Effect of *Moringa oleifera* Lam. Seeds Extract on Uptake of Heavy Metals in Micropropagated *Oryza sativa* L.

THESIS

Submitted in Partial Fulfillment of the
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In

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(Basic Environmental Science)

By

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B.Sc. in Science (Zoology - Chemistry), Faculty of Science, Cairo University (1999)

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SUPERVISION SHEET

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ABSTRACT

Effect of *Moringa oleifera* Lam. Seeds Extract on Uptake of Heavy Metals in Micropropagated *Oryza sativa* L.

Heavy metals have passive effects on plant growth and some physiological parameters in plant. The present investigation was done to investigate the effect of lead (Pb) and cadmium (Cd) on *in vitro* and *ex vitro* grown rice plantlets. A promising phytoremediation technique was used to remediate the negative effect of heavy metals. *Moringa oleifera* seed was used as natural and low cost phytoremediator, effective in heavy metals remediation in agriculture. Growth parameters were affected by the coagulation effect of *Moringa oleifera*. In addition, the physiological parameters as chlorophyll content, antioxidant activity, ascorbic acid, flavonoids content, indoles content were affected positively by *Moringa* seed extract under heavy metals effect. Also, glutilen bands of rice became denser under the effect of heavy metals and *Moringa* seed extract. Polyphenol oxidase, catalase and peroxidase activities exhibited increases in plants exposed to heavy metals and *Moringa* seed extract. The effect of

Moringa seed powder on growth parameters, carbohydrates content and heavy metals content of greenhouse grown rice under Pb and Cd stress were studied. In conclusion, the present investigation confirms the suitability and effectiveness of *Moringa* seed extract and powder as simple and cheap phytoremediation agents.

Keywords: *Moringa oleifera*, phytoremediation, rice, cadmium, lead, coagulants, antioxidants, polyphenol oxidase, catalase, peroxidase, ascorbic acid, flavonoids

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LIST OF ABBREVIATIONS

BA	Benzyl Adenine
CAT	Catalase
Cd	Cadmium
CPE	Crude Protein Extract
DPPH	ά- ά – diphenyl – β – picryl - hydrazyl
$HgCl_2$	Mercuric chloride
2iP	Iso pentenyladenine
Kin	Kinetin
MS	Murashige and Skoog
NaOCl	Sodium hypochlorite
SDS	Sodium Dodecyl Sulphate
Pb	Lead
PGRs	Plant Growth Regulators
POD	Peroxidase
PPO	Polyphenol oxidase

INTRODUCTION

INTRODUCTION

Excessive release of heavy metals into the environment due to industrialization and urbanization possess a great problem worldwide. Unlike organic pollutants, which are susceptible to biological degradation, heavy metal ions do not degrade into harmless end products **(Gupta *et al*., 2001)**. The increase heavy metals usage in industrial activities resulted in their existence in wastewater.

Some methods employed in the removal of heavy metals from wastewater; include ion exchange, precipitation, ultra-filtration, reverse osmosis, phytoremediation *etc*. In these methods, ion exchange has proved to be the most effective, although it is expensive. Though it is very cheap to phytoremediate polluted soils and water bodies, yet the method is slow **(Fourest and Roux, 1992)**. A promising method for heavy metal removal from wastewater is biosorption. Biosorption can be defined as the ability of biological materials to adsorb heavy metals from wastewater through metabolically mediated uptake and/or physicochemical uptake **(Fourest and Roux, 1992 and Adelaja *et al*., 2011)**.

Moringa oleifera Lam. (*Moringa*) is one of the natural coagulants that have been tested over the years as an alternative to the use of inorganic and synthetic coagulants. The plant is one of the best known and most widely distributed and naturalized

species of a monogenic family Moringaceae **(Ramachandran and Peter, 1980)**. It is a well-documented world renewed plant help for its extraordinary nutritional and medicinal properties. Also, it is a natural, antihelmintic, antibiotic, detoxifer, outstanding immune builder. *Moringa oleifera* tree grows in tropical and subtropical regions around the world.

Almost all parts of *Moringa*: root, bark, gum, leaf, fruit (pods), flowers, seed and seed oil have been used for various ailments in the indigenous medicine of South Asia, including the treatment of inflammation and infectious diseases along with cardiovascular, gastrointestinal, hematological and hepatorenal disease **(Morimitsu *et al.*, 2000**).

The seeds of *Moringa oleifera* have been traditionally used for the clarification of drinking water in rural areas of Sudan and Malawi **(Okuda *et al.*, 2000)**. Also, it is used in water purification and helps in reducing the incidence of water borne disease. Moreover, the *Moringa oleifera* seeds possess effective coagulation properties **(Muyibi and Evison, 1995)** and they are not toxic to human and animals **(Berger *et al.* 1984 and Grabow *et al.*, 1985)**. The coagulant in the seed is a protein that acts as a cationic polyelectrolyte. The soluble particles in the water attaches to the active agent that binds them together creating large flocks in the water.

Rice (*Oryza sativa* L., Gramineae) is a model plant for its economic importance and is used as a poplar crop in Egypt. Also, it needs a plenty of water for cultivation, which is much higher than other crops. The cultivation of rice in Egypt has expanded significantly, with an average annual increase of 6.7% **(Oad and Azim, 2002)**.

To overcome the increasing demand of water from expanding rice production, it is important to reduce the amount of water required for cultivation by the reuse of the agricultural wastewater after purification, mainly from heavy metals such as Pb and Cd, for water conservation.

The aim of the current work was to study the effect of *Moringa* seed coat protein extract and seed powder on heavy metals adsorption (Pb and Cd) and its ability to reduce their harmful effect on rice plantlets, either through *in vitro* culture or *ex vitro* in the greenhouse. Rice was chosen for the present study as an economical crop needs high quantity of water irrigation. Some growth and physiological characteristics were determined to evaluate the ability of *Moringa* seeds to precipitate heavy metals.

REVIEW
OF
LITERATURE

REVIEW OF LITERATURE

The review of literature contains the previous studies on the effect of heavy metals on the environment, human and plant with special emphasis on lead (Pb) and cadmium (Cd). Also, the use of plants as phytoremediators and *Moringa* (*Moringa oleifera* Lam.) as one of the natural coagulants. The cultivation problems of rice (*Oryza sativa*) are also included, in addition to the previous *in vitro* and *ex vitro* experiments based on phytoremediation.

1. Effect of heavy metals on human and environment

The presence of heavy metals in the environment due to industrialization and urbanization is becoming a severe environmental and public health problem.

Van Assche and Clijsters (1990) stated that in human, toxicity of heavy metals might result from the binding of heavy metals to sulphydryl groups in proteins, leading to inhibition of activity or disruption of structure, or from displacement of an essential element, resulting in deficiency effects. In addition, **Dietz *et al*. (1990)** discovered that excess of heavy metal might stimulate the formation of free radicals and excessive oxygen species, resulting in oxidative stress.