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Application of the Long-Term Delayed Luminescence for Study of Natural Water Environments

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1. Introduction

A part of photon energy absorbed by a photosynthetic apparatus of photosynthetic organisms and some organic compounds in water or bottom sediments is wasted for luminescence. Depending on the period elapsing from the exposure of the material until registration of its luminescence we may distinguish fluorescence (Fl) registered during exposure of the material to light and delayed luminescence (DL) registered in darkness some time after the exposure to light is terminated.
Despite the fact that the quantity of energy lost due to luminescence is not major, it provides important information, since it reflects processes related to the absorption of light, intramolecular energy transfer and structural changes in a given material.
For technical reasons, fluorescence and constituent parts of long-term DL registered $t > 0.1$ s after excitation are the most suitable for registration. Findings obtained so far suggest that using DL for testing water environments creates opportunities for developing fast and accurate luminescence assessment methods for photosynthetic properties of active algae cells and properties of bottom sediments.

2. Basic methods for registering long-term luminescence in suspensions

At the moment, suspension is excited for luminescence primarily using radiation of a visible light spectrum whereas luminescence is usually tested by registering its kinetic decay or changes in intensity during exposure of a sample (induction kinetics).
Determining the DL decay kinetics involves exposing a sample to light for a specified time and then registering intensity of decaying light in darkness in a continuous manner or at predefined intervals.
In luminometers designed to determine decay kinetics of long-term DL, a sample is placed in a light proof camera and exposed to light of specific properties with photodetector covered for a predetermined period. Then, the source of light is blocked and photodetector opened and connected to a recording system to register intensity of decaying light. The need to cover the photodetector during exposure of a sample to light of high density of photons and uncovering it when measuring DL signals of low density of photons stream results from limited dynamics of light-sensitive detectors and systems cooperating with them. It is

necessary to separate streams of photons exciting luminescence from those emitted in the form of luminescence. When we use photomultipliers to detect luminescence instead of using shutters, we block their operation electronically during excitation luminescence of a sample (Lavorel et al., 1986).

In the case of solutions and suspensions, the intensity of delayed luminescence in a given time after exposure as well as kinetics of its induction can be registered continuously using flow methods (Strehler & Arnold, 1951; Krause et al., 1982; Krause & Gerhardt, 1984; Prokowski, 1991; Wiltshire et al., 1998). The methods involve exciting luminescence in a tested suspension beyond the place of detection and then pumping illuminated suspension to the photodetector which registers DL. Streams of exciting and emitted photons are separated by light traps. The kinetics of DL decay can be determined by halting the flow of the suspension.

To determine DL intensity in a given time span after exposure the suspension is pumped through an exposure tray to a light proof camera which contains measuring (emission) cuvette and photodetector combined with a system registering intensity of luminescence (Fig. 1) (Krause et al., 1982; Krause & Gerhardt, 1984; Wiltshire et al., 1998). By using monochromatic light for exciting of luminescence and placing an additional photodetector covered with a filter before the excitation cuvette, it is possible to measure Fl and DL at the same time (Yacobi et al., 1998).

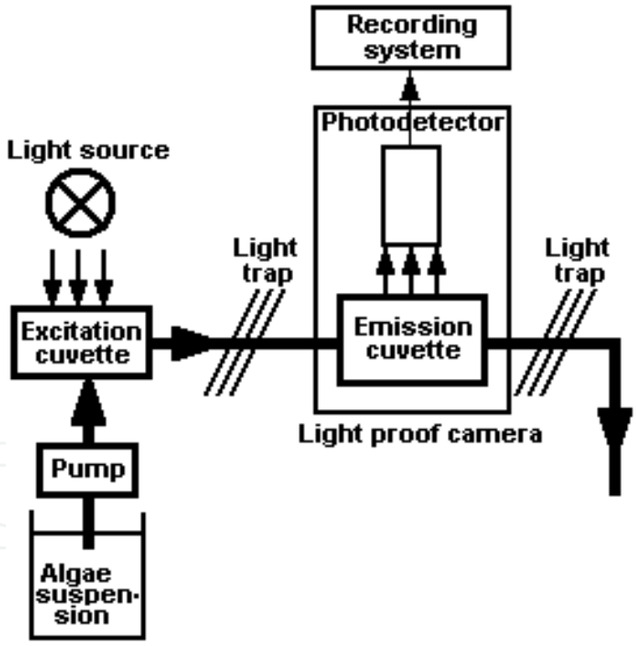

Fig. 1. Schematic diagram flow luminometer for registering intensity of long-term delayed luminescence in suspensions.

Exposure and DL registration times for suspension of algae depend on capacity of cuvette and flow. In the case of a fixed flow time between the end of exposure and DL registration depends on the capacity of pipes linking the two cuvette. Using monochromatic light of controlled wavelength to excite luminescence enables us to test the excitation spectrum of suspension delayed luminescence (Krause et al., 1982). The application of a special exciting

cuvette liked with a measuring cuvette in the system simplifies the construction of such luminometers (Prokowski, 2001).
In the case of suspensions for which DL intensity changes during their excitation it is possible to register the induction kinetics for this type of luminescence. In order to determine induction kinetics for long-term DL using a flow method, the suspension is placed in a transparent container – excitation cuvette (Fig. 2) (Strehler & Arnold, 1951; Prokowski, 1991) – Fig. 2.

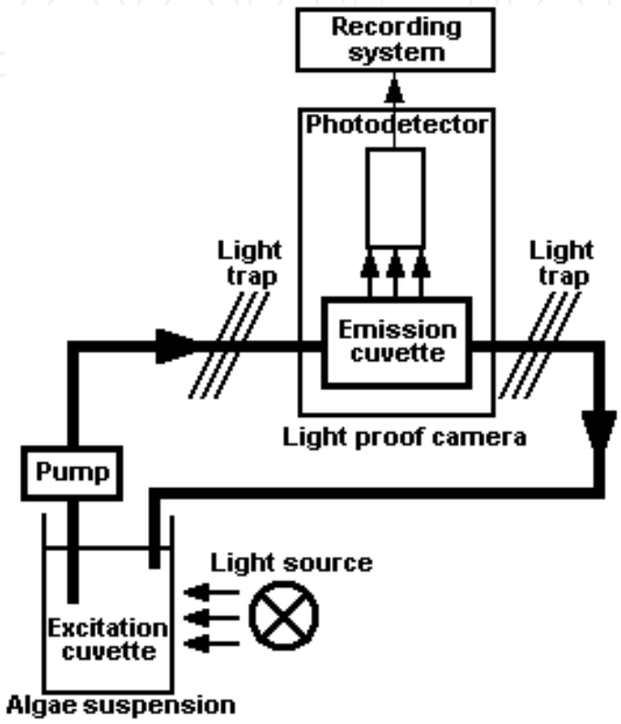

Fig. 2. Schematic diagram of flow luminometer used to determine induction kinetics for long-term delayed luminescence of suspensions.

The suspension is pumped from the container through non-transparent pipes to a light proof camera which contains measuring cuvette and photodetector. The suspension after leaving the measuring cuvette returns to the container. After the exposure of the suspension the photodetector registers changes in DL caused by the exposure.
Certain modifications of the flow method enabled registering a number of induction kinetics, both fluorescence and delayed luminescence using a single sample of algae suspension after different intervals after excitation (Prokowski, 1991, 1999). For this purpose, algae suspension after exposure in a transparent container is pumped through a series of connected light proof cameras with measuring cuvettes and detectors.
The intensity of luminescence registered is usually expressed in relative units. This is related to the fact that the registered value of emitted light from a given sample depends on a number of factors, such as geometry of the optic part of the luminometer and sample, emission intensity and spectrum of the excitation light source, optical properties of filters, mirrors and monochromators, as well as sensitivity spectrum of the photodetector.

3. Luminescence of chlorophyll *a* of the algal photosynthetic apparatus

In light-harvesting complexes, pigments in the photosynthetic apparatus of photoautotrophic organisms absorb light and while transforming absorbed photons into chemical energy some of the energy is reemitted in the form of luminescence. The excitation spectrum of the luminescence depends on the kind of pigments in light defusing complexes. In the case of land plants and algae, particles of chlorophyll *a* (Chl*a*) in photosystem II of their photosynthetic apparatus are responsible for the emission with its maximum at wavelength of $\lambda \approx 685$ nm. Luminescence Chl*a* accompanies light absorption by pigments in light-harvesting complexes and stabilisation of excitation energy in the photosystem. The luminescence excitation spectrum depends on the composition of pigments which accompany Chl*a* in the photosynthetic apparatus. The Chl*a* luminescence signal reflects particular stages of primary photosynthesis as well as influence of various biotic and abiotic factors on those reactions. Moreover, when exposure of photosynthetic organisms changes, their photosynthetic apparatus adjusts to new conditions. The processes are reflected in characteristic changes of the intensity of Chl*a* luminescence. The changes are described as luminescence induction and are observed during the first several minutes after new exposure to light. After that, the level of luminescence intensity stabilises (Prokowski, 1991).
Fluorescence (Fl_{Chla}) which accompanies photosynthesis is mainly the effect of the loss of photon energy absorbed by light-harvesting complexes during the photophysical stage of photosynthesis, whereas the delayed luminescence (DL_{Chla}) is related to the imperfection of photon energy transformation into chemical energy in the photosynthetic apparatus. Contrary to fluorescence, the emission of DL_{Chla} may occur from Chl*a* contained in the photosynthetic apparatus which has the capacity to promote primary photosynthesis. It occurs at some stages of primary photosynthesis and therefore DL_{Chla} has several components produced by various mechanisms (Jursinic, 1986). Particular DL_{Chla} constituent parts can be distinguished by registering the drop of luminescence after excitation light is turned off. The decay curve can then be divided into a number of exponential parts which last from several microseconds to several minutes (Jursinic, 1986).
In the case of long-term DL_{Chla}, its parts are registered within a range between hundreds of milliseconds to several dozens of seconds. This results from a recombination of negative charges accumulated at electron carriers Q_A and Q_B with positive charges in the water decomposition system (Jursinic, 1986). During that period we may distinguish at least three DL_{Chla} constituent parts: one lasting about 0.5 s (fast component DL_F) related to deactivation of reduced Q_A acceptor and two other of 2.5 s (middle component DL_M) and 14.5 s (slow component DL_S), both related to the deactivation of reduced Q_B acceptor (Prokowski, 1991, 1999). Those parts of delayed luminescence react to physical and chemical factors differently (Prokowski, 1991, 1993, 1999). In comparison with Fl_{Chla}, intensity of the DL_{Chla} component is more than two orders of magnitude smaller.
Figure 3 presents sample changes of luminescence intensity registered simultaneously for Fl_{Chla} (IFl) and three long-term component of DL_{Chla} (IDL_F, IDL_M and IDL_S) for test algae *Scenedesmus quadricauda* Brèb. in arbitral units – different for particular iterations, whereas figure 4 shows normalised values of those iterations in a semi-logarithmic scale.
Those iterations were registered for algae of 150 mgChl*a*·m^{-3} adapted in darkness for 2 h and then exposed to monochromatic light of wavelength λ_{max} = 660 nm and photons flux of 100 µmol(quanta)·m^{-2}·s^{-1}. Fluorescence was registered at wavelength λ_{max} = 690 nm during the exposure of algae suspension, whereas delayed luminescence intensity of particular

components was registered at λ > 660 nm at the following intervals after excitation: IDL_F – from 0.3 to 0.7 s, IDL_M – from 3.0 to 3.6 s and IDL_S – from 12.0 to 12.6 s. F_0, F_I, F_D, F_P, F_M, F_S, F_T are used to mark intensity of fluorescence in specific points of the induction curve; DL_{Fm} – transient maximum of changes intensity in DL_F.

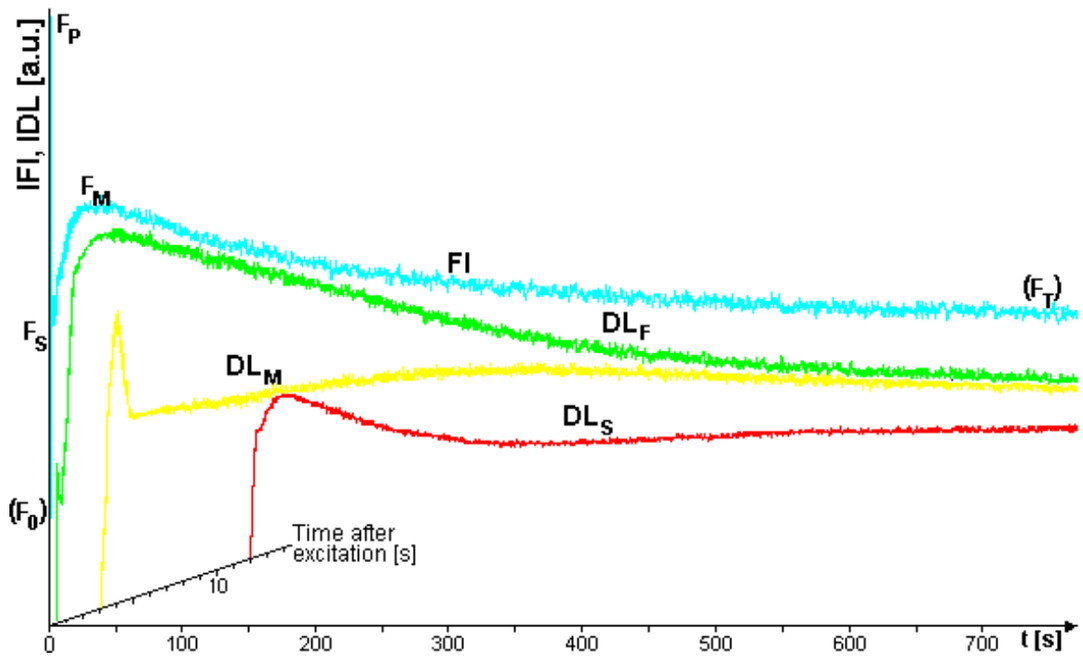

Fig. 3. Sample changes of fluorescence and three components of long-term delayed luminescence of test algae suspension.

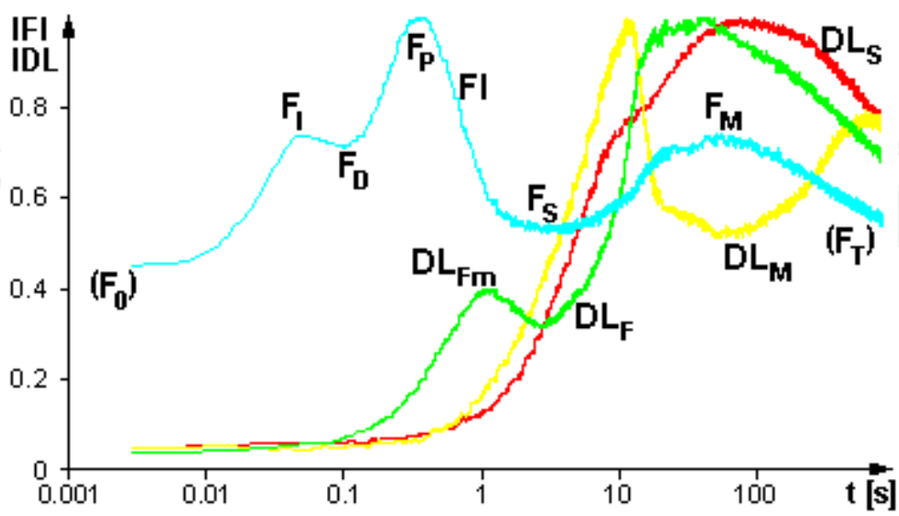

Fig. 4. Comparison of normalised changes of fluorescence and three components of long-term delayed luminescence of test algae suspension.

While analysing changes of luminescence Chl*a*, we notice that in the case of Fl_{Chla} typical changes of intensity occur during induction, namely abrupt increase of intensity to F_0 immediately after the excitation of the algae suspension. We also record fast increase to F_I after which there is a temporary slowdown to F_D and another increase to maximum F_P. During further exposure of the suspension, Fl_{Chla} intensity drops to F_S, then it increases again to F_M to drop later to the level of stationary intensity F_T.

In the case of DL_{Chla}, no temporary changes of intensity typical for Fl_{Chla} in the initial period of induction are observed. In the case of DL_F only, similarly to Strehler & Arnold (1951) we also observed a temporary maximum. The maximum was registered when intensity of Fl_{Chla} dropped from F_P to F_S, whereas during further exposure of the algae suspension changes of particular components of DL_{Chla} were different. Intensity of DL_F and DL_S reached maximum values and then decreased to a stable level after long time exposure of algae suspension whereas changes of DL_M before reaching that level showed two temporary increases in luminescence intensity. Usually during free excitation, several temporary changes of intensity occurred in delayed luminescence components. These changes were preceded by minor changes of fluorescence intensity. Moreover, both Fl_{Chla} and DL_{Chla} components react differently to physical and chemical factors (Prokowski, 1991, 1993, 1999).

Some research register Fl intensity changes during exposure of a sample to constant light with additional strong light impulses used to determine fluorescence parameters (for reviews see Seppälä, 2009). The technique used previously did not allow for testing reaction to such excitation in case of long-term delayed luminescence components. Figure 5 presents the induction kinetics of test algae suspension luminescence (C) registered for three levels of photon flux density (I) of exposure to continuous light and effect of the influence of a temporary increase of intensity of monochromatic light on luminescence of Chl*a*.

The research presented in the figure used photon stream impulses of 1000 µmol (quanta) $m^{-2} \cdot s^{-1}$ and duration of 0.6. The first impulse occurred 55 s before continuous excitation light was switched on. The next impulse occurred at the drop of Fl_{Chla} intensity to F_S, and further after every 60 s of exposure.

In the case of test algae luminescence, their Fl_{Chla} increased with the increase of photon flux density of light illuminating the algae suspension, whereas no major changes were registered in intensity of DL_{Chla} components and changes of conditions of illuminating algae (Fig. 5).

Additional light impulses cause different reactions as regards intensity of fluorescence and delayed luminescence components. In the case of fluorescence Chl*a* we observe temporary increase in intensity when an impulse occurs. The increase depends on the induction moment in which an impulse occurred. The largest increase was recorded for an impulse which occurred in darkness, whereas the smallest for an impulse which occurred when Fl_{Chla} amplitude for test algae changed from F_P to F_S. Impulses occurring every 60 s did not cause any visible changes of the fluorescence induction curve comparing a flash and the reference value.

In the case of DL_{Chla}, the increase in intensity of all components after an impulse in darkness was much lower than its intensity registered immediately after excitation light was switched on. Continuous excitation light causes various reactions of particular delayed luminescence components depending on the excitation light intensity.

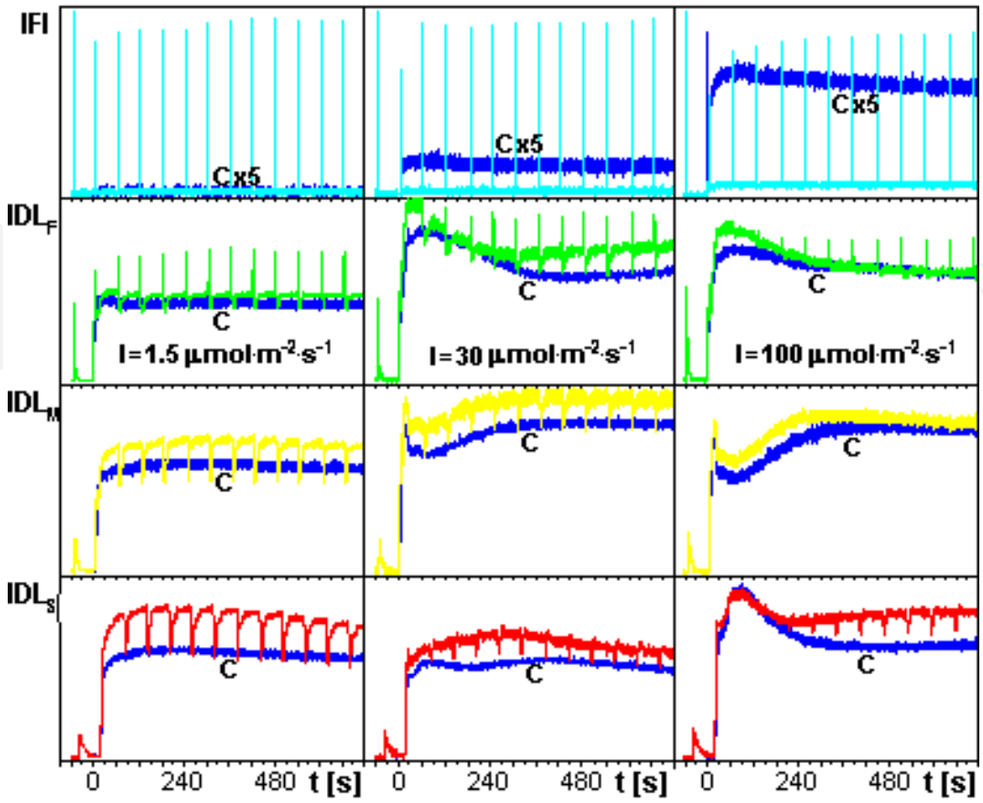

Fig. 5. Influence of additional light impulses on changes of test algae luminescence.

The first impulse at the beginning of the induction period caused small increase in intensity of all components. In the initial period of induction, we registered the smallest changes of intensity in components of delayed luminescence for all continuous light intensity levels tested. The nature of those changes depended on the excitation light intensity.

At the stream of continuous light photons exciting luminescence of 1.5 µmol(quanta)·m^{-2}·s^{-1}, consecutive light impulses caused temporary increase in DL_F intensity in the algae suspension. After that we usually recorded a small temporary drop followed by another increase to the actual stationary level. The changes of intensity of the delayed luminescence component were correlated with temporary drops of component intensity registered after a longer period from the exposure of the suspension.

After increasing continuous light photons exciting luminescence to 30 µmol(quanta)·m^{-2}·s^{-1} the amplitude of DL_F decreased and the drop amplitude increased. The intensity relaxation time also increased for DL_{Chla} to the stationary level. At the same time, the intensity of continuous light exciting third, fourth and fifth impulse caused larger temporary drop of intensity for DL_M and DL_S after the impulse. Then impulses caused a drop of amplitude and stabilised for those components of the delayed luminescence. However, in the case of DL_S, after a temporary drop of intensity, its temporary increase could be observed (decaying slowly).

Due to the increase in the stream of continuous light photons which excited luminescence to 100 µmol(quanta) m^{-2}·s^{-1}, the increase of DL_F was smaller after the impulse and decay of intensity changes for DL_M. DL_S at the third, fourth and fifth impulse reacted with a small increase in intensity above the stationary level. However, after further consecutive impulses was could observe a temporary drop of intensity DL_S followed by a small temporary increase.

4. The use of long-term delayed luminescence of chlorophyll *a* of the algal photosynthetic apparatus for testing phytoplankton

The interdependence of Chl*a* luminescence parameters and different biotic and abiotic factors is a basis for the luminescent methods for assessing properties of land plants and algae. However, the dependence of the phenomenon on conditions in which recording takes place creates problems in terms of measuring, as well as interpreting changes registered. We should remember, however, that luminescence methods provide information about luminescent properties of phytoplankton and we need to analyse a luminescence signals obtained in predefined conditions to determine other parameters. An additional difficulty while using long-term DL_{Chla} for phytoplankton testing is small intensity, which is particularly important when an *in situ* population of algae has small concentration.

Contrary to Fl_{Chla}, long-term DL_{Chla} intensity reflects concentration of reactive centres of photosystem II with separated charges. Findings of previous research show that in specific measurement conditions we may determine an important static relationship between DL_{Clha} intensity and primary productivity of phytoplankton (Krause & Gerhardt, 1984; Yacobi et al., 1998, Wilhelm et al., 2004).

Moreover, the use of monochromatic light of several wavelengths for exciting of Chl*a* luminescence enables us separating algae belonging to specific taxonomic from the population of phytoplankton (Krause et al., 1982; Gerhardt & Boedemer, 2005). However, from a practitioner point of view, the most desired is the use of luminescence for precise assessment of on line content of biomass of phytoplankton.

4.1 The use of long-term delayed luminescence for assessing the content of chlorophyll *a in situ*

The main difficulty while using luminescence for assessing the content of Chl*a* is the dependence of measurement results on several factors, such as specie composition of phytoplankton, temperature and growth conditions. This requires a frequent calibration of devices and adjustment of results. Some of those factors can be limited by incubating a sample of phytoplankton before luminescence measurement in the environment of constant temperature and light (Wiltshire et al., 1998). An additional way of limiting the impact of some of those factors is blocking electron transport in the photosynthetic apparatus of algae by adding a specific electron transport inhibitor to photosystem II of the sample, e.g. atrazine or 3-[3,4-dichlorophenyl]-1,1-dimethylurea (DCMU) (Prokowski, 1991, 2009). The inhibitors increase intensity of the DL_{Chla} fast components and reduce changes during excitation and eliminate DL_{Chla} slow components (Prokowski, 1991, 1999).

Previously, luminometers registering intensity of the DL_{Chla} fast component after a predefined exposure of algae suspension incubated in darkness were used for assessing Chl*a* content in phytoplankton (Wiltshire et al., 1998). Alternatively white light of small photons flux was used for that purpose (Prokowski, 2009). The exposure time is adjusted to register intensity of the component during temporary maximum at the induction curve (Fig. 4). White or monochromatic light is used for DL_{Chla} excitation. The signal registered is DL intensity (Prokowski, 2009; Wiltshire et al., 1998) or, in the case of luminometers capable of determining the excitation spectrum of phytoplankton, total intensity for particular wavelength of the excitation light (Yacobi et al., 1998). It was proved that in the case of a defined algae culture its DL_{Chla} intensity is a linear function of Chl*a* concentration through 5 order magnitudes (Krause et al., 1982). It was also proved that in the case of phytoplankton

incubated in darkness at predefined temperature there is a linear relationship between $DL_{Chl\,a}$ intensity and Chl*a* content in samples collected in a given measurement point at the same time but at different depths (Witshire et al., 1998). However, the relationship between $DL_{Chl\,a}$ intensity and Chl*a* concentration depends on the date of sampling (Wiltshire et al., 1998).

Since the sensitivity of existing luminometers designated for determining the relationship between $DL_{Chl\,a}$ intensity and Chl*a* concentration is limited, they are used to test the population of freshwater phytoplankton in which the concentration of phytoplankton is considerably high (Wiltshire et al., 1998; Istvánovics et al., 2005). Sensitivity can be enhanced by changing the construction of the optical part of the luminometer (Prokowski, 2001). Figure 6 includes a flow chart of a luminometer of improved sensitivity designed for continuous *on line* registration of delayed luminescence of phytoplankton incubated in predefined temperature and light with and without electron transport inhibitor.

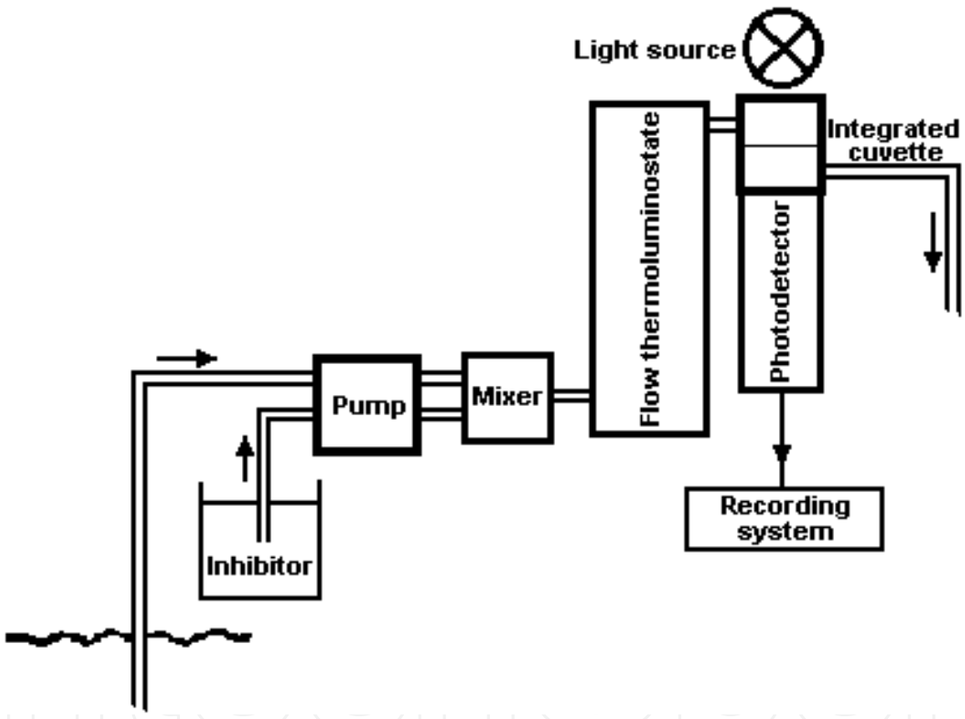

Fig. 6. Schematic diagram of flow luminometer for *on line* registration of delayed luminescence intensity of phytoplankton incubated in predefined temperature and light with and without electron transport inhibitor.

Figure 7 presents findings of research focusing on the relationship between $DL_{Chl\,a}$ intensity of sea phytoplankton registered *in situ* and extracted Chl*a* content ([Chl*a*]). Long-term delayed Chl*a* luminescence of phytoplankton was registered in time period of 0.3 s to 0.9 s for the suspension of algae incubated continuously for 3 min at 20 °C and in white light of 1 μmol(quanta)PAR·m^{-2}·s^{-1} of photon flux. The suspension was exposed to white light for 0.6 s with photons flux of 75 μmol(quanta)PAR·m^{-2}·s^{-1}. $DL_{Chl\,a}$ was detected using sounder with photomultiplier, which enabled registering light within the red part of the spectrum.

The study was conducted for phytoplankton from the depth of 1 m to 15 m sampled in 6 measurement points at the Gulf of Gdańsk. Chl*a* content was determined using a spectrophotometric method (Lorenzen, 1967). The ratio of chlorophyll *b* (Chl*b*) and Chl*a* determined using the spectrophotometric method by Jeffrey & Humphrey (1967) for the phytoplankton population tested was 0.263 ± 0.027, whereas the ratio of chlorophyll *c* (Chl*c*) and Chl*a* ranged 0.175 ± 0.018.

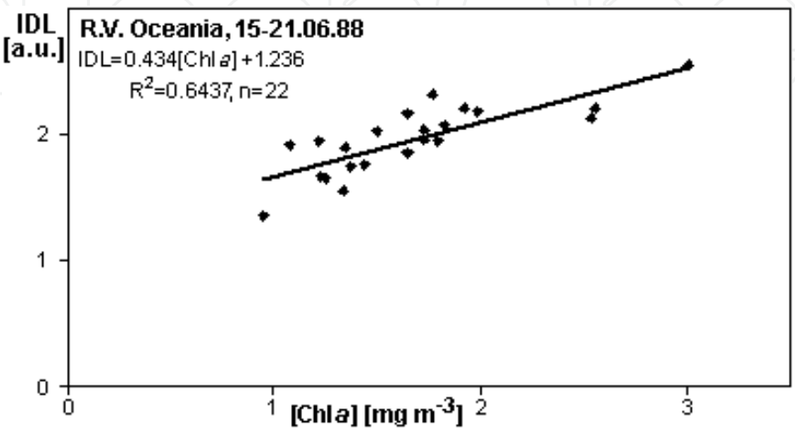

Fig. 7. Relationship between long-term delayed luminescence and concentration of chlorophyll *a* for sea phytoplankton samples.

While analysing data in figure 7, we may determine a statically significant linear relationship between delayed luminescence of phytoplankton from the Gulf of Gdańsk, which was incubated at constant temperature and lights, and concentration of Chl*a*. However, despite using incubation of samples at predefined temperature and light the value of determination coefficient R^2 for the tested population of phytoplankton was relatively low. Moreover, due to considerably small number of samples collected, it was not possible to determine whether there were any major differences of the relationship found between measurement points.

Also carried out studies to determine the relationship between DL_{Chla} intensity of sea phytoplankton registered *in situ* in the above mentioned conditions for the suspension of algae incubated continuously with the inhibitor of electron transport and the content of Chl*a* extracted using the spectrophotometric method. Karmex, urea herbicide, with DCMU as an active substance was used as inhibitor of electron transport. A saturated solution of herbicide was added to the suspension of phytoplankton to reach the concentration of the suspension 5 μmol·dm^{-3}. Tests were carried out in 4 measurement points at the South Baltic Sea: R6, G2, 92A and B13. Additionally, Fl_{Chla} intensity was determined for the analogical incubated suspension of phytoplankton. The Fl_{Chla} level was recorded using a spectrophotometer with an attachment for measuring fluorescence with photomultiplier as a photodetector. Chl*a* fluorescence was excited using light of wavelength λ_{max} = 436 nm and its intensity registered at wavelength λ = 690 nm. Table 1 shows the location of measurement points, range of the sampling depth and ratios Chl*b*/Chl*a* and Chl*c*/Chl*a*.

Test points	Geographic coordinates	h [m]	Chl*b*/Chl*a*	Chl*c*/Chl*a*
R6	λ = 54° 57′ N; φ = 18° 24′ E	1-27	0.202 ± 0.043	0.269 ± 0.023
92A	λ = 54° 35′ N; φ = 18° 40′ E	1-30	0.188 ± 0.025	0.269 ± 0.022
G2	λ = 54° 50′ N; φ = 19° 20′ E	1-40	0.119 ± 0.017	0.237 ± 0.020
B13	λ = 54° 04′ N; φ = 14° 15′ E	1-10	0.204 ± 0.012	0.190 ± 0.015

Table 1. Location of measurement points, sampling depths and of chlorophyll ratios.

Figure 8 presents relationship between the intensity of long-term DL_{Chla} (IDL) and Fl_{Chla} (IFl) for samples of sea phytoplankton with inhibitor of photosynthesis and concentration of Chl*a* extracted in particular measurement points. Inclination coefficients marked with different letters differed significantly from others ($P < 0.01$).

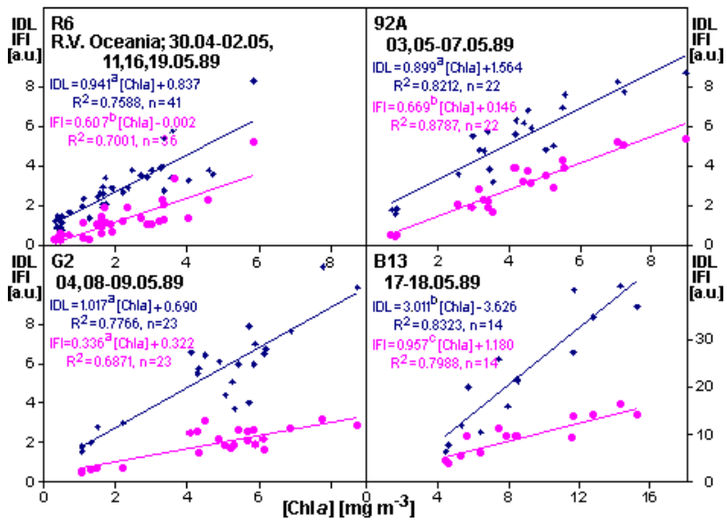

Fig. 8. Relationship between intensity of long-term delayed luminescence and fluorescence and concentration of chlorophyll *a* for samples of sea phytoplankton with inhibitor of electron transport in particular measurement points.

Comparing data in figure 8 we may notice major statistical differences between certain values of inclination coefficients determined for relations between luminescence from [Chl*a*] in particular sampling points. However, no major statistically significant differences were found for particular dates of sampling in a given measurement point.

In the case of linear relationship IDL = f([Chl*a*]) a major statistical difference in inclination values was found in the case of phytoplankton sampled at the estuary of the Oder to the Baltic Sea (point B13) if compared with corresponding relations registered in the case of phytoplankton collected in other points. Moreover, at the same point, the highest value of the coefficient and the lowest level of Chl*c*/Chl*a* (Tab. 1). In the case of linear relationship IFl = f([Chl*a*]), statistically significant differences in the inclination coefficient were recorded between phytoplankton sampled at 92A and R6 and that sampled at other stations. The lowest value of the coefficient was recorded at G2 for which Chl*b*/Chl*a* was also the lowest, whereas the highest coefficient was recorded at B13, for which Chl*c*/Chl*a* was the lowest.

Figure 9 shows relationship between Fl_{Chla} intensity (IFl) and long-term DL_{Chla} intensity (IDL) for samples of sea phytoplankton with photosynthesis inhibitor at particular measurement points. The coefficients marked with different letters show significant differences ($p < 0.01$).

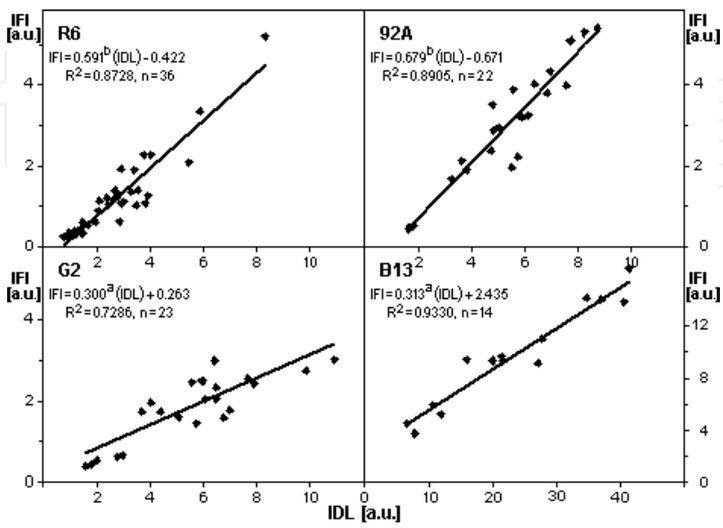

Fig. 9. Relationship between long-term DL_{Chla} intensity and Fl_{Chla} intensity for samples of sea phytoplankton with photosynthesis inhibitor.

In the case of linear relationship IDL = f(IFl), no statistically significant differences were recorded between directional coefficients of simple regression for phytoplankton sampled at R6 and 92A. Such differences were not found also for phytoplankton sampled at G2 and B13, whereas statistically significant differences were recorded between phytoplankton sampled at R6 and 92A and phytoplankton sampled at G2 and B13.

The findings show that despite incubating samples phytoplankton at identical temperature and light, with Karmex, both taxonomic composition of the phytoplankton suspension sampled at different stations and differences in conditions may have different impact on Fl_{Chla} and DL_{Chla} intensity of phytoplankton suspension samples.

In the case of IDL = f([Chl*a*]), statistically significant differences between directional coefficients of simple regression were only recorded for phytoplankton sampled at B13 and other stations. It may indicate that in this particular case the taxonomic composition of phytoplankton suspension sampled at R6, 92A and G2 situated at the Gulf of Gdańsk as well as differences of conditions there did not influence DL_{Chla} of the phytoplankton suspension set at measurement conditions. However, the delayed luminescence of phytoplankton sampled at the estuary of the Oder River (B13) influenced factors which did not differentiate the population of phytoplankton tested at the Gulf of Gdańsk.

5. Long-term delayed luminescence of sediments

Sediments are important elements of aquatic ecosystems. They comprise a natural filter and indicate the degradation of the natural environment. Comprehensive analysis of sediments is crucial while deciding about measures aimed at protecting lakes and their reclaiming; it may also be used for more detailed chemical analysis of the aquatic environment.

An important component of such sediments is organic matter, mainly humic substances. Such substances are produced by complex chemical and microbiological processes of decomposition and secondary synthesis of plant and animal residues in water. Properties of humic substances are determined by the type of organic matter humified in a specific environment as well as various habitat related and anthropogenic factors which determine the direction of their transformation.

In the case of certain types of multiple molecular organic matter, including substances in sediments, we also observe long-term delayed luminescence (DL_{om}) (Prokowski, 2001; Istvánovics et al., 2005; Mielnik, 2009). The luminescence is emitted within the whole spectrum. Excitation spectrum shows its maximum at short wavelength (Istvánovics et al., 2005). Mechanisms leading to generating delayed luminescence in organic matter in sediments have not been sufficiently examined. Findings of some research show that in specific excitation conditions, conditions of decomposition of primary organic matter may influence DL_{om} parameters of humic substances contained in sediments (Prokowski, 2001; Mielnik 2009).

The study was conducted for sediments from ten Lobelia lakes of different trophy: 5 - oligotrophic and 5 - dystrophic. Bottom sediments were sampled during summer stagnation from the surface layer (~15 cm) at the maximum depth in a lakes. Sediment samples were lyophilised, grinded and sieved using a sieve of 1 mm mesh size. Next humic substances were extracted using two solutions: (i): alkaline solution of 0.1 $mol \cdot dm^{-3}$ NaOH and (ii): bi-distilled water. The weight ratio of sediment to solution was 1:10.

Research on excitation and recording of intensity of the DL_{om} of humic substances solutions were carried out with the use of a device for continuous recording of photo-induced luminescence of liquids and suspension (Prokowski, 2001). DL_{om} was excited for 0.6 s by halogen light of density of a photon flux 3000 µmol(quanta)$PAR \cdot m^{-2} \cdot s^{-1}$. DL_{om} intensity was registered 0.1 s to 0.7 s after the excitation at wavelength range from 400 nm to 600 nm. All measurements were done at 20°C.

The statistically significant differences in DL_{om} intensity between the examined humic substances were observed. The relatively lower values of the DL_{om} intensity for humic substances extracted from oligotrophic lakes sediments were registered, compared to humic substances isolated from sediments deposited at the bottom of dystrophic lakes. It may be evidence of the differences in structure of studied humic substances. In addition obtained differences may be evidence of the variable quantitative and qualitative contribution of photoluminophors in the structure of the examined humic substances molecules as well as their different photochemical reactivity. The quality of the organic matter is an important feature in respect of the physico-chemical conditions at the lake bottom. It is generally accepted that the quality or the conditions for decomposition are determined by the ratio of organic carbon content to total nitrogen – C/N (Meyers, 1997).

Figure 10 presents the relationship between ratios of organic carbon and total nitrogen in bottom sediments (Co/No), water extract (C/N H_2O) and alkaline extract (C/N NaOH) and the value of DL_{om} intensity registered for water extract to DL_{om} intensity for alkaline extract (IDL H_2O/IDL NaOH).

While analysing data in figure 10, we may notice highly statistically significant linear relationship between C/N in bottom sediments and alkaline extract and IDL H_2O/IDL NaOH. Moreover, in the case of sediments sampled from oligotrophic lakes, IDL H_2O/IDL NaOH was the lowest. The findings may indicate the impact of the primary composition of organic matter and conditions of its decomposition on DL_{om} registered.

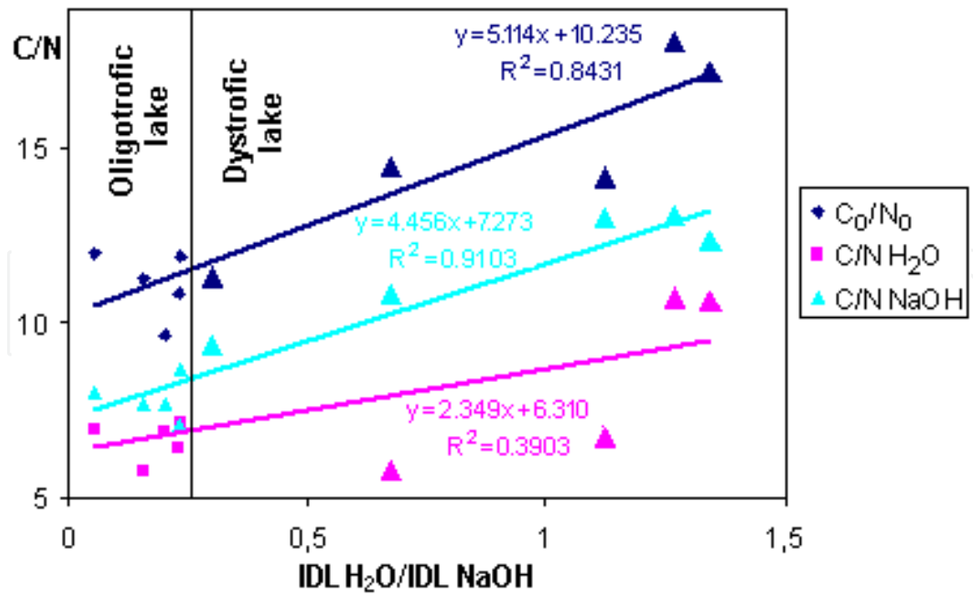

Fig. 10. Relationship between ratios of organic carbon and total nitrogen and ratios of intensity of delayed luminescence of humic substances in the water and alkaline extracts.

6. Conclusion

While studying the aquatic environment, luminescence can be a useful tool to determine certain properties of phytoplankton and assess threats to aquatic ecosystems, including the use of algae tests for assessing water quality. According to the research, in comparison with the delayed luminescence, mechanisms generating excited Chl*a* particles in the case of fluorescence are different and therefore the kinetics of their induction and reaction for a given factor is different as well.

Changes of fluorescence during exposure of the phytoplankton suspension and changes of intensity of long-term components of delayed luminescence provide information respectively about processes taking place in the photosynthetic apparatus immediately after light absorption and electron transport in the photosynthetic apparatus.

Registering changes in fluorescence and several component of delayed luminescence during exposure of a given sample provides for selective determination of primary mechanisms of photosynthesis. Relationship between changes of luminescence emission also enables testing the influence of various stress factors on photosynthesis. Moreover, the method can be used to examine *in situ* the suspension of phytoplankton and provides much more information about a given object. At the same time, the method enables defining several luminescence indicators and can be particularly useful in algae tests for diagnosing the status of the environment.

Findings indicate that humic substance exposed to light undergo photochemical processes accompanied by creation of long-term fluorophores capable of producing electromagnetic radiation in the process of luminescence. The use of luminescence in testing organic substances of various origin may provide information about the structure and nature of organic links, as well as photoreactivity of those substances. Additionally, based on findings from luminescence tests it is possible to determine changes which occur in those substance in time, in particular in the process of humification.

The method of using long-term delayed luminescence can play a major role in testing humic substances from various ecosystems, especially that the method is exceptionally sensitive, easy and quick. Eventually, this should save time and cost of such tests. The use of the method may significantly reduce the number of analyses and loss of information necessary to draw conclusions.

Therefore, while researching water environments, luminescence may provide important information concerning specific conditions in the environment. It is necessary, however, to develop new methods to register on line luminescence properties of phytoplankton and sediments found in water bodies. It seems that on line methods enabling simultaneous registration of fluorescence and delayed luminescence have the largest potential in terms of information provided.

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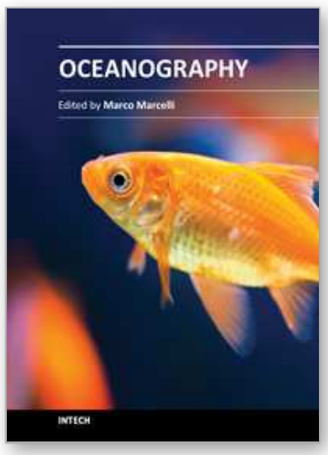

Oceanography

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How inappropriate to call this planet Earth when it is quite clearly Ocean (Arthur C. Clarke). Life has been originated in the oceans, human health and activities depend from the oceans and the world life is modulated by marine and oceanic processes. From the micro-scale, like coastal processes, to macro-scale, the oceans, the seas and the marine life, play the main role to maintain the earth equilibrium, both from a physical and a chemical point of view. Since ancient times, the world's oceans discovery has brought to humanity development and wealth of knowledge, the metaphors of Ulysses and Jason, represent the cultural growth gained through the explorations and discoveries. The modern oceanographic research represents one of the last frontier of the knowledge of our planet, it depends on the oceans exploration and so it is strictly connected to the development of new technologies. Furthermore, other scientific and social disciplines can provide many fundamental inputs to complete the description of the entire ocean ecosystem. Such multidisciplinary approach will lead us to understand the better way to preserve our "Blue Planet": the Earth.

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