

Ain Shams University Women's College for Arts, Science and Education

### Microbial Interaction in Relation to Plant Growth Performance in Heavy Metals Contaminated Soil

## A Thesis Submitted in Partial Fulfilment of the Requirements for the Degree of Master of Science

In

**Microbiology** 

By

Safa Shaaban Hafez Mohamed B. Sc. Microbiology and Chemistry 2004

To

Botany Department, Women's College for Arts, Science and Education, Ain Shams University 2010

## Approval Sheet

Name: Safa Shaaban Hafez Mohamed

Title: Microbial Interaction in Relation to Plant Growth Performance in Heavy Metals Contaminated Soil

Supervisors:	Approved
1- Prof. Dr. Azhar Abd El-Karim Hussein, Prof. of Microbiology, Botany Department, Women's College, Ain Shams University.	
2- Late Prof. Dr. Magdy Ismaïl Mostafa, Prof. of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University.	
3- Prof. Dr. Atef Fathallah Mohamed Abd El-Wahab, Prof. of Agricultural Microbiology, Soils, Water and Environment Institute, Agricultural research Center, Ministry of Agriculture.	
4- Dr. Hoda Hassan Ahmed Abo Ghalia, Ass. Prof. of Microbiology, Botany Department, Women's College, Ain Shams University.	

Name: Safa Shaaban Hafez Mohamed

Title: Microbial Interaction in Relation to Plant Growth Performance in Heavy Metals Contaminated Soil

#### **Examiners:**

- 1- Prof. Dr. El-Shahat Mohamed Ramadan,Prof. of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University.
- 2- Prof. Dr. Aziz Mohamed Aziz Higazy, Prof. of Agricultural Microbiology, Faculty of Agriculture, Cairo University.
- 3- Prof. Dr. Azhar Abd El-Karim Hussein, Prof. of Microbiology, Botany Department, Women's College, Ain Shams University.
- 4- Prof. Dr. Atef Fathallah Mohamed, Prof. of Agricultral Microbiology, Soils, Water, and Environment Institute, Agricultural Research Center, Ministry of Agriculture.

I would like to dedicate this work	to
My fa	ather
	My mother
	and
	My husband
For supporting me through this w	ork.

This thesis

has not been submitted

for a degree at this or any other

University

Safa Shaaban Hafez



Women's College for Arts, Science and Education Botany department

## Microbial Interaction in Relation to Plant Growth Performance in Heavy Metals Contaminated Soil

#### **A Thesis**

**Submitted in Partial Fulfilment of the Requirements** for the Degree of Master of Science (Microbiology)

#### By

### Safa Shaaban Hafez Mohamed B. Sc. Microbiology and Chemistry 2004

### Supervised by

Prof.Dr.Azhar Abd El-Karim Hussein, Prof. of Microbiology, Botany Department, Women's College, Ain Shams University.

Late Prof. Dr. Magdy Ismaïl Mostafa, Prof. of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University. Prof. Dr. Atef Fathallah Mohamed Abd El-Wahab, Prof. of Agricultural Soils, Water and Environment Institute, Agricultural research Center, Ministry of Agriculture.

Dr. Hoda Hassan Ahmed Abo Ghalia, Ass. Prof. of Microbiology, Botany Department, Women's College, Ain Shams University.

2010



M.Sc. Thesis

Microbiology

Safa Shaaban Hafez

2010

## Acknowledgement

First and foremost, I feel always indebted to **God**, the most beneficent and merciful. I praise **Allah** for all the gifts which he has gave me.

I would like to express my great thanks and sincere gratitude to my supervisors; **Prof. Dr.Azhar Abd El Karim Hussein**, Prof. of Microbiology Botany Department Women's College, Ain Shams University, **Prof. Dr. Atef Fathallah Mohamed**, Prof. of Agricultural Microbiology; Soil, Water and Environment Institute, Agriculture Research Center, **Late Prof. Dr. Magdy Ismail Mostafa**, Prof. of Agricultural Microbiology Faculty of Agriculture, Ain Shams University, **Ass. Prof. Dr. Hoda Hassan Ahmed Abo Ghalia**, Ass. Prof. of Microbiology, Botany Department, Women's College, Ain Shams University for their suggestion the points of research, kind supervisions, encouragement, valuable guidance and continuous advice through this work.

My best thanks are also extended to **the Head of Botany Department**, all staff members and my colleagues at Botany

Department, Women's College, Ain Shams University.

I wish also express my great thanks to **Dr. Ahmed Ahmed Khalafallah**, Lecturer of Plant Ecology, Botany Department, Women's College, for his kind help in preparing this work.

I would like to express my sincere and deepest thanks to my father, mother, sister, brother and my husband for their kind help and support during this work.

Safa S. Hafez

#### Abstract

Isolation, enumeration and identification of arbuscular mycorrhizal fungi and actinomycetes were carried out. The isolates were obtained from soil and *Zea mays* plant roots irrigated with water effluents from three locations contaminated with heavy metals.

A pot experiment was conducted using the most resistant actinomycetes (*Streptomyces catenulae*) and AM fungi to study the heavy metals tolerance in faba bean and peanut plants grown on sand culture amended with a common heavy metals in the collected samples (Zn, Mn and Fe) as individually, mixture as well as sewage sludge at different concentrations.

The yield parameters determined in the two used plants included nodulation status, photosynthetic pigments, biochemical constituents, antioxidant enzymes, NPK content, mycorrhizal colonization, spores number, tolerance index and biological yield.

The obtained results showed that application of AM fungi and *S. catenulae* can lead to significant increase for most of the previous yield parameters of the two plants treated with the heavy metals and sewage sludge but not exceed to concentrations 250 ppm and 2% respectively.

Keywords: Heavy metals, sewage sludge, Arbuscular mycorrhizae, *Streptomyces catenulae*, faba bean, peanut, bioremediation.

# **Contents**

	Page
Abstract	A
Introduction	I
REVIEW OF LITERATURE	
1-Importance of legumes	1
1.1-Faba bean	١
1.2-Peanut	٣
2. Sources of heavy metals and their pollution aspects	٤
2.1-Zinc	٦
2.2-Manganese	٧
2.3-Iron	٨
3-Remediation technique for contaminated soils	٩
4-Heavy metal detoxification and tolerance mechanisms	11
4.1-The cell wall and root exudates	18
4.2-Plasma membrane	١٤
4.3-Heat shock proteins	10
4.4-Phytochelatins	10
4.5-Vacuolar compartmentalization	١٦
5-Role of soil microbes in the rhizospheres of plants	١٧
growing on heavy metal contaminated soils	
6-Mycorrhizae	١٨
6.1-Morphology of mycorrhizae	19
6.1.1-Vesicular arbuscular mycorrhizas	۲.
6.1.2- Ectomycorryhizal fungi	۲.
6.1.3- Ectendo-, arbutoid – and mono-tropoid	71
mycorrhizal associations	
6.1.4-Orchid mycorrhizal fungi	77
6.1.5-Ericoid mycorrhizal fungi	77
7. Vesicular-Arbuscular (VA) Mycorrhizal Fungi	77
7.1. Soil hyphae and hyphal proferation in the cortex	77
7.2 Arbuscules	77

	Page
7.3 Vesicles	77
7.4 Spores	۲ ٤
8.Taxonomy of Arbuscular Mycorrhizal (AM) fungi	24
9. Functions of Endomycorrhizal Fungi	70
10. Role of mycorrhizae in phytoremediation	49
10.1 Heavy metal-tolerant AM fungi	32
10.2 Contribution of AM fungi to uptake of heavy metals	٣٣
11. Actinomycetes	30
12. Mycorrhizae and rhizobacteria	٣٧
13. Rhizobacteria and heavy metals	٣9
14. Sewage sludge as sources of heavy metals	٤١
MATERIALS & METHODS	
1. Samples collection	٤٥
1.1-Soil and plant roots	٤٥
1.2-Soil and water analysis	٤٥
2- Microbiological studies	٤٩
2.1-Media used for isolation of bacteria, fungi and Actinomycetes	٤٩
2.2 Enumeration of the major soil microbial populations	01
2.3 Qualitative assays of heavy metals resistant soil microorganisms	٥١
2.4 Semiquantitative assays of heavy metal resistant soil microorganisms	٥٢
2.5 Determination of phosphate dissolving actinomycetes	٥٣
2.6 Determination of the total acidity	٥٣
2.7 The interaction between the <i>Rhizobuim</i> and actinomycetes	٥٣
2.8 Identification of the resistant actinomycetes	٥٤
2.8.1 Morphological characteristics	0 8
2.8.2 Characterization of spore chains	0 8

	1
	Page
2.8.2 Physiological characteristics	00
2.9 Production of AM fungi	٥٧
2.9.1 Extraction and estimation of AM spores	٥٧
2.9.2 Multiplication of AM inoculum	58
2.9.3 Staining of AM infected roots	٥٨
2.9.4 Estimation of AM infection	٥٩
2.9.5 Identification of the isolated Arbuscular mycorrhizal fungi	٥٩
3. Pot experiment	71
3.1 Plant material	71
3.2 Soil used	71
3.3 Sewage sludge	٦٣
3.4 Heavy metals	7 £
3.5 Microorganisms used	70
3.6 Experimental design for treatment with individual	70
heavy metal	
3.7 Experimental design for treatment with mixture	٦٦
of heavy metals	
3.8 Experimental design for Sewage sludge treatment	77
4. Measurements	77
4.1 Nodulation status	77
4.2 Assay of nitrogenase activity	٦٨
4.3 Estimation of photosynthetic pigments	٦٨
4.4 Estimation of total soluble sugars	٦٩
4.5 Estimation of total soluble proteins	79
4.5.1 Extraction and preparation of protein samples	79
4.6 Estimation of proline content	٧.
4.7 Determination of total free amino acids	٧١
4.8 Estimation of crude protein	77

	Page
4.9 Estimation of antioxidant enzymes	77
4.9.1 Method of enzymes extraction	<b>٧</b> ٢
4.9.2 Assay of peroxidase activity	72
4.9.3 Assay of catalase activity	٧٣
4.9.4 Assay of superoxide dismutase activity	٧٣
4.10 Estimation of mineral content	74
4.11 Estimation of root surface area	٧٤
4.12 Tolerance index (Ti)	٧٥
4.13 Estimation of the heavy metals	٧٥
4.14 Yield parameters	٧٥
5. Statistical analysis	<b>٧٦</b>
RESULTS	
Section 1(Laboratory experiments)	<b>YY</b>
1.1- Isolation and enumeration of the major soil microbial	<b>YY</b>
population	
1.2- Qualitative assays of heavy metals resistant	<b>YY</b>
microorganisms	
1.3- Semiquantitative assays of heavy metals resistant	41
microorganisms	
1.4- Phosphate dissolving actinomycetes	۸١
1.5- Total acidity	۸١
1.6- Actinomycetes characteristics	٨٢
Section 2 (Pot experiment)	٨o
2.1-Nodulation status	٨o
2.2-Photosynthetic pigments	9 £
2.3 Antioxidant enzymes	١
2.4 Biochemical constituents	1.9
2.5 Total dry weight	119
2.6 Root surface area	170

	Page
2.7 Mineral contents	177
2.8 AM fungi infection and spores number	177
2.9 Tolerance index	139
2.10 Biological yield and its components	1 2 .
2.11 Heavy metals contents	100
DISCUSSION	101
CONCLUSION AND RECOMMENDATION	١٧٤
Summary	١٧٦
References	١٨٣
Arabic summary	