THE LOSS OF PESTICIDES FROM SOIL

AND SOIL COMPONENTS

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by

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To Janice for her indefatigable

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ABSTRACT

The biological and non-biological breakdown of 2,4-D, diallate and malathion was assessed in both a silt loam soil and in its separated inorganic and organic fractions. The information derived was used in an attempt to identify the major influence affecting the decay of each pesticide.

The breakdown of 2,4-D in soil was preceded by a brief lag phase of microbial enrichment. In the organo-mineral fraction breakdown was somewhat faster whilst no loss occurred from the silt, sand and clay fractions. Negligible loss occurred in the microbially-sterile soils and soil components.

In contrast the disappearance of diallate appeared to occur mostly via non-biological means (i.e. volatilization). Rates of loss increased in the coarser soil fractions (sand and silt) and neither irradiation nor autoclaving reduced these rates.

The insecticide, malathion, was degraded rapidly in both non-sterile, irradiated and azide treated soils but not in autoclaved soil. Breakdown was also rapid in separated organic and organo-mineral fractions, somewhat slower in sand and silt and negligible in all autoclaved fractions.

From comparisons of the breakdown of these three pesticides it was clear that different mechanisms were involved in each instance. 2,4-D was predominantly microbial; diallate due to volatilization; malathion a combination of microbial and exo-enzyme metabolism.

The results from experiments involving recombinations of sand, silt and organo-mineral components to produce artificial soils indicated that the organo-mineral complex again played a dominant role in determining the rates of loss of these three pesticides.

Finally, it was demonstrated that the amount of this organo-mineral component was important in controlling 2,4-D and malathion persistence in other soils.

However, in the case of diallate no correlation between rate of loss
and percentage organo-mineral content was observed.

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INTRODUCTION

A. Factors Affecting Pesticide Loss from Soil

I. Introduction

For the most effective, economic and environmental use of a pesticide, its persistence and that of its toxic by-products must be known. This data is important so that the amount of chemical which will perform a specific function for a required period of time can be calculated with precision.

Before attempting to study the persistence of a pesticide in the soil environment it is necessary to understand the factors which influence pesticide behaviour.   Three major degradative processes contribute to the breakdown of a pesticide once it has been introduced into the soil, namely: photo-, biological and chemical decomposition. In addition, a number of non-degradative factors influence the efficacy of a pesticide (Table 1).

Table 1

Non-degradative factors influencing pesticide efficacy

1. Volatilization

2. Adsorption onto soil particles

3. Transfer processes in soil

   (a) Leaching

   (b) Diffusion

   (c) Capillary movement

   (d) Lateral movement

   (e) Surface run-off

4. Adsorption and retention by plants and microorganisms

The way in which a particular pesticide will behave in the soil environment is also governed by the physical, chemical and biological properties of both the pesticide and the soil. In addition, various edaphic factors, including the nutrient and pH status, temperature and moisture conditions of the soil are of considerable importance.

II. Nature of the Pesticide

In this section the physical and chemical properties of a pesticide will be considered vis-a-vis its subsequent adsorption, volatilization and degradation.

(a) Adsorptive behaviour

The overall properties of each pesticide will govern its adsorptive behaviour in a soil system. The nature of each pesticide is a general function of its molecular structure, which is expressed by such factors as molecular size, ionizability, water solubility, lipophilicity and polarizability.

Weber (1972), has attempted to categorize the majority of pesticides into two broad groups, i.e. ionic and non-ionic according to their behaviour in aqueous solution (Table 2). This property of certain pesticides to ionize in aqueous solution has important consequences for determining the extent of adsorption and ease of subsequent desorption by colloidal systems.

The pK_a or dissociation constant indicates the degree of acidity or basicity that a compound will exhibit. Adsorption studies with acidic pesticides (Newman and Way 1966; Harris and Warren 1964; Weber, Perry and Upchurch 1965; Weber, Ward and Weed 1968; Donaldson and Foy 1965), have shown that these compounds were readily adsorbed by anion exchange mechanisms. These particles contain carboxylic or phenolic groups which may ionize in aqueous solution to produce organic anions. This tendency to ionize in aqueous solution {I} is described by the acid dissociation constant, K_a {II} :

Table 2

Classification of Ionic and Non-Ionic Pesticides (Weber and Weed 1974)

Ionic				Non-ionic
Cationic	Basic	Acidic	Miscellaneous	
Bipyridyls Paraquat Diquat Chlormequat	Triazines Atrazine Atratone Simazine Propazine Prometone Triazoles Amitrole	Aliphatic acids TCA Dalapon Aromatic Acids 2,4-D 2,4,5-T 2,4-DB 2,4,5-TB MCPA MCPB 2,3,6-TBA Mecoprop Picloram Fenac Dicamba Chloramben Endothall Phenols Dinoseb DNOC Hydroxybensonitriles Bromoxynil Ioxynil	Uracils Bromacil Isocil Terbacil Organic Arsenicals DSMA	Chlorinated hydrocarbons Aldrin,Dieldrin,DDT,Heptachlor,Lindane Organophosphates Diazinon,Malathion,Parathion,Phorate, Disulfoton Substituted Anilines Trifluralin, Nitralin Phenyl Ureas Diuron, Fenuron, Linuron Substituted Anilides Propachlor, Dicryl, Solan Phenylamides Diphenamide Thiocarbamates EPTC,CDEC,Pebulate,Vernolate,Cycloate Carbothioates Molinate Acetamides CDAA Benzonitriles Dichlobenil Esters 2,4-D
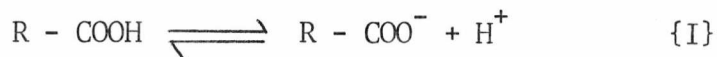
$$R - COOH \rightleftharpoons R - COO^- + H^+ \qquad \{I\}$$

$$pK_a = pH - \log \frac{\{RCOO^-\}}{\{RCOOH\}} \qquad \{II\}$$

where: $R - COOH$ = undissociated molecule; $R - COO^-$ = anion; H^+ = hydrogen ion.

The degree of ionization is pH dependent, the anionic species predominating in neutral or alkaline aqueous systems. This predominance of anionic species in soil aqueous systems, together with the presence of predominantly negatively charged soil colloids, generally means that these compounds are not adsorbed in significant amounts. In adsorption studies (Weber *et al.* 1965), the phenomenon of negative adsorption has been observed. This occurs at pH levels greater than 7.0, whereas positive adsorption of molecular species will occur in strongly acid systems where the degree of dissociation is small. According to Weber *et al.* (1965) negative adsorption occurred when dry montmorillonitic clay adsorbed water in preference to 2,4-D anions, causing an increase in the concentration of 2,4-D in the bulk of the solution. This phenomenon has been extensively studied by Bolt and Warkentin (1958); Frissel (1961). Frissel noticed that repulsion of the anion occurred until the pH of the clay water system approached the pK_a for the particular compound studied. Thereafter positive adsorption commenced and gradually increased as the pH of the system decreased. Harris and Warren (1964); Ogle and Warren (1954); Donaldson and Foy (1965); Hamaker, Goring and Youngson (1966), have observed the adsorption of acidic pesticides onto organic soil colloids. Adsorption was pH dependent, being greater under acid conditions, where pesticides were adsorbed in the molecular form. Weber (1974) states that molecular adsorption may include weak physical forces or hydrogen bonding involving the undissociated hydrogen. Adsorption of the anionic species will occur on those soil colloids possessing some anion exchange properties

at neutral and basic pH's (Frissel 1961; Frissel and Bolt 1962; Burnside and Lavy 1966), such as kaolinite and illite clay minerals and hydrous metallic oxides.

Adsorption of acidic compounds occurs in relatively low amounts compared to the basic and cationic herbicides. In consequence, acidic compounds are mobile and leached to a greater extent than the cationic or basic pesticides.

Basic pesticides, such as the s-triazines and triazoles (Table 2), behave as weak bases in aqueous systems. They readily associate with hydrogen ions to form protonated species (Allcock 1967) as shown below {I}:

$$R + H^+ \rightleftharpoons RH^+ \qquad \{I\}$$

where: R = molecular species; H^+ = hydrogen ions; RH^+ = cationic species. The amount of each species in solution is governed by an equilibrium expression {II}:

$$pK_a = \log \frac{\{RH^+\}}{\{R\}} + pH. \qquad \{II\}$$

The pK_a values represent the pH level at which half of the species in solution is present as the cationic form and half as the molecular form. The molecular form predominates at neutral or alkaline pH's and the cationic species predominates under acid conditions. This protonation under acid conditions may occur in several ways, as described by Bailey and White (1970) and Mortland (1970), in order that adsorption can occur via a negative site. Adsorption of s-triazines by organic matter through ion exchange processes has been suggested by the work of Weber, Weed and Ward (1969). Weber (1966); Bailey and White (1965), studied the basicicity of s-triazines, as expressed by pK_a, showing this to be a major factor governing adsorption. Weed and Weber (1974) state that at least three mechanisms are operating in the adsorption of basic compounds; (i) ion exchange

dependent on protonation and therefore sensitive to pH; (ii) hydrogen bonding; and (iii) hydrophobic bonding.

Cationic herbicides (Table 2) are readily inactivated when applied to soil (Calderbank 1968; Scott and Weber 1967). The water solubility of these compounds is high and they dissociate readily in aqueous solution to form cationic species. Diquat and parquat are readily adsorbed from aqueous solutions by soil particles (Scott and Weber 1967), montmorillonite (Dixon, Moore, Agnihotri and Lewis 1970; Weed and Weber 1968), kaolinite (Weber and Scott 1966), organic matter (Khan 1974; Burns, Hayes and Stacey 1973) and cation exchange resins (Weber et al. 1968). In addition to their cationic nature these two herbicides differ in the positive charge distribution which has been a factor attributed to their difference in adsorptive behaviour (Weber et al. 1968). The equilibrium adsorption and desorption behaviour of parquat and clay minerals (Weber et al. 1965; Weber and Weed 1968; Knight and Denny 1970) demonstrate that it is readily and strongly bound to an extent approaching the cation exchange capacity of the clay system studied. This dissociation of cationic pesticides is independent of pH, in contrast to the aforementioned groups.

A large number of pesticides exist which do not ionize to any significant degree (Table 2) and little information is available on the mechanisms of their adsorption.

The chlorinated hydrocarbon insecticides, including DDT, endrin and dieldrin, are probably adsorbed through physical adsorption (Barlow and Hadaway 1955; Harris 1964; Thurston 1953) by organic matter, their performance often being correlated with the organic matter content of soils (Edwards, Beck and Lichtenstein 1957). Pierce, Olney and Felbeck (1971) proposed that the bonding of non-polar pesticides to soil organic matter was a pesticide-lipid interaction.

The phenyl urea herbicides (Table 2) may have a mechanism of adsorption

related to their soluble nature and size of molecule (Weber 1972). Evidence is available to show that their adsorption and efficacy is related to soil organic matter and clay contents. Adsorption of phenyl ureas is essentially independent of pH (Hance 1969b; Yuen and Hilton 1962), suggesting that adsorption occurs through van der Waals forces (Hance 1969a,b).

The retention of organo-phosphates by soils is related both to clay and organic matter (Getzin and Chapman 1959, 1960; Konrad, Chesters and Armstrong 1969). Weed and Weber (1974) conclude that adsorption consisted of van der Waals forces between hydrophobic portions of the pesticide molecules and the organic matter and clay surface.

Generally, adsorption by soils of the non-ionic classes of pesticides is positively correlated with organic matter content, the varying extent of adsorption being related to differing solubilities and polarities.

(b) Volatilization

The vapour pressure of a molecule is an indication of the relative ease with which it changes from the solid or liquid state into the vapour phase. The higher the vapour pressure the more volatile the pesticide. In practical terms this means that for efficient use of volatile thiol-carbamates, carbothioates, acetamides and benzonitriles they must be incorporated into the soil or formulated as slow release granules.

(c) Formulation

The formulation of a pesticide will influence its subsequent behaviour, for example, the acidic herbicides may be formulated as the acid, salt or ester forms. The salts have a greater solubility than the acids; the esters are less soluble but more volatile. These differences will obviously effect the mobility and volatility of the pesticides in soil.

Different physical formulations, such as wettable powders, granules, dusts, water soluble or oil soluble liquids, emulsifiable concentrates and

gases may influence pesticide behaviour in soil, as will any additives.

(d) Degradation

The nature of a pesticide will also affect its susceptibility to the various degradative processes that operate in the soil environment. The chlorinated hydrocarbon insecticides are generally persistent in soil (Edwards 1966) and are ⟩ detoxified at a very slow rate (Table 3).

Table 3

Disappearance of persistent organochlorine

insecticides from soils (Edwards 1966)

Insecticide	Time for 95% disappearance	Average (years)
Aldrin	1 - 6	3
Chlordane	3 - 5	4
DDT	4 - 30	10
Dieldrin	5 - 25	8
Heptachlor	3 - 5	3.5
Lindane	3 - 10	6.5
Telodrin	2 - 7	4

In contrast, two other major groups of insecticides (the organo-phosphates and carbamates) do not present serious residue problems. Although variation exists within each group, most of them are readily degraded in soil (Matsumura and Boush 1971).

Alexander (1965) stated that a number of chemical characteristics give rise to persistence because of the apparent inability of microorganisms to metabolize specific molecular types. Unfortunately, in only a few

instances is it evident which structural characters or substituents impart resistance to microbial decay.

Alexander (1965) looked at the effect of chemical structure on the persistence of chlorinated phenoxy compounds. The data obtained suggested that two chemical characteristics were associated with the microbial resistance of these herbicides; the type of linkage of the aliphatic acid to the ring, and either the position or the number of chlorines on that ring.

Other examples of the relationship between chemical structure and persistence have been demonstrated for the triazines (Sheets, Crafts and Drewer 1962), for benzoates (MacRae and Alexander 1965), and for the phenyl-ureas (Sheets 1964).

III. Nature and Characteristics of Soil Components

The nature of the soil components, particularly those colloidal constituents having highly reactive surfaces such as the organo-clay complex and the crystalline and amorphous oxides and hydroxides, will have an important bearing on the behaviour and subsequent persistence of a pest-icide in the soil environment.

These soil colloids may be divided into a mineral fraction containing crystalline clay, crystalline and amorphous oxides and hydroxides, and an organic fraction consisting of largely uncharacterised polymeric humic materials.

(a) Clay

Clays are broadly subdivided into kaolinitic types having a 1:1 lattice arrangement, and a montmorillonitic group possessing a 2:1 arrangement of silicon tetrahedra and aluminium octahedra respectively. The kaolinitic clays as a result of their structural and physical properties, have non-expanding lattices, only an external adsorption surface and a low cation

exchange capacity   (2-10 meq./100g.), whilst the montmorillonitic clays have expanding lattices, internal and external adsorptive surfaces and a high cation exchange capacity (80-120 meq./100g.).

(b) Organic matter

Organic matter is made up of undecayed plant and animal tissue and a degraded and probably re-synthesised component, humus. These humic materials are described more fully by Kononova (1966). Included in these humic materials are humic acids which are usually regarded as polymers of aromatic compounds with carboxyl, phenolic and hydroxyl groups present. It is these functional groups that produce a pH dependent cation-exchange capacity, in contrast to the clays which have a permanent negative charge due to iso-morphous replacement, and a positive edge charge dependent on pH.

(c) Organo-mineral complex

The organo-mineral complex is described by Kononova (1966). It is stated here that in mineral soils most of the organic matter is colloidal in form and intimately associated with the inorganic fraction to form the organo-mineral complex. This humus-clay microenvironment is a site of high biological and non-biological activity (Burns 1972) and it is at this site that we may need to look for the basic information concerning soil/pesticide interactions.

(d) Adsorption processes

Bailey and White (1964) stated that the nature and properties of soil colloids directly influenced the adsorption-desorption processes of pesticides in soil systems.

In order to describe the extent of adsorption, isotherms have been found which fit the empirical Freundlich relationship (Bailey and White 1970)

{I}, where

$$\frac{x}{m} = kc^n \hspace{4cm} \{I\}$$

$\frac{x}{m}$ is the amount of pesticide adsorbed per unit weight of soil, k and n are constants and c is the equilibrium concentration in solution. The constant k gives an indication of the extent of adsorption and n reflects the degree of non-linearity of the adsorption (n would be = 1 for truly linear adsorption). The values of k vary widely for different soils and pesticides.

If the organic matter content of the soil is determined, the extent of adsorption (k) per unit mass or organic matter can be calculated (Osgerby 1970). This is represented by Q. {II}

$$Q = \frac{100 \times k}{\text{\% organic matter}} \hspace{3cm} \{II\}$$

This data has been used by Furmidge and Osgerby (1967), to compare the behaviour of different herbicides in soils. These adsorption studies are important when a comparison between herbicidal activity and organic matter or clay content, in relation to adsorption is needed. Adsorptive studies with clays have been reviewed by Weber (1970) and those involving organic matter by Hayes (1970).

Weber *et al.* (1974) and Moyer, Hance and McKone (1972) suggested that decreasing herbicidal activity in soil can be correlated with increasing organic matter contents. Geissbuhler, Haselbach and Aebi (1963); McCormick and Hiltbold (1966) and Smith and Meggitt (1970) concluded that adsorption by soil organic matter was exerting a protective effect on pesticides. Grover (1966) showed that the addition of organic matter reduced the phytotoxicity of simazine to oats grown in a heavy clay soil, whilst Edwards *et al.* (1957) found that the amounts of aldrin and lindane remaining in soil were closely correlated with organic matter content. Roberts and Wilson (1965); Lichtenstein and Schulz (1959); Lichtenstein, DePew,

Eshbaugh and Sleesman (1960) and Konrad *et al.* (1967) and many others agree that organic matter is the single most important soil characteristic influencing persistence of pesticides.

Moyer *et al.* (1972), looked at the effect of a range of adsorbents on the rate of degradation of herbicides incubated in soil. The presence of clay slightly increased the persistence of all compounds studied (atrazine, chlorthiamid and linuron), whereas charcoal, although increasing persistence of atrazine and chlorthiamid several fold, did not affect that of linuron. Charcoal reduced decomposition rates less than it reduced the herbicide concentration in soil solution.

Bailey and White (1970) have described the mechanisms of adsorption in relation to soil and pesticide properties and these include:-

1. Physical adsorption - due to van der Waals forces.

2. Hydrogen bonding.

3. Co-ordination complexes.

4. Chemical adsorption - including ion exchange and protonation.

(e) Volatilization

Harris and Lichtenstein (1961) found that insecticides volatilized at different rates from different soil types. Aldrin and Lindane volatilized much more rapidly from a quartz sand than from a muck soil. Wheatley (1962) estimated that dieldrin had a half-life of four years in a mineral soil whereas in an organic soil the half-life was 5 - 7 years.

It can be assumed that in many instances loss by volatilization is related to the organic matter content of soil. This correlation is probably related to increased adsorption with increasing organic matter as discussed elsewhere (p 7).

(f) Degradative processes

Having given consideration to the possible role of organic matter

(and clay) immobilisation in increasing pesticide persistence it should be realized that the converse has been observed, that is persistence in soils has decreased with increasing organic matter (Ogle and Warren 1954; Rahn and Baynard 1958; Dubey, Sigafus and Freeman 1966). It was suggested that this was due to a direct relationship between microbial activity and organic matter.

The complex relationship between organic matter and pesticide persistence has not yet been satisfactorily explained but it has been suggested (Burns 1972) that a minimum level of organic matter (possibly greater than 1%) is necessary to support an active and diverse microbial population to allow the decay of any organic substrate. Below the 1% level considerable enrichment would be needed for microbial decay i.e. indigenous microbial numbers may be insufficient. In high organic matter soils (greater than 15%) adsorption may be acting as an immobilizing agent thus increasing persistence. Soil with median levels of organic matter may be optimum for the rapid breakdown of some pesticides (Fig 1).

Koren, Foy and Ashton (1968) proposed this relationship with the thiolcarbamate herbicides suggesting that inactivation occurred by adsorption in soils with a high organic matter content, volatilization in low organic matter soils and microbial activity in soils with median levels of organic matter.

Different soil types may possess different microbial numbers and species which will in turn be determined by the nature of the organo-mineral complex, the environment at the colloid surface and other factors. Marshall (1971) mentions that the variety of organic and inorganic soil components offer numerous opportunities for sorption processes involving actual microorganisms. Since the degree of sorption between microorganisms and soil particles is related to their surface charge, it seems likely that the organo-mineral complex may be the most important area for these sorption
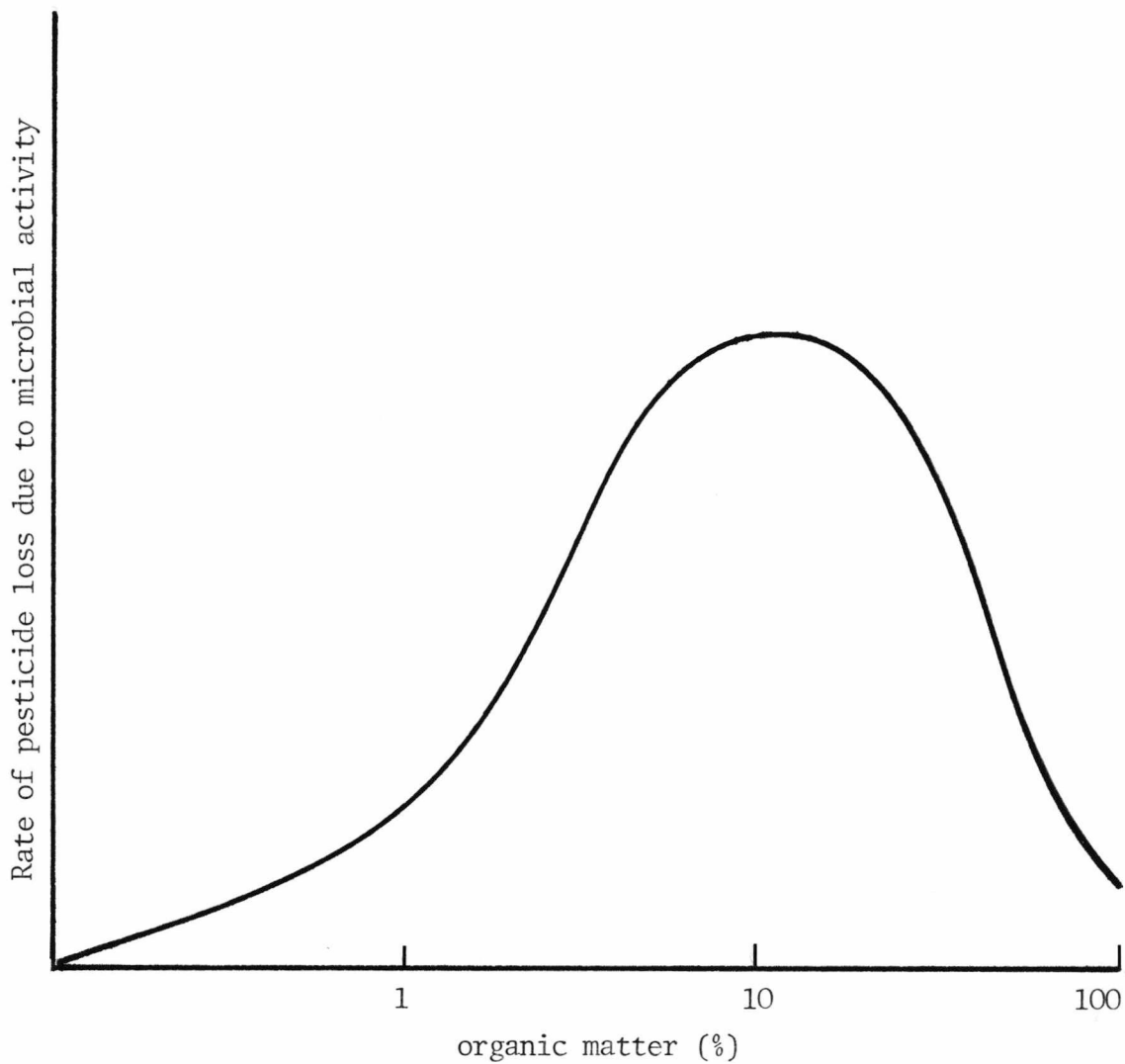
Fig. 1

Microbial decay of pesticides versus soil

organic matter content (Burns 1972)

interactions of microbes. Therefore the type rather than the quantity of clay present may be more important in terms of comparative microbial activity in soils. Microbial attachment to soil colloids is poorly understood but the obvious advantages of an association with the organo-mineral complex include a resident supply of organic matter augmented by adsorption from the soil water (either onto the colloid or directly onto the microbe) of additional substrates, such as pesticides (Burns 1972).

Adsorption may serve to increase persistence when the pesticide is adsorbed some distance away from a degradative source such as a microbe or soil extracellular enzyme, or decrease persistence if adsorbed in the vicinity of a degradative source (Burns 1972).

Temperature, pH and moisture will broadly influence the nature of the microbial population which will determine persistence if breakdown is microbial in origin.

Purely chemical decay may be influenced by the nature and proportion of different soil components. It is not easy to distinguish between biological and chemical decay without the use of systems that have under-gone considerable physical or chemical alteration, however, there is evidence for chemical decay in soil systems. Armstrong *et al.* (1967,1968) showed that atrazine hydrolysis was increased on contact with sterilized soil systems, implying catalysis by one of the soil components. Briggs and Dawson (1970) considered that soil organic matter provided protection against chemical hydrolysis in soils. Russell, Cruz, White, Bailey, Payne, Pope and Teasley (1968) observed that the chemical hydrolysis of a chloro-*s* triazine following interaction with a montmorillonite clay was facilitated by proton-ation at the clay surface. Evidence that soil components may enhance the degradation of organophosphate insecticides is provided by Mortland and Raman (1967) and Konrad *et al.* (1967). Kearney and Helling (1969) propose the presence of free radicals in soil organic matter may be involved in the

degradation of certain pesticides.

IV. Climatic Conditions.

Pesticide persistence will depend on climatic conditions due to the effect of climate on the soil macro and micro-environment. Temperature will influence volatilization of pesticides from the soil surface and pore spaces, whilst evaporation of water may carry pesticide residues into the atmosphere. This latter phenomenon is known as co-distillation. Temperature may also have important consequences for the rate of decay. Burnside, Schmidt and Behrens (1961) observed that in arctic and temperate zones triazines disappeared more slowly during winter than in summer. Microbial decay of dicamba (Hahn, Burnside and Lavy, 1969), and atrazine (Roeth, Lavy and Burnside 1969) increases with temperature as does the non-biological decay of DDT (Guenzi and Beard 1970) and atrazine (Obien and Green 1969). Temperature can also affect exothermic processes such as adsorption, and an increase in temperature will decrease adsorption and increase availability of the pesticide to degradative and various transfer processes (Bailey and White 1964). However, McGlamery and Stife (1966), reported that adsorption of triazines by humic acids increases with increasing temperature. Reports such as these may be due to temperature induced changes in the structure and porosity of the organic matter (Hayes, Stacey and Standley 1968; Guenzi and Beard 1970).

Rainfall will affect movement of pesticides in the soil profile depending on the formulation of pesticide and the soil and pesticide types. The greater the quantity of water entering the soil, the more rapid are the leaching rates (Upchurch and Pierce 1957, Burnside *et al.* 1961). Many studies on leaching under laboratory conditions bear little relation to an *in vivo* environment since varying soil structure, alternating drainage

and evaporation and soil moisture status upon application, will produce situations under field conditions that cannot realistically be reproduced by *in vitro* soil column studies.

The moisture regime, itself influenced by climate, may affect adsorptive processes, as water molecules compete with organic molecules for adsorption sites on the soil colloid. Lichtenstein and Schulz (1961), found that aldrin volatilized faster from a wet soil than from a dry soil. This contrasts with a dry soil system when adsorption can be almost irreversible. Good competitors are known to be poor soil pesticides and the effectiveness of DDT and heptachlor is due at least, in part, to their lack of competitiveness with water (Burns 1975). Harris (1964b) notes that the reason for the increase in toxicity of diazinon compared to parathion in a more moist soil was that parathion was more competitive for active sites on the soil particles.

The water regime of the soil may also determine which component of the soil is active in sorption reactions. Harris (1966) has stated that while the mineral factor appeared to be primarily responsible for adsorption in the dry state the presence of increasing moisture resulted in a decline in its importance. In contrast, the organic fraction becomes increasingly active at higher moisture levels and under these conditions the toxicity of the insecticide was related to the organic matter rather than the clay.

The presence of water influences decay because at the micro-environment level, it acts as a medium for transport of microbes, substrates, enzymes and products. Water levels may affect the persistence of a pesticide by altering oxygen levels, which will determine the ratios of aerobic to anaerobic activities. Lichtenstein and Schulz (1964) showed that parathion was most persistent in dry soils and least persistent in soils with a high moisture content. Increased microbial activity in the wet soil was suggested as the dominant factor.

Photochemical transformations may occur on or near the soil surface due to the effects of solar radiation. Watkins (1974) states that a variety of products have been identified from the photochemical decay of such pesticides as dieldrin, heptachlor, DDT, carbamates and dithiocarbamates. Shortwave uv light (253.7nm) has been reported to cause degradation of picloram (Bovey, Ketchersid and Merkle 1970), paraquat and diquat (Funderburk, Negi and Lawrence 1966), atrazine and simazine (Comes and Timmons 1965), monuron and diuron (Jordan, Coggins, Day and Clerx 1964; Weldon and Timmons 1961) and many other pesticides. Baur and Bovey (1974) have investigated the effects of both heat and long wave radiation on the losses of urea type compositions of 2,4-D and dicamba in order to identify losses due to degradation and those caused by volatilization. These volatility losses, together with enhanced degradation rates at higher temperatures are indirect effects of sunlight on the dissipation of pesticides.

V.   Cultural Practices

The cultivation of soil, following pesticide application, may stimulate degradation of pesticides due to the increase in microbial activity with aeration. Also any pesticides previously incorporated into the soil may be exposed to the atmosphere, facilitating volatilization and photochemical decomposition. Conversely, cultivations can decrease volatility of pre-emergence herbicides as they become incorporated. Additions of organic matter i.e. manure, may stimulate microbial activity and hasten the decay of pesticides but may also decrease breakdown by inducing anaerobiosis. Additions of certain pesticides may give rise to a long term microbial enrichment, which will, in turn, hasten the decay of subsequent applications of the same chemical (Audus 1964; Kirkland and Fryer 1972).

Finally, uptake and detoxification by crops, or harvesting of contaminated plants may be an important route of pesticide loss from soil.

VI. Conclusions

The processes involved in the disappearance of pesticides from the soil (Fig.2) are complex, being dependent on the influence of physical and chemical properties of the pesticide, soil characteristics, weather conditions and management. All these factors interact, making it difficult to quote the persistence of any pesticide in absolute terms.
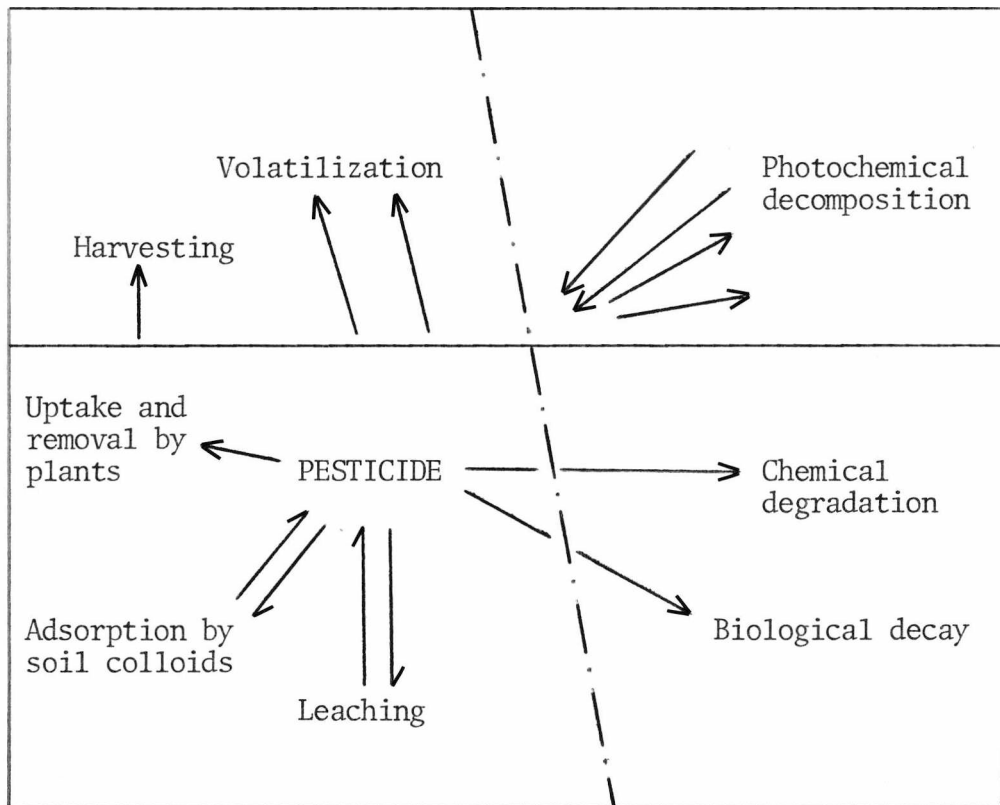
Fig. 2

Processes influencing the behaviour and fate

of pesticides in the environment

B. Factors Affecting 2,4-D,Diallate and Malathion

Loss from Soil

I. 2,4-Dichlorophenoxyacetic acid (2,4-D)

(a) Introduction

The phenoxyalkanoic acids are a large group of herbicides that find extensive use in the selective control of broadleaf weeds. They are commonly used either as parent acids or as their salts and esters.

Their structures consist of an aliphatic acid bonded to a benzene ring by an ether linkage. The best known member of this class of compounds and the one investigated here is 2,4-D {I}.

Cl ─────⟨benzene ring⟩───── O-CH$_2$-COOH   {I}
                    |
                    Cl
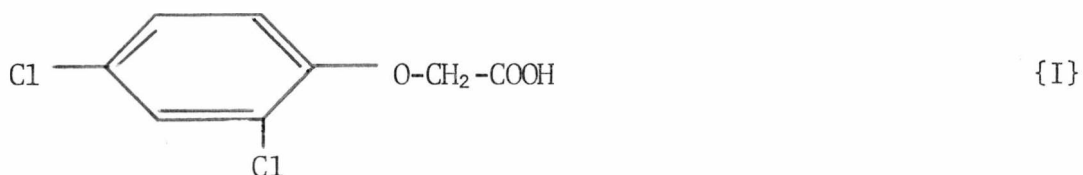
Other herbicides in this series are analogues of 2,4-D with, either chlorine and methyl substitutions on the benzene ring, or aliphatic moieties of different chain lengths. The more common members are listed below:

    2,4-D   - 2,4-Dichlorophenoxyacetic acid

    MCPA   - 2,Methyl-4-chlorophenoxyacetic acid

    2,4,5-T - 2,4,5-Trichlorophenoxyacetic acid

    2,4-DP   - 2-(2,4-Dichlorophenoxy)propionic acid

These compounds are growth regulators, bringing about abnormal growth responses in regions distant from the point of application.

(b) Degradative processes

Early workers recorded correlations between the speed of disappearance of 2,4-D and the occurrence of conditions favourable for bacterial growth. Such conditions were high moisture, temperature and organic matter (Brown and Mitchell 1948; Hernandez and Warren 1949, 1950; Kries 1947; Mitchell and Martin 1946). However, more definite evidence was required to establish breakdown as a purely microbial event.

This evidence was obtained by comparing breakdown in nonsterile soil with that in soil which had been autoclaved (Brown and Mitchell 1948; DeRose and Newman 1948; Audus 1951, 1952). Subsequently, in order to eliminate the destructive effect on soil properties of autoclaving, metabolic blocking agents such as sodium azide were added to soil and shown to prevent breakdown (Audus 1951; Brownbridge 1956). The results of Newman and Thomas (1949), and Audus (1951), also indicated that before degradation of 2,4-D there was a brief lag or microbial enrichment period followed by rapid metabolism of the herbicide. Further evidence which supports the explanation that the proliferation of adapted bacteria is involved in 2,4-D decay is the continuing ability of enriched soil to decompose additional doses of herbicide rapidly and without a further lag (Newman and Thomas 1949; Newman, Thomas and Walker 1952; Audus 1951; Brownbridge 1956). A state of enrichment has been shown to be retained for at least one year in stored, moist soil (Audus 1960).

Direct evidence for the role of bacteria in these detoxification processes was obtained by Audus (1950) who isolated *Bacillus globiforme* and grew it on agar containing 2,4-D as the sole carbon source. This organism was shown to induce on immediate enrichment of soil when a suspension of it was added to an herbicide-containing perfusate being passed through a soil column.

The mechanism by which a soil microbial population develops the capacity to degrade a pesticide is not completely understood. Audus (1960) has suggested that either chance mutation or adaptivity of microbial enzymes occurs; mechanisms which have been discussed at length elsewhere (Audus 1960; Alexander 1965; Kearney, Kaufmann and Sheets 1965).

Similar patterns of decay for other phenoxy herbicides, such as MCPA and 2,4,5-T have been recorded by Audus (1951), who has also observed that MCPA persists somewhat longer than 2,4-D and that 2,4,5-T treated soils may retain their toxicity for as much as nine months. This difference in susceptibility to microbial degradation is to some extent governed by the chemical structure of the herbicide (Alexander 1965).

Lag periods in 2,4-D breakdown vary from a few days to 4 weeks (Audus 1964); half-lives from 4 - 49 days (Newman and Downing 1958; Altom and Stritzke 1973; Foster and McKercher 1973); and the time for total disappearance may be as little as 7 days or as much as 14 weeks (Akamine 1951).

This internal variability may be due to a variety of factors including incubation conditions, which will in turn influence the activity of soil microorganisms, and the considerable variation in experimental technique between workers. In addition, different soils will have varying microbial numbers and species whose ability to degrade 2,4-D will also vary. Considerable variability may also be caused by the formulation of 2,4-D used, be it a salt (Na, K, NH_4 trimethylamine), alkyl ester (methyl, isopropyl, butyl) or a low volatile ester (butoxyethanol, tetrahydrofurfuryl).

Further support, if needed, for microbial involvement comes from the positive correlation between microbial numbers and rate of breakdown of 2,4-D (Foster and McKercher 1973), and there are numerous instances since the first achievement of Audus (1951) in which 2,4-D degrading microbes have been isolated from soil (Loos 1969). Despite this evidence, additional degradative

processes have been invoked to explain the decay of 2,4-D in soil. For example chemical hydrolysis of the esters, such as the iso-propyl, n-butyl and iso-octyl types, to the acid form in both aqueous solutions and in soils, has been reported (Smith 1972; Burcar, Wershaw, Goldberg and Kahn 1967).

The photolysis of halogenated herbicides, including the phenoxyacetic acids, has been reviewed by Plimmer (1970). Bauer and Bovey (1974) showed that 2,4-D formulated as a urea-type "polymer" was readily destroyed by long wave uv (356nm) irradiation, whereas conventional 2,4-D was more resistant. They also suggest that the "polymer" structure acts as a trap for uv photons whose energy is subsequently transferred to several 2,4-D molecules within the polymer. Aly and Faust (1964) investigated the effect of uv irradiation on 2,4-D in natural surface waters in an effort to elucidate the oxidation effects of solar radiation. They discovered that ultraviolet light decomposed 2,4-D esters at a rate dependent upon pH. These workers also conclude that under the experimental conditions used, the energy per photon was considerably greater than that encountered in sunlight. It was concluded that due to the shielding effects of suspended inorganic and organic matter and the lower energy of sunlight in these environments, the decay of 2,4-D by photolysis in natural surface waters would be insignificant.

(c) Adsorption

Adsorption of 2,4-D acid may have a significant influence on both the method and the ultimate rate of its decay. In fact, adsorption may account at least, in part, for some difference in recorded disappearance times.

2,4-D is a weak acid with a pKa of 2.73 (Nelson and Faust 1969), thus in aqueous solution it exists in molecular and ionic forms; the ratio of the two, depending on the pH of the solution. Thus at the pH's frequently

encountered in soil (pH 4.0), it will exist largely in a dissociated anionic state. The low adsorptive capacity of the 2,4-D acid for clays, including negative adsorption, has been reported (Frissel 1961; Weber *et al* 1965; Grover 1971, 1973).

Weber *et al* (1965) looked at 2,4-D adsorption in montmorillonitic and kaolinitic systems. It was apparent that dry montmorillonite and kaolinite adsorbed water in preference to 2,4-D anions, causing a concentration of 2,4-D in the bulk of the solution i.e. negative adsorption was occurring. Bolt and Warkentin (1958) and Frissel (1961), have also studied this phenomenon on clay systems. Positive adsorption at very low pH values, when the herbicides are in molecular form, has been reported (Harris and Warren 1964; Frissel 1961; Frissel and Bolt 1962; Bailey, White and Rothberg 1968; Hamaker *et al* 1966). Weber (1972) states that adsorption of molecular species through physical adsorption is probably a primary adsorptive mechanism, but that adsorption of anionic species has been found to occur with kaolinite and illite clay minerals, and hydrous metallic oxides i.e. those soil constituents that possess some anion exchange properties. Harter and Ahlrichs (1969) showed that 2,4-D was adsorbed onto dry clay by withdrawing the water from the system leaving the chemical behind to adhere to the silicate surfaces. These investigators have also suggested that the pesticide would adsorb to clay particles under field conditions because the chemicals are sprayed onto a dry surface. However, this adsorption would be weak and probably of very short duration, since small additions of moisture would quickly displace any 2,4-D into soil solution.

The adsorption and desorption of 2,4-D has also been investigated onto various resins (Weber *et al* 1965, 1968; Grover and Smith 1974), and has revealed that the compounds were readily adsorbed in large amounts by anion exchange reactions {I}

$$R - COO^- + X - RESIN \rightleftharpoons R - COO - RESIN + X^- \qquad \{I\}$$
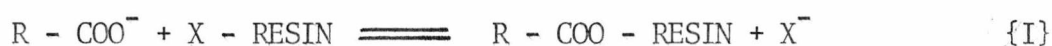
where: $R - COO^-$ = pesticide anion, $X - RESIN$ = anion exchange resin and
X = exchangeable anions such as Cl^-, OH^-, NO_3^- etc. The reaction was shown
to be reversible as 100% of the 2,4-D adsorbed by a strongly basic anion exch-
anger was desorbed with three extractions of 1.0M sodium chloride
solution.

Moderate amounts of 2,4-D have been found adsorbed to charcoal and
organic soil colloids. Grover and Smith (1974) showed that the dimethyl-
amine salt (which behaved after dissociation like the acid form), was
adsorbed to various adsorbents in the order: activated charcoal > anion
exchange resin > peat > cellulose triacetate. Weber (1972) stated that
adsorption to organic matter and charcoal was pH dependent, being greater
under acid conditions where pesticides were adsorbed in the molecular form.
Weber (1968) mentions that acidic pesticides, including 2,4-D, were readily
desorbed from the adsorbents with water, the initial adsorption probably
occurring through hydrogen bonding or weak physical adsorption.

Adsorption of 2,4-D from aqueous solution by organo-clay complexes
has been studied. Miller and Faust (1972), prepared their organo-clay
complexes by treating dimethylbenzyl octadecylammonium chloride and various
benzyl and aliphatic amines with a bentonite clay. Therefore the adsorp-
tive behaviour of these complexes may differ significantly from those
found in soils. Khan (1974) used a fulvic acid-clay complex which appeared
to be similar to the fine clay fraction of some soils (Kodama and
Schnitzer 1971). Their equilibrium data followed the Freundlich type iso-
therm and the relatively low values of the 'isosteric' heat of adsorption
indicated a physical van der Waals adsorption. Haque and Sexton (1968)
produced similar isotherms for the adsorption of 2,4-D onto humic acid.
However, Khan (1974) showed that the adsorption capacity of the fulvic acid-
montmorillonite complex was smaller than that of humic acid (Haque and
Sexton 1968), but greater than those reported for montmorillonite (Haque

and Sexton 1968).

From the various adsorbents studied it becomes obvious that the mobility and persistence of 2,4-D in soils is partly a function of pH and partly due to the nature of the adsorbent. Generally it can be said, (Weber 1972), that acidic pesticides, like 2,4-D, are not readily adsorbed by clay minerals but are adsorbed in limited amounts by organic matter and iron and aluminium hydrous oxides.

(d) Volatilization

Volatility of 2,4-D salts and esters has been investigated as another mechanism of loss from soil. Leonard (1961), reported volatility in the following descending order: isopropyl ester, isooctyl ester, butoxy-ethyl ester, emulsifiable acid and acid crystals. The sodium and alkanol amine salts were non-volatile. Que Hee and Sutherland (1974) looked at the degree and rate of volatilization of the methyl, n-butyl, n-octyl esters of 2,4-D at ambient temperatures, using a kinetic system approaching field conditions. They found that the initial surface area/applied mass ratio, as well as the temperature and type of compound, determined the volatility. Only when the initial surface area/applied mass ratio values were constant did volatility decrease with increasing chain length and decreasing vapour pressure. Similar results were obtained for the much less volatile amine salts. Finally, it was reported by Que Hee and Sutherland (1974), that if vapour drift was a significant drawback in the use of 2,4-D type compounds then it could be essentially eliminated by substituting amine salts for esters. Anderson, Linder and Mitchell (1952) found no loss of 2,4-D acid when the compound was applied to glass slides. Weber (1972) states that the vapour pressures of acidic pesticides in general are very low or negligible.

II. Diallate {S-(2,3-dichloroallyl)diisopropylthiolcarbamate

(a) Introduction

The thiolcarbamate group of herbicides are based on simple derivatives of the thiocarbamic acid molecule {I}:

$$NH_2 - \overset{\overset{\textstyle O}{\|}}{C} - SH \hspace{4cm} \{I\}$$
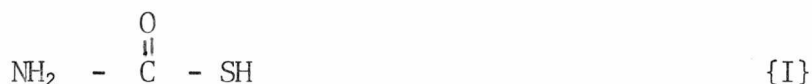
The thiolcarbamate herbicides include butylate, cycloate, EPTC, molinate, pebulate, triallate and vernolate. These have the basic chemical structure shown below {II}:

$$\overset{R_1}{\underset{R_2}{\diagdown}} N - \overset{\overset{\textstyle O}{\|}}{C} - S - R_3 \hspace{3cm} \{II\}$$
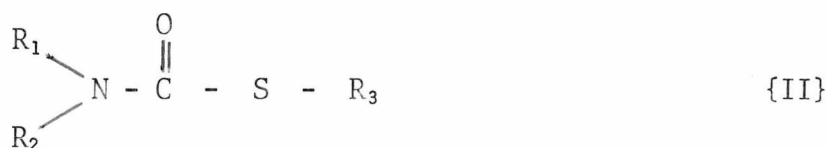
The herbicide investigated in this project is diallate, which consists of cis and trans isomers (Vernetti and Freed 1964; Harman and D'Amico 1967; Onley and Yip 1971; Rummens 1975), and has the following general structure {III} fitting into that shown previously {II}.

$$
\begin{array}{c}
\overset{\textstyle CH_3}{\underset{|}{}} \\
R_1 R_2 = CH_3 - CH \\
\underset{\overset{|}{C}}{Cl} \;\; \underset{\overset{|}{C}}{Cl} \\
R_3 = H - C = C - CH_2
\end{array}
\hspace{3cm} \{III\}
$$
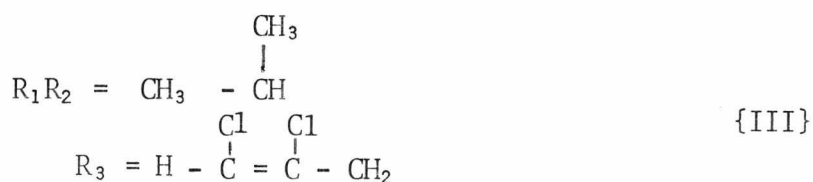
Diallate is a mitotic poison used for the pre-emergence control of wild oats (*Avena fatua*) in various crops (Banting 1970).

(b) Volatilization

In order to extend the residual life in soil and therefore herbicidal activity, diallate must be incorporated immediately after application. This is due to its volatility, a characteristic shown to be a major influ-

ence on the behaviour of many of the thiolcarbamate group (Ashton and Dunster 1961; Ashton and Sheets 1959; Fang 1969; Hauser 1965; Horowitz 1966; Sheets 1959).

Retention of EPTC (Fang et al 1961) and pebulate (Horowitz 1966) was greater on dry soils than wet soils and this is reflected in poorer weed control on moist soils. Sheets et al (1964) state that water films around soil particles either compete with EPTC to prevent adsorption or shield the adsorption sites. Similar observations have been made for CDAA and sulfallate (Deming 1963; Taylorson 1966).

Further evidence for the volatile nature of diallate has been provided by Banting (1967), who suggested that diallate vapour may account, to a large extent, for its phytotoxicity . Smith (1970) looked at volatiliz-ation losses from dry soils incubated at $50 \pm 2^{O}C$, $42 \pm 2^{O}C$ and $22 \pm 2^{O}C$. He concluded that the extent of vapour loss depended on the temperature; neglible loss occurring at $22 \pm 2^{O}C$ over a four week period and the greatest loss at $50 \pm 2^{O}C$ in all soils. The amount of loss varied from different soil types, indicating that additional factors were involved as reported for other thiolcarbamates (Gray and Weierich 1965; Koren, Foy and Ashton 1969).

(c) Adsorption

Correlations between soil organic matter contents and phytotoxicity of diallate (Banting 1967; Koren et al 1968) are indicative of the influence of adsorption. Smith (1970) found that adsorption ranged from 40-80% depending on soil type, there being a correlation between the degree of adsorption and organic matter content. Weber (1972); Gray and Weierich (1968); Koren et al (1969) stated that the adsorption of thiolcarbamates was related to their water solubilities and to soil organic matter and clay.

Since the thiolcarbamate herbicides are non-ionic and their adsorption

has been seen to be dependent on temperature and moisture contents of soils,

the mechanisms involved are probably physical involving dipole-dipole

or ion-dipole interactions (Weber 1972).

(d) Degradative processes

Limited data indicates that soil microorganisms contribute significantly

to the disappearance of thiolcarbamate herbicides in soil (Danielson,

Gentner and Jansen 1961; Kaufman 1966, 1967; MacRae and Alexander 1965;

Banting 1967). Banting (1967) attributed the loss of diallate to microbial

decay. This was indicated by a 6-7 day lag period prior to breakdown in

unsterilized soil compared to negligible loss from autoclaved soil. The

work of Smith (1969a, 1970), suggests that diallate can undergo microbial

degradation in non-sterile soils. Smith (1970) found that the half-life of

diallate varied from 4-8 weeks depending on the moisture status of the soil

i.e. most rapid degradation occurred at a moisture content around wilting

point and slower loss occurred at higher moisture contents. At moisture

levels considerably below the wilting point degradation was significantly

reduced. Smith (1970), also confirmed the work of Banting (1967), in that

only 10% of the applied diallate was lost from sterile soils after six weeks.

Further evidence of microbial involvement in diallate loss from soils has

been provided by Anderson and Domsch (1976). They recorded a loss of 50%

from microbiologically active soils over a 4 week period in contrast to

losses of less than 50% after 20 weeks in sterile soils. Degradation of

diallate by isolated soil microorganisms has been recently reported by

Kaufman and Blake (1973), who found that pure cultures of the fungus

Fusarium oxysporum dehalogenated the compound within a 20 day period.

Smith and Fitzpatrick (1970) compared the degradation rates of five

thiolcarbamates, including diallate, in two soils, to their hydrolysis in

acidic and basic media. Diallate was not degraded in soil as fast as in acidic and basic media, therefore it was concluded that biological break-down was not only related to chemical stability of the compound i.e. ease of hydrolysis at the thiolcarbamate linkage could not be correlated with the rate of biological degradation. However,he mentions that other factors such as adsorption to soil colloids and water solubility of the compound could account for the lack of correlation obtained.

Anderson 1975, attempted to resolve the various routes of diallate dissipation and stated that at low temperatures (less than $22^{O}C$) the major contribution was microbial decay, whilst above $22^{O}C$ volatilization became increasingly important.

III. Malathion O,O-dimethyl-S-(1,2-dicarbethoxyethyl) phosphorodithoate

(a) Introduction

Organophosphorus insecticides have gradually replaced all but the
most important organochlorine compounds (DDT, aldrin and dieldrin).
They are, as a group, less persistent and consequently present a smaller
number of toxic residue problems. Examples of organophosphorus compounds
in common use are malathion, parathion, diazinon, trichlorfon, phorate,
carbophenothion, disulfotan, dimethoate, thionazin, dyfonate and chlor-
fenvinphos. It can be seen from Table 4 that the persistence times for
organophosphorus compounds do vary but that none is very persistent in the
environment. Initial studies on this group of insecticides has followed
considerable work on organochlorines where correlations have been made
between residue times and soil properties such as organic matter and clay
content (Fleming and Maines 1953, 1954; Edwards *et al* 1957, Lichtenstein
et al 1960; Harris 1964, 1966).

Work on organophosphate loss from different soil types is more
limited due to the more recent introduction of these compounds as insect-
icides. Burns (1971) looked at the loss of phosdrin and phorate from a
range of soil types and concluded that more studies would be needed until
clearer relationships between soil type and persistence (as affected by
volatilization, adsorption and microbial activity) could be proposed.
Harris (1966) showed that soil type and moisture were important in the
persistence of diazinon and parathion. Beynon (1966) reported that diethyl
1-(2,4-dichlorophenyl)-2-chlorovinylphosphate was most persistent in peat
and least persistent in a sandy loam. Getzin and Chapman (1959, 1960),
obtained the same result with phosdrin and concluded that organic matter
content was primarily responsible for insecticide binding in the soil.

Table 4

Insecticide	Formula	Persistence		Reference
		% remaining	time (days)	
Malathion	CH_3O—P(=S)(—$CH_2COOC_2H_5$)—S-CH COO_2H_5 with CH_3O	3	1	Getzin and Rosefield (1968)
Parathion	C_2H_5O—P(=S)—O—C_6H_4—NH_2 with C_2H_5O	65	14	Getzin and Rosefield (1968)
Dimethoate	CH_3O—P(=S)—S-$CH_2CONHCH_3$ with CH_3O	23	14	Getzin and Rosefield (1968)
Phorate	C_2H_5O—P(=S)—S-$CH_2SC_2H_5$ with C_2H_5O	0	28	Parker and Dewey (1965)
Diazinon	$(CH_3)_2CH$ pyrimidine ring with CH_3, $OP(OC_2H_5)_2$, S	50	14-28	Getzin and Rosefield (1968)
Chlorfenvinphos	$HCCL$=C—O—P(=O)$(OC_2H_5)_2$ with C_6H_4—CL	50	84	Bro-Rasmussen et al (1970)

Studies on the degradation of chlorfenvinphos (Williams 1973) in mineral

and peat soils demonstrated that greater persistence occurred in the

organic soil. It was suggested that the insecticide was strongly adsorbed

and therefore inaccessible to both microbial decay and leaching.

The insecticide investigated in the work reported here is malathion

0,0-(dimethyl-S-(1,2-dicarbethoxyethyl)phosphorodithioate, having the

structure {I}, being based on the general structure {II}, both being shown

below:

$$
\begin{array}{lll}
\underset{RO}{\overset{O(S)}{\underset{RO}{\overset{\|}{\searrow}}}} \!\!\! P - O - x \quad \{II\} & \quad S = P \overset{\nearrow OCH_3}{\underset{\underset{CH_2COOC_2H_5}{|}}{\searrow}} OCH_3 \quad \{I\} \\
\end{array}
$$
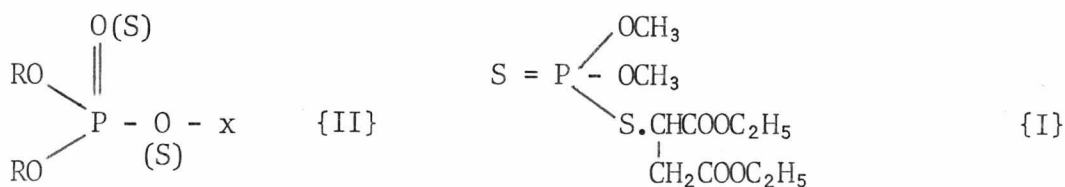
Where R is an alkyl group and x is an organic radical. Four types of

general structure are observed when either oxygen or sulphur is substituted

in the indicated position. This insecticide has been widely used in both

agricultural and domestic contexts, since its introduction in 1950.

(b) Degradation processes

Reports on the mechanism of malathion degradation that have appeared

recently, propose either a chemical or biological mechanism depending on

the system studied.

Konrad *et al* (1969) compared loss from non-sterile and 5 Mrad irradi-

ated soils and suggested that degradation was primarily a non-biological

hydrolytic process, related to adsorption and alkalinity. Spiller (1961)

mentions that malathion is readily hydrolysed in highly alkaline or mildly

acid media; at pH 12 hydrolysis was almost immediate, at pH 9, 50% was

hydrolysed in 12 hours with no hydrolysis occurring over 12 days at pH 5-7.

Weiss and Gakstatter (1965), broadly confirmed these observations with such compounds as parathion, diazinon, guthion as well as malathion. Cowart, Bonner and Epps (1971) stated that hydrolysis was the principle detoxification mechanism for the organophosphate pesticides and demonstrated that hydrolysis of malathion occurred in a slightly acid medium. Faust and Suffet (1966) presented a general review of the hydrolysis of organophosphates in natural water environments. These workers conclude that at pH values higher than 5.0 and at temperatures greater than $20^{o}C$, the dissipation of organophosphorus compounds, including malathion, would occur within a relatively short period of time, the rate of hydrolysis and type of product being dependent on the hydroxyl ion concentration.

It can be seen from these reports that the predominating view is that the rate of hydrolysis increases with increasing alkalanity of the system, although some workers have reported acidic hydrolysis occurring.

Others have proposed that the hydrolytic decay of organophosphates is related to amino acids (Gatterdam, Casida and Strontamire 1959), copper ions (Mortland and Raman 1967) and iron salts (Fleck 1966).

Other workers, studying the breakdown of organophosphates in soils (Konrad *et al* 1967; Getzin and Chapman 1960; Getzin 1973; Menn, McBain, Adelson and Patchett 1965), have indicated that non-biological hydrolysis is a major factor in the degradation of certain organophosphates. Bowman, Adams and Fenton (1970), looked at the stability of malathion on montmorillonite in order to see if the clay was able to catalyse the degradation of malathion. It was found that the interlammelar adsorption of malathion prevented its degradation. In contrast, others have reported that highly sorptive clays, such as montmorillonite, actually promoted degradation to a greater extent than those with low adsorptive capacities, such as kaolinite (Polon and Sawer 1962; Yost, Frederick and Migrdichian 1955).

Konrad *et al* (1969), showed that the rate of malathion degradation in soil was directly related to the extent of adsorption, suggesting that degradation occurred by a chemical mechanism catalysed by adsorption.

Evidence for the involvement of soil microorganisms in the decay of organophosphates is also available (Ahmed and Casida 1958; Lichtenstein and Schulz 1964; Getzin and Rosefield 1968). Konrad *et al* (1969) provided evidence that in aqueous soil-free systems (incubated with a soil extract), a lag phase followed by rapid loss indicated microbial adaptation to malathion. Supportive evidence was provided by Matsumura and Boush (1966), who found that malathion was metabolized by a soil fungus, *Trichoderma viride* and a bacterium *(Pseudomonas* sp*)* isolated from soil, whereas autoclaved soils showed a low capacity to breakdown the insecticide. Tiedje and Alexander (1967), also obtained evidence for microbial degradation of a malathion monoacid, a product initially formed from a non-enzymatic hydrolysis of malathion.

Not surprisingly, both chemical and microbiological mechanisms have been suggested as dominant factors involved in the decay of malathion in soil and aqueous systems. Walker and Stojanovic (1973), attempted to quantify the contribution of each degradative mechanism to total malathion decay by using non-sterile and autoclaved soils, together with aqueous dilutions. They found in all cases that breakdown was more rapid under non-sterile conditions, concluding that loss was mainly microbiological (77-95%), yet depended upon soil type.

Getzin and Rosefield (1968) were concerned that quantitative results may be affected by autoclaving,as a means of sterilization, since this alters the physical and chemical properties of the soil. Therefore, they examined malathion persistence in not only non-sterile and autoclaved soils but also in 4M rad irradiated soils,
/since this form of sterilization causes only minor fluctuations in temper-

ature. They subsequently discovered that decomposition was considerably faster in the non-sterile and irradiated soils, which had similar rates of decay, than in the heat sterilized samples and concluded that a temperature sensitive substance, which accelerated the breakdown of malathion, was present in soil. They were further able to extract from soil a heat labile, water soluble organic substance, which later partial purification and examination (Getzin and Rosefield 1971; Satyanarayana and Getzin 1973) showed to have typical enzymic properties. They were able to extract from non-irradiated and irradiated soils, although, four to five times more enzymic activity was obtained from non-irradiated than from irradiated soils. Getzin and Rosefield (1971) provided a method for partial purification of the substance and presented evidence that it was a stable, cell free soil enzyme which hydrolysed malathion to its monoacid. After partial purification the enzyme was shown to contain protein and was stable, only being denatured at temperatures above $70^{O}C$ and by 24 hour exposures of pH's <2.0 and > 10.0. No loss of activity occurred after extended storage at $4^{O}C$ or $-10^{O}C$. When the partially purified enzyme was applied to soil, activity was detected for the duration of the 8 week experimental period providing further evidence for its stability in soil. Following its application to soil the enzyme was shown to be adsorbed rapidly since its re-extraction was not possible using a neutral buffer. Satyanarayana and Getzin (1973) reported on further purification of a stable extracellular phenyl esterase. The esterase was not susceptible to enzymatic proteolysis nor easily inactivated by metal ions. The characteristic U.V. 280nm adsorption peak for proteins was not present unless the enzyme was first hydrolysed in $6\underline{N}$ HCl or digested with testicular hyaluronidase. This digestion increased the esterase activity almost 2-fold but decreased the stability of the enzyme. On the basis of its chemical composition and response to hyal-

uronidase treatment the enzyme was classified as a glycoenzyme. It was suggested that the properties of this carbohydrate-protein complex may account for its unusually persistent and stable nature in soil as a cell free enzyme. Gibson and Burns (1976) looked at the mechanism of malathion decay in soil and soil components, and suggested that the colloidal organic matter, or the fraction associated with it, contained a catalytic component which was the single most important agent in effecting a rapid decay of malathion in the soil studied. Other mechanisms of decay, i.e. microbial and hydrolytic were suggested as occurring on organic matter free-sand, silt and clay fractions. From their experimental evidence the catalytic component was suggested to be a stable exoenzyme in agreement with Getzin and co-workers.

Reviews clearly indicate the biological significance of enzymes in soil (Skujins 1967; Kiss 1975). Burns, Pukite and McLaren (1972), postulated that at least some soil extracellular enzymes function from an immobilized position within the colloidal organic matter. It is in this location that substrates can diffuse to and products away from the enzyme, whilst the enzyme itself is protected from denaturation. Experiments with urease (Pettit, Smith, Freedman and Burns 1976) have indicated that it may have similar properties to malathion esterase, at least in relation to its location in soil, persistence and resistence to gamma irradiation. Burns (1975) suggested that additional extracellular urease unassociated with soil colloids can occur in addition to the normal intracellular enzyme.

Cawse (1969), and Kiss (1975) showed that the activity of many enzymes persist after sterilization of the soil by gamma irradiation, which may again be due to the protected position of any extracellular bound enzyme in the organo-mineral complex.

(c) Adsorption.

Harris (1969) ranks the organophosphates along with the chlorinated

hydrocarbons as having high adsorption and low mobility in soil systems. Adsorption of organophosphates has been related to both the organic matter and clay contents of soils (Harris 1966; Konrad *et al.* 1969; Swoboda and Thomas 1968; Whitney 1967; Graham-Bryce 1967; Getzin and Chapman 1959, 1960). Macnamara and Toth (1970) noted that; (1) adsorption of malathion was related to the cation exchange capacity of clays, (2) humic acid adsorbed more malathion than the clays and (3) that malathion adsorption was higher in soils with increasing organic matter. Berigari (1967), studied the adsorption of malathion in aqueous solutions of montmorillonite and obtained evidence that malathion adsorption was via the carboxyl group. Bowman *et al.* (1970), found that adsorption occurred by way of hydrogen bonding between the carbonyl oxygen atoms of the malathion molecule and the water of hydration shells surrounding metallic cations on the clay surface. Adsorption in dehydrated systems occurred through a direct ion-dipole interaction involving the carboxyl oxygen atoms of malathion and the saturating cations on the clay.

This adsorptive behaviour of malathion is important in relation to persistence, since adsorption may retard breakdown (Bowman, Adams and Fenton 1970) or promote it (Polan and Sawyer 1962; Yost, Frederick and Migrdichian 1965).

(d) Volatilization.

Adsorption of organophosphates by soils has been shown to greatly reduce their volatilization (Bertagna 1959; Whitney 1967; Getzin and Chapman (1960). Lichtenstein and Schulz (1964) however, found no evidence of volatilization of parathion in confirmation with Harris and Lichenstein (1961). Getzin (1958) found that within one hour of phorate treatments, 18 - 25% of insecticide was lost depending on soil type, by volatilization. Harris and Lichenstein (1961) however, discovered no such relationship with malathion.

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MATERIALS AND METHODS

I.   Soil Characterisation.

A Hamble series silt loam was used for the majority of the basic experiments described herein.   In addition, soils of the Winchester, Barming and Hothfield series were used in the later persistence experiments.   All soils were collected from the surface 20cm, an additional sample being taken from the Winchester series soil at a depth of 50-60cm.

Table 5

Soil properties

Soil type	Series	pH	% Carbon	% Nitrogen	% Clay	% Sand	% Silt	WHC[3]	CEC[4]
Silt loam	Hamble	5.4	2.20	0.19	20.0	16	64	0.65	14.9
Clay	Winchester[1]	6.5	3.81	0.36	55.0	33	12	0.76	N.D.
Clay	Winchester[2]	6.5	0.78	0.00	65.0	30	5	0.59	N.D.
Sandy clay loam	Barming	6.8	1.00	0.00	23.0	62	15	0.41	N.D.
Sandy loam	Hothfield	3.8	1.72	0.00	11.0	79	10	0.41	N.D.

[1] 0-20 cm   [2] 50-60cm   [3] $ml.g^{-1}$ [4] $meq.100g^{-1}$

Soils were air dried, passed through a 2mm sieve and stored in screw top jars at $20^{o}C \pm 2$.   Their properties are described in Table 5 and the methods used for analysis outlined below.

1. Mechanical analysis.

Texture was determined by sedimentation, using a hydrometer (Bouyoucos 1927). For dispersion into primary particles, 50g of oven dried soil was homogenised with 50ml of sodium hexametaphosphate for 5 minutes. The contents of the blender were transferred to a sedimentation cylinder which was then filled to its lower mark. The temperature of the suspension was noted and the contents resuspended by turning the cylinder, base over apex, approximately 4 times. After 30 seconds the hydrometer was inserted into the cylinder and a reading taken 10 seconds later (40 seconds). The hydrometer was then removed. After 2 hours (2 hours 40 seconds) a second hydrometer and temperature reading were taken. For each degree above $20^{o}C$, 0.36 was added and 0.36 subtracted for each degree below.

2. Percentage carbon and nitrogen.

The percentage of carbon and nitrogen in an air dry sample of soil was determined using an automatic analyser (Hewlett Packard F and M 185).

3. pH.

pH was determined using a glass-calomel electrode (Pye Model 292) in a soil:distilled water slurry of 1:2.5. The slurry was allowed to equilibriate for 15 minutes prior to measurement.

4. Water holding capacity.

The water holding capacity of the soil was measured using a Hilgard cup (Pramer and Schmidt 1964). A circle of filter paper was moistened and placed in the cup, which was then weighed. The cup was three-quarters filled and the oven-dried soil compacted by dropping the cup from a height of one inch. The cup was re-weighed and then placed in a beaker containing water

to a depth that enabled saturation to occur via capillary action. The cup was removed from the water after saturation, all the gravitational water allowed to drain, and re-weighed.

5. Cation exchange capacity.

In order to measure the cation exchange capacity of soil, the indigenous cations are replaced by a cation not normally present to any appreciable extent. In this method ammonium (in ammonium acetate) was used as the replacing ion. Ammonium acetate has the advantage of being strongly buffered at pH 7.0 and the adsorbed ammonium is readily determined. The full details of the cation exchange capacity (c.e.c.) measurement (Wahhab and Ahmed 1956) are detailed below.

10g of air-dried soil, together with 250mls of neutral 1N ammonium acetate solution, were placed in a 500ml conical flask. The flask was then shaken for 5 minutes on a shaker and allowed to stand overnight. The soil was filtered through a Buchner funnel, using mild suction and subsequently leached with 1N ammonium acetate applied in several increments, allowing each successive addition to drain thoroughly before adding the next. When about 500ml of the ammonium acetate solution had passed through, the leachate was transferred to another beaker, the next 10ml collected in a test tube and tested for calcium. This was achieved by adding a few drops of 1N ammonium chloride, 10% ammonium acetate and concentrated ammonium hydroxide to the tube and heating. The presence of calcium is indicated by a white precipitate of calcium oxalate. This first stage of the leaching process was continued until the leachate was shown to be free of calcium.

The soil was then leached four times with neutral 1N ammonium chloride and once with 0.25N ammonium chloride. The electrolyte was then washed out with 150-200mls of 99% isopropyl alcohol (Fisons A.R.). When the test for chloride, the presence of which produces a white precipitate

on addition of dilute silver nitrate solution, indicated no chloride to be present in the leachate, the soil was allowed to drain thoroughly.

The soil was quantitatively transferred, using about 300mls of distilled water, into a 1 litre flask, which was then attached to a distillation apparatus. A 250 ml conical receiving flask containing 50 ml of 0.1N sulphuric acid and a drop of methyl red indicator was placed under the delivery tube, such that the tube dipped below the surface of the acid. Then 3-4g of freshly ignited magnesium oxide was added to the distillation flask, together with a small quantity of anti-bump granules. After about 20 minutes of steady boiling, the outside of the delivery tube was rinsed with distilled water and the distillate tested for ammonia with red litmus paper. Heating was continued until all the ammonia had distilled over.

The excess acid was titrated against 0.1N sodium hydroxide. The number of mls of 0.1N sulphuric acid neutralized by the ammonia distilling over was thus calculated, and from this the cation exchange capacity (in milligram equivalents.100g soil^{-1} of the soil) was determined.

II. Separation of Soil Components.

The Hamble series silt loam soil was used as a starting material.

1. Sand, silt and organo-mineral complex.

50g samples of soil, suspended in 250 mls of water, were subjected to ultrasonication (6μ amplitude, M.S.E. Ultrasonic disintegrator) for 15 minutes. The suspension was cooled in an ice bath throughout the treatment. This method was used in preference to the more normal method of dispersion into primary particles using sodium hexametaphosphate, in order to minimize any biological changes caused by excess inorganic phosphate.

The sonicated material was re-suspended in distilled water in a 1 litre

sedimentation cylinder. The settling times used for separating the components were those of Tinsley (1970). After 1 min 26 secs (the time for the separation of sand from silt and organo-mineral complex at $23^{\circ}C$) the top 20cm of the supernatant was siphoned off. This procedure was then repeated, i.e. the sedimentation cylinder was re-filled with water, the contents re-suspended and after 1 min 26 secs the top 20cm of the supernatant siphoned off. The sand fraction ($50-2000\mu$) was thoroughly washed to remove any plant debris and subsequently air dried. The silt and organo-mineral fractions were transferred to a 10 litre container and left to settle for 14 hours 52 minutes. After this period had elapsed the colloidal supernatant ($<2\mu$) was siphoned off from the top 20cm. This procedure of sedimentation followed by siphoning was repeated until the supernatant was clear. The sedimented silt ($2-50\mu$) fraction was air dried. The organo-mineral fraction (Greenland 1965) was removed from the supernatant by continuous centrifugation at 1500 rev.min^{-1} (Alfa Laval Separator Lab 102B. 20), and dried and ground prior to use.

2. <u>Clay</u> (free of organic matter).

Clay, free of organic matter, was prepared from the organo-mineral complex using sodium hypobromite as the oxidant (Brewer 1964). This process was chosen in preference to organic matter removal by hydrogen peroxide since the latter causes more drastic structural damage to the clay (Brewer 1964).

10 mls of bromine (Fisons, S.L.R.) was dissolved in 400 mls of 0.9N sodium hydroxide. 100g of the organo-mineral fraction was added to this solution and the mixture left for 2 hours. 410 ml of the sodium hydroxide/bromine reagent was then added and the suspension left to stand for a further 14 hours. After this period the sodium hypobromite solution, now

containing the organic matter, was decanted off. The residual clay
fraction (pH 10.1) was transferred to 28mm diameter visking size dialysis
tubing (Medicell Int.Ltd.) and dialysed against running tap water for 5
days. This reduced the pH to a constant figure of 7.4. The clay was
sedimented by centrifuging at 21,000g for 30 minutes (M.S.E.18), dried
at room temperature ($20^{o}C \pm 2$) and ground before use.

In all, seven oxidizing cycles were found to be necessary to reduce
the nitrogen content to zero and the carbon content to a constant figure
of less than 8% of that in the starting material.   (Table 6).

TABLE   6.

Successive removal of carbon and nitrogen using sodium hypobromite.

Number of treatments	% carbon after treatment	% nitrogen after treatment	carbon remaining as % of that in original sample
0	7.58	0.82	100
1	6.38	0.66	84.17
3	4.62	0.51	60.95
4	2.48	zero	32.72
5	1.47	zero	19.40
6	0.59	zero	7.78
7	0.57	zero	7.52

3. <u>Organic matter fraction.</u>

The organic matter extraction described previously by Brewer(1964) was consider-ed far too harsh to produce a biologically and chemically undisturbed fraction. Therefore an extension of the method of Burns, Pukite and McLaren (1972) was employed.

25g of Hamble soil was added to 250mls of distilled water contained in an ice-cooled beaker. The resultant suspension was sonicated for 15 minutes at 6μ amplitude (M.S.E. Ultrasonic disintegrator). Sodium citrate (69.85g), sodium dihydrogen orthophosphate (1.95g), glycine (0.92g) and sodium chloride (29.25g) were added, the former two components acting as humic acid extractants and buffers, the sodium chloride and glycine as stabilizing agents for proteins. Following this the pH was adjusted to 6.3. The toluene additions and bacteriological filtering steps, as described in the original method, were omitted so as to retain the microbial population of the extract. The resultant dispersed soil was agitated at 4°C for 2 hours prior to centrifugation (21,000g for 30 min). The supernatant was decanted off and retained.

The remaining pellets of soil were resuspended in 250mls of water and re-sonicated. Sodium dihydrogen orthophosphate (9.75g) and glycine (0.19g) were added and the pH was again adjusted to 6.3. The suspension was shaken at 4°C for 30 minutes, centrifuged and the resulting supernatant combined with the first one. The soil pellet was subjected to a third extraction cycle (adding 1.95g of sodium dihydrogen orthophosphate and 0.19g glycine) and the supernatant combined with the previous two extracts.

The pooled extracts were concentrated and the salts/removed using a 300ml ultra-filtration cell (Chemlab) containing a membrane filter (Amicon Diaflow PM30). The organic matter extract was then freeze dried and stored in a vacuum-sealed dessicator.

III. Soil Component Characterization.

The components, sand, silt, clay, organo-mineral complex and organic matter and their properties are listed in Table 7.

TABLE 7

Soil component properties

	% Carbon	% Nitrogen	pH	CEC (meq.$100g^{-1}$)	WHC (ml g^{-1})
Sand	0.17	0	5.4	1.76	0.29
Silt	0.71	0	3.7	2.12	0.31
Clay	0.59	0	7.4	133.20	0.95
Organo-mineral	7.58	0.82	5.8	62.60	1.01
Organic matter	16.70	1.24	6.8	329.00	-

Methods of characterization of the soil components were similar to those used for the parent soil. The one exception to this being the measurement of organic matter cation exchange capacity. The method of Wahhab and Ahmed (1956) was unsuitable since the soluble nature and limited amounts of organic matter made the leaching stage difficult. Instead the exchange capacity was estimated by determining the active hydrogen and total acidity of the extract. This method of functional group analysis is based on that of Schnitzer and Khan (1972) and is as follows. To 50g of organic matter extract in a 125ml ground glass stoppered Erylynmeyer flask, 20ml of 0.20N barium hydroxide was added. A blank was set up simultaneously, consisting of 20ml of 0.20N barium

hydroxide only. The air was displaced in each flask using nitrogen, the flasks stoppered and the system shaken for 24 hours at room temperature ($20^{\circ}C \pm 2$). Following this the suspension was filtered, the residue washed thoroughly with CO_2-free distilled water, and the combined filtrates titrated potentiometrically (glass calomel electrode) with standard 0.5N hydrochloric acid (end point:pH 8.4). The results were calculated as shown below:

$$\frac{\text{(titre for blank-titre for sample)} \times \text{N acid} \times 1000}{\text{Weight of sample in mg}}$$

$$= \text{meq. total acidity. g humic preparation}^{-1}.$$

From this the meq. $100g^{-1}$ organic matter was calculated so as to be comparable with other soil components.

IV. Estimation of Microbial Activity.

Numbers were estimated from soil dilutions on nutrient agar plates, incubated for 3 days at $25^{\circ}C$, and are shown in Table 8.

There was no growth on the clay fraction dilution plates after 3 days incubation but after a further 7 days a few bacterial colonies appeared, which were suspected to be contaminants.

V. Persistence Experiments.

1. Pesticides.

2,4-D (2,4-dichlorophenoxyacetic acid), 95% purity, was supplied by Shell Research Ltd; diallate {S-(2,3-dichloroallyl)diisopropylthiocarbamate}, 95% purity, by Monsanto, and malathion {0-0-dimethyl S-(1,2-dicarboxyethyl) phosphorodithioate}, 95% purity, by Cyanamid

2. Pesticide application.

TABLE 8

Microbial numbers

Soil or soil component	Microorganisms g soil^{-1} (x 10^{-3})
Hamble	84.6
Sand	1.30
Silt	40.6
Clay	-
Organo-mineral	15700
Organic matter	98.6
Winchester	152
Winchester (50-60cm)	32.6
Barming	209
Hothfield	68.6

(i) 2,4-D.

2,4-D was dissolved in water to provide a solution of 50ppm. Table 5 shows that 0.65ml of water was required to bring 1g of Hamble soil to 100% water holding capacity (w.h.c.) and from this it was calculated that 1.56mls of water was needed to bring 2.4g of Hamble soil to 100% w.h.c. In order to avoid application via organic solvents it was calculated from the above data that 1ml of 2,4-D solution ($47.5\mu g$ ml^{-1} a.i.) would be required to bring 2.4g of soil to 65% w.h.c. This produced 19.8 ppm ai w/w in our soil contained in a closed 100ml Erylynmeyer flask. Each flask containing 2.4g was extracted directly to avoid any inaccuracies that might occur when removing samples from a large volume of soil in such experiments.

For experiments using 2.4g of sedimented soil fractions, artificial and test soils, $47.5\mu g$ ai of 2,4-D were added. The herbicide was dissolved in either 1.0ml (50ppm) or 0.4ml (125ppm) of distilled water depending on the quantity of water required to bring the respective 2.4g sample to 65% w.h.c. The amounts of water required were calculated from data on w.h.c. (mlg^{-1}) in Tables 5 and 7, and are shown in Table 9.

Persistence experiments with the extracted organic matter used only 30mg per sample; a figure determined by the number of experiments and the difficulty in extracting large quantities of organic matter from soil. Preliminary work (N.M. Pettit - personal communication) has shown that 25g of Hamble soil yields approximately 1.8g of organic matter (freeze dried weight) using the extraction procedure previously described (Burns *et al.* 1972). 1ml of 2,4-D ($46.5\mu g$ ml^{-1} a.i.) was added to a 100ml Erylynmeyer flask followed by 30mg of organic matter in 9mls of distilled water. The resulting solution contained 30mg of organic matter in 10mls of distilled water (3000ppm of organic matter) and $47.5\mu g$ a.i. of 2.4-D (4.75 ppm).

When 2,4-D was added to the sterile soil and its components, filter

TABLE 9

Data on water and pesticide additions to soils or soil components

Soil or soil component	mls of 2,4-D solution added containing 47.5 μg ai	Distilled water added after additions of 2,4-D solution to bring to 65% WHC	mls of water required to bring 2.4 g sample to 65% WHC
Hamble	1.0	-	1.0
Sand	0.4	0.30	0.7
Silt	0.4	0.34	0.74
Clay	1.0	1.28	0.48
Organo-mineral	1.0	1.42	0.59
Artificial soils			
(a) Organo-mineral:sand			
(3:1) 1.8g : 0.6g	1.0	0.29	1.29
(1:1) 1.2g : 1.2g	1.0	-	1.0
(1:3) 0.6g : 1.8g	0.4	0.33	0.73
(b) Organo-mineral:silt			
(3:1) 1.8g : 0.6g	1.0	0.31	1.31
(1:1) 1.2g : 1.2g	1.0	-	1.00
(1:3) 0.6g : 1.8g	0.4	0.36	0.76
Winchester	1.0	0.19	1.19
Winchester (50-60cm)	0.4	0.52	0.92
Barming	0.4	0.24	0.64
Hothfield	0.4	0.24	0.64

sterilized herbicide was used and amendments were made aseptically in an innoculation cabinet (Microflow). Samples were raised to and maintained at 65% w.h.c. by the addition of sterile distilled water in both sterile and non-sterile experiments.

(ii) Malathion and diallate.

50µg quantities, containing 47.5µg a.i. of malathion and diallate, were applied in 0.5ml of acetone (Fisons D.D.S.) to 2.4g of soil, sand, silt, organo-mineral complex, clay or artificial soil contained in 100ml Erylynmeyer flasks. The final concentation of malathion was 19.5 ppm a.i. and of diallate 19.8 ppm a.i. The solvent was allowed to evaporate for 30 minutes, the flask contents homogenised, brought to their respective 65% w.h.c. and the flask plugged. Samples were maintained at this level of hydration throughout the experiments by periodic additions of sterile distilled water (aseptic additions in the case of sterile soils and soil fractions).

In experiments involving the organic matter the solvent was allowed to evaporate as described previously, and 30mgs of organic matter in 10mls of water added to the pesticide residue. Preliminary experiments had indicated that the malathion and diallate were not irreversably adsorbed onto the glass surface of the flask and were in fact transferred to the colloidal suspension upon its addition. This method of pesticide addition to the organic matter was considered desirable in view of the slowness and difficulty in detecting acetone evaporation from an aqueous system. Negligible volatilization of the pesticides occurred during solvent evaporation.

All treatments, for the three pesticides, were run in duplicate and incubated at 25°C either stationary or on an orbital shaker, in the case of the colloidal organic matter suspension.

3. Sterilization procedures.

Sterilization procedures which were identical for all three pesticides, included autoclaving, gamma irradiation, filtering and addition of sodium azide. Autoclaving involved 3 x 45 minute sessions (24 hour intervals between each) at 15 p.s.i. and 121°C. Gamma irradiation was used as a sterilizing agent for the complete soil at two levels (2.5 and 5.0 Mrads) for 2,4-D and diallate experiments and at five levels (2.5, 5.0, 10.0, 15.0 and 20.0 Mrads) for malathion experiments. The organic matter extract was filter sterilized using a 0.45μ membrane filter (Oxoid Nuflow) in addition to being autoclaved. Finally, sodium azide (1000ppm) was added to the complete soil to act as a partial bacteriostatic agent. In the case of 2,4-D, 1ml of sodium azide (containing 47.5μg ml^{-1} a.i.) was added to the soil to bring it to 65% w.h.c. at the start of the experiment. In malathion and diallate experiments 1ml of 1000ppm sodium azide solution was added to the soil after evaporation of the pesticide/acetone solution. Thereafter 0.3mls of sodium azide was added every two days to act both as a bacteriostatic agent and to maintain the soil at 65% w.h.c.

Autoclaving, irradiation and filter sterilization proved to be 100% effective in removing viable microbial cells(as shown by subsequent dilution plates). Soils and soil fractions also maintained their sterility throughout the experimental periods.

The effect of sodium azide was not directly observable on dilution plates because inhibited soil microbes would grow into sodium azide-free nutrient agar. However, sodium azide was shown to be an effective biostatic agent by incorporating it into agar at 0.1% concentration. Untreated soil innoculum failed to produce any microbial growth on these amended agar plates. The inhibiting effects of sodium azide have been confirmed by Skipper and Westerman (1973).

4. Extraction of pesticides.

(i) 2,4-D.

Foster and McKercher (1973) investigated the extraction of ^{14}C-labelled 2,4-D to determine the effect of different acidified solvents. Acidification was effected by 0.675N phosphoric acid. They concluded that diethyl ether and benzene:2-propanol (2:1) were the most efficient extractants, recoveryrates being 86 ± 6 .0 and 86 ± 2 .0% respectively. Purkayastha (1974) used an alkali-chloroform extraction mixture, whilst Gutenmann *et al.* (1964) employed a method based on acetone-phosphoric acid for 2,4-D extraction from soil. Earlier reports, Rooney (1947), Warshowsky and Schantz (1950) and Woodham *et al.* (1971), suggested the use of diethyl ether-acid extractions. Woodham *et al.* (1971) reported that recoveries ranged from 84.3 - 102.6%. Purkayastha (1974) achieved recoveries (for the methyl ester) of 86 and 110% from two soils containing low levels of herbicide (0.05 - 0.4ppm). Grover (1973) reported recoveries in the 95 - 100% range.

It was decided to adopt an acid-ether method based on that of Grover (1973). Soils, soil components and artificial soils were agitated on a flask shaker for 1.5 hours in the presence of 4mls of 3M sulphuric acid and 30mls of diethyl ether (Fisons A.R.). The solvent was decanted into a separating funnel. Subsequent washings of the remaining soil were also added to those in the separating funnel via a fluted filter paper to avoid any contamination with soil. The extract was washed by shaking twice with 10ml aliquots of distilled water. The ether phase was then dried by passing it through anhydrous sodium sulphate. Finally, the solvent was reduced almost to dryness in a stream of nitrogen using a water bath at 50°C

Esterification was the final stage before measurement of the free acid by gas chromatography. Erickson and Hield (1962), Gutnick and Zweig

(1964), Howard and Yip (1971), Purkayastha (1974), Khan (1975) and many others have used diazomethane for the methylation of chlorophenoxy acids and have shown this method to be both rapid and reliable. Since a rapid methylation procedure was imperative because of the large number of samples involved, this method was adopted in preference to refluxing 2,4-D with BCl_3 or BF_3/alcohol mixtures (Horner, Que Hee and Sutherland 1974; Gutenmann and Lisk 1964; Woodham *et al*. 1971).

Diazomethane/ether solution for methylation was prepared (de Boer and Backer 1954) as follows: 25ml of ethanol (95%) was added to a solution of KOH (5g) in water (8ml) contained in a 100ml distillation flask attached to a dropping funnel and condenser. The condenser was connected to two receiving flasks in series, the second of which contained 20-30mls of diethyl ether (Fisons A.R.). The inlet tube of the second receiver dipped below the surface of the ether and both flasks were ice-cooled. The flask containing the alkali solution was heated in a water bath to $65^{\circ}C$ and 21.5g (0.1M)N-methyl-N-nitroso-p-toluenesulfonamide (Diazald(R)) in about 200mls of ether was added through the dropping funnel over a period of 30 minutes, such that the rate of addition was equivalent to the rate of distillation. When the dropping funnel was empty, another 40ml of ether was added slowly and the distillation continued until the distillate was colourless. The combined distillates were carefully transferred to a stoppered 500ml bottle and stored at $4^{\circ}C$. All joints in the distillation apparatus were teflon lined to avoid the risk of explosion.

Samples of 2,4-D were methylated with a few drops of methanol plus 0.5mls of diazomethane/ether solution for 10 minutes. The resulting solution was evaporated, as described on page 53 , and made up to 10mls with dried, distilled methanol (Fisons DDS). All methylated samples were stored at $-22^{\circ}C$ prior to analysis.

Preliminary experiments showed recovery rates were of the order 87 ± 6% for all the soils and the soil inorganic components and 95 ± 4% for the organic matter.

(ii) Diallate

Detailed procedures for thiolcarbamate extraction from soil have been published by other workers. McKone and Hance(1967) reported that 2,2,4-trimethylpentane and isopropyl alcohol gave extraction rates in excess of 90%. Smith (1969), used a mixture of benzene and isopropanol for diallate extraction and obtained recoveries ranging from 86 - 97% depending on soil type. Acetonitrile : hexane extractants have been reported as giving recoveries in excess of 90% (A.E. Smith - personal communication).

TABLE 10

Percentage recovery of diallate

Solvent mixture	Ratio	% Recovery
Acetone : hexane	1 : 2.5	90
Isopropanol : benzene	1 : 2	83
Isopropanol : trimethylpentane	1 : 2	80
Aqueous acetonitrile : hexane	-	75

A comparison of extraction rates was carried out to determine the method providing best recovery rates under our conditions. An acetone : hexane mixture (as used in the extraction of malathion) was also investigated to determine its effectiveness as a possible extractant.

It was finally decided to use the mixture of acetone and hexane since this gave highest recovery rates (Table 10). The extraction of diallate was identical to that described for malathion and will be des-scribed under this heading. Recovery rates were subsequently found to be 90 ± 5% for all soils and soil inorganic components and 99 ± 1% for the organic matter.

(iii) Malathion.

Walker and Stojanovic (1973) used a 1:1 acetone:hexane mixture to extract malathion from soil whilst we have based our method on that used by Getzin and Rosefield (1968) who employed a 1:2 acetone:hexane mixture.

Preliminary tests involved shaking 10ml of dried acetone (Fisons DDS) with three samples of malathion containing soil for 15 minutes. Subsequent-ly 25ml aliquots of hexane (Fisons Distol) were added to each of the three samples and the resultant mixtures shaken either for one, two or three hours. After each period the solvent mixture and subsequent washings were decanted into a separating funnel. There was no requirement for filtering since the soil remained in the flask when washed with hexane. The hexane phase was then passed through anhydrous sodium sulphate to remove any water. The resulting samples were placed in a $50^{\circ}C$ waterbath and reduced almost to dryness in a stream of nitrogen. Finally, the extract was made up to 10ml with hexane and stored at $-22^{\circ}C$ prior to analysis.

It was noted that no increase in extraction of malathion occurred if a greater than one hour shaking period was used. Re-extraction of soil, after decanting off the solvent mixture, only produced a further 2% mal-athion and was considered unnecessary. In order to determine volatility losses some samples were evaporated to dryness, as described above, whilst others were reduced to a low volume. These losses were shown to be minimal

in both instances. Negligible volatilization was also observed on addition of the pesticide/acetone solution to an Erylynmeyer flask and subsequent evaporation of the solvent. These preliminary experiments were performed with diallate as well as malathion. The times adopted for the extraction of diallate and malathion were 15minutes shaking with 10ml of acetone (Fisons D.D.S.) and 1 hour shaking after the addition of hexane (Fisons Distol).

Recovery rates for malathion were of the order $91 \pm 5\%$ from the soil and inorganic components and $99 \pm 1\%$ from the organic matter.

5.   Analysis of pesticides.

Quantitative determination of the three pesticides was achieved using a Pye 104 gas chromatograph. Parameters for the assay of individual pesticides are described in Table 11.

A standard curve of peak height (in the linear response region) vs. concentration was prepared, the concentration of the standard dilutions ranging from $0.2 - 1\,ng.ul^{-1}$ for all pesticide standards. All samples were diluted to fall within this standard curve. The column used for diallate determinations did not resolve the cis and trans isomers of diallate.

6.   Effects of temperature on persistence.

Persistence times for the three pesticides were also studied at $4^{o}C$ and $37^{o}C$ for the Hamble soil under non-sterile conditions. All procedures for analysis were as described previously for experiments at $25^{o}C$.

7.   Artificial soils.

Artificial soils (2.4g) were composed of varying proportions of either sand or silt with the organo-mineral complex. For 2,4-D experiments these

Table 11.

Gas chromatographic analysis of 2,4-D, diallate and Malathion

Pesticide	Detector	Column	Carrier gas (N_2)*	H_2*	Air*	Column temp	Detector temp
2,4-D	Electron capture	1.5m x 4mm 3% SE 30 on 100-200 mesh diatomite CQ	65	-	-	200°C	300°C
Diallate	Electron capture	1.5m x 2mm 5% DC 200 on 70-80 mesh diatomite C-AW	65	-	-	190°C	300°C
Malathion	Thermionic	1.5m x 2mm 5% DC 200 on 70-80 mesh diatomite C-AW	27	19	150	210°C	250°C

* Gas flow rates expressed in ml min^{-1}

proportions were 100:0, 3:1, 1:1, 1:3 and 0:100. With diallate and mal-athion they were 100:0, 19:1, 3:1, 1:1 and 0:100. All experiments were carried out under non-sterile conditions at 25°C.

8. **Diallate loss from glass surfaces, autoclaved sand and organic matter.**

Loss of diallate was monitored from an open Erylynmeyer flask. The diallate was added as described previously (page 51) and the acetone all-owed to evaporate prior to incubating the flasks at 25°C. Herbicide loss from a closed system involving the use of a glass Bijou bottle (12ml capacity) with a screw cap was also determined at 25°C. Similar experiments to determine loss of diallate from autoclaved sand and organic matter were performed using closed systems.

Diallate was extracted from the open Erylynmeyer flask using 10mls of hexane. The hexane was then decanted, together with subsequent washings (5ml) into a storage bottle, evaporated almost to dryness and made up to volume prior to storage at -22°C.

For extraction from the soil components the contents of the Bijou were transferred (using a Pasteur pipette) to an Erylynmeyer flask using 10ml of acetone. 25ml of hexane was added and the extraction performed as des-cribed previously for malathion.

VI. **Adsorption Experiments.**

1. **2,4-D.**

Adsorption was measured from aqueous slurries of soil over a range of concentrations. 1g of soil was equilibrated for 24 hours with 10mls of water containing the pesticide. The range of concentrations used was 10, 20, 30, 40 and 50 ppm of 2,4-D. During incubation the tubes were shaken gently at 25°C to keep the soil in suspension.

After shaking, the soil was separated by centrifuging (21,000g for 15 minutes) and 5ml of the supernatant removed for extraction and analysis. Standard curves were drawn over the linear range of the detector and triplicate samples diluted accordingly. Simultaneous extraction of 2,4-D was carried out from control solutions containing no adsorbent. Extraction rates were 97 $\pm$ 2%.

2. Diallate.

(i) Preliminary studies.

A saturated aqueous solution of diallate was prepared as a standard dilution. However, subsequent extractions of diallate from 10ml aliquots of this solution to determine its concentration produced extremely variable results, suggesting that some undissolved diallate was present in solution. As a result the aqueous suspension was filtered through a 0.23μ filter (Millipore) which effectively adsorbed all the diallate. Finally, the saturated solution was passed through a 0.23μ filter (Millipore) previously saturated with diallate. The resulting diallate solution was of a constant concentration (3.6 ppm) and produced reproducible extraction rates.

(ii) Adsorption studies.

Samples of air dried soils (1g) were equilibrated for 24 hours at 25°C in glass, stoppered, tubes containing 10mls of aqueous solutions with 7.2, 13.8, 23.2, 29.4 and 36.0μg of diallate. All controls and treatments were run in triplicate. The glass tubes were treated with a water repellent (2% solution of dimethyldichlorosilane in carbon tetrachloride) to prevent diallate adsorption to their surfaces. After centrifugation (21,000g) 5mls of the clear supernatant was removed from each tube and extracted twice with 10ml of hexane (Fisons DDS). Details of extraction and analysis have already been outlined.

3. Malathion.

Adsorption of malathion to soil was measured using a 300ml ultra-filtration cell (Chemlab) containing a membrane filter (Amicon Diaflow PM 30). The method followed was essentially that of Grice and Hayes (1972). 99ml of distilled water was placed in the cell, the outlet sealed and the stirrer turned on. 1ml of 100ppm malathion in acetone and 1g of soil were then added. At 1,2,4 and 5 hours, 10ml samples were removed through the outlet. Incubation time was considerably shorter than for 2,4-D and diallate since persistence experiments had shown that some malathion loss was liable to occur within the 24 hour period used to study adsorption. Difficulties were experienced in dissolving malathion in water (as occurred with diallate) and so it was added in acetone. Malathion was extracted from these 10ml samples twice with 10ml of hexane (Fisons Distol). The remainder of the procedure has been detailed previously. Samples were diluted to fit a standard curve of 0-15ppm, since the maximum possible amount of pesticide obtained in one 10ml control sample (no soil) was 10ppm.

VII. Volatilization Experiments.

1. 2,4-D.

From the reported data it appears that volatility of the 2,4-D acid is minimal when compared with its ester forms. Since volatility appeared negligible from results of persistence experiments, no specific experiments were implimented to determine the volatility of the 2,4-D acid from soil.

2. Diallate and Malathion.

Volatilization of these two pesticides was assessed after the method of Spencer and Cliath (1973). Air at 100% relative humidity was blown

through flasks containing either sand or soil treated with 50µg of pesticide, at 65% water holding capacity. The air at a flow rate of 200ml min^{-1} was passed through the flask, samples being taken at 1,2,3 and 5 hours. Any volatilized pesticide was trapped in ice cooled hexane and measured directly with the gas chromatograph.

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<center>RESULTS</center>

<center>A.   Persistence Experiments</center>

I.   2,4-D.

1. Hamble Series soil.

Fig.3 (Appendix Table 1), compares the loss of 2,4-D from the non-sterile soil at three temperatures. At 25°C 2,4-D exhibited a typical lag phase, in this case 8 days, before rapid decline. The half-life of this herbicide was 10 days (2 days not including the lag) and the time required for total disappearance was 16 days. At 4°C and 37°C no loss of 2,4-D was observed over the 18 day experimental period. Lag periods of between 0 and 4 weeks have been reported (Audus 1964), however, in the experiments described here a relatively short lag phase was observed in all instances. Further experiments involving 2,4-D were carried out at 25°C since rapid decomposition was recorded at this temperature.

Fig.4 (Appendix Table 2) shows the effect of three sterilization procedures on 2,4-D loss at 25°C. The three sterilization processes were autoclaving, irradiation (2.5 and 5.0 Mrads) and sodium azide additions. No loss of 2,4-D was observed in any of these treated soils.

A microbial re-enrichment of sterile soils was carried out by the addition of 0.5mls of a 10^{-2} dilution of a soil water suspension to the soils. In the autoclaved soil profuse fungal growth was observed after a few days, yet no loss in 2,4-D was seen after 18 days in either this or the irradiated soils.

2. Soil components from Hamble soil.

The loss of 2,4-D from non-sterile soil components is shown in Fig.5

Fig. 3.

2,4-D breakdown at 4°C (O); 25°C (▲) and 37°C (■)
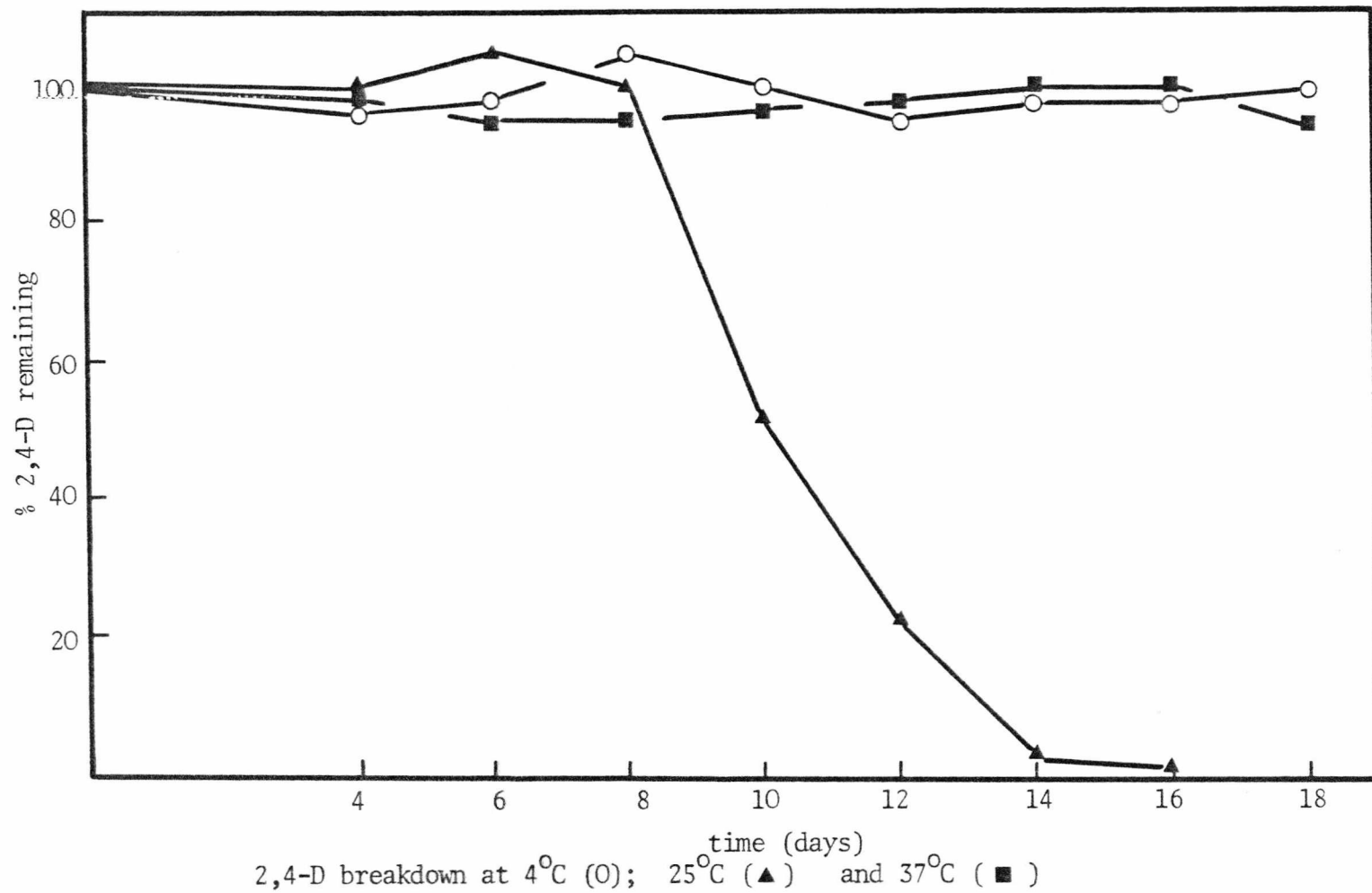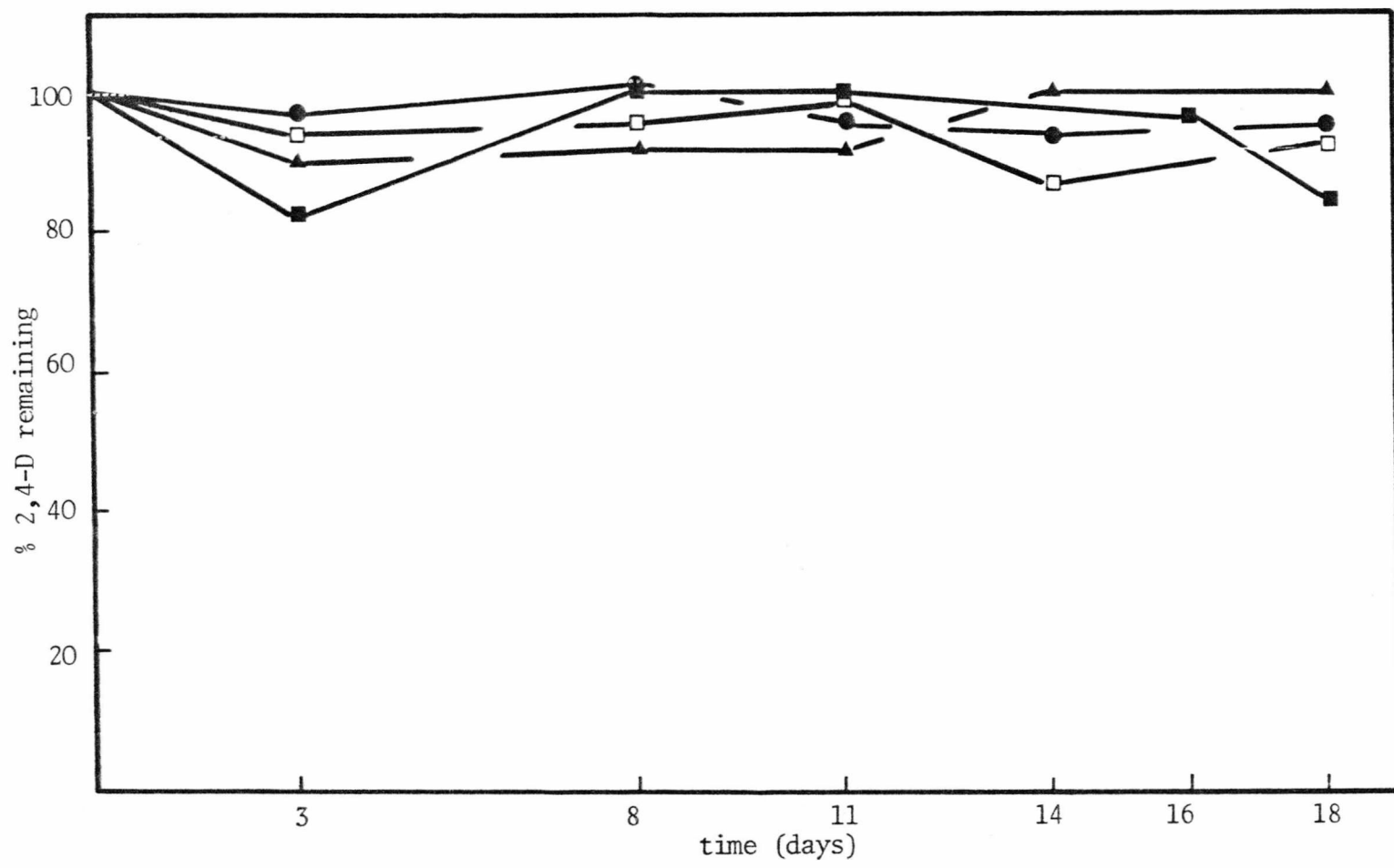
Fig. 4.

2,4-D breakdown in sterile soil - □ = 2.5Mrads; ● = 5.0Mrads;

■ = autoclaved; ▲ = NaN₃-treated
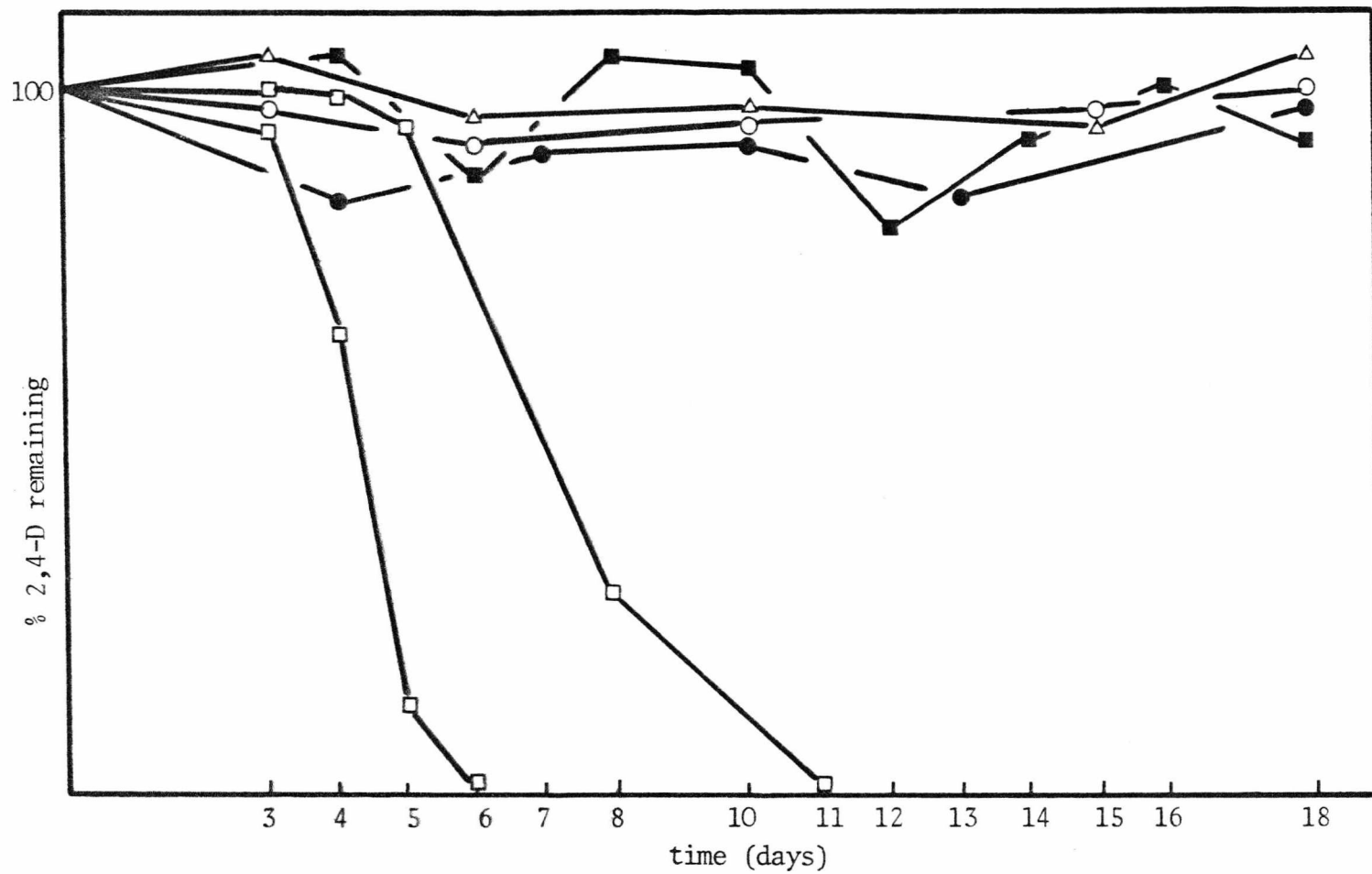
Fig. 5.

2,4-D breakdown in non-sterile soil components - □ = organo-mineral.

O = sand;   △ = silt;   ■ = clay;   ● = organic matter.

(Appendix Table 3). No loss was recorded from the sand, silt, clay and organic matter fractions, only from the organo-mineral complex. However, some variation in the lag phase was observed with the organo-mineral component, unlike the parent soil. Three experiments produced lag phases of 3 days and two experiments, lag phases of 5 days. An initial experiment demonstrated a lag phase of 10 days in the organo-mineral fraction, however this was not repeatable and so has been omitted from this series of results. Consistently rapid declines in pesticide concentration were seen in the case of the organo-mineral complex i.e. within 3 days after the lag for both examples cited in Fig.5. It is noticeable that shorter lag phases and more rapid decline phases of 2,4-D were observed in the organo-mineral complex, than in the complete soil.

No loss of 2,4-D from the remaining fractions shown in Fig.5 was recorded, even though microbial counts demonstrate the presence of viable populations on all these fractions, except for the clay.

Autoclaving was used to sterilize the soil components and the results are shown in Fig.6 (Appendix Table 4). No breakdown occurred in any of the components. The capacity of the organo-mineral complex to facilitate 2,4-D breakdown was also removed on autoclaving as it was in the parent soil.

There was no consistent decline in pesticide levels eliminating non-biological decay or volatilization as major processes contributing to 2,4-D loss in this soil.

3. Artificial soils.

The results in Fig.7 (Appendix Table 5) illustrate the significant effect that the addition of the organo-mineral component to the sand or silt components has on 2,4-D breakdown. With 100% sand or silt no breakdown of 2,4-D was observed, but on addition of the organo-mineral component loss of
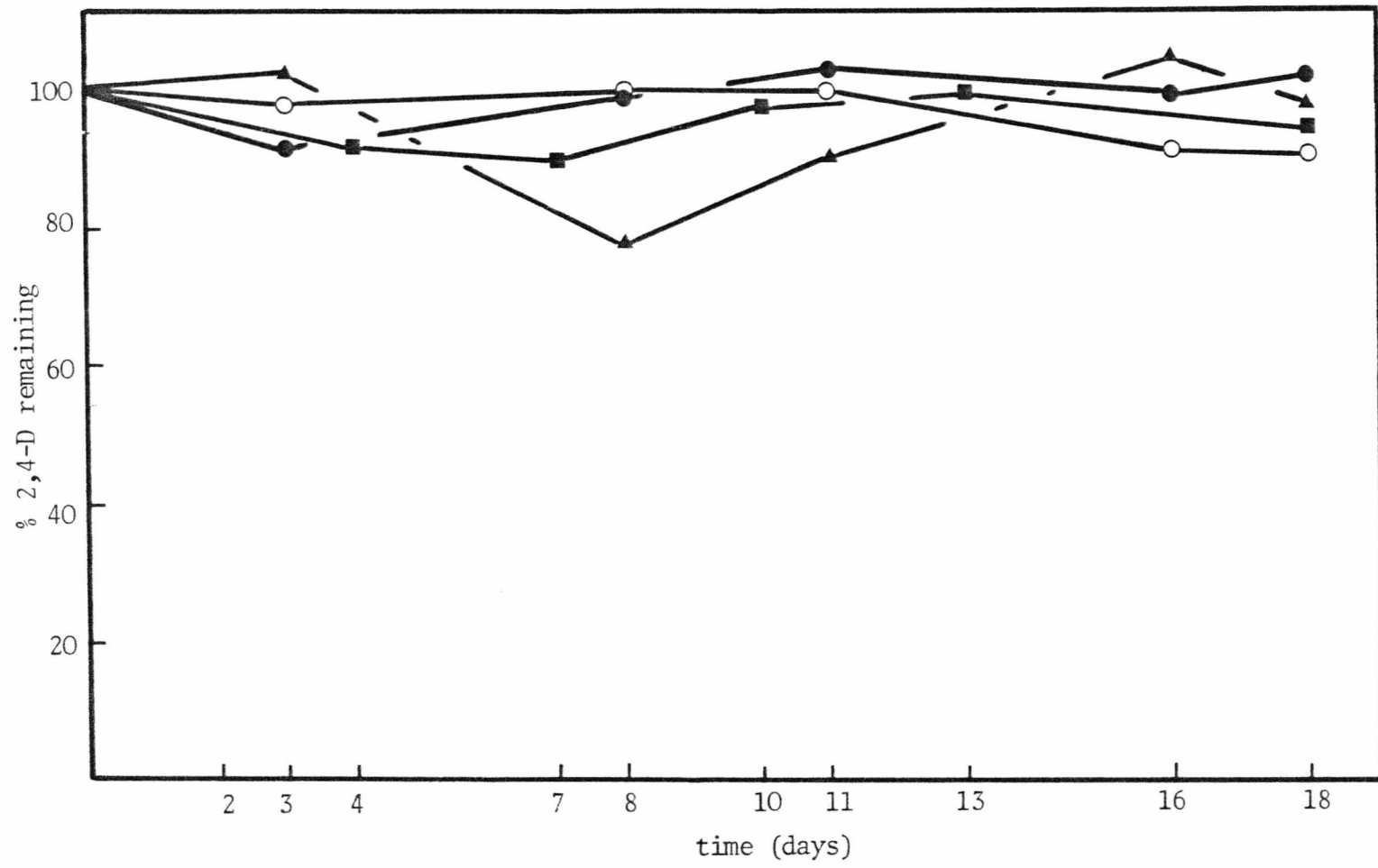
2,4-D breakdown in autoclaved soil components - ■ = organic matter;
● = sand;   ▲ = silt;   O = organo-mineral.

Fig. 6
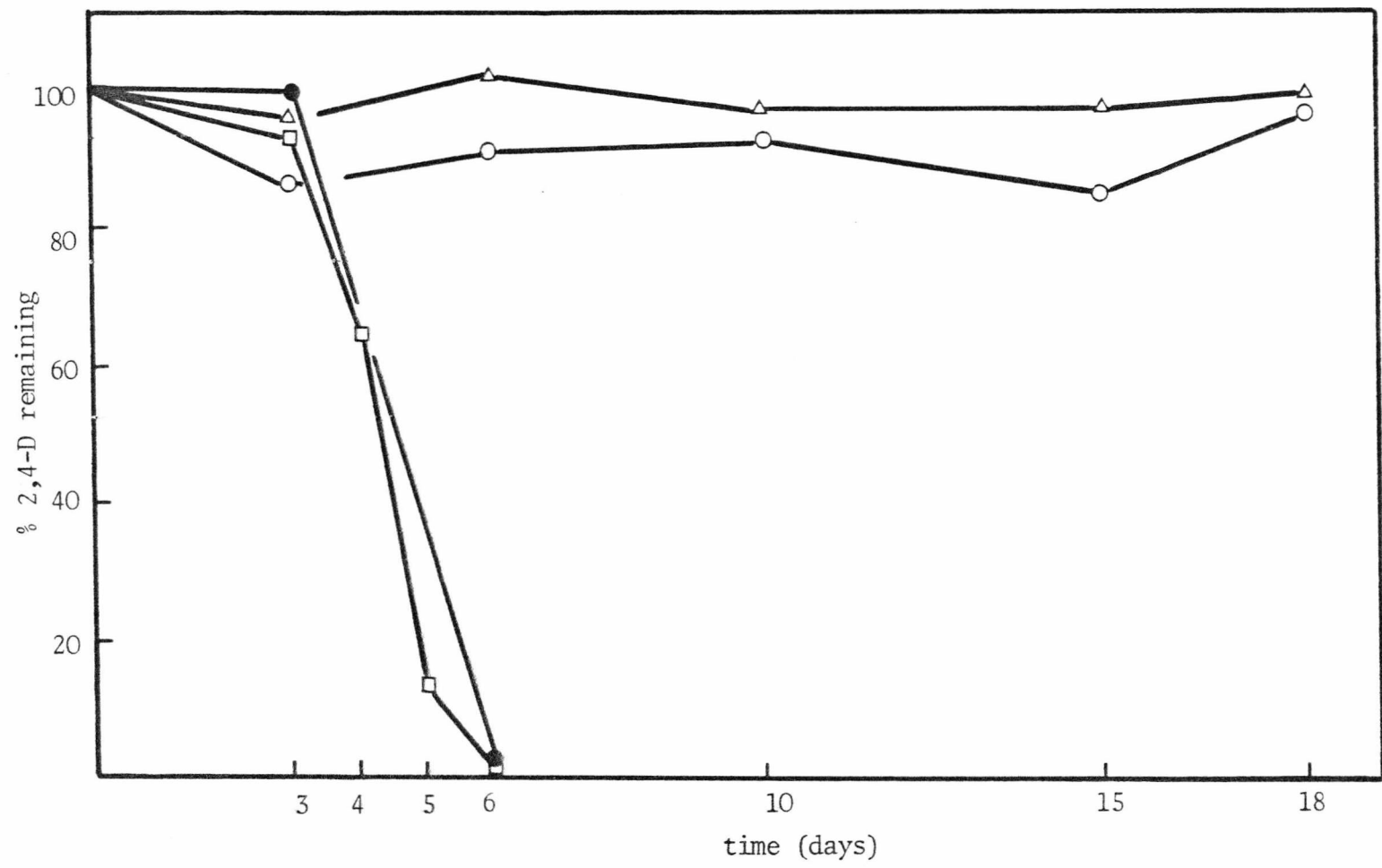
2,4-D breakdown in silt or sand : organo-mineral complex artificial soils -
□ = organo-mineral (100%);   0 = sand;   △ = silt;   ● = all combinations

Fig. 7

2,4-D showed a similar pattern to that seen in 100% of the organo-mineral complex. With the ratios, 3:1, 1:1 and 1:3 of silt or sand:organo-mineral complex, the stimulatory effect was similar in all cases, there being no alteration in lag phase with increasing sand and silt contents. Clearly the organo-mineral complex was demonstrated as being the active component in soil responsible for 2,4-D degradation.

4. Test soils.

Loss of 2,4-D in Barming, Hothfield and Winchester soils was investigated to determine any correlation between the percentage of active organo-mineral complex in the soil and the subsequent half-life. Microbial counts at time zero were also recorded.

The results shown in Fig.8 (Appendix Table 6) are summarized in Table 12.

TABLE 12

Loss of 2,4-D from 4 test soils

Soil	Depth (cm)	Lag (days)	Half-life including lag (days)	% organo-mineral complex	Bacteria g soil^{-1} (x 10^{-3})
Winchester	0-20	2	5	55	152
Winchester	50-60	-	-	65	32.6
Barming	0-20	6	$8\frac{1}{2}$	23	209
Hothfield	0-20	13	14	11	68.6
Hamble	0-20	8	10	20	84.6

The results show a considerable variation in lag phases and half-lives for these different soils. It appears that an high organo-mineral content,
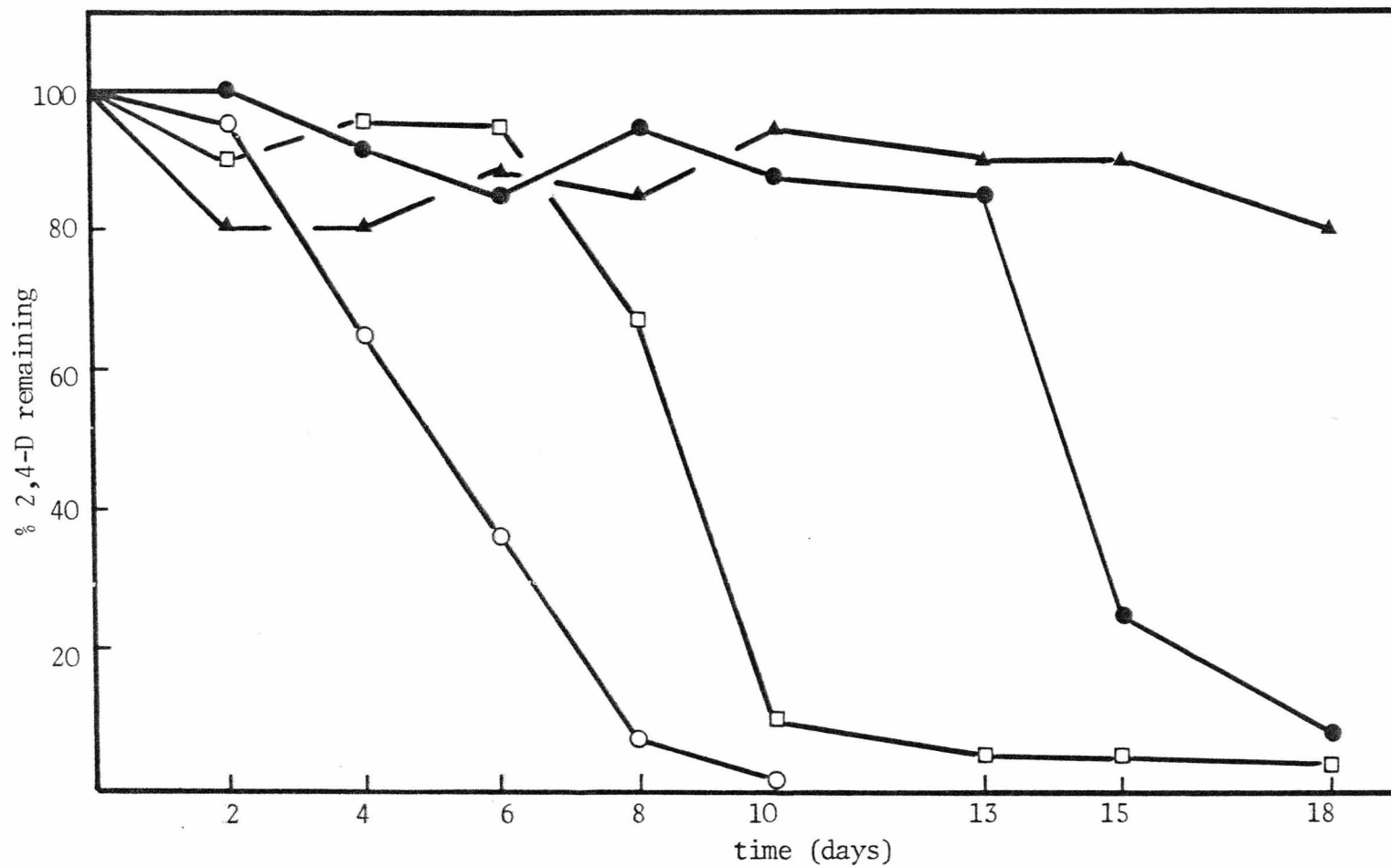
Fig. 8.

2,4-D breakdown in four test soils - o = Winchester (0-20cm);
Δ = Winchester (50-60cm);   □ = Barming;   ● = Hothfield.

together with an high initial microbial population facilitates the most rapid loss of 2,4-D in these soils, as results from artificial soil experments also suggest.

II. Malathion.

1. Hamble Series soil.

The loss of malathion from non-sterile soil at $4^{\circ}C$, $25^{\circ}C$ and $37^{\circ}C$ was compared. (Fig.9, Appendix Table 7). The half-lives at these temperatures were $11\frac{1}{4}$, $3/4$ and $3/4$ of a day respectively. It can be seen that at $4^{\circ}C$ a considerable increase in the half-life of malathion occurred compared to the half-life of $3/4$ day at both $25^{\circ}C$ and $37^{\circ}C$.

Fig.10 (Appendix Table 8) illustrates the effect of three sterilization procedures on malathion loss from Hamble soil. It can be seen that 2.5 and 5.0 Mrad doses decreased the rate of decay only slightly compared with the non-sterile soil. Addition of sodium azide still enabled the soil to catalyse malathion decay, malathion having a half-life of $1\frac{1}{4}$ days in this treated soil. However, in complete contrast, disappearance from auto-claved soil was negligible. Also no lag phases were observed in the soil treatments where breakdown occurred.

Fig.11 (Appendix Tables 8 and 9)illustrates the effect of varying levels of gamma irradiation on the ability of the treated soil to degrade malathion. It has previously been shown that 2.5 and 5.0 Mrads dosage did not significantly decrease the rate of decay of malathion. However, at higher levels (10, 15 and 20 Mrads) a more marked effect was seen, although far from total inhibition occurred. It is interesting to note that the two lowest levels of irradiation had similar effects (half lives of $1\frac{1}{4}$ and $1\frac{1}{2}$ days) as did the three highest levels (half lives of $8\frac{1}{2}$, 6 and 9 days). Therefore, there appears to be no obvious direct relationship between dosage and inhibition.
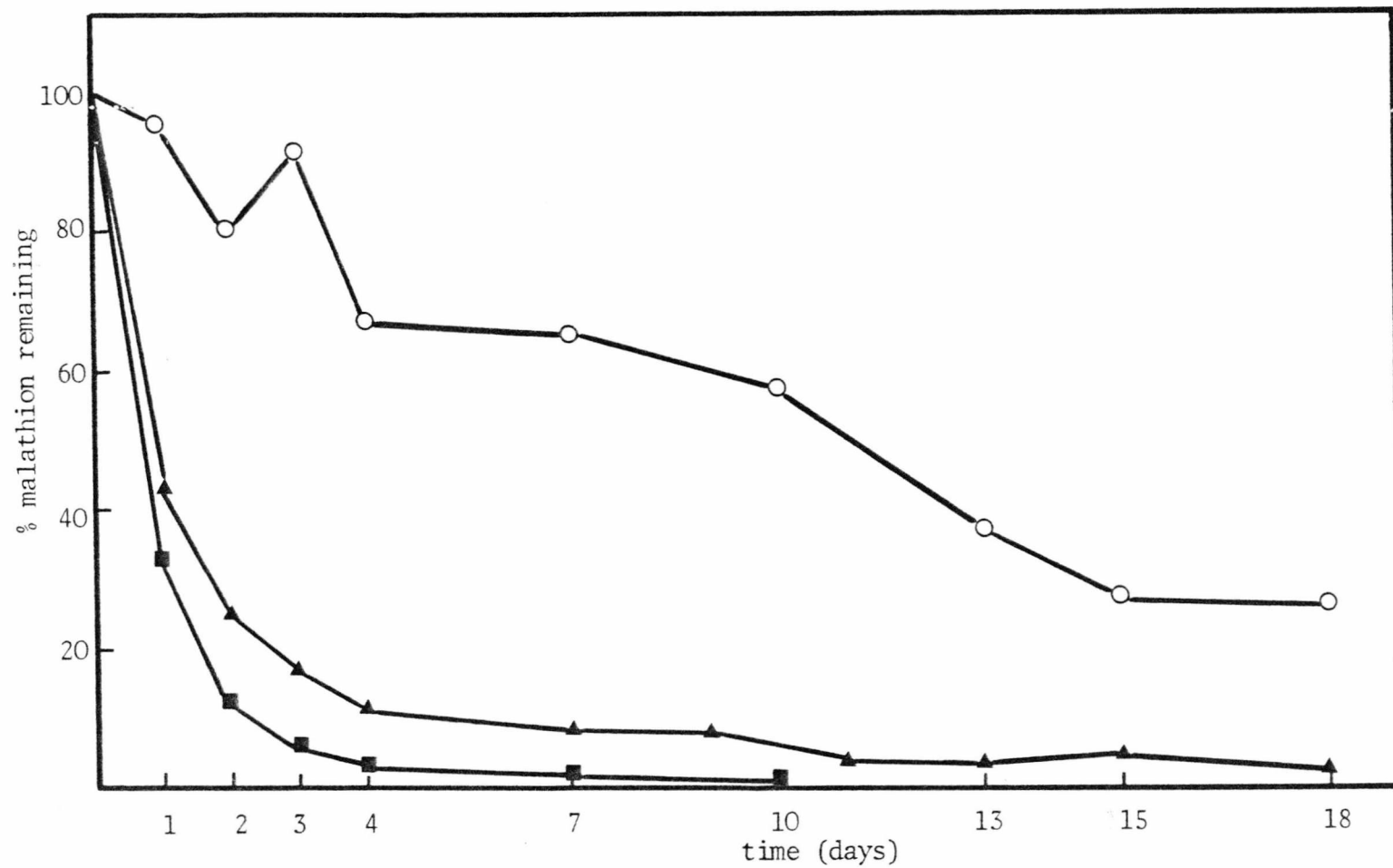
Malathion breakdown at $4^{\circ}C$ (O); $25^{\circ}C$ (▲); and $37^{\circ}C$ (■).

Fig. 9.

Fig. 10

Malathion breakdown in sterile soil - □ = 2.5Mrads; ● = 5.0Mrads;
■ = autoclaved; ▲ = NaN₃-treated

74.
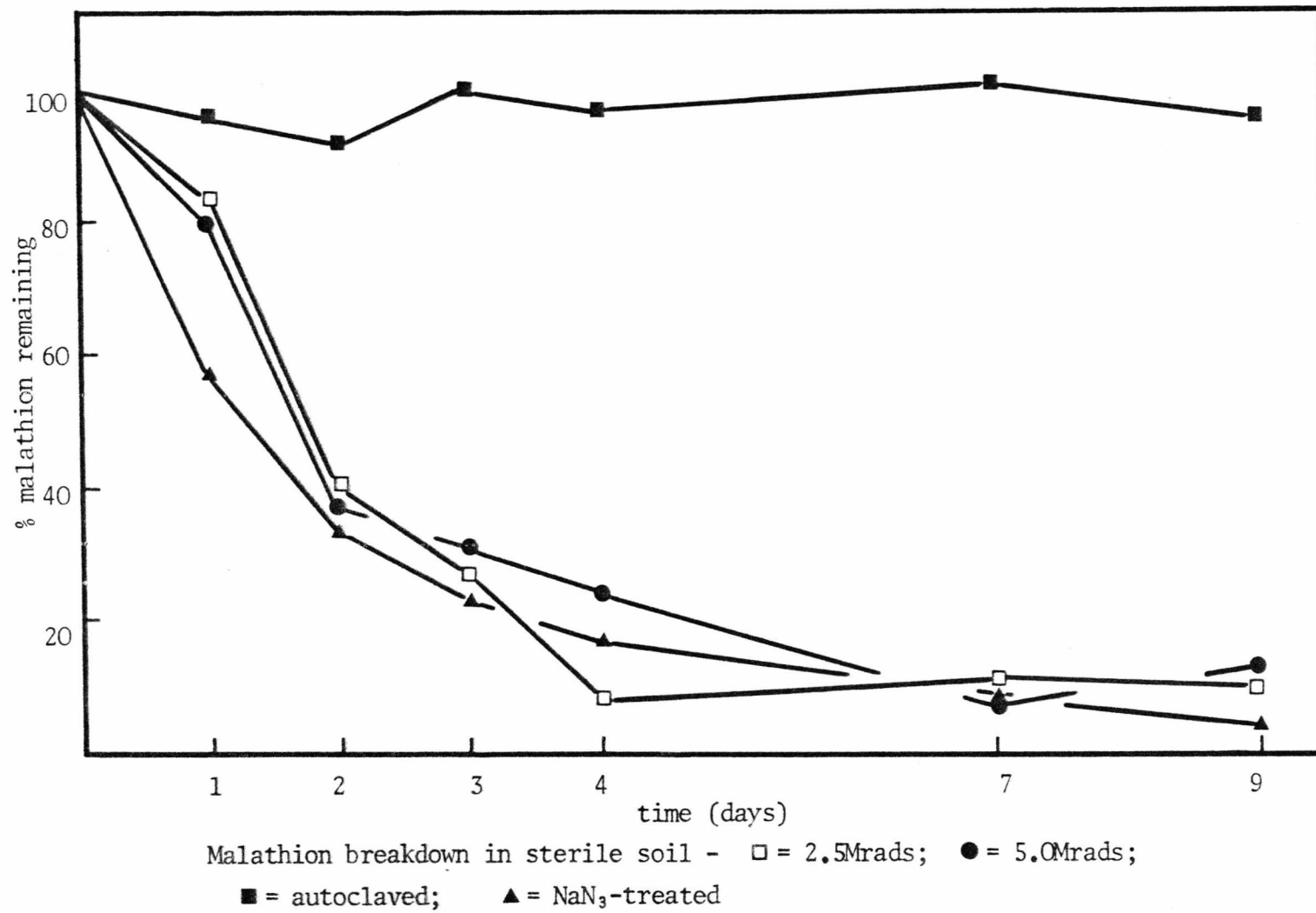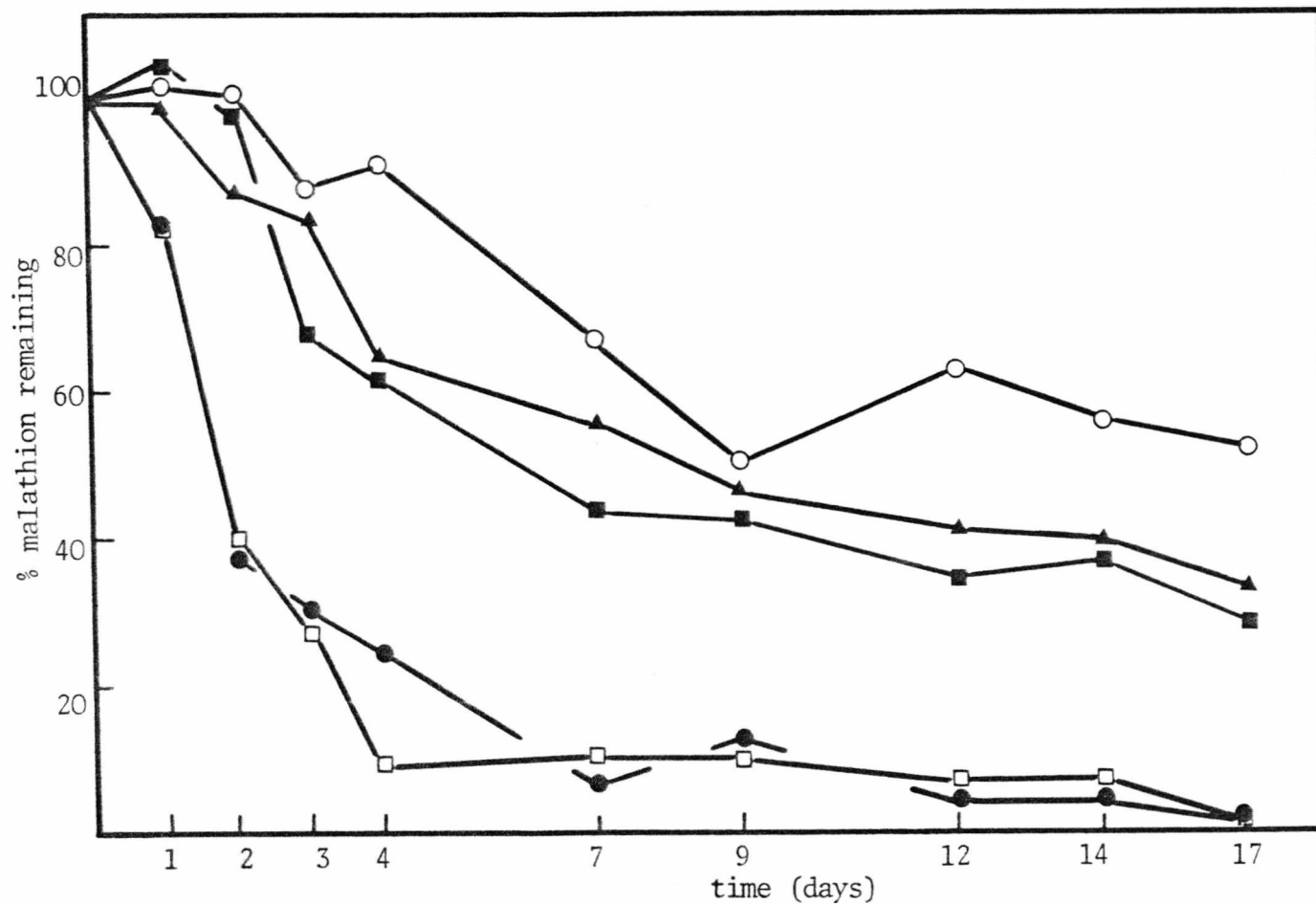
Fig. 11

Malathion breakdown in irradiated soils - □ = 2.5Mrads; ● = 5.0Mrads;
▲ = 10Mrads; ■ = 15.0Mrads; O = 20.0Mrads.

2. Soil components.

The breakdown of malathion in non-sterile sedimented soil components;
(App.Table 10)
clay, silt, sand and organo-mineral complex, is shown in Fig.12/. It is
noticeable that the organo-mineral complex (half-life $^3/4$ day) was as
efficient as the whole soil in promoting breakdown. Silt, sand and clay
were significantly less effective (half-lives $7\frac{1}{2}$, 11 and 18 days). Break-
down in silt and sand fractions also demonstrate a 3-4 day lag period, while
in the clay fraction there was a linear rate of disappearance over the 17
day period. The organo-mineral complex showed no lag phase, in common with
the parent soil.
(App.Table 11)
In contrast, Fig.13/illustrates the effect of autoclaving the soil
components on breakdown. There was as much as 20% variation between diff-
erent sampling times in this series of experiments. However, at day 18 in
the clay and organo-mineral complex greater than 90% of applied malathion
remained. In the sand and silt fractions 75-80% of applied malathion
remained, there being a general trend of 20% loss over the 18 day period.
In comparison to non-sterile fractions breakdown was retarded to a signifi-
cant extent by heat sterilization, as was also shown for the parent soil.

The degradation of malathion in separated organic matter fractions is
shown in Fig.14 (Appendix Table 12). Non-sterile organic matter rapidly
effected degradation of the insecticide (half-life $1\frac{3}{4}$ days) and removal of
microorganisms from this fraction by filtering slowed down the process
(half-life $4\frac{1}{2}$ days). No breakdown occurred in autoclaved organic matter.

3. Artificial soils.

The effect of additions of the 'active' organo-mineral complex to
either sand or silt is described in Fig.15 and 16. (Appendix Tables 13 and 14).

All artificial soils demonstrated the stimulatory influence of the
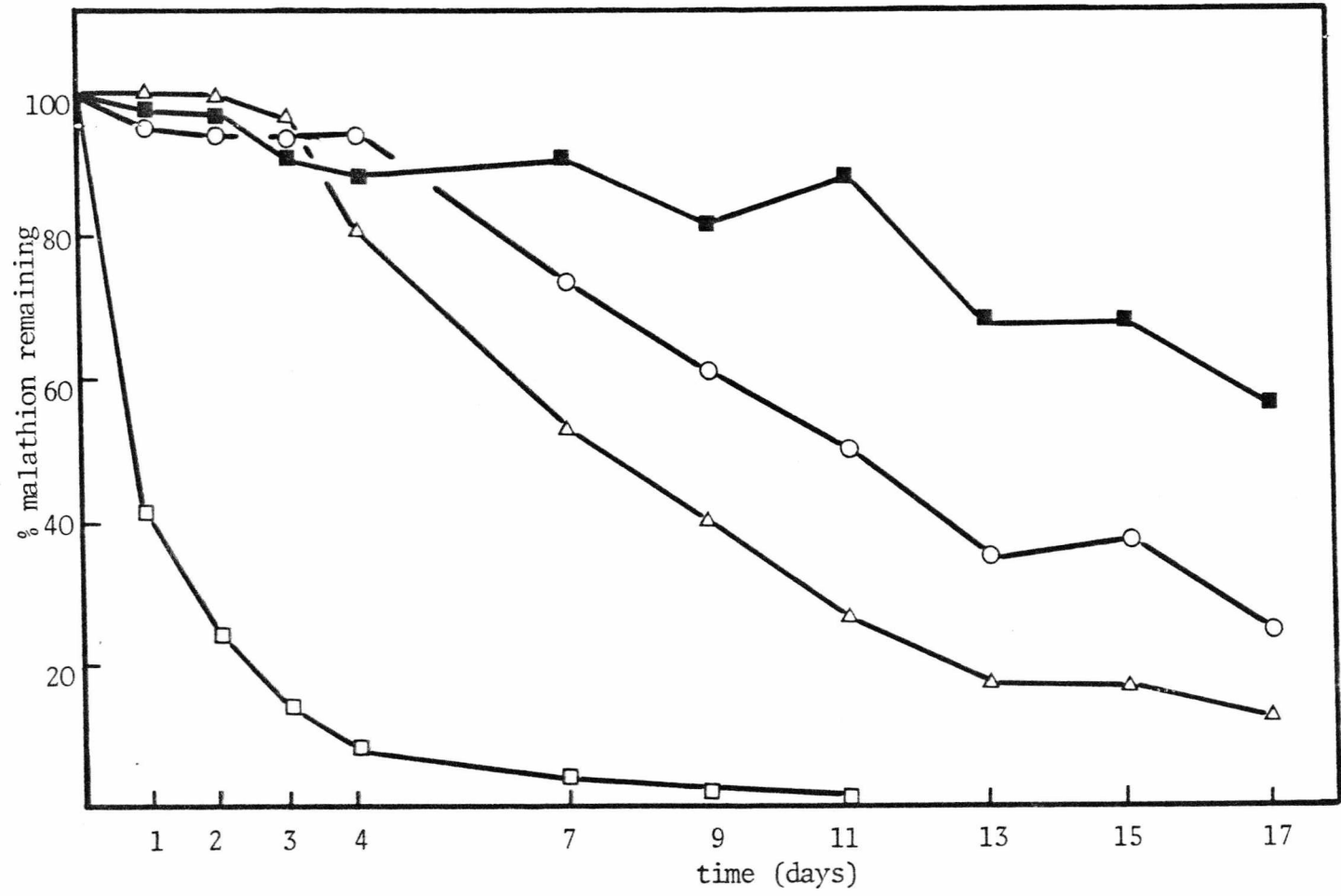
Malathion breakdown in non-sterile soil components -   □ = organo-mineral; O = sand;
Δ = silt;   ■ = clay.

Fig. 12

Malathion breakdown in autoclaved soil components - ○ = organo-mineral; ● = sand; ▲ = silt; ▽ = clay.

Fig. 13
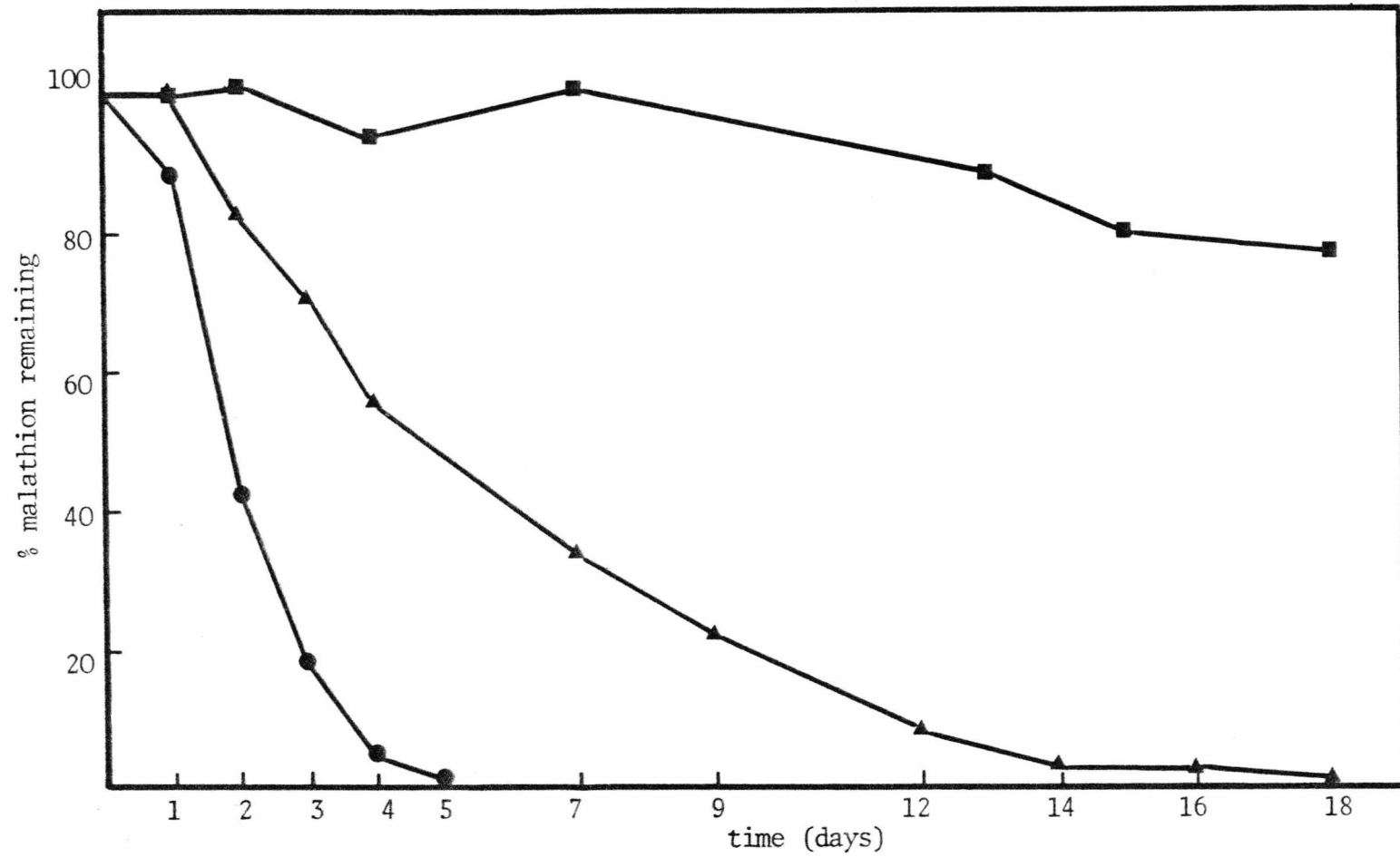
Malathion breakdown in sterile and non-sterile organic matter -   ● = non-sterile;

▲ = filtered;   ■ = autoclaved.

Fig. 14

Fig. 15

Malathion breakdown in sand : organo-mineral complex artificial soils -

□ = organo-mineral (100%);   ● = organo-mineral (50%) : sand (50%);   ▲ = organo-mineral (25%) : sand (75%);   ■ = organo-mineral (5%) : sand (95%);   O = sand.
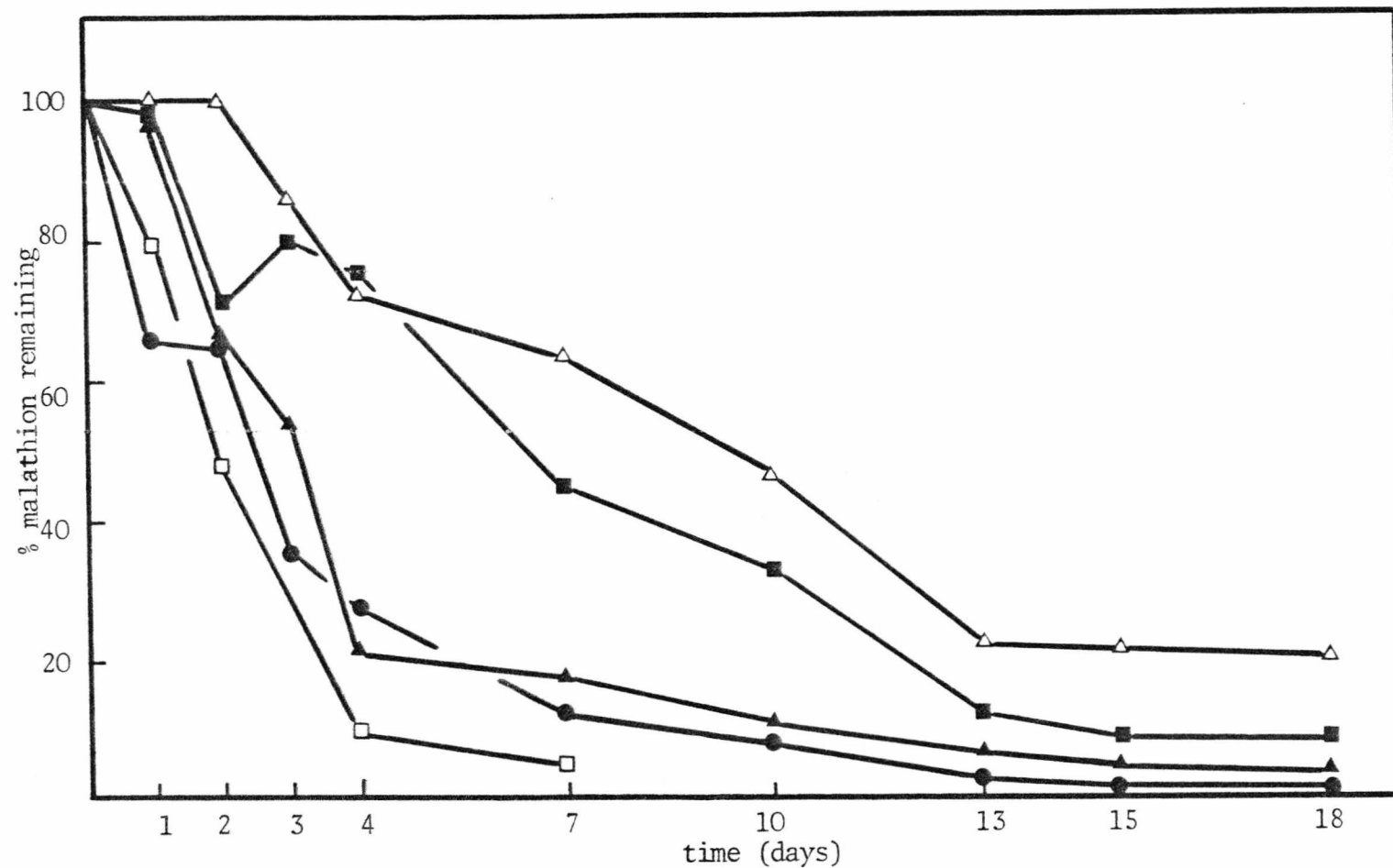
Fig. 16

Malathion breakdown in silt : organo-mineral complex artificial soils.

□ = organo-mineral (100%);   ● = organo-mineral (50%) : silt (50%);   ▲ = organo-
mineral (25%) : silt (75%);   ■ = organo-mineral (5%) : silt (95%);   △ = silt.

organo-mineral complex. In the 19:1 mixtures the 5% of organo-mineral complex decreased the half-life of malathion from 9 to 6 days (in silt) and from $11\frac{3}{4}$ to $6\frac{1}{2}$ days (in sand). In the 3:1 mixtures the 25% organo-mineral complex decreased the half-life of malathion from 9 to 3 days (in silt) and from $11\frac{3}{4}$ to $2\frac{1}{2}$ days (in sand). The rate of breakdown in the 1:1 mixtures were little different from the 3:1 mixtures. In both combinations the rate of breakdown was similar to the 100% organo-mineral complex control.

4. <u>Test soils</u>.

Winchester, Barming and Hothfield Series soils were again used as test soils. From the previous experiments it was concluded that the organo-mineral complex exhibited a stimulatory effect on malathion breakdown. It was therefore the aim to see if soils containing differing amounts of organo-mineral complex might exhibit different rates of loss according to the content of organo-mineral complex in the total soil.

Fig.17 (Appendix Tables 15 and 16) shows the loss of malathion from these soils, including the originally investigated Hamble soil, over an 18 day period. The half-life times for malathion were $6\frac{1}{2}$ days in Hothfield soil, $\frac{1}{2}$ day in Winchester soil and $\frac{3}{4}$ day in the Barming soil.

Due to rapid breakdown, loss of malathion was also studied over the 18 hour period (Fig.18, Appendix Tables 15 and 16), the level after 24 hours being obtained from Fig.17 and inserted for comparison purposes. Most rapid loss occurred in the 0-20cm depth of the Winchester soil where the half-life of malathion was 8 hours. The half-lives for malathion in the 50-60cm depth of Winchester soil and 0-20cm depth of the Barming soil were 14 and 18 hours respectively. For the Hamble soil a half-life of $22\frac{1}{2}$ hours for malathion was recorded.
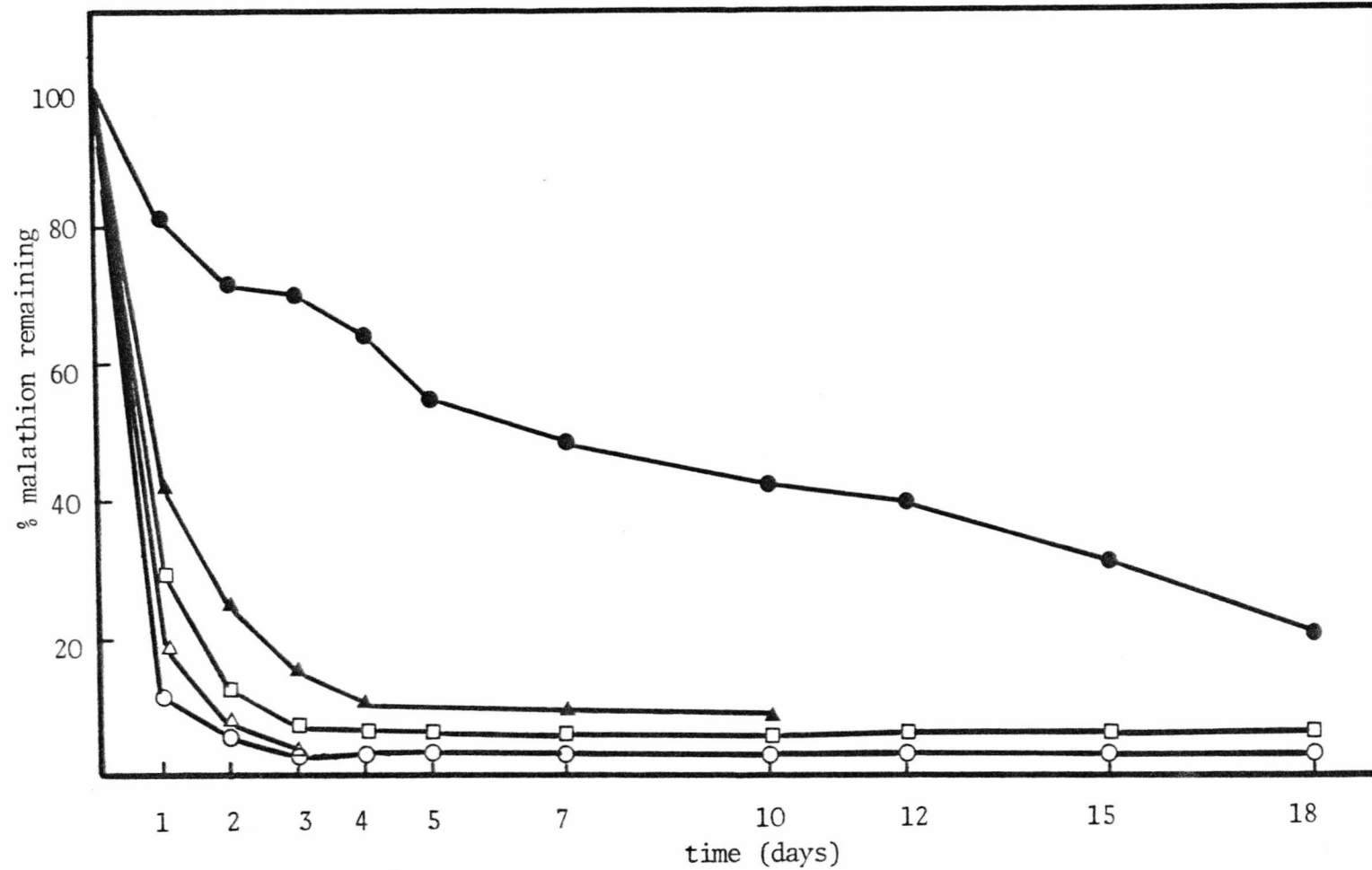
Fig. 17

Malathion breakdown in Hamble soil and four test soils - ○ = Winchester (0-20 cm); △ = Winchester (50-60 cm); □ = Barming; ● = Hothfield; ▲ = Hamble.
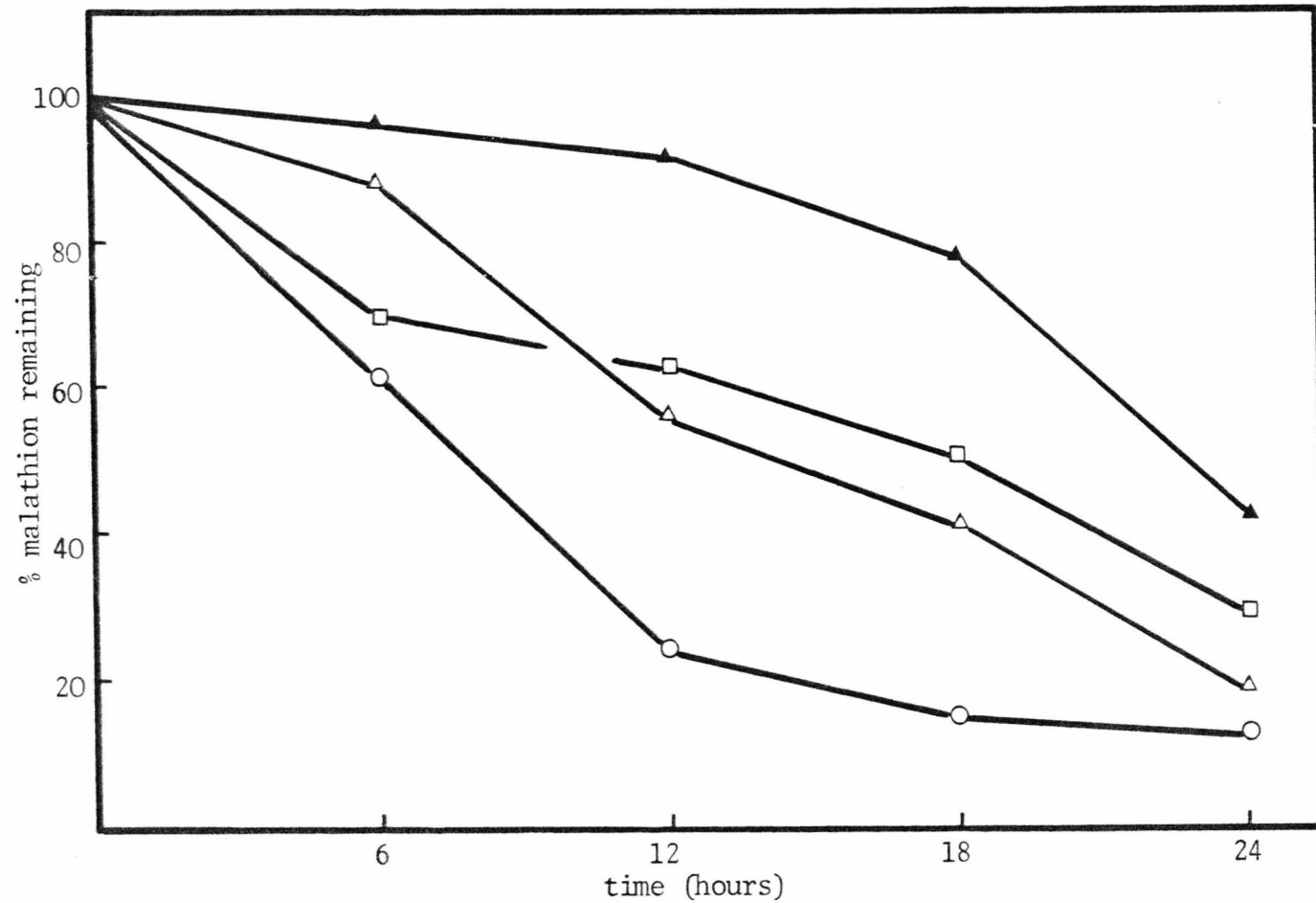
Fig. 18

Malathion breakdown over 24 hours in Hamble soil and three test soils -

▲ = Hamble;   □ = Barming;   ○ = Winchester (0-20cm);   △ = Winchester (50-60cm)

III. Diallate.

1. Hamble Series soil.

The loss of diallate at three temperatures, $4^{o}C$, $25^{o}C$ and $37^{o}C$ is shown in Fig.19 (Appendix Table 17). This compound exhibited negligible loss at $4^{o}C$ after 70 days. At $25^{o}C$ diallate was seen to be comparatively persistent, with a half-life of approximately 70 days, and at $37^{o}C$ was less persistent with a half-life of 25 days.

Fig.20 (Appendix Table 18) demonstrates the effect of three steriliz-ation procedures on the loss of diallate at $25^{o}C$ and the effect of auto-claving on loss at $37^{o}C$. The sterilization procedures had little effect on diallate loss, half-lives for diallate were around the 70 day period, similar to that in the non-sterile soil at $25^{o}C$. At $37^{o}C$ diallate showed a half-life of 25 days in the autoclaved soil, which was identical to that exhibited in the non-sterile soil at $37^{o}C$.

2. Soil components.

The loss of diallate from non-sterile soil components i.e. organo-mineral complex, sand, silt and clay is shown in Fig.21 (Appendix Table 19). Loss appeared to be related to the particle size of each component, the coarser the particle the greater the rate of loss. Half-lives of 10 days were recorded in the sand, 27 days in the silt and greater than 70 days in both the clay and organo-mineral components.

Fig.22 (Appendix Table 20) illustrates the loss of diallate from autoclaved soil components, a similar pattern being recorded to that in the non-sterile components. Half-lives for diallate being 15 days in the sand, 21 days in the silt, and greater than 70 days in the clay and organo-mineral complex. Therefore, we see that autoclaving does not have a significant

Fig. 19

Diallate loss at 4°C (O); 25°C (▲) and 37°C (■).
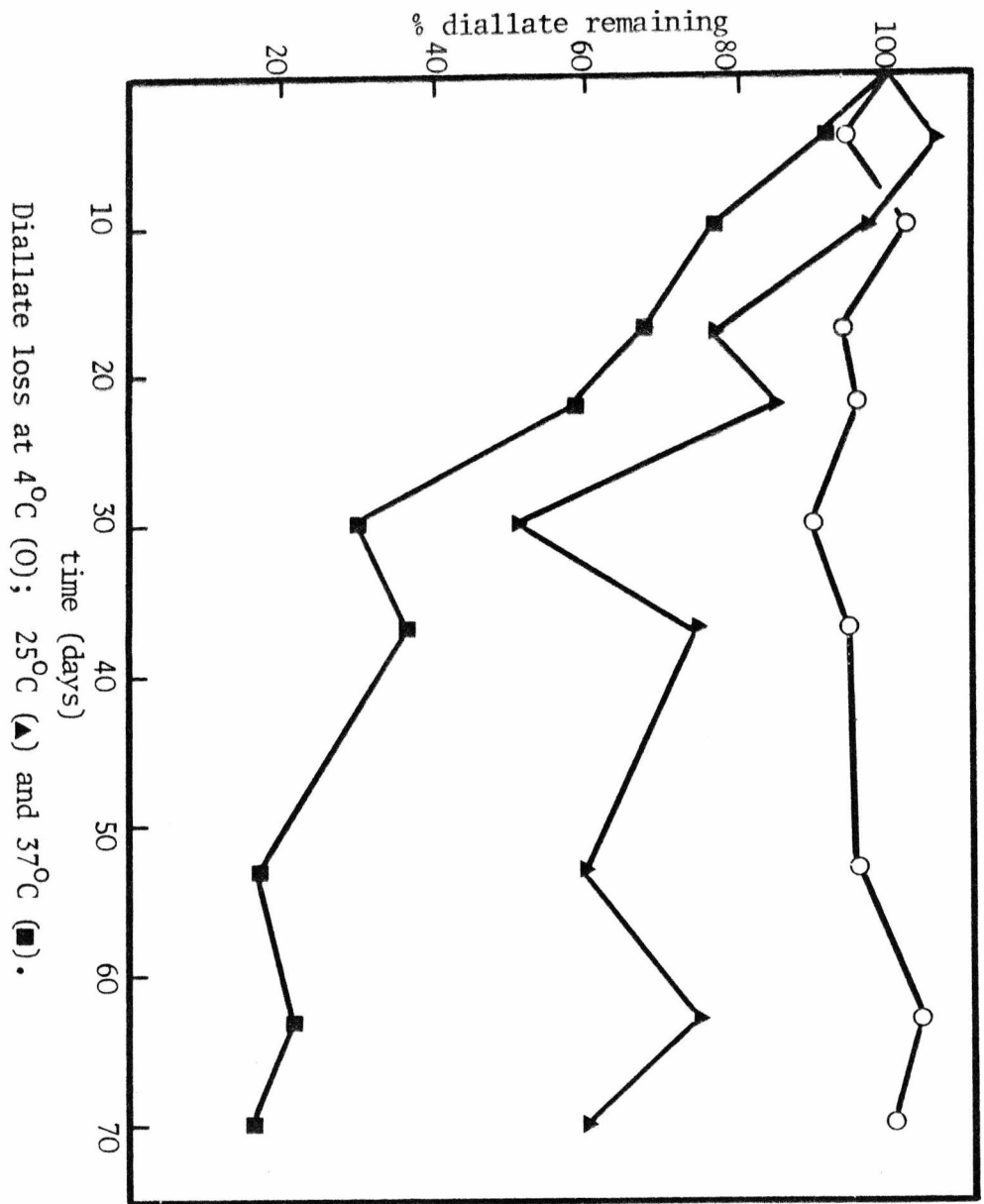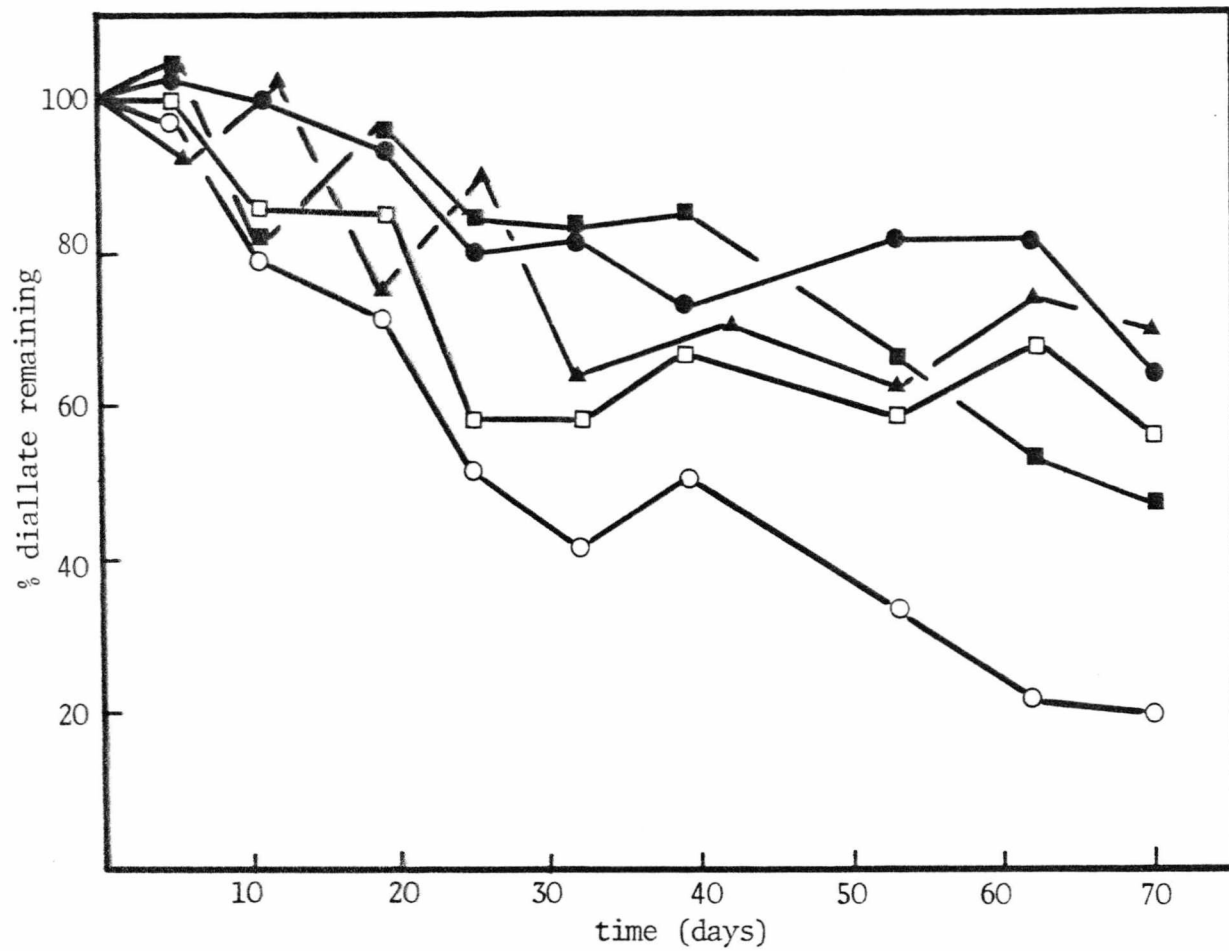
Diallate loss in sterile soil - □ = 2.5Mrads; ● = 5.0Mrads; ■ = autoclaved; O = autoclaved (37°C); ▲ = NaN₃- treated.

Fig. 20

87.
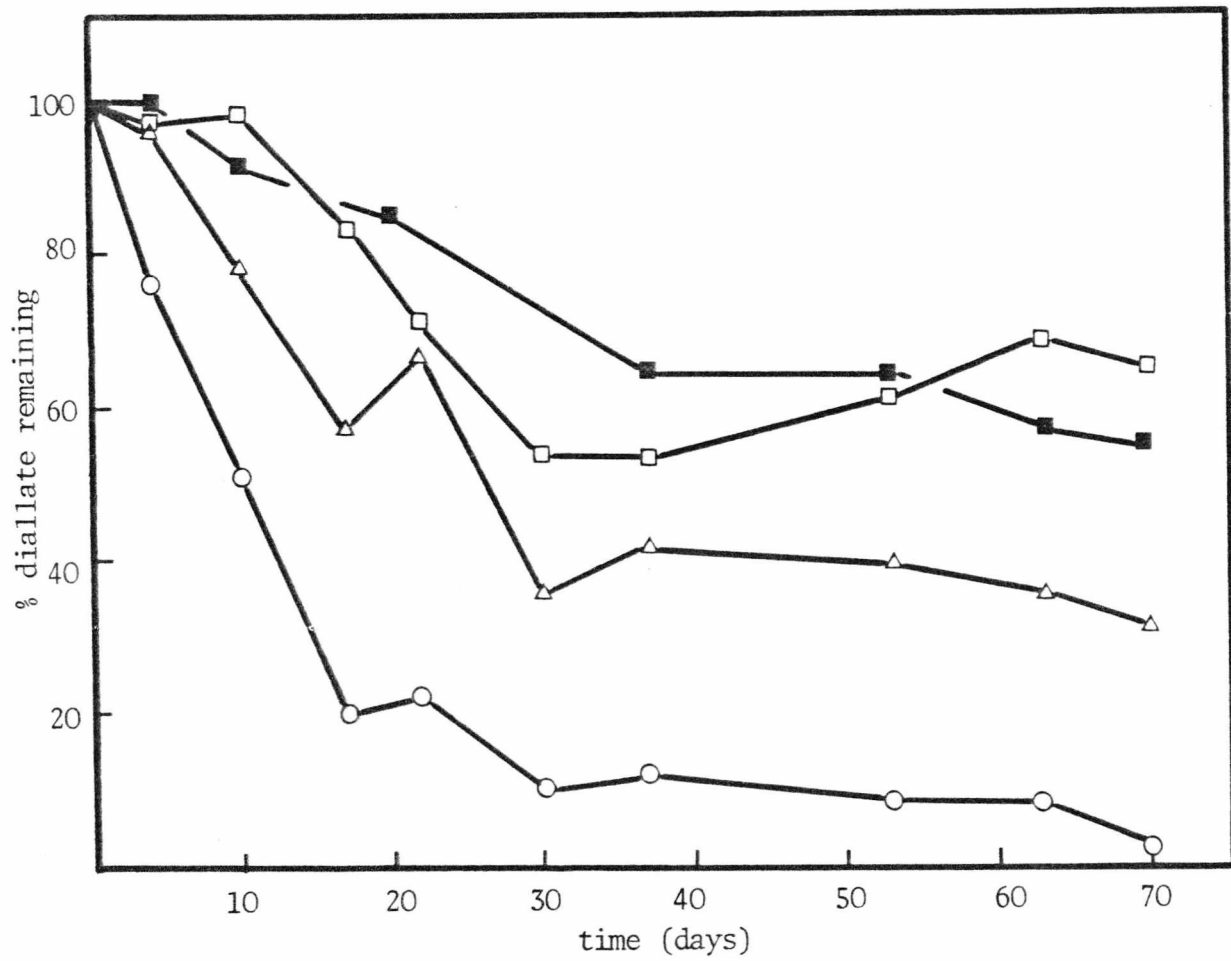
Diallate loss in non-sterile components - □ = organo-mineral; O = sand;
△ = silt; ▦ = clay.

Fig. 21
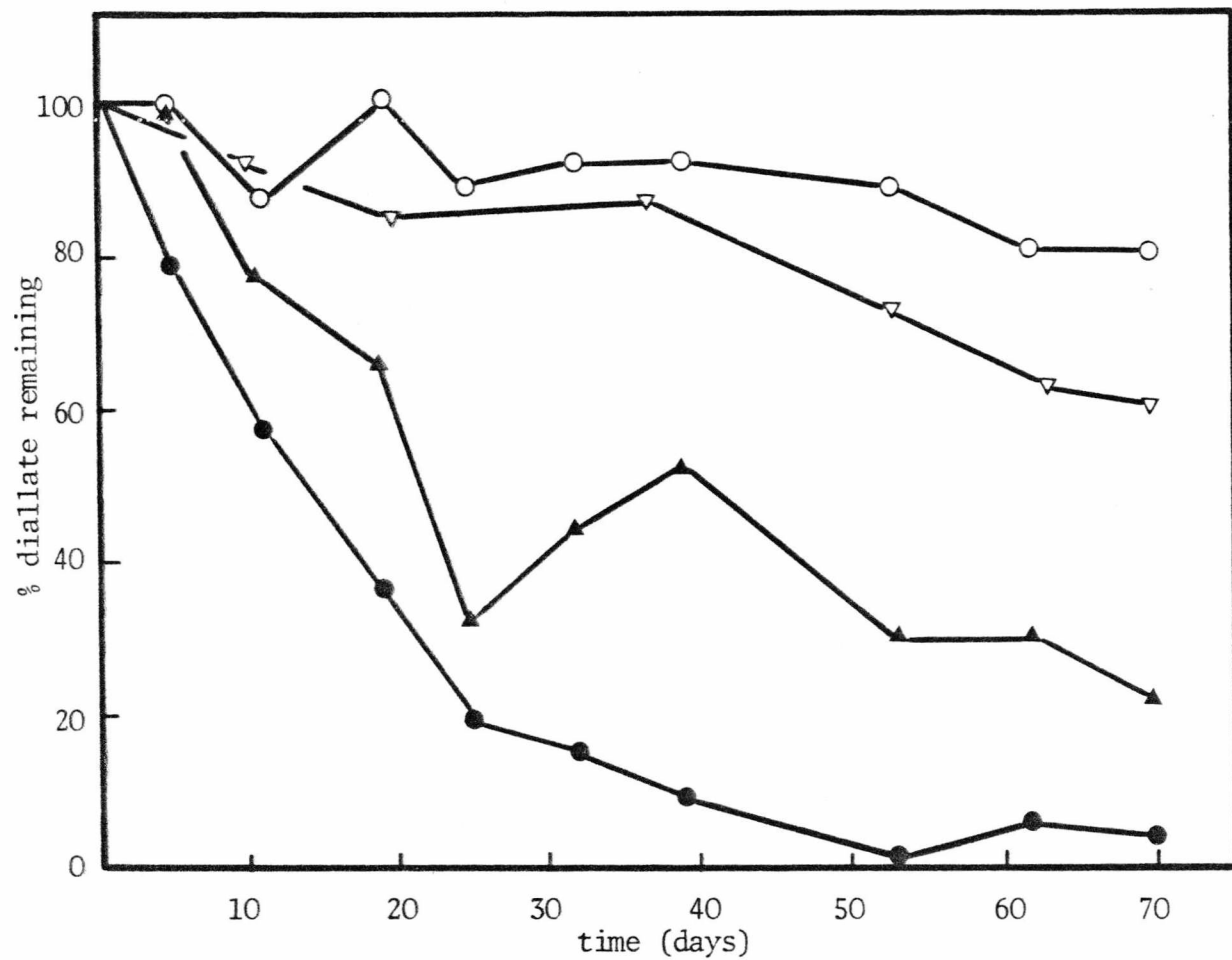
Diallate loss in autoclaved soil components - O = organo-mineral;
● = sand;   ▲ = silt;   ▽ = clay.

Fig. 22

89.

effect on loss of diallate from these soil components. Slight differences in half-life values may be due to variation between samples and extraction rates.

Loss of diallate from non-sterile and autoclaved organic matter fractions was extremely rapid, both treatments exhibiting half-lives of 5 days. An initial rapid loss was observed with a further gradual loss over 20 days. Fig.23 (Appendix Table 21) illustrates the loss of diallate from these organic matter components, together with the loss of diallate from open and sealed glass surfaces. Loss was most rapid (half-life 2 days) from an open glass surface of an Erylynmeyer flask at 25°C. The loss of diallate was considerably reduced if a sealed system was used, in this case a 12ml Bijoux bottle with a screw top, and 70% of the applied diallate remained after 70 days.

Fig.24 (Appendix Table 22) demonstrates the effect of sealing the system (as described above) on rates of diallate loss from autoclaved sand and organic matter fractions. It can be seen that sealed systems do retard the rate of loss from these components. A half-life for diallate of greater than 70 days was recorded in the sand. However, in the case of organic matter, although the half-life was considerably lengthened i.e. 18 days compared to 5 days in unsealed organic matter systems, fairly rapid loss was still occurring in comparison to other sealed systems.
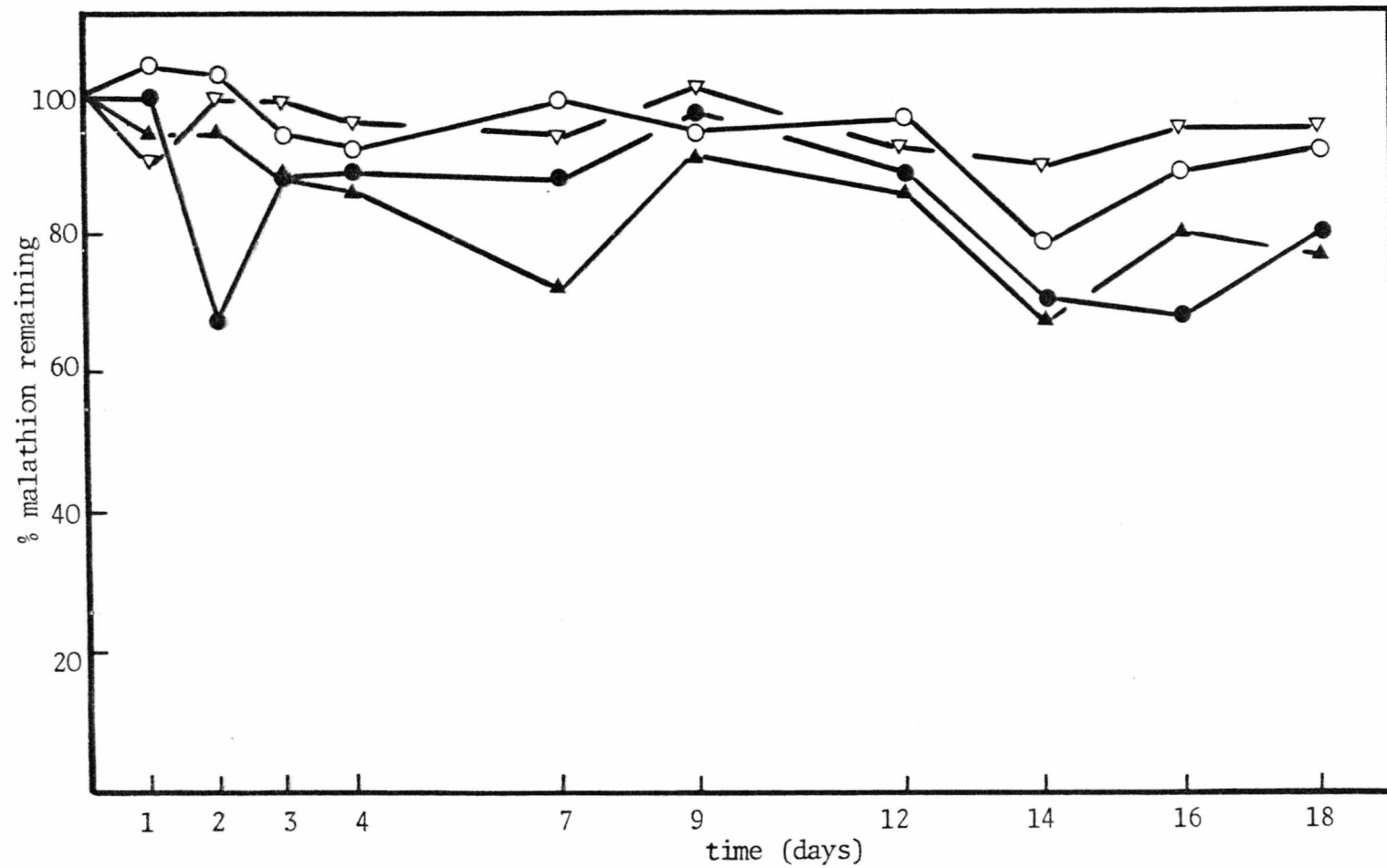
3. Artificial soils.

The effect of additions of the organo-mineral complex to either the sand or silt component is described in Figs.25 and 26 (Appendix Tables 23 and 24). A retardation in loss was observed with increasing organo-mineral complex compared to the rate for 100% of sand and silt controls. In the 19:1 mixtures the half-life of diallate increased from 11 to 15 days (in sand:
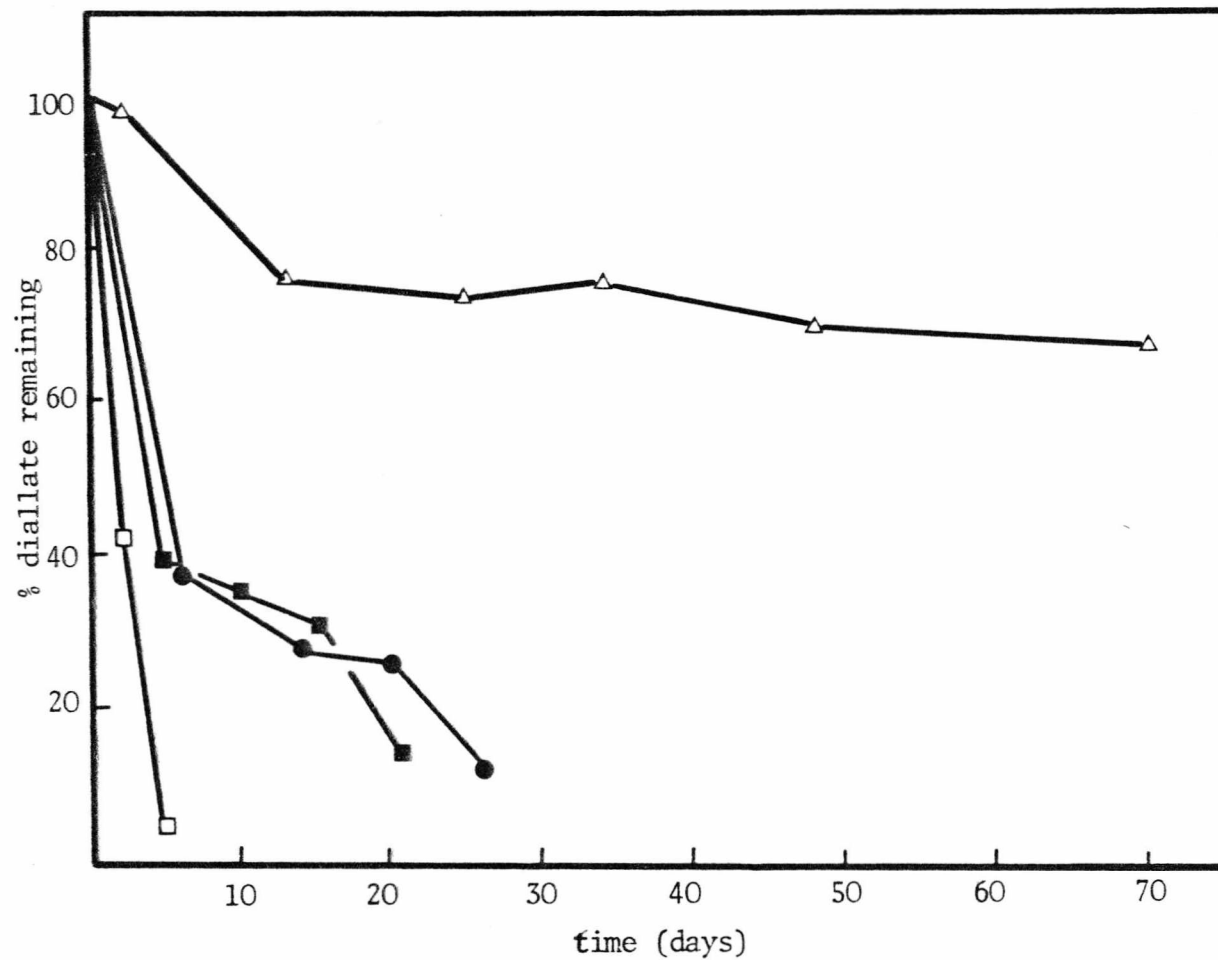
Diallate loss from glass surfaces and sterile and non-sterile organic
matter -   ● = non-sterile organic matter;   ■ = autoclaved organic-
matter;   △ = closed glass surface; .   O = open glass surface.
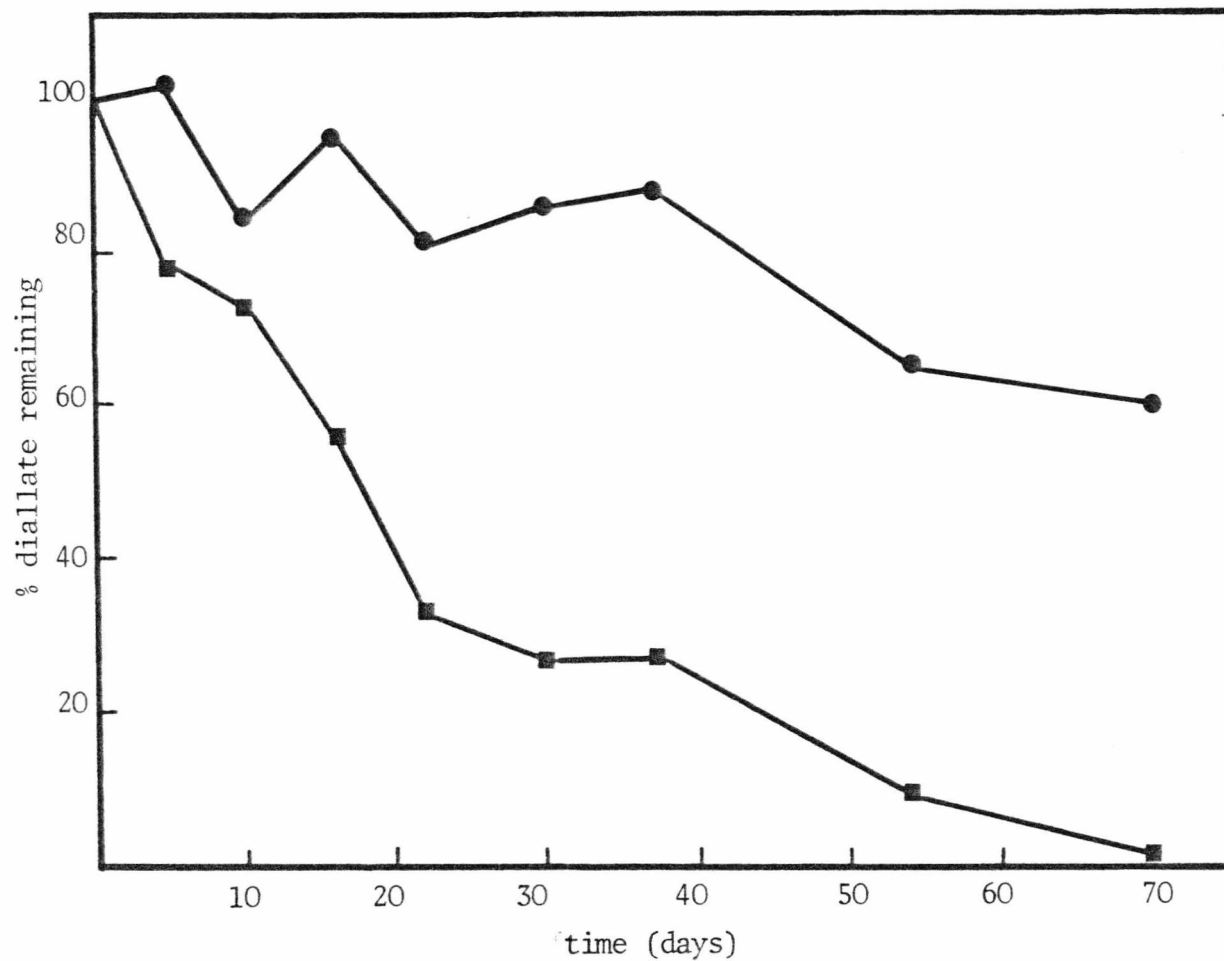
Diallate loss from autoclaved and sealed sand and organic matter systems.

● = sand; ■ = organic matter.

Fig. 24
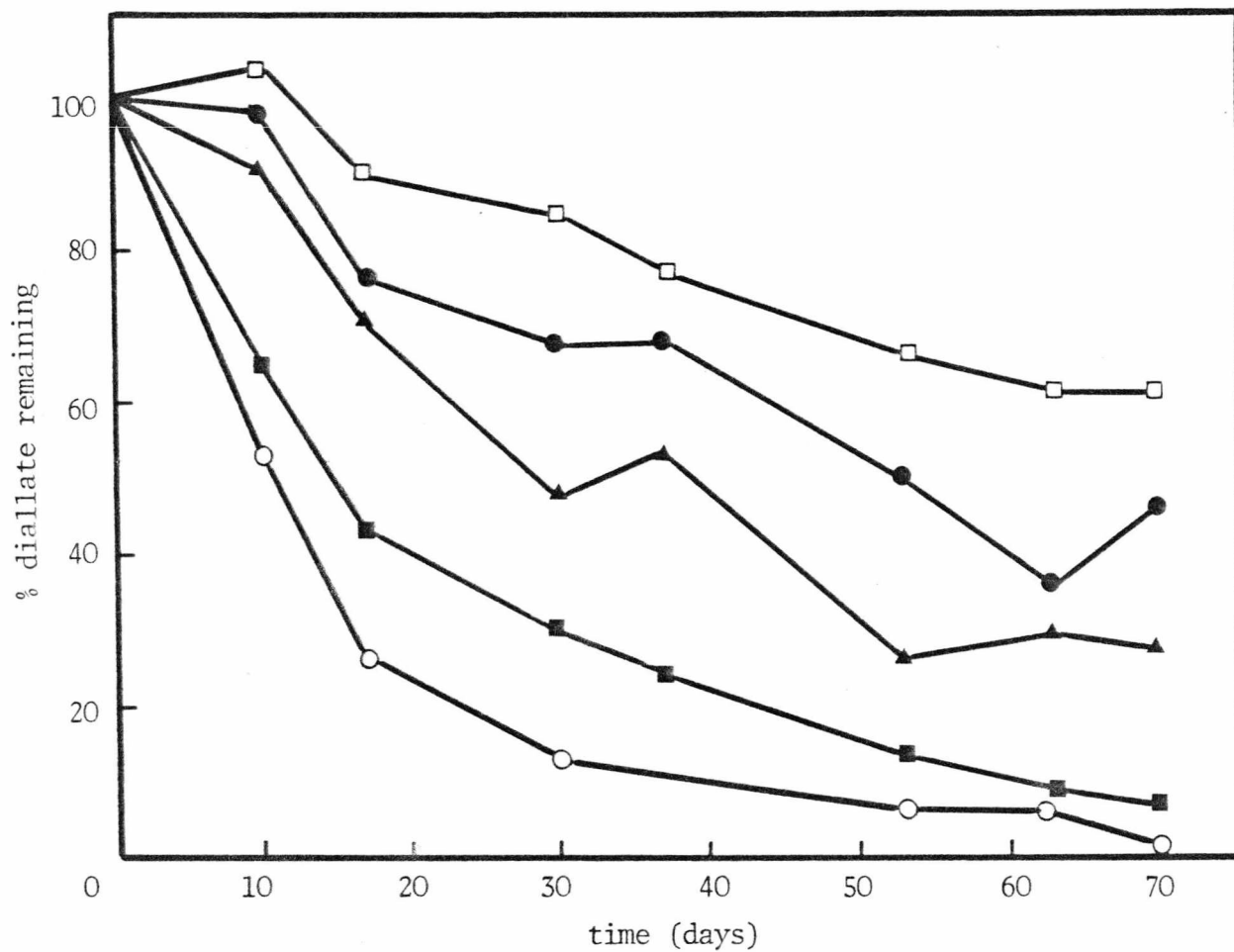
Fig. 25

Diallate loss from sand:organo-mineral complex artificial soils -

□ = organo-mineral (100%);   ● = organo-mineral (50%) : sand (50:);

▲ = organo-mineral (25%) : sand (75%);   ■ = organo-mineral (5%) :

sand (95%);   ○ = sand.

93.
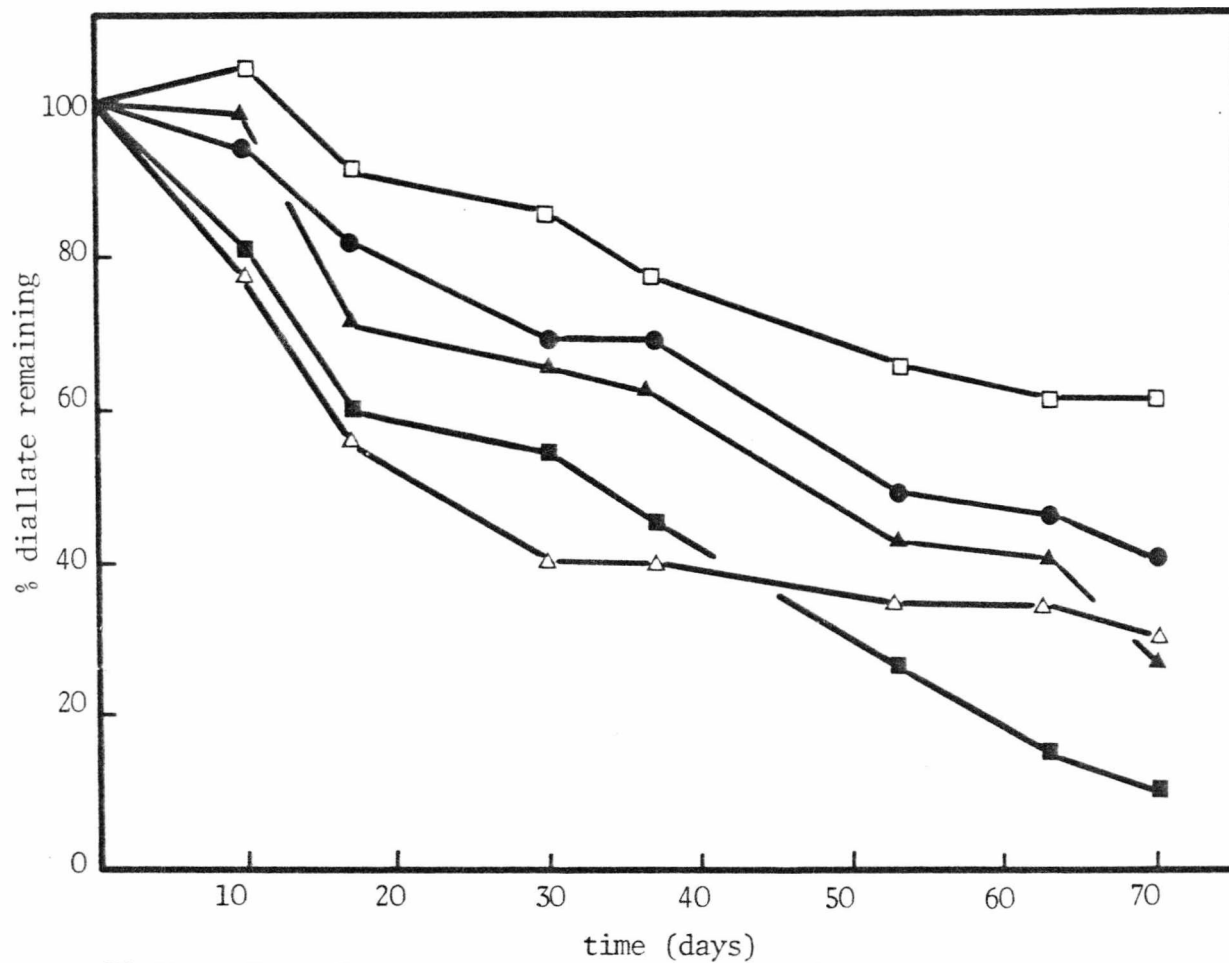
Fig. 26

Diallate loss from silt: organo-mineral complex artificial soils -

□ = organo-mineral (100%);   ● = organo-mineral (50%): silt (50%);

▲ = organo-mineral (25%): silt (75%);   ■ = organo-mineral (5%):

silt (95%);   △ = silt.

94.

organo-mineral complex) and from 22 days to 33 days (in silt:organo-
mineral complex). In the 3:1 mixture half-lives were extended from 11 to
27 days (in sand) and 22 days to 41 days (in silt). Finally in the 1:1
mixtures the half-lives were considerably extended, 11 to 53 days (in sand)
and 22 to 53 days (in silt). As the artificial soil increased in organo-
mineral content so the rate of loss decreased, gradually approaching that
of the 100% organo-mineral complex control. This effect was more marked
with the coarser sand component than with the finer particle sized silt
components.

4. Test soils.

Winchester, Barming and Hothfield Series soils were used as test soils.
Fig.27 (Appendix Tables 17 and 25) shows the loss of diallate from these
soils at $25^{O}C$, as compared with the Hamble soil. Half-lives for diallate
were 5, 15, 20 and 55 days for Winchester (50-60cm), Barming, Winchester
(0-20cm) and Hothfield soils respectively.

In contrast to the results expected from data on artificial soils,
there was no decrease in rate of loss as the organo-mineral complex
increased in any of these soils since the slowest rate of loss for diallate
was from the soil with the lowest organo-mineral content and the greatest
rate of loss from the soil with the highest organo-mineral content. Clearly
in these test soils other factors in addition to volatilization, as indic-
ated by the Hamble soil, were involved.
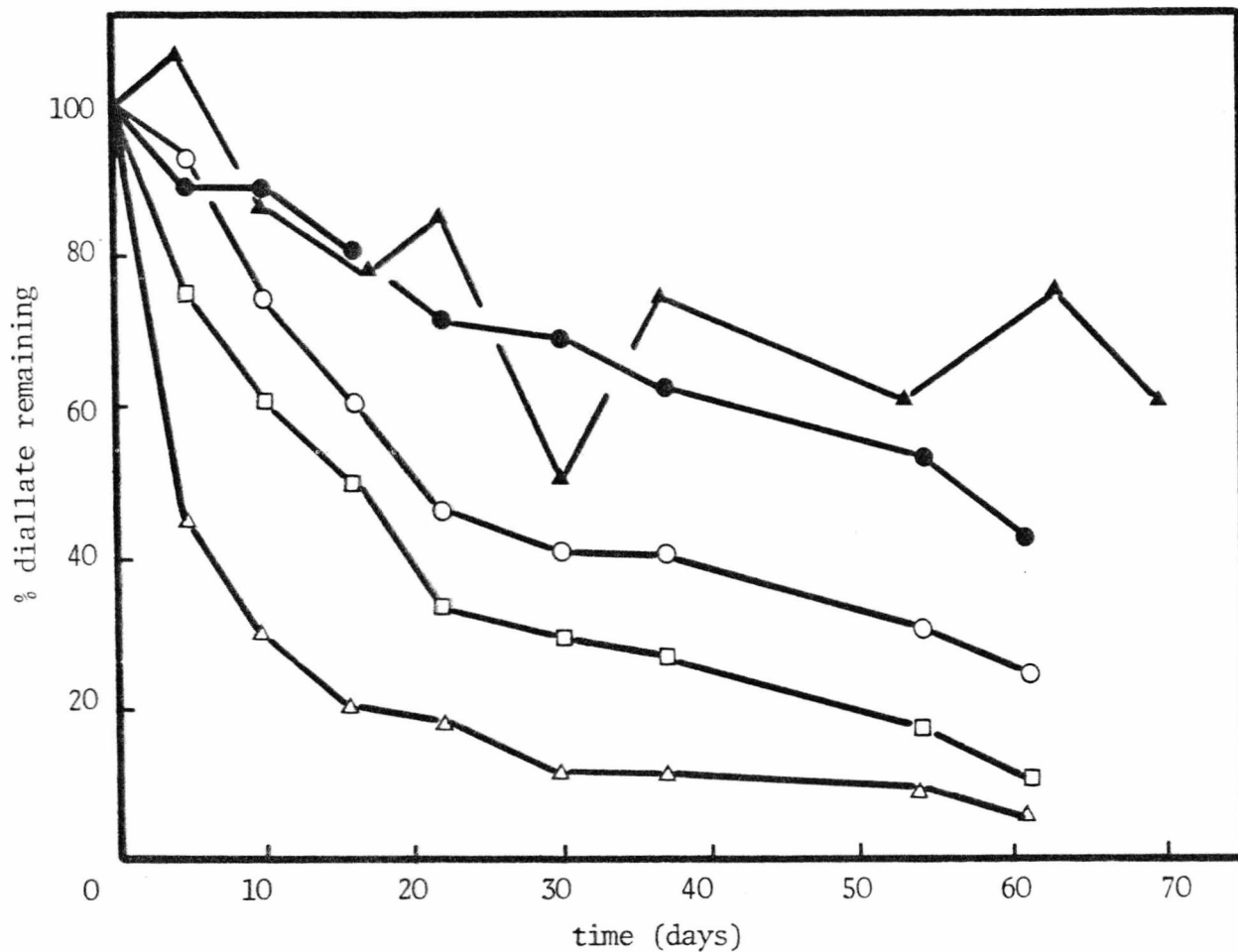
Diallate loss from Hamble soil and four test soils -
▲ = Hamble; □ = Barming;   O = Winchester (0.20cm); △ = Winchester (50-60cm);
● = Hothfield.

Fig. 27

B. Volatilization Experiments

I. Malathion

No malathion was detected from Hamble soil or its sand component under the experimental conditions previously described (p.61). Thus volatilization does not appear to be a significant factor with regard to malathion loss in these experiments, in agreement with Harris and Lichtenstein (1961).

II. Diallate

Volatilization of diallate was based on the method of Spencer and Cliath (1973) with modifications, as discussed previously (p.61). Volatilization was measured from Hamble soil and from the component with the largest particle size, i.e. the sand. These results are shown in Table 13. The results clearly demonstrate that diallate volatilized at a greater rate from the sand component than from the parent soil.

TABLE 13

Volatilization of diallate from soil and sand

Time (hours)	Soil (% loss)	Sand (% loss)
1	4.0	16.4
2	7.4	25.0
3	10.8	34.4
4	11.8	41.8
5	12.4	47.8

C. Adsorption Experiments

I. 2,4-D and Malathion.

No adsorption of 2,4-D was observed on the Hamble soil at pH 6.0 in the system studied.

The preliminary adsorption studies with malathion showed that none of this insecticide was adsorbed by the Hamble soil over the 5 hour equilibrium period. However, further experiments are needed to confirm this result, since reports of malathion adsorption in soils have been made (Macnamara and Toth, 1970).

II. Diallate

The results are expressed as the quantity of dillate adsorbed in μg/g soil plotted against equilibrium solution concentration in μg/ml (Fig.28). The mean value for percentage adsorption of diallate from Hamble soil as obtained from Appendix Table 26, being 41.3.

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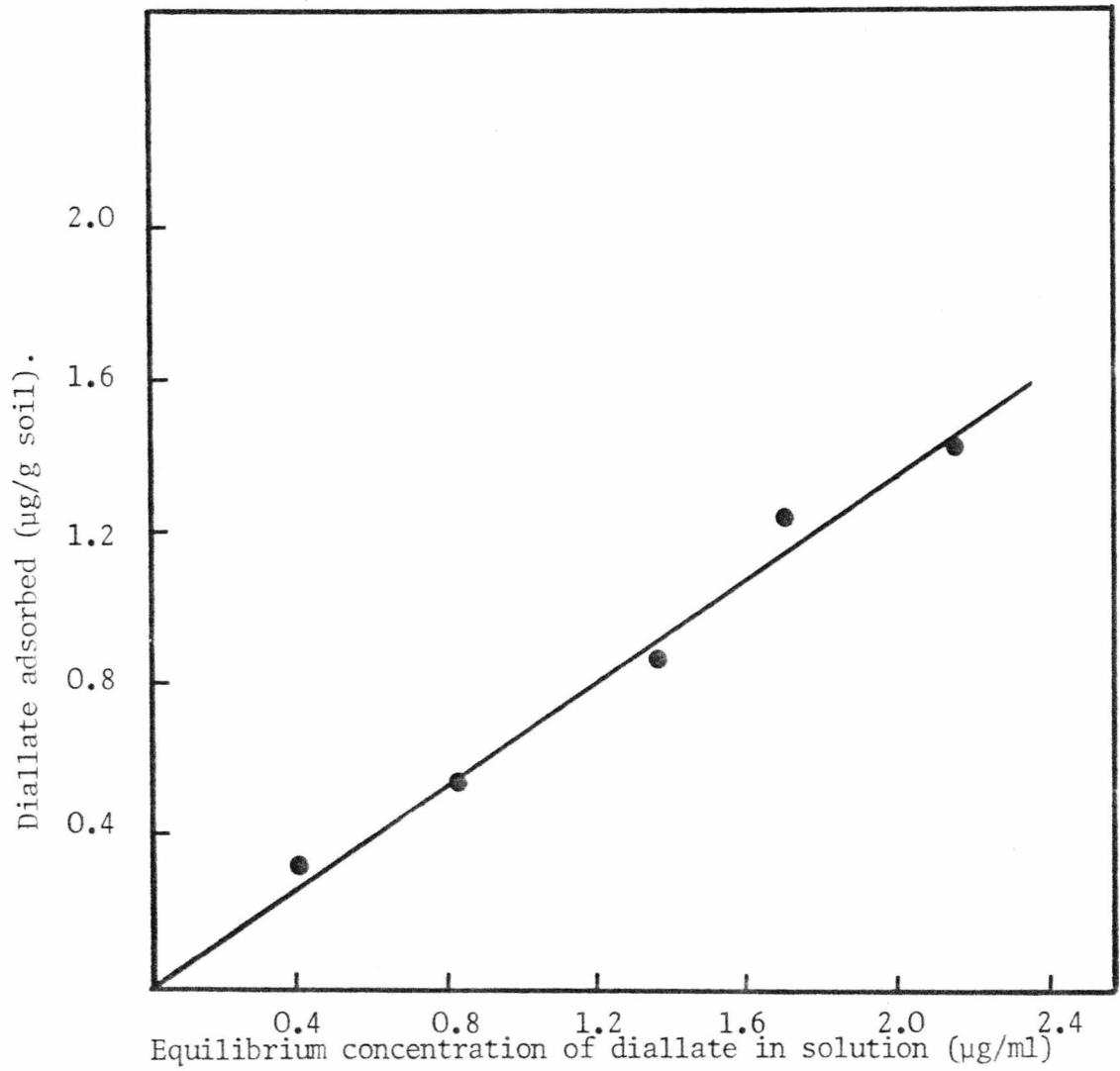
Fig. 28

Adsorption Isotherm of diallate in Hamble Soil

DISCUSSION

I.   Introduction

Pesticides disappear from the soil using a variety of routes (Burns 1975), all of which have been discussed earlier in this thesis. The *invitro* experiments reported here were performed in closed systems in the dark so as to eliminate losses through two of these routes, leaching and photodecomposition.

The three pesticides were selected on the basis of their contrasting chemical and physical properties, their differing mechanisms of loss as reported by other workers, and their relatively short persistence times in soil. The ultimate object was to obtain a range of detailed information concerning the relative importance of the major soil components in the decay of each pesticide. Experiments were directed towards using this information to measure disappearance rates from a variety of artificial and natural soils. The work discussed here sheds at least some light on these ambitous objectives and serves as an evaluation of a novel approach to the complex problem of soil-pesticide interactions.

II.   Statistical Treatment of Data

Detailed statistical treatment of the data is far from easy since only duplicate values were recorded; due to the difficulty in handling a large number of samples within the volume of experiments performed. Calculations of standard deviation or analysis of variance were not possible because the means are the result of only duplicate values. It was therefore decided to estimate variance using the t-test which is applicable to populations with unknown variances. Specifically, this method was used to test for significant differences between pesticide loss in different soils and components (and their respective sterilization treatments) at the 5% level.

The formula used is shown in {I}.

$$t = \frac{\bar{x}\ (-\mu)}{s/\sqrt{n}} \qquad \{I\}$$

where: n = number of values

$\bar{x}$ (mean difference) $= \frac{1}{n} \Sigma \times$

$S = \frac{1}{n-1} \left(\Sigma \times^2 - \frac{1}{n} (\Sigma \times)^2 \right)$

$\frac{S}{\sqrt{n}} =$ standard error

μ = zero (in our case)

An alternative method for plotting data graphically was to use a regression analysis. The data was tested for 'goodness of fit' to either cubic, quadratic, exponential or linear expressions, the theoretical shape of the former three parabolic forms being shown in Fig.29. The cubic and quadratic curves take into account any lag during the initial incubation period. The regression coefficients indicate the best fit; the theoretical ideal value being - 1.0. Rates of change (dy/dx), as determined by differentiation, are also shown (Appendix Tables 31-36). This enables a comparison to be made of malathion loss over any part of the experimental period.

Correlation coefficients were calculated using the formula {II} shown below.

$$rxy = \frac{N\Sigma XY - \Sigma X\Sigma Y}{\sqrt{N\Sigma X^2 - (\Sigma X)^2}\sqrt{N\Sigma Y^2 - (\Sigma Y)^2}} \qquad \{II\}$$

where N = numbers of pairs of X and Y

rxy = correlation coefficient.

III. Experiments on Hamble Soil

(i) 2,4-D

A — cubic
B — quadratic
C — exponential

y
axis

x axis
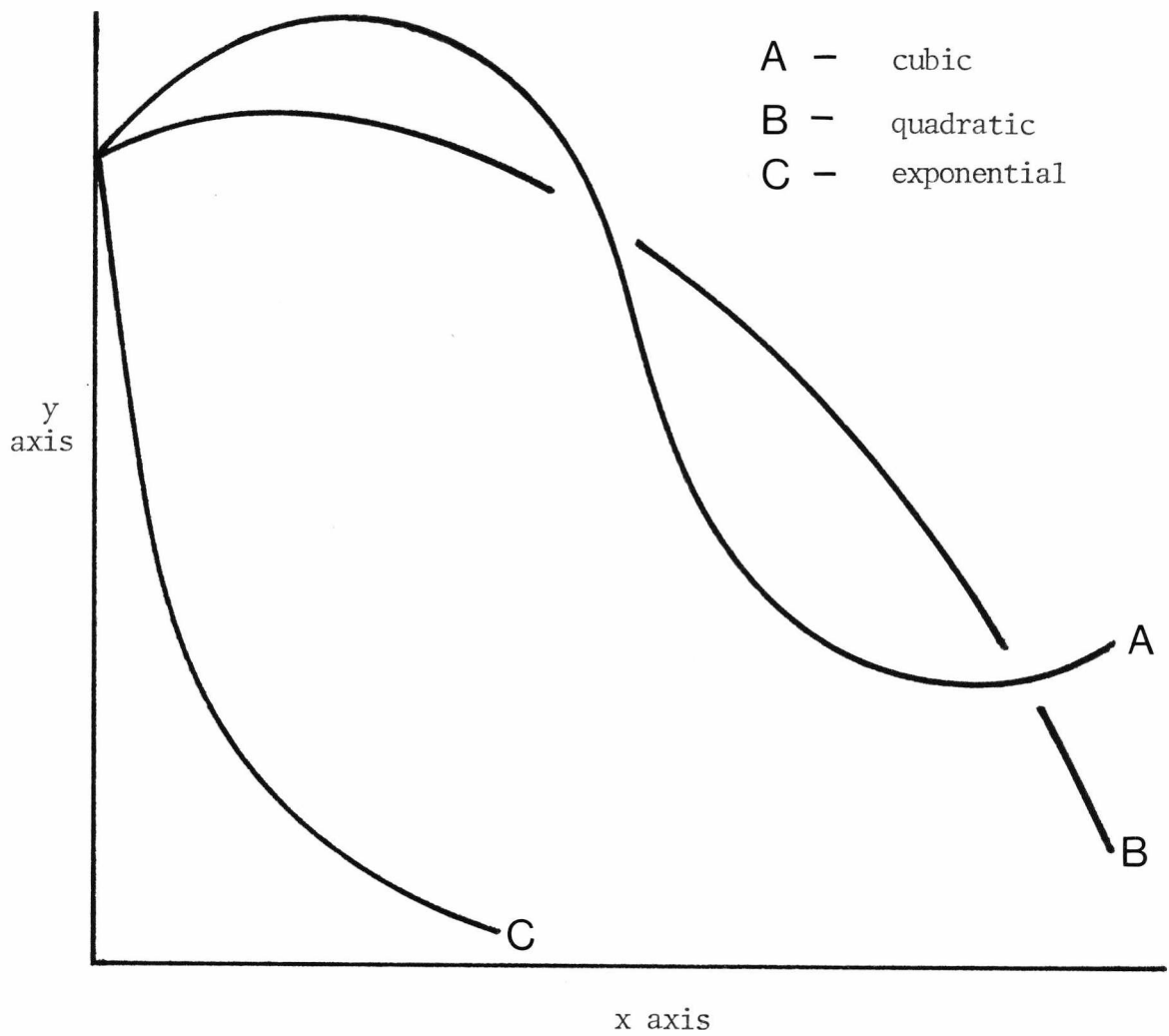
Fig.29

Theoretical cubic, quadratic and exponential regression curves

Fig. 3 shows that at 25°C rapid breakdown was preceded by a lag period. This is the typical pattern of 2,4-D degradation and has been demonstrated by many other workers. This temperature, together with the 65% w.h.c., would favour rapid microbial growth. At both 4°C and 37°C there was no breakdown, the obvious conclusion being that mesophilic microorganisms are the dominant degraders of 2,4-D in the Hamble soil.

The sterilization of soils using gamma-irradiation has been reviewed by Cawse (1975) and he suggests that levels of 1-2 Mrad can be effective in inactivating the majority of soil bacteria without drastically altering the physical and chemical properties of the soil environment. Since the effects of heat sterilization by autoclaving are more drastic, soil was sterilized using a variety of methods including gamma-irradiation and the addition of a bacteriostatic agent, sodium azide.

Loss of 2,4-D from all sterilized soils was negligible as shown in Fig. 4, there being no significant differences between any of the treatments. Dilution plate tests indicated that the soil remained sterile throughout the experimental period. Sterilization by autoclaving has been shown to greatly reduce the capacity of soil to degrade 2,4-D (Brown and Mitchell 1948; Hernandez and Warren 1950; DeRose and Newman 1948) and our results are in agreement with these workers. Experiments using the bacteriostatic agent, sodium azide, confirmed that the breakdown of 2,4-D was essentially a microbial process.

Experiments to restore the degradative ability of sterile soil using a soil inoculum were unsuccessful. Other workers have also experienced difficulty in the recolonisation of sterilized soil. Peterson (1962) found that *Myxococcus fulvus* would not re-establish itself in irradiated soil and grew poorly in an autoclaved sample. Jenkinson, Nowakowski and Mitchell (1972) observed that fresh soil inoculation of 2.5 Mrad irradiated soil

did not re-establish nitrification. In contrast, Peterson (1962b) demon-
strated that *Arthrobacter* spp, *Bacillus megaterium*, *Pseudomonas* spp, and
Xanthomonas upsicatoria could rapidly become established in irradiated
soil but that extended lag phases were often needed in autoclaved soils
prior to recolonization. Cawse (1975) states that it is difficult to
account for the nature of the inhibiting factor that is sometimes present
in heat-sterilized and radiation-sterilized soils. Stanier (1942) reported
that heat-sterilized glucose solutions inhibited cultures of *Cytophaga* spp
and Meiklejohn (1951) noticed a similar effect on nitrifying bacteria.
Schubert, Watson and White (1967) detected toxic hydroxy alkyl peroxides
produced by the interaction of hydrogen peroxide with the carbonyl compounds
occurring during the radiolytic breakdown of sucrose. Berry (1965) has shown
that when hexose sugars are exposed to 2 Mrad irradiation they develop cyto
toxicity. If this is the case the resistance of a sterilized soil to rec-
colonization may depend on the nature and amount of carbohydrates present.
Gupta (1967) has shown that glucose represents as much as 42-54% of the
total sugars present in some mineral and organic soils. Changes in the
composition of the soil solution have also been reported after steam
sterilization (Bowen and Cawse 1962; Eno and Popenoe 1964). Salonius,
Robinson and Chase (1967) reported that an autoclaved clay soil contained
more soluble organic matter, soluble carbohydrate and water extractable
electrolytes than did soil treated with 5 Mrad irradiation. Griffiths and
Burns (1968) described the depolymerization of microbial polysaccharides
in irradiated soil aggregates.

In addition to the possible appearance of inhibitors, our attempts to
recolonize sterilized soil may also have been unsuccessful simply because
the inoculum was too dilute to rapidly establish a viable microbial popul-
ation. It is possible, referring back to the work of Peterson (1962b), that

a longer incubation time would have revealed that the lag period for enrichment of 2,4-D degraders is considerably in excess of the 3 weeks allowed for this experiment.

(ii) Malathion

Loss of malathion occurred at all three temperatures, the rate being temperature dependent (Figs. 9, 30 and Appendix, table 31). Loss was exponential in all cases. With this breakdown pattern no lag period was noted suggesting either a lack of direct microbial involvement or the presence of an established population of malathion degraders. At 4°C bacterial proliferation would not be expected to occur yet significant loss was occurring from day 4 onwards. The rate of malathion loss at 25°C and 37°C was not significantly different over the 10 day period.

Breakdown of malathion has been investigated by various workers and the involvement of extracellular enzymes has been suggested by some workers. Breakdown of malathion was significantly affected by a low temperature; compare loss at 4°C with that at 25°C (Fig.9).

Breakdown at 4°C may be operating via extracellular enzymes since Getzin and Rosefield (1971) have provided evidence that a stable malathion degrading 'enzyme' is not inactivated at these temperatures. However, further evidence based upon the kinetics of malathion esterase activity at 4°C is needed before conclusive statements can be made. A purely chemical hydrolytic decay seems unlikely since no loss in sterile autoclaved soil was recorded, even at higher temperatures.

At 25°C no volatilization of malathion occurred, any loss being attributable to the degradative ability of the soil biological component. However, experiments to determine volatility losses at 37°C were not carried out so that any increase in rate of loss at this temperature may be partly due to volatilization as well as to an increase in extracellular

% malathion remaining

Malathion breakdown at 4°C (○); 25°C (▲) and 37°C (■)

time (days)
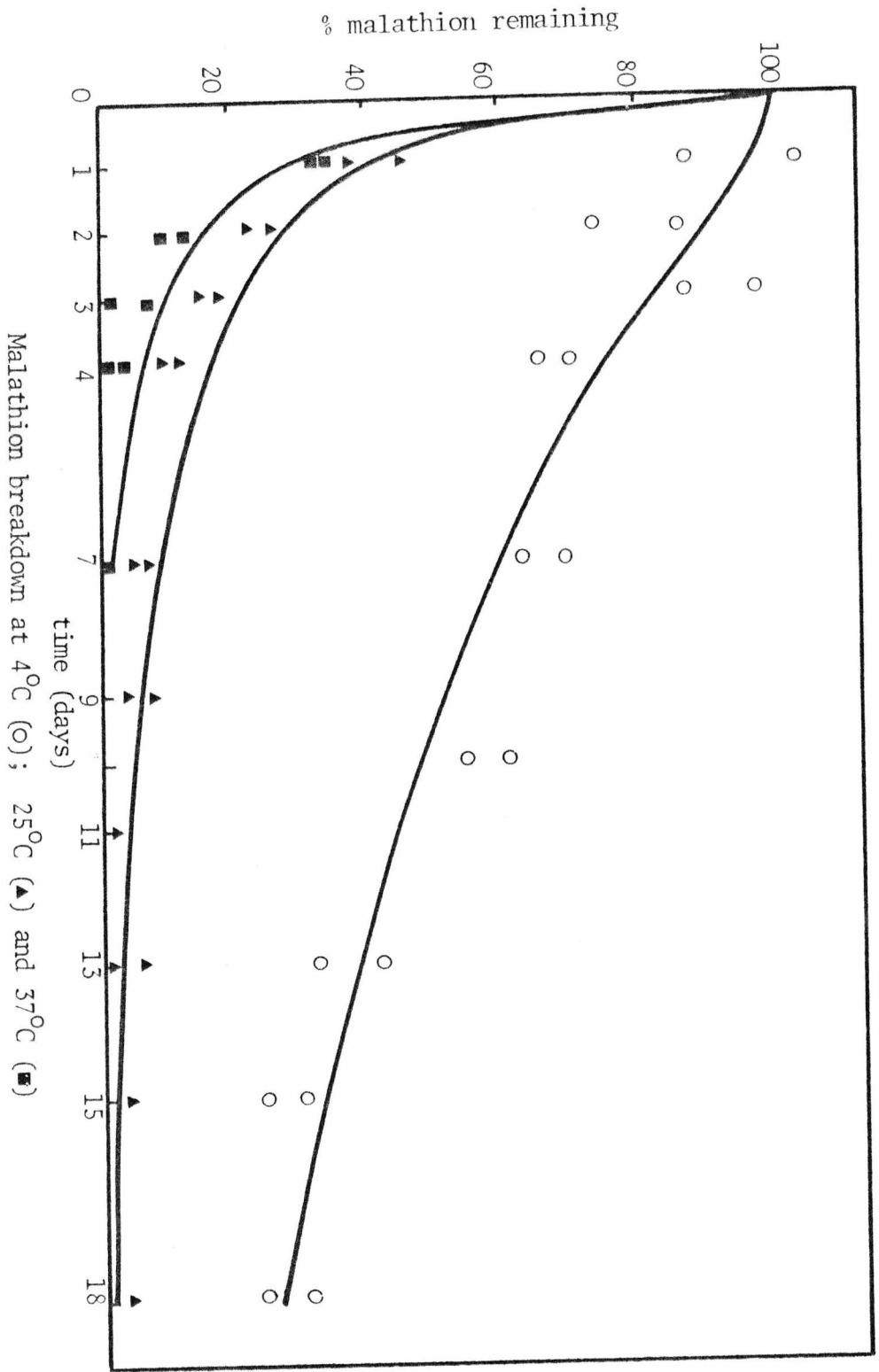
Fig. 30

enzymatic activity.

Heat sterilization prevented any major loss of malathion from soil, and what loss that occurred was linear over the experimental period (Figs. 10, 31 and Appendix table 31). Irradiation and chemical sterilization were ineffectual in preventing this loss. The rate of breakdown in irradiated soil (2.5 and 5.0 Mrads) showed an initial lag when compared to untreated soil. (Figs. 9 and 10). This suggests either the operation of microbial decay in the non-sterile soil or some denaturation of unprotected extracellular enzymes if microbial metabolism is insignificant. The loss of malathion in the sodium azide-treated soil was exponential, the rate being similar to that observed in non-sterile soil (Fig. 31). However, a significant difference between the % of malathion remaining in this and the nonsterile soil at $25^{\circ}C$ is seen, indicating at least the possibility of some microbial involvement in non-sterile soil. Although lag phases were discernable in the irradiated soils no significant differences between these soils and the non-sterile soil were observed over the 9 day period. Differences between 2.5 Mrad and 5.0 Mrad were not significant and the only significant treatment was autoclaving.

These results suggest that direct microbial degradation is not a major factor in malathion decay since the removal of the microbial population does not have a significant effect on malathion loss. Other workers, such as Konrad *et al* (1969); Spiller (1961); Cowart *et al* (1971) and Faust and Suffet (1966), have demonstrated a non-biological hydrolytic decay which may be contributory to breakdown in this instance. However, the loss of degradative ability in autoclaved soils tends to favour an alternative explanation since other workers have indicated that the rate of hydrolysis is dependent on pH. The change in pH due to autoclaving was not great enough to produce a significant reduction in the rate of hydrolysis.
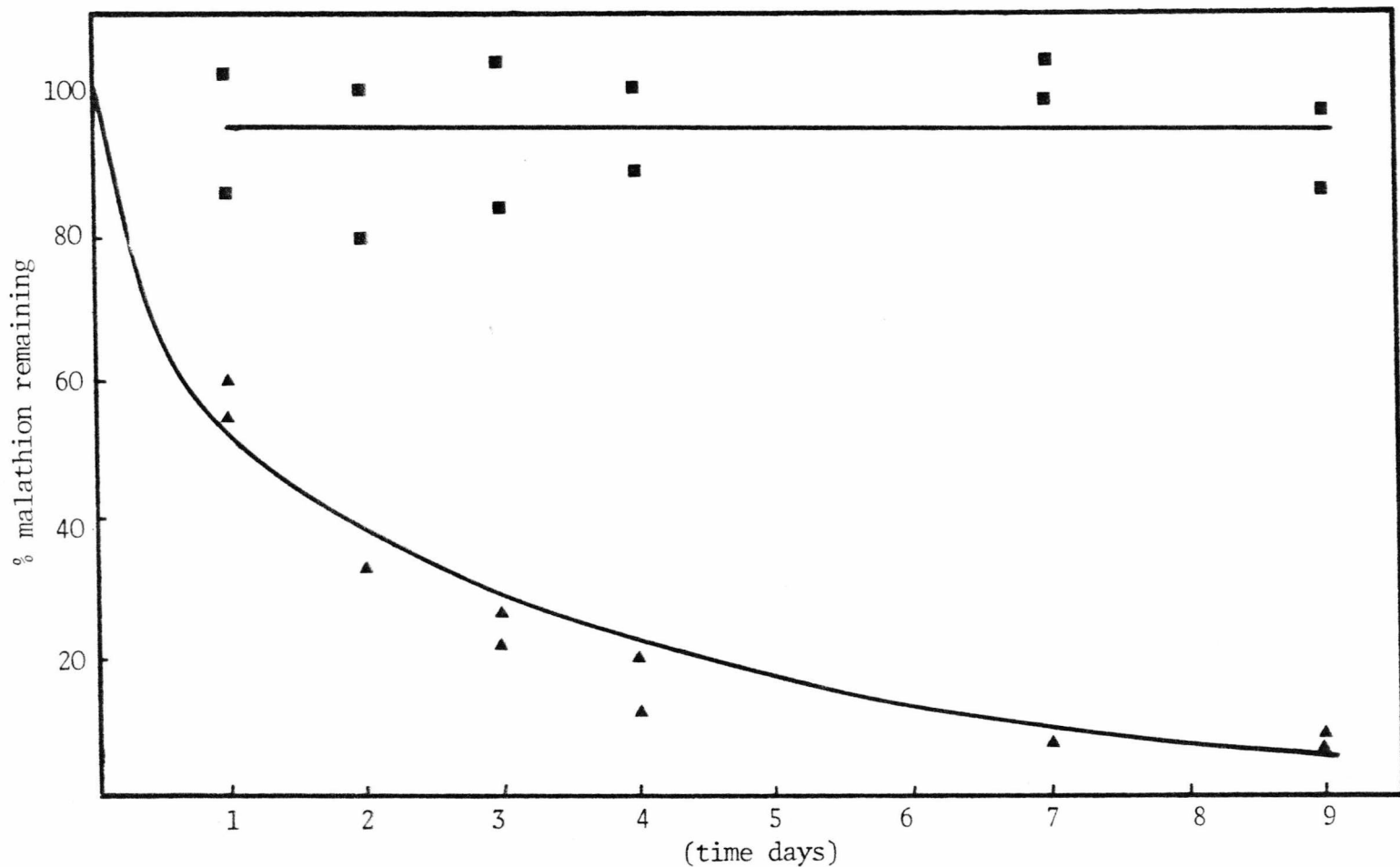
Malathion breakdown in autoclaved (■) and sodium azide treated soil (▲).

Fig. 31.

Skipper and Westerman (1972) recorded decreases as little as 0.2 units after autoclaving. Also the Hamble soil has an acid pH whereas most reports agree that an alkaline pH is important to the hydrolytic decay of malathion.

It has been reported that malathion adsorption onto clay can catalyse hydrolytic decay (Polon and Sawyer 1962) and it is arguable that the presence of an undisturbed (non-autoclaved) clay structure may be required to facilitate this in the Hamble soil.

Studies on the isolated soil components were performed in order to elucidate further the mechanisms of degradation in soil. Results on the unaltered clay fraction (after removal of organic matter) suggest that if hydrolysis is catalysed by unaltered clay then it is occurring at a slower rate than in the parent soil. The results suggest that with the elimination of both microbial metabolism and non-biological hydrolysis another factor is playing a dominant role in malathion decay. If this is so then the properties of this factor include heat lability and irradiation resistance (2.5 and 5.0 Mrads). These characteristics are supportive of the idea of a stable exoenzyme soil component, previously described by Getzin and co-workers. In a broader context, the activity of many soil enzymes shows little decline after a radiation dose sufficient to remove microbial activity (McLaren *et al* 1957, 1962, 1969). Studies on one soil enzyme urease, have indicated a two tier response to increasing irradiation dosage (Pettit *et al* 1976). These workers believe that the initial decline in urease activity is due to the cessation of microbially induced substrate hydrolysis and the denaturation of the unprotected exoenzyme component. Further declines in activity have been suggested as due to the destruction of the colloidal organic matter which protects a proportion of the extracellular urease. Experiments at similar irradiation doses, (2.5, 5.0. 10.0, 15.0 and

20.0 Mrads) were carried out and malathion degradation shows a similar two-tier response. These results (Figs. 11, 32-36 and Appendix table 32) show that at 10, 15 and 20.0 Mrads the rate is much slower than at 2.5 and 5.0 Mrads and provides further indirect evidence for the involvement of an extracellular enzyme in malathion decay. It might follow from this that the enzyme responsible may occupy a similar niche to urease in the soil micro-environment. The influence of irradiation on soil enzyme activities has been discussed in recent reviews by Cawse (1975); Kiss (1975) and Skujins (1976).

In order to investigate further the nature and association of the biological agent involved in malathion degradation experiments were performed using the separated soil components. It was hoped that the resulting information, together with that derived from the parent soil,would form a basis for the prediction of malathion behaviour in different soil types.

(iii) Diallate

Evidence for the volatile nature of this herbicide, together with examples of its microbial decay *in vitro* have been reported by other workers. However, information about diallate loss from soil is limited. Fig. 19 demonstrates the effect of temperature on diallate loss from soil, which was negligible at $4^{o}C$ over the 70 day period. However, higher temp-eratures do have a significant effect although there was no lag phase prior to loss at any temperature. At $37^{o}C$ a linear decline of 60% was observed up to day 30 with only a further 20% being lost subsequently. A similar, although less marked trend, occurred at $25^{o}C$.

Experiments with sterile soils showed that at $25^{o}C$ there were no significant differences between the non-sterile and sterile soils. The pattern of loss at $37^{o}C$ in autoclaved soil was similar to that in non-sterile
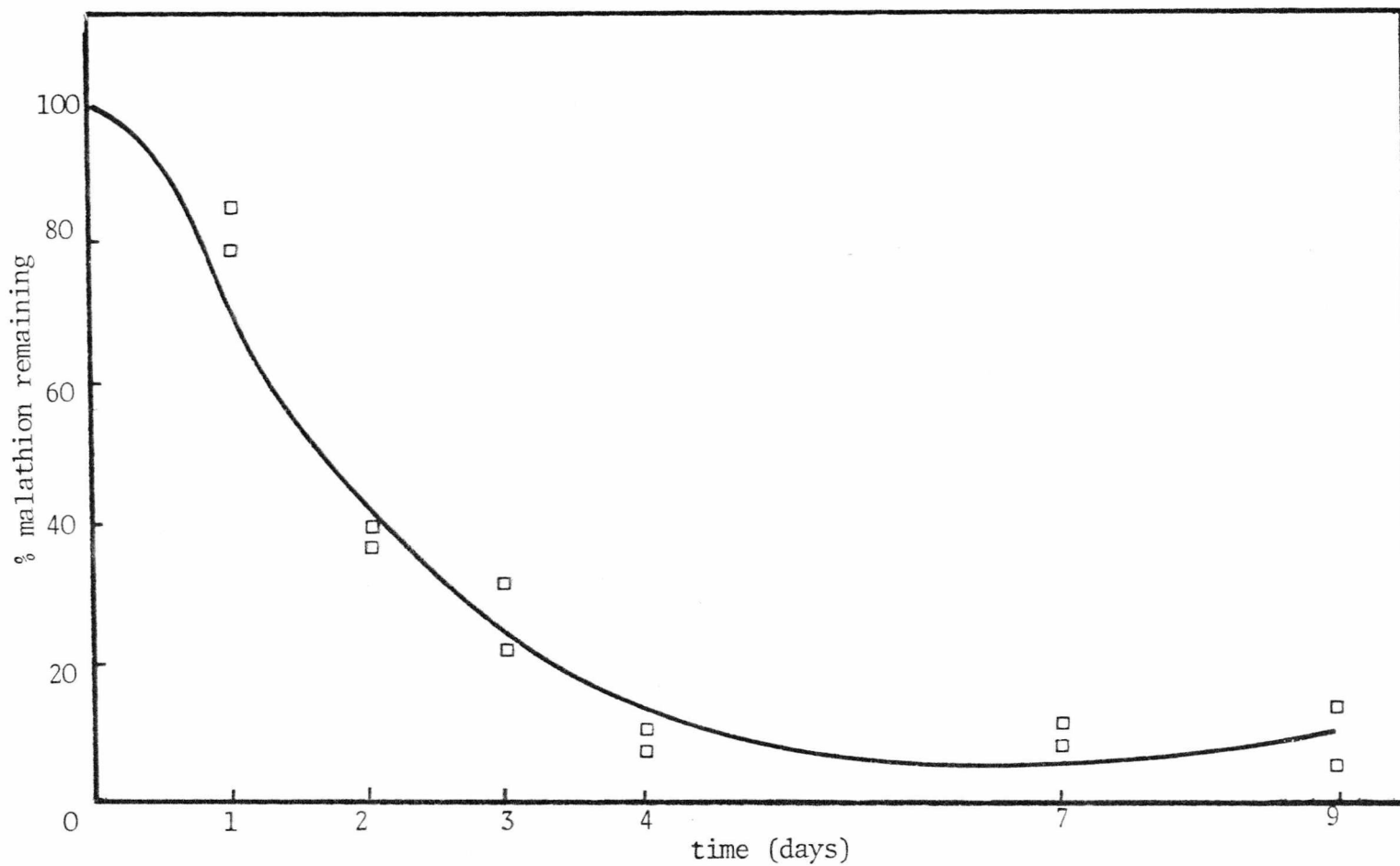
Malathion breakdown in 2.5 Mrad irradiated soil

Fig. 32
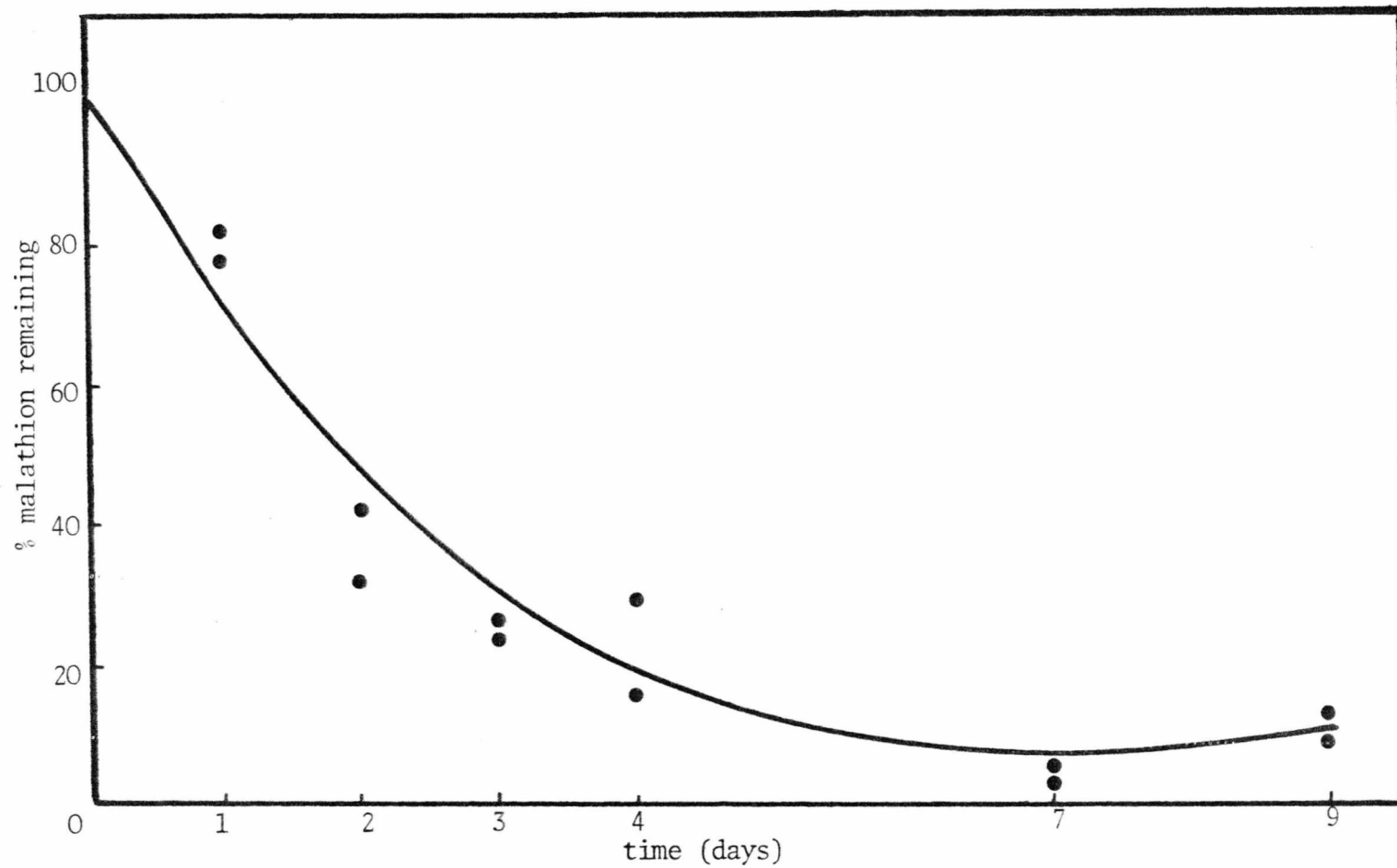
Malathion breakdown in 5.0 Mrad irradiated soil

Fig. 33
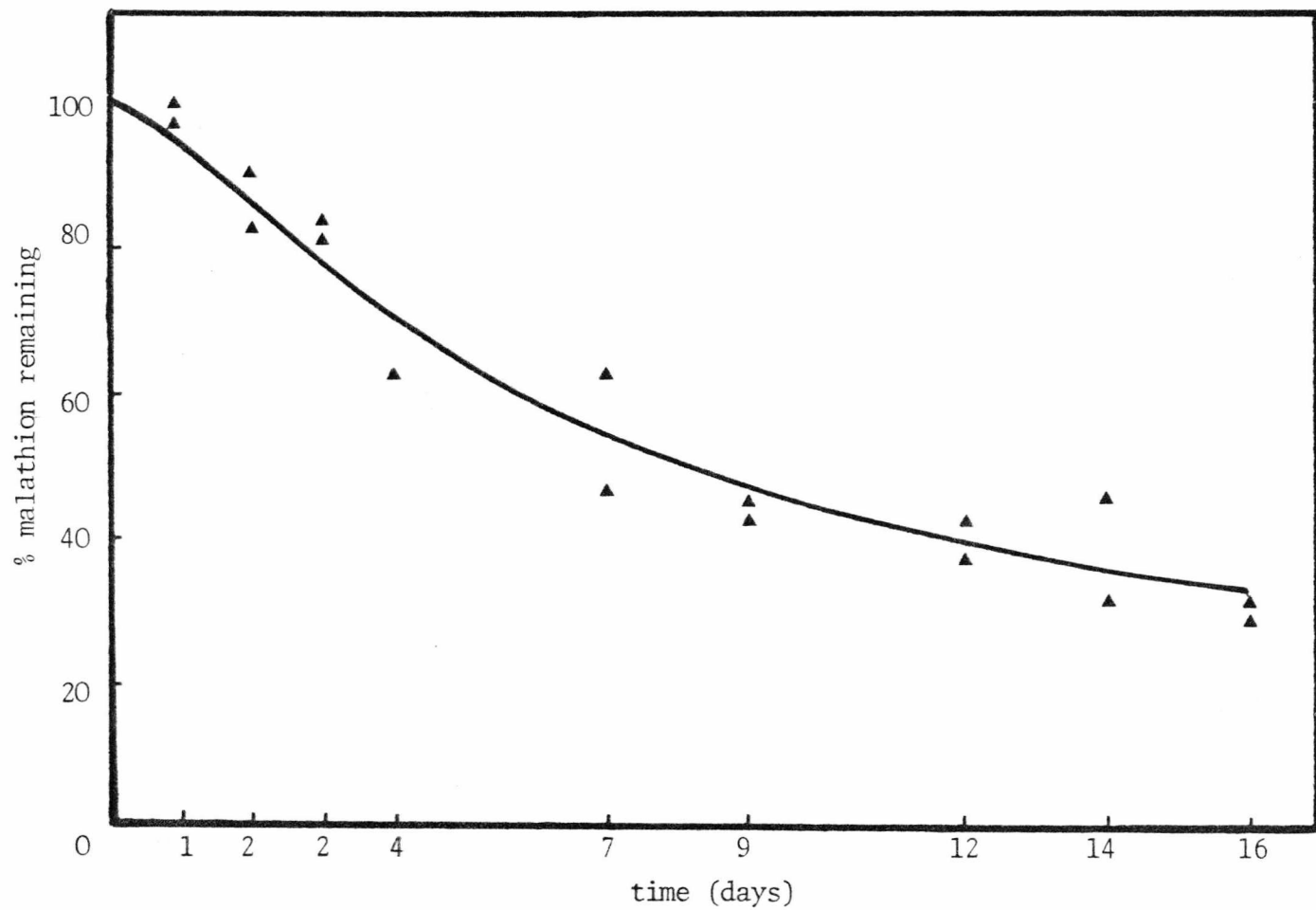
Fig. 34

Malathion breakdown in 10.0 Mrad irradiated soil.
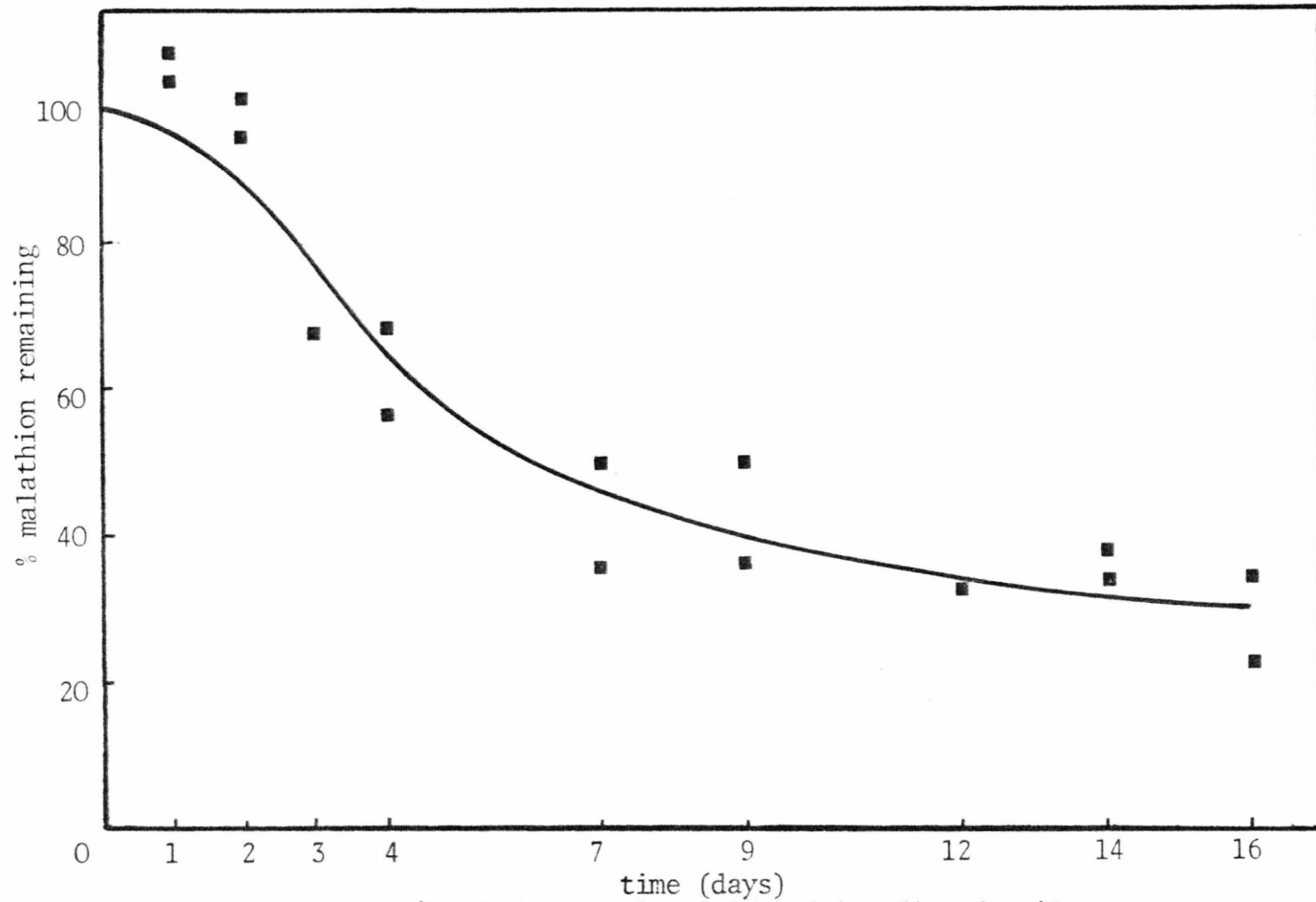
Fig. 35.

Malathion breakdown in 15.0 Mrad irradiated soil.
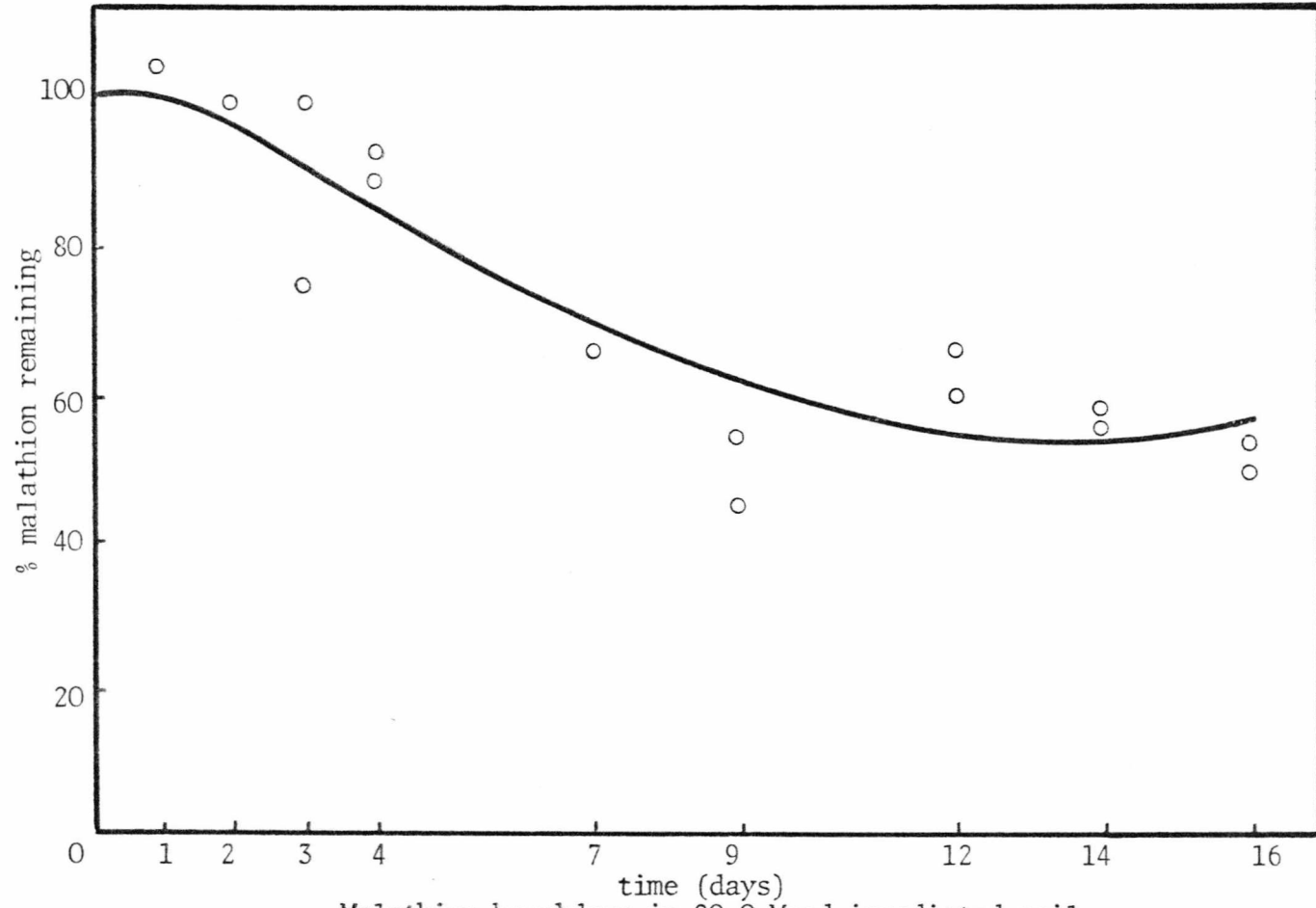
Fig. 36

Malathion breakdown in 20.0 Mrad irradiated soil.

soil, although a significant difference was recorded between the two. There may be a contribution by the microbial population to diallate loss at this higher temperature but it seems more probable that experimental variation could account for the slight differences.

These results favour either volatilization or chemical decay as major routes of diallate loss in Hamble soil. Loss from the various soil components was investigated to determine further the primary mechanism since it is less clear from experiments on the parent soil than it was with 2,4-D and malathion.

From a comparison of the loss of these three pesticides from Hamble soil it appears that different mechanisms predominate in each instance. The primary degradative agent in the case of 2,4-D was microbial; malathion breakdown appeared to be related to a stable exoenzyme whilst volatilization or chemical hydrolysis were responsible for diallate loss.

IV. Soil Components

(i) 2,4-D

On the preliminary results one could hypothesise that 2,4-D breakdown was predominantly associated with the soil fraction with a high surface area and possessing sufficient carbon and nitrogen to support an active microbial population. All the separated soil components, with the exception of the clay, retained a microbial population (Table 8) the counts varying from 1.30 to 15700×10^{-3} bacteria g.soil^{-1}. The microbial population was resistant to the sonication treatment used for separating the soil components. Clearly the largest population was associated with the fraction containing the highest organic matter content, the organo-mineral complex.

The organo-mineral complex was

in fact the only fraction to effect 2,4-D decay (Fig.5). It is possible that, on separation of the soil components, the microbes associated with the organo-mineral complex were the least disturbed but that those remaining in the coarser fractions, i.e. sand and silt, were unable to metabolize 2,4-D in their new environment. Growth on nutrient agar does not necessarily imply that the microorganisms are active in the medium from which they were isolated. Additionally, the lower microbial population may require a longer lag period to enrich for 2,4-D degraders, than was allowed for in these experiments. Perhaps, surprisingly, no breakdown was recorded in the organic matter extract, even though it contained microorganisms and was extended to 40 days. This indicates the possible importance of the physical association between organic matter and clay in providing the correct micro-environment for microbial growth. Alternatively, the extraction procedure may in some way have affected the enrichment procedure by providing an unsuitable growth medium for 2,4-D degraders in this extract.

All batches of parent soils used in these experiments exhibited a lag phase of 8 days although variations were observed when monitoring breakdown in the organo-mineral complex. The shorter lag phase may have been due to the increased density of microorganisms compared to the complete soil. In addition, without the diluent effect of sand and silt, the diffusion of the 2,4-D substrate to the site of microbial proliferation may have been considerably faster. Akamine (1951) and others have noted that bacterial numbers were related to 2,4-D persistence, high counts leading to a more rapid loss than low counts. The clay fraction, free of organic matter, lacked 2,4-D degradative ability because of the removal of microorganisms during the harsh extraction procedure. 2,4-D was not degraded at all in the sand and silt components over the experimental period of 18 days. These organic fractions had the lowest percentage carbon and nitrogen levels and

very low cation exchange capacities; all factors indicating unsuitability for microbial proliferation. The alteration in the ecological balance of the natural soil environment may preclude the development of 2,4-D degraders, even if they survive the sonication treatment. The ability of the organo-mineral complex to retain a viable 2,4-D degrading population during fractionation suggests that this component exerts a protective effect on its resident microflora.

No breakdown occurred in autoclaved soil components (Fig.6) providing additional evidence for the purely microbial decay of 2,4-D in Hamble soil.

(ii) Malathion

The loss of malathion from the organo-mineral complex was significantly faster than from the parent soil; $\frac{3}{4}$ day and 1 day respectively. The degradative pattern on the organo-mineral complex was of the exponential type (Fig. 37) the same as it was in the parent soil; the data on the rate of degradation is given in Appendix table 33. The sand and silt fractions were significantly less effective in degrading malathion and required a 3-4 day lag period before the onset of degradation, the pattern of loss being expressed by cubic regression coefficients (Figs.38 and 39). Unlike 2,4-D the loss of malathion on these inorganic fractions occurred at significantly different rates.

The presence of a lag period implied that microbial enrichment was taking place and thus, together with the information that a microbial population is associated with these fractions, provides indirect evidence of microbial breakdown. The clay fraction exhibited an even slower rate of malathion decay (Figs. 12, 37 and Appendix table 33) than the other fractions. The silt had a larger microbial population than did the sand and this alone may have accounted for its greater efficiency in degrading malathion and the shorter

Malathion breakdown in non-sterile clay (■) and
organo-mineral (□) soil components.

Fig. 37

Fig. 38

Malathion breakdown in the non-sterile sand component

Fig. 39

Malathion breakdown in the non-sterile silt component
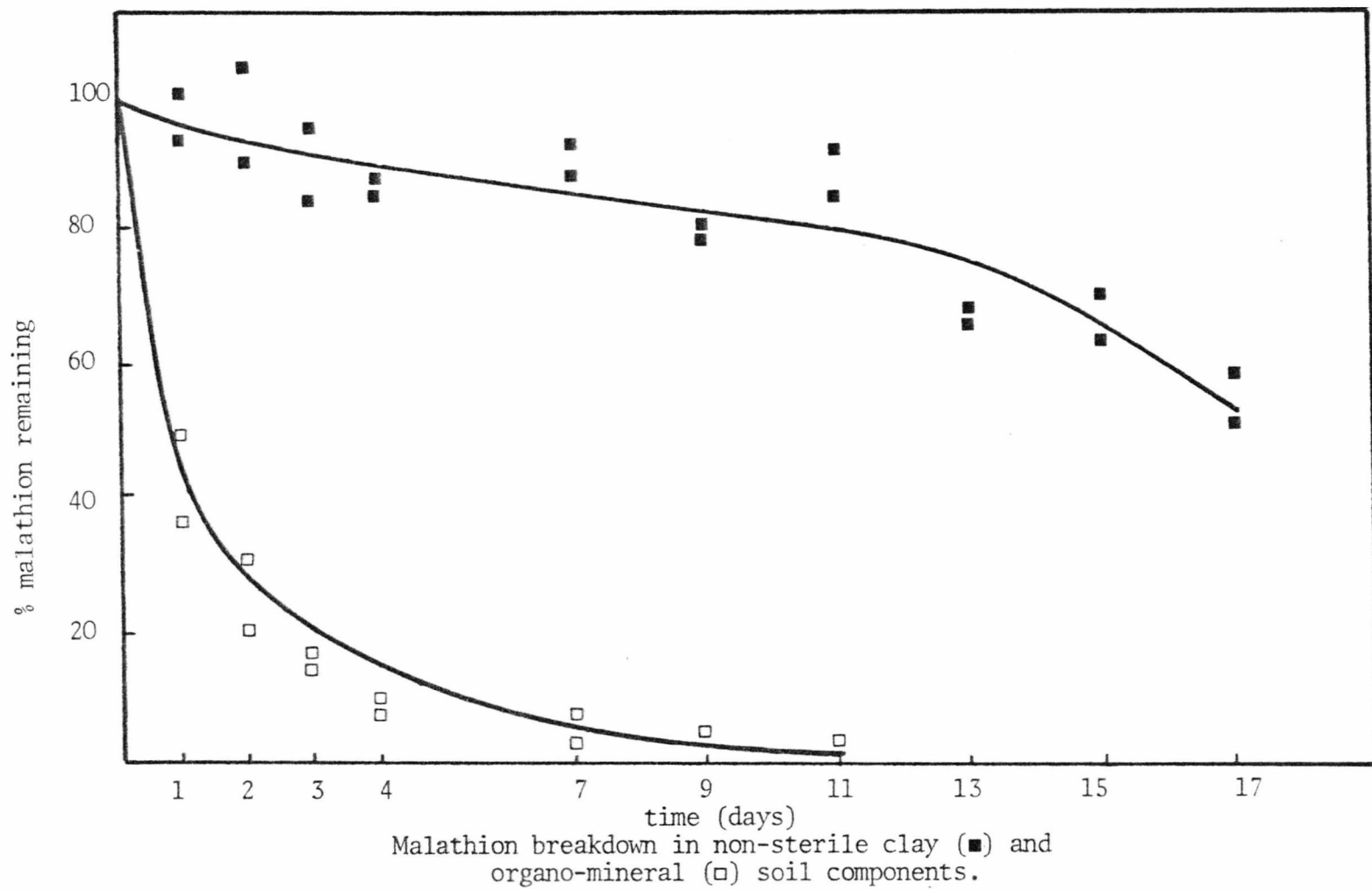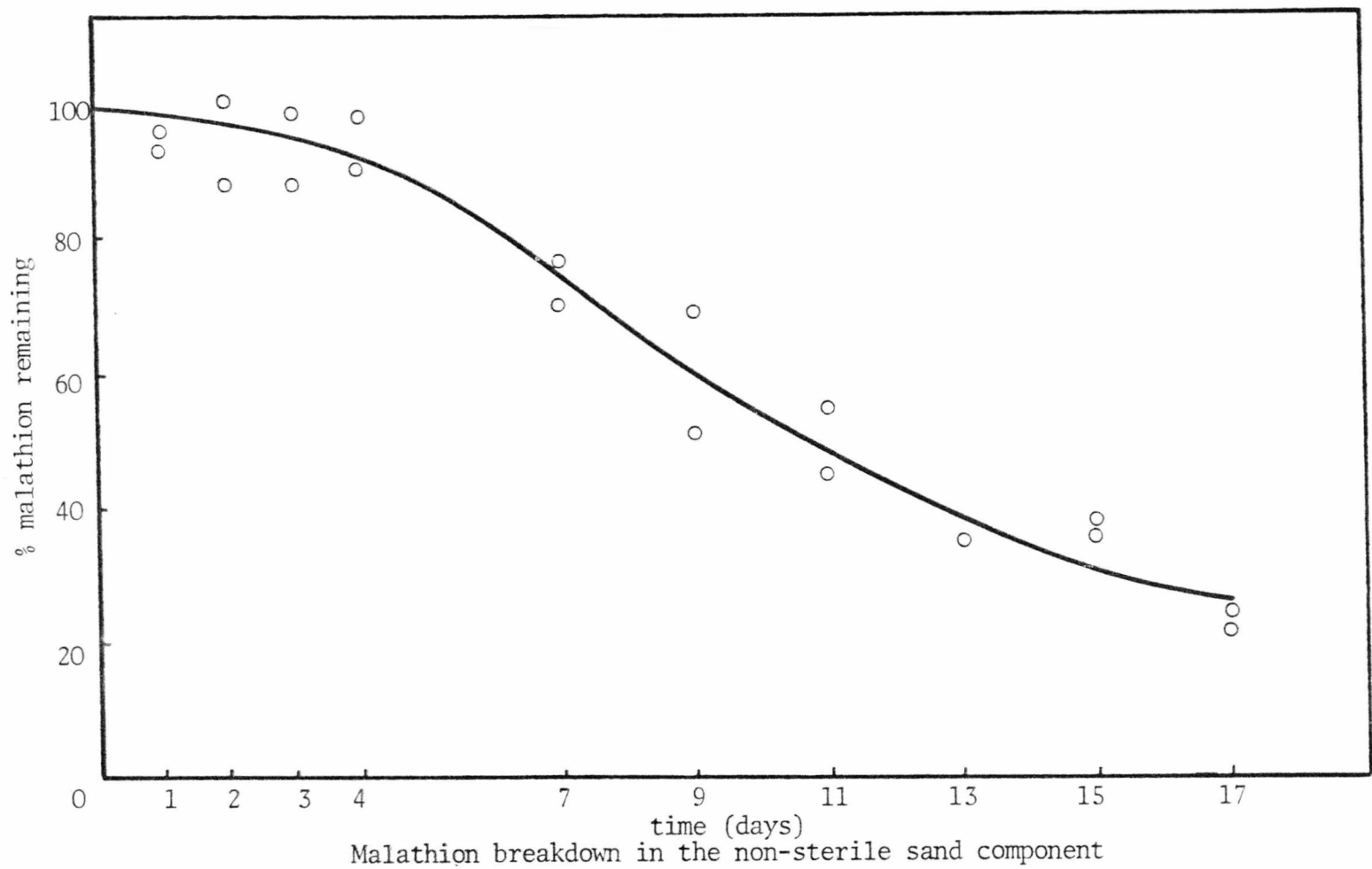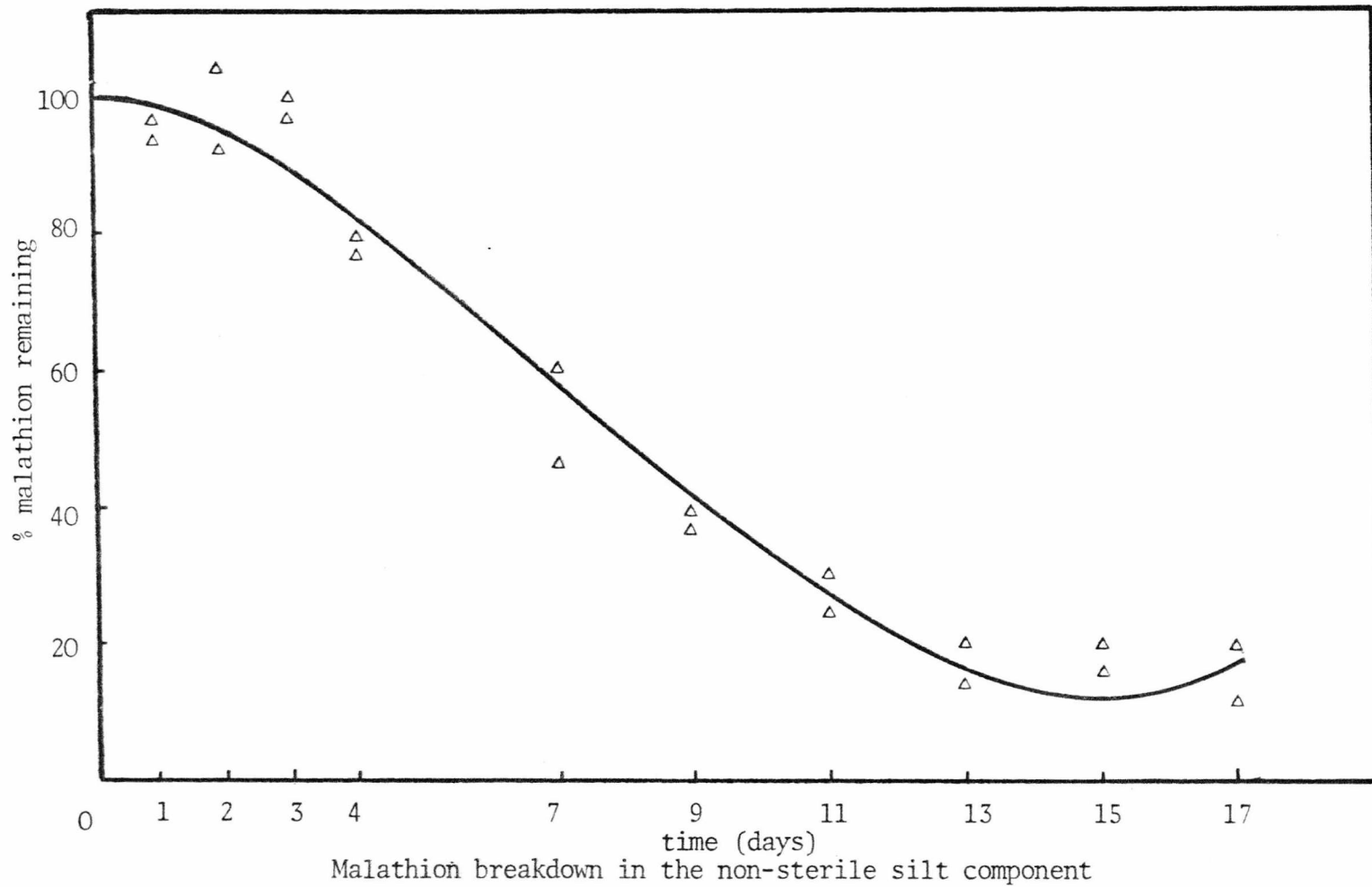
lag period. Haig (1955) mentioned the fractionation of a sandy loam to obtain information on the localization of enzymatic activity on soil particles. The results obtained showed that the greatest activity was associated with the organo-clay fraction, considerable but less activity with the silt fraction and very little with the sand fraction. Hoffmann (1959), after a similar separation process, showed urease activity to be greatest in the organo-clay fraction.

The removal of organic matter and the subsequent decline in malathion degradation on the residual clay fraction suggests that the catalytic agent is associated with the organic matter. Getzin and Rosefield (1971) provided evidence to show that a catalytic agent responsible for malathion decay was extractable with methods used for the extraction of organic matter from soils and suggested that it was this fraction that contained the extracellular enzyme. This is again consistent with the interpretations of Pettit *et al* (1976) concerning the location of another soil enzyme, urease. The slow decay of malathion observed on the clay fraction was probably due to a non-biological hydrolysis considering the alkalinity of the system, the lack of any distinct lag phase and the sterility of the fraction.

The effect of autoclaving (Figs. 13, 40-43 and Appendix table 34) dramatically reduced the degradative ability of all the soil components. Figs. 40-43 clearly show the linear nature of malathion decay. The linear regression coefficients are acceptable for these components and help produce a clearer picture of the behaviour of malathion than that shown in Fig.13. A significant difference in malathion loss from the clay fraction is observed when examining the linear pattern of degradation (autoclaved) and the cubic pattern of degradation (non-autoclaved) on the clay components. This difference is not easily accounted for at this stage unless one assumes that an unaltered clay structure is required for hydrolysis at this pH. Further
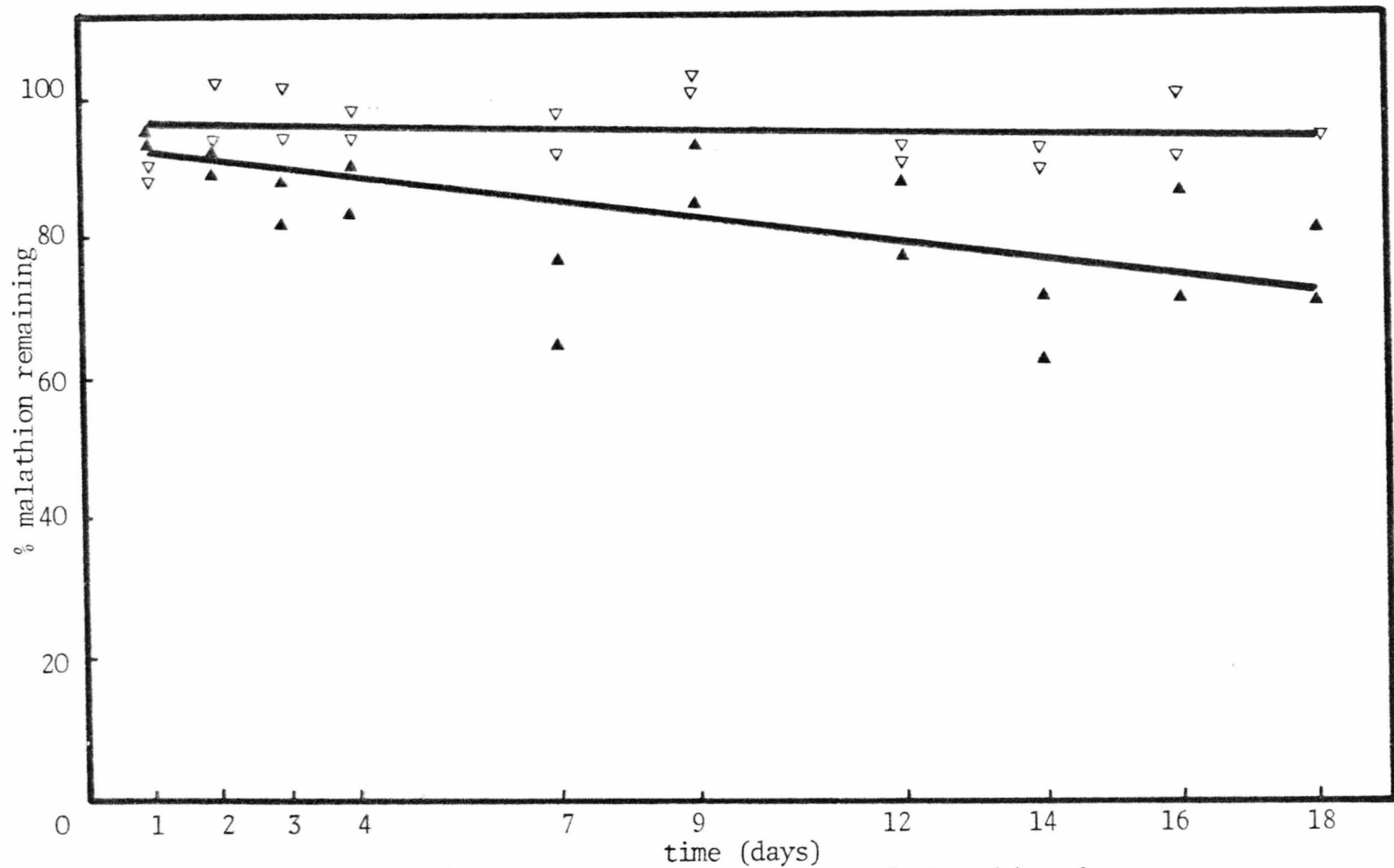
Fig. 40

Malathion breakdown in the autoclaved clay (▽) and
silt (▲) soil components.

Fig. 41

Malathion breakdown in the autoclaved organo-mineral component

Fig. 42

Malathion breakdown in autoclaved sand component

Fig. 43

Malathion breakdown in sterile and non-sterile organic matter

● = non-sterile; ▲ = filtered; ■ = autoclaved

126.
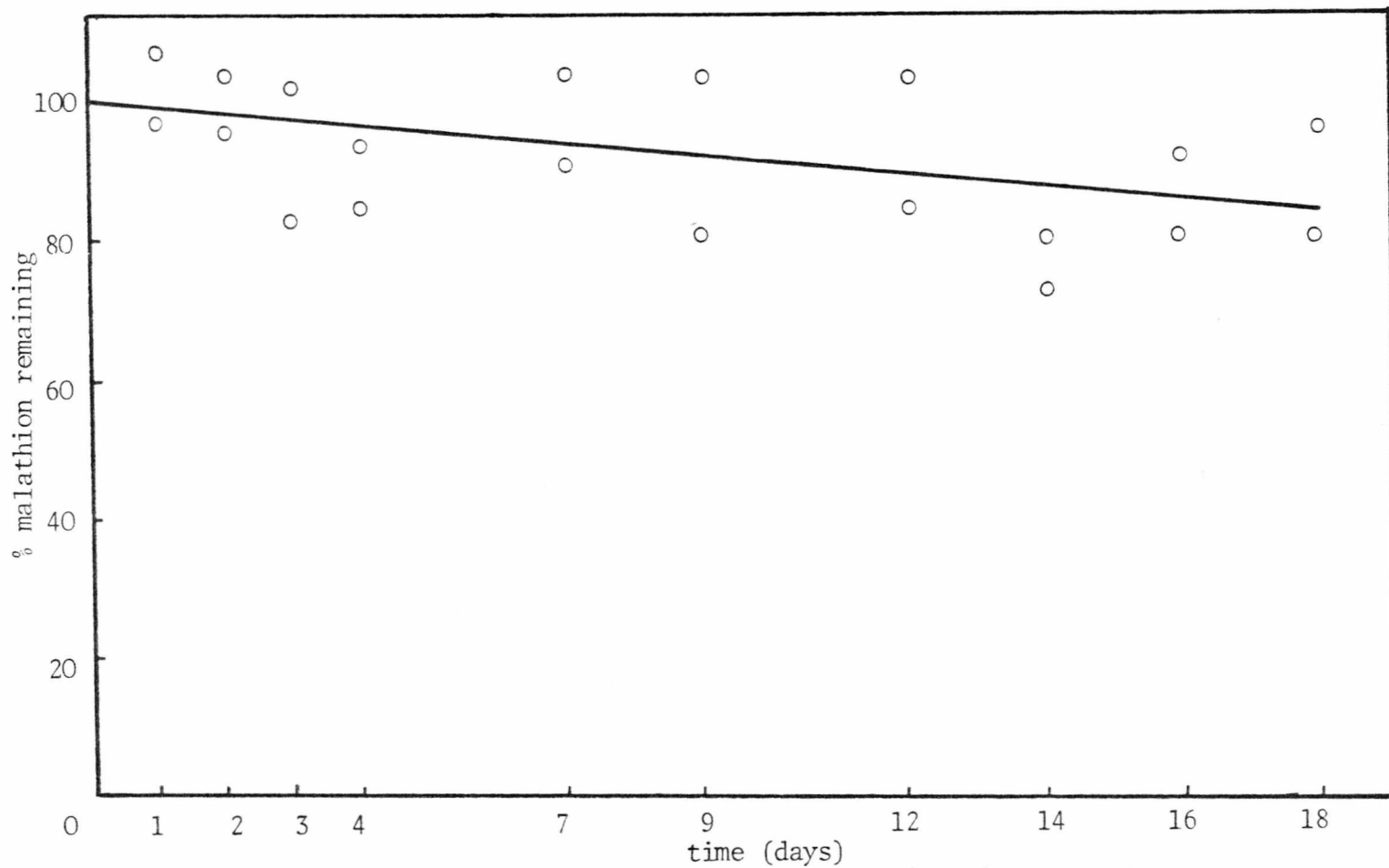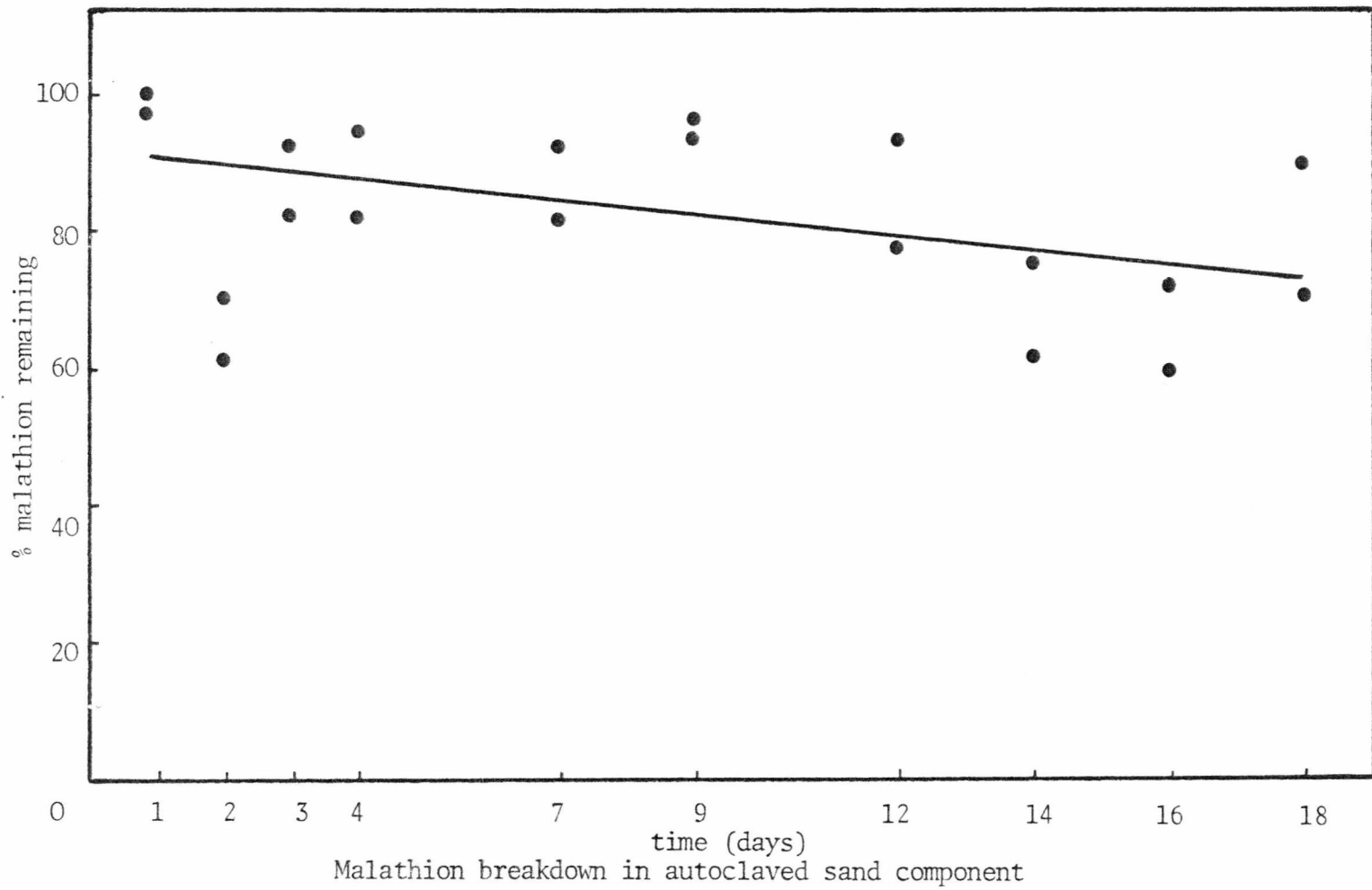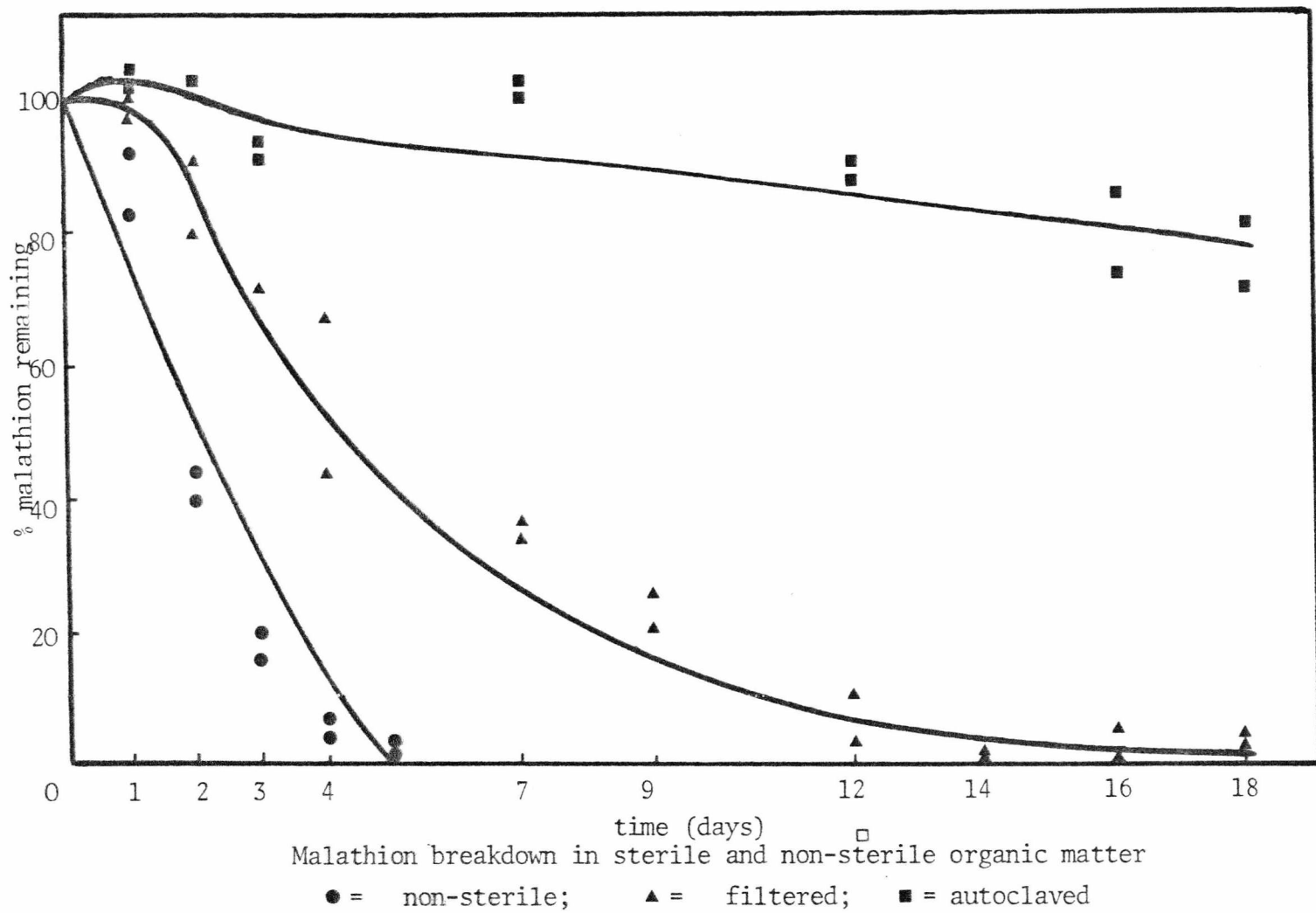
experiments are needed to totally describe the mechanisms involved in the decay on this component.

Non-sterile organic matter (Figs. 14 and 43) was confirmed as the major degradative agent, although some slight decrease was observed in comparison to the organo-mineral complex; this decrease was not, however, significant. The rate of decay was linear with a high rate of malathion loss over the 5 day period, (Appendix table 33). The less rapid initial decline may have been due to the destruction of some of the enzyme during extraction and the inefficiency of the extraction process itself. Filter-sterilized organic matter was also effective in catalysing malathion decay, although filtering (Figs. 14 and 43) produced a significant difference in rate of disappearance. The pattern of loss was exponential, which is an increasingly consistent characteristic of this enzymatic decay. The significant difference in rate of loss may be due to the removal of the microbial population, and the organic matter together with any associated exoenzyme. It seems probable that breakdown was catalysed by extracellular enzymes associated with the extracted organic matter, since a longer lag period might be expected if breakdown was purely microbial. This decline in the rate of breakdown may be due to the removal of stable extracellular enzymes associated with organic matter on filtering.

The rate of malathion loss from the organic matter extract after auto-claving was linear (Appendix table 34 and Fig. 43) and differed from the filtered and non-sterile organic matter extracts (Appendix tables 33 and 34) providing further evidence for the heat-labile nature of this catalytic agent.

(iii) Diallate

The rate of diallate loss from the soil components was related to their

particle size. Measurement of loss at $25^{\circ}C$ showed that the coarser the

particle size the more rapid the rate of loss. This, together with experiments

concerned with adsorption and volatilization, suggested that loss was due to

volatilization; itself determined by the extent of diallate adsorption. It

is highly unlikely that chemical hydrolysis occurred since this would not be/ related to

particle size. The evidence also suggests that the clay component adsorbed

diallate to the same degree as the organo-mineral complex and the parent soil,

even though the former contained a low percentage of organic matter (Table 15).

However, significant differences in loss also occurred from the components

containing no clay.

If volatilization was the primary route of loss, the pattern of loss

from the autoclaved components should have been comparable to that from the

non-sterile components. This was shown to be the case; similar half-lives were

recorded for all components regardless of treatment. Due to the large varia-

tions in the amount of diallate remaining at different sampling times,

regression analysis would have produced a clearer picture of the rates of loss

for comparison with the non-sterile components.

A comparison between autoclaved and non-sterile fractions does reveal

(Figs. 21 and 22) that the rate of loss decreased significantly in the organo-

mineral complex after autoclaving but that there was no significant difference

between treatments in either the clay components or in the parent soil. The

differences observed in the organo-mineral complex may be due to experimental

variation as the experiments were carried out at different times. In addition,

any real differences would surely have been revealed in the parent soil (non-

sterile versus autoclaved).

Direct evidence indicated a greater rate of volatilization from the

sand than from the parent soil (Table 13). It therefore seems reasonable to

suggest that the greater adsorption to the clay by diallate (cf. sand and silt) is the primary reason for its varying persistence in the different soil components.

Loss of diallate from the colloidal organic suspension was extremely rapid during the 5 days after its addition (Fig.23); thereafter the rate declined. A similar pattern was observed in the sand and silt components although the initial rate was somewhat slower. Hance, Holroyd and McKone (1973) have indicated that increased rates of triallate volatilization occur from wet soils. This herbicide is closely related to diallate and has been reported to be more strongly adsorbed by soil (Smith 1970). A similar effect may occur with diallate, its low solubility combined with competition with water for adsorption sites, may increase the initial rate of volatilization, especially in the aqueous organic matter extract. A comparison of diallate loss from sealed and open systems endorsed the importance of volatilization. However, in this instance the sealed organic matter suspension also effected loss of diallate (half-life = 18 days) indicating the additional possibility of an organic matter-catalyzed hydrolytic decay which assumed prominance when volatilization was restricted.

The information reported here concerning the possible mechanisms of degradation of these three pesticides in the Hamble soil are summarized in Table 14. 2,4-D and malathion loss is primarily biological, with microbial degradation being solely responsibile in the case of 2,4-D and extracellular enzymes in the case of malathion. Other workers have reported microbial involvement in malathion degradation and this may be a secondary mechanism in our soil. Diallate loss is due to volatilization not microbial decay; this latter mechanism possibly playing a secondary role only after the initial volatility losses.

In order to ascertain the feasibility of prediction based on texture,

	2,4-D		Malathion		Diallate	
	Mechanism of Loss		Mechanism of Loss		Mechanism of Loss	
	Primary	Secondary	Primary	Secondary	Primary	Secondary
Soil	Microbial	–	Exoenzymes	Microbial	Volatilization	Microbial
Sand	No loss	–	Microbial	Exoenzymes	Volatilization	–
Silt	No loss	–	Microbial	Exoenzymes	Volatilization	–
Clay	No loss	–	Chemical hydrolysis	–	Volatilization	–
Organo-mineral	Microbial	–	Exoenzymes	Microbial	Volatilization	Microbial
Organic matter	No loss	–	Exoenzymes/ Microbial	Hydrolysis	Volatilization	–

Table 14.

Proposed mechanisms of loss for 2,4-D, diallate and malathion in Hamble soil.

the production of artifical soils with varying proportions of sand, silt and clay (o.m.complex) was carried out. From this it was hoped that a quantitative relationship between the responsible component and rate of loss could be observed.

V. Artificial Soils

(i) 2,4-D.

Since the principal component in the degradation of 2,4-D was the microbially-active organo-mineral complex, it was hypothesised that a quantitative relationship might exist between, on one hand the proportion of organo-mineral complex in a sand or silt mixture and, on the other hand, the length of lag and rate of subsequent breakdown. However, in all combinations the stimulatory effect of the organo-mineral complex was similar irrespective of the actual amount present (Fig. 7). It is therefore probable that even the artificial soil containing the least amount of active component had sufficient numbers of microorganisms to effect a rapid degradation at these concentrations of 2,4-D.

It may be that the variation in lag periods between soils is rather more dependent on a variation in the microbial species capable of degrading 2,4-D rather than on total numbers, in which case artificial soils constructed from one parent soil may not illustrate any variation.

It appears likely that the variations in microbial species between soil types is more important since experiments using four different soil types show a lack of correlation between lag phases and microbial numbers (Table 17).

From this data one might predict that those soils which support an adequate microbial population (say $> 1 \times 10^5$) will rapidly degrade 2,4-D. This is in contrast to a microbially sparse sand or silt soil in which 2,4-D

may display both prolonged lag and breakdown periods. However, further work is necessary to determine whether microbial numbers or types are important before any predictions based on texture are possible.

(ii) Malathion

We have recorded that the most active component in malathion degradation was the organo-mineral complex. When this colloid was added to the less active sand and silt components an increase in degradation rates was observed (Figs. 15, 16, 44-49 and Appendix table 35). In the case of the sand/organo-mineral combinations the pattern of breakdown was seen to be exponential, as opposed to the linear relationship in the 100% sand control. With the silt/organo-mineral combinations all were exponential except the combination with the lowest clay content (Appendix table 35). The rates of breakdown in all sand/organo-mineral combinations were significantly different from each other. In the silt/organo-mineral combinations significant differences were only observed when the level of organo-mineral complex in the artificial soil reached 5%. This may be due to contamination of the silt fraction with rather more of the organo-mineral complex due to an overlap in particle size.

These experiments illustrate a quantitative relationship in that the rate of degradation was dependent on the amount of enzymatically-active soil component present. They also suggest that soils which have a high percentage of organo-mineral complex possess a greater malathion degradative ability than those with a high percentage of sand or silt. Microbial metabolism may assume a dominant degrading role only when the organic matter is removed. The breakdown of malathion in the sand and silt fractions was indicative of this. Non-biological hydrolysis does not appear to play an important part in the Hamble soil but may prove important in those soils
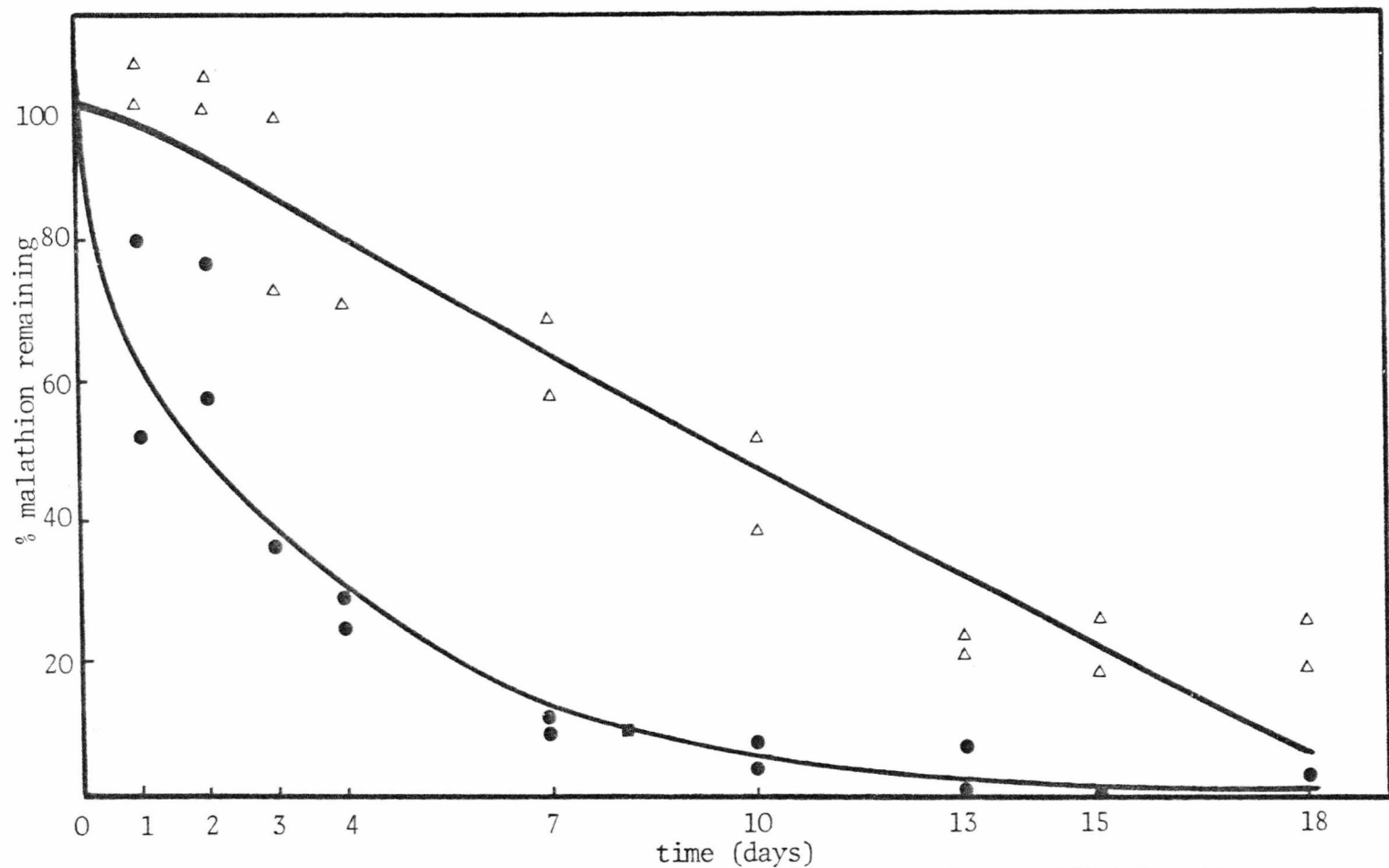
Fig. 44

Malathion breakdown in silt: organo-mineral complex artificial soils -
● = organo-mineral (50%): silt (50%);   △ = silt (100%)
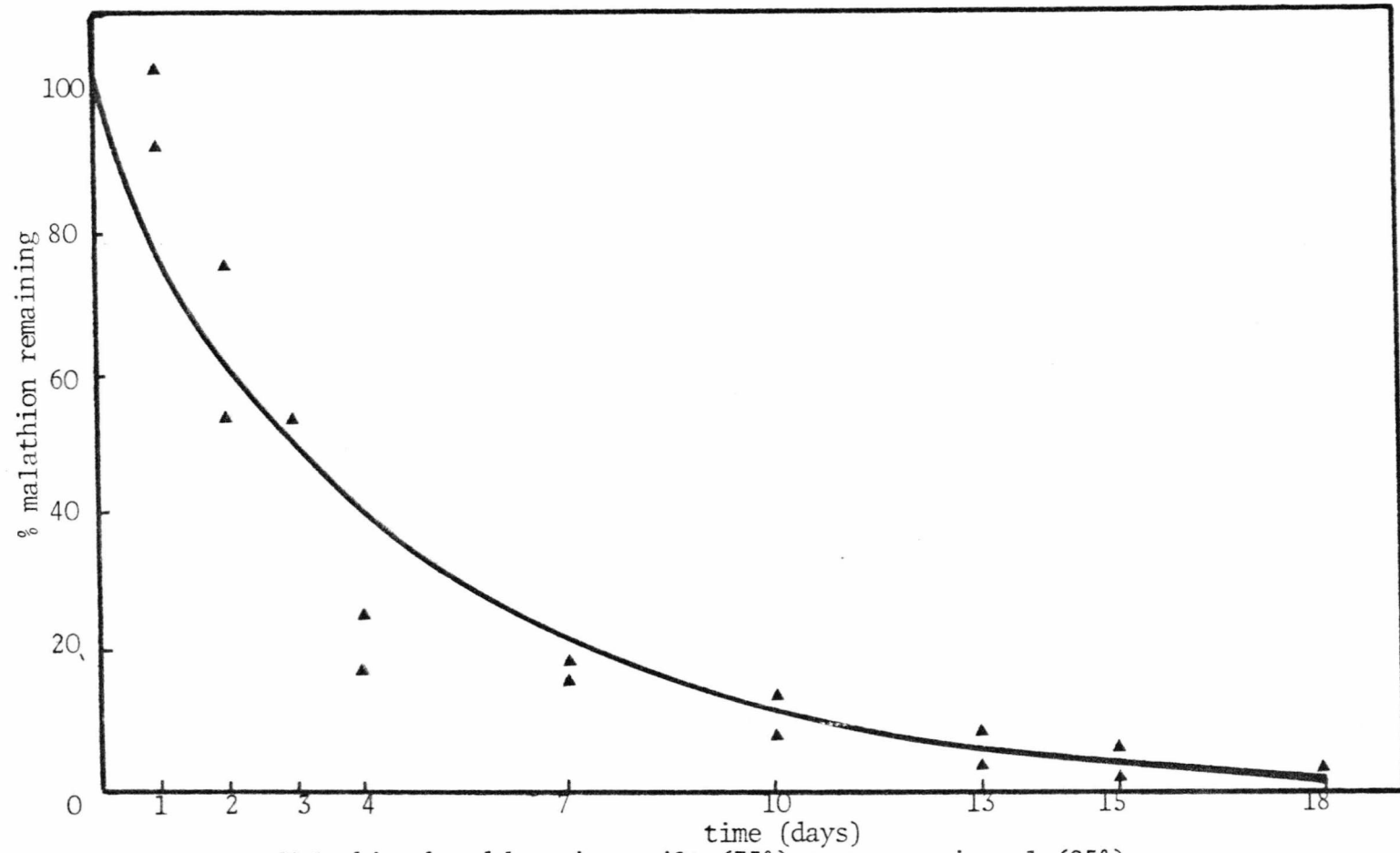
Malathion breakdown in a silt (75%) : organo-mineral (25%)
artificial soil.

Fig. 45
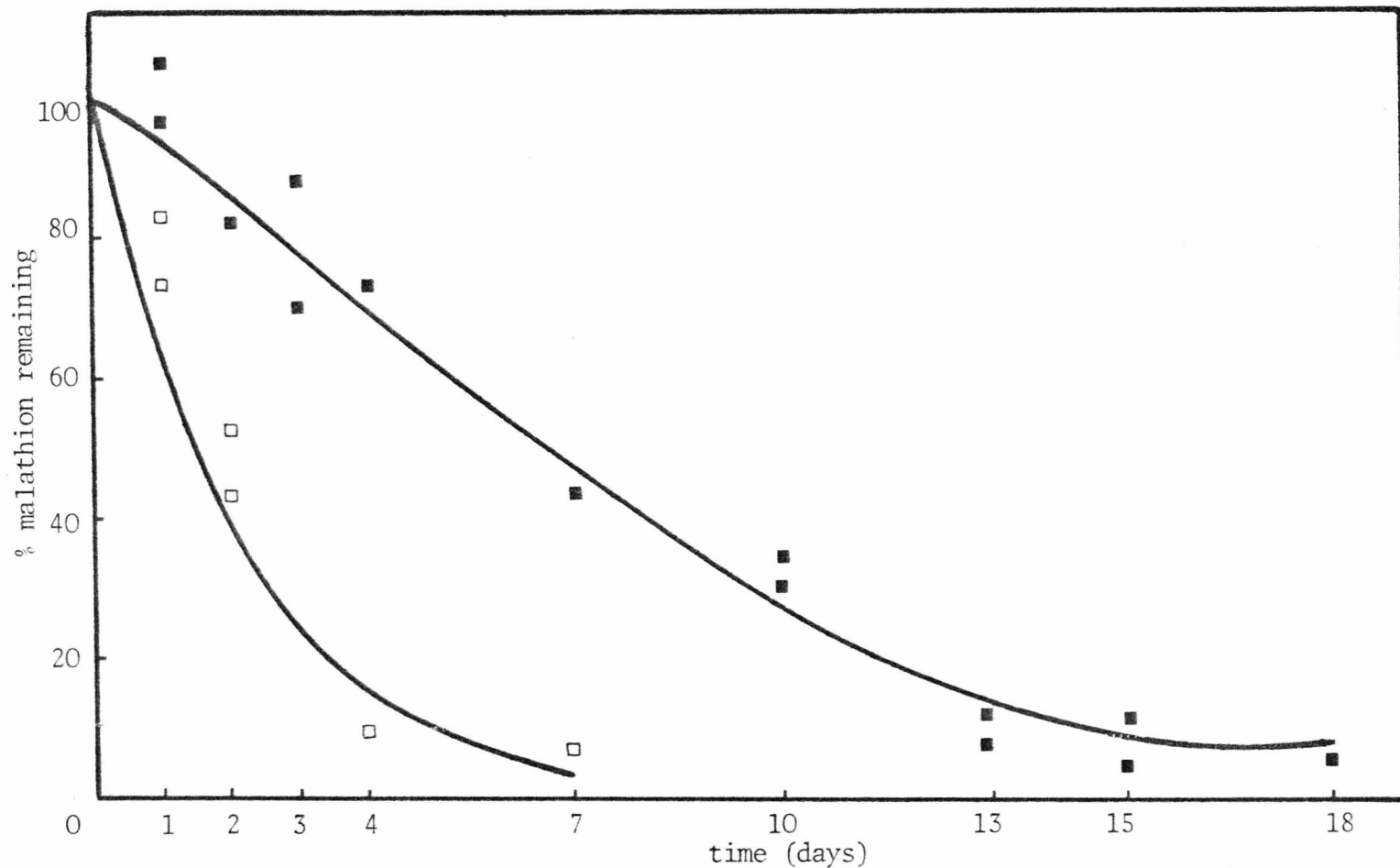
Fig. 46

Malathion breakdown in silt : organo-mineral complex artificial soils -
■ =   organo-mineral (5%) : silt (95%);   □ =   organo-mineral (100%)
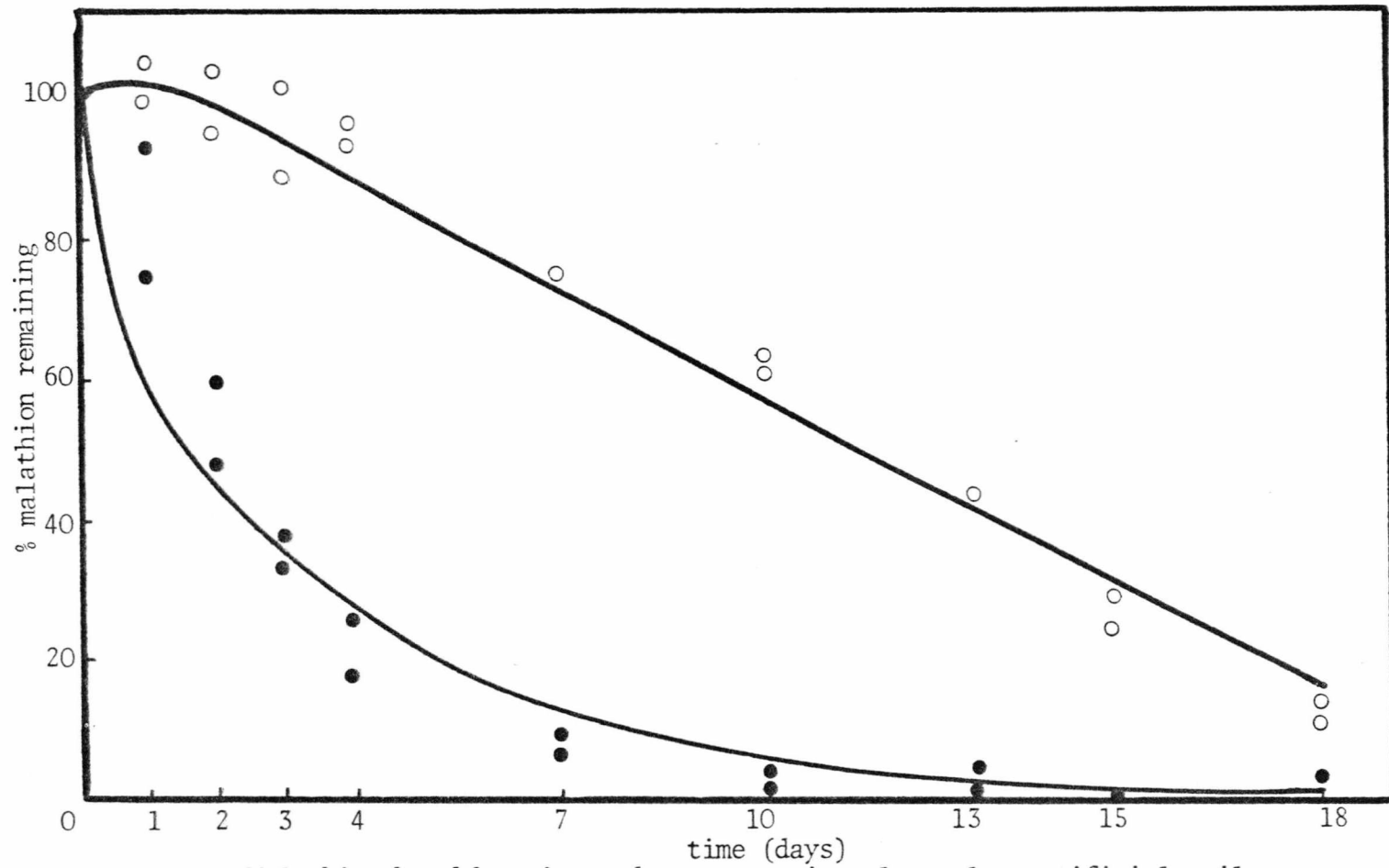
Fig. 47

Malathion breakdown in sand : organo-mineral complex artificial soils -
● = organo-mineral (50%) : sand (50%); ○ = sand (100%).
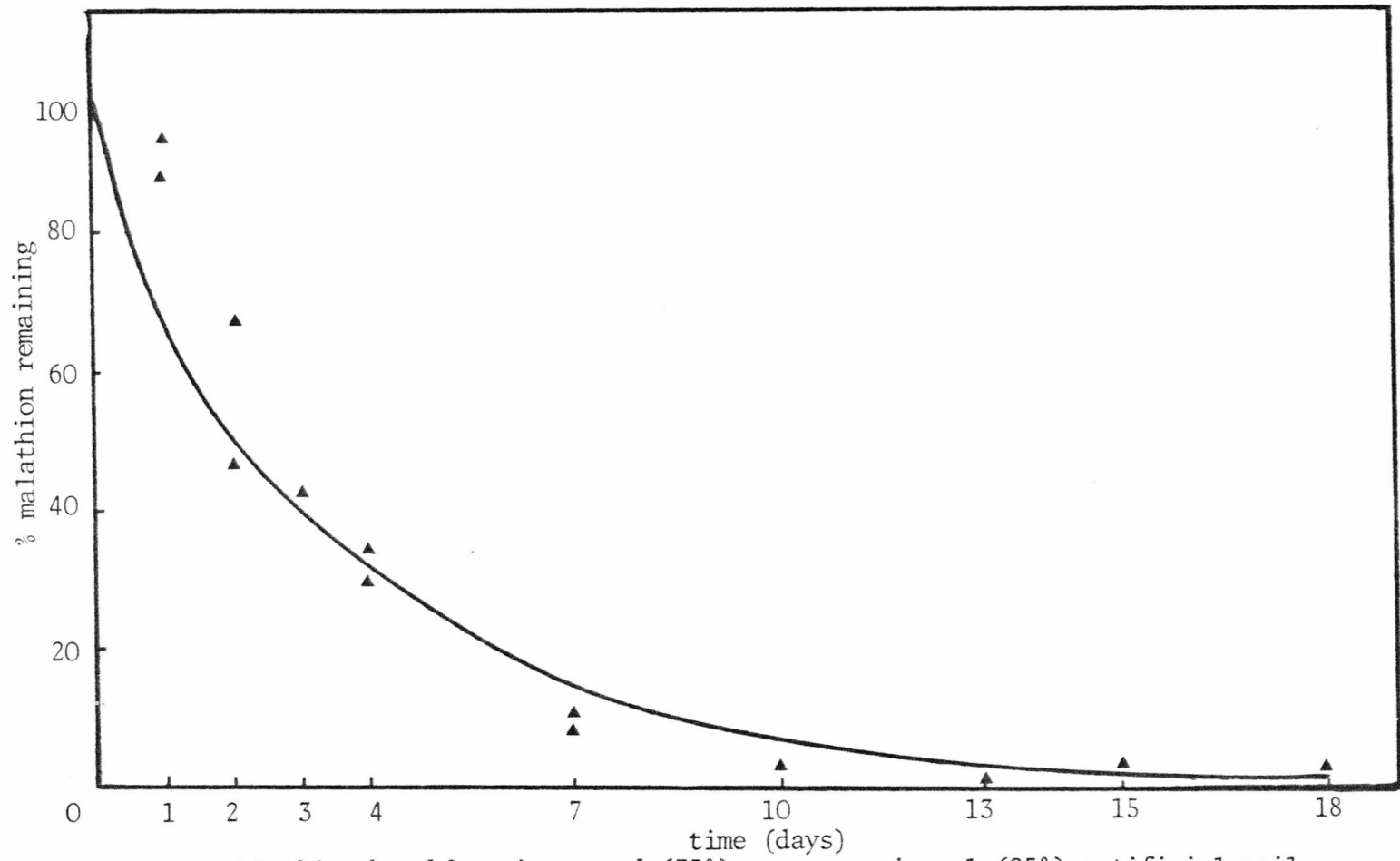
Malathion breakdown in a sand (75%) : organo-mineral (25%) artificial soil.

Fig. 48
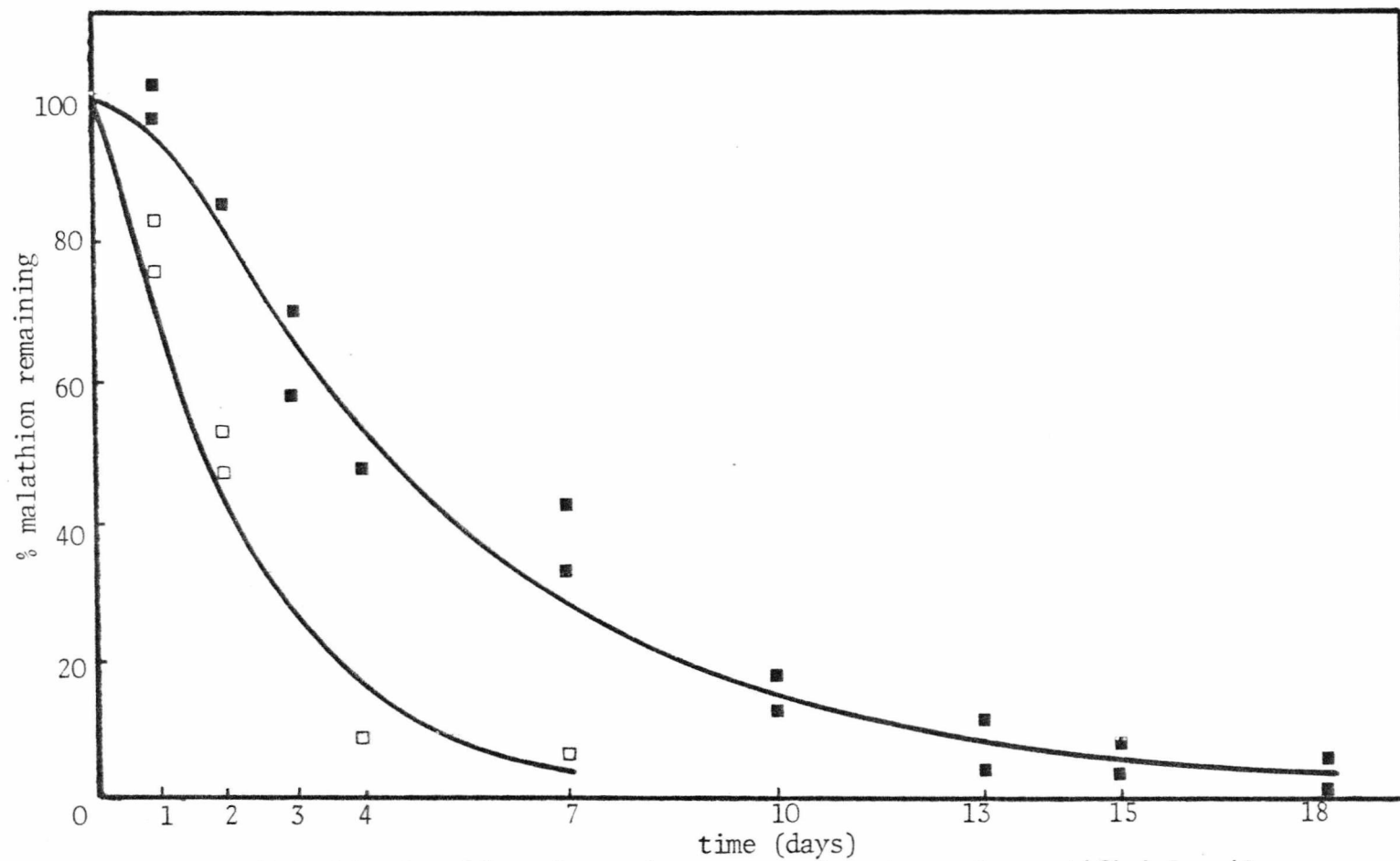
Malathion breakdown in sand : organo-mineral complex artificial soils -
■ =   organo-mineral (5%) : sand (95%);   □ =   organo-mineral (100%).

Fig. 49

which support small or unsuitable microbial populations, do not contain the bound exo-enzyme and have an alkaline pH.

The broader implications of controlling pesticide persistence using immobilized enzyme additions has been mentioned by Burns (1976).

(iii) Diallate

If volatilization is the major and constant contributor to loss, the rate should be dependent on texture in other natural and artificial soils. In the sand/organo-mineral complex combinations significant differences were observed in diallate loss, the rate decreasing with increasing organo-mineral content. In the silt/organo-mineral complex no significant retardation in the rate of loss was seen with a 5% organo-mineral complex artificial soil.

Further increases in organo-mineral content demonstrated a significant reduction in loss from the 100% silt control and between artificial soils as they increased in organo-mineral content. (Figs. 25, 26).

These results indicate that volatilization from coarse textured soils may be a more significant route of loss than microbial metabolism, since upon application a rapid loss is most likely prior to any microbial enrichment of the soil for diallate. In less sandy soils such as the Hamble Series the slow rate of volatilization may be followed by microbial degradation after a long lag period.

All the results have indicated that the organo-mineral complex was active in the breakdown of 2,4-D and malthion yet inhibitive to diallate loss.

Table 15 shows the variation in organic matter content between the soil and its components. Loss of 2,4-D and malathion was effected by the component containing the largest organic matter content. In the case of diallate, particle size and the nature of the component seem to influence

loss, clay being as important in diallate adsorption as the organic matter.
Table 15 shows clearly that the organic matter is associated with the

Table 15

Organic matter content of soil and its components

Soil or component	% Organic matter (% Carbon x 1.724)
Soil	3.79
Sand	0.29
Silt	1.22
Clay	1.02
Organo-mineral complex	13.07

clay to form the organic matter complex. Williams (1975) states that
increases in the organo-clay content of soil is usually associated with
increasing organic matter and has listed soils in order of increasing
organic matter content, from sands containing less than 1.0% organic matter
to heavy soils with 2.5-3.5% organic matter. Thus, it seems possible that if
the organo-mineral complex is important in the loss of these pesticides then
an assessment of soil texture may provide a guide to their loss from any
natural soil.

VI. Test Soils

The percentage organo-mineral and organic matter contents of the test
soils are shown in Table 16. Winchester soil from 0-20cm is only really
comparable with the other 0-20cm samples. It can be seen that the organic
matter does increase with a large increase in organo-mineral content but
that at lower levels no relationship is apparent.

Table 16

Percentage organo-mineral and organic matter contents

of the test soils.

Soil	% Organo-mineral complex	% Organic matter
Winchester (0-20cm)	55.0	6.57
Winchester (50-60cm)	65.0	1.34
Barming	23.0	1.72
Hothfield	11.0	2.97
Hamble	20.0	3.79

(i) 2,4-D

With experiments on 2,4-D, samples from a 0-20cm depth were compared, and with an increase in the organo-mineral content our results (Fig. 8) showed a decrease in both lag period and half-life of 2,4-D. Some correlation coefficients are listed in Table 17.

Fig. 17
Correlation Data.

Factors to be correlated	Correlation coefficient
Decreasing Lag phase length versus increasing organo-mineral content.	= 0.91
Decreasing half-life versus increasing organo-mineral content.	= 0.92
Decreasing Lag phase length versus increasing microbial numbers.	= 0.43

A good correlation was obtained between the lag phase and half life compared to the organo-mineral content, but not to microbial numbers. These results suggest that the amount of organo-mineral complex present is more important than microbial numbers which, as mentioned before, do not necessarily indicate the proportion of active 2,4-D degraders in the population. The larger organo-clay contents may provide more diverse populations and larger areas for pesticide/microbe interactions to take place. The correlation coefficient between the organic matter contents and half-lives of 2,4-D was -0.62 indicating that soil texture may again give a more realistic idea of 2,4-D persistence. Experiments with a wider range of different soil types with different organic matter and organo-mineral contents need to be carried out before any conclusive observations can be made. The type of clay present may also have an important bearing on persistence in all three pesticides studied.

The higher organo-mineral content of the 50-60cm layer of the Winchester soil yet its lack of degradative ability, demonstrates that the microbial degradation of 2,4-D must occur in the surface layers of this soil where favourable environmental conditions exist for microbial growth and proliferation.

(ii) Malathion

Results derived from malathion breakdown over a 24 hour period show a high degree of correlation (-0.974) between the organo-mineral contents of Barming, Hamble and Winchester (0-20cm) soils and the half-lives of malathion. A large difference in rate of loss was seen in the Hothfield soil (Figs.50 and 51) which possessed the lowest organo-clay content of the test soils. Significant differences were seen in rates of malathion loss over the 24 hour period. Any comparisons after this period were of limited value since only

Fig. 50

Malathion breakdown in Hothfield soil
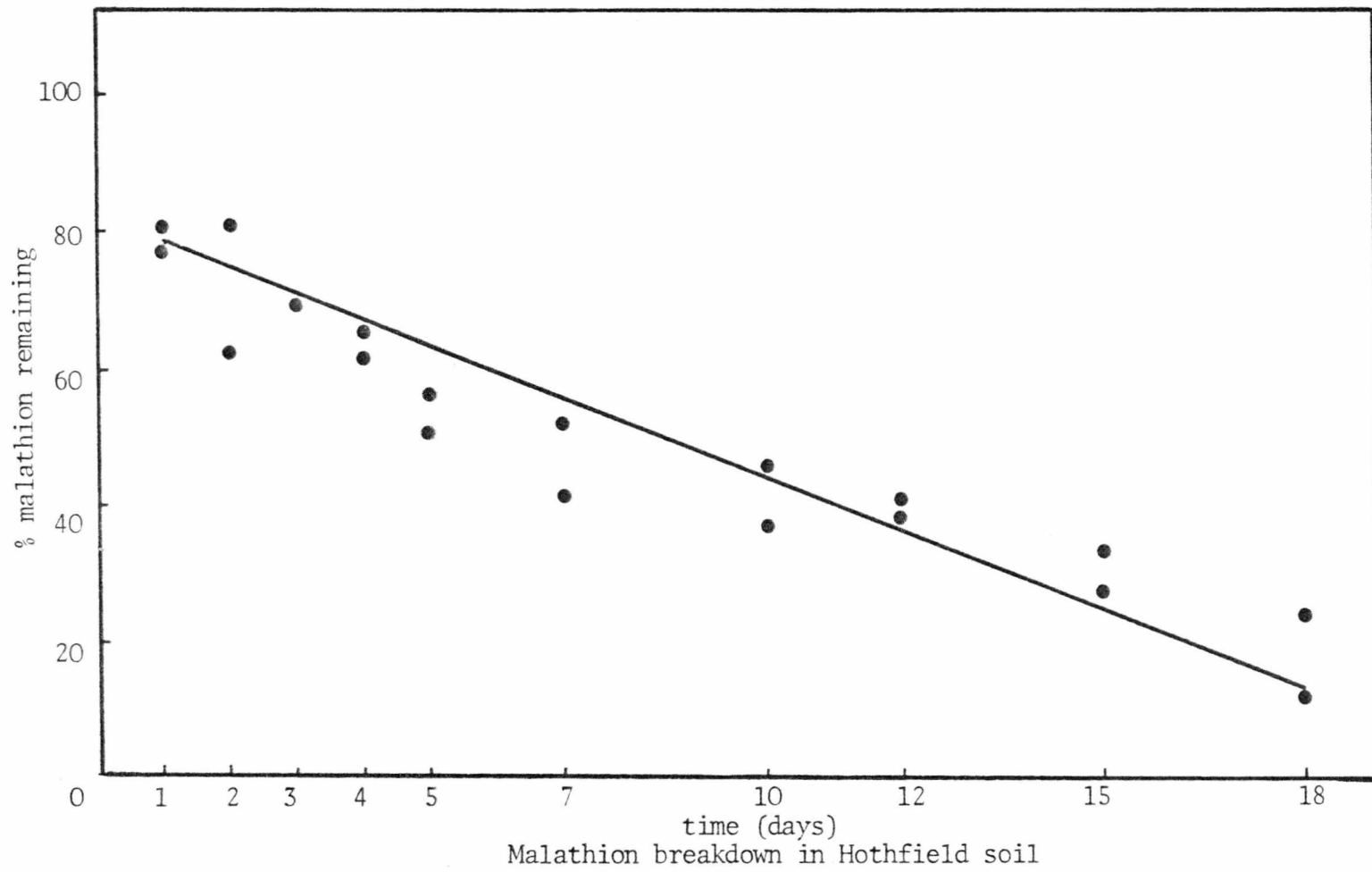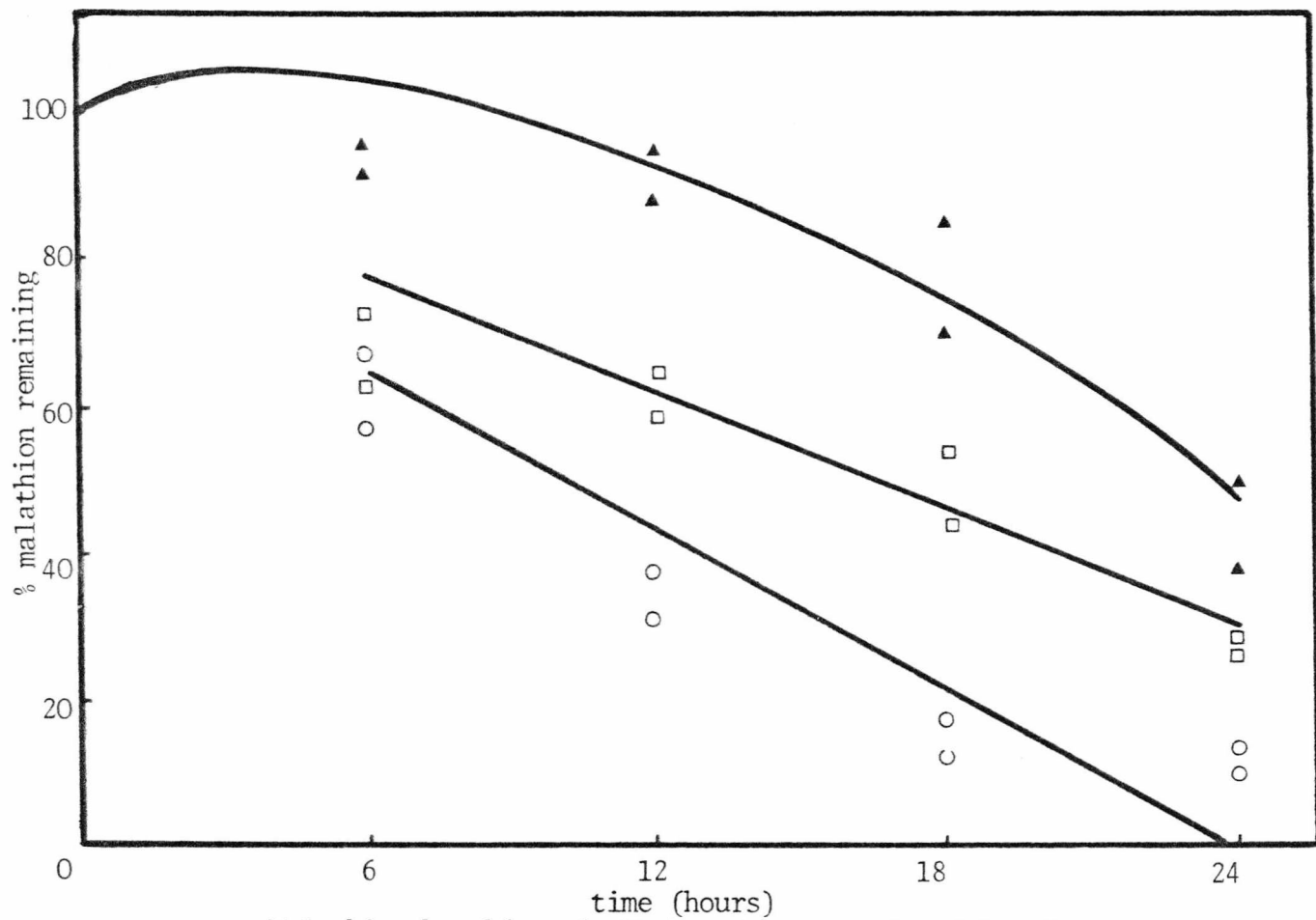
Malathion breakdown in Hamble (▲); Barming (□) and
Winchester (0-20cm) (o) soils.

Fig. 51

residual levels were found. The rates of loss in two soils over the 24 hour period (Barming and Winchester) were seen to be linear and quadratic in the case of the Hamble soil. (Fig. 51). However, over the 18 day period three soils exhibit a typical exponential pattern (Fig.17). Breakdown in the Hothfield soil was linear (Fig. 50 and Appendix Table 36).

The rapid loss patterns suggest mechanisms of decay similar to those observed in the Hamble soil. Loss from Hothfield soil over the 18 day period was linear, in contrast to the normal exponential patterns, suggesting that another mechanism may be operating in this instance.

(iii) Diallate

Any prediction of diallate behaviour based on previous experiments depends on the assumption that volatilization is the major source of loss. However, in the test soils, the rate of loss did not decrease with increasing organo-mineral content as had been expected (Fig. 27). At the concentrations of diallate used in these experiments there is always enough clay to cause immobilization and it is possible that, at this concentration, organo-mineral contents considerably lower than that in the Hothfield soil are required before retention is decreased and volatilization significantly increased. Therefore other mechanisms of decay were operating in these test soils; mechanisms which complicate the extrapolation of data derived from the Hamble soil to diallate behaviour in other soils.

VII. Conclusion.

The breakdown of pesticides in soil will continue to be of major scientific and economic interest in the next decade. A fundamental approach to the subject along the lines described in this thesis, may well prove rewarding in an attempt to increase our understanding of pesticide-soil interactions.

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APPENDIX

Table 1 - 2,4-D.

| Time | Non-sterile Hamble soil | | | | | | | | |
| | 4°C | | | 25°C | | | 37°C | | |
(days)	I	II	Mean	I	II	Mean	I	II	Mean
0	100	100	100	100	100	100	100	100	100
4	94.0	98.0	96.0	97.7	102.3	100	97.8	98.8	98.3
6	99.7	95.6	97.7	102.3	106.8	104.5	93.5	97.7	95.6
8	103.3	106.5	104.9	102.3	97.7	100.0	95.0	97.0	96.0
10	102.0	98.0	100.0	52.3	52.3	52.3	93.5	101.0	97.3
12	91.5	98.5	95.0	29.5	15.9	22.7	95.8	99.4	97.6
14	93.0	102.0	97.5	1.14	3.4	2.3	99.0	103.7	101.3
16	92.3	103.7	98.0	1.1	1.1	1.1	98.0	102.0	100.0
18.	98.0	101.5	99.8	0.0	0.0	0.0	90.5	98.5	94.5

Time (days)	Sterilized Hamble Soil											
	2.5 Mrad			5.0 Mrad			Autoclaved			NaN$_3$-treated		
	I	II	Mean	I	II	Mean	I	II	Mean	I	II	Mean
0	100	100	100	100	100	100	100	100	100	100	100	100
3	97.8	89.1	93.4	97.8	95.7	96.7	86.7	77.7	82.2	93.0	87.0	90.0
8	93.5	97.8	95.7	97.8	97.8	97.8	84.4	115.5	100.0	89.3	93.7	91.5
11	95.7	104.3	100.0	93.5	97.8	95.7	91.1	106.7	100.0	91.0	93.0	92.0
14	89.1	87.0	88.0	91.3	95.7	93.5				96.6	103.0	99.8
16							88.9	100.0	94.5			
18	93.5	93.5	93.5	97.8	91.3	94.5	77.8	88.9	83.3	100.0	100.0	100.0

Table 2 – 2,4-D

| Time (days) | Non-sterile Soil Components ||||||||||||||||||
| | Organo-mineral ||| Organo-mineral ||| Sand ||| Silt ||| Clay ||| Organic matter |||
	I	II	Mean	I	II	Mean	I	II	Mean	I	II	Mean	I	II	Mean	I	II	Mean
0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
3	91.5	97.5	94.5	99.6	103.0	101.3	97.8	97.8	97.8	102.3	109.1	105.7						
4	61.3	68.7	65.0	98.7	98.7	98.7							100.0	97.5	99.0	80.0	88.0	84.0
5	15.5	11.5	13.5	93.0	97.0	95.0												
6	2.5	0.5	1.5				93.5	95.7	94.6	104.5	88.6	96.6	75.0	100.0	87.5			
7																80.0	102.0	91.0
8				25.0	31.6	28.3							102.5	107.5	105.0			
10							97.8	93.5	95.6	102.3	93.2	97.8	105.0	100.0	102.5	92.0	96.0	94.0
11				1.0	3.0	2.0												
12													82.5	77.5	80.0			
13																90.0	84.0	87.0
14													95.0	95.0	95.0			
15							97.8	95.7	96.7	97.7	93.2	95.5						
16													97.5	102.5	100.0			
18							102.2	97.8	100.0	109.1	102.3	105.7	95.0	90.0	92.5	100.0	98.0	99.0

Table 3 – 2,4-D.

Table 4 – 2,4-D

Time (days)	Autoclaved Soil Components											
	Organo-mineral			Sand			Silt			Organic matter		
	I	II	Mean	I	II	Mean	I	II	Mean	I	II	Mean
0	100	100	100	100	100	100	100	100	100	100	100	100
3	100.0	93.2	96.6	91.7	89.6	90.7	102.3	102.3	102.3			
4										96.1	88.2	92.2
7										92.2	86.3	89.3
8	91.0	109.1	100.1	97.9	102.1	100.0	63.6	88.6	76.1			
10										100.0	94.1	97.1
11	102.3	97.7	100.0	104.2	97.9	101.1	95.5	109.1	102.3			
13										102.0	98.0	100.0
16	88.6	91.0	89.8	95.8	97.9	96.9	102.3	106.8	104.6			
18	79.5	102.3	91.0	97.9	104.2	101.1	91.0	104.5	97.8	94.1	94.1	94.1

Time (days)	Artificial Soil											
	Organo-mineral			Sand			Silt			All artificial soils		
	I	II	Mean	I	II	Mean	I	II	Mean	I	II	Mean
0	100	100	100	100	100	100	100	100	100	100	100	100
3	96.4	92.1	94.3	78.7	95.7	87.2	95.4	100.0	97.6	97.6	101.4	99.5
4	77.7	52.3	65.0									
5	12.2	15.4	13.8									
6	0.5	1.5	1.0	85.1	97.9	91.5	102.3	102.3	102.3	0.5	1.5	1.0
10				89.4	97.9	93.6	100.0	96.0	98.0			
15				80.9	89.4	85.1	102.3	97.0	99.7			
18				97.9	93.6	95.7	104.0	96.0	100.0			

Table 5 - 2,4-D.

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Time (days)	Winchester (0-20cm)			Winchester (50-60cm)			Barming			Hothfield		
	I	II	Mean	I	II	Mean	I	II	Mean	I	II	Mean
0	100	100	100	100	100	100	100	100	100	100	100	100
2	104.5	81.8	93.2	72.0	92.0	82.0	93.8	83.3	88.6	104.2	100.0	102.1
4	50.0	75.0	62.5	80.0	80.0	80.0	95.8	100.0	97.9	100.0	87.5	93.8
6.	27.3	40.9	34.1	92.0	80.0	86.0	97.7	97.9	94.8	87.5	83.3	85.4
8	6.8	6.8	6.8	90.0	80.0	85.0	83.3	47.9	65.6	97.9	91.7	94.8
10	2.3	2.3	2.3	94.0	94.0	94.0	6.3	14.6	10.5	93.8	79.2	86.5
13				92.0	88.0	90.0	6.3	4.2	5.3	89.6	79.2	84.4
15				92.0	88.0	90.0	6.3	4.2	5.3	27.1	20.8	24.0
18				76.0	84.0	80.0	4.2	2.1	3.2	12.5	4.2	8.4

Test Soils

Table 6 – 2,4-D

Table 7 - Malathion

Time	Non-sterile Hamble Soil								
	4°C			25°C			37°C		
(days)	I	II	Mean	I	II	Mean	I	II	Mean
0	100	100	100	100	100	100	100	100	100
1	104.3	87.0	95.7	38.6	45.5	42.0	34.8	30.4	32.6
2	73.9	87.0	80.5	27.3	22.7	25.0	13.0	10.9	11.9
3	97.8	87.0	92.4	18.2	15.9	17.1	6.5	4.3	5.4
4	69.6	65.2	67.4	11.4	11.4	11.4	4.3	2.2	3.2
7	60.9	69.6	65.3	9.1	6.8	8.0	2.2	2.2	2.2
9				9.1	6.8	8.0			
10	54.3	60.9	57.6				2.2	2.2	2.2
11				4.5	4.5	4.5			
13	41.3	32.6	37.0	4.5	2.3	3.4			
15	23.9	30.4	27.2	4.5	4.5	4.5			
18	23.9	30.4	27.2	2.3	2.3	2.3			

| Time | Sterilized Hamble Soil | | | | | | | | | | | |
| (days) | Autoclaved | | | NaN$_3$-treated | | | Irradiated(2.5Mrads) | | | Irradiated (5.0Mrads) | | |
	I	II	Mean	I	II	Mean	I	II	Mean	I	II	Mean
0	100	100	100	100	100	100	100	100	100	100	100	100
1	87.2	104.3	95.7	55.6	60.0	57.8	85.4	79.2	82.3	78.3	82.6	80.5
2	80.9	102.1	91.5	33.3	33.3	33.3	41.7	37.5	39.6	32.6	43.5	38.0
3	95.7	106.4	101.1	22.2	26.7	24.4	22.9	31.3	27.1	28.3	26.1	27.2
4	89.4	102.1	95.8	20.0	13.3	16.7	10.4	8.3	9.4	30.4	17.4	23.8
7	106.4	100.0	103.2	8.9	8.9	8.9	12.5	8.3	10.4	8.7	6.5	7.6
9	97.9	87.2	92.6	8.9	6.7	7.8	6.3	14.6	10.4	10.9	13.0	12.0

Table 8 - Malathion

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Table 9 - Malathion

Time	Irradiated Hamble Soil								
	10.0 Mrads			15.0 Mrads			20.0 Mrads		
(days)	I	II	Mean	I	II	Mean	I	II	Mean
0	100	100	100	100	100	100	100	100	100
1	100.0	97.9	98.9	102.3	106.8	104.6	104.4	100.0	102.2
2	87.2	85.1	86.2	100.0	95.5	97.7	100.0	100.0	100.0
3	83.0	80.9	81.9	68.2	68.2	68.2	100.0	75.6	87.8
4	63.8	63.8	63.8	68.2	56.8	62.5	93.3	88.9	91.1
7	46.8	63.8	55.3	50.0	38.6	44.3	66.7	66.7	66.7
9	46.8	44.7	45.7	50.0	36.4	43.2	55.6	44.4	50.0
12	38.3	42.6	40.4	34.1	34.1	34.1	60.0	66.7	63.3
14	31.9	46.8	39.4	34.1	38.6	36.4	55.6	57.7	56.7
16	34.0	31.9	33.0	34.1	22.7	28.7	55.6	48.9	55.2

Time (days)	Organo-mineral			Sand			Silt			Clay		
	I	II	Mean	I	II	Mean	I	II	Mean	I	II	Mean
0	100	100	100	100	100	100	100	100	100	100	100	100
1	46.9	36.7	41.8	93.0	97.7	95.3	97.7	95.3	96.5	93.8	100.0	96.9
2	20.4	28.6	24.5	88.4	100.0	94.2	93.0	104.2	98.6	89.6	104.2	96.9
3	14.3	16.3	15.3	88.4	97.7	93.0	100.0	97.0	98.5	95.8	85.4	90.6
4	10.2	8.2	9.2	90.7	97.7	94.2	81.4	79.1	80.2	85.4	87.5	86.5
7	6.1	4.1	5.1	69.8	76.7	73.3	60.5	46.5	53.5	93.8	87.5	90.7
9	4.1	4.1	4.1	51.2	69.8	60.5	41.9	39.5	40.7	79.2	83.3	81.3
11	2.0	2.0	2.0	44.2	55.8	77.9	30.2	25.6	27.9	91.7	85.4	88.6
13				34.9	34.9	34.9	20.9	16.3	18.6	68.8	66.7	67.7
15				39.5	38.4	38.4	16.3	20.9	18.6	70.8	66.7	68.7
17				23.3	24.4	24.4	11.6	18.6	15.1	54.2	58.3	56.3

Non-sterile Soil Components

Table 10 – Malathion

Time	Autoclaved Soil Components.											
(days)	Organo-mineral			Sand			Silt			Clay		
	I	II	Mean	I	II	Mean	I	II	Mean	I	II	Mean
0	100	100	100	100	100	100	100	100	100	100	100	100
1	108.3	97.9	103.1	96.0	100.0	98.0	95.9	93.9	94.9	88.7	91.3	90.0
2	104.2	97.9	101.1	62.0	70.0	66.0	95.9	89.8	92.9	102.9	94.5	98.7
3	102.1	83.3	92.7	82.0	92.0	87.0	83.7	89.8	86.7	97.6	101.0	99.3
4	85.4	95.8	90.6	94.0	82.0	88.0	83.7	89.8	86.7	94.8	98.0	96.4
7	91.7	104.2	98.0	82.0	92.0	87.0	77.6	65.3	71.5	92.3	97.3	94.8
9	104.2	81.3	92.7	96.0	94.0	95.0	96.0	83.7	89.8	101.6	103.0	102.3
12	104.2	85.4	94.8	80.0	94.0	87.0	79.6	89.8	84.7	95.0	90.5	92.8
14	81.3	72.9	77.1	76.0	62.0	69.0	71.4	63.3	67.3	93.2	89.2	91.2
16	81.3	93.8	87.5	72.0	60.0	66.0	71.4	87.8	79.6	99.5	91.5	95.5
18	97.9	81.3	89.6	70.0	90.0	80.0	81.6	71.4	76.5	95.0	95.0	95.0

Table 11 - Malathion

Table 12 - Malathion

Time (days)	Organic Matter Extract								
	Non-sterile			Filtered			Autoclaved		
	I	II	Mean	I	II	Mean	I	II	Mean
0	100	100	100	100	100	100	100	100	100.
1	92.0	82.0	87.0	102.2	97.8	100.0	102.1	100.0	101.1
2	40.0	44.0	42.0	80.0	86.7	83.3	100.0	100.0	100.0
3	20.0	16.0	18.0	71.1	71.1	71.1			
4	4.0	6.0	5.0	66.7	44.4	55.6	91.5	95.7	93.6
5	2.0	0.0	1.0						
7				33.3	35.6	34.4	102.1	100.0	101.1
9				24.4	20.0	22.2			
12				11.1	4.4	7.8	87.2	91.4	89.3
14				4.4	2.2	3.3			
16				2.2	4.4	3.3	74.5	85.1	79.8
18				1.1	2.2	1.7	72.3	80.9	76.6

Time (days)	Organo-mineral (100%)			Organo-mineral (50%) Sand (50%)			Organo-mineral (25%) Sand (75%)			Organo-mineral (5%) Sand (95%)			Sand (100%)		
	I	II	Mean	I	II	Mean	I	II	Mean	I	II	Mean	I	II	Mean
0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
1	82.9	73.2	78.0	93.2	75.0	84.1	93.0	88.4	90.7	102.2	95.6	98.9	104.3	100.0	102.3
2	53.7	41.5	47.6	59.1	47.7	53.4	67.4	48.8	58.1	84.4	84.4	84.4	95.7	104.3	100.0
3				36.4	34.1	35.2	44.2	44.2	44.2	66.7	57.8	62.2	102.2	89.1	95.7
4	9.8	9.8	9.8	27.3	18.2	22.7	30.2	32.6	31.4	48.9	48.9	48.9	97.8	93.5	95.6
7	4.9	4.9	4.9	6.8	9.1	7.9	11.6	9.3	10.5	33.3	42.2	37.8	73.9	73.9	73.9
10				6.8	2.3	4.5	4.7	4.7	4.7	17.8	13.3	15.6	60.9	56.5	58.7
13				4.5	2.3	3.4	2.3	2.3	2.3	11.1	4.4	7.8	43.5	43.5	43.5
15				2.3	2.3	2.3	2.3	2.3	2.3	6.7	4.4	5.6	23.9	30.4	27.2
18				2.3	2.3	2.3	2.3	2.3	2.3	4.4	2.2	3.3	10.9	13.0	12.0

Artificial Soils

Table 13 - Malathion

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Time (days)	Organo-mineral(100%)			Organo-mineral(50%) Silt (50%)			Organo-mineral(25%) Silt (75%)			Organo-mineral (5%) Silt (95%)			Silt (100%)		
	I	II	Mean	I	II	Mean	I	II	Mean	I	II	Mean	I	II	Mean
0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
1	82.9	73.2	78.0	52.3	79.5	65.9	104.7	93.0	98.9	93.3	104.7	100.0	96.6	105.7	101.2
2	53.7	41.5	47.6	75.0	56.8	65.9	76.7	55.8	66.3	58.1	81.4	69.7	103.4	98.9	101.2
3				36.4	36.4	36.4	53.5	53.5	53.5	88.4	69.8	79.1	97.7	72.7	85.2
4	9.8	9.8	9.8	25.0	31.8	28.4	18.6	25.6	22.1	74.4	69.8	72.1	72.4	72.4	72.4
7	4.9	4.9	4.9	9.1	15.9	12.5	16.3	18.6	17.5	44.2	44.2	44.2	69.0	57.5	63.3
10				6.8	9.1	7.9	11.6	9.3	10.5	34.9	30.2	32.6	50.6	38.0	44.3
13				2.3	4.5	3.4	4.7	9.3	7.0	9.3	14.0	11.6	22.7	20.5	21.6
15				2.3	2.3	2.3	4.7	2.3	3.5	11.6	7.0	9.3	18.4	24.1	21.3
18				2.3	2.3	2.3	2.3	2.3	2.3	9.3	9.3	9.3	25.3	16.1	20.7

Artificial Soils

Table 14 - Malathion

Time	Test Soils											
	Winchester (0-20cm)			Winchester (50-60cm)			Barming			Hothfield		
(days)	I	II	Mean	I	II	Mean	I	II	Mean	I	II	Mean
0	100	100	100	100	100	100	100	100	100	100	100	100
1	14.6	10.4	12.5	17.0	21.3	19.1	29.2	27.1	28.1	80.9	78.7	79.8
2	6.3	6.3	6.3	6.4	6.4	6.4	12.5	10.4	11.5	80.9	63.8	72.4
3	4.2	4.2	4.2	4.3	4.3	4.3	6.3	6.3	6.3	70.2	70.2	70.2
4	4.2	4.2	4.2	4.3	2.1	3.2	6.3	6.3	6.3	66.0	61.7	63.9
5	4.2	4.2	4.2	4.3	4.3	4.3	6.3	6.3	6.3	51.1	57.4	54.2
7	4.2	4.2	4.2	4.3	2.1	3.2	6.3	4.2	5.3	42.6	53.2	47.9
10	4.2	4.2	4.2	2.1	2.1	2.1	6.3	4.2	5.3	38.3	44.7	41.5
12	4.2	2.1	3.2	4.3	4.3	4.3	6.3	4.2	5.3	38.3	40.4	39.4
15	4.2	2.1	3.2	4.3	2.1	3.2	6.3	6.3	6.3	34.0	27.7	30.8
18	4.2	4.2	4.2	4.3	4.3	4.3	6.3	6.3	6.3	14.9	25.5	20.2

Table 15 - Malathion

Test	Test Soils											
(days)	Winchester (0-20cm)			Winchester (50-60cm)			Barming			Hamble		
	I	II	Mean	I	II	Mean	I	II	Mean	I	II	Mean
0	100	100	100	100	100	100	100	100	100	100	100	100
6	56.1	65.9	61.0	85.4	87.8	86.6	73.8	64.3	69.1	95.7	93.5	94.6
12	22.0	26.8	24.4	56.1	56.1	56.1	64.3	59.5	61.9	87.0	93.5	90.3
18	17.1	12.2	14.6	43.9	36.6	40.2	54.8	45.2	50.0	71.7	84.8	78.2
+24	14.6	10.4	12.5	17.0	21.3	19.1	29.2	27.1	28.1	38.6	45.5	42.0

+ Figures from appendix table 7 and 15

Table 16 - Malathion

Table 17 - Diallate

Time (days)	Non-sterile Hamble Soil								
	4°C			25°C			37°C		
	I	II	Mean	I	II	Mean	I	II	Mean
0	100	100	100	100	100	100	100	100	100
4	93.8	93.8	93.8	106.4	106.4	106.4	93.2	90.9	92.1
10	104.2	97.9	101.1	97.9	97.9	97.9	70.5	84.1	77.3
17	91.7	93.8	92.7	76.6	76.6	76.6	54.5	79.5	67.0
22	93.8	95.8	94.8	85.1	83.0	84.0	63.6	52.3	57.9
30	83.3	93.8	88.5	51.1	51.1	51.1	27.3	31.8	29.6
37	93.8	93.8	93.8	83.0	66.0	74.5	29.5	40.9	35.2
53	91.7	100.0	95.9	55.3	61.7	58.5	18.2	11.4	14.8
63	104.2	104.2	104.2	72.3	76.6	74.5	22.7	15.9	19.3
70	100.0	100.0	100.0	59.6	59.6	59.6	11.4	20.5	15.9

Time (days)	Sterilized Hamble Soil														
	Autoclaved			Autoclaved (37°C)			NaN$_3$-treated			Irradiated(2.5Mrads)			Irradiated (5.0Mrads)		
	I	II	Mean	I	II	Mean	I	II	Mean	I	II	Mean	I	II	Mean
0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
5	102.1	102.1	102.1	91.1	106.7	98.9				100.0	97.9	99.0	106.8	97.7	102.3
6							88.6	95.5	92.0						
11	102.1	63.8	83.0	84.4	75.6	80.0				83.3	87.5	85.4	97.7	102.3	100.0
12							111.4	93.2	102.3						
19	100.0	89.4	94.7	55.6	86.7	71.1	68.2	79.5	73.9	91.7	79.2	85.4	88.6	95.5	92.0
25	68.1	97.9	83.0	55.6	47.7	51.1				58.3	58.3	58.3	95.5	61.4	78.4
26							86.4	95.5	91.0						
32	61.7	100.0	80.9	44.4	37.8	41.1	63.6	61.2	62.4	60.4	54.2	57.3	75.0	86.4	80.7
39	87.2	83.0	85.1	46.7	53.3	50.0				72.9	58.3	65.6	79.5	63.6	71.6
42							68.2	72.2	70.5						
53	61.7	70.2	66.0	33.3	31.1	32.2	52.3	72.2	62.5	62.5	52.1	57.3	95.5	65.9	80.7
62	53.2	51.1	52.1	20.0	20.0	20.0	68.5	77.2	72.9	60.4	75.0	67.7	72.7	86.4	79.5
70	42.6	51.1	46.8	22.2	17.8	20.0	63.6	75.0	69.3	41.7	70.8	56.3	63.6	63.6	63.6

Table 18 – Diallate

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Time (days)	Non-sterile Soil Components											
	Sand			Silt			Organo-mineral			Clay		
	I	II	Mean	I	II	Mean	I	II	Mean	I	II	Mean
0	100	100	100	100	100	100	100	100	100	100	100	100
4	77.6	77.6	77.6	98.0	91.8	95.0	93.9	98.0	95.9	94.0	96.0	95.0
10	51.0	49.0	50.0	83.7	69.4	76.5	98.0	98.0	98.0	89.7	95.7	92.5
17	16.3	22.4	19.4	59.2	55.1	57.2	83.7	77.6	80.6			
20										85.1	83.0	84.0
22	20.4	22.4	21.4	71.4	61.2	66.3	71.4	71.4	71.4			
30	10.2	8.2	9.2	34.7	34.7	34.7	53.1	51.0	52.1			
37	14.3	10.2	12.3	46.9	38.8	42.8	49.0	57.1	53.1	70.2	60.0	66.0
53	8.2	8.2	8.2	36.7	38.8	37.7	57.1	65.3	61.2	63.8	61.7	62.8
63	8.2	8.2	8.2	38.8	30.6	34.7	65.3	73.5	69.4	60.0	53.2	56.6
70	2.0	2.0	2.0	30.6	30.6	30.6	61.2	69.4	65.3	53.2	57.4	55.3

Table 19 - Diallate

Time (days)	Autoclaved Soil Components											
	Sand			Silt			Organo-mineral			Clay		
	I	II	Mean	I	II	Mean	I	II	Mean	I	II	Mean
0	100	100	100	100	100	100	100	100	100	100	100	100
5	78.7	78.7	78.7	89.4	108.5	99.0	95.7	102.1	98.9			
10										91.5	89.4	90.4
11	57.4	55.3	56.4	76.6	74.5	75.5	87.2	87.2	87.2			
19	46.8	25.5	36.2	78.7	51.1	64.9	102.1	97.9	100.0			
20										89.4	80.9	85.1
25	14.9	21.3	18.1	31.9	31.9	31.9	89.4	89.4	89.4			
32	8.5	21.3	14.9	36.2	51.1	43.6	91.5	89.4	90.4			
37										85.1	80.9	83.0
39	6.4	10.6	8.5	42.6	63.8	53.2	91.5	93.6	92.6			
53	-	-	-	29.8	27.7	28.7	89.4	89.4	89.4	68.1	74.5	71.3
62	6.4	6.4	6.4	31.9	25.5	28.7	80.9	89.4	85.1	61.7	59.6	60.6
70	0	6.4	3.2	21.3	21.3	21.3	74.5	85.1	79.8	61.7	57.4	59.6

Table 20 - Diallate

Time (days)	Glass Surfaces						Organic Matter					
	Sealed			Open			Non-sterile			Autoclaved		
	I	II	Mean	I	II	Mean	I	II	Mean	I	II	Mean
0	100	100	100	100	100	100	100	100	100	100	100	100
2	96.0	98.0	97.0	42.0	42.0	42.0						
5				6.0	2.0	4.0				34.0	42.0	38.0
6							34.0	40.0	37.0			
9										34.0	34.0	34.0
13	78.0	74.0	76.0									
14							28.0	28.0	28.0			
15										26.0	32.0	29.0
20							26.0	24.0	25.0			
21										12.0	16.0	14.0
25	70.0	78.0	74.0									
26							12.0	12.0	12.0			
34	78.0	72.0	76.0									
48	68.0	72.0	70.0									
73	74.0	62.0	68.0									

Table 21 - Diallate

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Table 22 - Diallate

Time (days)	Autoclaved Sealed Systems					
	Sand			Organic Matter		
	I	II	Mean	I	II	Mean
0	100	100	100	100	100	100
5	109.3	95.3	102.3	80.0	75.6	77.8
10	83.7	83.7	83.7	77.8	68.9	73.3
16	97.7	93.0	95.4	53.3	55.6	54.4
22	79.1	83.7	81.4	33.3	33.3	33.3
30	81.4	88.4	84.9	28.9	24.4	26.7
37	93.0	83.7	88.4	28.9	24.4	26.7
54	51.2	79.1	65.1	11.1	6.7	8.9
70	62.8	58.1	60.5	0	2.2	1.1

Time (days)	Artificial Soils														
	Organo-mineral(100%)			Organo-mineral(50%) Sand (50%)			Organo-mineral (25%) Sand (75%)			Organo-mineral (5%) Sand (95%)			Sand (100%)		
	I	II	Mean	I	II	Mean	I	II	Mean	I	II	Mean	I	II	Mean
0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
10	106.4	102.1	104.3	102.1	91.5	96.8	89.4	95.7	92.6	60.9	70.0	65.5	61.7	42.6	52.1
17	89.4	95.7	92.6	76.6	83.0	79.8	72.3	70.2	71.3	47.8	39.1	43.5	23.4	25.5	24.5
30	85.0	83.0	84.0	70.2	63.8	67.0	48.9	42.6	45.7	26.1	32.6	29.4	14.9	10.6	12.8
37	74.5	78.7	76.6	70.2	66.0	68.1	51.1	53.2	52.1	21.7	26.1	23.9	-	-	-
53	63.8	68.1	66.0	48.9	53.2	51.0	29.8	21.3	25.5	13.0	10.9	11.9	6.4	4.3	5.3
63	63.8	59.6	61.7	38.3	34.0	36.2	31.9	25.5	28.7	8.7	8.7	8.7	4.3	6.4	5.3
70	59.6	61.7	60.7	46.8	42.6	44.7	29.8	25.5	27.7	6.5	4.3	5.4	2.1	2.1	2.1

Table 23 - Diallate

Time (days)	Organo-mineral (100%)			Organo-mineral (50%) Silt (50%)			Organo-mineral (25%) Silt (75%)			Organo-mineral (5%) Silt (95%)			Silt (100%)		
	I	II	Mean	I	II	Mean	I	II	Mean	I	II	Mean	I	II	Mean
0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
10	106.4	102.1	104.3	98.0	89.8	93.9	97.9	97.9	97.9	76.6	83.0	79.8	83.7	69.4	76.5
17	89.4	95.7	92.6	81.6	81.6	81.6	72.3	70.2	71.3	57.4	61.7	59.6	59.2	55.1	57.2
30	85.0	83.0	84.0	65.3	71.4	68.4	68.1	63.8	66.0	57.4	53.2	55.3	42.9	38.8	40.8
37	74.5	78.7	76.6	67.3	71.4	69.4	63.8	59.6	61.7	46.8	42.6	44.7	36.7	38.8	37.7
53	63.8	68.1	66.0	51.0	46.9	49.0	42.6	40.4	41.5	27.7	21.3	24.5	38.8	30.6	34.7
63	63.8	59.6	61.7	44.9	46.9	45.9	48.9	31.9	40.4	17.0	12.8	14.9	34.7	34.7	34.7
70	59.6	61.7	60.7	40.8	40.8	40.8	27.7	25.5	26.6	10.6	8.5	9.6	32.7	28.8	30.6

Artificial Soils

Table 24 - Diallate

Time	Test Soils											
	Winchester (0-20cm)			Winchester (50-60cm)			Barming			Hothfield		
(days)	I	II	Mean	I	II	Mean	I	II	Mean	I	II	Mean
0	100	100	100	100	100	100	100	100	100	100	100	100
5	82.0	102.0	92.0	43.2	47.7	45.5	79.2	70.8	75.0	84.4	91.1	87.8
10	72.0	76.0	74.0	31.8	31.8	31.8	58.3	62.5	60.4	91.1	86.7	88.9
16	62.0	56.0	59.0	15.9	25.0	20.5	52.1	45.8	49.0	75.6	82.2	78.9
22	50.0	42.0	46.0	20.5	13.6	17.1	31.3	33.3	32.3	73.3	66.7	70.0
30	42.0	36.0	39.0	13.6	9.1	11.4	27.1	29.2	28.2	68.9	66.7	67.8
37	42.0	38.0	40.0	9.1	11.4	10.2	25.0	29.2	27.1	64.4	60.0	62.2
54	36.0	22.0	29.0	11.4	6.8	9.1	14.6	18.8	16.7	48.9	55.6	52.2
61	24.0	22.0	23.0	6.8	2.3	4.5	12.5	8.3	10.4	40.0	44.4	42.2

*Figures in Tables 1-25 represent % pesticide remaining.

Table 25 - Diallate

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Table 26 - Adsorption of diallate by Hamble Soil

Original concentration (μg/ml)	Equilibrium concentration (μg/ml)	Adsorption of diallate by soil (μg/g)	% Adsorption of diallate
0.72	0.40	0.32	44.4
1.38	0.84	0.54	39.13
2.32	1.36	0.96	41.40
2.94	1.70	1.24	42.20
3.60	2.18	1.42	39.40

Comparison of treatments I & II		Malathion		Diallate		2,4-D	
I	II	t-value	Significance at 5% level	t-value	Significance at 5% level	t-value	significance at 5% level
$4^{o}C$	$25^{o}C$	8.33	S	3.38	S	3.06	S
$4^{o}C$	$37^{o}C$	-	-	-	-	0.65	NS
$25^{o}C$	$37^{o}C$	7.32	S	5.87	S	-	-
$25^{o}C$	NaN_3	2.67	S	0.58	NS	-	-
$25^{o}C$	2.5Mrads	1.79	NS	1.65	NS	-	-
$25^{o}C$	5.0Mrads	2.34	NS	1.77	NS	-	-
$25^{o}C$	Autoclaved	1.08	NS	0.21	NS	-	-
$37^{o}C$	$37^{o}C$ Autoclaved	-	-	2.46	S	-	-
NaN_3	Autoclaved	8.72	S	0.06	NS	0.55	NS
2.5Mrads	5.0Mrads	0.63	NS	4.98	S	1.08	NS
Autoclaved	5.0Mrads	5.70	S	1.38	NS	-	-
Autoclaved	Autoclaved $37^{o}C$	4.88	S	5.66	S	-	-
Autoclaved	2.5Mrads	4.32	S	1.43	NS	0.58	NS
10.0Mrads	15.0Mrads	1.08	NS	-	-	-	-
10.0Mrads	20.0Mrads	4.88	S	-	-	-	-
15.0Mrads	20.0Mrads	4.32	S	-	-	-	-

Table 27 - Students t-test on Hamblesoil treatments

Comparison of Soil Components		Malathion		Diallate	
I	II	t-value	Significance at 5% level	t-value	Significance at 5% level
Organo-mineral	Soil	4.05	S	1.32	NS
Organo-mineral	Sand	15.86	S	10.45	S
Organo-mineral	Silt	7.10	S	4.79	S
Organo-mineral	Clay	19.13	S	0.49	NS
Sand	Silt	2.69	S	11.40	S
Sand	Clay	2.91	S	8.44	S
Silt	Clay	3.43	S	5.57	S
Organo-mineral autoclaved	Organo-mineral	-	-	3.45	S
Clay autoclaved	Clay	-	-	0.90	NS
Soil autoclaved	Soil	-	-	0.21	NS
Sand autoclaved	Sand	-	-	1.16	NS
Silt autoclaved	Silt	-	-	0.10	NS
Sand autoclaved	Silt autoclaved	-	-	7.18	S
Organo-mineral autoclaved	Clay autoclaved	-	-	3.72	S
Organic matter filtered	Organic matter autoclaved	4.17	S	-	-
Organic matter	Organic matter filtered	5.30	S	-	-
Organic matter	Organo-mineral	1.40	NS	-	-

Table 28. Students t-test on Soil Components treatments

Comparison of Artificial Soils I & II				Malathion		Diallate	
I		II		t-value	Significance @ 5% level	t-value	Significance @ 5% level
Sand % : Organo-mineral %		Sand % : Organo-mineral %					
50	50	75	25	2.58	S	4.84	S
75	25	95	5	4.07	S	10.04	S
50	50	95	5	4.07	S	17.46	S
100	–	95	5	5.29	S	3.71	S
–	100	50	50	3.31	S	6.41	S
Silt % : Organo-mineral %		Silt % : Organo-mineral %					
50	50	75	25	1.60	NS	2.83	S
75	25	95	5	3.01	S	9.13	S
50	50	95	5	4.16	S	8.38	S
100	–	95	5	3.63	S	0.65	NS
–	100	50	50	1.12	NS	8.27	S

Comparison of Soils I & II		Malathion	
I	II	t-value	Significance @ 5% level
Winchester (0-20 cm)	Barming	3.32	S
Barming	Hamble	7.00	S

S = Significant)
NS = Not Significant) Tables 27 - 30
– = not determined)

Soil treatment	Calculated values	Days													
		I	2	3	4	7	9	10	11	12	13	14	15	16	18
Non-sterile 4°C R = 0.92 Exponential	Regression values	95.2	88.1	81.6	75.5	59.9		47.6			37.7		32.3		25.7
	Rate of change	67.6	45.4	31.4	22.1	8.9		4.0			2.0		1.4		0.8
Non-sterile 25°C R = 0.85 Exponential	Regression values	33.3	27.9	23.4	19.7	11.6	8.2		5.8		4.1		2.9		1.7
	Rate of change	-18.4	-9.4	-5.2	-3.1	-0.9	-0.4		-0.3		-0.2		-0.09		-0.05
Non-sterile 37°C R = 0.70 Exponential	Regression value	23.0	16.2	11.4	8.0	2.8		1.0							
	Rate of change	-8.2	-2.8	-1.2	-0.6	-0.1		-0.03							
Autoclaved R = 0.17 Linear	Regression value	97.3	97.2	97.2	97.4	97.3	97.3								
	Rate of change	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07								
Azide treated R = 0.95 Exponential	Regression value	51.9	39.2	29.6	22.4	9.7	5.5								
	Rate of change	-18.6	-6.5	-2.7	-1.3	-0.3	-0.1								

Table 32

Regression and differentiation of irradiated Hamble soil

Soil treatments	Calculated value	Days													
		1	2	3	4	7	9	10	11	12	13	14	15	16	18
Irradiated(2.5 Mrads) R = 0.98 Cubic	Regression value	70.5	44.4	26.1	14.4	6.4	11.9								
	Rate of change	-30.1	-21.2	-13.0	-5.7	+11.6	+19.1								
Irradiated(5.0 Mrads) R = 0.98 Cubic	Regression value	70.8	46.8	29.7	18.5	9.0	11.6								
	Rate of change	-27.9	-20.4	-13.9	-8.6	+0.7	+1.3								
Irradiated(10.0Mrads) R = 0.98 Cubic	Regression value	94.2	85.2	77.3	70.3	54.5	47.3			40.2		37.0		34.4	
	Rate of change	-9.5	-8.4	-7.4	-6.5	-4.2	-3.0			-1.8		-1.3		-1.2	
Irradiated(15.0Mrads) R = 0.96 Cubic	Regression value	96.4	84.9	75.0	66.5	48.2	40.9			34.7		32.5		30.7	
	Rate of change	-12.2	-10.7	-9.2	-7.8	-4.5	-2.9			-1.4		-0.9		+0.9	
Irradiated(20.0Mrads) R = 0.93 Cubic	Regression value	100.2	95.1	89.9	84.7	69.9	61.7			54.1		53.1		56.4	
	Rate of change	-5.0	-5.2	-5.2	-5.2	-4.5	-3.6			-1.4		+0.5		+2.8	

Table 33.
Regression and differentiation of non-sterile soil components

Component	Calculated values	Days														
		1	2	3	4	5	7	9	11	12	13	14	15	16	17	18
Organo-mineral complex Non-sterile R = 0.92 Exponential	Regression value	39.1	28.4	20.8	15.2		5.9	3.1	1.7							
	Rate of change		-14.2	-7.0	-4.1		-1.7	-1.3	-1.2							
Sand - Non-sterile R = 0.98 Cubic	Regression value	97.9	96.1	93.2	89.4		74.0	62.0	50.1		39.7		32.0		28.3	
	Rate of change	-1.2	-2.3	-3.4	-4.2		-5.8	-6.1	-5.7		-4.6		-2.9		-0.6	
Silt - Non-sterile R = 0.99 Cubic	Regression value	99.6	95.3	89.6	82.9		58.8	42.3	27.8		17.5		13.6		18.2	
	Rate of change	-3.5	-5.1	-6.2	-7.2		-8.4	-7.9	-6.4		-3.7		-0.0		+4.8	
Clay - Non-sterile R = 0.93 Cubic	Regression value	96.7	94.2	92.1	90.5		86.8	84.3	80.8		75.5		67.4		55.7	
	Rate of change	-2.9	-2.3	-1.8	0.0		-1.1	-1.4	-2.1		-3.3		-4.9		-6.9	
Organic matter Non-sterile R = 0.98 Linear	Regression value	74.9	53.1	31.2	9.4	-12.5										
	Rate of change	-21.9	-21.9	-21.9	-21.9	-21.9										

Component & treatment	Calculated values	Days														
		1	2	3	4	5	7	9	11	12	13	14	15	16	17	18
Organo-mineral complex Autoclaved R = 0.71 Linear	Regression value	98.9														
	Rate of change	-0.8	-0.8	-0.8	-0.8		-0.8	-0.8		-0.8		-0.8		-0.8		-0.8
Sand Autoclaved R = 0.68 Linear	Regression value	90.6														73.8
	Rate of change	-0.9	-0.9	-0.9	-0.9		-0.9	-0.9		-0.9		-0.9		-0.9		-0.9
Silt Autoclaved R = 0.81 Linear	Regression value	92.5														72.9
	Rate of change	-1.8	-1.8	-1.8	-1.8		-1.8	-1.8		-1.8		-1.8		-1.8		-1.8
Clay Autoclaved R = 0.50 Linear	Regression value	97.2	97.0	96.9	96.7		96.2	95.8		95.3		94.9		94.6		94.2
	Rate of change	-0.2	-0.2	-0.2	-0.2		-0.2	-0.2		-0.2		-0.2		-0.2		-0.2
Organic matter Filtered R = 0.98 Exponential	Regression value	107.4	84.2	66.0	51.7		24.9	15.3		7.4		4.5		2.8		1.7
	Rate of change	-37.1	-12.6	-5.2	-2.4		-0.5	-0.2		-0.08		-0.04		-0.02		-0.01
Organic matter Autoclaved R = 0.94 Linear	Regression value	101.2	99.9		97.3		93.3			86.8				81.5		79.1
	Rate of change	-1.3	-1.3		-1.3		-1.3			-1.3				-1.3		-1.3

Table 35

Regression and differentiation of artificial soils

Artificial soil	Calculated values	Days								
		1	2	3	4	7	10	13	15	18
Organo-mineral (100%) R = 0.98 Exponential	Regression value Rate of change	65.3 -11.5	40.9 -2.4		16.0 -0.3	3.9 -0.04				
Sand (100%) R = 0.99 Linear	Regression value Rate of change	103.9 -5.2	98.7 -5.2	93.5 -5.2	88.2 -5.2	72.5 -5.2	56.8 -5.2	41.1 -5.2	30.6 -5.2	14.9 -5.2
Silt (100%) R = 0.98 Linear	Regression value Rate of change	95.9 -5.2	90.8 -5.2	85.5 -5.2	80.3 -5.2	64.7 -5.2	49.0 -5.2	33.3 -5.2	22.9 -5.2	7.2 -5.2
Organo-mineral (5%) Sand (95%) R = 0 .98 Exponential	Regression value Rate of change	95.6 -39.8	78.0 -15.9	63.7 -7.2	51.9 -3.7	28.2 -0.8	15.3 -0.2	8.3 -0.1	5.5 -0.06	3.0 -0.03
Organo-mineral (25%) Sand (75%) R = 0.97 Exponential	Regression value Rate of change	66.3 -25.6	52.1 -9.6	41.0 -4.2	32.2 -2.1	15.7 -0.4	7.6 -0.1	3.7 -0.05	2.3 -0.3	1.2 -0.01
Organo-mineral (50%) Sand (50%) R = 0.95 Exponential	Regression value Rate of change	56.3 -23.6	44.8 -9.4	35.7 -4.3	28.4 -2.2	14.3 -0.5	7.2 -0.2	3.6 -0.07	2.3 -0.04	1.2 -0.02

Organo-mineral (5%) Silt (95%) R = 0.98	Regression value	92.0	84.0	76.1	68.3	46.4	28.0	14.8	9.6	8.6
	Rate of change	-8.05	-7.9	-7.9	-7.7	-6.8	-5.4	-3.4	-1.8	+1.1
Cubic										
Organo-mineral (25%) Silt (75%) R = 0.98	Regression value	73.6	59.5	48.1	38.8	20.5	10.8	5.7	3.7	2.0
	Rate of change	-30.9	-12.4	-5.7	-2.9	-0.6	-0.2	-0.08	-0.05	-0.02
Exponential										
Organo-mineral (50%) Silt (50%) R = 0.98	Regression value	62.8	50.2	40.1	32.0	16.3	8.3	4.3	2.7	1.4
	Rate of change	-26.1	-10.3	-4.7	-2.4	0.5	-0.1	-0.07	-0.04	-0.02
Exponential										

Test Soil	Calculated values	Hours				Days									
		6	12	18	24	2	3	4	5	7	10	12	15	18
Hothfield R = 0.97 Linear	Regression values Rate of change				78.7 -3.7	75.0 -3.7	71.2 -3.7	67.5 -3.7	63.8 -3.7	56.4 -3.7	45.2 -3.7	37.8 -3.7	26.6 -3.7	15.4 -3.7
Hamble R = 0.97 Quadratic	Regression value Rate of change	104.0 -0.6	90.9 -2.2	72.8 -3.8	44.7 -5.5									
Barming R = 0.98 Linear	Regression value Rate of change	78.2 -2.7	62.0 -2.7	45.8 -2.7	29.6 -2.7									
Winchester (0-20cm) R = 0.99 Linear	Regression value Rate of change	64.6 -3.7	42.5 -3.7	20.4 -3.7	-1.8 -3.7									
Winchester (50-60cm) R = 0.99 Linear	Regression value Rate of change	81.2 -3.5	60.4 -3.5	39.6 -3.5	18.8 -3.5									

Regression values expressed as percentage malathion remaining.

Regression and differentiation of test soils

Table 36