

DOCTORAL THESIS

Risk assessment and mycorrhizal remediation of cadmium contamination in vegetable farms around the Pearl River Delta, China

Hu, Junli

Date of Award:
2013

[Link to publication](#)

General rights

Copyright and intellectual property rights for the publications made accessible in HKBU Scholars are retained by the authors and/or other copyright owners. In addition to the restrictions prescribed by the Copyright Ordinance of Hong Kong, all users and readers must also observe the following terms of use:

- Users may download and print one copy of any publication from HKBU Scholars for the purpose of private study or research
- Users cannot further distribute the material or use it for any profit-making activity or commercial gain
- To share publications in HKBU Scholars with others, users are welcome to freely distribute the permanent URL assigned to the publication

**Risk Assessment and Mycorrhizal Remediation of Cadmium
Contamination in Vegetable Farms around the
Pearl River Delta, China**

HU Junli

**A thesis submitted in partial fulfillment of the requirements for
the degree of
Doctor of Philosophy**

Principal Supervisor: Prof. WONG Ming Hung

Hong Kong Baptist University

August 2013

Abstract

This study aimed to (1) evaluate the contamination status and potential health risks of Cd and other major heavy metals (HMs), including Cu, Cr, Ni, Pb, and Zn, in market vegetables available in Hong Kong, (2) investigate the transfer pattern of Cd from soil to edible vegetables in farms around the Pearl River Delta (PRD), China, (3) determine the role of arbuscular mycorrhizal (AM) fungi in Cd and P accumulation by remedial plants, i.e. hyper-accumulating (HA) and fast-growing (FG) plants, as well as by neighboring vegetables in intercropping systems, and (4) apply biochar and mycorrhizal intercrops on vegetable production in Cd-contaminated soils.

A systematic survey of HM concentrations and their bioaccessibilities in market vegetables in Hong Kong were carried out for assessing potential health risk to local inhabitants. The average concentrations of Cd, Pb, Cr, Ni, Cu, and Zn in nine major groups of fresh vegetable varied within 0.007–0.053, 0.05–0.17, 0.05–0.24, 0.26–1.1, 0.62–3.0, and 0.96–4.3 mg kg⁻¹, and their average bioaccessibilities varied within 21–96, 20–68, 24–62, 29–64, 30–77, and 69–94%, respectively. The bioaccessible estimated daily intakes (BEDIs) of Cd, Pb, Cr, Ni, Cu, and Zn through consumption of vegetables were far below the tolerable limits set by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The total bioaccessible target hazard quotient (TBTHQ) of the six HMs was 0.18 and 0.64 for average and high consumers, respectively, with Cd and leafy vegetable being the major risk contributors.

Five random vegetable farms (marked as DDH, SHH, DHH, HG, and SD) around the PRD were selected to investigate Cd bioaccumulation risks. Amongst the major HMs in soils, only total Cd concentrations (1.4–1.8 mg kg⁻¹) were higher than the permissible limit of China (≤ 0.3 mg kg⁻¹). Soil DTPA-extractable (phytoavailable) Cd

concentrations varied within 0.017–0.17 mg kg⁻¹. About 28.0% of vegetable samples were contaminated with Cd (>0.05 mg kg⁻¹), 71.4% of which belonged to stem/leaf vegetables. The average bioaccumulation factors of Cd from cultivated soil to stem/leaf vegetables and melon/fruit/bean vegetables varied within 0.021–0.050 and 0.005–0.020 (soil total Cd basis), and 0.50–2.01 and 0.13–0.53 (soil DTPA-extractable Cd basis), respectively. Redundancy analysis (RDA) showed that soil DTPA-extractable Cd concentration, which negatively but significantly correlated ($P<0.05$) to soil pH, was the key factor in influencing Cd bioaccumulation, notably stem/leaf vegetables.

A pot experiment was conducted to compare Cd phytoextraction efficiencies by Alfred stonecrop (*Sedum alfredii* Hance) and perennial ryegrass (*Lolium perenne* L.) from a Cd-contaminated (1.6 mg kg⁻¹) soil. The FG ryegrass and the HA stonecrop were harvested after growing for 9 and 27 weeks, respectively. Weekly Cd extraction by stonecrop (8.0 µg pot⁻¹) was 4.3 times higher than that by ryegrass (1.5 µg pot⁻¹). Both species of AM fungi *Glomus caledonium* (*Gc*) and *G. mosseae* (*Gm*) increased P acquisitions, shoot biomasses, and Cd acquisitions of stonecrop and ryegrass in most cases, while only *Gc*-inoculated stonecrop significantly accelerated ($P<0.05$) the phytoextraction efficiency of Cd by 78%. In addition, both *Gc* and *Gm* inoculations significantly decreased ($P<0.05$) soil DTPA-extractable Cd concentrations by 21–38% via elevating soil pH, causing Cd stabilization effects besides phytoextraction.

An 8-week pot trial was conducted to study Cd acquisition by upland kangkong (*Ipomoea aquatica* Forsk.) intercropped with stonecrop in the Cd-contaminated soil. Stonecrop tended to decrease kangkong Cd acquisition via competition of phyto-accessible Cd. Both species of AM fungi *Gc* and *G. versiforme* (*Gv*) significantly elevated ($P<0.05$) soil acid phosphatase activities and phytoavailable P, while only *Gc* increased ($P<0.05$) shoot biomass and Cd acquisition of the host (stonecrop), and

hence further lowered Cd concentration as well as Cd acquisition of kangkong. *Gv* significantly increased ($P<0.05$) P acquisition and shoot biomass of the neighboring kangkong rather than the host, causing a significant dilution ($P<0.05$) effect on kangkong shoot Cd concentration. In addition, both *Gc* and *Gv* significantly decreased ($P<0.05$) soil DTPA-extractable Cd by elevating soil pH, and thereby significantly lowered ($P<0.05$) kangkong Cd concentrations in a 6-week post-harvest experiment.

A 10-week pot experiment was conducted to investigate growth performance and Cd accumulation of kangkong intercropped with stonecrop (IS) in a Cd-contaminated soil inoculated with *Gc* (+M) and/or applied with biochar. Regardless of IS and +M, biochar addition (+B) significantly increased ($P<0.05$) kangkong yield via elevating phytoavailable P, and decreased ($P<0.05$) soil Cd phytoavailability and kangkong Cd concentration via increasing soil pH. Compared with the monocultural control, there was a significantly higher shoot yield (+25.5%) with a substantially lower Cd concentration (-62.7%) of kangkong under the treatment of IS+M+B. In addition, *Gc* generated additive effects on soil alkalization and Cd stabilization to biochar, causing substantially lower soil DTPA-extractable Cd and post-harvest transfer risks.

In summary, human health risk assessment of HMs from vegetables should be modified by taking bioaccessibility into account. Cd was the primary metal of risk in vegetable farms around the PRD, and stem/leaf vegetables posed higher health risks associated with exposure to Cd than melon/fruit/bean vegetables. For *in situ* treatment of Cd-contaminated soil, the hyper-accumulating plant (Alfred stonecrop) associated with AM fungi (notably *Gc*) showed a potential application for both extraction and stabilization of Cd. In addition, AM fungi and biochar played totally different but additive roles in the intercropping systems of kangkong and stonecrop for the dual purposes of vegetable production and phytoremediation of Cd-contaminated soils.

Table of Contents

Declaration.....	i
Abstract.....	ii
Acknowledgements.....	v
Table of Contents.....	vi
List of Tables.....	xii
List of Figures.....	xiii
Symbols and Abbreviation.....	xv
Chapter 1 General Introduction.....	1
1.1. Background of research.....	1
1.1.1. Cadmium contamination in agricultural soils and terrestrial foods.....	1
1.1.2. Phytoremediation and mycorrhizal performance in contaminated soils.....	3
1.1.3. Intercropping and biochar application in revitalizing contaminated soils.....	6
1.2. Literature review.....	8
1.2.1. Cadmium in natural environment and anthropogenic sources.....	8
1.2.2. Environmental toxicology and public health impacts of Cd.....	13
1.2.3. Sources of human exposure to environmental Cd.....	17
1.2.4. Occurrence and remediation of soil Cd contamination.....	22
1.2.5. Roles of arbuscular mycorrhizal fungi in phytoremediation.....	26
1.3. Objectives of the present research.....	29
1.4. Contributions and significance of the present research.....	30
1.5. Framework of research.....	31
Chapter 2: Concentration, Bioaccessibility, and Exposure Risk of Heavy Metals	

	from Market Vegetables to the General Public in Hong Kong Revealed with an <i>In Vitro</i> Gastrointestinal Model	33
2.1.	Introduction.....	33
2.2.	Materials and methods.....	38
2.2.1.	Vegetable consumption data analysis	38
2.2.2.	Sampling, preparation and chemical analysis.....	39
2.2.3.	Bioaccessibility test of heavy metals	41
2.2.4.	Health risk assessment of heavy metals	42
2.3.	Results	43
2.3.1.	Heavy metal concentrations in market vegetables in Hong Kong	44
2.3.2.	Estimated daily intakes and target hazard quotients for heavy metals from vegetables	44
2.3.3.	Bioaccessibility and bioaccessible target hazard quotients for heavy metals from vegetables	48
2.3.4.	Total bioaccessible target hazard quotients for heavy metals from different vegetables	48
2.4.	Discussion.....	51
2.5.	Conclusions.....	56

Chapter 3: Accumulation, Phytoavailability, and Transfer Risk of Heavy Metals

	from Soil to Vegetables, Focusing on Cd-Contaminated Farms around the Pearl River Delta, China.....	57
3.1.	Introduction.....	57
3.2.	Materials and methods.....	59
3.2.1.	Soil and vegetable sampling	59
3.2.2.	Soil sample analysis	61
3.2.3.	Vegetable sample analysis.....	62
3.2.4.	Statistical analysis and redundancy analysis	62
3.3.	Results	63

3.3.1. Soil pH and heavy metal accumulation in selected farms	63
3.3.2. Heavy metal accumulation in collected edible vegetables	63
3.3.3. Bioaccumulation factor of cadmium from soil to edible vegetables ...	65
3.3.4. Redundancy analysis of vegetable Cd accumulation, soil parameter, and farm site.....	69
3.4. Discussion.....	71
3.5. Conclusions.....	74

Chapter 4: Cadmium Phytoextraction Efficiency and Response of Alfred

Stonecrop (Sedum alfredii Hance) and Perennial Ryegrass (Lolium

perenne L.) to Arbuscular Mycorrhizal Fungal Inoculation..... 77

4.1. Introduction.....	77
4.2. Materials and methods.....	80
4.2.1. Soil preparation.....	80
4.2.2. Pot experiment and harvest.....	82
4.2.3. Mycorrhizal colonization and plant analysis	84
4.2.4. Soil chemical and biochemical property analysis	85
4.2.5. Statistical analysis	86
4.3. Results	86
4.3.1. Root mycorrhizal colonization rate and soil acid phosphatase activity	86
4.3.2. Shoot and root biomass, Cd concentration, and P concentration.....	88
4.3.3. Total acquisition and translocation efficiency of Cd and P, and the P/Cd molar concentration ratio	88
4.3.4. Weekly Cd extraction efficiency and average P consumption for extracting Cd	91
4.3.5. Soil pH, EC, total Cd, DTPA-extractable Cd, and available P concentrations	91
4.4. Discussion.....	93

4.5. Conclusions.....	100
-----------------------	-----

Chapter 5: Arbuscular Mycorrhizal Fungi Induce Differential Cd and P Uptake

by Alfred stonecrop (*Sedum alfredii* Hance) and Upland Kangkong

(*Ipomoea aquatica* Forsk.) in an Intercropping System101

5.1. Introduction.....	101
5.2. Materials and methods.....	104
5.2.1. Soil preparation.....	104
5.2.2. Intercropping experiment and harvest.....	105
5.2.3. Post-harvest experiment and harvest.....	106
5.2.4. Mycorrhizal colonization and plant analysis	107
5.2.5. Soil chemical and biochemical property analysis	108
5.2.6. Statistical analysis and redundancy analysis	109
5.3. Results	109
5.3.1. Mycorrhizal colonization, plant biomass, Cd and P concentrations, and Cd and P acquisitions of Alfred stonecrop.....	109
5.3.2. Plant biomass, Cd and P concentrations, and Cd and P acquisitions of upland kangkong from the intercropped system.....	111
5.3.3. Soil pH, EC, total Cd, DTPA-extractable Cd, available P, and acid phosphatase activity after the intercropping experiment.....	111
5.3.4. Shoot and root biomass, Cd and P concentrations, and Cd and P acquisitions of post-harvest upland kangkong	113
5.3.5. Soil pH, EC, total Cd, DTPA-extractable Cd, available P, and acid phosphatase activity after the post-harvest experiment.....	116
5.3.6. Redundancy analysis of experimental treatments, and soil and plant parameters.....	116
5.4. Discussion.....	119
5.5. Conclusions.....	125

Chapter 6: Application of Biochar and Intercropping of Mycorrhizal Alfred	
Stonecrop (<i>Sedum alfredii</i> Hance) Lower Cd Phytoavailability and	
Accessibility to Upland Kangkong (<i>Ipomoea aquatica</i> Forsk.)127
6.1. Introduction.....	127
6.2. Materials and methods.....	130
6.2.1. Biochar preparation.....	130
6.2.2. Soil preparation.....	131
6.2.3. Pot experiment and harvest.....	132
6.2.4. Mycorrhizal colonization and plant analysis	133
6.2.5. Soil chemical and enzymatic property analysis	134
6.2.6. Statistical analysis	135
6.3. Results	135
6.3.1. Mycorrhizal colonization, plant dry biomass, and Cd/P concentration	
and acquisition of Alfred stonecrop	135
6.3.2. Shoot and root fresh biomass and Cd/P concentration, and total Cd/P	
acquisition of upland kangkong	137
6.3.3. Rhizosphere competition in Cd and P acquisition between upland	
kangkong and Alfred stonecrop	139
6.3.4. Soil pH, EC, DTPA-extractable Cd, available P, and acid phosphatase	
activity	140
6.4. Discussion.....	144
6.5. Conclusions.....	149
Chapter 7 General Discussion and Conclusions.....	151
7.1. Introduction.....	151
7.2. Risk assessments of heavy metal contamination in edible vegetables.....	153
7.2.1. Heavy metal contamination in edible vegetables available in Hong	
Kong markets and Pearl River Delta farms	153
7.2.2. Potential health risks of heavy metals via consumption of market	

vegetables in Hong Kong.....	154
7.2.3. Difference in heavy metal accumulation and potential health risk of different vegetables	155
7.3. Mycorrhizal remediation of Cd contamination in vegetable farm soils.....	158
7.3.1. Faster removal of Cd by hyper-accumulating plants than fast- growing plants.....	158
7.3.2. Effects of AM fungi on Cd phytoextraction and stabilization in the soil.....	159
7.3.3. Effects of intercropping of Alfred stonecrop on Cd accumulation by vegetable	160
7.3.4. Effects of biochar amendment on the yield and safety of edible vegetables.....	161
7.3.5. Long-term effects of AM fungal inoculation and biochar a mendment.....	163
7.4. Inter-relationships and general conclusions of the present study	166
7.5. Limitations of the present study.....	169
7.6. Future work.....	170
References... ..	173
List of Publications and Presentations.....	203
Curriculum Vitae.....	205