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In situ Phytoremediation of azo dyes (methylene blue) by the plant *Azolla Pinnata*

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Abstract: Increased global industrial revolution has become a reason for them to get polluted. One of the major industrial sectors that are textile industry is one of the major industries that is responsible for water pollution. The textile effluents mainly consist of azo dyes which are used as colorants. Evan a small amount of azo dye present in wastewater gives colour to water bodies which may affect the physiochemical characters of water such as transparency, pH and Oxygen levels that may lead to the death of sensitive species. The dyes have the adverse health effects on human health also. Some dyes are carcinogenic also and also cause other serious health effects. Several water treatment methods are available in order to treat the azo dyes present in wastewater which includes biological, chemical and physical processes. Phytoremediation is a biological process that uses plants to treat the contaminated water and soil. It is a well liked treatment method because of its affordability, ethnic appeal, long term applicability and direct application to the contaminated site In this study we study the Phytoremediation potential of the plant *Azolla pinnata* which is a hyper accumulative plant. It is known for its high growth rate and nutritive value which can either be used as cattle feed. In this experiment we study the effects of dye concentration on rate of reaction and effects of methylene blue on plant growth rate and plant pigments.

1. Introduction

As a result of the global industrial revolution, more effluents comprising many harmful compounds were released into the surface water. The textile industry uses a lot of water and dyes, making it one of the most water-intensive industrial sectors (Uddin *et al.*, 2023; Medjahed *et al.*, 2013). Despite the fact that water usage varies greatly depending on the fabric and manufacturing process, an average textile mill is expected to use 200 L/kg fabric per day (Chandrakant *et al.*, 2016). Worldwide, more than 100,000 commercial dyes totalling 7 108-1 109 kg/year have been documented. In 1856, William Henry Perkin identified the first synthetic dye as an organic aniline dye and gave it the name Mauveine. The substrates are dyed to give them a permanent hue that will not fade in the presence of water, light, oxidising chemicals, sweat, or microbial attack (Khan *et al.*, 2022). More than 4 to 12% of Azo dyes are washed out during dying process in textile manufacturing. On estimation 2,80,000 tonnes of azo dyes are discharged into water sources annually (Chengji *et al.*, 2021). Furthermore, the textile sector is thought to be the source of 54% of dye wastewater (Samsami et al. (2002).

Methylene Blue (MB), which is used for cotton and silk painting, is one of the high-consumption substances in the dye industry (Zahra *et al.* (2013). The chemical structure of MB is shown in Figure 1. Due to both its advantages and disadvantages, MB is one of the dyes that has been the most extensively researched. It has numerous and extensive uses as a colouring and staining agent in the pharmaceutical and textile industries, respectively (Sharifi *et al.* (2018). Due to its widespread usage in the textile industry for colouring leather, cotton, printing, and tanning (Gupta. (2009), methylene blue (MB) is a basic aromatic heterocyclic cationic dye that is frequently found in dye-based industrial effluent. Humans who consume MB dye may have dizziness, shortness of breath, nausea, increased blood pressure, a range of allergic reactions, and cancer (Alshekhil *et al.*, 2020). Its detrimental effects on the environment qualify it as a hazardous pollutant (Karaghool, 2020; Aaddouz *et al.*, 2023). The biological, chemical, textile, and medical sectors all employ MB, a heterocyclic aromatic (Contreras. 2019). When eaten by humans, MB dye causes major side effects as headache, nausea, and elevated blood pressure (Muhammad *et al.* (2019).

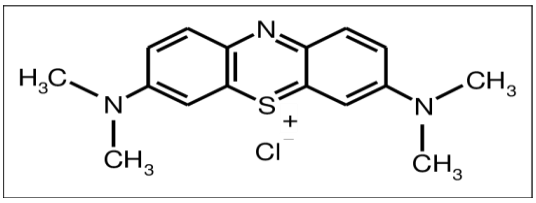


Figure 1: Chemical structure of methylene blue (Karen et al. (2016)

Due to its high molar absorption coefficient (8.4104 molcm^{1} at 664 nm), it has a negative impact on the photosynthetic activity of aquatic life, decreases oxygen solubility, diminishes sunlight transmittance and aesthetics of the biological community. The Figure 2 explains Toxic effects of MB on humans and other animals **(**Sharifi *et al.*, 2018**).**

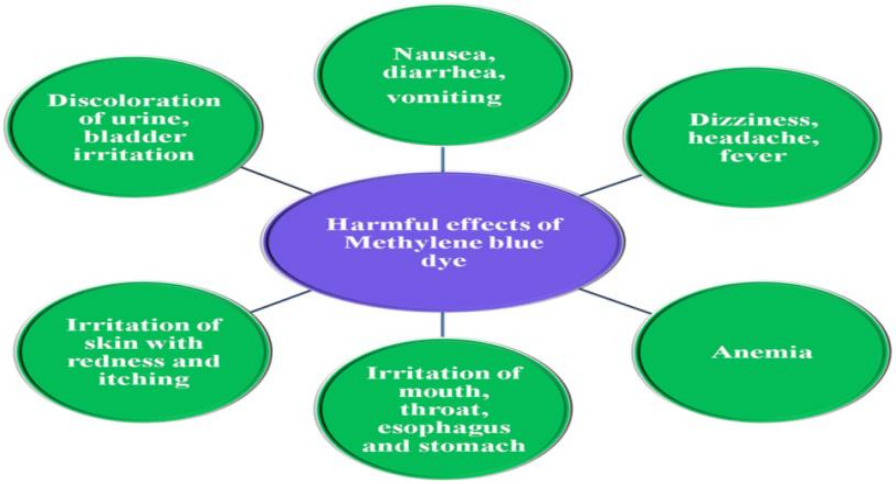


Figure 2: Toxic effects of MB on humans and other animals

A biological technique for cleaning up the environment by employing plants is called Phytoremediation. It is well-liked because it offers many benefits, including affordability, aesthetic appeal, long-term applicability, and direct application at polluted sites where other treatment options are prohibitively expensive. Additionally, plants provide protection against wind and water erosion, halting the spread of contaminants. Its autotrophic system, which has a big biomass and requires minimal fertiliser input, makes it simpler to handle. Grasses and other rapidly developing plants with deep, fibrous roots are excellent for Phytoremediation (Cluis. (2004).

The plant *Azolla pinnata* has a high potential for accumulation and functions well as a Phytoremediation agent (Sapna and Lingaraju, 2022). Nodes on the ventral surfaces of the rhizomes are the origin of numerous unbranched, adventitious roots that dangle into the water. In shallow water, the roots may touch the soil and draw nutrients from it. They also absorb minerals straight from the water (Kollah *et al.*, 2016). It is an aquatic fern with bearing roots that hang in the water and a small, branching, floating stem. Each leaf is placed in an alternating pattern and is made up of a thin, floating, colorless ventral lobe that is slightly bigger than the thick aerial dorsal lobe that contains green chlorophyll. Numerous bioactive substances, including vitamins, minerals, beta-carotene, essential amino acids, saponin, and flavonoids, are present in *A. Pinnata* (Ahmed *et al.*, 2020).

The aim of this study is to investigate Azolla pinnata's potential for azo dye extraction from wastewater. This study investigates the relationship between the rate of plant growth and the dye removal efficiency at different dye concentrations. In this study it is also examined how different plant masses affected dye removal rates as well as how contact area affected dye removal rates. This research also looks to understand how the build-up of dye within the structure of the plant influences the plant's chlorophyll level.

Objectives of the study:

- To study the Phytoremediation potential of *Azolla pinnata* in removal of an azo dye Methylene blue under different
    - To study and compare growth rate of *Azolla pinnata* in different dye concentrations
    - To study the effect of dye concentration on chlorophyll content of *Azolla pinnata* under different dye concentrations.
    - To study the effect of varying plant weight on rate of reaction.

2. Methodology

2.1. Stock Solution preparation:

Stock solution of methylene blue is prepared by serial dilution method. At first 1 g of methylene blue powder is mixed with 1l double distilled water. The above solution is used as stock solution. From that 5 ml of stock solution is diluted in 2l water to prepare 2.5mg/l solution. In the same way 10 ml, 20 ml and 30 ml stock solution is taken in order to prepare 5mg/L, 10Mg/l and 15 mg/l methylene blue solution.

2.2. Phytoremediation experiment

Parameters like Concentration of dye and plant dosage was altered during the study. The duration of experiment is about 6 days while absorbance was checked at the interval of 2 days.

A plastic container holding a 2-liter dye solution was used for the phytoremediation experiment. We chose four buckets with dye concentrations of 5 mg/l, 10 mg/l, 15 mg/l, and 20 mg/l. Water was used to wash the azolla plants, and then the extra water was drained away before 10 grammes of the plant were added to the container as shown in the Figure 3. According to Wagner's findings, all of the dye solutions included the vital mineral nutrients that have been shown to support optimal Azolla development, including 1 mM KNO_3, 2 mM $CaCl_2$, 1.3 mM K_3PO_4, 3.3 mM Mg $(NO_3)_2$, 0.038 M Zn $(NO_3)_2$, 5.4 M $FeCl_3$, 0.004 M $(NH_4)_6$ Mo_7O_{24}, and 0.016 M $Cu(NO_3)_2$). To reduce mistakes, the experiment was run three times, or triplets.

Same experimental setup was used to determine the effect of weight difference of azolla plant. Four buckets containing already weighed plant biomass. 5g, 10g, 15g and 20g of fresh azolla were used with constant dye concentration of 10 mg/L. the change in concentration was analyzed after 6 days. The initial and final concentrations of methylene blue are determined by spectrophotometer by checking absorbance at 663nm.

a b c d

Figure 3. The experimental setup a) 2.5mg/L, b) 5mg/L, c) 10mg/L, d) 15mg/L

2.3. Determination of removal efficiency

The removal efficiency is given by the formula

$$Removal\ efficiency\ \% = \frac{A_0 - A}{A_0} \times 100$$

A_0 = Initial Dye concentration
A = Final dye concentration

2.4. Growth rate estimation

Before the Phytoremediation experiment, the plants which were taken from pond were washed with water and the excess water was removed with the help of blotting paper. The growth of azolla pinnata was observed throughout the days of experiment. The plants were harvested after phytoremidiation experiment, and then died with blotting paper. The final plant mass was taken. Plant growth rate is given by the formula

$$Plant\ Growth\ rate = \frac{\ln(Wf) - \ln(Wi)}{Time}$$

Wf: Final plant weight
Wi: Initial plant weight

2.5. Determination of plant pigments

The plant photosynthetic pigments namely: chlorophyll a (Chl_a) and chlorophyll b (Chl_b) were determined spectrometrically using spectrophotometer at wavelength ranging from 350 nm to 750 nm. The AP was ground with a mortar and pestle and the pigment was extracted using 10 mL of 80% acetone in a dark area. All extractions were analyzed within 30 min and the estimation of plant pigments were based on the Arnon's method. Arnon's expressions of chlorophyll estimation are as follows:

Chl_a: [(12.7 X A_{663}) – (2.6 X A_{645})] X/m
Chl_b: [(22.9 X A_{645}) – (4.68 X A_{645})] X/m`

where **V (mL)**: volume of 80% acetone, **m (mg):** mass of plant and **A_{663}** and **A_{645}** represent the absorbance at wavelength 663 nm and 645 nm, respectively.

3. Results and Discussion

3.1 Effect of dye concentration of Decolourization rate

Compared to control average 80% of the methylene blue was removed from the water. The concentration of dye is inversely proportional to the rate of absorption because of chemical stress that is produced by the methylene blue concentration. Also, dead plants were observed at 15mg/L dye concentration. Since it is an acidic dye, the increased acidity causes death of plants.

Figure 4a. Observation at day 0 (initial) at different dye concentrations

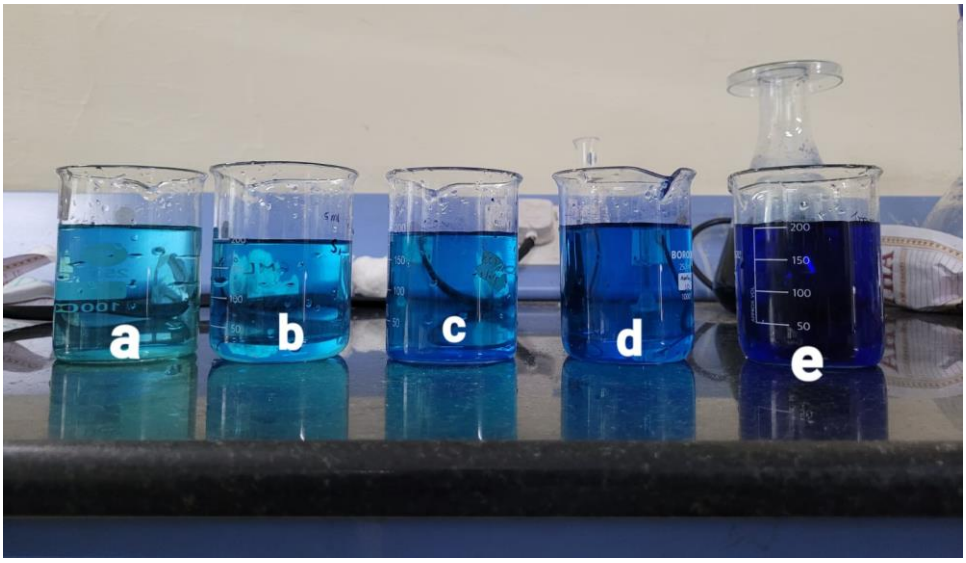


Figure 4b. Observation at day 6 (Final) at different dye concentrations
a) 2.5 mg/L, b) 5 mg/L c) 10 mg/L, d) 15 mg/L e) Control

Over the period of 6 days the highest removal efficiency was observed 5 mg/L dye concentration at 85.2 %. And the lowest was observed in 15mg/L dye concentration at 72.47 %. While in the control the remaining dye amount was same as initial dye concentration with 97.1 was still remaining. This observation indicates that more the dye concentration, the process is slow. It is also observed that the more than 50% of the process is done by the period of 3 days. The Decrease in the colour intensity can be seen in the Figure 4a and 4b. The changes in absorbance over a period of 6 days with varying dye concentration and fixed plant weight is given in the Table 1. The percentage decrease is represented in the Figure 5.

Table 1: Change in absorbance and remaining dye concentration over the period of 6 days

Dye concentration	Days							
	0		2		4		6	
	Absorbance at 663 nm	Concentration in mg/L	Absorbance at 663 nm	Concentration in mg/L	Absorbance at 663 nm	Concentration in mg/L	Absorbance at 663 nm	Concentration in mg/L
Control (10 mg/L)	1.8643	10	1.8631	9.98	1.8215	9.77	1.8112	9.71
2.5 mg/L	0.2768	2.5	0.1468	1.32	0.098	0.88	0.0445	0.4
5 mg/L	0.7608	5	0.3633	2.39	0.2267	1.49	0.113	0.74
10 mg/L	1.8643	10	0.8308	4.45	0.5908	3.16	0.4312	2.31
15 mg/L	3.3323	15	1.9232	8.64	1.155	5.19	0.9508	4.28

*Plant weight was kept constant at 10 grams

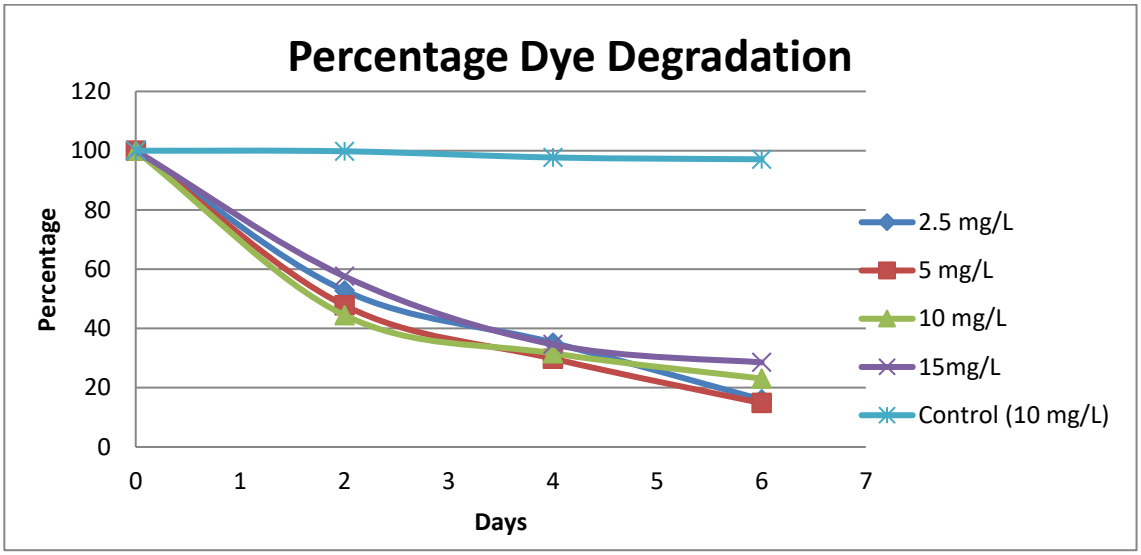


Figure 5: Percentage removal of dye for the span of 6 days with varying dye concentration and fixed plant weight

3.2. Effect of plant weight of Decolourization rate

On observing over the period of 6 days under different plant weight, the maximum removal efficiency was observed in 20 gram plant weight and lowest was in 5 gram plant weight. The weight of the plant is directly proportional to the rate of dye removal. The changes in absorbance over a period of 6 days with varying plant weight and fixed dye concentration is given in the Table 2. The Figure 6 shows the decrease in dye concentration compared to control. The percentage degradation is represented in Figure 7.

2.3. Effect on plant thallus

Under observation of the Plant thallus in microscope, the dye is seen to be stored in plant body and roots. As seen in the Figure 8a and 8b the dye is stored in the ventral surface not on dorsal surface because of presence of protective layer on the dorsal surface.

Table 2: Reduction in dye concentration over the period of 6 days with different plant weights

		Days							
		0		2		4		6	
Plant weight		Absorbance at 663 nm	Concenrtation in mg/L	Absorbance at 663 nm	Concenrtation in mg/L	Absorbance at 663 nm	Concenrtation in mg/L	Absorbance at 663 nm	Concenrtation in mg/L
	Control	1.8643	10	1.8622	9.98	1.821	9.9	1.798	9.4
	5	1.8643	10	1.2715	6.82	0.7388	3.96	0.6228	3.34
	10	1.8643	10	0.4887	2.66	0.4559	2.44	0.4198	2.25
	15	1.8643	10	0.4054	2.17	0.3312	1.77	0.3226	1.73
	20	1.8643	10	0.31	1.66	0.3035	1.62	0.2038	1.09

*Dye concentration was kept constant at 10 mg/L

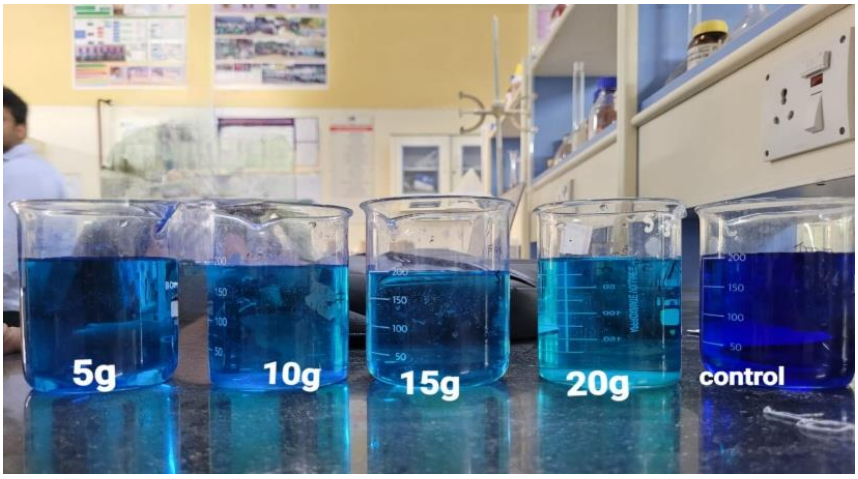


Figure 6: Observation at day 6 (Final) at different plant weight compared to control

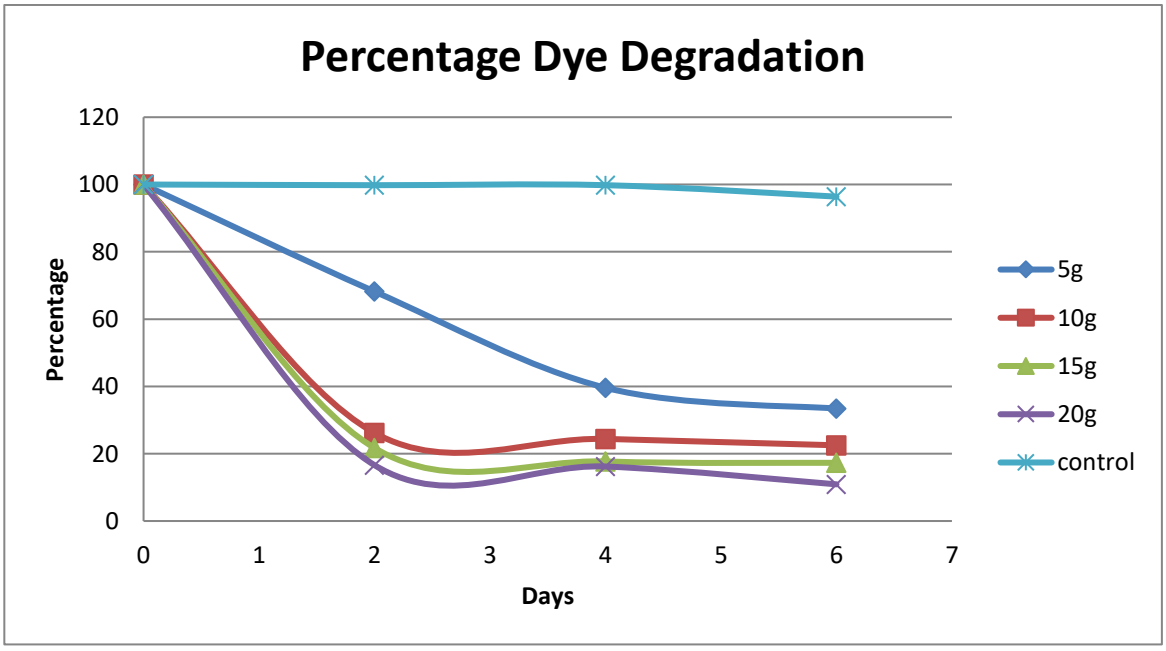
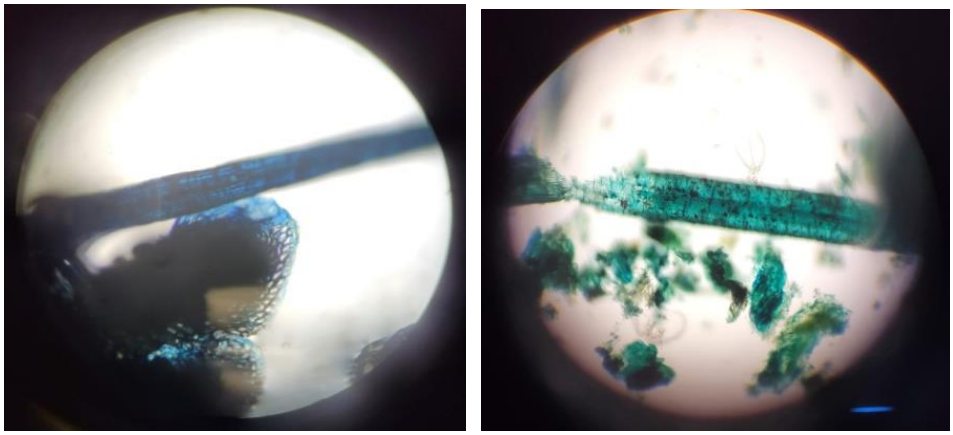


Figure 7: Percentage removal of dye for the span of 6 days with fixed dye concentration and varying plant concentration.



a b

Figure 8a: Effect of Methylene blue on Plant Leaves,
Figure 8b: Effect of Methylene blue on Plant thallus

3.4. Effect on Plant pigments

The plant pigments are reduced as the dye concentration increases because of the MB getting stored in cells. The figure 9 shows the chlorophyll content in each experimental setup. The Observed chlorophyll in samples is 275 µL/Grams, 201 µL/Grams, 172 µL/Grams, 150 µL/Grams compared to 300 µL/Grams in stock.

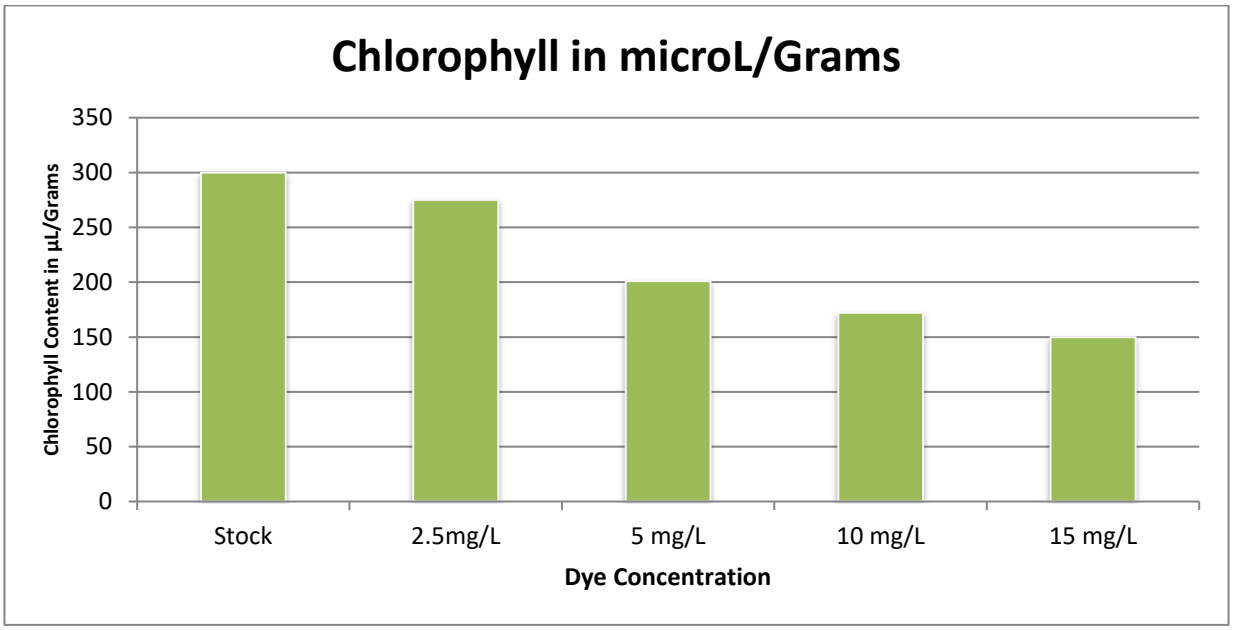


Figure 9: Effect of methylene blue on Chlorophyll content

3.5. Effect on Plant growth rate

The figure 10 shows effect of MB on growth rate of Azolla Under different dye concentrations. The normal plant growth rate of azolla is 0.104 per day. The observed growth rates are 0.053, 0.023, 0.011 and -0.083 per day. The negative plant growth rate was observed in 15mg/L dye concentration because of increased acidity. In this experiment on an average 80% of methylene blue was transformed in the period of 6 days. In this experiment it is seen that the dye concentration is directly proportional to the rate of the transformation. In a study conducted by Kah *et al.* 2016), the plant *Eichhornia crassipes*

showed the removal efficiency of 92% of methylene blue. The Phytoremediation potential of methylene blue is slightly higher than other azo dyes compared to other azo dyes because of its lower molecular weight. The slightest decrease in the control is because of dye degradation due to the light, a phenomenon called phototransformation, in which dye molecules brake down due to the light.

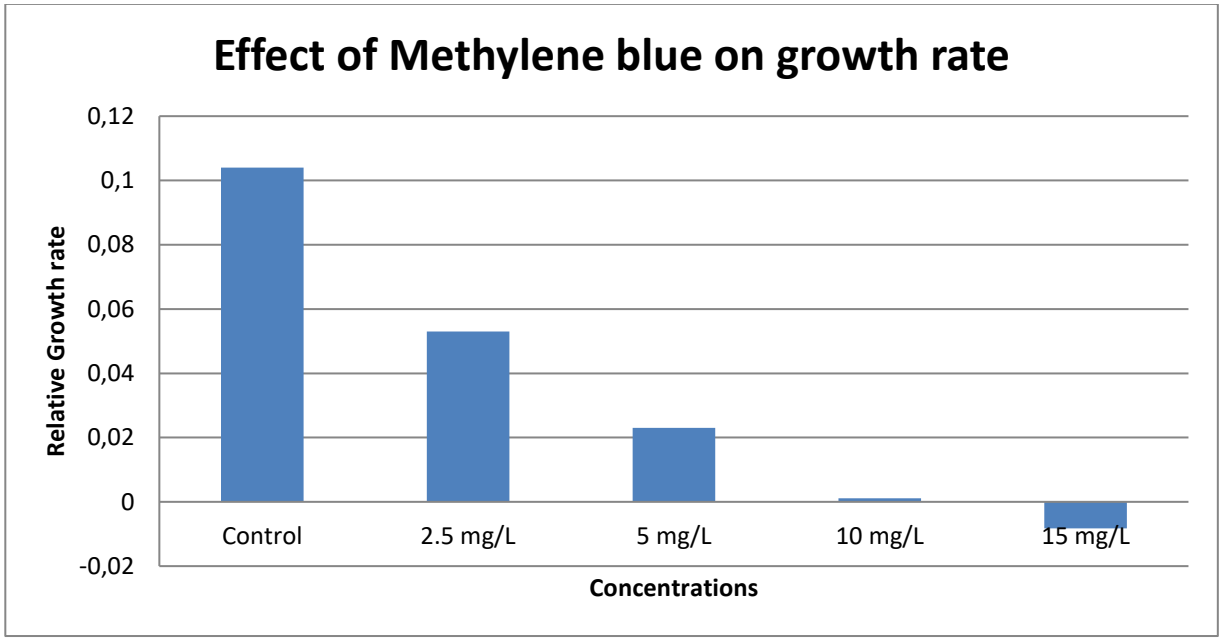


Figure 10: Effect of methylene blue on Growth rate

Manghabati and Pazuki (2014), who studied the Phytoremediation of MB dye by Spirodela polyrrhiza, discovered that the exposure time was the most important factor. According to Al-Baldawi *et al.*, 2018, MB dye has a maximum degradation efficiency of 90%, which may be achieved by Azolla pinnata over five days at a starting concentration of 25 mg/L. Following 24 hours of dye exposure, physical examination indicated definite evidence of bio-decolorization by duckweed (Lemna minor), according to Imron *et al.* (2019). According to research by Ewadh (2020) the aquatic plant Coontail (*Ceratophyllum demersum*) has a great potential as a Phytoremediation agent to remove MB dye from the wastewaters, as the removal percentage of MB dye reached up to 96% after five days.

Yaseen and Scholz (2018) examined the treatability of Basic Red 46 and Reactive Blue 198 combinations in shallow ponds containing L. minor in a follow-up investigation. While experiments with a different combination combining 8 mg/L Reactive Blue 198 and 2 mg/L Basic Red 46 produced a removal efficiency of 33%, the removal efficiency of a solution containing 2 mg/L Reactive Blue 198 and 8 mg/L Basic Red 46 dye mixture was 66%. As a consequence, it was discovered that a mixture's higher Reactive Blue 198 level reduced the solution's treatability. Phytoremediation effectiveness of L. minor was examined by Khataee *et al.* (2012) utilising artificial neural network modelling. They looked at how Acid Blue 92 removal efficiency was affected by beginning dye concentration, initial pH, working time, temperature, and weight of L. minor. At a pH of 3.5, a temperature of 25 C, a plant weight of 2 g, and an initial dye concentration of 10 mg/L over the course of 6 days, the highest removal efficiency was 91.0%. By combining triacontanol hormone with L. minor, Nur *et al.* (2010) investigated the elimination of four dyes (Reactive Orange 14, Reactive Red 120, Reactive Black 5, Brilliant Blue R, and Reactive Brilliant Blue R). With 1 mg/L of the hormone, the highest dye removal efficiency was reported to be 59.6%.

The method of using living plant compared to the other method called phytoadsorption uses dried mass of azolla. In this method the plants are dried powdered and made to contact with dye using rotary shaker. This requires lesser contact time. In an experiment conducted by (Aytaf *et al.*, 2020) using Phyto adsorption he was able to remove 90 of methylene blue from the dye solution**.** In an experiment conducted by (Muhammad *et al.*, 2016), used the plant duckweed (*Lemma minor*) he was able to remove 80% methylene blue from 50 mg/L methylene blue**.** The similar observations were taken by (Reema *et al.*, 2011, Khatau *et al.*, 2012) respectively in the period of 3 days (72 hours).

Several other technologies are available to degrade the azo dyes in wastewater. In an experiment conducted by Yang *et al.* (2020) using Electro coagulation method, they found out that the rate of removal varies with change in concentration. For 20 minutes of time, they were able to remove 90.7% of methylene blue and 95 % of Aniline yellow from the water. Lucas et al. (2022) reported that in different studies have done in order to estimate the effectiveness of using microbes to degrade the industrial dyes. They used *Pseudomonas sp*. Strains which were able to remove 90% azo dyes. The bacterial strain *Bacillus sp.* was able to remove upto 92% of azo dye in the period of 48 hours.

In the same experiment the relative growth rate was estimated. *Lemma minor* or duckweed showed the relative growth rate of 0.011 per day as compared to control unit which showed the growth rate of 0.24 per day (Reema *et al.*, 2011). In our experiment we observed the reducing growth rate as the dye concentration increased. The observed growth rate of azolla is was about 0.053 in 2.5 mg/l, 0.023 In 5 mg/l, 0.011 in 10 mg/l, and -0.0083 in 15 mg/l per day. In control it was 0.104 per day in azolla. The reduced growth rate compared to normal azolla cultivation is due to the larger ratio of azolla used in small amount of dye solution which contained nutrients.

Also, the contact time also plays an important role in dye removal. In the *study Lemma minor* removed 80% dye by the plant in 3 days. In this experiment 80 % of the process is done in the period of 3 days here the contact time and area of contact between plant and dye solution also plays important roles. From estimating the chlorophyll content of the plant, it was seen that the chlorophyll content of the azolla plant is reduced by 10 to 50 percent as the dye concentration increases. According to a study by Buy et al in 2022 dyes reduce the chlorophyll content in plants more than heavy metals because of similar characteristics of dye and plant pigments (Bai *et al.*, 2021). The azo dye gets stored in plant cells beak down the chlorophyll content in plant cells.

Conclusion

In this study the efficiency of Phytoremediation of azolla is studied. It was found that plant azolla pinnata removes up to 85% of dyes from contaminated water. It was observed that the factors such as dye concentration and plant weight affect the rate of reaction. The effect of methylene blue on plant body was studied. It was studied that more than half of the process is completed by period of 3 days. After 5 days the process reaches equilibrium state. It was observed that the death of plants occur at 15 mg/L dye concentration which indicates its threshold value. The methylene blue gets accumulated in the plant's body. Most of the dye is stored in root system and ventral thallus of azolla. Dorsal surface is not affected by methylene blue because of protective layer on the upper part of the body. The increasing concentration of MB has adverse effect on plants growth rate. It was seen that plants show negative growth i.e. death of plants occur in concentrations more than 15mg/L dye concentration because of increased acidity. This process is an effective process that uses zero machinery and far lesser cost and a simple mechanism. It's an easier process compared more modern and complex process that is useful in treatment of textile wastewater and heavy metal contamination in wastewater.

References

Aaddouz M., Azzaoui K., Akartasse N., Mejdoubi E., Hammouti B., Taleb M., Sabbahi R., Alshahateet S.F. (2023) Removal of Methylene Blue from aqueous solution by adsorption onto hydroxyapatite nanoparticles, *Journal of Molecular Structure*, 1288, 135807, https://doi.org/10.1016/j.molstruc.2023.135807

Al-Baldawi I.A., Abdullah S.R., Anuar N., Hasan H.A. (2018). Phytotransformation of methylene blue from water using aquatic plant (Azolla pinnata). *Environ. Technol. Innov*., 11, 15–22

Alshekhli, A.F., Hasan, H.A., Muhamad, M.H., Abdullah (2020), S.R.S. Development of Adsorbent from Phytoremediation Plant Waste for Methylene Blue Removal, *J. Ecol. Eng.*, 21, 207–215. doi: https://doi.org/10.12911/22998993/126873

Atyaf Farooq Alshekhli, Hassimi Abu Hasan, Mohd Hafizuddin Muhamad, Siti Rozaimah Sheikh Abdullah (2020). Development of Adsorbent from Phytoremediation Plant Waste for Methylene Blue Removal. *Journal of Ecological Engineering*, 21(8), 207-215. doi: https://doi.org/10.12911/22998993/126873

Bai, Y., Zhou, Y. & Gong, J. (2021). Physiological mechanisms of the tolerance response to manganese stress exhibited by Pinus massoniana, a candidate plant for the phytoremediation of Mn-contaminated soil. *Environ. Sci. Pollut. Res.* 28, 45422–45433, https://doi.org/10.1007/s11356-021-13912-8

Can-Terzi B., Goren A.Y., Okten H.E., Sofuoglu S.C. (2021). Biosorption of methylene blue from water by live Lemna minor, *Environmental Technology & Innovation*, 22, 101432, https://doi.org/10.1016/j.eti.2021.101432

Chandrakant R. Holkar, Ananda J. Jadhav, Dipak V. Pinjari, Naresh M. Mahamuni, Aniruddha B. Pandit (2016), A critical review on textile wastewater treatments: Possible approaches, *Journal of Environmental Management*, 182, 351-366, https://doi.org/10.1016/j.jenvman.2016.07.090.

Chengji Zhang, Hong Chen, Gang Xue, Yanbiao Liu, Shanping Chen, Chuan Jia (2021). A critical review of the aniline transformation fate in azo dye wastewater treatment, *Journal of Cleaner Production*, 321, 128971, https://doi.org/10.1016/j.jclepro.2021.128971

Cluis, C. (2004) Junk-Greedy Greens: Phytoremediation as a New Option for Soil Decontamination. *BioTeach Journal*, 2, 61-67,

Contreras, Margarita, Carlos David Grande-Tovar, William Vallejo, and Clemencia Chaves-López. (2019). Bio-removal of methylene blue from aqueous solution by galactomyces geotrichum KL20A, *Water*, 11, no. 2: 282. https://doi.org/10.3390/w11020282

Elrasoul A. S. A., Mousa A. A., Orabi S. H., Mohamed M. A. E-G., Gad-Allah S. M., Almeer R., Abdel-Daim M. M., Khalifa S. A. M., El-Seedi H. R., Eldaim M. A. A. (2020). Antioxidant, Anti-Inflammatory, and Anti-Apoptotic Effects of Azolla pinnata Ethanolic Extract against Lead-Induced Hepatotoxicity in Rats. *Antioxidants.* 9(10), 1014. https://doi.org/10.3390/antiox9101014

Ewadh H. M. (2020). Removal of methylene blue by coontail (Ceratophyllum demersum) using phytoremediation concept, *Plant Arch.*, 20, 2677–2681

Gupta. V, (2009). Application of low-cost adsorbents for dye removal–a review. *J. Environ. Manag.*, 90, 2313–2342. https://doi.org/10.1016/j.jenvman.2008.11.017

Imron MF, Kurniawan SB, Soegianto A, Wahyudianto FE (2019). Phytoremediation of methylene blue using duckweed (Lemna minor). *Heliyon*, 5(8), E02206. https://doi.org/10.1016/j.heliyon.2019.e02206

Karaghool H. (2021) Biodecolorization of methylene blue using aspergillus consortium. *IOP Conference Series: Earth and Environmental Science.* 779. 012111. 10.1088/1755-1315/779/1/012111.

Karen E. Gerhardt Perry D. Gerwing Bruce M. Greenberg (2016). Opinion: Taking Phytoremediation from Proven Technology to Accepted Practice. *Plant Science.* V256. 170-185. https://doi.org/10.1016/j.plantsci.2016.11.016

Khan I, Saeed K, Zekker I, Zhang B, Hendi AH, Ahmad A, Ahmad S, Zada N, Ahmad H, Shah LA, (2022). Review on Methylene Blue: Its Properties, Uses, Toxicity and Photodegradation. *Water*, 14(2):242. Doi: https://doi.org/10.3390/w14020242

Khataee A.R., Movafeghi A., Torbati S., Salehi Lisar S.Y., Zarei M. (2012), Phytoremediation potential of duckweed (Lemna minor L.) in degradation of C.I. Acid Blue 92: Artificial neural network modeling, *Ecotoxicology and Environmental Safety*, 80, 291-298, https://doi.org/10.1016/j.ecoenv.2012.03.021

Kollah, B., Patra, A.K., Mohanty, S.R. (2016) Aquatic microphylla Azolla: a perspective paradigm for sustainable agriculture, environment and global climate change. *Environ. Sci. Pollut. Res.* 23, 4358–4369. https://doi.org/10.1007/s11356-015-5857-9

Liu Y, Li C, Bao J, Wang X, Yu W, Shao L (2022). Degradation of Azo Dyes with Different Functional Groups in Simulated Wastewater by Electrocoagulation. *Water*, 14(1):123. https://doi.org/10.3390/w14010123

Manghabat H., Pazuki G., (2014). A study on the decolorization of methylene blue by Spirodela polyrrhiza: Experimentation and modelling, *RSC Adv.*, 4, 30137–30144

Medjahed, K., Tennouga, L., Mansri, A., Chetouani, A., Hammouti B., Desbrières, J. (2013), Interaction between poly(4-vinylpyridine-graft-bromodecane) and textile blue basic dye by spectrophotometric study, *Res. Chem. Intermed.*, 39 N°7, 3199-3208.

Muhammad Fauzul Imron, Setyo Budi Kurniawan, Agoes Soegianto, Febri Eko Wahyudianto, Phytoremediation of methylene blue using duckweed (Lemna minor), *Heliyon*, 5, Issue 8, 2019, https://doi.org/10.1016/j.heliyon.2019.e02206

Nur Koçberber Kiliç, Ergin Duygu, Gönül Dönmez (2010). Triacontanol hormone stimulates population, growth and Brilliant Blue R dye removal by common duckweed from culture media, *Journal of Hazardous Materials*, 182(1-3), 525-530, https://doi.org/10.1016/j.jhazmat.2010.06.063

Pinheiro L.R.S., Gradíssimo D.G., Xavier L.P., Santos A.V. (2022). Degradation of Azo Dyes Bacterial Potential for Bioremediation. *Sustainability* 214, https://doi.org/10.3390/su14031510

Reema R. M., Saravanan P., Dharmendira Kumar M. & Renganathan S. (2011) Accumulation of Methylene Blue Dye by Growing Lemna minor, *Separation Science and Technology*, 46(6), 1052-1058, doi: 10.1080/01496395.2010.528503

Samsami, S., Mohamadi, M., Sarrafzadeh, M.H., Rene, E.R., Firoozbahr, M. (2020). Recent advances in the treatment of dye-containing wastewater from textile industries: Overview, *Process Safety and Environmental Protection*, 143, 138-163, http://dx.doi.org/10.1016/j.psep.2020.05.034

Sapna and Lingaraju H. G. (2022) Azolla: A organic feed for fish farming – Review, *J. Mater. Environ. Sci.*, 13, Issue 03, 236-251

Uddin M.A., Begum M.S., Ashraf M., Azad A.K., Adhikary A.C., Hossain M.S. (2023) Water and chemical consumption in the textile processing industry of Bangladesh. *PLOS Sustain Transform* 2(7), e0000072. https://doi.org/10.1371/journal.pstr.0000072

Yaseen D.A., Scholz M (2018). Treatment of synthetic textile wastewater containing dye mixtures with microcosms. *Environ. Sci. Pollut. Res.*, 25, 1980–1997. https://doi.org/10.1007/s11356-017-0633-7

Zahra Derakhshan, Mohammad Ali Baghapour, Mojdeh Ranjbar, Mohammad Faramarzian (2013). Adsorption of Methylene Blue Dye from Aqueous Solutions by Modified Pumice Stone: Kinetics and Equilibrium Studies. *Health Scope.*, 2(3), 136-144. Doi: https://doi.org/10.17795/jhealthscope-12492