Al-Azhar University,
Faculty of Science,
(Girls Branch)

Bioremediation of Contaminated Soil with Petroleum Hydrocarbons

A thesis Presented
By

El-Shaymaa El-Sayed Mussa Mohamed

Assistant Researcher, Soils, Water and Environment
Research Institute , Agricultural Research Center
B.Sc. in Botany, Faculty of Science, Al-Azhar Univ. (2000)
M.Sc. in Botany, Faculty of Science, Al-Azhar Univ. (2005)

Submitted for the award of Degree of Doctor Philosophy (Ph.D) in Microbiology

To
Botany and Microbiology Department
Faculty of Science (Girls)
Al-Azhar University

SUPERVISED BY

Prof. Dr. Rawheya Abd El-Latif Salah El-Din

Professor in Botany & Microbiology Department,
Faculty of Science (Girls), Al-Azhar University.

Prof .Dr Ahmed Ghoneim Rahal

Professor (Emeritus) of Agricultural Microbiology, Agricultural Microbiology Department,
Soils, Water & Environment Research Institute
Agricultural Research Center, Giza, Egypt.

Prof. Dr.Lobna Abd EL-Aziz Ahmed Moussa

Professor of Agricultural Microbiology, Agricultural Microbiology Department,
Soils, Water & Environment Research Institute,
Agricultural Research Center, Giza, Egypt.

Dr. Maie Ahmed Younes El-khawaga

Assistant Professor of Microbiology, Botany & Microbiology Department,
Faculty of Science (Girls), Al-Azhar University.

2016

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PICTURES

ABSTRACT

Attention has been paid in this study to use both bioremediation and phytoremediation to return back the petroleum contaminated soil to its origin condition. The current study are divided into three parts, the first was for the isolation of the most effective bacterial isolates in degrading the hydrocarbons from oil contaminated soil. These isolates were identified as *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* biotype A. In this study both *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* proved to secret lipase enzyme, indole acetic acid, gibberellic acid and biosurfactant. The second part was for the use of these bacterial isolates in degradation (bioremediation) of the petroleum oil in their culture medium amended with 1% v/v petroleum oil. Results revealed that both *P. aeruginosa* & *P. fluorescens* biotype A strains can grow in presence of the petroleum oil due to the increase of bacterial counts and the values of fluorescein diacetate hydrolysis (FDA) both along with increasing the incubation period. The slightly decreases in their cultures pH values refers to the degradation of petroleum oil. This trend revealed that both *P. aeruginosa* & *P. fluorescens* strains were able to degrade the petroleum oil and utilize it as carbon sole source for growth, energy and reproduction. Results also revealed that *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* biotype A were the best petroleum oil degraders. The bioremediation behavior of *Pseudomonas aeruginosa* in laboratory was better than *Pseudomonas fluorescens*. Generally the degradation rate of petroleum oil was gradually increased with increasing the time of incubation with the two species, hence *P. aeruginosa* and *P. fluorescens* degrade 62.46 and 55.53 % of added petroleum oil, respectively, at the end of the experimental period. The third part was a greenhouse experiment to study the effect of both bioremediation and phytoremediation and their interactions on the petroleum oil contaminated soil through the response of common bean growth grown on this soil. The highest values of shoot heights, dry weight, chlorophyll and carotenoids contents were observed in plants cultivated in contaminated soil and inoculated with *P. fluorescens*. The phytoremediation by using common bean plant can be degraded petroleum oil in its rhizosphere. The plants cultivated in contaminated soil showed high significant proline amounts and peroxidase activity. Plants cultivated in contaminated soils and inoculated with *P. aeruginosa* or *P. flouresence* showed lower values of proline and peroxidase activity than that planted in contaminated and uncontaminated soil. The growth of common bean (*Phaseolus vulgaris* L.) and addition of petroleum hydrocarbon degraders ***viz***. *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* biotype A in petroleum oil contaminated soils improves the physical, chemical and biological properties of the soil and enhanced the degradation of petroleum oil. The study showed that the phytoremediation of crude oil contaminated soil by *Phaseolus vulgaris* only was not sufficient to degrade the hydrocarbons in soil whereas the multi-technique by using any of the petroleum oil degrading tested bacteria in combination with common bean plant showed the best degradation of petroleum oil in the contaminated soil. The bioremediation behavior of *Pseudomonas fluorescens* biotype A in the greenhouse was better than *Pseudomonas aeruginosa*.

Key words: bioremediation - phytoremediation - *Phaseolus vulgaris* - *Pseudomonas aeruginosa* - *Pseudomonas fluorescens* biotype A - petroleum contaminated soil.