CHAPTER 4

DISTRIBUTION OF LINDANE IN A MODEL MUDFLAT ECOSYSTEM

4.0 Distribution of Lindane in a Model Mudflat Ecosystem

4.1 General

The increasing use of hazardous man-made chemicals in developing countries has become a matter of great concern. OC pesticides are of particular concern due to their persistent characteristic as well as their toxic effects and accumulative properties. The OC insecticide lindane has been used for decades for agricultural and horticultural purposes in Malaysia. The mudflats along the western coast of Peninsular Malaysia are commonly used for the culture of the economically important marine bivalve, cockles (*Anadara granosa*). Some of the major agricultural areas are located in coastal plains which are in the vicinity of the culture beds. Hence, applied pesticides, including lindane may enter the mudflats as a result of spray drift and runoff from agricultural land. Information on the fate and distribution of pesticides in the mudflats is important not only with respect to possible ecological impacts but also in the interest of public health when shellfish from this area are available for consumption.

The present investigation examines the distribution of lindane in a model mudflat ecosystem comprising estuarine water,

sediment and cockles. The ecosystem was exposed to low concentrations of the insecticide under semistatic condition for a period of 30 days. Estuarine water , sediment and cockles used in this study were collected from the mudflats at Kuala Selangor, Peninsular Malaysia.

4.1.1 Preparation of a model mudflat ecosystem.

Estuarine water, sediment and cockles collected from Kuala Selangor were maintained in a fibre-glass tanks with aeration for a period of one and a half month prior to the commencement of the experiments.

The model ecosystem were prepared in a 50 litre glass tank (60cmx30cmx30cm) with approximately 3 kg of sediment, cockles (50 in number, biomass 30g) and 9 litre of estuarine water. The sediment used in this experiment were mainly a mixture of sand, silt and clay with the following particle size distribution, expressed as percentage(%) of total dry weight : 2.0mm (0.1%), 1.0-2.0mm (0.4%), 0.5-1.0mm (1.1%), 0.25-0.5mm (16.7%), 0.125-0.25mm (15.3%), 0.063-0.125mm (30.9%), 0.063mm (35.5%). Organic content was determined as loss on ignition of 20g of freeze-dried sediment for three hours at 550^{0}C. A mean value 8.9% of dry weight was obtained. Some physico-chemical properties of the

estuarine water were as follows (mean values) : temperature, 24.4^{0}C ; alkalinity, 110mg $CaCO_3$/L ; pH, 8.6 and salinity, 32.5 ppt. Prior to the experiment, estuarine water, sediment and cockles were also analyzed for the possible presence of lindane , none was detected.

Stock solutions were prepared with analytical-grade lindane in analytical-grade acetone. Appropriate quantities of the stock solution were added to 9 litre of estuarine water to achieve concentrations of 5 μg/L and 15 μg/L, stirred for 30 minutes, and then carefully poured into two different tanks containing sediment and cockles. The experiments were carried out under semistatic conditions, whereby the solutions were renewed every 24 hours.

4.1.2 Sampling and sample analysis.

4.1.2.1 Estuarine water

Estuarine water (500ml) were sampled in triplicate, just before pouring into the tanks, and 24 hours later, prior to each renewal. The extraction, clean up and analysis were carried out as described in Sections 2.5.1, 2.6 and 2.7.

4.1.2.2 Sediment

Sediment samples were collected using a metal hand corer with minimal disturbance to the ecosystem at 4, 6, 8, 11, 15, 19, 25 and 30 days time interval, wrapped with precleaned aluminium foil and kept at -20^{0}C. For the analysis, sediment samples were removed from the freezer and allowed to warm to room temperature. Excess water were removed by suction filtration followed by freeze-drying. Triplicate freeze-dried sediment samples (20g) were extracted, passed through a clean-up silica column and analyzed as described in Sections 2.5.2, 2.6 and 2.7.

4.1.2.3 Tissue

Cockles were removed by metal forceps with minimal disturbance to the ecosystem at 4, 6, 8, 11, 15, 19, 25 and 30 days time interval. Pooled samples (4-5 animals per replicate) wrapped with precleaned aluminium foil and were maintained at -20^{0}C prior to analysis. For the analysis, cockles were removed from freezer and were deshelled using a stainless steel tweezer and spatula. The deshelled cockles were rinsed thoroughly with distilled water and excess water were removed by suction filtration. The cockles tissue were cut into small pieces with the aid of a stainless steel knife and scissors and then freeze-dried. Freeze-dried tissue (5 -

10 g) were extracted in triplicate, passed through a clean-up silica column and analyzed in accordance to Sections 2.5.3, 2.6 and 2.7.

4.1.3 Results and discussion

Table 4.1.1 and 4.1.2 show the levels of lindane detected in water, sediment and cockles at the various intervals and at the exposure concentrations of 5 μg/L and 15 μg/L, respectively.

Table 4.1.1: Concentration of lindane in water, sediment and cockles exposed to 5 μg/L of insecticide under semistatic condition

Exposure Period (days)	Concentration of lindane[a]		
	water[b] (μg/L)	sediment[c] (μg/kg)	cockles[c] (μg/kg)
4	<0.01	4.89	8.07
6	<0.01	9.24	8.76
8	0.24	12.99	12.60
11	<0.01	15.45	16.37
15	0.02	16.46	24.02
19	<0.01	22.90	25.77
25	0.15	30.06	33.59
30	0.01	46.12	33.05

[a] mean value of triplicate determinations

[b] concentration at the end of 24 hours

[c] dry weight basis

Table 4.1.2: Concentration of lindane in water, sediment and cockles exposed to 15 μg/L of insecticide under semistatic condition

Exposure Period (days)	Concentration of lindane[a]		
	water[b] (μg/L)	sediment[c] (μg/kg)	cockles[c] (μg/kg)
4	<0.01	19.19	13.32
6	<0.01	26.50	16.50
8	0.08	30.67	22.13
11	0.12	32.40	25.79
15	0.06	32.60	43.30
19	0.73	45.80	50.68
30	0.50	82.19	56.19

[a] mean value of triplicate determinations

[b] concentration at the end of 24 hours

[c] dry weight basis

Water in the model ecosystem were analyzed just before pouring into the tank and 24 hours later before renewal. Measured initial concentration in water before pouring into tanks ranged from 81.4% to 95.3% and 90.4% to 95.3% for the exposure concentrations of 5 μg/L and 15 μg/L, respectively. At the end of 24 hours, prior to renewal of the solution , the concentration of lindane in water ranged between <0.01 μg/L to 0.24 μg/L at the exposure

concentration 5 μg/L and <0.01 μg/L to 0.73 μg/L at the exposure concentration 15 μg/L. The rapid disappearance of lindane from water for the two exposure concentrations (95% to 100% of applied concentration) over the 24 hour-period is thought to be due to volatilization of the chemical, in addition to sorption into sediment and bioconcentration in the cockles. A small amount of sorption to the glass surface of the tanks may also have occurred [1].

For the duration of the experiment (30 days), the concentrations of lindane in both sediments and cockles increased with time. When exposed to 5 μg/L of the insecticide, the concentration of lindane in sediment was 4.9 μg/kg while 8.1 μg/kg of the insecticide was detected in cockles at the end of the fourth day. However, when exposed to 15 μg/L, the measured concentration in sediment (19.2 μg/kg) was higher compared to cockles (13.3 μg/kg). Hence, there was no discernible trend with respect to the relative concentrations of the insecticide in sediment and cockles with period of exposure. However, at the end of the study the concentration of lindane in sediments were significantly higher than in cockles for both exposure concentrations. At the end of the experiment the concentrations of the chemical in sediment and cockles were found to be 46.1 μg/kg and 33.1 μg/kg. respectively at a exposure concentration 5 μg/L. When exposed to

15 μg/L of lindane, the measured concentration in sediment and cockles at the end of the experiment were 82.2 μg/kg and 56.2 μg/kg, respectively.

When taking into consideration the total amount of lindane that would be present at the end of 30 days under the semistatic conditions of the exposure, (which amounted to 1350μg at an exposure concentration of 5μg/L and 4050μg at an exposure concentration of 15μg/L) then the percentage of the chemical in water was negligible while in sediment it amounted to 10.25% and in biota 0.07% for a exposure concentration of 5μg/L at the end of 30 days. For the exposure concentration of 15μg/L, 0.11% was present in water, 6.09% in sediment and 0.04% in biota at the end of 30 days. It would therefore appear that much of the pesticide was lost presumably by volatilization.

The presence of lindane in sediment was more significant than in water, consistent with its lipophilic properties, characteristic of OC insecticides. The sediments used in this study are very rich in organic matter (8.9% on a dry basis), and this can act as a very good organochlorine insecticide reservoir. Choi W.W. and Chan K.Y. also demonstrated that humic substances which are important components of organic matter in sediment play a major role in the accumulation of chlorinated hydrocarbons [2]. Numerous

studies have shown relatively high amount of lindane in sediment compared to water [3-7].

The ability of aquatic organism to concentrate organochlorine chemical many times above levels found in water has been widely demonstrated and documented. In studies involving bioaccumulation of lindane in bivalves, bioaccumulation factors ranging from 110 in the mussel *Mytilus edulis* [8] to 2610 for the freshwater clam *Carbicula manilensis* [9] have been reported.

In the present study, with the semistatic nature of the exposure, the concentrations of lindane in the cockles increased with increasing time. As no steady state was reached during this period, bioconcentration factors could not be estimated as the ratio of the concentration of the insecticide in cockles and water at equilibrium. However, it is noteworthy that no mortalities were observed throughout the duration of the study, providing evidence of some amount of tolerance, as a result of the chemical. Bioconcentration of lindane was observed to increase with continued exposure, presenting some degree of risk to human health from the consumption of the bivalve [10]. The cockles are normally marketed immediately after harvest without depuration.

The results of this study clearly demonstrated the roles of adsorption and bioconcentration in determining the fate of lindane in the model mudflat ecosystem. Furthermore, volatilization and

possible biodegradation may also have occurred. The following experiments were conducted in order to elucidate the relative importance of volatilization, biodegradation and adsorption in determining the fate of lindane in the mudflats.

4.2 The persistence of lindane in estuarine water.

4.2.1 General

OC insecticides are known to be both chemically and biologically more stable in the environment than other classes of pesticides.

The following experiments were conducted to elucidate the persistence of lindane in estuarine water and distilled water. The experiments were conducted using identical conditions to the model ecosystem but without sediment or cockles. The experiments were conducted in both aerated and non-aerated conditions under natural light. Physico-chemical properties of the estuarine water used were similar to those determined in the semistatic studies. The experiments were also conducted in conjuction with distilled water, in place of estuarine water. Some physicochemical properties of the distilled water used in this study, are as follows (mean value): temperature, 25.0^{0}C; pH, 5.37 and salinity, 3 ppt.

4.2.2 Sampling and sample analysis.

Aliquots (500ml) were removed in triplicate at the beginning of the experiment and at the end of 24, 48, 72 and 96 hours. The aliquots were extracted, passed through a clean-up silica column and analyzed as described in Section 2.5.1, 2.6 and 2.7.

4.2.3 Results and Discussion.

Figure 4.1.2 shows the concentrations of lindane (expressed as percent remaining) as a function of duration of the study, in distilled and estuarine water and under aerated and non-aerated conditions. These results demonstrated that the persistence of lindane in estuarine water was reduced substantially by aeration. The half lives of lindane in estuarine water under aerated and non-aerated conditions were 21.9 hours and 43.0 hours, respectively. Under aerated conditions, lindane showed a dissipation of 54.3% at the end of 24 hours and a loss of 91.5 % at the end of the experiment (96 hours). When non-aerated, only 20% loss was observed at the end of 24 hours ,while at the end of 96 hours, 84.3% had dissipated. Lindane is one of the most volatile organochlorine insecticides with a vapour pressure of $9.4x10^{-6}$mmHg at 25^{0}C [2]. Volatilization was thought to be further enhanced by the high atmospheric temperature (28-31^{0}C), and as seen by this experiment aeration also appeared to be a factor.

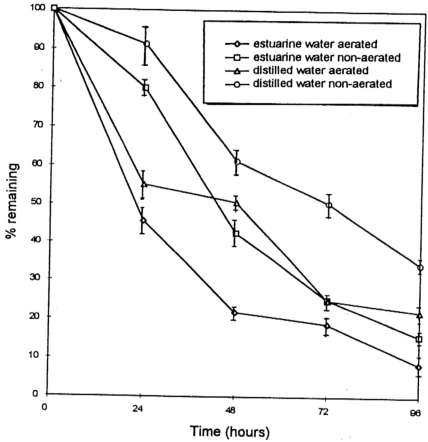


Figure 4.1.2 : Persistence of lindane in estuarine and distilled water under aerated and non-aerated conditions. Each point represents a mean of triplicate determinations. The percent recovery was calculated relative to the initial amount measured and corrected to 100%.

The half lives of lindane in distilled water under aerated and non-aerated conditions were 49.4 hours and 73.4 hours, respectively. When compared to the corresponding half lives of the chemical in estuarine water (21.9 hours and 43.0 hours, respectively), a significant difference was noted and was tentatively attributed to possible microbial degradation activity in estuarine water compared to the biologically-inactive distilled

water. In a previous study conducted by Matsumura, both microbial activity and alkalinity were implicated in the degradation of lindane [11].

Results obtained in the present studies were consistent with a study conducted using a model aquatic ecosystem, where volatilization accounted for more than 50% of the loss of the applied lindane [9]. Similarly, in another study conducted in the Vellor estuary in South India , most of the HCH applied in the paddy field was observed to be removed by volatilization [12].

4.3 Volatilization of [^{14}C]-lindane from the model mudflat ecosystem.

4.3.1 General

Volatile pesticides are more rapidly lost in the tropical agroecosystem compared to the temperate environment, due to the high temperatures associated with this region, where maximum temperatures may be as high as 34^{o}C. Pesticides such as lindane which has a vapor pressure of 9.4 x 10^{-6} mmHg have been shown to undergo rapid volatilization from tropical agroecosystems [12,13], as well as other environments [14]. In excess of 90% of

the HCH applied to a paddy field in India dissipated within two weeks by atmospheric transport [12].

Preliminary investigations with the model mudflat ecosystem (Section 4.1 and 4.2) revealed the significance of volatilization as a route of dissipation for lindane [15]. It is the aim of this study to further elucidate the process of volatilization in a model mudflat ecosystem consisting of soil and water taken from the mudflats of Kuala Selangor.

4.3.2 Measurement of volatilization

A diagram of the manifold assembly system used to study the volatilization, based on the method described by Kearney and Kontson is shown in Figure 4.1.3 [16]. It consisted of five Fernbach flasks and a 5 litre round bottom flask containing the model ecosystem ; two flasks contained 0.5M KOH to remove CO_2 from the incoming air and another contained water to maintain a saturated moist atmosphere in the model ecosystem. The model ecosystem flask was connected to a Fernbach flask, containing 2-ethoxyethanol to absorb volatile compounds. This in turn was connected to a NaOH (0.25M) trap to absorb released $^{14}CO_2$. Volatile organic compounds including parent HCH, as well as any degradation products would be trapped by the polyurethane plug

placed in the model ecosystem as well as in the 2-ethoxyethanol. The air flow was maintained at about 5ml min^{-1} based on observations from a preliminary experiment where a steady flow of air was maintained at this rate without disturbance to the model ecosystem. Physico-chemical properties of the mudfalt soil used are similar to those used in Section 4.1.1.

The model ecosystem consisted of soil (1000g) and estuarine water (1litre) taken from the mudflats in Kuala Selangor, allowed to acclimatized in the manifold assembly for a period of 2 weeks after which was added [^{14}C]-lindane (specific activity 27.40 mCi/mmol obtained from the IAEA, Marine Environment Laboratory, Monaco) prepared in ethanol to a final concentration of 1.5mgL^{-1}. A control experiment was also conducted without the insecticide.

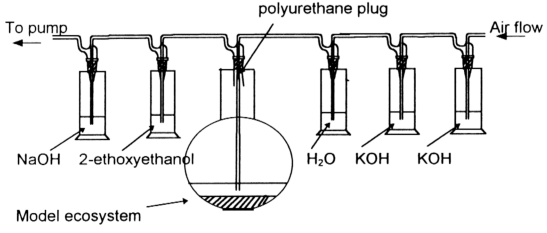


Figure 4.1.3: Diagram of the manifold system used to measure volatilization of lindane in the mudflat ecosystem.

Data on the formation of volatile organic compounds and $^{14}CO_2$ evolution were obtained at the end of 7 days, 14 days and 21 days. At the end of the appropriate incubation periods, the sampled soil was filtered. The filtered soil was extracted with acetone (250ml) followed by hexane (250ml) in a Soxhlet apparatus. The resulting extractable residues were analyzed by liquid scintillation counter (LS). The corresponding bound material was determined by combustion in a biological oxidizer. Water samples were extracted with hexane (50mlx2) and the radioactivity was directly measured by LS counting.

A 0.5ml aliquot from the NaOH trap was added to 10ml of scintillation cocktail (PPO, 5.5g ; POPOP, 0.1g ; toluene, 667ml ;triton X-100, 330ml) in triplicate. Similarly, a 0.5ml aliquot from the 2-ethoxyethanol trap was added to 10ml of scintillation cocktail. Both aliquots were then analysed by LS. The polyurethane plugs were extracted in a Soxhlet apparatus with hexane (150ml) and ^{14}C activity determined by LS counting. All samples were corrected for background and quenching.

4.3.3 Results and Discussions

The distribution of [^{14}C]activity in the various components of the manifold assembly expressed as percentage of the applied activity is shown in Table 4.1.3.

% of Applied Activity							
Time (days)	$^{14}CO_2$	Volatile Compounds		Water	Soil		Total
		2-Ethoxyethanol	Polyurethane plug		Extractable	Bound	
7	0.02 (0.04)*	0.1 (0.2)	6.64 (11.5)	0.05 (0.09)	48.06 (83.1)	2.99 (5.2)	57.86
14	0.12 (0.2)	0.14 (0.2)	6.57 (10.5)	0.03 (0.05)	51.63 (82.4)	4.23 (6.75)	62.72
21	0.2 (0.4)	0.16 (0.2)	13.46 (16.8)	0.03 (0.04)	61.42 (76.7)	4.95 (6.17)	80.22

*Figures in parentheses indicate the actual value corrected to 100%.

Table 4.1.3. Distribution of ^{14}C recovered from [^{14}C]-lindane-treated model ecosystem

The maximum amount of volatile organic compounds was observed at the end of 21days. The polyurethane plugs were observed to be able to trap the volatile labeled materials more than the 2-ethoxyethanol. Kearney and Kontson in their study with butralin (a pre-emergence herbicide) which has a vapor pressure of 1.3×10^{-5} mmHg (25^{o}C) noted that , polyurethane plugs were very efficient in trapping the volatile chemical [16]. 100% of the butralin

volatilized from the bottom of the flask was recovered in the first few centimeter of the polyurethane plugs.

TLC analyses of the hexane extracts of the polyurethane plugs and 2-ethoxyethanol traps showed that all the radioactivity spotted had disappeared from the plate. Thus, it appeared that the compounds trapped in the polyurethane plugs and in the 2-ethoxyethanol were extremely volatile. Furthermore the GCMS analysis of the hexane extracts and 2-ethoxyethanol did not show the presence of lindane. These observations are consistent to a previous study conducted by Drego and co-workers with lindane in green manure amended and unamended flooded soils [5].

The level of $^{14}CO_2$ detected in the present study did not exceed 0.2% of the applied activity, at the end of 21 days. The levels of $^{14}CO_2$ in these experiments were significantly lower compared to those reported in previous studies. In an investigation using labeled lindane in a paddy ecosystem, Drego and co-workers reported that a total of 12.3 and 10.7% of the applied radioactivity was found to be evolved as $^{14}CO_2$ in green manure-amended and unamended soils, respectively [5]. Brahmaprakash and co-workers also reported that the evolution of $^{14}CO_2$ from flooded soils planted and unplanted with rice amounted to 1-2% of

the originally applied ^{14}C at 30 days [17]. Kohnen and co-workers reported that 6% of the applied [^{14}C-lindane was evolved as $^{14}CO_2$ after 71 days and 17.8% as $^{14}CO_2$ after 140 days from submerged soil [18]. Scheunert and co-workers reported 3% of the applied [^{14}C]-γ-HCH was mineralized to $^{14}CO_2$ at the end of 42 days in the water phase of the paddy-field ecosystem [19].

In the present investigation the radioactivity levels in the water were 0.05, 0.03 and 0.03 % of the applied activity at the end of 7 days, 14 days and 21 days respectively. These low levels were consistent with the earlier experiments with non-labeled lindane where less than 0.05% were recovered after 4 days (Section 4.1.3). Residue levels of lindane in the water phase of paddy- field ecosystems have also revealed relatively low levels of the parent compound soon after introduction of the insecticide [5,19].

The soil was found to contain most of the applied activity in ranging from 51.1 to 66.4%, consistent with the characteristics of OC compounds. The extractable radioactivity from the soil were 48.1, 51.6 and 61.4 % of the applied activity at the end of 7 days , 14 days and 21 days, respectively (Table 4.1.3). The extractable radioactivity level are higher than those reported earlier by Drego

and co-workers where 30.0% and 4.7% of the applied activity in unamended soils and green-manure amended soils, respectively were attributed to extractable residues [5]. TLC and GCMS analysis also confirmed the presence of lindane in the soil extracts. Soil-bound residues of lindane constituted 2.99, 4.23 and 4.95% of the applied radioactivity at the end of 7 days, 14 days and 21 days, respectively. The formation of bound residues from ^{14}C-γ-HCH was also noted to increase with time, as did the extractable residues.

The ratio of pesticide volatilised and pesticide metabolised provide an indicator to assessing the relative importance of volatilization and metabolism in the mudflats. In this investigation the observed ratios were 332 and 56 for the 7-day and 14-day period respectively, while at the end of the 21-day period a ratio of 68 was observed. Hence, the amount lost as volatile organic materials tended to decrease significantly from 7 days to 21 days. From these observations it is evident that lindane tended to volatilized soon after introduction of the insecticide. However, it should be realised that the ratio of pesticide volatilized to pesticide metabolized will depend on many factors such as soil properties, moisture, air exchange rate and others [16].

From Table 4.1.3, it can be seen that 57.86, 62.72 and 80.22% of the applied activity could be accounted for at the end of 7 days, 14 days and 21 days, respectively. The unaccounted radioactivity could be due to losses incurred during soil filtration. The results obtained from this experiment indicated that volatilization is one of the important pathways involved in the dissipation of lindane from the mudflat environment. Furthermore the observation of $^{14}CO_2$ was taken as indication of metabolism, probably arising from microbial degradation occurring in the soil.

4.4 Adsorption of Lindane in Several Malaysian Soils.

4.4.1 General

Adsorption of pesticides in soils is an important factor affecting the fate of pesticides in the soil environment. The extent of adsorption depends primarily on the chemical nature of the pesticide and soil properties. For non-polar organic compounds the role of organic matter in the soil has been shown to be significant [20-25].

Preliminary investigation with the model mudflat ecosystem revealed the importance of adsorption as a route of dissipation for lindane [15]. In this study the adsorption of lindane in the mudflats and two agricultural soils were examined

4.4.2 Determination of adsorption isotherms.

The organic matter content and pH of the soils studied is shown in Table 4.1.4.

Table 4.1.4: Organic matter content and pH of the soils studied.

Sample	pH (Average)	Organic Matter (%)
Mudflat soil (M)	5.05	8.9
Agricultural soil (A)	4.45	7.6
Agricultural soil (B)	4.70	8.4

Soil M was collected from the mudflats in Kuala Selangor while soil A and soil B were from two different agricultural areas. Samples were air dried and sieved through 2.5mm mesh. Organic matter content was determined as loss on ignition of 20g of air-dried soil for three hours at 550^{o}C.

The lindane adsorption isotherms for the different soils were obtained by treating triplicate samples of soils (20g) with 20ml of an aqueous solution of the lindane at a concentration of

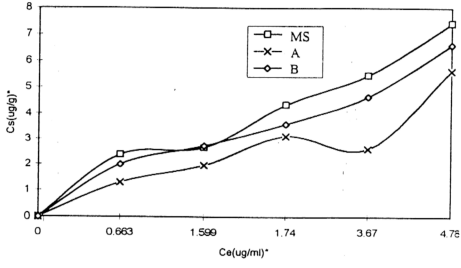
2.4, 3.4, 4.8, 7.3 and 9.8 μg/ml. Formulated lindane (active ingredient: lindane: 20.3%; inert material: 79.7%. supplied by Wesco Agencies, Malaysia) used was prepared in water. The suspensions were kept at 30°C for 24 hours in a thermostat-controlled chamber with circulatory shaking motion. Samples of 20g soil with 20ml deionized water were also maintained as control blanks.

At the end of 24 hours the soil suspensions were centrifuged and the supernants were extracted with hexane (5mlx2) , and finally the extract was concentrated for silica column chromatography clean-up as described in Section 2.6.

The elutes were collected and then concentrated using rotary evaporator and the final volume adjusted to 5ml with GC-grade hexane. The extracts were directly analyzed by GCMS in the following procedures as described in Section 2.5 and 2.7. The ionization potential was 70eV. lindane was quantified using selected ion monitoring (m/z: 111, 181, 183 and 219). Calibration was carried out using external standards. Extractions carried out on spiked samples (1μg/ml of lindane in 20ml of water) gave recoveries averaging 96% while the standard deviations of the method employed was less than 4 %.

4.4.3 Results and Discussion

Figure 4.1.4 shows the adsorption isotherms of lindane in the various soils.



* Mean of triplicate estimations.

Figure 4.1.4: Adsorption isotherms of lindane on soils.

The adsorption isotherms of pesticides by soils show different forms according to soil characteristics. Soils with an organic matter (OM) content not greater than 2.4% are classified as S-type (initial convex curvature) according to Giles and co-workers, reflecting a low adsorbent-adsorbate affinity at low concentrations [26]. Soils with organic matter content above 2.4% exhibit L-type isotherms (initial concave curvature), typical of a high adsorbent-adsorbate affinity [26]. The adsorption isotherms of all

the soils examined in this study exhibited the L type, attributed to the high OM content of these soils.

The isotherms obtained generally conformed to the Freundlich adsorption equation with a correlation coefficient $r > 0.86$ This equation can be expressed in linear form as :

$$\mathbf{logCs = logK + (1/n)logCe}$$

Where Cs(μg/g) is the amount of adsorbed pesticide, Ce(μg/ml) is the equilibrium concentration of dissolved pesticide and K and n are two constants characteristic of the pesticide adsorption capacity. K is the amount of pesticide adsorbed at an equilibrium concentration of 1μg/ml, hence representing adsorption at low concentrations. On the other hand n reflects the extent of dependence of pesticide adsorption on concentration. The K and n values obtained from isotherms, as well as K_d and Log K_{OM} values, are given in Table 4.1.5. The distribution coefficient, K_d , represents adsorption at equilibrium concentration higher than K and is defined as the ratio between the pesticide concentration in the soil and that in the equilibrium solution at a given equilibrium concentration. (K_d was calculated for Ce = 5μg/ml, K_{OM} is the K value normalized to 100% OM, K_{OM} = 100 x K / %OM.)

Table 4.1.5: Constants and Correlation Coefficients of the Freundlich Adsorption equation (K, n and r), Distribution Coefficient (K_d) and $logK_{OM}$.

Soil	K	n	r	K_d[a]	$LogK_{OM}$
M	1.40	1.05	0.97	1.40	1.20
A	0.76	0.99	0.97	0.83	1.00
B	1.25	1.03	0.86	1.21	1.17

[a] Ce = 5 μg/ml

Among the soils (M, A and B), the largest K values (Table 4.1.5) corresponded to soil M. K and K_d[a] values were very similar since n was essentially unity in all cases. $LogK_{OM}$ values, which is frequently used as a measure of the pesticide adsorption capacity of soils were 1.20, 1.00 and 1.17 for soil M, A and B, respectively. The order of adsorption was hence determined to be M > B > A, consistent with the organic matter content in the soils. The results obtained in this study indicated the role of OM content in the adsorption of lindane by the soils.

4.5 Biodegradation of Lindane in a Model Mudflat Ecosystem.

4.5.1 General

Microbial degradation has long been recognized as a primary route of dissipation for many pesticides in soil and water

ecosystems. The persistent nature of many OC insecticides have been attributed to the formation of a complex with some components of the environment which is largely resistant to microbial attack [27].

In the present study, the degradation of lindane in sterilized and non-sterilized soils taken from the mudflats was examined to assess the role of microbial degradation in the loss of lindane in the mudflats.

4.5.2. Soil treatment

Soil from the mudflat was collected from Kuala Selangor and maintained in a fibre-glass tanks with aeration for a period of one and a half months prior to the commencement of the experiment. To evaluate the significance of biodegradation of lindane in mudflat soil, a comparison of the persistence of lindane in sterilized soils and in nonsterilized soils was made. The soil was sieved (2.5mm. diameter) and sterilized by heating at 110 ^{0}C for 24 hours. Soil sample were treated with formulated lindane (lindane: 20.3%; inert material: 79.7%, supplied by Wesco Agencies, Malaysia) at the rate of 2.85 μg/g of soil and thoroughly agitated to ensure uniformity . They were then placed in incubator jar and maintained (30 ± 0.5 ^{0} C) for the duration of the experiment.

4.5.3. Sampling and sample analysis

At appropriate intervals, soils were sampled and Soxhlet-extracted in triplicate (20grams each) with analytical-grade hexane (250ml) for 8 hours followed by dichloromethane for another 8 hours. The dichloromethane was evaporated to dryness, the residue redissolved with the hexane phase, and then concentrated to about 5ml. Sulfur was removed by treatment with mercury, and finally the extract concentrated for silica column chromatography clean-up. The elutes were collected and then concentrated using rotary evaporator to a final volume of 5ml. This extract was directly analyzed by GCMS. The mean recovery rates of lindane from mudflat soil were 93%. Using samples spiked with 20 μg lindane, standard deviations of the methods employed was found to be less than 11% for mudflat soil samples.

4.5.4. Determination of microbial biomass.

The microbial biomass was determined in accordance to the method of Jenkinson and Powlson [28]. The soil were put through a 2.5mm sieve. Eight portions of soil each containing 25g moist soil were placed in glass beakers (100ml). Four portions were fumigated with $CHCl_3$ (purified by shaking AR grade $CHCl_3$ three times with concentrated H_2SO_4 , then washed three times with distilled water and dried over anhydrous K_2CO_3) and four left

unfumigated. The fumigation was conducted in a desiccator maintained in a alcohol-free $CHCl_3$ atmosphere for 24 hours, at the end of which, the soils were transferred to another clean and $CHCl_3$-free desiccator. $CHCl_3$ vapour from the soils were then removed by repeated evacuation in the desiccator by an aspirator followed by a high vacuum oil pump [28].

Each portion of fumigated soil was inoculated with 2.5g of moist unfumigated soil mixed evenly by spatula. After inoculation 10ml of distilled water was added to each soil. The unfumigated portions of soil were not inoculated. The portions of soil were incubated for 21days at 30^0C and CO_2 evolution measured. Soils from each beaker were transferred into wide neck glass bottle (500ml). A small glass sample bottle (18ml) containing 10ml of 0.25M of NaOH was placed in the center of the bottle. The wide neck bottle were closed tightly. Blank controls composed of 10ml of NaOH and soil were included in this experiment.

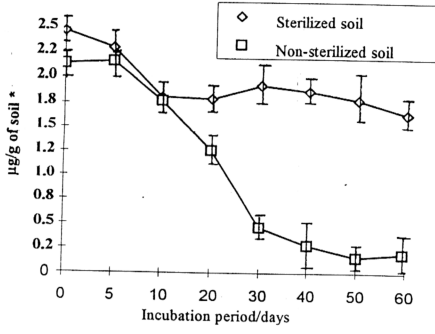
At the end of the incubation period, 10ml NaOH was made up to 25ml with distilled water and placed in a conical flask (100ml) and a few drop of fenolthelin (prepared by dissolving 0.5g of fenolthelin in 50ml methanol) and 2ml of 1M $BaCl_2$ was added. The solutions were then titrated with 0.2M HCl and the amount of CO_2

evolved during incubation calculated from the volume of acid required to neutralize the alkali.

4.5.5 Results and Discussion

Lindane disappeared more rapidly from non-sterilized soil compared to the sterilized soil (Figure 4.1.5). Less than 0.2μg/g of soil were detected in non-sterilized samples 60 days after application compared to 1.6μg/g from sterilized soil at the end of the study. The greater loss of the insecticide from non-sterilized soil indicated active participation of the microflora in the degradation of lindane.

The half live of lindane in non-sterilized soil was estimated to be 20.3 days. These results suggest the establishment, of a microbial population which is able to degrade lindane and indicate that biodegradation is one of the most important factors governing the persistence of lindane in soil.



* Mean of triplicate estimations

Figure 4.1.5. Persistence of lindane in sterilized and non-sterilized mudflat soil from Kuala Selangor.

Lindane was first observed to be less persistent in submerged tropical soils by Raghu and MacRea [4], who found only a small percentage of the applied insecticide remaining in the soil at the end of 90 days. Previous observation by Macrae and co-workers based on the biodegradation of lindane in submerged soils also showed that lindane disappeared more rapidly from non-

sterilized soil compared to sterilized soil [3]. Only trace amounts were detected in non-sterilized soil samples 70 days following application, while more than 65% of the applied insecticide were still present in sterilized soils at the end of the same period. In another study by Macrae and co-workers, an anaerobic bacterium isolated from flooded soils, a species of *Clostridium* were observed to be responsible for the biodegradation of lindane in a flooded soil [29]. Only 0.5% of the insecticide present at 1 hour of incubation were detected after 27 hours incubation with the microorganism. The bacterium *Sphingomonas paucimobilis* isolated from the surface layer of HCH-treated flooded soil was observed to cause rapid degradation of the four isomers of HCH in the order $\alpha > \gamma > \delta > \beta$ under aerobic conditions [30]. Hetrick and co-workers and Lichtenstein and co-workers reported that for flooded soils, the anaerobic species of the soil microflora were more active than aerobic species in the degradation of lindane [31,32].

The degradation of lindane has been observed to occur predominantly under anaerobic soil conditions. No biodegradation was observed under aerobic conditions in a clay soil, while in excess of 60% degradation was observed in 15 days in the same soil under submerged and anaerobic conditions. The rate of biodegradation of lindane in submerged soil has also been shown

to be dependent on soil properties and temperature [6]. The effect of temperature on lindane degradation was observed in a flooded clay soil in which degradation occurred more rapidly at 35°C compared to 20°C [6]. The insecticide was also seen to undergo rapid degradation in soils with high organic content as illustrated by the complete disappearance of lindane in a Casiguran clay loam soil with an organic content of 4.4% in 1 month, compared to Pila clay loam soil (1.5% organic matter content) in which more than half of the applied pesticide persisted after the same period [6].

The biomass content of mudflat soil calculated from the increase in CO_2 evolution is shown in Table 4.1.6.

Table 4.1.6. Biomass content of mudflat soil.

Mudflat soil	CO_2-C evolved mg/g soil*			Biomass* mgC/g soil
	Untreated soil 0-10 days	Untreated soil* 10-20days	Fumigated soil* 0-10days	
	0.27	0.11	0.33	3.84
S.D.	0.03	0.01	0.02	0.62

* Mean of four determinations.
S.D. : Standard deviation

The initial respiration rate and biomass (0.27 CO_2-C evolved mg/g soil and 3.84 mgC/g of mudflat soil respectively), indicated a

significant microbial population in the mudflat soils. The observed microbial biomass was also found to be significantly higher than those observed in microbially-active agricultural soils where 1.18 mgC/g were observed [28]. This observation was consistent with the earlier observation of the rapid loss of lindane from nonsterilized soil compared to sterilized soil [4].

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