The Detection of Calcium and Sodium Using Green Algae Spirogyra

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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 \( \lambda = 663 \text{ nm} \) 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 chlorophyll 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](image)

**Figure 1**: The responses of chlorophyll 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 ionic 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 tolerate 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.

<table>
<thead>
<tr>
<th>LDR (mg/L)</th>
<th>R² in each exposure time (hours)</th>
<th>Sensitivity (slope value) in each exposure time (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Ca Chlorophylls</td>
<td>0.001 - 0.100</td>
<td>0.331</td>
</tr>
<tr>
<td>Carotenoids</td>
<td>0.001 - 0.100</td>
<td>0.154</td>
</tr>
<tr>
<td>Na Chlorophylls</td>
<td>0.001 - 0.100</td>
<td>0.891</td>
</tr>
<tr>
<td>Carotenoids</td>
<td>0.001 - 0.100</td>
<td>0.961</td>
</tr>
</tbody>
</table>

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 $\lambda = 663$ nm), and carotenoids (measured at $\lambda = 450$ 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.

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References


