USAGE OF TRITUM LABELED HUMIC SUBSTANCES FOR THE STUDY OF THEIR INTERACTION WITH BIOMEMBRANES UNDER OPTIMUM AND SALT STRESS CONDITIONS

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Humic substances (HS) are the natural organic compounds comprising 50 to 90% of the organic matter of peat, lignites, sapropels, as well as of the non-living organic matter of soil and water ecosystems. The beneficial effects of HS on living organisms have been numerously reported. The mechanism of their action remains unclear, and the ways of HS usage by organisms are also sophisticated. Problem of bioavailability of HS and their penetration into the cells is even more sophisticated due to difficulties in HS determination in the presence of other organic substances. In spite of many studies demonstrating HS adsorption onto different biological membranes, there are only a few data on quantitative estimation[1,2] or influence of the environment conditions on this process. The most convenient way is a use of labeled compounds, and the availability of isotope-labeled HS is an important prerequisite to elucidate the fate of the heterogeneous organic matter in complex environments.

Labeling of HS can be accomplished by the addition of a labeled precursor to an unlabeled soil sample during composting[3] or by the synthesis of model polymeric compounds under defined conditions. The latter are synthesized either by enzyme mediated (usually initiated by adding H_2O_2 in the presence of horseradish peroxidase) oxidative polymerization of phenolic compounds[4], or by their spontaneous polymerization in the presence of oxygen or other oxidants, usually at alkaline pH.[5] Usage of those techniques, however, does not allow producing labeled preparation completely identical in their properties to the native preparations. Some methods of the direct labeling of HS were therefore developed including labeling of HS with ^3H.[6] The main advantage of the direct labeling of HS is an opportunity to produce a broad spectrum of isotope-labeled native humics varying significantly in both their origin and properties. The objective of the study was to quantify HS interaction with biomembrane using tritium labeled HS and bacteria *Escherichia coli* as a model under optimum and salt stress conditions.

Six samples of HS originated from coal, peat and soil were examined. Observed values of bioconcentration factor of HS by *Escherichia coli* under optimum and salt stress conditions varied in the range 0.9-13.1 or 0.2-130 l kg^{-1}, respectively, depending considerably on the HS preparation. Surface activity of HS was demonstrated to be a leading factor determining their both sorption and uptake by bacteria cells.

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References

1. L. Parent, M.R. Twiss, P.G. Campbell, P.G., *Environ. Sci. Technol*. 1996, **30**, 1713-1720.
2. J.B. Fein, J.-F. Boily, K. Guclu, E. Kaulbach, *Chem. Geol*. 1999, **162**, 33-45.
3. K.M. Haider, J.P. Martin, *Soil Biol Biochem*. 1988, **20**, 425–429.
4. A. Kappler, R. Ji, A. Brune *Soil Biol Biochem*. 2000, **32**, 1271–1280.
5. K.T. Steffen, A. Hatakka, M. Hofrichter, *Appl. Environ. Microbiol*. 2002, **68**, 3442-3448.
6. G.A. Badun, V.Yu. Pozdnyakova, A.V. Kudryavtsev, I.V. Perminova, *Bulg. J. of Ecol. Sci*. 2003, **2(3-4)**, 26-27.