

## Effect of Parathion Exposure towards Green Microalgae *Chlorella vulgaris* on the Changes of Physicochemical Parameters in Water

Cheng Wan Hee<sup>1</sup>, Wong Ling Shing<sup>1</sup> and Chong Kah Chi<sup>1</sup>

Faculty of Health and Life Sciences, INTI International University,  
Persiaran Perdana BBN, 71800 Nilai, Negeri Sembilan, Malaysia

**Email:** wanhee.cheng@newinti.edu.my

### Abstract

Agricultural activities has caused the release of pesticides such as parathion into the environment, which threatened the freshwater ecosystem. Therefore, the objective is to observe for the changes of physicochemical parameters (pH, conductivity and dissolve oxygen) caused on *Chlorella vulgaris* due to parathion exposure. The cell count of *C. vulgaris* was done using hemocytometer and this alga was exposed to different concentrations of parathion (1.000 mg/L, 0.100 mg/L, 0.001 mg/L, and 0.000 mg/L (negative control)). The values of each parameter were recorded before the exposure and after 1 hour, 2 hours, 6 hours and 24 hours of exposure by using physicochemical parameter. The results showed a fluctuation of all physicochemical parameters (pH, conductivity and dissolved oxygen) values in the surrounding water at the first 5 hours of exposure and decreased before reaching a plateau after the fifth hour of exposure. The reason of this phenomenon could be due to the inhibition effects of parathion towards the photosynthesis activities of the algae and thus lowering the physicochemical parameters. Therefore, *C. vulgaris* serves as a good bioindicator of parathion pollution.

### Keywords

*Chlorella vulgaris*, bio-indicator, parathion

### Introduction

Freshwater pollution is a global issue (Martonas, 2017) which is mainly due to human activities such as deforestation, inappropriate discharge of industrial waste and agricultural activities. The agricultural sector, in particular, has contributed to an enormous amount pesticides released into the environment leading to major environmental degradation (Rinkesh, 2009). One of these major pesticide pollutants were parathion, is a highly toxic substance that causes deleterious effect on the nervous system upon exposure (Aktar et al., 2009). Therefore it is crucial to establish an early detection system on the levels of parathion in the freshwater ecosystem. Many studies have concluded that fresh water algae were good bioindicators of pesticides due to their sensitivity

International Conference on Innovation and Technopreneurship 2019

Submission: 1 June 2019; Acceptance: 2 December 2019



**Copyright:** © 2019. All the authors listed in this paper. The distribution, reproduction, and any other usage of the content of this paper is permitted, with credit given to all the author(s) and copyright owner(s) in accordance to common academic practice. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license, as stated in the website: <https://creativecommons.org/licenses/by/4.0/>

towards environmental changes and they are ideally used in water quality assessment (Holy and Miller, 2010; Omar, 2010; Didem, 2016).

In this study, the freshwater algae, *Chlorella vulgaris* was used to detect the presence of parathion based on the changes of physicochemical parameters in the surrounding water. *Chlorella vulgaris* is a unicellular photosynthetic green algae in the fresh water ecosystem. Like many other photosynthetic algae, its photosynthetic activities will be disrupted by environmental pollutants such as parathion (Rosko & Rachlin, 1977). This hindrance on the metabolic activities of the algae causes a change of metabolic products released in to the surrounding and thus, changes in physicochemical parameters such as pH value, conductivity and dissolved oxygen of in the water body will ensue (Jeroen & Susan, 1987; Donmez et al., 1999; Celine et al., 2003). Therefore, the objective of this study is to determine the effects of different concentrations of parathion exposure to *C. vulgaris* towards the changes in pH, conductivity and dissolved oxygen in the water body.

## Methodology

### *Mother culture preparation*

The culture media for *C. vulgaris*, Bold Basic stock medium (10x), were purchased from Sigma-Aldrich, Malaysia. Cultivation of the alga was performed by adding 4 mL to 196 mL of distilled water for the preparation of a 200 mL mother culture and the culture was maintained at room temperature under fluorescent lamp (16 hours of light exposure and 8 hours without light).

### *Cell count & data analysis*

Cell count was conducted once in every three days by using the hemocytometer (Neubauer, Marienfeld) and observed under the light microscope (E100, Nikon Eclipse). Cell density calculation was performed by using the following equation:

$$\text{Cell Density (cells/mL)} = \text{Average cells per large square} \times 10^4 \times \text{Dilution factor}$$

### *Exposure of Pb towards algae and physicochemical parameter analysis*

The *C. vulgaris* cells, 5 mL of cultured cell in each exposure, from day 4 culture with approximately  $1.3 \times 10^6$  cells/mL was exposed to 1.000 mg/L, 0.100 mg/L, 0.001 mg/L, and 0.000 mg/L (negative control) of parathion. The culture of each exposure were then analyzed for pH, conductivity and dissolved oxygen in every 0<sup>th</sup> hour, 1<sup>th</sup> hour, 2<sup>nd</sup> hour, 6<sup>th</sup> hour and 24<sup>th</sup> hour by using the portable multiparameter meter (HandyLab 680 FK, SI Analytics). All the exposure tests were conducted in triplicate. The statistical values including percentage change of three parameters (pH, conductivity and dissolved oxygen), percentage of response, mean and standard deviation were calculated by Microsoft Excel. These statistical values were used to plot the response curve.

### Results and discussions

Growth curve were plotted based on the growth of *C. vulgaris* for 15 days (Figure 1) and two visible growth phases of the algae were observed, which are the exponential phase (day 1 to day 7) and the stationary phase (day 7 to day 15).

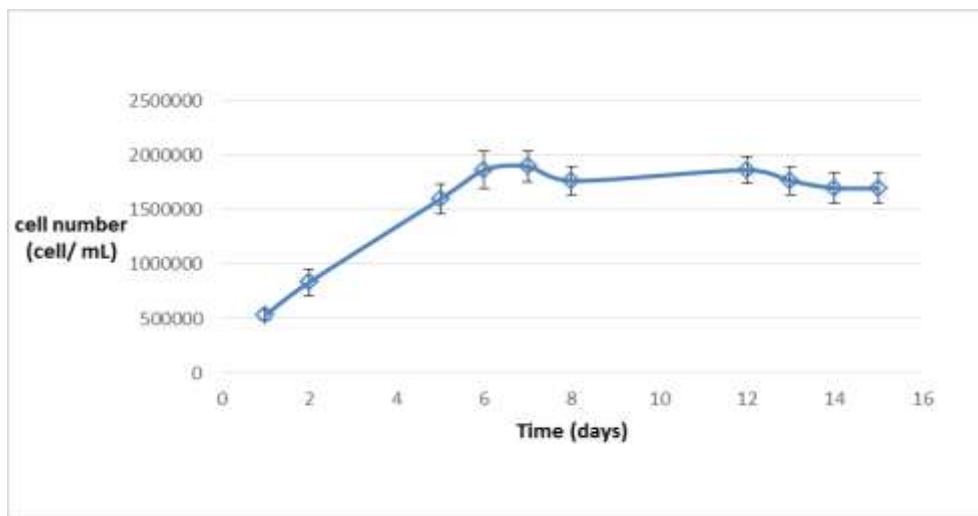


Figure 1. Growth curve of *Chlorella vulgaris*

Initial cell number of green algae in BBM was recorded at  $5.3 \times 10^5$  cell/mL. The growth of the algae gradually increased and reached the maximum cell density of  $1.9 \times 10^6$  cell/mL at day 7. Algae utilized the nutrients in BBM and started to reproduce (Zuliyana et al., 2014) causing increment in cell count in the first 7 days. Stagnant growth of the algae observed from day 7 to day 15, which could be due to the limitation of nutrients (Navarro et al., 2010) in BBM, which indicated that the algae proceed to the stationary stage. Day 4, which lies in the middle of the exponential phase, were chosen to be the parathion exposure day for the algae as they are still optimally growing. Figure 2, 3 and 4 show the changes of pH, conductivity and dissolved oxygen values when *C. vulgaris* was exposed to different concentrations of parathion by the number of hours.

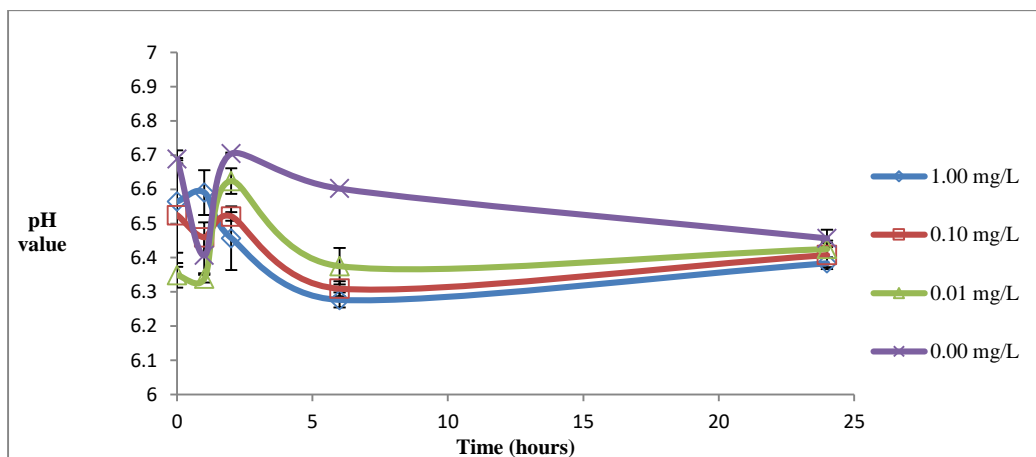


Figure 2. pH value of culture after cells exposed to parathion

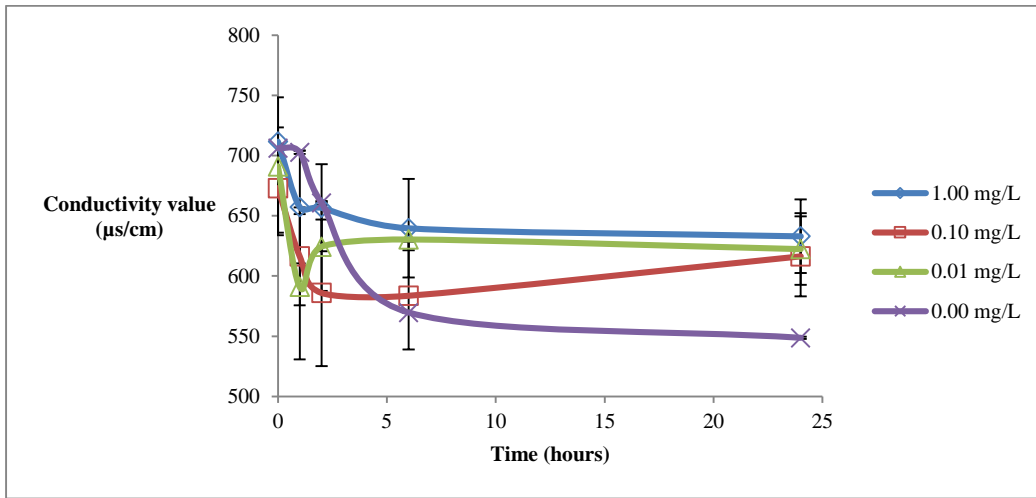


Figure 3. Conductivity of culture after cells exposed to parathion

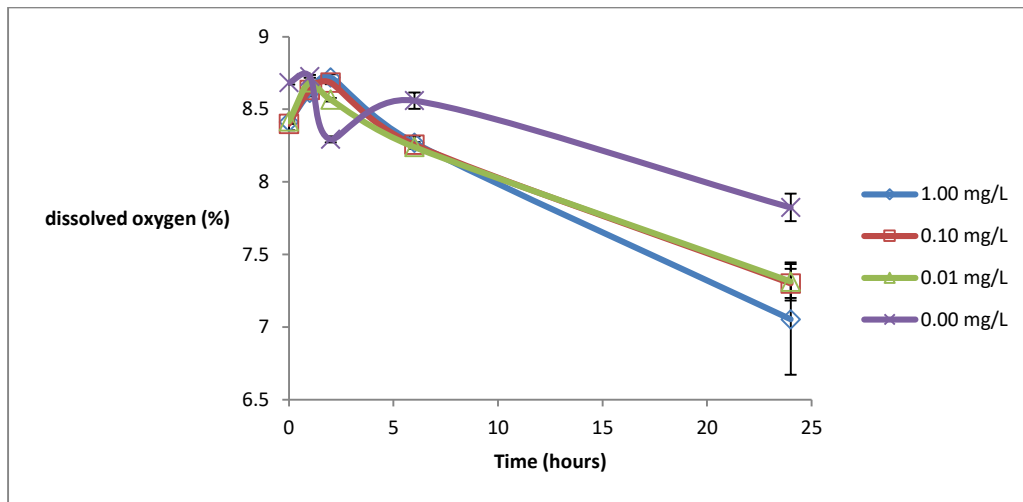


Figure 4. Dissolved oxygen of culture after cells exposed to parathion

All three parameters showed fluctuating values at the first 5 hours of exposure while reaching a plateau after the fifth hour. Based on Figure 2, the sudden drop and rise of pH values from 0 to 2 hours of exposure could be due to the transition between log phase and exponential phase of the growth due to adaptation to parathion. The decrease of pH values after the second hours, before reaching a plateau after the fifth hour, could be due to the inhibition of the growth and photosynthetic activities of the algae which led to a decrease in pH values (Tang et al., 1997).

As for dissolved oxygen (Figure 4), the dissolved oxygen values for all concentrations of parathion exposure were between 8.0-9.0 %. After 5th hour of exposure, the reading for all concentration of exposure decreased. The increased parathion concentrations exposure will increase the inhibitory stress toward the photosynthesis of algae due to the destruction of chlorophyll pigments essential for photosynthesis (Al-Hejuje, 2008). When oxygen producing photosynthetic pigments have been destroyed, dissolved oxygen will reduce as well (Raul and Benoit, 2006).

All different concentrations of parathion exposure showed no correlation among each other which could be due to the different toxicity responds based on the specific concentrations. However, further studies are required to further confirm this phenomenon.

### Conclusions

As a conclusion, *C. vulgaris* are able to detect the presence parathion in water through the changes of pH, conductivity and dissolved oxygen based on their respond towards the pollutant. Thus, the growth rate of algae will make changes on the parameter we test in this experiment which are pH, conductivity and dissolved oxygen.

### Acknowledgement

The authors wish to acknowledge the financial support provided through INTI International University's final year project student funding.

### References

- Aktar, W., Sengupta, D., & Chowhury, A. (2009). Impact of pesticides use in agriculture: their benefits and hazards. *Journal of Toxicology*, 2(1), 1-12.
- Al-Hejuje (2008). Effect of some heavy metals ions on the chlorophyll a pigment of *Nostoc linkia* and *Hapalosiphon aureus*. Department of Biology, College of Science, University of Basrah. 3(2), 136-146.
- Celine, C., Sergei, D., Chovelon, J. M., & Claude, D. (2003). Development of novel conductometric biosensor based on immobilized whole cell *Chlorella vulgaris* microalgae. *Biosensor and Bioelectronics*. 19(9), 1089-1096.
- Didem, G. (2016). *Organisms for Imminent Biotechnology*: IntechOpen.
- Donmez, G. C., Aksu, Z., Ozturk, A., & Kutsal, T. (1999). A comparative study on heavy metal biosorption characteristics of some algae. *Process Biochemistry*, 34(9), 885-892.
- Holy, E. A. & Miller, S. W. (2010). Bioindicators: Using organism to measure environmental impacts. *Nature Education Knowledge*. 3(10), 8.
- Jeroen, G., & Susan, W. B. (1987). Electrophoretic mobility of natural particles and cultured organisms in freshwater. *Limnology and Oceanography*, 32(5), 1987, 1049-1058.
- Marie, E. D., Geoffrey, I. S., & Philippe, E. R. (2001). Toxicity of pesticides to aquatic microorganisms. *Environmental Toxicology and Chemistry*. 10(1), 84-98.
- Martonas, J. (2017). The effect of environments. *Journal of Effect of Human Activities on the Environment*. Retrieved from <http://education.seattlepi.com/effect-human-activities-environment-3653.html>.
- Navarro, L. J., Tormo, A., & Martinez-Garci, A. E. (2010). Stationary phase in gram-negative bacteria. *FEMS Microbiology*, 34, 476-495.
- Omar, W. M. W. (2010). Perspectives on the use of algae as biological indicators for monitoring and protecting aquatic environments, with special reference to Malaysian freshwater ecosystems. *Tropical Life Science Research*, 21(2), 51-67.

- Raul, M., & Bennoit, G. (2006). Algal–bacterial processes for the treatment of hazardous contaminants: A review. *Water Research*, 40(15), 2799-2815.
- Rinkesh (2009). What is Pollution? Retrieved from [http://www.conserve-energy-future.com/pollutiontypes.php#abh\\_posts3](http://www.conserve-energy-future.com/pollutiontypes.php#abh_posts3).
- Rosko, J. J., & Rachlin, J. W. (1977). The effect of cadmium, copper, mercury, zinc and lead on cell division, growth, and chlorophyll a content of the chlorophyte *Chlorella vulgaris*. *Bulletin of the Torrey Botanical Club*, 104(3), 226-233.
- Tang, J. X., Hoagland, D. D., & Siegfried, B. D. (1997). Differential toxicity of atrazine to selected freshwater algae. *Bulletin of Environmental Contamination and Toxicology*, 59, 631-637.
- Zuliyana, N., Shukri, M., Hasnun, N. I., & Jani, M. (2014). The growth performance of freshwater *Chlorella* sp. and *Scenedesmus* sp. in different media. *Journal of Applied Science and Agriculture*, 9(11), 119-125.