

**STUDY ON MICROBIAL SUCCESSION WITH RESPECT TO CROP
PRODUCTIVITY IN RECLAIMED AGRICULTURAL SOIL OF EAST
KOLKATA WETLANDS**

***THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY (SCIENCE) IN MICROBIOLOGY***

Submitted by

SANJANA GHOSH

PHD/20/MCB/01 (2020-2025)



POST GRADUATE AND RESEARCH DEPARTMENT OF MICROBIOLOGY

ST. XAVIER'S COLLEGE (AUTONOMOUS), KOLKATA

AFFILIATED TO

UNIVERSITY OF CALCUTTA

Under the Supervision of

Dr. Sudeshna Shyam Choudhury (Supervisor)

Associate Professor

Post Graduate and Research Department of Microbiology

St. Xavier's College (Autonomous), Kolkata

Dr. Ayan Chandra (Joint Supervisor)

Assistant Professor

Department of Statistics

St. Xavier's College (Autonomous), Kolkata

Dr. Arup Kumar Mitra (Joint Supervisor)

Associate Professor

Post Graduate and Research Department of Microbiology

St. Xavier's College (Autonomous), Kolkata

THIS THESIS IS DEDICATED
TO MY LOVELY PARENTS
WHOSE UNWAVERING
SUPPORT, SACRIFICE, AND
GUIDANCE HAVE BEEN THE
FOUNDATION OF MY
JOURNEY.

ACKNOWLEDGEMENTS

My heartfelt thanks and deepest gratitude to Almighty, whose blessings, guidance, and grace have been my constant companions throughout this journey. It is only by His benevolence that I have been able to embark upon this academic endeavour and overcome the numerous challenges that come with a doctoral pursuit. In moments of doubt, uncertainty, and difficulty, it was His blessings that provided me with strength, clarity, and perseverance. This PhD journey, with all its highs and lows, has been a humbling experience that reminded me of the importance of faith, patience, and dedication. I am eternally grateful to the Almighty for giving me the wisdom, resilience, and opportunity to complete this work and for surrounding me with remarkable mentors, colleagues, and family who have made this achievement possible.

I owe my deepest and most sincere gratitude to **Rev. Father Dr. Dominic Savio, SJ., Principal, St. Xavier's College (Autonomous), Kolkata**, whose visionary leadership and unwavering support have been a beacon throughout my academic journey. Rev. Father Dr. Dominic Savio has created an environment where students can pursue excellence with confidence, where intellectual curiosity is nurtured, and where perseverance is recognized and encouraged. His belief in holistic education, combining academic rigor with moral and ethical guidance, has shaped not just the institution but also the lives of students like me. During moments of doubt, his encouragement reminded me of the larger purpose of this journey, reinforcing the importance of diligence, integrity, and resilience. I am deeply grateful to him for his trust, guidance, and for fostering a supportive atmosphere that enabled me to pursue my doctoral research with dedication and focus.

I am equally indebted to **Prof. Bertram Da Silva, Vice-Principal, Arts and Science, St. Xavier's College**, for his constant encouragement, thoughtful advice, and mentorship. Prof. Da Silva's approachability and readiness to assist have made the complex administrative and academic pathways navigable, and his insights into research methodology, academic planning, and scholarly rigor have greatly influenced my journey. His support has not only facilitated the smooth conduct of my research but has also motivated me to uphold the standards of excellence that this institution represents.

I would like to express my sincere gratitude to **Rev. Dr. Joseph Kulandai, SJ, Vice Principal, Department of Commerce, Rev Dr. Peter Arockiam, Vice Principal, Department of Management Studies and Commerce Evening, and Rev Fr. Johnson Padiyara, SJ, Vice Principal, Raghampur Campus** for their encouragement, guidance, and support. Their willingness to engage with students, their constructive feedback, and their constant reassurance provided a strong sense of direction, particularly during periods when balancing research and administrative requirements was challenging. Each of them, through their professional mentorship and personal encouragement, has significantly contributed to my academic growth.

I am profoundly thankful to the **administrative staff** and the **IQAC (Internal Quality Assurance Cell) team** of St. Xavier's College. Their efficiency, prompt assistance, and supportive attitude have ensured that the operational aspects of my research were handled seamlessly. From managing resources to facilitating timely approvals, their consistent support has been invaluable, allowing me to focus fully on the scientific and intellectual aspects of my work.

My heartfelt gratitude goes to my esteemed guides: **Dr. Sudeshna Shyam Choudhury, Dr. Ayan Chandra** and **Dr. Arup Kumar Mitra**,

I am grateful to **Dr. Sudeshna Shyam Choudhury**, whose guidance, patience, and constructive criticism have enriched my research experience. Her meticulous attention to detail and her insistence on clarity and rigor in scientific work helped refine my experiments and analyses. Dr. Choudhury's encouragement during challenging stages of research, her insightful discussions, and her readiness to assist have made a meaningful difference in my academic development.

I would like to extend my heartfelt thanks to **Dr. Ayan Chandra** for his thoughtful advice, guidance, and constant support. His feedback, suggestions, and encouragement helped me broaden my perspective, ensured that my research-maintained depth, precision, and coherence. The collaboration and guidance of my mentors have been instrumental in shaping the trajectory of my research and my growth as a scholar.

Dr. Arup Kumar Mitra has an exceptional role, since he has had a very important role in my academic journey. Dr. Mitra has been far more than a supervisor; he has been a mentor, a guide, and a constant source of inspiration. His profound knowledge, meticulous approach to scientific research, and ability to provide clarity in the most challenging situations have been a guiding light throughout my PhD journey. From conceptualizing research ideas to troubleshooting experimental

setbacks, from patiently reviewing multiple drafts of my work to offering personal guidance and encouragement, Dr. Mitra's support has been unwavering.

His ability to combine academic rigor with empathy, encouragement, and motivation has made a lasting impact on me. During moments of doubt, his unwavering belief in my potential gave me the strength to persist. His insightful discussions, critical analyses, and constructive suggestions have not only improved the quality of my research but have also nurtured my scientific thinking and problem-solving abilities. Dr. Mitra's guidance has transcended academic mentorship; he has inspired me to cultivate perseverance, resilience, and intellectual curiosity. I consider myself extremely fortunate to have had the privilege of working under such a mentor whose dedication, vision, and encouragement have shaped my doctoral journey in immeasurable ways.

I am profoundly grateful to the members of my **Research Advisory Committee** for their expert guidance and invaluable contributions. **Dr. Satadal Das, Consultant Microbiologist, Peerless Hospital & B.K. Roy Research Centre, Kolkata**, enriched my research with his expertise, especially in microbiology, and offered suggestions that greatly enhanced the scope and impact of my study. I am also deeply thankful to **Dr. Sugata Sen Roy, Professor, Department of Statistics, Calcutta University**, for his expert guidance in statistical analyses and methodology, which ensured the robustness and reliability of my research findings. I am grateful to **Dr. Samrat Roy, Ph.D. Co-ordinator, St. Xavier's College (Autonomous), Kolkata**, who has been a pillar of support, providing constructive feedback, insightful discussions, and guidance at every stage.

I am sincerely thankful to **Dr. Debjani Dutta, Head of the Department of Microbiology**, for her unwavering support, encouragement, and guidance throughout my doctoral journey. I extend my gratitude to other faculty members of the Department of Microbiology, including **Dr. Mahashweta Mitra Ghosh, Dr. Anindita Banerjee, Dr. Madhumita Maitra, Dr. Jaydip Ghosh, Dr. Kasturi Sarkar, and Dr. Riddhi Majumdar**, for their mentorship, guidance, and encouragement. The collective wisdom, expertise, and dedication of the faculty members have been a constant source of inspiration, fostering an academic environment that is vibrant, intellectually stimulating, and supportive of research excellence.

I am profoundly grateful to **Dr. Rina Ghosh, Director, Central Research Facility, St. Xavier's College (Autonomous), Kolkata**, and her team, including **Mr. Arup Mondal and Mr. Pallab Palta**, for their technical support, cooperation, and assistance during my experiments. Their readiness to provide guidance, troubleshoot challenges, and facilitate research processes ensured that my experiments progressed smoothly, even during technically demanding stages.

I would also like to extend my heartfelt thanks to the laboratory assistants, **Mr. Debasish Das** and **Mr. Bittu Baidya**, whose patience, helpfulness, and cooperation significantly facilitated my day-to-day research activities. Their contributions to maintaining an organized and efficient laboratory environment have been invaluable to the smooth progress of my experiments.

I am extremely grateful to my lab mates: **Debapriya Maitra, Bedaprana Roy, Bikram Dhara, Souradip Basu, Bidisha Chatterjee, Rupsha Karmakar, Smritikana Paul, Aratrika Samajdar, Atreyee Banerjee, Reshmi Paul, and Tanusree Sengupta**. The camaraderie, mutual support, and collaborative spirit shared within the lab have made the research journey both intellectually enriching and personally fulfilling. Their encouragement, willingness to share knowledge, and companionship during long hours of experimentation created a nurturing and inspiring environment. The discussions, debates, and collaborative problem-solving with these colleagues have sharpened my scientific thinking and made this journey memorable.

I owe my deepest gratitude to my parents, whose love, encouragement, and unwavering support have been the foundation of my perseverance and determination. Their sacrifices, patience, and belief in my abilities have been a source of strength throughout this journey. During moments of uncertainty and challenges, their faith in me provided the confidence to persist and succeed. I am eternally grateful to them for standing by me, supporting me, and motivating me through every stage of this endeavour. Their constant encouragement and love have made this achievement possible, and I dedicate a part of this accomplishment to them.

In addition to my parents, I am grateful to all my friends, colleagues, and well-wishers who have, directly or indirectly, contributed to this journey. Their encouragement, moral support, and motivation have made this rigorous process not only manageable but also enriching and memorable.

As I reflect on this journey, I realize that completing a PhD is not merely an academic achievement; it is a transformation shaped by the guidance, mentorship, encouragement, and support of a network of extraordinary individuals. I am humbled and privileged to have had the guidance of mentors who are not only experts in their fields but also genuinely invested in the growth and success of their students. The encouragement from my guides, the support from faculty, the camaraderie of my lab mates, and the love of my family have collectively enabled me to overcome challenges, embrace learning, and reach this significant milestone.

In conclusion, I would like to express my deepest gratitude to all those mentioned here and many others who may not have been explicitly named but have influenced my academic and personal journey. The collective support, mentorship, encouragement, and love I have received have shaped not only this research but also the person I have become through this process. Every word of guidance, every gesture of encouragement, and every act of support has contributed to this accomplishment.

LIST OF ABBREVIATIONS

- ❖ 16S rRNA: 16 Svedberg unit ribosomal ribonucleic acid
- ❖ ABA: Absciscic acid
- ❖ ACC: Amino cyclopropane 1-carboxylic acid
- ❖ Al: Aluminium
- ❖ AMF: Arbuscular Mycorrhizal Fungi
- ❖ ANOVA: Analysis of variance
- ❖ B: Boron
- ❖ BHI: Brain Heart Infusion
- ❖ BOD: Biological oxygen demand
- ❖ BNF: biological nitrogen fixation
- ❖ BSI: Botanical Survey of India
- ❖ C: Carbon
- ❖ Ca: Calcium
- ❖ CAS: Chrome Azurol S
- ❖ Cfu: Colony forming unit
- ❖ Chl: Chlorophyll
- ❖ Cl: Chlorine
- ❖ cm: Centimetre
- ❖ Co: Cobalt
- ❖ CO₂: Carbon dioxide
- ❖ CFS: cell free supernatant
- ❖ CRA: Congo Red Agar
- ❖ Cu: Copper
- ❖ DF media: Dworkin-Foster media
- ❖ DNA: Deoxy ribonucleic acid
- ❖ EKW: East Kolkata Wetlands
- ❖ EKWMA: East Kolkata Wetland Management Authority
- ❖ ESV: Ecosystem Service Value
- ❖ FAO: Food and Agricultural Organisation
- ❖ Fe: Iron
- ❖ FeCl₃: Ferric chloride

- ❖ FW: Fresh weight
- ❖ GA: Gibberellic acid
- ❖ H₂O: Water
- ❖ H₂O₂: Hydrogen peroxide
- ❖ HDTMA: hexadecyltrimethylammonium bromide
- ❖ HCl: Hydrochloric acid
- ❖ Hydrogen (H)
- ❖ I: Iodine
- ❖ IAA: Indole acetic acid
- ❖ ICAR: Indian Council of Agriculture Research
- ❖ ISR: Induced Systemic Resistance
- ❖ I.U.: International Unit
- ❖ JA: Jasmonic acid
- ❖ K: Potassium
- ❖ KNO₃: Potassium nitrate
- ❖ KOH: Potassium hydroxide
- ❖ KSB: Potassium solubilizing bacteria
- ❖ L.: Linnaeus
- ❖ LB broth: Luria Bertani broth
- ❖ LPS: Lipo-polysaccharides
- ❖ MDA: Malondialdehyde
- ❖ Mg: Magnesium
- ❖ Mn: Manganese
- ❖ Mo: Molybdenum
- ❖ µg: Microgram
- ❖ µg L⁻¹: Microgram per litre
- ❖ µM gm⁻¹: Micromolar per gram
- ❖ µg ml⁻¹: Microgram per millilitre
- ❖ mg kg⁻¹: Milligram per kilogram
- ❖ Mg L⁻¹: Milligram per litre
- ❖ mM: Millimolar
- ❖ mm: Millimetre
- ❖ µS: Micro Siemen

- ❖ N: Nitrogen
- ❖ NA: Nutrient agar
- ❖ Na: sodium
- ❖ NaCl: Sodium chloride
- ❖ NB: Nutrient broth
- ❖ NBS: nature-based solutions
- ❖ NGS: Next generation sequencing
- ❖ Ni: Nickel
- ❖ nBLAST: Nucleotide BLAST
- ❖ NCBI: National Centre for Biotechnology Information
- ❖ Ni: Nickel
- ❖ nm: Nanometre
- ❖ NO₃⁻: nitrate
- ❖ NH₄⁺: ammonium
- ❖ O: Oxygen
- ❖ O₂: Molecular Oxygen
- ❖ O₂[°] -: Superoxide radical
- ❖ OD: Optical Density
- ❖ OTU: Operational taxonomic unit
- ❖ P: Phosphorus
- ❖ PCR: Polymerase Chain Reaction
- ❖ PDA: potato dextrose agar
- ❖ PGP: plant growth promoting
- ❖ PGPB: Plant Growth Promoting Bacteria
- ❖ PGPM: Plant growth-promoting microorganisms
- ❖ PGPR: Plant Growth Promoting Rhizobacteria
- ❖ pH: Potential of Hydrogen (negative base 10 logarithm of H⁺ ion activity)
- ❖ PO₄³⁻: Phosphate ion
- ❖ PSB: Phosphate solubilizing bacteria
- ❖ ROS: reactive oxygen species
- ❖ S: Sulphur
- ❖ SA: Salicylic acid
- ❖ Se: Selenium

- ❖ Si: Silicon
- ❖ SOC: Soil organic carbon
- ❖ SMC: Soil microbial community
- ❖ SOM: Soil organic matter
- ❖ TOC: Total organic carbon
- ❖ VOC: Volatile Organic Compound
- ❖ W.B.: West Bengal
- ❖ W.H.O.: World Health Organization
- ❖ WRR: Waste Recycling Region
- ❖ Zn: Zinc
- ❖ ZSI: Zoological Survey of India

LIST OF FIGURES

Figure Number Caption

<i>Figure 2.1</i>	Cumulative number of Wetlands of International Importance, 1974–2023
<i>Figure 2.2</i>	Cumulative area of Wetlands of International Importance, 1974–2023
<i>Figure 2.3</i>	Classification of wetlands that correlated with Canadian classification of wetland system.
<i>Figure 2.4</i>	Classification of India’s wetland system. (The codes of the wetlands are as per the nomenclature provided the Government of India)
<i>Figure 2.5</i>	Services provided by the wetlands
<i>Figure 2.6</i>	Ramsar Sites of India
<i>Figure 2.7</i>	Services provided by East Kolkata wetlands
<i>Figure 2.8</i>	Risks assessed in East Kolkata Wetlands
<i>Figure 2.9</i>	Satellite image shows the waterbodies of the East Kolkata Wetlands (EKW) between 1989 and 2022, as determined using the MNDWI method.
<i>Figure 2.10</i>	Flow chart showing the Policy Efficiency and Enforcement Strategies in EKW
<i>Figure 2.11</i>	Basic methodology of soil metagenome
<i>Figure 2.12</i>	Mechanisms of Plant growth promoting bacteria
<i>Figure 2.13</i>	Direct and indirect mechanisms by which plant growth-promoting bacteria (PGPB) enhance plant performance through nutrient acquisition, stress alleviation, and pathogen suppression.
<i>Figure 2.14</i>	Derivatives of IAA
<i>Figure 2.15</i>	Chemical structure of some gibberellic acids

Figure 2.16	Lead uptake from different sources by plants
Figure 2.17	Time series plots
Figure 4.1	Map representing the study location at Chowbaga area of East Kolkata Wetlands
Figure 4.2	The soil sample collection was done during the initial, peak and intercropping period
Figure 4.3	Cultivation of <i>Cucurbita maxima</i> as seen in the agricultural fields of Chowbaga area of East Kolkata Wetlands
Figure 4.4a	Analysis of physico-chemical parameters of <i>Cucurbita maxima</i> soil collected from Chowbaga area of East Kolkata Wetlands over a two-year period.
Figure 4.4b	Analysis of Inorganic parameters (macro-elements) of <i>Cucurbita maxima</i> soil collected from Chowbaga area of East Kolkata Wetlands over a two-year period.
Figure 4.4c	Analysis of Inorganic parameters (micro-elements) of <i>Cucurbita maxima</i> soil collected from Chowbaga area of East Kolkata Wetlands over a two-year period.
Figure 4.5	Cultivation of <i>Zea mays</i> as seen in the agricultural fields of Chowbaga area of East Kolkata Wetlands
Figure 4.6a	Analysis of physico-chemical parameters of <i>Zea mays</i> soil collected from Chowbaga area of East Kolkata Wetlands over a two-year period.
Figure 4.6b	Analysis of Inorganic parameters (macro-elements) of <i>Zea mays</i> soil collected from Chowbaga area of East Kolkata Wetlands over a two-year period.
Figure 4.6c	Analysis of Inorganic parameters (microelements) of <i>Zea mays</i> soil collected from Chowbaga area of East Kolkata Wetlands over a two-year period.

Figure 4.7	Metagenomic analysis of soil sample involving <i>Cucurbita maxima</i> (Year 1)
Figure 4.8	Metagenomic analysis of soil sample involving <i>Cucurbita maxima</i> (Year 2)
Figure 4.9	Metagenomic analysis of soil sample involving <i>Zea mays</i> showing the predominant bacteria between the three cropping periods (Year 1).
Figure 4.10	Metagenomic analysis of soil sample involving <i>Zea mays</i> showing the predominant bacteria between the three cropping periods (Year 2).
Figure 4.10a	Venn Diagram representing the variations and similarity of microbial community in <i>C.maxima</i> soil during the three cropping periods (PI- Initial; PP- Peak, Pin-Intercrop) (Year 1)
Figure 4.10b	Venn Diagram representing the variations and similarity of microbial community in <i>Z. mays</i> soil during the three cropping periods (CI- Initial; CP- Peak, Cin-Intercrop) (Year 1)
Figure4.10c	Venn diagram of Corn & Pumpkin variations of Year 1
Figure 4.10d	Venn Diagram representing the variations and similarity of microbial community in <i>C. maxima</i> soil during the three cropping periods (PI- Initial; PP- Peak, Pin-Intercrop) (Year 2)
Figure 4.10e	Venn Diagram representing the variations and similarity of microbial community in <i>Z. mays</i> soil during the three cropping periods (CI- Initial; CP- Peak, Cin-Intercrop) (Year 2)
Figure 4.10f	Combined Venn diagram of Corn & Pumpkin variations of Year 2
Figure 4.11	Quantification of heavy metal contents in soil
Figure 4.12	Antibiotic assay of bacterial isolates
Figure 4.13	Streaking of bacterial isolates on blood agar and their analysis
Figure 4.14	Streaking of bacterial isolates on Hi chrome media

<i>Figure 4.15</i>	The phylogenetic tree of isolate DPP(A) along with total score and query cover comparison of top 10 hits from NCBI BLAST results indicating the phylogenetic identification and evolutionary positioning of the isolate DPP(A).
<i>Figure 4.16</i>	The phylogenetic tree of isolate DPP(B) along with total score and query cover comparison of top 10 hits from NCBI BLAST results indicating the phylogenetic identification and evolutionary positioning of the isolate DPP(B).
<i>Figure 4.17</i>	The phylogenetic tree of isolate DPP(C) along with total score and query cover comparison of top 10 hits from NCBI BLAST results indicating the phylogenetic identification and evolutionary positioning of the isolate DPP(C).
<i>Figure 4.18</i>	The phylogenetic tree of isolate DCP(A) along with total score and query cover comparison of top 10 hits from NCBI BLAST results indicating the phylogenetic identification and evolutionary positioning of the isolate DCP(A).
<i>Figure 4.19</i>	The phylogenetic tree of isolate DCI(D) along with total score and query cover comparison of top 10 hits from NCBI BLAST results indicating the phylogenetic identification and evolutionary positioning of the isolate DCI(D).
<i>Figure 4.20</i>	Qualitative analysis of macronutrient synthesizing ability of bacterial isolates using modified Jensen media for nitrogen fixing ability. The positive nitrogen fixing bacteria have shown change in colour of the media from blue to yellow.
<i>Figure 4.21</i>	Qualitative analysis of macronutrient synthesizing ability of bacterial isolates using modified Aleksandrow media for testing the potassium solubilizing activity. The positive potassium solubilizing bacteria have shown change in colour of the media from green to yellow.
<i>Figure 4.22</i>	Qualitative analysis of macronutrient synthesizing ability of bacterial isolates using modified Pikovskaya media for testing the

	phosphate solubilizing activity. The positive phosphate solubilizing bacteria have shown change in colour of the media from green to yellow.
<i>Figure 4.23</i>	Quantification of phosphate content of bacterial isolates
<i>Figure 4.24</i>	Quantification of IAA content in the bacterial isolates
<i>Figure 4.25</i>	Quantification of Gibberellic acid content in the bacterial isolates
<i>Figure 4.26</i>	Qualitative evaluation of ACC deaminase of bacterial isolates
<i>Figure 4.27</i>	Quantitative estimation of ACC deaminase of bacterial isolates
<i>Figure 4.28</i>	Qualitative analysis of urease production by bacterial isolates
<i>Figure 4.29</i>	Quantitative analysis of urease production by bacterial isolates
<i>Figure 4.30</i>	Qualitative analysis of cellulase production by bacterial isolates. The cellulase positive bacterial isolates, showed a zone of clearance in the media.
<i>Figure 4.31</i>	Quantitative analysis of cellulase production by bacterial isolates
<i>Figure 4.32</i>	Qualitative analysis of laccase production by bacterial isolates
<i>Figure 4.33</i>	Quantitative analysis of laccase production by bacterial isolates
<i>Figure 4.34</i>	Qualitative analysis of lignin peroxidase production by bacterial isolates showing zone clearance
<i>Figure 4.35</i>	Quantitative analysis of lignin peroxidase production by bacterial isolates
<i>Figure 4.36</i>	Qualitative analysis of amylase production by bacterial isolates, showing zone clearance
<i>Figure 4.37</i>	Quantitative analysis of amylase production by bacterial isolates
<i>Figure 4.38</i>	Qualitative analysis of pectinase production by bacterial isolates, showing a clear zone in the media.
<i>Figure 4.39</i>	Quantitative analysis of pectinase production by bacterial isolates

Figure 4.40	Quantitative analysis of metallothionein content of bacterial isolates
Figure 4.41	Qualitative analysis of HCN production of bacterial isolates. The bacterial isolates with positive HCN production have showed brown colour development on the filter paper, dipped in picrate solution.
Figure 4.42	Quantitative analysis of HCN production by bacterial isolates.
Figure 4.43	Qualitative analysis of ammonia production by bacterial isolates
Figure 4.44	Quantitative analysis of ammonia production by bacterial isolates
Figure 4.45	Qualitative analysis of the siderophore production by bacterial isolates and identification of the type of siderophore.
Figure 4.46	Quantitative analysis of the siderophore production by bacterial isolates.
Figure 4.47	Lead concentration in soil and its uptake by bacterial isolates. Graphical representations showing: (a) Lead tolerance by bacterial isolates. (b) Percentage of lead uptake by bacterial isolates. (c) Percentage of lead adsorbed by bacterial isolates
Figure 4.48	Interaction study between the bacterial isolates
Figure 4.49	Isolation of <i>Curvularia</i> sp and <i>Exserohilum</i> sp from the infected leaves of Zea mays
Figure 4.50	Interaction study between bacterial isolates A- DPP(A) , B- DPP(B), C- DPP(C), D-DCP(A) and E-DCI(D) with the fungus <i>Curvularia</i> sp.
Figure 4.51	Interaction study between bacterial isolates A- DPP(A), B- DPP(B), C- DPP(C), D-DCP(A) and E-DCI(D) with the fungus <i>Exserohilum</i> sp.
Figure 4.52	Field trial of <i>Cucurbita maxima</i> plants in East Kolkata Wetlands
Figure 4.53	Field trial of <i>Cucurbita maxima</i> plants in East Kolkata Wetlands

Figure 4.54	Field trial of <i>Zea mays</i> plants in East Kolkata Wetlands
Figure 4.55	Vegetative(A-D) and Reproductive(E-F) growth parameters of <i>Cucurbita maxima</i> , with arrows pointing towards highest growth in treated set-up.
Figure 4.56	Vegetative (A-E; G-I) and Reproductive(F) growth parameters of <i>Zea mays</i> , with arrows pointing towards highest growth in treated set-up.
Figure 4.57	Chlorophyll content in the leaves of <i>Cucurbita maxima</i> during the initial(A) and peak (B) period of cultivation.
Figure 4.58a	Chlorophyll content in the leaves of <i>Zea mays</i> during the initial(A) and peak (B)period of cultivation.
Figure 4.58b	Soil Enzyme Profile
Figure 4.58c	The fluorescence microscopy images acquired following SYBR Green staining clearly illustrates variations in microbial abundance between control and treated samples of both soil and plant tissues.
Figure 4.59	- Metagenomic analysis of soil sample of <i>Cucurbita maxima</i> rhizosphere, upon addition of consortia.
Figure 4.60	- Heatmap representation of metagenomic analysis of soil sample of <i>Cucurbita maxima</i> rhizosphere, upon addition of consortia.
Figure 4.61	- Krona chart of <i>Cucurbita maxima</i> rhizosphere and control soil upon addition of consortia, showing the increment of firmicutes.
Figure 4.62	- Metagenomic analysis of soil sample of <i>Zea mays</i> rhizosphere, upon addition of consortia.
Figure 4.63	- Heat map representation of soil sample of <i>Zea mays</i> rhizosphere, upon addition of consortia
Figure 4.64	- Krona chart of <i>Zea mays</i> treated rhizosphere and control soil upon addition of consortia, showing the increment of firmicutes.

Figure 4.65	- Krona chart of <i>Zea mays</i> treated rhizosphere soil (peak stage) and control soil upon addition of consortia, showing the increment of firmicutes.
Figure 4.66	Lead concentration and their subsequent accumulation in plant parts
Figure 4.67	Lead concentration and their subsequent accumulation in plant part
Figure 4.68, 4.69, 4.70	: Quantitative assay of catalase (A), ascorbate peroxidase (B) and proline (C) content of <i>Cucurbita</i> sp plants.
Figure 4.71, 4.72, 4.73, 4.74	: Quantitative assay of catalase(A), peroxidase(B), ascorbate peroxidase(C) and proline content of <i>Zea mays</i> sp plants
Figure 4.75	Calculation of the statistically significant (P) values of soil parameters of <i>Cucurbita maxima</i> and <i>Zea mays</i>
Figure 4.76	Time series analysis of the soil parameters
Figure 4.77	(1-10): Statistical analysis of crop productivity in <i>Cucurbita maxima</i>
Figure 4.78	T-test, Wilcoxon test for <i>Cucurbita maxima</i>
Figure 4.79	(1-8): Statistical analysis of crop productivity in <i>Zea mays</i>
Figure 4.80	T-test, Wilcoxon test for <i>Zea mays</i>

LIST OF TABLES

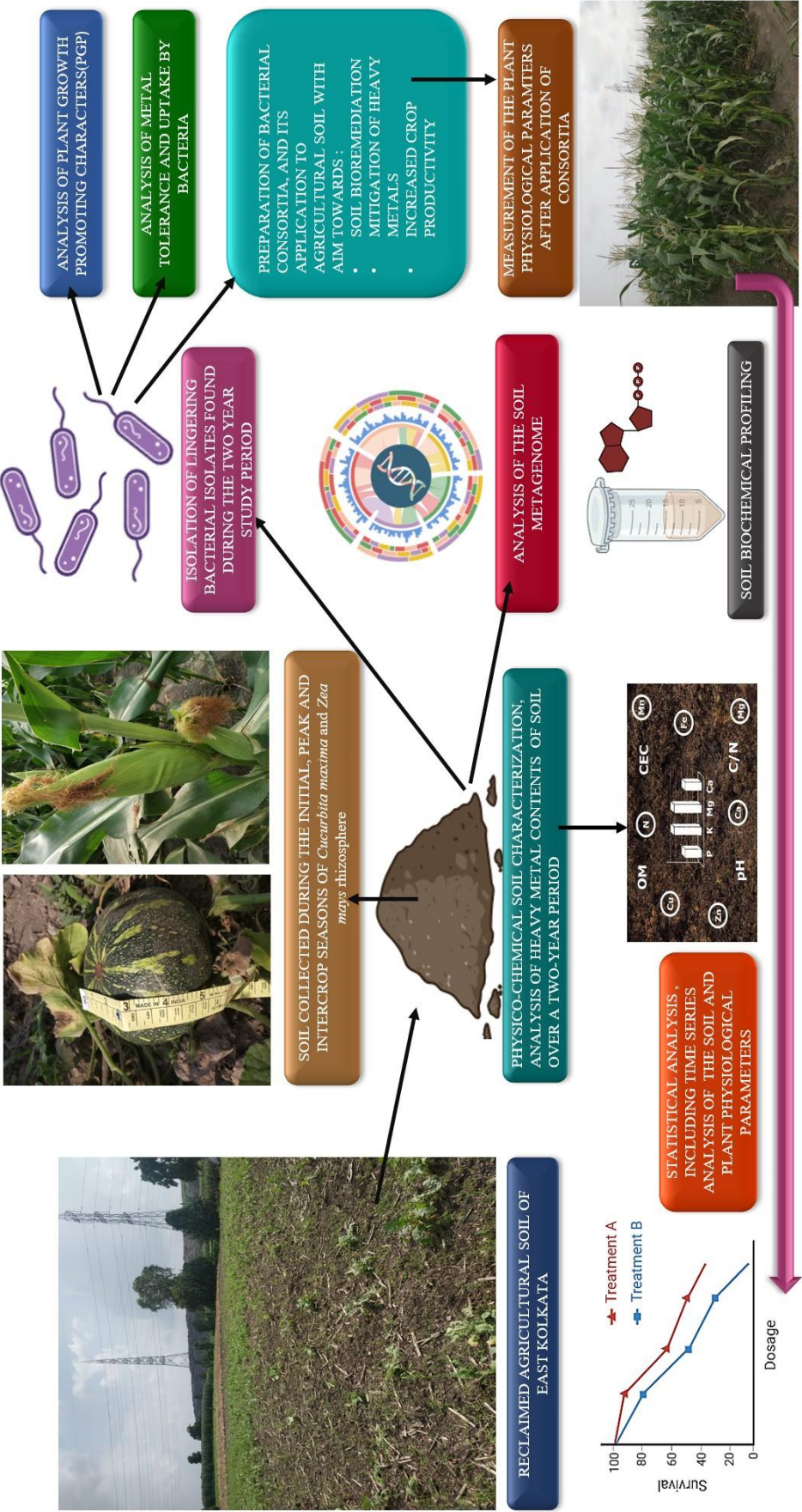
Table No.	Caption
Table 3.1	List of media used
Table 3.2	List of chemicals used
Table 3.3	List of crop varieties used for experiments
Table 3.4	List of instruments used
Table 3.5	List of software used
Table 4.1	Gram staining and colony morphology of bacterial isolates
Table 4.2	Presumptive identification of bacterial isolates
Table 4.3	Lead content of control and treated soil
Table 4.4-4.6	ANOVA for plant Length of <i>Cucurbita maxima</i>
Table 4.7-4.9	ANOVA for Number of leaves of <i>Cucurbita maxima</i>
Table 4.10-4.12	ANOVA for Leaf Lamina length of <i>Cucurbita maxima</i>
Table 4.13-4.15	ANOVA for Leaf Lamina width of <i>Cucurbita maxima</i>
Table 4.16-4.18	ANOVA for Number of nodes of <i>Cucurbita maxima</i>
Table 4.19-4.21	ANOVA for Number of tendrils of <i>Cucurbita maxima</i>
Table 4.22-4.24	ANOVA for Tendril length of <i>Cucurbita maxima</i>
Table 4.25-4.27	ANOVA for Number of flowers of <i>Cucurbita maxima</i>
Table 4.28-4.30	ANOVA for Number of fruits of <i>Cucurbita maxima</i>
Table 4.31-4.33	ANOVA for plant Length of <i>Zea mays</i>
Table 4.34-4.36	ANOVA for number of leaves of <i>Zea mays</i>
Table 4.37-4.39	ANOVA for leaf lamina length of <i>Zea mays</i>
Table 4.40-4.42	ANOVA for leaf lamina width of <i>Zea mays</i>

Table 4.43-4.45	ANOVA for number of nodes of <i>Zea mays</i>
Table 4.46-4.48	ANOVA for number of internodes of <i>Zea mays</i>
Table 4.49-4.51	ANOVA for plant girth of <i>Zea mays</i>
Table 4.52-4.54	ANOVA for number of fruits of <i>Zea mays</i>
Table 4.55	Analysis of soil parameters in both treated and control set of <i>Cucurbita maxima</i> and <i>Zea mays</i> in East Kolkata Wetlands

LIST OF CONTENTS

<i>SL.NO</i>	<i>CONTENT</i>	<i>PAGE NO</i>
1.	ACKNOWLEDGEMENT	I-V
2.	ABBREVIATIONS	VI-IX
3.	LIST OF FIGURES	X-XVII
4.	LIST OF TABLES	XVIII-XIX
	GRAPHICAL ABSTRACT	XXI
	ABSTRACT	XXII
5.	CHAPTER 1: INTRODUCTION AND OBJECTIVES	1-6 7
6.	CHAPTER 2: REVIEW OF LITERATURE	8-81
7.	CHAPTER 3: MATERIALS AND METHODS	82-122
8.	CHAPTER 4: RESULTS	123-275
9.	CHAPTER 5: DISCUSSIONS	276-310
10.	CHAPTER 6: SUMMARY	311-318
11.	CONCLUSION AND FUTURE PROSPECT	319
12.	REFERENCES	320-364
13.	LIST OF PUBLICATIONS	365-376
14	LAURELS ACHIEVED	377-378

GRAPHICAL ABSTRACT



ABSTRACT

Wetlands, refers to the confluence of land and water having a rich ecological diversity with multitudinous functions. As per Ramsar Convention, framed in the year August 2002, East Kolkata Wetlands have been considered to be of intercontinental importance. Although improper handling of these wetlands, has led to the gradual degeneracy of this wetland. In this present study, the soil sample was collected from these reclaimed agricultural lands of Chowbaga area, of East Kolkata Wetlands with coordinates of 22.5296°N, 88.4207°E, and their physico-chemical parameters during the initial, peak and inter cropping periods of *Cucurbita maxima* and *Zea mays* were analysed. The dissimilitude in the values of their physico-chemical parameters like organic carbon, calcium, magnesium, cation exchange capacity etc indicate their influence in controlling crop productivity. Moreover, they also result in the variation of rhizospheric bacteria. The plant growth promotion properties of the lingering bacterial flora present in the soil surrounding the rhizospheric region of *Cucurbita* sp and *Zea mays* were analysed. These PGPR which included some species of *Bacillus*, that can augment plant growth by different methodologies like nitrogen fixation, phosphate solubilization, phytohormone synthesis, siderophore production. These agricultural lands were found to have very high quantities of heavy metals, especially lead. Amongst the bacterial isolates, two of them were found to have great potential in lead mitigation. The desired bacterial isolates were used as a microbial consortium and applied to *Cucurbita maxima* and *Zea mays* grown in the reclaimed soil of East Kolkata Wetlands and they were found to reduce the lead content in the fruit of the above plants. The prospective potential of use of PGPR has gradually escalated, since it is one of the best substitutes against the constant usage of chemical fertilizers as well as pesticides. The use of these PGPR is one of the most reliable methods for ensuring sustainable agriculture. Subsequently, the PGPR population will be correlated with the nutrient availability and productivity of the plants.

Keywords: East Kolkata Wetlands, *Cucurbita* sp, *Zea mays*, physico-chemical parameters, soil, microbial flora, PGPR, lead mitigation.

A close-up photograph of several large, vibrant green corn leaves. The leaves are arranged vertically, with some in sharp focus and others slightly blurred in the background. The background is a clear, bright blue sky. A purple rectangular box with rounded corners is centered over the middle of the image, containing white text.

CHAPTER-1: INTRODUCTION

INTRODUCTION

Wetlands are effective ecosystem that have huge relevance in ensuring sustainable development of biodiversity and are responsible for maintaining the stability of ecological environment in the biosphere. As per Ramsar Convention framed in the year 1971, wetlands can be defined as “area of marshes, ferns, peatlands or the water source in case of artificial or be it natural, permanent either temporary, with water, *i.e.*, static or flowing, fresh, brackish or salt including areas of marine water, the depth of which at low tide must not transcend six meters.” (Mondal *et al.* 2022). The wetlands across the globe envelope only 6% of earth’s surface that encompasses 40% of freshwater biodiversity. Wetlands of India extend across an area of 111,204.56 km² with the presence of rare species of flora and fauna that are pivotal for the biodiversity hotspots.

The East Kolkata Wetlands (EKW), are referred as a Ramsar Site. In August 2002, East Kolkata Wetlands were given the global status of Wetlands of International Importance by the Ramsar Convention Authority. This was mainly because the EKW had the distinct identity as a Waste Recycling Region (WRR). In November 2002, the East Kolkata Wetlands (EKW) attained Ramsar Site status, encompassing an expansive area of approximately 127.41km² (Bhattacharya *et al.* 2008). Areas like agricultural lands, sewerage/wastewater fed fisheries, landfill along with horticulture are involved in this sheltered area, encompassing an area of approximately 125 km². It is of paramount importance in West Bengal, India. EKW functions as a kidney for the areas in and around Kolkata and helps to preserve as well as maintain the balance in ecology and environmental sustainability. This wetland has the ability to dissipate huge quantities of pollutants that are released from the urban regions of Kolkata. The delicate balance of ecology in this area has been affected due to land conversions, thereby leading to improper sustainable development (Mondal *et al.* 2022). The expansive area of EKW has been known to include intertidal marshes, salt marshes with noteworthy ability of treatment of waste water like ponds provincially referred to as known as Bheries and Jheels. Presence of certain dumping grounds, followed by green zones and cultivable lands are the typical characteristic features visible in East Kolkata Wetlands (Chaudhuri *et al.*,2012).

Functioning in various capacities, EKW serves as a flood control plain, a highly productive region, and a notable waste recycling area. Positioned within deltaic region of the Hooghly River, EKW along with the coastal rivers and creeks play a crucial role in shaping the ecotone of southern Bengal. The wetlands play a pivotal role in maintaining ecological equilibrium, contributing to overall environmental system's enhancement. (Bhattacharya *et al.* 2008). Wetland conversion, is a common phenomenon in urban areas due to anthropogenic stress on land and it emphasizes the need to understand land policies for identifying potential geographic risks during such conversions. The reclamation of wetlands, particularly EKW, has resulted in inadequate treatment of sewage and wastewater from Kolkata, leading to continual degradation. EKWs are categorized into water bodies, agriculture lands, garbage dumping sites, and encampment areas, further subdivided into rural and urban regions (Kundu *et al.* 2008). It has been reported to have approximately 364 fisheries, that are sewage fed, followed by agricultural lands and waste farms. The major types of land use motif include: a) Wetland area of approximately 58.5214 km² b) Agricultural area of approximately 47.1856km² c) Productive Farming area (reclaimed lands) of approximately 6.0278km², d) Urban and Rural encampment area of approximately 13.2652 km². Thus it is responsible for feeding the residents of Kolkata with natural zero waste mechanism.

These areas are severely affected by human activities like urbanisation, pollution, land use changes. Other threats like climatic variations, bio-invasions, overutilization of resources, microplastics are constantly found to have a detrimental impact upon the environment. Activities like urban expansion and their insistence for land have been found to transform the natural lands into urban corridor, that has been severely found to affect the services provided by ecosystem. This therefore had drastically resulted in the depreciation of ecosystem service value (ESV), and has concurrently deteriorated the health of ecosystem, followed by sweltering climatic conditions, although wetlands are known to generate indispensable services like regulation of floods, preservation of biodiversity followed by water filtration (Yadav *et al.*, 2024).

As Kolkata's wastewater treatment plant and reservoir, the EKW is directly related to the city. Being an "Ecologically Subsidized City," Kolkata's population pressure (about 1.50 million in 2021) jeopardizes the ecosystem functions of the EKW. Some of the regions of Dhapa of East Kolkata wetlands, it has been seen that the municipal solid waste and garbage laden soil are being used for agriculture. On a daily basis approximately 2000-3000 Megagram (Mg) of solid wastes have been found to be dumped in these areas. These garbage or waste-based agriculture have been found to yield nearly 3,70,650kg/ha/yr vegetables, which has resulted in production of nearly

150 Megagram (Mg) of vegetables on a daily basis. Huge quantities of garbage from the city of Kolkata are been usually released without any pretreatment into the inland waterways via sewage pumping stations. These are also known to discharge effluents from tanneries, and other industries like rubber, battery, electroplating containing heavy metals including lead (Pb) into the canals, that has been found to eventually emit it in the river Kultigong, that is located at a distance of 40km from the city of Kolkata. Water from these regions is being used for irrigation purposes of agriculture farms. (Chattopadhyay *et al.*,2002).

Solid wastes from industries and municipal areas are being used as a sanitary landfill in agriculture. Wastes from tanneries are being used as a fish food and the byproducts are often used as a fertilizer. Therefore, the ecological community in East Kolkata Wetlands are under a probable threat of contamination by heavy metals (Chattopadhyay *et al.*,2002). The safety, quality, and dependability of the farming process that uses treated solid and soluble waste to cultivate green leafy crops at EKW are controversial and ambiguous (Chaudhuri *et al.*,2006). The studies from the recent literature have also revealed bioaccumulation followed by biomagnification of these heavy toxic metals in the wetland regions (Chattopadhyay *et al.*,2002). Soil ecosystem has been found to be greatly affected by the presence of heavy metals, which in turn has its influence over the food hierarchy and the bionomics of the members of food chain, also impacting the land use for agriculture purpose. Heavy metals encompass compounds that usually involve metalloids, actinides, transition metals, and lanthanides. Heavy metals usually include lead, mercury, cadmium, which even in low concentrations possess high toxicity and affects the wellbeing of the ecosystem (Sarah *et al.*, 2021; Zhao *et al.*, 2022). The wetlands are affected by the wastewater from tanneries, that usually gets contaminated with the toxic effluents that are released from the industries.

These wastewaters are used for irrigation of the agricultural lands. This enactment over the years has led to the deposition of heavy metals in the soil. The heavy metal analysis of reclaimed agricultural soils of East Kolkata Wetlands have been found to have high quantities of heavy metals like lead, cadmium, chromium, and so on (Chatterjee *et al.*, 2011). The concentration of metals depends on the nature of industry in the vicinity. A metalloid like arsenic has also been found to be present in soil, which might be due to geological reason (Sengupta *et al.*, 2013).

High concentrations of heavy metals like chromium, arsenic, lead, manganese, copper have been reported from the soils of East Kolkata Wetlands (Kumar *et al.*,2023). Analysis of metal contents in different plant parts of *Amaranthus* sp, *Raphanus sativus*, *Brassica oleracea botrytis*,

Canna indica of East Kolkata Wetlands showed the presence of heavy metals like chromium, lead, copper. The organic matter along with the clay content of the soil can also regulate the level of metal bioaccumulation in the soil and subsequently in the plant parts. A quantity of 436.45 mg/kg, 716.37 mg/kg of lead in roots and stems of *Canna indica*, followed by 299.34 mg/kg of lead in the leaves of *Raphanus sativus* were reported. Lead is known to imitate the behaviour of calcium ions and thereby hinder the enzymatic reactions; also, organic lead is known to migrate faster into the plants as compared to Pb^{2+} (Chattopadhyay *et al.*, 2002).

In ecological science, identification of the various environmental factors and their spatio-temporal discrepancies is an important factor, since the productiveness of an ecosystem is influenced by these components (Pal, *et al.*, 2016). Some of the soil physico-chemical parameters measured in EKW include pH value between 7.5 and 8, electrical conductivity of approximately 1500 μ S indicating moderate solute movement, nitrogen of about 700 kg/ha and phosphorous content of about 2000 mg/kg (Firdous *et al.* 2018). Pal. *et al.*, 2016, has noted that total organic matter content in the soil was higher in the areas closer to the regions of the tanneries, sodium concentrations were high in the soil closer to the regions of Kultigong river and were found to be within a range of 315.45mg/kg-1285.63 mg/kg in the Chowbaga region. The sodium concentrations were high in the soil during the pre-monsoon regions as compared to the post monsoon regions, which may be due to the washing effect after the rainy season. High quantities of ammonium were found in the soil regions closer to the tanneries and municipal waste dumping grounds, with a range between 82.81–238.98 mg/kg of soil. Calcium levels were abundant in waste water rich regions. Each component's efficient functioning is essential for the establishment of a well-balanced ecosystem. Potassium concentrations were found to be present within a range of 152.88mg/kg–249.99 mg/kg in Chowbaga regions. Therefore, it was seen that the agricultural soils were highly affected with the wastewaters released from tanneries and thereby had resulted in the rise of anion content of soil. Additionally, pressure of these anions may alleviate their uptake by plants. Therefore, all of these negative consequences can be minimized by controlling the release of waste products into the EKW environment.

These wetlands have been found to function as an exceptional agro-ecosystem that had satiated its function as a resource recovery system beyond a timeline of 70 years. The process of bioremediation in EKW is based on microbes and is therefore found to contemplate important soil microbes. The dynamic activity in the soils is domineered by micro-organisms. The advantageous role of the micro-organisms involves the properties of nitrogen fixation, phosphate and potassium

solubilization, followed by decomposition of organic matter and metabolic by products. The soil is an area of paramount repertoire for microorganisms, as it comprises of diverse kinds of microbial communities, that have indispensable roles in the local terrestrial ecosystem. Plants are greatly benefitted by these microbial floras that inhabit the rhizospheric regions (Munees *et al.*, 2013). These microbial floras are mentioned as the plant growth promoting rhizobacteria (PGPR). The presence of bacteria like Actinobacteria and Firmicutes stipulate the breakdown of nitrophenol, nitroaromatic compounds, followed by pesticides and herbicides. Due to the continuous agricultural activity and the certitude that EKW has been known to be subjected to both solid and liquid waste from the entire city, their capacity to degrade these compounds have been studied. These microorganisms contribute to the creation of humus and decomposition. In order to bioremediate heavy metals from both liquid and solid sources, the EKW have been seen to depend on the group Proteobacteria. They would result in the biodegradation of soil tainted with oil and harmful substances, as well as the breakdown and recycling of plant woody tissue. They work with the bheri's cyanobacteria to fix nitrogen and subsequently are known to help with metal bioremediation (Chaudhuri *et al.*, 2006).

The bacterial isolates of EKW like *Bacillus* sp were found to synthesise enzymes important for agricultural activity like pectinase, amylase, laccase, cellulase and also fix nitrogen and solubilize phosphorous. They were also seen to produce insecticidal toxins extracellularly, that could be easily utilized in biopesticides, and thereby known to promote plant growth. The bacterial isolates were found to be of mesophilic character in many cases, thereby having ample applications in the industries of Indian subcontinent (Ghosh *et al.*, 2007).

Metagenomic sequencing of the soil samples have revealed the bacterial composition, that have a key role in nutrient cycling along with bioremediation (Chakraborty *et al.*, 2025). Metagenomics, a fusion of “meta-analysis” and analysis of genetic material (genomes), focuses on revealing the identities and functional information of microbial communities. A metagenomic analysis provides insights into functional potential of the microbial community. In EKW, some of the microorganisms found in sewage/sludge were *Deftuviicoccus* sp, *Lactococcus chungangensis*, *Rhodoligotrophos* sp, and *Trichococcus* sp were observed. Apart from Proteobacteria, Acidobacteria, Chloroflexi, Planctomycetes, and Cyanobacteria were observed EKW. It has also been discovered that many members of these phyla are essential for certain processes, such as the oxidation of ammonia in sewage, the detoxification, decomposition, and eutrophication of metal-

ion-contaminated soil. Additionally, Verrucomicrobia were more abundant in EKW, which may be related to the increased availability of nutrients (Kumari *et al.*,2021).

Therefore, in this investigation, the physico-chemical analysis of the soil parameters, metagenome study, followed by the isolation of the bacterial isolates and their PGPR property of the predominant/lingering microbial flora isolated from soil during initial, peak and intercropping periods of *Cucurbita maxima* and *Zea mays* from the reclaimed agricultural lands of East Kolkata Wetlands has been studied. The ability of the bacterial isolates to mitigate the levels of heavy metals like lead has been analysed, followed by their ability to enhance the productivity of crops. The capacity of lead uptake by the bacterial isolates were tested. Out of all the bacterial isolates, the best ones with higher PGP activities and metal uptake properties were selected. The consortia were prepared and applied to the plants grown on field, to check the viability of the bacterial consortia.

Metagenomic study of rhizospheric soil of *Cucurbita* sp. and *Zea mays* was conducted to gain an overview of microbial community present within the soil and understand their reciprocal relationship with soil parameters. Also, metagenomic analysis was done after the addition of microbial consortia, to find out the correlation with the soil biochemistry and the bacterial flora, followed by their concomitant role in plant growth promotion.

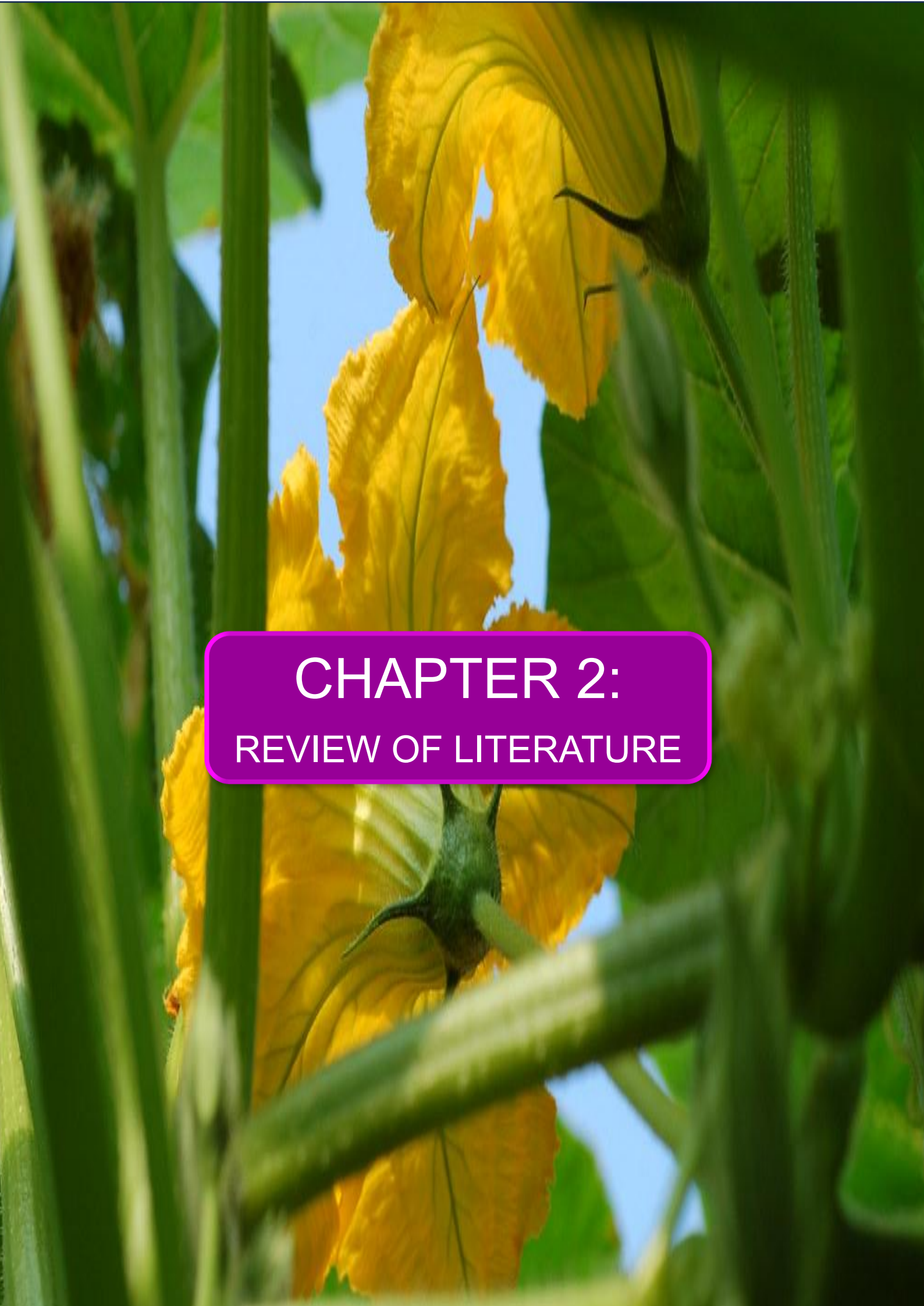
Statistical analysis of the data was performed. Time series analysis was conducted to analyse the differences in the physico-chemical soil parameters over the two-year study period. Representation of crop productivity, upon addition of bacterial consortia was done using linear trend model.

Therefore, this research emphasizes the collective impact of physico-chemical soil characteristics, microbial diversity, and the traits of plant growth-promoting rhizobacteria (PGPR) on crop yield in the reclaimed areas of the East Kolkata Wetlands. The application of time series statistical analysis was essential for understanding the dynamic shifts in soil biochemistry and microbial relationships throughout various cropping phases. The findings demonstrated that the chosen bacterial consortia alleviated lead pollution and enhanced soil quality along with crop productivity, thereby establishing a significant link between microbial activity, soil characteristics, and plant growth over time.

OBJECTIVES

The objectives of the work were laid down in the following ways:

1. To characterize soil, with respect to physical, chemical, microbiological and metagenomic data of designated plot involving *Cucurbita* sp and *Zea mays* over a two-year period.
2. To perform physical isolation, purification and characterization of potential microbes having dynamic role in soil development, and their role in mitigation of pollution (lead).
3. To prepare an ideal consortium to be supplemented all through the year suitable for any crop, either singly or with chemical fertilizers (in-vivo study).
4. To perform statistical analysis, including time series study of physico-chemical and microbiological property vis a vis crop productivity.



CHAPTER 2:

REVIEW OF LITERATURE

REVIEW OF LITERATURE

2. WETLANDS:

Wetlands serve as transitional areas between land and water ecosystems, remaining flooded or saturated with elevated groundwater or surface water levels for part or whole of the year. In tropical regions, wetlands account for 3% of the total land area (Junk *et al.*, 2013). Wetlands consist of a diverse array of types, defined by their hydrological and biological features: for instance, fens, bogs, marshes, swamps, shallow lakes, mangroves, and saltmarshes.

2.1: Definition of Wetlands:

At the international level, the Ramsar Convention (1971) (Article 1.1), with 169 signatory countries, played a leading role with respect to the conservation and wise use of wetlands. The Ramsar definition of wetlands states: “They are areas of marsh, fen, peatland or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine water the depth of which at low tide does not exceed six meters.” Article 2.1 of Ramsar Convention provides with a modified definition of wetlands that states that “In addition, to protect coherent sites especially waterflow habitat, wetlands may include riparian and coastal zones adjacent to the wetlands, and islands or bodies of marine water deeper than six meters at low tide lying within the wetlands.” Semeniuk *et al.*, 1995 defined wetlands as areas of seasonally, intermittently or permanently waterlogged soils or inundated land, which can be natural or otherwise, fresh or saline in nature. In Western Australia, the permanently inundated part of the wetland is traditionally considered as wetland area, and thus correctly extended the definition of peripheral wetlands. Cowardin *et al.* 1979 defined wetlands in the USA as “Lands transitional between terrestrial and aquatic systems, where the water table is usually at or near the surface or the land is covered by shallow water”. Wetlands must have one or more of the following three attributes: (1) at least periodically, the land supports predominantly hydrophytes; (2) the substrate is predominantly undrained, hydric soil; and (3) the substrate is non-soil and is saturated or covered by shallow water at some time during the growing season of each year. (Junk ;2024)

2.2 Classification of wetlands:

One of the earliest widely recognized wetland classification systems, created by Cowardin *et al.* in 1979, categorized wetlands into several types: marine (coastal wetlands), estuarine (which includes deltas, tidal marshes, and mangrove swamps), lacustrine (lakes), riverine (found along rivers and streams), and palustrine (referring to marshes, swamps, and bogs). This classification was based on their hydrological, ecological, and geological features. In total, there are 1052 identified Ramsar sites in Europe; 289 in Asia; 359 in Africa; 175 in South America; 211 in North America; and 79 in the Oceania region, all recognized as wetlands of international significance (Ramsar Secretariat, 2013). The number of wetlands and their cumulative areas have been represented in **Figure 2.1 and 2.2**.

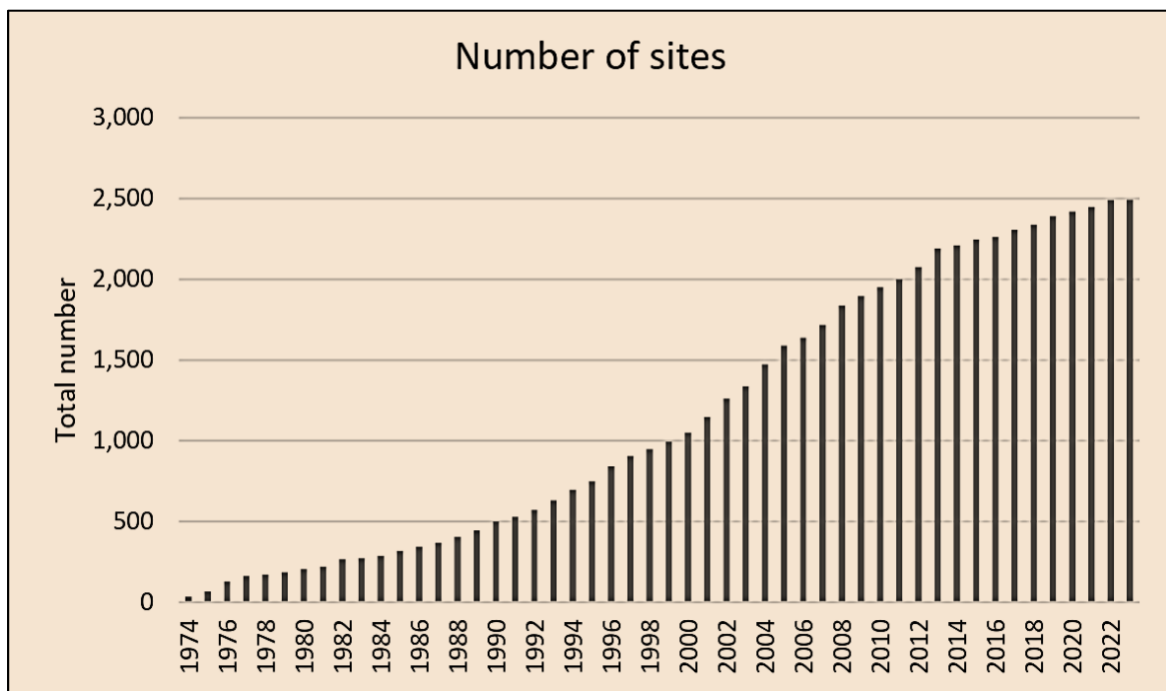


Figure 2.1: Cumulative number of Wetlands of International Importance, 1974–2023

Source: THE CONVENTION ON WETLANDS 62nd meeting of the Standing Committee Gland, Switzerland, 4-8 September 2023

(chromeextension://efaidnbnmnnibpcajpgclclefindmkaj/https://www.ramsar.org/sites/default/files/documents/library/sc62_22_status_ramsar_list_e.pdf)

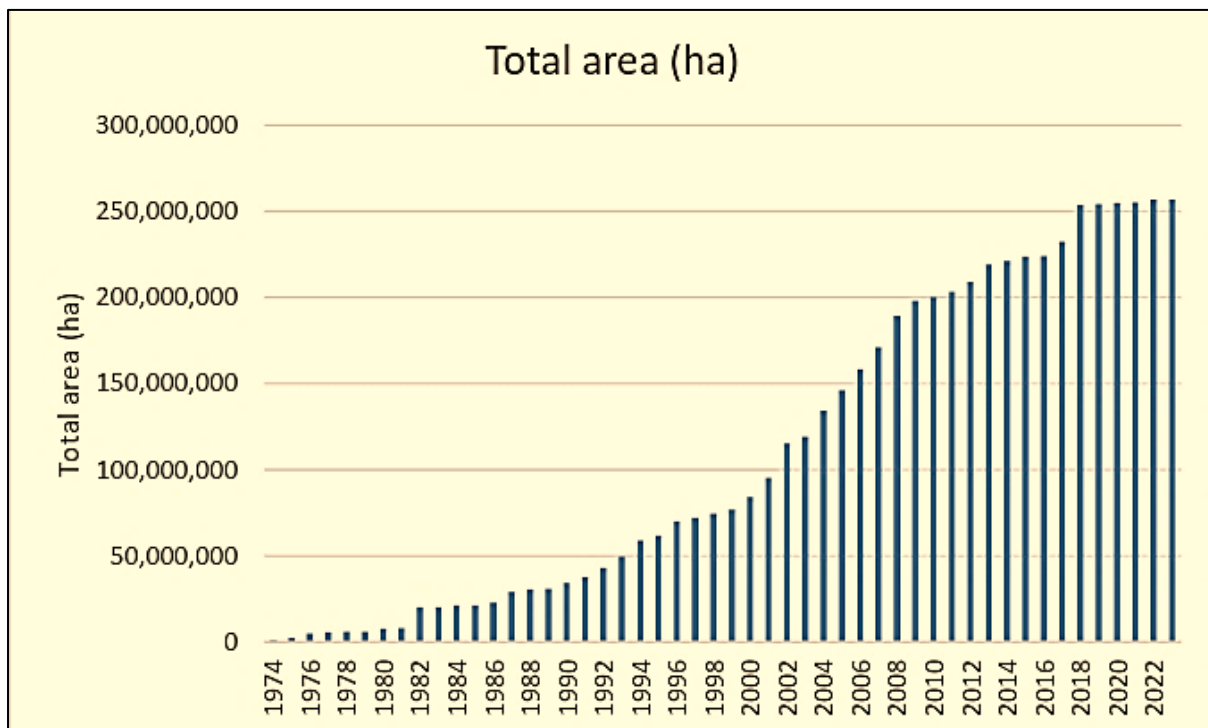


Figure 2.2: Cumulative area of Wetlands of International Importance, 1974–2023

**Source: THE CONVENTION ON WETLANDS 62nd meeting of the Standing Committee
Gland, Switzerland, 4-8 September 2023**

(chromeextension://efaidnbmninnibpcapjpcglclefindmkaj/https://www.ramsar.org/sites/default/files/documents/library/sc62_22_status_ramsar_list_e.pdf)

2.2.1 Classification of wetlands as per Ramsar Convention:

Wetlands are typically categorized into five fundamental systems: Lacustrine, Riverine, Palustrine, Marine, and Estuarine (Frazier, 1996). These systems encompass intricate wetland and deepwater environments that are influenced by similar hydrologic, geomorphologic, chemical, or biological factors. As per Vasumathi *et al.*, 2022, the Ramsar Classification of Wetland Type, which is currently utilized, was adopted by the Conference of the Parties in 1990. The codes for classifications are derived from the Ramsar Classification System for "Wetland Type," which was endorsed by Recommendation 4.7 and modified by Resolution VI.5 during the Conference of the Contracting Parties. The categories presented here are designed to offer a general framework to facilitate the quick identification of the primary wetland habitats found at each location. It classifies wetlands into three primary categories: marine and coastal wetlands, inland wetlands, and artificial wetlands as mentioned below.

Marine/coastal wetlands:

The order and abbreviation are maintained as per the original Ramsar List (1990)

- A). Permanent shallow marine waters, typically less than six meters deep at low tide; this includes sea bays and straits.
- B). Marine subtidal aquatic beds; encompassing kelp beds, seagrass beds, and tropical marine meadows.
- C). Coral reefs.
- D). Rocky marine shores; which include rocky offshore islands and sea cliffs.
- E). Sand, shingle, or pebble shores; covering sand bars, spits, and sandy islets; also includes dune systems and humid dune slacks.
- F). Estuarine waters; the permanent water found in estuaries and the estuarine systems of deltas.
- G). Intertidal mud, sand, or salt flats.
- H). Intertidal marshes; which consist of salt marshes, salt meadows, saltings, and raised salt marshes; also includes tidal brackish and freshwater marshes.
- I). Intertidal forested wetlands; comprising mangrove swamps, nipah swamps, and tidal freshwater swamp forests.
- J). Coastal brackish/saline lagoons; these are brackish to saline lagoons that have at least one relatively narrow connection to the sea.
- K). Coastal freshwater lagoons; which include freshwater delta lagoons.
- Zk (a). Karst and other subterranean hydrological systems, marine/coastal.

Inland wetlands

- L). Permanent inland deltas.
- M). Permanent rivers, streams, or creeks; including waterfalls.
- N). Seasonal, intermittent, or irregular rivers, streams, or creeks.
- O). Permanent freshwater lakes (over 8 ha); which include large oxbow lakes.
- P). Seasonal or intermittent freshwater lakes (over 8 ha); including floodplain lakes.
- Q). Permanent saline, brackish, or alkaline lakes.
- R). Seasonal or intermittent saline, brackish, or alkaline lakes and flats. .
- Sp). Permanent saline, brackish, or alkaline marshes or pools.
- Ss). Seasonal or intermittent saline, brackish, or alkaline marshes or pools.
- Tp). Permanent freshwater marshes, pools, and ponds (under 8 ha), as well as marshes and swamps situated on inorganic soils, are characterized by emergent vegetation that remains water-logged for the majority of the growing season.
- Ts). Seasonal or intermittent freshwater marshes and pools on inorganic soils encompass sloughs, potholes, seasonally flooded meadows, and sedge marshes.
- U). Non-forested peatlands consist of shrub or open bogs, swamps, and fens.
- Va). Alpine wetlands comprise alpine meadows and temporary waters resulting from snowmelt.
- Vt). Tundra wetlands include tundra pools and temporary waters from snowmelt.
- W). Shrub-dominated wetlands feature shrub swamps, shrub-dominated freshwater marshes, shrub carr, and alder thickets on inorganic soils.
- Xf). Freshwater, tree-dominated wetlands consist of freshwater swamp forests, seasonally flooded forests, and wooded swamps on inorganic soils.
- Xp). Forested peatlands are represented by peat swamp forests.
- Y). Freshwater springs and oases are included in this category.
- Zg). Geothermal wetlands are also recognized.

Zk (b). Karst and other subterranean hydrological systems are found inland.

Human-made wetlands

- 1). Aquaculture ponds (such as those for fish or shrimp).
- 2). Ponds, which include farm ponds, stock ponds, and small tanks (less than 8 ha).
- 3). Irrigated land, which encompasses irrigation channels and rice fields.
- 4). Seasonally flooded agricultural land, including intensively managed or grazed wet meadows or pastures.
- 5). Salt exploitation sites, such as salt pans and saline.
- 6). Water storage areas, including reservoirs, barrages, dams, and impoundments (generally over 8 ha).
- 7). Excavations, which include gravel, brick, and clay pits, as well as borrow pits and mining pools.
- 8). Wastewater treatment areas, such as sewage farms, settling ponds, and oxidation basins.
- 9). Canals, drainage channels, and ditches.

Zk (c). Karst and other subterranean hydrological systems, human-made.

2.2.2 Classification of wetlands in the western countries

In 1974, the U.S. Fish and Wildlife Services (FWS) launched the National Wetlands Inventory (NWI) Program to carry out a comprehensive inventory of wetlands across the United States. US National Wetlands Inventory (NWI) established a national wetland classification system (Cowardin *et al.* 1979), which has been followed for wetland classification and has been adopted by other nations. NWI classification system has a refined system of categorization of wetland types, encompassing both wetlands and deepwater habitats. The classification relies primarily on factors such as vegetation, hydrologic regime, soil characteristics, salinity, and the geographical positioning of wetlands. It employs a hierarchical structure, consisting of five fundamental levels, ranging from general to specific range like: system, subsystem, class, subclass, and modifiers. The five principal systems include: Marine (the open ocean and its related coastline), Estuarine (the estuary along with its tidal and adjacent wetlands), Riverine (wetlands and deepwater habitats located within the banks of rivers and streams), Lacustrine (permanently flooded lakes and reservoirs, intermittent lakes, and tidal lakes), and Palustrine (inland vegetated wetlands, including marshes, swamps, bogs, fens, ponds, and prairie wetlands) (Tiner 2009a)(Wu ; 2018).

Mitsch and Gosselink (2015) summarized the classification systems of wetlands in the United States and drew comparisons with those of Canada and the Ramsar Convention. Classification of the wetlands conducted by Ducks Unlimited Canada (DUC), that have been developed using remote sensing and aerial surveys classified, that conform with Canadian mode of classification (Merchant *et al.*,2024) has been represented in **Figure 2.3**

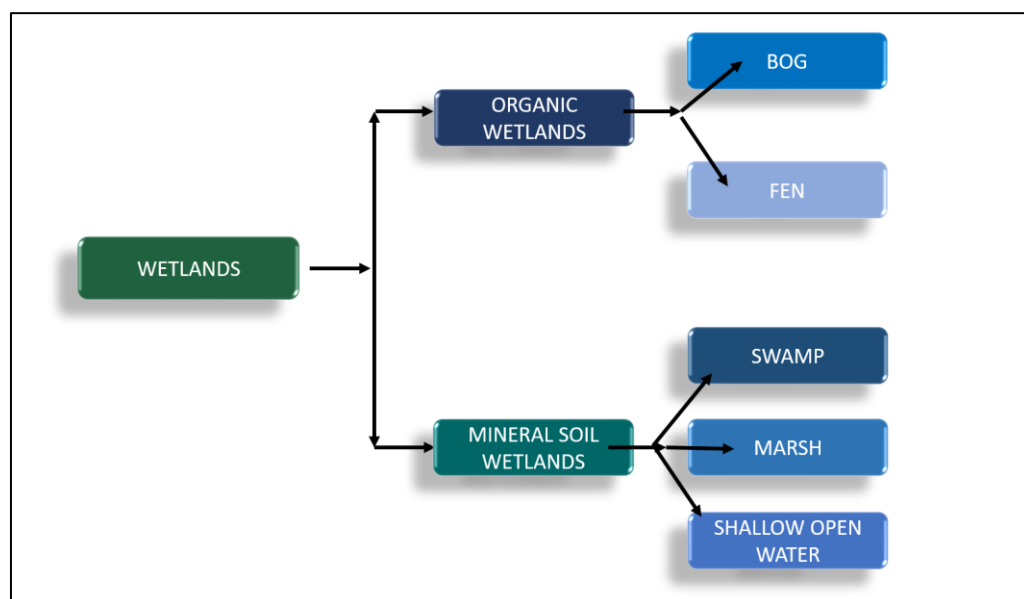


Fig 2.3: Classification of wetlands that correlated with Canadian classification of wetland system

2.2.3 Classification of wetlands in India

A wetland classification system that follows the IUCN/RAMSAR definition in India and is compatible with remotely sensed data is employed to categorize wetlands. This classification distinguishes inland and coastal wetlands as level-I, followed by natural and man-made wetlands as level-II. These are further organized into 20 types of wetlands as level-III classes, excluding rice fields. Each type of wetland displays significant diversity in aspects such as shape, size, distribution, and water quality, all of which are effectively captured through satellite imagery. The methodology for developing the wetland database complies with National Wetland Inventory Assessment (NWIA) Atlas technical standards. At level II, Indian wetlands are divided into two primary categories: Natural and Man-made. According to the Wetland Decadal Change Atlas (2006-07 to 2017-18), the total area of natural wetlands is 10.64 Mha (66.6%), while the area classified as man-made is 5.34 Mha (33.4%).

- i) Natural wetlands: These include high-altitude Himalayan lakes, wetlands located in the floodplains of major rivers, saline and temporary wetlands in arid and semi-arid regions, as well as coastal wetlands such as lagoons, backwaters, estuaries, mangrove swamps, and coral reefs (**Figure 2.2**).
- ii) Man-made Wetlands: These wetlands are constructed for specific purposes, such as water storage for irrigation and drinking, fish production, or recreational use. Examples of human-made wetlands include reservoirs, aquaculture ponds, salt pans, dams, barrages, inland lakes, impoundments, and traditional village ponds (**Figure 2.4**).

Level-I	Level-II Code	Level-III Code
Inland Wetlands	Natural (1100)	1101: Lakes
		1102: Ox-Bow Lakes/Cut-off Meanders
		1103: High altitude Wetlands
		1104: Riverine Wetlands
		1105: Waterlogged (natural)
		1106: River/Stream
	Man-made (1200)	1201: Reservoirs/Barrages
		1202: Tanks/Ponds
		1203: Waterlogged (man-made)
		1204: Salt Pans (inland)
		1205: Aquaculture ponds (inland)
Coastal Wetlands	Natural (2100)	2101: Lagoons/Backwaters
		2102: Creek
		2103: Sand/Beach
		2104: Intertidal mud flats
		2105: Salt marsh
		2106: Mangroves
		2107: Coral Reefs
	Man-made (2200)	2201: Salt Pans (Coastal)
		2202: Aquaculture ponds (coastal)

Figure 2.4: Classification of India's wetland system. (The codes of the wetlands are as per the nomenclature provided the Government of India)

Source: Garg J K and Patel J G, (2007). National Wetland Inventory and Assessment, Technical Guidelines and Procedure Manual, Technical Report, SAC/EOAM/AFEG/NWIA/TR/01/2007, June 2007, Space Applications Centre, Ahmedabad

2.3 RECLAIMED WETLANDS:

United Nations defined wetland reclamation as ‘the gain of land from wetlands and restoration of productivity or use to lands that have been degraded by human activities or impaired by natural phenomena.’ (GES 1997). Similarly, in Earth sciences wetland reclamation (they are used in the opposite sense – as a synonym for wetland restoration) is defined as ‘the rehabilitation of degraded wetlands habitat, reconstructing the impacted habitat as closely as possible to its original condition with respect to hydrological, ecological (vegetation, habitats), and morphological (soil) components’ (Koroglu 2015).

Wetlands are referred to as reclaimed wetlands since they undergo significant transformations in their landscapes to meet human requirements, primarily for agriculture, but occasionally for residential purposes too. Although reclamation initiatives have been carried out under various geographic, socio-political, and socio-economic contexts. (de Haas *et al.*,2022).

2.3.1 PURPOSE OF RECLAIMED WETLANDS:

Agricultural wetlands are vital for managing water resources, enhancing biodiversity, and delivering essential ecosystem services like nutrient cycling and carbon sequestration. Wetlands serve as crucial ecosystems for human well-being, satisfaction, and ecological health. It is imperative to promote the sustainable use of wetland resources and mitigate wetland degradation. It was suggested that enhancing the role of wetlands was essential in ecotourism and biodiversity, thereby advocating for a sustainable approach to wetland resource use that integrates wetlands with ecotourism. It was found that public awareness regarding the significance of wetlands is continually growing, encouraging more active and direct involvement in the formulation and execution of wetland management strategies. Reclamation of wetlands would cease the services provided by them to certain extent. Agricultural practices in wetlands, have impacted the ecological integrity of these areas and the services they offer. Difference has been observed between intensive agriculture, characterized by significant soil improvement, drainage, and fertilizer application, and extensive agriculture, which occurs in relatively undisturbed ecosystems, without the use of any machinery or chemicals (Verhoeven *et al.*,2009).

MA (Millennium Ecosystem Assessment) can be defined as the ecosystem services that refers to the benefits that people derive from ecosystems. The reclamation of wetlands (such as floodplains, peatlands, and wet depressions) for agricultural purposes has led to the complete destruction of the ecological integrity and the associated ecosystem services. Therefore, agricultural practices in

extensive wetland areas can coexist with high biodiversity and other ecosystem services (Verhoeven *et al.*,2009).

Water-centred agricultural wetland utilization involves leveraging wetlands within the agricultural production framework, utilizing the ecological properties of water to grow crops, cultivate aquatic products, and raise livestock and poultry in these areas, thereby fulfilling human demands for food and ecological services (Kitouni *et al.* 2018). This approach contrasts with traditional agricultural practices, which primarily enhance yield through methods such as land reclamation, water and soil conservation, weeding, fertilization, and irrigation. The focus has been on preserving and restoring the natural ecological functions of wetland ecosystems to achieve efficient and sustainable agricultural production (Kesikoglu *et al.* 2019). Additionally, the use of agricultural wetlands offers numerous ecological services, including water quality improvement, climate regulation, and biodiversity conservation, contributing to the advancement of ecological civilization and sustainable development. Agricultural wetlands serve as vital habitats for wildlife and contribute to the conservation of biodiversity. The services provided by the wetlands have been represented in **Figure 2.5**

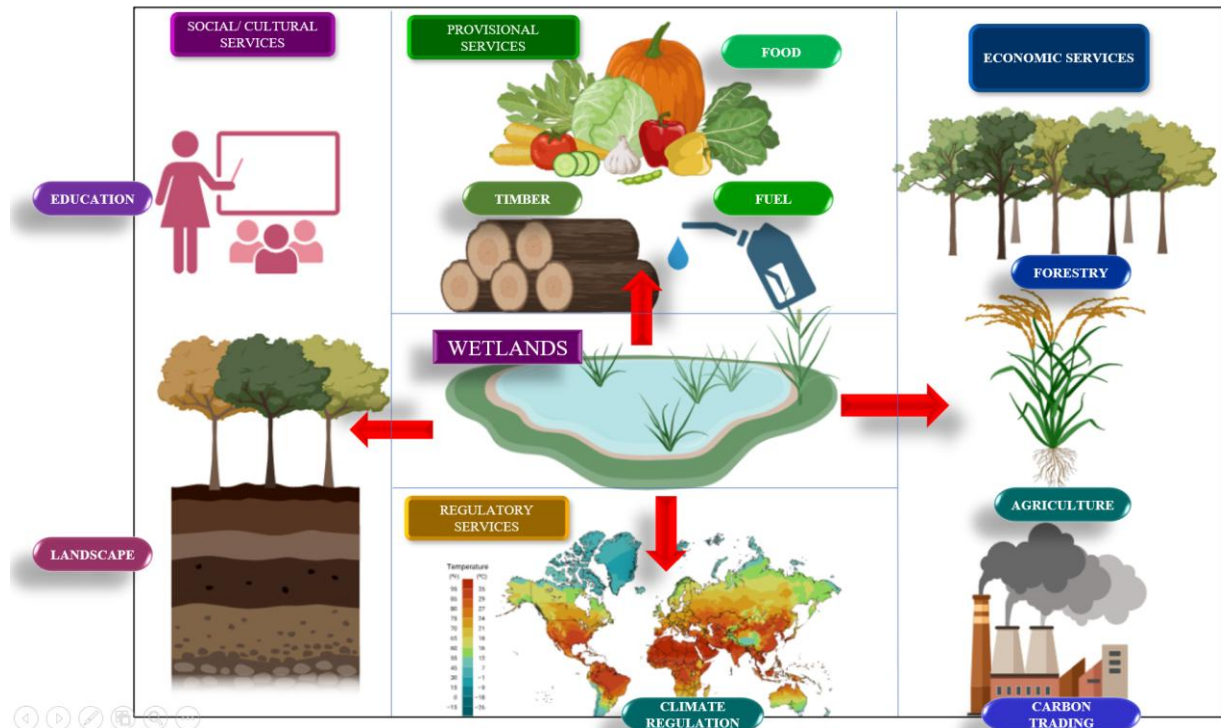


Figure 2.5: Services provided by the wetlands

Agricultural wetlands play a vital role in the interconnectedness of water, food, and energy, offering numerous ecological functions and services that support both natural and human systems. Effective water management is essential for the sustainable use of agricultural wetlands. Proper management of water resources is indispensable for ecological, economic, and social benefits of wetlands, that are vital for sustainable agricultural development and the establishment of ecological civilization. Water is a vital component of wetlands, and the wetland ecosystem relies heavily on sufficient water to sustain its life processes. Effective water management plays a key role in regulating both the quantity and quality of water in agricultural wetlands, ensuring the responsible use and protection of wetland water resources. The implementation of water-focused strategies in agricultural wetlands can significantly enhance agricultural productivity and the sustainability of farming ecosystems, while also mitigating environmental problems such as soil erosion and water pollution (Verhoeven *et al.*,2009).

Human activities and climate change have adversely impacted the availability and quality of water resources, complicating the effective use of these wetlands. Moreover, managing and maintaining agricultural wetlands demands considerable investment in both human and material resources, particularly under extreme weather and geographical conditions, which exacerbates the challenges. Lastly, the socio-economic factors and policy constraints impose certain limitations on the practical application of water-centred agricultural wetland utilization. To address these issues, the adoption of innovative technologies and the implementation of effective policy measures are crucial. The causes of water scarcity in agricultural wetlands can be due to both natural and anthropogenic interventions. Natural influences encompass climate change, inadequate rainfall. Human influences involve over-cultivation, poor management of water resources, etc. Water scarcity causes severe impacts upon agricultural wetlands, whereby it can directly affect crop growth, resulting in reduced yields or even crop failure. Secondly, water scarcity can lead to drying of soil, thereby reducing its innate capacity to retain water. Furthermore, water scarcity can disrupt the balance of wetland ecosystems, causing a decline in biodiversity and impairing the ecological functions of these wetlands. Similarly, it can also impact human livelihoods and production, hindering local economic development (Wu *et al.* 2023)

The creation of strategies and policies aimed at reducing water scarcity is important to secure agricultural wetlands and thereby encourage their sustainable utilization. The strategies include efficient water resource management framework, advocating for water conservation technologies, and enhancing water resource monitoring.

Prevention of water pollution has been extremely important for the proper functioning of wetlands. The methods of prevention of water usually encompass the effective management of chemicals like pesticides and fertilizers, regulating waste discharge from the aquaculture sector, and improving the oversight of industrial wastewater release. These processes are vital to bolster water quality monitoring and management, and at the same time expeditiously handle any identified water pollution problems, thereby safeguarding ecological environment and human health within agricultural wetlands (Marconi *et al.*,2022).

The consequences of agricultural wetland degradation include the loss of ecosystems, where wetland degradation can result in the extinction of numerous animal and plant species, disrupting the equilibrium of wetland ecosystems; decline in land quality, as degraded wetland areas may become infertile and incapable of supporting crop and vegetation growth, leading to a reduction in land quality; and loss of water resources, where wetland degradation can cause the depletion and contamination of water resources, thereby impacting the availability of water resources in adjacent areas.

Agricultural wetlands play a crucial role. The essence of this strategy is to enhance human satisfaction while safeguarding ecosystem health, through comprehensive research on wetland use and management, along with detailed field studies employing both quantitative and qualitative techniques, determined that not all wetlands are appropriate for food crop cultivation. Significant degradation is observed and high cultivation costs are required, if the food crops are grown on improper wetlands. Global population growth and economic development progression, shows the disparity between the supply and demand for food, water, and energy, with water scarcity emerging as a critical global issue (Maithya *et al.*,2020).

Concurrently, wetland ecosystems, recognized as some of the most diverse on the planet, are vital for sustaining the global water cycle and preserving biodiversity. The imbalance in the water-food-energy interconnections has resulted in various challenges and problems in the management and utilization of agricultural wetlands, such as changes in land use, degradation of wetlands, and unsuitable agricultural practices (Wu *et al.* 2023).

2.4 Distribution and Extent of Wetlands in India

India, characterized by its diverse topography and climatic conditions, hosts a variety of unique wetland ecosystems. These wetlands are found to support one-fifth of the known biodiversity of the area. These wetland areas are found across various geographical regions, from the Himalayas to the Deccan Plateau. According to the Country Report of the Directory of Asian Wetlands and the Directory of Indian Wetlands 1993, the total area of wetlands in India was approximately 58.3 million hectares. The paddy fields comprised nearly 71% of this wetland area. However, estimates from the Ministry of Environment and Forests (1990) indicated that wetlands cover about 4.1 million hectares, excluding mangroves. The first scientific mapping of the country's wetlands was conducted using satellite data from 1992 to 1993 by the Space Applications Centre (SAC) in Ahmedabad. This mapping classified wetlands according to the Ramsar Convention definition, estimating the total area of wetlands to be around 7.6 million hectares. These estimates did not account for paddy fields, rivers, canals, and irrigation channels. (Arya *et al.*,2020). The "National Wetland Inventory and Assessment - 2nd cycle" was conducted by the Space Applications Centre (SAC) in Ahmedabad at the behest of the Ministry of Environment, Forests and Climate Change in 2019 to understand the distribution of wetlands across India, whereby the total wetland area estimated is 15.98 million hectares (Mha), which is around 4.86 percent of the total geographic area of the country (Gupta *et al.*,2021). The Ramsar sites of India have been represented in **Figure 2.6**.

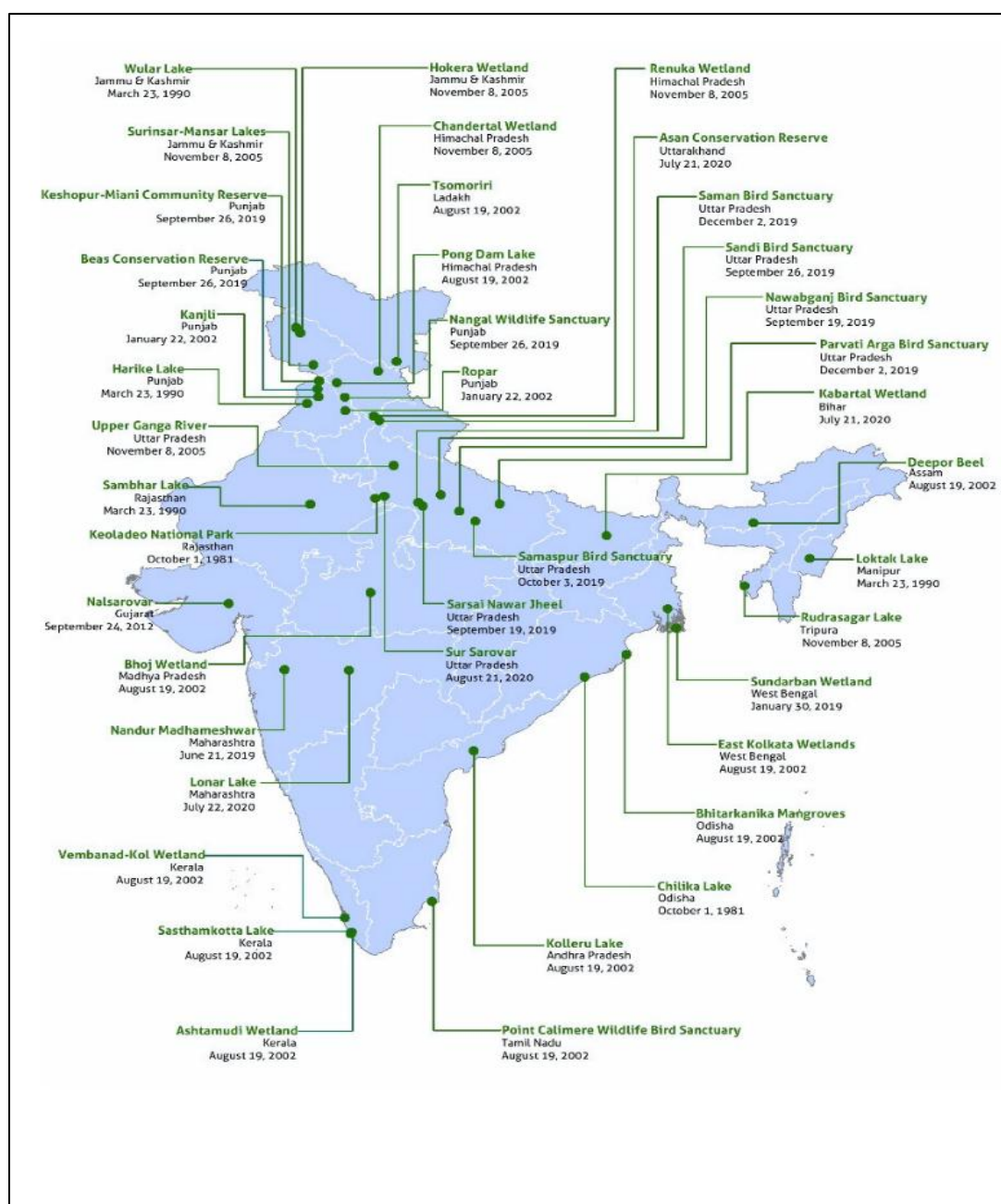


Figure 2.6: Ramsar Sites of India

Source: <https://indianwetlands.in/our-work/implementing-the-ramsar-convention/> (Ministry of Environment, Forest and Climate Change, Government of India)

2.4.1 India's wetland extent according to the most recent wetland inventory

Among all regions, if we consider the state-wise wetland distribution, Gujarat stands out with the largest wetland area in the country, amounting to 3,417,553.7 hectares. This is significantly higher than any other state and suggests that Gujarat plays a major role in India's wetland ecosystem. Other states with large wetland areas include West Bengal with 1,180,543.1 ha, Andhra Pradesh has 1,197,707.15 ha and Karnataka with 1,070,518.2 ha. These states contribute substantially to India's ecological and hydrological balance. Several other states also report substantial wetland coverage. For example, Assam has 867,150.15 ha, and Odisha has 835,863 ha of wetlands. These regions, often rich in biodiversity and influenced by riverine and coastal systems, highlight the environmental significance of wetlands in supporting wildlife, agriculture, and livelihoods. On the other hand, some regions show relatively smaller wetland areas. Delhi, for instance, has only 1,770.6 ha, while Sikkim records the lowest value with 6,778.7 ha. Other UTs like Chandigarh, Daman and Diu, and Lakshadweep also report wetland areas under 10,000 ha, which can be attributed to their limited geographic area and high levels of urbanization or insular nature (National wetland statistics, 2020).

Distribution of Inland wetlands in India

Natural inland wetlands account for roughly 43.9% of the total, while man-made systems contribute about 30.2% (National wetland statistics, 2020). Gujarat leads all states in overall wetland area, with inland wetlands representing a large share of its ~3.5 million ha (~21.9% of the national wetland area). Though Gujarat is known for its massive coastal mudflats, its inland reservoirs and salt pans also form a significant component. States rich in inland wetlands also include Maharashtra (1.15 million ha), Andhra Pradesh (1.14 million ha), Uttar Pradesh (1.10 million ha), West Bengal (1.13 million ha), and Madhya Pradesh (0.86 M ha)—all predominantly inland systems comprising river floodplains, reservoirs, tanks, and ponds (Government of India, Press Information Bureau, 2023). Tamil Nadu also has significant inland wetlands: lakes/ponds, tanks and riverine systems cover approximately 761,867 ha, about 84% of the state's wetland area (~0.92 million ha) (Environment, Climate Change and Forest Department - Tamil Nadu State Wetland Authority, 2025).

Distribution of Coastal wetlands in India

Coastal wetlands make up about 4.14 million ha, or around 27% of India's entire wetland area. These encompass natural ecosystems like lagoons, intertidal mudflats, salt marshes, mangroves, creeks, and coral reefs and human-made coastal areas such as salt pans and aquaculture ponds (National wetland statistics, 2020).

Gujarat has coastal wetlands, especially intertidal mudflats that cover over 2.26 million ha, supplemented by substantial areas of mangroves (90,475 ha), salt marshes and lagoons

West Bengal and Odisha also host extensive coastal wetlands: West Bengal's Sundarbans mangrove (209,330 ha) and Odisha's Bhitarkanika mangrove (65 000 ha) alongside Chilika Lake, a major brackish estuarine lagoon (246,044 ha in Odisha overall).

Tamil Nadu has coastal wetlands worth approximately 122,373 ha (13.6% of its total wetlands), including lagoons, mangroves, salt marshes, intertidal flats, salt pans, and aquaculture ponds (Environment, Climate Change and Forest Department - Tamil Nadu State Wetland Authority, 2025). Andhra Pradesh also shows a significant coastal component: mangrove cover (41,486 ha), lagoons (47,407 ha), and inland aquaculture ponds contribute to its total 1.14 million ha wetland area (Government of India, Press Information Bureau, 2023, <https://www.pib.gov.in/indexd.aspx>).

2.4.2 The significance of wetlands in India

Wetlands are recognized for their distinct ecological characteristics, offering a variety of products and services to humanity. The ecosystem goods provided by wetlands primarily consist of: irrigation water; fisheries; non-timber forest products; water supply; and recreational opportunities. Key services include: carbon sequestration, flood control, groundwater recharge, nutrient removal, toxic retention, and the maintenance of biodiversity. These services arise from the functioning of wetland ecosystems and are thus referred to as 'wetland functions'. In addition to the intensive agricultural practices that have compromised wetland functions and services globally, many wetlands have been subjected to extensive land uses. These uses often integrate food production with other roles, such as enhancing water quality, flood management, or promoting biodiversity. Examples include traditional crop farming methods that avoid chemical fertilizers and pesticides, livestock grazing schemes, and conventional water management practices aimed at boosting fish production and improving fish yields. (Arya *et al.*,2020).

The services provided by wetlands have been classified mainly into three categories, namely

1. Ecological services
2. Commercial service
3. Wetland reclamation services

1.Ecological services include:

a) Carbon Sequestration

Wetlands sequester carbon through high rates of organic matter input and lower rates of decomposition. The carbon content in wetland soils can be up to 200 times greater than that in the vegetation. However, the drainage from the extensive wetland areas and their subsequent cultivation in many regions have turned them into a net source of CO₂. Restoring wetlands can transform them back into a sink for atmospheric CO₂. Estimates suggest that the carbon sequestration potential of restored wetlands over a 50-year period is about 0.4 tonnes C/ha/year (IPCC, 2000). In India, coastal wetlands are also known to contribute towards carbon sequestration. The total area of coastal ecosystems, including mangroves, in India is approximately 43,000 km². Mangrove wetlands in eastern India serve as more significant carbon sinks compared to those on the west coast, due to their larger size, greater diversity, and more complex structures created by tidal creeks and canal networks (Lal.,2008). In general, mangroves can sequester around 1.5 metric tonnes of carbon per hectare annually, and the upper layers of mangrove sediments are rich in

carbon, with conservative estimates suggesting levels of 10%. It has been observed that mangroves emit methane (CH₄), a major greenhouse gas, which accounts for about 19% of their carbon sequestration capacity. This can be due to plant respiration and microbial decomposition, which emit CO₂ and CH₄ (methane)(Wylynko 1999). They also function as important carbon sinks by sequestering substantial quantities of carbon in their soils, owing to the gradual breakdown of organic matter in the anaerobic environment. Likewise, tropical coastal wetlands, such as Vembanad Lake—a lagoon located along the West Coast of India (Kerala)—have been found to release as much as 193.2 mg/m²/h of CH₄. The role of wetlands as either net carbon sequesters or greenhouse gas producers is influenced by their biogeochemical processes and hydrology (Arya *et al.*,2020).

b) Pollution Mitigation

Wetlands serve as a filter for pollutants in various agricultural and urban environments. Economically, wetlands are proposed as a cost-effective solution for mitigating both point and non-point source of pollution. Natural wetlands, including riparian types, help in the decrease of the nutrient load in flowing water by extracting nitrate and phosphorus from both surface and sub-surface runoff. The maximum potential rates for nitrogen and phosphorus removal by wetlands in temperate regions are estimated to be between 1000 to 3000 kg N/ha/year and 60 to 100 kg P/ha/year, respectively (Bystrom *et al.*, 2000). These natural wetlands may deteriorate due to increased pollution loads, leading to eutrophication, which can negatively impact wildlife habitats and recreational opportunities. On the other hand, well-designed restored or constructed wetlands can function effectively as pollution sinks, provided that the costs of abatement are low enough to encourage the restoration or creation of wetlands as part of a cost-efficient pollution reduction strategy. In India, wetlands are similarly affected by agricultural runoff and the discharge of untreated sewage and other urban waste. Under typical conditions, wetlands usually filter pollutants from both surface and subsurface runoff from their catchment areas, preventing these contaminants from entering streams and rivers. However, due to increased urbanization and changes in land use, the nutrient loading in wetlands often surpasses their ability to retain and remove pollutants through processes such as nitrification, sedimentation, adsorption, and uptake by aquatic vegetation. This negatively impacts the quality of water in wetlands and their biodiversity. Such wetlands experience significant alterations in nutrient cycling rates, leading to species loss. *Typha*, *Phragmites*, *Eichhornia*, *Azolla*, and *Lemna* are some of the identified effective wetland plants for the removal of heavy metal (Bassi *et al.*,2014).

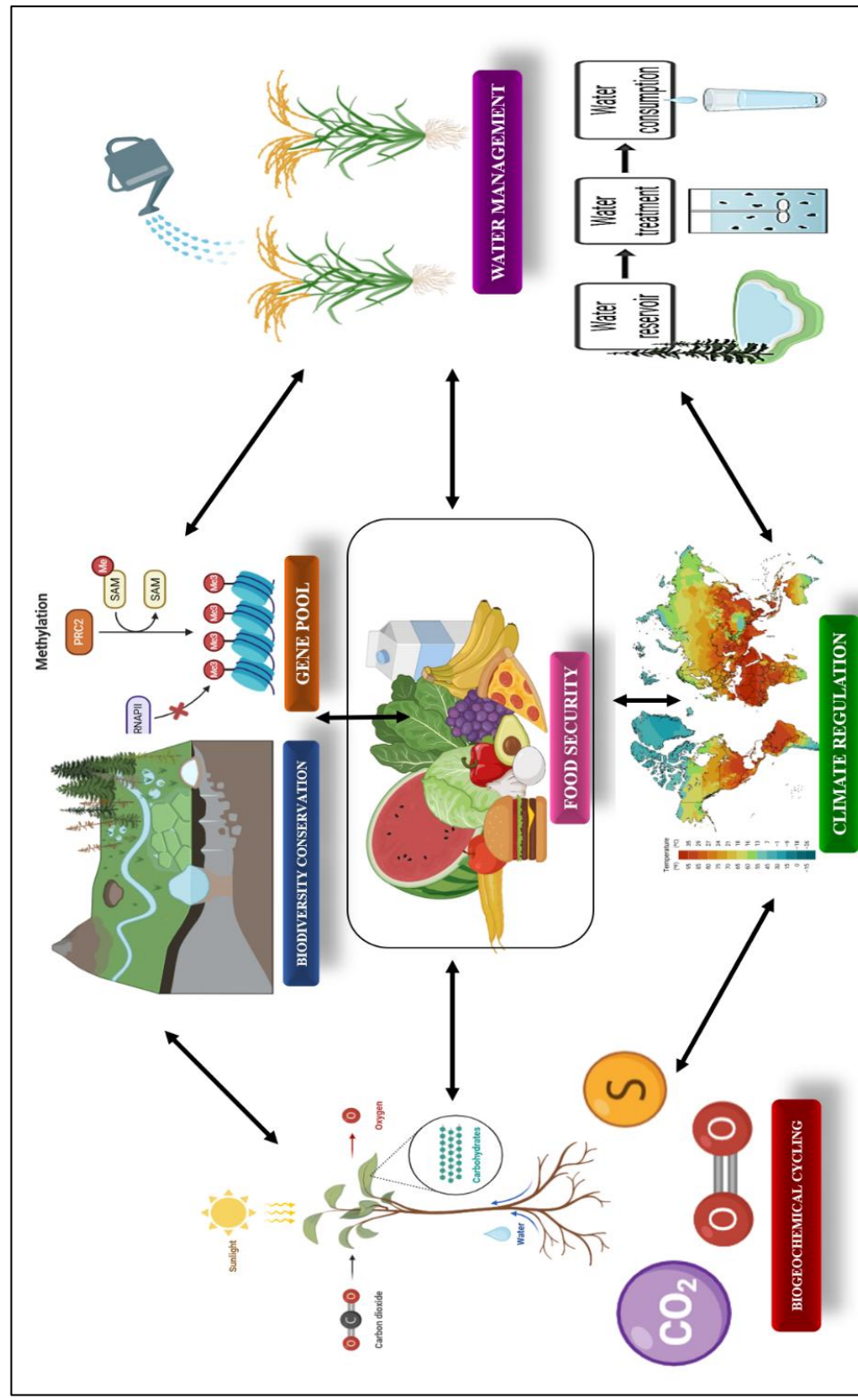


Figure 2.7: Services provided by East Kolkata wetlands

c) Flood Management

Wetlands are crucial for managing floods. They mitigate flooding effects by absorbing excess water and slowing down the flow of floodwaters. Additionally, during flooding events, wetlands capture suspended solids and nutrient loads. Consequently, streams that pass-through wetlands before reaching rivers, carry fewer suspended solids and nutrients compared to those that flow directly into the rivers. Since the wetlands are effective in preventing flood damage, they are being viewed as a natural alternative to traditional flood control measures like dykes, dams, and embankments. A primary factor contributing to flood-related disasters is the reduction in wetland area due to conversion for agricultural purposes, such as rice cultivation and fish pond aquaculture (Boyd *et al.*, 2007). Moreover, increased groundwater extraction for agriculture in eastern India, particularly in West Bengal, may negatively affect wetlands, as they also depend on inflows from shallow aquifers. The lowering of the water table in these shallow aquifers during the winter-summer seasons, when agricultural water demand rises, can lead to the temporary desiccation of shallow wetlands (Bassi *et al.*, 2014).

2. Commercial Service:

Wetlands inclusive of tanks, ponds, lakes, and reservoirs, have historically provided multiple-use water services, which encompass water for irrigation, domestic purposes, fisheries, and recreational activities; groundwater recharge; flood control; and silt capture. The southern states of Andhra Pradesh, Karnataka, and Tamil Nadu host the largest number of irrigation tanks, totalling to 0.12 million and account for nearly 60% of India's tank-irrigated area. These tanks are crucial for capturing surface runoff during the monsoon season, allowing for its later use. Beyond irrigation, they serve multiple purposes including fisheries, providing water for domestic use, enriching soils with nutrients, collecting fodder grass, and facilitating brick production. These functions significantly enhance household income, nutrition, and health for the most impoverished communities. From an ecological standpoint, tanks are essential as they aid in the conservation of soil, water, and biodiversity. Additionally, they play a role in groundwater recharge, flood management, and silt retention. Water sourced from tanks is also utilized for both domestic and livestock needs. Likewise, ponds in the northeastern states of India are utilized for fisheries and for irrigating home gardens (Central Ground Water Board, 2011) (Parra *et al.*, 2025). Lakes such as Carambolim (Goa), Chilika (Orissa), Dal Jheel (Jammu and Kashmir), Deepor Beel (Assam), Khabartal (Bihar), Kolleru (Andhra Pradesh), Loktak (Manipur), Nainital (Uttarakhand), Nalsarovar (Gujarat), and Vembanad (Kerala) have historically provided services for recreation,

tourism, fisheries, irrigation, and domestic water supply. These lakes also aid in groundwater recharge and support a diverse array of aquatic life. Furthermore, surface reservoirs have been instrumental in ensuring irrigation and domestic water security in both rural and urban settings (Arya *et al.*,2020). The effects of tourism on the ecological functions of wetlands arise due to the transport and other infrastructure projects related to tourism, as well as the construction, maintenance, and operation of tourist facilities (Daryadel *et al.*,2014).

3. Reclamation services

The growth of the world population and the rising demand for food, coupled with a shortage of fertile agricultural land over the past century, have led to the drainage and conversion of wetlands for agricultural purposes, thereby leading to the reclamation of wetlands for agricultural purposes. It has been clearly shown that drainage and transforming wetlands into croplands significantly affects the characteristics and functioning of these ecosystems. The capacity of wetlands to mitigate and cleanse stormwater is severely diminished, which in turn impacts downstream water users, including humans and other aquatic systems. The complete transformation of wetland habitats into croplands results in their total loss. Additionally, drainage can adversely influence water flow and supply during dry seasons, leading to serious consequences for downstream organisms and water quality due to the accumulation of pollutants. The degradation of wetlands and riparian zones accelerates sediment transport through streams, which reduces habitat diversity and disrupts ecological corridors within the landscape. Streams connected to wetlands carry increased sediment loads, causing significant environmental harm (Daryadel *et al.*,2014).

2.5 Escalating Threat to Wetland Ecosystems

Freshwater wetland ecosystems rank among the most heavily utilized, relied upon, and exploited systems for sustainability and human well-being. Over 50% of certain wetland types in regions of North America, Europe, Australia, and New Zealand were transformed during the twentieth century (MEA, 2005, <https://wedocs.unep.org/handle/20.500.11822/8735>).

In Asia, approximately 5000 km² of wetland area is lost each year due to agriculture, dam construction, and other activities. Additionally, the reliance on water and other resources in these environments has imposed significant pressures on ecosystems globally, leading to direct consequences for species diversity and populations. Consequently, many species that depend on wetlands, including 21% of bird species, 37% of mammal species, and 20% of freshwater fish species, are either extinct or face global threats (MEA, 2005). The reduction in wetland area adversely affects the essential functions (ecosystem goods and services) that wetlands provide (Zedler *et al.*, 2005). Globally, the primary drivers of wetland loss include urbanization, changes in land use, drainage for agricultural purposes, infrastructure development, pollution from industrial waste and agricultural runoff, as well as climate change and variability (Chaudhuri *et al.*, 2012).

a) Urbanization and Changes in Land Use

In between 1951 and 2011, India's total population surged from 0.4 billion to 1.2 billion, with an average growth rate of about 22%. This level of growth has placed immense pressure on wetlands and floodplain areas to satisfy the water and food needs of the expanding population. Between 1950 and 1951, and again from 2008 to 2009, the total area of cultivated land in India rose from approximately 129 million hectares to 156 million hectares. Additionally, the land designated for non-agricultural purposes, such as commercial or residential use, expanded from 9 million hectares to 26 million hectares. In many of India's major river basins, the growth in land for both agricultural and non-agricultural purposes came at the expense of converting floodplain regions, primary forests, grasslands, and related freshwater ecosystems to satisfy the needs of a growing population. For example, around 34,000 hectares of the water spread area of Kolleru Lake in Andhra Pradesh have been reclaimed for agricultural use in recent years (MoEFCC., 2018). Moreover, there has been extensive development of irrigation and water supply infrastructure across the country, which has changed the inflows and water spread areas of numerous water bodies. By 2007, approximately 276 major and 1,000 medium irrigation projects had been completed in India (Central Water Commission, 2010), with an estimated total water storage capacity of about 225 billion cubic

meters, representing 12% of India's total water resources potential. While large reservoir projects have been essential for water supply, flood control, irrigation, and hydroelectric power generation, the rapid increase in artificial water impoundment structures, often without adequate hydrological and economic planning (such as the construction of small dams in semi-arid and arid regions with limited runoff capacity), has led to significant loss and fragmentation of freshwater habitats (Kumar *et al.*, 2008; Zhao *et al.*, 2006) and a decrease in environmental flows due to the over-allocation of water primarily for agricultural and industrial needs. Currently, the majority of river basins in southern and western India are facing environmental water scarcity. This indicates that the water discharge in these basins has been diminished by withdrawals to such an extent that the remaining water is insufficient for the freshwater-dependent ecosystems (Smakhtin *et al.*, 2004) (Bassi *et al.*, 2014).

Urbanization significantly impacts the structure and function of wetlands, primarily by altering hydrological and sedimentation patterns, as well as the dynamics of nutrients and chemical pollutants. The effects of urbanization on natural water bodies within cities are equally concerning. Research revealed that among 629 water bodies identified in the National Capital Region (NCR) of Delhi, 232 are beyond revival, due to extensive encroachments (Khandekar, 2011) (Bassi *et al.*, 2014).

In a similar vein, from 1973 to 2007, the Greater Bengaluru Region lost 66 wetlands, covering approximately 1100 hectares, as a result of urban expansion. Moreover, inadequate management of water bodies, the absence of effective conservation strategies, increasing pollution levels, and a rapid rise in localized water demands are driving these vital ecological balance maintainers towards extinction (Bassi *et al.*, 2014).

b) Agricultural, municipal, and industrial pollution

Water quality in most Asian rivers, lakes, streams, and wetlands has significantly deteriorated, primarily due to agricultural runoff containing pesticides and fertilizers, as well as discharges from industrial and municipal wastewater, all of which contribute to widespread eutrophication [Liu *et al.*, 2005; Prasad *et al.*, 2002].

As a consequence of the intensification of agricultural practices over the last forty years, fertilizer usage in India has surged from approximately 2.8 million tonnes in 1973–1974 to 28.3 million tonnes in 2010–2011. Estimates suggest that 10–15% of the nutrients applied to soils via fertilizers ultimately enter the surface water system. Elevated nutrient levels promote algal proliferation,

resulting in the eutrophication of surface water bodies. Research shows that concentrations of 0.5 mg/l of inorganic nitrogen and 0.01 mg/l of organic phosphorus in water typically trigger undesirable algal growth in surface waters. Runoff from agricultural lands is the primary source of non-point source pollution affecting Indian rivers that traverse the Indo-Gangetic plains (Jain *et al.*, 2007a, b).

Water from lakes that suffer from algal blooms incurs higher purification costs for drinking or industrial applications. Eutrophication can diminish or eradicate fish populations (Verhoeven *et al.*, 2006) and may also lead to the loss of numerous cultural services provided by lakes. In addition to runoff from agricultural fields, untreated wastewater significantly exacerbates the pollution of water bodies. Less than 31% of domestic wastewater from urban centres in India is treated, in contrast to 80% in developed countries. Among 35 metropolitan cities, treatment facilities are available for only 51% of the sewage produced (Central Pollution Control Board, 2009., Verhoeven *et al.*, 2006).

c) Additional threats

Global climate change is anticipated to be a significant factor in the loss and transformation of wetland ecosystems (MEA, 2005; UNESCO, 2007). These insights are particularly relevant for the Indian subcontinent, where the average atmospheric temperature and the frequency of intense rainfall events have risen, while the number of rainy days and the total annual precipitation have declined due to the increased concentration of greenhouse gases like CO₂, CH₄, and N₂O in the atmosphere. Limited studies on the effects of climate change on wetlands in India indicate that high-altitude wetlands and coastal wetlands (including mangroves and coral reefs) are among the most vulnerable types that will be impacted by climate change (Patel *et al.*, 2009). For example, the rising levels of glacial-fed high-altitude lakes, such as Tsomoriri in Ladakh, have submerged crucial breeding islands in the lake where endangered migratory birds like the Black-necked Crane and Bar-headed Goose would nest (Chandan *et al.*, 2008). In the case of coastal wetlands, such as the Indian portion of the Sunderbans mangrove, increasing sea surface temperatures and rising sea levels due to thermal expansion could disrupt fish distribution and lead to the destruction of a significant portion of the mangrove ecosystem. The further degradation of the Sundarbans mangroves would undermine their essential role as natural barriers against tropical cyclones, resulting in loss of life and livelihoods (Centre for Science and Environment, 2012; UNESCO, 2007). The limited analysis appears to indicate that inland natural wetlands, particularly those located in arid and semi-arid areas, will be affected by changes in their hydrological regime due to

variations in precipitation, runoff, temperature, and evapo-transpiration. The rise in temperature and the decline in rainfall patterns caused by climate change pose a significant threat to the already vanishing lakes in the Gangetic plains (Sinha, 2011). Runoff in river basins, such as the Ganges, is expected to intensify with global warming, meaning that wet seasons will become wetter and dry seasons drier (World Bank, 2012, Bassi *et al.*, 2014). This shift could have severe negative impacts on affected communities, particularly if the changes in runoff seasonality do not align with water demand. Estimates suggest that India could lose approximately 84% of its coastal wetlands and 13% of its saline wetlands due to a climate change-induced sea level rise of 1 meter. Consequently, there will be detrimental effects on wetland species, especially those unable to migrate to suitable habitats, as well as on migratory species that depend on various wetland types throughout their life cycles. However, it is important to note that projections regarding the extent of wetland loss and degradation are not yet well-defined, as the climate models used for these predictions lack robustness. It remains unclear how the temporal and spatial variability of rainfall in the region is represented in these models (Arya *et al.*, 2020) (Bassi *et al.*, 2014).

Institutional strategies implemented for wetland management in India

In India, wetlands are often viewed in isolation and rarely included in water resource management and development plans. The Ministry of Environment and Forests (MoEF), Government of India, holds the main responsibility for managing these ecologically sensitive ecosystems. Despite India's commitment to both the Ramsar Convention on Wetlands and the Convention on Biological Diversity, a clear regulatory framework for wetland conservation appears to be lacking (Arya *et al.*, 2020).

2.5 EAST KOLKATA WETLANDS(EKW)

This investigation involves an intensive study of East Kolkata Wetland; therefore, a critically deep review of literature has been done on East Kolkata Wetlands, in order to establish the current knowledge and the applications of the work.

EKW (22° 27' N 88° 27' E) encompasses an area of 12,500 hectares of inter-tributary marshes formed by the shifting of the Hooghly River. Originally, these were saltwater lakes that stretched between the Bidyadhari River to the east and the Hooghly River to the west. Since the 18th century, there have been significant alterations in land use patterns primarily due to reclamation, river silting, and the diversion of both domestic and industrial wastewater, which has caused the marshes to become stagnant and severed their connection with the Bay of Bengal. The geographical positioning of EKW allows it to function as a spill basin for Kolkata, serving both rainwater storage and flood management. Consequently, this leads to the accumulation of various pollutants in EKW. The integrated resource recovery efforts at EKW trace back to the mid-19th century, initiated by Mr. Bhabanath Sen, a Bengali entrepreneur. He began by acquiring one square kilometre of wetland known as Dhapa for vegetable farming. It was in 1983 that the term 'East Calcutta Wetland' was introduced by Dr. Dhrubajyoti Ghosh (Former South Asia Chair of the IUCN Commission on Ecosystem Management), and brought the wetland to global attention. (Chaudhuri *et al.*, 2012). Cultivable lands have emerged from former solid waste dumping sites. Each day, garbage farms yield 150 tonnes of fresh vegetables. The paddy fields produce 16,000 tonnes of winter paddy varieties grown during the post-monsoon season each year. There is considerable debate and uncertainty regarding the safety, quality, and reliability of the farming methods that utilize treated solid and soluble waste for the cultivation of green leafy vegetables at EKW [Gupta *et al.*, 1990; Bhattacharyya *et al.*, 2003].

Socio-economic significance of East Kolkata Wetland: This wetland plays a vital role in safeguarding the environment and provides a stable urban fringe for the growing metropolis of Kolkata, serving as a lifeline for approximately 150,000 people. It is home to the world's largest aquaculture system fed by wastewater. Sewage from the city is directed to the East Kolkata Wetland, where it undergoes solar purification followed by natural oxidation, making the water suitable for the growth of algae and phytoplankton, which are essential producers in the aquatic ecosystem. The East Kolkata Wetland consists of 272 bheries, covering an area of 4,000 hectares. Sewage is transported to the wetland via DWF channels (Bantala gate) and reaches the bheries through several secondary canals lined with dense vegetation of water hyacinth (*Eichhornia*

crassipes), which plays a crucial role in the phytoremediation of city sewage. The bheries, with depths ranging from 0.7 to 3 meters, function as large natural oxidation ponds that facilitate extensive wastewater purification and integrated resource recovery. The overflow from these ponds is utilized in agricultural areas for vegetable cultivation, ultimately leading to the excess water from the wastewater-fed aquaculture system. The East Kolkata Wetland offers numerous aquatic plant resources that are utilized for green manure, compost, herbal medicine, vegetables, paper pulp, rhizo-filtration, and fodder. However, the East Kolkata Wetland is currently experiencing significant environmental pressures due to the influx of toxic industrial effluents containing hazardous pollutants such as heavy metals (Pb, Cr, Cu, Ni, etc.) and oil grease (Chaudhuri *et al.*,2012) (Mandal.,2021).

2.5.1 THREATS TO EAST KOLKATA WETLANDS

The wetlands play a vital role as a habitat for a wide variety of plant and animal species, as well as functioning as a natural sewage treatment system for the Kolkata Megacity. The East Kolkata Wetlands (EKW) repurposes Kolkata's wastewater for use in fisheries, agriculture, and paddy fields, establishing an economic and ecological urban waste recycling system. This area supports a rich diversity of flora and fauna, including fish, birds, and aquatic plants, which are essential for maintaining ecological balance and biodiversity. This remarkable system not only sustains a variety of living organisms, but also provides livelihoods for thousands of local residents involved in agriculture and aquaculture [Chaudhuri *et al.*,2012; Mundoli *et al.*,2023].

However, despite their significance, the EKW faces numerous challenges that jeopardize their ecological integrity and sustainability.

Land encroachment, pollution, habitat degradation, and biodiversity loss are among the most urgent issues threatening this crucial ecosystem. Over the past few decades, urbanization has led to a significant decrease in wetland area (Mundoli *et al.*,2023). The influx of untreated sewage and solid waste has drastically affected water quality, reducing the wetlands' natural ability to purify, thereby resulting in elevated pollutant levels (Kumar *et al.*,2023). Additionally, habitat destruction and human activities have led to a decline in biodiversity, while land subsidence and salinization present further risks to the ecological health of the EKW [Sahu *et al.*,2011; Hettiarachchi *et al.*,2014; Saha *et al.*,2021].

Land encroachment: The reports of encroachment of EKW, driven by urban expansion, has drastically affected the land conditions. A reduction in the size of EKW could result in harmful effects, such as alterations in ecosystem characteristics (flora and fauna), a decline in livelihoods, and a decrease in revenue stemming from a smaller wetland area. The overall impact would be catastrophic for the city of Kolkata (Chaudhuri *et al.*,2012).

Pollution continues to pose a significant threat to the East Kolkata Wetlands (EKW), jeopardizing its ecological integrity. The West Bengal Pollution Control Board reported that approximately 750 million litres of untreated sewage flow into the wetlands each day, surpassing their natural ability to purify [Saha *et al.*,2021; Kumar *et al.*,2023]. This surge leads to elevated levels of organic pollutants, nutrients, and heavy metals, which critically impair water quality and ecological health (Dutta *et al.*,2021). Furthermore, the issue of solid waste dumping intensifies the pollution challenges. The Dhapa landfill, situated within the EKW, serves as a primary waste disposal facility for Kolkata. Covering more than 60 hectares, this landfill plays a crucial role in the city's waste management strategy, but significantly disrupts the wetland ecosystem. Although the EKW's fisheries and agricultural fields, nourished by sewage, illustrate natural waste recycling, the landfill undermines these beneficial processes. With over 4,000 metric tons of waste deposited daily, it leads to severe contamination of soil, air, and water [Kundu *et al.*,2017; Banerjee *et al.*,2017]. Beyond ecological risks, the landfill has been expanding in response to the rising volume of urban waste. It poses health risks to surrounding communities due to air pollution from spontaneous fires and methane emissions. During the outbreak of Covid, in the year 2019, it was observed that, there was a significant decline in ecological landscapes of EKW, including vegetation cover and water bodies, as well as the loss of the East Kolkata Wetland (EKW), leading to a deterioration in environmental health [Das *et al.*,2020; Das *et al.*,2021]. A pathogenic bacterium like *Lasiodiplodia theobromae* was also found from the agriculture soil of EKW, known to be a nosocomial pathogen and has a significant role as an opportunistic pathogen to humans, causing infections (Ghosh *et al.*,2024). Moreover, recent research has revealed concerning levels of plastic debris and other pollutants in the water and sediment throughout the EKW [Kundu *et al.*,2017; Saha *et al.*,2021].

Decline in biodiversity and Habitat Degradation: The combined impacts of pollution, habitat degradation, and human-induced disturbances have led to a significant decrease in biodiversity within the EKW (Banerjee *et al.*,2017). The introduction of non-native species has magnified the decline of native biodiversity, leading to changes in species composition and disrupted ecosystem functions. Furthermore, the increasing demand for fish, along with unsustainable fishing practices

in fragmented Bheris, has resulted in overfishing, which impedes the natural recovery of fish populations. This overexploitation further reduces biodiversity and disrupts the food web within the wetlands. Habitat loss is closely associated with urban encroachment and escalating pollution levels. The reduction of wetland areas and the decline in water quality have devastated crucial habitats for numerous species. This has impacted the vital ecological services that the wetlands offer, such as water filtration and flood control. This persistent decline in habitat quality represents a significant threat to both local wildlife and the overall ecological integrity of the EKW (Kundu *et al.*,2017). The risks as assessed in EKW has been mentioned in **Figure 2.8**.

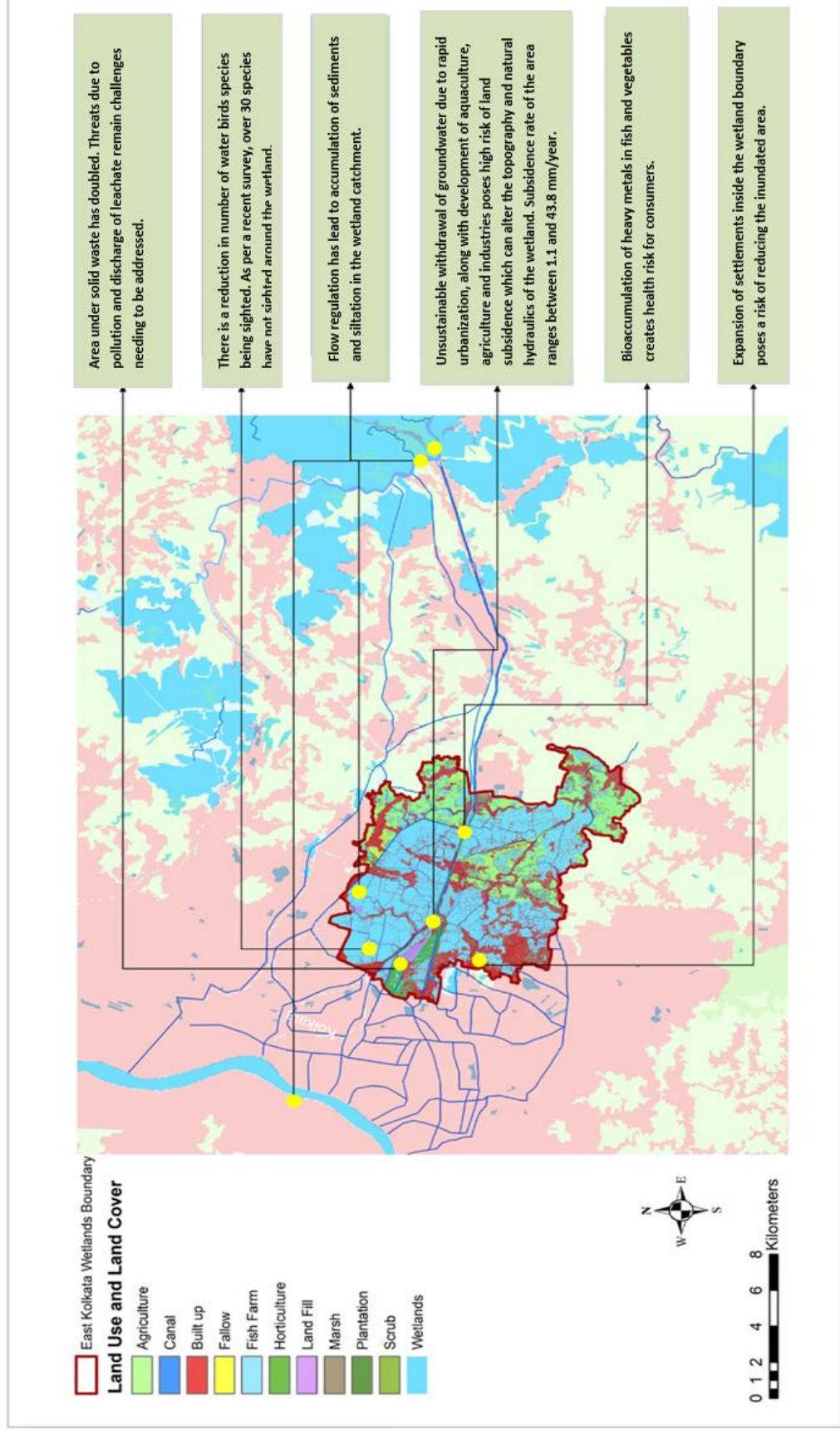


Figure 2.8: Risks assessed in East Kolkata Wetlands

Source: East Kolkata Wetland Management Plan 2021-2026(Department of Environment, Government of West Bengal.

2.5.2 SHRINKAGE OF THE WETLAND OVER THE YEARS

Analysis of satellite imagery has shown a notable reduction in wetland areas over the last 35 years, with approximately 34% of the total wetlands in EKW being lost due to urban development, infrastructure projects, and market-driven agricultural practices. This habitat loss poses a threat not only to the ecological health of the wetlands but also jeopardizes the livelihoods of local communities that rely on these resources.

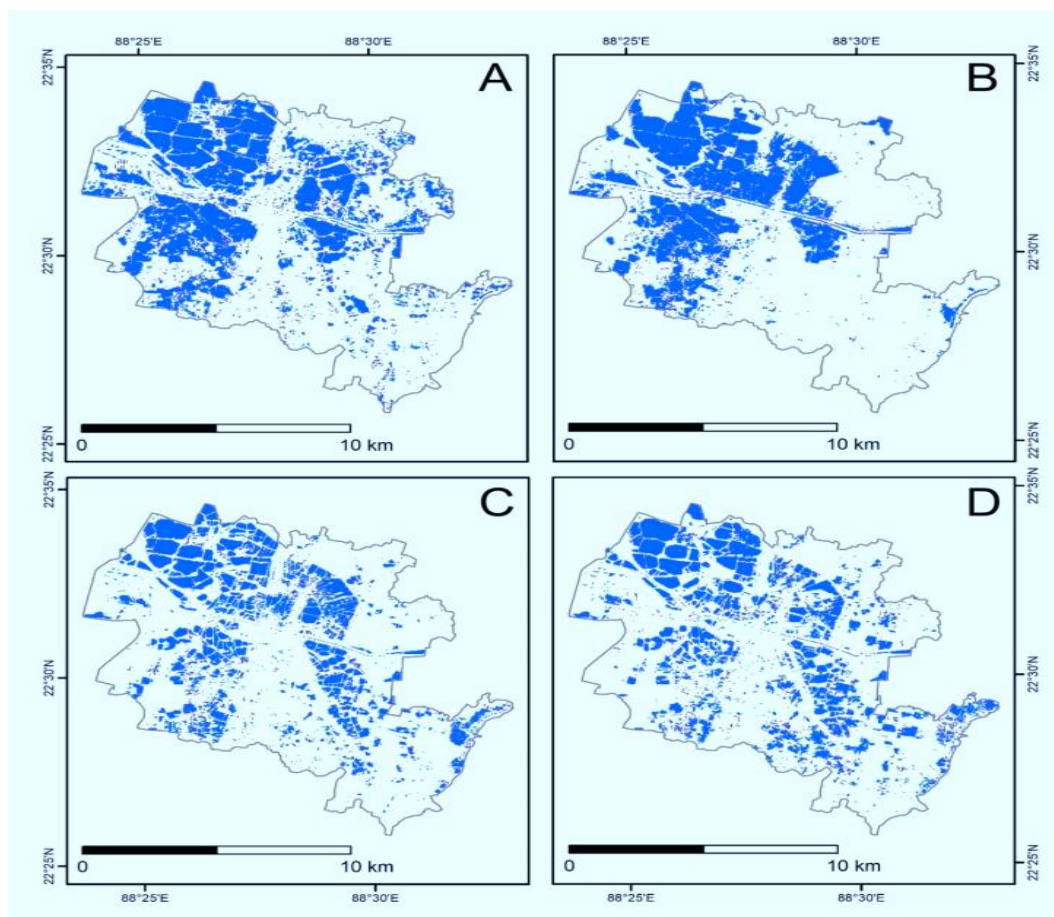


Figure 2.9: Satellite image shows the waterbodies of the East Kolkata Wetlands (EKW) between 1989 and 2022, as determined using the MNDWI method.

The images are sourced from: **A:** Landsat-5 TM pan-sharpened image, Path 138, Row 044, acquired on 28 February 1989. **B:** Landsat-7 ETM+ pan-sharpened image, Path 138, Row 044, acquired on 26 April 2001. **C:** Landsat-8 OLI pan-sharpened image, Path 138, Row 044, acquired on 8 March 2015. **D:** Landsat-9 OLI-2 pan-sharpened image, Path 138, Row 044, acquired on 3 March 2022 (Sourced from Das.,2025). The Landsat images are satellite generated images. (Source: Das,2025)

From the **figure 2.9**, the following conclusions can be drawn:

Panel A (1989, Landsat-5 TM):

The blue regions indicate waterbodies as identified in 1989. During this period, water coverage was quite extensive, showcasing numerous large and small wetland patches.

Panel B (2001, Landsat-7 ETM+):

In comparison to 1989, there is a clear decrease in some scattered small waterbodies, although the major water pools remain unaffected. The reduction in blue patches suggests an expansion of developed or land-reclaimed areas.

Panel C (2015, Landsat-8 OLI):

By 2015, a further reduction and fragmentation of the wetlands is apparent. While the larger central waterbodies persist, the smaller and peripheral ones have significantly diminished. This trend reflects the pressures of urbanization and changes in land use on EKW.

Panel D (2022, Landsat-9 OLI-2):

The latest image indicates a continuation of this trend. Some larger consolidated waterbodies are still visible, but the number of smaller wetland patches has further decreased. The fragmentation and conversion of waterbodies into land areas are distinctly observable.

The conversion of farmland or fallow land to water bodies has significantly decreased from 572 hectares between 1989-2001 to 365 hectares from 2015-2022. In contrast, the area converted from water bodies back to farmland has seen a drastic decline, from 765 hectares in 1989-2001 to merely 115 hectares by 2015-2022. The transition from farmland to urban areas has escalated from 719 hectares to a peak of 928 hectares during 2001-2015, before falling to 507 hectares in 2015-2022. The area transitioning from water bodies to urban land increased from 472 hectares to 732 hectares, but then experienced a decline to 192 hectares in 2015-2022(Das.,2025).

2.5.3 REASONS OF HUMAN SETTLEMENT IN EAST KOLKATA WETLANDS

Over the years, the urban growth of Kolkata has led to a reduction in wetlands. The swift industrialization and urban development aimed at transforming land use into built-up areas have a considerable environmental effect. The East Kolkata Wetland serves as a natural sewage treatment system, that is quickly declining due to urban expansion. The land utilized for the East Kolkata wetland spans 3,898 hectares, which is approximately two-thirds of the total 5,852 hectares of water area, dedicated to 364 sewage-fed fisheries. The remaining one-third of the water area, of around 2,000 hectares, is allocated for agricultural activities, including rice cultivation, ornamental flowers, and vegetables. Over-exploitation and various human activities have led to alterations in land use and land cover patterns due to increasing urbanization and industrialization (Banerjee *et al.*,2017). The most substantial conversion to built-up areas has been noted in the northwestern section of the east marsh, which is adjacent to Kolkata city and the satellite suburb of Newtown Rajarhat. The industrial growth in Kolkata and the Newtown region has increased opportunities for real estate development within the East Kolkata wetland. Over the past three decades, fisheries have experienced a decline. Fisheries have decreased from 29% to 16%. The primary reason for this decline is the sale of aquaculture ponds and their conversion into developed areas. As the population has increased over the decades, urban landfills have expanded. The East Kolkata wetland is utilized for the disposal of urban waste.

In the Dhapa Manpur area of East Kolkata Wetland, organic farming is practiced due to the rich soil fertility. The development of metro railways, concrete highways, and residential complexes has led to a reduction in aquaculture farms and open spaces. Consequently, the rural communities that depend on the East Kolkata wetland are significantly affected. The utilization of this region for both agriculture and aquaculture support the livelihoods of rural communities, showcasing an effective land use strategy. (Sardar.,2023).

2.5.4 RECLAMATION OF EAST KOLKATA WETLANDS

As per the land use and land cover map from 2019, the East Kolkata Wetlands (EKW) encompasses 5,852 hectares of waterbodies, 47.80 hectares of agricultural fallow land, 5,520 hectares of agricultural land, which includes garbage farming, and 777 hectares of built-up area within the Wastewater Reuse Region (WRR) of EKW. The Buffer zone surrounding this WRR spans an area of 50,505 hectares and currently comprises: 923 hectares of waterbodies, 13,667 hectares of agricultural land, 25,371 hectares of agricultural fallow land, and 10,554 hectares of built-up area. In 2019, the predominant land use within the WRR was attributed to waterbodies, while in the Buffer zone, agricultural fallow land constituted the largest share of land use. Currently, the leading land uses in this buffer zone include urbanization, infrastructure development, and recreational initiatives. Furthermore, the increase in real estate activities has significantly influenced this zone, with numerous township projects being proposed to meet the demands of future population growth (Haque.,2020). Currently, land is primarily being utilized for urbanization, infrastructure development, and recreational projects, reflecting the increasing influence of real estate interests in the buffer zone. Numerous township projects are underway, further intensifying the economic pressures on this ecologically sensitive area. Land encroachment remains a significant threat to the wetland region. The reclaimed land of EKW is not only under strain, but the bheris (fish ponds) and agricultural land are also at risk due to encroachment. The abuse of administrative authority by ruling political parties has expedited this encroachment, posing serious risks to the ecological and economic sustainability of the region. EKW shows a notable increase in built-up area and a 33.33% decrease in agricultural area, alongside a rise in garbage farming activities. Nevertheless, recent extensive land reclamation efforts have resulted in the reduction of this area, jeopardizing the environmental services it consistently offers to nearby regions. Consequently, the land use pattern following reclamation is of paramount importance for both the populace and the ecosystem [Roy *et al.*,2020]

2.5.5 CROPS GROWN IN EAST KOLKATA WETLANDS

The East Kolkata Wetlands are home to a diverse range of vegetable farming, due to their nutrient-dense, sewage-fed ecosystem. Numerous locally significant vegetables are cultivated here, which play a vital role in the livelihoods of local communities and the ecological sustainability of the area. Among the frequently grown vegetables is Bhindi (*Abelmoschus esculentus*, family Malvaceae), recognized for its edible green pods. Various species of Amaranthus, including Lalsag (*Amaranthus cruentus*), Kanta Note (*Amaranthus spinosus*), Dengua (*Amaranthus tricolor*), and Note (*Amaranthus viridis*), all part of the Amaranthaceae family, are widely cultivated for their leafy greens. Pui (*Basella alba*, family Basellaceae) is another favored leafy vegetable. Cruciferous vegetables such as Phul Kophi (*Brassica oleracea* var. *botrytis*, family Brassicaceae) and root vegetables like Mulo (*Raphanus sativus*, family Brassicaceae) are also grown. Fruit-bearing vegetables are well represented by Lanka (*Capsicum annuum*, family Solanaceae), Tomato (*Lycopersicon esculentum*, family Solanaceae), and Begun (*Solanum melongena*, family Solanaceae). Leafy greens such as Bethu (*Chenopodium album*, family Chenopodiaceae), Pat (*Corchorus olitorius*, family Tiliaceae), and Palak (*Spinacia oleracea*, family Amaranthaceae) are commonly cultivated. Gourds like Kundri (*Coccinia grandis*), Kumro (*Cucurbita maxima*), and Lau (*Lagenaria siceraria*), all from the Cucurbitaceae family, are staple crops. Aquatic and semi-aquatic vegetables such as Kochu (*Colocasia esculenta*, family Araceae) and Kalmi (*Ipomoea aquatica*, family Convolvulaceae) flourish in the wetland environment. Moreover, Sim (*Lablab purpureus*, family Fabaceae), Uchhe (*Momordica charantia*, family Cucurbitaceae), Sojne (*Moringa oleifera*, family Moringaceae), and Tulsi (*Ocimum tenuiflorum*, family Lamiaceae) are grown for both their nutritional and medicinal properties. Staple crops like Dhan (*Oryza sativa*, family Poaceae) and seasonal crops such as Bhutta (*Zea mays*, family Poaceae) are also cultivated (ZSI, BSI, East Kolkata Wetland Management Authority).

2.5.6 APPARENT THREATS FROM THE CROPS GROWN

As per the analysis done by Agarwal *et al.*, 2023, for crops like *Zea mays*, *Lagenaria siceraria*, *Abelmoschus esculentus* that were grown in East Kolkata Wetlands, it was found that they could absorb toxic metals like Pb, Cd, Cr, Hg, from highly contaminated soils. These crops may act as gateways for heavy metals (HMs) to enter the human food chain, resulting in prolonged accumulation and potential toxicity. The farmers use high quantities of insecticides and pesticides for agriculture purpose, thereby affecting the soil quality. The intake of tainted vegetables may lead to harm in the central nervous system, developmental setbacks in children, cancer, kidney problems, bone abnormalities, respiratory complications, and mental health issues.

2.5.7 MITIGATION STRATEGIES

The East Kolkata Wetland holds significant socio-economic value. Ecologically, it is crucial as it contributes to resource recovery, environmental protection, biodiversity conservation, and pollution control through phytoremediation.

The versatile function of EKW corresponds with numerous United Nations Sustainable Development Goals (SDGs), such as SDG 2 (Zero Hunger), SDG 6 (Clean Water and Sanitation), SDG 8 (Decent Work and Economic Growth), SDG 11 (Sustainable Cities and Communities), SDG 12 (Responsible Consumption and Production), SDG 13 (Climate Action), SDG 14 (Life Below Water), and SDG 15 (Life on Land). By fostering biodiversity, improving climate resilience through flood mitigation and carbon capture, and ensuring livelihoods and food security, EKW plays a significant role in achieving global sustainability objectives [Chaudhuri *et al.*,2012; Kumar *et al.*,2020]. Nevertheless, the realization of these goals necessitates tackling the challenges posed by urbanization and fragmented governance via focused policy measures, enhanced enforcement, and comprehensive management strategies [Mondal *et al.*,2017; Mukhopadhyay *et al.*,2022].

The East Kolkata Wetlands (Conservation and Management) (Amendment) Act, 2017, updates the 2006 Act to improve the administration and oversight of the EKW. Enacted by the West Bengal Legislature and receiving the governor's assent on March 17, 2017, this amendment restructured the East Kolkata Wetlands Management Authority (EKWMA) to promote inclusivity and diversity. The updated committee now includes the Minister-in-Charge of the Environment Department as Chairperson, the Chief Secretary of West Bengal, and senior officials from various departments. A significant aspect is the appointment of four experts in wetland ecology, hydrology, fisheries, and socio-economics, chosen by the State Government to provide technical and scientific guidance.

This incorporation of multidisciplinary expertise seeks to enhance governance and tackle the intricate ecological and socioeconomic issues related to wetland conservation. This amendment is in line with Ramsar's focus on scientific knowledge, adaptive management, and cross-sector collaboration. Nonetheless, its effectiveness should be assessed based on conservation results, funding, departmental coordination, and the execution of expert recommendations. The varied expertise at EKWMA aims to strengthen its ability to tackle ecological issues, encourage sustainable practices, and harmonize environmental and developmental objectives. Nevertheless, the successful execution relies on efficient collaboration among departments, the role of experts in the decision-making process, and adequate funding for conservation initiatives. The 2017 amendment was designed to strategically revise the management framework to safeguard West Bengal's ecological integrity by incorporating professional insights and administrative assistance. Despite these improvements, challenges like enforcement gaps, overlapping jurisdictions, and a lack of community engagement continue to hinder effective policy execution (Yadav *et al.*,2025).

A comparative analysis of the effectiveness and enforcement mechanisms of significant policies and legislation that govern the EKW has been represented in **Figure 2.10**. It assesses how well these policies align with conservation objectives, the robustness of their enforcement mechanisms, and their capacity to tackle challenges like urban encroachment, pollution, and habitat degradation.

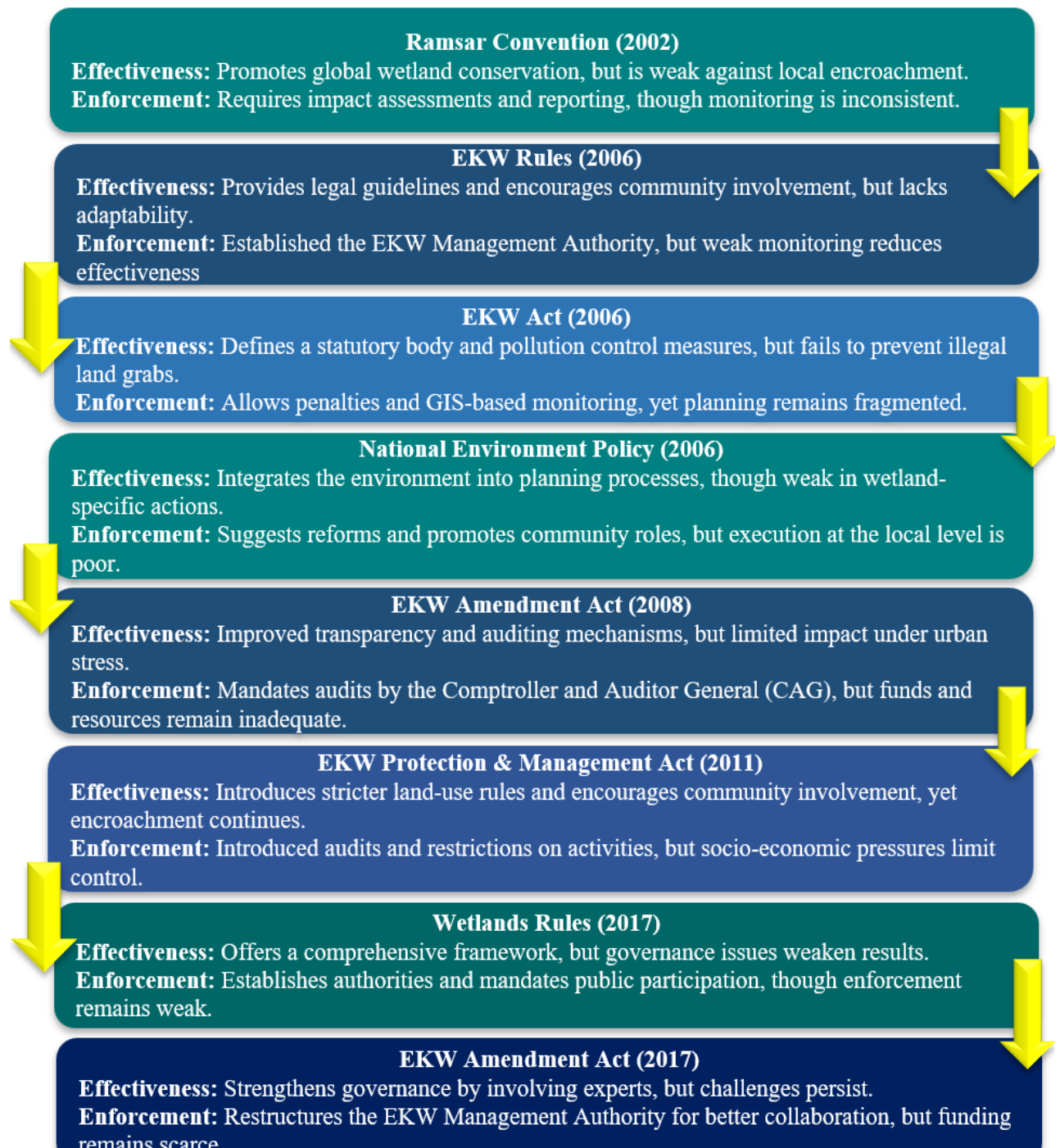


Figure 2.10: Flow chart showing the Policy Efficiency and Enforcement Strategies in EKW

2.6 WETLAND SOIL PHYSICO-CHEMICAL PROPERTIES and WETLAND SOIL METAGENOME

Since a section of this thesis focuses on soil physico-chemical properties and metagenomics, a comprehensive literature review on soil characters and metagenome studies has been undertaken to establish the current knowledge, methodologies, and applications relevant to this work.

Different types of wetlands, characterized by varying inputs of litterfall, root exudates, and human disturbances, significantly influence soil physicochemical properties [Zhang *et al.*,2020; Liu *et al.*,2020]. Studies have indicated that these factors are essential for maintaining the ecological health and functions within estuarine wetlands, including carbon sequestration and wetland restoration. Litter is a significant source of soil organic matter in the Liaohe Estuary Wetland. The litter decomposition rates and the nutrient contents of various plant species exhibit considerable variation, which directly influences the nutrient cycling within the soil [Liu *et al.*,2022]. Furthermore, agricultural and industrial activities can introduce pollutants, which alter the chemical composition of wetland soils. For instance, applying chemical fertilizers and pesticides may result in elevated concentrations of heavy metals in the soil, thereby seriously threatening the health of wetland ecosystems [Yu *et al.*,2022]. In this study done by Zheng *et al.*,2024, the total carbon and nitrogen contents in the reed and suaeda wetlands (wetlands dominated by suaeda plants) were significantly higher than those observed in the ponds and tidal flat wetlands, which aligns with existing research findings [Ding *et al.*,2023; Wang *et al.*,2024]. This disparity may result from the continuous input of organic matter, root exudate deposition, and nutrient release and recycling in the reed and suaeda wetlands. Contrasting with the observed patterns of total carbon and nitrogen, the C/N ratio was significantly lower in the reed wetland than in the suaeda and tidal flat wetlands. Previous research [Zhang *et al.*,2016] reported that the nitrogen content was considerably lower in suaeda than in reeds, which may explain the low carbon-to-nitrogen ratio in the reed wetland. A lower soil C/N ratio correlates with a higher nitrogen mineralization rate, which is advantageous for the absorption of nitrogen by microorganisms and plants. Studies have demonstrated that the high carbon-to-nitrogen ratio in reed wetlands is advantageous for soil organic carbon sequestration. Additionally, the soil salinity was significantly higher in tidal flat wetlands than in reed and suaeda wetlands, which aligns with existing research findings [Dai *et al.*,2024]. However, the soil salinity levels in these three types of wetlands were significantly lower than those observed in pond returning wetlands, likely due to the substantial addition of feed from human aquaculture activities before the abandonment of the ponds for restoration. Furthermore,

the salinity of the soil was notably greater in tidal flat wetlands compared to reed and suaeda wetlands, which is consistent with previous research findings. Nevertheless, the soil salinity levels in these three wetland types were considerably lower than those recorded in pond wetlands, probably due to the significant influx of feed from human aquaculture practices prior to the ponds being abandoned for restoration.

The soil microbiome plays a vital role in sustaining life on Earth, as these microbial communities promote healthy plant growth, maintain soil health, and are essential for the effective operation of key biogeochemical cycles. This process ensures the proper recycling of both organic and inorganic materials within the terrestrial ecosystem. Therefore, researchers have concentrated on environmental solutions and agricultural practices that enhance soil diversity and the microbes that improve soil quality, aiming to secure long-term food availability (Rachid *et al.*, 2023).

Despite the vast number of microbes present in the soil microbiome, only a small percentage (0.1 - 51%) can be cultivated under laboratory conditions. This limitation highlights the necessity for advanced technologies to study the microbiome comprehensively [Torsvik *et al.*, 1990; Torsvik *et al.*, 2002; Mocali *et al.*, 2010; Chaudhary *et al.*, 2019]. These “uncultivable” microorganisms represent unknown species with distinct biological and chemical properties. In their natural environments, these uncultivable bacteria require energy but are unable to thrive in laboratory media (Mu *et al.*, 2021), resulting in a significant knowledge gap regarding their habitats, interactions with organic and inorganic substances, and their biological functions in soil (Gwenzi, 2021).

Research has been done in invention of innovative techniques for accessing the vast information concealed within the entire genome of the soil microbiome. This "collective DNA of all the native soil biota" is known as the metagenome (Handelsman *et al.*, 1998), and the method of studying the metagenome extracted from all organisms, without the necessity for isolation and cultivation in laboratory settings, is termed "Metagenomics." (Chaudhary *et al.*, 2019). Recent molecular techniques utilized for the identification of target genes include direct genomic DNA extraction, the preparation of metagenomic libraries, heterologous gene expression, and next-generation high-throughput sequencing of environmental samples. The advancement of sustainable agriculture necessitates the ongoing exploitation and characterization of PGPRs (Bakshi *et al.*, 2020).

In the same way, metagenome of wetland can play a significant role in the maintenance of fertility of the area and reduce the menace of pollution (Barman *et al.*, 2024)

2.6.1 BASIC METHODOLOGY OF SOIL METAGENOME:

The basic steps involved in analysis of soil metagenome are:

a) Collection of soil sample and DNA extraction

Soil samples (approximately 10 g) are collected under sterile, with DNA being extracted through the modified CTAB method. The yield and quality of DNA are significantly influenced by the lysis technique used. Variations in microbial activity and the efficiency of DNA extraction can distort the representation of the community. Methods such as stable isotope probing (SIP) and PAGE are utilized to fractionate DNA according to microbial physiology [Chauhan *et al.*, 2009; Penton *et al.*, 2016]

b) Metagenomic Library Construction

It is done using, high molecular weight DNA, that undergoes partial digestion using restriction enzymes, followed by the fractionation of the partially digested fragments through gel electrophoresis, and then the elution of fragments ranging from 2 to 10 Kb from the gel. Subsequently, these partially digested DNA fragments are cloned into a vector and transformed.

c) Screening of Metagenomic Library

After the metagenomic clones are established in the form of a library, the selection of clones expressing a specific phenotype takes place. The selection process for metagenomic clones can be conducted using either a “phenotype-based” approach or functional metagenomics (Daniel 2004), or through a “genotypic” or “sequence-driven metagenomics” method. The latter approach is particularly beneficial when phenotypic screening opportunities are limited; however, some sequence information is required to design primers and probes [Daniel *et al.*, 2004; Jindal *et al.*, 2020].

d) Next-Generation Sequencing of Metagenomic DNA

The sequencing of metagenomic DNA is performed using available next-generation sequencing (NGS) platforms, such as Roche 454 and Illumina HiSeq Genome Analyzer, among others. The sequencing data is analysed using the QIIME (Quantitative Insights into Microbial Ecology) 1.9.0 pipeline, which encompasses the assembly of sequencing data and the identification of operational taxonomic units (OTUs) within the sequencing reads. These OTUs are then taxonomically classified into phylum, class, order, family, genus, and species, utilizing green-genes as a reference. This classification method, based on referential data, is also referred to as binning. It plays a crucial

role in metagenome analysis by offering insights into novel genomes that are otherwise challenging to uncover, as well as the number and types of taxa; it provides a means to simplify the complexity of metagenomic analyses (Mackelprang *et al.* 2011) (**Figure 2.11**)

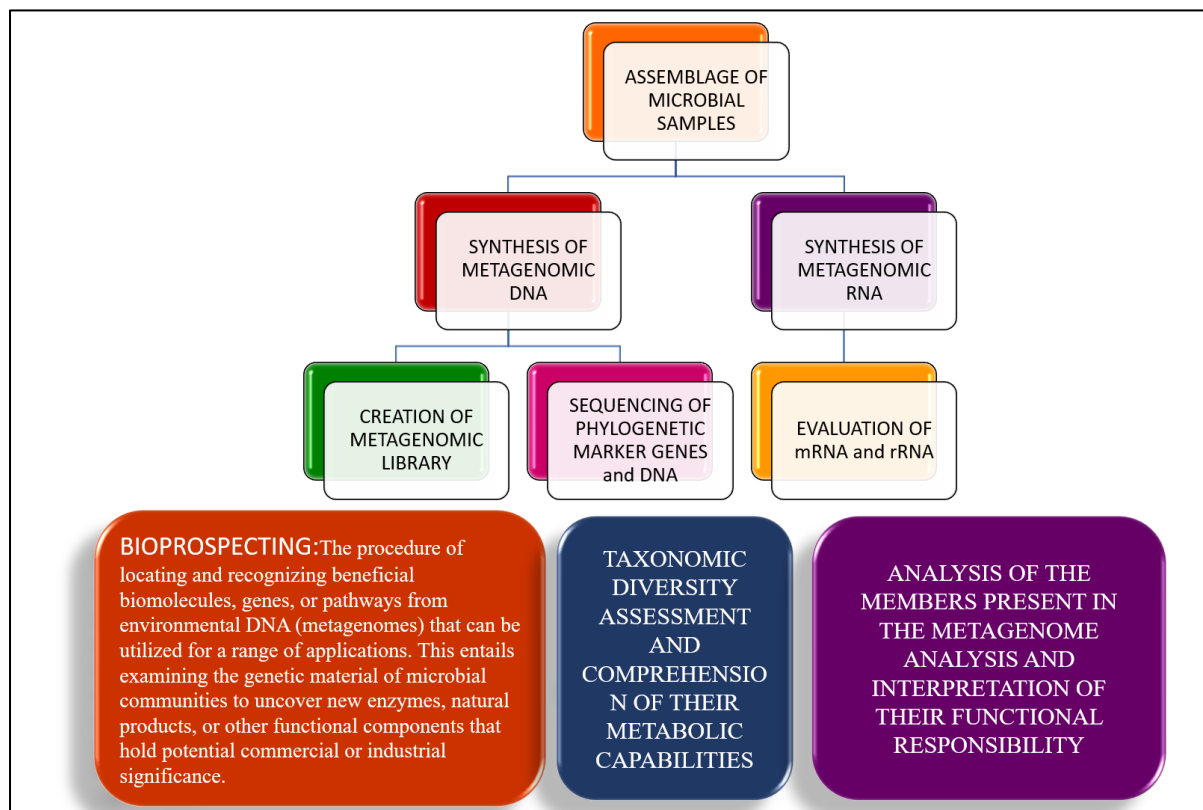


Figure 2.11: Basic methodology of soil metagenome

2.6.2 MICROBIAL MOVEMENTS ACROSS THE SOIL DOMAINS USING METAGENOMIC ANALYSIS.

The idea of microbial migration through soil domains not only illustrates the dispersal and colonization processes of soil microorganisms but also offers insights into their ecological functions, metabolic abilities, and their contributions to soil health and sustainability. Therefore, this thesis aligns its goals with the wider context of soil metagenomics, where microbial movements act as a crucial indicator of functional diversity, nutrient cycling, and ecosystem resilience.

a) Normal Agriculture Soil

Numerous studies have been conducted across various crops, including maize (Chauhan *et al.* 2011), rice (Somenahally *et al.* 2011), and biofuel-specific crops such as soybean, canola, and switchgrass (Jesus *et al.* 2010), to investigate the diversity of rhizosphere microbes. The predominant genera identified in the rhizosphere of these crops were Proteobacteria and Acidobacteria, highlighting their significant roles in plant growth and stress responses. A metagenomic analysis of oil-contaminated soil cultivated with barley and alfalfa indicated an increase in known oil-degrading genera, including *Alcanivorax* and *Aequorivita*, (Kumar *et al.* 2018).

b) Wetland soil

Wetland soils, also includes peat soils, that are significant reservoirs of organic carbon (C). The microbial breakdown of Carbon(C) and nitrogen (N) stocks can result in considerable emissions of greenhouse gases (GHGs), such as nitrous oxide (N₂O). N₂O is a highly effective GHG, possessing a global warming potential that is 265 times greater than that of CO₂. Additionally, N₂O is recognized as the leading ozone-depleting substance. Research on microbial communities linked to N₂O emissions in global wetland soils has correlated N₂O fluxes with the relative abundance of different microbial lineages, utilizing 16S and 18S rRNA gene metabarcoding techniques (Bahram *et al.*,2022). As per the studies conducted by Siljanen *et al.*,2019, the microbial phyla Proteobacteria, Acidobacteriota, and Chloroflexi are the most prevalent worldwide. These groups did not show a significant correlation with N₂O fluxes ($p > 0.05$). In contrast, the relative abundance of ammonia-oxidizing archaea (AOA) from the phylum Thaumarchaeota was identified as the group, that was most strongly associated with N₂O emissions.

Wetland ecosystems worldwide support a variety of microbial communities that facilitate crucial biogeochemical processes, and metagenomic research has offered enhanced understanding of these intricate microbial groups. In peatlands, which serve as important carbon sinks, metagenomic investigations have shown a prevalence of methanogenic archaea like *Methanobacterium* and *Methanosarcina*, especially in the deeper, anoxic layers, along with genes linked to methane production (*mcrA*) and the breakdown of complex organic matter (Tveit *et al.*, 2013). In mangrove wetlands, characterized by salinity and periodic flooding, shotgun metagenomics has uncovered a significant presence of sulfur-reducing and sulfur-oxidizing bacteria, with genes such as *dsrA*(sulphate reduction), *soxB*(sulfide oxidation), and *aprA*(sulfite reduction) being abundant, indicating active sulfur cycling in response to changing redox conditions (Zhou *et al.*, 2020, Qian *et al.*, 2023). These microbial communities also contain genes for hydrocarbon degradation, emphasizing their potential role in natural bioremediation. In rice paddy wetlands, which are artificially created yet replicate the functions of natural wetlands, metagenomic studies have shown dynamic changes in microbial composition throughout flooding cycles, maintaining a balance between methane-producing and methane-oxidizing organisms like *Methanosaeta* and *Methylocystis*, influenced by water management practices and fertilizer application (Lee *et al.*, 2015). Likewise, constructed wetlands intended for wastewater treatment display enriched microbial genes related to xenobiotic degradation, nitrate reduction, and heavy metal resistance, highlighting the adaptability of microbial consortia in engineered settings (Wang *et al.*, 2016; Zhao *et al.*, 2016). Collectively, these findings emphasize how microbial metagenomes in wetlands illustrate the interaction between environmental factors, land-use practices, and ecosystem services, providing essential insights for climate mitigation, pollution management, and biodiversity preservation.

In another study conducted by Zhang *et al.*, 2024, the examination of the soil metagenome from the Sanjiang Plain Wetlands (Northeast China) uncovered that *Bradyrhizobium* (1.62%) was the most prevalent genus across all soil samples. The leading 15 genera based on relative abundance included *Bradyrhizobium* (3.21%), *Candidatus sulfopaludibacter* (0.96%), *Candidatus sulfotelmatobacter* (0.79%), *Anaeromyxobacter* (0.45%), *Candidatus udaeobacter* (0.29%), *Usitatibacter* (0.28%), *Rhodoplanes* (0.25%) etc. A notable increase in shrub expansion intensity led to a significant rise in the relative abundances of *Bradyrhizobium*, *Rhodoplanes*, and *Reyranella*, while it decreased the abundances of *Anaeromyxobacter* and *Ktedonobacter*.

2.7 PLANT GROWTH PROMOTING BACTERIA

Plant growth-promoting bacteria (PGPB) are essential for sustaining soil health, improving nutrient availability, and promoting plant resilience during stressful conditions. Their capabilities to fix nitrogen, solubilize phosphates, generate phytohormones, and inhibit pathogens render them crucial for sustainable agriculture. Since the thesis lays emphasis upon PGPB, incorporating this into literature review framework highlights the functional significance of soil microbial communities and their direct effects on crop productivity and ecological equilibrium.

Kloepper and Schroth (1981) first coined the term plant growth promoting rhizobacteria (PGPR) to describe these beneficial microbes. Plant growth promoting rhizobacteria (PGPR) represent a varied group of soil bacteria that play a crucial role in soil-plant systems, engaging in a complex network of interactions within the rhizosphere, thereby influencing plant growth and yield (Vessey, 2003). PGPB encompasses free-living bacteria, those that establish specific symbiotic relationships with plants (such as *Rhizobia* spp. and *Frankia* spp.), and can exist as bacterial endophytes that can inhabit certain interior tissues of plants. They can enhance plant growth directly, typically by aiding in resource acquisition or adjusting plant hormone levels, or indirectly by mitigating the negative impacts of various pathogenic agents on plant growth and development, effectively acting as biocontrol bacteria (Glick, 2012). PGPR operates through various direct and indirect mechanisms, such as the generation of bacteriocins, lytic enzymes, siderophores, and the solubilization of insoluble phosphate and zinc. Additionally, it is involved in the synthesis of phytohormones. Furthermore, PGPR plays a role in regulating bio-geochemical nutrient cycles, which helps maintain nutrient availability for both plants and soil microbes. It also enhances essential physiological functions such as water and nutrient absorption, photosynthesis, and the dynamics of source-sink relationships that facilitate growth and development. Bacteria get associated with soil particles via exchange of ions (Goswami *et al.*, 2016). The studies done by Gray *et al.*, 2005 demonstrated the major types of PGPR associations that includes, colonization of bacteria within the roots, followed by survival and reproduction in the microhabitats linked to the root surface while competing with other microbiota for the necessary time to carry out their plant promotion/protection functions, and they must actively promote plant growth [Kloepper, 1994; Lucy *et al.*, 2004; Jeyanthi *et al.*, 2018].

Wetlands are an area of active plant-microbe interactions. The metabolites and inorganic ions are released by the plant roots, that provides the associated micro-organisms with carbon resources

and energy. They also regulate microbial activity, behaviour and their community structure (Song *et al.*, 2014; Chen *et al.*, 2021).

The interactions between plants and rhizosphere microorganisms are categorized based on their effects. Certain rhizosphere bacteria enhance the availability of nutrients by making insoluble minerals more accessible (Teng *et al.*, 2018; Chawngthu *et al.*, 2020). PGPR promotes biocontrol effects that plant–microbe interactions have on plants in the wetlands. (Saha *et al.*, 2019). Microorganisms in the rhizosphere can aid wetland plants in degrading, absorbing, or immobilizing external pollutants, thereby enhancing their tolerance to pollution stress (Man *et al.*, 2020; Wang *et al.*, 2021). Additionally, wetland microorganisms can increase plant resilience to salt stress, as they stimulate the physiological metabolic processes of plants and mitigate oxidative damage induced by salt stress.

The ways in which PGPB promote plant growth include the availability of nutrients derived from genetic processes like biological nitrogen fixation and phosphate solubilization, alleviation of stress through the regulation of ACC deaminase expression, and the synthesis of phytohormones and siderophores, among other factors, as represented in **Figure 2.12**. These bacteria are not only active in normal soil, but can also perform with similar efficacy in agriculture wetland soil.

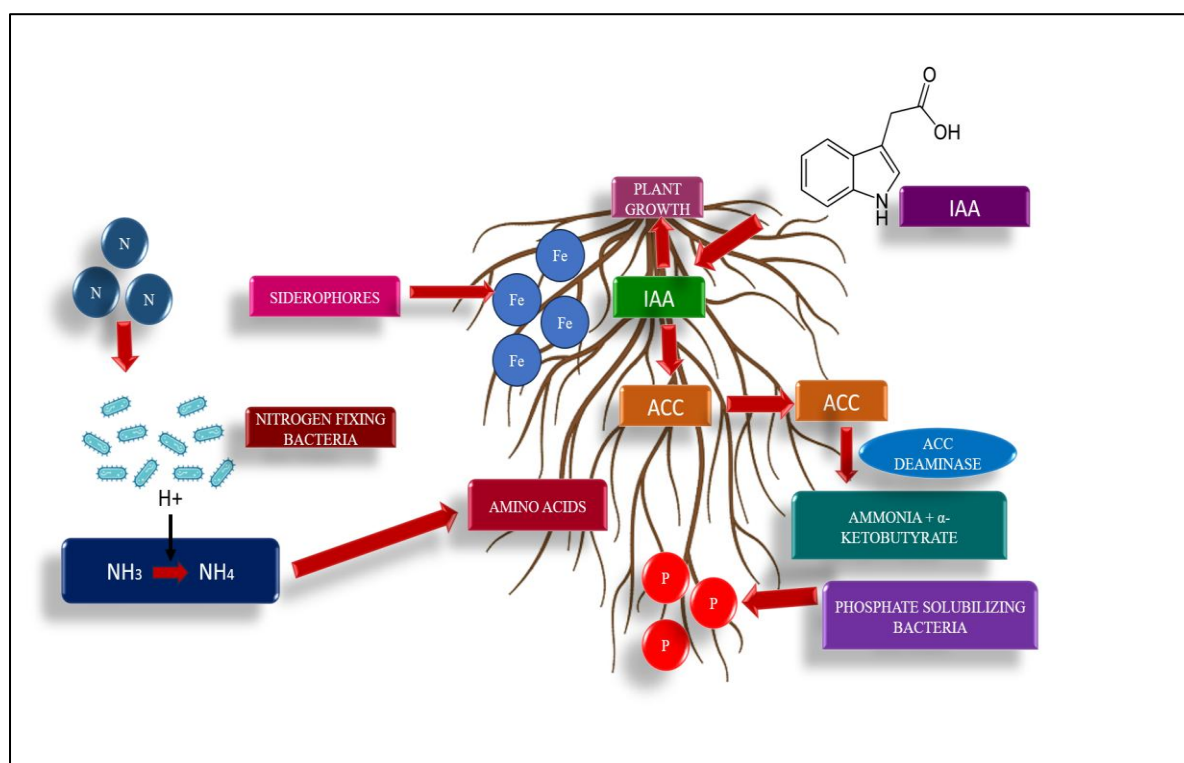


Figure 2.12: Mechanisms of Plant growth promoting bacteria

2.7.1 Mechanisms of Plant Growth Promotion

The nutrient acquisition is greatly helped by these rhizobacteria not only in normal soil condition, but also in wetland soil. The ongoing paragraphs indicate this nutrient uptake through microbial help.

Nitrogen is a crucial nutrient necessary for the growth of all living organisms, including plants and bacteria (Zhang *et al.* 2015). The micro-organisms require nitrogen for synthesis of proteins and nucleic acids. The nitrogen deficiency in soil has resulted in the application of substantial quantities of nitrogenous fertilizers to fulfil the essential requirements of plants for achieving optimal yields in most soils. Although nitrogen is abundant in the earth's atmosphere, comprising about 78%, this gaseous form of nitrogen [N₂] is not easily accessible to most organisms, i.e it cannot be assimilated by plants until it is converted into ammonia [Olanrewaju *et al.*,2017]. During the process of biological nitrogen fixation (BNF) N₂ is reduced to ammonia (NH₃) and is carried out by diazotrophic microorganisms, especially bacteria and archaea in the wetlands. Diazotrophic microorganisms execute BNF using nitrogenase, a highly conserved enzyme made up of two metalloproteins: FeMo-protein and Fe-protein.

A wide variety of nitrogen-fixing bacteria have been identified, including several organisms that symbiotically fix nitrogen with specific plants, primarily legumes. Notable examples of symbiotic nitrogen fixers include *Rhizobium* sp, *Sinorhizobium* sp, *Azorhizobium* sp, *Allorhizobium* sp, *Mesorhizobium* sp, *Bradyrhizobium* sp, *Frankia* sp, *Burkholderia* sp, and *Bacillus* sp [Babalola 2010; Pérez-Montaña *et al.* 2014; Turan *et al.* 2016]. Rhizobial bacteria colonize the root cells of plants and trigger a complex series of developmental changes that result in root nodule formation. In the root nodule, bacteria exist as bacterioides devoid of a cell wall, utilizing the nitrogenase enzyme to fix atmospheric nitrogen and generate ammonia. In this mutualistic association, the plant supplies the necessary nutrients for the bacterium's growth while it continues to fix nitrogen within the nodules [Olanrewaju *et al.*,2017]. A problematic side reaction in the nitrogenase enzyme's nitrogen fixation activity is the reduction of H⁺ to H₂ (hydrogen gas), as the hydrogen gas produced escapes into the atmosphere, resulting in a waste of the ATP used in its production. This side reaction significantly reduces the overall efficiency of the nitrogen-fixing process by about 30%. In contrast, certain strains of rhizobia possess an enzyme known as hydrogenase, which can capture the lost H₂ from the atmosphere and convert it back into H⁺ for ATP production, which can then be utilized for additional nitrogen fixation. These strains contribute to energy conservation while simultaneously fixing nitrogen [Adams *et al.* 1981; Olanrewaju *et al.*,2017]. Reclaimed wetlands

may serve as a sink for N_2O , due to the presence of nitrogen-fixing bacteria, especially those that participate in denitrification.

Phosphorus is a vital nutrient for plants, serving as a structural element in nucleic acids, phospholipids, and adenosine triphosphate (ATP). It plays a crucial role in various metabolic and biochemical pathways, especially in biological nitrogen fixation (BNF) and photosynthesis. Plants take up P in two soluble forms: monobasic (H_2PO_4^-) and dibasic (HPO_4^{2-}). Phosphate-solubilizing bacteria and fungi make up approximately 1-50% and 0.1-0.5%, respectively, of the total cultivable microbial population in the soil. Bacteria that are capable of solubilizing phosphorus are known as phosphate solubilizing bacteria. They provide phosphate to plants in a more accessible form. These bacteria transform insoluble organic and inorganic phosphate into a form that is easily available to plants. The most effective phosphate solubilizers belong to the genera *Bacillus* sp, *Rhizobium* sp, and *Pseudomonas* sp, along with non-symbiotic nitrogen-fixing bacteria such as *Azotobacter* sp and *Azospirillum* sp. Phosphate-solubilizing bacteria can solubilize inorganic soil phosphates, such as $\text{Ca}_3(\text{PO}_4)_2$, FePO_4 , and AlPO_4 , through the production of organic acids, siderophores, and hydroxyl PGPB ions in agricultural soils. Some bacteria are limited to solubilizing calcium phosphate, while others can solubilize different forms of inorganic phosphates with varying effectiveness. Bacterial isolates from the genera *Enterobacter* sp, *Pantoea* sp, and *Klebsiella* sp are particularly effective at solubilizing $\text{Ca}_3(\text{PO}_4)_2$ compared to FePO_4 and AlPO_4 [Souza *et al.*,2020].

The primary mechanism through which inorganic phosphate is solubilized involves the use of mineral-dissolving agents like hydroxyl ions, organic acids, protons, siderophores, and carbon dioxide (CO_2). Organic acids, which include gluconic, keto gluconic acids, citric, gluconic, lactic, succinic, and propionic acids. It has been proposed that the capacity of rhizobia to solubilize inorganic phosphate is linked to the acidification of the rhizosphere are produced and secreted by phosphate solubilizing bacteria, leading to the acidification of both the microbial cells and their environment, thereby releasing phosphate ions. The decrease in pH is a result of proton/bicarbonate release and the exchange of gaseous " O_2/CO_2 " (Sharma *et al.*,2017). Consequently, there is an inverse relationship between the pH of the rhizosphere and the availability of phosphorus. The primary sources of organic phosphorus in the soil are organic materials such as inositol hexaphosphate (phytate) that the PGPB are capable of solubilizing. Other organic phosphorus compounds include phosphomonoesters, phosphodiester, phospholipids, nucleic acids, and phosphotriesters. (Rodríguez *et al.*,1999; Olanrewaju *et al.*,2017].

In agricultural wetlands, phosphorus-solubilizing bacteria (PSB) play a vital role in enhancing ecosystem health by managing phosphorus runoff and mitigating eutrophication (the excessive enrichment of nutrients) in water bodies. PSB are capable of transforming insoluble phosphorus found in the soil into a form that plants can readily absorb, thereby enhancing the uptake and utilization of phosphorus by plants. In natural wetland, rhizospheres were primarily composed of Acidobacteria. The rate at which the isolates solubilized and released inorganic phosphorus significantly exceeded that of organic phosphorus, highlighting their distinct effects on both organic and inorganic phosphorus (Cao *et al.*,2018).

Potassium is a vital nutrient for plants. Microorganisms play a crucial role in the natural recycling of potassium. Plant growth promoting bacteria transform insoluble sources of potassium into forms that are accessible and usable by plants, in comparison to other soil-dwelling microbes such as fungi and actinomycetes. Rhizospheric microorganisms, particularly bacteria, possess the ability to solubilize potassium from minerals, facilitating its uptake by plants. A diverse array of microorganisms has been reported to release potassium from minerals, making it available in a form that plants can utilize. This includes bacteria such as *Acidithiobacillus ferrooxidans*, *Aminobacter* spp., *Arthrobacter* sp., *Bacillus amyloliquefaciens*, *Bacillus cereus*, *Bacillus circulans*, *Bacillus coagulans*, *Bacillus edaphicus*, *B. megaterium*, *Bacillus mucilaginosus*, *Bacillus subtilis*, *Burkholderia* spp., *Cladosporium*, *Enterobacter hormaechei*, *Flectobacillus* spp., *Paenibacillus* spp., and *Pseudomonas* spp. (Olaniyan *et al.*,2022).

Potassium-solubilizing bacteria (KSB) are microorganisms that enhance the growth and productivity of plants by converting complex potassium compounds into simpler forms, thereby making them accessible to plants. These KSB act on potassium-bearing minerals through three main mechanisms. The first is acidification, which decomposes the potassium aluminosilicate complex by releasing organic or inorganic acids, resulting in potassium silicate or aluminium silicate that is available for plant uptake. KSB employs various mechanisms, which may involve the generation of different organic acids for solubilization of complexes. The process of potassium solubilization by KSB entails the mobilization and solubilization of insoluble potassium-bearing minerals such as micas, muscovite, feldspar, biotite, illite, and orthoclase. One mechanism involves the release of potassium nutrients through the production of various organic acids. These secreted acids lead to the acidification of the rhizosphere, thereby enhancing the solubilization of potassium from minerals. It has been reported that a low pH promotes the production of different organic acids and protons by these microorganisms. The second mechanism involves the production of

siderophores; organic acids create chelating compounds with Fe^{2+} , Si^{4+} , Ca^{2+} , and Al^{3+} , which in turn releases K^+ into the exchangeable potassium pool. The third mechanism includes the synthesis of certain extracellular polymers, such as proteins or exopolysaccharides (EPS), by microbes that form biofilms around mineral rocks, altering their morphology and thus releasing K^+ in a form that plants can utilize (Nawaj *et al.*,2023) Potassium-solubilizing bacteria are important microorganisms in agriculture, especially in wetland areas, because of their capacity to mobilize potassium, enhance soil health, and boost crop productivity sustainably. Potassium-solubilizing bacteria found in wetlands convert potassium from insoluble mineral sources into soluble forms that are accessible to plants. They accomplish this through a process that includes the production of organic acids, chelation, and the formation of biofilms on mineral surfaces.

2.7.2 Siderophores

Siderophores are small peptide molecules characterized by side chains and functional groups that can bind ferric ions (Goswami *et al.* 2016). They act as iron chelators, functioning as carriers of iron, and exhibit a strong affinity for certain ligands. A considerable number of these molecules have been isolated and utilized from microbial sources, and they can also exhibit species-specific characteristics (Sandy *et al.*,2009). Certain bacteria, fungi, and plants release specialized low molecular weight iron-binding molecules known as siderophores, which have a molecular weight of approximately 400–1000 daltons(Da), into the soil to capture iron. Microbes that produce siderophores can inhibit or reduce the proliferation of pathogens by limiting the availability of iron to them (Shen *et al.* 2013). Plant Growth-Promoting Bacteria (PGPB) that synthesize siderophores hinder the growth of phytopathogens by secreting siderophores that have an exceptionally high affinity for iron. These siderophores effectively bind to most of the Fe^{3+} present in the rhizosphere of the host plant, allowing the bound iron to be absorbed either by the PGPB or the host plant itself. This action prevents fungal and bacterial pathogens in the rhizosphere of the host plant, where the biocontrol PGPB is located, from obtaining sufficient iron for their growth.

Typically, siderophores generated by PGPB possess a significantly higher affinity for iron compared to those produced by plants or fungi, enabling PGPB siderophores to sequester even trace amounts of iron. Each functional group of siderophore is connected to two oxygen atoms, or occasionally nitrogen, which bind to iron. These functional groups are bidentate, and trivalent ferric iron can effectively coordinate with three of these groups, resulting in a six-coordinate complex. The functional groups found on microbial siderophores are primarily hydroxamates or catecholates, along with other groups such as carboxylate, citrate, or ethylenediamine moieties

(Laschat *et al.* 2017). These functional groups may exist in a combined state within a single siderophore molecule. Hydroxamate-type siderophores are frequently found in fungi, whereas catecholates, which have a stronger binding affinity for iron compared to hydroxamates, are prevalent in bacterial siderophores (Saha *et al.* 2016) (Olanrewaju *et al.*, 2017) (**Figure 2.13**).

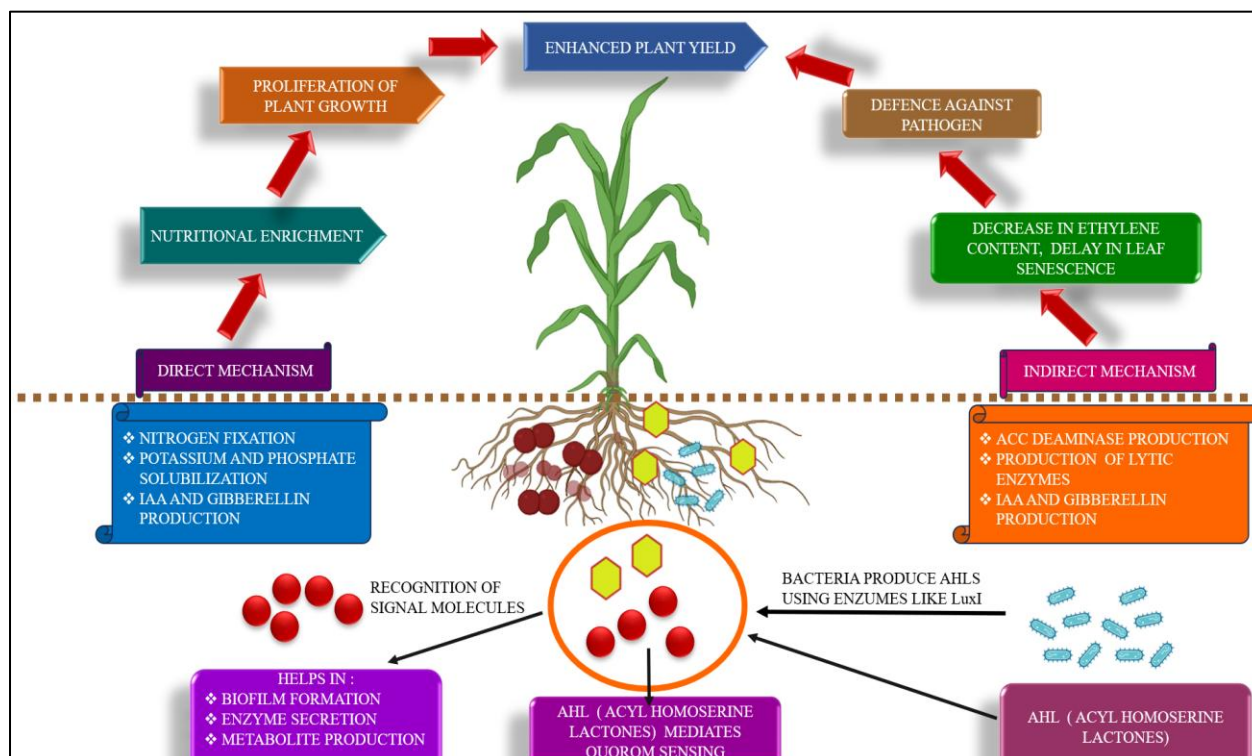


Figure2.13: Direct and indirect mechanisms by which plant growth-promoting bacteria (PGPB) enhance plant performance through nutrient acquisition, stress alleviation, and pathogen suppression.

Direct mechanisms encompass nitrogen fixation, phytohormone production, and nutrient solubilization, while indirect mechanisms consist of ACC deaminase activity, antibiotic production, siderophore release, and the induction of systemic resistance. Signal molecules, such as N-acyl-homoserine lactone (AHLs), facilitate quorum sensing (QS), affecting processes like biofilm formation, enzyme secretion, and metabolite production. The plant microbiome comprises various functional groups, AHL molecules that participate in QS signalling; mutant bacteria that cannot produce AHLs; and other signalling compounds (such as LuxR-binding mimics and QS intermediates) that influence plant–microbe communication.

2.7.3 PHYTOHORMONES

a) AUXIN

The primary mechanism used to elucidate the beneficial effects of plant growth-promoting bacteria (PGPB) on plant development is their capacity to generate the phytohormone namely auxin. Research indicates that approximately 80% of microbes in the rhizosphere can synthesize and release auxin as a secondary metabolite. Numerous naturally occurring auxins exist, with indole-3-acetic acid (IAA) being the most significant (Spaepen *et al.* 2007b). The terms auxin and IAA are often used interchangeably. Other compounds that exhibit auxin-like activity, some of which play a role in the synthesis of IAA, include indole-3-acetamide, indole-3-pyruvate, and indole-3-acetaldehyde. Auxins are involved in processes such as geotropism and phototropism, differentiation of vascular tissues, apical dominance, initiation of roots (both lateral and adventitious), cell division, and elongation of stems and roots (Grobelač *et al.* 2015). The IAA produced by rhizobacteria alters the auxin levels in plants, leading to increased root length and surface area, which in turn enhances the quantity of root exudates available for plant uptake. The auxin carrier proteins, that have been identified usually include components like PINs, ABCB, and AUX1/LAX, which are activated through a signalling cascade initiated by the F-box protein Transport Inhibitor Response 1 (TIR1) auxin receptor (Petrášek *et al.*, 2009).

Most auxin/IAA is synthesized from the amino acid tryptophan present in plant root exudates at varying low concentrations based on the plant's genotype. IAA appears to be synthesized by at least three different biosynthetic pathways with each pathway being named for a key intermediate within the pathway. These pathways include: the indole pyruvic acid (IPyA) pathway, the indole acetamide (IAM) pathway, the indole acetaldoxime (IAOx)/indoleacetonitrile (IAN) pathway (Duca *et al.* 2014), the indole acetaldehyde (IAH) pathway, and the tryptamine pathway. It should be noted that various PGPB can have one, two or even three functional IAA biosynthesis pathways. The *ipdC* gene, which has a role in IAA synthesis in plant growth-promoting bacteria (PGPB), is regulated by the quantity of IAA produced, creating a positive feedback loop that enhances the gene's expression. [Spaepen *et al.*, 2011; Olanrewaju *et al.* 2017]. In wetland ecosystems, bacteria that produce auxins can be utilized to improve the establishment and growth of vegetation, which is essential for the restoration and functionality of wetlands. These bacteria also contribute to phytoremediation, a process that employs plants to eliminate pollutants from soil and water, by promoting plant growth and enhancing nutrient absorption. The derivatives of IAA have been represented in **Figure 2.14**

In a study conducted by Yadav *et al.*,2021, in constructed wetlands, phytohormones like auxin enhance nutrient absorption and promote plant growth. Cassán *et al.* (2020) indicate that the plant growth-promoting bacteria *Azospirillum brasiliense* affects plant metabolism and encourages growth and productivity.

In general, the production of auxin by bacteria that promote plant growth is a vital mechanism for leveraging microbial–plant interactions to promote ecosystem resilience. In wetland environments, where plant life is essential for sustaining ecological equilibrium and facilitating bioremediation efforts, the existence of auxin-producing bacteria offers a natural approach to improve plant establishment, root growth, and nutrient absorption.

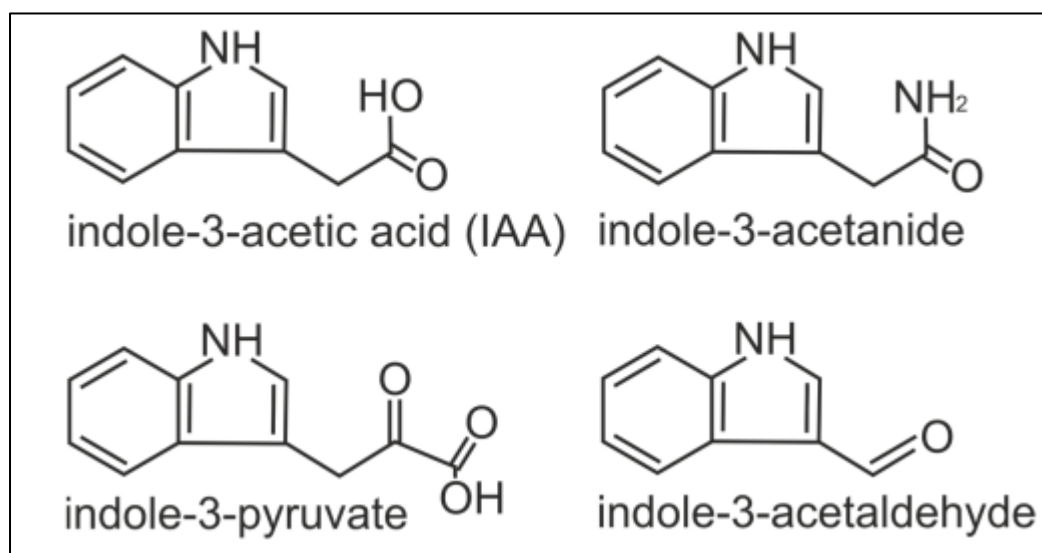


Figure 2.14: Derivatives of IAA

Source: Timofeeva et al.,2024 (<https://www.mdpi.com/2223-7747/13/17/2371>)

GIBBERELLIN

Gibberellin (GA) refers to a broad category of tetracyclic diterpenoid carboxylic acids characterized by either C₂₀ or C₁₉ carbon frameworks. They are named after the fungus *Gibberella fujikuroi*, which is now classified as *Fusarium fujikuroi* (Woo *et al.*, 2023). These compounds were first isolated because of their capacity to promote the elongation of plant shoots. A total of 136 distinct gibberellin structures has been recognized, labelled as GA₁–GA₁₃₆. Among these, only four GAs have been discovered in bacteria: GA₁, GA₃, GA₄, and GA₂₀, with GA₁ and GA₄ exhibiting the highest levels of activity. Chemical structure of some of the gibberellic acids have been represented in **Figure 2.15**. Gibberellins are recognized for their role in promoting growth and activating key growth processes such as stem elongation, seed germination, flowering, fruit setting, enhancing photosynthesis rates, and increasing chlorophyll content. In conjunction with other phytohormones, they serve as transducers of elicitor signals. The production of GAs by plant growth-promoting bacteria (PGPB) has been documented in genera such as *Achromobacter xylosoxidans*, *Gluconobacter diazotrophicus*, *Acinetobacter calcoaceticus*, *Rhizobia*, *Azotobacter* spp., *Bacillus* spp., *Herbaspirillum seropedicae*, and *Azospirillum* spp. Gibberellins can stimulate shoot growth and development while simultaneously inhibiting root growth through the mechanisms of the gibberellin signalling system, particularly the DELLA repressor, which activates genes that induce gibberellin responses [Olanrewaju *et al.*, 2017]. Bacteria that produce gibberellins are crucial in agriculture, especially within wetland ecosystems, as they facilitate plant growth and improve stress resilience. Located in the rhizosphere—the soil surrounding plant roots—these bacteria generate gibberellins (GAs), which are plant hormones that control several developmental processes, including seed germination, stem elongation, and flowering.

Some bacteria present in wetlands are capable of producing gibberellins, which are hormones that encourage plant growth. These bacteria, typically located in the rhizosphere (the region surrounding plant roots), can emit gibberellins that enhance the growth and development of plants. In a study done by Kang *et al.*, 2014, a bacterial isolate from soil, identified as *Pseudomonas putida* H-2-3 was applied to soyabean plants present in abiotic stress condition. Upon application, it was observed that, it improved the length, fresh and dry weight of shoots, as well as chlorophyll content in gibberellins (GAs) deficient mutant Waito-c rice seedlings compared to the control, potentially due to the presence of GA₁, GA₄, GA₉, and GA₂₀. *P. putida* H-2-3 significantly promoted the shoot length and fresh weight of plants affected by salt and drought stress.

Therefore, in wetland ecosystems, gibberellin-producing bacteria hold significant potential for strengthening vegetation establishment, resilience, and ecological restoration. By promoting processes such as stem elongation, seed germination, and chlorophyll synthesis, these microbes can accelerate plant growth in nutrient-variable and stress-prone wetland environments.

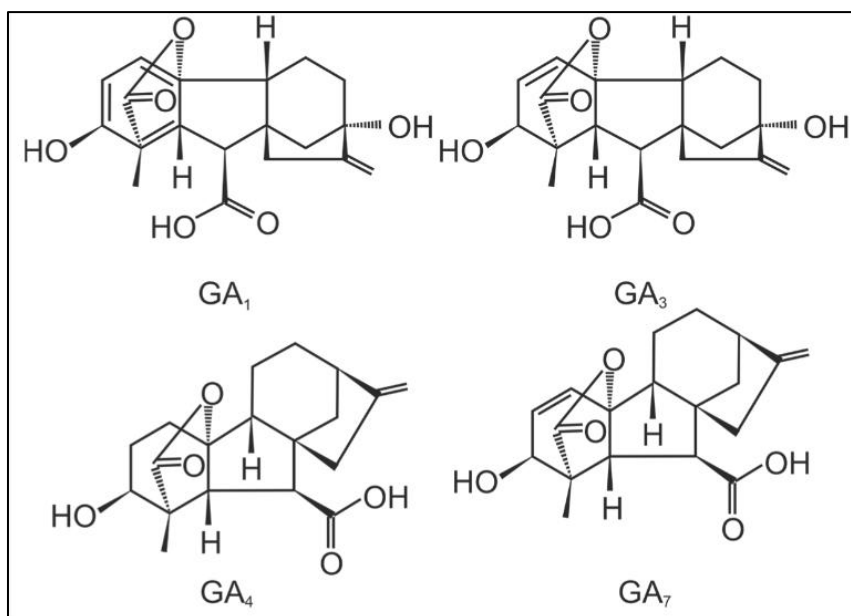


Figure 2.15: Chemical structure of some gibberellic acids

Source: Timofeeva et al.,2024 (<https://www.mdpi.com/2223-7747/13/17/2371>)

2.7.4 PLANT PATHOGENESIS AND THEIR CONTROL

Several biocontrol plant growth-promoting bacteria (PGPB) possess the capability to produce hydrogen cyanide (HCN). The PGPB capable of HCN production generate various antibiotics or enzymes that degrade cell walls (Ramette *et al.* 2006). Furthermore, studies have shown that the minimal amount of HCN produced by the bacterium enhances the efficacy of antifungal agents targeting fungal pathogens, thereby preventing the fungi from developing resistance to specific antifungals. Consequently, HCN produced by PGPB seems to work synergistically with other biocontrol strategies utilized by the same bacterium. The toxicity of HCN is attributed to its ability to inhibit cytochrome c oxidase and other crucial metalloenzymes (Nandi *et al.* 2017). Numerous bacterial genera, including *Rhizobium*, *Pseudomonas*, *Alcaligenes*, *Bacillus*, and *Aeromonas*, have been identified as HCN producers (Olanrewaju *et al.*, 2017)

Plant Growth-Promoting Rhizobacteria (PGPRs) generate various allelochemicals, including metabolites, antibiotics, and lytic enzymes, which help mitigate the impact of plant pathogens that hinder plant development. Factors such as water availability, nutrient levels, bacterial population density, soil composition, and texture influence the allelopathic effect. Lytic enzymes, including chitinases, cellulases, proteases, and lipases, have been utilized to break down the cell walls of numerous phytopathogenic fungi, including those from the genera *Fusarium*, *Phytophthora*, *Rhizoctonia*, *Sclerotium*, *Aspergillus*, and *Pythium*, among others [Balvantin *et al.*, 2011; Medina-de la Rosa *et al.*, 2016]. In wetland conditions, these pathogens are common and can be controlled organically using PGPR that contains HCN, as well as other lytic enzymes.

2.7.5 ACC DEAMINASE:

Ethylene is a gaseous phytohormone produced internally that works at low concentrations, and has a role in regulating all aspects of plant growth, development, and aging [Shaharoon *et al.*, 2006; Saleem *et al.*, 2007]. Beyond its role as a growth regulator, ethylene has also been recognized as a stress-related phytohormone. In response to abiotic and biotic stresses—such as pathogen damage, flooding, drought, salinity, and both organic and inorganic pollutants—there is a significant increase in the production of endogenous ethylene, which negatively impacts root growth and, consequently, the overall growth of the plant. Various mechanisms have been explored to lower ethylene levels in plants. One such mechanism involves the action of the bacterial enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase [Glick, 2005; Jalili *et al.*, 2009]. This enzyme regulates ethylene production in plants by converting ACC (the direct precursor of ethylene biosynthesis in higher plants) into α -ketobutyric acid and ammonia [Arshad *et al.*, 2007; Saleem *et*

al., 2007]. A considerable amount of ACC may be released from plant roots and subsequently absorbed by soil microorganisms, where it can be hydrolyzed by ACC deaminase, thereby reducing the environmental levels of ACC. When in association with plant roots, soil microbial communities that possess ACC deaminase activity may exhibit enhanced growth compared to other free-living microorganisms, as these microbes utilize ACC as a nitrogen source (Glick, 2005).

Bacterial ACC deaminase activity can be categorized into two main groups based on their enzymatic activity levels: high and low. Microorganisms that express high levels of ACC deaminase tend to bind non-specifically to various plant surfaces, including those found in the rhizosphere, phyllo sphere, and endophytes. In contrast, microorganisms with low ACC deaminase expression are limited to binding specific plants or are found only in particular tissues. Another study revealed that a mutation in the ACC deaminase pathway affected the physiology of the endophytic strain *Burkholderia phytofirmans* PsJN2, resulting in the loss of ACC deaminase activity, increased IAA synthesis, reduced siderophore production, and the inability to promote canola root growth (*Brassica napus* L.; Sun *et al.*, 2009). Similar results were obtained, indicating that a strain of *Pseudomonas* spp. possessing ACC deaminase activity partially mitigated the impact of drought stress on pea growth (*Pisum sativum* L.). Furthermore, selecting endophytes with ACC deaminase activity may serve as an effective strategy for developing a successful phytoremediation approach, considering the ability of these bacteria to alleviate plant stress [Souza *et al.*, 2015; Gallegos *et al.*, 2019]. Bacteria that produce ACC deaminase are essential in agriculture, especially in wetland environments, as they alleviate the adverse impacts of stress on plants. These microorganisms assist plants in coping with environmental challenges such as flooding, salinity, and heavy metal pollution by degrading 1-aminocyclopropane-1-carboxylic acid (ACC), which is a precursor to the stress hormone ethylene. By doing so, they diminish the detrimental effects of stress-induced ethylene on plant growth and development, thereby fostering healthier and more robust crops.

In case of wetlands, research conducted by Ravanbakhsh *et al.* (2017) explored the impact of ACC deaminase-producing rhizosphere bacteria on plant reactions to flooding, specifically examining the riparian wetland species *Rumex palustris*. Typically, flooding results in the accumulation of ethylene in submerged tissues, which is crucial for adaptive mechanisms such as shoot elongation that allow plants to access the water surface for oxygen absorption. The authors inoculated *R. palustris* with *Pseudomonas putida* UW4 (wild-type, ACC deaminase-positive) alongside its isogenic mutant (ACC deaminase-deficient) to clarify the microbial influences on ethylene

signalling. Their findings revealed that ACC deaminase activity diminished ethylene accumulation, thus inhibiting the elongation response vital for survival during flooding, while other bacterial characteristics (like auxin production) had a beneficial effect on biomass under stress conditions. This showed that although ACC deaminase-producing bacteria can alleviate ethylene stress in crops, they may also obstruct adaptive flood responses in wetland plants by disrupting ethylene-mediated survival strategies.

Therefore, in the realm of wetlands, bacteria that produce ACC deaminase works as a mode for the survival and resilience of plants. Their capability to break down ACC and reduce stress-induced ethylene levels proves advantageous in situations like salinity, drought, or pollution stress. However, their effect can be harmful to flood-adapted wetland species that depend on ethylene-mediated elongation to avoid submersion.

2.7.6 MICROBIAL ENZYMES

Enzymes like cellulase is an extracellular enzyme that functions as a glycoside, cleaving the β -1,4-D glucan bonds found in the structure of cellulase, which is a crucial component of the cell walls in certain fungi, bacteria, and all plants. This enzyme facilitates the degradation of cell walls, resulting in the production of cellobiose, glucose, and cello-oligosaccharides. Cellulase is classified into three primary types: endo- β -1,4 glucanase, exoglucanase, and β -glucosidase, all of which are involved in the hydrolysis of cellulase. Amylase is also produced by biocontrol bacteria. They are divided into three primary categories: β -amylases, which function as exo-hydrolases that hydrolyse α -1,4-glucan bonds into maltose units; α -amylases, which generate maltose and glucose by catalysing the hydrolysis of α -1,4-glycosidic linkages; and γ -amylases, which produce glucose by breaking down both α -1,6 and α -1,4 glycosidic bonds. They can tolerate acidic conditions and need calcium to stabilize their conformations (Riseh *et al.*,2024). Ureases are essential in the rhizosphere as they catalyse the hydrolysis of urea into ammonium and carbon dioxide, resulting in ammonium (NH_4^+), which serves as an easily absorbable nitrogen source for plants. Additionally, ureolytic bacteria aid in biomineralization through the precipitation of calcite, a process that is facilitated by an increase in pH and the production of carbonate ions. This mechanism is for enhancing soil nutrients (Rodriguez *et al.*,2025) In agricultural wetlands, bacteria that produce enzymes such as amylase, urease, pectinase, lignin peroxidase, and cellulase are vital for nutrient cycling and decomposition processes, enhancing soil fertility and the overall health of the ecosystem. These enzymes decompose complex organic materials, releasing key nutrients like

sugars, ammonia, and phenolic compounds, which can be absorbed by plants and other microorganisms.

During the degradation of wetlands, the alterations in plant community structures, soil moisture content, and aeration conditions differ markedly among various land use types. This, in turn, impacts the community structure, growth, and survival of microorganisms in the rhizosphere soil. It indicates that the shifts in aboveground vegetation and belowground soil microorganisms during wetland degradation may contribute to the observed variations in soil extracellular enzyme activity in this research [Sinsabaugh *et al.*,2010; Li *et al.*,2024].

The bacterial enzymes most essential in wetlands includes amylase, urease, cellulase etc. The roles of the enzymes are:

- A. **Amylase:** Decomposes starch, influencing the cycling of carbon in the soil.,
- B. **Phosphatase:** Facilitates the cycling and mineralization of phosphorus.,
- C. **Urease:** Transforms urea into ammonia, impacting nitrogen cycling.,
- D. **Dehydrogenase:** Participates in soil respiration and the breakdown of organic matter.,
- E. **Hydrolases** (such as glucosidases and cellulases): Decompose complex carbohydrates.,
- F. **Oxidoreductases** (like oxidases): Engage in oxidation-reduction reactions, which are crucial for nutrient cycling.

In summary, bacterial enzymes are essential in wetland soil as they promote decomposition, nutrient cycling, and the overall health of the soil. Their functions are affected by various factors and can act as indicators of the state of the wetland ecosystem.

2.8 HEAVY METAL POLLUTION OF SOIL

The ongoing global industrialization and urbanization has led to heavy metal pollution, which poses a significant threat to both ecosystems and human health (Mazzei *et al.*, 2013; Khan *et al.*, 2015;). Heavy metals are prevalent in the environment and are recognized as significant contaminants in food. This category of heavy metals encompasses both essential elements for normal metabolic functions, known as micronutrients (Fe, Mn, Cu, Zn, Mo), which can be more detrimental to plants in excessive amounts than to animals, as well as toxic elements like As, Hg, Pb, or Cd, which are harmful to humans and animals even at low concentrations, while having a lesser impact on plant growth and development. Based on their toxicity to living organisms, heavy metals can be ranked in the following order: Hg > Cu > Zn > Ni > Pb > Cd > Cr > Sn > Fe > Mn > Al. The dynamics of heavy metals in the 'soil' and their absorption by plants are influenced by soil characteristics, which are crucial for the bioavailability of these metals. The extent of accumulation of these compounds in plants is determined by various factors, including soil type, pH, moisture levels, micronutrient content, and the timing of crop harvesting [Właśniewski and Hajduk 2012; Yang-Guang *et al.* 2016] showed that soil age significantly influences the modulation of metal bioavailability in plants.

2.8.1 HEAVY METAL ACCUMULATION IN WETLANDS

Wetlands have maintained a significant and essential relationship with human civilization since ancient times, providing numerous benefits and services to humanity (Li *et al.*,2018). The wetland ecosystem plays a crucial role in supporting the hydrological cycle, regulating climate change, and offering a variety of ecosystem services that benefit biodiversity (Hu *et al.*,2017). Furthermore, it contributes both direct and indirect value to humans by facilitating various economic services (Constanza *et al.*,1997). When considering land area as a unit, the wetland ecosystem can be characterized as a leading provider of ecosystem services, accounting for 47% of the global ecosystem value. This statistic underscores the importance and productivity of this ecosystem compared to others (Li *et al.*,2018). Additionally, this ecosystem acts as a sink for heavy metals due to its significant role in various physical, chemical, and biological processes. In contemporary society, human activities are primarily responsible for the accumulation of heavy metals in this sink (Garg *et al.*,2015) (Lu *et al.*,2016). Common heavy metal pollutants resulting from urbanization, industrialization, and agricultural practices include lead (Pb), chromium (Cr), cadmium (Cd), copper (Cu), mercury (Hg), nickel (Ni), zinc (Zn), manganese (Mn), and arsenic (As) (Kumar *et al.*,2020).

Concentrations of heavy metals found in bottom sediments serve as indicators of environmental pollution across various ecosystems, including rivers (Han *et al.*, 2018) (Kumar *et al.*, 2010) streams (Wojciechowska *et al.*, 2019), wetlands (Prusty *et al.*, 2019) (Dash *et al.*, 2021) (Panda *et al.*, 2020), forests (Prusty *et al.*, 2009), grasslands (Prusty *et al.*, 2012), and marine ecosystems. In wetlands, the presence of heavy metals in bottom sediments can reflect both natural origins and anthropogenic activities, such as industrial waste discharged via streams, rivers, and agricultural runoff. Due to their persistence and the rising intensity of agricultural practices heavy metals accumulate in wetland soils over time, threatening both the environment and human health as they move through different trophic levels (Naz *et al.*, 2020).

In older flat wetlands, a greater concentration of heavy metals was detected, attributed to prolonged discharges of municipal and industrial wastewater. A multivariate analysis indicated that bedrock contributes Fe and Mn, whereas Cu, Zn, Cd, Cr, Ni, and Pb originate from anthropogenic activities. In comparison to wet ditches and reclaimed wetlands, certain heavy metals like Pb, Cd, and Zn are found in much higher concentrations in wetlands located in younger regions, as these wetlands are more abundant in mangrove soils. Elevated toxic levels of heavy metals in flat and reclaimed wetlands in older regions are noted due to their extensive reclamation history, especially when contrasted with lowland wetlands (Bai *et al.* 2011; Zwolak *et al.*, 2019]

The cultivation of food crops in soils that are contaminated with heavy metals results in the accumulation of these metals in the edible portions of the plants, which are then consumed by humans (Amin *et al.*, 2013). The contamination of food crops with heavy metals is a significant issue because these metals are non-biodegradable, possess long biological half-lives, and some can be toxic even at very low concentrations [Arora *et al.*, 2008; Khan *et al.*, 2008].

Exposure to cadmium, lead, and methylmercury compounds poses significant risks during prenatal development and infancy, leading to irreversible alterations in the central nervous system. Lead is also linked to cardiovascular diseases, interferes with heme biosynthesis and vitamin D metabolism, and results in kidney and liver dysfunctions, as well as disorders affecting the immune and reproductive systems. Additionally, it disrupts the metabolism of iron, zinc, and copper. Cadmium is known to be carcinogenic, neurotoxic, and nephrotoxic, contributing to skeletal disorders, liver damage, cardiovascular diseases, dysfunctions of the sexual glands, and imbalances in the body's mineral levels. Mercury, particularly in the form of methylmercury compounds, primarily accumulates in brain tissue, inflicting damage on the central nervous system, especially in the developing foetal brain. In adults, mercury exposure can lead to hearing, speech, and visual

impairments, cardiovascular diseases, and paralysis of limb muscles. Arsenic is associated with carcinogenic, neurotoxic (notably hearing disorders), and genotoxic effects, and is responsible for cardiovascular diseases, peripheral vascular disorders, anaemia, and dysfunctions of the reproductive system.

The transfer of heavy metals from soil to livestock and humans can happen either through the direct consumption of contaminated crops or via bioaccumulation within the food chain. These processes are influenced by various factors that exhibit distinct spatiotemporal variability. The environmental quality of wetlands can be assessed using the heavy metal content in sediments as an indicator. Evaluating and analysing heavy metal concentrations has become crucial for monitoring pollution in wetlands.

Studies have been done to investigate the accumulation of Pb, Cr, Cu, and Zn in the soil of wetlands with varying spatial distribution in Odisha, India. It was found that the concentration of Pb was highest at the Hirakud sampling site, measuring $51.25 \pm 4.46 \mu\text{g/g}$, with all sampling locations showing Pb levels greater than those reported in earlier studies. At the Hirakud site, the Cr concentration reached $266 \pm 6.95 \mu\text{g/g}$, which is considerably higher than findings from previous studies in Odisha. The Bhadrak site (Odisha) recorded the highest Cu concentration at $34.27 \pm 2.2 \mu\text{g/g}$, with all other sampling sites, except for Chandaneswar, showing Cu levels that exceeded those in earlier studies. The concentration of heavy metals in wetlands can be evaluated by measuring their levels in water and sediments, which are observed to be low in water and high in sediments due to accumulation. The presence of potentially harmful heavy metals in sediment poses a risk of bioaccumulation and biomagnification [Anthony 2023] [Panda *et al.*, 2023]

An investigation was done in Ropar wetland, Rupnagar district of Punjab. The Ropar wetland, recognized as a RAMSAR site, is located along the banks of the Sutlej River, nestled in the Shivalik foothills ($31^{\circ} 01'N$ $76^{\circ} 30'E$). It covers an area of 1365 hectares (Tiwana *et al.*, 2008). The wetland experiences significant human interference as a result of nearby agricultural and industrial activities, including a thermal power plant and a cement factory. Additionally, several villages are located within the study area. The inhabitants of these villages primarily consist of farmers who cultivate food crops like wheat, mustard, rice, and maize in their fields. It was discovered that all soil samples contained Cd and Co levels exceeding the safe thresholds of 0.06 mg/kg and 8.00 mg/kg, respectively. This may be due to the deposition of fly ash from thermal power plants onto the soil surface in agricultural fields (Aggarwal, 2009). In every crop sample analysed, the levels of Co and Pb were found to surpass the safe limits of 0.48 mg/kg and 2.50 mg/kg (Awasthi, 2000;

Singh et al., 2010), respectively. This indicates a greater absorption of Co and Pb from the soil, which can be attributed to the deposition of fly ash and the application of inorganic pesticides, insecticides, and fertilizers in the fields via plants. Rice grains were identified as hyper-accumulators of Cr and Cu, while maize grains were recognized as hyper-accumulators of Cu. This phenomenon may be due to the differing physiological needs and uptake mechanisms of various crops, as well as the heavy metal content in the soil samples (Sharma *et al.*,2019).

An evaluation was performed in East Kolkata Wetlands (22° 33' 13.40"N; 88° 26' 41.34"E), West Bengal, India showed the presence of heavy metals like Pd, Cd, Cr and Hg, when tested in edible parts of crops like *Abelmoschus esculentus* and *Lageneria siceraria*. Heavy metal analysis of the crops showed the heavy metals (HMs) Pb, Cd, Cr, and Hg found in the edible portions of *L. siceraria* varied from 23.59 to 35.45 ppm, 5.88 to 13.98 ppm, 2.04 to 7.63 ppm, and 0.88 to 2.04 ppm, respectively, throughout the entire study duration at the selected site. In the edible parts of *A. esculentus*, the concentrations of Pb, Cd, Cr, and Hg were recorded at 13.51–29.67 ppm, 3.42–9.64 ppm, 1.86–5.56 ppm, and 0.00–1.04 ppm, respectively. For *Z. mays*, the levels were 31.78–42.33 ppm, 11.93–18.43 ppm, 9.83–17.21 ppm, and 0.45–3.52 ppm for Pb, Cd, Cr, and Hg, respectively. Therefore, the heavy metal concentration in the soils of East Kolkata wetland followed the trend Pb>Cd>Cr>Hg (Agarwal *et al.*,2022).

Since the present study has focus on lead (Pb) contamination and its absorption by plants, there is a heightened focus on comprehending Pb-specific transport pathways, the physiological responses of plants, and mechanisms assisted by microbes.

Studies show that soil microbes aid in Pb uptake via biosorption, bioaccumulation, and solubilization (Sharma *et al.*, 2005; Collin *et al.*, 2022).

2.8.2 Uptake of lead

Lead (Pb) is immobilized in the soil due to formation of complexes. It is a non-essential element and thus has no specific channels for its uptake. Several other strategies are involved in Pb uptake and its transport between the various cells of a plant body. Chemical properties of the soil, including organic content, pH, and redox potential. Other factors, including root surface area, root exudates, the presence of mycorrhizae, and the rate of transcription, are some important aspects. In addition to this the soil microbes also play an important role in the uptake of Pb by regulating biosorption, bioaccumulation and solubilization of the heavy metals. Plants take up Pb either by the capillary action of the roots or by cellular respiration through the stomata, The latter depends on the leaf morphology. The immobilization of Pb in roots is attributed to the complex-forming ability of histidine. In contrast, the transportation of Pb to the aerial parts depends on the interaction of Pb with other organic acids. [Sharma, *et al.*, 2005; Aslam, *et al.*, 2021; Collin, *et al.*, 2022; Rahman, *et al.*, 2024]. [Fig:2.16].

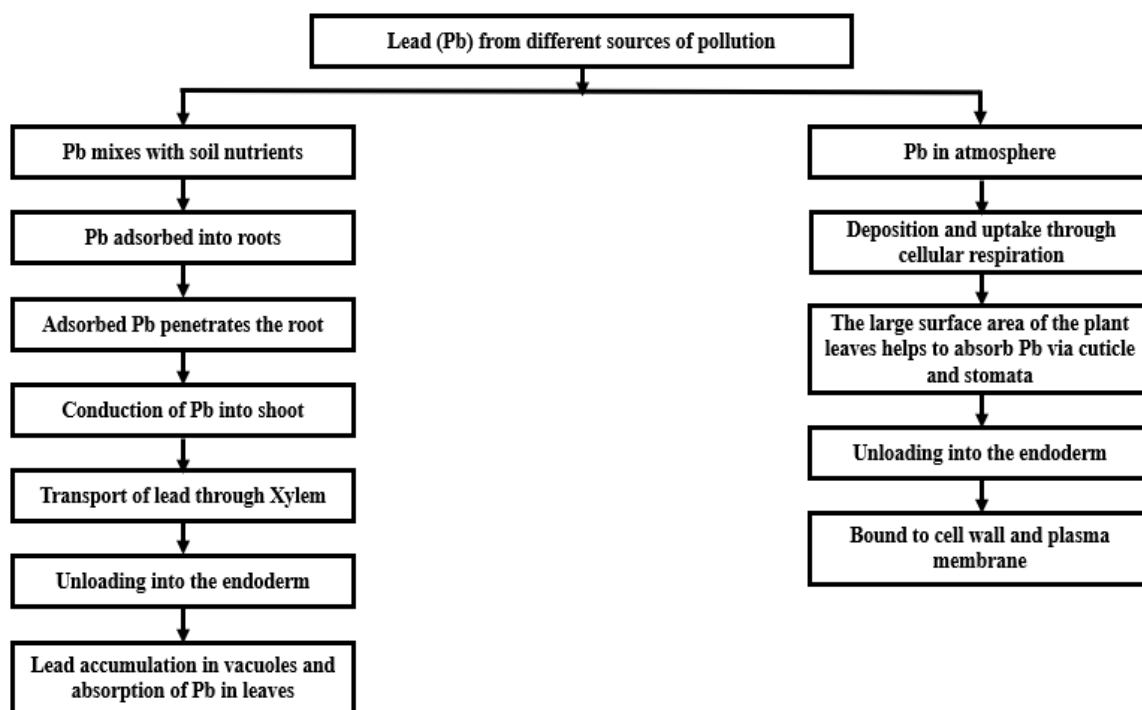


Fig:2.16 Lead uptake from different sources by plants.

2.8.3 Bioremediation of Lead (Pb) with Bacteria

In terms of bioremediation, many bacteria play an important role in mitigation of Pb accumulation in soil and plants. Specific some bacterial species has the ability of immobilizing or detoxify Pb through multiple mechanisms, for example bioaccumulation, biosorption, and bioprecipitation. Biosorption through bacteria includes the binding of Pb ions to the surface of cell and effectively causing removal of the metal from soil and preventing uptake of Pb by plants. Bioaccumulation is the internalization of Pb inside the bacterial cells, where it can be degraded or converted into some other less toxic forms [Sevak., *et al* 2021]. Bioremediation through microbes in the EKW area is a viable strategy to reduce Pb contamination in soil and as well as crops. The diversity of microbes in the wetland includes several species of bacteria that are capable of tolerating and detoxifying heavy metals, including Pb. *Proteobacteria*, especially, is recognised as an important contributor in bioremediation of heavy metal in EKW. These bacteria is capable of degrading complex organic molecules as well as recycling multiple nutrients and immobilizing heavy metals through more than one metabolic process. With increasing the activity of Pb tolerance of bacteria in wetland, it is very much possible to reduce the presence of Pb in the soil and reduce its uptake by plants. Also, combination of bacterial bioremediation with phytoremediation is also a pathway that uses plants capability of absorbing and detoxifying pollutants, which might be easier approach to reduce Pb contamination in the EKW [Saha, *et al* 2010].

The incredible efficiency of bacteria in the rhizosphere arrives from the high concentration of nutrients provided by the host plant, along with the different metabolic processes and special adaptations of bacteria themselves. This mutualistic behaviour is a reason of improved plant growth, high nutrient uptake, increase in stress tolerance, making rhizosphere bacteria crucial for plant health as well as productivity. Lead (Pb) contamination causes a severe environmental and health risk in public due to its widespread occurrence and toxicity. Bioremediation, utilizing bacteria, has emerged as a promising strategy to remove or neutralize lead in contaminated environments. Certain bacterial strains have evolved mechanisms to tolerate and detoxifying lead by binding it to their cell walls, accumulating it intracellularly, or transforming it into less toxic forms. Bacteria such as *Bacillus*, *Pseudomonas*, and *Rhizobium* were recognised for their resistance to Pb properties, making them potential candidates for the use in bioremediation efforts. These microorganisms are not only able survive in high concentration of Pb but also are able to help in increasing plant growth properties by reduction of lead toxicity in the soil. Incorporation of such bacteria into contaminated sites, will help in reduction of the availability of lead, minimizing

Pb uptake by plants and other organisms, hence providing a solution that is sustainable to lead pollution [Gupta, *et al* (2012); Mahmood, *et al* (2013); Zuo, *et al* (2022);]

2.9 Time series Analysis and its application in Agriculture

Introduction to Time Series Analysis:

Time series analysis refers to a specialized method for examining a series of data points gathered over a specific time frame. In this type of analysis, analysts systematically collect data points at regular intervals throughout a defined period, and not capturing them sporadically or at random. It is a technique used to investigate time within parametric series data to derive significant statistics and other attributes of the data (Esling *et al.*, 2012).

Time series or temporal data analysis refers to the process of examining time on a parametric level to extract informative statistics and additional characteristics of the data. Various methods of time series analysis exist, including frequency and time domain approaches, which can be categorized as linear or non-linear, parametric or non-parametric, and univariate or multivariate (Caiado *et al.*, 2006).

Time series analysis is closely connected to the present investigation, since it helps in monitoring changes in soil physicochemical properties and the growth patterns of crops during various cropping phases of *Cucurbita maxima* and *Zea mays*. Utilizing statistical models, like linear trend model, it helps us to know about the variations, and random fluctuations in soil characteristics, which subsequently affect plant productivity. This methodology enhances the relationship between soil dynamics and crop yield, providing predictive insights that support the specific goal of sustainable agricultural management.

Objectives of Time Series Analysis:

Primarily, there are four main objectives: Description, Explanation, Prediction, and Control.

Description of the data series

The initial steps in the analysis involve plotting the data and obtaining basic descriptive measures of the fundamental characteristics of the series. These measurements can range from simple trend observations to complex seasonal change analyses. A graph helps in the identification of 'outlier' observations which do not align with the rest of the data. By graphing the time series, it becomes possible to identify turning points where the upward trend suddenly shifts to a downward trend. If such a turning point exists, different models may need to be applied to the two segments of the

series. The two segments refer to the initial segment, that represents the part of the series preceding the turning point, during which the data may indicate an upward trend (for instance, growth or increase). The subsequent segment refers to the part of the series following the turning point, where the data may transition into a downward trend (for example, decline or decrease) (Chatfield *et al.*,2019).

Explanation of the data series

The variation in one time series can be used to explain the variance in another series, as observations are made on two or more variables. This can lead to enhanced understanding. In this context, a multiple regression model may prove beneficial. Multiple regression is a statistical method used to predict the values of a response (dependent) variable based on a set of predictor (independent) variable values. The most widely utilized form of this technique is multiple linear regression. As a predictive instrument, it helps to clarify the relationship between several independent variables (X_1, X_2, X_k) and the dependent variable being analysed (Y) [Sellam *et al.*,2016]. The coefficient of determination, R^2 , indicates the proportion of variability in the dependent variable that is accounted for by the model. In simpler terms, it serves as a gauge of the model's goodness-of-fit. The computational challenge of multiple regression involves fitting a straight line to a collection of data points. The least squares method is the most commonly employed technique for this purpose. This method allows for the optimization of the regression equation parameters, ensuring that the total of the squared distances from the measurement points to the fitted straight line is minimized.

The equation for the regression line is expressed as: $Y = \beta_0 + \beta_1 \times X_1 + \beta_2 X_2 + \beta_k X_k + \epsilon$

(1) where Y —dependent variable (explained variable), $X_1, X_2 \dots X_k$ —independent variables (explanatory variables), $\beta_0, \beta_1, \beta_2 \dots \beta_k$ —parameters of the equation, ϵ —random component (the residual of the model).

Prediction of time series data

One may wish to predict future values of the time series based on the observed data. Analysing economic and industrial time series is an essential task in sales forecasting. The terms forecasting and prediction are often interchangeable (Box *et al.*,2015).

Control of data series

When time series are developed to assess the quality of a manufacturing process (with the ability to adjust the process), several control techniques are available. During quality control, the observations are displayed on a control chart, and the controller reacts based on the information presented in the charts. A stochastic model is employed to fit the series. The expected future values of the series are utilized to modify the input process variables and ensure the process remains on course. A stochastic model is one that incorporates randomness and uncertainty within the data. In the realm of time series analysis, models such as ARIMA, Exponential Smoothing, or State-Space models are considered stochastic because they capture deterministic trends and also integrate the random error (noise) that is a natural part of real-world data (Jose *et al.*,2022)

Components of Time Series Analysis:

A time series fundamentally consists of four key components.

Trend of a Statistical data

A trend indicates a consistent pattern within the data. Over a prolonged period, it may rise, fall, ascend, or descend. The trend represents a stable, long-term general direction of data movement. It is not necessary for the data to move uniformly for a trend to exist. Over an extended timeframe, the movement or direction may vary, yet the fundamental tendency in a trend should remain unchanged. Examples of trends include the number of schools, agricultural production, and population growth. It is important to note that the trend can shift direction across different timeframes, whether upward, downward, or stable. A trend can be classified as either linear or non-linear (Qi *et al.*,2008).

Seasonal Variations of statistical data

Seasonal variations refer to short-term changes in time series that typically occur within a year. They are not visible close to the equator, but ideal variations are observed in this context, because of their closeness to the tropics. Throughout the 12-month duration of the time series, these variations usually follow a consistent trend of growth, whether upward or downward. To monitor these fluctuations, schedules based on hourly, daily, weekly, quarterly, and monthly intervals are commonly employed. Seasonal differences can arise from both natural and artificial factors. The multiple seasons and human-made changes significantly impact seasonal variations. For instance, the demand for umbrellas surges during the rainy season, air conditioner sales rise in the summer,

and harvests depend on the season, all of which exemplify seasonality. Additionally, human-created norms clearly reflect seasonal changes. Factors such as holidays, traditions, styles, habits, and various events like weddings influence these seasonal differences. It is important to note that the period of seasonal variation should not be used as a measure of a business's performance (Box *et al.*,2015).

Cyclical Variations of statistical data

Cyclical variations are changes in time series that occur over a period exceeding one year. These oscillatory movements in time series often last longer than a year. A complete operational period is referred to as either a cycle or a 'Business Cycle.' Cyclical variations consist of four distinct phases: prosperity, recession, depression, and recovery. They can be either regular or irregular in nature. Typically, cyclical variations result from the interplay of two or more economic forces and their interactions (Box *et al.*,2015).

Random Variations of statistical data

There exists a different type of movement observable in time series analysis. This is known as pure Irregular and Random Movement. As implied by the term, no hypothesis or trend can be applied to predict irregular or random movements within a time series. Such outcomes are unexpected, erratic, unpredictable, and beyond control. Examples of random components in time series include earthquakes, wars, famines, and floods (Chatfield *et al.*,2019).

Time Series Plots

It is a visual representation that displays data collected over time from any given process. This chart can help identify historical trends within the data and assess whether the data points are random or exhibit specific patterns. Additionally, a time series graph can be utilized to visually identify indications of stability in a process, helping to evaluate its overall stability (Jose *et al.*,2022) (**Figure 2.17**).

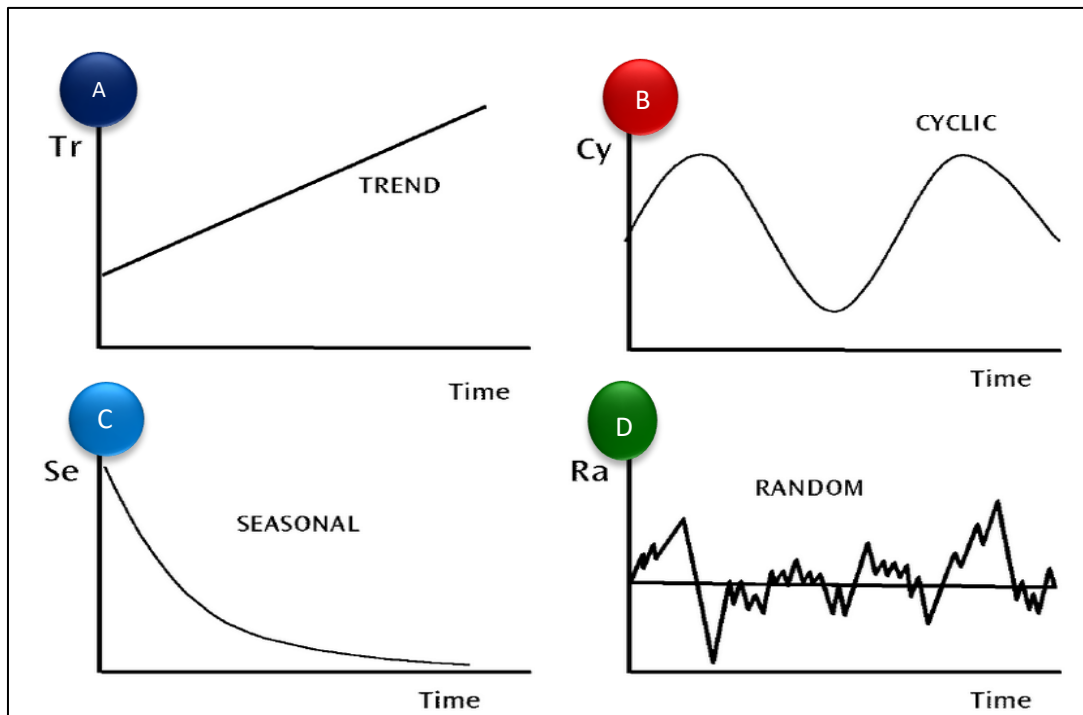


Fig 2.17: Time series plots

The **(A) trend** indicates the long-term trajectory of the data, illustrating whether values consistently rise or fall over time, exemplified by a gradual increase in crop yields due to enhanced practices.

The **(B) cyclic** component captures long-term wave-like variations that occur sporadically, often affected by broader economic or ecological influences, such as the periodic fluctuations in agricultural commodity prices.

The **(C) seasonal** component showcases patterns that recur at regular intervals, typically associated with the calendar or climatic cycles—for example, predictable surges in fertilizer application prior to the monsoon.

Lastly, the **(D) random** component addresses unforeseen and irregular changes that cannot be accounted for by the other three components, such as sudden pest infestations or unexpected rainfall.

Collectively, these four components offer a comprehensive view of the underlying structure and variability present in a time series.

Correlation of Time Series Analysis with crop yields:

By considering the dependent variable yield as a function of time, the correlation between crop yield and time can be determined. Researchers have utilized time-series datasets to forecast yields under typical climatic conditions (Schlenker and Roberts, 2009; Lobell and Burke, 2010).

The stability and growth of crop yield are essential in this context. With technological advancements, machine learning techniques have been increasingly utilized in agriculture, offering innovative methods and strategies for predicting crop yield. Accurate predictions of crop yield not only assist farmers in planning their planting schedules and effectively optimizing resource allocation but also provide scientific backing for governments in developing agricultural policies. Time-series analysis, a vital area of prediction techniques, presents considerable potential in agriculture. By utilizing time-series analysis, one can reveal the dynamic relationships between climate changes, agricultural inputs, and variations in crop yield, identifying trends, cyclical fluctuations, and key influencing factors. In contemporary agricultural production, the effects of climate factors (such as rainfall, temperature, and humidity) and agricultural inputs [like fertilizers (organic and inorganic) and irrigation] on crop yields demonstrate complex nonlinear patterns, which are often difficult for individual prediction models to capture thoroughly (Yan *et al.*, 2025).

The growth rates of various crops are primarily estimated using parametric models that assume either linear or exponential functional forms. Numerous researchers (Panse, 1964; Dey, 1975; Reddy, 1978; Narain *et al.*, 1982; Patel *et al.*, 1986; Kumar and Rosegrant, 1994; Kumar, 1997; Borthakur and Bhattacharya, 1998; Joshi and Saxena, 2002; Sharma, 2005; Patil *et al.*, 2009; Kumar *et al.*, 2021) have employed these parametric models to estimate growth rates, which are currently utilized by planners and policymakers in the country. However, the data may not conform to these linear or exponential models and might necessitate the fitting of higher-degree polynomials or non-linear models. Additionally, these models often lack econometric considerations, such as the normality and randomness of residuals. In such cases, it becomes essential to adopt a nonparametric regression approach, which relies on fewer assumptions.

In a study conducted by Parmar *et al.*, 2017, it was observed that linear model (part of time series model) was deemed appropriate for fitting the trends in the area and production of maize crops, while non-parametric regression proved to be the best-fitting trend function for productivity. The compound growth rate values for the years 1949-50 to 2007-08 in terms of area, production, and productivity of maize indicated that production increased at a rate of 0.49 percent per annum,

attributed to the combined effects of area and productivity growth rates of 0.30 and 0.21 percent per annum, respectively.

Therefore, time series analysis is a robust statistical method used to examine data gathered at regular time intervals, allowing for the detection of trends, seasonal behaviours, cyclical changes, and random variations. Time series analysis, especially when combined with contemporary machine learning techniques, offers considerable promise for enhancing crop yield forecasts, streamlining farm management, and guiding agricultural policy, although the training of the database with machine learning is essential.

A low-angle, upward-looking photograph of several vibrant green corn leaves against a clear, bright blue sky. The leaves are long, narrow, and pointed, with visible veins. They are arranged in a way that creates a sense of height and growth. A bright yellow rectangular box with rounded corners is centered in the middle of the image, containing the chapter title in white text.

CHAPTER 3:

MATERIALS AND METHODS

3.MATERIALS AND METHODS

Sl. no.	Name of the Media	Manufacturing company
1.	Aleksandro Agar medium	HiMedia, India
2.	Brain Heart Infusion	HiMedia, India
3.	Dworkin Foster Medium	HiMedia, India
4.	HI chrome UTI Agar	HiMedia, India
5.	HI chrome Bacillus Agar	HiMedia, India
6.	Jensen's broth	HiMedia, India
7.	King's B Agar Media	HiMedia, India
8.	Luria Bertani broth	HiMedia, India
9.	Minimal salt Media	HiMedia, India
10.	MRS media	HiMedia, India
11.	Nutrient broth	HiMedia, India
12.	Peptone	HiMedia, India
13.	Pikovskaya Agar	HiMedia, India
14.	Potato Dextrose Agar	HiMedia, India,
15.	Simmon's Citrate Agar	HiMedia, India
16.	Stuart's broth	HiMedia, India
17.	Tributyrin Agar	HiMedia, India
18.	Tryptone Soy broth	HiMedia, India
19.	Yeast Extract	HiMedia, India

Table 3.1: List of Media used.

CHEMICALS USED

Sl No.	Name of the Chemical / Reagents	Manufacturing company
1.	2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2H tetrazolium chloride (INT)	HiMedia (India)
2.	2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid	Sigma Aldrich (India)
3.	3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide	Sigma Aldrich (India)
4.	Acetone	Merck Millipore (India)
5.	Agarose	Sigma Aldrich (India)
6.	Amino cyclopropane carboxylic acid (ACC)	Sigma Aldrich (India)
7.	Ammonium metavanadate	Merck (India)
8.	Ammonium sulphate	Merck Millipore (India)
9.	Ammonium molybdate tetrahydrate	Merck Millipore (India)
10.	Aluminium chloride	HiMedia (India)
11.	Barton's reagent	HiMedia (India)
12.	Benedict's reagent	SRL (India)
13.	Bradford's reagent	SRL (India)
14.	Bromothymol Blue	Merck Millipore (India)
15.	Boric Acid	HiMedia (India)
16.	Butanol	Merck Millipore (India)
17.	Calcium Chloride	HiMedia (India)
18.	Carboxymethyl cellulose	HiMedia (India)
19.	Casein	HiMedia (India)
20.	Christensen's urea agar	HiMedia (India)
21.	Chrome Azurol S (CAS)	HiMedia (India)
22.	Citric Acid	Merck Millipore (India)
23.	Congo red	Merck Millipore (India)
24.	Cobalt nitrite	HiMedia (India)
25.	Crystal Violet	HiMedia (India)
26.	Dinitro salicylic Acid	Merck Millipore (India)
27.	Diphenyl amine	Merck Millipore (India)

Sl No.	Name of the Chemical / Reagents	Manufacturing company
28.	Dipotassium hydrogen phosphate	SRL (India)
29.	DMSO (Dimethyl sulfoxide)	HiMedia (India)
30.	Disodium hydrogen phosphate	SRL (India)
31.	Ethanol	Merck Millipore (India)
32.	Elution buffer	HiMedia (India)
33.	Ethelene-diamine tetra acetic acid (EDTA)	SRL (India)
34.	Ethidium bromide (EtBr)	SRL (India)
35.	Ferric Chloride	SRL (India)
36.	Ferrous sulphate	SRL (India)
37.	Gallic acid	SRL (India)
38.	Folin Ciocalteau reagent	Merck Millipore (India)
39.	Gram's iodine	HiMedia (India)
40.	Gelatin	SRL (India)
41.	Glacial acetic acid	Merck Millipore (India)
42.	Glutaraldehyde	Merck Millipore (India)
43.	Glucose	SRL (India)
44.	Gluconic acid	HiMedia (India)
45.	Glycerol	Merck Millipore (India)
46.	Glycin	HiMedia (India)
47.	Guaiacol	Merck Millipore (India)
48.	Hexadecyl trimethyl ammonium bromide (HDTMA)	HiMedia (India)
49.	Hydrochloric Acid	Merck Millipore (India)
50.	Hydrogen peroxide	Merck Millipore (India)
51.	Laminarin	Sigma Aldrich
52.	Lysis Buffer	HiMedia (India)
53.	Sulphuric acid	Merck Millipore (India)
54.	Magnesium chloride	Merck Millipore (India)
55.	Magnesium sulphate	Merck Millipore (India)
56.	Methanol HPLC grade	HiMedia (India)
57.	Mercuric Chloride	Merck Millipore (India)

Sl No.	Name of the Chemical / Reagents	Manufacturing company
58.	Methylene Blue	Merck Millipore (India)
59.	Nessler's Reagent	Merck Millipore (India)
60.	Ninhydrin	HiMedia (India)
61.	Pectin	HiMedia (India)
62.	Picric acid	HiMedia (India)
63.	Peptone	SRL (India)
64.	Perchloric Acid	Merck Millipore (India)
65.	Phenyl-methyl-sulfonyl-fluoride (PMSF)	Merck Millipore (India)
66.	Piperazine-1,4-bis (2-ethane sulfonic acid) (PIPES)	HiMedia (India)
67.	Potassium hydroxide	HiMedia (India)
68.	Potassium dihydrogen phosphate	Merck Millipore (India)
69.	Potassium permanganate	SRL (India)
70.	Potassium phosphate dibasic	HiMedia (India)
71.	Potassium ferrocyanide	Merck Millipore (India)
72.	Potassium persulfate	HiMedia (India)
73.	Proteinase K	HiMedia (India)
74.	RNase A solution	HiMedia (India)
75.	Safranin	HiMedia (India)
76.	Skimmed milk powder	HiMedia (India)
77.	Sodium nitrate	Merck Millipore (India)
78.	Sodium acetate	Merck Millipore (India)
79.	Sodium Bicarbonate	Merck Millipore (India)
80.	Sodium dihydrogen phosphate	Merck Millipore (India)
81.	Sodium hydroxide	Merck Millipore (India)
82.	Sodium molybdate	HiMedia (India)
83.	Sodium potassium tartrate	SRL (India)
84.	Starch	HiMedia (India)
85.	Streptomycin	HiMedia (India)
86.	Tetra-methyl- p-phenylenediamine dihydrochloride	HiMedia (India)

Sl No.	Name of the Chemical / Reagents	Manufacturing company
87.	Thiobarbaturic acid (TBA)	HiMedia (India)
88.	Tricalcium Phosphate	Merck Millipore (India)
89.	Tris	Sigma Aldrich (India)
90.	Trichloroacetic acid (TCA)	HiMedia (India)
91.	Triton X	Merck Millipore (India)
92.	Tryptophan	Merck Millipore (India)
93.	L-Tyrosine	SRL (India)
94.	Urea	Merck Millipore (India)
95.	Zinc acetate	SRL (India)
96.	Zinc sulphate	HiMedia (India)

Table 3.2: List of Chemicals used.

CROPS USED

S. No.	Crop	Variety	Manufacturing Company
1.	<i>Cucurbita maxima</i>	Jack 323	Libertas Agri tech, India
2.	<i>Zea mays L</i>	4226	VNR Seeds, India

Table 3.3: List of Crop varieties used for the experiments

INSTRUMENTS USED

SL NO.	NAME OF INSTRUMENT	NAME OF MANUFACTURER AND MODEL NUMBER	COUNTRY OF MANUFACTURE
1.	-80°C freezer	Eppendorf, CryoCube	India
2.	B.O.D Incubator	N.R Scientific	India
3.	Bio-safety cabinet	Biocoction Manufacturing pvt ltd.	India
4.	Bright-field compound light microscope	Dewinter, DIG1510, 5.1 MP 1/ 2.5” CMOS sensor	India
5.	Cooling centrifuge	Eppendorf Centrifuge 5810R	India
6.	Digital colony counter	N.R Scientific	India
7.	Digital weighing balance	Sartorius	India
8.	DNA sequencer	Thermo Fisher,ABI 3730xl Genetic Analyzer, using BDT v3.1 Cycle sequencing kit	United States of America
9.	Hot air oven	N.R Scientific	India
10.	Incubator	N.R Scientific	India
11.	Lyophiliser	Hahntech corporation 110N	South Korea
12.	Microwave oven	Electrolux	India
13.	Nanodrop	Thermo-Fisher Scientific, Multiscan sky	United States of America
14.	PCR thermal cycler	Bio-Rad T100	India

SL NO.	NAME OF INSTRUMENT	NAME OF MANUFACTURER AND MODEL NUMBER	COUNTRY OF MANUFACTURE
15.	Transilluminator	N.R Scientific	India
16.	UV-Visible spectrophotometer	Optizen POP	South Korea
17.	Ultra sonicator	QSonica	United States of America
18.	UV-Visible spectrophotometer	Thermo-Fisher Scientific,	United States of America
19.	Water Quality Analyzer	Elico PE 138	India
20.	Atomic Absorption Spectrophotometer	PinAAcle 500	Massachusetts, USA.

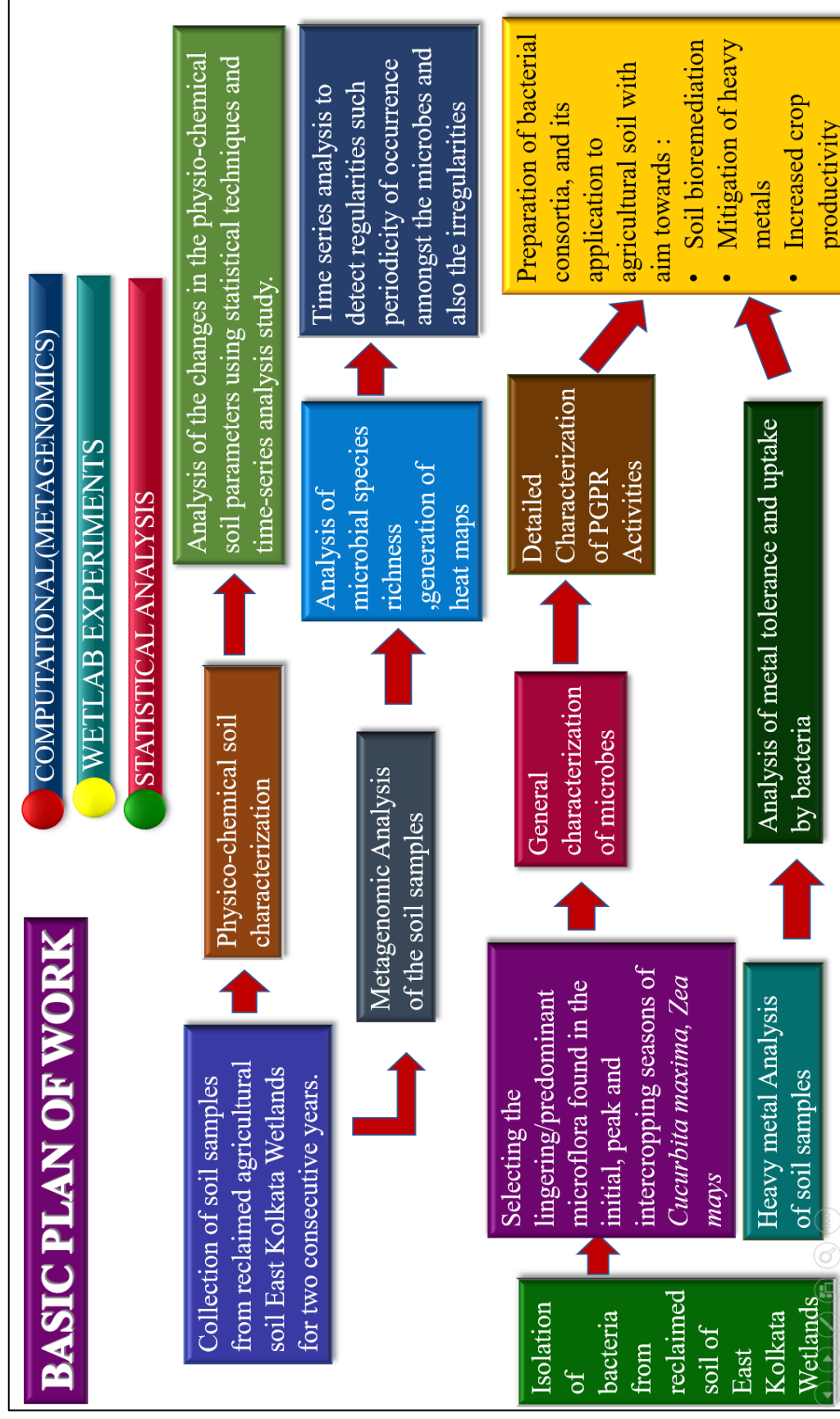
Table 3.4: List of Instruments used.

SOFTWARE USED

SL NO.	NAME OF THE SOFTWARE USED	VERSION AND COMPANY NAME	COUNTRY OF MANUFACTURE
1.	Metagenome sequencing	Illumina, Novaseq 6000 platform	United States of America
2.	Phylogenetic tree construction	MEGA 11	United States of America
3.	Python	3.1	Open software

Table 3.5: List of software used

INVESTIGATION FRAMEWORK IN THIS RESEARCH



OBJECTIVE 1

3.1 To characterize soil, with respect to physical, chemical, microbiological and metagenomic data of designated plot involving *Cucurbita* sp and *Zea mays* over two-year period.

3.1.1 Soil characterization with respect to physico-chemical parameters of the designated plot involving *Cucurbita maxima* and *Zea mays* (YEAR 1+ YEAR 2)

Soil samples were collected from a selected plot at Chowbaga in Dhapa, South 24 Parganas, a part of East Calcutta Wetlands with coordinates of 22.5296°N, 88.4207°E. The sample assemblage was conducted during the time interval between December, 2020- February, 2021, and December 2021- February 2022 whereby *Cucurbita maxima* was grown as main crop in the field. Soil samples were also collected from March, 2021-June, 2021 and March, 2022-June, 2022 whereby *Zea mays* was grown as the main crop.

The collection was done at three phases namely: **Initial** (when the seeds were sowed in soil, during their growing season); **Peak** (Plants in the reproductive phase with flowers and fruit structures); and **Intercrop** (Residual soil after harvesting of the old crops). The determination of the physico-chemical parameters of the soil samples included : pH , Electrical Conductivity (dsm^{-1}) , Organic Carbon(O.C) (%), Nitrogen(N) (kg/ha) , Phosphate(P) (kg/ha) , Potassium(K)(kg/ha) , Calcium(Ca) (mg/kg) , Magnesium(Mg) (mg/kg) ,Sulphur (S) (mg/kg) ,Boron (B) (mg/kg) , Copper(Cu) (mg/kg),Zinc (Zn) (mg/kg),Iron(Fe)(mg/kg), Manganese (Mn)(mg/kg), Cation Exchange Capacity (CEC) (meq/100 g) were done. The Keen Raczkowski Box measurement was used to determine physical properties such as water holding capacity of soil. This was done by filling a perforated metal box with oven-dried soil, saturated with water, and then permitting free drainage until only capillary water was left. The soil's water-holding capacity was determined by calculating the difference between the saturated weight and the oven-dry weight. (Piper 1966). pH and electrical conductivity were ascertained using pH meters (Jackson 1967) and conductivity meters (Basak 2000). Olsen's approach (In this method, soil was treated using a 0.5 M sodium bicarbonate solution (pH 8.5) to extract the inorganic phosphate that was available to plants. The phosphorus that was extracted, was subsequently quantified through colorimetric analysis, typically by producing a blue colour with the molybdate reagent) and the Walkley and Black method (Jackson 1967) [In this approach, potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) was used to oxidize soil organic matter in the presence of concentrated sulfuric acid, which produced heat and helped

in the oxidation process. The remaining dichromate was subsequently titrated with ferrous ammonium sulphate, allowing for the calculation of organic carbon based on the dichromate that has been consumed] were used to detect phosphorus and organic carbon, respectively.

Alkaline potassium permanganate solution was used to measure the amount of nitrogen that was available, and the amount of ammonia that was released was also quantified. The soil was treated with 1N ammonium acetate to determine the amount of calcium, magnesium, and traces of sodium and potassium present. The versene titration method was used to determine the amount of calcium and magnesium in solution [In the Versene titration method, a specific aliquot of soil extract was taken, the pH was adjusted to 10 using an ammonium buffer, and Eriochrome Black T indicator is introduced; the solution was subsequently titrated with standardized EDTA until the colour shifted from wine-red to blue, indicating the total calcium and magnesium content. In a separate aliquot, the pH was elevated to approximately 12, murexide indicator was added, and the solution was titrated with EDTA from pink to purple, determining the calcium content, while the magnesium content was derived by subtracting the calcium value from the total calcium plus magnesium measurement.], and the Systronics flame photometer model (Systronics fame photometer 128) was used to determine the amount of sodium and potassium present (Jackson 1967).

3.1.2 Metagenomic analysis of the soil rhizosphere of *Cucurbita maxima* and *Zea mays* during the three cropping periods, namely: Initial, Peak and Intercrop (YEAR 1+YEAR 2)

The soil samples were collected during the initial, peak and intercrop phase from the soil rhizosphere of *Cucurbita maxima* and *Zea mays* and the metagenome analysis was done.

Fast QC v.0.11.9 was used to evaluate the quality of the raw data (default parameters). BBDuk v.38.86 was used to preprocess the data (parameters: qtrim=rl trimq=18 minlength=100 minavgquality=20). After filtering, Fast QC was used to re-evaluate the cleansed data. DADA2 and QIIME2, which were implemented in the nf-core framework and support data analysis, adhered to FAIR principles, which were used to process pre-processed Illumina paired-end reads through the amplicon sequencing analysis pipeline (ampliseq v1.1.2). First, Fast QC was used to analyse the raw data quality control, and Multi QC was used to summarize the results. Using the cut adapt plugin, primer fragments of matching sequences were eliminated. The DADA2 plugin was then used to quality filter, denoise, merge, and eliminate the chimera from the sequences.

After downloading and extracting the sequences flanking the forward [CCTAYGGGRBGCASCAG] and reverse [GGACTACNNGGGTATCTAAT] primers, the QIIME2 Naïve Bayes feature classifier was trained. The fitted classifier was used to classify the representative sequences by taxon. Standard parameters were used to run the nf-core ampliseq pipeline, with DADA2 quality settings using the --truncclenr options of 245 and 242, respectively. Since ASVs labelled as mitochondria or chloroplasts are generally regarded as undesirable, they were eliminated using nf-core/ampliseq. The taxonomic taxonomy was used to compute relative abundance. A bar plot was used to plot the taxa's relative abundance in relation to their samples.

The Ampvis2 R package was used to plot the heatmap. The quality of quantified DNA was confirmed on 1% agarose gel (Zhang *et al.* 2020). In this process, about 2 µl of DNA was mixed with 2 µl of 6 times loading dye (Invitrogen) and subjected to electrophoresis at 120 V for tentatively 30 min (Malla *et al.* 2022). Qubit 4.0 fluorometer was used for quantification of final library (Seshadri. 2020). To identify the insert size of library, query was placed in Tapestation 4150 (Freeman *et al.* 2022). Finally, paired-end sequencing was performed with Illumina Novaseq 6000 platform with PE150 as sequencing parameter. Raw data quality assessment was performed using Fast QC v.0.11.9 (default parameters) (Andrews *et al.* 2010). The data was pre-processed using BBDuk v.38.86 (Kechin *et al.* 2017). Post filtering cleaned data were re-assessed using Fast QC. Data quality control analysis was done using Fast QC and summarized using Multi QC (Ewels *et al.* 2016).

3.1.3 Measurement of heavy metal contents in soil

The soil samples were oven dried at 50°C for a period of 24 hours, in order to remove moisture from soil. Analysis of heavy metals namely lead, chromium, cadmium, nickel and metalloid like arsenic was done by acid digesting the soils. Acid digestion of the soil samples was performed by treating 1 g of oven dried sample, with 15 mL of aquaregia, comprising of 35% HCl and 70% nitric acid in 3:1 ratio. The samples were digested at 70°C, and finally filtered using Whatman filter paper no. 42 (Maurya *et al.*, 2018). Atomic Absorption Spectroscopy (AAS) was used for probing the contents of heavy metal and metalloid in soil (Wong *et al.*, 2003)

OBJECTIVE 2:**3.2 To perform physical isolation, purification and characterization of potential microbes having dynamic role in soil development, and their role in mitigation of pollution (lead).****3.2.1 Isolation**

The isolation of bacterial strains was carried out via standard serial dilution methods followed by spread plating on Nutrient Agar medium. After initial observation of bacterial colonies, 12 bacterial isolates were selected based on their prevalence/ lingering nature, out of which five bacterial isolates namely DPP(A), DPP(B), DPP(C), DCP(A) and DCI(D) were selected on the basis of their persistence and prevalence in all the dilution plates. The names were conjured with the initials of the collection place, along with the first letter of crop name and collection time and an alphabetical indicator of the isolate.

3.2.2 General Characterization of isolated bacterial strains**3.2.2a Gram Staining and Colony morphology**

The pure cultures of the bacterial isolates DPP(A), DPP(B), DPP(C), DCP(A) and DCI(D) were first analysed for their colony morphologies. Then Gram staining was carried out following established procedures (Benson, 2001)

3.2.2b Antibiotic sensitivity

An antibiotic sensitivity assay of the five strains against common antibiotics was performed following the Kirby-Bauer disk diffusion assay, so that the antibiotic-resistant strains are not set free in nature. (Rath *et al.*, 2015).

3.2.2c Streaking of bacterial isolates on Blood Agar plates

The bacterial isolates were streaked on blood agar plates to check the toxicity levels of isolates, through degradation of haemoglobin. (Gerhardt *et al.*, 1994)

3.2.2d Presumptive identification of isolated bacterial strains

Presumptive identification of the bacterial strains was performed whereby the 5 selected strains were streaked on HI-Chrome Agar medium. The media was prepared using a standard protocol from HI-Media, colony colours were observed within 18 to 24 hours of incubation at 37°C (Alippi., 2019).

3.2.3. Molecular identification of the isolated strains

The phylogenetic characterization of the five isolates was done using the 16SrRNA gene sequences. 16S rRNA genes were amplified using the universal primers 27F and 1429R. Sequences, thus generated were used for BLAST analysis at NCBI database and 10 highest identity scores were matched for likely identification of the strain. On the basis of the identification, distance matrix and phylogenetic trees were constructed. Analyses were conducted using the Maximum Composite Likelihood model (Kimura, 1980). Evolutionary analyses were conducted in MEGA11. The sequencing results were submitted to NCBI GenBank database. [Kumar *et al.*, 2018., Kumar., 2015; Felsenstein., 1985., Kimura., 1980;].

3.2.4. Characterization of plant growth promoting properties

3.2.4. Assay for macro-micro nutrients

3.2.4a Nitrogen fixation: The streaking of bacterial isolates DPP(A), DPP(B), DPP(C), DCP(A) and DCI(D) were done on modified Jensen's media, where bromothymol blue was added as an indicator. The entire set up was incubated at a temperature of 37°C for 3–8 days. The alteration in coloration from greenish blue to dark blue and finally yellowish zones indicated the ability of the bacterial isolates to fix nitrogen (Jain *et al.*, 2017).

3.2.4b Potassium solubilization: The bacterial isolates DPP(A), DPP(B), DPP(C), DCP(A) and DCI(D) were streaked on modified Aleksandrow's medium that had bromothymol blue as an indicator, and was placed under incubation at 27°C for 3–6 days. The change in coloration if any would show the capability of the bacterial isolates to solubilize potassium (Verma *et al.*, 2016).

3.2.4c Phosphate solubilization: The bacterial isolates DPP(A), DPP(B), DPP(C), DCP(A) and DCI(D) were streaked on modified Pikovskaya's phosphate solubilizing agar medium comprising of bromothymol blue and were placed in incubator with the temperature being maintained at 28°C $\pm$ 0.2°C for 6 days (Joe *et al.*, 2018). The quantification of phosphate was done, whereby 0.1ml of bacterial isolate was inoculated into 10ml of autoclaved Pikovskaya broth. The set up was incubated at 28 °C $\pm$ 2.0 °C, for a time period of 8 days. The inoculated broth was centrifuged at 10,000 rpm for a time period of 20 minutes. Next, 0.1ml of the supernatant was mixed with 0.25ml of Barton's reagent [Composition: - Solution A: 25 grams of ammonium molybdate was dissolved in 400ml of distilled water. Solution B: 1.25 grams of ammonium metavanadate was added to 300ml of boiling distilled water and allowed to cool, after which 250ml of concentrated HNO₃ was incorporated. Solutions A and B were combined, and the total volume was adjusted to 1000ml with

distilled water]. The set up was allowed to stand for a time period of 10 minutes and the concentration of the phosphate was analysed using UV–VIS Spectrophotometer at 430 nm.

A standard curve of phosphate was prepared, whereby 0.02195 grams of potassium dihydrogen orthophosphate (dried at 60 °C for one hour and then were consequently cooled in desiccators) were dissolved in 100 ml of double-distilled water (and labelled as stock P solution) in order to create a standard curve. A stock solution of 15ml was prepared and was further diluted. Then the volume was increased to 25 ml using double distilled water and designated as the working solution. Each flask was filled with aliquots of 0.2, 0.3, 0.5, 0.8, 1.0, 1.5, 2.0, 2.5, and 3.0 ml of working solution and 0.25 ml of Barton's reagent. The volume was then adjusted to 5 ml using distilled water. The intensity of the yellow colour that appeared after 10 minutes was measured spectrophotometrically at 430 nm.

3.2.5. Plant Growth regulator production

3.2.5a Indole Acetic Acid

Every pure isolate that was obtained had its capacity to produce IAA assessed. Tryptic Soya Broth (TSB) liquid media (20 ml) comprising of L tryptophan (200 µg/ml) was used to measure the presence of IAA within the bacterial isolates. The bacterial isolates DPP(A), DPP(B), DPP(C), DCP(A) and DCI(D) were inoculated into the TSB medium and kept in shaking conditions at 37°C for 72 hours. Then they were centrifuged at 8000 rpm for a time period of 10 minutes. Four ml of Salkowski reagent (dissolved in 2% 0.5M FeCl₃ in 35% perchloric acid) was combined with one ml of supernatant, and the mixture was then kept steady at room temperature for twenty minutes. A shift in hue to pink signified the synthesis of indole. The spectrophotometer was set at 530 nm and the amount of IAA produced was measured from the absorbance of the supernatant. The IAA standard curve was then used to calculate it. Uninoculated TSB media with Salkowski's solution served as the blank treatment. Three replicates for each treatment were made.

IAA standard curve was charted. In this process, in a test tube, 10 ml of IAA standard of 100 ppm was diluted into 80 ppm, 40 ppm, 10 ppm, and 5 ppm concentrations. A test tube containing two ml of each concentration was filled with two ml of Salkowski reagent (ratio 1:1). After that, it was left to incubate at room temperature for a time period of sixty minutes. The solution turned pink after incubation. A standard curve was drawn to illustrate the connection between the absorbance (y) of IAA generation and the concentration of the standard IAA solution (x) after the absorbance of the IAA standard solution was measured at 530 nm. IAA's production yield is expressed in µg/ml (Suliasih *et al.*, 2020).

3.2.5b Gibberellic acid (GA₃)

Colorimetric estimation of gibberellic acid (GA₃) was done by a modified method of Hollbrook *et al.*, 1961 and Sharma *et al.*, 2017. After five days of incubation of the bacterial isolates DPP(A), DPP(B), DPP(C), DCP(A) and DCI(D) in a 50 ml Erlenmeyer flask with 10 ml of nutrient broth media, bacterial culture supernatant was extracted using centrifugation at 10,000 rpm for 15 minutes. After that, 40 ml of distilled water were used to dilute the sample. A volume of 1ml of glacial acetic acid along with 2 ml of zinc acetate reagent (21.9 grams of zinc acetate was combined, and the volume was made up to 100 ml using distilled water). Following two minutes of incubation, 2ml of potassium ferrocyanide (10.6% in distilled water) was added. Addition of distilled water was done to bring the volume down to 50 ml. The mixture was left to stand for five

minutes at room temperature. Using a 0.45 μ M milipore syringe filter, the contents were filtered. Eight ml of 100% alcohol were added to 100 ml volumetric flasks containing a 10-ml aliquot of each sample's filtrate. After adding equal amounts of 30% HCl to the aliquot, it was incubated at 20 °C for 75 minutes and measured at 254nm. Five ml of 5% HCl were used to set up the blank reading. For the culture set estimated after seven days of incubation, the identical procedure was carried out.

3.2.6. Estimation of abiotic stress-responsive enzyme

Qualitative evaluation of ACC Deaminase (ACCD):

The ACC deaminase activities of the bacterial isolates DPP(A), DPP(B), DPP(C), DCP(A) and DCI(D) were examined. After being freshly cultured in Nutrient broth, the bacterial cultures were centrifuged for 8 minutes at 10,000 rpm. The supernatant was discarded and the pellets were washed twice with sterile 0.1 M Tris-HCl (pH 7.5). The modified Dworkin and Foster (DF) minimal salts agar medium was used [DF salts per litre consist of: 4.0 g of KH_2PO_4 , 6.0 g of Na_2HPO_4 , 0.2 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 2.0 g of glucose, 2.0 g of gluconic acid, and 2.0 g of citric acid, along with trace elements including: 1 mg of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 10 mg of H_3BO_3 , 11.19 mg of $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 124.6 mg of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 78.22 mg of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, and 10 mg of MoO_3 , maintaining a pH of 7.2.]. This media was amended with 3 mM ACC as the only nitrogen source. DF minimum media augmented with 0.2% ammonium sulphate served as the positive control, whereas DF medium without any nitrogen source was prepared as the negative control. After being kept in the incubator for a time period of 72 hours at 30°C, the growth of the isolates on DF medium augmented with ACC was measured and contrasted with the control. Growth on ACC-modified DF plates was used to choose the ACCD producers. After 48 to 72 hours, the isolates developing on DF minimum medium were also streaked on nitrogen-free media, and growth of the bacterial isolates were observed (Maheshwari *et al.*, 2020).

Quantitative Estimation of ACC Deaminase:

The bacterial isolates DPP(A), DPP(B), DPP(C), DCP(A) and DCI(D) were inoculated in Dworkin Foster broth, centrifuged at 10,000 rpm for a time period of 5 minutes in order to evaluate the ACCD activity. The supernatant was discarded and the cell pellets were washed. The cell pellets were further resuspended in 600 μL 0.1M Tris HCl (pH 8.5). Toluene (30 μL) was added to labilize the cells. The mixture was vortexed for a time span of 30 seconds. Twenty μL of 0.5M ACC were added to 200 μL of tolueinized cell suspension, that was further incubated with 0.5M ACC at 30°C for 15 minutes. After incubation, 1 mL of 0.56 N HCl was added, centrifuged at 12,000 rpm for a time period of 5 minutes. HCl (0.56N) (800 μL) and freshly made 300 μL of DNPH reagent (0.1 g 2,4-dinitrophenyl hydrazine in 100 ml of 2 N HCl) were combined with 1 mL of culture supernatant, vortexed, and incubated for 30 minutes at 30°C. Following incubation, 2ml of NaOH (2N) was added, and a UV-Vis spectrophotometer (Thermo Scientific GENESYS 10S UV-VIS, Germany) was used to measure absorbance at 540 nm. The quantity of α -ketobutyrate generated

was ascertained by comparing it to a standard that ranged from 0.1 to 1 mM (Maheshwari *et al.*,2020)

3.2.7. Estimation of enzymes of bacterial isolates

3.2.7a Urease:

For qualitative test:

The qualitative test for urease was done using Christensen's urea agar [1 g of peptone, 5 g of NaCl, 1 g of dextrose, 2 g of KH_2PO_4 , 0.012 g of phenol red (6 ml of 1:500 solution), 20 g of urea, 15 g of agar in 1000 ml of distilled water] by streaking the bacterial isolates DPP(A), DPP(B), DPP(C), DCP(A) and DCI(D) on the slant surface of the test tube. The slant surface of the test tubes was checked for colour changes every twenty-four hours, till a time period of seven days. A bright pink (fuchsia) tint colour on the slant was a sign of urease production. A control medium devoid of urea is necessary to rule out protein hydrolysis as the reason for a positive test. (MacFaddin *et al.*,2000):

For quantitative estimation:

For quantitative estimation, a rapid spectrophotometric method was used following Onal *et al.*, 2013, whereby the overnight grown bacterial broth cultures were centrifuged at 150 rpm, washed and re-suspended in 0.01M PBS (phosphate buffer at pH 7.4). Resuspended bacterial cultures of 30 μl (O.D600 0.5) were inoculated in 300 μl of freshly prepared urea broth in a 96-well microtiter plate. The rate of colour changes from yellow to pink due to hydrolysis of urea over time, was measured at 430nm and 560nm at an interval of 24 and 48 hours (Bogale *et al.*,2021).

The rate of colour change was calculated by the formula:

T1= 430nm, T2= 560nm

Rate of colour change = (Absorbance at T2 – Absorbance at T1)/ T2–T1

3.2.7b Cellulase:**A) For qualitative estimation:**

The bacterial isolates DPP(A), DPP(B), DPP(C), DCP(A) and DCI(D) were streaked on the Cellulose Congo-Red media and the colonies showing discoloration of Congo-Red were considered to be cellulase positive. The composition of the media was 0.5 g of KH_2PO_4 , 0.25 g of MgSO_4 , 2 g of cellulose, 15 g of agar, 0.2 g of Congo-Red, and 2 g of gelatin; dissolved in 1 L of distilled water, and a pH of 6.8–7.2. By streaking on cellulose Congo Red agar media, the bacterial isolates' capacity to degrade cellulose was confirmed. A quick and accurate screening method for cellulolytic bacteria is based on the use of Congo-Red as an indicator for cellulose degradation in an agar medium. The colonies exhibiting Congo-Red discoloration to clear zones were selected for further research since they were cellulose-degrading bacterial colonies (Gupta *et al.*, 2012).

B) For quantitative estimation:

The amount of reducing sugar generated from filter paper was measured in order to calculate the total cellulose activity. Endoglucanase activity (EC 3.2.1.4, β 1-4 endoglucanase) of the bacterial isolates was determined. The amount of reducing sugar from amorphous cellulose was measured in order to assess activity. By incubating 0.5 ml of supernatant with 0.5 ml of 2% amorphous cellulose in 0.05 ml sodium citrate buffer (pH 4.8) at 50°C for 30 minutes, endoglucanase activity was ascertained.

Three ml of 3, 5-dinitrosalicylic acid (DNS) reagent were added to one ml of reaction mixture to stop the reaction after it had been incubated for an hour at 50°C. Using glucose as a standard, reducing sugars were quantified spectrophotometrically using 3, 5-dinitrosalicylic acid. International units (IU) were used to measure the enzymatic activity of endoglucanase. The quantity of enzyme that releases one μmol of reducing sugars (measured as glucose) per ml per minute is known as one unit of enzymatic activity (Gupta *et al.*, 2012).

3.2.7c. Laccase:

A) For qualitative estimation:

The bacterial isolates DPP(A), DPP(B), DPP(C), DCP(A) and DCI(D) were streaked on Nutrient agar plates supplemented with 0.5mM guaiacol, and incubated at 30°C for 96 hours (Sheikhi *et al.*,2012). The bacterial isolates showing change in the colour of bacterial isolates to dark brown, followed by formation of decolorization zone were considered to be laccase positive.

B) For quantitative estimation:

The Bacterial cultures DPP(A), DPP(B), DPP(C), DCP(A) and DCI(D) were centrifuged at 10,000rpm for a time period of 20 minutes at 4°C. The supernatant was discarded and the pellets were washed with 0.1M phosphate buffer maintained at a pH 6.5. The buffer had 10 mM of phenylmethanesulphonyl fluoride (PMSF), that was added to inhibit the protease activity in the supernatant before sonication (5 times, 45 seconds each time with 30 seconds interval, 20 MHz). The cell extract was obtained by centrifugation (10,000 rpm) at 4 °C for 20 min and used as crude intracellular enzyme. To eliminate the potential impact of H₂O₂ generated by the bacteria, catalase (1,000 U/mL; Sigma-Aldrich) was added to the assay solution and incubated for one hour at 37°C. The laccase activity was measured spectrophotometrically at 436 nm, using 2,2-azino-di-[3-ethylbenzo-thiazolin-sulphonate] (ABTS) as a substrate. The quantity of enzyme that oxidized one mole of ABTS per minute at 25°C was known as one activity unit (U), and the activities were reported in U/L. Using 2 mM guaiacol as a substrate in a reaction mixture with 50 mM phosphate buffer (pH 6.5), laccase activity was also measured spectrophotometrically at 465 nm. One unit of enzyme activity was defined as the quantity of enzyme that elevated the absorbance by 0.001 units per minute at 55°C (Sheikhi *et al.*,2012).

3.2.7d. Lignin peroxidase:

A) For qualitative estimation:

The bacterial isolates DPP(A), DPP(B), DPP(C), DCP(A) and DCI(D) were screened on Luria Bertani agar plates containing NaCl: 1g/l, tryptone: 2g/l, yeast extract: 1g/l, agar: 2g/l, and methylene blue dye: 0.25g/l, was used as an indicator. The plates were kept in the incubator at 30 °C for a time period of 72 hours. The bacterial isolates showing decolorization zone were considered to be lignin peroxidase positive (Rath *et al.*,2022).

B) For quantitative estimation:

One ml of culture broth of each bacterial isolates were centrifuged at 4°C at 7000 rpm. In 1ml of 50mM sodium potassium tartrate (pH-4) buffer, 0.1ml of 0.1mM H₂O₂ inducer was added. Then to this solution, 32µM methylene blue and 10 µl of enzyme supernatant were added. The final set up was incubated for 1hour at 37°C and absorbance was measured at 650nm (Rath *et al.*,2022).

The results were represented as the percentage of decolourization of methylene blue dye, exhibited by the enzyme lignin peroxidase in comparison with the control set up. The calculation was done using the formula:

$$[(A_{650}\text{CONTROL}-A_{650}\text{TEST})/ A_{650}\text{CONTROL}] \times 100$$

A₆₅₀ = Absorbance at wavelength 650 nm.

3.2.7e. Amylase

A) For qualitative estimation:

Bacterial isolates DPP(A), DPP(B), DPP(C), DCP(A) and DCI(D) were streaked on starch agar [beef extract: 3g/l, soluble starch 10g/l, agar (2%)] and incubated at 37 °c, at pH 7 for 7 days. Next, the plates were flooded with Gram's iodine. Bacterial isolates showing formation of clear zone surrounding the bacterial isolates were considered to be amylase positive (Luang *et al.*,2019).

B) For quantitative estimation:

The DNS (3,5-dinitrosalicylic acid) method was used to determine the amylase activity of each bacterial isolate; one mL of crude enzyme was mixed with 11% starch solution in 0.05 M sodium phosphate buffer. The samples were incubated at 37°C for 30 minutes. After incubation, 0.5 mL DNS solution was added to each sample to stop the reaction and boiled at 100°C for 5 minutes in the water bath. The enzyme activity was measured at 575 nm. The observed values were compared to the blank solution. The OD value at T₃₀ min. was consequently subtracted from T₀ min, considering that glucose persisted in the sample at T₀ min. The glucose standard curve was used to determine the amount of glucose generated for each solution. Amylase activity was computed using the value obtained from glucose determination. Under test circumstances, one unit of amylase activity is equal to one µmol of glucose per minute, which is the quantity of amylase needed to catalyse the synthesis of reducing sugar (Luang *et al.*,2019).

3.2.7f. Pectinase

A) For qualitative estimation:

Pectinase Screening Agar Medium (PSAM) was prepared. It comprised of NaNO₃: 2g/l, KCl: 0.5g/l, MgSO₄ 0.5g/l, K₂HPO₄ 1g/l, tryptone 0.5g/l, agar (2%) and pectin: 10g/l. PSAM was poured in petri plates and bacterial isolates DPP(A), DPP(B), DPP(C), DCP(A) and DCI(D) were streaked on the medium. They were incubated at 37°C for 24 hours and checked for clear zone formation (Bibi *et al.*,2018).

B) For quantitative estimation:

Measurement of pectinase activity was done using 0.5% pectin dissolved in 0.1M Glycine buffer and 0.3ml of crude enzyme. The reaction mixture was placed in the shaking water bath at 70°C for 10 minutes. One ml of Di Nitro Salicylic Acid (DNS) was added to the mixture and boiled in water bath 100°C for 10 minutes, and measured at 575 nm (Bibi *et al.*,2018).

3.2.8 Analysis of Metallothionein content:

Tryptone glucose broth was prepared, that was augmented with $\text{Pb}(\text{NO}_3)_2$, concentrations of values 200 mg/l and 500mg/l. The broths were inoculated with bacterial isolates DPP(A), DPP(B), DPP(C), DCP(A) and DCI(D), which were further incubated in the shaker at 37°C for a time period of 48 hours. Next the broths were centrifuged at 10,000 rpm for 20 minutes and was further solubilized in lysis buffer (0.5 M sucrose, 20 mM Tris-HCl buffer, pH 8.6, having 0.01% mercapto-ethanol as solvent). The pellets obtained after centrifugation, were sonicated for a time period of 2 minutes (45 seconds each time with 30 seconds interval, 20 MHz). After sonication, the samples were centrifuged at 10,000 rpm for a time period of 20 minutes, to acquire a supernatant comprising of metallothionein. For every 1 ml of the resultant supernatant, 1.05 ml of cold (-20°C) absolute ethanol and 80 µl of chloroform were added. Three times the volume of cold ethanol were added to the supernatant after the samples were centrifuged at 4°C at a speed of 8000 rpm for 10 minutes. The samples were then refrigerated at -20°C for one hour. The samples were centrifuged once more for 10 minutes at 8000 rpm. After being cleaned with ethanol, chloroform, and homogenization buffer (87:1:12), the resultant pellets were centrifuged once more for ten minutes at 8000 rpm for a time period of 10 minutes. The pellets were further dried and resuspended in a mixture of 5 mM Tris-HCl, 1mM Ethylene-diamine-tetra-acetic acid (EDTA) at pH 7. The resuspended metallothionein's were added to a mixture of 4.2 ml of 0.43 mM 5, 5'-dithiobis (nitrobenzoic acid), dissolved in 0.2 M phosphate buffer at pH 8 and was placed at room temperature for a tenure of 30 minutes. Finally, the quantity of reduced sulfhydryl was assessed at an absorbance of 412 nm using spectrophotometer. Accurate quantification of metallothionein in the bacterial samples were done by using a standard reference curve of glutathione (GSH). Since GSH only contains one cysteine per molecule, it served as a standard for measuring cysteines in protein analyses. At 412 nm, the absorbance of solutions with different concentrations of GSH was measured. The amounts of metallothionein in the samples were estimated using the GSH standard, assuming that 1 mol of MT contains 20 mol of cysteine (Murthy *et al.*,2011).

3.2.9 Determination of HCN production:

A) Qualitative Analysis:

The bacterial isolates DPP(A), DPP(B), DPP(C), DCP(A) and DCI(D) were streaked on Kings B media (K_2HPO_4 : 20g/l, $MgSO_4 \cdot 7H_2O$: 1,5 g/l, Peptone: 2 g/l, glycerol: 15ml/l, agar: 2%, distilled water), that was supplemented with glycine at 4.4g/l. Picric acid solution having a composition of 2.5 g of picric acid; 12.5 g of Na_2CO_3 and 1000 ml of distilled water was prepared. The filter papers were made sterile and dipped in picric acid solution, and consequently was placed on the underside of the upper lid of the petri plate. Then, they were sealed using parafilm strip and kept in the incubator at 28°C for a time period of 48 hours. A change in the colouration from yellow to light brown or brown to reddish brown was considered to be HCN positive (Reetha *et al.*, 2014).

B) Quantitative Analysis:

The bacterial isolates DPP(A), DPP(B), DPP(C), DCP(A) and DCI(D) were inoculated in Kings B broth, that was modified using glycine 4.4g/l, and similar size strips of sterile filter paper were immersed in alkaline picrate solution. They were hung inside a conical flask, and incubated at $28 \pm 2^\circ C$ for a tenure of 48 hours. The change in colouration of sodium picrate to reddish colouration signified the quantity of production of HCN, that was eluted in 10ml of distilled water present within a test tube. The absorbance was measured at 625nm (Reetha *et al.*, 2014).

3.2.10 Determination of Ammonia production:

Qualitative Analysis:

The presence of ammonia in the bacterial isolates DPP(A), DPP(B), DPP(C), DCP(A) and DCI(D) were tested by inoculating the bacterial isolates in peptone broth (4%) for a duration of seven days at 30°C followed by the addition of 0.5ml of Nessler's reagent. The change in colouration from brown to yellow, indicated the presence of ammonia (Bhattacharyya *et al.*, 2020).

Quantitative Analysis:

The absorbance of the ammonia concentration within the bacterial isolates was measured at 450nm and was correlated with a standard curve produced with a solution of ammonium sulphate. (Bhattacharyya *et al.*, 2020).

3.2.11 Estimation of iron chelating compounds

3.2.11a Estimation of Siderophore content

One of the methods of siderophore detection in bacterial isolates was by using the O-CAS method (Pérez *et al.*, 2007). CAS medium was prepared following the standard protocol as per Schwyn *et al.* (1987). The medium for overlay comprised of Chrome azurol S (CAS) 60.5 mg/l, hexadecyl trimethyl ammonium bromide (HDTMA) 72.9 mg/l, Piperazine-1,4-bis (2-ethanesulfonic acid) (PIPES) 30.24 g/l, and 1 mM FeCl₃·6H₂O in 10 mM of HCl, Agar (2%). In O-CAS method, the bacterial isolates DPP(A), DPP(B), DPP(C), DCP(A) and DCI(D) were inoculated as spots on the media and overlaid with CAS agar. After a duration of 30 minutes, observation of colour changes in the overlaid medium encircling the bacterial isolate confirmed the siderophore production by bacterial isolates. The quantitative estimation of the siderophore content in the bacterial isolates were done in the similar process, whereby the bacterial isolates were inoculated in Luria Bertani broth at 30°C, centrifuged at 120 rpm for 72 hours and spectrophotometrically screened for siderophore production.

Percent siderophore production (psu) = $(Ar - As) \times 100 / Ar$;

Ar = absorbance of CAS solution at 630nm

As= absorbance of sample, CAS solution and cell free supernatant at 630nm

3.2.11b Analysis of the type of siderophore

The selected strains were tested for understanding the type of siderophore they were producing. The hydroxamate type of siderophore was detected by using Tetrazolium salt. This test is based on hydroxamic acid's capacity to hydrolyse the hydroxamate group in the presence of a strong alkali, causing the lowering of tetrazolium salts. The cell free supernatant would instantly become a deep red hue when tetrazolium salt and NaOH were added, which was considered as a proof of a hydroxamate siderophore, while the catecholate nature of siderophore was detected by following the standard Arnow's Method, whereby in association with nitrous acids, alkali along with a molybdate, it would generate pink chromogen (Radhakrishnan *et al.*, 2014).

3.2.12 Analysis of Lead tolerance by bacterial isolates

3.2.12a Analysis of percentage of lead uptake by bacterial isolates

Bacterial isolates were grown in media with lead. Bacterial biomass was centrifuged, and the supernatant was analysed for the residual concentration of metal using flame atomic absorption spectrophotometer (PinAAcle 500). For each of the setup, three replicates were prepared. The capacity of the biosorption by the bacterial isolates were determined as per the protocol mentioned in Oves *et al.* (2012) and was calculated using the formula:

$Q = [V (C_i - C_f)]/M$ whereby,

Q = metal uptake capacity (mg/g)

V = Volume of the solution

C_i = Initial concentration of metal in the solution before biosorption (mg/g)

C_f = Final concentration of metal in solution after biosorption (mg/g)

M = weight of the biomass.

3.2.12b Analysis of percentage of lead adsorbed by bacterial isolates:

The quantity of lead associated with bacterial cells was done using Atomic Absorption Spectroscopy. The bacterial cultures were grown in Luria Bertani (LB) broth overnight, comprising of the IC₅₀ (half maximal inhibitory concentration) value of lead specific for each bacterial isolate. They were incubated at 37°C in shaker at 150 rpm for approximately 18 hours. The bacterial cultures were centrifuged at 3000 rpm for 30 minutes at a temperature of 4°C. The cellular pellets were then lyophilized and acid digested (nitric acid and HCL in the ratio of 3:1) as per the specifications mentioned in Rodriguez *et al.* (2017).

$[(C_i - C_f)/C_i] \times 100$ whereby,

C_i = Initial concentration of metal in the solution (mg/l)

C_f = Final concentration of metal in solution (mg/l) (Parungao *et al.*, 20

OBJECTIVE 3**3.3 To prepare an ideal consortium to be supplemented all through the year suitable for any crop, either singly or with chemical fertilizers (in-vivo study).****Testing of the efficacy of the formulation under in vivo conditions.****3.3.1. Interaction study between the bacterial strains**

The interaction between the selected strains were assessed by streaking the isolates using modified cross streak plate method (Velho-Pereira *et al.*,2011).

3.3.2 Pathogen isolation from the leaves of *Zea mays*, followed by their interaction with bacterial isolates:

Diseased leaves of *Zea mays*, were collected from the Chowbaga regions of East Kolkata wetlands, and the fungal pathogen were isolated following the standard isolation procedure. The diseased leaves of the plants were collected and kept at a temperature of 4°C in a sterilized, air-tight zip lock bag. Sterilized scalpel was used to randomly cut 1cmx1cm infected pieces from the diseased portions of the leaves in accordance with conventional isolation procedures. After immersing the samples in 70% ethyl alcohol for two to three seconds, the infected pieces were surface sterilized by dipping them in a HgCl₂ solution [0.1% HgCl₂(w/v) in autoclaved distilled water] for 30 seconds. The sterilized leaf pieces were cleaned in sterile distilled water and patted dry using sterile blotting paper. Potato dextrose agar (PDA) plates augmented with streptomycin (HIMEDIA Streptomycin sulphate (TC035-5G) of 100 ppm concentration) were prepared. The surface sterilized leaf pieces were placed on the PDA, and incubated at a temperature of 28°C in a B.O.D incubator. The plates were kept under observation till the appearance of the pure fungal culture. The colony growth pattern of the fungal culture, was observed followed by their colour, filament shape and spore morphology were examined in a Dewinter compound light microscope. Spore size was estimated by an average of 10 spore sizes (Dhar *et al.* 2024).

The interaction study between bacterial isolates DPP(A), DPP(B), DPP(C), DCP(A) and DCI(D) and fungal strains was carried out following a modified protocol as mentioned in Slama *et al.*,2019. In this process, 5 mm agar disc from previously grown fungal lawns on the PDA plates of the isolated fungal strains were cut using sterilized cork borer and forceps and placed on a combination of Potato Dextrose Agar (PDA) and Nutrient agar plate (1:1) that had been spotted with 20 µl of bacterial suspension (10^6 cells/ml) at four equidistant corners of the plate, surrounding the centre.

The setup was incubated 28°C for a week and spread of bacteria over the fungal mycelia were regularly monitored.

3.3.3. Standardization of Treatment Mode

The treatment dosage standardization study was conducted by procuring seeds of *Cucurbita maxima* (Variety: Jack 323) and *Zea mays* (Variety :4226). The treatment dosages were standardized by following Roy *et.al.*, 2023 and Chakraborty *et al.*, 2013.

Two bacterial isolates namely DPP(B) and DCP(A), that were selected on the basis of their PGPR results were inoculated in Nutrient broth, that was sterilized by autoclaving. The inoculated broth was incubated at 37°C for 24 hours in shaking conditions. The dosage for a single plant was designed as per the following composition: Ten ml of 24-hour-old culture half diluted to form 20 ml of treatment

For first mode of application, the bacteria were cultured in Nutrient broth and the half-diluted culture was then directly mixed with water and applied around the plant rhizosphere. For the second mode of application, water suspension treatment was used, whereby, the bacterial isolates DPP(B) and DCP(A) were centrifuged at 10,000 rpm for a time span of 30 minutes. The supernatant was discarded and the pellet was dissolved in water. The pellets obtained from 20 ml volume of 24-hour culture was dissolved in 40 ml of water to get the half dilution. The treatment was applied to the plants after 5 weeks of growth.

Mode of Application: 1 – (Garden soil + Bacterial consortia application)

Crops tested: *Cucurbita maxima* and *Zea mays*

In separate pots, a single plant of each crop was placed and the dosage for each plant was designed, whereby 20ml treatment of bacterial consortia mixed directly with water was applied in each pot. The upper layer of the soil was loosened without creating any damage to the roots and the bacterial consortia treatment, directly mixed with water was added to the pots encircling the stem portion of the plants.

Mode of Application:2 – (Garden soil + Water suspension treatment of Bacterial consortia)

Crops tested: *Cucurbita maxima* and *Zea mays*

One plant of each crop was placed in separate pots, and the dosage for each plant was determined by applying 20 ml of bacterial consortium, whereby upon centrifugation, only the pellets were mixed directly with water and applied to each pot (water suspension treatment). The treatment was applied carefully to the plant rhizosphere, encircling the stem portion.

Data collection and determination of the best mode of treatment.

The data on plant length, leaf number, leaf length, leaf width were noted on a weekly basis after the application of the treatment application. The best treatment was selected on the basis of the obtained data, which was used in the further set of experiments

In-vivo field trial of *Cucurbita maxima* (Variety: Jack 323) and *Zea mays* (Variety:4226)

The in vivo-field trial was conducted at Chowbaga field of East Kolkata Wetlands. The method of administration was determined from the outcome of the results obtained from the trial study. The bacterial strains DPP(B) and DCP(A) were applied together as a consortium, on test crop *Cucurbita maxima* (Variety: Jack 323). The treatment was carried out between the months of December - February and similar treatment was applied to *Zea mays* (Variety :4226) on the same field during the months of March-June. During each of the field trial study, a total of 20 plants of each crop was taken, whereby 10 plants were taken in 1 plot and administered with the treatment and the remaining 10 plants were taken in the adjacent plot, where no treatment was applied, and was thus referred to as the control set-up. The plant-to-plant distance for was 80cm (Molina-Romero *et al.*,2021) In the water suspension treatment administered to the treated plants in Chowbaga area, cell pellets of bacterial isolates DPP(B) and DCP(A) obtained upon centrifugation, were dissolved in water and was used as the mode of treatment. Bacterial suspensions (24 hours old, with optical densities of the cultures were 0.7-0.8 at OD 600nm containing 10^{6-7} CFU per ml of bacterial culture), diluted to 20 ml with water, was applied to each plant in the rhizosphere region circumscribing the stem region of the plants. The first treatment was applied at the end of 4th week of the plant growth (Average length of *Cucurbita maxima* plant was 25 cm, *Zea mays* plant was 50 cm) and then 2 more treatments were applied at 1month interval, for a total number of three times in the 90 days lifespan of the plant. (Roy *et.al.*, 2023). Physical data of the plant physiological parameters of both *Cucurbita maxima* and *Zea mays* was collected every week interval with respect to a control where no treatment was applied. The yield parameters of *Cucurbita maxima* and *Zea mays* were documented once the fruiting was completed

3.3.4 Plant Pigments

Chlorophyll content of the plant leaves of both *Cucurbita maxima* and *Zea mays* was determined according to Porra *et al.* (1989) method. For the measurement of chlorophyll content, plant leaves of 0.5 g were put into test tube containing 10 ml of methanol and kept in dark for 24 hours. After this the supernatant solution from each test tube was taken and the absorbance was measured at 470 nm, 652 nm and 665 nm with the help of dual beam UV visible spectrophotometer (Thermo Fisher Scientific) (Pradhan *et al.*, 2014)

Calculation ($\mu\text{g/ml}$):

$$\text{Chl a} = 16.29 A_{665} - 8.54 A_{652}$$

$$\text{Chl b} = 30.66 A_{652} - 13.58 A_{665}$$

$$\text{Chl (a+b)} = 22.12 A_{652} + 2.71 A_{665}$$

$$\text{Total carotenoid} = (1000 A_{470} - 2.86 \text{ Chl a} - 129.2 \text{ Chl b}) / 245$$

3.3.5. Analysis of lead content of soil

Absorption Spectroscopy (AAS) was used for probing the contents of heavy metal and metalloid in soil. In this process, samples of soil were powdered, thoroughly mixed, allowed to air dry at room temperature, and then stored in plastic (polyethylene) sealed lock bags for further metal analysis. Soil samples were digested using acid hydrolysis. A sample of 1 g of soil was broken down using the wet acid digestion method using HNO_3 and HCl in a 3:1 (v/v) ratio, and the mixture was heated on the surface of a hot plate for two hours until it turned clear in order to measure the Pb content of the soil. After cooling, distilled water was added to get the volume up to 50 ml. After passing the solution through Whatman's filter paper number 42, atomic absorption spectrophotometer (PinAAcle 500) was used for detecting lead (Wong & Li, 2003).

3.3.6. Statistical analysis of the subsequent data Analysis of variance was measured for the parameters that were considered

The yield parameters for both *Cucurbita maxima* and *Zea mays* were subjected to statistical analysis using Python 3 software, where an Analysis of Variance (ANOVA) (Yan *et al.*,2025) was conducted to assess the significance of variation among various treatments and their interactions. This methodology allowed for the identification of the effects of treatments on crop performance concerning growth and yield characteristics. Furthermore, a time series analysis was performed on the plant parameters utilizing a linear trend model, which aided in the assessment of temporal changes and trends throughout different cropping stages. The combination of ANOVA with time series modelling thus offered a thorough understanding of both the variability induced by treatments and the dynamic development of plant growth over time (Parmar *et al.*,2024).

3.3.7 Soil Health, Enzyme and Microbial Activity upon addition of Bacterial Consortia

3.3.7a. Improvement in the Soil Health Parameters after application of bacterial consortia.

The soil samples were collected from the field in Chowbaga, where the field trial was done. Soils present in the close proximity of the plant (*Cucurbita maxima* and *Zea mays*) regions of approximately 4cm away from the plant stems and at a depth of approximately 5 cm below the ground surface were collected. The collected soil was then analysed for its different macro and micronutrient content, as well as other physical characteristics for both the test and control set up of *Cucurbita maxima* and *Zea mays*. The soil samples were collected in triplicates. The soil samples were collected in triplicates. Physico-chemical analysis of the soil parameters was done. The parameters included : pH , Electrical Conductivity (dsm-1) , Organic Carbon (%), Nitrogen (kg/ha) , Phosphate (kg/ha) , Potassium(kg/ha) , Calcium (mg/kg) , Magnesium (mg/kg) ,Sulphur (mg/kg) ,Boron (mg/kg) , Copper (mg/kg),Zinc (mg/kg),Iron (mg/kg), Manganese(mg/kg), Cation Exchange Capacity (meq/100 g) . Electrical conductivity and pH were determined by conductivity meter (Basak 2000) and pH meters, respectively. Organic carbon and phosphorous were measured using Walkley and black method (Jackson 1967), as described in **section 1.1**

3.3.7b. Soil Enzyme Profiling

The soil samples of both treated and control set up plots were analysed for Beta-D Glucosidase, Amidase, Urease, Phosphatase and sulphatase was done.

Analysis of Beta-D Glucosidase activity in soil was done by extraction and colorimetric analysis of the p-nitrophenol released, when 1 g of soil was incubated with 5 ml of buffered p-nitrophenyl glycoside solution at 37°C for 1 hour. The reagents [0.5 M CaCl₂ and 0.1 M Tris (hydroxymethyl)aminomethane THAM, pH 12] were utilized for the extraction of p-nitrophenol (Eivazi *et al.*,1988).

Soil amidase activity pertains to the speed at which enzymes present in the soil decompose amide compounds, a vital process for the nitrogen cycle. The amidase activity was measured using the indophenol method at 630nm (Frankenberger *et al.*,1981; Kay *et al.*,2000)

Urease activity of the soil was measured by incubating soil with a solution of aqueous or buffered urea, extracting ammonium using 1 N KCl and 0.01 N HCl, and determining NH₄⁺ colorimetrically through a modified indophenol reaction (Kandeler *et al.*,1988).

Phosphatase activity of the soil was measured by colorimetric assessment of p-nitrophenol liberated through phosphatase activity when soil is incubated with a buffered sodium p-nitrophenyl phosphate solution at pH 6.5 and toluene at a temperature of 37° C for a duration of 1 hour (Tabatabai *et al.*,1969).

Sulphatase activity of the soil was examined, whereby 1 g of soil was pre-incubated for 1 hour at 20°C with 0.2 ml of toluene to suppress enzyme activity from microbial growth and new enzyme production. Subsequently, 4 ml of 0.5 M sodium acetate buffer (pH 5.8) and 1 ml of 0.05 M p-nitrophenyl sulfate were introduced, and the mixture was incubated for 1 hour at 37°C. The reaction was halted by cooling to 0°C in an ice bath, and the samples were centrifuged at 10,000 rpm for 10 minutes to obtain the supernatant. Three ml of the supernatant liquid were mixed with 2 ml of 0.5 M NaOH, and the absorbance of the yellow product was recorded at 400 nm using UV Vis Spectrophotometer (Whalen *et al.*,1996).

3.3.7c. SYBR Green Staining and Fluorescence Microscopy of Soil and Plant Samples

The soil sample was incubated and sonicated on ice for 1 min before incubation with the Sybr green dye, then the sample was pipetted into a FinStar staining reactor containing 4 ml of phosphate buffered saline PBS pH 7, following filtration with (Whatman filter paper 25-mm diameter). The liquid sample was washed twice with 4 ml PBS buffer (pH 7). Then the filter was coated with 1.5 ml of Sybr green solution in pH 7 PBS buffer following the incubation for 15 min. Then the filter paper was mounted into a drop of immersion oil on a microscope slide after drying and a drop of immersion oil was put on the surface. The sample was viewed under blue excitation light (Nikon B-2A, excitation 450–490 nm, dichroic mirror 50). Root pieces of both treated and control samples of *Cucurbita maxima* and *Zea mays* were taken for preparing transverse sections, and then sonicated. The same procedure was done similar to that of soil samples and have been applied for the analysis of root samples of plant parts. (Lunau *et al.*,2005).

3.3.8. Metagenomic Analysis of the Soil: The soil samples were collected from the control and treated soil of both the crops, namely, *Cucurbita maxima* and *Zea may*, when both the crops were approximately 60 days old (peak period). The soil samples were collected at a distance of approximately 4cm from the plant stem and at a depth of 5cm. The detailed process of metagenome analysis has been explained in **section 1.2**.

3.3.9 Analysis of Lead content of plant parts:

3.3.9 Quantification of lead analysis and accumulation in *Cucurbita maxima* and *Zea mays* plant parts

Lead (Pb) accumulation in various plant tissues of *Cucurbita maxima* and *Zea mays* (i.e., root, stem, leaf, and fruit) was assessed by carefully rinsing harvested plant parts with tap water, followed by deionized (DI) water to remove any soil particles that had stuck, and then oven-drying at 80 °C for approximately 1 hour. The oven-dried plant tissues were crushed using mortar and pestle. Then they were subjected on a heated plate, whereby, 12 mL of 3:1 (v/v) HNO₃/HClO₄ solutions were used to digest the 0.5 g of ground plant tissue samples for two hours. Following cooling, Whatman's filter paper number 42 was used to filter the digested solution, and deionized (DI) water was added to get the volume up to the 50 ml level. Under ideal analytical circumstances for lead estimation, the atomic absorption spectrometer (PinAAcle 500) fitted with a lead cathode lamp at a wavelength of 283.3nm and slit size of 2 nm was used to quantify the lead (Pb) in the corresponding tissues. Standard calibration procedure was used for the quantification of the results (Amin *et al.*, 2018).

The Pb concentration and accumulation in plant root and shoot were calculated by the following formula: (Monni *et al.*, 2000)

Pb Concentration (mg/ kg) = AAS interpretation (reading) × dilution factor dry weight of plant tissues

Pb Accumulation (µg/ plant) = Pb concentration × dry weight of plant tissues

3.3.10 Analysis of plant defence related enzyme assay:

3.3.10a. Quantitative Analysis of Catalase Activity in *Cucurbita maxima* and *Zea mays* leaves

In this process, 1g of fresh leaves were taken of both the control and treated set up, the enzyme was extracted with 50mM sodium phosphate buffer (pH 7.5) containing 1% Poly-vinyl-pyrrolidone (PVP). Reaction was initiated by addition of 1ml H₂O₂ solution to 0.2ml of the prepared enzyme extract and the decomposition of H₂O₂ was recorded at an interval of 30 seconds for 3 minutes, by measuring the absorbance at 240nm. The expression of catalase activity is done in micromoles of H₂O₂ decomposed/min/g fresh weight of tissue. H₂O₂ has an extinction coefficient of 43.6 mM⁻¹ cm⁻¹ (Das *et al.*, 2018)

3.3.10b. Quantitative Analysis of Ascorbate Peroxidase Activity in *Cucurbita maxima* and *Zea mays* leaves

The approach used to estimate ascorbate peroxidase (APX) was as per Nakano and Asada (1987) protocol. In this process, 1g of fresh leaves were taken of both the control and treated set up, 0.2 ml of enzyme was extracted using one ml of 50 mM sodium phosphate buffer (pH 7.0), 0.8 ml of 0.5 mM ascorbate, and 1 ml of H₂O₂ solution made up the reaction mixture, which had a total volume of 3 ml. The decrease in absorbance at 290 nm was used to evaluate the enzyme activity. According to the extinction coefficient of MDA, which is 2.8 mM⁻¹ cm⁻¹, ascorbate peroxidase was expressed as nano moles of MDA formed/min/g fresh weight of tissue (Das *et al.*, 2018).

3.3.10c. Quantitative Analysis of Proline content in *Cucurbita maxima* and *Zea mays*, leaves and roots

In this process, 100mg of sample of both treated and control set up was taken in a tube, 3% sulfosalicylic acid was added and the plant material was grounded and centrifuged for 5 minutes at 10,000 rpm. The composition of the mixture for protein content analysis encompassed 100 µl of 3% sulfosalicylic acid, 200 µl glacial acetic acid, 200 µl acidic ninhydrin (1.25 grams of 1,2,3-indantrione monohydrate, 30 ml of glacial acetic acid, and 20 ml of 6M orthophosphoric acid are combined to create acidic ninhydrin, which is then dissolved by vortexing and slowly heating), along with 100 µl from the supernatant of the plant extract were incubated at 96°C for 1 hour. In addition to this, the samples were extracted with toluene whereby 1ml toluene was added to the mixture, vortexed for 20 seconds and allowed to settle for a duration of 5 minutes, that helped in the separation of organic and water phases. The chromophore containing toluene was separated into a fresh test tube and the absorbance was measured at 520nm (Ábrahám *et al.*, 2010).

3.3.10d. Quantitative Analysis of Peroxidase Activity in *Zea mays* leaves

In this process, 1g of fresh leaves of both the control and treated set up were taken. The reaction mixture contained 2.8 ml of 0.05 M guaiacol in 0.1 M phosphate buffer (pH 6.5), 0.1 ml of enzyme extracts, and 0.1 ml of 0.8 M H₂O₂ were all present in the reaction mixture. As a blank, the reaction mixture devoid of H₂O₂ was measured. H₂O₂ was added to start the reaction, and for three minutes, at 30-second intervals, the rate of change in absorbance at 470 nm was measured. The definition of peroxidase activity is the change in absorbance/min/g fresh weight of tissue (Das *et al.*,2018).

OBJECTIVE 4**3.4 Statistical analysis, including time series study of physico-chemical and microbiological property vis a vis crop productivity.****3.4.1 Calculation of the statistically significant (P) values of soil parameters of *Cucurbita maxima* and *Zea mays***

Each soil sample was replicated three times, with all measurements taken independently. Graphs were created using average values, while statistical analysis was conducted based on raw data. To evaluate significant changes in soil physico-chemical parameters during the initial, peak, and intercrop phases of *Cucurbita maxima* and *Zea mays*, a t-test was utilized. This test was selected for its capability to manage unidentified variances and to assess deviations against the null hypothesis.

A p-value of less than 0.05 was deemed statistically significant, suggesting the rejection of the null hypothesis, while a p-value exceeding 0.05 indicated a non-significant deviation, leading to the acceptance of null hypothesis (Di Leo *et al.*, 2020).

3.4.2 Time series analysis of the soil parameters over two-year period

Statistically, time-series analysis would likely find strong annual periodicity with little net trend over just 2 years. Autocorrelations would highlight the 12-month cycle. Existing trend in the 2-year data (e.g. a slight year-to-year change), it would be small compared to seasonal amplitudes (Velicer *et al.*,2003). The soil data of both *Cucurbita maxima* and *Zea mays* were recorded and time series analysis was done to understand the trend of the macronutrients and micronutrients in the soil.

3.4.3 Statistical Analysis of the crop productivity upon application of consortia.

General Testing Procedure: H (null hypothesis): There is no significant difference in the average values of the parameter 0: under consideration in plants in the control group and the treated group.

H_1 (alternate hypothesis): The average value of the parameter under consideration is: significantly higher in plants in the treated group, than the control group.

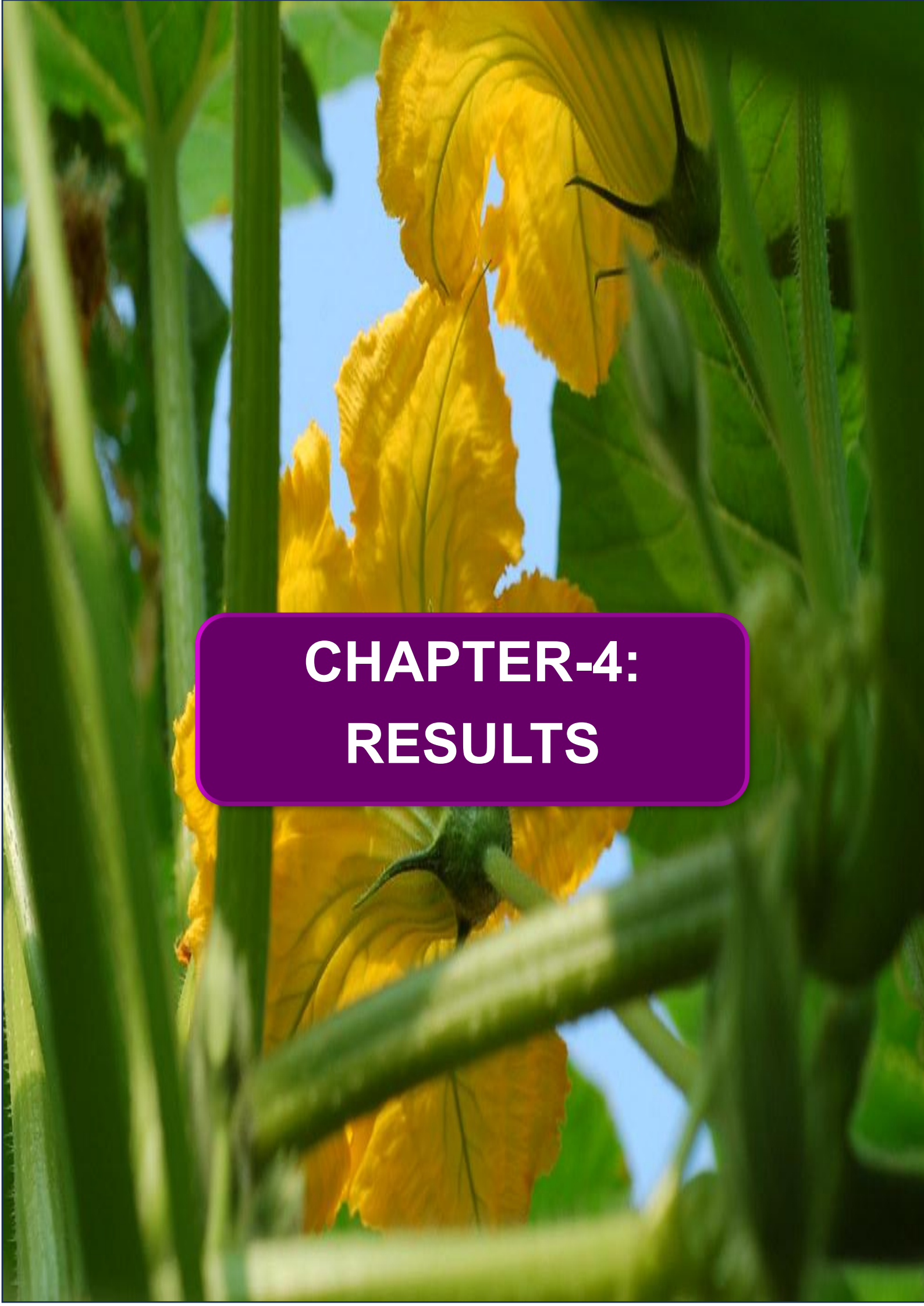
Level of significance (l.o.s): 0.05

Critical Region: Reject H_0 at 0.05 l.o.s if computed p-value < 0.05

Outline of the analysis:

- For the given data, the mean value of the data were plotted week-wise for both treatment and control for visual comparison. A linear trend has been fitted for both (which has been shown in the graph on the right-side of the panel for each parameter), that is, it has been assumed that the parameter under consideration increases linearly with time. The linear equations reflect the change in the value of the parameters with time in an ideal situation, i.e., excluding the effect of irregular factors. Multiple R values are reported in 2% for each fitting. Higher the R value, better is the linear fit.
- Then, tests have been carried out week-wise to study the differences between the control and treated group of plants for each parameter. Two tests, one parametric (Student's t-test), and the other non-parametric (Wilcoxon's rank-sum test) were opted. The p-values and the decisions of the tests have been tabulated for each parameter. The lower is the p-value, the more difference in the parameter between the treated and controlled groups is suggested by the data.
- The decision of any statistical testing procedure is conventionally made with respect to the null hypothesis H . "Accept" refers to accepting , i.e., it can be concluded that in the light of

the given data, there is no significant difference between the average values of the parameter under consideration in the control group and the treated group, whereas “Reject” refers to rejecting H_0 , i.e., where it can be concluded that in the light of the given data, the 0 value of the parameter under consideration is significantly higher in the treated group of plants than in the control group of plants.



CHAPTER-4: RESULTS

4. RESULTS

4.1 To characterize soil, with respect to physical, chemical, microbiological and metagenomic data of designated plot involving *Cucurbita* sp and *Zea mays* over two-year period.

The soil was collected from Chowbaga area of East Kolkata Wetlands as represented in **Figure 4.1**, and the three cropping periods of soil collection of *Zea mays* have been represented in **Figure 4.2**. The cultivation of *Cucurbita maxima* as done in Chowbaga, has been represented in **Figure 4.3**.

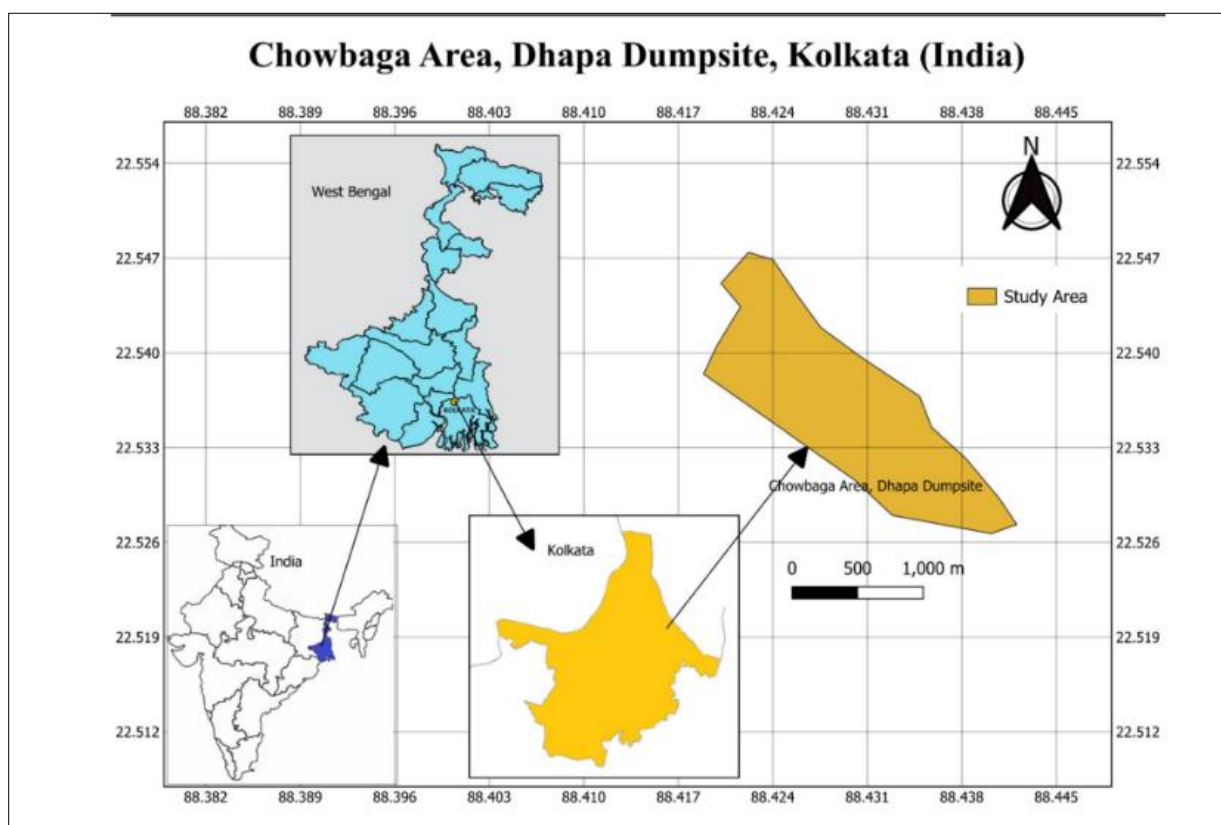


Figure 4.1: Map representing the study location at Chowbaga area of East Kolkata Wetlands



Figure 4.2: The soil sample collection was done during the initial, peak and intercropping period



Figure 4.3: Cultivation of *Cucurbita maxima* as seen in the agricultural fields of Chowbaga area of East Kolkata Wetlands

4.1.1a Soil characterization with respect to physico-chemical parameters of the designated plot involving *Cucurbita maxima*

The primary aim of this study was to comprehensively analyse and compare the soil physico-chemical and inorganic nutrient parameters associated with the cultivation of *Cucurbita maxima* in the Chowbaga area of the East Kolkata Wetlands over two consecutive cropping years. The investigation focuses on examining temporal variations of soil across three critical cropping stages—initial, peak (flowering/fruiting), and intercrop periods.

The soil physico-chemical parameters like pH, electrical conductivity (EC), organic carbon (OC), nitrogen (N), potassium (K), phosphorous (P), cation exchange capacity (CEC), soil moisture, calcium (Ca), Magnesium (Mg), Manganese (Mn), sulphur(S), Boron (B), zinc (Zn), Iron (Fe), copper (Cu) were analysed. The results are displayed in figure 4.4a, 4.4b and 4.4c

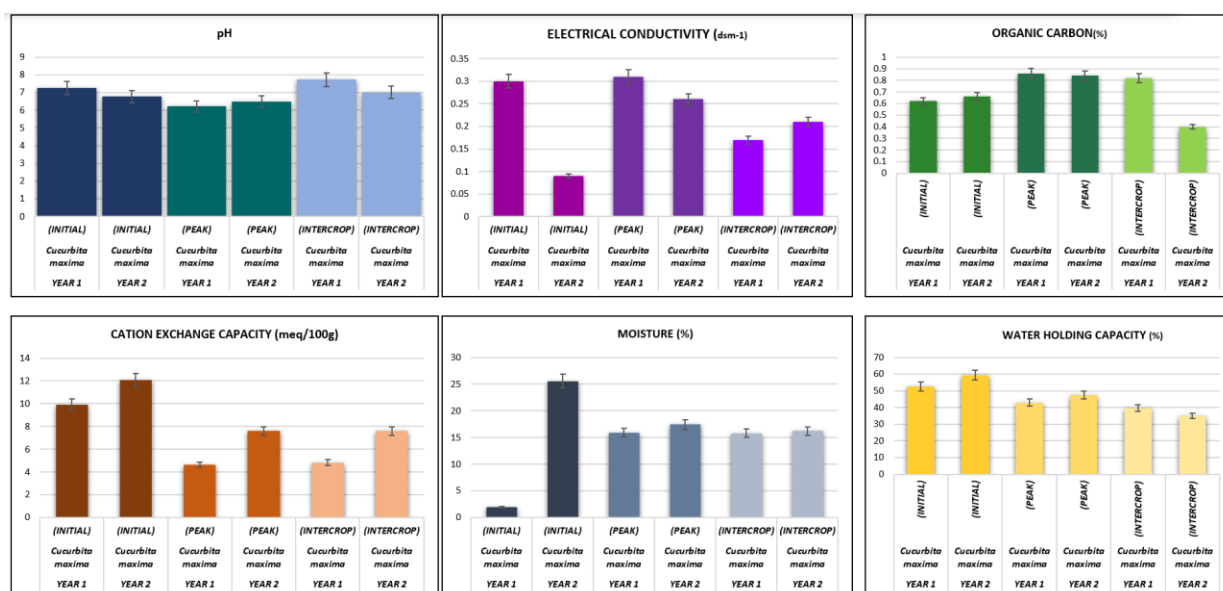


Figure 4.4a: Analysis of physico-chemical parameters of *Cucurbita maxima* soil collected from Chowbaga area of East Kolkata Wetlands over a two-year period.(In the graph, X- axis represents the cropping seasons and Y-axis represents the unit of the parameters)

The inorganic parameters of soil were analysed. They were as follows:

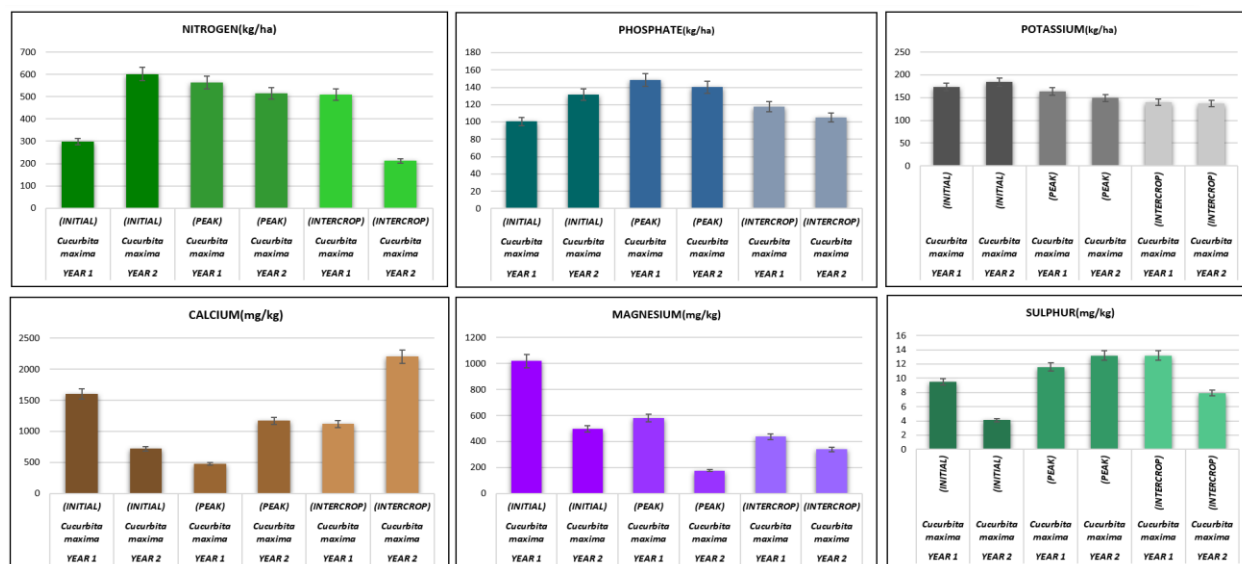


Figure 4.4b: Analysis of Inorganic parameters (macro-elements) of *Cucurbita maxima* soil collected from Chowbaga area of East Kolkata Wetlands over a two-year period. (In the graph, X-axis represents the cropping seasons and Y-axis represents the unit of the parameters)

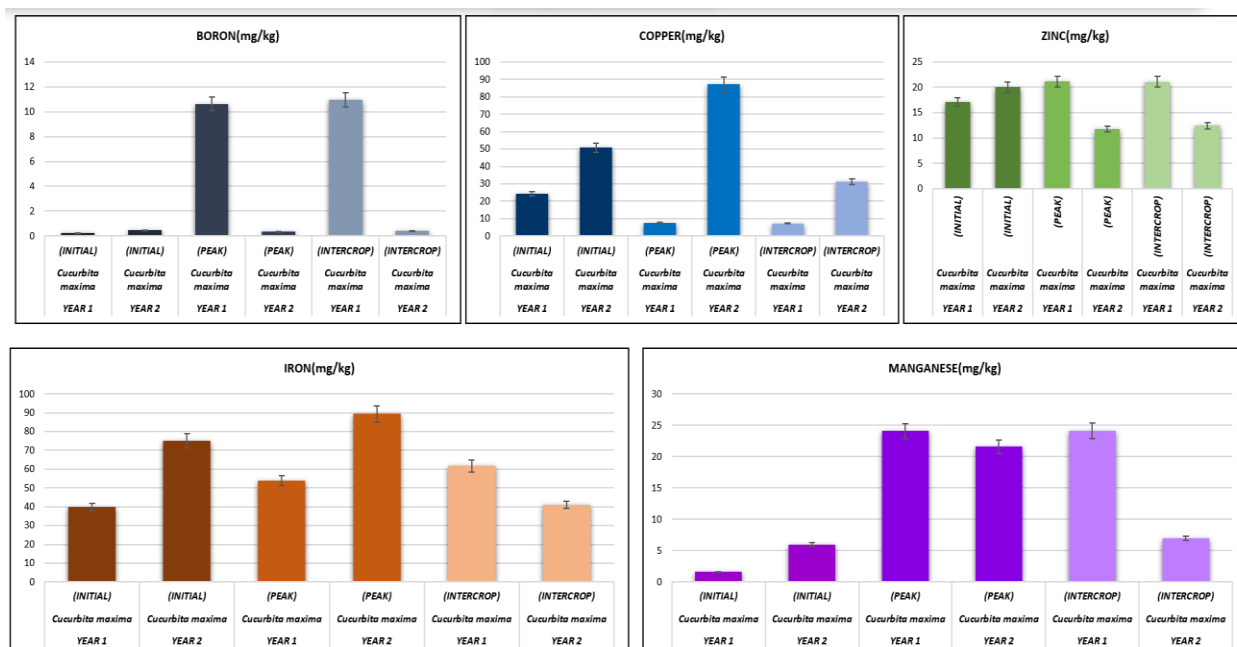


Figure 4.4c: Analysis of Inorganic parameters (micro-elements) of *Cucurbita maxima* soil collected from Chowbaga area of East Kolkata Wetlands over a two-year period. (In the graph, X-axis represents the cropping seasons and Y-axis represents the unit of the parameters)

KEY FINDINGS from 4.1.1a

- ❖ **Soil pH** remains near neutral in all stages, dipping into slightly acidic range (6.0–6.5) at the crop's peak stage. In Year 1, pH escalated to 7.73 by the intercrop stage, indicating an increase in the alkalinity levels of soil. In Year 2, the pH remained between 5.6–6.52 during the entire period.
- ❖ **Electrical conductivity (EC)** value for both the years is below 0.35 dS/m, indicating non-saline conditions. Year 1 has a higher EC level as compared to the Year 2.
- ❖ **Organic carbon (OC)** content peaked during the flowering/fruiting stage in both years (0.85–0.90%), indicating an increase in organic matter, of around 5.88% in the peak period of second year.
- ❖ **Nitrogen(N)** levels in Year 2 have a much higher nitrogen content level of about 602.11kg/ha as compared to Year1, 298.5 kg/ha during the initial stage. By peak stage, both years have roughly similar N (563.7kg/ha–514.22kg/ha). During the intercrop season, the Nitrogen content of the soil is 510.7kg/ha, as compared to year 2, whereby the nitrogen level declines to approximately 212kg/ha.
- ❖ **Phosphorus(P)** levels were found to rise from initial season to peak season then declined during the intercrop season in both the years. They reached to their optimum value of approximately 150 kg/ha and 140 kg/ha during the peak season of first year and second year respectively. The phosphate level declined during the intercrop season, where they were found to be 117.57kg/ha during first year and 105.33kg/ha in the second year.
- ❖ **Potassium(K)** levels were found to decline from 173.37 kg/ha in the initial stage to 139.77kg/ha in the intercrop season. The potassium content of the soil in the initial stage of year 2 (184.12 kg/ha) was higher in the soil as compared to first year.
- ❖ **Cation exchange capacity (CEC)** in Year 2 is higher at 12.08 meq(milliequivalents)/100g in the initial stages as compared to the first year at 9.92 meq/100g. The decline in the CEC level during the intercrop season to 4.84 meq/100g in the first year and 7.6 meq/100g in the second year suggests loss of exchangeable bases (Ca, Mg, K) as the crop grows.
- ❖ **Calcium (Ca)** levels during year 1 were found to vary from 1603.2 mg/kg of soil during the initial stages to 1122.24 mg/kg of soil in the intercrop season, and were found to dip to 480.96 mg/kg of soil in the peak season, indicating uptake of nutrients by plants. In the second year, the levels of calcium escalated, that can be due to addition of fertilizers.
- ❖ **Magnesium (Mg)** levels in year 1 were much were 40.5% higher as compared to year 2.

- ❖ **Sulphur (S)** levels increased during the peak stage (Year 1: 10 to 12 mg/kg; Year 2: 4 to 14mg/kg), then decreased at intercrop.
- ❖ **Copper (Cu)** contents in Year 2 reached an extremely high peak of 87.14 mg/kg compared to Year 1 with a value of 7.59 mg/kg.
- ❖ **Zinc (Zn)** levels in both the years have been present in the contents of 15–25 mg/kg – generally sufficient for plant growth.
- ❖ **Iron (Fe)** levels in soil is higher in Year 2 across all stages. Both years peak around flowering (53.85mg/kg and 89.33mg/kg), and has been found to decline to 41.05mg/kg of soil in the second year during the intercrop season.

4.1.1b Soil characterization with respect to physico-chemical parameters of the designated plot involving *Zea mays*

The objective of this study is to investigate and compare the temporal variability of soil physico-chemical and nutrient parameters associated with the cultivation of *Zea mays* in the Chowbaga region of the East Kolkata Wetlands across two consecutive years. The research aims to evaluate soil health and nutrient dynamics at three key cropping stages—initial, peak, and intercrop periods—with a focus on both macro- and micronutrient profiles.



Figure 4.5: Cultivation of *Zea mays* as seen in the agricultural fields of Chowbaga area of East Kolkata Wetlands

The soil physico-chemical parameters like pH, electrical conductivity (EC), organic carbon (OC), nitrogen (N), potassium (K), phosphorous (P), cation exchange capacity (CEC), soil moisture, calcium (Ca), Magnesium (Mg), Manganese (Mn), sulphur(S), Boron (B), zinc (Zn), Iron (Fe), copper (Cu) were analysed. The results are displayed in figure 4.6a, 4.6b and 4.6c

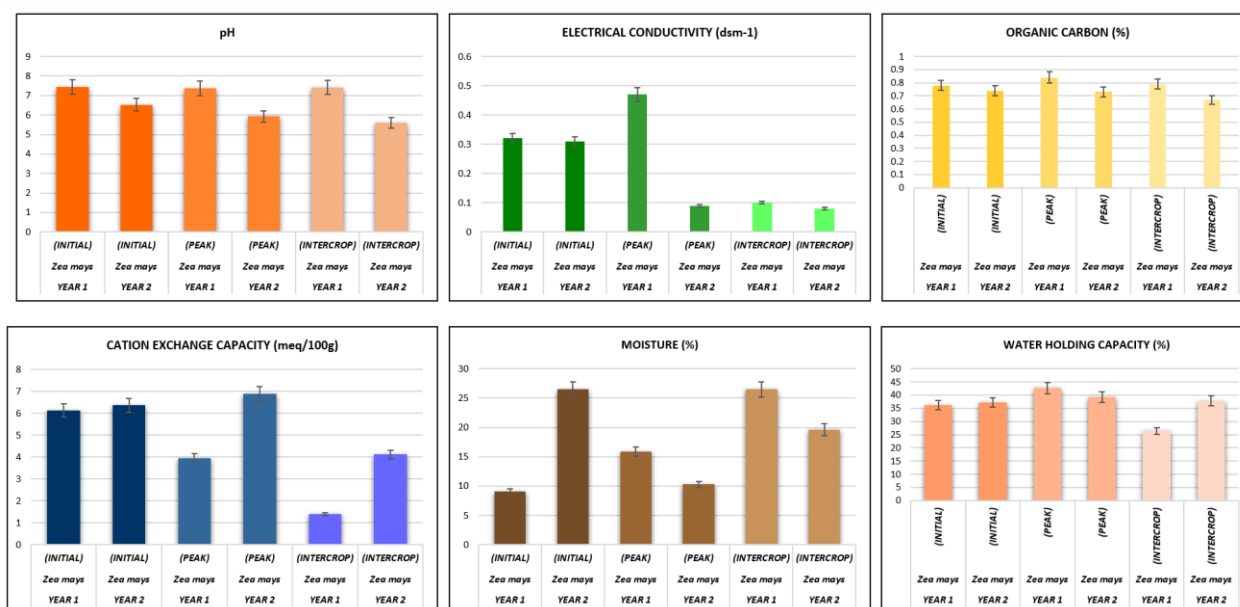


Figure 4.6a Analysis of physico-chemical parameters of *Zea mays* soil collected from Chowbaga area of East Kolkata Wetlands over a two-year period. (In the graph, X- axis represents the cropping seasons and Y-axis represents the unit of the parameters)

The **inorganic parameter** of soils was analysed. They were as follows:

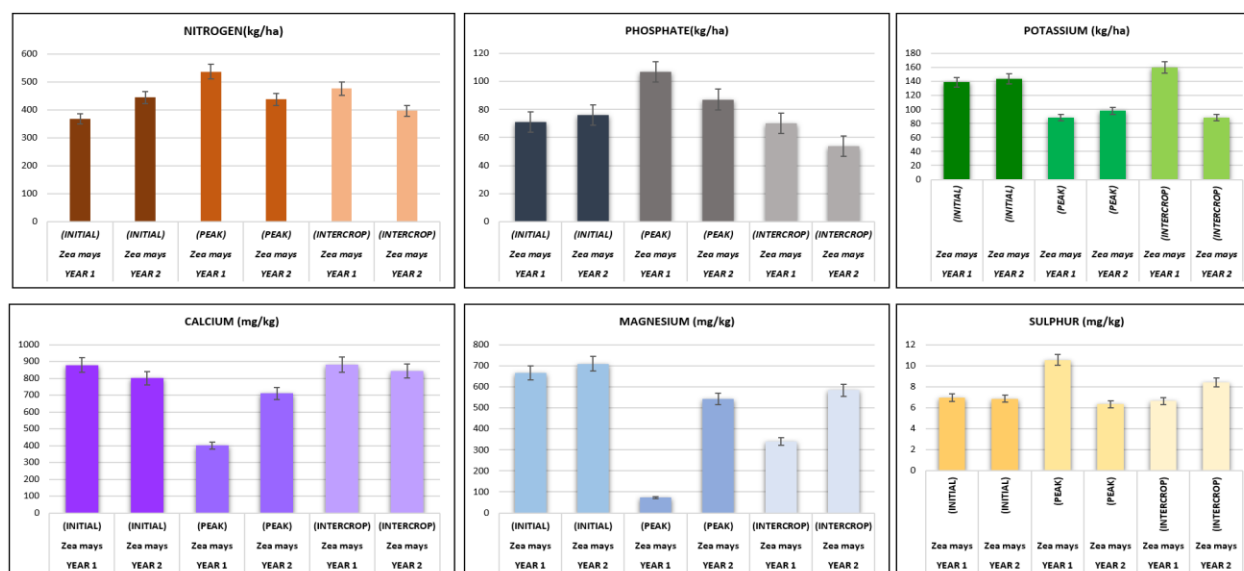


Figure 4.6b Analysis of Inorganic parameters (macro-elements) of *Zea mays* soil collected from Chowbaga area of East Kolkata Wetlands over a two-year period. (In the graph, X- axis represents the cropping seasons and Y-axis represents the unit of the parameters)

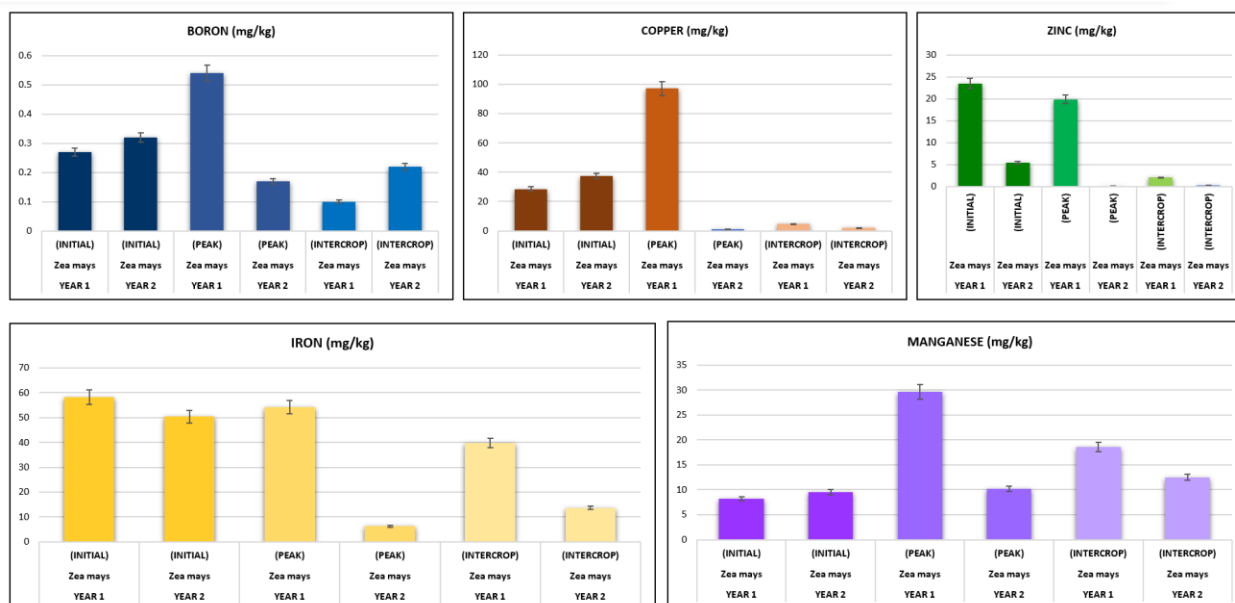


Figure 4.6c Analysis of Inorganic parameters (microelements) of *Zea mays* soil collected from Chowbaga area of East Kolkata Wetlands over a two-year period. (In the graph, X- axis represents the cropping seasons and Y-axis represents the unit of the parameters)

KEY FINDINGS from 4.1.1b:

- ❖ **Soil pH** in Year 1 was slightly alkaline to near-neutral (approximately 7.45 at the initial stage, dipped to 7.37 in the peak and increased to 7.47 at intercrop), whereas in Year 2, pH was lower and increasingly acidic (about 6.77 initial, 6.5 peak, and increased to 7.5 intercrop)
- ❖ **Electrical conductivity (EC)** remained very low in all cases: 0.32 dS/m at initial stages, and increased to 0.48 dS/m in Year 1, and then dropped to 0.1 ds/m during intercrop season. In Year 2, the EC values dipped to 0.09 ds/m during the peak season and dipped further to 0.08 ds/m in intercrop stages.
- ❖ **Organic carbon** levels in year 1 peaked slightly higher (0.84% organic carbon) than Year 2 (0.73%) at the peak season, with both years having marginally lower values (~0.7%) at the initial stage.
- ❖ **Cation exchange capacity (CEC)** levels in Year 2 soils were higher as compared to the Year 1, with values at 6.88 meq/100 g at peak and reduced 4.12 meq/100 g during intercrop stages.
- ❖ Year 1 had a higher **nitrogen** content buildup of 525 kg/ha consistent with plant uptake and mineralization, especially during the peak season as compared to year 2 (430 kg/ha).

- ❖ **Phosphorous** levels in soil during Year 1 was about 138.81 kg/ha during the initial stage and was lowest in Year 2 intercrop season at 53.81 kg/ha. The peak values of 106.76kg/ha during year 1 as compared to 87.12kg/ha in year 2 indicate more phosphate availability in Year 1. The decline by intercrop (especially in Year 2) likely reflects plant uptake and possible fixation, and therefore could limit the rate of plant growth.
- ❖ Available **potassium(K)** showed a different pattern, whereby in Year 1, the levels of potassium peaked to 159.94 kg/ha at intercrop season, whereas in Year 2, the highest value of potassium levels in the soil were found to be 143.81 kg/ha during the initial stage and declined to 88.7 kg/ha by intercrop season.
- ❖ In Year 1, **Calcium** dropped from 879.5 mg/kg initially to only 400.8 mg/kg at peak, and then escalated to 881.76 mg/kg during the intercrop period. During the second year, the calcium levels were 801.6mg/kg during the initial stages, dipped to 710.2 mg/kg during the peak season and again increased to 843.68 mg/kg during the intercrop season.
- ❖ **Magnesium** levels in the soil during year1 was about 664.54 mg/kg during the initial stage, declined sharply to 72.9 mg/kg at peak, then increased to 340.2 mg/kg at intercrop season. In Year 2, soils had higher levels of Mg at 542.58mg/kg of soil during the peak period as compared to year 1.
- ❖ Boron (B) peaked in Year 1 at the initial stage (0.55 mg/kg) but was low (0.1–0.3 mg/kg) during the other stages. Copper (Cu) was extremely high at Year 1 peak (100 mg/kg) but nearly absent by Year 1 intercrop; Year 2 started with moderate levels of Cu (40 mg/kg) and declined to 2 mg/kg by peak. Zinc (Zn) was present at a quantity of 25 mg/kg at Year 1 initial, and was nearly absent during the intercrop stage of Year 1; Year 2 Zn was very low and was present at an approximate quantity of 5 mg/kg throughout. Iron (Fe) was present at a quantity of 60 mg/kg at Year 1 during the initial period, declined to 55mg/kg at peak period, then reduced to 35mg/kg at intercrop; Year 2 had approximately 50mg/kg of Fe initially, but declined to 5 mg/kg by peak period.

4.1.2a Metagenomic analysis of the soil rhizosphere of *Cucurbita maxima* during the three cropping periods, namely: Initial, Peak and Intercrop (YEAR 1)

The objective of this study was to investigate the composition and dynamics of the rhizospheric microbial community associated with *Cucurbita maxima* soil across three distinct cropping periods—Initial, Peak, and Intercrop—during Year 1, through metagenomic analysis using the Illumina Novaseq 6000 platform, with the objective of identifying key bacterial taxa involved in soil health maintenance and plant growth promotion. The results have been represented in **Figure 4.7**. The sample data were uploaded to NCBI data base. The dataset includes Submission Id: SUB11494487, BIOPROJECT ID: PRJNA839190, BIOSAMPLE: SAMN24537899, SRA.: SRR19261839. BIOSAMPLE: SAMN28513088, SRA: SRR19261839. The microbial abundance of certain bacterial genus like *Candidatus* sp, *Pirellula* sp , *Gemmata* sp were visble via the heatmaps generated from the analysis of soil metagenome.

CHAPTER 4 - RESULTS

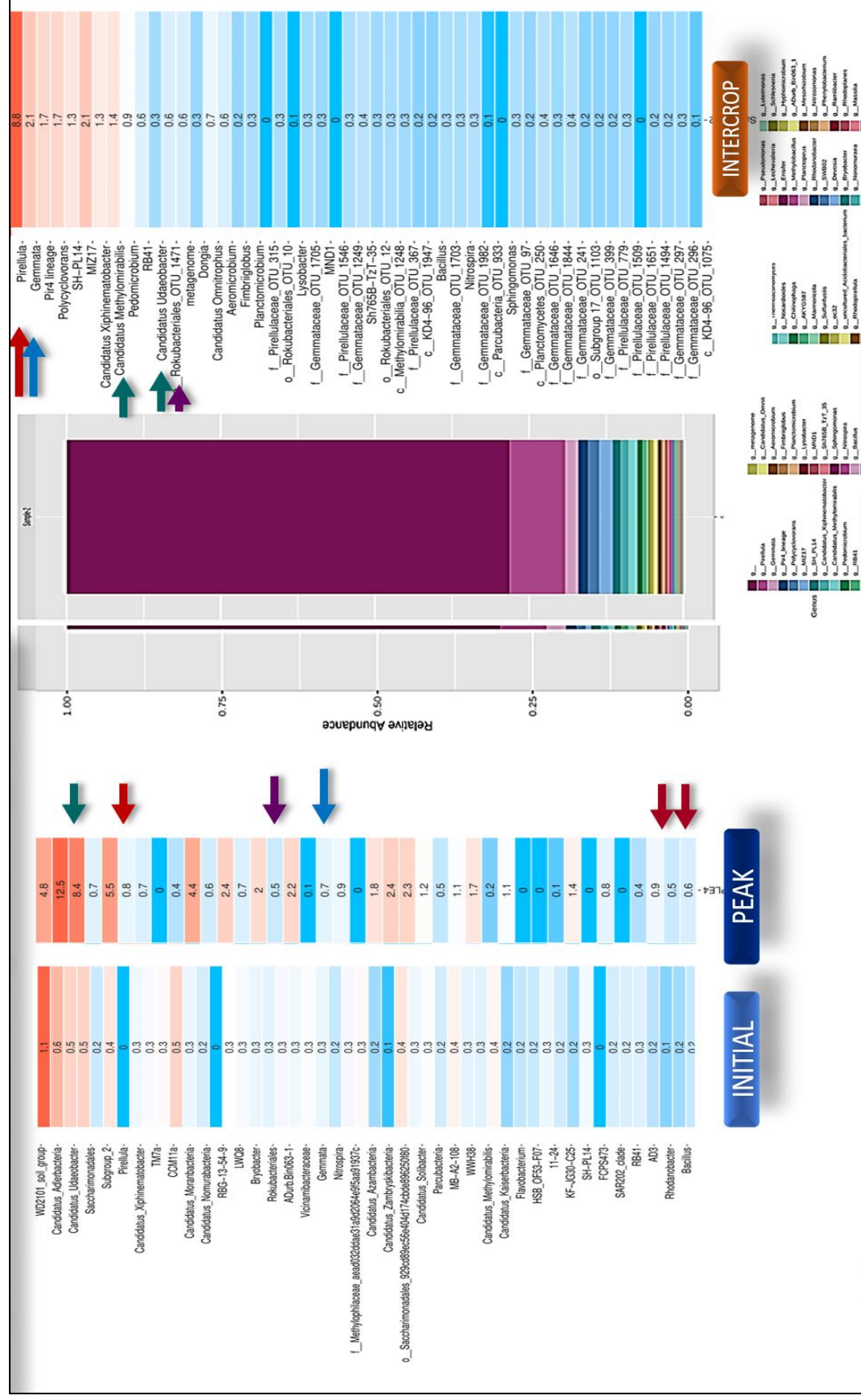


Figure 4.7: Metagenomic analysis of soil sample involving *Cucurbita maxima* (Year 1)

Key Findings from 4.1.2a:

- Metagenomic analysis of *Cucurbita maxima* rhizospheric soil was conducted across three cropping periods: Initial, Peak, and Intercrop (Year 1), using the Illumina Novaseq 6000 platform.
- Bacteria were found to be the predominant microbial domain in the rhizosphere during all three cropping stages.
- At the phylum level, the most abundant bacterial groups included:
Acidobacteriota, *Patescibacteria*, *Verrucomicrobiota*, *Planctomycetota*, *Proteobacteria* and *Chloroflexi*
- At the genus level, the most abundant taxa identified were:
Candidatus sp. – emerged as the most dominant genus throughout all stages, *Bryobacter* sp, *Bacillus* sp .
- Notably, *Bacillus* sp. and *Bryobacter* sp. are recognized for their plant growth-promoting traits and active roles in enhancing soil health and microbial interactions.
- The bacterial genera *Pirellula* sp., *Gemmata* sp., and *Nitrospira* sp. were consistently present (lingering) during all three cropping periods, suggesting their potential role in maintaining microbial stability in the rhizosphere.
- Overall, the analysis highlighted a diverse and dynamic bacterial community with several genera known for beneficial plant-microbe interactions and rhizospheric support

4.1.2b Metagenomic analysis of the soil rhizosphere of *Cucurbita maxima* during the three cropping periods, namely: Initial, Peak and Intercrop (YEAR 2)

Analysis of soil metagenome from *Cucurbita* sp rhizosphere was done in the second year. The predominance of lingering bacteria like *Candidatus* sp., *Polycyclovorans* sp

Submission Id: SUB14176048, BIOPROJECT ID: PRJNA1069712, BIOSAMPLE: SAMN39621110, EXP.: SRR27743398; SAMN39620623, SRR27743398. The heatmap representing the data has been represented in **Figure 4.8**.

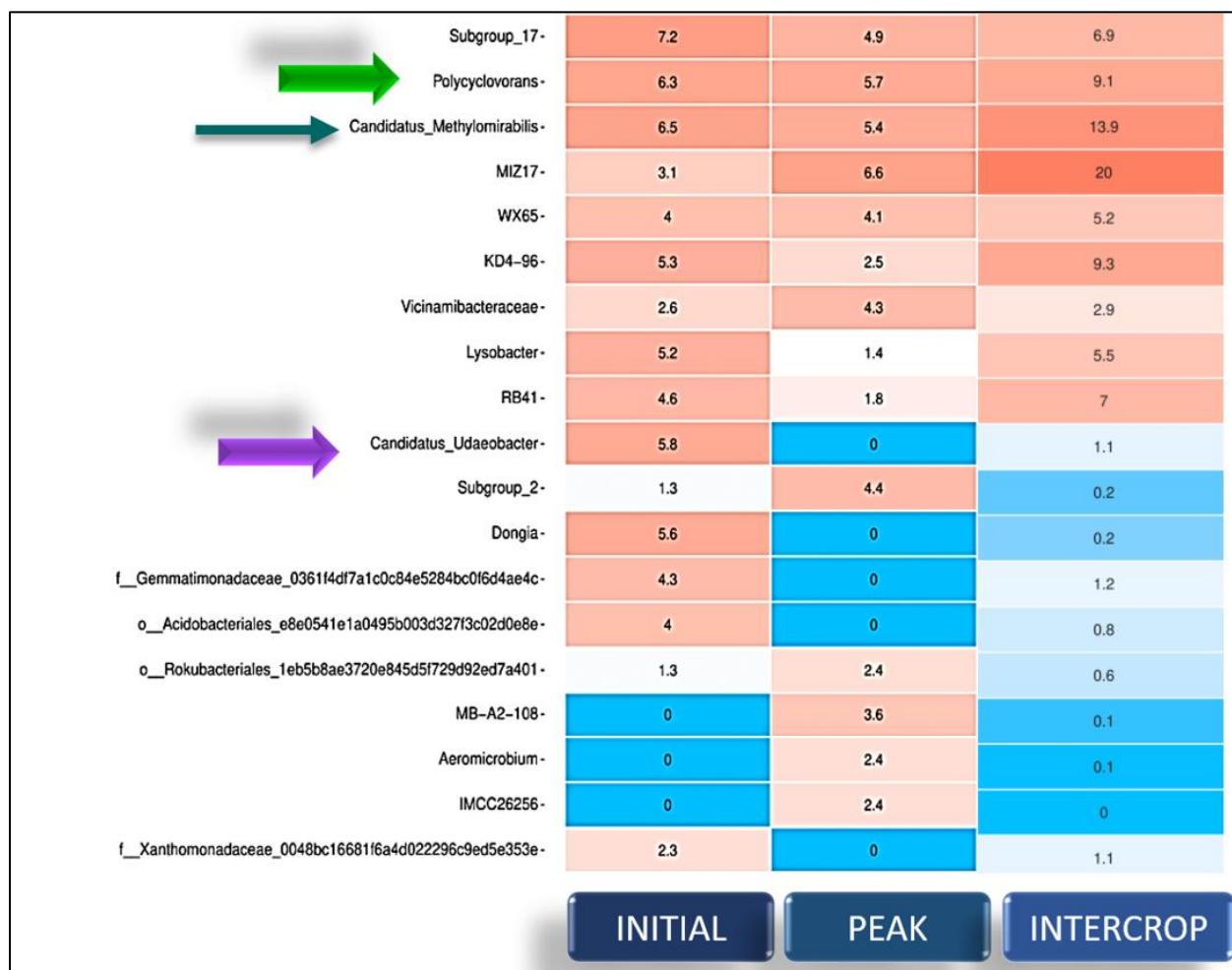


Figure 4.8: Metagenomic analysis of soil sample involving *Cucurbita maxima* (Year 2)

Key Findings from 4.1.2a:

- Metagenomic analysis of the rhizospheric soil of *Cucurbita maxima* during the three cropping periods (Initial, Peak, and Intercrop) in Year 2 was conducted using the Illumina Novaseq 6000 platform.
- The study revealed a set of dominant and consistently lingering bacterial genera across all three cropping stages.
- The key bacterial genera identified in high abundance throughout all periods were: *Polycyclovorans* sp, *Paenibacillus* sp, *Candidatus* sp, *Gemmata* sp, *Bacillus* sp. *Polycyclovorans* sp, *Candidatus* sp occupied the top 20 genera
- These genera were present consistently across all cropping phases, indicating their stable association with the *Cucurbita maxima* rhizosphere.
- Many of these bacterial groups (e.g., *Bacillus* sp, *Paenibacillus* sp, *Lysobacter* sp) are known for their plant growth-promoting properties, such as nitrogen fixation, pathogen suppression, and nutrient solubilization.
- The presence of *Candidatus* sp. and *Gemmata* sp. suggests involvement in specialized microbial interactions and potential roles in biogeochemical cycling within the rhizosphere.
- Overall, the findings indicate a resilient and beneficial core microbiome associated with *Cucurbita maxima* that persists through different cropping stages, supporting soil health and plant productivity.

4.1.2c Metagenomic analysis of the soil rhizosphere of *Zea mays* during the three cropping periods, namely: Initial, Peak and Intercrop (YEAR 1)

The objective of this study was to investigate the composition and dynamics of the rhizospheric microbial community associated with *Zea mays* soil across three distinct cropping periods—Initial, Peak, and Intercrop—during Year 1, through metagenomic analysis using the Illumina Novaseq 6000 platform, with the objective of identifying key bacterial taxa involved in soil health maintenance and plant growth promotion. All the sample data were uploaded to NCBI data base. The dataset includes SAMN21474479, SRR15931637; SAMN28462514, SRR17395637. The results have been represented in **Figure 4.9**.

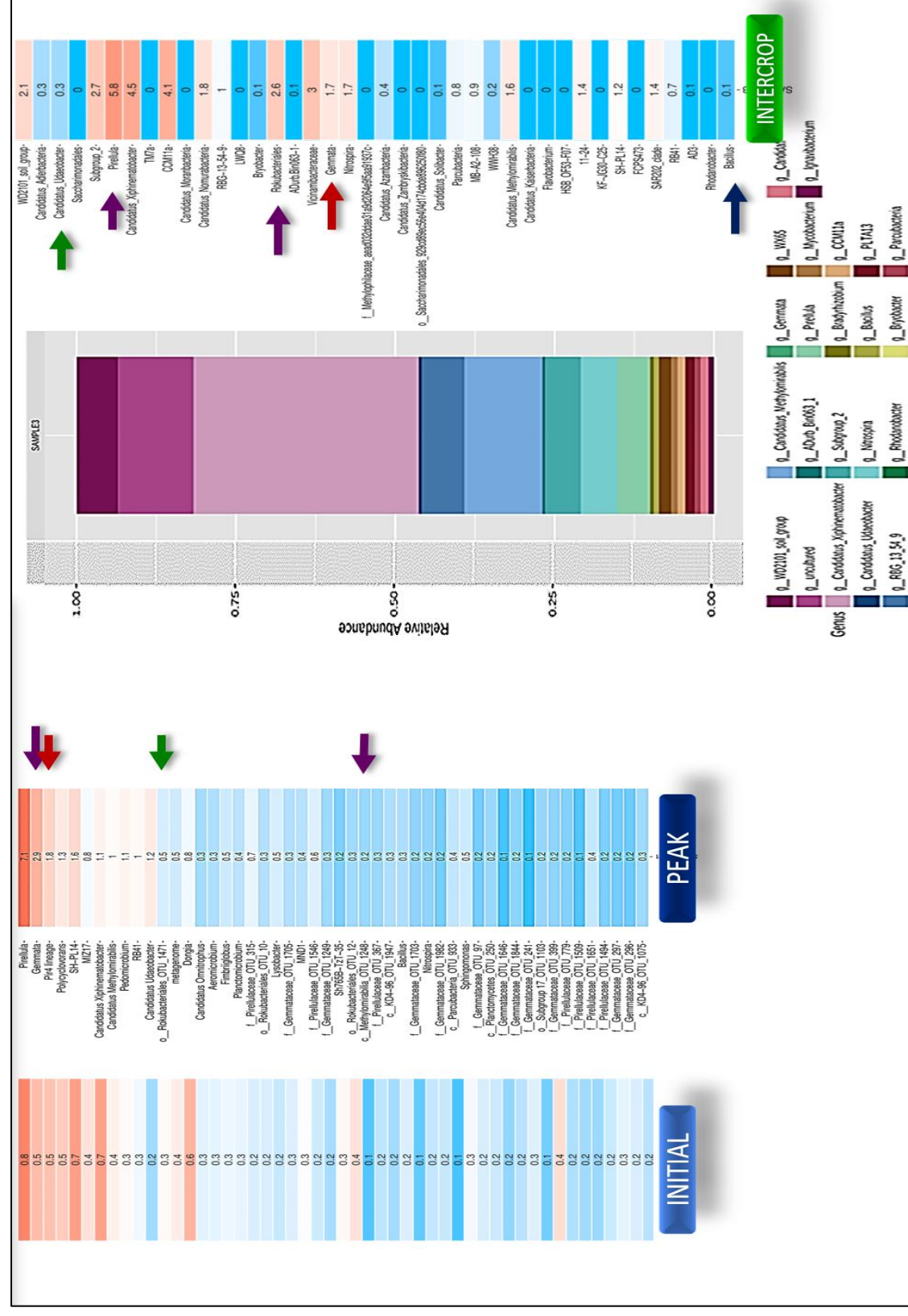


Figure 4.9: Metagenomic analysis of soil sample involving *Zea mays* showing the predominant bacteria between the three cropping periods (Year 1).

Key Findings from 4.1.2c:

- Metagenomic analysis of *Zea mays* rhizospheric soil during the Initial, Peak, and Intercrop periods of Year 1 was conducted using the Illumina Novaseq 6000 platform.
- The analysis revealed several bacterial genera that consistently lingered throughout all three cropping stages, indicating their stable association with the *Zea mays* rhizosphere.
- The dominant lingering bacterial genera identified were: *Gemmata* sp, *Nitrospira* sp, *Pirellula* sp and *Candidatus* sp.
- These bacterial groups were present across all cropping phases, suggesting important ecological roles such as nutrient cycling, soil structural enhancement, and plant-microbe interactions.
- *Nitrospira* sp. is especially noted for its role in nitrification, contributing to nitrogen availability in the soil.
- *Candidatus* sp. and *Gemmata* sp. indicate a presence of complex and potentially uncultivable microbial taxa involved in specialized soil functions.
- Notably, *Bacillus* sp. was specifically observed during the Intercrop season, highlighting its possible role in soil recovery, organic matter breakdown, and plant growth promotion during post-harvest or transitional phases.
- Overall, the rhizosphere of *Zea mays* in Year 1 harboured a core microbial community with functional diversity and adaptability across different cropping stages.

4.1.2d Metagenomic analysis of the soil rhizosphere of *Zea mays* during the three cropping periods, namely: Initial, Peak and Intercrop (YEAR 2)

Analysis of soil metagenome from *Zea mays* soil rhizosphere was done in the second year. The predominance of lingering bacteria like *Pseudomonas sp*, *Lactobacillus sp*, *Ralstonia sp*, *Proteus sp* were majorly observed. The dataset includes SAMN28462514, SRR17395637; SAMN31008114, SRR21700514. The representation of the soil metagenome data has been done using a heatmap, as represented in **Figure 4.10**.



Figure 4.10: Metagenomic analysis of soil sample involving *Zea mays* showing the predominant bacteria between the three cropping periods (Year 2).

Key Findings from 4.1.2d:

- Metagenomic analysis of the *Zea mays* rhizospheric soil during the Initial, Peak, and Intercrop periods of Year 2 was performed using the Illumina Novaseq 6000 platform.
- The analysis identified a set of dominant and lingering bacterial genera consistently present across all three cropping stages.
- The bacterial genera found to be abundant and persistent in the rhizosphere were: *Pseudomonas sp*, *Lactobacillus sp*, *Ralstonia sp*, *Proteus sp*, *Candidatus sp*. These genera were observed in the heatmap showing the highest microbial abundance.
- These genera remained consistently abundant especially during the Peak and Intercrop periods, indicating their adaptability and potential ecological importance in supporting plant health during active growth and post-harvest phases.
- Overall, the findings suggest a resilient, functionally diverse microbial community in the *Zea mays* rhizosphere that supports soil health and plant productivity across different cropping phases.

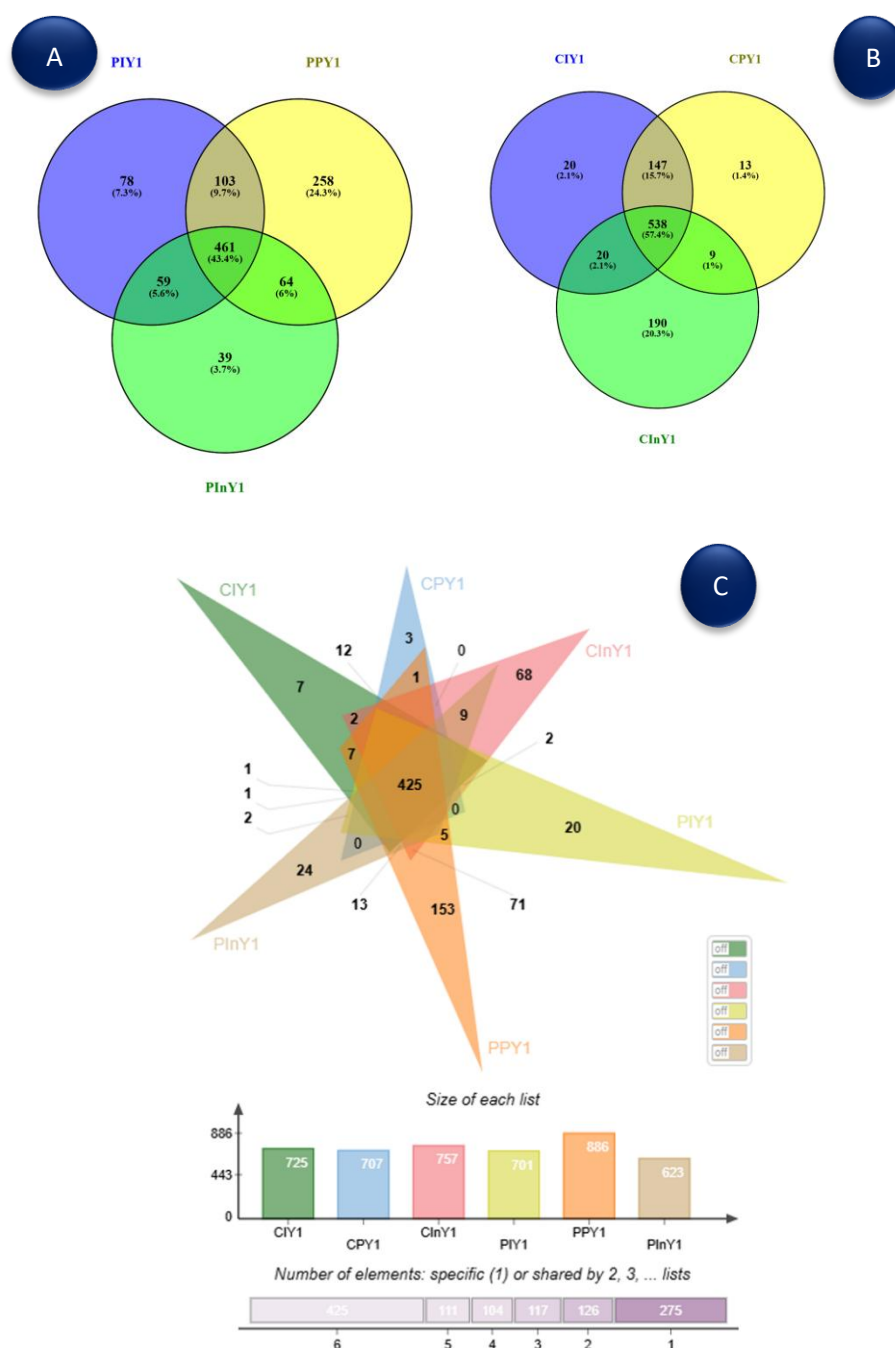


Figure 4.10a: Venn Diagram representing the variations and similarity of microbial community in *C. maxima* soil during the three cropping periods (PI- Initial; PP- Peak, Pin-Intercrop) (Year 1)

Figure 4.10b: Venn Diagram representing the variations and similarity of microbial community in *Z. mays* soil during the three cropping periods (CI- Initial; CP- Peak, Cin-Intercrop) (Year 1)

Figure 4.10c: Venn diagram of Corn & Pumpkin variations of Year 1

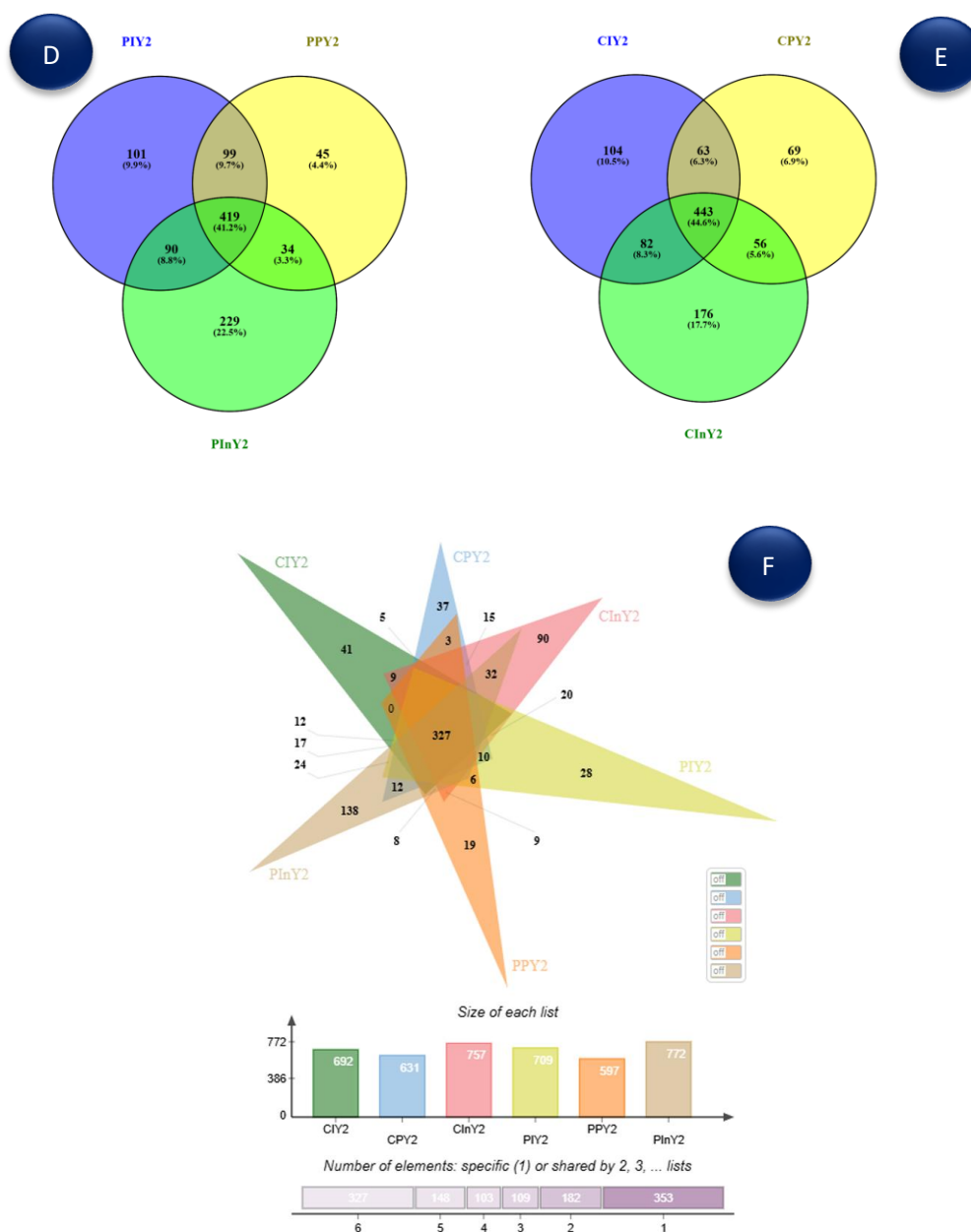


Figure 4.10d: Venn Diagram representing the variations and similarity of microbial community in *C. maxima* soil during the three cropping periods (PI- Initial; PP- Peak, Pin-Intercrop) (Year 2)

Figure 4.10e: Venn Diagram representing the variations and similarity of microbial community in *Z. mays* soil during the three cropping periods (CI- Initial; CP- Peak, Cin-Intercrop) (Year 2)

Figure 4.10f: Combined Venn diagram of Corn & Pumpkin variations of Year 2

KEY FINDINGS from Figures 4.10a, b, c, d, e, f:

- Soils associated with pumpkin and corn support both distinct and shared microbial communities.
- The most significant overlaps, illustrated in the center of the Venn and star plots, indicate stable and enduring microbial taxa that persist across various crops and their growth stages — potentially crucial members of the soil microbiome that contribute to resilience.
- The unique areas emphasize the varying impacts of cropping stages (initial, peak, intercrop) on microbial dynamics for each specific crop.

4.1.3 Measurement of heavy metal contents in soil

The objective of this work was to assess the extent of heavy metal contamination in the soil by quantifying the concentration of toxic elements such as lead, chromium, nickel, arsenic, and cadmium, and to evaluate their potential impact on soil health, plant growth, and ecological safety, with a view to find out the remediation strategies of the impacts of heavy metals on soil. The heavy metal measurements in soil have been done using Atomic Absorption Spectrophotometer PinAAcle 500. The results have been displayed in **Figure 4.11**.

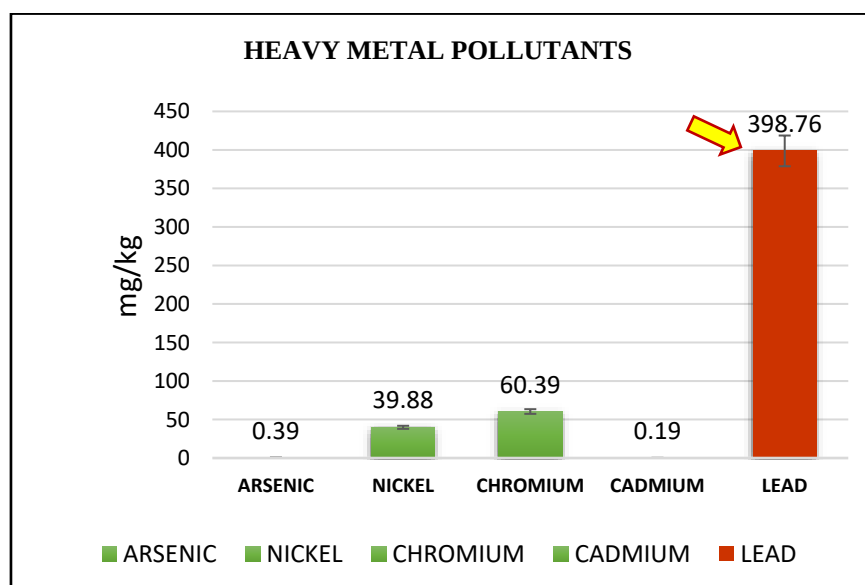


Figure 4.11: Quantification of heavy metal contents in soil

KEY FINDINGS (From Figure 4.1.3):

- The soil was found to contain a significantly high concentration of lead (Pb) at 398.76 mg/kg, indicating severe lead contamination.
- Chromium (Cr) was the second most abundant heavy metal detected at 60.39 mg/kg, suggesting potential industrial or anthropogenic input.
- Nickel (Ni) was present at 39.88 mg/kg, which may also contribute to soil toxicity if above permissible limits.
- Arsenic (As) was detected at a lower concentration of 0.39 mg/kg, but even small quantities of arsenic can be toxic depending on bioavailability and accumulation in crops.
- Cadmium (Cd) was found in trace amounts at 0.19 mg/kg, though cadmium is highly toxic and bio-accumulative even at low levels.
- Overall, the soil shows signs of heavy metal contamination, especially due to elevated levels of lead, which could pose risks to plant health, soil microbiota, and human exposure through the food chain.

IMPORTANT FINDINGS FROM OBJECTIVE 1:

- The study was conducted in the Chowbaga region of East Kolkata Wetlands and focused on analysing soil health and microbial ecology associated with *Cucurbita maxima* and *Zea mays* over two consecutive years.
- Soil samples were collected during three distinct cropping periods: initial, peak, and intercrop stages for both crops.
- In Year 1, the pH of soil under *Cucurbita maxima* cultivation decreased from 7.26 (initial) to 6.22 (peak) and then increased to 7.73 during the intercrop phase, indicating significant pH fluctuations. The minor decrease in pH of soil, during the peak growth period of *Cucurbita maxima* may be due to the liberation of H⁺ ions from plant roots, thereby leading to the changes in soil pH.
- Electrical conductivity (EC) remained stable (~0.3 dS/m) during initial and peak stages but dropped to 0.17 dS/m in the intercrop period, showing a loss in ionic activity. Macronutrients like nitrogen, phosphorus, and potassium decreased notably in the intercrop phase, indicating nutrient uptake by the crop and possible soil depletion. Calcium and magnesium showed reductions during the peak growth phase but calcium increased sharply from 480.96 to 1122.24 mg/kg during the intercrop stage, likely due to mineral return from plant residues or reduced uptake. Micronutrients such as iron, copper, boron, and zinc were observed to decline, particularly in the intercrop phase, potentially leading to soil nutrient imbalances.
- In Year 1, *Zea mays* soil pH declined slightly from 7.45 (initial) to 7.37 (peak), while EC peaked at 0.47 dS/m during peak growth and dropped sharply to 0.1 dS/m in the intercrop phase.
- *Zea mays* soil had peak nitrogen at approximately 537.2 kg/ha and phosphorus at 106.76 kg/ha during active growth, both of which reduced considerably in the intercrop period. Addition of fertilizers rich in nitrogen during the peak period of plant growth resulted in enhanced levels of nitrogen in soil.
- Calcium and magnesium also declined in the peak phase, supporting evidence of plant nutrient absorption. Micronutrients including boron, copper, zinc, iron, and manganese

were higher in the peak phase but declined during intercrop, indicating post-harvest nutrient leaching.

- In Year 2, *Cucurbita maxima* soil pH ranged from 6.77 to 7.01, fluctuating between acidic and neutral across the cropping phases. EC increased from 0.09 dS/m (initial) to 0.26 dS/m (peak) before slightly decreasing to 0.21 dS/m in the intercrop stage. Macronutrients continued to decline during the intercrop phase in Year 2, while micronutrients such as copper, zinc, and iron also reduced, reinforcing concerns of nutrient depletion.
- *Zea mays* soil in Year 2 became more acidic, with pH values ranging from 5.93 to 5.6 during the peak and intercrop periods, respectively. EC dropped drastically from 0.31 dS/m (initial) to 0.08 dS/m (intercrop), suggesting declining soil ionic concentration. Nitrogen and potassium levels were highest during the initial stage at 444.44 kg/ha and 143.81 kg/ha respectively, followed by steep declines through the crop cycle. Micronutrients like zinc, copper, iron, boron, and manganese decreased consistently, highlighting risks of soil nutrient imbalance over time.
- Metagenomic analysis in Year 1 for *Cucurbita maxima*, soil rhizosphere showed dominant phyla such as *Acidobacteriota*, *Patescibacteria*, *Planctomycetota*, and *Proteobacteria*. Genus-level diversity in *Cucurbita maxima*, soil rhizosphere included *Candidatus* sp, *Bryobacter* sp, *Bacillus* sp, with *Bacillus* sp and *Candidatus* sp being functionally important for plant growth.
- In *Zea mays*, the microbial community of soil metagenome showed consistent presence of *Gemmata* sp, *Nitrospira* sp, *Pirellula* sp, and *Candidatus* sp across all three cropping stages. *Bacillus* sp. appeared dominantly in the intercrop period in *Zea mays* soil, suggesting its resilience and potential beneficial role.
- Year 2 soil metagenomics in *Cucurbita maxima* rhizosphere revealed the continued presence of *Lysobacter* sp, *Polycyclovorans* sp, *Paenibacillus* sp, *Candidatus* sp, *Gemmata* sp, and *Bacillus* sp, thereby revealing them to be the lingering species.
- In *Zea mays* Year 2, the lingering microbial communities included *Rhodobacter* sp, *Rhodoplanes* sp, *Reyranella* sp, *Nitrospira* sp, *Paenibacillus* sp, *Candidatus* sp, *Gemmata* sp, and *Bacillus* sp across cropping phases.

- These bacterial genera are associated with plant growth-promoting properties, indicating healthy rhizospheric interactions despite physical nutrient losses.
- Heavy metal analysis revealed significantly high lead content at 398.76 mg/kg, posing a serious ecological and crop safety concern.
- Other detected heavy metals included chromium at 60.39 mg/kg, nickel at 39.88 mg/kg, arsenic at 0.39 mg/kg, and cadmium at 0.19 mg/kg, were well within the limits.
- The presence of these metals, especially lead, exceeds standard safety limits and suggests anthropogenic contamination that may harm soil microbial balance and crop health.
- Increase in diversity of micro-organisms in soil and biomass is also associated with the increased levels of organic carbon. Reduction of organic carbon is due to crop harvesting
- The findings underscore the need for targeted soil management strategies, such as nutrient replenishment, microbial consortia application, and remediation of heavy metal contamination to sustain long-term agricultural productivity.

4.2 To perform physical isolation, purification and characterization of potential microbes having dynamic role in soil development, and their role in mitigation of pollution (lead).

4.2.1. Isolation

The collected soil samples of initial, peak and intercrop of crops of all seasons were studied to identify the lingering ability of the bacterial isolates. The bacterial strains were isolated using spread plate method, whereby the colonies were selected on the basis of their predominance in different dilution plates. Initially 12 bacterial isolates were selected from different dilution plates (10^{-3} , 10^{-4}) on the basis of their prominence. From 12 bacterial isolates, 5 of the bacterial isolates were selected based on their lingering ability in all the sampling seasons, along with their diversity in their colony characters and relative abundance for further studies. They were found to be lingering flora during the initial, peak and intercropping seasons of both *C. maxima* and *Z. mays*, that were used for further characterization. They were named as DPP(A), DPP(B), DPP(C), DCP(A), and DCI(D). *Cucurbita maxima* (December-February) and *Zea mays* (March-May)

4.2.2. General Characterization of isolated bacterial strains

4.2.2a Gram Nature and Colony Morphology:

The bacterial isolates were subjected to determination of their Gram nature and analysis of their colony morphology was done. The data has been represented in **Table 4.1**.

BACTERIAL ISOLATES	GRAM NATURE	COLONY MORPHOLOGY
DPP(A)	Gram positive	Rod shaped, greyish white colony , margin entire
DPP(B)	Gram positive	Rod shaped, creamy-white colony ,margins are entire
DPP(C)	Gram negative	Rod shaped, light yellow colony, smooth margins
DCP(A)	Gram negative	Rod shaped, Creamy yellow in colony, margins are flat edged.
DCI(D)	Gram positive	Rod shaped, white colony, margin entire

Table 4.1: Gram staining and colony morphology of bacterial isolates

4.2.2b. Antibiotic sensitivity test:

The bacterial isolates were subjected to 10 antibiotics, namely Ciprofloxacin (CIP5), Rifampicin (RIF 5), Gentamycin (CN 30), Vancomycin (VA 30), Chloramphenicol (C10), Metronidazole (MET5), Meropenem (MRP 10), Tetracycline (TE 30), Bacitracin (B10), Polymyxin B (PB 300) and their sensitivity was tested as represented in **Figure 4.12**. The objective of this work was to evaluate the antibiotic susceptibility profile of the bacterial isolates in order to assess their resistance or sensitivity patterns for potential application in microbial consortia and safe agricultural or environmental use. All the bacterial isolates were found to be sensitive to the antibiotics used for testing.

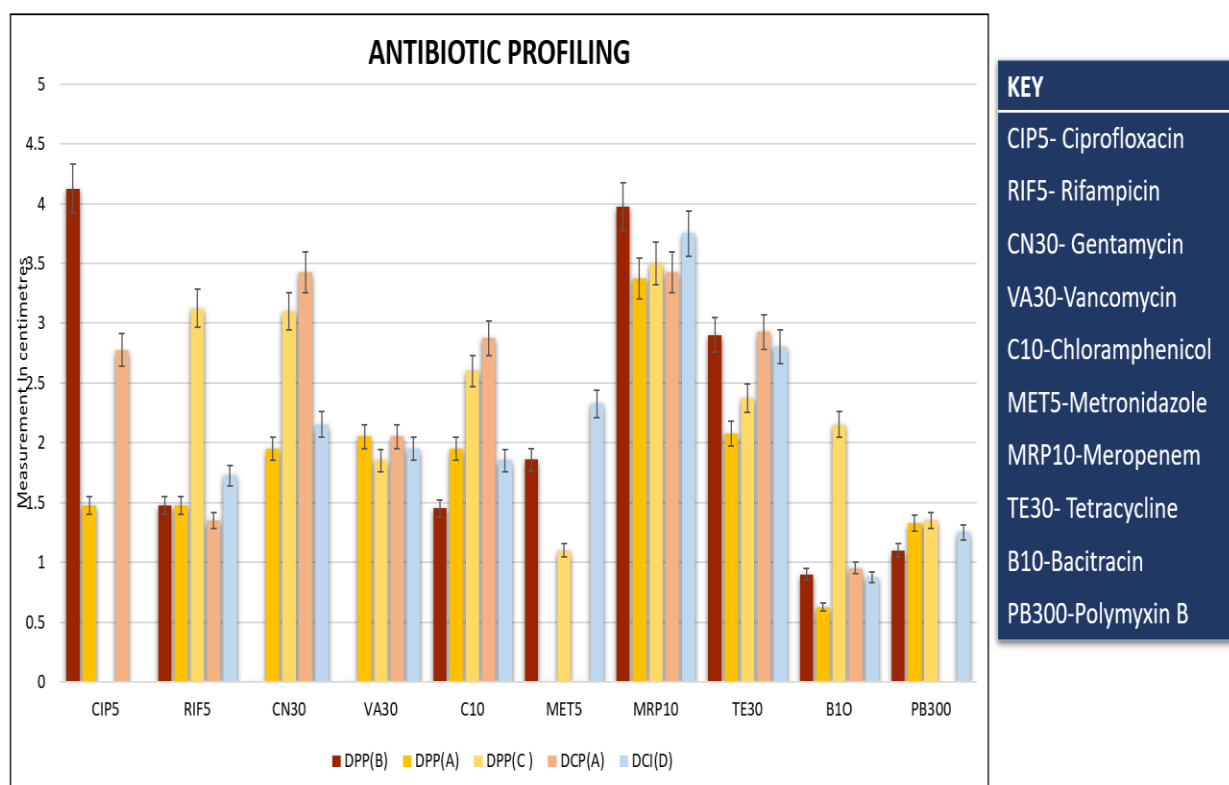


Figure 4.12: Antibiotic assay of bacterial isolates

4.2.2c Blood Agar Test: The bacterial isolates were streaked on blood agar media. The aim of this work was to assess the haemolytic activity of the bacterial isolates, by culturing them on blood agar media, in order to evaluate their potential toxicity and biosafety for agricultural or environmental applications, based on their haemolysis patterns (α , β , or γ). It was found that DPP(A) showed β haemolysis. DPP(B), DCP(A) and DPP(C) showed γ -haemolysis, while DCI(D) showed α -haemolysis. The results have been represented in **Figure 4.13**.

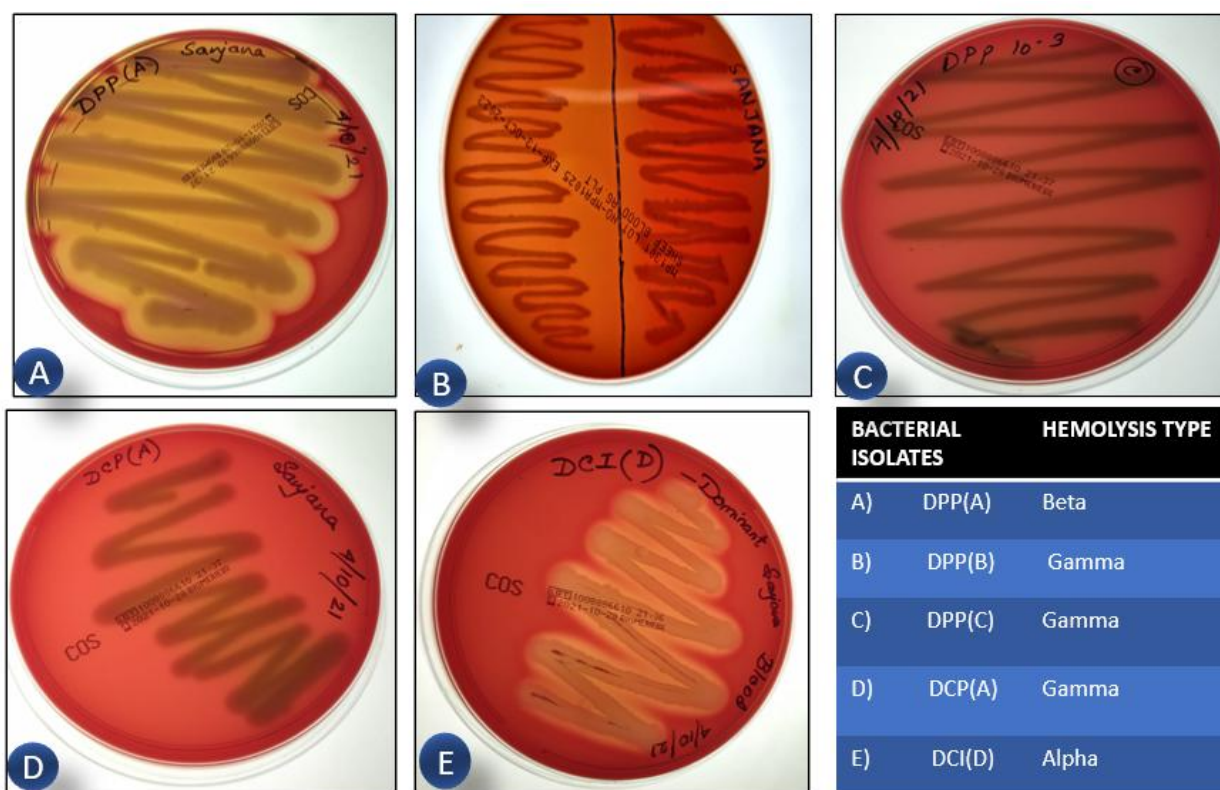


Figure 4.13: Streaking of bacterial isolates on blood agar and their analysis

4.2.2d Hi Crome Media

The bacterial strains when streaked on Hi Chrome UTI agar (**Figure 4.14 A, B, C**) and Hi Chrome *Bacillus* agar (**Figure 4.14 D, E, F**). **DPP(A), DPP(B), DCI(D)** showed similarity to the colour indexing found in *Bacillus* sp strains, whereas **DPP(C)** and **DCP(A)** showed similarity to *Pseudomonas* sp and *Proteus* sp respectively as represented in **Table 4.2**. The multitudinous variety of the colours visualised after 24 hours of incubation at 37°C in the respective chrome mediums (especially *Bacillus* chrome) indicated a variety of enzyme activities by the strains, which were responsible for the generation of colours (the basic scientific principle of a chromogenic medium).

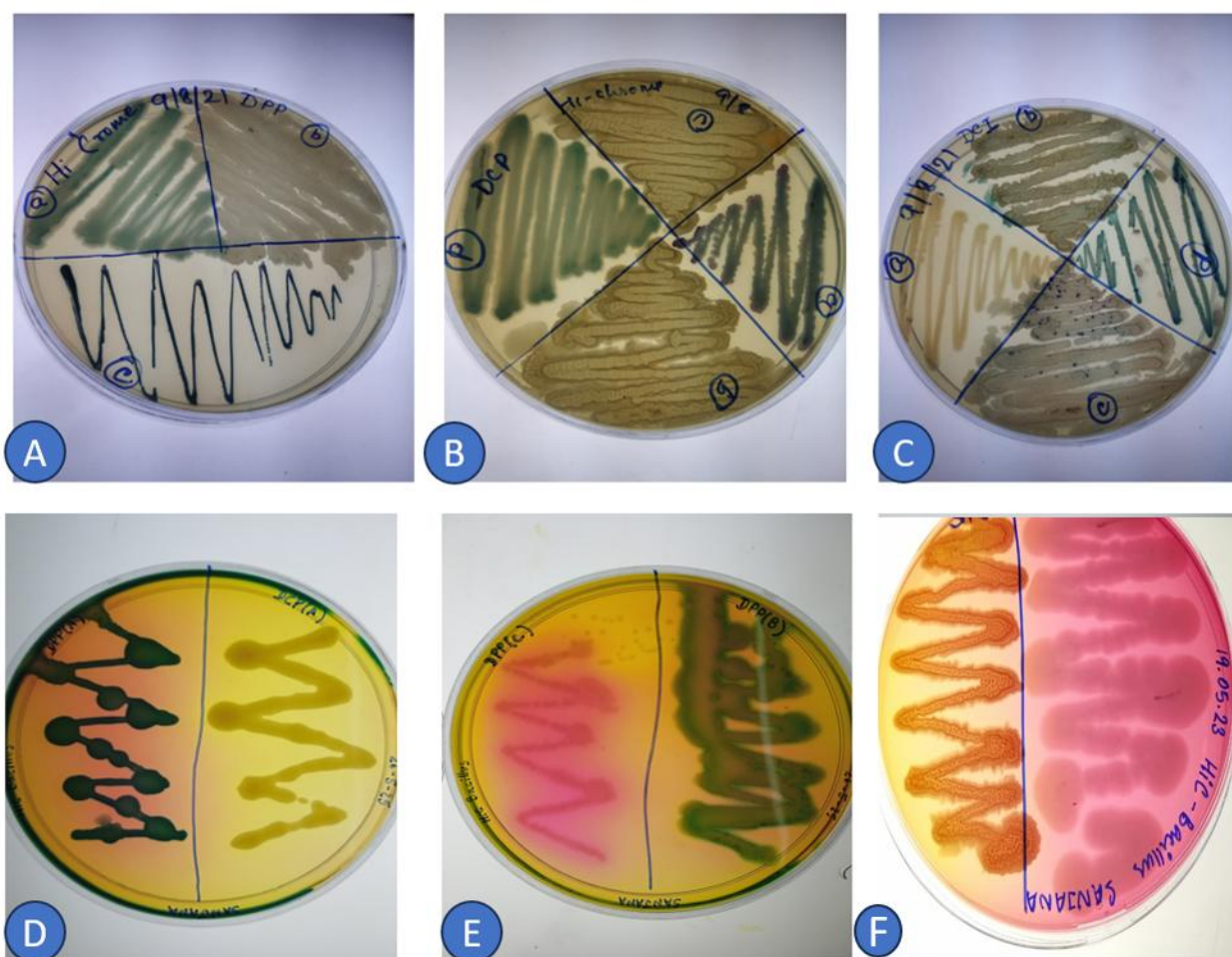


Figure 4.14: Streaking of bacterial isolates on Hi chrome media

BACTERIAL ISOLATE	HI-CROME BACILLUS	HI-CROME UTI
DPP(A)	<i>Bacillus</i> sp	
DPP(B)	<i>Bacillus</i> sp	
DPP(C)		<i>Pseudomonas</i> sp
DCP(A)		<i>Proteus</i> sp
DCI(D)	<i>Bacillus</i> sp	

Table 4.2: Table representing the presumptive identifications of bacterial isolates

Key Findings from 4.2.1 and 4.2.2

- A total of 12 bacterial isolates were initially obtained from the rhizospheric soil samples, out of which 5 isolates were found to be consistently present (lingering flora) across the Initial, Peak, and Intercrop seasons in both *Cucurbita maxima* and *Zea may* soils.
- These five key isolates were selected for further characterization and were designated as: DPP(A), DPP(B), DPP(C), DCP(A) and DCI(D)
- Gram staining and colony morphology studies were conducted as part of the general characterization of these isolates.
- The antibiotic susceptibility test of bacterial isolates was carried out to evaluate their safety and resistance patterns for agricultural and environmental applications.
- DPP(A) and DCP(A) were found to be slightly resistant to Metronidazole, which has a role in inhibition of protein synthesis by interacting with DNA. DPP(C) was found to be resistant to Ciprofloxacin, with a role in inhibiting DNA replication in bacteria.
- Blood agar streaking and analysis showed haemolytic patterns, contributing to phenotypic profiling of the strains. The bacterial strains were cultured on HI Chrome UTI and *Bacillus* chromogenic media, whereby all five isolates showed distinct colour development, confirming diverse enzyme activity, a hallmark of metabolically active bacteria.
- The 5 bacterial strains showed colorations in Hi-chrome UTI agar, due to their abilities to cleave the chromogenic substrates by production of enzymes such as β -glucosidase and β -D-galactosidase or both.
- Colour profiles on HI Chrome Bacillus agar indicated that: DPP(A), DPP(B), and DCI(D) closely matched *Bacillus* sp. based on chromogenic reaction patterns, DPP(C) was identified as *Pseudomonas* sp and DCP(A) showed similarity with *Proteus* sp.

- The chromogenic response confirmed the presence of multiple functional enzyme activities across the isolates, supporting their biochemical versatility.
- Overall, the five selected isolates represent a diverse and potentially beneficial bacterial consortium, with three *Bacillus* spp., one *Pseudomonas* sp., and one *Proteus* sp., all known for important ecological roles in soil health, nutrient cycling, and plant growth promotion.

4.2.3. Molecular identification of the isolated strains

The objective of the molecular identification of the isolated bacterial strains was to determine their taxonomic identity accurately through 16S rRNA gene sequencing. This was done to confirm their genus and species-level classification, establish their novelty or similarity to known strains, and to enable deposition of their genetic information in the NCBI database for global accessibility, scientific validation, and future reference in agricultural or environmental applications.

Molecular identification of all the bacterial isolates namely DPP(A), DPP(B), DPP(C), DCP(A) and DCI (D) were submitted to NCBI.

The identified bacterial isolates were as follows:

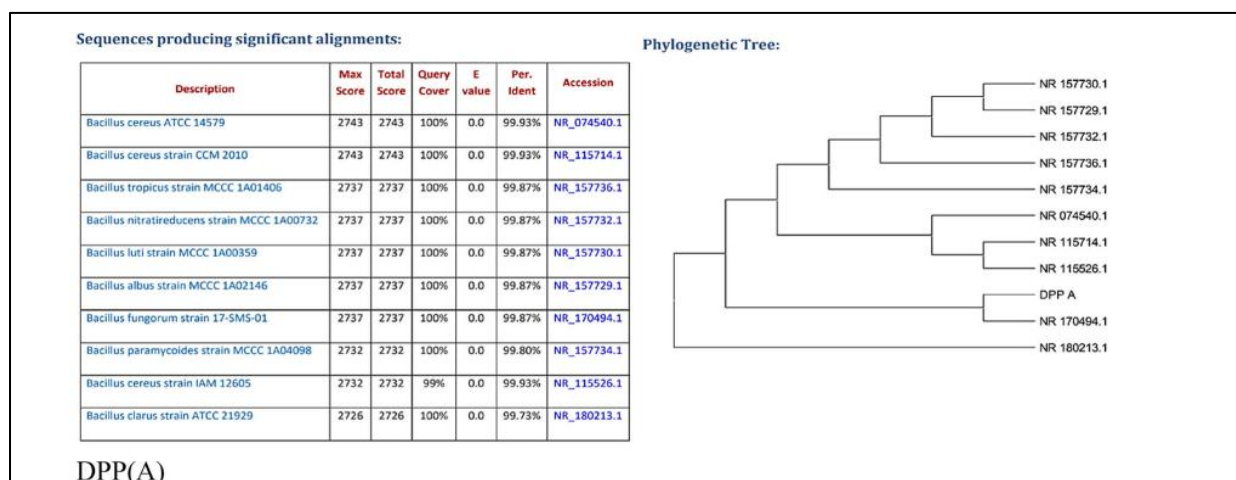
DPP(A): *Bacillus cereus strain DPP(A)_SGAM_SXC* (Accession number OQ216888)

DPP(B): *Bacillus velezensis strain DPPB_SGAM_SXC* (Accession number ON384543)

DPP(C): *Stenotrophomonas maltophilia strain DPP(C)_SGAM_SXC* (Accession number OQ217020)

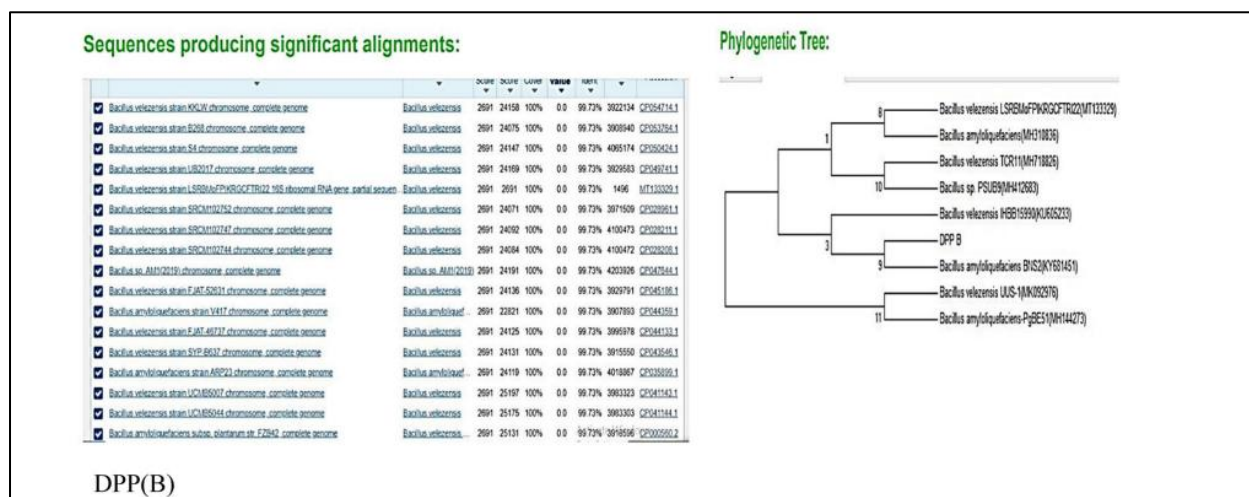
DCP(A): *Cupriavidus necator strain DCPA_SGAM_SXC* (Accession number ON384541)

DCI(D): *Bacillus cereus strain DCI(D)_SGAM_SXC* (Accession number OQ217005)



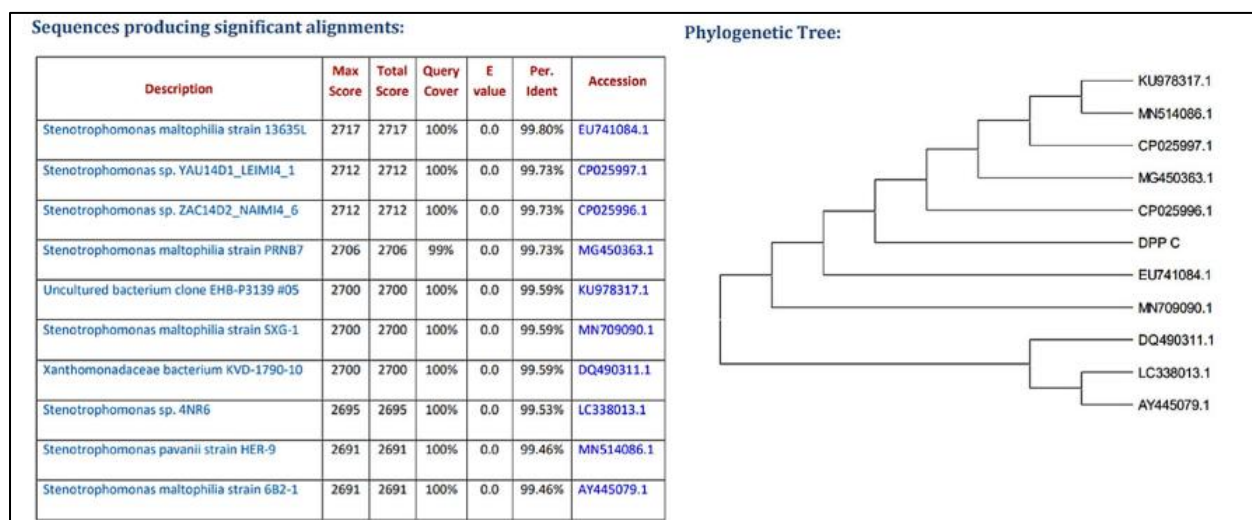
DPP(A): *Bacillus cereus*

Figure 4.15: The phylogenetic tree of isolate DPP(A) along with total score and query cover comparison of top 10 hits from NCBI BLAST results indicating the phylogenetic identification and evolutionary positioning of the isolate DPP(A).



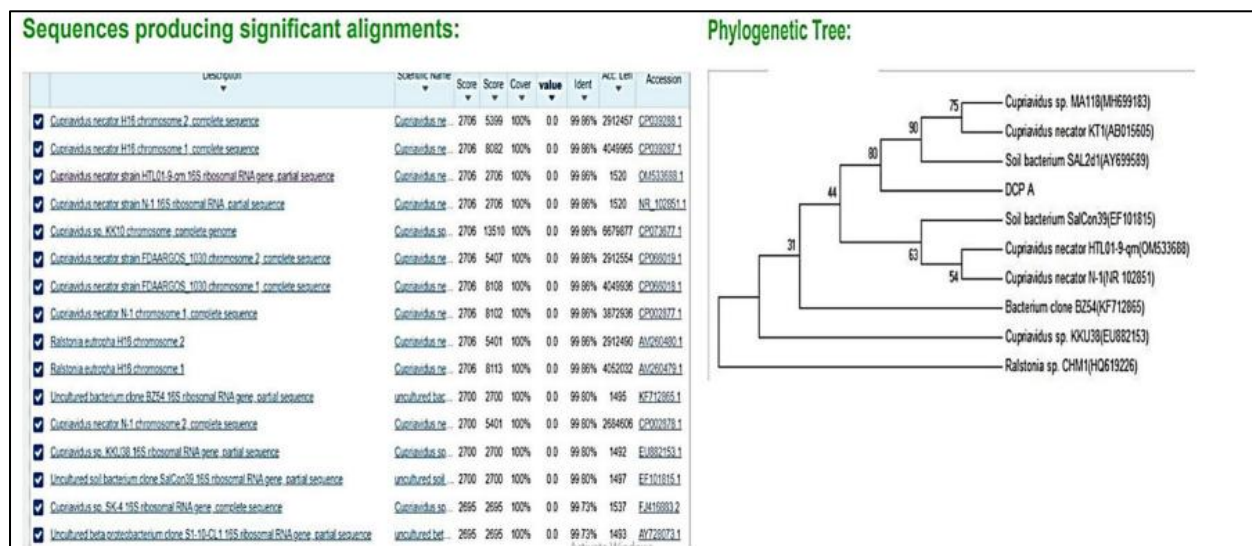
DPP(B): *Bacillus velezensis*

Figure 4.16: The phylogenetic tree of isolate DPP(B) along with total score and query cover comparison of top 10 hits from NCBI BLAST results indicating the phylogenetic identification and evolutionary positioning of the isolate DPP(B).



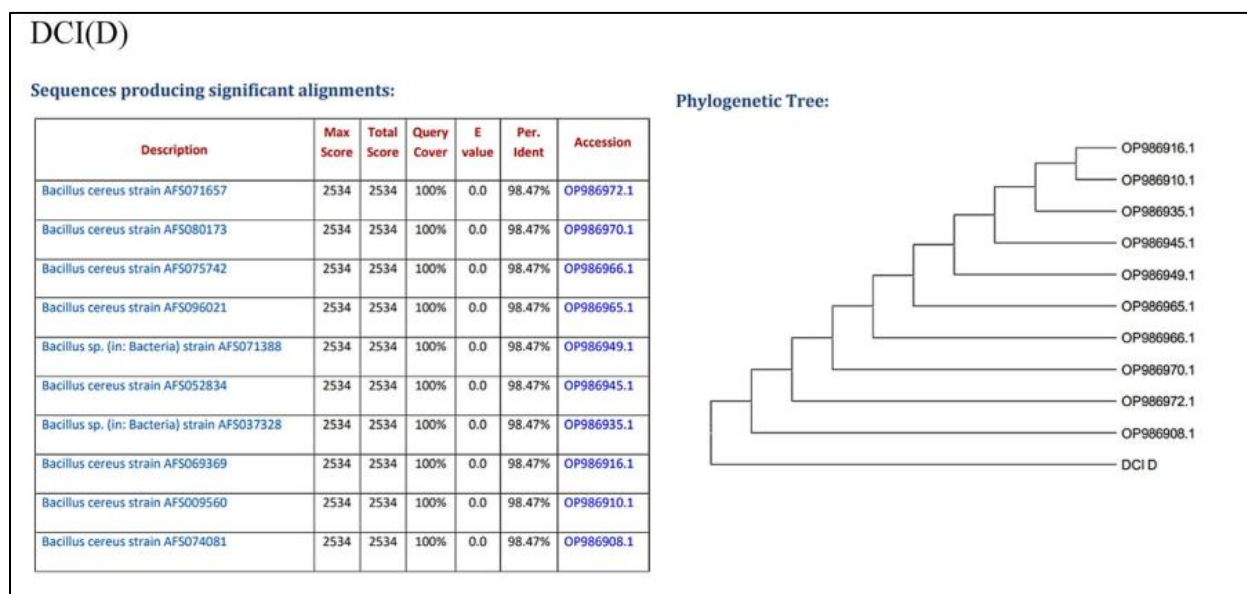
DPP(C): *Stenotrophomonas maltophilia*

Figure 4.17: The phylogenetic tree of isolate DPP(C) along with total score and query cover comparison of top 10 hits from NCBI BLAST results indicating the phylogenetic identification and evolutionary positioning of the isolate DPP(C).



DCP(A): *Cupriavidus necator*

Figure 4.18: The phylogenetic tree of isolate DCP(A) along with total score and query cover comparison of top 10 hits from NCBI BLAST results indicating the phylogenetic identification and evolutionary positioning of the isolate DCP(A).



DCI(D): *Bacillus cereus*

Figure 4.19: The phylogenetic tree of isolate DCI(D) along with total score and query cover comparison of top 10 hits from NCBI BLAST results indicating the phylogenetic identification and evolutionary positioning of the isolate DCI(D).

KEY FINDINGS from Section 4.2.3:

- Molecular identification of the five selected bacterial isolates was performed, and the sequences were successfully submitted to the NCBI database.
- The identified strains and their corresponding NCBI accession numbers are as follows:
 - a) DPP(A) was identified as *Bacillus cereus* strain DPP(A)_SGAM_SXC, Accession number: OQ216888
 - b) DPP(B) was identified as *Bacillus velezensis* strain DPPB_SGAM_SXC, Accession number: ON384543
 - c) DPP(C) was identified as *Stenotrophomonas maltophilia* strain DPP(C)_SGAM_SXC, Accession number: OQ217020
 - d) DCP(A) was identified as *Cupriavidus necator* strain DCPA_SGAM_SXC, Accession number: ON384541
 - e) DCI(D) was identified as *Bacillus cereus* strain DCI(D)_SGAM_SXC, Accession number: OQ217005

- The molecular characterization confirmed that three of the five isolates belong to the genus *Bacillus*, including two distinct strains of *Bacillus cereus* and one strain of *Bacillus velezensis*.
- The presence of *Stenotrophomonas maltophilia* and *Cupriavidus necator* among the isolates adds functional diversity to the consortium, as both are known for bioremediation potential and metabolic versatility (Harshitha *et al.*,2024)
- These results provide a precise taxonomic identity for the rhizospheric isolates and strengthen their potential applications in plant growth promotion, biocontrol, and soil health restoration.

4.2.4. Characterization of plant growth promoting properties

4.2.4a Assay for nitrogen fixing abilities of the selected strains:

The objective of this study was to evaluate the plant growth-promoting (PGP) properties of the five bacterial isolates— two variants of *B. cereus* (DPP-A; DCI-D), followed by *B. velezensis* (DPP-B), *S. maltophilia* (DPP-C) and *C. necator* (DCP-A) by assessing their functional abilities in key nutrient transformation processes. The aim of the work was to determine the ability of bacterial isolates to fix atmospheric nitrogen using nitrogen-deficient medium with bromothymol blue as an indicator. This characterization was intended to establish the potential of these isolates for use as biofertilizers in sustainable agriculture by enhancing nutrient availability and promoting plant growth. The results are represented in **Figure 4.20**.

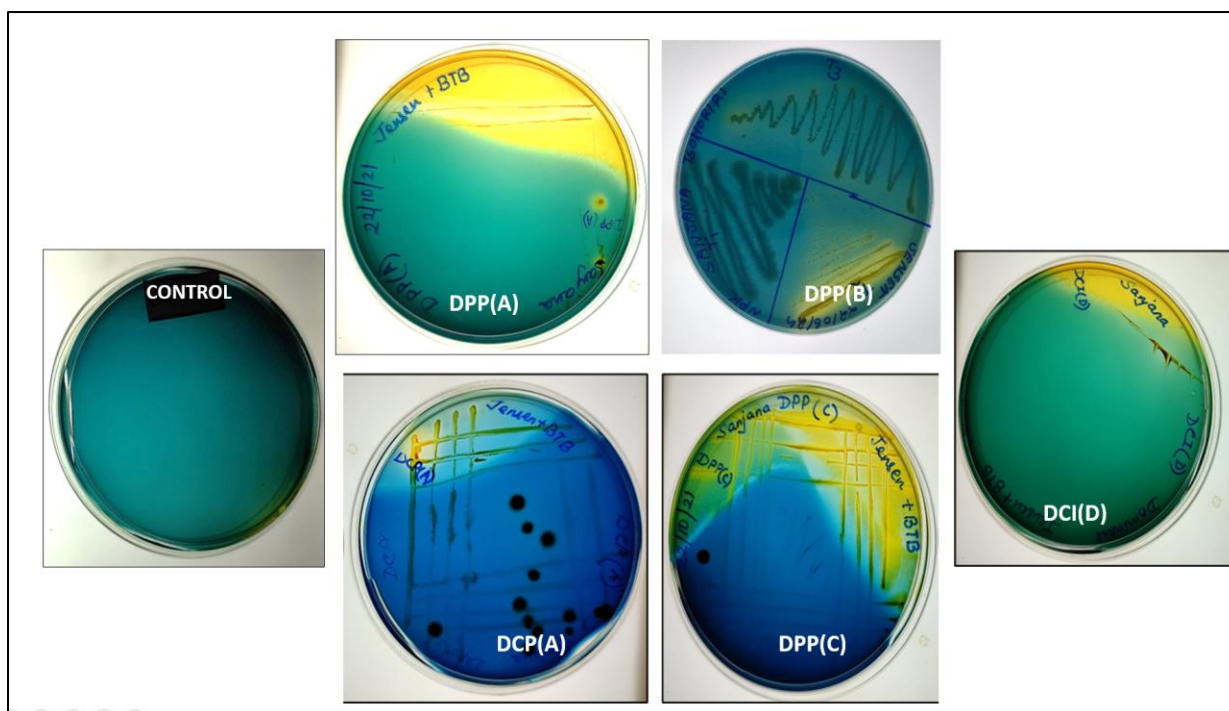


Figure 4.20: Qualitative analysis of macronutrient synthesizing ability of bacterial isolates using modified Jensen media for nitrogen fixing ability. The positive nitrogen fixing bacteria have shown change in colour of the media from blue to yellow.

KEY FINDINGS from Figure 4.2.4a

- The 5 bacterial strains were tested for nitrogen fixing abilities. All the strains namely, two variants of *B. cereus*, followed by *B.velezensis*, *S.maltophilia* and *C.necator* grew luxuriantly on nitrogen-free modified Jensen's medium supplemented with Bromothymol blue (indicator).
- The change in the colour of the supplemented indicator from bluish green to blue to greenish yellow to yellow indicated changing pH of the media due to the production of acid. This change in the pH of media due to acid production indicates steps of ammonification and nitrification in case of nitrogen fixing bacterial strains.
- *B.cereus*(DPP(A) and *S.maltophilia* (DPP-C) showed sharp decrease in pH value after 48 hours and 72 hours of observation, leading to yellowing of the media indicating rapid nitrification. by *B.velezensis*(DPP-B), *B.cereus*(DCI(D)) and *C.necator*(DCP-A) showed a slow change in the colour of media indicating ammonification after 48 hours of observation and slow nitrification after 72 hours of observation.

4.2.4b Assay for potassium solubilization of the selected strains

The determination of potassium solubilization potential of the isolates, were done by streaking the bacterial isolates on insoluble potassium containing Aleksandrow agar media supplemented with bromothymol blue (indicator). The aim of the work was to evaluate the potassium solubilization potential of bacterial isolates by using Aleksandrow agar supplemented with bromothymol blue, and to assess the acidification of the medium as an indicator of organic acid production responsible for the solubilization of insoluble potassium compounds. The results have been represented in **Figure 4.21**.

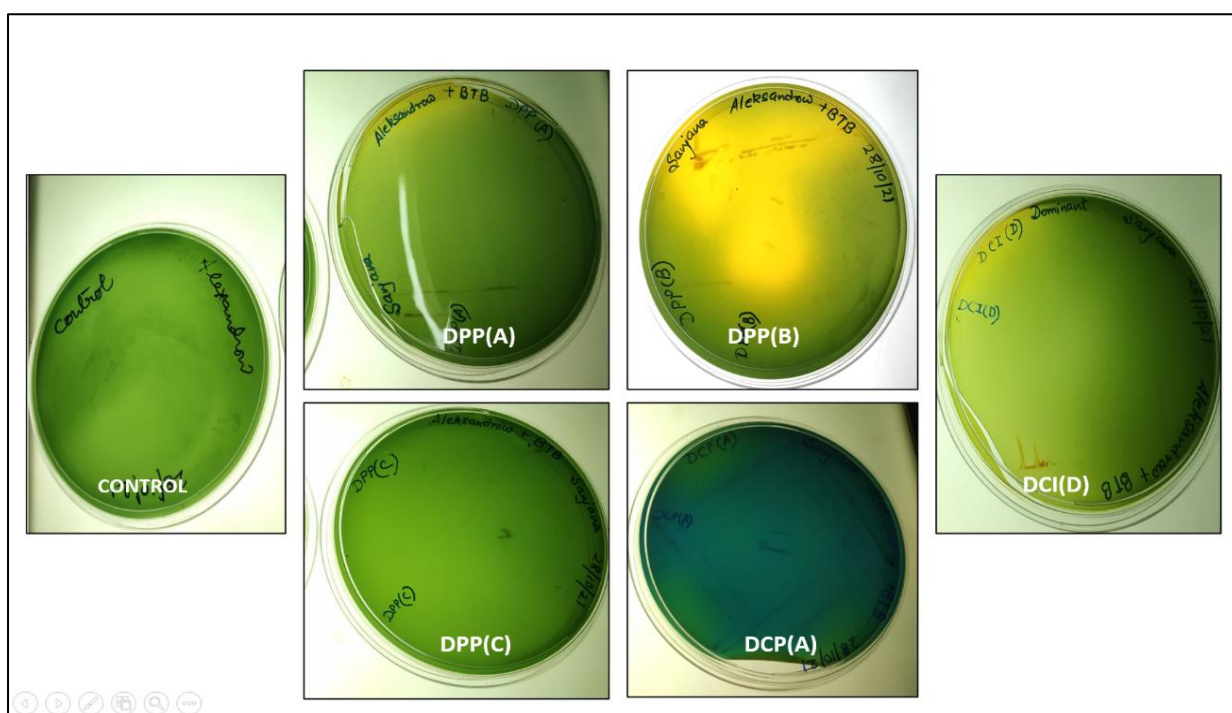


Figure 4.21: Qualitative analysis of macronutrient synthesizing ability of bacterial isolates using modified Aleksandrow media for testing the potassium solubilizing activity. The positive potassium solubilizing bacteria have shown change in colour of the media from green to yellow.

KEY FINDINGS from Figure 4.2.4b:

- The strain *B. velezensis* (DPP-B) showed a sharp decrease in the pH of the media leading to colour change from green to yellow, showing its intense ability to solubilize potassium.
- The two variant strains of *B. cereus* DPP(A), DCI(D) showed faint colour change in the colour of the media, indicating a decline in pH.
- The pH drop indicates in the media indicates production of different organic acids including α -ketogluconic acid, succinic acid, oxalic acid, citric acid, etc. that results in the solubilization of inorganic potassium.
- The bacterial isolate *S. maltophilia* (DPP- C) did not show any colour change in the media.

4.2.4c Assay for phosphate solubilization of the selected strains

The inorganic phosphate solubilization ability of the 5 strains were studied by streaking the strains on Pikovskaya agar medium. After 48 hours of incubation, luxuriant growth was observed in all cases (**Figure 4.22**). For conclusive determination of the phosphate solubilization ability, the concentration of phosphate solubilization was measured at 430 nm after 3 days of incubation (**Figure 4.23**). The aim of the work was to evaluate the phosphate solubilization potential of bacterial isolates by using modified Pikovskaya agar supplemented with bromothymol blue, and to assess the acidification of the medium as an indicator of organic acid production responsible for the solubilization of insoluble potassium compounds.

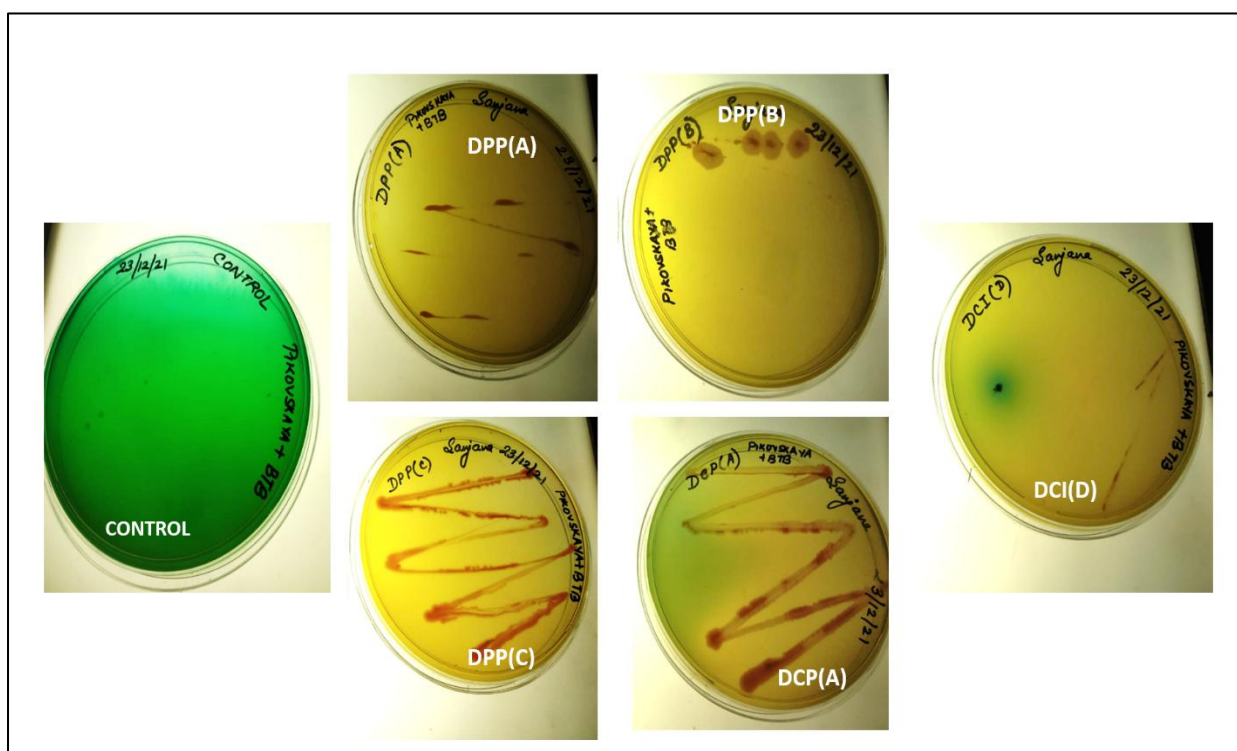


Figure 4.22: Qualitative analysis of macronutrient synthesizing ability of bacterial isolates using modified Pikovskaya media for testing the phosphate solubilizing activity. The positive phosphate solubilizing bacteria have shown change in colour of the media from green to yellow.

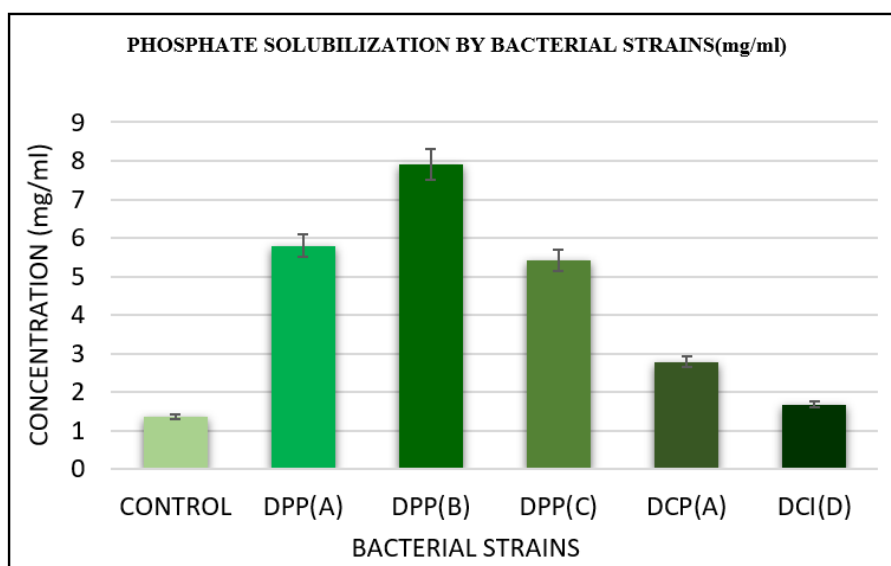


Figure 4.23: Quantification of phosphate content of bacterial isolates

Key Findings from 4.2.4c:

- Plant Growth promoting attributes of bacterial isolates were tested. It was found that all bacterial isolates namely two variants of *B. cereus* (DPP-A; DCI-D), followed by *B. velezensis* (DPP-B), *S. maltophilia* (DPP-C) and *C. necator* (DCP-A) grew luxuriantly on phosphate deficient media.
- All the bacterial isolates streaked on Pikovskaya media, each modified with bromothymol blue (pH indicator), showed colour change, thereby they were found to have the ability to solubilize phosphorous (**Figure 4.22**).
- The ability of the bacterial isolates to solubilize phosphorous was quantified, whereby the bacterial isolates, namely *B. cereus* (DPP(A)) followed by *B. velezensis* (DPP-B), *S. maltophilia* (DPP-C), *C. necator* (DCP-A) and *B. cereus* (DCI(D)) were able to solubilize 5.79mg/ml, 7.91 mg/ml, 5.41 mg/ml, 2.78 mg/ml and 1.98 mg/ml of phosphate respectively (**Figure 4.23**). The control set up showed low phosphate solubilizing property in comparison to the test set up.

4.2.5 Plant growth regulator production

4.2.5a Quantitative estimation of Indole Acetic Acid production

The objective of this work was to evaluate the plant growth-promoting potential of the isolated bacterial strains by assessing their ability to biosynthesize phytohormones—specifically Indole-3-acetic acid (IAA) in the presence and absence of tryptophan, under laboratory conditions, and to compare their production levels with control setups to determine their efficacy as bioinoculants for sustainable agriculture. Auxin production is required to enhance plant growth and development by directly stimulating root elongation and proliferation, which in turn improves nutrient and water uptake and strengthens the plant-microbe association. This bacterial production of auxin strengthens the plant-microbe relationship and is a major. The results for estimation of IAA have been represented in **Figure 4.24**.

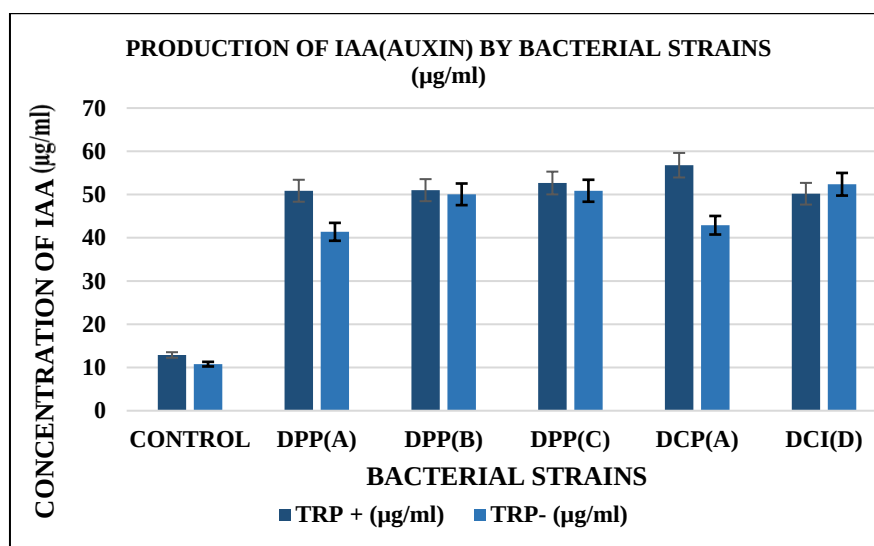


Figure 4.24: Quantification of IAA content in the bacterial isolates

KEY FINDINGS from Figure 4.2.5a:

- All the 5 bacterial strains could produce Indole Acetic Acid (auxins) in both presence and absence of the precursor tryptophan.
- All the bacterial isolates namely *B. cereus* (DPP-A), followed by *B. velezensis* (DPP-B), *S. maltophilia* (DPP-C), *C. necator* (DCP-A) and *B. cereus* (DCI-D) were able to produce 50.875 µg/mL, 51.012 µg/mL, 52.665 µg/mL, 56.785 µg/mL and 50.187 µg/mL of IAA. The production of IAA declined in absence of tryptophan. The control setup, showed relatively less amount of IAA production both in presence and absence of tryptophan, as compared to the test setup.
- *C. necator* (DCP-A) produced the maximum amounts of IAA in presence of tryptophan compared to the rest of the bacteria.

4.2.5b Quantitative estimation of Gibberellic acid (GA₃) production

The objective of this work was to evaluate the plant growth-promoting potential of the isolated bacterial strains by assessing their ability to biosynthesize phytohormones—specifically Gibberellin under laboratory conditions, and to compare their production levels with control setups to determine their efficacy as bioinoculants for sustainable agriculture. This phytohormones govern essential growth processes including seed germination, stem elongation, and flowering. The results for estimation of Gibberellin have been represented in **Figure 4.25**.

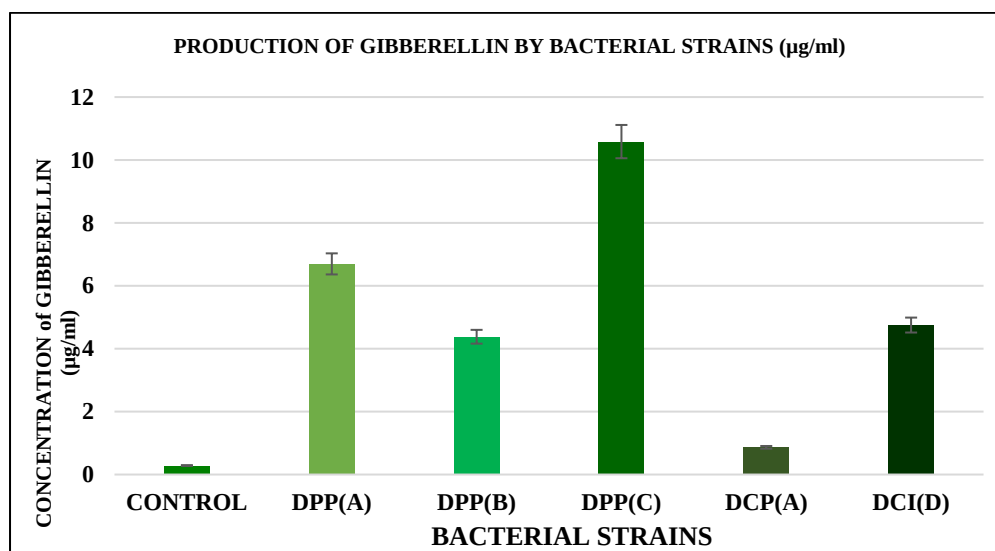


Figure 4.25: Quantification of Gibberellic acid content in the bacterial isolates

Key Findings from Figures 4.2.5b:

- Gibberellin production by bacterial isolates were tested for all the bacterial isolates. They showed the ability of production of gibberellin, wherein bacterial isolates *B. cereus* (DPP-A), followed by *B. velezensis* (DPP-B), *S. maltophilia* (DPP-C), *C. necator* (DCP-A) and *B. cereus* (DCI(D) were able to produce 6.696, $\mu\text{g/mL}$ 4.38 $\mu\text{g/mL}$, 10.584 $\mu\text{g/mL}$, 0.864 $\mu\text{g/mL}$ and 4.752 $\mu\text{g/mL}$ of gibberellin. Gibberellin production ability of the test set up was higher, when compared to the control set.
- All the 5 bacterial strains produced considerable amounts of gibberellins, out of which *S. maltophilia* (DPP-C) produced the highest quantity of gibberellin. It was quantified on 7th day of incubation, which also exhibits the continued and sustained production of the growth regulators, which would promote plant growth for a longer period of time.

4.2.6. Estimation of abiotic stress-responsive enzyme

Ethylene production is triggered by physical stresses like, drought, high temperature, heavy metals etc. 1-aminocyclopropane-1-carboxylic acid (ACC deaminase) is a non-protein amino acid that is the direct precursor of Ethylene. ACC deaminase plays a crucial role in promoting plant growth and reducing stress responses by breaking down the ethylene precursor, 1-aminocyclopropane-1-carboxylic acid (ACC). This enzyme helps to mitigate the negative effects of ethylene, a plant hormone involved in stress responses and senescence.

The bacterial isolates were tested for their ability to produce ACC Deaminase. The objective of the work was to assess the ability of the isolated bacterial strains to produce ACC deaminase both qualitatively and quantitatively, in order to evaluate their potential in mitigating plant stress by lowering ethylene levels, and to identify the most efficient strains for use as plant growth-promoting rhizobacteria (PGPR). The results have been represented in **Figure 4.26** and **Figure 4.27**

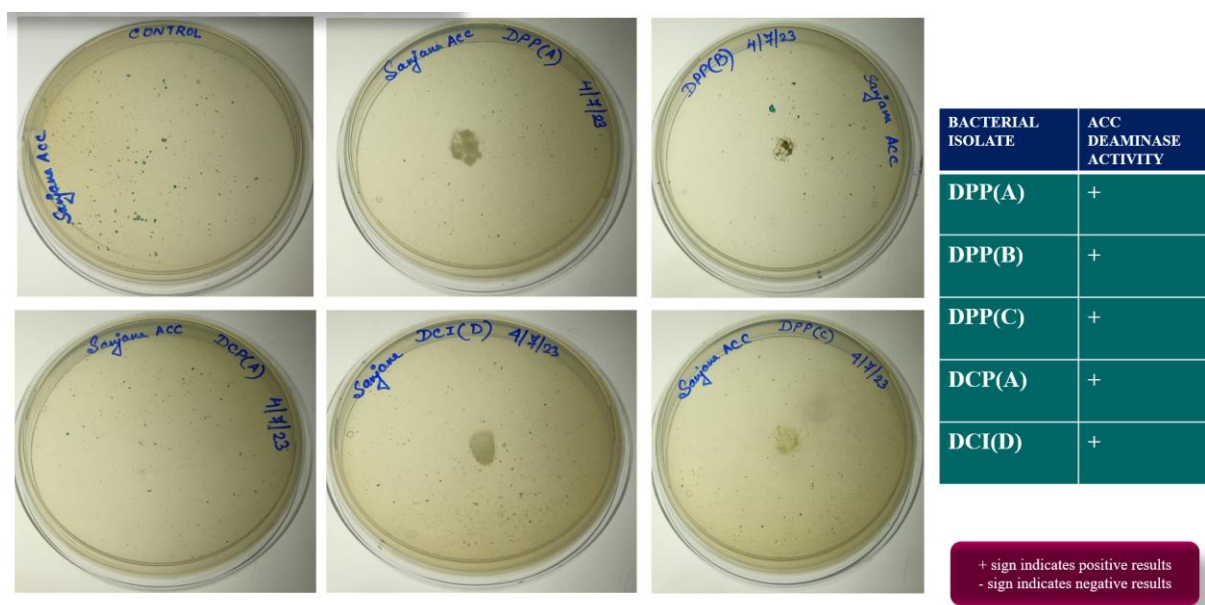


Figure 4.26: Qualitative evaluation of ACC deaminase of bacterial isolates

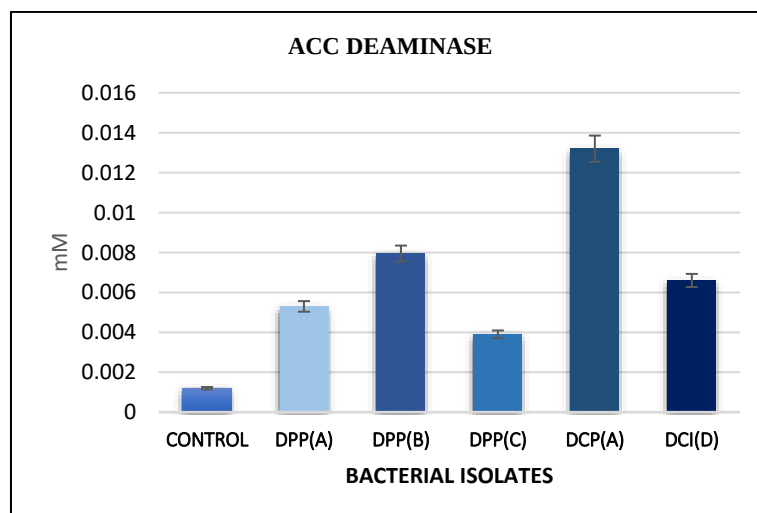


Figure 4.27: Quantitative estimation of ACC deaminase of bacterial isolates

The control set up showed very low activity (0.001 mM), indicating that there is no intrinsic ACC deaminase present. Among the isolates, *C.necator* (DCP-A) demonstrated the highest activity (0.013 mM), which implies a significant capacity to regulate ethylene levels and possibly improve plant stress tolerance. *B.velezensis* (DPP-B) exhibited moderate activity (0.008 mM), followed by *B. cereus* DCI(D) with 0.0065 mM and *B. cereus* DPP(A) at 0.005 mM, while *S.maltophilia* had the least activity among the isolates at 0.0035 mM.

Key Findings from 4.2.6:

- Ethylene is a plant stress response hormone. It causes senescence in the plant cells, thus causing stress in the plant body and eventual death.
- All the bacterial isolates showed the ability of production of ACC Deaminase qualitatively; upon quantitative analysis it was found that bacterial isolates *B.velezensis* (DPP-B) and *C.necator* (DCP-A) produced increased quantity of ACC Deaminase as compared to the other bacterial isolates
- Plant growth promoting bacteria can produce the enzyme ACC deaminase by which they can utilise ACC as a Nitrogen source thereby decreasing ethylene production and enhancing plant survival rate in times of physical stress.

4.2.7. Estimation of enzymes of bacterial isolates

All the bacterial isolates were tested for their enzyme production activity. The objective of the work was to evaluate the enzymatic potential of the isolated bacterial strains by assessing their ability to produce key hydrolytic and oxidative enzymes such as urease, cellulase, laccase, lignin peroxidase, amylase, and pectinase, with the aim of understanding their possible roles in nutrient cycling, organic matter decomposition, and plant growth promotion.

4.2.7a Estimation of urease activity:

Urease plays a crucial role in the breakdown of urea, a common nitrogen fertilizer into ammonia and carbon dioxide. This process is vital for nutrient cycling and plant growth, as it makes nitrogen readily available for uptake by microorganisms and plants. The aim of the work was to assess the urease activity of bacterial isolates in order to evaluate their potential in converting urea into plant-available forms of nitrogen, thereby contributing to nitrogen cycling and promoting plant growth. The results have been represented in **Figure 4.28** and **Figure 4.29**.

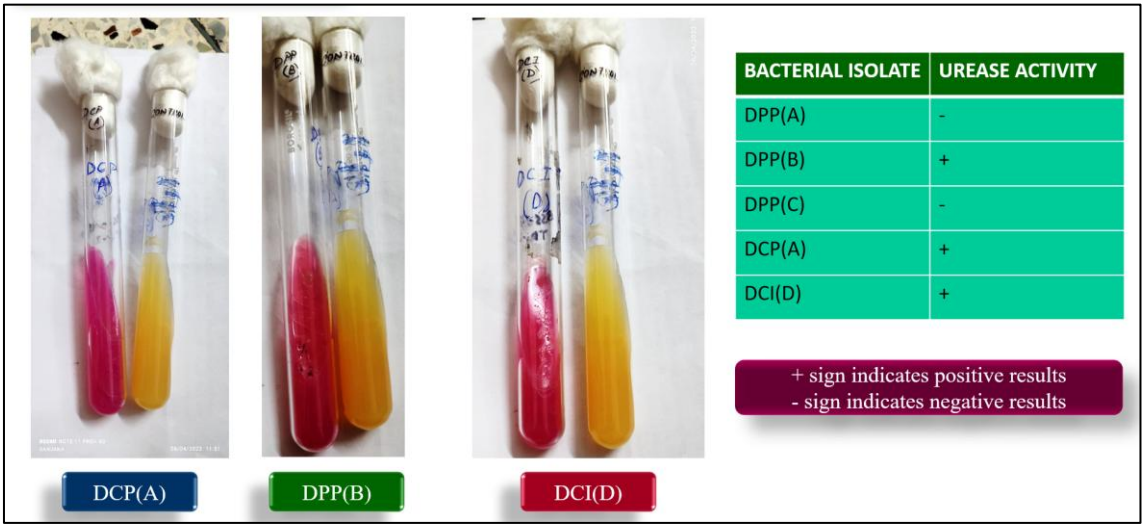


Figure 4.28: Qualitative analysis of urease production by bacterial isolates

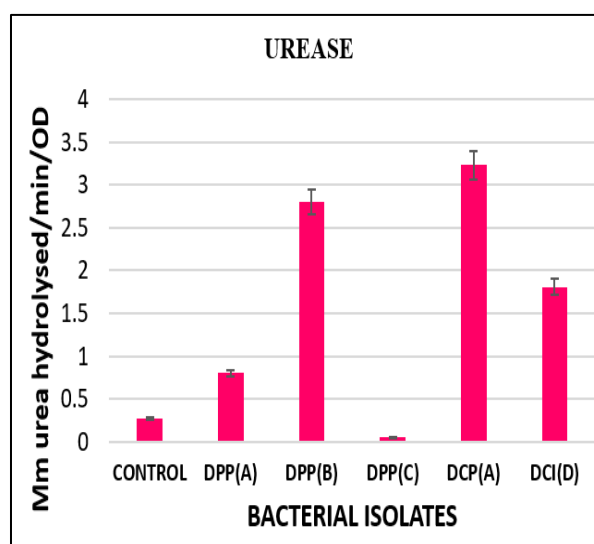


Figure 4.29: Quantitative analysis of urease production by bacterial isolates

KEY FINDINGS from 4.2.7a:

- Urease plays a crucial role in the breakdown of urea, a common nitrogen fertilizer, into ammonia and carbon dioxide. This process is vital for nutrient cycling and plant growth, as it makes nitrogen readily available for uptake by microorganisms and plants. The highest ability for urease production was seen in *C.necator* (DCP-A) with 3.23 mM urea hydrolyzed/min/OD

4.2.7b Estimation of cellulase activity

Cellulase plays a crucial role in breaking down cellulose, a major component of plant cell walls. This process is essential for nutrient cycling and decomposition of organic matter in the soil ecosystem. The aim of the work was to evaluate the cellulase activity of bacterial isolates for their ability to degrade cellulose, thereby assessing their role in organic matter decomposition and nutrient cycling within the soil ecosystem. The results have been represented in **Figure 4.30** and **4.31**.

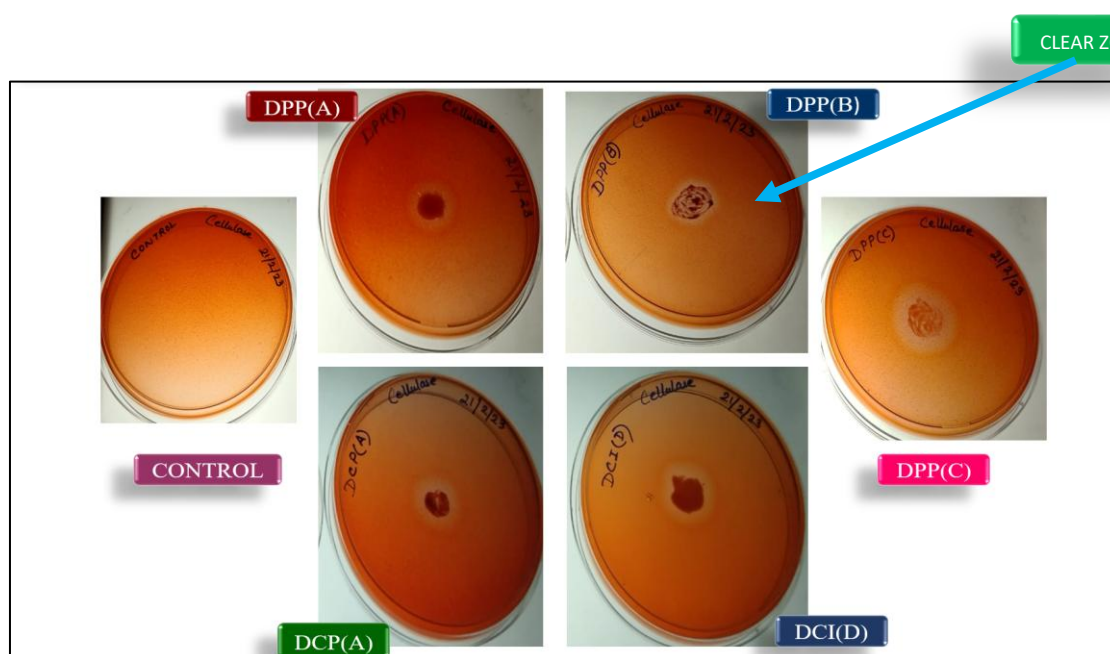


Figure 4.30: Qualitative analysis of cellulase production by bacterial isolates. The cellulase positive bacterial isolates, showed a zone of clearance in the media.

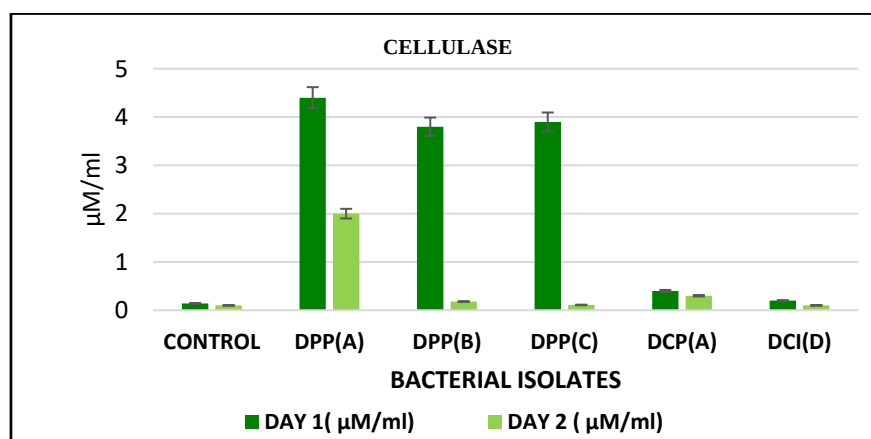


Figure 4.31: Quantitative analysis of cellulase production by bacterial isolates

KEY FINDINGS from 4.2.7b

- The quantification of cellulase production by bacterial isolates, showed that *B.cereus* DPP(A) and *C. necator* DCP(A) showed highest ability of cellulase production at 4.4µM/ml and 3.9 µM/ml respectively

4.2.7c Estimation of laccase activity:

Laccase, a copper-containing oxidase enzyme, plays a vital role in soil ecology, particularly in the degradation of organic matter and xenobiotics, and in microbial interactions. Bacterial laccases are involved in the transformation of lignin, polyphenols, and humic substances, contributing to the carbon cycle and nutrient cycling in soils. The objective of the work was to assess the laccase activity of bacterial isolates to evaluate their role in the degradation of lignin, polyphenols, and humic substances, thereby determining their contribution to organic matter transformation, carbon cycling, and overall soil nutrient dynamics. The results have been represented in **Figure 4.32 and 4.33**.

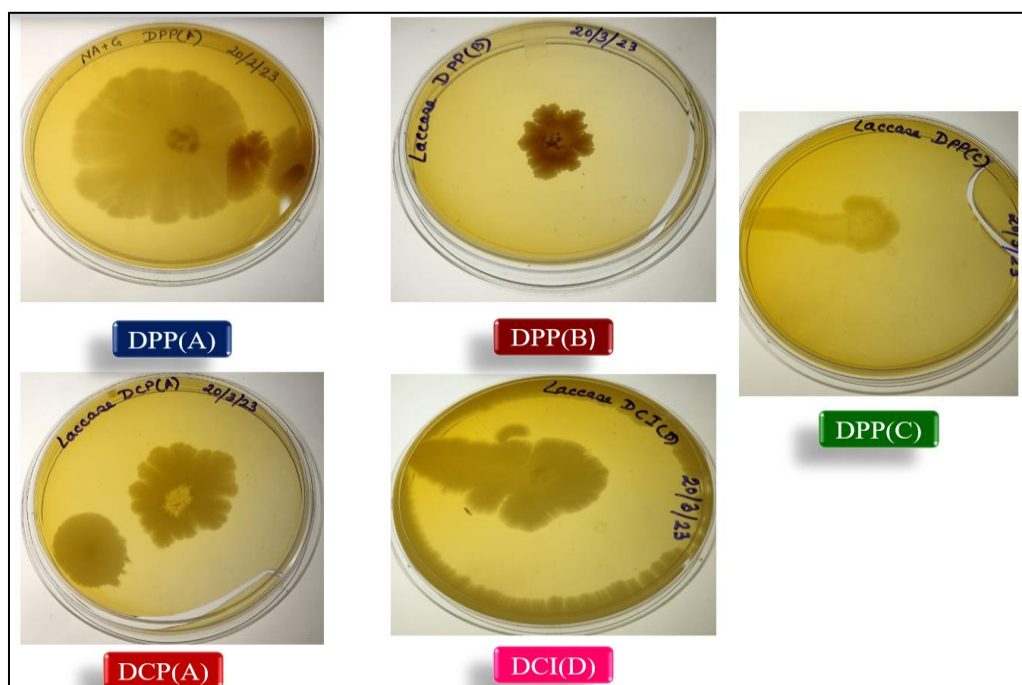


Figure 4.32: Qualitative analysis of laccase production by bacterial isolates

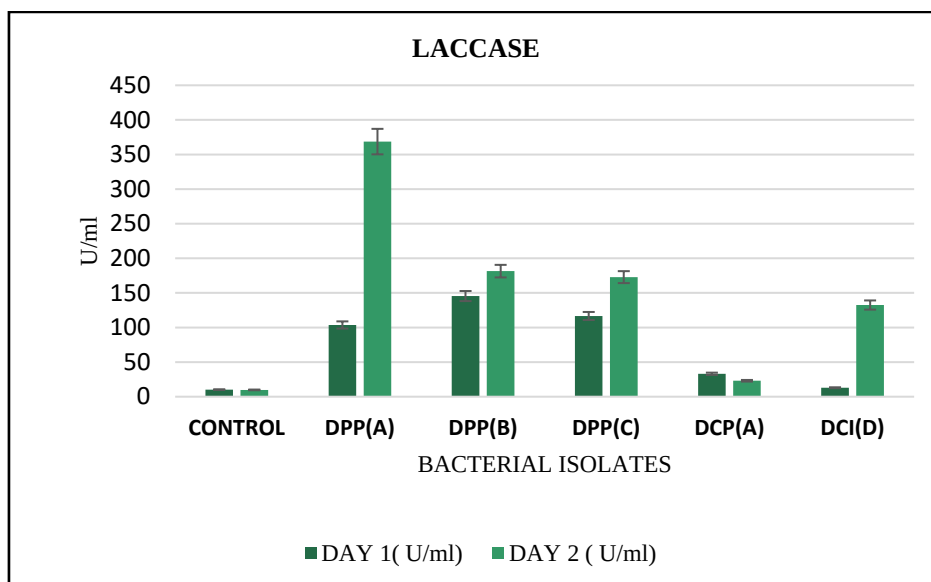


Figure 4.33: Quantitative analysis of laccase production by bacterial isolates

KEY FINDINGS from 4.2.7c:

- The strain *B.cereus* DPP(A) is the most efficient laccase-producing strain, with a major increase from Day 1 to Day 2. It stands out as the most potent laccase producer, potentially making it a strong candidate for applications in lignin degradation, bioremediation, or organic matter transformation in soil.
- *B.velezensis* (DPP-B) and *S.maltophilia* (DPP-C) show moderate and comparable activity with steady increase.
- The strain *B.cereus* DCI(D) shows promising potential with increasing trend of laccase production.
- *C.necator* (DCP-A) has the lowest laccase activity, showing a decline over time.
- The control validates that the observed enzyme activities are isolate-specific.

4.2.7d Estimation of lignin peroxidase activity:

Lignin peroxidase (LiP), produced by some soil bacteria, plays a crucial role in the degradation and detoxification of lignin, a complex polymer found in plant cell walls, and other recalcitrant compounds in soil. This enzyme, along with other ligninolytic enzymes like laccases and manganese peroxidase, contributes to the breakdown of organic matter, the carbon cycle, and the reduction of pollution in soil ecosystems. The objective of the work is to evaluate the lignin peroxidase (LiP) activity of bacterial isolates for their potential in the degradation of lignin and other recalcitrant organic compounds, thereby assessing their role in organic matter decomposition, carbon cycling, and soil pollutant reduction. The results have been represented in **Figure 4.34 and 4.35**

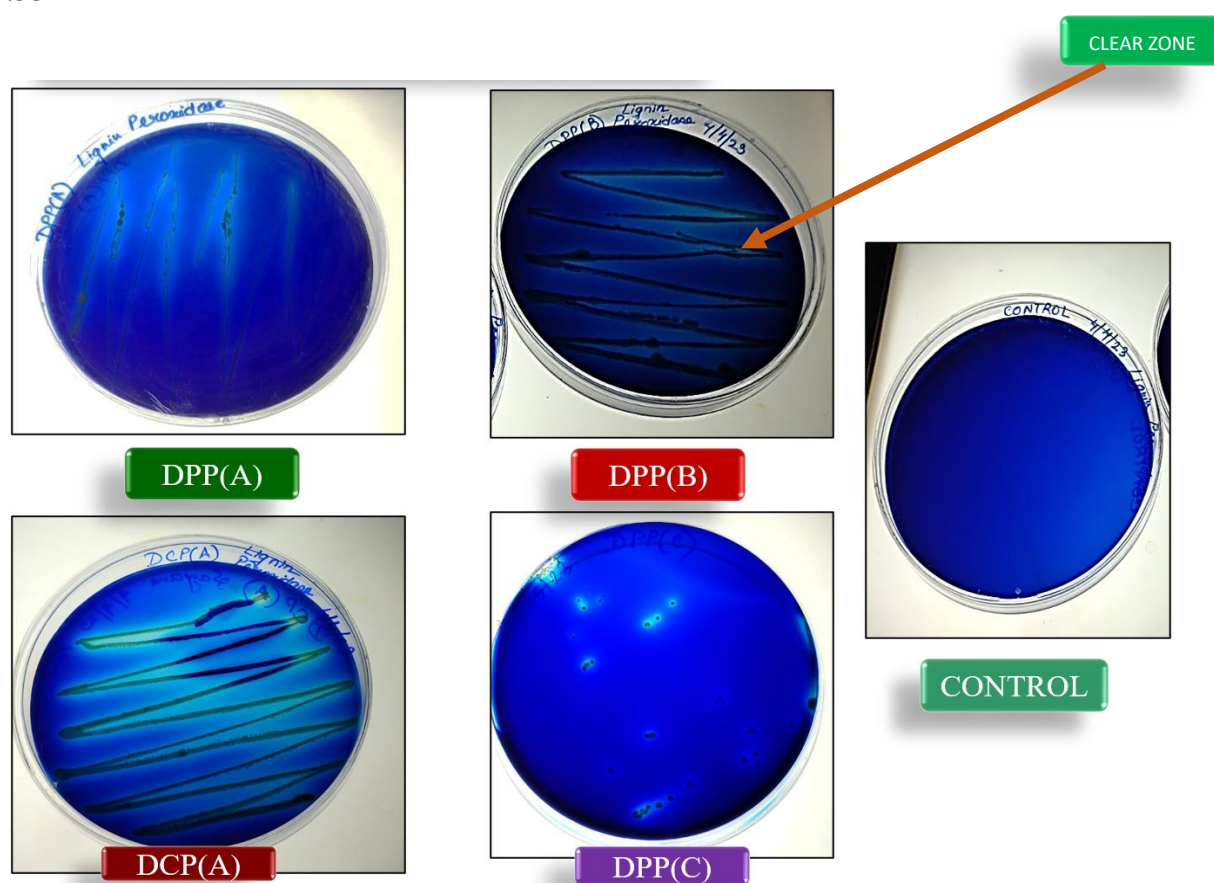


Figure 4.34: Qualitative analysis of lignin peroxidase production by bacterial isolates showing zone clearance

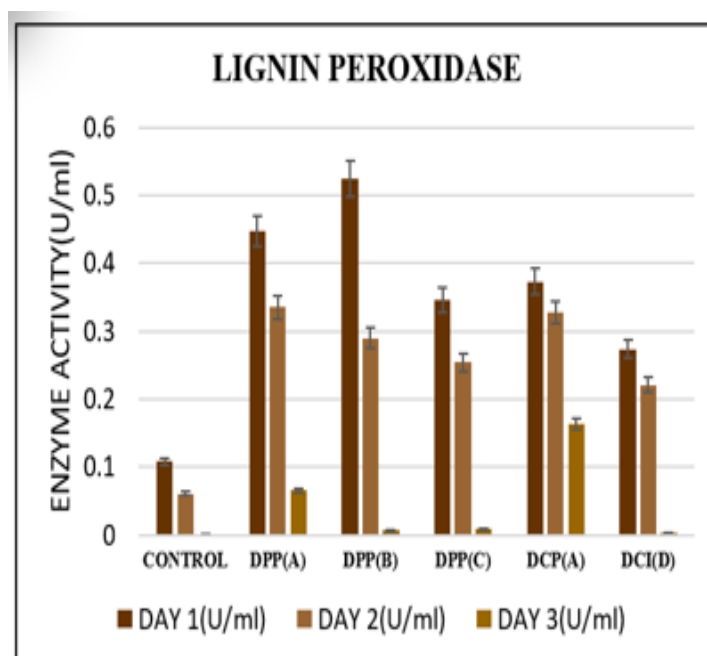


Figure 4.35: Quantitative analysis of lignin peroxidase production by bacterial isolates

KEY FINDINGS from 4.2.7d:

- The bacteria exhibited a time dependent assay of the enzyme, whereby *B. velezensis* (DPP-B) showed the highest lignin peroxidase activity on Day 2. The highest abilities of lignin peroxidase production were seen in *B. velezensis* (DPP-B), *B. cereus* DPP(A) and *C. necator* (DCP-A) at 0.524 U/ml, 0.447 U/ml and 0.373U/ml on Day 1. On Day 3, the bacterial isolate *C. necator* DCP(A) showed 0.163 U/ml of enzyme activity, the highest amongst the other bacterial isolates
- Both *B. cereus* DPP(A) and *B. velezensis* (DPP-B) exhibited peak activity on Day 2, followed by sharp declines by Day 3, suggesting early but transient expression.
- The bacterial isolate *B. cereus* DCI(D) maintains the most consistent activity across all three days, possibly suitable for longer-term applications.
- *C. necator* (DPP-C) also shows relatively steady performance.

4.2.7e Estimation of amylase activity

Soil bacteria utilize amylase to break down starch into smaller, more readily usable sugars like glucose. This process plays a crucial role in nutrient cycling and organic matter decomposition within the soil ecosystem. Amylase, produced by various soil bacteria, aids in the breakdown of complex carbohydrates, making nutrients accessible for plant uptake and contributing to overall soil health. The aim of the work was to assess the amylase activity of bacterial isolates in order to evaluate their ability to degrade starch into simpler sugars, thereby determining their role in organic matter decomposition, nutrient cycling, and enhancement of soil fertility. The results have been represented in **Figures 4.36 and 4.37**.



Figure 4.36: Qualitative analysis of amylase production by bacterial isolates, showing zone clearance

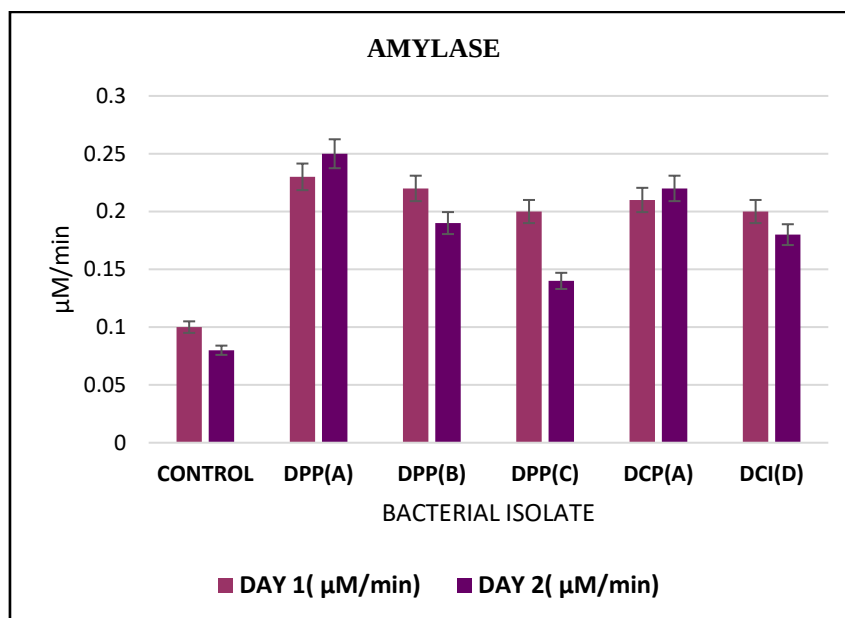


Figure 4.37: Quantitative analysis of amylase production by bacterial isolates

KEY FINDINGS from 4.2.7e:

- The bacterial strain *B. cereus* DPP(A), *B. velezensis* (DPP-B) and *C. necator* (DCP-A) were able to produce higher amylase content at 0.23 μM/min, 0.22 μM/min and 0.21 μM/min amylase, much higher as compared to the other bacterial isolates
- The bacterial strain *B. cereus* DPP(A) is the most effective amylase-producing isolate, suitable for applications in starch degradation and organic matter cycling in soil.
- *C. necator* (DCP-A) also holds strong potential due to its consistent activity.
- Isolates *B. velezensis* (DPP-B), *S. maltophilia* (DPP-C) and *B. cereus* DCI(D) demonstrate moderate efficiency, but may require optimization for sustained activity.

4.2.7f Estimation of pectinase activity

Pectinase plays a crucial role in the decomposition of plant material in soil, helping to recycle carbon compounds. Many soil bacteria produce pectinases, which are enzymes that break down pectin, a complex polysaccharide found in plant cell walls. This process aids in the breakdown of organic matter, making nutrients available for other organisms and contributing to soil health. The aim of the work was to evaluate the pectinase activity of bacterial isolates to determine their role in the degradation of plant-derived pectin, thereby assessing their contribution to organic matter decomposition, carbon recycling, and overall soil health. The results have been represented in **Figure 4.38** and **4.39**.

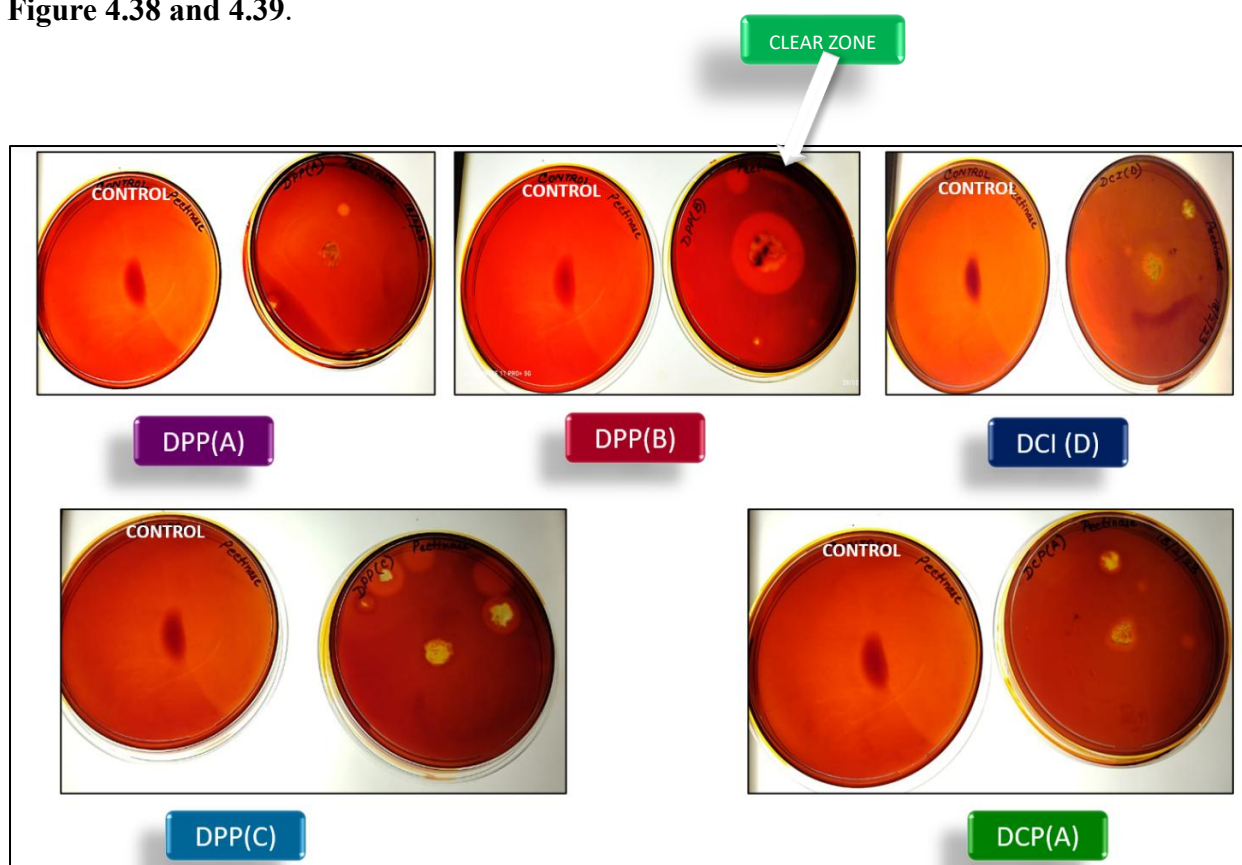


Figure 4.38: Qualitative analysis of pectinase production by bacterial isolates, showing a clear zone in the media.

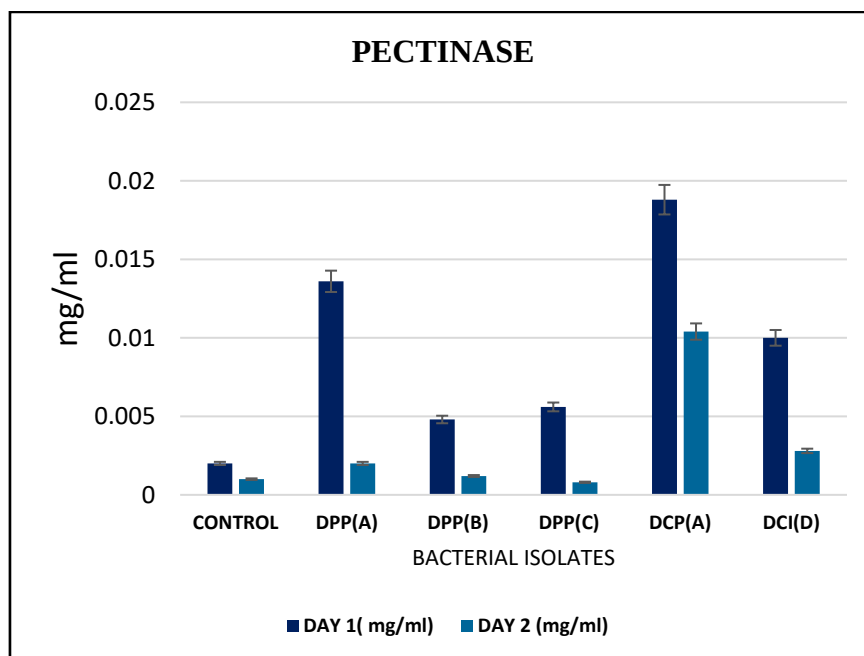


Figure 4.39: Quantitative analysis of pectinase production by bacterial isolates

KEY FINDINGS from 4.2.7f:

- Pectinase production in *C.necator* (DCP-A) was of about 0.0188 mg/ml followed by *B.cereus* DPP(A), *S.maltophilia* (DPP-C), *B.velezensis* (DPP-B) and *B.cereus* DCI(D) produced 0.0136 mg/ml, 0.0056mg/ml 0.0048mg/ml and 0.0028 mg/ml pectinase, much higher as compared to the control set up
- *C.necator* (DCP-A) is the most promising strain for pectin degradation, potentially useful in decomposition of plant residues and carbon cycling.
- The bacterial strain *B.cereus* DPP(A) may also have pectinase potential but may require optimization to stabilize enzyme expression.

4.2.8 Analysis of Metallothionein content

Metallothionein (MTs) in bacteria play a crucial role in metal homeostasis and detoxification, particularly against heavy metal toxicity. The objective of the work was to investigate the ability of bacterial isolates to produce metallothionein in response to heavy metal stress, specifically at 200 ppm and 500 ppm lead concentration, in order to assess their potential role in metal detoxification and tolerance mechanisms for applications in bioremediation of lead-contaminated environments. They act as a buffer, binding to and sequestering metals, preventing them from interacting with essential enzymes and other cellular components, thus protecting the cell from the detrimental effects of excess metals. The results have been represented in **Figure 4.40**

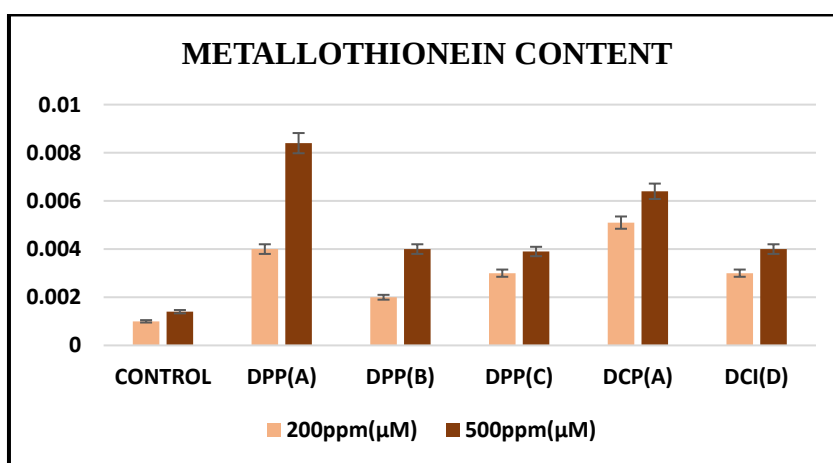


Figure 4.40: Quantitative analysis of metallothionein content of bacterial isolates

Key Findings from 4.2.8:

- The bacterial isolates *B.cereus* DPP(A), *B.velezensis* (DPP-B), *S.maltophilia* (DPP-C), *C.necator* (DCP-A) and *B.cereus* DCI(D) showed the ability to produce higher metallothionein in 500ppm of lead amount as compared to 200ppm of lead (**Figure 4.40**)
- In the control setup, very low metallothionein content is observed (no lead exposure), confirming that production is stress-induced.
- The bacterial isolate *B.cereus* DPP(A) shows the highest ability to produce metallothionein at 500ppm, as compared to other bacterial isolates.

4.2.9. Determination of HCN production:

Hydrogen cyanide (HCN) produced by soil bacteria plays a significant role in the soil ecosystem. The aim of the work was to evaluate the ability of soil bacterial isolates to produce hydrogen cyanide (HCN) and assess their potential role in plant growth promotion and biocontrol activity against soil-borne pathogens, thereby exploring their significance in sustainable agriculture and soil health management. The qualitative and quantitative estimation of HCN production by bacterial isolates were done and represented in **Figure 4.41**.

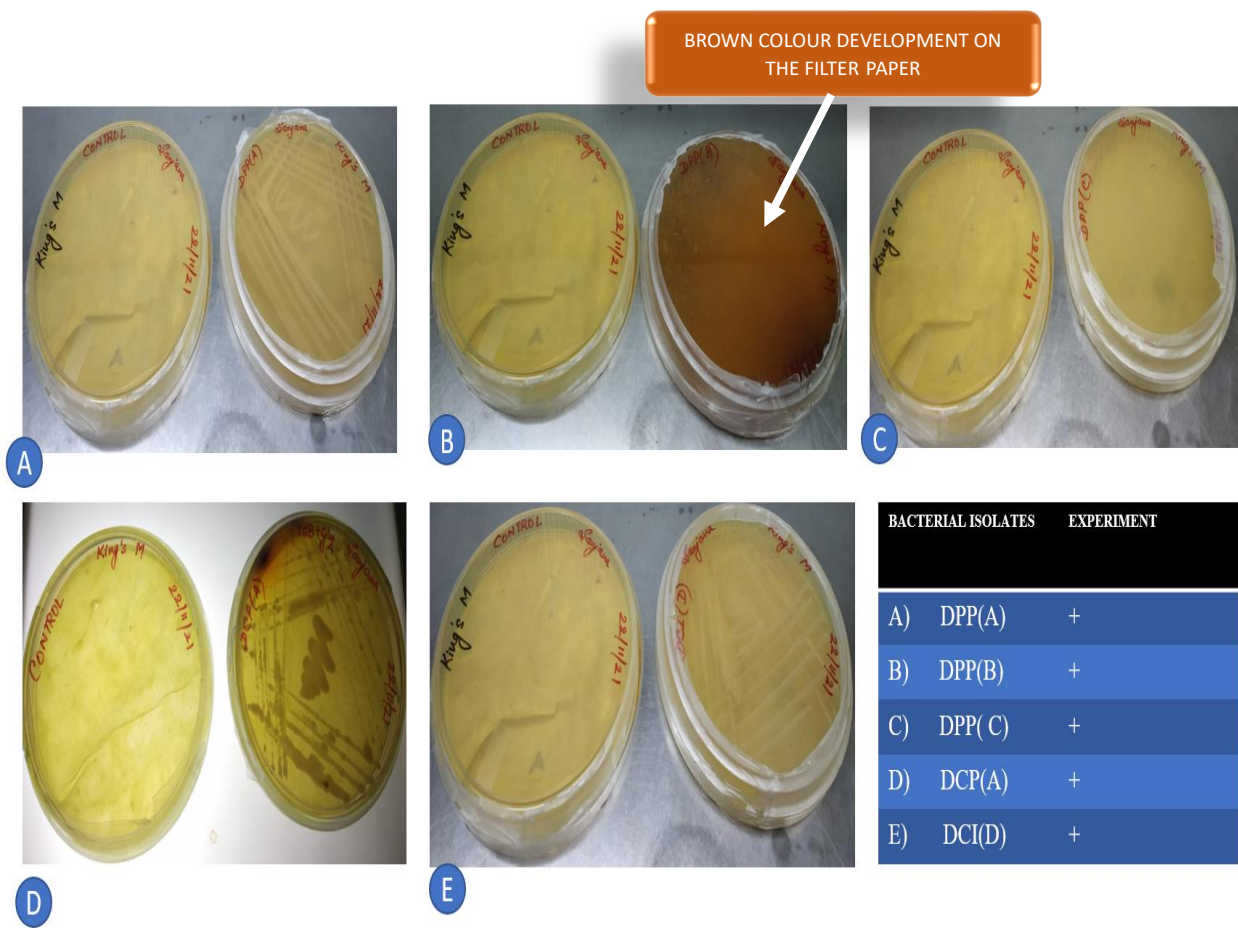


Figure 4.41: Qualitative analysis of HCN production of bacterial isolates. The bacterial isolates with positive HCN production have showed brown colour development on the filter paper, dipped in picrate solution.

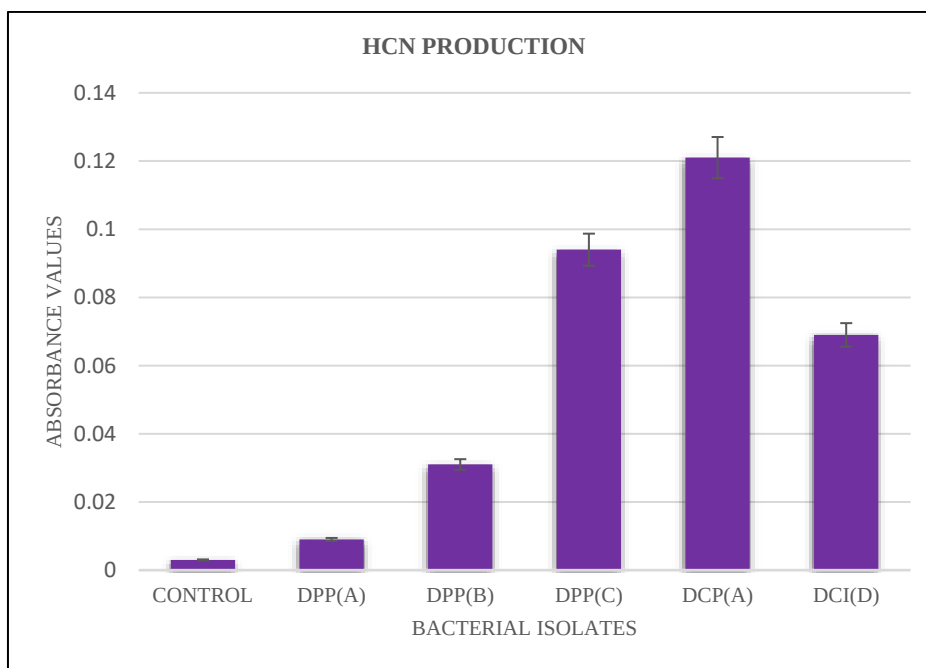


Figure 4.42: Quantitative analysis of HCN production by bacterial isolates.

KEY FINDINGS from 4.2.9:

- Qualitative analysis of all the bacterial isolates showed their ability to synthesise HCN, since the filter papers dipped in picrate solution turned brown.
- Upon quantitative analysis, it was found that *C. necator* (DCP-A) had the highest ability to synthesise HCN in comparison to the other bacterial isolates.
- Isolates with moderate to low production of HCN, such as *B. cereus* DCI(D) and *B. velezensis* (DPP-B), contribute to plant-microbe interactions, while *B. cereus* DPP(A) shows negligible HCN activity.

4.2.10. Determination of ammonia production:

Bacterial ammonia production plays a crucial role in various ecosystems and processes. The objective of the work was to assess the ability of bacterial isolates to synthesize ammonia and evaluate their potential role in plant growth promotion through nitrogen supplementation, thereby contributing to sustainable agricultural practices and nutrient cycling in the soil ecosystem. The results have been represented in **Figure 4.43 and 4.44**.



Figure 4.43: Qualitative analysis of ammonia production by bacterial isolates

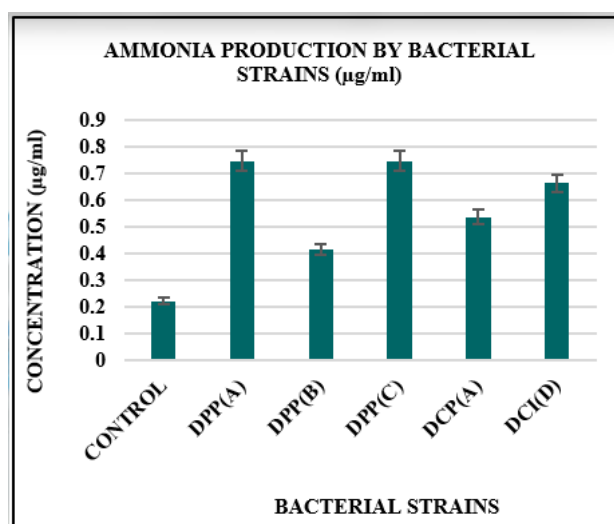


Figure 4.44: Quantitative analysis of ammonia production by bacterial isolates

Key Findings from 4.2.10:

- Ammonia acts as a readily available nitrogen source, contributing to plant growth and development.
- These bacteria oxidize ammonia, releasing energy, which will be used to convert carbon dioxide into organic molecules.
- Each of the bacterial isolates, showed the ability of production of ammonia.
- The highest capacity was seen among *B.cereus* DPP(A) and *S.maltophilia* (DPP-C) with 0.747 µg/mL. *B.velezensis* (DPP-B), *C.necator* (DCP-A), and *B.cereus* DCI(D) were able to produce 0.415 µg/mL, 0.537 µg/mL, and 0.664 µg/mL of ammonia respectively (**Figure 4.44**).

4.2.11 Estimation of iron chelating compounds

The strains were tested for their ability to synthesise siderophores. It was found to produce iron chelating compounds (Siderophores) detected by the orange halo formation by the cell free supernatants, when placed in O-CAS agar medium. Siderophore also plays an important role in control of phytopathogenic fungi. The objective of the work was to evaluate the ability of bacterial isolates to produce siderophores, determine their siderophore type, and assess their potential role in iron acquisition and biocontrol activity against phytopathogenic fungi, with a focus on identifying strains capable of producing hydroxamate-type siderophores. The siderophore production by the strains were quantified and typed, and it was found that all the 5 bacterial isolates showed orange to pink media coloration indicating the presence of a hydroxamate type of siderophore.

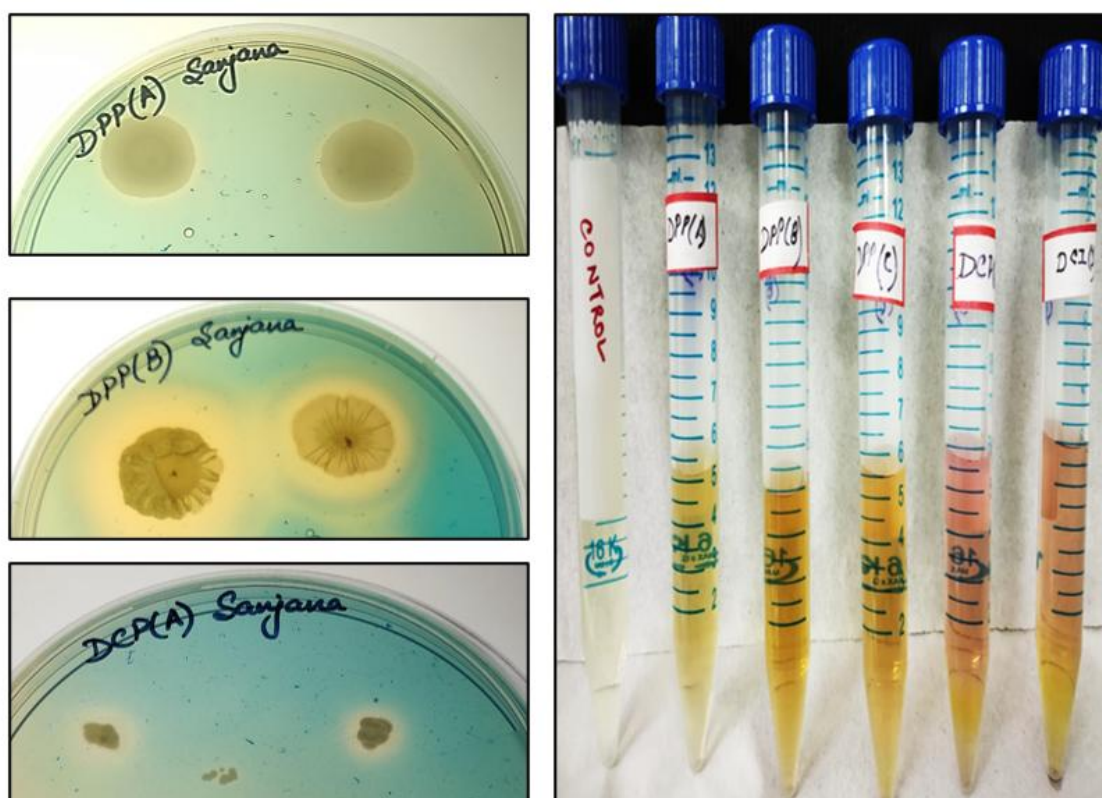


Figure 4.45: Qualitative analysis of the siderophore production by bacterial isolates and identification of the type of siderophore.

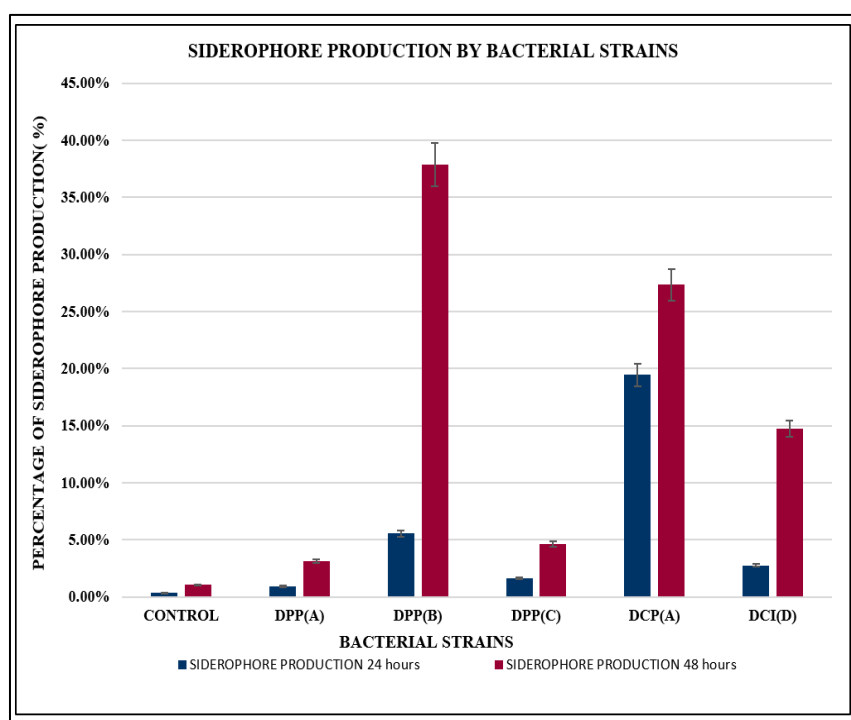


Figure 4.46: Quantitative analysis of the siderophore production by bacterial isolates.

Key findings from 4.2.11:

- Siderophores are iron chelating compounds produced by bacterial cells for the purpose of iron sequestration.
- The O-Cas agar plates show siderophore production and the orange halo zone formation around the wells in which culture supernatants were introduced.
- Quantification study revealed the percentage of siderophore production was recorded after 24 and 48 h, whereby the *B.cereus* DPP(A), *B.velezensis* (DPP-B), *S.maltophilia* (DPP-C) and *C.necator* (DCP-A) and *B.cereus* DCI(D) showed 3.1%, 4.62%, 37.89%, 27.36%, and 14.73%, respectively, that was much higher compared to its production in 24 h, both in test and control set up (**Figure 4.46**).
- The siderophores were further characterised for their types based on their chemical structures, and it was found that all the bacterial isolates showed hydroxamate type of siderophore (**Figure 4.45**).

4.2.12 Analysis of Lead tolerance by bacterial isolates

The bacterial isolates were tested for their ability to uptake lead. The aim of the work was to evaluate the lead (Pb) tolerance and uptake efficiency of selected bacterial isolates through quantitative screening using optical density (OD600) and metal uptake assays, with the objective of identifying the most efficient lead-utilizing strains for potential bioremediation of heavy metal-contaminated soils. The results have been represented in **Figures 4.47 a, b, c**

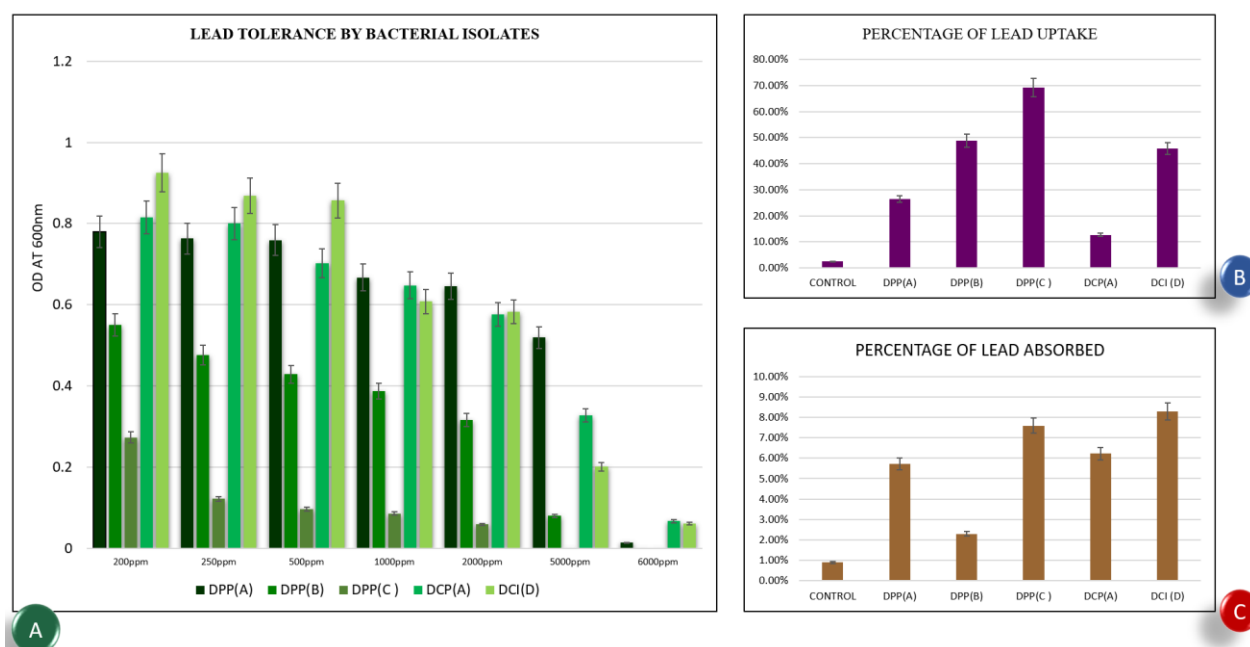


Figure 4.47: Lead concentration in soil and its uptake by bacterial isolates. Graphical representations showing: **(a)** Lead tolerance by bacterial isolates. **(b)** Percentage of lead uptake by bacterial isolates. **(c)** Percentage of lead adsorbed by bacterial isolates

Key Findings from 4.2.12:

- Most of the bacterial isolates were able to tolerate about 2000ppm of lead. The bacterial strains *B.velezensis* (DPP-B) and *C.necator* (DCP-A) show highest OD values across all tested Pb concentrations up to 2000 ppm, indicating strong lead tolerance.
- Growth drops significantly beyond 2000 ppm, with minimal or no growth at 5000 ppm and 6000 ppm, indicating toxic threshold.
- The bacterial strains *S.maltophilia* (DPP-C) and *B.cereus* DCI(D) show relatively higher OD even at 5000 ppm compared to others, suggesting better survival capacity under extreme Pb stress.
- The percentage of lead uptake in bacterial isolates was calculated and it was found that *S.maltophilia* (DPP-C) had the highest capacity for lead uptake at 69.2%, followed by *B.velezensis* (DPP-B) and *B.cereus* DCI(D) at 48.84% and 45.85%, respectively. The bacterial isolates were further tested for their metal uptake capacity. It was found to have 0.848 µg/g, 0.976 µg/g, 0.173 µg/g, 0.383 µg/g, 0.917 µg/g for *B.cereus* DPP(A), *B.velezensis*, *S.maltophilia* (DPP-C), *C.necator* (DCP-A) and *B.cereus* DCI(D), respectively (**Figure 4.47 b**).
- The bacterial isolates namely *B.cereus*(DPP-A), *B.velezensis* (DPP-B), *S.maltophilia* (DPP-C), *C.necator* (DCP-A) and *B.cereus* DCI(D) were found to have the following percentage of lead adsorbed, that is, 5.73%, 2.30%, 7.6%, 6.22%, 8.28%, respectively (**Figure 4.47c**)

SUMMARISED KEY FINDINGS FROM OBJECTIVE 2:

- From the collected soil samples, twelve bacterial isolates were initially selected, out of which five—DPP(A), DPP(B), DPP(C), DCP(A), and DCI(D)—were consistently present across different cropping phases of the two crops and accordingly chosen for further analysis.
- Gram staining revealed that DPP(A), DPP(B), and DCI(D) were Gram-positive, while DPP(C) and DCP(A) were Gram-negative.
- On blood agar, DPP(A) exhibited β -hemolysis, DPP(B), DPP(C), and DCP(A) showed γ -hemolysis, and DCI(D) showed α -hemolysis.
- Presumptive identification using Hi-chrome media indicated that DPP(A), DPP(B), and DCI(D) resembled *Bacillus* spp., DPP(C) resembled *Pseudomonas*, and DCP(A) resembled *Proteus*.
- Molecular identification using 16S rRNA sequencing confirmed the strains as follows:
 - ❖ DPP(A): *Bacillus cereus* (OQ216888), DPP(B): *Bacillus velezensis* (ON384543), DPP(C): *Stenotrophomonas maltophilia* (OQ217020), DCP(A): *Cupriavidus necator* (ON384541), DCI(D): *Bacillus cereus* (OQ217005)
- All five bacterial isolates were generally sensitive to a wide range of antibiotics, although *B.cereus* DPP(A) and *C.necator* (DCP-A) showed slight resistance to Metronidazole with a diameter of less than 0.5cm, and *S.maltophilia* (DPP-C) showed resistance to Ciprofloxacin.
- All isolates demonstrated plant growth-promoting properties, including nitrogen fixation, phosphate solubilization, and potassium mobilization.
- Quantitative phosphate solubilization results showed that *B.velezensis* (DPP-B) showed highest ability to produce phosphate (7.91 mg/mL)
- All strains were capable of producing Indole Acetic Acid (IAA), with maximum production by *C.necator* (DCP-A) at 56.785 $\mu\text{g/mL}$ in the presence of tryptophan.
- All isolates produced gibberellins, with *S.maltophilia* (DPP-C) showing the highest production at 10.584 $\mu\text{g/mL}$
- All strains produced ACC deaminase, which helps reduce ethylene-induced stress in plants; *B.velezensis* (DPP-B) and *C.necator* (DCP-A) showed the highest activity

- The isolates synthesized several extracellular enzymes with notable results as follows:
- Urease: *C. necator* (DCP-A) produced 3.23 mM urea hydrolyzed/min/OD
- Cellulase: *B. cereus* DPP(A) and *C.necator* (DCP-A) produced 4.4 $\mu\text{M/mL}$ and 3.9 $\mu\text{M/mL}$ respectively
- Laccase: *B. velezensis* (DPP-B) and *S.maltophilia* (DPP-C) produced 145.44 U/mL and 116.64 U/mL respectively.
- Lignin peroxidase: Highest production seen in *B.velezensis* (DPP-B), *B.cereus* DPP(A), and *C.necator* (DCP-A)
- Amylase: *B.cereus* DPP(A), *B.velezensis* (DPP-B) and *C.necator* (DCP-A) had similar high production around 0.22–0.23 $\mu\text{M/min}$
- Pectinase: *C.necator* (DCP-A) had the highest production at 0.0188 mg/mL
- All isolates produced metallothionein when exposed to 500 ppm lead, indicating heavy metal resistance.
- All strains were able to produce hydrogen cyanide (HCN), with *C.necator* (DCP-A) being the most potent HCN producer.
- Ammonia production was confirmed in all strains; *B.cereus* DPP(A) and *S.maltophilia* (DPP-C) had the highest levels at 0.747 $\mu\text{g/mL}$.
- All isolates synthesized siderophores of the hydroxamate type, which were visualized by orange halo zones on O-CAS agar.
- Highest siderophore production was recorded for *S.maltophilia* (DPP-C) (37.89%), followed by *C.necator* (DCP-A) (27.36%).
- All strains tolerated up to 2000 ppm of lead, with *S.maltophilia* (DPP-C) showing the highest uptake (69.2%), followed by *Bacillus velezensis* (DPP-B) (48.84%) and *B.cereus* DCI(D) (45.85%).
- Metal accumulation analysis showed: *Bacillus velezensis* (DPP-B) :0.976 $\mu\text{g/g}$, *S.maltophilia* (DPP-C) :0.173 $\mu\text{g/g}$, *B.cereus* DCI(D): 0.917 $\mu\text{g/g}$
- Lead adsorption percentages were: *S.maltophilia* (DPP-C) :7.6%, *C.necator* (DCP-A) :6.22%, DCI(D): 8.28%
- In summary, all five bacterial strains demonstrated non-pathogenic, environmentally safe profiles with strong plant growth-promoting and bioremediation potentials, making them suitable candidates for sustainable agriculture and soil detoxification strategies. The best bacterial isolates were DPP(B) -*Bacillus velezensis* and DCP(A)- *Cupriavidus necator*.

OBJECTIVE 3

4.3 To prepare an ideal consortium to be supplemented all through the year suitable for any crop, either singly or with chemical fertilizers (*in-vivo* study).

Testing of the efficacy of the formulation under in vivo conditions.

The primary aim of this objective is to prepare a novel bacterial formulation based on the selected bacterial isolates, and then subsequently test the newly designed formulations under in vivo conditions. The bacterial strains in the novel formulations were chosen on the basis of their nature of interaction with each other

4.3.1. Interaction study between the bacterial strains

The aim of the work was to evaluate the mutual compatibility and potential synergistic interactions of the selected plant-growth-promoting bacterial strains by employing the T-streak plate method, thereby determining their suitability for inclusion in a multi-strain consortium aimed at enhancing soil health and crop performance. The interaction between the selected strains were assessed by streaking the isolates in cross-streak plate method following standard procedure (Sharma *et al.*,2013). All the strains showed positive interaction amongst each other as represented in **Figure 4.48**

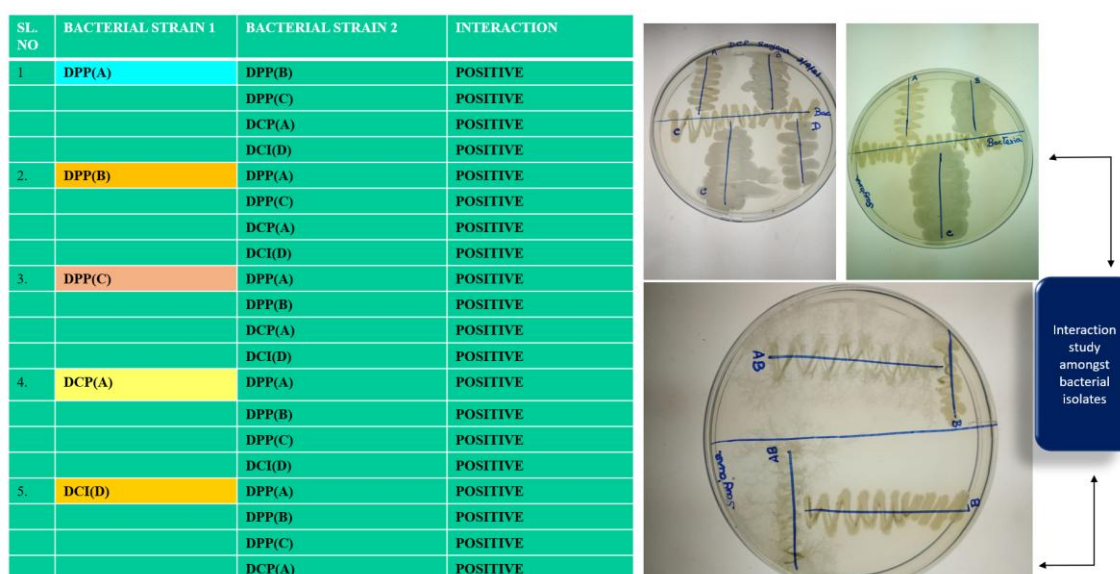


Figure 4.48: Interaction study between the bacterial isolates

KEY FINDINGS from 4.3.1:

- The study was aimed to determine mutual compatibility and potential synergistic interactions among selected plant growth-promoting bacterial (PGPB) strains through cross-streak plate method.
- Five bacterial strains labelled as *B.cereus* DPP(A), *B.velezensis* DPP(B), *S.maltophilia* DPP(C), *C.necator* DCP(A), and *B.cereus* DCI(D), were tested against all others in paired combinations.
- All strain pairs demonstrated positive interaction, indicating no signs of antagonism (e.g., inhibition zones or growth suppression) between any of the isolates.
- The successful interaction among all tested strains highlights their potential to be co formulated as a microbial consortium.

4.3.2. Pathogen isolation from leaves of *Zea mays* followed by their interaction with bacterial isolates

The aim of the work was to isolate and identify fungal pathogens infecting *Zea mays* leaves based on morphological and microscopic characteristics, and to evaluate the antagonistic potential of selected bacterial isolates against the prospective pathogens with the objective of assessing their efficacy as biocontrol agents for sustainable disease management. The infected leaves with small, round, light brown coloured lesions on leaves with a brown border, and a yellow halo around them were observed in *Zea mays*. The fungal pathogen was isolated, and observed under the microscope. The first one had a spore diameter between 23.2 μm -25 μm . With literature reference based on disease symptom and spore characteristics (Moparthy *et al.*,2023), the pathogen was found to be *Curvularia* sp. The second one exhibited a cream-coloured lesion, that was observed on the *Zea mays* leaf. Upon isolation of fungal pathogen, followed by microscopic analysis and spore diameter measurement and literature evidence by Aggarwal *et al.*,2025, it was found to be *Exserohilum* sp (Figure 4.49). An interaction test that showed the physical adhesion of bacterial isolates with fungus was conducted. It was found that all the bacterial isolates were able to restrict the fungal growth, therefore, they were found to be useful as a bio-control agent. The results have been represented in Figure 4.50 and Figure 4.51

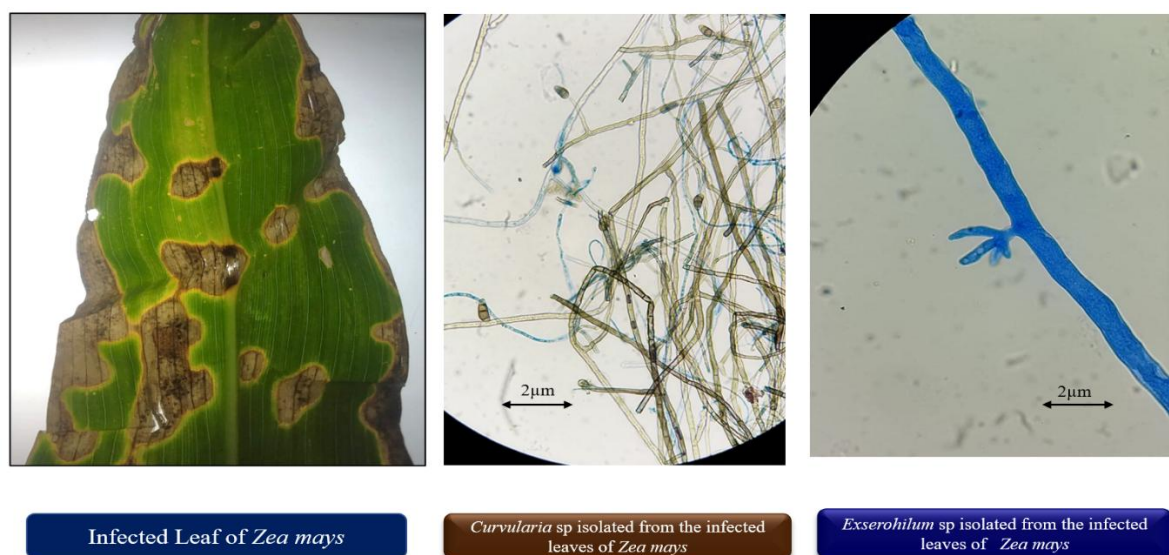


Figure 4.49: Isolation of *Curvularia* sp and *Exserohilum* sp from the infected leaves of *Zea mays*

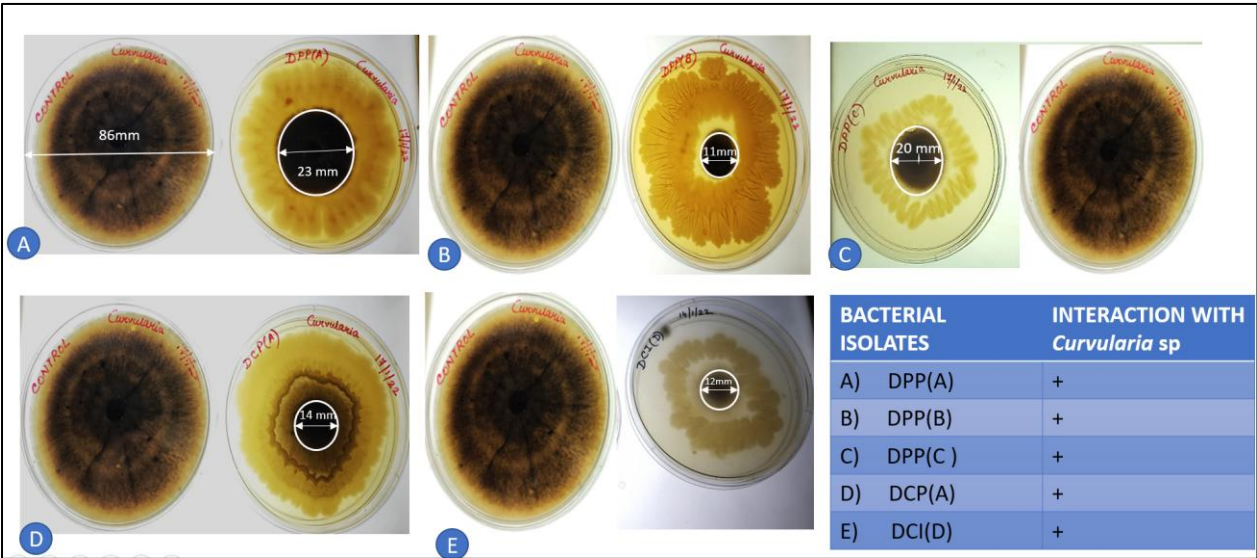


Figure 4.50: Interaction study between bacterial isolates A- DPP(A), B-DPP(B), C- DPP(C), D-DCP(A) and E-DCI(D) with the fungus *Curvularia* sp.

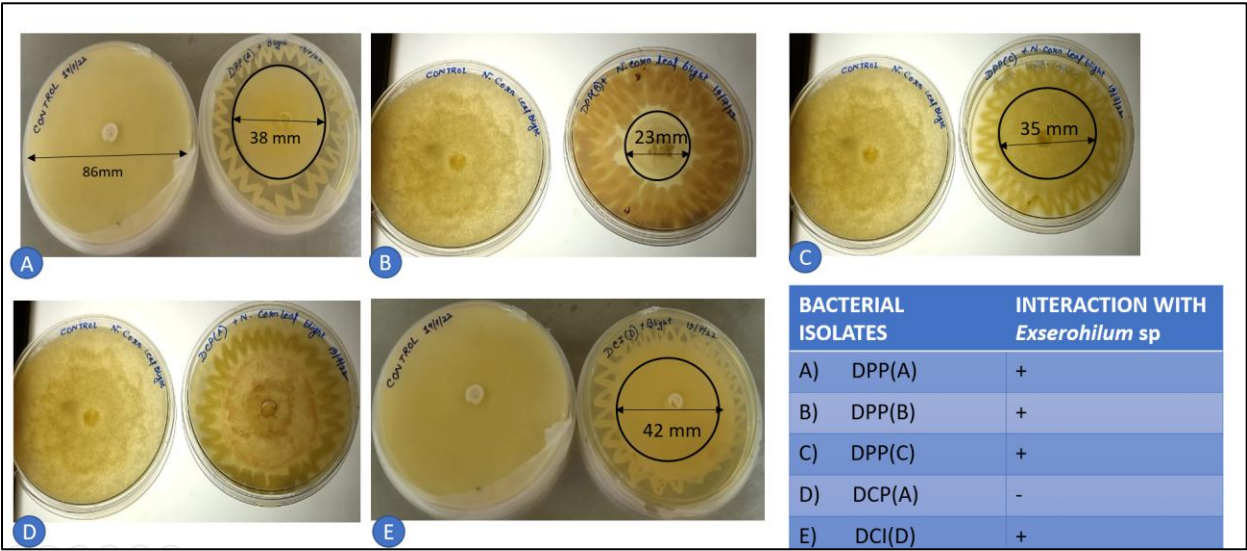


Figure 4.51: Interaction study between bacterial isolates A- DPP(A), B-DPP(B), C- DPP(C), D-DCP(A) and E-DCI(D) with the fungus *Exserohilum* sp.

KEY FINDINGS from 4.3.2:

- The fungal pathogens were isolated from infected *Zea mays* leaves and identified using morphological and microscopic features.
- The antagonistic potential of selected plant growth-promoting bacterial (PGPB) isolates against the pathogens (*Curvularia* sp. and *Exserohilum* sp.) were tested as a part of bio-control strategy.
- All the bacterial isolates namely *B.cereus* DPP(A), *B.velezensis* (DPP-B), *S.maltophilia* (DPP-C), *C.necator* (DCP-A) and *B.cereus* DCI(D) showed antagonistic effects against *Curvularia* sp by creating fungal zone inhibition diameters of 23 mm, 11 mm, 20 mm, 14 mm and 22 mm respectively.
- All the bacterial isolates namely *B.cereus* DPP(A), *B.velezensis* (DPP-B), *S.maltophilia* (DPP-C), and *B.cereus* DCI(D) showed antagonistic effects against *Exserohilum* sp by creating fungal zone inhibition diameters of 38 mm, 23 mm, 35 mm, and 42 mm respectively.
- *C.necator* (DCP-A) showed no visible inhibition, suggesting lack of antagonistic activity against *Exserohilum* sp.

4.3.3 Standardization of Treatment Mode

The aim of the work was to evaluate the effectiveness of a selected bacterial consortium characterized by their plant growth-promoting and biocontrol properties—on the vegetative growth, reproductive development, and yield of *Cucurbita maxima* and *Zea mays* under field conditions. The field trial was conducted by procuring seeds of *Cucurbita maxima* (Variety: Jack 323) and *Zea mays* (Variety: 4226). Out of all the five bacterial isolates, *B.velezensis* DPP(B) and *C.necator* DCP(A) were found to be most potent, on the basis of their PGPR properties, phytohormone properties, bio-control as well as the enzymatic properties. The other bacterial isolates were not used since literature study supported that *S. maltophilia* (DPP-C) was a nosocomial pathogen (Said *et al.*,2023), and strains of *B. cereus* were also reported to pathogenic in nature (Bottone.,2010). Therefore, DPP-B and DCP-A were used as a consortium and applied to the *Cucurbita maxima* and *Zea mays* 1 month old plants in Chowbaga plots of East Kolkata Wetlands. The treatment dosages were standardized by Chakraborty, *et al.*, 2013. The pictorial representation field trial of *Cucurbita maxima* and *Zea mays* have been shown in **Figure 4.52, 4.53 and 4.54.**

The results indicated that the water suspension treatment was the best mode as it was cost effective and used the concept of whole cell application, thus more scientifically acceptable.

The data was collected after every week till 12th week and is been represented in **Figure 4.54 and Figure 4.55.**

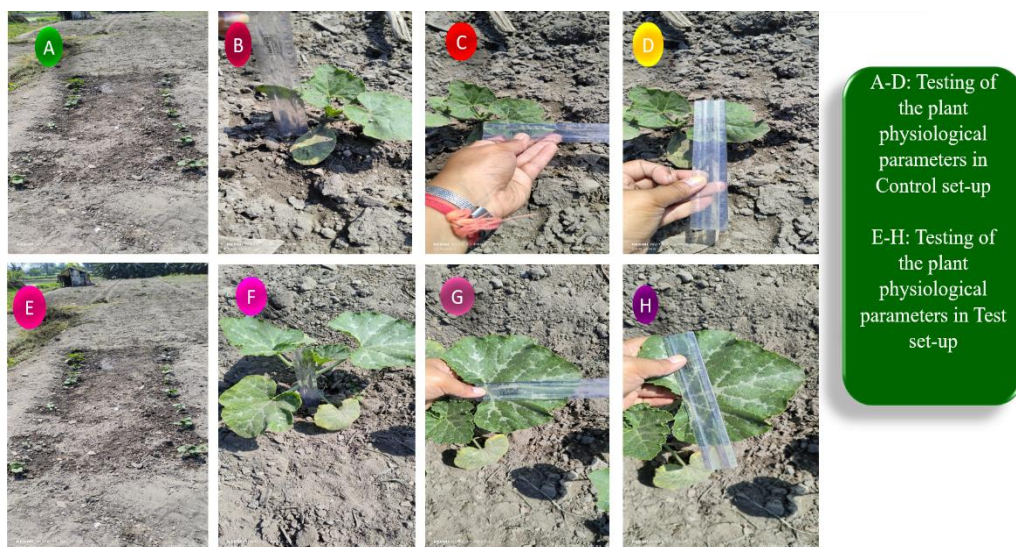


Figure 4.52: Field trial of *Cucurbita maxima* plants in East Kolkata Wetlands

Control Set-up

A- Agriculture field, B- Length of the plant, C- Length of the leaf, D- Width of the leaf

Treated Set-up (Bacterial consortia was applied)

E- Agriculture field, F- Length of the plant, G- Length of the leaf, H- Width of the leaf



Figure 4.53: Field trial of *Cucurbita maxima* plants in East Kolkata Wetlands



Figure 4.54: Field trial of *Zea mays* plants in East Kolkata Wetland

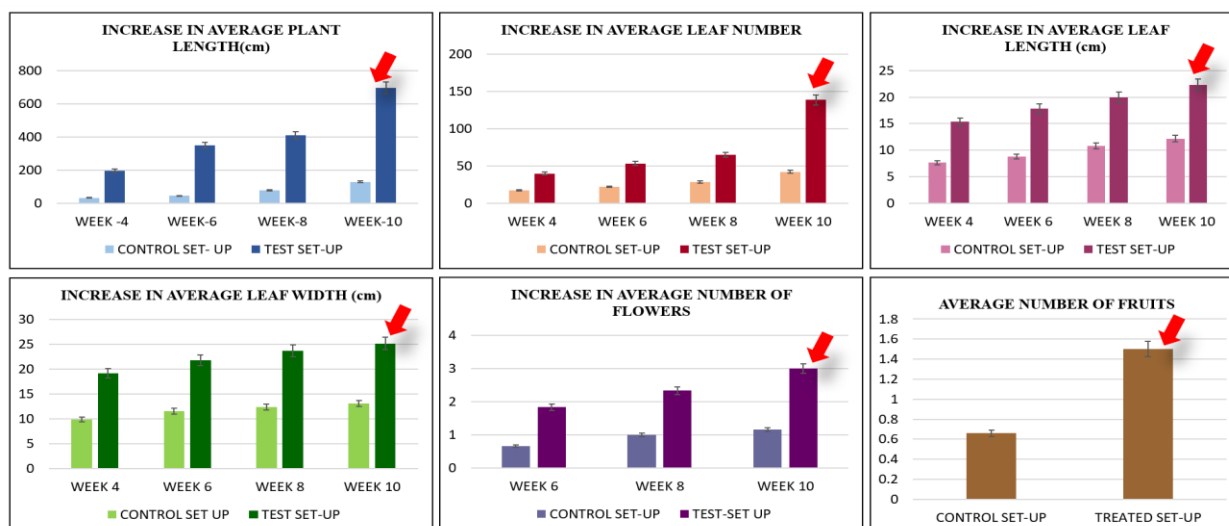


Figure 4.55: Vegetative(A-D) and Reproductive(E-F) growth parameters of *Cucurbita maxima*, with arrows pointing towards highest growth in treated set-up.



Figure 4.56: Vegetative (A-E; G-I) and Reproductive (F) growth parameters of *Zea mays*, with arrows pointing towards highest growth in treated set-up.

Key Findings from 4.3.3:

- A field study was conducted to determine the most effective mode of treatment application using bacterial inoculants.
- Three treatment application methods were tested, and vegetative parameters such as plant length, number of leaves, leaf length, and internodal length were monitored weekly.
- The graphical representations (Figures 4.52 and 4.53) showed that water suspension was the most effective treatment method. The water suspension method was found to be both cost-effective and scientifically valid, as it used whole cell application.
- Seeds of *Cucurbita maxima* (Variety: Jack 323) and *Zea mays* (Variety: 4226) were used for the field trial study with 20 plants.
- Among the five bacterial isolates, *B.velezensis* (DPP-B) and *C.necator* (DCP-A) demonstrated the most potent plant growth-promoting, phytohormonal, and enzymatic properties.
- These two strains were selected to create a bacterial consortium, which was applied to one-month-old plants in Chowbaga plots of the East Kolkata Wetlands.
- Based on the pilot results, the water suspension method was chosen for the field trial due to its scientific effectiveness and practical advantages.
- Vegetative data was collected weekly from the date of application up to the 12th week to assess treatment impact.
- The treated set up showed better vegetative and reproductive parameters as compared to the control setup.

4.3.4. Plant Pigments

The chlorophyll contents of the leaves were measured, and in all the treated set ups of both *Cucurbita maxima* and *Zea mays* plants. The aim of the work was to evaluate the effect of bacterial treatments on the chlorophyll content in the leaves of *Cucurbita maxima* and *Zea mays* plants, and to compare the treated setups with untreated controls in order to assess the potential plant growth-promoting capabilities of the applied bacterial strains. The chlorophyll content was found to be much higher in treated set up as compared to the control set up of the plants. The data has been represented in **Figure 4.57, 4.58**

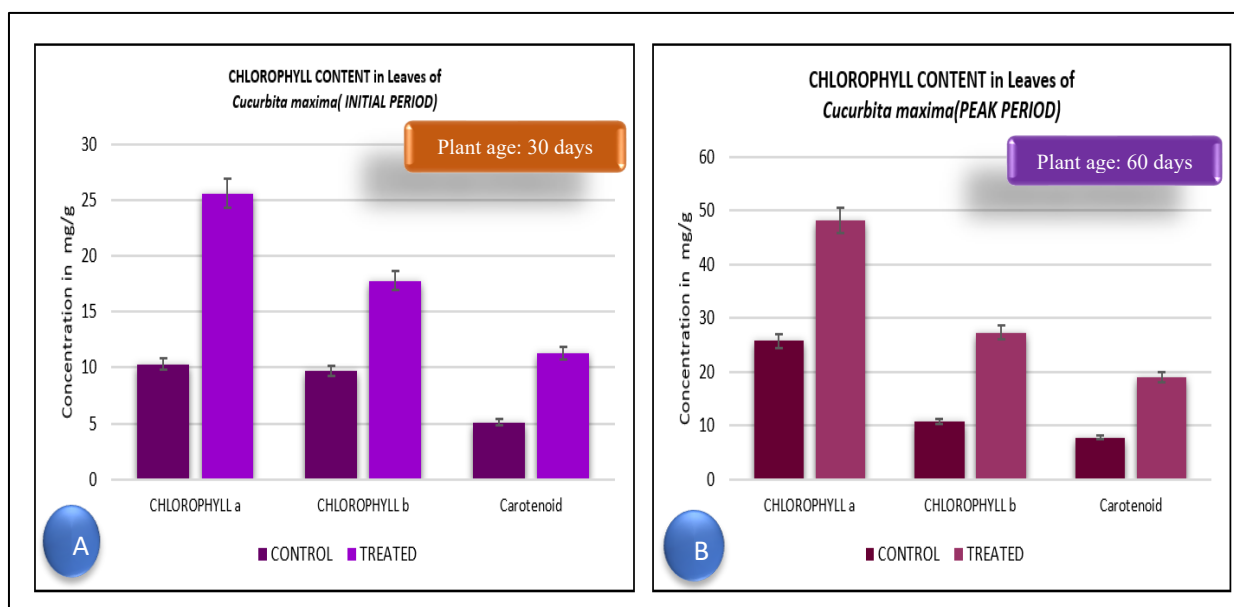


Figure 4.57: Chlorophyll content in the leaves of *Cucurbita maxima* during the initial(A) and peak (B) period of cultivation.

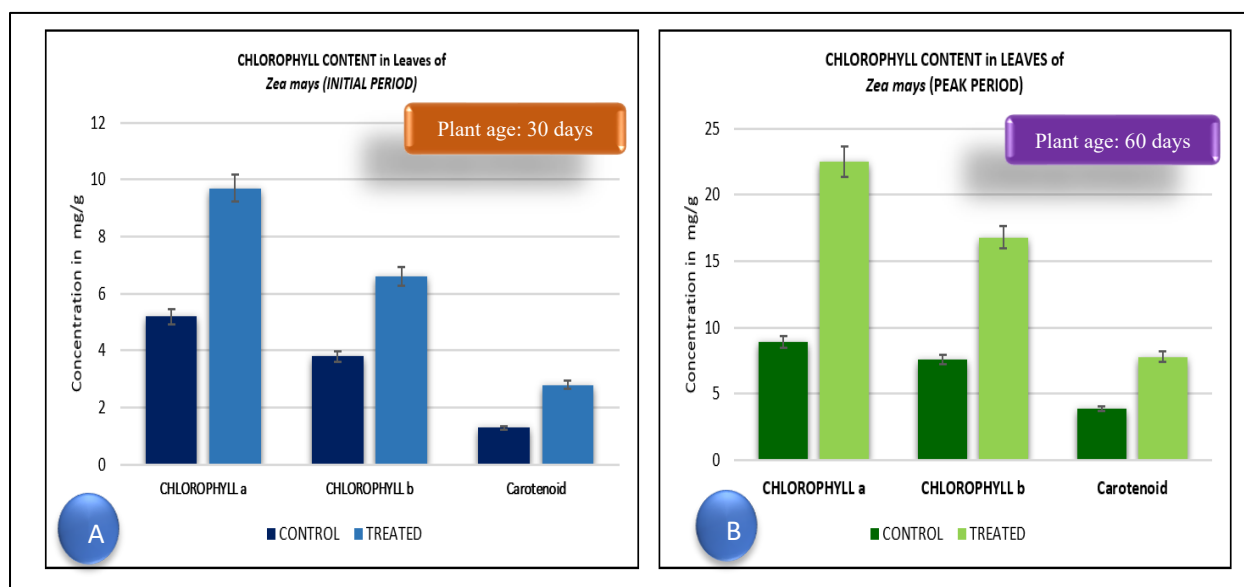


Figure 4.58: Chlorophyll content in the leaves of *Zea mays* during the initial(A) and peak (B)period of cultivation.

Key Findings from 4.3.4:

- In both the plant types of *Cucurbita maxima* and *Zea mays*, significantly enhanced Chlorophyll a, Chlorophyll b and carotenoid contents were observed in the treated set-up as compared to the control set-up.
- The peak period showed a more pronounced difference in pigment content, reflecting enhanced photosynthetic capacity and possibly improved plant health and growth.
- *Cucurbita maxima* showed higher absolute pigment content than *Zea mays*, though both exhibited substantial percentage increases due to treatment.
- Treated plants exhibiting higher chlorophyll content compared to control plants are significant because it indicates an enhanced ability to perform photosynthesis. This leads to greater energy capture, potentially boosting growth, biomass, and overall productivity.
- This suggests that the applied treatments positively influenced chlorophyll biosynthesis or retention in the plants, potentially enhancing their photosynthetic efficiency and overall vitality.

4.3.5 Analysis of lead content in the soil

The lead content of the soil upon application of consortia was found to be 12.28 mg/kg of soil much less, when compared with the control soil.

SAMPLE	CONTROL SOIL	TREATED SOIL
SOIL SAMPLE	106.1±0.02	12.28±0.01

Table 4.3: Lead content of control and treated soil

Key Findings from 4.3.5:

- The use of bacterial consortia comprising of *Bacillus velezensis* (DPP-B) and *Cupriavidus necator* (DCP-A) led to a significant decrease in the lead (Pb) content of the soil.
- The lead concentration in the control soil was measured at 106.1 ± 0.02 mg/kg, while the treated soil showed a significantly reduced level of 12.28 ± 0.01 mg/kg.
- This indicates an approximate reduction of 88.4% in lead levels, highlighting the effectiveness of the bacterial consortia in reducing heavy metal contamination and showcasing their potential for soil bioremediation.

4.3.6a. Statistical analysis of the subsequent data Analysis of variance was measured for the plant parameters of *Cucurbita maxima* (Variety: Jack 323)

Analysis of Variance (ANOVA) was carried out, with respect to the control set-up and the treatment set up. The aim of the work was to statistically evaluate the effects of bacterial consortia treatment and plant growth stages on various physiological parameters of *Cucurbita maxima* (Variety: Jack 323) using two-way ANOVA, in order to determine the significance of treatment and its interaction with time on overall plant development. Analysis was done using Python 3. Factor A is considered as control and Factor B as treated set up.

Two-way ANOVA was used for plant parameters, since it enables the simultaneous assessment of both temporal growth (in weeks) and the treatment effect (comparing control and treated groups), as well as testing if the treatment effect varies with the age of the plants (interaction).

Abbreviation and Explanation of terms:

C.D. - Critical Difference

This represents the smallest difference necessary between treatment means for them to be deemed statistically significant, typically following an ANOVA (often computed using the LSD test).

SE(D) - Standard Error of Difference

This denotes the standard error linked to the difference between two treatment means.

SE(m) -Standard Error of Mean (occasionally referred to as SE of a mean)

This indicates the standard error of the treatment mean, illustrating how much the sample mean is anticipated to fluctuate from the actual population mean.

PLANT LENGTH

The aim of the work was to analyse the overall growth enhancement, statistically using ANOVA in plant height upon both control and treated set up.

Two-way ANOVA was used for plant length, since it enables the simultaneous assessment of both temporal growth (in weeks) and the treatment effect (comparing control and treated groups), as well as testing if the treatment effect varies with the age of the plants (interaction).

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Replication	12	148,510.514			
Factor A	1	133,007.232	133,007.232	62.050	< 0.001
Factor B	7	1,308,159.053	186,879.865	87.182	< 0.001
Interaction A X B	7	52,914.198	7,559.171	3.526	0.001
Error	180	385,840.481	2,143.558		
Total	207	2,028,431.478			

Table 4.4: Analysis of Variance Table for plant length

	B1	B2	B3	B4	B5	B6	B7	B8	Mean A
A1	26.762	67.346	100.692	147.692	187.308	217.923	264.231	322.769	166.840
A2	26.892	39.538	57.538	107.154	141.462	166.462	180.077	211.000	116.265
Mean B	26.827	53.442	79.115	127.423	164.385	192.192	222.154	266.885	

Table 4.5: Two Way Mean Table for Plant length

Factors	C.D.	SE(d)	SE(m)
Factor(A)	12.680	6.420	4.540
Factor(B)	25.360	12.841	9.080
Factor (A X B)	35.864	18.160	12.841

Table 4.6: TABLE OF SEM, SED AND C.D

NUMBER OF LEAVES

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Replication	12	75.616			
Factor A	1	37.468	37.468	24.290	< 0.001
Factor B	7	631.911	90.273	58.523	< 0.001
Interaction A X B	7	13.850	1.979	1.283	0.261
Error	180	277.656	1.543		
Total	207	1,036.501			

Table 4.7: Analysis of Variance Table

	B1	B2	B3	B4	B5	B6	B7	B8	Mean A
A1	3.944	4.949	5.673	6.589	7.873	8.526	9.061	9.991	7.076
A2	3.941	4.558	5.001	6.160	6.766	7.249	7.671	8.468	6.227
Mean B	3.942	4.754	5.337	6.375	7.319	7.887	8.366	9.229	

Table 4.8: Two-way Mean Table

Factors	C.D.	SE(d)	SE(m)
Factor(A)	0.340	0.172	0.122
Factor(B)	0.680	0.344	0.244
Factor (A X B)	N/A	0.487	0.344

Table 4.9: TABLE OF SEM, SED AND C.D.

LEAF LAMINA LENGTH

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Replication	12	176.627			
Factor A	1	0.281	0.281	0.064	0.800
Factor B	7	3,666.687	523.812	119.889	< 0.001
Interaction A X B	7	43.554	6.222	1.424	0.198
Error	180	786.446	4.369		
Total	207	4,673.595			

Table 4.10: Analysis of Variance table for Leaf length

	B1	B2	B3	B4	B5	B6	B7	B8	Mean A
A1	9.192	9.500	11.231	11.708	13.231	17.615	20.885	21.115	14.310
A2	9.423	10.746	11.731	12.346	13.308	16.323	19.246	20.769	14.237
Mean B	9.308	10.123	11.481	12.027	13.269	16.969	20.065	20.942	

Table 4.11: Two Way Mean Table

Factors	C.D.	SE(d)	SE(m)
Factor(A)	N/A	0.290	0.205
Factor(B)	1.145	0.580	0.410
Factor (A X B)	N/A	0.820	0.580

Table 4.12: TABLE OF SEM, SED AND C.D

LEAF LAMINA WIDTH

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Replication	12	300.245			
Factor A	1	77.902	77.902	14.397	< 0.001
Factor B	7	2,821.544	403.078	74.492	< 0.001
Interaction A X B	7	115.054	16.436	3.038	0.005
Error	180	973.981	5.411		
Total	207	4,288.727			

Table 4.13: Analysis of Variance Table

	B1	B2	B3	B4	B5	B6	B7	B8	Mean A
A1	11.700	12.108	13.654	14.746	15.769	20.823	22.800	22.962	16.820
A2	11.215	12.769	13.923	14.346	15.062	17.946	19.200	20.308	15.596
Mean B	11.458	12.438	13.788	14.546	15.415	19.385	21.000	21.635	

Table 4.14: Two Way Mean Table

Factors	C.D.	SE(d)	SE(m)
Factor(A)	0.637	0.323	0.228
Factor(B)	1.274	0.645	0.456
Factor (A X B)	1.802	0.912	0.645

Table 4.15: TABLE OF SEM, SED AND C.D.

NUMBER OF NODES

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Replication	12	9,414.894			
Factor A	1	7,500.005	7,500.005	28.008	< 0.001
Factor B	7	55,129.072	7,875.582	29.411	< 0.001
Interaction A X B	7	2,555.803	365.115	1.363	0.223
Error	180	48,200.183	267.779		
Total	207	122,799.957			

Table 4.16: Analysis of Variance Table

	B1	B2	B3	B4	B5	B6	B7	B8	Mean A
A1	5.846	12.231	17.846	34.846	45.538	42.462	52.769	64.615	34.519
A2	5.154	8.538	12.462	17.615	26.000	27.308	37.077	45.923	22.510
Mean B	5.500	10.385	15.154	26.231	35.769	34.885	44.923	55.269	

Table 4.17: Two Way Mean Table

Factors	C.D.	SE(d)	SE(m)
Factor(A)	4.482	2.269	1.605
Factor(B)	8.963	4.539	3.209
Factor (A X B)	N/A	6.418	4.539

Table 4.18: TABLE OF SEM, SED AND C.D.

NUMBER OF TENDRILS

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Replication	12	82.889			
Factor A	1	96.623	96.623	34.081	< 0.001
Factor B	7	1,072.040	153.149	54.019	< 0.001
Interaction A X B	7	21.755	3.108	1.096	0.367
Error	180	510.315	2.835		
Total	207	1,783.622			

Table 4.19: Analysis of Variance Table

	B1	B2	B3	B4	B5	B6	B7	B8	Mean A
A1	3.204	5.173	6.620	7.932	9.509	9.132	9.436	10.321	7.666
A2	2.806	3.846	4.661	5.572	7.503	7.877	8.693	9.465	6.303
Mean B	3.005	4.510	5.640	6.752	8.506	8.504	9.064	9.893	

Table 4.20: Two Way Mean Table

Factors	C.D.	SE(d)	SE(m)
Factor(A)	0.461	0.233	0.165
Factor(B)	0.922	0.467	0.330
Factor (A X B)	N/A	0.660	0.467

Table 4.21: TABLE OF SEM, SED AND C.D.

TENDRIL LENGTH

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Replication	12	930.126			
Factor A	1	725.642	725.642	85.826	< 0.001
Factor B	7	4,068.459	581.208	68.743	< 0.001
Interaction A X B	7	178.664	25.523	3.019	0.005
Error	180	1,521.872	8.455		
Total	207	7,424.763			

Table 4.22: Analysis of Variance Table

	B1	B2	B3	B4	B5	B6	B7	B8	Mean A
A1	6.400	9.308	12.154	13.385	15.423	18.738	20.500	21.192	14.638
A2	5.308	7.577	10.508	7.792	9.208	13.669	15.938	17.215	10.902
Mean B	5.854	8.442	11.331	10.588	12.315	16.204	18.219	19.204	

Table 4.23: Two Way Mean Table

Factors	C.D.	SE(d)	SE(m)
Factor(A)	0.796	0.403	0.285
Factor(B)	1.593	0.806	0.570
Factor (A X B)	2.252	1.141	0.806

Table 4.24: TABLE OF SEM, SED AND C.D.

NUMBER OF FLOWERS

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Replication	12	1.003			
Factor A	1	3.277	3.277	23.062	< 0.001
Factor B	7	12.434	1.776	12.502	< 0.001
Interaction A X B	7	3.542	0.506	3.561	0.001
Error	180	25.574	0.142		
Total	207	45.829			

Table 4.25: Analysis of Variance Table

	B1	B2	B3	B4	B5	B6	B7	B8	Mean A
A1	1.000	1.000	1.519	2.144	1.496	1.633	1.527	1.064	1.423
A2	1.000	1.000	1.120	1.337	1.159	1.385	1.279	1.096	1.172
Mean B	1.000	1.000	1.320	1.741	1.328	1.509	1.403	1.080	

Table 4.26: Two Way Mean Table

Factors	C.D.	SE(d)	SE(m)
Factor(A)	0.103	0.052	0.037
Factor(B)	0.206	0.105	0.074
Factor (A X B)	0.292	0.148	0.105

Table 4.27: TABLE OF SEM, SED AND C.D.

NUMBER OF FRUITS

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Replication	12	4.767			
Factor A	1	1.911	1.911	20.167	< 0.001
Factor B	7	5.493	0.785	8.281	< 0.001
Interaction A X B	7	1.120	0.160	1.689	0.114
Error	180	17.058	0.095		
Total	207	30.350			

Table 4.28: Analysis of Variance Table

	B1	B2	B3	B4	B5	B6	B7	B8	Mean A
A1	1.000	1.000	1.120	1.458	1.332	1.377	1.420	1.679	1.298
A2	1.000	1.000	1.032	1.032	1.088	1.223	1.127	1.349	1.106
Mean B	1.000	1.000	1.076	1.245	1.210	1.300	1.274	1.514	

Table 4.29: Two Way Mean Table

Factors	C.D.	SE(d)	SE(m)
Factor(A)	0.084	0.043	0.030
Factor(B)	0.169	0.085	0.060
Factor(A X B)	N/A	0.121	0.085

Table 4.30: TABLE OF SEM, SED AND C.D.

KEY FINDINGS from 4.3.6a

PLANT LENGTH:

KEY FINDINGS from Table 4.4, 4.5 and 4.6:

- Treated plants showed marked increases in plant height ($F = 87.182$; $p < 0.001$).
- Comparing control and treated conditions also showed a highly significant difference ($F = 62.050$), affirming the growth-promoting potential of the bacterial consortia.

LEAVES

a) NUMBER OF LEAVES

KEY FINDINGS from Table 4.7, 4.8 and 4.9:

- Treated setups (Factor B) had significantly more leaves than control ($F = 58.523$; $p < 0.001$).
- The comparison between treated and control (Factor A) was also significant ($F = 24.290$), showing treatment enhanced leaf proliferation.

b) LEAF LAMINA LENGTH

KEY FINDINGS from Table 4.10, 4.11 and 4.12:

- Leaf length was not significant in control groups ($F = 0.064$; $p = 0.800$) with respect to the treated set up.
- The treated set up (Factor B) had a very strong impact ($F = 119.889$; $p < 0.001$), indicating that bacterial consortia inherently promote longer leaves.

c) LEAF LAMINA WIDTH

KEY FINDINGS from 4.13, 4.14 and 4.15:

- The treated set up (Factor B) was highly significant ($F = 74.492$; $p < 0.001$) as compared to the control set up ($F = 14.397$; $p < 0.001$).
- A significant interaction ($F = 3.038$; $p = 0.005$) reveals that treated set up are more effective when applied as compared to control set up.

NODES

KEY FINDINGS from Table 4.16, 4.17 and 4.18:

- Treated plants showed higher number of nodes with respect to the control set up. ($F = 29.411$; $p < 0.001$).
- The comparison between control and treated (Factor A) was also significant ($F = 28.008$), indicating treatment enhanced nodal development.

TENDRILS

KEY FINDINGS FROM Table 4.19, 4.20 and 4.21:

- Treatment significantly enhanced tendril production ($F = 54.019$; $p < 0.001$), compared to the control.

TENDRIL LENGTH

KEY FINDINGS from Table 4.22, 4.23 and 4.24:

- Tendril length increased significantly in the treated setup ($F = 68.743$; $p < 0.001$).
- The control vs treated difference (Factor A) was also highly significant ($F = 85.826$), showing strong effect in the treated plants.

FLOWERS

KEY FINDINGS from Table 4.25, 4.26 and 4.27:

- The treated setup (Factor B) showed a significant increase in flower number compared to the untreated control (Factor A), supported by an F-value of 12.502 ($p < 0.001$).
- The overall effect of control vs treated (Factor A) was also significant ($F = 23.062$), indicating that treatment improves flowering.

FRUITS

Key Findings from Table 4.28, 4.29 and 4.30:

- The treated plants (Factor B) produced significantly more fruits than the control group ($F = 8.281$; $p < 0.001$).
- Treatment vs control comparison (Factor A) was also significant ($F = 20.167$), confirming positive impact of treatment on fruiting.

4.3.6b. Statistical analysis of the subsequent data Analysis of variance was measured for the plant parameters of *Zea mays* (Variety: 4226)

Analysis of Variance (ANOVA) was carried out, with respect to the control set-up and the treatment set up. The aim of the work was to statistically evaluate the effects of bacterial consortia treatment and plant growth stages on various physiological parameters of *Zea mays* (Variety: 4226) using two-way ANOVA, in order to determine the significance of treatment and its interaction with time on overall plant development. Analysis was done using Python 3. Factor A is considered as treated set-up and Factor B as control set up.

Two-way ANOVA was used for plant parameters, since it enables the simultaneous assessment of both temporal growth (in weeks) and the treatment effect (comparing control and treated groups), as well as testing if the treatment effect varies with the age of the plants (interaction).

- Significance levels: A p-value less than 0.05 is generally considered significant. For example, $p < 0.001$ (seen in many results) means there is less than 0.1% chance that the observed effect is due to random variation. Smaller p-values indicate stronger evidence against the null hypothesis (no effect).
- Critical Difference (C.D.): This is essentially the *least significant difference* (LSD) for comparing treatment means. It explains that the difference between two treatment means must be to be statistically significant. For instance, for plant height, the C.D. for Factor A is 4.895 (units cm). Any mean difference above this (e.g. $181.19 - 144.33 = 36.86$) is considered significant.
- Standard Error of the Mean [SE(m)]: This measures how precisely the sample mean estimates the true population mean. Smaller SE(m) means more precise estimate. For example, SE(m) for plant height under Factor A is 1.748, indicating the mean height is estimated with that standard error. In general, SE(m) tells us the typical sampling error of the mean.
- Standard Error of Difference [SE(d)]: This quantifies the variability in the difference between two means. In practice, SE(d) is used to compute the LSD (critical difference). For instance, $SE(d) = 2.472$ for plant height under Factor A, which together with a t-value gives the C.D. The larger the SE(d), the larger the difference needed to declare significance.

Each of these values [C.D., SE(d), SE(m)] comes from the ANOVA error term and shows the precision of comparisons. In summary, the ANOVA indicates that treatment (Factor A) and time

(Factor B) both significantly affected all parameters except internode count (where A was marginal). Only plant height and leaf number showed a significant A×B interaction. The statistical significance (very low p-values) implies the treatment had real, practical effects (larger plants, more leaves/nodes, etc.), not just random fluctuations.

PLANT HEIGHT

A is treated and B is control

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Replication	9	26,158.931			
Factor A	1	54,353.756	54,353.756	222.286	< 0.001
Factor B	7	288,285.044	41,183.578	168.425	< 0.001
Interaction A X B	7	8,647.394	1,235.342	5.052	< 0.001
Error	135	33,010.369	244.521		
Total	159	410,455.494			

Table 4.31: Analysis of Variance Table

	B1	B2	B3	B4	B5	B6	B7	B8	Mean A
A1	102.700	104.700	173.200	182.900	201.400	214.600	228.700	241.300	181.188
A2	87.500	90.100	129.800	155.500	158.600	169.100	176.600	187.400	144.325
Mean B	95.100	97.400	151.500	169.200	180.000	191.850	202.650	214.350	

Table 4.32: Two Way Mean Table

Factors	C.D.	SE(d)	SE(m)
Factor(A)	4.895	2.472	1.748
Factor(B)	9.791	4.945	3.497
Factor (A X B)	13.846	6.993	4.945

Table 4.33: TABLE OF SEM, SED AND C.

NUMBER OF LEAVES

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Replication	9	1.696			
Factor A	1	2.633	2.633	80.517	< 0.001
Factor B	7	5.668	0.810	24.762	< 0.001
Interaction A X B	7	0.891	0.127	3.894	0.001
Error	135	4.415	0.033		
Total	159	15.303			

Table 4.34: Analysis of Variance Table

	B1	B2	B3	B4	B5	B6	B7	B8	Mean A
A1	3.327	3.388	3.643	3.751	3.802	3.855	3.916	4.041	3.715
A2	3.140	3.250	3.518	3.545	3.586	3.614	3.599	3.419	3.459
Mean B	3.233	3.319	3.580	3.648	3.694	3.734	3.757	3.730	

Table 4.35: Two Way Mean Table

Factors	C.D.	SE(d)	SE(m)
Factor(A)	0.057	0.029	0.020
Factor(B)	0.113	0.057	0.040
Factor (A X B)	0.160	0.081	0.057

Table 4.36: TABLE OF SEM, SED AND C.D.

LEAF LAMINA LENGTH

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Replication	9	906.195			
Factor A	1	1,825.877	1,825.877	38.310	< 0.001
Factor B	7	1,183.273	169.039	3.547	0.002
Interaction A X B	7	405.986	57.998	1.217	0.298
Error	135	6,434.130	47.660		
Total	159	10,755.461			

Table 4.37: Analysis of Variance Table

	B1	B2	B3	B4	B5	B6	B7	B8	Mean A
A1	72.050	71.400	70.000	73.150	73.350	73.600	75.300	76.300	73.144
A2	58.800	60.700	65.800	68.600	69.500	68.550	69.350	69.800	66.388
Mean B	65.425	66.050	67.900	70.875	71.425	71.075	72.325	73.050	

Table 4.38: Two Way Mean Table

Factors	C.D.	SE(d)	SE(m)
Factor(A)	2.161	1.092	0.772
Factor(B)	4.322	2.183	1.544
Factor (A X B)	N/A	3.087	2.183

Table 4.39: TABLE OF SEM, SED AND C.D.

LEAF LAMINA WIDTH

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Replication	9	66.295			
Factor A	1	31.507	31.507	21.374	< 0.001
Factor B	7	340.144	48.592	32.964	< 0.001
Interaction A X B	7	20.789	2.970	2.015	0.058
Error	135	199.006	1.474		
Total	159	657.741			

Table 4.40: Analysis of Variance Table

	B1	B2	B3	B4	B5	B6	B7	B8	Mean A
A1	8.080	8.720	9.360	10.220	10.260	10.620	11.080	11.830	10.021
A2	6.150	6.530	8.430	9.810	10.170	10.030	10.810	11.140	9.134
Mean B	7.115	7.625	8.895	10.015	10.215	10.325	10.945	11.485	

Table 4.41: Two Way Mean Table

Factors	C.D.	SE(d)	SE(m)
Factor(A)	0.380	0.192	0.136
Factor(B)	0.760	0.384	0.271
Factor (A X B)	N/A	0.543	0.384

Table 4.42: TABLE OF SEM, SED AND C.D.

NUMBER OF NODES

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Replication	9	2.005			
Factor A	1	1.332	1.332	15.404	< 0.001
Factor B	7	21.192	3.027	35.017	< 0.001
Interaction A X B	7	0.038	0.005	0.063	1.000
Error	135	11.672	0.086		
Total	159	36.239			

Table 4.43: Analysis of Variance Table

	B1	B2	B3	B4	B5	B6	B7	B8	Mean A
A1	2.426	2.503	2.885	2.979	3.196	3.354	3.432	3.447	3.028
A2	2.298	2.341	2.716	2.807	2.995	3.113	3.239	3.254	2.845
Mean B	2.362	2.422	2.801	2.893	3.096	3.233	3.336	3.351	

Table 4.44: Two Way Mean Table

Factors	C.D.	SE(d)	SE(m)
Factor(A)	0.092	0.046	0.033
Factor(B)	0.184	0.093	0.066
Factor (A X B)	N/A	0.131	0.093

Table 4.45: TABLE OF SEM, SED AND C.D

NUMBER OF INTERNODES

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Replication	9	0.682			
Factor A	1	0.160	0.160	3.730	0.056
Factor B	7	26.588	3.798	88.299	< 0.001
Interaction A X B	7	0.230	0.033	0.763	0.619
Error	135	5.807	0.043		
Total	159	33.468			

Table 4.46: Analysis of Variance Table

	B1	B2	B3	B4	B5	B6	B7	B8	Mean A
A1	2.210	2.253	2.857	3.068	3.102	3.217	3.299	3.343	2.919
A2	2.118	2.245	2.958	2.995	3.062	3.096	3.176	3.192	2.855
Mean B	2.164	2.249	2.907	3.031	3.082	3.157	3.237	3.268	

Table 4.47: Two Way Mean Table

Factors	C.D.	SE(d)	SE(m)
Factor(A)	N/A	0.033	0.023
Factor(B)	0.130	0.066	0.046
Factor (A X B)	N/A	0.093	0.066

Table 4.48: TABLE OF SEM, SED AND C.D.

PLANT GIRTH

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Replication	9	59.853			
Factor A	1	63.884	63.884	21.384	< 0.001
Factor B	7	804.090	114.870	38.450	< 0.001
Interaction A X B	7	5.027	0.718	0.240	0.974
Error	135	403.312	2.987		
Total	159	1,336.166			

Table 4.49: Analysis of Variance Table

	B1	B2	B3	B4	B5	B6	B7	B8	Mean A
A1	7.850	8.050	10.030	11.600	11.850	12.400	13.400	13.650	11.104
A2	6.060	6.210	8.720	10.850	10.850	11.320	12.300	12.410	9.840
Mean B	6.955	7.130	9.375	11.225	11.350	11.860	12.850	13.030	

Table 4.50: Two Way Mean Table

Factors	C.D.	SE(d)	SE(m)
Factor(A)	0.541	0.273	0.193
Factor(B)	1.082	0.547	0.386
Factor (A X B)	N/A	0.773	0.547

Table 4.51: TABLE OF SEM, SED AND C.D.

NUMBER OF FRUITS

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Replication	9	1.873			
Factor A	1	0.581	0.581	9.806	0.002
Factor B	7	6.146	0.878	14.811	< 0.001
Interaction A X B	7	0.535	0.076	1.290	0.260
Error	135	8.003	0.059		
Total	159	17.139			

Table 4.52: Analysis of Variance Table

	B1	B2	B3	B4	B5	B6	B7	B8	Mean A
A1	1.363	1.427	1.541	1.695	1.781	1.767	1.850	2.017	1.680
A2	1.302	1.302	1.302	1.700	1.719	1.749	1.749	1.653	1.560
Mean B	1.333	1.365	1.422	1.698	1.750	1.758	1.799	1.835	

Table 4.53: Two Way Mean Table

Factors	C.D.	SE(d)	SE(m)
Factor(A)	0.076	0.038	0.027
Factor(B)	0.152	0.077	0.054
Factor (A X B)	N/A	0.109	0.077

Table 4.54: TABLE OF SEM, SED AND C.D.

KEY FINDINGS from 4.3.6b**PLANT LENGTH****KEY FINDINGS from table 4.31, 4.32 and 4.33:**

- The ANOVA analysis revealed a very strong effect of the bacterial consortia treatment on plant height, with an F-value of 222.29 and a highly significant p-value (< 0.001).
- This indicates that the treated plants were significantly taller than the control plants across all observed weeks.

LEAVES**a) NUMBER OF LEAVES****KEY FINDINGS from Table 4.34, 4.35 and 4.36:**

- A highly significant treatment effect was observed on the number of leaves, with an **F-value of 80.52** and **$p < 0.001$** .
- This confirms that the application of bacterial consortia led to a noticeable increase in the number of leaves per plant.
- Additionally, the interaction effect with time was significant (**$F = 3.89$, $p = 0.001$**), indicating that the rate of increase in leaf number varied over the weeks depending on the treatment.

b) LEAF LAMINA LENGTH**KEY FINDINGS from Table 4.37, 4.38 and 4.39:**

- The treatment had a statistically significant impact on leaf length, as shown by an F-value of 38.31 and a p-value < 0.001 .
- This demonstrates that treated plants had significantly longer leaves compared to untreated controls.

c) LEAF LAMINA WIDTH**KEY FINDINGS from Table 4.40, 4.41 and 4.42:**

- A significant effect of treatment on leaf width was observed, with an F-value of 21.37 and $p < 0.001$.
- This indicates that the bacterial consortia significantly increased the breadth of leaves.

NODES

KEY FINDINGS from Table 4.43, 4.44 and 4.45:

- The control set up showed an F-value of 15.40 and a p-value < 0.001 , the treated set up showed an F -value of 35.017 and a p-value < 0.001 .
- This shows that the structural complexity of treated plants was higher than that of the untreated ones.
- No interaction with time was found, implying that the increase in node formation was consistent across the weeks.

INTERNODES

KEY FINDINGS from Table 4.46, 4.47 and 4.48:

- The effect of bacterial consortia on internode length was highly statistically significant, with an F-value of 3.73 ($p = 0.056$) for control set up and an F-value of 88.299 ($p < 0.01$) for treated set up.
- Treated plants developed higher number of internodes, which are often associated with better structural strength, nutrient transport, and stress tolerance.

PLANT GIRTH

KEY FINDINGS from Table 4.49, 4.50 and 4.51:

- Plant girth showed a significant increase under bacterial consortia treatment, as reflected by an F-value of 21.38 ($p < 0.001$) of control set up as compared to the F-value of 38.450 ($p < 0.001$) of treated set up.
- Treated plants developed thicker stems, which are often associated with better structural strength, nutrient transport, and stress tolerance.
- This treatment effect remained consistent over time, with no significant interaction observed.

FRUITS

KEY FINDINGS from Table 4.52, 4.53 and 4.54:

- Fruit production was positively affected by the bacterial consortia, with a statistically significant F-value of 9.81 ($p = 0.002$) for control set up and F-value of 14.811 ($p < 0.001$) for treated set up.

4.3.7 Soil Health, Enzyme and Microbial Activity upon addition of bacterial consortia

4.3.7a. Analysis of the soil parameters after application of bacterial consortia:

The application of bacterial consortia of *B. velezensis* DPP(B) and *C. necator* DCP(A) to the *Cucurbita maxima* crop and *Zea mays* soil crops was done. The soil was collected for analysis of physico-chemical parameters.

The aim of the work was to evaluate the effect of bacterial consortia treatment on soil physicochemical properties and nutrient availability, and to assess its influence on the growth and development of *Cucurbita maxima* (Pumpkin) and *Zea mays* (Maize), by comparing treated and untreated (control) setups under field conditions. The treated and untreated plots were present adjacent to each other. The bacterial consortia were applied to both *Cucurbita maxima* crops and *Zea mays* crops, when the plants were of 30 days age and 60 days age (peak stage). The soil parameters of the treated and control soil were measured when the plants were at the peak stage.

SOIL PARAMETERS	<i>Cucurbita maxima</i> soil (Control)	<i>Cucurbita maxima</i> soil (Treated)	<i>Zea mays</i> soil (Control)	<i>Zea mays</i> soil (Treated)
pH	8.8±0.002	8.3±0.007	8.7±0.001	8.2±0.005
Electrical Conductivity	50±0.010	194±0.003	64±0.001	102±0.07
Nitrogen(kg/ha)	234±0.004	225±0.001	221±0.03	189±0.006
Potassium(kg/ha)	157±0.07	151±0.001	160±0.002	141±0.002
Phosphorous(kg/ha)	465±0.001	415±0.021	459±0.02	364±0.04
Organic carbon (%)	2.52%	0.49%	0.39%	0.42%
Magnesium(mg/kg)	114±0.003	2.49±0.002	72.8±0.011	61.7±0.08
Calcium(mg/kg)	0.77±0.005	0.63±0.003	0.76±0.09	0.61±0.09
Iron(mg/kg)	4.84±0.018	4.25±0.010	4.75±0.004	4.17±0.02
Manganese(mg/kg)	1.83±0.006	2.34±0.003	3.44±0.01	2.54±0.004
Sulphur(mg/kg)	7.01±0.014	6.83±0.001	7.24±0.012	6.75±0.001
Copper(mg/kg)	0.42±0.003	0.4±0.016	0.4±0.005	0.38±0.011
Boron(mg/kg)	5.21±0.002	5.18±0.011	5.16±0.006	5.02±0.015
Zinc(mg/kg)	5.49±0.001	5.46±0.003	4.85±0.07	4.69±0.021

Table 4.55: Analysis of soil parameters in both treated and control set ups of *Cucurbita maxima* and *Zea mays* in East Kolkata Wetlands

Key Findings from Table 4.3.7a:**For *Cucurbita maxima***

- The pH in treated soil (8.3) is lower than in control soil (8.8), making the environment less alkaline and more suitable for nutrient uptake. Application of bacterial consortium to the plant rhizosphere, generates organic acids, CO₂ (carbonic acid), and metabolites that contribute to a slight acidification of the rhizosphere. These acids facilitate the chelation of cations (Ca²⁺, Mg²⁺, Fe³⁺) and release bound nutrients. Root exudates from *Cucurbita maxima* additionally decrease the local pH in the rhizosphere, improving nutrient uptake efficiency.
- Electrical Conductivity (EC) is significantly higher in treated soil (194 µS/cm) compared to control (50 µS/cm), indicating increased nutrient and salt availability within optimal range. A positive relationship exists between electrical conductivity (EC) and the availability of nutrients in treated soil, where increased EC indicates enhanced soil fertility and nutrient interactions that support plant growth.
- Nitrogen content is higher in treated soil (225 kg/ha) than in control (234kg/ha), which indicated that it supported better vegetative growth and development.
- Phosphorus level in treated soil was (415 kg/ha), which was lower compared to control (465 kg/ha), enhancing root development, flowering, and energy transfer.
- Calcium content is slightly lower in treated soil (0.63 mg/kg) than in control (0.77 mg/kg), showing better uptake of nutrients by plants and thereby improving structural support and root health.
- Iron content marginally decreased in treated soil (4.25 mg/kg) versus control (4.84 mg/kg), thereby showing that treated plants had better capability of chlorophyll formation and enzymatic functions.
- Manganese shows a clear increase in treated soil (2.34 mg/kg) compared to control (1.83 mg/kg), aiding photosynthesis and nitrogen metabolism.
- Sulphur is slightly lower in treated soil (6.83 mg/kg) than control (7.01 mg/kg), thereby contributing to amino acid synthesis and plant metabolism in treated plants.
- Zinc in treated soil (5.46 mg/kg) is nearly the same as control (5.49 mg/kg), with no significant loss in this essential micronutrient.
- Copper content in treated soil (0.4 mg/kg) is very close to control (0.42 mg/kg), showing better uptake abilities of treated plants to utilize copper.

- Boron levels in treated soil (5.18 mg/kg) and control (5.21 mg/kg) are nearly identical, ensuring maintained micronutrient balance.
- Potassium is slightly reduced in treated soil (151 kg/ha) compared to control (157 kg/ha), which may be due to solubilization of potassium by bacterial isolates, thereby promoting better uptake and utilization of potassium by bacterial isolates.

For *Zea mays*

- The pH of treated soil (8.2) is lower than that of control soil (8.7), reducing alkalinity and improving nutrient solubility and uptake.
- Electrical Conductivity (EC) is higher in treated soil (102 $\mu\text{S}/\text{cm}$) than in control (64 $\mu\text{S}/\text{cm}$), indicating greater availability of soluble salts and nutrients.
- Nitrogen content is higher in treated soil (189 kg/ha) than in control (221 kg/ha), thereby showing greater ability of bacterial isolates to fix nitrogen and make it available to the plants. This also supported enhanced vegetative growth and chlorophyll synthesis.
- Phosphorus content was found to decrease in treated soil (364 kg/ha), when compared to control (459 kg/ha), thereby showing that the bacterial isolates were able to solubilize more amounts of phosphorous in treated plants. This supported the functions of root development and energy transfer processes in treated plants.
- Potassium levels decreased in treated soil to 141mg/kg, when compared to control soils (160 kg/ha). This represented that upon addition of bacterial consortia to the treated plants, the bacterial isolates were able to solubilize potassium better and made it easier for plant uptake.
- Calcium content was found to be lower in treated soil (0.61 mg/kg) than in control (0.76 mg/kg), contributing to better cell wall development and root health.
- Iron content is slightly decreased in treated soil (4.17mg/kg) compared to control (4.75 mg/kg), aiding in chlorophyll formation and redox reactions.
- Zinc decreased in treated soil (4.69 mg/kg) when compared to control (4.85 mg/kg), which enhanced enzyme function and auxin synthesis.
- Manganese significantly decreases in treated soil (2.34 mg/kg) compared to control (3.44 mg/kg), which may slightly impact photosynthetic enzyme activity.
- Sulphur decreases in treated soil (6.75 mg/kg) from control (7.24 mg/kg), but remains within acceptable range for protein synthesis.

- Copper levels in treated soil were 0.38mg/kg, which was marginally less from control soils (0.4 mg/kg), ensuring continued enzyme activity and plant metabolism.
- Boron content in treated soil reduced to 5.02mg/kg when compared with control soils (5.16 mg/kg), thereby maintaining proper cell wall structure and reproductive growth.
- Organic carbon content increases slightly in treated soil (0.42%) compared to control (0.39%), improving soil structure and microbial activity.
- Magnesium content is slightly lower in treated soil (61.7 mg/kg) than control (72.8 mg/kg), but still adequate for chlorophyll production and enzyme activation.

4.3.7 b. Soil Enzyme Profile:

Enzyme profiling of both treated and control soil samples of *Cucurbita maxima* and *Zea mays* were done. In all the soil enzymatic parameters, it was observed that the values during the peak cropping period of the treated soil were much higher compared to the other cropping periods, as represented in Figure 4.58b.

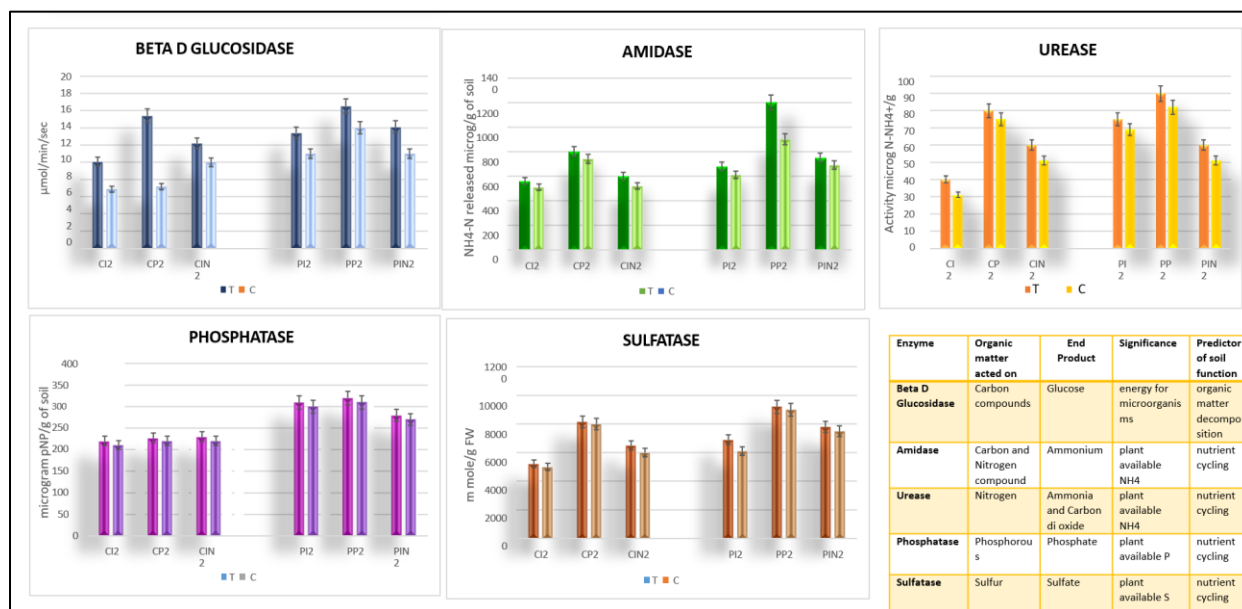


Figure 4.58b: Soil Enzyme Profile [CI- CORN INITIAL, CP-CORN PEAK, CIN-CORN INTERCROP; PI-PUMPKIN INITIAL, PP-PUMPKIN PEAK, PIN-PIMPKN INTERCROP]

Key Findings from Figure 4.3.7b:

- Beta-D-Glucosidase

The activity level is consistently greater in treated soils (T) when compared to controls (C) throughout all cropping stages. This indicates a boost in organic matter decomposition and carbon cycling within treated soils.

- Amidase

Treated soils exhibit a higher release of ammonium (NH₄⁺) than controls, particularly during the PI2 and PP2 stages. This suggests an enhancement in nitrogen mineralization and its availability for plants under treatment.

- Urease

The enzyme activity is increased in treated soils in comparison to controls, demonstrating a greater conversion of urea to NH_4^+ . This highlights an improved efficiency in nitrogen cycling under treated conditions.

- Phosphatase

There is a slight yet consistent enhancement in treated soils compared to controls. This suggests an increase in phosphate availability for plants, aiding in nutrient uptake.

- Sulfatase

The levels are significantly higher in treated soils, especially during the PI2 and PP2 stages. This demonstrates an improvement in sulfur cycling and sulfate availability as a result of the treatment.

4.3.7c. SYBR Green Staining and Fluorescence Microscopy of Soil and Plant Samples:

The staining of soil samples as well as the root samples of both the plants showed, that there was higher invasion of bacteria within the treated samples as compared to the control set-up, thereby signifying the fact that upon application of bacterial consortia, they directly determine the existence of bacterial colony in the soil as well as the root samples, as represented in **Figure 4.58c**.

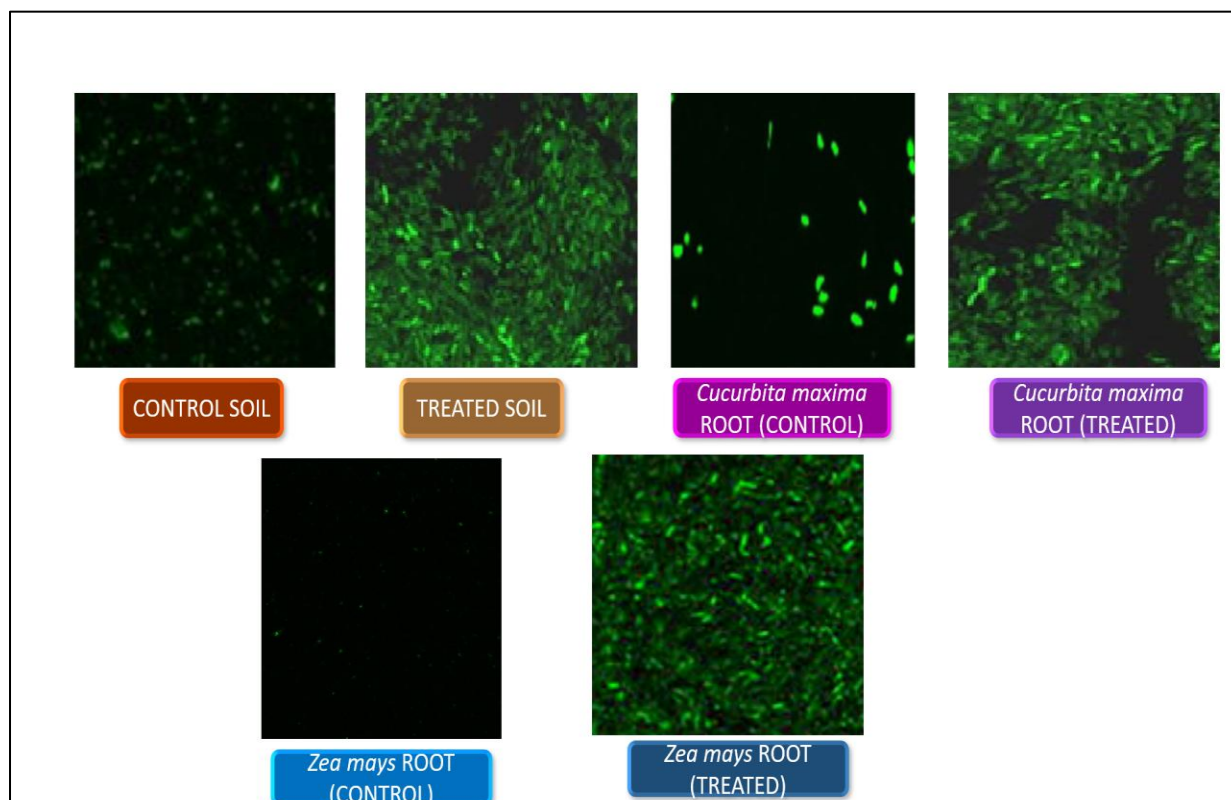


Fig4.58c: The fluorescence microscopy images acquired following SYBR Green staining clearly illustrate variations in microbial abundance between the control and treated samples of both soil and plant tissues

Key Findings from Figure 4.3.7c:**Soil Samples**

Control Soil: A limited number of scattered fluorescent green spots were observed, indicating a lower microbial density.

Treated Soil: A significantly higher density of fluorescent cells was noted, suggesting increased microbial colonization/activity post-treatment

***Cucurbita maxima* Root Samples**

Control Root: Sparse and distinct green fluorescent signals were detected, indicating minimal microbial association.

Treated Root: Numerous and dense fluorescent signals were recorded, indicating robust microbial colonization in root tissues under treatment conditions.

***Zea mays* Root Samples**

Control Root: Very faint and scattered fluorescence was noted, indicating a minimal microbial presence.

Treated Root: Bright, dense, and clustered fluorescence patterns were observed, indicating a significantly enhanced microbial colonization in the root tissue compared to the control.

4.3.8a. Metagenomic Analysis of *Cucurbita maxima* Soil upon addition of bacterial consortia

Metagenome analysis of the soil was done. The aim of the work was to investigate the colonization potential of applied plant growth-promoting bacterial (PGPB) strains in the rhizosphere of *Cucurbita maxima* by conducting a metagenomic comparison between control soil and treated soil, with a focus on tracking population shifts in *Bacillus* sp. and their associated taxonomic groups. The results have been represented in **Figure 4.59, 4.60 and 4.61**

In metagenomics, heatmaps are utilized to illustrate the compositions of microbial communities by showcasing the abundance of particular taxa or genes across various samples through color-coded cells. This method uncovers patterns and relationships among samples and organisms. Conversely, Krona charts employ a radial, space-filling hierarchy to represent the abundance and distribution of taxa within a phylogenetic tree, facilitating an intuitive exploration of intricate community structures and their hierarchical connections.

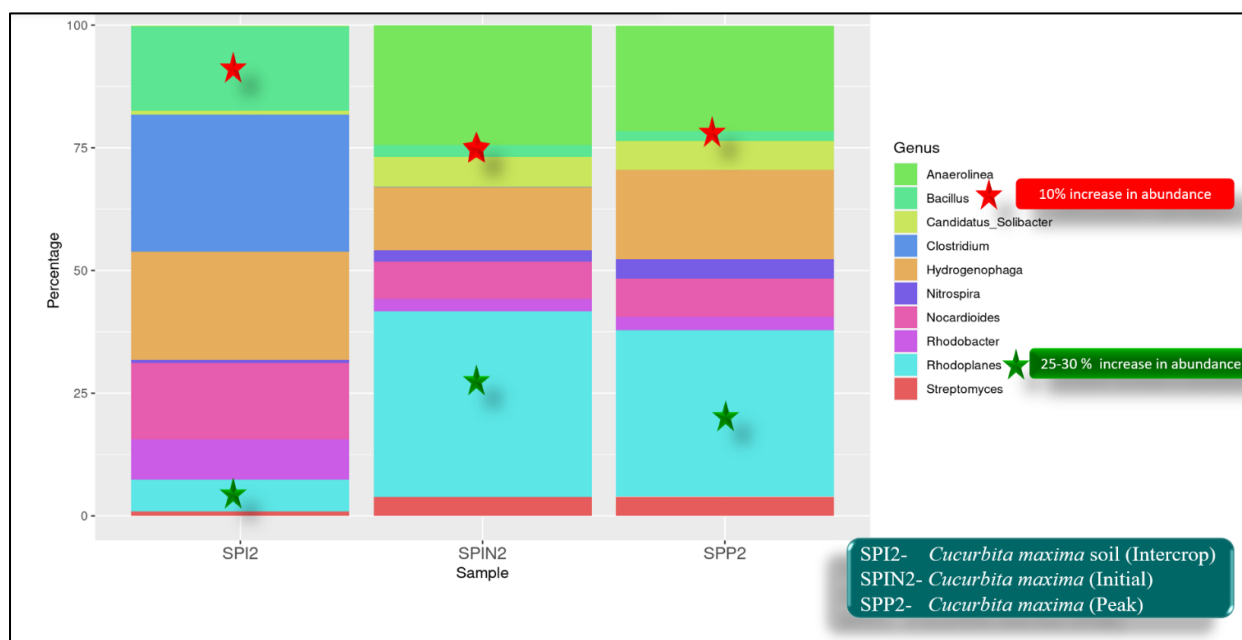


Figure 4.59- Metagenomic analysis of soil sample of *Cucurbita maxima* rhizosphere, upon addition of microbial consortia.

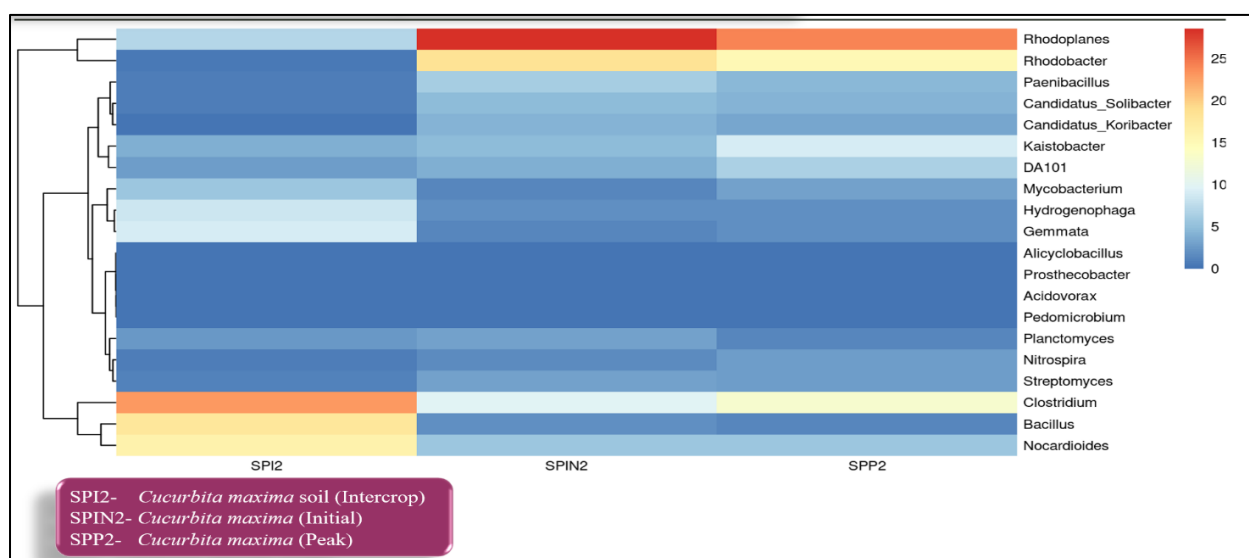


Figure 4.60- Heatmap representation of metagenomic analysis of soil sample of *Cucurbita maxima* rhizosphere, upon addition of bacterial consortia.

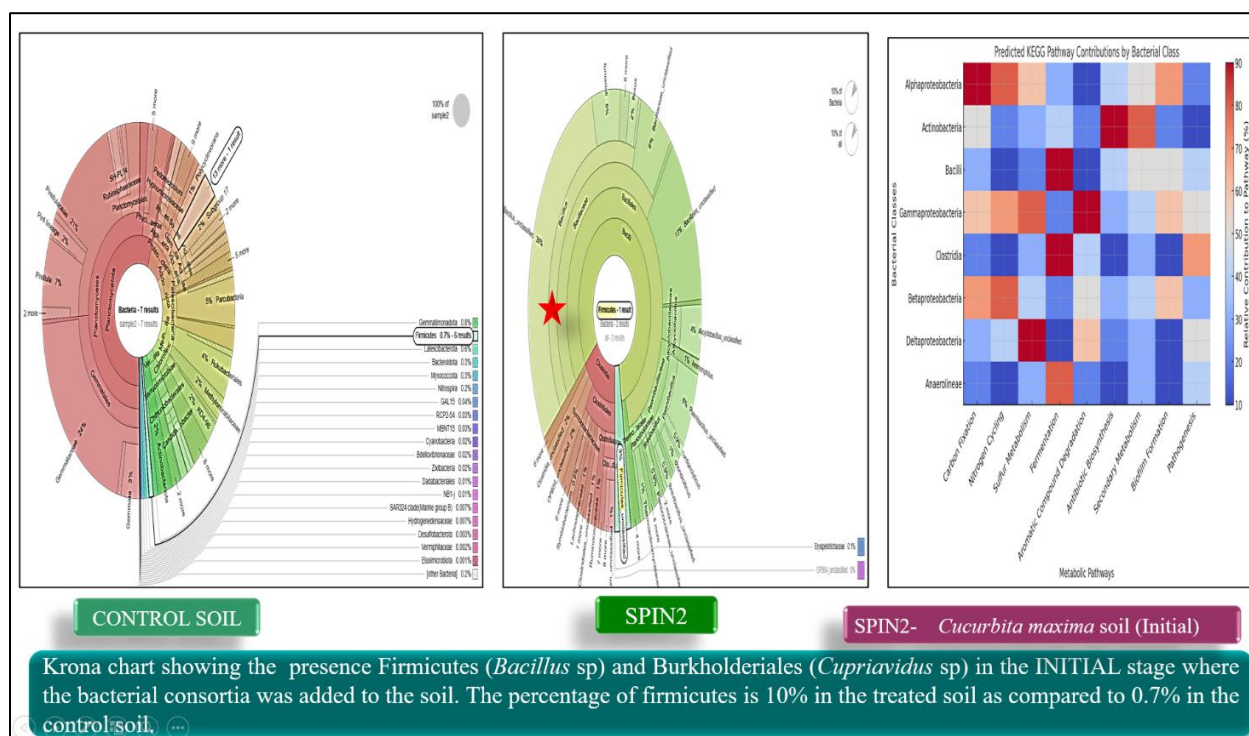


Figure 4.61- Krona chart of *Cucurbita maxima* rhizosphere and control soil upon addition of consortia, showing the increment of firmicute

KEY FINDINGS from 4.3.8a:

- The metagenomic comparison between control soil and treated soil revealed a 10% increase in the population of *Bacillus* sp., indicating effective colonization of the introduced bacterial strains, since the bacterial consortia included *Bacillus velezensis* and *Cupriavidus necator*.
- Heat map analysis showed a notable percentage increase in both the phylum Firmicutes and the family Bacillaceae from the initial to the final soil sample, further confirming successful establishment of the applied bacterial consortia in the rhizosphere of *Cucurbita maxima*.
- During the intercrop period, the abundance of *Bacillus* sp. increased by approximately 15%, demonstrating sustained colonization and activity of the applied strains.
- Other bacterial genera also showed increased abundance during the intercrop stage, including: *Clostridium* sp. (25% increase), *Hydrogenophaga* sp. (20% increase), suggesting possible synergistic interactions or shifts in microbial community structure driven by treatment. This can be because the introduction of bacterial consortia and interactions between plants and microbes probably changed the rhizosphere environment by enhancing the availability of root exudates, organic acids, and enzymatic by-products.
- During the peak crop period, a significant 25% increase in the abundance of *Rhodoplanes* sp. was observed, thereby indicating a dynamic microbial population supporting plant development. *Rhodoplanes* sp play a significant role in promoting plant growth mainly by converting atmospheric nitrogen into a usable form for plants. Additionally, certain species are known to synthesize plant hormones such as indole-3-acetic acid (IAA), which directly encourages plant growth. These advantageous processes contribute to improved plant development, increased yield, and enhanced resistance to pathogens and environmental stress.

4.3.8b. Metagenomic Analysis of *Zea mays* Soil upon addition of bacterial consortia

Metagenome analysis of the soil was done. The aim of the work was to investigate the colonization potential of applied plant growth-promoting bacterial (PGPB) strains in the rhizosphere of *Zea mays* by conducting a metagenomic comparison between control soil and treated soil, with a focus on tracking population shifts in *Bacillus* sp. and their associated taxonomic groups. The results have been represented in **Figure 4.62, 4.63, 4.64 and 4.65.**

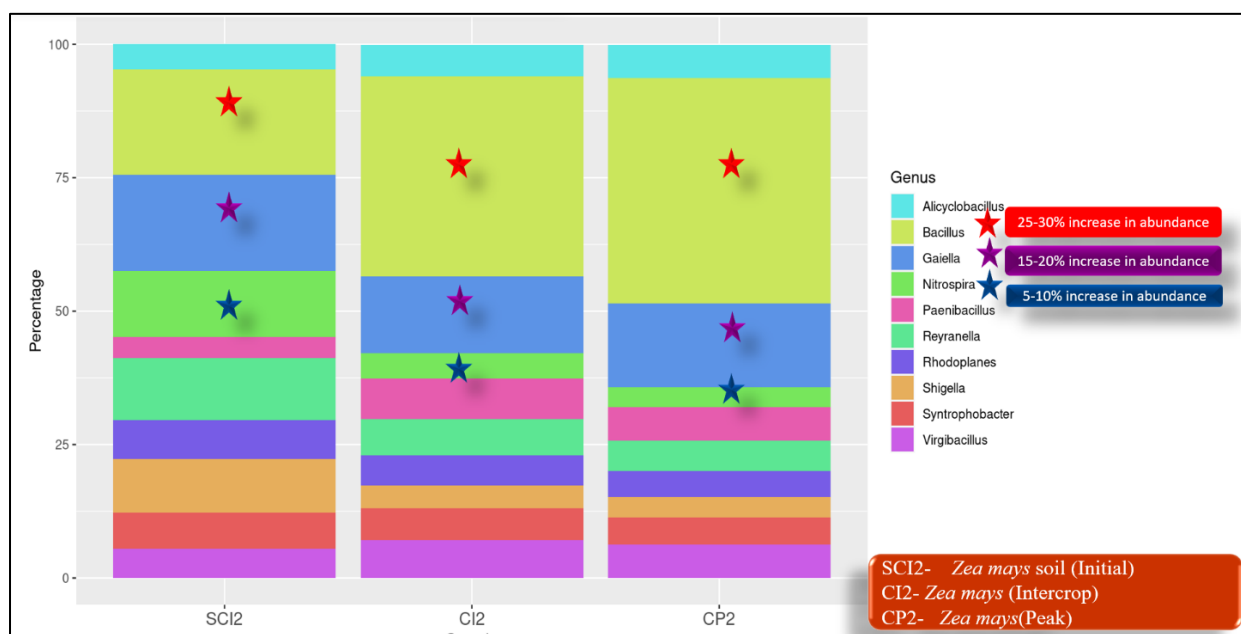


Figure 4.62- Metagenomic analysis of soil sample of *Zea mays* rhizosphere, upon addition of consortia.

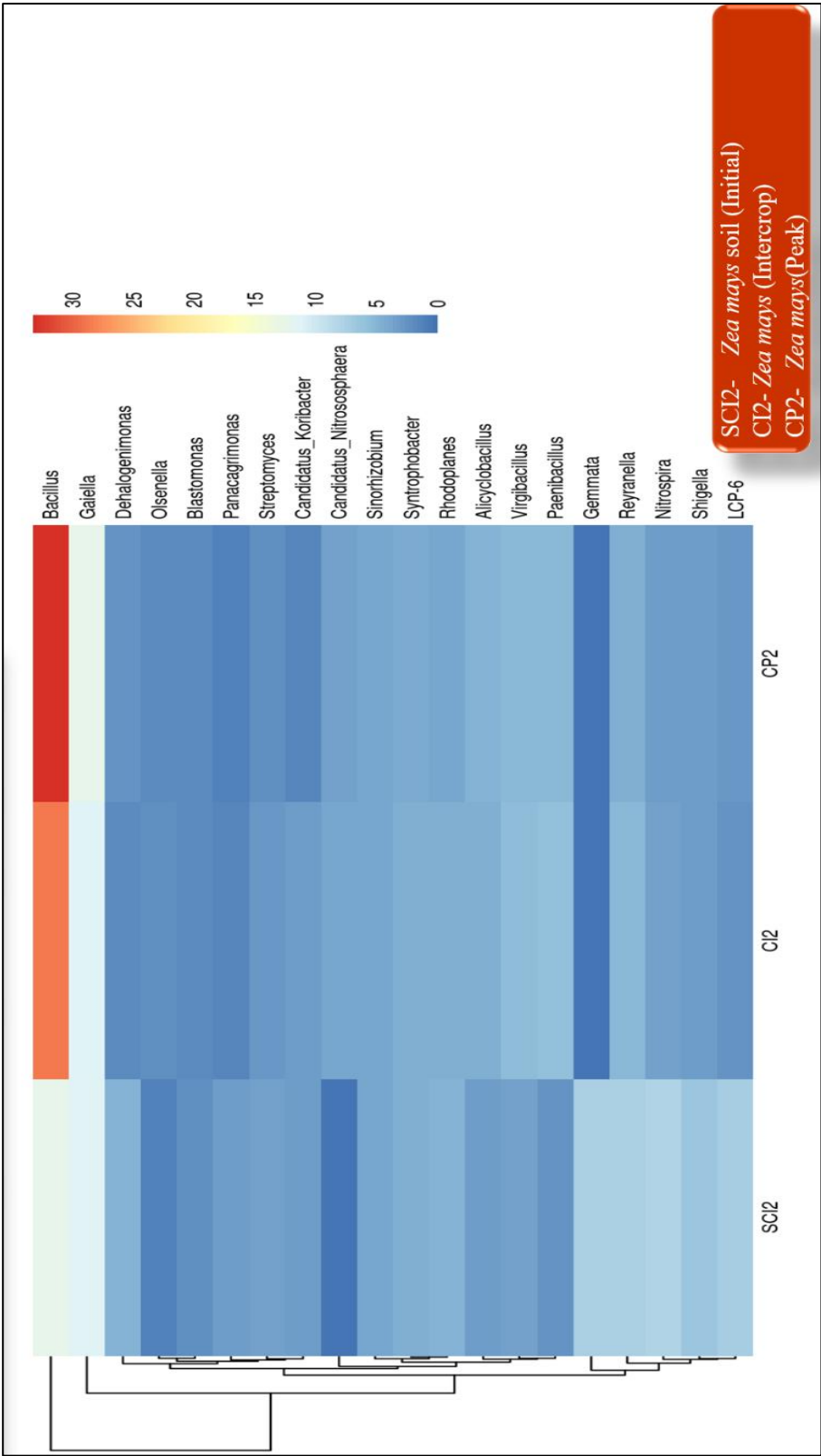
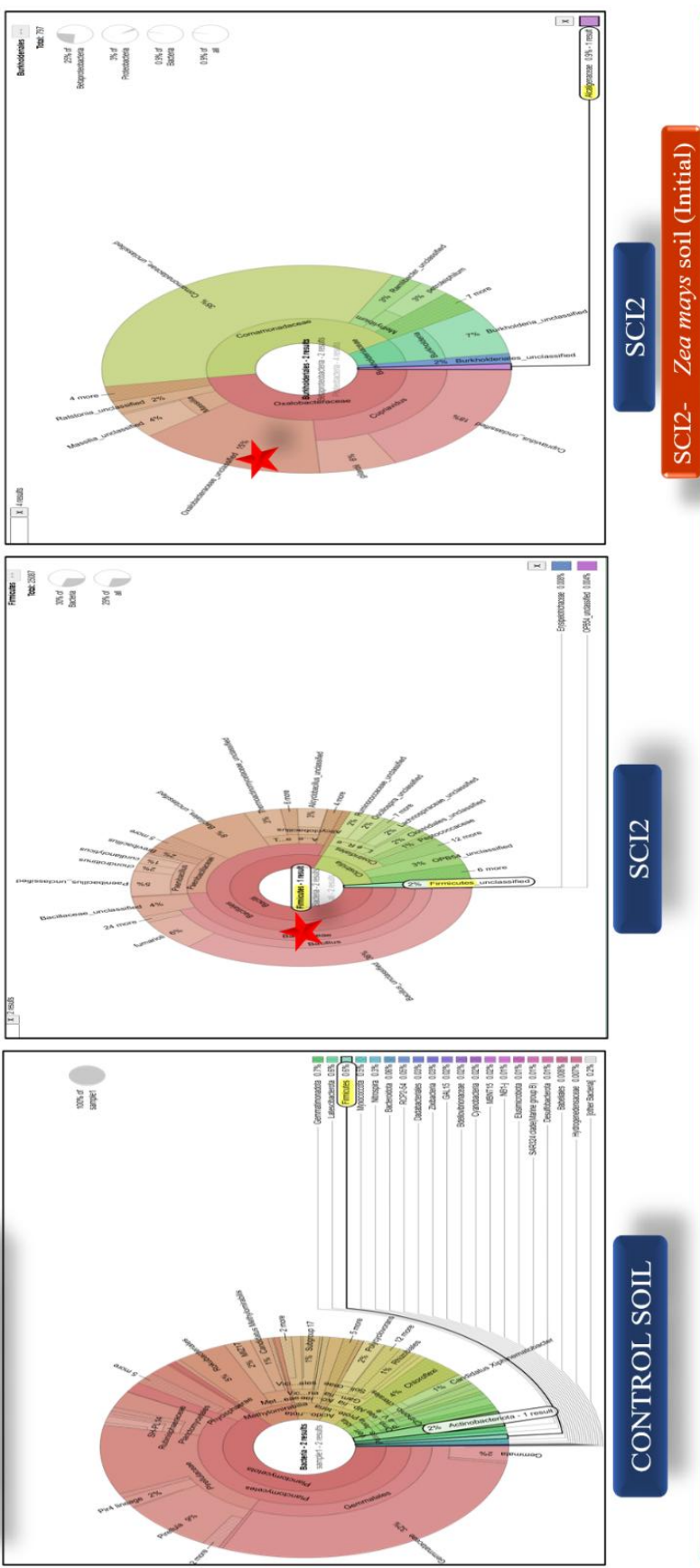


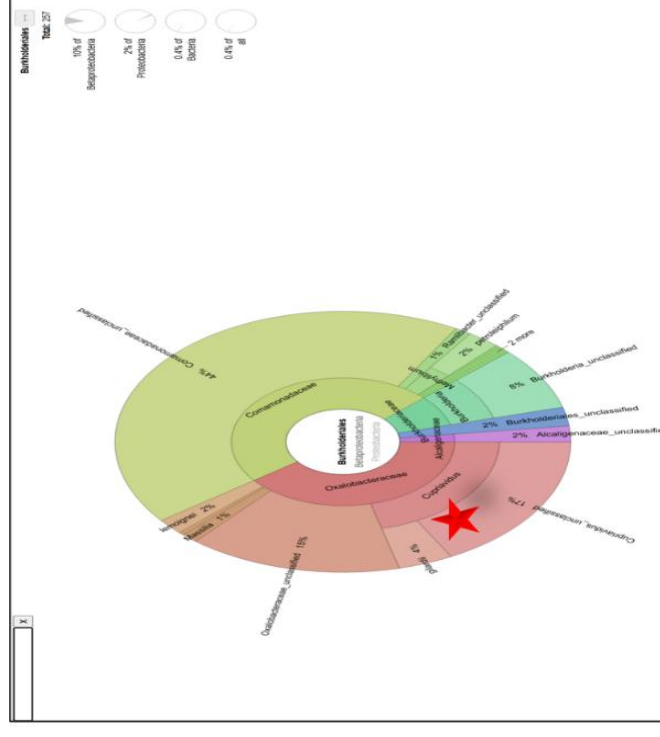
Figure 4.63- Heat map representation of soil sample of *Zea mays* rhizosphere, upon addition of bacterial consortia



Krona chart showing the presence Firmicutes (*Bacillus* sp) and Burkholderiales (*Cupriavidus* sp) in the initial stage where the bacterial consortia was added to the soil. The percentage of firmicutes is 30% in the treated soil as compared to 0.6% in the control soil.

Figure 4.64- Krona chart of *Zea mays* treated rhizosphere and control soil upon addition of consortia, showing the increment of firmicutes

CHAPTER 4 - RESULTS

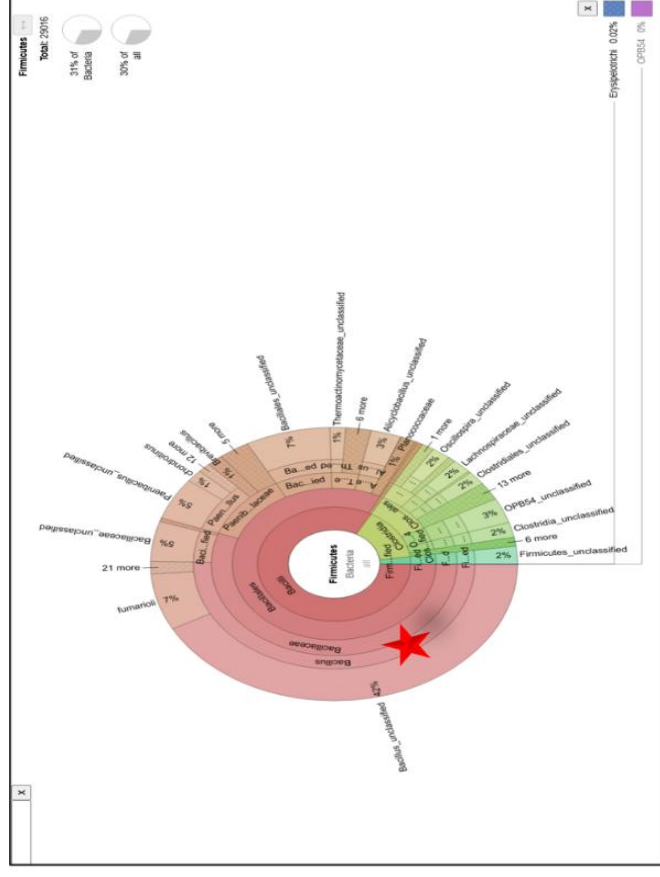


CP2

CP2- *Zea mays*(Peak)

Krona chart showing the presence Firmicutes (*Bacillus* sp) and Burkholderiales (*Cupriavidus* sp) in the PEAK stage where the bacterial consortia was added to the soil. The percentage of firmicutes is 31% in the treated soil as compared to 0.6% in the control soil.

Figure 4.65a–Krona chart of *Zea mays* treated rhizosphere soil (peak stage) and control soil upon addition of consortia, showing the increment of firmicutes.



CP2

KEY FINDINGS from Figure 4.3.8b:

- The metagenomic analysis revealed a significant increase in the abundance of *Bacillus* sp. in the rhizosphere of *Zea mays* following the application of the bacterial consortia.
- During the intercrop period, the abundance of *Bacillus* sp. increased by approximately 30%, indicating early colonization and establishment of the applied strains.
- A more pronounced 40% increase in *Bacillus* sp. abundance was observed during the peak growth period, demonstrating sustained and dominant colonization over time.
- In addition to *Bacillus*, the bacterial genus *Gaiella* sp. showed an approximate 15% increase in abundance during the intercrop period, suggesting possible shifts in microbial community dynamics and potential microbial synergy. *Gaiella* sp are a type of plant growth-promoting rhizobacteria (PGPR) that play various roles in enhancing plant growth. They contribute to soil carbon cycling, decompose organic matter, and may facilitate nutrient uptake by forming associations with plant roots. These bacteria are strictly aerobic chemo-organotrophs that coexist with plants, showcasing their adaptability to different soil conditions. The exudation of sugars, amino acids, and secondary metabolites from roots, which can serve as substrates for the proliferation of *Gaiella*. Additionally, the presence of *Bacillus* and other advantageous taxa may lead to synergistic effects, as *Bacillus* mediated nutrient solubilization and stress relief can indirectly promote the growth of *Gaiella*.

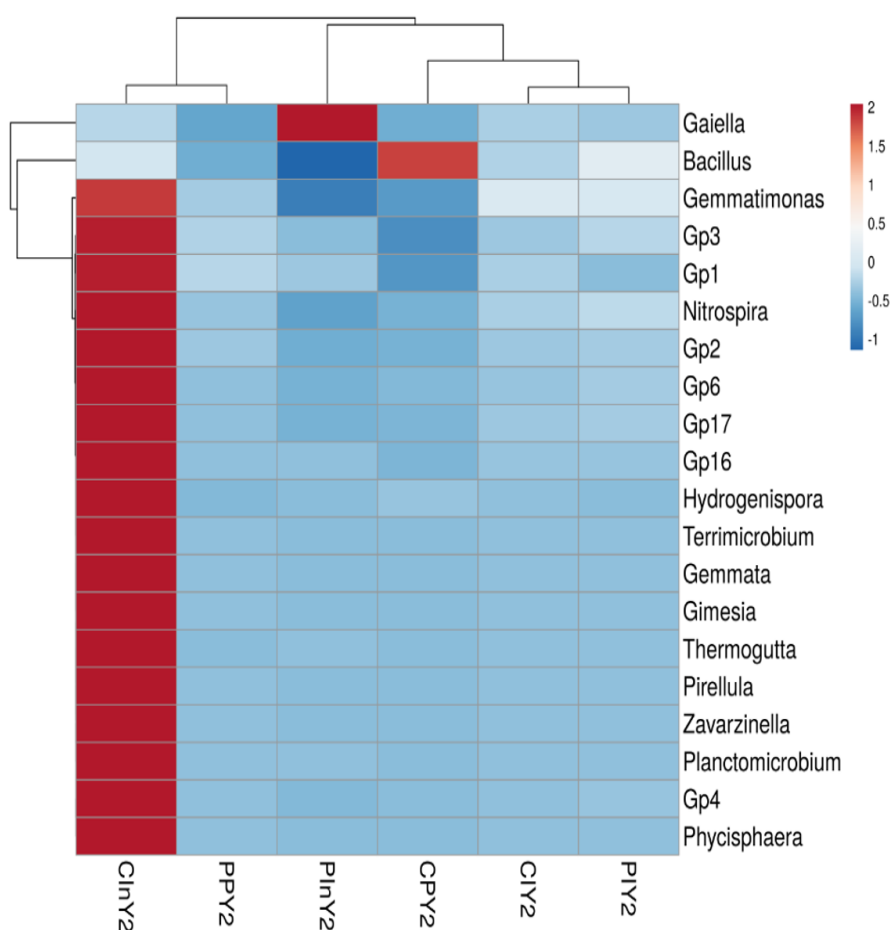


Figure 4.65b: Heatmap representing the top 20 genera of *Cucurbita maxima* (PI, PIn, PP) soil rhizosphere and *Zea mays* (CI, CIn, CP) soil rhizosphere.

KEY FINDINGS from 4.65b:

- Upon addition of bacterial consortia, CInY2 and PPY2 have consistently high relative abundance (red) for most taxa, suggesting richer microbial presence in these conditions.
- Other samples (PInY2, CPY2, CIY2, PIY2) show mostly light blue (neutral/average abundance), with occasional variations.
- Some taxa show sample-specific enrichment like:

Bacillus sp have been observed to be highly enriched in CInY2 and CPY2.; *Gaiella* sp are found to be present in the highest abundance in PPY2. Other genus like *Nitrospira* sp have also been observed in the top 20 genera.

4.3.9a. Quantification of lead analysis and accumulation in *Cucurbita maxima* plant parts

Estimation of lead content in the plant parts were analysed. The concentration of lead was found higher in roots and stems of treated plants as compared to the control plants. The values were found to decline drastically in leaves, flowers and fruits of treated plants as compared to the control plants, upon the application of bacterial consortia. The data has been represented in **Figure 4.66**

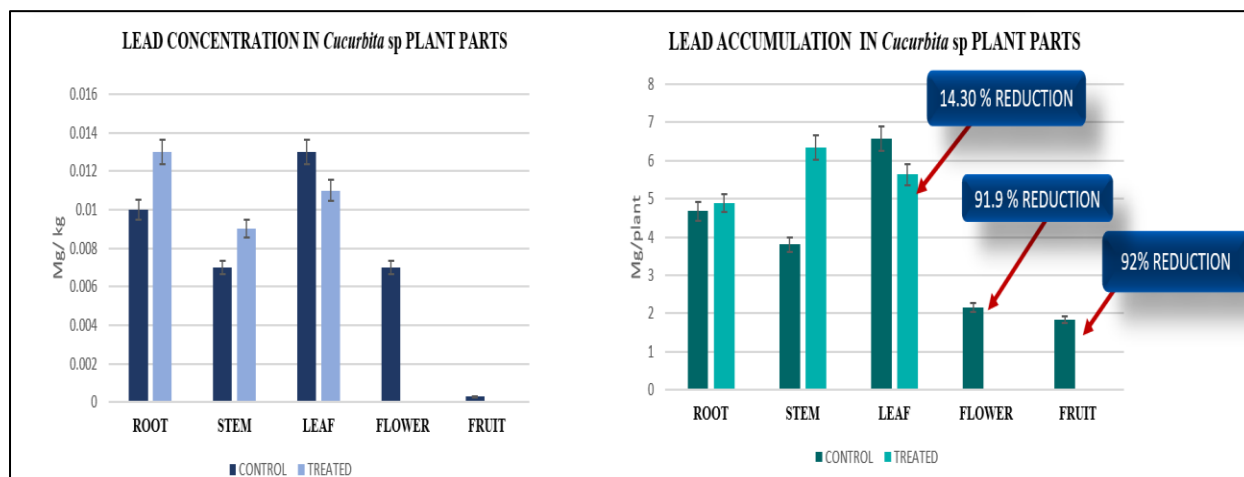


Figure 4.66: Lead concentration and their subsequent accumulation in plant parts

KEY FINDINGS from Figure 4.3.9a:

- Lead concentration was higher in roots and stems of treated plants compared to control, indicating initial uptake and immobilization in lower plant parts.
- However, lead levels declined significantly in the leaves, flowers, and fruits of treated plants compared to control, suggesting that the bacterial consortia effectively restricted upward translocation of lead into edible or aerial parts.
- In *Cucurbita* sp., lead accumulation was significantly reduced in treated plants. The flower showed a 91.9% decrease, while the fruit had the highest reduction at 92%. The leaf exhibited a moderate 14.3% decline

4.3.9b. Quantification of lead analysis and accumulation in *Zea mays* plant parts

Estimation of lead content in the plant parts were analysed. Concentration of lead was found to be much lower in roots, stems, leaves and cob grain of the treated plants as compared to the control set up, upon the application of bacterial consortia. The data has been represented in **Figure 4.67**

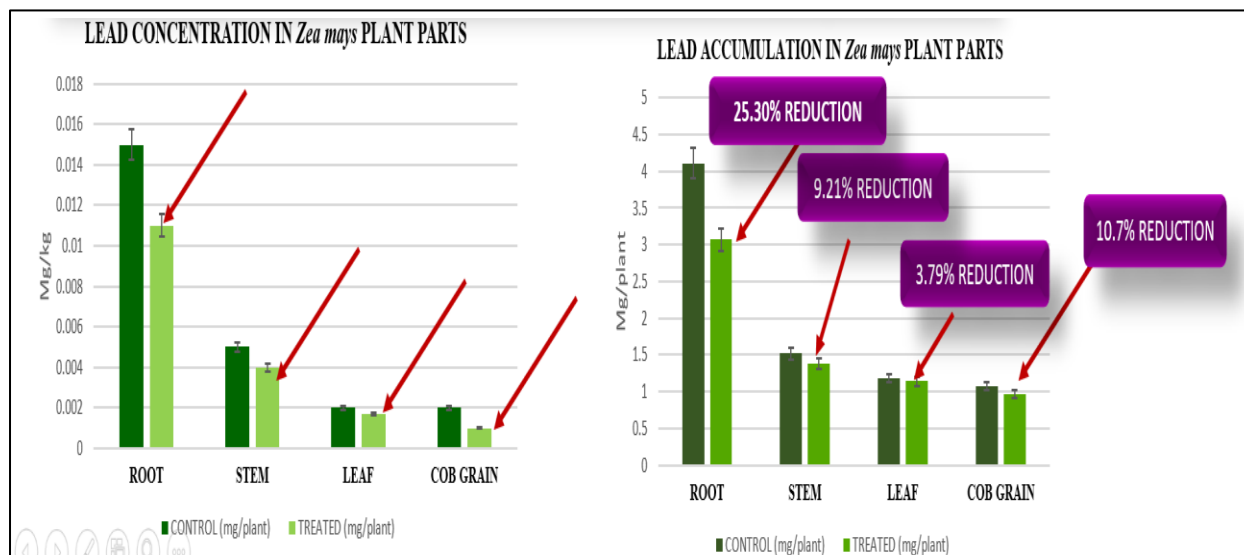


Figure 4.67: Lead concentration and their subsequent accumulation in plant part

KEY FINDINGS from Figure 4.3.9b:

- The treated plants showed a substantially lower lead concentration in roots, stems, leaves, and cob grains compared to the control.
- This demonstrates that the application of bacterial consortia was effective in reducing overall lead uptake by the plant.
- Lead accumulation (mg/plant) showed the most significant reduction in the root, with a 25.3% decrease in treated plants compared to control.
- In the stem, lead accumulation reduced by 9.21%, suggesting a moderate effect of the treatment on translocation to aerial parts.
- The leaf showed a 3.79% reduction, indicating limited improvement in foliar lead load.
- The cob grain, which represents the edible part, exhibited a 10.7% reduction in lead accumulation, highlighting an important improvement in food safety.
- The reduction in lead accumulation in the cob grain is particularly important for food safety and crop quality.

4.3.10: Analysis of plant defence enzymes

4.3.10a: Analysis of plant defence related enzyme assay in *Cucurbita maxima*

The aim of the work was to evaluate the impact of bacterial consortia treatment on oxidative stress response in *Cucurbita maxima* by assessing changes in catalase activity, ascorbate peroxidase activity, and proline content in different plant parts compared to untreated controls. The results have been represented in **Figure 4.68, 4.69 and 4.70**.

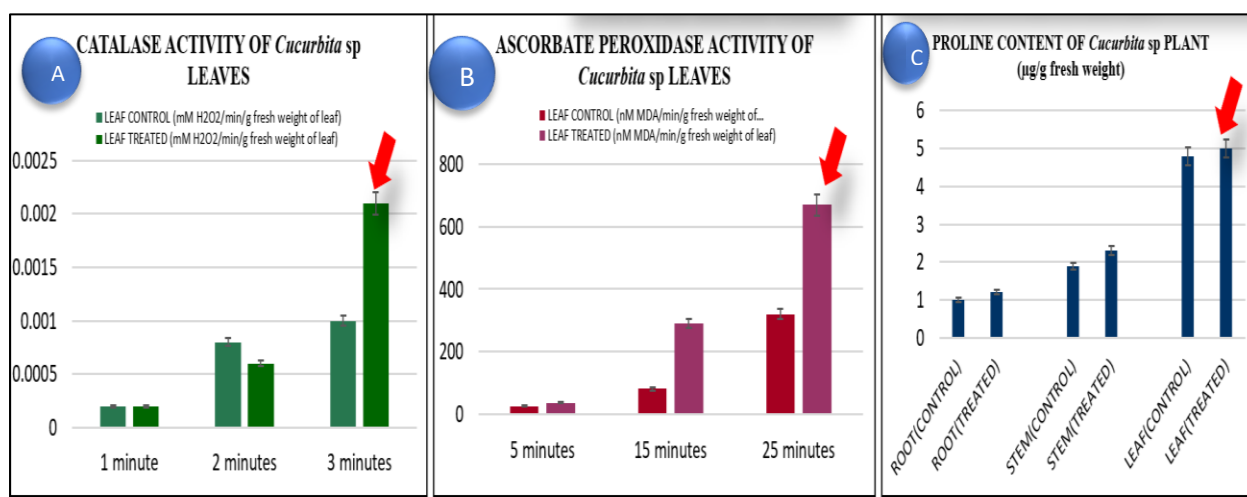


Figure 4.68, 4.69, 4.70: Quantitative assay of catalase (A), ascorbate peroxidase (B) and proline (C) content of *Cucurbita* sp plants.

KEY FINDINGS From 4.3.10a:

- Catalase activity, Ascorbate peroxidase activity was much higher in *Cucurbita maxima* treated leaves as compared to the control set up. In treated set up, the leaves showed a catalytic activity at 0.0021mM/ H₂O₂/min/g fresh weight of leaves and the control set up showed catalytic activity at 0.001mM/ H₂O₂/min/g fresh weight of leaves respectively at 3 minutes. (**Figure 4.68**).
- The ascorbate peroxidase activity in treated leaves, showed value of 669 nM/ Malondialdehyde (MDA)/min/g fresh weight of leaves and the control setup showed value of 321 nM/ MDA/min/g fresh weight of leaves respectively at 25 minutes. (**Figure 4.69**).
- Proline content was measured in roots, stems and leaves in both the treated and control set up. At each stage, the proline levels were higher in treated set up as compared to the control set up, with values of 1.2, 2.3 and 5µg/g fresh weight in treated root, stem and leaf respectively as compared to the values of 1,1.9 and 4.8 µg/g fresh weight control root, stem and leaf respectively (**Figure 4.70**).

4.3.10b: Analysis of plant defence related enzyme assay in *Zea mays*

The aim of the work was to evaluate the impact of bacterial consortia treatment on oxidative stress response in *Zea mays* by assessing changes in catalase activity, ascorbate peroxidase activity, and proline content in different plant parts compared to untreated controls. The results have been represented in **Figure 4.71, 4.72 ,4.73 and 4.74.**

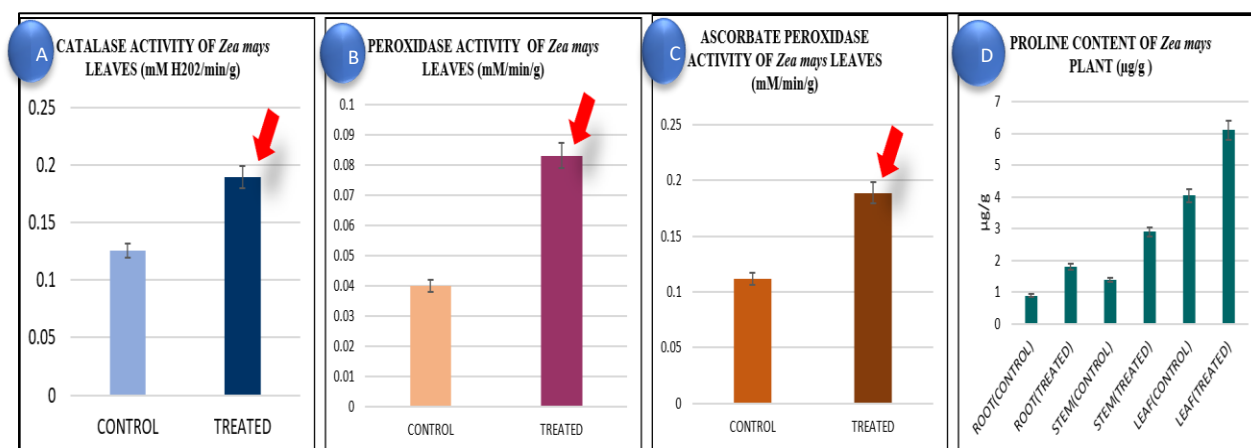


Figure 4.71, 4.72, 4.73, 4.74: Quantitative assay of catalase(A), peroxidase(B), ascorbate peroxidase(C) and proline content(D) of *Zea mays* sp plants respectively.

KEY FINDINGS from Figure 4.3.10b:

- Catalase activity was found to be 0.1895 mM/ H₂O₂/min/g fresh weight of leaves for treated set up and 0.1255mM/ H₂O₂/min/g fresh weight of leaves for control set up (**Figure 4.71**).
- Peroxidase activity in treated set up was found to be 0.08mM/min/g fresh weight of leaves and in control set up, the activity was found to be 0.03mM/min/g fresh weight of leaves (**Figure 4.72**).
- Ascorbate peroxidase activity in treated set up showed value of 0.18Mm/min/g fresh weight of leaves and control set up showed 0.11 mM/min/g fresh weight of leaves respectively (**Figure 4.73**).
- Proline content was measured in roots, stems and leaves in both the treated and control set up. At each stage, the proline levels were higher in treated set up as compared to the control set up, with values of 1.8, 2.9 and 6.1µg/g fresh weight in treated root, stem and leaf respectively as compared to the values of 0.9,1.4 and 4.05 µg/g fresh weight control root, stem and leaf respectively. Proline is essential for osmotic adjustment, helping to stabilize

cellular components such as membranes and proteins, neutralizing reactive oxygen species (ROS) to safeguard against damage, and binding heavy metals during stressful situations like drought, salinity, and heavy metal toxicity (**Figure 4.74**).

Therefore, catalase activity, peroxidase activity, and ascorbate peroxidase activity were much higher in *Zea mays* treated leaves as compared to the control set up

IMPORTANT FINDINGS FROM OBJECTIVE 3:

- ❖ Interaction study of the bacterial isolates was done.
- ❖ A bacterial consortium comprising of bacterial isolates DPP(B) and DCP(A) was prepared, the treatment was standardized.
- ❖ Bacterial consortia were then applied, to the *Cucurbita maxima* and *Zea mays* plants grown in East Kolkata Wetlands (field trial)
- ❖ All the plant physiological parameters; namely plant length, number of leaves, length and width of leaves, no of tendrils, followed by reproductive parameters of number of flowers and fruits were measured.
- ❖ It was observed, that the mean values of the physiological and reproductive parameters of the treated plants were higher than the control set-up.
- ❖ Quantification of lead content in plant parts of both treated and control set-ups were done, and it was observed that treated plants had much less lead content than control plants.
- ❖ It was observed, that the plant defence enzymes in treated plants were higher, than the control set ups. An elevation in enzymes like ascorbate peroxidase (APX) and other peroxidases in plant tissues following the introduction of bacterial consortia suggests the reaction of plant to oxidative stress, which may be triggered by the bacteria or a related environmental factor. These enzymes mitigate excess hydrogen peroxide (H₂O₂) by converting it into water, thereby safeguarding the plant from cellular harm and maintains the redox balance. This increase in antioxidant defences can improve the plant's resilience to different stresses and foster overall growth and biomass development.

4. To perform statistical analysis, including time series study of physico-chemical and microbiological property vis a vis crop productivity.

4.4.1 Calculation of the statistically significant (P) values of soil parameters of *Cucurbita maxima* and *Zea mays*

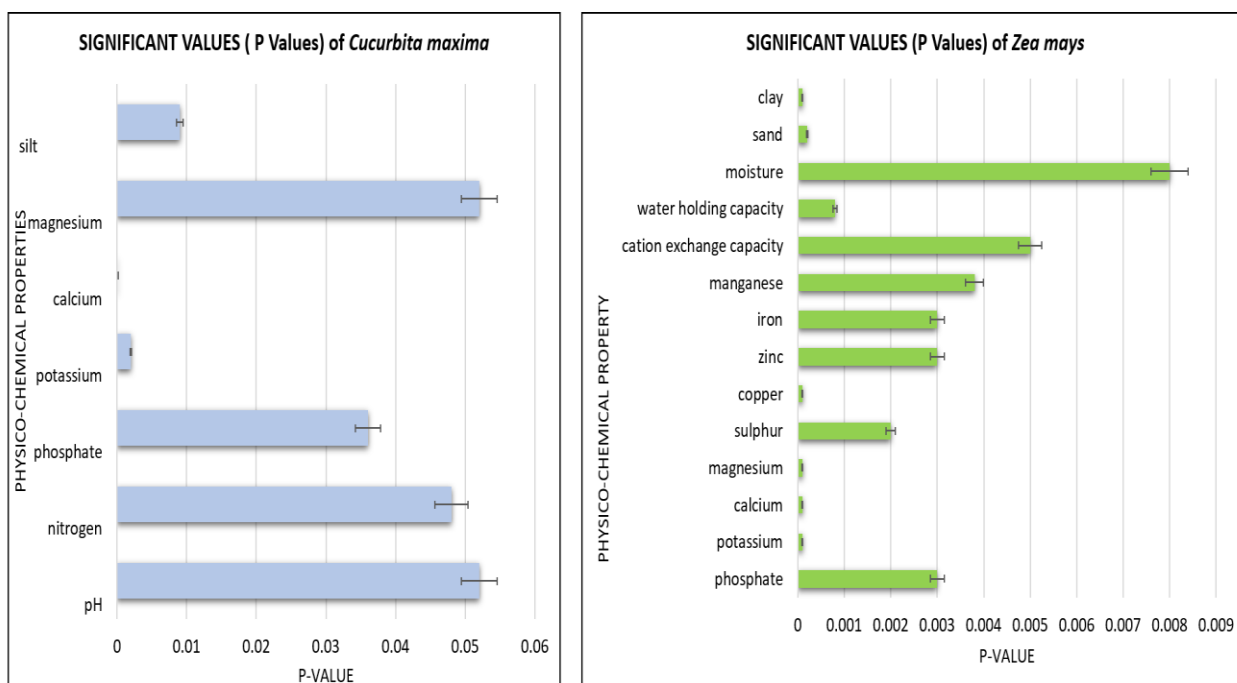


Figure 4.75 Calculation of the statistically significant (P) values of soil parameters of *Cucurbita maxima* and *Zea mays*

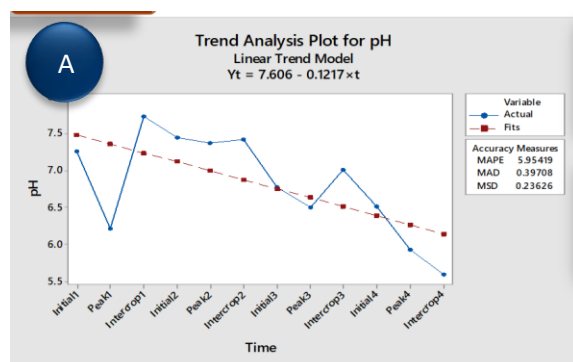
Each sample had been replicated three times i.e. each measurement was taken thrice, independently. Graphs have been constructed using mean values, while statistical analysis was done on the basis of raw observations. A t-test has been performed to test whether there have been any significant changes in soil physico-chemical parameters between initial, peak and intercrop phases of *Cucurbita maxima* and *Zea mays*. T-test has been used for analysing the unidentified variances and also the comparison to null hypothesis has been tested

KEY FINDINGS from 4.4.1

- P-value less than 0.05 is typically considered to be statistically significant, whereby the null hypothesis should be rejected.
- A p-value greater than 0.05 means that deviation from the null hypothesis is not statistically significant, and the null hypothesis is not rejected.
- From the soil, where *Cucurbita maxima* was grown as a crop, physico-chemical parameters like silt, calcium, potassium, phosphate, nitrogen were found to have P-values less than 0.05, and therefore, considered to be statistically significant
- . From the soil, where *Zea mays* was grown as a crop, physico-chemical parameters like clay, sand , moisture, water holding capacity and cation exchange capacity, followed by macro and micronutrients like manganese, iron, zinc, copper, sulphur, magnesium, calcium, potassium, phosphate were found to have P-values less than 0.05 and therefore, considered to be statistically significant.
- In soils cultivated with *Cucurbita maxima*, parameters such as silt, calcium, potassium, phosphate, and nitrogen presented p-values below 0.05, thus being statistically significant. Conversely, soils where *Zea mays* was cultivated showed significant changes ($p < 0.05$) in clay, sand, moisture, water-holding capacity, and cation exchange capacity, along with several macro- and micronutrients including manganese, iron, zinc, copper, sulphur, magnesium, calcium, potassium, and phosphate

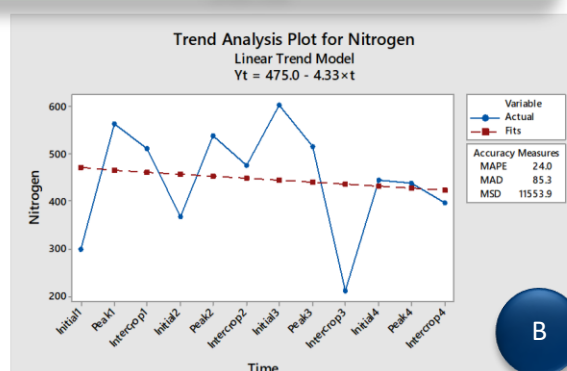
4.4.2: Time series analysis of the soil parameters:

The soil parameters that were found to be statistically significant like pH, nitrogen, calcium, phosphate, potassium and magnesium were analysed as per the linear trend model of time series analysis.



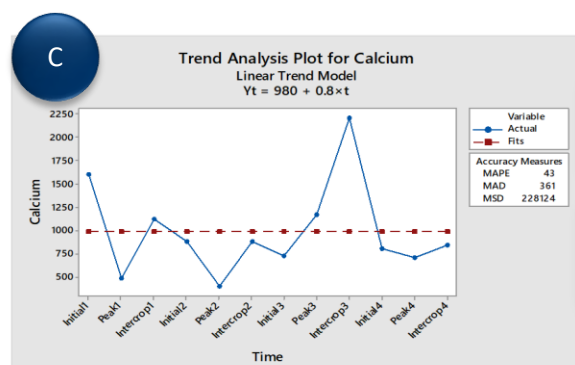
- ❖ YEAR 1: Decline in soil pH, meant that there was a higher production of *Cucurbita maxima*
- ❖ YEAR 2: *Cucurbita maxima* production was affected due to higher pH, the impact of Covid on agriculture and soil quality was also responsible for decline in crop production

- ❖ YEAR 1: Alkaline pH might have affected the production of *Zea mays*, with slight dip during the peak period.
- ❖ YEAR 2: Decline in pH towards ideal condition for *Zea mays*, would have best suited its production and there was a noticeable higher production of corn in the year.



- ❖ YEAR 1: Increase in nitrogen content of soil was seen during the peak period of *Cucurbita maxima* production, due to addition of fertilizers rich in nitrogen like urea.
- ❖ YEAR 2: Decline in nitrogen production in soil during intercrop period, represented better uptake of nitrogen by plants.

- ❖ YEAR 1: Soil nitrogen content increased during peak period, due to addition of fertilizers and with corn production, soil nitrogen content declined during intercrop period.
- ❖ YEAR 2: Soil nitrogen content increased significantly in the initial period, due to the addition of fertilizers.

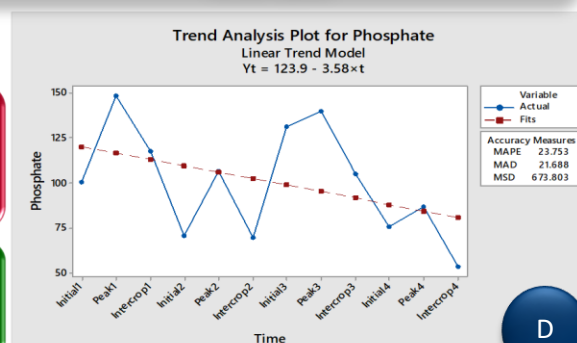


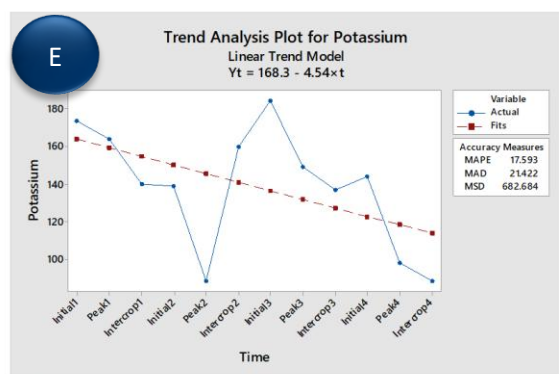
- ❖ YEAR 1: Decline in calcium content in soil during peak period, represented better uptake of calcium in plants. Maintaining soil pH in 6.0-6.8 range improves the availability of calcium
- ❖ YEAR 2: *Cucurbita maxima* production was affected due to higher pH, reduced moisture content of soil and the impact of Covid on agriculture and soil quality was also responsible for decline in crop production

- ❖ YEAR 1: Decline in calcium content of soil, represented better absorption of calcium in plants. Higher calcium in plants, helps to improve the absorption of nutrients by roots and their translocation in plants
- ❖ YEAR 2: Decline in calcium content of soil, represented better absorption of calcium in plants

- ❖ YEAR 1: Phosphorous is most available between pH 6.0-6.5. Lesser amount of phosphorous in plant would signify lower energy(ATP) to form buds and fruit. Decline of the phosphorous content of the soil during intercrop period, represents utilization of phosphorous during peak period.
- ❖ YEAR 2: The trend of phosphorous content in soil, was same like YEAR 1

- ❖ YEAR 1: Increase in phosphorous content in soil was observed during peak period, due to addition of chemical fertilizers.
- ❖ YEAR 2: Decline in phosphorous content in soil, signifies better absorption of phosphorous by the plants.





- ❖ YEAR 1: Decline in potassium content of soil, represented better absorption of in plants during peak period. It declined further in intercrop period.
- ❖ YEAR 2: Higher amount of potassium in the soil was found, due to addition of fertilizers, that declined during the peak period, showing uptake of potassium in plants

- ❖ YEAR 1: Sharp decline in the level of potassium in soil, represented better uptake of potassium by *Zea mays*.
- ❖ YEAR 2: The trend of phosphorous content in soil, was same like YEAR 1

- ❖ YEAR 1: Decline in magnesium content in soil during peak period, represented better uptake of magnesium within the plants. Magnesium is required by the *Cucurbita maxima* plants as a main component of chlorophyll
- ❖ YEAR 2: Soil showed a considerable decrease in the amount of magnesium in soil, during the peak period of *Cucurbita maxima* plant production. It was affected due to higher pH, reduced moisture content of soil and the impact of Covid on agriculture and soil quality was also responsible for decline in crop production

- ❖ YEAR 1: Decline in magnesium content in soil during peak period, represented better uptake of magnesium in plants. Maintaining soil pH in 6.0-6.8 range improves the availability of the compound.
- ❖ YEAR 2: *Zea mays* production was affected due to higher pH, reduced moisture content of soil and the impact of Covid on agriculture and soil quality was also responsible for decline in crop production

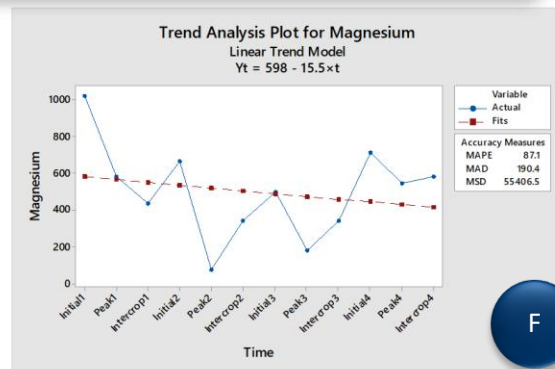


Figure 4.76(A, B, C, D, E, F): Time series analysis of the soil parameters

KEY FINDINGS from 4.4.2:**A. pH**

- Year 1: Slightly alkaline pH levels may have hindered crop yield, particularly for *Zea mays*.
- Year 2: A decrease in pH towards optimal levels promoted enhanced corn growth.
- Elevated pH negatively influenced the yield of *Cucurbita maxima*, in conjunction with low moisture and a decline in soil quality.

B. Nitrogen

- Nitrogen levels rose during peak production periods, primarily due to fertilizer application, especially urea.
- A decrease during intercrop periods indicated improved plant uptake.
- Year 1: Elevated nitrogen levels supported the production of *Cucurbita maxima*.
- Year 2: A reduction in nitrogen levels reflected uptake efficiency during intercrop periods.

C. Calcium

- A decrease in calcium levels during peak periods suggested enhanced absorption by plants.
- The ideal soil pH range of 6.0–6.8 enhances the availability of calcium.

D. Phosphate

- Phosphorus availability peaked at a pH of 6.0–6.5.
- Increased phosphorus levels in the soil were noted during peak periods as a result of fertilizer application.
- A reduction in phosphorus during intercrop periods indicated improved plant absorption.

E. Potassium

- A decrease in potassium levels during peak periods indicated improved plant absorption.
- The initial addition of fertilizer raised potassium levels, but these subsequently fell due to crop uptake.
- Significant declines were associated with enhanced uptake in *Zea mays*.

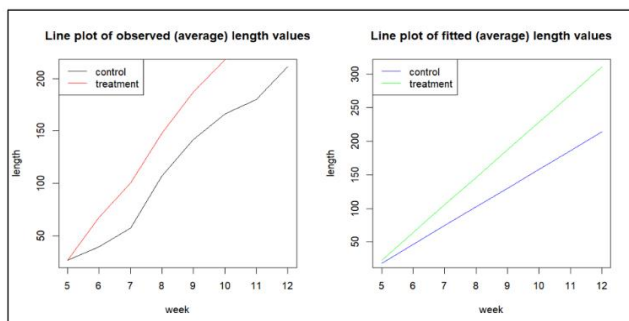
F. Magnesium

- A reduction in magnesium levels during peak periods indicated absorption by plants, which is particularly crucial for chlorophyll synthesis in *Cucurbita maxima*.
- Higher soil pH diminished magnesium availability; maintaining a pH of 6.0–6.8 optimizes absorption.
- Year 2: A lower crop yield was associated with high pH, low soil moisture, and issues related to soil quality due to Covid.

4.4.3 STATISTICAL ANALYSIS OF CROP PRODUCTIVITY

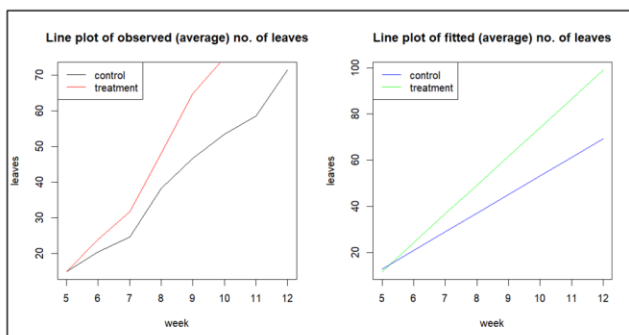
A. For *Cucurbita maxima*

1. Plant length



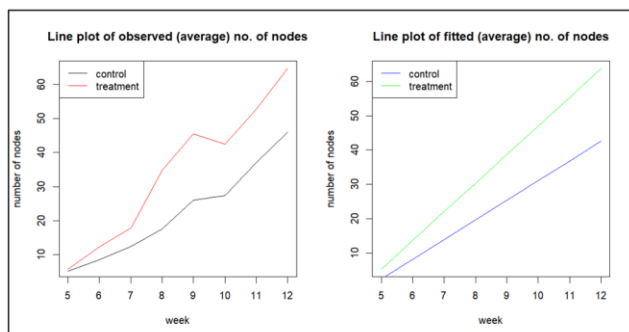
GROUP	FITTED LINEAR EQUATION	(MULTIPLE) R ² VALUES
CONTROL	$-9.763 + 28.006 \cdot t$ $t = 1(1)8$	98.02%
TREATED	$-17.862 + 41.045 \cdot t$ $t = 1(1)8$	99.53%

2. Number of leaves



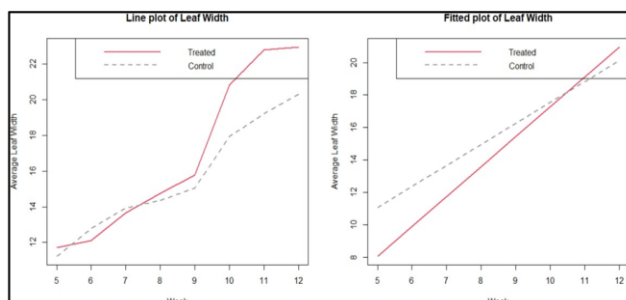
GROUP	FITTED LINEAR EQUATION	(MULTIPLE) R ² VALUES
CONTROL	$4.6071 + 8.0980 \cdot t$ $t = 1(1)8$	98.61%
TREATED	$-0.6868 + 12.4625 \cdot t$ $t = 1(1)8$	99.08%

3. Number of nodes



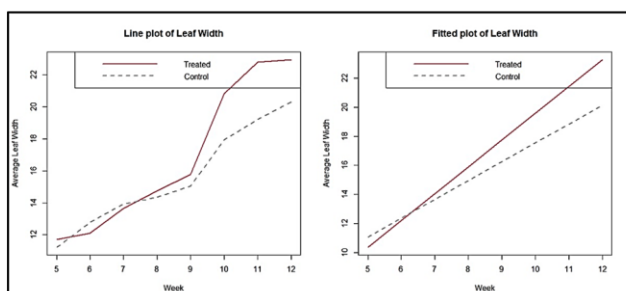
GROUP	FITTED LINEAR EQUATION	(MULTIPLE) R ² VALUES
CONTROL	$-3.2582 + 5.7262 \cdot t$ $t = 1(1)8$	97.19%
TREATED	$-2.9066 + 8.3168 \cdot t$ $t = 1(1)8$	96.19%

4. Leaf Lamina Length



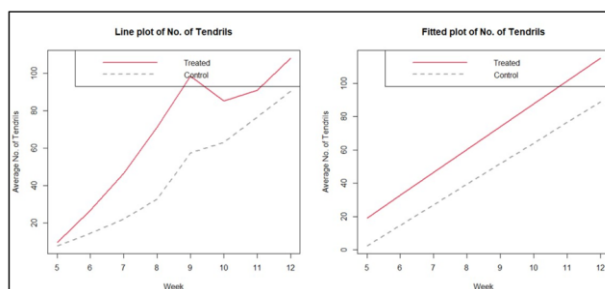
TREATMENT	FITTED LINEAR TREND EQUATION (ON WEEKLY AVERAGES)	MULTIPLE (R-SQUARED) VALUES
TREATED	$-1.9883+1.9174 \times t$	92.34%
CONTROL	$0.4077+1.6269 \times t$	94.06%

5. Leaf Lamina width



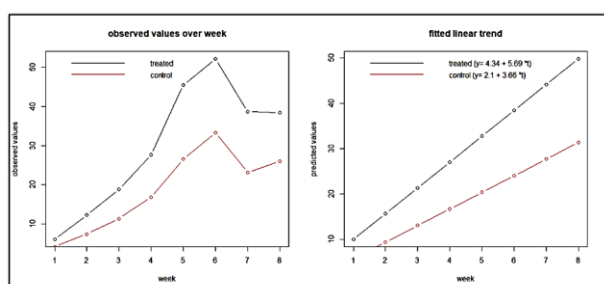
TREATMENT	FITTED LINEAR TREND EQUATION (ON WEEKLY AVERAGES)	MULTIPLE (R-SQUARED) VALUES
TREATED	$1.1536+1.8431 \times t$	93.04%
CONTROL	$4.60842+1.29267 \times t$	96.75%

6. Number of Tendrils



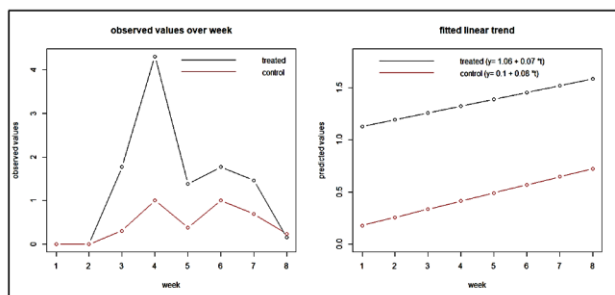
TREATMENT	FITTED LINEAR TREND EQUATION (ON WEEKLY AVERAGES)	MULTIPLE (R-SQUARED) VALUES
TREATED	$-49.598+13.730 \times t$	88.68%
CONTROL	$-59.2198+12.3324 \times t$	97.92%

7. Number of Buds



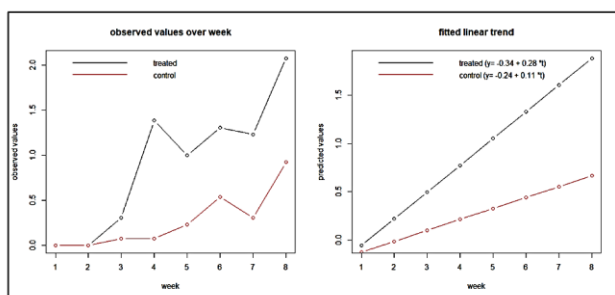
TREATMENT	FITTED LINEAR TREND EQUATION (ON WEEKLY AVERAGES)	MULTIPLE (R-SQUARED) VALUES
TREATED	$4.34+5.69 \times t$	71.3%
CONTROL	$2.1+3.66 \times t$	75.48%

8. Number of Flowers



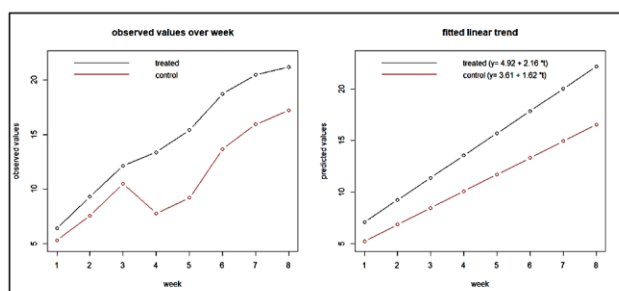
TREATMENT	FITTED LINEAR TREND EQUATION (ON WEEKLY AVERAGES)	MULTIPLE (R-SQUARED)VALUES
TREATED	$1.06 + 0.07 \times t$	1.25%
CONTROL	$0.1 + 0.08 \times t$	22.3%

9. Number of Fruits



TREATMENT	FITTED LINEAR TREND EQUATION (ON WEEKLY AVERAGES)	MULTIPLE (R-SQUARED)VALUES
TREATED	$-0.34 + 0.28 \times t$	83.45%
CONTROL	$-0.24 + 0.11 \times t$	75.02%

10. Length of Tendril



TREATMENT	FITTED LINEAR TREND EQUATION (ON WEEKLY AVERAGES)	MULTIPLE (R-SQUARED)VALUES
TREATED	$4.92 + 2.16 \times t$	98.43%
CONTROL	$3.61 + 1.62 \times t$	86.14%

Figure 4.77 (1-10): Statistical analysis of crop productivity in *Cucurbita maxima*

T-TEST AND WILCOXON VALUES FOR *Cucurbita maxima*

WEEK	t-TEST P VALUE	DECISION	WILCOXON P VALUE	P	DECISION	WEEK	t-TEST P VALUE	DECISION	WILCOXON P VALUE	DECISION	WEEK	t-TEST P VALUE	DECISION	WILCOXON P VALUE	DECISION
5	0.5116957	Accept hypothesis	Null	0.6112	Accept hypothesis	Null	5	0.5	Accept	Accept	5	0.219475483	Accept	0.25	Accept
6	0.002181404	Reject hypothesis	Null	0.005553	Reject hypothesis	Null	6	0.105690833	Accept	Accept	6	0.001367694	Reject	0.002408	Reject
7	0.003109751	Reject hypothesis	Null	0.00741	Reject hypothesis	Null	7	0.024833498	Reject	Reject	7	0.001387608	Reject	0.002277	Reject
8	0.06102977	Accept hypothesis	Null	0.1092	Accept hypothesis	Null	8	0.148369121	Accept	Accept	8	0.003513790	Reject	0.0064	Reject
9	0.03508167	Reject hypothesis	Null	0.05647	Accept hypothesis	Null	9	0.047181638	Reject	Accept	9	0.011604460	Reject	0.00741	Reject
10	0.02139207	Reject hypothesis	Null	0.04057	Reject hypothesis	Null	10	0.027425244	Reject	Accept	10	0.033979636	Reject	0.02126	Reject
11	0.001965804	Reject hypothesis	Null	0.003275	Reject hypothesis	Null	11	0.006799655	Reject	Reject	11	0.052483028	Accept	0.065	Accept
12	0.00004783904	Reject hypothesis	Null	0.0003549	Reject hypothesis	Null	12	0.003538430	Reject	Reject	12	0.030230997	Reject	0.03051	Reject

WEEK	t-TEST P-VALUES	DECISION	WILCOXON P-VALUES	DECISION	WEEK	t-TEST P-VALUES	DECISION	WILCOXON P-VALUES	DECISION
5	0.6277	ACCEPT	0.7566	ACCEPT	5	0.17426	ACCEPT	0.1624	ACCEPT
6	0.9737	ACCEPT	0.9702	ACCEPT	6	0.001737	REJECT	0.0017	REJECT
7	0.7967	ACCEPT	0.8561	ACCEPT	7	0.00749	REJECT	0.00297	REJECT
8	0.7979	ACCEPT	0.7078	ACCEPT	8	0.02153	REJECT	0.01649	REJECT
9	0.5416	ACCEPT	0.5522	ACCEPT	9	0.03928	REJECT	0.02874	REJECT
10	0.1471	ACCEPT	0.1515	ACCEPT	10	0.03838	REJECT	0.0323	REJECT
11	0.08395	ACCEPT	0.08582	ACCEPT	11	0.13106	ACCEPT	0.1648	ACCEPT
12	0.3713	ACCEPT	0.4077	ACCEPT	12	0.08689	ACCEPT	0.1239	ACCEPT

Figure 4.78(A-F): Tabular representation of T-test and Wilcoxon test for plant physiological parameters of *Zea mays*, upon application of bacterial consortia.

week	t test VALUE	t test DECISION	Wilcoxon Value	DECISION	week	t test VALUE	t test DECISION	Wilcoxon Value	DECISION	week	t test\$ pval	t test\$ dec	wilcox\$ pval	wilcox\$ dec
5	0.0570276	Accept	0.05987	Accept	5	1	Accept	1	Accept	5	1	Accept	1	Accept
6	0.0010077	Reject	0.001901	Reject	6	1	Accept	1	Accept	6	1	Accept	1	Accept
7	0.0048669	Reject	0.005491	Reject	7	0.025574	Reject	0.1048	Accept	7	0.119993	Accept	0.1438	Accept
8	0.0035314	Reject	0.002379	Reject	8	0.002938	Reject	0.006385	Reject	8	0.0120373	Reject	0.002167	Reject
9	0.0084649	Reject	0.003021	Reject	9	0.006953	Reject	0.009714	Reject	9	0.0854126	Accept	0.03315	Reject
10	0.0022486	Reject	0.005153	Reject	10	0.031557	Reject	0.03537	Reject	10	0.1496273	Accept	0.6464	Accept
11	0.0039057	Reject	0.003499	Reject	11	0.020994	Reject	0.03652	Reject	11	0.0458682	Reject	0.01953	Reject
12	0.0257654	Reject	0.01758	Reject	12	0.682348	Accept	0.7004	Accept	12	0.0458682	Reject	0.04177	Reject

- A. Plant length
- B. Number of leaves
- C. Number of nodes
- D. Leaf length
- E. Leaf width
- F. Number of tendrils
- G. Number of buds
- H. Number of Flowers
- I. Number of Fruits

Figure 4.78(A-I): Tabular representation of T-test and Wilcoxon test for plant physiological parameters of *Cucurbita maxima*, upon application of bacterial consortia

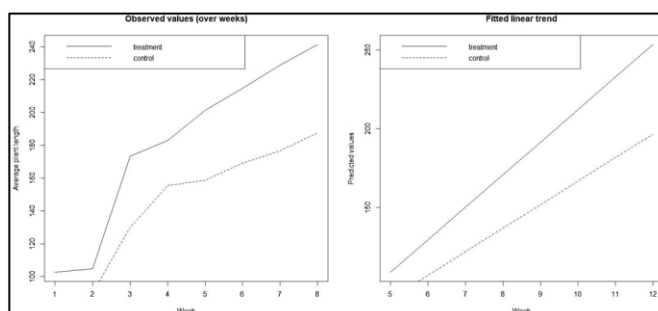
Interpretations from 4.4.3A:

- From the **plots** (both observed and fitted), it was observed that plant physiological parameters like average length of the plants, number of leaves, no. of nodes, leaf width, no of tendrils, tendril length, fruits of the plants for the treated group is higher than that of the control group for all the weeks.
- Additionally, from the **fitted linear equations**, it can be said that the rate of increase of average length of the plants, number of leaves, no. of nodes, leaf, leaf lamina width, no of tendrils, tendril length, fruits of plants in the treated group is higher than that of the control group, as indicated by the slope coefficients of the fitted lines.
- The plot of observed mean values of number of flowers of treated and controlled plants reveal that the number of flowers first increases and then decreases in both the groups. But the rate of increase in the number of flowers over time in treated plants is much more than that in controlled plants.
- From the plot of observed mean values the trend presents in both the time series (treated and controlled) seem not to be linear. The R^2 values of the linear fit reveal the same. Hence no conclusion can be made from the fitted linear plots.
- Both t-test (parametric) and Wilcoxon Rank-sum test indicate that the average length of the plant is almost always consistently higher for the treated group, compared to the control group, except for the 5th, 8th week for both tests, and the 9th week for Wilcoxon's test only. It also indicated that the average number of leaves of the plant for the treated group is almost always consistently higher compared to the control group, except for the 5th and 6th week for both tests, and 9th and 10th week for Wilcoxon's test only. The differences in the results of the t-test and Wilcoxon's test are due to marginal differences in the p-values (about the value 0.05) computed in both procedures. It showed that the average number of nodes of the plant for the treated group is almost always consistently higher than that of the control group, except for the 5th and 11th week.
- Based on the t-test and Wilcoxon Rank Sum test, it seemed that the treatment doesn't significantly affect the width of the leaf lamina initially. It starts affecting the width of the leaf lamina from 10th week onwards. Thus, the treatment results in significant increase in the width of leaves after 5 weeks of application.

- In light of the given data, as per t-test (parametric) and Wilcoxon Rank-sum test there is no significant difference in the no. of buds in the control group and the treated group initially, whereas the no. of buds becomes significantly higher in the treated group of plants than in the control group of plants as time progresses.
- Both t-test (parametric) and Wilcoxon Rank-sum test indicates that in light of the given data there is no significant difference in the no. of flowers in the control group and the treated group initially, whereas the no. of flowers becomes significantly higher in the treated group of plants than in the control group of plants as time progresses. It is also to be noted that since the number of flowers decreases in the end in both the groups, hence there is no significant difference in the no. of buds in the controlled group and the treated group in the 12th week.

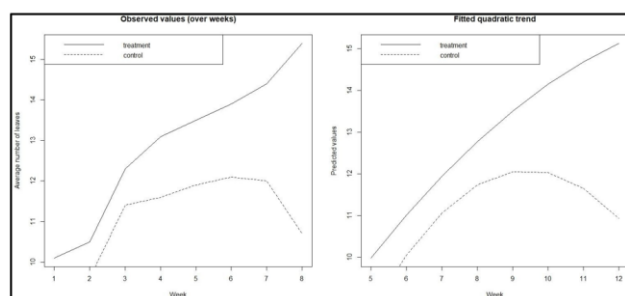
B. *Zea mays*

1. Plant length



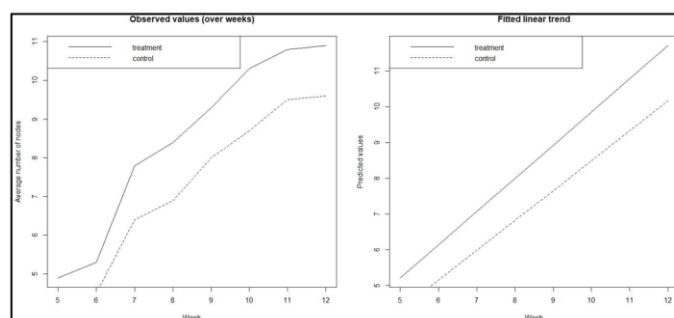
Treatment group	Fitted linear trend equation	Multiple R squared value
Treated	$88.354 + 20.360 \cdot t$ $t = 1(1)8$	91.79%
Control	$77.211 + 14.914 \cdot t$ $t = 1(1)8$	91.41%

2. Number of Leaves



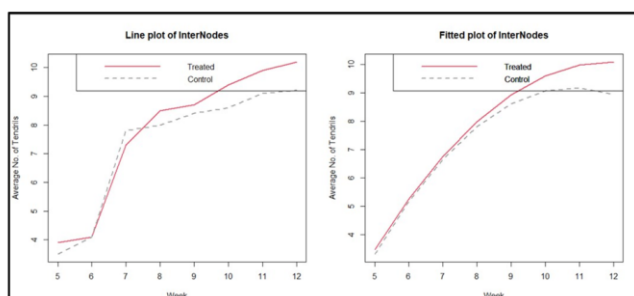
Treatment group	Fitted linear trend equation	Multiple R squared value
Treated	$10.771 + 19.732 \cdot t$ $t = 1(1)7$	87.69%
Control	$7.929 + 14.254 \cdot t$ $t = 1(1)7$	87.12%

3. Number of Nodes



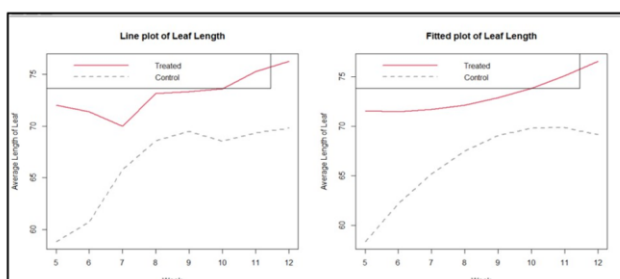
Treatment group	Fitted linear trend equation	Multiple R squared value
Treated	$4.28929 + 0.92738 \cdot t$ $t = 1(1)8$	93.53%
Control	$3.48214 + 0.83452 \cdot t$ $t = 1(1)8$	96.35%

4. Number of Internodes



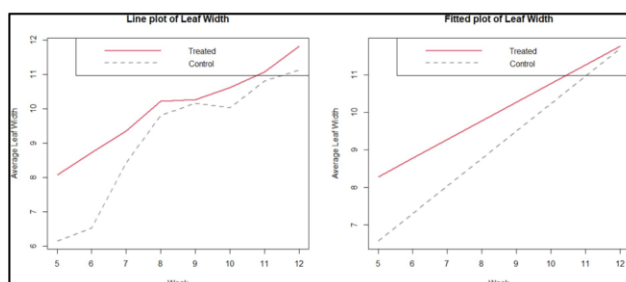
TREATMENT	FITTED QUADRATIC TREND EQUATION (ON WEEKLY AVERAGES)	MULTIPLE R ² VALUES
TREATED	$-9.63690 + 3.31548 \times \text{time} - 0.13929 \times \text{time}^2$	94.94%
CONTROL	$-11.11845 + 3.75060 \times \text{time} - 0.17321 \times \text{time}^2$	91.93%

5. Leaf Lamina Length



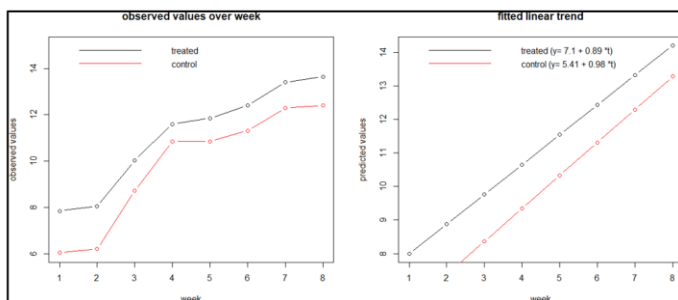
TREATMENT	FITTED QUADRATIC TREND EQUATION (ON WEEKLY AVERAGES)	MULTIPLE R ² VALUES
TREATED	$75.76101 - 1.49375 \times \text{time} + 0.13006 \times (\text{time}^2)$	84.36%
CONTROL	$28.04881 + 7.94583 \times \text{time} - 0.37679 \times (\text{time}^2)$	95.06%

6. Leaf Lamina Width



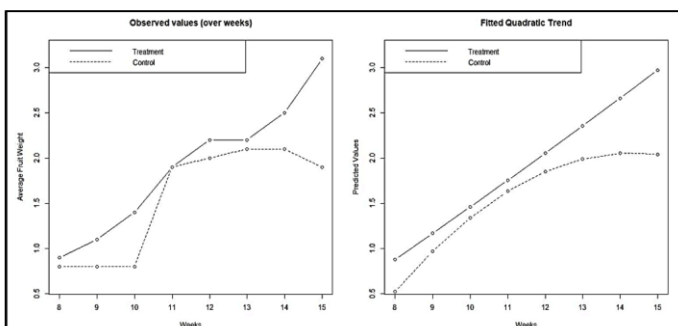
TREATMENT	FITTED LINEAR TREND EQUATION (ON WEEKLY AVERAGES)	MULTIPLE R ² VALUES
TREATED	$5.78440 + 0.49845 \times \text{time}$	97.1%
CONTROL	$2.9115 + 0.7320 \times \text{time}$	88.79%

7. Plant Girth



TREATMENT	FITTED QUADRATIC TREND EQUATION (ON WEEKLY AVERAGES)	MULTIPLE R ² VALUES
TREATED	$7.1 + 0.89 \times t$	94.11%
CONTROL	$5.41 + 0.98 \times t$	89.26%

8. Number of Fruits



Treatment group	Fitted quadratic trend equation	Multiple R squared value
Treated	$0.594643 + 0.282738 \times t + 0.001786 \times t^2$ $t = 1(1)8$	96.95%
Control	$0.003571 + 0.559524 \times t - 0.038095 \times t^2$ $t = 1(1)8$	80.86%

Figure 4.79 (1-8): Statistical analysis of crop productivity in *Zea mays*

T-TEST AND WILCOXON VALUES FOR *Zea mays*

Week	T test p value	T test decision	Wilcoxon p value	Wilcoxon decision
5	0.01087329	Reject	0.03045	Reject
6	0.01295363	Reject	0.048	Reject
7	0.0004914412	Reject	0.001304	Reject
8	0.005126921	Reject	0.009082	Reject
9	0.0004694252	Reject	0.004571	Reject
10	0.00004296222	Reject	0.0007615	Reject
11	0.00001276334	Reject	0.0004375	Reject
12	0.00000823592	Reject	0.000211	Reject

Week	T test p value	T test decision	Wilcoxon p value	Wilcoxon decision
5	0.02576972	Reject	0.06908	Accept
6	0.05853325	Accept	0.1388	Accept
7	0.06357483	Accept	0.1495	Accept
8	0.01018627	Reject	0.03748	Reject
9	0.01849507	Reject	0.06431	Accept
10	0.009433532	Reject	0.02907	Reject
11	0.004958483	Reject	0.01051	Reject
12	0.000002497569	Reject	0.0004252	Reject

Week	T test p value	T test decision	Wilcoxon p value	Wilcoxon decision
5	0.022635229	Reject	0.05606	Accept
6	0.031224097	Reject	0.06115	Accept
7	0.108739305	Accept	0.03339	Reject
8	0.109908136	Accept	0.03402	Reject
9	0.046129875	Reject	0.1094	Accept
10	0.005221097	Reject	0.006585	Reject
11	0.002060408	Reject	0.004743	Reject
12	0.001661632	Reject	0.00483	Reject

WEEK	t TEST P-VALUES	DECISION	WILCOXON TEST P-VALUES	DECISION
5	0.05992005	ACCEPT	0.06798	ACCEPT
6	0.50000000	ACCEPT	0.3903	ACCEPT
7	0.72015318	ACCEPT	0.8018	ACCEPT
8	0.24777889	ACCEPT	0.3629	ACCEPT
9	0.33002783	ACCEPT	0.3901	ACCEPT
10	0.079994562	ACCEPT	0.1685	ACCEPT
11	0.02798705	REJECT	0.04045	REJECT
12	0.01280497	REJECT	0.02177	REJECT

WEEK	t TEST P-VALUES	DECISION	WILCOXON TEST P-VALUES	DECISION
5	0.001184191	REJECT	0.002255	REJECT
6	0.002535692	REJECT	0.005497	REJECT
7	0.074928158	ACCEPT	0.03171	REJECT
8	0.035677826	REJECT	0.02217	REJECT
9	0.057885420	ACCEPT	0.02229	REJECT
10	0.068910760	ACCEPT	0.03131	REJECT
11	0.058417842	ACCEPT	0.01839	REJECT
12	0.048452363	REJECT	0.02873	REJECT

WEEK	t TEST P-VALUES	DECISION	WILCOXON TEST P-VALUES	DECISION
5	0.0053664810	REJECT	0.01265	REJECT
6	0.0003623503	REJECT	0.0009137	REJECT
7	0.0690004222	ACCEPT	0.07346	ACCEPT
8	0.2534941881	ACCEPT	0.234	ACCEPT
9	0.4337128511	ACCEPT	0.454	ACCEPT
10	0.2139900783	ACCEPT	0.2718	ACCEPT
11	0.3337651790	ACCEPT	0.2691	ACCEPT
12	0.1119430329	ACCEPT	0.04082	REJECT

Figure 4.80(A-F): Tabular representation of T-test and Wilcoxon test for plant physiological parameters of *Zea mays*, upon application of bacterial consortia.

Week	T test p value	T test decision	Wilcoxon p value	Wilcoxon decision
5	0.0002929569	Reject	0.0005959	Reject
6	0.0007366745	Reject	0.0011	Reject
7	0.0949219598	Accept	0.07948	Accept
8	0.1860611608	Accept	0.161	Accept
9	0.1238443801	Accept	0.1352	Accept
10	0.1254672533	Accept	0.09743	Accept
11	0.1248990960	Accept	0.1512	Accept
12	0.0832424730	Accept	0.142	Accept

A. Plant length

B. Number of leaves

C. Number of nodes

D. Number of internodes

E. Leaf lamina length

F. Leaf lamina width

G. Plant girth

H. Number of fruits

Week	T test p value	T test decision	Wilcoxon p value	Wilcoxon Decision
8	0.00000263	Reject	0.0001037	Reject
9	0.028853136	Reject	0.004239	Reject
10	0.043730382	Reject	0.006052	Reject
11	0.042228258	Reject	0.005602	Reject
12	0.022735811	Reject	0.002961	Reject

Figure 4.80(G-H): Tabular representation of T-test and Wilcoxon test for plant physiological parameters of *Zea mays*, upon application of bacterial consortia.

INTERPRETATIONS from 4.4.3B:

- From the two sets of graphs (with and without differencing), each containing both observed and fitted values, it seems that in parameters like plant height, no. of nodes, no. of internodes, leaf width, tassel length the treated group of plants dominates over the control group.
- The fitted lines also reveal that the slope of the treated group exceeds the control group.
- However, both parametric (t test) and non-parametric (wilcoxon's) tests suggest that in case of plant length, number of leaves and nodes the difference increases much more during the latter weeks.
- Both t-test (parametric) and Wilcoxon Rank-sum test (non parametric) indicate that in light of the given data there is no significant difference in the number of internodes of the control group and the treated group from week 5 to week 10. Based on the given data, there seems to be an increase in the growth of internodes in 11th and 12th week.
- Even though in case of number of leaves, plant girth it seems from the initial graph of averages (without taking differences) that the treatment group performs much better than the control group, this is not actually the case, as suggested by the results obtained from the differenced data. In the graph containing the observed and fitted differences, we can see that the control group actually initially dominates the treated group. The two graphs meet at some point between the 2nd and 3rd tabulated point, after which the treated group dominates the control group. Both t and Wilcoxon's tests suggest that there is no significant difference between the treated and control groups, except for the last week (week 12 after differencing).
- In case of T-test and Wilcoxon test, most of the plant physiological parameters like plant length, number of leaves, number of odes, number of fruits were found to be extremely significant and therefore rejected Null Hypothesis.

IMPORTANT FINDINGS FROM OBJECTIVE 4:

- ❖ On the basis of the time series analysis of the significant soil physiological parameters, over a two-year period, variations in their quantity were observed, which may be due to uptake of the macro and microelements by the plants or addition of fertilizers to the soil.
- ❖ Time series analysis of the plant parameters showed that upon application of the consortia, the treated set up showed better results and goodness of fit as compared to the control set-up.
- ❖ In the model we are assuming that the plant factor is a function of time (let's call this the explanatory variable), but in reality, there may be other factors that affect the factor. these are commonly referred to as noise. the variation in data due to these factors is called unexplained variation.
- ❖ r^2 basically tells us how good the chosen model is, based on ONLY time as the explanatory variable.
- ❖ If $R^2 = 98.02\%$ for the control group, it means that the fitted equation is responsible for 98.02% of the total variation in the data. The remaining
- ❖ 1.98% is due to other factors not captured by the equation (noise).
- ❖ If $R^2 = 99.53\%$ for the treated group, the equation does an even better job, explaining 99.53% of the changes in the data. Only 0.47% is left unexplained. Therefore, higher R^2 is to 100%, the better the fit.
- ❖ In case of T-test and Wilcoxon test, most of the plant physiological parameters like plant length , number of leaves, number of odes, number of fruits were found to be extremely significant and therefore rejected Null Hypothesis.



CHAPTER- 5: DISCUSSION

5. DISCUSSION

The discussion represented in this dissertation is as per the objectives of this investigation. The ongoing paragraphs represent a comparative analogy between this investigation and the work carried out by others, and also the basis of the particular experiments carried out to ensure the progress of the work.

5.1 To characterize soil, with respect to physical, chemical, microbiological and metagenomic data of designated plot involving *Cucurbita* sp and *Zea mays* over two-year period.

This study was aimed to characterize the soil that was collected from the selected plot at Chowbaga in Dhapa, South 24 Parganas, a part of East Calcutta Wetlands with coordinates of 22.5296°N, 88.4207°E (**Chapter 4, Figure 4.1**). The soil samples were collected during the time interval between December, 2020- February, 2021, whereby *Cucurbita maxima* were grown as main crop in the field. Another set of soil was collected during the tenure of March, 2021- June, 2021, whereby *Zea mays* was grown as the main crop. The collection was done at three phases namely: **Initial** (when the seeds were sowed in soil); **Peak** (Plants in their peak reproductive phase with flowers and fruit structures); and **Intercrop** (Residual soil after the removal of the crop plants). The soil sample collection was done over a two-year period involving years 2021 and 2022. The determination of the physico-chemical parameters of the soil samples included : pH , Electrical Conductivity (dsm-1) , Organic Carbon(O.C) (%), Nitrogen(N) (kg/ha) , Phosphate(P) (kg/ha), Potassium(K)(kg/ha) , Calcium(Ca) (mg/kg) , Magnesium(Mg) (mg/kg) , Sulphu(S)r (mg/kg), Boron (B) (mg/kg) , Copper(Cu) (mg/kg), Zinc (Zn) (mg/kg), Iron (Fe)(mg/kg), Manganese (Mn)(mg/kg), Cation Exchange Capacity (CEC) (meq/100 g)

In present investigation, for *Cucurbita maxima* soil rhizosphere, pH of soil remained nearly neutral in all stages, dipping into slightly acidic range (6.0–6.5) at the crop's peak stage. In Year 1, pH escalated to 7.73 by the intercrop stage, indicating an increase in the alkalinity levels of soil. In Year 2, the pH remained between 5.6-6.52 during the entire period. In case of *Zea mays* rhizosphere, soil pH in Year 1 was slightly alkaline to near-neutral (approximately 7.45 at the initial stage, dipped to 7.37 in the peak and increased to 7.47 at intercrop), whereas in Year 2, pH was lower as compared to year 1 (about 6.77 initial, 6.5 peak, and increased to 7.5 intercrop)(**Chapter 4, Figure 4.4a, b, c**). Soil is considered acidic with pH range below 6 and alkaline with a pH above 8.5 (Kekane *et al.* 2015). In present study, one of the factors for minor decrease in pH of wetland

soil, may be due to the liberation of H^+ ions from plant roots, thereby leading to changes in soil pH (Salem *et al.* 2020). The alkalinity of the wetland soil can be attributed to the utilization of wastewater for agriculture purpose and the existence of carbonates within the soil (Paul *et al.*,2014).

Electrical conductivity (EC) of soil is a quantification mechanism indicating the presence of ions in soil. It refers to the quantification of all the desired soluble salts in soil, therefore it is also a measure of soil salinity (soil with EC value less than 2ds/m indicates non saline soils and if it exceeds 4 ds/m, it is referred as a saline soil). During Year 1, electrical conductivity of *Cucurbita maxima* soil was found to be 0.30 dsm^{-1} , 0.31 dsm^{-1} and 0.17 dsm^{-1} in initial, peak and intercropping seasons. During second year, EC value was highest during the peak period of *Cucurbita maxima* rhizosphere, with value at 0.27 dsm^{-1} . For *Zea mays* soil, Electrical conductivity (EC) remained very low in all cases at 0.32 ds/m at initial stages, and increased to 0.48 dS/m in Year 1, and then dropped to 0.1ds/m during intercrop season. This can be attributed to fertilizer application and biological activity, whereas the significant drop during the intercrop season results from nutrient absorption by maize, along with leaching and dilution of salts due to a lack of replenishment. In Year 2, the EC values dipped to 0.09 ds/m during the peak season and dipped further to 0.08 ds/m in intercrop stages. Microbial process like respiration and nitrification reduce EC of the soil since, microbial respiration modifies soil chemistry, nitrification leads to acidification and enhances leaching, while microbial immobilization captures ions in biomass — collectively, these processes lower the concentration of soluble ions in the soil solution, resulting in a decrease in electrical conductivity (EC). In present study, initial EC of soil indicated that it was non-saline in nature. Rise in EC values of soil, during peak period may be due to the addition of organic manure and fertilizers (Shahid *et al.* 2018). Reduction in E.C values during the intercropping season, is due to reduced salt content of soil that resulted during the process of irrigation, also, the sandy texture of soil may be allocated for decrease in E.C values (Salem *et al.* 2020).

In *Cucurbita maxima* soil rhizosphere, **organic carbon** content of the soil was found to be 0.62% in initial cropping period, followed by a rise in its content to 0.86% and a minor decrease to 0.82% in the intercropping season during the first year. Organic carbon content increased by approximately 5.88% in the peak period of second year. In case of *Zea mays* soil rhizosphere, organic carbon levels in year 1 peaked slightly higher (0.84% organic carbon) than Year 2 (0.73%) at the peak season, with both years having marginally lower values (0.7%) at the initial stage

(Chapter 4, Figure 4.6a, b, c). In present investigation, rise in organic carbon percentage may be due to the microbial degradation of plant litter (Arumugam *et al.* 2015). Increase in diversity of micro-organisms in soil and its biomass is also associated with increased levels of organic carbon. Reduction of organic carbon during the initial season, can be due to crop harvesting and tillage, it leaves no biomass that can be returned to soil (Nyawira *et al.*,2021). EKW receives large amount of wastewater discharge from the tannery industry. Certain parts of wetlands close to the tannery areas contain high amount of organic carbon, expanding from 1.95 to 2.25% due to the discharge of chemicals like ammonium bicarbonate, sodium bicarbonate or calcium carbonate, that have been used in these industries, and upon discharge finds its way, through the canals of EKW (Pal *et al.* 2016). On the contrary, Chowbaga region has small and large canals for agricultural runoffs, therefore, the organic carbon content of soil is much less as compared to one close to the tannery industry.

Wetlands serve as effective sinks for nitrate and have also been recognized as a source of nitrous oxide, which results from two microbially controlled processes: nitrification and denitrification. Nitrogen is most essential element derived by plants from soil. It is essential for plant growth, since it provides nutrition and enhances their development (Dandwate 2020). **Nitrogen** levels of *Cucurbita maxima* soil rhizosphere in Year 2 have a much higher N level of about 602.11kg/ha as compared to Year1, 298.7 kg/ha during the initial stage. By peak stage, both years have roughly similar N (563.7kg/ha–514.22kg/ha). During the intercrop season, the Nitrogen content of the soil was 510.7kg/ha, as compared to year 2, whereby the nitrogen level declined to approximately 212kg/ha.

In this research, it was found that for *Zea mays* soil rhizosphere, Year 1 had a higher nitrogen content buildup (525 kg/ha) consistent with plant uptake and mineralization, especially during the peak season as compared to year 2 (430 kg/ha). In this case, addition of fertilizers rich in nitrogen during the peak period of plant growth resulted in enhanced levels of nitrogen in soil. Sandy texture of soil leads to the reduction of nitrate in soil (Kaur *et al.* 2014). Upon application of urea-based fertilizers, the urea undergoes hydrolysis to form ammonia-N within a few days. The plants that are incapable of taking up urea, ammonia-N is converted to nitrate-N within a month. Nitrate, being a chemically stable form of nitrogen, can easily leach from the soil profile. Tillage practices have been shown to significantly influence nitrate leaching. The impact of this leaching is notably variable, with the most pronounced effects occurring when tillage is performed just prior to a period of high-water recharge. Increase in nitrogen content of the soil, favours the rate of carbon

sequestration as well, although excessive content of nitrate may result in nitrate toxicity (Pal *et al.*,2016). Wetlands serve as biofilters that assist in removing particulate matter, act as sinks for nutrient accumulation, and also transform these nutrients into various gaseous nitrogen forms (Yousaf *et al.*,2021).

Phosphorous is another core constituents of cells, acquired from soil as well. Phosphorous is highly effective in enhancing the seed, fruit and root growth of plants (Dandwate 2020). In this study, it was observed that the phosphorus levels of *Cucurbita maxima* soil rhizosphere were found to rise from initial season to peak season then declined during the intercrop season in both the years. They reached to their optimum value of approximately 150 kg/ha and 140 kg/ha during the peak season of first year and second year respectively. The phosphate level declined during the intercrop season, where they were found to be 117.57kg/ha during first year and 105.33kg/ha in the second year. In case of *Zea mays* soil rhizosphere, phosphorous levels in soil during Year 1 was about 138.81 kg/ha during the initial stage and was lowest in Year 2 intercrop season at 53.81 kg/ha. The peak values of 106.76kg/ha during year 1 as compared to 87.12kg/ha in year 2 indicated more phosphate availability in Year 1. The decline during intercrop (especially in Year 2) likely reflects plant uptake and possible fixation, and therefore could limit the rate of plant growth.

Phosphorous restricts the nutrients within plant nuclei and thereby acts as a storehouse of energy (Kekane *et al.* 2015). It is one of the major nutrients required for production of good quality crops. It is usually catalytic in nature (Dandwate 2020). In present investigation, enhanced phosphate levels in soil during peak period, may due to the use of fertilizers rich in phosphate. The rise in phosphate levels of soil can also be due to the decomposition of organic matter by micro-organisms (Song *et al.*,2012). The decline in the phosphate levels of the soil can be due to the plant uptake for their biomass.

Potassium has a crucial role in plant development. It is associated with several metabolic reactions, whereby the formation of structural components of plant cells requires lignin and cellulose, for modulation of photosynthesis and formation of plant sugars (Kekane *et al.* 2015). In this investigation, it was observed that the quantity of potassium in soil ranges from 173.37 kg/ ha in initial growth period of *Cucurbita maxima*, decreases to 163.96 kg/ha in peak growth period and decreases further to 139.77 kg/ha in the intercropping season. During the second year, the levels of potassium in soil rhizosphere is approximately 184.12kg/ha, declines gradually in the peak period and reduces to approximately 140.5 kg/ha in the intercrop period. In case of *Zea mays* soil rhizosphere, available potassium(K) showed a different pattern, whereby in Year 1, the levels of

potassium peaked to 159.94 kg/ha at intercrop season, whereas in Year 2, the highest value of potassium levels in the soil were found to be 143.81 kg/ha during the initial stage and declined to 88.7 kg/ha by intercrop season. Increase in potassium ion uptake by plant occurs with an increase in the level of cation exchange content (Sparks 1980). It regulates synthesis of proteins and plant water balance. The mobility of potassium ions in soil is very slow and is also known to displace other ions like calcium, magnesium or sodium (Addis and Abebaw 2015). Excessive use of fertilizers also increases the levels of potassium in soil as seen in initial period of growth of *Cucurbita maxima* and *Zea mays*.

In this investigation, the quantity of **calcium** (Ca) in soil ranges from 1603.2 mg/kg in initial growth period of *Cucurbita maxima*, decreases to 480.96 mg/kg in peak growth period of *Cucurbita maxima* soil, and further increases to 1122.24 mg/kg in the intercropping season. In case of *Zea mays* soil rhizosphere, in Year 1, Ca dropped from 879.5 mg/kg initially to only 400.8 mg/kg at peak, and then escalated to 881.76 mg/kg during the intercrop period. During the second year, the calcium levels were 801.6mg/kg during the initial stages, dipped to 710.2 mg/kg during the peak season and again increased to 843.68 mg/kg during the intercrop season.

Calcium in soil results through deposition of minerals like limestone and gypsum. It has a cardinal responsibility in cell wall composition and formation of protoplasm. It is found to be correlated with several forms of carbohydrates and organic acids as well (Addis *et al.*,2015). In present investigation, decrease in calcium levels could be due to utilization by the plants, decline in soil pH, followed by presence of higher content of magnesium ions in soil (Wallace *et al.*,2008). Increase in calcium levels in soil during the intercropping period may be due to the addition of phosphate fertilizers or fertilizers containing limestone and gypsum that has probably resulted in the increment of calcium level (von Willert *et al.*,2003).

Magnesium (Mg^{2+}) plays a crucial role in the health, productivity, and quality of agricultural crops. As a key element in plant physiology, Mg^{2+} is essential for photosynthesis, serving as a vital part of chlorophyll, which enhances growth and biomass production. In addition to supporting fundamental growth, it significantly influences various quality aspects of crops, including chlorophyll production, texture, and shelf life (Ahmed *et al.*,2023). In this investigation, it was found that the quantity of magnesium in soil, during the first year is about 1020.6 mg/kg during initial cropping period *Cucurbita* sp, decreases to about 583.2 mg/kg in peak season and further decreases to 437.4 mg/kg in intercropping season. Soil has high levels of magnesium. It adheres to the colloidal complex of soil. Magnesium levels in soil during the initial period of second year

were less by 40.5%. In case of *Zea mays* soil rhizosphere, magnesium levels in the soil during year1 was about 664.54 mg/kg during the initial stage, declined sharply to 72.9 mg/kg at peak, then increased to 340.2 mg/kg at intercrop season. In Year 2, soils had higher levels of Mg at 542.58 mg/kg of soil during the peak period as compared to year 1.

The amount of magnesium in soil is related to the levels of cation exchange capacity of soils. Availability of magnesium ions in soil is strongly correlated with the levels of potassium in soil (Levy *et al.* 1988). Magnesium gets adsorbed in the soil at higher level over potassium (Sposito *et al.* 1986). It is a water-soluble cation and is essential for formation of green coloured chlorophyll pigments in plants (Addis *et al.*,2015). Therefore, in present investigation, decrease in magnesium levels during peak cropping period may be due to utilization by the plants. One of the reasons due to which the values further decreased in the intercropping season, may be due to irrigation during tillage practices. Most important source of nitrogen in plants is nitrate. Major sources of nitrate are from air, organic content of soil, excessive use of fertilizers, wastewater from the municipal areas etc., that are been taken up by the plants and utilized for their growth.,

The biogeochemical processes involving **sulphur** are crucial in wetland ecosystems, contributing to acidification, carbon mineralization, pyrite formation, the cycling of metallic elements, and the release of sulphur into the atmosphere [Reddy *et al.*, 2008; Lu *et al.*,2020].In this research, *Cucurbita maxima* soil rhizosphere showed that the sulphur contents increased in the peak stage (Year 1: 10 to 12 mg/kg; Year 2: 4 to 14mg/kg), then decreased at intercrop stage. In case of *Zea mays* soil rhizosphere, the sulphur content in Year 1 was highest at 11mg/kg and decreased rapidly to around 6.8mg/kg of soil during the intercrop period. During Year 2, the sulphur content in the soil was highest during the intercrop stage at about 8.2mg/kg of soil. Sulphate content of the soil has increased, because of the rise in the levels of organic carbon content of the soil. Presence of sulphate, has been known to be a promoter of chlorophyll production, as well as formation of nodule in legumes (Pal *et al.* 2016).

In the present investigation, in case of *Cucurbita maxima* soil rhizosphere, **copper** levels in Year 2 have an extremely high peak (87.14 mg/kg) compared to Year 1 (7.59 mg/kg). **Zinc** levels in both the years have Zn around 15–25 mg/kg – generally sufficient for plant growth. **Iron** levels in soil are higher in Year 2 across all stages. Both years peak around flowering (53.85mg/kg and 89.33mg/kg), and has been found to decline to 41.05mg/kg of soil in the second year during the intercrop season.

In case of *Zea mays* soil rhizosphere, the levels of micro-nutrients showed large stage-to-stage fluctuations across both the years. **Boron** (B) peaked in Year 1 at the initial stage (0.55 mg/kg) but was low (0.1–0.3 mg/kg) during the other stages. **Copper** (Cu) was extremely high at Year 1 peak (100 mg/kg) but nearly absent by Year 1 intercrop; Year 2 started with moderate levels of Cu (40 mg/kg) and declined to ~2 mg/kg by peak. **Zinc** (Zn) was present at a quantity of 25 mg/kg at Year 1 initial, and was nearly absent during the intercrop stage of Year 1; Year 2, Zn was very low and was present at an approximate quantity of 5 mg/kg throughout. **Iron** (Fe) was present at a quantity of 60 mg/kg at Year 1 during the initial period, declined to 55mg/kg at peak period, then reduced to 35mg/kg at intercrop; Year 2 had approximately 50mg/kg of Fe initially, but declined to 5 mg/kg by peak period. Micronutrients play a vital role in achieving balanced crop growth, just like macronutrients do. Micronutrients, including boron (B), copper (Cu), iron (Fe), zinc (Zn) have a positive impact on plant development. Nearly every cellular and metabolic function in plants is affected by these micronutrients. A deficiency in any essential micronutrient leads to reduced biomass production in plants [Hänsch *et al.*,2009; Mir *et al.*,2021].

Metagenome of the soil was studied to analyse the type of micro-organisms that were prevalent through all the cropping phases, and the presence of micro-organisms in the soil is also determined by the contents of physico-chemical parameters of soil. Metagenomic analysis of *Cucurbita maxima* (Chapter 4, Figure 4.7, 4.8) and *Zea mays* (Chapter 4, Figure 4.9, 4.10) rhizosphere soil was conducted across three cropping periods: Initial, Peak, and Intercrop, for Year 1 and 2, using the Illumina Novaseq 6000 platform. During the 1st year, at the phylum level, the most abundant bacterial groups included: *Acidobacteriota*, *Patescibacteria*, *Verrucomicrobiota*, *Planctomycetota*, *Proteobacteria* and *Chloroflexi*. The most abundant taxa at the genus level included *Candidatus* sp, *Bryobacter* sp and *Bacillus* sp. The bacterial genera *Pirellula* sp., *Gemmata* sp., and *Nitrospira* sp. were consistently present (lingering) during all three cropping periods, suggesting their potential role in maintaining microbial stability in the rhizosphere. During the 2nd year, the key bacterial genera identified in high abundance throughout all periods were: *Lysobacter* sp, *Polycyclovorans* sp, *Paenibacillus* sp, *Candidatus* sp, *Gemmata* sp, *Bacillus* sp. The presence of *Candidatus* sp. and *Gemmata* sp. suggests involvement in specialized microbial interactions and potential roles in biogeochemical cycling within the rhizosphere (Mujakić *et al.*,2022). The genus *Bryobacter* sp encompasses acid tolerant bacteria, which are aerobic in nature. Chemo-organotrophic bacteria are also a part of *Bryobacter* sp, that are known to inhabit wetlands. These bacteria also possess the capability to break down esculin, alginate, casein, chondroitin sulfate, gelatin, pectin, peptone, and protein hydrolysate. The capacity to metabolize sorbose, arabinose,

dulcitol, sorbitol, fucose, pyruvate, and lactate varies among different strains. Nitrogen sources utilized include ammonia, nitrate, peptone, yeast extract, alanine, valine, asparagine, isoleucine, lysine, ornithine, and methionine. However, the ability to fix dinitrogen is lacking (Deydsh *et al.* 2019). *Bacillus* sp has varied genetic makeup and heterogeneity in their metabolism. They have the ability to support plant growth by fixing nitrogen from soil, thereby making it readily available to the plants, also they help in synthesis of potassium and phosphorous. *Bacillus* sp were known for their ability to produce phytohormones that enhances the plant growth promoting property (Saxena *et al.* 2020). In wetland soils, the genus *Gemmata*, which belongs to the Planctomycetes group of bacteria, is involved in microbial interactions and nutrient cycling, especially concerning carbon and nitrogen. They engage in the breakdown of organic matter derived from plants and aid in the cycling of elements such as carbon and nitrogen. Their presence and activity are affected by various factors, including soil conditions, water levels, and the presence of other microbial species [Bahram *et al.*,2022.]

In the present investigation, the analysis of the heavy metal contents was done of the collected soil samples. The soil was found to contain a significantly high concentration of lead (Pb) at 398.76 mg/kg (**Chapter 4, Figure 4.11**), indicating severe lead contamination. Chromium (Cr) was the second most abundant heavy metal detected at 60.39 mg/kg, suggesting potential industrial or anthropogenic input. Nickel (Ni) was present at 39.88 mg/kg, Arsenic (As) was detected at a lower concentration of 0.39 mg/kg. Cadmium (Cd) was found in trace amounts at 0.19 mg/kg. The agricultural soil of the selected plot was found to be highly contaminated by lead, having a value of 398.76 mg/kg of soil, much higher than the permissible limit. Overall, the soil shows signs of heavy metal contamination, especially due to elevated levels of lead, which could pose risks to plant health, soil microbiota, and human exposure through the food chain. Presence of heavy metal like lead, even in the lower layers in the soil possess huge problems and may induce permanent damage to the organisms (Gupta *et al.*, 2020). As per World Health Organisation (1996) the permissible limit of lead in agricultural soil was about 85 mg/kg of soil.

Therefore, it can be safely concluded that long term cultivation of specific crops usually set in a specific group of bacterial population that actually determines the nutrient availability and crop production.

5.2 To perform physical isolation, purification and characterization of potential microbes having dynamic role in soil development, and their role in mitigation of pollution (lead).

In this objective, the aim of the work was to isolate, identify and characterize lingering soil bacteria i.e the bacteria prevailing in the soil for long period of time with antibiotic susceptibility, plant growth promoting properties and abilities of heavy metal absorption.

Evidences of identifying bacterial isolates from wetlands were studied, whereby 38 bacterial isolates were obtained from the soil samples of Yellow river wetland basin, China and further analysis was done for their ability to solubilize phosphorous and their other PGP properties (Sun *et al.*,2023). Another study was conducted in wetland rice fields of peninsular Malaysia, whereby 9 bacterial strains from 6 soil sites were tested for their ability to fix nitrogen, solubilize potassium and phosphorous. Their abilities to synthesise siderophores, IAA (indole acetic acid) and organic acids were also tested, thereby qualifying as efficient biofertilizers (Othman *et al.*,2022). In this investigation a total of 12 bacterial isolates were initially obtained from the rhizospheric soil samples of Chowbaga area of reclaimed agricultural soil of East Kolkata Wetlands. Out of 12 bacterial isolates, 5 isolates were found to be consistently present (lingering flora) across the Initial, Peak, and Intercrop seasons in both *Cucurbita maxima* and *Zea mays* which was confirmed through wetland studies. These five key isolates were designated as, DPP(A), DPP(B), DPP(C), DCP(A) and DCI(D). Gram staining and colony morphology studies were conducted as part of the general characterization of these isolates (**Chapter 4, Table 4.5,4.6**). The antibiotic susceptibility test of bacterial isolates (**Chapter 4, Figure 4.12**) was carried out, followed by their ability to function as plant growth promoters. Specific use of lingering bacterial isolates from reclaimed agricultural soil of wetlands as biofertilizers is limited.

All the five bacterial strains were studied elaborately for their various plant growth promoting biocontrol properties and enzymatic properties. The initial screening of the bacterial isolates was done based on their Gram characterization, whereby three strains were found to be Gram positive in nature and two strains were Gram negative in nature (**Chapter 4, Table 4.5**). Screening of their antibiotic sensitivity was carried out (**Chapter 4: Figure 4.12**). Further studies on bacterial chromogenic nature indicated all the 3 strains to be part of genus *Bacillus* (**Chapter 4: Table 4.6**) and two strains showed similarities with *Pseudomonas* sp and *Proteus* sp. Phylogenetic identification of all the 5 isolates showed that DPP(A): *Bacillus cereus* strain DPP(A)_SGAM_SXC (Accession number OQ216888) , DPP(B): *Bacillus velezensis* strain DPPB_SGAM_SXC (Accession number ON384543) , DPP(C): *Stenotrophomonas maltophilia*

strain DPP(C)_SGAM_SXC (Accession number OQ217020) , DCP(A): *Cupriavidus necator* strain DCPA_SGAM_SXC (Accession number ON384541) and DCI(D): *Bacillus cereus* strain DCI(D)_SGAM_SXC (Accession number OQ217005) [Chapter 4, Figure 4.15,4.16,4.17, 4.18, 4.19]

As per literature studies, *Bacillus velezensis* (strain CR-502T and strain CR-14b) was initially isolated from environmental samples collected from the mouth of the Vélez River in Torredelmar, located in the province of Málaga, Spain (Rabbee *et al.*,2019). *Bacillus velezensis* has been acknowledged as an excellent plant growth-promoting rhizobacterium (PGPR). It has shown remarkable efficacy in fostering the growth and safeguarding various crop plants such as cucumber, pepper, wheat, barley, soybean, and cotton. It has been noted for its ability to trigger a plant immune response known as induced systemic resistance (ISR), which provides defence against above-ground pathogens and insect pests. Additionally, a significant discovery was the initial identification of its bacterial volatile compounds, which are recognized for their role in enhancing plant growth and activating ISR. Studies have suggested the use of *B.velezensis* as an environmentally friendly alternative to traditional pesticides and fertilizers (Jang *et al.*,2023).

Evidences supporting the use of *Cupriavidus necator* as plant growth promoting bacteria are limited, although certain reports (Pereira *et al.*, 2015a, Pereira *et al.*, 2015b), showed that rhizobacterial strains *Cupriavidus necator* sp, 1C2 (B1) were chosen due to their plant growth-promoting ability which included production of siderophores and indole acetic acid (IAA), as well as 1-aminocyclopropane-1-carboxylic acid (ACC)-deaminase activity. Additionally, they have demonstrated the ability to improve maize growth in stressful environments, such as those affected by metal pollution [Moreira *et al.*, 2016b, 2019; Pereira *et al.*,2020].

As per literature studies, *Stenotrophomonas maltophilia* strains BCM and BCM_F exhibited exceptional antifungal properties, highlighting their potential for biocontrol. Pre-treating seeds with these strains facilitated nitrogen fixation and phosphate solubilization within the wheat rhizosphere, demonstrating their biofertilizer capabilities. Their presence in the rhizosphere also positively influenced plant health metrics, such as increased sugar and nitrite levels, and significantly improved crop yield ($P < 0.05$). The enhanced growth parameters and superior crop yield observed in *S. maltophilia* pre-inoculated seeds under field conditions suggest their viability as a sustainable option for boosting wheat production (Sharma *et al.*,2025). In another study conducted by Alexander *et al.*,2019, the growth of plants was enhanced when co-cultivated with

PGPR (Plant Growth Promoting Rhizobacteria) like *S. maltophilia* was observed in both normal and nitrogen-deficient stress conditions.

Bacillus cereus has been examined for properties that promotes the production of indole-3-acetic acid (IAA) and aminocyclopropane-1-carboxylic acid (ACC) deaminase, as well as phosphate solubilization, which facilitates direct promotion of plant growth. This encompasses enhancements in biometric traits, the content of chemical elements (e.g., N, P, and K), and the levels or activity of biologically active substances, such as antioxidant enzymes and total soluble sugars. Consequently, *B. cereus* has aided the growth of various plant species, including soybean, maize, rice, and wheat. Notably, certain strains of *B. cereus* can also enhance plant growth in the face of abiotic stresses, such as drought, salinity, and heavy metal contamination.

In spite of the plant growth promoting properties of *Bacillus cereus* and *Stenotrophomonas maltophilia*, several reports suggest [McDowell *et al.*, 2023;] that *B. cereus* leads to gastrointestinal disorders characterized by nausea, vomiting, and diarrhoea. Nevertheless, it has been linked to severe infections in individuals with weakened immune systems and can result in septicemia and endophthalmitis, potentially resulting in vision impairment. Similarly strain like *Stenotrophomonas maltophilia*, a common nosocomial pathogen was found to have risk factors associated with chronic respiratory conditions, particularly cystic fibrosis, hematologic cancers, neutropenia induced by chemotherapy, patients with organ transplants, individuals infected with the human immunodeficiency virus (HIV), those on haemodialysis, and newborns. Additionally, environments within hospitals, extended stays in intensive care units, mechanical ventilation, tracheostomy procedures, the presence of central venous catheters, severe traumatic injuries, major burns, mucositis or factors that damage the mucosal barrier, as well as the administration of broad-spectrum antibiotics, have been identified as increasing the likelihood of infection [Paez *et al.*, 2008; Kwa *et al.*, 2008; Abbott *et al.*, 2011; Willsey *et al.*, 2019; Said *et al.* 2023]

All the bacterial isolates were tested for their plant growth promoting potential, that included tests for nitrogen fixation, potassium and phosphate solubilization. Nitrogen, a crucial nutrient component in wetland soil, significantly contributes to the prevention or mitigation of environmental degradation. A deficiency in fixed nitrogen may restrict ecosystem productivity [Li *et al.* 2015; Zhang *et al.*, 2020]. In another study conducted by Li *et al.*, 2024, research showed that nitrogen fixing bacteria improve crop yields via nitrogen fixation (Shameem *et al.*, 2023), and also form symbiotic or mutualistic associations with crop root systems. Their interactions boost the release of growth hormones in the roots, which aids in root development and nutrient uptake,

ultimately enhancing the crops nutritional quality and resilience to stress conditions (Islam *et al.* 2013). In this investigation, all the 5 bacterial strains were tested for nitrogen fixing abilities. All the strains namely, DPP(A) [*Bacillus cereus*], DPP(B) [*Bacillus velezensis*], DPP(C) [*Stenotrophomonas maltophilia*], DCP(A) [*Cupriavidus necator*] and DCI(D) [*Bacillus cereus*], were potential fixers of nitrogen and grew luxuriantly on nitrogen-free modified Jensen's medium supplemented with Bromothymol blue (indicator) [Chapter 4, Figure 4.20].

Potassium is an essential macronutrient, having an essential role in plant growth promotion. Soil potassium in wetlands has a crucial role in plant photosynthesis, including the production of adenosine triphosphate (ATP), the translocation of sugars, the transport of water and nutrients, starch synthesis, nitrogen fixation in legumes, as well as enzyme and protein synthesis (Wolde, 2016). Although it is abundant, less than 2 to 3% of soil potassium is available to plants in a free soluble form, as the majority remains bound to other soil minerals, accounting for approximately 95% of total soil potassium (Etesami *et al.*, 2017). The potassium solubilizing micro-organisms facilitate the solubilization of potassium by generating organic acids like tartaric acid, citric acid, and oxalic acid, which help release potassium from its mineral forms (Olaniyan *et al.*, 2022). In this investigation, all the bacterial isolates were tested for their ability to solubilize potassium. It was observed that DPP(B) showed a distinct change in the colour from green to yellow in Aleksandrow media. The strains DPP(A) [*Bacillus cereus*], DCI(D) [*Bacillus cereus*] showed faint colour change, showing medium abilities for potassium solubilization. However, the bacterial isolate DPP(C) [*Stenotrophomonas maltophilia*] did not show any colour change in the media, suggesting their inability to synthesise potassium [Chapter 4, Figure 4.21].

Another essential macronutrient having pivotal role in plant growth promotion is phosphorous. In the soil, it is mainly taken up and used by plants in the form of orthophosphate (Pi). Microorganisms that solubilize phosphate convert insoluble phosphorus in the wetland soil into forms that are bioavailable by producing phytase to mineralize organic phosphorus or by secreting organic acids to dissolve inorganic phosphorus. [Khan *et al.*, 2009; Teng *et al.*, 2018;]. Furthermore, phosphate-solubilizing microorganisms (PSMs) can chelate heavy metal ions in the soil using exopolysaccharides, thereby releasing phosphate ions that are readily available to plants. Some PSMs also influence plant growth by modifying the levels of various plant hormones such as indole-3-acetic acid (IAA) (Wani *et al.*, 2007), or by producing siderophores that facilitate either symbiotic or non-symbiotic nitrogen fixation (Yang *et al.*, 2014). In addition, PSMs can generate hydrogen cyanide (HCN), and 1-Aminocyclopropane-1-carboxylic acid (ACC) deaminase (Yang

et al.,2008), which assist in managing soil-borne diseases and decreasing heavy metal toxicity [Zhu *et al.*,2024]. In this investigation, it was found that all bacterial isolates namely DPP(A)[*Bacillus cereus*], DPP(B)[*Bacillus velezensis*], DPP(C)[*Stenotrophomonas maltophilia*], DCP(A)[*Cupriavidus necator*] and DCI(D) [*Bacillus cereus*] grew luxuriantly on phosphate deficient media, i.e Pikovskaya media, modified with bromothymol blue (pH indicator), showed colour change from green to yellow, thereby they were found to have the ability to fix phosphorous [Chapter 4, Figure 4.22]. The ability of the bacterial isolates to solubilize phosphorous was further quantified, whereby the bacterial isolates DPP(A), DPP(B), DPP(C), DCP(A), and DCI(D) were able to synthesize phosphate within a range of 1.68 mg/mL – 7.91 mg/ml [Chapter 4, Figure 4.23].

Presence of phytohormones namely Indole Acetic Acid (IAA) and Gibberellic Acid (GA₃) are essential properties of plant growth promotion for bacterial isolates. In wetland soils, bacteria that generate Indole-3-acetic acid (IAA) and Gibberellic acid (GA₃) are vital for the growth and development of plants. These bacteria, typically located in the rhizosphere (the region surrounding plant roots), produce these phytohormones, which are critical for numerous plant functions. IAA, a primary auxin, affects root development, nutrient absorption, and stress resilience (Etesami *et al.*,2024), whereas GA₃ facilitates germination, stem elongation, and flowering (Gusmaity *et al.*,2019). Bacterial isolates were tested for their ability to synthesise phytohormones namely Indole Acetic Acid (IAA) and Gibberellic Acid (GA₃). It was found from the investigation that all the 5 bacterial strains could produce Indole Acetic Acid (auxins) in both presence and absence of the precursor tryptophan. DPP(A)[*Bacillus cereus*], DPP(B)[*Bacillus velezensis*], DPP(C)[*Stenotrophomonas maltophilia*], DCP(A)[*Cupriavidus necator*] and DCI(D) [*Bacillus cereus*] were able to produce IAA within a range of 50.187 µg/mL - 56.785 µg/mL and it was observed that DCP(A)[*Cupriavidus necator*] produced the highest amount of IAA at 56.785 µg/mL [Chapter 4, Figure 4.24]. Similarly, gibberellin production by bacterial isolates were tested for all the bacterial isolates. They showed the ability of production of gibberellin, whereby DPP(A) [*Bacillus cereus*], DPP(B) [*Bacillus velezensis*], DPP(C) [*Stenotrophomonas maltophilia*], DCP(A) [*Cupriavidus necator*] and DCI(D) [*Bacillus cereus*] were able to produce gibberellin within a range of 0.864 µg/mL-10.584 µg/mL. It was observed that DPP(C) [*Stenotrophomonas maltophilia*] produced the highest amount of gibberellin at 10.584 µg/mL. Gibberellin production ability of the test set up was higher, when compared to the control set [Chapter 4, Figure 4.25].

Plant Growth Promoting Rhizobacteria (PGPR), are known for their ability to produce ACC deaminase, an enzyme that reduces ethylene levels in plants. This process helps to alleviate stress responses and ultimately enhances crop productivity. (Saikia *et al.*, 2018; Ojuederie *et al.*, 2019). ACC deaminase plays a crucial role in promoting plant growth and reducing stress responses by breaking down the ethylene precursor, 1-aminocyclopropane-1-carboxylic acid (ACC). This enzyme helps to mitigate the negative effects of ethylene, a plant hormone involved in stress responses and senescence. Plant growth promoting bacteria can produce the enzyme ACC deaminase by which they can utilise ACC as a Nitrogen source thereby decreasing ethylene production and enhancing plant survival rate in times of physical stress (Orozco-Mosqueda *et al.*, 2020). All 5 bacterial strains namely DPP(A) [*Bacillus cereus*], DPP(B) [*Bacillus velezensis*], DPP(C) [*Stenotrophomonas maltophilia*], DCP(A) [*Cupriavidus necator*] and DCI(D) [*Bacillus cereus*] used in this study could produce ACC deaminase, whereby DPP(B) [*Bacillus velezensis*] and DCP(A) [*Cupriavidus necator*] being the highest of the entire set at 0.013mM and 0.08mM respectively [Chapter 4, Figure 4.26, 4.27].

Urease facilitates the hydrolysis of urea, leading to the formation of ammonia and carbon dioxide. This enzyme is vital for the nitrogen cycle. The indiscriminate use of urea as a nitrogen source in agricultural regions results in the buildup of residual urea in the soil, which plants cannot effectively utilize. Consequently, this leads to residual toxicity. Bacterial urease is instrumental in decomposing residual urea and reestablishing the nitrogen balance in the soil (Mekonnen *et al.*, 2021). In this investigation, it was found that the highest ability for urease production was seen in DCP(A) [*C. necator*] with 3.23 mM urea hydrolyzed/min/OD. [Chapter 4: Figure 4.28, 4.29].

Endophytic enzymes, including laccase (Chauhan *et al.*, 2016), pectinase (Haile *et al.*, 2022), and cellulase (Demissie *et al.*, 2024), are essential for the recycling of lignocellulosic waste in agricultural fields and for enhancing the organic carbon content of the soil. This process, in turn, improves soil quality. The tested bacterial strains also produce significant amounts of these enzymes, which further promote soil health. Moreover, these enzymes assist in breaking down root cell walls, enabling bacteria to infiltrate plant tissue. Once inside, the bacteria form a symbiotic relationship with the plant, fostering its growth and providing biocontrol against pathogens. In exchange, the bacteria obtain nutrition and shelter from the plant.

In the present investigation, the quantification of cellulase production by bacterial isolates, showed that DPP(A) [*Bacillus cereus*] and DCP (A) [*Cupriavidus necator*] showed highest ability of cellulase production at 4.4µM/ml and 3.9 µM/ml respectively (Chapter 4, Figure 4.30, 4.31). In the

present work, analysis of the bacterial isolates for enzyme analysis was done, whereby in case of **laccase** production, DPP(A) [*Bacillus cereus*] is the most efficient laccase-producing strain, as compared to DPP(B) [*Bacillus velezensis*] and DPP(C) [*Stenotrophomonas maltophilia*] that showed moderate activity of laccase production(Chapter 4, Figure 4.32,4.33). **Lignin peroxidase**, a heme-containing enzyme, utilizes hydrogen peroxide to oxidize both phenolic and non-phenolic compounds found in tea pruning litter, which disrupts soil microorganisms and leads to soil quality degradation (Kumar *et al.*, 2020). In the present investigation, the bacterial isolates were tested for their ability to produce lignin peroxidase. It was found that **DPP(B)** [*Bacillus velezensis*], showed the **highest lignin peroxidase activity** on Day 2 of the experimental set-up. The highest abilities of lignin peroxidase production were seen in DPP(B) [*Bacillus velezensis*], DPP(A) [*Bacillus cereus*], and DCP(A)[*Cupriavidus necator*] at 0.524 U/ml, 0.447 U/ml and 0.373U/ml on Day 1 (Chapter 4, Figure 4.34,4.35). Bacteria that produce **amylase** are essential in soil as they decompose starch, a complex carbohydrate, into simpler sugars such as glucose. This process is important for nutrient cycling and the growth of microbes within the soil ecosystem. The sugars produced serve as a food source for other microorganisms, thereby helping to thrive in the environment (Morbias *et al.*,2024). In this analysis, it was found that DPP(A) [*Bacillus cereus*], DPP(B) [*Bacillus velezensis*], and DCP(A) [*Cupriavidus necator*] were able to produce higher amylase content at 0.23 $\mu\text{M}/\text{min}$, 0.22 $\mu\text{M}/\text{min}$ and 0.21 $\mu\text{M}/\text{min}$ amylase, much higher as compared to the other bacterial isolates (Chapter 4, Figure 4.36, 4.37). **Pectinase** production in DCP(A) [*Cupriavidus necator*] was of about 0.0188 mg/ml. This was followed by DPP(A) [*Bacillus cereus*], DPP(C) [*Stenotrophomonas maltophilia*], DPP(B) [*Bacillus velezensis*] and DCI(D) [*Bacillus cereus*] that produced 0.0136 mg/ml, 0.0056mg/ml 0.0048mg/ml and 0.0028 mg/ml pectinase, much higher as compared to the control set up (Chapter 4, Figure 4.38,4.39).

Metallothionein (MT) is a low molecular weight protein that lacks histidine and is abundant in cysteine and non-aromatic amino acids (Romero-Isart *et al.*, 2002). It is commonly present in bacteria, fungi, as well as in a variety of animals and plants (Sun *et al.*, 2016). In bacterial strains, the intracellular sequestration of metals primarily relies on the presence of metallothionein, which plays a significant role in various cellular functions, especially in the transport, storage, and detoxification of metals (Chudobova *et al.*, 2015). As a result, a higher concentration of metallothionein can be vital for maintaining homeostasis and detoxifying metals, thereby enabling resistance to heavy metals in bacterial strains isolated from surface water. Numerous studies have indicated that metallothionein are essential for sustaining metal homeostasis and providing

protection against heavy metal toxicity through intracellular sequestration (Chudobova *et al.*, 2015).

In this investigation, the bacterial isolates DPP(A) [*Bacillus cereus*], DPP(B) [*Bacillus velezensis*], DPP(C) [*Stenotrophomonas maltophilia*], DCP(A) [*Cupriavidus necator*] and DCI(D) [*Bacillus cereus*] showed the ability to produce higher metallothionein in 500ppm of lead as compared to 200ppm of lead [**Chapter 4, Figure 4.40**]. DPP(A) [*Bacillus cereus*] shows the highest ability to produce metallothionein at 500ppm, as compared to other bacterial isolates.

HCN plays crucial role in biocontrol of phytopathogens as well as encouraging plant development (Sehrawat *et al.*, 2021). In this investigation, upon quantitative analysis, it was found that DCP(A) [*Cupriavidus necator*] had the highest ability of HCN production, with an absorbance value of 0.12 [**Chapter 4, Figure 4.41, 4.42**]. The degradation of protein rich compounds like manure or fertilizers in nature usually resulted in release of **ammonia** from bacteria that would alter the pH of the soil and promote interaction between the plant and microbes, followed by their ability to enhance plant growth (Weise *et al.*, 2013). The highest capacity was seen among DPP(A) and DPP(C) with 0.747 µg/mL. DPP(B)[*Bacillus velezensis*], DCP(A)[*Cupriavidus necator*], and DCI(D)[*Bacillus cereus*] were able to produce 0.415 µg/mL, 0.537 µg/mL, and 0.664 µg/mL of ammonia respectively [**Chapter 4, Figure 4.43, 4.44**]

In other studies, it was reported that the plant growth-promoting rhizobacteria colonize the roots of plants by enhancing their growth and competitively reducing the population of detrimental soil microorganisms through various mechanisms. These include the production of hydrogen cyanide, solubilization of phosphate, ammonia production, antibiotic synthesis, siderophore production, and the stimulation of phytohormones such as IAA (Olanrewaju *et al.*, 2017; Meena *et al.*, 2017). The diverse plant growth-promoting capabilities of *Bacillus* spp. identified in the study aligned with findings from other researchers (Altaf *et al.* 2016). The bacterial isolates examined in this research were found to produce IAA, which is one of the mechanisms utilized by PGPR to exert beneficial effects on plants, aligning with previous findings (Rana *et al.* 2011). The production of IAA by plant growth-promoting rhizobacteria enhances the elongation of the plant root system, facilitating the uptake of water and nutrients essential for plant growth (Egamberdieva *et al.* 2017). All bacterial isolates demonstrated the ability to produce ammonia (NH₃), supporting the results of Geetha *et al.* (2014), who reported ammonia production by rhizobacteria isolated from the rhizosphere of green gram. Additionally, Kandjimi *et al.* (2015) noted that ammonia production by rhizobacteria promotes plant growth by making nitrogen accessible for plant development.

Similarly, Bhattacharyya *et al.*, 2012 reported that ammonia production by rhizobacterial isolates aids in plant growth by inhibiting the proliferation of fungal pathogens.

Siderophores are referred to belong to the class of chelating groups. They have the ability to facilitate transport of iron inside the cell and also bind to the heavy metals that are present on the outside of the cell. They have high predilection for ferric compounds and compounds containing ferric complexes as well. The lone pair of electrons of nitrogen and oxygen atoms of the functional group, has been known to enhance the metal chelation capacity, thereby forming stable polycyclic bonds with heavy metals (Mitra *et al.*, 2021). In the present investigation all the bacterial isolates were found to have siderophore production capacities. Quantification study revealed the percentage of siderophore production was recorded after 24 and 48 h, whereby DPP(A)-*B.cereus*, DPP(B)-*B.velezensis*, DPP(C)- *S.maltophilia*, DCP(A)-*C.necator*, and DCI(D)-*B.cereus* showed 3.1%, 4.62%, 37.89%, 27.36%, and 14.73%, respectively, that was much higher compared to its production in 24 hours, both in test and control set up [Chapter 4, Figure 4.45,4.46]. This signified the fact that, since they had high siderophore production abilities, they would also have better metal chelation properties and therefore could be used successfully for soil bioremediation.

In another study by Shen *et al.*, 2013 it was showed that siderophore-producing microbes enhance plant growth by facilitating iron acquisition, suppress the growth of other microorganisms and restrict the development of fungal phytopathogens by limiting the iron available for their growth (Fasusi *et al.*, 2021).

The isolation and characterization of *Bacillus* spp. and *Pseudomonas* spp. from various maize rhizospheres in this study have been documented by Figueroa-López *et al.* (2016) for their potential in promoting plant growth and their biocontrol activities.

The accumulation of lead in soils poses a significant threat to agricultural production, primarily due to its detrimental impact on soil microflora, crop development, and food safety. In the soil environment, the speciation of lead plays a crucial role in determining its bioavailability and, consequently, its toxicity to plants and microorganisms. Numerous plants and bacteria have adapted by developing detoxification mechanisms to mitigate the harmful effects of lead. Several factors influence lead speciation, including soil pH, organic matter content, the presence of various amendments, clay minerals, as well as organic colloids and iron oxides (Kushwaha *et al.*, 2018).

The process of biosorption of heavy metal usually referred to the process, whereby, the metal ions get adhered or adsorbed to the cell surface, without the requirement of any form of energy (Li *et*

al., 2016). Ability of *Bacillus cereus* to remediate lead has been reported earlier as well, whereby the papers have also suggested that lead uptake capacity was directly proportional to the biomass of the bacteria (Gupta *et al.*, 2020).

Safeguarding mechanisms used by bacteria to mitigate the pernicious effect of lead, encompasses mechanisms like biosorption, extracellular sequestration, efflux, bioaccumulation in intracellular matrix, and so on. Biosorption has been referred to as the process, whereby microbial cell wall usually has a role as a shield to prevent the entry of toxic compounds. The metal cations in the cell walls have been known to bind to the negative ions of both Gram-positive and Gram-negative bacteria and at the same time monitor the entry of heavy metal ions into the cell, by restricting them on the outer surface. Reports have suggested, that the presence of amide along with sulphonamide groups in association with hydroxyl and carboxyl groups in *Bacillus* sp. have immobilized the lead on cell surface. *Klebsiella* sp. 3S1, was found as an active biosorbent of lead and was therefore proposed to be used as a biosorbent in lead contaminated soil (Mitra *et al.*, 2021).

In the current study, all the five bacterial isolates namely DPP(A) [*Bacillus cereus*], DPP(B)[*Bacillus velezensis*], DPP(C)[*Stenotrophomonas maltophilia*], DCP(A)[*Cupriavidus necator*], and DCI(D)[*Bacillus cereus*] were found to have high lead biosorption capacity, therefore they could be significantly used for lead bioremediation in the extremely lead contaminated agricultural soil of East Kolkata Wetlands. The bacterial isolates were further tested for their metal uptake capacity. It was found to have 0.848 µg/g, 0.976 µg/g, 0.173 µg/g, 0.383 µg/g, 0.917 µg/g for DPP(A), DPP(B), DPP(C), DCP(A), and DCI(D), respectively [**Chapter 4, Figure 4.47 b**]. The bacterial isolates namely DPP(A), DPP(B), DPP(C). DCP(A) and DCI(D) were found to have the following percentage of lead adsorbed, that was within a range of 2.30%-8.28% [**Chapter 4, Figure 4.47c**]. This inferred, that the selected bacterial isolates had good ability of soil bioremediation, since they had the ability to mitigate the problem of heavy metal pollution in the wetland soils.

The existence of these various compounds demonstrates the considerable efficacy of the chosen isolates in promoting plant growth, boosting soil fertility, alleviating the effects of abiotic stressors, and acting as a holistic biological control agent that reduces plant pathogens while strengthening the plants' capacity to withstand them.

5.3 To prepare an ideal consortium to be supplemented all through the year suitable for any crop, either singly or with chemical fertilizers (in-vivo study).

The main goal of this objective was to develop a new bacterial formulation using the chosen bacterial isolates, followed by testing the newly created formulations in vivo conditions.

Preparation of bacterial consortia can be successfully initiated, after checking the interaction between the bacterial isolates by cross streaking. Bacterial interactions within a consortium can be categorized into three types based on their mutual effects: (i) stimulatory or positive, (ii) inhibitory or negative, and (iii) neutral [Singh *et al.*,2019]. Positive interactions typically establish a network that supports individual members through cross-feeding, where one bacterium utilizes the metabolic byproducts of another consortium member. Negative interactions, on the other hand, result in the suppression of certain bacterial members within the consortium, undermining the community structure and its functionality [Loccoz *et al.*,2015]. In agricultural context, it is essential for consortium members to interact positively, as mutualistic growth is crucial for maintaining stable performance over extended cultivation periods to achieve the desired positive outcomes when applied to crops [Liu *et al.*,2012; Mahmud *et al.*,2020]. This form of communication is based on the production, detection, and response to extracellular signalling molecules that regulate and influence the bacterial population within the consortium, ensuring that only compatible microbes participate in modifying the plant defence response, which in turn affects overall plant health and growth [Mukherjee *et al.*,2019]. The communication within the consortium is heavily reliant on molecular signals, with quorum sensing being particularly important for bacterial compatibility in consortium formulations [Liu *et al.*,2012; Santoyo *et al.*,2021].

In this investigation, interaction test between the bacterial isolates namely DPP(A) [*B.cereus*], DPP(B)[*B.velezensis*], DPP(C)[*S.maltophilia*], DCP(A)[*C.necator*] and DCI(D)[*B.cereus*] were tested and it was observed that all the bacterial isolates showed positive interactions amongst them. This observation ensured that the bacterial isolates could be used together as a bacterial consortium. [Chapter 4, Figure 4.48]

Analysis of the biocontrol properties of the bacterial isolates were essential to know about the effectiveness of the bacterial consortia in pathogen control. *Curvularia* leaf spot (CLS) of maize (*Zea mays* L.), caused by *Curvularia lunata* var. *aeria*, is among the most prevalent diseases affecting maize. In India, this disease has been noted in maize across all states during the rainy and winter seasons (Choudhary *et al.* 2011). It leads to a decrease in yield both directly and indirectly, as the pathogen can infect both leaves and seeds. Reports indicate that this disease can result in

yield losses of 20–60% in China, up to 33.3% in Nigeria, and 21–23% in India (Dai *et al.* 1998). *Curvularia lunata* produces leaf spots that typically manifest as small, light brown circular or ovoid lesions, encircled by a brown ring. The size of these lesions can range from over 1 mm to 3 mm in diameter for the circular type. In advanced stages of infection, these small spots can cover the entire leaf area by either merging or increasing in number. Eventually, the leaf takes on a blighted appearance and may die before reaching senescence. The pathogen can attack any part of the plant at any growth stage due to seed-borne inoculum. The disease spreads to new areas through infected seeds. Secondary infections in standing crops are caused by air-borne conidia under favourable weather conditions in the field (Rathore *et al.* 2005). It is particularly prevalent in regions where the climate is warm and humid, with temperatures ranging from 28 to 32 °C (Garhwal *et al.*,2022). The application of *Bacillus subtilis*, isolated from rhizosphere was used for biological control of the pathogenic fungus *Curvularia gudauskasii* affecting sugarcane seedlings. The wild type strain SR/B-16 demonstrated over 70% inhibition of the in vitro growth of this plant pathogenic fungus, likely due to a combination of two biological control strategies: competition for nutrients and the secretion of antifungal lipopeptides as secondary metabolites (Ratón, *et al.*,2012). A study conducted by Santoso *et al.*,2022 showed that extracts of *Bacillus siamensis* could block the growth of *Curvularia lunata*. In a study conducted by Sartori *et al.*, 2015, it was observed that *Bacillus* sp isolates were able to suppress the rust disease caused by *Puccinia sorghi* (Schwein) and the northern leaf blight disease caused by *Exserohilum turcicum* (Pass.) These diseases are among the most significant diseases impacting the crop, leading to a reduction in yield.

In this investigation, the fungal pathogens were isolated from infected *Zea mays* leaves and identified using morphological disease pattern and microscopic features. The antagonistic potential of selected plant growth-promoting bacterial (PGPB) isolates against the pathogens (*Curvularia* sp. and *Exserohilum* sp.) was tested as a part of biocontrol strategy. All the bacterial isolates namely DPP(A) [*B.cereus*], DPP(B) [*B. velezensis*], DPP(C)[*S.maltophilia*], DCP(A)[*C.necator*] and DCI(D)[*B.cereus*] showed antagonistic effects against *Curvularia* sp by creating fungal zone inhibition diameters of 23 mm, 11 mm, 20 mm, 14 mm and 22 mm respectively. All the bacterial isolates namely DPP(A) [*B.cereus*], DPP(B) [*B. velezensis*], DPP(C)[*S. maltophilia*], and DCI(D)[*B.cereus*] showed antagonistic effects against *Exserohilum* sp by creating fungal zone inhibition diameters of 38 mm, 23 mm, 35 mm, and 42 mm respectively. DCP(A)[*C.necator*] showed no visible inhibition, suggesting lack of antagonistic activity against *Exserohilum* sp[Chapter 4, Figure 4.49, 4.50,4.51].

Determination of the most effective treatment application method for improving plant yield and promoting soil health, a small-scale pilot study was carried out. The formulation designs and treatment dosages were refined based on evidence gathered from pilot studies and earlier research conducted by Chakraborty *et al.* in 2013 and Roy *et al.* in 2023. In a small-scale pilot study, Chakraborty *et al.* (2013) carried out a trial over a period of 3 months, utilizing broth-based inoculation at one-month intervals. This study proposed a water-suspension based direct application of bacterial strains as a cost-effective alternative. In this investigation, a field study was conducted to determine the most effective mode of treatment application using bacterial inoculants. Three treatment application methods were tested, and vegetative parameters such as plant length, number of leaves, leaf length, and internodal length were monitored weekly. Seeds of *Cucurbita maxima* (Variety: Jack 323) and *Zea mays* (Variety: 4226) were used for the field study with 20 plants, whereby 10 plants were taken in 1 plot and administered with the treatment and the remaining 10 plants were taken in the adjacent plot, where no treatment was applied, and was thus referred to as the control set-up. The plant-to-plant distance for was 80cm. Among the five bacterial isolates, DPP(B) [*Bacillus velezensis*] and DCP(A) [*Cupriavidus necator*] demonstrated the better plant growth-promoting, phytohormonal, and enzymatic properties. These two strains were selected to create a bacterial consortium, which was applied to one-month-old plants in Chowbaga plot of 30 m² of the East Kolkata Wetlands. Based on the field results, the water suspension method was chosen for the field trial due to its scientific effectiveness and practical advantages. Vegetative and reproductive data was collected weekly from the date of application up to the 12th week to assess treatment impact. The treated set up showed better vegetative and reproductive parameters as compared to the control setup (**Chapter 4, Figure 4.52,4.53,4.54,4.55,4.56**).

Application of bacterial consortia to the plants has an impact on the chlorophyll content. Co-inoculation results in the most substantial and reliable enhancements in shoot weight, root weight, total biomass, lengths of both shoots and roots, total chlorophyll, shoot width, and grain yield. The findings indicated that the multi-strain consortium of PGPR had a significant positive impact on the biochemical characteristics of wheat, as evidenced by a reduction in electrolyte leakage and an increase in chlorophyll content, relative water content (RWC), and K/Na ratio (Khan *et al.*,2020). In this investigation, upon addition of bacterial consortia, in both the plant types of *Cucurbita maxima* and *Zea mays*, significantly enhanced Chlorophyll a, Chlorophyll b and carotenoid contents. The **peak period** showed a more pronounced difference in pigment content, reflecting enhanced **photosynthetic capacity** and possibly improved **plant health and growth**. Treated set up of *Cucurbita maxima* and *Zea mays* showed **higher pigment content** than control set up.

Treated set up of *Cucurbita maxima* had 48mg/g of Chl a, 27mg/g of Chl b and 19mg/g of carotenoid content. Upon comparison with control set up there was 25mg/g, 10mg/g and 9 mg/g of Chl a, Chl b and carotenoid content, during the peak period. During the peak growth period 22mg/g, 16mg/g and 7 m/g of Chl a, Chlb and carotenoid content was observed in the treated set up of *Zea mays*, as compared to 8mg/g of Chl a, 7mg/g of Chl b and 4mg/g of carotenoid content was observed in the control set-up of *Zea mays* (Chapter 4, 4.57, 4.58). Soil enzyme profiling was conducted of both treated and control set-up. It was observed that enzyme contents of soil administered with treatment had higher enzyme contents as compared to the control set-up. Research has been done to observe the presence of bacteria using SYBR stains in plant systems. In a study done by Jim et al.,2022, the population density of *P. odoriferum* , a pathogen was assessed by utilizing SYBR Green-based real-time quantitative PCR with a primer set designed for plants. Consequently, this newly established diagnostic technique facilitated swift and precise diagnosis as well as ongoing monitoring of soft-rot disease in Kimchi cabbage, eliminating the requirement for further procedures involving the plant tissue. Similarly, in this investigation, upon addition of SYBR green stain, microbial abundance in the treated soil and plant roots were higher as compared to the control set-up (Chapter 4, 4.58b, 4.58c).

ANOVA, which stands for Analysis of Variance, is a statistical technique employed to examine the differences between the means of various groups. When applied to vegetative plant parameters, ANOVA helps identify whether there are statistically significant variations in characteristics such as plant height, leaf count, or biomass across different treatment groups (for instance, varying levels of watering, types of fertilizers, or different plant varieties) (Abbas *et al* 2012). In the present analysis of *Cucurbita maxima* and *Zea mays*, a two-way ANOVA was utilized, considering treatment and time as factors. For *C. maxima*, plant height exhibited highly significant main effects: treatment (Factor A, $F \approx 62.05$, $p < 0.001$) and week (Factor B, $F \approx 87.18$, $p < 0.001$). The interaction between treatment and time was less pronounced but still significant ($F \approx 3.53$, $p = 0.001$). Similarly, leaf number in *C. maxima* demonstrated significant effects from treatment ($F \approx 24.29$, $p < 0.001$) and time ($F \approx 58.52$, $p < 0.001$), but no significant interaction was observed ($F \approx 1.28$, $p = 0.261$). In *Z. mays*, plant height revealed even more pronounced treatment and time effects – treatment ($F \approx 222.29$, $p < 0.001$) and week ($F \approx 168.43$, $p < 0.001$) – along with significant interaction ($F \approx 5.05$, $p < 0.001$). The leaf count of *Zea mays* also showed highly significant main effects (treatment $F \approx 80.52$, $p < 0.001$; time $F \approx 24.76$, $p < 0.001$) and a weaker interaction ($F \approx 3.89$, $p = 0.001$). In all instances, the two-way ANOVA framework facilitated the simultaneous evaluation of inoculation

and plant age, confirming that beneficial consortia significantly enhanced growth metrics over the weeks.

These results align broadly with findings from other crop studies. For instance, *Lactuca sativa* (lettuce) subjected to drought stress was examined using two-way ANOVA. Both *Bacillus* spp. inoculation and the watering regime had highly significant impacts on shoot fresh weight and water content ($p < 0.05$), with a significant interaction noted for leaf relative water content (Santander *et al.*, 2024). Leaf nitrogen and phosphorus levels were greatly affected by inoculation and the interaction between inoculation and water ($p < 0.0001$). Likewise, in *Solanum lycopersicum* (tomato) under lead stress, a two-way ANOVA (lead level vs PGPR inoculation) revealed that PGPR inoculation significantly alleviated the reduction of chlorophyll caused by lead, and ANOVA confirmed a significant interaction ($p < 0.05$) regarding chlorophyll content. (Anjum *et al.*, 2025)

In the present research involving *C. maxima* and *Zea mays* was performed in field trials where weekly measurements were taken, which facilitated time-series ANOVA. In contrast, the tomato/Pb investigation was a greenhouse pot experiment featuring 4 replicates for each treatment combination (six Pb levels with two inoculation treatments). The lettuce research employed a fully factorial pot design where strains of *Bacillus* sp was used as a bacterial consortium. (Anjum *et al.*, 2025). A field trial focusing on maize under drought conditions examined water (well vs drought) and microbial consortium factors within a greenhouse lysimeter ($n=4$) (Yim *et al.*, 2025). In the maize/drought research, inoculated plants grown in well-watered loam exhibited approximately 20–41% greater shoot fresh mass compared to the control group, and ANOVA indicated $F \approx 5.57$ ($p < 0.01$) for the effect of consortia on shoot dry mass. Drought significantly reduced shoot mass ($F \approx 68.90$, $p < 0.001$ in loam; $F \approx 6.82$, $p < 0.05$ in sand). In all instances, the experiments were factorial and randomized, employing similar ANOVA and post-hoc testing methods. In another study conducted by Pacheco da Silva *et al.*, 2022, it was observed that in soybean (*Glycine max*), treating seeds with *Bacillus velezensis* and pectin-rich orange peel across different cultivars resulted in notable enhancements in both root and shoot biomass. The two-way ANOVA indicated highly significant effects of inoculation and cultivar on nodule number and area ($p < 0.01$).

Therefore, the statistical and experimental patterns observed in this investigative study of *C. maxima* and *Z. mays* closely correspond with those found in various crops: two-way ANOVA consistently identifies significant advantages of microbial treatments (frequently with

interactions), providing assurance that the treatment effects noted are both robust and biologically significant.

Analysis of the soil physico-chemical parameters were done, to understand the changes in the soil parameters upon application of bacterial consortia. The pH level of the soil is the most critical factor due to its influence on all other soil characteristics. Consequently, pH is considered when analysing any type of soil. A soil is classified as acidic if the pH is below 6, normal if the pH ranges from 6 to 8, and alkaline if the pH exceeds 8.5[More *et al.*,2022].

Another vital property of soil is **electrical conductivity**, which is utilized to evaluate the quality of the soil. It quantifies the number of ions present in a solution (Schoenholtz *et al.*,2000). **Nitrogen** is the most essential element that plants can obtain from the soil, acting as a growth limiting factor for them (Gorde *et al.*,2013). **Phosphorus** is a vital component found in every biological cell (Schoenholtz *et al.*,2000). It is one of the most important micronutrients necessary for plant growth. Phosphorus often limits the retention of nutrients within the plant nucleus and acts as an energy reserve. **Potassium** is an essential element for plant growth and plays a significant role in various physiological processes within plants .It is involved in a wide array of metabolic activities, from the synthesis of plant sugars for different metabolic needs to the regulation of photosynthesis and the formation of lignin and cellulose, which are utilized in structural support.

The introduction of organic material into the soil can enhance its buffering capacity and facilitate the chelation of Al^{3+} , leading to an increase in soil pH (Baolan et al., 2014). Variations in pH across different soil depths can be linked to the increased downward movement of solutes and soil bases within the profile due to leaching (Assen et al., 2005), as well as elevated levels of carbonate ions and reduced weathering rates in the soil. It was observed a relatively higher organic carbon content in the upper layer (0–20 cm) of the soil, attributing this rise to an enhanced return of soil biomass at the surface resulting from microbial decomposition. In organically managed soils, phosphorus availability increases due to the rise in organic compounds, which leads to the formation of organophosphate complexes that are readily assimilated by plants. Additionally, organic matter in the soil can facilitate the maximum dissolution of inorganic phosphorus by minimizing its fixation and enhancing its availability (Selassie and Ayanna, 2013).

In the current investigation, bacterial consortia comprising of (DPP-B)*Bacillus velezensis* and (DCP-A) *Cupriavidus necator* was applied to the soil in the designated plot of *Cucurbita maxima* and *Zea mays* in their respective growing season. Another adjacent plot of land was taken, whereby seeds of *Cucurbita maxima* (December-February) and *Zea mays* (March-June) were sown. No

bacterial consortia were added to the plot at that time. In *Cucurbita maxima* soil rhizosphere, it was observed that the **pH** in treated soil (8.3) is lower than in control soil (8.8), making the environment less alkaline and more suitable for nutrient uptake. The **electrical conductivity** of a soil solution increases with the concentration of ions. This method is a quick, simple, and cost-effective means of assessing soil health. It measures the number of ions in a solution. The electrical conductivity of a soil solution rises as the concentration of ions increases. The analysis of the present investigation showed that the electrical conductivity (EC) was significantly higher in treated soil (194 $\mu\text{S}/\text{cm}$) compared to control (50 $\mu\text{S}/\text{cm}$), indicating increased nutrient and salt availability within optimal range. The present investigation showed that nitrogen content was lower in treated soil (225 kg/ha) than in control (234 kg/ha), which supports better vegetative growth and development, and enhanced uptake and utilization of nitrogen by treated plants. **Phosphorus** level is lower in treated soil (415 kg/ha) compared to control (465 kg/ha), showing better ability of uptake by treated plants and thereby promoting root development, flowering, and energy transfer. **Calcium** content is slightly lower in treated soil (0.63 mg/kg) than in control (0.77 mg/kg), improving structural support and root health. Iron is marginally decreased in treated soil (4.25 mg/kg) versus control (4.84 mg/kg), promoting chlorophyll formation and enzymatic functions. **Sulphur** is slightly lower in treated soil (6.83 mg/kg) than control (7.01 mg/kg), contributing to amino acid synthesis and plant metabolism. **Zinc** in treated soil (5.46 mg/kg) is nearly the same as control (5.49 mg/kg), with no significant loss in this essential micronutrient. **Copper** content in treated soil (0.4 mg/kg) is very close to control (0.42 mg/kg), with no significant difference. Boron levels in treated soil (5.18 mg/kg) and control (5.21 mg/kg) are nearly identical, ensuring maintained micronutrient balance. In this investigation, it was also observed that potassium was slightly reduced in treated soil (151 kg/ha) compared to control (157 kg/ha), which may be due to solubilization by bacterial isolates.

In case of *Zea mays*, the **pH** of treated soil (8.2) is lower than that of control soil (8.7), reducing alkalinity and improving nutrient solubility and uptake. **Electrical Conductivity** (EC) is higher in treated soil (102 $\mu\text{S}/\text{cm}$) than in control (64 $\mu\text{S}/\text{cm}$), indicating greater availability of soluble salts and nutrients. **Nitrogen** content was lower in treated soil (189 kg/ha) than in control (221 kg/ha), supporting enhanced vegetative growth and chlorophyll synthesis. **Phosphorus** was found to slightly decrease in treated soil (364 kg/ha) compared to control (459 kg/ha), which showed that treated plant could uptake and utilize the macro nutrient. It also supports root development and energy transfer processes. **Potassium** levels decreased in treated soil (141kg/ha) as compared to control soils (160 kg/ha). **Calcium** was found to decrease in treated soil (0.61 mg/kg) than in

control (0.76 mg/kg), contributing to better cell wall development and root health. **Iron** content decreased in treated soil (4.17 mg/kg) compared to control (4.75 mg/kg), aiding chlorophyll formation and redox reactions. **Zinc** was found to decrease in treated soil (4.69 mg/kg) compared to control (4.85 mg/kg), which showed enhanced enzyme function and auxin synthesis in treated plants. **Manganese** significantly decreases in treated soil (2.54 mg/kg) compared to control (3.44 mg/kg), which may have an impact upon photosynthetic enzyme activity. Sulphur decreased in treated soil (6.75 mg/kg) from control (7.24 mg/kg), but remains within acceptable range for protein synthesis. **Copper** levels were closeby in both treated (0.38 mg/kg) and control soils (0.4 mg/kg), ensuring continued enzyme activity and plant metabolism. **Boron** content decreased in treated soil to 5.02mg/kg and in control soils, it was present in a quantity of 5.16 mg/kg, thereby ensuring maintenance of proper cell wall structure and reproductive growth. **Organic carbon** content increases slightly in treated soil (0.42%) compared to control (0.39%), improving soil structure and microbial activity. **Magnesium** content is slightly lower in treated soil (61.7 mg/kg) than control (72.8 mg/kg), but still adequate for chlorophyll production and enzyme activation.

Soil metagenome of the treated as well as control soil was done, to understand the colonization of bacterial strains, that were applied as bacterial consortia. Metagenomics is a method that does not rely on culture for characterizing microbial diversity in soil samples [Hardwick *et al.*,2018]. It identifies the variety of uncultured soil microbial species by examining the direct extraction of genetic materials (DNA) from specific habitats [Stevenson *et al.*,2004; Rivera *et al.*,2015]. Rawat *et al.*,2019 stated that Next-Generation Sequencing technology has become an essential tool for studying biodiversity in metagenomic samples and metabolic pathways. Recently, this next-generation technique has been widely applied for analysing microbial diversity to tackle environmental issues [Gupta *et al.*,2017]. Next-generation sequencing (NGS) technologies that highlight conserved regions of evolutionary markers to identify operational taxonomic units (OTUs) enhance the study of complex microbial communities [Almeida *et al.*,2019]. These methods differentiate viruses, bacteria, fungi, parasites, and archaea from previously unknown and uncultivable organisms [Chiang *et al.*,2020]. Soil microbial communities represent the highest concentration of prokaryotic organisms in the environment [Zethof *et al.*,2020]

In this investigation, the colonization of introduced bacterial strains was confirmed through the soil metagenomic investigations and analyses, that was done at the peak period of crop growth for both the crops at a time of 60 days, from the day of initial growth. The increased quantity and distribution of class and phylum categories in the bacterial treatment, in comparison to the original

control soil, suggested successful colonization. Throughout the short 3-month cropping period, there was significant colonization of the applied bacteria, as illustrated in **Chapter 4: Figures 4.59 and 4.60**.

The metagenomic comparison between control soil and treated soil of *Cucurbita maxima* revealed a 10% increase in the population of *Bacillus* sp., indicating effective colonization of the introduced bacterial strains. Heat map analysis showed a notable percentage increase in both the phylum Firmicutes and the family Bacillaceae from the initial to the final soil sample, further confirming successful establishment of the applied bacterial consortia in the rhizosphere of *Cucurbita maxima*. During the intercrop period, the abundance of *Bacillus* sp. increased by approximately 15%, demonstrating sustained colonization and activity of the applied strains. The Krona charts distinctly showed an increase in the percentage of Firmicutes in the treated soil at 10%, in comparison to control soil at 0.7% (**Chapter 4: Figure 4.61**). This suggests that the improved growth of plants in the treated soils can be attributed to the incorporation of bacterial treatments into the maize rhizosphere.

In case of *Zea mays*, the metagenomic analysis revealed a significant increase in the abundance of *Bacillus* sp. in the rhizosphere of *Zea mays* following the application of the bacterial consortia. During the intercrop period, the abundance of *Bacillus* sp. increased by approximately 30%, indicating early colonization and establishment of the applied strains. A more pronounced 40% increase in *Bacillus* sp. abundance was observed during the peak growth period, demonstrating sustained and dominant colonization over time. Krona chart showed that the percentage of firmicutes and Burkholderiales (*Cupriavidus* sp) was 30% in the treated soil as compared to 0.6% in the control soil, when collected at the initial stage [**Chapter 4, Figure 4.64,4.65**]

Reactive oxygen species (ROS), which include free radicals and non-radical molecules [Sharma *et al.*,2006], play a crucial role in the network of signalling pathways. They serve as primary regulators of cellular responses and the physiological processes of plants in response to environmental factors [Bhattacharjee *et al.*,2012]. At low levels, H₂O₂ functions as a signalling molecule that plays a role in regulating various biological and physiological processes, including photosynthesis, the cell cycle, growth and development, as well as plant responses to both biotic and abiotic stresses [Cuypers *et al.*,2012].

Excess accumulation of H₂O₂ can lead to oxidative stress and ultimately cell death in plants. Stress factors can trigger an increased production of H₂O₂, which may result in severe damage to biomolecules due to the elevated levels of non-metabolized cellular H₂O₂. To combat this, plants

possess H₂O₂-metabolizing enzymes such as catalases (CAT), ascorbate peroxidases (APX), certain peroxiredoxins, glutathione/thioredoxin peroxidases, and glutathione sulfo-transferases. Among these, CAT and APX are particularly noteworthy; CAT is primarily found in peroxisomes and does not need a reductant to catalyse a dismutation reaction. Notably, APX has a greater affinity for H₂O₂, reducing it to water in chloroplasts, cytosol, mitochondria, and peroxisomes, as well as in the apoplastic space, using ascorbate as a specific electron donor [Apel *et al.*,2004].

In this investigation, in case of *Cucurbita maxima* leaves, it was observed that Catalase activity, Ascorbate peroxidase activity was much higher in *Cucurbita maxima* treated leaves as compared to the control set up. In treated set up, the leaves showed a catalytic activity at 0.0021mM/ H₂O₂/min/g fresh weight of leaves and the control set up showed catalytic activity at 0.001mM/ H₂O₂/min/g fresh weight of leaves respectively at 3 minutes. **(Chapter 4, Figure 4.68)**. The ascorbate peroxidase activity in treated leaves, showed value of 669 nM/ MDA/min/g fresh weight of leaves and the control setup showed value of 321 nM/ MDA/min/g fresh weight of leaves respectively at 25 minutes.

In case of *Zea mays*, Catalase activity was found to be 0.1895 mM/ H₂O₂/min/g fresh weight of leaves for treated set up and 0.1255mM/ H₂O₂/min/g fresh weight of leaves for control set up. Peroxidase activity in treated set up was found to be 0.08mM/min/g fresh weight of leaves and in control set up, the activity was found to be 0.03mM/min/g fresh weight of leaves. Ascorbate peroxidase activity in treated set up showed value of 0.18Mm/min/g fresh weight of leaves and control set up showed 0.11 mM/min/g fresh weight of leaves respectively **(Chapter 4, Figure 4.71,4.72, 4.73, 4.74)**.

5.4 To perform statistical analysis, including time series study of physico-chemical and microbiological property vis a vis crop productivity.

The present study evaluated the change in soil physicochemical (and by extension microbiological) properties across the initial, peak, and intercrop phases of *Cucurbita maxima* and *Zea mays*. It also correlated these changes with time-series patterns in plant growth. Two-sample *t*-tests were used to compare mean soil parameters between the cropping phases, interpreting *p*-values below 0.05 as evidence of statistically significant change.

Research done by Huang *et al.*, 2025, in a maize amendment with application of bacterial consortia experiment, *t*-tests were done upon soil nutrient data and the differences were marked as significant when $p < 0.05$. In this investigation, it was observed that *t*-tests in *C. maxima* plots, several soil components differed significantly over time – notably silt content and key nutrients (Ca, K, phosphate, N) showed $p < 0.05$. In the *Z. mays* plots, an even broader set of parameters changed significantly (clay and sand fractions, moisture, water-holding capacity, CEC, and many macro/micronutrients such as Mn, Fe, Zn, Cu, S, Mg, Ca, K, P; $p < 0.05$). These findings imply substantial soil nutrient changes during crop growth, operated by plant uptake and the applied microbial consortia. Similar patterns have been reported in other cropping systems. For instance, cover-cropping studies in orchards found that introducing legumes or grasses can raise topsoil nutrients (soluble carbon, microbial C/N, nitrate/ammonium N, etc.) by 20% on average relative to fallow, with associated increases in soil enzyme activities (Ma *et al.*, 2024). Cover cropping is an agricultural technique in which farmers cultivate specific crops, known as cover crops, to mainly enhance and safeguard the soil. Cover crops are integrated into the farming system during fallow periods or in between the cycles of main crops. Likewise, organic amendments in maize have been shown to significantly boost soil N, P, K levels (and soil organic C) compared to untreated controls (Huang *et al.*, 2025).

The use of *t*-tests (and, where appropriate, nonparametric tests like Wilcoxon's test) exhibited which soil properties changed in a statistically reliable way. In an experiment done by Huang *et al.*, 2025; one-way ANOVA followed by *t*-tests was used to identify significant differences in soil pH, organic C, N, P, K between treatments and the nutrients increased significantly under biochar and seaweed amendment treatments. In this investigation, it was observed that, *p*-value results showed that nutrient concentrations in *Cucurbita maxima* and *Zea mays* soils shifted at each growth

stage, likely reflecting nutrient uptake by roots and microbial transformation. Nutrient availability has an impact upon crop growth and are connected with each other.

Time series analysis is essential as it can show patterns in variable observations, thereby enhancing forecasting (Chatfield, 2004).

Time series forecasting models were utilized to examine the production trends. The most appropriate models were chosen based on their performance metrics, which include the Bayesian Information Criterion (BIC), Root Mean Square Error (RMSE), Mean Absolute Percentage Error (MAPE), and Mean Absolute Error (MAE) (Sandeep *et al.*, 2025).

In this investigation, time-series analysis is primarily utilized to understand and predict the growth patterns of *Cucurbita maxima* and *Zea mays* upon addition of bacterial consortia to the soil rhizosphere. Among various agronomic parameters, the number of branches and leaves directly correlate with the overall yield of the plant. The present study revealed that upon addition of bacterial consortia composed of *Bacillus velezensis*(DPP(A)) and *Cupriavidus necator* (DCP(A)) the plant growth of both the crops increased substantially as compared to the control set up.

Machine learning serves as an effective method for enhancing yield prediction by considering multiple factors. It falls under the umbrella of artificial intelligence (AI) that focuses on the process of learning. Machine learning (ML) can derive insights from data by identifying patterns and relationships. To be effective, the models need to be trained on datasets that reflect past experience-based results. Linear Regression is a machine learning method and a foundational model for analysing yield trends (Ashfaq *et al.*, 2024).

Linear regression models in agriculture include crop-specific case studies (e.g. maize, wheat, barley, cotton) and diverse regions (North America, Europe, Asia, Africa). Therefore, linear regression remains a fundamental tool in agricultural time-series analysis – from modelling yield–weather relationships and growth indices to mapping soil nutrients – and provides a baseline against which, more complex models are compared [Conradt *et al.*, 2022; Ashfaq *et al.*, 2025]

Linear Regression is a machine learning algorithm that operates on the principles of supervised learning. The objective of linear regression is to identify a straight line that reduces the error (the discrepancy) between the actual data points and the predicted values. This line enables us to forecast the dependent variable for new, previously unseen data. Regression aims to model a target prediction value using independent variables. This method is primarily employed to determine the

relationship between variables and for forecasting. Additionally, it is utilized to predict a dependent variable based on one or more independent variables (Murugan *et al.*,2020).

Simple Linear Regression Formula: $y=mx+c$

Here, y is referred to as the dependent or target variable, while x is known as the independent variable, which serves as the predictor for y . There are numerous types of functions or modules available for regression analysis. A linear function represents the most basic form of function. In this context, x can consist of a single feature or multiple features that characterize the problem. Various evaluation metrics can be employed to assess the effectiveness of any linear regression model. These evaluation measures typically provide insight into how accurately the model is generating the observed outputs.

For *C. maxima*, parameters such as plant height, leaf count, node number, leaf size, tendril length, flowering, and fruit set were analysed on a weekly basis. The observed time-series plots showed that treated plants performed consistently better than the control set up, the difference between the control and treated became more pronounced across the weeks and for most traits (height, leaves, nodes, etc.) the treated group had higher means at each week. Linear regression models of each trait versus time (week) were fitted, and compared with the slopes and goodness-of-fit. In nearly all cases, the fitted slope (growth rate) was steeper for the treated plants than for controls, indicating accelerated growth under the consortia treatment. Moreover, the coefficient of determination was very high. For example, the linear fit to plant height data produced an R^2 in excess of 0.98 for both groups. An R^2 of 0.98 implies that about 98% of the variation in height was explained by the time trend; the remainder (2%) is “noise” or unexplained variation. Such high R^2 values indicate that a simple linear growth trend describes the data extremely well. This finding aligns with large-scale yield studies: Zulauf *et al.* (2023) reported R^2 values of 0.96 for linear corn-yield trends over four decades. Therefore, observation of strong linear patterns (high R^2) is common in agronomic time series when growth occurs steadily at each period.

Although, all traits did not show simple linear trajectories. The number of flowers per plant in *C. maxima* rose and then declined towards the end of the season, producing a parabolic curve. A linear fit to this data was poor (low R^2), correctly signalling that linear regression was not appropriate. Instead, statistical tests on the number of flowers were calculated at each week to compare treated set up vs. control set up. These tests showed no early-season difference in flower count, but a significant difference emerged in later weeks (treated > control). By the final measurement, treated

plants had significantly more flowers than controls ($p < 0.05$), even though flower count in both groups was declining.

In general, the time-series and significance results for *C. maxima* point to a clear outcome: soil consortia application led to enhanced vegetative growth and fruiting. Similar conclusions have been drawn in other crop systems using analogous methods. For instance, Huang *et al.* 2025 observed that organic amendments (biochar and seaweed) significantly increased maize biomass and also raised soil nutrient levels (C, N, P, K) in tandem.

For *Z. mays*, growth metrics were also monitored (height, leaf number, node count, internode count, girth, tassel length, etc.) over time for treated versus control plots. The raw data showed that the treated maize grew taller and develop more nodes/internodes than controls as the season progressed. In early growth (weeks 6–8), the differences were small and statistically non-significant, but by mid to late season the treated plants accelerated past the controls. This definitely indicated that the bacterial consortia gradually got settled down in the soil amidst other microflora and gave positive impact to the growth of the crop plants. A linear regression of height versus time again gave very high R^2 values (0.98–0.99) for both groups, indicating a nearly perfect fit of the linear trend. Notably, the slope of the treated regression line exceeded that of the control, evidencing a higher rate of height gain. However, the timing of significant difference was delayed: statistical tests (both *t*-test and Wilcoxon) found no significant height difference between groups until the final measurement (week 12). A similar delay to statistical significance is reported in other maize studies: for example, a maize–legume intercropping trial found that plant height differences between treatments became statistically significant only by weeks 5–7 of growth (with *p*-values dropping below 0.05 after mid-season) (Kiponda *et al.*, 2023). In that study, early-week differences were non-significant ($p > 0.05$), showcasing the pattern of divergence over time (i.e after 6 weeks).

Other productivity traits in maize followed comparable dynamics. Initial graphs suggested the treated seedlings had more leaves and larger girth than controls set up, but time-series analysis showed that initially control set up had higher number of leaves and larger girth control. After about two to three measurement intervals, the lines crossed and treated plants led thereafter. Only the last data point showed a statistically significant advantage in leaf number and internode count for treated maize. This outcome highlights that growth delays or accelerations can mask or reveal significance only later. Despite this, the fitted regressions still had very high R^2 values, reinforcing that time was the dominant factor in explaining variation.

Huang *et al.* 2025, reported that biochar and organic fertilizer significantly raised maize biomass (up to 17% gain) along with higher soil nutrient levels. In this investigation, it was observed that near-linear growth (high R^2) is consistent with large-scale trends: linear models often capture >90% of maize yield variation over time (Zulauf *et al.*, 2023), due to the fact that maize yield is significantly influenced by time and exhibits a nearly linear response to enhancements in inputs and management practices over the years. The combination of very high R^2 and late-emerging significance in treated vs. control is also in line with field variability and multiple influences on growth (weather, genotype, etc.), which create “noise” around the strong time trend. In this context, the R^2 values of 0.98–0.99 indicate our models fit the data almost perfectly, leaving only a few percent of variance unexplained.

In both *Cucurbita maxima* and *Zea mays*, inoculation with *Bacillus velezensis* and *Cupriavidus necator* boosted vegetative growth. The two-way ANOVA showed that *Cucurbita maxima* plants were longer than controls (ANOVA $F = 87.18$, $p < 0.001$), with far more leaves. Similar effects are observed in other crops: for instance, application of *Bacillus* sp significantly enhanced tomato shoot height and biomass (one-way ANOVA, Tukey $p < 0.05$) and *B. velezensis* strains approximately doubled the trunk diameter in 30 d-old peach rootstock ($p < 0.05$). The growth curves fitted (slope over time) consistently indicate higher growth rates in treated plants (Álvarez *et al.*, 2021). The linear fits to weekly plant-height data (from our maize trial) produce slopes of ~20 cm/week under inoculation compared to ~17 cm/week for controls (both with $R^2 \approx 0.92$), suggesting a quicker increase in treated plants. This trend whereby a steeper slope following a lag was observed has been frequently documented. Kifle and Laing (2016) inoculated maize with bacterial isolates and noted no height increase at 30 days or 90 days, but a significant spike by 60 d (treated biomass then notably surpassed control). Across various studies, ANOVA results consistently reveal very low p-values (often $p < 0.001$) for inoculation effects on vegetative traits, affirming that treated plants ultimately exhibit faster and taller growth.

In this investigation, *C. maxima* showed significantly more flowers and fruits in treated vs control (flower count $F = 12.50$, $p < 0.001$; fruit count $F = 8.28$, $p < 0.001$). Trends in other crops are similar. For example, PGPR consortia increased flowering and fruit set in legumes and vegetables in multiple reports (often tested by ANOVA or t-tests with $p < 0.05$). Yield improvements have been noted: one study found *B. subtilis* inoculation raised wheat grain yield by ~33% (highly significant) (Shi *et al.*, 2022)

From a biological perspective, this indicates that microbes require time to establish themselves and exhibit growth-enhancing functions, after which their impact accumulates. Therefore, the experiments and existing literature concur that beneficial microbes enhance both vegetative and reproductive growth over time, resulting in steeper slopes (rates) in the treated groups. Statistical analyses (such as t-tests or ANOVA) consistently validate these improvements as highly significant (frequently $p \ll 0.01$) (Shi *et al.*, 2022).

Therefore, the combination of statistical tests with time-series modelling highlights that microbial consortia enhance soil quality while also fostering reliable and predictable advancements in crop growth, providing a data-driven basis for precision and sustainable agricultural practices.



CHAPTER-6: SUMMARY

SUMMARY

Objective 1: To characterize soil with respect to physical, chemical, microbiological, and metagenomic data of designated plots involving *Cucurbita sp.* and *Zea mays* over a two-year period

IMPORTANT FINDINGS FROM OBJECTIVE 1:

- ❖ Soil samples were collected from a selected plot at Chowbaga in Dhapa, South 24 Parganas, a part of East Calcutta Wetlands with coordinates of 22.5296°N, 88.4207°E.
- ❖ The sample assemblage was conducted during the time interval between December, 2020-February, 2021, and December 2021-February 2022 whereby *Cucurbita maxima* was grown as main crop in the field. Soil samples were also collected from March, 2021-June, 2021 and March, 2022-June, 2022 whereby *Zea mays* was grown as the main crop.
- ❖ Analysis of the major soil physico- chemical parameters was done for parameters like pH, Electrical conductivity, nitrogen, potassium, phosphorous, calcium, magnesium. Analysis of micronutrients like copper, boron, zinc etc were also done.
- ❖ The minor decrease in pH of soil, during the peak growth period of *Cucurbita maxima* and *Zea mays* may be due to the liberation of H⁺ ions from plant roots, thereby leading to the changes in soil pH.
- ❖ Increase in diversity of micro-organisms in soil and biomass is also associated with the increased levels of organic carbon. Reduction of organic carbon is due to harvesting of the respective crops.
- ❖ Addition of fertilizers rich in nitrogen during the peak period of plant growth resulted in enhanced levels of nitrogen in the soil, like Nitrabo, 10/26, humi gold etc
- ❖ The decrease in calcium levels during peak cropping period could be due to utilization by the plants and the increase in intercropping period may be due to addition of phosphate fertilizers

like Phosphid or fertilizers containing limestone and gypsum that has probably resulted in the increment of calcium level.

- ❖ Metagenomic analysis of the soil samples, showed the predominance of certain bacteria, like *Bacillus* sp, *Nitrospira* sp, *Reyranella* sp, *Candidatus* sp .
- ❖ An increase in relative abundance of *Bacillus* sp was observed
- ❖ The soil had very high quantity of heavy metal like lead at a value of 398.76mg/kg of soil, which might be due to leaching from the dumping sites of Dhapa, or due to its location close to the tanneries in Bantala.

Objective 2: To perform isolation, purification, and characterization of potential microbes having dynamic roles in soil development and mitigation of pollution (lead)

IMPORTANT FINDINGS FROM OBJECTIVE 2:

- A total of 12 bacterial isolates were initially obtained from the rhizospheric soil samples, out of which 5 isolates were found to be consistently present (lingering flora) across the Initial, Peak, and Intercrop seasons in both *Cucurbita maxima* and *Zea mays* during the entire two-year period of study.
- These five key isolates were selected for further characterization and were designated as: DPP(A), DPP(B), DPP(C), DCP(A) and DCI(D)
- ❖ All the bacterial isolates, showed antibiotic sensitivity.
- ❖ Molecular identification of the five selected bacterial isolates was performed, and the sequences were successfully submitted to the NCBI database.

The identified strains and their corresponding NCBI accession numbers are as follows:

- f) DPP(A) was identified as *Bacillus cereus* strain DPP(A)_SGAM_SXC, Accession number: OQ216888
 - g) DPP(B) was identified as *Bacillus velezensis* strain DPPB_SGAM_SXC, Accession number: ON384543
 - h) DPP(C) was identified as *Stenotrophomonas maltophilia* strain DPP(C)_SGAM_SXC, Accession number: OQ217020
 - i) DCP(A) was identified as *Cupriavidus necator* strain DCPA_SGAM_SXC, Accession number: ON384541
 - j) DCI(D) was identified as *Bacillus cereus* strain DCI(D)_SGAM_SXC, Accession number: OQ217005
- ❖ The bacterial isolates were capable of nitrogen fixation, potassium solubilization, phosphate solubilization, thereby showcasing PGPR role.
 - ❖ The ability of the bacterial isolates to solubilize phosphorous was quantified, whereby the bacterial isolates, namely *B.cereus*(DPP-A) followed by *B.velezensis* (DPP-B), *S.maltophilia*

(DPP-C), *C.necator* (DCP-A) and *B.cereus* (DCI-D) were able to solubilize 5.79mg/ml, 7.91 mg/ml, 5.41 mg/ml, 2.78 mg/ml and 1.98 mg/ml of phosphate respectively

- ❖ They were found capable of siderophore production. Quantification study revealed the percentage of siderophore production, that was recorded after 24 and 48 h, whereby the *B.cereus* DPP(A), *B.velezensis* (DPP-B), *S.maltophilia* (DPP-C), *C.necator* (DCP-A) and *B.cereus* DCI(D) showed 3.1%, 4.62%, 37.89%, 27.36%, and 14.73%, respectively,
- ❖ All the bacterial isolates were found to be capable of synthesis of phytohormones- namely: IAA and GA. All the bacterial isolates namely *B. cereus* (DPP(A)), followed by *B.velezensis*, *S.maltophilia* *C.necator* and *B.cereus* (DCI(D)) were able to produce 50.875 µg/mL, 51.012 µg/mL, 52.665 µg/mL, 56.785 µg/mL and 50.187µg/mL of IAA.
- ❖ Bacterial isolates *B. cereus* (DPP-A), followed by *B.velezensis* (DPP-B), *S.maltophilia*(DPP-C) , *C.necator* (DCP-A) and *B.cereus*(DCI-D) were able to produce 6.696, µg/mL 4.38 µg/mL, 10.584 µg/mL, 0.864 µg/mL and 4.752µg/mL of gibberellin.
- ❖ Bacterial isolates were found to be proficient in the synthesis of enzymes like cellulase, laccase, pectinase, lignin peroxidase, amylase, urease and ACC deaminase.
- ❖ Metallothionein content of the bacterial isolates were tested, whereby the bacterial isolates *B.cereus* DPP(A), *B.velezensis* (DPP-B), *S.maltophilia* (DPP-C), *C.necator* (DCP-A) and *B.cereus* DCI(D) showed the ability to produce higher metallothionein in 500ppm of lead amount as compared to 200ppm of lead
- The lead adsorption capacity and uptake of the individual bacterial isolates were studied. The percentage of lead uptake in bacterial isolates was calculated and it was found that *S.maltophilia* had the highest capacity for lead uptake at 69.2%, followed by *B.velezensis* and *B.cereus* DCI(D) at 48.84% and 45.85%, respectively. The bacterial isolates were further tested for their metal uptake capacity. It was found to have 0.848 µg/g, 0.976 µg/g, 0.173 µg/g, 0.383 µg/g, 0.917 µg/g for *B.cereus* DPP(A), *B.velezensis*, *S.maltophilia*, *C.necator* and *B.cereus* DCI(D), respectively.
- ❖ The bacterial isolates namely *B.cereus* DPP(A), *B.velezensis*, *S.maltophilia*, *C.necator* and *B.cereus* DCI(D) were found to have the following percentage of lead adsorbed, that is, 5.73%, 2.30%, 7.6%, 6.22%, 8.28%, respectively.

Objective 3: To prepare an ideal consortium to be supplemented all through the year, suitable for any crop, either singly or with chemical fertilizers (in-vivo study)

IMPORTANT FINDINGS FROM OBJECTIVE 3:

- ❖ Interaction study of the bacterial isolates was done using cross – streak analysis.
- ❖ Seeds of *Cucurbita maxima* (Variety: Jack 323) and *Zea mays* (Variety: 4226) were used for the field trial study in the Chowbaga area of East Kolkata wetlands with 20 plants in the plot. The control set-up plot was present adjacent to the treated set-up plot.
- ❖ Among the five bacterial isolates, *B.velezensis* (DPP-B) and *C.necator* (DCP-A) demonstrated the most potent plant growth-promoting, phytohormonal, and enzymatic properties. As per the studied literature *Stenotrophomonas maltophilia* is a nosocomial pathogen, in spite of its good plant growth promoting properties. Therefore DPP-C was not included in the consortia. Similarly, there were studies that suggested that *B.cereus* was pathogenic to humans. Moreover DPP-A (*B.cereus*) was haemolytic in nature.
- ❖ Both DPP-B (*Bacillus velezensis*) and DCP-A (*Cupriavidus necator*) had better plant growth promoting properties in most of the cases as compared to the other bacterial isolates. Thus, they were used in bacterial consortia.
- ❖ These two strains were selected to create a bacterial consortium, which was applied to one-month-old *C.maxima* and *Z.mays* plants in Chowbaga plots of the East Kolkata Wetlands. No bacterial consortia were applied to the field designated as control set up
- ❖ Based on the pilot results, the water suspension method was chosen for the field trial due to its scientific effectiveness and practical advantages.
- ❖ All the plant physiological parameters; namely plant length, number of leaves, length and width of leaves, no of tendrils, followed by reproductive parameters of number of flowers and fruits were measured.
- ❖ In both the plant types of *Cucurbita maxima* and *Zea mays*, significantly enhanced Chlorophyll a, Chlorophyll b and carotenoid contents were found in treated set-up.
- ❖ The peak period showed a more pronounced difference in pigment content, reflecting enhanced photosynthetic capacity and possibly improved plant health growth.

- ❖ It was observed, that the mean values of the physiological and reproductive parameters of the treated plants were higher than the control set-up.
- ❖ A two-way ANOVA system was used to find out the efficacy of the applied consortium. It was found that treated plants showed better efficacy than control plants, since the level of significance was found to be less than 0.05 in most of the physiological parameters of the treated set-up.
- ❖ The lead concentration in the control soil was measured at 106.1 ± 0.02 mg/kg, while the treated soil showed a significantly reduced level of 12.28 ± 0.01 mg/kg.
- ❖ This indicates an approximate reduction of 88.4% in lead levels, highlighting the effectiveness of the bacterial consortia in reducing heavy metal contamination and showcasing their potential for soil bioremediation.
- ❖ Quantification of lead content in plant parts of both treated and control set-ups were done, and it was observed that treated plants had much less lead content than control plants.
- ❖ In *Cucurbita* sp., lead accumulation was significantly reduced in treated plants. The flower showed a 91.9% decrease, while the fruit had the highest reduction at 92%. The leaf exhibited a moderate 14.3% decline.
- ❖ In case of *Zea mays*, Lead accumulation (mg/plant) showed the most significant reduction in the root, with a 25.3% decrease in treated plants compared to control. In the stem, lead accumulation reduced by 9.21%, suggesting a moderate effect of the treatment on translocation to aerial parts. The leaf showed a 3.79% reduction, indicating limited improvement in foliar lead load. The cob grain, which represents the edible part, exhibited a 10.7% reduction in lead accumulation, highlighting an important improvement in food safety.
- ❖ It was observed, that the plant defence enzymes in treated plants were higher, than the control set ups. An elevation in enzymes like ascorbate peroxidase (APX) and other peroxidases in plant tissues following the introduction of bacterial consortia suggests the reaction of plant to oxidative stress, which may be triggered by the bacteria or a related environmental factor. These enzymes mitigate excess hydrogen peroxide (H_2O_2) by converting it into water, thereby safeguarding the plant from cellular harm and maintains the redox balance. This increase in antioxidant defences can improve the plant's resilience to different stresses and foster overall growth and biomass development.

Objective 4: To perform statistical analysis, including time series study of physico-chemical and microbiological properties vis-à-vis crop productivity

IMPORTANT FINDINGS FROM OBJECTIVE 4:

- ❖ On the basis of the time series analysis of the significant soil physiological parameters, over a two-year period, variations in their quantity were observed, which may be due to uptake of the macro and microelements by the plants or addition of fertilizers to the soil.
- ❖ Time series analysis of the plant parameters showed that upon application of the consortia, the treated set up showed better results and goodness of fit as compared to the control set-up.
- ❖ In the model we are assuming that the plant factor is a function of time (let's call this the explanatory variable), but in reality, there may be other factors that affect the factor. these are commonly referred to as noise. the variation in data due to these factors is called unexplained variation.
- ❖ r^2 basically tells us how good the chosen model is, based on only time as the explanatory variable.
- ❖ If $R^2 = 98.02\%$ for the control group, it means that the fitted equation is responsible for 98.02% of the total variation in the data. The remaining 1.98% is due to other factors not captured by the equation (noise).
- ❖ If $R^2 = 99.53\%$ for the treated group, the equation does an even better job, explaining 99.53% of the changes in the data. Only 0.47% is left unexplained.

Therefore, higher R^2 is to 100%, the better the fit.

CONCLUSION AND FUTURE PROSPECTS

The thesis comprehensively addressed the interplay of soil physico-chemical characteristics, microbial communities, and crop productivity within the reclaimed agricultural soils of the East Kolkata Wetlands. Objective-wise investigations revealed that these soils, though heavily impacted by waste inputs and heavy metal contamination, harbour a diverse and resilient microbial population with immense functional potential. Metagenomic insights highlighted the dominance of Proteobacteria, Actinobacteria, and other functional groups capable of bioremediation and nutrient cycling. Isolation and characterization of PGPR strains confirmed their dual role in promoting plant growth and mitigating lead pollution. The development and field application of microbial consortia demonstrated remarkable success in improving soil fertility, reducing heavy metal bioavailability, and enhancing yields of *Cucurbita* and *Zea mays*. Statistical and time series analyses provided robust evidence of the temporal dynamics governing soil health and validated the positive impact of microbial consortia on productivity. Collectively, the findings underline the potential of microbial-based strategies in sustainable agriculture, especially in pollution-affected reclaimed lands. The study thus bridges ecological restoration with food security, establishing microbial consortia as a viable tool for environmental remediation and enhanced agricultural output in the East Kolkata Wetlands and similar ecosystems worldwide.

As per the future scope of the work, the prepared microbial consortia can be optimized and used for testing on other cereals, pulses and horticultural crops. Organic amendments can be added to the prepared microbial consortia to study their effective role. Multi-omics approach can also be applied to study the pathways used by bacteria for heavy metal detoxification and nutrient utilization.

Therefore, the work could create a bridge for agriculture with microbial ecology and aim for a better approach towards cleaner environment and food secure future.



REFERENCES

REFERENCES

1. (chromeextension://efaidnbmnnnibpcajpcglclefindmkaj/https://www.ramsar.org/sites/default/files/documents/library/sc62_22_status Ramsar_list_e.pdf)
2. Abbas, A., Naveed, M., Azeem, M., Yaseen, M., Ullah, R., Alamri, S., ... & Siddiqui, M. H. (2020). Efficiency of wheat straw biochar in combination with compost and biogas slurry for enhancing nutritional status and productivity of soil and plant. *Plants*, 9(11), 1516.
3. Abbott IJ, Peleg AY. *Stenotrophomonas*, *Achromobacter*, and nonmelioid *Burkholderia* species: antimicrobial resistance and therapeutic strategies. *Semin Respir Crit Care Med*. 2015 Feb;36(1):99-110
4. Abbott IJ, Slavin MA, Turnidge JD, Thursky KA, Worth LJ. *Stenotrophomonas maltophilia*: emerging disease patterns and challenges for treatment. *Expert Rev Anti Infect Ther*. 2011 Apr;9(4):471-88
5. Adams M, Mortenson L, Chen J (1981) Hydrogenase. *Biochim Biophys Acta* 594:105–176
6. Addis W, Abebaw A (2015) Analysis of selected physicochemical parameters of soils used for cultivation of garlic (*Allium sativum* L). *Sci Technol Arts Res J* 3(4):29–35
7. Agarwal, S., Mukherjee, P., Pramanick, P., & Mitra, A. (2022). Seasonal variations in bioaccumulation and translocation of toxic heavy metals in the dominant vegetables of East Kolkata Wetlands: A case study with suggestive ecorestorative strategies. *Environmental Monitoring and Assessment*.
8. Agarwal, S., Mukherjee, P., Pramanick, P., & Mitra, A. (2023). Seasonal variations in bioaccumulation and translocation of toxic heavy metals in the dominant vegetables of East Kolkata Wetlands: A case study with suggestive ecorestorative strategies. *Applied Biochemistry and Biotechnology*, 195(4), 2332–2358.
9. Ahmed, N., Zhang, B., Bozdar, B., Chachar, S., Rai, M., Li, J., Li, Y., Hayat, F., Chachar, Z., & Tu, P. (2023). The power of magnesium: unlocking the potential for increased yield, quality, and stress tolerance of horticultural crops. *Frontiers in Plant Science*, 14, Article 1285512.

10. Alengebawy, A., Abdelkhalek, S. T., Qureshi, S. R., & Wang, M. Q. (2021). Heavy metals and pesticides toxicity in agricultural soil and plants: Ecological risks and human health implications. *Toxics*, 9(3), 42. <https://doi.org/10.3390/toxics9030042>
11. Alexander, A., Singh, V. K., Mishra, A., & Jha, B. (2019). Plant growth promoting rhizobacterium *Stenotrophomonas maltophilia* BJ01 augments endurance against N₂ starvation by modulating physiology and biochemical activities of *Arachis hypogea*. *PloS one*, 14(9), e0222405.
12. Ali, Sk A., & Ahmad, A. (2019). Analysis of chemical and heavy metal concentrations of leachates and impact on groundwater quality near Dhapa dumping ground, Kolkata. *Asian Profile*, 47, 19–37.
13. Alippi, A. M., & Abrahamovich, E. (2019). HiCrome Bacillus agar for presumptive identification of *Bacillus* and related species isolated from honey samples. *International Journal of Food Microbiology*, 305, Article 108245.
14. Almeida O.G.G., De Martinis E.C.P. Bioinformatics tools to assess metagenomic data for applied microbiology. *Appl. Microbiol. Biotechnol.* 2019;103:69–82. doi: 10.1007/s00253-018-9464-9
15. Altaf, M.M., Ahmad I., “Plant Growth Promoting activities, biofilm formation and root colonization by *Bacillus* spp. Isolated from rhizospheric soils,” *Journal of Pure and Applied Microbiology*, 10. 109-20. 2016.
16. Amin G.A., Badr E.A. & Afifi M.H.M., 2013. Root yield and quality of sugar beet (*Beta vulgaris* L.) in response to biofertilizer and foliar application with micronutrients. *World Appl. Sci. J.*, 27(11), 1385-1389, doi.org/10.5829/idosi.wasj.2013.27.11.13732
17. Amin, H., Arain, B. A., Jahangir, T. M., Abbasi, M. S., & Amin, F. (2018). Accumulation and distribution of lead (Pb) in plant tissues of guar (*Cyamopsis tetragonoloba* L.) and sesame (*Sesamum indicum* L.): profitable phytoremediation with biofuel crops. *Geology, Ecology, and Landscapes*, 2(1), 51–60.
18. Andrews S, Krueger F, Segonds-Pichon A, Biggins L, Krueger C, Wingett S (2010) FastQC. A quality control tool for high throughput sequence data, 370

19. Anjum, S., Sarwar, M., Mukhtar, A., Hussain, T., Liaquat, M., Batool, A., Alam, M. W., Ali, Q., & Ashraf, M. I. (2025). Impact of rhizobacterial inoculation in plant growth medium to mitigate lead stress in Tomato (*Solanum lycopersicum* L.). *Scientific reports*, 15(1), 707.
20. Apel K, Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Ann Rev Plant Biol* 55:373–399.
21. Arora, M., Kiran, B., Rani, S., Rani, A., Kaur, B., & Mittal, N. (2008). Heavy metal accumulation in vegetables irrigated with water from different sources. *Food Chemistry*, 111(4), 811–815.
22. Arumugam, A., & Gomathinayagam, M. (2015). Comparative studies on physico-chemical variations of two major mangrove wetlands in East Coast of Tamil Nadu, India. *Current Life Sciences*, 1(4), 134–139.
23. Arunprasath A, Gomathinayagam M (2015) Comparative studies on physico-chemical variations of two major mangrove wetlands in East coast of Tamilnadu. *Current Life Sciences, India*, p 124
24. Arya, A. K., Joshi, K. K., Joshi, A. B., & Vashishth, D. (2020). A review on distribution and importance of wetlands in the perspective of India. *Journal of Applied and Natural Science*, 12(4), 710–720. <https://doi.org/10.31018/jans.v12i4.2412>
25. Ashfaq, M.; Khan, I.; Alzahrani, A.; Tariq, M.U.; Khan, H.; Ghani, A. Accurate Wheat Yield Prediction Using Machine Learning and Climate-NDVI Data Fusion. *IEEE Access* 2024, 12, 40947–40961.
26. Aslam, M., Aslam, A., Sheraz, M., Ali, B., Ulhassan, Z., Najeeb, U., ... & Gill, R. A. (2021). Lead toxicity in cereals: Mechanistic insight into toxicity, mode of action, and management. *Frontiers in plant science*, 11, 587785
27. Assen, M., le Roux, A. L., Barker, C. H., and Heluf, G. (2005). Soils of jelo micro-catchment in the chercher highlands of eastern ethiopia: I. Morphological and physiochemical properties. *Ethopian J. Nat. Res.* 7, 55–81.
28. Ábrahám, E., Hourton-Cabassa, C., Erdei, L., & Szabados, L. (2010). Methods for determination of proline in plants. In R. Sunkar (Ed.), *Plant Stress Tolerance (Methods in Molecular Biology, Vol. 639, pp. 317–331)*. New York, NY: Humana Press.

29. Álvarez-Maldini C, Acevedo M, Estay D, Aros F, Dumroese RK, Sandoval S and Pinto M (2022) Examining physiological, water relations, and hydraulic vulnerability traits to determine anisohydric and isohydric behavior in almond (*Prunus dulcis*) cultivars: Implications for selecting agronomic cultivars under changing climate. *Front. Plant Sci.* 13:974050. doi: 10.3389/fpls.2022.974050
30. Babalola, Olubukola & Olanrewaju, Oluwaseyi & Dias, Teresa & Ajilogba, Caroline & Kutu, Funso & Cruz, Cristina. (2017). Biological Nitrogen Fixation: The Role of Underutilized Leguminous Plants. 10.1007/978-981-10-6241-4_20.
31. Bahram, M., Espenberg, M., Pärn, J., Lehtovirta-Morley, L., Anslan, S., Kasak, K., Kõljalg, U., Liira, J., Maddison, M., Moora, M., Niinemets, Ü., Öpik, M., Pärtel, M., Soosaar, K., Zobel, M., Hildebrand, F., Tedersoo, L., & Mander, Ü. (2022). Structure and function of the soil microbiome underlying N₂O emissions from global wetlands. *Nature communications*, 13(1), 1430.
32. Bahram, M., Hildebrand, F., Forslund, S. K., Anderson, J. L., Soudzilovskaia, N. A., Bodegom, P. M., et al. (2018). Structure and function of the global topsoil microbiome. *Nature* 560, 233–237. doi: 10.1038/s41586-018-0386-6
33. Bai, J., Xiao, R., Cui, B., Zhang, K., Wang, Q., Liu, X., Gao, H., & Huang, L. (2011). Assessment of heavy metal pollution in wetland soils from the young and old reclaimed regions in the Pearl River Estuary, South China. *Environmental Pollution*, 159(2), 817–824.
34. Bakshi, M., et al. (2020). Plant growth-promoting rhizobacteria: A sustainable approach to enhance soil fertility and crop productivity. *Environmental Sustainability*, 3(4), 347–358.
35. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. *J Pharm Anal.* 2016 Apr;6(2):71-79. doi: 10.1016/j.jpha.2015.11.005. Epub 2015 Dec 2. PMID: 29403965; PMCID: PMC5762448.
36. BalvantinGarcia, C., M., K., Nava-Reyna, E., I., A., L., J., Rodriguez-Martinez, J., & Ilyi, A. (2011). Biocatalysts in Control of Phytopatogenic Fungi and Methods for Antifungal Effect Detection. *InTech*. doi: 10.5772/22675
37. Banerjee, S. 2012. Overseeing the Disappearance of an Ecosystem: Governance in West Bengal and Wetlands to the East of Kolkata In Bandyopadhyay, J., Chopra, K., Ghosh, N.

- (eds.) Environmental Governance: Approaches, Imperatives and Methods, INSEE, Bloomsbury, New Delhi. pp 190-222.
38. Banerjee, S., & Dey, D. (2017). Eco-system complementarities and urban encroachment: A SWOT analysis of the East Kolkata Wetlands, India. *Cities and the Environment (CATE)*, 10(1), Article 2.
 39. Bank, W. (2012). Turn Down The heat: why a 4 C warmer world must be avoided. A Report for the World Bank by the Potsdam Institute for Climate Impact Research and Climate Analytics. Washington DC, USA.
 40. Baolan, H., Shuai, L., Wei, W., Lidong, S., Liping, L., Weiping, L., et al. (2014). pH dominated niche segregation of ammonia-oxidising microorganisms in Chinese agricultural soils. *FEMS Microbiol. Ecol.* 90, 290–299. doi: 10.1111/1574-6941.12391
 41. Barde, M. P., & Barde, P. J. (2012). What to use to express the variability of data: Standard deviation or standard error of mean? *Perspectives in Clinical Research*, 3(3), 113–116.
 42. Barman, S., et al. (2024). Metagenomics of wetland soils: Implications for fertility and pollution mitigation. *Environmental Microbiology Reports*, 16(2), 234–245.
 43. Basak RK (2000) Soil testing and recommendation. Kalyani Publishers, Ludhiana, New Delhi
 44. Bassi, N., Kumar, M. D., Sharma, A., & Pardha-Saradhi, P. (2014). Status of wetlands in India: A review of extent, ecosystem benefits, threats and management strategies. *Journal of Hydrology: Regional Studies*, 2, 1–19.
 45. Benson, T. (2001) Microbiological Applications Laboratory Manual in General Microbiology. 8th Edition, The McGraw-Hill, New York.
 46. Bhattacharjee S (2012) An inductive pulse of hydrogen peroxide pretreatment restores redoxhomeostasis and oxidative membrane damage under extremes of temperature in two rice cultivars. *Plant Growth Regul* 68(3):395–410.
 47. Bhattacharyya A, Sen S, Roy PK, Mazumdar A (2008) A critical study on status of East Kolkata Wetlands with special emphasis on water birds as bio-indicator. In *Proceedings of Taal2007: The 12th World Lake Conference* (Vol 1561, p 1570).

48. Bhattacharyya PN, Jha DK (2012) Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *W J Microbiol Biotechnol* 28:1327–1350
49. Bhattacharyya, C., Banerjee, S., Acharya, U., Mitra, A., Mallick, I., Haldar, A., Haldar, S., Ghosh, A., & Ghosh, A. (2020). Evaluation of plant growth promotion properties and induction of antioxidative defense mechanism by tea rhizobacteria of Darjeeling, India. *Scientific Reports*, 10, 15536. <https://doi.org/10.1038/s41598-020-72439-z>
50. Bhattacharyya, P. N., & Jha, D. K. (2012). Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture. *World Journal of Microbiology & Biotechnology*, 28(4), 1327–1350.
51. Bibi, N., Ali, S., & Tabassum, R. (2016). Statistical optimization of pectinase biosynthesis from orange peel by *Bacillus licheniformis* using submerged fermentation. *Waste and Biomass Valorization*, 7(2), 467–479.
52. Bogale, E., Kebede, A., Nigussie, A., Bekele, G., & Tafesse, M. (2021). Isolation and characterization of urease-producing soil bacteria. *International Journal of Microbiology*, 2021, 1–11.
53. Borthakur, S. and Bhattacharya, B. K. (1998). Trend analysis of area, production and productivity of potato in Assam: 1951-1993. *Economic Affairs* 43: 221-226.
54. Bottone E. J. (2010). *Bacillus cereus*, a volatile human pathogen. *Clinical microbiology reviews*, 23(2), 382–398. <https://doi.org/10.1128/CMR.00073-09>
55. Box GE, Jenkins GM, Reinsel GC, Ljung GM. *Time Series Analysis: Forecasting and Control*. 5th ed. New Jersey: Wiley; 2015
56. Boyd, J., & Banzhaf, S. (2007). What are ecosystem services? The need for standardized environmental accounting units. *Ecological Economics*, 63(2–3), 616–626.
57. Bystrom, O., Andersson, H., & Gren, I.-M. (2000). Economic criteria for using wetlands as nitrogen sinks under uncertainty. *Ecological Economics*, 35(1), 35–45.
58. Caiado, J., Crato, N. and Peña, D. (2006). "A periodogram-based metric for time series classification", *Computational Statistics & Data Analysis*, 50, 2668-2684.
59. Cao, Y., Fu, D., Liu, T., Guo, G., & Hu, Z. (2018). Phosphorus Solubilizing and Releasing Bacteria Screening from the Rhizosphere in a Natural Wetland. *Water*, 10(2), 195.

60. Cassán F. & Diaz-Zorita M., 2016. Azospirillum sp. in current agriculture: from the laboratory to the field. *Soil Biol. Biochem.*, 103, 117-130, doi.org/10.1016/j.soilbio.2016.08.020
61. Cassán F. et al., 2020. Everything you must know about Azospirillum and its impact on agriculture and beyond. *Biol. Fertil. Soils*, 56, 461-479, doi.org/10.1007/s00374-020-01463-y
62. Central Pollution Control Board (CPCB) Status of Water Supply, Wastewater Generation and Treatment in Class-I Cities and Class-II Towns of India .Central Pollution Control Board, Ministry of Environment and Forests, Government of India, New Delhi (2009)
63. Central Water Commission (CWC), Financial Aspects of Irrigation Projects in India, Central Water Commission, New Delhi (2010)
64. Chakraborty, U., Chakraborty, B. N., Chakraborty, A. P., Sunar, K., & Dey, P. (2013). Plant growth promoting rhizobacteria mediated improvement of health status of tea plants. *Indian Journal of Biotechnology*, 12(1), 20–31.
65. Chandan, P., Chatterjee, A., & Gautam, P. (2007). Management planning of Himalayan high altitude wetlands: a case study of Tsomoriri and Tsokar wetlands in Ladakh, India. *Proceedings of Taal*, 1446-1452.
66. Chatfield C, Yar M. Holt-winters forecasting: Some practical issues. *Journal of the Royal Statistical Society Series D: The Statistician*. 1988;37(2):129-140.
67. Chatfield C. *The Analysis of Time Series: Theory and Practice*. London: Chapman and Hall; 2013
68. Chatfield, C. (2003). *The Analysis of Time Series: An Introduction* (6th ed.). Chapman & Hall / CRC.
69. Chatfield, C. (2004). *The Analysis of Time Series.- An Introduction*. Chapman and Hall, London, UK. Sixth Edition.
70. Chatterjee, S., Singh, L., Chattopadhyay, B., Datta, S., & Mukhopadhyay, S. K. (2011). A study on the waste metal remediation using floriculture at East Calcutta Wetlands, a Ramsar site in India. *Environmental Monitoring and Assessment*, 184(8), 5139–5150.

71. Chattopadhyay, Buddhadeb & Chatterjee, Asitava & Mukhopadhyay, Subhra. (2002). Bioaccumulation of metals in the East Calcutta Wetland ecosystem. *Aquatic Ecosystem Health & Management*. 5. 191-203. 10.1080/14634980290031848.
72. Chaudhuri, S., Mukherjee, I., Ghosh, D., & Thakur, A. (2012). East Kolkata Wetland: A multifunctional niche of international importance. *OnLine Journal of Biological Sciences*, 12(2), 80–88. <https://doi.org/10.3844/ojbsci.2012.80.88>
73. Chaudhuri, Shaon & Mukherjee, Indranil & Ghosh, Drishti & Thakur, Ashoke. (2012). East Kolkata Wetland: A Multifunctional Niche of International Importance. *OnLine Journal of Biological Sciences*. 12. 80-88. 10.3844/ojbsci.2012.80.88.
74. Chauhan NS, Ranjan R, Purohit HJ, Kalia VC et al (2009) Identification of genes conferring arsenic resistance to *Escherichia coli* from an effluent treatment plant sludge metagenomic library. *FEMS Microbiol Ecol* 67:113–130
75. Chauhan P, Chaudhry V, Mishra S et al (2011) Uncultured bacterial diversity in tropical maize (*Zea mays* L.) rhizosphere. *J Basic Microbiol* 51:15–32
76. Chauhan, P. S., Goradia, B., & Saxena, A. (2017). Bacterial laccase: recent update on production, properties and industrial applications. *3 Biotech*, 7(5), 323.
77. Chawngthu, L., Hnamte, R., & Lalfakzuala, R. (2020). Isolation and Characterization of Rhizospheric Phosphate Solubilizing Bacteria from Wetland Paddy Field of Mizoram, India. *Geomicrobiology Journal*, 37(4), 366–375.
78. Chiang A.D., Dekker J.P. From the Pipeline to the Bedside: Advances and Challenges in Clinical Metagenomics. *J. Infect. Dis.* 2020;221:S331–S340. doi: 10.1093/infdis/jiz151.
79. Choudhary O P, Trivedi A, Bunker R N and Mathur K. 2011. Factors affecting development of *Curvularia* leaf spot of maize (*Curvularia pallescens*) and its management. *Indian Phytopathology* 64(4): 371–73
80. Chudobova, D, S. Dostalova, B. Ruttkay-Nedecky, R. Guran and M. A. M. Rodrigo, *Microbiol. Res.*, 2015, 170, 147–156.
81. Colette, A. (2013). Case studies on climate change and World Heritage. UNESCO Publishing.

82. Collin, S., Baskar, A., Geevarghese, D. M., Ali, M. N. V. S., Bahubali, P., Choudhary, R., & Swamiappan, S. (2022). Bioaccumulation of lead (Pb) and its effects in plants: A review. *Journal of Hazardous Materials Letters*, 3, 100064
83. Costanza, R., d'Arge, R., de Groot, R., Farber, S., Grasso, M., Hannon, B., ... & van den Belt, M. (1997). The value of the world's ecosystem services and natural capital. *Nature*, 387(6630), 253–260.
84. Cowardin LM, Carter V, Golet FC, LaRoe ET. 1979. Classification of wetlands and deepwater habitats of the United States. U.S. Fish & Wildlife Service Pub. FWS/OBS-79/31, Washington, DC.
85. Cuypers A, Hendrix S, dos Reis RA, De Smet S, Deckers J, Gielen H, Jozefczak M, Loix C, Vercampt H, Vangronsveld J, Keunen E (2016) Hydrogen peroxide, signaling in disguise during metal phytotoxicity. *Front Plant Sci* 7(APR2016).
86. Dai F C, Xiao M W, Zhen D Z, Wei D G, Naxin H, Xiao H J, Wang X M, Zhu Z D, Gao W D, Huo N X and Jin X H. 1998. *Acta Phytopathologica Sinica* 28: 123–29
87. Dai, J., Liu, X., & Zhang, Y. (2024). Soil salinity in tidal flat wetlands. *Estuarine, Coastal and Shelf Science*, 258, 107475.
88. Dandwate SC (2020) Analysis of soil samples for its physicochemical parameters from Sangamner city. *GSC Biol Pharma Sci* 12(2):123–128
89. Daniel R (2004) The soil metagenome – a rich resource for the discovery of novel natural products. *Curr Opin Microbiol* 15:199–204
90. Daryadel, E., & Talaei, F. (2015). Analytical study on threats to wetland ecosystems and their solutions in the framework of the Ramsar Convention. *Procedia – Social and Behavioral Sciences*, 109, 109–114.
91. Das, M., Das, A., Pereira, P., & Mandal, A. (2021). Mapping the Impact of COVID-19 Lockdown on Urban Surface Ecological Status (USES): A Case Study of Kolkata Metropolitan Area (KMA), India. *Remote Sensing*, 13(21), 4395.
92. Das, M.; Das, A.; Mandal, A. Research note: Ecosystem Health (EH) assessment of a rapidly urbanising metropolitan city region of eastern India—A study on Kolkata Metropolitan Area. *Landsc. Urban Plan.* 2020, 204, 103938.

93. Das, P., & Barman, S. (2025). Perspective chapter: An overview of time series decomposition and its applications. In *Applied and Theoretical Econometrics and Financial Crises*. IntechOpen.
94. Das, S. (2025). The transformation and associated ecological problems of the East Kolkata Wetlands, West Bengal, India: Observations between 1989–2024. *IOP Conference Series: Earth and Environmental Science*, 1443, 012013.
95. Das, S., Borah, N., Sangha, M. K., Das, A., & Saikia, S. P. (2018). Activities of antioxidant enzymes and phytochemistry of stress tolerance in grains of oats and barley. *International Journal of Tropical Agriculture*, 36(2), 393–404. ISSN 0254-8755.
96. de Haas, T., & Schepers, M. (2022). Wetland Reclamation and the Development of Reclamation Landscapes: A Comparative Framework. *Journal of Wetland Archaeology*, 22(1–2), 75–96.
97. Dedysh SN (2019) *Bryobacter Bergey's Manual of Systematics of Archaea and Bacteria*, 1–5. <https://doi.org/10.1002/9781118960608.gbm01667>
98. Demissie, M., Legesse, N., & Tesema, A. (2024). Isolation and characterization of cellulase producing bacteria from forest, cow dung, Dashen brewery and agro-industrial waste. *PLOS ONE*, 19(4), e0301607.
99. Dey, A. K. (1975). Rates of growth of agriculture and industry. *Econ. Political Weekly* 10 : A26-A27,A29-A30.
100. Dhar J, Hazra A, Patra R, Kumar V, Subramaniyan V, Kumarasamy V, Mitra AK, Sayed AA, Aleya L, El-Demerdash FM, Almutairi MH, Akash S, Abdel-Daim MM, Kant A, Dhara B. Unveiling *Curvularia tuberculata*-induced leaf anomalies in *Rhododendron ferrugineum*: implications in cultural-ecological conservation and harnessing microbial intervention in socio-economic advancement. *Front Microbiol.* 2024 Jan 11;14:1280120. doi: 10.3389/fmicb.2023.1280120. PMID: 38274748; PMCID: PMC10808759.
101. Di Leo G, Sardanelli F. Statistical significance: p value, 0.05 threshold, and applications to radiomics-reasons for a conservative approach. *Eur Radiol Exp.* 2020 Mar 11;4(1):18. doi: 10.1186/s41747-020-0145-y. PMID: 32157489; PMCID: PMC7064671.

102. Di Leo G, Sardanelli F. Statistical significance: p value, 0.05 threshold, and applications to radiomics-reasons for a conservative approach. *Eur Radiol Exp*. 2020 Mar 11;4(1):18. doi: 10.1186/s41747-020-0145-y. PMID: 32157489; PMCID: PMC7064671.
103. Ding, L. J., Su, J. Q., Li, H., Zhu, Y. G., and Cao, Z. H. (2017). Bacterial succession along a long-term chronosequence of paddy soil in the Yangtze River Delta. *Soil Biol. Biochem.* 104, 59–67. doi: 10.1016/j.soilbio.2016.10.013
104. Ding, W., Zhang, L., & Li, Y. (2023). Carbon and nitrogen characteristics and sediment organic matter in reed and Suaeda wetlands. *Science of The Total Environment*, 811, 152290.
105. Duca D, Lorv J, Patten C, Rose D, Glick B (2014) Microbial indole3-acetic acid and plant growth. *Anton Van Leeuwenhoek* 106:85–125
106. Dutta, S., Gupta, B., Srivastava, S. K., & Gupta, A. K. (2021). Recent advances on the removal of dyes from wastewater using various adsorbents: A critical review. *Materials Advances*, 2(14), 4497–4531. <https://doi.org/10.1039/D1MA00354B>
107. East Kolkata Wetlands: Management Action Plan 2021 – 2026. East Kolkata Wetlands Management Authority and Wetlands International South Asia.
108. Egamberdieva, D., Wirth, S. J., Alqarawi, A. A., Abd_Allah, E. F., & Hashem, A. (2017). Phytohormones and beneficial microbes: essential components for plants to balance stress and fitness. *Frontiers in microbiology*, 8, 2104.
109. Eivazi, F., & Tabatabai, M. A. (1988). Glucosidases and galactosidases in soils. *Soil Biology and Biochemistry*, 20(5), 601–606. [https://doi.org/10.1016/0038-0717\(88\)90141-1](https://doi.org/10.1016/0038-0717(88)90141-1)
110. Esling, P., & Agon, C. (2012). Time-series data mining. *ACM Computing Surveys*, 45(1), Article 12.
111. Etesami, H., Emami, S., & Alikhani, H. (2017). Potassium solubilizing bacteria (KSB): Mechanisms, promotion of plant growth, and future prospects – A review. *Journal of Soil Science and Plant Nutrition*, 17(4), 897–911. <https://doi.org/10.4067/S0718-95162017000400005>
112. Ewels P, Magnusson M, Lundin S, Käller M (2016) MultiQC: summarize analysis results for multiple tools and samples in a single report. *Bioinformatics* 32(19):3047–3048

113. Fasusi, O. A., Cruz, C., & Babalola, O. O. (2021). Agricultural Sustainability: Microbial Biofertilizers in Rhizosphere Management. *Agriculture*, 11(2), 163.
114. Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, 39(4), 783–791.
115. Figueroa-López, A.M., Cordero-Ramírez, J.D., Martínez-Álvarez, J.C. et al. Rhizospheric bacteria of maize with potential for biocontrol of *Fusarium verticillioides* . *SpringerPlus* 5, 330 (2016).
116. Firdous K, Chakraborty N, Swati S (2018). Correlation of different soil parameters and evaluation of its effect in a Ramsar site: East Kolkata Wetland.
117. Frankenberger, W. T., Tabatabai, M. A. (1981) Amidase Activity in Soils: III. Stability and Distribution. *Soil Science Society of America Journal*, 45 (2). 333-338 doi:10.2136/sssaj1981.03615995004500020021x
118. Frazier, S. 1996. An overview of the world's Ramsar sites. *Wetlands International Publ.*29. 58 p.
119. Freeman CN, Herman EK, Abi Younes J, Ramsay DE, Erikson N, Stothard P, Links MG, Otto SJ, Waldner C (2022) Evaluating the potential of third generation metagenomic sequencing for the detection of BRD pathogens and genetic determinants of antimicrobial resistance in chronically ill feedlot cattle. *BMC Vet Res* 18(1):1–15
120. Garg J K and Patel J G, (2007). National Wetland Inventory and Assessment, Technical Guidelines and Procedure Manual, Technical Report, SAC/EOAM/AFEG/NWIA/TR/01/2007, June 2007, Space Applications Centre, Ahmedabad.
121. Garg, D., Patel, N., Rawat, A., & Rosado, A. S. (2024). Cutting edge tools in the field of soil microbiology. *Current Research in Microbial Sciences*, 6, 100226.
122. Garhwal, P. D., Sharma, S. S., Dahima, V., Yadav, K., Khokhar, M. K., & Gupta, R. (2018). Physiopathological studies, characterization and yield loss assessment of *Curvularia* leaf spot of maize (*Zea mays*). *Indian Phytopathology*, 71(2), 287–293

123. Geetha K, Rajithasri A, Bhadraiah B (2014) Isolation of plant growth promoting rhizo bacteria from rhizosphere soils of green gram, biochemical characterization and screening for antifungal activity against pathogenic fungi. *Int J Pharm Sci Investig* 3:47–54
124. Gerhardt, Philipp. *Methods for General and Molecular Bacteriology*. Washington, D.C: American Society for Microbiology, 1994.
125. GES. 1997. ‘Glossary of Environment Statistics’, Studies in Methods Series F, 67. United Nations, New York.
126. Ghosh, A., Maity, B., Chakrabarti, K. et al. Bacterial Diversity of East Calcutta Wet Land Area: Possible Identification of Potential Bacterial Population for Different Biotechnological Uses. *Microb Ecol* 54, 452–459 (2007).
127. Ghosh, D., 2005. Ecology and Traditional Wetland Practice: Lessons from Wastewater Utilisation in the East Calcutta Wetlands. 1st Edn., Worldview, Kolkata, pp: 120 (PDF) East Kolkata Wetland: A Multifunctional Niche of International Importance.
128. Ghosh, S., Mitra, A. K., Choudhury, S. S., & Chandra, A. (2024). Unleaded agriculture: Myth to reality by wetland microbial flora. *Environmental Quality Management*, 1–12.
129. Glick B. R. (2005). Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. *FEMS microbiology letters*, 251(1), 1–7.
130. Glick, B. R. (2012). Plant growth-promoting bacteria: mechanisms and applications. *Scientifica*, 2012, Article ID 963401.
131. Glick, B. R. Plant growth-promoting bacteria: mechanisms and applications. *Scientifica* 2012, 1–15 (2012)
132. Gorde, S. P., & Jadhav, M. V. (2013). Assessment of Water Quality Parameters: A Review. *International Journal of Engineering Research and Applications*, 3, 2029-2035.
133. Goswami M., Deka S. (2020). Plant growth-promoting rhizobacteria—alleviators of abiotic stresses in soil: a review. *Pedosphere* 30, 40–61. doi: 10.1016/S1002-0160(19)60839-8
134. Gray, E.J. and Smith, D.L., “Intracellular and extra cellular PGPR: Commonalities and distinctions in the plant bacterium Signaling processes.” *Soil Biology and Biochemistry*, 37.395-412. 2005.

135. Grobelak A, Napora A, Kacprzak M (2015) Using plant growth-promoting rhizobacteria (PGPR) to improve plant growth. *Ecol Eng* 84:22–28
136. Gupta P, Samant K, Sahu A. Isolation of cellulose-degrading bacteria and determination of their cellulolytic potential. *Int J Microbiol.* 2012;2012:578925. doi: 10.1155/2012/578925. Epub 2012 Jan 18. PMID: 22315612; PMCID: PMC3270400.
137. Gupta S., Kumar M., Kumar J., Ahmad V., Pandey R., Chauhan N.S. Systemic analysis of soil microbiome deciphers anthropogenic influence on soil ecology and ecosystem functioning. *Int. J. Environ. Sci. Technol.* 2017;14:2229–2238. doi: 10.1007/s13762-017-1301-7
138. Gupta, D., Ghosh, R., Mitra, A., Roy, S., Sarkar, M., Chowdhury, S., Bhowmik, A., Mukhopadhyay, U., Maskey, S., & Ro, C. (2011). Nondestructive characterization of municipal-solid-waste-contaminated surface soil by energy-dispersive X-ray fluorescence and low-Z (atomic number) particle electron probe X-ray microanalysis. *Journal of the Air & Waste Management Association*, 61(11), 1102–1114. <https://doi.org/10.1080/10473289.2011.604286>
139. Gupta, K., Chatterjee, C. & Gupta, B. Isolation and characterization of heavy metal tolerant Gram-positive bacteria with bioremedial properties from municipal waste rich soil of Kestopur canal (Kolkata), West Bengal, India. *Biologia* 67, 827–836 (2012).
140. Gupta, S., Surendran, A., & Thatheyus, A. (2020). Biosorption of lead using the bacterial strain, *Bacillus subtilis*(MTCC 2423). *Journal of Biotechnology and Biomedical Science*, 2(3), 1–14.
141. Gwenzi, W. (2021). The uncultured microorganisms: Novel technologies and applications. *Frontiers in Microbiology*, 12, 865222.
142. Haile, S., & Ayele, A. (2022). Pectinase from Microorganisms and Its Industrial Applications. *Scientific World Journal*. Hindawi Limited.
143. Handelsman J. Metagenomics: Application of genomics to uncultured microorganisms. *Microbiol. Mol. Biol. Rev.* 2004;68:669–685. doi: 10.1128/MMBR.68.4.669-685.2004
144. Handelsman, J., et al. (1998). Molecular biological access to the chemistry of unknown soil microbes: A new frontier for natural products. *Chemistry & Biology*, 5(R245–R249).

145. Haque, S. M. (2020). Assessing infrastructural encroachment and fragmentation in the East Kolkata Wetlands. In *Contemporary South Asian Studies* (pp. 233–257). Springer.
146. Hardwick S.A., Chen W.Y., Wong T., Kanakamedala B.S., Deveson I.W., Ongley S.E., Santini N.S., Marcellin E., Smith M.A., Nielsen L.K., et al. Synthetic microbe communities provide internal reference standards for metagenome sequencing and analysis. *Nat. Commun.* 2018;9:3096. doi: 10.1038/s41467-018-05555-0.
147. Harshitha, C. P., Narendra Kumar, H. K., Chandra Mohana, N., & Satish, S. (2024). Biodegradation of monocrotophos by *Stenotrophomonas maltophilia* and its potential *in vitro* plant growth promoting activities. *The Microbe*, 4, 100101. <https://doi.org/10.1016/j.microb.2024.100101>
148. He, Y. C., Ma, C. L., Xu, J. H., and Zhou, L. (2011). A high-throughput screening strategy for nitrile-hydrolyzing enzymes based on ferric hydroxamate spectrophotometry. *Appl. Microbiol. Biotechnol.* 89, 817–823. doi: 10.1007/s00253-010-2977-5
149. Hettiarachchi M, McAlpine C and Morrison TH 2014. Governing the urban wetlands: a multiple case-study of policy, institutions and reference points. *Environ. Conserv.* 41(3) 276–289.
150. Holbrook, A. A., Edge, W. J. W., & Bailey, F. (1961). Spectrophotometric method for determination of Gibberellic acid. *The Advances in Chemistry series*, 28, 159–167.
151. <https://www.pib.gov.in/indexd.aspx>
152. Hu B, Guo P, Wu Y, Deng J, Su H, Li Y, Nan Y (2021) Study of soil physicochemical properties and heavy metals of a mangrove restoration wetland. *J Clean Prod* 291:125965
153. Hu, S., Li, Z., & Wang, X. (2017). Functions and services of wetlands: Hydrological regulation and biodiversity support. *Environmental Science and Pollution Research*, 24(11), 10120–10132.
154. Huang, X., Yin, T., Sun, W., Ge, G., & Wei, W. (2025). Organic Amendments Enhance Maize Growth by Improving Chemical and Microbial Properties in Coastal Saline–Alkali Soils. *Plants*, 14(14), 2217.
155. Hänsch, R., & Mendel, R. R. (2009). Physiological functions of mineral micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B, Cl). *Current opinion in plant biology*, 12(3), 259–266.

156. Igiehon, B. C., Babalola, O. O., & Hassen, A. I. (2024). Rhizosphere competence and applications of plant growth-promoting rhizobacteria in food production – A review. *Scientific African*, 23, e02081.
157. Ilangumaran, G., & Smith, D. L. (2017). Plant Growth Promoting Rhizobacteria in Amelioration of Salinity Stress: A Systems Biology Perspective. *Frontiers in plant science*, 8, 1768.
158. Islam M. R., Rahman M. M., Mohi-Ud-Din M., Akter M., Zaman E., Keya S. S., et al. (2022). Cytokinin and gibberellic acid-mediated waterlogging tolerance of mungbean (*Vigna radiata* L. Wilczek). *PeerJ* 10:e12862. 10.7717/peerj.12862
159. Jackson ML (1967) Soil chemical analysis. Pentice Hall of India, New Delhi
160. Jain (b), S. K., Agarwal, P. K., & Singh, V. P. (2007). Hydrology and water resources of India (Vol. 57). Springer Science & Business Media.
161. Jain D, Kaur G, Bhojiya AA, et al. Phenetic Characterization of Nitrogen Fixing Azotobacter from Rhizospheric Soil of Southern Rajasthan. *J Pure Appl Microbiol.* 2021;15(1): 428-436. doi:10.22207/JPAM.15.1.40
162. Jain, A., Jain, A., & Durve, A. (2017). Isolation, characterization and appguptacation of endophytic bacteria isolated from medicinal plants. *International Research Journal of Natural and Applied Sciences*, 4(8), 4–20.
163. Jain, C.K., Singhal, D.C. & Sharma, M.K. Estimating nutrient loadings using chemical mass balance approach. *Environ Monit Assess* 134, 385–396 (2007).
164. Jalili, F., Khavazi, K., Pazira, E., Nejati, A., Rahmani, H. A., Sadaghiani, H. R., & Miransari, M. (2009). Isolation and characterization of ACC deaminase-producing fluorescent pseudomonads, to alleviate salinity stress on canola (*Brassica napus* L.) growth. *Journal of plant physiology*, 166(6), 667–674.
165. Jang, S., Choi, S. K., Zhang, H., Zhang, S., Ryu, C. M., and Kloepper, J. W. (2023). History of a model plant growth-promoting rhizobacterium, *Bacillus velezensis* GB03: from isolation to commercialization. *Front. Plant Sci.* 14. doi: 10.3389/ fpls.2023.1279896

166. Jesus E, Susilawati E, Smith SL et al (2010) Bacterial communities in the rhizosphere of biofuel crops grown on marginal lands as evaluated by 16S rRNA gene pyrosequences. *Bio Energy Res* 3:20–27
167. Jeyanthi, V & Kanimozhi, S. (2018). Plant Growth Promoting Rhizobacteria (PGPR) - Prospective and Mechanisms: A Review. *Journal of Pure and Applied Microbiology*. 12. 733-749. 10.22207/JPAM.12.2.34.
168. Jindal, S. (2020). Microbes in Soil and Their Metagenomics. 10.1007/978-981-15-4099-8_5.
169. Jin, Y. J., Jo, D., Kwon, S. W., Jee, S., Kim, J. S., Raman, J., & Kim, S. J. (2022). A New Approach Using the SYBR Green-Based Real-Time PCR Method for Detection of Soft Rot *Pectobacterium odoriferum* Associated with Kimchi Cabbage. *The plant pathology journal*, 38(6), 656–664. <https://doi.org/10.5423/PPJ.OA.09.2022.0138>
170. Joe, M. M., Deivaraj, S., Benson, A., Henry, A. J., & Narendrakumar, G. (2018). Soil extract calcium phosphate media for screening of phosphate-solubilizing bacteria. *Agriculture and Natural Resources*, 52(3), 305–308. <https://doi.org/10.1016/j.anres.2018.09.014>
<https://www.sciencedirect.com/science/article/pii/S2452316X16301946>
171. Jose, J. (2022). *Introduction to time series analysis and its applications*
172. Joshi, P. K. and Saxena, R. (2002). A profile of pulses production in India. Facts, trends and opportunities. *Ind. J. Agric. Econ.* 57 : 326-339.
173. Junk, W.J. World wetlands classification: a new hierarchic hydro-ecological approach. *Wetlands Ecol Manage* 32, 975–1001 (2024).
174. Kandeler, E., Gerber, H. Short-term assay of soil urease activity using colorimetric determination of ammonium. *Biol Fert Soils* 6, 68–72 (1988).
<https://doi.org/10.1007/BF00257924>
175. Kandjimi OS, Uzabakiriho J, Chimwamurombe PM (2015) Isolation and characterization of culturable bacteria from bulk soil samples and the rhizosphere of arid-adapted *Tylosema esculentum* (Burchell). A. Schreiber (Marama bean) in Namibia. *Afri J Biotechnol* 14:944–952
176. Kang, S. M., Radhakrishnan, R., Khan, A. L., Kim, M. J., Park, J. M., Kim, B. R., Shin, D. H., & Lee, I. J. (2014). Gibberellin secreting rhizobacterium, *Pseudomonas putida* H-2-3

- modulates the hormonal and stress physiology of soybean to improve the plant growth under saline and drought conditions. *Plant physiology and biochemistry : PPB*, 84, 115–124. <https://doi.org/10.1016/j.plaphy.2014.09.001>
177. Kaur M, Soodan RK, Katnoria JK, Bhardwaj R, Pakade YB, Nagpal AK (2014) Analysis of physico-chemical parameters, genotoxicity and oxidative stress inducing potential of soils of some agricultural fields under rice cultivation. *Trop Plant Res* 1(3):49–61
 178. Kay-Shoemake, J., Watwood, M., Sojka, R. *et al.* Soil amidase activity in polyacrylamide-treated soils and potential activity toward common amide-containing pesticides. *Biol Fertil Soils* **31**, 183–186 (2000). <https://doi.org/10.1007/s003740050643>
 179. Kechin A, Boyarskikh U, Kel A, Filipenko M (2017) cutPrimers: a new tool for accurate cutting of primers from reads of targeted next generation sequencing. *J Comput Biol* 24(11):1138–1143
 180. Kekane SS, Chavan RP, Shinde DN, Patil CL, Sagar SS (2015) A review on physico-chemical properties of soil. *Int J Chem Stud* 3(4):29–32
 181. Kesikoglu M. H., Atasever U. H., Dadaser-Celik F. & Ozkan C. 2019 Performance of ANN, SVM and MLH techniques for land use/cover change detection at Sultan Marshes wetland, Turkey. *Water Science and Technology* 80 (3), 466–477.
 182. Khan A, Singh P, Srivastava A (2018) Synthesis, nature and utility of universal iron chelator–Siderophore: A review. *Microbiol Res* 212:103–111.
 183. Khan, S., Cao, Q., Zheng, Y. M., Huang, Y. Z., & Zhu, Y. G. (2008). Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. *Environmental pollution* (Barking, Essex: 1987), 152(3), 686–692.
 184. Khandekar, N. (2011). Delhi water bodies go under, almost. *Hindustan Times* February.
 185. Kifle, M. H., & Laing, M. D. (2016). Effects of selected diazotrophs on maize growth. *Frontiers in Plant Science*, 7, 1429.
 186. Kimura, M. (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16, 111–120. Kloepper, J. W., & Schroth, M. N. (1978). Plant growth-promoting rhizobacteria

- on radishes. Proceedings of the 4th International Conference on Plant Pathogenic Bacteria. Gilbert-Clarey, Tours, 879–882.
187. Kitouni I., Benmerzoug D. & Lezzar F. 2018 Smart agricultural enterprise system based on integration of internet of things and agent technology. *Journal of Organizational and End User Computing* 30 (4), 64–82.
 188. Kloepper JW, Leong J, Teintze M, Schroth MN (1980) Enhanced plant growth by plant growthpromoting rhizobacteria. *Nature* 286:885–886
 189. Kloepper JW, Rodriguez-Kabana R, Zehnder GW, Murphy JF, Sikora E, Fernandez C (1999) Plantroot- bacterial interactions in biological control of soil borne diseases and potential extensionto systemic and foliar diseases. *Austral Plant Pathol* 28:21–26 (PDF) Role of Plant Growth Promoting Rhizobacteria in Biocontrol of Plant Diseases and Sustainable Agriculture.
 190. Koroglu, A. 2015. “Wetland Reclamations.” In *Encyclopedia of Estuaries*, edited by M. Kennish, 741–742. Dordrecht: Springer.
 191. Kumar M. et al., 2018. Influence of bio-fertilizers application on growth, yield and quality attributes of cucumber (*Cucumis sativus* L.): a review. *Plant Arch.*, 18(2), 2329-2334
 192. Kumar V, AlMomin S, Al-Aqeel H, Al-Salameen F, Nair S, Shajan A (2018) Metagenomic analysis of rhizosphere microflora of oil-contaminated soil planted with barley and alfalfa. *PLoS One* 13 (8):e0202127
 193. Kumar, A., & Chandra, R. (2020). Ligninolytic enzymes and its mechanisms for degradation of lignocellulosic waste in environment. *Heliyon*, 6(2).
 194. Kumar, M., Shekhar, C., & Hasija, R. C. (2021). Trend analysis of cotton crop in Gujarat. *International Journal of Agriculture Sciences*, 13(19), 10539–10542.
 195. Kumar, N., Chandan, N. K., Bhushan, S., Singh, D. K., & Kumar, S. (2023). Health risk assessment and metal contamination in fish, water and soil sediments in the East Kolkata Wetlands, India, Ramsar site. *Scientific reports*, 13(1), 1546.
 196. Kumar, P. (1997). Food security : Supply and demand perspective. *Indian Farming* 12 : 4-9.
 197. Kumar, P. and Rosegrant, M. W. (1994). Productivity and sources of growth for rice in India. *Econ. Political Weekly* 29 : A183-A188.

198. Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, 35, 1547–1549. Li, X.,
199. Kumar, V., R. D. Parihar, A. Sharma, et al. 2019. “Global Evaluation of Heavy Metal Content in Surface Water Bodies: A Meta-Analysis Using Heavy Metal Pollution Indices and Multivariate Statistical Analyses.” *Chemosphere* 236: 124364. .
200. Kumari K, Naskar M, Aftabuddin M, Das Sarkar S, Ghosh BD, Sarkar UK, Nag SK, Jana C, Das BK. Evaluation of Three Prokaryote Primers for Identification of Prokaryote Community Structure and Their Abode Preference in Three Distinct Wetland Ecosystems. *Front Microbiol.* 2021 Jul 14;12:643945. doi: 10.3389/fmicb.2021.643945. PMID: 34335488; PMCID: PMC8317468.
201. Kumari, S., Sharma, A., Shilpa, & Lohia, M. (2025). Forecasting of rice production in India using linear time series models. *Biological Forum*, 17(5a), 19–23.
202. Kundu N and Chakraborty A 2017. Dependence on Ecosystem Goods and Services: A Case Study on East Kolkata Wetlands, West Bengal, India. Prusty, B., Chandra, R. and Azeez, P. (eds) *Wetland Science* (Springer, New Delhi) pp. 381-405.
203. Kundu N, Pal M, Saha S (2008) East Kolkata Wetlands: a resource recovery system through productive activities. In *Proceedings of Taal2007: the 12th world lake conference* (Vol. 868, p 881).
204. Kushwaha, A., Hans, N., Kumar, S., & Rani, R. (2018). A critical review on speciation, mobilization and toxicity of lead in soil-microbe-plant system and bioremediation strategies. *Ecotoxicology and environmental safety*, 147, 1035–1045.
205. Kwa AL, Low JG, Lim TP, Leow PC, Kurup A, Tam VH. Independent predictors for mortality in patients with positive *Stenotrophomonas maltophilia* cultures. *Ann Acad Med Singap.* 2008 Oct;37(10):826-30.
206. Lal, R. (2008). Carbon sequestration. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 363(1492), 815–830
207. Laschat S, Bilitewski U, Blodgett J, Duhme-Klair A-K, Dallavalle S, Routledge A, Schobert R (2017) Chemical and biological aspects of nutritional immunity-perspectives for new anti-infectives targeting iron uptake systems. *Angew Chem Int Ed*

208. Lee H.J., Jeong S.E., Kim P.J., Madsen E.L., Jeon C.O. (2015). High resolution depth distribution of Bacteria, Archaea, methanotrophs, and methanogens in the bulk and rhizosphere soils of a flooded rice paddy. *Frontiers in Microbiology*, 6:639. doi:10.3389/fmicb.2015.00639.
209. Lee Y. S., Kim K. Y. (2016). Antagonistic potential of *Bacillus pumilus* L1 against root-knot nematode, *Meloidogyne arenaria*. *J. Phytopathol.* 164, 29–39. doi: 10.1111/jph.12421
210. Levy GJ, Van der Watt HVH, Du Plessis HM (1988) Effect of sodium-magnesium and sodium-calcium systems on soil hydraulic conductivity and infiltration. *Soil Sci* 146(5):303–310 .
211. Li X, Mitra C, Marzen L and Yang Q 2016. Spatial and Temporal Patterns of Wetland Cover Changes in East Kolkata Wetlands, India from 1972 to 2011. *Int. J. Appl. Geosp. Res.* 7(2) 1-13.
212. Li, L., Wang, S., Li, J., & Yang, Z. (2018). Wetland ecosystem services and their contribution to human well-being: A review. *Ecological Indicators*, 95, 19–29.
213. Li, P., Tian, Y., Yang, K. et al. Mechanism of microbial action of the inoculated nitrogen-fixing bacterium for growth promotion and yield enhancement in rice (*Oryza sativa* L.). *Adv. Biotechnol.* 2, 32 (2024).
214. Li, Y., Jin, J., Li, S., Xia, S., & Wei, J. (2024). A Study of the Effects of Wetland Degradation on Soil-Microbial-Extracellular Enzyme Carbon, Nitrogen, and Phosphorus and Their Ecological Stoichiometry. *Agronomy*, 14(12), 3008.
215. Liu, F.; Bian, Z.; Jia, Z.; Zhao, Q.; Song, S. The GCR1 and GPA1 participate in promotion of *Arabidopsis* primary root elongation induced by N-Acyl-homoserine lactones, the bacterial quorum-sensing signals. *Mol. Plant. Microbe Interact.* 2012, 25, 677–683.
216. Liu, F.; Bian, Z.; Jia, Z.; Zhao, Q.; Song, S. The GCR1 and GPA1 participate in promotion of *Arabidopsis* primary root elongation induced by N-Acyl-homoserine lactones, the bacterial quorum-sensing signals. *Mol. Plant. Microbe Interact.* 2012, 25, 677–683.
217. Liu, J., Diamond, J. China's environment in a globalizing world. *Nature* 435, 1179–1186 (2005).

218. Liu, J., Zhao, L., & Zhang, Y. (2022). Litter decomposition and nutrient cycling in the Liaohu Estuary Wetland. *Science of The Total Environment*, 803, 149937.
219. Lloyd, K. G., et al. (2020). Culturing of 'unculturable' subsurface microbes: Natural organic carbon as a key factor. *Frontiers in Microbiology*, 11, 610001.
220. Lobell, D.B. and Burke, M.B. (2010) On the Use of Statistical Models to Predict Crop Yield Responses to Climate Change. *Agricultural and Forest Meteorology*, 150, 1443-1452. <https://doi.org/10.1016/j.agrformet.2010.07.008>
221. Loccoz, Y.M.; Mavingui, P.; Combes, C.; Normand, P.; Steinberg, C. Microorganisms and Biotic Interactions. In *Environmental Microbiology: Fundamentals and Applications*; Springer: Dordrecht, The Netherlands, 2015; pp. 395–444. ISBN 9789401791182.
222. Lu-Yao Liu, Guo-Jun Xie, De-Feng Xing, Bing-Feng Liu, Jie Ding, Guang-Li Cao, Nan-Qi Ren. Sulfate dependent ammonium oxidation: A microbial process linked nitrogen with sulfur cycle and potential application. *Environmental Research* 2021, 192 , 110282.
223. Luang-In, V., Yotchaisarn, M., Saengha, W., Udomwong, P., Deeseenthum, S., & Maneewan, K. (2019). Isolation and identification of amylase-producing bacteria from soil in Nasinuan Community Forest, Maha Sarakham, Thailand. *Biomedical and Pharmacology Journal*, 12(2), 1061–1068.
224. Lucy, M., Reed, E. and Click, B.R. (2004) Application of Free Living Plant Growth-Promoting Rhizobacteria. *Antonie Van Leeuwenhoek*, 86, 1-25.
225. Lunau, M., Lemke, A., Walther, K., Martens-Habbena, W., & Simon, M. (2005). An improved method for counting bacteria from sediments and turbid environments by epifluorescence microscopy. *Environmental Microbiology*, 7(7), 961–968. <https://doi.org/10.1111/j.1462-2920.2005.00767.x>
226. MacFaddin, J. F. 2000. Biochemical tests for the identification of medical bacteria, 3rd ed. Lippincott Williams & Wilkins, Philadelphia, PA.
227. Mackelprang R, Waldrop MP, DeAngelis KM, David MM, Chavarria KL, Blazewicz SJ, Rubin EM, Jansson JK (2011) Metagenomic analysis of a permafrost microbial community reveals a rapid response to thaw. *Nature* 480(7377):368–371.

228. Maheshwari, R., Bhutani, N., & Suneja, P. (2020). Isolation and characterization of ACC deaminase producing endophytic *Bacillus mojavensis* PRN2 from *Pisum sativum*. *Iranian Journal of Biotechnology*, 18(4), e2308.
229. Mahmood, R., Sharif, F., Ali, S., & Hayyat, M. U. (2013). Bioremediation of textile effluent by indigenous bacterial consortia and its effects on *Zea mays* L. CV C141
230. Mahmood, R., Sharif, F., Ali, S., & Hayyat, M. U. (2013). Bioremediation of textile effluent by indigenous bacterial consortia and its effects on *Zea mays* L. CV C1415.
231. Mahmud, K.; Makaju, S.; Ibrahim, R.; Missaoui, A. Current Progress in Nitrogen Fixing Plants and Microbiome Research. *Plants* 2020, 9, 97.
232. Maithya J. K., Ming'ate F. L. M. & Letema S. C. 2020 A review on ecosystem services and their threats in the conservation of Nyando Wetland, Kisumu County, Kenya. *Tanzania Journal of Science* 46 (3), 711–722.
233. Malla MA, Dubey A, Kumar A, Yadav S (2022) Metagenomic analysis displays the potential predictive biodegradation pathways of the persistent pesticides in agricultural soil with a long record of pesticide usage. *Microbiol Res* 261:127081
234. Mandal, D. (2021). A review on the significance of East Kolkata Wetland: A Ramsar site with integrated resource recovery activities. *Ecology, Environment and Conservation*, 27(Supplement), 244–247
235. Mangosongo HM, Lyaruu HV, Mneney EE (2019) Assessment of soil physico-chemical properties in selected natural habitats of the wild rice (*Oryza Longistaminata*) and their effects on the species morphological characters. *Huria: J Open Univ Tanzania* 26(1):12–29
236. Marconi J. 2022 Urban water pollution prevention and protection based on convolutional neural network. *Water Pollution Prevention and Control Project* 3 (4), 29–37. .
237. Markovich, N. A., & Kononova, G. L. (2003). Lytic enzymes of *Trichoderma* and their role in protecting plants from fungal diseases. *Prikladnaia biokhimiia i mikrobiologiia*, 39(4), 389–400.
238. Maurya, A., Kesharwani, L., & Mishra, M. K. (2018). Analysis of heavy metal in soil through atomic absorption spectroscopy for forensic consideration. *International Journal for Research in Applied Science and Engineering Technology*, 6(6), 1188–1192.

239. Mazzei, V., Longo, G., Brundo, M. V., Copat, C., & Oliveri Conti, G. (2013).
240. McDowell, R. H., Sands, E. M., & Friedman, H. (2023, January 23). *Bacillus cereus*. In StatPearls [Internet]. StatPearls Publishing. Retrieved from
241. Medinade la Rosa, G., LópezReyes, L., Carcaño, M., Olguín, J. F., Hernández, M., & Rivera, A. (2016). Rhizosphere bacteria of maize with chitinolytic activity and its potential in the control of phytopathogenic fungi. *Archives of Phytopathology and Plant Protection*, 49(1), 1–12.
242. Mekonnen, E., et al., Isolation and Characterization of Urease-Producing Soil Bacteria. 2021. 2021(1): p. 8888641.
243. Merchant, M. A., Battaglia, M. J., French, N., Smith, K., Singer, H. V., Armstrong, L., Harriman, V. B., & Slattery, S. (2024). Species Abundance Modelling of Arctic-Boreal Zone Ducks Informed by Satellite Remote Sensing. *Remote Sensing*, 16(7), 1175.
244. Millenium Ecosystem Assessment(MEA 2005) Millennium Ecosystem Assessment (MEA)Ecosystems and Human Well-being: Wetlands and Water Synthesis. World Resources Institute, Washington, DC (2005)
245. Ministry of Environmental Forests (MoEF). (2011). National Wetland Atlas: As a Part of the Project on National Wetland Inventory and Assessment (NWIA). pp: 215.
246. Mir AR, Pichtel J, Hayat S. Copper: uptake, toxicity and tolerance in plants and management of Cu-contaminated soil. *Biometals*. 2021;34(4):737–59
247. Mitra, A., Chatterjee, S., Katak, S., Rastogi, R. P., & Gupta, D. K. (2021). Bacterial tolerance strategies against lead toxicity and their relevance in bioremediation application. *Environmental Science and Pollution Research*, 28, 14271–14284.
248. Mitsch WJ, Gosselink JG (2015) *Wetlands*, 5th edn. Wiley, Hoboken, p 456
249. Mocali, S., & Benedetti, A. (2010). The challenge of metagenomics in soil microbiology. *Research in Microbiology*, 161(6), 497–505.
250. Molina-Romero, D., Juárez-Sánchez, S., Venegas, B., Ortiz-González, C., Baez, A., Morales-García, Y., & Muñoz-Rojas, J. (2021). A bacterial consortium interacts with different varieties of maize, promotes the plant growth, and reduces the application of chemical fertilizer under field conditions. *Frontiers in Sustainable Food Systems*, 4, 616757.

251. Mondal BK, Kumari S, Ghosh A, Mishra PK (2022) Transformation and risk assessment of the East Kolkata Wetlands (India) using fuzzy MCDM method and geospatial technology. *Geogr Sustain* 3(3):191–203
252. Mondal, B. K., Dolui, G., Pramanik, M., Maity, S., Biswas, S. S., & Pal, R. (2017). Urban expansion and wetland shrinkage estimation using a GIS-based model in the East Kolkata Wetland, India. *Ecological Indicators*, 83, 62–73.
253. Monni, S., Salemaa, M., & Millar, N. (2000). The tolerance of *Empetrum nigrum* to copper and nickel. *Environmental Pollution*, 109, 221–229. [10.1016/S0269-7491\(99\)00264-X](https://doi.org/10.1016/S0269-7491(99)00264-X)
254. Montgomery DC. *Introduction to Statistical Quality Control*. United Kingdom: John Wiley & Sons; 2013
255. Moparthi, S., & Kleczewski, N. (2023). First report of *Curvularia* leaf spot on *Zea mays* caused by *Curvularia lunata* in North Carolina. *Plant Disease*, 107(10), 2306. <https://doi.org/10.1094/PDIS-10-22-2306-PDN>
256. Morbia, M. M., Pandey, A. A., Mahla, P. K., & Gohil, S. N. (2024). *Production of amylase by solid state fermentation using agricultural waste*. *Nature Environment and Pollution Technology*, 23(3), 1597–1604. <https://doi.org/10.46488/NEPT.2024.v23i03.029>
257. Moreira H., Pereira S.I.A., Marques A., Rangel A., Castro P. Selection of metal resistant Plant Growth-Promoting Rhizobacteria for the growth and metal accumulation of energy maize in a mine soil – effect of the inoculum size. *Geoderma*. 2016;278:1–11
258. Moreira H., Pereira S.I.A., Marques A.P.G.C., Rangel A.O.S.S., Castro P.M.L. Effects of soil sterilization and metal spiking in plant growth promoting rhizobacteria selection for phytotechnology purposes. *Geoderma*. 2019;334:72–81
259. Mu, Y., et al. (2021). Cultivation of uncultured marine microorganisms. *Frontiers in Microbiology*, 12, 610001.
260. Mujakić, I., Piwosz, K., & Kobližek, M. (2022). Phylum Gemmatimonadota and Its Role in the Environment. *Microorganisms*, 10(1), 151. <https://doi.org/10.3390/microorganisms10010151>
261. Mukherjee, S.; Bassler, B.L. Bacterial quorum sensing in complex and dynamically changing environments. *Nat. Rev. Microbiol.* 2019, 17, 371–382

262. Mukhopadhyay, C. (2022). Climate change adaptation and built environment resilience: East Kolkata wetland strategies. *Built Environment*, 48(4), 594–612.
263. Mundoli, S., Sanfui, A., & Nagendra, H. (2023). Pestilential or productive? Tracking two centuries of environmental change and current perceptions about ecosystem services of the East Kolkata Wetlands. *Urbanisation*, 8(1), 1–21.
264. Munees, A., & Mulugeta, K. (2013). Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. *Journal of King Saud University - Science*, 26, 1–20.
265. Murthy, S., Bali, G., & Sarangi, S. (2011). Effect of lead on metallothionein concentration in lead-resistant bacteria *Bacillus cereus* isolated from industrial effluent. *African Journal of Biotechnology*, 10(63), 13713–13718.
266. Murugan, R., Thomas, F. S., GeethaShree, G., Glory, S., & Shilpa, A. (2020). Linear regression approach to predict crop yield. *Asian Journal of Computer Science and Technology*, 9(1), 40–44.
267. Nakano, Y., Asada, K. Purification of Ascorbate Peroxidase in Spinach Chloroplasts; Its Inactivation in Ascorbate-Depleted Medium and Reactivation by Monodehydroascorbate Radical, *Plant and Cell Physiology*, Volume 28, Issue 1, January 1987, Pages 131–140, <https://doi.org/10.1093/oxfordjournals.pcp.a077268>
268. Nandi M, Selin C, Brawerman G, Fernando WGD, de Kievit T (2017) Hydrogen cyanide, which contributes to *Pseudomonas chlororaphis* strain PA23 biocontrol, is upregulated in the presence of glycine. *Biol Control* 108:47–54
269. Narain, P., Pandey, R. K. and Sarup, S. (1982). Perspective plan for foodgrains. *Commerce* 145 : 184-191.
270. Naz, A., A. Chowdhury, R. Chandra, and B. K. Mishra. 2020. “Potential Human Health Hazard due to Bioavailable Heavy Metal Exposure via Consumption of Plants With Ethnobotanical Usage at the Largest Chromite Mine of India.” *Environmental Geochemistry and Health* 42: 4213–4231. .
271. Nyawira, S.S.; Hartman, M.D.; Nguyen, T.H.; Margenot, A.J.; Kihara, J.; Paul, B.K.; Williams, S.; Bolo, P.; Sommer, R. Simulating soil organic carbon in maize-based systems under improved agronomic management in Western Kenya. *Soil Tillage Res.* 2021, 211, 105000.

272. Ojuederie, O. B., Olanrewaju, O. S., & Babalola, O. O. (2019). Plant Growth Promoting Rhizobacterial Mitigation of Drought Stress in Crop Plants: Implications for Sustainable Agriculture. *Agronomy*, 9(11), 712.
273. Olaniyan, F.T., Alori, E.T., Adekiya, A.O. et al. The use of soil microbial potassium solubilizers in potassium nutrient availability in soil and its dynamics. *Ann Microbiol* 72, 45 (2022).
274. Olanrewaju, O. S., Glick, B. R., & Babalola, O. O. (2017). Mechanisms of action of plant growth promoting bacteria. *World journal of microbiology & biotechnology*, 33(11), 197.
275. Orozco-Mosqueda, M. del C., Glick, B. R., & Santoyo, G. (2020). ACC deaminase in plant growth-promoting bacteria (PGPB): An efficient mechanism to counter salt stress in crops. *Microbiological Research*, 235, 126439.
276. Oves, M., Khan, M. S., & Zaidi, A. (2013). Biosorption of heavy metals by *Bacillus thuringiensis* strain OSM29 originating from industrial effluent contaminated north Indian soil. *Saudi Journal of Biological Sciences*, 20(2), 121–129.
277. Pacheco da Silva, M. L., Moen, F. S., Liles, M. R., Feng, Y., & Sanz-Saez, A. (2022). The Response to Inoculation with PGPR Plus Orange Peel Amendment on Soybean Is Cultivar and Environment Dependent. *Plants (Basel, Switzerland)*, 11(9), 1138.
278. Paez JI, Tengan FM, Barone AA, Levin AS, Costa SF. Factors associated with mortality in patients with bloodstream infection and pneumonia due to *Stenotrophomonas maltophilia*. *Eur J Clin Microbiol Infect Dis*. 2008 Oct;27(10):901-6.
279. Pal S, Manna S, Chattopadhyay B, Mukhopadhyay SK (2016) Carbon sequestration and its relation with some soil properties of East Kolkata Wetlands (a Ramsar Site): a spatio-temporal study using radial basis functions. *Modelling Earth Syst Environ* 2:1–12
280. Pal, S., Manna, S., Chattopadhyay, B. et al. Carbon sequestration and its relation with some soil properties of East Kolkata Wetlands (a Ramsar Site): a spatio-temporal study using radial basis functions. *Model. Earth Syst. Environ.* 2, 80 (2016).
281. Panda BP, et al. Heavy metal accumulation in some fishes preferred for consumption by egrets in Odisha, India. *Nat. Environ. Pollut. Technol.* 2019;18:975–979.

282. Panda, B. P., Mohanta, Y. K., Paul, R., Prusty, B. A. K., Parida, S. P., Pradhan, A., Saravanan, M., Patowary, K., Jiang, G., Joshi, S. J., & Sarma, H. (2023). Assessment of environmental and carcinogenic health hazards from heavy metal contamination in sediments of wetlands. *Scientific reports*, 13(1), 16314.
283. Panse, V. G. (1964). Yield trends of rice and wheat in first two five-year plans in India. *J. Ind. Soc. Agric. Statistics* 16 : 1-50.
284. Parmar, R. D., Varsani, V. G., Parmar, V., Vyas, S., & Dudhagara, D. (2024). Multidimensional evaluation of salt tolerance in groundnut genotypes through biochemical responses. *Oil Crop Science*, 9(2), 102–110.
285. Parmar, R. S., Chaudhari, G. B., & Parmar, D. K. (2017). Application of regression models for area, production and productivity trends of maize (*Zea mays*) crop for Panchmahal region of Gujarat state, India. *International Journal on Recent and Innovation Trends in Computing and Communication*, 1(3), 303–308.
286. Parra-López, C., Ben Abdallah, S., Garcia-Garcia, G., Hassoun, A., Trollman, H., Jagtap, S., Gupta, S., Aït-Kaddour, A., Makmuang, S., & Carmona-Torres, C. (2025). Digital technologies for water use and management in agriculture: Recent applications and future outlook. *Agricultural Water Management*, 309, Article 109347.
287. Parungao, M. M., Tacata, P. S., & Tanayan, G. C. (2007). Biosorption of copper, cadmium and lead by copper-resistant bacteria isolated from Mogpog River, Marinduque. *Philippine Journal of Science*, 136, 155–165.
288. Patel, J. G., Murthy, T. V. R., Singh, T. S., Panigrahy, S., Panigrahy, S., Shankar Ray, S., & Parihar, J. S. (2009, December). Analysis of the distribution pattern of wetlands in India in relation to climate change. In *Proceedings of the workshop on impact of climate change on agriculture*. Ahmedabad, India (pp. 17-18).
289. Patel, R. H., Patel, G. N. and Patel, J. B. (1986). Trends and variability in area, production and productivity of tobacco in India. *Ind. Tobacco J.* 18 : 3-5.
290. Patil, B. N., Bhonde, S. R. and Khandikar, D. N. (2009). Trends in area, production and productivity of groundnut in Maharashtra. *Financing Agriculture*, March-April. pp. 35-39.
291. Peng, W., Jia, Y., Lu, L., & Fan, W. (2016). Bioremediation of lead contaminated soil with *Rhodobacter sphaeroides*. *Chemosphere*, 156, 228–235.

292. Penton CR, Gupta VVSR, Yu J and Tiedje JM (2016) Size Matters: Assessing Optimum Soil Sample Size for Fungal and Bacterial Community Structure Analyses Using High Throughput Sequencing of rRNA Gene Amplicons. *Front. Microbiol.* 7:824. doi: 10.3389/fmicb.2016.00824
293. Pereira S.I.A., Castro P.M.L. Diversity and characterization of culturable bacterial endophytes from *Zea mays* and their potential as plant growth-promoting agents in metal-degraded soils. *Environ. Sci. Pollut. Res.* 2014;21:14110–14123. doi: 10.1007/s11356-014-3309-6.
294. Pereira, S. I. A., Abreu, D., Moreira, H., Vega, A., & Castro, P. M. L. (2020). Plant growth-promoting rhizobacteria (PGPR) improve the growth and nutrient use efficiency in maize (*Zea mays* L.) under water deficit conditions. *Heliyon*, 6(10), e05106.
295. Petrášek J, Friml J (2009) Auxin transport routes in plant development. *Development* 136:2675–2688
296. Piper CS (1966) Soil and plant analysis. Hans Publishers, Bombay, India
297. Porra, R. J., Thompson, W. A., & Kriedemann, P. E. (1989). Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: Verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, 975(3), 384–394.
298. Pradhan, Chinmay & Satapathy, Kunja. (2014). Decontamination of Lead from aquatic environment by exploitation of floating macrophyte *Azolla microphylla* Kauf. *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)*. 8. 10.9790/2402-081231723.
299. Prasad, S. N., Ramachandra, T. V., Ahalya, N., Sengupta, T., Kumar, A., Tiwari, A. K., ... & Vijayan, L. (2002). Conservation of wetlands of India-a review. *Tropical Ecology*, 43(1), 173-186.
300. Prusty BAK, Chandra R, Azeez PA. Chemical partitioning of Cu, Pb and Zn in the soil profile of a semi-arid dry woodland. *Chem. Speciat. Bioavailab.* 2009;21:141–151.

301. Prusty, P., and S. H. Farooq. 2021. “Understanding the Effects of Periodic Freshening and Salinization on Coastal Water Quality and Aquifer Sediments Through Laboratory-Based Column Experiments.” *Journal of Hydrology* 603: 127060. .
302. Prusty, P., S. H. Farooq, H. V. Zimik, and S. S. Barik. 2018. “Assessment of the Factors Controlling Groundwater Quality in a Coastal Aquifer Adjacent to the Bay of Bengal, India.” *Environmental Earth Sciences* 77: 762. .
303. Pérez-Miranda, S., Cabirol, N., George-Téllez, R., Zamudio-Rivera, L. S., & Fernández, F. J. (2007). O-CAS, a fast and universal method for siderophore detection. *Journal of Microbiological Methods*, 70(1), 127– 131. <https://doi.org/10.1016/j.mimet.2007.03.023>
304. Pérez-Montaña F et al (2014) Plant growth promotion in cereal and leguminous agricultural important plants: from microorganism capacities to crop production. *Microbiol Res* 169:325–336
305. Pérez-Montaña, F., Alías-Villegas, C., Bellogín, R. A., del Cerro, P., Espuny, M. R., Jiménez-Guerrero, I., López-Baena, F. J., Ollero, F. J., & Cubo, T. (2013). Plant growth promotion in cereal and leguminous agricultural important plants: From microorganism capacities to crop production. *Microbiological Research*, 169(5-6), 325–336.
306. Qian, L., Yu, X., Gu, H. et al. Vertically stratified methane, nitrogen and sulphur cycling and coupling mechanisms in mangrove sediment microbiomes. *Microbiome* 11, 71 (2023).
307. Rabbee, M. F., Ali, M. S., Choi, J., Hwang, B. S., Jeong, S. C., & Baek, K. H. (2019). *Bacillus velezensis*: A Valuable Member of Bioactive Molecules within Plant Microbiomes. *Molecules* (Basel, Switzerland), 24(6), 1046.
308. Rachid, C. T. C., et al. (2023). Soil microbiome: An intrinsic driver for climate-smart agriculture. *Science of the Total Environment*, 855, 158630.
309. Radhakrishnan, M., Samshath, K. J., & Balagurunathan, R. (2014). Hydroxamate siderophore from *Bacillus* sp. SD12 isolated from iron factory soil. *Current World Environment*, 9(3), 990.
310. Rafrafi Y, Trably E, Hamelin J, Latrille E, Meynial-Salles I, Benomar S, Giudici-Orticoni MT, Steyer JP (2013) Sub-dominant bacteria as keystone species in microbial communities producing biohydrogen. *Int J Hydrogen Energy* 38(12):4975–4985

311. Rahman, S. U., Qin, A., Zain, M., Mushtaq, Z., Mehmood, F., Riaz, L., ... & Shehzad, M. (2024). Pb uptake, accumulation, and translocation in plants: Plant physiological, biochemical, and molecular response: A review. *Heliyon*.
312. Ramachandra T.V. and Ahalya N., Conservation and Management Of Wetland Ecosystems In Karnataka
313. Ramachandra TV, Sudarshan PB, Mahesh MK, Vinay S. Spatial patterns of heavy metal accumulation in sediments and macrophytes of Bellandur wetland, Bangalore. *J. Environ. Manag.* 2018; 206:1204–1210. doi: 10.1016/j.jenvman.2017.10.014.
314. Ramette A, Moëgne-Loccoz Y, Défago G (2006) Genetic diversity and biocontrol potential of fluorescent pseudomonads producing phloroglucinols and hydrogen cyanide from Swiss soils naturally suppressive or conducive to *Thielaviopsis basicola*-mediated black root rot of tobacco. *FEMS Microbiol Ecol* 55:369–381
315. Ramsar Convention. (2013). The Ramsar Convention Bureau, Rue Mauverney 28, CH1196, Gland, Switzerland (accessed 13.12.13).
316. Ramsar Convention Secretariat, 2013. The Ramsar Convention Manual: a guide to the Convention on Wetlands (Ramsar, Iran, 1971), 6th ed. Ramsar Convention Secretariat, Gland, Switzerland.
317. Rana, A., Saharan, B., Joshi, M. et al. Identification of multi-trait PGPR isolates and evaluating their potential as inoculants for wheat. *Ann Microbiol* 61, 893–900 (2011).
318. Rath, S., & Padhy, R. N. (2015). Antibacterial efficacy of five medicinal plants against multidrug-resistant enteropathogenic bacteria infecting under-5 hospitalized children. *Journal of Integrative Medicine*, 13(1), 45–57.
319. Rath, S., Paul, M., Behera, H. K., & Thatoi, H. (2022). Response surface methodology mediated optimization of lignin peroxidase from *Bacillus mycoides* isolated from Simlipal Biosphere Reserve, Odisha, India. *Journal of Genetic Engineering and Biotechnology*, 20(1), 2.
320. Rath, Shakti & Padhy, Rabindra. (2015). Antibacterial efficacy of five medicinal plants against multidrug resistant enteropathogenic bacteria infecting under-5 hospitalized children. *Journal of Integrative Medicine (Elsevier)*. 13. 45-57.. 10.1016/S2095-4964(15)60154-6.

321. Rathore R S, Trivedi A and Mathur K. 2005. Stresses on Maize in Tropic, pp. 195–97. Zaidi P H and Singh N N (Eds). Directorate of Maize Research, ICAR, New Delhi.
322. Ravanbakhsh, M., Sasidharan, R., Voesenek, L. A. C. J., Kowalchuk, G. A., & Jousset, A. (2017). ACC deaminase-producing rhizosphere bacteria modulate plant responses to flooding. *Journal of Ecology*, 105(4), 979–986.
323. Rawat N., Joshi G.K. Bacterial community structure analysis of a hot spring soil by next generation sequencing of ribosomal RNA. *Genomics*. 2019;111:1053–1058. doi: 10.1016/j.ygeno.2018.06.008.
324. Reddy, K. R., & DeLaune, R. D. (2008). *Biogeochemistry of wetlands: Science and applications*. CRC Press.
325. Reetha, A. K., Pavani, S. L., & Mohan, S. (2014). Hydrogen cyanide production ability by bacterial antagonist and their antibiotics inhibition potential on *Macrophomina phaseolina* (Tassi.) goid. *International Journal of Current Microbiology and Applied Sciences*, 3(5), 172–178.
326. Riseh, R. S., Gholizadeh Vazvani, M., Vatankhah, M., & Kennedy, J. F. (2024). Chitin-induced disease resistance in plants: A review. *International Journal of Biological Macromolecules*, 266, 131105.
327. Rivera M.C., Izard J. In: *Promises and Prospects of Microbiome Studies*. 1st ed. Izard J., Rivera M.C., editors. Elsevier; London, UK: 2015. pp. 144–159
328. Rodríguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnol Adv* 17:319–339
329. Rodríguez-Sánchez, V., Guzmán-Moreno, J., Rodríguez-González, V., Floresde la Torre, J. A., Ramírez-Santoyo, R. M., & Vidales-Rodríguez, L. E. (2017). Biosorption of lead phosphates by lead-tolerant bacteria as a mechanism for lead immobilization. *World Journal of Microbiology & Biotechnology*, 33(8), 150.
330. Romero-Isart, N., & Vašák, M. (2002). Advances in the structure and chemistry of metallothioneins. *Journal of Inorganic Biochemistry*, 88(3–4), 388–396.
331. Roy, B., Maitra, D., Biswas, A., Chowdhury, N., Ganguly, S., Bera, M., Dutta, S., Golder, S., Roy, S., Ghosh, J., & Mitra, A. K. (2023). Efficacy of high-altitude biofilm-forming novel

- Bacillus subtilis species as plant growth-promoting rhizobacteria on Zea mays L. Applied Biochemistry and Biotechnology, 196(2), 643–666.
332. Roy, M., Basu, A., & Chakraborty, P. (2020). Adaptive co-management model for the East Kolkata wetlands: A sustainable solution to manage the rapid ecological transformation of a peri-urban landscape. Science of The Total Environment, 698, 134203.
 333. SAC (2011). National wetland atlas. Space application center (SAC). Indian Space Research Organisation (ISRO) Ahmedabad, India.
 334. Saha M, Sarkar S, Sarkar B, Sharma BK, Bhattacharjee S, Tribedi P (2016) Microbial siderophores and their potential applications: a review. Environ Sci Pollut Res 23:3984–3999
 335. Saha, M., Sarkar, A., & Bandyopadhyay, B. (2010). East Kolkata Wetland-A Rich Source of Microbes for Bioremediation and Environmental Protection. The Journal of Plant Science Research, 26(2), 145
 336. Saha, M., Sarkar, S., Sarkar, B., Sharma, B. K., Bhattacharjee, S., and Tribedi, P. (2016). Microbial siderophores and their potential applications: a review. Environ. Sci. Pollut. Res. 23, 3984–3999. doi: 10.1007/s11356-015-4294-0
 337. Sahu, P., & Sikdar, P. (2011). Threat of land subsidence in and around Kolkata City and East Kolkata Wetlands, West Bengal, India. Journal of Earth System Science, 120(3), 435–446.
 338. Said, M. S., Tirthani, E., & Lesho, E. (2023). *Stenotrophomonas maltophilia*. In StatPearls [Internet]. StatPearls Publishing. <https://www.ncbi.nlm.nih.gov/books/NBK572123/>
 339. Saikia, J., Sarma, R.K., Dhandia, R. et al. Alleviation of drought stress in pulse crops with ACC deaminase producing rhizobacteria isolated from acidic soil of Northeast India. Sci Rep 8, 3560 (2018).
 340. Salem MA, Bedade DK, Al-Ethawi L, Al-Waleed SM (2020) Assessment of physiochemical properties and concentration of heavy metals in agricultural soils fertilized with chemical fertilizers. Heliyon 6(10):e05224
 341. Salem MA, Bedade DK, Al-Ethawi L, Al-Waleed SM (2020) Assessment of physiochemical properties and concentration of heavy metals in agricultural soils fertilized with chemical fertilizers. Heliyon 6(10):e05224

342. Sandy M, Butler A (2009) Microbial iron acquisition: marine and terrestrial siderophores. *Chem Rev* 109:4580–4595
343. Santander C, González F, Pérez U, Ruiz A, Aroca R, Santos C, Cornejo P, Vidal G. Enhancing Water Status and Nutrient Uptake in Drought-Stressed Lettuce Plants (*Lactuca sativa* L.) via Inoculation with Different *Bacillus* spp. Isolated from the Atacama Desert. *Plants*. 2024; 13(2):158.
344. Santander, C., González, F., Pérez, U., Ruiz, A., Aroca, R., Santos, C., Cornejo, P., & Vidal, G. (2024). Enhancing Water Status and Nutrient Uptake in Drought-Stressed Lettuce Plants (*Lactuca sativa* L.) via Inoculation with Different *Bacillus* spp. Isolated from the Atacama Desert. *Plants*, 13(2), 158.
345. Santos, R. M., Diaz, P. A. E., Lobo, L. L. B., & Rigobelo, E. C. (2020). Use of plant growth-promoting rhizobacteria in maize and sugarcane: Characteristics and applications. *Frontiers in Sustainable Food Systems*, 4, 136. <https://doi.org/10.3389/fsufs.2020.00136>
346. Santoyo, G., Guzmán-Guzmán, P., Parra-Cota, F. I., Santos-Villalobos, S. d. I., Orozco-Mosqueda, M. d. C., & Glick, B. R. (2021). Plant Growth Stimulation by Microbial Consortia. *Agronomy*, 11(2), 219.
347. Sarah, G., & Thaura, G. (2021). Heavy metals in soils and the remediation potential of bacteria associated with the plant microbiome. *Frontiers in Environmental Science*, 9, 604216.
348. Sardar, S. (2023). Changing land use scenarios and biodiversity of East Kolkata Wetland. *International Journal of Recent Scientific Research*, 14(10), 4213–4217.
349. Saxena AK, Kumar M, Chakdar H, Anuroopa N, Bagyaraj DJ (2020) *Bacillus* species in soil as a natural resource for plant health and nutrition. *J Appl Microbiol* 128(6):1583–1594
350. Schlenker, W., & Roberts, M. J. (2009). Nonlinear temperature effects indicate severe damages to U.S. crop yields under climate change. *Proceedings of the National Academy of Sciences of the United States of America*, 106(37), 15594–15598.
351. Schoenholtz, S. H., Van Miegroet, H., & Burger, J. A. (2000). A review of chemical and physical properties as indicators of forest soil quality: Challenges and opportunities. *Forest Ecology and Management*, 138(1–3), 335–356.

352. Schwyn, B., & Neilands, J. B. (1987). Universal chemical assay for the detection and determination of siderophores. *Analytical Biochemistry*, 160, 46–56.
353. Sehrawat, Sindhu & Glick (2022).Sehrawat A, Sindhu SS, Glick BR. Hydrogen cyanide production by soil bacteria: biological control of pests and promotion of plant growth in sustainable agriculture. *Pedosphere*. 2022;32(1):15–38. doi: 10.1016/S1002-0160(21)60058-9
354. Selassie, Y. G., and Ayanna, G. (2013). Effects of different land use systems on selected physico-chemical properties of soils in Northwestern Ethiopia. *J. Agric. Sci.* 5, 112–120. doi: 10.5539/jas.v5n4p112
355. Semeniuk CA, Semeniuk V (1995) A geomorphic approach to global wetland classification for inland wetlands. *Vegetation* 118:103–124
356. Sengupta, D., Mitra, A., & Choudhury, S. S. (2013). Identification and characterization of the effectively arsenic tolerant bacterial strains from the potential arsenic contaminated site in 24-Parganas (North) district of West Bengal, India. *Nature Environment and Pollution Technology*, 12, 303–308.
357. Seshadri S (2020) The analogy between the effect of various dyes for DNA quantification in QUBIT 4.0
358. Sevak, P. I., Pushkar, B. K., & Kapadne, P. N. (2021). Lead pollution and bacterial bioremediation: a review. *Environmental Chemistry Letters*, 19(6), 4463-4488
359. Shaharoona, B., Arshad, M., & Zahir, Z. A. (2006). Effect of plant growth promoting rhizobacteria containing ACC-deaminase on maize (*Zea mays* L.) growth under axenic conditions and on nodulation in mung bean (*Vigna radiata* L.). *Letters in applied microbiology*, 42(2), 155–159.
360. Shahid SA, Zaman M, Heng L (2018) Introduction to soil salinity, sodicity and diagnostics techniques. In: Zaman Mohammad, Shayadavhid Shabbir A, Heng Lee (eds) *Guideline for salinity assessment, mitigation and adaptation using nuclear and related techniques*. Springer International Publishing, Cham, pp 1–42
361. Shameem MR, Sonali JM, Kumar PS, Rangasamy G, Gayathri KV, Parthasarathy V. *Rhizobium mayense* sp. Nov., an efficient plant growth-promoting nitrogen-fixing bacteria isolated from rhizosphere soil. *Environ Res*. 2023;220:115200.

362. Sharma S, Kumar V, Tripathi RB (2017) Isolation of phosphate solubilizing microorganism (PSMs) from soil. *J Microbiol Biotechnol Res* 1:90–95
363. Sharma S. Risk assessment and mitigation measures on the heavy metal polluted water and sediment of the kolleru lake in Andhra Pradesh, India. *Pollution*. 2019;5:161–178
364. Sharma SS, Dietz KJ (2006) The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. *J Exp Bot* 57(4):711–726. [https://doi.org/ 10.1093/jxb/erj073](https://doi.org/10.1093/jxb/erj073)
365. Sharma, P., & Dubey, R. S. (2005). Lead toxicity in plants. *Brazilian journal of plant physiology*, 17, 35-52.
366. Sharma, P., Pandey, R., & Chauhan, N. S. (2025). *Stenotrophomonas maltophilia* promotes wheat growth by enhancing nutrient assimilation and rhizosphere microbiota modulation. *Frontiers in bioengineering and biotechnology*, 13, 1563670.
367. Sheikhi F, Roayaei Ardakani M, Enayatizamir N, Rodriguez-Couto S. The Determination of Assay for Laccase of *Bacillus subtilis* WPI with Two Classes of Chemical Compounds as Substrates. *Indian J Microbiol*. 2012 Dec;52(4):701-7. doi: 10.1007/s12088-012-0298-3. Epub 2012 Aug 29. PMID: 24293734; PMCID: PMC3516662.
368. Shen X, Hu H, Peng H, Wang W, Zhang X (2013) Comparative genomic analysis of four representative plant growth-promoting rhizobacteria in *Pseudomonas*. *BMC Genomics* 14:1471–2164
369. Shi, H., Lu, L., Ye, J., & Shi, L. (2022). Effects of Two *Bacillus Velezensis* Microbial Inoculants on the Growth and Rhizosphere Soil Environment of *Prunus davidiana*. *International journal of molecular sciences*, 23(21), 13639.
370. Siljanen HMP, et al. Archaeal nitrification is a key driver of high nitrous oxide emissions from arctic peatlands. *Soil Biol. Biochem*. 2019;137:107539
371. Singh, N., Singh, V., Rai, S. N., Vamanu, E., & Singh, M. P. (2022). Metagenomic Analysis of Garden Soil-Derived Microbial Consortia and Unveiling Their Metabolic Potential in Mitigating Toxic Hexavalent Chromium. *Life (Basel, Switzerland)*, 12(12), 2094.
372. Singh, R.; Ryu, J.; Kim, S.W. Microbial consortia including methanotrophs: Some benefits of living together. *J. Microbiol*. 2019, 57, 939–952.

373. Sinsabaugh, R.; Hill, B.; Follstad Shah, J. Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. *Nature* 2010, 462, 795–798
374. Slama, H. B., Cherif-Silini, H., Chenari Bouket, A., Qader, M., Silini, A., Yahiaoui, B., Alenezi, F. N., Luptakova, L., Triki, M. A., Vallat, A., Oszako, T., Rateb, M. E., & Belbahri, L. (2019). Screening for *Fusarium* antagonistic bacteria from contrasting niches designated the endophyte *Bacillus halotolerans* as plant warden against *Fusarium*. *Frontiers in Microbiology*, 9, 3236.
375. Smakhtin, V., Revenga, C., & Döll, P. (2004). A Pilot Global Assessment of Environmental Water Requirements and Scarcity. *Water International*, 29(3), 307–317.
376. Solano-Alvarez, N., Valencia-Hernández, J. A., Rico-García, E., Torres-Pacheco, I., Ocampo-Velázquez, R. V., Escamilla-Silva, E. M., Romero-García, A. L., Alpuche-Solís, Á. G., & Guevara-González, R. G. (2021). A Novel Isolate of *Bacillus cereus* Promotes Growth in Tomato and Inhibits *Clavibacter michiganensis* Infection under Greenhouse Conditions. *Plants (Basel, Switzerland)*, 10(3), 506.
377. Somenahally AC, Hollister EB, Loeppert RH et al (2011) Microbial communities in rice rhizosphere altered by intermittent and continuous flooding in fields with long-term arsenic application. *Soil Biol Biochem* 43:1220–1228
378. Song, L., Gong, J., Zhang, Z., Zhang, W., Zhang, S., Dong, J., Dong, X., Hu, Y., & Liu, Y. (2023). Changes in plant phosphorus demand and supply relationships in response to different grazing intensities affect the soil organic carbon stock of a temperate steppe. *The Science of the total environment*, 876, 163225.
379. Souza, R.D., Ambrosini, A., Passaglia, L.M.P. Plant growth-promoting bacteria as inoculants in agricultural soils. *Genet. Mol. Biol.*, 2015; **38**, (4): 401-419.
380. Space Applications Centre (SAC). (2021). National Wetland atlas. pp: 310.
381. Spaepen S, Vanderleyden J (2011) Auxin and plant-microbe interactions. *Cold Spring Harb Perspect Biol* 3:a001438
382. Spaepen S, Vanderleyden J, Remans R (2007b) Indole-3-acetic acid in microbial and microorganism plant signaling. *FEMS Microbiol Rev* 31:425–448

383. Sparks DL (1980) Chemistry of soil potassium in Atlantic Coastal Plain soils: a review. *Commun Soil Sci Plant Anal* 11(5):435–449
384. Sposito G, LeVesque CS, Hesterberg D (1986) Calcium-magnesium exchange on illite in the presence of adsorbed sodium. *Soil Sci Soc Am J* 50(4):905–909
385. Stevenson B.S., Eichorst S.A., Wertz J.T., Schmidt T.M., Breznak J.A. New strategies for cultivation and detection of previously uncultured microbes. *Appl. Environ. Microbiol.* 2004;70:4748–4755. doi: 10.1128/AEM.70.8.4748-4755.2004.
386. Suliasih, & Widawati, S. (2020). Isolation of Indole Acetic Acid (IAA) producing *Bacillus siamensis* from peat and optimization of the culture conditions for maximum IAA production. *IOP Conference Series: Earth and Environmental Science*, 572, 012025. <https://doi.org/10.1088/1755-1315/572/1/012025>
387. Sumathy, J. (2017). A comparative study on the toluene degrading bacteria isolated from polluted environments. *World Journal of Pharmaceutical Research*, 1025–1045.
388. Sun, Y., Cheng, Z., & Glick, B. R. (2009). The presence of a 1-aminocyclopropane-1-carboxylate (ACC) deaminase deletion mutation alters the physiology of the endophytic plant growth-promoting bacterium *Burkholderia phytofirmans* PsJN. *FEMS microbiology letters*, 296(1), 131–136. <https://doi.org/10.1111/j.1574-6968.2009.01625.x>
389. Sun, X., Niu, Y., Du, Y., Geng, C., Guo, C., & Zhao, L. (2023). Isolation and Identification of New Soil Strains with Phosphate-Solubilizing and Exopolysaccharide-Producing Abilities in the Yellow River Wetland Nature Reserve of Luoyang City, China. *Sustainability*, 15(4), 3607.
390. Tabatabai, M. A., & Bremner, J. M. (1969). Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biology and Biochemistry*, 1(4), 301–307. [https://doi.org/10.1016/0038-0717\(69\)90012-1](https://doi.org/10.1016/0038-0717(69)90012-1)
391. Teng ZD, Shao W, Zhang KY, Huo YQ, Li M (2019) Characterization of phosphate solubilizing bacteria isolated from heavy metal contaminated soils and their potential for lead immobilization. *J Environ Manage* 231:189–197 DOI:
392. Timofeeva, A. M., Galyamova, M. R., & Sedykh, S. E. (2024). How Do Plant Growth-Promoting Bacteria Use Plant Hormones to Regulate Stress Reactions? *Plants*, 13(17), 2371.

393. Tiner, R. W. 1999. *Wetland Indicators: a Guide to Wetland Identification, Delineation, Classification, and Mapping*. Lewis Publishers
394. Tiner RW (1999) *Wetland Indicators: a guide to wetland identification, delineation, classification, and mapping*. CRC press, Boca Raton
395. Tiner RW (2018) *Wetland delineation: Overview*. In: Finlayson CM, Everard M, Irvine K, McInnes R, Middleton BA, van Dam AA, Davidson NC (eds) *The wetland book I: structure and functions, management, and methods*. Springer, Cham, pp 1469–1482
396. Tiner RW, Milton GR (2018) *Estuarine marsh: an overview*. In: Finlayson CM, Milton GR, Prentice RC, Davidson NC (eds) *The wetland book II: distribution, description, and conservation*. Springer, Cham, pp 55–72
397. Torsvik, V., et al. (1990). Microbial diversity in soil: A metagenomic approach. *Science*, 250(4983), 1443–1449.
398. Turan M, Kıtır N, Alkaya Ü, Günes A, Tüfenkçi Ş, Yıldırım E, Nikerel E (2016) Making soil more accessible to plants: the case of plant growth promoting rhizobacteria. *Plant growth. InTech*, Rijeka. doi:10.5772/64826
399. Tveit, A., Schwacke, R., Svenning, M. et al. Organic carbon transformations in high-Arctic peat soils: key functions and microorganisms. *ISME J* 7, 299–311 (2013).
400. Upadhyay, P., Shrivastava, R. & Agrawal, P.K. Bioprospecting and biotechnological applications of fungal laccase. *3 Biotech* 6, 15 (2016).
401. Vasumathi V., Kalpana R., Pazhanivelan S., Priya M.V., Arasan Pon A. (2025). Classification, Distribution, Features, Biodiversity and Functions of Wetlands: A Review . *Agricultural Reviews*. 46(3): 359-367. doi: 10.18805/ag.R-2559..
402. Velho-Pereira S, Kamat NM. Antimicrobial Screening of Actinobacteria using a Modified Cross-Streak Method. *Indian J Pharm Sci*. 2011 Mar;73(2):223-8. doi: 10.4103/0250-474x.91566. PMID: 22303068; PMCID: PMC3267309.
403. Velicer, W. F., & Fava, J. L. (2003). Time Series Analysis. In J. Schinka & W. F. Velicer (Eds.), *Research Methods in Psychology* (pp. 581–606). *Handbook of Psychology*, Vol. 2. New York: John Wiley & Sons.

404. Verhoeven, J. T., & Setter, T. L. (2010). Agricultural use of wetlands: opportunities and limitations. *Annals of botany*, 105(1), 155–163.
405. Verma, A., Patidar, Y., & Vaishampayan, A. (2016). Isolation and purification of potassium solubilizing bacteria from different regions of India and its effect on crop's yield. *Indian Journal of Microbiology Research.*, 3(4), 483– 488.
406. von Willert, F. J., & Stehouwer, R. C. (2003). Compost and calcium surface treatment effects on subsoil chemistry in acidic minespoil columns. *Journal of Environmental Quality*, 32(3), 781–788. <https://doi.org/10.2134/jeq2003.7810>
407. Wadhwa, R. R., & Marappa-Ganeshan, R. (2023). T Test - StatPearls. StatPearls Publishing. Weise, T., Kai, M., & Piechulla, B. (2013). Bacterial ammonia causes significant plant growth inhibition. *PLoS ONE*, 8(5), e63538. <https://doi.org/10.1371/journal.pone.0063538>
408. Wang H, Shao W, Hu Y, Cao W, Zhang Y (2023) Assessment of Six Machine Learning Methods for Predicting Gross Primary Productivity in Grassland. *Remote Sensing* 15(14):3475.
409. Wang R, Cui L, Li J and Li W (2023) Factors driving the halophyte rhizosphere bacterial communities in coastal salt marshes. *Front. Microbiol.* 14:1127958. doi: 10.3389/fmicb.2023.1127958
410. Wang T, Deng Z, Zhang C, Zou Y, Xie Y, Li F, Xiao F, Peng C (2024) Vegetation types and flood water level are dominant factors controlling the carbon sequestration potential in Dongting Lake floodplain, China. *Sci Total Environ* 921:171146–171146.
411. Wang, M.; Wang, Y.; Yao, Y. Analysis on Pattern Change and Driving Force of Lake-marsh Wetlands in Aerxiang. *China Resour. Compr. Util.* 2022, 40, 83–85
412. Wang, Q., Garrity, G.M., Tiedje, J.M. and Cole, J.R. (2007) Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol* 73, 5261-5267.
413. Wang, Q., Xie, H., Ngo, H.H. et al. Microbial abundance and community in subsurface flow constructed wetland microcosms: role of plant presence. *Environ Sci Pollut Res* 23, 4036–4045 (2016).

414. Wani, P. A., Khan, M. S., & Zaidi, A. (2007). Co-inoculation of nitrogen-fixing and phosphate-solubilizing bacteria to promote growth, yield and nutrient uptake in chickpea. *Acta Agronomica Hungarica*, 55(3), 315–323. <https://doi.org/10.1556/AAgr.55.2007.3.8>
415. Wang, X., Xiao, X., Xu, X., Zou, Z., Chen, B., Qin, Y., et al. (2021). Rebound in China's coastal wetlands following conservation and restoration. *Nat. Sustain.* 4, 1076–1083. doi: 10.1038/s41893-021-00793-5.
416. Weise T, Kai M, Piechulla B (2013) Bacterial Ammonia Causes Significant Plant Growth Inhibition. *PLoS ONE* 8(5): e63538.
417. Whalen, J.K., Warman, P.R. Arylsulfatase activity in soil and soil extracts using natural and artificial substrates. *Biol Fertil Soils* **22**, 373–378 (1996). <https://doi.org/10.1007/BF00334586>
418. Willsey GG, Eckstrom K, LaBauve AE, Hinkel LA, Schutz K, Meagher RJ, LiPuma JJ, Wargo MJ. *Stenotrophomonas maltophilia* Differential Gene Expression in Synthetic Cystic Fibrosis Sputum Reveals Shared and Cystic Fibrosis Strain-Specific Responses to the Sputum Environment. *J Bacteriol.* 2019 Aug 01;201(15)
419. Wojciechowska E, Nawrot N, Walkusz-Miotk J, Matej-Łukowicz K, Pazdro K. Heavy metals in sediments of urban streams: Contamination and health risk assessment of influencing factors. *Sustainability.* 2019; 11:5–10.
420. Wojciechowska, E., Nawrot, N., Walkusz-Miotk, J., Matej-Łukowicz, K., & Pazdro, K. (2019). Heavy Metals in Sediments of Urban Streams: Contamination and Health Risk Assessment of Influencing Factors. *Sustainability*, 11(3), 563.
421. Wolde, Z. (2016). A review on evaluation of soil potassium status and crop response to potassium fertilization. *Journal of Environment and Earth Science*, 6(12), 54–66.
422. Wong, C. S. C., & Li, X. (2003). Analysis of heavy metal contaminated soils. *Practice Periodical of Hazardous, Toxic, and Radioactive Waste Management*, 7(1), 12–18.
423. World Health Organization (WHO). (1996). Permissible limits of heavy metals in soil and plants. World Health Organization.

424. Wu J., Pichler D., Marley D., Wilson D., Hovakimyan N. & Hobbs J. 2023 Extended Agriculture-Vision: An Extension of a Large Aerial Image Dataset for Agricultural Pattern Analysis. arXiv preprint arXiv:2303.02460.
425. Wu, H., Wang, X., He, X., Zhang, S., Liang, R., & Shen, J. (2017). Effects of root exudates on denitrifier gene abundance, community structure and activity in a micro-polluted constructed wetland. *The Science of the total environment*, 598, 697–703.
426. Wu, C., Chen, W., Cao, C. *et al.* Diagnosis of Wetland Ecosystem Health in the Zoige Wetland, Sichuan of China. *Wetlands* **38**, 469–484 (2018). <https://doi.org/10.1007/s13157-018-0992-y>
427. Wylynko, D. (1999). Prairie wetlands and carbon sequestration. *Assessing Sinks under Kyoto Protocol*, 12–13
428. Właśniewski S, Hajduk E (2012) The accumulation of cadmium in soils and selected vegetables cultivated in the allotment gardens of Rzeszów. *Soil Sci Ann* 63:55–60.
429. Yadav A, Kansal ML, Singh A. Land use and land cover dynamics in the Upper Ganga Riverine Wetland: unraveling ecosystem services over two decades. *Environ Monit Assess.* 2024 May 31;196(6):590. doi: 10.1007/s10661-024-12748-2. PMID: 38819716.
430. Yadav AN (2021) Microbial biotechnology for bio-prospecting of microbial bioactive compounds and secondary metabolites. *J Appl Biol Biotechnol* 9:1–6.
431. Yadav, P. K., Jha, P., Joy, M. S., Bansal, T., Rawat, P., Begam, S., & Singh, A. K. (2025). Investigating the role of policies in the sustainable management of East Kolkata wetlands. *Discover Global Society*, 3(1), 79.
432. Yan J, Du J, Su F, Zhao S, S, Feng P. Reclamation and Ecological Service Value Evaluation of Coastal Wetlands Using Multispectral Satellite Imagery. *Wetlands (Wilmington)*. 2022;42(3):20. doi: 10.1007/s13157-022-01537-7. Epub 2022 Feb 19. PMID: 35228770; PMCID: PMC8865732.
433. Yan, Y., Hong, S., Chen, A., Peñuelas, J., Allen, C. D., Hammond, W. M., Munson, S. M., Myneni, R. B., & Piao, S. (2025). Satellite-based evidence of recent decline in global forest recovery rate from tree mortality events. *Nature Plants*, 11(4), 731–742.

434. Yan, Y., Wang, Y., Li, J., Zhang, J., & Mo, X. (2025). Crop yield time-series data prediction based on multiple hybrid machine learning models. *Applied and Computational Engineering*, 133, 215–221. DOI: 10.54254/2755-2721/133/2025.20800
435. Yang-Guang, G., Wang, X.-N., Lin, Q., Du, F.-Y., Ning, J.-J., Wang, L.-G., & Li, Y.-F. (2016). Fuzzy comprehensive assessment of heavy metals and Pb isotopic signature in surface sediments from a bay under serious anthropogenic influences: Daya Bay, China. *Ecotoxicology and Environmental Safety*, 126, 38–44.
436. Yim, B., Heider, M. A., Bloem, E., Vetterlein, D., Behr, J. H., Babin, D., & Smalla, K. (2025). Exploring the potential of seed inoculation with microbial consortia to mitigate drought stress in maize plants under greenhouse conditions. *Plant and Soil*.
437. Yim, B., Heider, M.A., Bloem, E. et al. Exploring the potential of seed inoculation with microbial consortia to mitigate drought stress in maize plants under greenhouse conditions. *Plant Soil* (2025).
438. Yousaf, A., Khalid, N., Aqeel, M., Noman, A., Naeem, N., Sarfraz, W., Ejaz, U., Qaiser, Z., & Khalid, A. (2021). Nitrogen Dynamics in Wetland Systems and Its Impact on Biodiversity. *Nitrogen*, 2(2), 196-217.
439. Yu, L., Li, X., & Wang, H. (2022). Impact of agricultural and industrial activities on wetland soil chemistry. *Environmental Pollution*, 300, 118932.
440. Zedler, J. B., & Kercher, S. (2005). Wetland resources: Status, trends, ecosystem services, and restorability. *Annual Review of Environment and Resources*, 30, 39–74.
441. Zethof J.H.T., Bettermann A., Vogel C., Babin D., Cammeraat E.L.H., Solé-Benet A., Lázaro R., Luna L., Nesme J., Woche S.K., et al. Prokaryotic Community Composition and Extracellular Polymeric Substances Affect Soil Microaggregation in Carbonate Containing Semiarid Grasslands. *Front. Environ. Sci.* 2020;8:51. doi: 10.3389/fenvs.2020.00051.
442. Zhang L, Lv J (2020) Metagenomic analysis of microbial community and function reveals the response of soil respiration to the conversion of cropland to plantations in the Loess Plateau of China. *Global Ecol Conserv* 23:e01067
443. Zhang S, Gao P, Tong Y, Norse D, Lu Y, Powlson D (2015) Overcoming nitrogen fertilizer over-use through technical and advisory approaches: a case study from Shaanxi Province, Northwest China. *Agric Ecosyst Environ* 209:89–99

444. Zhang, R., Wang, S., Zhong, H., Fu, X., Li, L., Wang, L., & Liu, Y. (2024). Microbial C and N Metabolism Alterations Based on Soil Metagenome and Different Shrub Invasion Stages in Sanjiang Plain Wetlands. *Microorganisms*, 12(8), 1648.
445. Zhang, R., Wang, S., Zhong, H., Fu, X., Li, L., Wang, L., & Liu, Y. (2024). Microbial C and N Metabolism Alterations Based on Soil Metagenome and Different Shrub Invasion Stages in Sanjiang Plain Wetlands. *Microorganisms*, 12(8), 1648. <https://doi.org/10.3390/microorganisms12081648>
446. Zhao, H., Wu, Y., Lan, X., Yang, Y., Wu, X., & Du, L. (2022). Comprehensive assessment of harmful heavy metals in contaminated soil in order to score pollution level. *Scientific Reports*, 12, 3552.
447. Zhao, S., Peng, C., Jiang, H., Tian, D., Lei, X., & Zhou, X. (2006). Land use change in Asia and the ecological consequences. *Ecological Research*, 21(6), 890–896.
448. Zheng, Y., Su, F., Li, H., Song, F., Wei, C., & Cui, P. (2024). Structure and function of soil bacterial communities in the different wetland types of the Liaohe Estuary Wetland. *Microorganisms*, 12(10), 2075.
449. Zhu, Y., Xing, Y., Li, Y., Jia, J., Ying, Y., & Shi, W. (2024). *The Role of Phosphate-Solubilizing Microbial Interactions in Phosphorus Activation and Utilization in Plant–Soil Systems: A Review*. **Plants**, 13(19), 2686. <https://doi.org/10.3390/plants13192686>
450. Zuo, W., Song, B., Shi, Y., Zupanic, A., Guo, S., Huang, H., ... & Yu, Y. (2022). Using *Bacillus thuringiensis* HM-311@ hydroxyapatite@ biochar beads to remediate Pb and Cd contaminated farmland soil. *Chem*
451. Zwolak, A., Sarzyńska, M., Szpyrka, E., & Stawarczyk, K. (2019). Sources of soil pollution by heavy metals and their accumulation in vegetables: A review. *Water, Air, & Soil Pollution*, 230(7), 164.



PUBLICATIONS

Received: 30 June 2023 | Revised: 3 April 2024 | Accepted: 11 May 2024

DOI: 10.1002/tqem.22259

RESEARCH ARTICLE

WILEY

Unleaded agriculture: Myth to reality by wetland microbial flora

Sanjana Ghosh¹  | Arup Kumar Mitra¹ | Sudeshna Shyam Choudhury¹ | Ayan Chandra²

¹Department of Microbiology, St. Xavier's College (Autonomous), Kolkata, West Bengal, India

²Department of Statistics, St. Xavier's College (Autonomous), Kolkata, West Bengal, India

Correspondence

Sanjana Ghosh, Department of Microbiology, St. Xavier's College (Autonomous), West Bengal, Kolkata.
Email: sanjana.ghosh2018@gmail.com

Abstract

Wetlands, refer to the confluence with regards to land and water having a rich biological diversity with multiple purpose. As per the Ramsar Convention, framed in August 2002, East Kolkata Wetlands have been considered to be of global significance. Although inappropriate supervision of these wetlands, has led to the gradual deterioration of this wetland. In this present study, the soil sample was collected from these reclaimed agricultural wetlands of Chowbaga area, of East Kolkata areas with coordinates of 22.5296°N, 88.4207°E. The predominant or lingering bacterial flora was isolated from the soil surrounding the rhizospheric region of *Cucurbita* sp. and *Zea mays* and their plant growth promotion properties of the lingering bacterial flora were analyzed. These plant growth promoting rhizobacteria (PGPR) included some species of *Bacillus* sp. that could augment plant growth by different methodologies like nitrogen fixation, phosphate solubilization, phytohormone synthesis, and siderophore production. One of the bacterial isolates DPP(C) showed highest ability of siderophore production of 37.89% at 48 hours, whereas DCP(A) showed better capability in IAA production of 56.785 µg/mL than rest of the bacterial isolates. These reclaimed agricultural lands were found to have very high quantities of heavy metals, especially lead. All the bacterial isolates, were found to have great potential in lead mitigation, whereby they could adsorb up to 8.28% of lead. Some studies have shown the capability of microbial degradation for bioremediation of organic forms of lead and thereby can be removed from agricultural field for longer period of time. The prospective potential use of PGPR has gradually escalated, since it is one of the best substitutes against the constant usage of chemical fertilizers as well as pesticides. The use of these PGPR is one of the most reliable methods for ensuring sustainable agriculture. Furthermore, in future, the PGPR population will be correlated with the nutrient availability and productivity of the plants.

KEYWORDS

Cucurbita sp, East Kolkata Wetlands, lead, microbial flora, PGPR, soil, *Zea mays*

Vegetos
https://doi.org/10.1007/s42535-024-01079-9



RESEARCH ARTICLES



Exploring soil fertility dynamics and microbial flora in the east kolkata wetlands: implications for sustainable agriculture and ecosystem conservation

Sanjana Ghosh¹ · Rahul Rautela^{2,3} · Arup Kumar Mitra¹ · Sudeshna Shyam Choudhury¹ · Ayan Chandra⁴ · Sunil Kumar² · Bikram Dhara^{5,6}

Received: 14 May 2024 / Revised: 27 September 2024 / Accepted: 18 October 2024
© The Author(s) under exclusive licence to Society for Plant Research 2024

Abstract

This study centres on internationally recognized East Kolkata Wetlands, renowned for their rich biodiversity and multifunctional ecosystem. Despite their Ramsar Convention designation, inadequate management has led to gradual degradation of these wetlands. Soil samples were collected from reclaimed agricultural lands within the wetlands, and an analysis of physico-chemical parameters was conducted during initial, peak, and inter-cropping periods of *Cucurbita maxima*. Notable variations were observed in nitrogen, ranging from 298.5 kg/ha to 563.7 kg/ha, phosphate levels varying from 100.49 kg/ha to 148.32 kg/ha, and potassium levels fluctuating between 139.77 kg/ha and 173.37 kg/ha. These variations signify a substantial impact on soil fertility. The wetlands harbour a diverse microbiome with distinct biochemical roles, influencing soil fertility and resource availability for plants. This investigation unveils the intricate relationship between metagenome and soil fertility parameters, shedding light on the presence of keystone bacterial species such as *Bacillus* sp. pivotal for maintaining agricultural productivity throughout the year. Future research aims to establish correlations between soil nutrient quality and plant age, offering insights into specific nutritional requirements of the soil.

Keywords East Kolkata Wetlands · *Cucurbita* sp · Physico-chemical parameters · Microbial flora · Metagenomic analysis

Introduction

Wetlands are effective ecosystem that have huge relevance in ensuring sustainable development of biodiversity and responsible for maintaining the stability of ecological environment in biosphere. As per Ramsar Convention framed in the year 1971, wetlands can be defined as “area of marshes, ferns, peatlands or the water source in case of artificial or be it natural, permanent either temporary, with water, i.e., static or flowing, fresh, brackish or salt including areas of marine water, the depth of which at low tide must not transcend six meters.” The East Kolkata Wetlands (EKW), are referred as a Ramsar Site. It is of paramount importance in West Bengal, India. EKW functions as kidney for the areas in and around Kolkata and helps to preserve as well as maintain the balance in ecology and environmental sustainability. This wetland has the ability to dissipate huge quantities of pollutants that are released from the urban regions of Kolkata. The delicate balance of ecology in this area has been affected due to land conversions, thereby leading to improper sustainable development (Mondal et al. 2022).

✉ Bikram Dhara
bikramdhara.smc@saveetha.com

¹ Department of Microbiology, St. Xavier's College (Autonomous), Kolkata, West Bengal 700 016, India

² CSIR-National Environmental Engineering Research Institute (CSIR-NEERI), Nehru Marg, Nagpur, Maharashtra 440 020, India

³ Academy of Scientific and Innovative Research (AcSIR), Ghaziabad, Uttar Pradesh 201 002, India

⁴ Department of Statistics, St. Xavier's College (Autonomous), Kolkata, West Bengal 700 016, India

⁵ Centre for Global Health Research, Saveetha Institute of Medical and Technical Sciences, Chennai, India

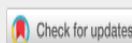
⁶ Department of Health Sciences, Novel Global Community and Educational Foundation, Hebersham, NSW, Australia

Research Articles

Potential of *Lasiodiplodia theobromae* in mitigating *Polyalthia suberosa* invasion in a semi-urban sanctuary

Swapneel Saha , Sanjana Ghosh , Ashim Chandra Roy , Krishna Ray , Sandip Kumar Basak , Rabishankar Sengupta [...show all](#)

Pages 725-735 | Received 18 Mar 2024, Accepted 27 Apr 2025, Published online: 29 May 2025

Cite this article  <https://doi.org/10.1080/11263504.2025.2500387> Full Article Figures & data References Citations Metrics Reprints & Permissions

Read this article

Abstract

Bethuadahari Wildlife Sanctuary (23°35'N, 88°23'E), situated in the Nadia district of West Bengal, India, is a habitat for various herbivores, notably the spotted deer (*Axis axis*). However, the sanctuary faces challenges due to invasive species like *Polyalthia suberosa*, which diminish grazing lands by outcompeting with local vegetation. This study focused on a fungal strain isolated from *P. suberosa* branches within the sanctuary. The fungus sourced from *P. suberosa* branches was examined for its enzymatic capabilities. This strain exhibited ligninolytic and cellulolytic activities, indicating its potential impact on *P. suberosa*. The fungus was inoculated onto *P. suberosa* wood to confirm the host-pathogen interaction, resulting in a substantial 47.6% weight loss over six weeks. Scanning electron microscopy revealed the extent of damage inflicted by the fungus on the wood. Molecular

Rela

Pec

Spec
of poSifar
Plant
with
Publi

Dete



A Holistic Treatise on Nature Driven Solution to Cancer

Sanjana Ghosh, Stootee Baruah, Suranjana Ray Chaudhuri,
Shoham Ghosh, Arup Kumar Mitra,
and Sudeshna Shyam Choudhury

Abstract

The World Health Organization (WHO) has mentioned cancer as the second-leading cause of death worldwide, resulting in the loss of ten million lives per year. Although, enormous strides in the fields of cancer research and medicine have helped in effective management of cancer, it is still not completely curable. Thus, the forage for more constructive, economical and safe treatments has led to the revelation of bioactive compounds. These naturally occurring molecules have been seen to produce medicative effects on various tumour cell lines. They are found in the form of secondary metabolites from different plants, fungi and bacteria and have shown response against cancer. This study, involves an in-depth study of the activity of different bioactive compounds against cancer. Plant-derived bioactive, such as curcumin, myricetin, geraniin and tocotrienols, have been found to affect the dictum of cell growth and apoptosis and possess antitumour possessions, which were found to inhibit augmentation in many cancer cell lines, like lung, pancreatic, breast, prostate, skin, stomach and colon cancers. As per the literature considered, the impacts of bioactive compounds, like curcumin with an IC_{50} value of $49 \pm 2.08 \mu\text{g/ml}$ against MDA-MB-231 cell lines and myricetin with an IC_{50} value of 690 mM , have been seen to prevent hFEN1 attachment, thereby inhibiting colon cancer, geraniin with a concentration of $10 \mu\text{M}$ reduced the NF- κB expression in C666-1 cells. Endophytic fungi like *Penicillium* sp. released compounds such as podophyllotoxin and diosgenin that are able to act at the transcriptional level and prevent the growth of metastatic cells. Fucoxanthin, a marine-derived bioactive, can induce cell growth arrest, apoptosis and autophagy in cells. Endophytic actinomycetes have shown to produce bioactive compounds that showed toxicity against human lung cancer



Anammox Process

Technological Advancement and Application in Industrial Wastewater Treatment Plant

2024, Pages 143-166



8 - Autotrophic nitrogen removal technology: A boon for Kolkata Ramsar sites

Debapriya Maitra, Bedaprana Roy, Sanjana Ghosh, Sanjana Lahiri, Gorky Guha,
Sudeshna Shyam Choudhury, Arup Kumar Mitra

Show more ▾

+ Add to Mendeley Share Cite

<https://doi.org/10.1016/B978-0-443-19209-8.00009-0> ↗

[Get rights and content](#) ↗

Abstract

Globally, wetlands are known to cover around 6.4% of the total area across the globe, with India having a coverage of 4.3% of its total land area. With an area coverage of about 12,500ha, the East Kolkata Wetlands (EWKs) are an ecological marvel serving as the world's largest organic sewage management system. An approximate amount of 250 million gallons of sewage effluents generated by the city per day along with additional

Chapter 1

Harnessing Carbon Nanomaterials: Applications and Innovations

Sanjana Ghosh

*St. Xavier's College (Autonomous),
Kolkata, India*

-5731

*St. Xavier's College (Autonomous),
Kolkata, India*

Debalina Saha

*St. Xavier's College (Autonomous),
Kolkata, India*

Arup Kumar Mitra

*St. Xavier's College (Autonomous),
Kolkata, India*

Stootee Baruah

 <https://orcid.org/0009-0002-5455-2014>

*St. Xavier's College (Autonomous),
Kolkata, India*

Sudeshna Shyam Choudhury

*St. Xavier's College (Autonomous),
Kolkata, India*

Ayan Chandra

*St. Xavier's College (Autonomous),
Kolkata, India*

Aniket Mookerjee

 <https://orcid.org/0009-0001-7945>

ABSTRACT

Carbon-based nanomaterials have a small size with a range between one to hundred nanometers. The unique structure has made them capable of incorporating highly specialized groups, thereby modifying their attribute and making them suitable for environmental sensing. They showcase unique mechanical, chemical, and electrical possessions, thereby exploring their capacity to develop highly sensitive sensors. The advent of recent technology has made these carbon nanomaterials capable to sequester and tackle water pollution. Therefore, in this chapter a detailed study has been done to interpret about the nature of carbon nanomaterials, followed by their types. The study has also included the impact of carbon nanomaterials upon

DOI: 10.4018/979-8-3693-8257-8.ch001

Copyright © 2024, IGI Global. Copying or distributing in print or electronic forms without written permission of IGI Global is prohibited.

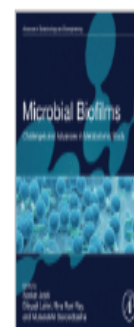


Microbial Biofilms

Challenges and Advances in Metabolomic Study

Advances in Biotechnology and Bioengineering

2023, Pages 289-314



Chapter 18 - Strategies to reduce microbial biofilm in medical prosthesis and other devices

Juhita Dhar, Anupriya Das, Soumyadip Biswas, Talaha Nishat Ahmed, Sanjana Ghosh,
Arup Kumar Mitra

Show more ▼

+ Add to Mendeley Share Cite

<https://doi.org/10.1016/B978-0-323-95715-1.00006-6> ↗

Get rights and content ↗

 Requires Authentication Published by De Gruyter
2023

Extremophilic pigments in eukaryotic microbes: a tool for survivability

From the book *Extremophiles*
*Riddhi Basu , Sanjana Ghosh , Bikram Dhara ,
Ishita Roy , Elizabeth Basumatary , Supriti Paul
and Arup Kumar Mitra*

<https://doi.org/10.1515/9783110788488-008>

Cite this

Share this

PATENT



Office of the Controller General of Patents, Designs & Trade Marks
Department for Promotion of Industry and Internal Trade
Ministry of Commerce & Industry,
Government of India



Application Details

APPLICATION NUMBER	202431101754
APPLICATION TYPE	ORDINARY APPLICATION
DATE OF FILING	22/12/2024
APPLICANT NAME	1 . St. Xavier's College, Kolkata 2 . SOUMYAJIT MODAK 3 . Arunabha Pal 4 . Adrish Sil 5 . Srinjoy Maitra 6 . Sanjana Ghosh 7 . Bedaprana Roy 8 . Ansh Arya 9 . Aarav Mittal 10 . Vedant Saboo 11 . Aditi Sanki 12 . Arup Kumar Mitra
TITLE OF INVENTION	A PROBIOTIC FORMULATION FOR ENHANCING IMMUNITY
FIELD OF INVENTION	FOOD
E-MAIL (As Per Record)	rupkathaganguly01@gmail.com
ADDITIONAL-EMAIL (As Per Record)	
E-MAIL (UPDATED Online)	
PRIORITY DATE	
REQUEST FOR EXAMINATION DATE	--
PUBLICATION DATE (U/S 11A)	08/08/2025

PATENT



Office of the Controller General of Patents, Designs & Trade Marks
Department for Promotion of Industry and Internal Trade
Ministry of Commerce & Industry,
Government of India



Application Details

APPLICATION NUMBER	202531068085
APPLICATION TYPE	ORDINARY APPLICATION
DATE OF FILING	16/07/2025
APPLICANT NAME	1 . SANJANA GHOSH 2 . Shoham Ghosh 3 . Arup Kumar Mitra
TITLE OF INVENTION	A METHOD FOR IDENTIFICATION AND FORMULATION OF BACTERIA FOR DEGRADATION OF PLASTIC
FIELD OF INVENTION	BIOTECHNOLOGY
E-MAIL (As Per Record)	rupkathaganguly01@gmail.com
ADDITIONAL-EMAIL (As Per Record)	
E-MAIL (UPDATED Online)	
PRIORITY DATE	
REQUEST FOR EXAMINATION DATE	--
PUBLICATION DATE (U/S 11A)	25/07/2025

(12) PATENT APPLICATION PUBLICATION

(21) Application No.202431016997 A

(19) INDIA

(22) Date of filing of Application :09/03/2024

(43) Publication Date : 10/05/2024

(54) Title of the invention : A BIOACTIVE MECHANIZED AEROBIC COMPOSTER

(51) International classification :C05F0017907000, C05F0017964000, C05F0017900000, C05F0017979000, C05F0017993000

(86) International Application No :NA
Filing Date :NA

(87) International Publication No : NA

(61) Patent of Addition to Application Number :NA
Filing Date :NA

(62) Divisional to Application Number :NA
Filing Date :NA

(71)Name of Applicant :

1)St. Xavier's College, Kolkata (Under Calcutta University)

Address of Applicant :30, Mother Teresa Sarani, Kolkata, West Bengal, India -----

2)Entrepreneurship Development Cell, St. Xavier's College Kolkata

3)Agnik Ghoshal

4)Dr. Arup Kumar Mitra

5)Bikram Dhara

6)Sanjana Ghosh

7)Debapriya Maitra

8)Bedaprana Roy

9)Rishab Dugar

10)Sahil Choudhary

11)Prashant Toshniwal

Name of Applicant : NA

Address of Applicant : NA

(72)Name of Inventor :

1)Jenifer Rajak

Address of Applicant :Indranita Apartment, Flat- C/3 27 B.N.Ghoshal Road, Belgharia Kolkata West Bengal India 700056 Kolkata -----

2)Ashok Kumar Das

Address of Applicant :40 Nityananda Nagar, 2nd Bye Lane , Danesh Sheikh Lane Howrah West Bengal India 711109 Kolkata - -----

3)Srinjoy Maitra

Address of Applicant :10, Raja road, Sukhchar, Kolkata-700015 Kolkata -----

4)Agnik Ghoshal

Address of Applicant :58, Dakshinayan, Ramkrishna Pally, Chandmari Road, Hanskhali Pole, Howrah, West Bengal, India Kolkata -----

(57) Abstract :

A BIOACTIVE MECHANIZED AEROBIC COMPOSTER The present invention describes an apparatus for aerobic composting. In an aspect of the invention, the aerobic composter comprises a tubular container, with a removable lid. The container comprises a stirrer system, with a central axis, from which stirrer rods are arranged in a typical pattern, having horizontal and vertical stirring rods at particular intervals along the length of the composting container, to ensure optimum mixing and aeration in the compost mass. A hollow operating arm connected to the central axis of the stirrers is present above the lid of the compost container, which serves the purpose of operating the stirrers externally, as well as the entry pathway of water and air. A perforated sheet at the bottom of the container, filters the leachate released from the composting mass which can be collected by means of a tap fitted outside the composter.

No. of Pages : 17 No. of Claims : 5

LAURELS ACHIEVED:

1. Won the best paper presentation award in Research scholar category in Khulna University, Bangladesh.
2. Won the best paper award in Calcutta University, 2022
3. Won the best paper award in Microbiology section of 1st Botanical Congress, held at Calcutta University, 2023
4. Won the second prize in oral presentation at SCALFE, Himachal Pradesh University, 2023
5. Won the best paper award at Biospectrum, UEM, 2023.
6. Won the best paper award at Regional State Science Congress, 2023 in Environmental Science Section.
7. Won the best paper award in Research Scholar category at ICEES, Ranchi, 2024
8. Won the second prize in oral presentation in Ram Krishna Mission, 2024
9. Won the best paper Award in International Conference on Plant Biotechnology for Sustainable Future, 2025 held at St. Xavier's Burdwan, 2025.
10. Won the best paper Award at CRISEA, Goa University, February 2025.

