

## NATURAL HUMIC ACIDS: MACROMOLECULES OR SUPRAMOLECULAR COMPLEX?

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### ABSTRACT

Despite in advances in chromatography, the soil HAs cannot be divided into their structural groups (such as aromatic lignin-derived fragments, fatty acids and hydrocarbons, carbohydrates and amino acids) or at least separated into fractions predominantly composed of aromatic or aliphatic compounds. So far soil HAs have been characterized only on the level of compound classes and presence of individual functional groups. On these reasons fractionation of soil HAs on their compound classes is a fundamental challenge given its diverse chemical complexity and its importance in many vital soil ecosystem processes. Different soil HAs were fractionated by tandem size exclusion chromatography - polyacrylamide gel electrophoresis (SEC-PAGE) and the resulting electrophoretic fractions were studied using modern physical-chemical methods. On the basis of different analytical tools studies we can conclude that SEC-PAGE setup allowed for the first time separated soil HAs into fractions predominantly composed of aromatic or aliphatic compounds. Moreover data showed that soil HAs electrophoretic fractions consist mainly of primary humic structures compounds and highly likely that similar marked fractions have similar structural organization. Findings are consistent with the hypothesis that the structure of soil HAs arises through supramolecular assembly of different compounds

### INTRODUCTION

The detailed molecular structure of soil HAs still remain unclear and research has not yet demonstrated convincingly whether they are macromolecules or supramolecular complexes. Major molecular structural components which are detected in soil HAs are aromatic lignin-derived fragments, fatty acids and hydrocarbons, carbohydrates and amino acids. Nevertheless, despite in advances in chromatography, the soil HAs cannot be divided into these structural groups or at least separated into fractions predominantly composed of aromatic or aliphatic compounds. So far soil HAs have been characterized only on the level of compound classes and presence of individual functional groups. On these reasons fractionation of soil HAs on their compound classes is a fundamental challenge given its diverse chemical complexity and its importance in many vital soil ecosystem processes.

As soil HAs contain negatively charged functional groups, electrophoretic techniques should be applicable for their separation on different electrophoretic fractions. An electrophoretic approach, which has been successfully used in the last 40 years is polyacrylamide gel electrophoresis (PAGE). During PAGE fractionation of soil HAs several zones with distinct electrophoretic mobilities (EM) were obtained and directed to demonstrate genetic differences in the organic matter of different soil types. However, yet the questions still remain as to what electrophoretic zones of soil HAs present and how they are related with the soil HAs structure. Are they artifact or primary humic structures? The purpose of these of twenty years investigations is to shed some light on these questions.

### EXPERIMENTAL

We have developed the new method of PAGE in the presence of denaturing agents (7M urea and 0.1% SDS) and used it in the combination with size exclusion chromatography (SEC). This procedure is called tandem SEC-PAGE (1-2).

## RESULTS AND DISCUSSION

The PAGE according to Trubetskoj et al. of bulk HAs, isolated from Chernozem (Russia), Ranker (France) and Elliot soil (IHSS standard 1S102H) is shown in Figure 1. Regardless of their origin, all HAs gave several zones (fractions) with distinct EM. Fraction that did not move into the polyacrylamide gel was named A, fraction in the mid part of the gel was named B, and all the fractions on the bottom of the gel were combined into the single fraction named C+D. Preparative quantities of electrophoretic fractions for their further studies were obtained by tandem SEC-PAGE. The novelty and advantage of this tandem rests primarily the presence of urea, which added at a level of 7M both in SEC and PAGE assist in the rupture of hydrogen bonds and prevents interaction between the fractionated humic material and the immobile phase. This is accompanied by the disaggregation of HAs, a process, we believe, is primarily related to the disruption of hydrogen bonds. The proposed procedure allows separation of primary disaggregated structural humic components, thereby solving some of the key problems occurring in fractionation of HS, e.g., irreversible/reversible chromatographic column adsorption. Using PAGE in combination with SEC makes possible to obtain preparative amounts of fractions differing in both EM and molecular size (MS). It should be stressed that only after application of tandem SEC-PAGE for fractionation of HS thorough study of a physical–chemical properties of soil HAs electrophoretic fractions was possible.

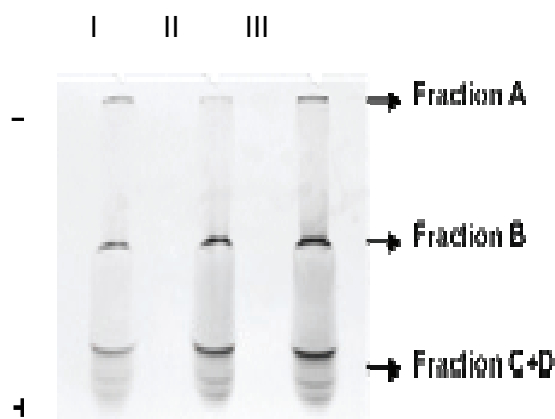


Figure 1: PAGE of ranker HA (I), chernozem HA(II) and IHSS soil HA standard (III).

We followed systematically this method over the last twenty years with different tools and its allowed us to obtain soil HA fractions independently from soil sources with clear differences in terms of:

1. electrophoretic mobility (1,2)
2. molecular size (2)
3. UV-vis absorption spectra (3)
4. amino acid weight content (4)
5. plant and microbial long chain fatty acids (>C22) distribution (6,9,12)
6. aromatic/aliphatic components (10,12)
7. hydrophobic components (12-14)
8. fluorescence intensity (5,7,11)
9. photosensitizing activity (5,8)
10. phytohormone-like activity (15)

On the basis of different analytical tools studies we can conclude that SEC-PAGE setup allowed for the first time separated soil HA into fractions predominantly composed of aromatic or aliphatic compounds. These soil HAs electrophoretic fractions consist mainly of primary humic structures compounds and highly likely that similar marked fractions have similar structural organization. Connection between fractions are based mainly on the hydrogen and hydrophobic bonds.

Obtaining of these fractions with different chemical structure by itself does not lead to structure of soil HAs however, it is clear that such data needs to be interpreted by considering the precursors of HA molecules and their chemical organization. Considering that high molecular size fraction A consisted mainly of aliphatic components (e.g., long chain fatty acids and amino acids), portions of which (e.g. long chain fatty acids) possess amphiphilic character, it is reasonable to suggest that these compounds are capable to organize micelles or vesicular structures, and amino acids in form of short peptides physically encapsulated within the structure of fraction A and so preserved from their further degradation. Findings are consistent with the hypothesis that the structure of soil HAs arises through supramolecular assembly of different compounds

## ACKNOWLEDGEMENTS

The work has been supported by Russian Foundation for Basic Research (project 15-04-00525-a).

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