

A New Method for Labeling Humic Substances with Tritium: Future Prospects for Biological Studies

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Contents

- ✱ Introduction
- ✱ How to produce ^3H labeled HS
- ✱ ^3H -HS for studying biological properties of HS
- ✱ ^3H -HS for quantification of interaction of HS with biota
- ✱ Visualization of humics-biota interaction using ^3H -HS
- ✱ Future prospects



What humics are?

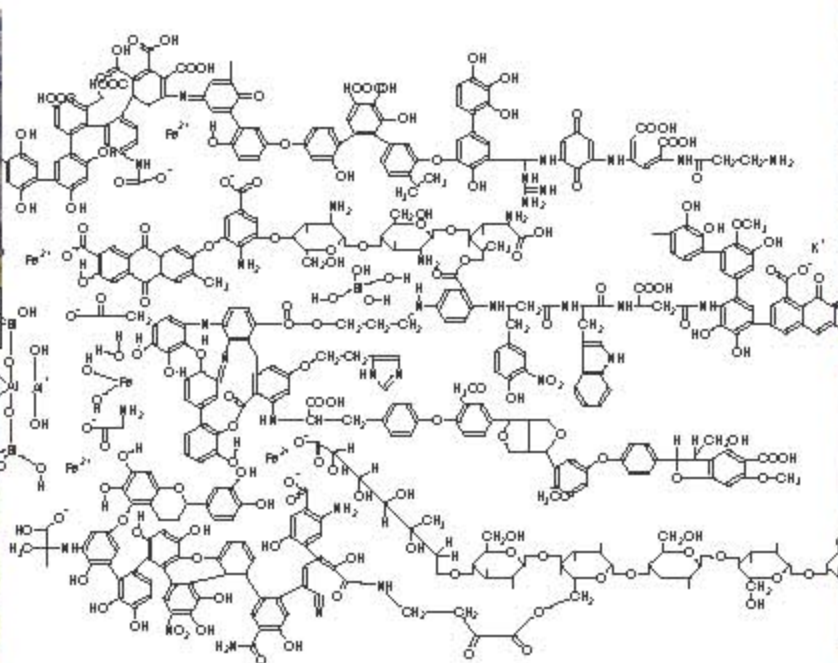
Humic substances are high-molecular-weight polymers originating from decomposition of organic matter



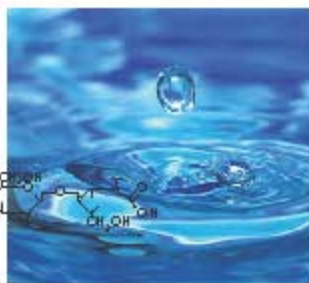
Coal: 15-60%



Soil: 85-90%



Peat: 70-80%



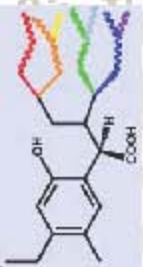
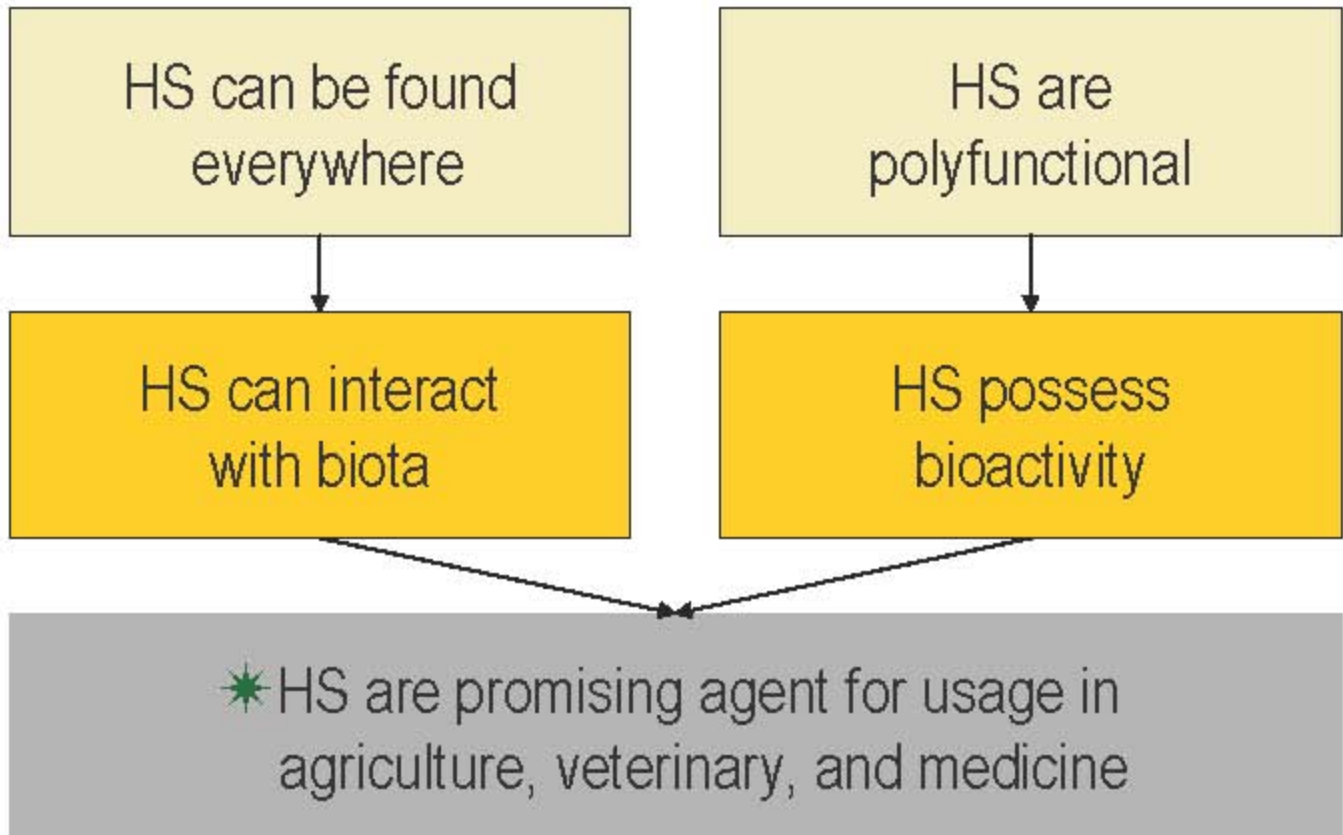
Water: 60-80%

HS properties

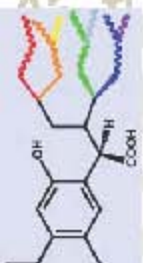
- ★ Polyfunctionality: $-\text{COOH}$, $\text{C}_{\text{Ar}}-\text{OH}$, $>\text{C}=\text{O}$, $-\text{CH}_n$
- ★ Presence of hydrophilic and hydrophobic moieties
- ★ High molecular weight
- ★ Surface activity
- ★ Redox activity



Humics: prerequisites for usage

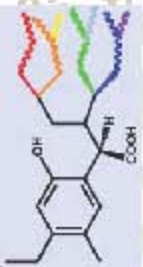


Humics as biological agents



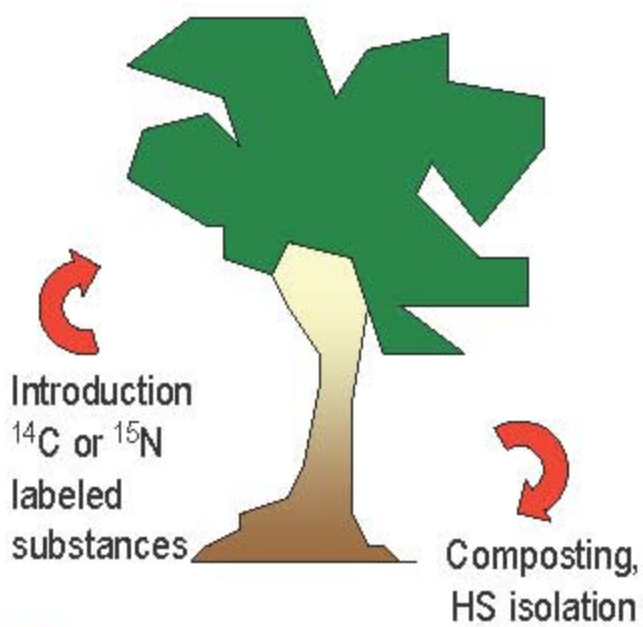
	Positive	Negative
Agriculture	Growth stimulators	Inhibiting activity
Veterinary	Antidiarrheal Analgesic Immunostimulatory Antimicrobial	Negative effects
Medicine	Anti-inflammatory Anti-neoplastic Anti-proliferative Anti-viral	Blackfoot disease Kashin-Beck disease Goiter Cancer

✳ HS possess bimodal activity

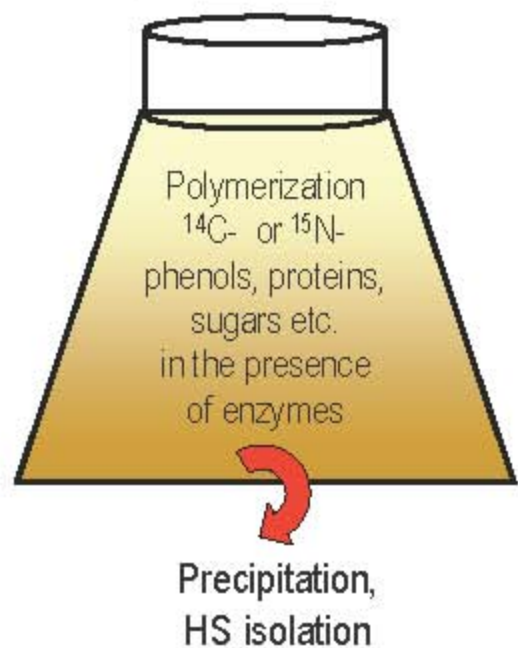


How to trace humics?

Isolation from labeled substrate



Synthesis from precursors



^{14}C or ^{15}N analogues of humic substances

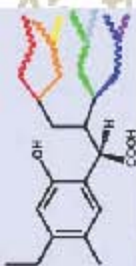
* Direct radioisotope labeling of HS is deadly needed

Why tritium?

- ✦ The decay is a simple one-step process

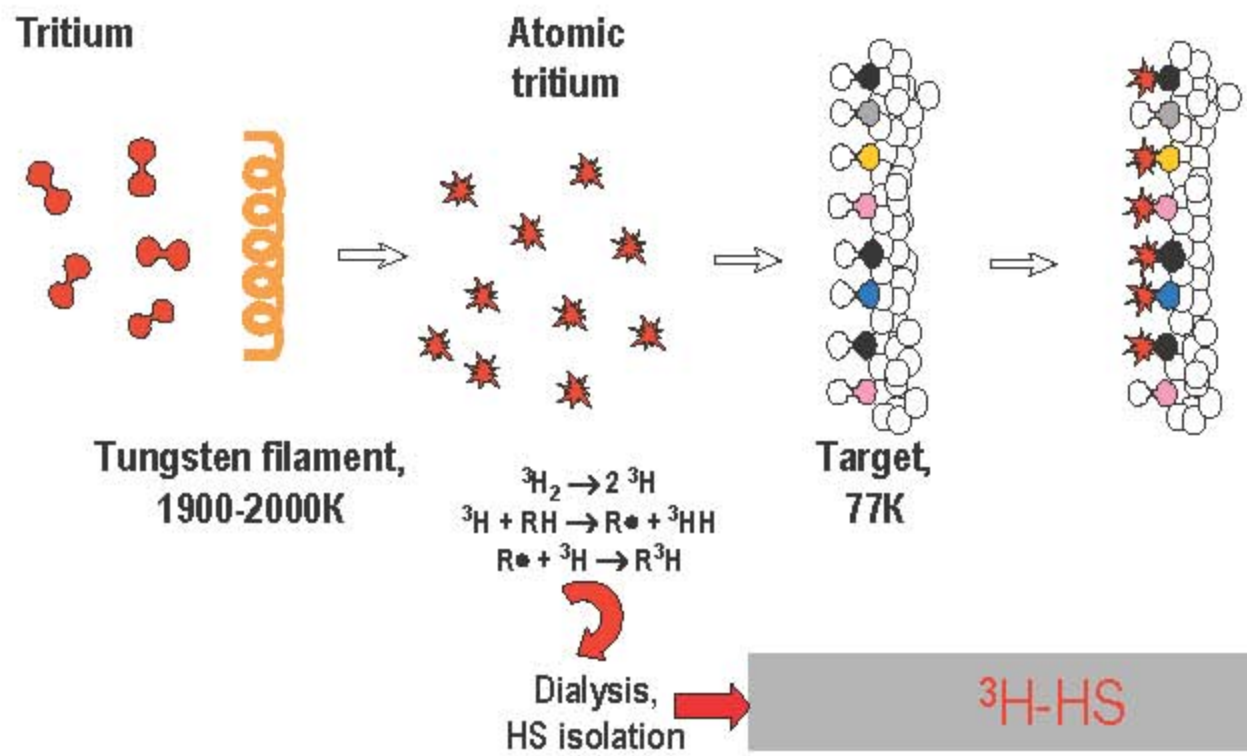


- ✦ Energy of β -radiation is 18 keV
- ✦ β -particles path length in condensed media reaches 1.6 μm
- ✦ Tritium that is bound to a substance is best measured by liquid scintillation counting



Preparation of ³H-HS

Tritium thermal activation method



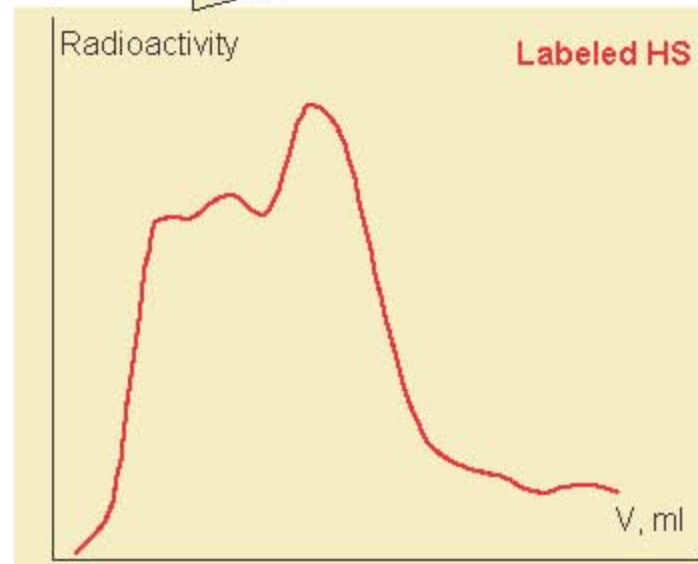
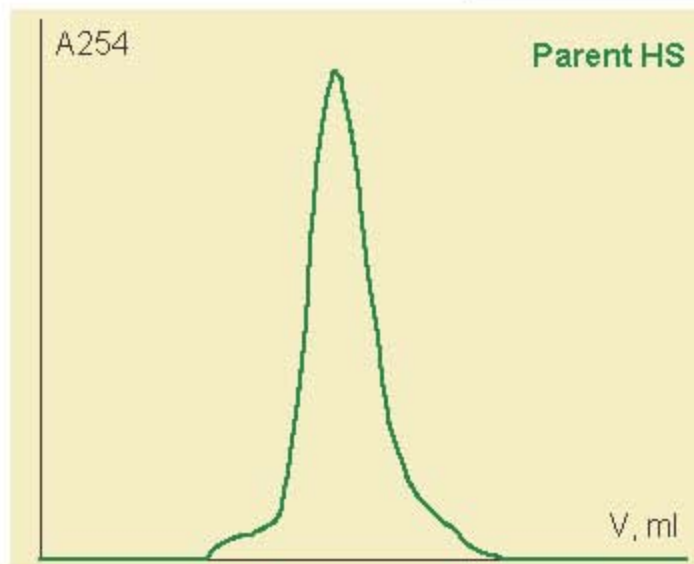
Prepared in accordance with the requirements of the Ministry of Education and Science of the Russian Federation

A new technique for tritium labeling of humic substances

By G. A. Bazant^{1,4}, M. G. Chernysheva¹, Z. A. Lyuzin¹, N. A. Kuzikova¹, A. V. Kostyakov² and L. V. Penzhenov¹

SEC analysis of ^3H -HS

Wrong conditions of tritium introduction

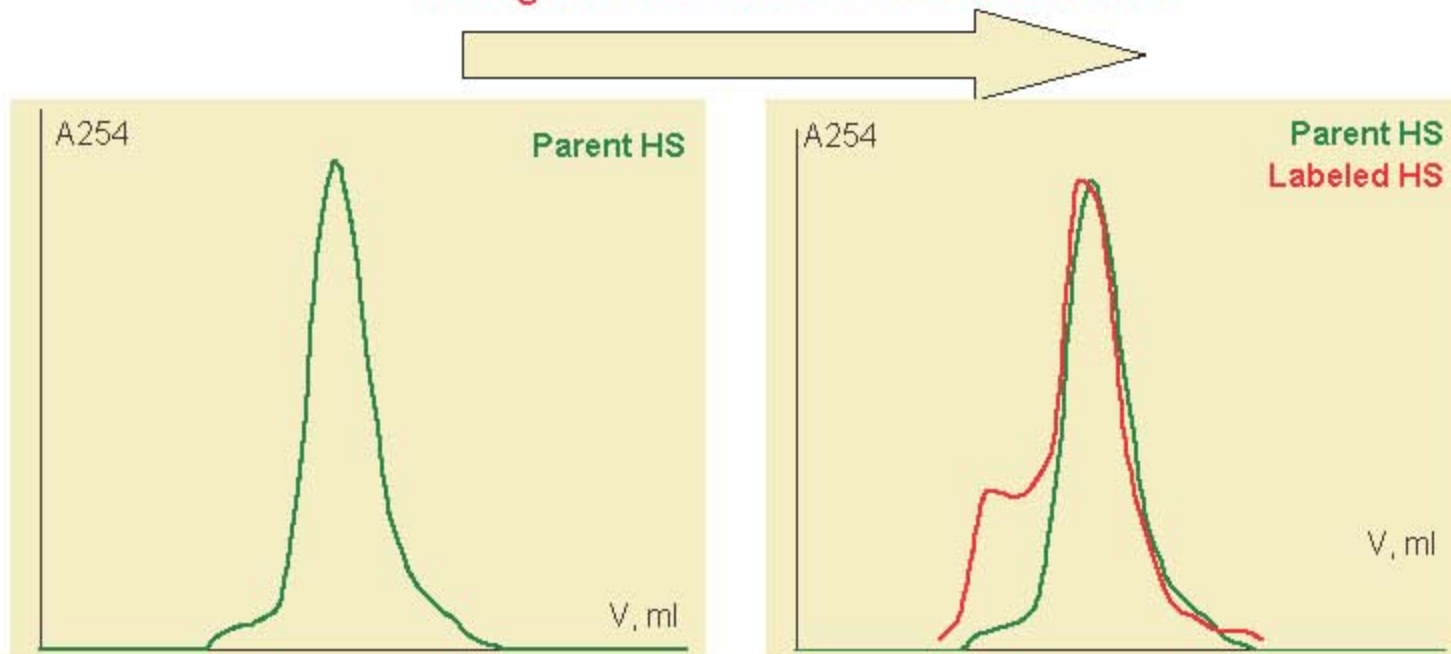


* SEC analysis can be used to study ^3H distribution



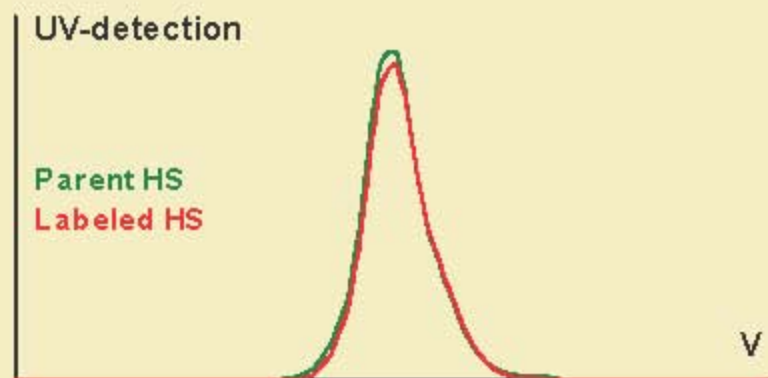
SEC analysis of ^3H -HS

Wrong conditions of tritium introduction

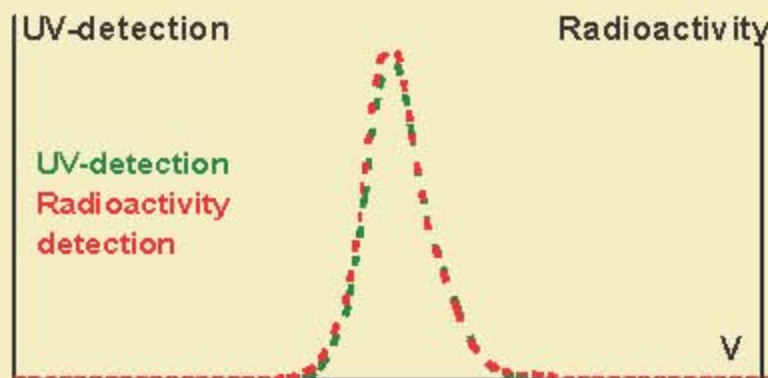


* SEC analysis can be used to study HS alteration

SEC analysis of ^3H -HS

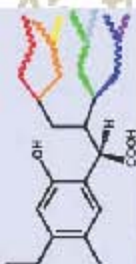


Labeled HS were identical to parent

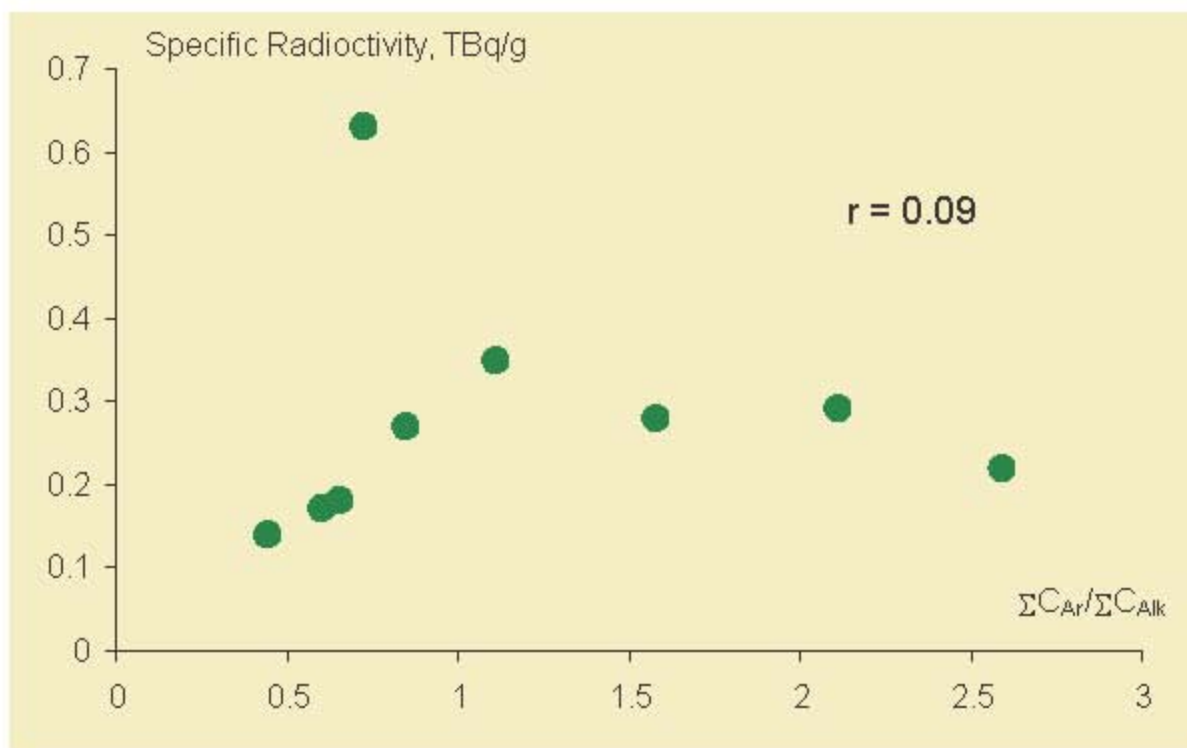


Tritium was uniformly distributed in HS

- ★ Tritium gas pressure 0.5 Pa, tungsten filament temperature 1950 K, duration of bombardment 10 s



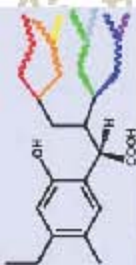
^3H introduction vs HS properties



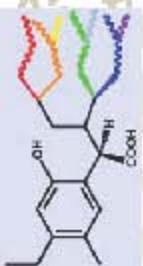
✦ Universality of the developed technique for radioactive labeling of HS

^3H -Humics: properties

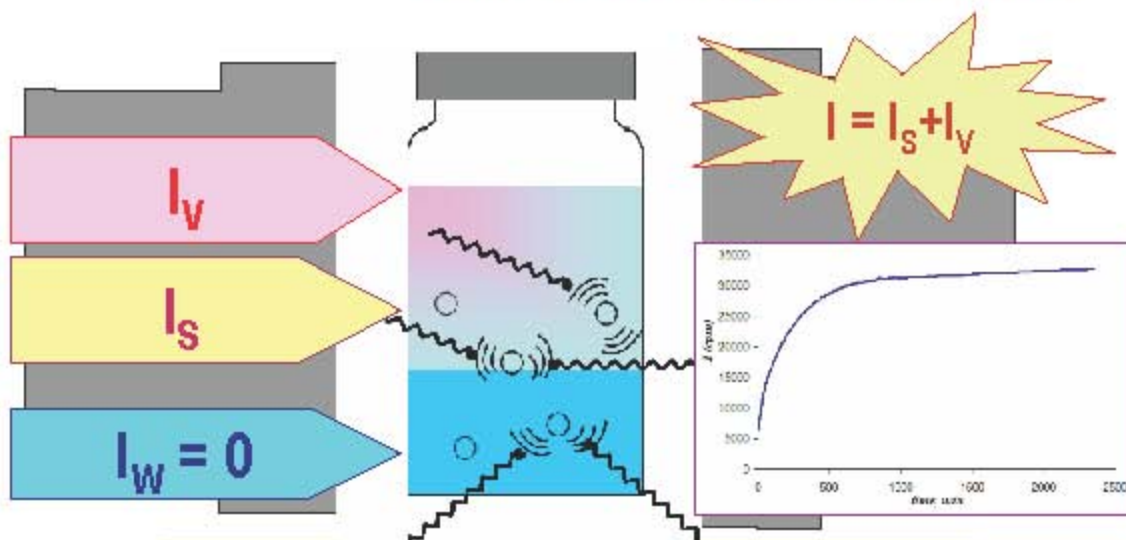
- ✦ High specific activity:
0.14-0.63 TBq/g HS
- ✦ Uniformly distributed radioisotope
- ✦ No significant changes as
compared to parent HS



H-HS: to learn new about humics



Scintillation phase method



$$K_{ow} = \frac{I_V / \epsilon_V}{V \cdot a_V \cdot C_{ow}}$$

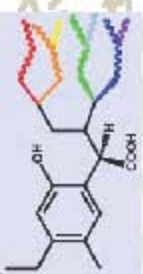
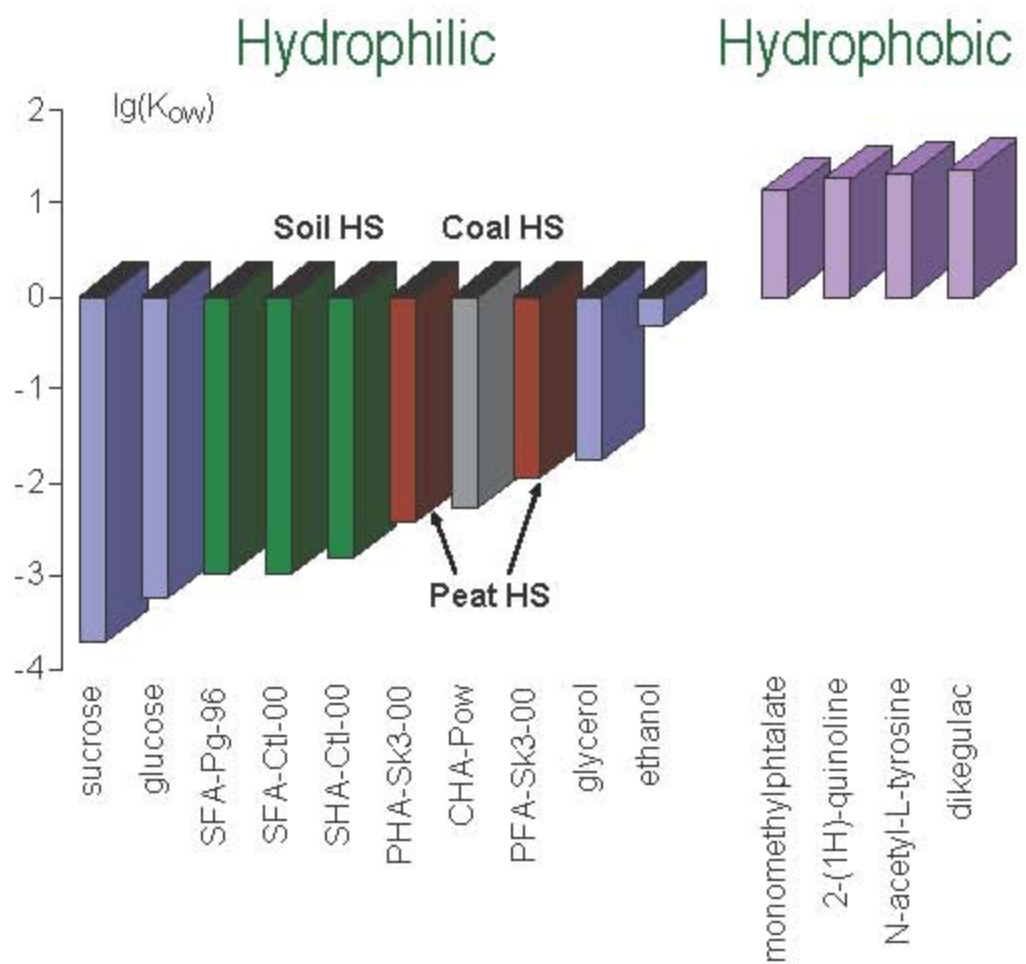
$$\Gamma = \frac{I_S}{\epsilon_S \cdot a_S \cdot S}$$

Liquid scintillation counter

ϵ - counting efficiency; a - specific radioactivity of HS

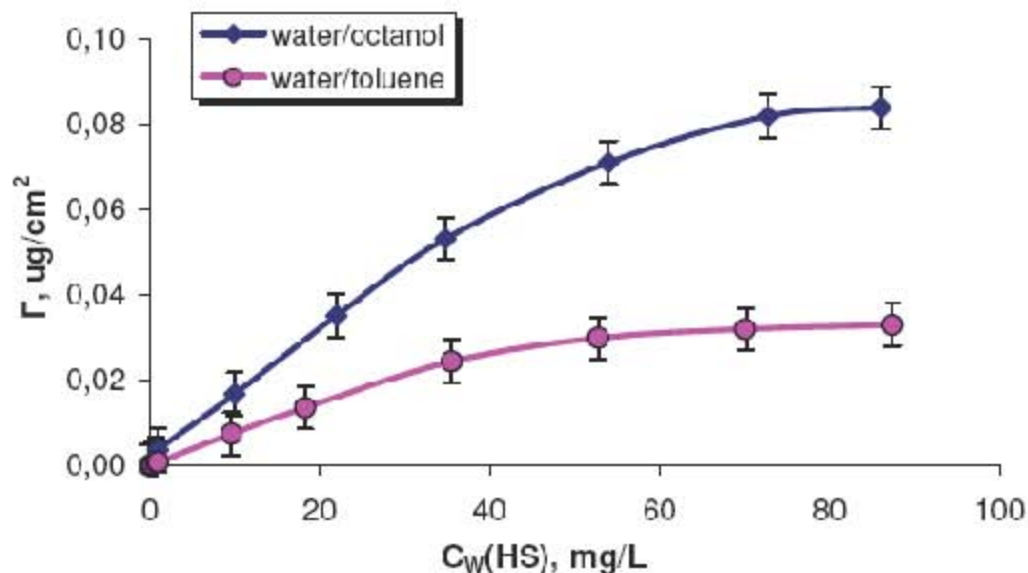
Hydrophobicity of HS

Kow – distribution coefficient between octanol-water phases



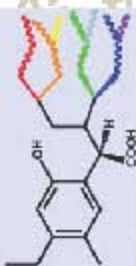
Surface activity of HS

Γ_{\max} – the maximal adsorption value on toluene-water interface



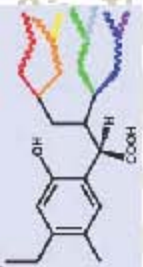
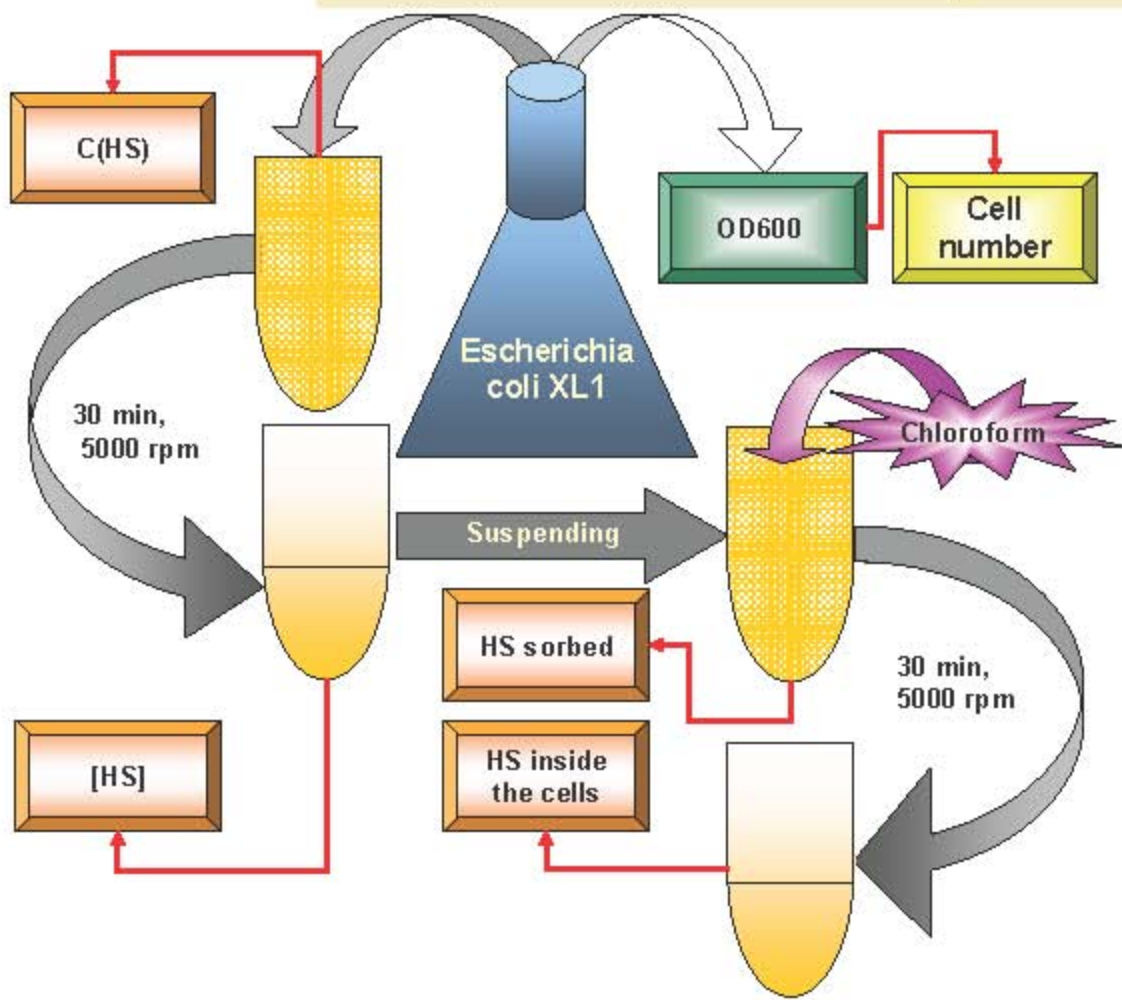
soil FA < soil HA < coal HA < peat FA < peat HA

* Γ_{\max} can be used as a quantitative characteristics of surface activity of HS

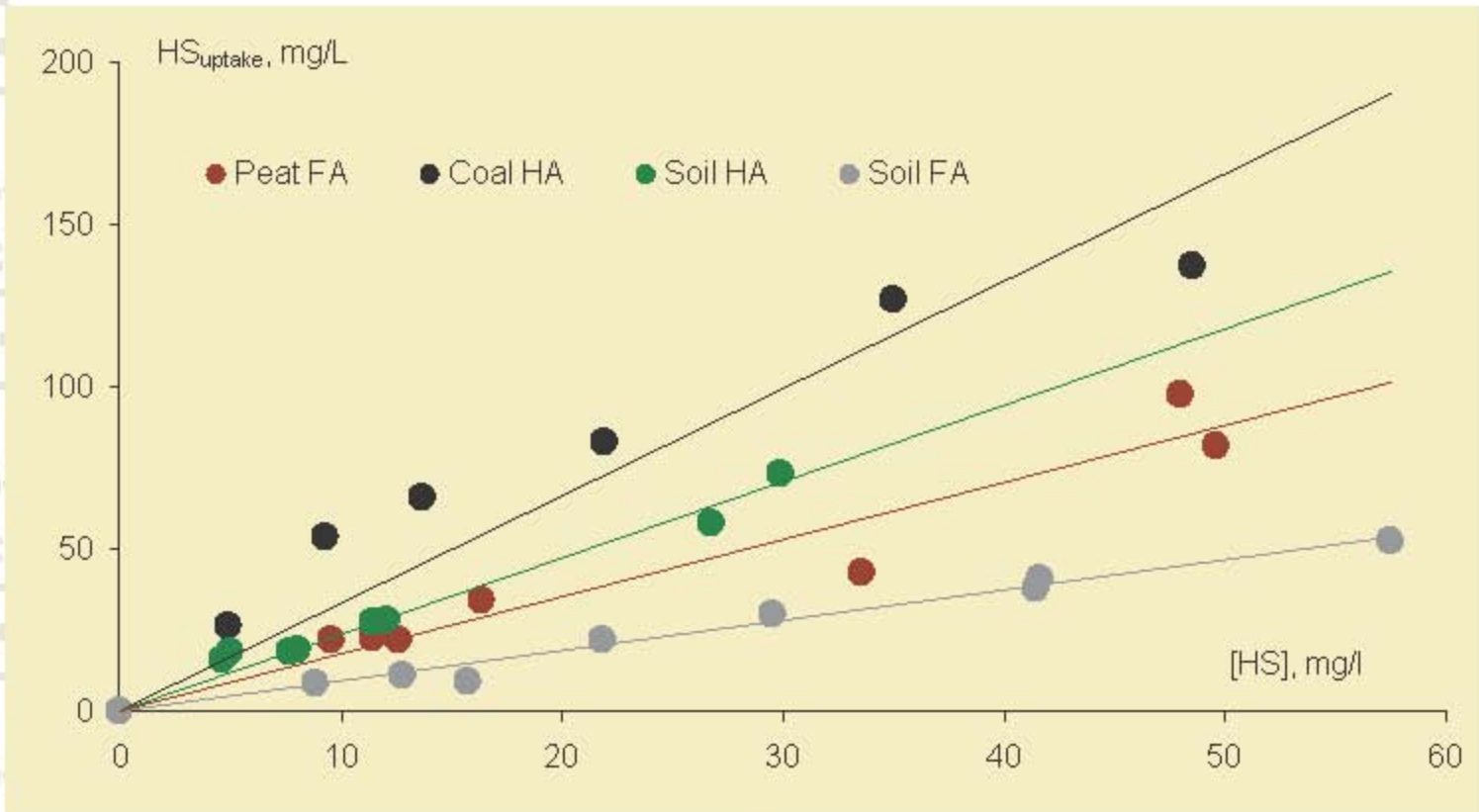


Interaction with bacteria: bioassay design

Test-object: *E. coli*



Interaction with bacteria: bioconcentration factor



$$HS_{\text{uptake}} = BCF \times [HS]$$

Interaction with bacteria: quantitative estimation

BCF, L/kg

0.9 – 13.1

HS sorbed at 50 mg/L,
mg/m²

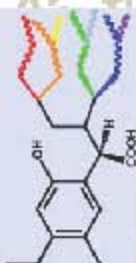
1 – 62

HS sorbed at 50 mg/L,
mg/(kg cells)

33 – 720

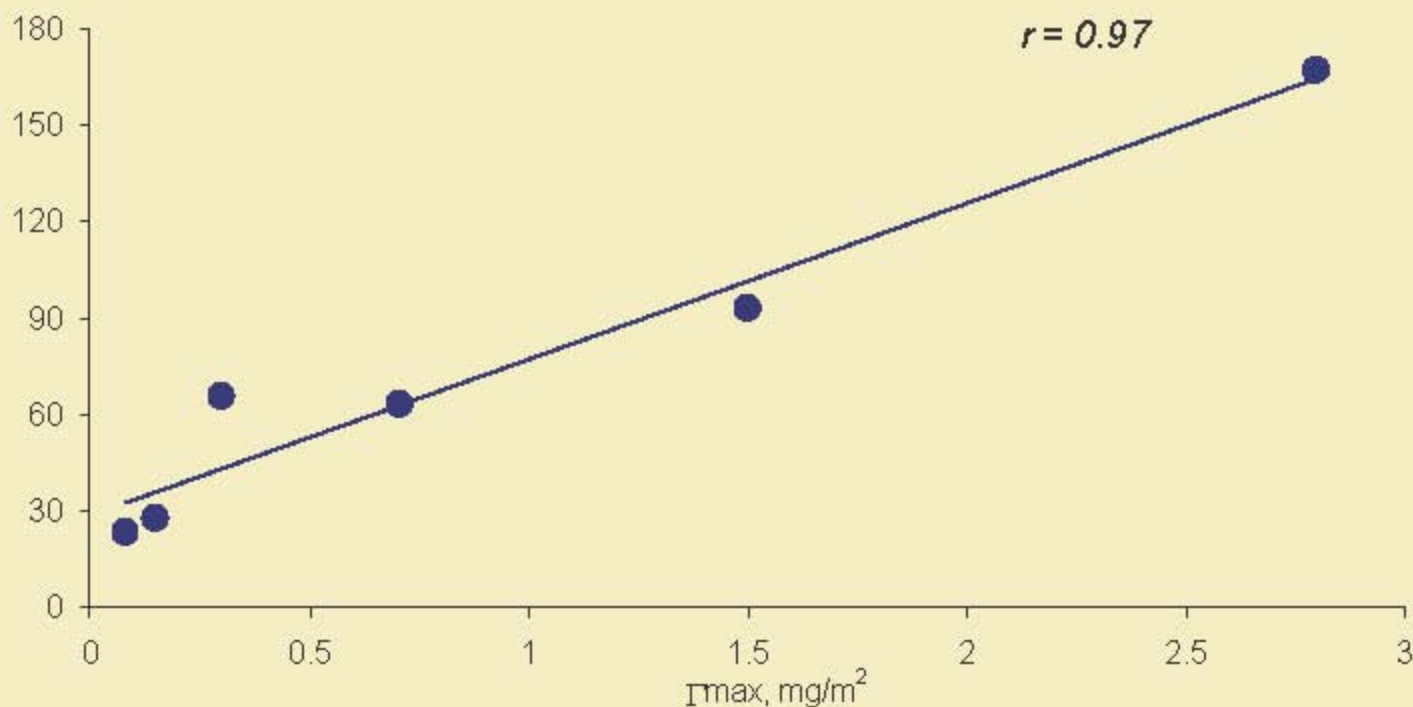
HS penetrated at 50 mg/L,
mg/(kg cells)

23 – 167

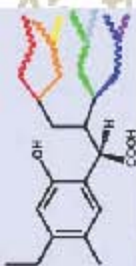


Interaction with bacteria: HS uptake vs. HS properties

Amount of HS penetrated, mg/kg

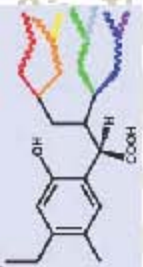
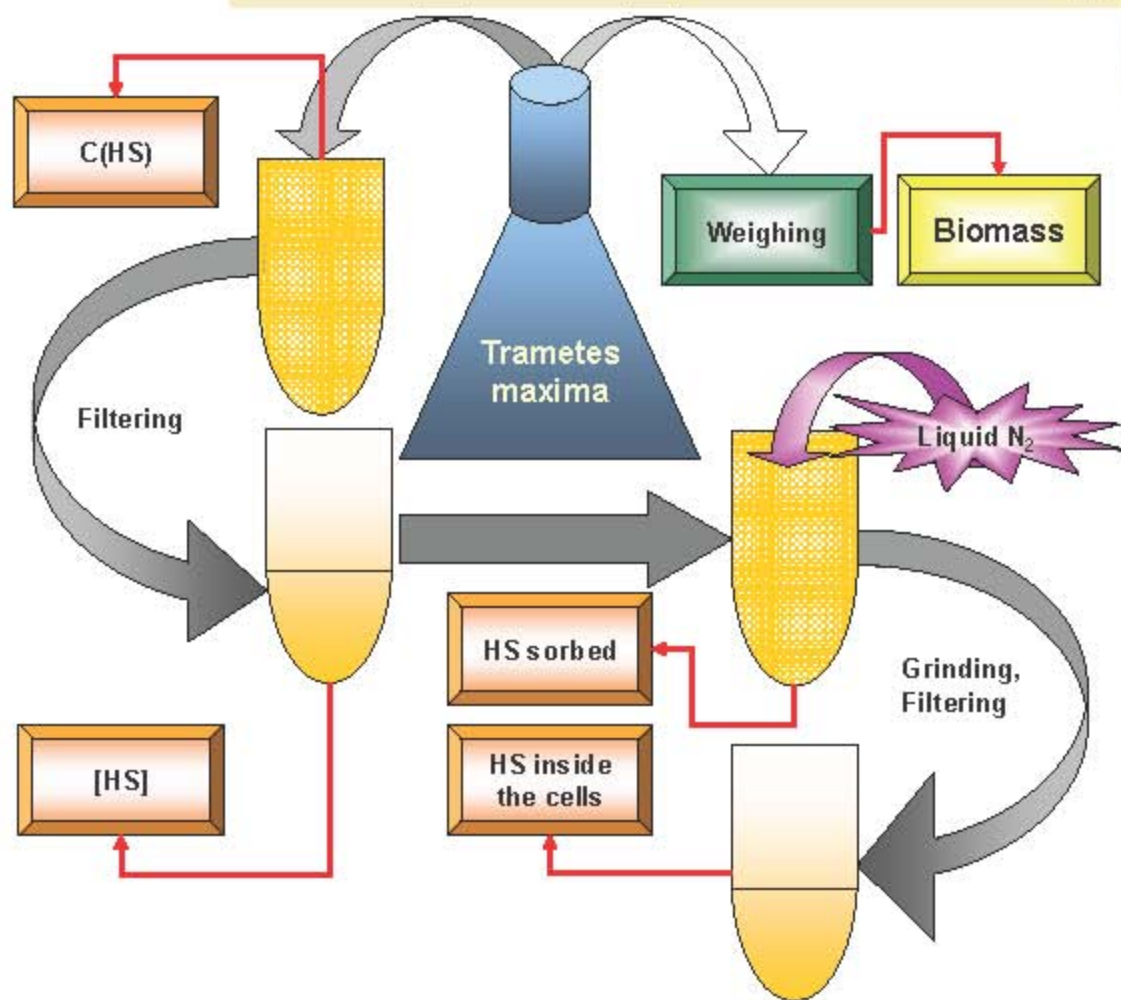


- * Surface activity was a leading property of HS influencing their interaction with *E. coli*.



Interaction with fungi: bioassay design

Test-object: white-rot fungi



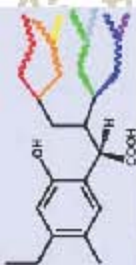
Interaction with fungi: quantitative estimation

Sorbed, mg/kg

190 – 800

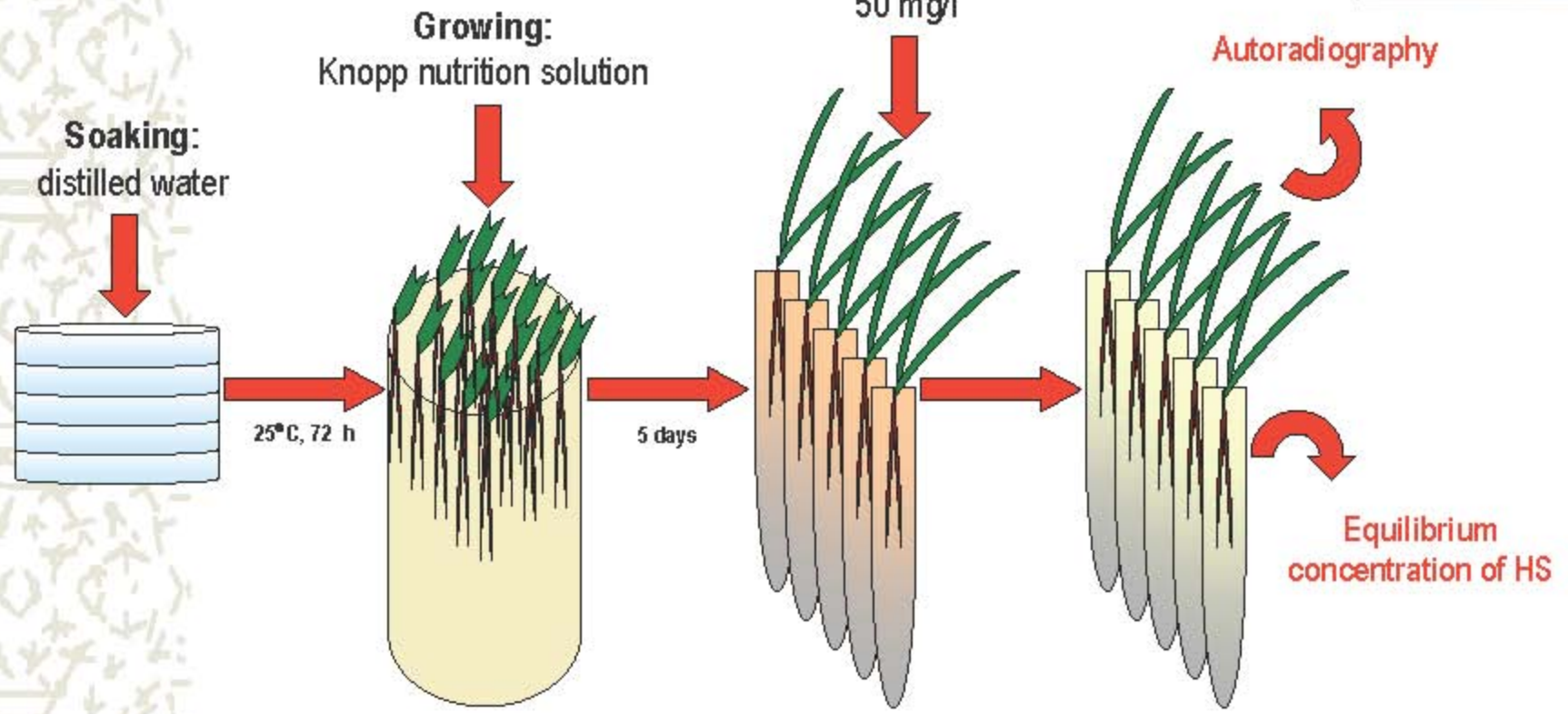
HS penetrated at 50 mg/L, mg/kg

70 – 150

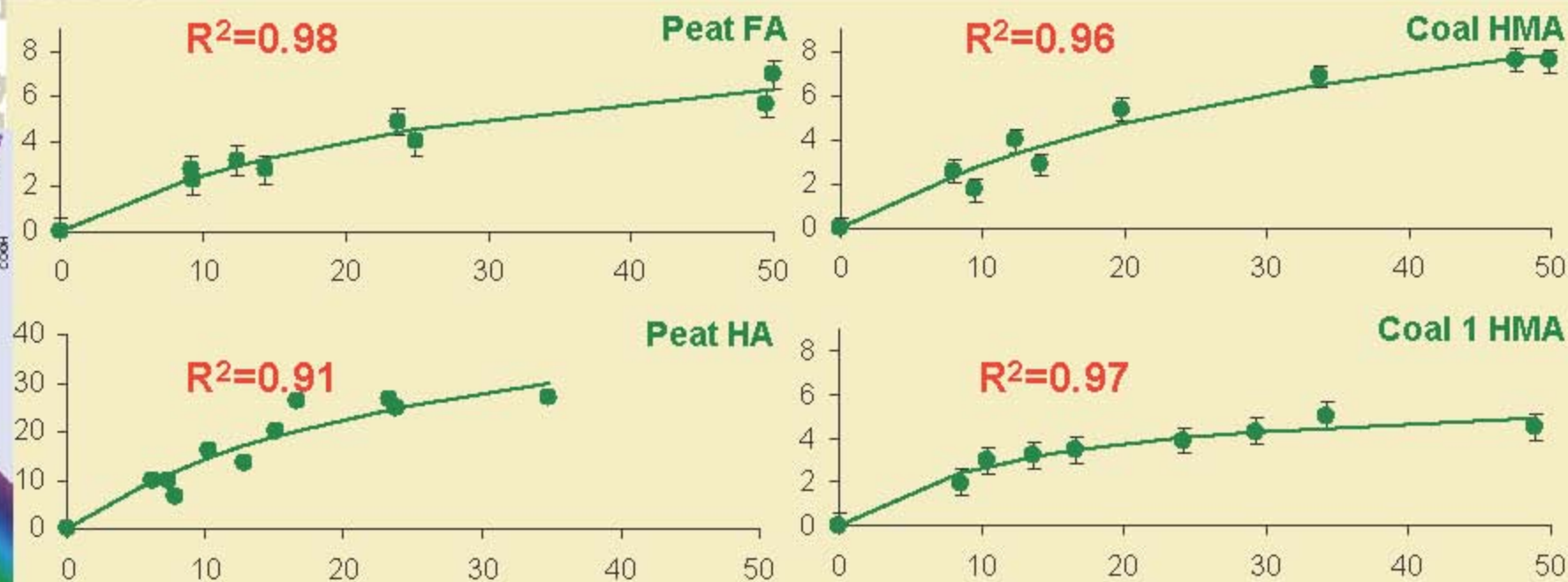


Interaction with plants: bioassay design

Test-object: wheat

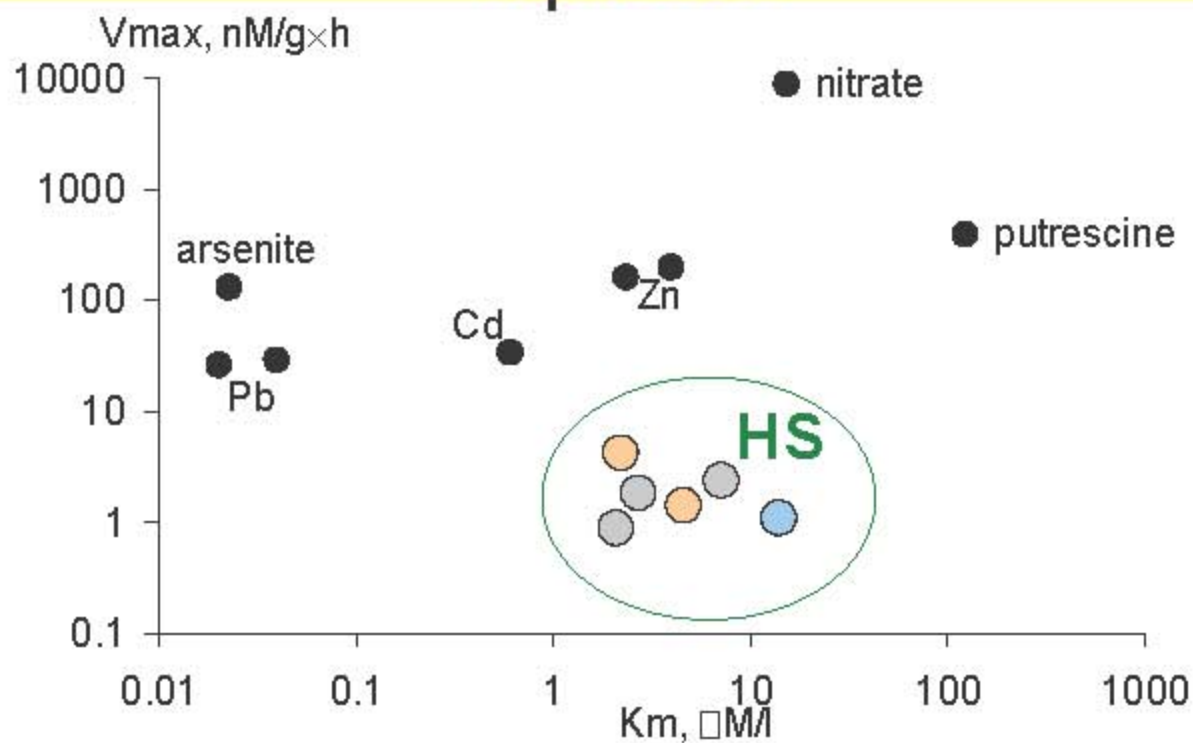


Interaction with plants: kinetics

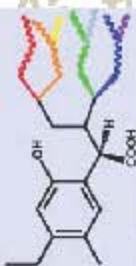


- ✱ Kinetics of HS uptake by plants can be described using Michaelis-Menten equation
- ✱ Uptake of HS by plants is hypothesized to be carrier-mediated (transporter-limited)

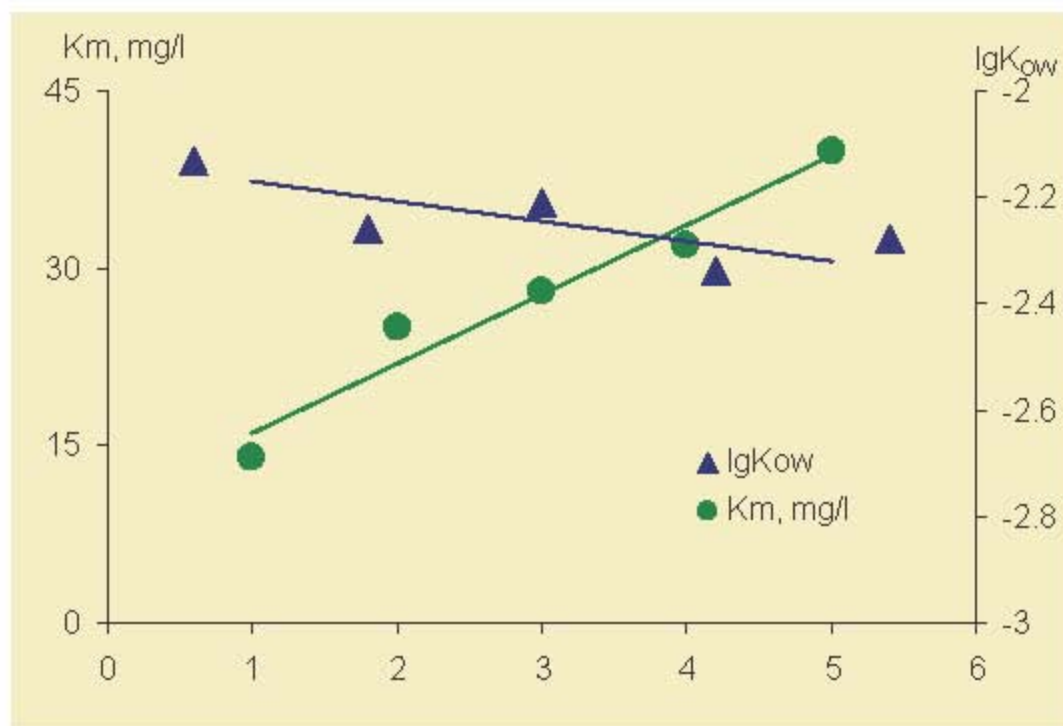
Interaction with plants: uptake



- * V_{max} of HS \ll V_{max} of individual compounds and ions
- * K_m of HS \approx K_m of individual compounds and ions



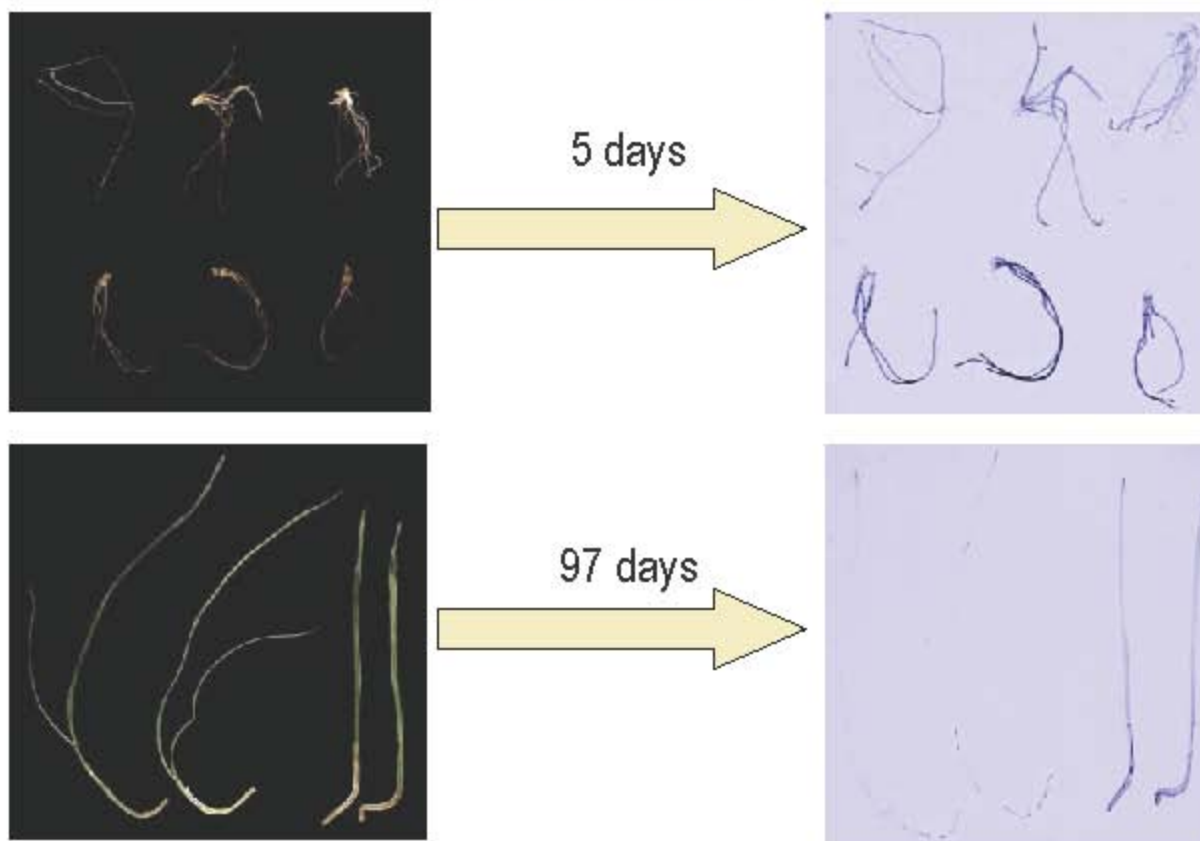
Interaction with plants: HS uptake vs. HS properties



- * Hydrophobicity was a leading property of HS influencing their interaction with wheat plants



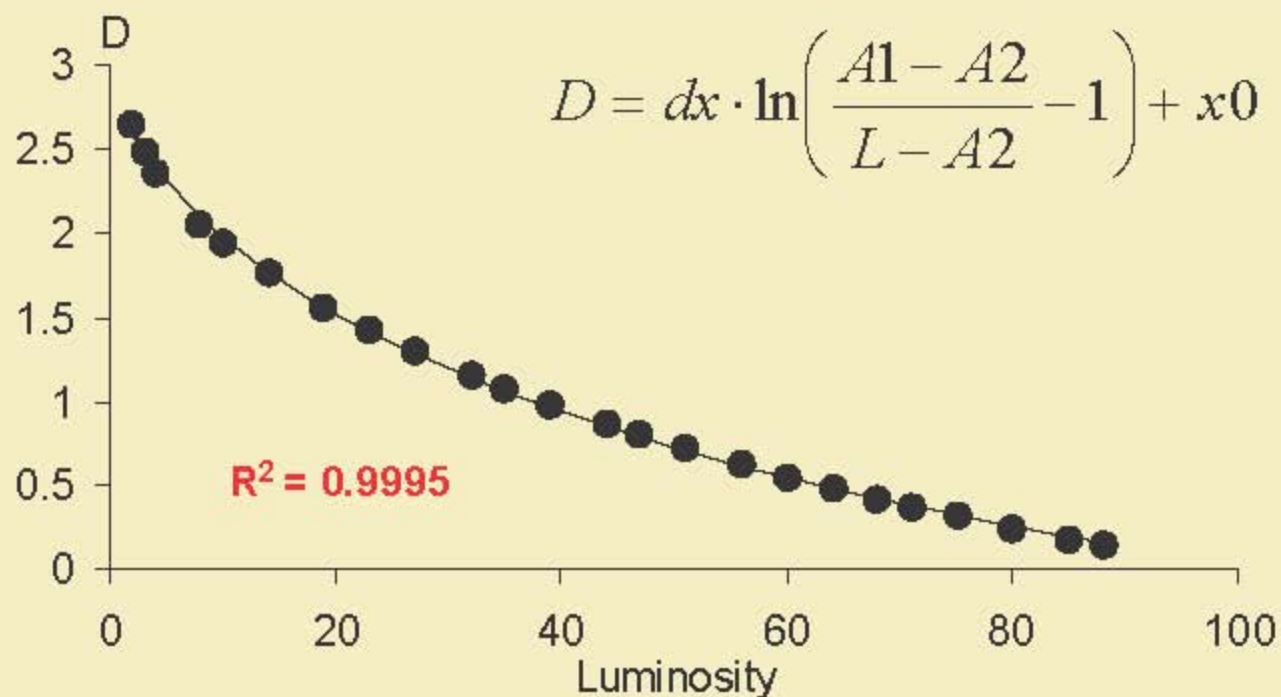
Interaction with plants: visualization



- ★ Tritium autoradiography can be used for visualization of HS interaction with plants



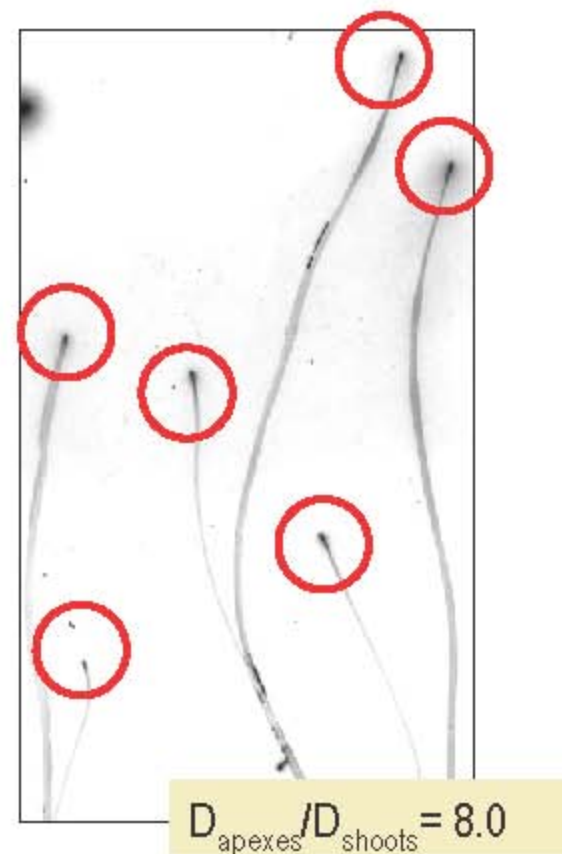
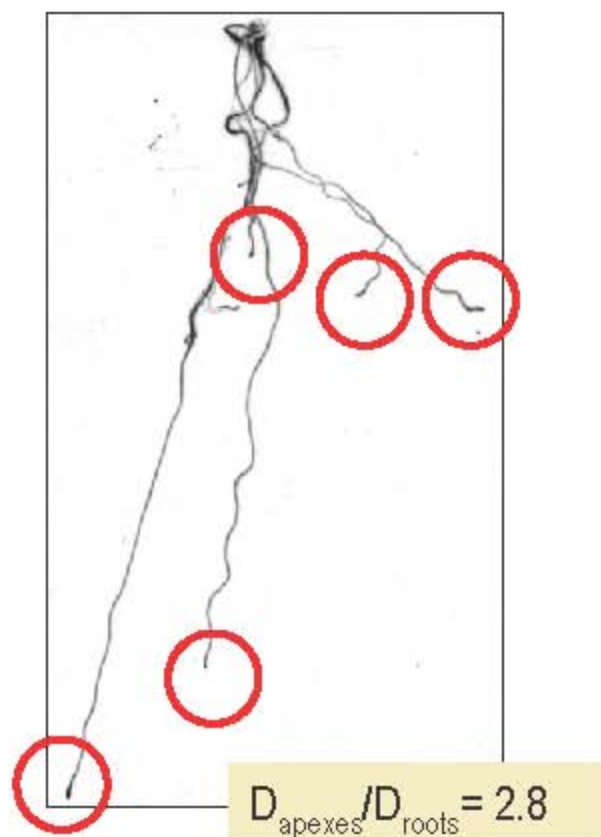
Autoradiography treatment: quantification



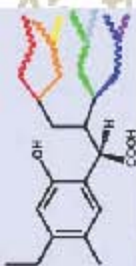
Kolotov et al., 2003

✱ Autoradiography results can be quantified

Interaction with plants: detailed visualization

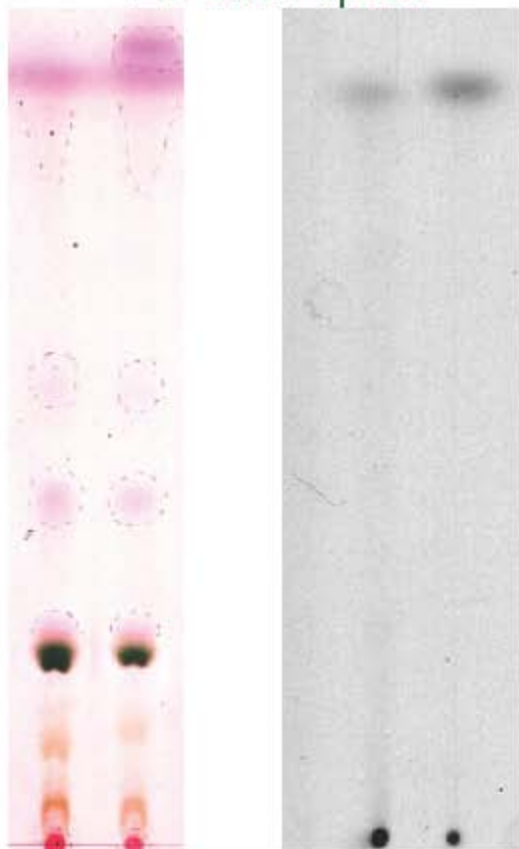


- ★ HS are mainly accumulated in the apexes of the both shoots and roots

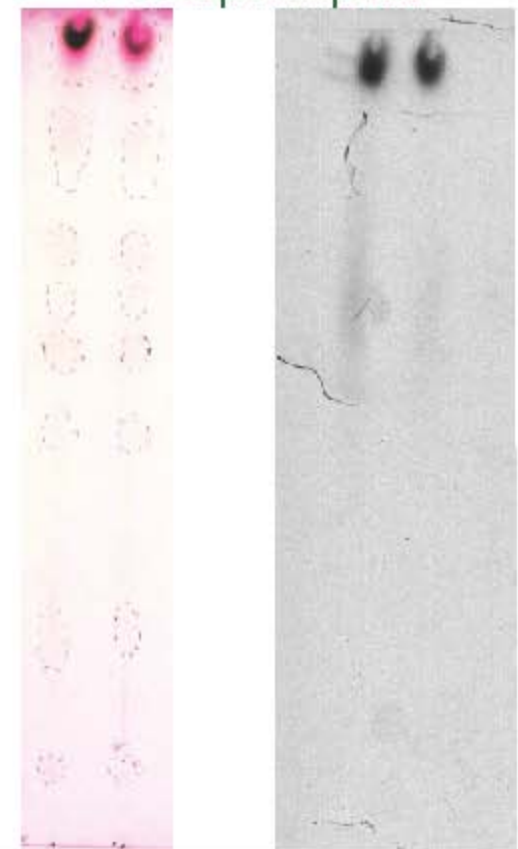


Interaction with plants: lipids extraction

Neutral lipids



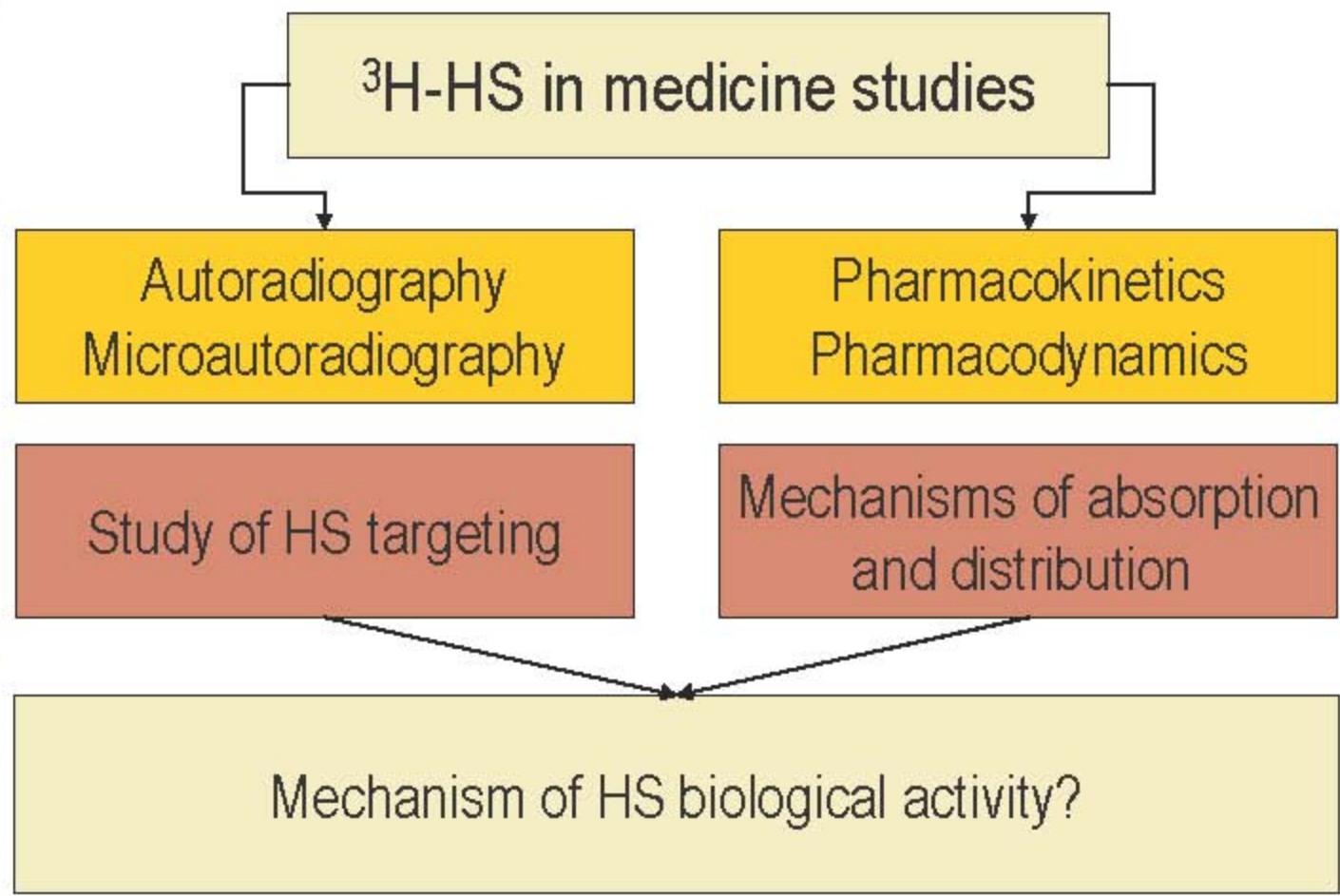
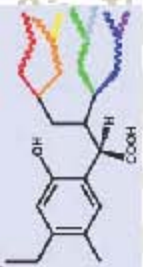
Phospholipids



* ^3H HS can be detected in plant extracts



^3H -HS: future prospects for medicine studies



The last!

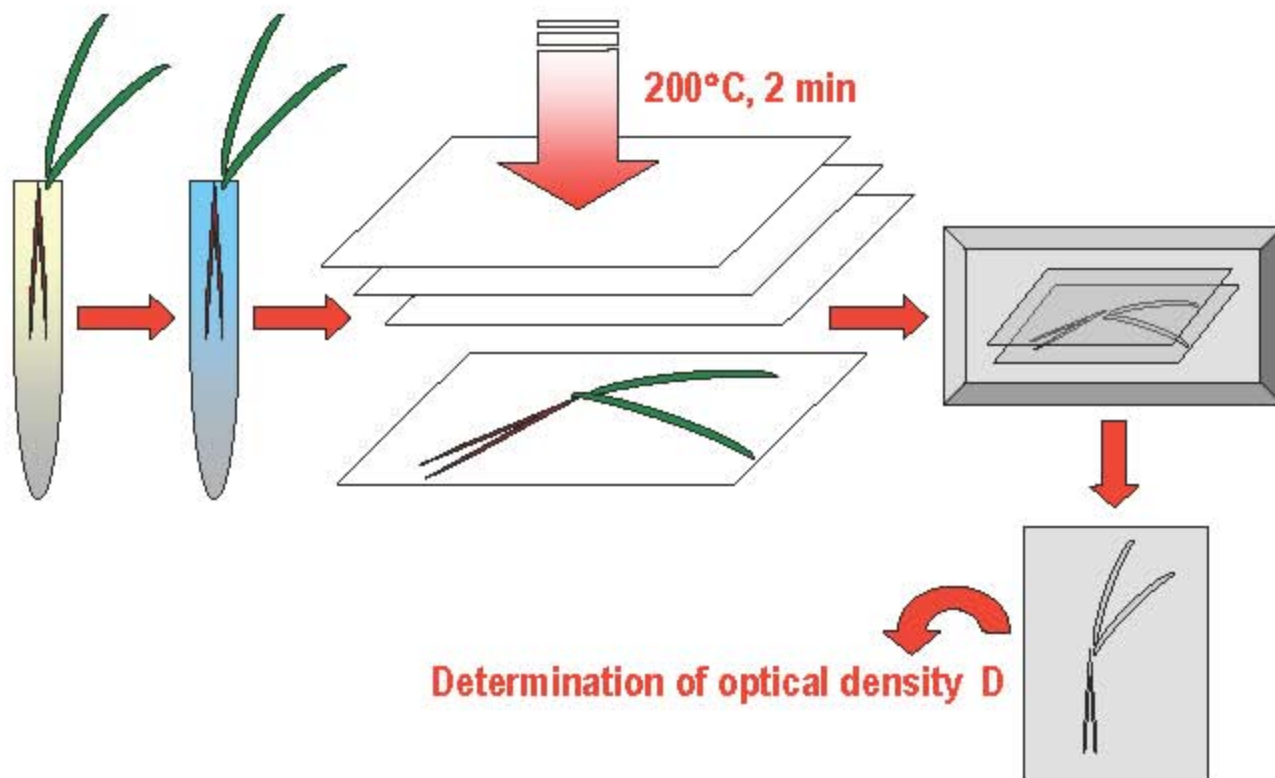


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Conference



Thank your very much for attention!

Tritium autoradiography



ISSN 0257-1724, Russ. Chem. Rev. 2010, 80, 10, 2297-2301. © IOP Publishing Ltd, 2010
 Russ. Chem. Rev. 80, 10, 2297-2301. doi:10.1088/0257-1724/80/10/2297. E-mail: rcr@iop.org

Tritium Labeling: A Unique Tool for Studying the Behavior of Humic Substances in Living Systems

G. A. Badun^a, N. A. Kullikova^a, M. G. Chirnyakova^a, Z. A. Tsastov^a, V. I. Korobkov^a,
 V. M. Fedoseev^a, F. A. Dvorkov^a, A. I. Konstantinov^a, A. V. Kudryavtsev^a, I. V. Perminova^a

