SCREENING FOR AND UPTAKE OF ARSENIC BY HYPERACCUMULATOR FERNS GROWN ON ORGANIC SOIL

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By

NUR AINI BINTI ABU BAKAR

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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DEDICATION

This thesis is dedicated to:

The love of my life
Mr. Abu Bakar bin Awang
Mrs. Nor Azmy bt. Hj. Amir

My other half
Muhammad Syahid bin Haron

My lovely siblings and in law
Muhammad Zaki bin Abu Bakar
Nur Yasmin binti Abu Bakar
Nur Adibah binti Abu Bakar
Muhammad Azri bin Abu Bakar
Nur Adilah Adha binti Abu Bakar
Muhammad Aiman Arif bin Abu Bakar
Norhidayah binti Rahim

My adorable niece and nephew
Auni Nadhirah
Muhammad Aniq Firdaus

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

SCREENING AND UPTAKE OF ARSENIC BY HYPERACCUMULATOR FERNS GROWN ON ORGANIC SOIL

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NUR AINI BINTI ABU BAKAR

June 2017

Chairman : Professor Che Fauziah Ishak, PhD
Faculty : Agriculture

A brake fern, *Pteris vittata* L. has been demonstrated to absorb heavy metals, especially arsenic (As) in soil. However, there was a limited study to investigate an interaction between phosphate and arsenic (As) in soil. Therefore, the objective of the study was to investigate suitable fern and condition for accumulation of As from As-rich soil. In the screening study, eight species of ferns, *Diplazium velutinum* H., *Diplazium esculentum* (Retz). Sw, *Pityrogramma calomenalos (L.*) Link, *Nephrolepis biserrata* (Sw). Schott, *Pteris vittata* L., *Christella arida* (D. Don) Holttum, *Didymochlaena truncatula* (Sw.) J. Sm. and *Angiopteris evecta* (G. Forst.) Hoffm in Peninsular Malaysia were evaluated on their potential in hyper accumulating As in soil. Uncontaminated soil was collected from Kampung Ulu Chuchoh was spiked with 200 mg AS kg^{-1} soil using sodium arsenate salt. Three ferns species were grouped as higher As accumulator - *P.calomenalos* (1552.67 mg kg^{-1}), *D.velutinum* (975.47 mg kg^{-1}) and *P.vittata* (947.07 mg kg^{-1}). Since, *P. vittata* is easily available in large numbers in nursery as compared to two other species, it was selected further in next experiment. In soil incubation study, naturally As-rich soil in Kampung Renal, Tangkak was sampled and incubated with 0 (control), 75 kg P/ha of TSP and KH_2PO_4 in the laboratory for ninety days at room temperature and at field capacity. Every ten days, the soil solution was collected using rhizon moisture sampler for determination of water-soluble As. A significant ($P \leq 0.0001$) increment in concentration of As was recorded with triple superphosphate (TSP) (76%) and potassium dihydrogen phosphate (KH_2PO_4) (69%) treatment compared to control. Three species of As present in the pore water with the amount of As(V) was higher than As(III) and undetectable value of MMA. In the glasshouse study, *P. vittata* was grown in soil added with 0 (control), 12.5, 25, 50 and 75 kg P/ha using TSP and KH_2PO_4 as P source. This study showed that addition of P in soil resulted in a significant increased in biomass of fronds. In contrast, a significant reduction in total concentration of As, up to 23 and 16% compared to the control. The present

study, showed the addition of TSP had increased frond biomass of *P .vittata* better (38.94%) than KH_2PO_4 and the same time increased the As uptake (6.60 mg pot^{-1}) in As rich soil. In conclusion, *P. vittata* was a suitable fern to be used for As accumulation in moderate concentration of As-rich soil with the addition of phosphate in soil because it can increase the availability of As for plant uptake due to the similarity in physico-chemical behavior.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PEMENCILAN DAN PENGAMBILAN ARSENIK OLEH PAKU PAKIS YANG BERKEUPAYAAN TINGGI MENGUMPUL BAHAN TERCEMAR PADA TANAH ORGANIK

Oleh

NUR AINI BINTI ABU BAKAR

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Pengerusi : Profesor Che Fauziah Ishak, PhD
Fakulti : Pertanian

Paku Uban Bukit, *Pteris vittata* L.telah menunjukkan keupayaan menyerap logam berat terutamanya arsenik (As) dalam tanah. Walaubagaimanapun, kajian untuk interaksi antara fosfat dan arsenik (As) adalah terhad. Oleh itu, objektif utama kajian ini adalah untuk menyiasat paku pakis dan keadaan yang sesuai untuk pengumpulan As daripada tanah yang kaya dengan As. Dalam kajian pemencilan, lapan spesies paku pakis iaitu *Diplazium velutinum* H., *Diplazium esculentum* (Retz). Sw, *Pityrogramma calomenalos (L.*) Link, *Nephrolepis biserrata* (Sw). Schott, *Pteris vittata* L., *Christella arida* (D.Don) Holttum, *Didymochlaena truncatula* (Sw.) J.Sm. dan *Angiopteris evecta* (G.Forst.) Hoffm di Semenanjung Malaysia telah dinilai melalui potensi dalam keupayaan mengumpul As. Tanah tidak tercemar telah dikumpul dari Kampung Ulu Chuchoh dan diaplikasikan dengan 200 mg kg^{-1} As daripada garam natrium arsenate. Tiga spesies paku pakis telah dikelaskan sebagai pengumpul As yang lebih tinggi - *P.calomenalos* (1552.67 mg kg^{-1}), *D.velutinum* (975.47 mg kg^{-1}) dan *P.vittata* (947.07 mg kg^{-1}). Memandangkan *P.vittata* lebih senang ada dalam jumlah yang banyak di nurseri berbanding dua lagi spesies, ia telah dipilih untuk kajian yang seterusnya. Dalam kajian inkubasi tanah, tanah yang kaya dengan arsenik secara semulajadi di Kampung Renal, Tangkak telah disampel dan diinkubasi dengan 0 (kawalan), 75 kg P/ha TSP dan KH_2PO_4 dalam makmal untuk sembilan puluh hari di suhu bilik pada kapasiti tanah. Setiap sepuluh hari, larutan tanah dikumpulkan menggunakan penyampelan kelembapan Rhizon untuk menentukan arsenik dari larutan air. Kenaikan ketara ($P \leq 0.0001$) dalam kepekatan As direkodkan dengan rawatan triple superphosphate (TSP) (76%) dan kalium dihidrogen fosfat (KH_2PO_4) (69%) berbanding dengan kawalan. Tiga spesies As terdapat dalam sampel air dengan nilai As(V) lebih tinggi daripada As(III) dan MMA yang tidak dapat dikesan nilainya,. Untuk kajian rumah hijau, *P.vittata* ditanam di dalam tanah yang ditambah dengan 0 (kawalan), 12.5, 25, 50 dan 75 kg

P/ha menggunakan TSP dan KH_2PO_4 sebagai sumber P. Kajian ini menunjukkan penambahan P dalam tanah mengakibatkan kenaikkan ketara dalam biojisim pelepah. Sebaliknya, pengurangan ketara dalam keseluruhan kepekatan As, sehingga 23% dan 16% berbanding kawalan. Dalam kajian terkini, menunjukkan penambahan TSP menambahkan biojisim pelepah *P.vittata* lebih baik berbanding KH_2PO_4 dan dalam masa yang sama menambahkan pengambilan As (6.60 mg pot^{-1}) dalam tanah yang kaya As. Kesimpulannya, *P. Vittata* adalah paku pakis yang sesuai digunakan untuk pengumpulan As dalam kepekatan sederhana pada tanah yang kaya dengan As dengan penambahan fosfat di tanah kerana ia boleh menambahkan ketersediaan As untuk diambil oleh tumbuhan kerana persamaan sifat fizikal-kimia

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"Winners are not those who never fail, but those who never quit"

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

ADP	Adenosine diphosphate
AFS	Atomic Fluorescence Spectrometry
ANOVA	Analysis of Variance
APL	Acute Promyelocytic Leukemia
AAS	Atomic Absorption Spectroscopy
As(V)	Arsenate
As(III)	Arsenite
ATP	Adenosine triphosphate
BCF	Bioconcentration factor
CCA	Chromated-copper-arsenate
CE	Common era
CRD	Completely Randomized Design
DMAA	Dimethylarsinic acid
DOC	Dissolved organic carbon
EDTA	Ethylenediaminetetraacetic acid
EDDS	Ethylenediamine disuccinate
Eh	Oxidation-reduction potential
EPA	Environmental Protection Agency
GC	Gas chromatography
HG-AFS	hydride generation atomic fluorescence spectrometry
HPLC	High Performance Liquid Chromatography
HSD	Honestly Significant Different
ICP-AES	Inductively coupled plasma atomic emission spectroscopy

ICP-MS	Inductively coupled plasma mass spectrometry
kPa	Unit of pressure measurement (kilopascal)
LOI	Loss of ignition
MMAA	Monomethylarsonic acid
MCL	Maximum contaminant level
NIPs	Nodulin-26-like intrinsic proteins
NIST	National Institute Standard and Technology
Pi	Inorganic phosphate
PHT1	Phosphate transport
PIPs	Plasma membrane intrinsic protein subfamily
PMF	Proton-motive force
RCBD	Randomized complete block design
RSD	Relative standard deviation
TF	Translocation factor
TMA	Trimethylarsine
TSP	Triple superphosphate
USDA	United States Department of Agriculture
V_{max}	Maximum influx velocity

CHAPTER 1

INTRODUCTION

Arsenic (As) has received great attention due to its chronic and epidemic toxic effects to humans. It can enter terrestrial and aquatic environments in both natural as well as anthropogenic activities. Primary anthropogenic inputs were derived from pesticides, fertilizers, wood preservatives, coal combustion and smelter waste (Ron, 2005). Remediation of the As contaminated sites has become an important issue to protect animal and human health. A specific phytoremediation approach, called phytoextraction, makes use of hyperaccumulating plants to remediate contaminated soils. Despite their ability to tolerate and extract the pollutant from soils, hyperaccumulator plants also can accumulate heavy metal to high concentrations in the harvestable parts (Lasat, 2002). This approach is being explored because of its cost efficiency and environmental friendliness (Fayiga and Ma, 2005).

A versatile and hardy arsenic hyperaccumulating plant, *Pteris vittata* L., also known as Chinese Brake Fern has been discovered from an As contaminated soils (Komar *et al.,* 1998). Its potential to accumulate extremely high concentration of arsenic in aboveground part was reported by many scientists (Chen *et al.,* 2002; Ma *et al.,* 2001a; Visoottiviseth *et al.,* 2002). Phytoremediation is not a rapid process. Thus, phytoremediation will be more efficient especially in phytoextraction if the phytoavailability of the pollutant is enhanced in soil for plant uptake. According to Schmöger *et al.* (2000), limitation factor especially in term of geological and climatic conditions of the contaminated site to be cleansed such as soil type, temperature, altitude and the accessessibility for agricultural equipments should be of concern. Contaminants must be in available form to be taken by plants and its root system thus, amendment with other chemicals may provide a feasible alternative to increase the phytoextraction abilities (Chen *et al.,* 2002; Pilon-Smits, 2005).

Researchers are currently improving the capabilities of *P.vittata* L. in accumulating arsenic by adding P-fertilizer to As-rich soil. Up to now, the chemically assisted arsenic phytoextraction has not been extensively examined. The types of chemical agents that can be used are still unclear, unlike other metals, in which the other literature reports a number of chemicals such as chelates and acids useful for inducing metal phytoextraction. As a new research interest, there is limited data and literature on phosphate interaction with arsenic in *P.vittata* L in Malaysia to remediate As-rich soil. In the ferns that hyperaccumulate arsenic, the interaction between phosphate and As(V) is important since optimum phosphate fertilization could be a key factor for optimum phytoextraction of arsenic. The ability of *P.vittata* L. to effectively take up phosphate in the presence of elevated arsenic may have helped its ability to resist arsenic.

1.1 Experimental hypothesis

The overall hypothesis of the study was addition of phosphorus in soil can enhance the ability of arsenic hyperaccumulator fern species to accumulate more arsenic in As-rich soil.

1.2 Justification of study

The finding that Histosol (Saprist) soil in a cultivated area in Johor had high concentration of Arsenic was of concern. Pteris vittata L. has been reported as a plant which can hyperaccumulate As and the capabilities can be further enhanced by adding P-fertilizer to As-rich soil. Previous research on phytoremediation technique has been implemented and documented widely in some countries, however, it is still in its infancy and considered as new technique in Malaysia. Besides, information regarding arsenic phytoextraction with the addition of phosphate in Malaysia is non-existence. Therefore, the data on *Pteris vittata* usage as phytoremediator in this country is warranted.

1.3 Objectives of study

The general objective of the study was to investigate suitable fern and condition for accumulation of As from As-rich soil. The specific objectives of the study were:

1) To screen arsenic hyperaccumulation capability of eight fern species.
2) To determine the effect of phosphate in different rates on plant-available As in organic As-rich soil.
3) To compare the effect of different rates and forms of phosphate (TSP and KH_2PO_4) on As uptake by *Pteris vittata* L. grown in naturally As-riched soil

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