Mansoura University
Faculty of Agriculture
Soils Department

Phytoremediation of Some Egyptian Soils Polluted with Heavy Metals

By

Mohamed Saad Abd El-Satar Ramadan

B.Sc. Agric. Sciences (Soils), Kafr El-Sheikh University, 2006
M.Sc. Agric. Sciences (Soils), Kafr El-Sheikh University, 2009

THESIS

Submitted in Partial Fulfillment of the
Requirements for the Degree

Of
DOCTOR OF PHILOSOPHY

In

Soil Science

SUPERVISORS

Prof. Dr.

Mohamed W .M. El- Agrodi.
Prof. of Soil Science, Soils Dept.,
Fac. of Agric., Mansoura University

Prof. Dr.
Farouk I. Zein
Head of Research
Soils, Water and Environment Research Institute, ARC

Asso. Prof.
Gomaa L. Ahmed.
Associate Professor of Soil Science, Soils Dept., Fac. of Agric., Mansoura University

2016

Mansoura University
Faculty of Agriculture
Soils Department

SUPERVISION

Title of Thesis:

Phytoremediation of Some Egyptian Soils Polluted with Heavy Metals

The Researcher:

Mohamed Saad Abd El-Satar Ramadan

Thesis Supervised by:

No	Name	Position	Signature
1	*Prof. Dr.* *Mohamed W .M. El- Agrodi.*	Prof. of Soil Science, Soils Dept., Faculty of Agric., Mansoura Univ.	
2	*Prof. Dr.* *Farouk I. Zein*	Head of Research, Soils, Water and Environment Research Institute, ARC.	
3	*Asso. Prof.* *Gomaa L. Ahmed.*	Associate Prof. of Soil Science, Soils Dept., Fac. of Agric., Mansoura Univ.	

Discussion date: **/ / 20**

Head of Dept.	Vice Dean of Higher Studies and Research	Dean
Prof. Dr. *Ayman M. El-Ghamry*	*Prof. Dr.* *Yasser M. Shabana*	*Prof. Dr.* *Yasser M. El-Hadidi*

2016

Mansoura University
Faculty of Agriculture
Soils Department

APPROVAL SHEET

Title of Thesis:

Phytoremediation of Some Egyptian Soils Polluted with Heavy Metals

The Researcher:

Mohamed Saad Abd El-Satar

Thesis Supervised by:

No	Name	Position	Signature
1	*Prof. Dr.* *Mohamed W.M. El- Agrodi.*	Prof. of Soil Science, Soils Dept., Faculty of Agric., Mansoura University.	
2	*Prof. Dr.* *Farouk I. Zein*	Head of Research, Soil, Water and Environment Research Institute, ARC.	
3	*Asso. Prof.* *Gomaa L. Ahmed.*	Associate Professor of Soil Science, Soils Dept., Fac. of Agric., Mansoura Univ.	

Thesis Approved By:

No	Name	Position	Signature
1	*Prof. Dr.* *Mohamed W.M. El- Agrodi.*	Prof. of Soil Science, Soils Dept., Faculty of Agriculture, Mansoura University.	
2	*Prof. Dr.* *Elsayd A. M. Awad.*	Prof. of Soil Science, Soils Dept., Faculty of Agriculture, Zagazig University.	
3	*Asso. Prof.* *Gomaa L. Ahmed.*	Associate Professor of Soil Science, Soils Dept., Fac. of Agric., Mansoura Univ.	
4	*Asso. Prof.* *Tarek M. El-Zehery.*	Associate Professor of Soil Science, Soils Dept., Fac. of Agric., Mansoura Univ.	

Discussion date: **/ / 20**

Head of Dept.	**Vice Dean of Higher Studies and Research**	**Dean**
Prof. Dr. ***Ayman M. El-Ghamry***	***Prof. Dr.*** ***Yasser M. Shabana***	***Prof. Dr.*** ***Yasser M. El-Hadidi***

2016

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

APCAs	Amino-PolyCarboxylic Acids
Approx.	Approximately
CA	Citric Acid
CDTA	1,2-CyclohexyleneDinitriloTetraAcetic acid
CTAB	cetyl trimethyl ammonium bromide
DGT	Diffusive Gradients in Thin films
EDDHA	EthyleneDiamine-N,N'-bis(2-HydroxyphenylAcetic acid)
EDDS	EthyleneDiamine-N,N'-Di-Succinic acid
EDTA	EthyleneDiamineTetraAcetic acid
EGTA	Ethylene Glycol-bis(β-aminoethyl ether)-N,N,N',N'-TetraAcetic acid
Fed.	Feddan
GA3	Gibberellic Acid
HA	Humic Acid
HEDTA	2-Hydroxyethyl Ethylene-Diamine-Triacetic Acid
IAA	Indole-3-Acetic Acid
MDA	MalonDiAldehyde
NLMWOA	Natural Low Molecular Weight Organic Acids
NTA	NitriloTriacetic acid

5. SUMMARY AND CONCLUSION

Three pot experiments were conducted in a wired greenhouse at Sakha Agricultural Research Station, Kafr El-Sheikh Governorate. The experiments aimed to investigate the phytoextraction efficiency of Cu, Zn and Pb from contaminated soils using sunflower (*Helianthus annuus*) and indian mustard (*Brassica juncea*) under chelators addition. Two experiments were carried out to study the effect of chelating agents, EDTA (0.0, 1.5, 3.0 and 4.5 mmol kg^{-1} soil), citric acid (CA) (0, 3, 6 and 9 mmol kg^{-1} soil) and humic acid (HA) (0.0, 0.2, 0.4 and 0.6 g kg^{-1} soil), on the phytoextraction efficiency of Cu, Zn and Pb by sunflower (summer season of 2013) and indian mustard plants (winter season of 2013/2014) and leaching behavior of these metals from Abu Rawash and Al-Gabal Al-Asfar contaminated soils. Third experiment (winter season 2013/2014) was conducted to study the residual effect of chelating agents added to sunflower experiment, without any addition of chelators, on phytoextraction of tested metals by indian mustard and leaching behavior of these metals from studied soils.

The main results can be summarized as follow:

5.1. Effect of chelators on the dry weight (g $plant^{-1}$) of sunflower and indian mustard plants grown on the two studied soils:

The plant organs of sunflower were increased due to CA and HA application (especially at 3 mmol kg^{-1} soil and 6 mmol kg^{-1} soil, respectively) compared to EDTA addition in both soils. Where, EDTA addition in both studied soils led to a significant decrease in dry matter production of sunflower plant as compared to control. In this respect, visual symptoms of toxicity were observed in the EDTA treatment especially at high levels.

The highest value of indian mustard dry weight were recorded due to CA and HA application, but this effect was higher for HA treatment. While, using EDTA reduced plant dry weight yield as compared to control in both soils. Besides, visual symptoms of toxicity were observed on indian mustard similar to that happened with sunflower.

5.2. Effect of chelators on Cu concentration (mg kg^{-1} DW) in plant organs of sunflower and indian mustard plants grown on the two soils under study:

Application of EDTA and CA increased Cu concentrations of sunflower and indian mustard plant organs as compared to HA, but this effect was higher for EDTA. Application of 4.5 mmol EDTA kg^{-1} soil gave the highest concentrations of Cu in roots, stems, leaves

and seeds of both plants as compared to control in both studied soils. The increments for sunflower were 4.02, 2.47, 3.12 and 1.37 folds in Abu Rawash soil and were 4.22, 2.64, 3.91 and 1.55 fold in Al-Gabal Al-Asfar soil, respectively. While, the increments for indian mustard were 2.08, 2.44, 2.87 and 1.30 folds in Abu Rawash soil and were 1.91, 2.88, 3.04 and 1.56 folds in Al-Gabal Al-Asfar soil, respectively.

5.3. Effect of chelators on Cu uptake (mg plant^{-1}) by sunflower and indian mustard plants grown on the two soils under study:

Each of EDTA and CA treatments significantly increased the uptake of Cu by roots, stems, leaves, seeds of sunflower plant and total uptake (mg plant^{-1}) by sunflower and indian mustard plants in both studied soils. Where, Cu uptake by sunflower and indian mustard plants as affected by using different chelator treatments was in the order: EDTA > CA > HA in both soils. No constant trend regarding to the effect of HA on Cu uptake by both plants in the both soils. EDTA at 4.5 mmol kg^{-1} soil achieved the highest values of Cu total uptake by plants in both studied soils. Where, the corresponding values increased as compared to control by 2.06 and 2.43 folds in case of sunflower and by 1.70 and 1.73 folds in case of indian mustard in Abu Rawash and Al-Gabal Al-Asfar soils, respectively.

5.4. Effect of chelators on Zn concentration (mg kg^{-1} DW) in plant organs of sunflower and indian mustard plants grown on the two soils under study:

Application of EDTA, CA and HA increased Zn concentrations in plant organs of sunflower and indian mustard plants. However, the order of different chelators on increasing Zn concentrations in plants was: EDTA > CA > HA in both studied soils. The concentrations of Zn in different plant parts increased with increasing EDTA and CA levels up to the highest level used (4.5 and 9.0 mmol kg^{-1} soil, respectively). Application of 4.5 mmol EDTA kg^{-1} soil recorded the highest concentrations of Zn in both plant parts in both soils as compared to control. These increases amounted by 1.21, 1.51, 1.39 and 1.15 folds in Abu Rawash soil and by 1.26, 1.96, 1.55 and 1.13 folds in Al-Gabal Al-Asfar soil, respectively, for sunflower plant. Whereas, these increases amounted by 26.28, 91.30, 77.00 and 15.75% in Abu Rawash soil and by 43.30, 90.82, 83.08 and 11.19% in Al-Gabal Al-Asfar soil, respectively, for indian mustard plant.

5.5. Effect of chelators on Zn uptake (mg plant^{-1}) by sunflower and indian mustard plants grown on the two soils under study:

Application of CA and HA treatment increased Zn uptake of sunflower plants (mg plant^{-1}) as compared to EDTA treatment in Abu Rawash soil. Whilst, a contrary trend was found in Al-Gabal Al-Asfar soil where EDTA increased Zn uptake (mg plant^{-1}) as compared to CA and HA. Application of 3 mmol CA kg^{-1} soil had the highest values of total uptake of Zn by plant in Abu Rawash soil (29.39% increase over control), while application of 3 mmol EDTA kg^{-1} soil had the highest uptake of Zn by sunflower in Al-Gabal Al-Asfar soil (32.45% increase over control).

Regarding to indian mustard plant, application of chelator treatments significantly increased the total uptake of different plant parts in both soils. However, application of EDTA and CA treatments increased total uptake of Zn (mg plant^{-1}) by plants as compared to HA treatment. Increasing the applied rate of EDTA and CA treatments up to the highest levels used (4.5 and 9 mmol kg^{-1} soil, respectively) resulted in increasing Zn uptake by indian mustard plants in both studied soils.

5.6. Effect of chelators on Pb roots concentration (mg kg^{-1} DW) of sunflower and indian mustard plants grown on the two soils under study:

Application of EDTA and CA treatments increased Pb concentrations in roots of both plants compared to HA treatment in both soils (the order was: EDTA > CA > HA). However, Pb concentrations in both plants roots increased with increasing EDTA and CA levels in both soils. The highest values of Pb concentration in roots of both plants were obtained due to using 4.5 mmol EDTA kg^{-1} soil in both studied soils, respectively. The increments for sunflower were 2.37 and 2.00 folds compared to control in Abu Rawash and Al-Gabal Al-Asfar soils, and the increments for indian mustard were 2.48 and 2.10 folds compared to control in Abu Rawash and Al-Gabal Al-Asfar soils.

5.7. Effect of chelators on Pb roots uptake (mg plant^{-1}) of sunflower and indian mustard plants grown on the two soils under study:

The uptake of Pb (mg plant^{-1}) by both plant roots due to EDTA and CA treatments application were increased compared to HA treatment in both soils. The uptake of Pb (mg plant^{-1}) by roots of both plant species due to application of chelator treatments were in the order: EDTA > CA > HA in both soils. The highest values of Pb uptake by indian mustard

and sunflower roots in both studied soils were realized by addition of 4.5 mmol EDTA kg^{-1} soil. Where, the increments in Abu Rawash and Al-Gabal Al-Asfar soils were 1.55 and 1.40 folds for sunflower roots and 1.80 and 1.58 folds for indian mustard roots as compared to control respectively.

5.8. Effect of chelators on Cu, Zn and Pb leaching (mg pot^{-1}) under cultivation of sunflower and indian mustard plants in the two soils under study:

EDTA had abnormal effects on Cu, Zn and Pb leaching than that of CA and HA in both soils, causing serious environmental threat due to the leaching of metals towards groundwater. CA also has a similar effect to that of EDTA, but to a lesser extent, on the leaching of these metals. HA levels did not affect any of metal leaching studied in both soil used. The highest values of Cu, Zn and Pb leaching (mg pot^{-1}) under cultivation of both plants were obtained using EDTA at a level of 4.5 mmol kg^{-1} soil. The corresponding increases were 53.44, 56.28 and 61.10 folds over control in Abu Rawash soil and 45.81, 38.81 and 54.72 folds over control in Al-Gabal Al-Asfar soil, respectively, under sunflower cultivation. Whereas, the corresponding increases were 50.83, 44.33 and 74.81 folds over control in Abu Rawash soil and 61.83, 38.30 and 62.92 folds over control in Al-Gabal Al-Asfar soil, respectively, under indian mustard cultivation.

5.9. Residual effect of chelators on indian mustard dry weight (g $plant^{-1}$) grown on the two studied soils:

the obtained results demonstrated that no significant or perceptible differences in dry weight of any indian mustard plant parts were found due to previous application of EDTA, CA and HA treatments in both soils.

5.10. Residual effect of chelators on Cu concentration (mg kg^{-1} DW) in plant organs and Cu uptake (mg $plant^{-1}$) by indian mustard grown on the two soils under study:

Previous application of EDTA has significant residual effects on Cu concentrations in some indian mustard organs in both studied soils. On the other hand no significant residual effect was found on Cu concentrations in different plant parts due to previous application of different levels of CA and HA in both soils. The highest concentrations of Cu in indian mustard organs were obtained when EDTA was previously applied at 4.5 mmol kg^{-1} soil in both soils, where the corresponding increases percentage for roots, stems, leaves and seeds

compared to control were 23.08, 29.07, 36.05 and 8.33% compared to control in Abu Rawash soil and by 9.63, 26.04, 40.38 and 13.60% in Al-Gabal Al-Asfar soil, respectively.

Previous application of EDTA significantly increased Cu uptake by indian mustard (mg plant^{-1}) in both tested soils. Anyway, previous application of 4.5 mmol EDTA kg^{-1} soil recorded the highest values of total Cu uptake (mg plant^{-1}) by plants in both soils, where the corresponding increases percentage compared to control were 15.70 and 15.59% in Abu Rawash and Al-Gabal Al-Asfar soils, respectively.

5.11. Residual effect of chelators on Zn concentration (mg kg^{-1} DW) in plant organs and Zn uptake (mg plant^{-1}) by indian mustard grown on the two soils under study:

Previous applications of EDTA treatment increased Zn concentrations in plants as compared to CA and HA treatments, and this effect was increased with increasing EDTA addition rates up to 4.5 mmol kg^{-1} soil in both soils. While, no significant residual effect on Zn concentration in plant parts were found due to previous application of CA or HA in both soils. However, previous application of 4.5 mmol EDTA kg^{-1} soil achieved the highest concentrations of Zn in plant parts in both soils, except for roots and seeds in Al-Gabal Al-Asfar soil.

Regarding to Zn uptake (mg plant^{-1}) by indian mustard, the highest values of total uptake of Zn by plants were obtained due to previous application of 4.5 mmol EDTA kg^{-1} soil and the corresponding increases as compared to control were 6.48 and 9.00% in Abu Rawash and Al-Gabal Al-Asfar soils, respectively.

5.12. Residual effect of chelators on Pb roots concentration (mg kg^{-1} DW) and Pb roots uptake (mg plant^{-1}) of indian mustard plant grown on the two soils under study:

Previous application of EDTA treatment increased the mean values of Pb concentrations in plant roots (mg plant^{-1}) compared with CA and HA treatments in both studied soils. However, this effect was insignificant in both soils. Previous application of different CA or HA levels did not prove any significant or obvious effect on Pb concentration in roots in both soils. The highest concentrations of Pb in plant roots were obtained when EDTA was previously applied at 4.5 mmol kg^{-1} soil in Abu Rawash soil (20.43% increase over control) and 1.5 mmol kg^{-1} in Al-Gabal Al-Asfar soil (6.42% increase over control).

Previous application of EDTA increased Pb uptake by roots (mg plant^{-1}) in both soils as compared with CA and HA treatments, but this effect was insignificant in both studied soil. Previous application of CA and HA levels in any soil did not prove any significant or perceptible effect on Pb uptake by plant roots.

5.16. Resedial effect of chelators on Cu, Zn and Pb leaching (mg pot^{-1}) under indian mustard cultivation in the two soils under study:

Previous application of EDTA and CA increased Cu, Zn and Pb in the leachate compared with HA treatment. The leaching of Cu, Zn and Pb due to previous applications of EDTA was many times higher than that of CA. The highest values of Cu, Zn and Pb leaching (mg pot^{-1}) were obtained when EDTA was previously added at the rate of 4.5 mmol kg^{-1} in both soils, where the corresponding increases as compared to control were 6.79, 6.29 and 3.31 folds in Abu Rawash soil and were 4.45, 6.22 and 4.36 folds in Al-Gabal Al-Asfar soil, respectively.

It can be concluded that:

- EDTA had the highest efficacy on enhancing Cu and Zn absorption by sunflower and indian mustard plants. Application of CA also increased Cu and Zn absorption by both plant species, while HA was only efficient to increase Zn absorption by both plant species. The high concentrations of Cu and Zn in the plant organs due to applying EDTA compared to the other chelators induced toxicity, so reduced the dry matter of these organs.

- The overall results demonstrated that sunflower was more efficient than indian mustard in the phytoextraction of Cu and Zn from polluted soils. Indian mustard had the ability to accumulate Pb in its roots than sunflower plant.

- Using CA for the phytoremediation of Cu as well as CA and HA for the phytoremediation of Zn is favorable than EDTA despite the high efficiency of EDTA, due to either its harmful effect of high rates on plant growth or its increment effect of groundwater contamination risk via metal leaching.

- Phytoextraction of Pb was failed even with EDTA, CA and HA chelators addition, where there were neither any perceptible concentrations of Pb in the above ground portion of indian mustard plant parts.

- EDTA can persist in soil for long periods of time because of its low biodegradability compared to CA and HA. Consequently, EDTA can enhance the phytoremediation of metals from contaminated soil in two successive seasons after addition once in the first season. However, its prolonged presence in the soil, and its non-selective nature, dramatically increase the leaching risk of heavy metals.