UPTAKE OF HUMIC SUBSTANCES BY WHEAT PLANTS: PREFERENTIAL ACCUMULATION IN LIPID FRACTION

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Humic substances (HS) have been the subject of numerous scientific studies due to their beneficial effects of HS on living organisms. The principal effects exhibited by HS onto living organisms include an increase in biomass accumulation, nutrient uptake, biosynthesis, antiviral activity, and others [1]. In spite of numerous studies on the biological effects of HS, the mechanism of their action remains unclear. The main reason seems to be the stochastic nature of HS. In contrast to common biological macromolecules, which are synthesized by a living organism according to the information encoded in DNA (nuclear acids, proteins, enzymes, antibodies etc.), HS are the products of stochastic synthesis. They are characterized as polydisperse materials having elemental compositions that are non-stoichiometric, and structures, which are irregular and heterogeneous. The above features hamper a use of common biological approaches to study biological activity of HS.

Several studies on the uptake of HS by higher plants have been performed. Earlier work relied on the color changes in the plant organs as an indication of HS uptake. The more recent study used ^{14}C-labeled humic-like substances or ^{3}H-labeled HS [2, 3]. Those studies also established that both humic-like substances and HS and were taken by the plants. Therefore, next goal in going deeper inside understanding mode of action of HS is a study distribution of HS in plant tissues. This study was aimed to find out uptake and distribution of HS in wheat seedlings.

For this study humic acids (HA) derived from leonardite and IHSS standard fulvic acids (FA) were used. To synthesize isotope-labeled HA, an approach developed in [4] for the preparation of tritium-labeled HS was used. The obtained ^{3}H-HA sample was dissolved in a phosphate buffer (0.028 M, pH 6.8) and purified by dialysis during a month at 4ºC. It allowed eliminating exchangeable tritium of OH^{-}, $COOH^{-}$, and NH_n groups of HS. Seeds of wheat *Triticum aestivum* L. were germinated at 24°C in the dark for 72 h followed by transferring seedlings into the 0.5 l tanks containing Knopp nutrition solution. After another 72 h plants were transferred into the vials containing HS at concentration 50 mg l^{-1} with specific radioactivity 0.1-0.2 Curie l^{-1}. Then lipid fraction was extracted according to [5] followed by radioactivity measurements.

Obtained results are presented in the Table 1.

Table 1. Contents of HS in lipid fraction of wheat plant

HS	Contents of HS in lipid fractions, % of taken up		
	Roots	Shoots	Total
Coal HA	82±6	16±2	98±8
Aquatic FA	49±10	16±8	64±5

As it can be seen from the Table, almost entire amount of HA that were taken up by plants were found in lipid fraction (98%); for FA that value decreased to 64%. Taking into account that contents of lipid fraction in plants were about 3-5%, one could conclude on preferential accumulation of HS in lipid fraction. Accumulation coefficient which was calculated as ratio of HS concentration in plant to concentration in lipids was as 43±2 and 25±2 for HA and FA respectively. On the other hand, lipid contents in HS usually vary in the range 3-8%. Therefore, preferential HS accumulation in lipid fraction was evident for preliminary transformation of HS on the root surface or nearby it before HS entrance into the plants. That finding could be also confirmed by equal amounts of HA and FA in lipid fraction of shoots (16%). The latter indicated that only specific fragments of HS, probably hydrophobic, were able to enter plants vascular system.

So, our experiments demonstrated that HS taken up by plants were preferentially accumulated in lipid fraction. Before entrance HS were seemingly to be partly transformed and mainly their hydrophobic fragments were adsorbed by plants. However, lipid fraction in plants is well known to be a wide group of natural compounds distinguishing greatly in both structure and functions. They are presented by several different compounds including pigments, fats, fatty acids, waxes, steroids, cutin, suberin and others. Therefore, further study of distribution of HS in lipid fraction of plants is required.

KEYWORDS: wheat, uptake, lipid, tritium, label