RESPONSES OF SOME VEGETABLE CROPS TO TOXIC HEAVY METALS

A Thesis Submitted to
Central Department of Botany
Institute of Science and Technology
TRIBHUVAN UNIVERSITY
Kirtipur, Kathmandu, Nepal
For the Degree of
DOCTOR OF PHILOSOPHY IN BOTANY

Baby Sharma (Kafle)
2008

Date: April, 29, 2008.

I have the pleasure of forwarding the thesis entitled "**RESPONSES OF SOME VEGETABLE CROPS TO TOXIC HEAVY METALS"** by **Ms. Baby Sharma Kafle** for the fulfillment of the degree of Doctor of Philosophy in Botany. The thesis is based on original research work carried out under my supervision and has not been submitted for a degree to any other university.

Mukesh Kumar Chettri, Ph.D.
Department of Botany, Amrit Campus
Tribhuvan University
Kathmandu, Nepal

DECLARATION

I hereby declare that the work presented in this thesis has been done by myself, and has not been submitted, elsewhere for the award of any degree. All sources of information have been specially acknowledged by reference to the authors or institutions.

Date: 29.04. 2008

MS. BABY SHARMA
Department of Botany, Amrit Campus
Tribhuvan University
Kathmandu, Nepal

ACKNOWLEDGEMENTS

This thesis would not have been accomplished without the invaluable guidance of my respected supervisor Dr. M. K. Chettri, Lecturer, Botany Department, Amrit Campus, Tribhuvan University, for whom I have the deepest regards and gratitude. I would like to thank him for his continuous inspiration, guidance, valuable suggestions and discussions during the course of this study. I would also mention that his continuous motivation and cooperation helped me to complete this thesis successfully.

I am also obliged to Prof. Dr Vishal Nath Uprety, Dean, Institute of Science and Technology, (IOST), TU, and Prof. Dr. G. P. Sharma Ghimire, former Dean, for granting me the study leave required for this research work. I am grateful to Prof. Dr. K. K. Shrestha, Head of the Central Department of Botany; Prof. Dr. P. K. Jha and Prof. Dr. S. D. Joshi, former Heads, Central Department of Botany, Tribhuvan University, for their invaluable support and encouragement.

I gratefully acknowledge Mr. Narayan Khadka, Campus Chief, and Mr. Madan Shakya, former Campus Chief, (Amrit Campus) TU, for granting recommendations for the study leave so I could undertake this research work. I would like to express my sincere thankfulness to Ms. Prabha Parajuli, Chairperson, Ms. Sharada Shrestha and Mr. Amrit Man Singh Bania, former Chairpersons, Botany Department, Amrit Campus, TU, for providing me their constant encouragement and laboratory facilities.

I would also like to thank the University Grant Commission, Thimi for providing me the partial research scholarship, Ph D Grant to carry out this research work. I would also like to thank the Nepal Academy of Science and Technology (NAST), Khumultar, Lalitpur for providing me the Ph D Fellowship.

I would like to acknowledge my sincere thanks to Dr. Vikash Satyal, Associate Professor, Department of Statistics, Amrit Campus for his valuable assistance in statistical analysis. I would also like to thank all my colleagues at Amrit Campus for their constant encouragement and substantial help. Especially, I would like to thank Dr. Bipana Acharya and Ms. Kumudini Shakya for their helpful advice, encouragement and constant support along the way. I would also like to

thank Dr. Anjana Bista, Dr. Kanta Poudyal and Dr. Govind Bahadur Khattri for their kind co-operation. I also acknowledge the supports of the administrative staff of my campus.

My special thanks go to Mr. Prakash Mani Adhikari, Mr. Shailesh K. Jha, and Mr. Surendra Subedi, Nepal Bureau of Standards and Metrology, Balaju for their valuable co-operation in performing careful and timely metal analysis for this research.

I am also grateful to Senoir Scientist, Dr. Yagya-Gajadhar Khadka and Soil Section, Nepal Agricultural Research Council, Khumaltar, Lalitpur, for their valuable co-operation during the soil testing required for this research.

This thesis would not have been possible without the persistent support and cooperation of my husband Dr. Purushottam Kafle and my children Atul Kafle and Anima Kafle.

I dedicate this work to my late mother Som Kumari Bhattarai.

Date April, 29, 2008**.**

Ms. Baby Sharma

ABSTRACT

Some common green vegetables (*Brassica juncea*, *Brassica rapa*, *Lactuca sativa*, *Lepidium sativ*um and *Daucas carota*) of Kathmandu valley have been investigated for the morphological changes after growing on different concentrations of heavy metals ($CdCl_2$, $CuCl_2$, $Pb(NO_3)_2$ and $ZnCl_2$). Morphological parameters such as root length (RL), shoot length (SL), fresh weight (FW), dry weight (DW), dry weight % and leaf size (leaf area/length) have been examined in potted plants grown on soil, artificially contaminated with heavy metal. Bioaccumulations of non-supplied micronutrients (Cu, Fe, Mn and Zn) after treatment with heavy metals were also observed in these vegetables. On the basis of FW of whole plant (matured), threshold concentration for Cd in *B. juncea*, *B. rapa*, *L. sativum*, *L. sativa* and *D. carota* were ascertained to be as 2.75, 0.25, 3.22, 1.25 and 1.0 mg kg^{-1} DW, respectively; for Cu were 18, 17.75, 14.75, 17.25 and 10.75 mg kg^{-1} DW; for Pb to be 46.5, 34.5, 52.7, 81.0 and 31.5 mg kg^{-1} DW; and for Zn were 173.5, 192, 172.5, 93.0, and 55.5 mg kg^{-1}, respectively. Among the studied vegetables, *D. carota* showed lowest accumulation of all heavy metals in roots and shoots. Highest accumulation of Cd was observed in the shoot of *L. sativum*; Pb in the root of *B. rapa*, Cu in both root and shoot of *B. juncea* and Zn in the shoot of *B. rapa*. Reduction in FW, SL and RL in *B. rapa, L. sativa* and *D. carota* have been recorded as symptoms of Cd toxicity; and reduction in FW and RL in *B. juncea, B. rapa* and *L. sativa* as symptom of Cu-toxicity. Similarly, reduction in FW in *B. rapa, L. sativum* and RL in *B. rapa, D. carota* have been observed as Pb-toxicity, reduction in FW and RL in *B. juncea* and *B. rapa*, and SL in *B. rapa, L. sativum* and *L. sativa* as symptoms of Zn- toxicity.

Impact of different concentrations of $CdCl_2$, $CuCl_2$, $Pb(NO3)_2$ and $ZnCl_2$ on chlorophylls after the increased uptake of Cd, Cu, Pb and Zn in *B. juncea, B. rapa, L. sativa*, *L. sativum* and *D. carota* were studied. Significant loss of Chl–a, Chl-b and total chlorophyll were observed in *B. juncea* and *L. sativum* with depletion of Cu, Zn and/or Mn after increased Cd uptake. Significant loss in total-chl after increased Cu uptake was observed in *B. juncea* and *D. carota* with depletion of essential Zn, Fe (in *B. juncea*) and or Mn (in *D. carota*). Total chlorophyll increased significantly in *B. juncea* up to 500 mg $Pb(NO_3)_2$ kg^{-1} soil treatment but it decreased insignificantly in *D. carota* only at 1000 mg kg^{-1} soil treatment. There was moderate increased in

Fe accumulation in all vegetables (except *L. sativum)* when grown on $Pb(NO_3)_2$ treated soil; but Zn, Cu and Mn depleted in *D. carota* grown on $Pb(NO_3)_2$ treatments. Insignificant change in chlorophyll was observed in Zn-treated vegetables. Present studies suggest that depletion of essential micronutrients like Zn and Fe; or Zn and Cu; or Fe and Mn in the plant body after heavy metal accumulation may be one of the causes for chlorophyll loss.

Evaluation of heavy metal in soil and vegetables grown from agricultural fields of Kathmandu valley was conducted in the present study. Highest accumulations of Cd (2 mg kg^{-1}), Cu (65.5 mg kg^{-1}) and Pb (46.75 mg kg^{-1}) from Shankhamul; Ni (29.25 mg kg^{-1}) from Nakhu; Co (15.25 mg kg^{-1}) and Mn (675 mg kg^{-1}) from Balkhu; Cr (73.75 mg kg^{-1}) from Banasthali, Zn (162 mg kg^{-1}) and Fe (75636 mg kg^{-1}) from Khusibun were recorded in soils of agricultural fields. On the basis of mean values, highest accumulation of Cu, Co and Zn were recorded in spinach and Pb in red radish; Ni, Cr and Fe were recorded in broad leaf mustard and Mn in cress leaf. Concentration of non –essential but toxic metal like Pb was higher than the normal plant value (0.1-10 mg kg^{-1}) in all the vegetables (except potato) collected from different sampling sites. Potato accumulated fewer amounts of heavy metals among the others.

As vegetables are one of the importance dietary items, it is cultivated widely in Kathmandu valley. The daily intake of heavy metals (Cd, Cu, Co, Cr, Fe, Mn, Ni, Pb and Zn) via vegetables was estimated in the present study. Intake estimation was based on vegetables availability data and analysis of vegetables contaminants. The mean daily intake of potentially toxic metals ranged about 33.4 % (for Cd), 40.38 % (for Ni) and 251.6 % (for Pb) of the provisional tolerable daily intakes (PTDI) for adults. Vegetables were found to contribute significantly to the recommended daily intake of essential elements such as Cr (97.4-24.35 %), Cu (15.45-7.7 %), Fe (41 %), Mn (65.71-26.28 %), and Zn (21.23 %).

To understand the phytoextraction of heavy metals from heavy metal contaminated soil, 18 different vegetable were grown on soil artificially contaminated with 300 mg $CuCl_2$ kg^{-1}, 500 mg $Pb(NO_3)_2$ kg^{-1}, 800 mg $ZnCl_2$ kg^{-1} or their mixed metal (1600 mg kg^{-1}). From the single metal contaminated soil, lowest Cu accumulation was

recorded in *B. juncea* and highest in *V. faba;* lowest Pb accumulation was recorded in *S. tuberosum,* and highest in *A. fistulosum*; and lowest Zn accumulation was recorded in A. *fistulosum* and *S. tuberosum* and highest in *V. faba*. From mixed metal soil treatments, lowest accumulation of Cu, Pb and Zn was recorded in *B. juncea*, *B. caulorapa* and *A. fistulosum*, respectively, and highest accumulation of Cu and Pb in *S. oleracea* (Deshi), and Zn in *S. oleracea* (Patane) was recorded.

Remedial measures using cow dung and lime at different treatments showed that Cu and Pb accumulation in vegetables are higher in lime treatments than in 20 % cow dung treatments. Zinc accumulation increased in *B. rapa* and *L. sativum* in both cow dung and lime than in control. Among the tested vegetables for remedial measures, accumulation of Cu, Pb and Zn from mixed metal treatment was highest in *L. sativum* (treated with lime 9 g for Cu and Pb, and 20 % cow dung for Zn).

Morphological changes such as FW, DW, SL and RL (except RL in *B. rapa*) increased significantly ($P \leq 0.01$) in all vegetables in Zn and cow dung treated soil, but not significant changes were observed with lime treatment. FW and DW increased only in *L. sativum* grown in 3 g lime treatment, whereas DW % increased significantly in *B. rapa* of both the doses of lime. Immobilization of Cu, Pb and Zn in both single and mixed metal treatments was found to be high in cow dung amended soil. But in single metal salts such as $CuCl_2$ or $Pb(NO_3)_2$ and lime treated soil, the concentration of Cu and Pb retained in the soil was lower than in control, indicating their free mobility in the plants. From this it can be ascertained that 20 % cow dung treatment is suitable for immobilization of supplied metals than lime treatment.

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ABBREVIATIONS

CBS	Central Bureau of Statistics
CDC	Centers for Diseases Control and Prevention
Chl-a	Chlorophyll a
Chl-b	Chlorophyll b
ctrl	control
DoAMDD	Department of Agricultural Marketing Development Directorate
DW	Dry weight
DW (%)	Dry weight percent
Ed	Edition
Ed's	Editors
e.g.	for example
et. al.	and others
fig, figs	Figure (s)
FW	Fresh weight
g	gram
h	hour
HMG	His Majesty's Government
i.e.	that is
kg	kilogram
mg	milligrams
no, nos,	number (s)
p., pp.,	page (s)
PTDI	Provisional Tolerable Daily Intakes
RDI	Recommended Daily Intake
RL	Root length
SL	Shoot length
t	tones
Total Chl	Total Chlorophyll
US	United States
US EPA	United States Environmental Protection Agency
WHO	World Health Organization

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