Bioaccumulation of Organophosphate Pesticides in Vetiver Grass (*Vetiveria zizanioides Nash.*)

Maliwan Boonsaner, Thamnoon Kaewumput and Arthorn Boonsaner
Dept. of Environmental Science, Faculty of Science, Silpakorn University,
Nakorn Pathom, Thailand

Abstract

Vetiver grass (*Vetiveria zizanioides* Nash.) was planted in 3 aluminum boxes, 2 boxes containing soils which have been pre-mixed with 5 organophosphate pesticides at different concentration sets and the last box was the control one. For the first concentration set, one kg of soil contained 30, 40, 2000, 400 and 1000 mg of Methamidophos, Ethoprophos, Methidathion, m-Parathion and EPN, respectively. The second concentration set, one kg of soil contained 20, 30, 1200, 40 and 500 mg of the same chemicals, respectively. Soil surface was covered with aluminum foil in order to prevent compound evaporation and phototransformation.

Soil and grass samples were collected at days 1, 3, 5, 7, 10, 15, 30, 40, 50, 60, 70, 80 and 90 after commencing the experiment. Soil samples were determined for the amount of test compounds, water and organic carbon contents while the root and stem of the grass samples were first separated and then analyzed for the amount of test compounds as well as lipid content. The results obtained from both experiments were similar. The common sequence for the uptake rate of test compounds in root was Methamidophos > Ethoprophos > Methidathion > m-parathion > EPN but the sequence was opposite in stem. The accumulation of test compounds in both root and stem increased with their hydrophobicity. The study found that the relationship between log RCF (root concentration factor) and log K_{ow} is linear and can be represented by log RCF = 0.29 log K_{ow} – 1.05, r^2 =0.99. It was also found that the accumulation of test compounds in stem was very low but still showed the significant linear relationship with log K_{ow}. The stem concentration factor (SCF) of test compounds in stem was related to their hydrophobicity by log SCF = 0.59 log K_{ow} – 1.06, r^2 = 0.95.

The log RCF and test compounds' physicochemical properties (aqueous solubility and molecular weight) were also found to have linear relationship. However, the highest significant relationship was found between log RCF and log K_{ow}. This indicated that the hydrophobicity of test compounds may play an important role in governing the partition process of the organic compounds especially organophosphate pesticides in soil-root-stem partitioning.

Keywords: bioaccumulation, partitioning, uptake, vetiver grass, root concentration factor, stem concentration factor

1. Introduction

When a chemical is discharged into the environment, it may distribute between various phases, such as air, water, soil, etc. Soil is known as a major repository for many hydrophobic chemicals. These chemicals can subsequently be released from soil to other phases including plants. Chemicals, taken up by plants, may accumulate in roots and translocate to upper plant parts depending on their hydrophobicity. Chemicals, which have high vapor pressure, may volatilize and accumulate in the leaves. Therefore, plants can accumulate chemicals from the soil either directly or indirectly.

For seedling and adult plants, the root system would probably be the most important site of chemical entry since it may be analogous to nutrient uptake and because the mass flow of water to root may bring chemical with it. The partitioning behavior of organic chemical in

root system is likely to be governed by its hydrophobicity. This is shown by the linear relationship between the mascerated barley root/water partition coefficient of o-methylcarbamoyloximes and substituted phenylureas and their log K_{OW} values [1].

For the aerial parts of the plant, including foliage, the chemical which volatilizes from soil may partition between air and the cutin or pass through the stomata and partition from air to the aqueous phase of the leaves or stems. The translocation of chemicals to other plant parts is also seen as the partitioning between the stem and the transpiration stream or xylem sap [2] However, Paterson et al. [3] concluded that organic chemicals of high aqueous solubility, volatile from soil and deposit on leaves, are more readily transported through the plant via the phloem than those that are more hydrophobic and tend to be sorbed to and remain in the waxy cuticle of leaves.

Another known chemical characteristic that can influence plant/soil partitioning is molecular weight. For groups of related compounds, properties such as aqueous solubility and K_{OW} are related to molecular weight. Topp et al. [4] showed an inverse relationship existed between the amount of chlorinated hydrocarbon compounds uptaken by barley root and foliage and the molecular weight of the compounds. They suggested that the molecular weight of a compound probably is a chemical characteristic which is more suitable to predict plant uptake than other characteristics because of the high correlation coefficient of this linear relationship, and the ease of calculation of molecular weight.

As well as the physicochemical properties which govern the partitioning process (or bioaccumulation process), plant species also influence the bioaccumulation of the organic compounds. Paterson et al. [3] suggested that the bioaccumulation of organic compounds in various plant and different species could be different. While Connell and Markwell [5] reported that the bioaccumulation of organic compounds in biota, either animal or plant, would depend upon the lipid content in that biota.

In this study, the behavior of organophosphate pesticides had been conducted in vetiver grass. Vetiver grass has been employed as a plant model since it is widely grown in the northern part of Thailand where soil erosion is a problem. In this part of the country, organophosphate pesticide is highly used and found contaminate in water and sediment [6]. Vetiver root contains 1-3% of volatile oil [7] and it may spend a relatively long time in soil, as a result, vetiver root may be able to absorb some organic chemicals as well as to prevent soil erosion. In the past, there were only a few studies that had been done on the accumulation of organophosphate pesticides in vetiver grass [6, 8, 9, 10]. However, those studies were conducted under the field situation where evaporation, degration and contamination during pesticide application may have caused some unpredictable variations. In Australia, some species of vetiver grass are also used as a feedstock [11]. Therefore, the study of pesticides' translocation in the upper part of vetiver grass becomes important. If it is found that organophosphate pesticides can translocate from root to upper plant part, vetiver grass may not be safe to be used as a feeding stock because it may link to human exposure.

The objective of this work is to study the bioaccumulation of organophosphate pesticides in each part of vetiver grass, either root or stem. The bioaccumulation factor, either root concentration factor (RCF) or stem concentration factor (SCF) will be related to the physicochemical properties of test compounds in order to determine if any physicochemical properties could govern the partitioning or bioaccumulation process.

2. Materials and Methods

Metamidophos, ethoprophos, methidation, m-parathion and EPN, obtained from Supelco, USA, were used as the test compounds. Hexane (Nanograde), acetone (AR), acetonitrile (AR) and florisil were purchased from Mallinckrodt Chemical, Inc., Kentucky, USA. Methanol (AR), chloroform (AR), toluene (AR) and diethyl ether (AR) were obtained from BDH Laboratory Supplies, England. Glass wool was bought from Ajax Chemical Company, Australia while anhydrous sodium sulfate (AR) was obtained from Merck KgaA, Germany. Glass fibre filter

paper (GF-C) was purchased from Whatman, England.

Anhydrous sodium sulfate and florisil were heated in a muffle furnace at 450°C and 650°C, respectively for at least 4 hours, then cooled in a dessicator, followed by deactivation with water (5% w/w) for florisil. Glass wool was prewashed with acetone and hexane, then air dried before use. All glassware was soaked in detergent overnight, washed and rinsed with deionized water. Before use, all glassware was rinsed again with hexane. Aluminum boxes (60X30X30cm) which were used to hold soil, were treated as for glassware.

Soil (100 kg) and vetiver grasses, used in this experiment, were obtained from Kund Kong Research Station, Chiengmai, Thailand. Both samples were determined for contamination prior to commencing the experiment.

The water content in soil and lipid content in vetiver grass were determined by methods of soil analysis, part 2 [1] and by Soxhlet Extraction [12], respectively. The extraction of test compounds in soil and roots and stem of vetiver grass were followed the method for analysis of pesticide residues [13]. Gas Chromatographs, Hewlett Packard, fitted with FID Detector and DB-wax column (30 x 0.32 x 0.25 um) were employed for the analysis of test compounds.

The bioaccumulation experiment of test compounds in a soil/vetiver grass system was performed with two different test concentrations in soil. The first test concentration was estimated based on the values of 10% of the test compounds'solubility [14]. Per kg of soil, it contained 30, 40, 2000, 400 and 1000 mg of Methamidophos, Ethoprophos, Methidathion, m-Parathion and EPN, respectively. This was designated to be experiment 1. The second test concentration was based on the values of 10% of the suggested used concentration. Per kg of soil, it contained 20, 30, 1200, 40 and 500 mg of Methamidophos, Ethoprophos, Methidathion, m-Parathion and EPN, respectively. This was designated to be experiment 2.

The experiments were conducted by preparing two soil samples which each contained a different concentration of test compounds as mentioned above. For each experiment, 50 kg of washed soil was mixed with test compounds in an aluminum box. The mixture of test compounds was prepared in 1000 ml hexane. After thorough mixing, soil was left with occasional stirring for two hours in order to remove the excess hexane. Then, soil was dampened with distilled water to give a moisture content about 25% (measured by a commercial moisture meter). However, when soil samples were taken, the determination of the moisture content in the soil was performed in the laboratory using a gravimetric method.

For the bioaccumulation experiment, 20 vetiver grasses were planted in prepared soil at 20 cm depth. The soil surface was covered with aluminum foil in order to protect test compounds from volatilization. In order to perform the quality control procedure, 20 of vetiver grass were also planted in the uncontaminated soil and covered with aluminum foil. Grass and soil samples were also taken for contamination check during the bioaccumulation experiment. All experiments were performed at a temperature between 27° to 29°C.

During the 90 day period for each experiment, grass samples together with one soil sample were taken at 1, 3, 5, 7, 10, 15, 30, 40, 70, 80, and 90 days after commencing the experiment. Each grass was separated into 2 portions, stem and root, and analyzed separately. The uptake for test compounds was monitored in each portion.

3. Result

Soil samples and vetiver grass sample had been analysed and found no contamination of any chemicals prior to the commencement of the bioaccumulation experiment. The organic carbon content in soil was 8% while the lipid content were 1.6 –1.8% and 2.4-2.8% in stem (leave) and in root, respectively. The high organic carbon content may result in the high sorption capacity of organic compounds in the soil [14]. The temperature during bioaccumulation study ranged from 27-29 °C and water content in the soil did not vary, since the soil was covered with aluminum foil.

3.1 Kinetics of test compounds in root

The kinetic behaviour of test compounds in vetiver grass was studied for a period of 90 days using two different test concentrations in soil (designated as Experiment ıand Experiment 2). The concentrations of test compounds in root obtained from both experiments are shown in Table 1.

For both experiments, it was found that the kinetic behaviour of each test compound in root were similar. The common observed sequence for the uptake rate of test compounds in root was Methamidophos > Ethoprophos > m-Parathion > Methidathion > EPN. This decreasing order of uptake rates is in accord with increasing compound molecular weight (Table 2). The sequence of uptake rate in which the test compounds with low hydrophobicity and low MW was higher than those with high hydrophobicity and high MW, suggests that the transportation of test compounds may be highly influenced by the molecular size more than the hydrophobicity of a compound. This result agrees with Topp et al., 1986 who showed that the uptake of ^{14} C-labelled organic chemicals (eg. chlorobenzenes, PCBs and pigments) by plants involved membrane penetration which is related to their molecular sizes.

Table 1 Test compound concentration in root and soil and RCF

Compounds	Concentration (mg/g) Experiment 1		RCF	Concentration (mg/g) Experiment 2		RCF
	Root (C_r)	Soil (C_{soil})		Root (C_r)	Soil (C_{soil})	
Methamidophos	0.001- 0.008	0.01 - 0.027	0.40	0.001 - 0.007	0.01 - 0.017	0.45
Ethoprophos	0.002 - 0.034	0.020 - 0.035	1.21	0.0020 - 0.03	0.015 - 0.026	1.31
Methidathion	0.003 – 2.2	1.52 - 1.98	2.04	0.003-12	0.85 – 1.10	1.89
m-Parathion	0.004 - 0.86	0.32 - 0.38	3.21	0.004 - 0.7	0.22 - 0.35	3.15
EPN	0.02 - 2.35	0.80 - 0.95	3.96	0.01 - 1.2	0.38 – 0.45	3.85

Note * average concentration for the whole experiments

Table 2 Physicochemical Properties of test compounds

Compounds	Molecular weight [4]	Aqueous solubility S (mg/l) [4]	log K_{ow}	Vapour Pressure (mPA) [4]	Persistence [4]
Methamidophos	141.3	2000000	0.08[1]	2.3	low
Ethoprophos	242.3	700	2.57[1]	46.5	low
Methidathion	302.3	240	2.91[1]	186	7 days
M - Parathion	263.2	60	3.52[2]	1.3	2-3 weeks
EPN	323.3	9.9[1]	3.91[3]	126	high

Note 1. log K_{ow} is calculated from : log K_{ow} (mg/l) = 4.62 – 0.72 log S , r = 0.964 [15]
2. experimnetal value [16]
3. Experimental value [17]
4. Worthing, 1997 [18]

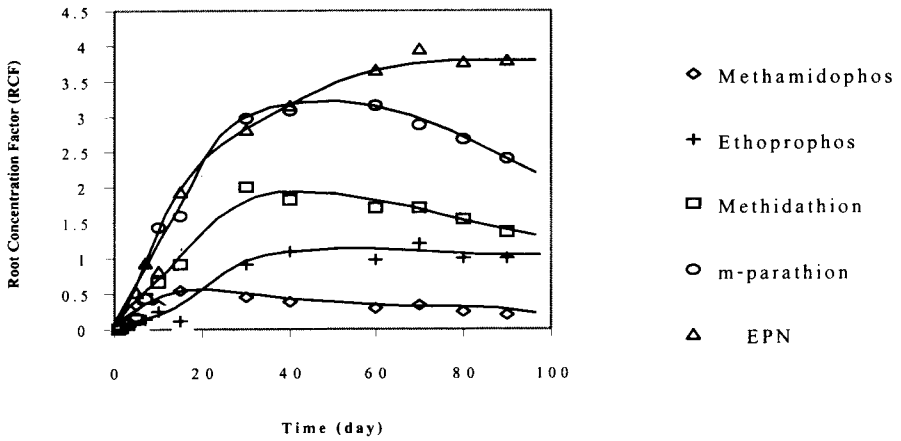


Figure 1 Accumulation of 5 organophosphate pesticides in root from soil (RCF) with time

3.2 Uptake of organophosphate pesticides by root

The uptake of chemicals to plants is partitioning process and by passive transport. The partitioning for hydrophobic chemicals occurs among soil, the surrounding aqueous phase and lipophilic components of roots. In the first 60 days of the experiment, the uptake of test compound in root which reflected by RCF (Root Concentration Factor) increases with time (Figure 1). The RCF was calculated from the concentration of test compound in root divided by the concentration of test compound in soil. Therefore, the concentration of test compounds in soil was still high while the equilibrium of partitioning process was not attained. The increasing of RCF with time for all test compounds were seen.

After 60 days, RCF started to decline. Because the compounds were degraded, soil concentrations were found to decrease with time and consequently affected the concentration profile of those compounds in root with time. This must mean a loss of compound from the root/soil system as a whole. For EPN, its concentration in soil did not decrease as fast as the other compounds because this compound has low aqueous solubility, hence, it highly persists in the environment [19, 20]. It should be noted that this compound may never reach the steady state because its molecular size is relatively large, which therefore diminishes their membrane permeation leading to a slower uptake rate. Table 1 showed that the accumulation of test compounds was quite low. This was not unusual since Connell and Markwell [5] had reported the bioaccumulation factor of m- Parathion from soil = 3.8 while this present study obtained 3.2. They suggested that organic compounds in soil had to partition to soil water before entering to plant root. Because soil water is limited, the amount of test compounds to enter the plant was limited as well.

3.3 The relationship between RCF and physicochemical properties of test compounds

The uptake and transportation of organic chemicals from soil to various plant parts are governed by hydrophobicity and molecular size of those compound [1, 21, 22, 23, 24]. The log K_{ow} value of any organic compound can reflect its hydrophobicity. Compound with high K_{ow} would have high accumulation [19]. In this study, it was found that the root uptake (RCF) of EPN > m-Parathion > Methidathion > Ethoprophos > Methamidophos (Table 1)

Relationship between RCF and log K_{ow}

The RCF of test compounds were related to their log K_{ow} value. The relationships between RCF and log K_{ow} obtained from both experiments were similar and were represented by

$\log RCF = 0.26 \log K_{ow} - 0.46 r^2 = 0.96$

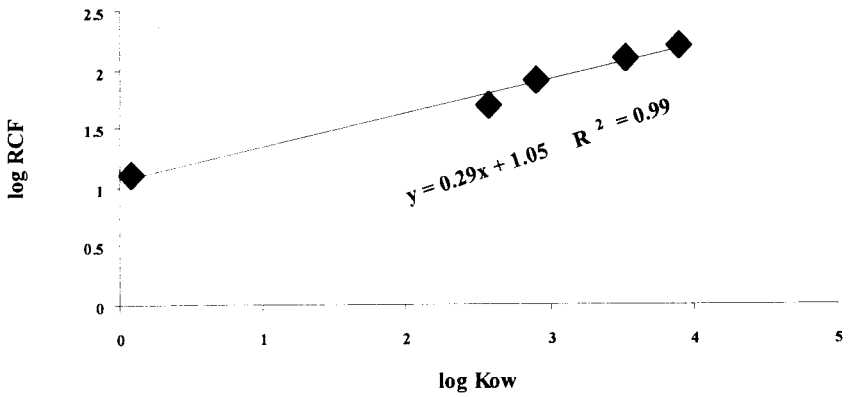
This linear relationship showed that the RCF and log K_{ow} of test compounds was closely related. RCF was also calculated in terms of root lipid weight since lipid plays an important part in the bioaccumulation process [5]. The equation which represented this relationship was (Figure 2)

$\log RCF = 0.29 \log K_{OW} + 1.05 \quad r^2 = 0.98$

This relationship was compared with that report by Briggs et al. [2]

$\log RCF = 0.77 \log K_{OW} - 1.52$

The relationships obtained from both studies showed the differences in slope and intercept. This may be explained by the differences of plant type and lipid content in root of each experiment. Briggs et al. [2] conducted the experiment on barley roots while this study employed vetiver root as a plant model. These two plants obviously have different lipid content in their roots. In addition, it should be noted that some of the test compounds (such as Methamidophos) may and some (such as EPN) may not reach their steady state in root/soil system. If all compounds attained the steady state, the RCF value might be changed and would change the slope of this relationship. However, Briggs et al. [2] had concluded that the uptake and translocation of o-methylcarbamoyloximes and substituted phenylureas, the compounds which have log K_{OW} values between - 0.57 to 4.5, are favored by increased hydrophobicity. With compounds of log K_{OW} greater than 4.5, amount uptaken was reduced. This result was compared to the present study which test compounds had log K_{OW} values between 0.08 – 3.91, the accumulation of 5 organophosphate pesticides in root increased with their hydrophobicity and agreed with that found by Briggs et al. From these studies, it may be concluded that the uptake of organic compounds in root is related to their hydrophobicity.



Relationship between RCF and Aqueous solubility

Root Concentration Factor was also related to the aqueous solubility (log S) of the test compound. It was found that log S is inversely related to RCF and the relationship can be represented by

$\log RCF = - 0.20 \log S + 1.91 \quad r^2 = 0.97$

From the previous study, it was found that log K_{OW} is inversely related to aqueous solubility [15, 25, 26]. As a result, it was as expected that the correlation between RCF obtained from this study and log S of test compounds showed an inverse relationship.

Relationship between RCF and Molecular weight

Many studies also suggested that the uptake of organic compound is also governed by compound molecular size [19, 27]. Molecular weight (MW) which reflects compound molecular size was related to the RCF. The relationship between RCF and MW showed linearity with high correlation and can be represented by

$$\log RCF = 2.88 \log MW - 5.07 \quad r^2 = 0.88$$

[19] concluded that the bioaccumulation of organic compound increases with molecular weight and start to decline if the compound has molecular weight >350. For this study, molecular weight of test compounds are smaller than 350, therefore, it was also expected that the bioaccumulation or RCF would increase with the molecular weight of test compounds.

3.4 Translocation of Test compound to vetiver stem

The translocation of organic compounds may occur by the transpiration stream directly from soil to roots, or by volatilization from soil, subsequent deposition on leaves and then absorption or uptake. As mentioned earlier, for this study, the soil surface was covered with aluminum foil in order to minimize the loss of test compounds via volatilization. In addition, all test compounds had low vapour pressure (Table 2). Therefore, in theory, any test compounds found in the upper parts of the vetiver should arise from translocation via the roots. However, for this study, the term "stem concentration factor"(SCF) would be used instead of "transpiration stream concentration factor" (TSCF) although both factors arise from the ratio of test compound concentration in leaves and stem (C_p) to the concentration in soil (C_s) [1, 21].

The SCF of test compounds obtained from Experiment 1 and 2 were similar (Table 3). The results showed the amounts of all test compounds in the stem and leaf of vetiver samples collected from both experiment were quite low although they increased with time. The concentrations of EPN, Methidathion and m-Parathion in stem increased faster than Methamidophos and Ethoprophos. The sequence was EPN > Methidathion > m-Parathion > Methamidophos > Ethoprophos. This is because the organic compound has to transport through the symplast of the endodermis before entering the xylem [22]. As a result, the compound with hydrophocity would be able to translocate to the stem faster than that with less hydrophobicity .

Table 3 Concentration of test compounds in stem, soil and calculated SCF

Compound	Concentration (mg/g) Experiment 1 stem (C_r)	soil (C_{soil})	SCF	Concentration (mg/g) Experiment 2 stem (C_r)	soil (C_{soil})	SCF
Methamidophos	ND - 0.00003	0.01 - 0.027	0.0012	ND - 0.00002	0.01 - 0.017	0.001
Ethoprophos	ND - 0.002	0.02 - 0.035	0.09	ND - 0.002	0.015-0.026	0.092
Methidathion	ND - 0.118	1.52 - 1.98	0.10	ND - 0.11	0.85 - 1.1	0.11
m-Parathion	ND - 0.05	0.32 - 0.38	0.14	ND - 0.03	0.22 - 0.35	0.11
EPN	ND - 0.17	0.80 - 0.95	0.19	ND - 0.08	0.38 - 0.45	0.20

ND = not detected

The relationship between SCF and log K_{ow}

In previous studies [1, 2, 21], it was found that the TSCF value of test compounds may be related to various physicochemical properties, eg. log K_{ow}.

For this study, the SCF which were calculated in terms of stem lipid content (1.6-1.8%) were related to log K_{ow} values. The correlation between SCF and log K_{ow} showed a linear relationship which was represented by

$$\log SCF = 0.59 \log K_{ow} - 1.06 \quad r^2 = 0.95$$

This relationship showed comparatively high linearity with $r^2 = 0.95$ (Figure 3). This study was compared to that by Briggs et al. [2] which reported that

$$\log SCF = 0.95 \log K_{ow} - 2.05$$

The relationship between SCF and log K_{ow} obtained from both studies suggests that the accumulation of test compounds in leaves and stem may depend upon test compound hydrophobicity. Briggs et al. also found that the translocation of 18 chemicals to barley (following uptake via roots) showed a Gaussian curve for the relationship between TSCF and log K_{ow}. The compound with log K_{ow} value higher than 4.5 would be accumulated lower than expected. Although that compound could enter the xylem, it may not be transported with the transpiration stream because of its high aqueous solubility. However, the compounds which were employed in this study have log $K_{ow} < 4.5$ (the highest log K_{ow} of test compounds is 3.91 for EPN), therefore, the correlation between SCF and log K_{ow} did not show the curvature relationship.

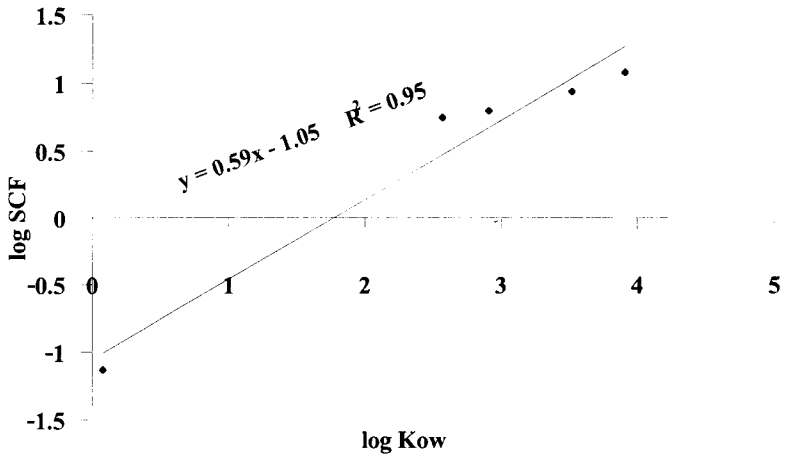


Figure 3 The relationship between SCF and log K_{ow}

4. Conclusions

The bioaccumulation study of 5 organophosphate pesticides in vetiver grass showed that the uptake of these compounds, either in root or stem, were related to their physicochemical properties. The uptake rate in root of test compounds with low hydrophobicity and low MW was larger than those with high hydrophobicity and high MW suggested that the transportation of test compounds may be highly influenced by the molecular size more than hydrophobicity of a compound.

The relationship between RCF and log K_{ow} showed linearity with high correlation and can be represented by log RCF = 0.29 log K_{ow} + 1.05, $r^2 = 0.98$. This relationship suggested that hydrophobicity of test compound may play an inportant role in governing the bioaccumulation or partitioning process.

The bioaccumulation of 5 organophosphate pesticides in stem (SCF) is also related to log K_{ow} by this equation log SCF = 0.59 log K_{ow} - 1.06, r^2 = 0.95. Although, this study had shown the relationship between log SCF and log K_{ow}, the amount of all test compounds found in stem were relatively low. In a normal situation, if soil was not covered by aluminum foil, those compounds may be degraded or volatilized before the accumulation in stem would occur. This may need further study.

5. Acknowledgements

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