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Using the Responses of Green Algae *Spirogyra* as Bioindicator for Metals and Pesticides Pollution

ABSTRACT

Metals and pesticides are common environmental pollutants. The presence of these pollutants in the environment need to be closely monitored because of its toxicity effects to human beings. In this study, the responses of Spirogyra in the form of changes in chlorophyll content due to the exposure to these pollutants were reported. The algae was collected from natural environment, immobilized with agarose gel, and then being exposed to lead (Pb), aluminium (Al), calcium (Ca), sodium (Na), atrazine and 2,4-dichlorophenoxyacetic acid (2,4-D). The changes of chlorophyll in the algae were measured for 48 hours using a spectrophotometer at 663 nm and 450 nm respectively. The content of the pigment was changed due to the presence of the pollutants at concentrations of 0.001 mg L^{-1} to 1.000 mg L^{-1}. The change might due to the biochemical reactions triggered by the pollutants. The response could potentially be used as whole cell bioindicator for the detection of the presence of metals and pesticides.

Key words: *Spirogyra, metals, pesticides, bioindicator, algae*

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INTRODUCTION

Rapid development in industry and agriculture leads to serious environmental pollution. Metals and pesticides are commonly found pollutants which pose a great threat to humans' health. The detection of these pollutants using conventional analytical tools e.g. atomic absorption spectrometer and high performance liquid chromatography require high expenditure and expertise, therefore, whole cell biosensors or bioindicators can be used an alternative for the detection of these pollutants.

Algae and cyanobacteria are sensitive to the metals and organic pollutants (T*eo and Wong 2014; Wan Jusoh et al. 2017; Wong et al. 2016*). These organisms are widely distributed in aquatic system and can be easily cultured in laboratory (*Omar 2010*). These characteristics make these organisms excellent candidates for bioindicator studies.

The toxicity responses of algae can be evaluated through changes in photosynthetic related activity (*Sáenz et al. 2012*), cell density (*Prasad et al. 1998*), antioxidant enzymes (*Awasthi 2012*; *Çelekli et al. 2013*; *Chia et al. 2015*; *Sabatini et al. 2009*) and cell growth (*Sáenz et al. 2012*). The responses of green algae e.g. *Chlorella* sp. and *Scenedesmus* sp. to metals and organic compounds had been well-studied (*Chia et al. 2015*; *Chia et al. 2013*; *Sabatini et al. 2009*). However, the responses of one of the most widely available green algae *Spirogyra* to the environmental pollutants were not much reported. In this study, the changes of chlorophyll content of *Spirogyra* to several types of metals and pesticides were reported. The responses were then analyzed to identify the potential of the algae to be used as bioindicator for these pollutants.

METHODOLOGY

Collection of *Spirogyra* and cell density determination

Spirogyra was collected from the pond at Green Foliage Nursery (Air Hitam, Kluang, Johor, Malaysia) and cultured in conical flasks. The water collected from the site was filtered and served as the medium for the algae. The culture was incubated in room temperature ($27 \pm 2^{\circ}C$) under cool-white fluorescent lights (20 Watts). Light and dark conditions were maintained at 16 hours and 8 hours respectively.

The cell density was determined using cell count and optical density (OD) measurement. Cell count was performed by using hemocytometer (Marienfeld-Superior, Neubauer), and light microscope (Eclipse E-100 LED, Nikon), while OD measurement was determined by using spectrophotometer (GeneQuant 1300, GE) at wavelength of 663 nm.

Immobilization of *Spirogyra*

Immobilization was carried out using 1% agarose gel (w/v) which was prepared by mixing 1 g agarose powder with 99 mL deionized water. A volume of 0.5 mL of cell culture at Day-1, (approximately 5.12 x 105 of cells) was mixed with 0.5 mL of agarose on a clear side of a cuvette at 45 °C. The cuvette was then sealed with plastic paraffin film (Parafilm M, Pechiney Plastic Packaging). The mixture was left to solidify in room temperature for 5 minutes.

Exposure test

Different concentrations of Pb, Ca, Na, Al, atrazine and 2,4-D solutions were prepared by serial dilution from $PbSO_4$, $CaCl_2$, NaCl, $Al_2(SO_4)_3.16H_2O$, altrazine and 2,4-D stock solutions. The immobilized cells were exposed to 0.001 mg L^{-1}, 0.010 mgL^{-1}, 0.100 mg L^{-1}, 1.000 mgL^{-1}, and 10.000 mg L^{-1} of Pb. Optical densities were measured with wavelengths of 663 nm and 450 nm using spectrophotometer, before the exposure. The values of OD were determined again after 1, 2, 6, 24 and 48 hours after the exposure. The exposure tests were repeated using Ca, Na, Al, atrazine and 2,4-D respectively. The cuvette containing clear agarose without immobilized cells was used as blank while the cuvette containing immobilized cells exposed to two mL of deionized water was used as control. All exposure tests were conducted in triplicates.

Statistical analysis of the exposure tests were conducted using Microsoft Excel 2010 version. The calculation of percentage of change in absorbance is shown in Equation 1.

$$\text{Change in OD (\%)} = [(OD_1 - OD_0) / OD_0] \times 100\% \quad (1)$$

Where:
OD_0 = OD reading before the exposure
OD_1 = OD reading after heavy metals or nutrients exposure at respective time

RESULTS AND DISCUSSION

The cell density of *Spirogyra* was determined using cell count and OD measurement. The $R^2 = 0.978$ indicated the cell number and the content of chlorophyll were well-correlated (**Figure 1**). Thus, OD at 663 nm which could be used to estimate the content of chlrophyll (*Mitchell and Kiefer 1988*) could be used to estimate the number of *Spirogyra* in the culture.

Lead was selected in this study for its characteristic as a kind of non-beneficial metal for photosynthetic organisms. Overall chlorophyll content in *Spirogyra* changed due to the presence of Pb (0.001 mg L^{-1} to 10.000 mgL^{-1}), which might be caused by the toxicity effect of Pb towards *Spirogyra* (**Figure 2**). The presence of Pb causes decomposition of chlorophyll or inhibition of the synthesis of chlorophylls (*Arunakumara 2009*; *Cao et al. 2015*). Lead interacts with functional sulfhydryl groups

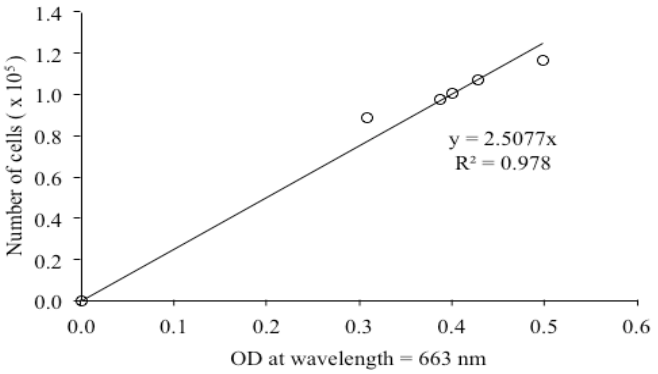

Figure 1. Correlation between cell density and OD at λ = 663 nm.

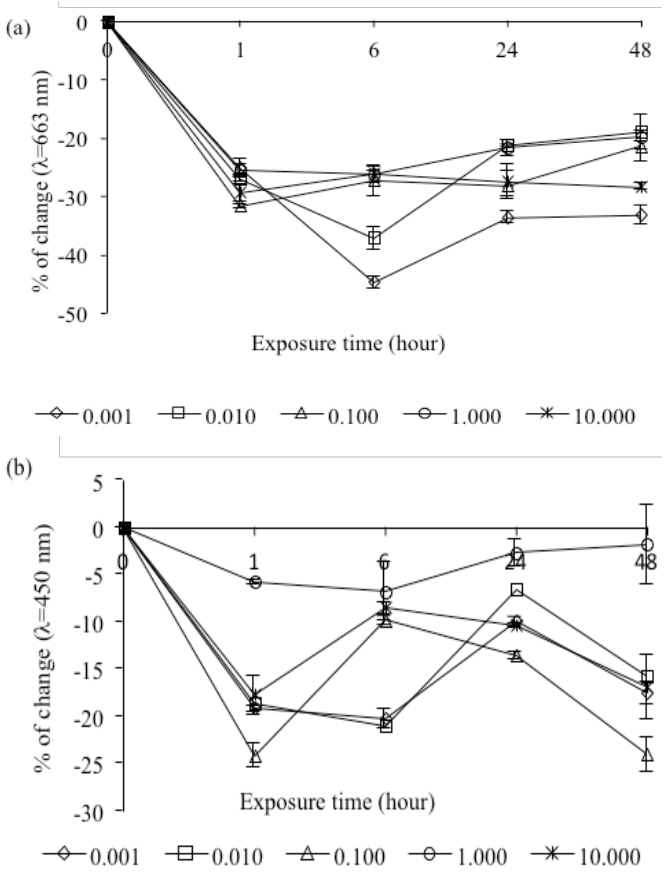

Figure 2. Response of *Spirogyra* to different concentrations of Pb as indicated by (a) λ = 663 nm and (b) 450 nm.

of the enzymes during biosynthesis of chlorophyll or substitute magnesium ions in the chlorophyll molecules (*Zakeri and Bakar 2013*). *Xiong et al.* (*2014*) reported that Pb decreased the expression of photosynthesis-related genes, thus the effect of Pb to both photosynthetic pigment was well anticipated.

The chlorophyll in *Spirogyra* decreased after the exposure to Al (**Figure 3**). Aluminium has no known biological function in living cells. Low concentration of Al had stimulatory effect on the cell growth of green algae *Dunaliella tertiolecta* (*Saçan et al. 2007*). At high concentration, it could cause inhibition of cell growth. Aluminum can disrupt the thylakoid membrane and lead to decrease of pigments content as well (*Pettersson et al. 1985*).

The content of chlorophyll in *Spirogyra* decreased in first six hour exposure to Ca while started bouncing back after that. Ca is needed as nutrient for algae (*CCAP 1999*) (**Figure 4**). The decrease might due to change of pH caused by $CaCl_2$. Ca ion might react with water to form calcium hydroxide and reduced hydroxide ions in water. *Spirogyra* might require some time to adapt new environment before the stimulation took effect on the content of chlorophyll.

The stress of Na caused decrease in chlorophyll content in *Spirogyra* (*Figure 5*). The cells adapted to the change of environment after long hours of exposure. Green algae *Scenedesmus quadricauda* produced more carbohydrates under NaCl treatment, while *Talebi et al.* (*2013*) confirmed the photosynthetic pigments might increase after the exposure of Na (*Kirrolia et al. 2011*).

The content of chlorophyll decreased slightly followed by a steady fluctuation within 48 hours exposure to altrazine for all concentrations below 10 mg L^{-1} (**Figure 6**). Atrazine is a kind of commonly used herbicide for agricultural industry. Low concentration of atrazine stimulated both growth and chlorophyll *a* content of the green algae *Chlamydomonas* sp. (*Tang et al. 1997*). Other studies indicated that low concentration did not inhibit the growth or photosynthesis of green algae (*Kabra et al. 2014*; *Lockert et al. 2006*).

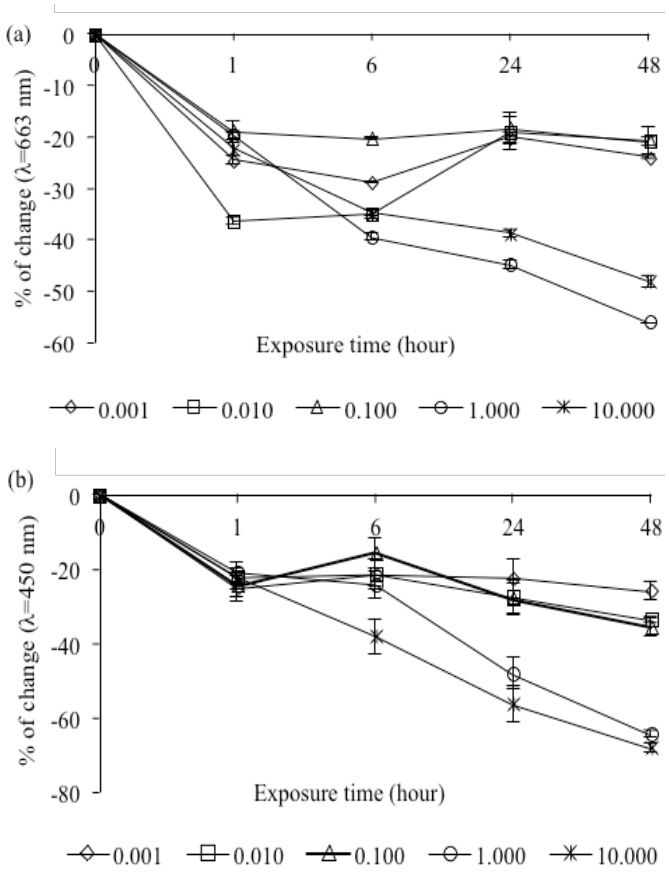

Figure 3. Response of *Spirogyra* to different concentrations of Al as indicated by (a) λ = 663 nm and (b) 450 nm.

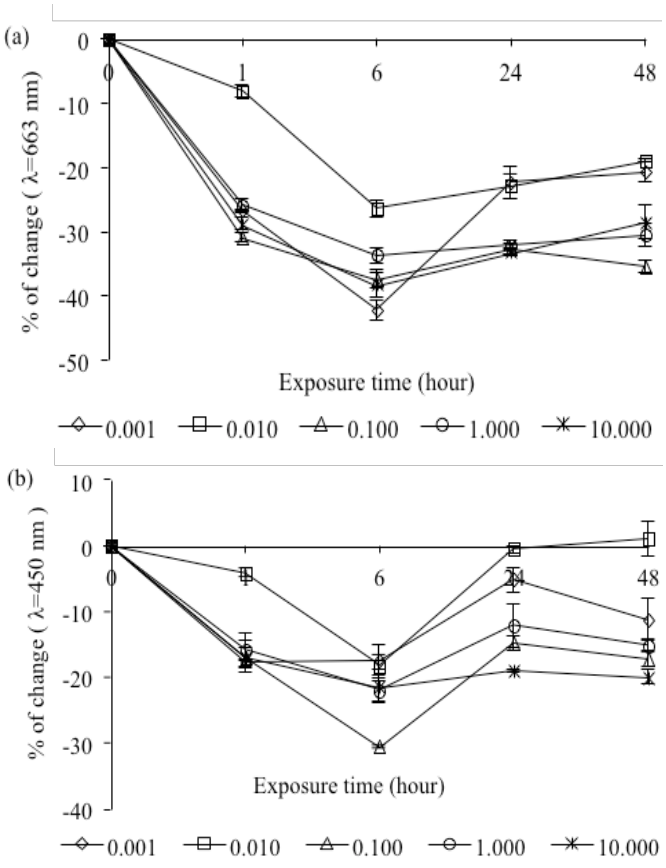

Figure 4. Response of Spirogyra to different concentrations of Ca as indicated by (a) λ = 663 nm and (b) 450 nm.

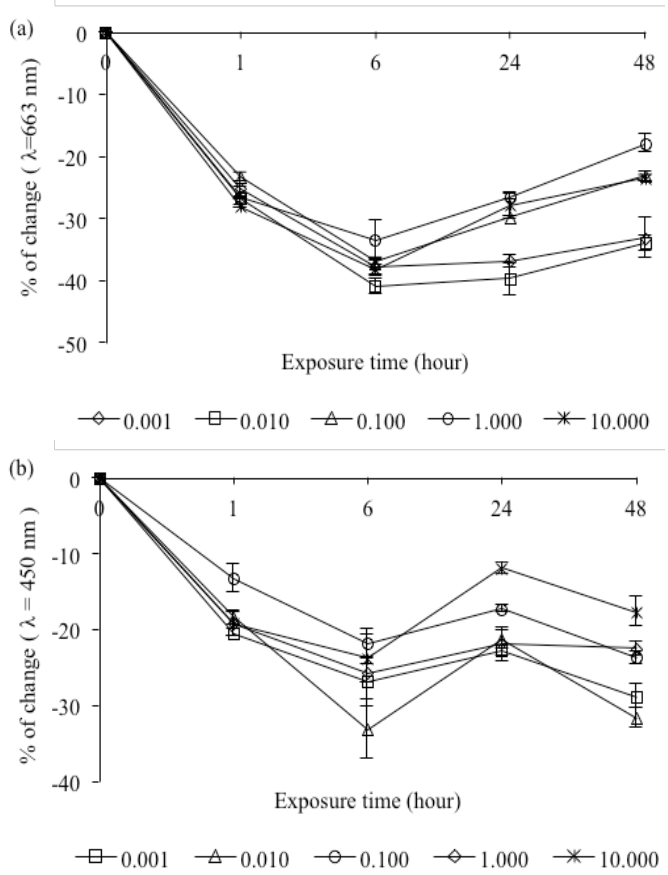

Figure 5. Response of *Spirogyra* to different concentrations of Na as indicated by (a) λ = 663 nm and (b) 450 nm.

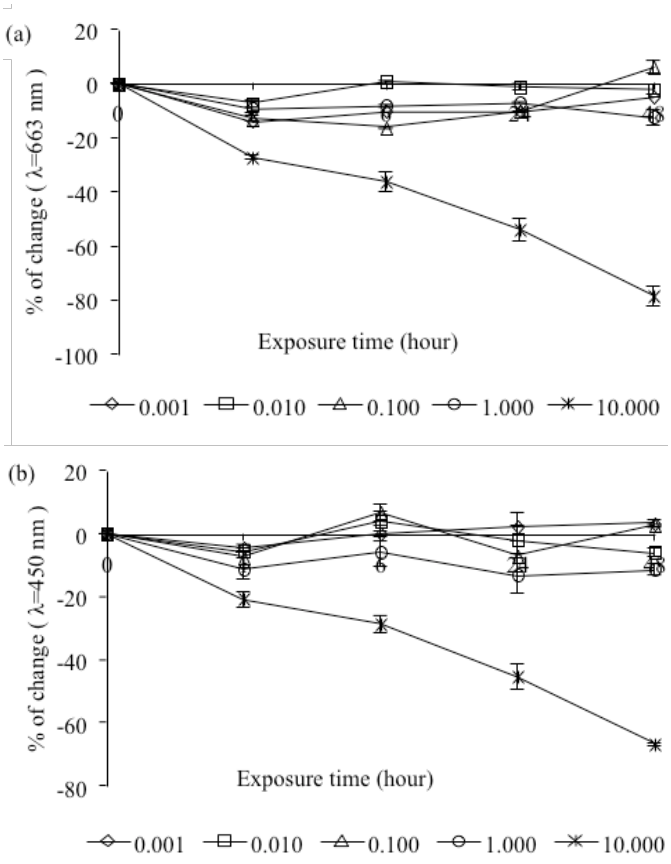

Figure 6. Response of *Spirogyra* to different concentrations of atrazine as indicated by (a) λ = 663 nm and (b) 450 nm.

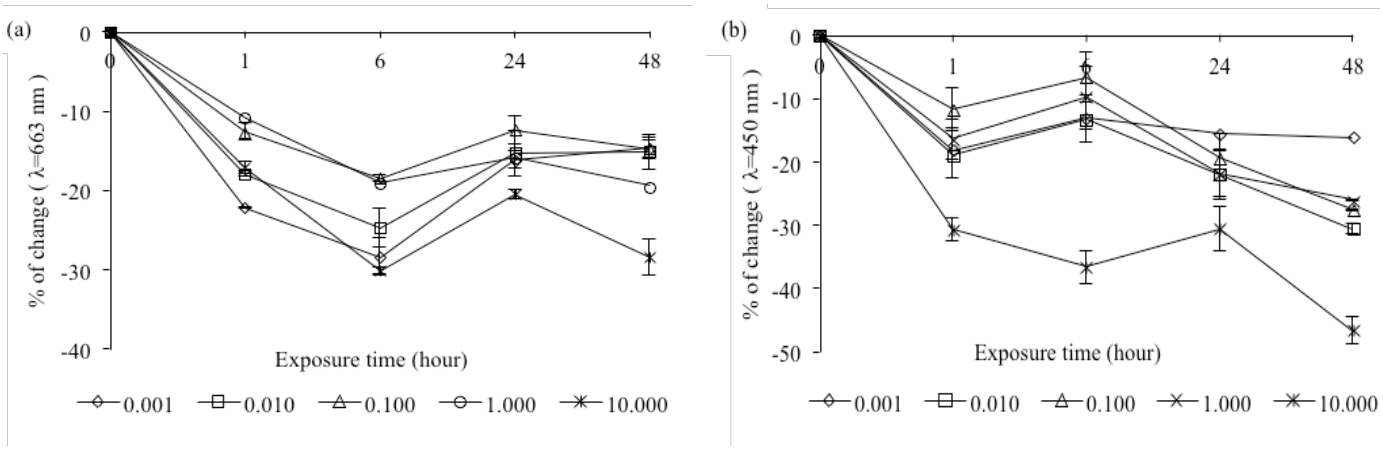

Figure 7. Response of *Spirogyra* to different concentrations of 2,4-D as indicated by λ = 663 nm and 450 nm.

At 10 mg L^{-1}, however, the OD reduced after exposing to atrazine for 48 hours. This indicated 10 mg L^{-1} of atrazine inhibited the growth of *Spirogyra*. The result was in agreement with previous studies that high concentration of atrazine will inhibit the growth of green algae, reduces protein synthesis, inhibits electron transfer in light reaction of photosynthesis, and reduces the expression of photosynthetic related genes (*El-Sheekh et al. 1994*; *Kabra et al. 2014*; *Mofeed and Mosleh 2013*; *Qian et al. 2008*; *Tang et al. 1997*). Inhibition of electron transfer further leads to generation of reactive oxygen species.

The chlorophyll content decreased in first six hours of exposure 2,4-D. Plant hormone 2,4-D might turn toxic in higher concentration. This indicates that the growth of cells which was reduced initially may be due to the toxicity of 2,4-D. The results are in agreement with earlier studies that 2,4-D could cause overproduction

Table 1. The best response time, possible linear detection ranges, R^2 values and slope of the linear equation for the responses of chlorophylls and carotenoids to Pb, Al, Ca, Na, atrazine and 2,4-D exposures.

Metals and organic compounds	Wavelength	Best response time (hour)	Linear detection range (mg L^{-1})	R^2	Slope
Pb	663 nm	48	0.010 – 10.000	0.933	0.86
	450 nm	24	0.001- 0.100	0.698*	53.58*
Al	663 nm	24	0.001 – 1.000	0.973	26.38
	450 nm	6	0.001 – 0.100	0.922	59.59
Ca	663 nm	48	0.001 – 0.100	0.970	162.79
	450 nm	24	0.001 – 0.100	0.844*	122.07*
Na	663 nm	48	0.001 – 0.100	0.977	108.24
	450 nm	24	0.001 – 0.100	0.964	50.74
Atrazine	663 nm	6	0.001 – 10.000	0.806*	29.09*
	450 nm	6	0.001 – 10.000	0.933	3.11
2,4-D	663 nm	24	0.001 – 0.100	0.979	35.71
	450 nm	6	0.001 – 0.100	0.986	68.14

* R2 < 0.9, which cannot be used as reference

of ROS in plant cells (*Pazmiño et al. 2012*; *Verma et al. 2010*). The production of ROS led to death of some cells.

The best response time for each exposure tests was identified by comparing the response curves of respective exposure time (**Table 1**). Chlorophyll content showed high correlation to the concentration of the pollutants, with three exceptions. Slope values obtained from the linear equations represent the sensitivity of each wavelength to the metals and pesticides. The sensitivity of *Spirogyra* using chlorophyll as marker increases following the sequence Pb < Al < Atrazine < 2,4-D < Ca for the wavelength 663 nm, and the sensitivity for wavelength 450 nm was Atrazine < Na < Al < 2,4-D.

CONCLUSION

Spirogyra collected from environment was successfully used for the indication of Pb, Al, Ca, Na, atrazine and 2,4-D through the changes of OD at the wavelengths of 663 nm and 450 nm. The sensitivity of *Spirogyra* to the pollutants and the presence of linear detection ranges showed that *Spirogyra* might be a good candidate as bioindicator for the presence the pollutants.

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