore directly interact with organisms. Major results that widened our perspective on humic substance ecology come from experiments with the compost nematode, *Caenorhabditis elegans*, which behaved contradictorily to textbook knowledge and provoked an in-depth re-consideration of some paradigms.

Approach. To overcome old paradigms on HS and their potential interactions with organisms, we reviewed recent international literature, as well as 'grey' literature. We also include results from own ongoing studies.

Results. This review focuses on direct interactions of dissolved HS with freshwater organisms and disregards indirect effects, such as under-water light quenching. Instead we show with some macrophyte and algal species that HS adversely interfere with photosynthesis and growth, whereby closely related algal species show different response patterns. In addition to this, HS suppress cyanobacteria more than eukaryotic algae. Quinones in the HS appear to be the effective structure.

Furthermore, HS can modulate the offspring numbers in the nematode *C. elegans* and cause feminization of fish and amphibians – they possess hormone-like properties. The ecological consequences of this potential remain obscure at present.

HS also have the potential to act as chemical attractants as shown with *C. elegans* and exert a mild chemical stress upon aquatic organisms in many ways: induction of molecular chaperons

(stress proteins), induction and modulation of biotransformation and anti-oxidant enzymes. Furthermore, they produce an oxidative stress with lipidperoxidation as one clear symptom or even stress defense strategy. Stronger chemical stresses by HS may even lead to teratogenic effects as shown with fish embryos; all physiological responses to HS-mediated stress require energy, which were compensated on the expense of yolk as shown with zebra fish embryos. One Finnish field survey supports the view of a strong chemical stress, as the weight yield in fish species decreases with increasing HS content in the lakes.

Discussion. HS exert a variety of stress symptoms in aquatic and compost organisms. According to current paradigms of ecotoxicology, these symptoms have to be considered adverse, because their compensation consumes energy which is deducted from the main metabolism. However, the nematode *C. elegans* looks actively for such stressful environments, and this behavior is only understandable in the light of new paradigms of aging mechanisms, particularly the Green Theory of Aging. In this respect, we discuss the mild HS-mediated stress to aquatic and compost organisms. New empirical findings with HS themselves and HS building blocks appear to be consistent with this emerging paradigm and show that the individual lifespan may be expanded. At present the ecological consequences of these findings remain obscure. However, a multiple-stress resistance may be acquired which improves the individual fitness in a fluctuating environment.

Conclusions. It appears that dissolved HS have to be considered abiotic ecological driving forces, somewhat less obvious than temperature, nutrients, or light.

Perspectives. The understanding of the ecological control by dissolved humic substances is still fragmentary and needs to be studied in more details.

Keywords: Chemical stress defense; dissolved humic substances; feminization; longevity; membrane irritation; multiple stress resistance; natural herbicides; natural organic matter (NOM); teratogenic effects

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Introduction

On the global scale with huge geological formations, such as the bedrock shields in the Northern Hemisphere or the nutrient-poor regions in the tropics, the non-calcareous, HS-rich freshwater type appears to predominate over the calcareous, HS-poor one. With up to 80%, humic substances (HS) comprise the majority of the organic carbon in any freshwater type, including all organisms. This figure even applies to non-eutrophicated freshwaters which do not have visible brownish colors (Jones 2005, Wetzel 2001). Yet, the knowledge of how HS control freshwater life is comparably scarce. Many HS-rich waters seem to have reduced biodiversity which applies to zooplankton, zoobenthos (e.g., Vuori and Muotka 1999), and fish [for instance, compare the species number of the Amazonian white-water Rio Solimões with its several thousand species with that of the HS-rich Rio Negro with its only ~1,000 species (Chao 2001)], phytoplankton and macrophytes (refer to Steinberg 2003, Steinberg et al. 2006). One explanation for the low diversity of primary producers is the poor underwater light climate (Jackson and Hecky 1980). From field observations, it is well understood that in HS-rich boreal lakes, nymphaeids and helophytes dominate (Nykänen et al. 2005). The same obviously applies also to tropical HS-rich freshwater systems, for instance the coastal lagoons [a major type of landscapes on the global scale, see Kjerfve (1994)] in the Restinga de Jurubatiba National Park, State of Rio de Janeiro, Brazil, in which *Nymphaea ampla* L., *Nymphoides indica* (L.) O. Kuntze, *Typha domingensis* Pers., and tropical *Eleocharis* spp. dominate (Fig. 1) (Gonçalves et al. 2004, Suhett et al. 2004).

Many studies deal with the interaction of natural organic water constituents (dissolved organic carbon, HS, etc.) with xenobiotic chemicals and/or heavy metals and show a natural attenuation/quenching of the potentially adverse chemicals [see papers by Pan et al. (2008a, b) in this series]. But what is the 'normal' function of these water constituents in a non-eutrophicated freshwater ecosystem? The quantitative aspect of these substances is well known: humic substances (HS) exceed the organic carbon in all living organisms by roughly one order of magnitude (Thurman 1985, Wetzel 2001); the concentrations lie between 0.1 and 8.5 mmol L⁻¹. With >14 mmol L⁻¹, extreme HS concentrations are found in one coastal lagoon in the Restinga de Jurubatiba National Park (Suhett et al. 2004). In obvious contrast to this quantitative significance of HS, the knowledge on the ecological function (qualitative significance) is still small. Only its func-

tion as an indirect external energy source has intensely been studied during the last two decades after the energy budget calculation of a humic lake by Sarvala et al. (1981) and the pioneering mechanistic studies of Geller (1985) and Tranvik and Höfle (1987) (see Farjalla et al., in press). Although the microbiological papers were the major opening of the ecological understanding of dead organic matter in freshwaters, this point of view remains still traditional, especially by disregarding HS as natural environmental chemicals. In freshwater systems, HS derive from peat and, the majority, from terrestrial plant debris, lignin building blocks, tannins and terpenoids being the main source material (Leenheer and Rostad 2004). The presence of functional groups such as carboxylic, phenolic, ketonic, aromatic, aliphatic etc. ones, enable HS to interact with both living and non-living matter.

This review will focus on various direct interactions of HS with freshwater organisms. In 1985, two papers appeared hypothesizing direct effects of HS. From studies of the electromobility of HS, Münster (1985) postulated the ability of HS to interact with biomembranes, which was also concluded by analogy by Visser (1985). Visser described how low HS concentrations could strongly stimulate, and high concentrations inhibit microorganism development – a so-called hormetic effect (Calabrese 2005). Meanwhile, it is accepted that HS are taken up (e.g., Ziehmman 1996, Wang et al. 1999, Beer et al. 2000, 2003, Steinberg et al. 2003, Kulikova et al. 2006a) and induce a variety of response reactions in the organisms. Once internalized, HS can exert specific as well as non-specific effects. So far, specific effects comprise reduction of the photosynthetic oxygen production, estrogenicity, or chemical attraction. Non-specific effects are physical and chemical membrane irritation, induction and modulation of biotransformation enzymes, induction of stress defense proteins (chaperons), or oxidative stress defense. Also programmed cell death reactions may be induced as observed with human cells (Cheng et al. 2003, Hseu et al. 2002, Yang et al. 2004). Furthermore, (artificial) HS have antiviral, anticoagulant, profibrinolytic, and anti-inflammatory properties (Meerbach et al. 2003, Helbig et al. 1997, 2003, Mahr et al. 2003).

This review starts with specific effects and then switches to the description of the non-specific ones. Concluding, we discuss the potential benefits of mild chemical HS-mediated stress.

Fig. 1. *Nymphoides indica* and *Typha domingensis* in the Cabiúnas Lagoon (1.7 mmol L⁻¹ DOC) (**left** and **center**) and *Eleocharis* spp. (**right**) in a desiccating arm of the Atoleiro Lagoon (>14 mmol L⁻¹ DOC), Restinga de Jurubatiba National Park, RJ, Brazil (Photos: C.E.W. Steinberg)

1 Approach

This review screens current papers on direct interactions of HS with organisms which have been published in accessible journals and refers to proceedings of recent meetings, too. Furthermore, it summarizes discussions and opinions which have been raised at these meetings. From ongoing studies we derive information and propose hypotheses for future research. New hypotheses are created by combining ecological findings with those from research on aging. Particularly the paradigms of the aging theories may put an innovative impetus into the stress ecological approaches.

2 Results

The results highlight recent findings of HS

- Interfering within the photosynthetic oxygen production,
- Interfering with info chemicals and hormones,
- Displaying hormone-like effects,
- Regulating a variety of genes in the compost nematode and attracting it into a stressful environment, whereby stress identified symptoms comprise:
 - Interaction with membranes,
 - Impact on ion regulation, and
 - Chemical stress defense reactions, such as Hsp, bio-transformation, MXR, and physiological costs.

2.1 Interference within photosynthetic oxygen production

From recent environmental and microbial studies, it is evident that HS have the potential to act as external electron acceptors for microbial respiration (Lovley et al. 1996). One may hypothesize that HS, once taken up, should interfere within all electron transport reactions in organisms or cells. This hypothesis has been validated with several freshwater plants and cyanobacteria by exposing them to different HS sources and measuring the photosynthetic oxygen release (Pflugmacher et al. 2006). Paul et al. (2003) showed that quinones may statistically count for the majority (>80%) of this effect. HS may directly quench electrons or bind to the bioquinones in photosystem II (PS II) and thereby block the electron transfer. Both mechanisms may be valid; however, the last one appears to be more likely, since Pflugmacher et al. (2006) found that PS II was most affected. PS II is responsible for the cleavage of water and the subsequent release of molecular oxygen. Another mode of action may also apply: internal oxidative stress (production of reactive oxygen species, see below). Future studies will reveal, which mode of action is prevalent and whether or not the two mechanisms are interrelated.

The HS-mediated stress may directly (via toxicity) or indirectly (via development of oxidative stress) result in the suppression of the more sensitive and the relative promotion of less sensitive species, as has been shown with three primary producers from different phyla (Steinberg 2003, pp. 285 ff) and more recently with two closely related species of the coccal green algal genus *Monoraphidium* (Karasyova et al. 2007). Interestingly, prokaryotic photosynthetic microorganisms appear to be much more sensitive than eukaryotic algae (Prokhotskaya and Steinberg 2007), probably because

of their simpler cell structure. Empirically, this fact has already been the basis for combating cyanobacterial growth and blooms by adding cheap natural phenolic compounds, such as leachates from straw and leaf litter (e.g., Welch et al. 1990, Ridge et al. 1999). The high sensitivity of cyanobacteria is in good agreement with field observations, which show that they are not able to develop in eutrophicated brown water lakes (Hehmann et al. 2001, Willén 2003) and cannot take advantage of their accessory pigments (for details, see Jones 1998).

If the assumption that prokaryotic photosynthetic microorganisms are more susceptible because of their simpler cell structure holds true, what is about the heterotrophic prokaryotes and their sensitivity? Microbiologists studying the biodegradation of HS by bacteria did not yet find direct adverse effects (L. Tranvik and M. Jansson, pers. comm.). However, some evidence is emerging that also heterotrophic microorganisms may suffer from HS exposure. Recently, Gryndler et al. (2005) found moderately antibiotic activity of HS in a hydroponic system. Experiments with 9 isolates from Scandinavian surface waters yielded significant growth-retarding effects on *E. coli* at environmental relevant concentrations (Paul et al. 2006): the stronger the humification the slower the growth.

2.2 Interaction with info chemicals and hormones

Pheromones play a determinant role in synchronization of the male–female reproductive physiology and behavior. With fish, many of the pheromones are steroidal in nature, and therefore barely soluble in water. Hence, one would expect that the hydrophobic pheromones bind to the hydrophobic fraction of HS; this binding, in turn, may cause their signaling function to be adversely affected (Hubbard et al. 2002). The authors recorded the response of the olfactory epithelium of the goldfish (*Carassius auratus* L.) to a steroid pheromone. Even at environmentally realistic concentrations, there is a significant attenuation of the amplitude of the response to the pheromone. These findings have been confirmed in a subsequent study (Mesquita et al. 2003). One may speculate that this effect may have deleterious impacts on the reproductive success of fish species and may be applicable to the chemical communication systems in general. More recently, Zenkevics et al. (2005) showed that HS have the potential to significantly decrease the sensitivity of frog oocytes to gonadotropic hormones. Furthermore, also the hormone molecule itself is adsorbed to the HS, thus producing deeper negative influence on the oocyte-hormone interaction. These results suggest that aquatic frog communities, and their reproduction, may be influenced by the concentration of HS in the water. However, how realistic all these phenomena are, is not as yet clear.

2.3 Hormone-like effects

Exposure to HS may induce several receptors which have been studied in relation to the so-called Blackfoot disease in Taiwan (see Gau et al. 2001). Among these were peroxisome proliferator activated receptors (Lee et al. 1999), α_2 -adreno receptors and D₂-dopamine receptors (Beer et al. 2002), and

Fig. 2: Sexual differentiation of fertilized eggs of the common carp, *Cyprinus carpio* L., exposed to 0.83 mmol L^{-1} of three different humic materials for 4 months (Meinelt et al. unpublished). Due to Berlin restrictions, only 10 individuals were allowed to be tested in each experiment; hence, no statistics can be applied. Nevertheless, the tendency of decreasing male numbers in favor of females or hybrids due to HS exposure is apparent. FuKu-NOM: reverse osmosis isolate from Lake Fuchskuhle, Brandenburg State, Germany; HS1500: artificial humic-like material with the main molecular mass of 1.5 kDa; HuminFeed®: commercial HS isolated from leonardite by alkaline extraction

nuclear factors (Gau et al. 2000). Also hormone-like effects of HS themselves have recently been recorded with the nematode *Caenorhabditis elegans* (Maupas) (Höss et al. 2001), the ornamental fish *Xiphophorus helleri* Heckel (Meinelt et al. 2004), and the clawed frog *Xenopus laevis* (Daudin) (Lutz et al. 2005). With the clawed frog, the estrogenic mode of action has been identified so far. Furthermore, preliminary results showed that also the thyroid system has been affected whereby the complexation of iodine by HS (reduced bioavailability) may be one likely mode of action. Similar conclusions were also provided by Danish scientists (Laurberg et al. 2003). Another, more direct, mode of HS action explaining a potential HS-induced goitrogenic effect can be the influence of HS on thyroid hormone metabolism by inhibiting hepatic thyroxine 5'-monodeiodinase activity (Huang et al. 1993, 1994). However in all these studies, the applied concentrations were not environmentally realistic. With three different HS preparations and 10 carp embryos each, the feminization experiment with fish has been repeated (Fig. 2): the tendency of the reduction in male numbers in favor of females and/or hybrids is obvious, although the results lack significance. The responsible structures for the hormone-like effects are not identified. At least with the nematode, there is a very strong guess that alkylphenolic structures were responsible (Höss et al. 2002); with the vertebrates, however, the effective structures remain obscure.

With a human cell line, also the anti-estrogenic mode of action has recently been shown with 8 out of 12 commercially available HS (Janošek et al. 2007). The possible explanations of this effect include sorption of 17- β -estradiol (E2) on HS, changes in membrane permeability for E2 or another specific, yet undiscovered mechanism.

2.4 Chemical attraction and gene regulation in *Caenorhabditis elegans*

To get an overview of the reactions upon HS-exposure, oligonucleotide-based whole genome DNA microarray experi-

ments (ecological genomics) were recently performed with the compost-dwelling nematode *C. elegans* (Menzel et al. 2005). Notable transcriptional up-regulations were identified in chemosensors and olfactory receptors. HS from different sources have the potential to act as environmental signals, and, in deed, adult worms migrated towards the humic material. This appears to be a genetically fixed property of *C. elegans*.

Besides the chemoreceptors, also transporters were induced which indicates that HS might have actively been taken up. Furthermore, a limited number of genes coding for enzymes involved in biotransformation were found to be differentially expressed, namely cytochromes P450, glutathione S-transferases, and UDP-glucuronosyltransferases. As we shall see, HS cause oxidative stress and other stress symptoms. Consequently, the question arises, why does *C. elegans* actively look for stressful environments? Does it only tolerate this stress, which some researchers consider adverse, or has the stress even positive effects to the worms? This aspect will be discussed in more detail after completing the display of the variety of stress phenomena.

2.5 Interaction with membranes

Exposed to HS, biomembranes are physically as well as chemically irritated. To identify the physical irritation, the resting potential of giant cells of the charophyte *Nitellopsis obtusa* (Groves) was tested. This potential gets depolarized upon exposure to HS (Steinberg et al. 2004). Chemical irritation may be identified by membrane (lipid) peroxidation as shown with red blood cells (Cheng et al. 1999) or amphipods (Timofeyev et al. 2006a,b; Fig. 3). The major underlying mechanisms may be the internal production of reactive oxygen and nitrogen species, ROS. Internal HS are processed with ROS as byproducts, because any activation and subsequent reduction of O_2 automatically produces ROS (Blokhina et al. 2003). It is interesting to note that in the exposed *Gammarus lacustris* Sars, lipidperoxidation starts clearly before free H_2O_2 accumulates in the tissue, indicating that the first process might even be part of the anti-oxidative stress defense.

Fig. 3: Development of internal H_2O_2 and lipidperoxidation, measured as diene conjugates, in *Gammarus lacustris* exposed to 1.2 mmol L^{-1} NOM of Lake Schwarzer See, Brandenburg State, Germany (from Timofeyev et al. 2006a, modified). * indicates statistically significant differences from controls. Note: Lipidperoxidation starts before free H_2O_2 accumulates in the tissues

2.6 Impact on ion regulation

Influence of HS on sodium (Na) metabolism in *Daphnia magna* Strauss was studied by (Glover et al. 2005, Glover and Wood 2005). Environmentally relevant levels of several, but not all tested HS were observed to significantly enhance transmembrane Na transport in *D. magna*. This effect was described as an uncompetitive stimulation of Na influx, characterized by an increased maximal Na transport rate and accompanied by a decreased uptake affinity. Also altered membrane permeability due to enhanced membrane binding of HS at low pH is considered as likely mechanism.

2.7 Further chemical stress defense reactions: Hsp, biotransformation, MXR

As one clear response to HS exposure, defense proteins (better known as heat shock proteins – Hsp) were expressed in freshwater animals (Wiegand et al. 2004, Timofeyev et al. 2004) and algae (Bierkens et al. 1998). Even environmentally realistic HS concentrations induced increased Hsp70 concentrations in the test organisms.

To get rid of the endogenous as well as exogenous chemical burdens (for instance with animals: cyclic production and degradation of hormones, exotic food chemicals, xenobiotics etc.), organisms have developed the biotransformation system. Also HS behave like chemical clues in this pathway. Since HS possess a variety of functional groups, we assume that particularly the Phase II enzymes of the biotransformation system are subject to induction and modulation upon HS exposure (Timofeyev et al. 2004, Wiegand et al. 2004, Menzel et al. 2005, Cazenave et al. 2006). An eco-genomic study with *C. elegans* showed that several transferases, representative of Phase II enzymes, are upregulated; furthermore, the enzyme activities are modulated (Timofeyev et al. 2004). With fish and cell lines, also the induction of CYP via the modulation of the Ah receptor (AhR) has been shown (Matsuo et al. 2006, Bittner et al. 2006, Janošek et al. 2007). Very recent studies show the HS-derived AhR agonists may be photo-stable as well as photo-labile (Bittner et al. 2007).

In addition to the biotransformation pathway, another mechanism for handling toxins and xenobiotic chemicals is active, the so-called multi-xenobiotic resistance transporter (MXR). This is a membrane-bound group of P-glycoproteins, which act as pump against xenobiotics and/or their metabolites (e.g., Smital et al. 2004). The MXR pump can be blocked or even inhibited by chemosensors. Chemosensitization of the MXR defense system could cause increases in intracellular accumulation and subsequent toxic effects of xenobiotic chemicals and heavy metals otherwise effluxed by the MXR system. Even HS may act in this manner and block the MXR pump: in a study with amphipods, Timofeyev et al. (2007) showed that the presence of HS increased the residual concentration of a fluorescent dye.

3 Discussion

All physiological responses to HS required energetic costs, which were compensated on the expense of the energy resources in zebra fish embryos (reduction of lipid contents, Cazenave et al. 2006), which in turn might affect the nor-

mal development of embryos. Exposure to non-contaminated HS in high concentrations led to teratogenic effects already within 48 h (Cazenave et al. 2006). These observations are in good agreement with a previous study of HS on energy metabolism of fish (Meshcheryakova et al. 2004), who stated that the adaptation of fish to humic-rich environments was accompanied by decreases in their fatness. As a field proof of the laboratory findings, we refer to the paper of Rask et al. (1999). In some Finnish lakes, the authors found significant negative correlation between total organic carbon content of the lakes and the body length of perch and roach. The authors ascribe this principally to indirect HS effects, such as poor light conditions, that result in less efficient predation on fish and invertebrates. Direct effects of HS, however, are not discussed, because unknown at that time. Yet, as shown above, they clearly do exist.

Albeit the obvious adverse energetic effects in the fish, there is increasing awareness that mild chemical stresses (perhaps milder than the above exposure with the zebra fish embryos) may even be beneficial (Calabrese 2005, Le Bourg 2003). In general, mild chemical stress to individuals means training of the chemical defense system (biotransformation enzymes, anti-oxidant enzymes, stress proteins) with interesting consequences.

3.1 Acquisition of multiple stress resistance

It is worthwhile to note that the *green theory of aging* claims that the biotransformation and the anti-oxidant defense systems have to be trained, and this training may lead to individual longevity (Bijlsma and Loeschke 1997, Minois 2002, Gems and McElwee 2003, Murphy et al. 2003). Murphy et al. (2003), Morrow et al. (2004), and others identified key molecules involved in longevity. Pro-longevity genes include some that encode antioxidant enzymes, biotransformation enzymes, and others encoding Hsp, particularly of low molecular weight (Hsp22). Genes of all enzyme families and Hsps have been found upregulated in *C. elegans* after exposure to natural and artificial HS (Menzel et al. 2005). More recent and ongoing studies with *C. elegans* exposure to HS preparations as well as HS-building blocks have revealed significant life-span extensions and up-regulation of several anti-stress genes, including two pro-longevity genes (Steinberg et al. 2007). The retardation of the aging process is often found to be combined with the acquisition of a multiple stress resistance. In deed, polyphenol exposed *C. elegans* had acquired a thermo-tolerance. On the phenomenological basis, increased stress-resistance was also found in fish with improved individual constitution, particularly slightly increased survival of fertilized eggs (*Danio rerio* Hamilton) and improved physical constitution after strong mechanical stress (*Xiphophorus helleri*) (Steinberg 2003, pp 318 ff, Meinelt et al. 2004). As a very recent quasi field validation of the *D. rerio*-results, Bertolo and Magnan (2007) published a study on logging-induced variations in dissolved organic carbon which affect the recruitment of yellow perch (*Perca flavescens* Mitchill) in Canadian Shield lakes. The authors found that the relative abundance of young-of-the-years increased after logging in proportion to the ratio between the area of the logged catchment and the lake volume

and the effect is likely explained by an increase in HS following logging. As one mode of action, the authors discuss an increased hatching success.

Even the reverse phenomenon may be found: animals from rather stable chemical and physical environments, such as the deeper strata of Lake Baikal, do not respond flexibly and adequately to HS stress, as proven with endemic amphipods. The animals were obviously never forced to adapt to any stressor (Timofeyev and Steinberg 2006).

The studies above refer to animals. The knowledge of potential benefits of mild stresses to plants is less extensive. Since most plants do not age in the strict gerontological sense (Thomas 2002), it can be expected that mild stresses do not necessarily lead to life-span extension of the complete plants, but of certain organs which means that these organs have developed a stress resistance. With respect to HS, there are first indications that HS-exposed wheat and apricot seedlings develop a water deficiency and salt resistance (Kulikova et al. 2006b, El-Shall et al. 2007). Furthermore, we assume that this acquisition might be a more widespread phenomenon: for instance, the plants, fish, and plankton organisms, remaining in the polyhumic coastal lagoons of the Restinga Jurubatiba National Park mentioned above (see Fig. 1) resist occasional salt water intrusion from the adjacent Atlantic Ocean (see Kozlowsky-Suzuki and Bozelli 2004), because they probably have developed a HS-mediated multiple stress resistance.

4 Conclusions

Due to their ubiquity and their variety of functional groups, dissolved HS have the potential to interfere within almost any biotic structure and biochemical pathway in freshwater organisms. Their role in indirect fueling the heterotrophic components of the freshwater ecosystems is well understood. However, HS are also environmental chemicals. Due to the low-molecular masses of their building blocks, they appear to be capable to easily pass biomembranes. The eco-genomic study with *C. elegans* shows that they have the chance to be actively taken up. Inside the organisms, HS are metabolized like xenobiotic chemicals with oxidizing oxygen and nitrogen species (ROS) as byproducts. They provoke a variety of non-specific and specific reactions in the organisms, starting from stress response, such as Hsp induction, to herbicide-like mode of action towards primary producers. They may modulate the activity of membrane-bound pumps for xenobiotic chemicals and, in turn, should change bioconcentration of xenobiotics and heavy metals. Strong chemical stress may lead to teratogenic effects and losses in lipid storages. However, mild chemical stress appears to be actively looked for, since the eco-genomic study with *C. elegans* revealed that also chemosensory and olfactory genes are switched on. In deed, the nematode actively migrated into the stressful environment. According to the *green theory of aging*, mild chemical stress equals the training of the antioxidant and the biotransformation systems. As shown with HS building blocks, the individual life-span can be prolonged and a multiple stress tolerance can be acquired. In general, exposure to HS may increase the general fitness of the individual to assert itself in a fluctuating stressful environment.

5 Perspectives

The above mosaic of describing the various direct effects of HS on aquatic and compost organisms has still some features of a simple stocktaking. However, by combining the seemingly adverse effects of being exposed to HS with recent paradigms of the aging research sheds new light into this kind of stress and hypothesize that natural mild stress may be beneficial to the exposed organisms which is supported by preliminary experimental findings. The ecological significance remains obscure. Nevertheless, even these rudimentary results and hypotheses show that HS directly interact with aquatic organisms and modify both the individual fitness and lifespan as well as the abiotic environment. Hence, dissolved HS have to be considered an abiotic ecological driving force – obviously more cryptic than light, nutrients, or temperature.

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