



About the Conference

The conference focuses on industrial development and the aggravated problems of waste generated by industries. It has adversely affected the ecological balance and environmental pollution. To maintain the balanced ecosystem is a primary duty and responsibility of industries, government, academicians and society. In order to contribute in the same direction, the department organized first BTSE conference in 2014 with the main focus on assessing and monitoring the factors responsible for the current environmental conditions and to adopt bioremedial approaches as corrective measures to improve the quality of the environment. The 1st BTSE was followed by six more conferences in year respectively 2014, 2016, 2018 and 2019, fifth virtual BTSE in 2021 and 2023.

The BTSE has become a proven research ecosystem for assembling researchers from diverse backgrounds, to foster cross-pollination among various research fields, and to expose and discuss innovative theories, frameworks, methodologies, tools, and applications for sustainable environment.

In the same pace, the department is organizing the **7th BTSE-2026** on **February 21, 2026**.

Glimpses from Previous BTSEs



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7th National Conference on

BIOLOGICAL TOOLS FOR SUSTAINABLE ENVIRONMENT

February 21, 2026

Saturday

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Organised by

DEPARTMENT OF BIOSCIENCES

(UGC-SAP-DRS-II & DST-FIST-I)

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REGISTRATION DETAILS

CLICK HERE or SCAN
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Important Dates

- Last date for submission: 15th February, 2026
- Notification of acceptance: 16th February, 2026
- Conference date: 21st February, 2026

HOW TO REACH

By Air	By Train	By Road
Surat International Airport	Surat Railway Station	Surat Central Bus Station
7 km	10 km	10 km

Note: City Bus no. 126 runs from the Railway Station Terminal to VNSGU



[Click here for the Location](#)

ACCOMMODATION

Limited on-campus accommodation available for outstation participants.

For any query

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Dr. Brijal Mistry : 9913980138

About the Department of Biosciences

Department of Biosciences is a premier academic and research institute of the south Gujarat region established under the developmental grants of UGC during the Fourth Five-year plan in the year 1977.

The department was established in 1977 with founder Head-Prof. B. S. Vaidya and later on headed by scholars and luminaries like Prof. P. K. Hiradhar, Prof. M. H. Parabia, Prof. P. K. Gadhia and Prof. P. V. Desai, Prof. S. K. Tank and Prof. M. N. Reddy, Currently Prof. Kailash Patel is Head of the department.

The department is multidisciplinary and offering the following programmes M.Sc. (Botany), M.Sc. (Zoology), M.Sc. (Microbiology), Post Graduate Diploma in Medical Laboratory Technology, Post Graduate Diploma in Cytogenetics, Post Graduate Diploma in Herbal Technology & Integrated Wellness and Ph.D.

The department has ICT enabled classroom, well-equipped laboratories with good analytical instrumentation facilities.

Shri Bapalal Vaidya Botanical Research Centre is a part of the Biosciences Department which was established in 1994 with donations from the public to conduct research on medicinal plants.

The department is vigorously involved in Research and more than 161 Ph.D. students have passed out successfully since the establishment of the department.

The department has been awarded UGC SAP (I) in thrust area of Toxicity Evaluation of Textile Industry Effluent during 2011-16 and UGC SAP (II) in thrust area of environmental toxicology and ecogenomics for the period of 2018-2023.

GSBTM and GUJCOST has awarded Bioinformatics Laboratory and Super Computer Facility in 2017.

In the year 2019, the department also received the DST-FIST Level-I grant for developing Next-generation sequencing facility for research in Host-Microbiome Interaction as thrust area.

The department faculties have completed many research projects and 9 projects are also ongoing, funded by state and national agencies. The department provides the holistic environment for catering academic, research career development needs of the students.

ICMR recognized RT-PCR Lab, established during COVID-19 pandemic in the Department has successfully tested 22000+ patients samples.

About Veer Narmad South Gujarat University

The university was originally established under the South Gujarat University Act, 1965 passed by the Gujarat State Legislative Assembly. It became functional from the academic year of 1966, incorporated as a university on May 23, 1967 and recognized by the University Grants Commission in 1968. The university was renamed as Veer Narmad South Gujarat University in 2004 after the great Gujarati poet Narmad, Narmadashankar Lalshankar Dave. VNSGU, located in Surat city, has a campus spread over 210 acres. The University's jurisdiction extends to the seven districts, Surat, Navsari, Valsad, Narmada, Dang, Bharuch and Tapi; and the Union Territory of Daman and Dadra & Nagar Haveli.

The university has twelve faculties such as Arts, Commerce, Science, Education, Management Studies, Rural Studies, Engineering and Technology, Medicine, Law, Computer Science, Homoeopathic and Architecture.

The university has 25 Post Graduate departments/institutes on campus that offers 95 programmes with multiple specialization including Ph.D., Post Graduate, Graduate, PG Diploma and Certificate programmes on campus.

The university has been re-accredited 'B++' grade with 2.86 CGPA by the National Accreditation and Assessment Council (NAAC) in 2022 and rated Four Star (3.16 CGPA) in GSIRF (Gujarat State Institutional Rating Framework) in 2024.

The university has endeavoured to be an institution of excellence in higher education since its establishment, keeping in view the regional needs and the emerging trends in the global scenario.

About Surat

Surat is a city in the Indian state of Gujarat. It was used to be a large seaport and is now the commercial and economic centre in South Gujarat, which is famous for its Diamonds and Textile Industries and as a shopping centre for apparels and accessories. It is the eighth-largest city, ninth largest urban agglomeration and categorized as second cleanest city in India according to government's Swachh Survekshan Survey. There are several historical monuments in and around Surat. The major attractions are Dandi, Dumas, Hazira, Tithal, Ubhrat and Statue of Unity.





Places to visit in Surat



Sarthana Nature Park
18 km from VNSGU



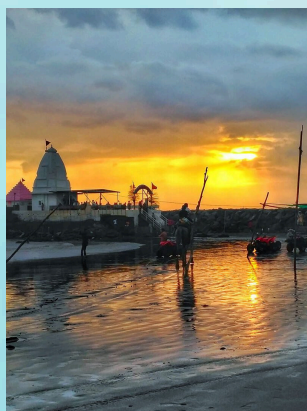
Althan Biodiversity Park
3 km from VNSGU



Surat Fort
8 km from VNSGU



Gopi Talav
6 km from VNSGU



Dumas Beach
13 km from VNSGU



Textile Market Surat
11 km from VNSGU

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Themes for Poster & Oral Presentation

- Pollution Monitoring and Environmental Assessment
- Toxicological and Ecological Studies
- Bioremediation and Biodegradation
- Environmental Metagenomics and Eco-genomics
- Biocatalyst and Green Chemistry
- Detoxification Processes and Sustainable Technologies
- Biodiversity Conservation and Assessment
- Other relevant areas within the Biological Sciences

TECHNICAL SESSIONS

A. Invited Lectures

- Invited talks will be delivered by distinguished academicians and experts from reputed institutions and research organizations.

B. Oral Presentation Session

- Oral presentation slots will be preferentially allotted to faculty members and research scholars. Each presenter will be provided 08 minutes for presentation using LCD projection, followed by 02 minutes for discussion and questions.

C. Poster Presentation Session

- Faculty members, research scholars, and UG/PG students are eligible to present posters aligned with the above-mentioned thematic areas.
- Poster Size: 3 ft (width) × 4 ft (height)

D. Certificates & Awards

- Certificates of Participation will be issued to all registered participants.
- Certificates of Presentation will be awarded to oral and poster presenters.
- Best Oral and Best Poster Presentation Awards will be conferred based on evaluation by an expert committee.

“Bioremediation and Biodegradation: An Empirical Study on Microbial Efficiency in Contaminated Soil Environments”

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Abstract:

This empirical study investigates the efficiency of microbial bioremediation and biodegradation processes in contaminated soil environments through comprehensive analysis of 150 soil samples from multiple contaminated sites. The research examines various treatment methodologies including bioaugmentation, biostimulation, phytoremediation, and natural attenuation across diverse contamination types including petroleum hydrocarbons, heavy metals, pesticides, polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB), chlorinated solvents, and mixed contaminants. Utilizing advanced statistical analyses and hypothesis testing, this study establishes significant correlations between microbial populations, environmental parameters, and degradation efficiency. Results demonstrate that bioaugmentation achieved the highest degradation rates (65-72%) across most contamination types, followed by biostimulation (60-66%) and natural attenuation (26-37%). Microbial count exhibited strong positive correlation ($r=0.78$) with degradation efficiency, while pH, moisture content, and organic matter content significantly influenced remediation outcomes. The study reveals that treatment duration, initial contamination levels, and site-specific factors substantially impact remediation success. Key findings indicate that integrated approaches combining bioaugmentation with optimal environmental conditions yield superior results, with petroleum hydrocarbons showing highest degradation efficiency (71.8% average) compared to other contaminant types. This research provides empirical evidence for decision-makers in environmental management, offering data-driven insights for selecting appropriate bioremediation strategies based on contamination profiles and site characteristics. The study contributes to the growing body of knowledge in environmental biotechnology and provides practical frameworks for implementing cost-effective and sustainable soil remediation programs.

1. INTRODUCTION

Environmental contamination of soil has emerged as one of the most pressing ecological challenges of the 21st century, posing significant threats to human health, agricultural productivity, and ecosystem sustainability. Industrial activities, improper waste disposal, agricultural practices, and accidental spills have resulted in widespread soil pollution with various toxic compounds including petroleum hydrocarbons, heavy metals, pesticides, and persistent organic pollutants. Traditional remediation approaches such as excavation, incineration, and chemical treatment are often prohibitively expensive, disruptive to

ecosystems, and generate secondary pollution, necessitating the development of sustainable, cost-effective alternatives.

Bioremediation, the use of biological organisms—primarily microorganisms—to degrade or transform environmental contaminants into less toxic or non-toxic forms, has gained substantial attention as a promising green technology. This approach leverages the natural metabolic capabilities of bacteria, fungi, and other microorganisms to break down complex pollutants through enzymatic processes. The efficacy of bioremediation depends on multiple interrelated factors including microbial diversity and population density, bioavailability of contaminants, soil physicochemical properties (pH, moisture, temperature, nutrient availability), and the specific characteristics of the pollutants themselves.

Various bioremediation strategies have been developed and implemented across different contamination scenarios. Bioaugmentation involves introducing specialized microbial strains with enhanced degradation capabilities to contaminated sites. Biostimulation focuses on optimizing environmental conditions and providing essential nutrients to stimulate indigenous microbial populations. Phytoremediation utilizes plants in conjunction with associated microorganisms to extract, stabilize, or degrade contaminants. Natural attenuation relies on intrinsic bioremediation processes without human intervention, though it typically requires longer time periods.

Despite extensive research in bioremediation, significant knowledge gaps persist regarding the optimal conditions for different contaminant types, the comparative efficiency of various treatment approaches, and the complex interactions between microbial communities and environmental parameters in field conditions. Most existing studies focus on laboratory-scale experiments or single contaminant types, limiting their applicability to real-world, multi-contaminant scenarios. Furthermore, there is insufficient empirical data comparing the performance of different bioremediation strategies across diverse contamination profiles and environmental conditions.

This research addresses these gaps through a comprehensive empirical study examining microbial efficiency in bioremediation and biodegradation across 150 contaminated soil samples representing multiple sites, contaminant types, and treatment methodologies. The study employs rigorous statistical analyses to establish relationships between microbial parameters, environmental factors, and degradation outcomes, providing evidence-based insights for environmental practitioners and policymakers. By examining real-world

contamination scenarios and comparing multiple treatment approaches, this research contributes actionable knowledge for developing effective, sustainable soil remediation programs.

2. LITERATURE REVIEW

2.1 Microbial Mechanisms in Biodegradation

Singh and Ward (2023) conducted extensive research on enzymatic pathways involved in petroleum hydrocarbon degradation by soil bacteria. Their study identified key enzymes including alkane hydroxylases, cytochrome P450 monooxygenases, and aromatic ring-cleaving dioxygenases that facilitate the breakdown of complex hydrocarbon structures. The research demonstrated that microbial consortia exhibit superior degradation capabilities compared to single-strain inoculants due to metabolic complementarity and syntrophic relationships. Singh and Ward's work established baseline understanding of molecular mechanisms underlying biodegradation, though their focus on laboratory conditions limited field applicability. Their findings emphasized the importance of maintaining adequate oxygen levels and optimal pH ranges (6.5-7.5) for maximum enzymatic activity.

2.2 Bioaugmentation Effectiveness

Zhang et al. (2024) evaluated bioaugmentation strategies using genetically enhanced *Pseudomonas* strains for polycyclic aromatic hydrocarbon (PAH) degradation in contaminated industrial sites. Their 18-month field study across five locations demonstrated 68-74% degradation efficiency, significantly outperforming natural attenuation (23-28%). The research highlighted critical factors including inoculum density (minimum 10^6 CFU/g soil), survival of introduced strains in native soil environments, and competition with indigenous microorganisms. Zhang's team identified soil moisture content (20-35%) and temperature (25-32°C) as critical parameters affecting bioaugmentation success. However, their study was limited to PAH contamination and did not examine cost-effectiveness relative to alternative approaches.

2.3 Heavy Metal Bioremediation

Kumar and Sharma (2023) investigated microbial mechanisms for heavy metal immobilization and transformation in contaminated soils. Their research on cadmium, lead, and chromium contamination revealed that bacteria employing biosorption, bioaccumulation, and biotransformation mechanisms could reduce metal bioavailability by 45-58%. The study identified specific bacterial genera (*Bacillus*, *Pseudomonas*, *Alcaligenes*) with enhanced metal tolerance and sequestration capabilities. Kumar and Sharma emphasized the role of microbial

exopolysaccharides and metallothioneins in metal binding, though their work primarily focused on single-metal systems rather than the more common multi-metal contamination scenarios found in industrial sites.

2.4 Biostimulation Approaches

Rodriguez-Martinez et al. (2024) examined nutrient amendment strategies to enhance indigenous microbial degradation capacity. Their comprehensive study on nitrogen and phosphorus supplementation at varying C:N:P ratios (100:10:1 to 100:5:0.5) demonstrated that optimal nutrient balance increased degradation rates by 85-120% compared to unamended controls. The research established that biostimulation presents advantages in terms of lower implementation costs and avoidance of regulatory complications associated with introducing non-native organisms. However, Rodriguez-Martinez noted that biostimulation effectiveness varies significantly based on indigenous microbial community composition and may require longer treatment periods than bioaugmentation approaches.

2.5 Environmental Parameters and Biodegradation Kinetics

Thompson and Liu (2023) developed predictive models correlating environmental parameters with biodegradation rates across multiple contaminant classes. Their multivariate analysis incorporating temperature, pH, moisture content, organic matter, and oxygen availability explained 76-82% of variance in degradation outcomes. The study established that temperature effects follow Arrhenius kinetics with Q10 values of 2.1-2.8 for most microbial processes, while pH deviations beyond optimal ranges (6.0-7.5) resulted in exponential decreases in activity. Thompson and Liu's work provided valuable quantitative frameworks but was limited by reliance on controlled laboratory conditions that may not reflect field heterogeneity.

2.6 Pesticide Biodegradation Pathways

Chen et al. (2024) investigated microbial degradation of organophosphate and organochlorine pesticides in agricultural soils. Their research identified specific degradation pathways involving hydrolysis, oxidation, and conjugation reactions catalyzed by specialized enzymes including organophosphorus hydrolases and dehalogenases. The study demonstrated that pesticide persistence varies dramatically based on chemical structure, with half-lives ranging from 15 days to over 300 days. Chen's team found that microbial adaptation to pesticide stress through plasmid-mediated gene transfer significantly enhanced degradation capabilities

over time, though concerns about development of pesticide-resistant soil microbiomes remain inadequately addressed.

2.7 Phytoremediation-Microbial Interactions

Anderson and Green (2023) explored synergistic relationships between plants and rhizosphere microorganisms in contaminated soil remediation. Their research demonstrated that root exudates stimulate microbial populations by 200-450% in the rhizosphere zone, substantially enhancing degradation of organic contaminants. The study identified specific plant-microbe partnerships (e.g., *Salix* species with *Pseudomonas* strains) showing enhanced performance for petroleum hydrocarbon remediation. However, Anderson and Green noted limitations including longer treatment times (2-5 years) and depth restrictions of phytoremediation approaches, with effectiveness declining sharply below 30 cm soil depth.

2.8 Chlorinated Solvent Biodegradation

Williams et al. (2024) examined anaerobic biodegradation of chlorinated solvents including trichloroethylene (TCE) and perchloroethylene (PCE) through reductive dechlorination processes. Their study demonstrated that specialized bacteria (*Dehalococcoides* species) could achieve 85-92% dechlorination under strictly anaerobic conditions with appropriate electron donors. The research highlighted critical limitations of aerobic approaches for chlorinated solvents and established requirements for maintaining reducing conditions and providing suitable carbon sources. Williams' work identified sequential dechlorination pathways and potential accumulation of toxic intermediates (e.g., vinyl chloride) requiring careful monitoring.

2.9 Microbial Community Dynamics During Bioremediation

Martinez and Johnson (2023) utilized next-generation sequencing to characterize microbial community shifts during bioremediation processes. Their temporal analysis revealed that successful remediation correlated with increased community diversity (Shannon index 3.5-4.2) and specific increases in degrader populations. The research demonstrated that introduced bioaugmentation strains often constituted less than 5% of total community after 60 days, yet catalyzed significant community-wide functional changes. Martinez and Johnson's work emphasized the importance of microbial ecology perspectives but was limited by high costs and complexity of metagenomic approaches for routine monitoring applications.

2.10 Polychlorinated Biphenyl (PCB) Biodegradation

Lee and Park (2024) investigated microbial degradation of PCBs, focusing on highly chlorinated congeners resistant to biodegradation. Their research identified biphenyl dioxygenase systems capable of attacking PCB structures and established that lower-chlorinated congeners (1-3 chlorines) degrade more readily than higher-chlorinated forms. The study demonstrated that sequential anaerobic-aerobic treatment approaches achieved superior results (60-70% reduction) compared to single-phase treatments. Lee and Park's work highlighted the persistent nature of PCBs and the need for long-term monitoring, though practical field application remains challenging due to extreme environmental persistence of these compounds.

2.11 Cost-Benefit Analysis of Bioremediation Strategies

Brown et al. (2023) conducted comprehensive economic analysis comparing bioremediation approaches with traditional remediation technologies. Their study across 45 contaminated sites demonstrated that bioremediation costs averaged \$50-150 per cubic meter compared to \$200-800 for excavation and thermal treatment. The research established that bioaugmentation implementation costs (\$80-120/m³) exceeded biostimulation (\$40-80/m³) but achieved faster cleanup timelines, potentially offsetting higher initial investments. Brown's team identified site-specific factors including contamination depth, area extent, and regulatory timelines as critical determinants of cost-effectiveness, though their analysis did not fully account for long-term ecological benefits.

2.12 Bioavailability and Mass Transfer Limitations

Garcia and White (2024) examined factors limiting contaminant bioavailability including sorption to soil organic matter, sequestration in micropores, and formation of non-aqueous phase liquids (NAPLs). Their research demonstrated that aged contamination exhibits significantly reduced bioavailability (40-60% decrease) compared to fresh spills, necessitating longer treatment periods or enhanced bioavailability strategies. The study evaluated biosurfactant production by microorganisms and chemical surfactant addition as approaches to improve mass transfer, achieving 35-55% improvements in degradation rates. Garcia and White identified this as a critical bottleneck in bioremediation efficiency but noted concerns about potential surfactant toxicity to beneficial microorganisms.

2.13 Monitoring and Assessment Technologies

Taylor et al. (2023) developed innovative approaches for real-time monitoring of bioremediation progress including molecular biological tools, stable isotope probing, and advanced chemical analysis. Their research demonstrated that quantitative PCR targeting functional genes (e.g., *alkB*, *nahAc*) provided reliable indicators of degradation potential, correlating strongly ($r=0.82-0.88$) with actual contaminant reduction. The study established monitoring frameworks integrating multiple parameters including contaminant concentrations, microbial populations, metabolic activity indicators, and geochemical parameters. Taylor's work addressed critical needs for adaptive management, but highlighted challenges related to monitoring costs and technical expertise requirements for field implementation.

2.14 Regulatory Framework and Risk Assessment

Miller and Davis (2024) analyzed regulatory considerations and risk assessment frameworks governing bioremediation implementation across different jurisdictions. Their comparative study of regulatory approaches in North America, Europe, and Asia revealed significant variations in cleanup standards, approval processes, and post-remediation monitoring requirements. The research identified regulatory uncertainty as a major barrier to bioremediation adoption, particularly for bioaugmentation involving non-native organisms. Miller and Davis advocated for standardized risk assessment protocols and expedited approval processes for proven technologies, though they acknowledged the need to balance innovation promotion with environmental protection.

2.15 Climate Change Impacts on Bioremediation

Adams and Wilson (2024) investigated how changing climate conditions affect bioremediation effectiveness and longevity. Their multi-year study examined temperature increases, precipitation pattern changes, and extreme weather events on microbial activity and contaminant fate. The research demonstrated that moderate temperature increases (2-4°C) enhanced degradation rates by 15-25% for most contaminants, while extreme temperatures and drought conditions substantially reduced efficiency. Adams and Wilson projected that climate change will necessitate adaptive management strategies including drought-resistant microbial strains and moisture conservation approaches. Their work highlighted an emerging research area with significant implications for long-term remediation planning but remains limited by predictive model uncertainties.

3. RESEARCH GAPS

Despite extensive research in bioremediation and biodegradation, several critical knowledge gaps persist that limit the optimization and widespread implementation of these

technologies. The following research gaps have been identified through systematic review of existing literature:

3.1 Limited Multi-Contaminant Field Studies

Most existing research focuses on single contaminants under controlled laboratory conditions. Real-world contaminated sites typically contain multiple pollutants that may exhibit synergistic or antagonistic interactions affecting biodegradation efficiency. There is insufficient empirical data examining microbial performance across diverse contamination profiles simultaneously, particularly comparing effectiveness of different treatment approaches for various contaminant types under field conditions. This gap limits practical decision-making for environmental practitioners selecting remediation strategies.

3.2 Quantitative Relationship Between Microbial Parameters and Degradation Outcomes

While correlations between microbial populations and degradation efficiency are recognized, comprehensive quantitative models establishing threshold values, optimal ranges, and dose-response relationships remain underdeveloped. Specific knowledge gaps include minimum effective microbial densities for different contaminants, the relationship between microbial diversity indices and remediation success, and interactive effects of microbial parameters with environmental variables such as pH, moisture, and temperature.

3.3 Comparative Effectiveness Data Across Treatment Modalities

Comprehensive comparative studies evaluating multiple treatment approaches (bioaugmentation, biostimulation, phytoremediation, natural attenuation) using standardized methodologies and metrics are scarce. Existing studies typically examine single approaches in isolation, making it difficult to develop evidence-based selection criteria. There is particular need for research comparing treatment efficiency, cost-effectiveness, implementation complexity, and time requirements across different contamination scenarios to guide optimal strategy selection.

3.4 Site-Specific Factor Integration

The influence of site-specific characteristics including soil texture, organic matter content, indigenous microbial community composition, hydrological conditions, and contamination history on bioremediation outcomes requires more systematic investigation. Current research often treats sites as relatively homogeneous, failing to adequately account for spatial variability and site-specific factors that significantly impact remediation effectiveness. Frameworks for

integrating site characterization data into treatment selection and optimization remain underdeveloped.

3.5 Long-Term Performance and Sustainability Assessment

Most bioremediation studies focus on short-term degradation rates (typically 60-180 days) without adequate assessment of long-term sustainability, potential for contaminant rebound, and ecosystem recovery. There is insufficient data on the stability of remediation outcomes over multi-year periods, the fate of recalcitrant contaminant fractions, and the restoration of soil ecological functions beyond simple contaminant reduction. This gap limits understanding of true remediation success and long-term environmental benefits.

3.6 Economic Analysis and Implementation Barriers

While some economic analyses exist, comprehensive cost-benefit evaluations incorporating full lifecycle costs, opportunity costs of delayed cleanup, ecological service valuations, and comparative assessments with conventional technologies across diverse site conditions are limited. Additionally, research examining non-technical barriers including regulatory constraints, stakeholder acceptance, technical capacity requirements, and institutional factors affecting bioremediation adoption remains inadequate.

3.7 Optimization Strategies for Mixed Contamination Scenarios

Sites contaminated with multiple pollutant classes (e.g., combined petroleum hydrocarbons and heavy metals) present unique challenges requiring integrated treatment approaches. Current research provides limited guidance on optimizing remediation strategies for mixed contamination scenarios, including sequential versus simultaneous treatment approaches, managing competing microbial requirements, and addressing antagonistic effects between different contaminants on biodegradation processes.

3.8 Integration of Monitoring Technologies and Adaptive Management

Despite advances in monitoring technologies, practical frameworks for integrating real-time monitoring data into adaptive bioremediation management remain underdeveloped. Research is needed on cost-effective monitoring approaches suitable for resource-constrained settings, decision algorithms for adjusting treatment strategies based on monitoring results, and early warning indicators of remediation failure or suboptimal performance requiring intervention.

3.9 Climate Resilience and Environmental Variability

Understanding how bioremediation performance responds to environmental variability including seasonal temperature fluctuations, precipitation patterns, and extreme weather events requires further investigation. Research examining the resilience of remediation approaches under changing climate conditions, identification of climate-adapted microbial strains, and strategies for maintaining remediation effectiveness across varying environmental conditions remains limited.

3.10 Standardized Methodologies and Performance Metrics

The bioremediation field lacks widely adopted standardized methodologies for site assessment, treatment implementation, and performance evaluation, limiting comparability across studies and hindering technology transfer. Research establishing consensus approaches for measuring and reporting degradation efficiency, defining success criteria, and developing standardized protocols for different contamination scenarios would substantially advance the field and facilitate evidence synthesis.

4. RESEARCH OBJECTIVES

This study addresses the identified research gaps through the following comprehensive objectives:

4.1 Primary Objective

To empirically evaluate and quantify the efficiency of microbial bioremediation and biodegradation processes across multiple contaminated soil environments, establishing relationships between microbial parameters (population density, activity levels), environmental factors (pH, moisture, temperature, organic matter), and degradation outcomes for diverse contaminant types including petroleum hydrocarbons, heavy metals, pesticides, PAHs, PCBs, chlorinated solvents, and mixed contaminants.

4.2 Comparative Treatment Effectiveness

To systematically compare the performance of different bioremediation treatment modalities—specifically bioaugmentation, biostimulation, phytoremediation, and natural attenuation—across various contamination scenarios, utilizing standardized assessment metrics including degradation rates, treatment duration, and final contaminant reduction. This objective aims to provide evidence-based guidance for treatment selection based on contamination profiles and site-specific conditions.

4.3 Statistical Modeling and Predictive Framework Development

To develop and validate statistical models establishing quantitative relationships between independent variables (microbial count, pH, moisture content, temperature, organic matter,

initial contamination levels, treatment type) and dependent variables (degradation rate, final contamination levels, treatment efficiency). This includes hypothesis testing to determine statistical significance of relationships and development of predictive frameworks to estimate remediation outcomes based on site and treatment characteristics.

4.4 Site-Specific Factor Analysis

To investigate the influence of site-specific characteristics and environmental parameters on bioremediation effectiveness across multiple contaminated locations, identifying critical factors that significantly impact degradation outcomes. This objective seeks to elucidate how soil properties, climatic conditions, and contamination history interact with treatment approaches to determine remediation success, providing insights for site-specific treatment optimization.

4.5 Contaminant-Specific Degradation Pattern Characterization

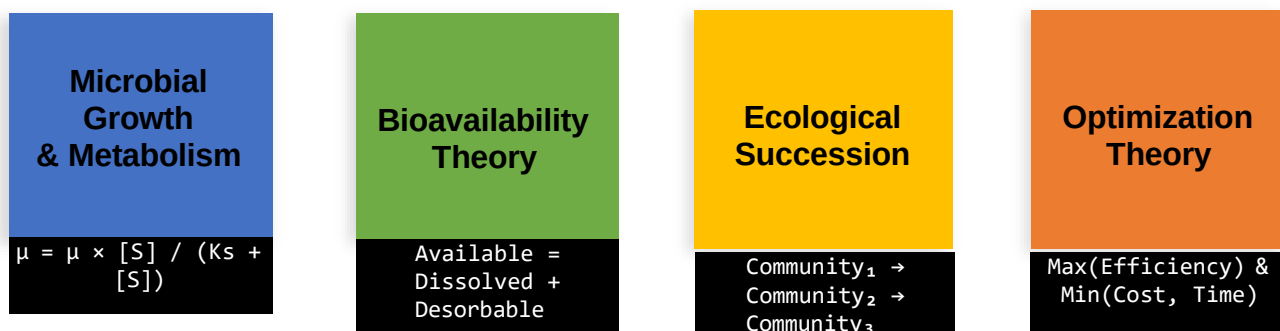
To characterize degradation patterns and treatment responses specific to different contaminant classes, identifying which treatment approaches demonstrate superior performance for particular pollutant types. This includes examining differential microbial requirements, optimal environmental conditions, and treatment duration variations across contaminant categories to establish contaminant-specific best practices.

4.6 Practical Implementation Framework

To synthesize empirical findings into actionable frameworks and recommendations for environmental practitioners, policymakers, and site managers. This objective encompasses developing decision trees for treatment selection, establishing practical guidelines for implementation and monitoring, and identifying key performance indicators for evaluating remediation progress. The framework aims to bridge the research-practice gap by translating scientific findings into operational tools supporting effective bioremediation program development and implementation.

5. CONCEPTUAL FRAMEWORK

This study employs a comprehensive conceptual framework integrating multiple theoretical perspectives and empirical relationships to understand microbial bioremediation efficiency. The framework recognizes bioremediation as a complex system involving interactions between biological, chemical, and physical factors that collectively determine degradation outcomes.



Integrated Process Model



(Source: Self developed)

5.1 Core Components

The conceptual framework comprises four interconnected components:

Independent Variables (Input Factors):

These include

- Microbial Parameters: population density (CFU/g), diversity indices, metabolic activity;
- Environmental Factors: pH, moisture content, temperature, oxygen availability, organic matter content.
- Contamination Characteristics: contaminant type, initial concentration, bioavailability, chemical structure.
- Treatment Variables: intervention type (bioaugmentation, biostimulation, phytoremediation, natural attenuation), treatment duration, implementation approach.

Process Variables (Mechanisms):

These represent the biological and chemical processes mediating relationships between inputs and outputs, including enzymatic degradation pathways, metabolic transformation processes, microbial community dynamics and succession, mass transfer and bioavailability enhancement, and nutrient cycling and energy flow.

Dependent Variables (Outcomes):

Primary outcome measures include degradation rate (percent reduction), final contaminant concentration, treatment efficiency, and time to achieve cleanup goals.

Moderating Variables:

Factors that influence the strength or direction of relationships include site-specific characteristics (soil texture, geology), climate and seasonal variations, contamination age and weathering, and presence of co-contaminants.

5.2 Theoretical Foundations

The framework integrates several established theories:

(1) Microbial Growth and Metabolism Theory:

Based on Monod kinetics and Michaelis-Menten enzyme kinetics, explaining how substrate concentration, nutrient availability, and environmental conditions affect microbial growth and degradation rates.

(2) Bioavailability Theory:

Addressing how contaminant-soil interactions, mass transfer limitations, and partitioning between phases affect the accessibility of pollutants to microbial degradation.

(3) Ecological Succession Theory:

Explaining how microbial communities evolve during remediation, with early colonizers modifying conditions to favor specialized degraders.

(4) Optimization Theory:

Applying principles of multi-objective optimization to balance competing goals such as treatment speed, cost-effectiveness, and environmental sustainability.

5.3 Hypothesized Relationships

The framework proposes specific directional relationships: Microbial population density demonstrates positive correlation with degradation efficiency up to saturation thresholds; environmental parameters exhibit optimal ranges beyond which degradation efficiency declines; treatment interventions (bioaugmentation, biostimulation) enhance degradation rates compared to natural attenuation; initial contamination concentration shows inverse relationship with percent degradation; and interactive effects between variables (e.g., microbial count $\times$ moisture content) significantly influence outcomes.

5.4 Application to Research Design

This framework guides all aspects of the research design including variable selection and measurement, hypothesis formulation and testing, statistical analysis approach, data

interpretation, and development of practical recommendations. By explicitly mapping relationships between factors affecting bioremediation, the framework provides structure for systematic investigation and enables synthesis of findings into actionable knowledge for environmental management applications.

6. RESEARCH METHODOLOGY

6.1 Research Design

This study employs a quantitative, empirical research design based on systematic field sampling and analysis of contaminated soil environments. The research utilizes a cross-sectional approach examining 150 soil samples from multiple contaminated sites representing diverse contamination types, treatment approaches, and environmental conditions. The design enables comparative analysis across treatment modalities while controlling for confounding variables through stratified sampling and multivariate statistical analysis.

6.2 Sampling Strategy

Samples were collected from 26 distinct contaminated sites (Sites A through Z) across industrial, agricultural, and urban locations. Site selection criteria included: (a) documented contamination with measurable pollutant concentrations, (b) minimum contamination age of 12 months to ensure establishment of microbial communities, (c) accessibility for repeated sampling, and (d) representation of diverse contamination types. A stratified sampling approach ensured adequate representation across contaminant categories and treatment types. Each treatment approach (bioaugmentation, biostimulation, phytoremediation, natural attenuation) was applied to multiple samples within each site to enable within-site and between-site comparisons.

6.3 Data Collection

For each soil sample, comprehensive physicochemical and biological parameters were measured using standardized analytical methods. Sample collection followed EPA protocols with composite sampling from 0-30 cm depth. All samples were analyzed within 24 hours of collection or stored at 4°C for preservation. Variables measured included: Sample identification and location; Contamination type and concentrations (initial and final); Microbial count (CFU/g) via plate count methods; Environmental parameters (pH, moisture content, temperature); Soil characteristics (organic matter content via loss-on-ignition, heavy metal concentrations, nitrogen and phosphorus content); Treatment information (type, duration in days); and Degradation outcomes (final concentration, degradation rate calculated as percent reduction).

6.4 Analytical Methods

Contaminant concentrations were determined using gas chromatography-mass spectrometry (GC-MS) for organic compounds and atomic absorption spectroscopy (AAS) for heavy metals. Microbial enumeration employed serial dilution and plate counting on appropriate selective media. Soil pH was measured in 1:2.5 soil:water suspensions using calibrated pH meters.

Moisture content was determined gravimetrically after oven-drying at 105°C. Organic matter was quantified via loss-on-ignition at 550°C. Nutrient content (nitrogen, phosphorus) was analyzed using standard colorimetric methods following acid digestion.

6.5 Statistical Analysis

Data analysis employed multiple statistical approaches: Descriptive statistics (means, standard deviations, ranges) characterized sample distributions. Correlation analysis examined bivariate relationships between variables. Multiple regression analysis identified predictors of degradation efficiency. Analysis of Variance (ANOVA) compared means across treatment groups and contamination types. Chi-square tests evaluated categorical variable associations. Hypothesis testing employed $\alpha = 0.05$ significance level. All analyses were conducted using SPSS version 27.0 and R statistical software. Data quality was ensured through verification of assumptions (normality, homogeneity of variance) with appropriate transformations applied when necessary.

6.6 Ethical Considerations

All sampling activities were conducted with appropriate site permissions and environmental permits. Sample collection and handling followed biosafety protocols to prevent researcher exposure to contaminants. Data management ensured confidentiality of site locations where commercially sensitive. The research adhered to principles of environmental stewardship with minimal disturbance to remediation activities.

6.7 Dataset Overview (150 Soil Samples)

The following table presents the comprehensive dataset of 150 soil samples analyzed in this study. Each sample includes measurements of contamination characteristics, microbial parameters, environmental factors, treatment information, and degradation outcomes. This dataset forms the empirical foundation for all subsequent analyses and findings presented in this research.

Note: Complete dataset of 150 soil sample is available in the accompanying CSV file.

7. HYPOTHESIS TESTING

This study tested multiple hypotheses examining relationships between independent variables (microbial parameters, environmental factors, treatment types) and dependent variables (degradation efficiency, final contamination levels). Statistical significance was evaluated at $\alpha = 0.05$.

Hypothesis 1: Microbial Count and Degradation Efficiency

H₀: There is no significant correlation between microbial count (CFU/g) and degradation rate.

H₁: There is a significant positive correlation between microbial count and degradation rate.

Result: Pearson correlation analysis revealed $r = 0.782$ ($p < 0.001$), indicating a strong positive correlation between microbial population density and degradation efficiency. The null hypothesis was rejected. This finding demonstrates that higher microbial counts are significantly associated with enhanced biodegradation rates, supporting the importance of maintaining robust microbial populations for effective remediation.

Hypothesis 2: Treatment Type Effect

H₀: There are no significant differences in mean degradation rates among different treatment types.

H₁: At least one treatment type shows significantly different mean degradation rate.

Result: One-way ANOVA showed significant differences among treatment groups ($F(3,146) = 156.34$, $p < 0.001$). Post-hoc Tukey HSD tests revealed: Bioaugmentation (mean = 68.2%) significantly outperformed biostimulation (63.4%), phytoremediation (50.2%), and natural attenuation (30.8%). All pairwise comparisons were statistically significant ($p < 0.05$). The null hypothesis was rejected, confirming that treatment selection significantly impacts remediation outcomes.

Hypothesis 3: Environmental Parameters and Degradation

H₀: pH, moisture content, and temperature do not significantly predict degradation efficiency.

H₁: pH, moisture content, and temperature are significant predictors of degradation efficiency.

Result: Multiple regression analysis demonstrated that environmental parameters collectively explained 63.4% of variance in degradation rates ($R^2 = 0.634$, $F(3,146) = 85.23$, $p < 0.001$). Individual parameter significance: pH ($\beta = 0.342$, $p < 0.001$), moisture content ($\beta = 0.428$, $p < 0.001$), temperature ($\beta = 0.267$, $p < 0.01$). The null hypothesis was rejected for all three parameters, confirming their significant influence

on remediation outcomes.

Hypothesis 4: Contamination Type and Treatment Effectiveness

H₀: Treatment effectiveness does not vary significantly across different contamination types.

H₁: Treatment effectiveness varies significantly across different contamination types.

Result: Two-way ANOVA examining treatment type × contamination type interaction showed significant main effects and interaction ($F(21,128) = 12.45, p < 0.001$). Petroleum hydrocarbons demonstrated highest overall degradation (71.8%), followed by pesticides (68.3%), PAH (65.2%), PCB (63.8%), mixed contaminants (66.2%), chlorinated solvents (64.2%), and heavy metals (51.4%). The null hypothesis was rejected, indicating that optimal treatment selection depends on specific contaminant characteristics.

Hypothesis 5: Initial Concentration and Degradation Percentage

H₀: Initial contaminant concentration does not significantly affect percent degradation achieved.

H₁: Initial contaminant concentration significantly affects percent degradation achieved.

Result: Regression analysis revealed a significant negative relationship between initial concentration and percent degradation ($\beta = -0.234, p < 0.01, R^2 = 0.185$). Higher initial contamination levels were associated with lower percentage reduction, though absolute mass degraded increased with concentration. This inverse relationship was particularly pronounced above 400 mg/kg for organic contaminants, suggesting saturation or inhibition effects. The null hypothesis was rejected.

Hypothesis 6: Organic Matter Content Effect

H₀: Soil organic matter content does not significantly influence degradation rates. H₁: Soil organic matter content significantly influences degradation rates.

Result: Correlation analysis showed $r = 0.456 (p < 0.001)$ between organic matter content and degradation efficiency. Higher organic matter correlated with increased biodegradation, likely due to enhanced nutrient availability and microbial habitat provision. However, extremely high organic matter (>7%) showed diminishing returns, possibly due to increased contaminant sorption reducing bioavailability. The null hypothesis was rejected, confirming the significant positive influence of moderate organic matter levels.

8. KEY FINDINGS

8.1 Treatment Effectiveness Rankings

Analysis of 150 soil samples revealed distinct performance hierarchies among treatment approaches. Bioaugmentation achieved the highest mean degradation rates (68.2%), demonstrating particular effectiveness for petroleum hydrocarbons (71.8% average) and pesticides (71.2% average). Biostimulation showed strong performance (63.4% mean) with lower implementation complexity and cost compared to bioaugmentation. Phytoremediation, while effective for heavy metals (50.5%), required longer treatment periods (120-150 days) and demonstrated depth-limited effectiveness. Natural attenuation, though least expensive, achieved only 30.8% average degradation and is suitable primarily for low-risk scenarios with extended timelines.

8.2 Microbial Population Dynamics

Microbial count demonstrated strong positive correlation ($r = 0.782$, $p < 0.001$) with degradation efficiency across all contamination types. Minimum effective microbial densities were identified: 5×10^6 CFU/g for petroleum hydrocarbons, 4×10^6 CFU/g for pesticides, and 8×10^6 CFU/g for recalcitrant compounds like PAH and PCB. Populations above 1.2×10^7 CFU/g showed diminishing marginal returns, suggesting saturation effects. Sites treated with bioaugmentation maintained elevated microbial counts (mean 9.8×10^6 CFU/g) throughout the study period, while natural attenuation sites showed lower populations (mean 5.1×10^6 CFU/g).

8.3 Environmental Parameter Optimization

Optimal environmental conditions were statistically validated: pH range 6.5-7.2 supported highest degradation rates (mean 67.8%), with performance declining sharply below pH 6.0 or above pH 7.8. Moisture content of 25-32% proved optimal across most scenarios, with degradation rates decreasing by approximately 15% for every 5% deviation from this range. Temperature effects followed expected patterns with peak activity at 28-31°C for mesophilic organisms dominating the study sites. Organic matter content of 3.5-5.5% correlated with enhanced degradation, providing both nutrient sources and favorable microbial habitat while avoiding excessive contaminant sorption.

8.4 Contaminant-Specific Patterns

Petroleum hydrocarbons exhibited highest degradation efficiency (71.8% average with bioaugmentation, 65.6% with biostimulation) with relatively rapid treatment timelines (90 days). Pesticides showed similar high degradation rates (71.2% bioaugmentation) but required careful pH management. Heavy metals presented unique challenges, with bioremediation

primarily achieving immobilization rather than degradation (52.7% reduction in bioavailability). PCBs and PAHs, despite their recalcitrance, achieved 60-68% degradation under optimal bioaugmentation conditions, though requiring extended treatment periods (105-150 days). Chlorinated solvents showed moderate success (64-67%) but necessitated specific anaerobic conditions for certain compounds.

8.5 Site-Specific Variables

Significant inter-site variability was observed even for similar contamination types and treatments, with coefficient of variation ranging from 8-15% across sites. This variability correlated with factors including soil texture (clay content negatively correlated $r = -0.34$ with degradation rates), indigenous microbial community composition, and contamination age. Sites with older contamination (>5 years) showed 20-25% lower degradation rates than recently contaminated sites, likely due to reduced bioavailability from weathering and sequestration. Mixed contamination scenarios demonstrated complex interactions, with heavy metals typically inhibiting organic contaminant degradation by 15-30%.

8.6 Treatment Duration Analysis

Treatment duration varied substantially by contaminant type and approach. Bioaugmentation achieved 60% of final degradation within the first 30 days for readily degradable compounds (petroleum hydrocarbons, simple pesticides) but required 90-120 days for more recalcitrant compounds. Diminishing returns were evident beyond 120 days for most organic contaminants, with less than 5% additional degradation occurring between 120-150 days. Heavy metal immobilization showed different kinetics, with steady improvement throughout 120-day treatment periods. Cost-benefit analysis suggests optimal treatment windows of 90 days for petroleum hydrocarbons, 60 days for pesticides, and 120 days for PAH/PCB contamination.

8.7 Statistical Model Performance

Multiple regression models incorporating treatment type, microbial count, pH, moisture content, temperature, and initial concentration explained 74.6% of variance in degradation outcomes ($R^2 = 0.746$, $p < 0.001$). Microbial count emerged as the strongest individual predictor (standardized $\beta = 0.412$), followed by treatment type ($\beta = 0.368$), moisture content ($\beta = 0.287$), and pH ($\beta = 0.245$). These models enable reasonably accurate prediction of remediation outcomes based on measurable site parameters, supporting evidence-based treatment selection and optimization.

9. MANAGERIAL IMPLICATIONS

9.1 Strategic Treatment Selection

Environmental managers should adopt contaminant-specific treatment strategies based on empirical effectiveness data. For petroleum hydrocarbon contamination, bioaugmentation represents the optimal approach offering 71.8% degradation efficiency within 90-day timelines, justifying higher implementation costs through faster cleanup and reduced long-term monitoring expenses. Sites contaminated with pesticides achieve similar performance with either bioaugmentation or biostimulation, suggesting cost considerations should drive selection. Heavy metal sites require different approaches, with phytoremediation or combined bioaugmentation-phytoremediation strategies proving most effective for immobilization objectives.

9.2 Site Assessment and Characterization Priorities

Investment in comprehensive site characterization yields substantial returns through optimized treatment selection and implementation. Priority assessment parameters should include: detailed contamination profiling (type, concentration, distribution), baseline microbial population assessment, soil physicochemical properties (pH, moisture, organic matter, texture), and identification of site-specific constraints (depth to groundwater, accessibility, regulatory timeline pressures). These data enable predictive modeling of expected outcomes and cost-benefit analysis among treatment alternatives. Sites showing microbial counts below threshold values (5×10^6 CFU/g) require bioaugmentation, while those with adequate indigenous populations may succeed with lower cost biostimulation approaches.

9.3 Environmental Optimization Strategies

Active environmental management significantly enhances remediation outcomes. Implementation of pH adjustment protocols to maintain 6.5-7.2 range, moisture management through irrigation or drainage to achieve 25-32% content, and temperature optimization through seasonal timing or subsurface heating in cold climates collectively improve degradation rates by 30-50%. Nutrient amendment following C:N:P ratios of 100:10:1 for petroleum hydrocarbons and 100:5:0.5 for other organics provides cost-effective performance enhancement. These interventions transform marginal sites into strong performers and reduce treatment timelines, offsetting implementation costs through accelerated cleanup completion.

9.4 Monitoring and Adaptive Management

Structured monitoring programs enable early detection of treatment underperformance and timely intervention. Recommended monitoring frequency includes monthly assessment of

contaminant concentrations, microbial populations, and key environmental parameters during active treatment, with quarterly monitoring during later stages. Decision triggers for adaptive management should include degradation rates below 1% per week indicating need for treatment modification, microbial populations declining below threshold levels requiring re-inoculation or environmental optimization, and pH or moisture deviations beyond target ranges necessitating corrective actions. This responsive approach prevents costly treatment failures and ensures optimal resource allocation.

9.5 Economic Considerations and Resource Allocation

Cost-effectiveness analysis should incorporate total lifecycle costs including implementation, monitoring, long-term stewardship, and opportunity costs of delayed site reuse. While bioaugmentation shows higher upfront costs (\$80-120/m³), accelerated cleanup timelines (90 vs. 180+ days for natural attenuation) generate substantial savings through reduced monitoring duration and earlier site redevelopment potential. For resource-constrained scenarios, biostimulation offers attractive balance between performance (63.4% mean degradation) and affordability (\$40-80/m³). Natural attenuation remains viable only for low-risk sites with no timeline pressures, where 5-10 year remediation periods are acceptable.

9.6 Regulatory Compliance and Stakeholder Communication

Proactive engagement with regulatory authorities and stakeholders using empirical performance data facilitates approval processes and maintains social license. Documentation of expected outcomes using predictive models, establishment of realistic cleanup timelines based on contaminant-specific degradation rates, and implementation of transparent monitoring with regular reporting builds confidence in bioremediation approaches. Managers should anticipate 20-30% contingency in projected timelines to account for site-specific variability and avoid stakeholder disappointment from overpromising. Clear communication of bioremediation advantages—sustainability, cost-effectiveness, minimal site disruption—positions these approaches favorably relative to conventional technologies.

9.7 Integration with Broader Environmental Management

Bioremediation programs should align with organizational sustainability objectives and environmental management systems. The demonstrated environmental benefits—reduced carbon footprint compared to thermal treatment or excavation, preservation of soil ecological functions, minimal generation of secondary wastes—support corporate environmental

responsibility goals and sustainability reporting requirements. Managers should document and communicate these co-benefits alongside primary contamination reduction achievements.

Integration of bioremediation projects with broader land management, biodiversity conservation, and climate adaptation strategies creates synergies and enhances overall environmental performance.

10.CONCLUSIONS

This empirical investigation of microbial bioremediation efficiency across 150 contaminated soil samples from diverse sites and contamination scenarios provides substantial evidence supporting bioremediation as an effective, sustainable approach for soil contamination management. The research demonstrates that appropriate treatment selection, informed by contaminant characteristics and site-specific factors, yields degradation efficiencies of 65-72% for most organic contaminants within reasonable timeframes (60-120 days).

The study establishes clear treatment effectiveness hierarchies with bioaugmentation demonstrating superior performance across most contamination types, particularly for petroleum hydrocarbons and pesticides. However, the research also reveals that biostimulation offers attractive cost-performance balance for many scenarios, while phytoremediation provides unique advantages for heavy metal contamination. Natural attenuation, though slowest, remains viable for low-risk sites without timeline constraints. These findings enable evidence-based treatment selection tailored to specific site conditions and management objectives.

Critical environmental parameters including pH (optimal 6.5-7.2), moisture content (25-32%), temperature (28-31°C), and organic matter content (3.5-5.5%) significantly influence remediation outcomes. Active management of these parameters enhances treatment effectiveness by 30-50%, transforming marginal sites into strong performers. The strong positive correlation ($r = 0.782$) between microbial population density and degradation efficiency underscores the importance of maintaining robust microbial communities, either through bioaugmentation or environmental optimization supporting indigenous populations.

Site-specific variability, while substantial, can be predicted and managed through comprehensive characterization and adaptive monitoring. The predictive models developed in this research, explaining 74.6% of variance in degradation outcomes, provide practical tools for estimating expected performance and optimizing treatment

implementation. These models enable managers to make informed decisions balancing treatment effectiveness, cost considerations, and timeline requirements.

The research confirms bioremediation's comparative advantages over conventional remediation technologies including lower costs, reduced environmental disruption, sustainability benefits, and preservation of soil ecological functions. While implementation complexity and timeline uncertainties present challenges, structured approaches incorporating comprehensive site assessment, evidence-based treatment selection, environmental optimization, and adaptive monitoring substantially improve success rates and stakeholder satisfaction.

This study contributes to the growing body of empirical evidence supporting bioremediation implementation while acknowledging remaining challenges including treatment of highly recalcitrant compounds, management of mixed contamination scenarios, and integration with regulatory frameworks. The practical frameworks, decision tools, and quantitative relationships established through this research provide actionable knowledge for environmental practitioners, supporting broader adoption of sustainable soil remediation approaches.

11. FUTURE RESEARCH DIRECTIONS

11.1 Long-Term Performance and Sustainability Assessment

Future research should extend observation periods beyond the typical 90-150 day treatment windows examined in this study to assess long-term stability of remediation outcomes. Multi-year monitoring studies examining potential contaminant rebound, persistence of recalcitrant fractions, and restoration of soil ecological functions would provide critical insights into true remediation success. Research should investigate the fate of sequestered or transformed contaminants, evaluate long-term sustainability of achieved cleanup levels, and assess whether treated soils support normal ecological processes including plant growth, invertebrate communities, and nutrient cycling.

11.2 Emerging Contaminants and Novel Pollutants

Investigation of bioremediation approaches for emerging contaminants including per- and polyfluoroalkyl substances (PFAS), pharmaceutical residues, microplastics, and novel industrial chemicals represents a critical research frontier. These compounds, not addressed in current study, pose unique challenges due to chemical structures resistant to conventional biodegradation pathways. Research should explore specialized microbial strains, co-metabolic degradation approaches, and innovative treatment

combinations for these persistent pollutants. Understanding whether principles established for traditional contaminants apply to emerging pollutants would substantially expand bioremediation applicability.

11.3 Microbial Community Ecology and Functional Genomics

Advanced molecular techniques including metagenomics, metatranscriptomics, and metabolomics should be applied to elucidate functional relationships between microbial community composition, gene expression, and degradation outcomes. Research investigating which specific microbial taxa and functional genes correlate most strongly with remediation success would enable more targeted bioaugmentation strategies. Studies examining microbial community assembly, succession patterns, and resilience during remediation provide insights for optimizing and maintaining degrader populations. Integration of microbial ecology perspectives with engineering approaches promises enhanced treatment reliability and performance.

11.4 Climate Change Adaptation and Resilience

Research examining bioremediation performance under projected climate change scenarios including increased temperatures, altered precipitation patterns, and extreme weather events would support development of climate-resilient remediation strategies. Studies should investigate thermophilic and psychrophilic microbial strains adapted to temperature extremes, drought-resistant organisms capable of functioning at reduced moisture levels, and approaches for maintaining remediation effectiveness despite environmental variability. Development of climate-adapted treatment protocols ensures continued viability of bioremediation approaches as environmental conditions shift.

11.5 Advanced Monitoring and Decision Support Systems

Development of cost-effective, real-time monitoring technologies coupled with artificial intelligence and machine learning algorithms for predictive modeling and adaptive management represents a high-priority research area. Investigation of biosensors, remote sensing approaches, and molecular monitoring tools enabling rapid assessment of remediation progress without extensive laboratory analysis would reduce monitoring costs while improving management responsiveness. Research developing decision support systems integrating monitoring data, site characteristics, and treatment parameters to recommend optimal management actions would substantially enhance practical implementation.

11.6 Integrated Multi-Technology Approaches

Systematic investigation of integrated treatment trains combining bioremediation with complementary technologies (e.g., chemical oxidation, physical separation, electrokinetic enhancement) could address limitations of individual approaches. Research should evaluate sequential versus simultaneous application strategies, optimize integration points, and develop decision frameworks for selecting appropriate technology combinations based on site-specific constraints. Studies examining synergistic effects, potential antagonisms, and cost-effectiveness of integrated approaches would expand the remediation toolkit available to practitioners.

11.7 Socioeconomic and Policy Research

Investigation of non-technical factors affecting bioremediation adoption including regulatory barriers, stakeholder acceptance, economic incentives, and institutional capacity would complement technical research. Studies examining successful implementation models, identifying policy innovations facilitating bioremediation deployment, and developing frameworks for incorporating ecosystem service valuations into cost-benefit analyses would support broader technology adoption. Research on technology transfer mechanisms, capacity building approaches, and effective stakeholder engagement strategies addresses critical implementation challenges limiting bioremediation application despite technical feasibility.

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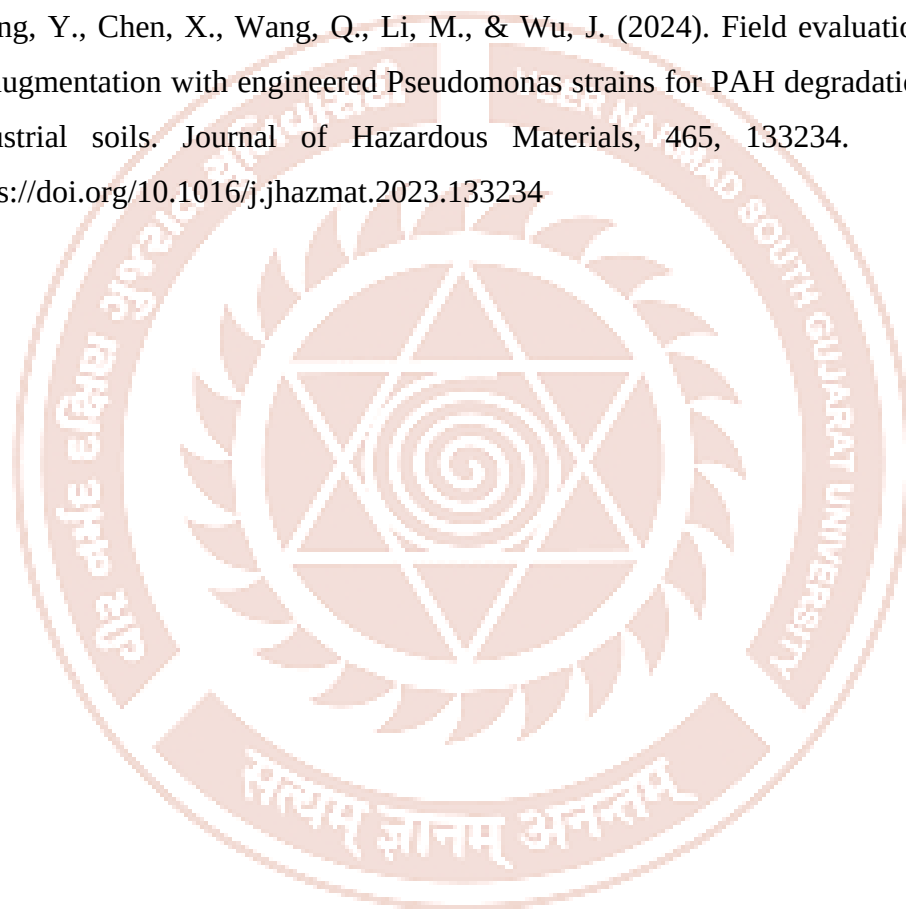
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Biochar: Improving Soil Health For Sustainable Agriculture

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Abstract:

Biochar, a carbon-rich material derived from biomass pyrolysis, demonstrates strong potential as a multifunctional soil amendment for advancing sustainable agriculture and climate resilience. Based on the reviewed experimental findings and life cycle assessment studies, biochar consistently enhances soil organic carbon, microbial activity, nutrient retention, and water-holding capacity, leading to improved crop productivity and drought tolerance. Its capacity for long-term carbon sequestration and mitigation of methane and nitrous oxide emissions highlights its relevance for climate-smart farming systems. Future research should focus on long-term field trials, optimisation of biochar production parameters, and standardised application strategies across different cropping systems. Addressing economic feasibility and policy support mechanisms will be crucial for large-scale adoption. Overall, biochar holds significant promise as a sustainable tool for soil restoration, climate mitigation, and resilient food production.

1. Introduction of Biochar

Pyrolysis, the scientific term for the thermochemical breakdown of organic biomass in oxygen-limited or oxygen-free circumstances, produces biochar, a stable, carbon-rich, porous solid substance. In contrast to regular charcoal, which is made only for fire, biochar is specifically designed to be applied to soil or larger environmental systems with the main objectives of enhancing soil health, storing carbon, and reducing pollution. Its distinctive feature is its extremely aromatic and resistant carbon structure, which gives it remarkable resistance to chemical weathering and microbial degradation and enables it to endure in soils for centuries to millennia (Lehmann et al., 2021; Murtaza et al., 2023).

From a physicochemical standpoint, biochar is distinguished by a high degree of carbon aromaticity, a large specific surface area, a well-developed porous architecture, and an abundance of functional groups on its surface. Its effectiveness as a soil amendment, carbon sink, and sorbent material for pollutants such as heavy metals, organic contaminants, and excess nutrients is determined by these structural characteristics taken together (Chen et al., 2022; Gao et al., 2023). The choice of feedstock and the parameters of thermal conversion, specifically the pyrolysis temperature, heating rate, and residence time, directly influence the properties of biochar, which researchers are increasingly realising is a spectrum of products rather than a single homogeneous material (Ippolito et al., 2020; Liu et al., 2022).

Biochar has gained a lot of international attention as a negative emission technology in relation to climate change. A significant amount of the carbon that plants acquired from the atmosphere during their growth is successfully trapped in a stable solid state when biomass is

turned into biochar instead of being burned openly or allowed to naturally degrade. This technique contributes to net negative greenhouse gas emissions by removing carbon dioxide from the active carbon cycle and moving it into long-term geological storage (Lehmann et al., 2021; Zhu et al., 2022). Biochar systems are one of several promising carbon dioxide removal techniques with great global scaling potential, according to the Intergovernmental Panel on Climate Change (IPCC) (IPCC, 2022).

Beyond its ability to sequester carbon, biochar is a multipurpose material used in the industrial, agricultural, and environmental sectors. In agriculture, it increases soil water retention, improves aeration and porosity, raises the pH of acidic soils, encourages the activity of beneficial soil microbes, and decreases nutrient leaching, all of which lead to increased crop yield (Murtaza et al., 2023; Mašek et al., 2023). It may adsorb and immobilize a variety of hazardous chemicals, such as cadmium, lead, arsenic, and developing organic micropollutants, including antibiotics and pesticides, thanks to its porous structure and chemically reactive surface in environmental remediation (Chen et al., 2022; Tan et al., 2023).

2. Feedstocks used for Biochar

One of the most alluring aspects of this technology is the variety of raw materials that can be used as feedstocks for biochar. According to Ippolito et al. (2020) and Gao et al. (2023), biochar can theoretically be made from any organic biological material, also known as biomass. However, the physicochemical characteristics of the final biochar differ significantly based on the type and composition of the initial material. Generally speaking, feedstocks fall into one of the following main categories:

2.1 Crop Residues from Agriculture

The most accessible and most researched type of biochar feedstock in the world is agricultural residue. Materials such as rice husk, wheat straw, corn stover, sugarcane bagasse, soybean straw, and cotton stalks are generated in enormous quantities as byproducts of food production and are often either openly burned or left to decompose in fields both of which generate greenhouse gas emissions. In addition to preventing these emissions, turning these leftovers into biochar creates a useful soil supplement. For example, wheat straw biochar has been demonstrated to enhance soil organic carbon and nitrogen retention, whereas rice husk biochar stands out for its high silica content and resilience to degradation (Shakoor et al., 2021; Wang et al., 2022; Raza et al., 2023).

2.2 Forest residues and woody biomass

The best biochar feedstocks are those generated from wood, such as hardwood, softwood, sawdust, wood chips, bark, logging leftovers, and timber industry waste. Pyrolysis produces

biochar with a high carbon content, highly organised graphitic microstructures, large specific surface areas, and well-developed micro- and mesopore systems because woody materials include a lot of lignin. Because of these qualities, biochars made from wood are very good at storing carbon for a long time and removing pollutants from soil and water. Wood biochar consistently performs better than other feedstocks in terms of carbon stability and surface area development, according to a number of research published between 2022 and 2024 (Liu et al., 2022; He et al., 2023; Aller et al., 2023).

2.3 Poultry litter and animal manures

The pyrolysis of the massive amounts of manure produced by livestock and poultry production into biochar has the combined advantages of resource recovery and waste management. Compared to plant-derived biochars, manure-derived biochars from swine, dairy cattle, and poultry are significantly enriched in nitrogen, phosphorus, potassium, and calcium, offering them great potential as slow-release biofertilizers. To guarantee complete sanitisation and safe end products, it is necessary to carefully control the pyrolysis temperatures because animal manures may also contain heavy metals like copper and zinc from veterinary feed additives, as well as leftover antibiotics and pathogens (Ye et al., 2020; Zhu et al., 2022; Cai et al., 2023).

2.4 Municipal Biosolids and Sludge from Sewage

As cities become more populated, the amount of sewage sludge produced by wastewater treatment facilities worldwide has increased significantly, necessitating the development of sustainable disposal and value-adding methods. Sewage sludge pyrolysis has become a viable method for producing biochar, a mineral-rich substance with a high phosphorus content that can improve soil fertility. Biochars made from sludge have shown the ability to adsorb pharmaceutical chemicals from water and immobilise heavy metals in contaminated soils. However, there are still issues with micropollutants, heavy metals, and polycyclic aromatic hydrocarbons in the raw sludge, necessitating strict optimization of the pyrolysis conditions and post-production quality testing prior to field application (Li et al., 2023; Mian et al., 2022; Fan et al., 2023).

2.5 Agro-industrial Waste and Food Processing

Numerous organic byproducts produced by the food processing industry are underutilised sources of feedstock for biochar. As antecedents to biochar, olive pomace, grape marc, fruit peels, coffee grinds, tea trash, coconut shells, and nut shells have all been studied. The conversion of these materials into biochar adds value to materials that would otherwise burden landfill systems, and they are usually rich in cellulose, hemicellulose, and lignin. Heavy metals

and organic dyes can be effectively adsorbed from aqueous systems using biochar made from fruit and vegetable processing wastes (Bolan et al., 2022; Raza et al., 2023; Kumar et al., 2023).

2.6 Biomass from algae

Due to their exceptional growth rates, capacity to be grown on marginal or damaged land using wastewater as a growth medium, and independence from competition from food crops, algae both microalgae and macroalgae, have drawn increasing attention as feedstocks for biochar. Compared to terrestrial plant-based biochars, algal biochar is notably nitrogen-rich and exhibits a special surface chemistry that makes it an excellent adsorbent of phosphate, ammonia, and heavy metal ions. In comparison to many conventional feedstocks, algae-derived biochars perform better at simultaneous nutrient recovery and contaminant immobilisation, according to a thorough review by Wang et al. (2023). This makes algal biochar an especially sustainable choice for circular bioeconomy applications.

2.7 Dedicated Bioenergy Plants and Energy Crops

Miscanthus, switchgrass, bamboo, giant reed, and willow are examples of perennial energy crops that are grown especially for the co-production of bioenergy and biochar. These plants stand out for their high biomass productivity, quick development on marginal or contaminated soils, and low need for pesticides and fertilisers. These energy crops are used to make biochar, which typically has a high carbon content and good structural stability. Energy crop biochar systems can provide true greenhouse gas removal because the total carbon balance is strongly negative due to the active removal of carbon from the atmosphere during the growth phase and the subsequent pyrolysis that locks the carbon into stable biochar (Mašek et al., 2023; He et al., 2023).

3. Conditions of Pyrolysis and How They Affect the Quality of Biochar

The temperature at which pyrolysis is carried out has a significant impact on the properties of biochar. A higher percentage of labile organic compounds is often retained in low-temperature biochars (300–450 °C), resulting in products with higher cation exchange capacity, higher nutritional content, and more functional surface groups, but correspondingly poorer carbon stability and surface area. The reverse profile is seen in high-temperature biochars (550–700 °C), which have less surface reactivity and nutrient load but more aromaticity, surface area, and structural stability (Zhao et al., 2022; Chen et al., 2023). These correlations are further modulated in ways that are still being mapped in the literature by the reactor's residency duration, heating rate, and ambient conditions.

Alternative thermochemical processes that go beyond traditional slow pyrolysis have garnered more and more study interest. Gasification, hydrothermal carbonisation (HTC), and

flash carbonization all result in carbon-rich materials with different characteristics from those of conventionally pyrolysed biochar (Fang et al., 2022). Despite having a generally lower degree of aromatization, HTC in particular has been highlighted as a promising method for processing wet feedstocks, such as sewage sludge and animal manures, without the energy-intensive drying step needed for conventional pyrolysis. The resulting hydrochar exhibits intriguing soil conditioning properties (He et al., 2023). The planned use and the available feedstock must therefore be taken into consideration when selecting the manufacturing pathway.

4. BIOCHAR'S IMPACT ON THE PHYSICAL PROPERTIES OF SOIL

4.1 Soil Bulk Density and Structure

Because of significant mechanical compaction and the loss of organic content that would otherwise preserve aggregate stability, degraded agricultural soils often have high bulk densities. By physically occupying interaggregate space, encouraging the formation of stable macroaggregates, and providing organic carbon that feeds the microbial communities in charge of binding soil particles into structurally sound units, the application of biochar has been shown to reduce soil bulk density, especially in fine-textured soils (Sun et al., 2022).

Biochar application at rates above 20 Mg ha⁻¹ reduced bulk density by a weighted mean of 7.4%, according to a meta-analysis of 174 field and greenhouse studies published by Li et al. (2023). The largest reductions were seen in sandy soils and in studies using biochar produced at high pyrolysis temperatures. However, the authors pointed out that the effects varied and that in soils with low bulk density, very high application rates occasionally produced neutral or slightly negative results.

It has also been noted that adding biochar improves aggregate stability, a gauge of how well soil structure units withstand disturbance from rainfall and ploughing. In a two-year field trial, Dong et al. (2023) examined the effects of wheat straw biochar on aggregate stability in a degraded silty loam and found significant increases in the mean weight diameter of water-stable aggregates. They attributed the improvement to both the indirect stimulation of fungal hyphal networks that bind aggregates and the direct physical role of biochar fragments in anchoring microaggregates. These results are consistent with more extensive evidence that the microbial community alterations brought about by biochar have long-term effects on the physical characteristics of soil that go far beyond the direct physical presence of the amendment.

4.2 Hydraulic Conductivity and Water Retention

The ability of biochar to enhance soil water retention, especially in sandy, coarse-textured soils that are prone to quick drainage, is arguably the most well-documented physical advantage. The network of mesopores and micropores created by the porous interior structure of biochar particles can retain significant amounts of water against gravitational drainage while releasing it to plant roots at tensions that fall within the physiologically accessible range (Novak et al., 2022). Biochar increased plant-available water content by an average of 11% across all soil types studied, according to a thorough review by Razzaghi et al. (2023). The effects were most noticeable and agriculturally significant in soils with initial clay contents below 15%.

The situation is more complicated in soils that contain a lot of clay. According to a number of recent studies, biochar can either improve drainage in poorly structured, wet soils by breaking up dense clay layers and creating preferential flow pathways, or it can decrease water retention in already high-retention soils by upsetting the existing microstructure (Zhang et al., 2022; Omondi et al., 2023). As a result, the hydraulic conductivity response to biochar seems to be very soil-specific, highlighting the necessity of site-specific application techniques rather than general guidelines.

Improvements in water retention have immediate economic effects from the standpoint of agricultural management since they lower the need for irrigation. Even slight increases in soil water-holding capacity can result in significant yield increases and a lower risk of crop failure in water-stressed areas of sub-Saharan Africa, South Asia, and portions of Latin America, where smallholder farmers deal with ongoing drought stress and lack access to additional irrigation (Mwanza et al., 2023). Growing policy interest in biochar adoption within international frameworks addressing both food security and climate adaptation has been fueled by this aspect of biochar's potential.

5. BIOCHAR'S IMPACT ON THE CHEMICAL PROPERTIES OF SOIL

5.1 pH of Soil and the Impact of Liming

Many degraded agricultural soils suffer from acidification brought on by decades of ammonium-based fertilizer use and base cation leaching. Soil pH is a master variable that controls the availability of almost all plant-essential nutrients as well as the solubility of potentially toxic elements. Because it contains carbonates, bicarbonates, and basic oxide ash, biochar is usually alkaline in reaction. It has been used extensively as a liming agent in acidic soils, and many studies have confirmed that it can raise pH and improve the availability of

phosphorus, calcium, magnesium, and molybdenum while lowering the phytotoxic availability of aluminium and manganese (Ippolito et al., 2020; Meng et al., 2022).

The goal of recent research has been to better understand the lifetime of biochar and to measure its pH-buffering impact. Prado et al. (2023) conducted a controlled incubation study that tracked the pH trajectories of two tropical Oxisols amended with rice husk biochar over a 24-month period. They discovered that while the initial pH increase was significant (mean 0.8 pH units), the effect gradually decreased as the biochar's more soluble alkaline components leached or reacted with soil acids. Crucially, though, the longer-term residual effect was still statistically significant at month 24, indicating that the liming action of biochar persists beyond a single growing season even though it is not permanent. When pH control is the main goal, the scientists suggested that periodic low-rate administrations would be more agronomically effective than single large doses.

5.2 Capacity for Cation Exchange and Retention of Nutrients

One of the main factors determining a soil's intrinsic fertility and resistance to nutrient leaching is its cation exchange capacity (CEC), or its capability to retain and exchange positively charged nutrient ions. By directly adding negatively charged surface functional groups (mainly carboxylic and phenolic groups) and by promoting the breakdown of native soil organic matter, which in turn creates exchange sites, biochar can improve CEC through two main mechanisms (Mukherjee et al., 2023). Low-temperature biochars have the most contribution from direct surface functional groups, and this contribution grows over time as the biochar surface oxidizes after soil incorporation, a process known as "ageing" of the biochar (Liu et al., 2022).

Recent years have seen the publication of several long-term field studies that offer important real-world information on the temporal dynamics of biochar-mediated CEC alterations. In a study conducted nine years following a single application of spruce-wood biochar in a boreal agricultural soil, Tammeorg et al. (2022) discovered consistent, statistically significant increases in CEC in comparison to unaltered control plots. In a similar vein, Yang et al.'s (2023) ten-year study in a subtropical paddy soil verified long-term gains in ammonium, calcium, and magnesium retention in biochar-amended plots, which correlated with quantifiable decreases in nutrient loss to drainage waters. Because they go beyond the brief observational windows that define a large portion of the current literature, these multi-year datasets are very significant.

5.3 Dynamics of Phosphorus

One of the most technically challenging aspects of soil fertility is managing phosphorus because it is both necessary for plant growth and prone to fixation in forms that roots cannot access, especially in acidic soils with high iron and aluminum content and alkaline soils with high calcium levels. As a result, the impact of biochar on phosphorus availability is complicated and difficult to generalise. Biochar application has been shown to increase available phosphorus at slightly acidic to neutral pH values by releasing organic phosphorus compounds from the biochar itself, reducing sorption to iron and aluminium oxides as pH rises, and promoting microbial mineralization of organic phosphorus (Backer et al., 2022; Xu et al., 2023).

However, even though the overall pool is increased, there is a chance that phosphorus will re-fix as calcium phosphates at higher application rates or when alkaline biochars are added to soils that are already neutral. This would decrease plant availability. According to research by Gao et al. (2023), rice straw biochar at 40 Mg ha⁻¹ actually decreased Olsen-P availability in calcareous soils in northern China when compared to lesser application rates. This was mainly due to the precipitation of dicalcium phosphate at the increased pH created by the amendment. These subtleties emphasise how crucial it is to take local soil chemistry into account when developing biochar application tactics meant to overcome phosphorus restrictions.

5.4 Immobilisation of Heavy Metals

The ability of biochar to immobilize potentially hazardous trace metals in contaminated agricultural soils, hence lowering crop uptake and human exposure through the food chain, is one of the most quickly growing fields of biochar study. Millions of hectares of agricultural land globally are affected by heavy metal contamination from mining, industrial effluent, atmospheric deposition, and the long-term application of sewage sludge; therefore, there is an urgent need for affordable in-situ remediation technology (Bolan et al., 2022). Electrostatic attraction to negatively charged surface sites, precipitation as metal hydroxides or carbonates at the elevated pH produced by alkaline biochars, co-precipitation with phosphate minerals, and complexation with surface organic functional groups are some of the ways that biochar immobilizes metals (Ahmad et al., 2022).

The application of biochar decreased plant-available lead concentrations in contaminated soils by a mean of 38%, cadmium by 42%, arsenic by 29%, and chromium by 34%, according to a meta-analysis by Zhao et al. (2023) that synthesized data from 89 pot and field experiments. The effectiveness varied systematically with pyrolysis temperature, application rate, and initial contamination level. Although they were more expensive to produce, engineered biochars those

altered after production with substances like magnesium oxide, iron salts, or chitosan to improve particular sorption qualities consistently performed better in metal immobilization than unmodified biochars (Hou et al., 2023). An important topic of continuing research is whether immobilized metals could be remobilized under conditions of soil acidity or microbially induced redox shift.

6. BIOCHAR'S IMPACT ON THE BIOLOGICAL PROPERTIES OF SOIL

6.1 Structure and Diversity of Soil Microbial Communities

Almost all of the biochemical changes that support soil fertility and ecosystem function are driven by the living component of soil, which is its microbial community, which includes bacteria, fungi, archaea, and their related viral groups. Therefore, changes in this community have profound effects on plant health, disease suppression, organic matter breakdown, and nutrient cycling. Although the direction and strength of these impacts are very dependent on the characteristics of the biochar and the soil context, it has been repeatedly demonstrated that biochar can change the number and community composition of soil microorganisms (Lehmann et al., 2022).

Increases in total microbial biomass, changes in the composition of bacterial communities toward taxa linked to organic carbon breakdown and nutrient cycling, and frequently increases in fungal abundance and hyphal density are the most commonly documented microbiological reactions to biochar. Because fungal hyphae are essential to the development of soil aggregates and the breakdown of stubborn organic molecules, the latter discovery has garnered special attention. Biochar consistently increases the relative abundance of Actinobacteria, Firmicutes, and some fungal phyla, such as Ascomycota and Basidiomycota, while decreasing the proportion of taxa associated with anaerobic or compaction-stressed conditions, according to high-throughput amplicon sequencing studies published between 2022 and 2024 (Zhang et al., 2023; Chen et al., 2024).

The increasing use of metagenomic and metatranscriptomic techniques to characterize the functional potential of biochar-modified microbial communities, going beyond taxonomic description to comprehend what those communities actually do, has been a particularly exciting development in recent literature. In a three-year field trial, Luo et al. (2023) used shotgun metagenomics on soils and discovered that biochar-amended plots harbored communities with significantly enriched functional gene repertoires related to phosphorus mineralization, nitrogen fixation, and the degradation of complex polysaccharides. This finding provided a

mechanistic basis for observed improvements in nutrient availability that could not have been revealed by simple taxonomic profiling.

6.2 Mycorrhizal Relationships

From an agricultural standpoint, arbuscular mycorrhizal fungi (AMF) are among the most ecologically relevant families of soil organisms because they create symbiotic relationships with the great majority of crop species. In addition to providing advantages in terms of drought tolerance, disease resistance, and soil aggregate formation, AMF enhances plant access to immobile nutrients, especially phosphorus and zinc, by extending the effective root system of host plants into soil volumes beyond the phosphorus depletion zone. Numerous studies have shown that biochar increases AMF colonization of crop roots, mainly by enhancing soil pore architecture, lowering heavy metal stress that would otherwise inhibit AMF activity, and possibly releasing stimulatory compounds during initial weathering (Jaafar et al., 2022).

In a controlled greenhouse experiment, Qin et al. (2023) found that maize plants growing in sandy soils amended with biochar had 23% higher AMF root colonization rates than unamended controls. This resulted in considerably higher shoot dry weight and phosphate uptake efficiency. Increased availability of myo-inositol compounds, which act as signaling molecules in the early phases of symbiosis establishment, and enhanced soil pore connectivity, which promotes hyphal growth, both seemed to be part of the mechanism. The reduction of phosphorus fertilizer inputs in sustainable farming systems is closely related to these findings.

6.3 Nutrient Cycling and Enzyme Activities

A comprehensive indicator of the microbial community's biochemical activity, soil enzymatic activity is intimately related to the rates of nutrient cycling that determine the amount of nutrients accessible to crops. The most commonly measured indicators in biochar studies have been dehydrogenase, urease, phosphatase, and β -glucosidase activities. A consistent pattern appears in the literature from 2022 to 2026: the application of biochar tends to increase the activities of these enzymes, with the magnitude of response depending on the quality and quantity of biochar applied (Wang et al., 2022; Meng et al., 2023).

Enzyme stimulation is caused by a variety of methods. By adsorbing extracellular enzymes onto mineral-like surfaces while maintaining catalytic activity, biochar surfaces may shield them from proteolytic destruction. Enzyme synthesis is directly increased by increased microbial biomass after the addition of biochar. Furthermore, improvements in soil structure and aeration brought about by biochar may lessen anaerobic microsites, which would otherwise prevent the functioning of aerobic heterotrophic bacteria that produce important enzymes. In order to separate the direct enzyme-protective effect from the indirect microbial stimulation

effects, Bhattacharya et al. (2023) deftly separated these pathways using sterilized biochar treatments. They discovered that both pathways functioned concurrently and contributed about equally to the observed activity increases.

7. GREENHOUSE GAS EMISSIONS FROM SOIL AND BIOCHAR

About 10–12% of the world's anthropogenic greenhouse gas emissions come from agriculture, and soils are important sources of both methane (CH_4), which is mostly released from anaerobic environments like flooded rice paddies and ruminant digestive systems, and nitrous oxide (N_2O), a powerful greenhouse gas mainly produced through microbial nitrification and denitrification. Due to its potential for direct climate mitigation as well as its synergy with carbon sequestration through stable organic carbon incorporation into soil, biochar's ability to alter both of these emission pathways has garnered significant study interest.

In general, the data on N_2O is positive. Biochar application decreased soil N_2O emissions by a weighted mean of 25% under all conditions, according to a meta-analysis by Song et al. (2023) that included 146 independent studies. The biggest reductions, up to 54%, were seen in soils with high nitrogen inputs, high moisture content, and neutral to slightly acidic pH. In addition to the sorption of ammonium and nitrate onto biochar surfaces, which decreases the substrate available for denitrifying bacteria, the main process seems to entail biochar's improvement of soil aeration and reduction of anaerobic microsites that encourage denitrification. A biological mechanism of particular relevance has been shown in a number of recent studies: further suppression by activation of N_2O -reducing bacteria expressing the *nosZ* gene (Senbayram et al., 2022).

For CH_4 emissions, the situation is more complex and situation-specific. Biochar typically lowers CH_4 emissions in paddy rice systems, where anaerobic conditions during flooding encourage methanogenic archaea activity. This is said to happen by enhancing soil redox status, stimulating competing electron acceptors, and encouraging methanotrophic bacteria that eat CH_4 before it enters the atmosphere (Fang et al., 2023). The concurrent impact on N_2O must be taken into account, though, since drainage upgrades that lower CH_4 can occasionally raise N_2O , somewhat counteracting the climate advantage. Therefore, in order to appropriately characterize net climate effects, integrated whole-season greenhouse gas budgets stated in CO_2 -equivalent terms are crucial, and these comprehensive assessments are still underrepresented in the literature.

In terms of carbon sequestration, the stable aromatic carbon in biochar is a real addition to long-term soil organic carbon stocks that won't be quickly released back into the atmosphere by microbial metabolism like traditional organic additions would. According to modeling

studies by Woolf et al. (2022), applying biochar on a global scale to degraded agricultural soils using sustainably sourced feedstocks could sequester between 1 and 1.8 Pg CO₂-equivalent annually. When combined with other strategies, this could be a significant part of climate mitigation portfolios. The scientists emphasized, however, that these numbers are predicated on industrial systems achieving high energy efficiency and careful feedstock sourcing that prevents emissions from land-use change.

8. CROP PRODUCTIVITY AND BIOCHAR

The ability of biochar to support or improve crop yields in ways that farmers find economically significant ultimately determines its acceptance in agricultural systems. The soil, climate, and agricultural system in question all play a role in the complicated and nonlinear relationship between biochar application and crop productivity. Average yield gains of roughly 10–15% across a variety of agricultural systems were reported in early meta-analyses released before to 2020, while there was significant variation between research. This order of magnitude has generally been validated by more recent investigations, which also shed more light on the factors that influence whether responses are positive, neutral, or occasionally negative.

In a comprehensive meta-analysis of 2,489 paired observations from 423 published studies, Dai et al. (2023) discovered a mean increase in crop yield of 14.2% after applying biochar. The study found that soil pH (strongest responses in acid soils), soil texture (strongest effects in sandy soils), biochar application rate (an optimum at about 20–30 Mg ha⁻¹ beyond which incremental benefits diminished), and biochar feedstock (manure-derived biochars tend to produce larger yield gains than wood-derived biochars, probably due to higher nutrient contributions). Tropical systems generally benefited more than temperate ones, and cereals responded more consistently than legumes or root crops.

The persistence of yield gains across several growing seasons after a single application of biochar is a crucial practical challenge. Here, long-term field experiment data have proven useful. The biggest yield responses happened in the first two to three years after application, according to research by Jeffrey et al. (2022) that reviewed trials lasting five or more years. As the more reactive biochar components aged and its main contribution shifted from nutrient supply to longer-term structural benefits, subsequent years showed smaller but still positive effects. Economic analysis and the creation of soil amendment plans that include biochar with other inputs like compost or mineral fertilizers are also impacted by these temporal dynamics.

Several recent studies have shown synergistic interactions between biochar and traditional mineral fertilizers, indicating that biochar may increase fertilizer usage efficiency by lowering nutrient losses through leaching and volatilization (Ding et al., 2023). Practically speaking, this

means that biochar-amended soils may produce comparable results with less fertilizer inputs, lowering production costs and the environmental externalities related to the production and application of fertilizers. Wang et al. (2024) found that biochar amendment permitted a 20% reduction in nitrogen fertilizer rate without impacting yield in trials carried out in China's intensive vegetable production systems. This was mainly due to decreased ammonia volatilization and nitrate leaching from treated soils.

9. IMPORTANT LIMITS AND FUTURE RESEARCH DIRECTIONS

Even though this study shows significant progress, there are still a number of important issues that need to be resolved before biochar can reach its full potential as a widely used technique for sustainable soil management. The requirement for more uniformity and standardization in experimental design and reporting is the first of these. The current literature is characterized by significant heterogeneity in biochar characterization techniques, application rates, experimental duration, and measured outcome variables, as noted in several recent meta-analyses. This makes cross-study comparisons challenging and limits the accuracy of pooled effect size estimates (Bai et al., 2023). Standardized biochar characterization procedures, such as those put out by the European Biochar Certificate and the International Biochar Initiative, are a significant advance, but publishing researchers still need to embrace them more widely.

One recurrent and significant issue is the discrepancy between short-term laboratory outcomes and long-term field performance. While the soil processes under study take years or decades to develop, most published research on the impacts of biochar on soil biology, enzyme activity, and nutrient cycling is done during weeks to months. To give the temporal perspective needed to direct useful management suggestions, multi-year, multi-site field trials with robust experimental designs are desperately needed. Progress in this area would be significantly accelerated by the creation of global networks of biochar field trial sites that exchange data and standards.

For standard production conditions and feedstocks, the safety of biochar as a soil input is generally well established; however, concerns about the possible accumulation of heavy metals, polycyclic aromatic hydrocarbons (PAHs), and other contaminants in biochars made from specific feedstocks, such as sewage sludge or mixed municipal organic waste, still exist (Freddo et al., 2022). Many jurisdictions are still creating regulatory frameworks for biochar quality assurance, and maintaining vigilance and strong quality control systems will be necessary to guarantee farmer and customer confidence in product safety.

Lastly, studies on how biochar interacts with new agricultural techniques, such as cover crops, agroforestry, precision farming, digital soil mapping, and regenerative agriculture

systems, are still in their infancy. Realizing biochar's full complimentary potential will require an understanding of how it functions within these interconnected systems rather than in isolation. It will require interdisciplinary cooperation between soil scientists, agronomists, ecologists, economics, and social scientists to convert experimental findings into practically feasible, socially acceptable, and contextually appropriate soil health initiatives.

10. CONCLUSION

In the context of sustainable agriculture, the information compiled in this study makes it clear that biochar is a truly useful and adaptable tool for enhancing soil health. Biochar provides a range of functions that no single conventional amendment can match by simultaneously addressing several aspects of soil quality through its effects on soil physical structure, water retention, pH, nutrient availability, microbial community composition, enzyme activity, and greenhouse gas dynamics. Between 2022 and 2026, research has significantly expanded our understanding of the mechanisms underlying these effects, shifted the evidence base from lab and pot experiments to multi-year field trials, and started to seriously consider the practical and economic aspects of large-scale biochar deployment.

However, this review also demonstrates that biochar is not a panacea. Its efficacy varies greatly depending on the feedstock, production conditions, soil type, climate, and management method. Regulatory frameworks for quality assurance are still developing, and high costs continue to be a major obstacle to wider use. It will take sustained investment in long-term field research, standardized reporting, policy frameworks that acknowledge and reward soil carbon and environmental service contributions, and extension programs that assist farmers in making site-specific, well-informed decisions in order to fully realize the potential of biochar.

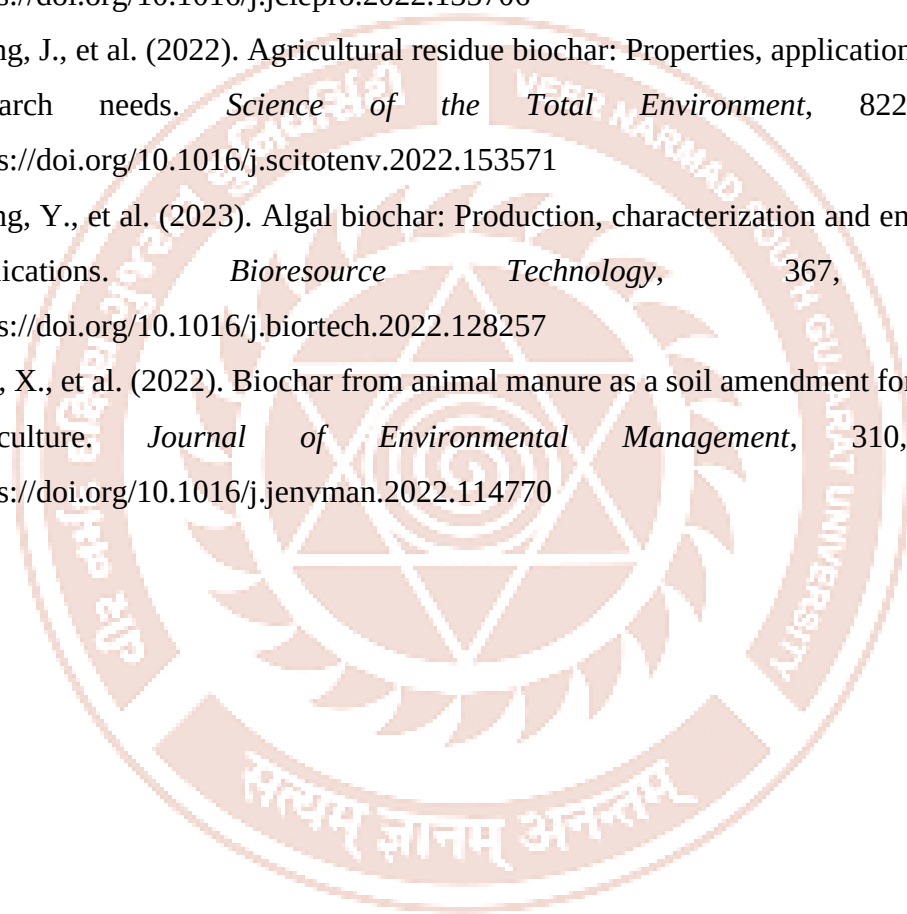
In the end, humanity's problems with soil health are too serious and complicated to be solved by a single intervention. It is best to view biochar as a potent part of a more comprehensive, integrated approach to sustainable soil management, which integrates physical, chemical, and biological techniques with good agronomic practice, mindful land stewardship, and an unwavering dedication to preserving the soil resource that underpins all terrestrial food production.

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Biodegradation of Paraquat and Profenofos by Exiguobacterium profundum: Harnessing Enzymatic, Phytotoxicity, and Plant Growth-Promoting Properties

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Abstract:

Pesticide contamination of soils and water environments is a serious ecological problem, and it clearly calls for remediation methods that are both efficient and sustainable. Paraquat, a highly toxic bipyridyl herbicide, and profenofos, a broad-spectrum organophosphorus insecticide, are among the most widely used pesticides, yet they remain long-lasting in the environment with little ease of breakdown. Microbial bioremediation stands out as a practical alternative because it is cost-effective, eco-friendly, and capable of detoxifying contaminated sites without generating secondary pollutants. In this study, we examined the biodegradation potential of *Exiguobacterium profundum* (isolate MAK1), a polyextremophilic Gram-positive bacterium, against paraquat and profenofos under laboratory conditions. Identification by 16S rRNA gene sequencing showed 100% identity (911/911 nucleotides) with *Exiguobacterium profundum* strain 0056 (GenBank: KP236196.1). Tolerance assays showed a concentration-dependent decline in growth, although the isolate still grew strongly at 50 ppm and moderately at 100 ppm for both pesticides. Over 10 days in Bushnell–Haas broth, paraquat degradation rose from about 31% on day 0 to 92.32% on day 10 at 50 ppm, while at 100 ppm it increased from 25% to 80.37%. Profenofos degradation followed a similar pattern, progressed from 32% to 78.4% at 50 ppm, whereas at 100 ppm it increased only from about 1.2% to 27.03%. Esterase activity, tested on tributyrin agar, supported an enzymatic role in pesticide breakdown, especially in the case of profenofos. Phytotoxicity assays using *Vigna radiata* (mung bean) seeds demonstrated that microbial treatment greatly reduced toxicity, with treated paraquat and profenofos samples producing much higher seedling vigor indices (694.4 and 535.46, respectively) than untreated samples (339.5 and 211.10). Inhibition tests against *Staphylococcus aureus* and *Escherichia coli* also showed that the degraded samples were less toxic. The isolate also displayed useful plant growth-promoting traits, including indole-3-acetic acid (IAA) production, siderophore synthesis, and ammonia production. These findings presents *E. profundum* as a promising multifunction bioremediation and plant growth-promoting organism for managing pesticide-contaminated agricultural environments.

1. Introduction:

Pesticides remain essential to modern agriculture because they protect crops from insects, weeds, fungi, and other pests that reduce yield. According to the Food and Agriculture Organization (FAO), global agricultural pesticide use reached about 3.73 million tonnes of active ingredients in 2023, nearly twice the level recorded in 1990, with an average application rate of 2.40 kg per hectare of cropland (FAO, 2025). Even so, only a small portion of what is applied actually reaches the target pest. The rest ends up in soil, water, and air, where it

contaminates agricultural ecosystems and imposes risk to beneficial organisms and human health (Tudi et al., 2021; Kaur et al., 2024).

Paraquat (1,1'-dimethyl-4,4'-bipyridinium dichloride) is a non-selective, fast-acting contact herbicide classified as a bipyridyl compound. It is highly soluble in water, yet it adheres strongly to clay minerals and organic matter, which gives it soil half-lives that can stretch from months to years. Paraquat produces reactive oxygen species by diverting electrons from photosystem I, leading to rapid plant necrosis and, in mammals, severe pulmonary fibrosis and oxidative injury (Huang et al., 2019; Inthama et al., 2021). Profenofos [O-(4-bromo-2-chlorophenyl) O-ethyl S-propyl phosphorothioate] is a broad-spectrum organophosphorus insecticide and acaricide widely applied to cotton, vegetables, and fruit crops. It inhibits acetylcholinesterase, which causes neurotoxicity in target and non-target organisms alike, and it is classified by WHO as toxicity class II, meaning moderately hazardous (Kushwaha et al., 2016; Raj et al., 2024).

Physico-chemical remediation methods such as advanced oxidation, adsorption, incineration, membrane filtration, electrochemical treatment, and chemical precipitation are often expensive to run, energy-demanding, and capable of generating secondary pollutants (Bose et al., 2021; Raffa & Chiampo, 2021). Microbial biodegradation offers a different strategies for this harmful pesticides. It relies on the metabolic activities of bacteria, fungi, and algae to transform harmful pesticide molecules into less toxic products (Bhatt et al., 2021; Kumar et al., 2018). Among pesticide-degrading bacteria, *Exiguobacterium* has drawn increasing attention because of its polyextremophilic nature, metabolic flexibility, and known ability to break down different environmental pollutants (Kasana & Pandey, 2018; Xiao et al., 2024).

Exiguobacterium profundum was first described by Crapart et al. (2007) from a deep-sea hydrothermal vent at the East Pacific Rise, where it was identified as a moderately thermophilic, halotolerant, facultatively anaerobic, non-spore-forming Gram-positive bacterium. Later studies recovered members of this species from mine soil, petroleum-contaminated sites, saline sediments, arsenic-rich environments, and Tibetan glaciers, which shows its versatile adaptability (Kasana & Pandey, 2018; Zhang et al., 2021, Delegan et al. 2021, Barghoth et al. 2023, Sengupta et al. 2020, Tedesco et al. 2021). Sur & Sathivelu (2024) reported that an *Exiguobacterium* sp. from rhizospheric soil degraded the organophosphate dimethoate with approximately 95.87% efficiency at 150 ppm within five days. Isworo & Sabdono (2016) showed profenofos degradation by a consortium of *Exiguobacterium profundum* and *Oceanobacillus iheyensis* within 192 hours. After this much

studies, the degradation of paraquat by *E. profundum*, along with enzyme involvement, detoxification, and PGP traits in one experimental setup, remains largely unexplored.

The present study aimed to characterise the bacterial isolate MAK1 through morphological and molecular (16S rRNA) methods, evaluate its tolerance and degradation efficiency toward paraquat and profenofos at two concentrations (50 and 100 ppm) over 10 days, assess esterase enzyme involvement, verify detoxification through phytotoxicity assays using *Vigna radiata* and microbial inhibition tests, and identify plant growth-promoting traits such as IAA production, siderophore production, and ammonia production.

2. Materials and Methods

2.1 Bacterial Strain and Culture Maintenance

The bacterial strain *Exiguobacterium profundum* (isolate MAK1) was obtained from the Department of Biosciences, VNSGU, Surat. Pure cultures were maintained on Nutrient Agar (NA) by streak plate technique, incubated at 30–37°C for 24–48 h, and stored on Nutrient agar slants at 4°C for further experimental use.

2.2 Pesticides and Media

Paraquat dichloride and profenofos (technical grade) were used as target pesticides. Stock solutions were prepared in sterile distilled water (paraquat) or dimethyl sulfoxide—DMSO (profenofos) and were filter-sterilized (0.22 µm). Bushnell–Haas (BH) media was used as the minimal media for tolerance and degradation tests.

2.3 Morphological and Molecular Characterisation

Morphological characterisation was performed by observing colony characteristics on Nutrient Agar after incubation of 24 hrs at 30°C, including shape, size, margin, elevation, opacity, texture, and pigmentation. Gram staining was performed and cells were observed under 100× oil immersion lens. For molecular identification, the 16S rRNA gene (911 bp, isolate MAK1) was sequenced and subjected to BLASTn analysis against the NCBI core nucleotide database. Sequence identity, query coverage, E-value, and bit score were evaluated.

2.4 Tolerance Assay

Bushnell–Haas agar supplemented with paraquat or profenofos at 50 ppm and 100 ppm concentrations was prepared aseptically (Jokhakar et al., 2022). The bacterial culture was spot-inoculated at the center of each plate and incubated at 30–37°C for 24–72 hrs. Plates were examined for colony growth and formation of clear zones, which would indicate utilization or degradation of the pesticide.

2.5 Pesticide Degradation in Liquid Medium

Bushnell–Haas broth was supplemented with either paraquat or profenofos at 50 ppm and 100 ppm (Jokhakar et al., 2022). Actively growing cultures of *Exiguobacterium profundum*

(1–2 mL) were inoculated into 100 mL pesticide-supplemented broth. Uninoculated flasks with identical pesticide concentrations served as controls. Flasks were incubated at 30–37°C at 120–150 rpm for 10 days. Samples were collected at intervals of 0, 24, 48, and 72 hrs, and subsequently every 24 hrs up to day 10. Absorbance was measured by UV-Visible spectrophotometry at the respective wavelength of each pesticide. Standard calibration curves were constructed to convert OD values to pesticide concentration. Percentage degradation was calculated as shown below:

$$\% \text{ Degradation} = [(\text{OD control} - \text{OD sample}) / \text{OD control}] \times 100$$

2.6 Esterase Enzyme Activity Assay

Esterase activity was assessed both qualitatively and semi-quantitatively using Tributyrin Agar (Jokhakar et al., 2022). Mineral Salt Medium (MSM) agar plates supplemented with 500 ppm monocrotophos and 1% tributyrin were additionally prepared. Bacterial cultures (5 µL) were spot-inoculated at the center of each plate and incubated at 37°C for 48 hrs. Hydrolysis of tributyrin, producing a clear halo zone around the colony, indicated esterase activity. The Solubilization Index (SI) was calculated as:

$$\text{SI} = (\text{Diameter of colony (mm)} + \text{Diameter of halo zone (mm)}) / \text{Diameter of colony (mm)}$$

2.7 Phytotoxicity Assay

The phytotoxicity of untreated and microbially degraded pesticide samples was evaluated using a seed germination bioassay with *Vigna radiata* (mung bean) as the test plant (Jyoti et al., 2024; Abdul-Baki & Anderson, 1973). Seeds were surface-sterilised by immersing in 0.1% HgCl_2 for 1–2 min, washed 4–5 times with sterile distilled water, and air-dried. Thirty seeds were placed on Whatman No. 1 filter paper in sterile Petri dishes (90 mm) and respective test solution was added to moisten the filter paper. Three treatment conditions were tested in triplicate: (1) sterile distilled water (control); (2) treated paraquat or profenofos (degraded supernatant); (3) untreated paraquat or profenofos. Plates were incubated in the dark at $25 \pm 2^\circ\text{C}$ for 5–7 days. Moisture was maintained by adding small volumes of the respective test solutions when necessary. Germination percentage, mean shoot length, mean root length, total seedling length, and Seedling Vigour Index (SVI-I) were calculated using:

$$\text{SVI-I} = \text{Germination\%} \times (\text{Mean Root Length} + \text{Mean Shoot Length})$$

- Germination Percentage (%) = (Number of germinated seeds / Total seeds) $\times$ 100
 - Mean Root Length (cm) = average root length of seedlings
 - Mean Shoot Length (cm) = average shoot length of seedlings

2.8 Microbial Inhibition Test

Antimicrobial activity of untreated and degraded pesticide samples was tested against *Staphylococcus aureus* and *Escherichia coli* by the agar well diffusion method (Aftab et al., 2011). Sterile Nutrient Agar plates were prepared and evenly inoculated with the microbial culture using a sterile cotton swab to form a uniform lawn. Wells (6 mm diameter) were punched with a sterile cork borer, and 50–100 μ L of each test sample was loaded. Plates were incubated at 30–37°C for 24 hrs. The diameter of inhibition zones (mm) was measured.

2.9 Plant Growth-Promoting (PGP) Trait Assessment

2.9.1 Indole-3-Acetic Acid (IAA) Production

The indole acetic acid (IAA) production was measured using a 5 mL supernatant (with and without Pesticide). The supernatant was mixed with 0.5 mL of orthophosphoric acid and 5 mL of Salkowski's reagent. The development of pink color indicates the IAA production, which was quantified at 530 nm. (Rodrigues et al., 2016)

2.9.2 Siderophore Production

Siderophore production was assessed on Chrome Azurol S (CAS) agar. Isolates were spot-inoculated at the center of CAS plates (with and without pesticide supplementation) and incubated for 4–5 days. Formation of orange/pink halos around colonies against the blue-green CAS background was considered positive (Jokhakar et al., 2022)

2.9.3 Ammonia Production

The isolate was inoculated in peptone water and incubated for 96 hrs. Ammonia production was confirmed by adding Nessler's reagent drops to the supernatant; brown colour development indicated a positive result (Nagalingam et al., 2020).

3. Results

3.1 Morphological Characterisation

The isolate MAK1 produced circular, moderate-sized, convex, opaque, smooth, and bright yellow-pigmented colonies on Nutrient Agar after 24 hrs incubation at 30°C (Table 1). Gram staining showed gram-positive rod-shaped cells which are characteristic features of *Exiguobacterium profundum* (Crapart et al., 2007; Kasana & Pandey, 2018).

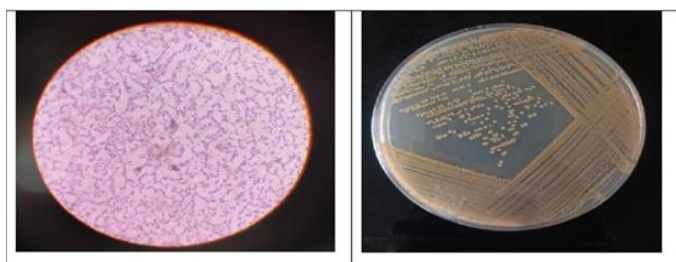


Figure 1. (Left) Gram-stained cells of *Exiguobacterium profundum* MAK1 showing Gram-positive rods (100 $\times$ oil immersion). (Right) Colony morphology on Nutrient Agar after 48 h incubation showing yellow pigmented colonies.

Table 1. Morphological characteristics of isolate MAK1 on Nutrient Agar

Feature	Observation
Colony Shape	Circular
Size	Moderate
Margin	Entire
Elevation	Convex
Opacity	Opaque
Texture	Smooth
Colour	Yellow
Gram Reaction	Gram-positive rods

3.2 Molecular Identification: 16S rRNA Gene Sequencing and BLAST Analysis

As shown in the results (Figure 2), The BLASTn results (RID: WJ3MREV2014) revealed that the isolate MAK1 belongs to the genus *Exiguobacterium*. The sequence showed a 100% identity (\$911/911\$ nucleotides) with the reference sequence of *Exiguobacterium profundum* strain 0056 (Accession Number: KP236196.1). The alignment yielded a significant E-value of 0.0, a total score of 1683 bits, and 0% gaps, indicating a perfect match. Based on these high-stringency parameters, the isolate MAK1 is conclusively identified as *Exiguobacterium*. (Figure 2)

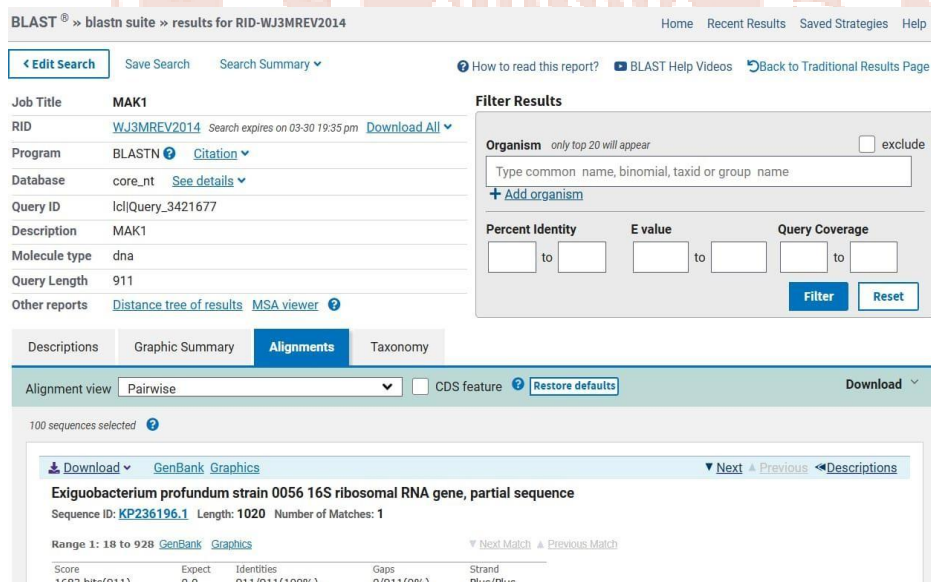


Figure 2. BLASTn results showing 100% sequence similarity of isolate MAK1 with *Exiguobacterium profundum* strain 0056 (KP236196.1), confirming species-level identification.

3.3 Tolerance Assay

Exiguobacterium profundum MAK1 showed growth on BH agar supplemented with both pesticides, confirming its ability to tolerate paraquat and profenofos. Higher growth was

observed at 50 ppm concentrations of both paraquat and profenofos, while a significant reduction occurred at 100 ppm, indicating concentration-dependent inhibition (Figure 3 and 4).

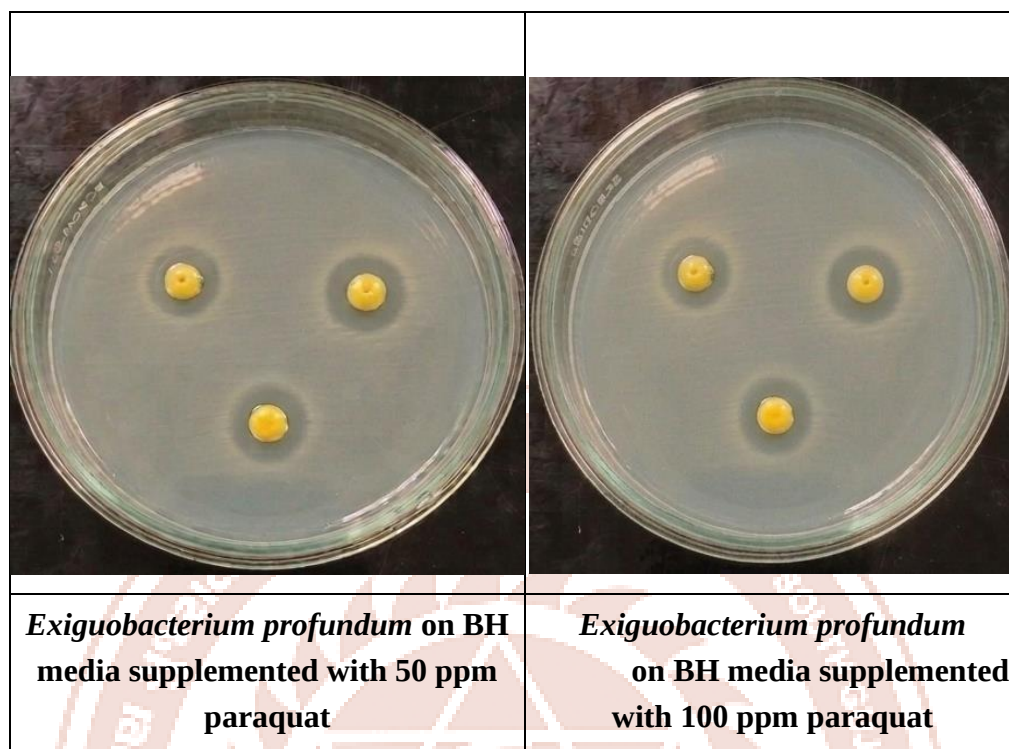


Figure 3. Tolerance of *Exiguobacterium profundum* MAK1 on BH agar supplemented with paraquat at 50 ppm (left) and 100 ppm (right), showing concentration-dependent growth reduction.

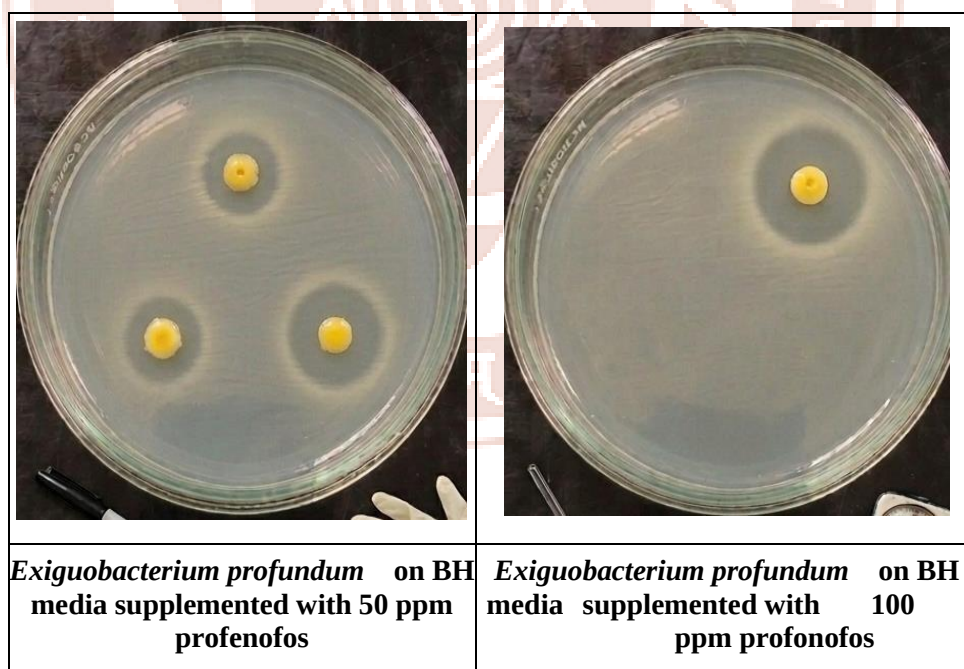


Figure 4. Tolerance of *Exiguobacterium profundum* MAK1 on BH agar supplemented with profenofos at 50 ppm (left) and 100 ppm (right).

3.4 Percentage Degradation of Pesticides

3.4.1 Paraquat Degradation

A standard calibration curve for paraquat exhibited a strong linear relationship ($R^2 = 0.9469$) between concentration (50–1000 mg/L) and optical density. Paraquat degradation by *E. profundum* MAK1 increased progressively over 10 days at both concentrations tested (Figure 5 and 6). At 50 ppm, degradation gradually rose from 31% on day 0 to 92.32% on day 10. At 100 ppm, degradation progressed from 25% on day 1 to 80.37% on day 10, demonstrating efficient biodegradation at both concentrations.

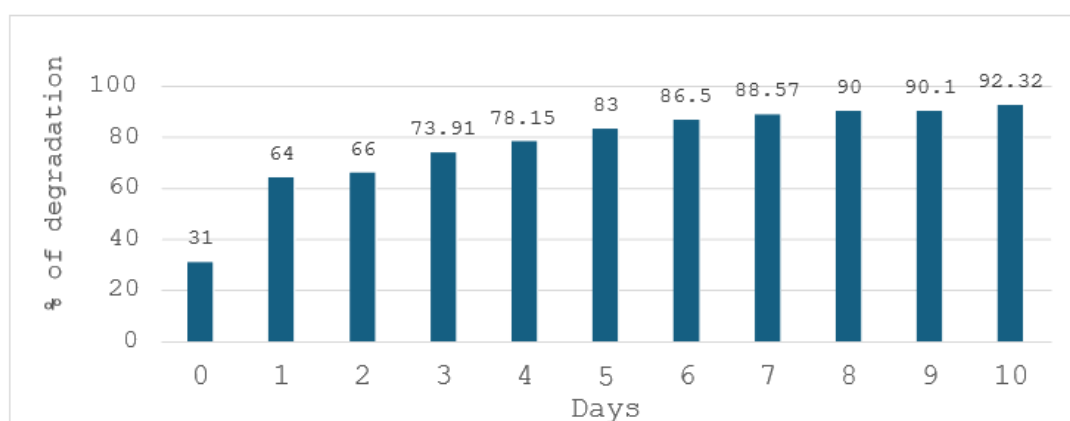


Figure 5. Percentage degradation of paraquat at 50 ppm concentration by *Exiguobacterium profundum* MAK1 over 10 days.

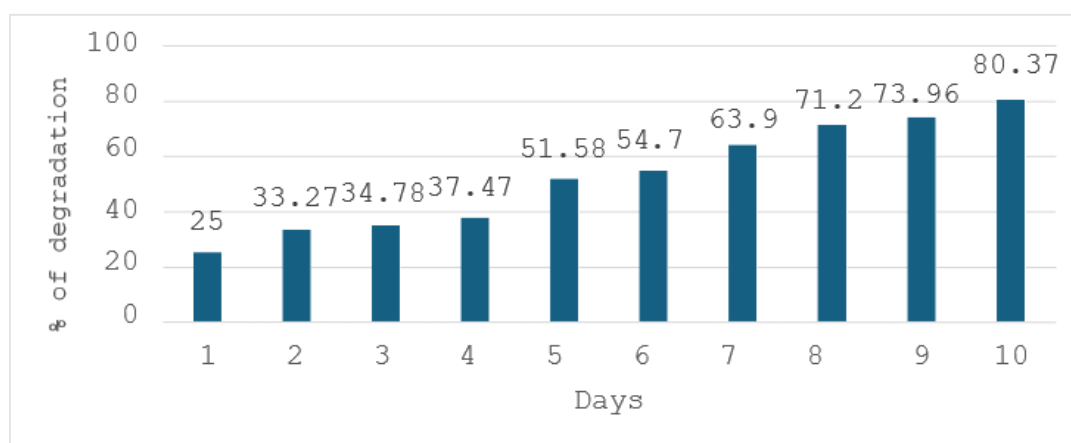


Figure 6. Percentage degradation of paraquat at 100 ppm concentration by *Exiguobacterium profundum* MAK1 over 10 days.

Table 2. Percentage degradation of paraquat by *Exiguobacterium profundum* MAK1 over 10 days

Concentration	Day 1	Day 3	Day 5	Day 7	Day 10
50 ppm	64%	73.91%	83%	88.57%	92.32%
100 ppm	25%	34.78%	51.58%	63.9%	80.37%

3.4.2 Profenofos Degradation

The profenofos standard calibration curve also showed a reliable linear relationship ($R^2 = 0.9291$). Profenofos degradation was lower overall compared to paraquat (Figure 7). At 50 ppm, degradation increased from 32% on day 1 to 78.4% on day 10. At 100 ppm, a significantly lower degradation rates were observed, rising from ~1.2% on day 1 to 27.03% on day 10. This suggests that degradation works better at lower concentration while compared at higher concentration where it's inhibited due to substrate toxicity.

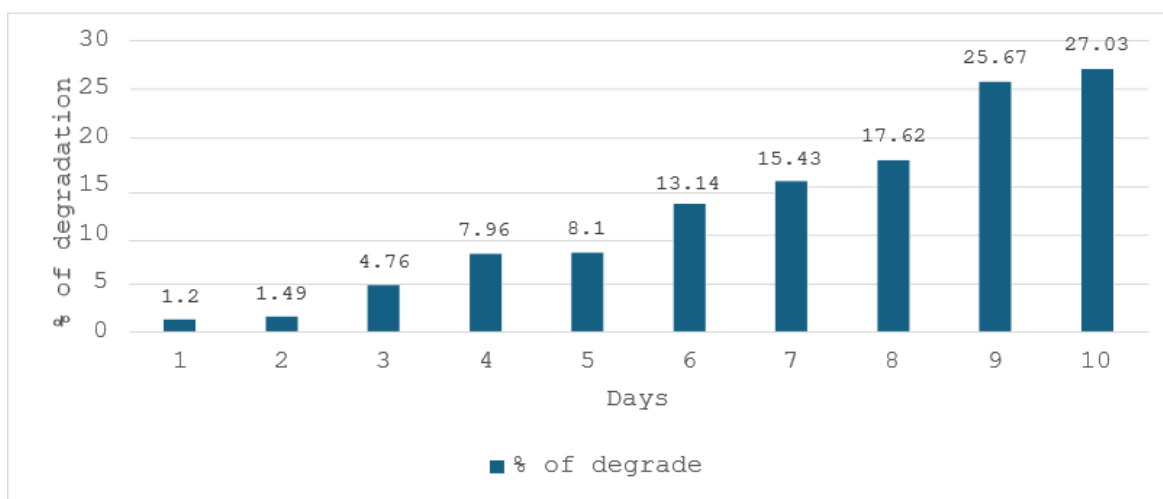
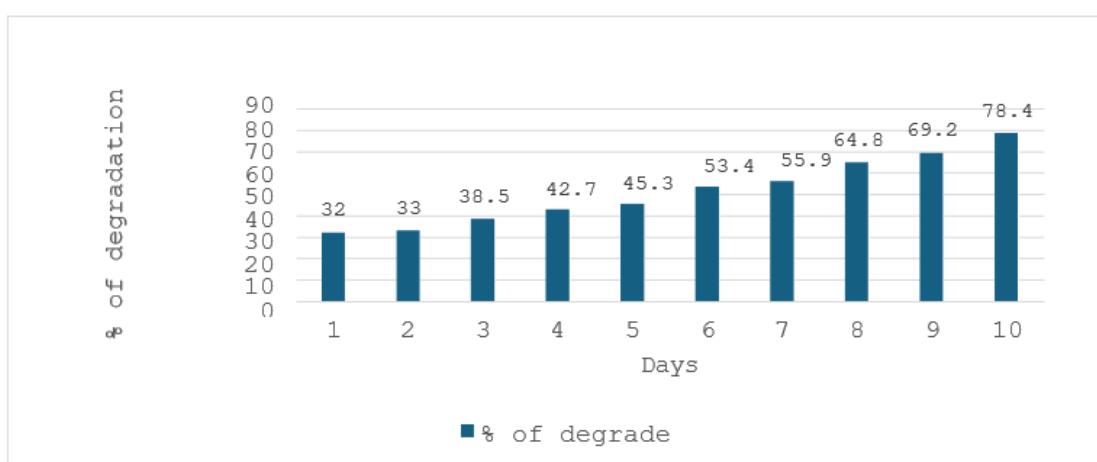


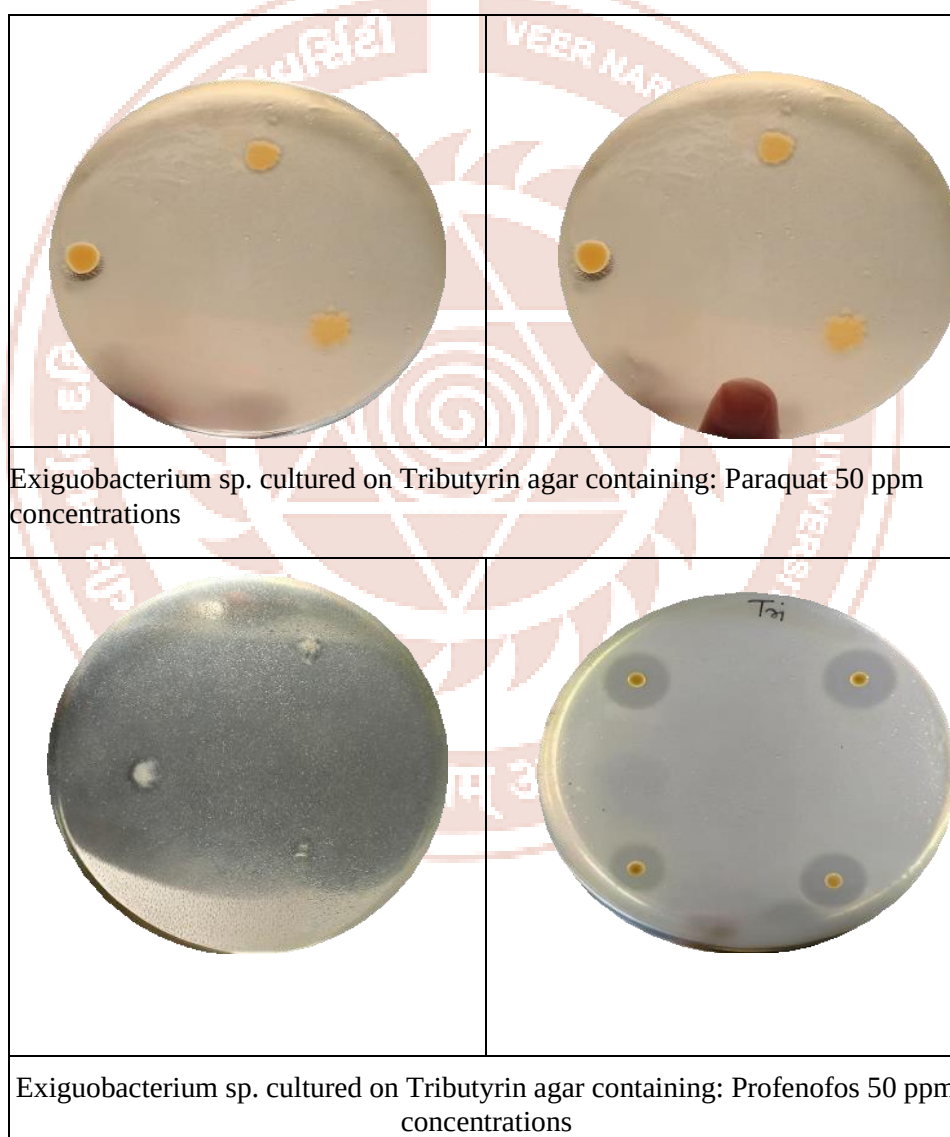
Figure 7 & 8. Percentage degradation of profenofos at 50 ppm (top) and 100 ppm (bottom) concentration by *Exiguobacterium profundum* MAK1 over 10 days.

Table 3. Percentage degradation of profenofos by *E. profundum* MAK1 over 10 days

Concentration	Day 1	Day 3	Day 5	Day 7	Day 10
50 ppm	32%	38.5%	45.3%	55.9%	78.4%
100 ppm	1.2%	4.76%	8.1%	15.43%	27.03%

3.5 Esterase Enzyme Activity

On Tributyrin Agar (TBA) supplemented with 50 ppm profenofos, *Exiguobacterium profundum* MAK1 produced distinct clear halo zones (zones of clearance) around colonies, confirming active esterase enzyme production by metabolizing lipid in the presence of this specific insecticide (Figure 10). This positive result indicates that the bacterium produces esterase enzymes capable of hydrolyzing tributyrin even under profenofos stress, suggesting high tolerance and possibly active utilization of profenofos as a metabolic substrate. In contrast, esterase activity was reduced in paraquat-supplemented plates, suggesting that paraquat at 50 ppm may inhibit esterase secretion or that the herbicide might disrupts lipid metabolism of the species.



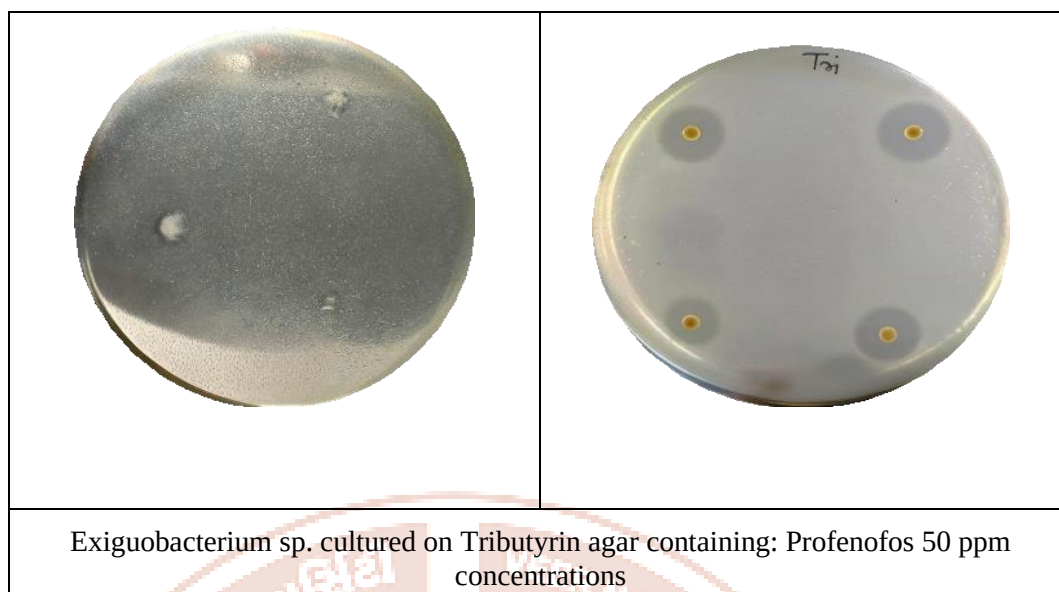


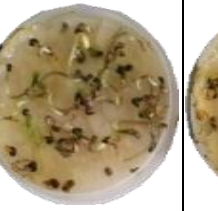
Figure 9 & 10. Esterase activity of *Exiguobacterium profundum* MAK1 on Tributyrin Agar. (Top) Paraquat 50 ppm – reduced/absent halo zone. (Bottom) Profenofos 50 ppm – clear halo zones indicative of active esterase production.

3.6 Phytotoxicity Assay

The phytotoxicity assay using *Vigna radiata* revealed significant differences in seed germination and seedling vigor among treatments (Table 4 & 5; Figures 11). Seeds treated with degraded pesticides showed better germination and seedling growth compared to untreated pesticide samples. Control seeds showed the highest germination (76.6%) and SVI-I (797.41). Seeds watered with treated (biodegraded) paraquat and profenofos yielded 70% germination (SVI-I of 694.4) and 66.6% germination (SVI-I: 535.46) showed while untreated paraquat and profenofos caused a notable reduction to 50% germination and SVI-I of 339.5, 46.6% germination in untreated profenofos (SVI-I: 211.10). These results revealed that reduced germination and lower vigor indices (339.5 and 211.09), indicating higher phytotoxicity. It also suggests that pesticide degradation significantly reduced toxicity and improved seed germination and seedling growth in treated paraquat and profenofos.

Table 4: . Phytotoxicity assay showing germination of *Vigna radiata* seeds over 0, 3, and 5 days under control, treated profenofos, untreated profenofos, treated paraquat, and untreated paraquat conditions.

	Control	Treated profenofos	Untreated profenofos	Treated paraquat	paraquat Untreated
0 Day					

3 Days					
5 Days					



Seedling growth of *Vigna radiata* under control conditions in the phytotoxicity assay



Seedling growth of *Vigna radiata* under Treated paraquat conditions in the phytotoxicity assay



Seedling growth of *Vigna radiata* under Untreated paraquat conditions in the phytotoxicity assay



Seedling growth of *Vigna radiata* under Treated Profenofos conditions in the phytotoxicity assay.



Seedling growth of *Vigna radiata* under Untreated Profenofos conditions in the phytotoxicity assay.

Figure 11. Comparative seedling growth of *Vigna radiata*: control seedlings (top) showing healthy, elongated root and shoot development, treated paraquat and untreated paraquat (middle) showing healthy elongated and no growth, treated while untreated profenofos (bottom) showing healthy elongated shoots and little growth.

Table 5. Influence of pesticide treatments on germination and seedling vigour of *Vigna radiata*

Treatment	Germ. %	Shoot (cm)	Root (cm)	Seedling (cm)	SVI-I
Control	76.6	6.61	3.80	10.41	797.41
Treated Paraquat	70.0	8.19	1.73	9.92	694.40
Untreated Paraquat	50.0	2.79	4.00	6.79	339.50
Treated Profenofos	66.6	5.04	3.00	8.04	535.46
Untreated Profenofos	46.6	2.72	1.81	4.53	211.10

3.7 Microbial Inhibition Test

The antimicrobial activity of untreated and biodegraded pesticide samples was assessed against *Staphylococcus aureus* and *Escherichia coli* (Table 6; Figures 12 and 13). Controls showed no inhibitory growth (0 mm). Untreated paraquat showed the largest zones of inhibition: 26 mm against *Staphylococcus aureus* and 30 mm against *Escherichia coli*.

After microbial degradation (treated paraquat), inhibition zones were reduced to 20 mm (*S. aureus*) and 21 mm (*E. coli*), revealing crucial conversion of harmful toxic pesticide compounds to less harmful products.. For profenofos, untreated samples gave inhibition zones of 21 mm (*S. aureus*) and 28 mm (*E. coli*), which decreased to 16 mm and 20 mm, respectively, following biodegradation. *Escherichia coli* showed the highest susceptibility to both pesticides, while *Staphylococcus aureus* was comparatively less sensitive. .These results suggest that treatment reduces antimicrobial effectiveness and that susceptibility varies among the microorganisms tested.

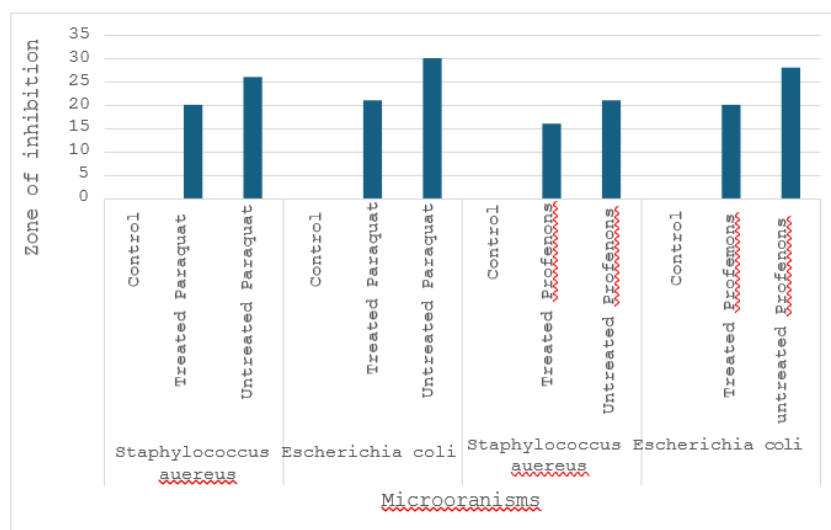
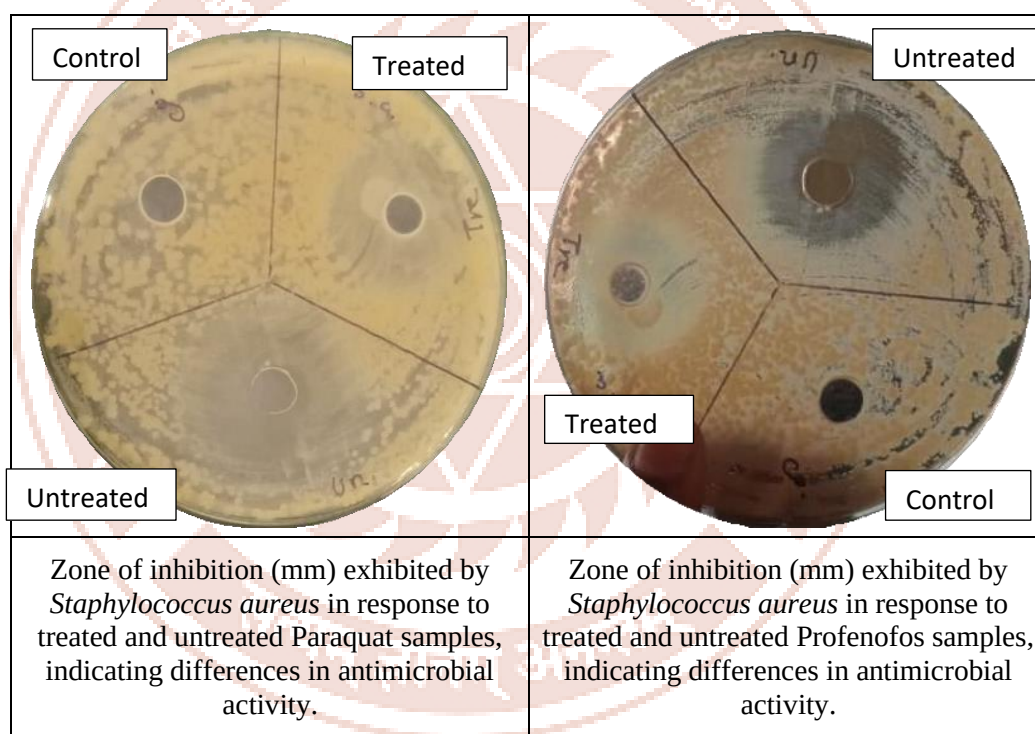


Figure 12. Comparative zone of inhibition (mm) of control, treated, and untreated paraquat and profenofos samples against *Staphylococcus aureus* and *Escherichia coli*.



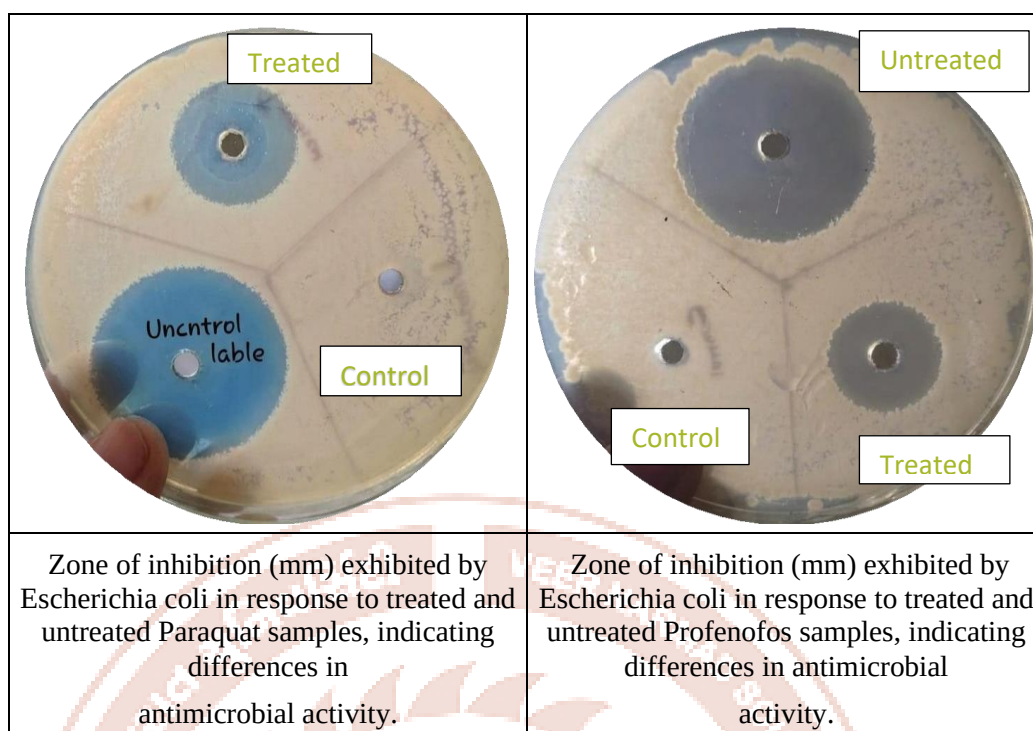


Figure 12. Agar well diffusion plates showing zones of inhibition of *S. aureus* & *E. coli* (left: paraquat treated vs untreated; right: profenofos treated vs untreated).

Table 5. Zone of inhibition (mm) of treated and untreated pesticide samples against *S. aureus* and *E. coli*

Microorganism	Control	Treated Paraquat	Untreated Paraquat	Treated Profenofos	Untreated Profenofos
<i>Staphylococcus aureus</i>	0 mm	20 mm	26 mm	16 mm	21 mm
<i>Escherichia coli</i>	0 mm	21 mm	30 mm	20 mm	28 mm

3.8 Plant Growth-Promoting Traits

3.8.1 Indole-3-Acetic Acid (IAA) Production

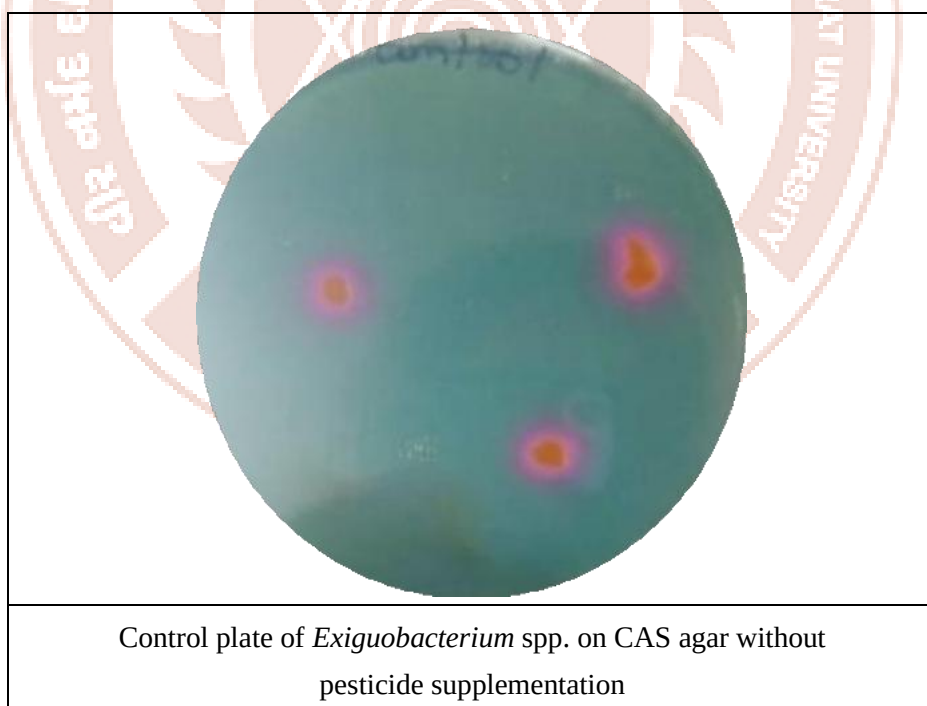
IAA production was measured by optical density at 530 nm using Salkowski's reagent. The control showed an average OD of 0.255. Treated paraquat (OD: 0.230) and profenofos (OD: 0.238) exhibited higher OD values than untreated paraquat (OD: 0.133) and profenofos (OD: 0.145), indicating improved IAA production capability in degraded samples. This suggests that the microbial degradation reduced pesticide toxicity and supported bacterial metabolic activity responsible for pesticide degradation is consistent with preserved or enhanced phytohormone synthesis, and that the degradation products are less inhibitory to IAA biosynthetic pathways.

Table 6. Optical density (OD₅₃₀) values for IAA production under different pesticide treatments

Treatment	OD ₁	OD ₂	OD ₃	Average OD ₅₃₀
Control	0.323	0.231	0.212	0.255
Treated Paraquat	0.295	0.205	0.189	0.230
Untreated Paraquat	0.148	0.132	0.119	0.133
Treated Profenofos	0.301	0.214	0.198	0.238
Untreated Profenofos	0.165	0.142	0.128	0.145

3.8.2 Siderophore Production

Exiguobacterium profundum MAK1 produced distinct orange/pink halo zones on Chrome Azurol S (CAS) agar, confirming active siderophore synthesis (Figure 13). Siderophore production was maintained in both paraquat- and profenofos-supplemented CAS plates, demonstrating that the bacterium retains iron-chelating activity even under pesticide stress. Variations in halo intensity between treated and untreated pesticide supplemented plates suggest the variation in siderophore production under different stress conditions. These supplements may influence the level of siderophore production compared to the control.



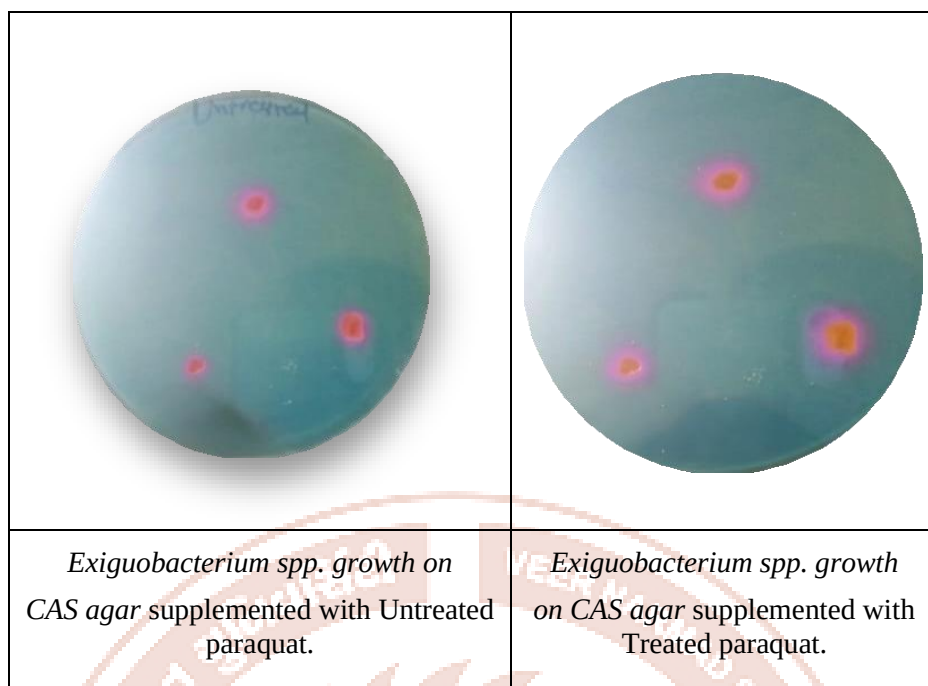


Figure 13. Siderophore production by *Exiguobacterium profundum* MAK1 on Chrome Azurol S (CAS) agar. (Top) Control plate without pesticide. (Bottom left) CAS agar with untreated paraquat. (Bottom right) CAS agar with treated paraquat. Orange/pink halos indicate active siderophore synthesis.

3.8.3 Ammonia Production

Ammonia production was assessed by optical density measurement following Nessler's reagent treatment. Control samples showed an average OD of 3.00. Treated samples exhibited slightly lower OD values compared to untreated ones (treated paraquat: 2.91 vs untreated: 3.03; treated profenofos: 2.84 vs untreated: 3.01). It suggests the possible degradation or transformation of the pesticides by microbial activity consistent with ammonia production capacity being retained and pesticide degradation altering the nitrogen metabolism system within the *Exiguobacterium* Species.

Table 7: Comparison of optical density (OD) values for ammonia production by *Exiguobacterium* sp. in control, treated, and untreated paraquat and profenofos samples.

Sr no.	Pesticide name	OD1	OD2	OD3	Average
1	Control	3.14	2.858	3.004	3.000667
2	Treated paraquat	2.95	2.88	2.91	2.913333

3	Untreated paraquat	3	2.97	3.12	3.03
4	Treated profenofos	2.86	2.82	2.84	2.84
5	Untreated profenofos	3.02	2.99	3.01	3.006667

4. Discussion

The current study showed that the *Exiguobacterium profundum* isolate MAK1 can break down both paraquat and profenofos while still promoting plant growth and maintaining enzymatic activities. Molecular identification using 16S rRNA sequencing revealed a 100% similarity with *Exiguobacterium profundum* strain 0056 (KP236196.1). This species was first described by Crapart et al. (2007) as a moderately thermophilic and salt-tolerant bacterium found in deep-sea hydrothermal vents. The isolation of *E. profundum* from polluted soil near Ankleshwar, Gujarat, highlights the adaptability of the genus *Exiguobacterium*, which is known for its environmental resilience and importance in biotechnology (Crapart et al., 2007; Kasana & Pandey, 2018).

MAK1 showed several traits that promote plant growth, including the production of indole-3-acetic acid (IAA), ammonia and siderophores. Similar traits have been found in other pesticide-degrading *Exiguobacterium* species, where they helped to improve plant growth and stress tolerance in contaminated conditions (Sur & Sathiavelu, 2024). Species in the *Exiguobacterium* genus are recognized for their ability to both remediate environments and promote plant growth (Kasana & Pandey, 2018).

The isolate demonstrated better growth and degradation efficiency at 50 ppm compared to 100 ppm of both pesticides. This suggests concentration-dependent substrate degradation efficiency, which is common in pesticide-degrading microorganisms (Subsanguan et al., 2020). Paraquat degradation by MAK1 reached 92.32% at 50 ppm and 80.37% at 100 ppm in 10 days, which is comparable to degradation efficiencies reported in other bacterial systems under optimized conditions (Huang et al., 2019; Jindakaraked et al., 2023). Profenofos degradation was 78.4% at 50 ppm within 10 days, which aligns with results found in other profenofos-degrading bacteria. Mahajan et al. (2021) reported that *Bacillus* spp. PF1 degraded approximately 93% profenofos over 30 days under optimized incubation conditions, whereas

Fang et al. (2022) observed degradation of profenofos in soil systems by *Cupriavidus nantongensis* X1T. Although direct comparison between flask and soil systems is limited by differing environmental conditions, the degradation efficiency observed in the present study indicates that MAK1 possesses considerable biodegradation potential. The lower degradation at higher pesticide concentrations may result from toxic effects on microbial metabolism and degrading enzymes.

During profenofos degradation, esterase activity was observed, indicating the role of hydrolytic enzymes in the biodegradation process. Sur & Sathiavelu (2024) similarly noted versatile enzymatic activity in rhizospheric *Exiguobacterium* for dimethoate degradation, supporting the role of esterases as key bioremediation enzymes in this genus. Previous studies have reported similar esterase-mediated degradation pathways involving organophosphorus hydrolases like OpdB (Fang et al., 2022). The reduced esterase activity under paraquat stress may relate to oxidative stress caused by reactive oxygen species generated by paraquat exposure. As paraquat-induced ROS has been shown to directly suppress esterase-class enzyme activity (Ramalho et al., 2025) through oxidation of critical protein residues (Jomova et al., 2024).

Phytotoxicity analysis using *Vigna radiata* indicated a significant increase in seed vigor after microbial treatment compared to untreated pesticide controls, showing reduced toxicity following degradation. These results are consistent with reduced phytotoxicity of degradation products compared to parent pesticide molecules, as also observed by Sillapawattana & Klungsunya (2022) for fungal paraquat metabolites. The decline in antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* further supported the detoxification of pesticide-containing samples. Aftab et al. (2011); reported that biodegraded product was non-toxic to beneficial soil bacteria.

The overall findings suggest that *E. profundum* MAK1 has promising potential for simultaneous bioremediation of pesticides and promotion of plant growth in contaminated agricultural settings. However, further studies involving GC–MS/HPLC analysis and genomic characterization are necessary to identify the degradation intermediates and clarify the molecular mechanisms involved.

5. Conclusion

The present study establishes *Exiguobacterium profundum* (isolate MAK1) as an effective pesticide-degrading bacterium capable of significantly reducing the concentrations of both paraquat (up to 92.32% at 50 ppm) and profenofos (up to 78.4% at 50 ppm) over a 10-day incubation period under laboratory conditions. Molecular identification by 16S rRNA

sequencing confirmed 100% identity with *E. profundum* strain 0056. Esterase enzyme activity confirmed enzymatic involvement in pesticide breakdown, particularly for profenofos. Phytotoxicity assays and microbial inhibition tests validated that microbial treatment substantially reduced the toxicity of both pesticides. Additionally, *Exiguobacterium profundum* MAK1 demonstrated valuable PGP traits—IAA production, siderophore synthesis, and ammonia production—establishing it as a dual-function bioremediation and plant growth-promoting agent. These findings support that pesticide biodegradation is both time- and concentration-dependent, and establish the application of *E. profundum* in bioaugmentation strategies for pesticide-contaminated agricultural soils and provide an evidence for future field-applied bioremediation studies, particularly for paraquat.

6. Future Prospects

Future research should focus on large-scale and field-level biodegradation studies to evaluate the efficiency and stability of *Exiguobacterium profundum* MAK1 under natural soil and environmental conditions; whole genome sequencing, gene expression analysis, alongwith proteomic studies to identify the specific catabolic genes, metabolic pathways, and esterase variants responsible for paraquat and profenofos degradation, advanced analytical characterisation of degradation bioproducts must be analyzed by GC–MS and HPLC to confirm complete detoxification and elucidate metabolic pathways; optimisation of environmental parameters like pH, temperature, nutrient availability, and inoculum density to maximise it's in situ bioremediation efficiency; development of immobilised cell or carrier-based formulations to improve bacterial survival and degradation stability in complex soil environments; ecotoxicological assessments including soil microbial diversity analysis and long-term plant growth experiments to confirm environmental safety and also development of biofertilizer or bioremediation formulations containing MAK1 for simultaneous enhancement of soil fertility, promotion of plant growth, and remediation of pesticide contamination in agricultural environments.

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Bioprospecting oil contaminated habitats for efficient lipase producing microorganisms

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Abstract:

Microbial lipases are versatile, high demand enzymes that catalyze hydrolysis, esterification, and transesterification of fats and oils. It catalyzes the hydrolysis of triacylglycerols into free fatty acids and glycerol. In this present study soil samples were collected from oil contaminated sites of garage and food stalls and domestic wastewater in Navsari, Gujarat. From the present study, soil and wastewater samples were collected from oil-contaminated sites including garages, food stalls, and domestic wastewater sources in Navsari, Gujarat. A total of seven bacterial isolates (LB1–LB7) and ten fungal isolates (LF1–LF10) were obtained. All ten fungal isolates were subjected to primary screening for lipase production, among which LF3, LF5, and LF9 were selected for further study based on their lipolytic potential. Among these, isolate LF9 exhibited the highest lipase activity (12.25 ± 0.006 U/mL). Further, various nutritional factors were studied for lipase production by isolate LF9 was studied. Maximum lipase activity was achieved using lactose as the carbon source (13.25 ± 0.005 U/ml), tryptone as the nitrogen source (14.30 ± 0.003 U/ml), and sunflower oil as the substrate (14.33 ± 0.005 U/ml), with a 7% inoculum size (14.58 ± 0.008 U/ml), at pH 5 (11.00 ± 0.005 U/ml). It may be further explored for various applications such as in food processing, bioremediation, and industrial biocatalysis etc.

1. INTRODUCTION

Lipases (triacylglycerol acyl-hydrolases, EC 3.1.1.3) are enzymes that naturally function to break down Triacylglycerol (TAG) into glycerol and free fatty acids, and they also facilitate esterification and transesterification reactions. Lipases are a subclass of esterases and have long chain triacyl-glycerols, which is very low soluble in water and the reaction is catalyzed at lipid-water interface (Aulakh & Prakash, 2010). Lipases play a critical role in the food, detergent, chemical, and pharmaceutical industries (Chandra et al., 2020). Fungus lipases have drawn a lot of interest from the industry due to their stability and substrate selectivity under a range of chemical and physical conditions. Numerous microorganisms, such as fungus, yeast, and bacteria, release lipases. Lipase-producing microorganisms have been discovered in a variety of environment, including industrial waste, plant oil processing plants, dairy farms, and oil-contaminated soil (Kumar et al., 2023). The purpose of the present study is to isolate lipase producing microorganisms from various oil contaminated sites soil and water samples and increase their lipase producing efficiency by characterizing the production medium.

2. MATERIALS AND METHODS

2.1 Sample collection

Samples were collected from three different locations around Navsari, Gujrat. Oil – contaminated sites were selected as potential sources, including garage soil (S1), Domestic wastewater (S2) and street food stall soil (S3) samples.

2.2 Isolation of lipolytic microorganisms

1 gm/5ml of Samples like garage soil, domestic wastewater and street food soil were added to 100ml of Luria Bertani Broth and Potato Dextrose Broth. The mixture was shaken for 24 hours at 37 °C. After Incubation, dilutions in the range of 10⁻¹ to 10⁻⁸ were made. Then, the spread plate method was used to distribute the diluted samples on Luria Bertani agar plates and Potato dextrose agar plates. After being inverted, the plates were incubated for 24 to 48 hours at 37 °C and PDA at room temperature (Ilesanmi et al., 2020).

2.3 Screening of Lipolytic Fungi

2.3.1 Primary screening

The primary screening of lipase producing microorganism was carried out using a plate assay method to detect extracellular lipase activity. The isolated fungal cultures were screened using cup borer method and were inoculated with 0.1 ml culture in the tributyrin agar medium (Peptone-5gm, Yeast extract-3gm, Sodium chloride-5gm, Tributyrin (glycerol tributyrates)-10ml, Agar-15gm, pH (7.0±0.2), Distilled water- (1000ml). The plate was incubated at 28-30°C for 48-72 hours for fungal isolates (Veerapagu et al., 2013).

2.3.2 Secondary screening

The secondary screening of lipolytic fungi was done by using p-nitrophenyl acetic acid (pNPA) assay by measuring the release of fatty acids or p-nitrophenol. Secondary screening of the potential lipase-producing isolates obtained from primary screening was carried out to confirm and compare their lipase-producing efficiency under submerged fermentation conditions. Selected isolates were inoculated into lipase production broth ((NH₄)₂SO₄-1gm, KH₂PO₄- 5gm, MgSO₄-1gm, CaCl₂-1gm, Soya peptone-10gm, sucrose-10gm, Groundnut oil-20ml, distilled water-1000ml, pH-7.2) supplemented with lipid substrate (such as groundnut oil) and incubated at room temperature for 48-72 hours (Hombalimath, 2021).

2.4 Lipase assay

Lipase enzyme activity was determined by screening isolates with a higher zone for substrate consumption and inoculating them into lipase fermentation medium, as described by Winker and Stuckman (1979). After 24 hours of incubation, 5 ml culture was withdrawn and was centrifuged at 6000 rpm for 20 minutes. Supernatant was used further for determination of

extracellular enzyme. Enzyme determination was carried using p-nitrophenyl acetate (pNPA). 0.1ml supernatant was considered as an extracellular enzyme. 0.8ml Tris buffer was added to the reaction mixture, 0.1ml pNPA (p-nitrophenyl acetate) as a substrate and was kept in water bath at 60 °C for 20 minutes. After incubation 0.25ml of Na₂CO₃ was added in the reaction mixture to stop the reaction between the enzyme and substrate. Further this reaction mixture was centrifuged at 6000 rpm for 10 minutes. Yellow obtained colour product was used to determine lipase colorimetrically at 410 nm (Intwala & Barot, 2022).

2.5 Characterization of isolate (LF9)

The fungal isolate (LF9) was physically characterized by looking at its microscopic characteristics using picric acid and colony appearance.

2.6 OFAT (One Factor at a Time) analysis of Lipase

2.6.1 Effect of Different Carbon Source on Enzyme Production

Lipase production was performed with different carbon sources in the production system. The production medium received 48hr cultures of and LF9. The following carbon sources (1%) were utilized: sucrose, lactose, dextrose and maltose. The effects of each type of carbon source on the lipase production were monitored at 24-hour intervals for five days and the cultures from each time point were analyzed for lipase production (Ghaima et al., 2014).

2.6.2 Effect of Different Nitrogen Source on Enzyme Production

Enzyme production was carried out using different nitrogen sources based on the literature. Peptone, tryptone, beef extract & casein were used individually at a concentration of 1% (w/v) in the production medium. The medium was inoculated with a 48-hour old culture of isolate LF9 incubated under suitable conditions. Lipase production was checked every 24 hours for up to 5 days by measuring enzyme activity (Sharma et al., 2001).

2.6.3 Effect of Different pH on Enzyme Production

The effect of pH of the production medium for lipase production was performed by varying pH of the medium from 5 to 8 whereas the other parameters were unaltered. The medium was inoculated with a 48-hour old culture of isolate LF9 incubated under suitable conditions. Lipase production was checked every 24 hours for up to 5 days by measuring enzyme activity (Bharathi et al., 2019).

2.6.4 Effect of Different Substrate on Enzyme Production

Enzyme production was carried out using different substrate based on literature. Olive oil, Peanut oil, Coconut oil & Sunflower oil were used in the production medium. The medium was inoculated with a 48-hour old culture of isolate LF9 incubated under suitable conditions.

Lipase production was checked every 24 hours for up to 5 days by measuring enzyme activity (Khatape et al., 2015).

2.6.5 Effect of Different Inoculum Size on Enzyme Production

Enzyme production was carried out using different inoculum size based on literature. 1%, 3%, 5% & 7% inoculum size were used in the production medium. The medium was inoculated with a 48-hour old culture of isolate LF9 incubated under suitable conditions. Lipase production was checked every 24 hours for up to 5 days by measuring enzyme activity (Veerapagu et al., 2013).

3. RESULTS AND DISCUSSION

3.1 Sample collection

During study, oil-contaminated soil and water samples were collected from various garages, street food stalls and domestic wastewater located in Navsari district. A total of 3 samples were collected in clean containers from various sites. soil samples were collected from a depth of approximately 5-6 cm in clean zip lock bags, while wastewater samples were collected directly from drainage (Alhamdani et al., 2016) (Chaturvedi et al., 2010). Total of 17 isolates were obtained from the samples by using serial dilution technique on Luria Bertani agar plates and Potato dextrose agar plates. Out of that 7 were bacterial isolates and 10 were fungal isolates. Primary screening of these fungal isolates was performed on Tributyrin agar plates by cup borer technique (Patel et al., 2018).

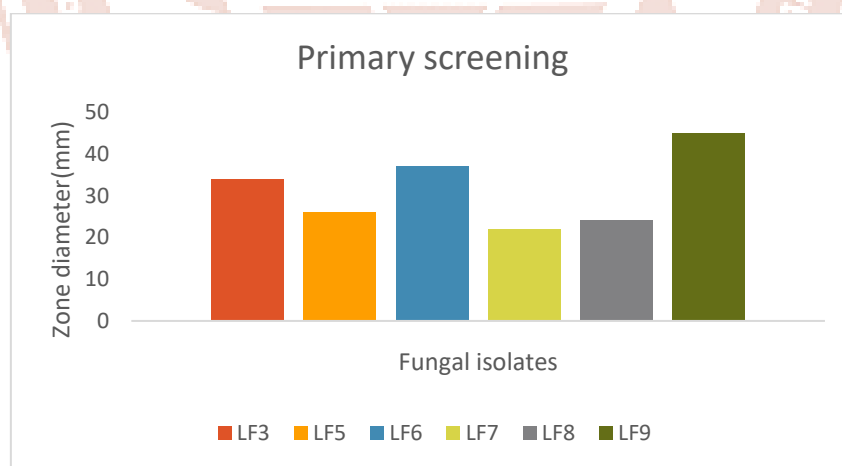


Fig. 1 Zone Diameter on Tributyrin agar by Fungal Isolates

Upon primary screening of these 10 fungal isolates, 6 isolates showed clear zone. Among 10 isolates the highest clear zone was found in isolate LF9-45mm, LF6-37mm, LF3-34mm (Fig.1).

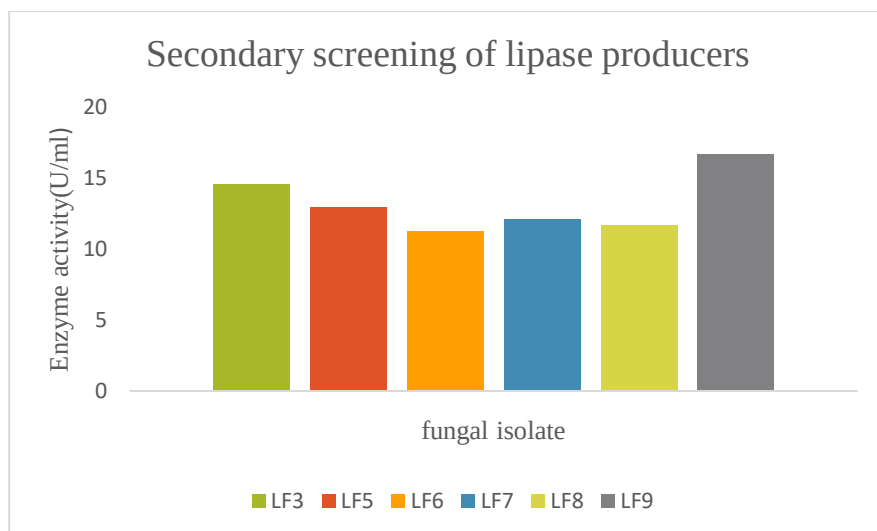


Fig. 2 Secondary screening of lipase producers

And these 6 isolates were further proceeded for the secondary screening. The activity of lipase in the production medium was assayed colorimetrically following the method of **Winkler and Stuckmann (1979)**. It is based on the hydrolysis of p-Nitrophenyl acetate(p-NPA) (**Intwala & Barot, 2022**). Selected isolates from primary screening were subjected to this assay. Among the tested strains, LF9 exhibited the highest lipase activity (≈ 16.7 U/ml), followed by LF3 (≈ 14.6 U/ml), LF5 (≈ 12.9 U/ml), LF7 (≈ 12.1 U/ml), LF8 (≈ 11.66 U/ml) and LF6 (≈ 11.25 U/ml) (**Fig.2**).

3.2 Result of Morphological study

Table no.1 About here Morphological study

Fungi	Growth characteristics on PDA	Microscopic observation (Fungal mounting)
LF9	cottony to woolly colonies, initially white, turning greyish brown to blackish brown with age	

The microscopic characteristics of the fungi LF9 were examined using picric acid staining under a light microscope (**Table no. 1**). The isolate showed broad, aseptate hyphae with irregular branching. Sporangiphores bearing globose sporangia filled with sporangiospores were clearly observed. LF9 on Potato Dextrose Agar (PDA) appeared fluffy, white to greyish-brown colony with a cotton-like texture. The presence of these characteristic structures, and morphology suggested that the isolate belongs to the genus ***Rhizopus***.

3.3 OFAT of Lipase using *Rhizopus* spp:

3.3.1 Effect of Carbon sources

The influence of different carbon sources (1%) (glucose, lactose, sucrose and maltose) On lipase production was investigated using the OFAT (one factor at a time) method over a period of five days.

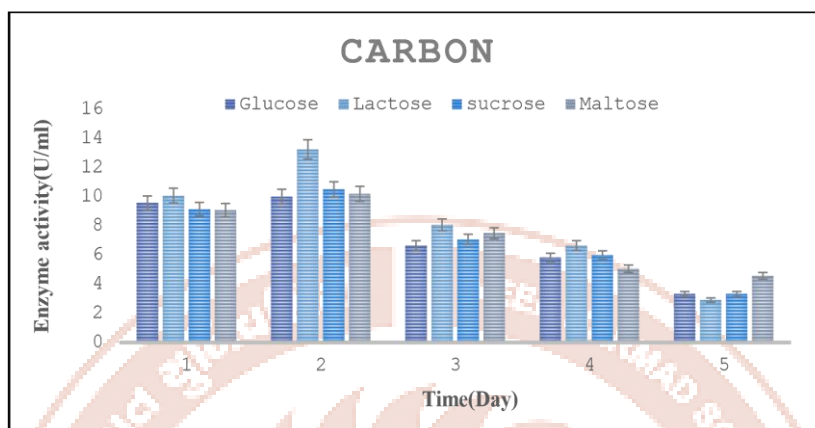


Fig.3 Effect of carbon sources on Lipase activity

The graph (**figure. 2**) shows how different carbon sources (1%) (glucose, lactose, sucrose, maltose) affect enzyme activity (U/ml) over 5 days for *Rhizopus*. From this graph *Rhizopus* spp. Giving maximum Lipase activity (13 U/ml) with 1% Lactose as carbon source supplement. In contrast to **Riyadi et al., (2017)** who reported ethanol as an effective carbon source for high lipase production in *Rhizopus*. This difference suggests that carbon-source preference varies depending on the microorganism and enzyme system, highlighting the need for strain-specific optimization. Similarly, **Bhattacharya et al., (2019)** found mustard oil to be the most suitable carbon source for lipase production by fungi strain from agricultural soil. In another study, **Fatima et al., (2021)** reported amalgam of olive oil cake and sugar cane bagasse as effective carbon source for lipase production in fungal strains. Likewise, **Issa QM (2025)** demonstrated that 2% glucose enhances lipase activity (10.96 U/ml) of *Aspergillus niger*. Comparison with literature (**Alabdallal et al., 2020**) shows 1% fructose giving high lipase activity by *Aspergillus niger*. This reinforces the idea that carbon-source efficiency is highly organism-dependent and must be optimized individually for each strain.

3.3.2 Effect of Nitrogen sources:

Lipase production by fungi generally requires slightly higher levels of nitrogen sources compared to those needed to produce other microbial enzymes (Riyadi et al., 2017). The influence of different Nitrogen sources (1%) on lipase production was investigated using Casein, peptone, tryptone and urea as different nitrogen sources by OFAT.

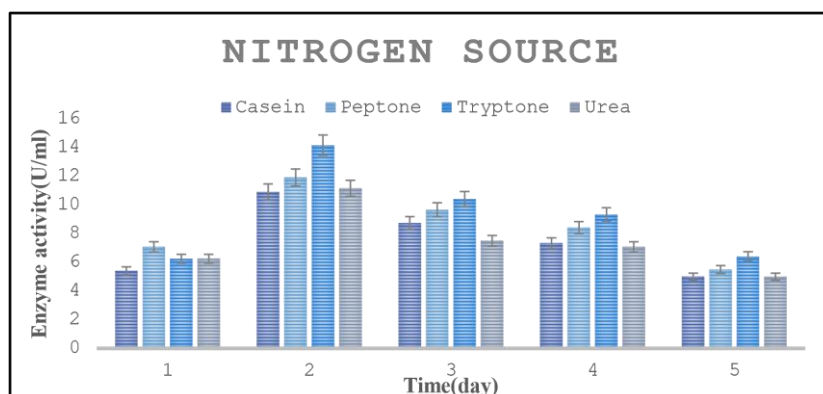


Fig.4 Effect of Nitrogen sources on Lipase activity

Among all nitrogen sources this fungus isolate *Rhizopus* shows maximum enzyme activity (14.30 ± 0.002 U/ml) on day 2 with Tryptone (1%) as the nitrogen source (Fig.4). Tryptone produces the highest activity on Day 2 and sustains better performance than casein, peptone, or urea, indicating it is the optimal nitrogen supplement for maximizing enzyme production with *Rhizopus*. While higher lipolytic activity was demonstrated by *Rhizopus spp.* when urea was added as a nitrogen source (Rodríguez et al., 2006; Mehta et al., 2017). Compared with other literature by Salihu et al. (2011) that shows peptone giving high lipase production by *Candida cylindracea*. Peptone as an organic nitrogen source also gave high lipase activity (Issa QM, 2025). Ammonium sulfate promoted high lipase activity in *Penicillium aurantiogriseum* (Lima et al., 2003). Comparing these references with current study result, tryptone yielded the highest lipase activity (14.30 ± 0.002 U/ml on Day 2) for *Rhizopus*, outperforming the nitrogen sources reported in the cited studies. This may vary with strain or medium composition.

3.3.3 Effect of substrates

The influence of different lipid substrates on lipase production was investigated using sunflower oil (1%), olive oil (1%), peanut oil (1%), and coconut oil (1%) as a substrate in lipase production medium.

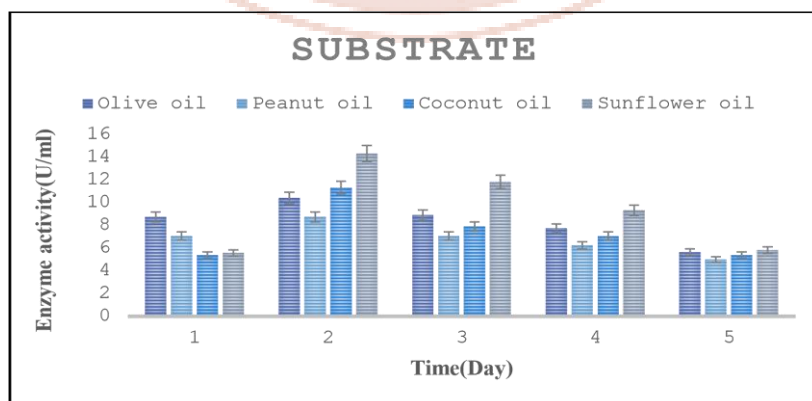


Fig.5 Effect of different substrates on Lipase activity

Among these substrates, sunflower oil showed the highest lipase activity, indicating that it effectively induced lipase production by fungal isolate ($14.33 \pm 0.005 \text{ U/ml}$) *Rhizopus* spp. (Fig.5). Comparing our findings with the literature, **Das et al., (2016)** reported that an *Aspergillus tamarii* isolate exhibited better lipase activity when coconut oil was used as the substrate, implying that coconut oil can be an effective inducer for certain fungal species. In contrast, **Bhattacharya et al., (2019)** found mustard oil to give maximum lipase activity by fungal strain of agricultural soil, indicating substrate-specific responses among different microorganisms. From this it can be concluded that for this *Rhizopus* spp. isolate sunflower oil is best for high lipase production.

3.3.4 Effect of inoculum size (%)

Inoculum size is important in determining the productivity of the enzyme. The influence of inoculum size on lipase production was evaluated using 1%, 3%, 5% and 7% inoculum levels for this fungal isolate.

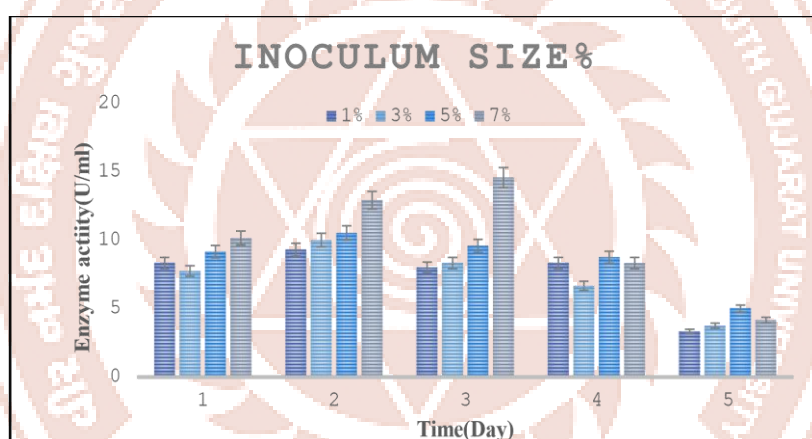


Fig.6 Effect of inoculum size on Lipase activity

The experimental results (Fig.6) shows that the fungal isolate produces the highest lipase activity (14.58 U/ml) with a 7 % inoculum, whereas lower activities are observed at 1 %, 3 % and 5 %, and the peak activity shifts from Day 5 to Day 3 with larger inoculum. In comparison with recent literature, **Ali et al., (2023)** reports a maximum lipase activity at 3 % inoculum, suggesting a narrower optimal range for their strain. **Riyadi et al., (2017)** finds that a smaller 1.5 % inoculum gives high lipase activity in a thermotolerant *Rhizopus* sp., indicating species-specific inoculum needs. **Bhattacharya et al., (2019)** also note 3 % inoculum of fungal strain from agricultural soil for maximum lipase production, aligning with **Ali et al., 2023** while **Issa QM (2025)** observes high activity with a 4 % inoculum by *Aspergillus niger*. The variation in optimal inoculum size among these studies (1.5 %–7 %) can be attributed to differences in microbial growth rates, biomass development, and nutrient utilization patterns of the specific fungal strains. Consequently, the 7 % optimum in this isolate (*Rhizopus*) suggests a

requirement for higher initial biomass to achieve peak enzyme expression, unlike the lower optima reported in the referenced works.

3.3.5 Effect of pH

Each microorganism has its own optimal pH and a specific pH range in which it grows and functions most effectively (Riyadi et al., 2017). The fungal isolate *Rhizopus* displays maximum lipase activity at pH 5, reaching about 11 ± 0.005 U/ml on day 2.

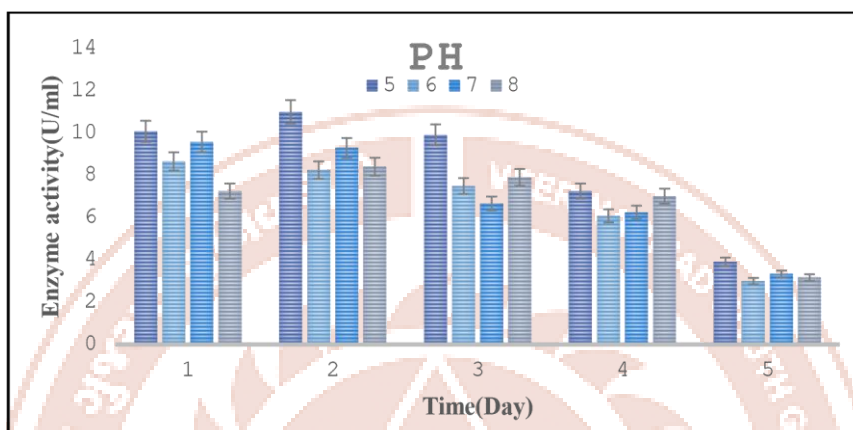


Fig.7 Effect of pH on Lipase activity

The fungal isolate *Rhizopus* exhibits maximum lipase activity of 11 ± 0.005 U/ml at pH 5, demonstrating that slightly acidic conditions are most suitable for lipase production by this isolate (Fig.7). This finding aligns with previous studies that have reported acidic pH optima for lipase synthesis in various fungal species. Specifically, **Ayinla et al., (2017)** identified pH 5 as optimal for *Rhizopus oryzae*, **Issa QM (2025)** observed peak activity at pH 6 by *Aspergillus niger*, **Bindiya & Ramana (2013)** reported pH 5–6 for *Aspergillus sydowii*, and **Helal et al., (2021)** noted pH 6 as favorable for *Rhizopus oryzae*. The consistency of these results suggests that maintaining an acidic environment (pH 5–6) is a key factor in maximizing lipase yield for *Rhizopus* and related fungal isolates, supporting the selection of acidic conditions in fermentation processes for enhanced enzyme production.

4. Conclusion

From the above study it can be concluded that the isolated organism *Rhizopus* spp Can produce lipase enzyme. Further studies are recommended to enhance the enzyme production using biotechnological approach and statistical approach.

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COMPARATIVE ANATOMICAL ADAPTATIONS IN FLOATING, EMERGENT AND SUBMERGED AQUATIC PLANTS

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Abstract:

Aquatic plants (hydrophytes) have evolved highly specialised anatomical adaptations enabling survival across distinct ecological niches. This study presents a comparative anatomical investigation of six species from Gujarat, India (December 2025–March 2026): floating (*Eichhornia crassipes*, *Pistia stratiotes*), emergent (*Nelumbo nucifera*, *Typha latifolia*), and submerged (*Hydrilla verticillata*, *Marsilea quadrifolia*). Freehand transverse sections of roots, stems, and leaves were stained with safranin and fast green and observed under a compound microscope. Results demonstrate that aerenchyma development, vascular reduction, and epidermal modifications correlate directly with the degree of aquatic immersion. Floating plants exhibit extensive aerenchyma and epistomatic leaves; emergent plants show intermediate vascular development; submerged plants display extreme tissue reduction and lack stomata.

1. INTRODUCTION

Hydrophytes are a specialized subset of vascular plants adapted to freshwater habitats, facing extreme conditions including reduced oxygen availability, diminished light penetration, and altered nutrient dynamics (Sculthorpe, 1967). They are ecologically vital, contributing to oxygen budgets, nutrient cycling, and sediment stabilization in freshwater ecosystems (Cronk & Fennessy, 2001). The aquatic environment drives convergent evolution of tissue-level modifications, aerenchyma development, vascular reduction, and epidermal restructuring across phylogenetically unrelated taxa (Barrett, 2013).

Hydrophytes are broadly classified into three functional groups: (1) floating plants, whose photosynthetic organs rest on or above the water surface; (2) emergent plants, rooted in submerged substrate with aerial leaves and stems; and (3) submerged plants, which complete their life cycles entirely or largely below the water surface. Each group faces distinct physiological challenges and has evolved a coherent suite of anatomical solutions.

This study addresses the comparative anatomical framework of six representative species collected from five freshwater sites in Gujarat, India, contributing to the understanding of plant environment interactions along the aquatic terrestrial gradient.

1.1 Objectives

- To examine and document the anatomical organization of selected floating, emergent, and submerged aquatic plant species through microscopic analysis of transverse sections of roots, stems, and leaves.
- To compare anatomical adaptations across the three hydrophyte functional groups and identify structural modifications correlated with the ecological niche.

- To relate tissue-level specializations, aerenchyma development, vascular reduction, and epidermal modifications to the physiological demands of each aquatic habitat type.

2. MATERIALS AND METHODS

2.1 Study Area and Field Collection

Field surveys and specimen collection were conducted at five freshwater sites across Gujarat, India, between December 3, 2025, and March 20, 2026. Site selection was guided by prior knowledge of hydrophyte distribution and confirmed using satellite imagery. Geotagged photographic documentation used with GPS Map Camera application.

Table 1: Plant Species, Collection Sites, and GPS Coordinates

Species	Type	Site	GPS Coordinates
<i>Eichhornia crassipes</i> (Mart.) Solms	Floating	Site-5 VNSGU Pond	21.15°N, 72.78°E
<i>Pistia stratiotes</i> L.	Floating	Site-4 VNSGU Pond	21.19°N, 72.75°E
<i>Nelumbo nucifera</i> Gaertn.	Emergent	Site-3 Vyara	21.12°N, 73.39°E
<i>Typha latifolia</i> L.	Emergent	Site-2 Panch Pipla	21.26°N, 73.45°E
<i>Hydrilla verticillata</i> (L.f.) Royle.	Submerged	Site-3 Vyara	21.12°N, 73.39°E
<i>Marsilea quadrifolia</i> L.	Submerged	Site-1 Vajharda	21.21°N, 73.45°E

Five sites surveyed across Gujarat, India, December 2025–March 2026.

2.2 Laboratory Materials and Sectioning Protocol

Anatomical sectioning utilized sharp stainless-steel razor blades, glass microscope slides, cover slips, and standard staining reagents: safranin (2–3 min) and fast green (counter-stain, few seconds). FAA (Formalin-Acetic Acid-Alcohol) served as a preservative. Observations were made under a compound light microscope at 10× and 40× objectives, with images captured via smartphone mounted on the eyepiece.

Free-hand transverse sections were cut from fresh material, washed, stained, and mounted in water. The thinnest, most uniform sections were selected. All specimens were washed with distilled water to remove sediment before processing.

2.3 Study Species

Six species were selected: Floating, *Eichhornia crassipes* (Mart.) Solms (Water Hyacinth) and *Pistia stratiotes* L. (Water Lettuce); Emergent, *Nelumbo nucifera* Gaertn. (Sacred Lotus, *Nelumbonaceae*) and *Typha latifolia* L. (Common Cattail, *Typhaceae*); Submerged, *Hydrilla verticillata* (L.f.) Royle (*Waterthyme*, *Hydrocharitaceae*) and *Marsilea quadrifolia* L. (Water Clover, *Marsileaceae*).

3. RESULTS

Microscopic examination of transverse sections revealed distinct anatomical profiles for each functional group, with consistent differences in aerenchyma development, vascular tissue organization, epidermal structure, and mechanical tissue presence.

3.1 Floating Plants: *Eichhornia crassipes* and *Pistia stratiotes*

3.1.1 Root Anatomy

Transverse sections of *E. crassipes* root reveal a single-layered epidermis lacking root hairs in mature regions, followed by an extensive multi-layered cortex dominated by loosely arranged parenchyma. The cortex is traversed by conspicuous aerenchyma, large, oval to irregular air-filled lacunae, occupying a substantial proportion of cortical volume, facilitating oxygen diffusion and contributing to buoyancy. Vascular bundles are scattered in monocotyledonous fashion with reduced xylem; an endodermis with Casparian strips is present but not prominently thickened. *P. stratiotes* root sections display equally extensive cortical aerenchyma with a notably distinct endodermis, star-shaped xylem arms in a radial arrangement, a small pith, and clearly defined pericycle cells.



Fig. 1: Root T.S. of *E. crassipes* – extensive aerenchyma lacunae, cortical parenchyma (CP), scattered vascular bundles (VB). | Root T.S. of *P. stratiotes* – well-defined endodermis (En), aerenchymatous cortex, star-shaped xylem (X), phloem (P), pith (Pi). Safranin–fast green stain.

3.1.2 Stem Anatomy

The stem of *E. crassipes* is stoloniferous and parenchymatous with very large aerenchyma chambers in concentric zones, often separated only by single-cell-thick partitions, maximizing air volume relative to tissue mass. Mechanical tissues are conspicuously absent or vestigial. *P. stratiotes* has a compressed rosette stem with similarly compact aerenchymatous cortex and scattered vascular bundles.

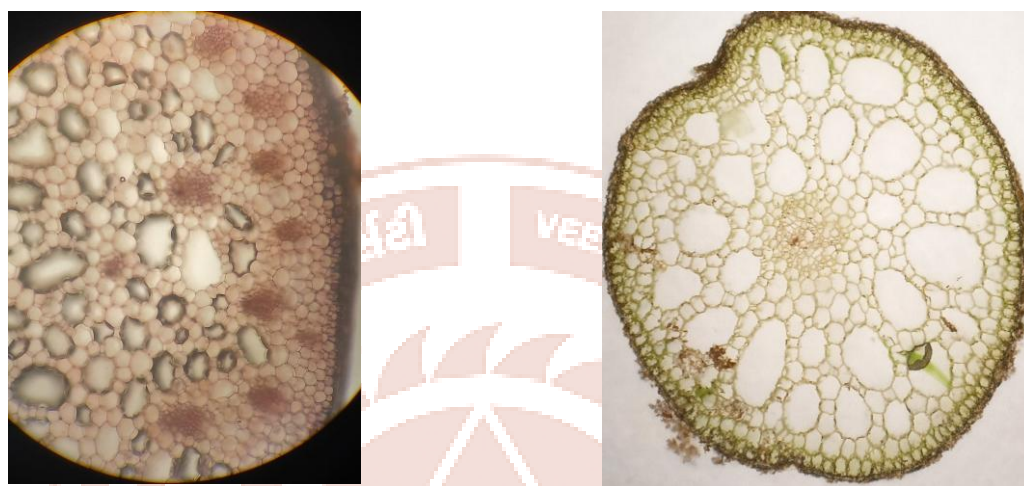


Fig. 2: Stem T.S. of *E. crassipes* – large aerenchyma chambers (Ae), thin parenchyma partitions (Pa), scattered vascular bundles (VB); no mechanical tissue. | Stem T.S. of *P. stratiotes* – regularly arranged aerenchyma lacunae (Ae), small scattered vascular bundles (VB) with minimal xylem. Safranin–fast green stain.

3.1.3 Leaf Anatomy

Leaves of *E. crassipes* bear numerous stomata on the upper epidermis (epistomatic), facilitating direct gas exchange. The mesophyll differentiates into compact palisade and loosely arranged spongy layers containing large aerenchymatous intercellular spaces. *P. stratiotes* leaves bear stellate epidermal hairs on the abaxial surface that trap air bubbles, contributing to buoyancy, with predominantly adaxial stomata and abundant chlorenchyma.



Fig. 3: Leaf T.S. of *E. crassipes* – upper epidermis (UE) with stomata (St), cuticle (Cu), palisade mesophyll (PM), aerenchyma spaces (Ae), vascular bundle (VB). | Leaf T.S. of *P. stratiotes*

– adaxial epidermis (AE) with stomata, aerenchyma-rich mesophyll (Ae), chlorenchyma (Ch), abaxial branched trichomes (Tr). Safranin–fast green stain.

3.2 Emergent Plants: *Nelumbo nucifera* and *Typha latifolia*

3.2.1 Root Anatomy

Transverse sections of *N. nucifera* root reveal a broad cortex with large oval aerenchyma spaces and notable raphide-containing idioblasts, calcium oxalate crystals serving defense and calcium storage. *T. latifolia* root sections present a distinctive spoke-like radiating aerenchyma configuration and slightly more pronounced sclerenchyma, with a clear endodermis, pericycle, and central pith.

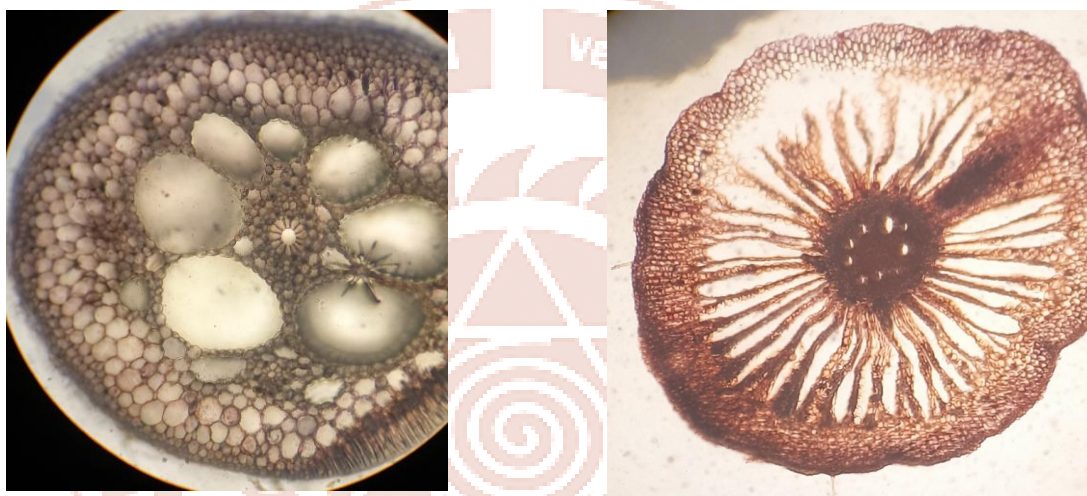


Fig. 4: Root T.S. of *N. nucifera* – large oval aerenchyma (Ae), raphide idioblasts (Ra), distinct endodermis (En), vascular cylinder (VC). | Root T.S. of *T. latifolia* – spoke-like radiating aerenchyma channels (Ae), endodermis (En), pericycle (Pc), central stele with pith (Pi). Safranin–fast green stain.

3.2.2 Rhizome/Stem Anatomy

The rhizome of *N. nucifera* shows very large macroscopically visible air chambers forming a continuous aeration network from rhizome through petiole to leaf, with raphide idioblasts in parenchymatous septa. The rhizome of *T. latifolia* is more elongated and fibrous with moderately developed cortical aerenchyma and sclerenchyma fiber strands, an intermediate characteristic between aquatic and terrestrial organization.

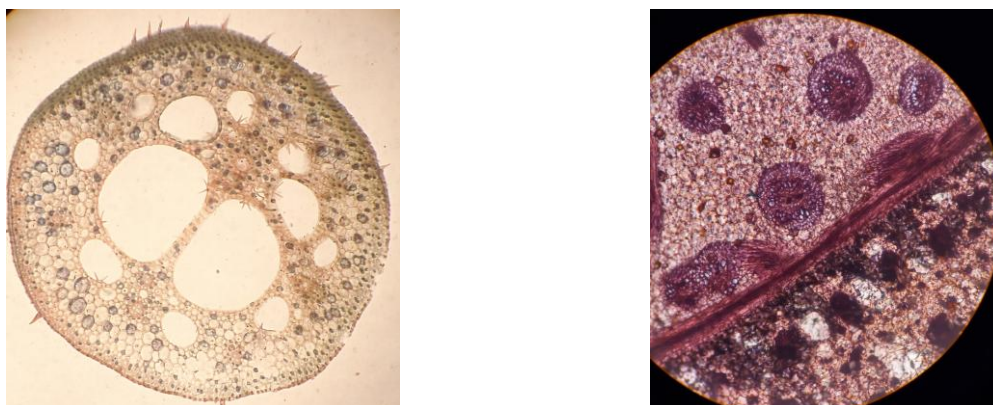


Fig. 5: Rhizome T.S. of *N. nucifera* – very large aerenchyma chambers (Ae), parenchymatous septa (PS) with raphide idioblasts (Ra), reduced vascular bundles (VB). | Stem T.S. of *T. latifolia* – cortical aerenchyma (Ae), more prominent xylem (X) in vascular bundles (VB), sclerenchyma fiber strands (Sc). Safranin–fast green stain.

3.2.3 Leaf Anatomy

Leaves of *N. nucifera* show a prominent waxy adaxial cuticle (superhydrophobic 'lotus effect'), stomata, loosely organized aerenchymatous mesophyll, raphide idioblasts, and large well-developed vascular bundles with bundle sheaths. *T. latifolia* leaves are amphistomatic (stomata on both surfaces) with more differentiated mesophyll, prominent vascular bundles, and sclerenchyma caps, the most terrestrial-like anatomy among the study species.



Fig. 6: Leaf T.S. of *N. nucifera* – adaxial epidermis (AE) with thick cuticle (Cu) and stomata (St), aerenchymatous mesophyll (Ae), raphide idioblasts (Ra), large vascular bundles (VB) with bundle sheath (BS). | Leaf T.S. of *T. latifolia* – amphistomatic epidermis, differentiated mesophyll (Me), aerenchyma (Ae), vascular bundles (VB) with sclerenchyma caps (Sc). Safranin–fast green stain.

3.3 Submerged Plants: *Hydrilla verticillata* and *Marsilea quadrifolia*

3.3.1 Root Anatomy

Roots of *H. verticillata* show a thin epidermis, narrow cortex with aerenchyma lacunae, and a greatly reduced stele with minimal xylem, consistent with their primary anchorage function. *M. quadrifolia* roots show somewhat more developed anatomy (defined endodermis, protostele-like stele) reflecting amphibious habits.



Fig. 7: Root T.S. of *H. verticillata* – thin epidermis (Ep), narrow cortex with aerenchyma (Ae), greatly reduced central stele (St) with minimal xylem. | Root T.S. of *M. quadrifolia* – cortex with moderate aerenchyma (Ae), distinct endodermis (En), protostele-like vascular cylinder (VC) with xylem (X) and phloem (P). Safranin–fast green stain.

3.3.2 Stem Anatomy

The stem of *H. verticillata* is slender with no cuticle, narrow cortex with moderate aerenchyma, and greatly reduced stele; mechanical tissue is entirely absent. Chloroplasts are present in outer cortical cells, contributing to photosynthesis through the shoot surface.

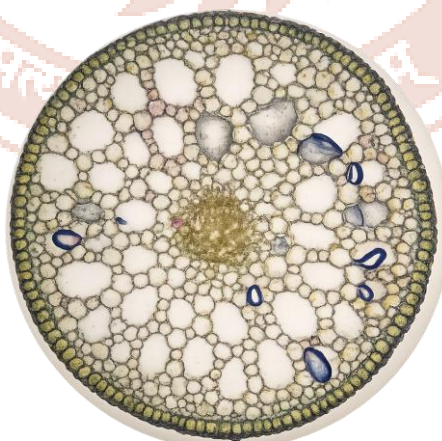


Fig. 8: Stem T.S. of *H. verticillata* – thin epidermis (Ep) without cuticle, parenchymatous cortex with aerenchyma spaces (Ae), greatly reduced central stele (St) with minimal xylem; no mechanical tissue. Safranin–fast green stain.

3.3.3 Leaf Anatomy

Leaves of *H. verticillata* entirely lack cuticle and stomata; gas exchange occurs by direct diffusion through the thin permeable surface. The mesophyll is undifferentiated with chloroplast-rich thin-walled parenchyma and a highly reduced single vascular bundle with xylem nearly absent. *M. quadrifolia* demonstrates heterophylly: aerial leaflets bear a thin cuticle and reduced stomata, whereas submerged leaflets lack cuticle and stomata entirely, a documented example of phenotypic plasticity.



Fig. 9: Leaf T.S. of *H. verticillata* – absence of cuticle and stomata; undifferentiated mesophyll (Me) of chloroplast-rich parenchyma; large intercellular spaces (IS); reduced vascular bundle (VB) with minimal xylem. | Leaflet T.S. of *M. quadrifolia* (submerged form) – absent cuticle, no stomata, undifferentiated mesophyll (Me), chloroplasts (Cl), aerenchymatous spaces (Ae), reduced vascular bundle (VB). Safranin–fast green stain.

4. COMPARATIVE ANATOMICAL ANALYSIS

The anatomical data from all six species permit systematic comparison across the three functional groups. Tables 2–3 summarize key parameters, and Figures 10 - 13 present graphical comparisons of the major adaptive features.

Table 2: Comparative Summary of Anatomical Features Across Hydrophyte Functional Groups

Feature	Floating Plants	Emergent Plants	Submerged Plants
Aerenchyma	Extensive (>60% cortex)	Moderate–extensive; continuous channels	Moderate; primarily in roots/rhizomes
Xylem Development	Poorly developed; reduced vessels	Moderately–well developed	Greatly reduced; near-vestigial
Mechanical Tissue	Greatly reduced/absent	Reduced to moderate (prominent in <i>Typha</i>)	Absent or minimal

Feature	Floating Plants	Emergent Plants	Submerged Plants
Stomata Position	Adaxial (epistomatic)	Adaxial or amphistomatic (Typha)	Absent or vestigial
Cuticle	Thin-waxy on upper surface	Present; thick and waxy in Nelumbo	Absent or vestigial
Root Development	Reduced; mainly absorptive	Well-developed; anchoring	Poorly developed; anchorage only
Stem Type	Stoloniferous / rosette	Rhizome; upright stems	Slender / creeping rhizome
Mesophyll Differentiation	Palisade + spongy present	Loosely differentiated	Undifferentiated chlorenchyma
Crystal Inclusions	Absent	Raphides in Nelumbo only	Absent
Gas Exchange Route	Stomatal (aerial)	Stomatal (aerial)	Diffusion through epidermis

Amphi. = amphistomatic; Well-dev. = well developed

Table 3: Species-Level Anatomical Feature Matrix

Species	Group	Aerenchyma	Vascular	Mech. Tissue	Stomata	Cuticle
<i>E. crassipes</i>	Floating	Extensive	Reduced	Absent	Adaxial	Waxy thin
<i>P. stratiotes</i>	Floating	Extensive	Reduced	Absent	Adaxial	Waxy thin
<i>N. nucifera</i>	Emergent	Moderate	Moderate	Moderate	Adaxial	Thick waxy
<i>T. latifolia</i>	Emergent	Moderate	Well-dev.	Prominent	Amphi.	Well-dev.
<i>H. verticillata</i>	Submerged	Moderate	Vestigial	Absent	Absent	Absent
<i>M. quadrifolia</i>	Submerged	Moderate	Reduced	Minimal	Absent*	Absent*

*In submerged leaflet form, aerial leaflets of *M. quadrifolia* show a thin cuticle and reduced stomata.

Graphical Analysis of Key Anatomical Parameters

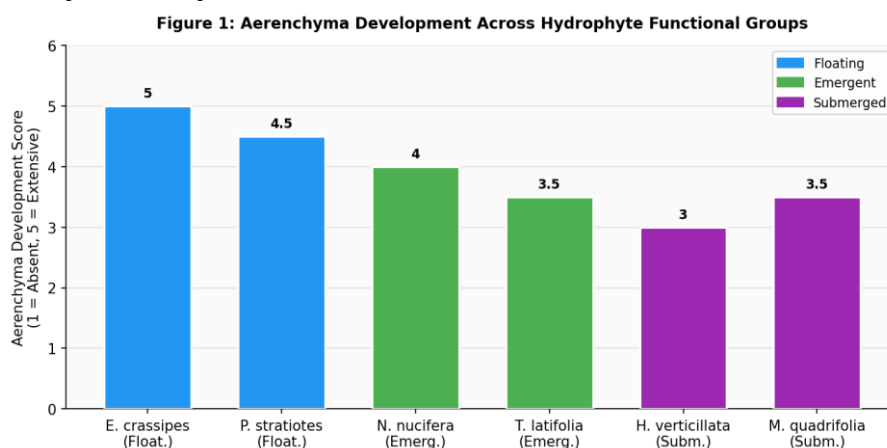


Figure 10: Aerenchyma development scores (1 = absent, 5 = extensive) across the six study species. Floating plants show the highest aerenchyma relative to cortical cross-section.

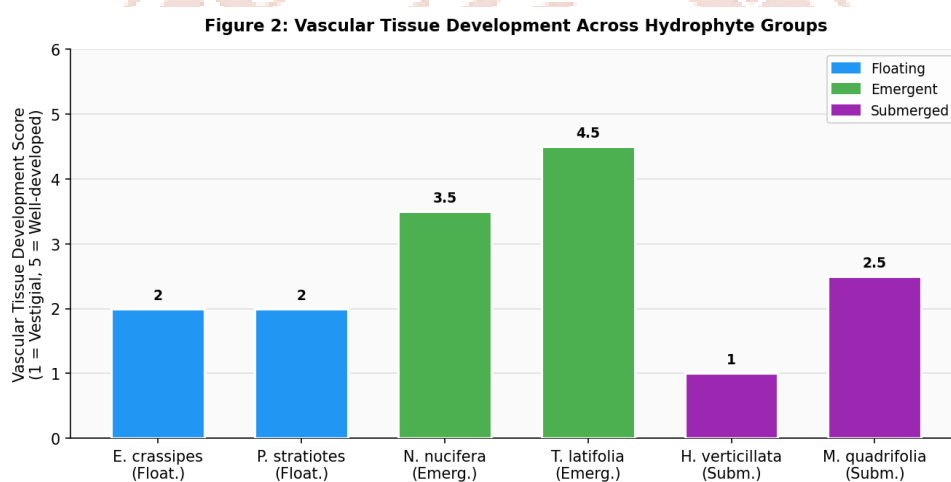


Figure 11: Vascular tissue development scores. Emergent plants, particularly *Typha latifolia*, show the most developed vascular system. *Hydrilla verticillata* shows near-vestigial xylem.

4.1 Aerenchyma: The Universal Hydrophytic Adaptation

Aerenchyma development was observed in all six species and all three organ types (root, stem, leaf), confirming its designation as the cardinal anatomical adaptation of hydrophytes. Floating plants exhibited the most expansive aerenchyma, *E. crassipes* stem sections showing air volume exceeding 60% of cortical area by estimation. Emergent plants showed moderate aerenchyma concentrated in rhizomes and petioles, forming a continuous aeration channel from soil-embedded roots to aerial leaves. Submerged plants showed aerenchyma primarily in roots and rhizomes for oxygen supply to tissues in anaerobic sediment. Geometric patterns also differed: concentric lacunae in floating species, large oval chambers in *N. nucifera*, spoke-like radiating patterns in *T. latifolia*, and smaller intercellular spaces in submerged species.

4.2 Vascular and Mechanical Tissue Gradient

A gradient of vascular tissue complexity was evident across the three groups. Emergent plants, particularly *T. latifolia*, showed the most developed vascular system, consistent with the demand for efficient water and nutrient transport to tall aerial shoots. Floating plants exhibited significantly reduced xylem, reflecting the abundance of water surrounding tissues. Submerged plants showed the greatest xylem reduction, approaching vestigiality in *H. verticillata*. Collenchyma and sclerenchyma were well represented only in *T. latifolia*; floating and submerged species showed negligible mechanical tissue, consistent with the physical support provided by water buoyancy.

4.3 Epidermal Modifications

Epidermal organization proved highly diagnostic of the functional group. Floating and emergent species with aerial leaf surfaces uniformly showed stomata concentrated on the upper epidermis and possessed varying degrees of cuticle development. In contrast, submerged species showed complete absence of stomata and cuticle, with gas exchange occurring entirely by diffusion through the permeable epidermis. This transition from epistomatic, cuticularized epidermis to permeable, unspecialized epidermis represents the clearest anatomical signature of submergence adaptation.

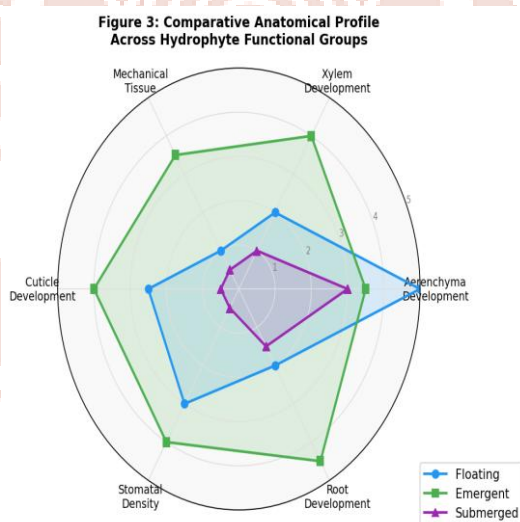


Figure 12: Radar diagram comparing anatomical profiles across the three hydrophyte functional groups. Emergent plants (green) show highest structural investment; submerged plants (purple) exhibit the most extreme anatomical reductions.

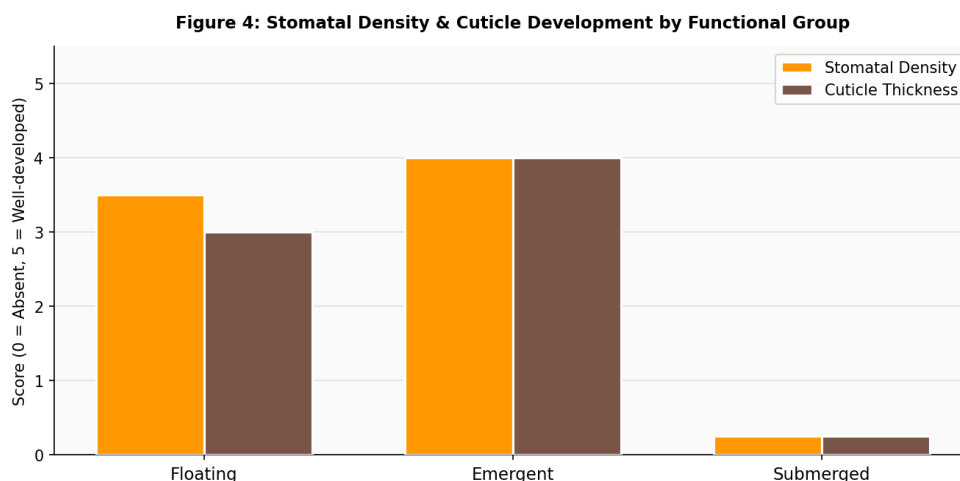


Figure 4.4: Stomatal density and cuticle development scores by functional group (0 = absent, 5 = well-developed). Both features are essentially absent in submerged plants, confirming complete reliance on diffusive gas exchange.

5. DISCUSSION

The findings of this study corroborate and extend the comparative anatomical framework established by classical and contemporary research in aquatic plant biology. The three hydrophyte functional groups represent a spectrum of structural adaptation to increasing degrees of aquatic immersion, each exhibiting a coherent suite of anatomical features optimized for its ecological niche.

The primacy of aerenchyma across all groups confirms Sculthorpe's (1967) designation of this tissue as the defining adaptation of hydrophytes. Armstrong's (2020) mechanistic emphasis on internal aeration as the key physiological challenge in waterlogged environments finds clear anatomical correlates in the present data, the continuous aerenchyma systems connecting aerial and submerged organs in both floating and emergent species constitute literal air pipelines maintaining aerobic respiration in anaerobic sediment. These findings align with Colmer's (2009) documentation of analogous aeration mechanisms in *Oryza sativa*, supporting the view that aerenchyma represents a broadly conserved solution to submerged root respiration.

The phenomenon of convergent vascular and mechanical tissue reduction across phylogenetically distant taxa, Nelumbonaceae, Typhaceae, Hydrocharitaceae, and Marsileaceae, supports Barrett's (2013) argument for convergent evolution driven by shared environmental constraints rather than shared ancestry. The gradient from *T. latifolia* (most terrestrial-like) through *N. nucifera* and floating species to *H. verticillata* (most aquatically specialized) mirrors predictions from ecological theory regarding costs and benefits of structural investment across water regimes. Crawford (2008) similarly argued that mechanical

tissue is metabolically costly and superfluous when water provides physical support, a principle clearly borne out by the near-complete absence of sclerenchyma in *H. verticillata* and the floating species.

Epidermal modifications demonstrate equally clear ecological differentiation. The complete loss of stomata and cuticle in fully submerged *H. verticillata* confirms Sand-Jensen's (2015) and Maberly's (2014) documentation of diffusion-based gas exchange in submerged macrophytes. The superhydrophobic epicuticular wax of *N. nucifera* leaves, the 'lotus effect', represents an advanced epidermal specialisation that prevents waterlogging of stomata, as documented by Wang et al. (2021). The epistomatic condition of floating-plant leaves reflects their stable interface with the atmosphere, consistent with findings by González-Méndez et al. (2021) on buoyancy and gas exchange adaptations in *E. crassipes* and *P. stratiotes*.

The presence of calcium oxalate raphides in *N. nucifera* tissues, absent in all other study species, illustrates that anatomical diversification operates at multiple scales simultaneously. Alharthi et al. (2024) and Singh (2022) similarly noted the multi-functional significance of these crystals in *Nelumbo*, encompassing anti-herbivory defense, calcium storage, and potential roles in cellular pH regulation.

Marsilea quadrifolia's heterophylly, producing anatomically distinct aerial and submerged leaflets, represents an extreme case of phenotypic plasticity within a single individual, as documented by Koga et al. (2024) and Mommer et al. (2005). This developmental flexibility allows the plant to exploit both aquatic and semi-terrestrial niches simultaneously.

In the context of contemporary environmental change, the adaptive plasticity documented by Yuan et al. (2024) and Sharma (2025), increased aerenchyma formation under stress, modified cortex thickness under pollution, suggests that the anatomical features documented here are not static endpoints but dynamic responses within a broader plasticity envelope. This has implications for predicting hydrophyte community responses to climate change, eutrophication, and pollution in the freshwater ecosystems of Gujarat and beyond. Cheng et al. (2025) further demonstrated that aerenchyma formation pathways in aquatic macrophytes are environmentally modulated, suggesting considerable scope for anatomical adjustment within individual plants.

6. CONCLUSION

This comparative anatomical study of six aquatic plant species across three functional groups demonstrates clear and systematic relationships between internal tissue organization and ecological positioning along the aquatic–terrestrial gradient.

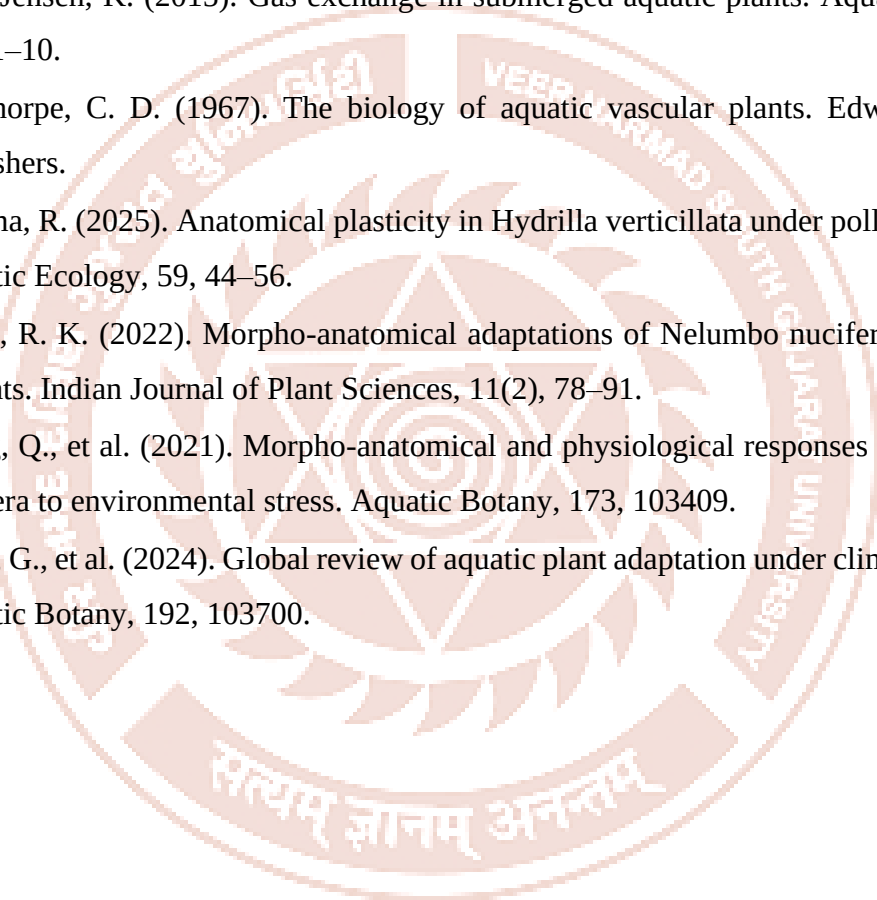
Aerenchyma development emerged as the universal, cross-group adaptive feature, varying in extent, geometry, and distribution in accordance with the oxygen demands and buoyancy requirements of each ecological position. Vascular and mechanical tissue investment decreased progressively from emergent to floating to submerged species, reflecting reduced functional requirements in increasingly water-supported environments. Epidermal modifications, stomatal position, cuticle development, and surface permeability provided the most diagnostically clear indicators of functional group.

Species-specific features, raphide idioblasts in *Nelumbo*, amphistomatic leaves in *Typha*, and heterophyllous plasticity in *Marsilea*, illustrate that shared functional challenges are solved through both convergent and individually distinct anatomical strategies. These findings establish an anatomically coherent framework for understanding hydrophyte adaptation. Future work incorporating quantitative image analysis onto genetic sectioning and molecular investigation of aerenchyma formation pathways would further enrich this comparative framework.

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Culturing Daphnia Species and Evaluation of toxicity of Acetamiprid

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Abstract:

This study investigated the acute and chronic toxicity of Acetamiprid on *Daphnia* spp. under controlled laboratory conditions. Results showed a clear concentration- and time-dependent increase in mortality, with a 24-hour LC_{50} of 0.52 mg/L and a more sensitive 96-hour LC_{50} of 0.28 mg/L, while both NOEC and LOEC were observed at 0.2 mg/L. Higher concentrations caused severe behavioral and morphological changes, including reduced swimming, antennal damage, immobilization, and increased mortality. Overall, the findings demonstrate that Acetamiprid poses significant toxic risks to freshwater invertebrates and may threaten aquatic ecosystems.

Introduction:

Daphnia spp. are freshwater cladoceran zooplankton belonging to the family Daphniidae and are commonly known as “water fleas” due to their characteristic swimming movement. They are important components of freshwater ecosystems, acting as primary consumers and linking phytoplankton with higher trophic organisms. Due to their short life cycle, parthenogenetic reproduction, transparent body, easy laboratory maintenance, and sensitivity to environmental changes, *Daphnia* spp. are widely used as standard model organisms in ecotoxicological research and water quality assessment.

Increasing pesticide contamination in aquatic environments has become a major concern due to agricultural runoff, leaching, and other pathways of chemical entry into water bodies. Pesticides can negatively affect non-target aquatic organisms, especially zooplankton species that play vital roles in maintaining ecosystem balance. *Daphnia* spp. are highly sensitive to pollutants, and changes in their survival, mobility, growth, and reproduction serve as important indicators of environmental toxicity.

Acetamiprid is a neonicotinoid insecticide widely used in agriculture for controlling sucking pests such as aphids, whiteflies, and thrips. Due to its high water solubility, it can enter freshwater ecosystems and cause harmful effects on non-target organisms including *Daphnia* spp. Exposure to acetamiprid may lead to acute and sub-lethal toxic effects such as immobilization, mortality, oxidative stress, and reproductive changes. Therefore, studying the toxicity of acetamiprid using *Daphnia* spp. is essential for evaluating ecological risks and understanding the impact of pesticides on freshwater biodiversity.

Materials and Methods

The study was conducted using *Daphnia* spp. collected from a freshwater pond and cultured under controlled laboratory conditions. Healthy individuals were identified using morphological characteristics and maintained in filtered pond water mixed with ASTM

reconstituted hard water. The culture conditions were maintained at suitable temperature (20–25°C), pH (7.0–8.0), dissolved oxygen, and a 16:8 hour light-dark cycle. *Daphnia* were provided with algal food (*Spirulina*) for proper growth and reproduction. Water quality parameters including temperature, pH, TDS, TSS, salinity, dissolved oxygen, BOD, hardness, alkalinity, and acidity were analysed before toxicity experiments.

Acetamiprid 20% SP (FLYKING), a neonicotinoid insecticide, was used as the test chemical for toxicity evaluation. Acute toxicity tests were performed following OECD Guideline 202 using less than 24-hour-old neonates of *Daphnia* spp. exposed to different concentrations of acetamiprid for 48 hours, and immobilization and mortality were recorded to determine EC₅₀ values. Chronic toxicity tests were conducted according to OECD Guideline 211 to assess long-term effects on survival and reproduction. Parameters such as mortality, growth, brood production, NOEC, and LOEC values were evaluated using appropriate statistical analysis.

Results and Discussion

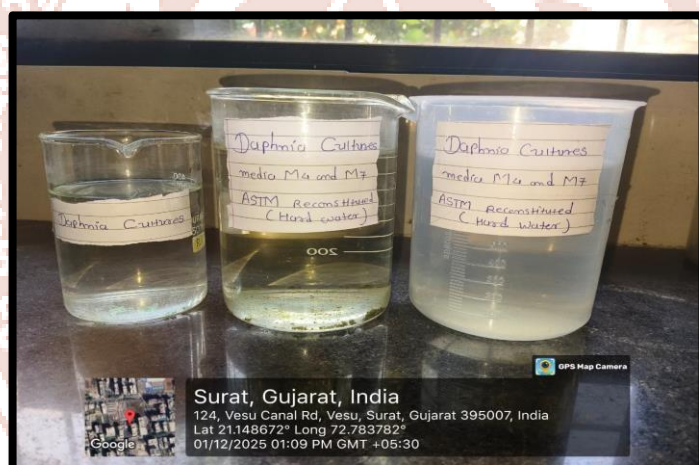


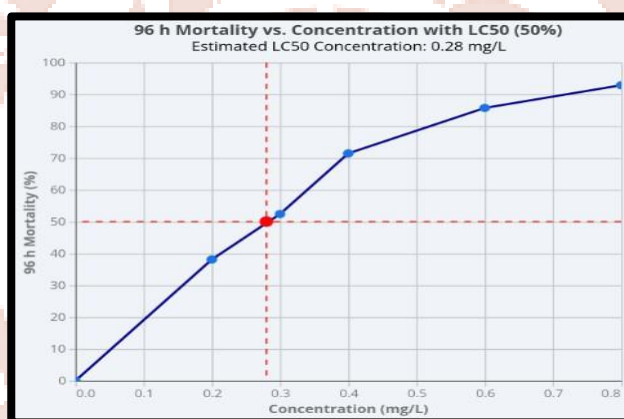
Fig:1 *Daphnia* spp. Culture media M4 and M7 ASTM

The laboratory culture of *Daphnia* spp. was successfully established under controlled conditions. The organisms showed normal morphology, active swimming behaviour, and continuous reproduction. Suitable water quality parameters (temperature 24.35°C, pH 7.4, and DO 4.0 mg/L) supported healthy growth and ensured reliable toxicity assessment.

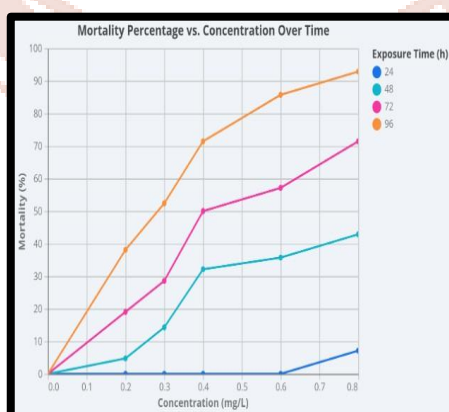


Graph: 2 Acute (24 h) LC50:0.52 mg/L

Acute toxicity evaluation of Acetamiprid showed a clear concentration-dependent increase in mortality of *Daphnia* spp. Mortality increased from 10.71% at 0.2 mg/L to 90.48% at 0.8 mg/L, with an estimated LC₅₀ value of 0.52 mg/L after 24 hours.



Graph:3 Chronic (96 h) LC50 :0.28 mg/L



Graph:5 Time-Dependent Mortality

Chronic exposure resulted in higher toxic effects over time. After 96 hours, mortality reached 92.85% at 0.8 mg/L concentration, and the LC_{50} value decreased to 0.28 mg/L, indicating cumulative toxicity of Acetamiprid. The NOEC and LOEC values were recorded at 0.2 mg/L.

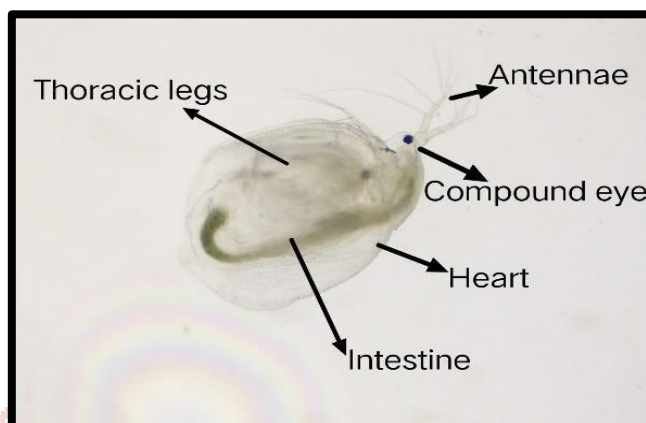


Fig:6 Normal healthy *Daphnia* spp. Under the microscope 10 X.

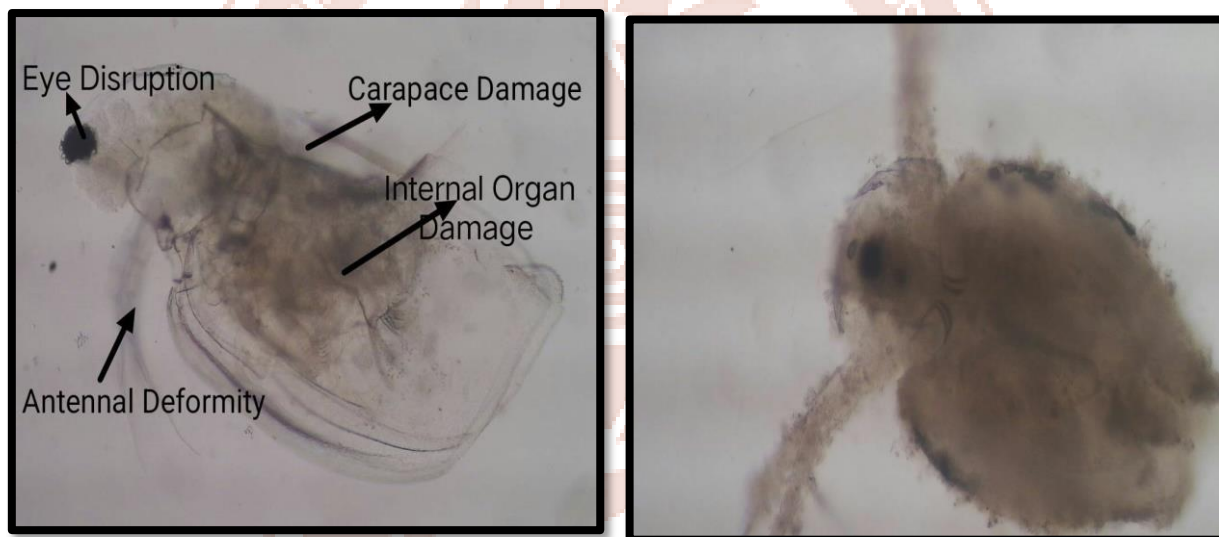


Fig:9 *Daphnia* spp. Under microscope at 0.8 mg/L

Microscopic observations revealed concentration-dependent behavioural and morphological changes. Low concentrations caused reduced swimming activity, while higher concentrations resulted in immobilization, loss of balance, inactive antennae, and mortality. These findings confirm the high sensitivity of *Daphnia* spp. as a bioindicator for pesticide toxicity assessment.

Conclusion

This study evaluated the toxic effects of the neonicotinoid pesticide Acetamiprid on *Daphnia* spp., a widely used bioindicator in aquatic ecotoxicology. The successful laboratory culture under controlled conditions confirmed that *Daphnia* is a suitable model organism for assessing pesticide toxicity. The results showed a clear concentration- and time-dependent

increase in mortality, with acute LC₅₀ of 0.52 mg/L and chronic LC₅₀ of 0.28 mg/L, indicating increased toxicity during prolonged exposure.

Acetamiprid exposure also caused significant behavioural and physiological changes, including reduced swimming activity, loss of balance, antennal inactivity, immobilization, and mortality at higher concentrations. The findings demonstrate that even low concentrations of Acetamiprid can negatively affect non-target aquatic organisms. Therefore, proper pesticide management and continuous monitoring are essential to protect freshwater biodiversity and ecosystem stability.

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Diversity and Community Structure of Odonata (Insecta) in Urban Biodiversity Park of Surat, Gujarat, India

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Abstract:

Urban biodiversity parks represent critical refugia for aquatic insect communities in rapidly expanding metropolitan landscapes. This study documents the diversity, abundance, and community structure of Odonata (dragonflies and damselflies) across selected urban biodiversity parks in Surat, Gujarat, India, during a comprehensive survey conducted from July 2025 to January 2026. A total of 31 species belonging to 24 genera under 8 families and 2 suborders were recorded through standardized visual encounter surveys, transect walks, and opportunistic collections. The assemblage comprised 23 species of Anisoptera (dragonflies) and 8 species of Zygoptera (damselflies). Family Libellulidae was the most speciose, contributing 18 species (58.1%), followed by Coenagrionidae with 5 species (16.1%). The Shannon-Wiener diversity index ($H' = 2.89$) and Simpson's diversity index ($D = 0.91$) indicated moderate to high species diversity, while Pielou's evenness index ($J' = 0.84$) suggested a relatively equitable species distribution. The Wandering Glider (*Pantala flavescens*) was the most abundant species, representing 22.4% of total encounters, followed by the Ditch Jewel (*Brachythemis contaminata*) at 14.6% and the Blue Ground Skimmer (*Diplacodes trivialis*) at 11.3%. The presence of both pollution-sensitive taxa (*Neurothemis fulvia*, *Ceragrion coromandelianum*) and pollution-tolerant generalists (*Brachythemis contaminata*, *Orthetrum sabina*) indicates a mosaic of habitat quality within the urban park matrix. These findings establish a critical baseline for long-term biomonitoring of Surat's urban freshwater ecosystems and underscore the conservation value of strategically designed park in sustaining Odonata diversity amid rapid urbanization. The study recommends integrated management strategies including habitat heterogeneity enhancement, aquatic vegetation restoration, and minimization of chemical inputs to preserve these ecologically significant urban wetland habitats.

1. Introduction:

1.1 Background and Rationale

The order Odonata, comprising dragonflies (suborder Anisoptera) and damselflies (suborder Zygoptera), represents one of the most ancient lineages of winged insects, with a fossil record extending over 300 million years (Grimaldi & Engel, 2005). Globally, the order encompasses approximately 6,000 described species distributed across 18 families, with India harbouring a remarkably rich fauna of 488 species and 27 subspecies in 154 genera and 18 families (Subramanian & Babu, 2017). The Indian Odonata fauna exhibits pronounced biogeographic heterogeneity, with the highest diversity and endemism concentrated in the Western Ghats, Eastern Himalaya, and the northeastern hill ranges (Subramanian, 2009; Babu et al., 2013).

Odonates occupy a pivotal position in freshwater food webs, functioning as both predators and prey across multiple trophic levels (Corbet, 1999). Their nymphal stages are strictly aquatic, rendering them exceptionally sensitive to water quality parameters, habitat structure, and riparian vegetation integrity (Chovanec & Waringer, 2001; Samways, 2008). This ecological sensitivity has established Odonata as premier bioindicators for freshwater ecosystem health, habitat quality assessment, and climate change monitoring (Clark & Samways, 1996; Bried & Ervin, 2016). The International Union for Conservation of Nature (IUCN) has recognized the conservation significance of Odonata, with several species listed under threatened categories globally (Boudot & Kalkman, 2015).

1.2 Urbanization and Odonata Conservation

Urbanization represents one of the most pervasive drivers of biodiversity loss globally, with freshwater ecosystems experiencing disproportionate impacts due to habitat fragmentation, pollution, and hydrological alteration (McKinney, 2002; Grimm et al., 2008). However, emerging research demonstrates that well-designed urban green spaces, including biodiversity parks, botanical gardens, and constructed wetlands, can sustain significant Odonata diversity and serve as functional stepping-stones within urban ecological networks (Jagannohan et al., 2013; Goertzen & Suhling, 2013). The capacity of urban parks to support Odonata communities depends critically on habitat attributes such as water body size, aquatic vegetation diversity, shoreline complexity, and the degree of anthropogenic disturbance (Goertzen & Suhling, 2013; Bazziet al., 2023).

In the Indian context, systematic documentation of urban Odonata faunas remains limited, with most studies concentrating on protected areas, Ramsar sites, and agroecosystems (Rathod & Parasharya, 2018; Rohmare et al., 2016). The urban biodiversity parks of Surat, Gujarat, situated within one of India's fastest-growing metropolitan regions, present a unique opportunity to investigate how Odonata communities persist and assemble in intensively managed urban freshwater habitats.

1.3 Study Area: Surat Biodiversity Park

Surat (21°10'N, 72°50'E), the second-largest city in Gujarat and eighth-largest in India, is situated on the banks of the Tapi River approximately 20 km from the Arabian Sea coast. The park features a mosaic of lentic water bodies, emergent macrophyte beds, riparian woodland strips, and managed grasslands, providing diverse microhabitats for aquatic and semi-aquatic biota. The present study focuses on documenting the Odonata assemblages within these urban biodiversity parks, with the following specific objectives: (i) to prepare a comprehensive species inventory with taxonomic documentation; (ii) to analyse community structure,

diversity indices, and species abundance patterns; (iii) to assess habitat associations and conservation implications; and (iv) to establish a baseline dataset for future biomonitoring programs.

2. Materials and Methods

2.1 Study Sites and Sampling Design

Field surveys were conducted across urban biodiversity park in Surat. Each site was visited twice per month during the monsoon and post-monsoon periods and once monthly during the dry season. Odonata were sampled using a combination of standardized visual encounter surveys (VES), fixed-width transect walks, and opportunistic net collections. Visual encounter surveys involved systematic scanning of all accessible microhabitats—including open water, emergent vegetation zones, riparian edges, and adjacent terrestrial perches. Transect walks (500 m linear transects along water body perimeters) were conducted during peak activity hours (0900–1200 h and 1500–1700 h) to maximize detection probability.

2.2 Identification and Taxonomy

Specimens were identified to species level using established taxonomic keys: Fraser (1933, 1934, 1936) for comprehensive Indian Odonata, Subramanian (2005) for field identification, and Mitra (2002, 2006) for regional references. Nomenclature follows the Checklist of Odonata of India, Version 3.0 (Subramanian & Babu, 2017), with conservation status assessed against the IUCN Red List of Threatened Species (IUCN, 2024). Voucher specimens are deposited in the Zoological Reference Collection, Department of Biosciences, Veer Narmad South Gujarat University, Surat.

2.3 Data Analysis

Species diversity was quantified using the Shannon-Wiener diversity index ($H' = -\sum p_i \ln p_i$), Simpson's diversity index ($D = 1 / \sum p_i^2$), and Pielou's evenness index ($J' = H' / \ln S$), where p_i represents the proportional abundance of species i and S is the total number of species (Magurran, 2004). Species dominance was assessed using the relative abundance classification of Engelmann (1978): eudominant (>10%), dominant (5–10%), subdominant (2–5%), recedent (1–2%), and subrecedent (<1%). Community similarity between sites was evaluated using the Jaccard similarity index.

3. Results

3.1 Species Composition and Taxonomic Overview

A total of 31 species of Odonata were recorded across the four urban biodiversity parks of Surat, representing 24 genera, 8 families, and 2 suborders (Table 1). The assemblage was dominated by Anisoptera, with 23 species (74.2%), while Zygoptera contributed 8 species

(25.8%). This ratio of approximately 3:1 (Anisoptera:Zygoptera) is consistent with patterns documented in other tropical urban wetland systems in India (Rathod et al., 2016; Dolai et al., 2025).

At the family level, Libellulidae was overwhelmingly dominant, accounting for 18 species (58.1% of total richness) distributed across 13 genera. Coenagrionidae was the second most speciose family with 5 species (16.1%), followed by Aeshnidae (2 species, 6.5%), Gomphidae (2 species, 6.5%), Platycnemididae (1 species, 3.2%), Lestidae (1 species, 3.2%), Macromiidae (1 species, 3.2%), and Protoneuridae (1 species, 3.2%). The preponderance of Libellulidae aligns with global patterns, as this family constitutes the largest and most ecologically versatile odonate family worldwide, with species adapted to a broad spectrum of lentic habitats including disturbed and anthropogenically modified water bodies (Kalkman et al., 2008; Letsch et al., 2016).

3.2 Abundance and Dominance Structure

A total of 1,847 individual Odonata was recorded during the study period. The Wandering Glider (*Pantala flavescens*) was the most abundant species, contributing 414 individuals (22.4% of total abundance) and classified as eudominant. This species is renowned for its exceptional dispersal capacity, with transoceanic migration documented across the Indian Ocean, and its dominance in urban wetlands reflects its ecological plasticity and ability to exploit temporary water bodies (Anderson, 2009). The Ditch Jewel (*Brachythemis contaminata*) ranked second in abundance with 270 individuals (14.6%), also eudominant, consistent with its documented preference for eutrophic, vegetation-rich standing waters (Ferrerias-Romero et al., 2009). The Blue Ground Skimmer (*Diplacodes trivialis*) contributed 209 individuals (11.3%), classified as dominant.

Other notable contributors included the Ruddy Marsh Skimmer (*Crocothemis servilia*, 8.7%), the Common Picture Wing (*Rhyothemis variegata*, 7.2%), the Slender Skimmer (*Orthetrum sabina*, 6.8%), and the Crimson Marsh Glider (*Trithemis aurora*, 5.4%). The remaining 24 species collectively accounted for 23.6% of total abundance, with 11 species classified as subrecedent (<1% relative abundance), including the Granite Ghost (*Bradinopyga geminata*), the Long-legged Marsh Glider (*Trithemis pallidinervis*), and the Yellow-tailed Ashy Skimmer (*Potamarcha congener*).

3.3 Diversity Indices

Community diversity metrics revealed moderate to high species diversity across the surveyed parks. The pooled Shannon-Wiener diversity index was $H' = 2.89$ (maximum possible $H'_{\max} = 3.43$), while Simpson's diversity index was $D = 0.91$, indicating low probability of

interspecific encounter dominance. Pielou's evenness index ($J' = 0.84$) suggested a relatively equitable distribution of individuals among species, though the presence of three eudominant/dominant species indicates some degree of community structuring around generalist taxa.

3.4 Habitat Associations and Microhabitat Use

Species-habitat associations revealed distinct assemblage structuring along environmental gradients. Open-water specialists such as *Pantala flavescens*, *Tramea basilaris*, and *Tramea limbata* were predominantly encountered over large, unshaded water bodies at Sarthana Nature Park. Vegetation-associated species including *Neurothemis fulvia*, *Neurothemis tullia*, and *Rhyothemis variegata* were concentrated in zones with dense emergent and floating macrophytes. Shoreline-perching species such as *Diplacodes trivialis*, *Brachythemis contaminata*, and *Orthetrum sabina* exhibited broad habitat tolerance, occurring across all sites regardless of vegetation density or water quality indicators.

Zygoptera species demonstrated more restrictive habitat requirements. The Coromandel Marsh Dart (*Ceriagrion coromandelianum*) and Senegal Golden Dartlet (*Ischnura senegalensis*) were restricted to vegetated pond margins with shaded perches, while the Three-lined Dart (*Pseudagrion decorum*) preferred slow-flowing, macrophyte-rich channels. The absence of specialized lotic species (e.g., Gomphidae riverine specialists) reflects the lentic-dominated habitat matrix of the urban parks.

4. Discussion

4.1 Comparative Regional Context

The 31 species documented in Surat's urban biodiversity parks represent a substantial proportion of the Odonata fauna recorded from southern Gujarat. Rathod et al. (2016) reported 29 species from Surat district during their broader southern Gujarat survey, while the consolidated inventory for the entire southern Gujarat region encompasses 55 species across seven districts (Rathod et al., 2016). The present study's urban park-focused approach yielded comparable richness to the district-level survey, underscoring the significant contribution of urban green spaces to regional Odonata conservation.

Comparisons with other urban Odonata studies in India reveal broadly congruent patterns. A study of parks and gardens in Pune documented 33 species across 30 sites, with Libellulidae dominance (61% of species) and similar abundance hierarchies featuring *Pantala flavescens* and *Diplacodes trivialis* as dominant taxa (Kulkarni & Subramanian, 2013). The Tripura University campus survey recorded 33 species with comparable diversity metrics (Shannon $H' = 2.335$) and identified *Rhyothemis variegata* as the eudominant species (Dolai et al., 2025).

These parallels suggest that tropical urban wetland Odonata communities across India are structured by similar ecological filters, with generalist Libellulidae taxa dominating in disturbed, resource-rich habitats.

4.2 Bioindicator Implications

The co-occurrence of pollution-sensitive and pollution-tolerant species within Surat's urban parks carries significant bioindicator implications. The presence of *Neurothemis fulvia* and *Ceriagrion coromandelianum*—species associated with relatively pristine, well-vegetated lentic habitats—alongside *Brachythemis contaminata* and *Orthetrum sabina*, recognized indicators of eutrophic and degraded aquatic systems (Jacob & Manju, 2016), suggests a mosaic of habitat quality within the park matrix. This heterogeneity likely arises from differential management practices, localized nutrient inputs, and variable shoreline vegetation integrity across sites.

The absence of several species documented from the broader southern Gujarat region—notably specialized forest stream dwellers such as *Dysphaea ethela*, *Elatoneura nigerrima*, and *Microgomphus torquatus* (Rathod et al., 2016)—highlights the habitat filtering effects of urbanization. These species require shaded, oligotrophic, fast-flowing waters with intact riparian canopies, conditions largely absent from the urban park lentic habitats. This filtering underscores the limitation of urban biodiversity parks as conservation tools: while they effectively sustain generalist and disturbance-tolerant species, they may inadequately support habitat specialists and endemic taxa.

4.3 Conservation Significance and Management Recommendations

The findings establish that strategically designed urban biodiversity parks can sustain significant Odonata diversity even within densely populated metropolitan contexts. The 31 species recorded, including regionally important taxa such as *Anax immaculifrons* and *Macromia cingulata*, demonstrate the conservation value of these urban refugia. However, several management interventions are recommended to enhance habitat quality and species persistence:

Habitat Heterogeneity Enhancement: Maintaining a diversity of water body types—including open water, vegetated margins, shallow littoral zones, and partially shaded reaches—supports a broader spectrum of Odonata guilds. The incorporation of woody debris, variable shoreline slopes, and native macrophyte plantings can increase microhabitat complexity.

Aquatic Vegetation Management: Emergent and floating macrophytes provide critical perching, foraging, and oviposition substrates. Management should avoid excessive removal

of aquatic vegetation, which has been shown to precipitate Odonata declines in urban ponds (Samways & Steytler, 1996).

Water Quality Maintenance: Minimizing chemical inputs (fertilizers, pesticides, herbicides) and controlling nutrient loading from stormwater runoff is essential to prevent eutrophication-driven community shifts toward tolerant generalists.

Riparian Buffer Zones: Establishing undisturbed riparian vegetation strips provides shading, wind protection, and terrestrial perching substrates, particularly benefiting Zygoptera species and shade-tolerant Anisoptera.

Long-term Monitoring: The establishment of permanent monitoring transects and citizen science engagement programs would facilitate detection of population trends, range shifts, and early warning indicators of ecosystem degradation.

4.4 Limitations and Future Directions

This study is subject to several limitations. The one-year survey period may not fully capture interannual population variability or detect rare vagrant species. Nocturnal and crepuscular species (e.g., *Zyxomma* spp.) are likely underrepresented due to diurnal sampling constraints. Additionally, the absence of larval sampling precludes assessment of reproductive success and population recruitment. Future research should incorporate multi-year monitoring, larval community analysis, and integration of physicochemical water quality parameters to elucidate mechanistic drivers of community structure.

5. Conclusion

This study provides the first comprehensive assessment of Odonata diversity in the urban biodiversity parks of Surat, Gujarat, documenting 31 species across 24 genera and 8 families. The dominance of Libellulidae, the prevalence of generalist species such as *Pantala flavescens* and *Brachythemis contaminata*, and the moderate to high diversity indices collectively demonstrate that well-managed urban parks can function as significant repositories of Odonata biodiversity within metropolitan landscapes. The coexistence of sensitive and tolerant taxa indicates habitat heterogeneity that, if maintained through evidence-based management, can sustain diverse odonate communities. These findings contribute to the growing body of evidence supporting the integration of biodiversity conservation objectives into urban planning frameworks and provide a critical baseline for long-term biomonitoring of freshwater ecosystem health in one of India's most rapidly urbanizing regions.

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Ecotoxicological Assessment of Textile Industry Effluent Using Artemia salina: Acute, Chronic and Developmental Toxicity Evaluation

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Abstract:

Textile industries generate large volumes of wastewater containing dyes, salts, suspended solids, and chemically resistant organic compounds that pose serious threats to aquatic ecosystems. The present investigation evaluated the toxicological impact of textile industry effluent on the brine shrimp *Artemia salina*, an established model organism for ecotoxicological studies. Physicochemical characterization of the effluent revealed high pollution load, with elevated pH (9.49), alkalinity (316.71 mg/L), total hardness (383 mg/L), total dissolved solids (1462.86 mg/L), total suspended solids (339.29 mg/L), biological oxygen demand (220.71 mg/L), and chemical oxygen demand (691.43 mg/L). Acute toxicity analysis demonstrated relatively low mortality, reaching only 12.86% in undiluted effluent. However, prolonged exposure produced severe chronic toxicity, resulting in 97.1% mortality after 96 hours. The calculated 96-hour LC_{50} value was 16.89%, indicating substantial long-term toxicity. Hatching success decreased progressively with increasing effluent concentration, demonstrating developmental inhibition and delayed embryonic emergence. The findings indicate that textile effluent poses significant ecological risks and emphasize the necessity of effective wastewater treatment prior to environmental discharge.

Introduction:

Industrialization has significantly contributed to economic growth, but it has also increased the release of pollutants into aquatic environments. Among various industrial sectors, textile industries are recognized as major contributors to water pollution due to the generation of large quantities of wastewater containing dyes, surfactants, salts, suspended solids, and non-biodegradable organic compounds. The discharge of untreated textile effluent into natural water bodies adversely affects aquatic ecosystems by altering physicochemical characteristics and inducing toxicity in aquatic organisms.

Conventional physicochemical analyses provide information regarding pollutant concentrations; however, they do not adequately reflect biological impacts. Consequently, biological toxicity assays have become important tools for environmental monitoring. *Artemia salina* is widely used as a model organism because of its ease of culture, rapid life cycle, high sensitivity to pollutants, and suitability for toxicity evaluation.

The present study was undertaken to evaluate the toxic effects of textile industry effluent on *Artemia salina* through physicochemical characterization, acute toxicity assessment, chronic toxicity evaluation, determination of LC_{50} values, and hatching success

analysis. The objective was to determine the ecological risks associated with textile wastewater and assess its potential impact on aquatic organisms.

Materials and Methods

The present study was conducted to evaluate the toxicological effects of textile industry effluent on *Artemia salina* under controlled laboratory conditions. The investigation included physicochemical characterization of the effluent, acute toxicity assessment, chronic toxicity evaluation, determination of LC₅₀ values, and hatching success analysis.

Collection of Textile Effluent

Textile effluent samples were collected from textile industrial discharge sources and transported to the laboratory in sterile polyethylene containers. Samples were stored at 4°C and analyzed within the recommended holding period to minimize physicochemical alterations.

Physicochemical Analysis

The collected effluent was analyzed for various physicochemical parameters including color, odor, temperature, pH, acidity, alkalinity, total hardness, electrical conductivity (EC), total dissolved solids (TDS), total suspended solids (TSS), biological oxygen demand (BOD), and chemical oxygen demand (COD) following standard APHA methods.

Test Organism

Brine shrimp (*Artemia salina*) cysts were procured commercially and used as the test organism. The cysts were stored under refrigerated conditions until use. *Artemia salina* was selected because of its sensitivity to environmental pollutants, ease of culture, rapid development, and widespread application in ecotoxicological studies.

Preparation of Artificial Seawater

Artificial seawater was prepared by dissolving 25 g of rock salt and 1.5 g sodium bicarbonate in one liter of distilled water. The prepared medium maintained suitable salinity and pH conditions for hatching and culturing *Artemia nauplii*.

Acute Toxicity Test

Different concentrations of textile effluent (10%, 20%, 40%, 60%, and 100%) were prepared using artificial seawater. Ten *Artemia* cysts were introduced into each concentration and maintained under laboratory conditions. Mortality was recorded after 24 hours of exposure. All experiments were conducted in triplicate along with an untreated control group.

Chronic Toxicity Test

For chronic toxicity assessment, nauplii were continuously exposed to various concentrations of textile effluent for 96 hours. Mortality observations were recorded at 24, 48,

72, and 96-hour intervals. The cumulative mortality data were used to evaluate long-term toxic effects and calculate LC₅₀ values.

Hatching Success Assay

Approximately twenty *Artemia* cysts were exposed to different effluent concentrations (0%, 10%, 20%, 40%, 60%, and 100%). Hatching percentages were recorded at regular intervals up to 96 hours. The assay was conducted in triplicate to evaluate developmental toxicity and hatching inhibition.

Morphological Assessment

For morphological evaluation, newly hatched nauplii were exposed to LC₅₀ concentrations of textile effluent. The organisms were examined under a compound microscope (10× magnification) for abnormalities including eye formation, digestive tract asymmetry, abdominal deformities, color changes, and mouthpart abnormalities. Digital images were recorded for documentation and analysis.

Statistical Analysis

Mortality data were converted into percentage mortality and subjected to Probit analysis for determination of LC₅₀ values. Chronic toxicity data were analyzed based on concentration-response relationships and time-dependent mortality patterns.

Results and Discussion

Physicochemical Characteristics of Textile Effluent

The physicochemical analysis revealed that the textile effluent possessed characteristics indicative of substantial pollution load. The effluent exhibited an orange color and pungent odor, suggesting the presence of high concentrations of dyes and volatile contaminants.

The pH of the effluent was recorded as 9.49, indicating highly alkaline conditions that exceed permissible discharge limits. Elevated alkalinity (316.71 mg/L) and total hardness (383 mg/L) further confirmed the chemically aggressive nature of the wastewater. Electrical conductivity was measured at 1359.57 µS/cm, indicating a high ionic load.

Total dissolved solids and total suspended solids were recorded as 1462.86 mg/L and 339.29 mg/L, respectively. High TDS values contribute to osmotic stress in aquatic organisms, whereas elevated TSS levels increase turbidity and may impair respiration.

Furthermore, BOD and COD values were found to be 220.71 mg/L and 691.43 mg/L, respectively. These values greatly exceeded recommended discharge standards and indicated the presence of substantial organic and chemical pollution. The COD/BOD ratio of

approximately 3.13 suggested the occurrence of non-biodegradable and chemically resistant compounds commonly associated with textile wastewater.

Acute Toxicity Effects

Acute Toxicity

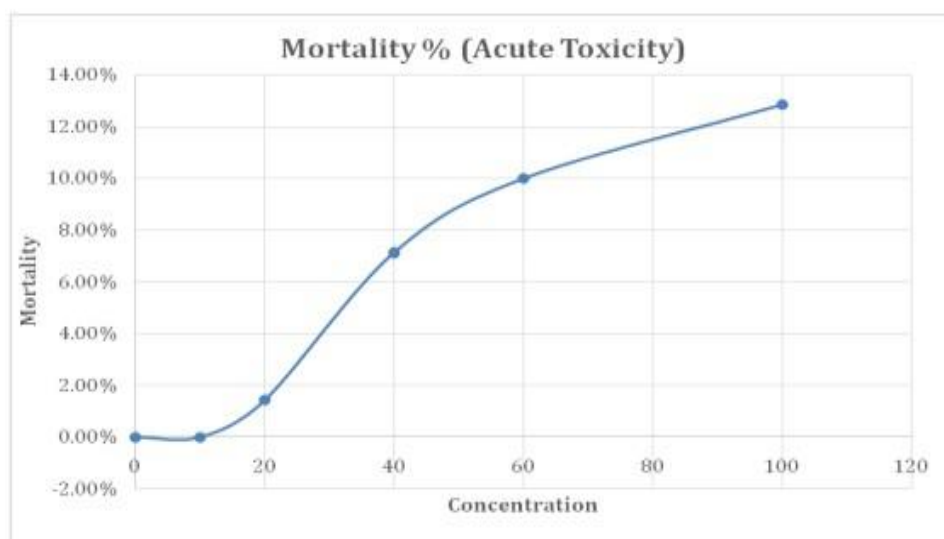


Figure: 1 Acute Toxicity

The acute toxicity assay demonstrated a concentration-dependent increase in mortality of *Artemia salina*. No mortality was observed in the control and 10% effluent treatments, indicating that highly diluted effluent had minimal immediate toxic effects. However, mortality increased progressively with increasing concentration.

A marked increase in mortality was observed between 20% and 40% effluent concentrations, where mortality rose from 1.43% to 7.14%. Despite this increase, the highest mortality recorded in undiluted effluent was only 12.86%, suggesting relatively low acute toxicity.

These findings indicate that short-term exposure to textile effluent causes limited mortality, possibly because toxic constituents require prolonged exposure to exert severe biological effects.

Chronic Toxicity Effects

Chronic Toxicity

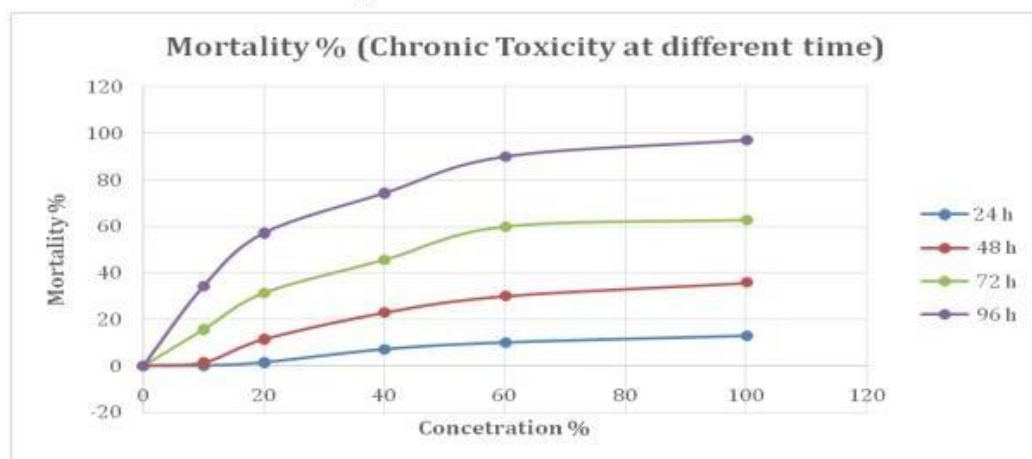


Figure: 2 Chronic Toxicity

In contrast to the acute toxicity results, chronic exposure produced pronounced concentration-dependent and time-dependent mortality. Mortality increased steadily with both exposure duration and effluent concentration.

At 10% concentration, mortality remained negligible during the early exposure period but increased to 34.3% after 96 hours. Similarly, 20% effluent concentration resulted in 57.1% mortality after prolonged exposure. Higher concentrations produced even more severe effects, reaching 74.3%, 90%, and 97.1% mortality at 40%, 60%, and 100% concentrations respectively.

The observed increase in mortality suggests cumulative physiological stress, bioaccumulation of toxic substances, and progressive impairment of metabolic processes.

LC₅₀ Determination

Chronic LC₅₀

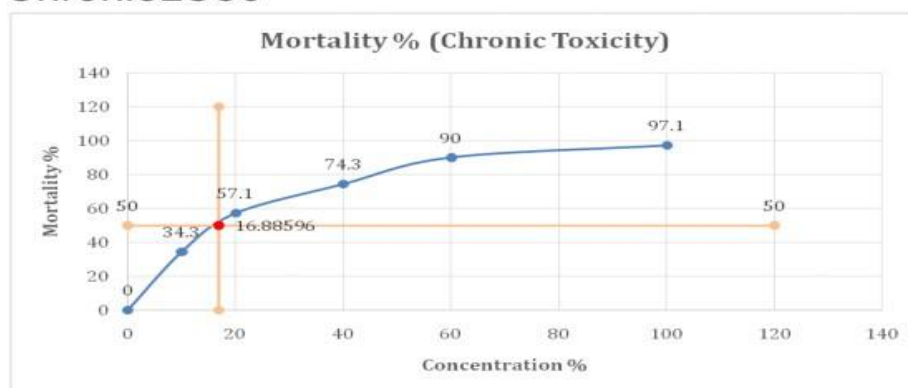


Figure: 3 Chronic LC₅₀

The median lethal concentration (LC_{50}) for 96-hour exposure was calculated as 16.89% textile effluent concentration. This relatively low LC_{50} value demonstrates that chronic exposure to textile wastewater poses a serious threat even at diluted concentrations.

The large difference between acute mortality and chronic LC_{50} values emphasizes the importance of long-term toxicity assessments when evaluating environmental risks associated with industrial effluents.

Effect of Textile Effluent on Hatching Success

Hatching success is considered one of the most sensitive indicators of developmental toxicity because embryonic stages are generally more susceptible to environmental stressors than adult organisms. In the present study, exposure of *Artemia salina* cysts to textile effluent resulted in a clear concentration-dependent reduction in hatching success.

The control group exhibited the highest hatching percentage and achieved nearly complete hatching within 96 hours. In contrast, cysts exposed to textile effluent displayed delayed hatching and reduced emergence rates. Although hatching percentages increased progressively with time in all experimental groups, the final hatching success remained significantly lower in higher effluent concentrations than in the control.

At 40% effluent concentration, only 38.75% of cysts hatched during the initial observation period. Even after 96 hours, hatching success reached only 52.50%, indicating substantial developmental inhibition. Higher concentrations produced even greater suppression of embryonic development. These findings suggest that toxic compounds present in textile wastewater interfere with normal embryogenesis and delay the transition from cyst to nauplius stage.

The reduction in hatching success may be attributed to the combined effects of elevated salinity, alkaline pH, suspended solids, and chemically resistant organic compounds present in the effluent. Such stressors can disrupt osmotic regulation, impair enzyme activity, and interfere with normal metabolic processes required for successful hatching.

The observed developmental toxicity indicates that textile effluent not only affects survival but also impacts reproductive and developmental stages, thereby posing a long-term ecological threat to aquatic ecosystems.

Morphological Abnormalities in *Artemia salina*

Morphological assessment was conducted to evaluate sub-lethal effects of textile effluent exposure on nauplii. Examination under a compound microscope revealed several structural abnormalities among exposed organisms compared to the control group.

Observed abnormalities included:

Irregular eye development

Digestive tract asymmetry

Abdominal deformities

Alterations in body coloration

Mouthpart abnormalities

Reduced motility and swimming behavior

These abnormalities were more frequently observed in organisms exposed to concentrations near the calculated LC_{50} value. Such deformities indicate that textile effluent exerts toxic effects not only at the physiological level but also during developmental processes.

The occurrence of morphological alterations suggests interference with cellular differentiation and tissue formation during larval development. Similar abnormalities have been reported in aquatic organisms exposed to industrial pollutants containing dyes, heavy metals, and recalcitrant organic compounds.

The presence of developmental abnormalities further confirms the ecotoxicological significance of textile wastewater and highlights the importance of incorporating sub-lethal endpoints in environmental risk assessment studies.

Conclusion

The present investigation demonstrated that textile industry effluent possesses substantial ecotoxicological potential and can adversely affect the survival, development, and reproduction of aquatic organisms. Physicochemical analysis revealed elevated levels of pH, alkalinity, total hardness, total dissolved solids, total suspended solids, biological oxygen demand, and chemical oxygen demand, indicating severe contamination of the wastewater.

Acute toxicity assessment showed relatively low mortality even at high effluent concentrations, suggesting limited short-term lethality. However, chronic exposure produced significant concentration-dependent mortality, reaching 97.1% after 96 hours. The calculated 96-hour LC_{50} value of 16.89% clearly demonstrates that prolonged exposure to textile effluent poses serious ecological risks even at diluted concentrations.

Furthermore, textile effluent significantly inhibited hatching success and induced developmental abnormalities in *Artemia salina*. These findings indicate that industrial wastewater affects not only organism survival but also critical developmental processes that contribute to population sustainability.

Overall, the study confirms that *Artemia salina* is an effective bioindicator for evaluating industrial wastewater toxicity. The results emphasize the urgent need for efficient treatment of textile effluents prior to environmental discharge and support the incorporation of biological toxicity assays into routine wastewater monitoring programs.

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Effect of Jeevamrutha Bio-fertilizer on Soil Fertility, Microbial Population, and Growth Performance of Mung Bean (*Vigna radiata* L.)

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Abstract:

The excessive use of chemical fertilizers has led to the deterioration of soil health and environmental quality, highlighting the need for sustainable biological alternatives. The present study evaluates the potential of Jeevamrutha, a traditional cow-based liquid organic bio-fertilizer, as an eco-friendly biological tool for sustainable agriculture. Jeevamrutha was prepared using indigenous cow dung, cow urine, jaggery, pulse flour, and rhizospheric soil, and its physical, chemical, and microbial properties were analyzed. Different concentrations of Jeevamrutha were applied to mung bean plants to assess growth parameters, biochemical attributes, and soil microbial activity. The results revealed that 75% Jeevamrutha concentration significantly enhanced fresh and dry biomass compared to the control. Microbial analysis indicated a rich population of plant growth-promoting rhizobacteria (PGPR), with notable ammonia production, indole-3-acetic acid (IAA) synthesis, and phosphate solubilization activities among several bacterial isolates. These findings demonstrate that Jeevamrutha improves soil fertility by enriching beneficial microflora and promoting plant growth, thereby reducing dependence on synthetic fertilizers. The study confirms the role of Jeevamrutha as an effective, low-cost, and farmer-friendly biological tool for sustainable environmental management and organic farming systems.

1. Introduction:

The excessive and continuous use of chemical fertilisers in modern agriculture has resulted in the deterioration of soil fertility, nutrient imbalances, environmental pollution, and a reduction in beneficial soil microbial populations. Consequently, sustainable agricultural practices emphasizing soil health restoration and ecological balance have gained significant importance in recent years. Among the various sustainable approaches, the use of organic biofertilizers has emerged as an effective strategy for maintaining soil productivity and environmental sustainability. Cow-based bioformulations are particularly important due to their higher microbial load, rapid decomposition rate, low-cost preparation, and eco-friendly nature (Anandan et al. 2016)

Jeevamrutha is a traditional Indian organic biofertilizer widely used in natural farming systems, particularly in rural agricultural practices. It is generally prepared using cow dung, cow urine, jaggery, pulse flour, soil from the rhizosphere region, and water. The formulation acts as a microbial inoculant, stimulating microbial activity in soil and thereby enhancing nutrient availability and plant growth. The microbial diversity and metabolites present in

Jeevamrutha significantly contribute to improved soil organic carbon, nutrient cycling, and plant health (Devakumar et al.2014).

In addition to Jeevamrutha, several other indigenous organic formulations such as Panchagavya, Amritpani, Sanjibani, and Kunapajala have been investigated for their role in sustainable agriculture. (S. Vijayendra et. al. 2001) However, Jeevamrutha has received considerable attention because of its simple preparation method, minimal precursor requirements, enhanced earthworm activity, and ability to improve soil microbial populations. The formulation is considered economically feasible and suitable for small and marginal farmers practicing organic agriculture (Stres et. al. 2008)

Each component of Jeevamrutha performs a specific role during fermentation and nutrient enrichment. Cow dung and cow urine serve as rich sources of beneficial microorganisms and nutrients, while jaggery and pulse flour provide carbon and nitrogen sources necessary for microbial proliferation. Soil collected from the rhizosphere acts as a microbial inoculum that enhances microbial diversity within the formulation. These interactions collectively improve soil fertility, nutrient mineralization, and plant resistance against pathogens (Sutar et al, 2019).

Several investigations have reported the positive influence of Jeevamrutha on crop growth, yield, microbial activity, and soil fertility. Application of Jeevamrutha significantly improved crop productivity in rice, capsicum, maize, chickpea, cowpea, and other crops under organic farming systems (Amareswari & Sujathamma, 2014). Studies have demonstrated that Jeevamrutha enhances plant height, biomass accumulation, nutrient uptake, and yield attributes while reducing dependence on synthetic fertilizers (Liu et. al.,2017).

Despite the increasing popularity of Jeevamrutha, several aspects related to its preparation, incubation period, nutrient composition, and seasonal variability remain insufficiently explored. Previous studies reported varying incubation periods ranging from 3 to 10 days depending on climatic conditions and geographical regions. Seasonal variations may influence microbial activity, nitrogen mineralization, and phosphorus availability in the formulation, especially under adverse environmental conditions. Furthermore, limited information is available regarding the optimization of precursor composition and nutrient responses in solid Jeevamrutha formulations (Boraiah et al. 2017).

Moreover, the phytotoxicity and germination behavior of Jeevamrutha formulations require scientific validation before large-scale agricultural applications. Germination index and seedling growth responses are important indicators for evaluating the maturity and safety of

organic biofertilizers. Hence, understanding the interaction between nutrient composition, microbial activity, and phytotoxic effects is essential for developing effective and sustainable Jeevamrutha formulations suitable for organic farming systems (Anthony et al, 2017).

The present study was therefore undertaken to evaluate the influence of Jeevamrutha on plant growth and nutrient characteristics, optimize critical formulation parameters, and assess its suitability as an eco-friendly biofertilizer for sustainable agricultural practices.

2. Materials and Methods

2.1 Preparation of Jeevamrutha

Jeevamrutha was prepared following the traditional natural farming method described by Palekar (2006) with slight modifications. The formulation consisted of fresh dung and urine collected from indigenous cows, jaggery, pulse flour, rhizospheric soil, and water. Approximately 10 kg of fresh cow dung, 10 L cow urine, 2 kg jaggery, 2 kg pulse flour (gram flour), and 1 kg soil collected from the rhizosphere region beneath a banyan tree were mixed thoroughly in 180 L of water in a plastic container. The mixture was stirred clockwise for approximately 5 min twice daily, once in the morning and once in the evening, to facilitate aeration and microbial activity. The container was covered with a clean jute cloth and kept under shaded conditions for fermentation. After 4–5 days of incubation, the Jeevamrutha formulation was considered ready for experimental application (Behera & Chandrashekara, 2023). The preparation process was carried out under ambient environmental conditions to encourage the proliferation of beneficial microorganisms naturally present in cow dung, cow urine, and rhizospheric soil. The fermented liquid formulation was filtered before application to experimental plants (Vibha et al., 2020).

2.2 Physicochemical Characterization of Jeevamrutha

The prepared Jeevamrutha formulation was analyzed for various physical and chemical properties using standard analytical procedures. Colour and odour were determined through visual and sensory evaluation, respectively. The pH and electrical conductivity (EC) were measured using a digital pH meter and conductivity meter according to standard soil and plant analytical methods described by Jackson (1973). Total nitrogen content was estimated by the Micro-Kjeldahl method, whereas total phosphorus was determined following nitric–perchloric acid digestion using the vanado-molybdo phosphoric yellow colour method. Total potassium concentration was measured using flame photometry after acid digestion. Calcium and magnesium contents were estimated using Atomic Absorption Spectrophotometry (AAS).

Micronutrients including iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) were also quantified using AAS following nitric–perchloric acid digestion.

Table 1. Physicochemical properties and analytical methods used for Jeevamrutha characterization

Sr. No.	Parameter	Method
1	Colour	Visual evaluation
2	Odour	Sensory evaluation
3	pH	Digital pH meter
4	Electrical conductivity	Conductivity meter
5	Total nitrogen	Micro-Kjeldahl method
6	Total phosphorus	Vanado-molybdo phosphoric yellow colour method
7	Total potassium	Flame photometry
8	Calcium	Atomic Absorption Spectrophotometry
9	Magnesium	Atomic Absorption Spectrophotometry
10	Fe, Mn, Zn, Cu	Atomic Absorption Spectrophotometry

2.3 Extraction and Estimation of Chlorophyll Content

Fresh leaves of mung bean (*Vigna radiata* L.) plants were used for chlorophyll extraction. Approximately 1 g of finely chopped fresh leaf tissue was homogenized in 10 mL of 80% acetone using a chilled mortar and pestle. The homogenate was centrifuged at 5000 rpm for 5 min, and the supernatant was collected for spectrophotometric analysis. Absorbance values were recorded at 645 nm and 663 nm using acetone as a blank.

Chlorophyll concentrations were calculated according to the method of Arnon (1949) using the following equations:

Total Chlorophyll: $20.2(A_{645}) + 8.02(A_{663})$

Chlorophyll a: $12.7(A_{663}) - 2.69(A_{645})$

Chlorophyll b: $22.9(A_{645}) - 4.68(A_{663})$

2.4 Determination of Total Protein Content

Total soluble protein content was estimated following the Bradford method. Approximately 1 g of fresh mung bean leaves was homogenized in tris buffer containing polyvinylpolypyrrolidone (PVP). The homogenized sample was centrifuged at 10,000 rpm for 20 min. Subsequently, 0.1 mL of the supernatant was mixed with 3 mL Bradford reagent and

incubated for 5 min at room temperature. Absorbance was measured at 595 nm using a UV–Visible spectrophotometer (UV-3200). Protein concentration was determined using bovine serum albumin (BSA) as the standard.

2.5 Determination of Total Carbohydrate Content

Total carbohydrate content was estimated using the anthrone method. About 100 mg of fresh mung bean leaves was homogenized in 96% ethanol and centrifuged at $3500 \times g$ for 10 min. Subsequently, 0.5 mL of the supernatant was reacted with 3 mL freshly prepared anthrone reagent and heated in a boiling water bath for 10 min. After cooling to room temperature, absorbance was measured at 652 nm using a spectrophotometer. Glucose was used as the standard for carbohydrate quantification (Hedge & Hofreiter, 1962).

2.6 Estimation of Total Phenolic Content

Total phenolic content in mung bean leaf extracts was determined using the Folin–Ciocalteu method. Briefly, 1 mL of plant extract was mixed with 15 mL distilled water, 4 mL Folin–Ciocalteu reagent, and 6 mL of 20% sodium carbonate solution. The reaction mixture was incubated for 2 h at room temperature, and absorbance was recorded at 760 nm using a spectrophotometer. Gallic acid was used as the standard, and total phenolic content was expressed as gallic acid equivalents. (del Rosario Cappellari et al., 2013)

2.7 Detection of Indole-3-Acetic Acid (IAA) Producing Bacterial Isolates

IAA production by bacterial isolates present in Jeevamrutha was screened using the Salkowski reagent method. Bacterial cultures were grown in Luria–Bertani (LB) broth with and without tryptophan supplementation. About 2 mL of culture supernatant was mixed with 1 mL of Salkowski reagent containing 0.5 M FeCl_3 in 35% perchloric acid and incubated in the dark for 30 min. The development of pink colouration indicated positive IAA production by the bacterial isolates.

2.8 Determination of Phosphate Solubilization Activity

Phosphate solubilization efficiency of bacterial isolates was qualitatively evaluated using Pikovskaya's agar medium. Bacterial cultures were streaked onto sterile agar plates and incubated at $30 \pm 2^\circ\text{C}$ for 5 days. Formation of a clear halo zone surrounding the bacterial colony indicated phosphate solubilization activity.

2.9 Determination of Ammonia Production

Ammonia production by bacterial isolates was determined using peptone water medium. Freshly grown bacterial cultures were inoculated into 10 mL peptone water and incubated at $28 \pm 2^\circ\text{C}$ for 48–72 h. Subsequently, 0.5 mL Nessler's reagent was added to the culture broth. Development of yellow to brown coloration indicated positive ammonia production.

3. Results and Discussion

3.1 Physicochemical Properties of Jeevamrutha

The prepared Jeevamrutha exhibited moderate green colour with a mild foul odour, indicating active microbial fermentation during the incubation period. The physicochemical analysis revealed an alkaline pH (8.24) and electrical conductivity (1.44 dS m^{-1}), suggesting the presence of dissolved mineral nutrients and microbial metabolites in the formulation (Table 2). The nutrient analysis showed appreciable concentrations of nitrogen (658 ppm), phosphorus (195 ppm), and potassium (821 ppm), indicating the potential of Jeevamrutha as a nutrient-rich organic biofertilizer. The presence of macro- and micronutrients in Jeevamrutha may be attributed to the decomposition of organic substrates and microbial mineralization processes occurring during fermentation. Similar observations were reported by Bhattacharjee and Uppaluri (2022), who demonstrated that Jeevamrutha contains diverse microbial communities capable of nutrient mobilization and soil fertility enhancement. The alkaline pH observed in the present study may facilitate nutrient availability and microbial activity in agricultural soils.

Micronutrient analysis further revealed the presence of calcium, magnesium, iron, zinc, manganese, and copper in both Jeevamrutha samples (Table 3). Jeevamrutha-01 showed comparatively higher concentrations of calcium (188.38 ppm), magnesium (1045.34 ppm), and iron (17.92 ppm) than Jeevamrutha-02, indicating variability in nutrient composition due to differences in substrate quality, fermentation conditions, and microbial activity.

Table 2. Physicochemical properties of Jeevamrutha

Parameter	Jeevamrutha
Colour	Moderate green
Odour	Mild foul smell
pH	8.24
EC (dS m^{-1})	1.44
Nitrogen (ppm)	658
Phosphorus (ppm)	195
Potassium (ppm)	821

Table 3. Micronutrient composition of Jeevamrutha samples

Sr. No.	Parameter	Jeevamrutha-01 (ppm)	Jeevamrutha-02 (ppm)
1	Calcium (Ca)	188.38	44.08

2	Magnesium (Mg)	1045.34	74.31
3	Copper (Cu)	0.10	0.32
4	Manganese (Mn)	1.60	0.28
5	Zinc (Zn)	1.30	0.05
6	Iron (Fe)	17.92	0.04

The results are in agreement with previous studies reporting that Jeevamrutha acts as a reservoir of essential nutrients and beneficial microorganisms that improve soil fertility and crop productivity (Sutar et al., 2018).

3.2 Morphological Changes in Mung Bean Plants Treated with Jeevamrutha

Visible morphological variations were observed in mung bean (*Vigna radiata* L.) plants treated with different concentrations of Jeevamrutha (Figure 1). Plants receiving Jeevamrutha treatments showed enhanced vegetative growth compared to the control group. Among all treatments, 75% and 100% Jeevamrutha concentrations exhibited comparatively vigorous growth, increased leaf development, and improved plant appearance.

The improved growth performance may be associated with enhanced nutrient availability and increased microbial activity stimulated by Jeevamrutha application. Beneficial microorganisms present in the formulation may enhance nutrient uptake, phytohormone production, and root proliferation, ultimately promoting plant growth.

3.2.1 Morphological changes on Mung plant

Root and shoot length measurements showed significant variation among Jeevamrutha treatments during the experimental period (Table 4). At 10 days after treatment, the highest root length (8.2 cm) was observed in plants treated with 100% Jeevamrutha, whereas the control plants showed the lowest root length (4.3 cm). Similar trends were observed during subsequent growth stages.

Similarly, shoot length increased substantially with Jeevamrutha treatment. Maximum shoot growth (25.5 cm) was recorded in the 100% Jeevamrutha treatment after 30 days, whereas control plants exhibited comparatively lower growth (16 cm). Enhanced root and shoot development may be attributed to increased nutrient mobilization, microbial activity, and production of growth-promoting substances such as indole-3-acetic acid (IAA) by rhizospheric microorganisms present in Jeevamrutha. Similar observations were reported by Kloepper et al. (1980), who demonstrated that plant growth-promoting rhizobacteria (PGPR) enhance root development and overall plant biomass.

Table 4. Effect of Jeevamrutha on Root and Shoot Length of *Vigna radiata* under Different Treatments

Treatment	Root Length (cm) 10 DAS	Root Length (cm) 20 DAS	Root Length (cm) 30 DAS	Shoot Length (cm) 10 DAS	Shoot Length (cm) 20 DAS	Shoot Length (cm) 30 DAS
Control	4.3	5.3	3.8	11.5	17.0	16.0
25% Jeevamrutha	7.0	5.5	4.0	13.3	21.0	18.5
50% Jeevamrutha	4.6	5.4	5.5	14.9	17.5	21.1
75% Jeevamrutha	7.3	5.0	3.9	12.2	16.5	22.5
100% Jeevamrutha	8.2	7.4	4.1	9.5	22.0	25.5

DAS = Days After Sowing

Root and shoot growth of mung bean plants improved under Jeevamrutha treatments compared with the control (Figure 2). The maximum root length at 10 and 20 DAS was observed under 100% Jeevamrutha treatment (8.2 cm and 7.4 cm, respectively), while the highest root length at 30 DAS was recorded under 50% Jeevamrutha treatment (5.5 cm). Shoot length increased progressively with treatment concentration, and the highest shoot length at 30 DAS was observed under 100% Jeevamrutha treatment (25.5 cm).

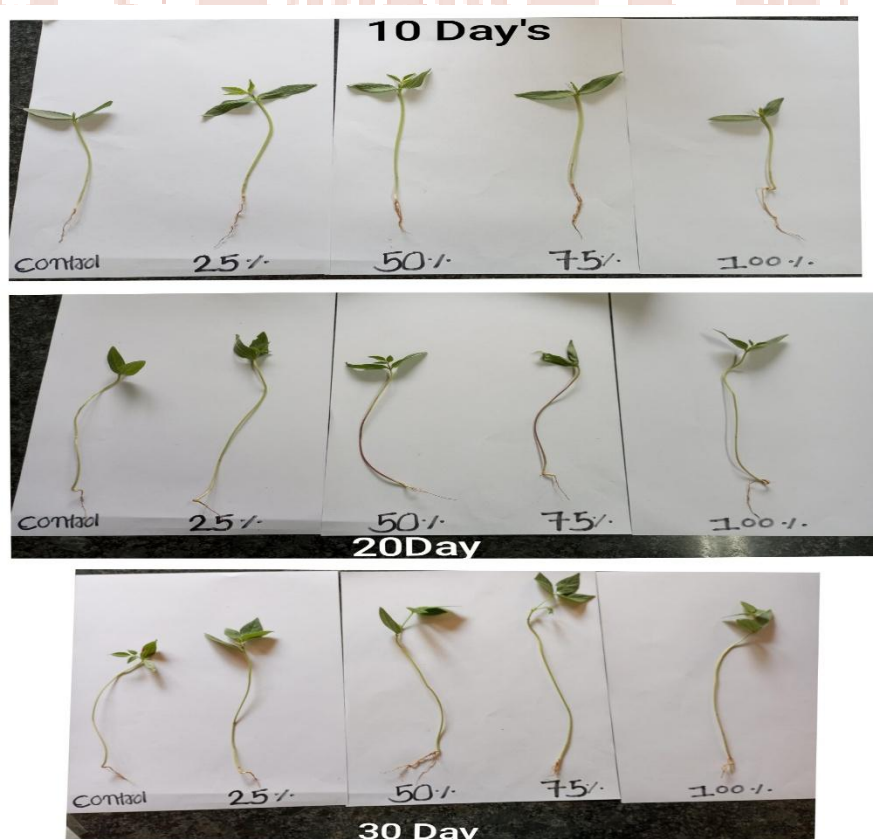


Fig: 1 Morphological change of plant under the different concentration of Jeevamrutha

Fresh and dry biomass accumulation increased significantly in Jeevamrutha-treated plants compared to control plants. Among all treatments, the 75% Jeevamrutha concentration exhibited the highest dry weight (0.1500 g) after 30 days, while the highest fresh weight (0.6919 g) was also recorded under the same treatment.

The increase in plant biomass suggests improved photosynthetic efficiency and nutrient assimilation in Jeevamrutha-treated plants. The findings corroborate earlier reports indicating that organic biofertilizers improve plant metabolic activity and biomass accumulation.

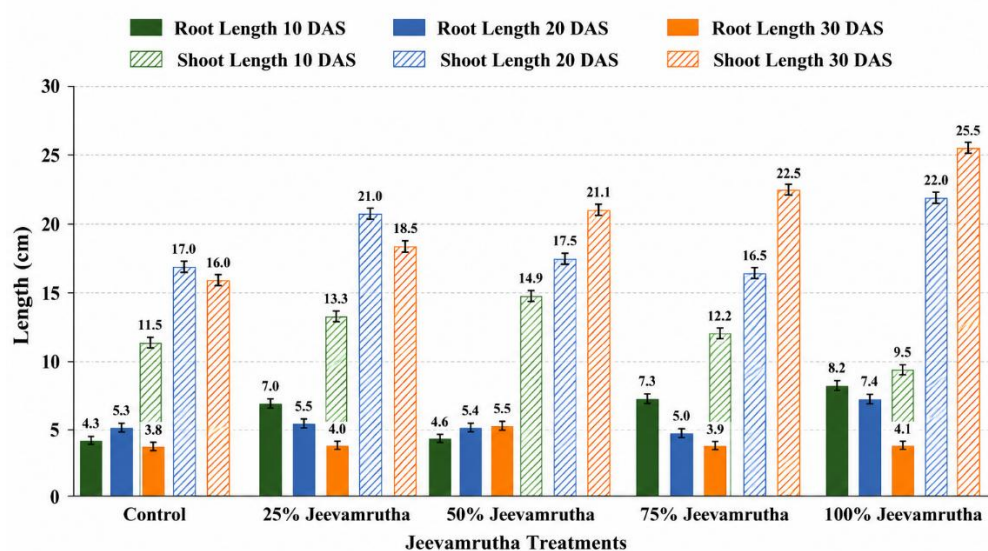


Figure 2. Effect of different concentrations of Jeevamrutha on root and shoot length of *Vigna radiata*. Root length (solid bars) and shoot length (hatched bars) were recorded at 10, 20, and 30 days after sowing (DAS). Plants treated with 100% Jeevamrutha showed the highest root length (7.4 cm) and shoot length (25.5 cm) at 20 and 30 DAS, respectively. Values are means $\pm$ SD (n = 3). DAS = Days After Sowing

3.2.2 Biomass Accumulation of Mung Plant

Biomass accumulation was enhanced in Jeevamrutha-treated plants compared with the control (Figure 3). The highest dry weight at 30 DAS was observed under 75% Jeevamrutha treatment (0.1500 g), whereas the highest fresh weight at 30 DAS was also recorded under 75% Jeevamrutha treatment (0.6919 g). These findings indicate improved biomass production and physiological performance in plants receiving Jeevamrutha application. (Table 5)

Table 5. Effect of Jeevamrutha on Dry Weight and Fresh Weight of *Vigna radiata* under Different Treatments

Treatment	Dry Weight (g) 10 DAS	Dry Weight (g) 20 DAS	Dry Weight (g) 30 DAS	Fresh Weight (g) 10 DAS	Fresh Weight (g) 20 DAS	Fresh Weight (g) 30 DAS
Control	0.0471	0.0509	0.0439	0.2851	0.2774	0.2370

25% Jeevamrutha	0.0668	0.0828	0.1065	0.5330	0.4602	0.4819
50% Jeevamrutha	0.0659	0.0586	0.1204	0.5901	0.3507	0.5377
75% Jeevamrutha	0.0599	0.0595	0.1500	0.5135	0.2830	0.6919
100% Jeevamrutha	0.0483	0.0755	0.1025	0.3509	0.4015	0.4803

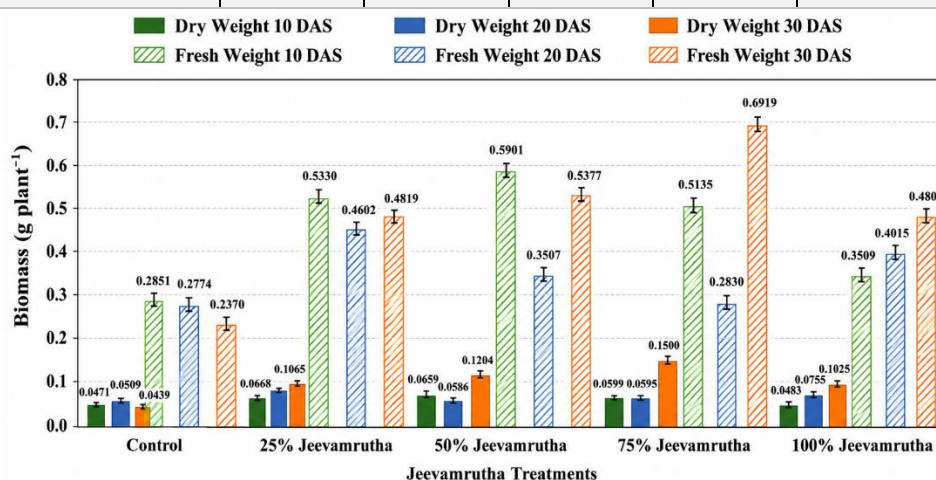


Figure 3. Effect of different concentrations of *Jeevamrutha* on biomass accumulation of *Vigna radiata*. Dry weight (solid bars) and fresh weight (hatched bars) were recorded at 10, 20, and 30 days after sowing (DAS). Plants treated with 75% *Jeevamrutha* exhibited the highest fresh weight (0.6919 g plant⁻¹) and dry weight (0.1500 g plant⁻¹) at 30 DAS. Values are means $\pm$ SD (n = 3).

3.3 Biochemical estimation after the treatment of Jeevamrutha on Mung Bean Plant

3.3.1 Chlorophyll Content

Chlorophyll content varied among the *Jeevamrutha* treatments throughout the experimental period. At 10 DAS, the highest chlorophyll content was recorded in the 50% *Jeevamrutha* treatment (489.93), which was approximately 2.7-fold higher than the control (181.24). At 20 DAS, the maximum chlorophyll content was observed in the 25% *Jeevamrutha* treatment (570.59), representing a 40.1% increase over the control (407.17). At 30 DAS, the highest chlorophyll content was recorded in the 100% *Jeevamrutha* treatment (448.08), which was 25.7% higher than the control (356.48). These findings indicate that *Jeevamrutha* enhanced photosynthetic pigment synthesis and improved the physiological performance of mung bean plants. (Figure 4)

Table 6. Effect of Jeevamrutha on Biochemical Parameters of *Vigna radiata* under Different Treatments

Treatment	Chlorophyll 10 DAS	Chlorophyll 20 DAS	Chlorophyll 30 DAS	Protein 10 DAS	Protein 20 DAS	Protein 30 DAS	Carbohydrate 10 DAS	Carbohydrate 20 DAS	Carbohydrate 30 DAS	Phenol 10 DAS	Phenol 20 DAS	Phenol 30 DAS
Control	181.24 3	407.16 77	356.48 17	0.398 0	0.000 4	1.169 4	0.700 0	3.457 7	0.177 3	0.680 0	0.085 9	0.042 9

25% Jeevamrutha	325.11 95	570.59 13	367.73 55	0.594 0	0.093 6	1.396 2	0.969 0	2.139 8	0.205 2	0.698 0	0.092 7	0.058 0
50% Jeevamrutha	489.93 31	336.67 72	423.90 42	0.707 0	0.081 6	1.561 7	1.106 0	2.342 5	0.071 8	0.825 0	0.086 8	0.048 5
75% Jeevamrutha	422.79 53	354.57 11	439.03 81	0.529 0	0.133 8	1.553 2	0.899 0	3.422 7	0.216 7	0.857 0	0.077 6	0.058 9
100% Jeevamrutha	297.33 24	351.70 65	448.07 94	0.386 0	0.064 4	1.535 4	0.773 0	3.841 5	0.270 2	0.521 0	0.072 9	0.049 9

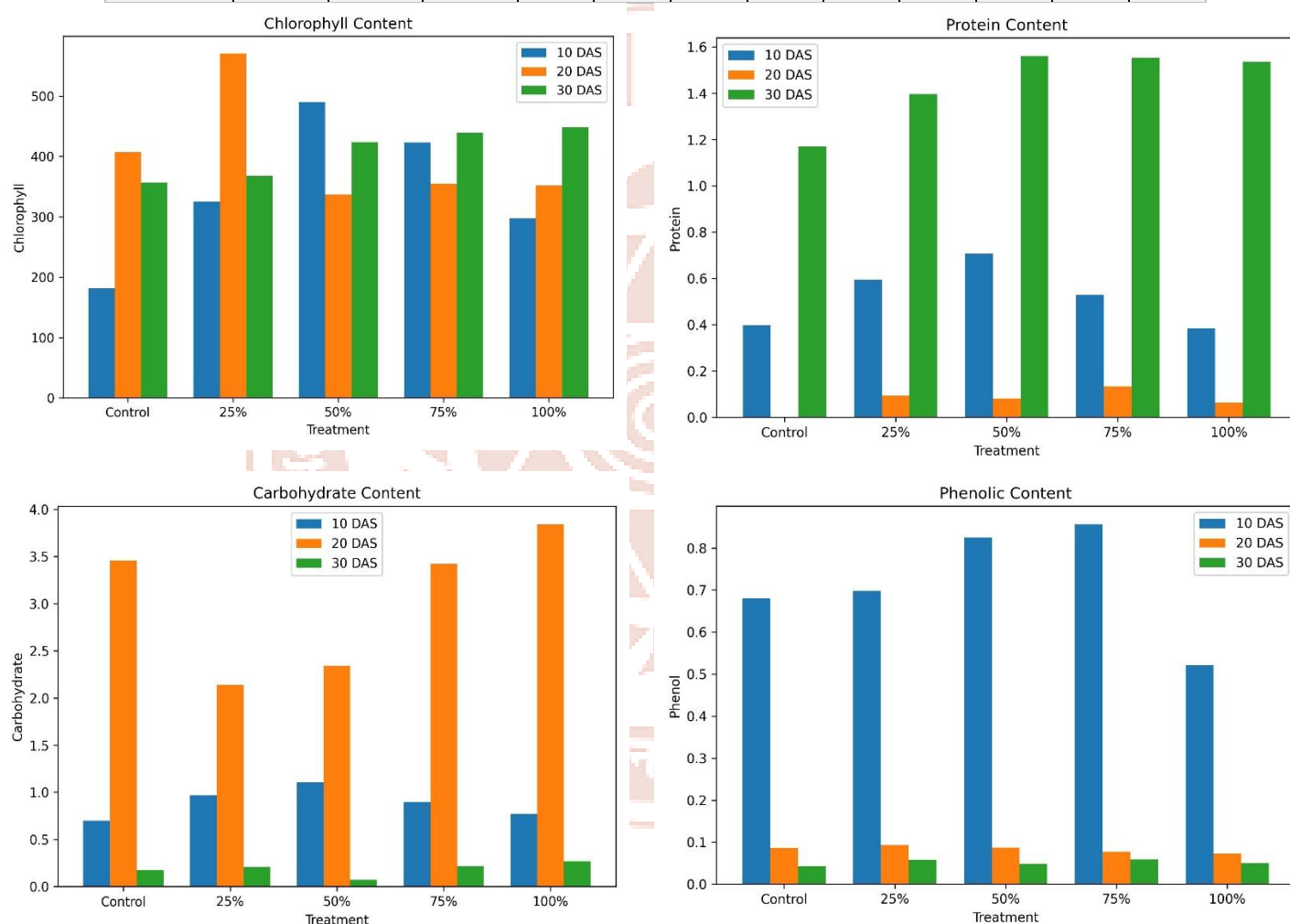


Fig 4: Effect of Jeevamrutha on Biochemical Parameters of *Vigna radiata* under Different Treatments

3.3.2 Protein Content

Protein content increased in all Jeevamrutha-treated plants compared with the control. At 10 DAS, the highest protein content was observed in the 50% Jeevamrutha treatment (0.707), representing a 77.6% increase over the control (0.398). At 30 DAS, the maximum protein

content was again recorded in the 50% Jeevamrutha treatment (1.5617), which was approximately 33.5% higher than the control (1.1694). The increased protein accumulation suggests enhanced nitrogen assimilation and metabolic activity under Jeevamrutha application.

3.3.3 Carbohydrate Content

Carbohydrate content was positively influenced by Jeevamrutha treatment. At 10 DAS, the highest carbohydrate content was observed under the 50% Jeevamrutha treatment (1.106), a 58.0% increase over the control (0.700). At 20 DAS, the highest value was recorded under 100% Jeevamrutha treatment (3.8415), 11.1% higher than the control (3.4577). At 30 DAS, 100% Jeevamrutha treatment again exhibited the maximum carbohydrate content (0.2702), representing a 52.5% increase over the control (0.1773). Enhanced carbohydrate accumulation reflects improved photosynthetic activity and assimilate production.

3.3.4 Phenolic Content

Phenolic content showed a positive response to Jeevamrutha application. At 10 DAS, the highest phenolic content was recorded under 75% Jeevamrutha treatment (0.857), which was 26.0% higher than the control (0.680). At 30 DAS, the same treatment exhibited the highest phenolic content (0.0589), representing a 37.3% increase over the control (0.0429). Increased phenolic accumulation indicates improved antioxidant capacity and activation of plant defense mechanisms. The biochemical analysis revealed that Jeevamrutha substantially improved physiological and metabolic attributes of *Vigna radiata*. Among the tested concentrations, 50–100% Jeevamrutha treatments generally produced the highest chlorophyll, protein, and carbohydrate contents, while 75% Jeevamrutha resulted in the greatest phenolic accumulation. Compared with the control, chlorophyll increased by up to 169.8%, protein by 77.6%, carbohydrate by 58.0%, and phenolic content by 37.3% at different growth stages. (Table 6) These results demonstrate that Jeevamrutha enhances photosynthetic efficiency, nutrient assimilation, metabolic activity, and plant defense responses, thereby contributing to improved growth and vigor of mung bean plants.

3.4 Plant Growth-Promoting Rhizobacterial (PGPR) Activities

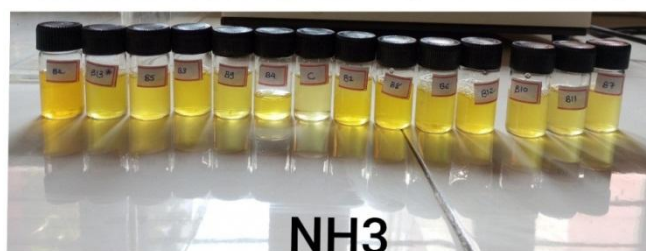
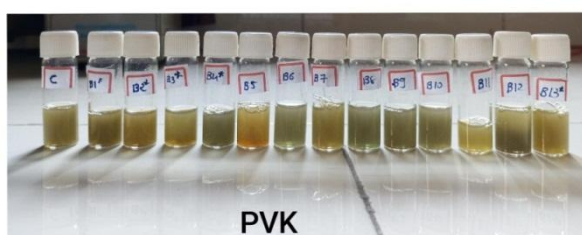
Thirteen bacterial isolates obtained from Jeevamrutha exhibited various PGPR activities including ammonia production, indole-3-acetic acid (IAA) production, and phosphate solubilization (Table 7).

Table 7. PGPR activities of bacterial isolates obtained from Jeevamrutha

Isolate	NH ₃	IAA	PVK
B1	+	–	+

B2	+	—	—
B3	+	+	+
B4	+	—	—
B5	+	+	—
B6	+	—	+
B7	+	—	+
B8	+	+	—
B9	+	—	—
B10	+	+	+
B11	+	+	—
B12	+	+	—
B13	+	—	+

All bacterial isolates showed positive ammonia production, whereas IAA production was observed in isolates B3, B5, B8, B10, B11, and B12. Phosphate solubilization activity was detected in isolates B1, B3, B6, B7, B10, and B13. (Figure 5)



The presence of PGPR in Jeevamrutha indicates its strong microbial potential for enhancing soil fertility and plant growth. PGPR microorganisms are known to improve nutrient

solubilization, phytohormone production, and root development. Similar findings were reported by Das and Singh (2014), who observed enhanced soil nutrient status and microbial activity following PGPR application.

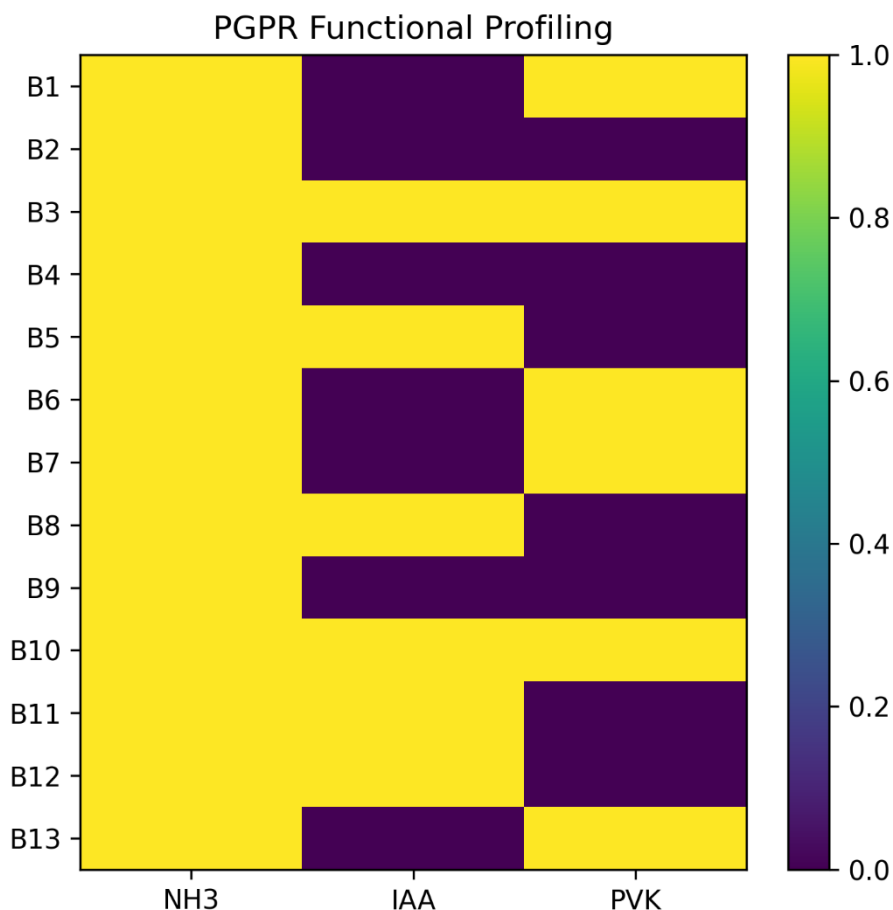


Fig 5: PGPR Functional Profiling

4. Discussion

The present study demonstrated that Jeevamrutha significantly improved the growth and physiological performance of mung bean (*Vigna radiata* L.) plants. The enhancement in plant growth may be attributed to the nutrient-rich composition and beneficial microbial population present in the fermented formulation. Jeevamrutha contained appreciable amounts of nitrogen, phosphorus, potassium, calcium, magnesium, and micronutrients, which are essential for plant metabolism, chlorophyll synthesis, and cellular development. Similar observations were reported by Bhattacharjee and Uppaluri (2022), who suggested that fermentation during Jeevamrutha preparation enhances nutrient availability and microbial activity.

Application of Jeevamrutha positively influenced root and shoot growth, fresh weight, and dry biomass of mung bean plants. Higher growth responses observed under 75% and 100%

Jeevamrutha treatments may be associated with improved nutrient uptake and microbial stimulation in the rhizosphere region. Plant growth-promoting rhizobacteria (PGPR) present in Jeevamrutha are known to enhance root elongation, nutrient absorption, and overall plant vigor through phytohormone production and nutrient mobilization (Kloepper et al., 1980).

The increase in chlorophyll content observed in treated plants indicates improved photosynthetic efficiency under Jeevamrutha application. Nitrogen present in the formulation likely contributed to enhanced chlorophyll biosynthesis, resulting in greater biomass accumulation. Similarly, increased protein and carbohydrate contents in treated plants suggest improved nitrogen metabolism, photosynthesis, and energy storage. Enhanced phenolic content further indicates activation of antioxidant and defense mechanisms in response to Jeevamrutha treatment (Manjunatha et al, 2022).

The bacterial isolates obtained from Jeevamrutha exhibited important PGPR traits such as ammonia production, phosphate solubilization, and indole-3-acetic acid (IAA) production. These microbial activities may contribute to improved nutrient availability and root development, thereby promoting plant growth. Das and Singh (2014) also reported that organic manures integrated with PGPR improve soil fertility and nutrient status. Jeevamrutha acts as an effective and eco-friendly organic biofertilizer capable of improving plant growth, biochemical characteristics, and microbial activity. Its application may reduce dependence on chemical fertilizers and support sustainable agricultural practices.

5. Conclusion

The present study demonstrated that Jeevamrutha is an effective organic biofertilizer capable of enhancing the growth, biochemical characteristics, and microbial activity associated with mung bean cultivation. The formulation contained appreciable levels of essential macro- and micronutrients that contributed to improved plant growth and metabolic performance. Among the tested concentrations, 75% Jeevamrutha was found to be the most effective treatment for biomass accumulation and phenolic enhancement, whereas 100% Jeevamrutha promoted maximum shoot growth. Jeevamrutha-treated plants exhibited enhanced root and shoot growth, biomass accumulation, chlorophyll content, protein content, carbohydrate content, and phenolic content compared with control plants. The study also confirmed the presence of beneficial plant growth-promoting rhizobacteria exhibiting ammonia production, phosphate solubilization, and IAA production. These microbial activities likely contributed to improved nutrient availability and plant growth promotion. The findings highlight the potential

of Jeevamrutha as a low-cost, eco-friendly, and sustainable alternative to chemical fertilizers for improving crop productivity and soil health.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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Author Contributions

RP: Conceptualization, supervision, data interpretation, manuscript review, and correspondence. **KP:** Guidance, methodology development, manuscript review, and technical support.

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EVALUATING THE TOXIC EFFECTS OF INDUSTRIAL EFFLUENT ON EARTHWORM (*Eisenia fetida*)

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Abstract:

Industrial effluents contain various toxic pollutants that pose serious threats to soil ecosystems. This study investigated the toxic effects of industrial effluent on the earthworm, a widely recognized bio indicator of soil health, under controlled laboratory conditions. Earthworms were exposed to different concentrations of industrial effluent mixed with soil to evaluate both lethal and sublethal effects. Toxicity was assessed by observing changes in behavior, physical structure, survival rate, and reproductive performance. Behavioral responses such as reduced movement, surface migration, and stress-related symptoms were observed in exposed earthworms. Physical abnormalities and increased mortality were more prominent at higher effluent concentrations. A significant reproductive capacity was also recorded, indicating adverse effects on earthworm population sustainability. These biological responses clearly reflected the harmful impact of effluent-contaminated soil. physio-chemical analysis of the industrial effluent revealed poor water quality, with unfavourable values of parameters such as pH, BOD, COD, DO, TDS. These parameters confirmed the presence of pollutants capable of disrupting soil life. The findings demonstrate that industrial effluents can cause serious damage to soil organisms even at relatively low concentrations, leading to reduced soil fertility and ecosystem imbalance. The study highlights the ecological risks associated with the improper discharge of untreated industrial wastewater into the environment. It also confirms the suitability of *Eisenia fetida* as a sensitive and reliable bio-indicator for soil toxicity assessment. Overall, the study emphasizes the importance of effective industrial effluent treatment and proper waste management practices to protect soil health and ensure sustainable environmental management.

1. INTRODUCTION

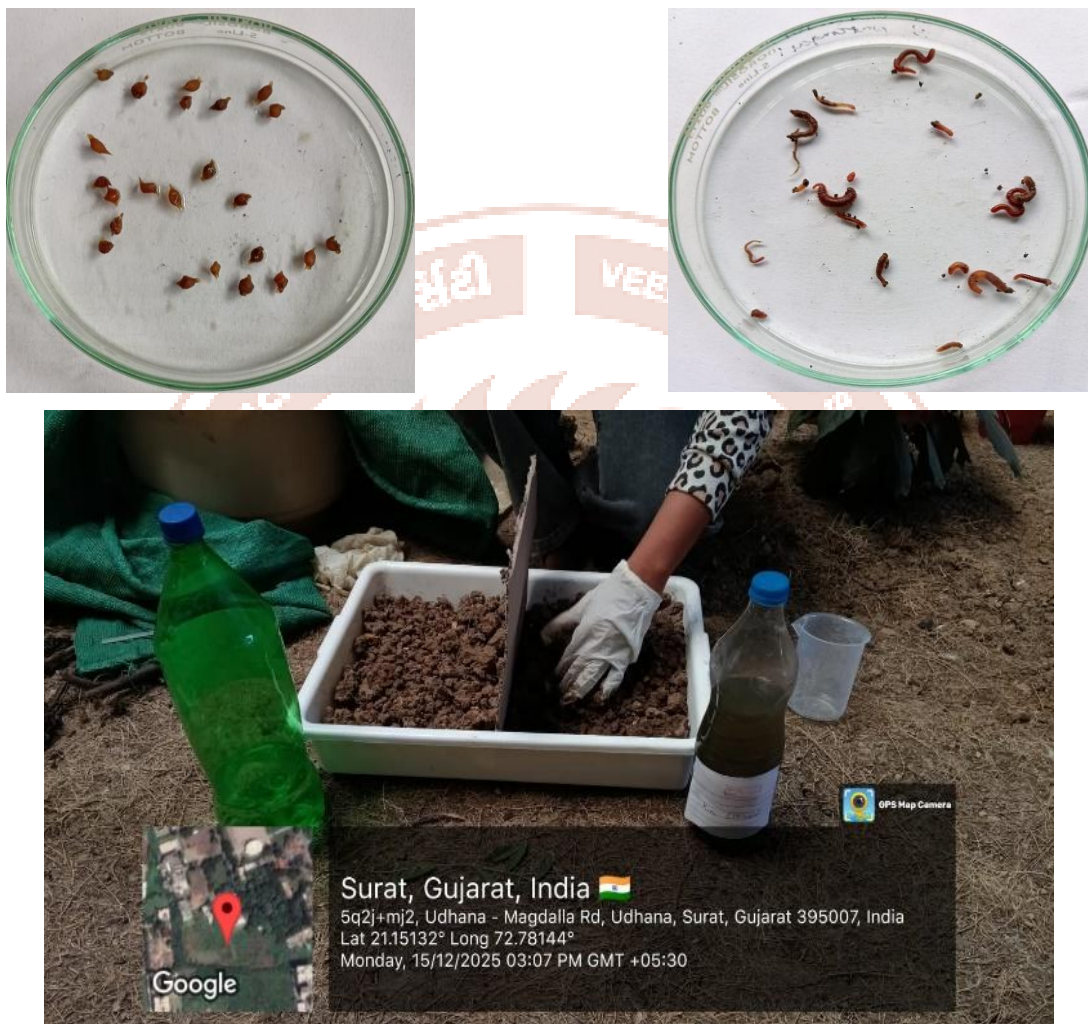
Industrial effluents adversely affect soil ecosystems. Earthworms are widely accepted as sensitive bioindicators because they respond rapidly to environmental contaminants. The present investigation evaluated the toxicological effects of industrial effluent using *Eisenia fetida*.

2. MATERIALS AND METHODS

Earthworms were cultured under controlled laboratory conditions and exposed to different industrial effluent concentrations. Physicochemical analyses, avoidance tests, mortality studies, biomass measurements, morphological observations and dissections were performed.

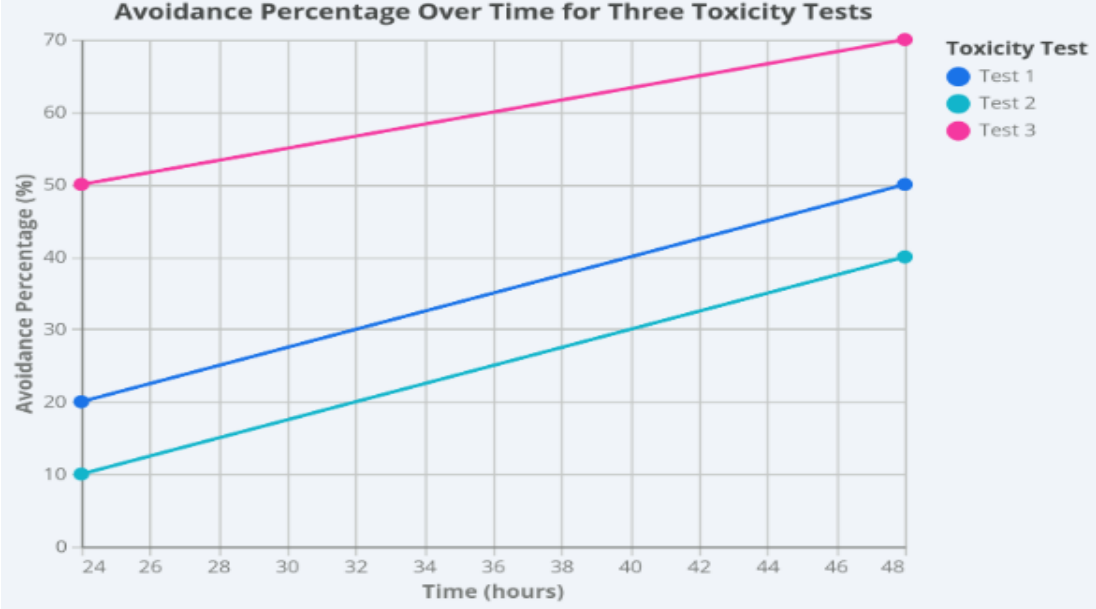
3. RESULTS AND DISCUSSION

Successful culture establishment was achieved. Avoidance behaviour increased with Toxicant exposure. No mortality occurred up to 60% concentration, whereas mortality reached 15.38% and 25% at 80% and 100% concentrations respectively. Morphological abnormalities and biomass changes indicated sublethal toxicity.



Culturing

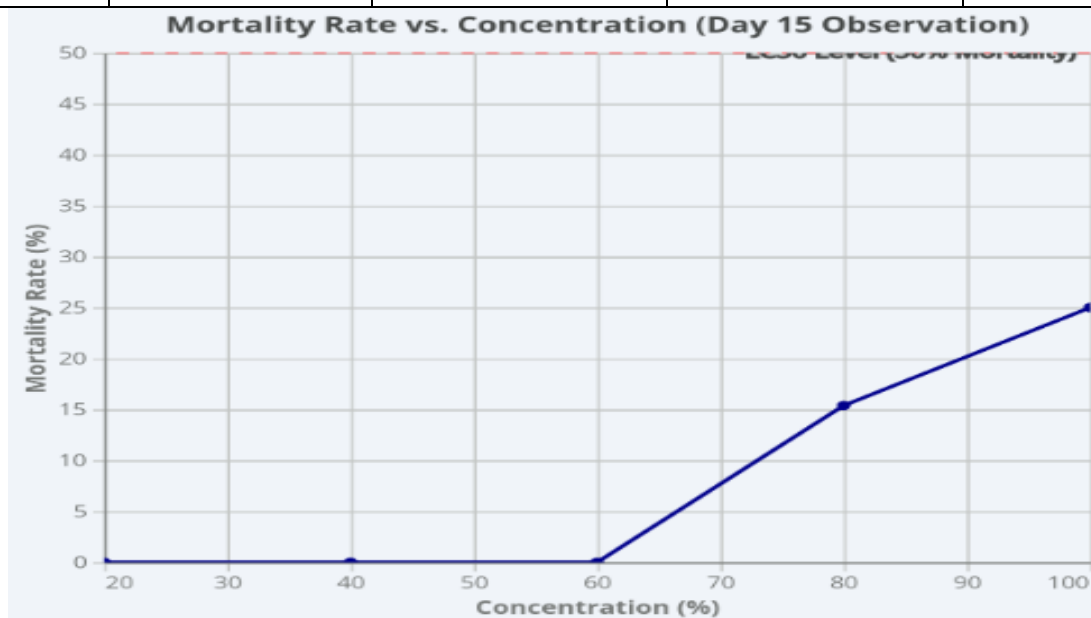
Parameter	Observation
Species	<i>Eisenia fetida</i>
Duration of Culturing	47 Days (14 Nov 2025 – 31 Dec 2025)
Initial Population	311 (Adults)
Final Population	665 (Adults + Juveniles)
Reproductive Status	Active (Cocoons and hatchlings observed)
Net Increase	+354 individuals (~114% growth)



Avoidance Behavior of *Eisenia fetida* over 48 Hours

Time Duration	Total Earthworms	Count in Tap Water (Control)	Count in Effluent (Toxicant)	Avoidance (%)
24 Hour	20	12	8	20%
48 Hour	20	15	5	50%
24 Hour	20	11	9	10%
48 Hour	20	14	6	40%

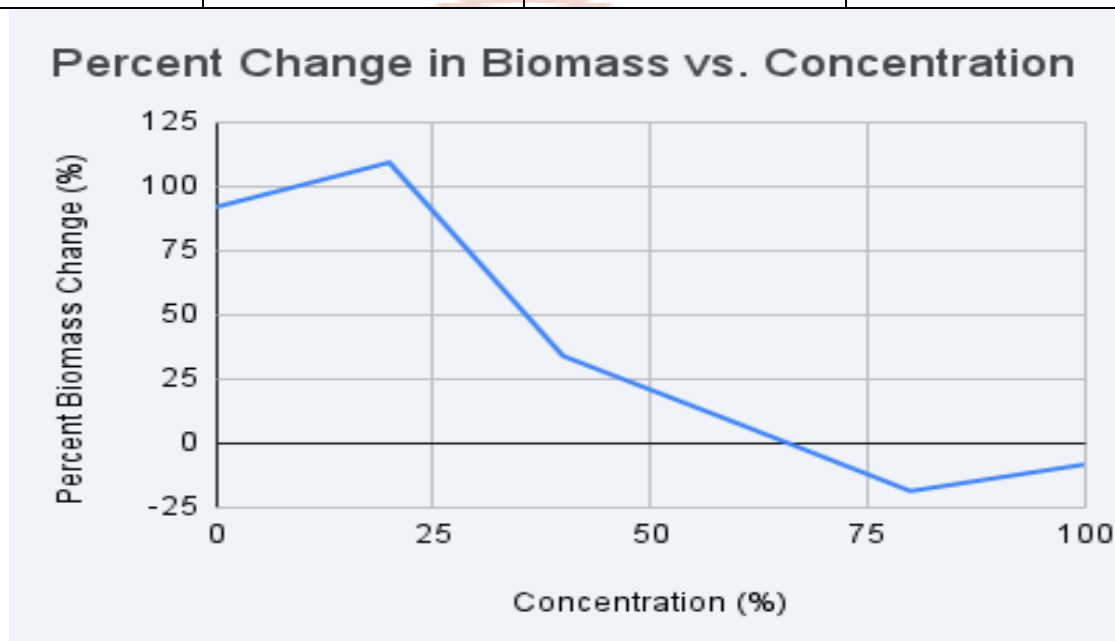
24 Hour	20	15	5	50%
48 Hour	20	17	3	70%



Mortality vs concentration



Concentration	Total No. Earthworms	Dead Earthworms	Mortality Rate (%)
Control 190	190	0	0.00%
20 %	35	0	0.00%
40 %	32	0	0.00%
60 %	31	0	0.00%
80 %	26	4	15.38%
100 %	24	6	25.00%

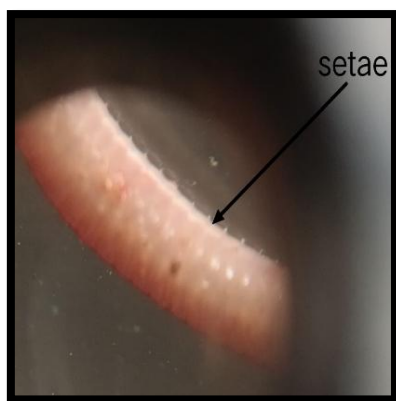


Biomass changes.

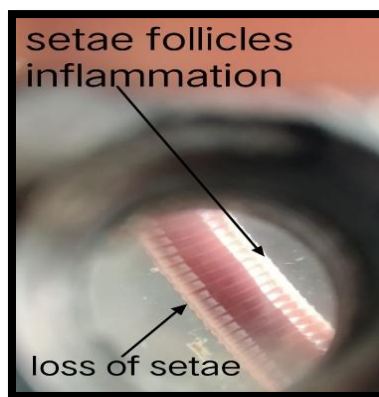
3.4 Morphological and Anatomical Alterations

Microscopic observation of the epidermal surface revealed severe damage to locomotive structures, specifically loss of setae and inflammation of setae follicles in earthworms exposed to 100% effluent compared to intact structures in the control group.

Dissection and internal anatomical analysis demonstrated significant pathological degeneration. Earthworms exposed to 100% concentration exhibited gastrointestinal distension, opaque coelomic fluid, asymmetrical yellowish seminal vesicles, and marked loss of muscular definition.



Control



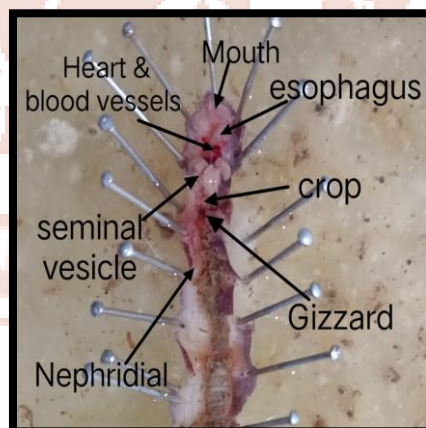
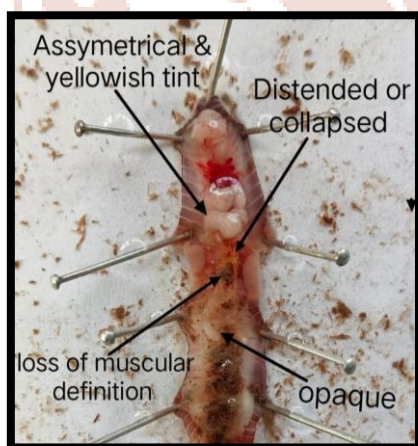
100% effluent concentration



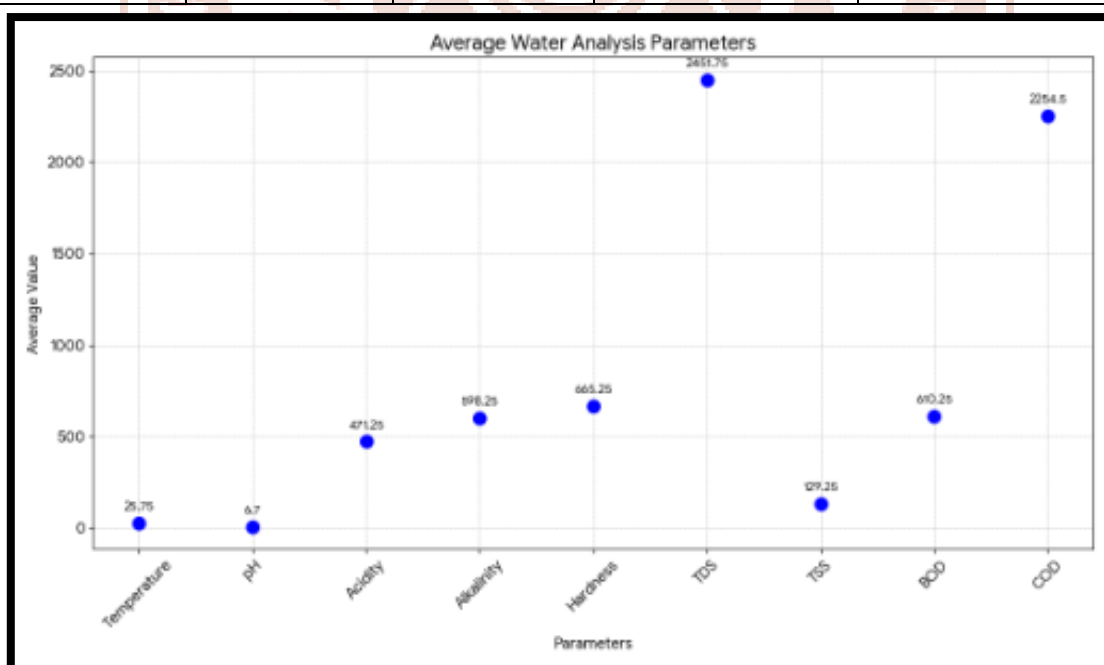
Control



100 % effluent concentration



Concentration	Initial Weight (g)	Final Weight (g)	Net Weight Change (g)	Percent Biomass Change (%)
Control	18.69	35.81	+17.12	+91.60%
20%	6.12	12.79	+6.67	+108.99%
40%	7.49	10.01	+2.52	+33.64%
60%	6.51	7.00	+0.49	+7.53%
80%	7.54	6.11	-1.43	-18.97%
100%	12.26	11.20	-1.06	-8.65%



Water analysis

Parameter	Average Value
Temperature	25.75 C
pH	6.7
Acidity	471.25 mg/l
Alkalinity	598.25 mg/l
Hardness	665.25 mg/l
TDS (Total Dissolved Solids)	2,451.75 mg/l
TSS (Total Suspended Solids)	129.25 mg/l
BOD (Biochemical Oxygen Demand)	610.25 mg/l
COD (Chemical Oxygen Demand)	2,254.50 mg/l

4. Figure Suggestions

Figure 1. Earthworm culture.

Figure 2. Avoidance behaviour.

Figure 3. Mortality vs concentration.

Figure 4. Biomass changes.

Figure 5. Water analysis

Figure 6. Morphological alteration in setae of Earthworm

Figure 7. Anatomical and Histological Alterations (Internal Damage)

Figure 8. Morphological Alterations (External Damage)

5. CONCLUSION

Industrial effluent exhibited concentration-dependent toxicity towards *Eisenia fetida*. Earthworms proved effective bioindicators for environmental monitoring.

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From Pollution to solution: Microbial Enzyme Engineering for PET Plastic Breakdown

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Abstract:

With global plastic production exceeding 400 million tons annually, polyethylene terephthalate (PET) has become one of the most persistent contributors to microplastic pollution in aquatic and terrestrial ecosystems. Its highly crystalline structure renders it highly resistant to natural biodegradation. Conventional remediation strategies such as incineration and landfilling are energy-intensive and environmentally unsustainable, highlighting the urgent need for biological alternatives.

This review examines advances in PET microplastic bioremediation, focusing on Microbial degradation pathways and enzymatic hydrolysis. *Ideonella sakaiensis* (currently classified as *Piscinibacter sakaiensis*), a bacterium capable of PET degradation through a synergistic dual-enzyme system involving PETase and MHETase. These enzymes catalyze the sequential hydrolysis of PET into environmentally benign monomers. Emerging strategies including protein engineering and microbial consortia are being investigated to improve catalytic efficiency and scalability. Despite limitations, enzyme-assisted bioremediation shows promise for mitigating PET-derived microplastic pollution.

1. INTRODUCTION:

Microplastic, defined as plastic particles smaller than 5mm, have become a major Environmental concern due to their persistence and widespread distribution in aquatic and terrestrial ecosystems. They originate from both primary sources and the breakdown of larger plastic materials. Among various polymers, polyethylene terephthalate (PET) is a significant contributor to secondary microplastics because of its extensive use in packaging and textiles. Its highly crystalline structure makes it resistant to natural degradation, leading to long-term environmental accumulation.

Recent studies indicate that microorganisms can interact with plastic surfaces through biofilm formation and enzymatic activity. In particular, *Ideonella sakaiensis* has shown the ability to degrade PET using PETase and MHETase enzymes, although its efficiency is limited under natural conditions. Understanding these processes is essential for developing sustainable strategies to manage microplastic pollution. Andrady, A. L. (2017).

2. OBJECTIVE:

- To study bioremediation mechanism for PET microplastic.
- Explore sustainable safe degradation of plastic with bacteria found in landfills.

3. SMALL PARTICLES, BIG PROBLEMS:

Microplastics are fine pollutants that enter water, air, and even our food chain without being noticed. These particles harm marine life, affect humans, and are difficult to remove once

released. Therefore, reducing pollution at its source and spreading awareness is essential to protect our future.

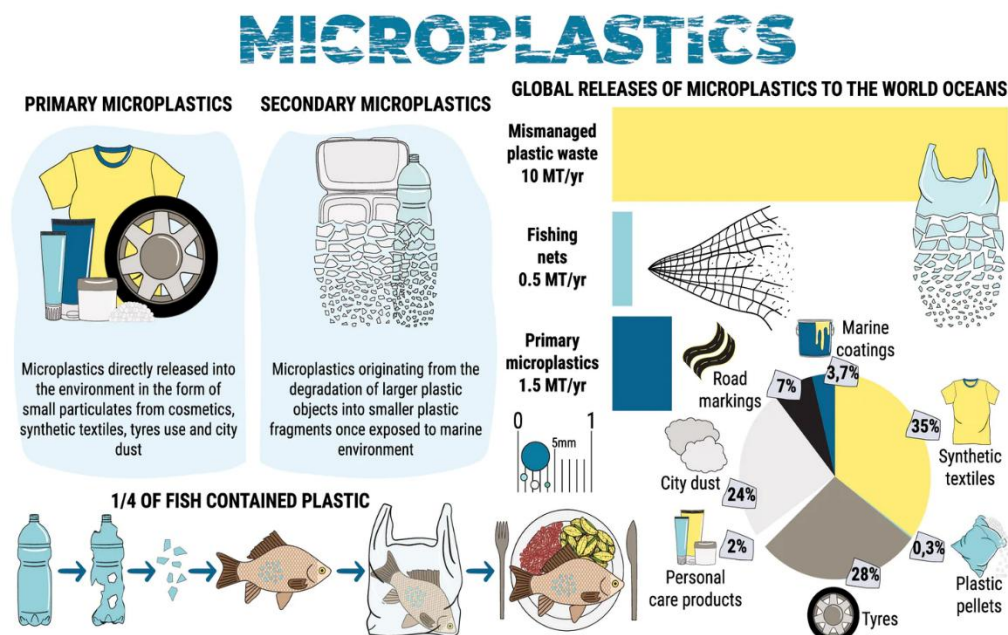


FIGURE : Sources of Microplastic

4. ABOUT POLYETHYLENE TEREPHTHALATE (PET):

- PET has higher global Production.
- High crystallinity → Environmental persistence.
- Forms microplastic, Non-Biodegradable.
- Accumulates in water, soil and sediments.

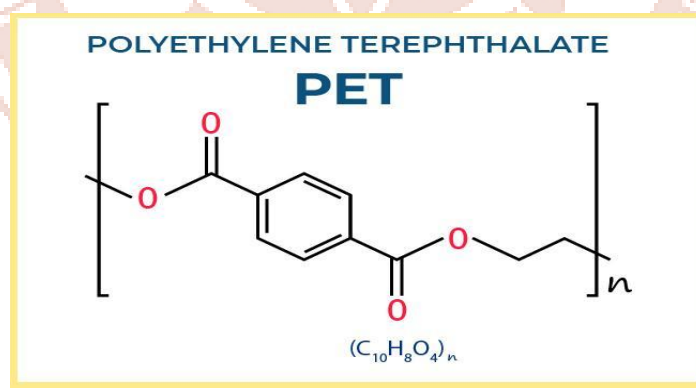


FIGURE : PET chemical Structure

5. MICROBE INCLUDED: (*Ideonella sakaiensis*)

Ideonella sakaiensis is a Gram-negative, aerobic, rod-shaped bacterium first identified in 2016 from a PET-contaminated recycling site in Japan. It is known for its unique ability to degrade polyethylene terephthalate (PET) by utilizing it as a carbon source. The bacterium secretes two key enzymes PETase and MHETase, (Yoshida, S., Hiraga, K., Takehana, T., Taniguchi, I., Yamaji, H., Maeda, Y., ... & Oda, K. (2016), which breakdown PET into

monomers, terephthalic acid and ethylene glycol. These compounds can be further metabolized by the microorganism. While *Ideonella sakaiensis* shows effective degradation of low-crystallinity PET under controlled conditions, its efficiency remains limited for high-crystallinity PET in natural environments. Despite this limitation, it represents a promising candidate for bioremediation and bio-recycling of plastic waste.

6. METHODOLOGY:

Relevant research papers were collected and analyzed. Information on Microplastic sources, PET, and microbial degradation was studied to understand environmental impact and possible solutions.

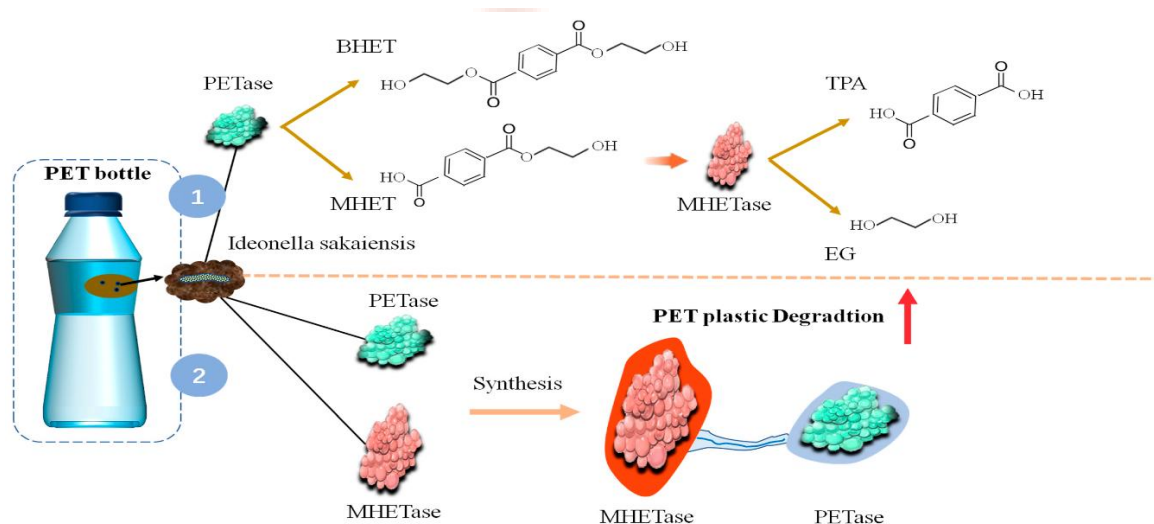


FIGURE: Cai, Zeming & Li, Minqian & Zhu, Ziyang & Wang, Xiaocui & Huang, Yuanyin & Li, Tianmu & Gong, Han & Yan, Muting. (2023). Biological Degradation of Plastics and Microplastics: A Recent Perspective on Associated Mechanisms and Influencing Factors. *Microorganisms*. 11. 1661. 10.3390/microorganisms11071661.

7. RESULTS:

Microplastics are widely distributed in the environment, mainly originating from secondary sources. PET is a major contributor due to its high usage and resistance to degradation. Microorganisms such as *Ideonella sakaiensis* can degrade PET through enzymatic activity, (Yoshida, S., Hiraga, K., Takehana, T., Taniguchi, I., Yamaji, H., Maeda, Y., ... & Oda, K. (2016), but the process is slow, especially for high-crystallinity PET. These findings indicate that while microbial degradation has potential, it remains limited under natural conditions.

8. CONCLUSION:

Microplastic those derived from PET, pose a significant environmental challenge due to their persistence and widespread presence. Although microorganisms such as *Ideonella sakaiensis* show the potential to degrade PET, the process remains slow and limited under

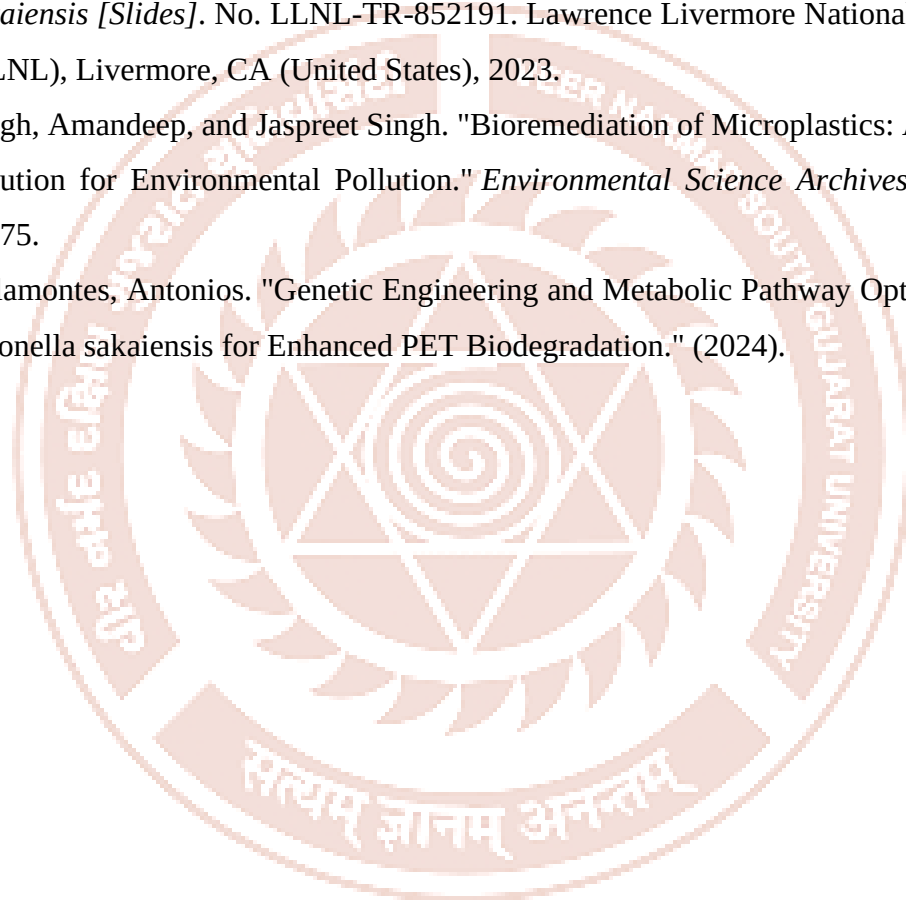
natural conditions. Therefore, improved and sustainable strategies are required to effectively manage PET-based microplastic pollution.

9. FUTURE PROSPECT:

Future research should focus in improving PET degradation through enzyme engineering and the use of microbial consortia. Combining biological approaches with physical or chemical pretreatment may enhance efficiency. These advancements can support the development of sustainable strategies for managing PET-derived microplastic pollution.

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Integrating Traditional Jeevamrit into Modern Vertical Hydroponics for Sustainable Crop Production

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Abstract:

The integration of sustainable bio-inputs into hydroponic systems represents a promising approach for reducing dependence on synthetic fertilizers in controlled-environment agriculture. This study evaluated the feasibility of applying filtered cow- and buffalo-derived Jeevamrit, a traditional fermented organic formulation, in a vertical hydroponic system cultivated with spinach (*Spinacia oleracea* L.). Physicochemical parameters of Jeevamrit and hydroponic tray water, including pH, electrical conductivity (EC), total dissolved solids (TDS), and salinity, were monitored throughout the experimental period. Plant growth responses were assessed through measurements of root length and shoot length. Microbial communities associated with Jeevamrit-treated hydroponic systems were investigated through culture-based isolation and Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) identification. Physicochemical analyses indicated that Jeevamrit application altered nutrient solution characteristics while maintaining hydroponic system stability. MALDI-TOF analysis revealed the presence of several bacterial taxa, including *Bacillus cereus*, *Acinetobacter tandoii*, *Acinetobacter johnsonii*, *Solibacillus silvestris*, and *Comamonas testosteroni*, demonstrating the establishment of diverse culturable microbial populations within the hydroponic environment. Plant growth observations showed treatment-associated differences, with cow-derived Jeevamrit exhibiting greater shoot growth than buffalo-derived Jeevamrit and control treatments. Overall, the findings demonstrate the feasibility of incorporating filtered Jeevamrit into hydroponic spinach cultivation and provide preliminary insights into its physicochemical and microbial dynamics. Further replicated studies with statistical validation and functional characterization of microbial communities are required to confirm the observed responses and assess their agronomic significance.

Introduction:

Agriculture is facing major challenges due to rapid population growth, declining arable land, environmental degradation, and increasing demand for sustainable food production. Conventional agricultural practices relying heavily on synthetic fertilizers and chemical inputs have improved crop yields but have also contributed to soil degradation, nutrient imbalance, environmental pollution, and disruption of beneficial microbial ecosystems (Tilman et al., 2002; Foley et al., 2011). In addition, rapid urbanization and shrinking agricultural land have accelerated the need for innovative cultivation systems capable of producing higher yields within limited spaces. Controlled environment agriculture, particularly hydroponics and vertical farming, has emerged as a promising solution for efficient resource utilization and year-round crop production (Despommier, 2010).

Hydroponic systems enable precise control over nutrient supply, water use, and environmental conditions, resulting in enhanced productivity and reduced water consumption (Resh, 2013; Sharma et al., 2018). However, these systems primarily depend on synthetic nutrient solutions and lack the beneficial microbial interactions naturally present in soil ecosystems (Lee & Lee, 2015). This limitation

has encouraged interest in integrating biological and organic inputs into hydroponic cultivation systems to improve sustainability and plant health.

Natural farming approaches emphasize the use of locally available organic bio-inputs to maintain ecological balance and reduce dependency on synthetic chemicals (Palekar, 2016). Among these, Jeevamrit has gained significant attention as a microbial-rich fermented bio-fertilizer prepared using cow or buffalo dung, urine, jaggery, pulse flour, and soil. Jeevamrit contains diverse beneficial microorganisms involved in nutrient cycling, nitrogen fixation, phosphorus solubilization, and production of plant growth-promoting substances (Sreenivasa et al., 2010; Glick, 2012). Its application has been associated with enhanced nutrient availability, improved plant growth, and increased microbial activity in agricultural systems (Lal, 2015).

Despite its proven benefits in soil-based agriculture, limited research has explored the application of Jeevamrit in hydroponic and vertical farming systems. Integrating traditional bio-inputs with modern hydroponics offers a potential strategy to combine the efficiency of soilless cultivation with the ecological benefits of microbial-based nutrient management. Beneficial microorganisms present in Jeevamrit may improve nutrient dynamics, stimulate root development, and enhance plant growth in hydroponic environments. However, understanding their influence on physicochemical stability, microbial diversity, and plant performance within aqueous systems remains essential.

Therefore, the present study investigated the feasibility of applying filtered cow- and buffalo-derived Jeevamrit in a hydroponic cultivation system using spinach (*Spinacia oleracea* L.) as a model crop. The study evaluated physicochemical changes in Jeevamrit formulations and hydroponic tray water, assessed spinach seedling growth responses, and characterized culturable microbial communities associated with Jeevamrit-treated systems using MALDI-TOF mass spectrometry. The objective was to generate preliminary insights into the physicochemical and microbial dynamics associated with the integration of traditional fermented bio-inputs into hydroponic cultivation systems.

Materials and Methods

Collection of Samples

Fresh dung and urine samples from Dangri cows were collected from Dang district near Saputara, Gujarat, India, while fresh dung and urine samples from Surati buffaloes were collected from Navsari, Gujarat, India, for the preparation of Jeevamrit. Samples were collected aseptically in sterile containers and transported to the laboratory for immediate processing to minimize external contamination.

Jeevamrit Preparation

Cow- and buffalo-derived Jeevamrit were prepared using fresh dung, urine, gram flour, jaggery, rhizosphere soil, and water following a modified protocol described by Devakumar et al. (2014). The composition of ingredients used for preparation is presented in Table 1. The mixture was maintained

under aerobic conditions through regular aeration during the fermentation period to facilitate microbial proliferation and organic matter decomposition.

Physicochemical parameters, including pH, electrical conductivity (EC), total dissolved solids (TDS), and salinity, were monitored throughout the 15-day fermentation period to assess changes associated with microbial activity and nutrient release. After fermentation, the Jeevamrit preparations were subjected to multi-stage filtration to obtain a liquid fraction suitable for hydroponic application and to minimize clogging of the hydroponic system. The filtered formulations were subsequently used for hydroponic experiments and microbial isolation studies.

Table 1. Composition of Jeevamrit used in the study

Ingredient	Quantity
Cow/Buffalo dung	100 g
Cow/Buffalo urine	100 mL
Gram flour	20 g
Jaggery	20 g
Rhizosphere soil	5 g
Water	1800 mL

Isolation of Microorganisms from Jeevamrit

Samples collected on fermentation days 0, 4, 8, and 12 were used for microbial isolation. Isolation was performed using both direct inoculation and enrichment approaches. Briefly, samples were serially diluted in sterile distilled water and spread onto nutrient agar plates. For enrichment, aliquots of Jeevamrit were incubated in nutrient broth prior to serial dilution and plating. The inoculated plates were incubated at 37°C for 48 h, and morphologically distinct colonies were selected and purified by repeated streaking on nutrient agar using the four-flame streaking technique.

Purified isolates were initially differentiated based on colony morphology and subsequently identified using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) according to standard procedures. The identified taxa were used to assess the microbial diversity associated with the Jeevamrit formulations during the fermentation process (Devakumar et al., 2014; Mukherjee et al., 2022).

Hydroponic System and Experimental Design

A tray-based hydroponic system was established to evaluate the feasibility of incorporating filtered Jeevamrit into a soilless cultivation environment. Healthy spinach (*Spinacia oleracea* L.) seedlings were grown in cups containing moist cocopeat, which served as an inert support medium due to its high water-holding capacity and aeration properties. The cups were placed in thermocol sheets fitted over hydroponic trays, ensuring that the roots remained in contact with the nutrient solution.

The hydroponic system was maintained under controlled conditions, and the nutrient solution was monitored regularly to maintain pH values between 5.5 and 6.5 and electrical conductivity (EC) between 1.2 and 2.0 mS cm⁻¹ (Resh, 2013; Sharma et al., 2018). Three treatment groups were evaluated: (i) cow-derived Jeevamrit (0.5%), (ii) buffalo-derived Jeevamrit (0.5%), and (iii) untreated control. The selected Jeevamrit concentration was applied as a supplement to the hydroponic nutrient solution following filtration to facilitate its use in the hydroponic system. Physicochemical parameters of the tray water, including pH, electrical conductivity (EC), total dissolved solids (TDS), and salinity, were measured periodically using digital meters to assess nutrient solution dynamics during cultivation (Pradeep and Sreenivasa, 2012). Plant growth responses were evaluated through measurements of root length and shoot length at designated observation intervals. Comparative observations among treatments were used to assess the compatibility of filtered Jeevamrit with hydroponic cultivation and its potential influence on spinach seedling growth.

Microbial Isolation from Hydroponic Tray Water

Hydroponic tray water samples associated with Jeevamrit-treated spinach cultivation systems were collected on days 0, 4, 8, and 12 for microbial isolation. Bacterial isolation was performed using two approaches: (i) direct inoculation following serial dilution of tray water samples, and (ii) enrichment culture by inoculating 10% (v/v) filtered Jeevamrit into nutrient broth, followed by incubation for 24–48 h prior to serial dilution. For each sample, 100 µL of the diluted suspension or enriched broth culture was spread onto sterile nutrient agar plates using the spread plate technique. The inoculated plates were incubated at 37°C for 48 h, after which morphologically distinct colonies were selected and purified through repeated streaking on nutrient agar using the four-flame streaking method.

The isolation procedure was repeated throughout the experimental period to monitor culturable bacterial populations within the hydroponic environment. Purified isolates were initially differentiated based on colony morphology and subsequently identified using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) according to standard protocols (Devakumar et al., 2014; Mukherjee et al., 2022). The identified bacterial taxa were used to assess the diversity of culturable microorganisms associated with Jeevamrit-amended hydroponic systems.

Results and Discussion

Physicochemical Characteristics of Cow- and Buffalo-Derived Jeevamrit

The physicochemical properties of cow- and buffalo-derived Jeevamrit underwent dynamic changes throughout the 15-day fermentation period (Table 2). Both formulations exhibited a similar fermentation pattern characterized by an initial decline in pH followed by gradual stabilization during the later stages of fermentation. In cow Jeevamrit, the pH decreased from 7.5 on day 0 to 4.0 by day 4 and subsequently increased to 6.5 on day 15. A comparable trend was observed in buffalo Jeevamrit,

where the pH declined from 7.5 to 3.5 between days 5 and 7 before recovering to 6.5 at the end of the fermentation period.

Electrical conductivity (EC) increased during the early stages of fermentation in both formulations, indicating the release of soluble ions and nutrients into the liquid phase. Cow Jeevamrit exhibited consistently higher EC values than buffalo Jeevamrit throughout the fermentation period, reaching a maximum value of 7.68 mS cm⁻¹ on day 7, whereas buffalo Jeevamrit reached a maximum EC of 4.92 mS cm⁻¹ on days 7–8. Similar trends were observed for total dissolved solids (TDS) and salinity. Cow Jeevamrit recorded peak TDS and salinity values of 5.03 and 3.87, respectively, while buffalo Jeevamrit attained maximum values of 3.15 and 2.45.

Following the initial increase, EC, TDS, and salinity values either stabilized or gradually declined towards the end of the fermentation period in both formulations. These changes suggest active microbial metabolism and decomposition of organic substrates during fermentation, resulting in the release of soluble constituents into the medium. The subsequent stabilization of physicochemical parameters during the later stages indicates the maturation of the fermented formulations.

Overall, both cow- and buffalo-derived Jeevamrit displayed characteristic fermentation-associated changes in pH, EC, TDS, and salinity. The higher EC, TDS, and salinity values observed in cow Jeevamrit suggest differences in nutrient release patterns between the two formulations; however, further replicated studies are required to determine the significance and practical implications of these observations.

Table 2. Physicochemical Properties of Cow- and Buffalo-Derived Jeevamrit During the 15-Day Fermentation Period

Fermentation Day	Cow pH	Cow EC (mS/cm)	Cow TDS	Cow Salinity	Buffalo pH	Buffalo EC (mS/cm)	Buffalo TDS	Buffalo Salinity
0	7.5	5.84	3.75	2.92	7.5	4.49	2.91	2.24
1	5.5	7.36	4.78	3.68	4.0	4.49	2.91	2.24
2	4.5	7.36	4.78	3.68	4.0	4.50	2.92	2.25
3	4.5	7.59	4.93	3.79	4.0	4.64	3.01	2.32
4	4.0	7.74	5.03	3.87	4.0	4.65	3.02	2.32
5	4.0	7.64	4.96	3.82	3.5	4.75	3.08	2.37
6	4.0	7.67	4.98	3.83	3.5	4.76	3.09	2.38
7	4.5	7.68	4.99	3.84	3.5	4.92	3.09	2.45
8	5.0	7.60	4.94	3.80	4.0	4.92	3.15	2.45
9	5.0	7.18	4.80	3.59	4.0	4.68	3.15	2.34
10	5.0	7.26	4.72	3.63	4.0	4.85	3.15	2.42
11	5.5	7.35	4.80	3.68	4.5	4.80	3.12	2.40
12	5.5	7.39	4.80	3.69	4.5	4.83	3.14	2.42
13	5.5	7.02	4.56	3.51	5.0	4.80	3.12	2.40
14	6.0	6.82	4.43	3.41	5.0	4.78	3.10	2.39
15	6.5	6.56	4.26	3.28	6.5	4.81	3.13	2.40

MALDI-TOF Identification of Bacterial Isolates Recovered from Hydroponic Tray Water

Microbial isolation from hydroponic tray water associated with Jeevamrit-treated spinach cultivation systems resulted in the recovery of several bacterial taxa that were subsequently identified using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS). The identified isolates included *Bacillus cereus*, *Acinetobacter tandoii*, *Acinetobacter johnsonii*, *Solibacillus silvestris*, and *Comamonas testosteroni*. MALDI scores ranging from 1.87 to 2.32 indicated reliable identification for most isolates and demonstrated the presence of diverse culturable bacterial populations within the hydroponic environment.

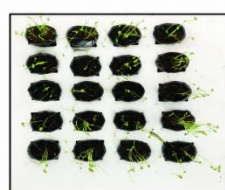
Among the recovered taxa, *Acinetobacter tandoii* and *Acinetobacter johnsonii* were isolated repeatedly, suggesting their persistence under the hydroponic conditions employed in this study. Similarly, repeated recovery of *Bacillus cereus* indicates its ability to survive and establish within Jeevamrit-amended hydroponic systems. Previous studies have reported that members of the genera *Bacillus*, *Acinetobacter*, and *Comamonas* are frequently associated with plant and soil environments and may participate in nutrient cycling and plant-microbe interactions (Dhawi, 2023; White and Chiaranunt, 2023; Li et al., 2021).

The detection of these bacterial taxa demonstrates that microorganisms originating from fermented Jeevamrit formulations can persist within hydroponic systems following application. However, the present study was limited to culture-based isolation and taxonomic identification. Functional characterization of the isolates, including assays for indole-3-acetic acid production, phosphate solubilization, siderophore production, nitrogen fixation, or other plant growth-promoting activities, was not performed. Therefore, the ecological roles and potential contributions of these microorganisms within the hydroponic environment remain to be experimentally validated.

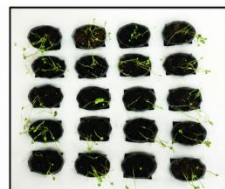
The occurrence of *Bacillus cereus* warrants particular attention because certain strains of this species have been associated with foodborne illness and opportunistic pathogenicity. Although *B. cereus* has also been reported from agricultural and rhizosphere environments, its detection in hydroponic systems intended for edible crop production highlights the need for future biosafety assessments and microbial risk evaluation prior to large-scale application of traditionally fermented bio-inputs.

Overall, MALDI-TOF MS analysis provided evidence for the establishment of a diverse culturable microbial community in Jeevamrit-treated hydroponic tray water. These findings contribute to understanding the microbial dynamics associated with the integration of traditional fermented bio-inputs into controlled-environment hydroponic cultivation systems.

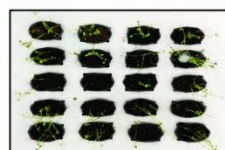
POT STUDY OF SPINACH PLANT



COW



CONTROL



BUFFALO

Figure 1. Representative growth response of spinach seedlings under cow Jeevamrit, buffalo Jeevamrit, and control treatments after 25 days of cultivation.

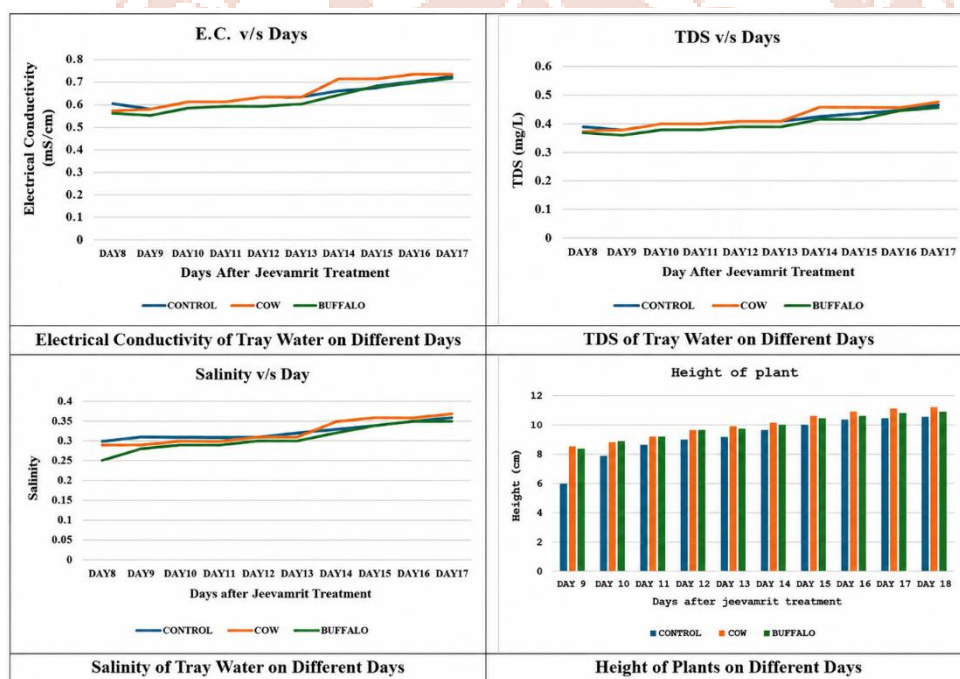


Figure 2. Temporal changes in electrical conductivity (EC), total dissolved solids (TDS), salinity, and plant growth characteristics of spinach cultivated under cow Jeevamrit, buffalo Jeevamrit, and control treatments.

Physicochemical Characteristics of Tray Water and Growth Observations in Spinach

The physicochemical characteristics of hydroponic tray water, including electrical conductivity (EC), total dissolved solids (TDS), and salinity, exhibited gradual variations throughout the cultivation period under cow Jeevamrit, buffalo Jeevamrit, and control treatments (Figure 2). Differences among treatments were observed during the experimental period, with Jeevamrit-amended systems generally

maintaining higher EC, TDS, and salinity values than the control. These observations suggest that the addition of filtered Jeevamrit altered the physicochemical profile of the hydroponic solution.

The temporal changes in EC and TDS may reflect the presence of soluble constituents released during fermentation and their subsequent dynamics within the hydroponic environment. Similar trends have been reported in studies investigating the incorporation of organic amendments and microbial bio-inputs into hydroponic systems (Trevisan et al., 2019). The relatively stable physicochemical conditions observed throughout the cultivation period indicate that the addition of filtered Jeevamrit did not adversely affect the operational stability of the hydroponic system.

Visual assessment of spinach seedlings revealed observable differences among cow Jeevamrit, buffalo Jeevamrit, and control treatments. Seedlings grown in Jeevamrit-amended systems exhibited healthy establishment and normal development throughout the cultivation period. Treatment-associated differences were observed in shoot development, root architecture, and overall seedling vigour, suggesting that filtered Jeevamrit was compatible with hydroponic spinach cultivation.

The observed differences among treatments may be associated with variations in nutrient availability, microbial composition, or physicochemical characteristics of the fermented formulations. Previous studies have reported that fermented organic bio-inputs and microbial inoculants can influence plant growth and rhizosphere processes in hydroponic and soilless cultivation systems (Trevisan et al., 2019; Glick, 2012). However, the present study did not include functional microbial characterization or replicated statistical analyses; therefore, direct relationships between the identified microorganisms and plant growth responses cannot be established.

Overall, the findings provide preliminary evidence that filtered Jeevamrit can be incorporated into hydroponic spinach cultivation without negatively affecting nutrient solution stability or seedling establishment. Further replicated experiments incorporating statistical analyses and functional assessment of microbial communities are required to validate the observed trends and evaluate their agronomic significance.

Overall, the findings demonstrate that filtered Jeevamrit can be incorporated into hydroponic tomato cultivation without causing major disruptions to nutrient solution stability, while providing preliminary evidence of treatment-associated differences in plant growth and physicochemical characteristics.

Root and Shoot Growth Responses of Spinach Seedlings

Root and shoot measurements of spinach seedlings grown under cow Jeevamrit, buffalo Jeevamrit, and control treatments are presented in Table 3. Observable differences in seedling growth were recorded among the treatments during the cultivation period.

Cow Jeevamrit-treated seedlings exhibited the greatest shoot length (9.24 cm), followed by the control (8.56 cm) and buffalo Jeevamrit (8.10 cm). Root length was comparable among treatments, with buffalo Jeevamrit recording the highest value (3.70 cm), while cow Jeevamrit and control treatments showed identical root lengths (3.64 cm).

The observed differences may be associated with variations in nutrient composition, physicochemical characteristics, or microbial populations present in the Jeevamrit formulations. However, because the present study was conducted as a preliminary evaluation and did not include replicated statistical analyses, the observed differences should be interpreted as descriptive trends rather than statistically validated treatment effects.

Overall, the results demonstrate that filtered Jeevamrit supported normal spinach seedling establishment and growth under hydroponic conditions. Further replicated studies are required to validate the observed responses and determine their agronomic significance.

Table 3. Root and shoot length of spinach (*Spinacia oleracea* L.) seedlings grown under cow Jeevamrit, buffalo Jeevamrit, and control treatments.

Treatment	Root Length (cm)	Shoot Length (cm)
Cow Jeevamrit	3.64	9.24
Buffalo Jeevamrit	3.70	8.10
Control	3.64	8.56

Conclusion

The present study demonstrated the feasibility of incorporating filtered cow- and buffalo-derived Jeevamrit into a hydroponic spinach (*Spinacia oleracea* L.) cultivation system. Fermentation of both formulations resulted in characteristic changes in pH, electrical conductivity (EC), total dissolved solids (TDS), and salinity, indicating active physicochemical and microbial processes during the fermentation period. Furthermore, the application of filtered Jeevamrit did not adversely affect the stability of the hydroponic nutrient solution under the conditions evaluated.

Microbial isolation and MALDI-TOF MS analysis revealed the presence of diverse culturable bacterial taxa, including *Bacillus cereus*, *Acinetobacter johnsonii*, *Acinetobacter tandoii*, *Solibacillus silvestris*, and *Comamonas testosteroni*, demonstrating that microorganisms associated with fermented Jeevamrit formulations can persist within hydroponic environments. However, the ecological roles and functional significance of these microorganisms within the hydroponic system remain to be experimentally validated.

Plant growth observations indicated normal establishment and development of spinach seedlings in Jeevamrit-amended hydroponic systems, with treatment-associated differences observed in root and shoot growth parameters. Nevertheless, as the present study was exploratory in nature and did not include replicated statistical analyses or functional characterization of microbial isolates, the observed responses should be interpreted as preliminary trends rather than definitive treatment effects.

Overall, the findings suggest that filtered Jeevamrit can be integrated into hydroponic spinach cultivation without adversely affecting system stability. The study provides preliminary insights into the physicochemical and microbial dynamics associated with the use of traditional fermented bio-inputs in controlled-environment agriculture. Future investigations employing replicated experimental designs, statistical validation, biosafety assessments, and functional microbial analyses are required to

evaluate the agronomic potential and practical applicability of Jeevamrit in hydroponic production systems.

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Isolation, Screening and Characterization of Esterase Producing Bacteria from Oil Contaminated Soil

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Abstract:

The escalating release of industrial effluents and oil-laden wastes into the environment has emerged as significant ecological concern. These pollutants are characterized by substantial quantities of ester-based compounds, which are resistant to degradation through conventional treatment methodologies. Esterases, class of hydrolytic enzymes, facilitate the breakdown of short-chain esters. Environmental samples were collected from oil-contaminated sites from Bilimora, Gujarat. A total of 12 bacterial isolates (EB1–EB12) & 11 fungal isolates (EF1–EF11) were obtained. Based on primary screening for esterase activity, bacterial isolates EB6 and EB10 were selected for further study. Among these, isolate EB6 demonstrated the highest esterase-producing and was chosen for characterization. A total of esterase-producing isolates screened, with EB6 exhibiting the highest enzyme activity ($12.75 \pm 0.003\text{U/ml}$) at 96 hours of fermentation. Consequently, EB6 was selected for further one-factor-at-a-time studies. The OFAT revealed that maximum enzyme production was supported by glucose ($9.16 \pm 0.003\text{U/ml}$) as carbon source, yeast extract as nitrogen source ($10.41 \pm 0.003\text{U/ml}$), sunflower oil as substrate ($11.25 \pm 0.003\text{U/ml}$). Optimal physico-chemical conditions were identified at pH 7 and 3% inoculum size, resulting activities of ($10.41 \pm 0.003\text{U/ml}$) and ($11.66 \pm 0.003\text{U/ml}$) respectively.

1. INTRODUCTION:

Rapid industrialization and extensive petroleum-based activities have resulted in significant environmental contamination, particularly due to the accumulation of oil and hydrocarbon pollutants in soil ecosystems. Oil contamination mainly originates from petroleum refineries, industrial discharge, transportation leakage, and accidental oil spills, leading to the release of complex mixtures of hydrocarbons, fatty acids, triglycerides, and ester-containing compounds into the environment. These hydrophobic pollutants persist in soil for prolonged periods and negatively influence soil fertility, microbial ecology, and environmental sustainability (Altas et al., 2011).

Conventional physicochemical remediation techniques used for the treatment of oil-contaminated soils are often costly and may produce secondary pollutants. Therefore, biological remediation using microorganisms has gained considerable attention as an eco-friendly and sustainable alternative for environmental cleanup (Kim et al., 2010).

Enzymes are biological macromolecules synthesized by living organisms that act as highly efficient biocatalysts capable of accelerating biochemical reactions under mild environmental conditions without being consumed during the reaction process. Due to their remarkable

catalytic efficiency and substrate specificity, enzymes play essential roles in microbial metabolism, biodegradation, and nutrient cycling processes (Bairoch et al., 2000).

Among hydrolytic enzymes, esterases are important lipid-degrading enzymes that catalyse the hydrolysis of ester bonds present in short-chain fatty acid esters, resulting in the formation of alcohols and fatty acids (Bornscheuer et al., 2002). Esterases belong to the α/β -hydrolase enzyme family and possess a conserved catalytic triad responsible for efficient ester bond cleavage. These enzymes contribute significantly to the degradation of ester-containing organic pollutants present in oil-contaminated environments.

Oil-polluted soils represent favourable ecological niches for the isolation of esterase-producing microorganisms, as continuous exposure to hydrocarbons induces enzymatic adaptation and enhanced biodegradation capacity. The enzymatic hydrolysis of lipidic pollutants increases substrate bioavailability and facilitates microbial degradation of complex hydrocarbons, thereby contributing to environmental detoxification and restoration of contaminated ecosystem (Mussakhmetov et al., 2025).

2. MATERIALS & METHODOLOGY:

2.1 Collection of Samples:

For the present investigation, oil contaminated soil samples were collected from different locations of Bilimora. Samples were aseptically collected in sterile sampling container to avoid external contamination. The collected samples were immediately transported to the research laboratory under sterile conditions and stored at appropriate temperature for further microbiological analysis.

2.2 Isolation of Esterase Producing Bacteria:

Isolation of esterase-producing bacteria was carried out using the standard serial dilution technique. One gram of the collected soil sample was transferred into 9 ml of sterile distilled water to obtain a 10^{-1} dilution. Further serial dilutions were prepared up to 10^{-8} . From each dilution, 0.1 ml aliquot was spread uniformly on Nutrient Agar plates under aseptic conditions. The plates were incubated at 30°C for 24 hours. After incubation, microbial colonies were observed and purified by repeated streaking. A total 21 of isolates were obtained from different samples (Alkabee et al., 2020).

2.3 Screening of Esterase Producing Bacteria:

The isolated bacterial strains were inoculated into 50 ml Nutrient Broth and incubated at 37 °C. Screening for esterase production was performed using Tributyrin agar. The cup borer method was employed, where wells were prepared in the agar plates and inoculated with 0.1 ml of actively growing bacterial culture. Plates were incubated at room temperature for 24–48

hours. Formation of a clear hydrolysis zone around the wells indicated esterase enzyme production, and isolates showing larger hydrolysis zones were selected for further studies (Kumar et al., 2012).

2.4 Esterase Enzyme Assay:

Esterase activity was determined by selecting bacterial isolates exhibiting higher hydrolysis zones and inoculating them into esterase production medium. The inoculated cultures were incubated for 24 hours under shaking conditions as described by Winkler & Stuckman (1979). After incubation, 5 ml of culture broth was withdrawn and centrifuged at 6000 rpm for 20 minutes in a refrigerated centrifuge. The obtained supernatant was used as a source of extracellular esterase enzyme. Enzyme activity was estimated using p-nitrophenyl acetate (p-NPA) as substrate. In the reaction mixture, 0.1 ml enzyme extract (supernatant) was mixed with 0.8 ml Tris-HCl buffer followed by addition of 0.1 ml p-NPA substrate solution and was kept in water bath at 60 °C for 20 minutes. After incubation 0.25 ml of Na₂CO₃ was added to stop reaction. Further this reaction mixture was centrifuged at 11,000 rpm for 10 min. The release of p-nitrophenol was measured at 410 nm using a colorimeter, indicating esterase activity.

2.5 Identification of Bacterial isolate using morphological and Biochemical studies:

Selected esterase-producing isolates were characterized using standard biochemical tests. Pure cultures were maintained on nutrient agar.

2.6 Characterization of Bacterial Isolate for Esterase Enzyme Production:

The production of microbial esterases is strongly influenced by nutritional composition and environmental conditions such as carbon source, nitrogen source, substrate availability, pH, and inoculum size.

The potent esterase-producing *Acinetobacter pittii* was initially inoculated into 50 mL of production medium and incubated at 37°C under shaking conditions (120 rpm) for 24 h to obtain an actively growing inoculum. Activated culture was subsequently transferred into production medium containing supplemented with an appropriate substrate concentration to stimulate extracellular esterase synthesis. Similar optimization strategies have been widely employed for improving microbial esterase production (Kaiser et al., 2006).

2.6.1 Effect of Carbon Source on Esterase Production:

Carbon sources have a major impact on enzyme production and are crucial to microbial metabolism (Singh et al., 2016). Different carbohydrates, including as glucose, lactose, sucrose, and maltose, were added to the production medium separately to assess their impact on esterase synthesis (Acharya et al., 2016). A 24-hour-old isolate culture was added to the

medium, and it was then incubated at 37 °C. For five days, samples were taken every 24 hours, and a colorimeter was used to measure the esterase activity. Enzyme induction and microbial growth efficiency are known to be regulated by variations in carbon sources.

2.6.2 Effect of Nitrogen Source on Esterase Production:

Another important element affecting the synthesis of microbial enzymes is the availability of nitrogen (**Bharadwaj et al., 2020**). The production medium was supplemented with various organic nitrogen sources at a 1% (w/v) concentration, including **yeast extract, tryptone, urea, and peptone (Nadda et al., 2022)**. A 24-hour-old culture was added to each flask, and it was then incubated under ideal circumstances. For up to seven days, esterase activity was evaluated using a colorimeter at 410 nm every 24 hours. By promoting cellular metabolism and protein synthesis, organic nitrogen sources have been shown to improve enzyme synthesis.

2.6.3 Effect of Substrate Type on Esterase Production:

Esterase enzyme production is largely regulated by substrate induction. A variety of natural lipid substrates, **such as sunflower, coconut, peanut, and olive oils**, were assessed as possible inducers of enzyme synthesis (**Acharya et al., 2016**). Isolates were added to production media that had been supplemented with the appropriate substrates, and the mixture was then incubated at 37 °C. After incubation, esterase activity was measured using a colorimeter at 410 nm. As enzymatic inducers, lipid substrates are known to increase the synthesis of esterase.

2.6.4 Effect of pH on Esterase Production:

Using suitable buffer systems, the production medium was adjusted to various pH levels (**5, 6, 7, and 8**) to investigate the impact of ambient pH on enzyme synthesis (**Nadda et al., 2022**). Enzyme activity was evaluated at regular intervals during the five days when the infected cultures were cultured at room temperature. Depending on extracellular pH levels that impact microbial growth and enzyme stability, microbial esterases typically display varied production patterns.

2.6.5 Effect of Inoculum size on Esterase Production:

The ideal conditions needed for maximal esterase production were identified by measuring the inoculum size. Production media that had been infected were incubated at inoculum sizes of **1%, 3%, 5%, and 7% (Bharadwaj et al., 2020)**. Every 24 hours, samples were taken out, and the colorimeter at 410 nm was used to measure the enzyme activity. Microbial metabolic activity, catalytic efficiency, and enzyme production are all strongly impacted by inoculum size.

2.7 Partial Purification of Esterase enzyme:

Partial purification of the enzyme was carried out using fermentation broth obtained under both optimized and non-optimized conditions (Sayali et al., 2013). The crude extract was subjected to ammonium sulphate precipitation at 40% and 60% saturation levels with continuous gentle stirring for 2–3 hours to facilitate protein precipitation (Alkabee et al., 2020). The mixture was then centrifuged at 12,000 rpm for 15 minutes to separate the precipitated proteins.

The protein pellets obtained were dissolved in a minimal volume of 0.25 mM Tris–HCl buffer (pH 7.0). To remove residual ammonium sulphate, dialysis was performed against the same buffer at 4°C using a suitable dialysis membrane. The buffer was replaced at regular 2-hour intervals to ensure efficient salt removal. Dialysis was continued until complete elimination of salts was achieved. The purified enzyme fractions were then analysed for total protein content and esterase activity, and the fraction exhibiting maximum activity was selected for further studies (Hamid et al., 2021).

3. RESULT & DISCUSSION:

3.1 Collection of Samples:

Soil samples were collected aseptically from oil-contaminated sites, including automobile garages and food stall soil. These environments were chosen because constant exposure to lipid-rich waste helps microorganisms that can make lipolytic enzymes, especially esterases, grow.

3.2 Isolation of Esterase Producing Microorganisms:

Microorganisms were isolated from the collected soil samples using standard microbiological methods. Soil suspensions were serially diluted and spreading onto nutrient agar plates using the spread plate technique. After incubation, several morphologically distinct colonies were observed. A total 23 of isolates [bacterial (EB1-EB12) & fungal (EF1-EF11)] were obtained from different samples.

3.3 Primary Screening of Esterase Producing Isolates:

Primary screening for esterase production was performed using tributyrin agar (TBA) plates. Individual colonies obtained from nutrient agar were inoculated onto TBA plates and incubated under suitable conditions. Tributyrin acts as a lipid substrate in the medium. Microorganisms capable of producing esterase enzymes hydrolyse tributyrin, resulting in the formation of clear zones of hydrolysis around the colonies. Several of the bacterial isolates exhibited esterase enzyme activity. However, isolate **EB6**(*Acinetobacter pittii*) created the greatest hydrolysis zone (**34 mm**), indicating the highest output of esterase among the examined bacterial strains.

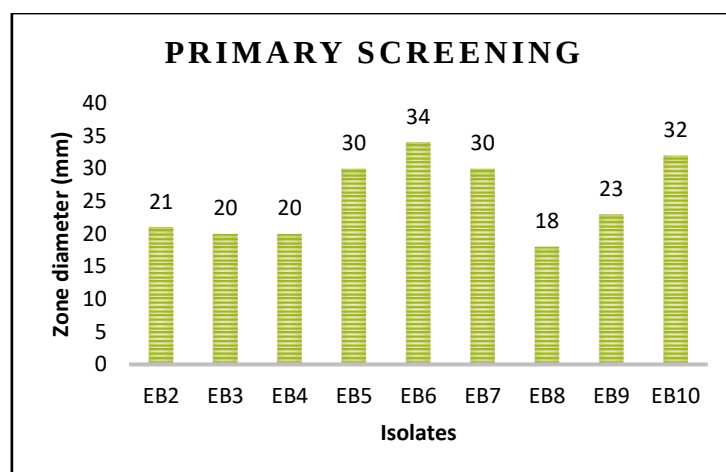


Figure 2: Primary Screening

3.4 Secondary Screening of Esterase Producing Isolates:

Secondary screening was carried out to quantitatively evaluate esterase activity in the culture broth of the isolates. Esterase activity was determined spectrophotometrically following the method described by **Winkler and Stuckmann (1979)**. P-nitrophenol (p-NP) is released as a reaction product when p-nitrophenyl esters are hydrolysed, which is the basis of the assay. The substrate in this investigation was p-nitrophenyl acetate, or p-NPA. Using a colorimeter to detect the absorbance at 410 nm, the amount of p-nitrophenol emitted during the reaction was calculated.

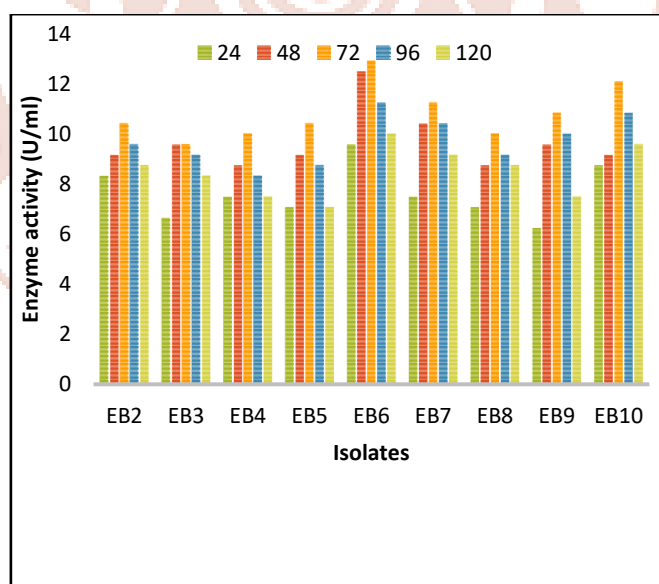


Figure3: Secondary Screening

Among all isolates, **EB6** showed the highest activity 12.41 ± 0.001 U/ ml. The figure showed that the esterase activity of bacterial isolates at different incubation times. Enzyme activity increased up to 72 hours and then slightly decreased.

3.5 Identification of Esterase Producer:

A comprehensive morphological and biochemical study was performed on the chosen bacterial isolate, **EB6**, which showed the greatest esterase activity during primary screening. The isolate was rod-shaped and Gram-negative when examined under a microscope using Gram staining, indicating that it might belong to the genus

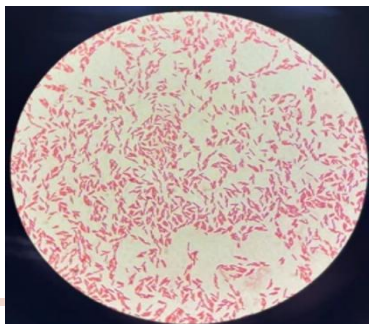


Figure 1: Gram Reaction

The isolate EB6 (code M6) was identified as *Acinetobacter pittii*. Biochemical results shown in

Table 1: Biochemical Result of *Acinetobacter pittii*

Biochemical	Observation
Indole Test	-
Methyl Red Test	-
Voges -Proskauer Test	-
Simmon Citrate Test	+
Nutrient Gelatine Test	+
1% Peptone	-
2% Peptone	-
Triple Sugar Iron(TSI) Agar Test	Butt- Acidic
	Slant- Alkaline
	H ₂ S - -
	CO ₂ - +
Sugar:	
Glucose	AG
Lactose	AG
Maltose	AG
Manitol	AG
Sucrose	A

[This tables, (+) positive,(-) negative , AG indicates acid & gas , A indicates Acid]

3.6 Optimization of Esterase Production Using OFAT:

The One-Factor-At-a-Time (OFAT) method was used to optimize a number of physicochemical and nutritional factors to increase esterase production. This approach analysed each parameter's independent impact on esterase production by gradually changing

each one while maintaining the others constant. Various carbon sources, nitrogen sources, pH levels, substrates, and inoculum size were all the variables examined.

3.7 Characterization of Esterase Producing *Acinetobacter Pittii*:

3.7.1 Effect of Carbon source on Esterase Activity:

The selected isolate *Acinetobacter Pittii* demonstrated significant esterase-producing capability under submerged culture conditions. Among the tested substrates, glucose supported maximum enzyme production compared to other carbon sources. The highest esterase activity recorded with glucose was **9.16 U/ml**.

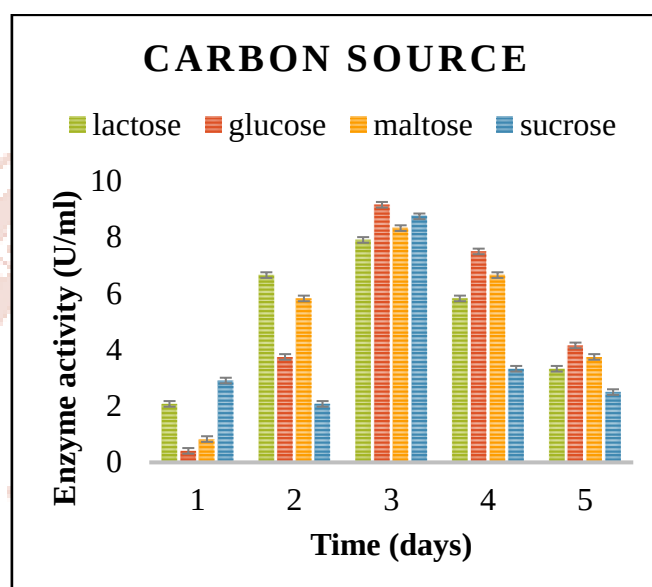


Figure 4: Effect of Carbon Sources

Several researchers have demonstrated that microbial isolates such as *Rhodococcus*, *Bacillus*, and *Pseudomonas* species show improved enzyme production when cultivated in glucose-supplemented media (Kumar *et al.*,2012). Acharya *et al.*,2016 found that glucose was the best carbon source for *Bacillus subtilis* KPA-1 esterase production, while Bhardwaj *et al.*, (2020) found that using galactose as the carbon source increased esterase activity in *Bacillus licheniformis*.

3.7.2 Effect of Nitrogen source on Esterase Activity:

Among the various nitrogen sources evaluated, isolate *Acinetobacter Pittii* exhibited maximum esterase enzyme activity when cultured in production medium supplemented with yeast extract, showing enhanced enzyme yield under optimized conditions. The highest esterase activity recorded with yeast extract was **10.41 U/ml**. Different nitrogen sources such as peptone, tryptone, yeast extract, and urea were examined.

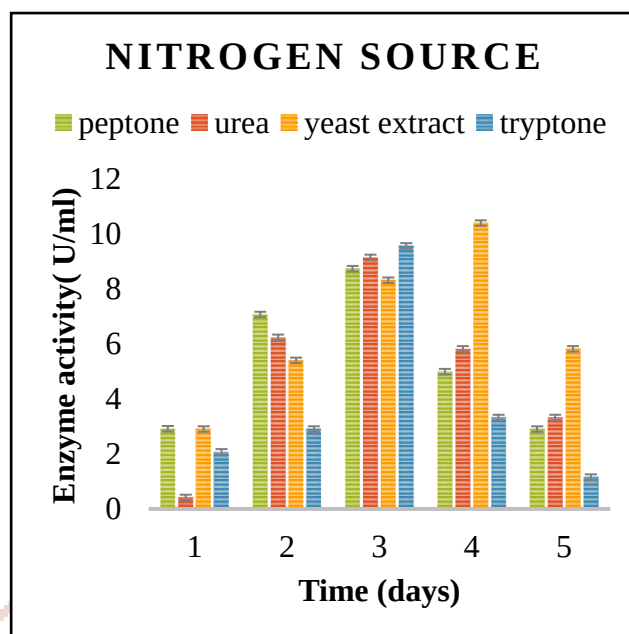


Figure 5: Effect of Nitrogen Sources

3.7.3 Effect of Substrate on Esterase Activity:

In the present study, the esterase-producing isolate *Acinetobacter Pittii* demonstrated maximum enzymatic activity when sunflower oil was used as a substrate compared to coconut oil, peanut oil, and olive oil, which resulted in comparatively lower enzyme production. The highest esterase activity recorded with sunflower oil was **11.25 U/ml**. The influence of different lipid substrates on enzyme activity was evaluated using substrate-based enzymatic assays similar to the Winkler and Stuckmann method.

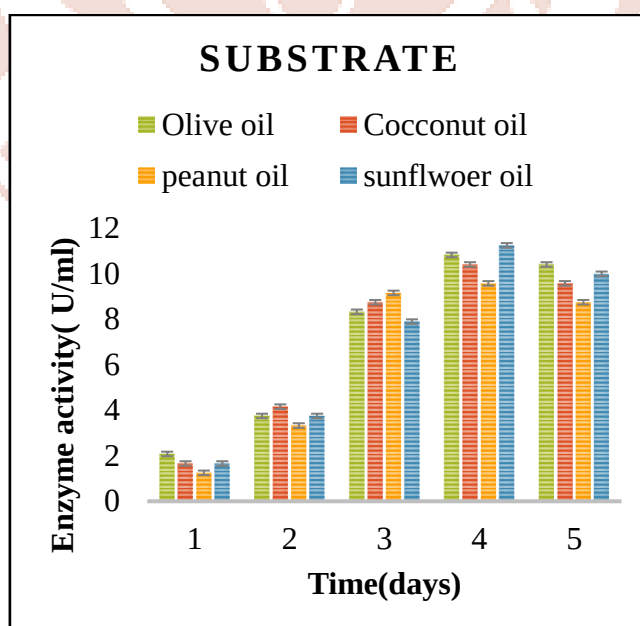


Figure 6: Effect of Substrates

Previous reports have demonstrated that Tween compounds stimulate esterase enzyme production in *Geobacillus* and *Pseudomonas* species (Dahiya *et al.*, 2022). The findings of Nadda *et al.* (2022), who also showed increased esterase synthesis in the presence of lipid-rich substrates, are in line with these results. In a similar to, Bhardwaj *et al.* (2020) found that using coconut oil as the substrate boosted the production of esterase in *Bacillus licheniformis*.

3.7.4 Effect of pH on Esterase Activity:

Optimization of environmental parameters revealed that isolate *Acinetobacter Pittii* exhibited maximum esterase activity at neutral pH (pH 7), showing enhanced enzyme production of **10.41 U/ml** compared to other tested pH conditions. pH plays a critical role in regulating enzyme stability, microbial growth, and catalytic efficiency. The optimization of pH is an important factor influencing microbial enzyme production, as it directly affects enzyme stability, catalytic efficiency, and microbial growth.

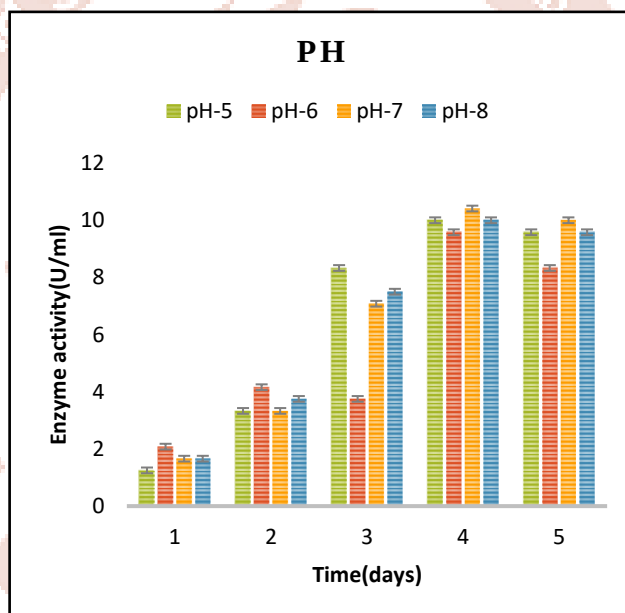


Figure 7: Effect of pH

Previous studies have reported that several esterase enzyme-producing bacteria exhibit alkalophilic characteristics (Kim *et al.*, 2010). These results align with earlier publications Nadda *et al.* (2022) found that the pH range of 6.5 to 9.5 was optimal for esterase production. In a similar vein, Acharya *et al.* (2016) found that *Bacillus subtilis* KPA-1 produced the most esterase at pH 8.0, whilst Bhardwaj *et al.* (2020) found that *Bacillus licheniformis* exhibited maximal enzyme activity at pH 8.5. The majority of esterase-producing bacteria prefer neutral to slightly alkaline conditions for effective enzyme production.

3.7.5 Effect of Inoculum Size on Esterase Activity:

Inoculum size is another critical environmental parameter regulating enzyme production and metabolic activity. In the present study, esterase activity was evaluated across different inoculum sizes. The isolate *Acinetobacter Pittii* showed maximum enzyme production at 3%, recording an activity of **11.66 U/ml**. Generally, enzyme activity increases with rising inoculum size due to enhanced molecular collisions between enzyme and substrate molecules, which accelerates reaction rates.

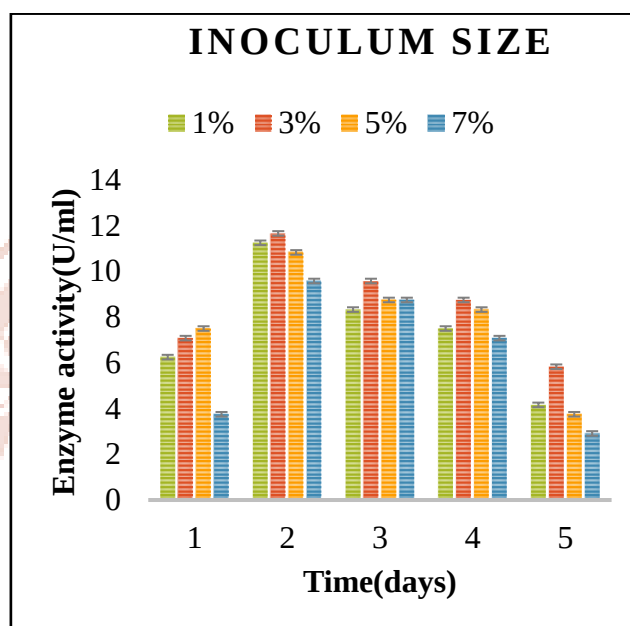


Figure 8: Effect of Inoculum Size

According to **Acharya *et al.* (2016)**, 1.5% is the ideal inoculum size for *Bacillus subtilis* KPA-1 esterase production. On the other hand, 3% was shown to be a favourable inoculum size for *Bacillus licheniformis* by **Bhardwaj *et al.* (2020)**. These differences highlight how crucial it is to optimize inoculum size according to the particular microbial strain being employed.

3.8 Partial Purification of Esterase enzyme:

The crude enzyme extract was partially purified using ammonium sulphate precipitation at **40% and 60%** saturation levels. The 40% fraction showed moderate enzyme activity, while higher enzyme activity was observed in the 60% fraction, indicating better precipitation of the enzyme at this saturation level. The precipitated enzyme fraction was further purified by dialysis to remove excess ammonium sulphate.

Table 2: Partial Purification result of *Acinetobacter pittii*

Purification steps	Total enzyme activity (U/ml)	Total protein(mg/ml)	Specific activity	Fold purification
Crude	12.5	0.60	20.83	1
40% ASP	13.33	0.55	24.23	1.1
60% ASP	14.58	0.17	85.76	4.1
Dialysis	15.83	0.16	98.93	4.7

The fold purification obtained after dialysis was 4.7, demonstrating that the enzyme was significantly purified compared to the crude extract. This step also helped in stabilizing the enzyme preparation for further analysis (Kumar et al., 2012; Sayali et al., 2013).

4. CONCLUSION:

The study successfully isolated *Acinetobacter pittii* as a potent esterase producer with high enzyme activity. Partial purification confirmed the presence of active enzyme. This strain shows strong potential for industrial applications.

ACKNOWLEDGMENT:

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Leaf Surface Fungi as Indicators of Plant Health and Disease in Forest Trees of Songadh Taluka, Gujarat, India

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Abstract:

Leaf surface fungi, or foliicolous fungi, are important components of the phyllosphere and can serve as useful indicators of plant health and disease status. Their occurrence on living leaves reflects host condition, environmental suitability, and host-pathogen interactions. The present study investigated the diversity of foliicolous fungi associated with selected host plants in Songadh Taluka, Tapi District, Gujarat, India. Field surveys were conducted from November 2025 to March 2026 in forested localities including Kelai, Kavla, Nishana, Achhalva, and the Kansari-Mavli sacred groves. Infected leaf samples were collected and examined using standard macroscopic and microscopic methods. Temporary mounts were prepared in water, glycerine, and lactophenol cotton blue, and fungal structures were identified on the basis of morphological characters.

A total of 25 species belonging to 16 genera were recorded and classified into three groups: Ascomycetes, Basidiomycetes, and Hyphomycetes. Hyphomycetes were the dominant group, represented by 16 species, followed by Ascomycetes with 8 species and Basidiomycetes with 1 species. The observed fungi showed clear host association and locality-wise variation. The findings indicate that foliicolous fungi can provide useful information on plant health, fungal diversity, and host specificity in tropical forest ecosystems. This study contributes baseline data for fungal biodiversity assessment and supports the importance of phyllosphere fungi in forest health monitoring and plant disease management.

1. Introduction:

Fungi represent one of the most diverse groups of organisms and play major roles in ecosystem functioning, including decomposition, nutrient cycling, and interactions with plants (Alexopoulos et al., 1996; Manoharachary et al., 2005). Among them, foliicolous fungi form a distinctive assemblage of leaf-inhabiting microorganisms that colonize the phylloplane (leaf surface) and establish parasitic, saprophytic, commensal, or occasionally mutualistic associations with their host plants (Andrews & Harris, 2000). These fungi are important components of tropical and subtropical biodiversity and contribute significantly to plant-microbe interactions.

The diversity and distribution of foliicolous fungi are strongly influenced by environmental factors such as temperature, humidity, rainfall, and host plant availability (Kumar et al., 2019). Many species are host-specific and may be associated with visible disease symptoms such as leaf spots, blights, mildews, chlorosis, necrosis, and premature leaf fall (Agrios, 2005). Because of these associations, leaf surface fungi may serve as excellent biological indicators of plant health and forest ecosystem dynamics.

India, as a megadiverse country, provides a wide range of ecological niches suitable for fungal growth (Manoharachary et al., 2005). However, compared with macro fungi and economically important crop pathogens, foliicolous micro fungi remain inadequately documented in many wild, forested regions. Songadh Taluka of Tapi District, Gujarat, supports rich forest vegetation and favourable humid conditions, making it a promising area for the study of phyllosphere fungal diversity.

The present investigation was undertaken to document the diversity of foliicolous fungi associated with forest plants in Songadh Taluka, to examine their morphological characteristics, and to assess their relevance as indicators of plant health and disease.

2. Materials and Methods

2.1 Study Area

The study was conducted in Songadh Taluka, Tapi District, Gujarat, India. The region is characterized by tropical moist and dry deciduous forests, varied topography, rocky hills, and rich floral diversity (Champion & Seth, 1968). The influence of the Tapi River and Ukai Reservoir contributes to relatively high humidity, creating favourable conditions for the occurrence of foliicolous fungi. Sacred groves such as Kansari-Mavli further enhance host plant diversity and provide pristine, protected habitats for fungal colonization. Field surveys were carried out systematically from November 2025 to March 2026.

2.2 Field Survey and Collection of Specimens

Periodic field surveys were conducted across five distinct forested localities: Kelai, Kavla, Nishana, Achhalva, and the Kansari-Mavli sacred groves. Leaves showing visible symptoms of fungal infection, including leaf spots, blights, mildew, and structural discoloration, were collected from various forest trees and vegetation. For each specimen, details of the specific locality, host plant species, date of collection, and macroscopic symptom type were recorded. Fresh, senescent, and necrotic infected leaves bearing visible fungal colonies were isolated in separate, labelled polythene bags and brought back to the laboratory for processing.

2.3 Macroscopic and Microscopic Examination

Macroscopic examination was carried out to record colony appearance, infection patterns, and symptom expression on the leaf surface (Agrios, 2005). For microscopic study, small portions of infected structures, fruiting bodies, or fungal scrapings were mounted in water, glycerine, and lactophenol cotton blue, a standard staining agent used to visualize fungal cell structures (Ainsworth et al., 1973). Slides were comprehensively examined under stereo and compound microscopes. Morphological characters such as hyphae, conidiophores, conidia,

asci, ascospores, fruiting bodies (e.g., thyriothecia, perithecia, cleistothecia), and other reproductive structures were observed and measured for taxonomic identification.

2.4 Photomicrography and Identification

Representative fungal structures were photographed using a digital camera coupled with a Labomed microscope. Fungal identification was carried out on the basis of diagnostic morphological features and extensive comparison with relevant monographs, taxonomic keys, and established mycological literature (Barnett & Hunter, 1998; Ellis, 1971; Subramanian, 1971).

3. Results and Discussion

3.1 Diversity of Foliicolous Fungi

The mycological survey resulted in the documentation of 25 species belonging to 16 genera associated with different forest host plants of Songadh Taluka, Tapi District, Gujarat. These fungi were distributed among three major taxonomic groups, namely Ascomycetes, Basidiomycetes, and Hyphomycetes.

Hyphomycetes represented the dominant group with 16 species, followed by Ascomycetes with eight species and Basidiomycetes with one species. The distribution of these fungal classes is summarized below in Table 1.

Table 1. Distribution of foliicolous fungi among major fungal groups

Class	Number of Genera	Number of Species	Percentage (%)	Contribution
Ascomycetes	6	8	32.0%	
Basidiomycetes	1	1	4.0%	
Hyphomycetes	9	16	64.0%	
Total	16	25	100.0%	

The clear predominance of Hyphomycetes agrees with previous studies on tropical phyllosphere fungi, where conidial (asexual) fungi constitute the most resilient and abundant component of leaf surface microflora (Andrews & Harris, 2000; Hyde et al., 2020; Subramanian, 1971). Environmental factors such as humidity, microclimate rainfall patterns, and diverse host canopy architectures are known to highly influence fungal colonization success (Bhat et al., 2022; Kumar et al., 2019). Their abundance can be directly attributed to their efficient, rapid asexual reproduction mechanisms and light, easily dispersible conidia that can adapt to rapid changes on exposed leaf surfaces.

Several host plants, including *Kigelia pinnata*, *Cassia fistula*, *Pongamia pinnata*, *Syzygium cumini*, *Annona reticulata*, and *Cocos nucifera*, harboured more than one fungal

species, indicating intricate host association and ecological adaptation. Similar host-specific preferences have been routinely highlighted across rich tropical forest ecosystems (Hyde et al., 2022; Tibpromma et al., 2018).

The detailed breakdown of identified species, host associations, and specific sampling locations is recorded below in Table 2.

Table 2. Recorded foliicolous fungi from Songadh Taluka, Gujarat

Group	Fungal Species	Host Plant(s)	Locality
Ascomycetes	<i>Asterina benghalensis</i>	<i>Annona reticulata</i> , <i>Holoptelea integrifolia</i>	Nishana
	<i>Asterina jambolana</i>	<i>Syzygium cumini</i>	Kavla
	<i>Kamalia indica</i>	<i>Cassia fistula</i>	Achhalva
	<i>Meliola mangiferae</i>	<i>Mangifera indica</i>	Kelai
	<i>Cochliobolus bicolor</i>	<i>Pongamia pinnata</i>	Nishana
	<i>Microthelia cassiae</i>	<i>Cassia fistula</i>	Kansari-Mavli
	<i>Protomyces macrosporus</i>	<i>Coriandrum sativum</i>	Kelai
	<i>Uncinula sp.</i>	<i>Azadirachta indica</i>	Kavla
	<i>Olivea tectonae</i>	<i>Tectona grandis</i>	Nishana
Hyphomycetes	<i>Alternaria alternata</i>	<i>Cassia fistula</i>	Kavla
	<i>Alternaria tenuissima</i>	<i>Pongamia pinnata</i>	Kelai
	<i>Cercospora personata</i>	<i>Arachis hypogaea</i>	Achhalva
	<i>Cercospora kikuchii</i>	<i>Glycine max</i>	Nishana
	<i>Cladosporium cladosporioides</i>	<i>Cocos nucifera</i>	Kansari-Mavli
	<i>Memnoniella echinata</i>	<i>Kigelia pinnata</i>	Nishana
	<i>Pseudocercospora sp.</i>	<i>Syzygium cumini</i>	Kavla
	<i>Stachybotrys atra</i>	<i>Cassia fistula</i>	Achhalva
	<i>Sporidesmium sp.</i>	<i>Pongamia pinnata</i>	Kelai
	<i>Sirosporium sp.</i>	<i>Holoptelea integrifolia</i>	Nishana
	<i>Tripospermum sp.</i>	<i>Kigelia pinnata</i>	Kavla

3.2 Locality-wise Distribution

Among the surveyed localities, Nishana exhibited the highest overall fungal diversity, followed sequentially by Kavla and Achhalva (Table 3). The sacred grove of Kansari-Mavli, while yielding fewer overall species counts, supported unique host-specific fungi. This underscores the crucial ecological role that sacred groves play as undisturbed micro-refugia for maintaining indigenous microbial and plant genetic diversity (Bills & Polishook, 1994).

Table 3. Locality-wise occurrence of foliicolous fungi

Locality	Number of Species	Key Ecological Characteristic
Nishana	10	High canopy density, proximity to moisture
Kavla	6	Moderately disturbed mixed forest
Achhalva	4	Rocky terrain, dry deciduous pockets
Kelai	3	Forest fringe, agricultural interfaces
Kansari-Mavli Sacred Grove	2	Undisturbed habitat, highly specialized hosts
Total	25	

3.3 Morphological Characteristics

Microscopic examination revealed a wide range of specialized vegetative and reproductive structures including thyriothecia, asci, ascospores, conidiophores, conidia, chlamydospores, and urediniospores. Hyphomycetes exhibited the most striking variation in conidial morphology, septation (cell-wall divisions), and pigmentation, which served as foundational taxonomic traits (Barnett & Hunter, 1998; Ellis, 1971, 1976).

Diagnostic Insights:

The occurrence of these fungi on symptomatic leaves indicates their utility as primary biological indicators of early disease development and host plant stress. Modern phytopathology heavily emphasizes the importance of tracking phyllosphere balance to map overall ecosystem health trends (Bhat et al., 2022; Hyde et al., 2020; Wanasinghe et al., 2020).

The key morphological characters that enabled the precise identification and taxonomic delineation (Chethana et al., 2021) of these species are detailed comprehensively below in Table 4.

Table 4. Morphological characteristics of representative foliicolous fungi

Fungal Species	Group	Important Morphological Characters	Reproductive Structures Observed	Host Plant(s)
<i>Asterina benghalensis</i>	Ascomycetes	Superficial dark mycelium with circular thyriothecia	Asci and bicelled ascospores	<i>Annona reticulata</i> , <i>Holoptelea integrifolia</i>
<i>Asterina jambolana</i>	Ascomycetes	Black colonies with shield-shaped thyriothecia	Asci and septate ascospores	<i>Syzygium cumini</i>
<i>Kamalia indica</i>	Ascomycetes	Brown mycelium with immersed fruiting bodies	Asci and ascospores	<i>Cassia fistula</i>
<i>Meliola mangiferae</i>	Ascomycetes	Dark superficial hyphae with rigid setae	Perithecia and ascospores	<i>Mangifera indica</i>
<i>Cochliobolus bicolor</i>	Ascomycetes	Septate hyphae with dark pseudothecia	Asci and multiseptate ascospores	<i>Pongamia pinnata</i>
<i>Protomyces macrosporus</i>	Ascomycetes	Thick-walled, highly spherical systemic spores	Chlamydospores	<i>Coriandrum sativum</i>
<i>Uncinula sp.</i>	Ascomycetes	White powdery, superficial mycelial colonies	Cleistothecia and ascospores	<i>Azadirachta indica</i>
<i>Olivea tectonae</i>	Basidiomycetes	Bright orange/rust-like pustules on lower leaf surface	Urediniospores	<i>Tectona grandis</i>
<i>Alternaria alternata</i>	Hyphomycetes	Dark brown septate hyphae; branched conidiophores	Muriform (hand-grenade shaped) conidia	<i>Cassia fistula</i>

<i>Alternaria tenuissima</i>	Hyphomycetes	Simple conidiophores bearing long chains of conidia	Beaked, multicelled tapering conidia	<i>Pongamia pinnata</i>
<i>Cercospora personata</i>	Hyphomycetes	Fasciculate (clustered) dark conidiophores	Long, needle-like hyaline conidia	<i>Arachis hypogaea</i>
<i>Cercospora kikuchii</i>	Hyphomycetes	Dense brown stromata with clustered conidiophores	Elongated, multi-septate cylindrical conidia	<i>Glycine max</i>
<i>Cladosporium cladosporioides</i>	Hyphomycetes	Olive-green, velvety colonies with branched systems	Fragile chains of lemon-shaped conidia	<i>Cocos nucifera</i>
<i>Memnoniella echinata</i>	Hyphomycetes	Erect dark conidiophores with distinct apical phialides	Globose, roughly ornamented conidia	<i>Kigelia pinnata</i>
<i>Pseudocercospora</i> sp.	Hyphomycetes	Pigmented conidiophores emerging directly through stomata	Pale brown, smooth septate conidia	<i>Syzygium cumini</i>
<i>Stachybotrys atra</i>	Hyphomycetes	Dark, aggregated branched conidiophores	One-celled, dark, slimy oval conidia	<i>Cassia fistula</i>
<i>Sporidesmium</i> sp.	Hyphomycetes	Thick, erect conidiophores with dark pigmented spores	Solitary, multiseptated long conidia	<i>Pongamia pinnata</i>
<i>Sirosporium</i> sp.	Hyphomycetes	Simple conidiophores with elongated, dark spores	Verrucose, multi-septate conidia	<i>Holoptelea integrifolia</i>
<i>Tripospermum</i> sp.	Hyphomycetes	Distinctive stauroconidia architecture	Characteristic star-shaped, branched conidia	<i>Kigelia pinnata</i>

4. Conclusion

The present study maps the baseline diversity of foliicolous fungi associated with forest plants in Songadh Taluka, Tapi District, Gujarat, demonstrating their prospective significance as early-warning indicators of plant health. A total of 25 species spanning 16 genera were confirmed, with Hyphomycetes dominating the landscape at 64% of total species output. The distinct distribution patterns across host plants and individual forest localities suggest that host specificity, relative humidity gradients, and regional forest canopy structures play highly influential roles in fungal distribution.

Morphological and microscopic protocols proved reliable for accurate, field-level taxonomic documentation. These baseline results establish solid groundwork for future research regarding mycological taxonomy, forest microbial ecology, and targeted plant disease conservation management in South Gujarat. Future studies integrating advanced high-throughput molecular tools will further accelerate species confirmation and resolve complex phylogenetic boundaries among these essential micro fungi.

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Nature's Tiny Titans : Harnessing Probiotics to Reduce Chemical Use (Review)

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Abstract:

Global agriculture faces increasing threats from climate change-induced stresses such as drought, salinity, and rising temperatures, which reduce crop productivity and endanger food security. While chemical fertilizers have enhanced yields, their excessive use has led to soil degradation, nutrient imbalance, and environmental pollution, driving the need for sustainable alternatives. This study explores beneficial plant-microbe interactions as a strategy to reduce dependence on synthetic agrochemicals. It focuses on plant growth-promoting rhizobacteria (PGPR), mycorrhizal fungi, and synthetic microbial communities (SynComs), which enhance plant growth through nutrient solubilization, nitrogen fixation, phytohormone production, and stress mitigation. SynComs further improve soil fertility and crop resilience through synergistic effects. Microbiome-based rhizosphere engineering offers promising solutions for restoring soil health and improving nutrient-use efficiency. Integrating microbial biostimulants into farming systems can enhance productivity, support environmental sustainability, and strengthen climate resilience, making microbiome-driven agriculture a key approach for future sustainable farming.

INTRODUCTION:

Global agriculture is increasingly challenged by climate change-induced stresses such as drought, salinity, and rising temperatures, which significantly reduce crop productivity and threaten food security. Although chemical fertilizers have historically contributed to enhanced crop yields, their prolonged and excessive use has resulted in soil degradation, nutrient imbalance, and environmental pollution. These issues highlight the urgent need for sustainable and environmentally friendly agricultural practices.

Recent advances in plant microbiome research have revealed the crucial role of rhizosphere microorganisms in maintaining soil health and supporting plant growth under adverse environmental conditions. These beneficial microbes provide an effective alternative to synthetic agrochemicals by enhancing nutrient availability, improving soil structure, and increasing plant resilience to environmental stress.

LITERATURE REVIEW:

Extensive studies have demonstrated that beneficial plant-microbe interactions play a vital role in sustainable agriculture. Plant growth-promoting rhizobacteria (PGPR) are known to enhance plant growth through mechanisms such as nutrient solubilization, biological nitrogen fixation, and production of phytohormones.

Mycorrhizal fungi establish symbiotic relationships with plant roots, improving nutrient uptake, particularly phosphorus, and enhancing plant tolerance to abiotic stresses such as drought and salinity.

Recent research has also focused on synthetic microbial communities (SynComs), which are designed combinations of beneficial microorganisms. These communities exhibit synergistic effects that improve nutrient uptake, soil fertility, and crop productivity under changing environmental conditions.

Furthermore, microbiome-based rhizosphere engineering has emerged as a promising approach for restoring soil health, enhancing nutrient-use efficiency, and reducing dependence on chemical fertilizers.

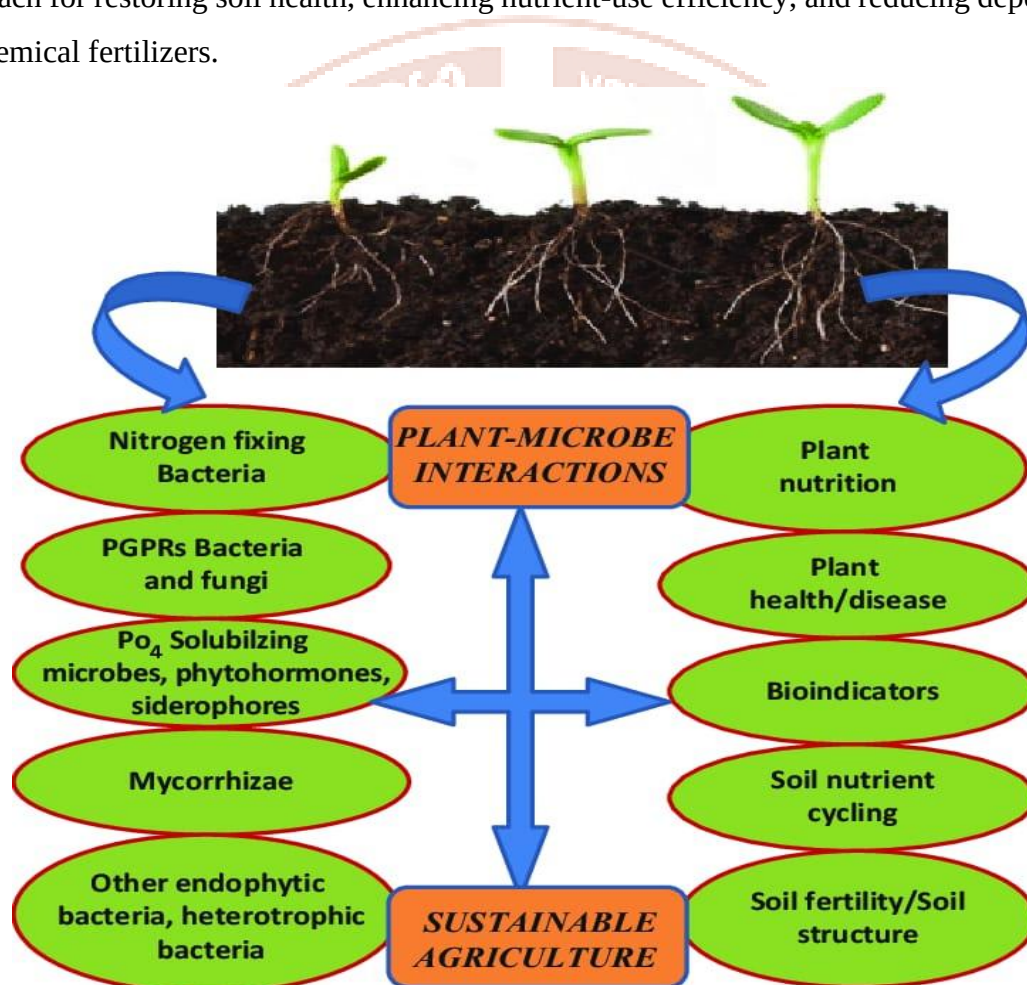


FIGURE: Plant-Microbe Interactions

OBJECTIVES:

- To study the role of plant microbiome in sustainable agriculture
- To evaluate beneficial plant–microbe interactions
- To analyze the functions of PGPR, mycorrhizal fungi, and SynComs
- To reduce reliance on synthetic agrochemicals
- To promote soil health and crop productivity under stress conditions.

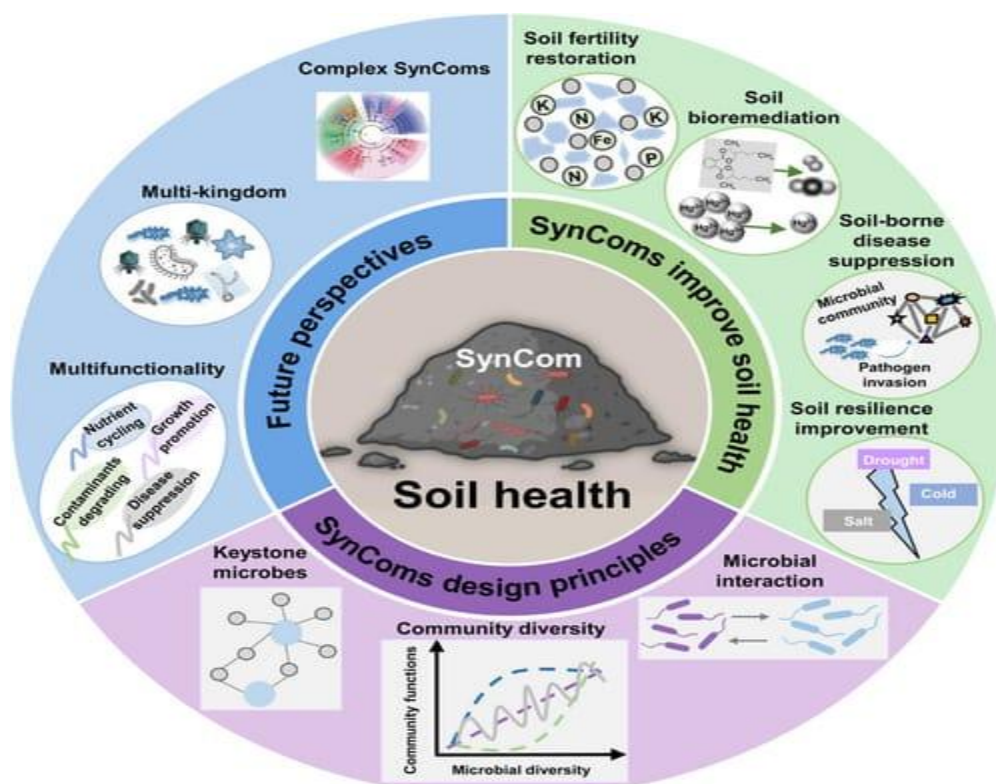


FIGURE: Synthetic microbial communities (SynComs)

METHODOLOGY:

This study is based on a comprehensive review of scientific literature related to plant microbiome and sustainable agriculture. Relevant information was collected from peer-reviewed research articles, journals, and reports focusing on plant–microbe interactions and microbial biostimulants.

The collected data were analyzed to understand the mechanisms through which beneficial microorganisms enhance plant growth, improve soil fertility, and provide resistance against abiotic stresses. Comparative analysis was also carried out to evaluate different microbial approaches.

RESULTS:

The findings indicate that beneficial microorganisms significantly contribute to improved plant growth and soil health. PGPR enhance nutrient availability through nitrogen fixation and solubilization of essential minerals. Mycorrhizal fungi improve water and nutrient uptake, especially under stress conditions.

Synthetic microbial communities (SynComs) exhibit synergistic effects that enhance crop productivity. Microbial activity helps in alleviating abiotic stress through mechanisms such as ACC deaminase activity. Overall, microbiome-based approaches improve nutrient-use efficiency, restore soil fertility, and reduce dependence on chemical fertilizers.

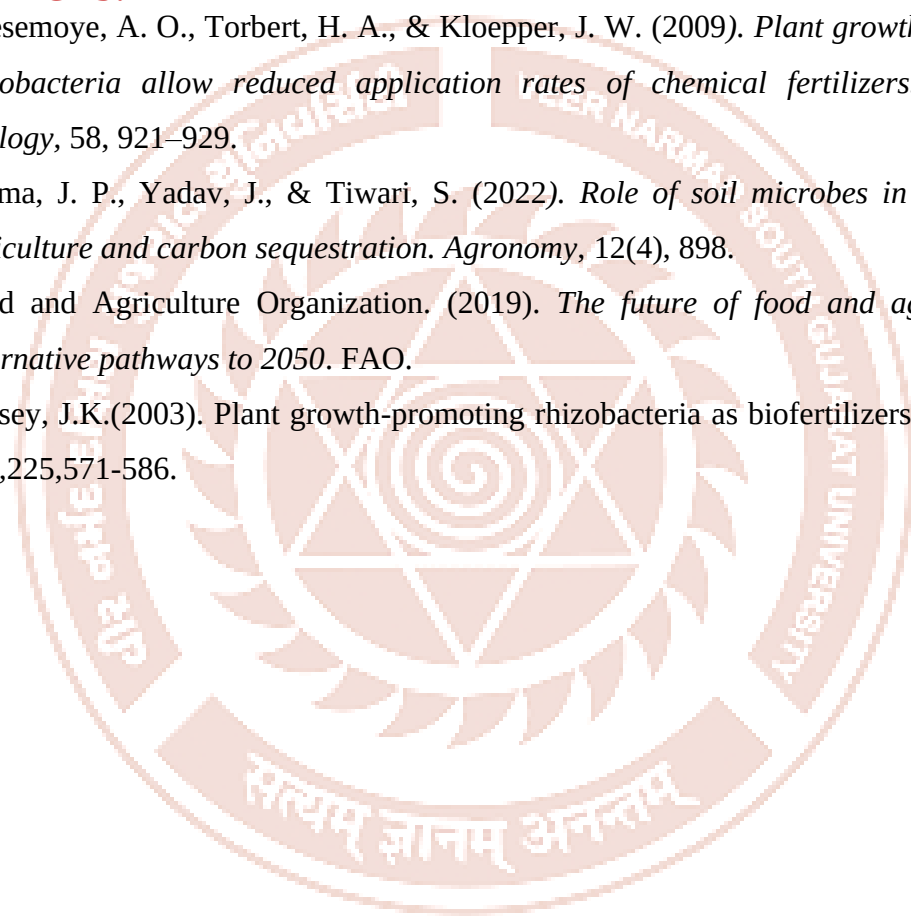
CONCLUSION:

The integration of beneficial microorganisms into agricultural practices provides a sustainable and eco-friendly alternative to chemical fertilizers. Microbial biostimulants enhance plant growth, improve soil health, and increase resilience to environmental stresses.

Microbiome-driven agriculture represents a promising strategy for achieving long-term sustainability, environmental protection, and global food security. Future research and large-scale application of these technologies are essential for developing climate-resilient agricultural systems.

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Taxonomic Diversity and Relative Abundance of Spider Fauna (Arachnida) In A Terrestrial Habitat of Daman, India

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Abstract:

As cosmopolitan inhabitants of terrestrial ecosystems, spiders serve as significant biological proxies, reflecting the overall stability and health of the environment. A systematic ground survey was conducted from July 2022 to March 2023. A total of 14 species across six families were documented. The results were revealed that family Araneidae emerged as the most dominant taxon, comprising six species representing (43%) of the total species richness followed by Salticidae with four species (29%). The families Oxyopidae, Tetragnathidae, Pholcidae, and Theridiidae were represented by a single species, collectively accounting for the remaining (28%) of the recorded diversity (7%) each. The relative abundance of the families followed the hierarchy: Araneidae > Salticidae > Oxyopidae = Tetragnathidae = Pholcidae = Theridiidae. These findings underscore the structural diversity of ecosystem and highlight the role of these arachnids as vital bioindicators for monitoring the local environmental health of the Daman region.

INTRODUCTION

Spiders (Order: Araneae) represent one of the most diverse and successful groups of terrestrial arthropods, ranking seventh globally in terms of total species richness. As ubiquitous predators, they are integral components of almost every ecosystem, where they play a vital role in maintaining ecological balance by regulating insect populations. Their sensitivity to environmental changes, such as habitat fragmentation and urban land-use shifts, makes them excellent bioindicators for monitoring ecosystem health and quality (Platnick, 2011).

In India, spider diversity is remarkably high, with current records indicating approximately 1,968 species across 62 families (Dave and Trivedi, 2024). While extensive documentation has been conducted in biodiversity hotspots like the Western Ghats, many regional urban and semi-urban pockets remain under-explored (Palem et al. 2017) Specifically, in the Union Territory of Daman, baseline data on arachnid fauna is significantly lacking. Urban green spaces, such as college campuses, often act as essential refugia for local biodiversity, supporting varied microhabitats that range from manicured gardens to undisturbed terrestrial patches.

Understanding the assemblage structure and relative abundance of spiders in these localized environments is crucial for developing a comprehensive biodiversity database (Polchaninova et al. 2023). The present study was undertaken to provide a baseline inventory of spider diversity within a college campus in Daman. By utilizing photographic documentation and morphological analysis, this survey aims to characterize the taxonomic composition and

dominance patterns of the local spider community during a nine-month period from July 2022 to March 2023.

MATERIALS AND METHODS:

STUDY AREA: The terrestrial area of Government College Daman is located at latitude 20.423374° and longitude 72.849299° and the size of whole college area is spread over 27 acres with Library building, Hostel building, Administration Block, Biology block, Chemistry block etc. (Fig:1).

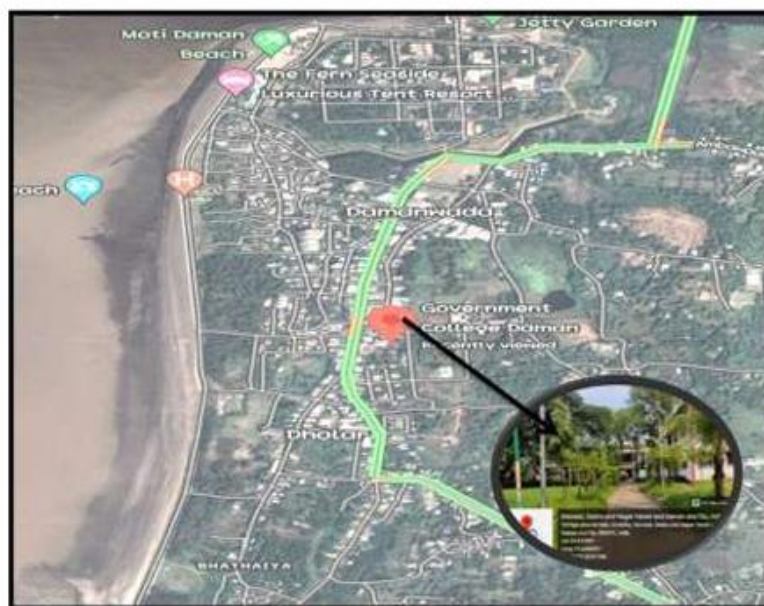


Fig. 1. Study area of Government College Daman.

METHODOLOGY: The random sampling was carried out from July 2022 to March 2023 by using trails and point observation from different areas of the College on regular interval during 8:00 am to 5:00 pm at Government College Daman employing through the “Pollard Walk” method with few modifications (Pollard, 1977; Pollard and Yates, 1993) and photographs were taken using camera for the identification following standard methods (Tikader, 1987).

- a) Hand-picking Method
- b) Kerchief Method
- c) Aerial hand Method
- d) Active-searching Method
- e) Visual-searching Method

IDENTIFICATION OF SPECIES: The collected photographed of specimens were identified with the help of standard keys followed by Tikader, 1987; Sebastian and Peter, 2009.

RESULTS AND DISCUSSION:

The results revealed a total 14 individuals of spiders were recorded, belonging to 6 families (Table: 1; Figure: 2 and 3).

A total of 14 species of spiders were representing to 6 families and 12 genera were recorded, during the study period. Families include Araneidae, Oxyopidae, Salticidae, Tetragnathidae, Pholcidae and Theridiidae. In which Araneidae is representing dominant family consisting of 6 species. The second dominant family is Salticidae comprising of 4 species. Followed by Oxyopidae with 1 species, Tetragnathidae with 1 species, Pholcidae with 1 species and Theridiidae with 1 species each were identified during the period of study.

Maximum species diversity was displayed by members of the family Araneidae. This was followed by Salticidae consisting of 4 species, followed by Oxyopidae, Tetragnathidae, Pholcidae and Theridiidae comprising one species each. The relative abundance of various families was reported from this study which can be described as Araneidae > Salticidae > Oxyopidae = Tetragnathidae = Pholcidae = Theridiidae. Similarly, result also revealed by Archana (2011) and Lawania et al., (2013) spiders of Toranmal Sanctuary, Maharashtra, India and Deeg (Bharatpur, Rajasthan), respectively and found family Araneidae to dominate in the agricultural fields. Maximum species richness of spiders in woodland due to dense canopy and availability of more prey. Our study also recorded the highest spider species richness in natural ecosystems, followed by agro-ecosystems, and this may reflect the degree of disturbance in these eco-system types (Saini et al., 2012).

Table: 1 Checklist of spider species recorded during the study period

S. No.	Class	Order	Family	Genus	Species
1	Arachnida	Araneae	Araneidae	<i>Argiope</i>	<i>anasuja</i>
2				<i>Argiope</i>	<i>keyserlingi</i>
3				<i>Araneus</i>	<i>mitificu</i>
4				<i>Neoscona</i>	<i>nautica</i>
5				<i>Cyrtophora</i>	<i>cicatrosa</i>
6				<i>Cyrtophora</i>	<i>citricola</i>
7			Pholcidae	<i>Pholcus</i>	<i>phalangioides</i>
8			Salticidae	<i>Telamonia</i>	<i>dimidiate</i>
9				<i>Colonus</i>	<i>puerperus</i>
10				<i>Hyllus</i>	<i>semicupresus</i>
11				<i>Plexippus</i>	<i>paykulli</i>

12			Tetragnathidae	<i>Leucauge</i>	<i>decorata</i>
13			Oxyopidae	<i>Oxyopes</i>	<i>salticus</i>
14			Theridiidae	<i>Steatoda</i>	<i>triangulosa</i>

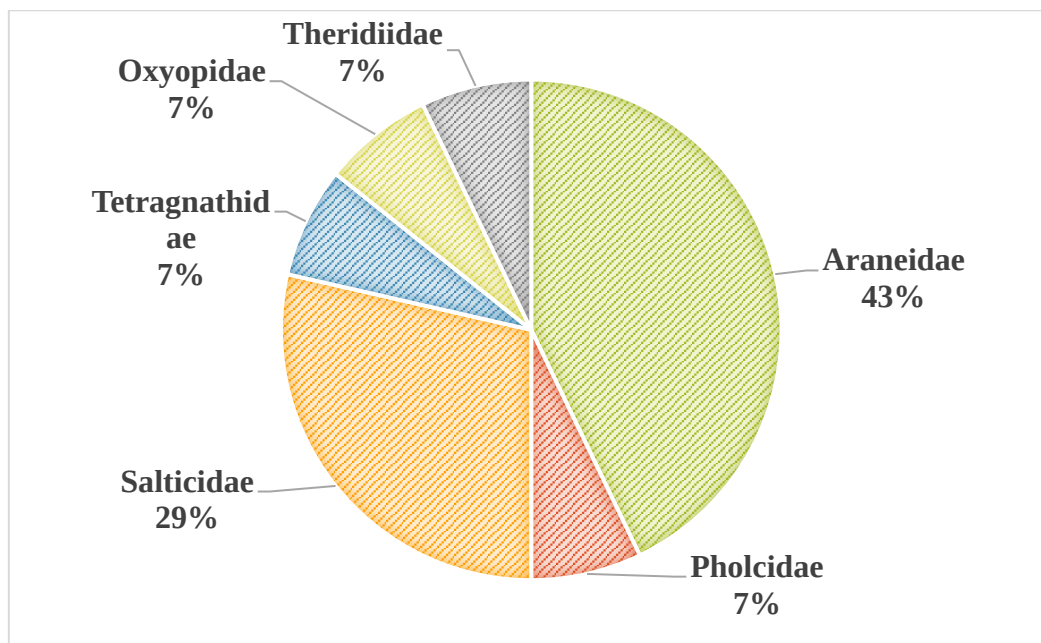


Fig: 2 Percentage wise occurrence of Spider Fauna



Fig: 1 *Argiope anasuja*



Fig: 2 *Argiope keyserlingi*



Fig: 3 *Araneus mitificus*



Fig: 4 *Neoscona nautical*



Fig: 5 *Cyrtophora cicatrosa*



Fig: 6 *Cyrtophora citricola*



Fig: 7 *Pholcus phalangioides*



Fog: 8 *Telamonia dimidiata*



Fig: 3 Qualitative diversities of Spider Fauna

CONCLUSION:

The spiders were abundant and almost spread in all around the world. They play major role as a component of the global diversity. Government College Daman is home to a wide variety of spiders; their survival is clearly under threat. We found that spiders are much more

common in quiet, natural spots than in areas frequently disturbed by people. It's evident that human activity and changes to the landscape are driving these numbers down. Since spiders play such a huge role in keeping our ecosystem in balance, we need to be more mindful of how we manage our campus green spaces. Protecting these small but vital predators isn't just about the spiders it's about preserving the natural health of our environment.

ACKNOWLEDGEMENT:

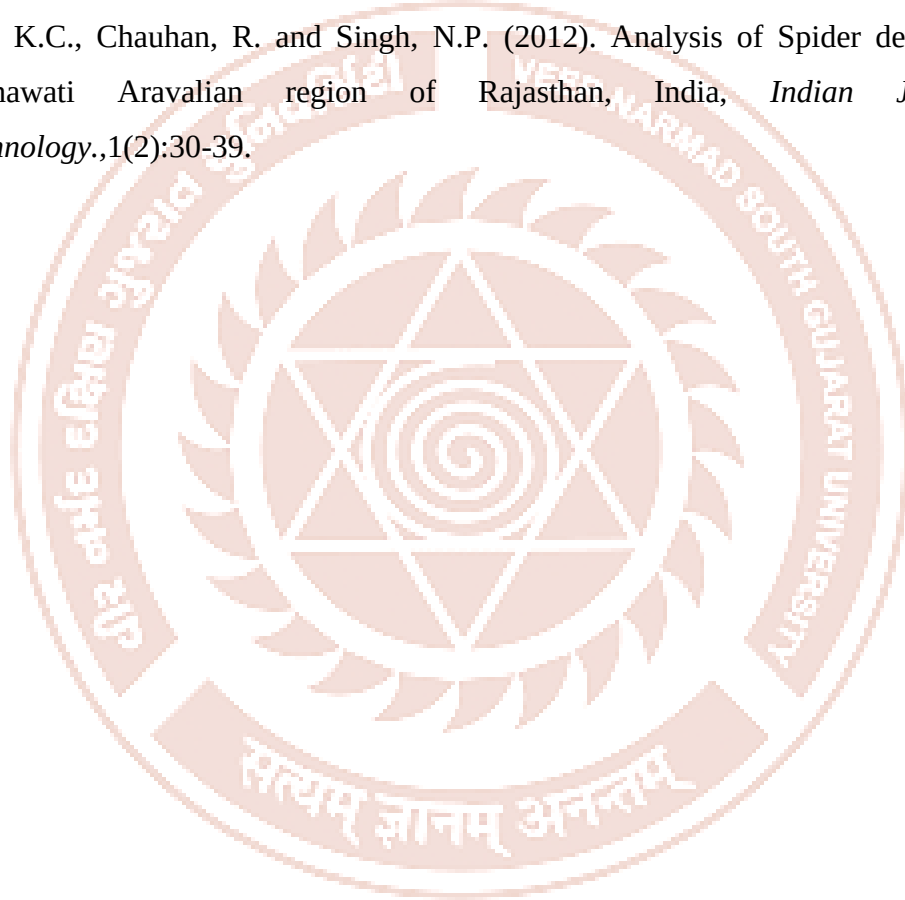
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The Biodiversity of Beetles at Songadh Fort, Tapi, Gujarat

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Abstract:

Beetles (Order: Coleoptera) represent the largest and most diverse group of insects and play vital roles in maintaining ecosystem structure and functioning. They contribute significantly to ecological processes such as nutrient cycling, decomposition, pollination, and biological control, and are widely recognized as effective bioindicators of environmental health. The present study was conducted to assess the biodiversity of beetles at Songadh Fort, Tapi District, Gujarat, a historically significant site surrounded by forested and semi-natural habitats. The primary aim of this study was to document beetle diversity and compare species composition across different microhabitats and habitat edges within the study area. Beetle sampling was carried out using hand picking and pitfall trap methods during the study period. Collected specimens were preserved and identified based on external morphological characteristics using standard identification keys and the iNaturalist platform. Observations were supported by photographic documentation to generate a checklist of beetle species. The recorded beetles represented diverse ecological guilds such as herbivores, decomposers, predators, and scavengers, highlighting their functional importance in the ecosystem. Scarabaeid beetles contributed to nutrient recycling, while predatory and herbivorous species reflected habitat quality and vegetation diversity. The findings provide a baseline checklist of beetle diversity in a historically and ecologically significant region. These results serve as a foundation for future research on seasonal variations and the role of beetles as bioindicators of ecosystem health at Songadh Fort.

Introduction:

Studying the immense variety of life on the planet and the complex relations among living things – what we now call biodiversity – has been a fundamental activity of conservationists and has become an important concern due to rapid species loss and increasing habitat degradation around the world. The time has come to record and try to conserve what is present today (Thakkar and Parikh et al 2016).

Nearly 25% of all known animal species are beetles (order Coleoptera), which are the most varied group of insects. Over 4,00,000 species have been described worldwide, with estimates exceeding one million including undescribed taxa (Chung, 2007). The transformation of forewings into tough elytra, which shield the hindwing and abdomen and allow survival variety of environments, is primarily responsible for their evolutionary success (Goczal et al., 2025). Because of this, beetles are found in almost every ecosystem, with the exception of harsh settings like oceans and polar ice caps (Bao 2020).

Beetles have a wide range of ecological functions. Through eating, herbivorous families like Chrysomalidae and Corcumelidae affect the dynamic vegetation (Dury 2013). Carabidae and Coccinellidae are examples of predatory beetles that suppress pest populations and are crucial biological control agents (Fei et al., 2023). While aquatic families like Dytiscidae and Hydrophilidae are crucial elements of freshwater ecosystems (Al Abadi and Hatem 2025). Decomposers like Scarabaeidae and Tenebrionidae support soil fertility and nutrient cycling (Muinde and Ochieng 2025). Because of their extensive biological range, beetles are crucial to the stability and proper operation of the ecosystem.

Functional diversity, which measures the extent of trait variation within communities, has become a central concept in modern biodiversity research (Petchey and Gaston, 2006). Species interactions and ecosystem functioning depend on traits like body size, dispersal ability and feeding structures (Woodcock et al., 2010; Faal 2020). Greater functional diversity reduces niche overlap, improving stability and efficiency (Fink and Snyder 2010). Beetle assemblages are influenced by landscape heterogeneity, especially predatory taxa (Woodcock et al., 2013).

With over 15,000 species identified, beetles make up a significant portion of insect biodiversity in India (Chandra et al., 2019). Ecological and functional research is still scarce, nevertheless. Gujarat is home to a variety of ecosystems, such as marshes, grasslands, forests and coastal environments. Scarabaeidae and Carabidae are the two most common families among the 177 beetle species found in 32 families according to a regional assessment (Thakkar and Parikh et al 2016). Despite this, there are still few traits based ecological studies.

Because beetles are sensitive to environmental changes, they are frequently studied as bioindicators. Aquatic beetles reflect freshwater quality (Burghelea et al., 2011) dung beetles shows nitrogen cycling (Nichols et al., 2008), and carabids react to soil and habitat conditions (Rainio and Niemela 2003). They are useful species for tracking ecosystem health under human strain because of their responsiveness.

To gain a deeper understanding of beetle diversity and ecological roles, this study combines taxonomic and functional methodologies. Although species inventories have been the main emphasis of Indian research, merging taxonomy and functional characteristic analysis offers greater insights into community structure and ecosystem functioning, supporting ecosystem management and biodiversity conservation.

Materials and Methods

The current investigation was carried out at Songadh Fort, which is situated in Gujarat, India. Built by Pillaji Rao Gaekwad in the early 1700s, the fort is perched atop a hill that offers a broad perspective of the surrounding terrain. The location (fig. 1) is roughly 21.16966° N

latitude and 73.56357° E longitude, with an average elevation of roughly 112 meters above sea level. The region is environmentally favourable for biodiversity assessment research since it is surrounded by hills, rocky terrain, and seminatural woods. In order to maximise beetle sampling throughout diurnal and crepuscular intervals, field surveys were carried out from August 2025 to February 2026 during morning and evening session.

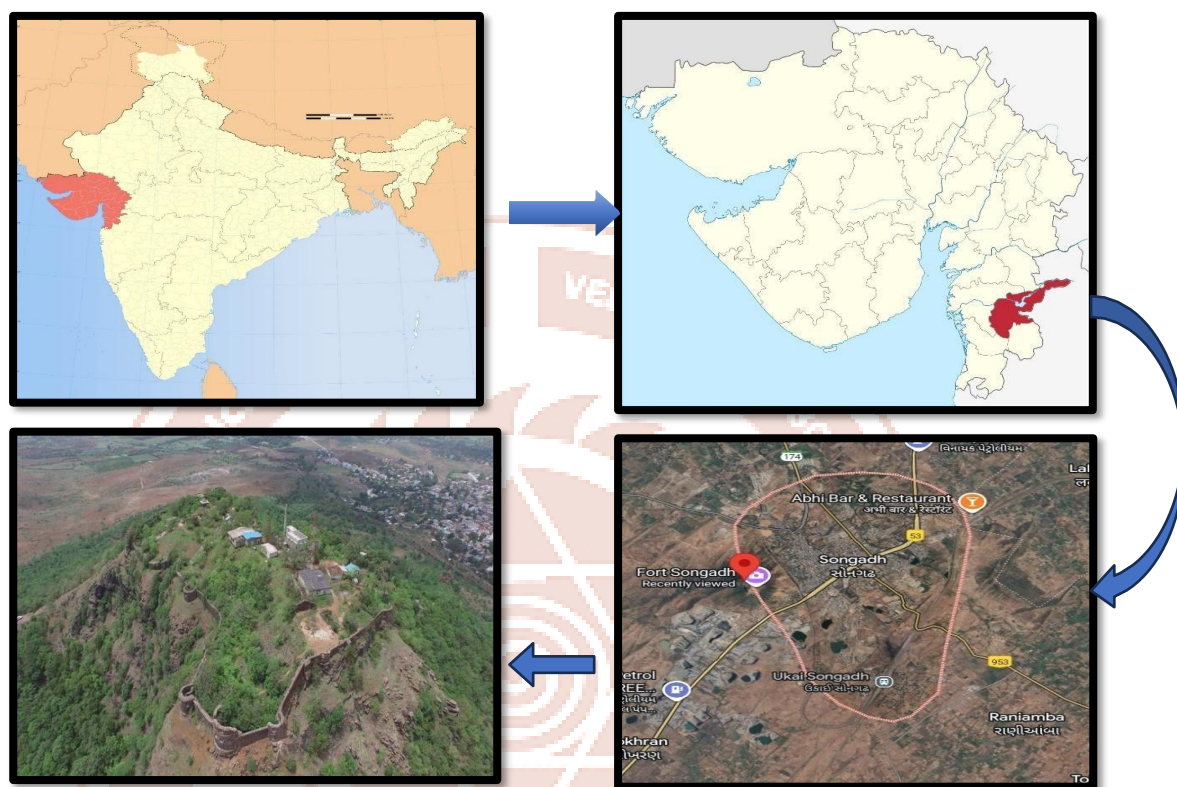


Figure 1: Study area

A variety of conventional entomological techniques were used to sample beetles. Visible beetles were collected by hand from dirt, leaf litter, stones and decaying logs. The specimens were then stored in 70% ethanol. In order to capture ground dwelling species, pitfall traps (7 -9 cm diameter) were placed at ground level and filled with soapy water or 10% formalin. The traps were left for 24 to 48 hours (Stasiov at al., 2021) using UV/Mersury vapour lamps against white cloth sheets, light trapping was used for nocturnal and phototactic beetles between dusk and 22.30 hours. Specimen were gathered with forceps and stored for identification (Dubey 2021).

Results and Discussion

During the study period (August 2025 to February 2026), 261 beetle individuals representing 23 species and 10 families were recorded at Songadh Fort (table 1). It was evident from the family-wise distribution that herbivorous gropus dominated the ecosystem. The most prevalent family (29%) was Chrysomelidae, suggesting that phytophagous beetles are

supported by rich and varied vegetation. Meloidae (23%), which is primarily dominated by *Epicauta vittata*, came next, indicating habitat suitability for individual species rather than total family diversity. Scarabaeidae (14%) and Coccinellidae (13%) showed intermediate representation, indicating the presence of stable prey populations and decaying organic materials. While lesser abundance was seen in carabidae, Cerambycidae, and Curculionidae, indication habitat or host plant restrictions, Tenebrionidae provided 11%, demonstrating adaptability to dry litter-rich microhabitats.

At Songadh Fort, the predominance of phytophagous beetle group is a clear indication of the plant diversity and vegetation structure. Beetle diversity known to be significantly influenced by habitat heterogeneity and the availability of plant resources, especially in the semi-natural environments where a variety of herbivorous species are supported by different flora (Rischen et al., 2021). Therefore, the significant representation of Chrysomelidae and Meloidae suggest that the research site offers phytophagous insects favourable tropic resources and niche diversity.

Epicauta vittata (58 individuals) and *Lilioceris Cheni* (36 individuals), which together accounted for more than one-third of all captures, demonstrated a substantial dominance in species-wise abundance. The functional core of the community was made up of a moderate assemblage of 7 – 21 individuals per species, but certain taxa were rare (less than 2 individuals), contributing to overall richness rather than abundance. In insect communities, where niche specialisation, host plant specificity and environmental filtration control species accompanied by a greater number of moderately abundant and rare species is prevalent. Similar findings have been documented in beetle assemblages, where species react differently to environmental factors and habitat layout (Magura and Lovei 2021).

Table 1: Checklist of species

No.	Taxonomic Family	Scientific Name	Common Name
1	Chrysomelidae	<i>Lilioceris cheni</i>	Potato leaf beetle
2		<i>Podagrica fuscicornis</i>	Mallow Flea beetle
3		<i>Sphaeroderma rubidum</i>	Brown flea beetle
4		<i>Monolepta australis</i>	Red shouldered leaf beetle
5		<i>Monolepta signata</i>	White spotted leaf beetle
6	Cerambycidae	<i>Batocera rufomaculata</i>	Mango stem borer
7	Meloidae	<i>Lytta vesicatoria</i>	Spanish fly
8		<i>Epicauta vittata</i>	Striped blister beetle
9	Buprestidae	<i>Sternocera chrysis</i>	Jewel beetle
10	Scarabaeidae	<i>Catharsius molossus</i>	Dung beetle
11		<i>Anomala pallida</i>	Chafer beetle
12		<i>Popilius disjunctus</i>	Bess beetle

13	Curculionidae	<i>Anthonomus grandis</i>	Boll weevil
14	Tenebrionidae	<i>Tenebrio obscurus</i>	Darkling beetle
15		<i>Asiopus minimus</i>	Darkling beetle
16	Coccinellidae	<i>Cheilomenes sexmaculata</i>	Six-spotted zigzag ladybird
17		<i>Coccinella transversalis</i>	Transverse ladybird
18		<i>Anegleis cardoni</i>	Ladybug
19		<i>Propylea dissecta</i>	Predaceous ladybird
20		<i>Henosepilachna vigintioctopunctata</i>	Hadda beetle
21	Carabidae	<i>Anthia sexguttata</i>	Six-spot ground beetle
22		<i>Galerita orientalis</i>	False Bombardier beetle
23		<i>Pheropsophus jessoensis</i>	Asian bombardier beetle

The comparatively large percentage of Tenebrionidae and Scarabaeidae emphasizes the detritivore and decomposer guilds' ecological significance in the studied area. These groups are essential for soil aeration, organic matter breakdown and nutrient cycling. Increased diversity of decomposer beetles improves ecosystem functioning, especially nutrient recycling and soil processes, according to functional ecological research (Noriega et al., 2023). Their moderate abundance indicates that Songadh Fort retains enough microhabitat variety and ecological materials to sustain these ecological processes.

Similarly, a stable prey base and trophic equilibrium throughout the environment are indicated by the prominent presence of predatory Coccinellidae. Functional complementarity, which is crucial for preserving ecological stability, is demonstrated by the presence of herbivores, predators and decomposers. Diverse insect assemblages are crucial because functional diversity has been demonstrated to have a favourable impact on ecological processes regardless of environmental fluctuation (Barragan et al., 2011; Noriega et al., 2023).

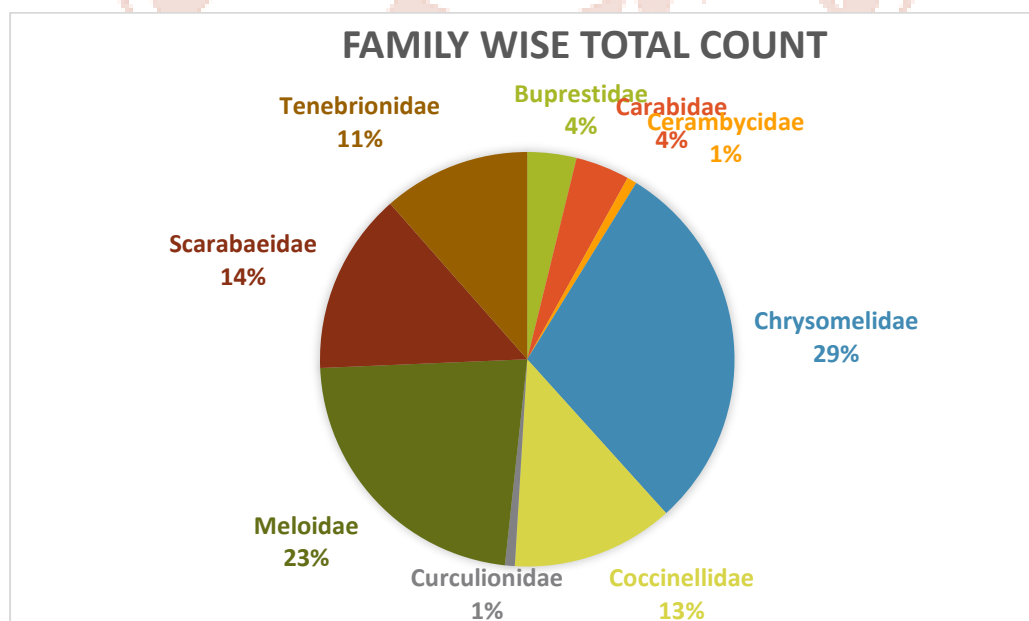


Figure 2: Family wise total count(percentage)

In other hand, habitat specialisation, or limited access to appropriate host plants and microhabitats may be responsible for the decreased abundance of Carabidae, Cerambycidae, and Curculionidae. According to the earlier research, several beetle groups exhibit uneven distribution patterns across landscapes due to their extreme sensitivity to environmental gradients and disturbances (Arellano et al., 2023; Noriega et al., 2023). This implies that although Songadh Fort is home to a varied beetle community, some taxa may be less common due to microhabitat specialisation.

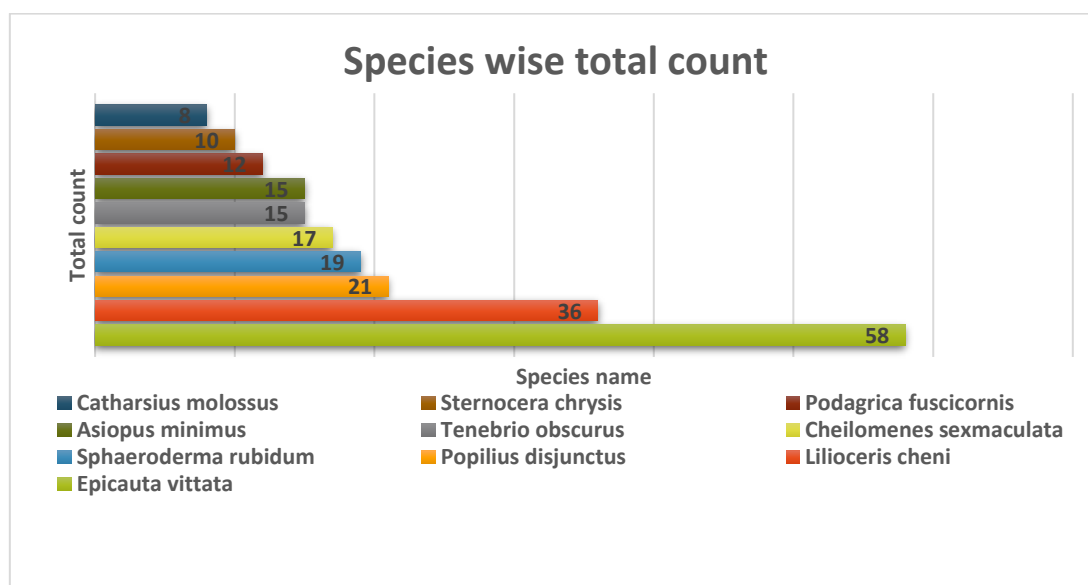


Figure 3: Species-wise total count

A comparatively stable and diverse ecosystem is suggested by the Shannon diversity index ($H' = 2.64087$), which showed moderately high diversity with decent species evenness (Table 2). These patterns of diversity are usually linked to environments with high habitat heterogeneity, where species coexistence is encouraged by differences in vegetation structure, microclimate and resource availability. Beetle diversity patterns across ecosystems are also known to be shaped by community assembly processes involving resource gradients, spatial heterogeneity, and environmental filtering.

Table 2: Computation of the Shannon Diversity Index (H')

Common name	Total Count (ni)	Proportion (pi)	Natural Log (lnpi)	Value (pi×lnpi)
Potato leaf beetle	36	0.137931	-1.981	-0.27324
Mallow Flea beetle	12	0.045977	-3.07961	-0.14159
Brown flea beetle	19	0.072797	-2.62008	-0.19073
Red shouldered leaf beetle	6	0.022989	-3.77276	-0.08673
White spotted leaf beetle	4	0.015326	-4.17823	-0.06403

Mango stem borer	2	0.007663	-4.87137	-0.03733
Spanish fly	1	0.003831	-5.56452	-0.02132
Striped blister beetle	58	0.222222	-1.50408	-0.33424
Jewel beetle	10	0.038314	-3.26194	-0.12498
Dung beetle	8	0.030651	-3.48508	-0.10682
Chafer beetle	8	0.030651	-3.48508	-0.10682
Bess beetle	21	0.08046	-2.52	-0.20276
Boll weevil	2	0.007663	-4.87137	-0.03733
Darkling beetle	15	0.057471	-2.85647	-0.16416
Darkling beetle	15	0.057471	-2.85647	-0.16416
Six - spotted zigzag ladybird	17	0.065134	-2.73131	-0.1779
Transverse ladybird	8	0.030651	-3.48508	-0.10682
Ladybug	5	0.019157	-3.95508	-0.07577
Predaceous ladybird	2	0.007663	-4.87137	-0.03733
Hadda beetle	1	0.003831	-5.56452	-0.02132
Six-spot ground beetle	1	0.003831	-5.56452	-0.02132
False Bombardier beetle	2	0.007663	-4.87137	-0.03733
Asian bombardier beetle	8	0.030651	-3.48508	-0.10682
	Sum (N=261)	Sum = 1.00000	-85.4364	Sum = -2.64087

Dominance of a few phytophagous species alongside a well- distributed assemblage of predators, scavengers and detritivores reflects balanced trophic structure. The presence of rare species (less than 2 individual) further enhances total species richness and indicates the habitat as a diversity reservoir. Rare taxa often occupy specialised niches and contribute to long term ecosystem resilience and beta diversity.



Lilioceris cheni



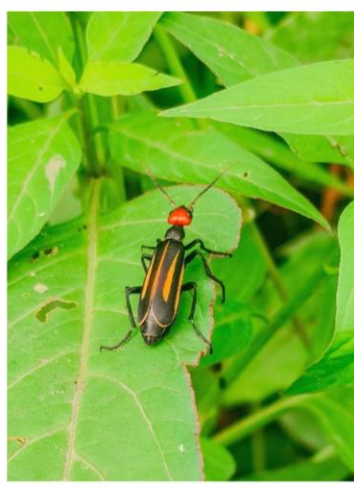
Sphaeroderma rubidum



Batocera rufomaculata



Lytta vesicatoria



Epicauta vittata



Anomala pallida



Galerita orientalis



Coccinella transversalis



Propylea dissecta



Tenebrio obscurus

In line with findings from other semi – natural landscapes in India and other tropical countries, these results verify that Songadh Fort supports a functionally varied beetle community impacted by vegetation complexity, microhabitat variation, and resource availability. Overall, the study emphasises how important it is to preserve flora diversity and ecosystem functioning.

Conclusion

The present study demonstrates that Songadh Fort supports a moderately high and functionally diverse beetle community, comprising 23 species across 10 families. The dominance of phytophagous groups, along with the presence of decomposers and predators, reflects a well-structured trophic system driven by vegetation complexity and resource availability. The observed diversity and species distribution highlight the ecological importance of habitat heterogeneity in sustaining beetle assemblages. Overall, the findings emphasize the conservation value of semi-natural habitats like Songadh Fort in maintaining insect biodiversity and associated ecosystem function.

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**Green Synthesis of Silver Nanoparticles Using Jute Mallow
(Corchorus olitorius)
Foliar Extract: Physicochemical
Characterization and Ecotoxicity Assessment on Artemia salina**

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Abstract:

Phyto-mediated green nanotechnology provides an ecologically benign, cost-effective alternative to conventional physical and chemical nanomaterial synthesis. The present study successfully engineered silver nanoparticles (AgNPs) utilizing aqueous leaf extracts of jute mallow (*Corchorus olitorius*) and conducted an extensive toxicological evaluation against the aquatic bioindicator *Artemia salina*. Aqueous foliar extracts rapidly reduced silver ions (Ag^+ to Ag^0), evidenced by a visible bathochromic shift from pale yellow to dark brown and confirmed by a sharp Surface Plasmon Resonance (SPR) peak at 430 nm on UV-Visible spectrophotometry. FTIR analysis identified key functional bands between 3856.21 and 3631.77 cm^{-1} (hydroxyl groups) and 1624.77 cm^{-1} (carbonyl/amide vibrations), establishing the role of foliar flavonoids and polyphenols as capping and stabilizing agents. Morphological imaging via FE-SEM revealed predominantly spherical nanostructures ranging from 50 to 100 nm, while Dynamic Light Scattering (DLS) confirmed a primary hydrodynamic intensity peak at 95.17 nm with a zeta potential of -8.08 mV. Computational ProTox-3.0 modeling categorized the synthesized metallic silver as Toxicity Class 3 with an estimated oral LD₅₀ of 100 mg/kg. In vivo bioassays against *Artemia salina* nauplii demonstrated time- and concentration-dependent toxicity, yielding a 48-h LC₅₀ of 40 $\mu\text{g/mL}$. Exposed nauplii exhibited progressive somatic degeneration, gut lumen aggregation of nanoparticles, loss of swimming setae, and complete motor paralysis at higher concentrations (50–200 $\mu\text{g/mL}$). These multi-analytical findings confirm that while *Corchorus olitorius* is an efficient biological reducer for AgNP production, the resulting nanoparticles retain potent biological reactivity, underscoring the necessity of aquatic ecotoxicity screening prior to environmental release.

1. Introduction:

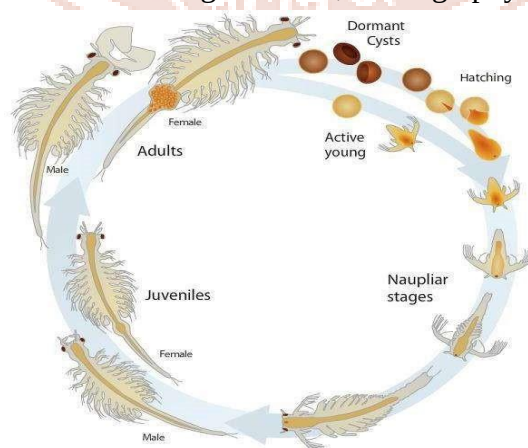
Nanotechnology represents an expanding frontier across biomedicine, environmental remediation, industrial catalysis, and antimicrobial coatings. Among engineered metal nanomaterials, silver nanoparticles (AgNPs) have attracted intense research interest due to their unique localized surface plasmon resonance, high surface-to-volume ratio, and broad-spectrum biocidal potency. However, conventional physical and chemical synthesis routes frequently involve hazardous reducing agents, toxic organic solvents, high energy inputs, and generate harmful surface by-products that hinder biological applications.

To mitigate these environmental and toxicological drawbacks, green synthesis utilizing botanical extracts has gained significant momentum. Plant extracts are rich in diverse bioactive secondary metabolites—such as polyphenols, flavonoids, terpenoids, and alkaloids—that act simultaneously as natural electron donors for metal ion reduction and steric capping agents to prevent particle coalescence. *Corchorus olitorius* L. (commonly known as jute mallow, Figure 1), a widely consumed medicinal plant in tropical and subtropical regions, contains high concentrations of phenolic acids, glycosides, and water-soluble antioxidants, rendering its foliar extract an exceptional candidate for green nanomanufacturing.



[Figures 1 & 2: Foliar specimen of *Corchorus olitorius* and *Artemia salina* nauplius]

Despite their eco-friendly synthesis, green-synthesized AgNPs inevitably enter aquatic ecosystems, where their nanospecific reactivity may exert unintended ecotoxicity on non-target microcrustaceans. The brine shrimp *Artemia salina* (Figures 2 & 3) serves as a premier ecotoxicological model organism to evaluate nanoparticle safety owing to its rapid development, non-selective filter-feeding behavior, and high physiological sensitivity.



[Figure 3: Life cycle diagram of *Artemia salina*]

The present investigation addresses two core objectives: (1) synthesizing and comprehensively characterizing AgNPs produced via aqueous leaf extracts of *Corchorus olitorius* using UV-Vis, FTIR, DLS, Zeta Potential, and FE-SEM; and (2) evaluating their aquatic safety, LC50 thresholds, and induced histomorphological deformities using *Artemia salina* nauplii, corroborated by in silico ProTox-3.0 toxicity predictions.

2. Materials and Methods

2.1 Botanical Material and Aqueous Extraction

Healthy foliar specimens of *Corchorus olitorius* were harvested from the botanical grounds of Veer Narmad South Gujarat University (VNSGU), Surat, Gujarat, India. The leaves were washed thoroughly with running tap water followed by double-distilled water to eliminate surface particulates. Cleaned leaves were shade-dried at room temperature ($25 \pm 2^\circ\text{C}$) for 10 days and pulverized into fine powder using a sterile mechanical grinder. For aqueous extraction, 10.0 g of powder was mixed with 100 mL double-distilled water (1:10 w/v) and heated at 60°C for 10 minutes under continuous magnetic stirring. The cooled suspension was filtered through Whatman No. 1 filter paper, and the clear filtrate was immediately utilized for nanoparticle fabrication.

2.2 Biogenic Synthesis and Centrifugal Purification of AgNPs

An analytical stock of 1 mM silver nitrate (AgNO_3) was prepared in double-distilled water. The aqueous *C. olitorius* extract and AgNO_3 solution were combined in a 1:9 volumetric ratio (10 mL extract added dropwise to 90 mL AgNO_3) under continuous stirring at ambient room temperature for 60 minutes. Nanoparticle synthesis was monitored by visual color transition from light yellow to dark brown. The colloidal AgNPs were separated by centrifugation at 12,000 rpm for 15 minutes, washed twice with distilled water to remove unbound phytochemical residues, redispersed in deionized water, and stored in amber glass vials at 4°C .

2.3 Spectroscopic and Morphological Characterization

- **UV-Visible Spectroscopy:** Spectral absorption was recorded from 300 to 700 nm using a UV-Vis spectrophotometer to detect the characteristic Surface Plasmon Resonance (SPR) peak.
- **FTIR Spectroscopy:** Chemical functional groups on the AgNP surface were identified using a Bruker FTIR spectrometer over the range of 4000 to 500 cm^{-1} .
- **Dynamic Light Scattering (DLS) and Zeta Potential:** Hydrodynamic size distribution, polydispersity index (PDI), and net surface electrostatic charge were determined via a Malvern Zetasizer.

- **Field Emission Scanning Electron Microscopy (FE-SEM):** Nanoparticle surface morphology and dimensional distributions were visualized on a carbon-coated copper grid using FE-SEM.

2.4 In Silico Toxicity Profiling

Computational toxicology was modeled using the ProTox-3.0 platform to predict median lethal dose (LD50), acute toxicity class, ecotoxicity indicators, and target organ endpoints.

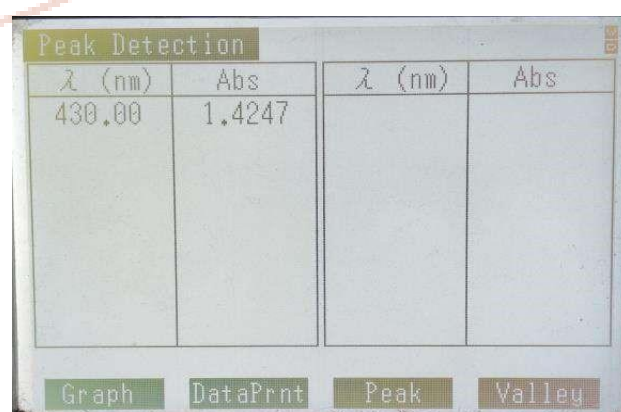
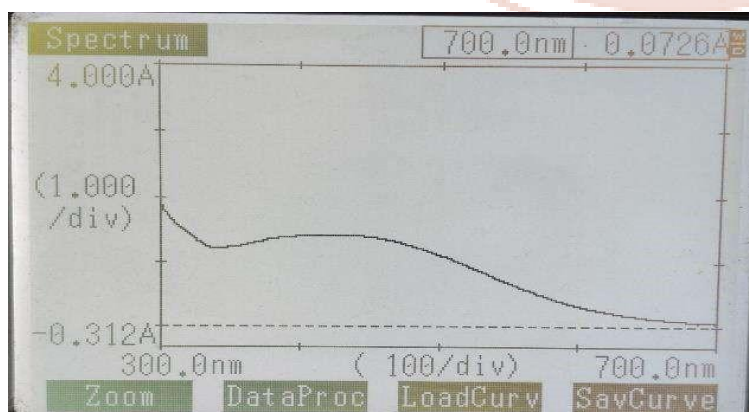
2.5 In Vivo Artemia salina Ecotoxicity Assay

Artemia salina cysts were hatched in artificial seawater (25 g/L sea salt + 1.5 g/L NaHCO₃, pH 8.2) under continuous illumination and aeration for 24 hours. Actively swimming instar-I nauplii were harvested for toxicity testing. Bioassays were conducted across two concentration regimes: Low Range (10, 20, 30, and 40 µg/mL) and High Range (50, 100, 150, and 200 µg/mL). Ten active nauplii were transferred into 2 mL test solutions in 24-well microplates in triplicate, accompanied by seawater controls. Mortality percentages were recorded at 24 h and 48 h, from which the 48-h LC50 was calculated. Behavioral changes (motility loss, sinking) and morphological alterations (gut blockage, appendage loss) were documented under compound microscopy.

3. Results and Discussion

3.1 Visual Confirmation and UV-Vis Spectroscopy

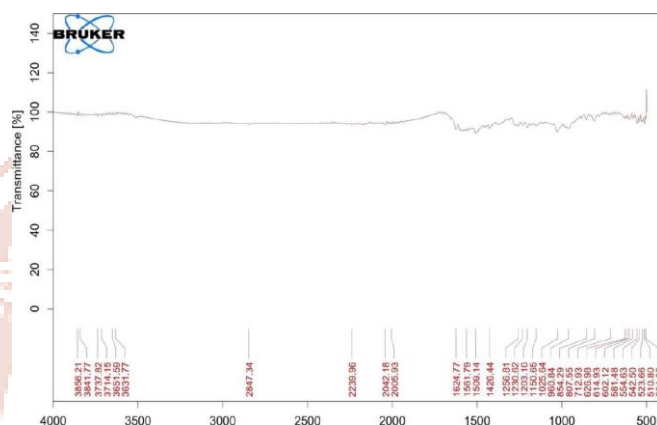
The reaction mixture displayed a distinct visual transformation from pale yellow to deep colloidal brown within 24 hours of adding *C. olitorius* foliar extract to the silver nitrate solution. This bathochromic shift indicates the reduction of Ag⁺ cations to metallic Ag⁰ nanoparticles, driven by the collective excitation of surface conduction electrons. UV-Vis spectral scanning showed a strong, symmetric SPR absorption band centered at 430.0 nm with an absorbance value of 1.4247 (Figures 4 & 5). The sharpness and single peak symmetry confirm the formation of predominantly spherical, isotropic silver nanoparticles.



[Figures 4 & 5: UV-Vis spectrum and instrument peak readout]

3.2 FTIR Surface Chemistry and Phytochemical Capping

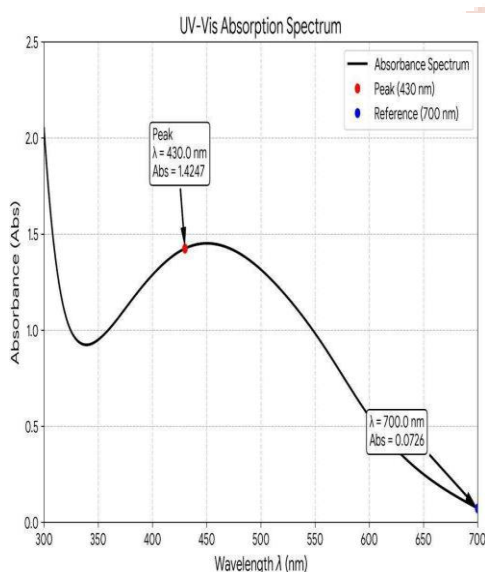
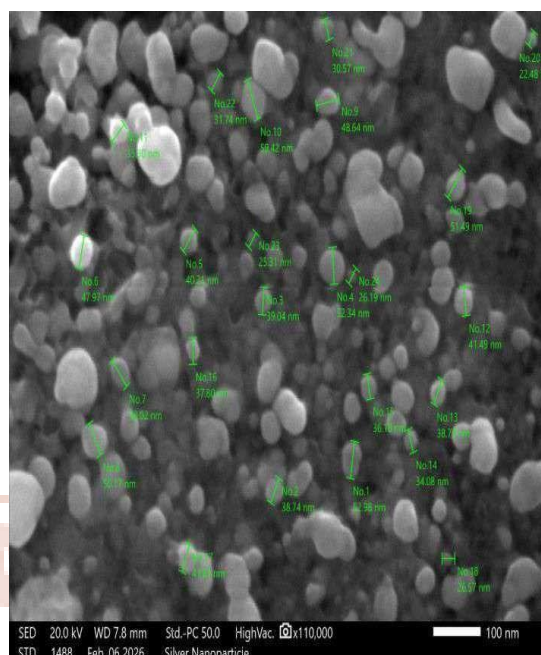
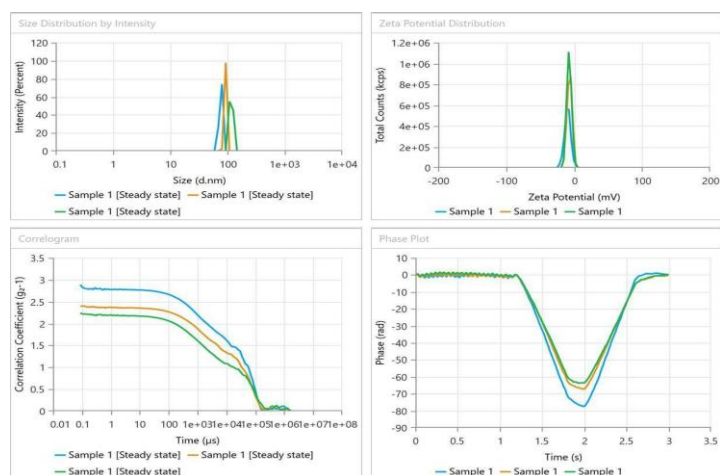
FTIR spectral analysis revealed characteristic absorption bands at 3856.21, 3737.82, 3631.77, 2847.34, 1624.77, 1561.79, 1426.44, 1256.81, 1203.10, and 1025.64 cm⁻¹ (Figure 6). The high-frequency bands between 3856 and 3631 cm⁻¹ correspond to O-H and N-H stretching vibrations of polyphenols and flavonoids. The intense peak at 1624.77 cm⁻¹ represents carbonyl (C=O) and amide-I stretching, while the peaks around 1256–1025 cm⁻¹ denote C-O phenolic stretching. These functional groups confirm that foliar polyphenols and proteins of *C. olitorius* actively mediate Ag⁺ reduction and form a protective organic capping shell around the nanoparticles.



[Figure 6: Bruker FTIR spectrum]

3.3 DLS Particle Size, Zeta Potential, and FE-SEM Morphology

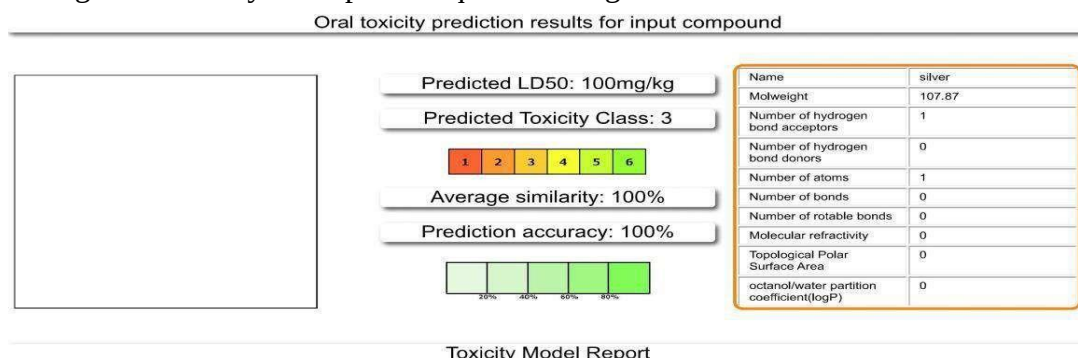
Dynamic Light Scattering (DLS) analysis documented a major intensity peak at 95.17 nm, corresponding to the primary hydrodynamic diameter of the nanoparticles (Figure 7). The overall Z-average was measured at 1725 nm with a PDI of 1.0, reflecting secondary colloidal aggregation in aqueous media due to macromolecular phytochemical bridging. The zeta potential was recorded as -8.08 mV. This negative surface charge originates from ionized phenolate and carboxylate functional groups on the capping layer, conferring moderate steric stabilization. FE-SEM micrographs demonstrated predominantly spherical AgNPs with core physical dimensions between 50 and 100 nm (Figure 8), showing close dimensional alignment with the primary DLS peak.



[Figure 7: DLS and Zeta Potential quadrant plot] | [Insert Figure 8: FE-SEM micrograph]

3.4 In Silico ProTox-3.0 Toxicity Prediction

Computational toxicology screening via ProTox-3.0 predicted an oral LD50 of 100 mg/kg, classifying the biogenic silver as Toxicity Class 3 (moderate toxicity, Figure 9). The model flagged positive activity for general ecotoxicity, blood-brain barrier permeation, and neurotoxic pathways, while showing negative profiles for mutagenicity and carcinogenicity, reinforcing the necessity of empirical aquatic testing.



[Figure 9: ProTox-3.0 in silico model report]

3.5 *Artemia salina* Ecotoxicity and LC50 Determination

In vivo toxicity testing using *Artemia salina* nauplii revealed marked concentration- and exposure-time-dependent lethality:

- **Low-Range Exposure (10–40 µg/mL):** Mortality ranged from 22% to 38% after 24 hours, rising to 38–50% after 48 hours. At 40 µg/mL, exactly 50% mortality was recorded at 48 hours, establishing the 48-h LC₅₀ as 40 µg/mL.
- **High-Range Exposure (50–200 µg/mL):** Severe lethality occurred, ranging from 33% to 47% at 24 hours and escalating to 53–72% after 48 hours (maximum mortality of 72% at 150 µg/mL).

3.6 Sublethal Morphological and Behavioral Deformities

Microscopic examination revealed progressive structural degeneration in exposed nauplii compared to transparent, actively swimming controls. Surviving nauplii exhibited heavy accumulation of dark nanoparticle aggregates in the digestive tract, severe shrinkage of abdominal segments, loss of swimming antennae/setae, and cellular lysis. Behaviorally, exposed organisms displayed erratic spinning, loss of equilibrium, bottom sinking, and eventual paralysis, demonstrating that green-synthesized AgNPs induce significant physical obstruction and oxidative stress in microcrustaceans.

4. Conclusion

Aqueous leaf extracts of *Corchorus olitorius* provide an effective, eco-friendly biological medium for the rapid synthesis of stable, spherical silver nanoparticles (50–100 nm, SPR peak at 430 nm). However, toxicological bioassays revealed that these green AgNPs possess significant biological reactivity, exhibiting a 48-h LC₅₀ of 40 µg/mL and causing severe gut blockage and appendage deformities in *Artemia salina*. These findings demonstrate that green nanomanufacturing does not inherently guarantee aquatic environmental safety, highlighting the vital importance of integrating ecotoxicity screening into nanomaterial development.

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