

# Analysis Of Phytoremediation Techniques As A Methodology For Removal Of Organic Pollutants From Water

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## Abstract

The wastewater treatment and its purification has been one of the key areas of focused research along with a critical demand keeping in view the current status of water table depletion and pollution. The purification of wastewater to be recycled for usage poses a practical solution which can be achieved through the phytoremediation techniques. The utilization of phytoactive plants to remove the organic pollutants from water using the phytodegradation and phytoextraction approaches through *Eichhornia crassipes*, an aquatic plant, commonly known as water hyacinth has yielded potentially significant results in water quality testing for wastewater recycling. This research targets the analysis of usage of species *E. crassipes* for removal of organic pollutants through the water and plant analysis with clear benefits of being non-intrusive, higher filtration capacity and cost effective in developing countries. The implementation of phytoremediation techniques with combined crude industrial approaches of decreasing wastewater production will result in effective reduction in water wastage and deprivation. The acceptance of green technologies in the public domain remains a crucial step in the implementation of this research.

**Keywords** – phytoremediation, wastewater treatment, water quality, phytoextraction, phytodegradation

## 1. Introduction

The removal of organic pollutants from the wastewater is a crucial step towards the quality and reusability parameter of water. In the crude industrial processes, around 100,000 chemicals along with industrial methods are available for this removal process which cleanses the water, including the zeolites which are majorly non-biodegradable (Schröder et.al, 2007). On the offset, freshwater sources are being contaminated every single day through the increment in fine and micro-sized particulate pollutants. Along with the dangerous impact of water bodies and marine organisms, such microparticles which have the tendency to pass industrial water filters, pose a deleterious impact towards the estrogenic imbalance in the body (Ramachandra and Solanki, 2007). The state of the art techniques have used the synthetic and naturally extracted chemicals to refine the quality of water to increase the reusability. In the current scenario, newer technologies aim towards super high filtration of water, with a more increased cost for installation, development and maintenance which becomes a major drawback for its practical implementation in the real world on a large scale. For the implementation of biological vegetation and microorganisms for phytoremediation, the crucial steps include the identification of a species which has inherent potency towards absorption of ions, organic salts and even mineral pollutants (Mirzaee et.al, 2021). This targeted issue has been explored to be solved through the use of

phytoremediation as a potentially upcoming technique which puts the plants of certain species to use, to extract the organic pollutants and other microparticles from water to render it pollutant free and safe for use.

The phytoremediation techniques focus on application of vegetation as well as microorganisms organically to either purify, recover nutrients and for the decontamination of the environment, including soil, water and other domains. This term phytoremediation has been an extension of a process called bioremediation which was highlighted to be the sustainable and futuristic approach towards environmental balancing (Mustafa and Hayder, 2021). The contaminated water purification is a crucial component for which the phytoremediation techniques are put to practice. The basis of utilizing this method is primarily volatilization, degradation, extraction, and purification of the environmental components by either plants, vegetation or microorganisms (Hu et.al, 2020). The aquatic plants, being a significant part of the wetlands are the commonest plants used for phytoremediation, one of which is *Eichhornia crassipes* or now known as *Pontederia crassipes* (Materac et.al, 2015). For the phytoremediation, not only aquatic plants, but halophiles, duckweeds, as well as algae have been used which recycle nitrates, phosphates, sulphates and even pathogenic bacteria from the wastewater, as well as cyanide and steel (Ceschin et.al, 2019, Ebel et al., 2007). The study on the Gorgan wastewater treatment plant has also established the inherent potential of Vetiver and pampas grass for extraction of problematic ions from the water and reducing the clogging effect (Mirazee et.al, 2021). The indoor and wetland studies on these aquatic plants have been verified through published researches in the domain of phytoremediation, wherein this study will focus on the plant growth in the sewage or wastewater as well as to establish and check for water quality after phytoremediation (Saha et al., 2018). The toxicity studies on this species have found that the effluents which have been purified by *Eichhornia crassipes* maintain the fish life beyond 24 hours (Victor et.al, 2016). The species of *Eichhornia* targeted in this research has a large root surface area and through the delicate root hairs which have a deep penetration inside the water, it is able to absorb the organic salts and ions from the wastewater for its own nutritional purposes (Kumar and Chopra, 2017).

The analysis and mechanism of action of *Eichhornia* has revealed a high concentration and deposition of toxic metals in the metal industry such as Cr, Cd, Pb and As in the roots and shoots of the plant (Agunbiade et.al, 2009). The usage of such methods through aquatic plants of *Eichhornia crassipes* is practical for an all year round treatment through creation of wetlands. (Anudechakul et al., 2015) The present investigation demonstrates phytoremediation potential of *Eichhornia crassipes* exposed to increasing concentration of organic pollutant waste water, even some organic dyes for recycling (Tan et.al, 2016). The effect and accumulation were studied with reference to selected biochemical parameters and physio-chemical parameters of the water. Growth characteristics, chlorophyll were studied so as to know the physiological changes associated with experimental species while water quality analysis was carried out to judge the natural ability to treat cadmium containing water (Zelekew et.al, 2021).. This was specially assessed with reference to pH, total dissolved solids (TDS), turbidity, chemical oxygen demand (COD), biological oxygen demand (BOD). Finally, the phytoremediation of *E. crassipes* was estimated to find out the Growth, kinetic mass, tolerance capacity and toxicity from liquid medium. These research findings will evaluate the plant and water characteristics after phytoremediation to identify changes in methodology and specifications for its implementation programs to be constructed for wastewater treatment.

## II. Materials and Methods

### 1.1 Phytoremediator, Sewage Collection and Assessment Parameters: Sampling Procedure

The study was initiated with the field visit for collection of the targeted phytoremediator *E. crassipes*, commonly known as water hyacinth. The collected plants were transported to the laboratory area and rinsed for debris removal. For the assessment of wastewater treatment, the samples of water from three different sites were taken. The three samples consisted of:

Sample 1 - Slaughterhouse waste water

Sample 2- Textile industry dye waste water

Sample 3- Pesticide waste water

The control for the experiment was the water taken from the borewell, accounting for the freshwater in the area. The water samples were filtered through gravity filtration method to remove large debris and floating dead leaves. Samples were filtered with filter paper and collected in Erlenmeyer flask. The collected plants of *E. crassipes* were acclimatized under sunlight for 3–5 days for adapting to the new environment.

## 1.2 Methodology for Treatment and Sampling

For the analysis of growth of plant and effective removal of organic matter from the water, the study was designed with inclusivity of the following parameters;

- Study of the plant growth analysis
- Photosynthetic pigmentation
- water parameters
- water analysis for solids
- measurement of water quality test

## 1.3 Parameter Assessment Procedure: Plant Analysis

### a. Study of the plant growth analysis

The plant growth analysis included the time interval efficient measurement of root length, fresh weight, dry weight and leaf area. The root length was calculated by using the 30 cm calibrated scale and compared on 0, 7, 14, 21, 28, 35, 42 days. The fresh weight was taken through sensitively calibrated weighing balance and hot air oven was used for measuring dry weight after 48 hours. For leaf area analysis in water hyacinth, a model of leaf growth in terms of photosynthetic rate was created.

### b. Photosynthetic pigmentation

Through centrifugation, dilution and spectrophotometry, the chlorophyll production and pigmentation was calculated utilizing the following:

$$\text{Chlorophyll a (milligrams/millilitre) (mg/ml)]} = 12.7 A_{663} - 2.69 A_{645}$$

$$\text{Chlorophyll b (MG/ML)} = 22.9 A_{645} - 4.68 A_{663}$$

where:

$A_{645}$  = Absorbance at a wavelength of 645 nm

$A_{663}$  = Absorbance at a wavelength of 663 nm

## 1.4 Parameter Assessment Procedure: Water Analysis

### a. Water parameters

The water parameters including temperature, pH, and turbidity were determined. The devices like thermometer, pH meter and Digital Turbidity Meter – INSIF'S IE 709 Model were used for determining the three variables for the three samples of water as well as the control.

### b. Water analysis for solids

The water analysis of solids for any residual particles after treatment by *E. crassipes* was done through determination of total dissolved solids (TDS). Pore size of 0.45  $\mu$ m was used to distinguish between microscopic particulate and dissolved material. The amount of TDS was determined by the weight difference after evaporation.

$$\text{TDS/L} = \frac{(A-B) \times 1000}{\text{Volume of sample in ml}}$$

The quality of water is expressed through two prime parameters which are the Biochemical Oxygen Demand (BOD) and the Chemical Oxygen Demand (COD). This test for BOD measured the oxygen that is needed to destroy organic chemicals (carbonaceous requirements) and also the oxygen that is used to extract the inorganic substances such as ferrous iron and sulfides. It also measured the oxygen which is used to release the decreased form of nitrogen (nitrogen requirement) unless the inhibitor inhibits its oxidation.

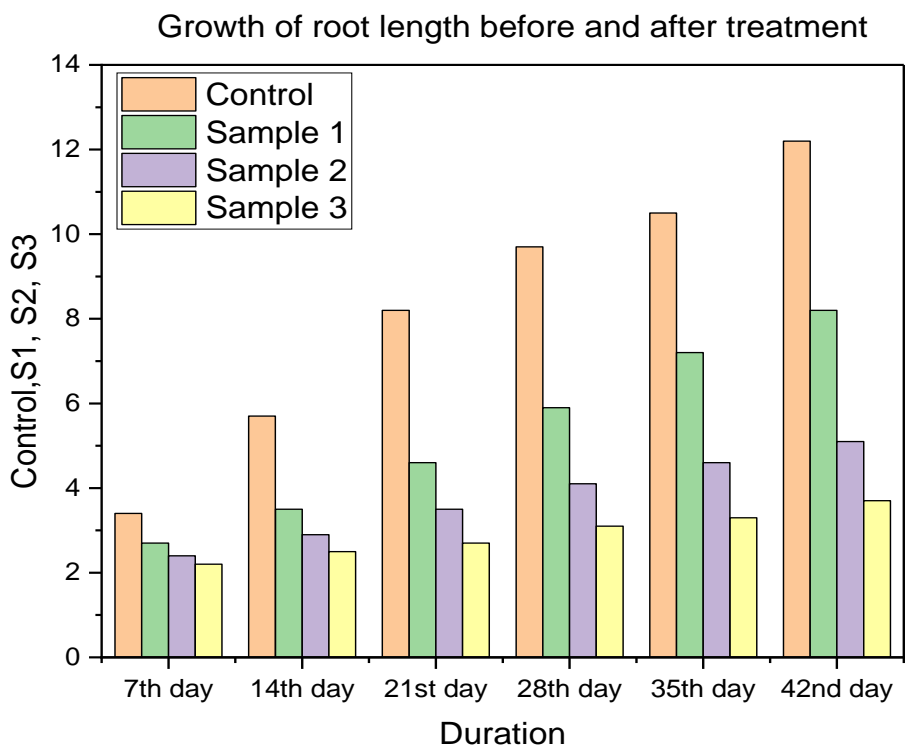
The amount of oxidant mentioned in response to a sample under controlled conditions is defined as COD. Under controlled conditions, the amount of oxidant used is expressed in terms of its oxygen balance. The organic and inorganic components of the sample are indeed susceptible to oxidation, but the organic component is generally more efficient and desirable. COD is frequently used to assess pollutants in the wastewater and the natural waters (APHA, AWWA and WEF, 1998).

### III. Results

#### I. Study of the plant growth analysis

##### 1. Root Length

The maximum length of the root was observed in control (0.2 cm) on 42nd day, while minimum length of the root (3.7 cm) was observed in sample 3(pesticide water) and in sample 2 the growth shown as (5.1 cm). The root length in sample 1 was (8.2 cm). This proves that the root length reduced as levels of organic pollutants increase, demonstrating a perfect negative correlation.

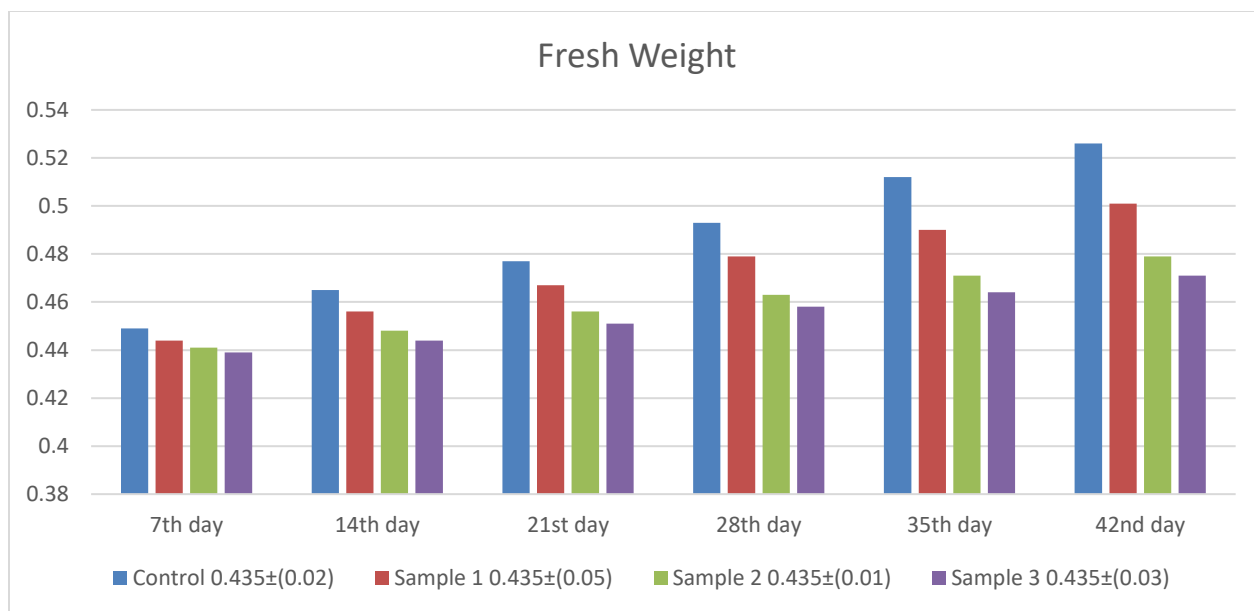


## 2. Fresh Weight

The maximum leaf weight was observed in control (0.526g) on 42nd day. In sample 1, the leaf weight was (0.501g), and in sample 2 the weight shown as (0.479g), while minimum weight attained in sample 3(pesticide water) was 0.471g. The fresh weight of leaf was gradually decreased when it is treated with different samples (s1, s2, s3).

Table: Bio Mass – Fresh Weight

Duration	Control	Sample 1	Sample 2	Sample 3
<b>0 day</b>	0.435±(0.02)	0.435±(0.05)	0.435±(0.01)	0.435±(0.03)
<b>7th day</b>	0.449 g	0.444 g	0.441 g	0.439 g
<b>14th day</b>	0.465 g	0.456 g	0.448 g	0.444 g
<b>21st day</b>	0.477 g	0.467g	0.456 g	0.451 g
<b>28th day</b>	0.493 g	0.479 g	0.463 g	0.458 g
<b>35th day</b>	0.512 g	0.49 g	0.471 g	0.464 g
<b>42nd day</b>	0.526 g	0.501 g	0.479 g	0.471 g

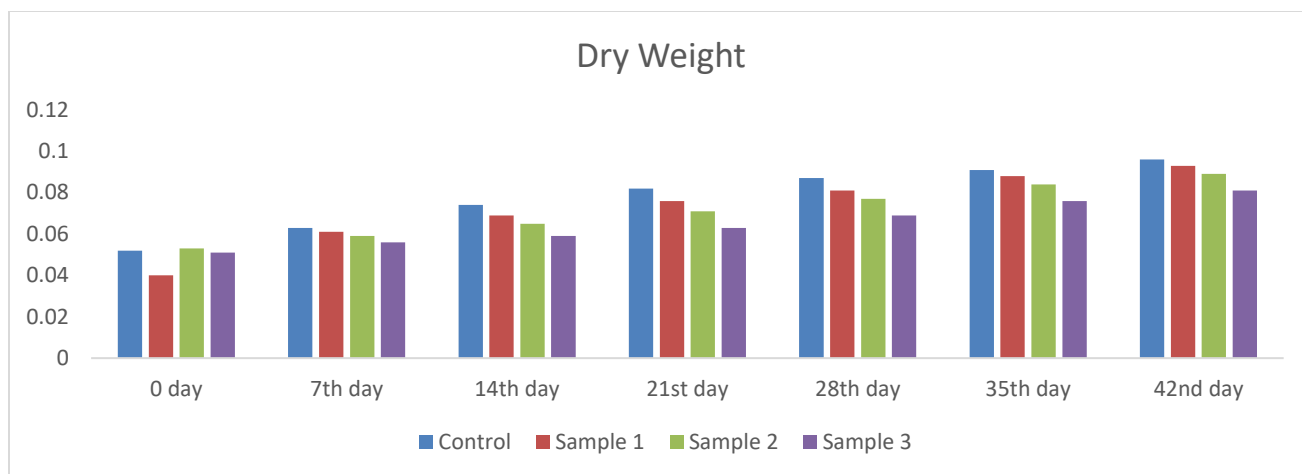


**Fig: Graphical Representation of Bio Mass – Fresh Weight in *Eichhornia crassipes***

### 3. Dry Weight

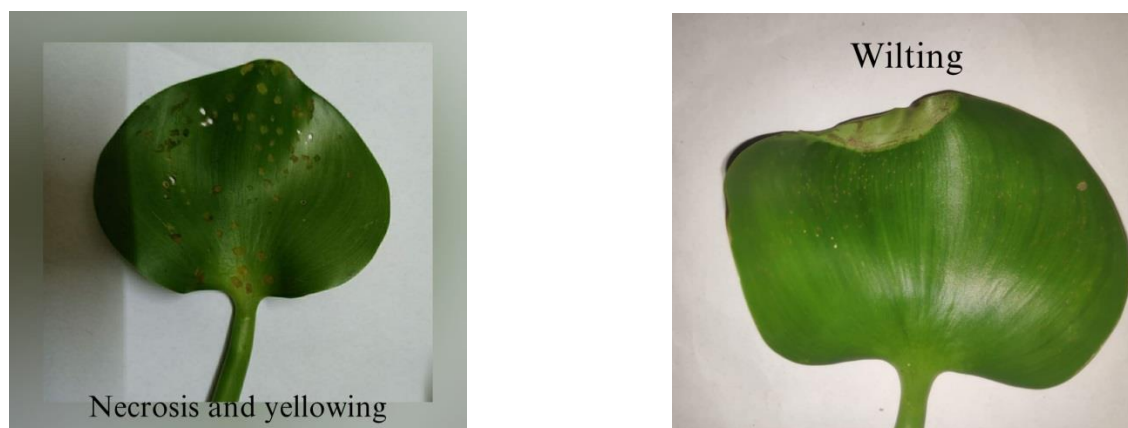
The dry weight of the leaves was 0.096 g on the 42nd day of control. The sample 3 weighed 0.081 g on the 42nd day of sample 3 treatment (pesticide water). Subsequently the weight was 0.091g and (0.089g when it was measured for sample 1 (slaughter house water) and sample 2 (industrial dye water) respectively. On the 42<sup>nd</sup> day the dry weight decreased serially with different samples (s1, s2, s3) showing perfect negative correlation.

Duration	Control	Sample 1	Sample 2	Sample 3
<b>0 day</b>	0.052 g	0.04 g	0.053 g	0.051 g
<b>7th day</b>	0.063 g	0.061 g	0.059 g	0.056 g
<b>14th day</b>	0.074 g	0.069 g	0.065 g	0.059 g
<b>21st day</b>	0.082 g	0.076 g	0.071 g	0.063 g
<b>28th day</b>	0.087 g	0.081 g	0.077 g	0.069 g
<b>35th day</b>	0.091 g	0.088 g	0.084 g	0.076 g
<b>42nd day</b>	0.096 g	0.093 g	0.089 g	0.081 g



**Fig: Graphical Representation of Bio Mass – Dry Weight in Eichhornia crassipes (E. crassipes)**

#### 4. Leaf Area



**Fig: Necrosis, Yellowing and Wilting in Eichhornia crassipes (E. crassipes)**

There were small pores seen in the leaves of waste water sample 2 and sample 3 which as shown above. It was also found to have few yellowish leaves during the experimental part. Wilting of leaves was also seen.

#### II. Photosynthetic pigmentation

**Table: Chlorophyll content value in Different Water Samples**

Serial No.	Samples	Chlorophyll a (mg ml <sup>-1</sup> )	Chlorophyll b (mg ml <sup>-1</sup> )	Total Chlorophyll Content (mg ml <sup>-1</sup> )	Chlorophyll ratio (mg ml <sup>-1</sup> )
1	Control	67.5	115.6	183.1	0.6
2	(Sample 1)	65.9	108.1	174	0.61
3	(Sample2)	65.7	103.6	169.3	0.63

4	(Sample 3)	61.6	98.7	160.3	0.62
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## Evaluation Of Chlorophyll Content

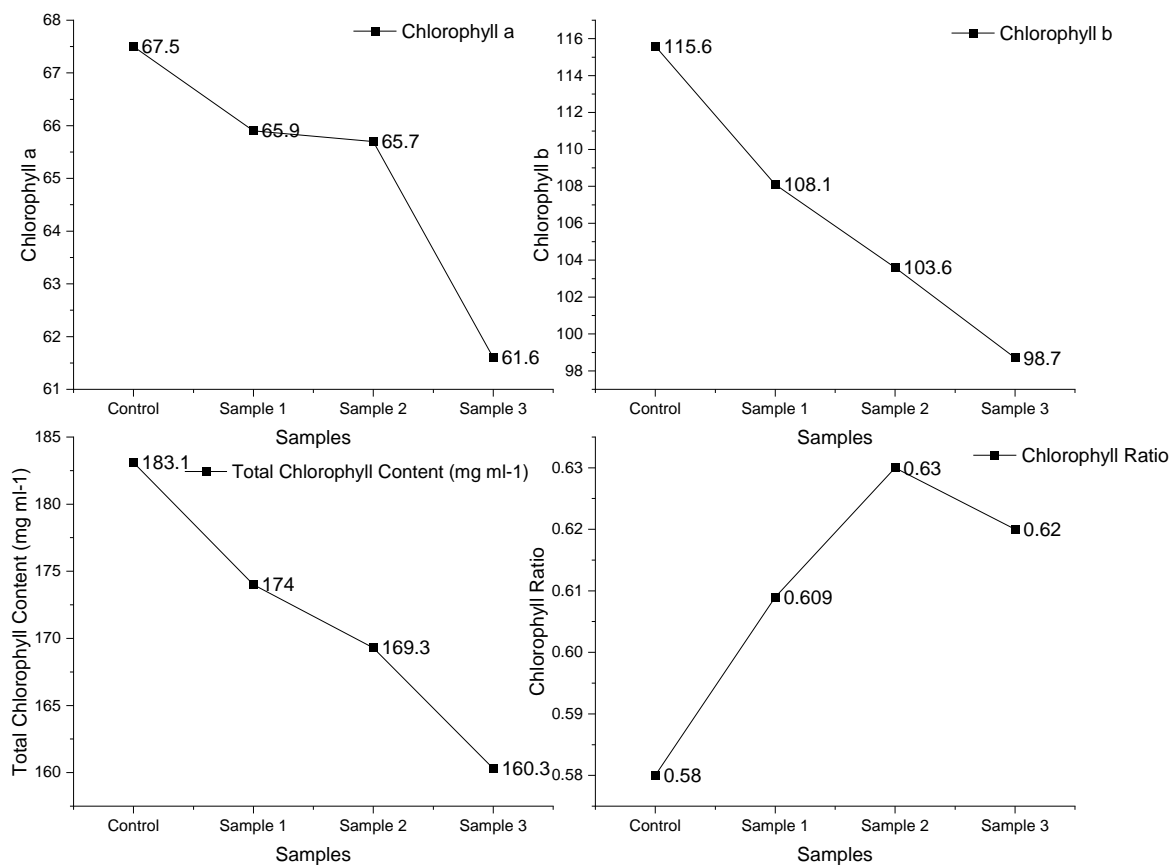


Fig: Graphical representation in Evaluation of Chlorophyll in *Eichhornia crassipes*.

The highest total chlorophyll content of *Eichhornia crassipes* was found in control water (79.06 ng ml<sup>-1</sup>) and the greatest decrease in plant chlorophyll content was found in sample 3 (pesticide water).

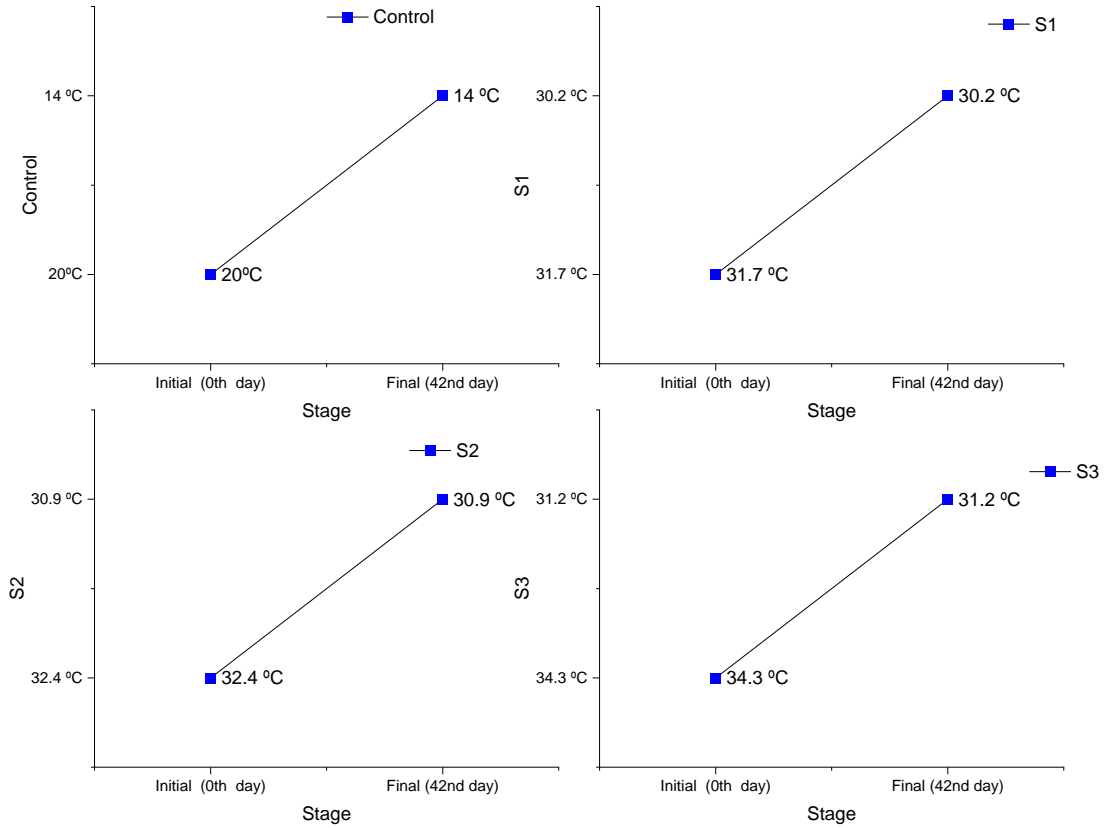
### III. Water parameters

#### 1. Temperature

Table: Temperature initial and final treatment

Stage	Control	S1	S2	S3
Initial (0 <sup>th</sup> day)	20 °C	31.7 °C	32.4 °C	34.3 °C
Final	14 °C	30.2 °C	30.9 °C	31.2 °C

### Temperature Initial And Final Treatment

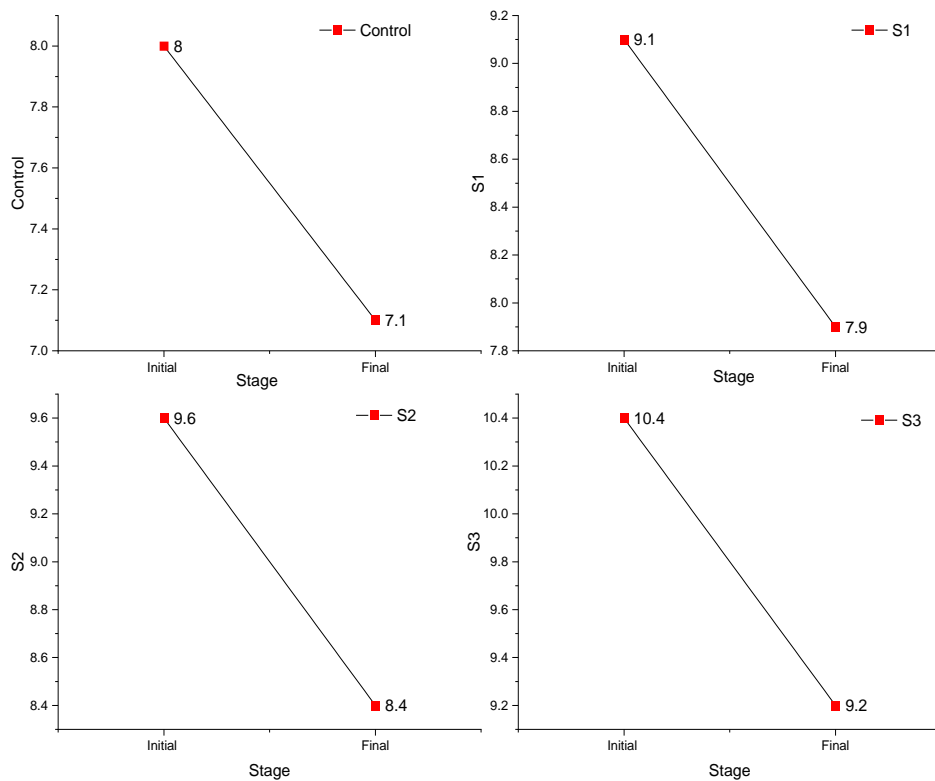


**Fig: Graphical Representation of temperature in initial and final stage of temperature in *Eichhornia crassipes*.**

During the phytoremediation treatment, the temperature was decreased. At the final stage of treatment, the temperature reduced to 14 °C, 30.2 °C, 30.9 °C and 31.2 °C due to the plant growth.

## 2. pH

## pH Initial And Final Treatment



**Graphical Representation of temperature in initial and final stage of temperature in *Eichhornia crassipes*.**

Table: pH - Initial and Final Treatment

Stage	Control	S1	S2	S3
<b>Initial (0<sup>th</sup> day)</b>	8.0	9.1	9.6	10.4
<b>Final (42<sup>nd</sup> day)</b>	7.1	7.9	8.4	9.2

All the samples taken were alkaline i.e. at high level pH. The level of alkalinity decreased after treatment with *E. crassipes*.

### 3. Turbidity

Table: Turbidity in initial and final stage of treatment

Stage	Control	S1	S2	S3
<b>Initial (0<sup>th</sup> day)</b>	0.9 NTU	4.7 NTU	5.4 NTU	5.9 NTU
<b>Final (42<sup>nd</sup> day)</b>	0.6 NTU	4.3 NTU	5.2 NTU	5.6 NTU

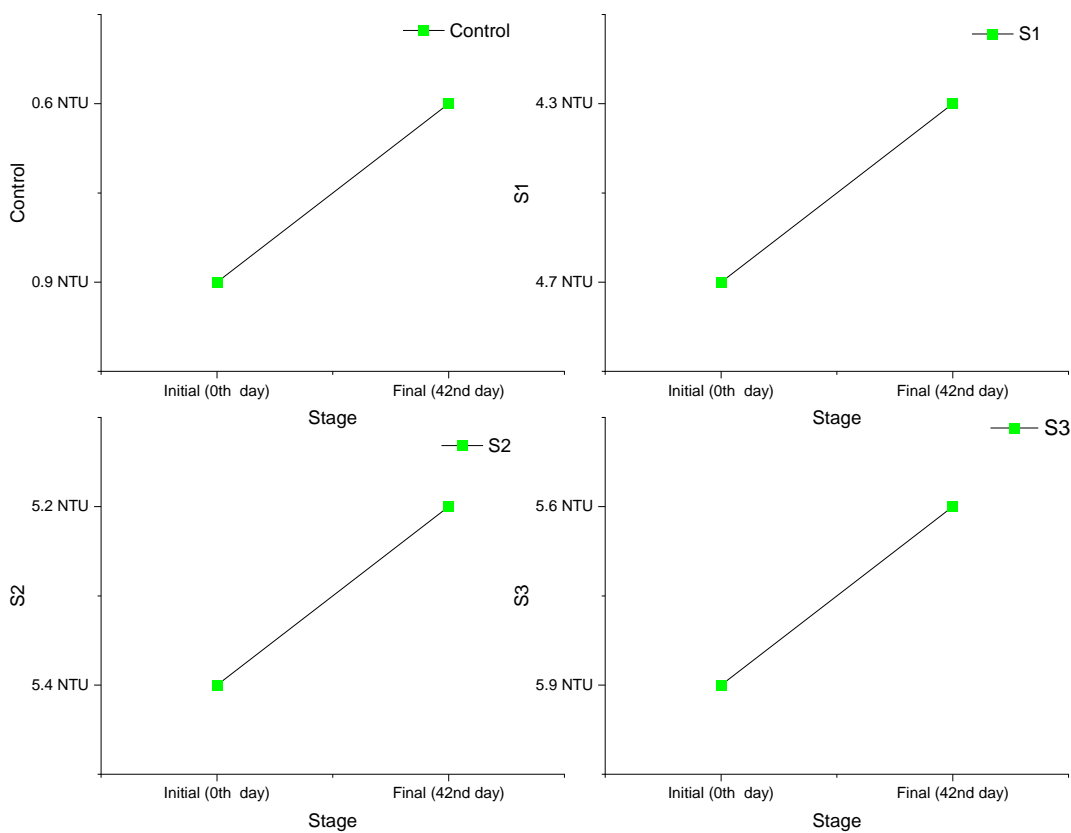


Fig: Graphical Representation of Turbidity in initial and final stage of treatment in *Eichhornia crassipes*.

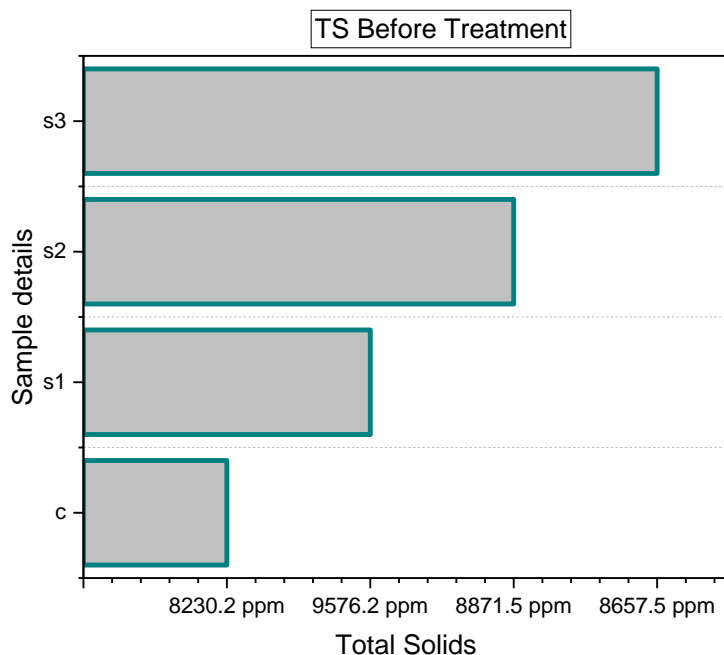
The difference between initial and final stage of the experiment was observed as turbidity in the water samples decreased in the range of 0.2 NTU – 0.4 NTU.

#### IV. Water Analysis for Solids

Table: TS before treatment

S.no	Sample name	Reading values in ppm
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1.	<b>C</b>	8230.2 ppm
2.	<b>s1</b>	9576.2 ppm
3.	<b>s2</b>	8871.5 ppm
4.	<b>s3</b>	8657.5 ppm

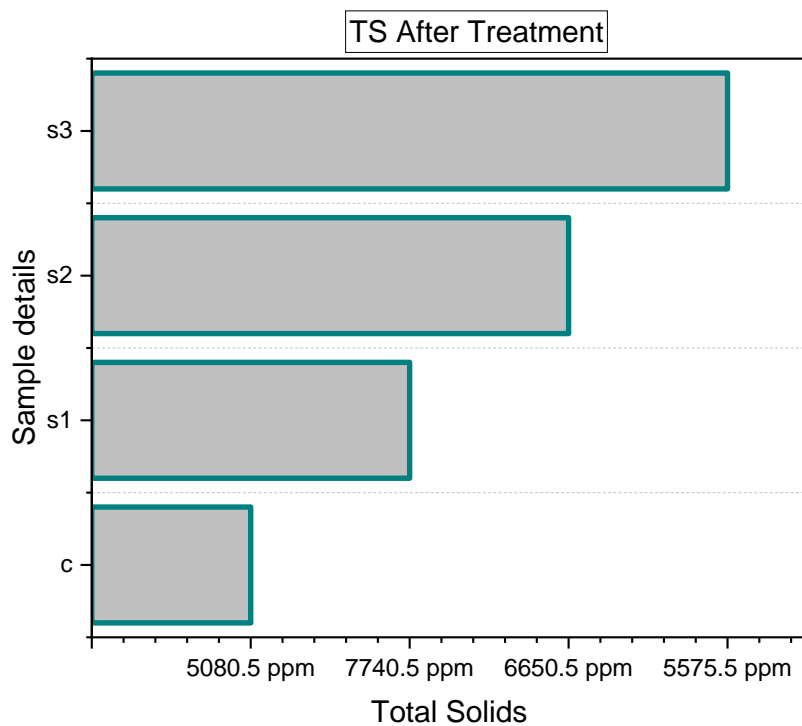


**Fig : Graphical representation of TS before treatment**

**Table : TS after treatment**

S.no	Sample name	Reading values in ppm
1.	<b>C</b>	5080.5 ppm
2.	<b>s1</b>	7740.5 ppm
3.	<b>s2</b>	6650.5 ppm
4.	<b>s3</b>	5575.5 ppm

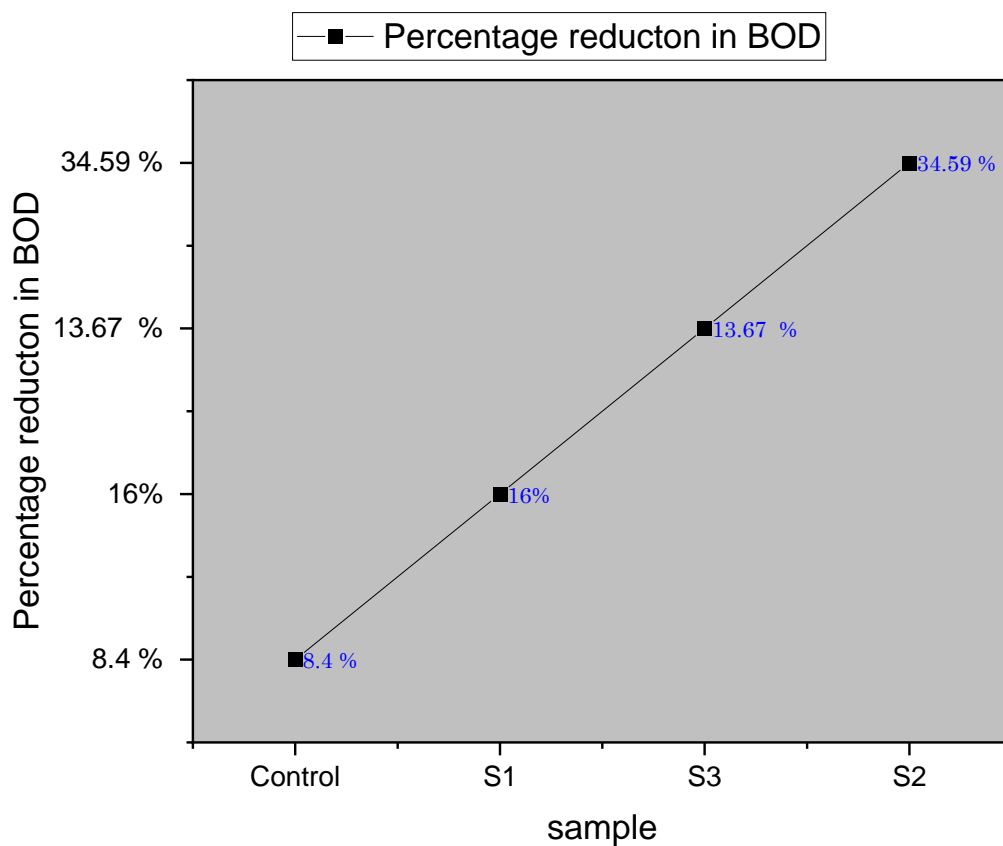
**Fig : Graphical representation of TS after treatment**



The initial stage experimental value of water samples was 980.2ppm in control, 1021.2ppm in S1, 1321.5ppm in S2 and 1532.5ppm in S3 respectively. After treatment with *E. crassipes*, the values were tested at regular intervals and lasted for 42 days. The TDS value decreased in the range of 855.5ppm in control, 990.5ppm in S1, 1125.5ppm in S2 and 1325.5ppm in S3.

## V. Measurement of water quality test

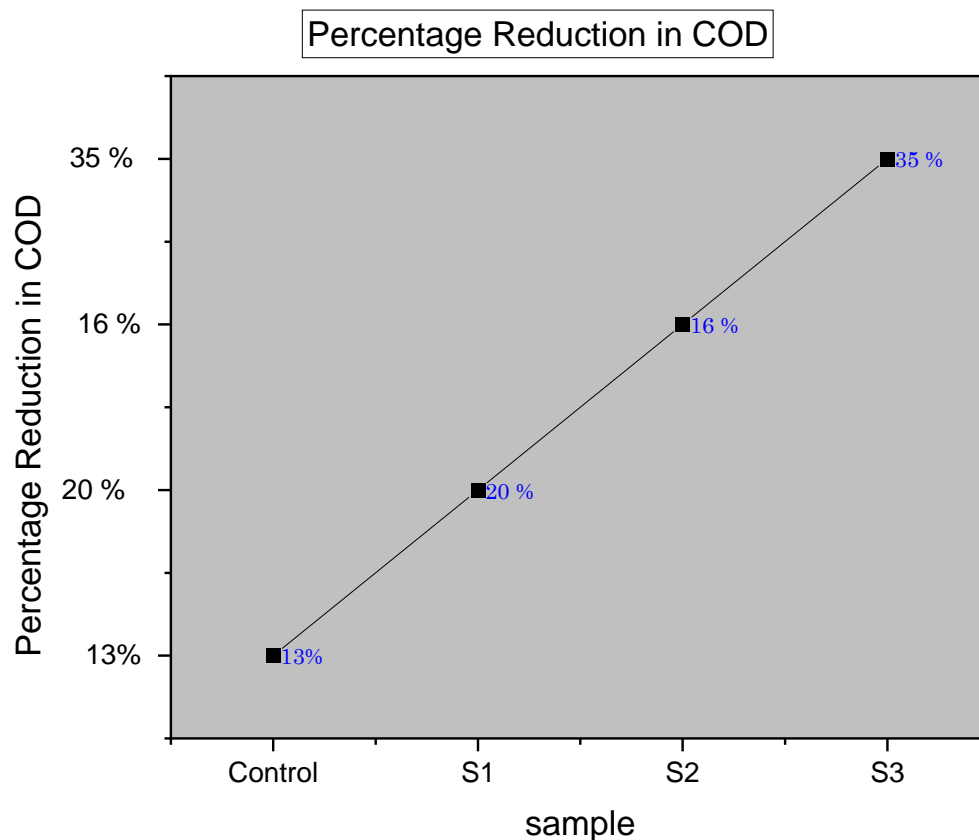
### 1. Biochemical Oxygen Demand



**Fig: Graphical representation of percentage reduction in BOD**

In the samples without treatment, the highest value of BOD was observed as 19 mg/ml in control, 152 mg/ml in (S1), 498 mg/ml in (S2) and 532 mg/ml (S3) respectively. Subsequently, with the exposure to *E. crassipes*, the pollutant level reduced in treated water samples at the range of 412 mg/ml, 448 mg/ml and 514 mg/ml in Sample 1, 2, and 3 respectively as 17.4 mg/ml in control, 131 mg/ml in S1, 370 mg/ml in S2 and 468 mg/ml in S3 respectively.

## 2. Chemical Oxygen Demand



The pre-treatment samples reported the COD values as 694mg/ml in sample 3, followed by 533 mg/ml, 494 mg/ml and 382 mg/ml in sample 2, sample 1 and control respectively. By analyzing the result treatment for COD, the values decreased at the range of 412 mg/ml, 448 mg/ml and 514 mg/ml in sample 1, 2 and 3 respectively and 339 mg/ml for control.

#### IV. Discussion

Through the analysis of plant growth characteristics, the root length of *E. crassipes* exhibited a negative correlation with respect to the amount of organic pollutants absorbed. The studies have established the absorption of Zn, Cd, Pb and Cu like metal pollutants being absorbed in range of 50-80 mg/kg of dry matter (Buta et.al, 2011). The fresh and dry weight has shown significant decrease with the time of treatment which is correlated towards the chlorosis and necrosis underwent by the plant, in the process of extraction of organic pollutants (Das and Goswami, 2015). The leaf area, through the process wilting and accumulation of dark, necropsied parts led to a substantial decrease. With an analysis of chlorophyll synthesis in water, the water enriched with pesticidal waste contained the least of chlorophyll content. Multiple correlations have been drawn towards the segregation for inhibition as the metals and organic pollutants in the water hamper the synthesis of chlorophyll, especially Selenium (Mane et.al, 2011). The analysis of water parameters including the temperature and pH exhibited expected changes which decreased and increased during the phytoremediation process respectively. The increased pH and alkalinity is a distinguishing component of water purification as the acidic substances, including organic pollutants have been extracted out. In the analysis of quality of water through total dissolved solids, the pre-treatment and post-treatment quality shows a significant difference, with water being completely unacceptable in the former, and poor for drinking, but suitable for use in the post-

treatment. The decreased BOD and COD of the post treatment water exhibited similar results, supporting the suitability of water for usage, other than drinking purposes. The techniques of phytoremediation have found immense application in the rural areas close to industrial setups and farmlands, for purification of water. The bioremediation techniques, although require cumbersome screening and analysis of species, but pose an effective solution to the rising water crisis and wastewater management programs.

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