

Fluorometric depth-profiling of chlorophyll corrected for yellow substances

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Summary:

The 'spectral groups' of algae (green, blue, brown, mixed) are each characterized by a specific composition of photosynthetic pigments, particularly relevant are: Chl a, phycocyanobilin, phycoerythrobilin, fucoxanthin, peridinin and, consequently, by a specific excitation spectrum of the Chl fluorescence. This was used in earlier approaches to determine the amount of chlorophyll and the algal group composition of phytoplankton. Yellow substances (coloured dissolved organic matter) may interfere with the measurement because of overlap in the excitation spectra with phytoplankton. In a new approach, built into a submersible instrument, we correct for the influence of yellow substances on the chlorophyll fluorescence.

The newly-developed probe is a submersible fluorometer which measures the emission intensity at six characteristic wavelength ranges employing pulsed light-emitting diodes. The submersible probe transfers all data on-line to a computer or stores them in the probe (fluorescence data plus the simultaneously measured water pressure for depth determination). The six-point excitation spectra are deconvoluted on the basis of norm spectra which have been obtained by analysis of several species of each spectral group. The usage of an uv-excitation source (370 nm LED) enables the differentiation between algal fluorescence and fluorescence of yellow substances.

Introduction:

In waters with a large content of organic compounds, determination of chlorophyll fluorescence as a measure of chlorophyll-concentration and as an indicator of phytoplankton quality can be perturbed by fluorescence of yellow substances. Traditionally chlorophyll-a fluorescence is measured at emission wavelengths around 685 nm (PSII-fluorescence) with various excitation wavelengths due to different absorption spectra of light-harvesting antenna pigments. According to the composition of the peripheral antenna pigments it is possible to sort phytoplankton to spectral algal groups (figure 1).

Yellow substances are part of the Dissolved organic carbon (DOC). DOC is organic matter which passes through filters with a pore size of 0.45 µm. Yellow substances is DOC which absorbs in the UV and blue spectral wavelength range. Yellow substances can be split up into two major groups - humic substances and non-humic substances, where by the lignins are prominent representatives.

Most organic material originates from land plants and soil. This means that freshwater lakes and coastal areas of the sea contain much more organic matter than water at the open sea.

Another characteristic of yellow substances is their fluorescence emission in the blue-green wavelength region. The emission spectra of yellow substances are not in a like a narrow band rather the emission is over in a wide wavelength range. Consequently, this includes the red wavelength region and this we made use of.

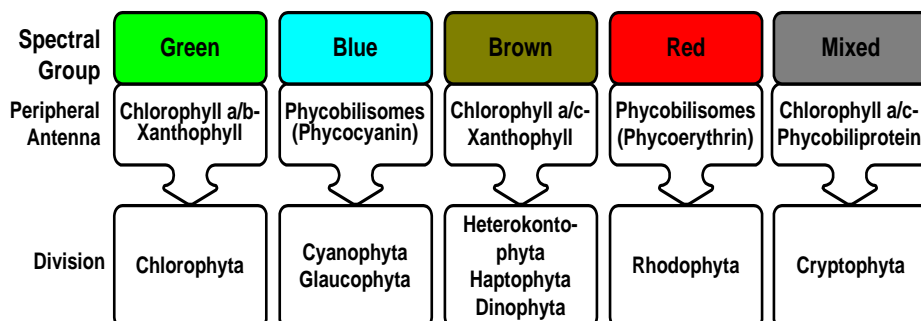


Figure 1: Assignment of several algal divisions in spectral groups

As a result of the pigment composition of the peripheral antennae contributing most fluorescence excitation spectra five main spectral groups can be recognised. In the 'Green Group' the peripheral antennae consist of chlorophyll-a, -b and xanthophyll. The phycobilisomes of the 'Blue Group' function as peripheral

antennae (mainly phycocyanin). The members of the 'Brown Group' contain chlorophyll-a and -c and xanthophyll (often fucoxanthin or peridinin). The peripheral antennae of the 'Red Group' are composed of phycobilisomes, as in the 'Blue Group'. But the phycobiliprotein phycoerythrin dominates in the 'Red Group' instead of the phycocyanin. However the 'Red group' generally plays an unimportant role among the planktonic. The 'Mixed Group' has a special pigment composition. Here there is a combination of chlorophyll-a, -c, with one phycobiliprotein which can be either phycoerythrin or phycocyanin. In this work just the phycoerythrin-containing members of the 'Mixed Group' are considered.

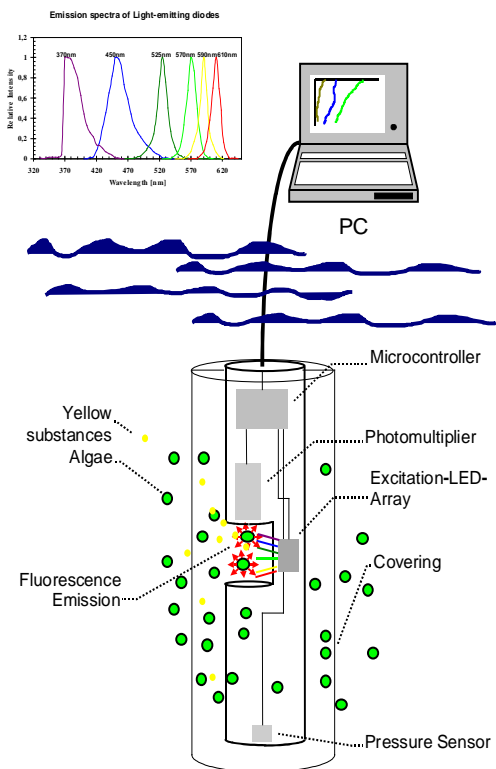


Figure 2: Set-up of the submersible fluorometer

Algal chlorophyll-a and yellow substances are excited with light of six LEDs (emission wavelength 370 nm, 450 nm, 525 nm, 570 nm, 590 nm, 610 nm, see inset for emission spectra). The LEDs are switched alternately by a microcontroller. Chlorophyll-a fluorescence with wavelengths between 690 nm and 710 nm is detected using a photomultiplier and the data is sent to the microcontroller. Data can be stored in the probe or transferred via RS 485 to a PC. A covering prevents the incidence of direct sunlight which could cause measurement perturbations.

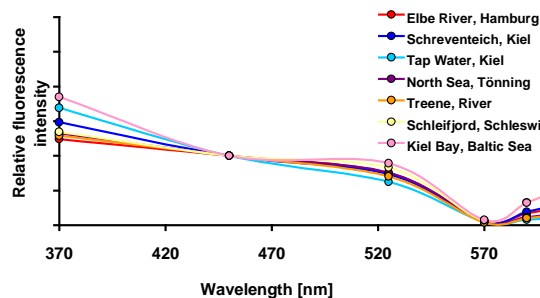


Figure 3: Fluorescence-offsets of natural water-samples

Fluorescence excitation spectra of different natural water samples filtered through glasfibre filters Whatman G/F (pore size 0.7 μm) were recorded using the set-up shown in figure above. Samples were collected from the surface (except tap water, Kiel) of the sampling location from different sites in Northern Germany. Spectra are normalised to fluorescence intensity at 450 nm. All spectra have similar characteristics and show high fluorescence intensity if excited by 370 nm light.

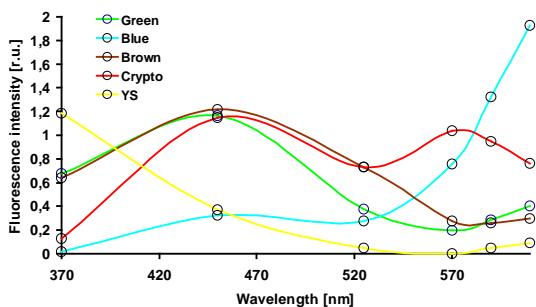


Figure 4: Norm spectra of spectral algal groups and yellow substances

Mean fluorescence intensity of four spectral algal groups and yellow substances at six excitation wavelengths in digits (photovoltage at the photomultiplier) normalised to chlorophyll-a concentration ($\mu\text{g L}^{-1}$) for spectral algal groups. A high signal is found at the 610 nm LED in the blue group. This is caused by phycocyanin. In addition the 'Brown Group' (here diatoms and dinoflagellates) has a high signal in the green wavelength region (525 nm) because of fucoxanthin (Diatoms) and peridinin (Dinoflagellates). High fluorescence intensity caused by phycoerythrin was found for the Cryptophyta (cryptomonas) at 570 nm. The shown spectrum of yellow substances in this case a sample of the Plußsee in Northern Germany from a depth of 1 m filtered with glas fibre filter Whatman G/F (pore size 0.7 μm). The characteristic fluorescence in the uv-region is obvious.

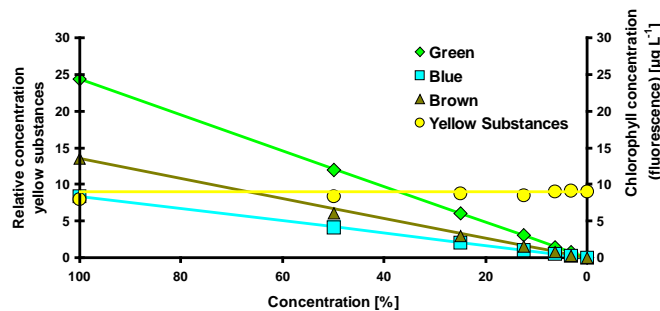


Figure 5: Dilution experiments

Fluorescence measurements were made of samples from a dilution experiment on algae and yellow substances.

Water of river Elbe (sample taken at Hamburg) was filtered with filter Whatman G/F (pore size 0.7 μm) fluorescence excitation spectrum was taken with set-up shown in Figure 2. Algae were added to the filtered water. Fluorescence excitation spectrum of this sample was determined and amount of chlorophyll and relative amount of yellow substances was calculated. This sample represents the 100%-concentration. The sample was then diluted to lower chlorophyll-concentrations by adding filtered water. Fluorescence measurements of the diluted samples show that the share of yellow substances was detected properly.

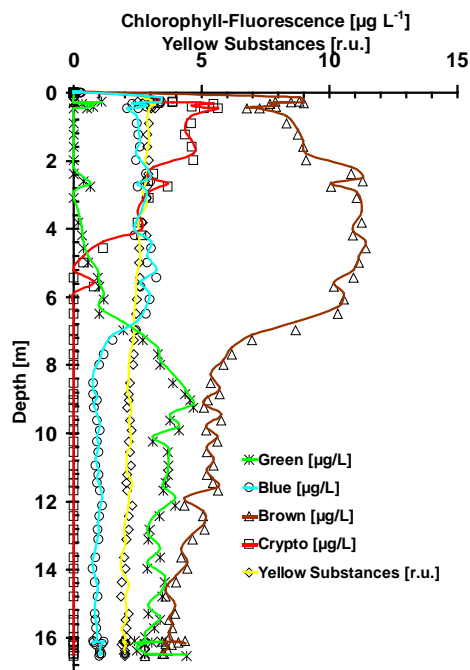


Figure 6: Depth profile of deconvoluted fluorescence excitation spectra of the Plußsee taken with the submersible instrument.

The fluorescence spectrum of filtered water from 1 m depth was taken as a yellow substance fingerprint. Fingerprints of algae shown in figure 2 were employed as norm spectra for the detection of spectral algal groups. The algae were stratified in this example. Fluorescence of yellow substances is distributed constantly with depth.