

Cadmium Immobilization in Rhizosphere and Plant-Cellular Detoxification: Role of Plant Growth-Promoting Rhizobacteria as a Sustainable Solution

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Doctor of Philosophy



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STATEMENT OF ORIGINALITY

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Different letters indicate the significant differences (a, b, c, and d, for pCHP, 1:3 CCHP, 1:1 CCHP, and 3:1 CCHP, respectively) among treatments at ($p \leq 0.05$) as per Duncan's multiple range test.

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Different letters indicate the significant differences (a, b, c, and d) among treatments at ($p \leq 0.05$) as per Duncan's multiple range test.

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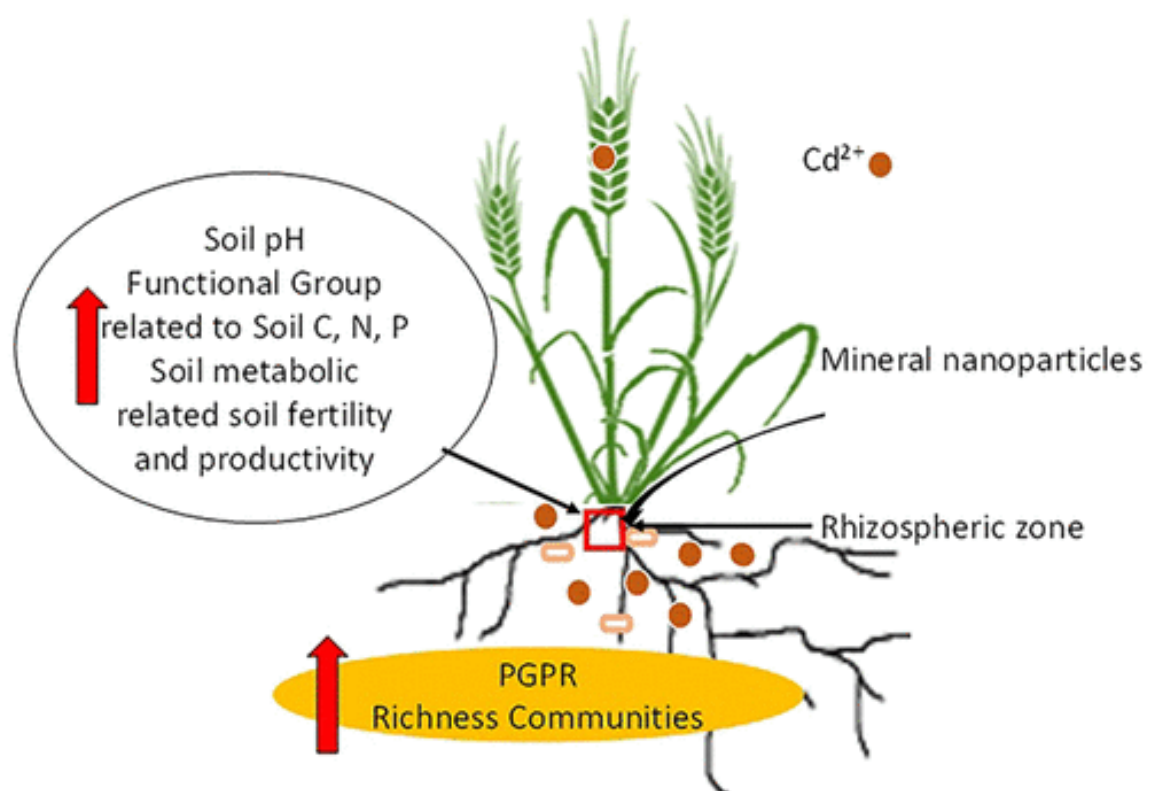
I look forward to telling the story in the pages of this dissertation which is titled **Cadmium Immobilization in the Rhizosphere: Implications in Sustainable Crop Improvement**. My memoir was a review published two and a half years ago in the *Journal of Agricultural and Food Chemistry*. I would like to thank my father, **Md Abdul Hye**, and Mother, **Firuja Khatun**, for their endless efforts in ensuring that I made my life a successful one. I deeply remember my maternal Grandfather, **Md Afaz Uddin Khan**, who shook my hand on a beautiful summer morning and told me that life is endless and beautiful if you can think independently. Finally, I would like to acknowledge all my colleagues and friends for their support, help with operating analytical instruments, brainstorming, advice, proofreading, enthusiasm, and much more.

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ILLUSTRATIVE SUMMARY OF THE THESIS



ABSTRACT

Food is the major pathway for exposure to cadmium (Cd) from agricultural soils to humans and other living entities; it is a substance that must be reduced urgently and effectively. Plants can select beneficial microbes such as plant growth-promoting rhizobacteria (PGPR) at the expense of root exudates in the rhizosphere. They are excellent and efficient bio-factories that have a significant ability to bio-reduce Cd by adsorption, precipitation, and bioaugmentation. This thesis aimed to explain the rhizo-immobilization of Cd in contaminated soils using a combination of PGPR and plant nutrient-containing fertilizers. In agricultural rhizosphere soil is prominent for controlling the PGPR, which is vital for the influence of Cd phyto-availability and developing the competition for different ions such as hydrogen ions and cations. The formation of positively charged sites can reduce the sorption of Cd at low pH, which might be toxic to PGPR and plant roots. The Cd sorption in this regard plays a crucial role. The distribution coefficient (K_d) of soil is closely linked to soil pH, SOM, and CEC in agricultural soil. The higher values of Freundlich parameters indicated a higher Cd sorption capacity with high pH soil (PTS) and lower in low pH soil (RTS), where PGPR can be applied for plant growth. The soil PTS is much better soil among others in the current experiment for the safe growing of different agricultural crops such as bread wheat, durum wheat, and maize and minimizes the Cd stress. The PTS soil Cd may reduce the plant growth parameters, which is predicted by 50% inhibition concentration (IC_{50}) values of 4.21 ± 0.29 and 4.02 ± 0.95 , respectively, whereas the maximum HRI index is 3.85 ± 0.05 and 5.32 ± 0.27 , respectively for *Triticum aestivum* L. cv. Mustang and *Triticum aestivum* L. cv. Lancer. The plant growth-promoting rhizobacteria (PGPR) (*Methylobacterium oryzae* CBMB20) increases the activities of plant Cd detoxification enzymes such as CAT, SOD, POD, AsA, GSH, TSS, TPH, and proline in the roots of wheat plants (Mustang) when they are grown in acidic and alkaline soil under Cd stress conditions.

Moreover, the PGPR improves the morphology and physiology of the roots of wheat (Mustang) seedlings and functions better in alkaline soil compared to its acidic counterpart. Novel

CMC-PEI-8 composites were synthesized to establish the effective reduction of Cd in plant parts in addition to PGPR. The CMC-PEI-8 showed significant adsorption of Cd^{2+} from an aqueous state and could be well described by the Langmuir model; adsorption capacity achieved $206.81 \pm 29.68 \text{ mg g}^{-1}$, which was better than what other reports documented. Simultaneously, good usability in different ranges of pH of the CMC-PEI-8 composites adsorbent offers good suggestions for applicability in agriculture. Then, the composite was synthesized by loading plant essential nutrients such as Ca and P while the micro-composites served to remediate Cd in plant parts. The slow and controlled release of P using these micro-composites was approximately 58.30 mg g^{-1} .

The phosphate release kinetics fitted well with the Elovich model. The essential plant nutrients loaded micro-composites were evaluated for Cd remediation from irrigation water, and their essence was assessed in terms of PGPR inoculation and multiplication. The micro-composites showed Cd^{2+} with 314.0 mg g^{-1} , in Langmuir isotherm equations. In addition, it has great potential for use with PGPR which illustrates the significant practical aspects under Cd stress conditions in the plant soil environment. The modified micro-composite CHP@CMC-PEI, which is regarded as CHP@CMC-PEI, reduces the DTPA-extractable Cd significantly by not altering pH considerably; this is due to the addition of PGPR (*Methylobacterium oryzae* CBMB20). The combined amendment benefits plant growth and can significantly diminish Cd accumulation in plants. Thus, combining the bacterial PGPR and biogenic CHP@CMC-PEI represents a potential method to reduce Cd in the soil and its accumulation in plants.

THESIS OUTLINE

Chapter 1 provides a detailed introduction to the utilization of plant growth-promoting rhizobacteria (PGPR) with mineral fertilizers to curtail Cd stress in plants, agricultural soil, and humans. The chapter also summarizes the current remediation strategies and global demand for PGPR, and polyethyleneimine modified carboxymethyl and Calcium hydrogen phosphate as novel fertilizers and their possibilities and challenges.

Chapter 2 is a review paper on Cd immobilization in the rhizosphere and plant-cellular detoxification: the role of plant growth-promoting rhizobacteria as a sustainable solution is explained here. **(Published)**

Chapter 3 is a research article seeking to demonstrate Cd retention and release kinetics in different agricultural soils for suitability. The objective is to reduce soil Cd-related health hazards.

Chapter 4 deals with Cd bioaccumulation and tolerance indices of wheat plants grown in Cd-contaminated soil. A health risk assessment is carried out here. **(Published)**

Chapter 5 describes the mitigation of Cd biotoxicity and stress through morphology, physiology, and antioxidative mechanisms of wheat (*Triticum aestivum* L.) roots in acidic and alkaline soils. To do this, Plant Growth-Promoting Rhizobacteria (PGPR) are employed.

Chapters 6-8 describe the various polymer-based composites which were developed and applied for their potential application in Cd adsorption from aqueous solution and agricultural soils. Additionally, the PGPR survivability was also assessed while those composites were applied in in-vitro growth medium.

Chapter 9 compiles the types of Plant Growth-Promoting Bacteria (PGPR) and modifies biogenic Calcium Hydrogen Phosphate on phytoavailable Cd remediation and immobilization in the rhizosphere of agricultural crops.

Chapter 10 is the summary of this thesis and suggests future research on Cd immobilization in the rhizosphere and plant-cellular detoxification. The role of plant growth-promoting rhizobacteria as a sustainable solution is concluded here.

LIST OF MANUSCRIPTS: PUBLISHED

1. Reprinted with permission from **Halim, M. A.**; Rahman, M. M.; Megharaj, M.; Naidu, R., Cadmium immobilization in the rhizosphere and plant cellular detoxification: role of plant-growth-promoting Rhizobacteria as a sustainable solution. *Journal of Agricultural and Food Chemistry* **2020**, 68 (47), 13497-13529. DOI: 10.1021/acs.jafc.0c04579. Copyright 2020 American Chemical Society.
2. **Halim, M. A.**; Rahman, M. M.; Mondal, D.; Megharaj, M.; Naidu, R., Bioaccumulation and Tolerance Indices of Cadmium in Wheat Plants Grown in Cadmium-Spiked Soil: Health Risk Assessment. *Frontiers in Environmental Science* **2021**, 623.
3. Rashid, M. H.; Rahman, M. M.; **Halim, M. A.**; Naidu, R., Growth, metal partitioning and antioxidant enzyme activities of mung beans as influenced by zinc oxide nanoparticles under cadmium stress. *Crop and Pasture Science* **2022**.

Conference Publications

4. **Halim, M. A.**; Rahman, M. M.; Megharaj, M.; Naidu, R., Human health risk investigation of potential heavy metals in wheat (*Triticum aestivum* L.) agricultural soil near industrial area: a methodological study. Clean Up Conference, Adelaide, Australia 2022.
5. **Halim, M. A.**; Rahman, M. M.; Megharaj, M.; Naidu, R., Root behaviour and nutrient medium's pH, EC AND Cd level of wheat grasses (ANTHOSACHNE SCABRA) under cadmium stress. Clean Up Conference, Adelaide, Australia 2019.

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I hereby certify that the work embodied in this thesis contains published papers/scholarly works of which I am a joint author. I have included as part of the thesis a written declaration endorsed in writing by my supervisor, attesting to my contribution to the joint publication work.

By signing below, I confirm that **Md Abdul Halim** contributed to developing the proposal, research tools, sample processing, analysis and interpretation of data and took primary responsibility for preparing, drafting and editing the text for the paper/ publication entitled:

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LIST OF ABBREVIATIONS

ACC	1-Aminocyclopropane-1-Carboxylic acid
ACCD	ACC deaminase
ADI	Average Daily Intake
ANOVA	analysis of variance
APS	Ascorbate peroxidase
AT	Averaging time
BW	Body Weight
Cd	Cadmium
CF	Continuous Flooding
CONTAM	Contaminants in Food Chain Panel
CRC	Cooperative Research Centre for Contamination Assessment and Remediation of the
CARE	Environment
CSF	Cancer slope factor
DL	Detection Limit
ED	Exposure duration
EF	Exposure frequency
EFSA	European Food Safety Authority
EIPS	Estimated intake per serving
EPA	Environmental Protection Agency
EPS	Bacterial exopolysaccharide
EU	European Union
EWI	Estimated Weekly Intake
FAO	Food and Agricultural Organization
Fe ²⁺	Iron
GCER	Global Centre for Environmental Remediation
GI	Gastrointestinal
HNO ₃	Nitric Acid
HPLC	High-Performance Liquid Chromatography
HQ	Hazard Quotient
IARC	International Agency for Research on Cancer
IC-ICP-	
MS	Ion chromatography inductively coupled plasma mass Spectrometry
ICP-MS	Inductively coupled plasma mass spectrometer
IR	Ingestion rate
ISIS	Integrated samples introduction system
JECFA	FAO/WHO Joint Expert Committee on Food Additives
K	Potassium
LCR	Lifetime cancer risk
MDA	Malondialdehyde
MTDI	Maximum tolerable weekly intake
MTH	Plant Metallothionine
N	Nitrogen
ND	Not detectable
NH ₃	Ammonia
NIST	National Institute of Standard and Technology

NO	Nitric oxide
P	Phosphorus
PTC	Plant Phytochelatin
PTWI	Provisional tolerable weekly intake
Rfd	Reference dose
RS	Recommended serving
HMRI	Health and Medical Research Institute
SAM	S-adenosyl methionine
SAU	Sher-e-Bangla Agricultural University
SF	Slope factor
Si	Silicon
SI	Supporting Information
SOD	Superoxide dismutase
SRM	Standard Reference Material
TDI	Total daily intake
TFA	Trifluoroacetic acid
US	United State
US EPA	US Environmental Protection Agency
ABW	Average body weight
WHO	World Health Organization

DEDICATION

This thesis is dedicated to my parents **Mrs. Firuja Khatun** and **Md Abdul Hye**, and to those who are working to mitigate Cd in Australia and elsewhere. It is also dedicated to those people who are suffering from any adverse health conditions related to Cd intoxication via food. I firmly believe that our collaborative efforts will bring about an effective and affordable mitigation strategy in the near future.