UNIVERSITI PUTRA MALAYSIA

In-Situ IMMOBILIZATION OF SELECTED HEAVY METALS USING DIFFERENT SOURCE OF PHOSPHATE AMENDMENTS FOR ORGANIC PRODUCTION OF MISAI KUCING (Orthosiphon stamineus Benth)

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By

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***In-Situ* IMMOBILIZATION OF SELECTED HEAVY METALS USING DIFFERENT SOURCE OF PHOSPHATE AMENDMENTS FOR ORGANIC PRODUCTION OF MISAI KUCING (*Orthosiphon stamineus* Benth)**

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Chairman : Professor Che Fauziah bt. Ishak, PhD
Faculty : Agriculture

Sustainable organic herbal production requires utilization of bio-waste materials as plant nutrient sources due to its economical aspect and would ensure continuous productivity. The usage of organic wastes in organic farming system (OFS) as fertilizers and soil amendments should be monitored as the accumulation of heavy metals has been reported in several studies. This study was set up to investigate the effect of chicken manure soil application on heavy metals concentration in organically grown *O. stamineus* and a potential approach to immobilize it using phosphate based amendments. Glasshouse trials has been conducted for study 1 and study 3 whilst incubation study was conducted for study 2 accordingly. Results in the study 1 indicated that the trace metals of lead (Pb) and Zn (Zn) exceeds the threshold value allowed by Pharmacopeia in herbs and by Malaysian Food Act (1983) and Food Regulations (1985) at 10 and 40 mg/kg respectively. This is postulated due to application of chicken manure and organic materials during cultivation. In study 2, 2 sources of apatite were tested: - 1) Egyptian rock phosphate (ERP) and 2) bone meal (BM) under incubation study. The results demonstrated that the dominant lead forms were mainly associated with residual, carbonates and also exist in exchangeable form. From this study, the suitable rate for reducing phyto-available lead in the soil system applied with 10 t/ha chicken manure is at 1 t/ha of Egyptian rock phosphate and 2 t/ha of bone meal. In the third study, the effectiveness of phosphorus on reducing heavy metals were evaluated under controlled condition in the glasshouse. Results demonstrated heavy metals accumulated in the aerial parts of *O. stamineus* with copper (18.3-37.7 mg/kg), zinc (60.9-92.0 mg/kg) and lead (1.9-3.6 mg/kg). From the fractionation study, the percentage of exchangeable fraction was reduced with application of P-amendments with the highest of 20.2% reduction recorded for 2 t/ha application of triple super phosphate. This is followed by reduction in exchangeable fraction for others treatments: 2 t/ha of bone meal (4.1%), 4 t/ha of bone meal (5.1%), 1 t/ha of Egyptian rock phosphate (8.1%) and 2 t/ha of Egyptian rock phosphate at 17.6%. Applications of P-amendments tested shows effectiveness of TSP in reducing

lead in soils. The best treatments are as follows: - 2 t/ha of triple super phosphate> 2 t/ha of Egyptian phosphate rock> 1 t/ha of Egyptian rock phosphate> 4 t/ha of bone meal> 2 t/ha of bone meal. These treatments were recorded as being able to stabilize the Pb as indicated in the percentage reduction of phytoavailable pools into a more stable form of residual pool.

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

IMMOBILISASI LOGAM BERAT SECARA *In-Situ* DENGAN MENGGUNAKAN SUMBER FOSFORUS BERBEZA BAGI PENGELUARAN SECARA ORGANIK MISAI KUCING *(Orthosiphon stamineus* Benth*)*

Oleh

KHAIRUN NAIM BIN MULANA@MAULANA

Januari 2017

Pengerusi : Profesor Che Fauziah bt. Ishak, PhD
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Pengeluaran herba secara lestari memerlukan penggunaan bahan bio-sisa sebagai sumber nutrien terutamanya dalam sistem pertanian secara organik. Secara amnya, penggunaan bahan sisa organik ini dalam pertanian organik sebagai baja atau peminda tanah perlu dipantau kerana pengumpulan logam berat telah dilaporkan dalam beberapa kajian terdahulu. Penyelidikan ini dijalankan bagi mengkaji kesan penggunakan tahi ayam terhadap pengambilan logam berat oleh *O. stamineus* dan pendekatan yang sesuai dalam mengawalnya menggunakan peminda berasaskan fosfat. Ujian rumah kaca telah dijalankan bagi kajian 1 dan kajian 3, manakala kajian pengeraman dijalankan bagi kajian 2. Keputusan dalam kajian pertama menunjukkan bahawa kandungan logam berat plumbum melepasi had yang dibenarkan oleh Pharmacoepia pada kadar 10 mg/kg bagi herba dan tumbuhan ubatan. Manakala, kandungan logam berat zink telah melepasi had yang dibenarkan oleh Akta Makanan Malaysia (1983) dan Peraturan-Peraturan Makanan (1985) pada kadar 40 mg/kg. Ini berkemungkinan disebabkan oleh penggunaan tahi ayam dan bahan-bahan organik semasa penanaman. Dalam kajian 2, 2 sumber apatite diuji: - 1) batuan fosfat dari Mesir (ERP) dan 2) serbuk sisa tulang (BM) dalam kajian pengeraman. Keputusan menunjukkan bahawa pecahan fraksi yang dominan berkait-rapat dengan fraksi sisa baki, karbonat dan dalam bentuk bertukar ganti. Daripada kajian ini, kadar yang sesuai untuk mengurangkan plumbum pada sistem tanah menggunakan 10 t/ha baja tahi ayam iaitu 1 t/ha batuan fosfat dari Mesir dan 2 t/ha sisa tulang. Dalam kajian yang ketiga, keberkesanan fosforus mengurangkan logam berat telah dinilai di dalam keadaan terkawal rumah kaca. Keputusan menunjukkan logam berat terkumpul di bahagian tisu *O. stamineus* adalah seperti berikut: tembaga (18.3-37.7 mg/kg), zink (60.9-92.0 mg/kg) dan plumbum (1.9-3.6 mg/kg). Daripada kajian pemeringkatan, peratusan pecahan ditukar-ganti telah dikurangkan dengan penggunaan peminda fosforus dengan yang tertinggi adalah pengurangan 20.2% yang dicatatkan pada aplikasi 2 t/ha triple super fosfat. Ini diikuti dengan pengurangan dalam pecahan ditukar-ganti pada rawatan lain: 2 t/ha sisa tulang terlerai (4.1%), 4 t/ha sisa tulang terlerai (5.1%), 1 t/ha

batuan fosfat dari Mesir (8.1%) dan 2 t/ha batuan fosfat dari Mesir pada kadar 17.6%. Penggunaan peminda fosforus diuji menunjukkan keberkesanan TSP dalam mengurangkan plumbum dalam tanah. Rawatan terbaik adalah seperti berikut: - 2 t/ha triple super fosfat> 2 t/ha batuan fosfat dari Mesir> 1 t/ha batuan fosfat dari Mesir> 4 t/ha sisa tulang terlerai> 2 t/ha sisa tulang terlerai. Rawatan ini dapat menstabilkan Pb seperti yang ditunjukkan dalam pengurangan peratusan fraksi yang boleh diambil tumbuhan ke dalam bentuk yang lebih stabil, fraksi sisa baki.

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I certify that a Thesis Examination Committee has met on 4 January 2017 to conduct the final examination of Khairun Naim bin Mulana@Maulana on his thesis entitled "*In-Situ* Immobilization of Selected Heavy Metals using Different Source of Phosphate Amendments for Organic Production of Misai Kucing (*Orthosiphon stamineus* Benth)" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS/NOTATIONS/GLOSSARY OF TERMS

AA	AutoAnalyzer
AAS	Atomic Absorption Spectrometer
ANOVA	Analysis of Variance
BM	Bone Meal
CRD	Completely Randomized Design
CRBD	Completely Randomized Block Design
EC	Electrical Conductivity
ERP	Egyptian Rock Phosphate
DOA	Department of Agriculture
MPL	Maximum Permissible Limits
rpm	Rotation per minute
TSP	Triple Super Phosphate

CHAPTER 1

INTRODUCTION

1.1 General background

Food safety and environmental concerns have gained attention among consumers recently which results in increasing demands of organic farming produce over those of conventional farming. Agronomic practices, such as application of soil amendment and organic fertilizer (types and rates) for organic production of herbs on Malaysian soils have not been well investigated. These practices should be reviewed due to potential of heavy metals accumulation in the herbal produce. Therefore, safety assurances of organic herbs products should be monitored along with the enhancement of biomass yield. Chicken manure and organic fertilizers might contribute to higher trace metals uptake in plant tissues particularly for lead. On the other hand, location of farms close to the roadside can also result in higher Pb content of soil due to automobiles emission using leaded gasoline. Through application of phosphorus-amendments, such as phosphate rock and bone meal, Pb can be immobilized or fixed in the soil system.

1.2 Justification & problem statement

Herbs production under organic farming system (OFS) claims to assure the safety of organic produce compared to conventional farming system (CFS). However, OFS heavy usage of organic amendments and organic fertilizers potentially contribute to accumulation of heavy metals. Through fractionation studies the information on reactivity and potential bioavailability of heavy metals could be obtained.

According to study 1, zinc (Zn) seemed to be the heavy metals that exceeded the maximum permitted concentration (MPC) by Malaysian Food Act (1983) and Food Regulations (1985) at 40 mg/kg. Similarly, lead (Pb) concentration surpassed the Pharmacopeia threshold value (10 mg/kg) in the organically grown misai kucing (*O. stamineus*). On the other hand, the copper (Cu) concentration can be a potential hazard as the amount indicated is near the 30 mg/kg maximum permitted concentration (MPC) by Malaysian Food Act (1983) and Food Regulations (1985). There are few reports related to copper, zinc and lead accumulations in *O. stamineus* being published. Abdu *et al.*, (2011) concluded that *O. stamineus* can be a potential phytoremediator for Pb and Zn, whilst Manan *et al.*, (2015) indicated that *O. stamineus* acts as a moderate accumulator for Zn, Cu and Pb. However, zinc and copper are essential for both plants and human, which unlike lead, possess threat even at minimum amounts. Thus, in order to reduce lead contents in the *O. stamineus* herbal produce, the application of P-amendments such as phosphate rock or bone meal could be a potential option. The formation of stable lead compounds and the process of immobilization through soil fractionation study in phosphate-amended soil will be investigated in this study.

For a sustainable herbal production, utilization of bio-waste materials as a nutrient source in organic farming is economical and would ensure continuous productivity. However, documented research on the effects of organic fertilizers towards safety and quality of herbs produced are still lacking.

1.3 Objectives

The general objective of this study was to investigate the effect of chicken manure application on heavy metals concentration in organically grown of *O. stamineus* and a potential approach to immobilize it using phosphate-based amendments.

The specific objectives of the study were:

1) To assess heavy metals (Cu, Zn, Pb, As, Cd and Hg) uptake in organically grown *O. stamineus* applied with different rates of chicken manure and biochar as soil amendments.
2) To determine the optimum rate of phosphate rock and bone meal in order to immobilize Pb in soil applied with high level of chicken manure based on incubation study.
3) To assess the effectiveness of selected rates and types of P-amendments indicated earlier to immobilize Pb in organically grown *O. stamineus* on soil applied with high level of chicken manure. Secondary assessment on Cu and Zn concentrations would be indicated accordingly.

1.4 Hypotheses

Organic farming system of herbs does not guarantee safety of the produce in terms of heavy metals accumulation. The phytoavailablity of heavy metals in the soil system can be confirmed through fractionation study. Lead contents in herbal produce can efficiently be reduced through land application of P amendments such as phosphate rock, bone meal and triple super phosphate to form a stable complex of lead phosphate minerals.

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