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PHYTOREMEDIATION OF ORGANIC DYES USING AQUATIC PLANTS

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Abstract:

The escalating augmentation of synthetic dyes in aquatic system poses a significant environmental challenge. Due to their non-biodegradable nature, these dyes persist in water, hindering light penetration and disrupting ecosystems. Crystal Violet (CV) exhibits mutagenic, teratogenic, or mitotic poisoning properties, while Methyl Orange (MO) is a carcinogenic water-soluble azo dye commonly found in industrial effluents. These dyes contribute to electrolyte imbalances and promote tumorigenesis in aquatic organisms. Various methods have been explored to mitigate the presence of dyes in aquatic environments. These include utilizing grapefruit peel, rice husk, jackfruit leaf powder, and ginger waste. However, biosorbents remain the most effective technique, whereby dyes are absorbed from wastewater. Thereby, the study focuses on the possibility of using aquatic plants such as Pistia stratiotes (water lettuce) and Cyperus papyrus (water cyperus) as phytoremediation to remove the industrial dye (here CV and MO) from the aquatic system. The study exemplified the biosorbent properties of these plants against the potential degradation of CV and MO.

Keywords: *Methyl Orange, Crystal Violet, Degradation, Phytoremediation, Cyperus Papyrus, Pistia Stratiotes.*

Introduction:

Wastewater or effluents from dyeing and biotechnology industries contain a lot of organic dyes, which are likely to be toxic. These dyes need to be broken down or removed before they reach aquatic ecosystems and irreversibly cause them harm. Crystal Violet (CV), extensively utilized in the dyeing industry, is highly toxic and recognized for its mutagenic and mitotic poisoning properties. Methyl orange (MO) is an azo dye known for its toxic, carcinogenic, tumorigenic, mutagenic, and genotoxic properties (Ali et al., 2019). It is frequently found in effluents released by industries such as textiles, printing, food processing, pharmaceuticals, paper manufacturing, and research laboratories. (Dutta et al., 2017). These dyes are some of the major contributors to dye contamination in water bodies due to textile industry-related effluents. Chemical methods to manage dye pollution produce solid waste, adding to environmental risks and necessitating safe disposal. Hence, there is a global exploration of alternative, eco-friendly approaches, and techniques. In addressing the escalating pollution, phytoremediation emerges as a crucial tool (Sharma et al., 2021).

Bioremediation involves harnessing biological processes to reduce pollution levels to safe thresholds

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OPEN ACCESS Original Research Article

through degradation, detoxification, mineralization, or modification. Environmental biotechnology utilizes natural elements such as microorganisms and plants to eliminate harmful organic pollutants, encompassing both bioremediation and phytoremediation (Mishel et al., 2023).

In recent period, phytoremediation has emerged as an eco-friendly, sunlight-driven and economically viable approach for the environmental cleanup compared to biological methods including physiochemical methods (Daneshvar et al., 2004).

Observations indicate that aquatic plants play a crucial role in enhancing the effectiveness of both artificial and natural wetland systems. They are naturally adapted to grow in liquid media, so this is very useful for biochemical studies since this media can be very precisely manipulated. *Pistia stratiotes* is a resilient and highly adaptable water plant, which can be utilized for various studies. It is also more convenient to take readings from water samples than soil samples, hence water plants are a preferred choice in biochemical studies (Ali et al., 2020; Schwantes et al., 2019).

Materials and Method:

1. **Plants -**

   Aquatic plants, water lettuce (Pistia stratiotes L.), and *Cyperus papyrus* were used for this study.

   Pistia stratiotes and *Cyperus papyrus* plants, sourced from the Tropical Nursery in Andheri, underwent thorough rinsing with water to eliminate any soil contaminants. Subsequently, the plants were placed in tap water and exposed to ample sunlight to adequately support photosynthesis.

2. **Dyes -**

   For *Cyperus papyrus*:

   Crystal Violet (Loba - Chemie, P.B. No. 6136) dye was obtained from and prepared in the Life science laboratory by dissolving 0.5 g of accurately weighed dye, on the electronic balance (Shimadzu Corporation, Type BL-220H, No. D55009408), in 1000 ml of tap water for a 500 ppm dye solution.

   The dye concentration was measured in the spectrophotometer at 580nm wavelength.

   Methyl Orange (SD Fine Chemicals) dye was obtained from and prepared in the Life science laboratory by dissolving 0.25 g of accurately weighed dye, on the electronic balance (Shimadzu Corporation, Type BL-220H, No. D55009408), in 1000 ml of tap water for a 250 ppm dye solution.

   The dye concentration was measured in the spectrophotometer at 450nm wavelength.

   For *Pistia stratiotes* (Water lettuce):

   Crystal Violet (Loba - Chemie, P.B. No. 6136) dye was obtained from and prepared in the Life science laboratory by dissolving 0.5 g of accurately weighed dye, on the electronic balance (Shimadzu Corporation, Type BL-220H, No. D55009408), in 1000 ml of tap water for a 500ppm dye solution.

   The dye concentration was measured in the spectrophotometer at 580nm wavelength.

   Methyl Orange (SD Fine Chemicals) dye was obtained from and prepared in the Life science laboratory by dissolving 0.25 g of accurately weighed dye, on the electronic balance (Shimadzu Corporation, Type BL-220H, No. D55009408), in 1000 ml of tap water for a 250ppm dye solution.

   The dye concentration was measured in the spectrophotometer at 450nm wavelength.

3. **Digital Spectrophotometer Readings** -The Spectrophotometer (Equiptronics EQ-820) readings were taken at 450 nm for Methyl orange and at 580 nm for Crystal Violet concentrations.

ISSN–2277- 8721

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Experimental setup –

Figure 1: The figure represents the experimental setup.

Results and Graphical Representation:

Visible Degradation of Dyes by *Cyperus papyrus*-

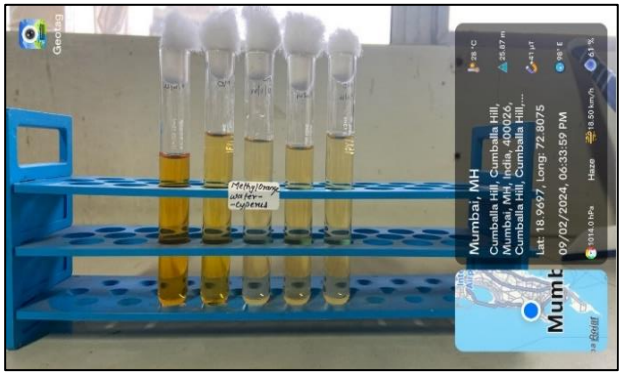


Figure 2: The figure represents the visible degradation of the Methyl Orange by *Cyperus papyrus*.

Figure 3: The figure represents the visible degradation of the Crystal Violet dye by *Cyperus papyrus*.

Figure 4: The figure represents the visible degradation of the Methyl Orange by *Pistia startiotes.*

Figure 5: the figure represents the visible degradation of the Crystal Violet by *Pistia startiotes.*

ISSN–2277- 8721

EIIRJ Electronic International Interdisciplinary Research Journal

Volume–XIII, Issues – III May – June 2024

OPEN ACCESS Original Research Article

Visible Degradation of Dyes by *Pistia stratiotes*-

Table 1: The values depicting the absorbance of CV by *Cyperus papyrus*:

Day	Crystal Violet					
	Control (DW+CV+ *Cyperus papyrus*)	Degraded CV (CV+ *Cyperus papyrus)* 1st Set	Control (DW+CV+ *Cyperus papyrus*) Duplicate Set	Degraded CV (CV+ *Cyperus papyrus)* Duplicate Set	Control (DW+CV+ *Cyperus papyrus*) Triplicate Set	Degraded CV (CV+ *Cyperus papyrus)* Triplicate Set
0	0.20	0.05	0.20	0.06	0.24	0.07
1	0.19	0.04	0.19	0.05	0.23	0.06
2	0.18	0.03	0.18	0.03	0.22	0.04
3	0.15	0.03	0.17	0.00	0.21	0.00
4	0.15	0.02	0.15	0.00	0.18	0.00

Table 2: The values depicting the absorbance of MO by *Cyperus papyrus*:

Day	Methyl Orange					
	Control (DW+MO+ *Cyperus papyrus*) (Dilution 1/10)	Degraded MO (MO+ *Cyperus papyrus*) (Dilution 1/10) 1st Set	Control (DW+MO+ *Cyperus papyrus*) (Dilution 1/10) Duplicate Set	Degraded MO (MO+ *Cyperus papyrus*) (Dilution 1/10) Duplicate Set	Control (DW+MO+ *Cyperus papyrus*) (Dilution 1/10) Triplicate Set	Degraded MO (MO+ *Cyperus papyrus*) (Dilution 1/10) Triplicate Set
0	0.95	0.55	0.94	0.56	0.98	0.68
1	0.94	0.54	0.93	0.55	0.97	0.67
2	0.66	0.40	0.56	0.46	0.53	0.32
3	0.50	0.28	0.50	0.43	0.49	0.26
4	0.30	0.14	0.31	0.14	0.41	0.25

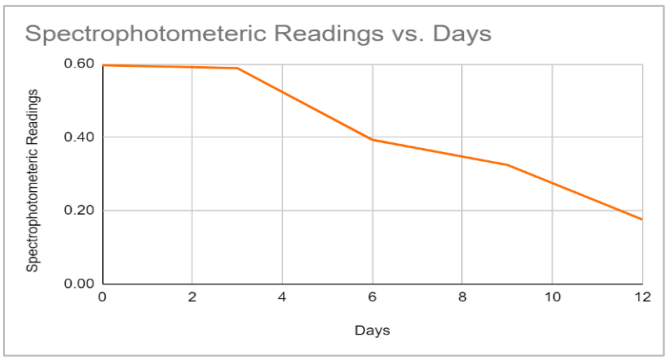


Figure 6: The graph represents the visible degradation of the Methyl Orange by *Cyperus papyrus.*

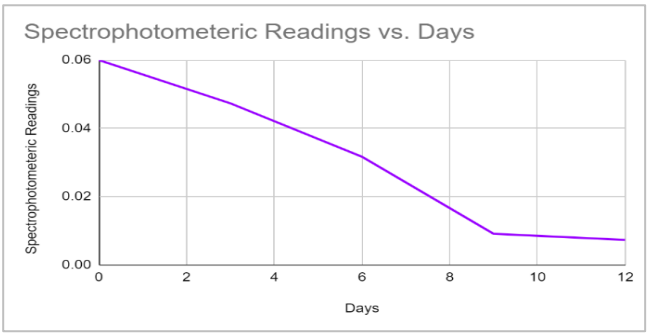


Figure 7: The graph represents the visible degradation of the Crystal Violet by *Cyperus papyrus.*

ISSN–2277- 8721

EIIRJ Electronic International Interdisciplinary Research Journal

Volume–XIII, Issues – III May – June 2024

OPEN ACCESS Original Research Article

Table 3: The values depicting the absorbance of CV by *Pistia stratiotes*

Day	Crystal Violet					
	Control (DW+CV+ *Pistia stratiotes*)	Degraded CV (CV+ *Pistia stratiotes)* 1st Set	Control (DW+CV+ *Pistia stratiotes*) Duplicate Set	Degraded CV (CV+ *Pistia stratiotes)* Duplicate Set	Control (DW+CV+ *Pistia stratiotes*) Triplicate Set	Degraded CV (CV+ *Pistia stratiotes)* Triplicate Set
0	0.22	0.05	0.20	0.06	0.22	0.05
1	0.21	0.04	0.19	0.05	0.21	0.04
2	0.19	0.04	0.18	0.03	0.21	0.03
3	0.16	0.02	0.17	0.01	0.19	0.02
4	0.14	0.02	0.15	0.00	0.18	0.00

Table 4: The values depicting the absorbance of MO by *Pistia stratiotes*

Day	Methyl Orange					
	Control (DW+MO+ *Pistia stratiotes*) (Dilution 1/10)	Degraded MO (MO+ *Pistia stratiotes*) (Dilution 1/10)	Control (DW+MO+ *Pistia stratiotes*) (Dilution 1/10) Duplicate Set	Degraded MO (MO+ *Pistia stratiotes*) (Dilution 1/10) Duplicate Set	Control (DW+MO+ *Pistia stratiotes*) (Dilution 1/10) Triplicate Set	Degraded MO (MO+ *Pistia stratiotes*) (Dilution 1/10) Triplicate Set
0	0.95	0.81	0.99	0.65	0.98	0.74
1	0.94	0.80	0.98	0.64	0.97	0.73
2	0.66	0.47	0.66	0.47	0.60	0.47
3	0.50	0.33	0.46	0.45	0.53	0.44
4	0.30	0.31	0.31	0.40	0.49	0.29

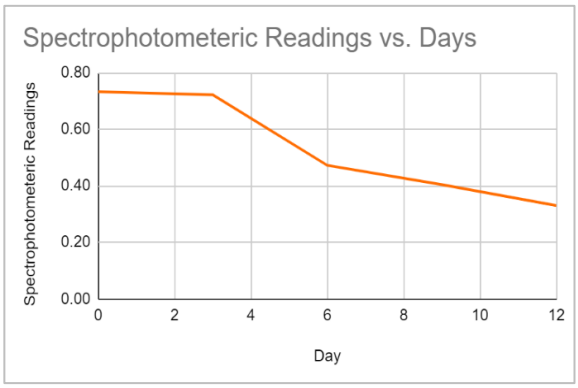


Figure 8: The graph represents the visible degradation of the Methyl Orange by *Pistia stratiotes.*

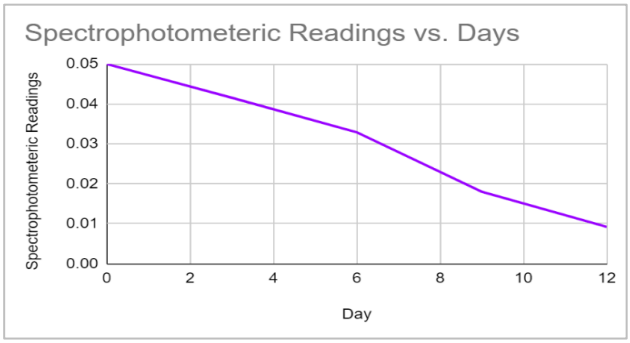


Figure 9: The graph represents the visible degradation of the Crystal Violet by *Pistia stratiotes.*

ISSN–2277- 8721

EIIRJ Electronic International Interdisciplinary Research Journal

Volume–XIII, Issues – III May – June 2024

OPEN ACCESS Original Research Article

Results and Calculations:

The efficiency of photodegradation was determined using the subsequent equation:

$$E = \left(\frac{C}{C_0}\right) \times 100\%$$

The concentration values of the dye solution at an initial time "t" are denoted as, C0 (mg/l) and C (mg/l)

To calculate the concentration of the dyes:

1. Methyl orange:

Concentration (mg/l):	Absorbance at 450 nm
0	0
250	0.6
500	0.94
Cyperus	0.18
Pistia stratiotes	0.33

Slope = $\frac{y_2 - y_1}{x_2 - x_1} = \frac{0.94 - 0.6}{500 - 250} = 0.00136$

Equation of line: y = 0.00136x + 0.16

A. Concentration of *Cyperus papyrus* (left in water) = 14.706 mg/l

Hence, Degradation/Taking up the efficiency of Methyl Orange by *Cyperus papyrus*.= 94.118 %

B. Concentration of *Pistia stratiotes* (left in water) = 125 mg/l

Hence, Degradation/Taking up the efficiency of Methyl Orange by *Pistia stratiotes* = 50 %

2. Crystal violet:

Concentration (mg/l):	Absorbance at 580 nm
0	0
250	0.05
500	0.06
Cyperus	0.01
Pistia stratiotes	0.03

Slope = $\frac{y_2 - y_1}{x_2 - x_1} = \frac{0.05 - 0.00}{250 - 0} = 0.0002$

Equation of line: y = 0.0002x

A. Concentration of *Cyperus papyrus* (left in water) = 50 mg/l

Hence, Degradation/Taking up the efficiency of Crystal Violet by *Cyperus papyrus* = 90%

B. Concentration of *Pistia stratiotes* (left in water) = 150 mg/l

Hence, Degradation/Taking up the efficiency of Crystal Violet by *Pistia stratiotes* = 70%

ISSN–2277- 8721

EIIRJ Electronic International Interdisciplinary Research Journal

Volume–XIII, Issues – III May – June 2024

OPEN ACCESS Original Research Article

Conclusion and Discussion:

The results show that the efficiency of degradation/taking up of Methyl Orange by *Cyperus papyrus* was observed to be 94.118%, more than that of water lettuce. The efficiency of degradation/taking up of Crystal violet by *Cyperus papyrus* was 90%. Accumulation of the crystal violet dye was observed at the roots of water lettuce plants. This could be potentially used for recovering the dye back by the industries. This would lead to less production of new dye due to recycling of the older product (via recovering it from the roots), and thus less pollution. Recovery of the dye and this re-cycling process could be beneficial for the environment (less pollution and degradation of ecosystems), as well as the industry (economical since less processing is involved).

Methyl orange was seen to be absorbed into the root and leaf tissues of the plants (as seen in the images below).An increase in plant growth was observed in both the dye solutions. As the experiment progressed, new growth emerged, indicating the plants' ability to reproduce, survive, and thrive in contaminated dye solutions. Conversely, plants in the aqueous dye solution exhibited symptoms of wilting, such as loss of rigidity, after approximately 17-20 days of exposure. Consequently, it can be concluded that *Pistia stratiotes* (Water lettuce) and *Cyperus papyrus* are suitable candidates for phytoremediation, demonstrating potential for use in water bodies contaminated with dye solutions.

Supplementary Data

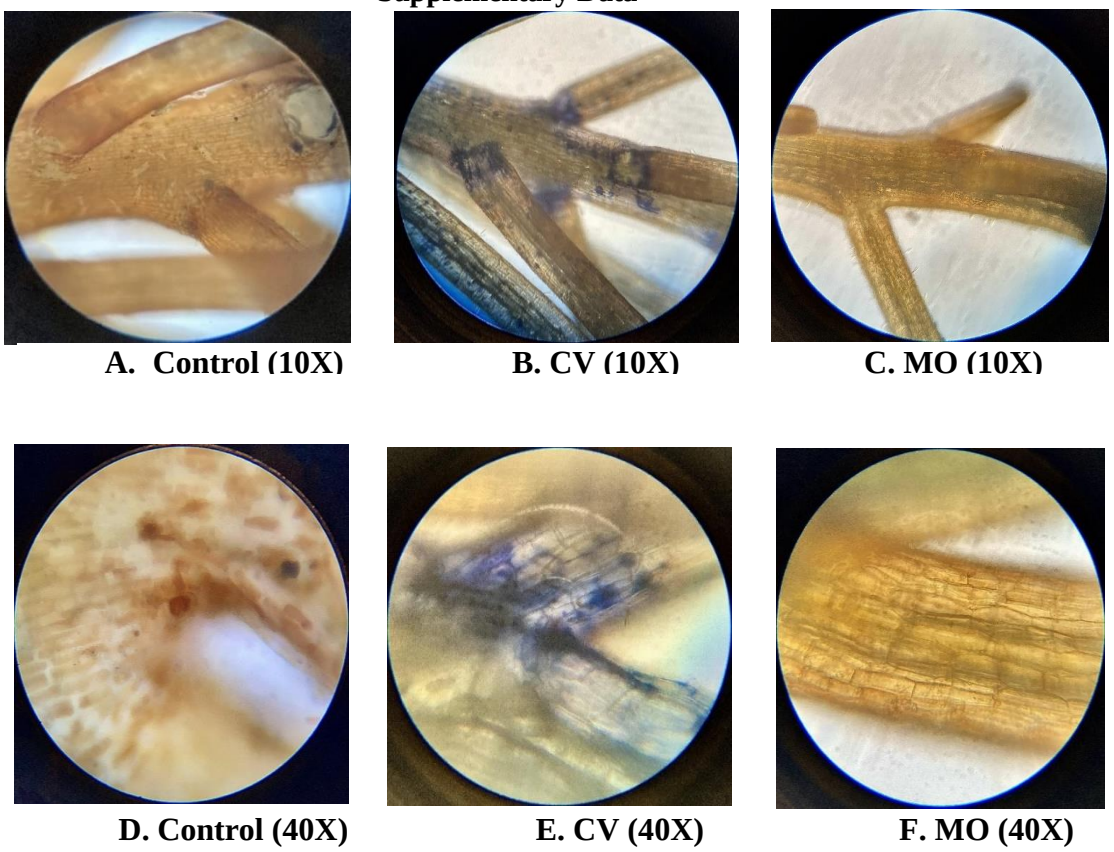


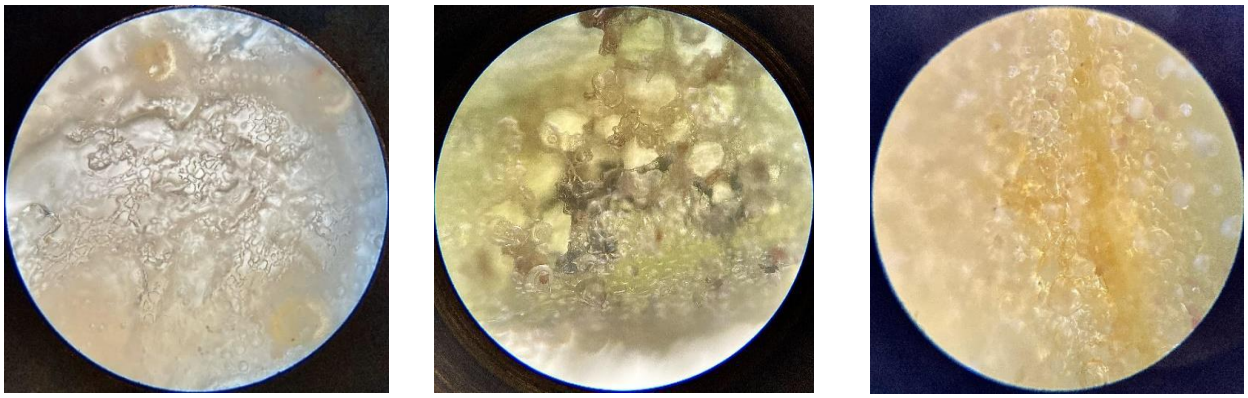
Figure S1: The image depicts the uptake of Crystal Violet (A-C) and Methyl Orange (D-F) in the roots of *Pistia stratiotes* (Water lettuce)

ISSN–2277- 8721

EIIRJ Electronic International Interdisciplinary Research Journal

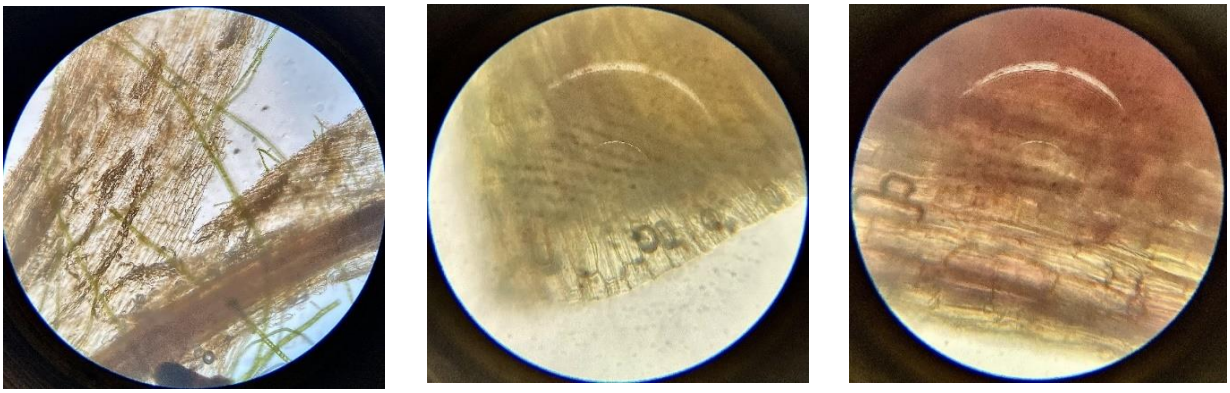
Volume–XIII, Issues – III May – June 2024

OPEN ACCESS Original Research Article

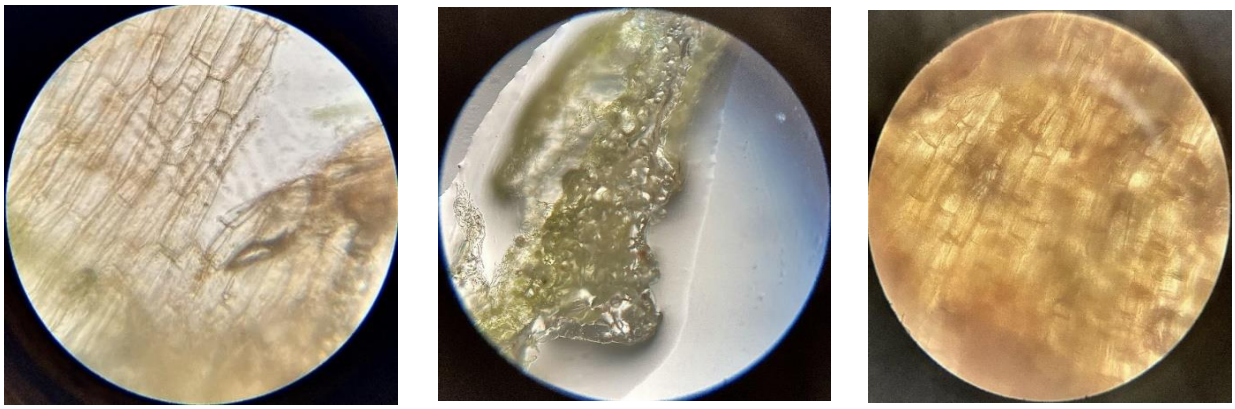


A. Control (40X) B. CV (40X) C. MO (40X)

Figure S2: The image depicts the uptake of Crystal Violet (B) and Methyl Orange (C) in the leaves of *Pistia stratiotes* (Water lettuce)



A. Control (10X) B. CV (10X) C. MO (10X)



D. Control (40X) E. CV (40X) F. MO (40X)

Figure S3: The image depicts the uptake of Crystal Violet (A-C) and Methyl Orange (D-F) in the roots of *Cyperus papyrus*.

ISSN–2277- 8721

EIIRJ Electronic International Interdisciplinary Research Journal

Volume–XIII, Issues – III May – June 2024

OPEN ACCESS Original Research Article

Scope for the Future:

Phytoremediation offers a promising solution for reclaiming heavily chemically polluted soil, enjoying widespread public acceptance, and presenting numerous advantages over other physicochemical methods. Nonetheless, phytoremediation encounters specific constraints, particularly its time-consuming process, especially in moderately to highly polluted areas where remediation can be prolonged. Fortunately, genetic engineering has emerged as a powerful tool for altering plants to showcase desired characteristics, such as accelerated growth, enhanced biomass production, resilience to harsh chemicals, and adaptability to various environmental conditions. In practice, a singular approach is neither feasible nor adequate for effectively cleaning up heavily chemically polluted soil. It is essential to combine multiple approaches, including genetic engineering, microbe-assisted techniques, and chelate-assisted methods, to ensure highly effective and thorough phytoremediation in the future.

Despite the availability of numerous chemical and mechanical remediation methods, these approaches only offer temporary solutions to our environmental challenges. Hence, it is imperative to implement long-term strategies for eradicating pollutants from the environment. Phytoremediation has demonstrated both environmental and economic benefits, presenting a reliable and safer alternative to other remediation techniques. With thorough research in this field and appropriate guidance and regulations from the Environmental Protection Agency, phytoremediation holds promise for achieving a cleaner, greener world.

Acknowledgements:

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