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Spectral laser scanning microscopy – an attempt for an objective determination of the degree of peat decomposition

Elke Bauriegel[1], Sabine Jordan[2], Jutta Zeitz[2], Albrecht Bauriegel[3] and Jörg Rühlmann[4]

[1] Leibniz Institute for Agricultural Engineering Potsdam-Bornim (ATB), Max-Eyth-Allee 100, D-14469 Potsdam, Germany
Phone: +49 (0)331 5699 414, fax: +49 (0)331 5699 849, e-mail: ebauriegel@atb-potsdam.de

[2] Humboldt Universität zu Berlin, Faculty of Agriculture and Horticulture, Institute of Crop Science, Division of Soil Science and Site Science, Invalidenstrasse 42, D-10115 Berlin, Germany
Phone: +49 (0)30 2093 8622 and 9030, fax: +49 (0)30 2093 8369, e-mail: sabine.jordan@agrar.hu-berlin.de, jutta.zeitz@agrar.hu-berlin.de

[3] State Office for Mining, Geology and Natural Resources Brandenburg, Stahnsdorfer Damm 77, D-14532 Kleinmachnow, Germany
Phone: +49 (0)33203 36 691, fax: +49 (0)33203 36 702, e-mail: albrecht.bauriegel@lbgr-brandenburg.de

[4] Leibniz-Institute of Vegetable and Ornamental Crops (IGZ) Großbeeren/Erfurt e.V., Theodor-Echtermeyer-Weg 1, D-14979 Großbeeren, Germany
Phone: +49 (0)33 701 78262, fax: +49 (0)33701 55391, e-mail: ruehlmann@igzev.de

Summary

It is known that the detection of the degree of peat decomposition (DPD) after von Post (1924) is described by a subjective method only providing a basis for a quick determination of the humification rate in the field. Accordingly, the class-variable and discontinuous DPD is influenced by the subjective evaluation of the researchers identifying the peat. For chemical evaluations like the estimate of phosphorus binding forms in fens influenced by DPD, it is suggested that an objective method is needed. Via spectral Laser Scanning Microscopy it is possible to characterise different DPD. Along certain wave lengths the spectra of 22 peat soil samples from fens in north-eastern Germany has been measured. The less humified the peat the higher are the auto-fluorescence intensities in the whole wavelength range. Complete humified peat has a low auto-fluorescence intensity caused by the stability of soil organic matter (SOM).

Key index words: fens, fluorescence, peat humification, spectra, soil organic matter

Introduction

For years, the detection of the degree of peat decomposition (DPD) after von Post (1924; von Post and Granlund, 1926) has been a common 'scientific' method. Like a convention it has been deemed to be representative. The evaluation of the DPD is based on a visual appreciation in the field considering colour, percentage of structured plant residuals and turbidity of the water by squeezing the peat through the fingers.

For chemical evaluations like the estimation of decomposition degree dependent potentials of phosphorus binding forms in rewetted fens (Jordan *et al.*, 2007, Zak and Gelbrecht, 2007) it is obvious we need to measure the DPD with a method that records those (surface) characteristics of peat being determined by humification.

Thus, the method after von Post, which subjectively classifies the DPD in terms of a ten-class scale, could be supported by Laser Scanning Microscopy as an objective and non-classifying method.

At an early stage the primary (or auto)-fluorescence was used for the fluorescence-microscopical determination of coal (Jacob, 1964) and of herbal tissues like roots (Melhuis, 1968) and lignified tissue (Babel, 1964), respectively. Later, fluorescence characteristics of different organic substances in the aquatic as well as in the terrestrial field were used to classify humification and organic material (Chen *et al.*, 2002; Corvasce *et al.*, 2006; Kalbitz *et al.*, 2000, 2005; Le Coupannec *et al.*, 1999; Zsolnay *et al.*, 1999).

In summary, it has been discovered that an increase in aromatic and long-chain compounds and a decrease in aliphatic compounds come along with an increasing degree of humification of SOM. This leads to a decrease in chemical reactions and fluorescence intensities as well as to a shift of the fluorescence emission-maxima from short to long wave spectral range.

Both peat-forming plants and their conversion products, the SOM, comprise fluorescence characteristics. To that effect, the fluorescence intensity should be higher the lesser the peat material is converted (humified).

If this is the case, the grades of degradation and DPD, respectively, should lead to different fluorescence intensities. Accordingly, the auto-fluorescence of the peat would be dependent on the degree of humification of the organic matter and could be measured.

Materials and methods

22 peat samples with different DPD from fens in north-eastern Germany have been chosen. On air-dried and coarsely ground samples the auto-fluorescence was determined by means of the META-detector of the Laser Scanning Microscope LSM 510 (Zeiss, Germany). The principle of measurement of this device is based on the stimulation with different wave lengths of the laser (λ=405 to 633nm). Thus, the emitted fluorescent light of each sample is spectrally partitioned at an optical grid and captured by 32 detectors (http://www.zeiss.de/mikro).

For determining the variance of the measuring device and the samples each of the samples has been measured twice and has been replicated five times. The samples were stimulated by an argon-laser with a wave length of 405 nm. The auto-fluorescence of the single peat samples has been determined in the entire range of emission from 421 to 751 nm, wherein the lowered emission values in the range of 502 to 523 nm could not be considered because of the instrument specific use of the colour splitter at a wave length of 514 nm. To provide for properly illuminated recordings the smallest possible laser power has been used on each single sample, which has been considered as a factor in the calculation of the relative fluorescence units. The median of each real sample repetition was calculated. The average value of the medians of the repeat determination became part of the consideration of the results.

Results

The measured (average) fluorescence intensities have been analysed with reference to site and DPD. Basically, correlations between the fluorescence intensities and the DPD (Fig. 1) have been observed. For example, at the site Kesselwiesen (Schlaubetal/Brandenburg) the poorly humified peat M13 (H3) shows a remarkably higher fluorescence intensity than the completely humified peat M12 (H10).

The results of the site Belzig/Brandenburg show an apparently ambiguous situation (Fig. 2).

Again, a gradation of the fluorescence intensities can be noticed from poorly humified M20 (H4-5) to fairly M19 (H5-6) and completely humified peat samples M18 (H10), respectively. In opposition to that, sample M16, which has been dedicated to the highest DPD, shows the highest fluorescence intensity at the site Belzig. This high intensity is an effect of the recent root penetration. The sample M16 represents the topsoil horizon from 0 to 15 cm below surface level. The recent plant roots insert fresh (non-humified) organic substance into the completely humified peat. Hence, the fluorescence intensity increases in this horizon.

In a site independent and decomposition degree related consideration the basic validity of the hypothesis is shown. Therefore the fluorescence intensities of the single peat samples have been assigned to three groups of decomposition degrees: H3 (poorly humified), H4-6 (fairly humified) and H7-10 (completely humified). Topsoil horizons have

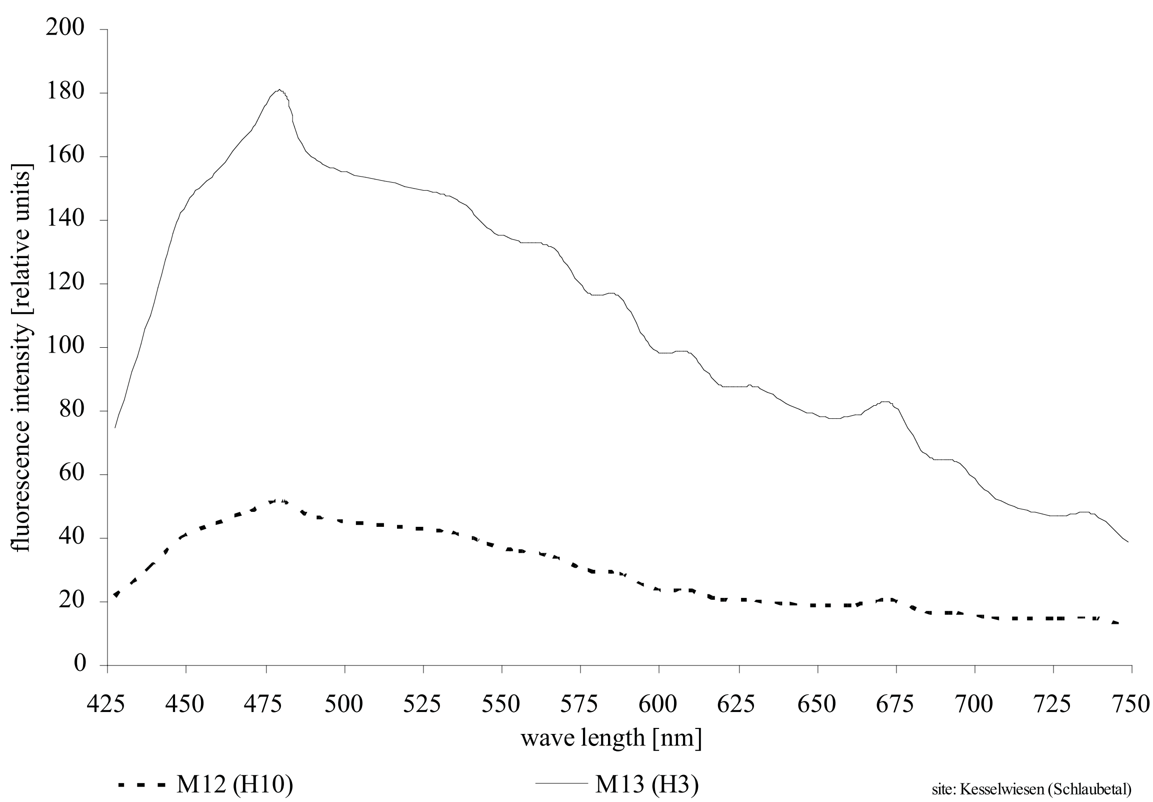

Figure 1. Comparison between the fluorescence intensities of poorly (M13, H3) and completely humified peat (M12, H10) by way of example at site Kesselwiesen (Schlaubetal/Brandenburg).

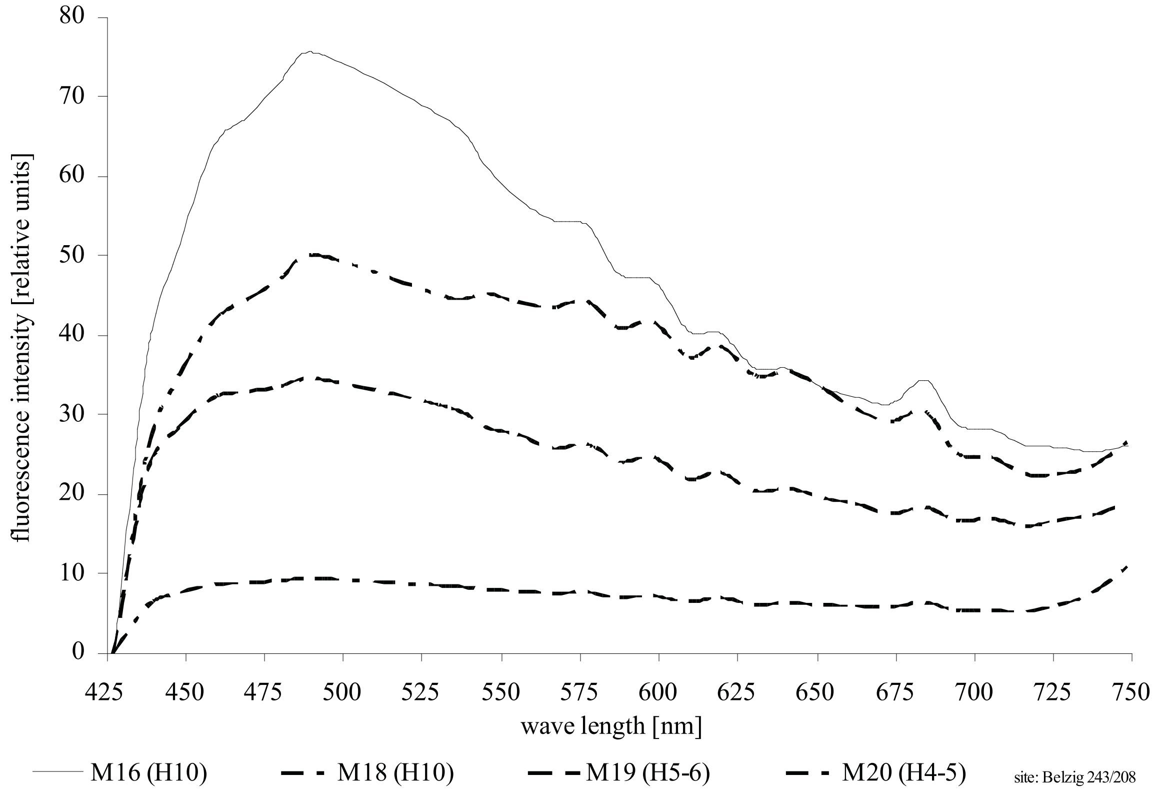

Figure 2. Comparison between the fluorescence intensities of peat soils with variedly DPD by way of example of a peat profile at the site Belzig.

not been taken into consideration, as the fluorescence of the plant roots superimposes the fluorescence intensity of the peat substance. The evaluation of the group average values (Fig. 3, black lines) proves a correlation between the fluorescence intensity and the DPD.

In a first step the influence of the quality of the SOM (DPD) on the characteristics of the fluorescence has been analysed. In a second step the quantity of the SOM was taken into consideration. It could be assumed that beside the recent root penetration intensity the percentage of both SOM and mineral addition affect the fluorescence intensity. For that reason all samples considered have been standardised with reference to their percentage of SOM by means of loss of ignition. The results proved that the relations do not change seriously. However, the intensities of the medium spectra diverge slightly (Fig. 3, grey lines). In relation to the considered samples, this effect significantly emerges in the group of the medium DPD.

Discussion/conclusion

The results show, that the DPD can be represented by the measurements of the fluorescence intensities on the LSM. That seems to prove the qualification of the criteria according to von Post and gives reason to hope for a valid metrological method by means of the fluorescence-spectroscopy. However, a direct evaluation of the single DPD and their dependencies on the fluorescence intensity requires a larger amount of samples. The latter was also necessary to evaluate the degree of subjectivity with reference to the DPD. The intensity of the recent root penetration and the percentage of mineral additions have to be seen as the main disturbances in the determination of the DPD by means of fluorescence intensity. The problem of the mineral additions can be solved methodically by a standardisation of the loss of ignition. The metrological elimination of the recent root penetration is far more problematic. The difficulties appear at first in the form of the identification and division, respectively, of peat-forming and non peat-forming plant residuals. For completely humified peat soils, which actually should not contain recognisable plant residuals any more, non peat-forming materials can be methodically removed. The less humified the peat, the more difficulties can occur to implement the removal of the non peat-forming materials. This fact will be relevant only in pristine peat areas. For most of the agricultural used fens in north eastern Germany completely humified peat soils exist in the effective root area.

Acknowledgements

This research was funded by the Deutsche Forschungsgemeinschaft within the priority programme SPP 1090 and by the Deutsche Bundesstiftung Umwelt within the scholarship programme. We thank Jan Fiedler and Sebastian Rossius for technical support.

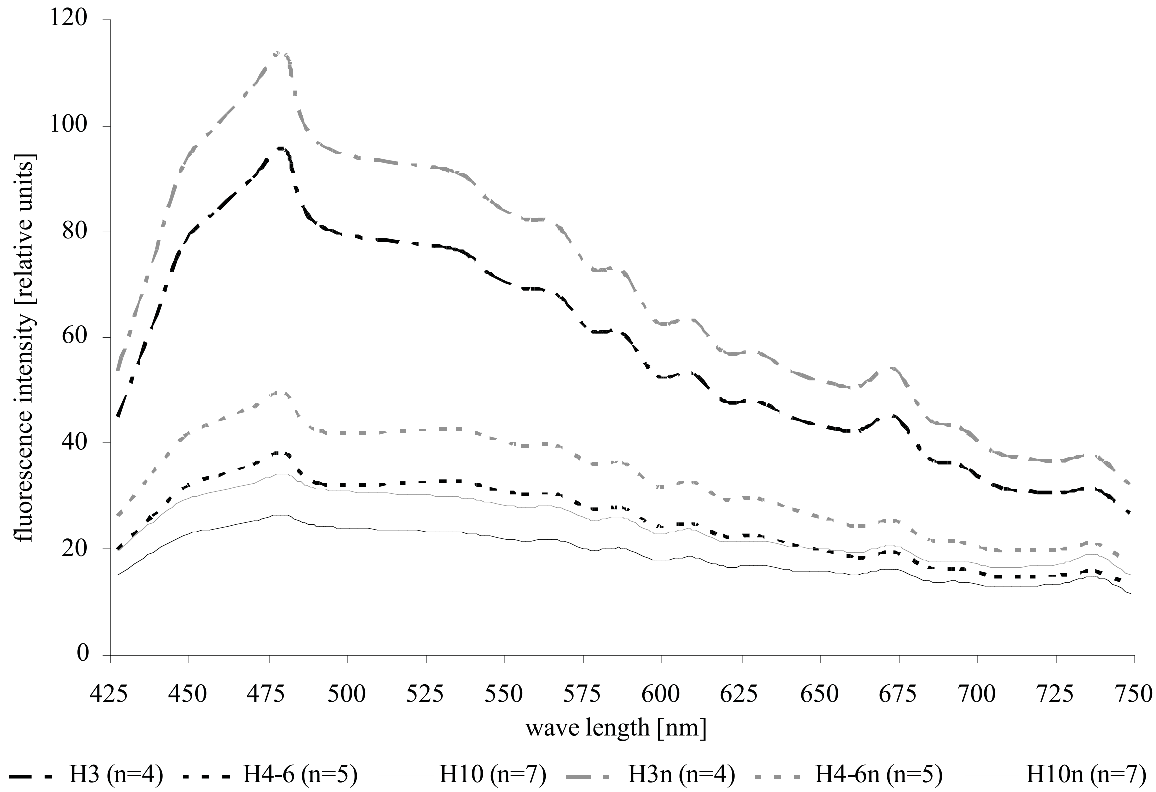

Figure 3. Mean values (black lines) and mean values standardised to loss of ignition (grey lines) of the DPD groups H3 (poorly humified), H4-6 (fairly humified) and H7-10 (completely humified).

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