



**UNIVERSITI PUTRA MALAYSIA**

**EFFECTS OF PHENOL ON THE GROWTH OF  
*Vigna angularis***

**SITI ROSLINA MUSTAPHA**

**FBSB 2015 130**

## PENGESAHAN

Dengan ini adalah disahkan bahawa projek yang bertajuk “Effects of Phenol on the Growth of *Vigna angularis*” telah disiapkan serta dikemukakan kepada Jabatan Mikrobiologi oleh SITI ROSLINA BINTI MUSTAPHA (164862) sebagai syarat untuk kursus BMY 4999 projek.

Disahkan oleh:

.....

Tarikh: .....

Prof. Madya Dr Janna Ong Abdullah

Penyelia

Jabatan Biologi Sel dan Molekul

Fakulti Bioteknologi dan Sains Biomolekul

Universiti Putra Malaysia

.....

Tarikh: .....

Prof. Madya Dr. Muhajir Hamid

Ketua

Jabatan Mikrobiologi

Fakulti Bioteknologi dan Sains Biomolekul

Universiti Putra Malaysia

## ABSTRACT

Rapid industrialisation since the recent past years has led to the increasing number of pollutions from industrial wastes, with phenol as one of the organic pollutant produced. Phytoremediation is one of the effective techniques available to clean up pollutant *in situ* by using plants as the main agent to absorb pollutants from targeted sites. *Vigna angularis*, which is a legume from Fabaceae family, is an annual plant that can be found mainly in East Asia and Himalaya countries such as Japan, and Malaysia. In this study, an experiment was conducted to investigate effects phenol had on *V. angularis*, and the plant's ability to absorb phenol contain in 4 L distilled water. Plants were exposed to different concentrations of phenol of 0.04, 0.05, 0.2, 0.3, and 0.4 g/L for 8 days and phenol remaining in the water was also being measured daily by using 4-aminoantipyrine assay method. The observations on changes on morphology of plants were made and followed by determination of rate of phenol degradation by plants. Changes on plants such as yellowing of leaves, changes of colour of roots from white to brown, and stems became droopy were recorded. The results obtained showed that *V. angularis* was able to tolerate variations of phenol concentrations, and can uptake 100% of phenol in the water for all concentrations with different rate of degaradations, except for in 0.4 g/L phenol.

## ABSTRAK

Pembangunan yang pesat sejak beberapa tahun kebelakangan telah menyebabkan peningkatan kadar pencemaran yang berpunca daripada sisa industri, dengan fenol sebagai salah satu ejen pencemaran organik yang terhasil. Fitopemuliharaan merupakan salah satu kaedah efektif yang digunakan untuk membersihkan kawasan pencemaran secara *in situ* dengan menggunakan pokok sebagai ejen utama untuk menyerap ejen pencemaran daripada tempat pencemaran. *Vigna angularis* merupakan sejenis legume daripada Famili Fabaceae dan boleh dijumpai kebanyakannya di kawasan Timur Asia dan Negara Himalaya seperti Jepun dan Malaysia. Dalam projek ini, satu eksperimen telah dijalankan untuk menyiasat kesan fenol terhadap *V. angularis* dan kebolehan pokok untuk menyerap fenol di dalam 4 L air suling. Pokok didedahkan kepada fenol dengan kepekatan yang berbeza-beza iaitu 0.04, 0.05, 0.2, 0.3, dan 0.4 g/L selama 8 hari dan baki fenol yang tinggal di dalam air juga telah diukur dengan menggunakan kaedah 4-aminoantipirina asai. Pemerhatian ke atas perubahan dalam morfologi pokok telah dibuat dan diteruskan dengan penentuan kadar degradasi fenol oleh pokok. Perubahan ke atas pokok seperti kekuningan pada daun, perubahan warna akar daripada putih kepada coklat, dan juga batang pokok menjadi layu telah dicatatkan. Keputusan yang diperolehi menunjukkan *V. angularis* boleh bertolak ansur di dalam kepekatan fenol yang pelbagai, dan boleh mengambil sehingga 100% fenol di dalam air untuk semua kepekatan dengan kadar degradasi fenol yang berlainan, kecuali di dalam 0.4 g/L fenol.

## ACKNOWLEDGEMENT

By the name of Allah s.w.t., The Most Gracious and Merciful, Alhamdulillah I finally managed to complete this dissertation. I am forever indebted especially to my supervisor, Prof. Madya Dr. Janna Ong Abdullah, and Dr. Aqlima Ahmad who had provided supports, guidances, stimulating suggestions, and encouragement from the beginning until the completion of this project.

Not to forget, many thanks to all members of lab 115 and remediation lab, especially Abang Rahim, Abang Ijan, dan also Kak Sabrina for teaching me all the techniques and new knowledges in order for me to carry out my project successfully. Special thanks also to my lab partners, Lee Siew Yi, Nurul Hafizah, and Syazwani for always giving me helping hands whenever I needed them. The completion of this project would have been almost zero probability if it weren't for all of you. Thank you so much.

A million thanks and loves to my supporting parents and siblings who were always there for me in providing their never ending physical and emotional support throughout the project. And last but not least, I would like to express my gratitude to all who gave me the possibility to complete this project. May God bless you and keep you always.

## TABLE OF CONTENTS

PENGESAHAN	i
ABSTRACT	ii
ABSTRAK	iii
ACKNOWLEDGEMENT	iv
LIST OF TABLES	vi
LIST OF FIGURES	vii
LIST OF ABBREVIATION	viii
CHAPTER 1	1
INTRODUCTION	1
CHAPTER 2	3
LITERATURE REVIEW	3
2.1 Phenol	3
2.1.1 Phenol toxicity	4
2.1.2 Degradation pathway of phenol	6
2.2 Phytoremediation	9
2.2.1 Phytoremediation technologies	10
2.2.2 Phytoremediation: advantages and limitations	11
2.3 <i>Vigna angularis</i>	12
CHAPTER 3	14
MATERIALS AND METHODS	14
3.1 Equipments	14
3.2 Materials	14
3.3 Methods and preparations	15
3.3.1 Plant material	15
3.3.2 Plant authentication	15
3.3.3 4-aminoantipyrene assay	15
3.3.4 Phenol standard curve	16
3.3.5 Statistical analysis of the data	17
CHAPTER 4	18
RESULTS AND DISCUSSION	18
4.1 Effects of phenol on <i>Vigna angularis</i>	18
4.2 Phenol effects on morphology of <i>Vigna angularis</i>	24
4.3 Phenol removal by <i>Vigna angularis</i>	27
4.4 Phenol content in plants	30
4.5 Overall discussion	31
CHAPTER 5	33
CONCLUSION AND RECOMMENDATIONS	33
REFERENCES	34
APPENDICES	41

## LIST OF TABLES

Table	Caption	Page
1	Equipments and manufacturers	14
2	Materials and manufacturers	14
3	Morphological observations of <i>Vigna angularis</i> bean subjected to different phenol concentrations.	22



© COPYRIGHT

## LIST OF FIGURES

Figures	Caption	Page
1	Chemical structure of phenol.	3
2	Scheme of the <i>meta</i> pathway of phenol degradation to produce pyruvate and acetyl-CoA.	8
3	Variation of root lengths in treated and control <i>Vigna angularis</i> at different phenol concentrations.	19
4	Variation of stem lengths in treated and control <i>Vigna angularis</i> at different phenol concentrations.	20
5	Variation of leaf area in treated and control <i>Vigna angularis</i> at different phenol concentrations.	20
6	Comparison between growth of <i>Vigna angularis</i> in 0.04 g/L and 0.4 g/L phenol on the 8 <sup>th</sup> days.	23
7	Changes in the leaf, root, and cotyledon of <i>Vigna angularis</i> after subjected to phenol.	26
8	Phenol remaining in the 4 L distilled water after 8 days.	28
9	Rate of phenol degradation by <i>Vigna angularis</i> for 8 days.	28



## LIST OF ABBREVIATION

%	Percentage
4-AAP	4-aminoantiprene
°C	Degree Celsius
et al.	And others
g	Gram
ml	Milimeter
nm	Nanometre
mg	Miligram
sp.	Species
var.	Variety
NH <sub>4</sub> C	Ammonium chloride
K <sub>3</sub> Fe(CN) <sub>6</sub>	Potassium ferric cyanide
μl	Micro liter
L	Litre
rpm	Revolutions per minute

## CHAPTER 1

### INTRODUCTION

The emergence of many industrial sectors has led to the increasing numbers of pollutions in the world. The high level of heavy metal toxics and organic contaminants that contaminate the air, soil and water has resulted in decreased quality of health in human and at the same time affected the ecosystem (Singh et al., 2014). Recent analysis by The Global Alliance on Health and Pollution (GAHP) also showed that pollution kills more than 8.4 million people each year.

Phenol is an aromatic organic compound and one of the contributors to the pollution issues. A report submitted to the government of Negeri Sembilan dated back in 1961 and 1979 (Abdullah and Nainggolan, 1991) showed that Linggi River was highly polluted by phenol and by WHO standards, can be classified as "heavily-polluted requiring extensive treatment. The anthropogenic sources of phenols are mainly originated from the domestic sewage and industrial effluents which include from the chemicals, petrol, pharmaceutical and textile industries, pulp mills, and pesticides (Michalowicz and Duda, 2007; Whitely and Bailey, 2000; Kumaran and Paruchuri, 1997).

Phytoremediation is a technology that utilises plants to remove or transform toxic chemicals present on soils, sediments, ground water, surface water, and even the atmosphere to clean up or reduce pollutions level (Susarla et al., 2002). Phytoremediation which is cost-effective as comparison to other technologies to clean up pollution may offer the only effective way to restore hundreds of thousands of square miles of land and water that have been polluted by industrial activities.

This project was focused on the effects of phenol on the growth of *Vigna angularis* in order to observe plant's tolerance towards phenol. The project was also intended to analyse the amount of phenol remaining in the water and relate it to the rate of degradation by the plants. The future application of the data obtained is to test the suitability of using *V. angularis* as a phytoremediator agent to reduce the level of phenol pollutions in the environment.

### **Objectives**

The objectives of this project are:

1. To determine the effects of phenol on the growth of *Vigna angularis*.
2. To assess *Vigna angularis* as a potential phytoremediator.

### **Hypotheses**

1. Different concentrations of phenol affect growth and morphological characteristics of *Vigna angularis*.
2. *Vigna angularis* has potential as a phytoremediator.

## REFERENCES

- Abdullah, P., & Nainggolan, H. 1991. Phenolic water pollutants in a Malaysian river basin. *Environmental Monitoring and Assessment* 19: 423-431.
- Agarry, S. E., Solomon, B. O., & Layokun, S. K. 2008. Optimization of process variables for the microbial degradation of phenol by *Pseudomonas aeruginosa* using response surface methodology. *African Journal of Biotechnology*, 7.
- Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for phenol. Atlanta, GA: US: Public Health Service; 2006.
- Ahmad, S. A., Shamaan, N. A., Arif, N. M., Koon, G. B., Shukor, M. Y. A., & Syed, M. A. 2012. Enhanced phenol degradation by immobilized *Acinetobacter* sp. strain AQ5NOL 1. *World Journal of Microbiology and Biotechnology* 28: 347-352.
- Alexieva, Z., Gerginova, M., Zlateva, P., & Peneva, N. 2004. Comparison of growth kinetics and phenol metabolizing enzymes of *Trichosporon cutaneum* R57 and mutants with modified degradation abilities. *Enzyme and Microbial Technology* 34: 242-247.
- Al-Khalid, T., & El-Naas, M. H. 2012. Aerobic biodegradation of phenols: a comprehensive review. *Critical Reviews in Environmental Science and Technology* 42: 1631-1690.
- Alkorta, I., & Garbisu, C. 2001. Phytoremediation of organic contaminants in soils. *Bioresource Technology* 79: 273-276.
- Arif, N. M., Ahmad, S. A., Syed, M. A., & Shukor, M. Y. 2013. Isolation and characterization of a phenol-degrading *Rhodococcus* sp. strain AQ5NOL 2 KCTC 11961BP. *Journal of Basic Microbiology* 53: 9-19.
- Banerjee, A., & Ghoshal, A. K. 2010. Phenol degradation by *Bacillus cereus*: pathway and kinetic modeling. *Bioresource Technology* 101: 5501-5507.
- Banuelos, G. S., Ajwa, H. A., Mackey, B., Wu, L., Cook, C., Akohoue, S., & Zambrozuski, S. 1997. Evaluation of different plant species used for phytoremediation of high soil selenium. *Journal of Environmental Quality* 26: 639-646.

- Bazrafshan, E., Mostafapour, F. K., & Mansourian, H. J. 2013. Phenolic compounds: health effects and its removal from aqueous environments by low cost adsorbents. *International Quarterly Journal* 2: 65-66.
- Bazrafshan, E., Mostafapour, F. K., & Mansourian, H. J. 2013. Phenolic compounds: health effects and its removal from aqueous environments by low cost adsorbents. *Health Scope* 2: 65-66.
- Biswas, D. K., Scannell, G., Akhmetov, N., Fitzpatrick, D., & Jansen, M. A. 2010. 2, 4, 6-trichlorophenol mediated increases in extracellular peroxidase activity in three species of Lemnaceae. *Aquatic Toxicology* 100: 289-294.
- Blum, U., & Gerig, T. M. 2005. Relationships between phenolic acid concentrations, transpiration, water utilization, leaf area expansion, and uptake of phenolic acids: nutrient culture studies. *Journal of Chemical Ecology* 31: 1907-1932.
- Bruce, W., Meek, M. E., & Newhook, R. 2001. Phenol: hazard characterization and exposure-response analysis. *Journal of Environmental Science and Health, Part C* 19: 305-324.
- Burken, J. G., & Schnoor, J. L. 1997. Uptake and metabolism of atrazine by poplar trees. *Environmental Science and Technology* 31: 1399-1406.
- Collantes, T. M., Rho, M. C., Kwon, H. J., Jung, B. G., Alfajaro, M. M., Kim, D. S., & Cho, K. O. 2012. Azuki bean (*Vigna angularis*) extract inhibits the development of experimentally induced atopic dermatitis-like skin lesions in NC/Nga mice. *Food Chemistry* 132: 1269-1275.
- Collins, L. D., & Daugulis, A. J. 1997. Biodegradation of phenol at high initial concentrations in two-phase partitioning batch and fed-batch bioreactors. *Biotechnology and Bioengineering* 55: 155-162.
- Cunningham, S. D., & Berti, W. R. 1993. Remediation of contaminated soils with green plants: an overview. *In Vitro Cellular and Developmental Biology-Plant* 29: 207-212.
- De Araujo, B. S., Dec, J., Bollag, J. M., & Pletsch, M. 2006. Uptake and transformation of phenol and chlorophenols by hairy root cultures of *Daucus carota*, *Ipomoea batatas* and *Solanum aviculare*. *Chemosphere* 63: 642-651.
- Dhir, B. 2013. *Phytoremediation: Role of Aquatic Plants in Environmental Clean-up*. Springer.

- Dong, Y., Wang, G., Jiang, P., Zhang, A., Yue, L., & Zhang, X. 2010. Catalytic ozonation of phenol in aqueous solution by CO<sub>3</sub>O<sub>4</sub> nanoparticles. *Bulletin of the Korean Chemical Society* 31: 2830-2834.
- Doty, S. L. 2008. Enhancing phytoremediation through the use of transgenics and endophytes. *New Phytologist* 179: 318-333.
- Dua, M., Singh, A., Sethunathan, N., & Johri, A. 2002. Biotechnology and bioremediation: successes and limitations. *Applied Microbiology and Biotechnology* 59: 143-152.
- Dushenkov, V., Kumar, P. N., Motto, H., & Raskin, I. 1995. Rhizofiltration: the use of plants to remove heavy metals from aqueous streams. *Environmental Science and Technology* 29: 1239-1245.
- Ettinger, M., Ruchhoft, C., & Lishka, R. 1951. Sensitive 4-aminoantipyrine method for phenolic compounds. *Analytical Chemistry* 23: 1783-1788.
- Ettinger, M., Ruchhoft, C., & Lishka, R. 1951. Sensitive 4-aminoantipyrine method for phenolic compounds. *Analytical Chemistry* 23: 1783-1788.
- Ferro, A. M., Sims, R. C., & Bugbee, B. 1994. Hycrest crested wheatgrass accelerates the degradation of pentachlorophenol in soil. *Journal of Environmental Quality* 23: 272-279.
- Flocco, C. G., Lo Balbo, A., Carranza, M. P., & Giulietti, A. M. 2002. Removal of phenol by alfalfa plants (*Medicago sativa* L.) grown in hydroponics and its effect on some physiological parameters. *Acta Biotechnologica* 22: 43-54.
- Gad, N. S., & Saad, A. S. 2008. Effect of environmental pollution by phenol on some physiological parameters of *Oreochromis niloticus*. *Global Veterinaria* 2: 312-319.
- Galíndez-Mayer, J., Ramon-Gallegos, J., Ruiz-Ordaz, N., Juárez-Ramírez, C., Salmeron-Alcocer, A., & Poggi-Varaldo, H. M. 2008. Phenol and 4-chlorophenol biodegradation by yeast *Candida tropicalis* in a fluidized bed reactor. *Biochemical Engineering Journal* 38: 147-157.
- Gao, J., Garrison, A. W., Hoehamer, C., Mazur, C. S., & Wolfe, N. L. 2000. Uptake and phytotransformation of organophosphorus pesticides by axenically cultivated aquatic plants. *Journal of Agricultural and Food Chemistry* 48: 6114-6120.
- Gao, J., Garrison, A. W., Hoehamer, C., Mazur, C. S., & Wolfe, N. L. 2000. Uptake and phytotransformation of O, P'-DDT and P, P'-DDT by axenically

cultivated aquatic plants. *Journal of Agricultural and Food Chemistry* 48: 6121-6127.

Ghosh, M., & Singh, S. P. 2005. A review on phytoremediation of heavy metals and utilization of its by-products. *Applied Ecology and Environmental Research* 3: 1-18.

Glick, B. R. 2003. Phytoremediation: synergistic use of plants and bacteria to clean up the environment. *Biotechnology Advances* 21: 383-393.

Gomez, J. L., Bodalo, A., Gómez, E., Bastida, J., Hidalgo, A. M., & Gómez, M. 2006. Immobilization of peroxidases on glass beads: an improved alternative for phenol removal. *Enzyme and Microbial Technology* 39: 1016-1022.

Gupta, G., & Rao, V. 1998. Biodegradation of phenol with poultry litter microorganisms. *Journal of Environmental Science and Health Part A* 33: 83-95.

Gupta, S., Ashrith, G., Chandra, D., Gupta, A. K., Finkel, K. W., & Guntupalli, J. S. 2008. Acute phenol poisoning: a life-threatening hazard of chronic pain relief. *Clinical Toxicology* 46: 250-253.

Ibanez, S. G., Alderete, L. G. S., Medina, M. I., & Agostini, E. 2012. Phytoremediation of phenol using *Vicia sativa* L. plants and its antioxidative response. *Environmental Science and Pollution Research* 19: 1555-1562.

Juahir, H., Zain, S. M., Yusoff, M. K., Hanidza, T. T., Armi, A. M., Toriman, M. E., & Mokhtar, M. 2011. Spatial water quality assessment of langat river basin (Malaysia) using environmetric techniques. *Environmental Monitoring and Assessment* 173: 625-641.

Kaga, A., Isemura, T., Tomooka, N., & Vaughan, D. A. 2008. The genetics of domestication of the azuki bean (*Vigna angularis*). *Genetics* 178: 1013-1036.

Khalafalla, M. M., El-Shemy, H. A., Mizanur, R. S., Teraishi, M., Teraishi, M., & Ishimoto, M. 2005. Recovery of herbicide resistant azuki bean (*Vigna angularis* Wild, Ohwi & Oshashi) plants via Agrobacterium-mediated transformation. *African Journal of Biotechnology* 4: 61-67.

Kranner, I., Minibayeva, F. V., Beckett, R. P., & Seal, C. E. 2010. What is stress? Concepts, definitions and applications in seed science. *New Phytologist* 188: 655-673.

- Król, A., Amarowicz, R., & Weidner, S. 2014. Changes in the composition of phenolic compounds and antioxidant properties of grapevine roots and leaves (*Vitis vinifera* L.) under continuous of long-term drought stress. *Acta Physiologiae Plantarum* 36: 1491-1499.
- Kumar, P. N., Dushenkov, V., Motto, H., & Raskin, I. 1995. Phytoextraction: the use of plants to remove heavy metals from soils. *Environmental Science and Technology* 29: 1232-1238.
- Kumaran, P., & Paruchuri, Y. L. 1997. Kinetics of phenol biotransformation. *Water Research* 31: 11-22.
- Lin, K., Pan, J., Chen, Y., Cheng, R., & Xu, X. 2009. Study the adsorption of phenol from aqueous solution on hydroxyapatite nanopowders. *Journal of Hazardous Materials* 161: 231-240.
- Loh, K. C., & Chua, S. S. 2002. Ortho pathway of benzoate degradation in *Pseudomonas putida*: induction of meta pathway at high substrate concentrations. *Enzyme and Microbial Technology* 30: 620-626.
- Lone, M. I., He, Z. L., Stoffella, P. J., & Yang, X. E. 2008. Phytoremediation of heavy metal polluted soils and water: progresses and perspectives. *Journal of Zhejiang University Science B* 9: 210-220.
- Mahammedilyas, B. K., Aravindan, R., & Viruthagiri, T. 2010. Recent advances in the biodegradation of phenol: a review. *The Journal of Experimental Biology* 1: 219-234.
- Mahiudddin, M., & Fakhruddin, A. N. M. 2012. Degradation of phenol via meta cleavage pathway by *Pseudomonas fluorescens* PU1. *ISRN Microbiology* 2012: 1-6.
- 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* 241: 61-70.
- Meagher, R. B. 2000. Phytoremediation of toxic elemental and organic pollutants. *Current Opinion in Plant Biology* 3: 153-162.
- Meena, M. C., Band, R., & Sharma, G. 2015. Phenol and its toxicity: a case report. *Iranian Journal of Toxicology* 8: 1222-1224.
- Michałowicz, J. and Duda, W. 2007. Phenols: sources and toxicity. *Polish Journal of Environmental Studies* 16: 347-362.



- Nair, C. I., Jayachandran, K., & Shashidhar, S. 2008. Biodegradation of phenol. *African Journal of Biotechnology* 7: 4951-4958.
- Pilon-Smits, E. 2005. Phytoremediation. *Annual Review of Plant Biology* 56: 15-39.
- Prieto, M., Hidalgo, A., Rodriguez-Fernandez, C., Serra, J., & Llama, M. 2002. Biodegradation of phenol in synthetic and industrial wastewater by *Rhodococcus erythropolis* UPV-1 immobilized in an air-stirred reactor with clarifier. *Applied Microbiology and Biotechnology* 58: 853-860.
- Prpich, G. P., & Daugulis, A. J. 2005. Enhanced biodegradation of phenol by a microbial consortium in a solid-liquid two phase partitioning bioreactor. *Biodegradation* 16: 329-339.
- Pulford, I. D., & Watson, C. 2003. Phytoremediation of heavy metal-contaminated land by trees-a review. *Environment International* 29: 529-540.
- Raskin, I., Smith, R. D., & Salt, D. E. 1997. Phytoremediation of metals: using plants to remove pollutants from the environment. *Current Opinion in Biotechnology* 8: 221-226.
- Saier Jr, M. H., & Trevors, J. T. 2010. Phytoremediation. *Water, air, and soil pollution* 205: 61-63.
- Santos, V. L., & Linardi, V. R. 2004. Biodegradation of phenol by a filamentous fungi isolated from industrial effluents-identification and degradation potential. *Process Biochemistry* 39: 1001-1006.
- Schwitzgubel, J. 2000. Potential of phytoremediation, an emerging green technology. *Ecosystem Service Sustain Watershed Management Science B* 9: 210-220.
- Shukla, K. P., Singh, N. K., & Sharma, S. 2010. Bioremediation: developments, current practices and perspectives. *Genetic Engineering and Biotechnology Journal* 3: 1-20.
- Singh, J. S., Abhilash, P. C., Singh, H. B., Singh, R. P., & Singh, D. P. 2011. Genetically engineered bacteria: an emerging tool for environmental remediation and future research perspectives. *Gene* 480: 1-9.
- Singh, S., Melo, J. S., Eapen, S., & D'souza, S. F. 2006. Phenol removal using *brassica juncea* hairy roots: role of inherent peroxidase and H<sub>2</sub>O<sub>2</sub>. *Journal of Biotechnology* 123: 43-49.
- Susarla, S., Medina, V. F., & Mccutcheon, S. C. 2002. Phytoremediation: an ecological solution to organic chemical contamination. *Ecological Engineering* 18: 647-658.

- Tangahu, B. V., Sheikh Abdullah, S. R., Basri, H., Idris, M., Anuar, N., & Mukhlisin, M. 2011. A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. *International Journal of Chemical Engineering* 2011: 1-31.
- Tsirogianni, I., Aivaliotis, M., Karas, M., & Tsiotis, G. 2004. Mass spectrometric mapping of the enzymes involved in the phenol degradation of an indigenous soil pseudomonad. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics* 1700: 117-123.
- Utas, J. E., Kritikos, M., Sandström, D., & Åkermark, B. 2006. Water as a hydrogen bonding bridge between a phenol and imidazole. A simple model for water binding in enzymes. *Biochimica et Biophysica Acta (BBA)-Bioenergetics* 1757: 1592-1596.
- Vangronsveld, J., Van Assche, F., & Clijsters, H. 1995. Reclamation of a bare industrial area contaminated by non-ferrous metals: in situ metal immobilization and revegetation. *Environmental Pollution* 87: 51-59.
- Wang, K., Cai, J., Feng, J., & Xie, S. 2014. Phytoremediation of phenol using *Polygonum orientale*, including optimized conditions. *Environmental Monitoring and Assessment* 186: 8667-8681.
- Weber, M., & Weber, M. 2010. Phenols. In *phenolic resins: a century of progress* (pp. 9-23). Springer Berlin Heidelberg.
- Whiteley, A. S., & Bailey, M. J. 2000. Bacterial community structure and physiological state within an industrial phenol bioremediation System. *Applied and Environmental Microbiology* 66:2400-2407.
- Yang, X. E., Long, X., Ni, W., & Fu, C. 2002. *Sedum alfredii* H: a new zinc hyperaccumulating plant first found in China. *Chinese Science Bulletin* 47: 1634-1637.
- Yusof, M. A., Hasan, N., & Abdullah, M. P. 2002. River water quality in Langat basin, Selangor, Malaysia. *Malaysian Journal of Environmental Management* 3: 125-142.
- Zhou, Q. X. and Song, Y. F. 2004. Remediation of contaminated soils: principles and methods. *China Environmental Science Press, Beijing*.