

Phytoremediation of Organophosphorus and Organochlorine Pesticides by *Acorus gramineus*

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Abstract

The performance of phytoremediation has proven effective in the removal of nutrients and metals from aqueous systems. However, little information is available regarding the behavior of pesticides and their removal pathways in aquatic environments involving plant-uptake. A detailed understanding of the kinetics of pesticide removal by plants and information on compound/plant partition coefficients can lead to an effective design of the phytoremediation process for anthropogenic pesticide reduction. It was determined that the reduction rates of four organophosphorus (OP) and two organochlorine (OC) pesticides (diazinon, fenitrothion, malathion, parathion, dieldrin, hexachlorobenzene [HCB]) could be simulated by first-order reaction kinetics. The magnitude of k was dependent on the pesticide species and found within the range of 0.409 – 0.580 d⁻¹. Analytical results obtained by mass balances suggested that differential chemical stability, including diversity of molecular structure, half-lives, and water solubility, would greatly influence the removal mechanisms and pathways of OPs and OCs in a phytoreactor (PR). In the case of OP pesticides, plant accumulation was an important pathway for the removal of fenitrothion and parathion from water, while pesticide sorption in suspended matter (SM) was an important pathway for removal of dieldrin and HCB. The magnitude of the pesticide migration factor ($M_p^{pesticide}$) is a good indication of determining the tendency of pesticide movement from below- to above-ground biomass. The uncertainties related to the different phenomena involved in the laboratory phyto-experiment are also discussed.

Keywords: Phytoremediation, Organophosphorus, Organochlorine, Removal kinetics, Pesticide accumulation, Migration factor

1. Introduction

Pesticides are chemicals used for crop protection and pest control and are speculated to be the most widely distributed contaminants in the environment over the last century. Although it is difficult to obtain precise quantities concerning their production and usage, millions of tons of pesticides are produced and spread annually all over the world.¹⁾ Given this widespread use, they are detected using various environmental matrices such as water,²⁾ soil,³⁾ and air.⁴⁾

Pesticides are generally classified according to the type of pest they are intended to control or kill, with many varieties that include insecticides, miticides, fungicides, herbicides, algicides, rodenticides, avicides, larvicides, and germicides.⁵⁾ Pesticides can be composed of inorganic substances, organometallic compounds, volatile organic compounds (VOCs), semi-VOCs, and non-VOCs, whereas the most conventional pesticides are semi-VOCs or non-VOCs.⁴⁾ Pesticides are divided into many classes, of which the most important are organochlorine (OC) and organophosphorus (OP). Due to the long residence time of these substances in the environment, many pesticides used in the fields end up in water resources. Surface water contamination may have ecotoxicological effects upon aquatic organisms, as well as human health, if used for public consumption.^{6,7)}

In order to minimize exposure, development of effective treatment technologies for direct remediation at the contaminated sites is paramount. Conventional techniques that include physical and/or chemical treatments⁸⁾ are typically used for pesticide removal. However, these are often expensive and produce hazardous by-products that must be shipped to landfills for disposal. In the past decade, the use of phyto-processes to remediate contaminated water has gained popularity as a new green evolution technology that is cost-effective and eco-friendly, as well as an efficient *in situ* technology for a variety of pollutants, including nutrients and heavy metals.⁹⁻¹¹⁾ Indeed, some plants possess a natural ability to absorb and hyperaccumulate trace elements in their tissues.^{12,13)} Phytoremediation is also being studied as a sound approach to degrade persistent organic pollutants (POPs), including pesticides.¹⁴⁻¹⁷⁾ Nevertheless, little information is available on the behavior of pesticides in aqueous solutions that involve the plant-uptake mechanism; information on compound plant/water partition coefficients and kinetic uptake parameters are only known for a very few species.

The objective of this work was to study the behavior of OP

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and OC pesticides in aquatic systems, including the remediation efficiency with plant and plant-pesticide uptake, as well as the behavior of pesticide translocation in the plant organs. The four OPs (diazinon, fenitrothion, malathion, parathion) and two OCs (dieldrin and hexachlorobenzene) were chosen as models because of their common use as active ingredients in pesticide products and their distinctive chemical structures that represent the variety of OP and OC pesticides. Although it is apparent that the initial behavior of a chemical in the environment is often affected by formulation, the effects of pesticide species on predicting its behavior in phytoremediation techniques have not been widely studied. The term “migration factor” of pesticides ($M_p^{pesticide}$: mass fraction ratio of pesticides between plant organs) was introduced in order to compare the mobility of pesticides in plants. In this research, the aquatic plant *Acorus gramineus* was chosen as the test model due to its widespread availability, portability, and manageability for indoor study and ornamental applications. Pesticide removal was assessed by estimating the kinetic parameters of pesticide reduction from the aqueous phase. The magnitude of the $M_p^{pesticide}$ values based on the types of pesticides was compared and discussed.

2. Experimental Methods

2.1. Aquatic Plant and Nutrients

Cultivated plants (*A. gramineus*) were taken from gardens and natural wetlands in Gyeonggi Province, Korea. After their arrival in the laboratory, the plants were irrigated with nutrient solutions until maturation for experimentation. Nutrient solutions were made with laboratory grade chemicals (Ca[NO₃]₂, K₂HPO₄, KH₂PO₄, MgSO₄, all assays >98%) in deionized water. The characteristics of the nutrient solutions are summarized in Table 1. Subsequently, the chemical pesticides (OPs or OCs) were added to the nutrient solution in order to prepare pesticide medium solutions for the phytoreactors (PRs).

Table 1. Characteristics of nutrient solution

| Parameters | Units | Quantity |
|-------------------------|---------------------|-----------|
| Stock nutrient solution | | |
| Nitrate as nitrogen | mg/L | 10 |
| Phosphate as phosphorus | mg/L | 2.5 |
| Sulfate as sulfur | mg/L | 3.0 |
| Magnesium | mg/L | 2.5 |
| Calcium | mg/L | 14 |
| Dissolved oxygen | mgO ₂ /L | 6.0 – 7.0 |
| Mixed OP solution | | |
| Diazinon | mg/L | 6.0 |
| Fenitrothion | mg/L | 3.0 |
| Malathion | mg/L | 7.0 |
| Parathion | mg/L | 3.0 |
| Mixed OC solution | | |
| Dieldrin | mg/L | 3.0 |
| HCB | mg/L | 6.0 |

OP: organophosphorus, OC: organochlorine, HCB: hexachlorobenzene.

2.2. Stock Pesticide Solutions

Analytical standard diazinon, fenitrothion, malathion, parathion, dieldrin, and HCB (all assays >99.9% purity) were

purchased from Sigma-Aldrich (Yongin, Korea). Individual pesticide stock solutions were prepared in a methanol solution (Sigma-Aldrich, assay >99.8%) and stored at -12°C until use as preparing pesticide medium solutions and standard pesticide solutions for gas chromatography/mass spectroscopy (GC/MS) calibration and interpretation. The physicochemical properties of the selected OP and OC pesticides are given in Table 2.

2.3. Pesticide Solutions for PRs

The OP solution (Table 1) consisted of a mix of diazinon, fenitrothion, malathion, and parathion and was prepared by diluting the appropriate volumes from the individual stock OP pesticide solutions with a nutrient solution (Section 2.1). The method was similar to a preparation of an OC solution consisting of mixed dieldrin and HCB.

2.4. Phytoreactors

The PRs were made of glass materials with dimensions of Ø105 mm × 150 mm (diameter × height), accommodating a volume of 0.5 – 1.0 L. Fig. 1 shows the design and setup of the PRs in the laboratory. *A. gramineus* (24 ± 1.0 g, wet weight) was introduced into the PR and filled with 0.5 L of pesticide medium solution. The reactors were operated by batch feed with water stirring twice a day. Pairs of the planted PRs, as well as the controls (PRs without plantation), were setup in the experiment. The experiment was repeated twice in sequence. Fluorescence lamps (FPL24EX-D; Kumho Electric Inc., Seoul, Korea) were used as a light source for photosynthesis. Both light and dark intervals inside the growth chamber were maintained at 12 h, respectively. The light intensity and ambient temperature inside the growth chamber were maintained at 1450 lx and 25 ± 2°C. Addition of deionized water to the PR and control reactor was sometimes required to maintain a constant water table level and prevent the level from falling due to water loss by evaporation.

2.5. Sample Extractions and Analysis

2.5.1. Extraction of Pesticides in Aqueous Solutions

The 5.0 mL aqueous samples inside the PRs were sampled daily and immediately extracted with 5.0 mL of *n*-hexane. The extracted solutions were dehydrated with sodium sulfate, filtered by syringe filters (Whatman glass microfiber filter, grade GF/C 0.45 µm), and analyzed by gas chromatography and mass spectroscopy (GC/MS-QP2010 Plus; Shimadzu Corp., Kyoto, Japan).

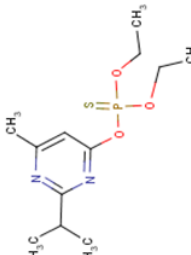
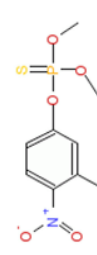
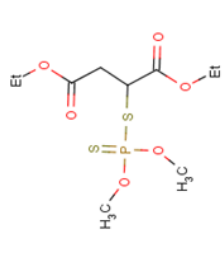
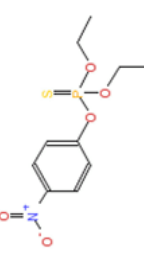
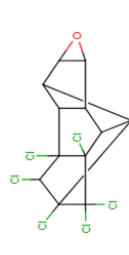
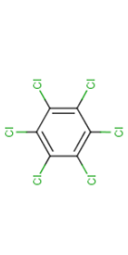
2.5.2. Extraction of Pesticides in Plants

In order to determine pesticide distribution in the plant tissues, one-third of the plants were sampled at the end of the first batch tests, while the remaining plants in the PRs were collected at the end of the second batch tests. The plants were separated into three parts (leaves, rhizomes, roots) and recorded for wet weight biomass. Subsequently, they were dried at 35°C for 72 h in an air recirculation oven (OF-22; Jeio Tech, Kimpo, Korea). The dried plant organs were recorded for their weights and ground into a fine powder. The pesticides in the powder samples were extracted by liquid-liquid (L-L) extraction using ethyl acetate (EA) as the solvent extractor. The extracted solutions were then filtered using Whatman GF/C filters and analyzed by GC/MS.

2.5.3. Extraction of Pesticides in Suspended Matter

At the end of first and second batch tests, the suspended matter inside the PRs were collected by filtering all aqueous solutions

Table 2. Physicochemical properties of selected pesticides

| Groups | Pesticides | MW | ρ^* (g mL ⁻¹) | $S_w^†$ (mg L ⁻¹) | $H^‡$ (atm m ³ mol ⁻¹) | $\log K_{ow}^§$ | $K_{oc}^ $ (mL g ⁻¹) | $t_{1/2}^¶$ (d) | Structures |
|--------|--------------|-------|--------------------------------|-------------------------------|---|-----------------|-----------------------------------|-----------------|---|
| OP | Diazinon | 304.4 | 1.11 | 60 | 6.01×10^{-7} | 3.30 | 643 | 40 - 185 |  |
| | Fenitrothion | 277.2 | 1.33 | 30 | 9.73×10^{-8} | 3.43 | 322 | 4 - 84 |  |
| | Malathion | 330.4 | 1.23 | 130 - 145 | 9.87×10^{-9} | 2.75 | 217 | 1 - 6 |  |
| | Parathion | 291.3 | 1.26 | 11 | 2.98×10^{-7} | 3.83 | 7,660 | 14 - 260 |  |
| OC | Dieldrin | 380.9 | 1.75 | 0.14 - 0.2 | 6.42×10^{-7} | 3.69 | 12,000 | >1,000 |  |
| | HCB | 284.8 | 2.04 | 0.005 | 1.02×10^{-4} | 5.31 | 50,000 | >1,000 |  |

OP: organophosphorus, OC: organochlorine, HCB: hexachlorobenzene, MW: molecular weight.

*Bulk density, †Solubility in water at 20 - 25°C, ‡Henry's law constant at 25°C, §Octanol-water partition coefficient,

|| Organic carbon-water partition coefficient and ¶Half-life at pH 7 and 20°C (adapted from Hornsby et al.,¹⁶⁾ Tomlin,¹⁹⁾ Agriculture & Environment Research Unit (AERU) at the University of Hertfordshire²⁰⁾).

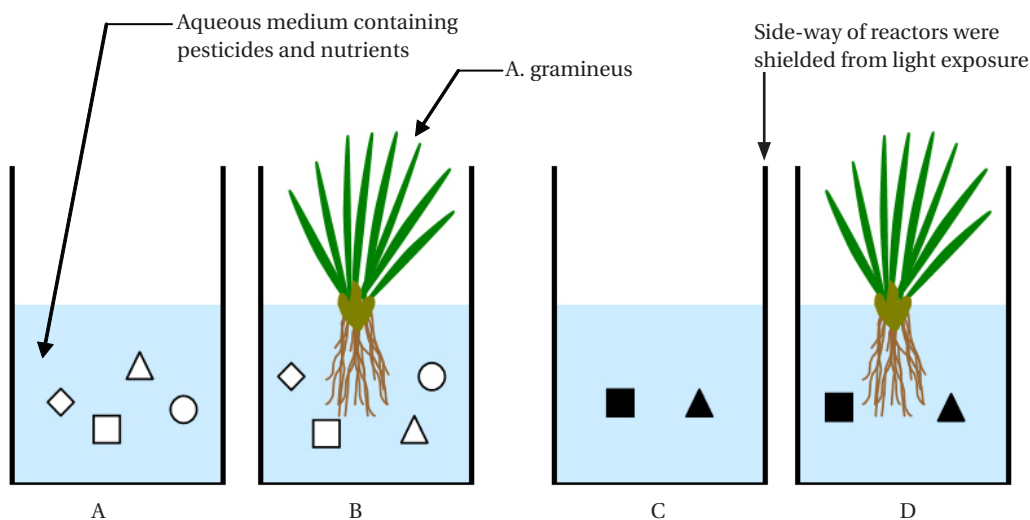


Fig. 1. Experimental setup for phyto-processes of organophosphorus (OP) and organochlorine (OC) removal by *Acorus gramineus*. Letters A, B, C, and D represent the reactors of OP control; OP with plant; OC control; OC with plant. Symbol: diazinon (○); fenitrothion (□); malathion (△); parathion (○); dieldrin (■); hexachlorobenzene (▲).

(500 mL/each PR) through Whatman GF/C filters. Collected samples were dried at 105°C for 1 h in an oven and weighed on an analytical balance (Ohaus Corp., Pine Brook, NJ, USA). They were subsequently extracted by L-L extraction. The extraction method was similar to that in section 2.5.2, but with *n*-hexane used instead of EA. Furthermore, after filtration, the remaining solutions were subjected to L-L extraction similar to that described in section 2.5.1.

2.5.4. Pesticide Analysis

One microlitre of extracted solution was injected automatically into the GC/MS by an auto-injector/auto-sampler (AOC-20i; Shimadzu). The operational conditions of the GC/MS analysis are given in Table 3. A selected ion-monitoring (SIM) mode was chosen for the GC/MS operation. A SIM table was constructed for the GC/MS quantification. The most abundant ion as a base peak (target ion), retention time, and other ions for confirmation (selected ions) were obtained from a standard pesticide calibration (Table 4).

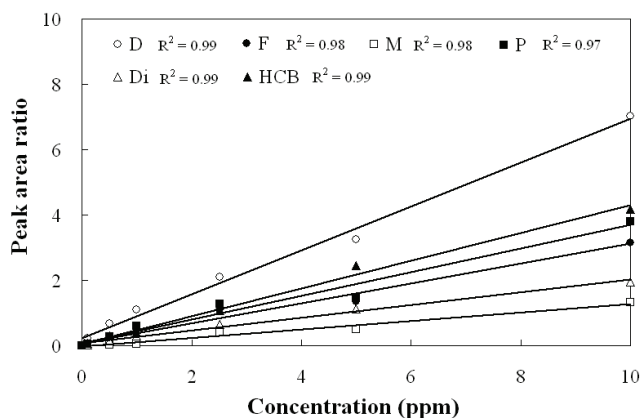


Fig. 2. Standard calibration plots for gas chromatography/mass spectroscopy (GC/MS) analysis of selected pesticides listed in Table 2. Symbols D, F, M, P, Di, and HCB represent diazinon, fenitrothion, malathion, parathion, dieldrin, and hexachlorobenzene, respectively.

Standard curves were created for quantification, using standard solutions of the selected analytes in *n*-hexane and Endosulfan I-*d*₄ as the internal analytical standard. Fig. 2 shows the linearity of the standard curves of each sample analyzed. The recovery study performed on blank samples (nutrient solution and plant samples with no pesticide contamination), spiked with known levels of the pesticide, is summarized in Table 5. Three replicates for each sample were carried out at three levels (25, 50, 100 µg L⁻¹ for water and 10, 50, 100 µg kg⁻¹ for plant, respectively), and the relevant recovery results, given as mean values, were 85 - 94% and 78 - 90% for water and plant, respectively. The RSD was 2 - 5% and 4 - 7% for water and plant, respectively.

3. Results and Discussion

3.1. Kinetics of Pesticide Removals

The change in OP and OC concentrations in the PRs operated by the batch mode are described by first-order reaction kinetics (Fig. 3) and mathematically expressed as:

$$C = C_0 \cdot e^{-kt} \quad (1)$$

where *C* is the pesticide concentration (mg/L) in the PRs at time *t* (d), *C*₀ the initial concentration (mg/L), and *k* the first-order kinetic constant (d⁻¹). A plot of $-\ln(C/C_0)$ versus *t* was linear and the slope equal to *k*. The experimental half-life (*t*_{1/2}, d), the time required to remove half of the pesticide concentration from the PRs, is given as:

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

Data obtained during the present investigation revealed that all experimental sets containing aquatic plants removed a substantial amount of pesticide. Approximately 88 - 93% of the OP and OC pesticides in the water were removed by planted PRs at a retention

Table 3. Parameters for operating gas chromatography and mass spectroscopy

| Gas chromatography | | Mass spectroscopy | |
|-----------------------------------|---|------------------------|---------|
| Chromatographic column | Solgel-1 (60 m × Ø0.25 mm i.d. × 1.0 µm film thickness) (Phenomenex, Inc., Torrance, CA, USA) | Interface temperature | 280°C |
| Carrier gas | He | Ion source temperature | 200°C |
| Flow rate | 1.3 mL/min | Operation mode | SIM |
| Injection mode | Splitless | Solvent cut time | 4.5 min |
| Injection volume | 1.0 µL | Ionization mode | EI |
| Injector port temperature | 250°C | Ionization voltage | 70 eV |
| Initial temperature and hold time | 60°C for 1 min | | |
| 1 st ramp | 20°C/min to 160°C | | |
| 2 nd ramp | 5°C/min to 280°C | | |
| Final hold time | 5 min | | |

SIM: selected ion-monitoring, EI:

Table 4. Chemical groups, base peaks, and selected ions used for gas chromatography/mass spectroscopy analysis

| Groups | Pesticides | MW | RT (min) | Base peak (m/z) | SI (m/z) |
|--------|--------------|--------|-------------|-----------------|---------------|
| OP | Diazinon | 304.35 | 17.6 – 17.8 | 137 | 152, 179, 304 |
| | Fenitrothion | 277.23 | 20.6 – 20.8 | 277 | 125, 260 |
| | Malathion | 330.36 | 20.9 – 21.1 | 173 | 93, 158 |
| | Parathion | 291.26 | 21.5 – 21.7 | 291 | 139 |
| OC | Dieldrin | 380.91 | 25.3 – 25.5 | 263 | 345, 237 |
| | HCB | 284.79 | 16.4 – 16.6 | 284 | 286, 249 |

OP: organophosphorus, OC: organochlorine, HCB: hexachlorobenzene, MW: molecular weight, RT: retention time, SI: selected ions.

Table 5. Average recoveries and relative standard deviations (RSDs, %) for OP and OC pesticides in water and plants fortified at 25, 50, and 500 (µg L⁻¹) in water and 10, 50, and 100 (µg g⁻¹) in plant

| Group | Pesticide | Water | | Plant | |
|-------|--------------|-------------------|---------|-------------------|---------|
| | | Mean recovery (%) | RSD (%) | Mean recovery (%) | RSD (%) |
| OP | Diazinon | 91 | ±2 | 86 | ±4 |
| | Fenitrothion | 92 | ±3 | 81 | ±4 |
| | Malathion | 89 | ±4 | 89 | ±7 |
| | Parathion | 94 | ±2 | 87 | ±6 |
| OC | Dieldrin | 85 | ±5 | 78 | ±4 |
| | HCB | 88 | ±3 | 90 | ±5 |

OP: organophosphorus, OC: organochlorine, HCB: hexachlorobenzene.

time (RT) of 5 d. The reduction rate of the pesticides in the PRs fit Eq. 1 (R^2 value >0.9 for all pesticides). The k and experimental $t_{1/2}$ values of selected OP and OC pesticides were obtained (Table 6). The magnitude of the k values of the OP pesticides was greater than the OC pesticides, with the k values ranging from 0.455 to 0.580 d⁻¹ for the OPs and 0.409 to 0.542 d⁻¹ for the OCs. Among the pesticides, malathion showed the highest k value, while HCB showed the lowest.

Estimates of the experimental $t_{1/2}$ of pesticides in the PRs

ranged between 1.2 to 1.5 d for OPs and 1.3 to 1.7 d for OCs. As a similar result with k values, malathion had the highest k value and shortest $t_{1/2}$ value, whereas HCB had the lowest k and longest $t_{1/2}$.

3.2. Plant Uptake of Pesticides

The weight-based pesticide accumulation in the plant organs of *A. gramineus* is illustrated in Fig. 4. Accumulation of pesticides in the plant organs varied widely depending on the pesticide species. From the data, the OP pesticides accumulated in *A. gramineus*

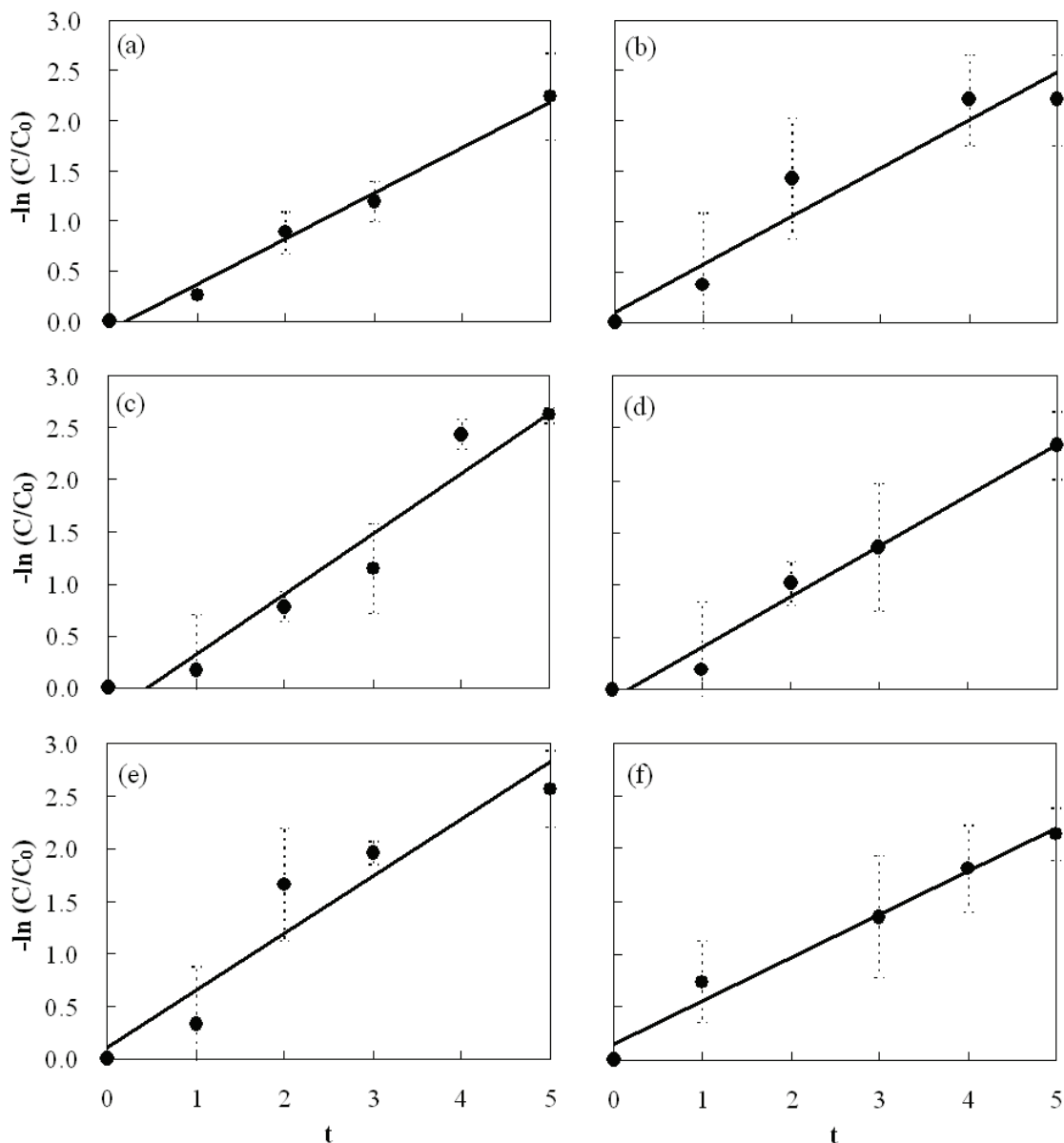


Fig. 3. Determination of first-order kinetic coefficient (k) for removal of pesticides in phytoreactors grown with *A. gramineus*: (a) Diazinon; (b) Fenitrothion; (c) Malathion; (d) Parathion; (e) Dieldrin; (f) Hexachlorobenzene. Points and dotted lines represent mean \pm SE ($n = 4$).

Table 6. Kinetic parameters of OP and OC pesticide removal from aqueous medium

| Groups | Pesticides | Efficiency* (%) | k (d^{-1}) | R^2 Value | Experiment $t_{1/2}^\dagger$ (d) |
|--------|--------------|-----------------|------------------|-------------|----------------------------------|
| OP | Diazinon | 89 \pm 9 | -0.455 | 0.99 | 1.5 |
| | Fenitrothion | 89 \pm 4 | -0.476 | 0.93 | 1.5 |
| | Malathion | 93 \pm 4 | -0.580 | 0.94 | 1.2 |
| | Parathion | 90 \pm 8 | -0.485 | 0.98 | 1.4 |
| OC | Dieldrin | 92 \pm 6 | -0.542 | 0.91 | 1.3 |
| | HCB | 88 \pm 14 | -0.409 | 0.98 | 1.7 |

OP: organophosphorus, OC: organochlorine, HCB: hexachlorobenzene.

*Removal efficiency of pesticides at retention time of 5 d (mean \pm SE, $n = 4$). † Values calculated by inserting k values (column 4) into Eq. 2.

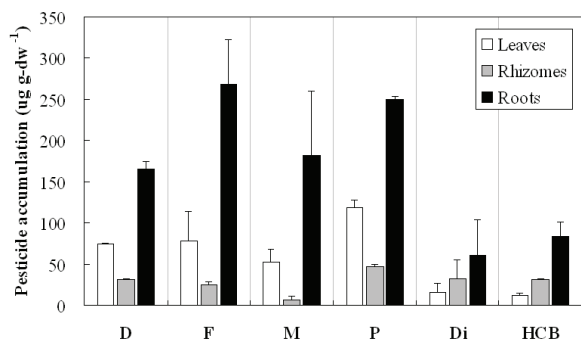


Fig. 4. Weight-based pesticide accumulation in plant organs. Symbols D, F, M, P, Di, and HCB represent diazinon, fenitrothion, malathion, parathion, dieldrin, and hexachlorobenzene, respectively. Bars and lines represent mean \pm SE ($n = 4$).

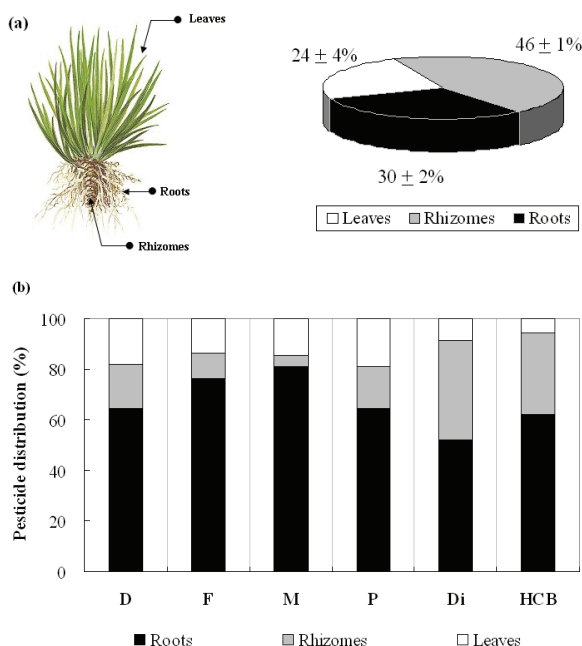


Fig. 5. Distribution of pesticides in plant organs of *A. gramineus* based on total plant biomass: (a) Percentages of plant organs; (b) Percentages of pesticides distributed in plant organ. Symbols D, F, M, P, Di, and HCB represent diazinon, fenitrothion, malathion, parathion, dieldrin, and hexachlorobenzene, respectively. Bars and lines represent mean \pm SE ($n = 4$).

more than the OC pesticides. The highest accumulation of both OPs and OCs was found in the roots. The leaves of the *A. gramineus* could accumulate more OP pesticides than the rhizomes, while the opposite result was found in the accumulation behavior of OC pesticides.

In addition, the overall distribution of pesticides based on plant biomass proportion is depicted in Fig. 5. According to the physiology of the plant, *A. gramineus* consists of approximately 30% roots, 46% rhizomes, and 24% leaves (Fig. 5a). The rooted portion of *A. gramineus* plays an important role in the total pesticide-uptake due to the relatively high weight-based accumulation of pesticides and its contribution to organ biomass proportion. Among the OP pesticides, malathion was an efficient accumulator (81%) in the roots, followed by fenitrothion (76%), diazinon (65%), and parathion (64%), respectively. Although, all OP pesticides had a relatively high weight-based pesticide accumulation in the leaves in comparison to the rhizomes, in terms of pesticide distribution in the whole plant, all OP pesticides, excluding malathion, bore no significant difference in the quantity of pesticide accumulation between leaves and rhizomes. In the case of OC pesticides, both roots and rhizomes played equal roles in the uptake of dieldrin and HCB, while all OCs distributed in the leaves took into account only 6 - 8% of total plant total biomass.

Furthermore, the weight-based pesticide accumulation rates in terms of biomass wet-weight were estimated by dividing the total amount of pesticide accumulated in the plant with the total biomass (wet-weight) and treatment time. Estimates of weight-based pesticide accumulation rates of OP pesticides were 6,725 $\mu\text{g kg}_{\text{wet plant}}^{-1} \text{d}^{-1}$ for diazinon, 9,557 $\mu\text{g kg}_{\text{wet plant}}^{-1} \text{d}^{-1}$ for fenitrothion, 6,488 $\mu\text{g kg}_{\text{wet plant}}^{-1} \text{d}^{-1}$ for malathion, and 9,084 $\mu\text{g kg}_{\text{wet plant}}^{-1} \text{d}^{-1}$ for parathion. Estimates of weight-based pesticide accumulation rates of OC pesticides were 4,157 $\mu\text{g kg}_{\text{wet plant}}^{-1} \text{d}^{-1}$ for dieldrin and 4,912 $\mu\text{g kg}_{\text{wet plant}}^{-1} \text{d}^{-1}$ for HCB.

3.3. Mass Balance

A mass balance of pesticides was performed on the PRs in order to determine pesticide removal pathways. For a batch mode of operation, the input amount of pesticides in the PRs must balance with the summation of total pesticides presented in the effluent treated water accumulated by plants and suspended matter (SM). However, all pesticides tested were organic substances likely to be degraded into other chemical breakdown-products as a function of time. The term "loss" is therefore inserted into the balance equation. A mass balance equation for distribution of a pesticide can be written as follows:

Total pesticides input to the PRs = Pesticides remaining in the water after treatment + Pesticides accumulated in plants + Pesticides accumulated in SM + Loss (3)

Table 7. Mass balances of pesticides in phytoremediation process

| Pathways | Pesticides | | | | | | |
|-------------------------------------|------------|--------------|-----------|-----------|----------|----------|--|
| | Diazinon | Fenitrothion | Malathion | Parathion | Dieldrin | HCB | |
| Input (mg) | 12 (100) | 6 (100) | 14 (100) | 6 (100) | 6 (100) | 12 (100) | |
| Remain in water (mg) | 1.2 (10) | 0.6 (10) | 1.0 (7) | 0.6 (10) | 0.6 (10) | 1.4 (12) | |
| Accumulate in plants (mg) | 2.6 (21) | 3.7 (61) | 2.5 (18) | 3.5 (58) | 1.6 (27) | 1.9 (16) | |
| Accumulate in suspended matter (mg) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 3.6 (60) | 7.6 (63) | |
| Losses | 8.2 (69) | 1.7 (29) | 10.5 (75) | 1.9 (32) | 0.2 (3) | 1.1 (9) | |

HCB: hexachlorobenzene.

The values in parentheses indicate percentages.

The results of the mass balance are shown in Table 7. The amount of pesticides removed from the water was much higher than that accumulated by plants in the PRs. After treatment (RT = 5 d), the residual pesticides in the water were within a similar range of about 7 – 12% of its initial concentrations, whereas the accumulation of pesticides in *A. gramineus* varied broadly depending on chemical species. Fenitrothion and parathion accumulated in *A. gramineus* at high levels (58 – 61% of total pesticides input to the PRs), while the other pesticides (diazinon, malathion, dieldrin, HCB) accumulated only in small portions (16 – 27%). Interestingly, nearly 60 – 63% of the total OCs input in the PRs accumulated in the SM, while there was no accumulation of OPs in the SM. Loss of OP pesticides in the PRs was relatively high in comparison to the OC pesticides. High losses (69 – 75%) occurred in diazinon and malathion, medium losses (29 – 32%) with fenitrothion and parathion, and low (3 – 9%) with dieldrin and HCB.

3.4. Plant Tolerance and Physical Toxicity Observation

In the first batch tests, all the experimental plants showed a slight reduction in plant growth, branching, leaf size, and root system. The mean biomass (wet-weight) after treatment increased only 0.8% in comparison with the original plant weight prior to treatment. The top portion of the leaves began to show yellow and dried, with portions of the roots beginning to show abnormal darkening and weakness. However, the plants seemed to acclimatize and endure the toxicity of the pesticides in the second batch tests. The dark green of the leaves was enhanced and observed in several experimental plants. The mean biomass increased 11.1% at the end of treatment.

4. Discussion

It appeared that *A. gramineus* had the ability to sorb many OP and OC pesticides (diazinon, fenitrothion, malathion, parathion, dieldrin, HCB), and assisted in their removal from the aquatic environment. In the natural environment, there are several factors and mechanisms both affected and influenced by pesticide dissipation in the water, including transport (volatilization, runoff, leaching) and transformation processes (hydrolysis, water/soil partition, photolysis, microbial degradation).²¹⁾ Factors that could affect aqueous concentration of pesticides in the present study include hydrolysis, water/organic matter partition, and uptake/metabolism by plant. This might also account for the disparity between the calculated $t_{1/2}$ herein and typical aqueous environmental half-lives (Table 2).

The presented experimental $t_{1/2}$ values of the pesticides in the PRs were much less than the $t_{1/2}$ values obtained from the literature (Table 2). For example, the $t_{1/2}$ of OC pesticides as nearly 1000-fold shorter than the values presented in the literature, suggesting that the presence of *A. gramineus* could shorten the lifetime of OC and OP pesticides in an aquatic environment. Furthermore, the plant species used in the treatment systems might influence the magnitude of the $t_{1/2}$ of the pesticide. Gao et al.¹⁴⁾ found the $t_{1/2}$ of malathion removal by three aquatic plants (parrot feather, duckweed, elodea) ranged between 2.0 to 4.8 d, whereas the required $t_{1/2}$ of *A. gramineus* (our experimental data) to mediate malathion in water was only 1.2 d (Table 6).

These results indicate that the chemical stability of pesticides used in the tests played an important role in the removal mechanisms and pathways in the PRs. Three main factors

involving pesticide stability in an aquatic environment were structural stability, volatilization, and solubility. Four OP pesticides selected for the tests had low to medium structural stability (typical half-lives in aquatic environment = 1 – 206 d), as well as a high water solubility ($S_w = 11 – 145$ mg/L at 20 – 25°C) (Table 2). However, two OC pesticides selected for the tests were extremely stable (typical half-lives in aquatic environment >1,000 d) and possessed an extremely low water solubility (0.005 – 0.2 mg/L) at 20 – 25°C. Obviously, the distinction of pesticide removal pathways between OPs and OCs in the PRs was the amount of pesticides sorbed by the SM. All OC pesticides were found primarily in the SM as 60 – 63% of the total pesticide input to the PRs, whereas there was no sorption phenomenon of the OP pesticides by the SM due to the large differences of the S_w and organic carbon-water partition coefficient (K_{oc}) between OP and OC pesticides (Table 2). Considering the OP and OC concentrations used in the tests (Table 1), all OP pesticides were very soluble in water and tended to not accumulate in the SM because of their strong polar nature. Contrary to the OC pesticides, both dieldrin and HCB were highly insoluble in water and much heavier than water (bulk density = 1.75 and 2.04 g mL⁻¹), with a relatively high K_{oc} (12,000 and 50,000 mL g⁻¹). Thus, they could easily bind together with settled SM. This phenomenon was similar to the behavior of 17 OPs and 18 OCs in the Jiulong River of China reported by Zhang et al.²⁾ where SM did not bear an obvious correlation with OPs due to their higher water solubility and weaker sorption capacity relative to OCs. Regardless, the required $t_{1/2}$ (experimental $t_{1/2}$ as seen in Table 6) for the phytoremediation of OPs and OCs in contaminated water were small. Unlike the OPs, the OC pesticides strongly sorbed by SM were likely to be more persistent as they were protected from phytoremediation (plant uptake/metabolism) by binding with SM.

Among the pesticides used in the tests, the OPs possessed high portions of unknown loss described by mass balances (Table 7). Loss of pesticides *via* volatilization might not be the main removal mechanism in the PRs given their extremely low vapor pressure (diazinon = 8.0; fenitrothion = 0.1; malathion = 1.1; parathion = 0.7; dieldrin = 0.4; HCB = 1.5 mPa).¹⁸⁾ Furthermore, all pesticides used in the test (HCB excepted) had a Henry's law constant (H) at 25°C less than 6.5×10^{-7} atm m³ mol⁻¹ (Table 2), indicating their diminished tendency to move into the air *via* volatilization. Fairbrother et al.²²⁾ suggested that chemical volatilization is significant in all water for a pollutant having an H value greater than 10^{-5} atm m³/mol, while a pollutant with a value smaller than 3.0×10^{-7} atm m³ mol⁻¹ is considered nonvolatile. Although the H value of HCB was estimated at about 1.02×10^{-4} atm m³ mol⁻¹ (Table 2), and considered as moderate volatilization in water, estimation of HCB loss in the PR by mass-balance calculation was relatively low (about 9%, Table 4) in comparison with other pesticides (diazinon, fenitrothion, malathion, parathion). This result supported that the high portion of HCB (63% of the total HCB input into the PR) bound into the SM could promote the movement of HCB into the air.

The other causes of loss might arise from their chemical structures that typically undergo rapid environmental degradation, when compared with OCs,²³⁾ and possibly also plant metabolic conversion or phytotransformation.¹⁴⁾ However, only the parent pesticide compounds were analyzed herein, not the breakdown products. Thus, further information regarding chemical intermediates was unavailable.

Malathion had the largest quantity of unknown loss in mass balance (75% of total malathion input into the PRs) due to its very small $t_{1/2}$ in comparison with other pesticides (see Table 2

for typical half-lives of pesticides). However, the typical $t_{1/2}$ of diazinon in an aquatic environment obtained from literature reviews was found in a moderate range (40 – 185 d) with a large quantity of unknown loss in mass balance (69% of total diazinon input). Since the $t_{1/2}$ value for diazinon removal in water obtained by kinetic calculation in the presented tests was only 1.5 d, the typical $t_{1/2}$ of diazinon degradation in an aquatic environment could subsequently vary greatly depending on environmental matrices, such as appearance/disappearance of plants and the physico-chemical properties of water. Additionally, it was found that diazinon rapidly hydrolyzed at low or high pH values since it is susceptible to both base- and acid-catalyzed hydrolysis.²⁴⁾ Drufovka et al.²⁵⁾ showed that the presence of microorganisms and the decrease in solution pH could enhance diazinon degradation. For example, microbial-assisted diazinon degradation at a neutral pH resulted in a 10-fold decreased half-life and a 30-fold increased degradation rate; the diazinon was completely degraded within 1 – 2 d at a solution pH less than 3.2. In our tests, all planted reactors had a slightly negative effect upon solution pH. Rapid reduction of solution pH was found at an early stage in the experiment. The solution pH during the PR treatment was found in the range of 6 to 6.7 (unpublished data). The reason for this might be formation of dissolved CO₂ and carbonic acid (H₂CO₃) in the water due to the degradation of organic substances (residual organic matter suspended in water and plant material) of aerobic microorganisms and plant root exudation of organic acids, resulting in pH reduction.^{26,27)} This reduction in pH in the PRs might plausibly enhance diazinon degradation rate.

Uptake and distribution of pesticides in plants were greatly influenced by pesticide species and plant physiology itself. The accumulation (in percent) of OP pesticides by *A. gramineus* varied broadly from a small (18 – 21% of total pesticide input into the PRs) to a large quantity (58 – 61%), and had an inverse proportion to the portions of unknown loss, while plant accumulation (in percent) of the OC pesticides was small in quantity and varied in a narrow range (Table 6). Although both OPs and OCs had a relatively high log K_{OW} , resulting in a great potential in plant accumulation, they were distinguished by chemical polarity and typical half-lives in the environment (Table 2) as mentioned above. In the case of OP pesticides, all OPs had high water solubility and were expected to efficiently accumulate in the plant. But only two OPs with a small quantity of unknown loss and a moderate and typical $t_{1/2}$ value, including fenitrothion and parathion, could accumulate in plants in high portions, whereas, the other two OPs with a large quantity of unknown loss and short typical $t_{1/2}$ value, including diazinon and malathion, could accumulate in plants in only small portions. In the case of OC pesticides, they possessed a hydrophobicity greater than the OPs, resulting in a greater tendency to partition out of the aqueous phase and inefficient uptake/assimilation by the plant.²⁸⁾

All pesticides accumulated and stored mainly in the plant below ground, especially in the roots (Figs. 4 and 5). This result was similar to the work reported by Bouldin et al.²⁹⁾ in that the roots of two aquatic plants, *Juncus effusus* and *Ludwigia peploides*, played an important role in storing toxic organic pollutants, including atrazine and lambda-cyhalothrin. Leaves and rhizomes of *A. gramineus* in our tests showed a distinguished capacity for accumulating OPs and OCs. All OPs accumulated in the leaves, more so than in the rhizomes of *A. gramineus*, while all OCs accumulated in the rhizomes over the leaves of *A. gramineus*. One of the reasons might be due to the higher water solubility of OPs than OCs, resulting in higher mobility of OP transportation

from roots to rhizomes and rhizomes to leaves. This result was in agreement with Wilson et al.,³⁰⁾ who investigated the uptake and distribution of metalaxyl in *A. gramineus*, *Canna hybrida*, *Myriophyllum aquaticum*, and *Pontederia cordata* using the ¹⁴C-labeling technique and reported a significant accumulation of ¹⁴C-metalaxyl primarily in the leaves, and to a lesser extent in the roots of *A. gramineus*. The most probable, highly water-soluble metalaxyl (8.4 g/L) would be easily taken up by plants and highly accumulate in the aboveground biomass.

The usage of pesticide-tissue concentrations as a criterion for identifying plant-pesticide uptake could not indicate the *mobile-capacity* of pesticides in plants. The term “migration factor” ($M_p^{pesticide}$) is introduced in this present work to evaluate the movement ability of pesticides in the plant. The $M_p^{pesticide}$ factor is the ratio of partitions of pesticides between rhizomes to leaves (V_b) and roots to rhizomes

$$M_p^{pesticide} = \frac{V_b}{V_a} = \frac{\text{Partition of pesticide between rhizome (or stem) to leaf}}{\text{Partition of pesticide between root to rhizome (or stem)}} \quad (4)$$

$$V_a = \frac{\left[\frac{\text{Pesticide in rhizome (or stem)}}{\text{Rhizome (or stem)'s biomass}} \right]}{\left[\frac{\text{Pesticide in root}}{\text{Root's biomass}} \right]} \quad (5)$$

$$V_b = \frac{\left[\frac{\text{Pesticide in leaf}}{\text{Leaf's biomass}} \right]}{\left[\frac{\text{Pesticide in rhizome (or stem)}}{\text{Rhizome (or stem)'s biomass}} \right]} \quad (6)$$

Table 8. Migration factor of selected pesticides in plant

| Groups | Pesticides | V_a^* | $V_b^†$ | M_p^\ddagger |
|--------|--------------|---------|---------|----------------|
| OP | Diazinon | 0.19 | 2.39 | 12.6 |
| | Fenitrothion | 0.09 | 3.12 | 33.4 |
| | Malathion | 0.04 | 7.91 | 215.1 |
| | Parathion | 0.19 | 2.53 | 13.5 |
| OC | Dieldrin | 0.53 | 0.49 | 0.9 |
| | HCB | 0.37 | 0.40 | 1.1 |

OP: organophosphorus, OC: organochlorine, HCB: hexachlorobenzene. *Values calculated by Eq. 5. †Values calculated by Eq. 6. ‡Values calculated by Eq. 4.

The $M_p^{pesticide}$ factor shows a different order in each pesticide species (Table 8). The magnitude of the $M_p^{OC\ pesticides}$ values was relatively small in comparison with $M_p^{OP\ pesticides}$. The highest M_p factor was with malathion (215.1) and the lowest dieldrin (0.9). Since malathion had the highest water solubility and moderate log K_{OW} among the pesticides used in the tests, it was probably taken up by *A. gramineus* with ease and translocated into its metabolic system. While dieldrin was less water soluble than malathion, about 650- to 1036-fold, it appeared as the highest molecular size (MW). This may result in the gradual movement of dieldrin in the plant. Accordingly, the M_p factor can be an indicator of a chemical's tendency to accumulate in plants. The M_p factor is also a good indicator of where a chemical will be distributed and used as criteria for plant selection in the phytoremediation process. If the M_p is high, the pollutants will be highly mobile throughout

the plant organs and have a high tendency to accumulate in the aboveground biomass. However, there are many possible factors that could plausibly influence the magnitude of the M_p factor, including plant species, RT/treatment period, and physico-chemical properties of water (such as pH and temperature). This lab is currently conducting additional work to investigate the relationships of those parameters with the $M_p^{pesticide}$ factor by using the same key design data as well as constructing a semi-pilot scale in order to demonstrate the technical viability in the real environmental field.

5. Summary

This study examined the detailed kinetics of six organic pesticides (diazinon, fenitrothion, malathion, parathion, dieldrin, HCB), along with the behavior of plant uptake, information on pesticide/plant partition coefficients, and removal mechanisms/pathways in PRs grown with *A. gramineus*. The *A. gramineus* was found to endure and acclimatize well with toxicity at high concentrations of the six pesticides, with only slightly adverse effects on growth. In batch tests, about 88 - 92% of the initial pesticide concentrations were removed by PR treatment at a RT of about 5 d. The presence of *A. gramineus* could shorten the lifetime of pesticides in the PRs. The $t_{1/2}$ values of pesticides in the PR treatment were found to range between 1.2 and 1.7 d.

According to mass balance analysis, the chemical stability of the pesticides, especially diverse molecular structures, solubility, and typical half-lives in an aquatic environment, clearly influenced the removal pathways in the PRs. The main distinction of the removal pathways between OP and OC pesticides in the PR treatment were the plant accumulation and sorption by SM. The plant accumulation of the pesticides varied broadly (16 – 61% of total pesticide input to PR) depending on the pesticide species. The highest accumulation rate of pesticides occurred with malathion (about $9084 \mu\text{g kg}_{\text{wet plant}}^{-1} \text{d}^{-1}$), while the lowest was with dieldrin (about $4157 \mu\text{g kg}_{\text{wet plant}}^{-1} \text{d}^{-1}$). All OPs had no sorption in the SM, while nearly 60 - 63% of the total pesticides input of the PR were accumulated by the SM. High portions of unknown loss were found in the mass balances of diazinon and malathion. This could be explained by the very small half-lives of the two pesticides in an aquatic environment.

Distribution of pesticides in *A. gramineus* was found mainly in the belowground biomass, especially the roots. The leaves of the *A. gramineus* accumulated more OPs than the OCs. The $M_p^{pesticide}$ factor was introduced in this experiment in order to describe the mobility of pesticides in plants and the tendency of pesticide transportation into the above-ground biomass. The highest M_p factor was achieved by malathion at 215.1, and the lowest dieldrin at 0.9, suggesting that malathion possesses the highest tendency to accumulate in the aboveground biomass of *A. gramineus*.

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