

CONTENTS

TITLE	PAGE NO.
CERTIFICATE	I
DECLARATION	II
ACKNOWLEDGMENT	III
LIST OF FIGURES	IV-IX
LIST OF TABLES	X-XI
ABBREVIATION USED	XII-XIV
CHAPTER I INTRODUCTION	1-7
1.1. Study Background	1-6
1.2. Scientific Limitation	7
1.3. Research Objectives	7
CHAPTER II REVIEW OF LITERATURE	8-41
2.1. Sources and Environmental Behavior of nitro-PAHs	8-10
2.2. Chemical Structure and Classification of nitro-PAHs	11-18
2.2.1. Solubility of nitro-PAHs and Environmental Implications	12-13
2.2.2. Melting Points of nitro-PAHs and their Structural Influence	13
2.2.3. Boiling Points of Nitro-PAHs and the Influence of Intermolecular Forces	14
2.2.4. Vapor Pressure of nitro-PAHs: Structural and Environmental Influences	14-15
2.2.5. Chemical Reactivity and Environmental Transformation of nitro-PAHs	15-16
2.3. Detection and Monitoring Challenges of nitro-PAHs in Environmental Matrices	17-22
2.3.1. Sampling Techniques for nitro-PAHs in Different Environmental Matrices	17-18
2.3.2. Extraction Methods for nitro-PAHs from Environmental Matrices	18-20
2.3.2.1. Liquid-liquid Extraction	18

2.3.2.2. Liquid-phase Microextraction (LPME)	18-20
2.3.2.3. Soxhlet Extraction for nitro-PAHs: Methodology and Applications	20
2.3.2.4. QuEChERS Approach for nitro-PAHs Extraction: Methodology and Applications	20-21
2.3.2.5. Solid-Phase Microextraction for nitro-PAHs Analysis: Principles and Advances	21-22
2.3.3. Analytical Techniques for nitro-PAHs Detection in Environmental Samples	22
2.4. Toxicological Effects and Human Exposure to nitro- PAHs	23-30
2.4.1. Genotoxic Mechanisms of nitro-PAHs	23-24
2.4.2. Oxidative Stress and Inflammatory Responses Induced by nitro-PAHs	24-25
2.4.3. Carcinogenic Potential of nitro-PAHs and Their Impact on Organisms	25-27
2.4.4. Impact of nitro-PAHs on Plant Health and Development	27
2.4.5. Impact of nitro-PAHs on Soil Microbial Communities and Ecosystem Dynamics	28
2.4.6. Persistence and Bioaccumulation of nitro- PAHs: Environmental and Health Implications	28-29
2.5. Regulatory Challenges and Advancements in Phytoremediation Strategies for nitro-PAHs	39-31
2.6. Microbial Degradation of nitro-PAHs	32-36
2.7. Factors Affecting Phytoremediation and Microbial Remediation Efficiency	36-37
2.8. Key Factors Influencing the Efficiency of Bioremediation Technologies for nitro-PAHs Degradation	37-39

2.9. Integrated Bioremediation: Enhancing Efficiency Through Synergistic Approaches	39-40
2.10. Conclusion and Future Prospects	40-41
CHAPTER III MATERIALS AND METHODS	42-67
3.1. Chemicals and Reagents	42-43
3.1.1. Analytical Standards and Solvents	42
3.1.2. General Chemicals	42
3.1.3. Microbiological Media and Culture Components	43
3.1.4. Acids and Sequencing Reagents	43
3.1.5. Other Materials	43
3.2. Isolation and Identification of Potential nitro-PAHs Degrading Rhizobacteria and nitro-PAHs Accumulating Plants	43-57
3.2.1. Site Description	43-44
3.2.2. Screening and Selection of nitro-PAHs Accumulating Plant Species	44-45
3.2.3. Pot Experiment for Quantitative Estimation of nitro-PAHs Accumulation	45-47
3.2.4. Isolation of Potential nitro-PAHs Degrading Rhizobacteria	47-48
3.2.5. Compatibility Assessment and Biodegradation Efficiency of nitro-PAH- Degrading Rhizobacteria	48-49
3.2.6. Maintenance and Storage of Bacterial Isolates	49
3.2.7. Biochemical Characterization	50-52
3.2.7.1. Gram Staining	50
3.2.7.2. Citrate Utilization	50
3.2.7.3. Methyl Red Test	50-51
3.2.7.4. Voges Proskauer Test	51
3.2.7.5. Urease Test	51
3.2.7.6. Catalase Test	51
3.2.7.7. Gelatine Test	52
3.2.7.8. Oxidase Test	52

3.2.8. Genomic DNA Extraction and 16S rDNA Sequencing	52
3.2.9. <i>In Vitro</i> Assessment of Plant Growth-Promoting Traits	53-56
3.2.9.1. Phosphate Solubilization Assay	53-54
3.2.9.2. Siderophore Production and Quantification	54
3.2.9.3. Ammonia Production Assay	54-55
3.2.9.4. Indole Acetic Acid (IAA) Production Assay	55
3.2.9.5. Hydrogen Cyanide (HCN) Production Assay	55-56
3.2.9.6. The Triple Sugar Iron (TSI) Fermentation Test	56
3.2.10. Rhizobacterial Inoculation and Growth Assessment in Plants	56-57
3.3. Preparation of Biostimulant from Agricultural Byproducts	57-59
3.3.1. Amino Acid Analysis Using an Amino Acid Analyzer	58
3.3.2. Greenhouse Experiment for Biostimulant Effectiveness	59
3.4. Formulation of Plant-Bacterial Co-Inoculum	60
3.5. Microcosm Soil Collection, Preparation, and Nitro-PAHs Spiking	60-67
3.5.1. Microcosm Experiment Setup and Plant Transplantation	61
3.5.2. Nitro-PAHs Extraction, Purification, and GC-MS Analysis	61-62
3.5.3. Evaluation of Soil Physicochemical Properties	62-64
3.5.3.1. pH	62
3.5.3.2. Electrical Conductivity	62
3.5.3.3. Organic Carbon	62-63

3.5.3.4. Available Nitrogen	63
3.5.3.5. Available Phosphorus	64
3.5.3.6. Available Potassium	64
3.5.4. Analysis of Plant's Antioxidant Enzyme	64-67
3.5.4.1. Peroxidase (POD)	65
3.5.4.2. Ascorbate Peroxidase (APX)	65-66
3.5.4.3. Superoxide Dismutase (SOD)	66-67
3.5.4.4. Catalase	67
3.6. Statistical Analysis	67
CHAPTER IV RESULTS AND DISCUSSION	68-156
4.1. Screening and Selection of nitro-PAHs Accumulating Plant Species	68-80
4.2. Isolation of Potential nitro-PAHs Degrading Rhizobacteria	80-83
4.3. Biochemical Characterization	83-87
4.3.1. Gram Staining	83
4.3.2. Citrate Utilization	84
4.3.3. Methyl Red Test	84
4.3.4. Voges Proskauer Test	84-85
4.3.5. Urease Test	85
4.3.6. Catalase Test	85
4.3.7. Gelatine Test	85-86
4.3.8. Oxidase Test	86-87
4.4. Molecular Characterization and Phylogenetic Analysis	88-93
4.5. Compatibility Assessment and Biodegradation Efficiency of nitro-PAH-Degrading Rhizobacteria	94-111
4.6. Characterization of Plant growth-promoting (PGP) activities	112-125
4.6.1. <i>In Vitro</i> Assessment of Plant Growth-Promoting Traits	112-119
4.6.1.1. Phosphate Solubilization	112-113
4.6.1.2. Siderophore Production	113-114
4.6.1.3. Ammonia Production	114-115
4.6.1.4. Indole Acetic Acid Production	115-116

4.6.1.5. HCN Production	116-117
4.6.1.6. The Triple Sugar Iron (TSI) Test	117-119
4.6.2. Rhizobacterial Inoculation and Growth Assessment in Plants	120-125
4.7. Preparation of Biostimulant from Agricultural Byproducts	125-131
4.7.1. Amino Acid Analysis Using an Amino Acid Analyzer	125-126
4.7.2. Greenhouse Experiment for Biostimulant Effectiveness	127-131
4.8. Nitro-PAH Degradation by Plant-Bacterial Co- Inoculum and Biostimulant in Microcosmic Soil	131-143
4.8.1. 1-nitropyrene Degradation Dynamics	131-136
4.8.2. 2-nitrofluorene Degradation Dynamics	136-143
4.9. Effect of Plant-Bacterial Co-Inoculum and Biostimulant on Soil Properties in nitro-PAH Microcosms	144-148
4.10. Effect of Plant-Bacterial Co-inoculum and Biostimulant Interactions on the Plant's Stress Response in nitro-PAH-Contaminated Microcosms	149-156
CHAPTER V	
CONCLUSION AND FUTURE PROSPECTS	157-158
REFERENCES	159-209
APPENDIX-I	209-217
APPENDIX-II	218-249
APPENDIX-III	250-251