## ผลของแคดเมียมต่อการตอบสนองทางสรีรวิทยาของพืชบางชนิด

## นายสุพัฒน์ โปษยะวัฒนากุล



วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต สหสาขาวิชาวิทยาศาสตร์สภาวะแวดล้อม บัณฑิควิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย ปีการศึกษา 2541 ISBN 974-639-683-8 ลิขสิทธิ์ของบัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย

# EFFECTS OF CADMIUM ON PHYSIOLOGICAL RESPONSES OF SOME PLANTS

Mr. Supat Posayawattanakul

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Environmental Science Inter-Department of Environmental Science

Graduate School
Chulalongkorn University
Academic Year 1998

ISBN 974-639-683-8

Thesis Title

Effects of Cadmium on Physiological Responses of Some Plants

By

Mr. Supat Posayawattanakul

Inter-Department

Environmental Science

Thesis Advisor

Associate Professor Thaweesakdi Boonkerd, Ph.D.

Accepted by the Graduate School, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

Dean of Graduate School

(Professor Supawat Chutivongse, M.D.)

Thesis Committee

Polony Chairman

(Assistant Professor Pipat Patanaponpaiboon, Ph.D.)

Thaweisakdi Soonkird Thesis Advisor

(Associate Professor Thaweesakdi Boonkerd, Ph.D.)

Ruda Bas Member

(Associate Professor Preeda Boon-Long, Ph.D.)

K Thinakhupt Member

(Assistant Professor Kumthorn Thirakhupt, Ph.D.)

#### ชิวเก่ตับภูบัวษาทลัดย่อวิทยานิพนธ์ภายในกรอบสีเขียวนี้เพียงแผ่นเดียว

สุพัฒน์ โปษยะวัฒนากุล : ผลของแคดเมียมต่อการตอบสนองทางสรีรวิทยาของพืชบางชนิด (EFFECTS OF CADMIUM ON PHYSIOLOGICAL RESPONSES OF SOME PLANTS) อาจารย์ที่ปรึกษา : รองศาสตราจารย์ ดร. ทวีศักดิ์ บุญเกิด, 108 หน้า, ISBN 974-639-683-8

งานวิจัยนี้ศึกษาการตอบสนองทางสรีรวิทยาของผักและวัชพืชอย่างละ 2 ชนิด ซึ่งได้แก่ ผักบุ้งจีน (Ipomoea aquatica Forsk.) ผักคะน้ำ (Brassica oleracea L. cv. Group Chinese Kale) กะเม็ง (Eclipta prostrata L.) และหญ้ารังนก (Chloris barbata Sw.) เมื่อได้รับแคคเมียมที่ความเข้มข้น 5 และ 20 มิลลิกรัมต่อ ลิตร โดยพืชจะได้รับแคดเมียมในช่วงเวลาที่ต่างกัน ได้ศึกษาการสะสมโพรลีน ปริมาณคลอโรฟิลล์ ปริมาณ น้ำในพืช การเจริญเติบโต ตลอดจนปริมาณแคดเมียมที่สะสมในส่วนรากและส่วนด้นของพืช

ผลการศึกษาแสดงให้เห็นว่าพืชมีการสะสมโพรถินเมื่อได้รับแคดเมียม อย่างไรก็ตามผลการ ศึกษาครั้งนี้ได้บ่งถึงความไม่เหมาะสมในการใช้ปริมาณโพรถินสะสมเพื่อเป็นดัชนีแสดงถึงการได้รับ แคดเมียม ในส่วนของปริมาณคลอโรฟิลล์ ปริมาณน้ำในพืช และการเจริญเติบโต พบว่ามีการลดลงซึ่งเป็นผล จากการได้รับแคดเมียม สำหรับปริมาณสะสมในส่วนรากและส่วนด้นนั้น พบว่ามีความสัมพันธ์กันอย่างมีนัย สำคัญทางสถิติที่ระดับความเชื่อมั่น 99 เปอร์เซ็นต์ ในการศึกษาการตอบสนองของพืชแต่ละชนิด แสดงให้เห็น ว่าผักคะน้ำมีความทนทานและมีการสะสมแคดเมียมมากที่สุด

| ภาควิชา    | สนุ ลำชา                | ลายมือชื่อนิสิต ผู้ส่อน โปรอะรัฒนาล       |
|------------|-------------------------|---|
|            |                         |   |
| สาขาวิชา   | วิทยาศาสตร์สภาวะแอดค้อม | ลายมือชื่ออาจารย์ที่ปรึกษา กรัดเรา นายด้ว |
| 61101711   | ••••                    |   |
| ปีการศึกษา | J541                    | ลายมือชื่ออาจารย์ที่ปรึกษาร่วม            |

#### - เองขัดของรับอุดคลักย่อวิทยานิพ**นธ์ภายในกรอบสีเขียวนี้เพียงแผ่น**เดียว

C826918 : MAJOR INTER - DEPARTMENT OF ENVIRONMENTAL SCIENCE

KEY WORD: CADMIUM STRESS / PLANT PHYSIOLOGICAL RESPONSES

SUPAT POSAYAWATTANAKUL: EFFECTS OF CADMIUM ON PHYSIOLOGICAL RESPONSES OF SOME PLANTS. THESIS ADVISOR: ASSOC. PROF. THAWEESAKDI BOONKERD, Ph.D. 108 pp. ISBN 974-639-683-8.

This study investigated the physiological responses of two vegetable species, Kangkong (*Ipomoea aquatica* Forsk.) and Chinese Kale (*Brassica oleracea* L. cv. Group Chinese Kale), together with two weed species, False daisy (*Eclipta prostrata* L.) and Swollen finger grass (*Chloris barbata* Sw.) under Cd stress. These plants were exposed to 5 and 20 ppm Cd-containing solution with different exposure time. Proline accumulation, chlorophyll contents, Relative Water Content (RWC) and plant growth were determined against Cd accumulation both in roots and in shoots.

The results have shown that proline is accumulated in Cd-stressed plants. However, the findings imply that proline accumulation is not an effective indicator of Cd stress. Unlike proline, chlorophyll contents, RWC and plant growth are adversely affected by Cd. Strong correlation (P≤0.01) between root and shoot Cd content were observed. As for the responses of each species, it was evident that Chinese Kale was the greatest Cd accumulator as well as the most tolerant species.

 ภาควิชา
 INTER - DEPARTMENT

 สาขาวิชา
 ENVI ACOMMENTAL
 SCIENCE

 ปีการศึกษา
 1998

ลายมือชื่อนิสิต Supat fosay amattanakul ลายมือชื่ออาจารย์ที่ปรึกษา Thawesakdi Boonkerd



I would like to express my deepest appreciation to my thesis advisor, Associate Professor Dr. Thaweesakdi Boonkerd for providing me a chance to carry out this thesis. Accomplishment was due to his strong encouragement and valuable advice.

I also would like to express my sincere appreciation to the thesis committee Assistant

Professor Dr. Pipat Patanaponpaiboon, Associate Professor Dr. Preeda Boon-Long and Assistant

Professor Dr. Kumthorn Thirakhupt for their encouragement and valuable suggestion.

I wish to express my appreciation to teachers who provided me the knowledge on plant physiology and Mr. Suwat Terapongtanakorn for his assistance.

It is a pleasure to acknowledge the Department of Botany, Faculty of science and Environmental Research Institute, Chulalongkorn University for offering laboratory facilities in this research and the Graduate School of Chulalongkorn University for financial support.

Thanks and admiration also extended to Mr. Chainamm Prempreechakul who always offered a help without hesitation.

Special thank to Miss Nantanit Wanichacheva, Mr. Likhit Sriprasertsuk, and C826xxx friends for their help, generosity and friendliness. Thanks also extended to other people from the Department of Botany and the Inter-Department Environmental Science.

Finally, I would like to express my deepest thanks to my family for their love and support.

#### CONTENTS

| Page   |
|--|
| THAI ABSTRACTiv  |
| ENGLISH ABSTRACTv  |
| ACKNOWLEDGEMENTvi  |
| CONTENTSvii  |
| LIST OF TABLESx  |
| LIST OF FIGURESxii   |
| ABBREVIATIONS AND UNITS xiv  |
| CHAPTER 1 INTRODUCTION   |
| 1.1 Cadmium and the environmental concerns                         |
| 1.2 Objective6   |
| 1.3 Scope of study6  |
| 1.4 Anticipated benefits6  |
| CHAPTER 2 LITERATURE REVIEW  |
| 2.1 Factors affecting plant uptake of cadmium                      |
| 2.1.1 pH   |
| 2.1.2 Cation Exchange Capacity (CEC)8                              |
| 2.1.3 Cadmium origin and its content8                              |
| 2.1.4 Organic matter8  |
| 2.1.5 Other ions   |
| 2.1.6 Mycorrhiza   |
| 2.1.7 Plant factors10  |
| 2.2 Cadmium uptake and translocation                               |
| 2.3 Effects of cadmium stress on physiological responses           |
| 2.3.1 Cell expansion as affected by cadmium                        |
| 2.3.2 Alteration of plant-water relation                           |
| 2.3.2.1 Water transport via plasma membrane                        |
| 2.3.2.2 Effect of cadmium on factors governing plant water balance |
| 2.3.3Translocation of other nutrients.                             |

## **CONTENTS (Cont.)**

| Pag   | e |
|---|---|
| 2.3.4 Proline accumulation  | ó |
| 2.3.5 Chlorophyll content   | 7 |
| 2.3.6 Cadmium accumulation.   | 8 |
| 2.4 Phytoremediation : an alternative method to ameliorate the Cd-polluted soil | 2 |
| CHAPTER 3 MATERIALS AND METHODS   |   |
| 3.1 Materials   | 4 |
| 3.2 Methods   |   |
| 3.2.1 Study on the physiology of plants22                                       | 7 |
| 3.2.2 Study on cadmium accumulation   | ) |
| 3.3.3 Statistical Analysis29  | ) |
| CHAPTER 4 RESULTS   |   |
| 4.1 Physiological responses of plants to cadmium stress                         |   |
| 4.1.1 Proline accumulation  | ) |
| 4.1.2 Chlorophyll contents  | 4 |
| 4.1.3 Relative Water Content (RWC)  | 1 |
| 4.1.4 The number of leaves  | ó |
| 4.1.5 Leaf area   | 5 |
| 4.1.6 Root to Shoot Ratio   | i |
| 4.1.7 Cadmium accumulation  | 1 |
| 4.2 Relationship of treatments and parameters6                                  | İ |
| CHAPTER 5 DISCUSSION  |   |
| 5.1 Physiological responses of plants under stress                              |   |
| 5.1.1 Relative Water Content (RWC) and proline accumulation6                    | 7 |
| 5.1.2 Chlorophyll contents  | 1 |
| 5.1.3 The growth of plants  | 3 |
| 5.1.4 Cadmium accumulation  | 5 |
| 5.2 Selection of metal tolerant species   |   |
| 5.2.1 Selection by determining metal accumulation                               | 6 |

#### **CONTENTS (Cont.)**

|   | PAGE |
|---|------|
| 5.2.2 Selection by root growth determination                  | 78   |
| 5.2.3 Selection by using proline accumulation                 | 79   |
| 5.2.4 Selection based on yield reduction                      | 80   |
| 5.3 Possibility of using plant materials for phytoremediation | 81   |
| CHAPTER 6 CONCLUSION AND RECOMMENDATION                       |      |
| 6.1 Conclusion  | 82   |
| 6.2 Recommendation.   | 83   |
| REFERENCES  | 84   |
| APPENDIX A  | 90   |
| APPENDIX B  | 92   |
| APPENDIX C  | 94   |
| APPENDIX D  | 97   |
| APPENDIX E  | 100  |
| DIOCDADHY   | 108  |

## LIST OF TABLES

| I | ABL  | E PAC   | ЭE  |
|---|------|---|-----|
|   | 2.1  | Cadmium accumulation by different cultivated plants                           | 19  |
|   | 4.1  | Proline accumulation of four plants species with 0 and 5 ppm Cd treatment     | .31 |
|   | 4.2  | Proline accumulation of four plants species with 0 and 20 ppm Cd treatment    | .32 |
|   | 4.3  | Chlorophyll a content of four plants species with 0 and 5 ppm Cd treatment    | 35  |
|   | 4.4  | Chlorophyll a content of four plants species with 0 and 20 ppm Cd treatment   | .36 |
|   | 4.5  | Chlorophyll b content of four plants species with 0 and 5 ppm Cd treatment    | .37 |
|   | 4.6  | Chlorophyll b content of four plants species with 0 and 20 ppm Cd treatment   | .38 |
|   | 4.7  | Chlorophyll a+b content of four plants species with 0 and 5 ppm Cd treatment  | .39 |
|   | 4.8  | Chlorophyll a+b content of four plants species with 0 and 20 ppm Cd treatment | .40 |
|   | 4.9  | Relative Water Content (RWC) of four plants species                           |     |
|   |      | with 0 and 5 ppm Cd treatment.  | .43 |
|   | 4.10 | Relative Water Content (RWC) of four plants species                           |     |
|   |      | with 0 and 20 ppm Cd treatment  | .44 |
|   | 4.11 | The number of leaves of four plants species with 0 and 5 ppm Cd treatment     | 47  |
|   | 4.12 | The number of leaves of four plants species with 0 and 20 ppm Cd treatment    | .48 |
|   | 4.13 | Leaf area of four plants species with 0 and 5 ppm Cd treatment                | .49 |
|   | 4.14 | Leaf area of four plants species with 0 and 20 ppm Cd treatment               | .50 |
|   | 4.15 | Root to Shoot Ratio of four plants species with 0 and 5 ppm Cd treatment      | .52 |
|   | 4.16 | Root to Shoot Ratio of four plants species with 0 and 20 ppm Cd treatment     | .53 |
|   | 4.17 | Cadmium content in root exposed to 0 and 5 ppm treatment                      | .54 |
|   | 4.18 | Cadmium content in root exposed to 0 and 20 ppm treatment                     | .55 |
|   | 4.19 | Cadmium content in shoot of four plants species                               |     |
|   |      | with 0 and 5 ppm Cd treatment   | .58 |
|   | 4.20 | Cadmium content in shoot of four plants species                               |     |
|   |      | with 0 and 20 ppm Cd treatment  | .59 |
|   | 4.21 | Correlation of each parameter with the 0 and 5 ppm Cd treatment               | 62  |
|   | 4.22 | Correlation of each parameter with the 0 and 20 ppm Cd treatment              | .63 |

#### LIST OF TABLES (Cont.)

| PAG  | E  |
|--|----|
| 4.23 Correlation between each parameter and root Cd content                      |    |
| of the 0 and 5 ppm treatment6  | 54 |
| 4.24 Correlation between each parameter and root Cd content                      |    |
| of the 0 and 20 ppm treatment6   | 54 |
| 4.25 Correlation between each parameter and shoot Cd content                     |    |
| of the 0 and 5 ppm treatment6  | 5  |
| 4.26 Correlation between each parameter and shoot Cd content                     |    |
| of the 0 and 20 ppm treatment  | 55 |
| 4.27 Correlation between RWC and proline accumulation at each studied period6    | 56 |
| 5.1 Percentage of shoot dry weight of stressed plants relative to the controls   | 30 |
| B-1 Shoot dry weight of four plant species exposed to 5 and 20 ppm Cd            | )3 |
| C-1 Cadmium accumulation in different plant parts after 0 and 5 ppm Cd exposure  | €  |
| C-2 Cadmium accumulation in different plant parts after 0 and 20 ppm Cd exposure | 96 |
| D-1 Proline accumulation at different RWC of four plant species9                 | 18 |
| D-2 Proline accumulation at various leaf Cd content of three plant species       |    |
| exposed to 0, 5 and 20 ppm Cd9   | )9 |

#### LIST OF FIGURES

| F | IGUI | PAGE   |
|---|------|--|
|   | 4.1  | Proline accumulation of four plant species exposed to 5 ppm Cd                           |
|   | 4.2  | Proline accumulation of four plant species exposed to 20 ppm Cd                          |
|   | 4.3  | Leaf chlorophyll a, b and a+b contents of B. oleracea and E. prostrata                   |
|   |      | at day 14 after Cd elimination of the 5 ppm treatment                                    |
|   | 4.4  | Leaf chlorophyll a, b and a+b contents of B. oleracea and                                |
|   |      | at day 14 after Cd elimination of the 20 ppm treatment                                   |
|   | 4.5  | RWC of four plant species exposed to 5 ppm Cd  |
|   | 4.6  | RWC of four plant species exposed to 20 ppm Cd   |
|   | 4.7  | Cadmium accumulation in roots of four plant species exposed to 5 ppm Cd56                |
|   | 4.8  | Cadmium accumulation in roots of four plant species exposed to 20 ppm Cd56               |
|   | 4.9  | Cadmium accumulation in shoots of four plant species exposed to 5 ppm Cd60               |
|   | 4.10 | Cadmium accumulation in shoots of four plant species exposed to 20 ppm Cd60              |
|   | E-1  | I. aquatica exposed to 0 ppm (A) and 5 ppm (B) Cd treatment                              |
|   |      | at day 20 of exposure  |
|   | E-2  | I. aquatica exposed to 0 ppm (A) and 20 ppm (B) Cd treatment                             |
|   |      | at day 8 of exposure   |
|   | E-3  | Normal growth of <i>B. oleracea</i> (the controls) of the 0 and 5 ppm Cd treatment102    |
|   | E-4  | Symptoms of Cd-treated B. oleracea (5 ppm Cd) of the 0 and 5 ppm Cd treatment102         |
|   | E-5  | Symptoms of Cd-treated B. oleracea (20 ppm Cd)   |
|   |      | of the 0 and 20 ppm Cd treatment   |
|   | E-6  | B. oleracea's root browning after 8 days of 20 ppm Cd treatment                          |
|   | E-7  | Normal growth of <i>E. prostrata</i> (the controls) of the 0 and 5 ppm Cd treatment104   |
|   | E-8  | Symptoms of Cd-treated <i>E. prostrata</i> (5 ppm Cd) of the 0 and 5 ppm Cd treatment104 |
|   | E-9  | Symptoms of Cd-treated <i>E. prostrata</i> (20 ppm Cd)                                   |
|   |      | of the 0 and 20 ppm Cd treatment   |

# LIST OF FIGURES (cont.)

| FIGURE PAGE  |   |
|--|---|
| E-10 E. prostrata's roots development after being transferred to Cd-free solution  |   |
| for 14 days of the 0 and 5 ppm Cd treatment  |   |
| E-11 Normal growth of C. barbata (the controls) of the 0 and 5 ppm Cd treatment106 |   |
| E-12 Symptoms of Cd-treated C. barbata (5 ppm Cd)                                  |   |
| of the 0 and 5 ppm Cd treatment  |   |
| E-13 Symptoms of Cd-treated C. barbata (20 ppm Cd)                                 |   |
| of the 0 and 20 ppm Cd treatment   | , |
| E-14 C. barbata's roots development after being transferred to Cd-free solution    |   |
| for 14 days of the 0 and 5 ppm Cd treatment107                                     | , |

#### ABBREVIATIONS AND UNITS

2d = 2 days of 5 or 20 ppm Cd exposure

11d = 11 days of 5 ppm Cd exposure

20d = 20 days of 5 ppm Cd exposure

7d (27) = 7 days of transfer to new Cd-free solution after 20 days of 5 ppm Cd exposure or the 27<sup>th</sup> day of treatment

14d (34) = 14 days of transfer to new Cd-free solution after 20 days of 5 ppm Cd exposure or the 34<sup>th</sup> day of treatment

5d = 5 days of 20 ppm Cd exposure

8d = 8 days of 20 ppm Cd treatment

7d (15) = 7 days of transfer to new Cd-free solution after 8 days of 20 ppm Cd exposure or the 15<sup>th</sup> day of treatment

14d (22) = 14 days of transfer to new Cd-free solution after 8 days of 20 ppm Cd exposure or the 22<sup>nd</sup> day of treatment

 $\mu g/g$  = Microgram per gram

 $\mu$ M or  $\mu$ mol = Micromole

ANOVA = Analysis of varience

C/N = Carbon per nitrogen

CEC = Cation Exchange Capacity

Chl = Chlorophyll

CRD = Completely randomized design

cm<sup>3</sup> = Cubic centimeter

DMRT = Duncan's Multiple Range Test

DW = Dry weight

EC = European Community

FW = Fresh weight

Fig = Figure

g = gram

Ha = Hectare

m = Meter

M = Mole

mg/l = Milligram per liter

mm = Millimeter

nm = Nanometer

rpm = round per minute

R/S Ratio = Root to Shoot Ratio

RH = Relative humidity

SL = Significance Levels

TW = Turgid weight