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Humic Substances as Mediators of Chemical Stress in Aquatic Environments.

Irina V. Perminova*, Christian E.W. Steinberg** and Fritz H. Frimmel***
*Department of Chemistry, Lomonosov Moscow State University, 119899 Moscow, Russia;
**Institute of Freshwater Biology and Fisheries, Mueggelseedamm 310, 12587 Berlin, Germany;
***Engler-Bunte Institute, University of Karlsruhe (TH), Department of Water Chemistry, Richard-Willstaetter-Alle 5, 76131 Karlsruhe, Germany

Humic substances (HS) account for 50-80% of dissolved organic matter in aquatic ecosystems. Their content in natural waters of 1 to 50 mg/L exceeds manifold those of any kind of contaminants and of low soluble organic xenobiotics (polycyclic (PAHs) and polychlorinated aromatic hydrocarbons, pesticides, etc.), in particular. Being high molecular weight polyfunctional compounds, HS are able of ionic, donor-acceptor and hydrophobic interactions, that determines their affinity for binding to heavy metals, hydrophobic organic chemicals, some of the pesticides and other compounds. Such a chemical binding leads to a reduction in concentration of free species of the contaminants. Its immediate ecotoxicological consequence - a decrease both in toxicity and bioavailability of the contaminants. Given the mentioned above abundance of HS, we have chosen to investigate a possible mediating role of humic substances in contaminated aquatic ecosystems. The objectives of the presented work were to (1) assess quantitatively binding affinity of HS for two different classes of the contaminants - PAHs and mercury(II), (2) assess quantitatively an impact of HS on toxicity and bioconcentration of PAHs and mercury (II), (3) conclude on the feasibility of the prediction of mediating action of HS in aquatic ecosystems.

To reach the goal, the following experimental design was adopted. The humic materials used in this study were isolated predominantly from different natural waters, as well as from peat and soil. Their chemical composition and structure was characterised by means of elemental and functional analyses, UV and ^{13}C NMR spectroscopy and size-exclusion chromatography. The chemical stressors used were PAHs and Hg(II) in form of Hg(OH)$_2$ and HgCl$_2$. The PAHs used were anthracene, fluoranthene, pyrene and benzo(a)pyrene. Binding affinity of HS for PAHs was quantified with a use of the "chemical" partition coefficients (K_{oc}^{ch}). They were determined with a use of fluorescence quenching technique. An impact of HS on toxicity of PAHs was estimated by means of calculation of the "toxicological partition coefficients (K_{oc}^{tox}) by fitting the experimental dependences between a residual toxicity of PAHs and concentration of HS in the test system. As a target organism, a cladoceran *Daphnia magna* was used, as a target function - grazing activity. An impact of humic substances on bioconcentration of PAHs was estimated by means of calculation of the "bioconcentration" partition coefficients" (K_{oc}^{bio}) by fitting the experimental dependences between the measured bioconcentration factors and concentration of HS. As a target organism the nematode *Caenorhabditis elegans* was used. Binding affinity of HS for Hg(II) was quantified with a use of stability constants (K_{HgHS}) calculated on the basis of the preliminary determined Hg(II) equivalent concentration of HS. An impact of HS on toxicity of Hg(II) was quantified as a concentration of HS that caused a 50% reduction in toxicity of Hg(II) (DC_{50}). An impact on bioconcentration was estimated by means of calculation of corresponding bioconcentration factors (BCF). As a target organism, green microalgae *Chlorella pyrenoidosa* was used, as a target- function - photosynthetic activity.

The experiments allowed to obtain the following data. "Structural set" which included atomic ratios, content of functional groups, carbon distribution and molecular weights of the humic substances. "Reactivity set" composed of the K_{oc}^{ch} and K_{HgHS} values for PAHs and Hg(II), respectively. "Toxicological set" composed of the K_{oc}^{tox} and DC_{50} values for PAHs and Hg(II), respectively. "Bioconcentration set", composed of the K_{oc}^{bio} and BCF values, for PAHs and Hg(II), respectively. An analysis of the "reactivity set" revealed high affinity of HS for binding both to PAHs and Hg(II): the values of K_{oc}^{ch} accounted for $0.1\text{-}2\times10^5$ L/kg of C, $\log K_{HgHS}$ varied from 13 till 15. This suggested that HS at the natural contents in aquatic ecosystems (1-10 mg/L) could play a substantial mitigating role owing to a reduction in biological activity of the given hazardous chemicals due to induced changes in their speciation. The toxicological and bioconcentration assays conducted in the presence of the mentioned above HS concentrations confirmed unambiguously mitigating effect of HS in relation to PAHs and Hg(II). An increase in concentration of HS lead both to a decrease in toxicity and bioconcentration of PAHs, as well as of Hg(OH)$_2$ and HgCl$_2$. QSAR-analysis of the data on structure, reactivity and mitigating ability of HS to both classes of the contaminants allowed to obtain for the PAHs the best empirical relationships of the general type: $K_{oc} = a + b \times C_{Ar}$, where K_{oc} represents all three types of the determined partition coefficients, C_{Ar} is a percentage of aromatic carbon. For $\log K_{HgHS}$, the right part of the corresponding equation represented a linear composition of the percentages of carbon in COOH, C_{Ar}-O and C_{Alk}, as well as N/C atomic ratio. The obtained relationships are of particular importance for the development of an unified approach for prediction of the mitigating impact of HS in the aquatic environments containing different types of humic materials. The corresponding experiments with the other chemical stressors are in need.