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Isolation of fluorescent constituents from soil humic and fulvic acids by hydrophilic interaction chromatography

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Humic acids (HAs) and fulvic acids (FAs) are the most abundant components of soil organic matter and exhibit fluorescence. Our previous studies using high performance size-exclusion chromatography (HPSEC) and polyacrylamide gel electrophoresis demonstrated that the fluorescence of soil HAs was mainly due to the minor constituents with relatively small molecular sizes. In order to clarify the nature of the fluorescence of soil organic matter, it is necessary to isolate the fluorescent constituents from HAs and FAs. I succeeded in isolating the fluorescent constituents from soil HAs and FAs by using hydrophilic interaction chromatography (HILIC).

When HILIC of soil HAs and FAs was carried out under isocratic conditions using a SeQuant ZIC-HILIC column and acetonitrile-water as a mobile phase, the complete separation of fluorescent and non-fluorescent peaks was achieved at the acetonitrile concentration of 90%. Another fluorescent peak was eluted with decreasing concentration of acetonitrile from 90% to 50%. The use of a TSKgel Amide-80 column gave the same results. The best resolution was obtained when HILIC was performed under gradient conditions from 90% to 50% acetonitrile using the ZIC-HILIC and Amide-80 columns linked in series. For both HAs and FAs, a sharp non-fluorescent peak (peak A) followed by a sharp fluorescent peak (peak B) and a broad fluorescent peak (peak C) were eluted under the above optimum operating conditions. The intensity of peak A relative to that of peak B was significantly less in the FAs than in the HAs. The fluorescent peaks (peaks B and C) of the FAs showed considerable UV absorption, whereas those of the HAs did little UV absorption. When the fluorescence emission spectra (excitation at 280 nm) were measured for the fluorescent peaks, two emission peaks were located at 460 and 520 nm for the HAs, while for the FAs, a broad emission peak at 400-450 nm with a small shoulder at around 500 nm was observed.

The peaks were collected separately and subjected to HPSEC and diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy for characterizing the constituents of the peaks. HPSEC showed that the molecular size of the constituents was larger in the non-fluorescent peak than in the fluorescent peaks. The fluorescent constituents in the peak B of HAs were further resolved into as many as 10 peaks or more by HPSEC. DRIFT spectroscopy revealed that the functional group compositions of individual peaks differed largely from those of whole HA and FA samples. For the HAs, the non-fluorescent constituents were characterized by an amide I band at 1670 cm^{-1} and an aromatic band at 1600 cm^{-1}, whereas the fluorescent constituents by the predominance of absorption bands in the region 1000 to 1250 cm^{-1}. For the FAs, the DRIFT spectra of all the peaks were similar to each other and were characterized by an intense amide I band at 1670 cm^{-1} and a strong absorption band at 1060 cm^{-1}.