



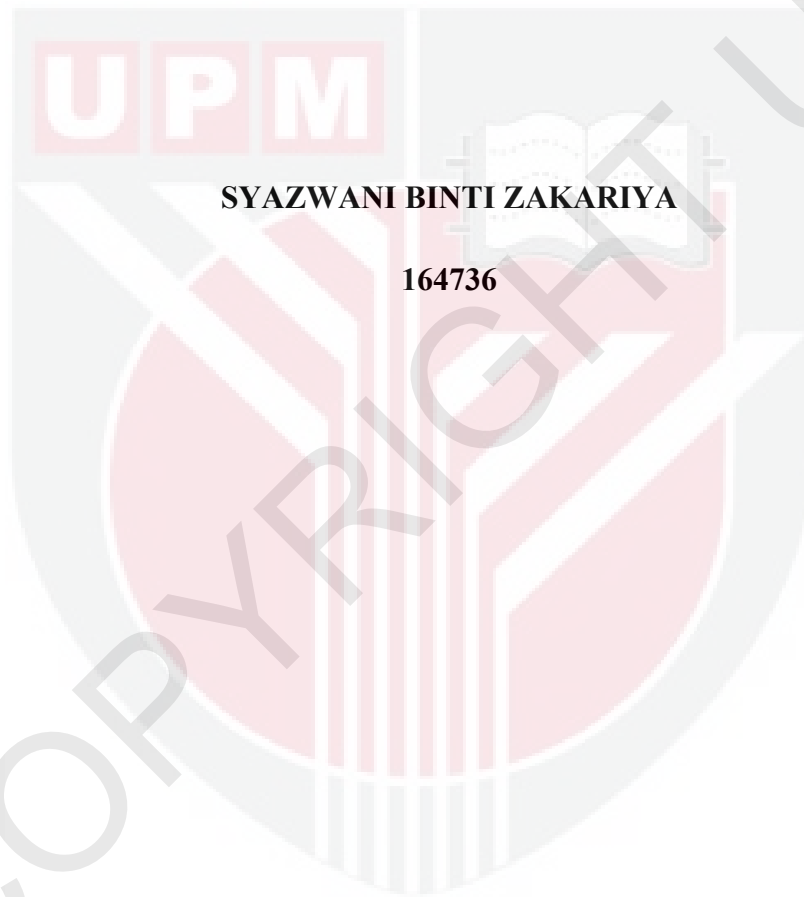
UNIVERSITI PUTRA MALAYSIA

***Glycine max* AS A POTENTIAL PHENOL PHYTOREMEDIATOR**

SYAZWANI ZAKARIYA

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SOYBEAN (*Glycine max*) AS A POTENTIAL PHENOL PHYTOREMEDIATOR



SYAZWANI BINTI ZAKARIYA

164736

**Dissertation submitted in partial fulfillment of the requirement for the course
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SOYBEAN (*Glycine ma*)x AS A POTENTIAL PHENOL PHYTOREMEDIATOR

SYAZWANI BINTI ZAKARIYA

164736

**DEPARTMENT OF MICROBIOLOGY
FACULTY BIOTECHNOLOGY AND BIOMOLECULAR SCIENCES
UNIVERSITI PUTRA MALAYSIA
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LETTER OF CONFIRMATION

Project paper title “*Glycine max* as a Potential Phenol Phytoremediator” prepared by Syazwani binti Zakariya (164736) for BMY4999 project and accepted as being qualified to get a degree from Faculty of Biotechnology and Biomolecular Sciences Universiti Putra Malaysia.

Confirmed by,

.....

Date:

Assoc. Prof. Dr. Janna Ong binti Abdullah

Supervisor

Department of Cell and Molecular Biology

Faculty of Biotechnology and Biomolecular Sciences,

Universiti Putra Malaysia

.....

Date:

Prof. Dr. Muhajir Hamid

Head of Department

Department of Microbiology

Faculty of Biotechnology and Biomolecular Sciences,

Universiti Putra Malaysia

ABSTRACT

There are many reported cases of phenol contamination worldwide. Malaysia itself is also facing the phenol pollution problems. *Glycine max* from the family Leguminosae and is commonly known as soybean is one of the potential plants that can be used to remediate phenol contaminated areas. The aims of this study were to analyse the potential of *G. max* in absorbing phenol from phenol- spiked water and to assess the shoots, roots and leaves growth of *G. max* grown in water spiked with different phenol concentrations. The soybean seeds were germinated and grown in designed system for each respective concentration for six days at room temperature. During the growth period, the elongation of roots, stems and leaves of the soybean plants were measured for every 24 hours by using a standard ruler. The plant morphologies throughout the 6 days were observed. One ml of water sample was collected and subjected to the phenol assay to quantify any remaining phenol in the water. The roots of plants grown in 0.04 g/ L phenol- spiked water were the longest averaging about 17.22 ± 1.23 cm. The stem length in 0.04 g/ L phenol was the longest measuring 8.87 ± 0.73 cm. The Control plants exhibited the longest leaves length (1.33 ± 0.60 cm) and the least (0.78 ± 0.17 cm) was in 0.05 g/ L phenol. No leaves formation could be observed in 0.4 g/ L phenol- spiked water. Phenol at 0.4 g/ L was concluded as high and toxic to the plants since their growths were distorted as early as Day 1. The seeds germinated normally in 0.01, 0.04 and 0.05 g/ L phenol water. Abnormal growth of the plants could be noted at three respective phenol water concentrations which were 0.1, 0.2 and 0.4 g/ L. Plants absorbed 100% phenol at different rates except for 0.04 g/ L, only half (0.20 g/ L) of the phenol was successfully absorbed by the plants on Day 3. The fastest absorption was detected at 0.2 g/ L whereby the plants absorbed all the phenols within 5 days at a rate of 0.00170 g/ L/ hour meanwhile, the slowest rate was at 0.01 g/ L. *G. max* was classified as a phytodegrader in group of phytoremediator since the phenol content in plant tissues after being treated with phenols showed reduction in amount.

ABSTRAK

Terdapat banyak kes pencemaran fenol yang berlaku di seluruh dunia. Malaysia juga turut menghadapi masalah yang sama. *Glycine max* daripada keluarga Leguminosae yang dikenali sebagai kacang soya adalah salah satu tumbuhan yang mempunyai potensi untuk digunakan bagi merawat fenol di kawasan yang tercemar. Tujuan kajian ini dilakukan adalah untuk menganalisis potensi *G. max* dalam penyerapan fenol dari air di samping mengukur dan menilai pertumbuhan pucuk, akar dan daunnya dalam kepekatan fenol yang berlainan. Benih kacang soya dibiarkan bercambah terlebih dahulu sebelum diletakkan ke dalam sistem hidroponik yang telah dicipta. Kajian dijalankan pada suhu bilik selama enam hari bagi setiap kepekatan. Dalam tempoh pertumbuhan, pemanjangan akar, batang dan daun pokok telah diukur bagi setiap 24 jam dengan menggunakan pembaris yang sama. Morfologi tumbuhan sepanjang 6 hari diperhatikan untuk mengenalpasti pertumbuhan yang normal dan tidak normal. Satu ml sampel air telah diambil dan esei fenol telah dijalankan untuk mengira fenol yang tertinggal di dalam air. Purata akar pokok yang ditanam dalam 0.04 g / L air mengandungi fenol adalah paling panjang iaitu 17.22 ± 1.23 cm. Panjang batang dalam 0.04 g / L fenol juga adalah terpanjang iaitu berukuran 8.87 ± 0.73 cm. Pokok kawalan menghasilkan daun paling panjang (1.33 ± 0.60 cm) manakala daun yang terpendek adalah yang didedahkan dalam 0.05 g / L fenol (0.78 ± 0.17 cm). Tiada pertumbuhan daun yang boleh diperhatikan dalam air yang mengandungi 0.4 g / L fenol. Kepekatan fenol 0.4 g / L disimpulkan sebagai tinggi dan toksik kepada pokok mulai terbantut seawal hari pertama. Benih bercambah secara normal dalam 0.01, 0.04 dan 0.05 g / L air mengandungi fenol. Pertumbuhan abnormal tumbuh-tumbuhan boleh diperhatikan dalam tiga kepekatan air fenol iaitu masing-masing merupakan 0.1, 0.2 dan 0.4 g / L. Pokok menyerap 100% fenol pada kadar yang berbeza kecuali 0.04 g / L, hanya separuh (0.20 g / L) fenol yang berjaya diserap oleh tumbuh-tumbuhan pada hari 3. Penyerapan paling cepat dikesan pada 0.2 g / L fenol di mana pokok menyerap semua fenol dalam masa 5 hari pada kadar 0.00170 g / L / jam. Sementara itu, kadar paling perlahan adalah pada 0.01 g / L. *G max* diklasifikasikan sebagai tumbuhan degradasi iaitu tergolong dalam tumbuhan remediasi kerana kandungan fenol dalam tisu pokok selepas dirawat dengan air mengandungi fenol menunjukkan pengurangan daripada jumlah asal.

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TABLE OF CONTENTS

LETTER OF CONFIRMATION	i
ABSTRACT	ii
<i>ABSTRAK</i>	iii
ACKNOWLEDGEMENT	iv
LIST OF TABLES	vii
LIST OF FIGURES	viii
LIST OF ABBREVIATIONS	ix
CHAPTER 1	1
INTRODUCTION	1
CHAPTER 2	3
LITERATURE REVIEW	3
2.1 <i>Glycine max</i>	3
2.2 Morphology of <i>Glycine max</i>	4
2.2.1 Leaves	4
2.2.2 Stems	5
2.2.3 Roots	5
2.3 Biological Remediation	7
2.4 Phytoremediation	7
2.4.1 Phytoextraction	8
2.4.2 Phytostabilization	8
2.4.3 Phytodegradation	9
2.5 Phenol	9
2.6 Phenol Toxicity in Malaysia	11
2.7 Potential of Phytoremediation Acted on Phenol	13
2.8 Variety of Plant Phenol	15
2.9 Health Benefits of Plant Phenol	15
2.9 Health Benefits of Plant Phenol	18
CHAPTER 3	19
MATERIALS AND METHODS	19
3.1 Materials and Chemicals	19
3.2 Preparation of System	20
3.3 Plant material (<i>Glycine max</i>)	20
3.4 Phenol Standard Curve	21
3.5 4- Aminoantipyrene (4- AAP) assay	21
3.6 Plant Growth	22
3.7 Grinding and Storing	22
3.8 Phytomarker or Phytoremediator Analysis	23
3.9 Statistical analysis	23
CHAPTER 4	24
RESULTS AND DISCUSSION	24
4.1 Growth of the soybean plants	24

4.2	Normal Growth Morphology of Soybean Seeds and Plants	27
4.3	Abnormal Growth Morphology of Soybean Seeds and Plants	29
4.4	Phenol Remaining in Spiked Water	32
4.5	Rate of Phenol Absorption by <i>Glycine max</i>	34
4.6	Verification the Category of Phytoremediator	35
CHAPTER 5		38
CONCLUSION AND RECOMMENDATIONS		38
REFERENCES		40
APPENDICES		45



LIST OF TABLES

Table	Caption	Page
1	Physical and chemical properties of phenol	10-11
2	Standard levels of chemicals allowed in IWK effluent	12-13
3	Sources and brands of equipments used throughout this study	19
4	Chemicals and their suppliers	19-20
5	Concentration of phenol inside the plant tissue before and after the treatment	38

LIST OF FIGURES

Figures	Caption	Page
1	Diagram of a young, vegetative soybean plant	4
2	Transverse section of an intact soybean	5
3	Transverse section of a soybean root after initiation of secondary growth	6
4	The production of phenylalanine	16
5	Precursors pathway of plant phenolic compounds	17
6	System preparation	20
7	Plant materials	21
8	Average length of plant roots exposed to concentrations of phenol- spike water	25
9	Average elongation of plant stems in different phenol water concentrations	25
10	Average elongation of plant leaves in different phenol water concentrations	26
11	Normal seed and plant morphologies from Day 1 until Day 6	28
12	Abnormal morphologies of plants in 0.1 g/ L phenol- spiked water.	30
13	Abnormal morphologies exhibited by germinated seedlings grown in 0.2 g/ L phenol-spiked water	30
14	Abnormal morphologies of beans (germinated and ungerminated) in 0.4 g/ L phenol spiked water	31
15	Amount of remaining phenols (g/ L) in water from Day 1 to Day 6 when treated with <i>Glycine max.</i>	33
16	Rate of phenol absorption by soybean plants at different phenol concentrations	35

LIST OF ABBREVIATIONS

Symbol	Definition
%	Percentage
µl	Microlitre
AAP	Aminoantiprene
Acetyl-CoA	Acetyl Coenzyme A
BOD	Biological Oxygen Demand
Co	Cobalt
Cu	Copper
DNA	Deoxyribonucleic Acid
EPA	Environmental Protection Agency
FAO	UN Food and Agricultural Organization
g	Gram
<i>G. max</i>	<i>Glycine max</i>
g/ cm ³	Gram per Cubic Centimetre
g/ L	Gram per Litre
g/ mol	Gram per mol
hPA	Hectopascal
IAA	Auxin
IWK	Indah Water Konsortium
L	Litre
LDL	Lipoprotein
Log K _{ow}	Log Octanol-Water Partition Coefficient
mg/ L	Miligram per Litre
Mo	Molybdenum

Ni	Nickel
°C	Degree Celcius
PAH	Polycyclic Aromatic Hydrocarbon
Pb	Lead
PCP	Pentachlorophenol
pKa	Negative base-10 logarithm of the acid dissociation constant of a solution
ppb	Part per Billion
ppm	Part per Million
<i>S. acuta</i>	<i>Sida acuta</i>
SBP	Soybean Peroxidase
TCA	Tricarboxylic Acid Cycle
TCE	Trichloroethylene
WHO	World Health Organization
Zn	Zinc

CHAPTER 1

INTRODUCTION

Water is a basic necessity for the existence of all living things. It plays a crucial role in the survival rate of living things. It carries a high risk to humans, animals and plants because water is a vehicle for transmittable diseases caused by microorganisms, or by chemicals released by human activities. One cause of water pollution is phenol.

Phenol is one of the pollutants produced by burning wood and petroleum fuel facilities. The presence of phenol in the environment is from a few sources such as refineries, wood reservation plants and multiple chemical industries (Paula and Young, 1998). Mostly, phenol originated from both industrial and natural activities and natural occurrence (Nair et al., 2007). People living near rivers contaminated with phenol often face detrimental health problems. Various traditional techniques such as thermal, physical and chemical had been used to treat the contaminants. However, the techniques used have many bad effects and need high maintenance cost (Lundstedt, 2003). Therefore, a few methods were proposed to treat the phenol pollutions.

Remediation is a process of waste and pollutants management by using organisms to break down contaminants through their natural activity (Merkl, 2005). It has been proven effective for a variety of contaminants such as heavy metals, radio nuclei and a wide range of organic pollutants (Schroder et al., 2002; Schnoor, 2002). Phytoremediation is pollutants biodegradation process by using plant. Plants may also contribute to changes in soil structure that benefit microbial degradation of

pollutants (Angers and Caron, 1998). Phytoremediation is a developing technology, which has most commonly been associated with inorganic compounds such as heavy metals (Meagher, 2000; Wong, 2003). More recently there have been several published studies on the use of phytoremediation to degrade hydrocarbon compounds, including recalcitrant compounds such as PAH (Lee et al., 2008; Palmroth et al., 2002). Moreover, phytoremediation which is an effective and economical way of treating recalcitrant contaminants also might be used to treat phenol (Trapp and Karlson, 2001).

Glycine max from the family Leguminosae and is commonly known as soybean is one of the potential plants that can be used to remediate phenol contaminated areas (Mcgrath and Zhao, 2003). Therefore, the potential of soybean to remediate phenol led to this study which was hypothesized that *G. max* was able to absorb and utilize phenol as nutrients for growth thus removing phenol in wastewater. This is suitable with nowadays critical issue where everyone is trying to find the solution regarding phenol pollution.

The objectives of this study were to analyse the potential of *G. max* in absorbing phenol from phenol- spiked water and to assess the shoots, roots and leaves growth of *G. max* grown in water spiked with different phenol concentrations

REFERENCES

- Ahmad, S., Syed, M, Arif, N., Shukor, M. & Shamaan, N. (2011). Isolation, identification and characterization of elevated phenol degrading *Acinetobacter* sp. strain AQ5NOL1. *Australia Journal Basic Applied Science*, 5(8), pp.1035-1045.
- Alkorta, I., & Garbisu, C. (2001). Phytoremediation of organic contaminants in soils. *Bioresource Technology*.
- Angers, D. A, Caron J. 1998. Plant-induced changes in soil structure: Processes and feedbacks. *Biogeochemistry*, 42, 55-72.
- Aransiola, S., Ijah, U. & Abioye, O. (2013). Phytoremediation of Lead Polluted Soil by *Glycine max L.* *Applied and Environmental Soil Science*.
- Arif, N., Ahmad, S., Syed, M. & Shukor, M. (2012). Isolation and characterization of a phenol-degrading *Rhodococcus* sp. strain AQ5NOL 2 KCTC 11961BP. *Journal of Basic Microbiology*, 53(1), pp. 9-19.
- Azizullah, A., Khattak, M., Richter, P. & Häder, D. (2011). Water pollution in Pakistan and its impact on public health — A review. *Environment International*, 37(2), pp. 479-497.
- Baker, E., Landrigan, P., Bertozzi, P., Field, P., Basteyns, B. & Skinner, H. (1978). Phenol Poisoning Due to Contaminated Drinking Water. *Archives of Environmental Health: An International Journal*, 33(2), pp. 89-94.
- Bhattacharya, A., Sood, P. & Citovsky, V. (2010). The roles of plant phenolics in defence and communication during *Agrobacterium* and *Rhizobium* infection. *Molecular Plant Pathology*, 11(5), pp 705-719.
- Carlson, J.B. & Lersten, N.R. (2004) Reproductive morphology. In: Boerma, H.R. and Specht, J.E. (eds) Soybeans: Improvement, Production, and Uses, 3rd edn. Agronomy Monograph 16. *American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America*, Madison, WI, USA, pp. 59-95.
- Carlson, J.B. (1973) Morphology. In: Caldwell, B.E. (ed.) Soybeans: Improvement, Production, and Uses. Agronomy Monograph 16. *American Society of Agronomy*, Madison, WI, USA, pp. 71–95.
- Chakraborty, S., Bhattacharya, T., Patel, TN., & Tiwari, KK. (2010). Biodegradation of phenol by native microorganisms isolated from coke processing wastewater. *Journal Environment Biology*, 31(3), pp. 293–296.
- Cunningham, S. D., & Berti, W. R. (1993). Remediation of contaminated soils with green plants: An overview. *In Vitro Cellular & Developmental Biology-plant*.
- Dec J., & Bollag J. M. (1994). Use of plant material for the decontamination of water polluted with phenols. *Biotechnology Bioengineering*, 44, pp. 1132–1139.

- Dueas M, Hernandez T, & Estrella I (2001). *Europe Food Research Technology*, 215, pp. 478–483
- Dura'n, N., & Esposito, E. (2000). Potential applications of oxidative enzymes and phenoloxidase-like compounds in wastewater and soil treatment: A review. *Applied Catalysis B: Environmental*, 28, pp. 83–99.
- Ettinger, M., Ruchhoft, C. & Lishka, R. (1951). Sensitive 4-Aminoantipyrine Method for Phenolic Compounds. *Analysis Chemistry*, 23(12), pp.1783-1788.
- Flocco, C., Lo Balbo, A., Carranza, M. & Giulietti, A. (2002). Removal of Phenol by Alfalfa Plants (*Medicago sativa L.*) Grown in Hydroponics and its Effect on Some Physiological Parameters. *Acta Biotechnology*, 22(1-2), pp.43-54.
- Flock, C., Bassi, A., & Gijzen, M. (1999). Removal of aqueous phenol and 2-chlorophenol with purified soybean peroxidase and raw soybean hulls. *Journal Chemistry Technology Biotechnology*, 74, pp. 303-309.
- Gami, A., Shukor, M.Y., Khalil, K. A., Dahalan, F. A., Khalid, A., & Ahmad, S.A. (2014). *Journal of Environmental Microbiology and Toxicology*, 2(1), pp.11--24.
- Gayathri, K. & Vasudevan, N. (2010). Enrichment of Phenol Degrading Moderately Halophilic Bacterial Consortium from Saline Environment. *Journal of Bioremediation & Biodegradation*, 01(01).
- Gibson, L., & Benson, G. (2005). Origin, History, and Uses of Soybean (*Glycine max*), Iowa State University, Department of Agronomy.
- Hall, J., Soole, K. & Bentham, R. (2011). Hydrocarbon Phytoremediation in the Family Fabacea —A Review. *International Journal of Phytoremediation*, 13(4), pp. 317-332.
- Hansch, C., McKarns, S., Smith, C. & Doolittle, D. (2000). Comparative QSAR evidence for a free-radical mechanism of phenol-induced toxicity. *Chemico-Biological Interactions*, 127(1), pp. 61-72.
- Hejri, S. & Saboora, A. (2009). Removal of phenolic compounds from synthetic wastewaters by enzymatic treatments. *Journal of Science*, 35, pp. 13-19.
- Hicks, D.R. (1978). Growth and development. In: Norman, G.A. (ed.) Soybean Physiology, Agronomy and Utilization. Academic Press, Inc., New York, USA, pp. 17-44.
- Hollman, P. (2001). Evidence for health benefits of plant phenols: local or systemic effects? *Journal of the Science of Food and Agriculture*, 81(9), pp.842-852.
- Hsieh, F., Huang, C., Lin, T., Chen, Y. & Lin, J. (2008). Study of sodium tripolyphosphate-crosslinked chitosan beads entrapped with *Pseudomonas putida* for phenol degradation. *Process Biochemistry*, 43(1), pp.83-92.
- Indah Water Consortium (IWK) 2012. Standard water effluent for Malaysia: standard A and B. 2012. <http://www.iwk.com.my.html>.

- Klibanov, A.M., Alberti, B.N., Morris, E.D., & Felshin, L.M. (1980). Enzymatic removal of toxic phenols and anilines from waste waters. *Journal Applied Biochemistry*, 2, pp. 414-421.
- Lagos, A. (2009). Phytoremediation of Crude Oil Contaminated Soil: The Effect of Growth of *Glycine max* on the Physico-chemistry and Crude Oil Contents of Soil.
- Lasat, M. M., Pence, N. S., Garvin, D. F., Ebbs, S. D., & Kochian, L. V. (2000). Molecular physiology of zinc transport in the Zn hyperaccumulator *Thlaspi caerulescens*. *Journal of Experimental Botany*.
- Lee, S., Lee, W., Lee, C. & Kim, J. (2008). Degradation of phenanthrene and pyrene in rhizosphere of grasses and legumes. *Journal of Hazardous Materials*, 153(1-2), pp. 892-898.
- Liu, Y., Zhang, A. & Wang, X. (2009). Biodegradation of phenol by using free and immobilized cells of *Acinetobacter* sp. XA05 and *Sphingomonas* sp. FG03. *Biochemical Engineering Journal*, 44(2-3), pp. 187-192.
- Lundstedt, S. (2003). Analysis of PAHs and their transformation products in contaminated soil and remedial processes.
- Ma, L., Komar, K., Tu, C., Zhang, W., Cai, Y. & Kennelley, E. (2001). A fern that hyperaccumulates arsenic. *Nature*, 409(6820), pp. 579-579.
- McCall, I. C., Betanzos, A., Weber, D. A., Nava, P., Miller, G. W., & Parkos, C. A. (2009). Effects of phenol on barrier function of a human intestinal epithelial cell line correlate with altered tight junction protein localization. *Toxicology and Applied Pharmacology*.
- McGrath, S., & Zhao, F. (2003). Phytoextraction of metals and metalloids from contaminated soils. *Current Opinion in Biotechnology*, 14(3), pp. 277-282.
- Meagher, RB. (2000). Phytoremediation of toxic elemental and organic pollutants. *Current Opinion Plant Biology*, 3, pp. 153-162.
- Merkl, N., Schultze-Kraft, R., & Infante, C. (2005). Phytoremediation in the tropics influence of heavy crude oil on root morphological characteristics of graminoids. *Environmental Pollution*.
- Nair, I., Jayachandran, K. & Shashidhar, S. (2007). Treatment of paper factory effluent using a phenol degrading *Alcaligenes* sp. under free and immobilized conditions. *Bioresource Technology*, 98(3), pp.714-716.
- Olujimi, OO., Fatoki, OS., Odendaal, JP., & Okonkwo, JO. (2010). Endocrine disrupting chemicals (phenol and phthalates) in the South African environment: A need for more monitoring. *Water SA. Africa Journal Online*, 36(5), pp.671-682.
- Palmroth, MRT., Pichtel, J., & Puhakka, JA. (2002) Phytoremediation of subarctic soil contaminated with diesel fuel. *Bioresource Technology*, 84(3), pp. 221-228.

- Panagos, P., Liedekerke, M.V., Yagini, Y., & Montanarella, L. (2013) Contaminated sites in Europe: review of the current situation based on data collected through a European network. *Journal Environment Public Health*, pp. 1-11.
- Parr, A. & Bolwell, G. (2000). Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. *Journal of the Science of Food and Agriculture*, 80(7), pp. 985-1012.
- Paula, M., Van, S., & Young, L., Y.(1998). Isolation and Characterization of Phenol Degrading Denitrifying Bacteria. *Journal of Applied Environmental Microbiology*, 64(7), pp. 2432-243.
- Pilet, P. (1977). Plant Growth Regulation. Berlin, Heidelberg: Springer Berlin Heidelberg.
- Ramarathnam, N., Osawa, T., Ochi, H., & Hawakishi, S. (1995). *Trends Food Science Technology*, 6, pp. 75- 82.
- Sarata, K. (2010). Soybean Growth and Development. In: Singh, G (ed) *The Soybean: Botany, Production and Uses*. Wallingford: CAB International. Pp. 48- 73.
- Schnoor, J.L. (2002). Phytoremediation of Soil and Groundwater: Technology Evaluation Report TE-02-01. Groundwater Remediation Technologies Analysis Centre (GWRTAC).
- Schroder, P., Harvey, P.J. & Schwitzguebel, J.P. 2002. Prospects for the phytoremediation of organic pollutants in Europe. *Environment Science Pollution Research*, 9(1), pp. 1-3.
- Shahidi, F., Chavan, U.D., Naczki, M., & Amarowicz, R. (2001). *Journal Agriculture Food Chemistry*, 49, pp. 926-933
- Sherameti, I. & Varma, A. (2010). Soil heavy metals. Heidelberg: Springer Verlag, p. 439.
- Shu, W., Ye, Z., Lan, C., Zhang, Z. & Wong, M. (2002). Lead, zinc and copper accumulation and tolerance in populations of *Paspalum distichum* and *Cynodon dactylon*. *Environmental Pollution*, 120(2), pp. 445-453.
- Singh, O.V., Jain, R.K.(2003). Phytoremediation of toxic aromatic pollutants from soil. *Applied Microbiology Biotechnology*, 63, pp. 128-135.
- Steinberg, D. (1997). Lewis A. Conner Memorial Lecture: Oxidative Modification of LDL and Atherogenesis. *Circulation*, 95(4), pp.1062-1071.
- Stephen, E1. & Ijah, U.J.J (2011). Comparison Of *Glycine Max* and *Sida Acuta* in the Phytoremediation Of Waste Lubricating Oil Polluted Soil. *Nature and Science*, 9(8).
- Strek, H.J., & Weber, J.B. (1982). *Environmental Pollution*, 28, pp. 291-312

- Tan, G. C., Vijayaletchumy, K., & Chee- Long, C. (1991). Trace Determination of Waterborne Organic Pollutants in the Klang River Basin. *Analytical Sciences*, 7.
- Trapp, S., & Karlson, U. (2001). Aspects of phytoremediation of organic pollutants. *Journal of Soils and Sediments*, 1, pp. 37-43.
- Troszynska, A., Estrella, I., Lopez-Amores, M. L. & Hernandez, T. (2002). Antioxidant activity of pea (*Pisum sativum L.*) seed coat acetone extract. *LWT Food Science and Technology*, 35, pp. 158-164
- Ucisik, A. & Trapp, S. (2006). Uptake, Removal, Accumulation, and Phytotoxicity of Phenol in Willow Trees (*Salix Viminalis*). *Environmental Toxicology and Chemistry*, 25(9), p. 2455.
- Ulrico, J., & López-Chuken. (2012). Hydroponics and Environmental Clean-Up. INTECH Open Access Publisher.
- Wasi, S., Tabrez, S. & Ahmad, M. (2012). Toxicological effects of major environmental pollutants: an overview. *Environmental Monitoring and Assessment*, 185(3), pp.2585-2593.
- Wattenberg, L.W. (1992). Inhibition of Carcinogenesis by Minor Dietary Constituents. *Cancer Research*, 52.
- Wei, G., Yu, J., Zhu, Y., Chen, W., & Wang, L. (2008). Characterization of phenol degradation by *Rhizobium* sp. CCNWTB 701 isolated from *Astragalus chrysopteru* in mining tailing region. *Journal of Hazardous Materials*, 151(1), pp.111-117.
- Wong, MH.(2003). Ecological restoration of mine degraded soils, with emphasis onmetal contaminated soils. *Chemosphere*, 50(6), pp. 775-780.
- Yi, H.,& Crowley, DE. (2007). Biostimulation of PAH degradation with plants containing high concentrations of linoleic acid. *Environment Science Technology*. 41, pp. 4382-4388.
- Yoon, J., Cao, X., Zhou, Q., & Ma, L. Q. (2006). Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. *Science of The Total Environment*.