Occurrence of heavy metal contamination and *in vitro* bioaccessibilitiy of heavy metals in selected green leafy vegetables (GLV) obtained from Colombo District

by

Thilini Chathurangi Kananke

Ph.D. **2016**

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We certify that the candidate has incorporated all corrections, additions and amendments recommended by the examiners to the final version of the PhD thesis.

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The work described in this thesis was carried out by me under the supervision of Dr. M.A. Jagath Wansapala and Professor D.M. Anil Gunaratne and a report on this has not been submitted in whole or in part to any university or any other institution for another Degree / Diploma.

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We certify that the above statement made by the candidate is true and that this thesis is suitable for submission to the University for the purpose of evaluation.

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Table of Contents

List of Tables

List of Figures

Abbreviations

AAS	Atomic Absorption Spectroscopy
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
APHA	American Public Health Association
A.R.	Analytical Reagent
ATSDR	Agency for Toxic Substances and Disease Registry
BAC	Bio Accumulation Factor
CEC	Cation Exchange Capacity
DL	Detection Limit
DNA	Deoxyribonucleic Acid
EDL	Electrodeless Discharge Lamp
EPA	Environmental Protection Agency
EU	European Union
FAAS	Flame Atomic Absorption Spectroscopy
FAO	Food and Agriculture Organization
GFAAS	Graphite Furnace Atomic Absorption Spectroscopy
GLV	Green Leafy Vegetables
GP	Gastric Phase
HCL	Hollow Cathode Lamp
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectroscopy
ICP-MS	Inductively Coupled Plasma Mass Spectroscopy
IP	Intestinal Phase
IUPAC	International Union of Pure and Applied Chemistry
L1 (S-R)	Soil to Root
L2 (R-S)	Root to Stem
L3 (S-L)	Stem to Leaf
MI	Mobility Index
MPL	Maximum Permissible Limit

n	Sample size
N/A	Not Available
ND	Not Detected
OM	Organic Matter
P	Probability
PBET	Physiologically Based Extraction Test
PFA	Prevention of Food Adulteration Act
pH	Power of Hydrogen
PTDI	Provisional Tolerable Daily Intake
PVC	Poly Vinyl Chloride
RF	Radio Frequency
RSD	Relative Standard Deviation
rpm	Revolutions per minute
SD	Standard Deviation
SF	Stir Fried
SLSI	Sri Lanka Standards Institute
TF	Translocation Factor
THQ	Target Hazard Quotients
TSP	Triple Super Phosphate
USA	United States of America
USDA	United States Department of Agriculture
US-EPA	United Stated Environmental Protection Agency
UK	United Kingdom
WHO	World Health Organization

Acknowledgement

I am exceptionally grateful to my supervisors, Dr. M.A. Jagath Wansapala and Professor D.M. Anil Gunaratne for their continuous guidance, encouragement, support and care given throughout this period. I am also thankful to the University Grants Commission, Sri Lanka, for providing the financial assistance during the first two years of my research project. In addition, my sincere gratitude goes to the Accredited Laboratories of Industrial Technology Institute and SGS Lanka (Pvt.) Ltd. for analyzing the samples and providing the results in due time. Further, I would like to thank Mr. Sasanka Ubesena, the former research assistant at the Instrument Laboratory of Faculty of Applied Sciences, University of Sri Jayewardenepura for providing his assistance in sample analysis during the research project. In addition, I would like to express my gratitude to all the academic, non-academic and technical staff members of the Department of Food Science and Technology of University of Sri Jayewardenepura, for providing their assistance in many ways through this entire period. My sincere gratitude also extends to the administration, academic and non-academic staff of Sabaragamuwa University of Sri Lanka for their immense support throughout the research project, to make it successful.

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ABSTRACT

This research aimed to investigate the occurrence of heavy metal contaminations (Ni, Cd, Cr, Pb and Cu) in five key Sri Lankan green leafy vegetables ["Mukunuwenna" (*Alternanthera sessilis*), "Nivithi" (*Basella alba*), "Thampala" (*Amaranthus viridis*), "Kankun" (*Ipomea aquatica*) and "Kohila Leaves" (*Lasia spinosa*)] grown and marketed in and around Colombo District (Piliyandala, Wellampitiya, Kolonnawa, Kottawa, Bandaragama, Kahathuduwa, Pettah and Delgoda), Sri Lanka. The study also focused on heavy metal contents in soils, irrigated water, fertilizer and animal manure types frequently used by the farmers, bio-accumulation and translocation of metals between different GLV species, the effect of different cooking practices on heavy metal contents in GLV and *in vitro* bioaccessibility of heavy metals in gastrointestinal fractions after dietary ingestion of raw and cooked forms of GLV.

The average concentrations of Cd, Cu, Ni, Cr and Pb in the tested soils were 1.45±1.15, 66.5±59.52, 51.5±45.51, 48.4±42.9 and 39.7±32.26 mg kg^{-1}, respectively in the tested areas. Unlike other elements, the levels of Ni detected in the irrigated water samples collected from Wellampitiya, Kolonnawa and Kahathuduwa areas have exceeded the WHO/FAO safe limit. The mean concentrations of heavy metals tested in all fields and market GLV exceeded the WHO/FAO safe limits, except for Cu. The Wellampitiya, Kolonnawa, Pettah and Kottawa areas showed significantly higher ($P<0.05$) metal contaminations in GLV compared to those of Piliyandala, Bandaragama, Kahathuduwa

and Delgoda areas. The GLV collected from the market sites have shown slightly increased levels of heavy metals compared with the production sites, but the differences were not significant at $P<0.05$. The soil pH and organic matter contents have negatively correlated with the heavy metal contents of GLV. The Pb concentrations in the soils and GLV grown closer to the road sides (5 m) were significantly higher than the samples taken farther away from the road sides (> 50 m). Except for Cd in TSP fertilizer, other analyzed heavy metals in manure and fertilizer samples complied with the SLSI standard limits. Irrespective of the species and the location, all the collected GLV showed the distribution pattern for the heavy metals as: roots>stems>leaves. The highest metal bioaccumulations were shown Kohila, while the least accumulations were observed in Thampala, with few exceptions. The magnitude of influence of cooking practices in reducing heavy metals in GLV varied as; Blanching>Stir-frying>Cooking>Raw, for each heavy metal tested in the study. The average bioaccessibility (%) of Ni, Cd, Cr and Pb in GLV were significantly higher (at $P<0.05$) in the gastric phase than in the intestinal phase, except for Cu. Despite the higher total heavy metal concentrations found in GLV, the bioaccessible fractions of heavy metals were significantly low (at $P<0.05$) in raw, cooked and stir-fried GLV samples. From the study, it became apparent that Kolonnawa and Wellampitiya areas are not suitable for GLV cultivation and more appropriate heavy metal remediation techniques should be sought in the respective areas to ensure consumer food safety.