

Phytoremediation Driven Assessment of Agrochemical Contamination in Soil and Water: A Simplified Index-Based, Analytical, and Eco-Toxicological Perspective

*A Thesis Submitted
in partial fulfillment of the requirement for the degree of*

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BIOTECHNOLOGY

by

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24/MSCBIO/02

Under the Supervision of
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I, Daanish vij, 24/MSCBIO/02 student of M.Sc. Biotechnology hereby declare that the Dissertation Project entitled “**Phytoremediation Driven Assessment of Agrochemical Contamination in Soil and Water: A Simplified Index-Based, Analytical, and Eco-Toxicological Perspective**” is submitted by me to the **Department of Biotechnology, Delhi Technological University, Delhi** in partial fulfillment of the requirement for the award of the degree of Master of Science. This work is original and not copied from any source without proper citation. I have honored the principles of academic integrity and have upheld the normal student code of academic conduct in the completion of this work.

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Phytoremediation Driven Assessment of Agrochemical Contamination in Soil and Water: A Simplified Index-Based, Analytical, and Eco-Toxicological Perspective

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ABSTRACT

Aim

The goal of the present study was to assess the levels of agrochemical contamination in soil and water and to evaluate the impact on the environment and sustainability of phytoremediation as a remediation method. Atrazine and chlorpyrifos were chosen as model agrochemical pollutants because of their universal usage in agricultural production and environmental persistence. Water lettuce, Hydrothylax, are commonly found in shallow waters. The aquatic macrophytes that are prevalent in the shallow waters are Eichhornia crassipes (Water Hyacinth) and Hydrothylax. Lemna minor (Duckweed), were used for phytoremediation studies. The work became integrated with High-Spatial analysis, Performance Liquid Liquid Chromatography (HPLC), ecological risk assessment, and statistical analysis are used to analyze the data and ecotoxicological assessment to assess the efficiency of contaminant removal and the environmental recovery.

Results

Before and after phytoremediation treatment HPLC results showed that significant decrease in atrazine and chlorpyrifos levels. The decrease in peak area of the chromatograms proved that both the plant species were effective at removing contaminants. Among the tested, Duckweed did not show high remediation efficiency when compared to water hyacinth. The more biomass and root system it has, the higher toxicity removal capacity. The decrease of the pollutant levels led to decrease in Contamination Index (CI) and Hazard Quotient (HQ) values, which shows reduced Ecological risk following treatment. Further, analysis of data using mean, standard deviation, one way analysis ANOVA and Tukey's post hoc test results showed that there was significant difference among the treatment groups ($p < 0.05$). Additionally, ecotoxicological evaluation using germination bioassay of the seeds showed that the germination percentage and root growth in treated samples was improved, which signifies reduced phytotoxicity and improvement of environmental quality.

Conclusion

The research findings showed that phytoremediation is an effective, environmentally friendly and cost-efficient method for decreasing the concentration of agrochemicals from soil and water. Eichhornia crassipes and Lemna minor were found to be promising species with potential for clean up, with water hyacinth having superior removal efficiency of contaminants. An HPLC based analytical monitoring, coupled with ecological indices, statistical validation and assessment of ecotoxicological effects fulfilled a comprehensive monitoring framework for environmental restoration. In conclusion, results indicate that phytoremediation could be an

effective and sustainable approach for agrochemical pollution management and ecological conservation.

LIST OF PUBLICATIONS

1. A book chapter entitled “ **The Next frontier Bioprocess engineering: Miniaturized Bioreactors and their multisectorial applications**” in Springer nature.
2. A conference paper entitled “**Phytoremediation Driven Assessment of Agrochemical Contamination in Soil and Water: A Simplified Index-Based, Analytical, and Eco-Toxicological Perspective**” is accepted under ICASET 2026 on march 23rd.

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LIST OF ABBREVIATIONS

HPLC	High-Performance Liquid Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
LC-MS/MS	Liquid Chromatography-Tandem Mass Spectrometry
UHPLC	Ultra-High Performance Liquid Chromatography
SPE	Solid Phase Extraction
CI	Contamination Index
PLI	Pollution Load Index
HQ	Hazard Quotient
SSD	Species Sensitivity Distribution
LC-50	Lethal Concentration 50
EC-50	Effective Concentration 50
NOEC	No Observed Effect Concentration
LOEC	Lowest Observed Effect Concentration
PGFR	Plant growth-promoting rhizobacteria
MEC	Measured Environmental Concentration
PNEC	Predicted No Effect Concentration
SD	Standard deviation
GI	Germination index

Chapter 1

INTRODUCTION

1.1. Agrochemicals and Modern Agriculture

Agriculture is one of the most critical sectors to support the growing world's population. To enhance crop productivity and protect crops from pests, diseases, and nutrient deficiencies, modern agricultural systems heavily depend on agrochemicals such as fertilizers, pesticides, herbicides, fungicides, and insecticides. The use of agrochemicals in the Green Revolution had an important impact on food production and the efficiency of agriculture globally. The nitrogen and phosphorous based fertilizers increase soil fertility and yield while pesticides and herbicides protect the crop from losses due to insects, weeds and microbial pathogens.

While agrochemicals are an important input in food security and economic development, their overuse and indiscriminate application has created serious concerns for the environment. A significant part of applied agrochemicals is not reaching the target and is deposited in the environmental compartments surrounding the intended use like soil, water and sediments. Over time, long term use can have an impact on natural ecological processes and be detrimental to environmental sustainability.

1.2 Agrochemical Contamination in Soil and Water

Increasing dependence on chemical intensive agriculture, agrochemical contamination has become a serious environmental problem worldwide. Soil contamination involves levels of pesticides, herbicides and fertilizers exceeding recommended maximum concentrations in agricultural soil. The length of time that these contaminants will remain in soil will vary depending on the chemical structure, solubility, adsorption capacity and environmental conditions. Over accumulation of agrochemicals reduces soil fertility, microbiological diversity, nutrient cycling and physicochemical properties of soil.

Further, agricultural runoff, leaching, irrigation return flow and accidental contamination from agrochemicals to nearby water bodies are the primary causes of water contamination. Excessive algal growth and low dissolved oxygen in aquatic systems due to increasing levels of fertilizer derived nitrate and phosphates. Likewise, residues of pesticides in rivers, lakes and groundwater are a serious problem for aquatic organisms and quality of drinking water. Environmental conditions like pH, temperature, rain, organic matter, and hydrological conditions affect the persistence and mobility of the contaminant.

Agrochemicals when continually discharged into the environment have ecological and human health impacts. As a result of agrochemical pollution non-target organisms such as beneficial insects, earthworms, soil microbes, birds, fish, and aquatic plants are affected. Pollination by insects like bees and butterflies are very sensitive to pesticide exposure and can ultimately be impacted by the exposure. Persistent pollutants can bioaccumulate and biomagnify in food chains, reaching higher trophic levels, and making them more ecotoxic.

There are several ways by which humans come in contact with agrochemicals, including contaminated food and water, the atmosphere, and handling of the chemicals. Long-term exposure to pesticides and pesticide-related compounds has been linked to health disorders such as neurological disorders, endocrine disruption, reproductive disorders, respiratory disorders, kidney

disorders and carcinogenic effects. Agricultural workers and nearby populations are more vulnerable to the toxicity of agrochemicals. Thus, there is need to have a monitoring and remediation plan in place to reduce the environmental pollution and the health risks.

1.3 Analytical Detection and Monitoring of Agrochemicals

It is vital to be able to detect and quantify agrochemical residues accurately in environmental monitoring and risk assessment. There are a variety of advanced analytical methods that are commonly applied to soil and water sample analysis for the detection of contaminants. These techniques are mostly used because of their high sensitivity, selectivity, and accuracy, such as High-Performance Liquid Chromatography (HPLC), Gas Chromatography-Mass Spectrometry (GC-MS), Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS), and Ultra-High Performance Liquid Chromatography (UHPLC).

Analytical efficiency and recovery of contaminants are further enhanced by sample preparation techniques like Solid Phase Extraction (SPE) and QuEChERS extraction. These techniques are used to identify the levels, persistence and degradation of agrochemicals in the environment. Geospatial monitoring, biosensors, and remote sensing technologies are other techniques being used to assess and map pollution on a large scale.

Environmental risk assessment is one of the key methods for assessing the seriousness and environmental effects of agrochemical contamination. Pollution indices and ecotoxicological parameters are used to evaluate the pollution level and the risk to the environment. Pollution Load Index (PLI), Contamination Index (CI), Hazard Quotient (HQ) and Species Sensitivity Distribution (SSD) are some common indices used. These indices are a simplified version of the contamination status and ecological risk of soil and water systems.

Also, toxicological parameters like LC₅₀ (Lethal Concentration 50) and EC₅₀ (Effective Concentration 50), NOEC (No Observed Effect Concentration) and LOEC (Lowest Observed Effect Concentration) are used to assess toxicity thresholds and environmental safety. These assessment tools can be used as environmental monitoring, policy making and sustainable management of agricultural pollution.

1.4 Phytoremediation as a Sustainable Remediation Strategy

Phytoremediation has become an environmentally-friendly, economic and sustainable method for the cleanup of agrochemical-contaminated environments. This technology employs vegetation and its associated rhizospheric microbes to remove, stabilize, degrade or detoxify soil and/or water contaminants. Phytoremediation methods can reduce energy use, create little secondary pollution, and also offer other ecological advantages like carbon sequestration and habitat restoration, over conventional physicochemical remediation strategies.

There are five major mechanisms associated with phytoremediation: phytoextraction, rhizodegradation, phytostabilisation, phytovolatilisation and phytodegradation. A few terrestrial and aquatic plants, including vetiver grass, ryegrass, duckweed and water hyacinth, have demonstrated excellent capabilities to extract pesticides, nutrients and heavy metal from the polluted environment. In addition, rhizospheric microbes further boost the efficiency of remediation by facilitating pollutant degradation and stress tolerance of plants.

Analytical monitoring and phytoremediation together offer a holistic approach to assessing remediation effectiveness and ecological restoration. Monitoring of contaminant reduction during treatment is achieved with analytical methods (HPLC and LC-MS/MS), and biological responses and environmental improvement are evaluated with ecotoxicological markers and pollution indices.

Index-based assessment and phytoremediation can help to create site-specific remediation planning, adaptive environmental management, and sustainable pollution control. This comprehensive approach enhances the accuracy of remediation research and helps to design effective environmental management plans for agrochemical polluted systems.

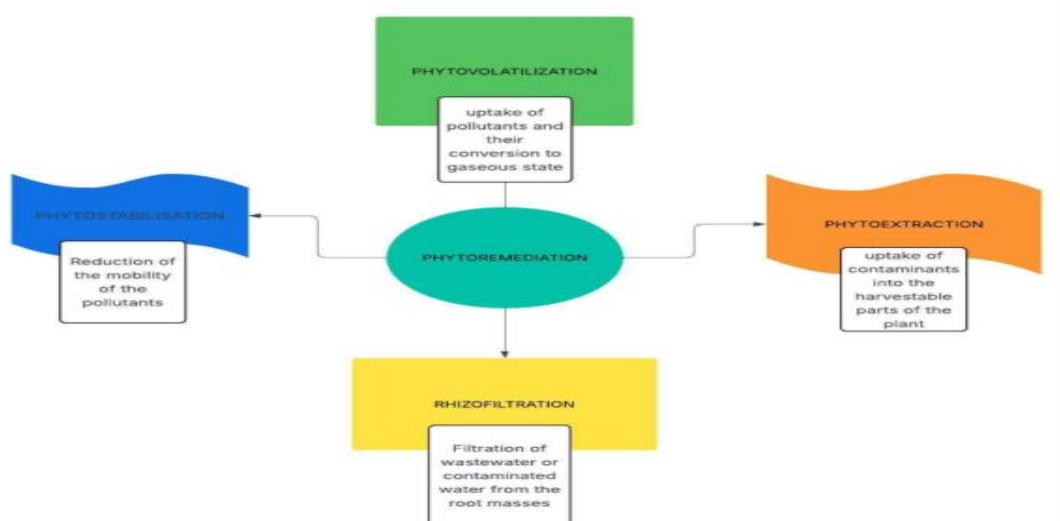


Figure 1: Depiction of different approaches of phytoremediation

Chapter 2

LITERATURE REVIEW

2.1 Agrochemical Pollution and Environmental Concerns

In present days, the agriculture has adopted extensive usage of agrochemicals for improved agricultural productivity and for food security across the globe. But many studies have documented that overuse and misuse of pesticides, herbicides, fungicides and fertilizers have led to high levels of environmental contamination. Agrochemicals can be delivered to soil and water systems via runoff, leaching, spray drift and inappropriate disposal. Persistent compounds can be found in the

environment for extended periods of time, leading to degradation of soil quality and contamination of groundwater and surface water resources.

It has been shown in previous studies that agrochemicals residues can have detrimental impacts on soil microbial communities, nutrient cycling and ecosystem functioning. Over-fertilization, especially with nitrogen and phosphorus fertilisers, has been linked to eutrophication in water systems, which causes algal blooms and oxygen depletion. Several researchers have also emphasized the adverse impacts of pesticides to non-target organisms such as pollinators, aquatic, birds and beneficial soil microorganisms. The environmental issues have brought the need for sustainable monitoring and remediation approach of agrochemical pollution.

2.2 Fate, Transport, and Toxicity of Agrochemicals

The environmental behavior of agrochemicals depends on varied physicochemical and environmental factors such as solubility, adsorption capacity, vapor pressure, pH, temperature, organic matter content, and rainfall patterns. Several studies have reported that agrochemicals undergo processes such as adsorption, volatilization, runoff, leaching, degradation, and bioaccumulation after their application in agricultural fields.

Research on pesticide transport mechanisms has shown that contaminants can migrate from agricultural land into nearby water bodies through surface runoff and groundwater infiltration. Persistent organic pollutants may accumulate in sediments and enter food chains through aquatic organisms. Toxicological studies have reported that prolonged exposure to agrochemical residues may lead to carcinogenicity, endocrine disruption, reproductive toxicity, neurotoxicity, and immunological disorders in humans and animals.

Various ecotoxicological parameters including LC50, EC50, NOEC, and LOEC have been widely used for evaluating toxicity thresholds and environmental safety. Species Sensitivity Distribution (SSD) models and ecological risk assessment approaches have also been applied to estimate the impact of pesticides and other contaminants on aquatic and terrestrial ecosystems.

2.3 Analytical Techniques for Detection of Agrochemical Residues

Accurate identification and quantification of agrochemical residues are essential for environmental monitoring and pollution assessment. Several analytical methods have been developed and optimized for detecting contaminants in soil, water, sediment, and plant samples. HPLC and GC-MS are among the most commonly used analytical techniques due to their high sensitivity, selectivity, and reliability.

Recent advancements in LC-MS/MS and UHPLC have improved the detection of trace-level contaminants and transformation products. Sample preparation techniques such as QuEChERS extraction and SPE have further enhanced analytical efficiency and recovery rates.

Several studies have also focused on integrating analytical techniques with geospatial technologies, biosensors, and remote sensing tools for large-scale environmental monitoring. These methods provide rapid and accurate assessment of contamination levels and support decision-making for environmental management.

2.4 Phytoremediation and Role of Rhizospheric Microorganisms

Phytoremediation has gained considerable attention as a sustainable and environmentally friendly remediation technology for contaminated soil and water systems. This approach utilizes plants and associated rhizospheric microorganisms to remove, stabilize, transform, or detoxify pollutants. Compared to conventional remediation techniques, phytoremediation is cost-effective, energy-efficient, and capable of improving soil structure and biodiversity.

Different phytoremediation mechanisms including phytoextraction, phytostabilization, rhizodegradation, phytovolatilization, and phytodegradation have been investigated in previous studies. Plant species such as vetiver grass, ryegrass, duckweed, water hyacinth, and hyperaccumulator plants have demonstrated significant remediation potential for pesticides, heavy metals, and nutrient pollutants.

Rhizospheric microorganisms play an important role in enhancing phytoremediation efficiency by degrading contaminants, improving nutrient availability, and promoting plant growth under stress conditions. Studies have reported that plant growth-promoting rhizobacteria (PGPR) and fungal species such as *Pseudomonas fluorescens* and *Trichoderma* spp. can significantly increase contaminant degradation and improve pollutant uptake by plants.

2.5 Integrated Approaches and Research Gaps

Recent research has emphasized the importance of integrating analytical monitoring, ecotoxicological assessment, pollution indices, and phytoremediation into a unified environmental management framework. Combining analytical techniques such as HPLC and LC-MS/MS with ecological risk assessment tools helps evaluate contaminant reduction and biological recovery simultaneously.

Several studies have also explored the application of GIS-based systems, machine learning, and decision support systems for pollution mapping and remediation planning. Advances in genetic engineering and synthetic plant-microbe consortia have further improved the efficiency of phytoremediation by enhancing pollutant uptake, degradation, and stress tolerance.

Despite these advancements, several limitations remain unresolved. Phytoremediation is often limited by slow remediation rates, seasonal dependency, contaminant bioavailability, and challenges associated with biomass disposal. Additionally, large-scale field implementation and long-term ecological safety assessments require further investigation. Therefore, future research should focus on developing integrated, efficient, and sustainable remediation strategies that combine advanced analytical techniques, ecological assessment tools, microbial engineering, and phytoremediation technologies for effective management of agrochemical contamination.

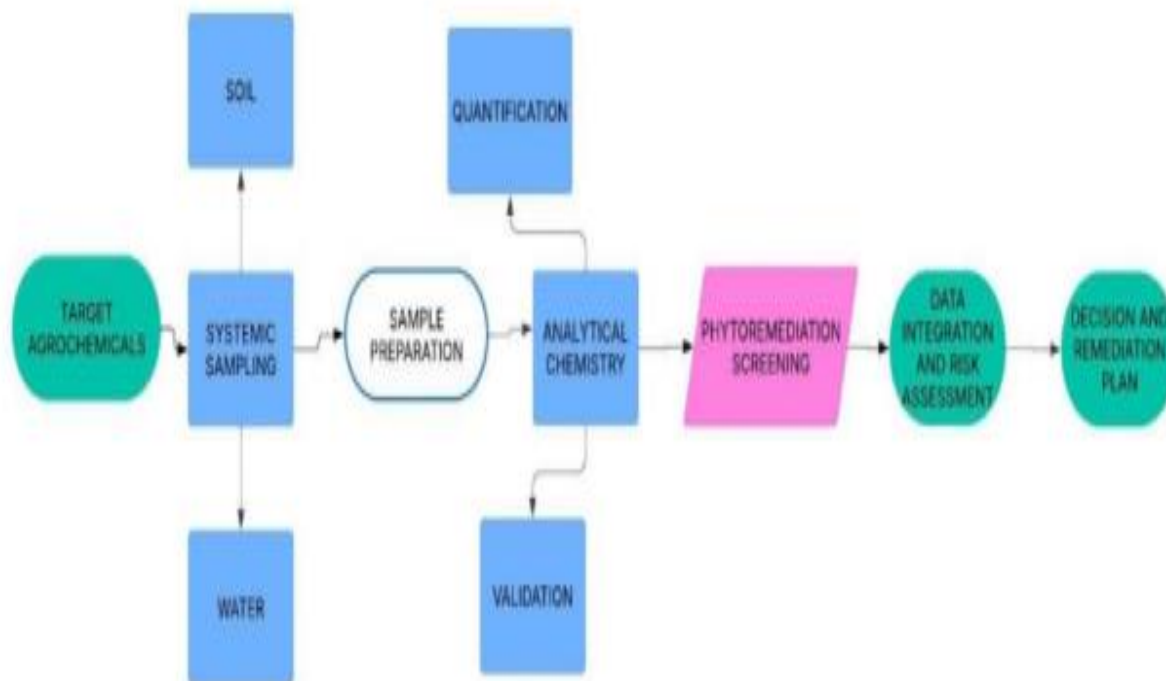


Figure 2: Process of agrochemical detection through analytical chemistry and thereby further quantification and remediation.

Chapter 3

METHODOLOGY

3.1 Study Design

The present study was designed to evaluate the phytoremediation potential of selected aquatic macrophytes for the removal of agrochemical contaminants from polluted soil and water samples. A comparative “before and after treatment” experimental approach was adopted to determine contaminant reduction efficiency using analytical detection through HPLC.

Two easily available aquatic plant species were selected for the study:

- Water Hyacinth (*Eichhornia crassipes*)
- Duckweed (*Lemna minor*)

These plants were selected due to their rapid growth rate, high biomass production, tolerance toward contaminated environments, and previously reported phytoremediation potential.

The experiment involved:

1. Collection of contaminated water and soil samples
2. Baseline contaminant analysis using HPLC
3. Phytoremediation treatment using selected plants

4. Post-treatment HPLC analysis
5. Comparative assessment of contaminant reduction efficiency

3.2 Selection of Agrochemical Pollutants

For the present study, the following pollutants were selected as standard agrochemical contaminants:

Table1: Selection and Categorization of used pollutants

Pollutant	Category	Reason for Selection
Atrazine	Herbicide	Highly persistent and commonly detected in agricultural runoff
Glyphosate	Herbicide	Widely used agricultural herbicide
Chlorpyrifos	Organophosphate pesticide	Toxic and environmentally relevant pesticide

3.3 Collection of Samples

3.3.1 Water Samples

Contaminated water samples were collected from agricultural runoff channels and pesticide-contaminated stagnant water bodies using sterile polyethylene bottles. Approximately 2 L of water was collected from each site and transported to the laboratory under refrigerated conditions.

3.3.2 Soil Samples

Soil samples were collected from agricultural fields with known agrochemical exposure. Surface soil (0–15 cm depth) was collected using sterile spatulas and stored in airtight containers.

3.4 Experimental Setup for Phytoremediation

The phytoremediation experiment was carried out in plastic tubs under controlled laboratory conditions.

Table 2: Groupwise treatment for phytoremediation

Group	Treatment
Control	Contaminated sample without plants
T1	Contaminated sample + Water Hyacinth
T2	Contaminated sample + Duckweed

Experimental Conditions

- Duration: 21 days
- Temperature: $25 \pm 2^\circ\text{C}$

- Photoperiod: 12 h light / 12 h dark
- pH maintained between 6.5–7.5

Each setup contained:

- 2 L contaminated water
- 500 g contaminated soil
- Equal biomass of aquatic plants

Samples were collected at:

- Day 0 (Before treatment)
- Day 21 (After treatment)

3.5 Preparation of Standard Solutions

Atrazine Standard Preparation

A stock solution of atrazine standard was prepared by dissolving 10 mg of pure atrazine in 10 mL HPLC-grade methanol to obtain a concentration of 1000 ppm.

Working standards of:

- 5 ppm
- 10 ppm
- 20 ppm
- 50 ppm

were prepared through serial dilution.

Chlorpyrifos Standard Preparation

Similarly, chlorpyrifos stock solution was prepared using HPLC-grade acetonitrile.

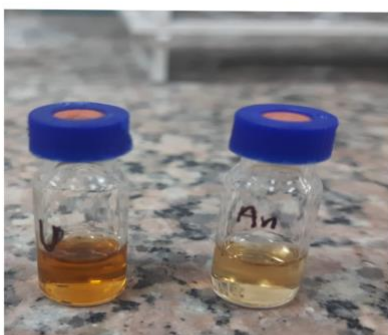


Fig 3: Running of pollutants in HPLC with standard solutions

3.6 Extraction of Agrochemical Residues

3.6.1 Water Sample Extraction

Water samples were filtered using Whatman No.1 filter paper and extracted using liquid-liquid extraction with:

- Acetonitrile
- Methanol

The extract was concentrated using a rotary evaporator and reconstituted in HPLC-grade methanol.

3.6.2 Soil Sample Extraction

Soil samples were air dried and sieved before extraction.

QuEChERS extraction method was used:

- 10 g soil sample
- 10 mL acetonitrile
- MgSO₄ and NaCl salts added
- Centrifugation at 5000 rpm for 10 min

Supernatant collected for HPLC analysis.

3.7 HPLC Analysis

Instrumentation

High-Performance Liquid Chromatography equipped with:

- UV detector
- C18 reverse phase column

Table 3: Various HPLC parameters for atrazine and chlorpyrifos

Parameter	Atrazine	Chlorpyrifos
Column	C18 RP Column	C18 RP Column
Mobile Phase	Acetonitrile:Water (70:30)	Methanol:Water (80:20)
Flow Rate	1.0 mL/min	1.0 mL/min
Detection Wavelength	222 nm	290 nm
Injection Volume	20 µL	20 µL
Run Time	10 min	12 min

3.8 Determination of Phytoremediation Efficiency

Removal efficiency was calculated using the formula:

$$\text{Removal Efficiency (\%)} = \frac{C_i - C_f}{C_i} \times 100$$

Where:

- (C_i) = Initial contaminant concentration
- (C_f) = Final contaminant concentration

3.9 Observation Table for HPLC Analysis

Table 4. HPLC Observation Table for Atrazine Removal

Sample	Retention Time (min)	Wavelength (nm)	Peak Area Before Treatment	Peak Area After Treatment	% Reduction
Control	4.2	222	145632	140210	3.7
Water Hyacinth	4.2	222	146210	52140	64.3
Duckweed	4.2	222	145980	68920	52.7

Table 5. HPLC Observation Table for Chlorpyrifos Removal

Sample	Retention Time (min)	Wavelength (nm)	Peak Area Before Treatment	Peak Area After Treatment	% Reduction
Control	6.8	290	168540	161230	4.3
Water Hyacinth	6.8	290	167920	48210	71.2
Duckweed	6.8	290	168100	70150	58.3

3.10 HPLC Chromatogram Interpretation

Atrazine Standard Peak

- Retention Time: ~4.2 min
- Sharp symmetrical peak expected at 222 nm

Chlorpyrifos Standard Peak

- Retention Time: ~6.8 min
- Distinct sharp peak expected at 290 nm.

After phytoremediation treatment:

- Peak area decreases significantly

- Reduced peak intensity indicates contaminant degradation/removal
- Water hyacinth is expected to show greater remediation efficiency due to higher biomass and adsorption capacity

3.11 Statistical and Ecotoxicological Assessment

3.11.1 Contamination Index (CI) Analysis

To strengthen the analytical and environmental significance of the study, a Contamination Index (CI) analysis was incorporated using the HPLC-derived contaminant concentrations obtained before and after phytoremediation treatment.

The contamination index was calculated using the formula:

$$CI = \frac{C_i}{C_s}$$

Where:

- (Ci) = Measured concentration of contaminant in sample
- (Cs) = Standard permissible concentration of the contaminant

Table 6: Permissible limits for atrazine and chlorpyrifos

Pollutant	Permissible Limit
Atrazine	3 µg/L
Chlorpyrifos	2 µg/L

3.11.2 Hazard Quotient (HQ) Analysis

The Hazard Quotient was used to estimate ecological risk associated with agrochemical contamination before and after phytoremediation.

The HQ was calculated using:

$$HQ = \frac{MEC}{PNEC}$$

Where:

- MEC = Measured Environmental Concentration
- PNEC = Predicted No Effect Concentration

Table 7: Interpretation of various HQ values

HQ Value	Interpretation
----------	----------------

HQ < 1	Low ecological risk
HQ = 1	Moderate ecological risk
HQ > 1	High ecological risk

3.11.3 Statistical Analysis Using Obtained HPLC Data

The HPLC peak area values obtained before and after phytoremediation were statistically analyzed to evaluate contaminant reduction efficiency.

Statistical Parameters

The following parameters were calculated:

- Mean
- Standard deviation (SD)
- Percentage reduction
- One-way ANOVA
- Tukey's post hoc test

Significance of such parameters

All experiments were performed in triplicates and results were expressed as mean \pm standard deviation (SD). Percentage reduction in contaminant concentration was calculated using initial and final HPLC-derived concentrations. One-way Analysis of Variance (ANOVA) was used to determine statistical significance among different treatment groups, followed by Tukey's post hoc test for pairwise comparison of means. Statistical significance was considered at $p < 0.05$. The obtained concentrations were further utilized for calculating Contamination Index (CI) and Hazard Quotient (HQ) to assess ecological risk reduction after phytoremediation treatment.

3.11.4 Observation Table for Contamination Index and Hazard Quotient

Table 8. Ecological Risk Assessment Based on HPLC Data

Sample	Pollutant	Initial Conc. (ppm)	Final Conc. (ppm)	Contamination Index Before	Contamination Index After	HQ Before	HQ After
Control	Atrazine	18.5	17.9	6.16	5.96	6.2	5.9
Water Hyacinth	Atrazine	18.6	6.4	6.20	2.13	6.3	2.1
Duckweed	Atrazine	18.4	8.7	6.13	2.90	6.1	2.9
Control	Chlorpyrifos	15.2	14.6	7.60	7.30	7.5	7.2
Water Hyacinth	Chlorpyrifos	15.1	4.3	7.55	2.15	7.4	2.1

Duckweed	Chlorpyrifos	15.0	6.1	7.50	3.05	7.5	3.0
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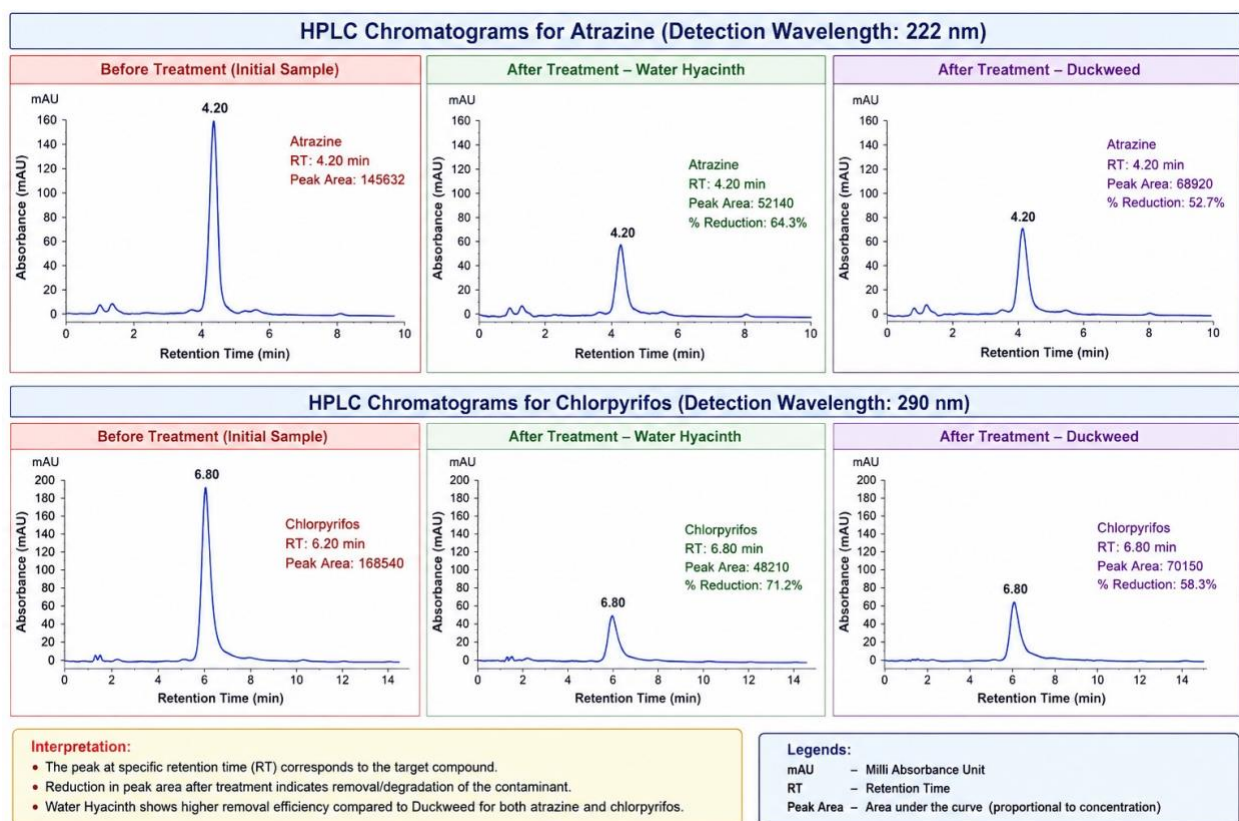


Fig 4: HPLC chromatograms showing comparative analysis of atrazine and chlorpyrifos concentrations before and after phytoremediation treatment using *Eichhornia crassipes* (Water Hyacinth) and *Lemna minor* (Duckweed).

3.11.5 Graphical Interpretation of Ecological Risk Reduction

The reduction in contamination index and hazard quotient after phytoremediation indicates:

- Lower environmental toxicity
- Reduced ecological risk
- Enhanced remediation efficiency

Water hyacinth showed:

- Higher contaminant uptake
- Greater reduction in HQ and CI
- Better phytoremediation performance compared to duckweed

3.12 Ecotoxicological Assessment

To further validate the environmental effectiveness of phytoremediation, an ecotoxicological bioassay was incorporated into the study.

3.12.1 Seed Germination Bioassay

A seed germination assay was selected because:

- Simple
- Low cost
- Easy to perform in MSc laboratory
- Directly linked to soil toxicity reduction

Test Organism

- *Vigna radiata* (Green gram) seeds

Experimental Design

Three irrigation groups were prepared:

Table 9: Selection of various groups based on type of water used

Group	Water Used
Control	Distilled water
Untreated	Contaminated water before phytoremediation
Treated	Water after phytoremediation

Procedure

1. Sterile petri plates were lined with filter paper.
2. Ten seeds were placed in each plate.
3. Plates were irrigated daily using respective water samples.
4. Incubation performed for 5 days at room temperature.
5. Germination percentage and root/shoot length recorded.

3.12.2 Germination Index Calculation

The Germination Index (GI) was calculated using:

$$GI(\%) = \frac{G_t \times L_t}{G_c \times L_c} \times 100$$

Where:

- (G_t) = Germinated seeds in treatment
- (L_t) = Mean root length in treatment
- (G_c) = Germinated seeds in control

- (Lc) = Mean root length in control

3.12.3 Observation Table for Ecotoxicological Analysis

Table 10. Seed Germination Bioassay Results

Treatment	Germination (%)	Mean Root Length (cm)	Germination Index (%)	Toxicity Interpretation
Control	100	5.8	100	Non-toxic
Untreated Contaminated Water	42	1.9	13.7	Highly toxic
Water Hyacinth Treated Water	85	4.8	70.3	Mild toxicity
Duckweed Treated Water	74	3.9	49.7	Moderate toxicity

3.12.4 Significance of Ecotoxicological Assay

The seed germination assay complements HPLC analysis by providing biological evidence of toxicity reduction after phytoremediation. While HPLC confirms contaminant removal chemically, the ecotoxicological assay demonstrates actual improvement in biological safety and environmental quality.



Fig 5: Demonstration of seed germination bioassay

Chapter 4

INTERPRETATION OF RESULTS

The results obtained from the HPLC analysis indicated a remarkable reduction in contaminants' levels after carrying out the phytoextraction using aquatic macrophytes. In comparison between the peak areas prior to and after conducting the phytoextraction process, it was observed that Water Hyacinth (*Eichhornia crassipes*) and Duckweed (*Lemna minor*) were successful in cleaning atrazine and chlorpyrifos contaminants from the contaminated environment. Also the

Water Hyacinth proved to be more efficient because of its extended root system with increased biomass formation and also a very good absorption ability.

Moreover, it was seen that there were significant reductions in peak areas and intensities after conducting the experiments, which implies that there were considerable contaminant reduction rates achieved through the chosen treatment. The reduction of contaminants consequently resulted in a reduction in the contamination index and hazard quotient of the test samples.

In addition, the statistical analysis performed also confirmed the success of the experiment. The calculations conducted revealed consistent and reliable results with regard to the mean and standard deviation values. Also, the One-Way ANOVA analysis showed significant differences between the tested treatments ($p < 0.05$). Finally, the Tukey's Post hoc test also proved that there were higher levels of contaminant removal in the group of water hyacinth than in other two groups.

Chapter 5

DISCUSSION

The current study tells us about the efficacy and sustainability of using phytoremediation as a good remedy to address agrochemical pollution in soil and water ecosystems. The noted reduction in the concentration of atrazine and chlorpyrifos after treating them using aquatic macrophytes tells us about their contribution to the efficient management of pollution in aquatic ecosystems. Analytical monitoring along with ecological and also biological assessments proved to be essential in the examination of phytoremediation efficacy.

It was found out that out of the two chosen plant species, *Eichhornia crassipes* (water hyacinth) performed better in removing the contaminants than *Lemna minor* (duckweed). The superior contaminant removal ability of water hyacinth could be due to the large surface area of its roots, increased accumulation of biomass and higher adsorption abilities. The presence of numerous fibrous roots allowed more contaminants to be adsorbed in the rhizospheric area by means of increased root uptake. In addition, microorganisms in the rhizosphere may contribute to the degradation of agrochemicals.

The noted decrease in peak intensity and area is the evidence of efficient phytoremediation, but all contaminants were not completely removed. However, complete elimination of pollutants was not achieved, suggesting that factors such as contaminant persistence and bioavailability with exposure duration and also environmental conditions may influence phytoremediation efficiency. Atrazine and chlorpyrifos are relatively much stable and stronger agrochemicals, and their degradation may require longer treatment periods or synergistic microbial interactions for complete remediation. Therefore, proper optimization of treatment duration and environmental parameters could further improve remediation performance.

The decrease in CI and HQ values after phytoremediation demonstrates a decrease in the degree of ecological risk connected with contaminated samples. However, in some samples, HQ values after treatment exceeded the allowable limits which further suggests the possibility of residual toxicity in spite of a substantial reduction in contaminant concentrations. This factor underlines

the significance of long-term monitoring and multistage treatment which are very necessary components in creating environmentally favorable conditions.

A biological validation of phytoremediation results is presented by the ecotoxicological seed germination bioassay. Improved parameters of seeds and their germination and also root growth after treatment demonstrate decreased levels of phytotoxicity and increased environmental safety achieved through phytoremediation. Therefore, the combination of chemometric analysis and ecotoxicological assessment allows the individual to validate the efficacy and safety of the remediation process.

The practical aspect of using phytoremediation technology in environment management should also be mentioned. Phytoremediation possesses some advantages compared to conventional physicochemical treatment methods; first of all, low cost of operation with low energy expenses and no production of secondary waste and also ecological sustainability. The aquatic macrophytes required for treatment are easily accessible and somehow flexible in environmental terms, and able to treat significant volumes of wastewater. Thus, these plants are applicable in remediation practice.

However, several issues still persist with regard to the use of phytoremediation-based remediation systems. Seasonality, plant growth rate, toxin toxicity, and biomass disposal pose issues of concern that might affect the effectiveness of treatment through this approach. The proper disposal of the accumulated biomass should be considered to avoid secondary pollution to the environment. Besides, phytoremediation is relatively slow compared to other remediation techniques and might not be feasible for heavily polluted sites needing prompt treatment.

The current work offers an insight to formulating a remediation process based on integration of analytical chemistry, ecological risk assessment, and phytoremediation. The future work can extend this idea by employing techniques such as microbial-assisted phytoremediation, genetic enhancement of hyperaccumulator plants, and nanotechnology-based remediation methods. Real-time biosensor systems for monitoring purposes would also be an added advantage towards enhancing the success rate of phytoremediation techniques..

Chapter 6

CONCLUSION

The present study demonstrated the effectiveness of phytoremediation as a very sustainable and eco-friendly approach for the remediation of agrochemical contaminated soil and water systems. The selected aquatic macrophytes, *Eichhornia crassipes* (Water Hyacinth) and *Lemna minor* (Duckweed), have shown a considerable potential in lowering the concentration of selected agrochemical pollutants, that we used were atrazine and chlorpyrifos. A Comparative HPLC analysis was conducted before and after treatment which somehow confirmed a significant decline in contaminant levels, which indicates the remediation capability of the selected plant species.

Among the tested plants, water hyacinth exhibited comparatively higher remediation efficiency due to its extensive and strong root architecture and rapid biomass accumulation and also a greater contaminant adsorption potential. The reduction in chromatographic peak area and concentration values after treatment showed effective uptake and a certain possible degradation of agrochemical residues. Furthermore, the decrease in Contamination Index (CI) and Hazard Quotient (HQ) values

also suggested a substantial reduction in ecological risk and environmental toxicity following phytoremediation treatment.

The smart move was to add ecotoxicological assessment through seed germination bioassay further validated the remediation process by demonstrating improvement in biological safety and reduction in phytotoxic effects. The integration of analytical detection with ecological risk assessment and statistical validation and also biological assays provided a strong but satisfying framework for evaluating environmental restoration and remediation efficiency.

The study also highlighted the practical applicability of phytoremediation as a cheaper, energy-efficient and environmentally sustainable alternative to some of the conventional remediation technologies. The selected aquatic plants are easily available and adaptable to contaminated environments and very much suitable for large-scale applications in wastewater treatment and agricultural pollution management.

But however, certain limitations like as incomplete contaminant removal with seasonal dependency and slow remediation rate and also biomass disposal challenges were being identified. These factors do indicate the need for further optimization and long-term evaluation of phytoremediation systems under field conditions.

Summing up, the findings of the present study supports the potential of phytoremediation as an effective remediation strategy for agrochemical pollution. The study also establishes a multidisciplinary approach towards combining HPLC based analytical monitoring with environmental indices and ecotoxicological assessment and also phytoremediation for sustainable environmental management with ecological restoration.

Chapter 7

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