International Journal of Environmental Engineering– IJEE
Volume 2: Issue 2 [ISSN: 2374-1724]
Publication Date: 30 October, 2015

The Changes of Carotenoid and Chlorophyll Contents in Green Algae Spirogyra Due to the Exposure to Pesticides

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***Abstract*— The accumulation of pesticides in the environment poses a major health concern to humans, plants and animals, thus the presence of these toxicants require close monitoring. Bioindicators such as green algae are widely researched for the detection of toxicants or pollutants and has been reported to have more advantages compared to the conventional detection methods. The objective of this study is to determine the response of carotenoids and chlorophyll in Spirogyra as a potential bioindicator in pesticides detection. Cells immobilized using agarose gel were exposed to 0.001 mg/L, 0.010 mg/L, 0.100 mg/L, 1.000 mg/L and 10.000 mg/L of 2,4-dichlorophenoacetic acid (2, 4-D) and atrazine for a period of 48 hours. Respond trends for chlorophyll and carotenoids were recorded. Carotenoids showed higher potential as the marker for 2,4-D detection while chlorophyll was the better one in atrazine detection.**

Keywords*—Chlorophyll, carotenoids, pesticides bioindicator, *Spirogyra

I. Introduction

The use of toxic pesticides to manage pest problems has become a common practice around the world. There are various types of pesticides that are used on a large scale basis such as insecticides, herbicides, rodenticides and fungicides. Pesticides are able to be enter into the environment in different ways whether direct or indirect such as disposal of waste, leachate from agriculture bases, or even accidents which tend to increase the pesticides amount found in the water and soil [1].

The severity of the pesticides polluting the environment is one of the factors in detecting pesticides in polluted soil or water. Some of the current conventional methods used to detect pesticides are gas chromatography coupled with mass spectrometry (GC/MS) and liquid chromatography coupled with mass spectrometry (LC/MS). Although both of the methods are sensitive to pesticides and able to detect in a short time, but the accuracy of both methods may be low due to the restricted range of pesticides that can be detected for both the instruments (chromatography and mass spectrometer) as different instrument may have different range of concentration limits [2].

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Bioindicator offers better alternatives to detect pesticides due to the rapid detection and are highly sensitive to the changes of samples, which allow measuring the real physiological of the impact to the pollutants [3]. The whole cells e.g. algae, cyanobacteria, and plant cells have a higher tolerance to the changes in environment, while sensitive to the changes. With the use of polluted soil or water as samples, the toxicity of pollutants can be evaluated through their metabolic outputs, e.g. bioluminescence or photosynthetic activity [4].

A number of studies have been reported on the use of algae in bioindication of pesticides [5, 6]. In algae, photosynthetic pigments such as chlorophyll and carotenoid and also biological macromolecules such as alkaline phosphatase and superoxide dismutase (SOD) are suitable to use as marker for light metal and pesticides detection [7, 8].

In this study, *Spirogyra* was used for the detection by studying the response of multi markers (chlorophyll a and carotenoids) towards pesticides (2, 4 –D, and atrazine). The purpose of using *Spirogyra* is due to the presence of multi marker which are able to detect different range of pesticides concentrations. The study is to observe the change of carotenoids and chlorophyll when the cells were exposed to the pesticides and evaluate the suitability of these markers as reporter group for the environment toxicity assessment.

II. Methodology

A. *Collection and Immobilization of the cells.*

The green algae was collected from an agricultural pond. The identification of *Spirogyra* was done morphologically using light microscope (Eclipse E-100 LED, Nikon). The cells were later being cultured in the water collected from the sampling site.

The algae cells were then immobilized with 0.5% of agarose into plastic cuvette, by mixing 0.5 mL of culture (with approximate 5 x 10^5 cells) and 0.5 mL of agarose solution. The number of cells per cuvette was determined through cell count with hemacytometer (Marienfeld-Superior, Neubauer).

B. *Pesticides Solutions*

2,4-D and atrazine were purchased from Sigma Aldrich, Malaysia. Stock solutions of 100 mg/L were prepared from the pesticides in powder form. Other concentrations of pesticides were obtained through serial dilution of the stocks.

C. *Exposure to Pesticides*

Two mL of deionized water was added to the cuvette with agarose gel as blank, and the cuvette with immobilized cells as negative control.

Two mL of 2,4-D with concentrations of 0.001 mg/L, 0.010 mg/L, 0.100 mg/L, 1.000 mg/L and 10.000 mg/L were added to cuvettes containing immobilized cells. OD readings were taken at 450 nm and 663 nm with UV-Vis spectrophotometer (GeneQuant 1300, GE) at time, t = 0 hour, 1 hour, 6 hours, 24 hours, and 48 hours respectively. The tests were repeated using atrazine replacing 2,4-D. All tests were conducted in five replicates unless stated otherwise.

III. Results and Discussion

The *Spirogyra* responded to the exposure of 2,4-D and atrazine as both chlorophyll and carotenoids concentrations changed over 48 hours of exposure as shown in Fig. 1, Fig.2, Fig. 3 and Fig. 4. The negative control yielded a higher increase in absorbance in comparison to pesticides treated cells, as the growth of cell was still carrying on after the immobilization [9].

However, the increase in absorbance to 0.001, 0.01 and 0.1 mg/L of 2, 4-D and atrazine shows the cells were still viable even though the increment was lower compared to control. When the concentration of pesticides reached 10 mg/L, the decreasing trend of chlorophyll and carotenoids showed the cells might not be able to mediate the toxicity of the pesticides.

Chlorophylls are green pigments and the most abundant pigments on earth, required for the photosynthesis [10]. Chlorophyll absorbs red and blue wavelength of light and green light cannot be absorbed which will be reflected making the plant appeared to be green in colour [11].

Apart from chlorophyll as the main photosynthetic pigments in algae, carotenoids also can be found widely in photosynthetic organisms [12]. Carotenoids have two main functions that are addition light harvest pigments to help chlorophyll in light harvest process and protect the organism from damage through photo-protection[13]. With the two functions mentioned above, photosynthetic organism able to adapt harsh changes of light intensity environment and it is highly essential for algae in nature.

As both pigments are involved in photosynthetic activities, the ability of the pesticides to redox sites in photosynthetic organisms will interrupt the process of photosynthesis, thus might affect the production of these pigments [14, 15].

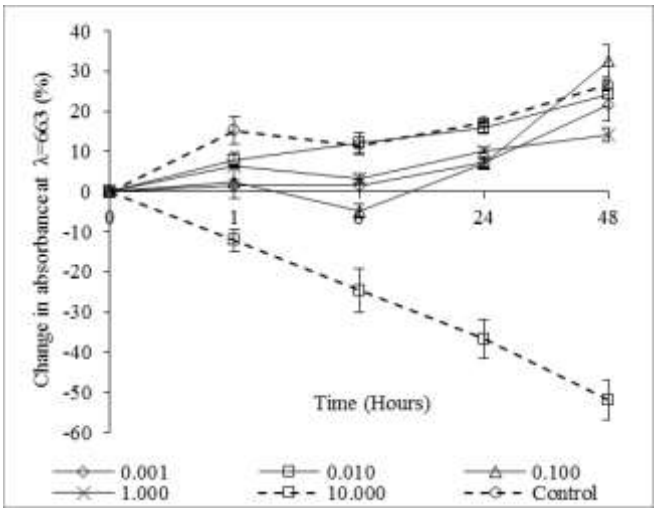

Figure 1. The change of chlorophyll content after exposed to different concentrations of 2,4-D for 48 hours.

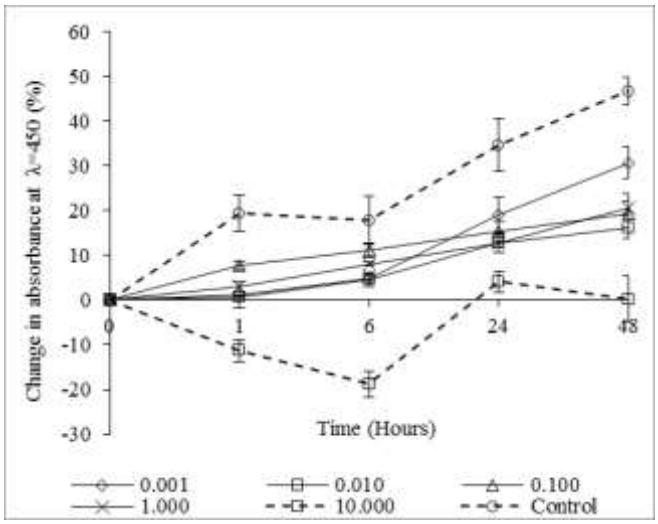

Figure 2. The change of chlorophyll content after exposed to different concentrations of atrazine for 48 hours.

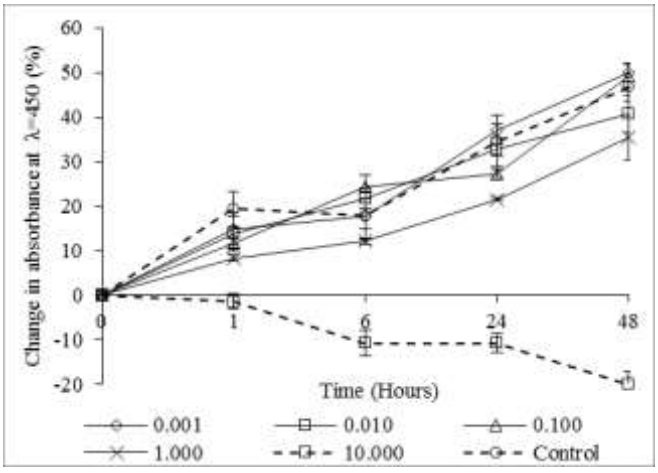

Figure 3. The change of carotenoids content after exposed to different concentrations of 2,4-D for 48 hours.

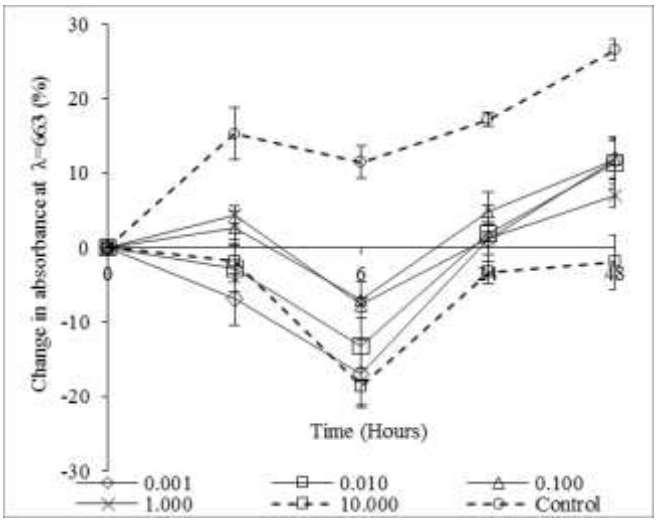

Figure 4. The change of carotenoids content after exposed to different concentrations of Atrazine for 48 hours.

Analysis on the responses based on the exposure time, carotenoids could be a better marker for 2,4-D detection, with the value of R^2 = 0.89 at the linear detection range of 0.001 mg/L – 10.000 mg/L. Chlorophyll is a better marker for atrazine detection, with value of R^2 = 0.96 at the linear detection range of 0.001 mg/L – 10.000 mg/L. Although both markers showed potential to be used in pesticides detection, more work need to be done to confirm the effect of pH, cell density, cell age, and a few other factors that might affect the performance of the indicator.

IV. Conclusion

The changes of chlorophyll and carotenoids in green algae *Spirogyra* to the exposure of two types of pesticides-2,4-D and atrazine have been studied. Both pigments showed potential as the markers with responses to 0.001 mg/L – 10.000 mg/L of pesticides, and good correlation to the dosage of the pesticides used within 24 hours of exposure. However, the applicability of using both pigments as markers in the field are yet to be determined through further research.

Acknowledgement

This project was supported by the Ministry of Education, Malaysia (FRGS-1-2014-SG03-INTI-02-1).

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