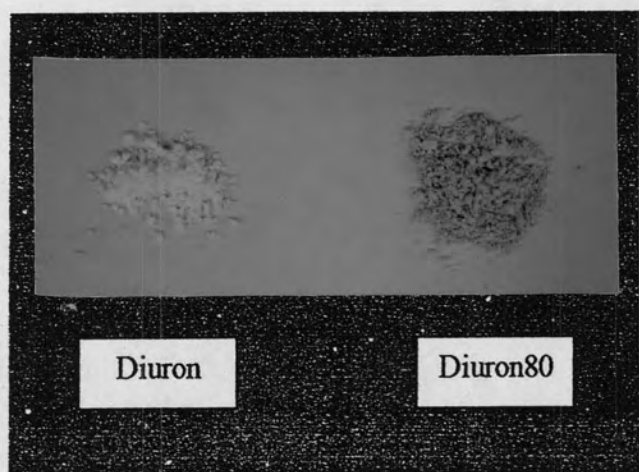# CHAPTER III

METHODOLOGY

3.1 Chemicals

1. Diuron (Figure 3.1A and 3.1B)

    1.1   Powder of diuron (98% purity) CAS 330-54-1 was purchased from Sigma Chemical, USA.

    1.2   Agriculture grade of a commercially available from of diuron (80% active ingredient) was purchased from Chemiphar Co. Ltd., Thailand.


Figure 3.1A Diuron (with a purity of 98%).

2.4   *sec*-Butanol (99.0%, Analytical grade, CAS 78-92-2) Merck, Germany.

2.5   *n*-Octanol (99.0%, Analytical grade, CAS 111-87-5) Sigma Chemical, USA.

2.6   Toluene (99.9%, Analytical grade, CAS 108-88-3) Fisher Scientific, UK

2.7   Benzene (99.7%, Analytical grade, CAS 71-43-2) Fisher Scientific, UK

2.8   Acetone (99.8%, Analytical grade, CAS 67-64-1) LAB-SCAN Co. Ltd., Thailand.

2.9   *n*-Hexane (99.0%, Analytical grade, CAS 110-54-3) from Merck, Germany.

2.10 Acetonitrile (99.9%, HPLC grade, CAS 75-05-8) LAB-SCAN Co. Ltd., Thailand.

3. Surfactant

3.1   Triton X-100 (100%, Analytical grade, CAS 9002-93-1) LAB-SCAN Co. Ltd., Thailand.

3.2   Tergitol NP-10 (100%, Analytical grade, CAS 127087-87-0) Sigma Chemical, USA.

3.3   Tween80 (100%, Analytical grade, CAS 9005-65-6) Sigma Chemical, USA.

3.4   Brij 35 (97.0%, Analytical grade, CAS 9002-92-0) Merck, Germany.

3.5   Sodium dodecyl sulphate (SDS) (95%, Analytical grade, CAS 151-21-3) Sigma Chemical, USA.

4. Calcium chloride ($CaCl_2.2H_2O$) (99%, Analytical grade, CAS 10035-04-8) Merck, Germany.

5. Titania was received from Department of Chemical Engineering, Faculty of Engineering, Center of Excellence on Catalysis and Catalytic Reaction Engineering, Chulalongkorn University, Thailand.

Deionized water (DI) with pH ~ 5.75±0.25 was also used in experiment.

3.2 Equipments

1. Glass column was made-to-order from Scientific and Technology Research Equipment Centre, Chulalongkorn University, Bangkok, Thailand.
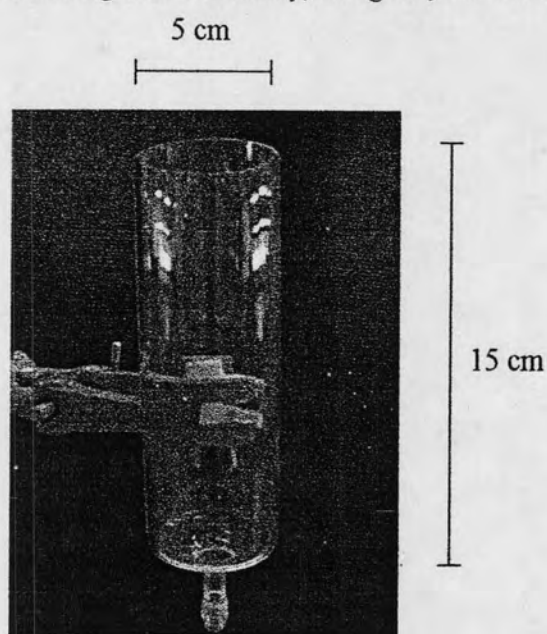
Figure 3.2 Glass column (5x15 cm) used in soil column experiment.

2. UV chamber for photocatalytic degradation was received from Center of Excellence on Catalysis and Catalytic Reaction Engineering, Department of Chemical Engineering, Faculty of Engineering, Chulalongkorn University, Thailand.
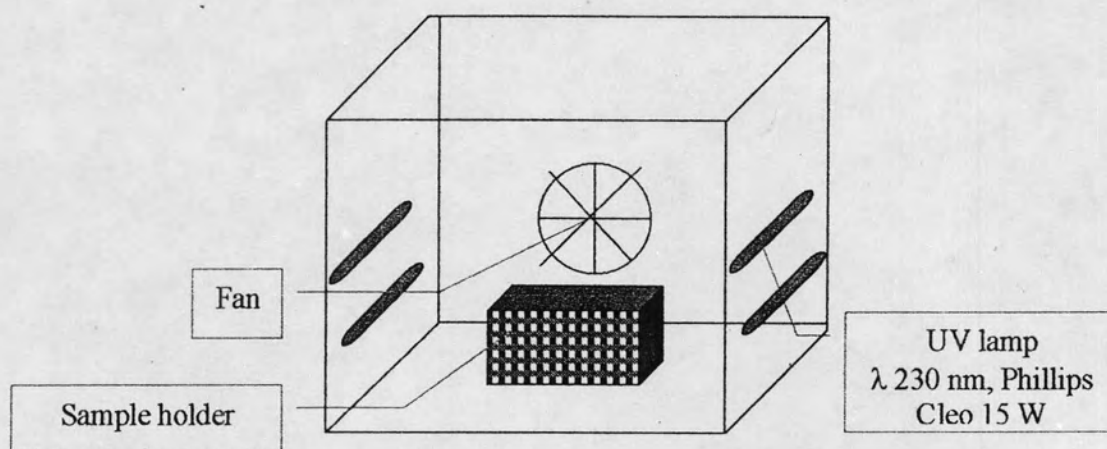
Figure 3.3 UV chamber.

3. High liquid performance chromatography (HPLC) LC-20AT, Shimazu, Japan

4. Microcentrifuge 5804R, Eppendorf, U.S.A.

5. Autoclave MLS-3020, Sanyo Electric, Japan

6. Incubator shaker, Innova4000, New Brunswick Scientific

7. Peristaltic pump SJ-1211, Bioinstrument ATTA, Japan

8. Balance AB204-S, Mettler Toledo, U.S.A.

9. pH meter MP220, Mettler Toledo, U.S.A.

3.3 Experiment Design

The experimental design was shown in Figure 3.6

Soil

Preparation and Analysis of soil properties

Soil contaminated with diuon   Soil contaminated with diuron 80

Adsorption isotherm   Leaching Efficiency

Shaking condition   Soil column

Aging   Type and concentration of surfactant   pH   Photooxidation

Type and concentration of organic solvent   Ionic strength

Combination of organic solvent and surfactant to ward the leaching efficiency

Figure 3.4 The experimental design.

3.4   Soil Sampling, Analysis and Preparation

Soil sample was collected from cassava agricultural area in Nakhonratchasima province, Thailand at the depth of 2-10 cm. It was analyzed for its physical properties and chemical compositions at Department of Soil Science, Faculty of agriculture, Kasetsart University and Soil and Water Group, Agricultural Chemistry Division. The soil properties including soil texture, pH, organic matter, available phosphorus,

moisture and surface area content were analyzed. Analysis methods for soil properties were as followed;

Table 3.1 The analysis methods for soil properties

Properties	Method
Soil texture	Hydrometer method (Gee and Bauder, 1979)
pH	Dilution soil: water (1:1) measured with Mettler Toledo pH meter (MP220)
Organic matter	Walkley-Black method (Walkley and Black, 1934)
Cation exchange capacity	Barium acetate method (Mehlich, 1948)
Phosphorus	Bray II method (Bray and Kurtz, 1945)
Soil moisture	Gravimetric method (Black, 1965)
Surface area	BET multipoint method (Brunauer et al., 1938)

The soils pretreatments were as followed: screening (to removed debris), air-drying, grinding, sieving (through 2 mm mesh) and sterilization twice (at 121°C, 20 psi for 15 min.). The soil samples were intentionally mixed with diuron or diuron 80 to the final concentration of 100 mg kg^{-1}soil through methanol carrier to ensure uniform concentration levels. The differences on contamination levels of the samples were minimized by contaminating a single batch of soil, and then stored equal amounts of the contaminated soil into individual sealed containers and placed in a dark, dry area at room temperature to prevent the occurrence photooxidation reactions for further studies. Soil sample which was not mixed with diuron was used as a

control. All glasswares used were cleaned with distilled water, sterilization (at 121°C, 20 psi for 15 min.) and dried at 110°C overnight.

3.5 Sorption Isotherm

A batch technique was used to measure diuron adsorption. Stock solution of either diuron or diuron80 was prepared by dissolving in methanol and was diluted with 0.01 M CaCl$_2$ aqueous solutions to obtain various initial pesticide concentrations (C$_0$) between 2.5 mg.l^{-1} and 50 mg.l^{-1}. In triplicate, 0.5 g of air-dried soil that had been sieved through 2-mm mesh was mixed with 10 ml of each herbicide solution and shaken in a rotary shaker for 24 hours at room temperature (~30°C). After shaking, the solutions were centrifuged at 6,000 rpm for 5 min and the concentration of diuron in the supernatant liquid was determined by HPLC. The amount of diuron sorbed was calculated from the difference between the amount initially added and that remaining in equilibrium solution. The measurements with blanks containing no diuron and diuron80 and no processes other than sorption contributed to the loss of solution-phase diuron (Gonzalez-Pradas et al., 1998).

The sorption isotherms were calculated using the K$_f$ parameter of the Freundlich's adsorption equation. The linear form of this equation is

$$\text{Log } C_s = \log K_f + n \log C_w,$$

Where C$_s$ is milligrams of diuron sorbed per kilogram of soil, C$_w$ is the equilibrium solution concentration (mg dm^{-3}) and K$_f$ and n are constants that characterize the sorption capacity for the pesticide. The constant K$_f$ is the amount of pesticide sorbed for an equilibrium concentration of 1 mg dm^{-3} and n is a measurement of the intensity

of adsorption and reflects the degree to which adsorption is a function of concentration. The K_f and n values were calculated from the least square method applied to the linear form of the Freundlich equation.

3.6 Diuron and Diuron80 Solubility

10 mg of diuron or diuron80 were placed in 20-ml test tube. Then, 10 ml of water or organic solvent (methanol, ethanol, n-butanol, sec-butanol, n-octanol, toluene, benzene, acetone and n-hexane) or surfactant (Triton X-100, Tergitol NP10, Tween80, Brij35 and SDS) was added in test tube and shaken at room temperature for 1 day (Higuchi and Connors, 1965). Solubilization of diuron or diuron80 was measured by HPLC and observed particulates. Solubility was calculated from ratio of diuron equilibrium concentration in aqueous solution and initial diuron concentration (1 mg.ml^{-1}) using standard curve in Appendix B and C. All experiments were performed in 6 replicates.

3.7 Determination of Leaching Conditions of Diuron-contaminated Soil

3.7.1 Pre-washing of diuron contaminated soil using 0.01 M CaCl$_2$

In the initial of soil washing experiment, the column volume of soil column was determined through 200 g of diuron contaminated soil packed in glass column, 0.01M CaCl$_2$ was slowly pumped at flow rate 2 ml.min^{-1} to cover the soil. The volume to cover the soil was noted as the column volume. This column volume was always consistently maintained in each experimental batch (Haberhauer et al., 2002).

To pretreat the soil column, of 0.01 M CaCl$_2$ was used to equilibrate soil column and leach unbound-diuron from soil. The volume of calcium chloride solution

used to leach diuron from soil until diuron concentration is constant was collected in fractions and identified as the void volume.

3.7.2 Leaching conditions of diuron and diuron80-contaminated soil

Leaching of diuron- or diuron80-contaminated soil was investigated in two conditions at room temperature:

3.7.2.1 Static condition (soil column)

Diuron-contaminated soil (200 g) was packed in the laboratory-scale vertical, downward-flowed soil column, which was constructed using 15-cm long glass column with 5-cm diameter. The surface of the soil column was then covered with a filter paper to minimize disturbance of the soil surface and to evenly distribute any solution applied over the soil surface. Glass column was plugged with glass wool at the base of each column before packed with 200 g of soil sample which resulted in a soil column of 10-cm length. The void volume of each soil column was maintained equally in each experiment using same amount of soil (200 g) and same height of soil packed in the column (10 cm). Then, the column was wrapped column using aluminium foil to avoid photooxidation. The soil columns was pre-treated with 5 times of the column volume (500 ml) of calcium chloride solution to saturate the column, to leach unbounded-diuron and to minimize variations in soil water content among repeated experiments. Next, diuron was leached from soil with 5-10 column volume of organic solvent or surfactant at flow rate of 2 ml min^{-1} using peristaltic pump controller. The leachate was collected in one column volume portion and determined for the concentration of diuron using HPLC.
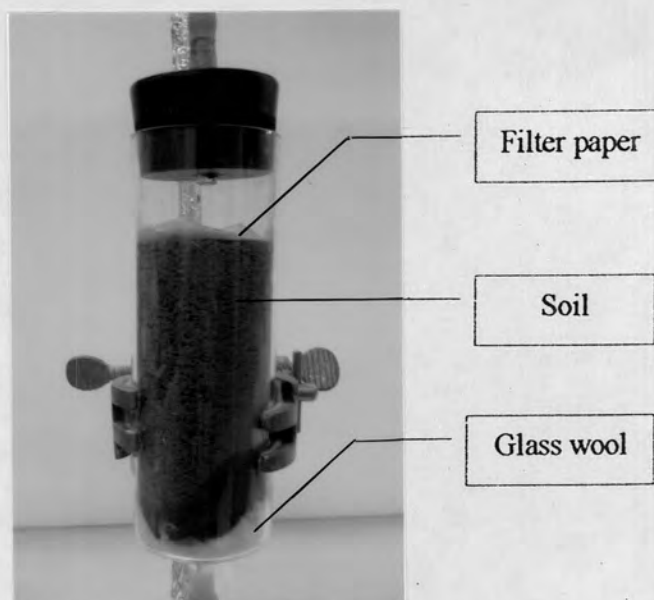
Figure 3.5 Packed soil column.
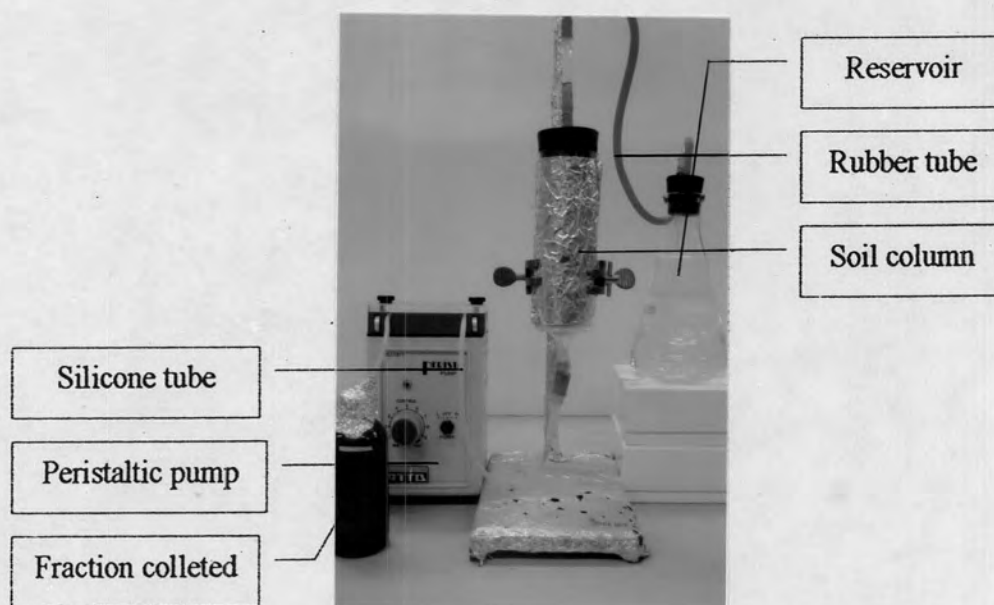
Figure 3.6 Laboratory-scale vertical, downward-flowed soil column.

3.7.2.2 Mixing condition (shaking-flask)

Conditions in this experiment was scale-down 10 times of static condition (Method 3.7.2.1) as follow; diuron-contaminated soil (20 g) was placed in Erlenmeyer flask (250 ml) which sealed by aluminium foil and pre-treated by shaken in a rotary shaker for 5 h at 150 rpm with 50 ml of either calcium chloride solution. The slurry

was centrifuged at 6,000 rpm for 5 min at 4°C. Supernatants were removed and the soils transfer to same flask. Thereafter, 50 ml of organic solvent or surfactant was added into flask containing the contaminated soil and shaken for 5 h at 150 rpm. The samples were centrifuged at 6,000 rpm for 5 min at 4°C. The soil was, then, removed and the concentration of diuron in the supernatant was measured by HPLC.
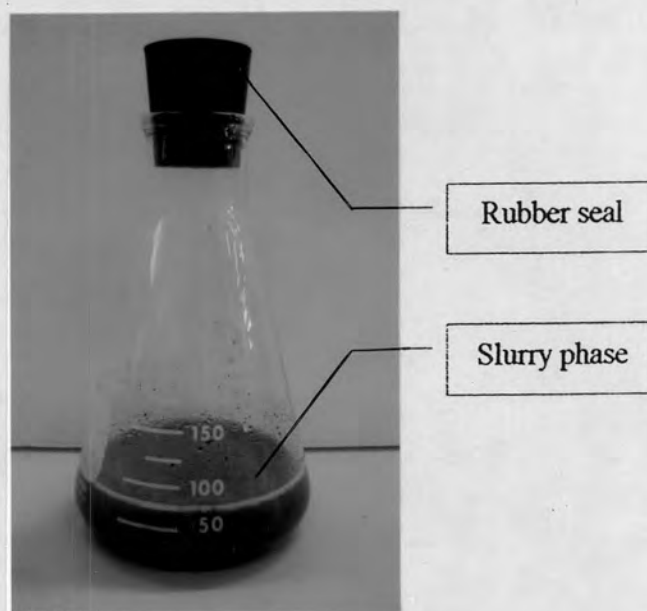
Figure 3.7 Mixing condition (shaking-flask).

3.7.3 Investigation of leaching conditions of diuron contaminated in soil by organic solvents and surfactants

Factors involving the leaching efficiency using organic solvents and surfactants were investigated as followed:

3.7.3.1 Effect of aging period of contaminated soil with diuron and diuron80

In Aging experiments, the soil samples that had been intentionally contaminated with diuron or diuron 80 to the final concentration of 100 mg kg^{-1} were divided to two

groups. The first group was stored in sealed container and placed in a dark, dry area at room temperature for 1 week, while the other was stored in similar condition for 1 month. Each group of diuron-contaminated soil sample was used to determine leaching efficiency of organic solvent and surfactant by measuring the amount of diuron leached from static- and mixing-condition (Method 3.7.2.1 and 3.7.2.2) by HPLC system.

3.7.3.2 Effect of type and concentration of organic solvents

In this experiment, various types of organic solvent (such as methanol, ethanol, *n*-butanol, *sec*-butanol, toluene, benzene and acetone) and various concentrations (1-10%v/v) were tested for leaching effectiveness of diuron or diuron80 contaminated in soil after pretreatment by calcium chloride solution by static- and mixing-condition (Method 3.7.2.1 and 3.7.2.2). Diuron concentration in leachate fractions were measured by HPLC.

3.7.3.3 Effect of type and concentration of surfactant on leaching of diuron

Anionic surfactant and nonionic surfactant were used in this study. Anionic surfactant was sodium dodecyl sulphate; SDS, and nonionic surfactant were Triton X-100, Tergitol NP-10, Tween80, Brij 35. Their characteristics are shown in Table 3.2. Various concentrations of surfactant (1, 2, 8 and 20 CMC) were tested for leaching effectiveness of diuron or diuron80 contaminated in soil after the pretreatment by calcium chloride solution by static- and mixing-condition (Method 3.7.2.1 and 3.7.2.2). Diuron concentration in leachate fractions were measured by HPLC.

Table 3.2 The CMC value and chemical structure of surfactants

	Symbol	Chemical structure	Mw	HLB*	CMC(mM)
Cationic surfactant					
SDS		$C_{12}H_{25}OSO_3^-Na^+$	288	40.0	1.25
Anionic surfactant					
Triton X-100	POE10**	$C_8H_{17}C_6H_4O(CH_2CH_2O)_{9.5}H$	625	13.5	170
Tergitol NP-10	POE10.5	$C_9H_{19}C_6H_4O(CH_2CH_2O)_{10.5}H$	683		
Tween80	POE20	$C_{24}H_{43}O_6(CH_2CH_2O)_{20}H$	1310	15.0	12
Brij 35	POE23	$C_{12}H_{25}O(CH_2CH_2O)_{23}H$	1200	17	92

*HLB = Hydrophilic-Lipophilic balance (High HLB = High water solubility)

**POE = Polyoxyethylene chain (Hydrophilic chain)

(Edwards et al., 1991)

3.7.3.4 Effect of combination of suitable organic solvent and surfactant

In this experiment, suitable types of organic solvent and surfactant from Method 3.7.3.2 and 3.7.3.3 were combined in various concentration ratios and tested for leaching effectiveness of diuron or diuron80 contaminated in soil after the pretreatment by calcium chloride solution by static- and mixing-condition (Method 3.7.2.1 and 3.7.2.2). Diuron concentration in leachate fractions were measured by HPLC.

3.7.3.5 Effect of pH on leaching of diuron in soil

The initial pH of soil and leachate from the soil is about 7. It was brought to pH 6 and 8 by adjusting pH of eluent solution with concentrated hydrochloric acid (HCl) and sodium hydroxide (NaOH) in very small amounts, therefore, such that changes in ionic strength of solution were negligible. Diuron concentration in leachate fractions from static- and mixing-condition (Method 3.7.2.1 and 3.7.2.2) were measured by HPLC. This method was modified from Sheng and coworker (2005).

3.7.3.6 Effect of ionic strength (μ) on leaching of diuron in soil

To study effect of ionic strength, test solutions were adjusted to have higher ionic strength ionic (5 and 10 times of the control) by 0.05 M $CaCl_2$ and 0.1 M $CaCl_2$, respectively. The pH was determined at the beginning and end of each experiment and the change was less than 0.05 units. Diuron concentration in leachate fractions from static- and mixing-condition (3.7.2.1 and 3.7.2.2) were measured by HPLC. This method was modified from Park and Bielefeldt (2003).

3.8 Photocatalytic Degradation of Diuron

The photocatalytic activity of the synthesized titania was employed to investigate the photodegradation of diuron in aqueous solution. The leachate samples were collected from the soil column (final fraction of the pretreatment using calcium chloride solution and five fractions leached from the column using organic solvent or surfactant) and mixed with titania in the ratio of 10 mg titania to 10 ml of solution in 20-ml PIREX test tube and kept in the dark for at least 15 min to allow the complete adsorption of diuron on the surface of titania. The photocatalytic reaction was initiated by exposing test tubes to light from 4 UV lamps (λ 230 nm, Phillips Cleo 15

initiated by exposing test tubes to light from 4 UV lamps (λ 230 nm, Phillips Cleo 15 W) for 24 h and test tubes were shaken every 30 min. Diuron degradation was periodically monitored every 4 h by using a reverse phase HPLC system. (Klongdee et al., 2005). Soil column-fractions sampling for photocatalytic degradation procedure was shown in Figure 3.9.
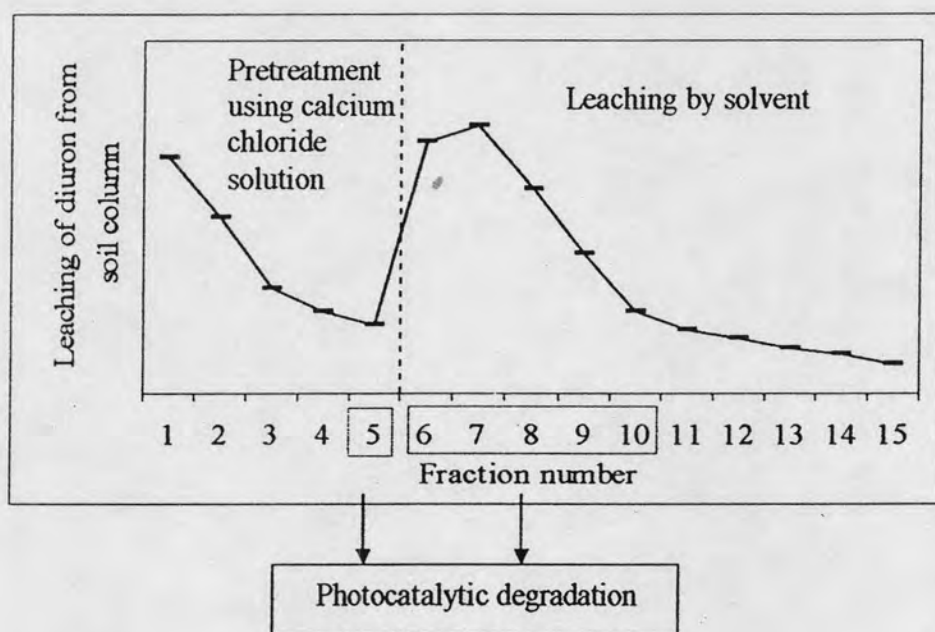
Figure 3.8 Soil column-fractions sampling for photocatalytic degradation procedure. Fraction 5-10 was presented by F5-F10 in text.

3.9 Analytical Method of Diuron

The samples were collected, centrifuge at 12,000 rpm for 10 min mixed with mobile phase (1:1 v/v) and filtered with 0.45μm diameter filter. Diuron concentration was analyzed directly by injection of 20μl onto high performance liquid chromatography (HPLC) system (Shimadzu Tokyo, Japan) equippd with a UV-Visible detector (SPD–20A diode array detector) at 240 nm. A 5-μm 4.6x250mm Inertsil® ODS-3 C-18 column (GL Sciences Ins. Japan) in a reverse phase system was used. With an isocratic method (70% acetonitrile, 29.75% water and 0.25%

phosphoric acid) at a flow rate of 1.0 ml min^{-1}, the injection volume was 20 µl and the retention time of diuron was 4.9 min. The column was backflushed with 80% aqueous acetonitrile to remove any strongly retained materials that might otherwise elute on later runs, with possibly deleterious effects on peak quantification (Gooddy et al., 2002). Examples of diuron and diuron80 HPLC peaks are shown in Appendix D.

3.10 Diuron and Diuron80 Calibration Curve

Diuron and diuron80 calibration curve were used for diuron analysis in soil. Both herbicides were prepared by dilution to various concentrations of diuron and diuron80 ranging from 1 µg.ml^{-1} to 20 µg.ml^{-1}. Then, the various concentration of diuron and diuron80 was analyzed in high performance liquid chromatography (HPLC) with a UV detector at a wavelength of 240 nm as describe in Method 3.9. The calibration curve of diuron and diuron80 were shown in Appendix B and C, respectively.