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Study on Phytoremediation and Biomass-Adsorption of Cationic and Anionic Dye using Azolla Pinnata

By

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The undersigned certify that they have read, and recommended to the Institute of Engineering for acceptance, a thesis entitled "**Study on Phytoremediation and Biomass-Adsorption of Cationic and Anionic Dye using Azolla Pinnata**" submitted by Manoj Gyawali, (Thesis No. :076/MSMSE/012) in partial fulfilment of the requirements for the degree of Master of Science in Material Science and Engineering.

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ABSTRACT

Waste water generated from the textile industry consists of non- biodegradable complex compounds such as dye. This waste water can be toxic to the living organism. Hence, the treatment of the waste water consisting dye is very important before it is released streams, lake, river etc. Among the several methods of dye waste water treatment such as adsorption, filtration, bio adsorption and photo degradation, Phytoremediation is suitable because it is naturally occurring process on which micro-organism or plant transforms or immobilize the complex compounds such as dye. Azolla Pinnata, phytoremediation plant can be used for extraction of the dye and complex compound from waste water.

This study focuses on the experimental investigation of the phytoremediation and biomass adsorption study of Azolla Pinnata using cationic and anionic dye. The phytoremediation investigation was carried out using methylene blue dye and Methyl orange under the varying concentration (10 – 30 mg/l) using the wet weight of 2-5 gm. The quantification of dye and chlorophyll content measurement were done by using UV-Vis spectrophotometer. The effect of plant doses, initial concentrations on the dye removal efficiencies were determined. The phytoremediation study showed that the azolla pinnata is able to remove the Methylene blue dye (cationic) from aqueous above 90% in 24 hours. Whereas it only able to remove 32-37% Methyl orange (Anionic) in aqueous solution for seven days of the exposure period. The FTIR study for the dry mass of azolla exposed to Methylene blue, Methyl orange and distilled water were done. FTIR study showed that the removal mechanism of the Methylene blue dye by the azolla was phyto-extraction through hydrogen bonding and electrostatic interaction of functional groups and dye molecules. Similarly, the biomass-adsorption study showed that adsorbed percentage of dye using biomass of azolla was found to be higher than 90% in lower concentration (50 mg/l to 300 mg/l) of Methylene blue dye. Langmuir isotherm and Pseudo second order kinetic models were best suited for describing Methylene blue dye adsorption. However, azolla biomass did not absorb the Methyl orange dye even in low concentration (3mg/l-15 mg/l.) Hence, it is concluded that the azolla pinnata and it’s biomass can be highly efficient for the removal of cationic dye than anionic dye.

Keywords: Phytoremediation, Azolla pinnata, Biomass, Cationic and Anionic dye, Phytoremediation mechanism

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LIST OF SYMBOLS, ACRONYMS AND ABBREVIATIONS

AP	Azolla Pinnata
Chl_a	Chlorophyll A
Chl_b	Chlorophyll B
C_x	Carotenoids
^{0}C	Degree Celsius
gm	Gram
gm/l	Gram per liter
hrs	Hours
mg	Milligram
mg/l	Milligram per liter
Mg/g	Milligram per litre
MB	Methylene Blue
MO	Methyl Orange
v	Volume

CHAPTER ONE: INTRODUCTION

1.1 Background

Azolla is a plant that is short, branched and have floating stem and roots which hang them in the water. This plant's leaves are arranged alternatively alternately and consist of a thick aerial dorsal lobe. The diameter of the plant is ranging from 1-2.5 mm. It produces high amount of a biomass in a short duration of time. It can be used as bio fertilizer, feed for animals and phytoremediator for waste water treatment. This plant has a protective cavity in a leaf for colonies of Anabaena in order to fix atmospheric nitrogen. Azolla provides the 80% of the nitrogen after fixing the biological nitrogen. It has a relationship with a cyanobacterium for fixing of nitrogen and provides the nitrogen to the interfaced crops. Azolla Pinnata has great potential as a bio-fertilizer, because it can assimilate atmospheric nitrogen efficiently and hence increased the productivity of the paddy crops (Kandel et al., 2020). Beside this, the product based on the Azolla is used for making the feed for the animals and birds. It provides the cattle or buffalo herders as a cheaper feed(Kumar et al., 2020). The nutrient enriched Azolla has good effect on growth performance of fish and decrease the feeding cost for a fish. In addition to this, Azolla Pinnata is a phytoremediator and can be used for remediate the complex organic compound such as dye.

1.2 Phytoremediation

Phytoremediation is the naturally occurring process via which micro-organism transform or immobilize the contaminants such as dye to the harmless products. It also absorbs heavy metal from the growing medium. It defines to the utilization of the plants and linked to the soil or water for reducing the toxic consequences of the contaminant for the environment. It is widely applied as cost effective technology for the environmental restoration. It is also known as the alternative to the engineering method that are more destructive to the soil(Yadav et al., 2015). It uses the metabolic activities of the plant for the absorption of a heavy metals and transformation and degradation of dyes and toxic chemicals. According to the phytoremediation process for remediate the toxic contaminant from soil and water, the overall process or mechanisms are defined as Phyto-volatilization, phytoextraction, phytodegradation, Phyto-stabilization and Rhizo-filtration(Kafle et al., 2022).

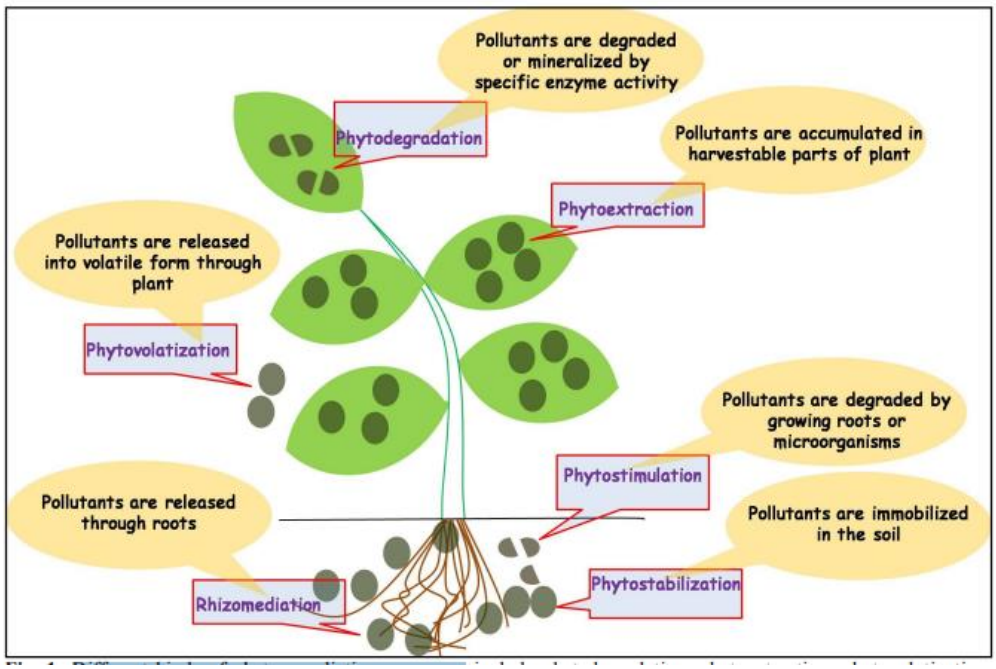

Figure 1.1: Schematic diagram of different approaches of phytoremediation (Pradip Kumar Prusty et al, 2020)

1.2.1 Phyto-extraction

It involves the absorption of the toxic metal by the plants stem, roots and stored into a cell wall, metabolically active parts and cell membrane. The plants which follows the phytoextraction process are called as hyperaccumulator that accumulates the toxic contaminant in the shoot and root's tissue.

1.2.2 Phyto-stabilization

The roots of a plant restrict the mobility of the contaminant and reduced to low toxic compound. It is the process of remediation of the toxicant by immobilization or inactivation with in the rhizosphere and roots. The plant's root stabilizing activity restrict the mobility and bio-availability of the contaminant so that the toxic effects are lowered.

1.2.3 Rhizo-filtration

It consists of removing the contaminants form the saturate zone such as ground water, waste water and surface water with the use of adsorption and precipitation mechanism by roots or other parts of the aquatic plant(Jadia & Fulekar, 2010).

1.2.4 Phyto-volatilization

It is the process of transforming the less volatile contaminants from soil or water to more volatile form. It finally exposed them to the atmosphere. These methods are mostly adopted by the plants for removal of the organic compounds(Limmer & Burken, 2016).

1.2.5 Phyto-degradation and Rhizo-degradation

Plant destroys and metabolizes the pollutants inside the tissue of the plant via phytodegradation process. The organic contaminants can be removed by the transformation or degradation through the various parts of a plants. In this methods or process, the plant extracted the pollutants from the soil or water and breaks them to the small and harmless compounds. It finally circulates with in the tissue of the plants. Not only this, this type of the organic compound is absorbed via the passive nature of extraction. When the pollutants are degraded in the rhizosphere and this method is known as rhizo-degradation

1.3 Azolla Pinnata as Phytoremediation

Azolla Pinnata utilizes biological technique called Phytoremediation that uses the plants to remediate contaminants for soil and surface water. (Din et al., 2020; Jacob, 2020; Parikh & Mazumder, 2015; Rai, 2008, 2010; Rezooqi et al., 2021) This technique can be highly efficient and useful for cleaning up the heavy and toxic metals from the surrounding environment. It is a cheap and suitable green technology for remediation of pollutant available in a water tanks and lakes. Azolla are known as the Hyper accumulators that have capability for absorbing the heavy metals without affecting the plant growth. Hence, this technique is used for removing of the heavy metals and industrial waste contaminants by using the Azolla(Akhtar et al., 2019).

1.4 Bio adsorption

It is the biomass adsorption method which recover or remove the in organic or organic compounds in the aqueous solution utilizing the biologic materials which includes the dead biomass of the vegetable waste, natural waste, agricultural waste as adsorptive medium. These methods are appears via the interaction of the dye, toxic contaminant with the active sites of the adsorbent such as hydroxyl groups, amino groups, sulphate groups etc(Costa et al., 2020).

1.5 Dyes

Dyes can be defined as coloring imparting agent. They are made up of two main components such as Chromophone and Auxophone that give the colours, water solubility and affinity of the dye to the clothes. The dye existing in a water bodies have adverse impact on the human health The range of commercially available dyes is more than 100,000 with higher than 7 to107 tons of dye related product generated worldwide annually. There are many classes of the dye which depends of the several factors. As per the classification relied on molecule carries charge, they are classified as Anionic and Cationic dye

1.5.1 Cationic Dye

Cationic dyes are broadly utilized for the dying of wool, nylon and silk. This kind of the dye consists of the various kind of the chemical structure relied on the replaced aromatic compounds. This type of the dye can cause the harmful effect on the human health and also produce the toxic colorants. Cationic dyes carry the positive kind of the charge in their molecules and soluble of the water and produce the coloured cations in the aqueous solution. Functionality of a Cationic is presented in the various kind of the dye like azo and methane dyes. Not only this, It is also found in an anthraquinone, tri and di-arycarbenium, phthalocyanine dyes, polycarbocylic and solvent dyes(Mahmoud et al., 2011).

1.5.1.1 Methylene Blue Dye

Methylene blue dye is a cationic, aromatic and heterocyclic chemical compound which has molecular formula of C16H18N3SCl. The dye has a greater molecular weight of a 319.85 g/mol. It is an dye of aniline-basic which generates the deep blue colour in alcohol or water. It is an organic compound having greenish blue in colour and generally utilized as a stain and indicator. It is also a man-made drug utilized for the medication of the psychiatric disorder, methemoglobinemia, malaria and nervous system. At the high doses, it causes the anaemia and other severe effects. MB dye is harmful for the human kind and can be carcinogenic because of a non- biodegradable in nature(Uddin & Nasar, 2020).

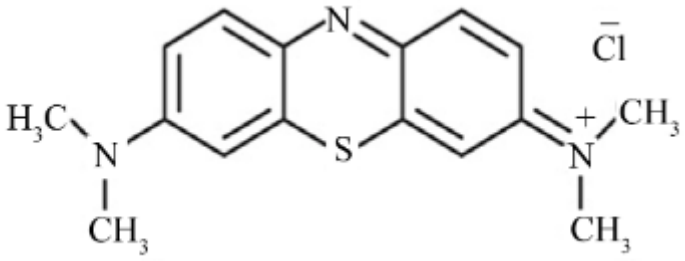

Figure 1.2: MB dye Chemical Structure (Elmorsi, 2011)

1.5. 2 Anionic Dye

Anionic dyes carry the negative ion. They consist of the various compounds from different classes. It exhibits the characteristic differences in the chemical structure which possess the azoic anthraquinone, nitro dyes, triphenylmethane etc. The anionic kind of the dye includes the direct dye, and reactive dyes.(Mahmoud et al., 2011).

1.5.2.1 Methyl Orange Dye

It is azo dye generally utilized as the pH indicator while performing titration. This was due to the fact that it gives the distinct colour variance when treated with different pH. It is a weak acid which breaks down into the neutral molecule of the MO when it dissolves in the water. MO called as dimethylaminoazobenzenesulfonate, is a general kind of the azo anionic dye. It gives the bright orange color when dissolved in the water. The MO consists of an– N= N- groups and aromatic in their chemical structure. They are also carcinogenic, teratogenic and highly toxic and. In addition to this, they are harmful to the organism and environment. The molecular diameter of the MO is to be 6-8 nm as per its structure and molecular weight(Wu et al., 2021).

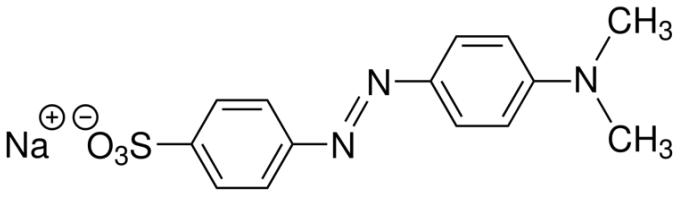

Figure 1.3: Chemical Structure of MO Dye(Attallah et al., 2016)

1.6 Problem Statement

With the industrialization, the discharge of the industrial waste water to the surface water has been increased. The contaminants such as dyes presented in the waste water or water streams has adverse effect of the human health and aquatic life. The synthetic

dyes made up of aromatic compounds are extensively used for in the paper printing, plastic, leather and textile industries for the colouration of the product. But due to the huge discharge of the dye contaminants in the water source can cause the adverse effect on the environment. Hence, there is requirement for determining effective and cost-effective methods for the dye removal from waste water. The various methods practiced for the treatment of the dye containing water are adsorption, filtration, photo degradation and phyto-remediation method. Among the various methods, the phytoremediation by aquatic plant can be cheapest and environmentally friendly. Azolla is available in the wet land, ponds and ditches in the tropical regions and warm temperate around the world. It can be used as bio- fertilizer, feed for chicken and animals, phytoremediation, etc. Here in Nepal, the Azolla Pinnata have been grown throughout the country for the chicken feed domestically and commercially. However, it's application concerning, phytoremediation has not practiced yet. The climatic conditions of the Nepal suits for the azolla growth due to it's diverse climatic conditions. Hence, Nepal has huge potential for the commercial production of the azolla and making the bio filter for the removal of the dye containing waste water. Hence, this project focuses on a Study of phytoremediation properties of azolla using cationic and anionic dye.

1.7 Research Questions

Some of the research question encountered at the very beginning of the project are:

- Does azolla participate in the phytoremediation of cationic and anionic dye?
- How much the anionic and cationic dye removal efficiencies in aqueous solution by using azolla pinnata.
- Does the dye effect on the growth of the azolla pinnata?

1.8 Objective of the Thesis

1.8.1 General Objectives

The main objective of the thesis is to understand the phytoremediation and biomass adsorption Anionic and cationic dye using Azolla Pinnata.

1.8.2 Specific Objectives

The Specific objectives are:

- To investigate the phyto-toxicity of the dye for the plant

- To evaluate the phytoremediation mechanism of azolla pinnata for the cationic and anionic dye.
- To provide the mechanism of dye absorption by the plant.
- To determine the cationic and anionic dye removal efficiencies using fresh Azolla Pinnata as phyto-remediator.

1.9 Limitation of the project

Some of the limitations of the project are as follow:

- Toxicity of the dye on the azolla plants is considered only for 7 days of exposure periods.
- The experiment is carried out in the artificial solution of the dye.
- Temperature effect on the phytoremediation is not considered.
- The Experiment is carried out under the laboratory conditions.

CHAPTER TWO: LITERATURE REVIEW

2.1 Previous Studies on the Phytoremediation process using various plants

(Al-Baldawi et al., 2018) investigated the phytoremediation properties of the azolla pinnata using the MB as dye. They prepared the three concentration of the dye and used the 3 g of fresh azolla pinnata. After that, they evaluated the absorbance of the dye from day 1 to day 5 using UV/Vis spectrophotometer. From the investigation, it was found that the highest decolorization of MB was found to be 85%. The conclusion was made in such a way that the azolla pinnata could be capable for transform the dye and suitable for the treatment of water.

(Akhtar et al., 2019) performed the experiment under the controlled condition for the investigation of the azolla growth and phytoremediation ability. The plants were exposed to the different concentration of the zinc and copper. They found that the lower concentration of the zinc and copper enhance the plant growth. But the higher concentration inhibited the growth. Azolla showed substantial metal removal capacity. The bio-concentration factor was high which indicated that the azolla pinnata can be utilized for a filtration of the heavy metals like Zn and Cu.

(Li et al., 2020) discussed the simultaneous effect of Zn and Se as micronutrient on the various factor such as growth indicator, bio concentration factor and metal removal efficiencies. The two aquatic plant such as azolla and lemina were grown in the hydroponic cultures having the Se and Zn concentration with the 1- 5 mg/l. When comparing to the effect of Se (VI) and Se (IV), it was found that the Se (IV) has high toxicity than the Se (VI) because there was remarkable decrease in the root length and biomass content when growing to the Se (IV). The accumulation of Zn and Se in the azolla and lemina leads to rapid growth and high protein content and nutrients in their biomass. Th Zn and Se enriched azolla pinnata biomass can be utilized as a crop fertilizer and nutrient rich feed products.

(Kooh, Dahri, et al., 2016) investigated the possible potential of the Azolla pinnata for the phytoextraction of the methyl violet dye waste water by using the ANN (Artificial neural network modelling). The included parameters effect the concentration of dye,

pH and volume of the dye. The greatest removal efficiency is obtained as 93% by using the plant dosage of 0.8g, volume of 600ml and dye concentration of 10 mg/l. The significant reduction in the RFN and chlorophyll showed the toxicity in the chlorophyll. The author concluded that the azolla pinnata was suitable for the phyto-extraction of the methyl violet and Artificial neural network method as most reliable method for representing the phytoextraction of methyl violet using azolla pinnata.

(Goala et al., 2021) analyzed the phytoremediation efficiency of the azolla pinnata for treating of the diary waste water. The experimentation method in batch mode was performed utilizing the various dilution for the waste water with the five healthy azolla pinnata leaflets. Not only this, the growth of the plant is monitored by using camera vision image recognition and modified Gompertz models. The phytoremediation experiment conducted for 14 days showed that the maximum number of leaflets which further helped for simulating the azolla pinnata growth pattern. The maximum dry biomass, chlorophyll content was observed using the 75% diluted waste water.

(Kaushal & Mahajan, 2022) performed the kinematic investigations for Salvina molesta Mitchell's phytoremediation ability using the Direct Red dye in their research work. The plant potential regarding the removal of the dye was analyzed using the various pH and various concentration of initial dye. Around 90% of the decolorization of the dye was achieved using the 50 mg/l dye consisting solution including the 4 gm S. molesta plant with the PH of 6.5. The results of the experiment evaluated using the various kinematic models. The most fitted kinematic models were pseudo second order having correlation coefficient of the 0.99. The FTIR results also depicted that the functional groups of a dye interacted with the dye molecules and hairy roots.

(Mahajan & Kaushal, 2020a) used the macroalgae *Chara vulgaris* L for examining the for the phytoremediation of the methyl red (MR) dye in the aqueous form. The experiment was conducted with the C vulgaris for predicting the different experimental process parameters such as concentration, dosage of micro algae, pH and contact time. The experiment results depicted that the decolorization percentage declined with the decrement of the initial dye concentration. The kinetics of the phytoremediation was

examined by utilizing the Elovich and pseudo first order & second order type kinetic models. More over to this, the phytoremediation data favors the Freundlich equilibrium isotherms. The FTIR was also investigated before and after dye degradation biomass and showed that the remediation of the dye using C.vulgaris.

2.2 Previous studies on the Biomass-Adsorption of MB dye using biomass

(Afroze et al., 2015) attempted to investigate the locally raw eucalyptus bark biomass for removing the MB from aqueous solution. The consequences of the various parameters like absorbent loading, concentration, temperature etc on to the MB dye adsorption containing solution were studied. The experimental investigation showed that the MB dye adsorption extent was increased with the MB dye concentration increment. The kinetic and isotherm studies depicted that an adsorption regarding MB dye adsorption using biomass was followed the second order type kinetics & Langmuir isotherm for adsorption. Hence, finally, they concluded that the Biomass of eucalyptus bark as better and novel adsorbent, can be better alternative to the use of adsorbent prepared from activated carbon For a MB dye removal.

(Safa & Bhatti, 2011) investigated the acid treated and CMC immobilised rice husk biomass regarding the adsorption of the direct blue & Ever direct orange dye. The optimum biosorption capacity acid treated and immobilized biomass was found to be 29.98 mg/g & 37.92 mg for Ever direct orange dye and direct blue-67 dye respectively. The Langmuir isotherm models are best fitted to the both dye adsorption. Not only this, they concluded that the Elvoch and second order models described the adsorption mechanism of a dyes by using the rick husk biomass.

(Zuorro et al., 2013) studied the low-cost absorbent produced from the Spent tea leaves for adsorption of the two azo dyes. The reactive violet and Reactive green dyes were used for this study. Primary experiments showed that the untreated spent tea leave biomass has very low dye removal efficiencies. By the thermal activation, SRL to 300 °C in a 1 hour enhance the dye removal efficiencies to 98.8% & 72.8% for the Reactive green and reactive violet dye respectively. They claimed that this was due to the chemical and physical changes occurred in the cellulosic materials. Hence, they concluded that STL can be low-cost alternative to the conventional absorbents.

(Paşka et al., 2014a) used the biomass of the corn husk as a replacement absorbent for the dye removal. The experimental parameters such as time of contact, temperature and concentration on the MB dye adsorption process were investigated. The experimental results depicted that the amount off the dye absorbed per absorbent decreased with the increment of the doses, temperature, and increased with the concentration and contact time. The experimental data are modelled by the pseudo first order, second order and Elovich kinetic models. The kinetic of the MB dye was best described by the models of Pseudo type second order kinetic. The results obtained from the MB adsorption experiments showed that the corn husk is the low-cost materials for the removing of the MB dye through an artificial solution.

(Garg et al., 2004) conducted the experiment to determine possible used of the Indian Rosewood saw dust for the MB dye removal from the waste water. They considered the several parameters like contact time, pH, Initial concentration of the dye, absorbent dosage while performing experiment. The experiment results depicted that the when the absorbent amount increased, the dye removal percentage was also increased accordingly. The MB adsorption by the MB dye followed the first order equation and suited to the lagergren equation. Hence the acid treated and formaldehyde treated Indian Rosewood dust can be suitable for removing of the dye from waste water.

2.3 Research Gaps

After reviewing the various research regarding the phytoremediation study of the different types of the dyes (Cationic and anionic dye), it was found that the algae, ornament plant, aquatic plant has highly involved in the dye removal. The phytoremediation study using dyes such as MB dye, rhodamine dye, methyl violet and Cango red dye are studied in various research articles. However, comparative study regarding the plant response toward the cationic and anionic dye are not seen in many research articles. The phytoremediation process involves the several approaches such as phytodegradation, phyto-violization, phyto-extraction and rhizoremediation. The state of the phytoremediation process of azolla pinnata is not explained in several research works. In addition to this, there are several researches have been found using biomass of Azolla filiculoide for removing of an Acid Blue 225, Acid black, Acid Green, reactive black 5 and acid dyes. The adsorption of various kind of the dye in the

research papers only focuses on using the biomass of Azolla filiculoides. Beside this, very limited research articles focus on the adsorption MB dye using biomass of the azolla pinnata. Not only this, the adsorption parameters, kinematics and adsorption mechanism under the various process conditions are not focused using biomass azolla pinnata. Hence, this study focuses on the phytoremediation and biomass adsorption of the MB dye and MO dye utilizing of an azolla pinnata available locally.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Materials Required

- Cationic dye (MB)
- Anionic dye (MO)
- Modified Hoagland solution
- Distilled water
- PH tablet

3.2 Apparatus Required

- Beaker
- Test tubes
- Plastic box
- Measuring Cylinder
- Weighing Balance
- Stir rod
- PH meter
- UV-VIS spectrometer
- Optical microscope

3.3 Methods of Phytoremediation Study

3.3.1 Methods and layout planning Phytoremediation Study

3.3.1.1 Methods

The wet azolla pinnata was collected from the farmer's ponds whom are cultivating it for feeding purpose for chicken and domestic animals. The absorption experiment was carried out in the at the laboratory condition of 25±4℃. The 48 plastic containers having diameter of 8 cm and capacity of 500 ml was used for the phytoremediation study of the dye using cationic and anionic dye such as MB dye and MO dye. The initial weight of the azolla was varied by 2gm, 3gm and 5gm respectively for both type of the dye experiment. The stock 1000 ppm solution was prepared by using the MB dye and MO dye powder of 1 gm in 1000 ml of the volumetric flask. Then after, 10, 20 and 30 mg/l of the dye solution was prepared. The 100ml of the dye solution was transferred to each bottle and azolla pinnata was transferred in each by weight of 2gm, 3gm and 5

gm respectively. Then, the azolla with dye solution was weighted in order to determine the evaporation losses during the experiment period. The azolla is harvested in the period of 1,3,5 and 7th day and Aqueous solution was filtered and filtered dye solution was utilized for the quantitation of dye in the solution using UV-Vis spectrophotometer.

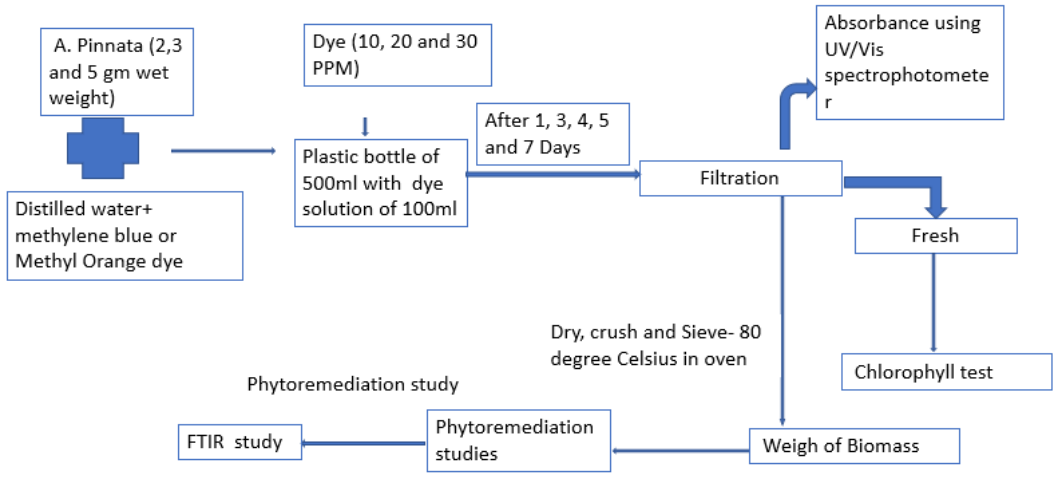

Figure 3.1: Methodology for phytoremediation examination

3.3.1.2 Layout planning

The layout of the MB and MO dye phytoremediation experiment adopted is shown in the figure 3.2 and 3.3 respectively. The bucket of the control samples for each day and plant doses were independent to each other. The evaluation was done independently. The quantitation of the dye in the aqueous solution was done in each day.

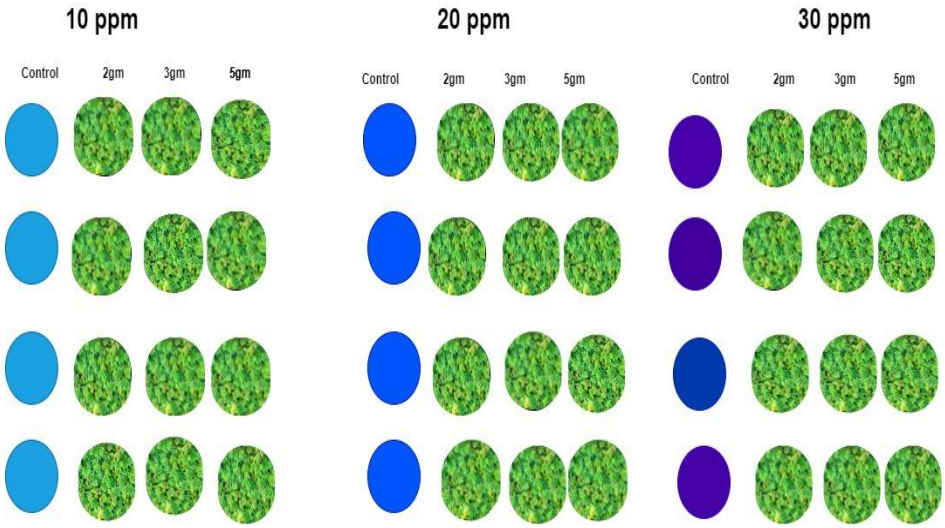

Figure 3.2: Layout of Phytoremediation of MB dye using azolla pinnata

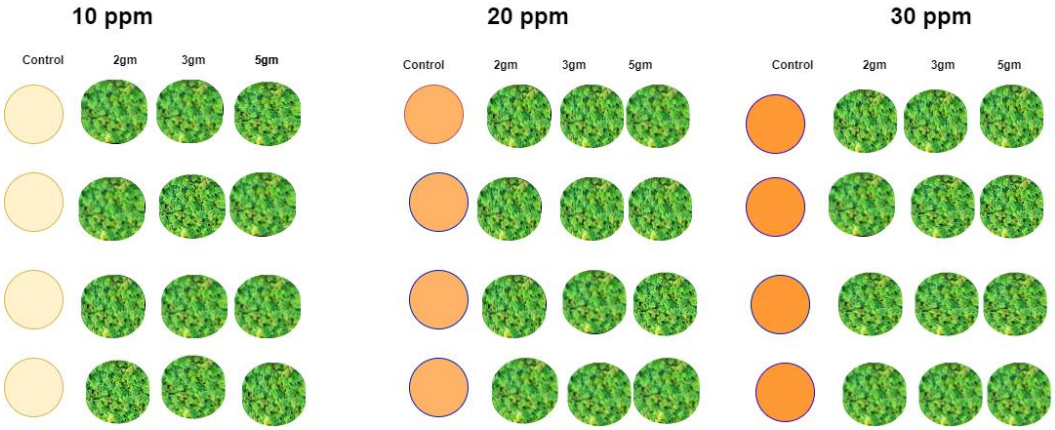

Figure 3.3: Layout of Phytoremediation of MO dye using azolla pinnata

3.4 Methods of Biomass-adsorption Study

3.4.1 Preparation of Adsorbent for Biomass-adsorption Study

Azolla pinnata was collected from the nearest agriculture farm located in Balaju, Kathmandu district in the month of late July 2022 as they cultivated it for feed to the chicken. The collected samples were washed using the deionized water till the impurities were clearly removed. The washed azolla pinnata were dried in the oven using temperature of 75-80 ℃. Then after, the dried sample was grounded to the fine powder using the mortar and pestle. Finally, sample were stored in the plastic sample bottle for the adsorption experiment

3.4.2 Adsorption experiments

The adsorption experiments are performed by altering the absorbent doses, dye concentration and time. The mixture was shaken in laboratory temperature condition 30±2 ℃. The dye of 25 ml having concentration of 50, 100, 200, 300, 500 and 600mg/l were filled in the Erlenmeyer flask. The doses of the absorbent were taken as 25 mg (1gm/l). Then shaking was done by using the 200 RPM in the rotatory shaker for the time of 180 minutes. The solutions with absorbent were filtered utilizing Wattman filter paper in order to separate the absorbent form the solution. Then after, the quantification of the residual dye solution was done by UV-Vis spectrophotometer and calibration curve. Similarly, the effect of the absorbent doses was determined by varying the doses from 10 mg to 60 mg in the dye volume of 25ml and 300 mg/l concentration

3.5 Testing and Evaluation

3.5.1 Testing

3.5.1.1 Dry mass

The dry mass of the azolla was determined by using oven drying method. In this method, the initial weight of the azolla (W_1) is measured and then the azolla plant was dried in the oven around 70-80 degree Celsius(Al-Baldawi et al., 2018). Then, weighted as(W_1) using the measuring balance.

3.5.1.2 Chlorophyll Estimation

The chlorophyll was estimated by weighting the 150 mg of the fresh azolla plant and making paste by using 80% acetone. The filtration was done using Whatman filter paper and made the solution to 25 ml. Then after, the absorbance was measured using the wavelength of 470 nm, 645 nm and 663 nm respectively. After that, the quantification of the chlorophyll was done by using following equations(Kooh, Lim, et al., 2016):

$$Chl_a(mg\ g^{-1}) = [(12.7 \times A_{663}) - (2.6 \times A_{645})] \times \frac{V}{M} \quad \text{Eq } 3.1$$

$$Chl_b(mg\ g^{-1}) = [(22.9 \times A_{645}) - (4.68 \times A_{645})] \times \frac{V}{M} \quad \text{Eq } 3.2$$

$$C_x(mg\ g^{-1}) = \left[\left(1000 \times A_{470} \times \frac{V}{M}\right) - (1.90 \times Chl_a) - 63.14 \times Chl_b\right] \times \frac{1}{214} \quad \text{Eq. } 3.3$$

3.5.1.3 Dye quantification

The quantification of the dye in the aqueous solution was done utilizing the UV-Vis-NIR spectrophotometer. The calibration was done by using the solution of 1-10 mg/l. The absorbance of MB dye and MO dye were determined by using the λ_{max}= 664nm and λ_{max}= 464 nm, respectively. The absorbance was determined for each solution. Then, the calibration curve was determined in term of concentration and absorbance. After that, the initial concentration absorbance value of samples and control solutions were determined. The absorbance of all samples was measured in the respective days of azolla harvesting.

3.5.1.4 FT-IR analysis

FT-IR analysis characterization to find the functional groups present in the azolla pinnata that are responsible for the dye adsorption. The FTIR analysis of samples was performed using the Perkin Elmer Spectrum IR. The dry mass of azolla that were used

for phytoremediation in the MB dye and MO dye of different concentration 10 mg/l, 20 mg/land 30 mg/l were used for the testing purpose. For the comparative purpose, the azolla sample that were exposed in the distilled water was utilized. In addition to this, the FT-IR analysis of the MB dye and MO dye was also done. FTIR analysis helps to determine the phytoremediation behaviour of the plant by comparing the functional groups present in the dye samples, sample of biomass in distilled water and sample of biomass in various dye concentrations.

3.5.2 Evaluation

3.5.2.1 Dye removal percentage

The dye removal efficiency was computed by using the formula given below:

$$\text{Dye removal efficiency}(\%) = \frac{(C_o - C_f)}{C_o} \times 100\% \qquad \text{Eq 3.4}$$

Were,

C_o = Initial Concentration

C_f = Final Concentration

Or,

$$\text{Dye removal efficiency}(\%) = \frac{(A_0 - A_f)}{A_0} \times 100\% \qquad \text{Eq 3.5}$$

were

A_0= Initial absorbance

A_f = Final absorbance

The dye removed quantity by the azolla pinnata was determined with help of equations below:

$$q_t = \frac{C_o - C_t}{W} \times v \qquad \text{Eq 3.6}$$

$$q_e = \frac{C_o - C_e}{W} \times v \qquad \text{Eq 3.7}$$

Where,

q_t (mg/g) and q_e (mg/g) are the dye removed amount with the respect to time t (hours or minutes) and equilibrium stages respectively

C_0 is the concentration at initial stage,

C_t is the concentration with the respect to 't'

$C_e$is the concentration of dye at equilibrium in mg /L

v is the volume (v)and

W is the plant weight in g

3.5.2.2 Plant's Relative Growth Rate (PRGR)

To understand the dye toxicity on the azolla pinnata, the plant relative growth rate was calculated. The plant growth rate value explained the dye impact on the plant growth after the dye solution exposure.

$$\mathrm{PRGR} = (\ln \mathrm{Wt} - \ln(\mathrm{Wo}))/\mathrm{t} \quad \text{Eq 3.8}$$

Where, the Wt and Wo are the wet weight of the plant taken after and before treatment of the azolla after exposure of the dye solution.

3.5.2.3 Kinetic Models

The kinetic studies of the phytoremediation of the azolla pinnata were performed with the different concentration of MB and MO dye. The kinetics of the azolla pinnata for the phytoremediation of the MB and MO dye was done by utilizing the first order, and Pseudo second order kinetics as per the following equation 3.9 and equation 3.10 (Imron et al., 2021)

$$Log\ (q_e - q_t) = log q_e - \frac{k_1}{2.303} t \quad \text{Eq 3.9}$$

$$\frac{t}{q_t} = \frac{1}{k_2 {q_e}^2} + \frac{1}{q_e} t \quad \text{Eq 3.10}$$

where k_1 and $k_2$are the rate constants of pseudo-first-order and pseudo-second-order with units1/hr and mg/g/hr, respectively

3.5.2.4 Isotherm Models

The adsorption Isotherm are expressed in two models such as Langmuir and Freundlich. These models are selected in order to explicate the dye interaction with biomass and also evaluate the adsorbent capacity. The Langmuir isotherm signifies a monolayer adsorption mechanism for a absorbent surface having similar energy of the adsorption, no interaction has been in the adsorbed molecules. Not only this, they have the homogenous binding sides. Beside this, the Freundlich isotherm signifies the multilayer adsorption on the heterogenous surfaces(Kaushal & Mahajan, 2022).

The linear equation of the Freundlich isotherm and Langmuir isotherm are expressed as in equation 3.11 and equation 3.12

$$Log\ (q_e) = logK_f + \frac{1}{n} logC_e \qquad \text{Eq 3.11}$$

$$\frac{C_e}{q_e} = \frac{1}{k_a q_m} + \frac{1}{q_m} \qquad \text{Eq 3.12}$$

Where K_f and K_a are the isotherm constant for Freundlich and Langmuir isotherm.

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Results of Phytoremediation study of the Cationic dye (MB dye)

In the experiment, the azolla pinnata has good probability for removing the MB dye in the solution with in 1 day of the experiment. The equilibrium is obtained after 24 hours of the experiments. As per these studies, the further studies were performed in order to understand the effect of concentration, contact time, and wet weight of the azolla pinnata on the dye concentration removal in the aqueous solution.

4.1.1 Effect of contact time, initial concentration and plant weight for Cationic dye

The impact of a contact time and amount of the azolla on the different dye concentration is studied. The dye concentration is reduced to less than 2.6 mg/l from all dye concentration after 24 hours of the dye exposure as shown in figure 4.1. The 3-gram wet weight of the azolla was sufficient for the removal of the dye up to 30 mg/l of the dye concentration. However, when considering the hourly removal of the dye using the azolla of different wet weight, the dye concentration of 30 mg/l was reduced to 5.16 mg/l by azolla of 2gm, around 3.11 mg/l by 3gram and 1.86 mg/l by 5 gram respectively after exposure of 8 hours as in figure 4.1- 4.2. Hence, the equilibrium is reached above 24 hours for the 30 mg/l and 20 mg/l in all absorbent doses of the azolla respectively.

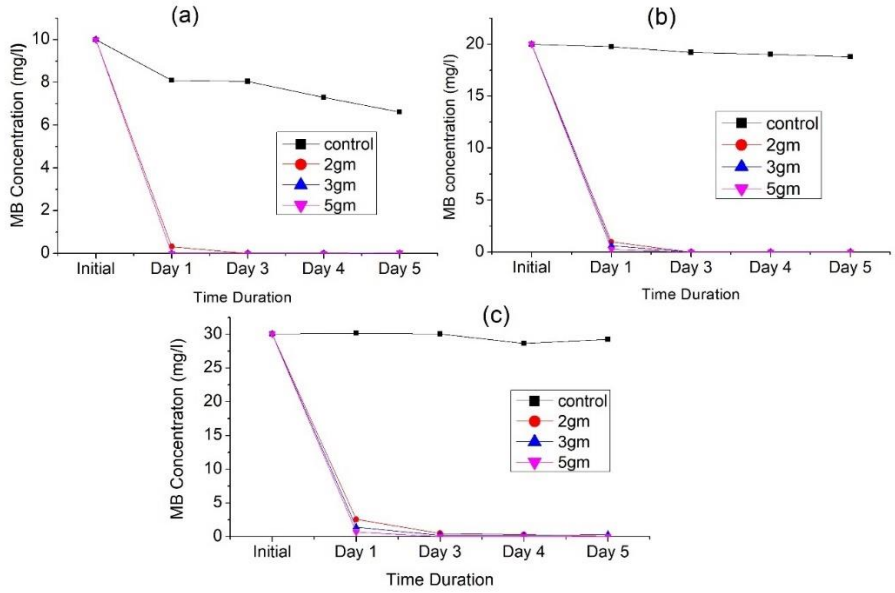

Figure 4.1: Variation of MB dye concentration after exposure of azolla pinnata of different doses (2, 3,5 gm) in dye of (a) 10 mg/l, (b) 20 (mg/l) and (c) 30 mg/l

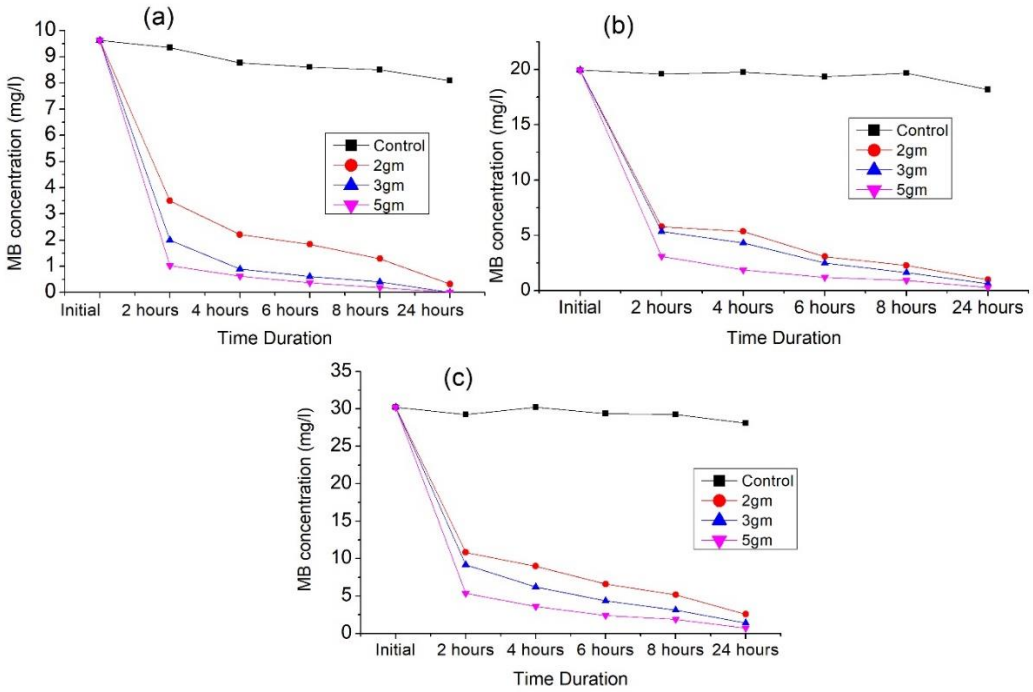

Figure 4.2: Hourly variation of MB dye concentration by azolla pinnata in aqueous solution of (a) 10 mg/l, (b) 20 mg/l dye and (c) 30 mg/l dye

4.1.2 Cationic dye removal efficiencies

The removal percentage of the dye (figure 4.3) was higher when exposed to the plant at 4 hours. It removes about 70.31% and 79.42% and 88.0968% of MB dye by 2gm, 3gm and 5 gm respectively when exposed to 30 mg/l of the dye. The removal efficiency of azolla in 30 mg/l dye were 91.513%, 95.375% and 97.6851% by the use of 2, 3 and 5 gram wet weight of azolla pinnata after 24 hours.

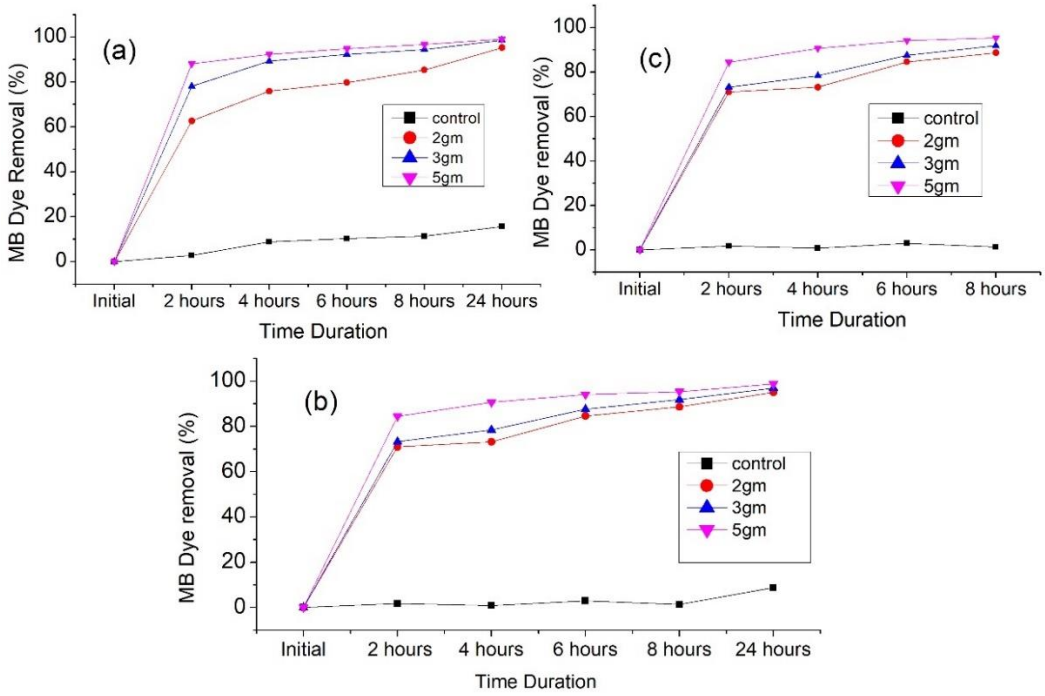

Figure 4.3: MB dye removal percentage for (a) 10 mg/l, (b) 20 mg/l and 30 mg/l under the varying azolla doses with the respect to time

4.1.3 Isotherm and Kinetic studies for MB dye removal using Azolla Pinnata

4.1.3.1 Isotherm studies for the MB dye removal using Azolla Pinnata

The outcomes of the adsorption isotherm of the MB dye by using the Azolla Pinnata are suggested in the figure 4.4 and figure 4.5 as Langmuir isotherm and Freundlich isotherm. Figure 4.4, figure 4.5, Table 4.1 and Table 4.2 depicts that the value of the R-squared was closed to 1 for both isotherms. The value of R^2 is 0.99289 and 0.99539 for Freundlich isotherm and 0.96852 and 0.97276 for Langmuir isotherm with the use of azolla wet weight as 3 gram and 5 grams respectively. This suggested that the adsorption isotherm of MB dye is fitted Freundlich isotherm. Hence, the adsorption process of the MB dye by azolla pinnata follows the multilayer adsorption on the heterogenous plant surface and roots. As per the, parameters of Langmuir isotherm in Table 4.1, Q_m and K_a values for 3 gram and 5 grams are 1.047998 mg/g and 1.08711 l/mg and 0.62385 mg/g and 2.217358 1/mg respectively. In addition to this, The table 4.2 shows that the value of $(1/n) \leq 0.5$ for 3 gram and 5 grams. This describes that the MB remediation by the azolla was faster and efficient.

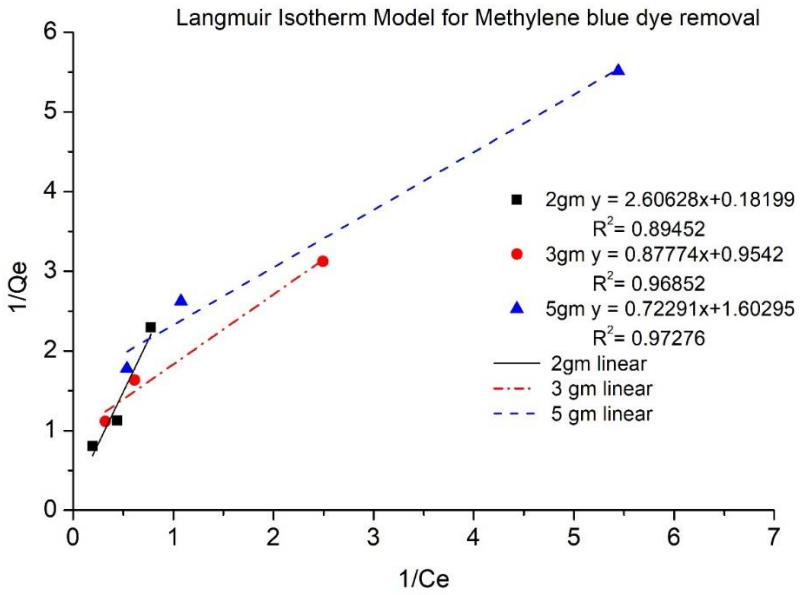

Figure 4.4: Langmuir isotherm for the MB dye removal

Table 4.1:Parameters for Langmuir isotherm for the MB dye removal

Doses	**K_a (L/mg)**	**Q_m (mg/g)**	**R^2**
2gm	0.069827	5.494807	0.89452
3gm	1.08711	1.047998	0.96852
5gm	2.217358	0.62385	0.97276

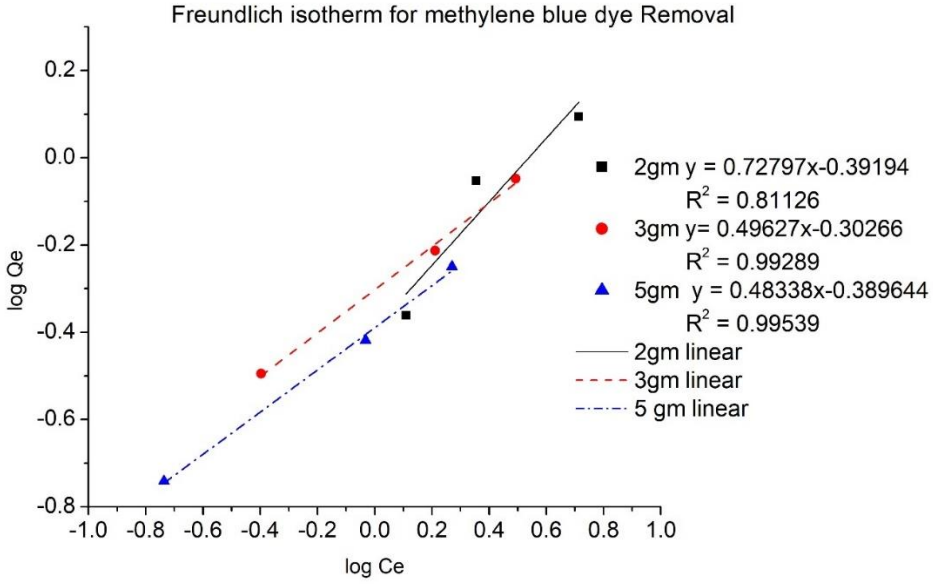

Figure 4.5: Freundlich Isotherm for the MB dye removal

Table 4.2: Parameters for Freundlich Isotherm for the MB dye removal

Doses	**K_f**	**n**	**R^2**
2gm	0.405565	1.373683	0.81126
3gm	0.498127	2.015032	0.99289
5gm	0.407718	2.068766	0.99539

4.1.3.2 Kinetic studies for MB dye removal using Azolla Pinnata

From the table 4.3 and table 4.4, it is found that the kinetic of the dye removal by the azolla pinnata found to be fitted well with the pseudo second order model as (R^2 greater or equal to 0.999 for all plant weight 2 gm, 3 gm and 5 grams respectively and also for 10 mg/l, 20 and 30 mg/l dye concentration. It shows that the of Azolla Pinnata for the removal process of MB dye at various plant mass signifies the chemisorption(Imron et al., 2021; Saber et al., 2018). As comparing the table 4.3 and 4.4, it is suggested that the K_2 value of the 5 grams showed the highest value of 17.68601 mg/g/hour, 6.162374 mg/g/hour and 3.474382mg/g/hour for 10 mg/l, 20 mg/l and 30 mg/l respectively. This indicate that, the MB dye absorption is faster when doses of the azolla pinnata is increased. (Tang et al., 2017). Beside this, the rate of reaction k_2 decreased with the increasing the dye initial concentration. Not only this, the higher value of initial dye concentration, the higher the Q_e values are obtained. This is because of an availability of the contact in between the MB molecules & surface of the plant(Khataee et al., 2012).

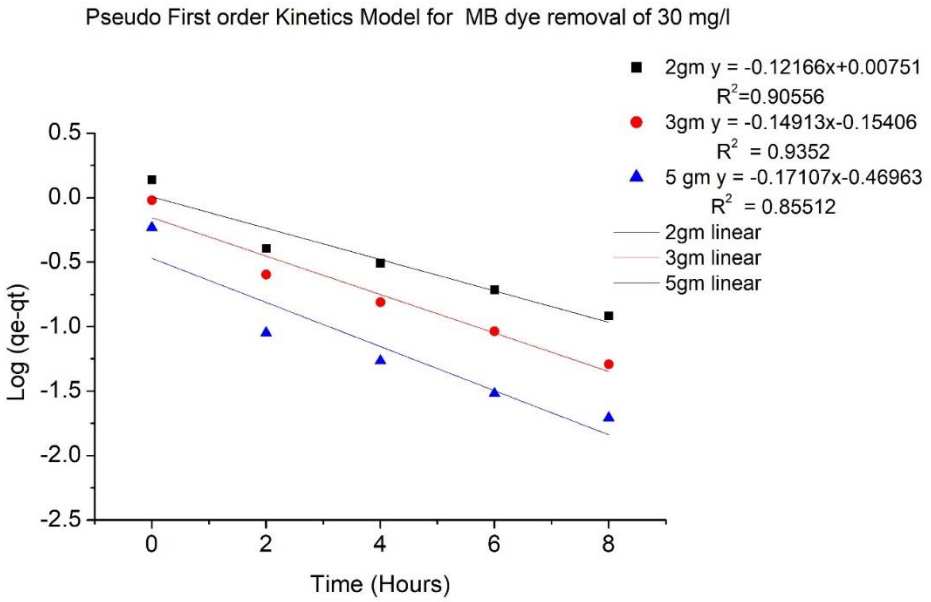

Figure 4.6: Pseudo first order kinetic model for MB dye removal of 30 mg/l

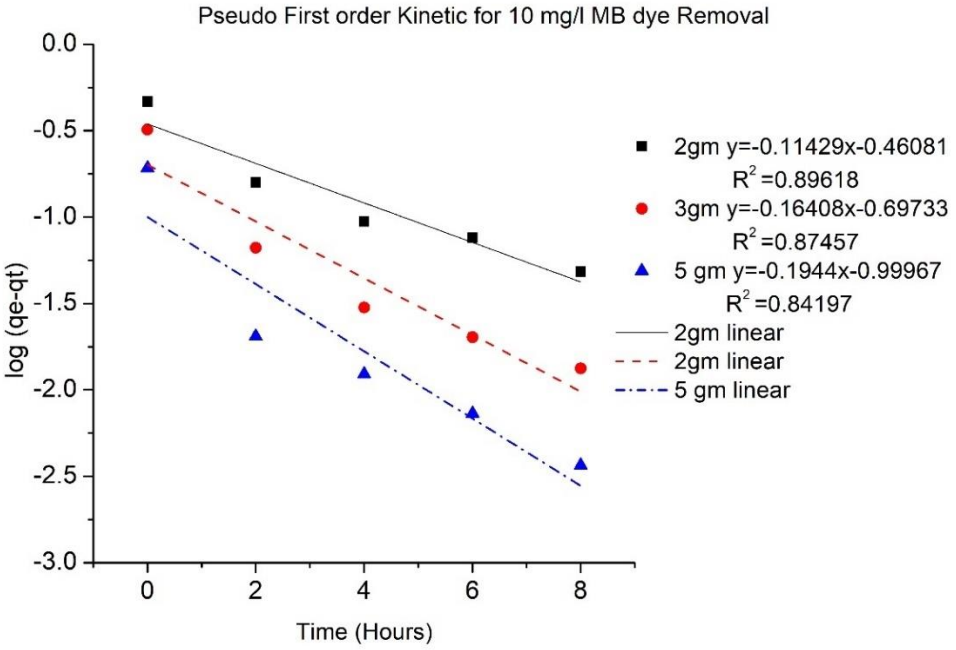

Figure 4.7: Pseudo first order kinetic model for MB dye removal of 10 mg/l

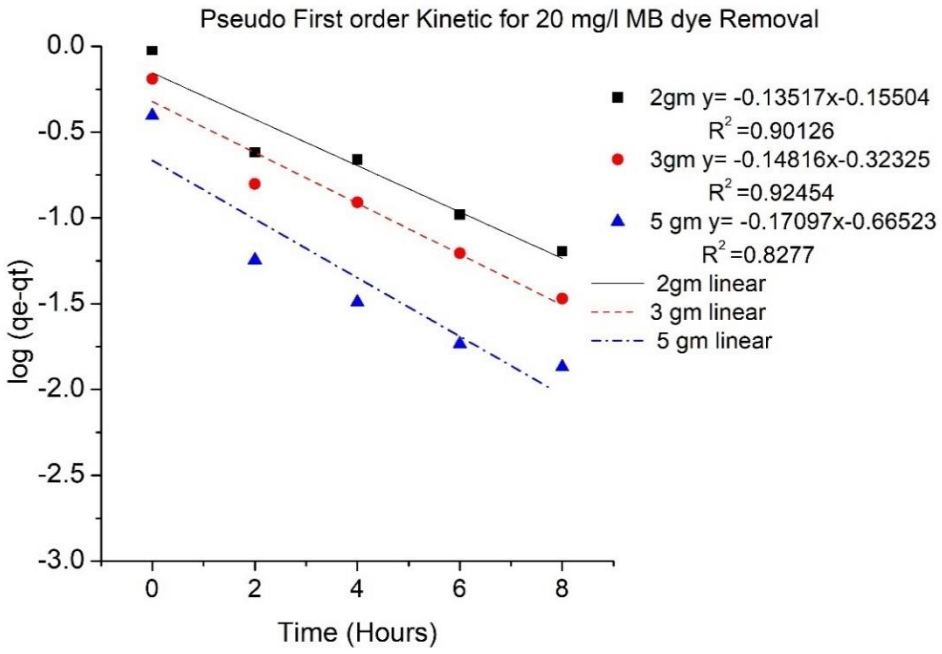

Figure 4.8: Pseudo first order kinetic model for MB dye removal of 20 mg/l

Table 4.3: Parameter of Pseudo first order for the MB dye removal

Dye Concentration	**Doses**	**Qe (mg/g)**	**K_1 (/hour)**	**R^2**
10	2gm	0.346091	0.26321	0.89618
	3gm	0.200757	0.377876	0.87457
	5gm	0.100076	0.447703	0.84197
20	2gm	0.699778	0.311297	0.90126
	3gm	0.475062	0.341212	0.92454
	5gm	0.216157	0.393744	0.8277
30	2gm	1.017443	0.280183	0.90556
	3gm	0.701358	0.343446	0.9352
	5gm	0.339133	0.393974	0.85512

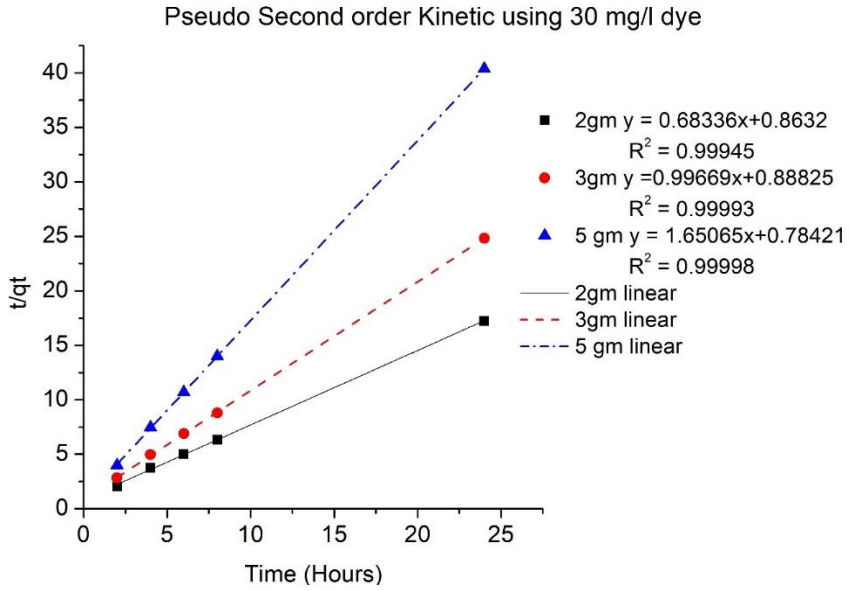

Figure 4.9: Pseudo Second order kinetic model for MB dye removal of 30 mg/l

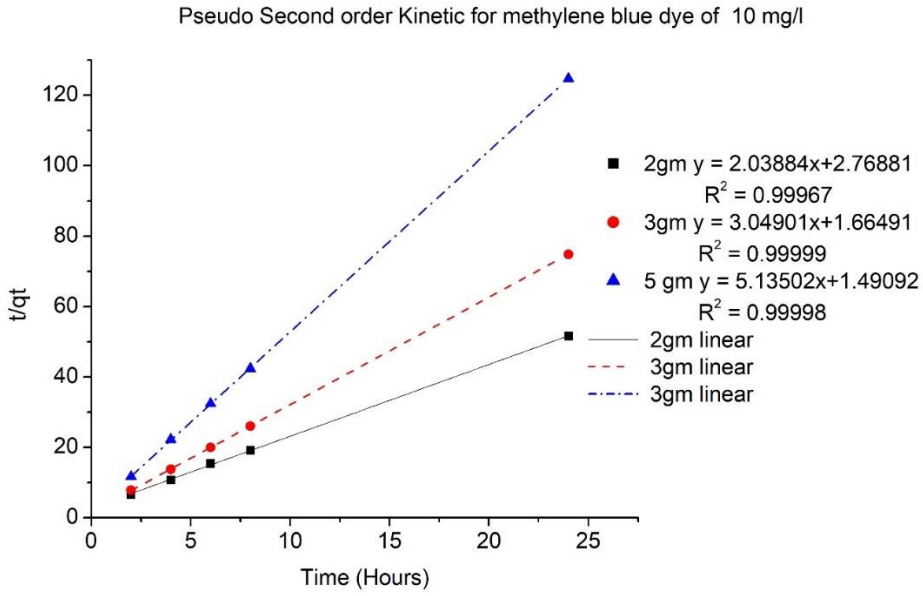

Figure 4.10: Pseudo Second order kinetic model for MB dye removal of 10 mg/l

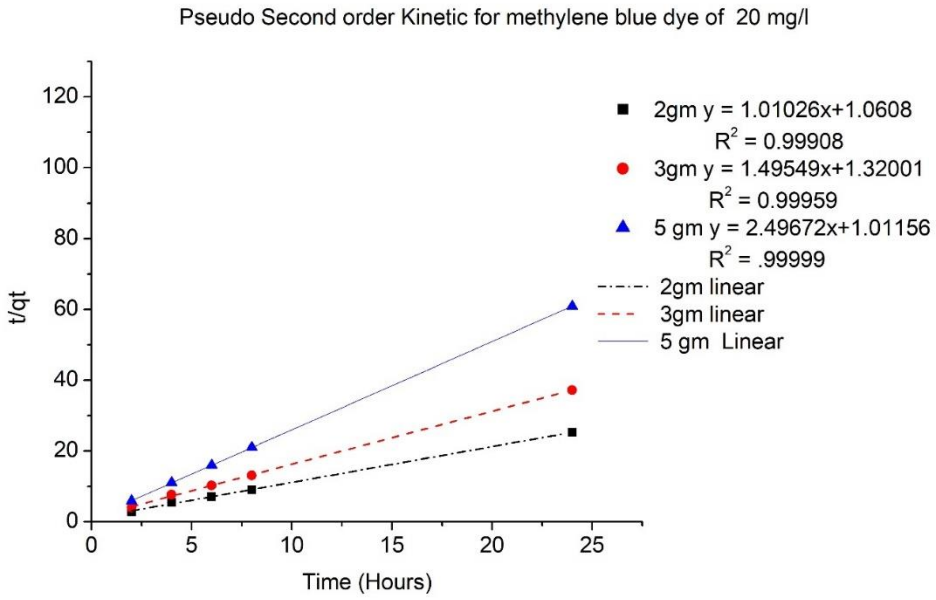

Figure 4.11: Pseudo Second order kinetic model for MB dye removal of 20 mg/l

Table 4.4: Parameters of the Pseudo Second order model for the MB dye removal

Dye Concentration	**Doses**	**Q_e (mg/g)**	**K_2 (mg/g/h)**	**R^2**
10	2gm	0.490475	1.50132	0.99967
	3gm	0.327975	5.583762	0.99999
	5gm	0.194741	17.68601	0.99998
20	2gm	0.989844	0.962128	0.99908
	3gm	0.668677	1.694298	0.99959
	5 gm	0.400525	6.162374	0.99999
30	2gm	1.463358	0.540988	0.99945
	3gm	1.003321	1.118369	0.99993
	5 gm	0.605822	3.474382	0.99998

4.1.4 Chlorophyll studies

The chlorophyll studies of the fresh azolla were done on the initial azolla, azolla exposed in 10 mg/l, 20 mg/l and 30 mg/l. The Chlorophyll A and Chlorophyll B are determined for each sample. The chlorophyll test was carried out for in the initial azolla samples and samples of each day. Then after the chlorophyll experiment was conducted after transferring the azolla nutrient solution(Li et al., 2020). The results (Figure 4.12)

showed that the Chlorophyll A is increased after exposure of the MB dye because the dye effect of the chlorophyll experiments. This is due to fact that the chlorophyll A is measured by considering the absorbance of wavelength of 663 and 645 nm which is also the wavelength where the MB showed maximum absorbance. The results showed that chlorophyll A and Chlorophyll B increased with the increment of the dye exposure time. This is because of a concentration of the dye on the leaves of the azolla and also simultaneously increment of the green pigment. Then the Chlorophyll A and Chlorophyll B evaluation of the plant exposed to the 7 days nutrient solution also give highest peak of chlorophyll A and Chlorophyll B which also showed that the azolla pinnata absorbs dye and remains in the plant after the exposure of 7 days in dye solution and 7 days in nutrient solutions. Hence, these results conclude that azolla absorbs the MB dye in shorter interval of the time and do not able to degrade it completely until the 15 days growing period.

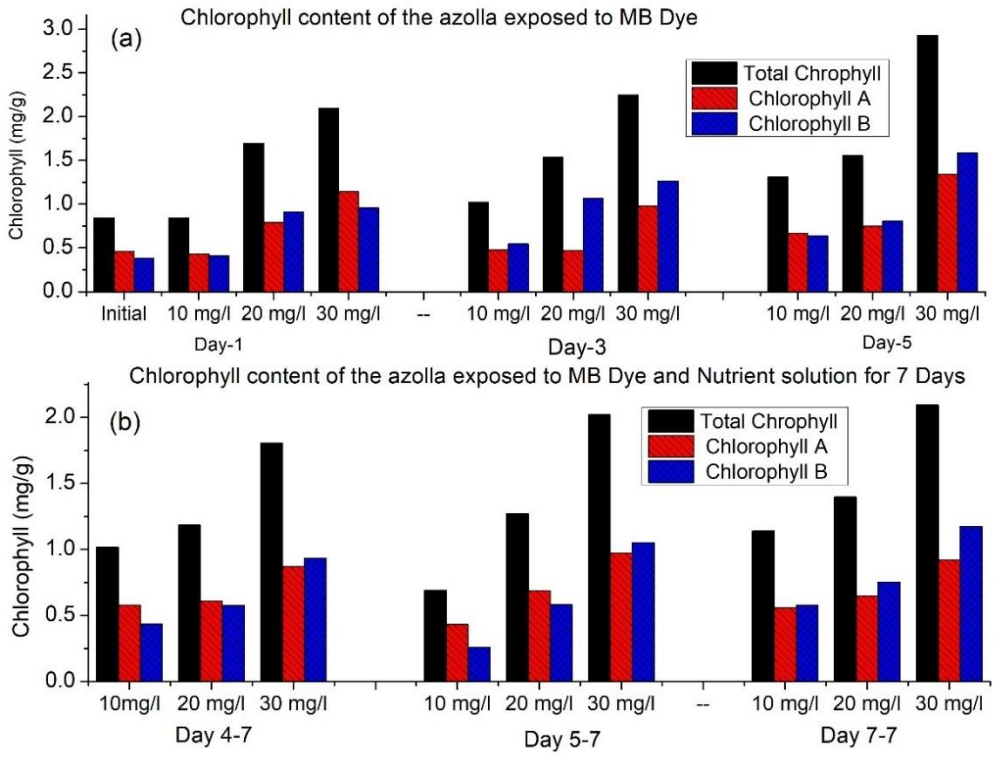

Figure 4.12: (a) Chlorophyll estimation of the fresh plant of the azolla under the different dye exposure and time exposure in the dye solution and (b) transferred to the 7 days nutrient solutions.

4.1.5 Plant's relative growth rate (PRGR) studies

The dry mass study of the azolla showed that the biomass is decreased when concentration of the dye solution is increased. Figure 4.13 shows that it is also decreased with the respect to time. However, the green pigment remains still in the plant. The plant is not died completely for 7 days of the dye exposure. The PRGR value for all dye concentration and plant doses are in the negative value.

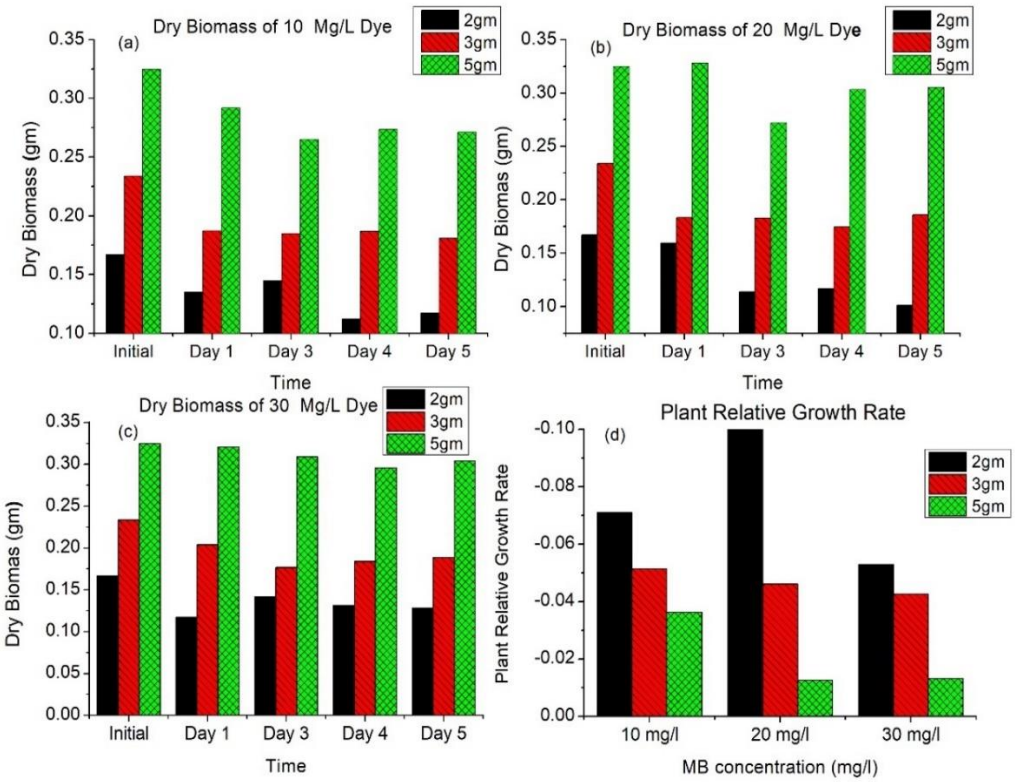

Figure 4.13: Effect of MB Dye concentration of (a) 10 mg/l, (b) 20 mg/l and (c) 30 mg/l on the Biomass of Azolla pinnata and (d) Plant relative growth Rate

4.2 Results of the Phytoremediation study of Anionic Dye (MO Dye)

4.2.1 Effect of initial concentration, Contact time and plant weight for anionic dye

The effect of contact time and amount of the azolla on the different dye concentration of MO dye and removal efficiencies were studied as depicted in a figure 4.14 and figure 4.15. The dye concentration is reduced to 6 -7 mg/l from 10 mg/l concentration after 7 days of the dye exposure. The removal efficiencies of the different weight of the azolla under the 10 mg/l dye concentration are 30, 37% and 31% respectively for 2gm, 3gm and 5gm azolla. The dye concentration is reduced to 12 - 13 mg/l from 20 mg/l

concentration after 7 days of the dye exposure. The removal efficiencies of the different weight of the azolla under the 20 mg/l dye concentration are 32, 35% and 37% respectively for 2gm, 3gm and 5gm azolla. In Addition to this, the dye concentration is reduced to 22-24 mg/l from 30 mg/l concentration after 7 days of the dye exposure. The removal efficiencies of the different weight of the azolla under the 30-mg/l dye concentration are 19%, 18% and 23% respectively for 2gm, 3gm and 5gm of azolla.

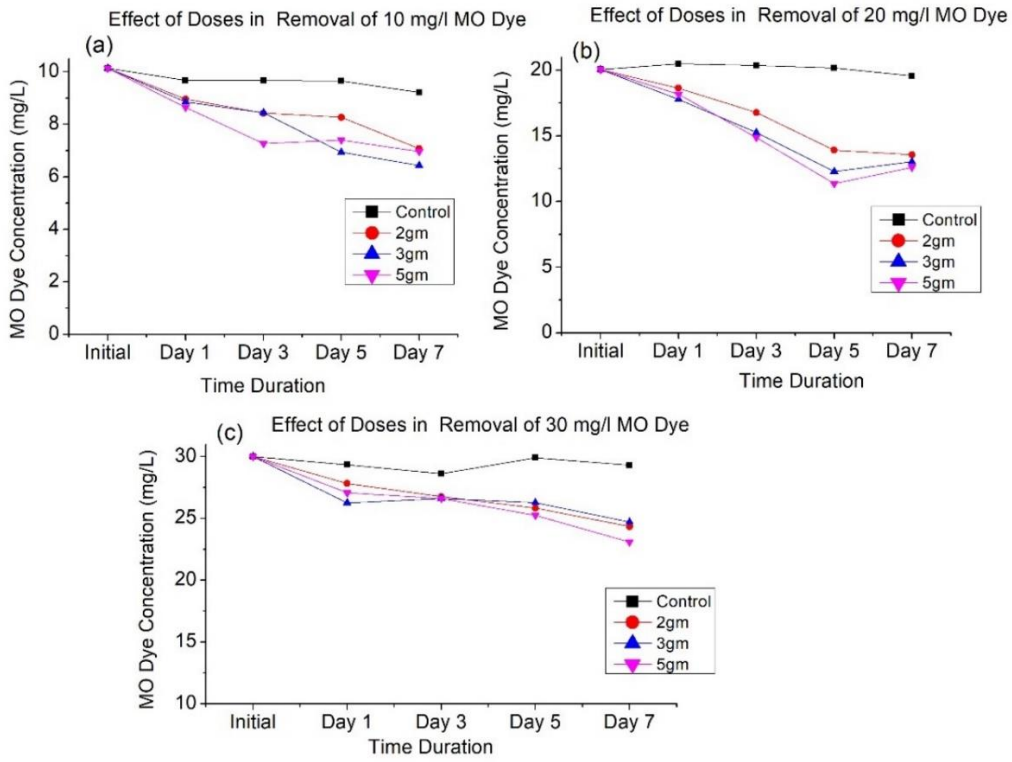

Figure 4.14: Concentration variation under the Different concentration (a) 10 mg/l, (b) 20 mg/l and (c) 30 mg/l of MO aqueous solution using azolla of different doses (2gm, 3gm and 5 gm)

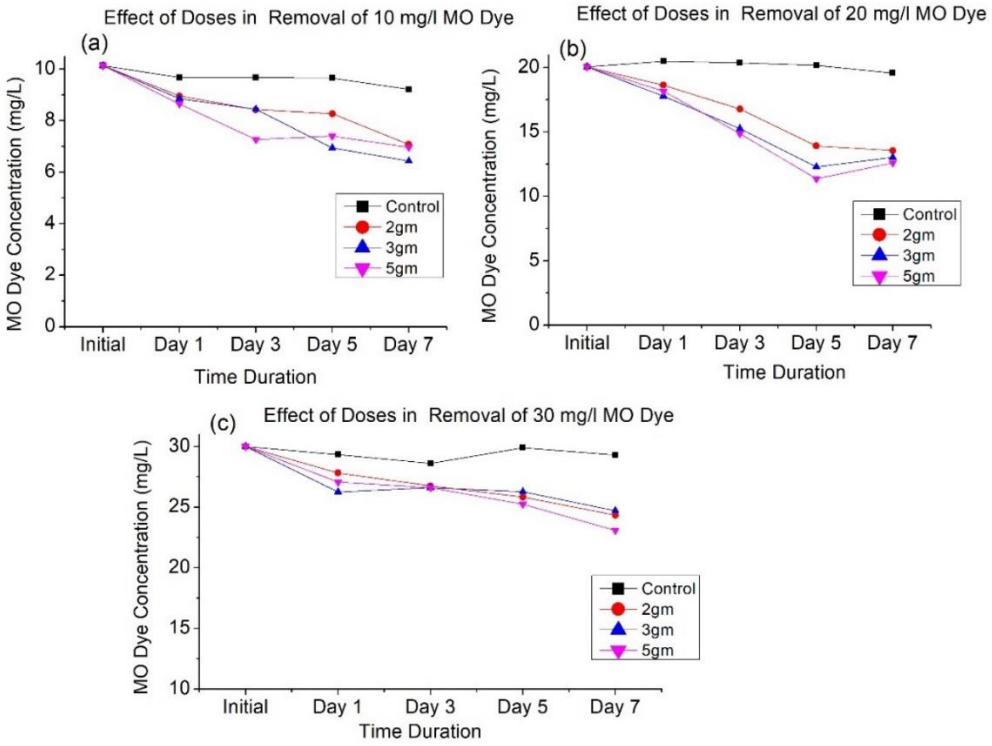

Figure 4.15: Dye removal percentage of a) 10 mg/l, (b) 20 mg/l and (c) 30 mg/l MO dye in aqueous solution using azolla of different doses (2gm, 3gm and 5 gm).

4.2.2 Isotherm and Kinetic studies for MO dye removal using Azolla Pinnata

4.2.2.1 Isotherm studies for the MO dye removal using Azolla Pinnata

The outcomes of the adsorption isotherm of the MO dye by using the Azolla Pinnata are suggested in the figure 4.16 and figure 4.17 as Langmuir isotherm and Freundlich isotherm. Figure 4.16, figure 4,17, Table 4.5 and Table 4.6 depicts that the value of the $R^2 < 0.70$ for 2 gm, 3gm and 5 gm respectively for both isotherms. Hence it is suggested that the Langmuir isotherm and Freundlich models are not fitted for describing the MO dye absorption process.

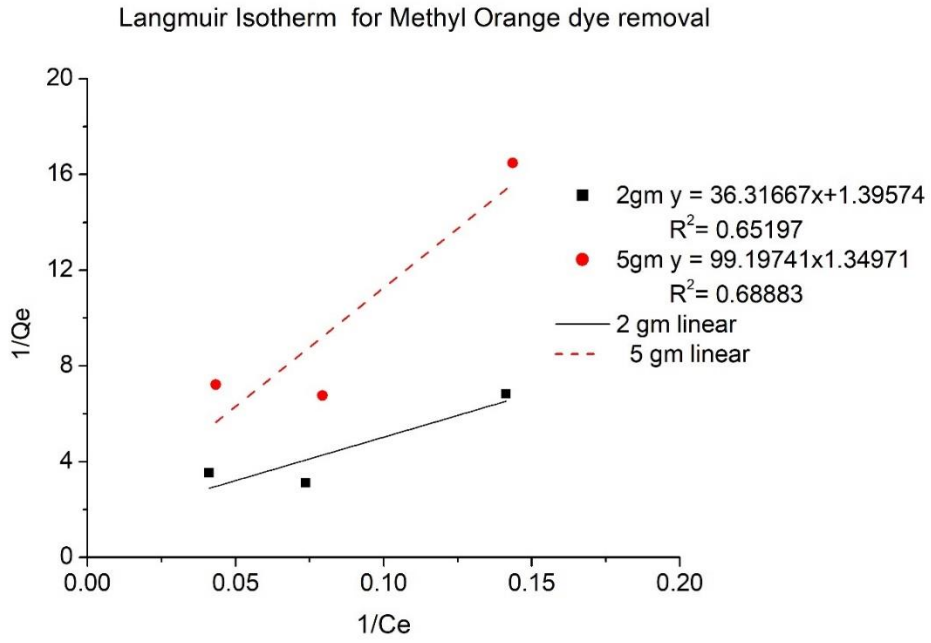

Figure 4.16: Langmuir isotherm for the MO dye removal

Table 4.5: Parameter of the Langmuir isotherm model for the MO dye removal

Doses	**K_a (1/mg)**	**Q_m (mg/g)**	**R^2**
2gm	0.038432	0.716466	0.65197
5gm	0.013606	0.7409	0.68883

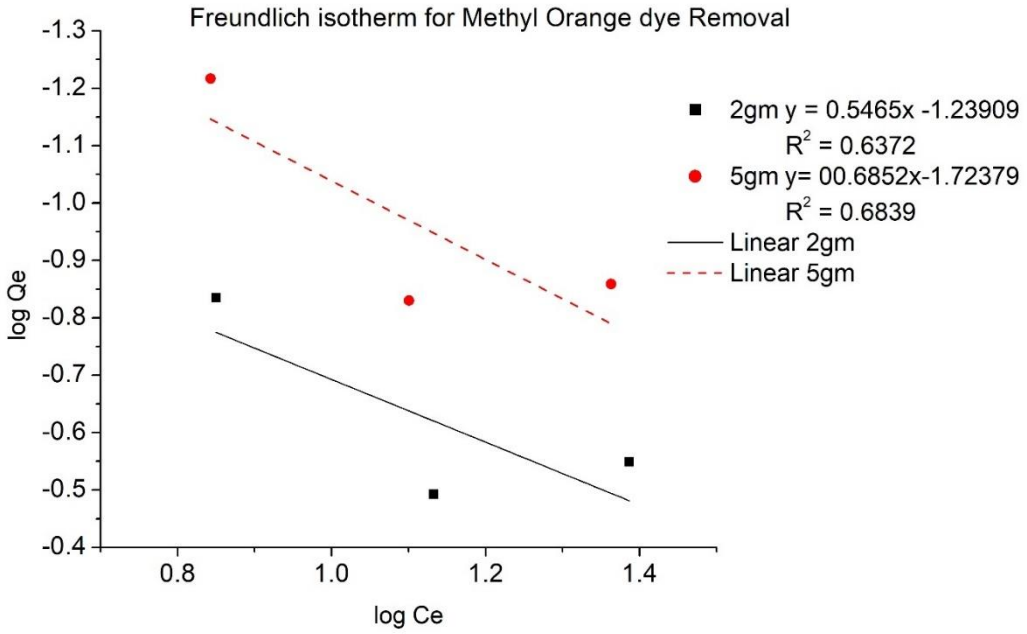

Figure 4.17: Freundlich isotherm for the MO dye removal

Table 4.6: Parameters of the Freundlich isotherm for the MO dye removal

Doses	**K_f**	**n**	**R^2**
2gm	0.057665	1.829826	0.6372
5gm	0.018889	1.459428	0.6839

4.2.2.2 Kinetic studies for MO dye removal

From the table 4.7 and table 4.8, it is found that the kinetic of the dye removal by the azolla pinnata found to be best described by a first order kinetic model as R^2 around and greater than 0.80 for 2gm and 3gm and also for 10 mg/l and 30 mg/l dye concentration. As comparing the table 4.7 and 4.8, it is suggested that the K_1 value of the 2 and 3 grams for 30 mg/l and 10 mg/l shows the highest value of 0.010502 1/hr and 0.015154 1/hr respectively.

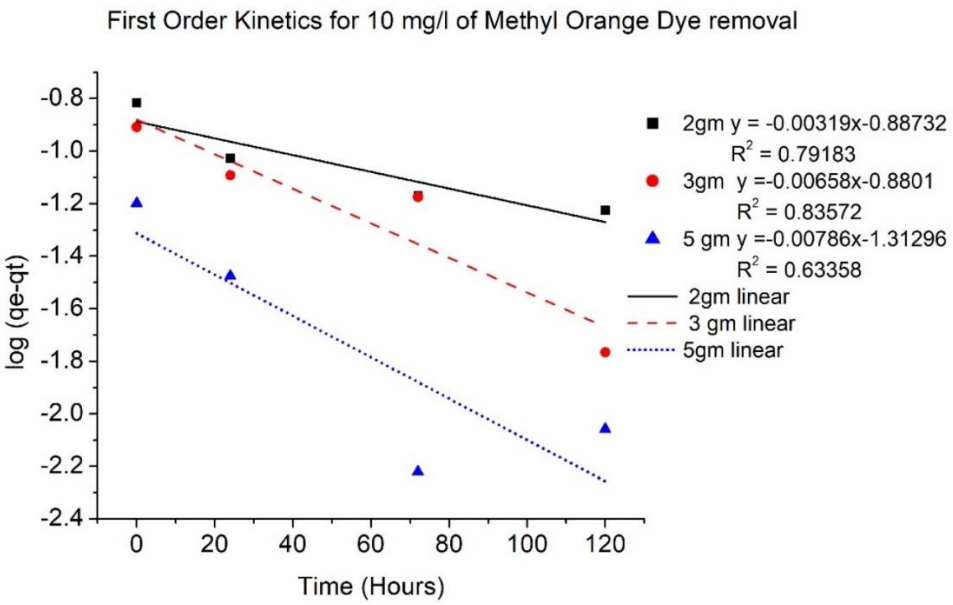

Figure 4.18: Pseudo first order kinetic model for the MO dye removal of 10 mg/l

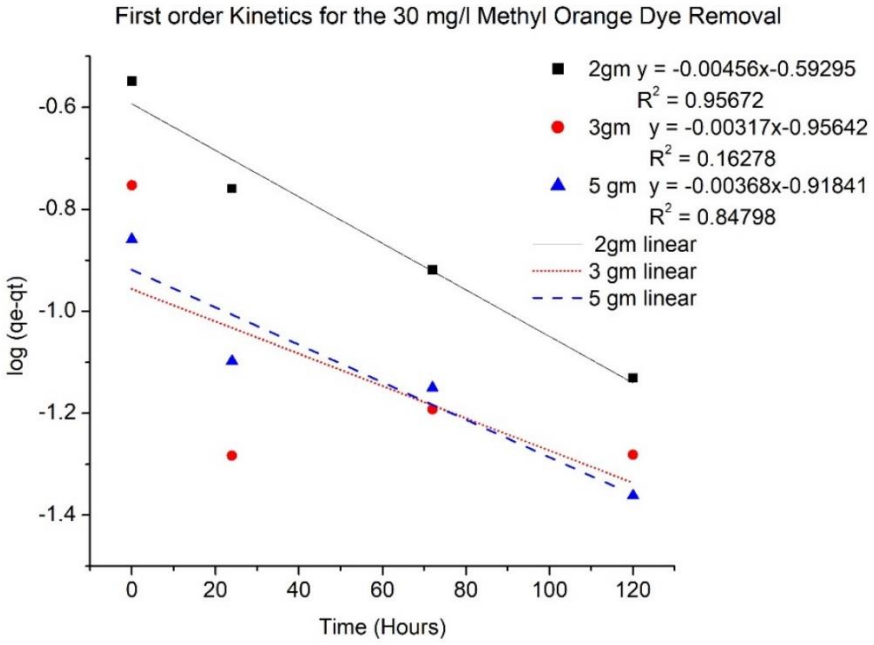

Figure 4.19: Pseudo first order kinetic model for the MO dye removal of 30 mg/l

Table 4.7: Parameters of Pseudo first order kinetic model For MO Dye Removal

Initial Dye Concentration	Doses	Qe (mg/g)	K_1 (1/hr)	R^2
10	2gm	0.129622	0.007347	0.79183
	3gm	0.131795	0.015154	0.83572
	5gm	0.048645	0.018102	0.63358
30	2gm	0.2553	0.010502	0.95672
	3gm	0.110555	0.007301	0.16278
	5gm	0.120667	0.008475	0.84798

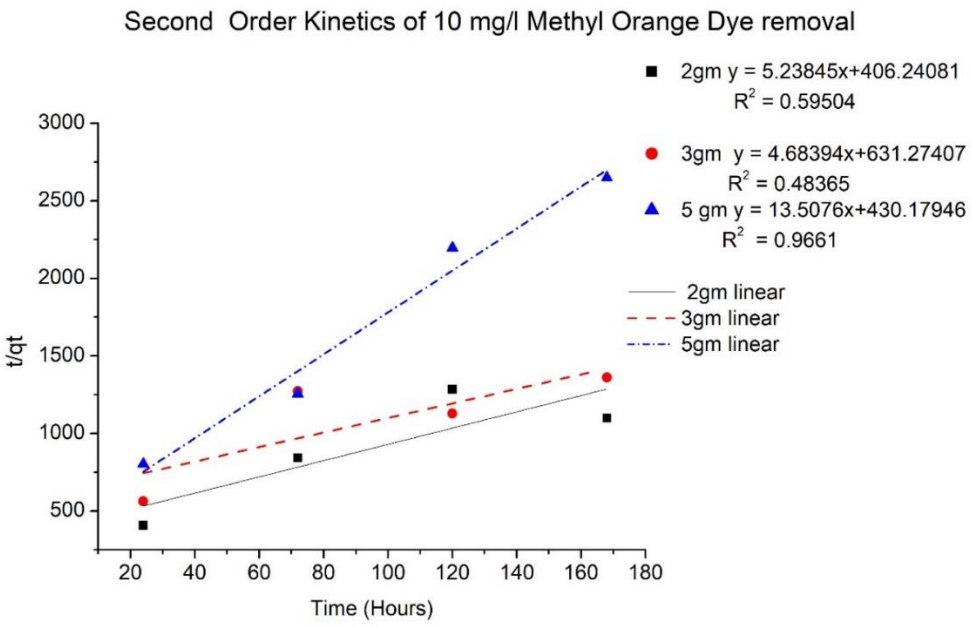

Figure 4.20: Pseudo Second order kinetic model for the MO dye removal of 10 mg/l

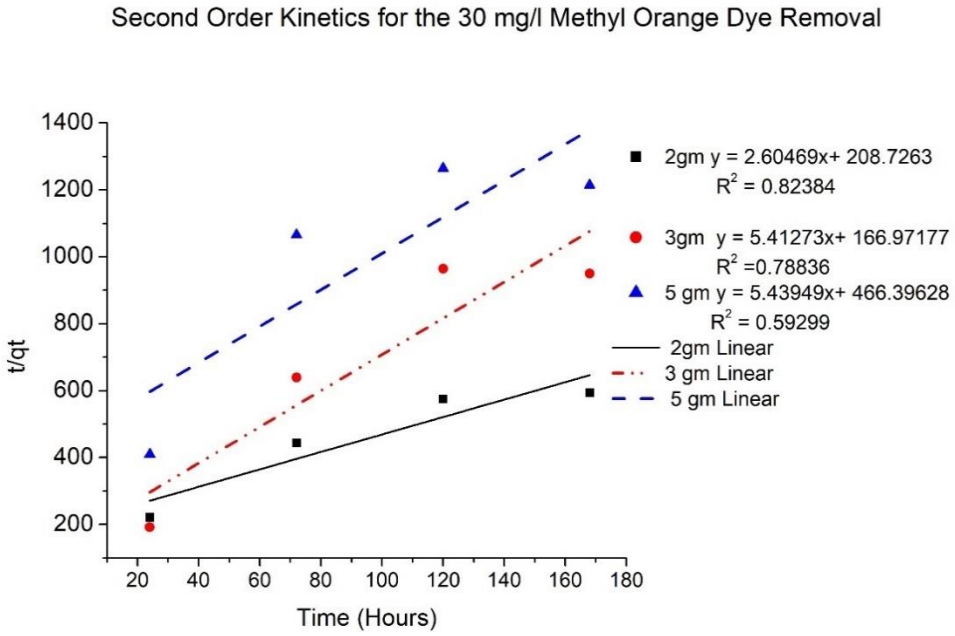

Figure 4.21: Pseudo Second order kinetic model for the MO dye removal of 30 mg/l

Table 4.8: Parameters of the Pseudo second order kinetic model For MO Dye removal

Initial Dye Concentration	**Doses**	**Q_e (mg/g)**	**K_2 (mg/g/hr)**	**R^2**
10	2gm	0.190896	0.067549	0.59504
	3gm	0.213495	0.034754	0.48365
	5gm	0.074032	0.424138	0.96661
30	2gm	0.383923	0.032504	0.82384
	3gm	0.18475	0.175465	0.78836
	5gm	0.183841	0.06344	0.59299

4.2.3 Plant's relative growth rate (PRGR) studies for Anionic Dye

The dry mass study of the azolla figure 4. 22 (a-c) showed that the biomass is decreased when concentration of the dye solution is increased. It is also decreased with the respect to time. However, the green pigment remains still in the plant and biomass of the azolla of 3gm slightly increased with the respect to time. The plant did not die completely for 7 days of the dye exposure. The PRGR value (figure 4.22(d)) for all dye concentration and plant doses are in the negative value. Beside this, the 3gm doses in 20 mg/l has higher PRGR value in comparison to the 3gm azolla exposed to the other dye

concentration. Figure 4.23 reflects that while performing the comparative analysis of the biomass obtained from 3gm azolla exposure in distilled water and dye of the different concentration and noted that the biomass of azolla in 20 mg/l is higher in 7-day exposure period.

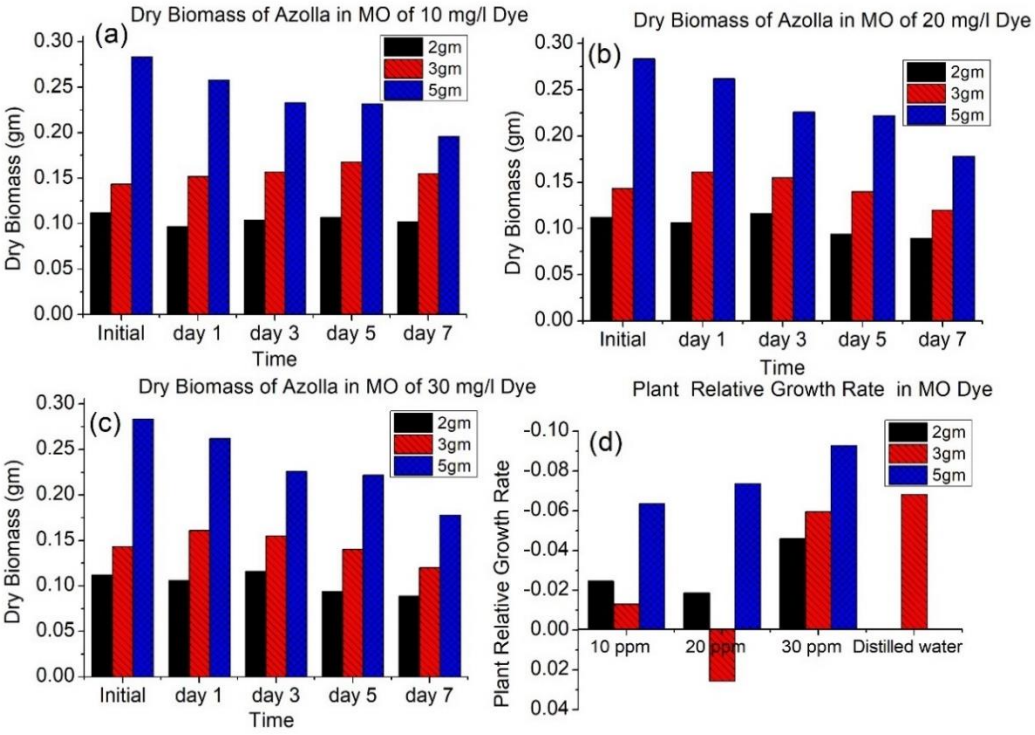

Figure 4.22: Effect of MO dye concentration of (a) 10 mg/l, (b) 20 mg/l and (c) 30 mg/l on the Biomass of Azolla pinnata and (d) Plant relative growth Rate

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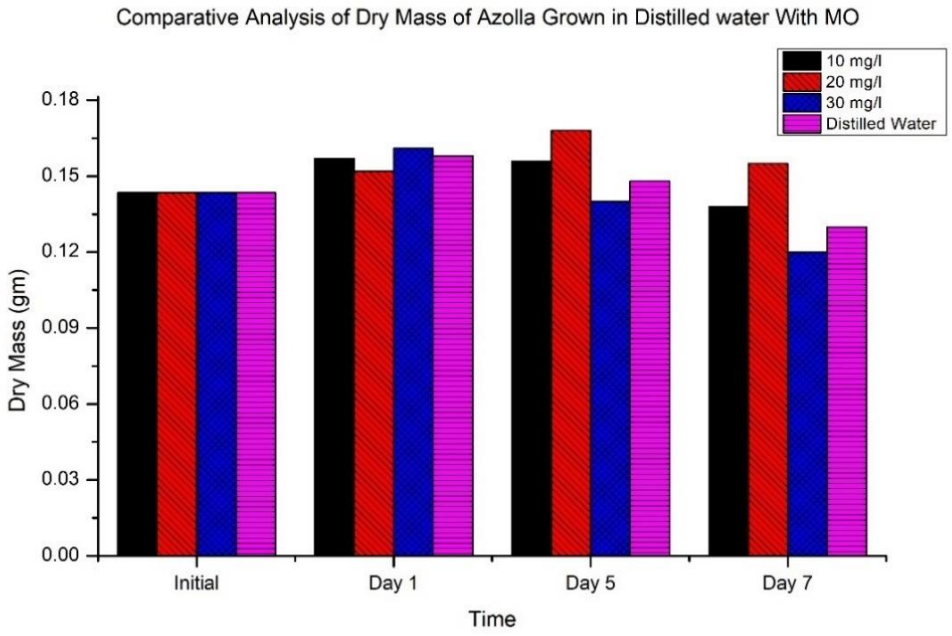

Figure 4.23: Comparative analysis of dry mass of azolla exposed in distilled water with MO dye

4.3 FT-IR Characterization of Biomass obtained from Phytoremediation Study

4.3.1 FTIR Characterization of Biomass of Azolla obtained from MB Remediation

The FT-IR spectra of the MB dye (MB) in figure 4.24 shows several peaks which representing the consisting of the of various functional groups. The broad peak at 3371.77cm^{-1} which indicate the presence of the OH or N-H stretch with alcohol and phenol or Amines. The peak at 2928cm^{-1} and 3050.12 cm^{-1} represent the C-H stretch alkenes and =C-H and =CH2 stretch alkenes. The sharp peaks at the 1596. 17 cm^{-1} and 1492.08cm^{-1} show the presence of a C=C, C=N and C-N bonds respectively. Not only this, sharp peaks at the 1066 cm^{-1} and 570 to 730 cm^{-1} signifies the C=S and C-S bonds. The FT-IR spectra of the biomass of the azolla pinnata grown in the distilled water in figure 4.24 depicts the several peaks which representing the several functional groups. The broad peak at 3292.17 cm^{-1} which indicate the presence of the OH/N-H stretch with alcohol and phenol/Amine. The peak at 2919.01cm^{-1} and 2850.74 cm^{-1}represent the C-H stretch alkenes and C=O aldehydes. The sharp peaks at the 1633.90 cm^{-1} and 1031. 06 cm^{-1} indicate the presence of the N-H bend primary amine and C-N stretch aliphatic amines. The peaks at 1409.78 cm^{-1} and 1244.00 cm^{-1} signifies the Bend Nitro and C-N stretch aromatic amines respectively.

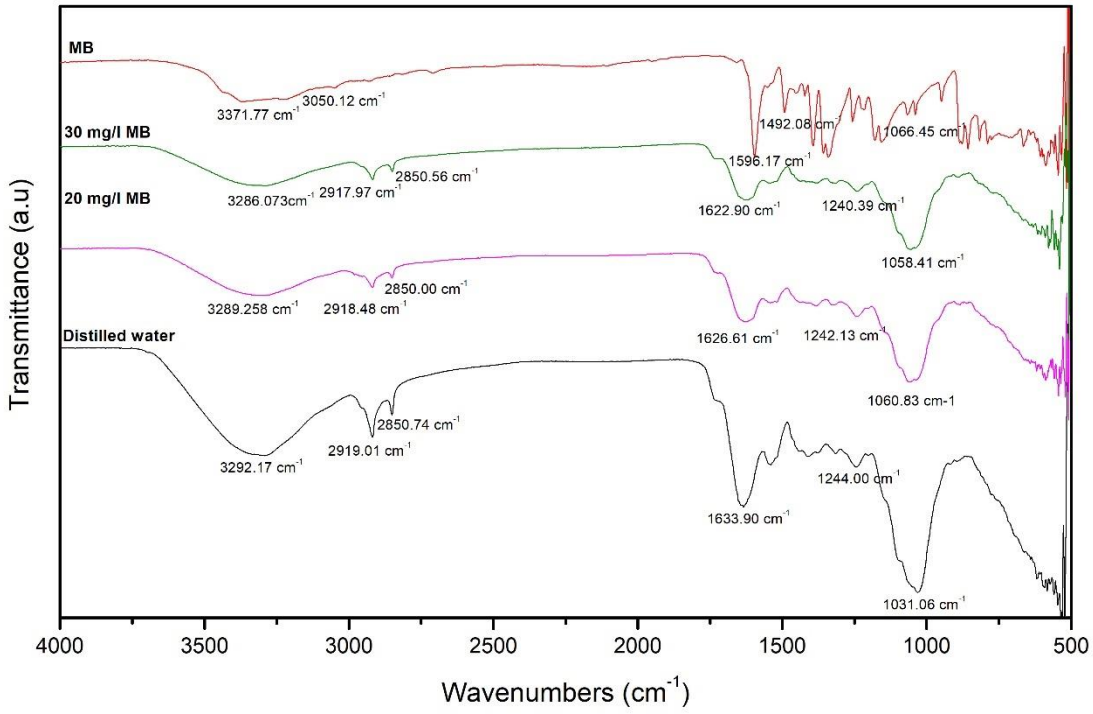

Figure 4.24: FT-IR results of the Azolla pinnata Biomass grown in the different concentration of the MB dye Solution

The FT-IR spectra of the biomass of the azolla pinnata grown in the 30 mg/l MB depicts the shifting of the several peaks when comparing the FTIR spectra of the biomass of the azolla grown in the distilled water. The broad peak shifted to 3286.073 cm^{-1} which shows the interaction of the MB dye with OH/N-H stretch of the alcohol and phenol/Amine. This is fact that the N^{+} in the MB molecules is attracted with the hydroxyl groups via hydrogen bonding(Mahajan & Kaushal, 2020b). There is very small shift in the peak of the C-H stretch alkenes and C=O aldehydes from 2919.01cm^{-1} and 2850.74 cm^{-1} to 2917.97 cm^{-1} and 2850.56 cm^{-1} which also signifies the electrostatic interactions between the O^{-} of a aldehyde groups with N^{+} of the MB dye. In addition to this, C-N and N=O peaks are shifted from 1031.06 cm^{-1} and 1409. 78cm^{-1} to a 1058.41 cm^{-1} and 1377 cm^{-1} which reflects the electrostatic interaction N^{+} and O^{-} (Ahmad et al., 2012; Imron et al., 2021). Besides that, there is no sharp peaks of C-S and C=S bonds are presents in the azolla pinnata biomass grown in the MB dye of 30 mg/l and other low concentration to which suggested that the desulfurization of the MB dye(Al-Baldawi et al., 2020; Patil & Jadhav, 2013). In addition to that when observing

the FT-IR results (Figure 4.24) of the azolla biomass grown in the low concentration of the dye (10 mg/l and 20 mg/l) signifies the changes and shift in the several peaks as like to the 30 ppm. Hence FT-IR comparative analysis showed that the MB dye is absorbed by the azolla pinnata plant through electrostatic, H- bonding and Vander wall force of attractions. Since, the plant absorb the methylene dye in their parts and remain un-degraded which is also indicated by the chlorophyll analysis.

4.3.2 FTIR characterization of Biomass of Azolla obtained from MO Dye Remediation

The FT-IR spectrum of the MO dye depicts that the presence of the various peaks such as 3432.73 cm^{-1} for a N-H stretch as the secondary amides. The peaks at the 2921.05 cm^{-1} and 1604.83 cm^{-1} show the C-H stretch form alkane and C-C stretch from the aromatic compounds. Again, the sharp peaks at 1365.41 cm^{-1} and 1200.01 cm^{-1} depicts C-N stretch for aliphatic amine and S-O and S=O bonds. The FT-IR spectrum (figure 4.25) of the azolla biomass grown in the distilled water and MO dye of the 30 mg/l have the several peaks because of a presence of the functional groups. The broad peaks at 3292.17 cm^{-1} which signifies NH- stretch from amides or OH- stretch from phenols and alcohol shifted to 3345.20 cm^{-1} that signifies the interaction of hydrogen bond of the phenol compound with the dye molecules. There is no significant shift in the peak of C-H stretch alkenes and C=O aldehydes by the interaction of the MO dye molecule. Hence, there is very small shift of the C-N, N-H and N=O bend peaks because of an interaction of a Nitrogen bond of the dye molecules to the azolla pinnata. The several peaks including the S-O and S=O bends are disappeared in the dye adsorbed biomass which indicates the split of the bond(Al-Baldawi et al., 2020). In addition to that, the adsorption process of the anionic dye (MO dye) is very low.

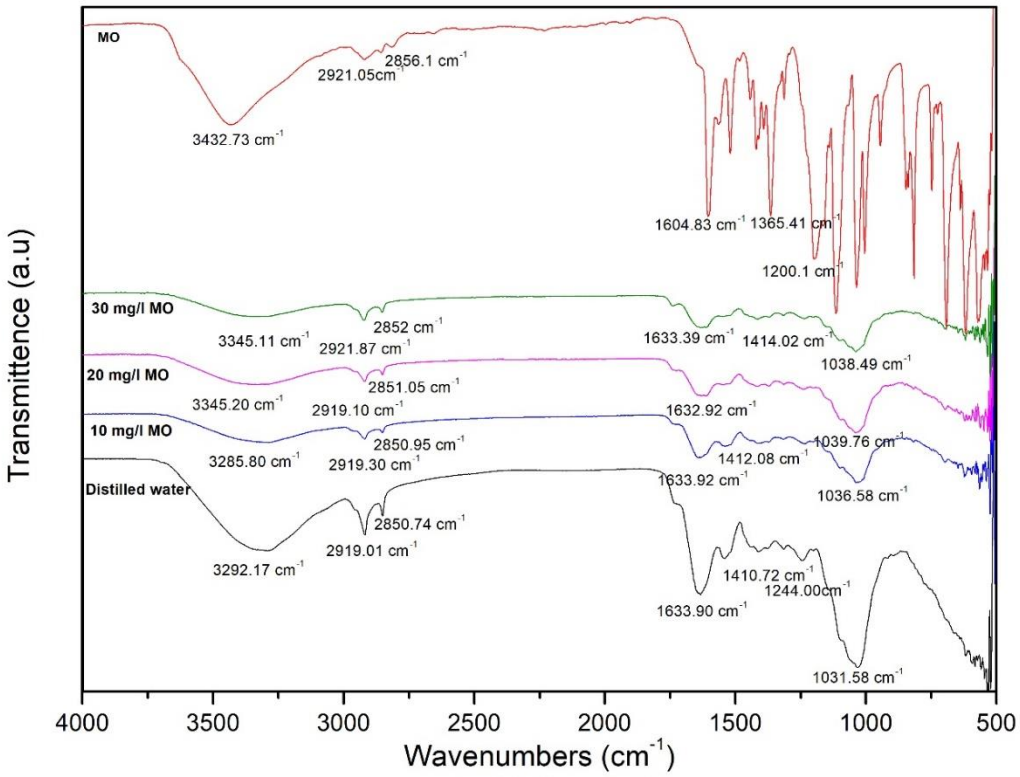

Figure 4.25: FT-IR results of the Azolla pinnata Biomass grown in the different concentration of the MO Dye Solution

4.4 Results of the Biomass-adsorption study of Cationic Dye (MB Dye)

4.4.1 FT-IR analysis of Azolla Biomass

The FT-IR spectra of the AP biomass (figure 4.26) shows several peaks which representing the consisting of the of various functional groups. The broad peak at 3329cm^{-1} which indicate the presence of the OH stretch with alcohol and phenol. The peak at 2920cm^{-1} and 2851cm $^{-1}$represent the C-H stretch alkenes and C=O aldehydes. The sharp peaks at the 1633cm^{-1} and 1039cm^{-1} indicate the presence of the N-H bond with Aromatic amine and aliphatic amines. The interaction of functional groups with the MB dye with the electrostatic interaction, H- bond and Vander wall force. This leads to the rapid adsorption of the cationic dye into it's pore and surfaces(Manna et al., 2017).

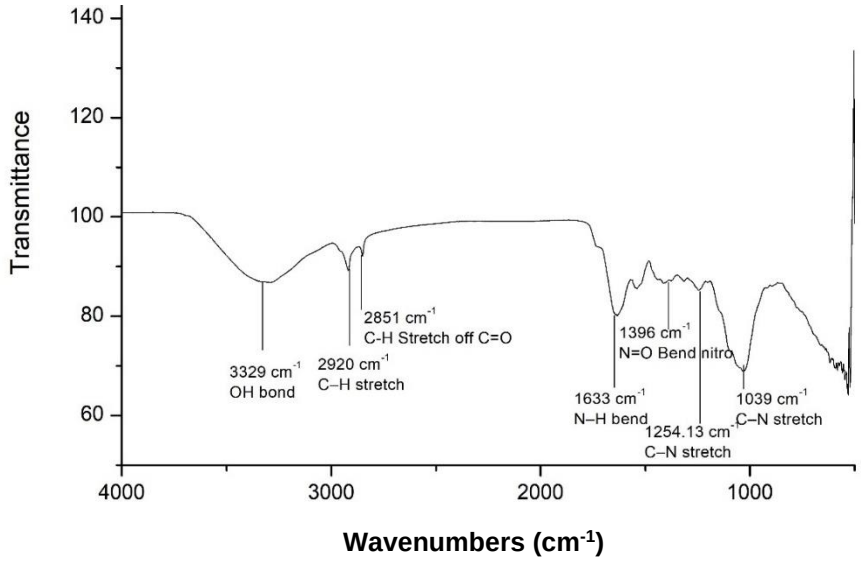

Figure 4.26: FTIR results of Azolla pinnata Biomass

4.4.2 Effect of initial Dye concentration on MB dye adsorption

For investigating the MB dye concentration effect, the experiment was performed by altering the concentration ranging from 50 mg/l to 600 mg/l by taking the absorbent doses as 1g/l. The figure 4.27 shows that the dye adsorption increased with increment of a dye concentration and later remains constant with the respect to higher dye concentration. However, the removal efficiency decreases with the from 97.10% − 55.03% when concentration of the dye augmented from 50 mg/l to 600 mg/l.

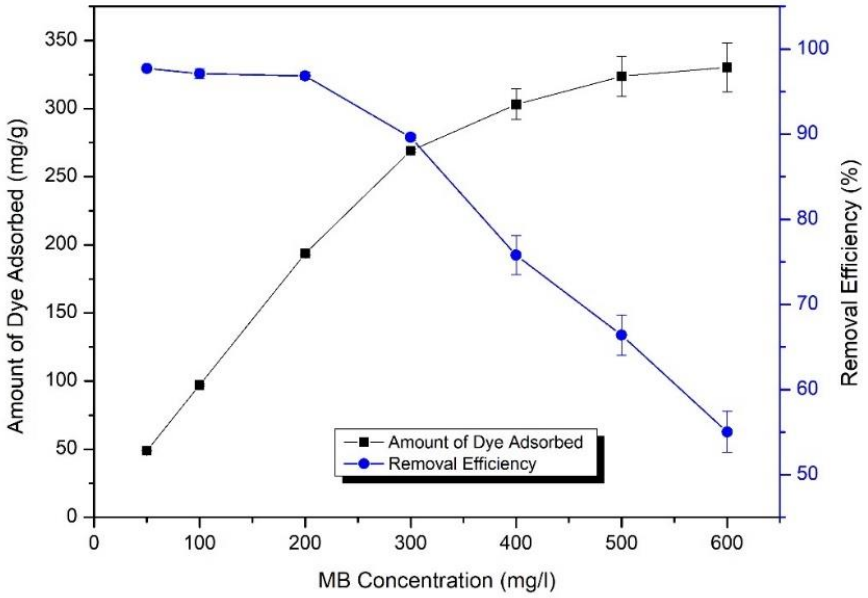

Figure 4.27: Effect of initial dye concentration on the amount of dye adsorbed and removal efficiency

4.4.3 Effect of absorbent doses on MB dye adsorption

The absorbent dosage is a crucial parameter due to fact that this factor evaluates the absorbent capacity at the specified dye concentration. The AP biomass were varied from 10 mg to 60 mg in a 25 ml of 300 mg/l dye solution. The graph (figure 4.28) between absorbent doses, removal efficiency and amount of dye adsorbed shows that as the absorbent dosage increased, dye removal efficiency also escalated. However, the amount of the dye adsorbed by an AP biomass is decreased considerably. This was due to the unsaturated of the adsorption sites during adsorption reactions.

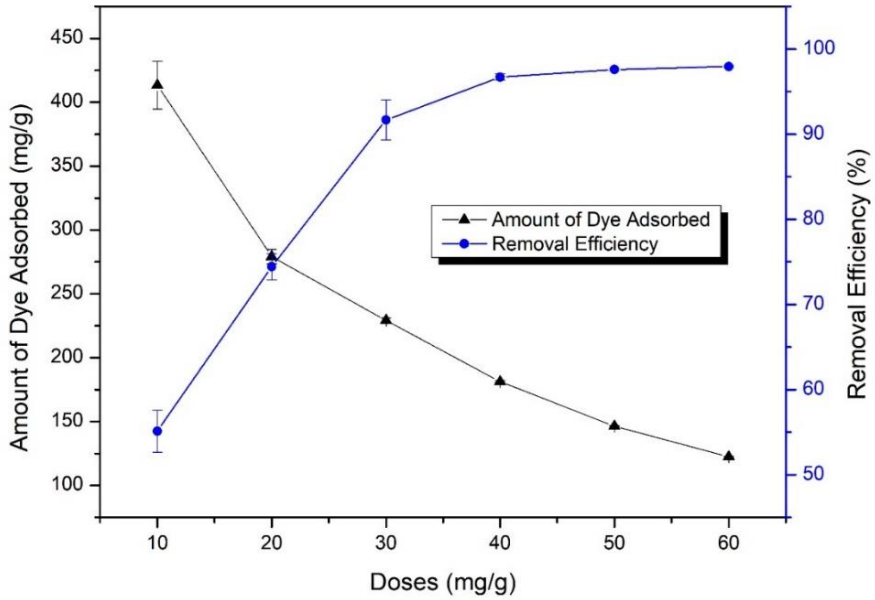

Figure 4.28: Effect of biomass doses on the amount of MB dye adsorbed and removal efficiency

4.4.4 Effect of contact time on MB dye adsorption

The adsorption of the MB dye using the biomass of the azolla pinnata was studied as the function of the time for determining equilibrium condition as shown in figure 4.29. For this, 1.2g/l (30 mg in 25 ml) of the absorbent, 300 mg/l dye concentration were taken and experiment was performed with different contact time 30 minutes to 180 minutes. The dye adsorption to the azolla pinnata increased from 84% to 93% when contact time augmented from 30 minutes to 180 minutes respectively. The adsorption rate is huge during the initial time of an experiment because of an availability of adsorption sites. The MB dye ion is efficiently adsorbed in these sites.

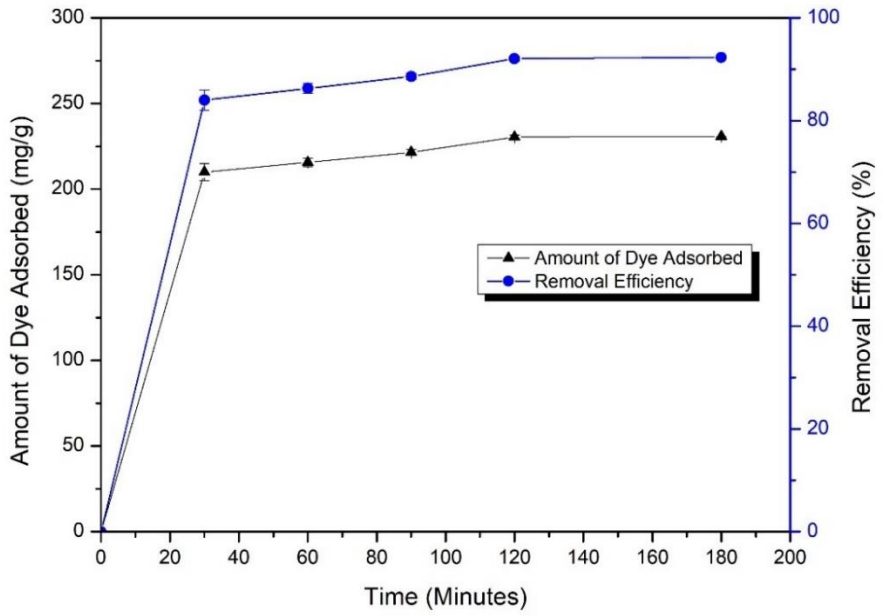

Figure 4.29: Effect of contact time on the amount of dye adsorbed and removal efficiency

4.4.5 Effect of pH on MB dye adsorption

The chemical surface of the adsorbent has huge influence on the absorbate adsorption. This can be done changing the pH value. Figure 4.30 depicts the effect of the Initial pH value on the MB dye absorption. The removal efficiency and amount of dye adsorbed uplifted with the increment of the pH ranging from 2-12. From the research, it is found that there can be an electrostatic repulsion with a positively charged absorbent and cationic MB molecules at small pH values. Hence, it inhibits the adsorption of the dye due to the completes of the hydrogen ion of the MB dye (Marrakchi et al., 2017; Pandimurugan & Thambidurai, 2016; Pang et al., 2017). When the pH increases, the biomass surface possesses the negative charge and facilitates the MB dye adsorption by biomass. Hence, the adsorption is more efficient when the negative surface charges is presented. Hence high pH value is suitable for the MB dye removal(Gao et al., 2016; Zhang et al., 2016). Hence,7-12 pH of the dye solution can be appropriate for the MB dye removal form the solution using biomass of azolla pinnata.

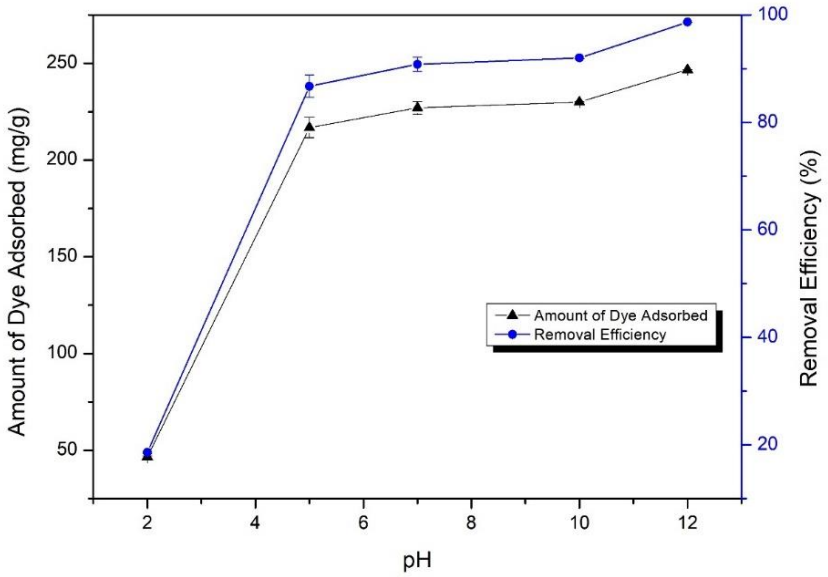

Figure 4.30:Effect of dye pH solution on the removal efficiency of MB dye

4.4.5 Isotherm and Kinetic Studies for the MB dye removal using Azolla biomass.

The adsorption isotherm gives the su fficient information about the dye molecules distribution in the absorbent at the equilibrium condition. These studies were performed for determining the correlation between the adsorption capacity and residual concentration of the absorbate. The table 4.9 and figure 4.31 and figure 4.32 show the isotherm model parameters including the regression coefficient. The parameters from the table shows that a Langmuir isotherm (R^2 = 0.9944) fits the experimental data better than a Freundlich isotherm model (R^2 = 0.8229). This suggests that the Azolla pinnata biomass flows the mono-layer adsorption mechanism on the homogeneous surface(Davoud Balarak et al., 2016).

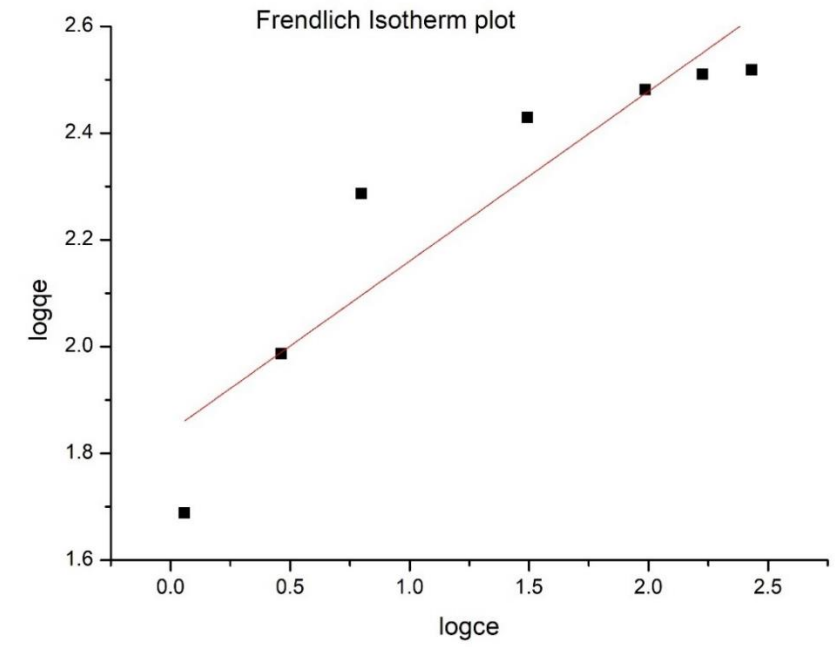

Figure 4.31: Plot of Freundlich isotherm for MB dye removal using azolla biomass

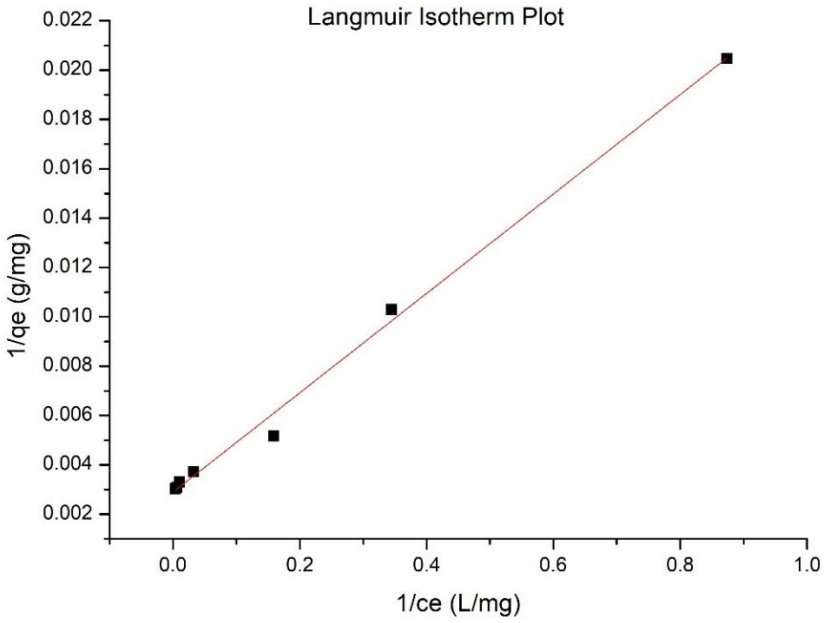

Figure 4.32: Plot of Langmuir isotherm for MB dye removal using azolla biomass

Table 4.9: Parameters of Langmuir and Freundlich isotherm

Langmuir Isotherm			**Freundlich isotherm**		
Q_m(mg/g)	**K_a**	**R^2**	**n**	**k_f**	**R^2**
344.8275862	0.144064	0.9944	3.14	69.554	0.8229

Similarly, for determining the rate controlling mechanism in an adsorption process, the two kinetic models such as Pseudo first and second order were used. From the table 4.10 and figure 4.33 and figure 4.34, it is found that the second order kinetic model with the R^2 of 0.999 represents the experimental data more precisely than first order kinetic model with the R^2 of 0.85602 (D. Balarak et al., 2015).

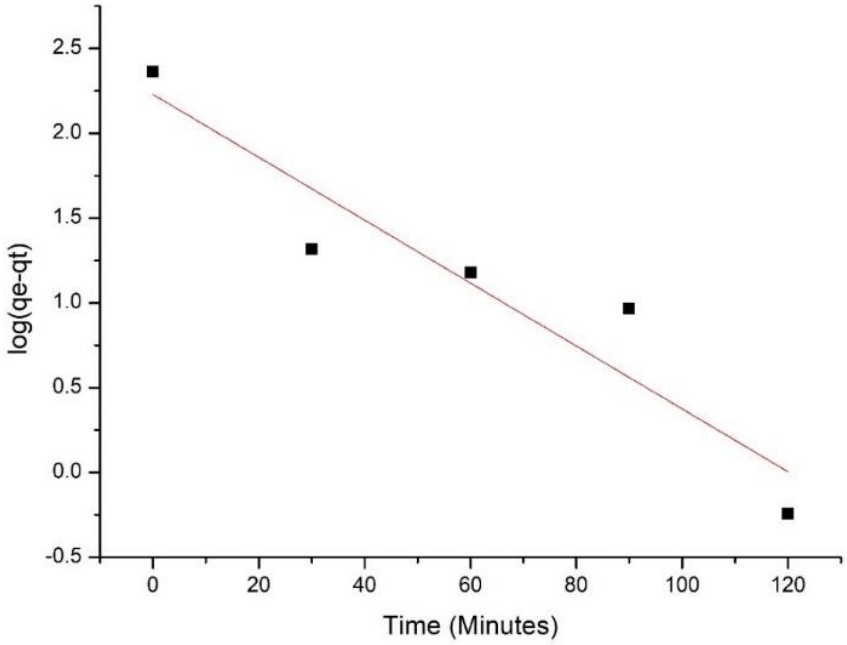

Figure 4.33: Plot of Pseudo First order model for the MB dye removal using azolla biomass

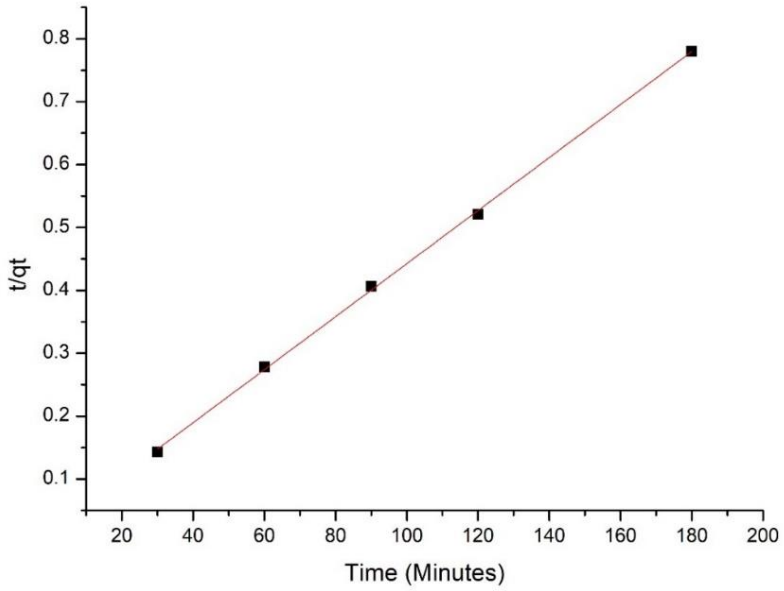

Figure 4.34: Plot of Pseudo Second order model for the MB dye removal using azolla biomass

Table 4.10: Parameters of Pseudo first order and Second order kinetic model for MB dye removal using Biomass

Model	**Q_e(mg/g)**	**K**	**R^2**
First order	169.457	K_1= 0.0426	0.85602
Second order	236.966	K_2 = 2.232	0.99941

4.5 Results of the Biomass-adsorption study of Anionic Dye (MO Dye)

From the adsorption experiments of the MO dye using 25 ml of solution and 25 mg of azolla pinnata biomass. The procedures used are same as biomass adsorption of MB dye. The graphs between the final and initial concentration of a dye solution are plotted as in figure 4.35. From the graphs, there is not significant adsorption of the MO dye by the biomass of a Azolla pinnata. This is because of that the functional groups of the azolla are not interacted with the MO anion. The absorbance spectrum graphs (figure 4.36) shows that the maximum absorbance for the MB dye solution and solution after adsorption at wavelength of 464 nm. In addition to this, absorbance is higher around the UV range which indicates the impurities presents in the adsorbed solution. This

affects the absorbance of the adsorbed MO solution leads to small increment in the concentration than initial solution.

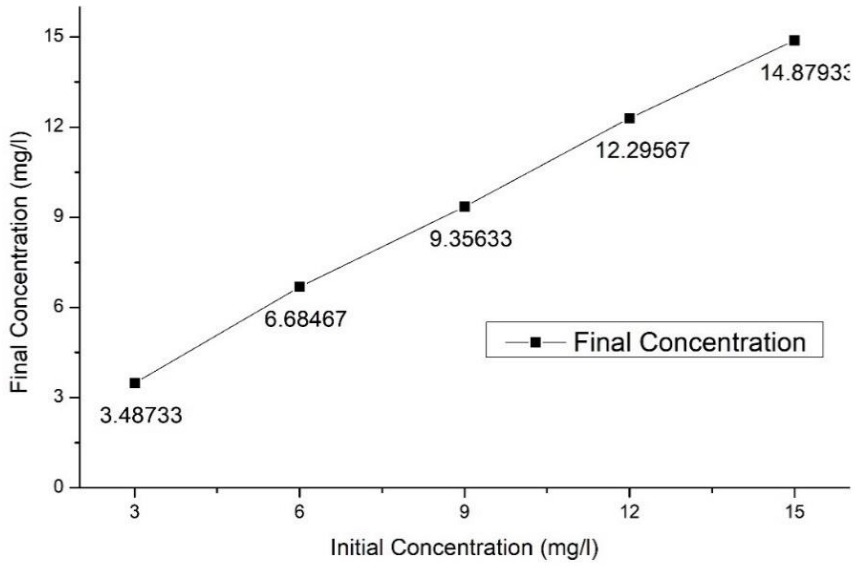

Figure 4.35: Adsorption study of th MO dye using azolla pinnata azolla biomass

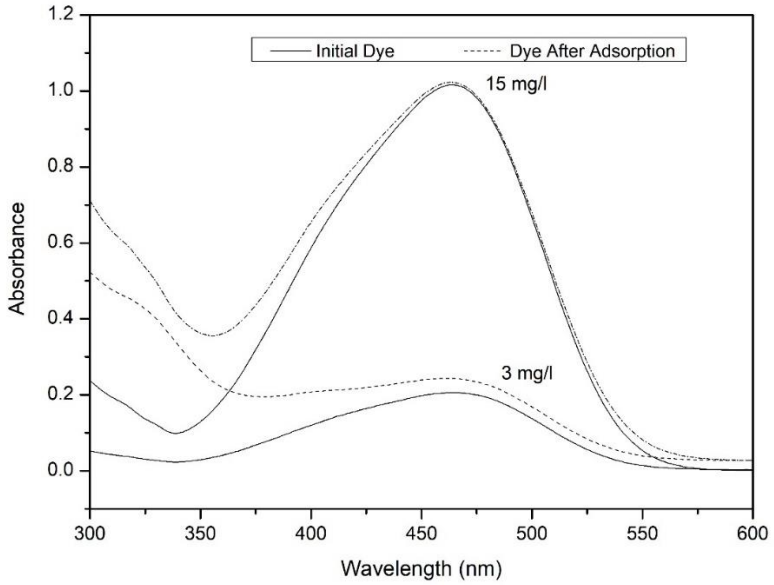

Figure 4.36: Absorbance Spectrum of the Initial MO dye solution and final dye solution after adsorption

4.6 Comparison of the Biomass -adsorption study and phytoremediation study of the azolla pinnata with previous research works.

The table 4.11 and 4.12 show that the comparative study of biomass-adsorption and phytoremediation of MB dye with other adsorbents and plants. When considering the phytoremediation study, the azolla pinnata has maximum adsorption capacity of the MB dye than other phytoremediation plants such as lemina minor and Trachyspermum ammi when considering the phyto-toxicity. In addition to this, adsorption capacity of the biomass of the azolla is higher than the biomass of the other plants such as Ecalyptus Sheathiana bark, Corn, Neem leaf, saw dust and canola residue. Hence, the biomass of the azolla pinnata is good adsorbent for the MB dye removal.

Table 4.11: Comparison of the biomass- adsorption of the MB dye by azolla biomass with other research woks

SNo	**Biomass**	**Maximum adsorption capacity (mg/g)**	**References**
1	Eucalyptus sheathiana bark	204.08	(Afroze et al., 2015)
2	Corn	47.95	(Paşka et al., 2014b)
3	Neem leaf	8.76–19.61	(Bhattacharyya et al., 2005.)
4	Banana Waste	243.90	(Hameed et al., 2008)
5	Indian Rosewood sawdust	56.4	(Garg et al., 2004)
6	Canola Residue	16.7	(Davoud Balarak et al., 2015)
7	Azolla Pinnata biomass	344.8275862	This study

Table 4.12: Comparison of the Phytoremediation of the MB dye by azolla with other research woks

SNo	Plants	Adsorption capacity (mg/g)	References
1	Lamina minor	1.14	(Imron et al., 2021)
2	Trachyspermum ammi	0.138	(Kaur et al., 2022)
3	Azolla Pinnata	1.68	This study

CHAPTER FIVE: CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The phytoremediation investigation was conducted on azolla pinnata to remediate the MB dye and MO dye from the artificial waste water. The plant weight, contact time and concentration for the MB dye removal were 3g, with efficiency of more than 90%, 24 hours and 30 mg/l initial concentration. Similarly, the MO dye remediation process was very slow and only able to remove 35-37% for the 2gm and 3gm azolla under the 10 mg/l and 20 mg/l dye concentration during the 7 days exposure. The Plant relative growth rate was negative for all dye concentration and doses which indicate the slightly toxic and plant relative growth rate for the MO dye for 2gm and 3 grams in 20 and 30 mg/l were higher in comparison to MB dye. Pseudo second order kinetics were good fit for describing the absorption mechanism of MB dye for various plant weight. Similarly, the first order kinetics was good fit for describing the absorption mechanism for MO dye for various plant weight. Depending on the FTIR results, the Azolla pinnata contained the O-H, C-H, C=O and C-N functional groups were directly involved and interacted through electrostatic and Vander wall forces and hydrogen bonding between the MB dye molecules. Chlorophyll studies of the Azolla pinnata exposed to MB dye showed that the phyto-extraction process is adopted by the azolla pinnata for the MB dye removal. In order to signify more about phytoremediation behaviour, biomass-adsorption experiment using azolla pinnata biomass was conducted by varying the azolla biomass doses (10-60 mg), pH (2-12), time (0-180 minutes) and concentration (50-600 mg/l) of MB dye. The biomass-adsorption study reveals the dye adsorbed percentage was found to be higher than 90% in lower concentration (50 mg/l to 300 mg/l). Pseudo second order kinetic and Langmuir isotherm models suit for the adsorption of a MB dye by azolla. The maximum adsorbent capacity of biomass for a MB dye was 344.82 mg/g. Similarly, there was no adsorption of the MO dye with azolla pinnata biomass under the very low concentration (3 mg/l-15 mg/l). Hence, it is concluded that the azolla pinnata and it’s biomass can be highly efficient for removal of the cationic dye than anionic dye.

5.2 Recommendation

The future enhancements for this research works are follow:

1. The phytoremediation pattern adopted during the dye removal was analyzed FT-IR analysis. The HPLC and GC-MS analysis of azolla pinnata exposed in dye may be done in future for determining the metabolic activities involved during cationic and anionic. dye absorption.
2. The effect of nutrient on the dye removal needs to be done in the future for the for enhancing the growth rate in dye solution.
3. The highly toxic dyes rather than MO and MB dye may be used in future for determining removal rate and toxicity towards plants etc.

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APPENDIX A

Table A1: Hourly Removal of MB Dye

10 mg/l					
Initial	2hours	4 hours	6 hours	8 hours	24 hours
9.620305	9.350352	8.763498	8.610915	8.505282	8.094484
9.620305	3.499413	2.208333	1.838615	1.286972	0.318662
9.620305	1.991197	0.899648	0.606221	0.400822	0.001761
9.620305	1.022887	0.617958	0.36561	0.183685	-0.05106

20 mg/L					
Initial	2hours	4 hours	6 hours	8 hours	24 hours
19.95246	19.60035	19.77641	19.35387	19.67077	18.1919
19.95246	5.782277	5.348005	3.071009	2.26115	0.981808
19.95246	5.336268	4.309272	2.478286	1.627347	0.612089
19.95246	3.094484	1.867958	1.175469	0.928991	0.254108

30 mg/l					
Initial	2hours	4 hours	6 hours	8 hours	24 hours
30.23415	29.24824	30.23415	29.38908	29.24824	28.10387
30.23415	10.82512	8.974765	6.592136	5.16608	2.566315
30.23415	9.186033	6.222418	4.344484	3.112089	1.398474
30.23415	5.353873	3.599178	2.396127	1.862089	0.700117

Table A2: Chlorophyll Estimation of the Azolla pinnata under the Exposure of MB dye

	Total Chlorophyll	chlorophyll A	chlorophyll B	Days
10 mg/l	1.015692968	0.578222307	0.437470661	4--7
20 mg/l	1.186090143	0.60984884	0.576241303	4--7
30 mg/l	1.806768959	0.87204253	0.934726429	4--7
10 mg/l	0.690105738	0.432486885	0.257618852	6--7
20 mg/l	1.270703863	0.688133047	0.582570815	6--7
30 mg/l	2.021421362	0.971740959	1.049680404	6--7
10 mg/l	1.138519045	0.558676754	0.579842291	7--7
20 mg/l	1.397953852	0.646278656	0.751675197	7--7
30 mg/l	2.094109389	0.920820754	1.173288636	7--7

Table A3: Chlorophyll Estimation of the Azolla pinnata under the Exposure of MB dye and Nutrient Solution

	Total chlorophyll	chlorophyll A	chlorophyll B	Day
Initial	0.845444656	0.461644	0.383800656	Day-1
10 mg/l	0.844794	0.4312	0.413594	Day-1
20 mg/l	1.697648	0.78847	0.909178	Day-1
30 mg/l	2.0991	1.1416	0.9575	Day-1
10 mg/l	1.023166667	0.476566667	0.5466	Day-3
20 mg/l	1.536556667	0.46765	1.068906667	Day-3
30 mg/l	2.247153333	0.9839	1.263253333	Day-3
10 mg/l	1.311303333	0.670566667	0.640736667	Day-5
20 mg/l	1.556086667	0.748333333	0.807753333	Day-5
30 mg/l	2.927876667	1.3397	1.588176667	Day-5

Table A4: Daily Wise Biomass of Azolla pinnata under the Exposure of MB dye

Biomass weight under the Dye of 10 mg/l (in gram)				
	Initial	Day 1	Day 3	Day 4
2gm	0.167	0.1352	0.145	0.1124
3gm	0.234	0.1872	0.185	0.1869
5gm	0.325	0.2917	0.265	0.2732

Biomass weight under the Dye of 20 mg/l (in gram)					
	Initial	Day 1	Day 3	Day 4	Day 5
2gm	0.167	0.1595	0.114	0.1167	0.10117
3gm	0.234	0.1833	0.183	0.1743	0.18582
5gm	0.325	0.3282	0.272	0.303	0.30512

Biomass weight under the Dye of 20 mg/l (in gram)					
	Initial	Day 1	Day 3	Day 4	Day 5
2gm	0.167	0.1174	0.142	0.1317	0.12812
3gm	0.234	0.204	0.177	0.1841	0.18902
5gm	0.325	0.3207	0.3089	0.2962	0.30412

Table A5: Daily variation MB dye Concentration of 10 mg/l, 20 mg/l and 30 mg/l

Daily variation MB dye Concentration of 10 mg/l				
	control	2gm	3gm	5gm
Initial	10	10	10	10
Day 1	8.0944836	0.318662	0.001760563	0
Day 3	8.04375	0	0	0
Day 4	7.29375	0	0	0
Day 5	6.60625	0	0	0.03728638

Daily variation MB dye Concentration of 20 mg/l				
	control	2gm	3gm	5gm
Initial	20	20	20	20
Day 1	19.7619048	0.981808	0.612089	0.254108
Day 3	19.2124542	0	0	0
Day 4	19.029304	0	0	0
Day 5	18.7851038	0	0.02175	0

Daily variation MB dye Concentration of 30 mg/l				
	control	2gm	3gm	5gm
Initial	30.05882	30.05882	30.05882	30.05882353
Day 1	30.17647	2.566315	1.398474	0.700117371
Day 3	30.05882	0.503573	0.23	0.02
Day 4	28.64706	0.313931	0.23	0.011393061
Day 5	29.23529	0.032139	0.33	0.014764371

Table A6: Daily variation MO dye Concentration of 10 mg/l, 20 mg/l and 30 mg/l

Daily variation MO dye Concentration of 10 mg/l				
	control	2gm	3gm	5gm
Initial	10.134	10.134	10.134	10.134
day 1	9.676888	8.957831	8.854682	8.641056
day 3	9.679534	8.427551	8.436452	7.266575
Day 5	9.653788	8.267073	6.943083	7.403405
Day 7	9.21489	7.07676	6.428948	6.965444

Daily variation MO dye Concentration of 20 mg/l				
	control	2gm	3gm	5gm
Initial	20.045	20.045	20.045	20.045
day 1	20.48531	18.63365	17.77544	18.16913
day 3	20.35623	16.78124	15.25339	14.88003
Day 5	20.16099	13.90771	12.28106	11.35781
day 7	19.55851	13.56547	13.04441	12.60099

Daily variation MO dye Concentration of 30 mg/l				
	control	2gm	3gm	5gm
Initial	30	30	30	30
day 1	29.34417	27.8311	26.2593	27.07287
day 3	28.60593	26.758	26.62211	26.62211
Day 5	29.91224	25.828	26.26623	25.25566
day 7	29.29995	24.34688	24.69627	23.08056

Table A7: Daily Wise Biomass of Azolla pinnata under the Exposure of MO dye

Biomass weight under the MO Dye of 10 mg/l (in gram)			
	2gm	3gm	5gm
Initial	0.112	0.1435	0.2835
Day 1	0.105	0.157	0.239
Day 3	0.096	0.146	0.221
Day 5	0.105	0.156	0.222
Day 7	0.099	0.138	0.206

Biomass weight under the MO Dye of 20 mg/l (in gram)			
	2gm	3gm	5gm
Initial	0.112	0.1435	0.2835
Day 1	0.097	0.152	0.258
Day 3	0.104	0.157	0.233
Day 5	0.107	0.168	0.232
Day 7	0.102	0.155	0.196

Biomass weight under the MO Dye of 30 mg/l (in gram)			
	2gm	3gm	5gm
Initial	0.112	0.1435	0.2835
Day 1	0.106	0.161	0.262
Day 3	0.116	0.155	0.226
Day 5	0.094	0.14	0.222
Day 7	0.089	0.12	0.178

APPENDIX B

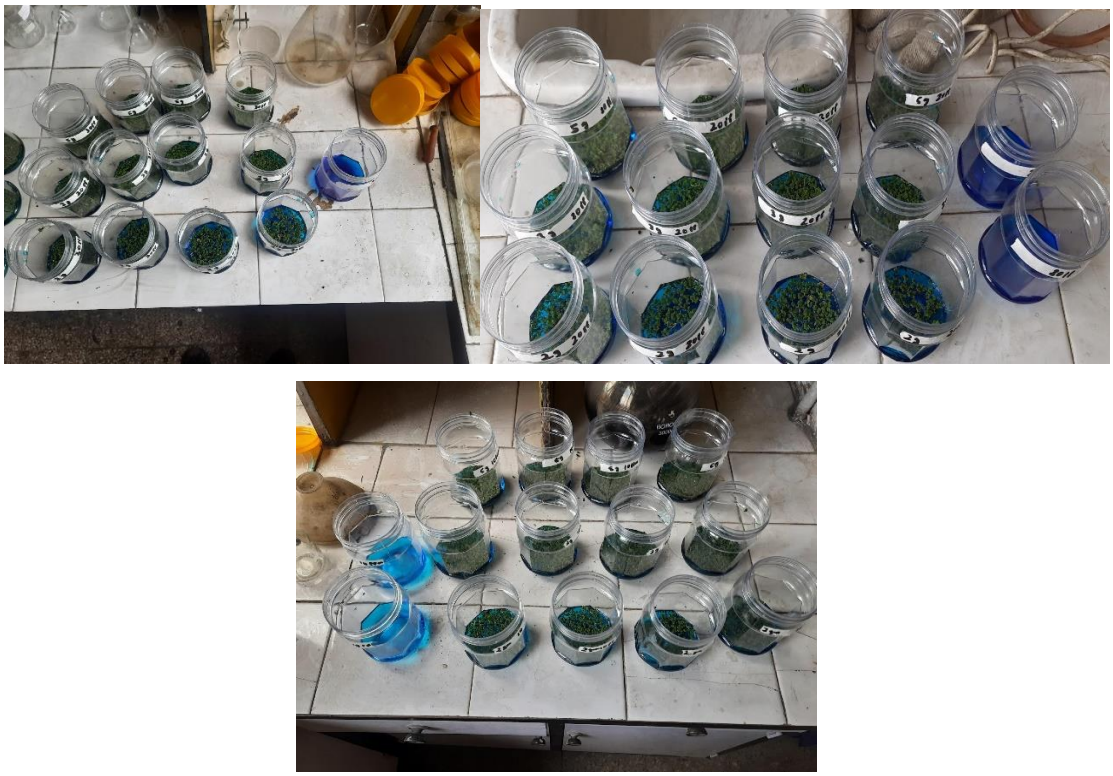

Figure B1: Layout for the phytoremediation of the MB dye

Figure B2: The Decolorization of MB dye after 24 hours

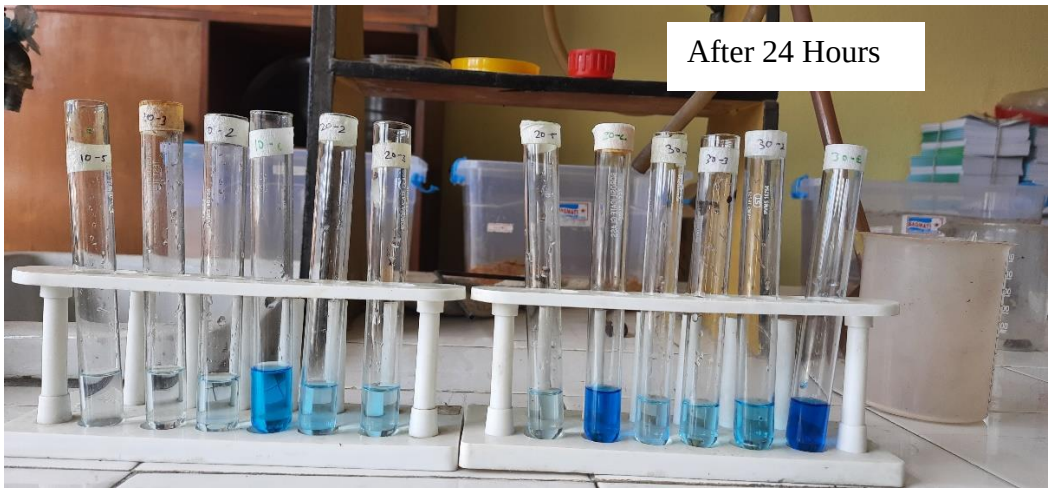

Figure B3: The Decolorization of MB dye after 24 hours

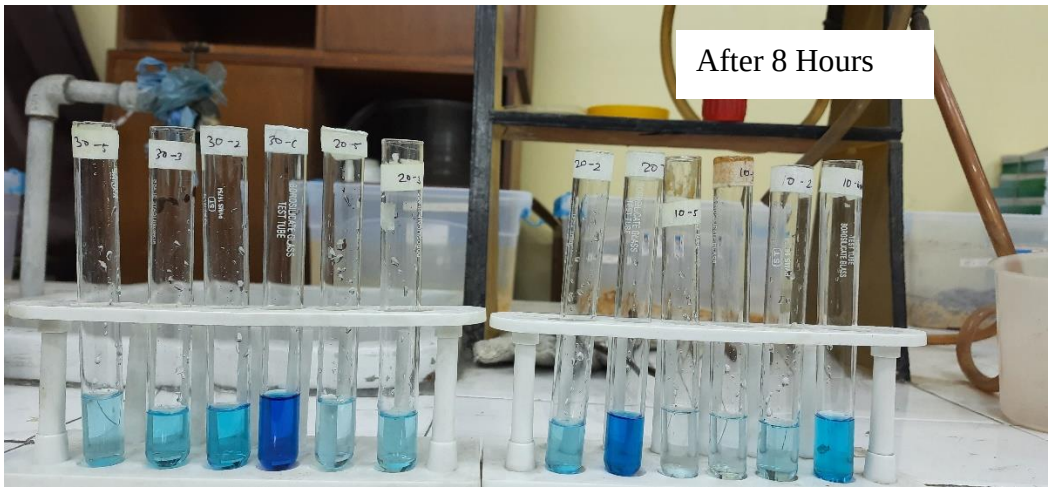

Figure B4: The Decolorization of MB dye after 8 hours

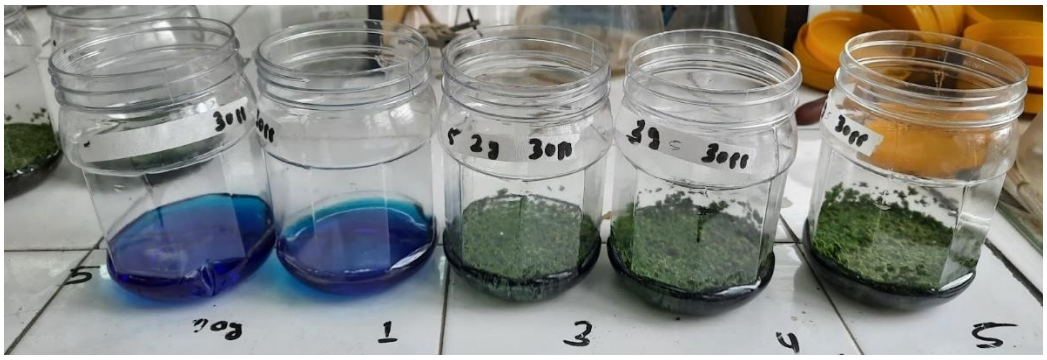

Figure B5: The Decolorization of MB dye after 5 days

Figure B6: Absorption of MB dye by Azolla Pinnata

Figure B7: Chlorophyll content measurement

Figure B8: Layout of Phytoremediation of MO dye by Azolla Pinnata

Figure B9: Phytoremediation of MO dye after exposing azolla for 7 days

Figure B10: Decolorizations of MO dye after exposing azolla for 7 days

Figure B11: Biomass adsorption of MB dye under varying pH

Figure B12: Biomass adsorption of MO dye