Assessment of the Efficacy of *Azolla pinnata* in Improving Textile Dye Wastewater Quality at Varying Concentrations

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Dedication

I would like to dedicate my project to my Parents. They always encourage me to gain knowledge about different aspects of life and makes sacrifices so I would have access to high quality education from an early age. Also, this is dedicated to my honorable supervisor Md. Arifur Rahman Bhuiyan, Assistant Professor, Department of Environmental Science, Bangladesh University of Professionals for his tremendous supports and guiding.

Acknowledgement

All praise goes to the Almighty Allah, the most merciful and the most gracious!

I express my deepest thanks and sincere gratitude to my honorable supervisor, MD. Arifur Rahman Bhuiyan, Assistant Professor, Department of Environmental Science, Faculty of Science and Technology, Bangladesh University of Professionals for his tremendous support and guiding. I am very grateful to all the faculties and concern authorities of Department of Environmental Science of Bangladesh University of Professionals for their cooperation during the research work. Besides, I am very much grateful to some of my friends for helping me at the time of sample collection and supporting me. I also want to give heartiest thanks to the authors whose articles I used as reference.

Lastly, I express my heartfelt gratitude to my parents and other members of the family for their blessings, encouragement and constant inspiration throughout my academic career.

Declaration

I do hereby declare that this dissertation titled **"Assessment of the Efficacy of *Azolla pinnata* in Improving Textile Dye Wastewater Quality at Varying Concentrations"** has been prepared as a part of my partial fulfillment of the requirements for the degree of BSc. in Environmental Science. The contents of this dissertation are original and have not been submitted in whole or in part for consideration for any other degree or qualification in this, or any other university. In this regard, I would like to mention that this dissertation is the result of my own research work and no part of this report was copied without proper acknowledgement.

Nishat Tasnim

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Certificate of the Supervisor

This is to certify that Nishat Tasnim carried out her Project under my guidelines and supervision, and hence prepared the project entitled **"Assessment of the Efficacy of *Azolla pinnata* in Improving Textile Dye Wastewater Quality at Varying Concentrations"**. So as far as I am aware, the researcher duly acknowledged the other researchers' materials and sources used in this work. Further, the project was not submitted to any other Universities or institutions for any other degree or diplomas.

It is thus recommended that the project be submitted to the Department of Environmental Science, Faculty of Science and Technology, Bangladesh University of Professionals, in fulfilment of the requirements for the award of the degree of BSc in Environmental Science.

Supervisor

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Abstract

In the past few decades, large-scale wastewater discharge from the textile industries into the water bodies have been a major threat to society and environment of Bangladesh. In particular, the discharged water which consists of active ingredients such as dyes and coloration for the finishing of different fibers is more detrimental to human health as well as aquatic biodiversity. This study was conducted to investigate the efficacy of *Azolla pinnata* in improving the water quality of various concentration of textile dye wastewater. Some physicochemical parameters such as Temperature, TSS, TDS, DO, pH, EC, Turbidity, Salinity, Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD_5) of the textile dye wastewater were observed before and after incorporation of *Azolla pinnata* without pH adjustment. Higher values of Temperature (35.53℃), pH (11.97), Biochemical Oxygen Demand (384.7 mg/l), Chemical Oxygen Demand (500.5 mg/l), Electrical Conductivity (10573 µS/cm), Total Suspended Solids (2601.3 mg/l), Total Dissolved Solids (5342 ppm), Salinity (3.8 psu), Turbidity (179 NTU) and Absorbance (1.096) were recorded in the collected textile dye wastewater comparing with the control sample and exceeded the standards for industrial wastewater prescribed by the Department of Environment (DoE). The concentration of Dissolved Oxygen (DO) in textile wastewater was 0.67 mg/l which was much lower than the control (5.76 mg/l). The efficiencies in improving the mentioned physicochemical parameters have been studied for 0%, 30%, 60% and 100% textile dye-wastewater. Results demonstrated that the maximum efficacy of *Azolla pinnata* was recorded for 30% wastewater concentrated treatment (T1). In T1 (30%), the improvement efficiencies were 36.84%, 66.67%, 55.05%, 53.40%, 46.85%, 58.60%, 52.21%, 76.86%, and 77.49% for pH, Biochemical Oxygen Demand (BOD_5), Chemical Oxygen Demand (COD), Electrical Conductivity (EC), Total Dissolved Solids (TDS), Total Suspended Solids (TSS), Salinity, Turbidity and Absorbance, respectively. Dissolved oxygen (DO) increased significantly in each of the treatment. So the findings indicate the most significant results for 30%, then 60% and lastly 100%. So this study's findings indicate that the selective treatment approach may hold great promise for the phytoremediation of textile dye wastewater and may also be feasible to use.

Keywords: *Textile dye-wastewater, Aquatic macrophyte (Azolla pinnata), Phytoremediation*

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CHAPTER ONE

INTRODUCTION

1.1 Introduction

The textile sector has been an important part of Bangladesh’s economy over the past few decades. In the fiscal year 2021-2022, Bangladesh exported garments worth US $42.613 billion, making it the second-largest apparel exporter in the world (Government of the People's Republic of Bangladesh, 2023). More than 84% of export revenue is generated from textiles and items that involve textiles (Hossain, 2023). The textile industry contributes about 13% of the GDP. Approximately 4.5 million people are employed by these industries, 60% of them are women (BTMA, 2023). The textile sector is well-known for its numerous beneficial contributions to the economy, but it is also well-known for its significant environmental and social challenges in terms of long-term sustainability (Roy et al., 2020). Despite significant economic contributions, Bangladesh textile industries cause a range of environmental problems; mostly it pollutes the water resources (Khan et al., 2011). The textile manufacturing sector is one of the major industrial water users in Bangladesh. The average amount of groundwater required to process 01 kilogram of textile materials was 164 liters (SD ~81.8); the amount of dyehouse water used was 136 liters (SD ~70.6), and the amount of wastewater produced was 119 liters (SD ~73.0) (Uddin et al., 2023).

This textile wastewater (TW) is one of the most hazardous wastewaters for the environment when discharged without any proper treatment. Wastewater originating from these industries is one of the major sources of pollution for surface and groundwater bodies in countries like Bangladesh where textile is the most growing industry. Depending on the specific textile process, such as scouring, bleaching, dyeing, printing, and finishing, the wastewater from the textile industry are complex, containing synthetic dyes, dispersants, bases, acids, detergents, salts, oxidants, surfactants, inhibitory compounds, grease and oil, and many other compounds and salts (Pereira & Alves, 2011). Moreover, these wastewaters have high temperatures and varying pH (Imran et al., 2015). In addition to being harmful to people, animals and plants, dyes can also cause water's dissolved oxygen levels to drop and creates anoxic conditions, which can have an lethal impact on aquatic life (Solís et al., 2012). So it’s necessary to treat the wastewater properly before disposal to prevent any significant undesirable or harmful environmental effect. Various physicochemical and biological strategies have been devised to remove contaminants from such wastewaters (such as precipitation, membrane filtering, adsorption and electrochemical procedures) (Roy et al., 2020). However, Phytoremediation approaches have attracted worldwide attention for their relative cost-effectiveness and environmentally friendly nature (Imran et al., 2015). Phytoremediation aims to safeguard the environment by utilizing plants and microbes associated with plant root systems by removing contaminants in the form of organic and inorganic wastes (Bharathiraja et al., 2017). Among all the mentioned procedures it is commonly known that adsorption is an economical, eco-friendly and easy to operate process for pollutant removal (Dotto et al., 2012). Aquatic macrophytes treat water by accumulating harmful organic and inorganic wastes. The uptake of pollutants from wastewater in aquatic plants occurs primarily through the root system because it has a large surface area that can absorb and store both non-essential toxins and the water and nutrients needed for their development (Rashid, 2021).

A variety of aquatic floats can be suggested for phytoremediation approach to treat wastewater. Such as water hyacinth (*Eichornia crassipes*), water lettuce (*Pistia atratoites*), salvinia (*Salvinia spp.*) and some species of duckweeds (*Lemna spp.*, *Spirodella spp.*, etc.) and fern have been extensively investigated in case of pollutant removal (Rashid, 2021). *Azolla pinnata* is an aquatic fern. Researchers have discovered that *Azolla pinnata* can reduce pH, Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD_5), nitrogen, phosphorus, and heavy metal concentrations in wastewater; therefore, it can be utilized for wastewater treatment (Jayasundara, 2022). According to the literature, *Azolla pinnata* has a high growth rate, with a doubling rate in 2-4 days. Its cell wall is composed of pectin, which has a high affinity for the absorption of organic substances. So it can be served as a "biofilter" during wastewater treatment in this way (Shiomi & Kitoh, 1987). *Azolla pinnata* is known to remove contaminants; dissolved and suspended by absorption and incorporate them into their own system or store them in a bound form. The present study was undertaken to investigate the effectiveness of aquatic macrophyte, *Azolla pinnata* in removing contaminants and improving textile dye-wastewater quality as well as reduce the adverse effects on environment.

1.2 Problem Statement

Over the past few decades, large discharge of dye containing wastewater from textile industries in water bodies has been posing serious threat to environmental safety and society. Therefore this red category industry is regarded as one of the main causes of environmental pollution and the world's second-biggest polluter of water (Kant, 2012). At present textile is the largest and fastest growing sector in Bangladesh. Again these industries consume a large amount of water and commonly use active ingredients such as dyes and coloration for the finishing of different fibers. These active ingredients enhance the Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD) and Total Organic Compounds (TOC) of water and show toxic effects toward the environment as well as human body (Siddiqui et al., 2018). The discharge of untreated or not properly treated textile wastewater into water bodies can lead to severe environmental and health degradation. Various physical, chemical and biological methods such as adsorption, photolysis, chemical precipitation, chemical oxidation and reduction, electrochemical precipitation have been employed for the removal of pollutants from wastewater. However, these technologies are usually not much effective in all pollutant removal, or are expensive and less adaptable to wide range of dye containing wastewaters (Azanaw et al., 2022). Numerous studies have previously been conducted on phytoremediation techniques for the treatment of wastewater from textile industries but still there is a lack in study of different aquatic macrophyte in wastewater treatment and specifically the efficiency of *Azolla pinnata* in different concentrated dye-wastewater in improving the water quality is still not well researched.

1.3 Rationale of the Study

The number of textile industry in Bangladesh is increasing day by day. The textile manufacturing process is characterized by the high consumption of different resources like water, energy and a variety of chemicals in a long process sequence and ultimately it generates huge quantities of complex chemical waste substances as a part of unused materials including dyes, metals contamination of the water bodies in the form of wastewater from almost every stages of the manufacturing process (Kabra et al., 2004). The common practices of low process efficiency result in substantial wastage of resources and a severe damage to the environment (Uddin et al., 2023). This discharge of untreated or partially treated wastewater raised much concern among the general public because of the potentiality of the hazards associated with the entry of these substances into the food chain of humans and other animals (Qadri et al., 2019).

Phytoremediation approaches have attracted worldwide attention for their relative cost-effectiveness and environmental friendly nature. By studying several related papers, we come to know that adsorption efficiency of *Azolla pinnata* have a great potential for removing contaminants in aqueous solutions of textile industry (Dotto et al., 2012). There are a few number of research publication have been found regarding the implementation of aquatic macrophyte, *Azolla pinnata*, in wastewater treatment. Hopefully this work will be very fruitful in evaluating the characteristics of dye-wastewater produced by textile industries, assessing the efficiency of *Azolla pinnata* in improving various concentration of textile dye-wastewater. From this study we can compare in which concentration of textile dye-wastewater *Azolla pinnata* is more efficient and could be highly promising for the phytoremediation of textile wastewater and can also be practically implementable.

1.4 Research Gap

From the literature review, it is found that limited research has been conducted on the application of *Azolla pinnata* in specifically the treatment of textile dye-wastewater. After completing this study, hopefully we can be able to determine the efficacy of *Azolla pinnata* in enhancing the water quality of textile dye wastewater and to determine the concentration of wastewater in which *Azolla pinnata* performs most efficiently as a phytoremediation approach in a more cost-effective and environmentally friendly approach.

1.5 Research Questions

- Can the quality of textile dye wastewater be enhanced by treating with *Azolla pinnata*?
- In which concentration of textile dye wastewater, *Azolla pinnata* works most efficiently to enhance the water quality?

1.6 Research Objectives

- To determine the efficacy of *Azolla pinnata* in enhancing the water quality of collected textile dye wastewater.
- To determine the concentration in which *Azolla pinnata* performs most efficiently to treat the textile dye wastewater.

1.7 Limitations of the Study

The key limitations of this study are summarized as follows:

- The study only incorporates one test species. The findings may differ depending on the physiological characteristics of the test species.
- The experiment was carried out in natural atmospheric conditions; not in a controlled chamber. So, the weather impacts may differ from those observed in a controlled laboratory setting.

1.8 Outline of the Report

The next chapters are organized as follows:

- **Chapter Two**:

  Literature Review

  Under this chapter the local and international articles which are related to the thesis topic are reviewed and summarized and the research gaps are mentioned.

- **Chapter Three**:

  Conceptual framework

  Under this section the abstract representation of the research study was delineated which is connected with the research study goal that direct the collection and analysis of data.

  Methodology

  Under this section the detail information about samples and data collection as well as the methodology of experiment and data analysis are described.

- **Chapter Four**:

Results and Discussions

Under this chapter the results obtained from the experimental work and data analysis are interpreted and discussed elaborately.

- **Chapter Five** :

  Conclusion

  Under this chapter some conclusions are given.

- **References**

- **Appendix**

  All supportive document, tables, figures are listed here.

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CHAPTER TWO

LITERATURE REVIEW

2.1 Literature Review

2.1.1 Textile Dye Wastewater

According to Hossain (2023), the most significant economic sector in Bangladesh is the textile industry. More than 84% of export revenue is generated from textiles and items that involve textiles.

It has been mentioned by Sultana et al. (1970) that the textile industry utilizes a lot of water in its production operations, and it's extremely hazardous and polluted wastewaters are thrown untreated into sewers and drains. Numerous physicochemical contaminants are found in discharged textile dye wastewater at unacceptable levels.

The textile dye wastewaters also include different concentrations of metals/metalloids, salts, and organic pollutants, stated by Imran et al. (2014). These wastewaters also have different pH levels and high temperatures.

From Roy et al. (2020) it can be said that the environment is seriously threatened by the large-scale discharge of these dyes. In addition to the aesthetic issues posed by contaminated waterways, colors in textile effluent have been shown to slow down aquatic plants' rate of photosynthesis.

According to a study of Yusuf (2018), certain dyes and the products of their degradation are also carcinogenic and mutagenic, endangering the health of people and animals.

According to another study conducted in 2021 by Desai et al., azo dye-containing textile effluent is frequently discarded and used to irrigate crops in impoverished nations like Bangladesh, which depletes agricultural soils of these dangerous colors. These dyes have the potential to change the biological characteristics of soil, such as the makeup of microbial communities and the activity of enzymes. Additionally, it has been noted that plants are poisoned by azo dyes.

2.1.2 Phytoremediation

As per the definition by Khan et al. (2022), phytoremediation as a new environmentally friendly method for identifying, dissolving, and eliminating different kinds of toxins from the environment. It is also mentioned that plant species are used in the removal of various toxins that have negative impacts on human health and other biological systems. These plant species absorb these toxins from the surroundings and eliminate their harmful effects. This method has an advantage over traditional procedures since it is environmentally benign, whereas traditional techniques have negative impacts on the environment and biological system.

Industrial wastewater was treated by Alam & Hoque (2018) by *Salvinia cucullata Roxb.* and *Trapa natans L.* in order to assess the phytoremediation capacity by measuring the effectiveness of pollutant and nutrient removal. They found that removal efficiencies of total phosphorus (TP), nitrate-N, ammonium-N, biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), and ammonium-N in cultures of *T. natans* and *S. cucullata* were 43.02%, 31.04%, 20.00%, 5.26%, and 81.25%, respectively.

According to an assessment of the metals removal efficiency from the wastewater by Rashid (2021) using the aquatic macrophytes cultures of *Salvinia cucullata*, *Pistia stratoites*, *Eichornia crassipes,* in textile-dying effluent. When comparing industrial effluents to controls, higher values were found for temperature (56 °C), pH (12.32), electrical conductivity (12375 µS/cm), biochemical oxygen demand (835 mg/l), total suspended solids (2187 mg/l), total dissolved solids (6952 mg/l), turbidity (89.53 NTU), and total organic carbon (421.6 mg/l). Compared to the control (5.65 mg/l), the lowest concentration of DO in industrial effluents (0.12 mg/l) was much lower.

Alam & Hoque (2018) treated the wastewater to measure the nutrient removal efficiency by *Eichhornia crassipes L.* Wastewater's nutrient content was assessed both before and after treatment and the removal efficiencies of TDS, COD, N, P, K, and S from wastewater were, on average, 70.89%, 78.86%, 63.28%, 58.54%, 85.89%, and 60.44% for the *E. crassipes* cultures.

Chlorella spp. Beij. was used by Lim et al. (2010) to assess the possibility of a high-rate pond system for the treatment of textile effluent. The holding tank, which is the final stage of treating textile wastewater before it is discharged, held the effluent from a Malaysian clothing manufacturer. According to the authors, the system worked well as a polishing stage in the post-final discharge treatment of wastewater.

2.1.3 An Overview on Aquatic Macrophyte, *Azolla pinnata*

Acero (2019) treated wastewaters from Estero de San Miguel with *A. pinnata* and *E. crassipes*; the result revealed that T1 (*A. pinnata*) lowered the pH and ammonia-N (mg/l) of. T3 (combination of *A. pinnata* and *E. crassipes*) has significantly lowered the Phosphorous level of the wastewaters. Thus both aquatic macrophytes can be used as phytoremediation agents.

Chuleemas (2010) treated wastewater from animal farm mostly from poultry industry with *Azolla pinnata* and found that it reduced BOD about 41% and produced biomass of *Azolla pinnata* 90, 167, 245%in 100%, 50% and 25%dilution of wastewater respectively.

Shiomi & Kitoh (1987) treated wastewater by the aquatic macrophyte, *Azolla pinnata*. Given *Azolla*'s capacity for N fixation, effective phosphorus elimination would be anticipated even after N is used. From May through October, *Azolla pinnata* in outdoor batch cultures actively absorbed nutrients from the secondary treated effluent. When the P level was greater than the N level, the greatest growth—with a 2.5-day doubling time and the maximum nutritional absorption—was seen. Compared to other aquatic plants that have been documented to far, *Azolla pinnata* has a lower ability for absorbing nitrogen and a higher capacity for absorbing P.

Jayasundara (2022) found that *Azolla* can be used for wastewater treatment since it can lower the quantities of nitrogen, phosphate, heavy metals, chemical oxygen demand (COD), and biochemical oxygen demand (BOD) in wastewater. Also *Azolla* generates a significant amount of biomass from wastewater. In addition to its other advantages, he also discussed the use of *Azolla* in lowering eutrophication in lakes and streams.

CHAPTER THREE

MATERIALS

& METHODS

3.1 Conceptual Framework

This conceptual framework defines the relevant objectives for the research and illustrates the expected relationship between the objective and the research process. The conceptual framework of this research is illustrated in Figure 1:

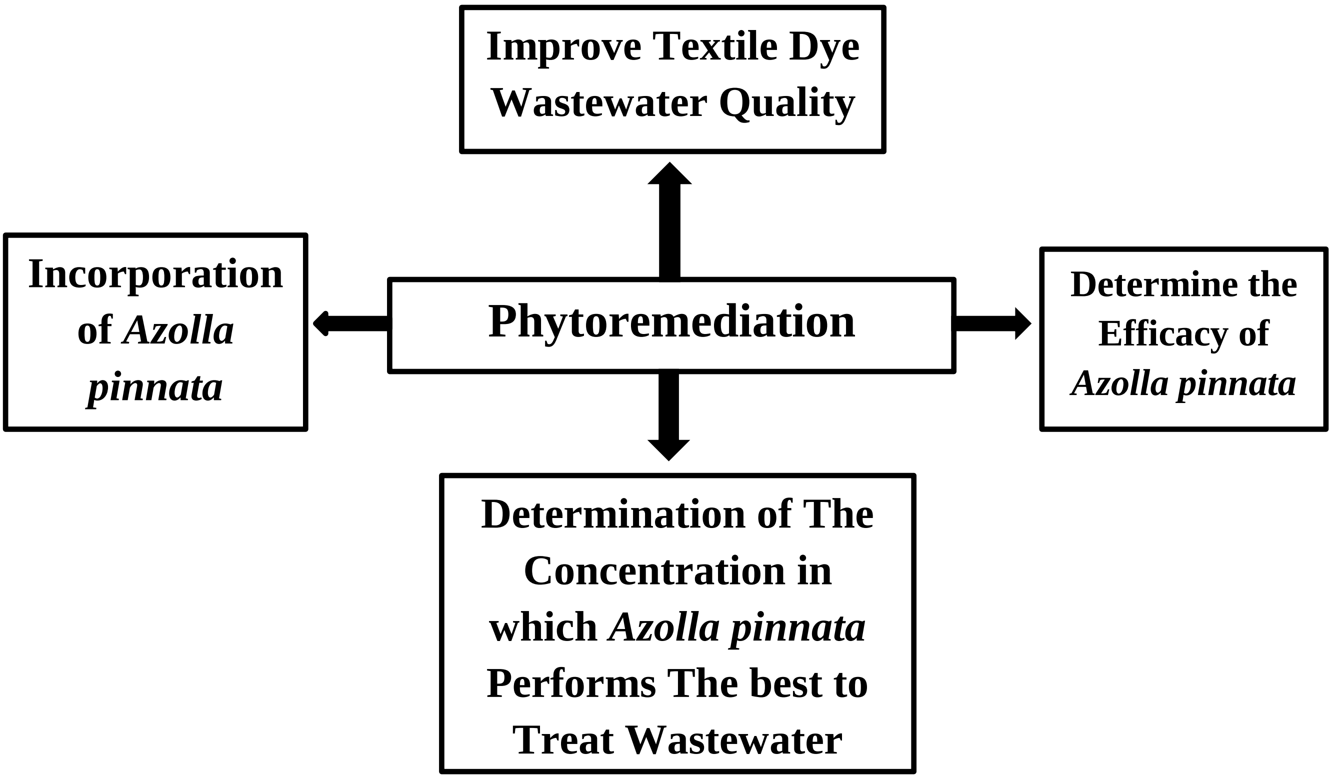

Figure 1: Conceptual Framework

3.2 Collection of Textile Dye Wastewater

The dye-wastewater sample was collected from a Textile Industry located in the BSCIC Industrial Estate, Tongi, Dhaka; on July 25th. For comparison, normal tap water was used as control sample that does not receive any industrial discharge. The sampling was done very cautiously using spot/grab sampling techniques (Rashid, 2021). The high density PVC containers (5liters capacity) were used for preserving sample water. They were thoroughly cleaned by washing with distilled water followed by repeated rinsing with water samples so as to avoid contamination. The bottles were kept air tight and labeled properly with location, collection time and date for later identification (Figure 2). Aeration during sampling was avoided as much as possible. Wastewater was dark red-colored and obnoxious unpleasant odor was found during collection.

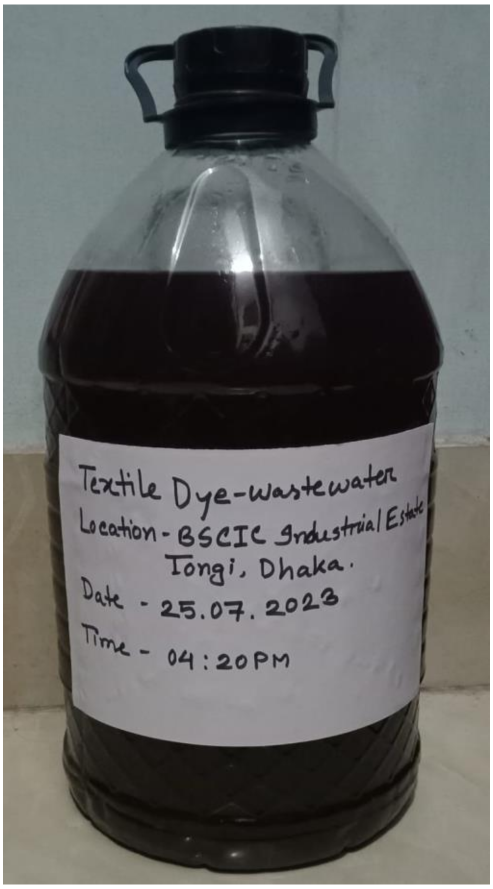

Figure 2: Collected Textile Dye-wastewater Sample

3.3 Collection of Aquatic Macrophyte (*Azolla pinnata*)

To carry out a Floating Aquatic Macrophyte based Treatment (FAMT) of textile dye-wastewater, *Azolla pinnata* was collected from paddy field with shallow water at Bangladesh Rice Research Institute, located in Joydebpur, Gazipur; that had no connection with domestic and industrial discharges. After collection, the macrophytes were washed thoroughly and maintained in a large surface container with fresh water (Figure. 3). The following aquatic macrophyte, *Azolla pinnata*, was taken as test species (Figure. 4).

Figure 3: Collected *Azolla pinnata*

Figure 4: *Azolla pinnata*

3.4 Analysis of Physicochemical Parameters of Textile Dye Wastewater

The sample waters were delivered to the laboratory within a day of being taken and analyzed the physicochemical parameters within one day. The samples were kept air tight without any chemicals addition.

The samples were put to examination in the laboratory to determine some physical and chemical parameters. At first 30%, 60% and 100% dilution samples were prepared with the collected dye wastewater. Analysis was carried out to determine various water quality parameters such as Temperature, pH, Dissolved Oxygen (DO), Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD_5), Electrical conductivity (EC), Total Dissolved Solids (TDS), Total Suspended Solids (TSS), Salinity, Turbidity and Absorbance by standardized methods.

3.4.1 Determination of Physical Parameters

3.4.1.1 Temperature

Temperature was measured using HI-9829 Multiparameter (Hanna instruments) (Figure. 5). For measuring these parameters at first the clean beaker was thoroughly rinsed with the sample water. After that the sample water was poured into the beaker. The probe was rinsed with sample water that would be tested and then immersed into the sample water in the beaker. Then the parameter was selected by enabling the required parameter (Temperature). When the value of the parameter became stable, the reading was taken.

3.4.2 Determination of Chemical Parameters

3.4.2.1 pH, Electrical Conductivity (EC), Total Dissolved Solids (TDS) and Turbidity

Some of the parameters such as pH, Electrical conductivity (EC), Total Dissolved Solids (TDS) and Turbidity were measured using HI-9829 Multiparameter (Hanna instruments). For measuring these parameters at first a clean beaker was taken and thoroughly rinsed with the sample water. Before pouring the sample water into beaker the sample bottle with sample water was shaken for 30 seconds so that the suspended materials spread into the whole sample water uniformly. After that 250 ml sample water was poured into the beaker. The probe was rinsed with sample water that would be tested and then immersed into the sample water in the beaker. Before immersing the probe the meter had to turn on by pressing the ON/OFF key. The meter will automatically recognize the probe and the sensors that are installed for measuring required parameters and identify them on the probe status screen. Then by accessing the measurement mode the parameters were selected by enabling the required parameters that were decided to determine. After submersing the multi sensor probe into the beaker, the probe was allowed to immerse into the sample water until the values of the parameters became stable. When the values of the parameters became stable the readings were taken. Then the sample water was again poured into the sample bottle and the beaker was washed with distilled water (APHA, 1976).

Figure 5: HI-9829 Multiparameter Device

3.4.2.2 Biological Oxygen Demand (BOD_5)

For measuring Biological Oxygen Demand (BOD_5) the portable Dissolve Oxygen Meter YSI Pro 20i was used. Before using the meter at first it was rinsed with sample water. Then a clean beaker was taken and thoroughly rinsed with the sample water. After that 300 ml sample water was poured into the beaker and then the tip of the probe was immersed in the sample to be tested. At the time of measuring DO the magnetic stirrer was used, to avoid any errors due to the presence of air bubbles on the membrane surface. For an accurate measurement, the probe was allowed to immersed three minutes for thermal equilibrium between the probe and the measurement sample. Then DO readings were noted from the display of the meter. For each sample three repetitive readings were taken. Then the sample water was again poured into the sample bottle as shown in Figure 6.

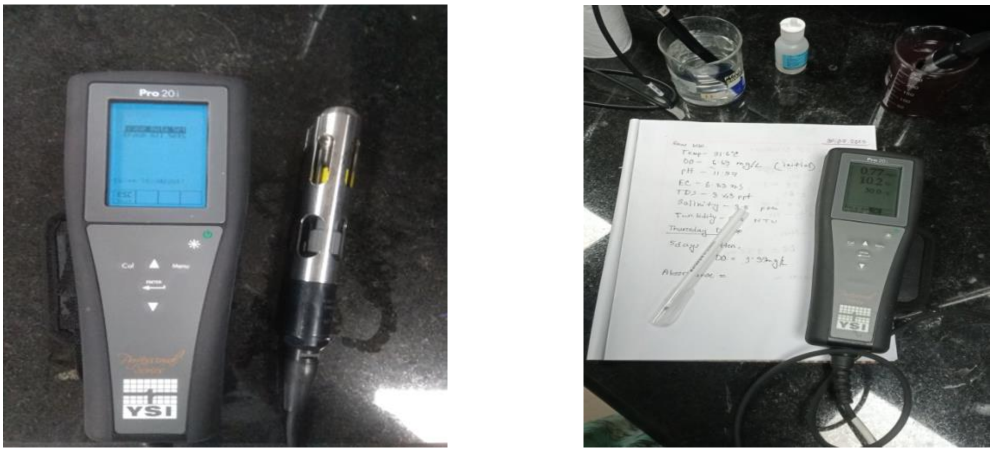

Figure 6: Dissolve Oxygen Meter (YSI Pro 20i)

After taking all the readings of DO the samples were tightly closed into the carbon paper wrapped BOD sample bottles (Figure 7). Then these samples were incubated at 20 degree Celsius for five days in dark chamber.

Figure 7: BOD Sample Bottle

After five days again the DO concentration of the samples were measured by same way. The change in DO concentration over five days represents the "oxygen demand" for respiration by the aerobic biological microorganisms in the sample. Then the final

DO (after 5 day incubation) reading was then subtracted from the initial DO (1st day reading) reading and found the BOD concentration (mg/l) result.

The general equation for the determination of a BOD_5 value is:

$$\mathbf{BOD_5\ (mg/l) = D_1 - D_2} \quad [01]$$

D_1= initial DO (dissolved oxygen level) of the sample,

D_2= final DO of the sample at the end of the 5 day incubation period

By this way the BOD of each sample was measured. (Delzer & McKenzie, 1999).

3.4.2.3 Chemical Oxygen Demand

Chemical Oxygen Demand (COD) of wastewater sample was measured using Lovibond RD 125 (Figure 8). At first 2 ml sample water was added to a 16 mm glass culture tube containing the digestion reagent and a catalyst. The tube is capped with a polypropylene, PTFE lined cap. Then the blank sample tube and the wastewater containing tube heated in a block digester at 150 degree Celsius for 2 hours. The orange Hexavawlentste chromium is reduced to a greenish trivalent chromium. After two hours, the tubes are removed from the oven or digester, cooled, and measured spectrophotometrically at 600 nm. Before measuring the COD of the sample waters at first the meter was calibrated with blank sample (Editor, 2021). By this way all the samples’ COD was measured.

Figure 8: Measurement of COD using Lovibond RD 125

3.4.2.4 Total Suspended Solid

For measuring Total Suspended Solid (TSS) at first the sample was shaken vigorously and 50 ml sample was transferred to the graduated cylinder. The filter paper (Whatman type Glass microfiber filter paper 47 mm) was weighted using analytical balance in gram (g) unit. Then that pre weighted filter paper was placed on clean funnel and that filter paper was wetted by small volume of distilled water to fix it. Then the funnel with filter paper was placed on conical flask and pour 50 ml sample through the filter paper. After that the empty graduated cylinder was rinsed with distilled water and poured through the filter 3 times to remove any dissolved solids trapped in and on the filter. Then the filter paper was transferred to the labelled aluminium dish and the dish with filter papers was placed in an oven and dry it at 104°C for one hour. After one hour the filter paper was brought out from the oven and place it in a desiccator until they reach room temperature. After that filter paper with dried solids was weighted on a balance and record the weight. Then this drying and cooling step was repeated as many as are necessary to obtain a reading ± 0.0005 g from the previous weight (Coleparmer, 2019).

Then the result was calculated with the following equation. 02:

Total Suspended Solids (mg/l) = {(F-I) × 1000}/ V [02]

Where:

F = Weight of filter + dried residue in g

I = Weight of filter in g

V = Volume of sample filtered in liter

3.4.2.5 Salinity

For measuring Salinity the High Accuracy Digital Portable Multiparameter Water Analyzer was used. Before using the meter at first it was rinsed with sample water. Then a clean beaker was taken and thoroughly rinsed with the sample water. After that 250 ml sample water was poured into the beaker. Before immersing the probe the meter had to turn on by pressing the ON/OFF key. The meter will automatically recognize the probe and the sensors that are installed for measuring required parameters and identify them on the probe status screen. Then by accessing the measurement mode the salinity was selected by enabling the required parameter that was decided to determine. After submersing the multi sensor probe into the beaker, the probe was allowed to immerse into the sample water until the values of the parameter became stable. When the values of the parameter became stable the readings were taken. For each sample three repetitive readings were taken. Then the sample water was again poured into the sample bottle and the beaker was washed with distilled water.

3.4.2.6 Absorbance

Absorbance was measured using UV-6300PC Double Beam Spectrophotometer. For measuring this parameter at first a clean beaker and cuvette was taken and thoroughly rinsed with the sample water. Before pouring the sample water into beaker the sample bottle with sample water was shaken for 30 seconds so that the suspended materials spread into the whole sample water uniformly. After that 250 ml sample water was poured into the beaker. Then 5 ml samples were withdrawn from the beaker and poured into the cuvette. After that the cuvette was placed into the spectrophotometer and maximum absorbance wavelength was observed for the sample and then absorbance value was noted down for that particular wavelength. For each sample three repetitive readings were taken (Hzdg, n.d.). Then other samples' absorbances were measured by following the same technique.

3.4.3 Experimental set up

Experiments were performed during August-September, 2023. Batch experiments were conducted in square plastic tubs with a working depth of 8 cm, a surface area of 12 cm^2 and a capacity of 900 ml. In this research, the plants were grown in three different percentage 30%, 60% and 100% of wastewaters. A control experiment was also carried out with wastewater free fresh water. After preparing the different dilutions with the dye-wastewater, experimental tubs were filled with 500 ml of each solution including the control one and 11 different physicochemical parameters were measured. Then 5 g (Fresh weight) of *Azolla pinnata* was added to each experimental tub as shown in Figure 9. During experiments, the phenotypic impacts on test species and physicochemical parameters were recorded with a 24 hours interval. Each experiment was repeated three times and the average of the data was collected. The total treatment period was 5 days with 3 replicates for each treatment.

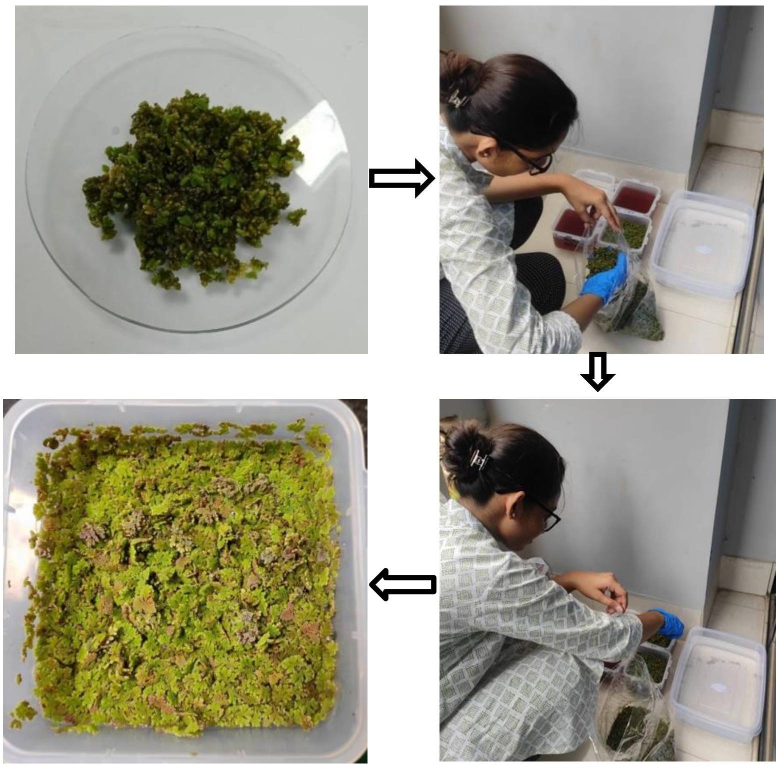

Figure 9: Set up of Treatment Tubs

3.4.4 Different Treatments and Doses

Table 1: Four different treatments with their doses

Treatments	Doses	Total Volume of Water
T_0 (0%)	500 ml fresh water + 5 g *Azolla pinnata*	500 ml
T_1 (30%)	150 ml dye-wastewater + 350 ml freshwater + 5 g *Azolla pinnata*	500 ml
T_2 (60%)	300 ml dye-wastewater + 200 ml freshwater + 5 g *Azolla pinnata*	500 ml
T_3 (100%)	500 ml dye-wastewater + 5 g *Azolla pinnata*	500 ml

3.4.5 Data Analysis:

3.4.5.1 Evaluation of Pollutant Removal Efficiency (%)

$$\text{PRE (\%)} = ((\boldsymbol{A} - \boldsymbol{B}))/\boldsymbol{A} \times \mathbf{100} \qquad [03]$$

Here,

A = Concentration at starting

B = Concentration at the end (Alam & Hoque, 2018)

3.4.5.2 Statistical Analysis

To detect significant differences between treatments at the 5% level of confidence, the data were processed using Microsoft Excel version 10 and the one-way ANOVA (Analysis of Variance) technique.

CHAPTER FOUR

RESULTS & DISCUSSIONS

4.1 Results

4.1.1 Background Analysis of the Samples

Table 2 displays the physicochemical characteristics of 100% dye-wastewater from the textile dying industry. Additionally, Tables 3, 4, and 5 provide the initial (before treatment) physicochemical parameters of 60%, 30%, and 0% dye-wastewater, respectively.

Table 2: Background analysis of the collected wastewater (100% wastewater)

Parameters	Units	Concentrations
Temperature	℃	35.6
pH	-	11.97
DO	mg/l	0.69
BOD	mg/l	384
COD	mg/l	500.5
EC	µS/cm	10573
TSS	mg/l	5343
TDS	ppm	2600
Salinity	psu	3.8
Turbidity	NTU	178
Absorbance	-	1.094

Table 3: Background analysis of the diluted wastewater (60% wastewater)

Parameters	Units	Concentrations
Temperature	℃	31.8
pH	-	10.89
DO	mg/l	1.32
BOD	mg/l	229.7
COD	mg/l	473.3
EC	µS/cm	7588
TSS	mg/l	801.3
TDS	ppm	4293.5

Salinity	psu	1.72
Turbidity	NTU	123
Absorbance	-	0.81

Table 4: Background analysis of the diluted wastewater (30% wastewater)

Parameters	Units	Concentrations
Temperature	℃	29.4
pH	-	10.58
DO	mg/l	3.26
BOD	mg/l	125
COD	mg/l	254
EC	µS/cm	4345
TSS	mg/l	339
TDS	ppm	2976
Salinity	psu	0.91
Turbidity	NTU	41.63
Absorbance	-	0.284

Table 5: Background analysis of the control (0% wastewater)

Parameters	Units	Concentrations
Temperature	℃	27.1
pH	-	7.53
DO	mg/l	5.76
BOD	mg/l	5.76
COD	mg/l	56
EC	µS/cm	275
TSS	mg/l	42.7
TDS	ppm	687.5
Salinity	psu	0.23
Turbidity	NTU	3.5
Absorbance	-	0.044

4.1.2 Physicochemical characteristics of wastewater before and after treatment

Tables 5, Table 4, Table 3, and Table 2 provide the initial values of the physicochemical parameters for the treatments, T0 (0%), T1 (30%), T2 (60%) and T3 (100%). The findings show that the initial values of the physicochemical parameters of the dye-wastewater containing treatments (T1, T2 and T3), with the exception of temperature, are significantly higher than the control water and exceed the DoE (2003) limit.

To ascertain the most important dose for treating wastewater containing dye and to assess the improvement of various water quality parameters of the four different treatments through standardized procedures, a comparison analysis was conducted between Initial and Final observations with standards for industrial wastewater prescribed by DoE.

4.1.2.1 Temperature (°C)

The average initial temperatures during sampling for T0 (0%), T1 (30%), T2 (60%) and T3 (100%) were 27.1°C, 29.4°C, 31.83°C and 35.53°C respectively. After 120hours of treatment, the average final temperatures for T_0 (0%), T1 (30%), T2 (60%) and T3 (100%) were 26.2°C, 28°C, 28.33°C and 28.5°C respectively. The following chart 1 depicts the changes in comparison with standards for industrial wastewater by DoE:

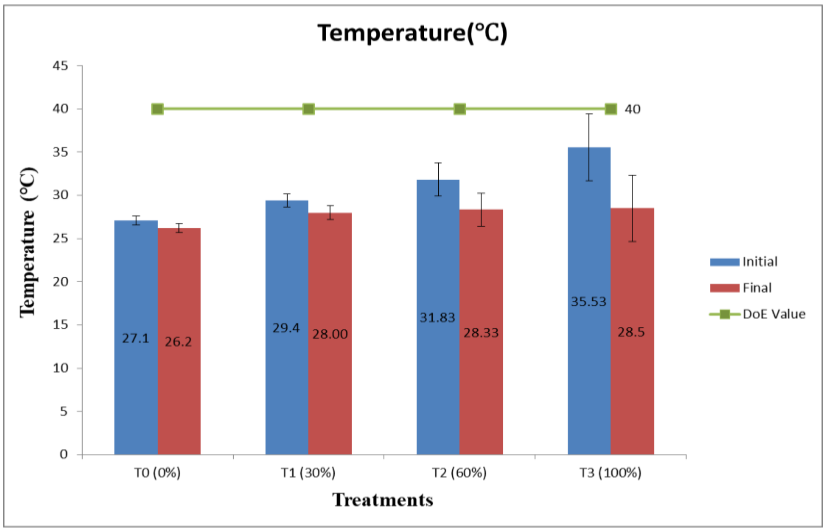

Chart 1: Comparison between initial & final temperature

Chart 1 shows that temperatures of all the treatments have decreased comparing to the initial values and also the final values are much lower than the limit of DoE (2003) which is 40°C.

4.1.2.2 pH

The average initial pH during sampling for T0 (0%), T1 (30%), T2 (60%) and T3 (100%) were 7.53, 10.58, 10.89 and 11.97 respectively. After 120hours of treatment, the average final pH for T0 (0%), T1 (30%), T2 (60%) and T3 (100%) were 6.7.08, 7.08, 8.64 and 9.81 respectively. The following chart.2 depicts the improvement in comparison with standards for industrial waste by DoE:

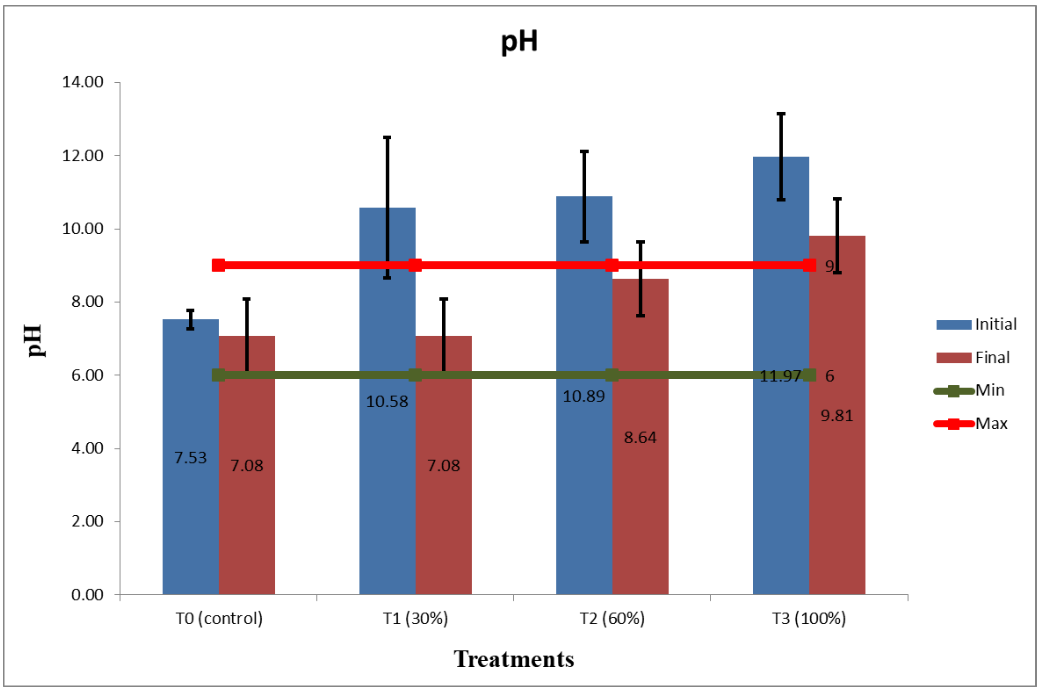

Chart 2: Comparison between initial & final pH

Chart 2 shows that the initial values of the pH of dye wastewater containing treatments (T1, T2 and T3) are much higher than the control water and exceeded the limit of DoE (2003). Incorporation of *Azolla pinnata* has improved the pH level of the treatments. T1 (30%) showed the most efficient result in improving pH, then comes the T2 (60%) and finally comes the T3 (100%). Even though T3 (100%) has improved the pH value but couldn't avail within the DoE range.

4.1.2.3 EC (µS/cm)

The average initial EC during sampling for T0 (0%), T1 (30%), T2 (60%) and T3 (100%) were 275.00, 4345.00, 7588 and 10573 µS/cm respectively. After 120hours of treatment, the average final pH for T0 (0%), T1 (30%), T2 (60%) and T3 (100%) were 256.67, 2024.67, 4739.33 and 7946.33 µS/cm respectively. The following chart 3 depicts the improvement in comparison with standards for industrial wastewater by DoE:

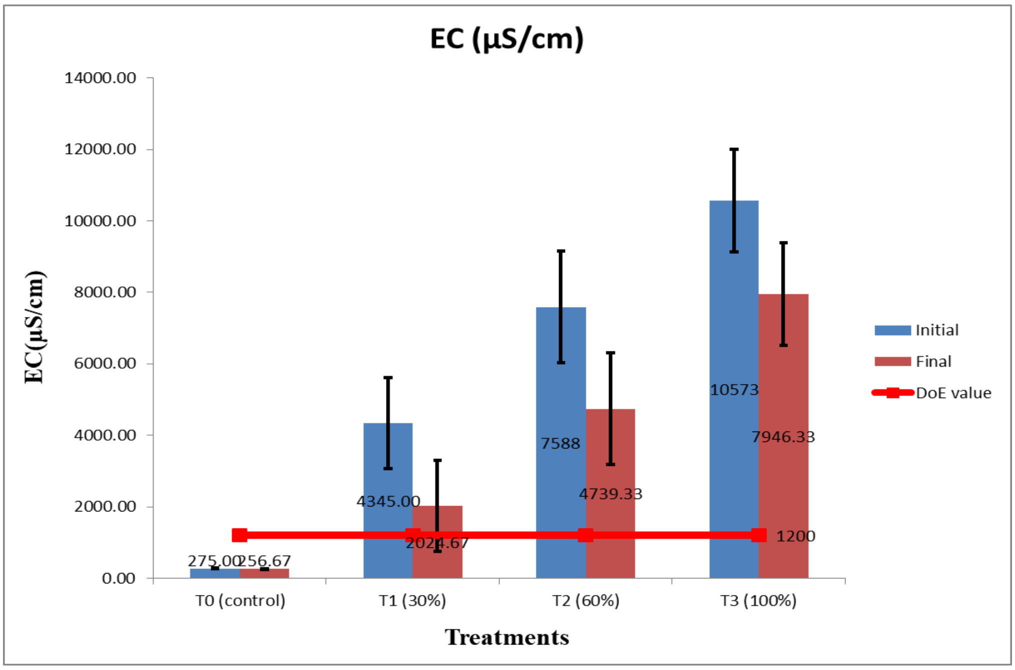

Chart 3: Comparison between initial & final EC

Char 3 shows that the initial values of EC of treatments in treatments that contains dye wastewater (T1, T2 and T3) are much higher than the control water and exceeded the limit of DoE (2003). Incorporation of *Azolla pinnata* has improved the EC level of the treatments. T1 (30%) showed the most efficient result in improving EC which is 53.40%, then comes the T2 (60%) with an improvement of 37.54% and finally comes the T3 (100%) that improved about 24.85% EC from the initial one. Even though all the three treatments have improved the EC value but couldn't avail the DoE Limit.

4.1.2.4 DO (mg/l)

The average initial DO during sampling for T0 (0%), T1 (30%), T2 (60%) and T3 (100%) were 5.76 mg/l, 3.26 mg/l, 1.32 mg/l and 0.67 mg/l respectively. After 120hours of treatment, the average final DO for T0 (0%), T1 (30%), T2 (60%) and T3

(100%) were 6.08 mg/l, 5.84 mg/l, 2.18 mg/l and 1.05 mg/l respectively. The following chart 4 depicts the improvement in comparison with standards for industrial wastewater by DoE:

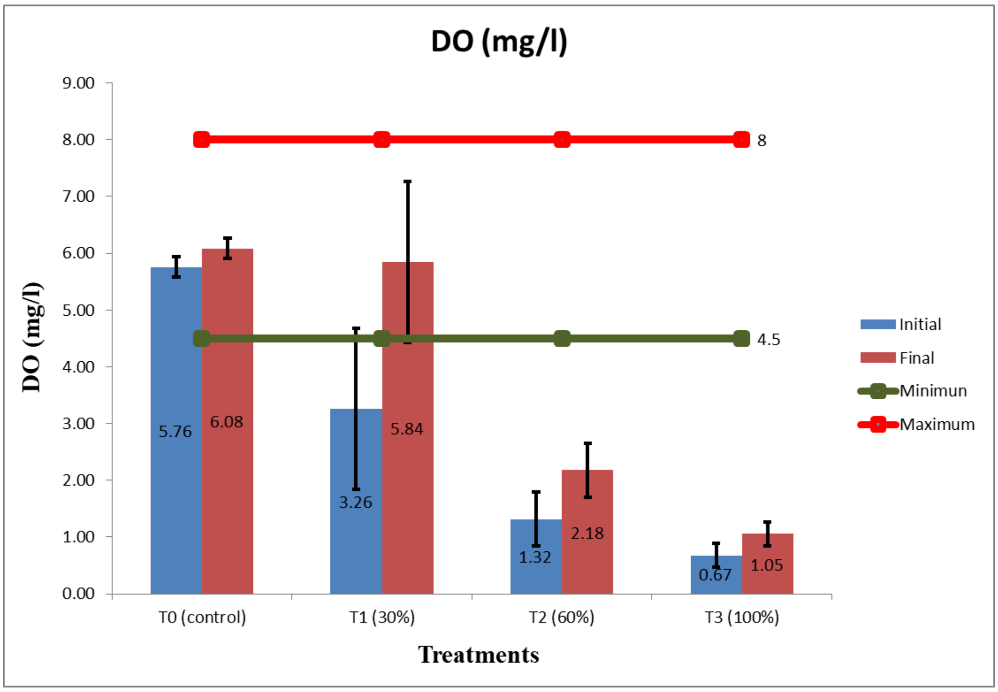

Chart 4: **Comparison between initial & final DO**

Chart 4 shows that the initial values of DO in dye wastewater containing treatments (T1, T2 and T3) are much lower than the control water and the limit of DoE (2003). Incorporation of *Azolla pinnata* has increased the DO level of the treatments. T1 (30%) showed the most efficient result in improving DO level which is 44.21% and reached within the DoE range. Then comes the T2 (60%) with an improvement of 39.60% and finally comes the T3 (100%) that increased about 36.08% DO from the initial values. Even though the T2 and T3 have increased the dissolved oxygen than before but couldn't reach within the DoE range.

4.1.2.5 BOD_5 (mg/l)

The average initial BOD during sampling for T0 (0%), T1 (30%), T2 (60%) and T3 (100%) were 5.76 mg/l, 125 mg/l, 229.67 mg/l and 384.67 mg/l respectively. After 120hours of treatment, the average final BOD_5 for T0 (0%), T1 (30%), T2 (60%) and T3 (100%) were 5.17 mg/l, 41.67 mg/l, 132 mg/l and 285 mg/l respectively. The following chart 5 depicts the improvement in comparison with standards for industrial wastewater by DoE:

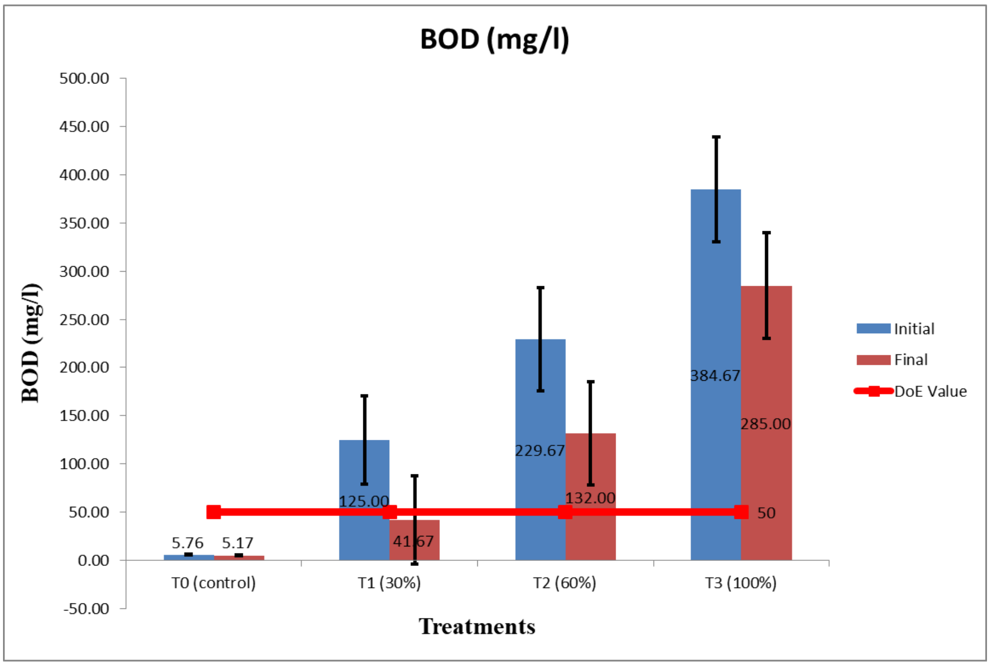

Chart 5: Comparison between initial & final BOD_5

Chart 5 shows that the initial values of BOD_5 of treatments in treatments that contains dye wastewater (T1, T2 and T3) are much higher than the control water and exceeded the limit of DoE (2003) which is 50 mg/l. Incorporation of *Azolla pinnata* has improved the BOD level of the treatments. T1 (30%) showed the most efficient result in improving BOD and reach a value within the DoE limit. Then comes the T2 (60%) and T3 (100%) with an improvement of 42.53% and 25.91% respectively. Even though the T2 and T3 have improved the BOD level but couldn't avail the DoE Limit.

4.1.2.6 COD (mg/l)

The average initial COD during sampling for T0 (0%), T1 (30%), T2 (60%) and T3 (100%) were 56 mg/l, 254 mg/l, 473.33 mg/l and 500.50 mg/l respectively. After 120hours of treatment, the average final COD for T0 (0%), T1 (30%), T2 (60%) and T3 (100%) were 50.13 mg/l, 114.17 mg/l, 286 mg/l and 355.33 mg/l respectively. The following chart 6 depicts the improvement in comparison with standards for industrial wastewater by DoE:

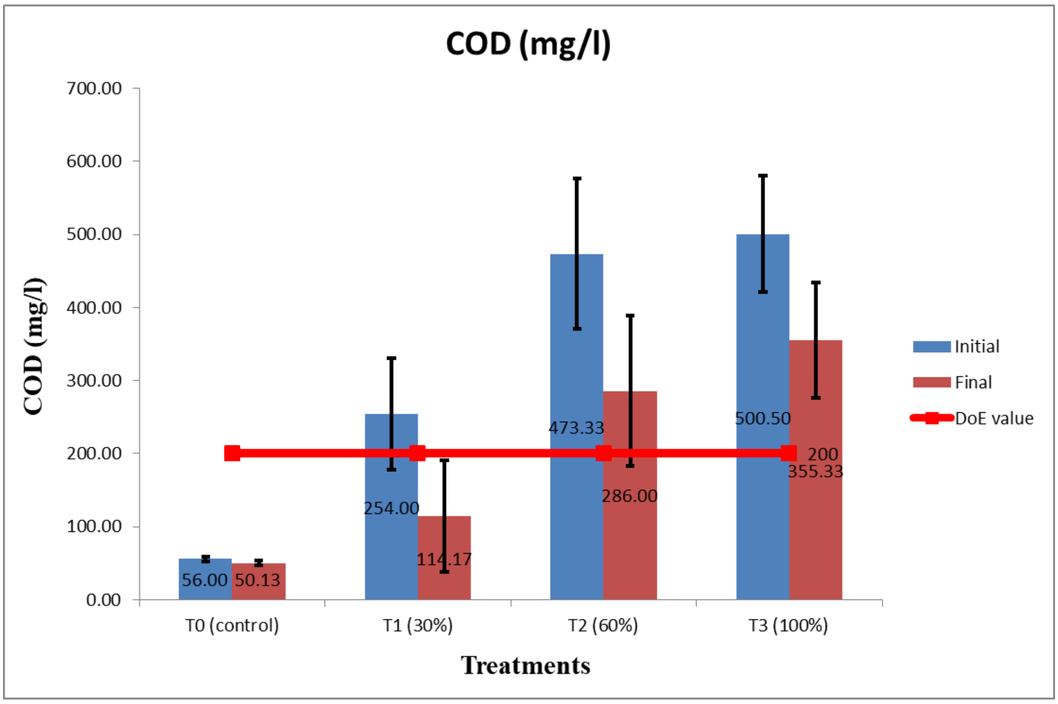

Chart 6: Comparison between initial & final COD

Chart 6 shows that the initial values of COD of dye wastewater containing treatments (T1, T2 and T3) are much higher than the control water and exceeded the limit of DoE (2003) which is 200 mg/l. Incorporation of *Azolla pinnata* has improved the COD level of the treatments. T1 (30%) showed the most efficient result in decreasing COD level and reach a value within the DoE limit. Then comes the T2 (60%) and T3 (100%) with an improvement of 39.58% and 29% respectively. Even though the T2 and T3 have improved the COD level but couldn't avail the DoE Limit.

4.1.2.7 TSS (mg/l)

The average initial TSS during sampling for T0 (0%), T1 (30%), T2 (60%) and T3 (100%) were 42.67 mg/l, 339 mg/l, 801.33 mg/l and 2601.33 mg/l respectively. After 120hours of treatment, the average final TSS for T0 (0%), T1 (30%), T2 (60%) and T3 (100%) were 39.10 mg/l, 140.33 mg/l, 477.33 mg/l and 1803.57 mg/l respectively. The following chart 7 depicts the improvement in comparison with standards for industrial wastewater by DoE:

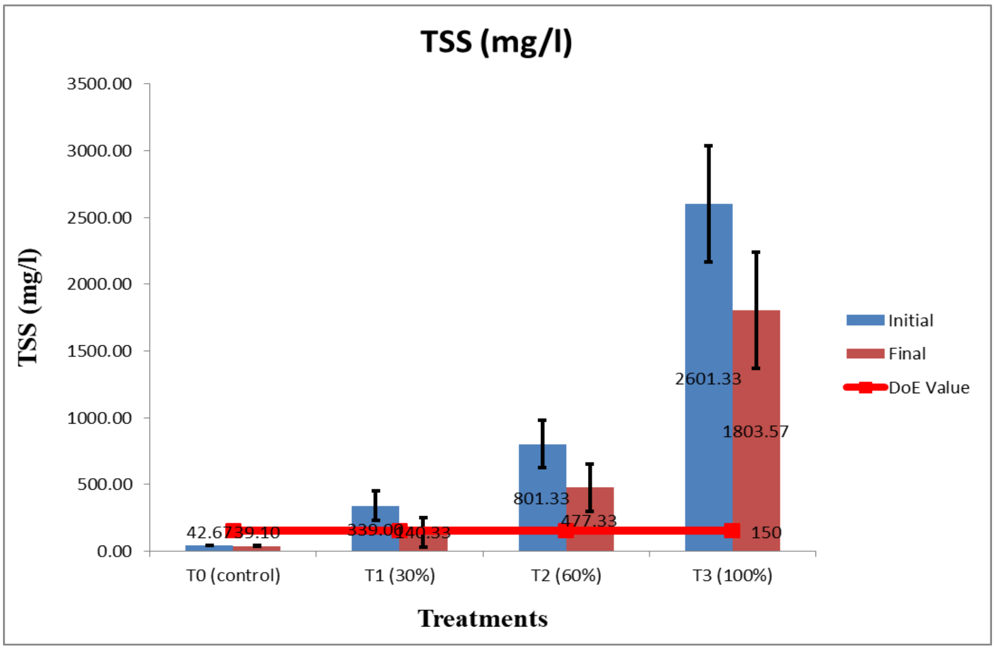

Chart 7: Comparison between initial & final TSS

Chart 7 shows that the initial values of TSS of dye wastewater containing treatments (T1, T2 and T3) are much higher than the control water and exceeded the limit of DoE (2003) which is 150 mg/l. Incorporation of *Azolla pinnata* has improved the TSS level of the treatments. T1 (30%) showed the most efficient result in decreasing TSS and reach a value within the DoE limit. Then comes the T2 (60%) and T3 (100%) with an improvement of 40.43% and 30.67% respectively. Even though the T2 and T3 have decreased the TSS in wastewater but couldn't avail the DoE Limit.

4.1.2.8 TDS (ppm)

The average initial TDS during sampling for T0 (0%), T1 (30%), T2 (60%) and T3 (100%) were 687.50 ppm, 2976 ppm, 4293.50 ppm and 5342.67 ppm respectively. After 120hours of treatment, the average final TSS for T0 (0%), T1 (30%), T2 (60%) and T3 (100%) were 595.67 ppm, 1581.83 ppm, 2546 ppm and 3386 ppm respectively. The following chart 8 depicts the improvement in comparison with standards for industrial wastewater by DoE:

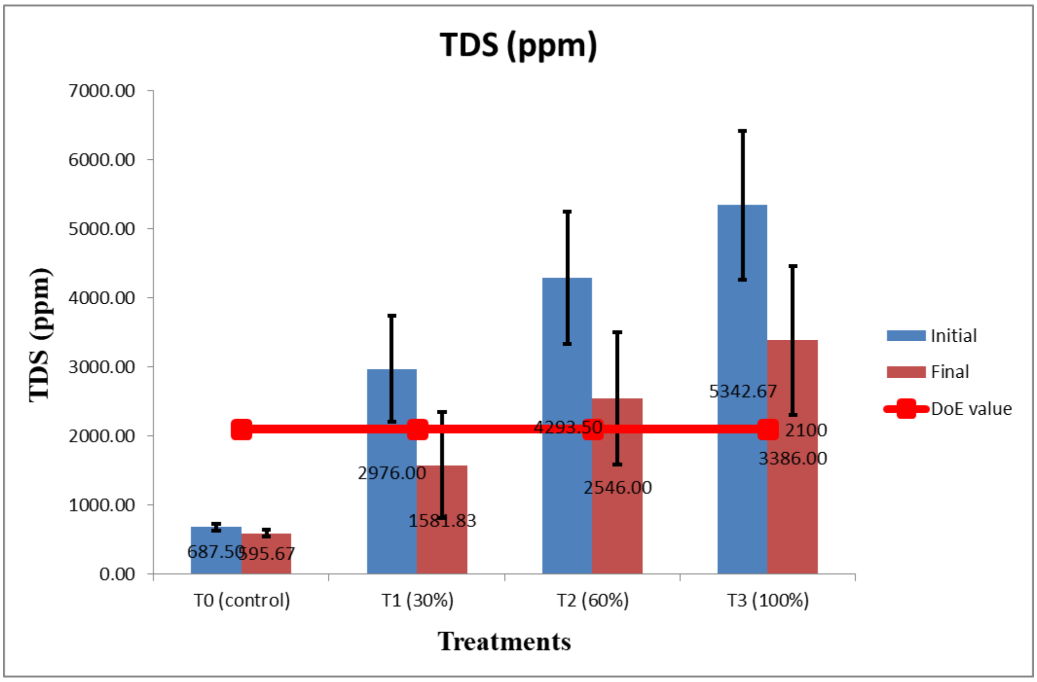

Chart 8: Comparison between initial & final TDS

Chart 8 shows that the initial values of TDS of treatments in treatments that contains dye wastewater (T1, T2 and T3) are much higher than the control water and exceeded the limit of DoE (2003) which is 2100 ppm. Incorporation of *Azolla pinnata* has decreased the TDS level of the treatments. T1 (30%) showed the most efficient result in decreasing TDS and reach a value within the DoE limit. Then comes the T2 (60%) and T3 (100%) with an improvement of 40.70% and 36.62% respectively. Even though the T2 and T3 have decreased the TDS in wastewater but couldn't avail the DoE Limit.

4.1.2.9 Turbidity (NTU)

The average initial Turbidity during sampling for T0 (0%), T1 (30%), T2 (60%) and T3 (100%) were 3.50 NTU, 41.63 NTU, 123 NTU and 179 NTU respectively. After 120hours of treatment, the average final TSS for T0 (0%), T1 (30%), T2 (60%) and T3 (100%) were 3 NTU, 9.63 NTU, 49.57 NTU and 108.53 NTU respectively. The following chart 9 depicts the improvement in comparison with standards for industrial wastewater of DoE:

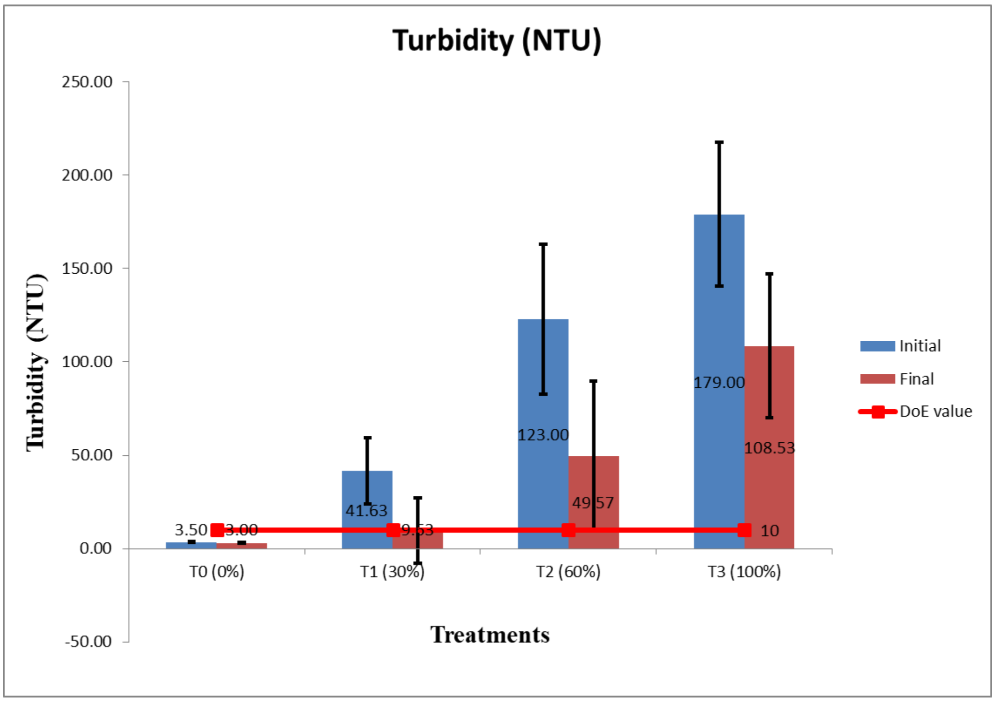

Chart 9: Comparison between initial & final Turbidity

Chart 9 shows that the initial level of Turbidity of treatments in treatments that contains dye wastewater (T1, T2 and T3) are much higher than the control water and exceeded the limit of DoE (2003) which is 10 NTU. Incorporation of *Azolla pinnata* has decreased this level in the treatments. T1 (30%) showed the most efficient result in decreasing Turbidity and reach a value within the DoE limit which is 9.63. Then comes the T2 (60%) and T3 (100%) with an improvement of 59.70% and 39.37% respectively. Even though the T2 and T3 have decreased the turbidity in wastewater but couldn't avail the DoE Limit.

4.1.2.10 Salinity (psu)

The average initial Salinity during sampling for T0 (0%), T1 (30%), T2 (60%) and T3 (100%) were 0.23 psu, 0.91 psu, 1.72 psu and 3.80 psu respectively. After 120 hours of treatment, the average final TSS for T0 (0%), T1 (30%), T2 (60%) and T3 (100%) were 0.21 psu, 0.43 psu, 1.17 psu and 2.93 psu respectively. The following chart 10 depicts the improvement in salinity level:

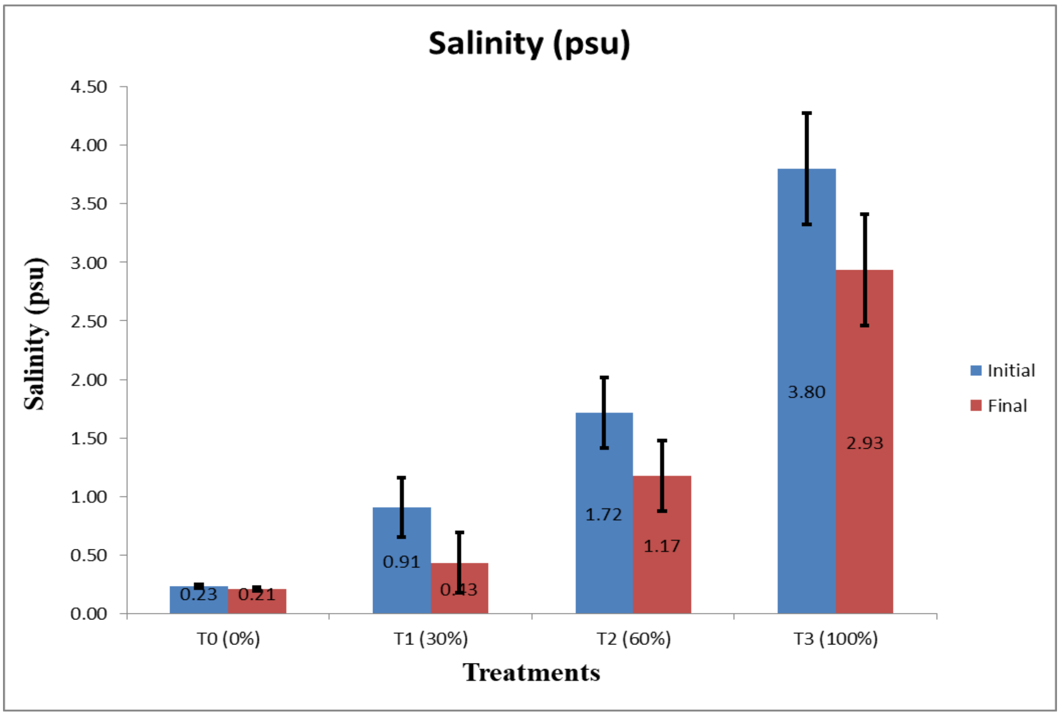

Chart 10: Comparison between initial & final Salinity

Chart 10 shows that the initial level of Salinity in treatments that contains dye wastewater (T1, T2 and T3) are much higher than the control water. Incorporation of Azolla has decreased this level in the treatments. T1 (30%) showed the most efficient result in decreasing salinity which is 52.21%. Then comes the T2 (60%) and T3 (100%) with an improvement of 31.65% and 22.81% respectively. Even though the T2 and T3 have decreased the salinity level in wastewater but couldn’t as efficiently as the T1 (30%).

4.1.2.11 Absorbance

The average initial Absorbance during sampling for T0 (0%), T1 (30%), T2 (60%) and T3 (100%) were 0.04, 0.28, 0.81 and 1.10 respectively. After 120hours of treatment, the average final TSS for T0 (0%), T1 (30%), T2 (60%) and T3 (100%) were 0.04, 0.06, 0.36 and 0.80 respectively. The following chart.11 depicts the improvement in salinity level:

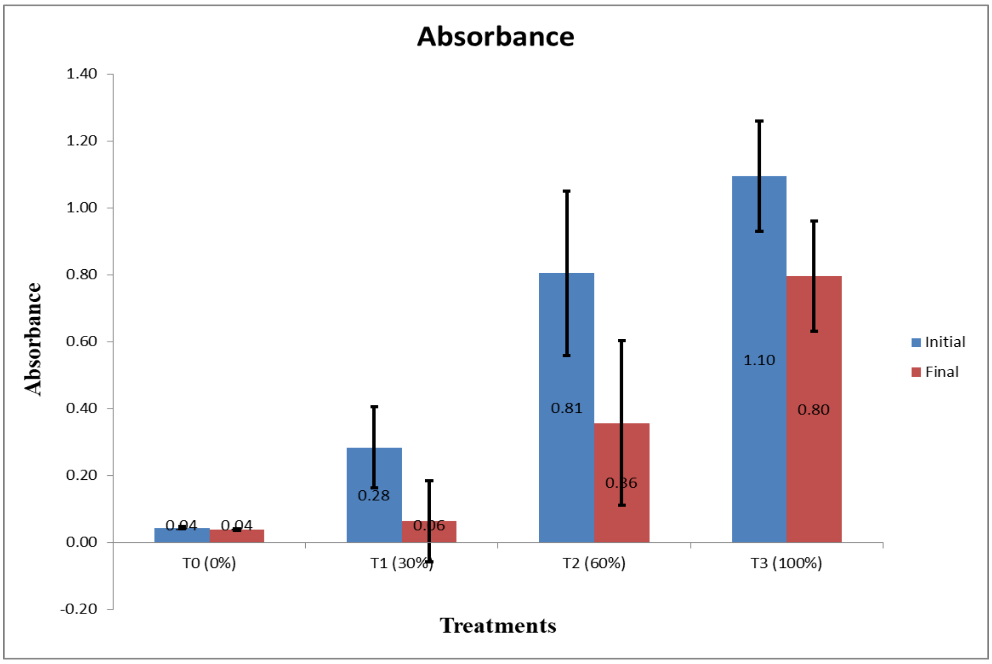

Chart 11: Comparison between initial & final Absorbance

Chart 11 shows that the initial level of Absorbance in treatments that contains dye wastewater (T1, T2 and T3) are much higher than the control water. Incorporation of Azolla has decreased this level in the treatments. T1 (30%) showed the most efficient result in decreasing absorbance which is 77.49%. Then comes the T2 (60%) and T3 (100%) with an improvement of 55.65% and 27.29% respectively. Even though the T2 and T3 have decreased the absorbance level in wastewater but couldn't as efficiently as the T1 (30%).

4.2 Discussion

4.2.1 Observation of overall Water Quality Improvement (%)

Chart 12 depicts the overall water quality improvement (%) in the four treatment set up.

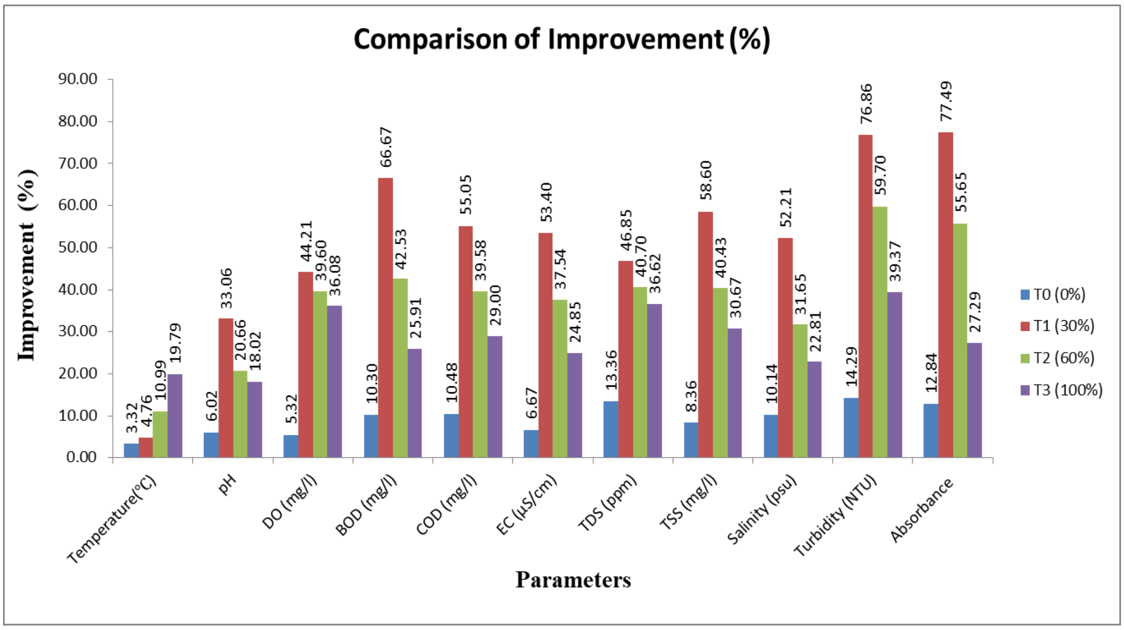

Chart 12: Comparison of Improvement (%)

Chart 12 depicts that the T1 (30%) treatment shows more improvement in most of the parameters comparing to the other two treatments that contained wastewater.

Higher values of Temperature (35.53°C), pH (11.97), Biochemical Oxygen Demand (384.7 mg/l), Chemical Oxygen Demand (500.5 mg/l), Electrical Conductivity (10573 µS/cm), Total Suspended Solids (2601.3 mg/l), Total Dissolved Solids (5342 ppm), Salinity (3.8 psu), Turbidity (179 NTU) and Absorbance (1.096) were recorded in the collected textile dye wastewater (Table 2).

The temperature of the collected textile dye wastewater (35.53°C) was close to the limit of DoE (40°C) but in control water it was much less than the DoE limit (Chart 1). Higher rate of pH is detrimental to aquatic life, including fish, microbes, and plants. The pH level of water affects the other characteristics of the aquatic system, the activity of the organisms, and the concentration of harmful compounds in the aquatic environment (Kant, 2012). The reported pH value of the wastewater was 11.97, which exceeds the limit of DoE (6-9) (Chart 2). The DO value of the textile dye wastewater was 0.67 mg/l, which is much below the limit of DoE (4.5-8) (Chart 4). The low DO value of the wastewater of the textile suggested that textile industry was creating a significant amount of organic chemicals, most likely dyes, which are wastes with a high oxygen demand (Imran et al., 2014). Biochemical oxygen demand (BOD) is an indicator of the amount of biodegradable organic matter in the system. The amount of oxygen needed by bacteria and other microorganisms to biochemically break down and change organic matter in wastewater in an aerobic environment is known as BOD. The investigated textile wastewater had a BOD value of 384.7 mg/l, which was much higher than the DoE limit of 50 mg/l (Chart 5). According to Kabir et al. (2002), an excessively high BOD_5 value can seriously harm aquatic flora and fauna, including fish and microorganisms. The Chemical Oxygen Demand (COD) value represents the extent of pollution of a water body; the more the COD value, the more the pollution is (Jayasundara, 2022). The value of COD of the wastewater was found 500.5 mg/l which exceeded the limit of DoE (200 mg/l) (Chart 6). The value of EC of the collected wastewater was found 10573 µS/cm, which exceeded the limit of DoE (1200 µS/cm) and indicates the presence of large amount of ionic substances in the textile dye wastewater. Higher value of EC is very harmful for aquatic life and irrigation purposes (Chart 3). The collected wastewater with textile dyes included 2601.3 mg/l of total suspended solids (TSS), more above the DoE's recommended limit of 150 mg/l (Chart 7). The term TSS refers to the suspended contaminants found in water. High TSS levels in water bodies can prevent aquatic plants from receiving the sunlight needed for photosynthesis. A high concentration of dissolved particles in water raises its density, affects freshwater species' ability to regulate their osmoregulation, and decreases the solubility of gases. It's possible that the sample's elevated pH caused low molecular mass organic bases from the dye industry to dissolve. Higher TDS values result from this as well (Moore et al., 1960). The wastewater from the textile industry had a high pH value, which was accompanied by a high TDS value (5342.6 mg/l) that exceeded the DoE requirement Chart 8). Plankton and other tiny creatures, silt, clay, and other suspended and colloidal particles, as well as finely split organic and inorganic debris, are the main causes of turbidity in water (Momtaz et al., 2013). The collected textile wastewater's turbidity was 178 NTU, over the DOE's permissible limit of 10 NTU (Chart 9). The presence of different dye contents and other substances increases the salinity level of the wastewater, which is 3.8 psu (Chart 10) and the absorbance value is 1.096 due to the presence of impurities (Chart 11).

Incorporation of *Azolla pinnata* in T3 (100%) has improved almost all the physicochemical parameters; though comparatively less efficiently than T2 (60%) and T1 (30%). The improvement values are Temperature (19.79%), pH (18.02%), Dissolved Oxygen (41.62%), Chemical Oxygen Demand (29%), Biochemical Oxygen Demand (41.62%), Electrical conductivity (24.85%), Total Dissolved Solids (36.62%), Total Suspended Solids (30.67%), Salinity (22.81%), Turbidity (39.37%) and Absorbance (27.29%) (Chart.12).

When *Azolla pinnata* was incorporated in 60% concentrated dye-wastewater, it also improved almost all the physicochemical parameters; though comparatively less efficiently than T1 (30%). The improvement values are Temperature (10.99%), pH (20.66%), Dissolved Oxygen (39.60%), Chemical Oxygen Demand (39.58%), Biochemical Oxygen Demand (42.53%), Electrical conductivity (37.54%), Total Dissolved Solids (40.70%), Total Suspended Solids (40.43%), Salinity (31.65%), Turbidity (59.70%) and Absorbance (55.65%) (Chart 12).

In 30% concentrated dye-wastewater, *Azolla pinnata* most efficiently improved all the physicochemical parameters. The improvement values are Temperature (4.76%), pH (33.06%), Dissolved Oxygen (44.21%), Chemical Oxygen Demand (55.05%), Biochemical Oxygen Demand (66.67%), Electrical conductivity (53.40%), Total Dissolved Solids (46.85%), Total Suspended Solids (58.60%), Salinity (52.21%), Turbidity (76.86%) and Absorbance (77.49%) (Chart 12).

4.2.2 Mortality (%) of *Azolla pinnata*

The following chart 13 depicts the mortality (%) of *Azolla pinnata* exposed to wastewater samples at varying concentrations:

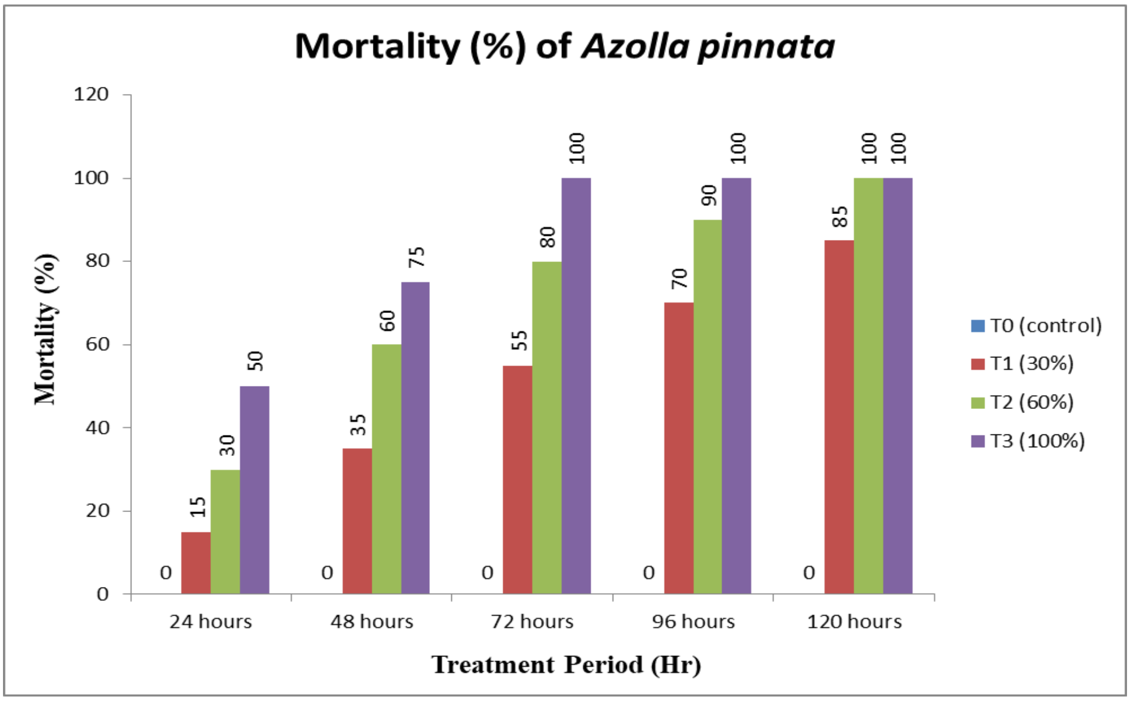

Chart 13: Mortality (%) of *Azolla pinnata*

The higher values of temperature, pH, EC, salinity, high load of TDS and TSS associated with low DO level in the wastewater samples increased water turbidity and caused death of the test species, *Azolla pinnata* (Momtaz et al., 2013). From chart 13, it is observed that *Azolla pinnata* survived 2-3 days in 100% concentrated dye-wastewater. However, it improved almost all the physicochemical parameters; though comparatively less efficiently than T2 (60%) and T1 (30%) (Chart 12). When *Azolla pinnata* was incorporated in 60% concentrated dye-wastewater, it survived about 3-4 days. However, it has also improved all the physicochemical parameters; but comparatively less efficiently than T1 (30%) (Chart 12). In 30% concentrated dye-wastewater, *Azolla pinnata* survived more than 5 days and most efficiently improved all the physicochemical parameters comparing to the other two wastewater containing treatments (Chart 12). The mortality (%) of *Azolla pinnata* increased with the percentage of textile dye wastewater in the treatments (Rashid, 2021).

Based on the above discussion, it can be said that the most efficient treatment was T1 (30%), where the maximum amount of improvement is observed. Whereas the T2 (60%) and T3 (100%) comparatively shown overall lesser improvement respectively (Chart 12).

4.3 Statistical Analysis

To determine the significance of the treatment alternatives used at a 5% level of confidence, a one-way ANOVA was performed in Microsoft Excel. One-way ANOVA is frequently used to determine if changes in one independent variable or its level have a statistically significant impact on the dependent variable when there is only one independent variable.

The p-values from one-way analyses ANOVA revealed that there were significant differences in all the water quality parameters of T1 (30%) and T2 (60%), with a p-value of 0.00749 for temperature. Additionally, the p-values of pH, Dissolved Oxygen (DO), Chemical Oxygen Demand (COD), Biochemical Oxygen (BOD), Electrical conductivity (EC), Total Dissolved Solids (TDS), Total Suspended Solids (TSS), Salinity, Turbidity and Absorbance are respectively 1.81×10^{-09}, 6.58×10^{-11}, 3.32×10^{-09}, 1.11×10^{-07}, 2.67×10^{-13}, 3.34×10^{-12}, 1.12×10^{-09}, 6.17×10^{-08}, 1.86×10^{-10} and 10^{-09}. All values were less than 0.05.

Similar findings were discovered in the case of T1 (30%) and T3 (100%). The p-values from oneway ANOVA for temperature, pH, Dissolved Oxygen (DO), Chemical Oxygen Demand (COD), Biochemical Oxygen (BOD), Electrical conductivity (EC), Total Dissolved Solids (TDS), Total Suspended Solids (TSS), Salinity, Turbidity and Absorbance were respectively 0.0037, 2.12×10^{-10}, 9.1×10^{-11}, 2.3×10^{-09}, 1.03×10^{-07}, 1.18×10^{-14}, 1.6×10^{-12}, 1.3×10^{-12}, 2.23×10^{-09}, 1.5×10^{-11} and 2.57×10^{-11}. All of the values were less than 0.05, implying that there were significant differences in the treatments used to observe water quality improvement. As a result of the statistical analysis, significant differences between treatment groups were discovered.

CHAPTER FIVE

CONCLUSION

5.1 Conclusion

Now-a-days, Aquatic Macrophyte based Systems (AMS) are a significant modern technique for treating wastewater and eliminating adverse substances. The cultures of *Azolla pinnata* in the wastewater that contains dye elements in it, effectively eliminated Temperature, pH, BOD, COD, EC, TSS, TDS, Turbidity, Salinity and Absorbance in comparison to the control treatment and standards for industrial waste prescribed by DoE. Furthermore, the treatments increased the dissolved oxygen (DO) level in the wastewater. However, among the three textile dye wastewater containing treatments, the T1 (30%) have shown the most efficient results in improving all the mentioned water quality parameters. This study suggests that *Azolla pinnata* can be a promising agent for the treatment of textile dye wastewater; which will ultimately benefit the surface water system. Again due to its cheap operational and maintenance costs compared to the existing conventional treatment systems, aquatic macrophyte based treatment systems like *Azolla pinnata* can be an efficient, costeffective as well as environmentally viable choice for wastewater treatment.

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APPENDICES

APPENDIX A

Table-1: Temperature (°C)

Treatments	Average		Improvement (%)	DoE value	Mean	SD (±)
	Initial	Final				
T0 (0%)	27.1	26.2	3.32	40	26.65	0.50
T1 (30%)	29.4	28.00	4.76	40	28.70	0.77
T2 (60%)	31.83	28.33	10.99	40	30.08	1.92
T3 (100%)	35.53	28.5	19.79	40	32.02	3.85

Table-2: pH

Treatments	Average		Improvement (%)	DoE range		Mean	SD (±)
	Initial	Final		Min	Max		
T0 (control)	7.53	7.08	6.02	6	9	7.31	0.25
T1 (30%)	10.58	7.08	33.06	6	9	8.83	1.92
T2 (60%)	10.89	8.64	20.66	6	9	9.77	1.23
T3 (100%)	11.97	9.81	18.02	6	9	10.89	1.18

Table-3: DO (mg/l)

Treatments	Average		Improvement (%)	DoE Range		Mean	SD (±)
	Initial	Final		Minimum	Maximum		
T0 (control)	5.76	6.08	5.32	4.5	8	5.92	0.18

T1 (30%)	3.26	5.84	44.21	4.5	8	4.55	1.42
T2 (60%)	1.32	2.18	39.60	4.5	8	1.75	0.47
T3 (100%)	0.67	1.05	36.08	4.5	8	0.86	0.21

Table-4: BOD (mg/l)

Treatments	Average		Improvement (%)	DoE value	Mean	SD (±)
	Initial	Final				
T0 (control)	5.76	5.17	10.30	50	5.46	0.33
T1 (30%)	125.00	41.67	66.67	50	83.33	45.66
T2 (60%)	229.67	132.00	42.53	50	180.83	53.51
T3 (100%)	384.67	285.00	25.91	50	334.83	54.67

Table-5: COD (mg/l)

Treatments	Average		Improvement (%)	DoE value	Mean	SD (±)
	Initial	Final				
T0 (control)	56.00	50.13	10.48	200	53.07	3.49
T1 (30%)	254.00	114.17	55.05	200	184.08	76.60
T2 (60%)	473.33	286.00	39.58	200	379.67	102.66
T3 (100%)	500.50	355.33	29.00	200	427.92	79.52

Table-6: EC (µS/cm)

Treatments	Average		Improvement (%)	DoE Value	Mean	SD (±)
	Initial	Final				
T0 (control)	275.00	256.67	6.67	1200	265.83	10.11

T1 (30%)	4345.00	2024.67	53.40	1200	3184.83	1270.90
T2 (60%)	7588	4739.33	37.54	1200	6163.50	1560.10
T3 (100%)	10573	7946.33	24.85	1200	9259.83	1438.87

Table-7: TSS (mg/l)

Treatments	Average		Improvement (%)	DoE value	Mean	SD (±)
	Initial	Final				
T0 (control)	42.67	39.10	8.36	150	40.88	2.57
T1 (30%)	339.00	140.33	58.60	150	239.67	108.82
T2 (60%)	801.33	477.33	40.43	150	639.33	177.47
T3 (100%)	2601.33	1803.57	30.67	150	2202.45	436.96

Table-8: TDS (ppm)

Treatments	Average		Improvement (%)	DoE value	Mean	SD (±)
	Initial	Final				
T0 (control)	687.50	595.67	13.36	2100	641.5833333	50.31
T1 (30%)	2976.00	1581.83	46.85	2100	2278.916667	763.62
T2 (60%)	4293.50	2546.00	40.70	2100	3419.75	957.15
T3 (100%)	5342.67	3386.00	36.62	2100	4364.33	1071.71

Table-9: Turbidity (NTU)

Treatments	Average		Improvement (%)	DoE value	Mean	SD (±)
	Initial	Final				
T0 (control)	3.50	3.00	14.29	10	3.25	0.29

T1 (30%)	41.63	9.63	76.86	10	25.63	17.53
T2 (60%)	123.00	49.57	59.70	10	86.28	40.23
T3 (100%)	179.00	108.53	39.37	10	143.77	38.60

Table-10: Salinity (psu)

Treatments	Average		Improvement (%)	Mean	SD (±)
	Initial	Final			
T0 (control)	0.23	0.21	10.14	0.22	0.02
T1 (30%)	0.91	0.43	52.21	0.72	0.26
T2 (60%)	1.72	1.17	31.65	1.50	0.30
T3 (100%)	3.80	2.93	22.81	3.46	0.48

Table-11: Absorbance

Treatments	Average		Improvement (%)	Mean	SD (±)
	Initial	Final			
T0 (control)	0.04	0.04	12.84	0.042	0.003
T1 (30%)	0.28	0.06	77.49	0.196	0.121
T2 (60%)	0.81	0.36	55.65	0.626	0.246
T3 (100%)	1.10	0.80	27.29	0.976	0.164

APPENDIX B

Table-12: ANOVA test between T1(30%) & T3(100%) for Temperature

Temperature		
	T1 (30%)	T3 (100%)
R1	28	28.4
R2	28.1	28.5
R3	27.9	28.6

Anova: Single Factor

SUMMARY				
Groups	Count	Sum	Average	Variance
Column 1	3	84	28	0.01
Column 2	3	85.5	28.5	0.01

ANOVA						
Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	0.375	1	0.375	37.5	0.003602233	7.708647422
Within Groups	0.04	4	0.01			
Total	0.415	5				

Table-13: ANOVA test between T1(30%) & T3(100%) for pH

pH		
	T1 (30%)	T3 (100%)
R1	7.07	9.81
R2	7.08	9.82
R3	7.09	9.81

Anova: Single Factor

SUMMARY				
Groups	Count	Sum	Average	Variance
Column 1	3	21.24	7.08	1E-04
Column 2	3	29.44	9.813333333	3.33333E-05

ANOVA						
Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	11.20666667	1	11.20666667	168100	2.12324E-10	7.708647422
Within Groups	0.000266667	4	6.66667E-05			
Total	11.20693333	5				

Table-14: ANOVA test between T1(30%) & T3(100%) for DO

DO		
	T1 (30%)	T3 (100%)
R1	5.84	1.04
R2	5.84	1.07
R3	5.85	1.05

Anova: Single Factor

SUMMARY				
Groups	Count	Sum	Average	Variance
Column 1	3	17.53	5.843333333	3.33333E-05
Column 2	3	3.16	1.053333333	0.000233333

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	34.41615	1	34.41615	258121.125	9.00519E-11	7.708647422
Within Groups	0.000533333	4	0.000133333			
Total	34.41668333	5				

Table-15: ANOVA test between T1(30%) & T3(100%) for BOD

BOD		
	T1 (30%)	T3 (100%)
R1	40	280
R2	42	289
R3	43	286

Anova: Single Factor

SUMMARY				
Groups	Count	Sum	Average	Variance
Column 1	3	125	41.66666667	2.333333333
Column 2	3	855	285	21

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	88816.66667	1	88816.66667	7612.857143	1.03437E-07	7.708647422
Within Groups	46.66666667	4	11.66666667			
Total	88863.33333	5				

Table-16: ANOVA test between T1(30%) & T3(100%) for COD

COD		
	T1 (30%)	T3 (100%)
R1	114.5	354
R2	113	355
R3	115	357

Anova: Single Factor

SUMMARY				
Groups	Count	Sum	Average	Variance
Column 1	3	342.5	114.1666667	1.083333333
Column 2	3	1066	355.3333333	2.333333333

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	87242.04167	1	87242.04167	51068.5122	2.30032E-09	7.708647422
Within Groups	6.833333333	4	1.708333333			
Total	87248.875	5				

Table-17: ANOVA test between T1(30%) & T3(100%) for EC

EC

	T1 (30%)	T3 (100%)
R1	2023	7946
R2	2025	7945
R3	2026	7948

Anova: Single Factor

SUMMARY				
Groups	Count	Sum	Average	Variance
Column 1	3	6074	2024.666667	2.333333333
Column 2	3	23839	7946.333333	2.333333333

ANOVA						
Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	52599204.17	1	52599204.17	22542516.07	1.18072E-14	7.708647422
Within Groups	9.333333333	4	2.333333333			
Total	52599213.5	5				

Table-18: ANOVA test between T1(30%) & T3(100%) for TSS

TSS		
	T1 (30%)	T3 (100%)
R1	140	1802.7
R2	139	1803
R3	142	1805

Anova: Single Factor

SUMMARY				
Groups	Count	Sum	Average	Variance
Column 1	3	421	140.3333333	2.333333333
Column 2	3	5410.7	1803.566667	1.563333333

ANOVA						
Source of Variatio	SS	df	MS	F	P-value	F crit

n						
Between Groups	52599204.17	1	52599204.17	22542516.07	1.18072E-14	7.708647422
Within Groups	9.333333333	4	2.333333333			
Total	52599213.5	5				

Table-19: ANOVA test between T1(30%) & T3(100%) for TDS

TDS		
	T1 (30%)	T3 (100%)
R1	1583	3388
R2	1581	3386
R3	1581.5	3384

Anova: Single Factor

SUMMARY				
Groups	Count	Sum	Average	Variance
Column 1	3	4745.5	1581.833333	1.083333333
Column 2	3	10158	3386	4

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	4882526.042	1	4882526.042	1920993.852	1.62591E-12	7.708647422
Within Groups	10.16666667	4	2.541666667			
Total	4882536.208	5				

Table-20: ANOVA test between T1(30%) & T3(100%) for Turbidity

Turbidity		
	T1 (30%)	T3 (100%)
R1	9.7	108.3
R2	9.6	108.6

R3	9.6	108.7

Anova: Single Factor

SUMMARY				
Groups	Count	Sum	Average	Variance
Column 1	3	28.9	9.633333333	0.003333333
Column 2	3	325.6	108.5333333	0.043333333

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	14671.815	1	14671.815	628792.0714	1.51751E-11	7.708647422
Within Groups	0.093333333	4	0.023333333			
Total	14671.90833	5				

Table-21: ANOVA test between T1(30%) & T3(100%) for Salinity

Salinity		
	T1 (30%)	T3 (100%)
R1	0.44	2.93
R2	0.44	2.95
R3	0.42	2.92

Anova: Single Factor

SUMMARY				
Groups	Count	Sum	Average	Variance
Column 1	3	1.3	0.433333333	0.000133333
Column 2	3	8.8	2.933333333	0.000233333

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	9.375	1	9.375	51136.36364	2.29422E-09	7.708647422
Within Groups	0.000733333	4	0.000183333			
Total	9.375733333	5				

Table-22: ANOVA test between T1(30%) & T3(100%) for Absorbance

Absorbance		
	T1 (30%)	T3 (100%)
R1	0.064	0.795
R2	0.063	0.798
R3	0.065	0.797

Anova: Single Factor

SUMMARY				
Groups	Count	Sum	Average	Variance
Column 1	3	0.192	0.064	0.000001
Column 2	3	2.39	0.796666667	2.33333E-06

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.805200667	1	0.805200667	483120.4	2.5706E-11	7.708647422
Within Groups	6.66667E-06	4	1.66667E-06			
Total	0.805207333	5				

APPENDIX C

Table-23: ANOVA test between T1(30%) & T3(60%) for Temperature

Temperature		
	T1 (30%)	T2 (60%)
R1	28	28.3
R2	28.1	28.4
R3	27.9	28.3

Anova: Single Factor

SUMMARY				
Groups	Count	Sum	Average	Variance
Column 1	3	84	28	0.01
Column 2	3	85	28.33333333	0.003333333

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.166666667	1	0.166666667	25	0.007490434	7.708647422
Within Groups	0.026666667	4	0.006666667			
Total	0.193333333	5				

Table-24: ANOVA test between T1(30%) & T3(60%) for pH

pH		
	T1 (30%)	T2 (60%)
R1	6.67	8.65
R2	6.68	8.63
R3	6.69	8.64

Anova: Single Factor

SUMMARY				
Groups	Count	Sum	Average	Variance
Column 1	3	20.04	6.68	0.0001
Column 2	3	25.92	8.64	1E-04

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	5.7624	1	5.7624	57624	1.80673E-09	7.708647422
Within Groups	0.0004	4	0.0001			
Total	5.7628	5				

Table-25: ANOVA test between T1(30%) & T3(60%) for DO

DO		
	T1 (30%)	T2 (60%)
R1	5.84	2.17
R2	5.84	2.19
R3	5.85	2.18

Anova: Single Factor

SUMMARY				
Groups	Count	Sum	Average	Variance
Column 1	3	17.53	5.843333333	3.33333E-05
Column 2	3	6.54	2.18	0.0001

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	20.13001667	1	20.13001667	301950.25	6.58068E-11	7.708647422
Within Groups	0.000266667	4	6.66667E-05			
Total	20.13028333	5				

Table-26: ANOVA test between T1(30%) & T3(60%) for BOD

BOD		
	T1 (30%)	T2 (60%)
R1	40	131
R2	42	133
R3	43	132

Anova: Single Factor

SUMMARY				
Groups	Count	Sum	Average	Variance
Column 1	3	125	41.66666667	2.333333333
Column 2	3	396	132	1

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	12240.16667	1	12240.16667	7344.1	1.11142E-07	7.708647422
Within Groups	6.666666667	4	1.666666667			
Total	12246.83333	5				

Table-27: ANOVA test between T1(30%) & T3(60%) for COD

COD		
	T1 (30%)	T2 (60%)
R1	114.5	287
R2	113	286
R3	115	285

Anova: Single Factor

SUMMARY				
Groups	Count	Sum	Average	Variance
Column 1	3	342.5	114.1666667	1.083333333
Column 2	3	858	286	1

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	44290.04167	1	44290.04167	42518.44	3.3184E-09	7.708647422
Within Groups	4.166666667	4	1.041666667			
Total	44294.20833	5				

Table-28: ANOVA test between T1(30%) & T3(60%) for EC

EC		
	T1 (30%)	T2 (60%)
R1	2023	4741
R2	2025	4739
R3	2026	4738

Anova: Single Factor

SUMMARY				
Groups	Count	Sum	Average	Variance
Column 1	3	6074	2024.666667	2.333333333
Column 2	3	14218	4739.333333	2.333333333

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	11054122.67	1	11054122.67	4737481.143	2.67335E-13	7.708647422
Within Groups	9.333333333	4	2.333333333			
Total	11054132	5				

Table-29: ANOVA test between T1(30%) & T3(60%) for TSS

TSS		
	T1 (30%)	T2 (60%)
R1	140	479
R2	139	476
R3	142	477

Anova: Single Factor

SUMMARY				
Groups	Count	Sum	Average	Variance
Column 1	3	421	140.3333333	2.333333333
Column 2	3	1432	477.3333333	2.333333333

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	170353.5	1	170353.5	73008.64286	1.12555E-09	7.708647422
Within Groups	9.333333333	4	2.333333333			
Total	170362.8333	5				

Table-30: ANOVA test between T1(30%) & T3(60%) for TDS

TDS		
	T1 (30%)	T2 (60%)
R1	1583	2545
R2	1581	2547
R3	1581.5	2546

Anova: Single Factor

SUMMARY				
Groups	Count	Sum	Average	Variance
Column 1	3	4745.5	1581.833333	1.083333333
Column 2	3	7638	2546	1

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1394426.042	1	1394426.042	1338649	3.34823E-12	7.708647422
Within Groups	4.166666667	4	1.041666667			
Total	1394430.208	5				

Table-31: ANOVA test between T1(30%) & T3(60%) for Turbidity

Turbidity		
	T1 (30%)	T2 (60%)
R1	9.7	49.4
R2	9.6	49.6
R3	9.6	49.7

Anova: Single Factor

SUMMARY				
Groups	Count	Sum	Average	Variance
Column 1	3	28.9	9.633333333	0.003333333
Column 2	3	148.7	49.56666667	0.023333333

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	2392.006667	1	2392.006667	179400.5	1.86418E-10	7.708647422
Within Groups	0.053333333	4	0.013333333			
Total	2392.06	5				

Table-32: ANOVA test between T1(30%) & T3(60%) for Salinity

Salinity		
	T1 (30%)	T2 (60%)
R1	0.44	1.17
R2	0.44	1.17
R3	0.42	1.18

Anova: Single Factor

SUMMARY				
Groups	Count	Sum	Average	Variance
Column 1	3	1.3	0.433333333	0.000133333
Column 2	3	3.52	1.173333333	3.33333E-05

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.8214	1	0.8214	9856.8	6.17143E-08	7.708647422
Within Groups	0.000333333	4	8.33333E-05			
Total	0.821733333	5				

Table-33: ANOVA test between T1(30%) & T3(60%) for Absorbance

Absorbance		
	T1 (30%)	T2 (60%)
R1	0.064	0.356
R2	0.063	0.357
R3	0.065	0.359

Anova: Single Factor

SUMMARY				
Groups	Count	Sum	Average	Variance
Column 1	3	0.192	0.064	0.000001
Column 2	3	1.072	0.357333333	2.33333E-06

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.129066667	1	0.129066667	77440	1.00042E-09	7.708647422
Within Groups	6.66667E-06	4	1.66667E-06			
Total	0.129073333	5				