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Three Dimensional Excitation-Emission Matrix Fluorescence Spectroscopy of Typical Japanese Soil Powders

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Highlights

- Front face fluorescence spectroscopy of typical Japanese soil powders were performed.
- The soil powders showed 3D-EEM fluorescence patterns.
- Inner filter effect-like fluorescence extinction by blackish compounds were suggested.
- The extinction could be partly corrected by incorporating diffuse reflection.

Abstract

Front face fluorescence spectroscopy of typical Japanese soil powders (soil A: Typic Hapludand; soil H: Typic Hydraquent; soil Y: Typic Paleudult) has been conducted. Three dimensional excitation-emission matrix fluorescence spectra of the 100 wt% soils showed similar fluorescence patterns to each other. The fluorescence patterns were similar between the soil samples and their residues after extraction by NaOH solution for 60 minutes. In order to examine fluorescence extinction from a view point of whiteness of the soils, the soil powders were mixed with white and black diluents (Al_2O_3 and Fe_3O_4) and fluorescence spectra of the mixtures were measured at 450 nm excitation. At low levels of dilution with Al_2O_3 (2–100 wt% of A; 50–100 wt% of H and Y), the fluorescence intensities increased with dilution. At high levels of dilution with Al_2O_3, the fluorescence intensities decreased with dilution. On the other hand, fluorescent intensities decreased by dilution with Fe_3O_4. These results suggested inner filter effect-like fluorescence extinction by (1) large amount of blackish organic compounds giving high Total Carbon value and (2) blackish non-fluorescent mineral compounds. In order to correct the fluorescence intensities of the mixtures containing the sample soils and the diluents, we preliminary applied a correction method based on Kubelka-Munk theory using diffuse reflectance. The corrected fluorescence intensities of samples with white diluents (Al_2O_3) were described by a simple fluorescence response model having saturation values.

Keywords: Soil organic matter; Humic substances; Front face fluorescence spectroscopy; Solid state; Fluorescence extinction.

1. Introduction

"Humic substance (HS)" is a general term of yellow-brownish polymerized high molecular weight organic matter, whose structure is not well defined. While HSs are sources of nutrients for microorganisms, plants and some insects, and sources of fossil fuels, they play key roles in the following environmental issues: complexation with toxic heavy metal ions using the functional groups as ligands; toxifying or detoxifying of heavy metal ions and organic chemicals; interactions with organic pollutants; formation of various products including CO_2 (Stevenson, 1994).

For examining chemical structures and properties of HSs, their extraction from soils, sediments and natural waters has been generally performed. The International Humic Substances Society (IHSS) proposed extraction methods of HS by using alkali solutions and resins (Aiken, 1985; Kuwatsuka et al., 1992; Swift, 1996), and this extraction method has been applied in most of HS research (Olk et al., 2019). However, Lehmann and Kleber (2015) recently pointed out that alkali extractable fractions might include laboratory artifacts, and the validity of alkali extraction of HS in soil organic matter (SOM) research is under debate (Janzen 2019; Kleber and Lehmann, 2019; Olk et al., 2019; Myneni, 2019).

On the other hand, transformation processes of organic matter to HS in the earth's surface environments is often called "humification". Milori et al. (2002) proposed determination of humification degrees of HSs in solution states extracted from natural soils by fluorescence spectroscopy with an excitation wavelength (Ex.) of 465 nm. Milori et al. (2006) then pioneered the use of laser-induced fluorescence spectroscopy (LIFS) for solid soil samples as a measurement method of humificaton degrees of SOM, where an Ar laser excites soil powder samples compressed into pellets. This soil LIFS method does not need any chemical and physical separation procedures from the mineral matrix, and its use as a simple method determining humification degrees of SOM is expanding (Senesi et al., 2018). These applications of fluorescence spectroscopy to solid soil samples can provide evidence of the effect of alkali extraction.

Three dimensional excitation-emission matrix (3D-EEM) fluorescence spectroscopy is one of the common HS measuring methods, which has been used for examining structures and functional groups of HS in solution states (Senesi, 1990; Nagao et al., 2003). In fluorescence spectroscopy of liquid samples, spectroscopic correction is needed for strongly absorbing materials because of fluorescence extinction by self-absorption of excitation light and/or re-absorption of emitted light (inner filter effects). For example, Terajima and Moriizumi (2013) applied a correction method by Lakowicz (1999) for 3D-EEM spectroscopy of natural water samples. On the other hand, Lagorio et al. (1998) proposed a correction for fluorescence spectroscopy of solid samples by modifying the Kubelka-Munk theory of diffuse reflectance. Verri et al. (2008) and Clementi et al. (2009) applied this model for correction of fluorescence spectra of paintings.

On the other hand, some studies have reported 3D-EEM fluorescence spectroscopy of solid samples for analyzing organic matter. For example, Muller et al. (2011) succeeded in characterization of some components in organic wastes by 3D solid phase fluorescence (3D-SPF) spectroscopy. However, they reported that 3D-SPF spectroscopy was not effective for samples with highly light-absorptive chemical structures. Ammari et al. (2014) conducted front face fluorescence spectroscopy (FFFS) for surfaces of model mixtures simulating natural soils. They indicated necessity of further studies to understand quenching and interactions among mixed compounds. Albrecht et al. (2015) monitored organic matter during evolution of compost processes by measuring 3D-SPF spectra, and they pointed out necessity of additional studies for direct application of 3D-SPF spectroscopy to soil samples.

Mounier et al. (2017) attempted FFFS for dark-brown HS powders, pure chemicals (tryptophan, quinine sulfate and boric acid) and their mixtures. One of their findings was that the HS powders showed no significant fluorescence, despite the presence of fluorescence in their solution states. They suggested extinction of fluorescence of the solid pure chemicals by dark brown HS powders in their mixtures with pure chemicals. On the other hand, the authors reported that fluorescence intensities by tryptophan and quinine sulfate increased to saturation values with increases of their concentrations in boric acid (as a scattering material).

Based on these pioneering works and suggestions, this paper reports FFFS of typical Japanese soil powders. We examined differences in 3D-EEM spectra among original soil powders, their alkali extract solutions and residue powders. Since fluorescence properties of organic components are the main target of the present study, effects of representative inorganic white and black materials (Al_2O_3 and Fe_3O_4) on organic fluorescence have been examined by using them as diluents of soils. Possible extinction effects are discussed from a view point of whiteness/darkness of the soils.

2. Samples and Methods

2.1 Soil samples

Representative soil samples [soil A: Typic Hapludand (Andosol); soil H: Typic Hydraquent (Gray lowland soil); soil Y: Typic Paleudult (Yellow soil)] were taken from artificial soil fields in the Central Region Agricultural Research Center (National Agriculture and Food Research Organization, Tsukuba, Ibaraki prefecture, Japan) and air-dried at 40 °C for 3 days. They were sieved through a 2 mm meshed sieve, hand-powdered in an agate mortar for 10 minutes, and preserved in a desiccator. In this paper, we refer to these samples as 100 wt% soil (A, H and Y) powders. Their total carbon (TC), total nitrogen (TN) and water contents are shown in Table 1.

Table 1. Total carbon (TC), total nitrogen (TN) and water contents (%) for Typic Hapludand (A), Typic Hydraquent (H) and Typic Paleudult (Y) soils.

Samples	TC (%)	TN (%)	Water (%)
(A) Typic Hapludand	7.29	0.43	10.2
(H) Typic Hydraquent	1.18	0.10	3.4
(Y) Typic Paleudult	0.71	0.06	2.2

2.2 Extract solutions from the soil samples and their residues

For alkaline extraction of the soils (A, H and Y), 0.1 mol L^{-1} NaOH aqueous solution was used. A mixture of 1 g of the soil and 20 mL of NaOH solution was shook up at 190 rpm for 60 minutes in a centrifuge tube. After centrifugation at about 20000 m s^{-2} (6000 rpm) for 40 minutes, the supernatants were filtered (Advantec No. 6) and extract solutions were obtained. The extract solution of soil A was 500 times diluted by pure water and those of soil H and Y were 20 times diluted for avoiding fluorescence extinction. The precipitates were water-washed and centrifuged again. After air-drying the precipitates, the residue powders were obtained. In this paper, the obtained extract solutions and residues for the soil samples are referred to as extract solutions (A, H and Y), and residue powders (A, H and Y).

2.3 Diluted soil powders

As for the white and black diluents, Al_2O_3 (alumina) and Fe_3O_4 (magnetite) were selected respectively. By using Al_2O_3, we prepared 0.1, 1, 2, 5 9.7, 10, 20 and 50 wt% of soil A, 10, 50 and 60 wt% of soil H, and 10 and 50 wt% of soil Y. It should be noted that 9,7 wt% of soil A, 60 wt% of soil H and 100 wt% of soil Y have similar carbon contents (TC: 0.71 %). By adding Fe_3O_4 powders to the soils, 80 and 90 wt% of soils A, H and Y were prepared.

2.4 FFFS

Front face 3D-EEM fluorescence spectroscopy was conducted for the powder samples (100 wt% soil powders, residue powders after alkali extraction, 10 wt% soil powders with 90 wt% Al_2O_3 and 90 wt% soil powders with 10 wt% Fe_3O_4). Before the measurement, the powders were ground again in an alumina mortar for 3 minutes for mixing them. The spectra were measured by using a 3D-EEM spectrometer, Fluorolog-3 spectrometer (HORIBA, Ltd., Japan) equipped with double-grating excitation and emission monochromators. We used a sample holder (solid-sample holder accessory with sample block, item number: 1933), which has a hollow that is about 76 cm^2 with 2 mm depth. The samples were hand-pressed in the holder by a glass plate to make the measurement surface flat. The incident light is applied to the sample surface with an angle of 60° and the emitted fluorescence is collected with an angle of 150° (fluorescence of 90° direction with respect to the incident light). 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 s at 5 nm intervals and photomultiplier voltage was 950 V.

For the 0–100 wt% soil powders diluted by Al_2O_3 and Fe_3O_4, their fluorescence spectra were measured in the soil sample holder at a fixed excitation of 450 nm in the 480–850 nm range with a long-pass filter (>520 nm), with 0.1 s accumulation time at 1 nm intervals. Photomultiplier voltage was 950 V. The measurement was performed 3 times by refilling the sample powders.

For the extract solutions, 3D-EEM fluorescence spectroscopy was conducted by using Fluorolog-3 with a fluorescence quartz cell (internal thickness: 2 mm, internal width:10 mm). Extract solutions were diluted 500 times by pure water (A: 500 times; H and Y: 20 times). Ex. was 250–800 nm (excitation slit: 4 nm band-pass) and Em. was 300–850 nm (emission slit: 4 nm band-pass). Signal accumulation time was 0.1 s at 5 nm intervals and photomultiplier voltage was 950 V. It should be noted that preliminary ultraviolet–visible spectroscopy by using the quartz cell indicated absorbance of the extract solutions less than 0.1, where spectral correction of fluorescence extinction is not needed (Holland et al., 1977).

2.5 Visible reflectance spectroscopy

For all the powder samples, visible reflectance spectra (400–760 nm) were measured by a colorimeter, PRISMO MIRAGE (FUSO PRECISION Co., Ltd., Japan). The spectra were measured at 5 points (spot diameter: 3 mm) of one sample in the sample holder of the 3D-EEM spectrometer (Fluorolog-3) just after the fluorescence measurements.

In order to quantitatively represent colors of the samples, the reflectance spectra were converted to L*, a* and b* values by the same calculation as in previous works concerning colors of rocks (Onga and Nakashima, 2014; Okada and Nakashima, 2019). L*a*b* color space was proposed by the International Commission on Illumination (CIE). The L* represents whiteness (L* = 0: black and L* = 100: white), and a* and b* represent chromaticity (+a*: reddish, -a*: greenish, +b*: yellowish and -b*: bluish).

The reflectance spectra [$R(\lambda)$] by the colorimeter were also converted to Kubelka-Munk (KM) spectra [$KM(\lambda)$] according to the previous works (Onga and Nakashima, 2014; Okada and Nakashima, 2019). The KM spectrum represents absorption spectrum of powdered sample, and it is calculated by Kubelka-Munk function:

$$KM(\lambda) = \frac{1 - R^2(\lambda)}{2R(\lambda)}$$

3. Results

3.1 3D-EEM fluorescence spectra

Fig. 1 shows 3D-EEM fluorescence spectra of the 100 wt% soil powders (A_{100}, H_{100} and Y_{100}), the residue powders after alkaline extraction (A_{res}, H_{res} and Y_{res}) and the extract solutions (A_{ex}, H_{ex} and Y_{ex}). The spectra of the 100 wt% soil powders (A_{100}, H_{100} and Y_{100}) are shown again in Fig. 2, where spectra of the 10 wt% soil powders diluted by Al_2O_3 (A_{10}^{+a}, H_{10}^{+a} and Y_{10}^{+a}), the 90 wt% soil powders diluted by Fe_3O_4 (A_{90}^{+m}, H_{90}^{+m} and Y_{90}^{+m}) and the 0 wt% soil powders [100 % of (Wh) Al_2O_3 and (Bl) Fe_3O_4] are shown. TC and L* values of the powder samples are also shown in corresponding spectra.

The fluorescence spectra of the powders of 100 % samples, 10 % samples and extraction residues have a peak around Ex. 450 nm/Em. 550 nm and a broad band around Ex. 250 nm. The peaks were at Ex. 465 nm/Em. 600 nm for soil A, Ex. 450 nm/Em. 545 nm for soil H and Ex. 450 nm/Em. 550 nm for soil Y. On the spectra of 90 % samples with Fe_3O_4, peak around Ex. 450 nm/Em. 550 nm was not recognized (Fig. 2). Sharp signals on straight lines rising to the right are due to Rayleigh and higher-order lights scattering of the excitation light. Sharp fluorescence at Ex. 350–600 nm/Em. 700 nm is possibly due to impurities in Al_2O_3, and a peak around Ex. 640 nm/Em. 770 nm is attributed to a second order spectrum by strong fluorescence of Al_2O_3 around Ex. 300 nm/Em. 770 nm [Fig. 2(A_{10}^{+a}, H_{10}^{+a} , Y_{10}^{+a} and Wh)].

The fluorescence intensity at Ex. 450 nm/Em. 550 nm is the largest for 100 wt% Y powder among the 100 wt% soils [Figs 1(A_{100}, H_{100} and Y_{100})]. The sample with smaller TC values show larger fluorescence intensities and L* values.

Fig. 1(A_{100} and A_{res}) indicates that the fluorescence intensity at Ex. 450 nm/Em. 550 nm for soil A became larger after alkaline extraction. However, those for soil H and Y became smaller after alkaline extraction [Fig. 1(H_{100}, Y_{100}, H_{res} and Y_{res})]. It should be noted that the TC values are considered to become small by alkaline extraction. The L* values of the residue powders were larger than those for 100 wt% soil powders.

Fig. 2(A_{100} and A_{10}^{+a}) indicates that the fluorescence intensity at Ex. 450 nm/Em. 550 nm for soil A became larger after dilution with Al_2O_3. However, those for soil H and Y became smaller after dilution [Fig. 2(H_{100}, Y_{100}, H_{10}^{+a} and Y_{10}^{+a})]. The L* values of the 10 wt% soil powders diluted by Al_2O_3 were larger than for 100 wt% soil powders. On the other hand, dilution with only 10 wt% of Fe_3O_4 greatly decreased the fluorescence intensity at Ex. 450 nm/Em. 550 nm and the L* values [Fig. 2(A_{100}, H_{100}, Y_{100}, A_{90}^{+m}, H_{90}^{+m} and Y_{90}^{+m})].

The fluorescence spectra of the extract solutions show peaks at similar positions to the powder samples [Fig. 1(A_{ex}, H_{ex} and Y_{ex})]. However, their overall spectral patterns were slightly different to those for the 100 wt% soil powders and the residue powders. For example, longer wavelength tails are more obscure than for the powder samples.

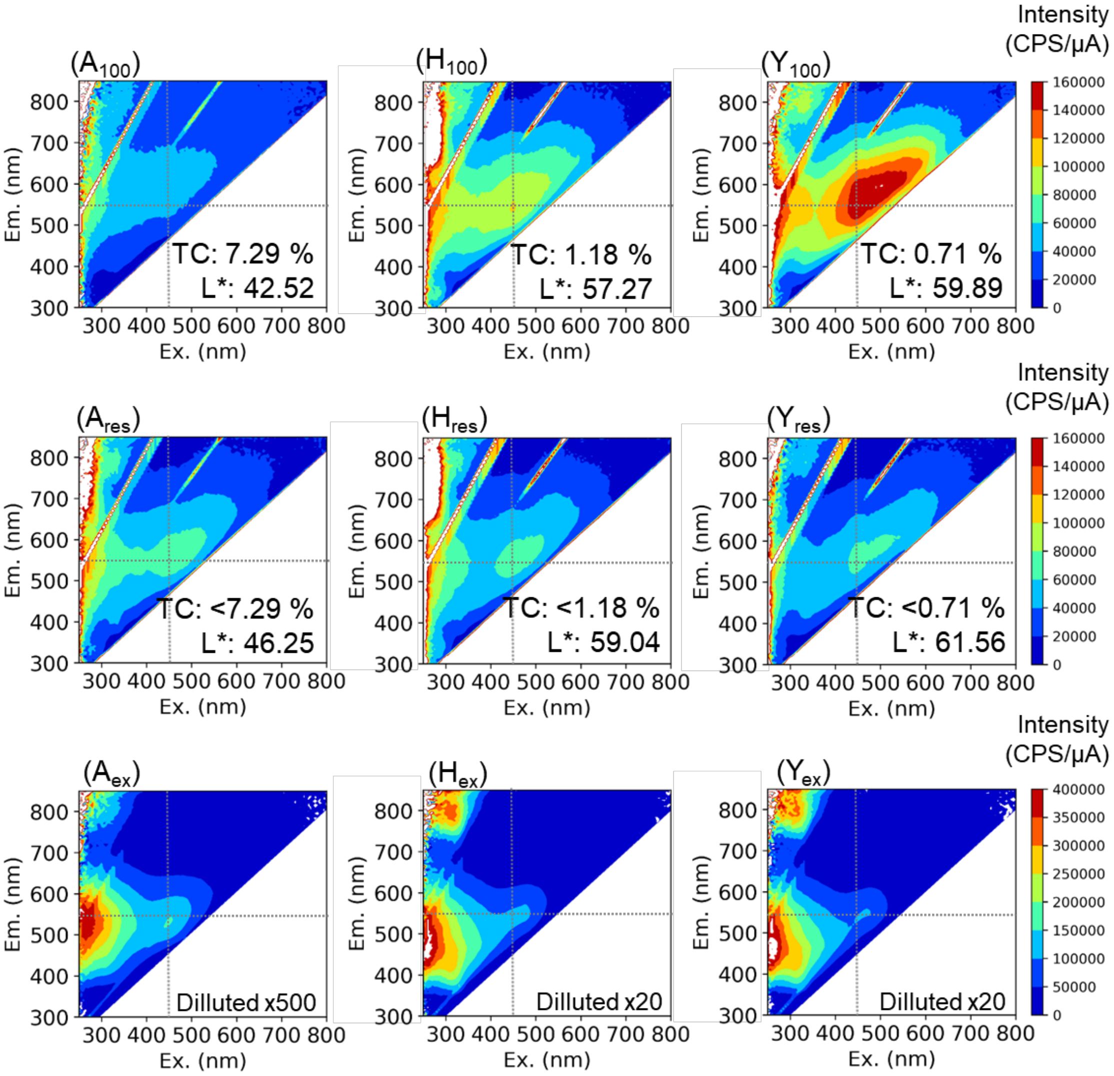

Fig. 1. 3D-EEM fluorescence spectra of the 100 wt% soil powders (A_{100}, H_{100} and Y_{100}), the residue powders after alkaline extraction (A_{res}, H_{res} and Y_{res}) and the extract solutions (A_{ex}, H_{ex} and Y_{ex}). The TC and L* values of the powder samples are also shown in the corresponding spectra. Positions at Ex. 450 nm/Em. 550 nm are indicated as crossed lines. White part on the spectral patterns is where the fluorescence intensity exceeds the upper limit of color map.

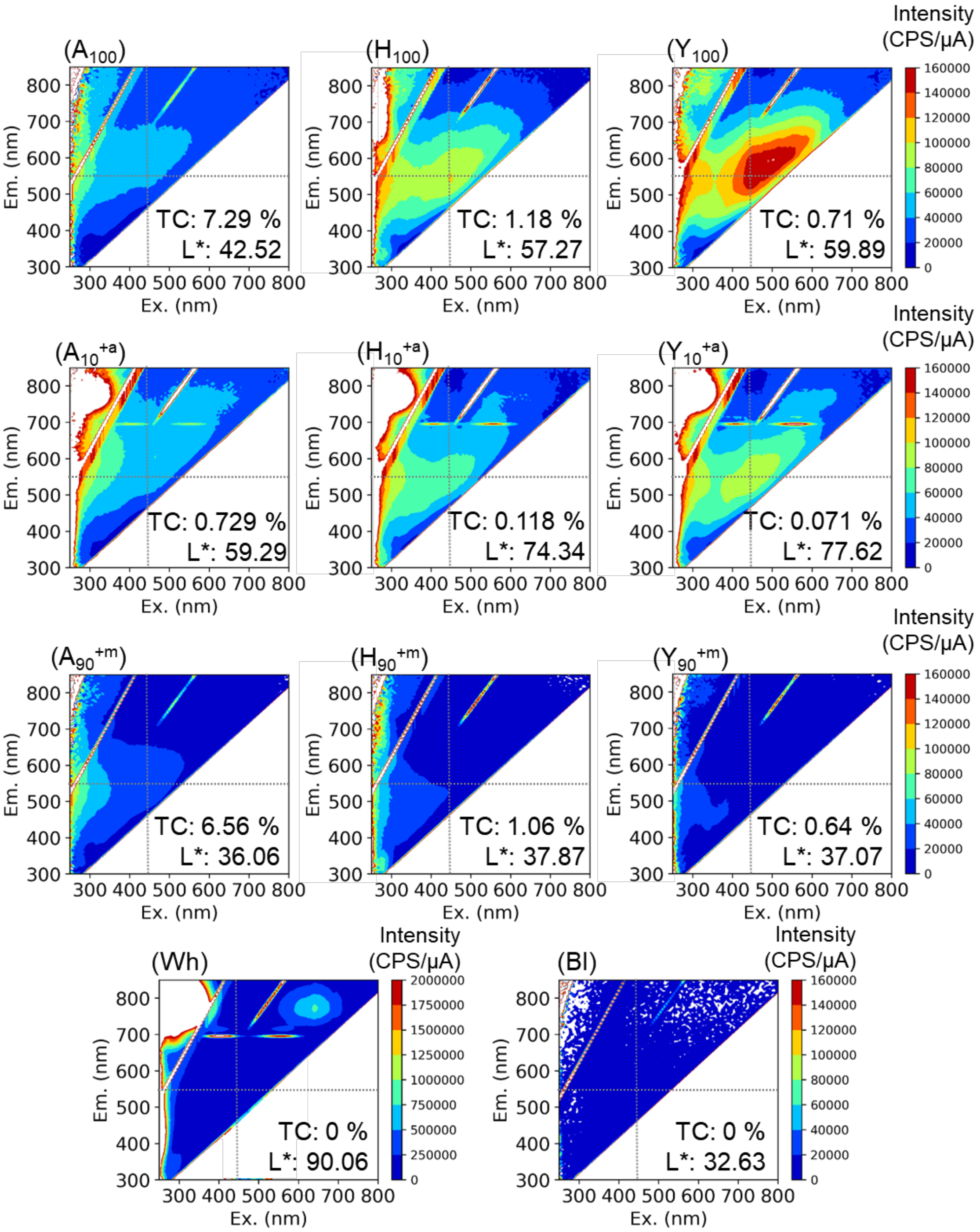

Fig. 2. 3D-EEM fluorescence spectra of the 100 wt% soil powders (A_{100}, H_{100} and Y_{100}), the 10 wt% soil powders diluted by Al_2O_3 (A_{10}^{+a}, H_{10}^{+a} and Y_{10}^{+a}), the 90 wt% soil powders diluted by Fe_3O_4 (A_{90}^{+m}, H_{90}^{+m} and Y_{90}^{+m}), and the 0 wt% soil powders composed of 100 % of (Wh) white diluent Al_2O_3 and (Bl) black diluent Fe_3O_4. The TC and L* values of the powder samples are also shown in the corresponding spectra. Positions at Ex. 450 nm/Em. 550 nm are indicated as crossed lines. White part on the

spectral patterns is where the fluorescence intensity exceeds the upper limit of color map.

3.2 Fluorescence spectra of the diluted powder samples excited at 450 nm

In order to compare fluorescence intensities at a specific Ex. wavelength for each diluting ratio, fluorescence spectra of the diluted powder samples excited at 450 nm were measured. Fig. 3 shows representative fluorescence spectra of the soil powders diluted by Al_2O_3 and Fe_3O_4 excited at 450 nm. Spectra of the 100 wt% soil powders and the 0 wt% soil powders (100 % Al_2O_3 and Fe_3O_4) are also shown in Fig. 3. Peaks around 700 nm due to Al_2O_3 fluorescence are recognized in the spectra of the samples containing Al_2O_3.

For the 2–100 wt% A powders, dilution with Al_2O_3 resulted in the strong fluorescence [Fig. 3(a)]. However, for the 0.1–2 wt% A powders, the dilution decreased their fluorescence and made their spectra similar to the spectrum of 100 % Al_2O_3 [Fig. 3(b)]. The 10–100 wt% H powders showed similar spectra to each other; the spectra did not change regardless of dilution with Al_2O_3 [Fig. 3(c)]. For the 10–100 wt% Y powders, the dilution decreased their fluorescence [Fig. 3(d)]. By dilution with Fe_3O_4, fluorescence for each soil was strongly decreased. For clear comparison of the fluorescence intensity for all the diluted samples, fluorescence intensities at Ex. 450 nm/Em. 550 nm against calculated TC values are shown in Fig. 4, where average intensities of 3 measurements for each sample are plotted with error bars. Some of the error bars are so small that they cannot be recognized in the plots.

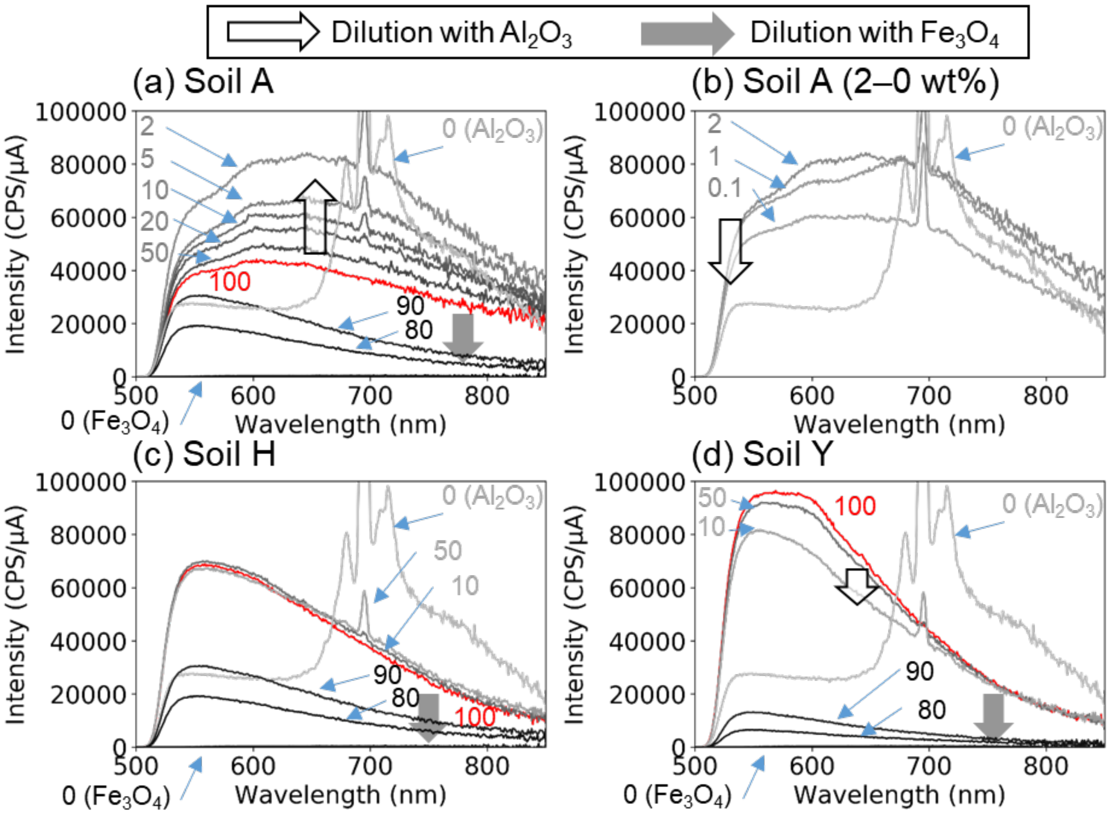

Fig. 3. Representative emission spectra by 450 nm excitation of (a) the 0–100 wt% soil A mixed with Al_2O_3 and Fe_3O_4, (b) the 0–2 wt% soil A mixed with Al_2O_3, (c) the 0–100 wt% soil H and (d) the 0–100 wt% soil Y mixed with Al_2O_3 and Fe_3O_4. Original (100 wt%) soils (A, H, Y) are in red curves, their dilutions by Al_2O_3 are in light gray curves and their dilutions by Fe_3O_4 are in dark gray curves. Each number in the windows indicates the concentration of soil (wt%). Increasing/decreasing trends of fluorescence intensities are also shown as wide arrows (white for Al_2O_3 and gray for Fe_3O_4).

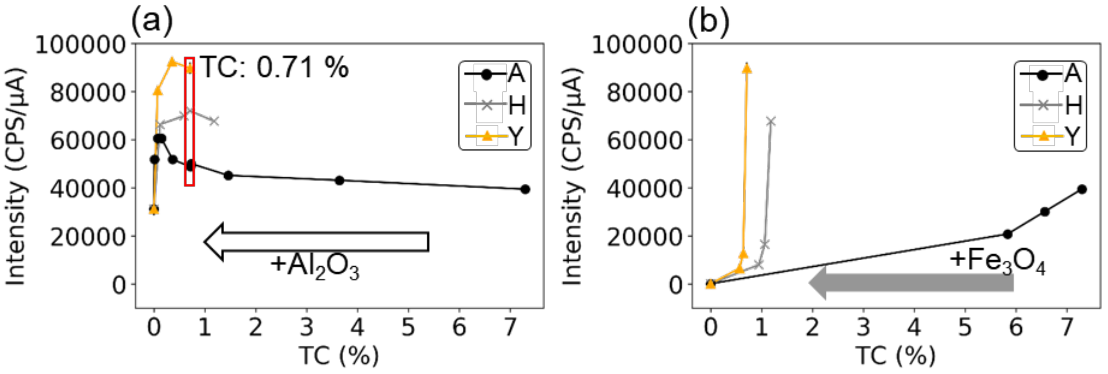

Fig. 4. Average fluorescence intensities with error bars at Ex. 450 nm/Em. 550 nm against the calculated TC values of the 0–100 wt% soil powders diluted by (a) Al_2O_3 and (b)

Fe_3O_4. Intensity values of the samples with 0.71 % of TC are indicated by a red frame in (a). The error bars are so small to be recognized.

3.3 L* values of diluted powders

Fig. 5(a) shows the obtained L* values against the calculated TC values of the soil powders diluted by Al_2O_3. The TC value and L* value had a negative correlation during dilution with Al_2O_3 of soils [Fig. 5(a)]. Fig. 5(b) shows the obtained L* values against the calculated TC values of the soil powders diluted by Fe_3O_4. It should be noted that average intensities of 3 measurements for each sample are plotted with error bars as well as in Fig. 4, and the error bars are so small that they cannot be recognized in the plots.

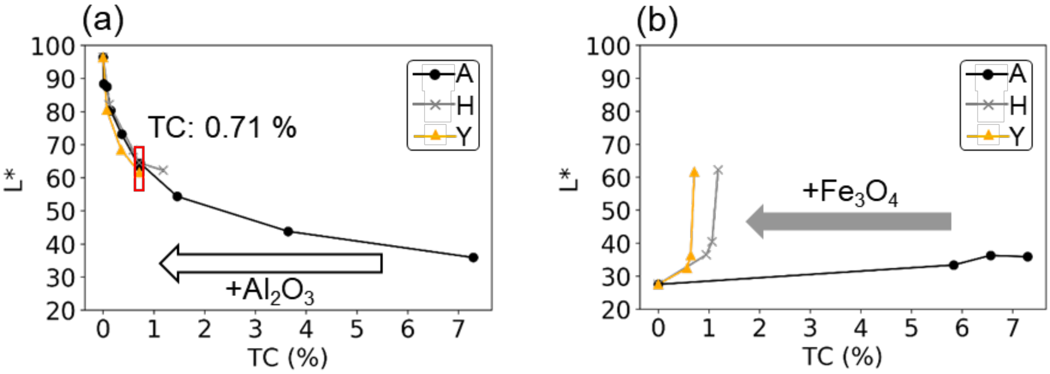

Fig. 5. Average L* values with error bars against the calculated TC values of the soil powders diluted by (a) Al_2O_3 and (b) Fe_3O_4. Average L* values of the samples with 0.71 % of TC are indicated by a red frame in (a). The error bars are so small to be recognized.

3.4 Reflectance at 450 and 550 nm

Fig. 6(a) and (c) respectively show obtained intensities of Kubelka-Munk spectra at 450 nm (KM_{450}) and 550 nm (KM_{550}) against the calculated TC values of the soil powders diluted by Al_2O_3. They had a positive correlation during dilution with Al_2O_3 of soils. Fig. 6(b) and (d) respectively show obtained KM_{450} and KM_{550} against the calculated TC values of the soil powders diluted by Fe_3O_4. It should be noted that average intensities of 3 measurements for each sample are plotted with error bars as well as in Fig. 4 and Fig. 5, and the error bars are so small that they cannot be recognized in the plots.

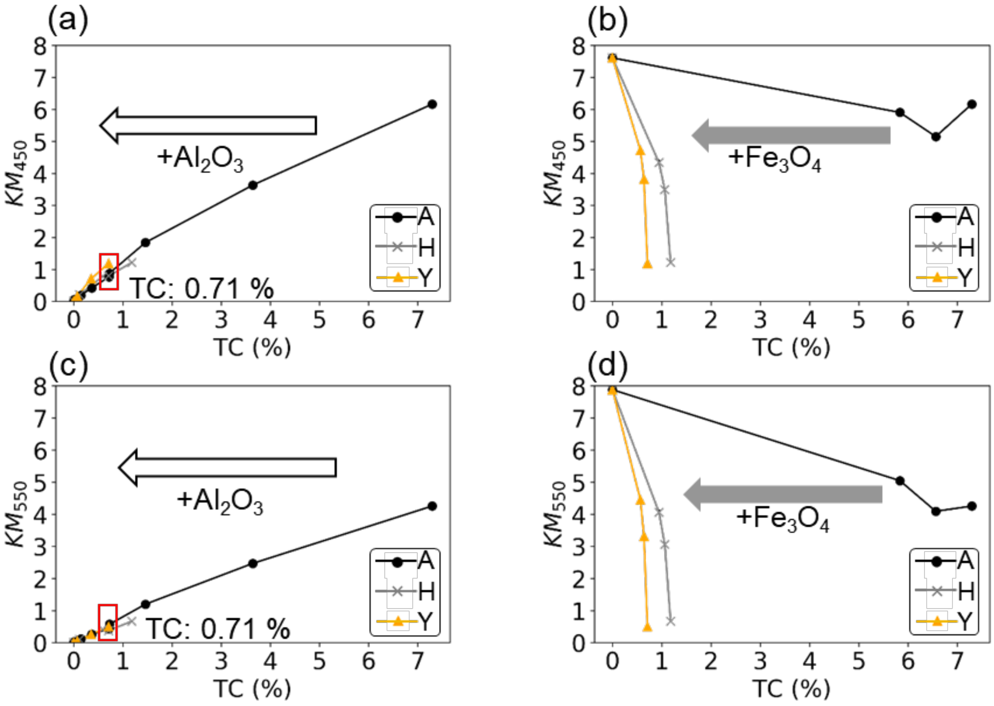

Fig. 6. Average (a, b) KM_{450} and (c, d) KM_{550} with error bars against the calculated TC values of the soil powders diluted by (a, c) Al_2O_3 and (b, d) Fe_3O_4. Average KM_{450} and KM_{550} for the samples with 0.71 % of TC are indicated by a red frame in (a) and (c). The error bars are so small to be recognized.

4. Discussion

4.1 Interpretation of results

The 3D-EEM fluorescence spectra of the 100 wt% soils [Fig. 1(A_{100}, H_{100} and Y_{100})] showed similar fluorescence patterns to each other. Although it is difficult to identify the fluorescent chemical species, the three typical Japanese soils possibly contain common fluorescent structures which can be detected by solid state fluorescence spectroscopy. Their fluorescence intensities and TC values showed a negative correlation: soil A is the carbon-richest sample with the weakest fluorescence intensity [Fig. 1(A_{100}, H_{100} and Y_{100})].

The fluorescence patterns were not significantly different between the 100 wt% soils [Fig. 1(A_{100}, H_{100} and Y_{100})] and the residues after extraction by NaOH solution [Fig. 1(A_{res}, H_{res} and Y_{res})]. This suggests that some fluorophores can remain in solid residues after the alkaline extraction. However, there is a possibility that alkali solution modifies some non-fluorescent components in the soil. The extracted solution can also contain alkali-modified components, and this may possibly be responsible to the difference of fluorescence pattern between the powder samples and the extracted solution [Fig. 1(A_{ex}, H_{ex} and Y_{ex})]. Modification of soil powder samples by alkali solution should be further examined in detail by combining the present fluorescence spectroscopy with other analyses such as infrared spectroscopy, nuclear magnetic resonance spectroscopy and mass spectrometry.

The fluorescence intensities of the original soils have a negative correlation with carbon contents (TC values) [Fig. 1(A_{100}, H_{100} and Y_{100})]. This suggests that inner filter effect-like fluorescence extinction by self-absorption of excitation light and/or re-absorption of fluorescence can occur in FFFS of soil powders with high TC and blackish color. On the other hand, decrease of organic components (TC) by the alkaline extraction can both increase [Fig. 1(A_{100} and A_{res})] and decrease [Fig. 1(H_{100}, H_{res}, Y_{100} and Y_{res})] fluorescence intensities. The increase in fluorescence intensity for soil A can be explained by cancellation of extinction in association with increase in whiteness (L* value) of soil A. For soils H and Y, although alkaline extraction gives more whitish colors, their fluorescence intensities decreased. This is possibly because simple loss of fluorophores by the alkaline extraction has lager impact on the fluorescence intensity for soil H and Y than the cancellation of fluorescent extinction.

Competition between simple loss of fluorophores and cancellation of fluorescence extinction is recognized in the dilution experiments with Al_2O_3 (white diluent). At low levels of dilution with Al_2O_3 (2–100 wt% of A; 50–100 wt% of H and Y), the fluorescence intensities increased with dilution [Fig. 4(a)]. This can be explained by cancellation of extinction by decrease in blackness of the soils (increase in L* value) due to mixing with Al_2O_3. At high levels of dilution with Al_2O_3, the fluorescence intensities decreased with dilution [Fig. 4(a)]. This is possibly because fluorophores decreased by the dilution. On the other hand, dilution with Fe_3O_4 (black diluent) decreased both the fluorescent intensities [Fig. 4(b)] and L* values [Fig. 5(b)]. This suggests that blackish mineral (Fe_3O_4) induces large inner filter effect-like fluorescence extinction.

In summary, front face 3D-EEM fluorescence spectroscopy of the typical Japanese soil powders suggested inner filter effect-like fluorescence extinction for (1) large amount of blackish (small L* value) organic compounds giving high TC value and (2) blackish non-fluorescent mineral compounds. Therefore, extinction by fluorescent organics and/or other dark organic/inorganic constituents should be considered in front face 3D-EEM for natural soil powders.

In the diluting experiments of soils with Al_2O_3, the three samples with 0.71 wt% TC showed similar L*, KM_{450} and KM_{550} values [Figs. 5(a) and 6(a and c), indicated by a red frame]. However, they showed different fluorescent intensities (50,000–90,000 counts)

[Fig. 4(a), indicated by a red frame]. This suggests that fluorescence intensity is not controlled only by TC values. Therefore, chemical structures, organic and mineral components with fluorescent centers should be considered.

On the other hand, the 3D-EEM fluorescence spectra of the extracted solutions [Figs. 1(A_{ex}, H_{ex} and Y_{ex})] indicated the presence of similar fluorophores to the original powder samples. Although their intensities cannot be compared directly because of different measuring methods (solution vs solid states), the spectra of the extracted solutions exhibit more clear peaks at shorter Ex. wavelength and less tails to longer Ex./Em. wavelength sides than those for the powder samples. Details of differences between solid and solution states should be investigated in future studies.

4.2 Spectral correction and comparison with previous researches

The pioneering research on soil LIFS by Milori et al. (2006) showed that soils with 1.00–3.84 % of carbon contents had positive correlations (1) between fluorescence intensities and carbon contents and (2) between fluorescence intensities over carbon contents and humification degrees. It should be noted that they used a laser for excitation of the fluorophores in contrast with our study using the xenon lamp and the double-grating monochromator. Our study shows the fluorescence intensities and TC values do not have positive correlations (1) for high TC soils (soil A: 0.15–7.29 wt%) and (2) for dark soils (small L* value) (Soil A and Fe_3O_4 -diluted samples). Therefore, specific soils with high organic contents (high TC) and/or blackish color (small L*) may not show correlations between fluorescence intensities and carbon contents. This problem possibly due to fluorescence extinction by dark-colored and highly light-absorptive materials and/or chemical structure is consistent with the indications in the previous researches (Muller et al., 2011; Ammari et al., 2014; Albrecht et al., 2015; Mounier et al., 2017), and spectral correction methods are needed. However, it should be noted again that fluorescence by the soils (mixtures of humic-like substances and minerals, etc.) showed the fluorescence patterns (Fig. 1), while the previous studies reported no specific fluorescence by humic substances' powders.

In order to correct the fluorescence intensities at Ex. 450 nm/Em. 550 nm of the mixtures containing the sample soils and the diluents (Fig. 4), we preliminary tried two correction methods using reflectance at 450 and 550 nm (Fig. 6).

First, we applied the method by Lakowicz (1999), which gives a corrected fluorescence (emission) intensity of liquid samples by multiplying an observed fluorescence intensity with a factor γ:

$$\gamma = 10^{\frac{A_{ex}+A_{em}}{2}}$$

where A_{ex} is the absorbance at excitation wavelength and A_{em} is the absorbance at emission wavelength. In this study, we replaced A_{ex} and A_{em} by KM_{450} and KM_{550}, in the above equation to obtain the correction factor γ_1:

$$\gamma_1 = 10^{\frac{KM_{450}+KM_{550}}{2}}$$

This correction factor γ_1 was multiplied to the un-corrected fluorescence intensities at Ex. 450 nm/Em. 550 nm for the diluted soil samples to obtain corrected fluorescence intensities. However, weak reflectance for soil A and soils with Fe_3O_4 gave so large γ_1 that the corrected fluorescence intensities became too large. Therefore, the Lakowicz method for liquid samples may not be effective for FFFS of the solid samples in this study.

Second, we applied the Kubelka-Munk theory-based correction method by reference to the previous researches on dyes (Lagorio et al., 1998) and paintings (Verri et al., 2008; Clementi et al., 2009). This method can be used under two assumptions: (1) scattering coefficient of sample is independent of wavelength; (2) emission light of the first fluorophore excited by a light source can excite the second fluorophore and generate re-emission light, which does not excite more fluorophores (Verri et al., 2008). In this study, fluorescence intensities at Ex. 450 nm/Em. 550 nm for the diluted soil samples were corrected by multiplying the un-corrected intensities with a factor γ_2 using KM_{450} and KM_{550}:

$$\gamma_2 = \left[1 + \sqrt{\frac{KM_{550}}{KM_{550}+2}}\right] \times \left[1 + \sqrt{\frac{KM_{550}(KM_{550}+2)}{KM_{450}(KM_{450}+2)}}\right]$$

Fig. 7 shows average fluorescence intensities at Ex. 450 nm/Em. 550 nm corrected by γ_2 against the calculated TC values of the 0–100 wt% soil powders diluted by (a) Al_2O_3 and (b) Fe_3O_4 . The trend is almost similar to the un-corrected fluorescence (Fig. 4), except for saturation at high TC mixtures with Al_2O_3[Fig. 7(a)]. Summarizing the above, after the spectral correction based on the modified Kubelka-Munk theory, the fluorescence intensities increased to constant values as concentrations of the soils increased, in the mixture systems with the soils and white diluents Al_2O_3. This is consistent with the fluorescence response model of mixture systems with the fluorescent materials (tryptophan and quinine sulfate) and the diluents (boric acid) by Mounier et al. (2017):

$$IF = \alpha\left(1 - e^{-\beta C}\right)$$

, where *IF* is the integrated area of emission spectrum, α is the constant including fluorescence quantum yield, probability of emission, strength of excitation light and experimental factor, β is the constant including absorption coefficient of excitation light and path length, and C is the concentrations of fluorophores. By considering the corrected fluorescence intensities and the TC values [Fig. 7(a)] as IF and C, their saturation tendencies for the three soils were fitted by the above model equation. The fitted curves [Fig. 7(a)] showed good reproducibility with high correlation coefficients (r^2=0.99). This suggests that corrected FFFS of soil powder samples diluted by Al_2O_3 can be considered as FFFS of materials showing almost no extinction effects, which has been assumed by the simple fluorescence response model by Mounier et al. (2017).

However, among the 100 % soil samples, negative correlation between the fluorescence intensities and TC values (fluorescence intensity: Y>H>A, but TC: A>H>Y) remained after the correction [Fig. 7(a)]. Moreover, the corrected fluorescence intensities of the mixtures of black diluents (Fe_3O_4) showing different trends from Fig. 7(a) cannot be explained by the simple response model [Fig. 7(b)]. Therefore, further studies are needed for soils containing black materials such as organic carbon and Fe_3O_4.

Moreover, identification of fluorescent components and examination of differences in fluorescence spectra by sample conditions (e.g. solid/liquid; presence of organic-inorganic interactions; varieties of dark-colored components, water contents, extraction time, particle sizes, surface areas and surface charges of soil components) are necessary. These improvements will expand the use of FFFS as a simple method for examination of fluorescent structure in soils and sediments without using any extraction procedures.

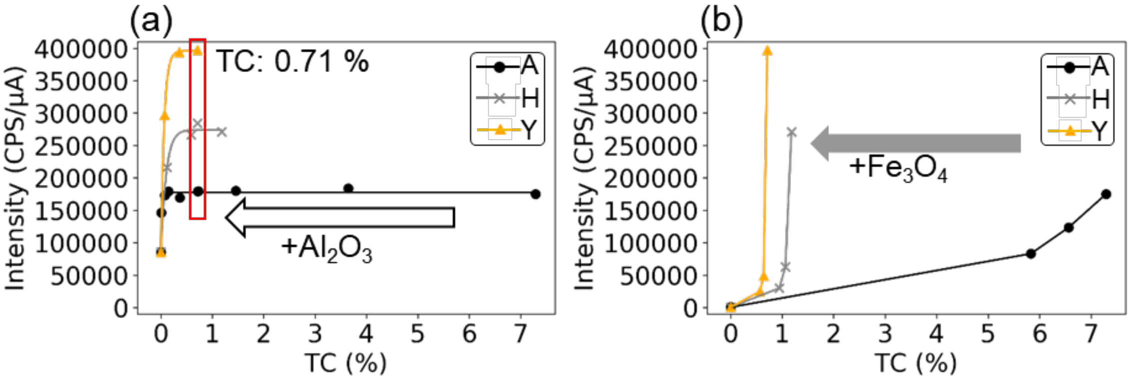

Fig. 7. Corrected fluorescence intensities at Ex. 450 nm/Em. 550 nm against the calculated TC values of the 0–100 wt% soil powders diluted by (a) Al_2O_3 and (b) Fe_3O_4. Intensity values of the samples with 0.71 % of TC are indicated by a red frame in (a). Solid curves of (a) are the fitted curves by the simple model by Mounier et al (2017), and solid lines of (b) are connecting the dots for eye guide.

5. Conclusion

3D-EEM fluorescence spectroscopy is one of the common HS measuring methods, and it has been used for examining structures and functional groups of HS in solution states. The previous works performed FFFS of organic materials and suggested necessity to understand fluorescence extinction by mixed compounds. This study reports FFFS of typical Japanese soil powders and examined differences in fluorescence spectra by alkaline extraction and dilution with white and black compounds (Al_2O_3 and Fe_3O_4). The obtained spectra suggested fluorescence by the soils, which are considered to be mixtures of humic-like substances and minerals, etc., while the previous studies reported no specific fluorescence by humic substances' powder. However, the results suggested inner filter effect-like fluorescence extinction by (1) large amount of blackish organic compounds giving high TC value and (2) blackish non-fluorescent mineral compounds. Therefore, specific soils with high organic contents (high TC) and/or blackish color (small L*) may not show correlations between fluorescence intensities and carbon contents. The fluorescence extinction in the mixture systems of the soils and white compounds (Al_2O_3) were corrected by the factor based on the Kubelka-Munk theory. The corrected fluorescence intensities could be explained by the simple fluorescence response model having saturation values proposed in the literature. Although further studies including identification of fluorescent components and examination of differences in fluorescence spectra by sample conditions are needed, these improvements will expand the use of FFFS as a simple method for examination of fluorescent structures in soils and sediments without using any extraction procedures.

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