การใช้ชีวมวลเป็นวัสดุดูดซับยาฆ่าแมลงกลุ่มออร์กาโนฟอสเฟตเพื่อป้องกันการปนเปื้อนสู่ดิน

นางสาวศิรประภา ร่มเย็น

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UTILIZATION OF BIOMASS AS SORBENT MATERIAL TO PREVENT ORGANOPHOSPHATE PESTICIDES CONTAMINATE TO SOIL

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ศิรประภา ร่มเย็น : การใช้ชีวมวลเป็นวัสดุดูดซับยาฆ่าแมลงกลุ่มออร์กาโนฟอสเฟตเพื่อป้องกันการปนเปื้อนสู่ดิน. (UTILIZATION OF BIOMASS AS SORBENT MATERIAL TO PREVENT ORGANOPHOSPHATE PESTICIDES CONTAMINATE TO SOIL) อ. ที่ปรึกษา: ดร.เบญจลักษณ์ กาญจนเศรษฐ์, อ.ดร.เอกวัล ลือพร้อมชัย, Prof. Darryl Hawker, Ph.D., 144 หน้า.

ชีวมวลซึ่งเป็นวัสดุเหลือใช้จากการเกษตร 4 ชนิด ได้แก่ ขุยมะพร้าว, แกลบ, เปลือกถั่ว และพีทมอส ถูกนำมาหาค่าความสามารถในการลดปริมาณยาฆ่าแมลงกลุ่มออร์กาโนฟอสเฟตของน้ำชะจากการพ่นต้นพืช ชีวมวลเหล่านี้มีองค์ประกอบของอินทรีย์คาร์บอน(35.4-45.4%)และมีสารอาหารที่เอื้อต่อการเจริญเติบโตของจุลชีพอยู่สูงเพราะฉะนั้นคาดว่ายาฆ่าแมลงจะถูกดูดซับไว้และเกิดการย่อยสลายทางชีววิทยาในเวลาเดียวกัน การศึกษาในครั้งนี้ชีวมวลจะถูกนำมาเปรียบเทียบกับตัวอย่างดินทรายจากสวนส้มในภาคเหนือของประเทศไทย ยาฆ่าแมลงที่ใช้ในการศึกษาคือ มาลาไทออน เมทิลพาราไทออน คลอไพริฟอสและโปรฟิโนฟอส จากการทดลองหาค่าการดูดซับแบบแบตช์พบว่าความสามารถในการดูดซับของยาฆ่าแมลงเรียงลำดับจาก โปรฟิโนฟอส>คลอไพริฟอส>เมทิลพาราไทออน>มาลาไทออน ในขณะที่ความสามารถในการดูดซับของวัสดุเหลือใช้ทางการเกษตรเรียงลำดับจาก พีทมอส>ขุยมะพร้าว>แกลบ>เปลือกถั่ว ดินมีค่าความสามารถในการดูดซับต่ำสุดในทุกชนิดของยาฆ่าแมลง พฤติกรรมของยาฆ่าแมลงในชีวมวลถูกควบคุมด้วยคุณสมบัติของยาฆ่าแมลง (โครงสร้างและความไม่ชอบน้ำ) ร่วมกับคุณสมบัติของชีวมวล (อินทรีย์คาร์บอน ขนาด พื้นที่ผิว) การศึกษานี้ยังศึกษาการสลายตัวของยาฆ่าแมลงหลังจากการดูดซับโดยวัดค่าครึ่งชีวิต ผลการทดลองพบว่าค่าครึ่งชีวิตของยาฆ่าแมลงแต่ละชนิดขึ้นอยู่กับชนิดของวัสดุดูดซับ เช่น ค่าครึ่งชีวิตของคลอไพริฟอสในขุยมะพร้าวมีค่า 21.6 วัน ซึ่งเร็วที่สุดในกลุ่มของชีวมวลที่ใช้ในการทดลอง ในขณะที่ดินมีค่าครึ่งชีวิต 63.6 วัน เช่นเดียวกับยาฆ่าแมลงชนิดอื่นที่สามารถย่อยสลายในชีวมวลได้ดีกว่าในดิน คลอไพริฟอสและขุยมะพร้าวจึงถูกเลือกมาทำการทดลองคอลัมน์ดิน การทดลองแบบชะทำโดยการบรรจุขุยมะพร้าวความสูง 4 ซม. ลงบนผิวหน้าคอลัมน์ดิน (250 มม. X 38.5 มม.) และพ่นคลอไพริฟอส 0.25 กก./เฮคเตอร์ ที่ผิวหน้า แล้วชะด้วยน้ำที่อัตรา 20 มล./ชม. ผลการทดลองพบว่ามวลน้อยกว่าร้อยละ 6.25 ของคลอไพริฟอสค้างอยู่ในชั้นของขุยมะพร้าว ในระยะเวลา 28 วัน ในขณะเดียวกันปริมาณจุลชีพที่ย่อยสลายคลอไพริฟอสก็เพิ่มขึ้นด้วย ซึ่งสามารถระบุได้ว่าคลอไพริฟอสที่ถูกดูดซับจะถูกย่อยสลายทางชีวภาพต่อไป นอกจากนี้ค่าครึ่งชีวิตโดยประมาณการของยาฆ่าแมลงอื่นๆ คือ 9.8, 6.0 และ 10.4 วัน ปริมาณยาฆ่าแมลงคงค้าง น้อยกว่าร้อยละ 12.5, 6.25 และ 12.5 ของเมทธิวพาราไทออน มาลาไทออนและโปรฟิโนฟอส ตามลำดับดังนั้นการคลุมหน้าดินด้วยขุยมะพร้าวจึงเป็นวิธีที่จะนำมาใช้ลดการปนเปื้อนจากน้ำชะยาฆ่าแมลงกลุ่มออร์กาโนฟอสเฟตที่พ่นจากต้นพืชสู่ดินได้

สาขาวิชาการจัดการสิ่งแวดล้อม
ปีการศึกษา 2549

ลายมือชื่อนิสิต.....
ลายมือชื่ออาจารย์ที่ปรึกษา.....
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

4689686020 : MAJOR ENVIRONMENTAL MANAGEMENT
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SIRAPRAPA ROMYEN: UTILIZATION OF BIOMASS AS SORBENT MATERIAL TO PREVENT ORGANOPHOSPHATE PESTICIDES LEACHING THROUGH SOIL. THESIS ADVISOR : BENJALAK KARNCHANASEST, Ph.D., EKAWAN LUEPROMCHAI, Ph.D., PROF.DARRYL HAWKER, Ph.D., 144 pp.

Four biomass which are agricultural by-products namely, coconut husk (CH), rice husk (RH), peanut shell (PS) and peat moss (PM) were evaluated for their ability to minimize the amount of organophosphate pesticides (OPs) in leachate after spraying onto plants. The biomass have high organic carbon content (35.4-45.4%) and contain various nutrients to support microbial growth; consequently they are expected to sorp OPs and simultaneously promote OPs biodegradation. In this study, the biomass were investigated in comparison to a sandy soil sample taken from tangerine orchard in northern Thailand. The OPs tested were malathion, methyl parathion, chlorpyrifos and profenofos. Sorption studies were carried out by the batch (equilibrium) method. Sorption strength of OPs were in the order that profenofos>chlorpyrifos>methyl parathion> malathion and for a given OP with different types of biomass PM>CH>RH>PS. Soil sorption obviously exhibits the lowest values for all OPs. OP behavior in the biomass-water systems is governed by the compound properties (structural features and hydrophobicity) together with biomass properties (organic carbon contents, size, and surface areas). The study also determined the degradation of OPs after sorption by measuring their half-life ($t_{1/2}$). The results showed that the half-life of each OPs was depended on the type of sorbed materials. For example, the half-life of chlorpyrifos in coconut husk was 21.6 days, which was the fastest among the tested biomass, whereas; its half-life was 63.6 days in soil. Similarly, malathion, methyl parathion and profenofos were degraded more rapidly in biomasss than in soil. Chlorpyrifos and coconut husks were later selected as a model for soil column study. Leaching experiments were carried out by packing 4 cm coconut husk on top of soil columns (250 mm x 38.5 id mm), and sprayed with 0.25 kg ha^{-1} chlorpyrifos to the column surface. Leaching tests were performed at a water flow rate of 20 ml hr^{-1}. Results indicated chlorpyrifos mass were retained in the coconut husk layer. The amounts of sorbed chlorpyrifos were retained less than 6.25% after 28 days. At the same time, the number of chlorpyrifos-degrading bacteria was increased, which suggested that the sorbed chlorpyrifos was biodegraded. Moreover, the half-life estimation for other OPs was 9.8, 6.0 and 10.4 days and amount of OPs were retained less than 12.5, 6.25 and 12.5% methyl parathion, malathion and profenofos, respectively. Therefore, covering of soil surface with coconut husk could be a feasible method to reduce contamination of OPs leachate from the sprayed plants through soil.

Field of study Environmental Management
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LIST OF ABBREVIATIONS

BTCs	=	Breakthrough curves
$cm^3\ g^{-1}$	=	Cubic centrimeter per gram
CH	=	coconut husk
Cs	=	The concentrations of the OPs sorbed by solid phase
Cw	=	The concentrations of the OPs dissolved in aqueous phase
EM	=	Effective Microorganisms
Foc	=	Fraction of total organic
K_D	=	Distribution coefficient
K_{oc}	=	Soil organic carbon-water partition coefficient
Kow	=	Octanol-water partition coefficient
M	=	Molar
$mg\ kg^{-1}$	=	Milligram per kilogram
$mg\ l^{-1}$	=	Milligram per litre
Mw	=	Molecular Weight
OC	=	Organic carbon
OM	=	Organic Matter
OPs	=	Organophosphate pesticides
P	=	Vapor pressure
PCP	=	Pentachlorophenol
PS	=	peanut shell
RH	=	rice husk
S	=	Water solubility
$t_{1/2}$	=	Degradation half-life
µECD	=	Electron capture detector

CHAPTER I

INTRODUCTION

1.1 Problem Identification

The increased use of various pesticides in modern agricultural land for protecting and growing crops has led to the possibility of serious environmental contamination. Recently, approximately 95,763 tons of pesticides are used in Thailand and this amount is continued to increase each year (Agricultural regulatory division, 2006). Organophosphate pesticides (OPs) as a class have become the most frequently used pesticides because of their rapid breakdown into environmentally safe products. Although most of OPs are nonpersistent pesticides (Breast Cancer Fund, 2007), they are applied regularly to ensure the effective insect control. Thus, it is inevitable that these pesticides become a major environmental problem because of run off and leaching events once they reach the soil surface. There are many types of OPs used on the agricultural land such as chlorpyrifos, malathion, methyl parathion, profenofos and etc. (Chiangmai, 2003). In Thailand, OPs residues have been found in soil, water and agricultural products (Thapinta and Hudak 2000). Recognizing the growing problem, this study aimed to develop an approach that can be used to protect the environment by preventing OPs transport from the soil surface to the water table.

Normally, sorption and degradation are key processes affecting the fate and transport of pesticides in the environment (Linn et al., 1993; Boivin et al., 2005). Sorption is the influential factor because it is the attraction of a pesticide to organic matter in soil surfaces, especially OPs which are the nonionic pesticides (Hamaker et al., 1972; Barriuso et al., 1998). According to Xu et al. (1997), the potential leaching of agrochemicals can be decreased by creating sorptive or immobilizing zones in the soil by the incorporation of the appropriate sorbent in the affected area of soil.

Biomass is a residue of agricultural process, widely generated in Thailand. The addition of agriculture biomass as exogenous organic matter to soil is being use as an alternative method for disposal. Due to the high organic matter, it can modify surface of soils and subsurface materials, and then promoting sorption of pesticides and

retarding their movement. For example, Cox et al. (2001) found herbicides sorption increased in soil adding with organic amendment. Organic amendment can also affect the biodegradation of pesticides by enhancing microbial activity and consequently, promoting biodegradation (Cox et al., 1997; Perrin-Ganier et al., 2001; Albarrain et al., 2004). Vischetti et al. (2004) constructed a biomassbed reactor as filter of discharge water contaminated with chlorpyrifos, metalaxyl and imazamox from sprayers after pesticide field treatment by mixing biomass e.g vine-branch and citrus pulp with urban waste and green compost. The main function of the biomassbed is to reduce the environmental pesticide concentrations by the strong sorption of the pesticide on the organic components and the rapid degradation by the active microbiological component.

Therefore, the objective of this study is to assess the feasibility of using biomass as sorbent material to prevent the discharge of OPs such as methyl parathion, malathion, chlorpyrifos and profenofos from soil. The application of biomass is interested because they are waste from agricultural activities which are abundance in local area. Morever, the biomass can be degraded biologically after used, thus there is no need for further remediation or special disposal. Initially, biomass i.e. rice husk, coconut husk, peat moss and peanut shell were studied as sorbent materials by covering on top soil. Partitioning behavior of OPs mixture containing chlorpyrifos, malathion, methyl parathion, and profenofos, which were widely used (Chiangmai, 2003) in these biomass were investigated in comparison to native soil from Tangerine orchard in Mae Ai, northern Thailand. The half-life of OPs in soil and biomass was assessed to evaluate degradation rate by bacteria. Finally, the actual leaching of selected OPs from sorbent was verified in soil columns.

1.2 The purpose of the study

The main goal was to explore the biomass potential in reducing OPs contamination to soil. There were three specific objectives in this study:

1.2.1 To determine sorption behavior of OPs in the biomass and soil.

1.2.2 To monitor biodegradation behavior of OPs in the biomass and soil.

1.2.3 To investigate OPs in the biomassbed and develop methods using characteristics in sorption, biodegradable and leaching to reduce pollutionfrom sprayed OPs.

1.3 Hypothesis

Biomass has higher sorption properties than soil due to the presences of high amount of organic carbon. The high sorption could decrease mobility of free pesticides. Furthermore, the sorped OPs were expected to be degradable by microorganisms in the biomass before leach down to soil surface.

1.4 Scope of the study

Four locally available biomass materials were used for this study including coconut shell, rice husk, peanut shell and peat moss. Background soil sample was taken from tangerine orchard area located in Chiangmai, Northern of Thailand. Four OPs were selected for this study because of their widespread use in tangerine orchard area located in Chiangmai. They were chlorpyrifos, methyl parathion, profenofos and malathion.

This study was devided into two parts. First part was set to determine the sorption and degradation behaviors of OPs in the biomass and soil. The batch partitioning was conducted to determine sorption capacity (K_D) of biomass and soil by calculating from sorption isotherm. Effect of pH, particle size, the correlation of sorption coefficient and OPs properties such as solubility, log Kow and Mw also investigated as well. After that, the degradation rate of OPs in biomass and soil was performed by kinetic equation. The criteria for selection of best sorbent and modeled OPs for leaching experiment were high sorption capacity, cheap and shortest half-life.

The selected biomass and OPs was employed to find the optimum covering depth on top soil for using in further part. The second part was set to investigate the efficiency of developed biomassbed method to reduce pollution from the sprayed OP to the environment. The leaching of a selected OP from sorbent was verified in soil columns. The biodegradation of sorped OP was also examined in microcosms and OP-degrading bacteria was counted by plate counting method. In addition, the effect of Effective microorganisms (EM) on degradation was investigated. The analysis of OPs residue in biomass, soil and leachate was conducted by gas chromatography with an electron capture detector (ECD).

CHAPTER II

LITERATURE REVIEWS

2.1 Organophosphates Pesticides

2.1.1 Sorption

The extent of sorption can be described in term of distribution coefficients or K_D ,which is an organic chemical distributes itself between an environmental solid and aqueous phase at equilibrium. It is generally derived from the slope of the sorption isotherm at the contaminant concentrations of interest. The greater the value of K_D, the greater the amount of sorption. These isotherms can be linear or nonlinear, depending on the properties of the chemical and solid phase as well as on the concentration in aqueous phase. Linear sorption isotherms often are observed if the equilibrium aqueous phase organic compound concentrations are below 10^{-5} M or one-half the aqueous phase solubility (whichever is lower) and the organic content of the solid is greater than 0.1% (LaGregra et al., 2001; Smith et al., 2003; Suthersan, 2002). In addition, isotherms of nonionic organic compounds (e.g. OPs) are often assumed to be linear (Chiou, 1989; Hamaker and Thomson, 1972; Karickhoff, 1984; Smith et al., 2003). Therefore a linear relationship is represented by the following equation;

$$K_D = Cs.\ C_w^{-1} \qquad (2.1)$$

Where, C_s and C_w are the concentrations of the OPs sorbed by solid phase (mg kg^{-1}) and dissolved in aqueous phase (mg l^{-1}), respectively. Units of K_D typically are given as l kg^{-1}, ml g^{-1}, or cm^3 g^{-1}.

As most of the pesticides and other contaminants that are found in soil are bound to the organic matter of the soil, the distribution coefficients between organic carbon and water (K_{oc}) is often calculated on the basis of sorption coefficient. The following equation gives the expression relating K_D to K_{oc}:

$$K_{oc} = K_D . f_{oc}^{-1} \quad (2.2)$$

or

$$K_{oc} = 100\ K_D . \%OC^{-1} \quad (2.3)$$

Where, K_{oc} is the soil sorption coefficient normalized by total organic carbon content, K_D is the linear sorption coefficient and f_{oc} is fraction of total organic carbon in equation 2.2, and % OC in equation 2.3 is the organic carbon content of sorbent material expressed in percent (Gawlik et al., 1997).

The extent and strength of sorption reaction, which governed by the chemical and physical properties of the soils and pesticides involved. There are many reports about factors that affect sorption behavior.

(1) Soil characteristics

Oliver et al. (2005) suggested soil organic matter properties affect the sorption of organic molecules. Organic matter provides the greatest number of binding sites; as a result it has an extremely large surface area and is very chemically reactive (Huddleston, 1996). Moreno et al. (2007) revealed greater sorption capacity of terbuthylazine in soil amended with olive cake which has high organic matter content. Riaz et al. (2006) also confirmed that organic carbon is the most important component of soil controlling sorption. While, Suter (2002) suggested that sorption was directly related to organic matter content and inversely related to clay content. However, sorption behavior is not only influenced by the organic carbon, but shape, and properties of soil, polarity, solubility of pesticides are also the factors that could affect this behavior (Joern and Lohman, 2007).

The texture of a soil is extremely important in the sorption process. If a soil is made up of mostly clay and organic matter, a significant amount of sorption will take place. Clay especially intermixed with organic particles, by far adsorbs the most out of the three main soil textures (clay, silt, and sand) because of its small particle size, high surface area, and high surface charge. In addition**, s**oil structure influences the movement of water and pesticides. Coarse textured sandy soils with large air spaces allow more rapid movement of water than fine textured or compacted soils with fewer

air spaces. Dry condition of soil may increase binding because water in the soil competes for the binding sites. Wu et al. (2003) and Zhou et al. (2004) investigated the influences of particle size of soil and sediment from Beizhai River and Guanting Reservoir (Beijing,China) on sorption. They reported the sorption capacities increase with decreased particle size.

The other factor is temperature, it can influence sorption, the strength and direction of the effect depends on the properties of the sorbent and sorbate and on the sorption mechanism. Sorption processes are generally exothermic, so the higher the temperature, the less the sorption. Other reviews also indicated that the influence of temperature on equilibrium sorption and have found that, in most cases, equilibrium sorption decreases with increasing temperature (Wiedemeier, et al, 1999; Suthersan, 2002).

(2) Pesticides characteristics

Solubility is defined as the maximum amount of a contaminant that dissolved in water at a specified temperature. The solubility of a compound tends to be inversely proportional to the amount of sorption that the contaminant can undergo. Ferrante (1996) reported that the higher a pesticide's water solubility, the more likely it will move with water. Pesticides with solubility of less than 1 ppm tend to remain on the soil surface. They tend not to be leached, but may move with soil sediment in surface runoff. Pesticides with solubility greater than 30 ppm are more likely to move with water (Ministry of Agriculture of British Columbia, 2007).

Soil sorption is measured by K_{oc} value which describes the tendency of pesticides to be attached to soil particles. Zytner (1994) suggested that the soil-water partition coefficient is a useful indicator for mobility: a K_{oc} of 100 L kg^{-1} indicates high chemical mobility in soil, while a K_{oc}, in excess of 1000 L kg^{-1} indicates chemical immobility in soil.

The pH of the fluid can affect sorption considerably because it can affect the solubility of a compound. Certain compounds dissolve better in fluids under certain pH, for example organic acids tend to adsorb better under acidic conditions and amino

compounds adsorb better under alkaline conditions (Fall, 1996). Sheng et al. (2005) reported influence of pH on diuron, bromoxynil and ametryne sorption in soil with and without wheat residue-derived char. The research demonstrated that the sorption of diuron was not influenced by pH, due to its electroneutrality. Bromoxynil was sorbed lower at pH 7 less than pH 3. While sorption of ametryne by char- amended soil at pH 3 was influenced by both the soil and char. Bras et al. (2005) studied the sorption of pentachlorophenol on pine bark. The results showed the neutral PCP distribution coefficient (K_D) of the linear sorption isotherm with the increasing of solution pH from 2 to 7.

The polarity of a compound plays a major role in the mobility of the compound. Polar substances tend to dissolve more readily in water than nonpolar substances and therefore adsorb to soil particles less.

2.1.2 Biodegradation

Biodegradation is the breakdown of pesticides by fungi, bacteria, and other microorganisms into smaller molecules. Most biodegradation of pesticides occurs in the soil. It is believed that degradation by microbes accounts for over 90% of all degradation reactions in the environment and it is the nearly exclusive breakdown pathway in most surface soils (Wheeler, 2002). The proficiency of microorganisms is due to their simplicity in absorbing chemicals from exogenous sources or excreting transformation products, and their diverse enzymatic content. Microorganisms transform the contaminants through metabolic or enzymatic processes. Biodegradation processes vary greatly, but frequently the final product of the degradation is carbon dioxide or methane. Biodegradation is also a key process in the natural attenuation of contaminants at hazardous waste sites. A related term is biotransformation.

Microbial degradation can be rapid and thorough under soil conditions favoring microbial activity. Those conditions include warm temperatures, favorable pH level, adequate soil moisture, aeration (oxygen), and fertility. Another optimum condition is abundance of organic matter. It has been revealed by Sánchez et al. (2004) that the increasing of organic matter is varied directly to microbial degrading

activities. The rate of most degradation catalyzed by enzymes tends to double for each 10ºC increase in temperature between 10 and 45ºC. The degree of adsorption also influences microbial degradation, because pesticides must be in solution in order to be absorbed and metabolized by microorganisms. Accelerated microbial degradation may occur when the same pesticide is used repeatedly in a field, because of a rapid build up of the organisms that are effective in degrading the chemical. As the population of these organisms increases, degradation accelerates and insufficient pesticide remains available to control the pest. Moreover, microbial degradation occurs at a higher rate in surface horizons, particularly in areas with higher organic matter and microbial numbers than in deep soil (Hua et al., 1997; Dai et al., 2001).

The pesticide degradation in soil can be described with the first-order kinetic equation as:

$$\frac{dC}{dt} = -kt \qquad (2.5)$$

From this equation, we can obtain the following equations for determining the half-life of pesticide;

$$\ln C = -kt + \ln C_o \qquad (2.6)$$

$$t_{1/2} = 0.693\, k^{-1} \qquad (2.7)$$

Where C is concentration of pesticide in soil, mg kg^{-1}, k is degradation rate constant, d^{-1} , Co is the initial concentration of pesticide in soil, mg kg^{-1}, t is time, days, and $t_{1/2}$ = half-life, days. Half-life is often expressed the persistence of pesticide in environment. Pesticides can be categorized into three groups based on half-lives: nonpersistent pesticides with a typical soil half-life of less than 30 days, moderately persistent pesticides with a typical soil half-life of 31 to 99 days, or persistent pesticides with a typical soil half-life of more than 100 days (Deer, 2004). Generally, the longer half-life results in the greater the potential for pesticide movement. A pesticide with a half-life greater than 21 days may persist long enough to leach or

move with surface runoff before its degradation (Ministry of agriculture, British Columbia, 2007).

The use of living organisms to degrade environmental pollutant into less toxic form is emerging as one of alternative technologies. Evidently, the introduction of specific bacterial strains also enhances pesticide degradation. The study of Fostor et al. (2004) showed that two bacterial isolates, *Pseudomonas* and *Azospillum* species were capable of degrading the organophosphate pesticide, Ethion rapidly and utilizes this pesticide as carbon source. While, Actinomycetes have been known for their potential in the biotransformation and biodegradation of pesticides with widely different chemical structures, including organochlorines, s-triazines, triazinones, carbamates, organophosphates, organophosphonates, acetanilides, and sulfonylureas (Schrijver and Mot, 1999). In general, only a limited number of these xenobiotic pesticides can be mineralized by single isolates, but often consortia of bacteria are required for complete degradation.

The selected OPs including methyl parathion, malathion, chlorpyrifos and profenofos have been showed to degrade successfully by microorganism. For instance, a degradative bacterium, *Plesiomonas* sp. M6, was isolated and found to hydrolyze methyl parathion to *p*-nitrophenol (Zhongli et al., 2001). Megharaj et al. (1994) studied the role of microalgae and cyanobacteria in biodegradation of methyl parathion by measuring the rate of disappearance of the insecticide and its major hydrolysis product, p-nitrophenol (PNP), from the culture media. They found the nitro-group reduction is another means of PNP degradation.

Hayatsu et al. (2000) studied the degradation of methyl parathion. They found NF100 was able to utilize 3-methyl-4-nitrophenol and 4-nitrophenol, which are hydrolysis products of methyl parathion. Zhang et al. (2004) investigated the degradation of methyl parathion in soil and Chinese chive by strain DLL-1. The results showed the amount of pesticide residues is significantly decreased through the application of high effective degrading microbial agents. Besides, the appropriate time for the application of the degrading microbe is 3 days after the application of the pesticide.

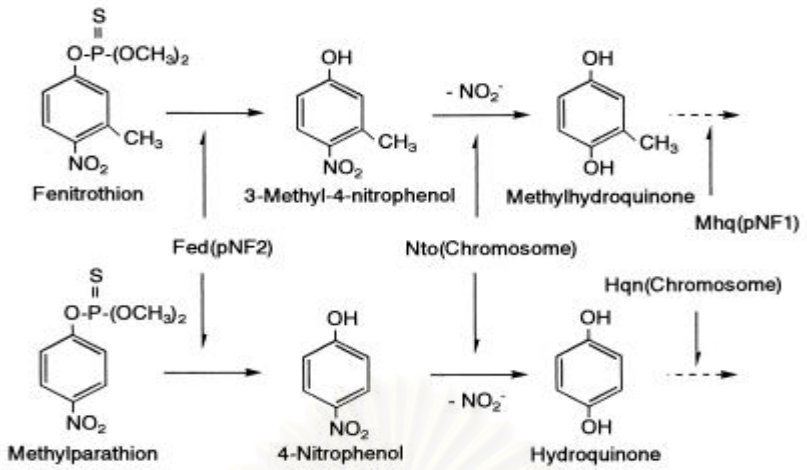


Figure 2.1 Degradation pathways of methyl parathion and fenitrothion (Hayatsu et al.,2000).

Malathion is metabolized rapidly by the soil fungus *Trichoderma viride* and the bacterium *Pseudomonas* sp. (Barlas, 1996). Hashmi and Kim (2003) investigated the potential of *Pseudomonas* by continuous cultivation using two different sets of conditions (with-culture: *Pseudomonas*, and without-culture: indigenous microorganisms) for malathion degradation. They revealed that the degradation potential of *Pseudomonas* was better than the degradation potential of indigenous microorganisms.

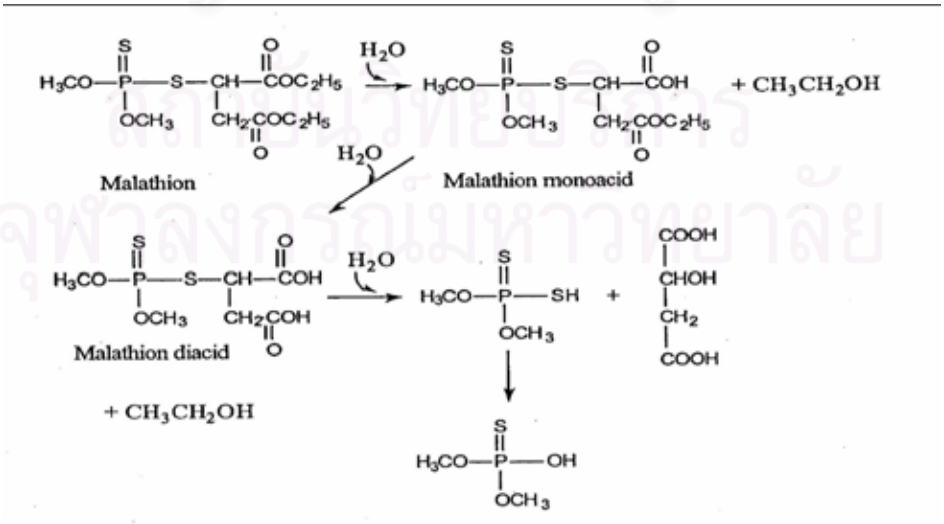


Figure 2.2 Degradation path way of malathion (Newhart, 2006)

Chlorpyrifos (O,O-diethyl O-3,5,6-trichloro-2-pyridinyl phosphorothioate) is used worldwide as an agricultural organophosphate insecticide. The successes of chlorpyrifos degradation in soil have been reported both by indigenous and augmented bacteria. Many reports show that chlorpyrifos-degrading bacteria can be found in soil of many countries e.g. Australia (Singh et al., 2004), India (Pandey and Singh, 2004), and China (Li, 2007). An addition of *Alcaligenes faecalis sp*. DSP3 (10^8 cells g^{-1}) or *Sphingomonas sp.* Dsp-2 to soil with chlorpyrifos (100 mg kg^{-1}) resulted in a higher degradation rate than the one obtained from noninoculated soils (Yang et al., 2006; Li et al., 2007). Yang et al. (2006) isolated an effective chlorpyrifos-degrading bacterium strain YC-1 from sludge of a wastewater treating system of an organophosphorus pesticides manufacturer. The isolate utilized chlorpyrifos as the sole source of carbon and phosphorus for its growth and hydrolyzed chlorpyrifos to 3,5,6-trichloro-2-pyridinol. Parathion, methyl parathion, and fenitrothion are also degraded by strain YC-1 when providing as the sole source of carbon and phosphorus. Theses results highlight the potential of this bacterium to be used in the cleanup of contaminated pesticide waste in the environment. Recently, Li et al. (2007) reported that strain Dsp-2 can be used as chlorpyrifos- and profenofos-degrading bacteria.

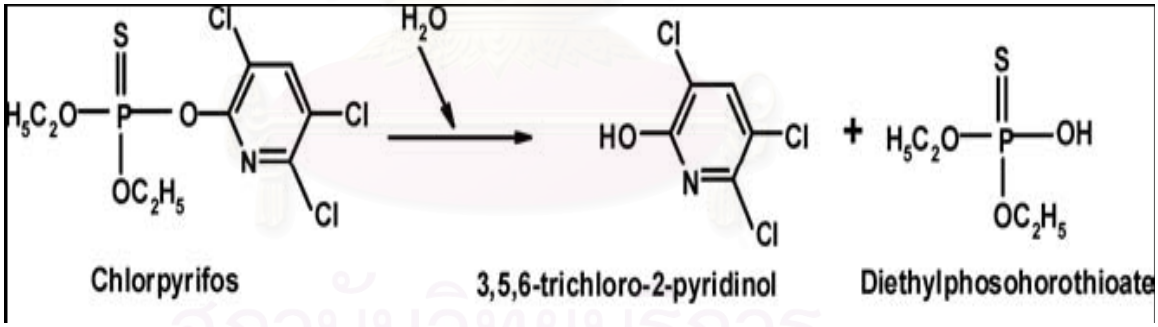


Figure 2.3 Degradation path way of chlorpyrifos (Shemer, 2005)

However, Silja (2004) suggested that breakdown pattern is through bacterial metabolism of many species, usually through a consortium of microbes rather than a single species.

Effective Microorganisms or EM is a mixed culture of beneficial micro-organisms used to create a favourable microbiological environment for plant growth for decades. According to Higa (1998), EM contains about 80 species of micro-

organisms, which can be divided into the following groups: (1) photosynthesizing bacteria;(2) lactic acid bacteria; (3) yeasts; (4) actinomycetes and (5) fermenting fungi such as *Aspergillus* and *Penicillium*. The application of EM has been found in various fields. He et al., (2005) used EM that isolated from landfill on degradation of municipal solid waste. They demonstrated that EM can increase the biodegradability of municipal solid waste and result in a high degree of waste stabilization. Some studies have shown that the inoculation of argo-ecosystems with EM cultures can improve soil and crop quality (Higa and Parr 1994; Hussain et al., 1999). Khaliq et al. (2005) studied the effects of integrated use of organic and inorganic nutrient sources with effective microorganisms on growth and yield of cotton. The results indicated that the application of EM increased the efficiency of both organic and mineral nutrient sources but alone was ineffective in increasing yield.

However, the impacts of EM on accelerating pesticide biodegradation in soil or biomass have rarely been done. Therefore, the aim of this research was to find out the effect of EM inoculation on OPs degradation in biomass and soil. These results would confirm the benefit of using EM and biomass on reducing pesticide contamination.

2.1.3 Hydrolysis

When malathion is released to soil, it may moderately sorb to the soil. Hydrolysis may be important based on the hydrolysis rate in water with a reported degradation of 50-90% in 24 hr in both sterile and non sterile soils. Biodegradation may be an important fate process, especially in soils at < pH 7 where the rate of hydrolysis may be slow relative to the rate of biodegradation. The major metabolite in soil is malathion beta monoacid. Reported half lives in soil range from approximately 4 days to a reported literature average of 6 days. Percent degradation, range (avg), in 4 West Bengal soils between 3 and 7 days after treatment with malathion in dry, moist, waterlogged soils, respectively, were: 33-86%(70%), 15-40%(33%), and 20-54%(37%); 100% overall degradation reported within 20 days for all 3 conditions . Based on the range of K_{oc} reported and the rapid degradation of malathion in soils, it should not leach to the groundwater.

Methyl parathion is rapidly degraded in soil at the low concentrations which are associated with its use as an insecticide. Loss is primarily due to biodegradation (half life 10 days - 2 months). Degradation increases with temperature and exposure to sunlight. Methyl parathion will mineralize; intermediate products are amino methyl parathion and O-methyl-O'-p-nitrophenylthiophosphoric acid. At very high concentrations such as might be associated with a spill, degradation is exceedingly slow and may be primarily due to photodegradation. Since methyl parathion demonstrates moderate soil adsorption and biodegrades rapidly, it is unlikely to leach into ground water under most circumstances. Evaporation should not be an important transport mechanism. In a terrestrial ecosystem the majority, 65%, of the residue from ring labeled methyl parathion was found in the air, 15 days after application which indicates that volatile degradation products such as CO_2 are formed. Degradation was more rapid under flooded conditions than under non-flooded conditions in soil. Hydrolysis was implicated under non-flooded and to a minor extent in flooded degradation under flooded conditions proceeded essentially by nitro group reduction. Mineralization of methyl parathion proceeded more rapidly in moist soils (which have lower soil-water tension values) than in dry soils.

Chlorpyrifos is released into the environment primarily from its application as an insecticide. If released to soil, chloropyrifos can degrade by a combination of chemical hydrolysis and microbial degradation. The chemical hydrolysis is clay catalyzed and yields a primary degradation product of 3,5,6-trichloro-2-pyridinol. Volatilization from soil surfaces is expected to contribute to its loss from soil. Chlorpyrifos is tightly absorbed by soil and not expected to leach significantly. Although a general soil persistence of 60-120 days has been reported, the persistence can vary greatly depending on soil type, climate, and conditions and has been experimentally measured to range from as little as 2 weeks to over 1 year. If released to water, chlorpyrifos partitions significantly from the water column to sediments. The measured hydrolysis half-life at 25ºC at (or near) neutral conditions is 35-78 days. The hydrolysis rate is relatively independent of pH from pH 1 to pH 7, increases significantly under alkaline conditions, decreases 2.5-3 fold with 10ºC temperature decrease, is markedly enhanced by the presence of Cu^{+2} ions in sufficient concentration, and is not affected by adsorption to sediments in acidic or neutral water. The hydrolysis products include 3,5,6-trichloro-2-pyridinol and various

trichloropyridyl phosphorothioates. The photolysis half-life at the water surface in the US during the mid summer is about 3 to 4 weeks; however, photolysis is not expected to be a very significant removal mechanism in relatively deep waters, in the winter-time, or in any natural waters containing sufficient light attenuating material. Microbial degradation may contribute to removal in some natural waters. The volatilization half-life from a river one meter deep flowing 1m sec^{-1} with a wind velocity of 3 m sec^{-1} is estimated to be 5.7 days; however, the significance of volatilization may be greatly decreased by aquatic sediment adsorption. Experimental and estimated log BCF values ranging from 2.50 to 3.54 indicate potential significant bioconcentration. The desorption from sediments can contribute to long term residual concentration in the water column (low ppb). If released to air, chlorpyrifos will react in the vapor-phase with photochemically produced hydroxyl radical half-life of 13.74 hours, but it is not expected to react with ozone. Photolysis in air may contribute to its transformation. Major general population exposure to chlorpyrifos will occur through consumption of contaminated food and inhalation of contaminated air. Occupational exposure by dermal and inhalation routes may be significant.

When released to soil, chlorpyrifos can degrade by a combination of chemical hydrolysis and microbial degradation. The chemical hydrolysis, which is catalyzed by clay and yields a primary degradation product of 3,5,6-trichloro-2-pyridinol, occurs in both dry and moist soils. Microbial degradation may be significant in various soils as indicated by significantly faster degradation rates in non-sterile versus sterile soil. Laboratory experiments have indicated that volatilization from soil surfaces under field conditions is expected to contribute to its loss from soil. Photodegradation on soil surfaces may occur, but is not expected to be competitive with other fate processes. Measured K_{oc} values ranging from 4,381 to 13,600 and various field studies indicate that chlorpyrifos is tightly absorbed to soil and not expected to leach significantly. A general soil persistence of 60-120 days has been reported. An initial half-life of 10 days was measured in a paddy soil with residual chlorpyrifos remaining after 60 days. The initial half-lives in field plots of sandy and muck soils were 2 and 8 weeks, respectively, with 4% and 9% remaining after one year, respectively. A persistence of 2-4 weeks was measured in a sandy loam soil. Persistence of 180 days was measured in a field soil receiving normal application rates. A field study with silt

loam soil showed that chlorpyrifos disappeared 2-3 times faster from generally dry surfaces than when incorporated or applied beneath the soil surface.

2.1.4 Photodegradation

Pehkonen and Zhang (2002) studied the photodegradation of OP. They reported that the direct photolysis can occur at UV region (240–310 nm), since the OPs exhibit maximum absorption. Burkhard and Guth (2006) investigated photolysis on soil surfaces of the organophosphorus insecticides diazinon, methidathion and profenofos under artificial sunlight conditions. All three compounds were readily degraded under the conditions used. The rate of degradation decreased in the order diazinon, profenofos, methidathion and was always greater in moist than in dry soil. The same order of stability was also observed from photolysis studies in aqueous solution. Profenofos, however, showed a different photolytic reaction in aqueous systems, forming *O*-(2-chlorophenyl) *O*-ethyl *S*-propyl phosphorothioate. While Shemer et al (2005) reported the primary degradation product of chlorpyrifos, by both hydrolysis and photolysis, is 3,5,6-trichloro-2-pyridinol (TCP). A phosphorus oxygen bond in the chlorpyrifos molecule is cleaved to generate TCP and diethylphosohorothioate.

2.1.5 Toxicity

OPs are generally acutely toxic. However, each pesticide within this group can pose varying degree of toxicity. Their primary mode of action on insects and other animals is by phosphorylation of the acetylcholinesterase enzyme. This enzyme is necessary for controlling nerve impulse transmission between nerve fibers. A loss of enzyme function results in an accumulation of acetylcholine, which causes unregulated nervous impulses. Symptoms of acute poisoning develop during or after exposure, within minutes to hours, depending on method of contact. Inhalation exposure results in the fastest appearance of symptoms, followed by the gastrointestinal route and then the dermal (skin) route (Fishel, 2005). The poisoning symptoms include: excessive sweating, salivation and lachrimation, nausea, vomiting, diarrhoea, abdominal cramp, general weakness, headache, poor concentration and tremors. In serious cases, respiratory failure and death can occur. Other consequences

may follow high acute exposures. From one to several weeks after exposure, organophosphate - induced delayed neuropathy (OPIDN) [nerve damage] may set in. This may begin with burning and tingling sensations and progress to paralysis of the lower limbs. Moreover, evidence suggests that OPs are mutagenic and teratogenic and that a large number of modern-day diseases of the nervous and immune system of mammals can be linked to these pesticides. These include BSE (mad cows disease), CJD, Gulf War syndrome, Parkinson's disease and multiple sclerosis (Ragnarsdottir, 2000).

The World Health Organisation classifies methyl parathion as a class I a “extremely hazardous” pesticide. It is highly toxic by inhalation and ingestion, and moderately toxic by dermal adsorption (it is also readily adsorbed through the skin). The oral LD_{50} in rats is 2.9 mg kg^{-1}, in mice is 33.1-119.5 mg kg^{-1}, in rabbits is 19-420 mg $kg^{-1}$and dogs are 50 mg kg^{-1}. The dermal rat LD_{50} is 44-67 mg kg^{-1} (PAN International Website, 2007). Short-term exposure to high levels of methyl parathion, an organophosphate, may affect the nervous system by inhibiting the activity of an enzyme called cholinesterase. At normal levels, cholinesterase breaks down a chemical called acetylcholine, which helps transmit signals in the nervous system. When cholinesterase is inhibited, an excess of acetylcholine builds up and impairs the proper functioning of the nervous system. Signs and symptoms of direct exposure to high levels of the more concentrated forms of methyl parathion may include headache, dizziness, loss of coordination, muscle twitching, tremor, nausea, vomiting, abdominal cramps, diarrhea and general weakness, blurred vision, excessive perspiration and salivation.

Malathion is slightly toxic via the oral route, with reported oral LD_{50} values of 1000 mg $kg^{-1}$to greater than 10,000 mg kg^{-1}in the rat, and 400 mg $kg^{-1}$to greater than 4000 mg kg^{-1}in the mouse. It is also slightly toxic via the dermal route, with reported dermal LD_{50} values of greater than 4000 mg kg^{-1}in rats. Effects of malathion are similar to those observed with other organophosphates, except that larger doses are required to produce them. It has been reported that single doses of malathion may affect immune system response. Symptoms of acute exposure to organophosphate or cholinesterase-inhibiting compounds may include the following: numbness, tingling sensations, incoordination, headache, dizziness, tremor, nausea, abdominal cramps,

sweating, blurred vision, difficulty breathing or respiratory depression, and slow heartbeat. Very high doses may result in unconsciousness, incontinence, and convulsions or fatality. The acute effects of malathion depend on product purity and the route of exposure (Extoxnet, 1996).

The acute toxic effects of chlorpyrifos exposure are primarily due to the inhibition of acetylcholinesterase (Kwong, 2002; Bicker et al., 2005). Chlorpyrifos is very toxic to humans, between 1 teaspoon and 1 ounce may be fatal. Chlorpyrifos toxicity is considerably greater if administered orally compared to dermal. Primary routes of exposure are inhalation, skin or eye contact. Inhalation exposure to high concentration may cause upper respiratory irritation, central nervous system depression headache, dizziness, increased sensitivity to epinephrine, irregular heartbeats, incoordination, muscle twitching, tremor, pinpoint pupils, blurred vision, tightness in chest, and convulsions. Eye contact may cause pain, moderate irritation. Poisoning also impairs Central Nervous System. The oral LD_{50} for chlorpyrifos in rats is 95 to 270 mg kg^{-1}. The LD_{50} for chlorpyrifos is 60 mg kg^{-}in mice, 1000 mg kg^{-1}in rabbits, 32 mg kg^{-1}in chickens, 500 to 504 mg kg^{-1}in guinea pigs, and 800 mg kg^{-1}in sheep. The dermal LD50 is greater than 2000 mg kg^{-1}in rats, and 1000 to 2000 mg kg^{-1}in rabbits. The 4-hour inhalation LC_{50} for chlorpyrifos in rats is greater than 0.2 mg L^{-1}(Extonet, 1996). Breathing the air in an area in which chlorpyrifos has recently been sprayed may produce a variety of effects on the nervous system including headaches, blurred vision, watering of the eyes (called lacrimation), excessive salivation, runny nose, dizziness, confusion, muscle weakness or tremors, nausea, diarrhea, and sudden changes in heart rate. The effect depends on the amount in the air and length of time exposed. Ingesting chlorpyrifos orally through contaminated food containers or, in the case of children, putting objects of hands in their mouth after touching chlorpyrifos, may cause similar symptoms. Exposure to high levels may cause severe sweating, loss of bowel control, severe muscle tremors, seizures, loss of consciousness (coma), or death. There is no information at present to show that chlorpyrifos either effects the ability of humans to reproduce or causes human birth defects (ATSDR, 2007).

Profenofos and its metabolites were determined in a case of fatal poisoning (Gotoh et al., 2001). Profenofos can cause cholinesterase inhibition in humans; that is,

it can overstimulate the nervous system causing nausea, dizziness, confusion, and at very high exposures (e.g., accidentsor major spills), respiratory paralysis and death Organophosphate mammalian toxicities (mg kg_{-1} of body weight) reported 358, 472 for Rat oral LD_{50} and Rabbit dermal LD_{50}, respectively.

2.2 Biomass

Biomass is plant residues left from agricultural process. There are various types of biomass which is locally available and inexpensive. Biomass if produce in large amounts is generally sent to factory to use as raw materials e.g. coconut husk used for mattress, peanut shell and rice husk for livestock feed. However, small amount of biomass is usually disposed as an agriculture waste.

Biomass have many useful purposes not only for industry, but also for agriculture, for instance; modify soil surface, decrease soil porosity, use as culture media, and etc. In this study, biomass will be used for pesticide sorption since it has high amounts of organic matter. The benefit of using biomass for pesticide sorption has not been studied in Thailand.

Rice husk is a by-product of rice milling. During the milling of paddy, about 78% is received as rice, broken rice and bran. The remaining 22% is received as husk which contains 38.43% of carbon (Assureira, 2002). Major components of rice husk which may be responsible for pesticide sorption are carbon and silica (Ahktar et al., 2005). This husk is used in many purposes such as fuel in the rice mills, fuel for household energy, animal food, soil amendment, plant growing medium, biofilter in gas treatment, and etc.

Philippine Coconut Authority (2005) reported the composition of coconut husks which are made of bristle fiber (10%), mattress fiber and coir dust (20%) and shorts or wastes (70%) They are used for making brushes, doormats, carpets, bags, ropes, yarn fishing nets, and mattresses, etc. It is also suitable for making pulp and paper, etc.

Peat moss is termed for partially decomposed organic matter that has accumulated in a moist environment. Different types of peat moss vary in their degree of decomposition. Plant species, climate, and quality of water affect the distinct characteristics of peat moss. It is used as a growing medium. The peat was a sphagnum moss peat and an element analysis was carried out; the results, on a dry basis, are carbon, 57.2%; hydrogen 5.7%; oxygen 36.0%; nitrogen 0.7%; and sulphur 0.4%. The BET surface area was determined to be 26.5 m^2 g^{-1} and the pore volume 0.73×10^{-6} m^3 g^{-1}. The absolute density was measured in paraffin oil and was found to be 1220 kg m^{-3}(Ho and Makay, 2000).

Peanut Shell was found to contain 34.56% lignin, 39.42% cellulose, 73.98% acid detergent fiber and 86.16% neutral detergent fiber. The hulls bound 2-3g HOH/g sample, exchanged 1.55 meq cations/g sample, and bound 2.28 + 0.87 µM sodium taurocholate/ 100 mg sample (Childs and Abajian, 1976).

2.2.1 Sorption

For the last decades, sorption of contaminants by sorbents of natural origin has gained important credibility due to the good performance and low cost of these complex materials (Chubar et al., 2003; Domingues et al., 2005). Biomass is a by-product from agricultural process, which is widely generated in Thailand. Utilization of agriculture biomass as exogenous organic matter is being used as an alternative method for disposal. Organic carbon or organic matter is one of key parameters in the sorption and degradation process of pesticides in soils (Dennis et al., 2004). According to the high organic carbon content, biomass show to be a good sorbent. The amendment of soil with biomass not only promotes sorption of the pesticides and retards their movement, but also enhances biodegradation of the pesticides by increasing soil microbial activity (Cox et al., 1997; Perrin-Ganier et al., 2001; Albarrain et al., 2004; Vischetti et al., 2004). However, the application of different biomass materials may have different effects on fate of certain pesticide.

2.2.2 Leaching

The major transfer process in this study is leaching, which is a physical process that describe transfer behavior of pesticides whereby the applied pesticides are moved from the surface through soil column and finally to groundwater. Biomass from agriculture processes can be used as sorbents for contaminant removal from the environment i.e. air, water and soil due to its high organic matter content. The organic matter content of soil increases retention of pesticide on soil particles, thus, leaching of pesticide in soil profile decreases (Graber et al., 1997; Singh, 2003; Majumda and Singh, 2007). Many researchers chose biomass in treatment process because they are abundant in the area, inexpensive and can be easily degraded in the environment afterward. Normally, biomass is used as soil amendment or air/wastewater filter for pollutant removal.

Soil amendment by organic material, commonly used to increase the amount of organic matter in soil, modify surfaces of soils and subsurface materials, increase porosity, decrease bulk density, increase sorption potential, and reduce pesticide contamination of groundwater (Zsolnay, 1992; Barriuso et al., 1996; Cox et al., 2001). Soil amendment also affects pesticide binding, which can affect pesticide transport and ultimate distribution in the soil profile (Senesi et al., 1997). For example, Cox et al. (2001) studied the effect of exogenous carbon materials, including compost from solid waste of the olive mill process, the corresponding liquid residue and compost from municipal waste on the movement of simazine and 2,4-D in sandy soil. They reported that herbicides sorption coefficient (K_D) was increased in the amended soil. Leaching studies indicated that further degradation affects movement to higher extent than sorption. Albárran et al. (2004) investigated the same herbicide in sandy loam soil amend with solid residue from olive-oil extraction. They found that simazine sorption increased after residue addition to soil. The sorption coefficients are 0.94, 1.69 and 2.34 mg l^{-1} in unamended, 5% amended and 10% amended respectively. In addition, breakthrough curves of simazine in handpacked soil columns confirmed the results that residue addition retarded the vertical movement of the herbicide through the soil and greatly reduced the amount of herbicide available for leaching. Selim et al. (2003) reported the significant amounts of herbicides such as atrazine, metribuzin and pendimethalin from mulch residue higher retained than soil. The presence of

mulch residue on the sugarcane rows was also minimizing run off of applied herbicides. The residue reduce run off – effluent concentrations as much as 50%. Moreover, loss of atrazine and pendimethalin in surface soil led to the lower decay rate in the presence of mulch residue. Singh (2003) showed that both cow manure and urea fertilizers amendments increase metolachlor sorption in soils and these amendments also reduced leaching losses. The sewage sludge in soil amendment was investigated by Graber et al. (2001). They found that atrazine, terbuthylazine and brommacil sorption was increased, and transport was retarded under sludge amendment condition. Yang et al. (2005) found that the presence of 1% of wheat char in soil resulted in a 7-80 times higher diuron sorption. Majumdar and Singh (2006) reported the effect of organic manure and fly ash amendments on metribuzin downward mobility in sandy loam soil columns. The Study indicated that both animal manure and fly ash were quite effective in reducing the downward mobility of metribuzin in packed soil columns of a sandy loam soil.

Another purpose of biomass is to remove pollutants from air and water. For example, Vischetti et al. (2004) used vine branch, citrus peel, and urban waste and public green compost for cleaning of water contaminated with pesticides. They concluded that biomass may be used as biofilter to reduce environmental contamination of pesticides. Adachi et al. (2001) used rice bran as an adsorbent and found that it is an efficient and cost-effective method for removal of organochlorine compounds and benzene from wastewater. Akhtar et al. (2005) also used rice bran to remove volatile organic compounds (VOCs), including benzene, toluene, ethylbenzene, and xylene (BTEX). Low cost agricultural waste such as rice bran, bagasse fly ash, *M. oleifera* pods, and rice husk can be effectively used to remove methyl parathion pesticide from water in the range of 70–90% (Akhtar et al., 2007). Khan et al. (2004) reported that the oil sorption ability of some biomass sorbents for water runoff (e.g. kapok fiber, cattail fiber, and *Salivinia* sp.) was not much different from the commercial sorbent (polyester fiber). Ho et al. (2005) studied the efficiency of sugarcane dust on removal of basic dyes from aqueous solution. The results revealed the potential of sugarcane dust, a waste material, as a low cost sorbent. While, five agricultural by-products available in Latin America including peanut shells, rice husk, coconut shells, cane baggasse and maize stubble can be used as biofilter for the treatment of polluted gas (López et al., 2003). Brás et al. (2005)

reported the sorption of pentachlorophenol on pine bark. Viraraghavan and Slough (1999) investigated the sorptive characteristics of pentachlorophenol on peat and bentonite mixtures. They concluded that peat-bentonite mixtures can be used to successfully remove pentachlorophenol from aqueous media and can be used effectively as a barrier to attenuate the migration of pentachlorophenol through soil and groundwater systems. Moreno et al. (2007) studied the application of two different rates (2 and 8% w/w) of olive cake to a Mediterranean calcareous soil and found an increased sorption of four triazine herbicides in soil.

From the previous studies, biomass has been applied as both soil amendment and pollutant filtered materials. Meanwhile, each biomass has different amounts of organic content and physical properties, which may differently influence the sorption and mobility of certain pollutants. The prediction of fate and transport of any pesticides in biomass bed have never been studied, thus prevent its application for agriculture area where various pesticides may be used. This study therefore used biomass locally available to study its efficiency in OPs sorption and the possibility of biomass application in Thailand.

2.2.3 Biodegradation

Organic amendment is generally added to soil surface to stimulate soil microbial activity, which could potentially lead to the accelerated degradation of pesticides and thereby reducing the total amount of chemical available for leaching or injuring crops (Felsot and Dzantor, 1995; Topp et al., 1996; Cox et al., 2001). According to the increase organic matter, microbial degrading activities will increase. For instance, Sanchez et al. (2004) investigated the influence of sewage sludge on ghd degradation of organophosphate pesticides. They observed the higher the organic matter content in sludge, the more rapid degradation of organophosphate pesticides such as fenittrothion and dimethoate. Vischetti et al. (2004) studied the degradation of chlorpyrifos, metalaxyl and imazamox in reactors filled with differing mixtures of vine-branch, citrus peel, urban waste and public green compost. The results showed that higher amount of organic content contributed to faster pesticide degradation in soil, especially when compared to the published values. The half life of all modeled pesticides in reactor was less than 14 days, compared to literature values of 60-70

days in soil. Sun et al. (2004) studied the degradation of aldicarb in sterile, non-sterile and plant-grown soils and the capability of different plant species to accumulate the pesticide. They found that pesticide degradation in soil followed first-order kinetics. Half-lives ($t_{1/2}$) of aldicarb in non-sterile soil were shorter than sterile soil, which indicated that microorganisms played important role in degradation. Plant uptake also enhanced removal aldicarb from soil due to plant-promoted degradation in the rhizosphere. Cox et al. (2001) also showed that half-life of Simazine was reduced upon amendment, mixed soil with compost from olive-mill process and compost from municipal waste.

2.2.4 Decomposition

Decomposition is the natural process of dead animal or plant tissue being rotted or broken down. This process is carried out by invertebrates, fungi and bacteria. The result of decomposition is that the building blocks required for life can be recycled. In turn, this will also decompose, eventually returning nutrients to the soil (Offwell Woodland & Wildlife Trust, 2007). When organic materials decompose in the presence of oxygen, the process is called "aerobic." The aerobic process is most common in nature. In aerobic decomposition, living organisms, which use oxygen, feed upon the organic matter. They use the nitrogen, phosphorus, some of the carbon, and other required nutrients. Much of the carbon serves as a source of energy for the organisms and is burned up and respired as carbon dioxide ($C0_2$). Since carbon serves both as a source of energy and as an element in the cell protoplasm, much more carbon than nitrogen is needed. Generally about two-thirds of carbon is respired as $C0_2$, while the other third is combined with nitrogen in the living cells. However, if the excess of carbon over nitrogen (C:N ratio) in organic materials being decomposed is too great, biological activity diminishes. Several cycles of organisms are then required to burn most of the carbon. When the ratio of available carbon to available nitrogen is in sufficient balance, nitrogen is released as ammonia. Under favorable conditions, some ammonia may oxidize to nitrate. Phosphorus, potash, and various micro-nutrients are also essential for biological growth.

CHAPTER III

EXPERIMENTAL PROCEDURE

3.1 Scheme for the overall experiments

The experiments are designed as shown on Figure 3.1. The strategies to obtain the objectives of the study are shown on Figure 3.2.

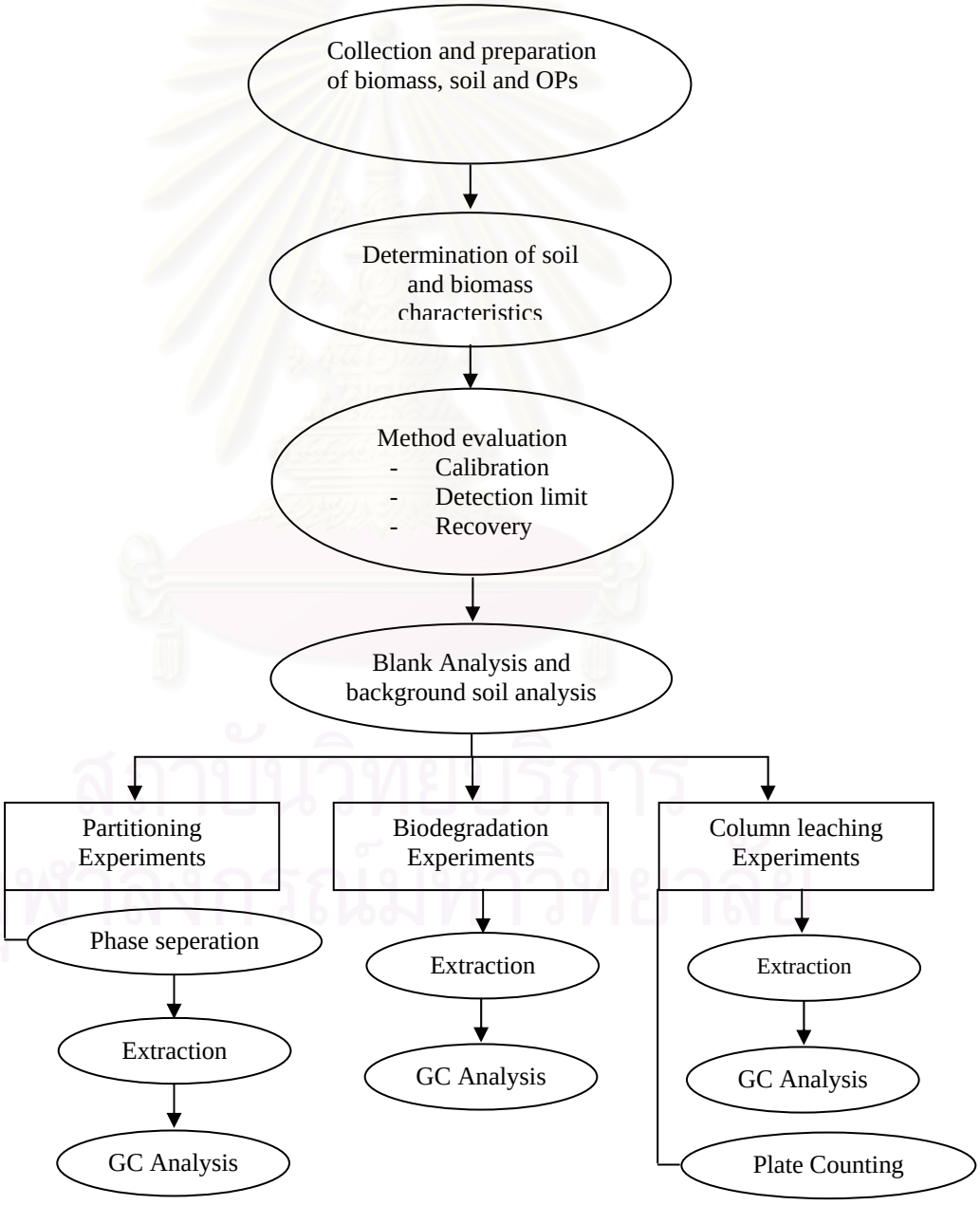


Figure 3.1 Scheme of the overall experimental procedure

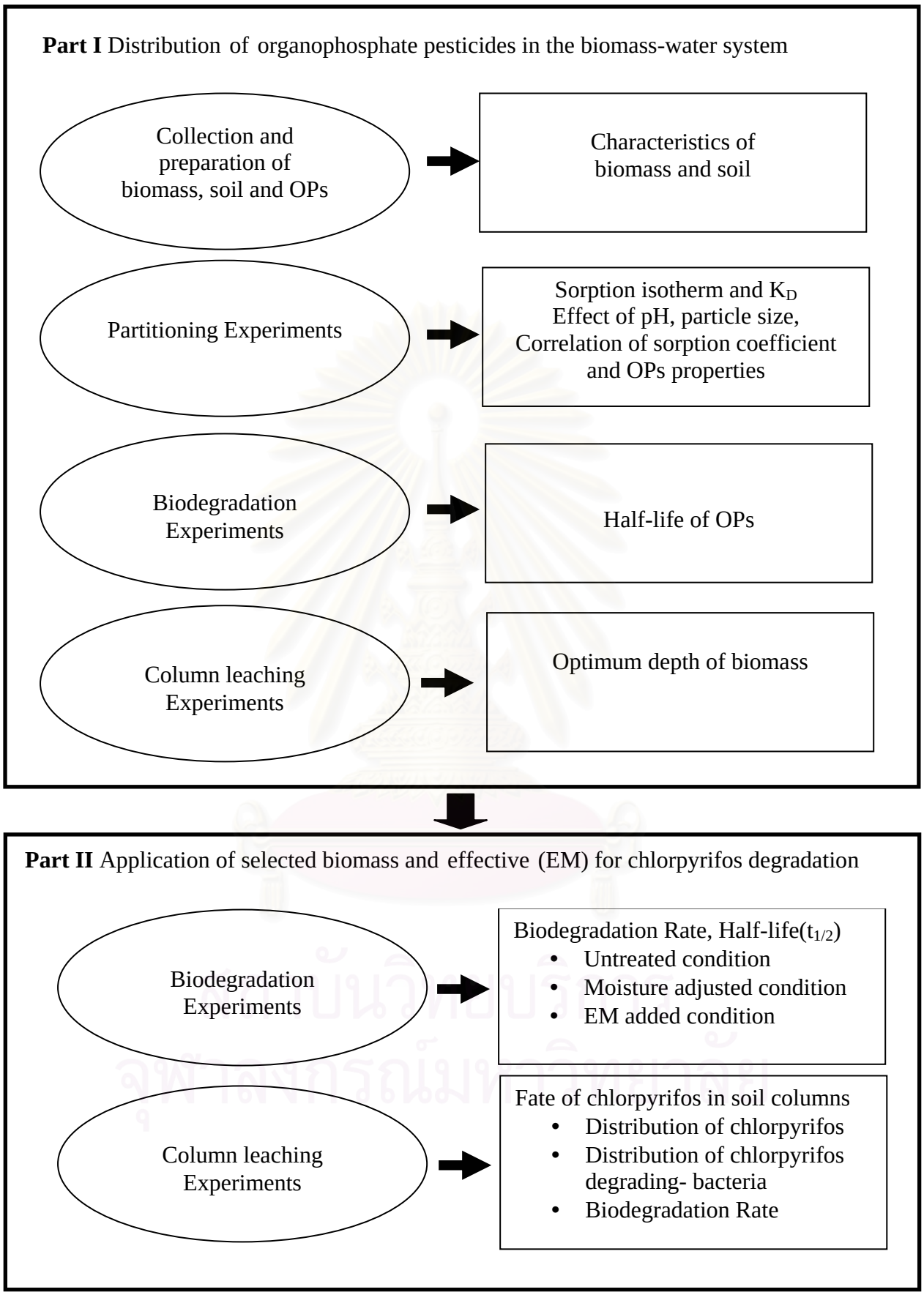


Figure 3.2 Strategies for the experiments

3.2 Materials

3.2.1. Biomass

Four types of biomass were employed, namely coconut husk (CH), rice husk (RH), peat moss (PM) and peanut shell (PS) (see APPENDIX C). They were purchased in one batch from a plant and fertilizer section available in supermarkets in Thailand. They were air dried, sieved through 500 μm mesh and stored in sealed plastic containers at room temperature. One sample of soil was collected from a tangerine orchard at Mae Ai, Chiangmai province. The top 15 cm of the soil was collected by using a shovel and sample scoop and stored in plastic bag. Stones and debris were removed from soil, and the remaining soil was sieved through mesh and ≤500 μm soil was obtained. Moreover the biomass and soil were ground and sieved to 4 sizes and the biomass/soil sizes obtained were ≤25, ≤125, ≤250 and ≤500 μm used for the dermination of particle size influence on sorption capacity.

Table 3.1 Properties of biomass and soil

	Coconut husk (CH)	Rice Husk (RH)	Peat moss (PM)	Peanut Shell (PS)	Soil (S)
Organic carbon (% dry weight)	45.39	35.44	49.42	41.61	1.29
Organic Matter (% dry weight)	78.62	61.10	85.20	70.73	2.22
Nitrogen (%)	4.63	4.01	4.77	3.42	0.07
Phosphorus (ppm)	369	118	310	373	229
C:N ratio	12:1	9:1	10:1	12:1	18:1
pH	5.30	6.30	5.20	5.50	4.40
Surface area ($m^2\ g^{-1}$)	17.77	1.89	6.98	4.80	7.69
CEC ($cmol_c kg^{-1}$)	34.5	9.3	51.1	51	5.3
Price kg^{-1} ($)	0.25	0.25	1.25	0.25	none

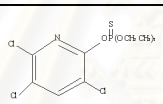
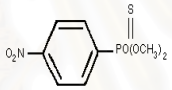
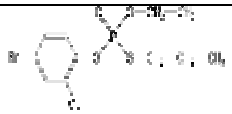
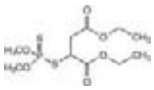
The biomass and soil of the size of ≤500 μm were analyzed for OC (wet oxidation method), pH (soil: water 1:1), N (Carlo Erba Combustion Method), P (Olsen Method), K (Sulfuric Acid Extraction), cation exchange capacity (Ammonium saturation method) and specific surface area (Quantachrome, Autosorb-1) (Table 3.1).

The results show the percentage of organic carbon (OC) in biomass with the same particle size in the range of 35.44-49.42 while the soil is of 1.29. PM has highest OC followed with CH (45.39), PS (41.61) and RH (35.44), respectively. Organic matter is important since it binds soil particles together into stable aggregates which are necessary for soil structural stability. Organic matter is 1.723 times of OC. It is also involved in sorption of cations from solution. The biomass exhibits acidic as well as the soil in water. The pH is an important chemical property because it affects the availability of nutrients and the activity of microorganisms in the soil. The most favorable range in organic soils is pH 5.4 to 6.2 (Communication and Educational Technology Services, 2004). Apparently, CH has highest surface area (17.77 m^2 g^{-1}) whereas RH shown the least (1.89 m^2 g^{-1}) because CH has porous characteristic. PM and S have similar surface area in one gram. C:N ratio indicate nutrient availability because carbon and nitrogen are both necessary for microbial growth. Organic carbon (which makes up about 50 percent of the mass of microbial cells) provides both an energy source and a basic cellular building block (www.Digitalseed.com, 1998). C:N ratio of biomass range is of 9:1-12:1 as compared to soil (18:1). Noticeably, CH and PS have the same C: N ratio (12:1) and close to the optimum ratio in soil organic matter is about 10 carbons to 1 nitrogen, or a C:N ratio of 10:1 (Washington University, 2007). C:N at lower ratios, nitrogen will be supplied in excess and will be lost as ammonia gas, causing undesirable odors. Higher ratios mean that there is not sufficient nitrogen for optimal growth of the microbial populations, so degradation will proceed at a slow rate. C.E.C indicates the amount of negative charge in biomass and soil that is available to bind positively charged ions (cations) from solution. C.E.C. is shown the highest (~51 $cmol_c kg^{-1}$) in PM and PS. This is as a result of the presence of negative charge in organic matter. Thus the higher organic matter content, the higher the C.E.C.(University of Minnesota Extension, 2004).

3.2.2 Test compounds and internal standard

Test compounds were 99% Chlorpyrifos (O,O-diethyl O-3,5,6-trichloro-2-pyridinyl phosphorothioate), 99.2% Malathion (O,O-dimethylS-[1,2-bis(ethoxycarbonyl)ethyl] dimethylphosphorothiolothionate,), 98% Methyl Parathion (O,O-dimethylO-(4-nitrophenyl) phosphorothioate) and 95.5% Profenofos (0-4-bromo-2-chlorophenyl-0-ethyl-s-propyl phosphorothioate). These were purchased from Chem Service Inc., U.S.A. as was 98.2 % 2-Fluorobiphenyl used as internal standard. The relevant physicochemical properties of the test compounds are shown on Table 3.2.

Table 3.2 Relevant physicochemical properties of OPs in this study.

Properties	Chlopyrifos	Methyl Parathion	Profenofos	Malathion
Molecular Formula	$C_9H_{11}Cl_3NO_3PS$	$C_8H_{10}NO_5PS$	$C_{11}H_{15}BrClO_3PS$	$C_{10}H_{19}O_6PS_2$
Molecular Structure				
log Koc	3.78[a]	3.47[b]	3.79[c]	2.84[b]
Molecular Weight (g/mol):	350.58	263.2	363.36	330.35
Water Solubility (mg/L)	2	60	28	130
log Kow	4.30[d]	3.40[e]	4.82[f]	2.84[g]
Vapor Pressure(Pa at 25ºC)	0.0025[h]	0.002[i]	$9X10^{-7}$[j]	$5.3x10^{-3}$[k]
Hydrolysis Half-life at pH 7, days	35[l]	40[m]	24-62[n]	6-7[o]

Source: http: www.pesticideinfo.org., 2004

[a] Dowed et al. 1993, [b] Karickhoff 1981, [c] Lyman 2004, [d] Sicbaldi and Finizio 1993, [e] Finizio et al.1997, [f] Denish EPA, 2004 , [g] Patil, 1994 , [h] Montgomery 1993, [i] Halfon et al. 1996, [j] US EPA, 2007 [k] Tomlin,1994, [l] Deer and Beard 2001, [m] US EPA, 2003, [n] US EPA, 1998, [o] ATSDR,2001

Commercial chlorpyrifos (40% w/v) purchased from Rojpanakij Co., Ltd. was applied to column leaching experiments for studying test compounds distribution in the biomass and soil.

Log Kow which indicates compounds capacity to sorb in the octanol relative to water shows that profenofos, chlorpyrifos, methyl parathion and malathion have the values of 4.82, 4.30, 3.40 and 2.84, respectively. Log Kow is also considered as one of the most important physicochemical characteristics related to sorption on soil since the soil sorption behaviour is similar to the octanol water partition behavior (Jailuk, 2003).

3.2.3 Chemicals

Chemicals used in the experiment were haxane, acetone, N,N-dimethylformamide, sodiumsulphate (Na_2SO_4), calciumchloride ($CaCl_2$), sodium hydroxide (NaOH), sulfuric acid (H_2SO_4), phosphate buffer pH 7, acetate buffer pH 5, acetate buffer pH 3. The pH selection was due to normal pH 7 and acid rain condition at pH 3 and 5. All chemicals were analytical grade.

3.2.4 Effective Microorganisms (EM)

EM is a mixed culture of beneficial microorganisms used to create a favourable microbiological environment (Lindros Whole earth consultants, 2006). EM solution was purchased from Kyosei Factory (Thailand) Co., Ltd. EM contains about 3 families of micro-organisms, which can be divided into the following groups: photosynthetic microorganisms, yeasts and lactic acid bacteria.

3.2.5 Glassware

Beaker, Duran glass bottle, weighing funnel, erlenmeyer flask, glass column, glass funnel, 22 ml. glass bottle with screw cap, glass plate, cylinder, glass funnel, syringe, GC vial, insert vial.

3.2.6 Equipment

Platform shaker (GFL 3017), pH meter, grounding machine, magnetic stirrer, hot plate, cartridge pump (Masterflex ®L/S™ Model 7519-15), analytical balance, sieve analyzer, and gas chromatography with μECD (Agilent Technologies 6890N). The use of μECD detector was selected due to its more sensitive 10-1,000 times than the FID (Library 4 Science, 2007). The μECD detector uses a beta particle emitter (electrons) to ionize the GC carrier gas. Organic molecules containing electronegative functional groups, including nitrogen groups, halogens, phosphorous, can be detected by this detector (Intertek group, 2007).

3.3 Method evaluation

3.3.1 Contamination and blank procedure

All biomass, soil and water samples were checked for any OPs contamination prior to use. Three blank analyses were carried out in each biomass, soil and water analyses. Blank analyses were determined in the same manner as the sample (section 3.4.1) determination, but without the tested OPs.

Two grams of biomass, 20 g of soil and 20 ml of water were used for contamination and interference analysis. Then the samples were extracted and prepared for GC analysis. The results were found that there were no tested OPs in the samples.

3.3.2 Calibration curves of OPs

The calibration curves were developed using six concentrations in solvent (hexane:acetone at ratio 8:2) of OPs (methyl parathion, malathion, chlorpyrifos and profenofos). A certain concentration of internal standard was also added. The compound peak areas over internal standard peak area were plotted against the compound concentrations and the linear relationship are obtained (see Appendix B). The slope, y-intercept and r^2 of each compound were presented in Table 3.3.

Table 3.3 The slopes, y-intercepts and correlation coefficients of the linear regression equations derived from the relationships between peak ratio and the concentrations

Test compounds	Slope	Y-intercept	r^2
Methyl parathion	4.0092	-10.955	0.99
Malathion	1.2856	-2.3207	0.99
Chlorpyrifos	6.0435	+0.5452	0.99
Profenofos	6.7584	-10.115	0.99

3.3.3 Recovery of OPs

Recoveries of OPs were determined and repeated three times to determine the extraction efficiencies. Percent recovery was determined according to the following.

$$\text{Percent recovery} = \frac{\text{Amount recovered x100}}{\text{Original amount spiked}}$$

As the results of the recovery of OPs in water, biomass and soil were all above approximately 90%.

3.3.4 Detection limit

The detection limit was determined by injection various concentrations of test compounds. The results obtained reflect the minimum level at which the analyte can be reliably detected by GC. The standard deviation (σ) was determined from triplicate analyses of each compound. The equation used for determination of the limit of detection is as follows:

$$\text{Detection limit} = \frac{2 \text{ x initial concentration x } \sigma}{\text{Average signal detected in GC}}$$

The results of detection limit were determined and reported in Table 3.4.

Table 3.4 Detection limits of the experimental procedure

Test compounds	Detection limit (μg l^{-1})
Methyl parathion	4.8
Malathion	2.0
Chlorpyrifos	0.5
Profenofos	1.2

3.4 Methods

3.4.1 Extraction

3.4.1.1 Extraction of water

Solvent extraction was used to extract test compounds from samples. This method is modified from extraction of organochlorine pesticides in soil and water (Kraijitmet, 2004). The solvent here is a mixture of hexane and acetone at 8:2 ratio. For water samples, 20 ml solution samples were transferred into a new 125 ml flask and then 20 ml solvent were added. The samples were mixed and shaken at 250 rpm for 4 hrs. After that, the flasks were frozen at -4°C to solidify the lower aqueous layer, and then solvent were transferred to vial where 2-3 g of Na_2SO_4 were added to dewater the sample. The volume was reduced using a stream of 99.5% nitrogen. Then, the extracted samples were transferred to gas chromatography vials for analysis using gas chromatography equipped with an electron capture detector (GC-ECD).

3.4.1.2 Extraction of soil/biomass

For soil samples, 20 g soil samples were mixed with 40 ml hexane and acetone at 8:2 ratio and 5 ml 15% Triton x-100. For biomass samples, 2 g of biomass samples were mixed with 40 ml of solvent and 5 ml of 15% Triton x-100. The samples were mixed and shaken at 250 rpm for 4 hrs. After that, the flasks were frozen at -4°C to solidify the lower aqueous layer, and then solvent were transferred to vial where 2-3 g of Na_2SO_4 were added to dewater the sample. The volume was reduced using a stream of 99.5% nitrogen. Then, the extracted samples were transferred to gas

chromatography vials for analysis using gas chromatography equipped with an electron capture detector (GC-ECD).

3.4.2 Quantifications

The concentrates were quantified by gas chromatographic technique using Hewlett Packard 6890 equipped with an electron capture detector (ECD) and a HP-5 (5% Phenyl Methyl Siloxane), fused-silica capillary column (30 m x 0.32 mm ID x 0.25 µm). Optimal conditions employed are shown on Table 3.5.

Table 3.5 Optimal conditions of GC

Injector	Column	Temperature program	Detector
Injection volume: 2.0 µl Split mode: ratio 5:1 Carrier gas: Helium at 20 ml/min	HP-5 (5% Phenyl Methyl Siloxane) fused-silica capillary column: (30 m x 0.32 mm ID; thickness 0.25 µm)	Initail 120°C, 15°C/min to reach 270°C for 3 min then 40°C/min to reach 300°C for 3 min	µECD, 350°C Nitrogen make up gas at 60 ml/min

3.4.3 Plate counting

Procedure was shown as follows:

(1) Agar plates, mark on the underside of each dilution that were added to it. e.g. 10^{-1}, 10-^{2}, 10^{-3}, 10^{-4}, 10^{-5},10^{-6}.

(2) Dip the L-shaped glass rod into a beaker of ethanol and then tap the rod on the side of the beaker to remove any excess ethanol.

(3) Briefly pass the ethanol-soaked spreader through the flame to burn off the alcohol, and allow it to cool on the agar surface.

(4) Add 0.1ml of each particular dilution to agar plate, and spread evenly across the surface using a spreader.

(5) Spread the bacterial sample evenly over the agar surface with the sterilized spreader, making sure the entire surface of the plate has been covered.

(6) Immerse the spreader in ethanol, tap on the side of the beaker to remove any excess ethanol, and reflame.

(7) Invert the plates and incubate for 5 days at room temperature.

(8) After incubation, count some representative bacterial colonies.

3.4.4 Equilibrium attainment test

Trials on equilibrium attainment for the partitioning experiments between biomass/soil and water was carried out by batch shaking between soil or biomass and water at time periods of 1, 2, 4, 6, 12, 24 and 48 hours using 1 g biomass and 10 g soil at the highest working concentration of OPs (0.25 times S). Biomass which have high OC were selected, i.e. CH and PM. The water extracts were analysed for the concentration of OPs. Equilibrium was reached within 48 hr (see Figure 3.3).

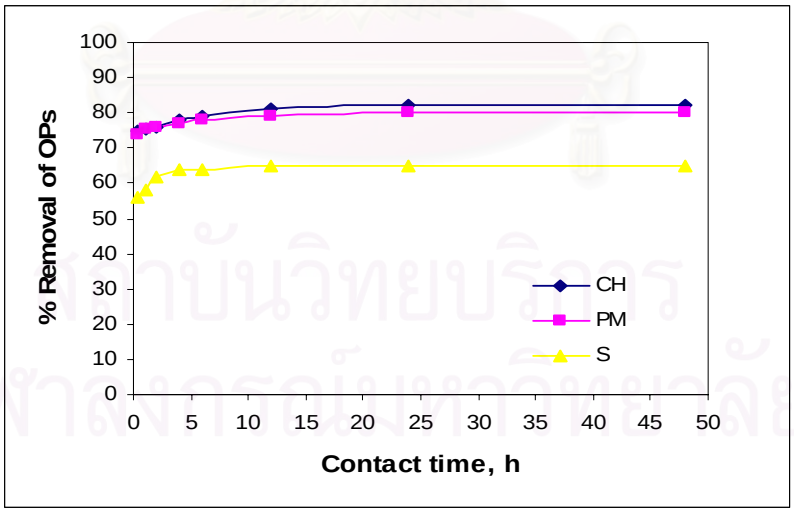
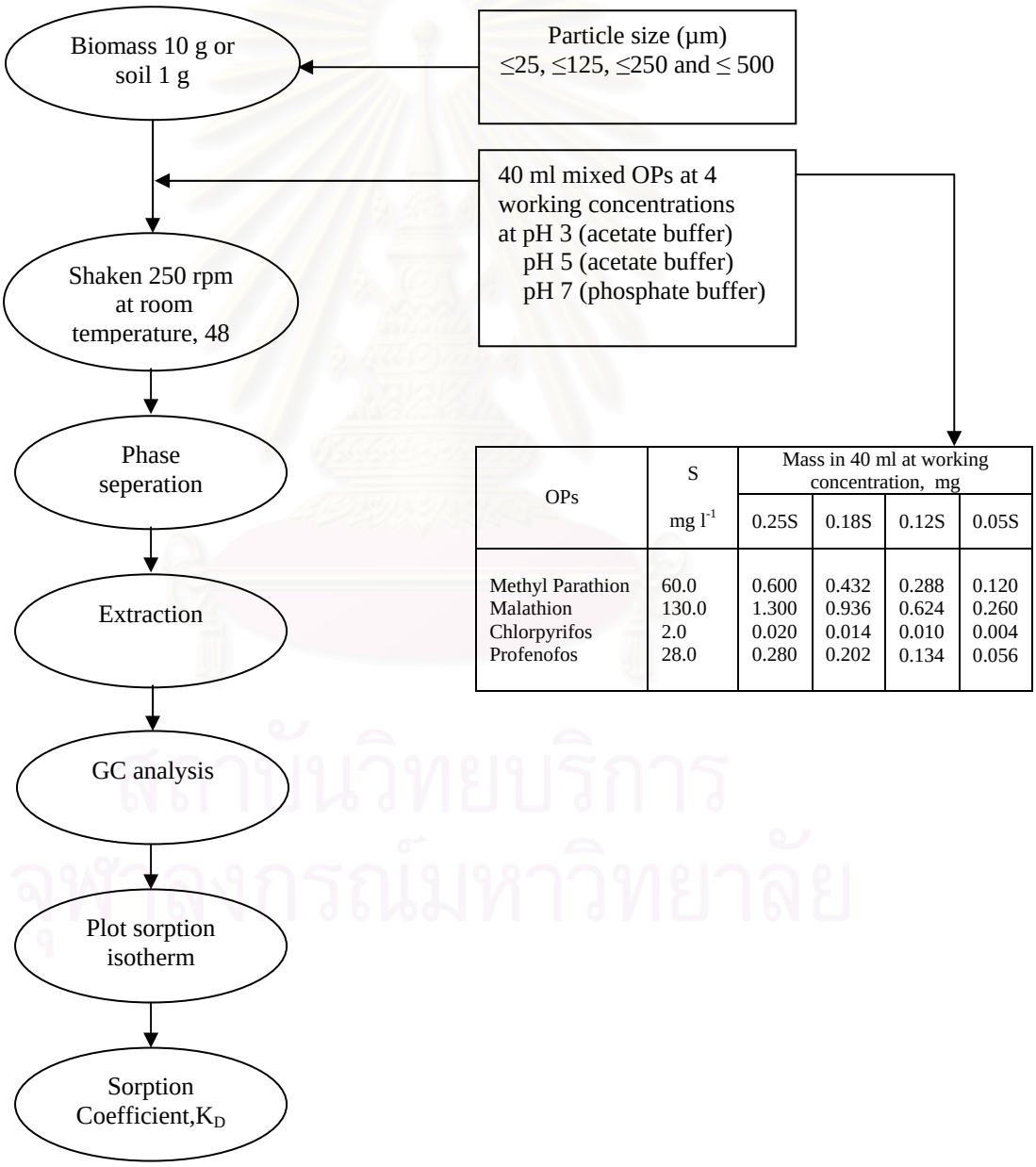


Figure 3.3 The equilibrium time of malathion in coconut husk (CH), peat moss (PM) and soil (S)

3.4.5 Partitioning Experiments

Mixed OP solutions were partitioned with 1 g biomass (in case of soil, 10 g used) using 4 working concentrations of compounds (0.05, 0.12, 0.18 and 0.25 times the maximum aqueous solubility of the test compounds) in Erlenmayer flasks under pH adjusted by the buffer at room temperature (see Figure 3.4). The flasks were shaken on Platform shaker. The pH of the solution remained constant even after 48 hr. Biomass was used in lesser amount due to its higher OC as compared to soil.



OPs	S mg l^{-1}	Mass in 40 ml at working concentration, mg			
		0.25S	0.18S	0.12S	0.05S
Methyl Parathion	60.0	0.600	0.432	0.288	0.120
Malathion	130.0	1.300	0.936	0.624	0.260
Chlorpyrifos	2.0	0.020	0.014	0.010	0.004
Profenofos	28.0	0.280	0.202	0.134	0.056

Figure 3.4 Diagram of partitioning experiments

According to equilibrium attainment test, the experiments were operated at 48 hrs. Differences between initial OPs concentration (Ci) and equilibrium OPs concentration (Ce) in OPs solution were assumed to be the amounts sorbed by biomass or soil. Sorption isotherms were fit to the linear equation and the distribution coefficients, K_D for each OP in biomass or soil were calculated from the concentration of test compounds in biomass or soil and concentration of solution at equilibrium state. Each partitioning experiments was carried out in triplicate. Additionally, partitioning experiments at pH 3 and 5 were also conducted using buffer solution.

3.4.6 Biodegradation experiments

3.4.6.1 Determination of compound half-life

Portions of 5 g of soil and 0.5 g each biomass at optimum particle size were packed and adjusted moisture at 70% of water holding capacity in 22 ml vial to make soil microcosm. OPs were spiked to give concentration of normal agricultural application rate of dry soil and biomass. The microcosms were maintained at room temperature. The change of OPs was monitored in soil and biomass from 0, 7, 14, 21, 28 and 35 days. OPs residues were extracted and determined by Gas Chromatography. Half-life of OPs under various conditions was investigated (see Figure 3.5).

3.4.6.2 Determination of EM capability on enhancing OP degradation

Portions of 5 g of soil or 0.5 g each biomass were packed in 22 ml vial to make soil microcosm. The three different conditions were set up into untreated, moisture adjusted and EM added. For untreated microcosms, the soil and biomass were used without adjusting moisture content. The rest of microcosms were adjusted moisture at 70% of water holding capacity by adding deionized water for moisture adjusted condition and EM solution for EM added condition. EM solution was prepared according to the manufacturer (Kyosei Co. Ltd.). Then, chlorpyrifos was spiked to all microcosms to give a concentration of normal agricultural application rate (0.25 kg ha^{-1}) or 500 ppm. The microcosms were maintained at room temperature. The changes in amount of chlorpyrifos were monitored in soil and biomass after 0, 7, 14, 21, and 28 days. Chlorpyrifos residues were extracted and

determined by Gas Chromatography. Half-life of chlorpyrifos was calculated by kinetic equation (see Figure 3.6).

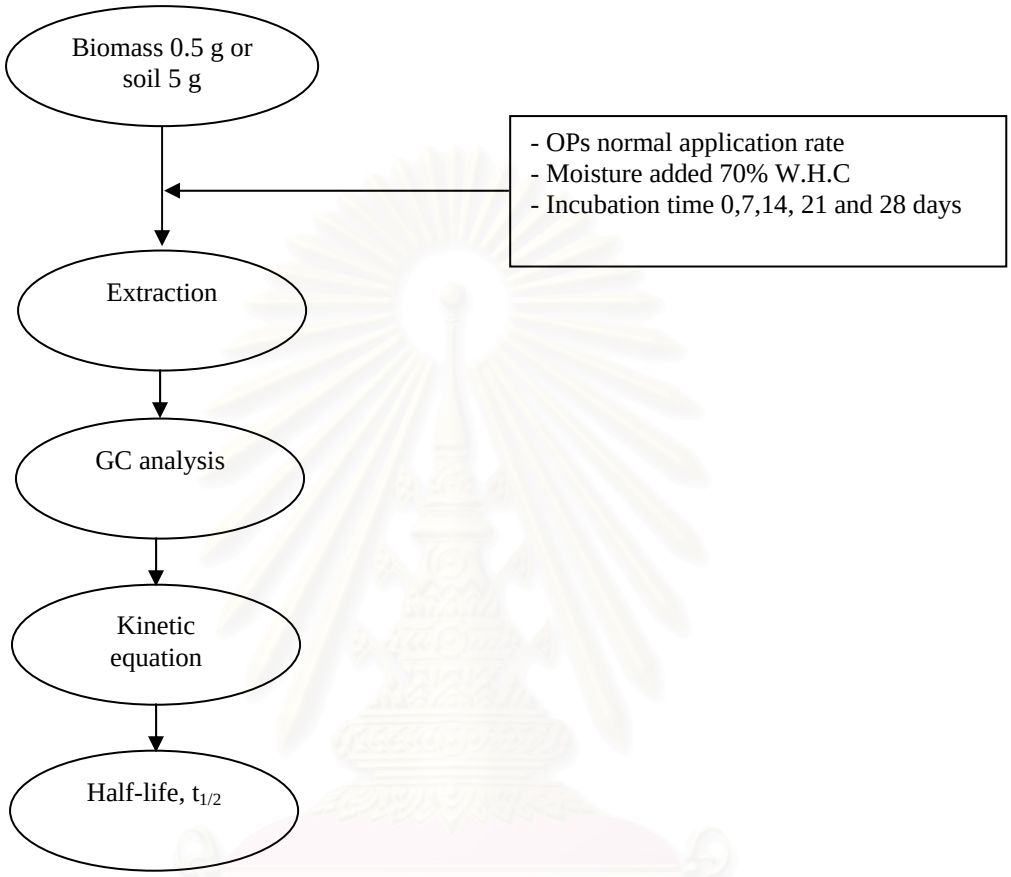


Figure 3.5 Diagram of biodegradation experiments for determination of compound half-life

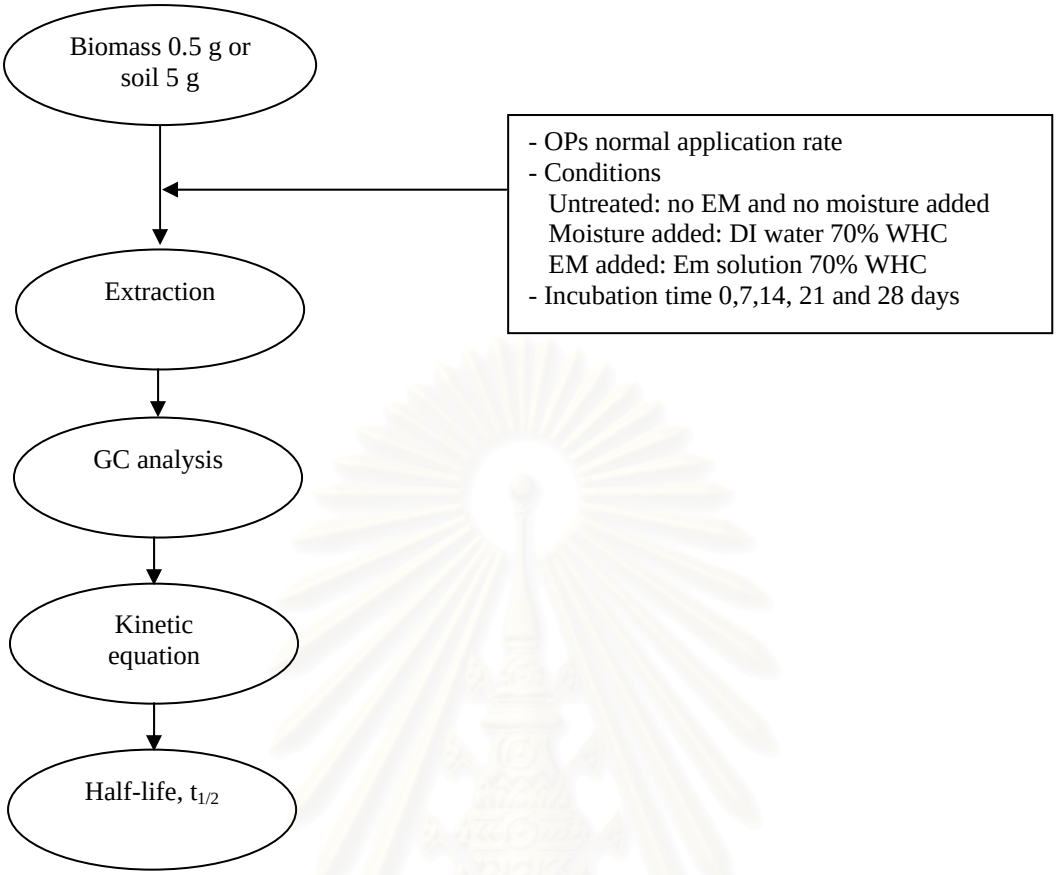


Figure 3.6 Diagram of biodegradation experiments for determination of EM capability on enhancing OP degradation

3.4.7 Column leaching experiments

3.4.7.1 Optimum of biomassbed depth

PS and CH was selected due to their high sorption strength and low in cost. 3.4 g PS and 1.5 and 3 g CH were added to 3 separate glass columns (70 mm x 38.5 id mm) as shown in Figure 3.7. The columns were lined below with glass wool to avoid biomass leakage and small glass beads on top in order to distribute water evenly over the biomass surface. Generally, they were spiked with an OPs mixture comprising 0.0728, 0.0829, 0.0291, 0.116 mg methyl parathion, malathion, chlorpyrifos and profenofos respectively. These masses are equivalent to the actual application rates (0.625, 0.713, 0.25 and 1.0 kg ha^{-1}) of methyl parathion, malathion, chlorpyrifos and profenofos employed on a tangerine orchard in Thailand.

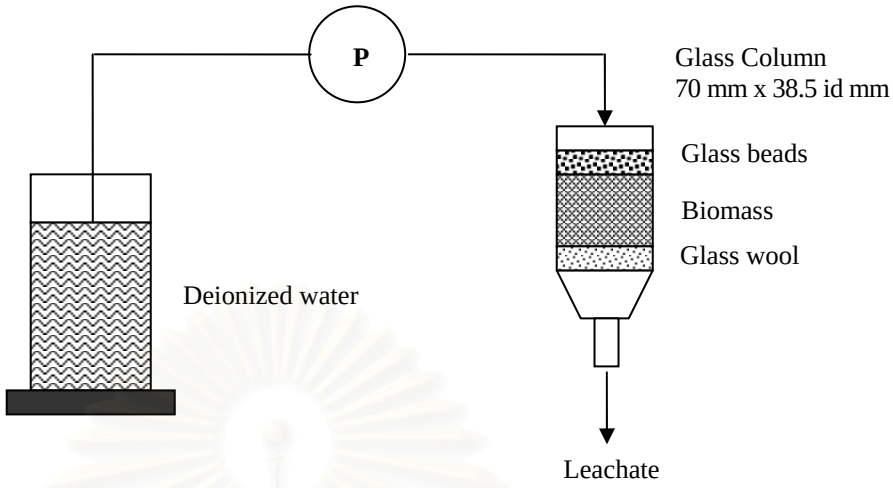


Figure 3.7 Diagram of leaching studies for the determination of optimum depth

The columns were then left 24 h before leaching. Deionized water was added at 20 ml h^{-1}, the current average watering regime in the orchard. Leachate collection started after about 30 ml drained out, and afterwards every pore volume until no sorbate occurred in the collected water. Following this, in the third column (3 g CH), OPs spiking and leachate collection was repeated.

3.4.7.2 Application of biomassbed

Soil columns were designed to determine the efficiency of selected biomass in minimizing chlorpyrifos leaching through soil column. Leaching behavior of chlorpyrifos in coconut husk and soil were investigated. The efficiency of EM inoculation on chlorpyrifos degradation was determined in coconut husk as well. The leaching studies were set into three treatments: (1) soil, (2) soil + biomass and (3) soil+biomass+EM. Soil columns (250 mm x 38.5 id mm) were prepared by connecting five 5-cm glass rings and sealed with parafilm and tape (Figure 3.8).

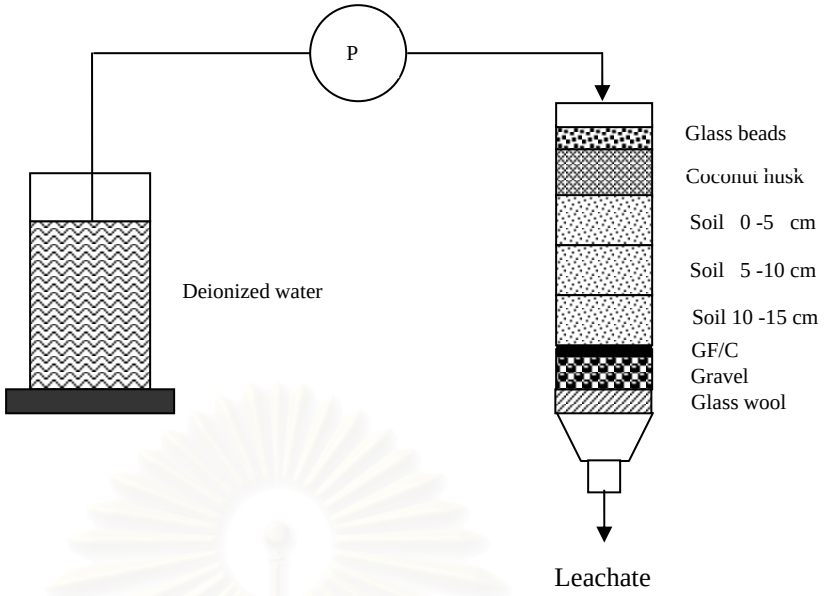


Figure 3.8 Diagram of leaching studies for determination for the application of biomass and effective microorganisms (EM)

The bottom ring was packed with gravel plus glass wool and GF/C, to minimize losses of biomass and soil via leachate. Then, 280 g of soil was packed to give a 15 cm depth according to its field moisture and density. The top ring of treatment (2) and (3) were covered with an optimum depth for retaining organophosphate pesticides in the biomass. One day before chlorpyrifos application, the columns were pretreated with 50 ml of deionized water for treatment (2) and EM solution for treatment (3) to minimize the variation in biomass water content between the columns. Treatment (1) was left without water or EM solution. All of columns were sprayed with chlorpyrifos at dose resembled to field application (0.25 kg ha^{-1}). Then, the columns were leached daily with deionized water at 40 ml hr^{-1} day^{-1} which is related to the normal agricultural application. The experiment was continued for 28 days. Columns were sacrificed and separated into certain depths periodically at 0, 7, 14, 21 and 28 days of leaching. The amount of chlorpyrifos in biomass, soil and leachate was monitored and determined by GC-ECD. Microbial numbers per gram soil were measured at the same time of sampling by spread plate technique (section 3.4.3) in mineral salt agar containing 100 mg l^{-1} chlorpyrifos. Colonies of bacteria were counted after 5 days. Consequently, half-life of chlorpyrifos at top layer of various columns was investigated by kinetics equation.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Distribution of organophosphate pesticides in the biomass-water system

Batch sorption studies were conducted to determine the distribution of OPs in the biomass-water system compare to sandy soil which collected from tangerine orchard in different pH at 3, 5 and 7. The obtained data may be used in models to predict the movement of OPs in the soil. The biomass is selected base on organic content including; coconut husk, peat moss, rice husk, and peanut shell.

4.1.1 Sorption isotherm

OPs in the aqueous solution were taken up into the biomass by binding to biomass surface via Van de Waals forces. The sorption isotherms of each OPs were determined over a range of concentrations (from 0.05 to 0.25 times the maximum aqueous solubility). At these concentrations, all sorption equilibrium data were fit with linear sorption isotherm (Figure 4.1). This conforms to the study of Chiou, 1989; Hamaker and Thomson, 1972; Karickhoff, 1984; Smith et al., 2003, that isotherm of nonionic organic compounds are often assumed to be linear. Particularly, nonionic compound in very low concentrations equivalent to 10^{-5} M or one-half the aqueous phase solubility (whichever is lower) and the organic content of the sorbent is greater than 0.1% (LaGregra et al., 2001; Smith et al., 2003; Suthersan, 2002).

4.1.1.1 The biomass-water sorption coefficients of OPs (K_D)

Sorption coefficients, K_D describe the extent to which an organic chemical distributes itself between an environmental solid and aqueous phase at equilibrium.

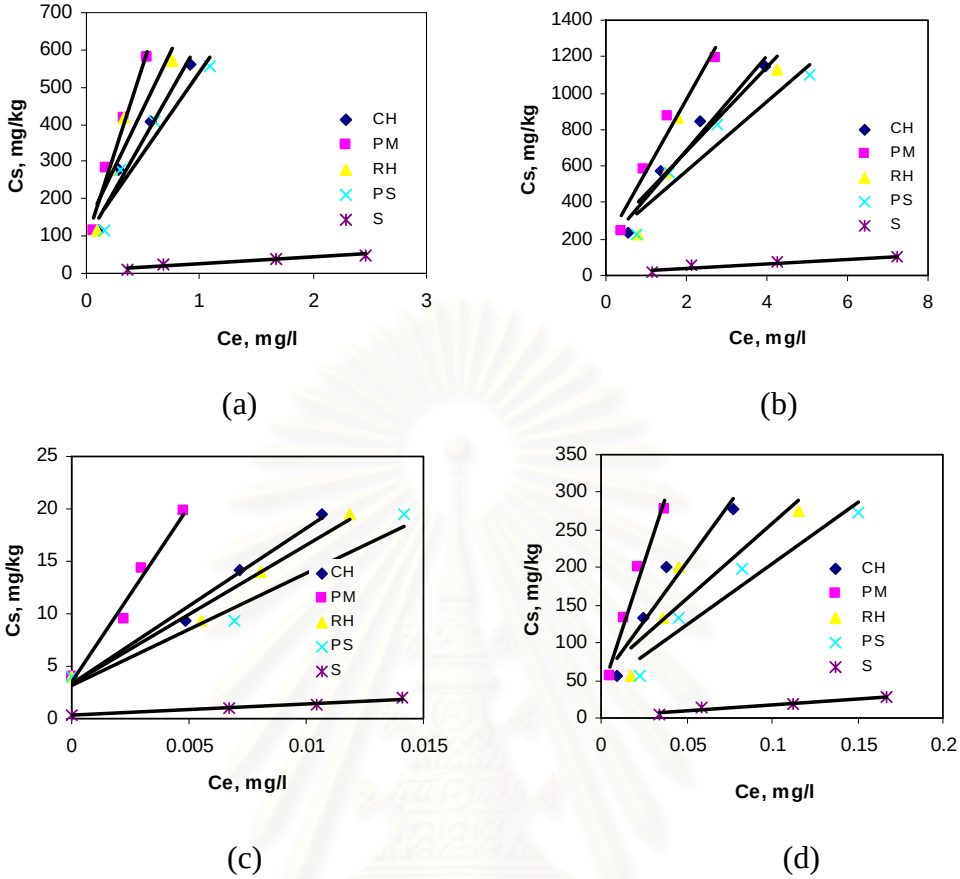


Figure 4.1 Sorption isotherm of sorbents for (a) methyl parathion (b) malathion (c) chlorpyrifos and (d) profenofos (Ce represents equilibrium aqueous concentrations and Cs represents OP concentrations in sorbents)

It is generally derived from the slope of the sorption isotherm at the contaminant concentration of interest (equation 4.1). K_D obtained in this study were shown in Table 4.1.

$$K_D = C_s C_w^{-1} \qquad (4.1)$$

Where, Cs and Cw are the concentrations of the chlorpyrifos sorbed by biomass or soil (mg kg^{-1}) and dissolved in aqueous phase (mg l^{-1}), respectively. Units of K_D are given as l kg^{-1}.

Table 4.1 Sorption Coefficient (K_D) of OPs in sorbents and soil at different pH

		Methyl parathion		Malathion		Chlorpyrifos		Profenofos	
	sorbents	K_D	r^2	K_D	r^2	K_D	r^2	K_D	r^2
pH 3	CH	480.0	0.98	190.4	0.69	1692.9	0.60	4770.6	0.92
	PM	914.8	0.96	371.4	0.83	1877.6	0.83	4929.0	0.75
	RH	342.4	0.96	181.0	0.63	954.8	0.55	2349.0	0.97
	PS	331.6	0.98	109.8	0.69	510.2	0.51	906.6	0.84
	Soil	16.4	0.86	9.3	0.89	118.4	0.92	138.9	0.86
pH 5	CH	417.0	0.88	196.8	0.68	959.5	0.94	2284.1	0.80
	PM	700.1	0.95	364.2	0.91	1865.6	0.90	6369.7	0.95
	RH	367.4	0.95	188.6	0.80	960.1	0.89	1146.4	0.86
	PS	314.3	0.91	131.8	0.79	951.4	0.87	1017.6	0.90
	Soil	8.9	0.89	7.5	0.85	143.7	0.93	98.7	0.94
pH 7	CH	532.1	0.98	259.1	0.97	1475.4	0.98	3116.5	0.93
	PM	957.0	0.98	393.5	0.96	3371.4	0.98	6942.1	0.98
	RH	644.3	0.88	230.5	0.80	1316.5	0.98	2020.1	0.84
	PS	440.2	0.93	190.4	0.93	1072.2	0.97	1613.9	0.94
	Soil	16.9	0.94	12.0	0.91	107.9	0.98	154.8	0.97

In all biomass types investigated, the K_D values of profenofos were the highest followed by chlorpyrifos, malathion and methyl parathion respectively. In term of biomass, the sorption increases in the order of PM>CH>RH>PS. Soil sorption obviously exhibits the lowest values.

Focusing on the biomass, while they have relatively high organic carbon contents (35.4-52.7 % dw), they do not sorb much as can be seen from their K_D values. This could be partly influenced by relative high aqueous solubility OP (2-130 mg l^{-1}) as a result of their molecular structures. In addition, hydrolysis of OPs is very limited at pH 7, and has no influence on the magnitude of the observed K_D value.

A paired t-test is applied to the values of K_D at pH 3, 5 and 7 (see APPENDIX D) to determine whether these sorptions differ from each other in a significant way. The result indicates that K_D values are not significantly different and thus independent of pH (acidic to neutral). This pH range as well as the short time period (24 h) for the determination of K_D in the laboratory permits very low hydrolysis. Other work by

Weber et al. (2004) is consistent with our study that K_D values are not related to soil pH for nonionizable pesticide families, including OPs. Liu et al. (2001) also suggested that chlorpyrifos in the sorbed state is much less susceptible to base-catalyzed hydrolysis. Nevertheless, Akhtar et al., 2006 investigated the sorption of methyl parathion by low cost sorbents (rice bran, bagasse fly ash, sugarcane pods and rice husk). They reported that pH plays an important role in the sorption onto these surfaces. Because pH affect the surface properties of the sorbent, i.e. surface charge of the cells present in the sorbent. At very low pH values, the surface of the sorbent would be surrounded by the hydronium ions, which may enhance the sorbate interaction with binding sites of the sorbents by greater attractive forces and hence improve its uptake on polar sorbent (Rengarag et al., 2002). On the other hand, Koleli et al (2006) who studied the sorption of methamidophos. They found methamidophos sorption to heterogeneous alluvial soils (OC 0.9-1.2%, pH 7.7-8.3) increases with increasing pH, and reach a maximum of 100% at around pH 11.5. Because methamidophos contains several reactive functional groups (e.g. P, O and NH_2) and the protonation of these groups may be responsible for the binding of methamidophos with organic matter and/or mineral surfaces in soils. An increase in sorption with increasing pH is usually indicative of sorption processes between positively charged ions (e.g. protonated amino groups) and metal oxides. Table 4.2 reported log Koc from this study compared to other researches. The variation from our study could be due to soil texture, OC and environmental conditions.

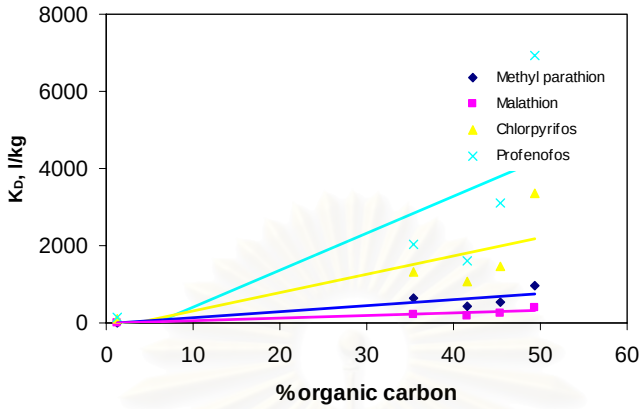
Table 4.2 The comparison of log Koc values from this study to previous studies

OPs	This study		Previous studies		
	Log Koc	sorbent	Log Koc	Sorbent	Ref
Methyl Parathion	3.12 3.26 3.02 3.07 3.29	Soil RH PS CH PM	3.71 3.00	Soil Soil	Hornsby et al (1996) González et al (2005)
Malathion	2.97 2.81 2.66 2.76 2.90	Soil RH PS CH PM	2.61 3.25	Lihue silty clay soil, pH 5.5, OC 2.66% Soil	Miles and Takashima (1991) Howard (1991)
Chlorpyrifos	3.92 3.57 3.41 3.51 3.83	Soil RH PS CH PM	3.93 3.77-4.13	Soil and sediment Soil	Racke (1993) Mongomory (1993)
Profenofos	4.08 3.76 3.59 3.84 4.15	Soil RH PS CH PM	3.79 3.30	Soil Soil	González et al (2005) Danish EPA, 2004

4.1.1.2 K_D in relation to biomass characteristics

Characteristics of biomass considered in this study are OC, surface area and particle size. K_D values for all OPs are correlated well with the OC of biomass (see Figure 4.2) and confirmed with other studies with soil (Weber et al., 2004, Rao and Davison, 1980; Jury et al., 1987; Vischetti et al., 2004). The K_D increased when organic carbon content increased. This relationship indicated that organic carbon played a major role in the sorption of organic chemicals in soil and biomass. Since organic carbon provides the greatest number of binding sites because it has extremely large surface area and is very reactive chemically (Huddleston, 1996). Brausseau (1995) also reported a positive linear correlation between sorption of non-ionic

organic chemicals and soil organic matter (OM) content. Table 4.2 reported log Koc from this study compare to other researches.



Methyl parathion ;	$K_D = 96.257(\%OC) - 563.890$	$r^2 = 0.53$
Malathion ;	$K_D = 47.271(\%OC) - 168.310$	$r^2 = 0.60$
Chlorpyrifos;	$K_D = 15.320(\%OC) - 12.4340$	$r^2 = 0.75$
Profenofos;	$K_D = 6.502(\%OC) - 8.063$	$r^2 = 0.83$

Figure 4.2 Relationships between sorption coefficients (K_D) and organic cabons in sorbents

Besides OC levels, the structure and composition of biomass components, i.e. cellulose, hemicellulose, and lignin, are involved partly in sorbing OPs since each has different binding forces to the same sorbates. As shown on Table 3.1. CH and PM which have OC of 45.4 and 49.4, respectively, CH exhibits less K_D values than PM although its surface area is higher than PM. However, deviated results occur to the RH and PS comparison. PS, though, has higher both OC and surface area as compared to RH shown less K_D values. This might be influenced by the faster decomposition of PS than RH. The results as shown on Figure 4.4 indicate the larger particle size, the lesser sorption are.

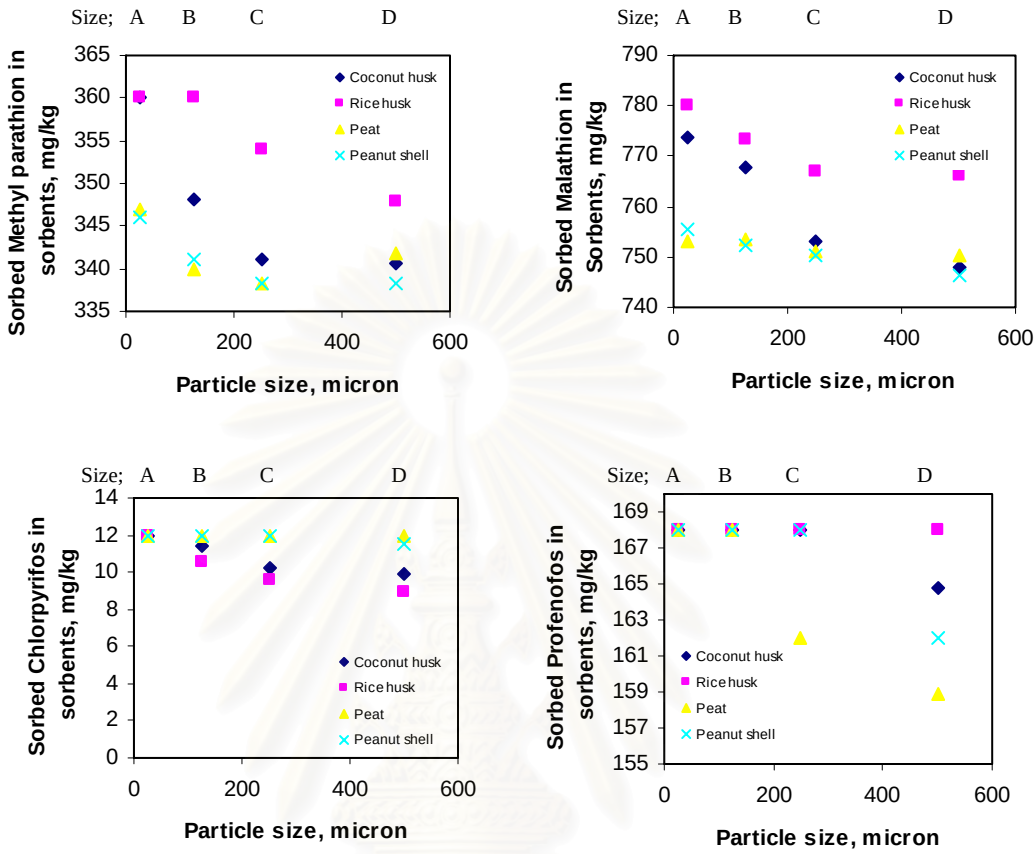
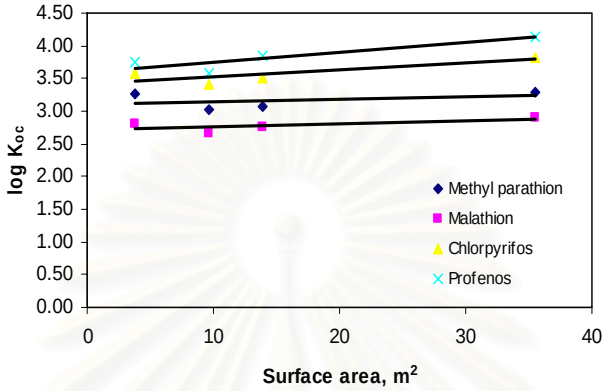


Figure 4.3 Sorption of the test compounds in relation to biomass particle sizes (a) methyl parathion (b) malathion (c) chlorpyrifos and (d) profenofos (size A ≤ 25µm, B ≤ 125µm, C ≤ 250µm and D≤ 500µm)

The enhanced sorption of sorbate by smaller particles has been reported previously for organic pollutants in urban soils by Krauss and Wilcke, 2002. This was most probably due to the increase in the total surface area which provided more sorption sites for the OPs. Corresponding plot for the surface area of biomass with K_{OC} (obtained from K_D normalized by OC (K_D /OC) is shown in Figure 4.4.



Methyl parathion ; $\log K_{oc} = 0.0040(\text{surface area}) + 3.0968$ $r^2 = 0.18$

Malathion ; $\log K_{oc} = 0.0150(\text{surface area}) + 3.5963$ $r^2 = 0.79$

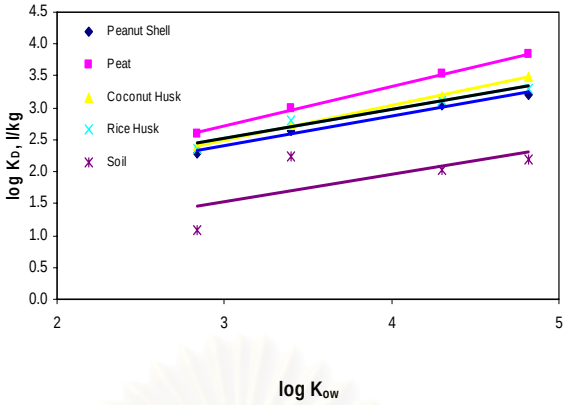
Chlorpyrifos; $\log K_{oc} = 0.0109(\text{surface area}) + 3.4096$ $r^2 = 0.71$

Profenofos; $\log K_{oc} = 0.0048(\text{surface area}) + 2.7070$ $r^2 = 0.44$

Figure 4.4 log K_{oc} of the test compounds in relation to biomass surface area

4.1.1.3 Magnitude of K_D in relation to OP properties

While the K_D values obtained are related closely to the similar systems of octanol-water partition coefficients (K_{OW}) (Figure 4.5), The octanol-water partition coefficient (K_{ow}) is a measure of the equilibrium concentration of a compound between octanol and water that indicates the potential for partitioning into soil organic matter (i.e., a high K_{ow} indicates a compound which will preferentially partition into soil organic matter rather than water). K_{ow} is inversely related to the solubility of a compound in water. Log Kow is used in models to estimate plant and soil invertebrate bioaccumulation factors. Sorption (log K_D/OC or log K_{oc}) follows a non-linear relationship with MW of the OPs investigated (Figure 4.6). The higher MW OPs exhibited enhanced sorption with minimal retention around MW = 300g mol^{-1}. El-Shahawi et al. (1995) studied the extraction capacity of polyurethane foam for removal of chlorpyrifos, malathion, and diazinon from water. Their work noted that



PS; $\log K_D = 0.4628 \log K_{ow} + 1.0133$ $r^2 = 0.98$

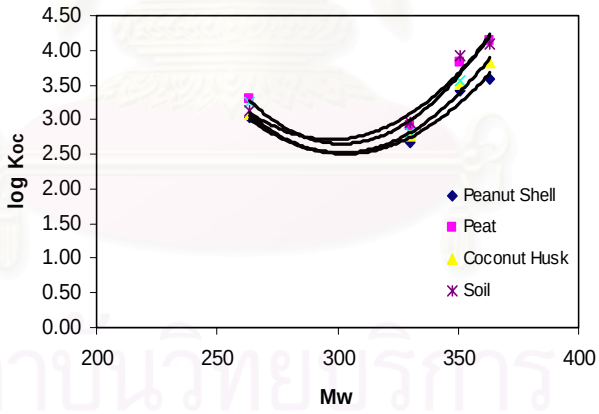
PM; $\log K_D = 0.6260 \log K_{ow} + 0.8913$ $r^2 = 0.99$

CH; $\log K_D = 0.5363 \log K_{ow} + 0.8324$ $r^2 = 0.99$

RH; $\log K_D = 0.4547 \log K_{ow} + 1.1533$ $r^2 = 0.96$

Soil; $\log K_D = 0.4319 \log K_{ow} + 0.2240$ $r^2 = 0.50$

Figure 4.5 Log K_D of the test compounds in 4 biomasses and one soil with log K_{ow} of test compound.



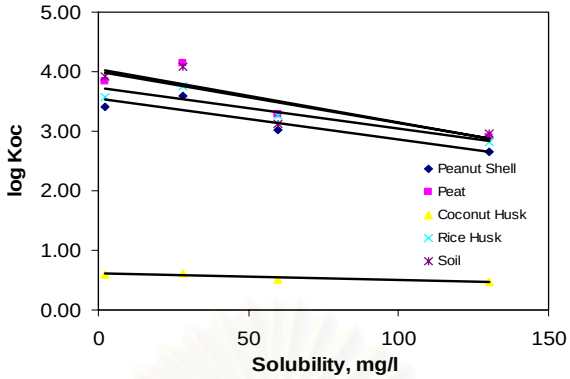
PS; $\log K_{OC} = 0.0003\ Mw^2 - 0.1954\ Mw + 32.1110$ $r^2 = 0.91$

PM; $\log K_{OC} = 0.0004\ Mw^2 - 0.2544\ Mw + 41.0450$ $r^2 = 0.94$

CH; $\log K_{OC} = 0.0004\ Mw^2 - 0.2199\ Mw + 35.7110$ $r^2 = 0.97$

Soil; $\log K_{OC} = 0.0003\ Mw^2 - 0.2028\ Mw + 32.8440$ $r^2 = 0.91$

Figure 4.6 Log K_{OC} of the test compounds in 4 biomasses and one soil in relation to Mw of the test compounds



PS; $\log K_{oc} = -0.0069S + 3.5484$ $r^2 = 0.84$

PM; $\log K_{oc} = -0.0088S + 4.0283$ $r^2 = 0.77$

CH; $\log K_{oc} = -0.0011S + 0.6071$ $r^2 = 0.81$

RH; $\log K_{oc} = -0.0069S + 3.7287$ $r^2 = 0.86$

Soil; $\log K_{oc} = -0.0087S + 3.9995$ $r^2 = 0.74$

Figure 4.7 log K_{oc} of the test compounds in 4 biomasses and one soil in relation to solubility of the test compounds

sorbate MW influenced the extraction. That is, the higher MW of the sorbates are likely to retain on the foam. Delgado et al (2003) developed a quantitative structure-property relationship (QSPR) model to predict the logarithm of the soil sorption coefficient of 82 organic compounds. They found the best correlation equation, containing only five constitutional descriptors (number of benzene rings, molecular weight, and number of N, O, and S atoms) for log Koc prediction.

Chiou et al. (1983) have previously demonstrated water solubility is also a factor governing the partitioning of nonionic organics between the organic sorbents and water in terms of retention in the sorbate. Figure 4.7 shows the plots of log K_{OC}, and S. As expected, K_{OC} decreases with hydrophilicity of the molecules.

4.1.2 OPs leaching characteristics

Biomass from agriculture processes can be used as sorbents for contaminant removal from the environment i.e. air, water and soil due to its high organic matter

content. The organic matter content of soil increases result in retention of pesticide on soil particles increases, thus, leaching of pesticide in soil profile decreases (Graber et al., 1997; Singh, 2003; Majumda and Singh, 2007). Thus, the experiments were set to determine the influence of biomass type and amount of organic carbon content in leaching of OPs behavior.

4.1.2.1 Influence of biomass type

Since CH which has high sorption and 5 times cheaper in cost than PM might be good for practical use and then selected for leaching experiment. PS is also carried out for comparison purpose as it is decomposed relatively faster. Figures 4.8, 4.9 and 4.10 depict the leaching profile of an OPs mixture spike with through 3.4 g PS, 1.5 and 3.0 g CH columns. Given the OC contents of the sorbents, this is equivalent to 1.4, 0.8 and 1.4 g OC respectively. While the same mixture was applied to all columns, the mass of each OPs in the mixture is different.

The initial breakthrough curves (BTCs) (Figure 4.8) in the first column (3.4g PS) occur at the same time (pore volumes 2-3) for all OPs. It also occurs at this time in the third column (3 g CH)(Figure 4.10) for methyl parathion and malathion. Comparing Figure 4.8 with Figure 4.10 where the sorbents have the same mass of OC, a much later occurrence of BTCs for chlorpyrifos and profenofos in CH is observed which confirms their greater sorption in CH than in PS. Evidently, it is not the amount of OC, but rather it's composition which is important. It was noted during the experiments that PS tended to degrade. CH composes of more lignin (40% approximately, Anto et al., 1997) which is relatively slower in decomposition.

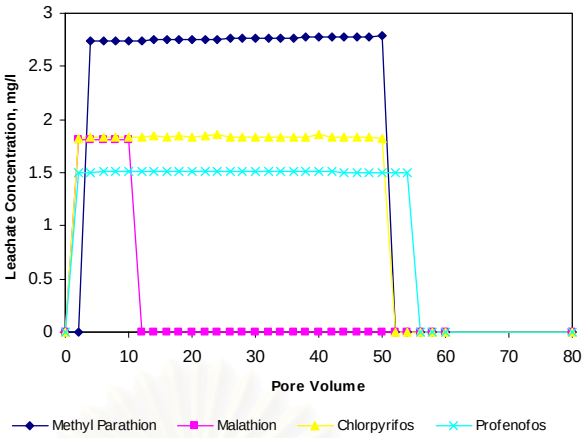


Figure 4.8 BTCs from first leaching through 3.4 g PS by spraying deionized water at 20ml h^{-1} on prior spiked OPs at normal application rate

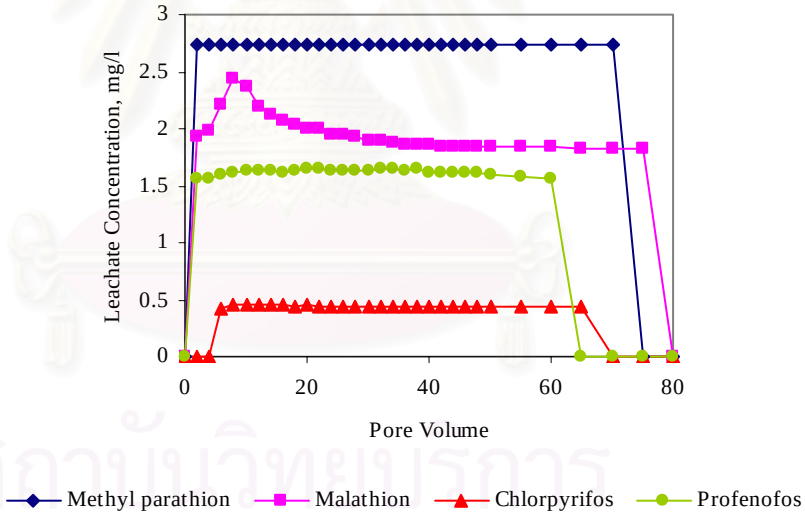


Figure 4.9 BTCs from first leaching through 1.5 g CH by spraying deionized water at 20ml h^{-1} on prior spiked OPs at normal application rate

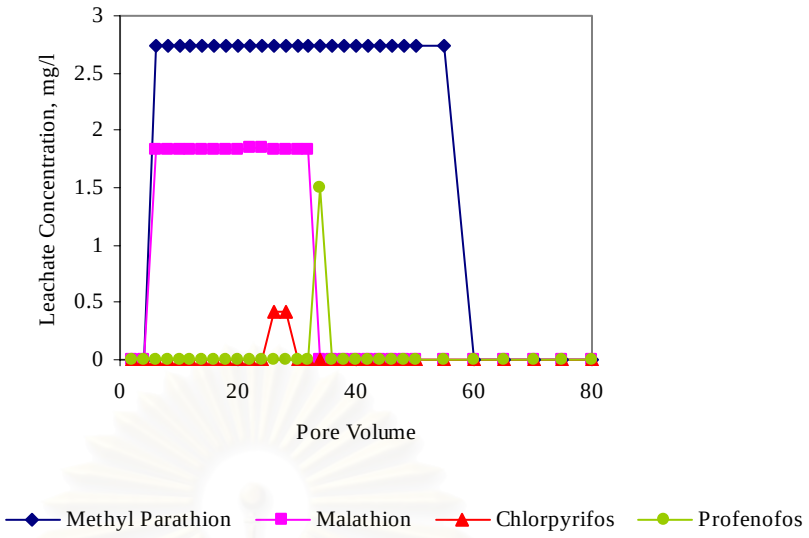


Figure 4.10 BTCs from first leaching through 3 g CH by spraying deionized water at 20ml h^{-1} on prior spiked OPs at normal application rate

4.1.2.2 Organic carbon influence

As clearly seen in Table 4.3, with the same spike mass for an OPs, 3 g CH can sorb 98.9-100% OPs using 1.8 liters (60 pore volumes) deionized water compared with sorption of 90.9-99% OP sorption when 1.5 g CH was used. Comparing Figures 4.9 and 4.10, representing CH columns with different masses, but the water flowing through at the same rate, breakthrough is prolonged in the column containing more OC allowing more time for the OPs to bind to OC. The difference of retained OPs between difference amount of OC indicated that the leaching of OPs is inversely related to sorption by organic matter controlled the mobility of OPs in biomass. The results obtained agree with the earlier observation with other chemicals. Vischetti et al (2004) investigated the retaintion chlorpyrifos, metalaxyl and imazamox in biomassbed (vine-branch, citrus peel, urban waste and public green compost) and found the test compounds were retained well and degraded afterwards. Si et al. (2006) investigated distribution of ethametsulfuron-methyl along amended soil profile. They found soil amended with peat moss and humic acid significantly reduced the leaching of ethametsulfuron-methyl.

Table 4.3 Comparison of the percentage of sorption by two amounts of coconut husk (CH) carried out by spraying deionized water at 20ml h^{-1} on prior spiked OPs (methyl parathion: malathion: chlorpyrifos: profenofos = 0.0728 : 0.0829: 0.0291: 0.116 mg) on CH

Mass (g)	OC (g)	methyl parathion	malathion	chlorpyrifos	profenofos
1.5	0.8	99.07	90.87	97.02	93.29
3.0	1.4	99.88	98.86	99.96	100

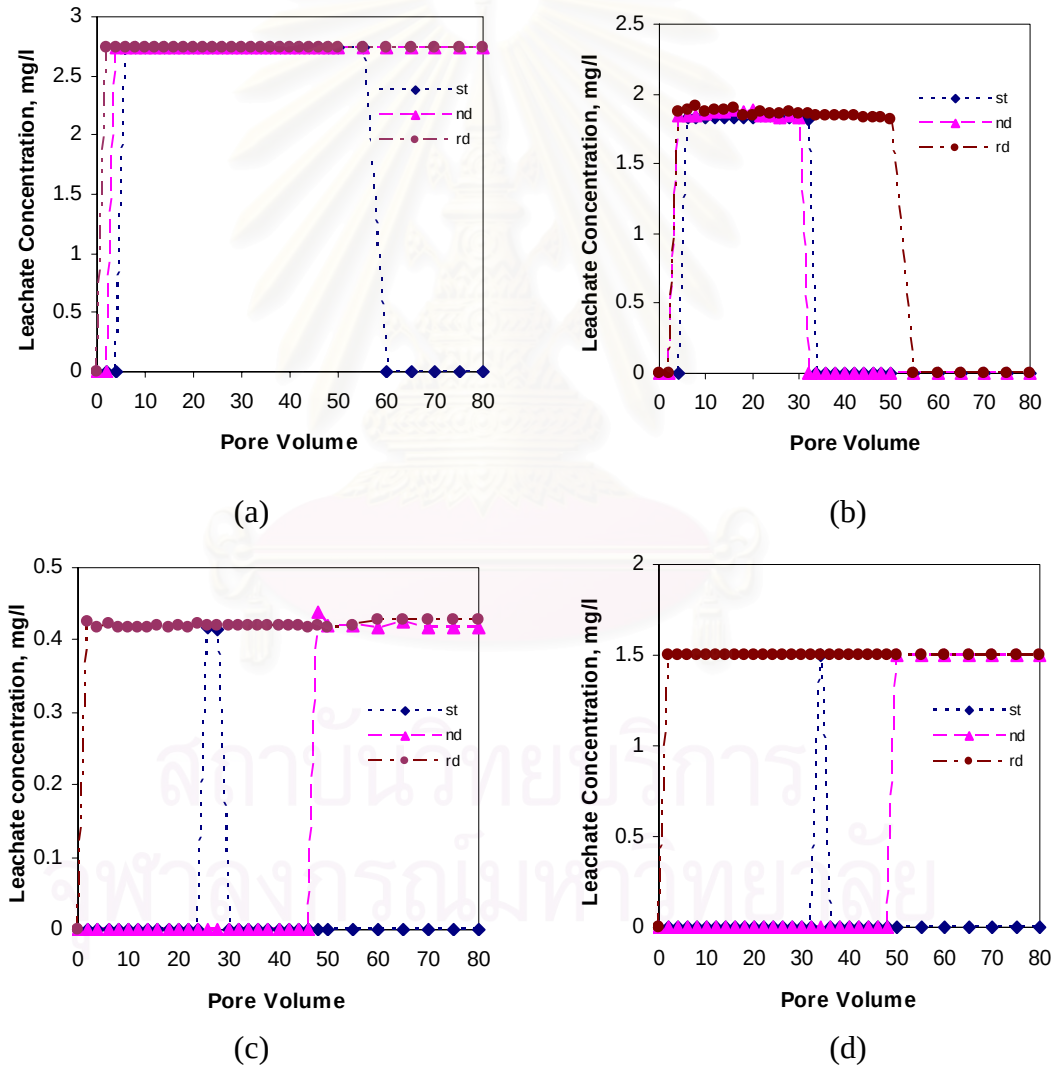


Figure 4.11 BTCs from first, second and third leachings through a column of 3 g CH which was 24 h prior spiked before starting leaching with (a) methyl parathion (b) malathion (c) chlorpyrifos and (d) profenofos

4.1.2.3 Sorption strength and water solubility influence

Figure 4.11 is produced from the second and third leaching on the same 3 g CH column. It is clearly shown that most added OPs are not leached in the second leachate, but accumulates on the CH until it reaches sorption capacity and can no longer sorb.

Added OP is then observed in the first pore volume of the third leaching. That sorption strength as represented by K_D as well as total added mass influences the process can be seen from the time of occurrence of BTCs and their shape. The steady concentration in the leachate indicates equilibrium has reached and the concentration drops when desorption is complete.

4.1.3 Biodegradation of OPs

It is believed that degradation by microbes accounts for over 90% of all degradation reactions in the environment and it is the nearly exclusive breakdown pathway in most surface soils (Wheeler, 2002). Biodegradation can be rapid and thorough under soil conditions favoring microbial activity. Those conditions include warm temperatures, favorable pH level, adequate soil moisture, aeration (oxygen), and fertility (Carlson, 2005). The aim of this experiment was set to determine the effect of moisture which is the one favorable condition for microbial activities.

4.1.3.1 Effect of moisture

The degradation studies were carried out to determine the effect of moisture on OPs degradation in soil and RH. The experiments were set in microcosms into different moisture content at 15%, 30% and 45% of soil and RH. OPs were spiked to give concentration of normal agricultural application rate of dry soil and biomass. The microcosms were maintained at room temperature. The change of OPs was monitored in soil and biomass from 7, 21 and 35 days. OPs residues were calculated the %removal. The results are given in Figure 4.12 and 4.13, respectively.

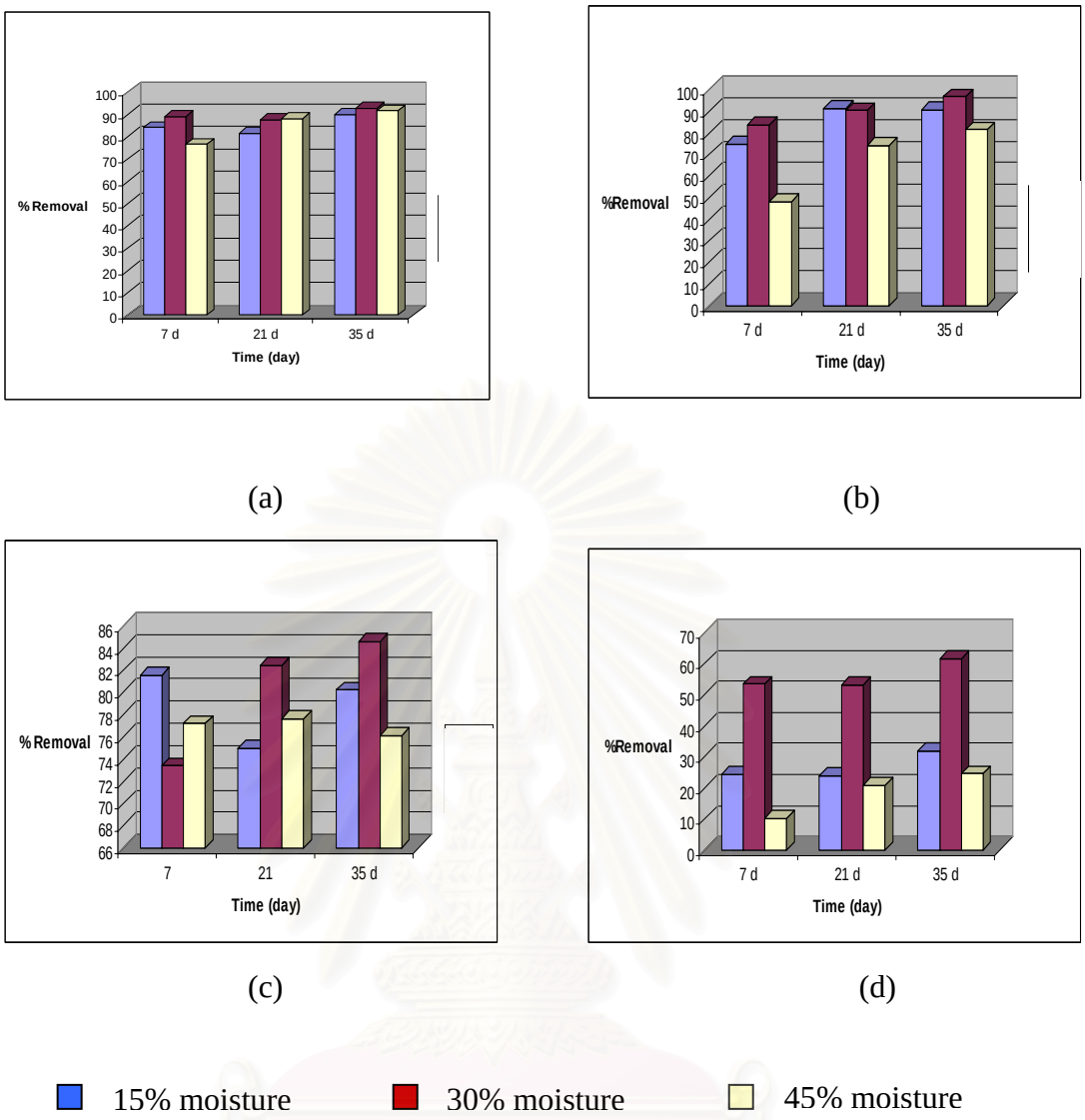


Figure 4.12 Effect of moisture on OPs degradation in soil (a) methyl parathion, (b) malathion, (c) chlorpyrifos and (d) profenofos

The figures showed the percent removal of OPs in soil and RH.. It was observed that the percent removal of OPs increased with increasing time. The optimum moisture content that makes the highest percent removal of organophosphate for soil is 30% in all OPs. Methyl parathion can be degraded most rapid in microcosm. While others can be degraded order by malathion, chlorpyrifos and profenofos , respectively.

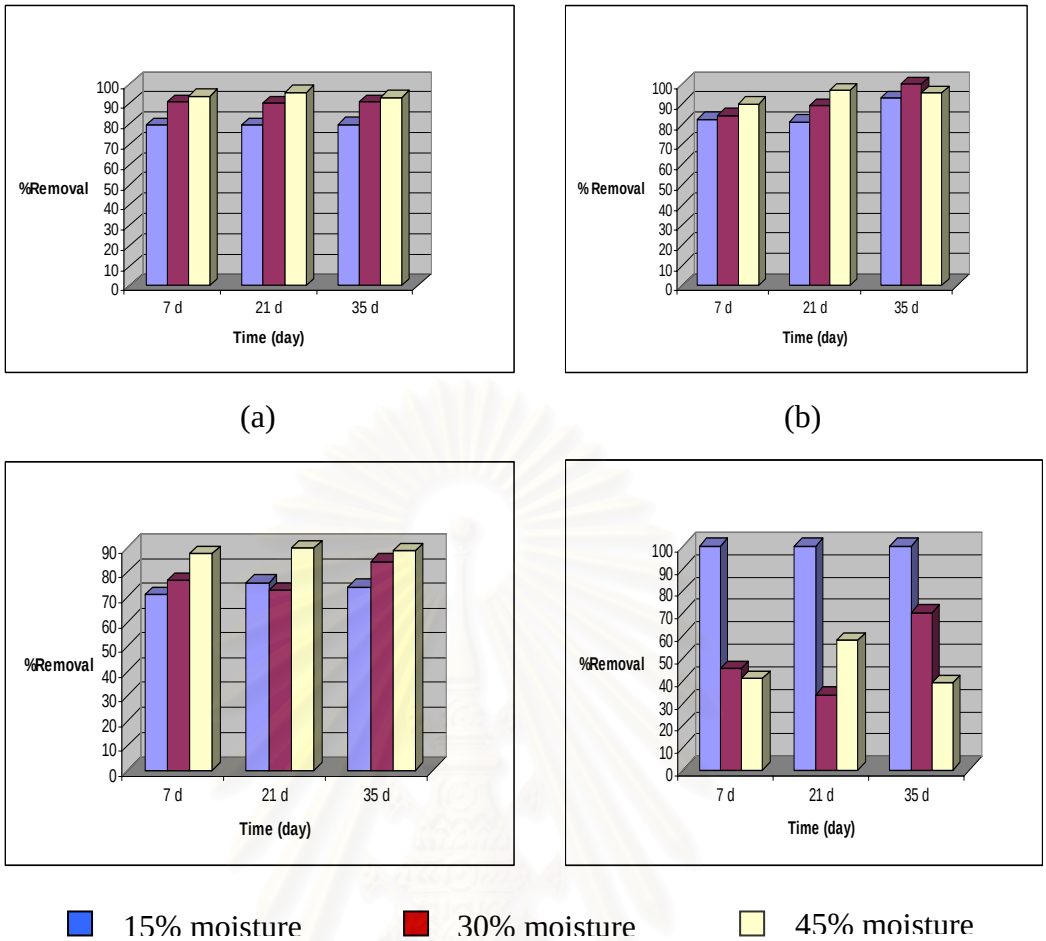


Figure 4.13 Effect of moisture on OPs degradation in RH (a) methyl parathion, (b) malathion, (c) chlorpyrifos and (d) profenofos

The figure 4.13 showed percent removal of OPs in RH. These results were not different from percent removal in soil. It was found that the removal efficiency increased with increasing time. The optimum moisture content that makes the highest percent removal of OPs for soil is 45% in methyl parathion, malathion and chlorpyrifos, respectively. Water availability is the one condition under which the bacteria can synthesize enzyme and degrade organic substances (Vischetti et al., 2004; Han and New, 1994, Carlson, 2005). Results in OPs in higher amount of moisture have higher percent removal.

4.1.3.2 OPs degradation in biomass and soil

The degradation of organic compounds in soil and biomass after moisture adjustment (70% water holding capacity) can be described with the first-order kinetic equation as dC/dt = -kt. From the equation, we can obtain the following equations:

$$\ln C = -kt + \ln C_o \quad (4.2)$$

$$t_{1/2} = 0.693/k \quad (4.3)$$

Where C is concentration of chlorpyrifos in soil or biomass, mg kg^{-1}, k is degradation rate constant, d^{-1}, C_o is the initial concentration of in soil or biomass, mg kg^{-1}, $t_{1/2}$ is half life, days.

Table 4.4 The kinetic equations of OPs in CH and soil

OPs	Biomass	k (d^{-1})	$t_{½}$ (days)	$t_{1/2}$(literature) (days)
Methyl Parathion	CH	0.0186	41.7	
	PM	0.0326	21.3	
	RH	0.0671	10.3	
	PS	0.0986	7.0	
	Soil	0.0195	35.5	44 [a], 1-25 [b]
Malathion	CH	0.0290	23.9	
	PM	0.0589	11.8	
	RH	0.0660	10.5	
	PS	0.0812	8.5	
	Soil	0.0347	20.0	17[c]
Chlorpyrifos	CH	0.0321	21.6	
	PM	0.0104	67.3	
	RH	0.0123	56.3	
	PS	0.0153	45.3	
	Soil	0.0109	63.6	60-129[d]
Profenofos	CH	0.0134	51.7	
	PM	0.0189	36.7	
	RH	0.0201	34.5	
	PS	0.0345	20.1	
	Soil	0.0164	42.3	7[d]

Source; [a] Pait et al.,1992 , [b] US EPA, 2001, [c] Gegenta et al., 2000[d]Tomlin 1995,

Soil microcosms were also determined for degradation rate of OPs by the plot of Ln C against incubation time (days). The degradation of OPs in biomass obtained was shown in Table 4.4.

It seems like $t_{1/2}$ in CH for OPs in this study have the longest except for chlorpyrifos and PM followed. Thus, CH and chlorpyrifos are selected for the application purpose. The results indicated malathion and chlorpyrifos can be degraded rapidly in RH and CH, respectively. Meanwhile, methyl parathion and profenofos can be degraded well in PS. The obtained half-life data from this study was in the range of previous study. However, the different of half-life from previous study could be occurred from different environmental conditions such as temperature, moisture content, amount of applied contaminant, photo, oxygen, and etc. Another optimum condition is abundance of organic matter. Noticeably, OPs in soil show moderate degradation and longer half-life than biomass due to its low organic matter content. This confirms by Sánchez et al. (2004) that the increasing of organic matter is varied directly to microbial degrading activities.

4.1.4 Overall evaluation of distribution of OPs biomass-water system and OPs biodegradation

Results suggest that sorption of OPs by the biomass in the presence of water is due to a partitioning process where not only the amount but the chemical nature of the OC is important. Sorption strength of OPs are in the order that profenofos>chlorpyrifos>methyl parathion> malathion and for a given OPs with different types of biomass PM>CH>RH>PS. OP behavior in the biomass-water systems is governed by the compound properties (structural features and hydrophobicity) together with biomass properties (organic carbon contents, size, surface areas). In leaching by water, additional controlling factors are water flow rate, OPs concentration as well as frequency of spiking and watering. Bacterial break down experiments on OPs with four biomass demonstrate likely that four OPs degrade relatively rapidly in PS and RH except for chlorpyrifos in CH the most rapid among all cases.

4.2 Application of biomass and effective microorganisms (EM) to minimize chlorpyrifos leaching through soil

The purpose of this study was to assess the possibility of using biomass and EM to prevent the leaching of sprayed chlorpyrifos to soil. Chlorpyrifos leaching behavior in biomass covered soil columns were determined. The effect of EM on degradation of chlorpyrifos was investigated as well. According to the sorption experiment, coconut husk was the best sorbent in term of highest sorption capacity and was very economical due to its lowest price compared to other biomass. Coconut husk also showed the shortest chlorpyrifos half-life.

4.2.1 Biodegradation of chlorpyrifos in soil and biomass

The experimental degradation of chlorpyrifos was set up in three different conditions: untreated, moisture adjusted and EM added. This experiment was conducted to evaluate degradation rate of chlorpyrifos and effect of EM on chlorpyrifos degradation. The results of untreated conditions were used to determine the efficiency of natural attenuation. While, moisture adjusted and EM added condition were used to verify efficiency of biostimulation and bioaugmentation. The degradation of chlorpyrifos from each treatment was shown in Table 4.5.

Table 4.5 Half-life of chlorpyrifos in biomass and soil at different conditions.

Sorbent	Untreated			Moisture adjusted			EM added		
	k	r^2	$t_{1/2}$ (day)	k	r^2	$t_{1/2}$ (day)	k	r^2	$t_{1/2}$ (day)
CH	0.0085	0.95	81.5	0.0321	0.77	21.6	0.0401	0.85	17.3
PM	0.0081	0.81	85.6	0.0104	0.94	67.3	0.0175	0.75	39.6
RH	0.0080	0.58	86.6	0.0123	0.90	56.3	0.0133	0.93	52.1
PM	0.0133	0.80	52.1	0.0153	0.88	45.3	0.0206	0.80	33.6
Soil	0.0122	0.91	56.8	0.0109	0.85	63.6	0.0151	0.94	45.9

When compared between each condition, the disappearance of chlorpyrifos in EM added condition was remarkably faster than the others. In EM added condition, $t_{1/2}$ was ranged from 17.3–52.1 days while it was 21.6–67.3 days in moisture adjusted condition. However, the $t_{1/2}$ of untreated condition was 56.8-86.6 days, which was related with the reported chlorpyrifos in soil (varies from 10 to 120 days) (Singh, 2003).

Environmental factors such as types of pesticide, characteristics of soil, temperature, pH, and water availability are the conditions under which the bacteria can synthesize enzyme and degrade organic substances (Vischetti et al., 2004; Han and New, 1994, Carlson, 2005). As a result, the untreated condition had the longest $t_{1/2}$ because of the lack of water availability which is an appropriate factor for bacterial growth.

According to the increase organic matter, microbial degrading activities were increased. Sanchez et al. (2004) also found that the higher the organic matter content in sludge, the more rapid degradation for organophosphate pesticides such as fenittrothion and dimethoate. Generally, microorganisms that decompose organic matter use carbon as a source of energy and nitrogen for building cell structure. C:N at lower ratios, nitrogen will be supplied in excess and will be lost as ammonia gas, causing undesirable odors. Higher ratios mean that there is not sufficient nitrogen for optimal growth of the microbial populations, so degradation will proceed at a slow rate. The C:N optimum ratio in soil organic matter is about 10 carbons to 1 nitrogen (Misra et al., 2003). Every biomass materials had C:N ratio closed to 10:1, which thereby resulted in faster degradation rate than soil sample. When compared between each type of biomass, CH contributed to the shortest half-life of chlorpyrifos. This was probably due to the high sorption capability of CH. Several reports showed that soil microorganisms could directly and more easily act on the surface–sorbed compounds (Verstraete and Devliegher, 1996; Charoenchang et al., 2003).

Besides one of the major ways of this breakdown pattern is through bacterial metabolism, usually though a consortium of microbes rather than a single species. In the case of EM added condition, the results showed the shortest half-life of chlorpyrifos. This finding was probably due to the presence of variety of

microorganisms in EM solution. According to Higa (1998), EM contains about 80 species of microorganisms, which can be divided into the following groups: (1) photosynthesizing bacteria; (2) lactic acid bacteria; (3) yeasts; (4) actinomycetes and (5) fermenting fungi like *Aspergillus* and *Penicillium*. Such microorganisms may be (1) capable of degrading target pollutants, (2) competitive and persistent after inoculation, and (3) degradation of specific compounds (Yu and Mohn, 2002; Loperena et al., 2005). When EM was inoculated into the soil or biomass, it enhanced the rate of chlorpyrifos degradation probably by directly degrading chlorpyrifos or indirectly improving soil quality and thereby promoting the activity of soil indigenous bacteria.

4.2.2 Fate of chlorpyrifos in soil columns during leaching studies

According to the sorption experiment, CH was the best sorbent in term of highest sorption capacity and economic with lower price than other biomass. In addition, CH also showed the shortest chlorpyrifos half-life. Therefore, CH was used as biomassbed to cover soil columns in the leaching studies. The experiments were set in columns of three treatments; soil, soil+biomass and soil+biomass+EM. The distribution of chlorpyrifos along soil profile was showed in Figure 4.14. The results demonstrated the highest amount of chlorpyrifos retained in 0-5 cm depth of soil columns. Related to Redondo et al. (2004) who studied the fate of pesticides in a citrus orchard and found that the pesticide concentrations were always highest in the upper layer (0–0.05 m) of soil. The obtained results also agreed with earlier observation. Si et al. (2006) investigated distribution of ethametsulfuron-methyl along amended soil profile. They found soil amended with PM and humic acid significantly reduced the leaching of ethametsulfuron-methyl.

The amount of chlorpyrifos at 0-5 cm decreased gradually over time. However, soil at the same depth still showed significantly higher amount of chlorpyrifos than soil+biomass and soil+biomass+EM columns, respectively. At the end of experiment, the remainings of chlorpyrifos in soil, soil+biomass and soil+biomass+EM columns at 0-5 cm depth were 7.75, 2.53 and 0.824 µg, respectively. The amounts of chlorpyrifos in these samples were lower than the initial concentration, thus the results suggested that the sorbed chlorpyrifos in biomass was

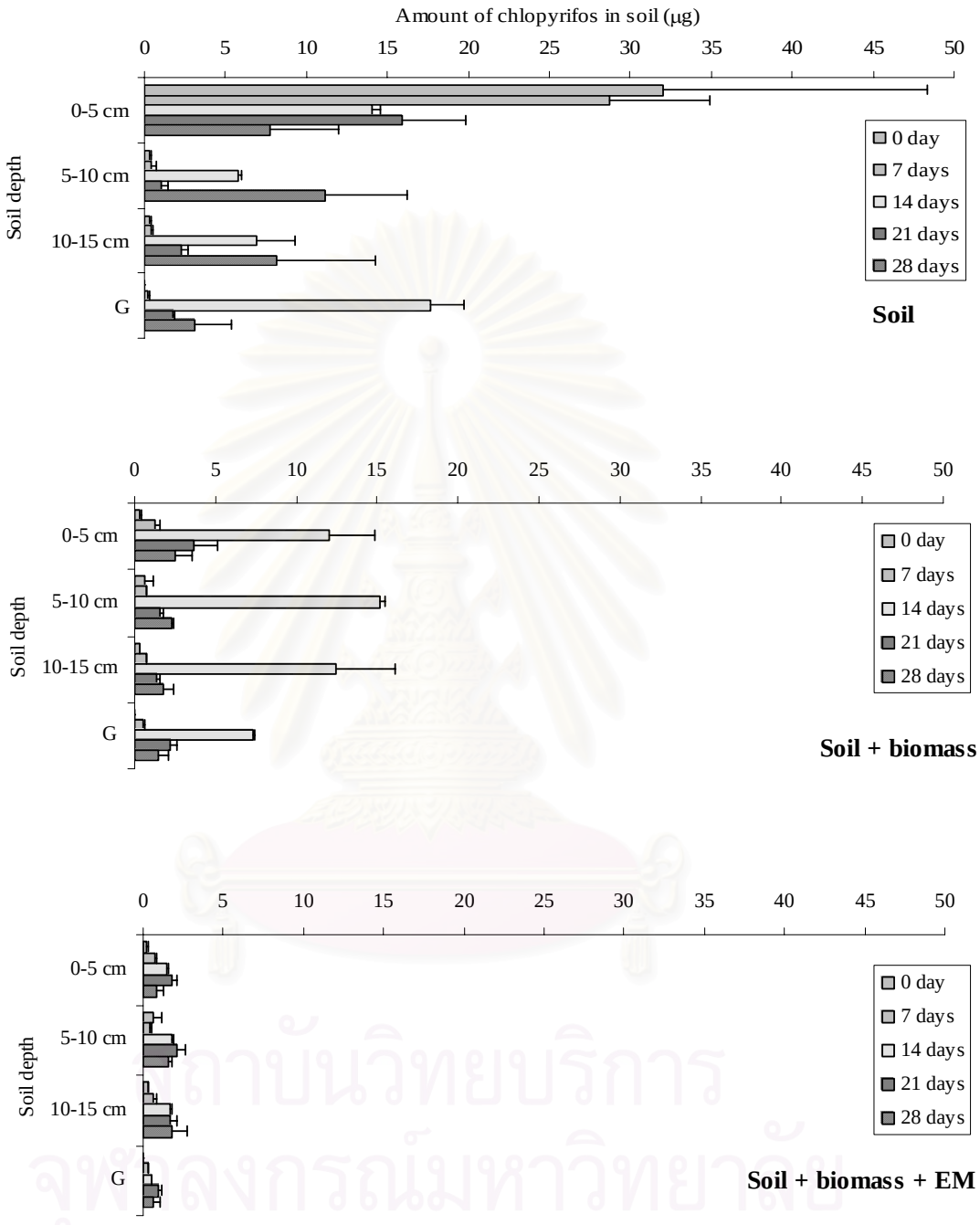


Figure 4.14 Distribution of chlorpyrifos in soil columns with different amendments. Chlorpyrifos were also monitored in the gravel layer (G) at bottom of the columns.

degraded later. Soil+biomass columns had less amounts of retained chlorpyrifos than the soil only columns. This was probably due to the present of organic matter in biomass, which has been found to increase the sorption of pesticides and decrease their subsequent mobility in the soil profile (Singh, 2003, Sluszny et al., 1999, Guo et al., 1993). The high amounts of chlorpyrifos were shown at 14 days. Because sorbed chlorpyrifos could be desorped from coconut husk layer instead of degradation. Then desorped chlorpyrifos were leached and presented along soil column. The least amounts of retained chlorpyrifos were found in soil+biomass+EM columns. After leached daily, chlorpyrifos was found in every depth of the soil columns. Especially, soil only columns showed higher amount of chlorpyrifos in lower depth than other biomass coverage columns. The results confirmed that chlorpyrifos was retained in biomass containing columns.

Figure 4.15 showed the numbers of chlorpyrifos-degrading bacteria in the soil columns. All of treatments found more number of chlorpyrifos-degrading bacteria at 0-5 cm soil than deeper depths. The results indicated that the decreased of chlorpyrifos was mainly due to the activity of microorganisms in the surface soil. In addition, many researchers have been reported that the degradation occurred mainly in surface soils (Hua et al., 1995; Dai et al., 2001).

At the 0-5 cm depth, the averaged numbers of chlorpyrifos-degrading bacteria were higher in biomass and biomass+EM soil columns than in soil only columns. The source of these bacteria was therefore suggested to be biomass and EM. Similarly, Kastner and Mahro (1996) reported that the addition of compost materials to soil was not only used as a source of nutrient and organic matter but also served as a source of microorganisms that might play a major role in PAHs degradation. In addition, Vischetti et al., 2004 found faster degradation of chlorpyrifos, metallazyl and imazamox in leachate with the mixture of biomass and compost than the degradation in soil. The distribution of chlorpyrifos-degrading bacteria through out biomass+EM columns also confirmed that some bacteria in EM could degrade chlorpyrifos.

The relationship between the amount of chlorpyrifos and number of chlorpyrifos-degrading bacteria in biomass layers was showed in Figure 4.16. Biomass with EM had higher amount of bacteria than biomass without EM. Thus, the

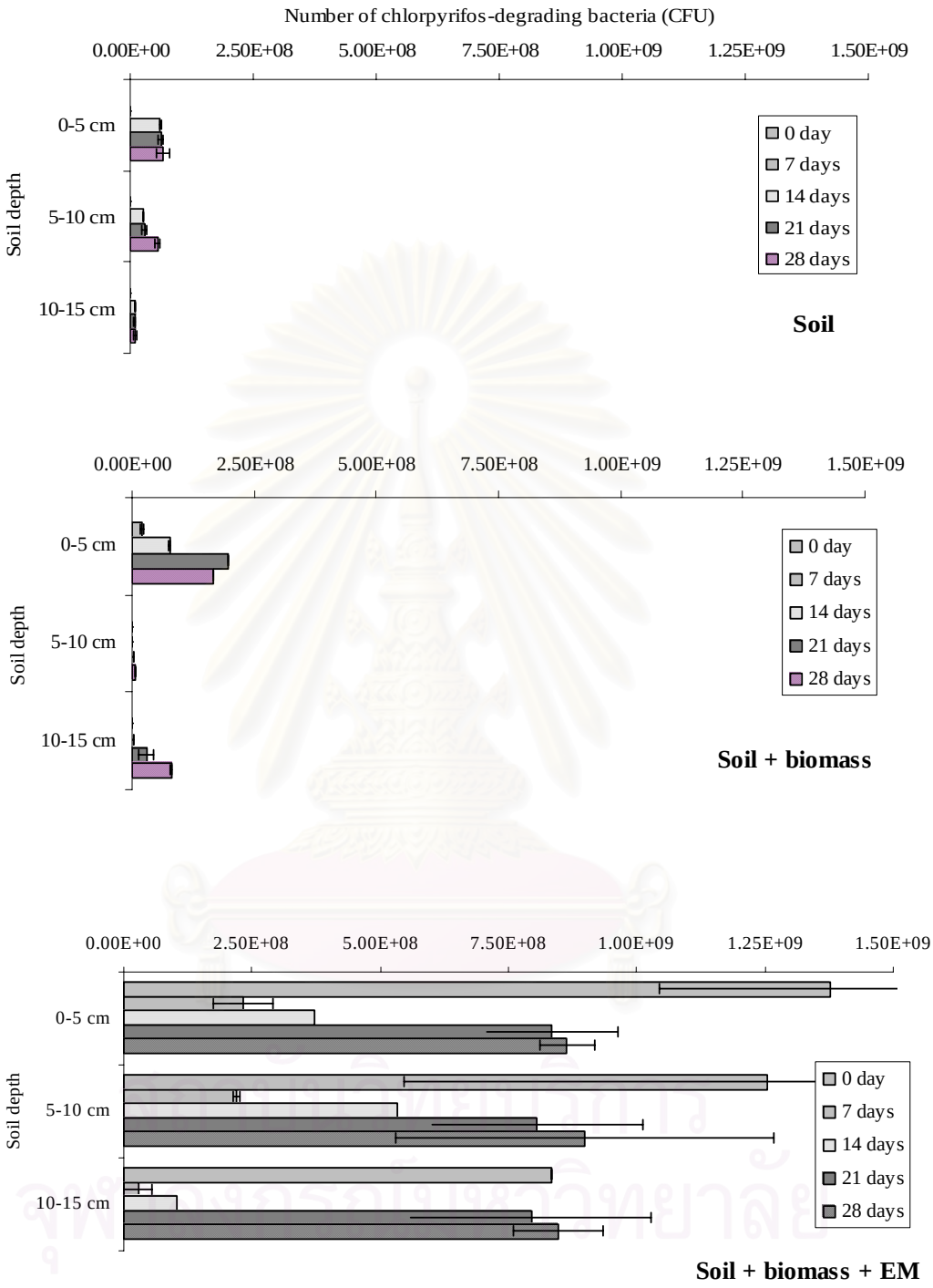


Figure 4.15 Distribution of chlorpyrifos-degrading bacteria in soil columns with different amendments.

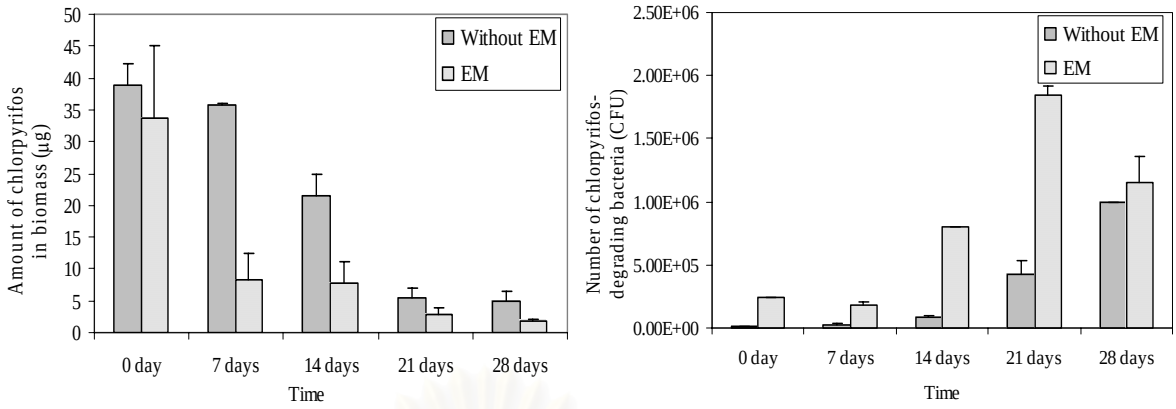


Figure 4.16 Amount of chlorpyrifos and chlorpyrifos-degrading bacteria in biomass layer.

addition of EM promoted the growth of chlorpyrifos-degrading bacteria in biomass. The amounts of chlorpyrifos decreased over time, while the number of chlorpyrifos-degrading bacteria increased at the same period.

Therefore, the interplay between reduction of chlorpyrifos and abundance of bacterial cells, could describe as growth-linked biodegradation, in which chlorpyrifos was served as a carbon source for the microorganisms.

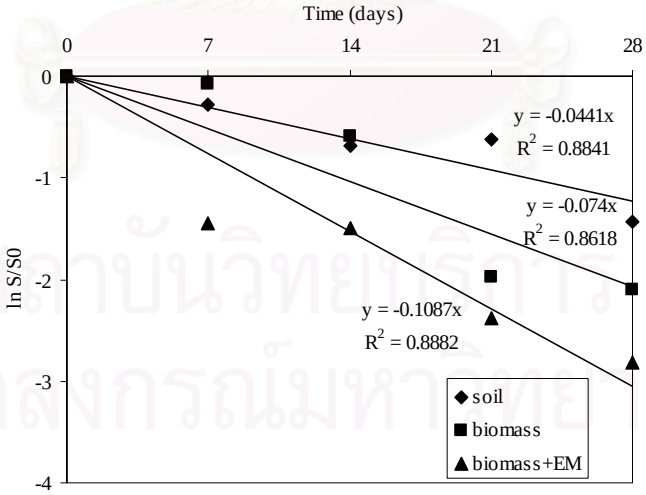


Figure 4.17 Degradation of chlorpyrifos in surface soil (0-5 cm depth) and biomass layer. The amounts of initial and remaining chlorpyrifos are presented as S_0 and S, respectively.

In addition, the chlorpyrifos degradation in surface soil and biomass layer could be described with the first-order kinetic equation (Figure 4.17). Biomass+EM showed the highest degradation constant rate (k) at 0.1087 d^{-1}. Biomass and soil showed lower k value at 0.074 d^{-1} and 0.044 $d^{-1,}$ respectively. The half-lifes were 6.4, 9.4 and 15.8 days in biomass+ EM, biomass and soil respectively. The half-life of chlorpyrifos in CH with or without EM was shorter than soil. It means that chlorpyrifos in biomass+EM was degraded faster than other conditions. The degrading bacteria probably used the retained chlorpyrifos as nutrient source. The results confirmed that the reduction of chlorpyrifos was due to biodegradation.

4.3 Evaluation of CH and EM application

These studies demonstrated that CH was effective to retard the mobility of chlorpyrifos and the retained chlorpyrifos could be degraded afterward. The presence of organic carbon in CH played a major role of chlorpyrifos sorption. Degradation of chlorpyrifos in CH was more rapidly than in other biomass and soil. Growth-linked biodegradation of chlorpyrifos was occurred as shown by the increased number of chlorpyrifos-degrading bacteria. The adding EM could increase the number of chlorpyrifos-degrading bacteria as well as the rate of chlorpyrifos degradation. The degradation was occurred mainly in the surface layers due to higher microbial numbers in the surface than deeper soil columns. The estimation results also showed the short half-life of OPs for Biomass + EM condition. Thus, the covering of soil surface with 4 cm depth-CH along with EM addition could be a feasible method to reduce contamination of OPs leachate from sprayed plants through soil. In addition, not only CH but also other biomass which have the similar properties such as high sorption capacity and ability to promote pesticide degradation may be applied by covering the soil surface or packing as barrier around plantation to prevent the pesticide contamination from run off at farm level.

4.3.1 Biodegradation of methyl parathion, malathion and profenofos at different condition

This experiment was conducted to evaluate degradation rate of OPs at different conditions. When compared between each condition, the amount of other OPs (methyl parathion, malathion and profenofos) faster in EM added condition than untreated and moisture adjusted condition, which was related with the degradation of chlorpyrifos in section 4.2.1.

Table 4.6 Half-life of OPs in biomass and soil in microcosms at different conditions

OPs	Sorbent	Untreated		Moisture adjusted		EM added	
		k (d^{-1})	$t_{1/2}$ (days)	k (d^{-1})	$t_{1/2}$ (days)	k (d^{-1})	$t_{1/2}$ (days)
Methyl parathion	Coconut husk	0.0184	37.6	0.0186	41.7	0.0261	26.6
	Peat moss	0.0111	62.4	0.0326	21.3	0.0709	9.8
	Rice husk	0.0091	76.2	0.0671	10.3	0.0500	13.9
	Peanut shell	0.0268	25.9	0.0986	7.0	0.0925	7.5
	Soil	0.0111	62.4	0.0195	35.5	0.0218	31.8
Malathion	Coconut husk	0.0155	44.7	0.0290	23.9	0.0427	16.2
	Peat moss	0.0125	55.4	0.0586	11.8	0.0931	7.4
	Rice husk	0.0190	36.5	0.0660	10.5	0.0801	8.7
	Peanut shell	0.0406	17.1	0.0812	8.5	0.0987	7.0
	Soil	0.0202	34.3	0.0347	20.0	0.0374	18.5
Profenofos	Coconut husk	0.0109	63.6	0.0134	51.7	0.0246	28.2
	Peat moss	0.0194	35.7	0.0189	36.7	0.0356	19.5
	Rice husk	0.0205	33.8	0.0201	34.5	0.0273	25.4
	Peanut shell	0.0245	28.3	0.0345	20.1	0.1154	6.0
	Soil	0.0199	34.8	0.0164	42.3	0.0258	26.9

4.3.2 Estimation of other OPs half-life on coconut husk bed

According to fate of chlorpyrifos in soil columns during leaching studies in section 4.2.2, CH was useful to retard the mobility of chlorpyrifos and the retained chlorpyrifos could be degraded afterward. The additional of EM in both microcosms (Table 4.6) and column leaching showed the higher disappearance of chlorpyrifos than other conditions. Therefore, the use of CH and EM should be appropriate to other OPs (i.e. methyl parathion, malathion and profenofos). The estimation is one methods

to determine the possibility for other OPs. Table 4.6 showed the estimation half-life and % remaining at 28 days after single spraying OPs and leached by water everyday.

Table 4.7 The estimation of OPs half-life in columns from EM added condition base on chlorpyrifos as tested compound

OPs	$t_{1/2}$[a] of EM added in microcosm (days)	Ratio[b]	$t_{1/2}$[c] Estimation in columns (days)	% Remaining[d] at 28 days
Chlorpyrifos	17.3[e]	1.0	6.4[f]	less than 6.25%
Methyl parathion	26.6	1.5	9.8	less than 12.50%
Malathion	16.2	0.9	6.0	less than 6.25%
Profenofos	28.2	1.6	10.4	less than 12.50%

a = half-life of OPs in microcosm at EM added condition

b = Ratio of each OPs half-life in CH compare to Chlorpyrifos half-life in CH, microcosms

c = Estimation of OPs half-life in CH bed from chlorpyrifos half-life in CH bed (section 4.2.2, chlorpyrifos half-life $t_{1/2}$ = 6.4 days) was calculated by Ratio x chlorpyrifos half-life $t_{1/2}$ in CH bed.

d = % Remaining at 28 days

e = Chlorpyrifos half-life (Table 4.4)

f = chlorpyrifos half-life in CH bed (section 4.2.2)

The estimation results shown profenofos had the longest half-life in CH with EM added at 10.4 days. Meanwhile malathion could be degraded fastest. After 28 days, methyl parathion and profenofos will be retained in media less than 12.5%. While, malathion and chlorpyrifos will be retained less than 6.5%. Since the obtained values were derived from estimation, the determination by laboratory should be conducted to verify before use in the real.

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

Sorption strength of OPs in four biomass in the presence of water was in the order that profenofos>chlorpyrifos>methyl parathion>malathion, while the sorption capacity of a given OPs with different types of biomass was in the order of PM>CH>RH>PS. Sorption capacity of soil obviously gave lowest values than all biomass due to its very low organic carbon content. The results indicated that organic carbon in biomass plays a major role of OPs sorption. However, the extent of sorption depends further on characteristics of the sorbents (i.e. size, porosity and surface areas) and properties of the sorbate (i.e. structural features and hydrophobicity). Hence, OPs in this study have sorption strength corresponding to the log Koc and log Kow values. The PM and CH relatively higher capacity for the sorption is due mainly to their OC. The exception of this is the biomass decomposition, i.e PS. Influencing factors for the sorption found are particle size and surface area. The K_D values also correspond to log Kow, and inversely to S. Particular second order regression equations are found for log Koc and Mw since OPs are more water soluble than PAHs or organochlorine compounds. In leaching biomass by water, additional factors are water flow rate, OP concentration as well as frequency of spiking and watering.

Experimental degradation rates under moisture added condition in soil and biomass which is equivalent to 70% water holding capacity showed that disappearance of chlorpyrifos in CH was remarkably the fastest ($t_{1/2}$=21.6 days). Particularly, chlorpyrifos was more degradable in coconut husk than soil (63.6 days) and other biomass (7.0-56.3 days) due to coconut husk alone was partly rich in organic matter and C:N very close to 10:1. The addition of compost materials to soil was not only used as a source of nutrient and organic matter but also served as a source of microorganisms that might play a major role in biodegradation. Noticeably most favorable biomass for methyl parathion, malathion and profenofos is PS but less for chlorpyrifos. Biodegradation experiments on OPs with four biomass demonstrate likely that four OPs degrade relatively rapidly in PS and RH except for chlorpyrifos in CH the most rapid among all cases. OPs in soil show most moderate degradation. This confirms the need for bioremediation in term of effective biomass and EM addition on

the soil. Repeatedly, the OPs in CH are obviously demonstrated the lowest breaking down with the exemption of chlorpyrifos in CH as mentioned earlier. However, as CH shown to be the best sorption and economical but less effective for methyl parathion, malathion and profenofos, EM addition could help accelerate those compounds to break down faster.

Three treatments for the biodegradation of OPs in four biomass were conducted for 28 days and shown that moisture and EM added treatments were favorable with the latter gave the shortest half life of the compounds in all biomass. The favorable biomass for the biodegradation was inconclusive and likely compound specific. However, based on biomass sorption capacity and economic, coconut husk was selected for further leaching in column experiments. The coconut husk with the volume of 46.54 cm^3 after spraying with chlorpyrifos of 0.0291 mg, adding with EM 50 ml (of 1:100 EM: molass solution) on top of the CH column and leaching with deionized water 40 ml h^{-1} d^{-1} for 28 days indicated half life of chlorpyrifos as low as 6.4 days. Thus, approximately 12.5% of chlorpyrifos sprayed was remained in the CH at the day of 28. Estimations of other OPs degration in leaching experiments were made based on ratio of half life of chlorpyrifos to half life of other OPs in the same EM added treatment, therefore methyl parathion, malathion, and profenofos degration ratio were 1.5, 0.9 and 1.6, respectively. Since leaching experiment of chlorpyrifos in CH is 6.4, then estimate leaching half life of methyl parathion, malathion, and profenofos were 9.8, 6 and 10.4, respectively. It is possible that during leaching within 28 days OPs will be remained approximately less than 12.5%, 6.25% and 25% for methyl parathion, malathion and profenofos, respectively. It should be noted rate of disappearance of OPs in biomass in this study based on certain amount of OPs sprayed by the farmers.

Further studies should be undertaken to investigate the sorption and degradation behavior of mixed type of biomass with other popular pesticide groups such as organochlorine and carbamate pesticides. Since, various types of biomass will give difference magnitude of sorption and degradation rate. The combination of biomass which high sorption and short half-life may reduce amounts of biomass in removal pollutant with high efficiency. The optimum ratio of mixed biomass should be investigated. Moreover, other factors that affect the fate of pesticides should be

studied. For instance, the efficiency of the biomassbed technique when using other type of biomass, the changes in sorption efficiency for applying single pesticide with repetition and/or mixed pesticides, the determination of the life time of other biomass, and the reliability of the biomass in order to maintain its sorption and degradation ability. Furthermore, the isolation and identification of OPs-degrading bacteria should be included to elucidate the mechanisms of OP degradation in soil and biomass. It is also important in running PCR-DGGE of 16S rRNA gene to determine and reveal the dominant bacteria present in the soil and biomass samples. With this method, it could be predicted whether the indigenous or augmented species are responsible for OPs degradation.

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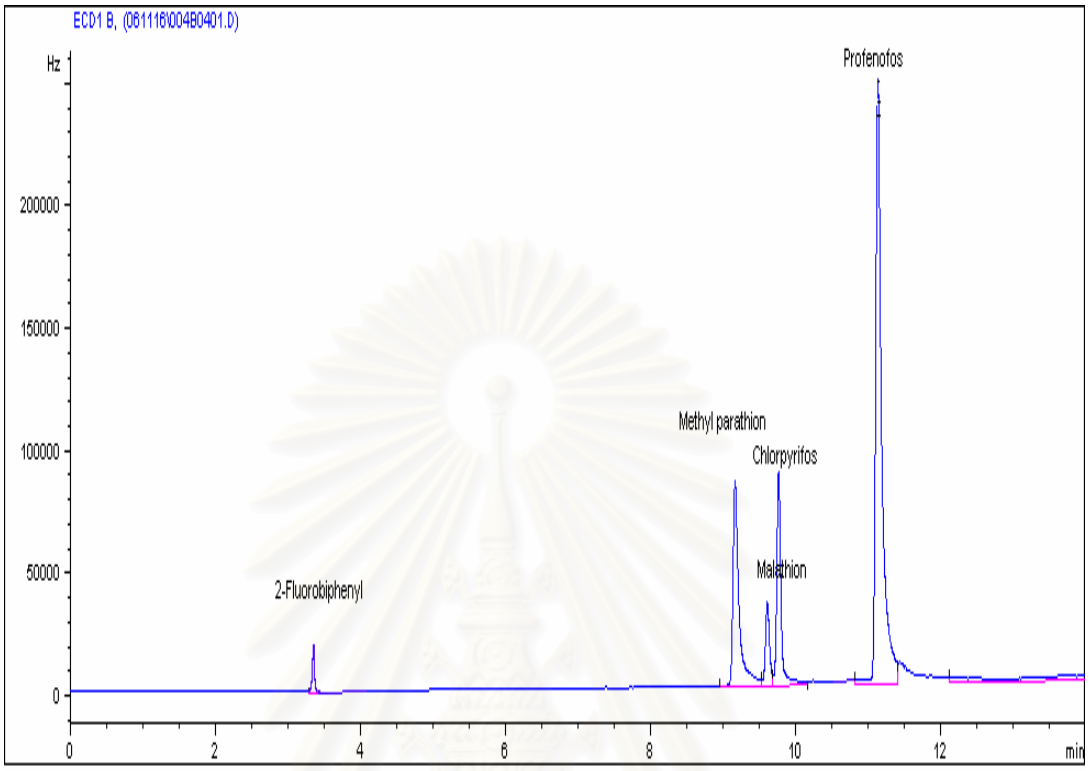
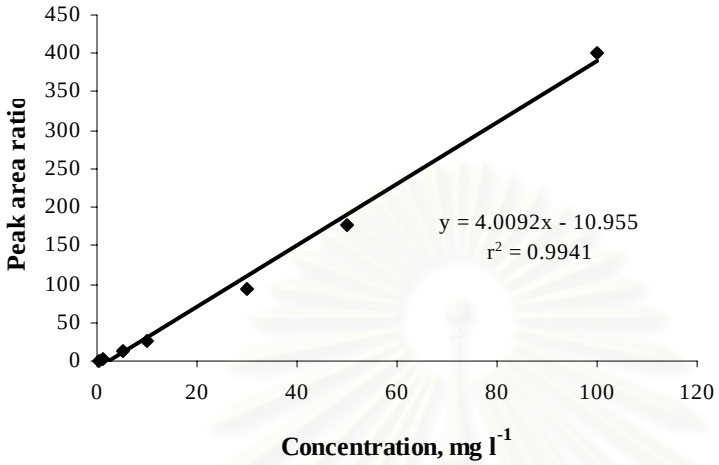
APPENDICES

APPENDIX A-Chromatogram of tested compounds

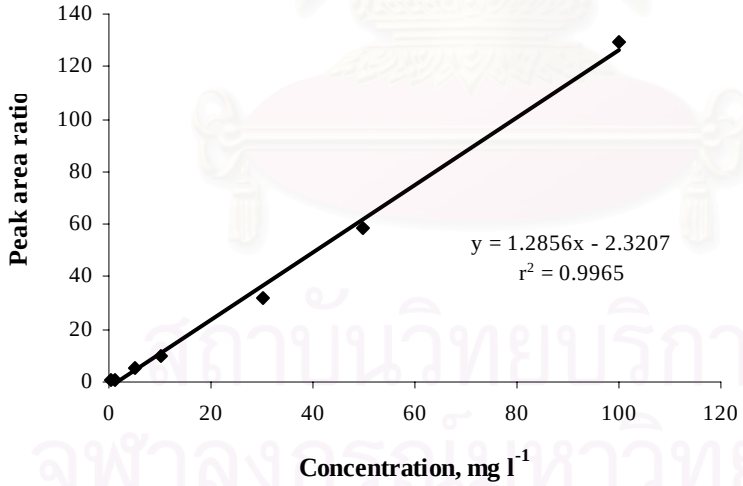


APPENDIX B-Calibration curve

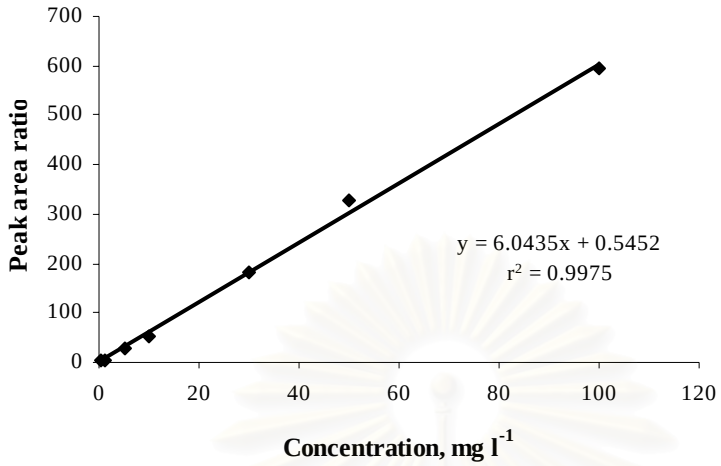
B.1 Methyl parathion



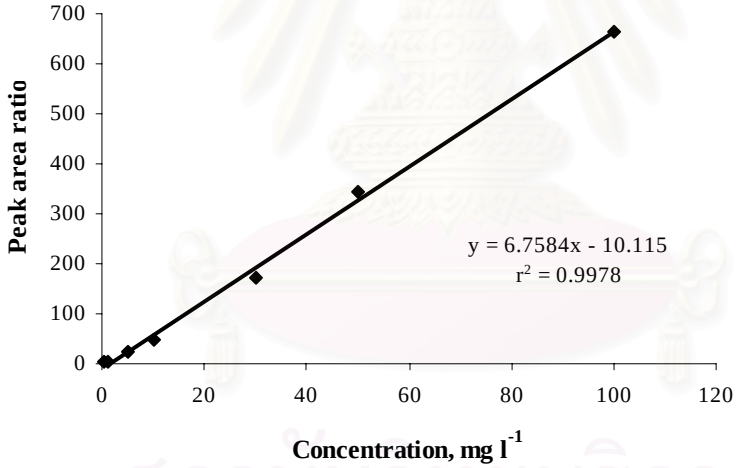
B.2 Malathion



B.3 Chlorpyrifos



B.4 Profenofos



APPENDIX C-Biomass

Rice husk

Coconut husk

Peanut Shell

Peat moss

APPENDIX D-Batch partitioning experiments

D.1 Preliminary experiment for effect of pH determination

D.1.1 DI water adjusted pH 7

D.1.1.1 Methyl parathion

Sorbent	Ce	Ci	Ci-Ce (mg/l)	Mass (g)	Solution (ml)	Media (mg/g)
Coconut husk	2.1545	15.00	12.8455	1.00	40.00	0.5138
	1.3983	10.80	9.4017	1.00	40.00	0.3761
	0.9425	7.20	6.2575	1.00	40.00	0.2503
	0.6605	3.00	2.3395	1.00	40.00	0.0936
Rice husk	2.3011	15.00	12.6989	1.00	40.00	0.5080
	1.8542	10.80	8.9458	1.00	40.00	0.3578
	1.1616	7.20	6.0384	1.00	40.00	0.2415
	0.7089	3.00	2.2911	1.00	40.00	0.0916
Peat moss	1.9708	15.00	13.0292	1.00	40.00	0.5212
	1.2581	10.80	9.5419	1.00	40.00	0.3817
	1.0477	7.20	6.1523	1.00	40.00	0.2461
	0.8465	3.00	2.1535	1.00	40.00	0.0861
Peanut shell	1.1785	15.00	13.8215	1.00	40.00	0.5529
	1.0925	10.80	9.7075	1.00	40.00	0.3883
	0.7730	7.20	6.4270	1.00	40.00	0.2571
	0.4175	3.00	2.5825	1.00	40.00	0.1033
Soil	3.1108	15.00	11.8892	10.00	40.00	0.0476
	2.6913	10.80	8.1087	10.00	40.00	0.0324
	1.3388	7.20	5.8612	10.00	40.00	0.0234
	0.5892	3.00	2.4108	10.00	40.00	0.0096

D.1.1.2 Malathion

Sorbent	Ce	Ci	Ci-Ce (mg/l)	Mass (g)	Solution (ml)	Media (mg/g)
Coconut husk	11.2384	32.50	21.2616	1.00	40.00	0.8505
	4.5244	23.40	18.8756	1.00	40.00	0.7550
	3.0647	15.60	12.5353	1.00	40.00	0.5014
	2.0599	6.50	4.4401	1.00	40.00	0.1776
Rice husk	14.3835	32.50	18.1165	1.00	40.00	0.7247
	6.3621	23.40	17.0379	1.00	40.00	0.6815
	4.2051	15.60	11.3949	1.00	40.00	0.4558
	2.4836	6.50	4.0164	1.00	40.00	0.1607
Peat moss	4.1197	32.50	28.3803	1.00	40.00	1.1352
	3.1779	23.40	20.2221	1.00	40.00	0.8089
	2.5394	15.60	13.0606	1.00	40.00	0.5224
	1.5271	6.50	4.9729	1.00	40.00	0.1989
Peanut shell	6.1913	32.50	26.3087	1.00	40.00	1.0523
	4.1389	23.40	19.2611	1.00	40.00	0.7704
	3.2014	15.60	12.3986	1.00	40.00	0.4959
	1.6819	6.50	4.8181	1.00	40.00	0.1927
Soil	22.6141	32.50	9.8859	10.00	40.00	0.0395
	13.0039	23.40	10.3961	10.00	40.00	0.0416
	6.2254	15.60	9.3746	10.00	40.00	0.0375
	4.1411	6.50	2.3589	10.00	40.00	0.0094

D.1.1.3 Chlorpyrifos

Sorbent	Ce	Ci	Ci-Ce (mg/l)	Mass (g)	Solution (ml)	Media (mg/g)
Coconut husk	0.0154	0.50	0.4846	1.00	40.00	0.0194
	0.0119	0.36	0.3481	1.00	40.00	0.0139
	0.0000	0.24	0.2400	1.00	40.00	0.0096
	0.0000	0.10	0.1000	1.00	40.00	0.0040
Rice husk	0.4680	0.50	0.0320	1.00	40.00	0.0013
	0.0301	0.36	0.3299	1.00	40.00	0.0132
	0.0000	0.24	0.2400	1.00	40.00	0.0096
	0.0000	0.10	0.1000	1.00	40.00	0.0040
Peat moss	0.0424	0.50	0.4576	1.00	40.00	0.0183
	0.0000	0.36	0.3600	1.00	40.00	0.0144
	0.0000	0.24	0.2400	1.00	40.00	0.0096
	0.0000	0.10	0.1000	1.00	40.00	0.0040
Peanut shell	0.0973	0.50	0.4027	1.00	40.00	0.0161
	0.0413	0.36	0.3187	1.00	40.00	0.0127
	0.0000	0.24	0.2400	1.00	40.00	0.0096
	0.0000	0.10	0.1000	1.00	40.00	0.0040
Soil	0.0542	0.50	0.4458	10.00	40.00	0.0018
	0.0000	0.36	0.3600	10.00	40.00	0.0014
	0.0000	0.24	0.2400	10.00	40.00	0.0010
	0.0000	0.10	0.1000	10.00	40.00	0.0004

D.1.1.4 Profenofos

Sorbent	Ce	Ci	Ci-Ce (mg/l)	Mass (g)	Solution (ml)	Media (mg/g)
Coconut husk	0.4538	7.00	6.5462	1.00	40.00	0.2619
	0.3652	5.04	4.6748	1.00	40.00	0.1870
	0.3503	3.36	3.0097	1.00	40.00	0.1204
	0.3160	1.40	1.0840	1.00	40.00	0.0434
Rice husk	0.5605	7.00	6.4395	1.00	40.00	0.2576
	0.4325	5.04	4.6075	1.00	40.00	0.1843
	0.3907	3.36	2.9693	1.00	40.00	0.1188
	0.3216	1.40	1.0784	1.00	40.00	0.0431
Peat moss	0.3521	7.00	6.6479	1.00	40.00	0.2659
	0.3304	5.04	4.7096	1.00	40.00	0.1884
	0.3214	3.36	3.0386	1.00	40.00	0.1215
	0.3160	1.40	1.0840	1.00	40.00	0.0434
Peanut shell	0.5066	7.00	6.4934	1.00	40.00	0.2597
	0.4362	5.04	4.6038	1.00	40.00	0.1842
	0.4243	3.36	2.9357	1.00	40.00	0.1174
	0.3322	1.40	1.0678	1.00	40.00	0.0427
Soil	0.5402	7.00	6.4598	10.00	40.00	0.0258
	0.4292	5.04	4.6108	10.00	40.00	0.0184
	0.3676	3.36	2.9924	10.00	40.00	0.0120
	0.3254	1.40	1.0746	10.00	40.00	0.0043

K_D of OPs in biomass and soil at pH 7

Sorbent	Methyl parathion		Malathion		Chlorpyrifos		Profenofos	
	K_D	r^2	K_D	r^2	K_D	r^2	K_D	r^2
Coconut	0.2753	0.96	0.0574	0.62	0.7502	0.85	1.5011	0.89
Rice Husk	0.2702	0.99	0.0386	0.62	0.0158	0.46	0.8901	0.95
Peat	0.3585	0.90	0.3660	1.00	0.2117	0.53	5.6837	0.91
Peanut Shell	0.5345	0.93	0.1933	0.98	0.0977	0.77	1.2542	0.94
Soil	0.0134	0.93	0.0010	0.35	0.0157	0.50	0.0959	0.95

D.1.2 DI water adjusted pH 5

D.1.2.1 Methyl parathion

Sorbent	Ce	Ci	Ci-Ce (mg/l)	Mass (g)	Solution (ml)	Media (mg/g)
Coconut husk	1.9572	15.00	13.0428	1.00	40.00	0.5217
	1.4573	10.80	9.3427	1.00	40.00	0.3737
	0.9898	7.20	6.2102	1.00	40.00	0.2484
	0.6506	3.00	2.3494	1.00	40.00	0.0940
Rice husk	2.0822	15.00	12.9178	1.00	40.00	0.5167
	2.0363	10.80	8.7637	1.00	40.00	0.3505
	1.2375	7.20	5.9625	1.00	40.00	0.2385
	0.7431	3.00	2.2569	1.00	40.00	0.0903
Peat moss	1.7403	15.00	13.2597	1.00	40.00	0.5304
	1.4992	10.80	9.3008	1.00	40.00	0.3720
	1.1260	7.20	6.0740	1.00	40.00	0.2430
	0.7648	3.00	2.2352	1.00	40.00	0.0894
Peanut shell	1.3966	15.00	13.6034	1.00	40.00	0.5441
	1.0957	10.80	9.7043	1.00	40.00	0.3882
	0.8329	7.20	6.3671	1.00	40.00	0.2547
	0.4116	3.00	2.5884	1.00	40.00	0.1035
Soil	2.8037	15.00	12.1963	10.00	40.00	0.0488
	2.7947	10.80	8.0053	10.00	40.00	0.0320
	0.9740	7.20	6.2260	10.00	40.00	0.0249
	0.5920	3.00	2.4080	10.00	40.00	0.0096

D.1.2.2 Malathion

Sorbent	Ce	Ci	Ci-Ce (mg/l)	Mass (g)	Solution (ml)	Media (mg/g)
Coconut husk	12.5319	32.50	19.9681	1.00	40.00	0.7987
	5.9243	23.40	17.4757	1.00	40.00	0.6990
	3.7994	15.60	11.8006	1.00	40.00	0.4720
	2.2336	6.50	4.2664	1.00	40.00	0.1707
Rice husk	14.0290	32.50	18.4710	1.00	40.00	0.7388
	8.3909	23.40	15.0091	1.00	40.00	0.6004
	5.3370	15.60	10.2630	1.00	40.00	0.4105
	2.8917	6.50	3.6083	1.00	40.00	0.1443
Peat moss	6.3621	32.50	26.1379	1.00	40.00	1.0455
	4.2479	23.40	19.1521	1.00	40.00	0.7661
	3.0904	15.60	12.5096	1.00	40.00	0.5004
	1.6339	6.50	4.8661	1.00	40.00	0.1946
Peanut shell	10.0994	32.50	22.4006	1.00	40.00	0.8960
	4.2265	23.40	19.1735	1.00	40.00	0.7669
	4.0941	15.60	11.5059	1.00	40.00	0.4602
	1.5830	6.50	4.9170	1.00	40.00	0.1967
Soil	18.3151	32.50	14.1849	10.00	40.00	0.0567
	13.2516	23.40	10.1484	10.00	40.00	0.0406
	4.2692	15.60	11.3308	10.00	40.00	0.0453
	0.6899	6.50	5.8101	10.00	40.00	0.0232

D.1.2.3 Chlorpyrifos

Sorbent	Ce	Ci	Ci-Ce (mg/l)	Mass (g)	Solution (ml)	Media (mg/g)
Coconut husk	0.0373	0.50	0.4627	1.00	40.00	0.0185
	0.0000	0.36	0.3600	1.00	40.00	0.0144
	0.0000	0.24	0.2400	1.00	40.00	0.0096
	0.0000	0.10	0.1000	1.00	40.00	0.0040
Rice husk	0.0168	0.50	0.4832	1.00	40.00	0.0193
	0.0133	0.36	0.3467	1.00	40.00	0.0139
	0.0109	0.24	0.2291	1.00	40.00	0.0092
	0.0082	0.10	0.0919	1.00	40.00	0.0037
Peat moss	0.0148	0.50	0.4852	1.00	40.00	0.0194
	0.0055	0.36	0.3545	1.00	40.00	0.0142
	0.0000	0.24	0.2400	1.00	40.00	0.0096
	0.0000	0.10	0.1000	1.00	40.00	0.0040
Peanut shell	0.0596	0.50	0.4404	1.00	40.00	0.0176
	0.0556	0.36	0.3044	1.00	40.00	0.0122
	0.0000	0.24	0.2400	1.00	40.00	0.0096
	0.0000	0.10	0.1000	1.00	40.00	0.0040
Soil	0.0642	0.50	0.4358	10.00	40.00	0.0017
	0.0447	0.36	0.3154	10.00	40.00	0.0013
	0.0000	0.24	0.2400	10.00	40.00	0.0010
	0.0000	0.10	0.1000	10.00	40.00	0.0004

D.1.2.4 Profenofos

Sorbent	Ce	Ci	Ci-Ce (mg/l)	Mass (g)	Solution (ml)	Media (mg/g)
Coconut husk	0.4776	7.00	6.5224	1.00	40.00	0.2609
	0.3997	5.04	4.6403	1.00	40.00	0.1856
	0.3692	3.36	2.9908	1.00	40.00	0.1196
	0.3245	1.40	1.0755	1.00	40.00	0.0430
Rice Husk	0.5666	7.00	6.4334	1.00	40.00	0.2573
	0.4821	5.04	4.5579	1.00	40.00	0.1823
	0.4321	3.36	2.9279	1.00	40.00	0.1171
	0.3473	1.40	1.0527	1.00	40.00	0.0421
Peat moss	0.3643	7.00	6.6357	1.00	40.00	0.2654
	0.3469	5.04	4.6931	1.00	40.00	0.1877
	0.3381	3.36	3.0219	1.00	40.00	0.1209
	0.3114	1.40	1.0886	1.00	40.00	0.0435
Peanut Shell	0.5874	7.00	6.4126	1.00	40.00	0.2565
	0.4647	5.04	4.5753	1.00	40.00	0.1830
	0.4192	3.36	2.9408	1.00	40.00	0.1176
	0.3312	1.40	1.0688	1.00	40.00	0.0428
Soil	0.4960	7.00	6.5040	10.00	40.00	0.0260
	0.4488	5.04	4.5912	10.00	40.00	0.0184
	0.3684	3.36	2.9916	10.00	40.00	0.0120
	0.3303	1.40	1.0697	10.00	40.00	0.0043

K_D of OPs in biomass and soil at pH 5

Sorbent	Methyl parathion		Malathion		Chlorpyrifos		Profenofos	
	K_D	r^2	K_D	r^2	K_D	r^2	K_D	r^2
Coconut husk	0.3203	0.99	0.0523	0.72	0.2460	0.54	1.5495	0.98
Rice husk	0.2546	0.88	0.0505	0.89	0.1158	0.47	1.0866	0.99
Peat moss	0.4206	0.98	0.1807	0.98	0.1898	0.52	4.2174	0.97
Peanut shell	0.4428	0.99	0.0738	0.72	0.1436	0.71	0.8451	0.98
Soil	0.0129	0.78	0.0025	0.58	0.0157	0.82	0.1212	0.97

D.1.3 DI water adjusted pH 3

D.1.3.1 Methyl parathion

Sorbent	Ce	Ci	Ci-Ce (mg/l)	Mass (g)	Solution (ml)	Media (mg/g)
Coconut husk	1.6148	15.00	13.3852	1.00	40.00	0.5354
	0.8321	10.80	9.9679	1.00	40.00	0.3987
	0.7280	7.20	6.4720	1.00	40.00	0.2589
	0.6506	3.00	2.3494	1.00	40.00	0.0940
Rice husk	1.9161	15.00	13.0839	1.00	40.00	0.5234
	1.7244	10.80	9.0756	1.00	40.00	0.3630
	1.2074	7.20	5.9926	1.00	40.00	0.2397
	0.7417	3.00	2.2583	1.00	40.00	0.0903
Peat moss	1.7189	15.00	13.2811	1.00	40.00	0.5312
	1.3568	10.80	9.4432	1.00	40.00	0.3777
	0.8706	7.20	6.3294	1.00	40.00	0.2532
	0.6846	3.00	2.3154	1.00	40.00	0.0926
Peanut shell	1.1762	15.00	13.8238	1.00	40.00	0.5530
	1.1848	10.80	9.6152	1.00	40.00	0.3846
	0.7238	7.20	6.4762	1.00	40.00	0.2590
	0.7131	3.00	2.2869	1.00	40.00	0.0915
Soil	2.3447	15.00	12.6553	10.00	40.00	0.0506
	2.2933	10.80	8.5067	10.00	40.00	0.0340
	1.3260	7.20	5.8740	10.00	40.00	0.0235
	1.2447	3.00	1.7553	10.00	40.00	0.0070

D.1.3.2 Malathion

Sorbent	Ce	Ci	Ci-Ce (mg/l)	Mass (g)	Solution (ml)	Media (mg/g)
Coconut husk	10.6760	32.50	21.8240	1.00	40.00	0.8730
	3.2014	23.40	20.1986	1.00	40.00	0.8079
	0.9740	15.60	14.6260	1.00	40.00	0.5850
	0.5075	6.50	5.9925	1.00	40.00	0.2397
Rice husk	11.6584	32.50	20.8416	1.00	40.00	0.8337
	6.8533	23.40	16.5467	1.00	40.00	0.6619
	4.9313	15.60	10.6687	1.00	40.00	0.4268
	3.5004	6.50	2.9996	1.00	40.00	0.1200
Peat moss	6.1272	32.50	26.3728	1.00	40.00	1.0549
	3.6499	23.40	19.7501	1.00	40.00	0.7900
	2.2169	15.60	13.3831	1.00	40.00	0.5353
	1.6744	6.50	4.8256	1.00	40.00	0.1930
Peanut shell	11.6860	32.50	20.8140	1.00	40.00	0.8326
	6.1251	23.40	17.2749	1.00	40.00	0.6910
	4.5084	15.60	11.0916	1.00	40.00	0.4437
	2.3971	6.50	4.1029	1.00	40.00	0.1641
Soil	15.5025	32.50	16.9975	10.00	40.00	0.0680
	15.2463	23.40	8.1537	10.00	40.00	0.0326
	8.0065	15.60	7.5935	10.00	40.00	0.0304
	4.5682	6.50	1.9318	10.00	40.00	0.0077

D.1.3.3 Chlorpyrifos

Sorbent	Ce	Ci	Ci-Ce (mg/l)	Mass (g)	Solution (ml)	Media (mg/g)
Coconut husk	0.0492	0.50	0.4508	1.00	40.00	0.0180
	0.0000	0.36	0.3600	1.00	40.00	0.0144
	0.0000	0.24	0.2400	1.00	40.00	0.0096
	0.0000	0.10	0.1000	1.00	40.00	0.0040
Rice husk	0.0392	0.50	0.4608	1.00	40.00	0.0184
	0.0000	0.36	0.3600	1.00	40.00	0.0144
	0.0000	0.24	0.2400	1.00	40.00	0.0096
	0.0000	0.10	0.1000	1.00	40.00	0.0040
Peat moss	0.0000	0.50	0.5000	1.00	40.00	0.0200
	0.0000	0.36	0.3600	1.00	40.00	0.0144
	0.0000	0.24	0.2400	1.00	40.00	0.0096
	0.0000	0.10	0.1000	1.00	40.00	0.0040
Peanut shell	0.0464	0.50	0.4536	1.00	40.00	0.0181
	0.0000	0.36	0.3600	1.00	40.00	0.0144
	0.0000	0.24	0.2400	1.00	40.00	0.0096
	0.0000	0.10	0.1000	1.00	40.00	0.0040
Soil	0.0298	0.50	0.4702	10.00	40.00	0.0188
	0.0293	0.36	0.3307	10.00	40.00	0.0132
	0.0000	0.24	0.2400	10.00	40.00	0.0096
	0.0000	0.10	0.1000	10.00	40.00	0.0040

D.1.3.4 Profenofos

Sorbent	Ce	Ci	Ci-Ce (mg/l)	Mass (g)	Solution (ml)	Media (mg/g)
Coconut husk	0.4338	7.00	6.5662	1.00	40.00	0.2626
	0.3456	5.04	4.6944	1.00	40.00	0.1878
	0.3069	3.36	3.0531	1.00	40.00	0.1221
	0.3134	1.40	1.0866	1.00	40.00	0.0435
Rice husk	0.7503	7.00	6.2497	1.00	40.00	0.2500
	0.4171	5.04	4.6229	1.00	40.00	0.1849
	0.4281	3.36	2.9319	1.00	40.00	0.1173
	0.3412	1.40	1.0588	1.00	40.00	0.0424
Peat moss	0.3550	7.00	6.6450	1.00	40.00	0.2658
	0.3307	5.04	4.7093	1.00	40.00	0.1884
	0.3180	3.36	3.0420	1.00	40.00	0.1217
	0.3077	1.40	1.0923	1.00	40.00	0.0437
Peanut shell	0.5967	7.00	6.4033	1.00	40.00	0.2561
	0.4309	5.04	4.6091	1.00	40.00	0.1844
	0.4856	3.36	2.8744	1.00	40.00	0.1150
	0.4273	1.40	0.9727	1.00	40.00	0.0389
Soil	0.5484	7.00	6.4516	10.00	40.00	0.0258
	0.5431	5.04	4.4969	10.00	40.00	0.0180
	0.4147	3.36	2.9453	10.00	40.00	0.0118
	0.4127	1.40	0.9873	10.00	40.00	0.0039

K_D of OPs in biomass and soil at pH 3

Sorbent	Methyl parathion		Malathion		Chlorpyrifos		Profenofos	
	K_D	r^2	K_D	r^2	K_D	r^2	K_D	r^2
Coconut husk	0.3618	0.72	0.0433	0.50	0.1768	0.51	1.4044	0.77
Rice husk	0.3389	0.95	0.0796	0.84	0.2331	0.53	0.4234	0.76
Peat moss	0.3886	0.96	0.1738	0.88	0.1898	0.52	4.5459	0.96
Peanut shell	0.6358	0.76	0.0533	0.55	0.2701	0.71	0.8289	0.76
Soil	0.0271	0.78	0.0037	0.65	0.0336	0.79	0.1078	0.79

D.2 Effect of pH

D.2.1 Phosphate buffer pH 7

D.2.1.1 Methyl parathion

Sorbent	Ce	Ci	Ci-Ce (mg/l)	Mass (g)	Solution (ml)	Media (mg/g)
Coconut husk	0.1027	3.00	2.8973	1.00	40.00	0.1159
	0.2891	7.20	6.9109	1.00	40.00	0.2764
	0.5629	10.80	10.2371	1.00	40.00	0.4095
	0.9170	15.00	14.0830	1.00	40.00	0.5633
Peat moss	0.0656	3.00	2.9344	1.00	40.00	0.1174
	0.1734	7.20	7.0266	1.00	40.00	0.2811
	0.3260	10.80	10.4740	1.00	40.00	0.4190
	0.5337	15.00	14.4663	1.00	40.00	0.5787
Rice husk	0.1026	3.00	2.8974	1.00	40.00	0.1159
	0.2245	7.20	6.9755	1.00	40.00	0.2790
	0.3272	10.80	10.4728	1.00	40.00	0.4189
	0.7509	15.00	14.2491	1.00	40.00	0.5700
Peanut shell	0.1559	3.00	2.8441	1.00	40.00	0.1138
	0.2950	7.20	6.9050	1.00	40.00	0.2762
	0.5978	10.80	10.2022	1.00	40.00	0.4081
	1.0883	15.00	13.9117	1.00	40.00	0.5565
Soil	0.3610	3.00	2.6390	1.00	40.00	0.0106
	0.6820	7.20	6.5180	1.00	40.00	0.0261
	1.6671	10.80	9.1329	1.00	40.00	0.0365
	2.4633	15.00	12.5367	1.00	40.00	0.0501

D.2.1.2 Malathion

Sorbent	Ce	Ci	Ci-Ce (mg/l)	Mass (g)	Solution (ml)	Media (mg/g)
Coconut husk	0.5449	6.50	21.2616	1.00	40.00	0.2382
	1.3714	15.60	18.8756	1.00	40.00	0.5691
	2.3223	23.40	12.5353	1.00	40.00	0.8431
	3.9530	32.50	4.4401	1.00	40.00	1.1419
Peat moss	0.3637	6.50	18.1165	1.00	40.00	0.2455
	0.9257	15.60	17.0379	1.00	40.00	0.5870
	1.5133	23.40	11.3949	1.00	40.00	0.8755
	2.7099	32.50	4.0164	1.00	40.00	1.1916
Rice husk	0.7913	6.50	28.3803	1.00	40.00	0.2283
	1.4797	15.60	20.2221	1.00	40.00	0.5648
	1.7812	23.40	13.0606	1.00	40.00	0.8648
	4.2518	32.50	4.9729	1.00	40.00	1.1299
Peanut shell	0.7610	6.50	26.3087	1.00	40.00	0.2296
	1.5703	15.60	19.2611	1.00	40.00	0.5612
	2.7867	23.40	12.3986	1.00	40.00	0.8245
	5.0640	32.50	4.8181	1.00	40.00	1.0974
Soil	1.1365	6.50	9.8859	10.00	40.00	0.0215
	2.1118	15.60	10.3961	10.00	40.00	0.0540
	4.2601	23.40	9.3746	10.00	40.00	0.0766
	7.2291	32.50	2.3589	10.00	40.00	0.1011

D.2.1.3 Chlorpyrifos

Sorbent	Ce	Ci	Ci-Ce (mg/l)	Mass (g)	Solution (ml)	Media (mg/g)
Coconut husk	0.0000	0.10	0.1000	1.00	40.00	0.0040
	0.0049	0.24	0.2351	1.00	40.00	0.0094
	0.0072	0.36	0.3528	1.00	40.00	0.0141
	0.0107	0.50	0.4893	1.00	40.00	0.0196
Peat moss	0.0000	0.10	0.1000	1.00	40.00	0.0040
	0.0023	0.24	0.2377	1.00	40.00	0.0095
	0.0030	0.36	0.3570	1.00	40.00	0.0143
	0.0048	0.50	0.4952	1.00	40.00	0.0198
Rice husk	0.0000	0.10	0.1000	1.00	40.00	0.0040
	0.0055	0.24	0.2345	1.00	40.00	0.0094
	0.0081	0.36	0.3519	1.00	40.00	0.0141
	0.0119	0.50	0.4881	1.00	40.00	0.0195
Peanut shell	0.0000	0.10	0.1000	1.00	40.00	0.0040
	0.0069	0.24	0.2331	1.00	40.00	0.0093
	0.0106	0.36	0.3494	1.00	40.00	0.0140
	0.0141	0.50	0.4859	1.00	40.00	0.0194
Soil	0.0000	0.10	0.1000	10.00	40.00	0.0004
	0.0067	0.24	0.2333	10.00	40.00	0.0009
	0.0105	0.36	0.3495	10.00	40.00	0.0014
	0.0141	0.50	0.4859	10.00	40.00	0.0019

D.2.1.4 Profenofos

Sorbent	Ce	Ci	Ci-Ce (mg/l)	Mass (g)	Solution (ml)	Media (mg/g)
Coconut husk	0.0097	1.40	1.3903	1.00	40.00	0.0556
	0.0243	3.36	3.3357	1.00	40.00	0.1334
	0.0383	5.04	5.0017	1.00	40.00	0.2001
	0.0777	7.00	6.9223	1.00	40.00	0.2769
Peat moss	0.0047	1.40	1.3953	1.00	40.00	0.0558
	0.0138	3.36	3.3462	1.00	40.00	0.1338
	0.0214	5.04	5.0186	1.00	40.00	0.2007
	0.0367	7.00	6.9633	1.00	40.00	0.2785
Rice husk	0.0175	1.40	1.3825	1.00	40.00	0.0553
	0.0366	3.36	3.3234	1.00	40.00	0.1329
	0.0451	5.04	4.9949	1.00	40.00	0.1998
	0.1151	7.00	6.8849	1.00	40.00	0.2754
Peanut shell	0.0222	1.40	1.3778	1.00	40.00	0.0551
	0.0456	3.36	3.3144	1.00	40.00	0.1326
	0.0823	5.04	4.9577	1.00	40.00	0.1983
	0.1509	7.00	6.8491	1.00	40.00	0.2740
Soil	0.0336	1.40	1.3664	10.00	40.00	0.0055
	0.0588	3.36	3.3012	10.00	40.00	0.0132
	0.1123	5.04	4.9277	10.00	40.00	0.0197
	0.1669	7.00	6.8331	10.00	40.00	0.0273

D.2.2 Acetate buffer pH 5

D.2.2.1 Methyl parathion

Sorbent	Ce	Ci	Ci-Ce (mg/l)	Mass (g)	Solution (ml)	Media (mg/g)
Coconut husk	0.3544	3.00	2.6456	1.00	40.00	0.1058
	0.4580	7.20	6.7420	1.00	40.00	0.2697
	0.7378	10.80	10.0622	1.00	40.00	0.4025
	1.3029	15.00	13.6971	1.00	40.00	0.5479
Peat moss	0.1604	3.00	2.8396	1.00	40.00	0.1136
	0.2456	7.20	6.9544	1.00	40.00	0.2782
	0.5351	10.80	10.2649	1.00	40.00	0.4106
	0.7484	15.00	14.2516	1.00	40.00	0.5701
Rice husk	0.4191	3.00	2.5809	1.00	40.00	0.1032
	0.5822	7.20	6.6178	1.00	40.00	0.2647
	1.0466	10.80	9.7534	1.00	40.00	0.3901
	1.5090	15.00	13.4910	1.00	40.00	0.5396
Peanut shell	0.4513	3.00	2.5487	1.00	40.00	0.1019
	0.5607	7.20	6.6393	1.00	40.00	0.2656
	1.0744	10.80	9.7256	1.00	40.00	0.3890
	1.6652	15.00	13.3348	1.00	40.00	0.5334
Soil	1.0388	3.00	1.9612	10.00	40.00	0.0078
	1.4372	7.20	5.7628	10.00	40.00	0.0231
	3.0723	10.80	7.7277	10.00	40.00	0.0309
	4.3813	15.00	10.6187	10.00	40.00	0.0425

D.2.2.2 Malathion

Sorbent	Ce	Ci	Ci-Ce (mg/l)	Mass (g)	Solution (ml)	Media (mg/g)
Coconut husk	2.1145	6.50	4.3855	1.00	40.00	0.1754
	2.5836	15.60	13.0164	1.00	40.00	0.5207
	2.7056	23.40	20.6944	1.00	40.00	0.8278
	5.6716	32.50	26.8284	1.00	40.00	1.0731
Peat moss	1.1085	6.50	5.3915	1.00	40.00	0.2157
	1.6093	15.60	13.9907	1.00	40.00	0.5596
	2.1129	23.40	21.2871	1.00	40.00	0.8515
	3.5554	32.50	28.9446	1.00	40.00	1.1578
Rice husk	2.7264	6.50	3.7736	1.00	40.00	0.1509
	3.6430	15.60	11.9570	1.00	40.00	0.4783
	3.9513	23.40	19.4487	1.00	40.00	0.7779
	6.8712	32.50	25.6288	1.00	40.00	1.0252
Peanut shell	2.5674	6.50	3.9326	1.00	40.00	0.1573
	3.5323	15.60	12.0677	1.00	40.00	0.4827
	4.3111	23.40	19.0889	1.00	40.00	0.7636
	8.0616	32.50	24.4384	1.00	40.00	0.9775
Soil	3.4788	6.50	3.0212	10.00	40.00	0.0121
	4.9045	15.60	10.6955	10.00	40.00	0.0428
	7.1301	23.40	16.2699	10.00	40.00	0.0651
	11.9461	32.50	20.5539	10.00	40.00	0.0822

D.2.2.3 Chlorpyrifos

Sorbent	Ce	Ci	Ci-Ce (mg/l)	Mass (g)	Solution (ml)	Media (mg/g)
Coconut husk	0.0000	0.10	0.1000	1.00	40.00	0.0040
	0.0060	0.24	0.2340	1.00	40.00	0.0094
	0.0074	0.36	0.3526	1.00	40.00	0.0141
	0.0161	0.50	0.4839	1.00	40.00	0.0194
Peat moss	0.0000	0.10	0.1000	1.00	40.00	0.0040
	0.0003	0.24	0.2397	1.00	40.00	0.0096
	0.0036	0.36	0.3564	1.00	40.00	0.0143
	0.0072	0.50	0.4928	1.00	40.00	0.0197
Rice husk	0.0000	0.10	0.1000	1.00	40.00	0.0040
	0.0097	0.24	0.2303	1.00	40.00	0.0092
	0.0104	0.36	0.3496	1.00	40.00	0.0140
	0.0155	0.50	0.4845	1.00	40.00	0.0194
Peanut shell	0.0064	0.10	0.0936	1.00	40.00	0.0037
	0.0097	0.24	0.2303	1.00	40.00	0.0092
	0.0114	0.36	0.3486	1.00	40.00	0.0139
	0.0214	0.50	0.4786	1.00	40.00	0.0191
Soil	0.0046	0.10	0.0954	10.00	40.00	0.0004
	0.0099	0.24	0.2301	10.00	40.00	0.0009
	0.0103	0.36	0.3497	10.00	40.00	0.0014
	0.0155	0.50	0.4845	10.00	40.00	0.0019

D.2.2.4 Profenofos

Sorbent	Ce	Ci	Ci-Ce (mg/l)	Mass (g)	Solution (ml)	Media (mg/g)
Coconut husk	0.0374	1.40	1.3626	1.00	40.00	0.0545
	0.0579	3.36	3.3021	1.00	40.00	0.1321
	0.0575	5.04	4.9825	1.00	40.00	0.1993
	0.1221	7.00	6.8779	1.00	40.00	0.2751
Peat moss	0.0110	1.40	1.3890	1.00	40.00	0.0556
	0.0226	3.36	3.3374	1.00	40.00	0.1335
	0.0271	5.04	5.0129	1.00	40.00	0.2005
	0.0460	7.00	6.9540	1.00	40.00	0.2782
Rice Husk	0.0644	1.40	1.3356	1.00	40.00	0.0534
	0.1227	3.36	3.2373	1.00	40.00	0.1295
	0.1228	5.04	4.9172	1.00	40.00	0.1967
	0.2427	7.00	6.7573	1.00	40.00	0.2703
Peanut Shell	0.0700	1.40	1.3300	1.00	40.00	0.0532
	0.1205	3.36	3.2395	1.00	40.00	0.1296
	0.1435	5.04	4.8965	1.00	40.00	0.1959
	0.2715	7.00	6.7285	1.00	40.00	0.2691
Soil	0.0869	1.40	1.3131	10.00	40.00	0.0053
	0.1493	3.36	3.2107	10.00	40.00	0.0128
	0.1796	5.04	4.8604	10.00	40.00	0.0194
	0.3017	7.00	6.6983	10.00	40.00	0.0268

D.2.3 Acetate buffer pH 3

D.2.3.1 Methyl parathion

Sorbent	Ce	Ci	Ci-Ce (mg/l)	Mass (g)	Solution (ml)	Media (mg/g)
Coconut husk	0.1971	3.00	2.8029	1.00	40.00	0.1121
	0.4774	7.20	6.7226	1.00	40.00	0.2689
	0.8658	10.80	9.9342	1.00	40.00	0.3974
	1.0669	15.00	13.9331	1.00	40.00	0.5573
Peat moss	0.1091	3.00	2.8909	1.00	40.00	0.1156
	0.2267	7.20	6.9733	1.00	40.00	0.2789
	0.4614	10.80	10.3386	1.00	40.00	0.4135
	0.5689	15.00	14.4311	1.00	40.00	0.5772
Rice husk	0.3194	3.00	2.6806	1.00	40.00	0.1072
	0.7561	7.20	6.4439	1.00	40.00	0.2578
	1.2820	10.80	9.5180	1.00	40.00	0.3807
	1.4833	15.00	13.5167	1.00	40.00	0.5407
Peanut shell	0.2115	3.00	2.7885	1.00	40.00	0.1115
	0.7223	7.20	6.4777	1.00	40.00	0.2591
	0.9586	10.80	9.8414	1.00	40.00	0.3937
	1.5291	15.00	13.4709	1.00	40.00	0.5388
Soil	0.3550	3.00	2.6450	10.00	40.00	0.0106
	0.9176	7.20	6.2824	10.00	40.00	0.0251
	1.1849	10.80	9.6151	10.00	40.00	0.0385
	2.6020	15.00	12.3980	10.00	40.00	0.0496

D.2.3.2 Malathion

Sorbent	Ce	Ci	Ci-Ce (mg/l)	Mass (g)	Solution (ml)	Media (mg/g)
Coconut husk	0.8470	6.50	5.6530	1.00	40.00	0.2261
	3.6759	15.60	11.9241	1.00	40.00	0.4770
	4.3545	23.40	19.0455	1.00	40.00	0.7618
	4.3981	32.50	28.1019	1.00	40.00	1.1241
Peat moss	0.5379	6.50	5.9621	1.00	40.00	0.2385
	1.9176	15.60	13.6824	1.00	40.00	0.5473
	2.6153	23.40	20.7847	1.00	40.00	0.8314
	2.6908	32.50	29.8092	1.00	40.00	1.1924
Rice husk	3.1946	6.50	3.3054	1.00	40.00	0.1322
	6.1042	15.60	9.4958	1.00	40.00	0.3798
	6.8470	23.40	16.5530	1.00	40.00	0.6621
	6.6561	32.50	25.8439	1.00	40.00	1.0338
Peanut shell	1.0751	6.50	5.4249	1.00	40.00	0.2170
	5.7217	15.60	9.8783	1.00	40.00	0.3951
	5.4564	23.40	17.9436	1.00	40.00	0.7177
	7.2632	32.50	25.2368	1.00	40.00	1.0095
Soil	1.5631	6.50	4.9369	10.00	40.00	0.0197
	5.8786	15.60	9.7214	10.00	40.00	0.0389
	6.5057	23.40	16.8943	10.00	40.00	0.0676
	9.3858	32.50	23.1142	10.00	40.00	0.0925

D.2.3.3 Chlorpyrifos

Sorbent	Ce	Ci	Ci-Ce (mg/l)	Mass (g)	Solution (ml)	Media (mg/g)
Coconut husk	0.0022	0.10	0.0978	1.00	40.00	0.0039
	0.0091	0.24	0.2309	1.00	40.00	0.0092
	0.0072	0.36	0.3528	1.00	40.00	0.0141
	0.0082	0.50	0.4918	1.00	40.00	0.0197
Peat moss	0.0000	0.10	0.1000	1.00	40.00	0.0040
	0.0052	0.24	0.2348	1.00	40.00	0.0094
	0.0067	0.36	0.3533	1.00	40.00	0.0141
	0.0049	0.50	0.4951	1.00	40.00	0.0198
Rice husk	0.0117	0.10	0.0883	1.00	40.00	0.0035
	0.0130	0.24	0.2270	1.00	40.00	0.0091
	0.0102	0.36	0.3498	1.00	40.00	0.0140
	0.0103	0.50	0.4897	1.00	40.00	0.0196
Peanut shell	0.0000	0.10	0.1000	1.00	40.00	0.0040
	0.0211	0.24	0.2189	1.00	40.00	0.0088
	0.0145	0.36	0.3455	1.00	40.00	0.0138
	0.0189	0.50	0.4811	1.00	40.00	0.0192
Soil	0.0000	0.10	0.1000	10.00	40.00	0.0004
	0.0126	0.24	0.2274	10.00	40.00	0.0009
	0.0098	0.36	0.3502	10.00	40.00	0.0014
	0.0123	0.50	0.4877	10.00	40.00	0.0020

D.2.3.4 Profenofos

Sorbent	Ce	Ci	Ci-Ce (mg/l)	Mass (g)	Solution (ml)	Media (mg/g)
Coconut husk	0.0524	1.40	1.3476	1.00	40.00	0.0539
	0.0612	3.36	3.2988	1.00	40.00	0.1320
	0.0701	5.04	4.9699	1.00	40.00	0.1988
	0.0965	7.00	6.9035	1.00	40.00	0.2761
Peat moss	0.0233	1.40	1.3767	1.00	40.00	0.0551
	0.0215	3.36	3.3385	1.00	40.00	0.1335
	0.0268	5.04	5.0132	1.00	40.00	0.2005
	0.0341	7.00	6.9659	1.00	40.00	0.2786
Rice husk	0.1147	1.40	1.2853	1.00	40.00	0.0514
	0.1528	3.36	3.2072	1.00	40.00	0.1283
	0.1658	5.04	4.8742	1.00	40.00	0.1950
	0.2103	7.00	6.7897	1.00	40.00	0.2716
Peanut shell	0.0856	1.40	1.3144	1.00	40.00	0.0526
	0.1907	3.36	3.1693	1.00	40.00	0.1268
	0.1693	5.04	4.8707	1.00	40.00	0.1948
	0.3105	7.00	6.6895	1.00	40.00	0.2676
Soil	0.0621	1.40	1.3379	10.00	40.00	0.0054
	0.0963	3.36	3.2637	10.00	40.00	0.0131
	0.1066	5.04	4.9334	10.00	40.00	0.0197
	0.2066	7.00	6.7934	10.00	40.00	0.0272

D.3 Pair t-test

pH	OPs	K_D (L g^{-1}) Peanut Shell	Peat	Coconut Husk	Rice Husk	Soil
3	Methyl parathion	0.3316	0.9148	0.4800	0.3424	0.0164
	Malathion	0.1098	0.3714	0.1904	0.1810	0.0093
	Chlorpyrifos	0.5102	1.8776	1.6929	0.9548	0.1184
	Profenofos	0.9066	4.9290	4.7706	2.3490	0.1389
5	Methyl parathion	0.3143	0.7001	0.4170	0.3674	0.0089
	Malathion	0.1318	0.3642	0.1968	0.1886	0.0075
	Chlorpyrifos	0.9514	1.8656	0.9595	0.9601	0.1437
	Profenofos	1.0176	6.3697	2.2841	1.1464	0.0987
7	Methyl parathion	0.4402	0.9570	0.5321	0.6443	0.0169
	Malathion	0.1904	0.3935	0.2591	0.2305	0.0120
	Chlorpyrifos	1.0722	3.3714	1.4754	1.3165	0.1079
	Profenofos	1.6139	6.9421	3.1165	2.0201	0.1548

D.3.1 pH 3 and pH 5

Methyl parathion	pH 3	pH 5	d	d-d'	(d-d')^2
Peanut shell	0.3316	0.3143	0.0173	-0.0429	0.0018
Peat moss	0.9148	0.7001	0.2147	0.1545	0.0239
Coconut husk	0.4800	0.4170	0.0630	0.0028	0.0000
Rice husk	0.3424	0.3674	-0.0250	-0.0852	0.0073
Soil	0.0164	0.0089	0.0075	-0.0527	0.0028
		Sum	0.2775		0.0358
		d'	0.0555		
		S	0.0945		
		Sd'	0.0473		
		t	1.1740		
		t=.05,df=4	2.7760		
		t>p	n-sig		

Malathion	pH 3	pH 5	d	d-d'	(d-d')^2
Peanut shell	0.1098	0.1318	-0.0220	-0.0822	0.0068
Peat moss	0.3714	0.3642	0.0072	-0.0530	0.0028
Coconut husk	0.1904	0.1968	-0.0064	-0.0666	0.0044
Rice husk	0.1810	0.1886	-0.0076	-0.0678	0.0046
Soil	0.0093	0.0075	0.0018	-0.0584	0.0034
		Sum	-0.0270		0.0220
		d'	-0.0054		
		S	0.0742		
		Sd'	0.0371		
		t	-0.1456		
		t=.05,df=4	2.7760		
		t>p	n-sig		

n-sig = not significantly

Chlorpyrifos	pH 3	pH 5	d	d-d'	(d-d')^2
Peanut shell	0.5102	0.9514	-0.4412	-0.5014	0.2514
Peat moss	1.8776	1.8656	0.0120	-0.0482	0.0023
Coconut husk	1.6929	0.9595	0.7334	0.6732	0.4532
Rice husk	0.9548	0.9601	-0.0053	-0.0655	0.0043
Soil	0.1184	0.1437	-0.0253	-0.0855	0.0073
		Sum	0.2736		0.7185
		d'	0.0547		
		S	0.4238		
		Sd'	0.2119		
		t	0.2582		
		t=.05,df=4	2.7760		
		t>p	n-sig		

Profenofos	pH 3	pH 5	d	d-d'	(d-d')^2
Peanut shell	0.9066	1.0176	-0.1110	-0.1712	0.0293
Peat moss	4.9290	6.3697	-1.4407	-1.5009	2.2527
Coconut husk	4.7706	2.2841	2.4865	2.4263	5.8869
Rice husk	2.3490	1.1464	1.2026	1.1424	1.3051
Soil	0.1389	0.0987	0.0402	-0.0200	0.0004
		Sum	2.1776		9.4744
		d'	0.4355		
		S	1.5390		
		Sd'	0.7695		
		t	0.5660		
		t=.05,df=4	2.7760		
		t>p	n-sig		

D.3.2 pH 5 and pH 7

Methyl parathion	pH 5	pH 7	d	d-d'	(d-d')^2
Peanut shell	0.3143	0.4402	-0.1259	-0.1861	0.0346
Peat moss	0.7001	0.9570	-0.2569	-0.3171	0.1006
Coconut husk	0.4170	0.5321	-0.1151	-0.1753	0.0307
Rice husk	0.3674	0.6443	-0.2769	-0.3371	0.1136
Soil	0.0089	0.0169	-0.0080	-0.0682	0.0047
		Sum	-0.7828		0.2842
		d'	-0.1566		
		S	0.2666		
		Sd'	0.1333		
		t	-1.1747		
		t=.05,df=4	2.7760		
		t>p	n-sig		

Malathion	pH 5	pH 7	d	d-d'	(d-d')^2
Peanut shell	0.1318	0.1904	-0.0586	-0.1188	0.0141
Peat moss	0.3642	0.3935	-0.0293	-0.0895	0.0080
Coconut husk	0.1968	0.2591	-0.0623	-0.1225	0.0150
Rice husk	0.1886	0.2305	-0.0419	-0.1021	0.0104
Soil	0.0075	0.0120	-0.0045	-0.0647	0.0042
		Sum	-0.1966		0.0517
		d'	-0.0393		
		S	0.1137		
		Sd'	0.0569		
		t	-0.6914		
		t=.05,df=4	2.7760		
		t>p	n-sig		

Chlorpyrifos	pH 5	pH 7	d	d-d'	(d-d')^2
Peanut shell	0.9514	1.0722	-0.1208	-0.1810	0.0328
Peat moss	1.8656	3.3714	-1.5058	-1.5660	2.4524
Coconut husk	0.9595	1.4754	-0.5159	-0.5761	0.3319
Rice husk	0.9601	1.3165	-0.3564	-0.4166	0.1736
Soil	0.1437	0.1079	0.0358	-0.0244	0.0006
		Sum	-2.4631		2.9912
		d'	-0.4926		
		S	0.8647		
		Sd'	0.4324		
		t	-1.1393		
		t=.05,df=4	2.7760		
		t>p	n-sig		

Profenofos	pH 5	pH 7	d	d-d'	(d-d')^2
Peanut shell	1.0176	1.6139	-0.5963	-0.6565	0.4310
Peat moss	6.3697	6.9421	-0.5724	-0.6326	0.4002
Coconut husk	2.2841	3.1165	-0.8324	-0.8926	0.7967
Rice husk	1.1464	2.0201	-0.8737	-0.9339	0.8722
Soil	0.0987	0.1548	-0.0561	-0.1163	0.0135
		Sum	-2.9309		2.5136
		d'	-0.5862		
		S	0.7927		
		Sd'	0.3964		
		t	-1.4789		
		t=.05,df=4	2.7760		
		t>p	n-sig		

D.3.3 pH 3 and pH 7

Methyl parathion	pH 3	pH 7	d	d-d'	(d-d')^2
Peanut shell	0.3316	0.4402	-0.1086	-0.1688	0.0285
Peat moss	0.9148	0.9570	-0.0422	-0.1024	0.0105
Coconut husk	0.4800	0.5321	-0.0521	-0.1123	0.0126
Rice husk	0.3424	0.6443	-0.3019	-0.3621	0.1311
Soil	0.0164	0.0169	-0.0005	-0.0607	0.0037
		Sum	-0.5053		0.1864
		d'	-0.1011		
		S	0.2159		
		Sd'	0.1079		
		t	-0.9363		
		t=.05,df=4	2.7760		
		t>p	n-sig		

Malathion	pH 3	pH 7	d	d-d'	(d-d')^2
Peanut shell	0.1098	0.1904	-0.0806	-0.1408	0.0198
Peat moss	0.3714	0.3935	-0.0221	-0.0823	0.0068
Coconut husk	0.1904	0.2591	-0.0687	-0.1289	0.0166
Rice husk	0.1810	0.2305	-0.0495	-0.1097	0.0120
Soil	0.0093	0.0120	-0.0027	-0.0629	0.0040
		Sum	-0.2236		0.0592
		d'	-0.0447		
		S	0.1217		
		Sd'	0.0608		
		t	-0.7352		
		t=.05,df=4	2.7760		
		t>p	n-sig		

Chlorpyrifos	pH 3	pH 7	d	d-d'	(d-d')^2
Peanut shell	0.5102	1.0722	-0.5620	-0.6222	0.3871
Peat moss	1.8776	3.3714	-1.4938	-1.5540	2.4149
Coconut husk	1.6929	1.4754	0.2175	0.1573	0.0247
Rice husk	0.9548	1.3165	-0.3617	-0.4219	0.1780
Soil	0.1184	0.1079	0.0105	-0.0497	0.0025
		Sum	-2.1895		3.0073
		d'	-0.4379		
		S	0.8671		
		Sd'	0.4335		
		t	-1.0101		
		t=.05,df=4	2.7760		
		t>p	n-sig		

Profenofos	pH 3	pH 7	d	d-d'	(d-d')^2
Peanut shell	0.9066	1.6139	-0.7073	-0.7675	0.5891
Peat moss	4.9290	6.9421	-2.0131	-2.0733	4.2986
Coconut husk	4.7706	3.1165	1.6541	1.5939	2.5405
Rice husk	2.3490	2.0201	0.3289	0.2687	0.0722
Soil	0.1389	0.1548	-0.0159	-0.0761	0.0058
		Sum	-0.7533		7.5061
		d'	-0.1507		
		S	1.3699		
		Sd'	0.6849		
		t	-0.2200		
		t=.05,df=4	2.7760		
		t>p	n-sig		

D.4 Effect of particle size

OPs	Size (μm)	Sorbed OPs in biomass, mg kg^{-1}			
		Coconut husk	Rice husk	Peat moss	Peanut shell
Methyl Parathion	≤25	360.00	360.00	347.07	346.09
	≤125	348.03	360.00	339.96	341.03
	≤250	341.03	353.92	338.34	338.34
	≤500	340.55	347.84	341.79	338.30
Malathion	≤25	773.85	780.00	753.29	755.49
	≤125	767.60	773.39	753.33	752.32
	≤250	752.94	766.87	750.92	750.46
	≤500	747.76	766.23	750.22	746.33
Chlorpyrifos	≤25	12.00	12.00	12.00	12.00
	≤125	11.44	10.57	12.00	12.00
	≤250	10.19	9.62	12.00	12.00
	≤500	9.94	8.93	12.00	11.54
Profenofos	≤25	168.00	168.00	168.00	168.00
	≤125	168.00	168.00	168.00	168.00
	≤250	168.00	168.00	161.99	168.00
	≤500	164.77	168.00	158.92	162.00

APPENDIX E-Columns Leaching studies

E.1 Optimum depths (Effect of OC)

E.1.1 Peanut Shell (Average concentration, mg l^{-1})

Pore Volume	Methyl Parathion	Malathion	Chlorpyrifos	Profenofos
0	0.000	0.000	0.000	0.000
2	0.000	1.805	1.826	1.505
4	2.737	1.806	1.832	1.504
6	2.739	1.806	1.833	1.506
8	2.741	1.806	1.835	1.507
10	2.743	1.806	1.834	1.507
12	2.745	0.000	1.831	1.507
14	2.747	0.000	1.843	1.508
16	2.749	0.000	1.840	1.510
18	2.751	0.000	1.845	1.511
20	2.753	0.000	1.837	1.511
22	2.755	0.000	1.848	1.512
24	2.757	0.000	1.860	1.513
26	2.759	0.000	1.839	1.510
28	2.761	0.000	1.839	1.510
30	2.763	0.000	1.838	1.509
32	2.765	0.000	1.837	1.509
34	2.768	0.000	1.837	1.508
36	2.770	0.000	1.838	1.508
38	2.772	0.000	1.835	1.507
40	2.774	0.000	1.858	1.512
42	2.776	0.000	1.835	1.507
44	2.778	0.000	1.831	1.506
46	2.780	0.000	1.830	1.505
48	2.782	0.000	1.833	1.505
50	2.784	0.000	1.827	1.505
52	0.000	0.000	0.000	1.505
54	0.000	0.000	0.000	1.497
56	0.000	0.000	0.000	0.000
58	0.000	0.000	0.000	0.000
60	0.000	0.000	0.000	0.000
80	0.000	0.000	0.000	0.000

E.1.2 Coconut husk 2 cm (Leachate concentration, mg l^{-1})

Pore Volume	Methyl Parathion	Malathion	Chlorpyrifos	Profenofos
0	0.000	0.000	0.000	0.000
2	2.733	1.933	0.000	1.566
4	2.733	1.991	0.000	1.568
6	2.733	2.214	0.418	1.598
8	2.733	2.438	0.458	1.617
10	2.733	2.362	0.455	1.632
12	2.733	2.195	0.452	1.627
14	2.733	2.127	0.450	1.633
16	2.733	2.062	0.449	1.606
18	2.733	2.040	0.447	1.637
20	2.733	1.993	0.448	1.647
22	2.733	1.995	0.446	1.647
24	2.733	1.949	0.445	1.638
26	2.735	1.952	0.445	1.627
28	2.734	1.922	0.445	1.640
30	2.734	1.897	0.444	1.632
32	2.734	1.888	0.446	1.648
34	2.733	1.876	0.446	1.641
36	2.734	1.865	0.444	1.630
38	2.734	1.856	0.447	1.641
40	2.735	1.853	0.442	1.611
42	2.736	1.850	0.446	1.622
44	2.735	1.845	0.445	1.612
46	2.735	1.849	0.447	1.611
48	2.734	1.845	0.446	1.608
50	2.735	1.844	0.447	1.602
52	2.735	1.844	0.442	1.577
54	2.733	1.845	0.442	1.565
56	2.733	1.831	0.436	0.000
58	2.733	1.833	0.000	0.000
60	0.000	1.827	0.000	0.000
80	0.000	0.000	0.000	0.000

E.1.3 Coconut husk 4 cm (Leachate concentration,mg l^{-1})

Pore Volume	Methyl Parathion	Malathion	Chlorpyrifos	Profenofos
0	0.000	0.000	0.000	0.000
2	0.000	0.000	0.000	0.000
4	2.733	1.836	0.000	0.000
6	2.733	1.835	0.000	0.000
8	2.733	1.836	0.000	0.000
10	2.733	1.834	0.000	0.000
12	2.733	1.834	0.000	0.000
14	2.733	1.833	0.000	0.000
16	2.733	1.834	0.000	0.000
18	2.733	1.833	0.000	0.000
20	2.733	1.840	0.000	0.000
22	2.733	1.848	0.000	0.000
24	2.733	1.837	0.418	0.000
26	2.734	1.833	0.414	0.000
28	2.734	1.829	0.000	0.000
30	2.734	1.823	0.000	0.000
32	2.734	0.000	0.000	1.497
34	2.734	0.000	0.000	0.000
36	2.734	0.000	0.000	0.000
38	2.734	0.000	0.000	0.000
40	2.734	0.000	0.000	0.000
42	2.734	0.000	0.000	0.000
44	2.734	0.000	0.000	0.000
46	2.734	0.000	0.000	0.000
48	2.734	0.000	0.000	0.000
50	2.734	0.000	0.000	0.000
52	0.000	0.000	0.000	0.000
54	0.000	0.000	0.000	0.000
56	0.000	0.000	0.000	0.000
58	0.000	0.000	0.000	0.000
60	0.000	0.000	0.000	0.000
80	0.000	0.000	0.000	0.000

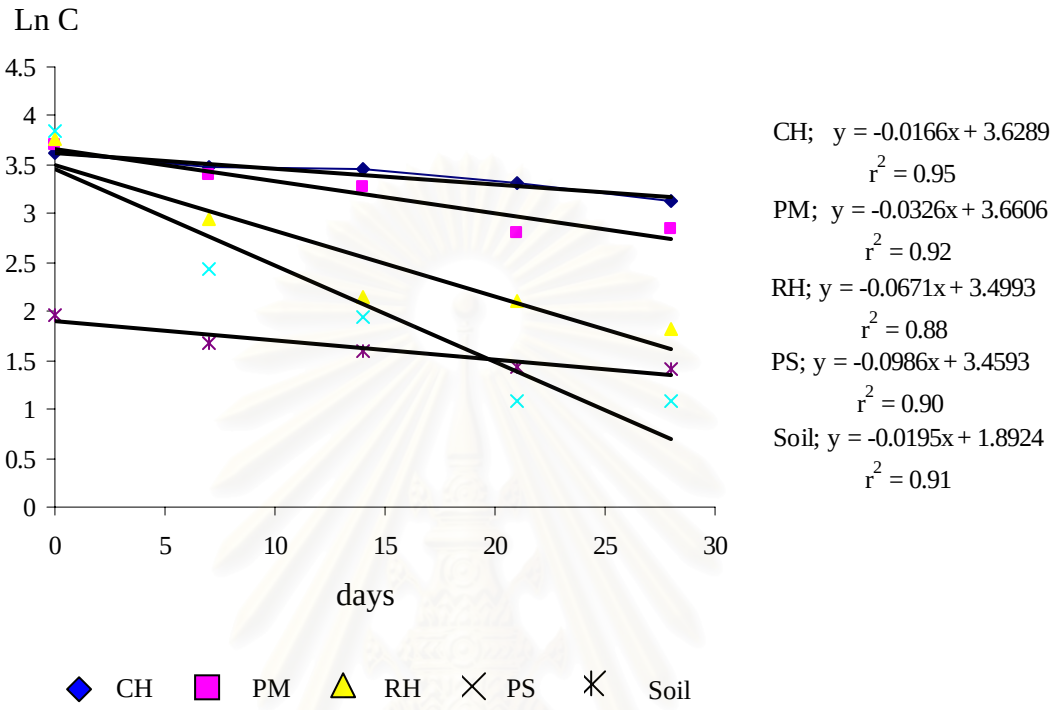
E.2 Average leachate concentration , mg l^{-1} for 3 times leaching

Pore Volume	Methyl Parathion			Malathion			Chlorpyrifos			Profenofos		
	1st leach	2nd leach	3rd leach	1st leach	2nd leach	3rd leach	1st leach	2nd leach	3rd leach	1st leach	2nd leach	3rd leach
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	0.000	0.000	2.735	0.000	0.000	0.000	0.000	0.000	0.426	0.000	0.000	1.499
4	0.000	2.734	2.734	0.000	1.840	1.865	0.000	0.000	0.417	0.000	0.000	1.500
6	2.734	2.733	2.734	1.836	1.845	1.888	0.000	0.000	0.422	0.000	0.000	1.500
8	2.734	2.734	2.734	1.835	1.851	1.908	0.000	0.000	0.417	0.000	0.000	1.500
10	2.734	2.734	2.735	1.836	1.860	1.871	0.000	0.000	0.418	0.000	0.000	1.500
12	2.734	2.734	2.735	1.834	1.866	1.888	0.000	0.000	0.418	0.000	0.000	1.500
14	2.734	2.734	2.735	1.834	1.872	1.889	0.000	0.000	0.418	0.000	0.000	1.500
16	2.734	2.734	2.736	1.833	1.882	1.901	0.000	0.000	0.419	0.000	0.000	1.504
18	2.734	2.734	2.734	1.834	1.869	1.849	0.000	0.000	0.418	0.000	0.000	1.501
20	2.734	2.734	2.734	1.833	1.880	1.846	0.000	0.000	0.419	0.000	0.000	1.500
22	2.734	2.734	2.734	1.840	1.842	1.867	0.000	0.000	0.418	0.000	0.000	1.501
24	2.734	2.733	2.735	1.848	1.841	1.860	0.000	0.000	0.422	0.000	0.000	1.500
26	2.734	2.734	2.734	1.837	1.837	1.858	0.418	0.000	0.419	0.000	0.000	1.500
28	2.734	2.734	2.734	1.833	1.839	1.866	0.414	0.000	0.419	0.000	0.000	1.500
30	2.734	2.734	2.735	1.829	1.833	1.858	0.000	0.000	0.420	0.000	0.000	1.500
32	2.734	2.734	2.734	1.823	0.000	1.854	0.000	0.000	0.420	0.000	0.000	1.500
34	2.734	2.734	2.734	0.000	0.000	1.849	0.000	0.000	0.419	1.498	0.000	1.500
36	2.734	2.734	2.734	0.000	0.000	1.848	0.000	0.000	0.419	0.000	0.000	1.500
38	2.734	2.734	2.734	0.000	0.000	1.847	0.000	0.000	0.418	0.000	0.000	1.500
40	2.734	2.734	2.734	0.000	0.000	1.847	0.000	0.000	0.419	0.000	0.000	1.500
42	2.734	2.734	2.734	0.000	0.000	1.844	0.000	0.000	0.418	0.000	0.000	1.500
44	2.734	2.734	2.734	0.000	0.000	1.837	0.000	0.000	0.419	0.000	0.000	1.501
46	2.734	2.734	2.734	0.000	0.000	1.831	0.000	0.000	0.418	0.000	0.000	1.501
48	2.734	2.735	2.734	0.000	0.000	1.824	0.000	0.439	0.418	0.000	0.000	1.501
50	2.734	2.734	2.734	0.000	0.000	1.819	0.000	0.420	0.418	0.000	1.500	1.500
55	2.734	2.734	2.734	0.000	0.000	0.000	0.000	0.419	0.419	0.000	1.500	1.500
60	0.000	2.734	2.735	0.000	0.000	0.000	0.000	0.418	0.428	0.000	1.500	1.501
65	0.000	2.734	2.734	0.000	0.000	0.000	0.000	0.426	0.427	0.000	1.500	1.500
70	0.000	2.734	2.734	0.000	0.000	0.000	0.000	0.417	0.427	0.000	1.499	1.500
75	0.000	2.734	2.734	0.000	0.000	0.000	0.000	0.417	0.427	0.000	1.499	1.499
80	0.000	2.734	2.734	0.000	0.000	0.000	0.000	0.417	0.427	0.000	1.500	1.501

APPENDIX F- Biodegradation experiment

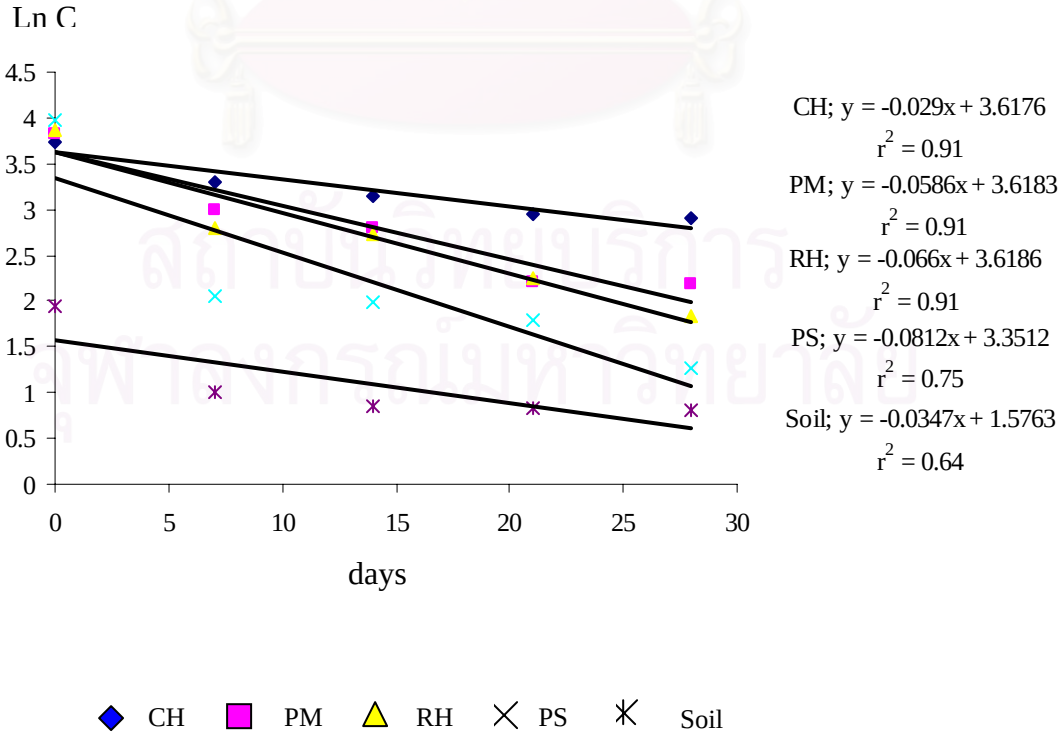
F.1 OPs degradation in biomass and soil

F.1.1 Methyl parathion



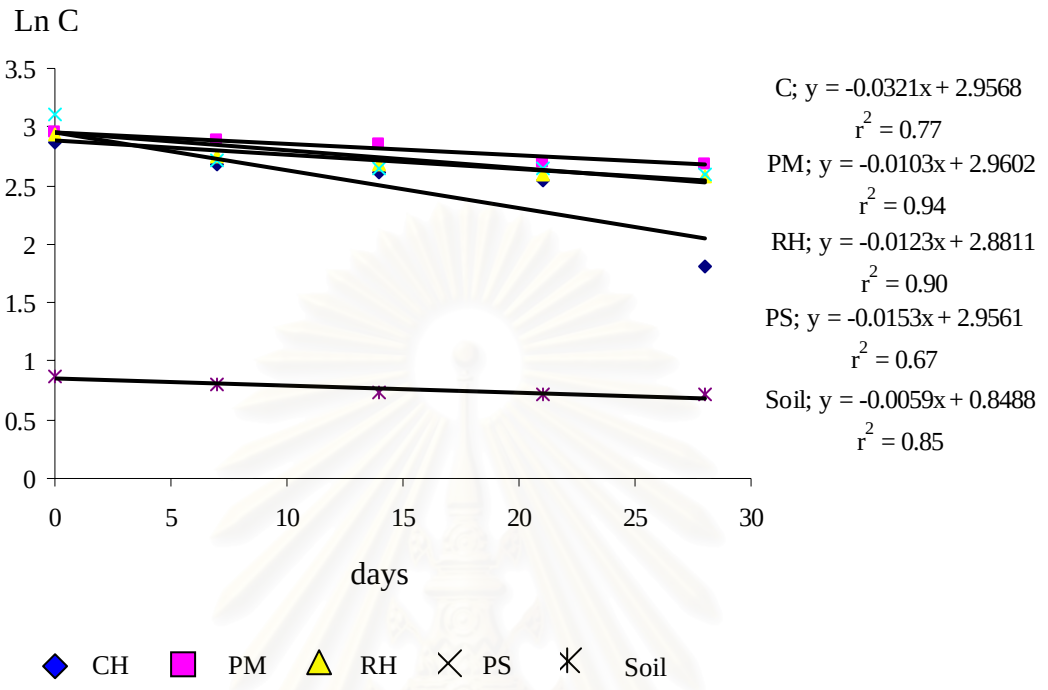
CH; y = -0.0166x + 3.6289
r^2 = 0.95
PM; y = -0.0326x + 3.6606
r^2 = 0.92
RH; y = -0.0671x + 3.4993
r^2 = 0.88
PS; y = -0.0986x + 3.4593
r^2 = 0.90
Soil; y = -0.0195x + 1.8924
r^2 = 0.91

F.1.2 Malathion

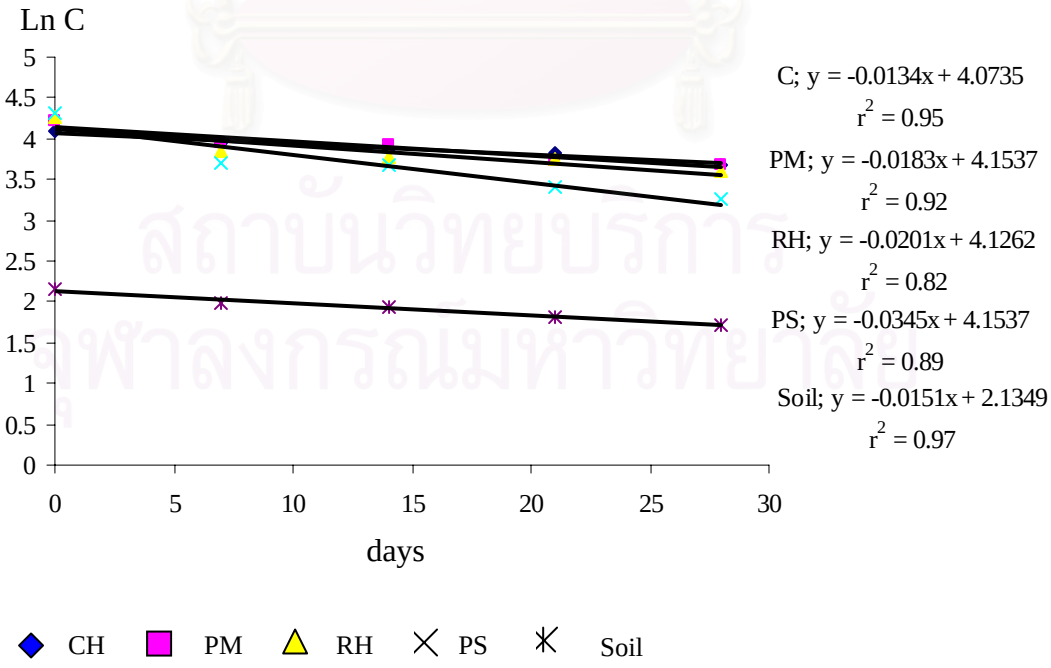


CH; y = -0.029x + 3.6176
r^2 = 0.91
PM; y = -0.0586x + 3.6183
r^2 = 0.91
RH; y = -0.066x + 3.6186
r^2 = 0.91
PS; y = -0.0812x + 3.3512
r^2 = 0.75
Soil; y = -0.0347x + 1.5763
r^2 = 0.64

F.1.3 Chlorpyrifos

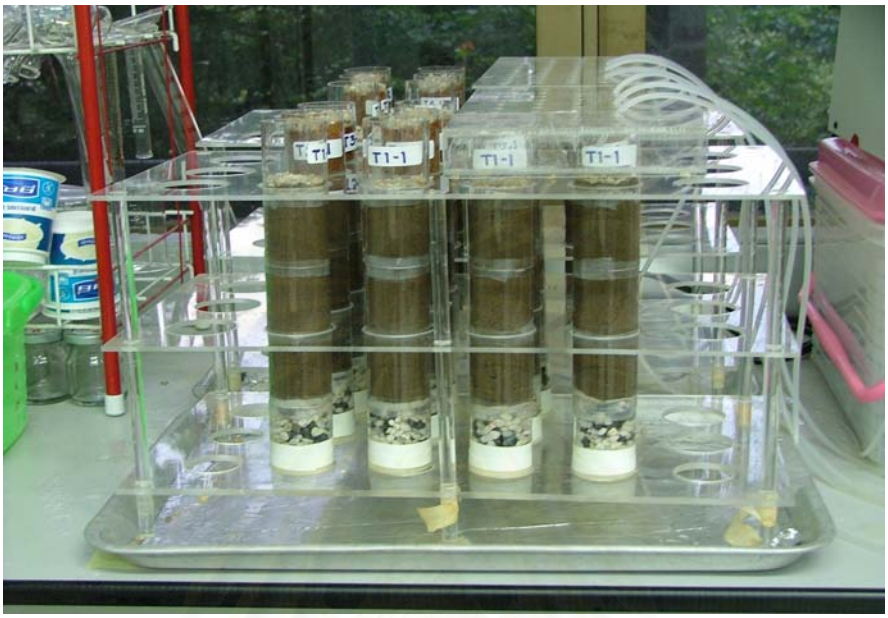


F.1.4 Profenofos



APPENDIX G-Column leaching experiments

G.1 Test compound distribution in the biomass and soil



G.2 Average mass of chlorpyrifos in soil

Treatment	Depth (cm)	Mass (µg)					SD				
		0 day	7 days	14 days	21 days	28 days	0 day	7 days	14 days	21 days	28 days
Soil	0-5	32.0353	28.6911	14.0796	15.9065	7.7467	16.3256	6.2713	0.5141	3.9182	4.1876
	5-10	0.3227	0.4510	5.8246	1.0603	11.1698	0.0767	0.2709	0.1948	0.3858	5.0258
	10-15	0.3180	0.4200	6.8710	2.3093	8.1461	0.0974	0.0945	2.4144	0.3428	6.0735
	G	0.0000	0.2200	17.6470	1.7798	3.1438	0.0000	0.0500	2.1343	0.1077	2.2435
Soil+Biomass	0-5	0.3492	1.2634	12.0367	3.7128	2.5299	0.0523	0.2874	2.8112	1.4356	1.0127
	5-10	0.5962	0.7714	15.1269	1.6055	2.3397	0.5045	0.0115	0.3624	0.1485	0.0830
	10-15	0.3025	0.7225	12.4053	1.3501	1.7624	0.0066	0.0265	3.6568	0.1946	0.6690
	G	0.0000	0.5047	7.2731	2.2203	1.5072	0.0000	0.1461	0.1616	0.3829	0.5507
Soil+Biomass+EM	0-5	0.2632	0.7315	1.4313	1.7504	0.8240	0.0699	0.1129	0.1129	0.3951	0.4407
	5-10	0.6474	0.4474	1.7962	2.0742	1.5970	0.4817	0.0365	0.0514	0.5550	0.2438
	10-15	0.2687	0.6039	1.7092	1.7399	1.7709	0.0726	0.2570	0.1102	0.3976	1.0154
	G	0.0000	0.2807	0.5047	0.9234	0.6702	0.0000	0.0084	0.0282	0.2229	0.3829

G.3 Average number of chlorpyrifos degrading bacteria in soil

Treatment	Depth (cm)	CFU					SD				
		0 day	7 days	14 days	21 days	28 days	0 day	7 days	14 days	21 days	28 days
Soil	0-5	1.0000E+03	1.2000E+04	7.4500E+05	7.5000E+05	8.0500E+05	0.0000E+00	2.8284E+03	2.1920E+05	7.1000E+04	1.4849E+05
	5-10	1.0000E+03	2.5000E+03	3.3500E+05	3.5000E+05	6.6500E+05	0.0000E+00	7.0711E+02	6.3640E+04	7.1000E+04	4.9497E+04
	10-15	0.0000E+00	0.0000E+00	1.0100E+05	1.0300E+05	1.3000E+05	0.0000E+00	0.0000E+00	1.1314E+04	2.8000E+03	4.2426E+04
Soil+Biomass	0-5	1.0000E+03	2.5000E+05	9.2500E+05	2.4000E+06	2.0000E+06	0.0000E+00	2.8284E+04	3.5355E+04	0.0000E+00	0.0000E+00
	5-10	0.0000E+00	1.3000E+04	1.0000E+04	5.0000E+04	1.0000E+05	0.0000E+00	2.8284E+03	0.0000E+00	1.4000E+04	0.0000E+00
	10-15	0.0000E+00	1.1000E+04	3.0000E+04	3.5500E+05	9.6500E+05	0.0000E+00	1.4142E+03	0.0000E+00	2.1000E+05	1.9092E+05
Soil+Biomass+EM	0-5	1.6700E+07	2.8000E+06	4.5000E+06	1.0100E+07	1.0450E+07	4.0305E+06	7.0711E+05	7.0711E+05	1.6000E+06	6.3640E+05
	5-10	1.5200E+07	2.6500E+06	6.4500E+06	9.7500E+06	1.0850E+07	8.5560E+06	7.0711E+04	7.7782E+05	2.5000E+06	4.4548E+06
	10-15	1.0100E+07	3.3500E+05	1.2500E+06	9.6000E+06	1.0250E+07	0.0000E+00	3.3234E+05	3.5355E+05	2.8000E+06	1.0607E+06

G.4 Average mass of chlorpyrifos in coconut husk

Treatment	Mass (µg)					SD				
	0 day	7 days	14 days	21 days	28 days	0 day	7 days	14 days	21 days	28 days
Without EM	38.8532	35.8450	21.4390	5.3185	4.8456	3.2824	0.2447	3.5392	1.7974	1.7577
With EM	33.6991	8.2122	7.6839	2.7505	1.9351	11.3742	4.1090	3.3618	1.2146	0.2281

G.5 Average number of chlorpyrifos degrading bacteria in coconut husk

Treatment	CFU					SD				
	0 day	7 days	14 days	21 days	28 days	0 day	7 days	14 days	21 days	28 days
Without EM	1.0000E+04	2.5000E+04	8.5000E+04	4.2500E+05	1.0000E+06	0.0000E+00	7.0711E+03	7.0711E+03	1.0600E+05	0.0000E+00
With EM	2.4000E+05	1.8000E+05	8.0000E+05	1.8500E+06	1.1500E+06	0.0000E+00	2.8284E+04	0.0000E+00	7.0700E+04	2.1213E+05

G.6 Mass of chlorpyrifos in leachate (mg l^{-1})

Time (day)	Soil	Soil+biomass	Soil+biomass+EM
1	0.0000	0.0000	0.0000
2	0.0000	0.0000	0.0000
3	0.0000	0.0000	0.0000
4	0.0000	0.0000	0.0001
5	0.0001	0.0002	0.0003
6	0.0004	0.0008	0.0008
7	0.0000	0.0000	0.0000
8	0.0000	0.0000	0.0000
9	0.0008	0.0000	0.0000
10	0.0000	0.0000	0.0000
11	0.0000	0.0000	0.0000
12	0.0000	0.0000	0.0000
13	0.0005	0.0005	0.0001
14	0.0008	0.0009	0.0004
15	0.0000	0.0002	0.0001
16	0.0001	0.0001	0.0000
17	0.0006	0.0006	0.0004
18	0.0001	0.0002	0.0002
19	0.0000	0.0006	0.0001
20	0.0008	0.0002	0.0002
21	0.0001	0.0002	0.0001
22	0.0001	0.0000	0.0001
23	0.0000	0.0000	0.0000
24	0.0000	0.0000	0.0000
25	0.0000	0.0000	0.0000
26	0.0000	0.0000	0.0000
27	0.0000	0.0000	0.0000
28	0.0000	0.0000	0.0000

APPENDIX-H Acceptance letter

May 05, 2007

National Research Center for
Environmental
and Hazardous Waste Management,
Chulalongkorn University Bangkok
10140,
Thailand

Subject: Acceptance letter for Article No. 301-JAS-DOI

Dear Dr. Siraprapa Romyen,

It's a great pleasure for us to inform you that below mentioned manuscript has been accepted for publication in "Journal of Applied Sciences" on the recommendation of the reviewers.

Title:	Potential of Agricultural By-Product In Reducing Chlorpyrifos Leaching Through Soil
Author's Name:	Siraprapa Romyen, Ekawan Luepromchai, Darryl Hawker and Benjalak Karnchanasest

Regards

Muhammad Imran Pasha
Publishing Editor
Journal of Applied Sciences

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APPENDIX I Proceeding paper

07R4-06

การใช้ประโยชน์จากวัสดุทางการเกษตรเพื่อการลดการชะของสารคลอไพริฟอสสู่ดิน

Utilization of Agricultural Biomass to Minimize Chlorpyrifos Leaching through Soil

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บทคัดย่อ

การศึกษานี้ใช้วัสดุทางการเกษตรเพื่อลดปริมาณสารคลอไพริฟอสซึ่งเป็นยาฆ่าแมลงกลุ่มออร์กาโนฟอสเฟตในน้ำชะภายหลังจากการฉีดพ่นต้นพืช เนื่องจากวัสดุดังกล่าวมีปริมาณสารอินทรีย์คาร์บอนสูงและมีจุลชีพหลายชนิดอาศัยอยู่ จึงคาดว่าจะสามารถดูดซับสารคลอไพริฟอสและในเวลาเดียวกันจะช่วยเพิ่มการย่อยสลายทางชีวภาพ ในการศึกษาครั้งนี้ได้นำวัสดุในท้องถิ่น ได้แก่ ขุยมะพร้าว พีทมอส แกลบ และเปลือกถั่ว มาศึกษาเปรียบเทียบกับตัวอย่างดินทรายจากสวนส้ม การทดลอง Batch partitioning เป็นการทดลองแรกที่นำมาหาค่าความสามารถในการดูดซับสารของวัสดุ โดยพีทมอสสามารถดูดซับสารคลอไพริฟอสได้สูงกว่าขุยมะพร้าว แกลบ และเปลือกถั่วตามลำดับ สำหรับดินจะมีค่าความสามารถในการดูดซับต่ำกว่าวัสดุทางการเกษตรชนิดอื่นๆ ค่าสัมประสิทธิ์การดูดซับ (K_d) จะเพิ่มขึ้นเมื่อเพิ่มปริมาณสารอินทรีย์คาร์บอนในวัสดุ ซึ่งบ่งชี้ได้ว่าปริมาณอินทรีย์คาร์บอนมีบทบาทสำคัญในการดูดซับสารคลอไพริฟอส ค่าครึ่งชีวิตของสารคลอไพริฟอสในขุยมะพร้าวมีค่าเท่ากับ 10 วันซึ่งเป็นค่าเร็วที่สุดในระหว่างกลุ่มวัสดุที่ใช้ทดสอบ ในขณะที่ค่าครึ่งชีวิตของดินมีค่าเท่ากับ 59 วัน ผลการทดลองกล่าวได้ว่าสารคลอไพริฟอสที่ถูกดูดซับในขุยมะพร้าวจะสามารถถูกย่อยสลายได้ในเวลาต่อมา เพื่อหาความสูงที่เหมาะสมของชั้นวัสดุก่อนการประยุกต์ใช้ในสนาม จึงทำการทดลอง Leaching test โดยบรรจุขุยมะพร้าวความสูง 2-4 ซ.ม. ในคอลัมน์ (เส้นผ่านศูนย์กลาง 3.85 ซ.ม.) แล้วพ่นสารคลอไพริฟอสปริมาณ 1.0 กก ต่อเฮคเตอร์ ที่ผิวหน้าของคอลัมน์ ทดลองการชะโดยให้น้ำที่อัตราการไหล 20 มล. ต่อชั่วโมง ผลการทดลองพบว่ามวลของสารคลอไพริฟอสถูกกักที่ขุยมะพร้าวความสูง 2 และ 4 ซ.ม. ร้อยละ 97.02 และ 99.96 ตามลำดับ ดังนั้น จึงแนะนำให้ใช้ขุยมะพร้าวเป็นวัสดุดูดซับเนื่องจากมีคุณสมบัติร่วมกันของความสามารถในการดูดซับที่สูงและเร่งการย่อยสลายทางชีวภาพ การคลุมหน้าดินด้วยขุย

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มะพร้าวที่ความสูง 4 ซ.ม. (29,209 ก.ก. ต่อ เฮคเตอร์) จึงเป็นวิธีที่มีความเป็นไปได้ในการลดการปนเปื้อนการชะของสารคลอไพริฟอสจากต้นพืชที่มีการฉีดพ่นสู่ดิน

คำสำคัญ : คลอไพริฟอส; การดูดซับ; ครึ่งชีวิต; การชะ

Abstract

This study utilized agricultural biomass to minimize the amount of chlorpyrifos, an organophosphate pesticide in leachate after spraying onto plants. Since, the biomass materials have high organic carbon content and contain various microorganisms; they are expected to sorp chlopyrifos and simultaneously promote its biodegradation. In this study, local biomass samples including coconut husk, peat moss, rice husk, and peanut shell were investigated in comparison to a sandy soil sample taken from orange grove. Batch partitioning experiments were first conducted to evaluate sorption capacity of these biomass. Peat moss could sorp chlorpyrifos higher than coconut husk, rice husk, and peanut shell, respectively. For soil, sorption gave obviously lower values than all biomass samples. Sorption coefficient (K_d) values were increased with increasing organic carbon contents in biomass, which indicated that organic carbon played an important role in chlorpyrifos sorption. Half-life ($t_{1/2}$) of chlorpyrifos in coconut husk was 10 days, which was the fastest among the tested biomass, whereas; its half-life was 59 days in soil. The results suggested that the sorped chlopyrifos could be degraded afterward. To find the optimum depth of biomass bed before use in the field, leaching experiments were carried out by packing 2-4 cm coconut husk in column (3.85 cm diameter), and sprayed with 1.0 kg ha^{-1} chlorpyrifos to the column surface. Leaching tests were performed at a water flow rate of 20 ml hr^{-1}. Results indicated that 97.02 % and 99.96% of chlorpyrifos mass could be retained in coconut husk at 2 and 4 cm depth, respectively. Therefore, coconut husk was recommended as sorbent material due to the combination of high sorption capacity and enhanced biodegradation properties. The covering of 4 cm depth-coconut husk (29,209 kg.ha^{-1}) on top of soil surface could be a feasible method to reduce contamination of chlorpyrifos leachate from the sprayed plants through soil.

Keywords : Chlorpyrifos; sorption; degradation; leaching

Introduction

In order to protect the environment and human health, it is important to develop techniques to prevent pesticide contamination from the point sources. Agricultural land is major source of pesticides due to the run off and leaching events affecting these chemicals once they reach the soil surface. In Thailand, organophosphate pesticides (OPs) residues have been found in soil, water and agricultural products [1]. Chlorpyrifos (O,O-diethyl O-3,5,6-trichloro-2-pyridinyl phosphorothioate) is used worldwide as an agricultural OP insecticide. The reported half-life of chlorpyrifos in soil varies from 10 to 120 days [2]. Recognizing the growing problem, the study focused on the protection of ground water by prevention of contaminant transport in infiltrating water from the soil surface to the water table. One method to minimize the potential leaching of those agrochemicals can be carried out by creating sorptive or immobilizing zones in the soil by the incorporation of the appropriate sorbent in the affected area of soil [3]. Sorption is the most influential factor on fate and transport of pesticide due to it is the attraction of a pesticide to organic matter in soil surfaces.

Biomass is a residue of agricultural process, widely generated in Thailand. Utilization of agriculture biomass which is exogenous organic matter to soil is being use as an alternative method for disposal. Due to the high organic matter, it can promote sorption of pesticides and retarding their movement [4]. Organic from biomass can also affect the biodegradation of pesticides by enhancing microbial activity and consequently, promoting biodegaradation [5,6,7].

The aim of this study was to assess the possibility of using agricultural by-product; namely coconut husk, peat moss, rice husk and peanut shells as sorbent material to prevent leaching from sprayed chlorpyrifos to soil. Sorptive property of chlorpyrifos was investigated by batch partitioning behavior in comparison with native soil from tangerine orchard in Mae Ai, Chiangmai Province. The degradation of chlorpyrifos after sorption was also determined. Finally, the appropriate depth of the selected biomass on soil to prevent chlopyrifos discharge was obtained from leaching test.

Materials and Methods

Materials

Four sorbents under study are coconut husk (C), rice husk (R), peat moss (P) and peanut shell (Ps). They were air dried, sieved through 500 µm and stored in the sealed plastic containers at room temperature. One sample of soil was collected from the tangerine orchard at Mae Ai, Chiangmai province. The top 15 cm of the soil was collected by using a shovel and sample scoop and stored in plastic bag before air dried, stones and debris were removed, and the remaining soil was sieved to 500 µm prior to use. The biomass and soil were analyzed for their physical and chemical properties as shown in Table 1.

Table 1 Properties of the four local biomass and soil

	Coconut husk (CH)	Rice husk (RH)	Peat moss (PM)	Peanut Shell (PS)	Soil (S)
Organic carbon (% dry weight)	45.39	35.44	49.42	41.61	1.29
Organic Matter (% dry weight)	78.62	61.10	85.20	70.73	2.22
pH	5.30	6.30	5.20	5.50	4.40
Surface area ($m^2\ g^{-1}$)	17.77	1.89	6.98	4.80	7.69
Nitrogen (%)	4.63	4.01	4.77	3.42	0.07
Phosphorus (ppm)	369.00	118.00	310.00	373.00	229.00
Price kg^{-1} ($)	0.25	0.25	1.25	0.25	-

Standard chlorpyrifos (O,O-diethyl O-3,5,6-trichloro-2-pyridinyl phosphorothioate(>99%), purchased from Chem Service Inc., West Chester, PA, U.S.A. Its solubility is 2 mg l^{-1}. Figure 1 shows the chemical structure of the test compound.

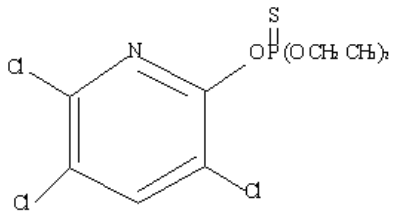

Figure 1 Structure of Chlorpyrifos

Batch Sorption Studies

Sorption studies were performed in triplicate partitioning experiments at room temperature. The partitioning experiments were carried out at the concentrations ranged from 0.05, 0.12, 0.18 and 0.25 times the maximum aqueous solubility of chlorpyrifos in Phosphate Buffer pH 7. The 20 g soil or 2 g of each biomass was treated with 40 ml of chlorpyrifos solution. This experiment operated at 48 hrs. Differences between initial chlorpyrifos concentration (Ci) and equilibrium chlorpyrifos concentration (Ce) were assumed to be the amounts sorbed by biomass and soil. Sorption isotherms were fit to the linear equation and distribution coefficients, Kd were calculated.

Biodegradation Studies

Portions of 5 g of soil and 0.5 g each biomass at optimum particle size were packed and adjusted moisture at 70% of water holding capacity in 22 ml vial to make soil microcosm. Chlorpyrifos was spiked to give concentration of normal agricultural application rate of dry soil and biomass. The microcosms were maintained at room temperature. The change of chlorpyrifos was monitored in soil and biomass from 0, 7, 14, 21, 28 and 35 days. Chlorpyrifos residues were extracted and determined by Gas Chromatography. Half-life of chlorpyrifos was calculated by kinetic equation.

Leaching Studies

Coconut husk were added to the separate glass columns (70 mm x 38.5 id mm) at 2-cm and 4-cm depths. The columns were lined below with glass wool to avoid biomass leakage and small glass beads on top in order to distribute water evenly over the biomass surface (see Figure 2). Generally, they were spiked with chlorpyrifos 0.0291 mg. Its mass is equivalent to the actual application rates at 0.25 kg ha^{-1} of chlorpyrifos employed on a tangerine orchard in Thailand. The columns were then left 24 h before leaching. Deionized water was added at 20 ml h^{-1}, the current average watering regime in the orchard. Leachate collection was started after about 30 ml drained out, and afterwards every pore volume until no chlorpyrifos was occurred in the collected water.

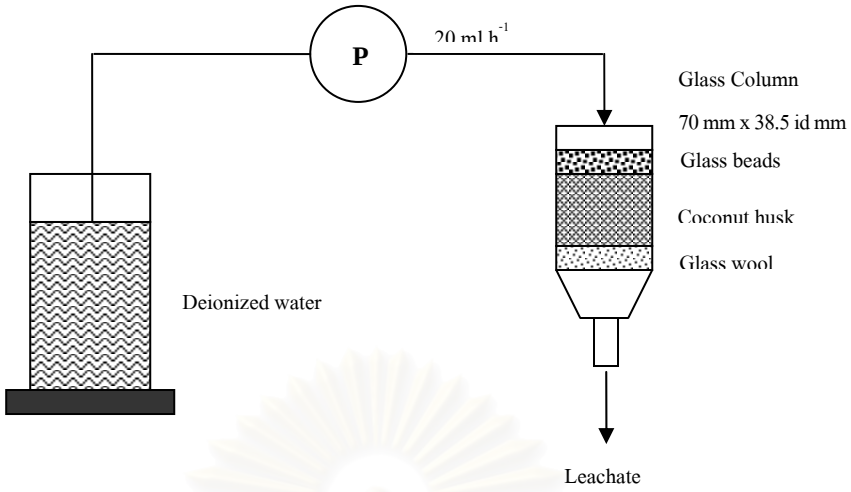

Figure 2 Diagram of Leaching Studies

Analysis of samples

Solvent extraction was used to extract chlorpyrifos from both liquid and solid samples. This method is modified from extraction of organochlorine pesticides in soil and water [8]. The solvent here is a mixture of hexane and acetone at 8:2 ratio. For liquid samples, 20 ml solution samples were transferred into a new 125 ml flask and then 20 ml of solvent were added, for soil samples, 20 g of soil samples were mixed with 40 ml of solvent and 5 ml of 15% Triton x-100. For biomass samples, 2 g of biomass samples were mixed with 40 ml of solvent and 5 ml of 15% Triton x-100. The samples were mixed and shook at 250 rpm for 4 hrs. After the samples are being shaken, the flasks were frozen at -4°C to solidify the lower aqueous layer, and then solvent were transferred to vial where 2-3 g of Na_2SO_4 were added to dewater the sample. Chlorpyrifos dissolved in solvent fraction were concentrated using nitrogen 99.5% blower. Then the extracted samples were transferred to gas chromatography vials for analysis using gas chromatography equipped with an electron capture detector (GC-ECD) and a HP-5 (5% Phenyl Methyl Siloxane) fused-silica capillary column (30 m x 0.32 mm ID; thickness 0.25 μm). The following operating conditions were used: injector temperature 250°C, detector temperature 250°C, initial column temperature 120°C then, programmed at 120°C to 250°C at a rate of 10°C min^{-1} (3min), and 250 °C to 300°C at a rate of min^{-1} (4 min). A post column temperature of 325°C were held for 3 min. The carrier gas is helium with gas flow 20 ml min^{-1}, and a 5:1 split ratio. The make up gas is N_2 at 60 ml $min^{-1.}$

Results and Discussion

Batch Sorption Studies

Batch sorption studies were conducted to determine sorption coefficient (Kd) of chlorpyrifos in each biomass and soil. In this experiment, Kd values were obtained from the slope of linear isotherm which often are observed if the equilibrium aqueous phase organic compound concentrations are below 10^{-5} M or one-half the aqueous phase solubility

(whichever is lower) and the organic content of the solid is greater than 0.1% [9, 10, 11]. In addition, isotherms of nonionic organic compounds (e.g. organophosphate pesticides) are often assumed to be linear [12, 13, 14, 15].

The sorption data fitted well to linear isotherm. All regression generated had $r^2 > 0.95$. The results were reported in Table 2. The sorption coefficient of soil was 107.9 $l.kg^{-1}$, while Kd of coconut husk, peat moss, rice husk and peanut shell were 1,475.4, 3,371.4, 1,316.5 and 1,072.2 $l.kg^{-1}$, respectively. As a result, sorption capacities of all biomass were higher than soil. These results showed K_d values were correlated well with organic carbon contents in biomass. Soil had

Table 2 Sorption coefficient (K_D) of chlorpyrifos in biomass and soil

Biomass	K_D ($l.kg^{-1}$)	r^2
Coconut husk	1475.4	0.98
Peat moss	3371.4	0.98
Rice husk	1316.5	0.98
Peanut shell	1072.2	0.97
Soil	107.9	0.98

lowest sorption coefficient value due to lower organic content than biomass. This relationship indicated organic carbon played a major role in the sorption of chlorpyifos in soil and biomass. Previous studies have shown organic carbon is the most important component of soil controlling sorption [16]. In addition, sorption behavior is not only influenced by the organic carbon, but there are also the other factors that effect on this case such as shape and properties of biomass, polarity, solubility of pesticides.

Degradation of chlorpyrifos

One of the major ways for chlorpyrifos breakdown is through bacterial metabolism, usually though a consortium of microbes rather than a single species. The pesticides degradation in soil and biomass could be described with the first-order kinetic equation as $dC/dt = -kt$. From the equation, we can obtain the following equations:

$$\ln C = -kt + \ln Co \quad (1)$$

$$t_{1/2} = 0.693/k \quad (2)$$

Where C is concentration of chlorpyrifos in biomass, mg/kg, k is degradation rate constant,d^{-1} , Co is the initial concentration of in biomass, mg/kg , $t_{1/2}$ is half life, days. The degradation of chlorpyrifos in biomass and soil were shown in Table 3.

Table 3 Half-life of Chlorpyrifos in Biomass

Biomass	Kinetic Equation	$t_{1/2}$
Coconut husk	ln C = -0.0697t + 3.3077	10
Peat moss	ln C = -0.0112t + 2.9685	62
Rice husk	ln C = -0.0126t + 2.8843	55
Peanut shell	ln C = -0.0145t + 2.9483	48
Soil	ln C = -0.0118t + 3.0223	59

The results indicated that chlorpyrifos was degraded more rapidly in coconut husk than peanut shell, rice husk and peat moss. Meanwhile, chlorpyrifos was degraded gradually in soil. The reported half-life of chlorpyrifos in soil varies from 10 to 120 days [2]. From table 1, every biomass had higher amount of nutrients (C, N, and P) than soil. This probably promoted microbial growth in biomass and thereby enhanced chlorpyrifos degradation in biomass.

Leaching Test

According to the sorption experiment, coconut husk was the best sorbent in term of highest sorption capacity and economic with lower price than other biomass. Coconut husk also showed the shortest chlorpyrifos half-life. Therefore, coconut husk was selected to determine the leaching behavior of chlopyrifos which described by breakthrough curve (BTC). Coconut husk columns were leached by DI water until zero discharge. Chlorpyrifos in leachate were showed in Figure 3.

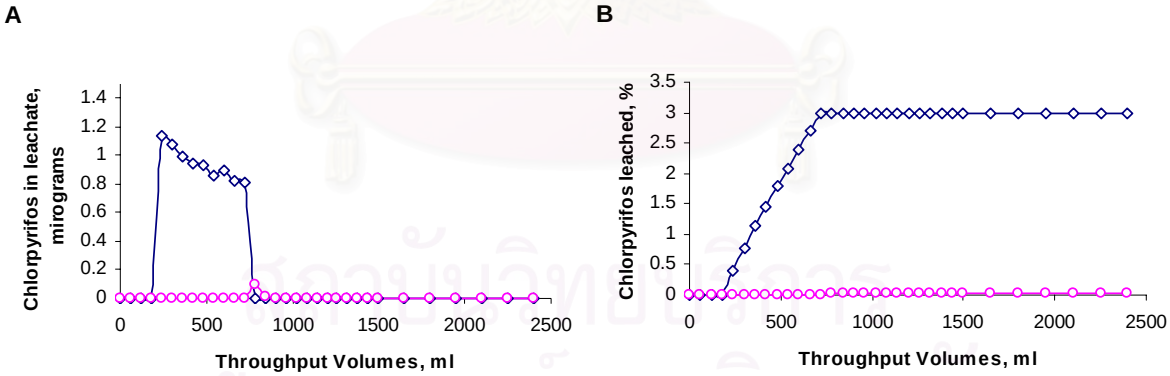

Figure 3 Chlorpyrifos breaktrough curves (BTCs) in coconut husk 2-cm depth (◇) and 4-cm depth (○): (A) relative BTCs and (B) cumulative BTCs

Relative breakthrough curves of chlorpyrifos (Figure 3A) demonstrated the amount of chlorpyrifos was came out from 2-cm depth coconut husk bed more than 4-cm depth. Approximately 0.1 µg of chlorpyrifos was leached from 4-cm depth. Figure 3B showed total amounts of chlorpyrifos leached from the 2-cm and 4-cm depth 2.98% and 0.04%. Thus, the sorbed chlorpyrifos in 2-cm depth and 4-cm depth were 97.02% and 99.96%, respectively. The breakthrough

was prolonged in the column containing higher depth, which allowed more time for chlorpyrifos to bind to organic carbon in coconut husk.

Conclusion

This work demonstrated that coconut husk was effective for retarding the mobility of chlorpyrifos and the retained chlorpyrifos could be degraded afterward. The presence of organic carbon in coconut husk played a major role of chlorpyrifos sorption. Degradation of chlorpyrifos in coconut husk was more rapidly than in other biomass and soil. Therefore, covering top soil with biomass may be useful to reduce the risk of groundwater contamination. The covering of soil surface with 4 cm depth-coconut husk (29,209 kg ha^{-1}) could be a feasible method to reduce contamination of chlorpyrifos leachate from the sprayed plants through soil.

Acknowledgement

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ทำเนียบวิทยากร

ชื่อบทความ การใช้ประโยชน์จากวัสดุทางการเกษตรเพื่อการลดการชะของสารคลอไพริฟอสสู่ดิน
Utilization of Agricultural Biomass to Minimize Chlorpyrifos Leaching through Soil

ผู้นำเสนอบทความ นางสาวศิรประภา ร่มเย็น

สถานที่ทำงาน มหาวิทยาลัยเทคโนโลยีราชมงคลล้านนา
ต.สุเทพ อ.เมือง เชียงใหม่

โทรศัพท์ 089-128-8752

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ตำแหน่งหน้าที่ปัจจุบัน อาจารย์ประจำภาควิชาวิศวกรรมสิ่งแวดล้อม มหาวิทยาลัยเทคโนโลยีราชมงคลล้านนา

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ประสบการณ์ - ควบคุมงานก่อสร้างระบบประปาสุขาภิบาล

ผลงาน
- Poster Presentation

Romyen, S., Karnchanasest, B., and Luepromchai, E., 2006. Sorption of Organophosphate by Biomass International Conference Hazardous waste Management for a Sustainable Future. Bangkok, Thailand 10-12 January, 2006.

BIOGRAPHY

Miss Siraprapa Romyen, was born on December 8, 1973 in Chiangmai, Thailand. She finished her secondary school from Prince's Royal College, Chiangmai. After that, she joined and graduated in Environmental Engineering, Faculty of Engineer at Khonkean University and graduated in 1996. Afterwards, she enrolled in Master's degree in Environmental Engineering at Khonkean University and graduated in 2000. Then, she worked as lecturer at Rajamankala University of Technology Lanna, Chiangrai campus since 2001 to 2003. After that she started her Ph.D. degree in International Programs in Environmental Management, Chulalongkorn University and completed the program in May 2007.