BIO-OPTICAL INVESTIGATION ON THE ALBANO LAKE

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ABSTRACT

Water quality parameters, such as Particulate and Dissolved Organic Matter and phytoplankton characteristics, have been investigated by optical (absorption and fluorescence) and biological techniques. The Italian Albano Lake, near Rome, has been chosen as a test site being a well-known place for tourism. Different water samples were collected monthly, from June 1999 to April 2000, at the same station and depth of the lake.

Filtration has played a crucial rule due to the necessity to prepare the samples for the subsequent analyses. Primary productivity and photosynthetic activity of the natural phytoplankton community were investigated by using a Pulsed Amplitude Modulated fluorometer. These analyses were carried out on a GF/F filter (0.7 µm). Concentrated samples on the filter were placed in a quartz cuvette and excited with red and white actinic light. A lamp spectrofluorometer was used to perform analysis, both on unfiltered and filtered samples by using filters of different pore size (0.7, 0.45, 0.22 µm). All the water samples were also analysed for phytoplankton taxonomic identification. Samples were fixed in formaline 2% and observed on a reverse optic-microscope, after sedimentation in a specific sedimentation chamber. Different optical techniques have been applied for chlorophyll pigments determination. Results have shown a good correlation coefficient (r=0.86) between absorption and fluorescence techniques. The Chlorophyll *a* detected by spectrofluorometer showed a significant correlation (r=0.71) with the phytoplankton biomass determined at the microscope. Dissolved Organic Matter and its components were observed and better analysed in filtered samples than in the unfiltered ones.

INTRODUCTION

Monitoring pollution in aquatic ecosystems is a main objective of ecological research. The present study was carried out in the Albano Lake, situated in Latium (Central Italy). This lake occupies two ancient volcanic craters, presents an area of 602 ha, a perimeter of 10 km and a maximum depth of 170 m. The water level is maintained constant by an artificial underground channel. Given the importance of the lake for tourism, monitoring water quality is crucial to the environment and to public health.

The phytoplankton, being the first level of the aquatic food chain, is considered an important indicator of water ecosystem salubrity . In fact the composition of the phytoplankton community, including its chlorophyll concentration, is one of the main parameters for assessing water quality. It has been demonstrated that the presence of Cyanophyceae colonies affects the water quality (1,2). In this respect the quantity and average composition of Dissolved Organic Matter (DOM), often originating from the natural degradation of microorganisms, is the second important parameter. Fluorescence, and in particular the Laser Induced Fluorescence (LIF) technique proves to be a good tool for detecting information about these families of compounds in waters, since both carry characteristic chromophoric groups (3). The Pulse Amplitude Modulate fluorescence (PAM) technique is another tool for measuring the *in vivo* primary productivity of the test area, in spite of large changes in fluorescence yields upon illumination by saturating light techniques (4).

Photosynthetic pigments in phytoplankton have the functional role to capture sunlight at specific UV and visible wavelengths for photosynthesis. Chlorophylls are green pigments which contain a stable porphyrin ring-shaped molecule, around which electrons are free to migrate. Since electrons move freely, the ring can easily gain (by direct absorption) or lose (by collision) excited electrons, thus supplying energised electrons to nearby molecules. In this process the chlorophyll captures the energy of sunlight and transfers it to the other molecules which are responsible for successive steps in the photochemical chain (5). Chlorophyll *a* is the main member of the chlorophyll family. In fact, all photosynthetic organisms, including phytoplankton, contain chlorophyll *a*. Usually, the chlorophyll *a* concentration is considered an expression of phytoplankton biomass. Other kinds of chlorophylls are characteristic for each phytoplanktonic family. Among the "accessory" pigments for example chlorophyll *b* is peculiar to green algae and chlorophyll *c* characterises Diatoms and Dinoflagellates. Conversely, in Cyanophyceae only chlorophyll *a* is present. Differences in chlorophyll content and distribution are one of the main clues for the identification of the phytoplankton groups, but only in a few cases was pigment analysis compared with direct observation of phytoplankton populations (6).

Phytoplankton, however, is characterised by a large number of different accessory pigments, including the families of carotenoids (e.g. fucoxanthin) and phycobilins. Carotenoids include the familiar compound named carotene, which with its long chain of unsaturated bonds is responsible for their orange dominating colour (varieties ranging from yellow to red tones can be found). Fucoxanthin is the brown pigment which gives their characteristic dark colour to Diatoms. Phycobilins are water-soluble pigments, and are therefore found in the cytoplasm, or in the stroma of the chloroplast. The latter pigments are found only in Cyanophyceae, which use them for soaking up light energy. However they present very peculiar optical properties since both members of the family (phycocyanin and phycoerythrin) are characterised by narrow absorption bands and very specific fluorescence wavelengths.

The present study was performed in order to compare different methods for pigment analysis *vs*. the phytoplankton microscope qualitative/quantitative investigation; namely optical absorption, fluorescence and biological techniques are considered. The results gave indications as to the phytoplankton composition in the investigated ecosystem and to its seasonal variations, directly related to water quality.

METHODS

Water samples from the Albano Lake were collected monthly from the same station and at 1 m depth, of, starting from June 1999 to April 2000.. The GPS position of the station is: 41° 45,60' N – 12°40,00' E. Different kinds of measurements on each sample, were performed (Table 1). The methodology adopted in each case is described briefly below.

Table 1: Analysis on Albano lake water samples.

Type of analysis	**Purpose**
Acetone pigments extraction	Pigments phytoplankton characterisation
Phytoplankton identification by microscope	Phytoplankton characterisation
Fluorescence spectra by spectrofluorometer	CDOM, UV components and phytoplankton pigments characterisation
Time resolved fluorescence by Pulsed Amplitude Modulated (PAM) fluorometer	Natural phytoplanktonic primary productivity

Pigment extraction

The determination of chlorophyll (mg/m^3) and carotenoid (mSPU/mc) concentrations in algae and phytoplankton requires measurements of absorbance by spectrophotometer at specified wavelengths and the use

of these values in the trichromatic equations (7-9). In the case of chlorophyll *a*, *b* and *c* the wavelengths selected for the measurements are 663, 645 and 630 nm, each corresponding to the peak of the respective absorbance at the "red end" of the spectra where the interference with other pigments is minimal (10). Carotenoids determination has been carried out at the characteristic blue wavelength (480 nm) (11).

A fixed volume (1 litre) of water sample was concentrated on cellulose-derivative membrane filters (Millipore membrane 0.45µm pore size). The filter containing the plankton was folded and placed in 10 ml of 90% acetone in a glass tube. The sample was placed at 4°C in the dark for 24 hours, then centrifuged (3000 rpm for 20 minute at 4ºC) and analysed at 480, 750, 663, 645 and 630 nm wavelength against a 90% acetone blank (7,8,12). For quantitative determination of pheopigments, which are chlorophyll degradation products, the extract was acidified with a drop of 1 N HCl and the absorbance at 663 and 750 nm was subsequently detected.

Phytoplankton identification

Quantitative analysis and taxonomic identification of phytoplankton were performed according to Zingone *et al*. (13). The water samples collected monthly were fixed with a 2% formaline solution for qualitative and quantitative analysis. Phytoplankton was counted using a CARL ZEISS Axiovert 135 inverted microscope. Depending on phytoplankton density, 50 or 100 ml water samples were sedimented for 36 to 72 h in sedimentation chambers. The complete list of the *taxa* identified by observing the entire chamber was performed, while for the quantitative analysis 100 fields for each chamber were examined. Quantitative results were expressed as individuals l^{-1}. In the case of colonial or filamentous algae, the counting unit was a predefined aggregate of cells.

Fluorescence measurements

A commercial lamp spectrofluorometer (PTI Quantamaster) was used to perform a multispectral analysis at different UV-VIS excitation wavelengths, collecting excitation and emission spectra of natural and filtered water samples. Excitation and emission scans were corrected for the spectral sensitivity of the instrument and the lamp intensity spectral distribution. Correction factors for excitation and emission spectra were supplied by the manufacturer. A list of the main fluorescence excitation and fluorescence channels is detailed in Table 2.

In order to discriminate the different dissolved organic compounds in water from phytoplankton, samples for spectrofluorometer analysis were filtered at 33.25 hPa with different cellulose membrane (0.22 µm, 0.45 µm) and GF/F (0.7 µm.) filters. Spectra of samples without filtration (natural lake water) were also measured. Before each determination, a blank analysis was performed on MilliQ water.

Examples of fluorescence spectra obtained at different excitation wavelengths for filtered and unfiltered lake waters, are reported in Figure 1. The fluorescence spectra excited at 230 nm (figures 1A and 1B) for the unfiltered and filtered water samples show that the intensity between 300 nm and 350 nm (protein-like fluorescence) increases after filtration. This effect on fluorescence intensity after the samples filtration was previously observed in the paper of Campanella (14) for dissolved organic matter (humic compounds) in water. For visible spectra (Fig. 1C - 1E), which did not show the same effect upon filtration, signatures of the main pigments and fluorescent substances are indicated in the spectra of the unfiltered samples shown.

Fluorescence spectra were processed with a background subtraction, a multiple Gaussian deconvolution of overlapping structures, and an integration of resolved peaks within a 10 nm bandwidth. A successive subtraction of the respective MilliQ water contribution and a normalisation to the relevant water Raman peak permitted to release data in "Raman units". The latter units are adopted in order to relate measurements performed on identical water samples using different local or remote instruments and to compare different sea or lake waters (15). Fluorescence intensities in these relative units can be successively converted into

absolute values of concentrations by calibrating them against chemical analytical determinations carried out upon the same water samples.

Table 2: Main excitation (λ_{exc}) and emission (λ_{em}) fluorescence bands used in the fluorescence determinations.

Natural Band	λ_{exc} [nm]	λ_{em} [nm]	Notes
Raman	230	249	Transparency
Tyrosine	230	305	Protein-like fluorescence
Tryptophan	230	345	Protein-like fluorescence
DOM	230	380	DOM fluorescence in the UV
Raman	355	403	Transparency
Humic and fulvic acids	355	445	DOM fluorescence in the Blue
Phycoerythrin	355	580	Algal pigment
Allophycocyanin	355	650	Algal pigment
Chlorophylls	355	680	Chl- algal pigments
Blue-Greenpigments	480	510	Degradation algal pigments
Humic and fulvic acids	480	545	DOM fluorescence tail
Raman	480	571	Transparency
Chlorophylls-a	480	680	Chl-a algal pigments
Phycoerythrin	530	585	Algal pigment
Phycocyanin	530	630	Algal pigment
Raman	530	644	Transparency

PAM Analysis

A Pulse Amplitude Modulated fluorometer (Walz 101 main body completed by Walz 103 accessory module which controls the saturating pulse) has been used to measure the fluorescence yield of discrete phytoplankton samples uptake from the natural community, so as to determine the relevant electron transport rates (16). Complete electron transfer rate curves were obtained by increasing in steps the actinic light from dark to 2000 [µmoles of quanta m^{-2} s^{-1}). We used the chlorophyll fluorescence nomenclature, abbreviations and formulas according to van Kooten and Snel (17). PAR is the Photosynthetically Active Radiation [µmoles quanta m^{-2} sec^{-1}] utilised in the experiment, while I_k and PAR_{SAT}, are physiologic indicators useful to characterise the primary productivity of the phytoplankton population in combination with other quantities (Chl-a content, absorption coefficient) at given environmental conditions (temperature, spectral radiance and intensity)(16).

In summer and autumn the natural phytoplankton community was characterised by PAM measurements. The low sensitivity of the instrument required to concentrate samples on GF/F filters. One litre of water sample was filtered and successively put in a quartz cuvette with 1 cm path length. The sample was adapted to the dark for 20 minutes (all photosynthetic reaction centres opened) and then analysed collecting the red fluorescence signal upon excitation either with red or white actinic light.

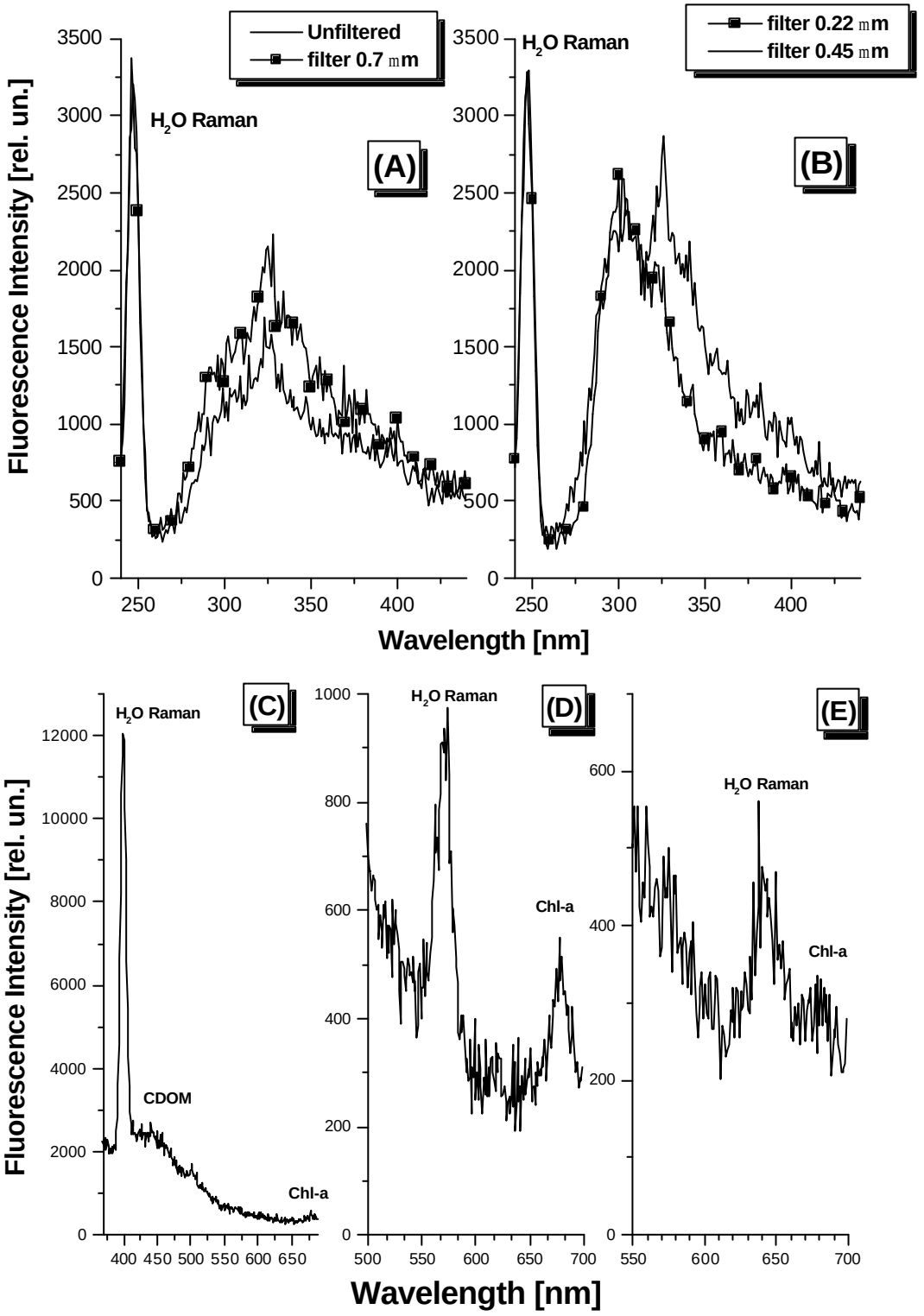

Figure 1: Fluorescence spectra of different lake water samples. Filtered and unfiltered samples at λ_{exc} = 230 nm (a, b). Unfiltered samples at λ_{exc} = 355 nm (c) ; λ_{exc} = 480 nm (d) ; λ_{exc} = 530 nm (e). Albano, 27 July 1999.

Data processing

SYSTAT software package (18) for correlating all the variables obtained was used, which performs Bartlett chi-square test for showing the significance of correlation in all considered pairs. In case of signifi-

cant correlation the Principal Components Analysis (PCA) (18) has been applied, which gives the "maximum likelihood" P for orthogonal Principal Components.

RESULTS AND DISCUSSION

A comparison between the spectrofluorometric and the spectrophotometric analysis with the phytoplankton microscope evaluation is shown in Table 3.

Table 3: Correlation between results of different methods for chlorophyll analysis and the identified phytoplankton classes.

	SpectrChl *a*	FluorChl *a*	SpectrChl *b*	SpectrChl *c*	Total Phytopl.	Diatoms	Greenalgae
FluorChl *a*	0.86**						
SpectrChl *b*	0.91***	0.92***					
SpectrChl *c*	n.s.	n.s.	n.s.				
Total Phytopl.	n.s.	0.72*	n.s.	n.s.			
Diatoms	n.s.	n.s.	n.s.	0.72*	n.s.		
Greenalgae	n.s.	n.s.	n.s.	n.s.	0.81**	n.s.	
Cyanophyceae	n.s.	0.81**	n.s.	n.s.	0.86**	n.s.	n.s.

n.s. = not significant
* = significant for $P \leq 0.05$
** = significant for $P \leq 0.01$
*** = significant for $P \leq 0.001$

The correlation between Chlorophyll *a* measured by the spectrophotometer (SpectrChl *a*) vs. Chlorophyll *a* measured by the spectrofluorometer (FluorChl *a*), is quite high (r=0.86), so that both methods appear suitable to determine phytoplankton biomass. . However, the correlation with the total phytoplankton determined by microscope counting is significant only for FluorChl *a* (r=0.72), thus indicating that the spectrofluorometer is a better tool than spectrophotometer for biomass investigation.

Chlorophyll *b*, peculiar pigment of Green algae, by using a spectrophotometer (SpectrChl *b*) is well correlated with both SpectrChl *a* and FluorChl *a* (respectively r= 0.91 and r=0.92), but it does not appear directly correlated with the Green algae class, characterised by the presence of this pigment together with Chlorophyll *a*. The rather unexpected result can be understood taking into account differences in the algae morphology in this family: namely the observed presence of colonial algae is responsible for an underassessment of Chl *b* concentration in the relevant macro-aggregates.

FluorChl *a* is correlated with Cyanophyceae (r=0.81), forming the major group of total phytoplankton (fig. 2), and thus a good correlation with the Total Phytoplankton (r=0.72) is also obtained. On the contrary, for Cyanophyceae,as well as for the other algae classes, no direct correlation with SpectrChl *a* is found, being probably much weaker when determined after acetone extraction with respect to the in vivo measurements. Diatoms are correlated only with SpectrChl *c*, and this is certainly due to the presence of this characteristic pigment in the class.

Figure 2 shows the total annual biomass of phytoplankton families. Total phytoplankton appears dominated by the Cyanophyceae class (the most representative species identified are *Oscillatoria* spp. and *Anabaena* spp.), followed by the Green algae (*Chlorella* spp. and *Ocystis* spp.) and finally by Diatoms (*Cyclotella ocellato* and *Fragilaria* spp). For each class only a few families are present throughout the year.

The dominating presence of Cyanophyceae and Green algae in the total phytoplankton population is confirmed by their correlation coefficient with the latter, r=0.86 for Cyanophyceae and r=0.81for Green algae respectively.

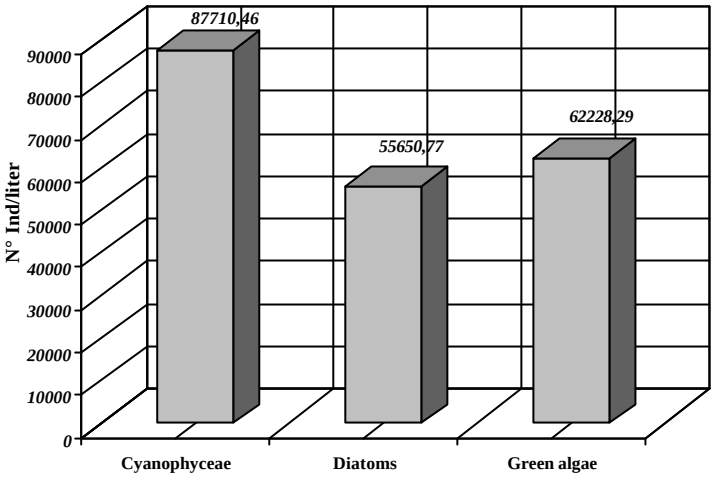

Figure 2: Total phytoplankton distribution after integrating results collected in all the examined period.

Figure 3 (a,b) shows the Principal Component Analysis results reporting on the axes two orthogonal Principal Components calculated as a linear combination of the variables given in table 3. Two different matrices, one for SpectroChl a vs. the other variables (3b) and the other for FluorChl a vs. the same variables (3a) are plotted. The spectrofluorometric analysis appears to be an especially suitable method for phytoplankton classification in fresh waters of temperate zones: a clear cluster is formed by fluorometer analysis (FluorChl a), total phytoplankton (TotPhyt), Green algae and Chl b concentration; conversely SpectroChl a (3b) is distant from l other variables. These results confirm that the fluorescence technique gives us more reliable data for biomass evaluation than the photometric one. The same conclusion is found in Friedrich et al. (19).

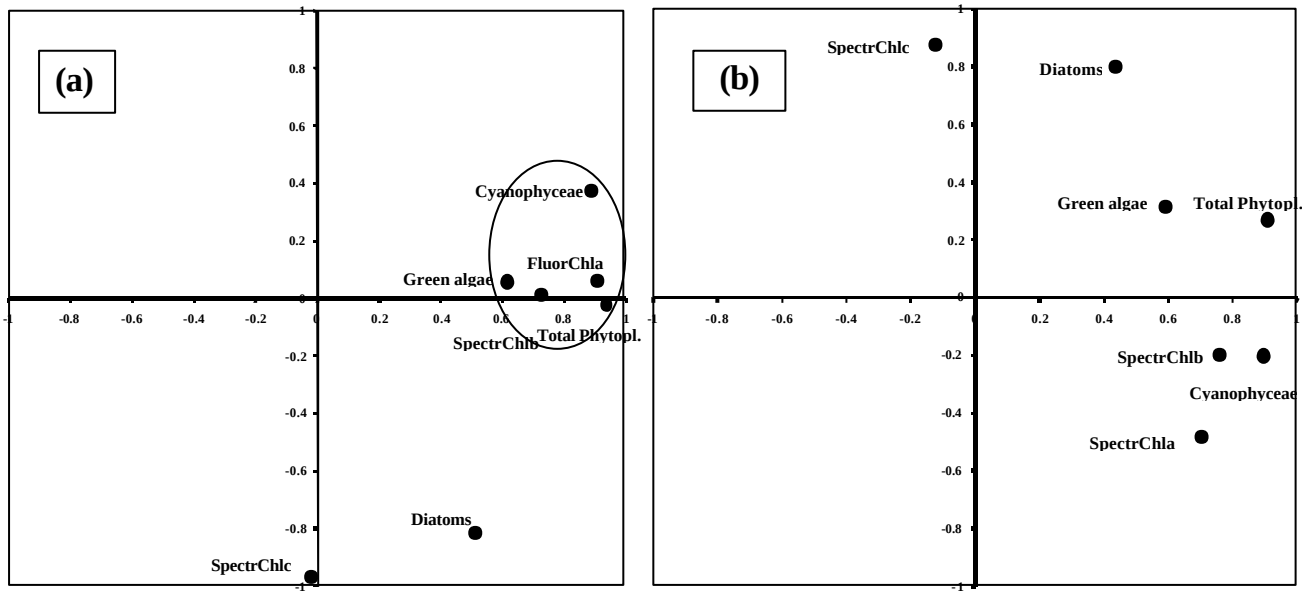

Figure 3: PCA analysis for FluorChl *a* (a) and SpectroChla *a* (b) vs other variables.

The results of 10 months of lake water monitoring by means of the spectrofluorometer are shown in Figure 4. The differences in chlorophyll seasonal variations, measured only for unfiltered samples, (Figure 4a) show the expected behaviour in analogy to the one of the chlorophyll *a* determined by the spectrophotometer (not shown). In summer (July), the growth of phytoplankton population investigated is at a minimum. In October there is a maximum phytoplankton biomass (Figure 4a) typical of the autumnal bloom in temperate zones. In spring 2000 a second bloom was observed. By comparing phytoplankton biomass with other components dissolved in water (mainly Tyrosine and Tryptophan-like substances) the opposite behaviour was obtained.(Figure 4a). The latter result can be explained since the substances, responsible for part of this emission, are utilised during phytoplankton blooms. On the contrary, during the after-bloom-phase (winter months), the amino acid-like spectral features appear at maximum intensity for different bio-organic degradation compounds dissolved in water released by algae (e.g. exudates), bacteria and aquatic plants (Figure 4a).

CDOM components, analysed in filtered and unfiltered samples, show a similar spectrum (rather flat) for all months observed (Figure 4a-d). Whereas features relevant to protein-like fluorescence channels (Tryptophan and Tyrosine) are more evident after 0.45 µm filtration than in natural lake water. In these cases, probably the chemical chromophoric groups of large natural organism living in lake, as phyto- and zooplankton, interfere heavily, especially at low concentrations (Figure 4 a,c,d, on January). The flat DOM distribution might be related to its main antropogenic origin in a suburban area, which is rather constant during the year.

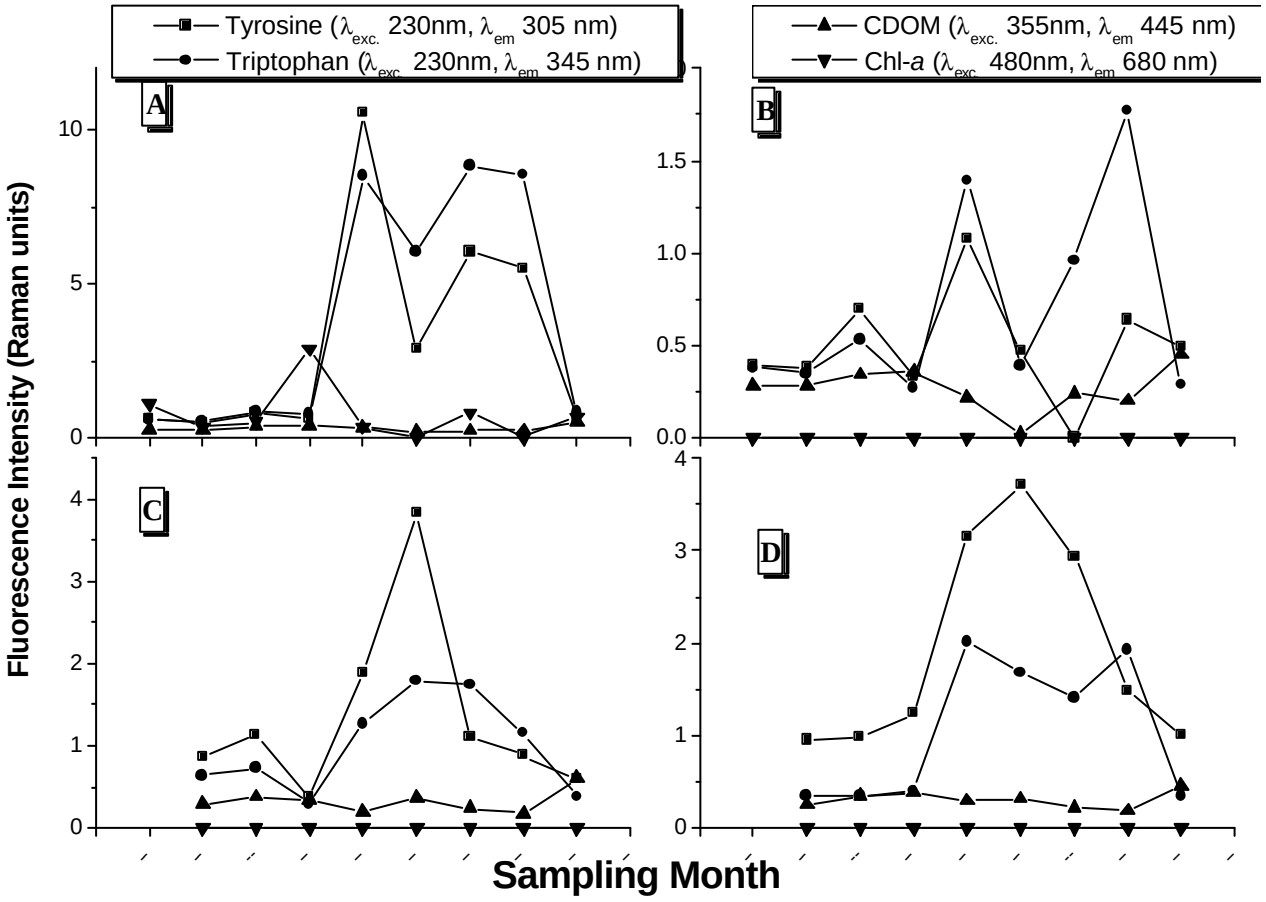

Figure 4: Summary of the different fluorescent channels intensity [Raman units]: a) natural lake water; b) 0.7 µm; c) 0.45 µm; d) 0.22 µm filtered samples.

For the autumn months the chlorophyll *a* and carotenoids excitation spectra were measured (Figure 5). Carotenoids and Chl *a* fluorescence intensity appear higher in October than in September. Comparable differences in both carotenoids and chlorophyll, corresponding to the total biomass increase due to October bloom, were observed. Lami (20) reported that the total carotenoids detection in natural water is a good tool for phytoplankton biomass evaluation, as well as chlorophyll *a* determination. The dynamic and metabolic activity of the phytoplankton population can be also described with monthly carotenoids variations (20) as observed in the months considered here (Figure 5).

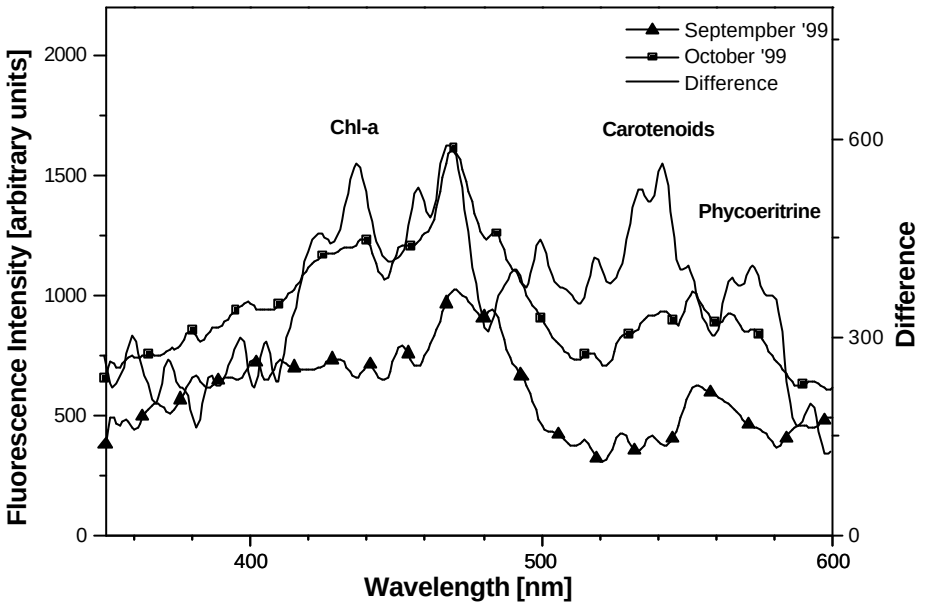

Figure 5: Excitation spectra of the natural Albano Lake waters for September and October 1999 sampling [λ_{em}=680nm]. The difference curve (right scale) refers to October and September spectra.

Significant PAM results obtained in pre and autumnal bloom are reported in Figure 6. The October bloom is characterised by low saturation PAR_{sat} (=143) compared with September PAR_{sat} (=244). The natural phytoplankton community, which is adapted to a low sun irradiation typical of autumn weather in a temperate zone, tends to show a lower saturation PAR than in summer periods (values comparable to September have been also obtained in July). The higher I_k value in September is an indication of a pre-bloom stage in which the phytoplankton community is at its maximum potentiality in terms of reactivity to environmental solicitations (sun irradiation in this case).

CONCLUSIONS

The phytoplankton communities of Albano lake have been fully characterised in their annual cycle by means of the reported bio-optical techniques. The results show the current critical condition of the lake, with a tendency towards a eutrophic condition. In fact we did not find a large biodiversity, having identified only 112 species always present throughout the year. The dominant taxa, Cyanophyceae, includes 20 species 14 of which are toxic species (1,2). In particular the presence of *Oscillatoria* spp. and *Anabaena* spp is an indication of organic pollution (1,2).

The possibility of remote use of some of the considered optical methods, especially of fluorescence techniques, offer perspectives to integrate data with non local monitoring campaigns which may interest the whole lake area. For instance in the latter case Laser Induced Fluorescence (LIF) can be used from a shipborne or airborne lidar fluorosensor in order to obtain information on the photosynthetic pigment distribu-

tion. A differential LIF technique, allowing for direct Electron Transfer Rate measurement has also been developed for remote application of a PAM-like method.

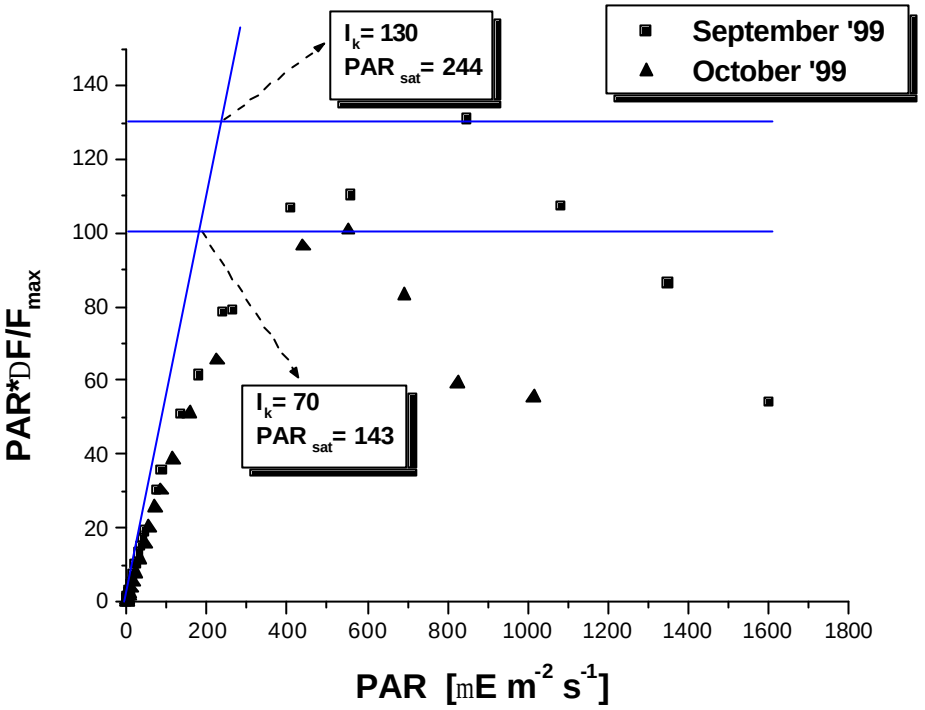

Figure 6: PAM measurements on lake water samples collected in September and October 1999.

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