Study of the Degradation of Carbofuran using Response Surface Methodology

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Carbofuran has been banned by the European Union in 1999, however, this insecticide continues to be widely used in Asia, Australia and South America, including Malaysia. The degradation of carbofuran under the effects of pH (4 - 7), moisture (30 - 70%) and temperature (30 – 70 °C) were studied using response surface methodology. Sterilized soil was incubated according to the inscribed central composite design with a total of 24 experiments. The percentage of carbofuran loss was calculated and fitted according to linear, interaction and quadratic models. The model quality was measured based on the root mean square error (RMSE) and regression coefficient, R^2. Linear model was the best model to describe the degradation of carbofuran with the lowest RMSE. All the three factors were found to correlate positively to the loss of carbofuran with pH demonstrating a significant effect ($p < 0.05$). This suggests that hydrolysis is the major pathway controlling the degradation of carbofuran. The model was experimentally verified revealing an error of 5.82%.

Key words: Central composite design, model quality root mean square error, regression coefficient, degradation pathway

The quality and quantity of crops are governed by the cultivars used, advances in agricultural practices and applications of pesticides [1]. In the last 50 years, pesticides have been increasingly utilised to maintain the farming productivity [2]. This has led to environmental and health concerns. However, the use of pesticides has undeniably reduced pernicious diseases in crops and plants [3-4]. Carbofuran (2,3-dihydro-2,2-dimethyl-benzofuranyl-N-methylcarbamate) is a pesticide commonly used in Malaysia. It is marketed under the trade name of Furadan, applied in granular form or as foliar spray [5]. Carbofuran is used to control rodents in rice fields and rhinoceros beetles in oil palm plantations [6-7].

The use of carbofuran has become an issue after several incidents of carbofuran detection in crops higher than the Maximum Residue Level (MRL). Carbofuran was banned by the European Union in 1991 due to its toxicity towards invertebrates and birds [8]. In 2004, a total of 187 vultures and hyenas were killed after scavenging the bird carcasses containing carbofuran residue and its metabolites. In 2002 – 2011, a total of 316 cases of bird poisoning associated to carbofuran were reported by the Royal Society for Protection of Birds. In another field study, silica-based carbofuran granules were reported to be mistaken by birds as food [9]. Carbofuran is often misused on the account that it is easily available and cheap. Although carbofuran is banned in many parts of the world, it continues to be widely used in Asia, Australia and South America.

The degradation of carbofuran could take place in plants, soil and water. Several studies showed that carbofuran can be dissipated through hydrolysis, oxidation, chlorination, photodegradation and biodegradation [10]. Under photodegradation, the free radical and photon energy from sunlight lead to cleavage of carbamate group, followed by opening of furan moiety to attain a substituted catechol that subsequently undergoes dehydration reaction to produce an alkene side group [11]. Carbofuran residue present in water, on the other hand, can be degraded during the water treatment process by the disinfection agents such as free chlorine and monochloramine [12]. Bacteria such as *Pseudomonas*, *Flavobacterium*, *Achromobacter*, *Novosphingobium* and *Paracoccus* were further found to be capable in transforming carbofuran into carbofuran-phenol via oxidative and hydrolysis conversion, with *Sphingomonas* sp. demonstrating the additional ability to catabolise both carbofuran and carbofuran-phenol [13].

It is widely established that the dissipation of pesticides is governed by pH, moisture, temperature

and clay and organic matter content, as well as the presence of microorganisms [14-16]. The hydrolysis of carbofuran was found to be faster at pH 10 than at pH 7 where the half-lives were 1.2 hr and 864 hr, respectively [17]. A study done by Swarcewicz and Gregorczyk [18] stated that soil moisture affects the dissipation and adsorption of pesticides depending on the type of soil and pesticide used. For polar pesticides, high soil water content would enhance dissipation through leaching [19]. Conversely for non-polar pesticides, the adsorption decreases with increasing moisture content; at a higher moisture level, water molecules tend to compete with pesticides for the adsorption sites on soil colloids [19-20]. The degradation of pesticides also depends on the microbial community involved where microbial activity has been found to be accelerated at 35°C [21-22]. Theadsorption and desorption behaviour of carbofuran is further controlled by soil organic matter and clay content. Soil high in organic matter and clay tend to slow down the movement of water, allowing adsorption of pesticides. High organic matter soil, richer in bacterial diversity and population, initiates break down of pesticides through microbial action [23-24]. Generally, in alkaline soil, base-catalysed hydrolysis is the major degradation pathway while microbial and chemical mechanisms predominate in acidic soil. Numerous studies have been done to evaluate the degradation behaviour of organochlorine and organophosphate pesticides, however, there are limited reports on the dissipation behaviour of carbofuran.

The degradation behaviour of pesticides is traditionally evaluated based on the design of one-factor-at-a-time where the effect of a factor is examined by keeping the other factors constant. This classical approach does not demonstrate the combined effects of all factors and the experiment is designed based on a discrete set of factors where the optimum point can be easily missed [25]. To assess the degradation of carbofuran under simultaneous effects of pH, temperature and moisture content, response surface methodology (RSM) integrated with central composite design (CCD) was used. RSM is a more effective mathematical statistics method for establishing models, evaluating the relative significance variable and determining optimal conditions of desirable responses [26]. The findings

of this study can be used to appraise the fate of carbofuran in the environment, predicting the degradation behaviour of the pesticide.

MATERIALS AND METHODS

Chemicals and Reagents

Carbofuran standards were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Stock solutions of carbofuran (500 mg/L, not validated) were prepared by dissolving appropriate amounts of standards in acetone. The stock solutions were diluted to standards of 0.01, 0.05, 0.1, 0.5, 1.0, 5.0, 10 and 50 mg/L of carbofuran. Analytical and residue grades of acetonitrile, acetone, glacial acetic acid, sodium chloride and anhydrous magnesium sulphate were purchased from J.T. Baker, Philipsburg, USA. Florisil (2% deactivated) was used as a sorbent for the extraction.

Central Composite Design

The degradation of carbofuran in sterilized soil under the influence of pH, temperature and moisture content was evaluated using RSM with circumscribed CCD. The coded and uncoded levels of the factors are summarised in Table 1 and the experimental design is shown in Table 2 with a total of 24 experiments. The pH was adjusted with NaOH and HNO3 and the soil was wetted according to mass ratio. To attain pH 2, 4 and 7, 2-10 M of HNO_3 in the range of 0.2 – 3 mL was added, whilst for pH 10 and 12, 1 M of NaOH in the range of 1.0 – 2.5 mL was used. The final pH was determined using a pH meter.

The laboratory incubation experiments were carried out according to [27]. Ten grams of dry soil was weighed into 50 mL glass vials and autoclaved for one hour at 121 °C for sterilization. The sterilized soil was wetted according to the designated moisture, spiked with 100 μL of 500 mg/L carbofuran to attain a concentration of 5 mg/kg and incubated for 24 hr in triplicates. Carbofuran residue was then extracted and analysed using GC-MS/MS. Soil samples spiked with carbofuran were analysed to determine the concentration before incubation.

Table 1. The coded and uncoded levels of pH, moisture and temperature.

Factors		Coded levels		
		-1	0	1
pH	x_1	4	7	10
Moisture (%)	x_2	30	50	70
Temperature (°C)	x_3	30	50	70

Table 2. The circumscribed central composite design and the corresponding percentage of carbofuran.
degradation

Experiment number (n)	Experiment design			Percentage of carbofuran degradation
	x_1	x_2	x_3	
1	-1	-1	-1	52.08
2	-1	-1	1	72.55
3	-1	1	-1	77.15
4	-1	1	1	81.56
5	1	-1	-1	84.03
6	1	-1	1	83.43
7	1	1	-1	80.49
8	1	1	1	70.34
9	-1.68	0	0	82.90
10	1.68	0	0	87.11
11	0	-1.68	0	50.97
12	0	1.68	0	75.42
13	0	0	-1.68	67.87
14	0	0	1.68	97.80
15	0	0	0	72.01
16	0	0	0	87.11
17	0	0	0	69.34
18	0	0	0	65.66
19	0	0	0	72.14
20	0	0	0	71.34
21	0	0	0	98.26
22	0	0	0	98.13
23	0	0	0	97.90
24	0	0	0	92.25

Sample Extraction

Carbofuran was extracted from the soil samples according to modified QuEChERS method (Quick Easy Cheap Effective Rugged Safe) [0]. Ten grams of samples were weighed into test tubes and 20 mL of acidified acetonitrile (1% acetic acid) (J.T Baker, Philipsburg, USA) was added, shaken vigorously and vortexed for 1 min. The samples were then added with 1.5 g of NaCl and 6.0 g of anhydrous MgSO$_4$. The mixtures were further vortexed and centrifuged (2500 g) for 1 min. Two millilitres of the supernatant was leached through a Pasteur pipette packed with 0.2 g of deactivated florisil for clean-up. The eluent was left to dry, added with 2 mL of acetone and transferred into a vial. The samples were analyzed in triplicates for carbofuran residue using GC-MS/MS. The concentration was reported in mg/kg fresh weight.

Apparatus and Instrumentation

An Agilent Model 7000 gas chromatography equipped with mass spectrometry (GC-MS/MS) was used to determine carbofuran residue present in the samples. A column HP-5MS 5% phenyl methyl Silox (30 m × 0.25 μm × 0.25 μm) was used with helium and nitrogen as carrier gases at the flow rates of 2.250 mL/min and 1.500 mL/min, respectively. The column temperature was maintained at 70 ℃ for 2 mins and subsequently increased to 150 ℃ at 25 ℃/min. The temperature was then increased to 200 ℃ at 25 ℃/min and further to 280 ℃ at 65 ℃/min. Temperature was held constant for 2 min.

Response Surface Methodology (RSM)

The percentage of carbofuran degradation was calculated according to the following formula:

$$\text{Percentage of degradation} = \frac{\text{Concentration after incubation}}{\text{Concentration before incubation}} \times 100\%$$

The model was fitted according to linear, interaction and quadratic models with the root mean square error (RMSE) computed. Equations 1 – 3 show the linear, interaction and quadratic models, respectively.

Linear $y = b_o + b_1x_1 + b_2x_2 + b_3x_3$ Equation 1
Interaction $y = b_o + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3$ Equation 2
Quadratic $y = b_o + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{11}x_1^2 +$ Equation 3
                  $b_{22}x_2^2 + b_{33}x_3^2$

where y = predicted degradation percentage; x_1, x_2 and x_3 = coded factors; b = coefficients

The fitness of the model was represented by the Root Mean Square Error (RMSE) and the regression coefficient (R^2) and adjusted R^2 values. The RMSE indicates the prediction performance; the smaller is the RMSE value, the better the model predicts the degradation of carbofuran. Correspondingly, R^2 and adjusted R^2 denote the model quality; values converging towards 1.0 postulates a good agreement between the predicted and experimental values. Two dimensional response surface plots were used to evaluate the relationship of carbofuran and the soil conditions using Matlab 2013b.

$$R^2 = 1 - \frac{\sum_{i=1}^{I}(y - \hat{y})^2}{\sum_{i=1}^{I}(y - \bar{y})^2}$$

$$\text{Adj } R^2 = 1 - \frac{(1 - R^2)(n - 1)}{n - P - 1}$$

$$\text{RMSE} = \sqrt{\frac{\sum_{i=1}^{I}(y - \hat{y})^2}{n - p}}$$

where
n = number of experiments;
p = number of predictors

Method Validation

Soil samples (n=3) were spiked with carbofuran to attain concentrations of 0.01, 0.1 and 1.0 mg/kg in triplicates. The samples were mixed and left for 15 min before extraction and analysis with GC-MS/MS. The recovery performance was calculated as the percentage of experimental concentration over expected concentration. The blank samples were also analyzed and the limit of detection (LOD) was evaluated based on the lowest recoverable concentration.
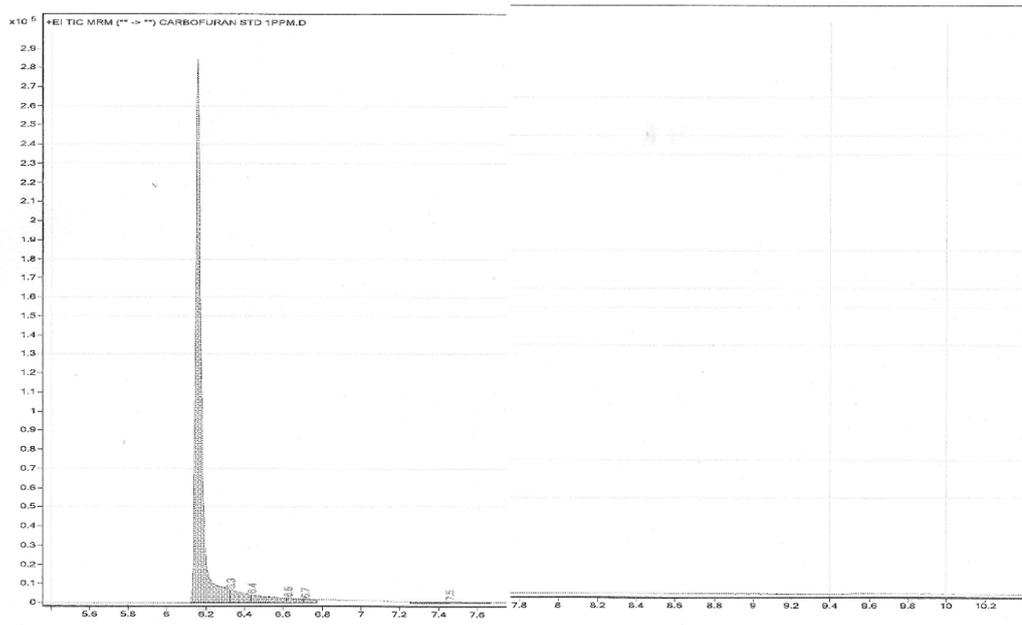
RESULTS AND DISCUSSION

Carbofuran was eluted at 5.68 min with no undesirable interference and impurities; Figure 1 shows the chromatogram of carbofuran at 1 mg/L. Carbofuran was satisfactorily recovered from soil spiked with 0.01, 0.1 and 1.0 mg/kg of carbofuran with recovery of 92.7%, 91.6% and 93%, respectively. The recovery performance is consistently more than 90% with the minimum detectable concentration established at 0.01 mg/kg (concentration less than 0.01 mg/kg is unrecoverable). The blank extract from soil also did not exhibit any interference on the target compound. Farahani et al. [7] reported similar range of recovery in sandy clay and clay soil at 98.7%.

Figure 1. Chromatogram of carbofuran at 1 mg/L.

The concentration of carbofuran attained before the incubation was 4.99 mg/kg. The percentage of carbofuran degradation corresponding to the experimental conditions is tabulated in Table 2. The response was fitted according to linear, interaction and quadratic models. The lowest RMSE was attained using the linear model with a value of 10.12 compared to the interaction and quadratic models at 10.91 and 10.46, respectively.

Linear model
$$y = 78.66 + 11.60x_1 + 2.26 x_2 + 2.02x_3$$

Interaction model
$$y = 78.66 + 11.60x_1 + 2.26x_2 + 2.02x_3 - 0.16x_1x_2 - 1.84x_1x_3 - 0.33x_2x_3$$

Quadratic model
$$y = 77.00 + 11.60x_1 + 2.26x_2 + 2.02x_3 - 0.16x_1x_2 - 1.84x_1x_3 - 0.33x_2x_3 + 3.68x_1^2 - 3.28x_2^2 + 2.52x_3^2$$

The R^2 values calculated for respective models were 0.489, 0.498 and 0.618 with the corresponding adjusted R^2 of 0.412, 0.318 and 0.373, respectively. The R^2 values tend to overestimate the model fit as the values increase with increasing number of predictors. Hence, adjusted R^2 is recommended as it is not affected by the number of predictors [25]. Table 3 summarizes the fitness of linear, interaction and quadratic models.

The results suggest that the degradation is more appropriately described with the linear model with pH, temperature and moisture content imposing positive effects on carbofuran degradation. Figure 2 illustrates the response surface plots of pH, temperature and organic matter on the degradation of carbofuran. The predictive model was verified experimentally, setting the pH at 2, moisture at 14% and temperature at 40 ℃ (coded level pH: -1.68; moisture: -1.80; temperature: 0.5). According to the model, the percentage of degradation at the afore-mentioned conditions was anticipated at 56.11%. The experimental loss was recorded at 59.38%, exhibiting an error of 5.82%. This implies that the model predicts the degradation of carbofuran with reasonable accuracy.

Table 3. The fitness of linear, interaction and quadratic models.

Model	RMSE	R^2	Adjusted R^2	p-value
Linear	10.12	0.489	0.412	$p < 0.05$
Interaction	10.91	0.496	0.318	$p < 0.05$
Quadratic	10.46	0.618	0.373	$p > 0.05$
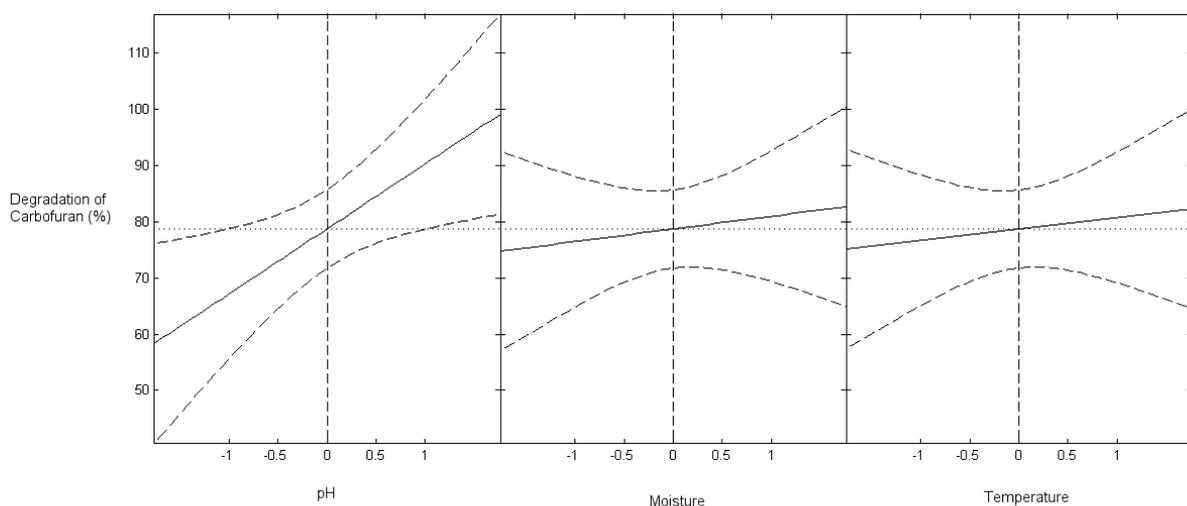
Figure 2. The response methodology plots of carbofuran degradation under the effects of pH, moisture and temperature.

The results demonstrated rapid breakdown of carbofuran in more alkaline soil with 98% degradation at pH 12 and 68% at pH 2. Statistically, pH demonstrates a significant effect on the degradation of carbofuran ($p < 0.05$). The sorptive behaviour of pesticides on clay and organic surfaces is profoundly influenced by soil pH, which eventually affects the chemical speciation, mobility and bioavailability of pesticides [29]. The degradation of pesticides depends greatly on whether a compound is susceptible to alkaline or acid hydrolysis. Evidently, most pesticides are found to degenerate rapidly at a higher pH [30-33]. This observation leads to the inference that soils under liming effect may experience elevated degradation rate whilst naturally acidic peat soil will retain pesticides longer.

Moisture is an important factor that governs pesticide dissipation in soil [34]. It was however found to exert no significant effect on the degradation of carbofuran ($p > 0.05$). The degradation percentage attained at different soil moisture regimes was comparable. Swarcewicz & Gregorczyk [35] stated that moisture affects the dissipation and adsorption of pesticides depending on the type of soil and pesticide used. For most pesticides, water acts as a hydrolytic agent that controls hydrolytic and photolytic degradation [34, 36]. Soil moisture also enhances biomass activity [36]. A study on the effect of moisture towards sorption of carbofuran found lower residue concentration with decreasing moisture. The finding was associated with the inhibitory effects on carbofuran-degrading micro-organisms [37].

The degradation of pesticides in soil is also found to be greatly affected by temperature [38-39], nonetheless its significance is not deduced in this study. The results obtained did demonstrate enhanced degradation when temperature was increased from 30 ℃ to 70 ℃. Singh et al. [40] observed accelerated degradation of carbofuran at 35 ℃ compared to 25 ℃. Likewise, Mora et al. [41] corroborated the findings revealing reduction in carbofuran concentration when temperature was altered from 30℃ to 40 ℃. Typically, the degradation rate of pesticides is promoted at a higher temperature, which in most cases reflecting escalated microbial activity in soil [42-43]. In microbial degradation, the mechanism involves adsorption of pesticides by bacteria, followed by enzymatic reaction and decomposition into carbon dioxide and water. The process is profoundly affected by temperature and moisture where the increase in temperature would promote growth and reproduction of bacteria, boosting the degradation rate [44].

In this study, the effect of moisture and temperature were appraised without interference from microorganisms as the soil samples were sterilised before use. Besides, the factor of sunlight was not considered. Both photodegradation and microbial degradation are closely related to moisture and temperature. In the absence of both, the factors of temperature and moisture did not demonstrate significant effects on carbofuran degradation.

CONCLUSION

Overall, the linear model was the best model to describe the degradation of carbofuran. The model was verified experimentally with an error of 5.82%. pH, moisture and temperature were found to exert positive effects on the loss with pH demonstrating predominating effect. This indicated that hydrolysis is the major pathway involved in the experimental study of carbofuran degradation. The factors of temperature and moisture did not demonstrate any significant effects on the loss of carbofuran. The contribution of sunlight and the presence of microorganisms were evicted in this study, hence the occurrence of photodegradation and microbial degradation were controlled. Both processes are profoundly governed by moisture and temperature. In the absence of both pathways, temperature and moisture do not contribute to significant loss of carbofuran.

CONFLICT OF INTEREST

The authors declare no conflict of interest in the publication of this work.

REFERENCES

1. Morais, S., Dias, E. and Pereira, M. (2012) Carbamates: Human exposure and health effects. In the Impact of Pesticides, eds. M. Jokanovic, *Academy Publish*, **21-38**.

2. Jeong, I. S., Kwak, B. M., Ahn, J. H. and Jeong, S. H. (2012) Determination of pesticide residues in milk using a quechers-based method developed by response surface methodology, *Food Chemistry,* **133(2)**, 473-481.

3. Singh, R., Kumari, K. and Singh, D. (1994) Influence of different factors on the adsorption of carbofuran (2, 3-dihydro-2, 2-dimethyl-7-benzofuranyl-n-methyl carbamate) on soils, *Ecotoxicology and Environmental Safety,* **29(1)**, 70-79.

4. Dalvie, M.A., Africa, A. and London, L. (2009) Change in the quantity and acute toxicity of pesticides sold in south african crop sectors, 1994–1999, *Environment International,* **35(4)**, 683-687.

5. Kale, S., Murthy, N. and Raghu, K. (2001) Degradation of 14 C-carbofuran in soil using a continuous flow system, *Chemosphere,* **44(4)**, 893-895.

6. Benitez, F. J., Acero, J. L. and Real, F. J. (2002) Degradation of carbofuran by using ozone, uv radiation and advanced oxidation processes. *Journal of Hazardous Materials,* **89(1)**, 51-65.

7. Farahani, G., Zakaria, Z., Kuntom, A., Omar, D. and Ismail, B. (2007) Adsorption and desorption of carbofuran in malaysian soils, *Advances in Environmental Biology,* **1(1)**, 20-26.

8. Otieno, P. O., Lalah, J. O., Virani, M., Jondiko, O. and Schramm, K. W. (2011) Carbofuran use and abuse in Kenya: Residues in soils, plants, water courses and the African white-backed vultures (gyps africanus) found dead, *The Environmentalist,* **31(4)**, 382-393.

9. Richards, N., Zorrilla, I., Lalah, J., Otieno, P., Fernandez, I., Calvino, M. and Garcia, J. (2017) Talons and beaks are viable but underutilized samples for detecting organophosphorus and carbamate pesticide poisoning in raptors, *Vulture News,* **72(1)**, 3-13.

10. De Bertrand, N. and Barceló, D. (1991) Photodegradation of the carbamate pesticides aldicarb, carbaryl and carbofuran in water, *Analytica Chimica Acta,* **254(1-2)**, 235-244.

11. Bachman, J. and Pattern H. H. (1999) Photodegradation of the carbamate pesticide carbofuran: Kinetics and the influence of dissolved organic matter. *Environmental Science & Technology*, **33(6)**, 874-881.

12. Chamberlain, E., Shi, H., Wang, T., Ma, Y., Fulmer, A. and Adams, C. (2011) Comprehensive screening study of pesticide degradation via oxidation and hydrolysis. *Journal of Agriculture and Food Chemistry*, **60(1)**, 354-363.

13. Yan, X., Jin, W., Wu, G., Jiang, W.,Yang, Z., Ji, J., Qiu, J., He, J., Jiang, J. and Hong, Q. (2018) Hydrolase CehA and Monooxygenase CfdC are responsible for carbofuran degradation in Sphingomonas sp. strain CDS-1. *Applied and Environmental Microbiology*, **84(16)**, e00805-18.

14. Roy, C., Gaillardon, P. and Montfort, F. (2000) The effect of soil moisture content on the sorption of five sterol biosynthesis inhibiting fungicides as a function of their physicochemical properties, *Pest Management Science,* **56(9)**, 795-803.

15. Sarmah, A. K., Müller, K. and Ahmad, R. (2004) Fate and behaviour of pesticides in the agroecosystem—a review with a New Zealand perspective, *Soil Research,* **42(2)**, 125-154.

16. Das, A. C., Chakravarty, A., Sen, G., Sukul, P. and Mukherjee, D. (2005) A comparative study on the dissipation and microbial metabolism of organophosphate and carbamate insecticides in orchaqualf and fluvaquent soils of west bengal, *Chemosphere*, **58(5)**, 579-584.

17. Seiber, J., Catahan, M. and Barril, C. (1978) Loss of carbofuran from rice paddy water: Chemical and physical factors. *Journal of Environmental Science & Health Part B*, **13(2)**, 131-148.

18. Swarcewicz, M. K. and Gregorczyk, A. (2012) The effects of pesticide mixtures on degradation of pendimethalin in soils. *Environmental monitoring and assessment*, **184(5)**, 3077-3084.

19. Laabs, V., Amelung, W., Pinto, A. and Zech, W. (2002) Fate of pesticides in tropical soils of Brazil under field conditions, *Journal of Environmental Quality,* **31(1)**, 256-268.

20. Ismail, B., Mazlinda, M. and Zuriati, Z. (2012) Effects of temperature, soil moisture content and soil type on the degradation of cypermethrin in two types of malaysian agricultural soils, *World Applied Sciences Journal,* **17(4)**, 428-432.

21. Van Eerd, L. L., Hoagland, R. E., Zablotowicz, M. and Hall, J. C. (2003) Pesticide metabolism in plants and microorganisms. *Weed Science*, **51(4)**, 472-495.

22. Mohamed, B., Rachid, M. and Amina, A. (2013) Dissipation processes of 14C-carbofuran in soil from northwest morocco as influenced by soil water content, temperature and microbial activity, *Journal of Environmental Chemistry and Ecotoxicology,* **5(5)**, 119-128.

23. Benavides, L. C. L., Pinilla, L. A. C., López, J. G. and Serrezuela, R. R. (2018) Electrogenic biodegradation study of the carbofuran insecticide in soil. *International Journal of Applied Engineering Research*, **13(3)**, 1776- 1783.

24. Pérez-Lucas, G., Vela, N., El Aatik, A. and Navarro, S. (2018) Environmental Risk of Groundwater Pollution by Pesticide Leaching through the Soil Profile. In Pesticides, Anthropogenic Activities and the Health of our Environment. IntechOpen.

25. Lee, T. Z. E., Krongchai, C., Lu, N. A. L. M. I.,

Kittiwachana, S. and Sim, S. F. (2015) Application of central composite design for optimization of the removal of humic substances using coconut copra, *International Journal of Industrial Chemistry, 6(3)*, 185-191.

26. Carlson, R. and Carlson, J. E. (2005) Design and Optimization in Organic Synthesis (Vol. 24), *Elsevier.*

27. Chai, L. K., Wong, M. H. and Hansen, H. C. B. (2013) Degradation of chlorpyrifos in humid tropical soils, *Journal of Environmental Management, 125*, 28-32.

28. Chai, L. K., Elie, F. and Jinang, C. (2014) Determination of 24 pesticides residues in mineral and peat soils by modified quechers method and gas chromatography, *International Journal of Environmental Analytical Chemistry, 94(5)*, 519-530.

29. Hussain, A., Tirmazi, S., Maqbool, U., Asi, M. and Chaughtai, F. (1994) Studies of the effects of temperatures and solar radiation on volatilization, mineralization and binding of 14c- ddt in soil under laboratory conditions, *Journal of Environmental Science and Health Part B, 29(1)*, 141-151.

30. Given, C. J. and Dierberg, F. E. (1985) Effect of pH on the rate of aldicarb hydrolysis, *Bulletin of Environmental Contamination and Toxicology, 34(1)*, 627-633.

31. Racke, K. D., Steele, K. P., Yoder, R. N., Dick, W. A. and Avidov, E. (1996) Factors affecting the hydrolytic degradation of chlorpyrifos in soil, *Journal of Agricultural and Food Chemistry*, **44(6)**, 1582-1592.

32. Singh, B. K., Walker, A., Morgan, J. A. W. and Wright, D. J. (2003) Effects of soil pH on the biodegradation of chlorpyrifos and isolation of a chlorpyrifos-degrading bacterium, *Applied and Environmental Microbiology, 69(9)*, 5198-5206.

33. Singh, B. K., Walker, A., Morgan, J. A. W. and Wright, D. J. (2003) Role of soil pH in the development of enhanced biodegradation of fenamiphos, *Applied Environmental and Microbiology, 69(12)*, 7035-7043.

34. Frank, M. P., Graebing, P. and Chib, J. (2002) Effect of soil moisture and sample depth on pesticide photolysis, *Journal of Agricultural and Food Chemistry, 50(9)*, 2607-2614.

35. Swarcewicz, M. K. and Gregorczyk, A. (2012) The effects of pesticide mixtures on degradation of pendimethalin in soils, *Environmental Monitoring and Assessment, 184(5)*, 3077-3084.

36. Jebellie, S., Prasher, S. and Clemente, R. (1996) Role of soil moisture content in reducing environmental pollution from pesticides, *Canadian Water Resources Journal, 21(4)*, 393-402.

37. Shelton, D. R. and Parkin, T. B. (1991) Effect of moisture on sorption and biodegradation of carbofuran in soil, *Journal of Agricultural and Food Chemistry, 39(11)*, 2063-2068.

38. Yen, J. H., Hsiao, F. L. and Wang, Y. S. (1997) Assessment of the insecticide carbofuran's potential to contaminate groundwater through soils in the subtropics. *Ecotoxicology and Environmental Safety, 38(3)*, 260-265.

39. Lee, Y. C., Ismail, B., Salmijah, S. and Halimah, M. (2013) Persistence of cyfluthrin in three Malaysian agricultural soils under laboratory conditions, *Journal of Environ-mental Biology*, **34(5)**, 957.

40. Singh, N., Sahoo, A. and Sethunathan, N. (1990). Accelerated degradation of carbofuran in standing water from carbofuran-retreated azolla plots. *Journal of Environmental Science and Health Part B, 25(2)*, 205-213.

41. Mora, A., Comejo, J., Revilla, E. and Hermosin, M. (1996) Persistence and degradation of carbofuran in spanish soil suspensions, *Chemosphere, 32(8)*, 1585-1598.

42. Abdullah, A., Sinnakkannu, S. and Tahir, N. (2001) Adsorption, desorption, and mobility of metsulfuron methyl in Malaysian agricultural soils, *Bulletin of Environmental Contamination and Toxicology*, **66(6)**, 762-769.

43. Gevao, B., Semple, K. T. and Jones, K. C. (2000) Bound pesticide residues in soils: A review, *Environmental Pollution, 108(1)*, 3-14.

44. Huang, Y., Xiao, L., Li, F., Xiao, M., Lin, D., Long, X. and Wu, Z. (2018) Microbial degratation of pesticide residues and an emphasus on the degradation of Cypermethrin and 3-phenoxy benzoic acid: A review. *Molecules*, **23(9)**, 23.