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EVOLUTION OF PARTICULATE CHLOROPHYLL IN THE GIRonde ESTUARY

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Résumé -
L'évolution spatio-temporelle de la chlorophylle particulaire en suspension dans l'estuaire de la Gironde a été étudiée par spectroscopie photo-acoustique.

On a observé la disparition de la chlorophylle dans la partie amont de l'estuaire et son absence tout le long de l'estuaire; cette disparition a été corrélée avec l'accroissement de la turbidité dans cette même zone. Une tentative d'évaluation quantitative a été réalisée à partir de mesures sur des milieux modèles de cultures de chlorelles de turbidité variables.

La chlorophylle est à nouveau détectable à proximité de la mer. Son environnement est alors différent de celui de la chlorophylle fluviale car son spectre photo acoustique est décalé de 4 nm vers les courtes longueurs d'onde. La spectroscopie photo acoustique est une des rares techniques permettant une telle observation puisqu'elle dispense de tout procédé d'extraction et étudie la matière particulaire in situ.

Abstract -
The spatio-temporal evolution of particulate chlorophyll in suspension in the Gironde estuary has been studied by photo acoustic spectroscopy. Disappearance of fluvial chlorophyll has been observed in the upper part of the estuary; it has been correlated with the increase in turbidity. Quantitative measurements have been tempted using calibration with algae cultures of varying turbidity.

Chlorophyll again appears near by the sea. Marine chlorophyll has a shifted spectrum relative to the fluvial one. Such an observation has been possible because photo acoustic technique does not necessitate any sample chemical preparation.

INTRODUCTION
The Gironde is generally considered by geologists and geochemists as a model of temperate zone estuary (1). As a result, it has been widely investigated with a great number of techniques in order to determine the specific evolution of the functional characteristics of such an eco-system.

The estimation of primary photo synthetic production constitutes a necessary step in such an investigation. Direct measurements have been attempted but due to the difficulty of such determination, a variety of indirect methods such as chlorophyll analysis have been used. Indeed a good correlation has been established between chlorophyll a and biomass estimates (2) as well as particulate organic carbon and nitrogen (3). Furthermore, chlorophyll measurements during the spring bloom in the Baltic sea have been compared with particulate metals: Cu, Fe, Zn analyzed by atomic absorption technique. Good correlation is only observed in the photic larger and not in deeper water (4).

Much effort has been devoted in the investigation of chlorophyll evolution in various environments. Temporal chlorophyll evolution has been studied during a tidal cycle in Buzzard Bay (5) making evident the influence of tidal resuspension in photosynthetic production during Spring and Summer. The spatial distribution of chlorophyll has been investigated in the estuary of Penze (France).
The observed disappearance of the chlorophyll in the upper part of the estuary has been correlated with the increase in salinity (6).

Only spectroscopic techniques have been used for these measurements. They all imply the solubilization of the particulate chlorophyll contained in the water. The experimental procedure may vary but it usually consists in a filtration on a Whatman GFC filter followed by an extraction of the particulate matter with acetone (7). The extract is then analysed either by fluorescence spectroscopy (10) or by transmission spectroscopy. In this latter case the amount of chlorophyll is calculated using formulae taking in account the optical density at various wavelengths (8,9). Such a calculation is made necessary in order to distinguish chlorophyll from other pigments and to account for partial destruction of chlorophyll during the extraction procedure.

The inconvenience of these spectroscopic techniques is that they necessitate extraction of the chlorophyll from the sample. As chlorophyll is a very unstable compound which is easily oxidized it is difficult to define a good compromise between an incomplete extraction and a too severe one that partially destroys the chlorophyll.

For these reasons photoacoustic spectroscopy which does not require extraction of the chlorophyll appears as a promising technic for such a study. We shall not describe the basic principles of this technique which are described in ref.14 to 18. Let us just indicate that the major advantages of this technique are the following:

- direct and surface observation of the particles after a simple filtration, of the estuarine water.
- non destructive measurement making possible the use of other techniques for the analysis of the same sample,
- technique which needs only a small volume (10 to 100 ml) of water.

In order to demonstrate the efficiency of photoacoustic spectroscopy for the study of chlorophyll in the Gironde estuary we have measured the spatial evolution of chlorophyll all along the estuary and the evolution as a function of tide and place in its upper part.

The major inconvenience of the photoacoustic technique is the difficulty in making quantitative evaluations, due to the complexity of the signal which depends on both the optical and thermal properties of the sample. In the case of estuarine particulate matter, the problem consists in the occurrence of both chlorophyll containing phyto plancton or particles and inorganic particulate matter. This latter part which results in the turbidity of the water contributes to the photoacoustic signal as it modifies the propagation of the thermal wave within the sample. We have thus made a calibration using samples obtained by filtration of a chlorella culture of varying turbidity.

EXPERIMENTAL

Samples were collected from the estuary by two different procedures. For spatial evolution measurements we have participated in a vast interdisciplinary sampling campaign: "Libellule III" on July 16, 1982. The samples were collected at one meter depth from an helicopter which moved along the estuary in order to always sample at low tide.

In the case of the spatio-temporal evolution, the samples have been taken at 0.5 m depth in a 50 km range which is the domain where chlorophyll disappears. The sampling has been done at low, midle and high tide on July 21 1982. The collected samples were immediately refrigerated and filtered within 12 h. on Whatman GFC (0.45μm) filters. Depending on the turbidity of the water, 10 to 100 ml water samples have been used.

The calibration samples were prepared from a chlorella culture. It is known that the amount of chlorophyll contained in algae cultures of same population may vary by an order of magnitude depending on the nutritional state of the algae (11). We have thus calibrated the culture by extracting the chlorophyll.
FIGURE 1: Photo acoustic spectrum filters obtained by filtration of 100 ml of water a/ La Réole, b/ Royan (see fig.2)

FIGURE 2: Evolution of particulate chlorophyll along the estuary. The quantitative estimation has been correlated with the turbidity of the water.
As chlorellas are known to have a strong pecto-cellulosic membrane, we first destroyed it by immersing the filters in liquid nitrogen and then extracted the chlorophyll with acetone under ultrasonic agitation. After centrifugation, the absorption spectrum of the extract was taken and the amount of chlorophyll calculated.

In order to study the influence of the water turbidity on the signal we added to the chlorella solutions a variable amount of mud particulate matter and thus we measured the variation of the photo acoustic signal as a function of chlorella concentration and sample turbidity. The PA spectra of the filters have been taken on a EDT instrument.

RESULTS AND DISCUSSION

The samples collected at low tide along the estuary allowed us to make the following observations :

- Chlorophyll is abundant in the river and is characterized by a visible band at 672 nm (fig.1). This band occurs at lower energy than in solution (13) as it is usually observed between 660 and 670 nm depending on the solvent. This makes evident the importance of the local environment of chlorophyll on its spectrum.

- The amount of chlorophyll decreases on entering the estuary. There is no detectable chlorophyll in the major part of the estuary (fig 2). Meantime the turbidity of the water strongly increases down the estuary. The disappearance of chlorophyll in the upper part of estuaries which is also accompanied by a decrease in particulate organic carbon (13) has already been observed. It is generally interpreted as being due to the clay in suspension which prevents lights from penetrating the water. As a result phyto plancton dies and chlorophyll in contact with water without its protecting environment is oxidized and disappears.

- The chlorophyll is again present near the sea (fig 2). It exhibits a spectrum slightly shifted relative to the fresh water chlorophyll as the visible band occurs at 668 (fig. 1). This makes evident the differences in chlorophyll environments in both type of waters. The shift might be due to different phyto plancton or different water characteristics. It has to be noted that photo acoustic spectroscopy is the only technique which allows such an observation as it does not require the extraction of the chlorophyll from the phyto plancton.

The samples collected between La Reole and Portets at various moments of the tide have shown that the disappearance of chlorophyll in the upper part of the estuary occurs within a sigmoid profile in a short distance of a few kilometers (fig.3). This concentration profile is displaced upstream at high tide and down stream at low tide.

The calibration procedure allowed us to make a quantitative estimation of particulate chlorophyll. The evolution of photo acoustic signal has been studied in a chlorella concentration range to C/200. Extraction has showed that the chlorella solution of concentration c contains 100ug chlorophyll per liter.

A linear relationship between the signal and the chlorella concentration is only observed in the concentration range C/200 to C/8. At higher chlorella concentration, saturation is observed and the signal is lower than expected(fig.4). We do not interpret this saturation as being due to the intrinsic photo acoustic saturation effect because the phase angle is not modified at these high concentration values. We propose that it only results from the existence of multi layers of chlorellas on the filter.

When increasing the chlorella suspension turbidity, by introducing inorganic particulate matter one does not observe any change in the signal as long as the turbidity does not exceed 10 mg/l. This corresponds to a 1 mg mud deposit on the filter as 100 ml samples have been filtered (fig. 5). At higher turbidity, the signal still depends linearly upon concentration but the slope of the curve decreases (fig.5)
We interpret these observations (fig.6) in the following way: as far as the chlorella deposit does not exceed a monolayer, linear relationship is observed. At low turbidity, mud is inserted on the filter in between the chlorellas and thus does not affect the signal. At higher turbidity values the mud covers the chlorellas and thus modifies the propagation of the thermal wave within the sample.

In order to make a quantitative evaluation of particulate chlorophyll from acoustic spectra, it is thus necessary to determine the amount of inorganic material on the filter by weighing it and then estimating from the appropriate calibration curve of fig. 5 the amount of chlorophyll.

CONCLUSION

We have demonstrated the possibility of using photo acoustic spectroscopy in environmental science in the case of the analysis of particulate chlorophyll. The major advantage of the technique is that it does not need any sample preparation. We have tried to overcome its major inconvenience which is the difficulty of making quantitative analysis by establishing a calibration procedure.
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