Effects of fluorescence quenching of soil powder samples in fluorescence spectroscopy

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Fluorescence spectroscopy has been widely used for examining quality and quantity of organic substances extracted from soils and sediments by using alkaline solutions. For soil samples without any extraction procedures, Laser-Induced Fluorescence (LIF) spectroscopy has been applied for assessing humic substances in soils (Milori et al., 2006). However, it has been pointed out that fluorescence quenching by sample itself can prevent quantitative analyses of fluorescence spectra (Ammari et al., 2014; Mounier et al., 2017). In this study, three dimensional excitation emission matrix (3D-EEM) fluorescence spectroscopy for Japanese typical soil powders is reported without using lasers. In order to examine fluorescence quenching, 3D-EEM spectra for the soils diluted by white alumina (Al_2O_3) and black magnetite (Fe_3O_4) powders were also measured.

Representative Japanese soil samples (Typic Hapludand, Typic Hydraquent and Typic Paleudult) were taken from artificial soil fields in Central Region Agricultural Research Center (National Agriculture and Food Research Organization, Tsukuba, Ibaraki prefecture, Japan) and air-dried at 40 ºC. They were sieved through a 2 mm meshed sieve and powdered in a mortar.

Typic Hapludand (Andosol) soil samples have 7.29 % of total carbon (TC), 0.43 % of total nitrogen (TN) and 10.2 % of moisture content. 0.8 g of the sample was preserved as a non-diluted sample, other was mixed with alumina powder (Al_2O_3, Wako) in several ratios to obtain 0.8 g of diluted soil samples having the soil content of 50, 20, 10, 5 and 2 wt%.

3D-EEM spectra for the 100 wt% (non-diluted), 10 wt% (diluted) and 0 wt% (alumina powder only) soil sample in powdered states were measured by a 3D-EEM spectrometer, Fluorolog-3 (HORIBA, Ltd., Japan) with a soil sample holder (fluorescence of 90º direction with respect to incidence). The samples were hand-pressed in the holder by a glass plate for making the measurement surface roughly flat. Excitation wavelength (Ex.) was 250-800 nm (excitation slit: 5 nm band-pass) and emission wavelength (Em.) was 300-850 nm (emission slit: 5 nm band-pass). Signal accumulation time was 0.1 sec. at 5 nm intervals.

Since fluorescence maxima were observed in the 3D-EEM spectra by 450 nm excitation, the soil samples with fine soil content increments were measured at a fixed excitation at 450 nm in the 480-850 nm range with a long-path filter (>520 nm), with 0.1 sec. accumulation time with 1 nm intervals.

3D-EEM spectra for the (a) 100 wt%, (b) 10 wt% and (c) 0 wt% soil samples (Typic Hapludand) diluted by Al_2O_3 are shown in Figure 1. Fluorescence maxima at Ex. 450 nm/ Em. 600 nm can be recognized for the 100 wt% and 10 wt% soil samples (Figure. 1(a), (b)). Sharp fluorescence at Ex. 350-600 nm/Em. 700 nm were possibly due to Al_2O_3 with some impurities (Figure 1(c)). A peak around Ex. 650 nm/Em. 800 nm is attributed to a second order spectrum by strong fluorescence of Al_2O_3 around Ex. 325 nm/Em. 800 nm.

Representative emission spectra by 450 nm excitation for the 0-100 wt% soil samples (Typic Hapludand) are shown in Figure 2(a). Peaks around 700 nm due to alumina fluorescence were recognized in the spectra for 0-50 wt% soil samples. These peaks were removed from the sample spectra by subtracting the alumina spectrum (0 wt% soil) so as for the 700 nm peak height (baseline: 686-703 nm) to become zero. Alumina-subtracted spectra are shown in Figure 2(b). Fluorescence intensities decreased for increasing soil contents. Since the Typic Hapludand (Andosol) soil sample is a dark colored soil, this result can be understood by fluorescence quenching through absorption of emitted lights by dark colored soil

components.

Other two Japanese soil samples (Typic Hydraquent and Typic Paleudult) contain less organic carbon and nitrogen and are more whitish than Typic Hapludand. Their fluorescence intensities increased for increasing soil contents in an opposite way to black Typic Hapludand. This can be explained by the absence of dark colored soil components absorbing fluorescence. In order to test this hypothesis of fluorescence quenching by dark colored components, a black material (magnetite: Fe_3O_4) will also be used to dilute these soil samples.

These fluorescence quenching effects due to dark colored components of soils should be evaluated for quantitative application of fluorescence spectroscopy for soil powder samples without extraction.

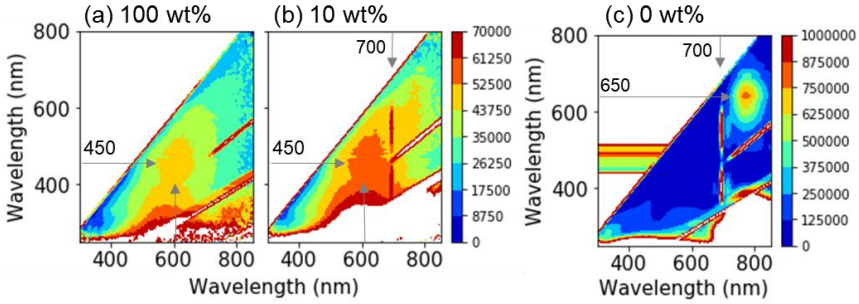

Fig. 1 3D-EEM spectra for (a) 100 wt%, (b) 10 wt% and (c) 0 wt% Typic Hapludand soil samples diluted by alumina (Al_2O_3).

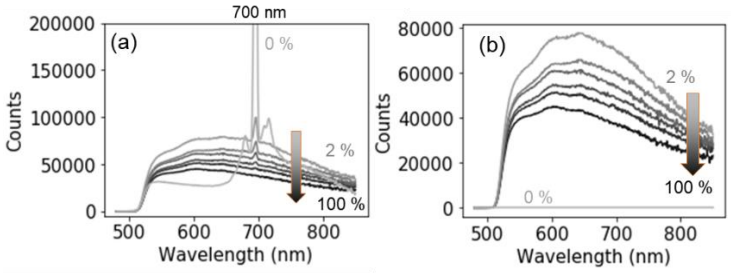

Fig. 2 (a) Representative emission spectra by 450 nm excitation for the 0-100 wt% Typic Hapludand soil samples and (b) alumina-subtracted spectra for the 0-100 wt% soil samples for eliminating fluorescence around 700 nm of alumina.

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