Article

Performance Evaluation of a Portable Laser-Induced Fluorescence Spectroscopy System for the Assessment of the Humification Degree of the Soil Organic Matter

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Laser-induced fluorescence spectroscopy (LIFS) has shown advantages for analyses of whole soil, providing results about chemical structure of soil organic matter (SOM) without the need for a chemical fractionating process; thus, allowing direct analysis of soils. Moreover, another advantage is the potential for the development of portable systems, which could be usable in field analyses. The aim of this paper is to evaluate the performance of a portable LIFS system to assess the humification degree (HD) of SOM. A good correlation (R > 0.8) was obtained for results (HD) achieved using the proposed portable system and a conventional fluorescence spectrometric method for humic acid. In addition, the results obtained using the portable LIFS system statistically agreed with the ones achieved using a benchtop LIFS system already described in the literature for such application (R = 0.953). Thus, the portable LIFS system constitutes an affordable option for the *in situ* characterization of SOM.

Keywords: laser-induced fluorescence spectroscopy, humic acid, soil organic matter, humification degree

Introduction

Nowadays, the concern about soil quality has increased considerably due to environmental problems related to land degradation and sustainable agricultural production. However, the definition of soil quality and the development of techniques for such evaluation is an important challenge for science. The impact of agriculture on soil quality may be assessed indirectly through the measurement of soil characteristics (indicator of quality), which may allow comparisons with soils under native vegetation or among different soil managements.[1]

Soil organic matter (SOM) represents one of the main pools of the carbon on earth and has a key role on the carbon cycling.[2-4] SOM comprises humic substances

(HS) and non-humic substances. However, an important information, often disregarded when studying SOM, is the soil humification degree (HD).

Among the different analytical techniques that could be used to characterize the humification degree of organic materials, absorption spectrophotometry in the UV-Vis region,[5-8] electron paramagnetic resonance (EPR),[9-13] Fourier transform infrared spectroscopy (FTIR),[8,14-16] [13]C nuclear magnetic resonance (NMR) and conventional fluorescence spectroscopy are able to provide information which have a straight correlation to humification degree of humic acid (HA).[14,15,17-24] For instance, EPR provides the semiquinone-type free radical concentration and ^{13}C NMR provides information of the degree of aromaticity.[8,25,26] However, the use of these techniques requires chemical extraction and fractionation of HS, which might modify fulvic acid, humic acid and humin content in respect to what

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is present of them in the original sample.[27,28] Furthermore, sample preparation is labor-intensive, increasing analysis time and cost.

With regard to agricultural and environmental issues, it is extremely important to develop an analytical method able to provide a high analytical frequency (number of samples analyzed *per* unit of time) and able to meet the demand for information on HD and SOM.

This study makes a contribution of special interest for analytical chemistry because the portable laser-induced fluorescence spectroscopy system (LIFS) is a very promising technique to assess the HD of organic matter (OM) in whole soil samples and OM insoluble, as the humin.

Currently, in the literature, there is no technique able to determine the humification degree of the whole OM and the most of existing studies use HA, which represents only a fraction of the bulk SOM. Information based on humin, insoluble fraction of HS and that compose more than 80% of the humic material from SOM, is not reliable as measurements made using ^{13}C NMR and EPR suffer interference due to the presence of high levels of clay materials, iron oxide and other minerals that are paramagnetic, causing reduction in the relaxation times and degrading the resolution of the spectra.[13,26,29] The advantage of LIFS is that it is a technique that enables the direct optical probing of substances in solid materials and can enable information on HD of the whole OM without the need for extraction and fractionation of HS from soil.

Several humification indexes based on fluorescence spectroscopy have been proposed.[21,22,24] Zsolnay *et al.*,[22] working with dissolved organic matter extracted from soil, proposed a humification index based on the ratio of the signals (integrated area) of the emission bands measured between 570 to 641 nm (A_4) and between 356 to 432 nm (A_1), using excitation as 240 nm. This index is referred as A_4/A_1. This definition is based on the fact that humification can be associated to an increasing of C/H ratio and a shift to longer fluorescence emission wavelengths.[8,30,31] The theoretical background can be found in Haken and Wolf[32] and are corroborated by the analysis of literature data.[33-35]

Kalbitz *et al.*[21] and Milori *et al.*[24] also defined humification indexes based on the spectral characteristics of humic acids in solubilized samples, they proposed another humification index denominated I_{400}/I_{360}, based on the ratio of fluorescence intensities measured respectively at 400 nm (I_{400}) and at 360 nm (I_{360}). Alternatively, it was used a similar index I_{470}/I_{360} with these intensities measures using fluorescence synchronous scanning in order to improve spectral selectivity. Milori *et al.*[24] presented the third humification index by measuring the fluorescence

emission spectra of solubilized humic acids using excitation at 465 nm, index denominated A_{465}.

LIFS has been used to assess the humification index of SOM in whole soil samples.[36] The method is promising because it enables fast acquisition of results relying on the direct probing of the solid sample, allowing the assessment of OM associated with minerals in the soil matrix.[26] The results are in agreement with the ones obtained using other spectroscopic approaches such as conventional fluorescence spectroscopy, EPR and ^{13}C NMR.[24,26,36] According to González-Pérez *et al.*,[26] LIFS is a promising technique for OM studies with whole Oxisol samples. It is sensitive and allows rapid analysis of samples without any prior chemical treatment.[36-40]

The proposed LIFS method relies on the excitation of SOM using a laser in the near ultraviolet-blue region (350-480 nm), which coincides with the absorption profile of more rigid and complex molecules of HS,[24,41] such as aromatics and heterocyclic compounds. The aim of this study was to evaluate the performance of a lab-made portable LIFS system for the assessment of the humification degree of the SOM. The results of the portable LIFS system were compared with the values of humification degree of HA extracted from the same samples and determined by fluorescence spectroscopy methods.[21,22,24] The performance of the portable system was also compared to a benchtop LIFS system similar to that described by Milori *et al.*[36]

Experimental

Experimental samples

Soil used in this study was the Typic Hapludults, cultivated with Tifton 85 Bermudagrass. The field experiment was located in the city of Lins, São Paulo. A set of soil samples with low carbon content was collected from six depths: 0-10 cm, 10-20 cm, 20-40 cm, 40-60 cm, 60-80 cm and 80-100 cm, being used a total of 36 samples (6 sets with 6 samples each). The samples were named A1 to A36.

This set of samples was chosen due to its variation of humification degree of SOM.

Total organic carbon (TOC)

In the laboratory, the soil samples were sieved to remove roots and ground to obtain particles smaller than 0.15 mm. For this analysis, aliquots of 100 mg of each of the six samples of soil were used for all treatments. These aliquots were oxidized at 900 °C using a flow rate of 0.3 L min^{-1} oxygen. The determination of the percentage of

TOC was performed in triplicate for samples of whole soil. The measurements were performed using a Total Organic Carbon Analyzer, TOC-V Model, Shimadzu Model, coupled to a Solid Sample Module, SSM-500A, Shimadzu.

Humification degree of humic acids assessed by conventional fluorescence spectroscopy

The HAs were extracted according to the procedure advised by the International Humic Substances Society (IHSS).[42]

The HAs were brought to a concentration of 20 mg L^{-1} and the pH was adjusted to 8.0 by diluting them in a solution of NaHCO$_3$ 0.05 mol L^{-1}. This concentration of 20 mg L^{-1} was used to minimize post-filter effect and guarantee that analytical response is within its linear range.

Normal fluorescence spectra and synchronous fluorescence spectra (excitation band) were acquired using a Perkin Elmer Luminescence Spectrometer LS 50 B spectrofluorophotometer (right angle geometry, 1 cm × 1 cm quartz cell) using a 1 cm optical pathlength quartz cuvette, spectral band pass of 10 nm and scan velocity of 500 nm min^{-1} for both excitation and emission.

To obtain the A$_4$/A$_1$ humification index, the emission spectra were scanned from 300 to 700 nm with excitation at 240 nm. The A$_4$/A$_1$ ratio was calculated from the integrated area measured in the last quarter of the emission spectrum (570-641 nm) and the first quarter (356-432 nm).[22] In this study, synchronous scan excitation spectra were acquired over a range of 220 to 650 nm using a Δλ of 55 nm and the humification index was calculated by the ratio of the fluorescence intensities measured at 468 and 374 nm (I$_{468}$/I$_{374}$).[21] To obtain the A$_{465}$ humification index, the emission spectra were obtained over a range of 480 to 800 nm using an excitation wavelength of 465 nm. In this case, the humification degree was calculated using the total area of emission (A$_{465}$).[24]

Humification degree of SOM assessed using LIFS

After the sample collection was performed, the samples were dried at room temperature, passed through a 100-mesh sieve and ground. After this, around 0.5 g from each sample was submitted to 10 ton cm^{-2} of pressure for 2 min to form pellets. For each soil sample, two pellets were prepared. Each pellet was placed in a sample holder with a quartz window and was measured using the benchtop and portable LIFS system.

The portable LIFS system is also a lab-made equipment developed by Embrapa Instrumentantion at the Lasers and Optics Laboratory for this application (Figure 1). It comprises a diode laser (Coherent - CUBE) emitting at 405 nm (50 mW), an optical shutter, a bifurcated optical fiber bundle with seven optical fibers in a stainless steel ferrule: six illumination fibers around one read fiber (Ocean Optics), a high sensitivity mini-spectrometer (USB4000 - Ocean Optics), an adjustable optical filter, and a notebook. The resolution of the system was around 10 nm for all acquisition ranges (475-800 nm). In addition, a software was developed to control the laser, the shutter, and spectrometer parameters such as integration time and number of averages for each measurement.

The benchtop LIFS equipment is a lab-made system similar to that described by Milori *et al.*[36] In this paper, LIFS operation parameters were: (*i*) lock-in amplifier 100 mW, (*ii*) photomultiplier tube type of detector operating at 850 V, (*iii*) the laser used is an argon laser manufactured by Coherent; model Innova 90, tuned for emission at 458 nm blue region to excite soil fluorescence and (*iv*) 400 mW laser power. The spectral resolution was adjusted to 4 nm for all acquisition ranges (470-650 nm).[36]

For both LIFS equipment, the ratio between the area under fluorescence emission spectrum (FEA) (range 475 and 800 nm) and TOC concentration in the sample was considered as an indicator of humification of SOM (HD).[36] The measurements were made in triplicate for each soil sample. The same soil pellets used in the benchtop system were measured to validate the portable system.
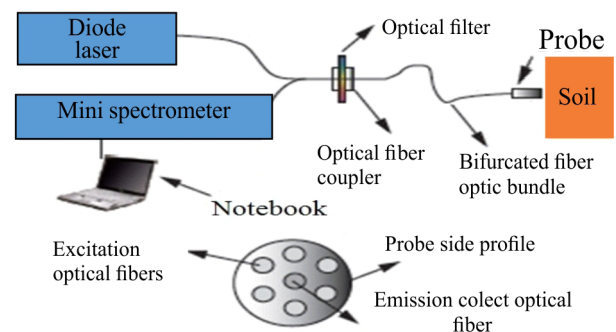

Figure 1. Experimental scheme of the portable LIFS system.

Comparison among methods

Fluorescence methods to assess humification degree of humic acid were compared: conventional fluorescence index[21,22,24] and laser induced fluorescence index.[36]

The humification degree of SOM assessed by portable LIFS system using whole soil was compared to humification degree of humic acid determined in solution by conventional methods of fluorescence spectroscopy.

Moreover, considering the benchtop LIFS system similar to that described by Milori *et al.*,[36] the portable

system was valued using basic statistical treatments. The calibration curve was fitted by the least squares method (LSM) using the HD obtained from the benchtop LIFS system as the independent variables and the HD obtained from the portable LIFS system as the dependent variables. The samples used to obtain the calibration model were the A1 to A30. For validation, the samples used were A31 to A36.

Results and Discussion

Organic carbon contents of samples are shown in Table 1. As can be seen, the used samples have low quantities of carbon, and the carbon content decreases with

Table 1. Carbon concentration of whole soil samples obtained by the elemental analysis (TOC)

Sample	Depth / cm	Carbon content / %
A1	0-10	0.82 ± 0.02
A2	10-20	0.69 ± 0.02
A3	20-40	0.65 ± 0.02
A4	40-60	0.58 ± 0.02
A5	60-80	0.51 ± 0.01
A6	80-100	0.41 ± 0.01
A7	0-10	0.79 ± 0.01
A8	10-20	0.62 ± 0.01
A9	20-40	0.62 ± 0.02
A10	40-60	0.53 ± 0.02
A11	60-80	0.40 ± 0.01
A12	80-100	0.32 ± 0.01
A13	0-10	0.81 ± 0.02
A14	10-20	0.68 ± 0.02
A15	20-40	0.63 ± 0.01
A16	40-60	0.64 ± 0.01
A17	60-80	0.48 ± 0.01
A18	80-100	0.38 ± 0.01
A19	0-10	0.83 ± 0.02
A20	10-20	0.69 ± 0.01
A21	20-40	0.65 ± 0.01
A22	40-60	0.63 ± 0.04
A23	60-80	0.47 ± 0.01
A24	80-100	0.36 ± 0.01
A25	0-10	0.84 ± 0.03
A26	10-20	0.85 ± 0.02
A27	20-40	0.75 ± 0.01
A28	40-60	0.65 ± 0.02
A29	60-80	0.45 ± 0.01
A30	80-100	0.37 ± 0.02
A31	0-10	0.83 ± 0.02
A32	10-20	0.72 ± 0.02
A33	20-40	0.65 ± 0.01
A34	40-60	0.62 ± 0.01
A35	60-80	0.50 ± 0.01
A36	80-100	0.40 ± 0.01

soil samples depth. Figure 2 presents typical spectra of fluorescence emission for whole soils and HA in solution.

For HA, it was possible to observe changes in spectral profiles and emission intensities. In the presented example, variations in intensity reached a factor of 5 (Figure 2a, 2b and 2c). From the data of carbon obtained by TOC (Table 1) and the fluorescence spectrum area, the HD was determined for the diferent methods mentioned above. A comparison among methods to assess humification degree of HA is shown in Figure 3. The correlation among indexes (A_{465}, A_4/A_1 and I_{468}/I_{374}, Figure 3) can be considered very strong (R ≥ 0.95, with *$p < 0.0001$).

It is important to highlight that the three methods that use fluorescence are basically different from each other. While the method of Zsolnay *et al.*[22] uses ultraviolet excitation, where several groups of HA are excited, the method proposed by Milori *et al.*[24] excludes a great portion of the groups, being, therefore, more selective. Although the emission spectrum with excitation at 240 nm is wide (Figure 2a), it presents changes in the spectral profile, while the emission spectrum with excitation at 465 nm is narrower (Figure 2b), possessing the same spectral profile. When the synchronous-scan is carried out, Kalbtiz *et al.*[21] method is also more selective than Zsolnay *et al.*[22] method, because it specifically shows a resonance whose difference between the excitation and emission is 55 nm (Figure 2c).

For analysis using whole soil, it was observed mainly changes in intensity of fluorescence emission. When excited at 458 nm, LIFS emission spectrum of whole soil samples shows a single broad band from 470 to 650 nm (Figure 2e), quite similar to those reported for HAs fluorescence spectra.[24] These spectra also show maximum fluorescence intensity around 520 nm, in all depths, increasing the fluorescence intensity in deeper depths. Other samples presented very similar spectra. Similarly, when excited at 405 nm (portable system), LIFS emission spectrum shows a single broad band from 475 to 800 nm (Figure 2d). It is interesting to emphasize that, for the presented spectra, variations in fluorescence intensity may reach a factor of 2. It means that the carbon structures are different. The quantum efficiency of fluorescence emission for samples A31 to A36 is bigger than for A7 to A12. In other words, there is a lot of carbon in samples A7 to A12, which are inside of unsaturated and rigid structures, like aromatic rings.

Figure 4 shows the correlation between the HD of humic acid determined by conventional fluorescence and the HD assessed by the portable LIFS system using pellets of whole soil samples.

The Pearson's coefficients between HD determined using the portable system and the conventional fluorescence spectroscopy methods were high (R = 0.85 for A_{465},
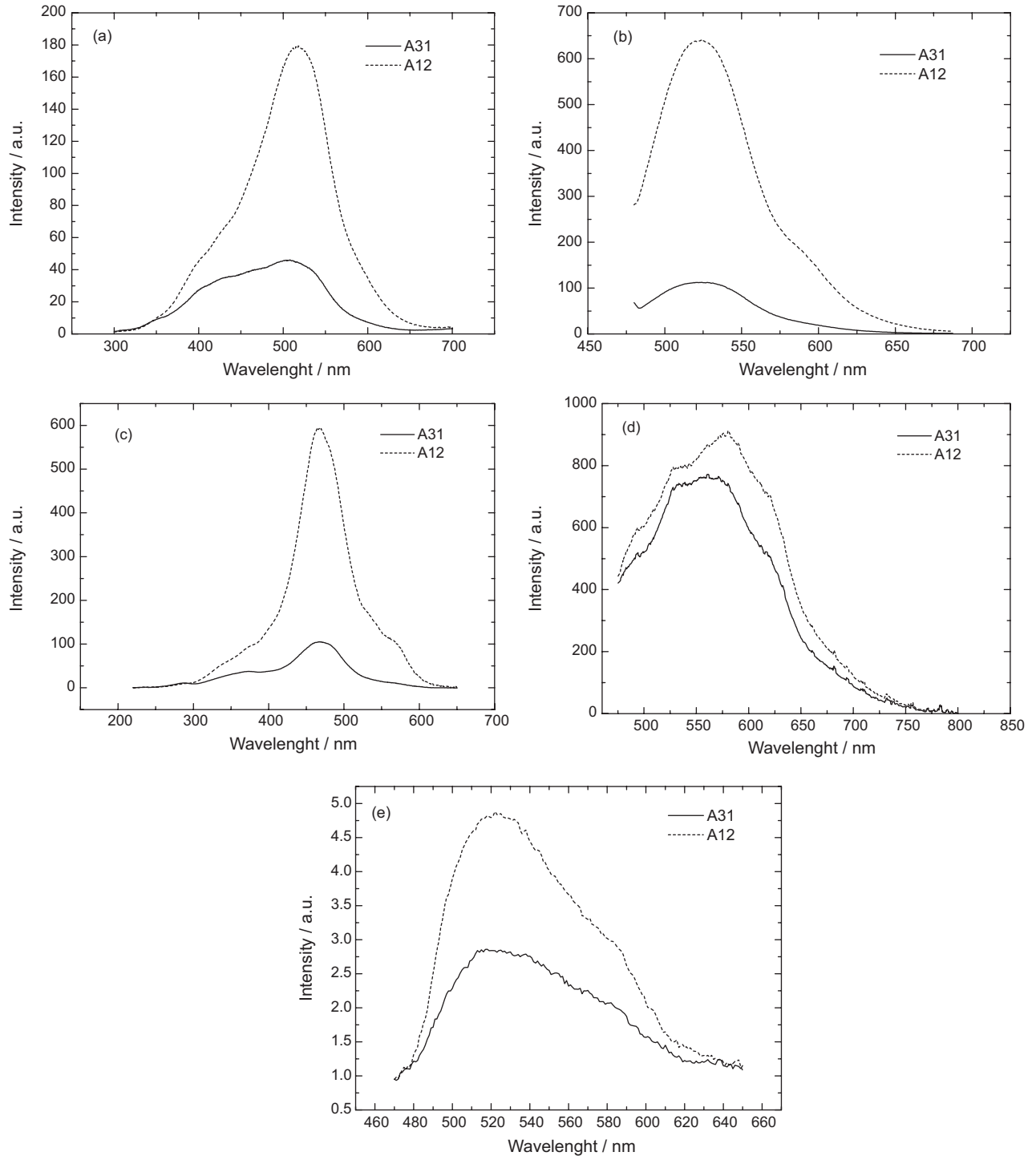
Figure 2. Fluorescence emission spectra of HA obtained with excitation in 240 nm (a), excitation in 465 nm (b), synchronous scan using a $\Delta\lambda$ of 55 nm (c) and fluorescence emission spectra of whole soil samples obtained by the portable LIFS (d) and by the benchtop LIFS systems (e) for the sample less humified (A31 sample: solid lines) and more humified (A12 sample: dashed lines).

R = 0.83 for A_4/A_1, and R = 0.80 for I_{468}/I_{374}, Figure 4a, 4b and 4c) and dispersion was low *p < 0.0001. Another interesting observation is the increase of dispersion in the HD of OM in deeper horizons (these are the horizons that have greater depth of soil samples and showed the highest humification degree) (Figure 4a, 4b and 4c). It is important

to emphasize that the measurements using conventional fluorescence methods analyze only the humic acid and the experiments using LIFS analyze the total OM. Thus, the insoluble fraction of humic substances (humin) and fulvic acid are also analyzed by LIFS. In light of this, the humin fraction can comprise over 80% of humic material of SOM.
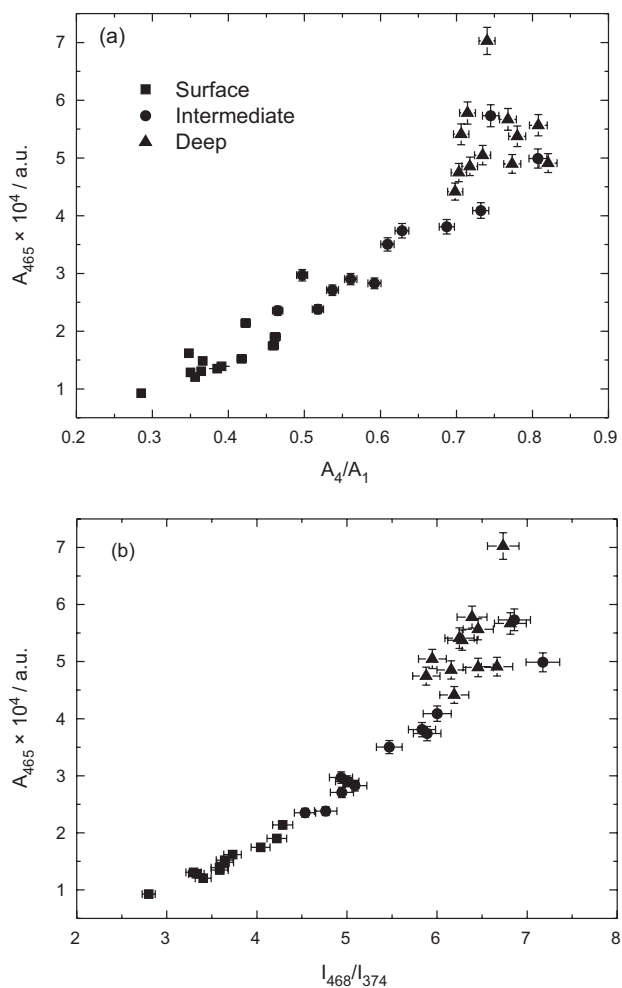
Figure 3. Correlation between the HD determined by conventional fluorescence: (a) A_{465} (Milori *et al.*[24]) and A_4/A_1 (Zsolnay *et al.*[22]) and (b) A_{465} (Milori *et al.*[24]) and I_{468}/I_{374} (Kalbitz *et al.*[21]).

The correlations among the data are high and in both cases, the results indicate the feasibility of using the portable LIFS system as a tool for investigating the humification of organic matter in whole soil samples, without the need of chemical extractions.

Notwithstanding, the portable LIFS system is easier to use because the measurement is conducted using optical fiber, thus avoiding the misalignment that may occur with the benchtop LIFS system. The portable equipment is an analytical method able to provide a high analytical frequency, allowing direct analysis of soil samples meeting the demand for information on HD, which substantially reduces the scanning time.[43] The correlation between the portable LIFS and the benchtop LIFS in estimating HD is shown in Figure 5a. The Pearson's coefficient (R = 0.953 with *p < 0.0001) indicates a very strong correlation.

The calibration model was fitted from the LSM using the HD determined by the benchtop LIFS system as independent variables and the portable LIFS system as dependent
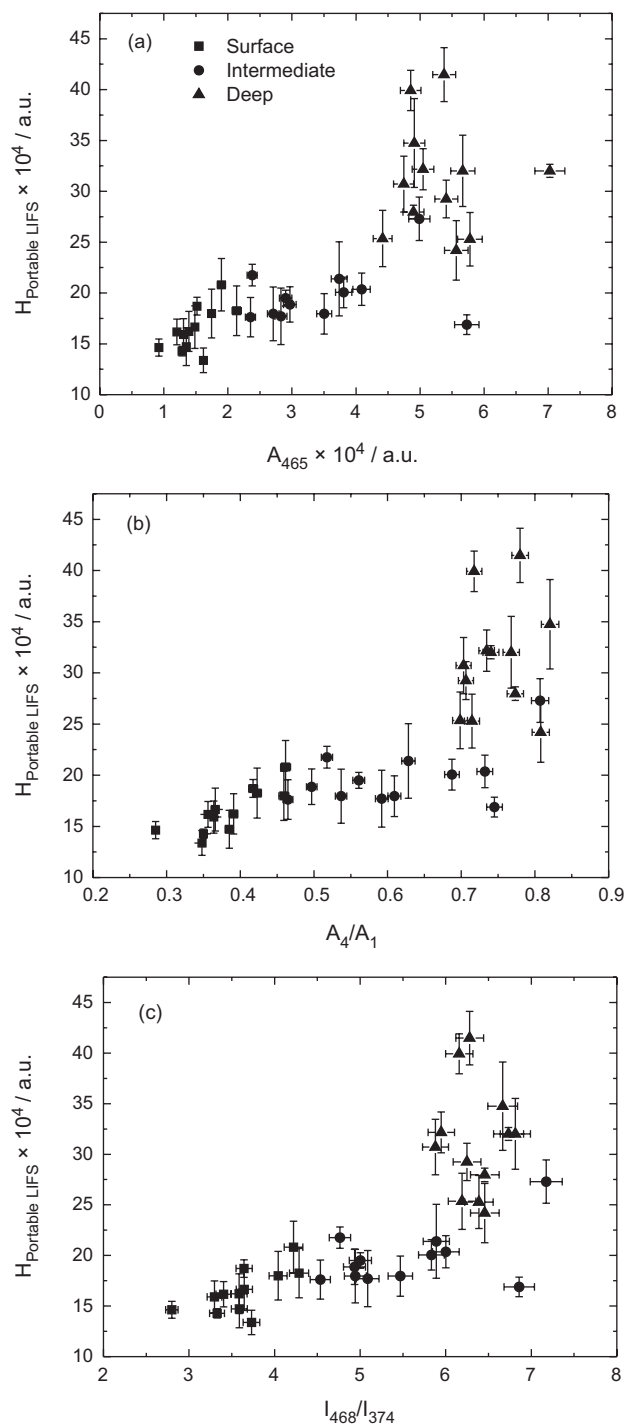

Figure 4. Correlation between the HD determined by portable LIFS system ($H_{portable\ LIFS}$) in whole soil samples and the HD determined by conventional fluorescence: (a) portable LIFS system ($H_{portable\ LIFS}$) and Milori *et al.*[24] (A_{465}); (b) portable LIFS system ($H_{portable\ LIFS}$) and Zsolnay *et al.*[22] (A_4/A_1) and (c) portable LIFS system ($H_{portable\ LIFS}$) and Kalbitz *et al.*[21]

variables. The equation obtained from this model curve was

$$H_{portableLIFS} = [(66 \pm 11) + (0.32 \pm 0.02)\ (H_{benchtopLIFS})] \times 10^3$$

(Figure 5a). The treatment samples used for the model validation were A31 to A36. Figure 5b shows the validation graphic, where the Pearson's correlation coefficient
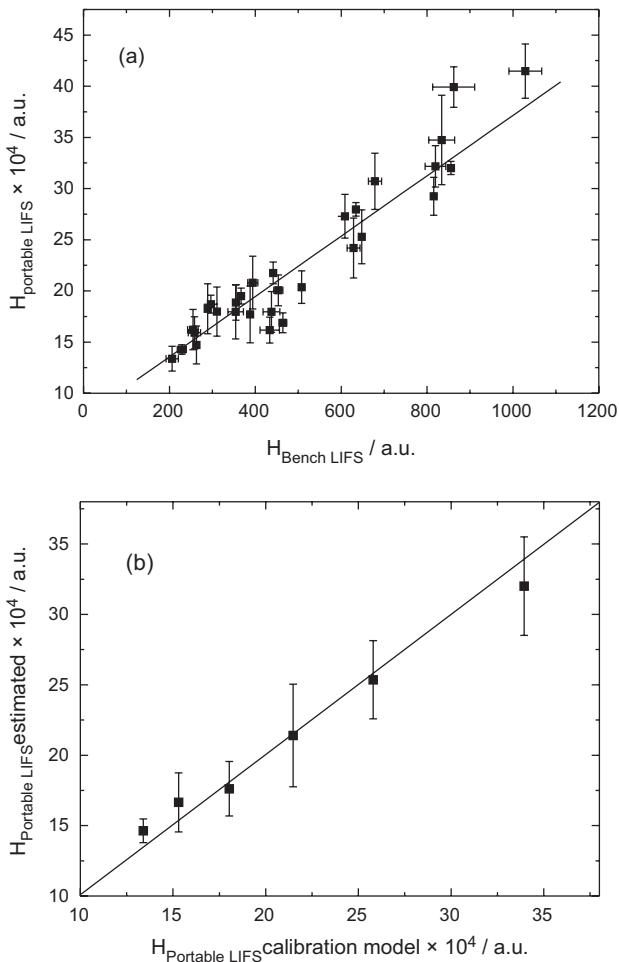
Figure 5. (a) Calibration model for portable LIFS using the benchtop LIFS systems already described in the literature by Milori *et al.*[36] for samples: A1 to A30. (b) Validation of the model using samples A31 to A36.

was very strong (R = 0.997) with low dispersion factor (*$p < 0.0001$*), indicating a low value of error in estimating the HD of SOM. Table 2 shows the HD values used in the calibration model and the values estimated by the portable LIFS system.

As can be seen in Figure 5b and Table 2, the results from the portable LIFS system are very similar to the ones obtained with the benchtop LIFS system, thus demonstrating the great application potential of portable

systems for the assessment of the HD of the SOM of whole soil samples. These results agree with those obtained by Segnini *et al.*[43] who used a portable LIFS system for assessing SOM stability in whole soil samples from permanent and seasonally flooded wetlands in the Peruvian Andes. Results from the portable LIFS showed significant correlation when compared with the benchtop LIFS system (R = 0.94), showing the feasibility of using a portable LIFS system and representing an improvement in speed and convenience for SOM stability evaluation across the landscape.

Coherence and concordance between the results obtained from the proposed calibration model and the HD estimated by the portable LIFS were observed; the relative error between them was lower than 11%.

The use of LIFS is based on the measurement of fluorescence of the whole soil through soil sample excitation with a laser in the violet or blue region (405-458 nm). This procedure privileged the fluorescence excitation of complex structures of OM associated with more recalcitrant part of HS (HA), such as aromatic and heterocyclic structures. Aliphatic structures should not show fluorescence signal in the violet or blue region as well as should not excite less humified structures of HS (fulvic acid).

It is important to report LIFS that generate semiquantitative information about the molecular complexity of SOM and thus provides a relative indication of its humification process. The equipment parameters are adjusted based on the strength of the signal produced by the whole soil samples and thus quantitative assessment are not feasible. Regarding the benchtop and portable LIFS systems used in this work, there were intrinsic differences of the experimental apparatus to generate specific signal intensity. Since spectroscopic techniques give no absolute humification degree, only relative comparisons are possible. The laser intensities used had to be different, for the obvious reasons; therefore, the electrical signals generated by those lasers are expected to be distinct.[43] However, high correlation between systems that use whole soils and solutions is very interesting from an analytical point of view.

Table 2. HD estimated by the calibration model and estimated from the portable LIFS system using the simple linear regression model for the sample of A31 to A36

Sample	Depth / cm	$H_{benchtopLIFS} \times 10^4$ / a. u.	$H_{portableLIFS} \times 10^4$ / a. u.	Error / %
A31	0-10	13.4 ± 1	14.6 ± 0.8	9
A32	10-20	15.3 ± 2	17 ± 2	13
A33	20-40	18.0 ± 2	18 ± 2	0
A34	40-60	21.5 ± 2	21 ± 4	2
A35	60-80	25.8 ± 3	25 ± 3	3
A36	80-100	33.9 ± 4	32 ± 4	6

This study can be a very interesting contribution for analytical chemistry, since this portable LIFS system is well suited to be applied *in situ*, enabling real time evaluation of soil quality through assessing of humification degree.

Conclusions

The performance of a portable LIFS system for assessment of the HD of the SOM was analyzed in this study. The correlation between HD determined using a portable system and that determined by conventional fluorescence spectroscopy methods for humic acid were high (R = 0.85 for A_{465}, R = 0.83 for A_4/A_1 and R = 0.80 for I_{468}/I_{374}), suggesting that whole soil fluorescence with excitation at 405 nm is strongly related to humified structures of SOM. The results obtained from the portable LIFS system agree with the benchtop LIFS system, showing very strong Pearson's correlation coefficient (R = 0.953, $*p < 0.0001$), and the coefficient between the calibration model and the estimates by the portable LIFS system (R = 0.997, $*p < 0.0001$) indicate that the model is well consolidated.

The portable system showed some advantages over conventional fluorescence spectroscopy and benchtop LIFS techniques, because it has been shown to be a fast technique with low cost sample analyses. Moreover, another advantage of the proposed method is to allow direct analysis of soil samples and the possibility to access significant information about the HD of the SOM without the need of chemical fractionation steps, which is also in accordance with the green chemistry concept. However, the main advantage of this new method is the feasibility to be integrated into an embedded system for application in precision agriculture.

Acknowledgements

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