Laser Remote Sensing of Gelbstoff and Chlorophyll a on the Wadden Sea Surface

T. Hengstermann, R. Reuter, H. Wang and R. Willkomm

Oldenburg University Physics Department
P.O. Box 25 03 - 2900 Oldenburg, Germany

ABSTRACT

Nutrients are necessary for the growth organisms in the Wadden Sea. However, a surplus of nutrients increases algae growth, my lead to oxygen deficiency and the death of marine organisms. In order to find a possibility of indicating the nutrients through chlorophyll a fluorescence of diatoms on the Wadden Sea surface, qualitative studies of fluorescence of water and sediment in an artificial Wadden Sea have been performed by taking samples and measuring in situ laser-induced fluorescence. It is shown that Gelbstoff and chlorophyll a are two dominant constituents of the Wadden Sea which correlate with the fluorescence. Meanwhile the structure of the fluorescence of chlorophyll a can be used to distinguish the wadden Sea surface with a diatom canopy from that without diatoms.

INTRODUCTION

The Wadden Sea is an area of tidal flats of the North Sea extending along the coasts of Denmark, Germany, and the Netherlands. It is a transiction zone between land and sea where complex environments and dynamic processes form a unique area of natural beauty and ecological importance.
In order to investigate oceans and coastal waters, remote sensing has been used intensively for fifteen years. Satellite data processing of the Wadden Sea was already mentioned by Doerffer (Doerffer, 1989). Approaches were made at the University of Oldenburg to interpret aerial imagery and data acquired with the Oceanographic Lidar System (OLS) (Reuter et al, 1986). However, no positive results have been obtained with laser fluorosensor so far.
From the study of data acquired with OLS, it is obvious that systematic spectral measurements of substances specifically present in the Wadden Sea are necessary if lidar is applied to investigate this coastal area. In this paper, laser fluorescence spectra of water and sediment in the Wadden Sea are presented and first results of in situ measurements, acquired with a mini - laser fluorosensor in an artificial Wadden Sea, are described.
Gelbstoff and chlorophyll a are studied because, on the one hand, they are relevant parameters which dominate the fluorescence spectra of Wadden Sea water and sediments. On the other hand, chlorophyll a mainly consists of diatoms correlating with the efflux of nutrients from the sediment (Hopner et al, 1983).
As shown in Fig. 1, nutrients reach the Wadden sea via river run-off, atmosphere, lands and the North Sea. These nutrients are necessary for the growth of organisms, however, a surplus of the nutrients increases algae growth, may lead to oxygen defficiency and the death of organisms. It is expected that information about the diatom distribution on the Wadden Sea surface is a useful parameter to speculate about the current status of the Wadden Sea ecosystem, or, strictly speaking, the nutrient efflux on the Wadden Sea surface.

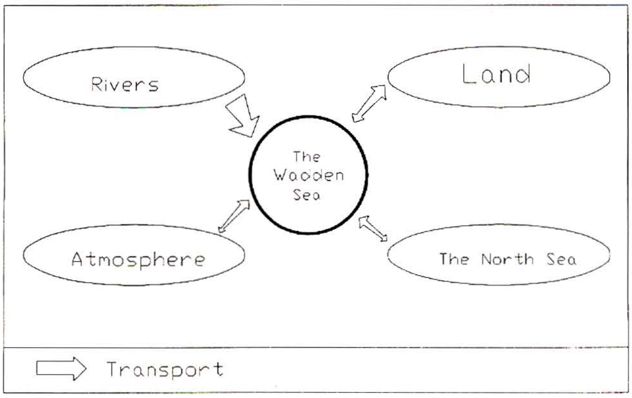

Fig. 1 - Exchange of nutrients between the Wadden Sea and its environments. Surplus of nutrients may lead to oxygen deficiency and death of the organisms in the Wadden Sea.

1. IN VITRO STUDIES OF WATER AND SEDIMENT IN THE WADDEN SEA

Fluorescence spectra of water and sediment in the Wadden Sea considerably change during the tidal cycles when compared to those in open sea. This kind of change could be representatively shown in Fig. 2. Fluorescence spectra of open seawater usually show two peaks, one broad peak for Gelbstoff and the other more narrow one for water Raman. There exists also chlorophyll a fluorescence if algae are present in the seawater. Gelbstoff fluorescence in coastal water and the Wadden Sea water is generally strong due to its high concentration. Compared to Gelbstoff fluorescence, water Raman and chlorophyll a fluorescence are relatively small and often not detectable. The Raman signal disappears in the fluorescence of Wadden Sea sediment and, instead of this, chlorophyll a fluorescence becomes more obvious. It is even dominant if diatoms grow on the Wadden Sea sediment.

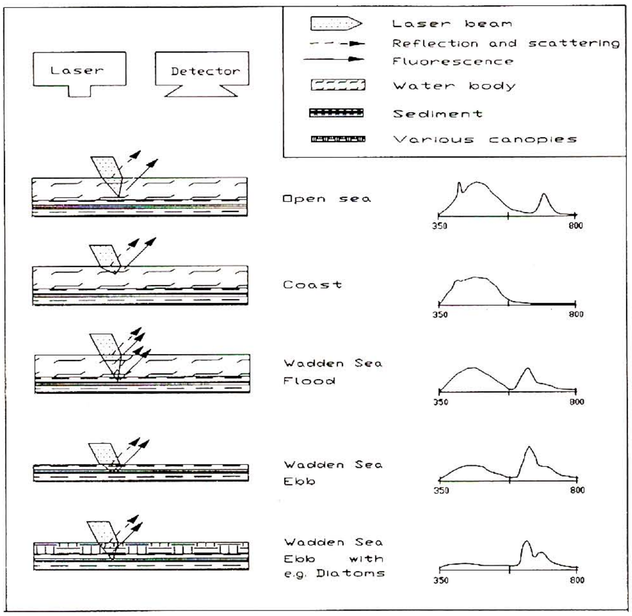

Fig. 2 - Simplified representations of the Wadden Sea and their related characteristic fluorescence spectra.

1.1 Sampling

The water and sediment samples were taken from an artificial Wadden Sea inside a glasshouse laboratory in which three basins were filled with Wadden Sea sediments. These basins can be controlled to simulate the conditions which daily occur in the Wadden Sea along the North Sea coast. Each basin, as shown in Fig. 3, has a size of 1.3 m x 1.3 m x 0.5 m and simulates different culture conditions (e.g. nutrient depression, without macro organisms, etc.).

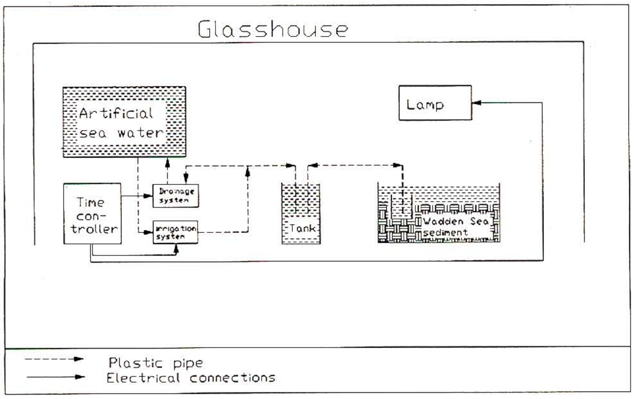

Fig. 3 - Principal diagram of the artificial Wadden Sea in a glasshouse laboratory (From Prof. Hopner, Department of Biology, University of Oldenburg).

Sediment samples were collected with glass containers (plate) which were put into the Wadden Sea sediment during flood and were taken out immediately after the flood.

Samples of various diatoms were made available by the Department of Biology, University of Oldenburg. Some mixed samples of sediments and diatoms were also prepared and placed into a light field to stimulate the growth of diatoms.

Purified water was taken from a SeraDest SP100. Artificial seawater was prepared according to the designation D1141-75*.

1.2 Measurement

A Perkin Elmer Luminescence Spectrometer LS5O was used for measuring fluorescence spectra of water samples. In order to measure fluorescence spectra of sediment covered with water, the set-up of the sample compartment was modified to obtain an excitation light beam downward, and to collect the fluorescence upwards. An UV cut-off filter and a 337 nm band pass filter were used to avoid the second order effect of the grating, and to reduce the stray light due to scattering at the excitation wavelength.

The depth of water coverage on the sediment samples was roughly estimated from the time elapsed between sampling and measurement.

* This specification is under the jurisdiction of ASTM Committee D-19 on Water and is the responsibility of Subcommittee D19.02 on General Specifications and Technical Resources.

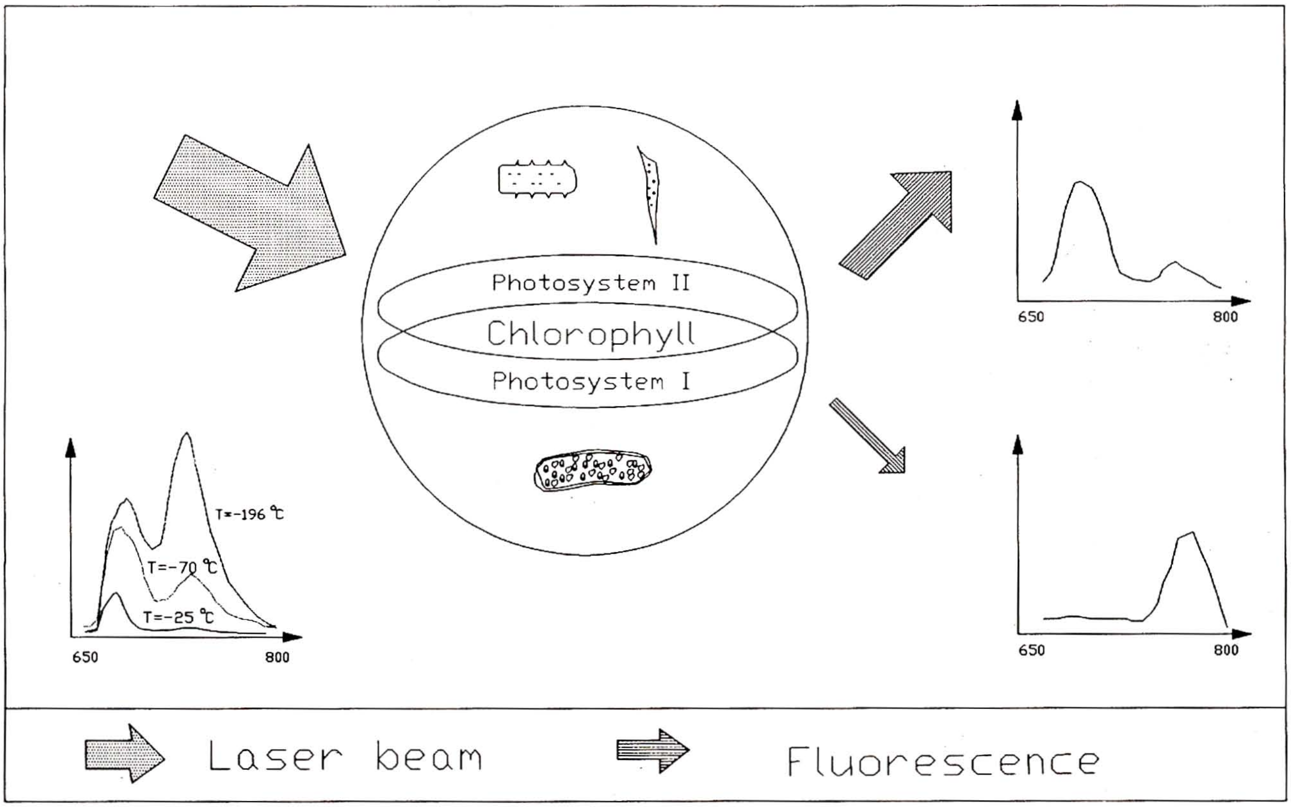

Fig. 4 - Typical fluorescence spectra of the Wadden Sea water, North Sea water, pure water, Wadden Sea sediment, diatomsion and diatoms on the surface of the Wadden Sea sediment.

1.3 Results

The most obvious difference between fluorescence spectra of the artificial Wadden Sea and of open seawater is the relative contribution of water Raman scattering at 381 nm. The Raman intensity becomes small and even disappears if water close to the shore line is considered (Fig. 4). However, water Raman is a very important parameter for the interpretation of laser fluorosensor data (Bristow, et al, 1980). This means that the water Raman normalization technique generally used in quantifying fluorescent matter in the laser remote sensing can not be applied directly to the Wadden Sea.

Compared with the fluorescence of hydrographic open seawater, Gelbstoff fluorescence around 430 nm of the Wadden Sea becomes stronger. This can be attributed to the high concentration of Gelbstoff in the Wadden Sea. The increased Gelbstoff fluorescence is also the reason why the Raman signal is not detectable in the fluorescence spectra of the Wadden Sea.

Referring to Fig. 4, there usually exists a chlorophyll a fluorescence peak around 685 nm (F_{685} for short) in the fluorescence spectra of open seawater. Another chlorophyll a fluorescence peak at 735 nm (noted as F_{735}) is observed in fluorescence spectra of the Wadden Sea sediment. The second peak becomes bigger if the concentration of chlorophyll a in sediment increases, e.g. in case of diatoms growing on sediment.

As shown in Fig. 5 and 6, chlorophyll a has both fluorescence peaks as stated above, which are attributed to photosystem I and II, respectively (Murata et al, 1986). Due to the big difference of quantum yield of chlorophyll a fluorescence at wavelengths 685 nm and 735 nm (Beddard et al, 1979), the first peak is so strong that the second one can hardly be seen if low concentrations of chlorophyll a as typically found in open sea are concerned.

Because of the interaction of the photosystems, and the self-absorption of chlorophyll a around its absorption band at 680 nm, chlorophyll a reabsorbs its fluorescence

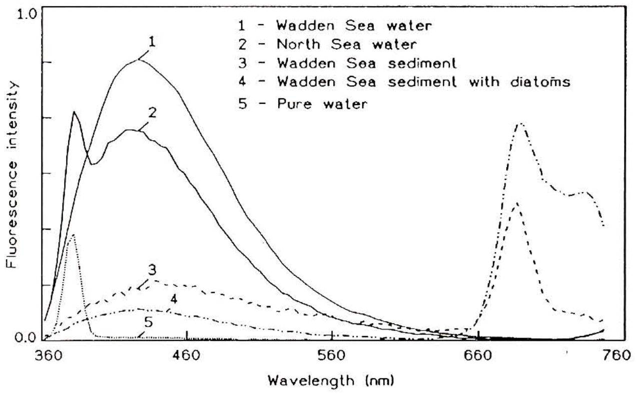

Fig. 5 - Fluorescence spectra correspond to photosystem I and photosystem II at various temperatures T (Gregory, 1989, Strasser et al, 1977). The fluorescence of photosystem I at room temperature and low chlorophyll a concentrations is not obvious when compared to that of photosystem II.

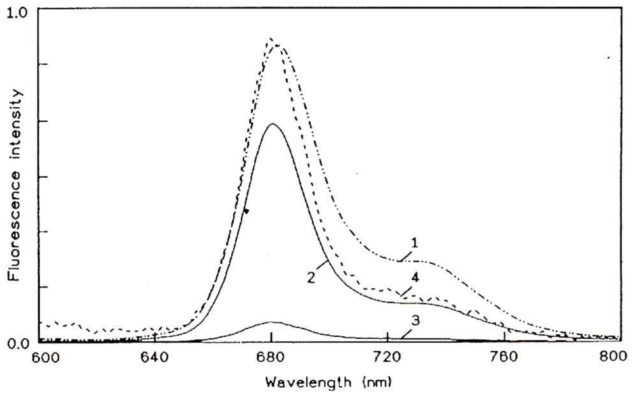

Fig. 6 - Variation of diatoms fluorescence at 685 nm and 735 nm due to change of chlorophyll a concentration. The concentration of diatoms increases from curves 1 to 3. Curve 4 is the curve 3 amplified by factor 50.

around 685 nm and shows strong fluorescence at 735 nm if concentrations become high (Belanger et al, 1988, Lichtenthaler, 1986). Very strong diatom canopy on the Wadden Sea surface is one example of such conditions. This means that F_{735} increases if the concentration of chlorophyll a in diatom increases. Compared to F_{735} F_{685} increases relatively slowly if the concentration of chlorophyll a increases. This indicates a possibility to use the ratio F_{735}/F_{685} to estimate the chlorophyll a content in Wadden Sea sediments, as it is usually done when monitoring green plant stress (Lichtenthaler, 1988).

Fig. 7 schematically shows that diatoms grow very quickly when imigrating from a deeper sediment layer to the surface if adequate conditions of light, temperature, and moisture content are provided (G. Cadee, 1984).

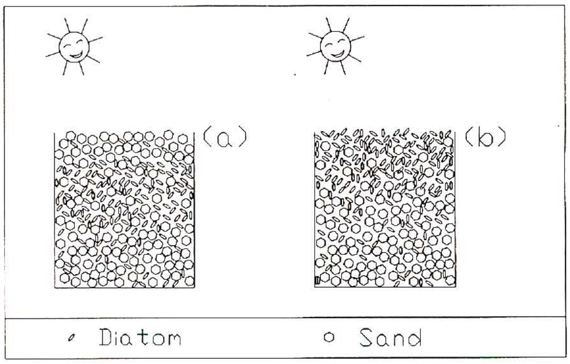

Fig. 7 - In order to get light, diatoms move to the surface of the Wadden Sea and grow very quickly on their way to the surface. This results in a diatom canopies on the Wadden Sea surface which correlate with the nutrients supply and can be investigated by the fluorescence spectroscopy technique.

Water layers on the sediment surface change the fluorescence signature essentially. As shown in Fig. 8, there is a tendency that increasing water coverage increases Gelbstoff fluorescence. Whereby chlorophyll a fluorescence is decreasing. In an extreme case the Gelbstoff fluorescence can be so dominant that chlorophyll a fluorescence is virtually disappearing. This suggests a way by which a fluorescence relationship between Gelbstoff and chlorophyll a fluorescence can be estimated to classify the Wadden Sea according to the depth of water coverage on sediment.

Other pigments besides chlorophyll a in the Wadden Sea can be revealed by changing the excitation wavelength (Yentsch et al, 1979). It is expected that non-chlorophyll a pigments can also be used as parameters for remote sensing of the Wadden Sea, as it was shown for the open sea (Hoge et al, 1986).

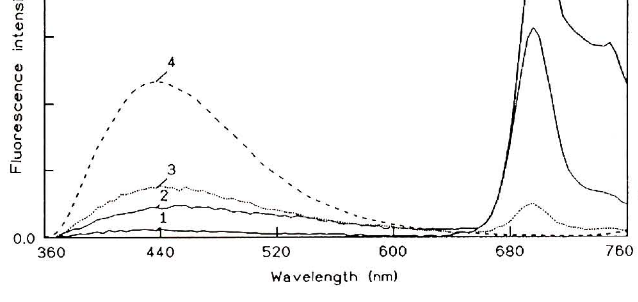

Fig. 8 - Influence of water quantity on fluorescence of the Wadden Sea sediment. The quantity of water on the sediment increases with the sample number.

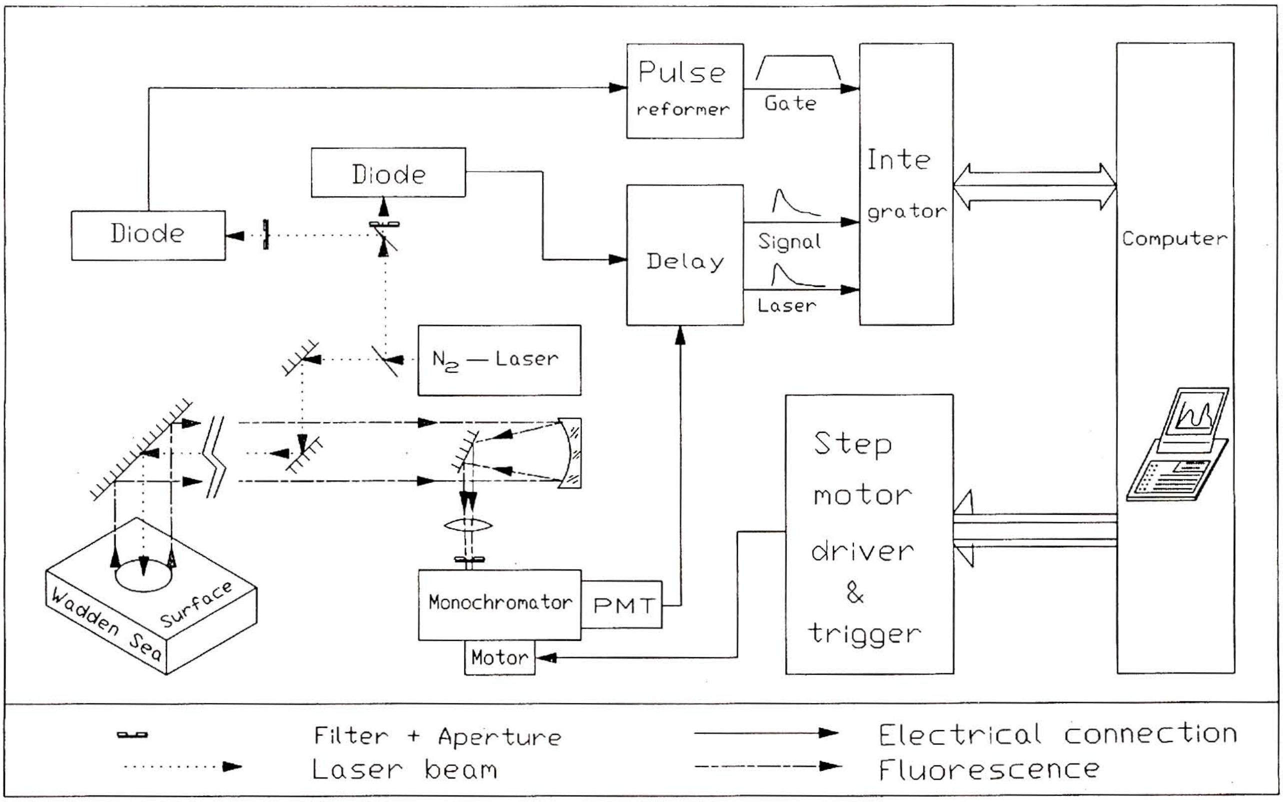

Fig. 9 - Block diagram of a Mini - N2 laser based fluorosensor (MiLF).

2. REMOTE SENSING MEASUREMENT

2.1 Instrument description

A mini - nitrogen laser based fluorosensor (MiLF) is partly developed to get field fluorescence spectra of Wadden Sea water and sediment. The MiLF, shown in Fig. 9, is mainly composed of a N_2-laser with 2 megawatt peak power and 0.6 ns pulse length, a Newton telescope, a monochromator, a LeCroy integrating A/D converter and a computer.

The N_2-laser beam is directed to a target on the artificial Wadden Sea surface to induce fluorescence in a group of substances. Fluorescence collected by the telescope is conveyed into the monochromator which is coupled with a photomultiplier. The photomultiplier signal is transferred to an A/D converter which is synchronized by a trigger signal from a photodiode. The system is equipped with a portable computer whose main task is to acquire and process data, to trigger the laser and to control the monochromator with wavelength setting in a range of 350 nm to 750 nm.

In the experiment a technique of multisample summation based on variance analysis has been used to get the best signal to noise ratio (H. Wang et al, 1991).

2.2 Results

The experiment was performed with the MiLF described above in the artificial Wadden Sea in a glasshouse. Additional fluorescence spectra were measured on sediment samples which were taken from the East Frisian Wadden Sea near Wilhelmshaven.

Almost the same results as those mentioned above can be obtained from the samples described above if the MiLF is used. With this instrument it was possible to measure fluorescence of the Wadden Sea without disturbing its living conditions. Fig. 10 shows fluorescence spectra of the artificial Wadden Sea during tide. The correlation between Gelbstoff fluorescence and chlorophyll a fluorescence is once more confirmed here.

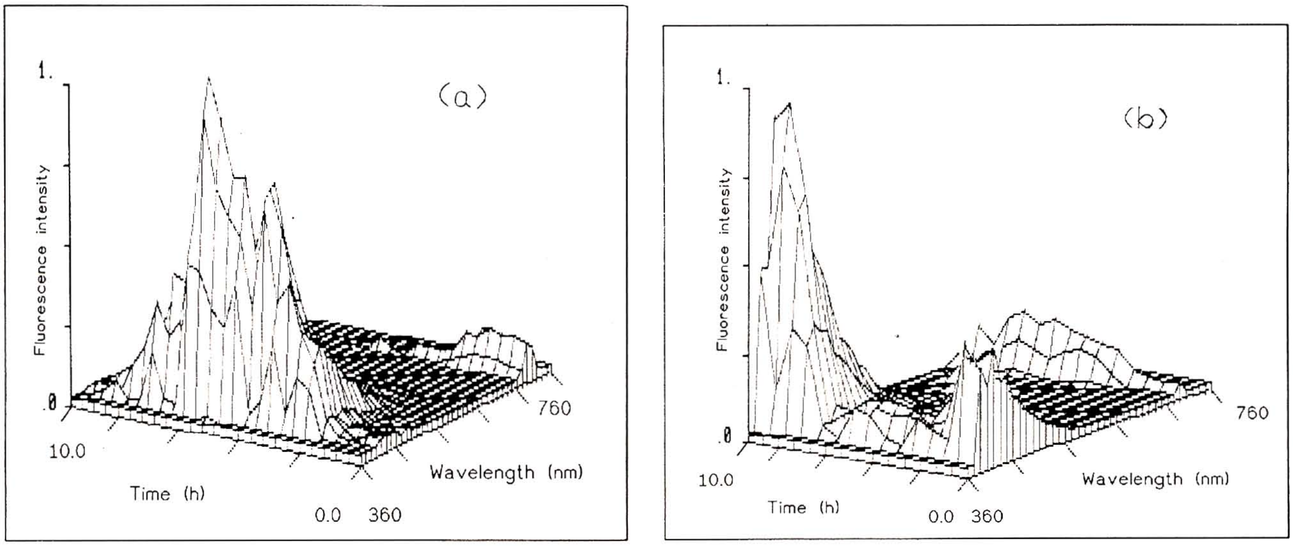

Fig. 10 - Fluorescence spectra of an artificial Wadden Sea during cycles of ebb - flood - ebb (a) and of flood - ebb - flood (b).

Due to high Gelbstoff concentrations in the water of the Wadden Sea a straightforward classification of water on different Wadden Sea surfaces is not obvious (see Fig. 11). The fluorescence spectra shown in Fig. 11 are normalized to the Gelbstoff fluorescence maximum to show the differences in shape; the intensity change alone is not stable enough to be used as a classification parameter.

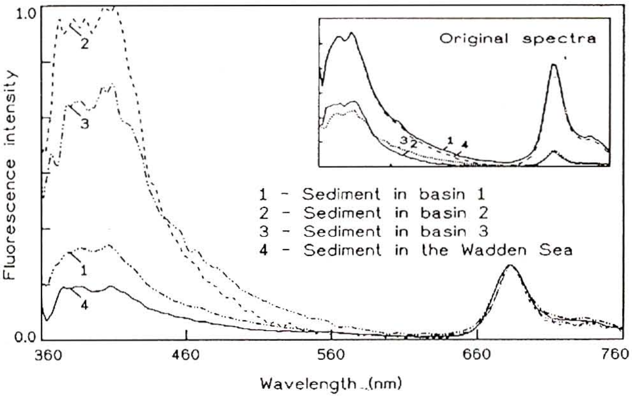

Fig. 11 - Fluorescence spectra of water in the different artificial-Wadden Sea (normalized with fluorescence maximum of Gelbstoff).

Fig. 12 shows that a classification of the Wadden Sea surface with thin water coverage is difficult. There exist very big differences between sediment fluorescence spectra which are normalized to the chlorophyll a fluorescence maximum. However, an obvious correlation between sediments with water coverage and fluorescence spectra is not found so far.

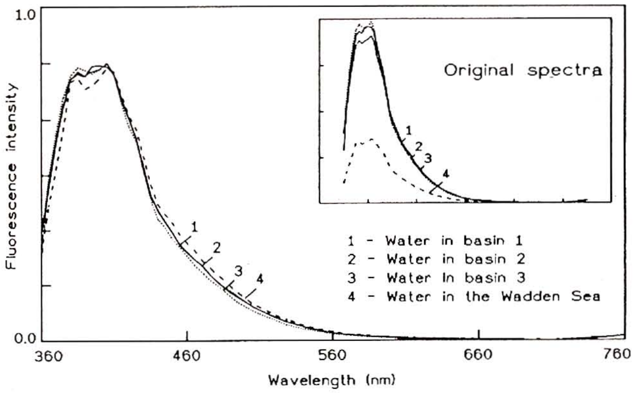

Fig. 12 - Fluorescence spectra of sediments with water cover in the different artificial Wadden Sea (normalized with fluorescence maximum of chlorophyll a).

A classification of the sediment with very thin water coverage is possible by multivariate statistics, which extract useful information from several variables. In the present case 40 variables were selected from fluorescence intensities at various wavelengths, weight centers of spectra, integrations of fluorescence intensities between two wavelength bands and ratios between two variables. Corresponding analysis and clustering analysis are performed with these variables (Davis, 1973).

Fig. 13 shows that three basins and Wadden Sea sample can be clustered into three groups C_1 C_2 and C_3. Here sample number 1 and 2 represent the measurement point 1 and 2 in basin 1, sample 3 and 4 for basin 2, sample 5 and 6 for basin 3, and 7 for the sample of the Wadden Sea.

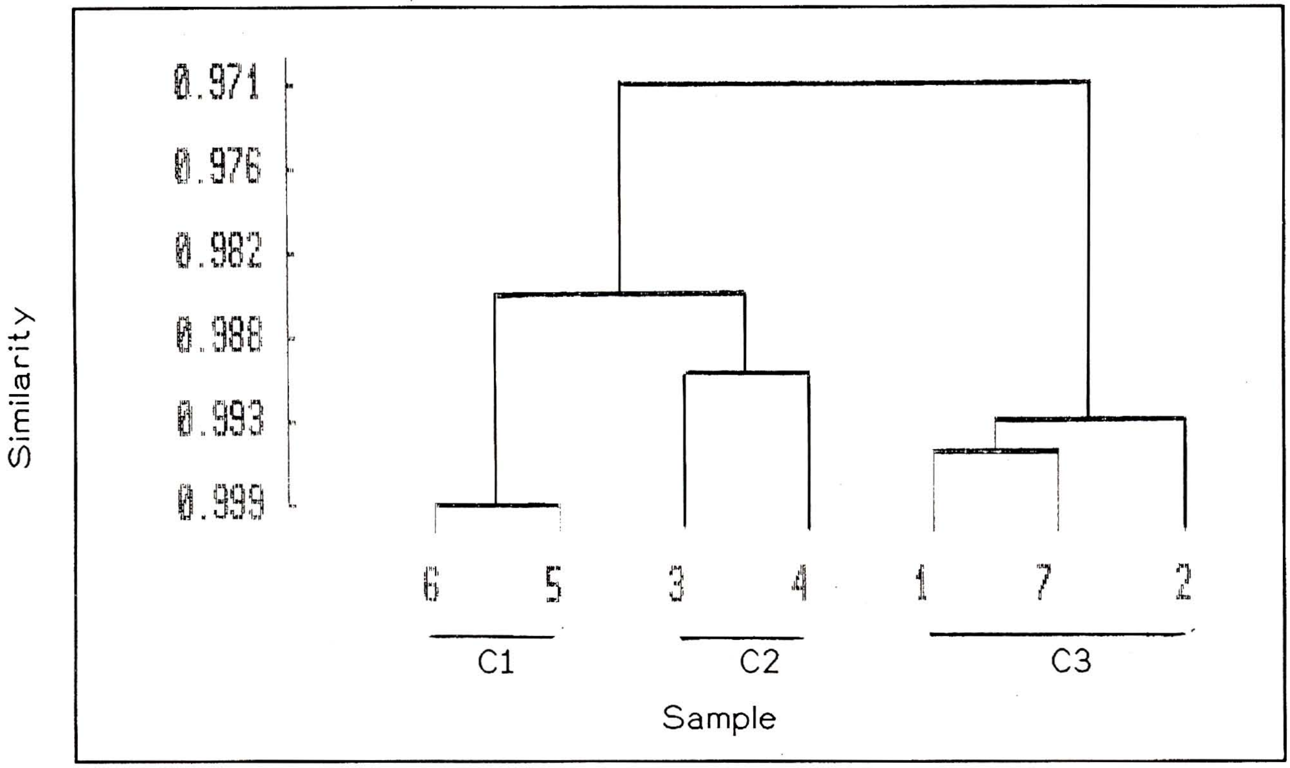

Fig. 13 - Result of clustering analysis with 7 samples and 40 variables extracted from the fluorescence spectra of the different artificial Wadden Sea. 7 samples are clustered into three groups named Cl C2 and C3 individually.

The diagram of the loading matrix of factor 1 versus factor 2 in Fig. 14 shows that in the cluster groups C_1 and C_2 Gelbstoff is more dominant as in group C_3. The covariance analysis of 30 spectra in all three groups also shows that the spectra in group C_1 and C_2 are more influenced by Gelbstoff, and the spectra in group C_3 are sensitive to chlorophyll a. This is reasonable because, on the one hand, basin 1 and 2 were in normal state of the Wadden Sea where there are only water and sediment correlated with Gelbstoff fluorescence and, on the other hand, basin 3 and the Wadden Sea sample were covered by diatoms and cyanic bacteria which produce very strong chlorophyll a fluorescence.

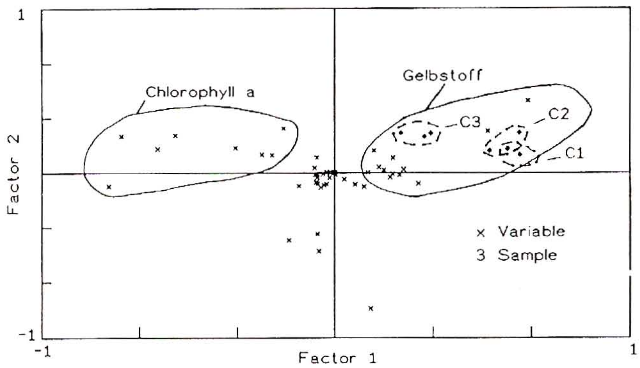

Fig. 14 - Result of corresponding analysis with the data used in clustering analysis in Fig. 13. The loading matrixes for the first two factors show that different cluster represents different status of the Wadden Sea surface.

3. DISCUSSION

According to these findings it can be stated that laser induced fluorescence can be used to study the Wadden Sea water and sediment. The definition of substances quantity needs to be proved which depends on the deduction of new algorithms instead of the Raman normalization technique. Three of these new algorithms are as follows.

3.1 The ratio F_{735}/F_{685}

The ratio F_{690}/F_{735} is proportional to the chlorophyll a content of plants and a direct indicator of plant stress (Lichtenthaler, 1988). This ratio also shows a very good correlation with chlorophyll a concentrations on the Wadden Sea surface, which represents the quantity of diatom canopy on the Wadden Sea surface. This fluorescence ratio can also be used to eliminate the influences of some factors such as laser power, sensitivity, and so on. Here we use F_{735}/F_{685} instead of F_{690}/F_{735} for plants to avoid the infinitive value of F_{690}/F_{735} because F_{735} could be too small in area with poor diatom coverage.

3.2 The ratio F_{685}/F_{max} or ratio F_{735}/F_{max}

F_{685}/F_{max} and F_{735}/F_{max} show water quantity over the Wadden Sea surface. Fmax represents here the maximum of Gelbstoff fluorescence. This provides a possibility to classify the Wadden Sea surface into the drying and flooding sediment surfaces. Taking into account F_{735}/F_{685}, a classification of the Wadden Sea sediment surface with a diatom canopy and that without diatom canopy is possible.

3.3 Multivariable combinations

Fluorescence spectra of the Wadden Sea strongly vary from place to place not only in amplitude but also in spectral shape. The change of spectral shape could indicate the change of substances in the Wadden Sea. In order to extract the message hidden in the spectral shape, a multivariable combination technique is necessary. Multivariables could be fluorescence intensities at various wavelengths, the slope of spectra at certain wavelengths, weighth centres of spectra, etc.

Besides that, several factors, combined by some variables in a statistical way, can also be used as classification parameters.

In this paper we show that ratio F_{685}/F_{max}, F_{735}/F_{max} and F_{735}/F_{685} can be used to classify the samples if they are measured with the LS50 in the labortory. A classification of the Wadden Sea with MiLF data is only possible if multivariable analysis is used. Modification of MiLF will be made so that a classification of the Wadden Sea through some single parameters or those ratios described above could be reached in the future.

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