Peculiarities of antioxidant activity of humic substances

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Keywords: antioxidant activity, enzymatic bioassay, humic substances, bioluminescence, reactive oxygen species

doi: 10.36291/HIT.2019.kudryasheva.072

Humic substances (HS) are known to function as natural toxicity attenuators in natural solutions, involving solutions of redox-active compounds. In our study we monitored toxicity of oxidizer solutions under addition of HS.

Bioluminescence bacteria-based and enzyme-based bioassays were used to monitor toxic and antioxidant effects at cellular and biochemical levels, respectively. The bioluminescence assays are widely used to monitor toxicity of media due to high rates of the assay procedures and simple registration of the luminescent signal. These features provide a large number of measurements under comparable conditions, which is extremely important for statistical processing. A suppression (or activation) of maximal bioluminescence intensity is a quantitative characteristic of general toxicity (GT) in the environment. A particular feature of the enzymatic bioassay is specificity to the group of toxic agents of oxidative nature: additionally to reducing maximum luminescence intensity, a bioluminescence delay period occurs; it depends non-additively on the oxidizer' concentration and redox potential. This feature is a basis for monitoring the oxidative toxicity (OxT) of the environment. Hence, the bioluminescent enzymatic bioassay can be used to determine both: changes of GT in high-concentration solutions of HS and ability of HS to change OxT of media, i.e. antioxidant activity of HS.

Toxicity and antioxidant activity of HS was studied in a wide range of their concentrations. The toxicity of HS solutions was evaluated with effective concentrations EC-50; detoxification coefficients D_{OxT} and D_{GT} were used to evaluate antioxidant activity in solutions of organic and inorganic oxidizers. The toxicity and antioxidant activity of HS were compared to those of the other bioactive compound, fullerenol-60 (F). Differences in the effects of HS and F were related to (A) the mobility of functional groups and fragments of HS, (B) their higher ability to reduce the content of reactive oxygen species (ROS) in the solutions. The peculiarities of the HS antioxidant effect were: the low active concentrations of HS, dependence of their antioxidant activity on time and oxidizer' hydrophobicity.

Change of ROS content under addition of HS was studied in tritiated water (Tritium is a beta-emitting radionuclide of low-specific radioactivity, product of a lot of radiochemical processes, which is intensively accumulated in environment now). Tritium and HS demonstrated synergetic activation effect on the bioluminescence enzymatic bioassay. Content of ROS was evaluated by chemiluminescence luminol assay. Correlations between bioluminescence response and ROS content were found in solutions of HS+ tritiated water, revealing molecular aspect of HS antioxidant function in diluted solutions. Additionally, statistical processing revealed a decrease of stochasticity in tritium low-dose effects under exposure to HS.

Acknowledgements. The work was supported by the PRAN-32, grant of RFBR N18-29-19003, RFBR and Krasnoyarsk Regional Foundation N18-44-242002, N18-44-240004.