Pol. J. Environ. Stud. Vol. 32, No. 3 (2023), 2589-2600
DOI: *10.15244/pjoes/161517* ONLINE PUBLICATION DATE: 2023-04-03

Original Research

Construction of a New Response Index for Sensitive Detection of the Toxicity of Photosynthetic Inhibitory Herbicides to Photosynthesis of *Chlorella pyrenoidosa* Based on Change Characteristics of Chlorophyll Fluorescence Rise Kinetics Curve

Tingting Gan[1, 2, 3], Nanjing Zhao[1, 2, 3]*, Gaofang Yin[1, 2, 3], Xiaoxuan Tan[1, 2, 3], Ying Wang[1, 2, 3]**

[1]Key Laboratory of Environmental Optics and Technology, Anhui Institute of Optics and Fine Mechanics, Hefei Institutes of Physical Science, Chinese Academy of Sciences, Hefei, 230031, China
[2]University of Science and Technology of China, Hefei, 230026, China
[3]Key Laboratory of Optical Monitoring Technology for Environment of Anhui Province, Hefei, 230031, China

Received: 10 December 2022
Accepted: 21 February 2023

Abstract

Photosynthetic inhibitory herbicides widely used to control weeds, such as triazine herbicides, will have toxic effects on the photosynthesis of microalgae once entering aquatic environment, thus posing serious threats to the ecological balance of aquatic systems. However, at present, in terms of toxicity detection of triazine herbicides to microalgal photosynthesis, the commonly used photosynthetic fluorescence parameters such as the maximum photochemical quantum yield of photosystem II (F_V/F_M) and performance index (PI_{ABS}) are not sensitive enough in response to the toxicity of triazine herbicides. In order to seek for a suitable response index which can be used to rapidly and sensitively predict the toxicity of triazine herbicides to the photosynthesis of microalgae, in this study, a common freshwater microalgae *Chlorella pyrenoidosa* was used as the test organism, and the impacts of four triazine herbicides atrazine, terbuthylazine, propazine and simazine on the chlorophyll fluorescence rise kinetics (OJIP) curve of *C. pyrenoidosa* were first investigated. On this basis, a novel photosynthetic response index (PI) for detecting the toxicity of triazine herbicides was successfully constructed according to the change characteristics of OJIP curve of exposed *C. pyrenoidosa*, then its response performance to triazine herbicide toxicity was further verified. The results indicate that the significant changes in OJIP curves of exposed *C. pyrenoidosa* compared with the control were mainly caused by the increases

*e-mail: njzhao@aiofm.ac.cn
**e-mail: gfyin@aiofm.ac.cn

of fluorescence intensity of O-step (F_o) and variable fluorescence intensity between J-step and O-step ($Fv_{(J-O)}$) and the reductions of variable fluorescence intensities between I-step and J-step ($Fv_{(I-J)}$) and between P-step and I-step ($Fv_{(P-I)}$) of OJIP curve. The constructed PI with F_o, $Fv_{(O-J)}$, $Fv_{(J-I)}$, $Fv_{(I-P)}$ of OJIP curve as variables showed very good Logistic curve concentration-response relationships with each triazine herbicide. By verification, the response sensitivity of PI to each triazine herbicide were all superior to that of F_V/F_M and PI_{ABS}, and PI showed good agreement with PI_{ABS} in predicting the toxicity ranking of different triazine herbicides to *C. pyrenoidosa* photosynthesis. Therefore, the constructed PI was a more suitable and reliable response index for sensitively detecting the toxicity of triazine herbicides to microalgae photosynthesis. This study provides a new and optional index for sensitive determination of the toxicity of triazine herbicides in water based on chlorophyll fluorescence induction kinetics technology.

Keywords: toxicity, triazine herbicide, microalgae, photosynthesis, OJIP curve

Introduction

In modern agricultural production, herbicides play an important role in improving the quality and output of agricultural products, so they have become essential agricultural inputs in agricultural development. Among the many commercially available herbicides, triazine herbicides have been extensively used in agricultural production to control the grassy and broadleaf weeds [1-3]. However, only a small part of the applied herbicides can be effectively used, and the remaining nearly 70% will enter the aqueous system through rainfall, irrigation, surface or underground runoff [4]. In recent years, triazine herbicides have been frequently detected in surface water and coastal seawater in many countries [5-7]. Due to the 1,3,5-triazine ring (C_3N_3) structure in their molecules, triazine herbicides have the characteristics of stable chemical property and long half-life [3, 8]. Moreover, triazine herbicides also have the property of biotoxicity [9, 10]. Therefore, the contamination of aquatic environments by triazine herbicides and their toxic effects on aquatic organisms have attracted worldwide extensive attentions.

The reason that triazine herbicides can be used to effectively control weeds in agriculture is because they belong to photosynthetic inhibitors, so triazine herbicides have inhibiting effect on the photosynthesis of plants. The mechanism of triazine herbicides inhibiting photosynthesis is that they target the D1 protein of plant photosystem II (PSII) so as to block the photosynthetic electron transfer and hinder the exciting energy transfer [1, 4, 11]. In aquatic ecosystems, microalgae as an important class of photosynthetic organisms, are at the forefront of aquatic food chain and are the most important primary producers and energy converters [12, 13]. So microalgae play a vital role in maintaining the normal structure and function of aquatic ecosystems. However, once triazine herbicides enter the aquatic environment, microalgae are the most vulnerable aquatic organisms not only due to their small cell size and sensitivity to toxicants [14], but also because the toxicity of triazine herbicides have severe impacts on their photosynthesis [3].

Photosynthesis as an important physiological process of microalgae, has multiple important functions for aquatic ecosystems. Through photosynthesis, microalgae can fix carbon and produce biological macromolecules such as proteins, lipids and carbohydrates, which form the basis for the primary productivity of aquatic ecosystems and the aquatic food webs [4, 15]. Therefore, the photosynthesis of microalgae is extremely important in maintaining the normal material circulation and energy flow of aquatic ecosystem. However, the toxic effects of triazine herbicides on the photosynthesis of microalgae seriously affect the primary productivity of aquatic ecosystems, and pose potential risks to the aquatic ecological environment [3, 5]. In this way, accurate detection of the toxicity of triazine herbicides in water to the photosynthesis of microalgae is of great significance for assessing the quality of aquatic environment and predicting the risk of aquatic ecosystem.

As we all know, chlorophyll fluorescence as a by-product of plant photosynthesis process, is closely related to the photosynthesis state of plant. When the photosynthesis of plants is affected by toxic substances or adverse environmental factors, the photosynthetic state of plants will change, and at this time chlorophyll fluorescence will also change accordingly. So chlorophyll fluorescence is vividly known as the probe of plant photosynthesis [16]. Based on this, intravital chlorophyll fluorescence induction kinetics technology, as a simple, fast, non-destructive and in vivo fluorescence detection technology, has become an important tool for studying the photosynthetic process and rapidly analyzing the photosynthetic state of living plants [17-20], because it can rapidly and conveniently obtain the fluorescence information such as chlorophyll fluorescence rise kinetic (OJIP) curve and various photosynthetic fluorescence parameters to characterize the photosynthetic state of plants. For this reason, chlorophyll fluorescence induction kinetics technique has also become a very favorable tool for rapid detection of the toxicity of pollutants to the photosynthesis of microalgae.

At present, in terms of detecting pollutant toxicity to microalgal photosynthesis based on chlorophyll fluorescence induction kinetics technique, although some photosynthetic fluorescence parameters, such as the maximum photochemical quantum yield of PSII (F_V/F_M), and the performance index of PSII photosynthetic activity (PI_{ABS}), have been often used as response indexes to evaluate the influence degree of pollutants on the photosynthesis of microalgae [12, 17, 21]. However, for chlorophyll fluorescence induction kinetics technology, the measured OJIP curve is the most direct and visualized reflection of plant photosynthetic state, which can display the detailed information on the structure and function of photosynthetic apparatus, such as the state and function of PSII reaction centers (RCs), the light-harvesting antenna complexes (LHC), and both the donor and acceptor sides of PSII [19, 22, 23]. When the toxic effects of pollutants on photosynthesis cause changes in the photosynthetic state of microalgae, the OJIP curve of microalgae will change accordingly. So compared with the commonly used photosynthetic fluorescence parameters, the OJIP curve is really the important basis for accurate determination of pollutant toxicity to microalgal photosynthesis based on chlorophyll fluorescence induction kinetics technology. The changes in OJIP curve of microalgae exposed to pollutants compared with the control are the key to truly and accurately reflect the impacts of pollutants on the photosynthesis of microalgae. In contrast, if the commonly used photosynthetic fluorescence parameters such as F_V/F_M are directly used as response indexes to detect the toxicity of pollutants, these parameters may not correspond well to the change characteristics of the OJIP curve of exposed microalgae compared with the control, so the toxicity of pollutants may be underestimated. Therefore, it is extremely necessary and important to detect the toxicity of pollutants based on the change characteristics of the OJIP curve of exposed microalgae to improve the accuracy and sensitivity of assessing the effect degree of pollutant toxicity on the photosynthesis of microalgae. However, at present, the studies on the toxicity detection of pollutants to microalgae based on the change characteristics of OJIP curve were few. In view of the toxic effect of triazine herbicides on the photosynthesis of microalgae in the aquatic ecosystem, finding a more suitable response index to accurately detect the toxicity of triazine herbicides to microalgae photosynthesis according to the change characteristics of OJIP curve after exposure is of great importance and significance for accurately evaluating the toxicity of triazine herbicides and predicting their risk to aquatic ecosystem.

In view of this, in this study, in order to seek for a suitable response index based on the change characteristics of OJIP curve of microalgae to sensitively and accurately detect the toxicity of triazine herbicides to the photosynthesis of microalgae, a common freshwater microalgae *Chlorella pyrenoidosa* was used as the test organism, and chlorophyll fluorescence induction kinetics technique was adopted to first investigate the effects of four triazine herbicides including atrazine, terbuthylazine, propazine and simazine on the OJIP curve of *C. pyrenoidosa*. Based on the change characteristics of the OJIP curve of *C. pyrenoidosa* exposed to the four triazine herbicides compared with the control, a new photosynthetic response index (PI) suitable for detecting the toxicity of triazine herbicides was constructed. Then the response performances of PI to the toxicity of triazine herbicides were further verified by compared with F_V/F_M and PI_{ABC}. The newly constructed response index in this study will be helpful to sensitively and accurately detect the toxicity of triazine herbicides and other herbicides with the same photosynthetic inhibition mechanism to microalgae and rapidly assess their risk to aquatic ecosystem.

Materials and Methods

Test Organism and Culture Conditions

The microalgae *C. pyrenoidosa* (FACHB-5), a common freshwater green algae, was purchased from the Freshwater Algae Species Bank of the Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China), and was used as the test organism in this study. BG11 medium (main components include $NaNO_3$, K_2HPO_4, $MgSO_4$, $CaCl_2$, ammonium ferric citrate, citric acid, EDTA, Na_2CO_3, H_3BO_3, $MnCl_2$, $ZnSO_4$, $CuSO_4$, $NaMoO_4$, $Co(NO_3)_2$) and 250 mL culture flasks were used for the culture of *C. pyrenoidosa* [24]. Before use, all the culture flasks and the medium were sterilized by autoclaving in a YM 50 pressure steam sterilizer (Shanghai Sanshen Medical Equipment Co., Ltd., China) at 121ºC for 30 min. After aseptically inoculated into sterile BG11 medium, *C. pyrenoidosa* was placed and cultured in a MQD-B3G constant temperature incubator (Shanghai Minquan Instrument Co., Ltd., China) with white cold fluorescent tubes as the light source. The culture conditions of *C. pyrenoidosa* were as follows: the culture temperature was (25±1) ºC; and the light intensity was 120 μmol m^{-2} s^{-1} with a light and dark cycle of 12 h : 12 h [25]. All the culture flasks were shaken 3 times by hand daily. The cell density of the algal culture was determined daily using an ECLIPSE Ni-U biological fluorescence microscope (Nikon Corporation, Tokyo, Japan). Then the healthy *C. pyrenoidosa* incubated for 3-4 days to reach the exponential growth phase was used for the exposure experiments of triazine herbicides [4, 26].

Triazine Herbicides

Four triazine herbicides used in this study, atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine, CAS: 1912-24-9, 97% purity), terbuthylazine (2-tert-butylamino-4-chloro-6-ethylamino-1,3,5-triazine,

CAS: 5915-41-3, 99% purity), propazine (2,4-bis(isopropylamino)-6-chloro-1,3,5-triazine, CAS: 139-40-2, 99% purity) and simazine (2,4-bis(ethylamino)-6-chloro-1,3,5-triazine, CAS: 122-34-9, 98% purity), were all analytical standard and were all purchased from Aladdin (Shanghai, China). Dimethyl sulfoxide (DMSO, CAS: 67-68-5, purity≥99.5%) was of analytical grade and was obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). For each triazine herbicide, a stock solution with a concentration of 100 mg L^{-1} was first prepared by dissolving the corresponding herbicide in DMSO. Then, a series of working solutions of each triazine herbicide with different concentrations were prepared by diluting different volumes of the corresponding herbicide stock solution with DMSO and were used for the exposure experiments of *C. pyrenoidosa* to triazine herbicides. For a series of atrazine, propazine and simazine working solutions, the concentrations of atrazine, propazine and simazine were in the range from 1.001 to 60.060 mg L^{-1}, respectively. The concentrations of terbuthylazine in a series of terbuthylazine working solutions ranged from 0.501 to 60.060 mg L^{-1}.

Triazine Herbicides Exposure Experiments

The exposure experiments of *C. pyrenoidosa* to each triazine herbicide were performed according to the method described by Majewska et al. (2018) [11] with minor modification. Before exposure to each triazine herbicide, the *C. pyrenoidosa* culture was diluted with sterile BG11 medium so that the initial cell density of the algal suspension reached 5×10^5 cells mL^{-1}. Then 0.05 mL of each triazine herbicide working solutions were added into aliquots of 50 mL of *C. pyrenoidosa* suspension, after that each mixture was shaken by hand to obtain a series of treatments by each triazine herbicide among atrazine, terbuthylazine, propazine and simazine. The controls were prepared by adding 0.05 mL of DMSO into aliquots of 50 mL of *C. pyrenoidosa* suspension. The final concentration of DMSO in each treatment and control was 0.1% (*v*/*v*) and it has been confirmed that this concentration had no significant effect on the photosynthesis and chlorophyll fluorescence of *C. pyrenoidosa* according to our pre-experiment and the results reported by Majewska et al. (2018) [11] and Aksmann et al. (2008) [27]. Three replicates were performed for each treatment and control. The initial concentrations of herbicides in the treatments of each triazine herbicide were as follows: the initial atrazine concentrations of atrazine treatments were in the range from 1 to 60 μg L^{-1}; the initial terbuthylazine concentrations of terbuthylazine treatments ranged from 0.5 to 60 μg L^{-1}; the initial propazine concentrations of propazine treatments were in the range from 1 to 60 μg L^{-1}; and the initial simazine concentrations of simazine treatments ranged from 1 to 60 μg L^{-1}. Then all the controls and treatments were placed in the incubator and incubated under the same culture conditions as before the exposure experiment. According to our previous works, trazine herbicides such as atrazine have rapid toxic effects on the photosynthesis of *C. pyrenoidosa* [25], so after a short-term exposure of 1 h, the OJIP curve of each test algae sample was measured.

OJIP Curve Measurement

All the test algae samples were dark-adapted for 15 min before the measurements of chlorophyll fluorescence rise kinetics OJIP curves in order to allow the PSII RCs to open (re-oxidize) and the electron transport chain to be fully oxidized [28]. Then the OJIP curve of each test algae sample was measured at room temperature by a AquaPen AP110/C Handheld Algae Fluorescence Meter (Photon Systems Instruments, Czech Republic) with a blue light source of 455 nm and a saturation light pulse intensity of 1800 μmol (photons) m^{-2} s^{-1} according to the method described by Pokara et al. (2017) [29]. During the measurement of OJIP curve, fluorescence intensity data from 20 μs to 2 s were recorded with a varying sampling rate, specifically: recording a data per 10 μs from 20 μs to 610 μs, per 100 μs from 1 ms to 13.9 ms, per 1 ms from 15 ms to 89 ms, and per 10 ms from 90 ms to 2 s. The recorded 360 fluorescence intensity data before 1000 ms were used to draw the OJIP curve of each test algae sample.

Several basic fluorescence intensity data in the OJIP curve as follows were used in this study: F_O was the initial fluorescence level of OJIP curve, corresponding to the fluorescence intensity of O-step at 20 μs in the OJIP curve at which all RCs were open; F_J was the fluorescence level of J-step in the OJIP curve, corresponding to the fluorescence intensity at 2 ms; F_I was the fluorescence level of I-step in the OJIP curve, corresponding to the fluorescence intensity at 30 ms; and F_M was the maximal fluorescence intensity of OJIP curve, which was equal to the fluorescence level of P-step in the OJIP curve, and was also denoted as F_P [23].

Some Photosynthetic Fluorescence Parameters Calculation and A New Photosynthetic Response Index PI Construction

Three variable fluorescence parameters $Fv_{(J-O)}$, $Fv_{(I-J)}$ and $Fv_{(P-I)}$ were calculated based on the above basic fluorescence intensity data to analyze the change characteristics of the OJIP curve of *C. pyrenoidosa* after treatment with each triazine herbicide. The variable fluorescence parameter $Fv_{(J-O)}$ refers to the variation in fluorescence intensity between J-step and O-step of OJIP curve and it was calculated according to Equation (1):

$$Fv_{(J-O)} = F_J - F_O \quad (1)$$

The variable fluorescence parameter $Fv_{(I-J)}$ refers to the variation in fluorescence intensity between I-step and J-step of OJIP curve and it was calculated as Equation (2):

$$Fv_{(I-J)} = F_I - F_J \quad (2)$$

The variable fluorescence parameter $Fv_{(P-I)}$ refers to the variation in fluorescence intensity between P-step and I-step of OJIP curve and it was calculate according to Equation (3).

$$Fv_{(P-I)} = F_P - F_I \quad (3)$$

Furthermore, the photosynthetic fluorescence parameters F_v/F_M was also calculated based on F_M and F_O according to Equation (4):

$$F_V/F_M = (F_M - F_O)/F_M \quad (4)$$

which represents the maximum photochemical quantum yield of PSII [30] and has previously been frequently used in the evaluation of photosynthetic state of plants under stress [31]. Another photosynthetic fluorescence parameters PI_{ABS} was also directly obtained from JIP-test according to equation (5), which refers to the performance index for energy conservation from photons absorbed by PSII to the reduction of intersystem electron acceptors:

$$PI_{ABS} = [\gamma_{RC}/(1-\gamma_{RC})]\times[\varphi_{po}/(1-\varphi_{po})]\times[\Psi_{Eo}/(1-\Psi_{Eo})] \quad (5)$$

where, γ_{RC} denoted the probability that a PSII chlorophyll molecule functioned as RC; φ_{Po} denoted the maximum quantum yield for primary photochemistry; and ψ_{Eo} denoted the probability that an electron moved further than Q_A^- [12].

Moreover, a new photosynthetic response index PI for response to the toxicity of triazine herbicides to *C. pyrenoidosa* was constructed based on the four key elements of OJIP curve, Fo, $Fv_{(J-O)}$, $Fv_{(I-J)}$ and $Fv_{(P-I)}$, and the calculation model of PI was shown as Equation (6):

$$PI = \frac{Fv_{(I-J)}\times Fv_{(P-I)}}{F_o\times Fv_{(J-O)}} = \frac{(F_I-F_J)\times(F_P-F_I)}{F_o\times(F_J-F_o)} \quad (6)$$

Then the constructed PI and the photosynthetic fluorescence parameters F_V/F_M and PI_{ABS} were used as response indexes to detect the toxicity of the four triazine herbicides to the photosynthesis of *C. pyrenoidosa*.

Date Processing and Statistical Analysis

Statistical analyses were carried out using SPSS 19.0 software. For the key elements of the OJIP curve such as Fo, $Fv_{(J-O)}$, $Fv_{(I-J)}$ and $Fv_{(P-I)}$ and the response indexes PI, F_V/F_M and PI_{ABS}, the statistical significance of the difference between the control group and each treatment group was determined by one-way ANOVA analysis of variance with Tukey post-hoc multiple test. $P<0.05$ (*) was considered that the treatment group was significantly different from the control group. In order to detect the toxicity of the four triazine herbicides to *C. pyrenoidosa* photosynthesis, the inhibitions rates of different response indexes of *C. pyrenoidosa* induced by each triazine herbicide with different concentrations were calculated according to following Equation (7):

$$I_t(\%) = [(R_{ct}-R_{tt})/R_{ct}]\times 100\% \quad (7)$$

where R_{tt} was the response index of treatment at the exposure time of t; R_{ct} was the response index of control at the exposure time of t; and $I_t(\%)$ was the inhibition rate of response index of *C. pyrenoidosa* after exposure to triazine herbicide for the time of t. All the concentration-response relationships between the response indexes and each triazine herbicide were fitted using logistic curve [32]. According to the concentration-response curves, the median effective concentration (EC_{50}) values of each triazine herbicide at the exposure time of 1 h were calculated [4].

Results and Discussion

Change Characteristics of OJIP Curves of *C. Pyrenoidosa* Exposed to Four Triazine Herbicides

The chlorophyll fluorescence rise kinetic OJIP curves (less than 100 ms) of *C. pyrenoidosa* exposed to different concentrations of atrazine, terbuthylazine, propazine or simazine for 1 h were shown in Fig. 1. For the control, the OJIP curve of *C. pyrenoidosa* had two obvious transients in the process of rising from O-step (the starting point of OJIP curve) to P-step (the time corresponding to the maximum fluorescence intensity of OJIP curve), namely J-step (about 1 ms) and I-step (about 20 ms), respectively. According to related reports, the O-step refers to that after a period of dark adaptation, all the electron acceptors of PSII of *C. pyrenoidosa* including the primary quinone electron acceptors (Q_A), secondary electron acceptors (Q_B) and plastoquinone (PQ) pools lost electrons and were oxidized. So the PSII RCs was in a completely open state and could receive light quantum to the greatest extent. At this time, the fluorescence emission was minimal [24, 32]. When *C. pyrenoidosa* was irradiated with strong light, the electrons generated by the excitation of PSII RCs were transferred to Q_A through demagnesium chlorophyll (Pheo) in order to reduce Q_A and generate Q_A^-. However, because Q_B could not accept electrons in time to oxidize Q_A^-, Q_A^- would accumulate in large quantities, which made the fluorescence rise rapidly with time, resulting in the generation of J-step. So J-step reflected the instantaneous large accumulation

of Q_A^- [17]. Q_B accepted electrons from Q_A^-, which caused Q_B^{2-} to accumulate continuously so that Q_B could not accept electrons from Q_A^- in time. Therefore, Q_A and Pheo were completely reduced, and at this time the PSII RCs were completely closed and no longer accepted light quantum, which resulted in a maximum fluorescence emission and the appearance of P-step [23, 32, 33]. Moreover, the appearance of I-step may be due to the heterogeneity of the PQ pools in the process of electron transfer from Q_A^- to Q_B [33]. Therefore, the OJIP curve of *C. pyrenoidosa* provided considerable information about the photosynthetic process and photosynthetic state of *C. pyrenoidosa*.

As photosynthetic inhibitors, it can be seen from Fig. 1 that all the four triazine herbicides atrazine, terbuthylazine, propazine and simazine had the same and important influence on the OJIP curve of *C. pyrenoidosa*. For the OJIP curves of *C. pyrenoidosa* exposed to each triazine herbicide, the most obvious changes were that the fluorescence intensity of J-step increased significantly, and the height of the overall OJIP curve was upraised compared with the control. Moreover, with the increase of the concentration of each triazine herbicide, the I-step of OJIP curve of *C. pyrenoidosa* became less and less obvious. When the concentrations of atrazine and terbuthylazine reached 60 μg L^{-1}, the I-step of OJIP curve of *C. pyrenoidosa* had completely disappeared. Chen el al. (2016) [34] reported that herbicides belonging to PSII inhibitors could cause a rapid increase in the fluorescence intensity of J-step, even make the fluorescence intensity of J-step reach that of P-step, and cause I-step to be vanished. So this reported phenomenon was very consistent with the findings in this study. Moreover, according to Sun et al. (2020) [12] and Chen et al. (2007) [35], a significant increase in the fluorescence intensity of J-step in the OJIP curves was the result of these four triazine herbicides as PSII inhibitor blocking the electron transfer from Q_A to Q_B or Q_B^- and leading to a rapid accumulation of Q_A^-. Furthermore, compared with the control, the fluorescence intensity of O-step in the OJIP curves of *C. pyrenoidosa* exposed to each

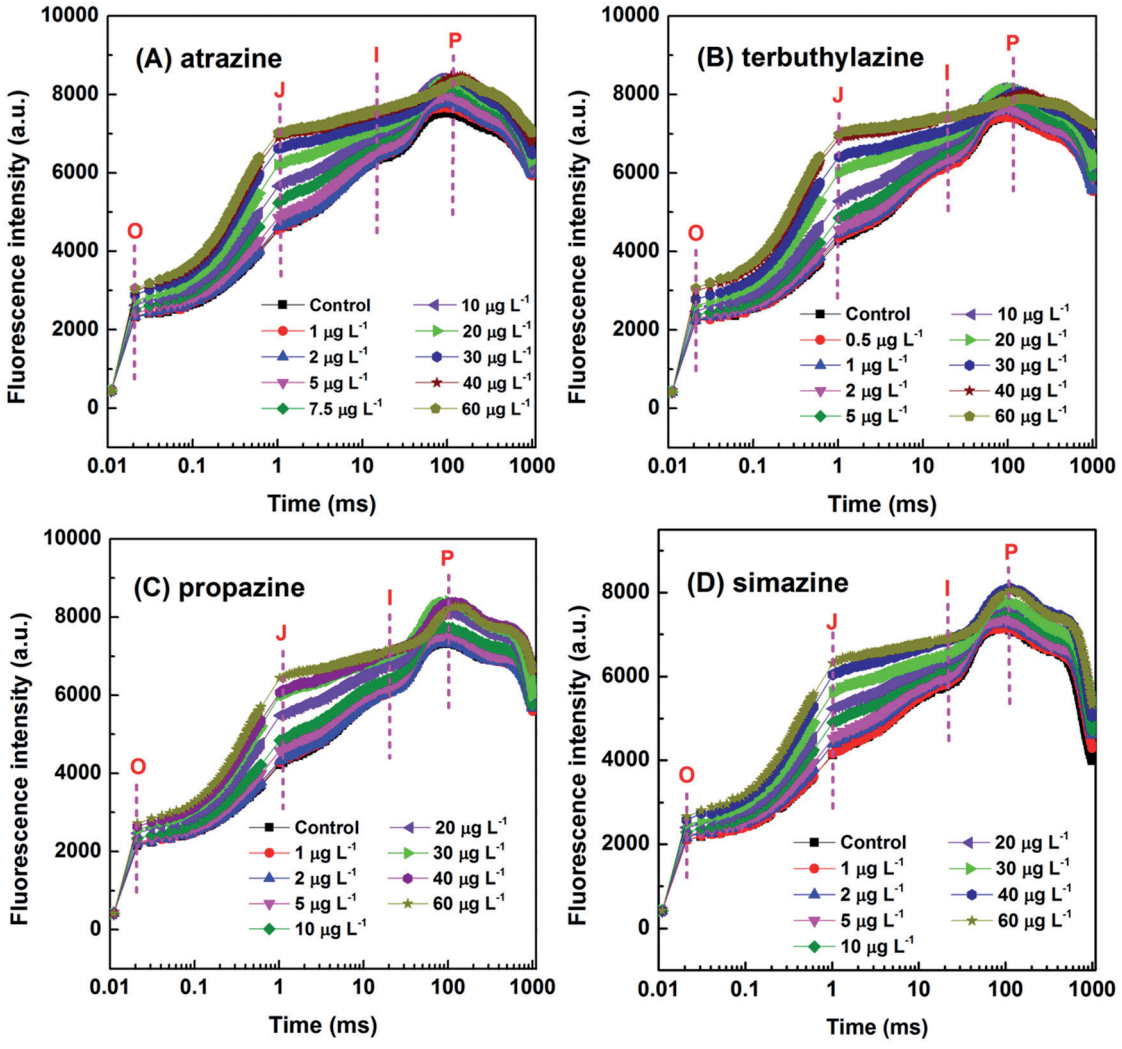

Fig. 1. OJIP curves of *C. pyrenoidosa* exposed to different concentrations of triazine herbicides for 1 h. a) atrazine; b) terbuthylazine; c) propazine; d) simazine.

triazine herbicide was also induced to increase, this may be due to the release of LHC from the PSII complex and the inactivation of PSII photochemical reaction of *C. pyrenoidosa* induced by triazine herbicides [22, 36].

According to Fig. 1, we found that for the *C. pyrenoidosa* exposed to atrazine, terbuthylazine, propazine or simazine, the differences in the OJIP curves between the treatments and the control were mainly reflected in four key aspects: the fluorescence intensity of O-step (F_O), the variation in fluorescence intensity between J-step and O-step ($Fv_{(J-O)}$), the variation in fluorescence intensity between I-step and J-step ($Fv_{(I-J)}$), and the variation in fluorescence intensity between P-step and I-step ($Fv_{(P-I)}$). As shown in Fig. 2, as the concentration of each triazine herbicide increased, both the values of F_o and $Fv_{(J-O)}$ shown a gradual increasing trend, while the value of $Fv_{(I-J)}$ gradually decreased. Moreover, compared with the control, for the *C. pyrenoidosa* exposed to low concentration of triazine herbicides, although some treatments showed a slight increase in $Fv_{(P-I)}$, and some treatments showed a slight decrease in $Fv_{(P-I)}$, the differences in $Fv_{(P-I)}$ between the treatments and the control were not very large. However, for the *C. pyrenoidosa* exposed to high concentrations of triazine herbicides (such as atrazine≥30 μg L^{-1}, terbuthylazine≥30 μg L^{-1}, propazine≥60 μg L^{-1} and simazine≥40 μg L^{-1}), the $Fv_{(P-I)}$ values were significantly lower than that of the control, and gradually decreased with the increase of the concentration of each triazine herbicide. For example, compared with the control, the $Fv_{(P-I)}$ values of *C. pyrenoidosa* treated with 60 μg L^{-1} of atrazine, terbuthylazine, propazine and simazine decreased by nearly 50%, 63%, 11% and 22%, respectively.

The above results indicate that all the four triazine herbicides atrazine, terbuthylazine, propazine and simazine had significant impacts on F_O, $Fv_{(J-O)}$, $Fv_{(I-J)}$ and $Fv_{(P-I)}$ of OJIP curve. Among them, F_o and $Fv_{(J-O)}$ were positively correlated with the concentration of each triazine herbicide, while $Fv_{(I-J)}$ and $Fv_{(P-I)}$ were negatively correlated with the concentration of each triazine herbicide. Therefore, for *C. pyrenoidosa*, it was attributed to the increases of F_O and $Fv_{(J-O)}$ and the reductions of $Fv_{(I-J)}$ and $Fv_{(P-I)}$ induced by atrazine, terbuthylazine, propazine and simazine that the OJIP curves of the treatments changed significantly compared with that of the control. So F_O, $Fv_{(J-O)}$, $Fv_{(I-J)}$ and $Fv_{(P-I)}$ were the four key elements of OJIP curve for the quantitative detection of the toxicity of atrazine, terbuthylazine, propazine and simazine to the photosynthesis of *C. pyrenoidosa*.

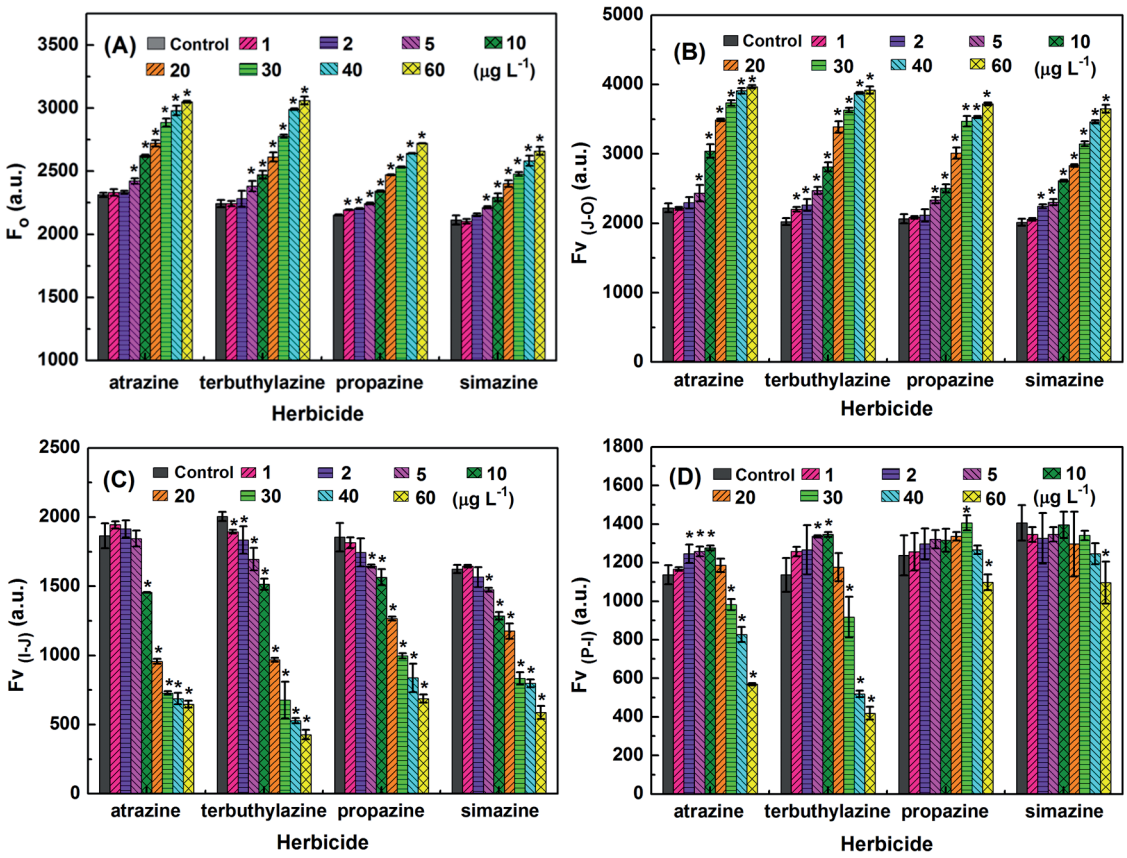

Fig. 2. Values of four key elements of the OJIP curves of *C. pyrenoidosa* exposed to different concentrations of atrazine, terbuthylazine, propazine and simazine for 1 h. a) F_o; b) $Fv_{(J-O)}$; c) $Fv_{(I-J)}$; d) $Fv_{(P-I)}$. * indicates there was a significant difference between the treatment and the control (*$p<0.05$, post-hoc multiple test).

Response Performance of PI Constructed Based on the Change Characteristics of OJIP Curve to Triazine Herbicides

Because OJIP curve contains important information about the photosynthetic process of microalgae. The changes in OJIP curve reflects the toxicity effects of hazardous substances on microalgal photosynthesis. In order to accurately detect the toxicity of triazine pesticides to the photosynthesis of *C. pyrenoidosa*, considering that the changes of the OJIP curves of *C. pyrenoidosa* treated by atrazine, terbuthylazine, propazine and simazine were mainly caused by the increases of F_O and $Fv_{(J-O)}$ and the decreases of $Fv_{(I-J)}$ and $Fv_{(P-I)}$, in this study, taking the four key elements F_o, $Fv_{(J-O)}$, $Fv_{(I-J)}$ and $Fv_{(P-I)}$ of OJIP curve as variables, a new photosynthetic response index (PI) for response to the toxicity of triazine herbicides was constructed employing equation (4). The PI values of *C. pyrenoidosa* exposed to different concentrations of atrazine, terbuthylazine, propazine or simazine for 1 h were shown in Fig. 3. We can see that with a increase in the concentration of each triazine herbicide, the PI value of *C. pyrenoidosa* gradually decreased. Moreover, for the *C. pyrenoidosa* exposed to each triazine pesticide, Logistic curve fitting was performed between the inhibition rates of response index PI and the concentration of each triazine herbicide, and the results were shown in Fig. 4. As can be seen that there was a very good Logistic curve relationship between the inhibition rate of PI and the concentration of each triazine herbicide, and the correlation coefficients R^2 of atrazine, terbuthylazine, propazine and simazine were 0.9973, 0.9960, 0.9984 and 0.9963, respectively. These results indicate that the constructed novel photosynthetic response index PI of *C. pyrenoidosa* had good concentration-response relationships with atrazine, terbuthylazine, propazine and simazine, so it could be used as a response index to quantitatively detect the toxicity of triazine pesticides to the photosynthesis of *C. pyrenoidosa*.

As an important photosynthetic fluorescence parameter, F_V/F_M can reflect the maximum photosynthetic capacity of plants [12], so it is a commonly used response index for detecting the toxicity of pollutants to microalgal photosynthesis. In order to further verify the response performance of PI to the toxicity of triazine herbicides, when *C. pyrenoidosa* was exposed to atrazine, terbuthylazine, propazine or simazine for 1 h, the response sensitivities

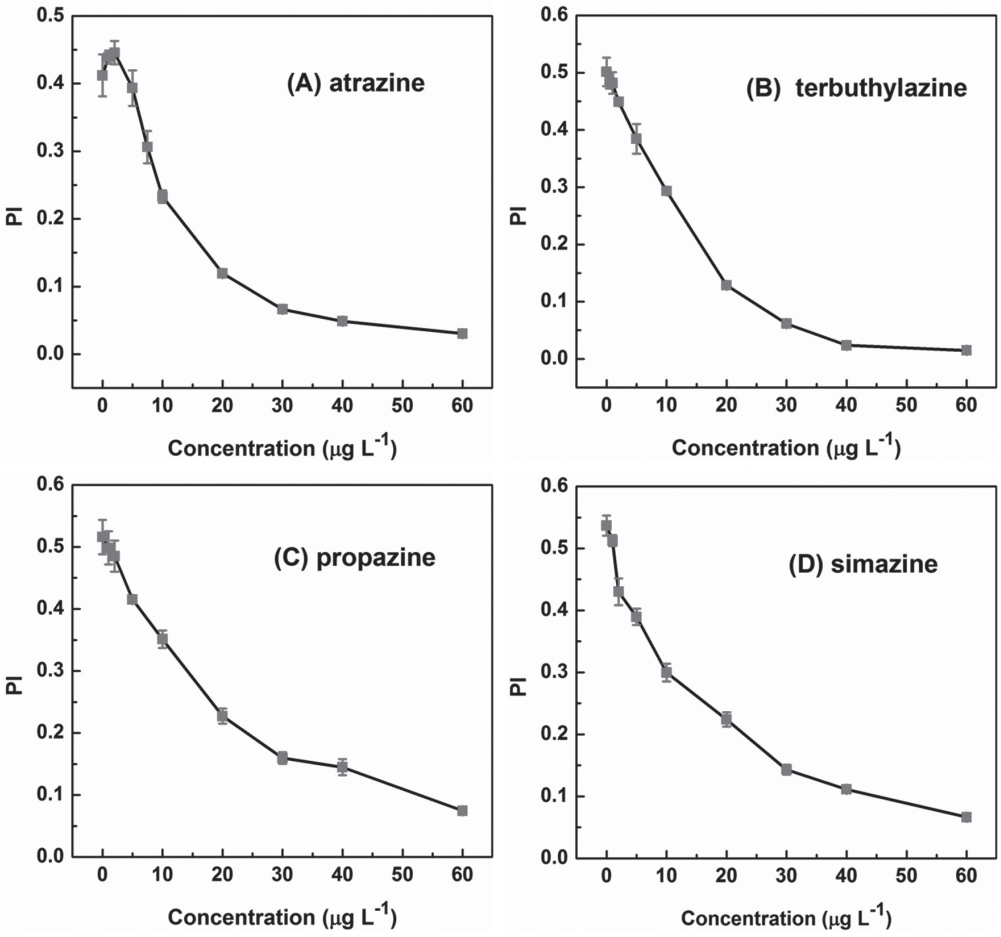

Fig. 3. Variation of constructed PI of *C. pyrenoidosa* with the concentration of triazine herbicides. a) atrazine; b) terbuthylazine; c) propazine; d) simazine.

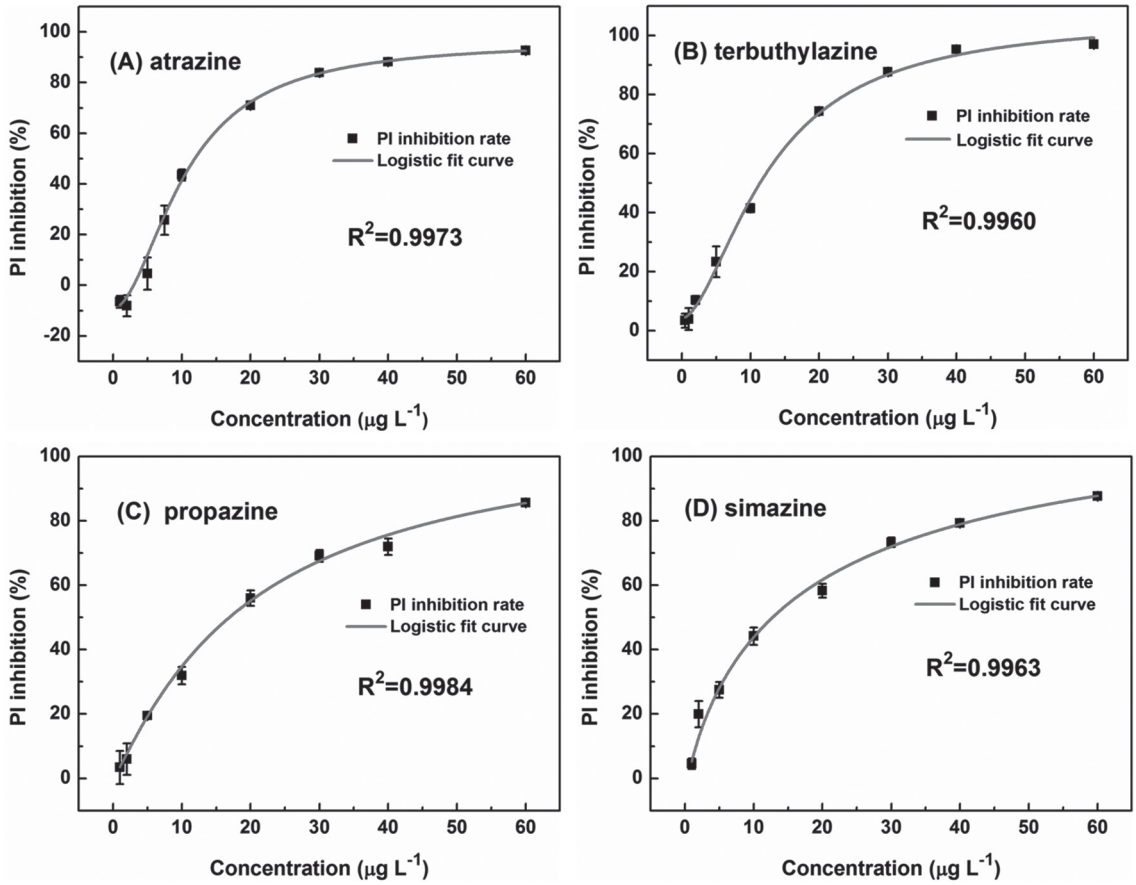

Fig. 4. Concentration-response relationships between constructed PI and triazine herbicide concentration at 1 h. a) atrazine; b) terbuthylazine; c) propazine; d) simazine.

of PI to the four triazine herbicides were compared with that of F_V/F_M, and the inhibition rates of PI and F_V/F_M of *C. pyrenoidosa* induced by each triazine herbicide in the concentration range of 1-60 μg L^{-1} were shown in Fig. 5. As can be seen that whether it was atrazine, terbuthylazine, propazine or simazine, for *C. pyrenoidea* exposed to each concentration of triazine herbicides, the inhibition rate of PI was always significantly greater than that of F_v/F_M. For example, when the concentrations of atrazine, terbuthylazine, propazine and simazine were 10 μg L^{-1}, the inhibition rates of PI of the exposed *C. pyrenoidosa* were 43.45%, 41.50%, 31.92% and 44.17%, respectively, and it can also be observed from Fig. 1 that the OJIP curves of *C. pyrenoidosa* with the same treatment had very significant differences compared with the control. But in this case, the inhibition rates of F_V/F_M were only 1.80%, 1.93%, 0.55% and 1.10%, respectively, which were significantly lower than those of PI. Similarly, when the concentrations of the four triazine herbicides were as high as 60 μg L^{-1}, the inhibition rates of PI of *C. pyrenoidosa* exposed to atrazine, terbuthylazine, propazine and simazine had reached 92.61%, 97.02%, 85.55% and 87.64%, respectively, but for the same treatments, the inhibition rates of F_V/F_M were only 10.96%, 15.54%, 5.53% and 7.00%, respectively. These results indicate that if F_V/F_M was used as a response index, the toxic effects of triazine herbicides on the photosynthesis of algae would be greatly underestimated, so F_V/F_M was not suitable for detecting the toxicity of triazine herbicides to *C. pyrenoidosa* photosynthesis. This result was consistent with the findings of Sun et al. (2020) [12] that for *Chlorella* sp exposed to atrazine, F_V/F_M was not an optimal indicator in response to atrazine toxicity because of its insensitivity to stress. Compared with F_V/F_M, the new photosynthetic response index PI constructed based on the change characteristics of OJIP curve had more sensitive characteristics in respond to the toxicity of triazine herbicides.

Sun et al. (2020) [12] reported that PI_{ABS} was a more sensitive parameter for evaluating the toxicity of atrazine to *Chlorella sp* by comparing the EC_{50} values of various indexes. Moreover, PI_{ABS} was also suggested as the most sensitive JIP-test parameter in terms of expressing overall photosynthetic activity of PSII of microalgae in previous report [34]. In order to further verify whether or not the sensitivity of PI constructed based on the change characteristics of OJIP curve in this study was better than that of PI_{ABS} in response to the toxicity of triazine herbicides, when *C. pyrenoidosa* was exposed for 1 h, the EC_{50} values of atrazine, terbuthylazine, propazine and simazine using PI as the response index were compared with those EC_{50} values calculated based on PI_{ABS}, and the results were shown

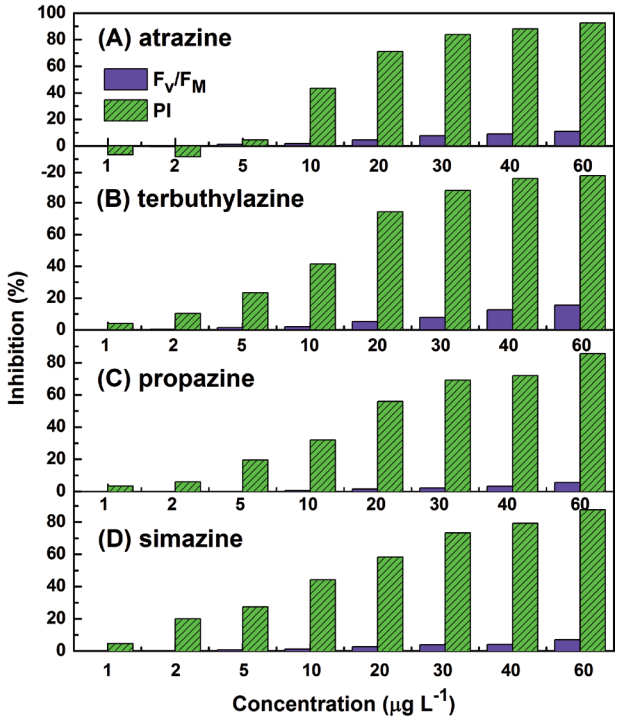

Fig. 5. Inhibition rates of F_V/F_M and PI of *C. pyrenoidosa* exposed to different concentration of triazine herbicides for 1 h. (A) atrazine; (B) terbuthylazine; (C) propazine; (D) simazine.

in Table 1. We can see that for atrazine, terbuthylazine, propazine and simazine, the EC_{50} values calculated based on PI_{ABS} were all higher than the EC_{50} values calculated based on PI, and were 1.13 times, 1.04 times, 1.26 times and 1.52 times of the EC_{50} values using PI as the response index, respectively. This result indicates that the constructed new response index PI was more sensitive than PI_{ABS} in detecting the toxicity of triazine herbicides to *C. pyrenoidosa* photosynthesis.

In addition, in order to further verify the reliability of PI in comparing the toxicity of different triazine herbicides to the photosynthesis of *C. pyrenoidosa*, when PI was used as the response index, the toxicity ranking of atrazine, terbuthylazine, propazine and simazine to *C. pyrenoidosa* photosynthesis was further compared with that when PI_{ABS} was used as a response index. As shown in Table 1, when response index RI was used to compare the toxicity of four triazine herbicides, the toxicity ranking of the four triazine herbicides to *C. pyrenoidosa* from high to low was in the order of te rbuthylazine>atrazine>simazine>propazine, which was consistent with the result when PI_{ABS} was used as the response index. So PI and PI_{ABS} had a good consistency in comparing the toxicity of different triazine herbicides to the photosynthesis of *C. pyrenoidosa*. This result further verified that PI as a response index could reliably and accurately distinguish the strength of toxicity between different triazine herbicides.

Table 1. EC_{50} values of atrazine, terbuthylazine, propazine and simazine at 1 h for *C. pyrenoidosa* using PI and PI_{ABS} as response indexes.

Herbicides	EC_{50} values of PI (mg L^{-1})	EC_{50} values of PI_{ABS} (mg L^{-1})
Atrazine	11.844±0.367	13.328±0.653
Terbuthylazine	11.379±0.324	11.840±0.455
Propazine	16.922±0.761	21.272±1.042
Simazine	12.755±0.523	19.331±0.909

Consequently, by verifying the response performance of PI to the toxicity of triazine herbicides, we can know that in terms of quantitatively detecting the toxicity of triazine herbicides, PI had a good concentration-response relationship with each triazine herbicide; in terms of response sensitivity to each triazine herbicide, PI was all significantly superior to F_V/F_M and PI_{ABS}; and in terms of comparing the toxicity of different triazine herbicides, PI was also in good agreement with PI_{ABS}. Therefore, the new response index PI constructed based on the change characteristics of OJIP curve in this study was a very suitable response index for sensitively and accurately detecting the toxicity of triazine herbicides to the photosynthesis of *C. pyrenoidosa*.

Because all triazine herbicides have the same triazine ring structure, halogen atom and amino group in their molecular, and different triazine herbicides have the same inhibition mechanism on photosynthesis [1, 4, 11]. Therefore, the effects of different triazine herbicides on the OJIP curve of *C. pyrenoidosa* are the same, and the OJIP curve of *C. pyrenoidosa* exposed to different triazine herbicides will have the same change characteristics. Although in this study only atrazine, terbuthylazine, propazine and simazine were used as the research objects, in addition to atrazine, terbuthylazine, propazine and simazine, the new response index PI constructed according to the change characteristics of OJIP curve will also have good applicability for the detection of the toxicity of all other triazine herbicides to *C. pyrenoidosa* photosynthesis. Moreover, there are some other herbicides or chemicals, which also have the same inhibition mechanism on photosynthesis as triazine herbicides. These herbicides or chemicals will also have the same effects as triazine herbicides on the OJIP curve of *C. pyrenoidosa*. Therefore, in addition to triazine herbicides, the new response index PI constructed in this study is also applicable to detecting the toxicity of other herbicides or chemicals with the same inhibition mechanism on photosynthesis as triazine herbicides. In addition, in this study, the response performance of the new response index PI to triazine herbicides was only compared with the most commonly used photosynthetic fluorescence parameters F_V/F_M and PI_{ABS}. However, there are many photosynthetic fluorescence parameters obtained by chlorophyll fluorescence induction kinetics technology. Although compared with F_V/F_M and PI_{ABS},

PI had better sensitivity in response to the toxicity of triazine herbicides, the current research results have not yet proved that PI was the most sensitive response index among numerous photosynthetic fluorescence parameters in detecting the toxicity of triazine herbicides. On the other hand, the test organism used in this study was only *C. pyrenoidosa*, which is a common species of freshwater green algae. Therefore, it is uncertain whether the new response index PI constructed in this study is also applicable to other species of microalgae, and this nondeterminacy will be further verified in our subsequent studies.

In general, this study provides an optional and more appropriate response index for the rapid, sensitive and accurate detection of the toxicity of herbicides to microalgae in the aquatic environment. Using this new response index to detect the toxicity of herbicides is of great significance to rapidly and accurately assess the quality of aquatic environment and predict the risk of aquatic ecosystem.

Conclusions

The toxicity of four triazine herbicides atrazine, terbuthylazine, propazine and simazine had the same effects on the OJIP curve of *C. pyrenoidosa*, and all those effects were reflected in the changes of the four key elements of OJIP curve, F_o, $Fv_{(J-O)}$, $Fv_{(I-J)}$ and $Fv_{(P-I)}$. Therefore, F_o, $Fv_{(J-O)}$, $Fv_{(I-J)}$ and $Fv_{(P-I)}$ were the four key factors used to detect the toxicity of triazine herbicides to *C. pyrenoidosa* photosynthesis according to the change characteristics of OJIP curve. The new photosynthetic response index PI constructed with F_o, $Fv_{(J-O)}$, $Fv_{(I-J)}$ and $Fv_{(P-I)}$ as variables had the performance of sensitive and accurate quantitative detection of the toxicity of triazine herbicides. So compared with F_V/F_M and PI_{ABS}, the newly constructed PI was a reliable and more suitable response index to detect the toxicity of trazine herbicides to *C. pyrenoidosa* photosynthesis based on chlorophyll fluorescence induction kinetic technology. The proposal of this new response index based on the change characteristics of OJIP curve is helpful to the rapid assessment of herbicide toxicity in water and the accurate prediction of the risk of aquatic ecosystem.

Acknowledgments

This work was supported by the National Key research and development Program of China (Grant number 2021YFC3200100, 2022YFC3103901); Anhui Province Science and Technology Major Special Project (Grant number 202003a07020007, 202203a07020002); National Natural Science Foundation of China (Grant number 61875254, 61875207, 61805254, 42206198).

Conflict of Interest

The authors declare no conflict of interest.

References

1. MANOUSI N., ALAMPANOS V., PRIOVOLOS I., KABIR A., FURTON K.G., ROSENBERG E., ZACHARIADIS G.A., SAMANIDOU V.F. Designing a moderately hydrophobic sol-gel monolithic Carbowax 20 M sorbent for the capsule phase microextraction of triazine herbicides from water samples prior to HPLC analysis. Talanta, **234**, 122710, **2021**.
2. KNISS A.R. Long-term trends in the intensity and relative toxicity of herbicide use. Nature Communications, **8**, 14865, **2017**.
3. DEBLOIS C.P., DUFRESNE K., JUNEAU P. Response to variable light intensity in photoacclimated algae and cyanobacteria exposed to atrazine. Aquatic Toxicology, **126**, 77, **2013**.
4. WANG Z., SUN X., RU S., WANG J., XIONG J., YANG L., HAO L., ZHANG J., ZHANG X. Effects of co-exposure of the triazine herbicides atrazine, prometryn and terbutryn on Phaeodactylum tricornutum photosynthesis and nutritional value. Science of the Total Environment, **807**, 150609, **2022**.
5. YANG L., LI H., ZHANG Y., JIAO N. Environmental risk assessment of triazine herbicides in the Bohai Sea and the Yellow Sea and their toxicity to phytoplankton at environmental concentrations. Environment International, **133**, 105175, **2019**.
6. VRYZAS Z., ALEXOUDIS C., VASSILIOU G., GALANIS K., PAPADOPOULOU-MOURKIDOU E. Determination and aquatic risk assessment of pesticide residues in riparian drainage canals in northeastern Greece. Ecotoxicology and Environmental Safety, **74**, 174, **2011**.
7. BACHETTI R.A., URSELER N., MORGANTE V., DAMILANO G., PORPORATTO C., AGOSTINI E., MORGANTE C. Monitoring of Atrazine Pollution and its Spatial-Seasonal Variation on Surface Water Sources of an Agricultural River Basin. Bulletin of Environmental Contamination and Toxicology, **106**, 929, **2021**.
8. JING X., CHENG X., ZHAO W., WANG H., WANG X. Magnetic effervescence tablet-assisted switchable hydrophilicity solvent-based liquid phase microextraction of triazine herbicides in water samples. Journal of Molecular Liquids, **306**, 112934, **2020**.
9. JIANG Y., QIN Z., LIANG F., LI J., SUN Y., WANG X., MA P., SONG D. Vortex-assisted solid-phase extraction based on metal-organic framework/chitosan-functionalized hydrophilic sponge column for determination of triazine herbicides in environmental water by liquid chromatography-tandem mass spectrometry. Journal of Chromatography A, **1638**, 461887, **2021**.
10. SAFARI M., YAMINI Y., TAHMASEBI E., EBRAHIMPOUR B. Magnetic nanoparticle assisted supramolecular solvent extraction of triazine herbicides prior to their determination by HPLC with UV detection. Microchimica Acta, **183**, 203, **2016**.
11. MAJEWSKA M., HARSHKOVA D., GUŚCIORA M., AKSMANN A. Phytotoxic activity of diclofenac: Evaluation using a model green alga *Chlamydomonas*

reinhardtii with atrazine as a reference substance. Chemosphere, **209**, 989, **2018**.
12. SUN C., XU Y., HU N., MA J., SUN S., CAO W., KLOBUČAR G., HU C., ZHAO Y. To evaluate the toxicity of atrazine on the freshwater microalgae *Chlorella sp.* using sensitive indices indicated by photosynthetic parameters. Chemosphere, **244**, 125514, **2020**.
13. GAO C., GAO L., DUAN P., WU H., LI M. Evaluating combined toxicity of binary heavy metals to the cyanobacterium *Microcystis*: A theoretical non-linear combined toxicity assessment method. Ecotoxicology and Environmental Safety, **187**, 109809, **2020**.
14. PASTORINO P., BROCCOLI A., BAGOLIN E., ANSELMI S., CAVALLO A., PREARO M., RENZI M. A multidisciplinary approach to evaluate the effects of contaminants of emerging concern on natural freshwater and brackish water phytoplankton communities. Biology, **10**, 1039, **2021**.
15. FUHRMAN J.A., CRAM J.A., NEEDHAM D.M. Marine microbial community dynamics and their ecological interpretation. Nature Reviews Microbiology, **13**, 133, **2015**.
16. KUMAR K.S., DAHMS H.U., LEE J.S., KIM H.C., LEE W.C., SHIM K.H. Algal photosynthetic responses to toxic metals and herbicides assessed by chlorophyll a fluorescence. Ecotoxicology and Environmental Safety, **104**, 51, **2014**.
17. XIA J., TIAN Q. Early stage toxicity of excess copper to photosystem II of Chlorella pyrenoidosa-OJIP chlorophyll a fluorescence analysis. Journal of Environmental Sciences, **21**, 1569, **2009**.
18. SUN C., LI W., XU Y., HU N., MA J., CAO W., SUN S., HU C., ZHAO Y., HUANG Q. Effects of carbon nanotubes on the toxicities of copper, cadmium and zinc toward the freshwater microalgae *Scenedesmus obliquus*. Aquatic Toxicology, **224**, 105504, **2020**.
19. DAO L., BEARDALL J. Effects of lead on two green microalgae *Chlorella* and *Scenedesmus*: photosystem II activity and heterogeneity. Algal Research, **16**, 150, **2016**.
20. SHIVAGANGAIAH C.P., SANYAL D., DASGUPTA S., BANIK A. Phycoremediation and photosynthetic toxicity assessment of lead by two freshwater microalgae *Scenedesmus acutus* and *Chlorella pyrenoidosa*. Physiologia Plantarum, **173**, 246, **2021**.
21. PERRON M.C., JUNEAU P. Effect of endocrine disrupters on photosystem II energy fluxes of green algae and cyanobacteria. Environmental Research, **111**, 520, **2011**.
22. KALAJI H.M., JAJOO A., OUKARROUM A., BRESTIC M., ZIVCAK M., SAMBORSKA I.A., CETNER M.D., LUKASIK I., GOLTSEV V., JADLE R.J. Chlorophyll a fluorescence as a tool to monitor physiological status of plants under abiotic stress conditions. Acta Physiologiae Plantarum, **38**, 102, **2016**.
23. YUSUF M.A., KUMAR D., RAJWANSHI R., STRASSER R.J., TSIMILLI-MICHAEL M., Govindjee, SARIN N.B. Overexpression of gamma-tocopherol methyl transferase gene in transgenic Brassica juncea plants alleviates abiotic stress: Physiological and chlorophyll a fluorescence measurements. Biochimica et Biophysica Acta-Bioenergetics, **1797**, 1428, **2010**.
24. DENG Y., ZHANG W., QIN Y., LIU R., ZHANG L., WANG Z., ZHOU Z., DIAO J. Stereoselective toxicity of metconazole to the antioxidant defenses and the photosynthesis system of *Chlorella pyrenoidosa*. Aquatic Toxicology, **210**, 129, **2019**.
25. CHEN M., YIN G., ZHAO N., GAN T., FENG C., GU M., QI P., DING Z. Rapid and sensitive detection of water toxicity based on photosynthetic inhibition effect. Toxics, **9**, 321, **2021**.
26. DAO L., BEARDALL J. Effects of lead on growth, photosynthetic characteristics and production of reactive oxygen species of two freshwater green algae. Chemosphere, **147**, 420, **2016**.
27. AKSMANN A., TUKAJ Z. Intact anthracene inhibits photosynthesis in algal cells: a fluorescence induction study on *Chlamydomonas reinhardtii* cw92 strain. Chemosphere, **74**, 26, **2008**.
28. COSTA C.H., PERREAULT F., QUKARROUM A., MELEGARI S.P., POPOVIC R., MATIAS W.G. Effect of chromium oxide (III) nanoparticles on the production of reactive oxygen species and photosystem II activity in the green alga *Chlamydomonas reinhardtii*. Science of the Total Environment, **565**, 951, **2016**.
29. POKORA W., AKSMANN A., BASCIK-REMISIEWICZ A., DETTLAFF-POKORA A., RYKACZEWSKI M., GAPPA M., TUKAJ Z. Changes in nitric oxide/hydrogen peroxide content and cell cycle progression: study with synchronized cultures of green alga *Chlamydomonas reinhardtii*. Journal of Plant Physiology, **208**, 84, **2017**.
30. OUKARROUM A., PERREAULT F., POPOVIC R., PHOTOCH J. Interactive effects of temperature and copper on photosystem II photochemistry in *Chlorella vulgaris*. Journal of Photochemistry and Photobiology B: Biology, **110**, 9, **2012**.
31. CONNAN S., STENGEL D.B. Impacts of ambient salinity and copper on brown algae: 1. Interactive effects on photosynthesis, growth, and copper accumulation. Aquatic Toxicology, **104**, 94, **2011**.
32. ZHAO G., SONG S., WANG C., WU Q., WANG Z. Determination of triazine herbicides in environmental water samples by high-performance liquid chromatography using graphene-coated magnetic nanoparticles as adsorbent. Analytica Chimica Acta, **708**, 155, **2011**.
33. CHEN S., STRASSER R.J., QIANG S. In vivo assessment of effect of phytotoxin tenuazonic acid on PSII reaction centers. Plant Physiology and Biochemistry, **84**, 10, **2014**.
34. CHEN S., XU X., DAI X., YANG C., QIANG S. Identification of tenuazonic acid as a novel type of natural photosystem II inhibitor binding in QB-site of *Chlamydomonas reinhardtii*. Biochimica et Biophysica Acta-Bioenergetics, **1767**, 306, **2007**.
35. CHEN S., YANG J., ZHANG M., STRASSER R.J., QIANG S. Classification and characteristics of heat tolerance in Ageratina adenophora populations using fast chlorophyll a fluorescence rise O-J-I-P. Environmental and Experimental Botany, **122**, 126, **2016**.
36. MATHUR S., JAJOO A., MEHTA P., BHARTI S. Analysis of elevated temperature-induced inhibition of photosystem II using chlorophyll a fluorescence induction kinetics in wheat leaves (T*riticum aestivum*). Plant Biology, **13**, 1, **2011**.