USE OF CHLOROPHYLL FLUORESCENCE TO EVALUATE THE EFFECT OF CHROMIUM ON ACTIVITY PHOTOSYSTEM II AT THE ALGA SCENEDESMUS OBLIQUUS

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ABSTRACT

Industrial activities are significant sources of contamination of aquatic environments by heavy metals such as chromium. The latter occurs in two forms, trivalent or hexavalent, trivalent Cr is a trace element essential to the human body but its hexavalent form is toxic and very harmful to human health. Unfortunately, this form is found in abundance in industrial effluents and urban areas due to industrial activities, such as chromium metal and wood preservation [17].

In industrialized countries such as Quebec the use of Cr is much smaller because of its toxicity, but this will not lead to its total disappearance in the ecosystem. Maybe it will disappear from the air, soil and end up in the sea then in the fish we eat. While in developing countries its use is still present.

The photosynthetic apparatus of plants represents a target sensitive to these pollutants. In this project we will study the mechanism of toxicity of chromium on chlorophyll synthesis and electron transfer between photosystem II and photosystem I in the alga Scenedesmus obliquus. The yield and kinetics of chlorophyll fluorescence were used to evaluate the photochemical alteration of photosystem II. Our results provide an indication of the degree of toxicity of chromium on the photosynthetic apparatus of algae and can also be used to develop biomarkers photosynthetic sensitive to the toxicity of heavy metals.

Keywords: *Florescence of chlorophyll, photosynthesis, chromium Hexavalent, alga Scenedesmus obliquus, toxicity, environmental.*

LIST OF ABBREVIATIONS

Fm	for a maximum fluorescence in dark-adapted plant
Fm’	Fluorescence maximum for a plant adapted in the light
Fo	Constant for Fo fluorescence in dark-adapted plant
Fo’	'Fluorescence constant for a plant adapted in the light
Fv	variable Fluorescence for a plant in dark-adapted
Fv’	variable fluorescence to a plant adapted to light
L A	The actinic light
L M	The modulated light
L S	The light saturation
Ms	Millisecond
O-P-I-J	Transitions of the rapid kinetics of fluorescence
P680	reaction center of PSII
PEA	Plant Efficiency Analyser
PAM	Pulse Amplitude Modulation
PQ	Plastoquinone
PSI et PSII	Photosystems I et II
QA	primary electron acceptor of PSII
RL	Red distant
ϕ_M:	PSII photochemical efficiency
ϕ'_M:	Operational photochemical efficiency of PSII

1. INTRODUCTION

Photosynthesis is the basis for maintaining all life on earth. Every living being needs energy for its life and growth, but only plants, algae and certain bacteria are capable of using solar energy for the synthesis of carbohydrates necessary for their growth. It is for this reason that photosynthetic organisms are of great importance on earth. They are primary producers of organic matter to the remains of terrestrial organisms. Mechanisms of photosynthesis are very sensitive to environmental pollution such as pollution of aquatic ecosystems with heavy metals, in particular, chromium (Cr). The latter occurs in two forms, trivalent or hexavalent. The trivalent Cr is a trace element essential to the human body; the hexavalent Cr is toxic and very harmful to human health. Unfortunately this form is found in abundance in the industrial and urban effluents due to industrial activities, such as leather tanning, the manufacture of paints and dyes, chromium metal and wood preservation [17].

The effluent from tannery may contain concentrations of Cr between 2 and 5 g / l [23]. In Quebec, the use of Cr is much smaller because of its toxicity, but this will not lead to its total disappearance in the ecosystem. Maybe it will disappear from the air, soil and end up in the sea then in the fish we eat.

The use of the mechanism of photosynthesis for the detection of such pollution can be considered as a bioassay very simple and fast that can compete with the first bioassay developed, such as lethal toxicity bioassays performed with fishes[7] or bioassays sub-lethal toxicity also performed on fishes[21].

Chlorophyll fluorescence is very useful to study various fundamental aspects of photosynthesis. It is indicative of the photosynthetic activity and status of the device of photosynthesis. When plants previously adapted to darkness are illuminated, the intensity of chlorophyll fluorescence kinetics shows highly dependent photochemical reactions of photosynthesis[13].

The inhibition of these reactions by a pollutant induces a decrease in the conversion of light energy into chemical energy which is reflected in the kinetics of fluorescence. Analysis of the kinetics of fluorescence is used to evaluate several parameters used as biomarkers of operation of the photosynthesis[17]. In higher plants, it was shown that Cr (VI) alters the photosynthetic apparatus by acting on several sites in the electron transport chain photosynthétics[16].

The objective of this study is to evaluate the toxic effect of Cr (VI) on the functioning and structure of photosystem II (PSII) by analyzing the fast kinetics and multiphase (OPJI) using the PEA and Analysis of the kinetics modulated using PAM.

In this study we used the alga Scenedesmus obliquus as a bioindicator because of its high sensitivity to heavy metals.

2. CHLOROPHYLL FLUORESCENCE INDICATOR OF PHOTOSYNTHESIS

2.1 Introduction

The absorbed light energy that is not used for photochemistry of PSII can be dissipated by non-radiative pathways or by chlorophyll a fluorescence of PSII. The amount of energy dissipated by the fluorescence depends on the competition between these three routes of dissipation of energy. Dissipation of energy through photochemical associated with electron transport between PSII and PSI determines the yield and loss of fluorescence. The fluorescence can be used to study how the electron transport and mechanisms of energy transfer in photosynthesis. This approach has been used repeatedly to study the effects of pollutants and environmental conditions on photosynthesis. The fluorescence of chlorophyll a (HPLC a) represents only part of the energy collected by harvesting antennae of PSII that is not converted into chemical energy by charge separation in PSII reaction center. When a molecule of HPLC or receives a photon excitation energy transmitted by a neighboring molecule, it becomes unstable in an excited state of high energy. This form of HPLC returns to a lower energy level (steady state) in several ways: either by transferring an electron to a pheophytin molecule to the reaction center (photochemistry) or by transferring the excitation energy to a molecule neighbor, or by dissipating the energy as heat (non-radiative de-excitation) or either by emitting a photon (fluorescence). The fraction of energy dissipated by fluorescence depends mainly on the competition between the process of de-excitation. The de-excitation by photochemical means, and therefore the electron transport between PSII and PSI, plays an important role in the level of fluorescence dissipated. For this reason, the fluorescence can be used to study how the electron transport and mechanisms of energy transfer in photosynthesis, since all the elementary processes which constitute photosynthesis are highly interdependent.

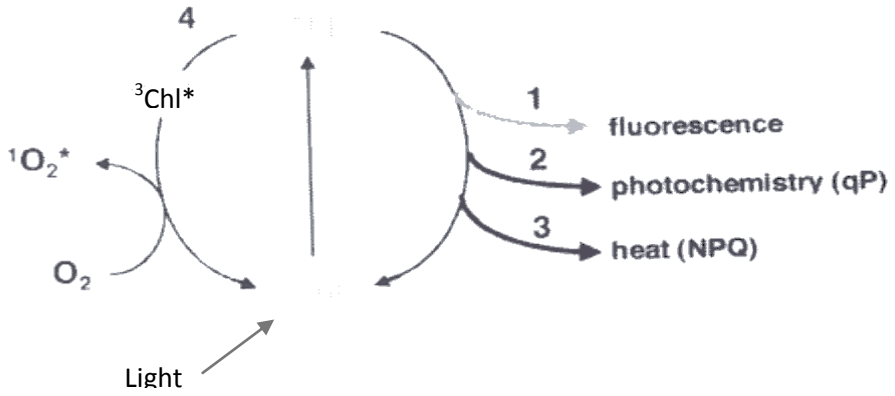

Figure1. Pathways of energy dissipation of excited chlorophyll (From Müller et al., 2001).

2.2 The fast kinetics and multiphase fluorescence

Kautsky and Hirsh (1931) had discovered that green algae, previously adapted to darkness, had a fluorescence yield which varied over time under continuous illumination. The variable fluorescence was then known until today as the "Kautsky effect". It is now well established that in vivo measurement of the fluorescence emission of HPLC (a) depends on the redox state of the primary electron acceptor of PSII, QA. When the fluorescence rises the QA is reduced QA and it decreases when the latter is re-oxide. Many transitions have been identified on the kinetics of fluorescence (OJIP), each representing different states of the photochemical activity of PSII and its interaction with the PSI.

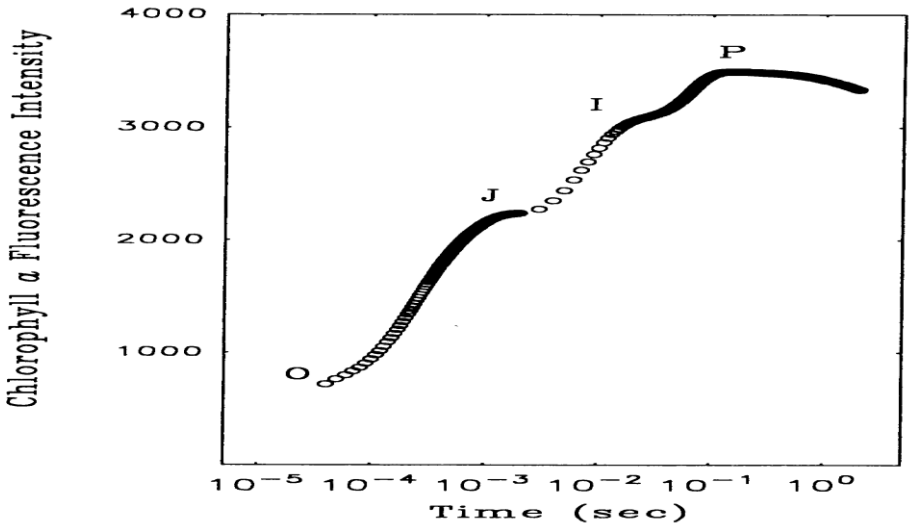

Figure2. Fluorescence induction curve showing transitions OJIP, measured on a logarithmic time scale (From Guissé et al. 1995).

2.3 The kinetics of the modulated fluorescence

The method for measuring the kinetics of fast polyphasic fluorescence is imprecise to determine the level of Fo (in an approximate way) which involves some error in this value and the resulting parameters. Furthermore, this measurement method does not detail the components of the fluorescence related to the energy dissipation (quenchings photochemical and non-photochemical).

By using a modulated fluorimeter introduced in the last twenty years, allows an adequate determination of Fo and a better understanding of the relationship between the fluorescence yield and photosynthetic functions. The principle

of this fluorometer based on the use of three types of light sources. The first light (analytical), used to measure the fluorescence intensity is too weak to change the state of redox PSII. This light source is modulated allowing the detection system to measure the fluorescence emitted only to the frequency and phase of the modulated light and so have no interference with other light sources. The second light source (actinic light) is used to induce photosynthesis. A third light source (light saturation) of very high intensity, used to close the reaction centers, allows the determination of yields and photochemical component of fluorescence on quenching photochemical and non-photochemical.

The constant fluorescence of a plant in dark-adapted (Fo) was measured using analytic modulated light (LM). The maximum fluorescence (Fm) is induced by a flash of light saturation (LS) which causes the reduction of all QA. Change in fluorescence level (F) under continuous illumination occurs with actinic light (LA) and which initiates transport of electrons. Simultaneously, the maximum level of fluorescence (F'M) is obtained using flashes of light saturation (LS) periodically every 40 seconds. In the steady state of electron transport, the actinic light is turned off and a far red light (RL) is used to obtain the level of Fo representing the fluorescence when all PSII reaction center is in an "open" state for a sample adapted to light (Figure.3).

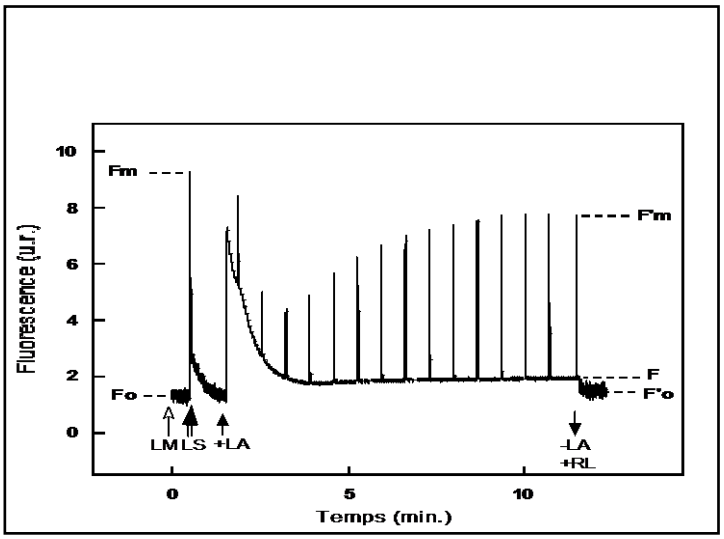

Figure 3. Modulated fluorescence kinetics obtained by the use of a fluorimeter WFP.

3. MATERIALS AND METHODS

The culture algae Scenedesmus obliquus was made in a two liter Erlenmeyer flask with an addition of one liter of sterile culture medium specific (Kessler). The flask is closed by a cap containing two holes, one for a long glass rod which is used to enter the air. This rod is connected by a pipe connected to a pump tank, and the other hole for a short glass rod which is used for air outlet. The two rods are provided with a syringe filter containing pores of 0.2 microns to avoid contamination of the culture.

3.1 Treatment in Chrome

Algae were exposed to different concentrations of chromium (K2Cr2O7)0.25, 0.50, 1, 5, 10 ppm for 24 and 48h.The cultures were incubated at 26°C under an illumination of 88 containing µmol.m-2.S-1.

After exposure, their chlorophyll were retrieved in methanol (by the method described below), then measured by spectrophotometry to calculate the volume of the solution of seaweed necessary for filtration. Algae collected on filters were measured by fluorimetry.

3.2 Measurement of chlorophyll fluorescence kinetics

Algae have been previously adapted to darkness for 30 min prior to fluorescence measurements. The kinetics of induction and rapid polyphasic chlorophyll fluorescence has been measured with a fluorometer Handy PEA (Plant Efficiency Analyser). The induction kinetics of fluorescence was obtained by a flash of light with an intensity of 3000 µmol.m-2.S-1.

The kinetics of modulated chlorophyll fluorescence was measured with a fluorometer PAM (Pulse Amplitude Modulation).The fluorescence Fo is measured by applying a continuous and modulated low intensity when all PSII reaction centers are open. A saturating flash was used to evaluate the maximum fluorescence Fm, where all reaction

centers are closed. Actinic light is used to measure the continuous fluorescence emission reaching a steady state and during which the maximum fluorescence Fm 'is evaluated periodically (every 40 seconds). A distant red light is applied to oxidize the electron transport chain to measure the level of fluorescence Fo '.

3.3 Protocol for extraction of chlorophyll in methanol

1. Take 1ml of the suspension of seaweed and put it in a centrifuge tube;
2. Centrifuge for 10 minutes at 9800 xg and 5 ° C;
3. Getting rid of the supernatant and resolubilized pellet with 2 ml of algae in 100% methanol;
4. Place in a bath at 65 ° C for 10 minutes with marbles to prevent evaporation of methanol;
5. Let the methanol stands until it reaches room temperature;
6. Centrifuge for 10 minutes at 9800 xg and 5 ° C;
7. Get about 1.5 ml of supernatant;
8. Take the absorbance at wavelengths of 652.4 nm and 665.2nm;
9. The formula for determining the total concentration of chlorophyll is (24.93* ABS652.4 +1.44* ABS665.2) * 2/1 (2/1: dilution factor)

4. RESULTS AND DISCUSSION

From the results obtained, we noticed that the change in fluorescence caused by chromium was proportional to the concentrations used.

4.1 Concentration of chlorophyll

After exposing the algae to different concentrations of Cr we calculated the concentration of chlorophyll solutions for our various algae; we found that the chlorophyll concentration decreased with increasing Cr concentration. This makes sense especially after 48hours. Therefore, the Cr exposure causes a decrease in the synthesis of chlorophyll in the alga Scenedesmus obliquus. With prolonged duration of exposure there is a significant decrease in the synthesis of chlorophyll.(Fig. 4)

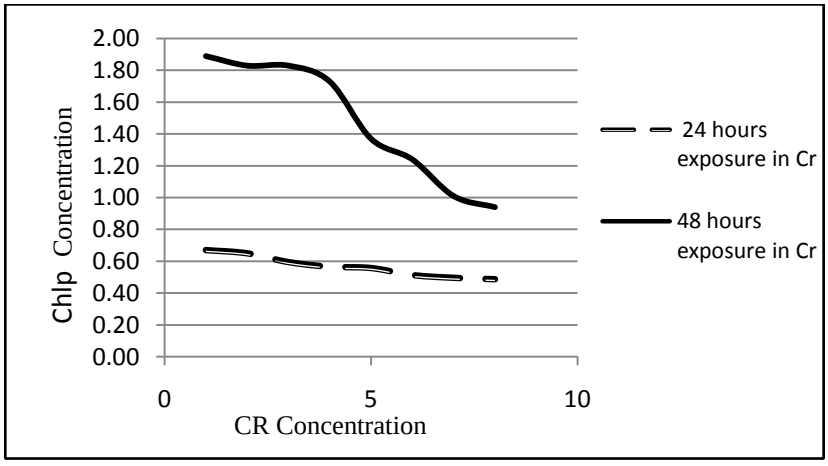

Figure 4. Concentration of chlorophyll after exposure to Cr at various concentrations for 24h and 48h

4.2 The measurement of fast kinetics

The measurement of fast kinetics and multiphase (OPJI) shows that after an adaptation of algae in the dark, the fluorescence yield for the transition O (Fo) is the emission of light energy by chlorophyll antenna of PSII, excitement before going to the reaction center. At this level, the reaction centers are open and the QA is completely oxidized, because the energy is not sufficient to induce the separation of charge[12]. Normally, the yield of Fo is constant, however, the performance change of Fo is an alteration of pigment-protein complexes associated with PSII. This was not observed after exposure to Cr for 24 h (Fig. 5), however, algae exposed for 48 h treated with 10 ppm Cr had an increase of Fo, Fo value increases when the protein II reaction center are damaged or when the chlorophyll antennae are disconnected from the PSII complex under the effect of environmental stress [2, 13]. This explains an alteration of light harvesting antennae of PSII to a concentration of 10 ppm after exposure for 48 h. (Fig. 6). When the excitation energy is sufficiently high collared to the reaction center of PSII charge separation may be

effected by the reaction of the photolysis of water (which is the electron donor), the primary acceptor electron (QA) is reduced causing an increase in the fluorescence to the transition (J) [20]. This indicates the efficiency of electron transport through the QA[5], [12].

Our results show that 24 hours exposure show a small decrease for the three elevated 1, 5and 10 ppm concentration of Cr. On the other hand, after 48hours of exposure, the decrease is very remarkable, proving that there was an alteration of the electron transport via the primary acceptor QA.

The level of fluorescence during the transition (JIP) determines the rate of reduction of plastoquinone pool (which will transfer the electrons to PSI) [4].

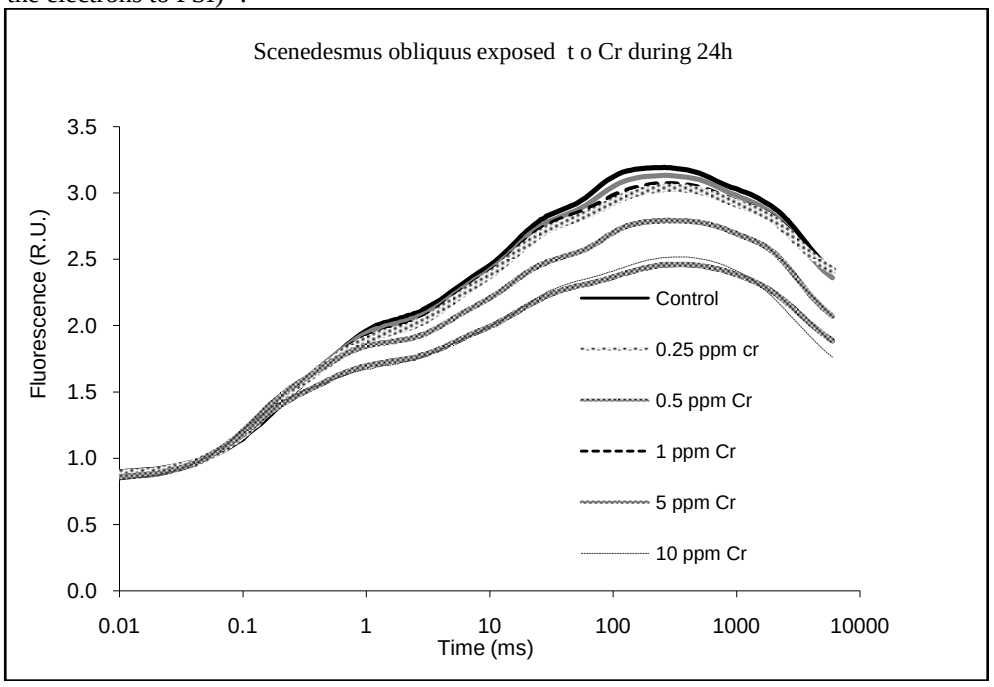

Figure 5. Induction of fluorescence affected by different concentrations of chromium after exposure for 24 hours

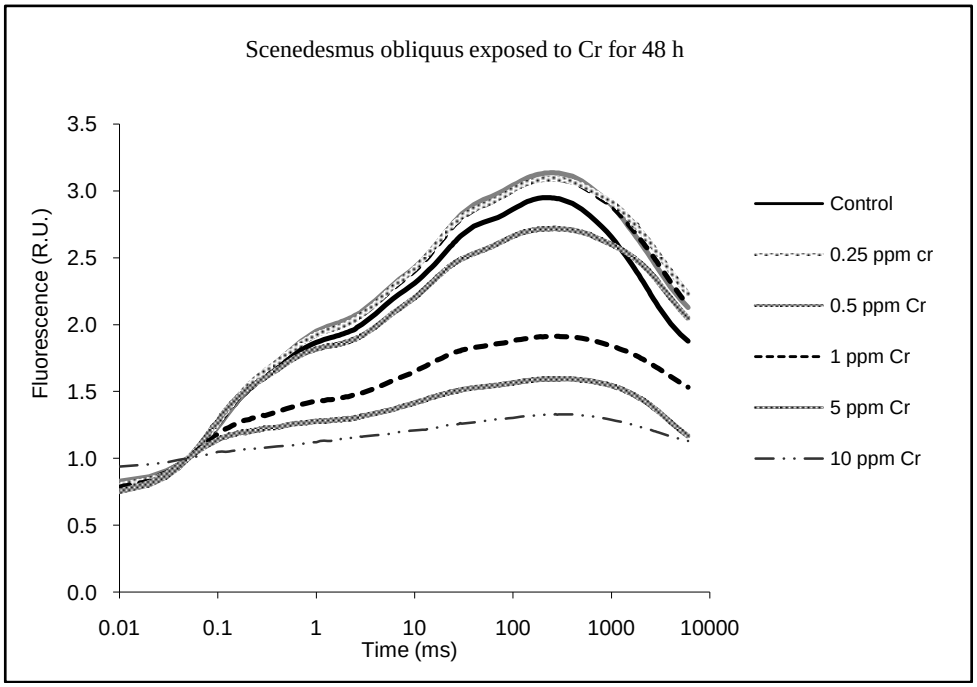

Figure 6. Induction of fluorescence affected by different concentrations of chromium after exposure for 48 hours

We note that the maximum fluorescence Fm decreases from levels 1, 5 and 10 ppm of Cr (Fig. 7) so the system of electron transport between QA and the PQ is affected.
For a plant adapted to darkness, the photochemical efficiency of PSII (ΦM) can be calculated as following: ΦM = Fm-Fo/Fm = Fv / Fm[1].
This parameter represents the quantum yield of electron transfer reaction center P680 to primary electron acceptor QA3. In this study we observed a significant decrease in the ratio Fv / Fm (Fig. 8), especially after exposure for 48 hours and this for the highest concentrations of Cr 1, 5 and 10 ppm which brings us to say that the electron transfer reaction center P680 and the primary acceptor QA begins to affect the concentration from 100uM.

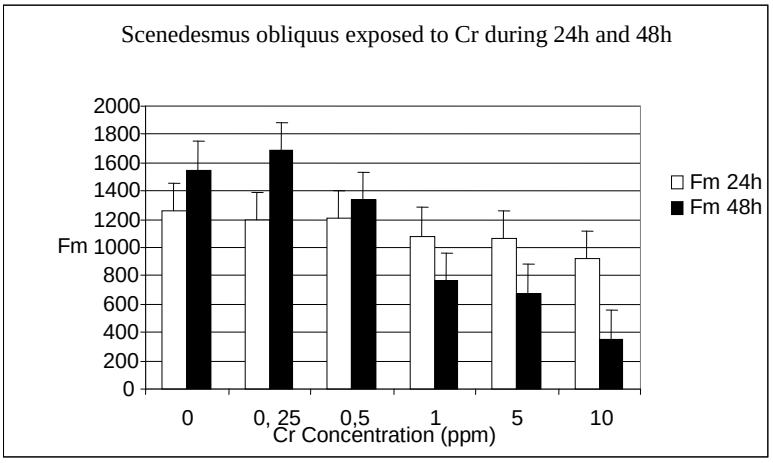

Figure 7.Effect of chromium at different concentrations on the maximum fluorescence Fm

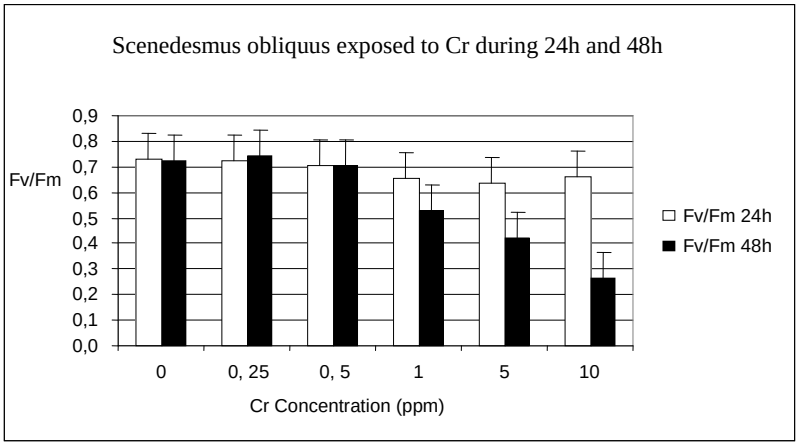

Figure 8. Effect of chromium at different concentrations on the ratio Fv / Fm

4.4 The measurement of the kinetics of modulated fluorescence

The technique of modulated fluorescence (PAM) allows a better understanding of the mechanisms of energy dissipation in photosystems[17,22].The use of a modulated light actinic and saturation, allows the analysis of chlorophyll fluorescence (a) at different stages of oxidation of electron carriers associated with PSII and PSI[20]. After adaptation of algae in the dark then submit them to a modulated light of low intensity, the absorbed energy is sufficient to induce a charge separation in reaction center (P680) which is in an open state (oxidized), so that modulated light will allow measurement of fluorescence Fo which is not always constant and depends on the state of energy transfer between the light harvesting antennae and reaction centers of PSII. In our results the Fo was not influenced by treatment with Cr, therefore,Cr concentrations used at an exposure of 24 hours (Fig. 9) do not affect the light harvesting antennae of PSII. It is the same conclusion that wehad when we were using the PEA.

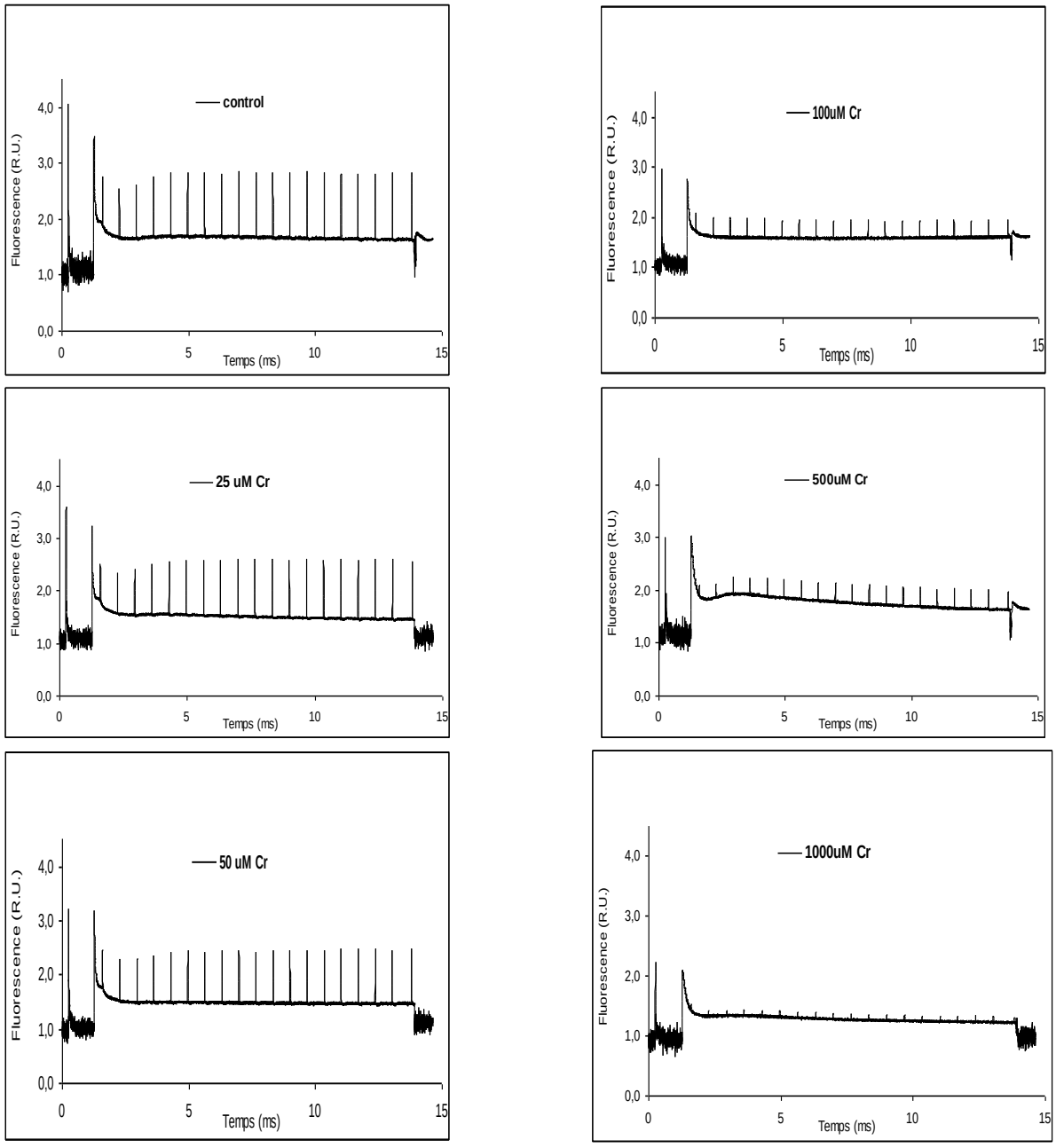

Figure 9. Kinetics of modulated fluorescence of Scenedesmus obliquus treated by the Chromium exposure of 24 hours, obtained by using a PAM Fluorometer

By saturating flashes of light it permits us to determine the maximum yield of fluorescence Fm, when PSII reaction centers are closed, so the QA is reduced. We noted that the Fm decreases as the Cr concentration increases; this proves that Cr affects electron transport system via the QA. The ratio Fv / Fm represents the quantum yield of electron transport in the reaction center primary acceptor QA (for algae adapted in the dark), the results show a decrease with increasing concentration of Cr (Fig. 10).
The illumination of the algae by actinic light (light energy in which the photosynthetic apparatus function normally) is used to analyze the kinetics of fluorescence linked to electron transport between PSI and PSII. Sending flashes of saturated light permits the fluorescence to obtain the yield of Fm ', where the reaction centers of PSII will closed [6, 11].
We observed a significant decrease in the Fm 'with increasing Cr concentration, especially at a concentration of 10 ppm, the Fm' is almost zero, this explains a complete alteration of the electron transport system

Actinic light is turned off and a distant red light is used to obtain the fluorescence yield of Fo' which represents the reaction center of PSII in the open state when the plant is adapted to the light[11, 20]. At this level, a proportion of PSII reaction centers closed in these lighting conditions, the photochemical efficiency of PSII quantum yield is proportional to the energy transfer between P680 and QA in the steady state transport of electrons. From the values of Fo ', Fm' representing distinct transitions on the kinetics of modulated fluorescence, we can calculate Avery significant parameter which is ΦM' qui; that represents the operational photochemical efficiency of PSII.
ΦM '= Fm'-Fo' / Fm '= Fv' / Fm'[8].
We find that the ratio Fv '/ Fm' decreases with increasing Cr concentration of 10 ppm (Fig. 11), this means that the balance in the regulation processes of electron transport between PSII and PSI is impaired.
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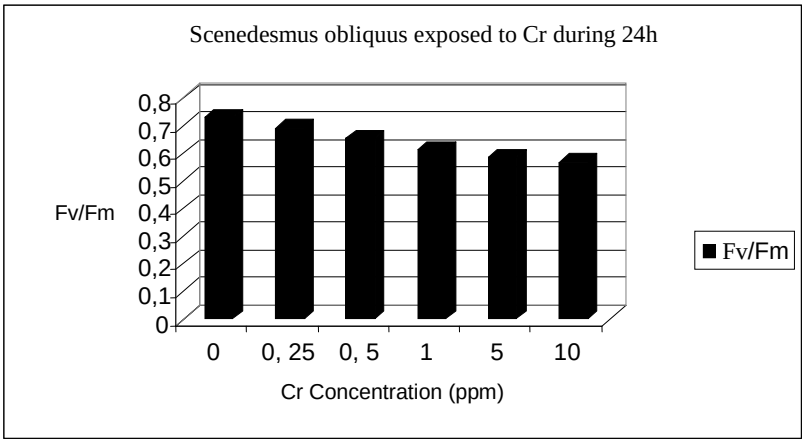

Figure 10. Effect of chromium at different concentrations on the ratio Fv / Fm

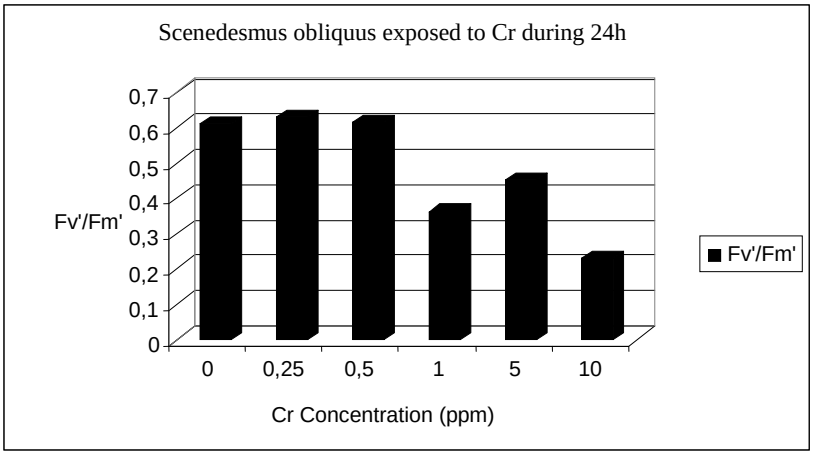

Figure 11. Effect of chromium at different concentrations on the ratio Fv '/ Fm'

5. CONCLUSION

The Cr affects the photosynthetic apparatus of algae:

- At 10 ppm Cr, there was an increase in the fluorescence Fo, therefore an alteration of light harvesting antennae of PSII.
- From 1 ppm Cr we noticed an important decrease in fluorescence, which shows a degradation of the electron transfer system.
- The Fv / Fm decreased when the concentration of Cr increases, as a consequence of damage to PSII.
- The Fv '/ Fm' decreases when the concentration of Cr increases, in fact impaired electron transfer between PSII and PSI.

6. GENERAL CONCLUSION

The use of chlorophyll fluorescence as an indicator of toxicity of chromium is a simple and fast method. It gives us an indication of the photosynthetic apparatus of the algae. Therefore, we could use this as bioassays to detect environmental stress.

7. ACKNOWLEDGEMENTS

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