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Spectral fluorescence variation of pollen and spores from recent peat-forming plants

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ABSTRACT

The fluorescence properties of spores and pollen grains examined under ultraviolet incident light are used to assess the maturity of sedimentary organic matter and may have other applications in relation to recent sediments, in areas such as paleoenvironmental research. In this study pollen grains and spores from 33 species common in peat ecosystems were mounted on a glass slide in accordance with standard palynologycal procedures for recent plants. The main objective of this work was to assess the variability of fluorescence spectra of pollens and spores within a single species or even within a single sample. A minimum of 10 spectra were recorded from each sample and were averaged to obtain a spectrum characteristic of each sample. Both the average scattering and the scattering in different spectral regions were calculated using the standard deviation (SD) and the coefficient of variation (CV). The effect of the preparation techniques was assessed on some samples of Ericaceae taxa. The results indicated similar spectra for alcohol-washed and distilled water-washed samples, whereas the application of an acetolysis solution caused an increase in intensity and a shift to longer wavelengths. The spectra corresponding to the *Sphagnum* spores had the lowest intensity of all the families studied and displayed their maxima at the lowest registered wavelengths. They often showed a peak in the red region of the spectra, causing a larger scatter in fluorescence in this region. This peak is probably the result of wax or cytoplasmic material attached to the exospore. A significant number of Ericaceae taxa had two fluorescing pollen populations: a blue one of high intensity and smaller size and a yellow-orange one of low intensity and larger size. This difference could be related to different degrees of maturity of the pollen grains. In the case of pollen grains of herbaceous, tree and bush plants the largest scatter was found in the tails of the spectra toward the blue and red regions. The decreasing trend of fluorescence intensity with the shift of the spectra toward red was not observed in the pollen and spores of fresh plants. A good correlation was found between the spectral maxima (λ_{max}) and the red-green quotient ($Q_{R/G}$) regardless of the type of plant.

1. Introduction

Pollen grains and spores are ubiquitously found in sediments and have been used in numerous applications including paleoenvironmental studies (determination of plant assemblage in a given place at a given moment — Traverse, 2007), basin formation and evolution (assessment of the distance to the coastline in a basin — Tyson, 1995), and petroleum exploration (determination of the degree of maturity of the sediment based on its color change — Teichmüller, 1986). Their ubiquity is essentially due to the high resistance of the outer layer of the walls known as exine in pollen grains and exospore in spores, which is crucial for the fertilization process in the former and for the survival of the latter under adverse climatic conditions (Brooks and Shaw, 1972). This outer layer, often sophisticatedly ornamented and sculptured (Bedinger, 1992), is the most distinctive feature of each species, and shows strong resistance to chemical and biological decay. The inner layer essentially consists of cellulose and pectin and degrades rapidly during fossilization (Ivleva et al., 2005), whereas a significant part of the chemical composition of the outer layer consists of a complex and highly resistant biopolymer known as sporopollenin (Zetsche and Kälin, 1931). A carotenoid nature has been suggested for sporopollenin (Brooks and Shaw, 1978), the composition of which varies for different species (Guilford et al., 1988). The use of degradative and pyrolysis techniques has revealed *n*-alkanes, *n*-alk-1-enes, α,ω-alkadienes, alkylphenols and benzaldehydes (Davis et al., 1985; Dungworth et al., 1971; Schenck et al., 1981; van Bergen et al., 1995) to be the main structural moieties of sporopollenin in different proportions. The composition of

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sporopollenin varies with the degree of fossilization which gives rise to different chemical and structural modifications (Yule et al., 2000). These modifications are reflected in the color change of the spores and pollen from pale yellow through orange, reddish brown to finally black with increasing temperature due to the depth of burial. The systematic changes have been widely used as a thermal indicator through either qualitative (Fisher et al., 1981; Pearson, 1982; Staplin, 1969) or quantitative scales (Marshall, 1991; Ujiié, 2001; Yule et al., 1998). The color transformation with increasing maturation corresponds to a decrease in O and H and an increase in the percentage of C, which is translated into a significant reduction of aliphatic groups and an increase in aromatic C═C bonds in the Fourier Transformed Infrared (FT-IR) spectra (Yule et al., 2000). The subtle changes in color in the immature stage correspond to a reduction of carbonyl/carboxyl groups and a relative increase in aliphatic $-CH_2-$ and $-CH_3$ groups. Since the early studies of van Gijzel (1963), it has become clear that the maturity transformations observable in spores and pollen under transmitted white light also have an effect on their fluorescence properties (van Gijzel, 1967a). The fluorescence phenomenon is due to the presence of "fluorophores" and "chromophores" (Lin and Davis, 1988) in a molecule. These possess electrons, which are susceptible to being excited by incident light to higher levels of energy, emitting fluorescence light when they return to their basal stage. FT-IR studies suggest that aliphatic unsaturated C═C double bonds and conjugated C═C double bonds adjacent to C–O groups are responsible for spore and pollen fluorescence properties (van Gijzel, 1971; Yule et al., 2000). Although the first studies on spore and pollen fluorescence were performed using palynological slides (Berger, 1934), it was soon acknowledged that polished pellets and blocks could also be used and therefore the same preparations as for vitrinite reflectance measurements could be employed. Moreover a good correlation was established between the maturity of the source rock determined from the bulk chemical results or by the vitrinite reflectance measurements and the spectral fluorescence properties of sporinite (Jacob, 1964; Ottenjann et al., 1982; Robert, 1979; Teichmüller, 1986; van Gijzel, 1967b). In general it has been found that the spectra decreased in intensity and shifted to a reddish color with increasing maturity.

The most widely used parameters extracted from the fluorescence spectra of sporinite are the wavelength of the spectral maximum (λ_{max}) and the red/green quotient ($Q_{R/G}$). Other parameters such as the total emission flux (F), the quotient between the areas at higher and lower wavelengths than 530 nm (Pradier et al., 1988), the area corresponding to the different spectral colors (Crelling, 1983), and the chromaticity coordinates using the CIE (International Commission on Illumination) chart (Hagemann and Hollerbach, 1981) have been used in various attempts to differentiate numerically the spectroscopic curves. The differences between the fluorescence spectra of different pollen/spores species tend to decrease with increasing age of the sediment (Van Gijzel, 1967b). This, apart from the difficulty of distinguishing between the various species in polished blocks, might be the reason why the fluorescence spectra of sporinite are recorded all together in maturity studies. Nevertheless, the spectra of various species in sediments of the same age have been differentiated and significant differences have been found between *Sphagnum* spores and pollen grains of the same age in peats (Van Gijzel, 1967b). Other aspects, which affect the fluorescence spectra, are acid treatment during the preparation of the samples (Mendonça Filho et al., 2010; Van Gijzel, 1967b), environmental changes (Yeloff and Hunt, 2005) and differential alteration and corrosion of the grains (Havinga, 1967).

The aim of the present study is to record the natural variation of fluorescence spectra from pollen and spores of peat-forming plants as a first step to study their characteristics and variability in peat deposits. Fluorescence spectra can record the effects of alteration caused by prolonged dryness or microorganismal action providing additional and highly useful paleoenvironmental information (Yeloff and Hunt, 2005).

2. Experimental

Strew mounts from pollen and spores (44) of 33 different species of peat forming plants, common in the peat bogs of northern Spain have been used in this study. Most of the *Sphagnum* samples were provided by the VIT Herbarium of the Natural Science Museum of Alava (Vitoria) while the other peat-forming plants were provided by the Faculty of Biology (BOS department) of the University of Oviedo. The samples were prepared in their respective storage locations. The *Sphagnum* spores from the VIT Herbarium were hand-picked from the capsule and spread on glass slides. The BOS samples were acetolyzed using the method devised by Erdtman (1960) to remove the cytoplasmic and cellulosic material in order to improve the transparency of the specimens.

The samples for microscopic examination were fixed with Kaiser's glycerol gelatin, after first making sure the fixer was free of fluorescence. The preparation technique used ensures that most of the grains are far enough apart inside the gel, to minimize the effects of different orientations or the presence of folded specimens in the fluorescence spectra.

An incident light optical microscope equipped with a LED light source, a combination of filters to provide ultraviolet illumination (excitation filter BP 360/40, dichroic mirror 400, suppression filter 470/40) and an oil immersion objective (50×) was used to record the spectra. Intensities were recorded in the 420–750 nm range at 5 nm intervals and were corrected for background noise. In order to be able to compare the intensities of the spectra a green 2941B007 standard with maximum intensity at 527 nm was employed. The maximum intensity of the standard was considered to be 1, and after measuring the standard through the optical system a factor was introduced to correct the spectral intensity of each spectrum. Fig. 1 shows the expected and recorded values of the standard. The spectra were only corrected for intensity. As the pollen and spore spectra cover a wider wavelength range than the certified values of the standard, no other functions for shape could be applied. Nevertheless comparison of the recorded and expected results for the standard indicated that a minor alteration of the spectra due to the transmittance of the optical system was to be expected, except for a small shoulder at 565 nm that was identified in the fluorescence spectra. The equipment also recorded images of the same specimen where the spectrum was taken. At least 10 spectra were recorded from each slide and they were averaged to produce a representative spectrum of the sample. The following spectral parameters – λ_{max} (spectral maximum), $Q_{R/G}$ (red green quotient) calculated as the intensity at a wavelength of 650 nm divided by the intensity at 500 nm, Q+ (Intensity of the spectral maximum divided by the intensity at 500 nm) and I_{max} (maximum intensity) – were used to compare the characteristics of the spectra. Additionally the standard deviation (SD) of the averaged intensity at each measured wavelength was calculated for each sample. As the intensities of the spectra were rather different and the SD is affected by the actual

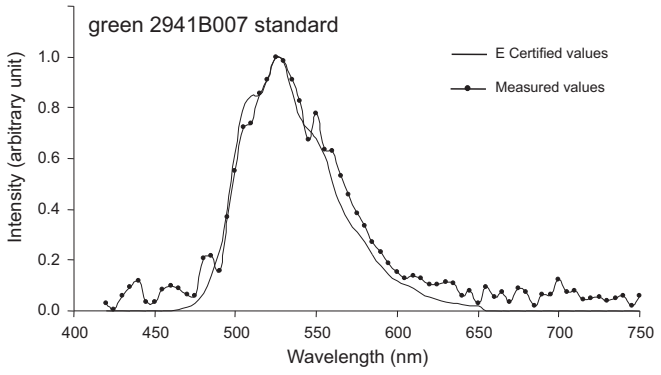

Fig. 1. Certified values and obtained values of the fluorescence standard 2941B007 used in this study.