Comparative Evaluation of Phytoremediation Efficiency of *Azolla pinnata* and *Pistia stratiotes* in Methylene Blue Contaminated Water

Ernie binti Zulkifli[1,2*], Ain Nurnajwa binti Anis[2], Ayman Zahran bin Mohd Amran[2], Ku Najwa binti Ku Azman[2]

1 Centre of Technology , Politeknik Sultan Idris, Sungai Lang 45100, Sungai Air Tawar, Selangor, Malaysia
2 Department of Civil Engineering , Politeknik Sultan Idris, Sungai Lang, 45100, Sungai Air Tawar, Selangor, Malaysia

* E-mail: erniezulkifli@psis.edu.my

ABSTRACT

Dye pollution occurs when synthetic dyes from industrial are released into waterways, often leading to environmental harm due to their persistence and potential toxicity and become significant environmental concern. The dye wastewater need to be treat before discharge to water body. However the current methods are costly and some of the treament can treat the various types of organic and inorganic compund in dye. Phytoremediation presents a promising eco-friendly approach for treating textile dye contaminants. This study evaluated the comparative performance and efficiency of Azolla Pinnata and Pistia Stratiotes in removing MB from contaminated water. Laboratory experiments were conducted to assess the uptake and absorption capabilities of both aquatic plants over a 12-day period. The reduction of the MB before and after phytoremediation process were analyze by using spectrophotometer. Results indicated that both Azolla pinnata and Pistia stratiotes effectively reduced MB concentrations, with achieved a removal efficiency of approximately 97.8 % for Pistia Stratiotes, 93.9% for Azolla pinnata and 95.1 % (Azolla pinnata and Pistia stratiotes) . The high removal of through this aquatic plant were due to the capability of the plant to absorb MB especially through the roots that rich with microbial activity. Overall, this comparative study highlights the potential of Azolla pinnata and Pistia stratiotes as efficient phytoremediators for treating Methylene Blue contamination in aquatic ecosystems, underscoring their application fullfill Sustainable Development Goal 6 (Clean Water and Sanitation).

KEYWORDS

azzola pinnata; methelyne blue; phytoremediation; pistia stratiotes; synthetic dye

1. INTRODUCTION

In Malaysia, the textile and batik industries play a vital role in the economy, particularly through Small and Medium-sized Enterprises (SMEs). This indusrty industry consumes a large volume of water and produces a large amount of wastewater during the boiling process and dyeing process. The release of colored effluents that contain a large number of dyes and chemicals can harm the environment and human health (Ramakreshnan, L. et.al, 2020). The discharge of synthetic dyes from textile and batik industries poses a significant environmental challenge due to their persistence and potential toxicity. Methylene Blue (MB), a commonly used dye in these industries, is known for its vibrant color and stability, making it difficult to degrade through conventional wastewater treatment processes . It is soluble in various solvents including methanol, 2-propanol, air, ethanol, acetone, and ethyl acetate (Idress Khan et al., 2023) that toxic that can give ecological implications because it affects the aquatic food web and ecosystem health (Li Si., et.al, 2023).

In order to comply with the textile industry wastewater discharge standard and legislation in Malaysia , these wastewater need to treat before discharge. The discharge of synthetic dyes, particularly MB, from the textile and batik industries poses significant environmental challenges globally, including in Malaysia. These industries, primarily comprised of small-medium enterprises (SMEs), often lack access to advanced wastewater treatment technologies capable of effectively mitigating dye pollution. Current process to treat this types of wastewater were using biological, physical and chemical treatment methods, for example precipitation, electroplating, chemical coagulation, ion-exchange, membrane separation, and electrokinetics methods. However, the processes involved are highly complex and often incur high costs. Some of these treatments may not achieve standard compliance due to the stable nature of the contaminants.

Phytoremediation, the use of plants to remove, degrade, or immobilize environmental contaminants, is a highly promising treatment for addressing dye wastewater, particularly Methylene Blue. The plant used in phytoremediation have high capability to absorb ionic compound through their roots systems. The use of phytoremediation technology can also provide good advantages because it was environment and eco-friendly that can reduce exposure of the pollutants to the environment and ecosystem and also economically feasible, therefore, simple to manage, also the cost of installation and maintenance is low (Jacob, et. al, 2018). Over the year, aquatic plant seem to be the best option for phytoremediation. Aquatic plants naturally develop an extensive root system, which enhances their capability and makes them optimal for accumulating contaminants in both their roots and shoots (Gorito, et.al, 2017).

Azolla pinnata (AP) and Pistia stratiotes (PS) have shown promises solution due to their ability to uptake and accumulate pollutants from water bodies. Both species are known for their rapid growth rates and efficient nutrient uptake, making them ideal candidates for remediation applications. Despite their potential, comparative studies evaluating the phytoremediation efficiency of AP and PS against MB contamination remain scarce in the literature. Understanding the comparative performance of these plants in removing MB can provide valuable insights into their applicability as eco-friendly solutions in textile and batik wastewater treatment strategies. Previous study from Gyawali et.al, 2023 by using AP as absorbent powder confirm that MB can be removed up to 90% while the study from Al-Baldawi et.al, 2018 that used Ap as biomass media, 25 mg/l were removed by AP up to 85%. A little study from previous research study the ability of PS to treat MB. Study from M.Fauzul Imran et.al, 2023 in capability of PS to remove hemical oxygen demand (COD), biological oxygen demand (BOD), ammonia, nitrate, nitrite, and phosphate (TP) showed good absorption of PS and reduce up to 99.8 %, 97.2 %, 46.4 %, 100 %, 100 %, and 80.4 %, respectively. The efficiency for reducing COD was 81.73%, and the efficiency for surfactant was 99.42% in N. Hendrasari and C.Redina, 2023 showed the capability of PS to tolarate with pollutant. Hence, to fulfill these research gap, this study can become futher reference to other researcher for finding ability of AP and Ps to treat MB in dye wastewater.

Addressing these gaps is crucial for developing tailored, sustainable wastewater management strategies were needed in this textiles and batik industry. By evaluating and comparing the phytoremediation efficiency of AP and PS, this study aims to provide empirical insights that can inform the adoption of environmentally-friendly practices within this vital sector of the Malaysian economy. The findings from this study on AP and PS phytoremediation efficiency in MB contaminated water have the potential to transform wastewater management practices for Malaysia's textile and batik SME sector. By addressing environmental challenges, promoting sustainable practices, and contributing to achieving sustainable development goals, this research provides a pathway towards a more sustainable and environmentally conscious industrial sector through a novelty phytoremediation by using both AP and PS to treat MB in dye wastewater.

2. METHODOLOGY

An experiment of phytoremediation were conducted for 12 day. The synthetic MB solution is prepared to a specific concentration in a 6 liter container. 120 mg MB powder were dilute with 6 liter of water. Each container is then populated with 20 g of three types of aquatic plants for testing: AP, PS, and a combination of both. These plants are left to interact with the MB-contaminated water for a period of 12 days in a well-lit environment suitable for their growth. At the end of the 12-day period, AP, PS, and combined AP-PS plants are removed from the containers. The Figure 1 and Figure 2 shows the design of this pythoremediation process:

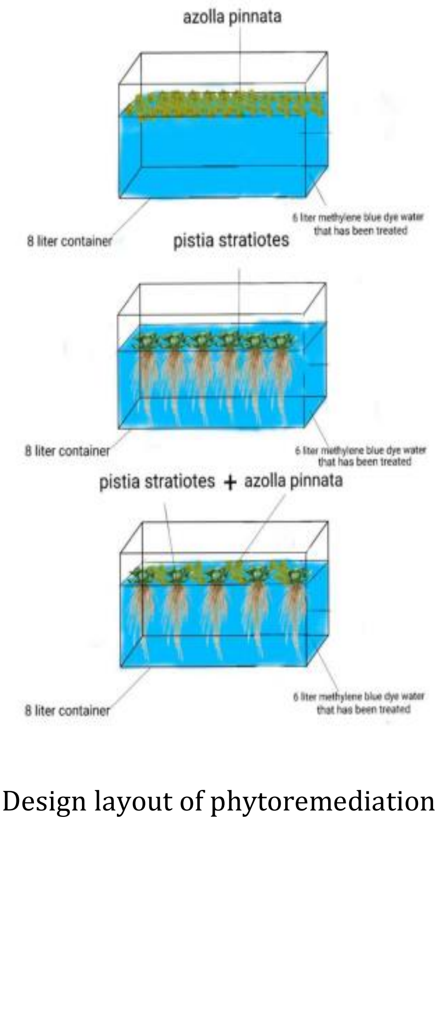

Figure 1. Design layout of phytoremediation process

Figure 2. The process of interaction of AP, PS, AP-PS for 12 days

The color measurement is conducted using the Platinum Cobalt standard method with the DR3900 spectrophotometer. Initially, a sample cell containing 6 ml of deionized water serves as the blank for calibration. After zeroing with the blank sample, the wastewater sample is then placed into the cell holder. The initial concentration of each dye in the wastewater is determined, and color readings after phytoremediation process are recorded every 3 days. The data were organized into a table, and the percentage of dye removal was meticulously recorded.

3. RESULTS AND DISCUSSION

3.1. Azolla Pinnata (AP)

Pyhtoremediataion process were conducted to evaluate the percentage of MB. Figure 3 shows the graph of MB percentage removal for day 3, 6,9 and 12. From the result, the AP, PS, AP-PS shows significantly increment of removing MB color. AP can remove MB color up to 94% after 12 day, PS 98% and AP-PS 95%.

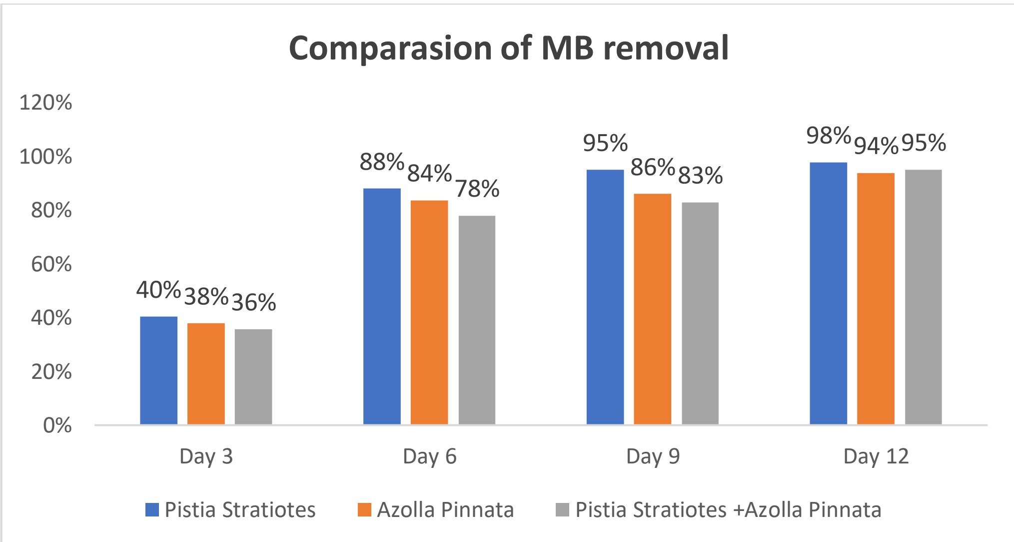

Figure 3. Comparison of MB removal from day 3 to day 12

Over the 12 days, Methylene Blue (MB) can be reduced by up to 94% (Figure 4), and Azolla Pinnata (AP) remains in good condition without showing signs of plant death. It's shows that This indicates that during the study, AP received adequate light exposure, maintained in optimum pH of 7, and had sufficient water depth of 20 cm (N. Adzman et.al, 2022) . The observation of pH shows the acidic water of MB pH 4.8 were increase to natural condition 7.5 from day 1 to day 12 (Table 1). This result shows neutralization of the water happen due to the absorption of MB to plant roots.

Figure 4. Decoloration of MB by using AP after 12 day of experiment

The result obtained shows that the capability of the aquatic plant were based on their physical characteristic. AP has long and dense roots. The increment of root length of AP were tabulate in Table 1. The observation of AP growth rate in 12 days (Figure 4) shows that the root of AP become longer thus provide larger surface for MB to adsorb. The length and branching nature of the roots increase the surface area available for absorption (H.W Tan, et.al, 2023). The roots of AP also contain various functional groups such as carboxyl, hydroxyl and amino groups that interact with MB molecule through electrostatic attraction mechanism. The hydroxyl group could interact with MB molecules by hydrogen bonding (Z. Liu, 2022). Beside, the microbial activity through AP roots helps transformation of MB into less toxic and non- toxic compound. at rhizosphere can synergistically. Therefore, plant-microbe interaction happen in plant root surface metabolize and degrade the organic contaminant (Niraj R.Rane et.al, 2022).

Table 1. Roots length grow from say to day 12 for AP

Plant/Roots length	Day 1	Day 3	Day 6	Day 9	Day 12
Azolla Pinnata (AP)	0.18 cm	0.2 cm	0.35 cm	0.6 cm	1.5 cm

Source: personal document

Figure 5. The picture of AP after 12 day of observation

Other than roots, AP is aquatic fern that easy to growth and highly productive (N. Adzman et.al, 2022). The observation shows, the MB adsorption does not affect the color of chlorophyl in the AP surface due to there is protective layer on the upper part of the body (Godwa B. and Lingaraju H.G., 2023), thus increase AP lifespan. This larger surface area and fast growing plants allows more interactions between the dye molecules in the water with the plant roots. The adsoption mechanism happen due to the pore size of AP was bigger than MB (Escoto et.al, 2019). As a result, the plant can effectively uptake and accumulate higher concentrations of the dye.

3.2. Pistia Stratotes (PS)

In term of capabilities of PS, PS shows the best removal result in this experiment (98%) and the MB water turn into clear water (Figure 6) . Its clearly notice that the size of PS and the root of PS are bigger and longer compare to AP (Table 2) (Figure 7) , thus enhancing the plant's ability to uptake MB with mechanism of uptakes by rhizofiltration process where the MB accumulate at the roots tissue (Z.Zaida et.al,2021) . PS utilizes a unique hanging root-biofilm network to create a biologically active surface area that supports various biochemical and physical processes. This network plays a crucial role in water purification by facilitating filtering and entrapment mechanisms. The submerged, elongated roots specifically exploit their interaction with contaminated water, enhancing their effectiveness in pollutant removal (Ali M. et.al, 2024).

By reducing water velocity, these roots promote increased microbial activity, sedimentation, and biological assimilation rates (Sharma et.al, 2023). The existence of microorganisms that are usually in plant roots such as Genus of Pseudomonas play importance role to degrade pollutant (N. Hendrasarie and C. Redina, 2023). This intricate process ultimately improves overall water quality through enhanced pollutant removal efficiencies.

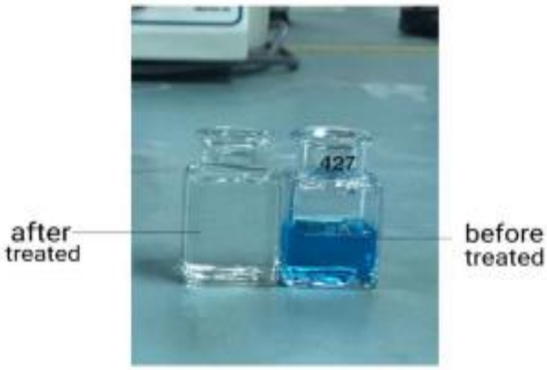

Figure 6. Decoloration of MB by using PS after 12 day of experiment

Table 2. Roots length grow from say to day 12 for AP

Plant/Roots length	Day 1	Day 3	Day 6	Day 9	Day 12
Pistia Stratiotes (PS)	10.3 cm	11 cm	13.5 cm	19 cm	25 cm

Source: personal document

Figure 7. The picture of PS after 12 day of observation

The physical examination of Pistia stratiotes after exposure to methylene blue for 12 days reveals a consistent and positive development throughout the observation period. Both root growth and leaf formation exhibited steady progress each day. This observation supports findings by Ali M. et al. (2024), suggesting that PS demonstrates rapid growth, potentially doubling in size in less than a months. By its larger root structure and more numerous root hairs, larger surface for MB adsorption were provide. As the surface area of PS increases, its capacity to adsorb molecules (MB) also increases.

3.3. Azolla Pinnata and Pistia Stratiotes

The good ability of both plant to absorb MB shown in their removal percentage up to 95% (Figure 6).

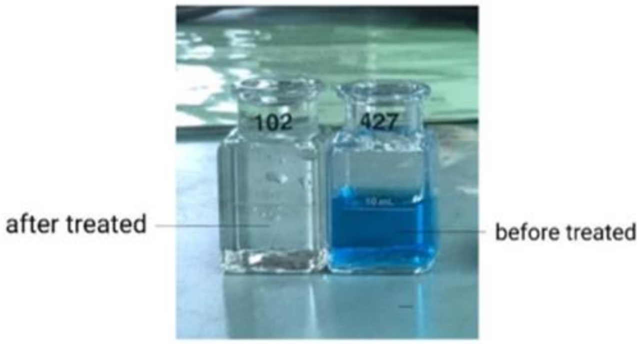

Figure 8. Decoloration of MB by using AP-PS after 12 day of experiment
(Source: personal document)

The combination of AP and PS showed, both plant species exhibited a 100% survival rate even though the size of the plants were different (Table 3). This result showed there is no competition for growth between the two plants. In this combination, AP with dense root systems concentrate the maximum concentration of the contaminants with the larger root adsorption area (Sharma et.al, 2018) while the PS that has long roots that can penetrate deep into the water for an efficient adsorption (V. Kumar et.al, 2019). Through the processes of rhizofiltration and rhizodegradation that occur, along with the respective advantages of each plant, a greater amount of MB can be absorbed. The combination of these two plants demonstrates a great ability and have large potential to be commercial to remediate MB.

Table 3. Roots length grow from day 1 to day 12 for PS

Plant/Roots length	Day 1	Day 3	Day 6	Day 9	Day 12
Azolla pinnata	0.1 cm	0.15 cm	0.2 cm	0.4 cm	0.7 cm
Pistia stratiotes (PS)	8.9 cm	10 cm	13 cm	17 cm	21 cm

Source: personal document

4. CONCLUSION

In conclusion, the AP and PS plants can be identified as hyperaccumulator species, demonstrated good efficacy in removing MB from wastewater, achieving removal rates of 95%, 94%, and 98% within just 12 days. Their rapid remediation capability were good compare to others phytoremediation plants, the specific morphology of the plant's roots, characterized by their length and branching, enhances its capacity to absorb and remove colorants like Methylene Blue from water, making it a suitable candidate for phytoremediation applications. These roots enable efficient MB accumulation through mechanisms such as rhizofiltration and rhizodegradation. Moreover, favorable environmental conditions including ample sunlight and optimal pH levels supported robust plant growth despite the high MB uptake. These findings highlight the considerable potential of AP and PS plants in effectively treating dye wastewater containing MB. This resilience underscores their potential as reliable candidates for treating dye wastewater contaminated with MB, showcasing their capacity to both tolerate and effectively remediate environmental pollutants.

REFERENCES

Al-Baldawi, Israa & Abdullah, Siti & Anuar, Nurina & Abu Hasan, Hassimi. (2018). Phytotransformation of methylene blue from water using aquatic plant (Azolla pinnata). Environmental Technology & Innovation. 11. 10.1016

Ali, M., Aslam, A., Qadeer, A. et al. (2024). Domestic wastewater treatment by Pistia stratiotes in constructed wetland. Sci Rep 14, 7553.

Bharat Godwa and Lingaraju H.G. (2023). In situ Phytoremediation of azo dyes (methylene blue) by the plant Azolla Pinnata,J. Mater. Environ. Sci., 2023, Volume 14, Issue 11, Page 1456-1467

Escoto, Dandara Gayer, Mateus Bianchini, Matheus Pereira, Geovana Roehrs, Rafael Denardin Elton. (2019). Use of Pistia Stratiotes for phytoremediation of water resources contaminated by Clomazone, Chemosphere.

Gyawali, Manoj Pant, Salina Kumari, Anshu Bhattarai, Deval Pant, Hem. (2023). Adsorptive Removal of Methylene Blue Dye Using Low-Cost Adsorbent Prepared From Azolla Pinnata, Proceedings of 12th IOE Graduate Conference.

Gorito, A.M.; Ribeiro, A.R.; Almeida, C.M.R.; Silva, A.M. (2017). A review on the application of constructed wetlands for the removal of priority substances and contaminants of emerging concern listed in recently launched EU legislation. Environ. Pollut. 2017, 227, 428–443.

Hui Wun Tan, Yean Ling Pang, Steven Lim, Woon Chan Chong. (2023). A state-of-the-art of phytoremediation approach for sustainable management of heavy metals recovery,Environmental Technology & Innovation,Volume 30, 103043.

Ideres Khan, Saeed K., Zekker I., Zhang B., Hendi A.H., Ahmad A., Ahmad S., Zada N., Ahmad H., Shah L.A. (2022). Review on Methylene Blue: Its Properties, Uses, Toxicity and Photodegradation. Water. 2022; 14(2):242

Jacob M., Karthik C., Saratale R. G., Kumar S. S., Prabakar D., Kadirvelu K., et al. (2018). Biological approaches to tackle heavy metal pollution: a survey of literature. J. Environ. Manage. 217 56–70. 10.1016

Li, S., Cui, Y., Wen, M., & Ji, G. (2023). Toxic Effects of Methylene Blue on the Growth, Reproduction and Physiology of Daphnia magna. Toxics, 11(7), 594

Muhammad Fauzul Imron, Abiyyu Armijn Firman Firdaus, Zhiella Octabriani Flowerainsyah, Dzikro Rosyidah, Nurina Fitriani, Setyo Budi Kurniawan, Siti Rozaimah Sheikh Abdullah, Hassimi Abu Hasan, Yudha Gusti Wibowo. (2023). Phytotechnology for domestic wastewater treatment: Performance of Pistia stratiotes in eradicating pollutants and future prospects,Journal of Water Process Engineering,Volume 51,103429

Niraj R. Rane, Savita Tapase, Aakansha Kanojia, Anuprita Watharkar, El-Sayed Salama, Min Jang, Krishna Kumar Yadav, Mohammed A. Amin, Marina M.S. Cabral-Pinto, Jyoti P. Jadhav, Byong-Hun Jeon. (2022). Molecular insights into plant–microbe interactions for sustainable remediation of contaminated environment, Bioresource Technology,Volume 344, Part B,2022,126246.

Nur Zaida Zahari, Ng Sean Fong, Fera Nony Cleophas, Sahibin Abd Rahim. (2021). The Potential of Pistia stratiotes in the Phytoremediation of Selected Heavy Metals from Simulated Wastewater, International Journal of Technology 12(3) 613-624

N. Adzman, S.J. Goh, A Johari, M.N. H. Zainal Alam and M . J. Kamaruddin. (2022). Preliminary study on Azolla cultivation and characterization for sustainable biomass source, Journal of Physics: Conference Series, Volume 2259, 9th Conference on Emerging Energy & Process Technology 2021 (CONCEPT 2021)

N. Hendrasarie and C. Redina (2023), Ability of Water Lettuce (Pistia stratiotes) And Water Hyacinth (Eichhornia crassipes) To Remove Methylene Blue Anionic Surfactant (MBAS) From Detergent Wastewater, Nature Environment and Pollution Technology Journal, Volume 22 (4), 1961-1970.

Ramakreshnan, L.; Rajandra, A.; Aghamohammadi, N.; Fong, C.S.; Nalatambi, S. A. (2020). Preliminary Insight into the Environmental Awareness of Community in the Vicinity of Batik Manufacturing Units in Kelantan, Malaysia. GeoJournal , 85, 1745–1753.

Sharma, S.; Tiwari, S.; Hasan, A.; Saxena, V.; Pandey, L.M. (2018). Recent advances in conventional and contemporary methods for remediation of heavy metal-contaminated soils, Biotech , 8, 216.

Sharma, R., Vymazal, J. & Malaviya, P. (2021). Application of floating treatment wetlands for stormwater runoff: A critical review of the recent developments with emphasis on heavy metals and nutrient removal , Sci. Total Environ. 777, 146044.

V. Kumar, J. Singh, A. Saini, P. Kumar. (2019). Phytoremediation of copper, iron and mercury from aqueous solution by water lettuce (Pistia stratiotes L.), Environmental Sustainability 2, 1–11,

Zhongchuang Liu, Md. Azharul Islam, Jiale Huang. (2022). Study of the adsorption of methylene blue by phytoremediation-plant biomass carbon, Journal of Molecular Liquids, Volume 366, 120273.