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IMMOBILISATION AND CHARACTERISATION OF CHLORELLA VULGARIS AS POTENTIAL BIO-INDICATOR FOR SELECTED HERBICIDES

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By

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Thesis Submitted in Fulfilment of the Requirement for the Degree of Doctor of Philosophy in the Faculty of Science and the Environmental Studies
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DEDICATION

This work is dedicated to Associate Professor Dr. Nor Aripin Shamaan for his invaluable guidance throughout my years in UPM. I also dedicate this achievement to my beloved husband Zuhaidi Mukrim for his support and believing in me; to my son Muhammad Danish and my daughter Nabel Darwish, for cheering me up during the blues; to my mother Mariyah Ali, for blessing me and her prayers for my success; to my father Desa Hj. Demat for his blessing; to my sisters Desmariatul Safinaz and Shabrinaz, my brother Kamal Affendi for their inspirations and motivations.

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Abstract of thesis submitted to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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Chairman: Associate Professor Dr. Nor Aripin Shamaan

Faculty: Science and Environmental Studies

Herbicides are one of the major contributing pollutants in water bodies. Several detection methods have been developed to monitor herbicide pollution including the use of bio-indicators. The competency of a bio-indicator in herbicide detection must comply with the sensitivity and efficiency of the method. In this study, *Chlorella vulgaris* as a bio-indicator was immobilised in alginate and compared with the free cell to determine its ability as a bio-indicator.

There were two immobilised conditions; immobilised cells of recommended cell concentration ($2x10^4$cells/ml) and immobilised cells based upon suitability test. In the suitability test, four bead concentrations were tested; 0.1%w/w, 0.2%w/w, 0.4%w/w and 0.8%w/w. 0.1%w/w was selected as test bead based on stability and

water and 26 days in calcium chloride. The other bead concentrations were stable for less than 20 days. The 0.1%w/w bead had constant growth rate and exponential rate pattern of oxygen production for 7 days, compared with the other beads.

Free cells and two immobilised conditions were compared using two methods; oxygen production rate inhibition test and 96 hour's toxicity test. Four herbicides were used in this study; Atrazine, Simazine, Diuron and Paraquat. The first three are photosystem II inhibitor and Paraquat is a photosystem I inhibitor.

Immobilised microalgae was dark incubated in herbicide for 30 minutes before measuring the oxygen production rate. 30 minutes was chosen as incubation time due to significant inhibition of oxygen production rate by herbicide at this period. Light and temperature values during detection were previously examined and selected for suitability. The selected light intensity was 90µmol/sec/m^2 and 28°C for sample chamber's temperature due to the production of oxygen at exponential rate.

Cells were incubated for 96 hours in herbicide with 12:12h light cycle for 96 hour's toxicity test. Cells were enumerated and compare to reference. For immobilised cells, cells were counted after dissolving the beads with trisodium citrate.

There were three significant findings in this study. First, the ability to immobilise *Chlorella vulgaris* as a 2mm bead, which can survive for more than three months. Second, immobilisation of the recommended cell number was the better choice as bio-indicator using oxygen production rate change compared to free cells or test bead. There was 50% inhibition using this condition at 0.12µM Atrazine, 5.8µM Simazine, 0.4µM Diuron and calculated value at 3.913 mM for Paraquat, while the other cell conditions needed higher concentration than 1000µM for 50% inhibition or could not exhibit 50% inhibition. Third, for toxicity testing, free cells is recommended compared to the immobilised cells. Toxicity of free cells at 1000µM was higher in Simazine > Atrazine > Diuron > Paraquat, while at 0.01µM: Diuron > Paraquat > Atrazine> Simazine. For the immobilised conditions, no 50% inhibition of cell number was observed, suggesting the cells were protected by alginate. In conclusion, immobilised cells are potential useful bio-indicator for herbicide or other pollutant that interfere with photosynthesis in water body. However, further research should be done to improve and simplify the method.

Abstrak tesis diserahkan kepada Senat Universiti Putra Malaysia
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IMMOBILISASI DAN PENCIRIAN *CHLORELLA VULGARIS* SEBAGAI BIO-PENUNJUK BERPOTENSI BAGI RACUN RUMPAI TERPILIH

OLEH

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Oktober 2001

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Racun rumpai adalah satu daripada bahan pencemaran di dalam air. Terdapat banyak kaedah pengesan yang dibangunkan bagi membantu pemantauan di lapangan. Kebolehan bio-pengesan di dalam mengesan racun rumpai mestilah selari dengan sensitiviti dan keberkesanan sesuatu kaedah. Di dalam kajian ini, *Chlorella vulgaris* sebagai bio-penunjuk dipegunkan di dalam alginate dan dibandingkan dengan keadaan bebasnya untuk mengetahui kebolehannya bertindak sebagai bio-penunjuk.

Terdapat dua keadaan pegun; memegun sel pada kepekatan yang disarankan ($2x10^4$ sel/ml) dan sel pegun kajian yang dipilih dari ujian kesesuaian. Di dalam ujian kesesuaian, empat kepekatan sel

dipilih sebagai sel pegun kajian berdasarkan kestabilan dan pertumbuhan yang sesuai. Sel pegun tersebut stabil selama 23 hari apabila disimpan di dalam air suling dan 26 hari di dalam kalsium klorida. Kepekatan sel pegun yang lain stabil selama kurang dari 20 hari. Sel pegun 0.1%w/w mempunyai pertumbuhan yang malar dan kadar eksponen bagi penghasilan oksigen selama 7 hari berbanding sel pegun yang lain..

Keadaan bebas dan pegun dibandingkan melalui dua ujikaji; Ujikaji perencatan kadar penghasilan oksigen dan Ujikaji ketoksikan 96 jam. Empat racun rumpai digunakan didalam kajian ini; Atrazine, Simazine, Diuron dan Paraquat. Tiga racun yang pertama adalah perencat fotosistem II manakala Paraquat adalah perencat fotosistem I.

Sel pegun dieram di dalam racun rumpai selama 30 minit di dalam keadaan gelap sebelum pengukuran kadar penghasilan oksigen dibuat. 30 minit dipilih sebagai masa pengeraman kerana perencatan oleh racun rumpai adalah signifikan pada jangkawaktu ini. Nilai cahaya dan suhu semasa pengukuran telah diuji dan dipilih mengikut kesesuaian terlebih dahulu. Nilai cahaya yang dipilih adalah 90µmol/sec/m^2 and 28°C bagi suhu kebuk sampel berdasarkan kadar penghasilan oksigen yang eksponen.

Sel dieram selama 96 jam di dalam racun rumpai dengan 12:12j kitaran cahaya untuk ujian ketoksikan 96 jam. Sel dikira dan

dibandingkan dengan kawalan. Bagi sel pegun, sel dikira selepas diurai menggunakan trisodium sitrat.

Terdapat tiga penemuan yang signifikan di dalam kajian ini. Pertama, kebolehan untuk memegun *Chlorella vulgaris* bersaiz 2 mm diameter dan mampu hidup lebih dari tiga bulan. Kedua, sel pegun merupakan pilihan yang terbaik berbanding keadaan lain sebagai bio-penunjuk kepada perubahan kadar penghasilan oksigen berbanding sel bebas atau sel pegun kajian. Terdapat 50% perencatan pada 0.12μM Atrazine, 5.8μM Simazine, dan 0.4μM Diuron dan nilai pengiraan 3.913mM bagi Paraquat., manakala dua keadaan yang lain memerlukan kepekatan yang lebih tinggi dari 1000μM bagi perencatan 50% atau tidak boleh merencat 50%. Ketiga, bagi ujian ketoksikan, sel bebas disarankan penggunaannya berbanding sel pegun. Ketoksikan terhadap sel bebas pada 1000μM adalah tinggi di dalam Simazine > Atarzine > Diuron > Paraquat, manakala pada 0.01μM: Diuron > Paraquat > Atrazine > Simazine. Bagi kedua dua sel pegun, tidak terdapat 50% perencatan, menunjukkan sel dilindungi oleh alginate. Kesimpulannya, sel pegun berpotensi sebagai bio-penunjuk yang berguna terhadap racun rumpai atau pencemar lain yang mengganggu fotosintesis di dalam air. Bagaimana pun, kajian lanjutan mestilah dijalankan untuk memperbaiki dan memudahkan kaedah tersebut.

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BBM	Bold basal media
EPA	Environmental Protection Agency
PSI	Photosystem I
PSII	Photosystem II

CHAPTER 1

INTRODUCTION

Pesticides And Environment

Rapid increase in world population has led to intensive farming as the better option for food production. Inevitably, a successful intensive farming requires extensive use of agrochemicals. Numerous studies have been carried out worldwide which indicated that the extensive use of pesticides has directly or indirectly caused adverse effects to the environment.

Most of the agrochemicals are inherently toxic to living organisms and inevitably affect human health. The indiscriminate use of pesticides not only affects human health but also creates serious environmental implication. However, the effects of herbicide contamination on aquatic systems depend on the characteristics of the herbicide, its concentration and the nature and biology of the aquatic systems.

The pesticide industry in Malaysia is heavily dependent on imported active ingredients and foreign product technology (Yeoh et al., 1991). This is because pesticides play an important role in crop

protection for the foreseeable future, as there are no practical alternatives at the moment. However, there are tremendous changes in some of the latest pesticides introduced; for instance the significant reduction in dosage rates and their reduced persistence in the environment.

Pesticides enter water body easily via soil leaching, spray drift or through ground water. Besides containing aquatic food chain, water is important and its scarce resource has been used for irrigation, aquaculture and human consumption. Therefore it is vital to keep water sources safe. Thus, pollution monitoring and treatment become an important issue. It brings on researchers throughout the world to develop methods of herbicide detection in water. Their goal is to produce a fast, cheap, easy-handling and reliable pesticides-detecting tool.

Herbicide Detection

Current herbicide detection methods are not perfectly suitable for large scale monitoring or field monitoring programme. These methods include gas chromatography (GC) and high performance liquid chromatography (HPLC) that is affordable by most laboratories. The techniques required large sample volumes, extensive extraction and clean up procedures for analyses. These techniques also require

solvents that will finally end up polluting the environment. The chemical analyses carried out in laboratory are laborious and expensive.

Nowadays there are high-speed techniques that may detect heavy metals and pesticides. For example, induced couple plasma mass spectrometry (ICPMS) and gas chromatography mass spectrometry (GCMS) are among the most reliable equipment in Malaysia. However, the drawback is the cost of the equipment. Detection can only be carried out by research institutes, government based monitoring team or private companies that owns the facilities. Therefore it is useful to develop a reliable detecting method that is affordable by all users.

The accuracy, reproducibility and sensitivity of conventional methods and biological based methods are almost of the same quality (Korpan and El'skaya, 1995; Pandard and Rawson, 1993 and Gaisford et al., 1991). Table I summarises the comparison of conventional methods and biological based analyses on several aspects. The biological based analyses mainly involved the usage of microalgae, plant organelles and bacteria.

The basis of any detection tool is accuracy, precision, repeatability, reproducibility, sensitivity and reliability. Therefore, development of any detection method for herbicides should consider the