



## **Degradation of the Pesticides Carbofuran and Diazinon in Tropical Soils from Sri Lanka**

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**CSIRO Land and Water Science Report 67/06**

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## Executive Summary

The rate of use of agrochemicals in Sri Lanka has been increasing markedly over the last 5 to 10 years in order to maintain or improve food production. However the residual levels of pesticides, and their derivatives, in soils, surface and ground waters are becoming an increasing public concern with respect to human health issues.

To help understand the environmental fate of pesticides, and consequently better manage farm practices in Sri Lanka, we studied the degradation of two commonly used pesticides (carbofuran and diazinon) in four soils from Sri Lanka. The soils were selected to give a range of chemical and physical properties from both the wet and dry regions of Sri Lanka. Small additions of  $^{14}\text{C}$  pesticide were added to each of the study soils and the %C mineralised by microbial activity was assessed over a period of about two months. In a separate experiment we monitored the general microbial health of the soils during the study period. Additions of  $^{14}\text{C}$  glucose were made to the soils and amount of carbon mineralised was measured.

The ranking of the soils for the rates of mineralisation was different for the  $^{14}\text{C}$  glucose and  $^{14}\text{C}$  pesticide treatments. The glucose treatments showed the Nuwara Eliya soil had 98.5% C mineralised after 2 months, compared to values ranging from 38.6-72.5% for the other Sri Lankan soils in the study. With the pesticide treatments mineralisation, after 62 days, ranged from 1.9 to 29.0% for diazinon and from 7.3 to 40.1% for carbofuran and for both pesticides the Nuwara Eliya soil had the lowest mineralisation rate. The degradation rate of diazinon was lower in acidic conditions and decreased as both % organic carbon and clay content increased. It is likely that the sorption of diazinon, and possibly its derivatives, onto soil organic matter and clay has a strong influence on the degradation rate of the pesticide diazinon.

The mineralisation rate for carbofuran increased for three out of the four Sri Lankan soils studied. The increase correlated with lower pH values and higher organic matter and clay contents. The reverse trend was found for diazinon.

The Nuwara Eliya soil, which had the lowest pH (4.5), highest organic carbon (7.6%) and clay content (40%) of the soils studied, gave the lowest mineralisation values for both carbofuran and diazinon.

The rate of microbial mineralisation of the pesticides, with respect to the pH of the soils, showed the opposite trend when compared to, for example, chemical hydrolysis, where acidic soil conditions favour diazinon degradation and carbofuran rapidly degrades in alkaline soils.

Although only a small number of soils were studied for rates of pesticide degradation and the study time was limited to about two months, significant mineralisation from microbial activity was measured for many of the soils. Local conditions, climate and physical properties of soils in Sri Lanka may well mean much higher degradation rates for both the pesticides studied and also for many of the other pesticides applied to agricultural land in Sri Lanka. An understanding of microbial mineralisation rates as well as chemical hydrolysis processes may lead to lower application rates of pesticides. This could also mean much lower residual concentrations of applied chemicals remaining in the environment.

Keywords : degradation, tropical soils, Sri Lankan soils, pesticides

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# 1. Introduction

The use of herbicides and fungicides has certainly helped to maintain, and in many instances, increase food production around the world. Agricultural practices now rely heavily on the use of pesticides but the contamination of soils and water supplies are now an ever increasing consequence of such farming activities.

Sri Lanka, as are many other developing countries, is still a largely agricultural based community, with the use of pesticides in such countries likely to suffer from higher than necessary application rates. This combined with only a limited knowledge as to what may happen to pesticides in Sri Lankan soils and the general environment, after application, is leading to more communities becoming exposed to pesticide residues in foods and drinking water. A large number of these pesticides are likely to pose a risk to human health and to ecosystems.

The use of pesticides in tropical ecosystems is rapidly increasing. However, although the fate of pesticides has been well researched in temperate soils, there is little data for tropical soils (1). Further to this, there have been only a limited number of studies on carbofuran and diazinon (2, 3, 4, 5,) with respect to their fate and behaviour in tropical soils and environments, including some work on submerged soils in the tropics, (6, 7). A limited degree of monitoring of surface and ground waters over the last few years in Sri Lanka has revealed some alarming increases of the residual levels of pesticides, (8), in these environments. In addition there are an increasing number of reports of people having diseases that may be linked with the pollution of water and soils in Sri Lanka.

Degradation of pesticides is usually a combination of a number of processes, including chemical hydrolysis and microbial degradation, and is also influenced by soil properties such as pH, organic carbon and moisture content(9). For Carbofuran, chemical hydrolysis is usually much faster, and is the primary degradation route, under alkaline soil conditions, whereas under acidic conditions microbial degradation plays a more important role (10). For Diazinon degradation by chemical hydrolysis usually occurs more rapidly under acidic conditions, but microbial degradation also occurs (6, 11).

Studying the ability of microbial populations to mineralise pesticides has been limited in tropical zones (3, 6, 7). <sup>14</sup>C substrate respiration and substrate induced respiration techniques have been used for a number of years to help evaluate and compare the general microbial health of soils (12, 13, 14). In this report we utilised those techniques to determine the rate of microbial mineralisation for the pesticides carbofuran and diazinon, both of which have been applied to the tropical soils of Sri Lanka over many years. The determined rates of mineralisation of the pesticides studied could help with better farming management practices, and therefore lead to a safer environment to live in for future generations.

## 2. Methods and Equipment

### 2.1. Soil properties

Four soils from Sri Lanka of different agricultural use and type were selected. A soil from Australia was included in the study in order to compare the mineralisation behaviour of the tropical soils from Sri Lanka to that of a temperate zone soil. Physical properties of the soils are given in Table 1. Samples were supplied as air dried at 40°C and ground to <2mm. Soils were adjusted to 60% of their maximum water holding capacity, (MWHC), with deionised water and then left to equilibrate at 28°C for four weeks. The temperature was selected on the basis of the average daily temperature in Sri Lanka and the period of four weeks was an estimated time to allow the natural soil microbial population to reach adequate population levels, prior to treating the soils with pesticides.

### 2.2. Glucose labelled compound

The <sup>14</sup>C Glucose was supplied as, C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>, D-[U-<sup>14</sup>C], with an activity, at time of use, of 9,246 kBq/ml.

A stock solution of this compound was made by adding 16µl of the supplied <sup>14</sup>C Glucose to 3.984ml of sterilised water (deionised water autoclaved for one hour prior to use), giving the stock solution an activity of 36,984Bq/ml (total activity, 147,940Bq/4ml).

Where applicable, 0.1ml of the stock solution, equivalent to 3,698Bq (0.10µCi) was added to soil samples.

### 2.3. Glucose stock solution

For the <sup>14</sup>C Glucose mineralisation study an additional pool of carbon was added to soils in the form of D-Glucose. For this study the extra pool was equivalent to 4,000 µgC/g of incubated soil.

The D-Glucose stock solution was prepared by dissolving 11.628g D-glucose into 25 ml deionised water. This gave a concentration of 186.05mg/ml. An addition of 0.215 ml was made to samples, this being equivalent to 40,000 µgC/10g.

### 2.4. Incubation with <sup>14</sup>C glucose

Utilising a substrate induced respiration technique, three Sri Lankan soils, Negombo, Nuwara Eliya and Pugoda the South Australian soil were used in the assessment of <sup>14</sup>C glucose mineralisation. All soils had been sampled within three months of commencing the incubation studies.

The incubated soil, (10g), was weighed into 40ml plastic vials. Additions of <sup>14</sup>C glucose stock, (0.1ml), glucose stock (0.215ml) and deionised water (up to 0.735ml) were made to the soils (Table 2). The volumes of deionised water were added so that the final water holding capacity was 75% of the soil's MWHC.

For convenience, and to reduce volume addition errors, the <sup>14</sup>C and glucose stocks, plus any additional water were mixed together, making a 'sample' stock solution and therefore all added in just one application (Table 3).

The treated soil samples, set up in quadruplicate, were placed into sealable 250ml plastic containers, together with a separate plastic vial containing 3mls of 1M NaOH, and then incubated in a controlled environment (temperature: 28°C, day and night light cycle: 14 and 10 hours respectively). At the following time intervals, 0.8, 28, 42, 58, 100 and 120 days, a 1 ml sub-sample from the NaOH trap was aliquotted into a 20ml scintillation vial, 10mls of scintillant was added, the vial sealed and shaken vigorously for about 10 seconds to ensure the two solutions were mixed thoroughly. After mixing, the samples were left to stand for about 5 hours, prior to being measured for activity. From the activity (count) data %C mineralised (as %CO<sub>2</sub>-C) was calculated.

At each sampling interval a fresh NaOH 'trap' (3ml) was placed next to the sample. The vessel with the sample and trap was then resealed and the incubation process continued.

Note that during the sampling period, samples were temporarily removed from the controlled environment to enable all sampling procedures to be performed safely in a radioisotope certified laboratory, and for one soil (Nuwara Eliya) sampling concluded at 58 days, as 98.5 % of added C had already been mineralised.

The treated soil samples were also checked for water loss every week, with small additions of deionised water usually necessary to maintain the samples at 75% of their MWHC. This process also meant the samples were exposed to fresh air on a weekly basis as well.

In conjunction with the treated soils, control soils and solutions were also prepared. They were sampled and measured for activity at the same intervals as the treated soil samples.

The controls were,

1. the incubated test soils, at 60% of their water holding capacity(WHC) were made to 75%(WHC) with deionised water. These were placed into 250ml vessels together with a vial containing 3ml 1M NaOH.
2. 10ml deionised water added to 20ml vial, placed into 250ml vessels together a vial containing 3ml 1M NaOH.
3. 3mls 1M NaOH in a 20ml vial, to which 0.1ml <sup>14</sup>C stock solution (3,700Bq) 0.215ml d-glucose stock solution,(40,000µgC), and 0.315ml deionised water was added. Vial then placed into a 250ml vessel. (Note: the three solutions added in one application.

The 250ml vessels for all of the controls were sealed and then incubated as per the treated soil samples.

## 2.5. Pesticide labelled compounds

Two radio labelled pesticides,  $^{14}\text{C}$  diazinon and  $^{14}\text{C}$  carbofuran were used in this study.

Diazinon,  $\text{C}_{12}\text{H}_{21}\text{N}_2\text{O}_3\text{PS}$  [Pyrimidinyl-6- $^{14}\text{C}$ ], is an organothiophosphate (used as an insecticide and acaricide). The activity of the supplied material, at time of use, was 7,400 kBq/ml. A  $^{14}\text{C}$  stock diazinon solution was prepared by adding 15 $\mu\text{l}$  of the supplied  $^{14}\text{C}$  Diazinon to 5.985ml of sterilised deionised water, giving the stock an activity of 18,500Bq/ml (total activity 111,000Bq/6ml).

Carbofuran,  $\text{C}_{12}\text{H}_{15}\text{NO}_3$ , [Benzene Ring-U- $^{14}\text{C}$ ], is a carbamate (used as an insecticide, acaricide and nematicide). The activity of supplied material, at time of use, was 9,250 kBq/ml. A  $^{14}\text{C}$  stock carbofuran solution was prepared by adding 16 $\mu\text{l}$  of the supplied  $^{14}\text{C}$  Carbofuran to 7.984ml of sterilised deionised water, giving the stock an activity of 18,500Bq/ml. An 0.1ml aliquot of the stock solution, equivalent to 1,850Bq (0.05 $\mu\text{Ci}$ ) was added to samples (total activity 148,000Bq/8ml).

Where applicable 0.1ml of the pesticide Diazinon and Carbofuran stock solutions, activity equivalent to 1,850Bq (0.05 $\mu\text{Ci}$ ), was added to soil and control samples.

## 2.6. Non labelled, ('cold'), pesticide stock solutions

Non-labelled pesticides were also added to the treated soils. A 0.2ml aliquot of 250 $\mu\text{g}/\text{ml}$  of each pesticide was added. This was to give a specific activity ratio for the labelled: non-labelled pesticide in the soil of 0.05 $\mu\text{Ci}$  (1850Bq) to 50 $\mu\text{g}/\text{ml}$  respectively for 10g of the incubated soil (pers. comm. I. Ferris, IAEA).

Note that the 'cold' pesticide application rate, equivalent to 5 $\mu\text{g}/\text{ml}/10\text{g}$  of incubated soil is similar to application rates used in general agricultural practices. (viz about 1kg/Ha).

## 2.7. Incubation with $^{14}\text{C}$ pesticides

The incubated soil, 10g, was weighed into 40ml plastic vials. Additions of  $^{14}\text{C}$  pesticide (0.1ml) and deionised water, (up to 0.735ml) were made to the soils (Table 4). The deionised water was added to make the final water holding capacity to 75% of MWHC for all of the soils.

For convenience, and to reduce volume addition errors, the  $^{14}\text{C}$  pesticide plus any additional water were mixed together, therefore making a 'sample' stock solution to be added in one application (Table 5).

The treated soil samples, set up in quadruplicate, were placed into sealable 250ml plastic containers, together with a separate plastic vial containing 3mls of 1M NaOH, and then incubated in a controlled environment (settings same as for Glucose incubation).

At the following time intervals, 0.4, 1, 3, 7, 14, 28, 42 and 62 days, a 1 ml sub-sample from the NaOH trap was aliquotted into a 20ml scintillation vial, 10mls of scintillant added, the vial sealed and shaken vigorously for about 10 seconds to ensure the two solutions were mixed thoroughly. After mixing, the samples were let stand for about

5 hours, prior to being measured for activity. From the activity (count) data %C (as %CO<sub>2</sub>-C mineralised) was calculated.

At each sampling interval a fresh NaOH 'trap', (3ml), was used. The vessel with the sample and trap was then resealed and the incubation process continued.

As with the glucose mineralisation experiment, samples were temporarily removed from the controlled environment for the sampling procedures etc., and the treated soil samples were checked for water loss every week, with small additions of deionised water being made to maintain the samples at 75% of their MWHC.

In conjunction with the treated soils, control soils and solutions were also prepared. They were sampled and measured for activity at the same intervals as the treated soil samples. The controls were :

1. 1M NaOH(3ml) in a 20ml vial, placed into a 250ml vessel which was then sealed.
2. Deionised water(10ml) added to 20ml vial, placed into a 250ml vessel together with a vial containing 3ml 1M NaOH.
3. Incubated test soils (10g) weighed into 40ml plastic vials, made to 75% MWHC with deionised water and placed into 250ml vessels together with a vial containing 3ml 1M NaOH.
4. Incubated test soils(10g) weighed into 40ml plastic vials, 0.2ug/ml 'cold' pesticide added, soils made to 75% MWHC with deionised water and placed into 250ml vessels together with a vial containing 3ml 1M NaOH.
5. From a 'stock' solution of 1.0ml <sup>14</sup>C pesticide, 2.0ml (250ug/ml) 'cold' pesticide and 3.15ml deionised water, 0.615ml (equivalent to 0.1ml <sup>14</sup>C pesticide, 0.2ml 'cold' pesticide and 0.315ml deionised water) were added in one application to 3ml 1M NaOH in a 20 ml plastic vial and placed into a 250ml plastic vessel. This control was made for each pesticide used, viz diazinon and carbofuran.

(Note: the addition of 0.615ml was chosen as an approximate median value to reflect the additions made to the treated soil samples)

The 250ml vessels for all of the controls were sealed and then incubated as per the treated soil samples.

## **2.8. Analytical Method for Sample Activity**

Sub-samples taken from the NaOH traps during the incubation periods had the 'scintillation cocktail', Optiphase 'hisafe' 3 supplied by the Perkin Elmer company, added to them in order to develop any fluorescence because of any <sup>14</sup>C present.

Samples were analysed for activity using a Wallac 1414 Win Spectral α/β liquid scintillation counter. The count time for each sample was ten minutes.

## 2.9 Calculations

### Substrate respiration

The substrate respiration response, measured as %C mineralised, for the diazinon and carbofuran treated samples was calculated from the total CO<sub>2</sub>-<sup>14</sup>C evolved (controls, blanks, samples) trap volume at each incubation time.

$$1) \text{ Ratio of } ^{14}\text{C (pesticide) trapped} = \frac{[\text{sample}(\text{counts})-\text{blank}(\text{counts})] \times 3^1}{\text{Total CPM}^2, \text{ added}}$$

$$2) \text{ CO}_2\text{-C evolved } (\mu\text{g/sample/sampling period}) = \frac{\text{ratio } ^{14}\text{C trapped}}{\text{sampling period}(\text{days})}$$

$$3) \% \text{CO}_2\text{-C mineralised} = (\text{CO}_2\text{-C) evolved} \times 100$$

<sup>1</sup> when 1ml sub sampled from the NaOH trap, (normal volume 3ml)

<sup>2</sup> CPM counts per minute

### Substrate Induced Respiration

The substrate induced respiration response measured as %C mineralised, for the diazinon and carbofuran treated samples was calculated from the total CO<sub>2</sub> – C<sup>14</sup> evolved (controls, blanks, samples) trap volume, at each incubation time.

$$1) \text{ Ratio of } ^{14}\text{C (pesticide) trapped} = \frac{[\text{sample, (counts)}-\text{blank, (counts)}] \times 3^1}{\text{Total CPM1, added}}$$

$$2) \text{ CO}_2\text{-C evolved } (\mu\text{g/sample/sampling period}) = \frac{\text{ratio } ^{14}\text{C trapped} \times 40,000^2}{\text{sampling period}(\text{days})}$$

$$3) \% \text{CO}_2\text{-C mineralised} = (\text{CO}_2\text{-C) evolved} \times 100$$

<sup>1</sup> when 1ml sub sampled from the NaOH trap, (normal volume 3ml)

<sup>2</sup> 40,000μg carbon added to each sample, (as glucose)

### 3. Results

#### 3.1 Mineralisation of <sup>14</sup>C glucose

The 'general health' of the microbial population of the soils was monitored up to 120 days, which encompassed the mineralisation period selected for the pesticides, viz about 60 days. The cumulative %C values were plotted against the sampling period number (Figure 1).

After 58 days the soils gave a range of 38.6 to 98.5% of the <sup>14</sup>C glucose mineralised (as %CO<sub>2</sub>-C) of the 40,000µgC added. The rate at which the added carbon was mineralised varied considerably between the soils, Negombo ranged from just 13% carbon mineralised, after 28 days, to 39% after 58 days, compared to Urrbrae, from 54% to 66%), Pugoda from 60% to 73% and Nuwara Eliya from 75% to 99%).

For three soils the monitoring period was extended to 120 days, with the total (cumulative) <sup>14</sup>C mineralised for Negombo, Urrbrae and Pugoda being 69%, 74% and 84% respectively (Table 6). The rate of mineralisation slowed significantly for all three soils after 100 days with this probably being directly correlated to the amount of the readily available added carbon pool left in each soil. Nuwara Eliya had showed the same trend at between 42 and 58 days.

To assess the time taken for 50% of the <sup>14</sup>C glucose pool to be mineralised  $I_n$  (fraction remaining) was plotted against time (days), (Figure 2).

The  $t^{1/2}$  values (days) calculated from the graphs were, Nuwara Eliya (12), Pugoda (23), Urrbrae(35) and Negombo (76), (Table 7).

#### 3.2 Mineralisation of <sup>14</sup>C diazinon

The mineralisation of the pesticide <sup>14</sup>C diazinon was measured over a period of 62 days, with samples being assessed for %C mineralised (as %CO<sub>2</sub>-C) at eight sampling intervals (Table 8).

The values for each soil were totalled and the cumulative %C mineralised plotted against sampling period number (Figure 3).

The total %C mineralised for diazinon was 1.9, 4.0, 7.1, 16.4 and 29.0% for the Nuwara Eliya, Urrbrae, Pugoda, Kalpitiya and Negombo soils respectively.

The time taken for 50% of the <sup>14</sup>C Diazinon pool to be mineralised was plotted against time (days), (Figure 4).

The  $t^{1/2}$  values calculated from the graphs were, Nuwara Eliya (2,302 days), Urrbrae (1153), Pugoda (631), Negombo (307) and Kalpitiya (276).

#### 3.3 Mineralisation of <sup>14</sup>C carbofuran

The mineralisation of the pesticide <sup>14</sup>C carbofuran was also measured over a period of 62 days. The cumulative %C mineralised (Table 9) was plotted against time (Figure 5).

The total %C mineralised for Carbofuran was 7.3, 15.5, 28.0, 32.5 and 40.1% for the Nuwara Eliya, Negombo, Urrbrae, Kalpitiya and Pugoda soils respectively.

The time taken for 50% of the  $^{14}\text{C}$  carbofuran was plotted against time (days), (Figure 6). The  $t^{1/2}$  values calculated from the graphs were, Nuwara Eliya (622 days), Negombo (272), Urrbrae (136), Kalpitiya (107) and Pugoda (87).

### **3.4 Controls**

For  $^{14}\text{C}$  glucose, water and untreated soil controls gave a mean count value of 32 compared to a mean value of 160,100 counts for  $^{14}\text{C}$  glucose+d-glucose+water.

For the  $^{14}\text{C}$  pesticides, water, NaOH, untreated soil, soil + 'cold' pesticide only, controls range from 29-33 counts compared to mean values for  $^{14}\text{C}$  diazinon and  $^{14}\text{C}$  carbofuran of 419,680 and 525,780 counts respectively.

## 4. Discussion

### Mineralisation of $^{14}\text{C}$ glucose

The ranking for the four soils studied for % $^{14}\text{C}$  glucose mineralised was Nuwara Eliya > Pugoda > Urrbrae > Negombo .

The rate of mineralisation varied considerably for the four soils tested. After 58 days, 99% of the added carbon had been mineralised by the Nuwara Eliya soil, whilst just 39% had been mineralised by the Negombo soil .

The Nuwara Eliya soil exhibited a very rapid phase in degradation - at just 28 days 75% of the available  $^{14}\text{C}$  pool had been mineralised. The Negombo soil showed a much slower, but generally a more constant rate of mineralisation, even up to 120 days. The Urrbrae and Pugoda soils both had fairly fast mineralisation phases, with about 50% of the C mineralised at 28 days, and then slowed a steady rate of degradation. It is interesting to note that the control soil, Urrbrae, an Australian soil from a Mediterranean climate, still had a large pool of carbon to mineralise, even though it showed a steady rate of  $^{14}\text{C}$  degradation during the study period.

Increases in both organic carbon and clay content directly correlated with increases in the total % $^{14}\text{C}$  mineralised. By contrast lower pH values was a strong contributing factor toward the total %C mineralised, with the highest amounts mineralised in the acidic soils of Nuwara Eliya and Pugoda. The constant environmental conditions would have also helped to maintain active microbial populations and therefore mineralisation processes.

The half life values ( $t^{1/2}$ ) for  $^{14}\text{C}$  glucose mineralisation for the Nuwara Eliya soil was just 12 days with the half life for the other three soils ranging from 23 to 73 days. The calculated half lives and the 'estimated' half life values from measured data corresponded very well.

The rate of mineralisation slowed after the first month in three of the soils and after about three months for the Negombo soil. However all soils showed that the microbial population was still quite active after four months of incubation. As this experiment was conducted under the same environmental conditions and time as set for the degradation experiment, it was clear the microbes in the re-wetted Sri Lankan soils and the Australian control soil, would remain active over the proposed degradation study period.

### Mineralisation of $^{14}\text{C}$ diazinon

The ranking for the five soils studied for  $^{14}\text{C}$  diazinon mineralisation was, Negombo > Kalpitiya > Pugoda > Urrbrae > Nuwara Eliya.

The rate of mineralisation between soils, and also for each soil, varied considerably. For example, at the end of the first week of incubation the fraction of the final total percentage of diazinon mineralised ranged from 0.09 to 0.79 for the Pugoda and Negombo soils respectively. For the Negombo soil, the fraction of the final total percentage of diazinon mineralised reduced from 0.79,(week one), to just 0.01 during the ninth week of incubation. The total cumulative percentage of diazinon mineralised ranged from 1.9% for the Nuwara Eliya soil to 29% for the Negombo soil after 62 days. The higher rates of degradation for diazinon were associated with increasing pH values (4.5-6.9) decreasing organic matter (7.6-0.15%), and decreasing clay content (40-0.15%). In our study the environmental conditions were set to a constant 28°C and soils were maintained at a constant 75% of their maximum water holding

capacity, which was conducive to microbial activity. However, diazinon degradation for all soils only reached a maximum of 29.0%, (average 11.7,+/-11.1%) after 62 days. Soil properties can certainly influence the degradation of pesticides as will regional temperatures and rainfall. These factors may have influenced a reversal of which soils had the highest and lowest mineralisation rates of the <sup>14</sup>C pesticides when comparing to the <sup>14</sup>C glucose work. viz Nuwara Eliya highest rate for glucose but lowest for diazinon.

The half life value for diazinon was 2,302 days for the Nuwara Eliya soil, which would certainly be of concern in agricultural systems if microbial mineralisation was the only pathway for degradation.

### **Mineralisation of <sup>14</sup>C carbofuran**

The ranking for the five soils studied for <sup>14</sup>C carbofuran mineralisation was, Pugoda > Kalpitiya > Urrbrae > Negombo > Nuwara Eliya.

The rate at which carbofuran mineralised was fairly uniform for each of the soils, however during the first week of incubation the rate of mineralisation, for the Sri Lankan soils, was about double the rate that was measured for each of the subsequent weeks. The values for the total % carbofuran mineralised were higher than for diazinon for all soils, except the Negombo soil. The ranking of the mineralisation for carbofuran showed no particular correlation or trend when compared with the soil properties that we measured.

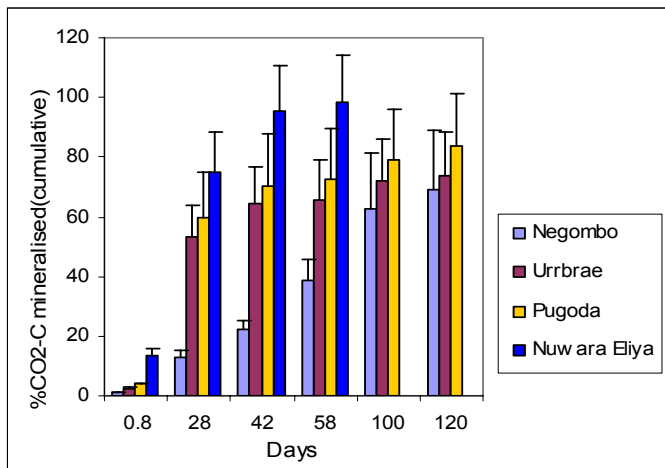
The half life values ranged from 87 to 622 days. Similarly to diazinon, the Nuwara Eliya soil had the longest  $t^{1/2}$  value, (622 days) and this could also be a problem in the environment if microbial mineralisation was the only degradation pathway.

## **5 Conclusions**

The degradation of the pesticides carbofuran and diazinon, commonly applied to soils in Sri Lanka, by microbial biota was found to occur in each of the soils studied. Mineralisation of both pesticides varied considerably for the soils, ranging from about 2-29% and 7-40% for diazinon and carbofuran respectively. The differences in mineralisation rates for the two pesticides studied was evident when looking at data for Negombo and Pugoda soils, (figures 7,8) and coupling this with both pesticides generally giving higher rates of mineralisation during the first week of incubation, compared to any other week, suggests that abiotic factors must also play a role in the break down of these pesticides. Other published work has shown that high organic content in soils generally leads to slower degradation rates for diazinon, the observations in this study agreed with this. However in other studies alkaline conditions were correlated with the rapid degradation of carbofuran but we observed for three of the Sri Lankan soils increasing acidic conditions resulted in higher mineralisation rates.

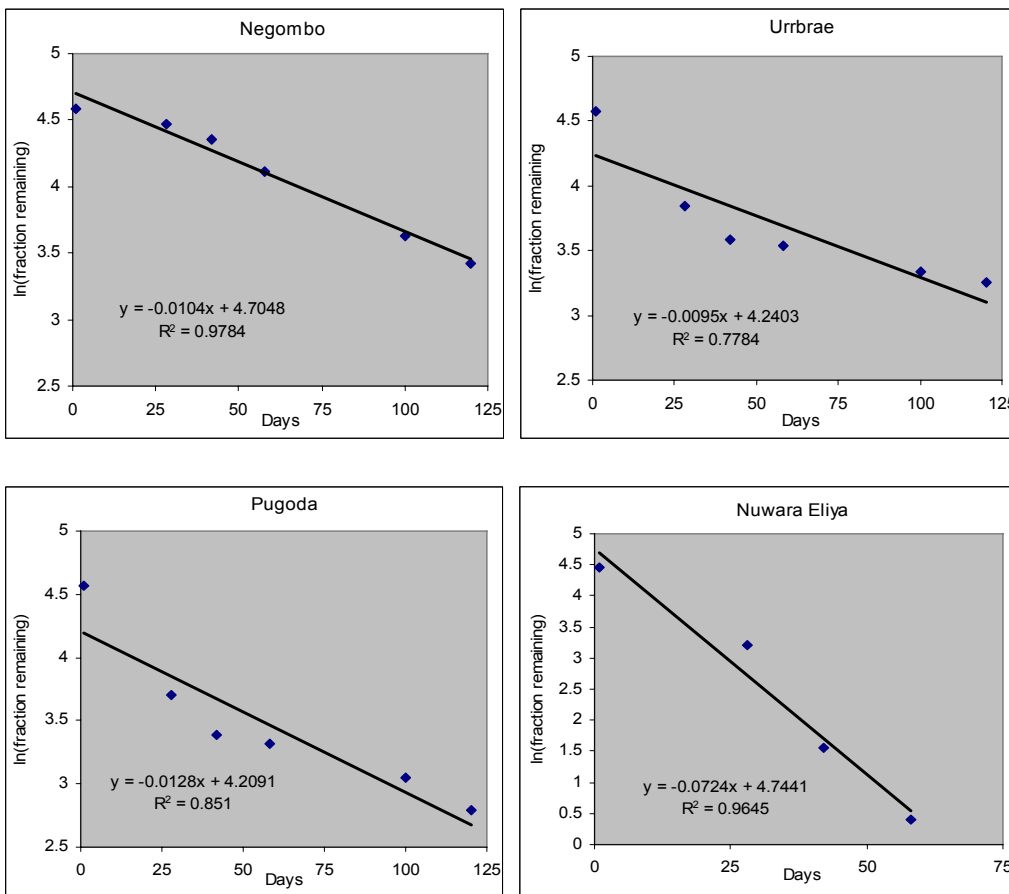
Half life data presented in this paper suggests there could be long retention times in the environment for the two pesticides studied, but the degradation processes of chemical hydrolysis and volatilisation generally are generally considered to be the major pathways for the breakdown of many pesticides, including carbofuran and diazinon, in the environment, ultimately giving half life values in the order of just 10-120 days. However our results show that the breakdown of pesticides by microbial mineralisation, over relatively short time periods, is an important pathway of the degradation process.

**Figure 1 %C mineralised<sup>1</sup>(cumulative) of <sup>14</sup>C glucose treated soils**

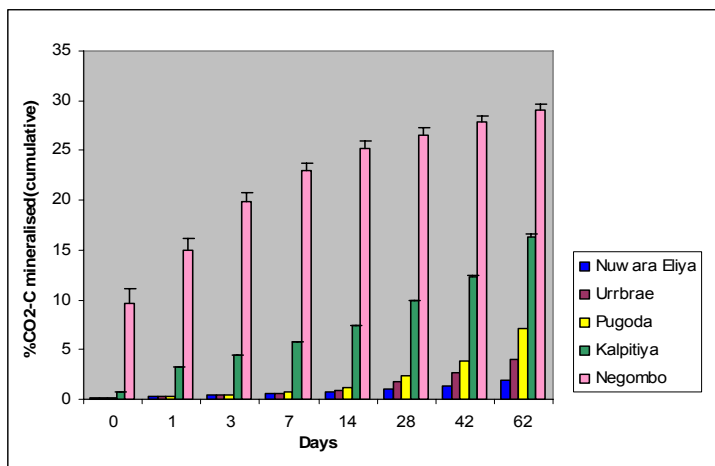


<sup>1</sup> %C as %CO<sub>2</sub>-C

**Figure 2 <sup>14</sup>C glucose mineralisation: I<sub>n</sub> (fraction remaining) vs time(days)**



**Figure 3 %C<sup>1</sup> mineralised,(cumulative) of <sup>14</sup>C diazinon treated soils**



<sup>1</sup> %C as %CO<sub>2</sub>-C

**Figure 4 <sup>14</sup>C diazinon mineralisation: I<sub>n</sub> (fraction remaining) vs time(days)**

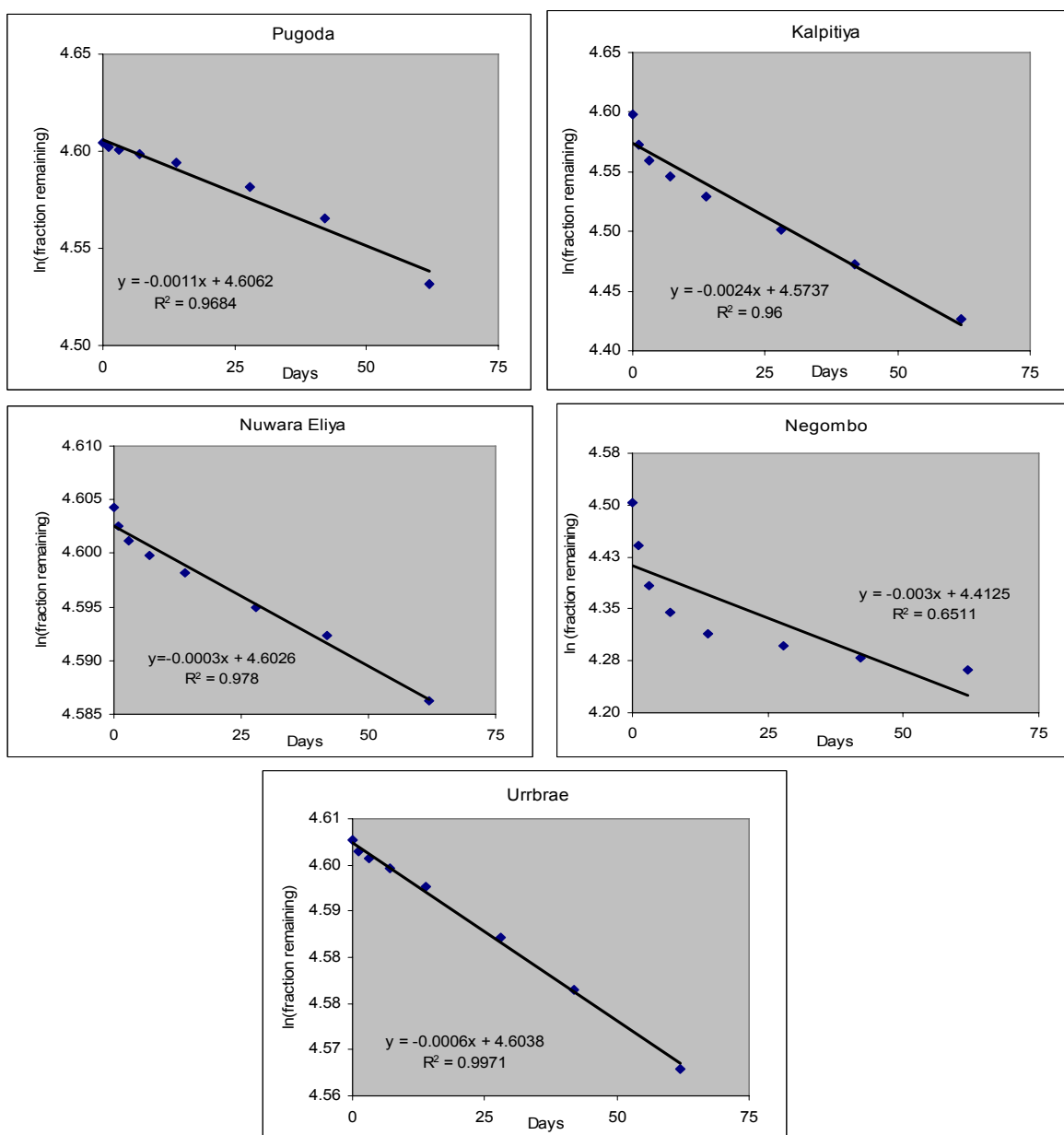
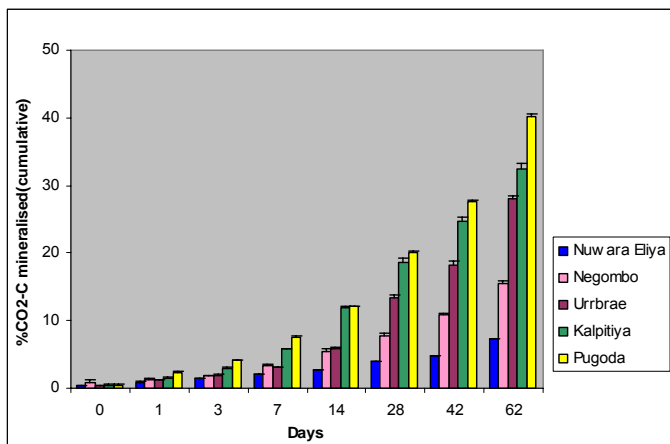
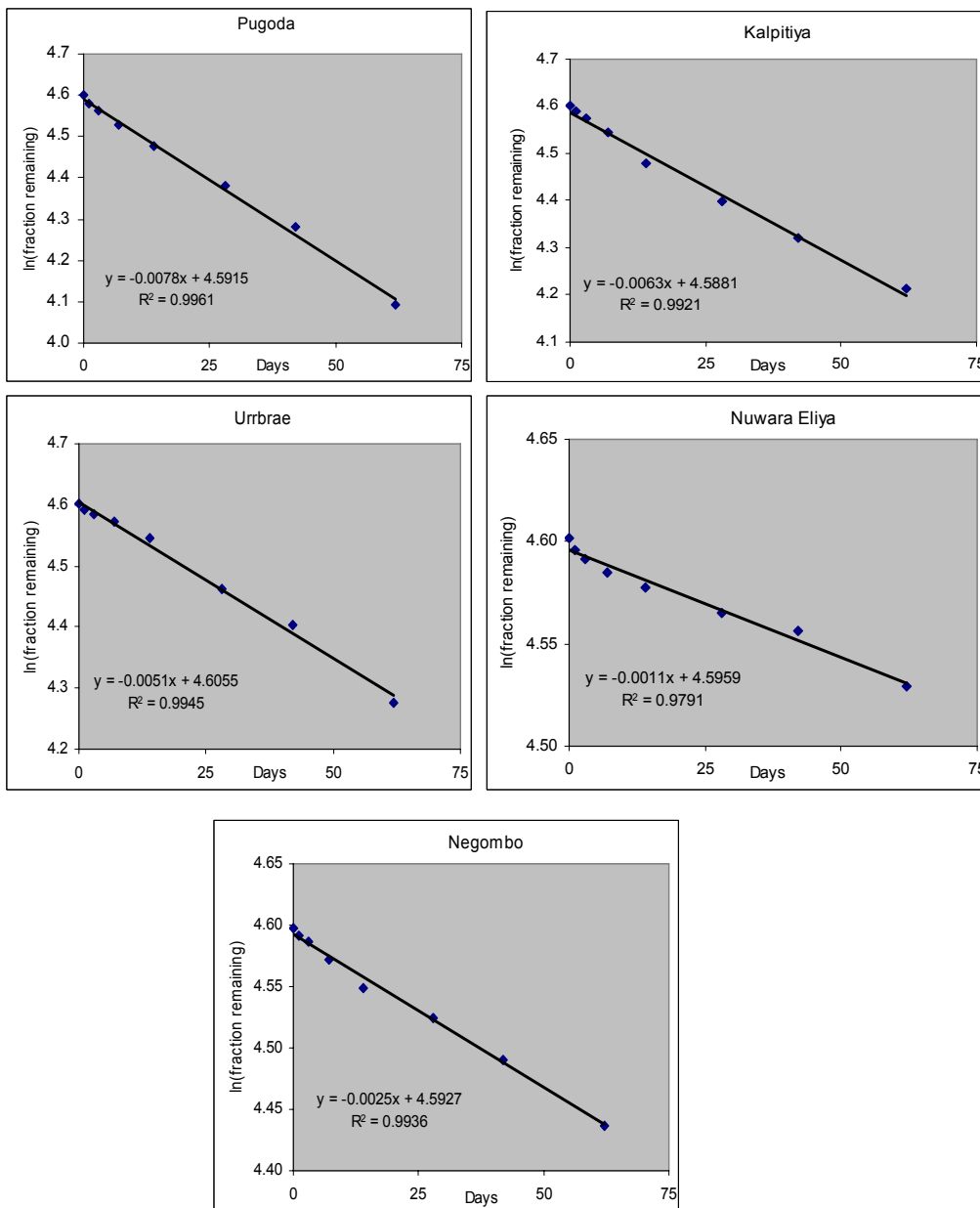


Figure 5 %C<sup>1</sup> mineralised, (cumulative), of <sup>14</sup>C carbofuran treated soils

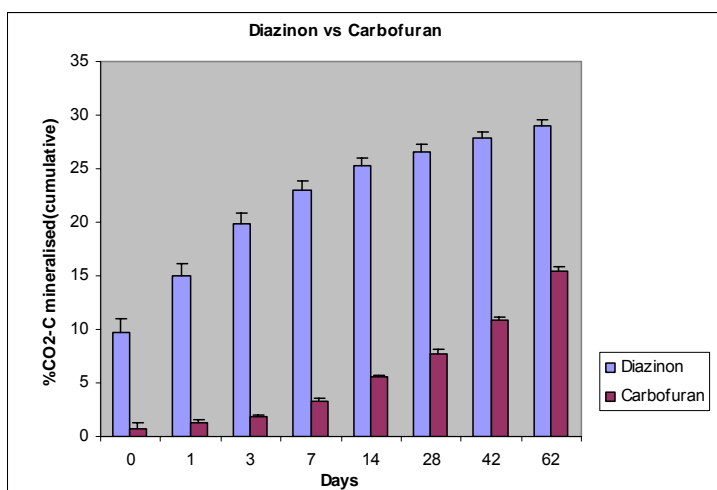


<sup>1</sup> %C as %CO<sub>2</sub>-C

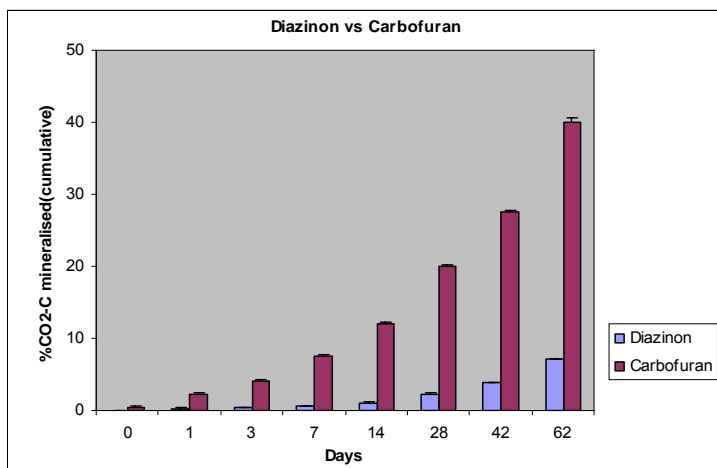
Figure 6 <sup>14</sup>C carbofuran mineralisation:  $\ln$  (fraction remaining) vs time(days)



**Figure 7 Comparison of %C<sup>1</sup> mineralised(cumulative) for Negombo**



**Figure 8 Comparison of %C<sup>1</sup> mineralised(cumulative) for Pugoda**



<sup>1</sup> %C as %CO<sub>2</sub>-C

**Table 1 Physical properties of the study soils**

Soil ID	Classification <sup>1</sup>	pH <sub>w</sub> (1:5, water)	EC (ms cm)	OC (%)	MWHC (%)	Clay (%) (<2 $\mu$ )
Negombo <sup>2</sup>	Ustic Quartzpsammet	6.9	0.04	0.15	21	<1
Kalpitiya <sup>2</sup>	(not yet classified)	6.7	0.29	1.8	21	11
Pugoda <sup>2</sup>	Typic Ustifluvent	5.5	0.02	2.3	69	37
Nuwara Eliya <sup>2</sup>	Typic Paleudult	4.5	0.06	7.6	70	40
Urrbrae <sup>3</sup>	Typic RhodoXeralfs	5.9	0.06	1.2	41	13

<sup>1</sup>USA, soil taxonomy classification

<sup>2</sup>Soils from Sri Lanka, collected by R.C.Watawala and A.G.P.Aravinna

<sup>3</sup>Reference Australian soil, collected from Waite Campus Arboretum, Adelaide, S.A.

pH and EC analysed using an Orion 940 meter, Organic Carbon determined with a Leco CNS-2000 analyser.

**Table 2 Additions<sup>1</sup> to the study soils for <sup>14</sup>C glucose mineralisation**

Sample	MWHC (gw <sup>2</sup> /10g)	60% MWHC (gw/10g)	75% MWHC (gw/10g)	<sup>14</sup> C glucose (ml)	Stock <sup>3</sup> glucose (ml)	Water <sup>4</sup> added (ml)	Total added (ml)
Negombo	2.1	1.26	1.575	0.100	0.215	0.000	0.315
Urrbrae	4.1	2.46	3.075	0.100	0.215	0.300	0.615
Pugoda	6.9	4.14	5.175	0.100	0.215	0.720	1.035
Nuwara Eliya	7.0	4.20	5.250	0.100	0.215	0.735	1.050

<sup>1</sup>Note addition (<sup>14</sup>C glucose, stock glucose, deionised water) made to soils that were pre-incubated at 60% water holding capacity.

<sup>2</sup> g water/sample

<sup>3</sup>Glucose Stock solution, equivalent to 100mg D-glucose or 40,000 $\mu$ gC per 0.215ml.

<sup>4</sup> additional water added to make sample to 75% MWHC

MWHC : maximum water holding capacity

**Table 3 Sample Stock<sup>1</sup> solutions used for single addition, <sup>14</sup>C glucose incubation**

Soil	<sup>14</sup> C glucose (ml)	d-glucose stock (ml)	DI <sup>2</sup> water (ml)	Total volume of stock (ml)	Volume added (ml)/sample
Negombo	0.70	1.505	0	2.205	0.315
Urrbrae	0.70	1.505	2.10	4.305	0.615
Pugoda	0.70	1.505	5.04	7.245	1.035
Nuwara Eliya	0.70	1.505	5.145	7.350	1.050
Control no.3	0.70	1.505	2.10	4.305	0.615

<sup>1</sup> stock solution volumes for 7 samples

<sup>2</sup> deionised water

**Table 4 Additions<sup>1</sup> to study soils for determining mineralisation of pesticides**

Sample	Pesticide <sup>2</sup> <sup>14</sup> C (ml)	Pesticide <sup>2</sup> 'cold' (ml)	DI water (ml)	Total Added (ml)	Total <sup>3</sup> vol in soil (ml)	MWHC (ml/10g)
Negombo	0.100	0.200	0.015	0.315	1.575	2.1
Kalpitiya	0.100	0.200	0.015	0.315	1.575	2.1
Urrbrae	0.100	0.200	0.315	0.615	3.075	4.1
Pugoda	0.100	0.200	0.735	1.035	5.175	6.9
Nuwara Eliya	0.100	0.200	0.750	1.050	5.250	7.0

<sup>1</sup>Note, additions made to soils after they had been incubated at 60% water holding capacity

<sup>2</sup>Pesticide added, <sup>14</sup>C Diazinon or <sup>14</sup>C Carbofuran

<sup>3</sup>Final total volume in soils equivalent to 75%, water holding capacity of 10g soil

**Table 5 Sample Stock solutions<sup>1</sup> used for single addition, <sup>14</sup>C pesticide incubation**

Soil	<sup>14</sup> C pesticide <sup>2</sup> (ml)	'cold' pesticide (ml)	DI <sup>3</sup> water (ml)	Total volume of stock (ml)	Vol. of sample stock added (ml)/sample
Negombo	1.00	2.00	0.15	3.15	0.315
Kalpitiya	1.00	2.00	0.15	3.15	0.315
Urrbrae	1.00	2.00	3.15	6.15	0.615
Pugoda	1.00	2.00	7.35	10.35	1.035
Nuwara Eliya	1.00	2.00	7.50	10.50	1.050
Control no. 5	1.00	2.00	3.15	6.15	0.615

<sup>1</sup> stock solution volumes for 10 samples

<sup>2</sup> <sup>14</sup>C pesticide, same additions for Diazinon stock and Carbofuran stock

<sup>3</sup> deionised water

**Table 6 %C<sup>1,2</sup> mineralised from substrate induced respiration of <sup>14</sup>C glucose**

Day	Negombo (%C)	Urrbrae (%C)	Pugoda (%C)	Nuwara Eliya (%C)
1 <sup>3</sup>	1.36	2.53	4.0	13.2
28	12.8	53.5	59.6	75.0
42	22.1	64.1	70.4	95.3
58	38.6	65.6	72.5	98.5
100	62.4	71.9	79.0	na
120	69.3	74.0	83.8	na

<sup>1</sup> %C value, calculated as %CO<sub>2</sub>-C

<sup>2</sup> values based on a 10g sample, dry weight basis

<sup>3</sup> day 1, sampled at 20 hours

na, not analysed

**Table 7  $t^{1/2}$  mineralisation values<sup>1</sup>**

Soil	Glucose	Diazinon	Carbofuran
Nuwara Eliya	12	2302	622
Pugoda	23	631	87
Urrbrae	35	1153	136
Negombo <sup>2</sup>	76	307	272
Kalpitiya	na	276	107

<sup>1</sup>  $t^{1/2}$  value : days

<sup>2</sup> Negombo value calculated from 7 to 62 day data, (plot was non-linear 0.4-3 days)

na, not analysed

**Table 8 diazinon : %C<sup>1</sup> mineralised, (cumulative)/ sample<sup>2</sup>/ sampling interval**

Day	Negombo	Pugoda	Nuwara Eliya	Kalpitiya	Urrbrae
0 <sup>3</sup>	9.71	0.09	0.09	0.73	0.09
1	15.03	0.30	0.26	3.20	0.27
3	19.84	0.47	0.40	4.45	0.41
7	22.95	0.67	0.54	5.71	0.57
14	25.22	1.12	0.70	7.35	0.90
28	26.61	2.32	1.02	9.87	1.77
42	27.86	3.89	1.28	12.38	2.64
62	29.00	7.10	1.87	16.35	3.96

<sup>1</sup> %C value, calculated as %CO<sub>2</sub>-C

<sup>2</sup> values based on a 10g sample, dry weight basis

<sup>3</sup> day 0, sampled at 4 hours

**Table 9 Carbofuran %C<sup>1</sup> mineralised (cumulative)/ sample<sup>2</sup>/ sampling interval**

Day	Negombo	Pugoda	Nuwara Eliya	Kalpitiya	Urrbrae
0 <sup>3</sup>	0.75	0.41	0.37	0.49	0.39
1	1.31	2.33	0.91	1.55	1.20
3	1.80	4.18	1.39	3.02	1.98
7	3.31	7.52	2.02	5.79	3.12
14	5.53	12.03	2.70	11.96	5.86
28	7.75	20.06	3.91	18.63	13.32
42	10.83	27.63	4.77	24.77	18.29
62	15.47	40.07	7.29	32.47	27.99

<sup>1</sup> %C value, calculated as %CO<sub>2</sub>-C

<sup>2</sup> values based on a 10g sample, dry weight basis

<sup>3</sup> day 0, sampled at 4 hours

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