Research Article

International Journal of Pharmaceutical Biological and Chemical Sciences

e-ISSN: 2278-5191

CHALLENGES OF A HPLC-UV ANALYSIS OF METHOMYL, CARBOFURAN AND CARBARYL IN SOIL AND FRESH WATER FOR DEGRADATION STUDIES

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ABSTRACT:

This study that explored on modified procedures of graphitized carbon black extraction and soxhlet extraction with HPLC-UV detection to analyse carbamate pesticides provides information on the persistence of carbamate residues in water and soil, respectively. There were challenges before achieving good recoveries in the determination of carbaryl, carbofuran, methomyl. This paper explains how these challengeswere overcome. Also, the effect of environmental conditions on carbaryl, carbofuran, methomyl degradation in soil and fresh water were examined using the modified procedures.

KEYWORDS:

carbamates; degradation; HPLC-UV; soxhlet extraction; solid-phase extraction.

INTRODUCTION

Carbamates are widely used as insecticides, fungicides and herbicides. Among the more popular carbamate pesticides being used in the Philippines are carbaryl, carbofuran and methomyl (Fertilizer and Pesticide Authority, 2001). They are applied on various crops at different concentrations. Farmers also use carbamate mixtures for crop protection.

Carbamates are reversible acetylcholinesterase enzymes (AchE) inhibitors (Baron, 1991; Lotti and Moretto, 2006). AchE are responsible for the nervous system functions, thus, overexposure of organisms including man to carbamates can lead to damage of the nervous and reproductive systems.

Many crops in the Philippines are planted near rivers, irrigation and water canals for easy access to water. Frequent use of pesticides can result to leaching into bodies of water close to the areas of application. During the rainy season, pesticide residues may be found in surface water in the area because of runoff. Contamination can also occur when pesticide containers are thrown into the water. Pesticides that contaminate the aquatic systems can affect the organisms that thrive in them. Most of the pesticide related death in Asia is due to organophosphate pesticides (OPs) (Eddleston et.al 2004). Carbamates, like the OPs are AchE inhibitors, thus it is also important to monitor carbamate residues in soil and water.

Analysis of carbamates involve several analytical procedures such as solvent drop microextraction (SDME) followed by gas chromatography photoionization detection (GC/PID) (Lopez-Blanco et al., 2005), an extraction step with methanol, centrifugation, 1:1 dilution with aqueous 10 mM ammonium acetate, filtration and direct analysis by high performance liquid chromatography triple quadrupole mass spectrometry (HPLC/MS) (Mayer-Helm et al., 2006), high performance liquid chromatography-ultra violet (HPLC-UV) or diode array detection (DAD) (Rosales-Conrado et al., 2005) and direct analysis by laser-induced fluorescence detection (Burel-Deschamps et al., 2006).

In this report, we present the challenges (and how it was overcome) of an analytical procedure for the determination of the carbamates carbaryl, carbofuran and methomyl in water using solid phase extraction (SPE) soxhlet extraction in soil and HPLC-UV detection. Using these techniques, we show the persistence of the carbamate pesticides in water and soil under controlled laboratory conditions. The effects of natural river or soil components, open air condition and sunlight were

examined. The natural pH of the water and soil were maintained. The moisture of the soil was kept constant.

MATERIALS AND METHODS

HPLC analysis of the carbamates

The HPLC system consisted of a LC-10AS Shimadzu liquid chromatography equipped with a SPD-10AV UV-Vis detector (Kyoto, Japan). The carbamates were separated using a 250 x 4.6 mm Thermohypersil column packed with 5µm HyPURITY C18 column. For the optimization of the mobile phase composition and λ_{max}, chromatograms of carbamate mix containing aldicarb sulfone, aldicarb sulfoxide, oxamyl, methomyl, 3-hydroxycarbofuran, aldicarb, propuxur, carbofuran, carbaryl and methiocarb and individual standards were taken at various wavelengths ranging from 195 to 254 nm using methanol or acetonitrile (JT Baker, Phillipsburg, NJ) and distilled deionized water as mobile phase at different concentrations. Temperature of 35°C and flow rate of 1.0 mL min^{-1} were used. Various wavelengths for detection and mobile phases were also examined. The optimized chromarographic conditions were used to analyse carbamates in soil and water extracts. The target analytes were identified and determined using mixed analytical standards of carbaryl (99.7%), carbofuran (99.9%), methomyl (99.9%), as well as the degradation products of carbaryl and carbofuran, 1-naphthol (99.9%), 3-hydroxycarbofuran (9.6 ± 5 ng μL^{-1}), respectively and internal standard diphenyl sulfone (99.2%) purchased from Riedel-de-Haen, Seelze, Germany.

Extraction of carbamates in water and soil samples

The SPE apparatus used in this study consisted of a 1-L suction flask attached to the vacuum pump that drew water samples from the containers of the water samples through tubings connected to tightly stoppered SPE cartridges. The 1-L suction flask was connected to the SPE manifold to collect the water that passed through the cartridges. The SPE graphitized carbon black cartridges (SupelClean™ CARB 250 mg, 6 mL tube, Supelco, Bellefonte, PA, USA) were conditioned to enhance retention of the analytes. Briefly, conditioning was done as follows: 5 mL MeOH, 5 mL 80:20 methylene chloride-MeOH, 5 mL MeOH and 20 mL 10% w/v ascorbic acid. Deionized water was used as the final solvent for conditioning. The water samples were then passed through the cartridge at a flow rate of 10 to 15 mL min^{-1}. The SPE cartridges were rinsed with deionized water and dried by suction for 5 minutes prior to elution. The carbamate pesticide residues were eluted with 3 mL MeOH, 2 x 3 mL 80:20 methylene chloride-MeOH. The eluate was evaporated to almost dryness using a gentle stream of N_2 gas. The internal standard was added to the residue before redissolving in methanol to a final volume of 1 mL. Percent recovery was checked by spiking 10 µg L^{-1} to 40 µg L^{-1} mixed carbamate standards in 1 L deionized water, extracted using SPE and analysed by HPLC-UV.

As a representative sample, agricultural sandy loam soil collected from Aurora, Isabela, Philippines was used in this study. The soil was cleaned by exhaustive circulation of acetonitrile through the soil by soxhlet extraction. A 2.5 µg g^{-1} mixed carbamate standards was spiked on previously soxhlet cleaned soil sample. A 20 g replicate sample of the soil was transferred to an extraction thimble and extracted for 12 hours. The soil samples were soxhlet extracted using different solvents such as acetonitrile, acetonitrile-methanol, acetonitrile-methanol-acetone, acetonitrile-methanol-hexane and acetonitrile-hexane. The extract was cooled and concentrated to almost dryness at 35°C using a rotary evaporator. The residue was dissolved in 1 mL hexane and cleaned in a silica gel column using 100 mL 50:50 acetonitrile-acetone. The eluate was evaporated to dryness and the residue was dissolved in MeOH and filtered through a 0.45 µm nylon membrane prior to HPLC-UV analysis.

Degradation experiments of carbamates in water and soil

A degradation study was carried out to determine the persistence of the carbamates. A 20 L filtered deionized water was spiked with a carbamate pesticide solution and mixed to make a 50 µg L^{-1} solution. The spiked deionized water was placed in a sealed glass container and allowed to stand for forty nine days at ambient indoor conditions. At days 0, 14 and 53, water samples were taken for analysis.

About 2 kg of previously soxhlet-cleaned soil material was crushed, sieved and homogenized. Standard carbamate pesticide mixed solution diluted in an appropriate amount of water was dosed onto the soil spread out on a stainless steel tray using a syringe to achieve a 2.5 µg g^{-1} concentration. After dosing, water was added to the soil and mixed. A control sample was prepared by repeating this procedure without the standard carbamate pesticides. The soil was placed in tightly covered 1.5 L glass jar and was studied under room conditions was studied for fifty three days. Replicate samples were taken for analysis at days 0, 14 and 49. The soil water content of the soil samples were routinely checked and brought up to initial level when necessary.

RESULTS AND DISCUSSION

HPLC with different wavelengths and mobile phases

Carbamates were reported and detected at different wavelengths specifically - 220 nm, 195 nm, 210 nm, 225 nm, 232 nm, 237 nm and 247 nm. As shown in Fig. 1, 225 nm has similar chromatogram with that of 220 nm; however, the peaks are smaller. The carbamates showed less sensitivity when using 232, 237 and 247 nm wavelengths. Although 195 and 210 nm wavelengths gave high sensitivities, it is noticed that there are only few carbamates detected as compared with 220 nm. The 220 nm wavelength, therefore, gave the best detection among the wavelengths tried. The chromatogram appeared to have good resolution and selectivity of the desired analytes.

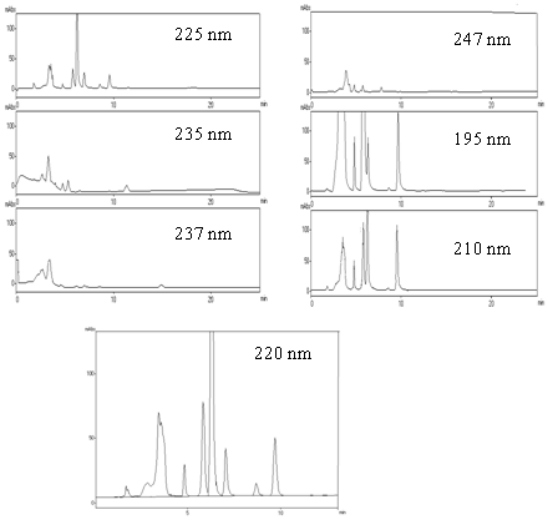

Fig. 1 HPLC-UV chromatograms of standard carbamate mix standard at various wavelengths

Among the different solvent combinations of acetonitrile-water, the resolution was better with 50% than 30%, 40%, 60%, 75% (fig. 2), or 0-50%, 40-60% and 50-100% acetonitrile in water. Using 50% MeOH-H_2O, 75% MeOH-H_2O, 100% MeOH and 25-55% MeOH-H_2O did not result to a stable baseline and good separation of the analytes. The probable cause of the observed negative peak is that the concentration of the mobile phase might be more absorptive than the sample components at 220 nm. The problem of the baseline drift in the chromatogram when using gradient as well as isocratic elution in methanol-water may be attributed to difficulty in solubility of analytes in methanol-water, poorly resolved peaks. Also, sensitivities of these compounds were less in the said parameters and possible slow column equilibration in methanol. Compounds were less in the said parameters and the possible slow column equilibration in methanol.

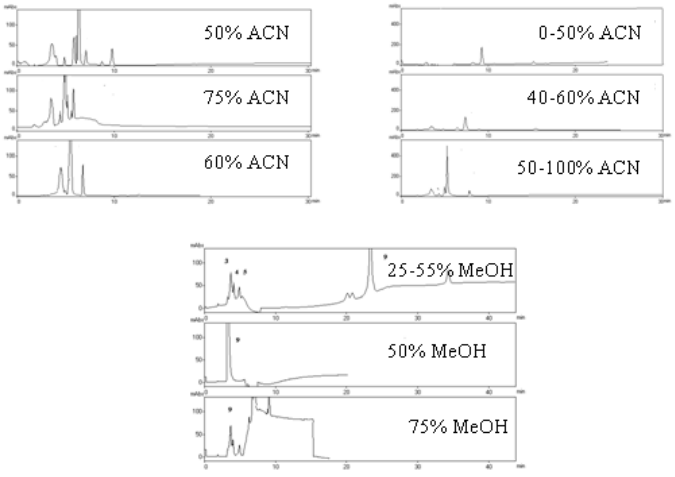

Fig 2: HPLC-UV chromatograms of standard carbamate mix standard using 220 nm wavelength at different mobile phase concentrations

In the chromatographic analysis, all of the analytes were detected using 220 nm and 50% ACN-water at 35 ºC and 1.0 mL/min flow rate. The carbamate peaks were identified against the specific retention times of the carbamate standards. The sequence of elution was as follows: methomyl, 3-hydroxycarbofuran, carbofuran, carbaryl and 1-naphthol. The internal standard (IS) diphenyl sulfone eluted last. The concentrations of the carbamates in the extracts were quantified against standard calibration solutions. The correlation coefficients of the analytes were within the range of 0.979 to 0.999. The method detection limits (MDL) of the carbamates spiked in deionized water were 3.90 μg L^{-1}, 3.90 μg L^{-1} and 2.30 μg L^{-1} in methomyl, carbofuran and carbaryl, respectively. The MDLs of these carbamates are higher than the reported LOQ value of 0.10 ppm (100 μg L^{-1}) for carbofuran determined using HPLC/positive electrospray ionization tandem mass spectrometry (Mayer Helm and Muller, 2006) and are similar to the detection limits of 0.2 to 5 μg L^{-1} in OPs and carbamates determined using SDME-GC (Lopez-Blanco et al, 2005). The MDLs of carbamates spiked in agricultural soil were 0.12 μg g^{-1}, 0.04 μg g^{-1} and 0.27 μg g^{-1} for methomyl, carbofuran and carbaryl, respectively.

Recovery Studies

In this study, graphitized carbon black was used to extract and preconcentrate the carbamates from the water samples. Water samples were filtered prior to extraction to avoid clogging of the large particles or impurities present in water. The graphitized carbon black was conditioned to create a sorbent environment compatible with the sample solvent and to remove impurities from the cartridge. Methanol and dichloromethane-methanol (80:20) were used to clean the sorbent while ascorbic acid was used to reduce quinones to less reactive hydroquinones found in the sorbent material. Graphitized carbon black contaminated with the quinone groups would lead to partial irreversible adsorption of compounds that reacts with them (Di Corcia and Marchetti, 1991).

The carbamate analytes were spiked in deionized water at various concentrations a nd extracted using graphitized carbon black. The recoveries of carbamates, as well as the metabolite 3-hydroxycarbofuran, in deionized water ranged from 78 to 116%. However, the recovery of the metabolite 1-naphthol was not within the acceptable range. 1-Naphthol might be unstable or strongly adsorbed to the sorbent.

The carbamate standards were spiked on previously soxhlet-cleaned soil sample. Acetonitrile and acetonitrile mixed solvents were tested (Fig. 3). One of the important considerations is the presence of water in soil that improved extraction. Soil sample should not be very dry because water aids in the breakdown of the composition of soil, which permits the solvents to work in a greater surface area. All solvents used gave relatively high recoveries ranging from 61.7 to 120.5%. 1-naphthol was not successfully recovered in all extraction solvents used. Acetonitrile was chosen as extraction solvent because of the high solubility of carbamates in this solvent while having low solubility of possible interfering nonpolar organic compounds. High recoveries of the pesticides confirm minimal loss of analytes in the succeeding steps and good extraction efficiency for methomyl, carbofuran and carbaryl spiked in agricultural soil. The presence of interferences and co-extractives in the chromatogram, however, made the quantification of methomyl difficult. The acetonitrile might have extracted substantial amount of humic material that were not removed even after the clean-up. Humic substances contain phenolic hydroxyl and carboxyl groups that make them soluble in acetonitrile. This problem may also be attributed to minimal solubility of analytes in methanol-water and poorly resolved peaks. The history of Lannate use in the soil used for the recovery experiments, which might not be thoroughly removed even after soxhlet cleaning, the possible soil matrix complexity, and the polarity of the compound. The former was confirmed by comparing the chromatogram of the blank with the spiked sample chromatogram. The sample peak (approx. retention time of 3.8 min) was also checked by isolating the sample from the extract by HPLC and analyzing with LC Q^{TM} Finnigan Mat MS detector. A mixture of the standards was passed through the HPLC and the 3.8 min retention time peak (methomyl) was collected manually. A similar procedure was done with the extract from the soil sample. The mass spectrum of the methomyl standard collected from HPLC and that of the sample was compared and found to be similar (Fig. 4). Ignoring the m/z 142 peak, which is a prominent contaminant during the analysis from the LC-MS used, m/z 160 is present in both spectra. The molecular weight of methomyl is known to be 162 g/mol. The molecular weight of the compounds isolated is approximately 161 (160 +1).

On the other hand, because the recoveries of 1-naphthol are low and sometimes zero, for all concentrations using acetonitrile, the recoveries of the carbamates were checked using combinations of different solvents without clean-up. None of the solvent mixtures, however, gave

acceptable results for 1-naphtol. Increasing the hours of extraction had very little effect on the recoveries of the carbamates. Acetone produced extracts with high level of extraneous soil constituents. When ACN-MeOH (2:1 v/v) was used as extracting solvent, carbaryl and carbofuran gave acceptable recoveries but not 1-Naphthol. The ACN-MeOH-Acetone (1:1:1 v/v) combined solvents gave slightly higher 1-naphtol recovery of about 1%. The ACN-MeOH-Hexane (1:1:1 v/v) combinations increased the recovery to about 10% for 1-Naphthol but lowered the recovery for carbaryl. ACN-Hexane (1:1 v/v) recovered only 4% of 1-naphthol.

The addition of polar solvents such as MeOH and MeOH-Acetone to acetonitrile slightly increased the percent recoveries of 3-hydroxy carbofuran and 1-naphthol. The addition of MeOH-hexane to acetonitrile lowered the recovery of carbaryl but increased the recovery of 3-hydroxy carbofuran and 1-naphthol. The addition of a nonpolar hexane that gave higher recovery for 1-naphthol is considered insufficient. The low recovery of 1-naphthol may be due to the instability of this metabolite to thermal heat or high temperature during soxhlet extraction, the rotary evaporation step, and the presence of humic acids and salts in the soil. The extraction of 1-naphtol in soil has not been reported in literature. For these reasons, the rest of the extraction was still carried out using acetonitrile.

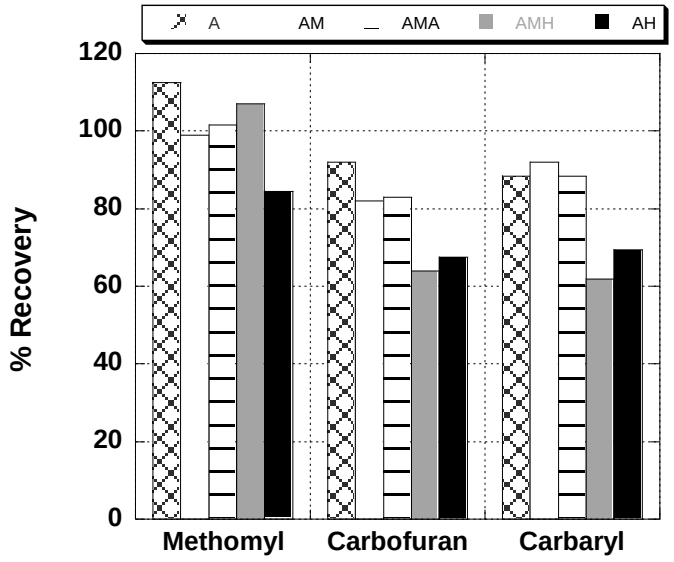

Fig 3: Recoveries of methomyl, carbofuran and carbaryl in soil by soxhlet extraction using acetonitrile (A), acetonitrile-methanol (AM), acetonitrile-methanol-acetone (AMA), acetonitrile-methanol-hexane (AMH) and acetonitrile-hexane (AH) as extracting solvent.

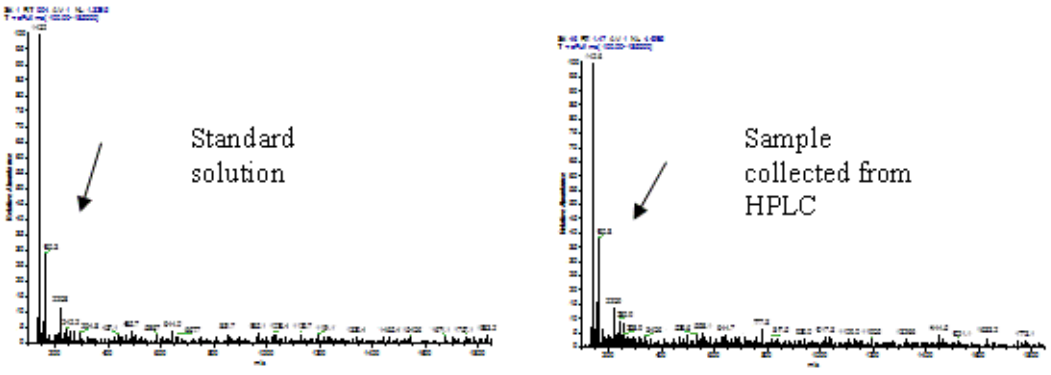

Fig 4: LC-MS chromatograms of methomyl standard and isolated fraction from the experiment.

Degradation Studies under Indoor Conditions

The results of the degradation study of methomyl, carbofuran, carbaryl and 1-naphtol in deionized water is shown in fig 5. The experimental conditions were kept constant; there was no significant change in pH and temperature during the monitoring period. Despite 1-naphthol's low recovery, its degradation in deionized water was observed. The concentration of 1-naphthol considerably increased during the monitoring period possibly due to the degradation of carbaryl as the carbamates may be converted into their degradation products. 1-naphthol was found to be persistent in deionized water. This behavior is attributed to the pH of the water where 1-naphthol was reported to be stable in slightly acidic water (average pH of 6.1) in the study of Mount and Oehme, 1981; hence, it was detected in water during the early days of the study. Its concentration was observed to slightly increase from day 14 to day 53, most likely due to the degradation of carbaryl. Among the carbamate pesticides studied, carbofuran was more persistent while carbaryl decomposed faster in water than methomyl and carbofuran.

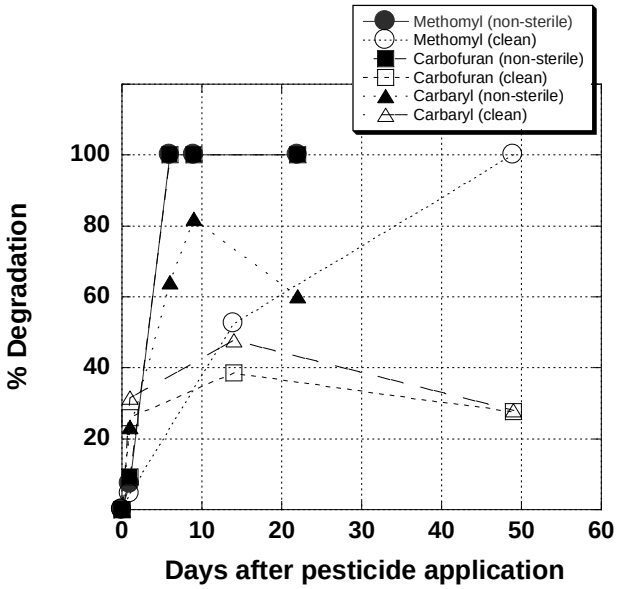

Fig 5: Degradation of methomyl, carbofuran and carbaryl in soil.

In the degradation study of carbamates in previously cleaned soil at indoor laboratory conditions, methomyl concentration in soil gradually decreased through time and was not detected after 49 days. The degradation of these carbamates cannot be attributed to photolysis caused by sunlight since the container was covered and maintained inside the laboratory. The degradation of this compound may be due to possible microbial activities, presence of humic acid, volatilization, hydrolysis and temperature changes. In addition, there was minimal change in pH during the monitoring period. The concentrations of carbaryl and carbofuran, however, increased from Day 14 to Day 49 of observation thus these compounds were relatively stable in the previously cleaned soil. This slight increase in concentrations of carbaryl and carbofuran may be due to the production of transformation products that have similar adsorption properties as carbaryl and carbofuran. These transformation products may be eluted at the same time during analysis and were simultaneously detected using wavelength 220 nm.

Methomyl was less persistent in clean soil than carbofuran and carbaryl under anaerobic conditions and in the absence of sunlight. Methomyl seemed unstable in soil when stored under the conditions tried. The rate of degradation of methomyl was faster than other pesticides studied. In this degradation study, 1-naphthol was not recovered and any transformation from carbaryl to 1-naphthol was not observed. In contrast, carbaryl and carbofuran persisted longer in soil. A study done on the degradation of carbofuran which was not easily degraded and regarded as possible leachate from soil spiked with carbamates including 3-ketocarbofuran and 3-hydroxycarbofuran at 2.5 mg Kg^{-1} was reported by Fava et al. (2007). These expected degradation products of carbofuran spiked onto soil were also not detected in their study. In a similar study, carbofuran was reported to be highly mobile in soil and completely leached from the soil (Rajagopal et al., 1984). In Rajagopal et al.'s (1984) study, it was demonstrated that the soil moisture was an important factor in the determination of carbofuran's persistence. The degradation of carbofuran

increased with soil moisture. In the present degradation study, soil moisture was kept as constant as possible that made carbofuran persist in the soil. Carbaryl, that also stayed in soil, was made stable due to the constant moisture content.

The results of the degradation study of methomyl, carbofuran, carbaryl and 1-naphtol in deionized water are shown in fig 6. There was no significant change in pH and temperature during the monitoring period. Methomyl was more persistence in river water than carbaryl and carbofuran.

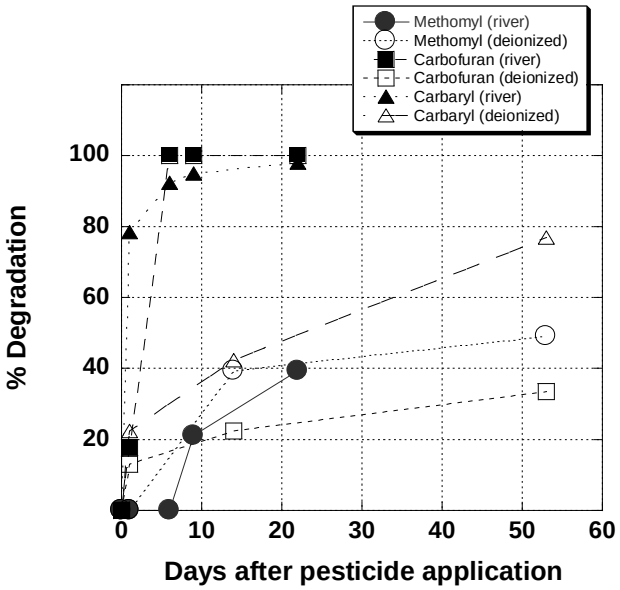

Fig 6: Degradation of methomyl, carbofuran and carbaryl in water.

The physicochemical properties also determine the environmental fate and behavior of carbamates (Rajagopal et al., 1984; Mount et al., 1981; Fukoto, 1987; Leistra and Smelt, 2001). Carbaryl has the lowest water solubility (120 mg L^{-1} at 20°C) as compared to that of carbofuran (320 mg L^{-1}) and methomyl (57.9 x 103 mg L^{-1}). Carbaryl does not readily leach into the aquatic systems and is more likely to bind in soil than carbofuran and methomyl. Carbaryl also has the highest measure of K_{oc} (205 to 457) and K_{ow} (log P: 1.59) indicating its high lipophilic properties. The K_{oc} and K_{ow} of carbofuran are 22 and l.52, respectively and for methomylthese are 72 and 1.24, respectively. Methomyl is highly soluble in water and it can drain more readily from the agricultural land into the canal or rivers. In addition, 3-hydroxycarbofuran and carbaryl are unstable under neutral and basic water as observed in the other studies (Barcelo, 1993). These physicochemical properties point to the observed persistence of methomyl in water and the persistence of carbaryl and carbofuran in soil in this degradation study. Due to insufficient data, $t_{1/2}$ calculations was not carried out.

CONCLUSIONS

The analytical procedures presented here are useful in studying carbaryl, carbofuran and methomyl in the different environmental matrices. Soxhlet extraction of soil, solid phase extraction of water and HPLC UV detection without post column derivatization of these compounds represents interesting options in carbamate determination.

Although carbamates are degraded rapidly in water and soil, the extensive (and excessive) use of these pesticides has lead to the increasing concern on their effects to human health and the environment. Monitoring studies are thus important. The results of this work can be used to predict the environmental fate of methomyl, carbaryl, carbofuran and 1-naphthol in soil and water.

ACKNOWLEDGMENTS

A.P. Cid thanks the Philippine Council for Advanced Science and Technology Research and Development (PCASTRD) of the Department of Science and Technology for the thesis grant; Natural Sciences Research Institute (NSRI) and Institute of Chemistry Analytical Services Laboratory (ASL) of the University of the Philippines Diliman for the use of the laboratory and instruments; Isabela State University Ilagan Campus for their assistance.

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