

**SELECTED CYANOBACTERIA AND GREEN ALGAE
SPECIES AS PHYCOREMEDIATION AGENT FOR
TOXIC METAL POLLUTANTS**

BY

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ABSTRACT

Water is an essential environmental element and vital in the Islamic value system for purification in performing *Ibadah*. Cleanliness is not only on application of water to worship but also from safety aspect for daily usage like drinking water and recreation. Regrettably, this tiny volume of water that available on earth is under severe stress because of speedy rise in populace, urbanisation, and untenable utilisation of water in agricultural and industrial setups. Thus, this research has aimed to explore various groups of selected algae that can be employed as potential biosorption agent for inorganic contaminants in polluted aquatic ecosystem. The research was conducted by developing two phases which were algae culturing and phycoremediation model system. In algae culturing, the research has been undertaken by investigating two green algae species of *Chlorella vulgaris* and *Pandorina morum* as well as two blue-green algae species (Cyanobacteria) of *Pseudanabaena* sp. and *Synechococcus* sp. cell culture growth exposed to different medium formulation, pH and photoperiod. In the second phase, phycoremediation model system was performed by assessing heavy metal sequestration of the same group of algae species exposed to various concentrations and period of time. Two species from group of green algae; *C. vulgaris* and *P. morum* and cyanobacteria; *Pseudanabaena* sp. and *Synechococcus* sp were tested in four different medium; Chu-10, Bold's Basal medium, Bristol, and BG-11 in different pH (3.8, 5.8, 6.8 and 7.8) in six different photoperiods (24:0, 12:12, 18:6, 6:18 and 0:24 day/dark cycle) condition. Range of green and blue green algae species cells density growth in different pH, photoperiod and medium formulation varies between species. The highest cells density growth for both *P. morum* and *C. vulgaris* were detected under photoperiod of 24 h light in BG11 medium at pH 7.8 with the amount of $170 \times 10^4 \text{ mL}^{-1}$ and $161 \times 10^4 \text{ mL}^{-1}$ respectively, whereas in cyanobacteria species cells density growth were found varied. *Pseudanabaena* sp. was identified to have yielded the highest amount of cells density growth at $115 \times 10^4 \text{ mL}^{-1}$ in BBM medium under photoperiod of 24 h light at pH 7.8. Meanwhile, *Synechococcus* sp. highest amount of cells density growth was detected in BG11 medium at pH 7.8 and 24 h light at $481 \times 10^4 \text{ mL}^{-1}$. Marked differences were observed between green and blue-green algae species in cells density growth rate. Therefore, all four selected algae species were analysed over bioconcentration factor (BCF) to further investigate the phycoremediation capability between green and blue green algae species. Three different concentrations of 1mg/L, 2mg/L and 3mg/L of heavy metals (Pb, Fe, Cr, Cd, Al and Cu) were tested for four weeks and the samples were analysed every week via Inductively Coupled Plasma (ICP). Highly significant differences were observed between the species, the heavy metals, the time duration, and all combinations of interactions, which emphasises that changes in BCF value are complex and the responses are not consistent across species. The magnitude of these effects depends on the algae species, time duration, and the concentration and type of heavy metal. Although these factors had an effect on the BCF value, the most influential factor appeared to be the algae species selection. Amongst the selected algae, *C. vulgaris* and *Synechococcus* sp. were great candidates as good heavy metal accumulator but the cyanobacteria species were the best alternative instead of green algae species for phycoremediation agent.

خلاصة البحث

الماء عنصر بيئي أساسي وحيوي في نظام القيم الإسلامية للتطهير في أداء العبادة. لا تقتصر النظافة على استخدام الماء للعبادة فقط، ولكن أيضاً من ناحية الأمان للاستخدام اليومي مثل مياه الشرب والاستحمام. للأسف، هذا الحجم الضئيل من المياه المتوفرة على الأرض يتعرض لضغوط شديدة بسبب الارتفاع السريع في عدد السكان، والتحضر، والاستخدام غير المستدام للمياه في المنشآت الزراعية والصناعية. وبالتالي، يهدف هذا البحث إلى استكشاف مجموعات مختلفة من الطحالب المختارة التي يمكن استخدامها كعامل امتصاص حيوي محتمل للملوثات غير العضوية في النظام البيئي المائي الملوث. لقد تم إجراء البحث من خلال تطوير مرحلتين هما نظام استزراع الطحالب ونموذج العلاج الطبيعي. في استزراع الطحالب، كما تم إجراء البحث عن طريق فحص نوعين من الطحالب الخضراء من *Pandorina* و *Chlorella vulgaris* و *Pseudanabaena morum* بالإضافة إلى نوعين من الطحالب الزرقاء والخضراء (*Cyanobacteria*) من *Pseudanabaena* و *Synechococcus sp* و *Synechococcus sp* نمو ثقافة الخلية المعرضة لتركيبية متوسطة مختلفة، ودرجة الحموضة وفترة الضوء. في المرحلة الثانية، تم إجراء نظام نموذج المعالجة الطبيعية عن طريق تقييم عزل المعادن الثقيلة لنفس المجموعة من أنواع الطحالب المعرضة لتركيزات مختلفة وفترة زمنية مختلفة. هناك نوعان من مجموعة الطحالب الخضراء. *C. vulgaris* و *P. morum* والبكتيريا الزرقاء؛ *Pseudanabaena sp* و *Synechococcus sp* تم اختبارها في أربعة وسائط مختلفة؛ *Chu-10* و *Bold's Basal medium* و *Bristol* و *BG-11* بدرجات حموضة مختلفة (3.8 و 5.8 و 6.8 و 7.8) في ست فترات ضوئية مختلفة (0:24، 6:18، 12:12، 18:6، 24:0 يوم / دورة مظلمة). توجد مجموعة من أنواع الطحالب الخضراء والزرقاء والخضراء كثافة خلايا النمو في مختلف الأس الهيدروجيني، وفترة الضوء والصياغة المتوسطة تختلف بين الأنواع. لقد تم الكشف عن أعلى نمو لكثافة الخلايا لكل من *P. morum* و *C. vulgaris* تحت فترة ضوئية 24 ساعة من الضوء في وسط *BG11* عند الرقم الهيدروجيني 7.8 بكمية 170×10^4 مل-1 و 161×10^4 مل على التوالي، بينما في خلايا أنواع البكتيريا الزرقاء تم العثور على نمو الكثافة المتنوعة. وتم التعرف على *Pseudanabaena sp* على أنها أسفرت عن أكبر قدر من نمو كثافة الخلايا عند 115×10^4 مل-1 في وسط *BBM* تحت فترة ضوئية 24 ساعة من الضوء عند الرقم الهيدروجيني 7.8. وفي الوقت نفسه، تم اكتشاف من *Synechococcus sp* على أنه أكبر قدر من نمو كثافة الخلايا في وسط *BG11* عند درجة الحموضة 7.8 و 24 ساعة في الضوء عند 481×10^4 مل. كما لوحظت اختلافات ملحوظة بين أنواع الطحالب الخضراء والزرقاء والخضراء في معدل نمو كثافة الخلايا. لذلك، تم تحليل جميع أنواع الطحالب الأربعة المختارة باستخدام عامل التركيز

الحيوي (BCF) لمزيد من البحث في قدرة المعالجة الطبيعية بين أنواع الطحالب الخضراء والزرقاء. تم اختبار ثلاثة تراكيز مختلفة من 1 مجم/لتر و 2 مجم/لتر و 3 مج/لتر من المعادن الثقيلة (الرصاص والحديد والكروم والكاديوم والألمنيوم والنحاس) لمدة أربعة أسابيع وتم تحليل العينات كل أسبوع عن طريق البلازما المقترنة بالحث (ICP). لوحظت أيضا فروق ذات دلالة إحصائية كبيرة بين الأنواع، والمعادن الثقيلة، والمدة الزمنية، وجميع مجموعات التفاعلات، مما يؤكد أن التغييرات في قيمة عامل التركيز البيولوجي معقدة والاستجابات ليست متسقة عبر الأنواع. يعتمد حجم هذه التأثيرات على أنواع الطحالب، والمدة الزمنية، وتركيز ونوع المعدن الثقيل. على الرغم من أن هذه العوامل كان لها تأثير على قيمة عامل التركيز الأحيائي، يبدو أن العامل الأكثر تأثيراً هو اختيار أنواع الطحالب. من بين الطحالب المختارة، *C. vulgaris* و *Synechococcus sp* كانت مرشحة رائعة كمركب جيد للمعادن الثقيلة، لكن أنواع البكتيريا الزرقاء كانت البديل الأفضل بدلاً من أنواع الطحالب الخضراء كعامل معالجة الطور.



APPROVAL PAGE

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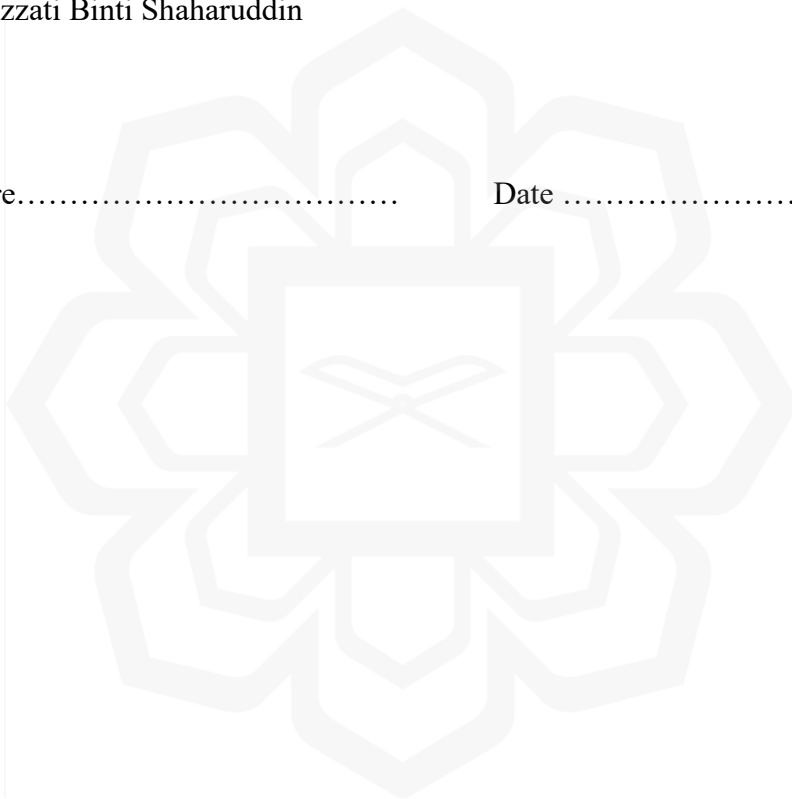
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DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

Ruhul 'Izzati Binti Shaharuddin

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**STUDIES ON CYANOBACTERIA AND GREEN ALGAE
SPECIES AS POTENTIAL OF PHYCOREMEDIATION AGENT
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First of all most importantly, Allah, the all Mighty, who gave me the strength and courage to carry-on throughout my studies, making it all possible. I hope that the finding of this research will be beneficial to others. I believe that future extension of the research will contribute significantly to the authority and relevant stakeholders.

“O mankind! Have fear of your Rabb, the One who created you from a single soul, from that soul He created its mate, and through them He spread countless men and women. Fear Allah, the One in whose name you demand your rights from one another and the ties of relationship; surely Allah is watching you very closely.” (An-Nisa’: 4)

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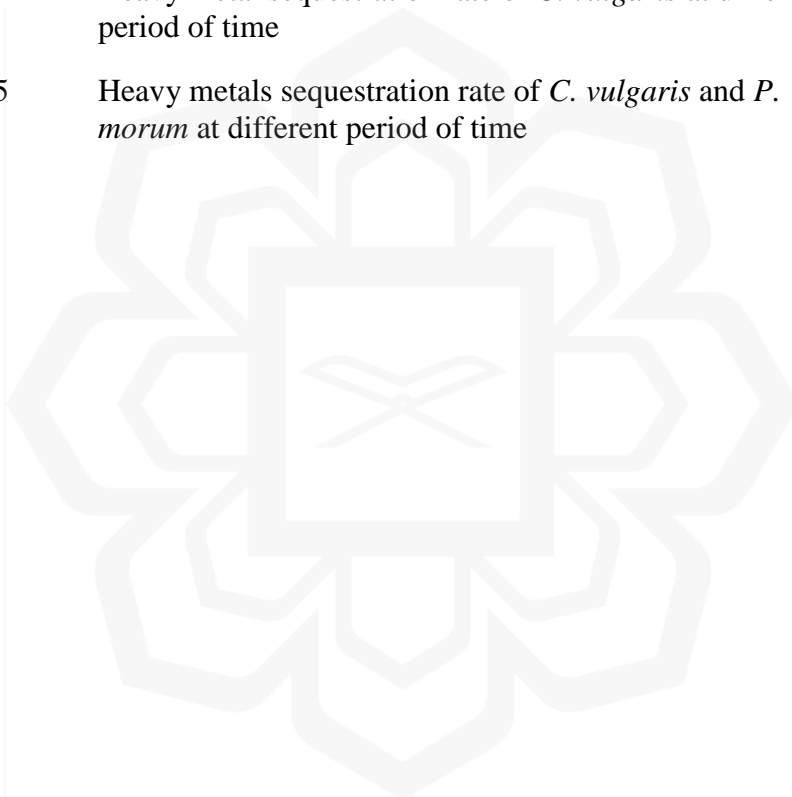
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LIST OF ABBREVIATIONS

| | | | |
|-------------------|---|--------------------------------|--|
| BCF | Bioconcentration factor | pH | Power of hydrogen |
| ml | Milliliter | HNO ₃ | Nitric acid |
| < | Less than | USEPA | United States Environmental Policy Agency |
| ICP- MS | Inductively coupled plasma mass spectrometry | HCL | Hydrochloric acid |
| > | More than | QC | Quality control |
| °C | Degree Celsius | Zn | Zinc |
| Kg | Kilogram | NAHRIM | National Institute of Hydraulic Research Malaysia |
| Al | Aluminum | DOE | Department of Environment |
| L | Liter | NWQS | National Water Quality Standard |
| ANOVA | Analysis of Variance | Co | Cobalt |
| A | | | |
| mg/kg | Milligram per kilogram | ppb | Part per billion |
| mg/L | Milligram per liter | mm | Millimeter |
| Mg | Magnesium | MS | Malaysia Standard |
| Cd | Cadmium | FAS | Ferrous ammonium sulphates |
| Mn | Manganese | g | Gram |
| Cr | Chromium | Ca | Calcium |
| Cu | Copper | K ₂ SO ₄ | Pottasium sulfate |
| DO | Dissolved oxygen | WQI | Water Quality Index |
| NH ₃ N | Ammoniacal Nitrogen | * | Highly significant |
| Ni | Nickel | MCL | Maximum contaminant level |
| Fe | Iron | KOC | Organic carbon partition coefficient |
| Pb | Lead | KOW | Octanol-water partition coefficient |

CHAPTER ONE

INTRODUCTION

1.1 RESEARCH BACKGROUND

With the advancements in urbanisation and industrialisation, the profusion of heavy metals in the environs has risen immensely in the last few decades, thus triggering substantial concerns across the globe (Ashraf et al., 2019). Heavy metals refer to a cluster of metallic chemical elements which possess comparatively greater atomic weights, densities, and atomic numbers. Mercury (Hg), arsenic (As), nickel (Ni), cadmium (Cd), lead (Pb), zinc (Zn), copper (Cu), and chromium (Cr) are some of the common heavy metalloids/metals. They come from anthropogenic or natural sources like generated water produced in the oil & gas sector (Pichtel, 2016), usage of phosphate fertilisers in farming (Hamzah et al., 2016; Rafique & Tariq, 2016), metal mining and smelting (Chen et al., 2016), sewage sludge (Farahat and Linderholm, 2015), pesticide use (Iqbal et al., 2016), electroplating, and burning of fossil fuels (Muradoglu et al., 2015). Malaysia has enforced the Environmental Quality Act 1974 and successfully decreased pollution to some extent, with regards to regulating the point and non-point sources. Nonetheless, there exist multiple challenges which have to be tackled for attaining an optimal quality of water. Considering the fact that Malaysia is vigorously growing and emerging as an industrial nation, several water bodies are fast turning into a trashcan for sewage disposal, chemicals, and contaminants. Around 60% of the 90 reservoirs and lakes shortlisted were eutrophic and mainly comprising phosphates and nitrates resulting from runoff fertilisers and usage of pesticides in farming (Wan Sulaiman, 2019). Such materials are said to be directed continually to the water bodies

and then diffused into organic and inorganic particles, pesticides, nutrients, and herbicides that directly impact the aquatic environment. Indeed, the water pollutants do not just damage the aquatic network but also impact the water safety for human usage (Wan Sulaiman, 2019). The aquatic environs are a sensitive portion of the planet and easily vulnerable to negative fluctuations in the environment. The environment can easily gather organic and inorganic contaminants (trace metal, heavy metal, etc.) from direct or indirect sources. These could be naturally appearing contaminants, like soil erosion and deposition, atmosphere, weathering of rock, floods, forest fires, and anthropogenic sources like agricultural, urban, and industrial releases. Because of increasing concerns regarding metal corruption and its likely accrual in the food chain, several research works on new taxons intend to ascertain their tolerance towards the toxic environs and their potential in phytostabilisation or phytoremediation (Abu Yazid, 2017). Until now, the usage of macrophytes for treating pollutants in water bodies has demonstrated favourable results, majorly led by the strategic usage of the natural and integral qualities of plants. Appropriate selection of plant species is vital for attaining an effective phytoremediation. As per Randrianarison & Ashraf (2017), one of the primary factors in the acceptance of phytoremediation is related to the measurement of its performance, eventual use of by-products, and its general economic feasibility.

Malaysia requires environmental technologies in several fields. Plant-based technology, called phytoremediation, is quite popular these days for environment cleaning. Several living beings can collect particular toxicants with concentrations quite higher compared to those in the environment, making them appropriate as phytoremediation agents (Yan et al., 2020). Different techniques were deployed for removing heavy metals from industrial discharges, such as membrane filtration, chemical precipitation, ion exchange, electro dialysis, reverse osmosis, solvent

extraction, oxidation, evaporation, and activated carbon adsorption. However, these approaches were futile and costly, particularly when the concentration of heavy metals was quite high (Vidu et al., 2020). Hence, phycoremediation is one of the likely technologies necessary for ensuring a better prospect for the water environment in the nation.

1.2 ISSUES

Environmental contamination related matters have become graver because of swift rise in populace, industrialisation, and urbanisation, and these are affecting the bionetwork services. Every year, huge volumes of wastes (liquid and solid) are produced globally. By 2050, the yearly generation of waste is projected to increase from 2.01 billion tonnes to 3.40 billion tonnes. Just a small part is recycled, and the remaining is abandoned or left unprocessed, which triggers a cascade of issues like water, land, air, thermal and visual contamination for humans and the environment (Koul & Taak, 2018; Koul et al., 2022; Renuka et al., 2014). Furthermore, there is a key concern about waste water treatment prior to its release into the environs in emerging nations. Hence, it is essential to determine ecologically viable, economical water treatment approaches which entail minimal infrastructure and involvements.

Water is a fundamental part of life. Water that comprises a considerable solvent power is continuously threatened and gets polluted easily. Contamination refers to any change that triggers unevenness in the natural quality of environs by means of chemical, physical, or biological practices. Such industrial contaminants vitiate the bionetwork, pollute the water bodies, harm the aquatic environment, and impair the soil fertility and subsystem. The effluent comprises different organic and inorganic materials in diverse concentrations that might impact the quality as well as nature of water. Only a minor

rate (0.01 %) of freshwater is available for human use, which contains 3% of the overall water on earth; the remaining is kept in ice caps, glaciers, and permafrost. Regrettably, this tiny volume of freshwater is under severe stress because of speedy rise in populace, urbanisation, and untenable utilisation of water in agricultural and industrial setups (Sayqal & Ahmed, 2021). Because of the anthropogenic activities, ecological contamination also rises and turns into a grave concern as they generate heavy metals in the environs and disrupt the biogeochemical cycles. Such metals are not biodegradable, i.e., they are not detached from water due to self-purification. After they are released into water bodies, they undergo adsorption on sediment particles, accrue in basins, venture into the food chain, and exhibit persistence in the bionetwork (Kapahi & Sachdeva, 2019). Fish and other tiny beings might take in the heavy metals and dissolved elements from adjacent food and water, which might then accumulate in different tissues in substantial amounts (Ali et al., 2019).

Manifold technologies have been introduced for remediating water polluted by heavy metals, like conventional membrane filtration, which is a typical substitute utilised for major water volume treatment by means of reverse osmosis, ion exchange, microfiltration, precipitation, or flocculation (Sayqal & Ahmed, 2021). Notably, these kinds of technologies could be vastly costly (Yule et al., 2015), particularly for emerging nations such as Malaysia, and frequently do not attain a sustainable outcome nor offer any aesthetic enhancements for the polluted sites (Kapahi & Sachdeva, 2019). Because of the high costs, several contaminated regions are left untouched, thereby decreasing water supply. In view of this, there is a pressing requirement for coming up with viable water treatment methods that can be accomplished by means of ground-breaking green technologies. Phycotechnology is presently an extensively deliberated option as an effectual and cost-effective technological solution for tracing ecological imbalance, and

immobilising, accruing, and degrading metal contaminants from polluted water. This environment friendly technology is not just good for the environment but also economical as it makes the most of the naturally arising relationships between microorganisms, plants, and the environment.

1.3 PROBLEM STATEMENT

The contamination of aquatic bionetworks in Malaysia has surfaced as a key ecological issue because of hasty urbanisation and industrialisation (Yule et al., 2015; Wan Sulaiman, 2019). Water contamination issues have become more and more evident and triggered grave biological and environmental concerns. Moreover, development and administration of water resources have been unsystematic and there is dearth of public consciousness on the necessity for ecological protection and an equivalent dearth of legal structures to endorse this protection. Consequently, grave conflicts have surfaced; industries are competing with farming for acquiring water, farming is competing with the environment, and upstream regions are competing for water with downstream regions (Ma et al., 2011; Abu Yazid, 2017).

There are three kinds of waste water treatment mechanisms utilised in treating discharges or contaminated water: chemical, physical, and biological. Based on the kind of contaminant, degree of contamination and the quantity to be treated, they might be utilised together or individually. The World Health Organization (WHO) has formulated the guiding principles for enhancing waste water management administrations and alleviating health concerns during all phases of treatment (Yan et al., 2020). Waste water treatment techniques are classified into primary, secondary, and tertiary. The primary method encompasses provisional storage of waste in a container, wherein heavy materials settle at the bottom whereas lighter ones (grease, oil, and solids) float on the

upper tier. Native, water-borne microorganisms frequently take on secondary treatment in a well-maintained setting, and it might be vital to isolate the microbes from the treated water prior to release (Rawat et al., 2011; Koul et al., 2022). Tertiary treatment is defined as something which is carried out along with primary and secondary treatments. As against biological treatment, physical and chemical treatments are costlier. Chemical processes that are part of the water treatment are organised to render certain variations by means of chemical reactions (Koul et al., 2022). Furthermore, the chemical technique increases the conductivity, pH of the treated water, and overall dissolved matter.

Thus, bio-treatment or biological treatment of waste water is termed as the most effective and viable process. Phycoremediation, or the usage of algae (macro or micro algae) to eliminate or bio transform contaminants such as nutrients, heavy metals, and other toxins (for e.g., xenobiotics) from contaminated water, seems to be a viable method (Koul and Taak, 2018; Upadhyay et al., 2018). Algae are an exceptional sink for carbon dioxide and can competently decrease the carbon footprint (atmospheric carbon dioxide resulting in global warming, pollution, and greenhouse effects) (Arbib et al., 2014; Ding et al., 2020). They are universal in nature and well-adjusted to an extensive range of habitats. They are generally categorised as microalgae, macroalgae, and marine algae, broadly called seaweeds. Microalgae are regarded to be rich in biodiversity, wherein there are almost 200,000–800,000 species globally with their outstanding adaptogenic ability enabling them to thrive in different environments, which make them optimum candidates to be employed for water treatment (Abinandan and Shanthakumar, 2015; Hussain et al., 2021; Poonia et al., 2022). Biological treatment not only helps to remove nutrients, toxins and heavy metals but also allows heterotrophic aerobic bacteria providing oxygen to mineralise the organic contaminants (Chaudhary et al., 2018; Jiang et al., 2011). Since the efficiency of phycoremediation is

regarded to be proportional to the growth of microalgae, factors that are regarded to facilitate the development of microalgae can also help increase algal biomass. Algal biomass offers benefits to human nutrition, biofuel production, aquaculture as well as pharmaceuticals. (Badwy et al., 2008; Chisti et al., 2008; Jais et al., 2017; Shields and Lupatsch, 2012; Sydney et al., 2011). Optimum N and P concentrations are needed for microalgae in order to synthesise nucleic acids, phospholipids and proteins (45–60 percent of microalgae dry weight) (Apandi et al., 2017). In addition, eliminating nutrients by employing microalgae can be regarded as a suitable strategy for the treatment of tertiary wastewater in order to remove PO_4^{3-} , NO_3^- as well as ammonium (Rawat et al., 2011). They also help eliminate hydrocarbons, HMs and pesticides from wastewater by making use of different mechanisms, including bioaccumulation, biosorption, biotransformation, assimilation and decay (Chekroun and Baghour, 2013; Rath, 2012; Leong and Chang, 2020).

By employing molecular and functional genomic methods, researchers have synthesised algal strains for performing bioremediation of water treatment, by enhancing their flexibility (resistance to severe climate), photosynthetic effectiveness as well as capacity to detoxify pollutants (Lutzu et al., 2020; Zeng et al., 2011). Phycoremediation provides several benefits when compared with other standard physiochemical methods because of its exceptional ability to remove nutrients (reverse osmosis, dialysis, membrane-mediated separation, ion exchange and electro-dialysis, chemical oxidation or reduction as well as activated carbon-mediated adsorption) (Abdel-Shafy & El Saharty, 2015; Hussain et al., 2021). These benefits also include acclimatisation of P and N into algal biomass, low operational expenses, oxygenation of effluent prior to its discharge into the water body as well as removing the need for sludge handling. Moreover, this process is regarded to be environment-friendly since

after the removal of nutrients, the algal biomass can be re-employed as fertiliser without producing any secondary contaminant (Liu et al., 2016). Microalgae have been found to efficiently extract both inorganic and organic contaminants even for agro-industrial wastewater containing high nutrient content (Das et al., 2019; López-Serrano et al., 2020). Different microalgae such as *Botryococcus* sp., *Phormidium* sp., *Scenedesmus* sp., *Chlorella* sp., *Chlamydomonas* sp., *Oscillatoria* sp., and *Botryococcus* sp. are utilised for treating diverse waste waters.

In Malaysia, there is a continuous rise in the number of contaminated urban water bodies that are left untreated because of high cost associated with the implementation of conventional water treatment as well as limited funding pertaining to water management (Abu Yazid, 2017). To make things worse, because of lack of proper sludge treatment facilities, untreated sludge gets directly discharged into the environment, which is detrimental to the quality of our water bodies. Consequently, contaminated urban water bodies tend to become unusable as well as less accessible because of health hazard risks towards humans and other living organisms.

Moreover, there are limited and selected plant species that have displayed potential to remediate unhealthy aquatic environments that have been contaminated with heavy metals. For instance, in Malaysia, implementing man-made wetland is generally limited to the usage of mangrove plant species, which does not seem to be practical with regards to water bodies in urban areas. Unlike more advanced and developed countries, in Malaysia, green technologies like phycotechnology have not been well exposed for the public (Abu Yazid, 2017). Thus, public understanding regarding the potential of green technology is lacking, which has led to limited number of sustainable greener alternatives being introduced to substitute the high-cost pertaining to conventional methods. This has been seen to discourage private and public