TRANSACTIONS ON SCIENCE AND TECHNOLOGY

The Detection of Calcium and Sodium Using Green Algae *Spirogyra*

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Received: *13 August 2015*
Revised: *26 August 2015*
Accepted: *1 October 2015*
In press: *2 October 2015*
Online: *1 April 2016*

Keywords:
Metals detection, bioindicator, *Spirogyra*, multi-markers.

Abstract

Continuous monitoring of metals is necessary due to the influx of metals into the environment from human activities. The presence of green algae *Spirogyra* in various water bodies can be used as an effective bioindicator for the detection of metals. In this paper, naturally available pigments in *Spirogyra*- chlorophylls and carotenoids were used to detect the presence of light metals by measuring the light absorbance at λ = 663 nm and 450 nm respectively, before and after the exposure to Ca and Na. The results showed both markers were sensitive to the presence of Ca and Na in aqueous environment, with carotenoids gave a better response. As all markers showed high correlation to the concentration of metals within 0.001 mg/L - 0.100 mg/L, *Spirogyra* had great potential to be as bioindicator for Ca and Na in aqueous environment.

Introduction

Metal pollutions have become a worldwide issue today because of its detrimental effects to many living organisms, especially aquatic life form. Although some of these light metals such as calcium (Ca) are essential nutrients for proper metabolism in living organisms, they are toxic at high concentrations (Qiu *et al.*, 2002). Other light metals such as aluminium (Al) which are currently thought as non-essential are toxic even at low concentration (Silva, 2012).

Naturally occurring whole cell can be good bioindicators for metals detection as they are available widely in natural environment, and able to provide simple, inexpensive detection and more accurate response on the toxic effect of various compounds (Buonasera *et al.*, 2011). Cyanobacteria, yeast, fungi, algae and plant cells are commonly used as bioindicators (Teo & Wong, 2014). They can detect the presence of metals by showing photosynthetic related responses. The toxicity level can then be estimated from the changes of absorbance, fluorescence, bioluminescence, or oxygen release from the cells.

To date, the study on *Spirogyra* for bioindicator applications was mainly focus on the effect of heavy metals on one single marker in cells. There are only little research has been done in using multi-markers, including pigments such as carotenoids together with chlorophyll. The study of *Spirogyra* as bioindicator of essential nutrients such as Ca and sodium (Na) is lacking as well. In this paper, the responses of *Spirogyra* obtained from natural environment towards the exposure of Ca and Na is reported. The potential of the *Spirogyra* to be used as natural bioindicator for these two light metals are discussed as well.

Methodology

The collection of algae cell

Spirogyra was collected from Green Foliage Nursery, Lot 8, Project Pertanian Moden, Air Hitam, Kluang, Johor, Malaysia, and subculture into 250 mL flasks with the water collected from the site served as the medium. The culture flasks were incubated in room temperature (27 ± 2˚C) under the illumination of cool-white fluorescent light. The dark and light ratio was maintained at 16 hours to 8 hours. All the subculture works were conducted in the laminar air flow with aseptic condition. The glassware and disposables-wares were autoclaved before use.

Identification of spirogyra cell and determination of cell density

Light microscope (Eclipse E-100, Nikon) was used to observe and identify the *Spirogyra* though their unique morphology and cell structures. Next, the cell count was conducted by haemocytometer (Marienfeld-Superior, Neubauer). Optical density (OD) determination of free *Spirogyra* cells was conducted at λ = 663 nm to correlate the number of cell in *Spirogyra* culture to OD. Day 1 cell culture was used to test the response of cells towards different concentrations of Ca and Na.

Immobilization of Cell

Spirogyra cells were immobilized in the cuvettes before the exposure to Ca and Na. The agarose gel for immobilization was prepared using agarose powder (Fisher Scientific, Malaysia). To make a 1.0% (w/v) agarose, 0.5 g of agarose was added to 50 mL of deionized water, followed by 3 minutes of boiling. Next, 0.5 mL of cell culture (with OD = 0.3 A – 0.5 A) and 0.5 mL of 1.0% agarose gel were mixed in a plastic cuvette. The mixture was left in room temperature to solidify on a clear side of the cuvette. The cuvette with immobilized cell was stored in room temperature.

Exposure to metals

Two mL of analytes was transferred into the cuvette with immobilized cells. The cell OD was measured with λ = 663 nm and 450 nm at 1, 6, 24 and 48 hours respectively. The responses of *Spirogyra* towards metals were determined by absorbance changes of the marker pigments (chlorophylls and carotenoids), which corresponding to the changes of OD with λ = 663 nm and 450 nm. The results obtained were then compared with negative controls to identify the responses of *Spirogyra* to Ca and Na. The blank used contain clear agarose without immobilized cells, while the negative control contained immobilized *Spirogyra* exposed to two mL of deionized water. All the exposure tests were conducted in triplicates unless specified otherwise.

Result and discussion

The OD for the *Spirogyra* used in this experiment fall within an interval of 0.30 A - 0.50 A, which was equivalent to 0.89 - 1.17 million cells/mL. Thus each cuvette contained approximately 0.445 –

0.585 million immobilized *Spirogyra* cells. The absorption wavelengths of chlorophylls and carotenoids were determined based on the literature, which chlorophylls produce absorption peak at 663 nm (Shoaf & Lium, 1976) and carotenoids have the maximum absorption around 450 nm (de Carvalho *et al*., 2012; Wong & Choong, 2014).

The responses chlorophyll and carotenoids to the exposures of 0.001, 0.010, 0.100, 1.000 and 10.000 mg/L of Ca and Na was showed in Figure 1. Both markers were predominately decreased in OD compared to the negative control, which reflected the decrease in the content of chloropyll and carotenoids, after been exposed to the metals within a period of 24 hours. Although Ca is required as nutrient for green algae, the decrease of chlorophyll carotenoids might cause by the ionic stress from these metals.

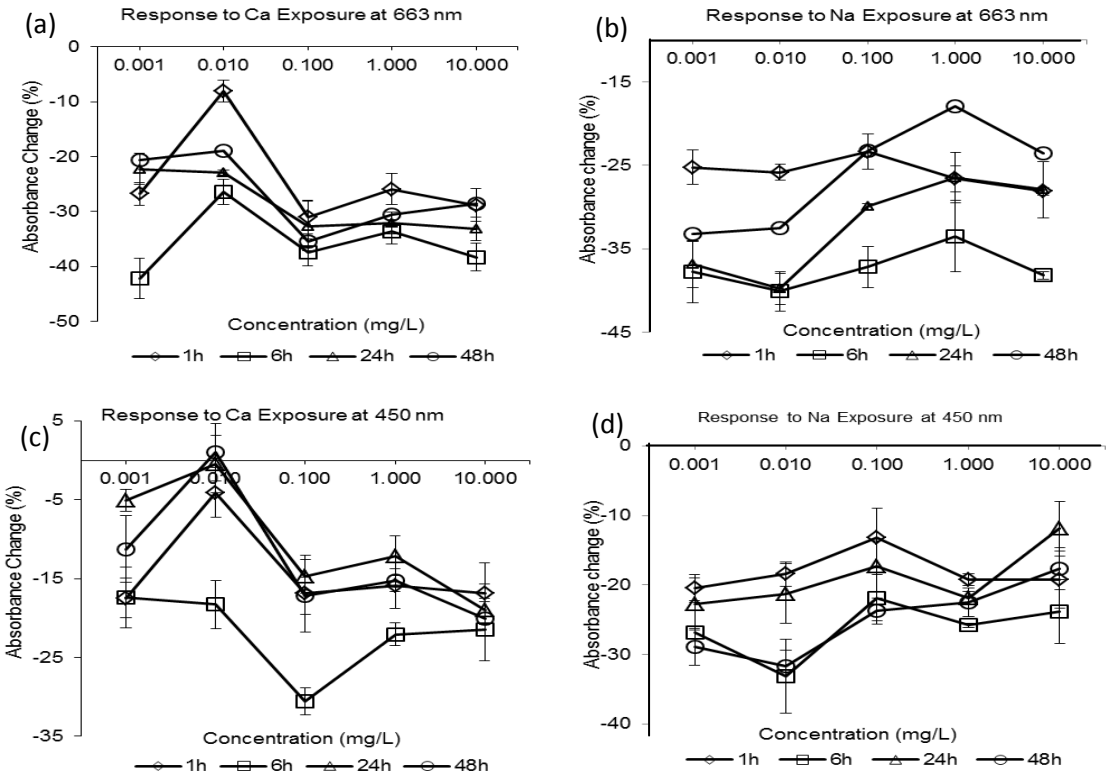

Figure 1: The responses of chloropyll to Ca (a) and Na (b) and the responses of carotenoids to Ca (c) and Na (d).

The *Spirogyra* native to natural environment might grow under the condition which lack of Ca was normal. When the additional Ca was introduced, the metal might interfere with the synthesis of chlorophyll and carotenoids (Brand & Becker, 1984). The iontic stress created by Ca might trigger the production of ROS, which would affect the level of the pigments (Sharma *et al.*, 2012). The introduction of Na altered the salinity of the medium, which creates stress to the algae (EL-Sheekh, 2004). The additional Na may lead to the changes of pottasium (K) and Na balance and further

disturbs the electron transfer chain in photosynthesis. The stress from Na might lead to the change in the concentration of photosynthetic pigments and antioxidant enzymes.

According to Xiong *et al*. (2002), plant cells could tolerant the ROS generated by ionic stress through the production of antioxidants (Sharma *et al*., 2012), thus might cause the "bounce-back" effects as shown in the 24 hours and 48 hours response curves. The production of ROS would further activate anti-oxidative pigments and enzymes (Sharma *et al*., 2012).

The effectiveness of *Spirogyra* as bioindicator was determined in terms of the best exposure time, value of correlation coefficient (R^2) and sensitivity which could be determined from the value of slope (Table 1). For Ca exposure, the best R^2 values were obtained within 0.001 mg/L - 0.100 mg/L at six hours of exposure for carotenoids (0.999), and 24 hours of exposure for chlorophylls (0.999). The slope values for carotenoids and chlorophyll at 134.11 and 106.55 respectively indicated that carotenoids were more sensitive towards Ca exposure. The carotenoids had a shorter response time of 6 hours towards Ca as well. However, both markers were suitable to be used to indicate the presence of Ca for the value of $R^2 > 0.950$.

For Na, the best R^2 values were obtained between 0.001 mg/L - 0.100 mg/L of Na with 24 hours of exposure for carotenoids (0.964) and 48 hours of exposure chlorophyll (0.999). The slope values calculated were 50.74 and 101.58 respectively. The results showed the carotenoids responded more rapidly to Na compare to chlorophyll, but chlorophyll was most sensitive to the Na exposure. *Spirogyra* is a widely available algae which the presence was reported all over the world. As native species, the algae can be a very useful tool to be used for environmental toxicant evaluation. Besides, the study of the responses of the algae to the toxicants might provide useful insights on these substances affect the biological entities.

Table 1: Summary of linear detection ranges (LDR), R^2 values for different exposure times, and sensitivity for chlorophyll and carotenoids exposed to Ca and Na over 48 hours.

		LDR (mg/L)	R^2 in each exposure time (hours)				Sensitivity (slope value) in each exposure time (hours)			
			1	6	24	48	1	6	24	48
Ca	Chlorophylls	0.001 - 0.100	0.331	0.021	0.999	0.970	127.9	21.2	106.5	162.8
	Carotenoids	0.001 - 0.100	0.154	0.999	0.844	0.482	54.4	134.1	122.0	118.0
Na	Chlorophylls	0.001 - 0.100	0.891	0.353	0.865	0.999	22.4	16.6	85.8	101.6
	Carotenoids	0.001 - 0.100	0.961	0.617	0.964	0.815	66.8	81.3	50.7	66.6

As this research progresses, tests for the responses of *Spirogyra* on more toxicants should be studied to observe the behavior of the native algae to these compounds. The effect of pH, different methods of immobilization, and the number of cells to the responses should be studied as well.

Conclusion

Spirogyra which is native to environment has been found to respond well to the presence of light metals. These responses were successfully captured using spectrophotometer through the changes of chlorophylls (measured at λ = 663 nm), and carotenoids (measured at λ = 450 nm) contained in the *Spirogyra*. Although the responses discovered the potential of *Spirogyra* to be used as environmental bioindicator for Na and Ca, more studies have to be done to optimize the parameters required for environmental toxicity analysis.

Acknowledgements

This project is funded by Ministry of Education Malaysia, Grant no: FRGS/1/2014/SG03/INTI/02/1.

References

[1] Brand, J. J., & Becker, D. W. (1984). Evidence for direct roles of calcium in photosynthesis. *Journal of Bioenergetics and Biomembranes*, **16**(4): 239-249.

[2] Buonasera, K., Lambreva, M., Rea, G., Touloupakis, E., & Giardi, M. (2011). Technological applications of chlorophyll a fluorescence for the assessment of environmental pollutants. *Analytical and Bioanalytical Chemistry*, **401**(4): 1139-1151.

[3] de Carvalho, L. M. J., Gomes, P. B., de Oliveira Godoy, R. L., Pacheco, S., do Monte, P. H. F., de Carvalho, J. L. V., Nutti, M. R., Neves, A. C. L., Vieira, A. C. R. A., & Ramos, S. R. R. (2012). Total carotenoid content, α-carotene and β-carotene, of landrace pumpkins (*Cucurbita moschata* Duch): A preliminary study. *Food Research International*, **47**(2): 337-340.

[4] EL-Sheekh, M. M. (2004). Inhibition of the water splitting system by sodium chloride stress in the green alga Chlorella vulgari. *Brazilian Journal of Plant Physiology*, **16**(1): 25-29.

[5] Qiu, D., Liu, X., & Guo, S. (2002). Regulation function of calcium on photosynthesis of *Dimocarpus longana* Lour. cv. wulongling under simulated acid rain stress. *The Journal of Applied Ecology*, **13**(9): 1072-1076.

[6] Sharma, P., Jha, A. B., Dubey, R. S., & Pessarakli, M. (2012). Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *Journal of Botany*, **2012:** Article ID 217037.

[7] Shoaf, W. T., & Lium, B. W. (1976). Improved extraction of chlorophyll a and b from algae using dimethyl sulfoxide. *Limnology and Oceanography*, **21**(6): 926-928.

[8] Silva, S. (2012). Aluminium toxicity targets in plants. *Journal of Botany*, **2012**: Article ID 219462.

[9] Teo, S. C., & Wong, L. S. (2014). Whole cell-based biosensors for environmental heavy metals detection. *Annual Research & Review in Biology*, **4**(17): 2663-2674.

[10] Wong, L. S., & Choong, C. W. (2014). Rapid detection of heavy metals with the response of carotenoids in *Daucus carota*. *International Journal of Environmental Science and Development*, **5**(3): 270-273.

[11] Xiong, L., Schumaker, K. S., & Zhu, J.-K. (2002). Cell signaling during cold, drought, and salt stress. *The Plant Cell Online*, **14**(suppl 1): S165-S183.