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ASSESSMENT OF 2,4-D RESIDUES IN THE MAJOR RICE SOILS OF KERALA

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree of

Doctor of Philosophy in Agriculture

Faculty of Agriculture
Kerala Agricultural University

DEPARTMENT OF SOIL SCIENCE & AGRICULTURAL CHEMISTRY
COLLEGE OF HORTICULTURE
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KERALA, INDIA

2002

DECLARATION

I hereby declare that this thesis entitled **'Assessment of 2, 4-D residues in the major rice soils of Kerala'** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship, associateship or other similar title, of any other University or Society.

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We, the undersigned members of the Advisory Committee of **Smt. K.M. Durga Devi** , a candidate for the degree of **Doctor of Philosophy in Agriculture** with major in Soil Science & Agricultural Chemistry, agree that the thesis entitled **'Assessment of 2,4-D residues in the major rice soils of Kerala'** may be submitted by Smt. K.M. Durga Devi in partial fulfillment of the requirement of the degree.

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Vellanikkara,

25-11-2002 K.M.DURGA DEVI

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Introduction

INTRODUCTION

More than 150 pesticides have been registered in India, under Insecticide Act 1968, of which insecticides hold a major share (37%) followed by fungicides (28%) and herbicides (21%). There was tremendous increase in the consumption of pesticides in our country during the period from 1953-54 to 1994-95. However, thereafter a declining trend in the use of pesticides was noticed primarily due to the better awareness of problems associated with the indiscriminate use of these chemicals. During 1995 – 1996, the total pesticide consumption in India was 73652 MT, which declined to 54135 MT during 1999 – 2000. This trend was not reflected in the case of herbicides, the consumption of which increased from 7393 MT to 7546 MT during the respective periods (Agnihotri, 2000).

Majority of herbicide use in our country is for wheat and rice cultivation. Though the per hectare use of herbicides in India is very much lower (13 % of total pesticides) than that of the developed countries (43 %), there is an increasing trend in the use of herbicides. (Bhan and Misra, 2001; Chhokkar, 2001). Among the 33 herbicides registered in India, isoproturon ranks first in consumption followed by butachlor and 2,4-D. Even though 2,4-D ranks third in consumption at national level, it occupies the first position in Kerala.

Chlorophenoxyacetic acids are systemic chemicals and 2,4-dichloro-phenoxy acetic acid (2,4-D) is widely used for the control of broad-leaved weeds

in cereal crops. Salt forms of 2,4-D are safer than its esters as the former do not release vapours to contaminate the environment.

The importance of chemical weed control has been increasingly realized by the farmers of Kerala, especially the rice growers, due to acute scarcity of labourers during the peak periods of cultivation and high wage rates. With noticeable shift from transplanting to direct sowing, rice weed management with herbicides is widely adopted. Fernoxone, the sodium salt of 2,4-D is the cheaper form and is most popular in the major rice growing areas of the state viz., Palakkad, kole and kuttanad.

Herbicides are applied in the early vegetative phase of rice crop where there is very little plant cover and significant quantity of the applied herbicide is likely to fall on the soil. For the rice crop, 2,4-D is recommended at the rate of one kg ha^{-1} at three weeks after sowing or transplanting (KAU, 1996). Hence the concentration of 2,4-D in the top 15 cm soil will be approximately 0.5 $\mu g\ g^{-1}$ soil.

India accounts one third of pesticide poisoning cases in the third world (Bhan and Misra, 2001). Due to severity of weed problem and lack of knowledge about the residual effect of herbicides there is a tendency for indiscriminate use of herbicides in rice fields.

Persistence of a herbicide in soil determines its effectiveness and pollution potential. If 2,4-D persists in the soil longer than the desired period it may cause several environmental problems. Adsorption by soil is the major factor

responsible for longer persistence of herbicides. Physicochemical properties of soil, viz; texture, pH, organic matter content, cation and anion exchange capacities exert a profound influence on the sorption of herbicides. As 2,4-D is weakly acidic in nature, both molecular and anionic adsorption of 2,4-D is possible under acidic soil conditions. The major rice soils of Kerala are highly acidic to slightly acidic in reaction and their properties are different from other soils of the country. A sound understanding of the relationship between soil properties and behaviour of 2,4-D in these soils is necessary for making appropriate recommendations.

Sodium salt of 2,4-D is highly soluble in water and is likely to migrate into ground water, which may lead to pollution of drinking water sources. Another risk associated with herbicide use is the alteration of biological ecosystem either by stimulating or inhibiting population of soil microflora. Continuous use of the same herbicide year after year in an area may lead to shift in weed flora. Increase in population of sedges like *Cyperus sp., Scirpus sp., Fimbristylis sp.* etc. have been noticed in rice fields in Punjab and Haryana due to continuous use of grass killers. It may also induce development of 2,4-D resistant weeds in rice fields.

Considering the above factors, studies on 2,4-D residues in the rice soils of Kerala are highly warranted. No information on 2,4-D residues in the rice ecosystem is presently available. Hence the present study was planned with the following objectives:

(i) to standardize method for the determination of 2,4-D residues in soil

(ii) to study the persistence, degradation and movement of 2,4-D in the major rice soils of Kerala, namely Palakkad, kole and kuttanad

(iii) to understand the influence of soil properties on the sorption characteristics of the herbicide

(iv) to monitor the 2,4-D residues in the soil and plant at various stages of rice growth and

(v) to find out the effect of 2,4-D application on soil microbial population.

Review of literature

2. REVIEW OF LITERATURE

In Kerala, low lands or coastal areas, made up of river deltas, backwater and the shore of Arabian sea are essentially the lands under rice cultivation. Kuttanad and kole, the two rice bowls of the state are located in this region. The main features of these two lands are (i) they lie at a level of 1.0 to 2.5 m below MSL and get flooded during the monsoons. Total area under rice cultivation in the state is 4.7 lakh ha, of which kuttanad occupies 1.0 lakh ha and kole occupies 11,000 ha (KAU, 1989a).

In kuttanad, the paddy lands are classified into five groups viz., (i) single crop lands (ii) kayal lands (iii) karappadam (iv) double crop lands and (v) kari lands, based on the soil characteristics and topography. Among these, kayal, karappadam and kari soils are the three major rice soils of this area.

Kayal soils are found in the reclaimed lake bed of Kottayam and Aleppey districts and occupy about 8,000 ha. They are situated below the sea level and slightly acidic to neutral in soil reaction, low in organic matter and poor in total and available nutrients. They are affected with salinity. The karappadam soils occur along the inland waterways and rivers spread over a large part of upper kuttanad covering an area of 41,000 ha. Soils are characterized by high acidity, high salt content and a fair amount of decomposed organic matter. They are generally poor in available plant nutrients, particularly phosphorus, and are highly deficient in lime.

The kari soils are peat soils found in large isolated patches in the Alleppey and Kottayam districts covering about 20,000 ha and exhibit characteristics of once submerged forest area. They are deep, black, heavy clay soils with poor aeration, bad drainage and low content of available nutrients. They are affected with saline inundation resulting in the accumulation of salts. These soils are highly acidic in reaction, the pH approaching 3.0 during the summer months (KAU, 1984).

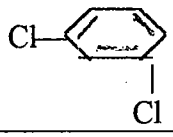
The kole area lies continuously along the coastal strip of Thrissur and Malappuram districts and cover an area of 11,000 ha. The lands are reclaimed lake beds. Acidity, salinity, poor drainage and presence of toxic salts are the characters of the region during the summer season. The fields are submerged during the rest of the period. Soil Survey Unit of Department of Agriculture in Kerala identified seven series in the kole area. They are (1) Manalur (Manalur village) (2) Ayyanthole (Ayyanthole village) (3) Anthikad (Paralam village) (4) Kizhpillikkara (Cherpu village) (5) Kattoor (Kattoor village) (6) Konchira (Venkitengu village) and (7) Perumpuzha (Manalur village). Manalur and Ayyanthole are located in the outer fringes of the kole and are at high geographic position and have better external drainage. The rest of five series are located in the interior regions of the kole areas and lie below mean sea level. Physico chemical characteristics and morphology of the kole soils are similar to those of kari soils of kuttanad (Johnkutty and Venugopal, 1993).

The Palakkad area comprises 23 % of the rice area of the state. Palakkad district comprise of five taluks viz., (1) Mannarghat (2) Ottapalam (3) Palakkad (4) Alathur and (5) Chittur. The major five rice growing areas are (1) Palakkad (2) Chittur (3) Alathur (4) Koyalmannam and (5) Nenmara and they are located in three taluks viz., (1) Palakkad (2) Alathur and Chittur. The soils are relatively old and are formed as a result of weathering of gneissic rocks. These soils are deep to very deep, moderately to excessively drained with moderate permeability. The textural range noticed is from loam to gravelly silty clay loam and colour varies from black to dark reddish brown. Black soils are found to occur in patches and are considered as extensions of black cotton soils observed in the adjacent Coimbatore district of Tamil Nadu. These soils are dark, low in organic matter, calcareous, neutral to moderately alkaline and high in clay content and cation exchange capacity (KAU, 1989b).

In kuttanad, farmers have been using 2,4-D for the last two decades and the total consumption of 2,4-D in this area during 1998-1999 was 40 MT (KAU, 1999). Eventhough relevant data on the use of 2,4-D in kole and Palakkad is not available; there are indications that the use of 2,4-D has become a regular practice in rice fields of these areas.

The technical information and toxicity data on 2,4-D are presented in Table 1.

Table 1. Technical information and toxicity data on 2,4-D

Chemical name	2, 4-dichloro phenoxyacetic acid
Chemical structure	
Chemical formula	$C_8H_6Cl_2O_3$
Molecular weight	221.00
Physical state, colour and odour	White crystals, odourless when pure
Specific gravity	1.565
Melting point	140.5°C
Boiling point	160°C at 0.4 mm Hg
Decomposition temperature	Stable at its melting point
Vapor pressure, mm Hg at 160°C	0.4
Solubility in water at 25°C	0.09 g/100g
Solubility in Benzene at 28°C	1.07 g/100g
Solubility in Carbon tetra chloride at 25°C	0.1 g/100g
Solubility in Ethyl ether at 25°C	27 g/100g
Acute toxicity (Acute oral LD_{50}) for rats	375 mg / kg
Chronic toxicity	Rats (2 years) 1250 ppm in diet – no effect Dogs (2 years) 500 ppm in diet – no effect
Dermal acute precutaneous LD_{50} for rats	> 1600 mg / kg
Inhalation LC_{50} 24h exposure for rat	> 1.79 mg l^{-1} air
Acceptable daily intake	0.3 mg/kg

Source: BCPC, 1979; WSSA, 1983; Gahukar, 1999

While reviewing the research works conducted in different parts of the world and in India on the residues of 2,4-D in soil, it was felt that the literature is so voluminous that the presentation of all the works is beyond the scope of this chapter. The major findings are classified under the following sections.

2.1 History of development of 2,4-D

During the mid 1930's when auxin properties of IAA were discovered synthetic compounds related to IAA were applied to a wide range of plants as rooting compounds by Zimmerman and Wilcoxin in 1935. Subsequent works showed that acetate side chain was essential for auxin behaviour and the chlorinated phenoxy compounds could act as analogues for indolyl compounds. Their lower toxicity and less polar nature led quickly to the successful demonstration of their herbicidal characteristics. The synthesis of 2,4-D and 2, 4, 5-T was first accomplished by Pokorny of the C. B. Dolge company, Westport, Conn in 1941 (Pokorny, 1941). For this, equimolar quantities of

2,4- dichlorophenol and 2, 4, 5-trichlorophenol were mixed separately with monochloracetic acid and heated with a slight excess of sodium hydroxide together with water and evaporating almost to dryness. Both were white odourless crystals almost insoluble in water. In 1944, Hamner and Tukey published a report on the selective herbicidal action of 2,4-D and 2, 4, 5-trichlorophenoxy acetic acid (2, 4, 5-T) on bindweed and on the superior effectiveness of 2, 4, 5-T as a bush killer. 2,4-D had a very definite specific toxicity towards broadleaved plants and it was needed in very small concentration (Kirby, 1980).

2.2 Dissipation of 2,4-D from soil

2.2.1 Mechanisms

Physical, chemical and biological processes are involved in 2,4-D dissipation from soil. Precipitation can move the herbicide from the surface soil layers to lower depths. The herbicide may also dissolve in run off water. Some quantity may be taken up from the soil by crops and weeds. With volatile esters, evaporative losses are possible immediately following their application. These mechanisms of loss do not degrade the herbicide molecule, but do remove residues from the surface soil and distribute them into the environment. Actual degradation of herbicide in soil is taking place by photochemical, chemical and biochemical processes (Smith, 1989).

Photochemical degradation of several phenoxyalkanoic acids and their esters have been reported in aqueous solution (Payne and Fults, 1947; Aly and

Faust, 1964; Crosby and Tutass, 1966; Boval and Smith, 1973; Binkley and Oaks, 1974; Soderquist and Crosby, 1975 and Zepp *et al.*, 1975). But their photolysis on soil surface has not been reported since they are foliar applied herbicides and the residues reaching the soil surface will be partially protected from sun's radiation by the crop canopy. Thus photochemical break down would not be expected to be a major mechanism for the loss of phenoxy alkanoic acids from soil. There is little breakdown of herbicidal acids in the absence of microbial activity and hence chemical mechanisms do not appear to result in any significant degradation of phenoxy alkanoic acids in the soil (Smith, 1989).

2.2.2 Degradation of 2,4-D by soil microorganisms

Microbial degradation is the major pathway of 2,4-D dissipation from soil. Shortly after their introduction, it was reported that they were degraded in the soil by warm and moist conditions that favored microbial activity. The importance of microorganisms in the breakdown of these herbicides was demonstrated by the pioneering studies of Audus (1951) using soil perfusion techniques, in which soil columns were continuously percolated with aerated solutions containing 100 ppm of the test herbicide. By monitoring the amounts of herbicide remaining in the eluate, it was shown that after an initial adsorption of the herbicides to soil colloids there was a lag phase, varying from 2 weeks for 2,4-D to 40 weeks for 2, 4, 5 T, during which little or no breakdown of the phenoxyacetic acids occurred. This lag phase was then followed by a period of rapid herbicide degradation. Further application of the phenoxyacetic acids to

the perfusing solution was rapidly degraded without a prior lag phase. During the lag phase microbes produce adaptive enzymes which presumably resulted in the gradual buildup of a population of organisms capable of metabolizing the herbicides and utilizing them as a carbon source for growth (Audus, 1951 and 1964). Once this population had reached a critical size, rapid breakdown of the phenoxyacetic acids occurred so that further additions of the herbicide to the enriched soil were rapidly metabolized.

Subsequent investigations confirmed that microbial degradation was the major mechanism resulting in the decomposition of the phenoxyalkanoic acids in the soil and various bacteria and actinomycetes capable of degrading phenoxyalkanoic acids in the soil, were isolated from soils and used in culture solution to investigate degradation pathways. These early studies have been extensively reviewed by Audus (1951 and 1964), Freed and Montgomery (1963), Kearney and Kaufman (1972), Sharpee (1973) and Loos (1975).

Much of this early microbiological work on the metabolism of phenoxyalkanoic acids was carried out in liquid media using microbial cultures. As with the perfusion studies, high herbicidal concentrations were frequently used to aid in the isolation and detection of both parent acids and metabolites. Thus, the relevance of the early investigations to an understanding of the biological degradation of low concentrations of phenoxyalkanoic acids in soils under laboratory and field conditions was limited (Smith, 1989).

Studies have been conducted to enumerate the microorganisms that are able to degrade MCPA and 2,4-D acids in soils. Less than ten 2,4-D degrading organisms per gram soil were reported in some soil, while in others over 1,000,000 per gram were enumerated (Tortensson *et al.*, 1975; Loos *et al.*, 1979; Fournier, 1980; Cullimore, 1981; Fournier *et al.*, 1981; Kunc and Rybatova, 1983, Ou, 1984; Soulas *et al.*, 1984).

Field plots receiving annual applications of approximately one kg ha^{-1} of 2,4-D over a period of 32 years contained higher populations of 2,4-D degrading organisms than similar plots receiving 0.42 kg ha^{-1} and untreated check soils (Cullimore, 1981).

In a study conducted at highly saline and alkaline lake site in southwestern Oregon, USA, contaminated with 2,4-D production wastes, three, 2,4-dichlorophenoxy acetic acid (2,4-D) degrading bacterial isolates were obtained. While similar in most respect, the three isolates differed significantly in 2,4-D degradation rates, with the most active strain, I-18, demonstrating an ability to degrade upto 3000 mg 2,4-D in 3 days (Maltseva *et al.*, 1996).

2.2.2.1 Pathway of 2,4-D degradation by soil microbes

From the early microbiological studies, which have been the subject of several reviews it was apparent that major metabolic mechanisms associated with phenoxyalkanoic acids were cleavage of the ether linkage, beta oxidation of the side chain aliphatic acid moiety, ring hydroxylation, and ring cleavage.

Depending on the complexity in the nature of soils and the presence and interactions of myrid soil organisms, the breakdown pathway of phenoxyalkanoic acids in soil would be different (Audus, 1964; Freed and Montgomery, 1963; Kearney and Kaufman, 1972; Loos, 1975).

2,4-dichlorophenol can undergo reactions with soil constituents (Bollag *et al.*, 1980 and Minard *et al.*,1981) that can lead to its incorporation into soil organic matter. However, such reactions probably do not occur in soils treated with 2,4-D, since it has been suggested that 2,4-D is degraded intracellularly (Stott *et al*; 1983). Therefore any 2,4-dichlorophenol formed by metabolic processes is likely to remain within the microbial cells and not come into contact with soil to undergo reactions with soil components.

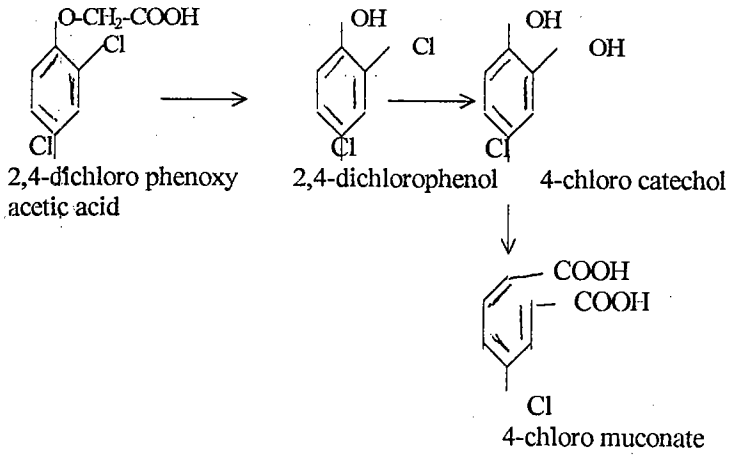
Both 2,4-dichlorophenol and 2,4-dichloroanisole were isolated in small amounts when the degradation of ring-labelled ^{14}C-2,4-D was investigated. At rates of 1.0ppm small amounts of ring-labelled ^{14}C-2,4-dichlorophenol were converted to the corresponding anisole, confirming that ^{14}C-dichloroanisole is formed by methylation of the phenol in soil (Smith, 1985).

Given the volatile nature of the phenols and anisoles, and the fact that the phenols will exist in the volatile molecular form rather than the nonvolatile anionic form in soil, it is doubtful whether these degradation products could accumulate in the field (McCall *et al.*, 1981 and Smith, 1985).

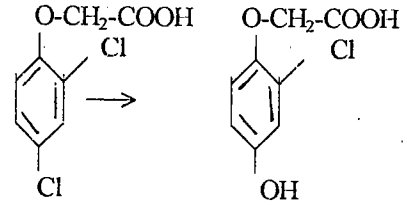
Laboratory studies conducted with ^{14}C-2,4-D specifically labelled in the carboxyl group, in the chain-2C, and with uniform labelling in the six ring carbon atoms, have indicated that in moist nonsterile soil there is rapid evolution of $^{14}CO_2$ in all cases. These data confirm that the ring and side chain of the 2,4-D molecule undergo cleavage, with major conversion of the eight carbon atoms to carbon dioxide. The evolution of $^{14}CO_2$ from the different labelled positions follow a classical sigmoidal curve with a slow initial release being followed by a rapid evolution. Finally a plateau is reached, when release of $^{14}CO_2$ is continued at a much reduced rate. 2,4-D, 2,4-DB and 2,4,5-T are easily degraded in soil to smaller molecules, which can themselves undergo further degradation to carbon dioxide and other small carbon-containing fragments. These are then incorporated into the microbial biomass and soil organic matter and buildup of phenolic degradation products in soil would not be expected (Smith, 1989). Gahlot and Narula (1996) reported that the resistant strains of *Azotobacter chroococcum* degraded 2,4-D to chlorocatechol, even at a concentration of 2500 ppm, in the presence of sucrose as a C source in liquid medium and without any additional C source in soil. Chlorocatechol formation occurred even at stationary phase of cells indicating co-metabolism of 2,4-D. The nitrogenase activity on these strains remained unaffected upto 50 ppm of 2,4-D in liquid medium. Accumulation of chlorocatechol showed that some strains may not be able to metabolise this intermediary product.

Fig. 1 Pathway of microbial degradation of 2,4-D in soil

2,4-D degradation by *Arthrobacterium* and *Pseudomonas*



2,4-D degradation by *Pseudomonas* through dehalogenation



2,4-dichloro phenoxy acetic acid 2-chloro 4-hydroxy phenoxy acetic acid

(Source: Rao, 1992)

2.2.2.2 Factors influencing microbial degradation of 2,4-D in soil

Concentration of the herbicide in soil, type of formulation, presence of other pesticides, repeated treatments, soil depth, soil pH, soil type, soil moisture and soil temperature are the major factors influencing rates of loss of 2,4-D from soils.

2.2.2.2.1 Concentration of 2,4-D in the soil

From the studies on the evolution of $^{14}CO_2$ from ^{14}C-2,4-D treated soils, and from the loss of radio labelled and non radioactive phenoxyalkanoic acids from fortified soils, it appeared that the breakdown of these herbicides partially depends upon their soil concentration. Thus the breakdown rate approximates first order kinetics below about 10 ppm (Altom and Strizke, 1973; Smith 1978; Walker and Smith, 1979; Smith and Hayden, 1980; Smith and Hayden, 1981; McCall *et al.*, 1981; Sattar, 1982 and Ou, 1984), whereas at higher soil concentrations up to about 500 ppm, degradation is biphasic with slow initial breakdown followed by an increased breakdown rate (Ou *et al.*, 1978; Fournier *et al.*, 1981; Parker and Doxtader, 1982 and 1983).

In laboratory experiments where low treatment rates (1 to 5 ppm) were incorporated in the soil the herbicide would provide insufficient nutrients and energy for an increase in the population of herbicide degrading organisms. Thus initial degradation would be mainly due to co-metabolism. At higher herbicide concentrations, the degrading microorganisms should be able to utilize carbon

from the herbicide to increase the number of the metabolizing organisms, thus eventually increasing the herbicide degradation rate (Altom and Strizke, 1973; Smith, 1978; Walker and Smith, 1979; Smith and Hayden, 1980; Smith and Hayden, 1981; McCall *et al.,* 1981; Sattar, 1982; and Ou, 1984),

It would appear that the persistence of high rates of applications of formulated 2,4-D and 2, 4, 5 T can be greater compared to that from normal rates (Stewart and Gaul, 1977 and Bovey, 1980). But even at field rates of 5-6 kg ha^{-1}, the n-butylesters of 2,4-D and 2, 4, 5 T slowly degraded over 3 years. At very high rates, the toxic effects of the additives in the herbicidal formulations would probably inhibit soil microorganisms more than the herbicides themselves. (Majka *et al.*, 1982).

2.2.2.2.2 Type of Formulation

Phenoxyalkanoic amine and metallic salts are salts of weak acids and would be expected to undergo dissociation to their respective phenoxyalkanoic anions in soils. These anions would presumably reassociate with a variety of inorganic cations present in the soil to maintain electrical neutrality before undergoing further degradation (Smith, 1989).

2.2.2.2.3 Presence of other pesticides

Croplands receiving annual treatments with phenoxyalkanoic acids often receive applications of a second or third pesticide for additional fungus, insect, or weed control. These chemicals can be applied as tankmixes, as separate pre and

postemergence treatments, or as seed treatments. In addition, residues of certain persistent pesticides can be carried over in the soil to the following growing season, so that phenoxyalkanoic acid treatments can come into contact with soil already containing residues of previously applied herbicides. It is possible that additional pesticides may adversely affect the soil organisms capable of degrading phenoxyalkanoic acids, which could perhaps result in increased soil persistence.

All studies designed to assess the persistence of phenoxyalkanoic acids in combination with other pesticides have been carried out under carefully controlled laboratory conditions, and not much investigations have been carried out to determine the significance of any changes under field conditions. These investigations under laboratory conditions have indicated that the soil persistence of 2,4-D can be prolonged in the presence of the insecticides carbaryl and parathion, or the herbicide dinoseb, but not in combination with the fungicide benomyl (Repiquet and Fournier, 1977). The breakdown of 2, 4, 5-T was not affected by 2,4-D in several different soil types (Smith, 1979). Similarly, the degradation rate of 2,4-D was unchanged in the presence of benzoylprop-ethyl, dicamba, diclofopmethyl, dichloroprop, difenzoquat, dinitramine, flamprop-methyl, nitrofen, picloram, TCA, 2, 4, 5-T, triallate, trifluralin, or a combination of triallate and triflurain (Smith, 1979 and 1980). Neither was the soil degradation of 2,4-D affected by the cereal seed dressing Vitaflow-DB (mixture of the fungicide carbathiin (14.6 % by weight) and the insecticides lindane (18.7 % by weight) and thiram (28.9 % by weight), the insecticide malathion or

by a mixture of Vitaflow-DB and malathion (Smith, 1980). The breakdown of MCPA (Smith, 1982) was unaltered by the presence of benzoylprop-ethyl, bromoxynil, bromoxynil with asulam, bromoxynil with difenzoquat, dicamba, dicamba with mecoprop, diclofop-methyl, flamprop-methyl, linuron, MCPB, metribuzin, propanil, TCA, triallate, trifluralin, triallate in combination with trifluralin, Vitaflow-DB, malathion or a mixture of Vitaflow-DB and malathion.

Interactions among herbicides and chemicals affect their persistence and residues in soil. In the presence of MCPA, degradation of 2,4-D was rapid and vice versa (Rao, 1992). The breakdown of 2,4-D is not influenced by commonly used herbicides or other pesticides under field condition. In uplands, rice treated with butachlor + 2,4-D @ 2.0 + 0.75 kg ha^{-1} both herbicides were degraded rapidly (Deka and Gogoi, 1993).

2.2.2.2.4 Repeated application

Increased rates of loss of 2,4-D and MCPA, following repeated use, have also been reported in field experiments (Kirkland, 1967; Fryer and Kirkland, 1970; and Tortensson *et al.*, 1975). Thus, it appears that once soils contain a microbial population adapted to phenoxyalkanoic acid herbicides, the rate of degradation will be higher for further applications of the same herbicides.

The findings from longterm plots at Saskatchewan, have indicated that crop fertility, as determined by wheat yields, has not been impaired by 25 repeated spring applications of amine and ester formulations of 2,4-D This

confirmed that no build up of the 2,4-D had occurred. Soil from these plots after 35 spring treatments with amino and ester formulations of 2,4-D indicated that less than 0.05 ppm of the herbicide was recoverable from the top 10 cm soil, six months after the last treatment. This confirmed that no build up of the 2,4-D had occurred (Smith and Hayden, 1981).

2.2.2.2.5 Soil characteristics

2.2.2.2.5.1 Soil depth

Since soil organic matter, temperature, and aeration are more favourable for microbial activity in topsoil than in subsoil, degradation rates might decrease if a herbicide is leached into the subsoil. However, over a five months period the degradation of 2,4-D, stored in field pits was rapid under aerobic soil conditions and it was completely dissipated at 15, 40 and 90 cm depths (Lavy *et al.*, 1973).

Vee *et al.* (1996) reported that soil depth and temperature are the important variables affecting microbial growth and degradation kinetics. Soil samples collected from 0-30, 30-60 and 60-120 cm depths of two Montana (USA) soils, when treated with ^{14}C labelled 2,4-D at temperatures of 10, 17 and $24^{0}C$, degradation rates of 2,4-D decreased significantly with soil depth and were positively correlated with microbial plate counts. An increase in temperature was shown to increase 2,4-D breakdown.

2.2.2.2.5.2 Soil pH

Soil acidity or alkalinity can affect degradation directly and indirectly via its effects on the adsorption of the herbicide to the soil. The pH of the soil also influence the composition of the soil microflora.

Conclusions, based on bioassy techniques, indicated that maximum degradation of 2,4-D occurred at a soil pH of 5.3 and Corbin and Upchurch (1967) inferred that this pH level was the most favourable for growth of specific microorganisms involved in the breakdown.

Degradation studies with 2,4-D and MCPA in natural soils of pH 3.8, 4.2, 4.8 and 6.3 had indicated that the most rapid breakdown occurred at the highest pH. However, as the microflora content of each soil could have been drastically different, no general conclusions can be made as to the effects of soil pH on persistence (Torstensson, 1975).

2.2.2.2.5.3 Soil organic matter content, temperature and moisture

Because soil microorganisms are involved in the degradation of phenoxyalkanoic acid herbicides, soil organic matter should influence their breakdown. There is usually more microbial activity in soils containing greater amounts of organic material than in mineral soils. On the other hand, adsorption of the herbicides to soil components could reduce their amounts in the soil solution and so provide protection from degradation. The K_d values obtained from Freundlich adsorption isotherm for 2,4-D in prairie soils ranged from 0.0 to

1.3 (Grover, 1973). Adsorption was correlated with soil organic matter content (Grover and Smith, 1974).

Since the rates of non biological reactions and biological processes are favoured by increasing temperature, herbicide degradation rates should also increase. Adequate moisture is also essential for microbiological activity. At a soil temperature of 20^0 C, the rate of breakdown of 2,4-D was similar at moisture levels between 65 and 100% of field capacity, but was much slower at 50% of field capacity. On the other hand, at a soil temperature of 25^0 C the breakdown of 2, 4, 5-T was similar at soil moistures ranging from 50 to 100% of field capacity (Smith, 1989).

The degradation rate of 2,4-D and the proliferation rate and maximum population of the 2,4-D degraders in soil were markedly different depending on the moisture conditions (Kuwatsuka and Miwa,1989). It was suggested that the kinds of degraders contributing to 2,4-D degradation differed with the soil types (Kuwatsuka and Miwa, 1989; Miwa and Kuwatsuka, 1990). The effect of soil water content on the bio degradation of 2,4-D and the degrading microorganisms was studied in soils whose water potentials were altered using different salt concentrations (Han and New, 1994). Higher rate of 2,4-D degradation were observed at field capacity (psi=0.1 mpa) and decreased to no degradation at psi = -22 mpa. 2,4-D degrading bacteria decreased more rapidly as soil water content decreased. *Azotobacter chroococcum* isolated from forest soils containing decaying wood efficiently cleaved 2,4-D. The cleavage was profoundly

influenced by bacterial cell concentration, moisture content of the soil and temperature at which the soil was incubated. An increase in cell concentration increased 2,4-D degradation. The conditions which favoured maximum degradation of 2,4-D were 30^0 C (58% degradation) and 50% moisture content (Balajee and Mahadeven, 1993).

Willems *et al.* (1996) studied the effects of temperature, moisture and initial pesticide concentration on mineralisation of 2,4-D and atrazine at different soil depths down to 1.5 m. Mineralization of 2,4-D was continued for 2.5 months and atrazine mineralisation for one month. Between 10 and 80 % of applied 2,4-D was completely mineralized depending on the test conditions and soil depth. 2,4-D mineralisation rates were generally highest at depths between 1.0 and 1.5 m and on average 4 times higher than in the surface soils. Variations in 2,4-D mineralization rates with depth could not be correlated to changes in soil organic C, soil biomass or chemical availability and are probably the result of complex interaction between microbial activity, nutrient status and soil chemical and physical properties.

Voos and Groffman (1997) found that 2,4-D dissipation in soil had a direct relationship with the microbial biomass and soil organic matter content. Hence the presence of 2,4-D in soil is a matter of little significance.

2.3 Persistence of 2,4-D in soil

Persistence of herbicides in soils is an important factor determining its suitability to a particular soil. Soil persistence data under controlled laboratory conditions for single application of individual phenoxyalkanoic acids indicated that the half life of 2,4-D in a variety of soils at different temperatures ranged from 2 to 28 days and it can be concluded that the phenoxyalkanoic acids are degraded rapidly in warm soils with adequate moisture (Foster and Mekercher, 1973; Mccall *et al.*, 1981). Breakdown of the phenoxyalkanoic acids is rapid under field conditions so that negligible amounts are carried over at the end of the year of application. Both 2,4-D and 2,4,5-T are capable of some leaching from the top soils and the amount depends upon soil type and rainfall. Half life of 2,4-D was 20.5 days under field capacity and the half life decreased with increasing soil moisture content (Foster and Mckercher, 1973).

Many studies have been conducted on the soil persistence of 2,4-D (De Rose and Newman, 1947; Brown and Mitchell, 1948; Newman *et al.*, 1952; Burger *et al.*, 1962; Norris, 1966; Altom and Stritzke, 1973; Foster and Mckercher, 1973; Plumb *et al.*, 1977; Smith and Aubin, 1991,; Sankaran *et al.*, 1993 and Cox, 1999). There is wide controversy on the persistence of 2,4-D in soils. Although the usual time for 2,4-D persistence was 2 to 4 weeks, detoxification varied from 14 to 94 days depending on the soil. As reported by Cox (1999) persistence of 2,4-D is variable at the half lives of 2-297 days.

Studies on persistence of 2,4-D conducted at Bangalore showed that 2,4-D persisted in red soil up to 3 months (Leela, 1986). At Faizabad, the 2,4-D Na salt persisted only for 42 days (Sankaran *et al.*, 1993).

2.4 Adsorption of 2,4-D by soil components

Adsorption of anions increases greatly with falling pH. It is also intimately connected with the nature and properties of soil colloids viz. type of clay minerals, contents of hydrous oxides, organic matter and the ratio of SiO_2 and R_2O_3 (Bear, 1964).

Adsorption by soil organic matter and clay colloids play an important role in determining the fate of pesticide in a soil. Adsorption will control the quantity of a pesticide in soil solution and thus determine its persistence, leaching, mobility and bioavailability. The extent of adsorption of pesticide depends upon the nature and properties of the chemical itself, the kind and amount of organic matter present and the environment provided in the soil. Once adsorbed by soil organic matter surface, it may be easily desorbed and it is particularly in the case of acidic pesticides. The adsorbed particles can readily be released to water (Harris and Warren, 1964). At low pH levels most of the weakly acidic herbicides are present in the molecular form than the anionic form. Thus they would be adsorbed to a greater extent than stronger acidic herbicides.

Increase in soil moisture content increased the adsorption of nonionic pesticides (Hance, 1967; Doherty and Warren, 1969).

Khan (1974) observed that fulvic acid clay complex adsorbed high amount of 2,4-D. The adsorption of 2,4-D is greater in topsoil than in the sub soil which has less organic matter (Wilson and Cheng, 1978). Adsorption was correlated with soil organic matter content (Grover, 1973; Grover *et al.*, 1985). Adsorption was dependent to some extent on the pH of the soils, which ranged from 3.25 to 6.91 (Moreale and Van-Bladel, 1980).

In general the adsorption of 2,4-D on to the individual model component of soils and natural soils is described by a Freundlich isotherm, although in some cases pseudo first order kinetics may be shown (QueHee and Sutherland, 1981). Most of the research in this field has been of artificial nature and do not approximate field conditions.

Adsorption of 2,4-D to soil components could reduce their amounts in the soil solution and so provide protection from degradation. The affinity of 2,4-D for the more acidic soils could be due to increased adsorption to organic matter by the less water-soluble molecular species rather than the water soluble anionic form (Smith, 1989).

From the solution phase studies of the adsorption of 2,4-D on soils from Palampur, Ludhiana and Habowal, Bhardwaj and Singh (1991) found that adsorption capacity increased with rise in activation temperature, decrease in pH and increase in organic matter content of the soil. Multiple linear regression analysis between K_d (adsorption coefficient) and various soil parameters

indicated that organic matter was strongly correlated ($r^2 = 0.650$) with K_d and pH had only a small additional effect on adsorption of 2,4-D (McGrath, 1994).

Studies on sorption and movement of 2,4-D in three New Zealand soils which were amended with different levels of exogenous carbon (poultry manure, sewage sludge, mushroom compost, peat and pig manure) using ^{14}C labelled compounds indicated that the sorption as measured by the distribution coefficient (K_d), increased with increasing C addition, and varied between the C-sources. The difference in the effect of C sources on the sorption of herbicides was related to the difference in the amount of dissolved organic carbon (DOC) and the pH. The increase in the K_d values per unit, C addition decreased with increasing amounts of both the exogenous C addition and the indigenous C in the soil material. In a separate study, the addition of DOC to solutions of herbicides prior to sorption measurements decreased the sorption of herbicides, where as the addition of DOC to soil increased the sorption of herbicides (Baskaran *et al.*, 1996).

Studies conducted in New Zealand by Bolan and Baskaran (1996) on adsorption desorption behaviour and the degradation of ionic herbicide 2,4-D using 10 soils of varying organic matter and clay content indicated that the adsorption isotherm tended to become linear ie. the values of the exponent 'n' of the Freundlich isotherm were close to 1 (0.92 to 0.98). The extent of adsorption as measured by the distribution coefficient (K_d) which increased with an increase in soil organic carbon. The rate of desorption of 2,4-D followed first order

kinetics with respect to surface concentration and decreased with an increase in the organic carbon content of the soils. The rate of degradation of 2,4-D, as measured by the half life ($t_{1/2}$) decreased with an initial increase in soil organic carbon, which is attributed to the increase in adsorption. With increasing adsorption, the rate of desorption decreased resulting in a low concentration of 2,4-D in the soil solution that is available for microbial degradation. When the organic carbon content was more than 12% both the adsorption and rate of degradation increased.

Working with Malaysian agricultural soils lower values of Freundlich adsorption distribution coefficients (Kads (f) were observed for 2,4-D (0.57 and 5.26) in a sandy loam and muck soil respectively. Desorption of 2,4-D from the muck soil occurred. Adsorption of pesticides was not affected by temperature (20^0 C/30^0 C), and pH (Chea-Uanbohl *et al.*, 1997).

2.5 Leaching and movement of 2,4-D in soil

The same factors for adsorption work inverse for leaching. Leaching into the soil is influenced by moisture levels and evapotranspiration rate (Hartley, 1964). Reports on surface water monitoring indicated that only those herbicides that had been applied very frequently viz., 2,4-D and atrazine were found in the river water. Since the herbicides are used rather early in the crop season where there is only little plant cover, much of the chemical gets into the soil where it is subjected to different translocation processes which leads to pollution of lakes and streams with these chemicals (Hurle, 1993).

Percolating water appears to be the principal means of movement of relatively nonvolatile compounds, and diffusion for these compounds in soil water is important only for transport of over very small distances (Hartley, 1964). This certainly holds for the 2,4-D anion and the diffusion coefficient for the anion in nine soils worked out by Lindstrom *et al.*, (1968) was generally found to be inversely, related to soil texture. 2,4-D was found to leach as deeply as 90 cm and degrade quickly under aerobic, but not under anaerobic conditions (Lavy *et al.*, 1973). European Economic Community Directive concerning the quality of water for human consumption established the maximum concentration of each pesticide at 0.1μg l^{-1} and the total pesticide concentration at 0.5 μg l^{-1} (Vettorazzi, 1979).

Studies on the leaching losses of the different herbicides in sandy loam soils of Karnataka revealed that 31 to 68 % of the herbicide is lost from the soil. 2,4-D moved up to 20 cm in the soil column. However, higher quantity was retained in the top 5 cm soil layer (Sankaran *et al.,* 1993). Rocco-Estrella *et al.*, (1993) reported that sorption of 2,4-D had a slight but significant effect on transport of 2,4-D under saturated (retardation factor 0.18) and unsaturated conditions (retardation factor 0.34).

Felding (1995) measured the content of phenoxy alkanoic herbicides in drainage water from three soils (sandy loams, clay loams and sandy clay loams) over a two year period. The herbicides content in 17 out of 65 water samples was greater than 0.1μg/litre, which is the maximum residue limit for drinking

water in Europe. Jorgensen *et al.*, (1995) evaluated the leaching of phenoxy herbicides to soil water, ground water and surface water. Transport of these pesticides occurred in fractures and macropores, largely bypassing the surrounding matrix. In approximately 30% of these samples, the maximum residue limit for drinking water (0.1 µg / litre) was exceeded.

Column studies with exogenous carbon sources on the sorption and movement of 2,4-D by soils of New Zealand showed that dissolved organic carbon enhanced the movement of herbicides in soils. The effect of DOC on the movement of herbicides varied between the soil materials and may be related to the difference on the sorption of both the herbicides and the DOC (Baskaran *et al.*, 1996).

Smith and Bridges (1996) reported that only small quantity of 2,4-D was found in the leachate from the green house and out side lysimeters. The concentrations of these herbicides did not exceed 61 µg l^{-1} and the total quantity to exit the lysimeters was less than 0.9 % of the applied herbicide. Over an 8 days period following the treatment, 9 % of the applied 2,4-D left the simulated fairways.

Leaching of 2,4-D was evident under a high water influx (200 mm) and comparable results were observed between laboratory studies and a VARLEACH model prediction, (Cheah-Uan Bol *et al.*, 1997).

Leaching, drain flow and surface runoff are the main pathways responsible for herbicide movement within soils. It is therefore necessary to understand the spatial characteristics of soils, their hydrology and the associated herbicide use patterns (Carter, 2000).

2.6 2,4-D residues in surface and groundwater

High application rates, particularly of pesticides characterized by high water solubility and persistence and low adsorptivity are the main factors responsible for high water pollution (Cohen *et al.*, 1984). In still water (ponds, lakes or reservoirs) 2,4-D persisted as much as 6 months after application (USEPA, 1988). 2,4-D is frequently found in rivers and streams. This is particularly due to 2,4-D' s wide use. It is also due to high solubility of 2,4-D in water (Albanis, 1992).

Felding (1995) measured the content of phenoxyalkanoic herbicides in drainage water from three soils (sandy loams, clay loams and sandy clay loams) over a two year period. The herbicide content in 17 out of 65 water samples was greater than 0.1 $\mu g\ l^{-1}$ which is the maximum residue limit for drinking water in Europe.

In a study by Jorgensen *et al.* (1995) leaching of phenoxy herbicides to soil water, ground water and surface water was evaluated and they found that in approximately 30 % of the samples, the maximum residue limit for drinking water was 0.1 $\mu g\ l^{-1}$.

Farm ponds in the Canadian Prairies are almost universally contaminated with 2,4-D and over 93 percent of ponds tested in Canada contained 2,4-D. Higher concentration was measured in the spring, but unlike other pesticides studied, 2,4-D residues persisted past to end of the growing season (Grover *et al.*, 1997).

In a national survey of river basins 2,4-D was found in 19 out of 20 basins samples. Over all, 10-13 % of the samples collected were contaminated (Cox, 1999). The amount of herbicide that moved away from the area of application was dependent on the physico chemical properties of the chemical and the agroclimatic characterisatics of the target site. Under average conditions the amount of herbicide lost by movement from a soil profile is typically less than 0.1 % of the applied mass, but under certain localized circumstances it can reach upto 5 % or greater.

The levels of pesticide residues in water depend on various factors related to the pesticide properties and their behaviour in the environment, the agricultural practice, and pesticide application rates, soil, geology and climatic characteristics (Cohen *et al.*, 1984). As per the report of Croll (1995) the quantity of 2,4-D detected in ground waters in East Anglia was 0.11 to 0.20 $\mu g\ l^{-1}$. Only 1 % of the total number of samples taken for analysis registered 2,4-D residues, while the herbicide atrazine was detected in 28% of the total samples, at a concentration range of 0.02 to 0.43 $\mu g\ l^{-1}$. Studies on the persistence of 2,4-D in ground water revealed that its half-life varied from 800 to 1900 days (Covalier *et al.*, 1991).

Ground water contamination is generally considered as the greatest threat posed by pollutants from diffuse sources (Harris and Skinner, 1992).

2,4-D contaminated wells because of its very high mobility in soils (Cheah-UanBol *et al.,* 1997). Contamination of ground water has been directly associated with roadside application of 2,4-D for noxious weed control and 2,4-D manufacturing plants (Cox, 1999).

A study on monitoring of pesticide contamination in ground and river water from Bulgarian Danube plain indicated that atrazine was the most frequently found active ingredient followed by alachlor and 2,4-D. 2,4-D was detected in 27.3 % of samples, while atrazine was detected in 54.5 % of the samples. Residues range was 0.02 to 0.09 $\mu g\ l^{-1}$ for atrazine and 0.08 to 0.140 $\mu g\ l^{-1}$ for 2,4-D (Balinova and Mondesky, 1999).

A study on "Evaluation of kuttand ecosystem for possible contamination by pesticides, herbicides and toxic heavy metals" was conducted at Regional Agricultural Research Station, Kumarakom during 1996-1999. In this study, 2,4-D residues were detected in 17 % of field water samples at a concentration range of 0.26 to 8.7 $\mu g\ l^{-1}$. Samples taken during January showed higher values and that of July showed lower values. Residues of 2,4-D were detected in 13% river water samples in the range of 0.50 to 5.40 $\mu g\ l^{-1}$ and the residue content was high during January and trace in July. In the case of ground water, residues were high during May and only 8% of the samples showed the presence of residues of 2,4-D upto a concentration of 0.09 $\mu g\ l^{-1}$. The maximum residue level of 2,4-D

detected in ground water samples of kuttanad was below the permissible limits of 0.1 $\mu g\ l^{-1}$ (Anon, 1999).

2.7 2,4-D residues in rice ecosystem

Post emergence herbicides 2,4-D and MCPA are the most widely used herbicides in rice in several countries and they have shown good performance in India (Bhan, 1978) at the rates ranging from 2 to 4 kg ha^{-1}. Studies conducted at Regional Agricultural Research Station, Pattambi (KAU, 1993), also showed that 2,4-D is highly effective in controlling weeds under semi dry rice. The grasses at the younger stages also are found to be controlled by 2,4-D particularly with its ester formulation .Being the cheaper form, sodium salt of 2,4-D is preferred to its ester form. Joint application of 2,4-D and fertilizer urea resulted in effective and economic weed control in transplanted rice (John and Sadanandan, 1995).

Audus (1951) found that in general 2,4-D persisted for 2 to 4 weeks. Sankaran *et al.* (1993) summarized the results of the herbicide residue studies conducted at different AICRP Centres in India. At Bangalore, 2,4-D persisted in red soil upto 3 months. At Faizabad, 2,4-D salt persisted only for 42 days. There is wide disagreement on how persistent the phenoxys are in soils. As reported by Cox (1999) persistence of 2,4-D in soil is variable at the half lives of 2-297 days.

The maximum residue limits (MRL) permitted for 2,4-D in rice grain and straw are 0.01 ppm and 20.00 ppm respectively (FCN, 1982). Tejada *et al.* (1995) reported the results of a multidisciplinary investigation to assess the

environmental and health impacts of pesticides in rice field ecosystem in Laguna, Philippines. The on and off paddy effects of pesticides on rice plants, soil, paddy water, rice field, flora and fauna, ground water and well water have been detected specifically and concluded that pesticide use in rice fields did not represent a significant environmental threat. Rice samples contained no residues at harvest time because of the usual practice of harvesting rice 30-45 days after the last application of pesticides.

When 2,4-D amine salt @ 2 kg ha^{-1} was applied at fully tillering stage in paddy, residues persisted in rice plants for one month but no residues were detected in harvested grain (Sokolov *et al.*, 1974) 2,4-D did not affect grain quality also (Pak and Pak, 1975). Laboratory studies and field trials conducted to determine the acute toxicity of 11 herbicides to common rice field fish in Malaysia indicated that bensulfuron, 2,4-D, metsulfuran and quincloram were mildly toxic (LC_{50} of > 10 ppm) to sepat siam (*Trichogaster pectoralis*) and or Keli (*Clorias batrachus*) (Ooi and Lo, 1992). Lavy *et al.* (1996) detected 2,4-D residues in rice ecosystem at trace levels and found that they did not build up in the reservoirs. 2,4-D was detected upto 2.6 µg l^{-1} in ponds of Canada (Grover *et al.*, 1997).

On evaluation of kuttanad ecosystem for possible contamination of pesticides 2,4-D residues were not detected in fish and clam samples. When the soil samples of the area were analysed for 2,4-D residues, 30% of the samples

recorded residues in the range 0.001 to 0.12 μg g^{-1}. Higher concentration was observed in the month of March (KAU, 1999).

In the above study no residues were detected of 2,4-D in the rice grain samples. However 2,4-D was detected in 7% straw samples upto a concentration of 0.0225 μg g^{-1}.

2.8 Metabolism of 2,4-D in plants

The transformations of phenoxy acids have been known since 1950's. The early investigations were on the metabolism of 2,4-D by *Phaseolus vulgaris* (Weintrub *et al.*, 1952) which suggested that 2,4-D is metabolized in plants by three mechanisms viz., degradation of acetic acid chain, hydroxylation of the aromatic ring and conjugation with a plant constituent.

2,4-dichlorophenoxy acetic acid is a systemic herbicide. Once absorbed by plants it undergoes various transformations which include degradation and detoxification and such processes occur both in tolerant and susceptible plant species, but in tolerant species they take place at a rate much faster than herbicide accumulation and before the chemical could disrupt the plant metabolic processes (Rao, 1992).

2.8.1 Degradation of side chain

During degradation of side chain CO_2 is released from 2,4-D and this was observed in bean, strawberry, corn, cotton and several weed species. This degradation takes place by oxidation at carboxyl and methylene carbons of the

side chain (Weintrub *et al.*, 1952; Luckwill and Lloyd-Jones, 1960 (a) and (b)). The resistant species were able to metabolise 2,4-D via oxidation and decarboxylation pathways than the susceptible species (Luckwill and Lloyed-Jones, 1960(a) and (b)).

When the side chain is lost from a phenoxy acetic acid without further metabolic changes to the molecule it may result in the formation of corresponding phenol.

2.8.2 Hydroxylation of aromatic ring

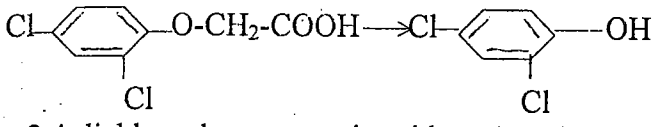
Bach (1961) reported that the degradation of 2,4-D molecules would involve hydroxylation of the ring and oxidation of the hydroxyls to carboxyls with a split in the ring. 2,4-D also undergoes β-oxidation followed by ring hydroxylation (Wilcox *et al.*, 1963) forming 4-hydroxyphenoxyacetic acid and this hydroxylates pathway is considered to be the mechanisms of detoxification in resistant species. The hydroxylated 2,4-D may conjugate with glucose to form the 4-0-β-D-glucosides.

2.8.3 Conjugation with plant constituents

Phenoxyacids form complexes with plant components proteins and amino acids particularly aspartic acid. The phenoxy acids with no hydroxy group conjugate with glucose during esterification to form glucose ester of 2,4-D.

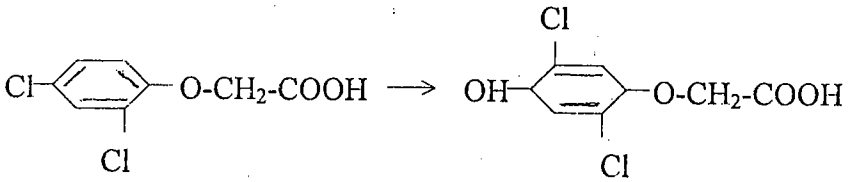
Fig. 2 Metabolic reactions of 2,4-D in plants

1. Degradation of acetic acid side chain



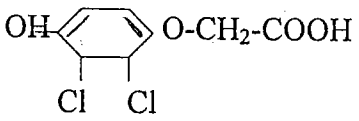
2,4-dichlorophenoxy acetic acid — 2,4-dichlorophenol + Acetic acid → CO_2

2. Hydroxylation of aromatic ring



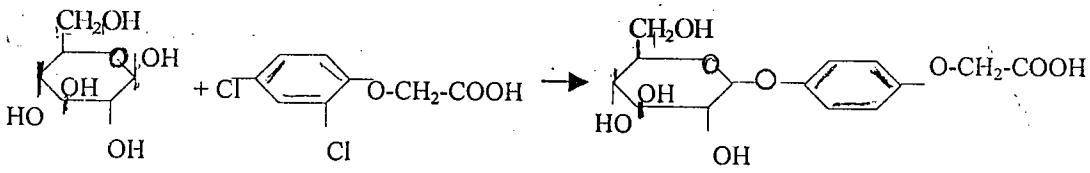
2,4-dichlorophenoxy acetic acid — 2,5-dichloro ,4- hydroxy phenoxy acetic acid

or



2,3-dichloro ,4-hydroxy phenoxy acetic acid

3.Conjugation with plant constituents



Glucose — 2,4-dichlorophenoxy acetic acid — Glucoside of 2, 4- dichloro phenoxy acetic acid

(Source: Weintrub, *et. al.*, 1952, Luckwill and Lloyd-Jones, 1980 (a) and (b); Bach, 1961)

2.9 Residue analysis techniques

2,4-D residues in soil can be estimated either by chemical or biological methods. Biological methods, commonly termed as bioassays, involve the measurement of the response of a living organism from which an estimate can be made of the biologically active compound present in the substrate. Measurement of root length of cucumber is a sensitive parameter for estimating 2,4-D residues in soil (NRCWS, 1987). Bioassays are not well suited for the determination of compounds in biological materials. Relatively narrow ranges of herbicide concentration only can be tested by bioassay and this technique measures only the amount of herbicide available to the test organism and not the quantity actually present in the medium.

Precise quantitative analysis can be done by chemical methods only. Since the quantity of residues in the substrate is of very small amounts, the chemical analysis involve a chain of procedures viz. extraction, clean up, concentration, storage and determination. The instrumentation methods include visible and ultraviolet spectrophotometry, thin layer chromatography, gas chromatography, high performance liquid chromatography, nuclear magnetic resonance spectroscopy (NMR), polarography etc. (Sankaran *et al.*, 1993).

Colorimetric methods were among the earliest instrumental methods used for the analysis of herbicides and even today it continues to be a useful technique wherever costlier modern facilities like gas chromatograph are not readily available. Freed (1948) developed a qualitative method in which, 2,4-D was

heated with chromotropic acid (1, 8 dihydroxy naphthalene3, 6-disulphonic acid) in concentrated sulphuric acid at 150^0 C and the development of wine purple colour was taken as a measure of 2,4-D concentration. The method depends on the splitting of formaldehyde from the phenoxy acetic acid and its subsequent reaction with chromotropic acid to give a wine-coloured product, the chemistry of which is uncertain. Subsequent modifications of Freed's (1948) procedure by Marquardt and Luce (1951 and 1955) helped in the quantification of 2,4-D residues in milk, seeds and grains. A method applicable to all the phenoxy acids was devised by Marquardt and Luce (1961) in which the ether linkage was cleared by pyridine hydrochloride and the phenol so released is determined colorimetrically after reaction with amino antipyrene and potassium ferricyanide. Colorimetric methods have a sensitivity of 50 ng (QueeHee and Sutherland, 1981) and all these procedures involve a large number of extractions, steps with a high volume of organic solvents for eliminating the analytical interferences from other compounds. Hence it is necessary to simplify the extraction and separation procedures for saving time and volume of costly chemicals.

In the residue analysis of 2,4-D, gas liquid chromatography is the most sensitive method (QueeHee and Sutherland, 1981). The free phenoxy acids and salts are too polar and nonvolatile to chromatograph easily. Hence derivatization is necessary. The free acids have been usually converted to methyl esters which are less polar and easier to analyse by GLC. Horner *et al.* (1974) compared several methods for 2,4-D methylation with respect to yield purity, concentration and stability of the esterifying reagent. The reagent of choice for producing a

high yield of GLC pure esters in the shortest time was the BF_3 alcohol reagent. Procedure for esterification of 2,4-D with BF_3/alcohol is well presented by QueeHee and Sutherland (1981).

In herbicide residue analysis, extraction of the residue with suitable solvents is the initial step. For the extraction of 2,4-D residues from plant tissue maceration with water followed by alkali and acid hydrolysis was the best method. The recovery was greater than 92% (QueeHee and Sutherland, 1981).

Extraction of the phenoxys from soils is dependent on soil type. Many extractants and methods have been tried [0.5% NaOH (Henkel, 1966), 1:1:1, 18N H_2 SO_4 ethanol/H_2O and 1:1 NaOH/CH Cl_3 (Purkayastha, 1974), 1:1 hexane/acetone and 2:1 hexane/diethyl ether (Renberg, 1974), acidic acetone (Khan, 1975)] etc. Shaking soil with acetonitrile: distilled water: glacial acetic acid (80: 20 : 2.5) on a mechanical shaker for one hour was found good for estimating 2,4-D residues in soil (Smith, 1978). Aqueous acidic acetonitrile was selected as extraction solvent by many workers since it has been shown to be an efficient extractant of 2,4-D, 2,4-DCP and 2,4-DCA from soils. (Smith and Muir, 1980; Smith and Aubin, 1991).

Co-extractives like pigments, fats, oils etc interfere with the method of analysis and hence must be removed by any method of separation viz. partition distribution, chromatographic method, precipitation method etc. Liquid clean up proposed by QueeHee and Sutherland (1981) was approximately 91% efficient. Gas Chromatography columns and the temperature used for detection vary

depending on the conditions. In general column temperature ranges from 175-230^0 C. The injector temperature is 30-50^0 C above the column temperature. Since the esters and amides of phenoxys tend to be thermally unstable above 250^0 C, the choice of the liquid phase is not really crucial (QueeHee and Sutherland, 1981).

Materials and methods

3. MATERIALS AND METHODS

A series of experiments were conducted to understand the behaviour of 2,4-D in the major rice soils of the state and to find out the extent of contamination of soil and crop with this herbicide. The project consisted of three laboratory studies and one field experiment. The laboratory studies were conducted during 1997-2001 at the Herbicide Residue Laboratory of the All India Co-ordinated Research Programme on Weed control, Thrissur Centre, located in the Radio Tracer Laboratory, College of Horticulture, Kerala Agricultural University, Vellanikkara. The field experiment was laid out in a farmer's field at Pidikkaparambu of kole area of Thrissur District during the second crop season (September to December) of 1998 and the residue estimation related to field experiment was carried out at Regional Agricultural Research Station, Kumarakam, under Kerala Agricultural University.

3.1 Materials

3.1.1 Soils

Bulk surface soil samples (0-0.15 m depth) were collected from five representative locations each from Palakkad (Palakkad district) and kuttanad (Alappuzha district) rice growing regions. From kole lands of Thrissur district, samples were collected from six locations (the additional one was from the area where field experiment was conducted). The site for field experiment was selected based on the feasibility for getting good water management. The details of the locations are given in Table 2.

Table 2. Details of the soil sampling locations

Rice growing region	Location	Name of padasekharam
Palakkad (Palakkad district)	Alathur	Ambalaparambu
	Chittur	Manchira
	Koyalmannam	Kannadipadam
	Nemmara	Kootakadavu-Nenmara
	Palakkad	Alampallam
kuttanad (Alappuzha district)	Moncompu-1	RRS Moncompu
	Moncompu-2	do.
	Moncompu-3	do.
	Karumady	Kavil Thekkumpuram
	Mathikayal	Mathikayal
kole (Thrissur district)	Anthikkad	Anthikkad kole padavu
	Cherpu	Jubilee
	Kattoor	Vellanipadam
	Manalur	Thazhampadavu
	Venkitengu	Elamutha
	Pidikkaparambu (Experimental field)	Pidikkaparambu padam

3.1.2 Processing of soil samples

The soil samples were brought to the laboratory and air dried in shade. The clods were broken with the help of a wooden hammer and screened through a 2 mm sieve. The sieved samples were stored in plastic containers until use. These samples were used for the determination of physico-chemical characteristics and for residue studies. For the estimation of organic carbon,

cation exchange capacity and anion exchange capacity, the soil samples were ground thoroughly so as to pass through 0.5 mm sieve.

3.1.3 Physico-chemical characteristics

All the 16 soil samples were analysed for physico-chemical characteristics viz., texture, pH, organic carbon, CEC, AEC and sesquioxides content. The analytical methods employed for each estimation are given in Table 3.

Table 3. Analytical methods employed in the determination of physico chemical characteristics of soils

Sl. No.	Soil property	Method of estimation	Reference
1	Texture	Mechanical analysis by International Pipette method	Chopra and Kanwar (1976)
2	pH	1:2.5 soil water suspension	Jackson (1958)
3	Organic carbon	Walkley and Black's rapid titration method	Chopra and Kanwar (1976)
4	Cation exchange capacity	Sodium acetate method	Hesse (1972)
5	Anion exchange capacity	Phosphorus fixing capacity	Hesse (1972)
6	Sesquioxide content	A.O.A.C. method	Chopra and Kanwar (1976)
7	Maximum water holding capacity	Keen-Raezkowski method	Chopra and Kanwar (1976)

3.1.4 Forms of 2,4-D used for the laboratory experiments

Details of the different forms of 2,4-D used for fortification of soil samples and the standards for residue analysis are given in Table 4.

Table 4. Technical information on forms of 2,4-D used for the study

Sl. No.	Name of chemical	Molecular weight (g)	Physical appearance	Assay (%)	Source
1	2,4-dichlorophenoxy acetic acid (2,4-D)	221.00	Dull white coloured powder	97.00	M/s Atul Limited, Agro & Pharma Division, Atul, Gujarat
2	Methyl ester of 2,4-dichlorophenoxy acetic acid	235.00	Viscous, colourless, odourless solid	99.50	M/s SUPELCO Inc, SUPELCO park Bellefente PA
3	2,4-dichlorophenoxy acetic acid sodium salt, monohydrate (for field application)	261.00	White crystalline powder with slightly phenolic odour	83.90	M/s Atul Limited, Agro & Pharma Division, Atul, Gujarat

3.1.5 Primary standard

A solution containing one gram of 2,4-D per 1000 ml of methanol was prepared and kept as primary standard.

3.1.6 Working standards

From the primary standard, working standards of 100 ppm and 10 ppm 2,4-D were prepared at the time of start of each experiment. 2,4-D standard viz., 10 ppm was used for fortifying soil samples and also for the preparation of calibration curve for colorimetric estimation of 2,4-D residues.

3.1.7 Reagents

In the laboratory studies, estimation of 2,4-D was carried out by colorimetry and all the reagents were of GR grade.

3.1.8 Glasswares

All items of glasswares were washed with detergent solution using brush and then rinsed with tap water, distilled water and acetone and were oven dried before use.

3.1.9 Colorimetric estimation of 2,4-D residues

Spectronic 20 GENESYS was the instrument used for estimation of 2,4-D residues in soils of the laboratory experiments.

3.2 Standardisation of method for determination of residues of 2,4-D in the soil samples

Standardisation of procedure involved fortification of soil samples, selection of extractants and soil : extractant ratios, clean up of the extract, estimation of 2,4-D residues and validation of method.

3.2.1 Fortification of soil samples

One kilogram of sample collected from each of the five locations in kole lands (as given in Table 2) was pooled, processed (as mentioned in 3.1.2) and ten gram each were fortified with 2,4-D at 1.00 ppm(1.00µg per gram) level.

3.2.2 Standardisation of extraction method

In order to standardize the extraction method, combinations of

(A) seven extractants viz., (i) acetonitrile, (ii) acetic acid, (iii) benzene, (iv) acetonitrile: distilled water: glacial acetic acid (80:20:2.5), (v) chloroform: ether: acetic acid (50: 50: 1), (vi) 1 % Na OH, (vii) 4% Na OH and

(B) two soil: extractant ratios of (i) 1:2 and (ii) 1:4 were tested.

The soil samples were taken in a 250 ml conical flask and fortified with 2,4-D at 1.00 μg g^{-1}. They were equilibrated for a period of half an hour and shaken with the extractants on an orbital shaking incubator for 30 minutes. The extract was filtered using Whatman no.1 filter paper. The extractant which gave maximum recovery of 2,4-D and minimum interference with co-extractives was selected as the best extractant for the determination of 2,4-D residues from soil samples.

3.2.3 Clean up of the extract

Clean up procedures were based on the methods suggested by Marquardt and Luce (1951 and 1955). Modifications were made to reduce the number of steps in clean up. The filtrate obtained after shaking the fortified samples with the extractants at various soil: extractant ratios were shaken with 100 ml of 1*N* NaOH followed by 15ml conc. HCl and 50 ml diethyl ether in a 250 ml separating funnel. The ether portion was separated and the aqueous layer was

shaken again with 50ml diethyl ether. The ether portions were pooled and extracted twice with 25 ml buffer solution (25.00 g Na_2HPO_4 $12H_2O$ and 10.00 g NaH_2PO_4 H_2O in one litre distilled water). The pooled buffer separate was shaken with one ml concentrated HCl and 10 ml carbon tetrachloride. The aqueous layer was separated and again shaken with 10 ml carbon tetrachloride. The pooled carbon tetrachloride layer was kept for evaporation.

3.2.4 Estimation of 2,4-D residues

3.2.4.1 Preparation of standard curve

From the working standard of 10 ppm 2,4-D, a series of standards ranging from 0.05 to 2.00 ppm herbicide in methanol were prepared. One ml of each of the standards was taken in 100 ml beaker and the solvent was allowed to evaporate. Then three ml chromotropic acid reagent (0.4 g chromotropic acid in 100 ml concentrated H_2SO_4) was added and kept in an oven at 135^0C for 20 minutes. The wine purple coloured complex obtained was cooled, diluted and made up to 50 ml with distilled water. Absorbance of the coloured complex was read in a visible spectrophotometer at 565 mμ against a methanol blank set at 100 percent transmission. Standard absorption curve was prepared plotting absorbance values on the Y-axis and 2,4-D concentration on the X-axis.

3.2.4.2 Estimation of 2,4-D residues in the fortified samples

The residue obtained after clean up was treated with 3 ml chromotropic acid developed the colour as in the case of standards. Methanol blank was set at 100 per cent transmission and absorbance of the coloured complex for each

sample was read on the spectrophotometer at 565 mμ. Absorbance of an unfortified sample for each soil extracted and separated for 2,4-D residues (as detailed in the above sections) were deducted from the absorbance of the fortified sample and 2,4-D residues were estimated.

3.2.4.3 Selection of extractant and soil extractant ratios for the extraction of 2,4-D from soil samples

The major criteria used for the selection of extractant and soil extractant ratio was the percentage recovery of 2,4-D residues from soil and the amount of co-extractives removed. The extractant which recorded highest recovery of 2,4-D residues and which exhibited least colour in the filtrate was selected as the best extractant.

3.2.4.4 Validation of the method

In order to confirm the reproductability of the method 10 g each of the fifteen soil samples (five each from Palakkad, kuttanad and kole) were fortified with 2,4-D acid at three levels viz., 0.50, 1.00 and 2.00 μg g^{-1} and the recovery of 2,4-D from the soil samples were estimated by adopting the procedure as standardized above.

3.3 Studies on persistence and degradation of 2,4-D in the major rice soils of the state

Ten gram soil sample from each of 16 locations were taken in 250 ml conical flasks and water was added to the level of maximum water holding capacity. Samples were fortified with 2,4-D at levels of 0.50, 1.00 and

2.00 μg g^{-1} and after incubation for different periods (0, 1, 3, 6, 9, 15, 30 and 60 days), the herbicide residues in the soil samples were estimated by colorimetry as given in section 3.2

Kinetics of 2,4-D degradation in Palakkad, kuttanad and kole soils were worked out separately by preparing graphs, taking period of incubation on the X-axis and average concentration of 2,4-D in each soil type on the Y-axis. Half lives were worked for each soil type using standard procedures explained by Reghupathy and Dhamu (1990). Simple exponential model of the form $C= C_0e^{-kt}$ [where C=concentration of herbicide(μg g^{-1}) remaining at time t (days); C_0 = initial concentration of herbicide (μg g^{-1}) and k =degradation rate constant] was used to calculate the half life of 2,4-D in the soils.. In the logarithmic form the above equation could be written as $\log C= \log C_0 - kt$. When logarithm to the base 10 of herbicide concentration (log C) was taken on the Y-axis and time t on the X-axis , a linear curve was obtained. The slope of the curve was multiplied by 2.303 for getting the degradation rate constant, k. Half life was worked out from the equation $t_{½} = 0.693/k$. Relationship between persistence of 2,4-D in each soil type and physicochemical characteristics was established by the determination of correlation coefficients.

3.4 Studies on adsorption of 2,4-D by soil

Adsorption of 2,4-D on the sixteen soil samples was studied using the method described by Adams and Li (1971). Separate standard solutions for each treatment viz., 0.50, 1.00 and 2.00 $\mu g\ g^{-1}$ soil were prepared and 25 ml of the standard solution was added to one gram each of the representative soil sample taken in centrifuge tubes, to which 2,4-D was added as per treatments. The tubes were shaken for different equilibration periods viz., 2, 4 and 6 hours, centrifuged and the quantity of 2,4-D in the aliquot was determined by the colorimetric procedure as described in section 3.2. The difference between the amount of herbicide added and the quantity remaining in equilibrium solution was taken as the amount of herbicide adsorbed by the soil. Correlation between physico chemical properties and the quantity of 2,4-D adsorbed by the soils were worked out.

Adsorption isotherms were prepared by plotting logarithm to the base 10 of the mass of 2,4-D adsorbed per unit mass of soil on the Y-axis and logarithm to the base 10 of equilibrium concentration on the X-axis. Freundlich equation of the form log x/m=log K + n logC (where x/m=quantity of 2,4-D adsorbed per unit mass of the soil; C =equilibrium concentration of 2,4-D; K and n are constants. Slope and intercept of the curve provided values of n and K respectively) was used for estimating the degree of adsorption of 2,4-D by the soils. The equilibration period at which maximum K and R^2 (coefficient of

determination) were obtained was selected and the extent of binding of 2,4-D by different soils was compared using the isotherm constant K.

3.5 Leaching and movement of 2,4-D in soil

A column leaching study was conducted to estimate the leaching losses and the pattern of movement of 2,4-D in the 16 representative soils. Tubes made up of PVC with dimensions 40 cm length and 3.5 cm internal diameter were driven into the paddy field to a depth of 20 cm so that soil columns having structure similar to that in the field was obtained. The PVC tubes with the representative soil columns were taken out, both ends of which tied up with cotton cloth and placed in plastic basins (60 cm diameter and 30 cm depth) containing water filled three fourth of its volume. This was done to simulate the submerged rice field condition. At the time of experiments the columns were taken out from the basins and kept on a stand to drain off excess water. Cotton cloth of the top end was removed and water was added continuously through the column and collected leachate in a beaker kept below the column. The time taken for collecting 200 ml water was noted and the percolation rate (ml min^{-1}) was calculated. Separate standards of 2,4-D sodium (technical) dissolved in water were prepared as per treatments (1.0 kg ha^{-1}, 2.0 kg ha^{-1} and 4.0 kg ha^{-1}) and one ml of the same were applied to the columns. Water was added in the same manner as done initially and 200 ml of the leachate was collected. The tubes were cut at 10 cm and 20 cm depths from the top and the herbicide content in the 0-10 cm and 10-20 cm soil layers and in the collected leachate was

estimated by colorimetry. The effect of water flux and physiochemical properties of the soils on the movement of 2,4-D in the sixteen soils was worked out by correlation.

3.6 Persistence of 2,4-D residues in soil and rice plant under field condition

An experiment was conducted in farmer's field at Pidikkaparambu in Thrissur district (representative of the kole region) to study the dissipation of 2,4-D residues in soil and rice plant under field situation.

3.6.1 Details of the experiment

Location : Pidikkaparambu paddy field, Thrissur
Crop : Rice
Variety : Jyothi
Season : Second crop season, 1998 (September – December)
Design : RBD

Number of treatments : 5

T_1 : 2,4-D @ 0.00 kg ha^{-1} (hand weeding 25 and 40 DAS)
T_2 : 2,4-D @ 0.50 kg ha^{-1}
T_3 : 2,4-D @ 1.00 kg ha^{-1}
T_4 : 2,4-D @ 2.00 kg ha^{-1}
T_5 : 2,4-D @ 4.00 kg ha^{-1}

Time of application : Three weeks after sowing
Number of replications : 4

Germinated rice seeds were sown in the puddled field and all the crop management practices were followed as per Package of Practices Recommendations Crops (KAU, 1996).

Appropriate quantities of 2,4-D sodium monohydrate (equivalent to 83.90% of 2,4-D acid) required for each plot as per treatments were weighed

accurately, dissolved in measured quantity of water (at the recommended spray volume of 500 l ha^{-1}) and sprayed in the plots at 21 days after sowing, using ASPEE knapsack sprayer fitted with a floodjet nozzle.

3.6.2 Observations

The following biometric observations were recorded

1. Species wise weed count and dry matter production at 60 days after sowing

   Quadrat of 0.25 m^2 was kept randomnly at three places in each plot. The weeds coming under these areas were removed and their species wise counts were recorded. Then they were kept in an electric oven at 80°C and their total dry weight of each replication was determined. The weed count as well as dry weight per quadrat were multiplied with four so as to express the data on square meter basis.

2. Height of the plant and tiller production (total and productive tillers) at 60 days after sowing

   Ten plants from each of the above quadrats were selected and their height, number of total and productive tillers were recorded and expressed the data on square meter basis.

3. Yield of grain and straw at the time of harvest

   At the time of harvest quadrat of 0.25 m^2 was kept randomnly at three places in each plot. All the rice plants coming under the area under the quadrats were cut at the ground level. The grains were removed and

dried. Dry weight of grains per square meter area was calculated for each plot and tabulated.

Straw portions from each quadrat were dried separately and expressed the dry weight of straw per square meter area.

3.6.3 Analysis of 2,4-D residues

3.6.3.1 Soil

3.6.3.1.1 Sampling

Two soil samples were collected from each plot at 0-15 cm depth using an aluminium scoop, at 0, 1, 3, 6, 9, 15 and 30 days after spraying. The soil samples collected in the scoop was spread on a filter paper and kept for one hour so as to drain excess water. The two samples collected from the same plot were pooled and 20g of the pooled sample was taken for residue analysis. Simultaneously another 20 g soil sample was weighed and kept for determination of moisture content gravimetrically.

3.6.3.1.2 Estimation of herbicide residues in soil by colorimetry

Soil samples collected up to 30 days after spraying were analysed for 2,4-D residues by colorimetry. The extraction, cleanup and final estimation were carried out by the procedure as standardized in 3.2.

3.6.3.1.3 Estimation of herbicide residues in soil by gas chromatography

Residue levels in the soil samples at 30 DAS were below the minimum detectable level of spectrophotometer and the 2,4-D residues in soil at 30 DAS were estimated by gas chromatography.

3.6.3.1.3.1 Extraction

For the estimation of residues of 2,4-D in soil by gas chromatography, the same extraction procedure as described in section 3.2 was followed. Twenty gram wet soil (after draining excess water by spreading over a filter paper) was shaken with 80 ml of extractant mixture (acetonitrile : distilled water : glacial acetic acid of 80 : 20: 2.5 ratio) for a period of 30 minutes and filtered through Whatman no.1 filter paper.

3.6.3.1.3.2 Separation and derivatization

The method described by Sankaran and coworkers (Sankaran *et al.*, 1993) was employed for separation and derivatization. The filtrate was acidified with concentrated HCl (15 ml) and separated by extracting twice each with 50 ml diethyl ether. The pooled ether was allowed to evaporate.

The phenoxy alkanoic acid cannot be analysed by gas chromatograph without derivatization. The free carboxyl groups are highly polar and gets adsorbed on the GC column and gives the acid a low volatility. By converting the carboxyl group to a methyl ester derivative, volatility is increased and polarity decreased resulting in quantitative symmetrical peaks in the chromatogram.

For derivatization of 2,4-D acid, 3.5 ml of boron trifluoride-methanol mixture was added to the residue left behind in the beaker after the evaporation of diethyl ether. Then the beaker was kept on a waterbath at 70^0C for 45 minutes, cooled and the residue was transferred to 120 ml separating funnel. To this 25 ml of saturated sodium chloride solution was added and the 2,4-D residues were

extracted 5 times with 5 ml each of hexane. The combined hexane portions were concentrated to about 1 ml .

3.6.3.1.3.3 Clean up

The residue was cleaned up by passing through a glass column of 30 cm length and 20 mm internal diameter. The column was plugged with cotton at the tail end and packed successively with 6 g silica and 2g anhydrous sodium sulphate. For this purpose silica and anhydrous sodium sulphate were placed in an oven at 105^0C to remove moisture. The residue as obtained in section 3.6.3.1.3.2 was eluted through the column using 50ml methylene chloride : hexane solvent mixture (30:70). The first 25 ml of the eluate was rejected and the remaining 25 ml was collected and the solvent was evaporated. The residue was dissolved in one ml n-hexane and a suitable aliquot was analysed by gas chromatograph.

3.6.3.2 Estimation of 2,4-D residues in whole plant samples and straw

The method suggested by QueeHee and Sutherland (1981) was used with suitable modifications.

3.6.3.2.1 Sampling

Five whole plants were collected randomly from each plot at 30 DAS. Root portion was removed and the above ground portions were pooled and chopped into small pieces. In the case of straw samples sampling was done at the time of harvest. Five plants were selected randomly and cut above the soil

surface. Grains were separated and the straw portions were pooled and cut into small pieces.

3.6.3.2.2 Extraction

Ten gram of the chopped sample was macerated with 100 ml water. Ten milliliter of 0.6*N* NaOH was added and refluxed for two hours. This was strained through an oil cloth and washed with 100 ml water. Filter cake was suspended in 50 ml of 2 *M* HCl, refluxed for two hours and extracted thrice each with 50 ml of diethyl ether. The ether and alkaline extracts were combined and the ether portion was separated and allowed to evaporate. Then 30 ml of 10% Na OH was added so that the solution pH was greater than thirteen.

The aqueous alkaline solution was refluxed for 20 minutes, cooled and extracted thrice each with 100 ml of diethyl ether. The caustic portion was acidified with conc. HCl (pH = 2) and extracted thrice each with 100 ml of diethyl ether. This procedure of acidic and alkaline hydrolysis separates 2,4-D from conjugates and other compounds present in the plant material (QueeHee and Sutherland, 1981). The ether portions were mixed and allowed to evaporate. The separation , clean up and derivatization procedures were same as in the case of soil samples.

3.6.3.3 Determination of 2,4-D residues in paddy grain

The grains separated from the five plants as given in section 3.6.3.2.1 were pooled and 10 g portions of the pooled grain sample was ground in a mixer

grinder and macerated with 150 ml water. The extract was acidified with conc. HCl (15 ml) and kept overnight. This was filtered and extracted five times each with 10ml diethyl ether. The ether portion was allowed to evaporate. Esterification, clean up and estimation procedures were same as in the case of soil samples. (Section 3.6.3.1.3.2 and 3.6.3.1.3.3.)

3.6.3.4 Recovery studies

Ten gram each of the soil, whole plant, straw and grain were fortified with 2,4-D acid at 0.001 ppm level and the residues were estimated using same procedure as mentioned above for soil, whole plant and straw and grain respectively (sections 3.6.3.1 to 3.6.3.3).

3.6.3.5 Conditions for gas chromatography

Detector : Electron Capture Detector
Column : HP-608 30 m X 0.53 mm X 0.5 μm

Temperature conditions:

Column : 180^0C
Injector : 210^0C
Detector : 300^0C
Carrier gas flow : 70 ml / min.
Retention time : 8 min.

3.6.4 Effect of 2,4-D on soil microbial population

Soil plate dilution method was employed for determining the population of microorganisms in soil. Two soil samples were taken from the treated plots of the field experiment at a depth of 0-15 cm at 3, 6, 15 and 30 days after treatment. The samples taken from each treatment were pooled and air dried. One gram of

the pooled sample was diluted with water at a dilution of 10^6 and 10^4 for bacteria and fungi respectively and replicated three times. They were separately plated on enrichment media designed to promote these organisms. Martin's Rose Bengal Streptomycin agar and soil extract agar were the enrichment media for fungi and bacteria respectively (Rangaswami, 1988). The counts of bacteria and fungi were taken daily up to one week after plating. From the observations on the total number of bacteria per 10^{-6} g soil and fungal colonies per 10^{-4} g soil, change in their population (percentage increase / decrease over control) was worked out. By taking the population in the control plot as 100 (normal) corresponding values in the treated plots were calculated. Graphs were plotted using the percentage deviation in the microbial population over control on the Y-axis and days after spraying on the X-axis.

3.6.5 Statistical analysis

Relationship between the residue data obtained in the laboratory experiments and the data on physicochemical characteristics of soils was determined by working out their correlation.

The data on biometric observations and residue analysis of the field experiment were statistically analysed by applying the analysis of variance.

Results

4. RESULTS

Three laboratory experiments and one field experiment were conducted at College of Horticulture, Vellanikkara to assess the magnitude of 2,4-D residue accumulation in the major rice soils of Kerala and the extent of contamination of paddy grain and straw with this herbicide. Effect of 2,4-D residues on microbial population in the rice field was also studied. Results of the experiments are presented in sections 4.3 to 4.6.

Major physicochemical characteristics of the 16 soils (5 samples each from Palakkad and kuttanad region and six from kole region) taken for laboratory experiments were estimated and the results are presented in section 4.1. Procedure for the determination of 2,4-D residues in soils was standardized and the results are presented in section 4.2.

4.1 Physicochemical characteristics of soils

The data on major physicochemical properties of the soils under study (mechanical composition, pH, organic carbon content, cation exchange capacity, anion exchange capacity, sesquioxide content, silica content and maximum water holding capacity) are presented in Table 5.

4.1.1 Soils of Palakkad region

Clay content of the soils was in the range of 24.60 to 50.10 per cent. The highest value of 50.10 per cent was shown by the soil collected from Alathur and

Table 5. Physico chemical characteristics of the soils under study

Rice growing region	Location	Sand %	Silt %	Clay %	pH	Organic Carbon %	Cation exchange capacity cmol(+) kg^{-1}	Anion exchange capacity cmol(-) kg^{-1}	Sesqui-oxides %	Silica %	Maximum water holding capacity, %
Palakkad	Alathur	44.60	4.80	50.10	5.38	0.58	4.73	7.80	5.00	69.00	36.99
	Chittur	34.50	19.80	45.00	6.44	0.81	6.53	8.23	3.00	67.00	37.10
	Koyalmannam	49.10	15.00	34.60	5.38	0.77	3.60	8.04	2.00	42.00	33.71
	Nemmara	48.90	25.20	24.60	5.38	0.45	3.60	8.28	2.00	58.00	33.44
	Palakkad	49.00	10.20	39.50	5.31	0.51	3.83	8.15	11.00	44.00	36.05
kuttanad	Moncompu 1	35.30	29.80	34.60	3.90	2.14	6.53	9.36	18.00	66.00	53.27
	Moncompu 2	35.10	24.00	40.00	4.00	1.83	6.53	9.59	13.00	79.00	48.56
	Moncompu 3	34.80	15.20	50.00	3.80	2.27	5.85	9.57	9.00	65.00	53.45
	Karumady	5.10	19.90	74.00	2.80	15.02	16.88	9.77	21.00	54.00	52.55
	Mathikayal	5.00	34.60	59.50	3.70	2.46	10.80	9.55	6.00	14.00	56.74
kole	Anthikkad	20.10	14.80	64.00	5.10	1.87	15.75	9.38	3.00	35.00	41.54
	Cherpu	20.00	14.90	64.10	4.40	1.85	8.10	9.45	9.00	53.00	45.35
	Kattoor	30.20	9.90	60.20	4.60	1.72	6.98	9.32	19.00	70.00	52.51
	Manalur	30.00	14.70	54.00	3.80	0.67	6.08	8.45	14.00	73.00	31.14
	Venkitengu	29.50	9.80	59.00	3.70	1.64	12.83	9.05	1.00	68.00	50.60
	Pidikkaparambu	55.10	4.80	39.60	5.15	0.90	3.15	9.40	4.00	65.00	31.81

the lowest value of 24.60 per cent was obtained in Nenmara sample. Except the soil at Chittur all the other soils recorded pH values in the range of 5.30 to 5.40. Highest pH value of 6.44 was recorded in Chittur sample. Organic carbon content was low to medium in all the samples and the highest value of 0.81 per cent was recorded by Chittur sample and lowest value by Nenmara sample. Cation exchange capacity varied from 3.60 to 6.53 per cent and anion exchange capacity was in the range of 7.80 to 8.28 per cent. Cation exchange capacity value was highest in Chittur sample and the highest anion exchange capacity value was shown by Nenmara sample. Sesquioxide content varied from 2.00 to 11.00 per cent and the Palakkad sample had shown highest sesquioxide content. Silica content was in the range of 42.00 to 69.00 per cent of which the highest value was shown by Alathur sample. Maximum water holding capacity was varying from 33.44 to 37.10 per cent and Chittur sample showed the highest value.

In general, the soils of Palakkad region are sandy clay in texture with medium organic carbon content and slightly acidic in soil reaction. Cation exchange capacity was low, while the anion exchange capacity was high. The data on properties of soils indicate that these soils are lateritic in origin and provides favourable environment for growth of rice.

4.1.2 Soils of kuttanad region

Among the different soils of the five locations in kuttanad, kari soil samples of karumady recorded the highest clay percentage (74.00 %) and the soil

sample of Mathikayal recorded the highest silt percentage (34.60 %). Conversely, the sand content of these two samples were very low (5.10 and 5.00 % for Karumady and Mathikayal respectively). Sand, silt and clay content of the other three samples were in the range of 34.80 to 35.30 per cent, 15.20 to 29.80 per cent and 34.60 to 50.00 per cent respectively. Silt content of Karumady sample was 19.90 per cent and clay content of Mathikayal was 59.50 per cent.

The pH of soil samples of kuttanad area was very low. The values ranged from 2.80 to 4.00. The lowest value (2.80) was registered by Karumady and the highest value (4.00) was shown by Moncompu-2.

Organic carbon content of all the five samples was very high, the range being very wide (1.83 to 15.02 %). The highest value of 15.02 per cent was registered by Karumady sample.

Cation exchange capacity of the samples ranged between 5.85 to 16.88 and anion exchange capacity ranged from 9.36 to 9.77. Both CEC and AEC values were higher in karumady sample which has recorded the maximum value for clay content and organic carbon content. This soil has recorded the lowest pH also.

The content of silica and sesquioxide in the soil samples were in the range of 14.00 to 79.00 per cent and 6.00 to 21.00 per cent respectively. Silica content was higher in Moncompu-1, 2, and 3 samples (66.00, 79.00 and 65.00 %

respectively) where as the sesquioxide content was higher in Karumady (21.00 %) and Moncompu-1 (18.00 %) samples. Lowest content of both sesquioxide and silica was registered by Mathikayal sample.

Water holding capacity of all the soils were very high and the values ranged between 48.56 to 56.74 per cent respectively. Among the five soil samples, Mathikayal sample recorded the highest value (56.74 %) and Moncompu-2 recorded lowest value (48.56 %).

In general soil samples of kuttanad region had greater amounts of organic carbon and sesquioxides than the samples of kole and Palakkad regions. pH was very low due to the presence of partially decomposed organic matter. CEC, AEC and water holding capacity of the kuttanad samples were comparable to kole samples.

4.1.3 Soils of kole region

Soils of kole area registered sand content in the range of 20.00 to 55.10 per cent. The lowest content was in Cherpu samples and the highest content was in Pidikkaparambu samples. Silt content was varying from 4.80 to 14.90 per cent, the highest value being recorded by Cherpu and the lowest by Pidikkaparambu. In the case of clay also highest value was recorded by Cherpu (64.10 %). The lowest value was shown by Pidikkaparambu (39.60 %).

All the soil samples of kole area were highly acidic in reaction. Soil samples of Venkitengu recorded the lowest pH (3.70) and the highest value of 5.15 was shown by the soil sample of Pidikkaparambu.

Organic carbon content of the soil samples were high (> 1.50 %) except in Manalur and Pidikkaparambu. Highest value (1.87 %) was recorded by Anthikkad sample. As in the case of organic carbon, CEC was highest (15.75 c mol(+) kg^{-1}) in Anthikkad and lowest (3.15 c mol(+) kg^{-1}) in Pidikkaparambu.

Anion exchange capacity was varying from 8.45 to 9.45 per cent which were recorded by Manalur and Cherpu respectively. Sesquioxide content was very high in the samples of Kattoor and Manalur (19.00 % and 14.00 % respectively) where as the other three samples recorded values in the range of 1.00 to 9.00 percent. The samples of Manalur, Kattoor and Venkitengu have shown higher values of silica (73.00 and 70.00 and 68.00 % respectively) and the soil samples of the Anthikkad, Cherpu and Pidikkaparambu recorded lower values of silica content viz., 35.00, 53.00 and 65.00 respectively.

Soil samples of Kattoor and Venkitengu were very high in maximum water holding capacity compared to Anthikkad, Cherpu and Manalur samples. Maximum WHC of Kattoor and Venkitengu samples were 52.51 per cent and 50.60 per cent respectively. Soils of Manalur and Pidikkaparambu have recorded lower values (31.14 and 31.18 % respectively).

Compared to the soils of Palakkad, kole soils had higher clay and organic matter contents. These soils are highly acidic in reaction and have greater CEC and AEC values which are indicative of their sedimentary origin.

4.2 Standardisation of method for the determination of residues of 2,4-D in soil

In order to develop a simple colorimetric technique for the estimation of 2,4-D residues in soil, methods of extraction (extractant and soil: extractant ratios) and clean up were standardized. The first step consisted of determination of minimum detectable level of the instrument and the same is summarized below.

4.2.1 Preparation of calibration curve and minimum detectable level of the instrument (MDL)

Standard absorption curve was prepared by taking varying concentrations of 2,4-D acid, ranging from 0.05 μg g^{-1} to 2.00 μg g^{-1} in methanol. The absorbance reading of each standard solution of 2,4-D was plotted against concentration and depicted in Fig. 3. The response curve showed a linear relationship across the range of 0.05 to 2.00 μg g^{-1} of 2,4-D acid. The MDL of the instrument for 2,4-D acid as obtained from the response curve was 0.01 μg g^{-1}.

4.2.2 Selection of extractant and soil: extractant ratios

Two criteria used for the selection of extractant and soil: extractant ratios were (i) percentage recovery of 2,4-D residues from fortified soil samples

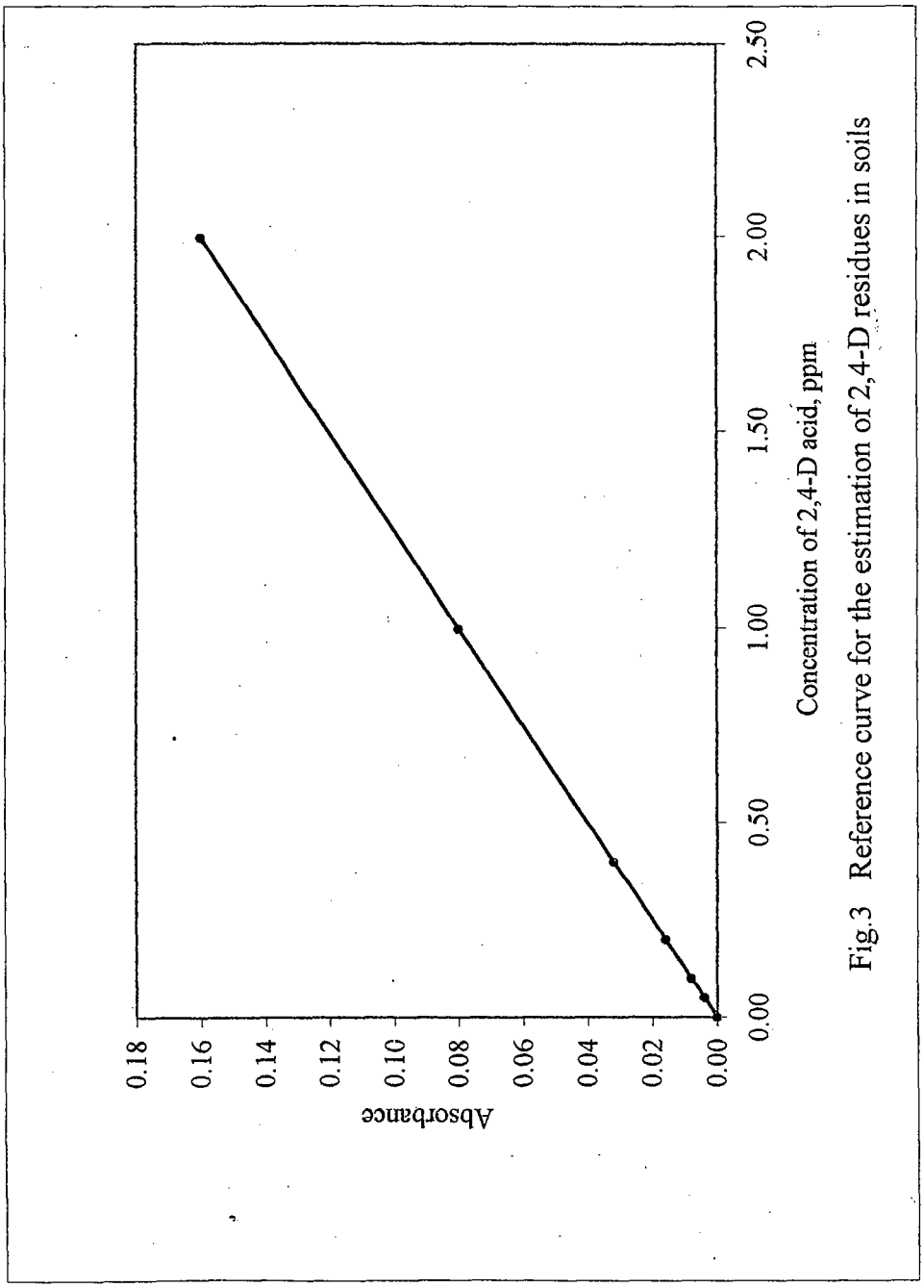


Fig.3 Reference curve for the estimation of 2,4-D residues in soils

(ii) amount of co-extractives removed. Among the seven extractants tested, highest recovery (94.00 %) of 2,4-D residues from soil was obtained with acetonitrile: distilled water: glacial acetic acid (80: 20: 2.5) at 1: 4 soil: extractant ratio (Table 6). The filtrate obtained by NaOH (1 % and 4 %) extraction was highly turbid and coloured. The colouring materials co-extracted with 2,4-D residues, when NaOH was used could not be eliminated by the clean up procedure employed in the study. The other four solvents recorded lower recovery percentage, which was in the range of 22.00 to 52.00 per cent only.

4.2.3 Recovery of 2,4-D from soil at different levels of fortification

Studies were conducted on the recovery of 2,4-D acid from the three major rice soils of Kerala, at three different levels of fortification viz., 0.50, 1.00 and 2.00 μg g^{-1} 2,4-D acid. Fifteen soil samples (five each from Palakkad, kole and kuttanad) were used for this purpose and the results are presented in Table 7.

In the soil samples of Palakkad region, percentage recovery of 2,4-D acid at 0.50, 1.00 and 2.00 μg g^{-1} levels of fortification were in the range of 74.00 to 100.00 per cent, 52.00 to 87.00 per cent and 60.00 to 86.00 per cent respectively. Kole soil samples registered values in the range of 40.00 to 100.00 per cent, 45.00 to 89.00 per cent and 39.00 to 77.00 per cent respectively for 0.50, 1.00 and 2.00 μg g^{-1} levels of fortification. Kuttanad soil samples recorded recovery values in the range of 86.00 to 100.00 per cent (at 0.50 μg g^{-1}), 72.00 to 100.00 per cent (at 1.00 μg g^{-1}) and 63.00 to 96.00 per cent (at 2.00 μg g^{-1}).

Table 6. Recovery of 2, 4-D acid from pooled soil sample of the kole region by different extractants at 1.00 μg g^{-1} level of fortification

Sl. No.	Extractants	Recovery percentage at soil extractant ratio	
		1:2	1:4
1	Acetonitrile	46.00	52.00
2	Acetic acid	45.00	48.00
3	Benzene	17.00	22.00
4	Acetonitrile: distilled water, glacial acetic acid (80:20:2.5)	86.00	94.00
5	Chloroform: ether: acetic acid (50:50:1)	46.00	46.00
6	1% NaOH (0.25 *M*)	555.00	566.00
7	4% NaOH (1.0 *M*)	566.00	600.00
	$CD_{(0.05)}$	12.44	9.31

Table 7. Recovery of 2,4-D acid from the soils under study at varying levels of fortification

Soil		Recovery of 2,4-D ($\mu g\ g^{-1}$ and percentage) at			Mean recovery (%)
		0.5 ($\mu g\ g^{-1}$)	1.00 ($\mu g\ g^{-1}$)	2.00 ($\mu g\ g^{-1}$)	
Palakkad	Alathur	0.39 (78.00)	0.65 (65.00)	1.72 (86.00)	76.33
	Chittur	0.42 (84.00)	0.87 (87.00)	1.44 (72.00)	81.00
	Koyalmannam	0.46 (92.00)	0.87 (87.00)	1.20 (60.00)	60.07
	Nenmara	0.50 (100.00)	0.80 (80.00)	1.67 (84.00)	88.00
	Palakkad	0.37 (74.00)	0.52 (52.00)	1.22 (61.00)	62.33
kuttanad	Moncompu 1	0.45 (90.00)	0.86 (86.00)	1.45 (73.00)	83.00
	Moncompu 2	0.49 (98.00)	1.00 (100.00)	1.68 (84.00)	94.00
	Moncompu 3	0.45 (90.00)	0.87 (87.00)	1.77 (89.00)	88.67
	Karumady	0.43 (86.00)	0.98 (98.00)	1.26 (63.00)	82.33
	Mathikayal	0.50 (100.00)	0.72 (72.00)	1.91 (96.00)	89.33
kole	Anthikkad	0.20 (40.00)	0.87 (87.00)	0.94 (47.00)	58.00
	Cherpu	0.45 (90.00)	0.71 (71.00)	0.78 (39.00)	66.67
	Kattoor	0.50 (100.00)	0.89 (89.00)	1.51 (76.00)	88.33
	Manalur	0.50 (100.00)	0.77 (77.00)	1.53 (77.00)	84.67
	Venkitengu	0.49 (98.00)	0.45 (45.00)	1.22 (61.00)	68.00
Mean recovery (%)		88.00	78.87	71.20	

* Percentage recovery of the applied herbicide is given in parenthesis

In general, recovery of 2,4-D acid from the fortified samples decreased at higher levels of fortification in all the soils which was obvious from the mean values of the samples taken for the study.

4.3 Persistence and degradation of 2,4-D in the major rice soils of the state

Persistence of 2,4-D in the sixteen soil samples (five samples each from Palakkad, kole and kuttanad and one from the trial field viz., Pidikkaparambu) was studied at three levels of fortification of 2,4-D acid viz., 0.50, 1.00 and 2.00 μg g^{-1}. Herbicide residues remaining in the soil samples at 0, 1, 3, 6, 9, 15, 30 and 60 days after 2,4-D application were estimated using the standardized colorimetric procedure as detailed in 3.2.

4.3.1 Persistence of residues in the soils of Palakkad region

At 0.50 μg g^{-1} level of fortification, the five soil samples of Palakkad region registered 2,4-D residues in the range of 0.24 to 0.47 μg g^{-1} on the same day of application (Table 8). Then the values decreased in all soils and attained non detectable level at nine DAS in Chittur soil. At this time the other soil samples recorded values in the range of 0.03 to 0.16 μg g^{-1}. At 15 DAS, only the Palakkad soil sample registered residues (0.02 μg g^{-1}). At 30 DAS, no soil registered detectable level of 2,4-D residues. At 1.00 μg g^{-1} level of fortification of 2,4-D, initial residue levels were in the range of 0.63 to 0.90 μg g^{-1}. As the time of incubation increased, the residues decreased and two soils viz., Chittur and Nenmara registered no 2,4-D residues at 15 DAS. At 30 DAS only the

Table 8. Residues of 2,4-D ($\mu g\ g^{-1}$) in the soils of Palakkad region at different levels of fortification and after different periods of incubation

Levels of fortification ($\mu g\ g^{-1}$)	Soil	Period of incubation (days)							
		0	1	3	6	9	15	30	60
0.50	Alathur	0.24	0.17	0.14	0.12	0.04	ND	ND	ND
	Chittur	0.36	0.33	0.19	0.18	ND	ND	ND	ND
	Koyalmannam	0.34	0.31	0.29	0.25	0.16	ND	ND	ND
	Nenmara	0.45	0.18	0.16	0.03	0.03	ND	ND	ND
	Palakkad	0.47	0.18	0.15	0.13	0.04	0.02	ND	ND
1.00	Alathur	0.90	0.38	0.33	0.23	0.09	0.04	ND	ND
	Chittur	0.63	0.50	0.32	0.07	0.02	ND	ND	ND
	Koyalmannam	0.89	0.70	0.46	0.32	0.17	0.09	0.06	ND
	Nenmara	0.85	0.48	0.37	0.17	0.017	ND	ND	ND
	Palakkad	0.85	0.34	0.30	0.25	0.20	0.02	ND	ND
2.00	Alathur	1.45	0.91	0.72	0.55	0.25	0.13	0.01	ND
	Chittur	1.20	1.16	0.73	0.73	0.56	0.64	0.47	ND
	Koyalmannam	0.98	0.94	0.82	0.68	0.48	0.68	0.37	ND
	Nenmara	1.35	1.08	0.87	0.28	0.16	0.13	ND	ND
	Palakkad	1.54	1.34	0.95	0.65	0.53	0.23	0.16	ND

ND – Not Detected

Koyalmannam soil samples registered 2,4-D residues (0.06 μg g^{-1}). 2,4-D residues dissipated from all the soils by 60DAS.

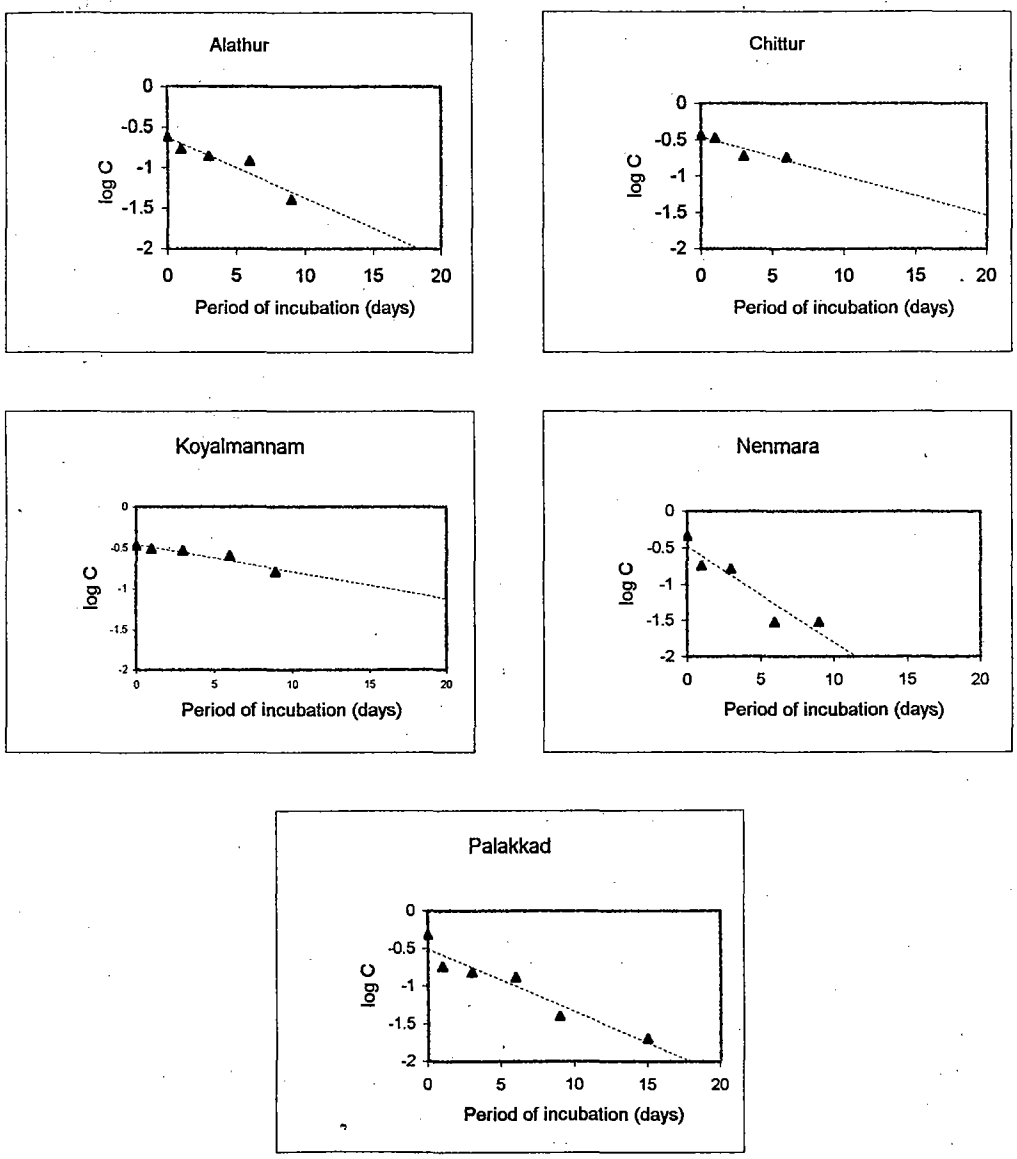
At the highest level of fortification of 2,4-D (2.00 μg g^{-1}), residues in the five soil samples at 0 DAS was in the range of 0.98 to 1.54 μg g^{-1}. The residue levels in the soil samples decreased with time and at 30 DAS it was in the range of non detectable level to 0.47 μg g^{-1}. At 30 DAS, the lower residue values were registered by Nenmara (non detectable level) and Alathur (0.01) samples. At the 60 day stage, 2,4-D residues could not be detected in any of the samples.

As the period increased the residues decreased at all the levels of application. The residue levels in the soil samples increased almost proportionately with the levels of application.

Kinetics of 2,4-D degradation in the soils of Palakkad region at different levels of fortification was worked out by plotting the logarithm to the base 10 of the quantity of 2,4-D residues on the Y-axis and period of incubation on the X-axis (Fig. 4, 5 and 6). The regression equations for the same are given in Table 9. The degradation rate constant (first order rate constant, k) varied from 0.0769 to 0.1893 depending upon the level of fortification and soil type.

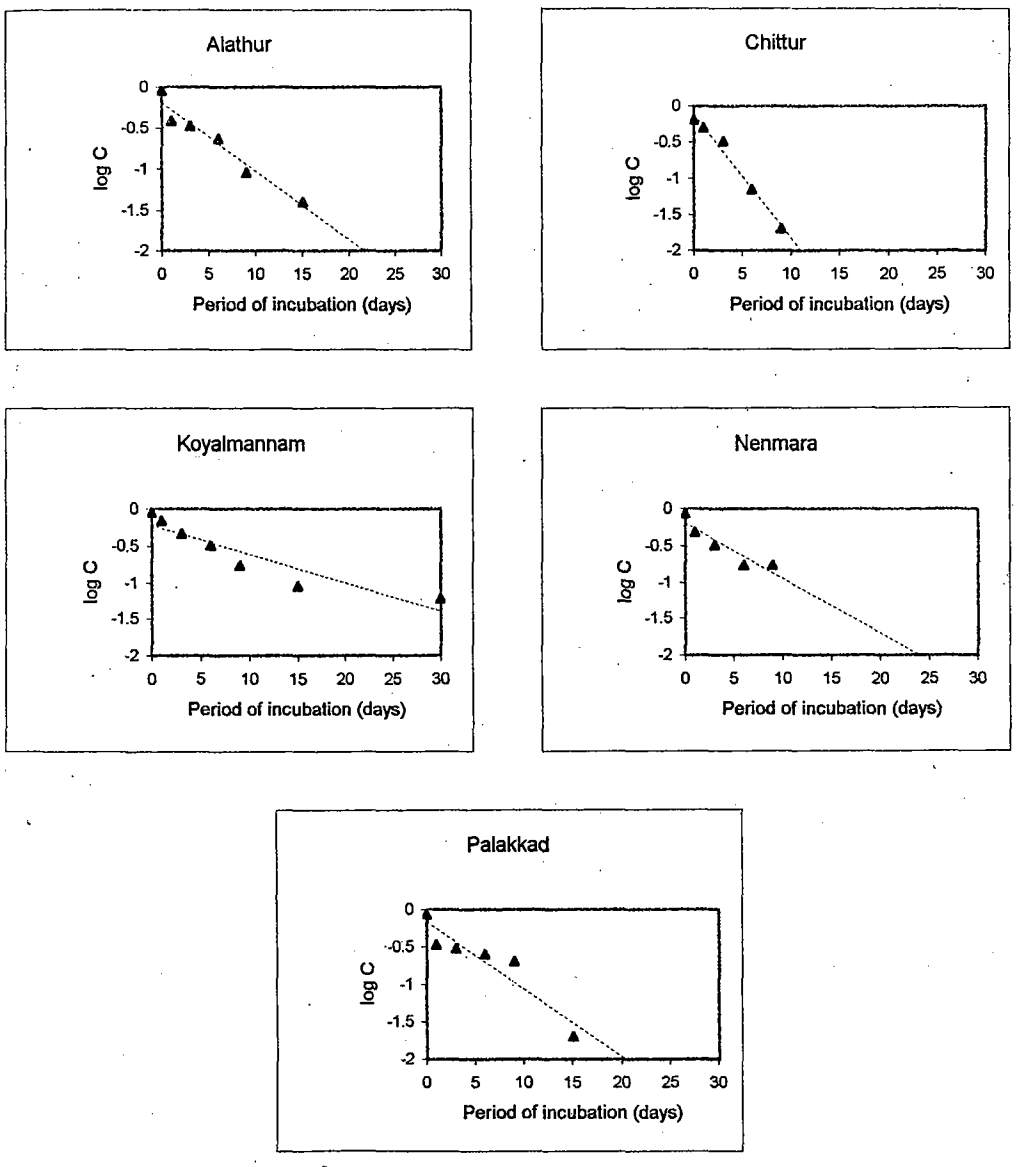
4.3.2 Persistence of residues in the soils of kuttanad region

At 0.50 μg g^{-1} level of fortification, 2,4-D residues in kuttanad soils at 0 DAS was in the range of 0.33 to 0.43 μg g^{-1} (Table 10). In the soil sample of Karumady, residues disappeared at a faster rate compared to other soils. Even at



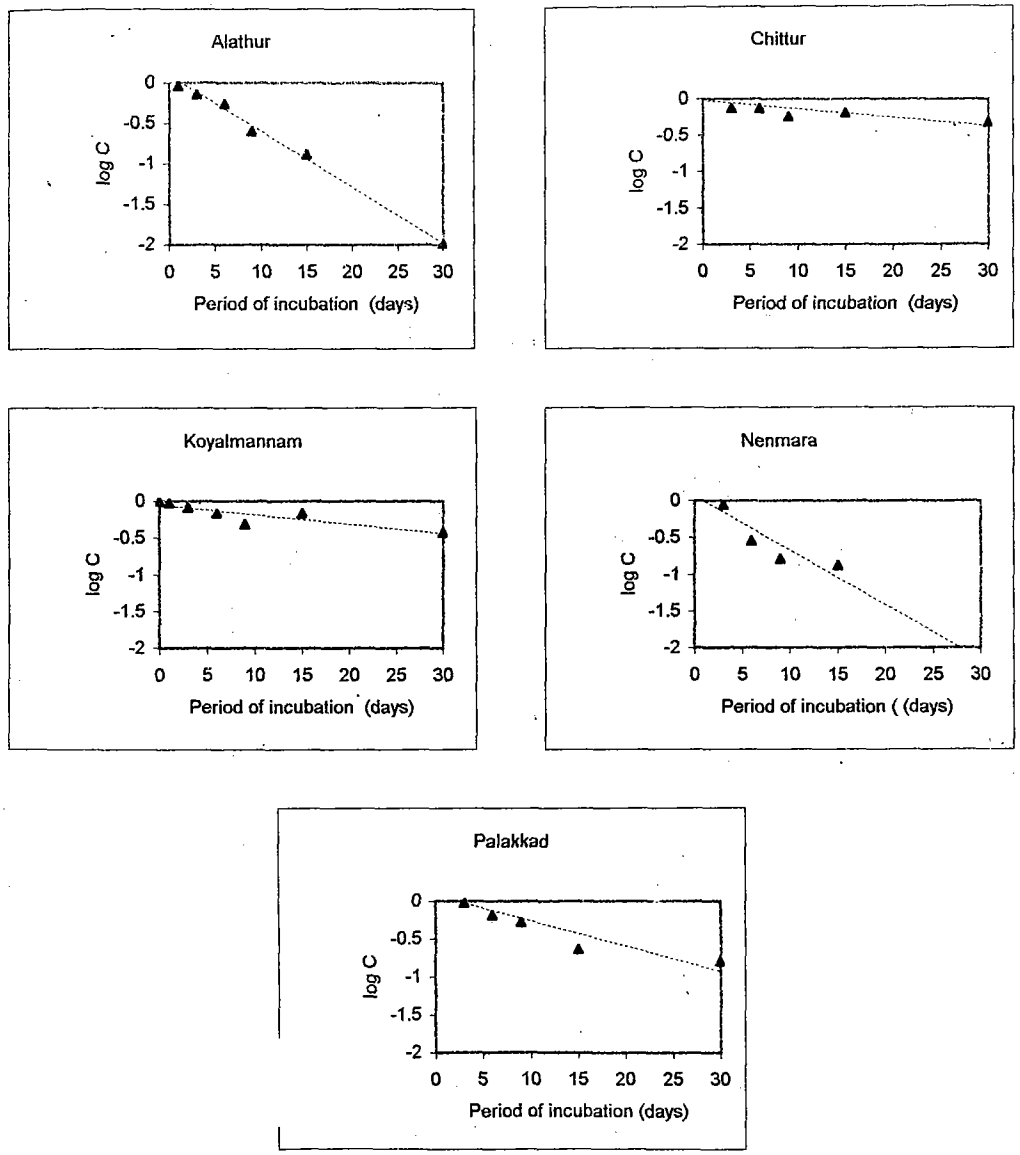
C = Concentration of 2,4-D residues in $\mu g\ g^{-1}$

Fig.4 Kinetics of 2,4-D residues in the soils of Palakkad region fortified at 0.50 $\mu g\ g^{-1}$



C = Concentration of 2,4-D residues in $\mu g\ g^{-1}$

Fig.5 Kinetics of 2,4-D residues in the soils of Palakkad region fortified at 1.00 $\mu g\ g^{-1}$



C = Concentration of 2,4-D residues in $\mu g\ g^{-1}$

Fig.6 Kinetics of 2,4-D residues in the soils of Palakkad region fortified at 2.00$\mu g\ g^{-1}$

Table 9. Regression equations for degradation of 2, 4-D in the soils of Palakkad region at varying levels of fortification

Levels of fortification ($\mu g\ g^{-1}$)	Soil	Regression equation	Coefficient of determination (R^2)
0.50	Alathur	log C = -0.0749 t – 0.6279	0.90
	Chittur	log C = -0.0541 t – 0.4626	0.83
	Koyalmannam	log C = -0.0334 t – 0.4557	0.92
	Nenmara	log C = -0.1319 t – 0.4853	0.88
	Palakkad	log C = -0.0834 t – 0.5076	0.92
1.00	Alathur	log C = -0.0831 t – 0.2006	0.95
	Chittur	log C = -0.1711 t – 0.1200	0.98
	Koyalmannam	log C = -0.0392 t – 0.2236	0.86
	Nenmara	log C = -0.0755 t – 0.1977	0.86
	Palakkad	log C = -0.0904 t – 0.1646	0.88
2.00	Alathur	log C = -0.0694 t – 0.0958	0.99
	Chittur	log C = -0.0119 t – 0.0205	0.67
	Koyalmannam	log C = -0.0129 t – 0.0548	0.77
	Nenmara	log C = -0.0743 t – 0.0658	0.90
	Palakkad	log C = -0.0335 t – 0.0768	0.89

C =Concentration of 2,4-D residue ($\mu g\ g^{-1}$)

t= time (days)

Table 10. Residues of 2,4-D ($\mu g\ g^{-1}$) in the soils of kuttanad region at different levels of fortification and after different periods of incubation

Levels of fortification ($\mu g\ g^{-1}$)	Soil	Period of incubation (days)							
		0	1	3	6	9	15	30	60
0.50	Moncompu 1	0.33	0.21	0.18	0.16	0.12	ND	ND	ND
	Moncompu 2	0.42	0.31	0.27	0.26	0.21	ND	ND	ND
	Moncompu 3	0.34	0.34	0.33	0.26	0.23	0.13	ND	ND
	Karumady	0.43	0.25	ND	ND	ND	ND	ND	ND
	Mathikayal	0.43	0.25	0.04	0.01	ND	ND	ND	ND
1.00	Moncompu 1	0.94	0.62	0.57	0.26	0.24	0.05	ND	ND
	Moncompu 2	0.82	0.71	0.67	0.43	0.09	ND	ND	ND
	Moncompu 3	0.81	0.76	0.64	0.61	0.50	0.30	0.13	ND
	Karumady	0.58	0.47	0.12	ND	ND	ND	ND	ND
	Mathikayal	0.94	0.47	0.45	0.29	0.12	0.07	ND	ND
2.00	Moncompu 1	1.30	1.21	0.69	0.34	0.28	0.22	0.22	ND
	Moncompu 2	1.50	0.94	0.86	0.67	0.59	0.42	0.29	ND
	Moncompu 3	1.34	1.32	0.74	0.59	0.59	0.43	ND	ND
	Karumady	1.28	1.22	0.76	0.60	0.42	0.20	0.04	ND
	Mathikayal	1.66	0.97	0.64	0.43	0.38	0.23	0.06	ND

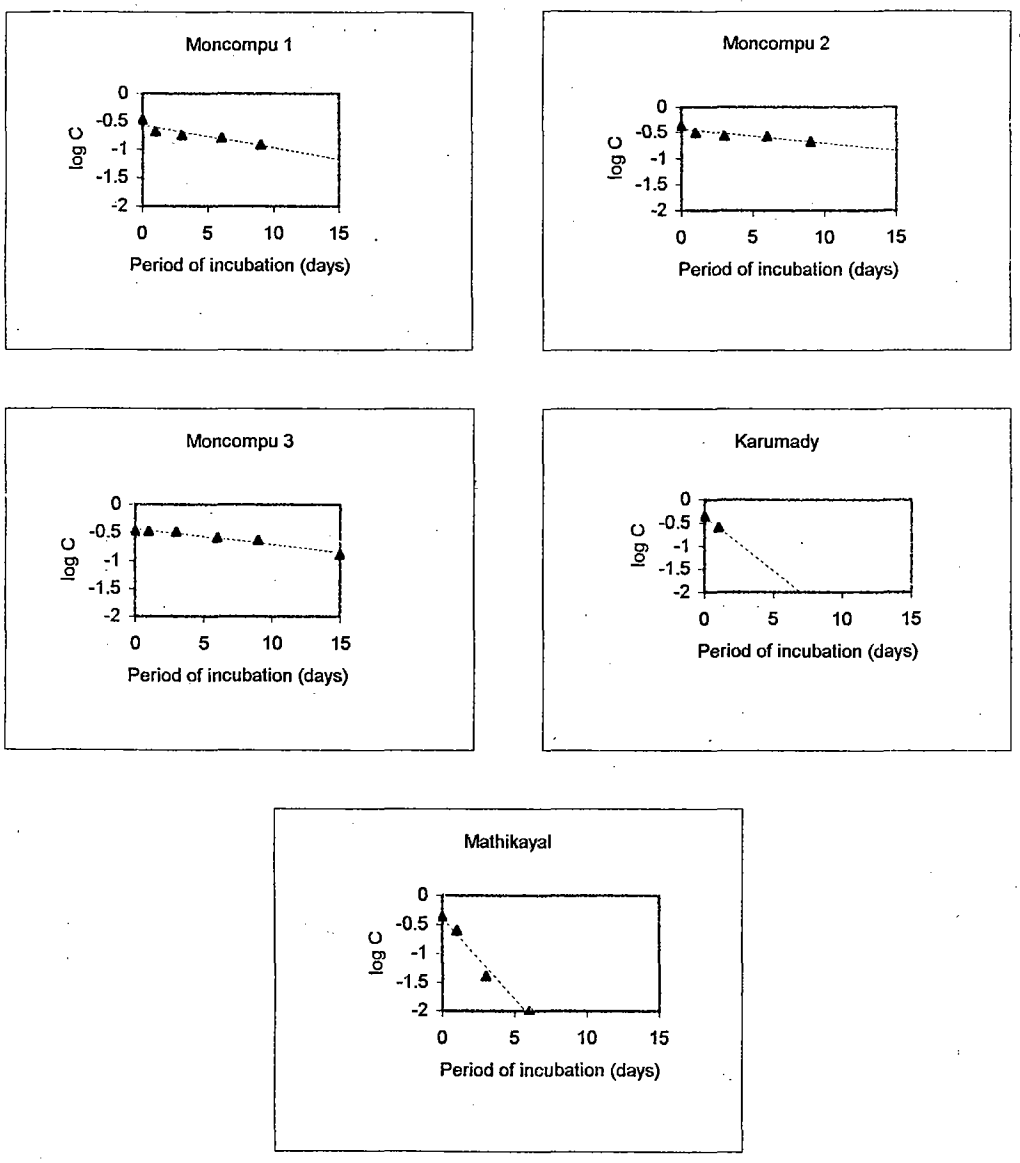
ND – Not Detected

3 DAS, 2,4-D residues in Karumady sample fell below the detectable level. In the soil sample of Moncompu-3, residue level was high at 15 DAS also. But in the other samples 2,4-D residues could not be detected at this time. Moncompu-3 registered non detectable level of 2,4-D at 30 DAS.

At 1.00 $\mu g\ g^{-1}$ level of fortification, the initial soil residues were in the range of 0.58 to 0.94 $\mu g\ g^{-1}$. As the application level increased, the Karumady soil showed detectable levels upto the third day only. In Moncompu-2 samples, residues could not be detected after 15 days, but in the other three samples the residues were in the range of 0.05 to 0.30 $\mu g\ g^{-1}$. At 30 DAS, only Moncompu-3 sample recorded residues of 2,4-D in soil (0.13 $\mu g\ g^{-1}$).

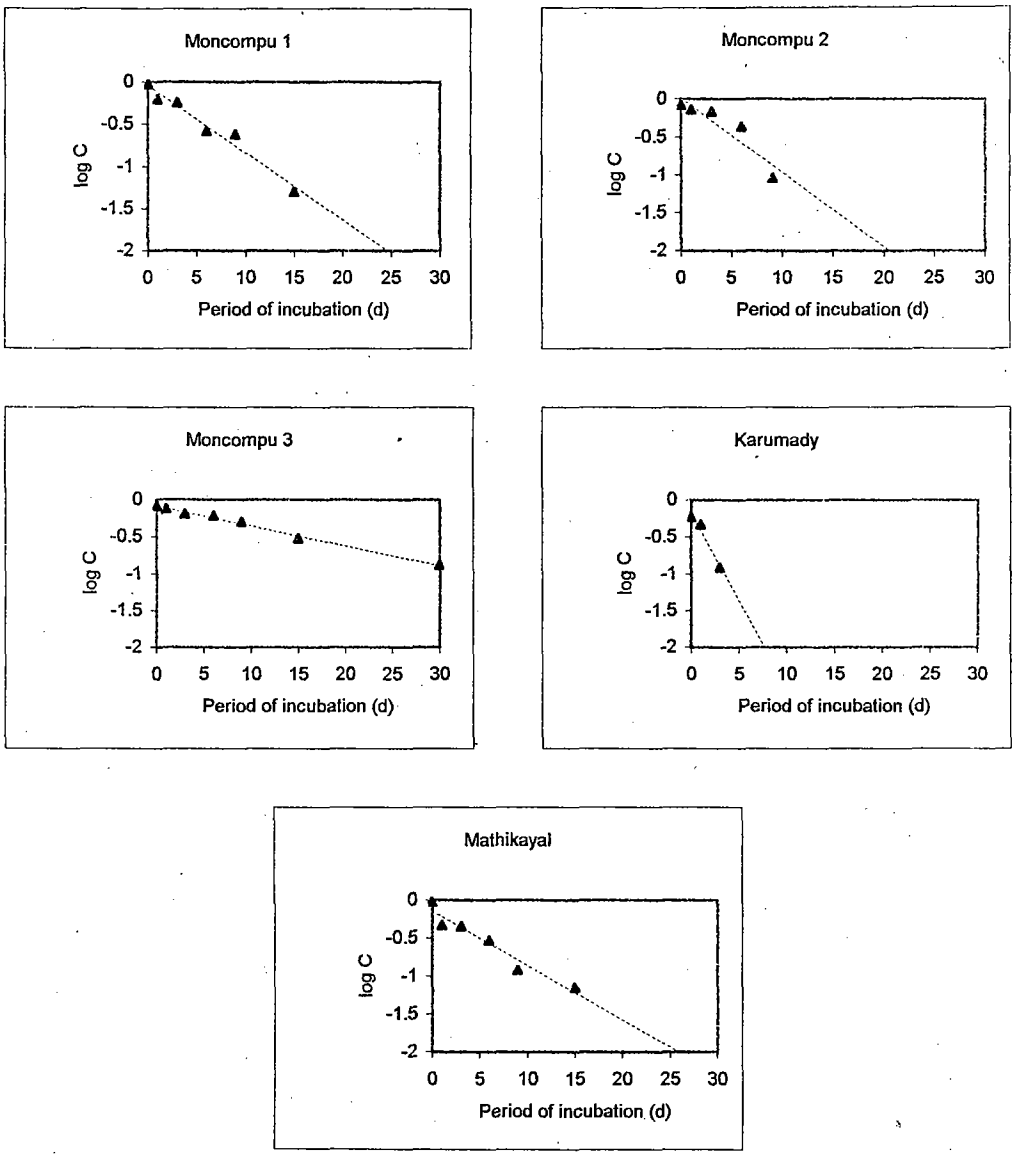
When the soil samples of kuttanad were fortified with 2,4-D at 2.00 $\mu g\ g^{-1}$, considerable increase in persistence was noticed in all the samples. The initial residues were in the range of 1.28 to 1.66 $\mu g\ g^{-1}$, which decreased to a range of non detectable to 0.29 $\mu g\ g^{-1}$ at 30 DAS. The non-detectable level was observed only in soil sample of Moncompu-3 soil on the 30^{th} day.

Kinetics of 2,4-D degradation in the soils of kuttanad region at different levels of fortification are depicted in figures 7, 8 and 9 and the regression equations for the same are given in Table 11. The degradation rate constant varied from 0.0644 to 0.2015 depending upon the level of fortification and soil type.



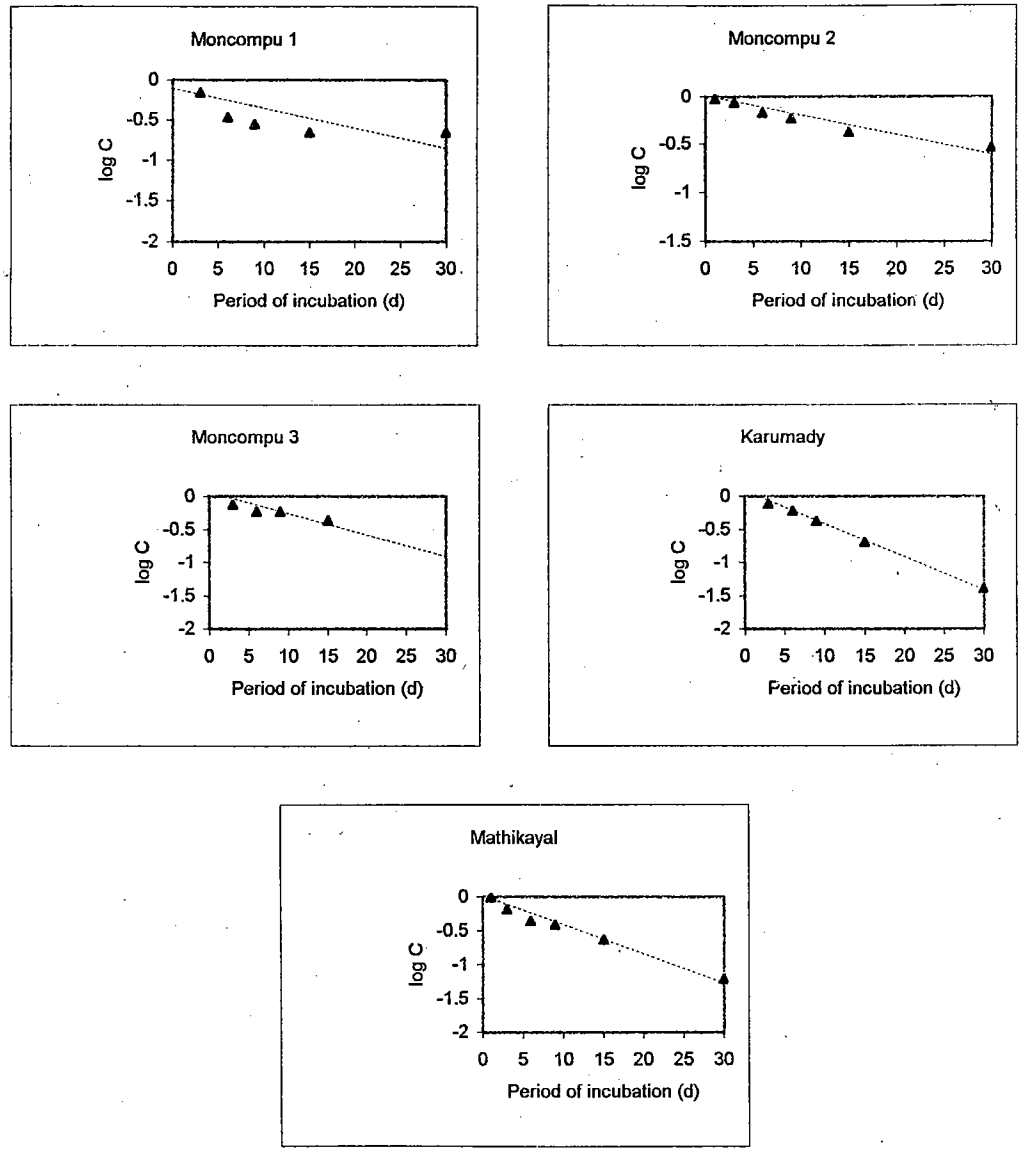
C = Concentration of 2,4-D residues in $\mu g\ g^{-1}$

Fig.7 Kinetics of 2,4-D residues in the soils of kuttanad region fortified at 0.50 $\mu g\ g^{-1}$



C = Concentration of 2,4-D residues in $\mu g\ g^{-1}$

Fig.8 Kinetics of 2,4-D residues in the soils of kuttanad region fortified at 1.00 $\mu g\ g^{-1}$



C = Concentration of 2,4-D residues in $\mu g\ g^{-1}$

Fig.9 Kinetics of 2,4-D residues in the soils of kuttanad region fortified at 2.00 $\mu g\ g^{-1}$

Table 11. Regression equations for degradation of 2,4-D in the soils of kuttanad region at varying levels of fortification

Levels of fortification ($\mu g\ g^{-1}$)	Soil	Regression equation	Coefficient of determination (R^2)
0.50	Moncompu 1	Log C = -0.0404 t – 0.5705	0.81
	Moncompu 2	Log C = -0.0274 t – 0.4393	0.83
	Moncompu 3	Log C = -0.0280 t – 0.4291	0.95
	Karumady	Log C = -0.2355 t – 0.3665	1.00
	Mathikayal	Log C = -0.2800 t – 0.3917	1.00
1.00	Moncompu 1	Log C = -0.0793 t – 0.0482	0.96
	Moncompu 2	Log C = -0.0978 t + 0.0075	0.84
	Moncompu 3	Log C = -0.0267 t – 0.0883	0.99
	Karumady	Log C = -0.2379 t – 0.1780	0.95
	Mathikayal	Log C = -0.0717 t – 0.1463	0.94
2.00	Moncompu 1	Log C = -0.0251 t – 0.1001	0.62
	Moncompu 2	Log C = -0.0207 t + 0.0134	0.86
	Moncompu 3	Log C = -0.0326 t + 0.0668	0.83
	Karumady	Log C = -0.0502 t + 0.0842	1.00
	Mathikayal	Log C = -0.0431 t + 0.0176	0.95

C =Concentration of 2,4-D residue ($\mu g\ g^{-1}$)
t= time (days)

4.3.3 Persistence of residues in the kole soils

The six soil samples collected from different locations of the kole area had residue levels in the range of 0.28 to 0.47 μg g^{-1} at 0 DAS, when the samples were fortified with 2,4-D at 0.50 μg g^{-1} level (Table 12). The residues disappeared in soil samples of Cherpu, Kattoor and Venkitengu by 30 DAS where as in samples of Pidikkaparambu no residues could be detected even at 15 DAS. The samples of Anthikkad and Manalur recorded values of 0.01 and 0.03 μg g^{-1} respectively at 30 DAS and no residues could be detected in any sample at 60 DAS.

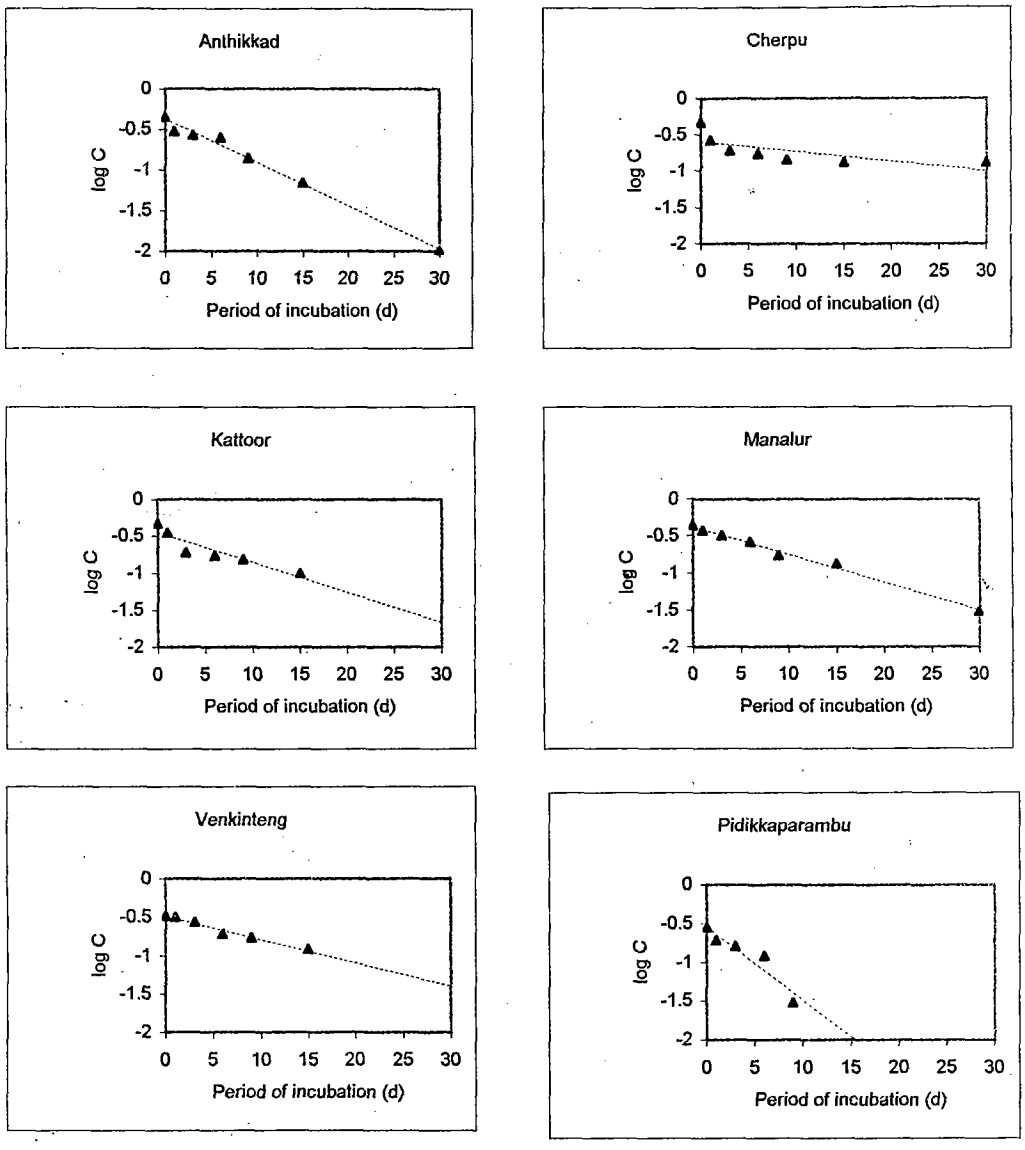
At the fortification level of 1.00 μg g^{-1}, 2,4-D residues estimated at 0 DAS was in the range of 0.76 to 0.90 μg g^{-1}. The residue levels decreased with time and attained non detectable level at 15 DAS in Pidikkaparambu sample and the other three soils viz., Anthikkad, Cherpu and Kattoor, residues reached non detectable level at 30 DAS. The soil samples of Manalur and Venkitengu retained residues of 0.13 and 0.17 μg g^{-1} respectively at 30 DAS. In these two samples also residues could not be detected at 60 DAS.

When the level of fortification was enhanced to 2.00 μg g^{-1} the initial residue values were in the range of 1.20 to 1.51 μg g^{-1} and the level decreased with time. Even then, all the six soil samples retained residues in the range of 0.05 μg g^{-1} to 0.36 μg g^{-1} at 30 DAS. Dissipation of residues to non detectable level occurred only at 60 DAS. It indicated clearly that persistence of 2,4-D

Table 12. Residues of 2,4-D ($\mu g\ g^{-1}$) in the soils of kole region at different levels of fortification and after different periods of incubation

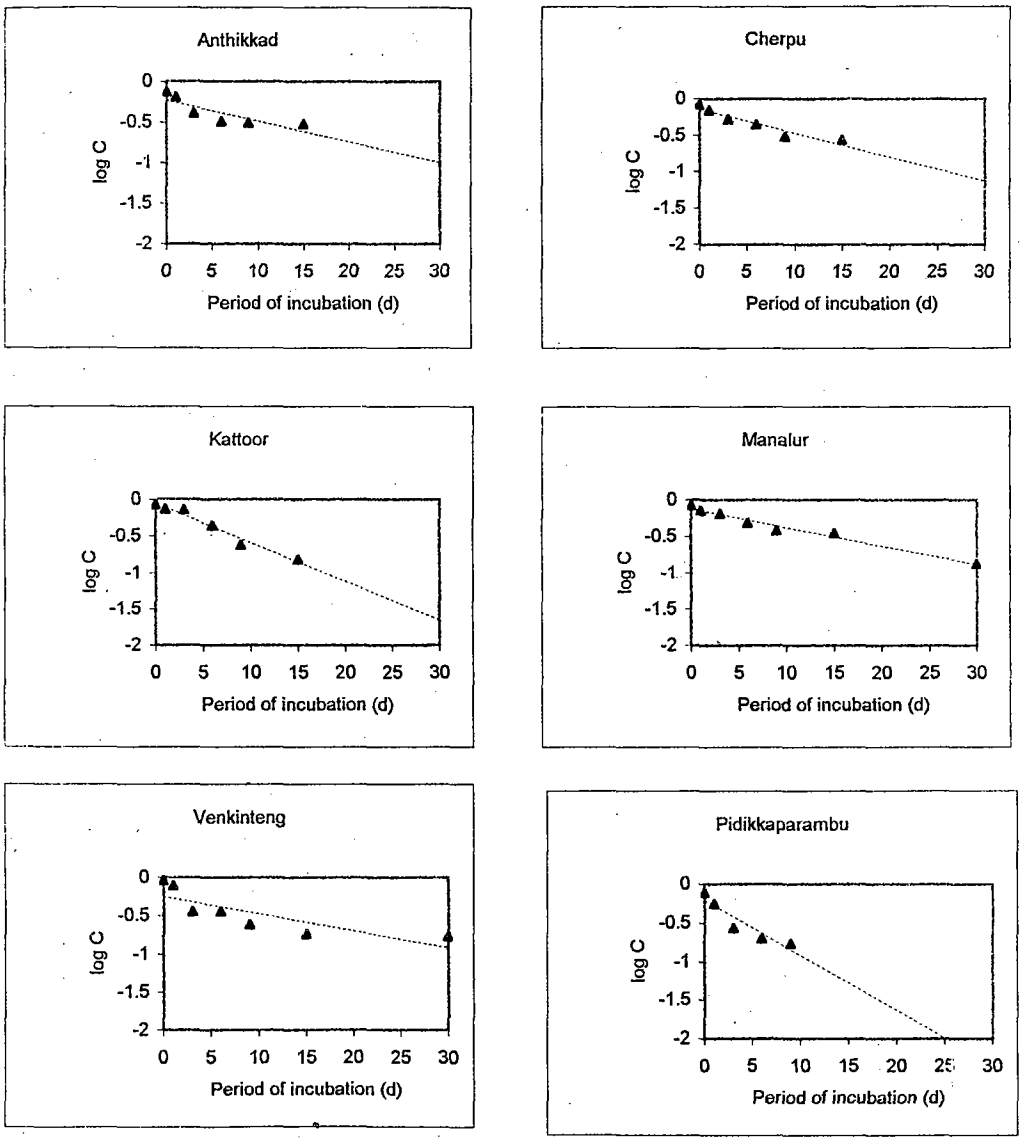
Levels of fortification ($\mu g\ g^{-1}$)	Soil	Period of incubation (days)							
		0	1	3	6	9	15	30	60
0.50	Anthikkad	0.45	0.30	0.27	0.25	0.14	0.07	0.01	ND
	Cherpu	0.46	0.26	0.19	0.17	0.14	0.13	ND	ND
	Kattoor	0.47	0.35	0.19	0.17	0.15	0.10	ND	ND
	Manalur	0.44	0.37	0.32	0.26	0.17	0.13	0.03	ND
	Venkitengu	0.33	0.32	0.27	0.19	0.17	0.12	ND	ND
	Pidikkaparambu	0.28	0.19	0.16	0.12	0.03	ND	ND	ND
1.00	Anthikkad	0.74	0.64	0.41	0.32	0.31	0.30	ND	ND
	Cherpu	0.84	0.70	0.52	0.44	0.30	0.27	ND	ND
	Kattoor	0.85	0.74	0.73	0.44	0.24	0.15	ND	ND
	Manalur	0.84	0.71	0.64	0.48	0.38	0.35	0.13	ND
	Venkitengu	0.90	0.78	0.36	0.36	0.24	0.18	0.17	ND
	Pidikkaparambu	0.76	0.55	0.27	0.20	0.17	ND	ND	ND
2.00	Anthikkad	1.20	0.80	0.60	0.56	0.55	0.40	0.36	ND
	Cherpu	1.43	0.70	0.59	0.58	0.47	0.45	0.27	ND
	Kattoor	1.36	0.96	0.78	0.78	0.53	0.33	0.31	ND
	Manalur	1.51	1.40	0.79	0.52	0.52	0.38	0.20	ND
	Venkitengu	1.46	1.05	1.49	0.48	0.35	0.16	0.05	ND
	Pidikkaparambu	1.42	1.36	0.52	0.39	0.38	0.25	0.05	ND

ND – Not Detected



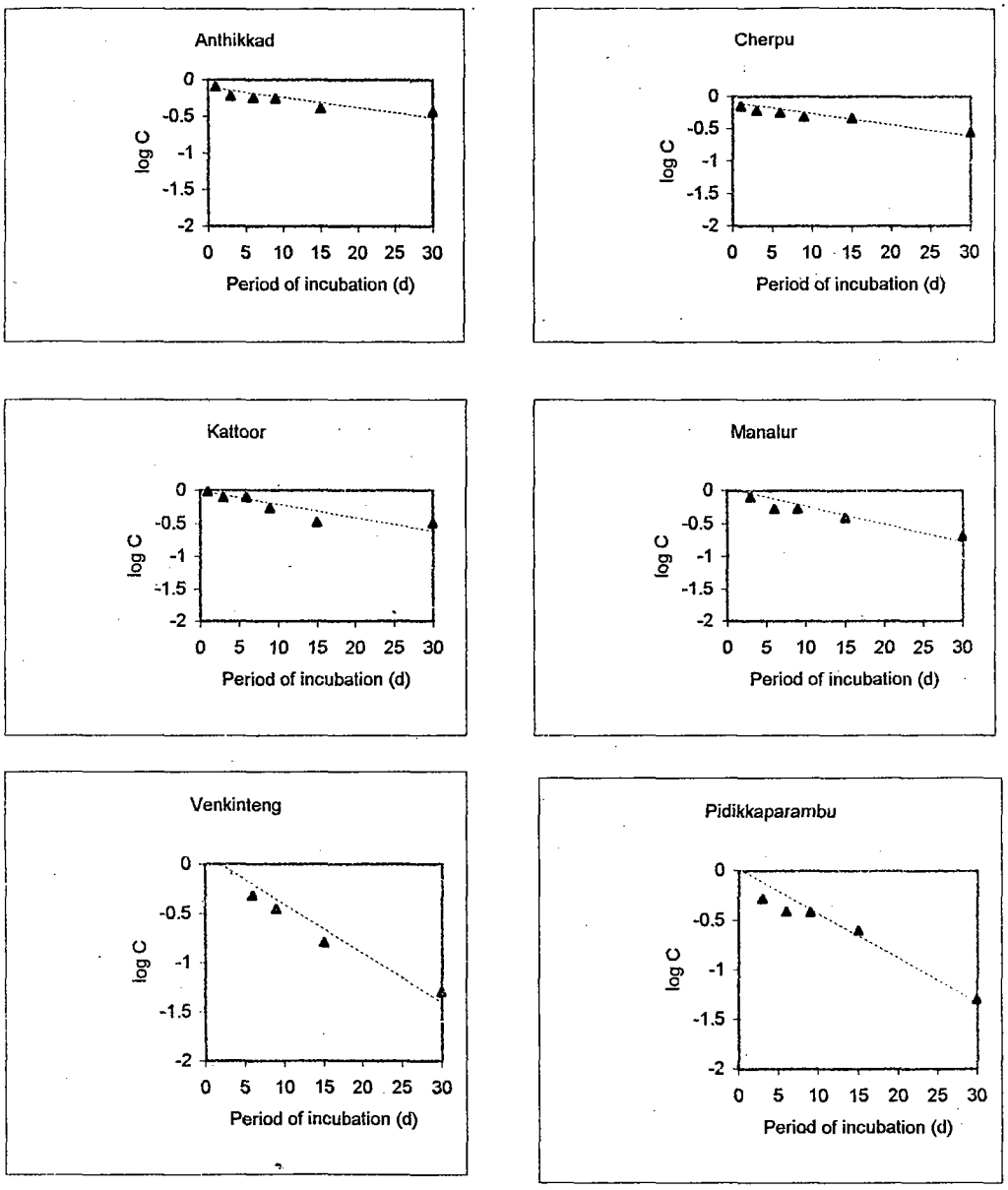
C = Concentration of 2,4-D residues in $\mu g\ g^{-1}$

Fig.10 Kinetics of 2,4-D residues in the soils of kole region fortified at 0.50 $\mu g\ g^{-1}$



C = Concentration of 2,4-D residues in $\mu g\ g^{-1}$

Fig.11 Kinetics of 2,4-D residues in the soils of kole region fortified at 1.00 $\mu g\ g^{-1}$



C = Concentration of 2,4-D residues in $\mu g\ g^{-1}$

Fig.12 Kinetics of 2,4-D residues in the soils of kole region fortified at 2.00 $\mu g\ g^{-1}$

Table 13. Regression equations for degradation of 2,4-D in the soils of kole region at varying levels of fortification

Levels of fortification ($\mu g\ g^{-1}$)	Soil	Regression equation	Coefficient of determination (R^2)
0.50	Anthikkad	log C = -0.0533 t – 0.3769	0.99
	Cherpu	log C = -0.0133 t – 0.5985	0.49
	Kattoor	log C = -0.0403 t – 0.4545	0.85
	Manalur	log C = -0.0378 t – 0.3756	0.99
	Venkitengu	log C = -0.0304 t – 0.4871	0.97
	Pidikkaparambu	log C = -0.0947 t – 0.5430	0.89
1.00	Anthikkad	log C = -0.0255 t – 0.2285	0.71
	Cherpu	log C = -0.0330 t – 0.1401	0.90
	Kattoor	log C = -0.0533 t – 0.0541	0.97
	Manalur	log C = -0.0255 t – 0.1240	0.97
	Venkitengu	log C = -0.0224 t – 0.2492	0.67
	Pidikkaparambu	log C = -0.0713 t – 0.2124	0.87
2.00	Anthikkad	log C = -0.0140 t – 0.0996	0.69
	Cherpu	log C = -0.0177 t – 0.0844	0.72
	Kattoor	log C = -0.0202 t – 0.0105	0.80
	Manalur	log C = -0.0274 t + 0.0417	0.66
	Venkitengu	log C = -0.0502 t + 0.0999	0.94
	Pidikkaparambu	log C = -0.0451 t + 0.0220	0.93

C =Concentration of 2,4-D residue ($\mu g\ g^{-1}$)

t= time (days)

residues in soil samples of kole area is comparatively higher than that of Palakkad area at all the levels of fortification.

Kinetics of 2,4-D degradation in the soils of kole region at different levels of fortification are depicted in figures 10, 11 and 12 and the regression equations for the same are presented in table 13. The degradation rate constant varied from 0.0760 to 0.1227 depending upon the level of fortification and soil type.

The results on degradation of 2,4-D in the three rice soils of Kerala indicated that degradation rate of the chemical is greater in kuttanad soils compared to Palakkad and kole soils.

4.4 Studies on adsorption of 2,4-D by the soils

Sorption of 2,4-D in the major rice soils of Kerala viz., Palakkad, kole and kuttanad was studied in the laboratory. The treatments consisted of three levels of 2,4-D viz., 0.50, 1.00 and 2.00 $\mu g\ g^{-1}$ and three periods of equilibration (2, 4 and 6 hours). The quantity of 2,4-D adsorbed by soil samples was determined by subtracting the quantity of residue obtained in the solution from the total quantity applied to the soil. The results are presented below.

4.4.1 Soils of Palakkad region

When the level of application was 0.50 $\mu g\ g^{-1}$ the variation in the quantity adsorbed between soils at two hours was from 0.29 to 0.50 $\mu g\ g^{-1}$ (Table 14). The variation in adsorption between soil samples was comparatively less at four

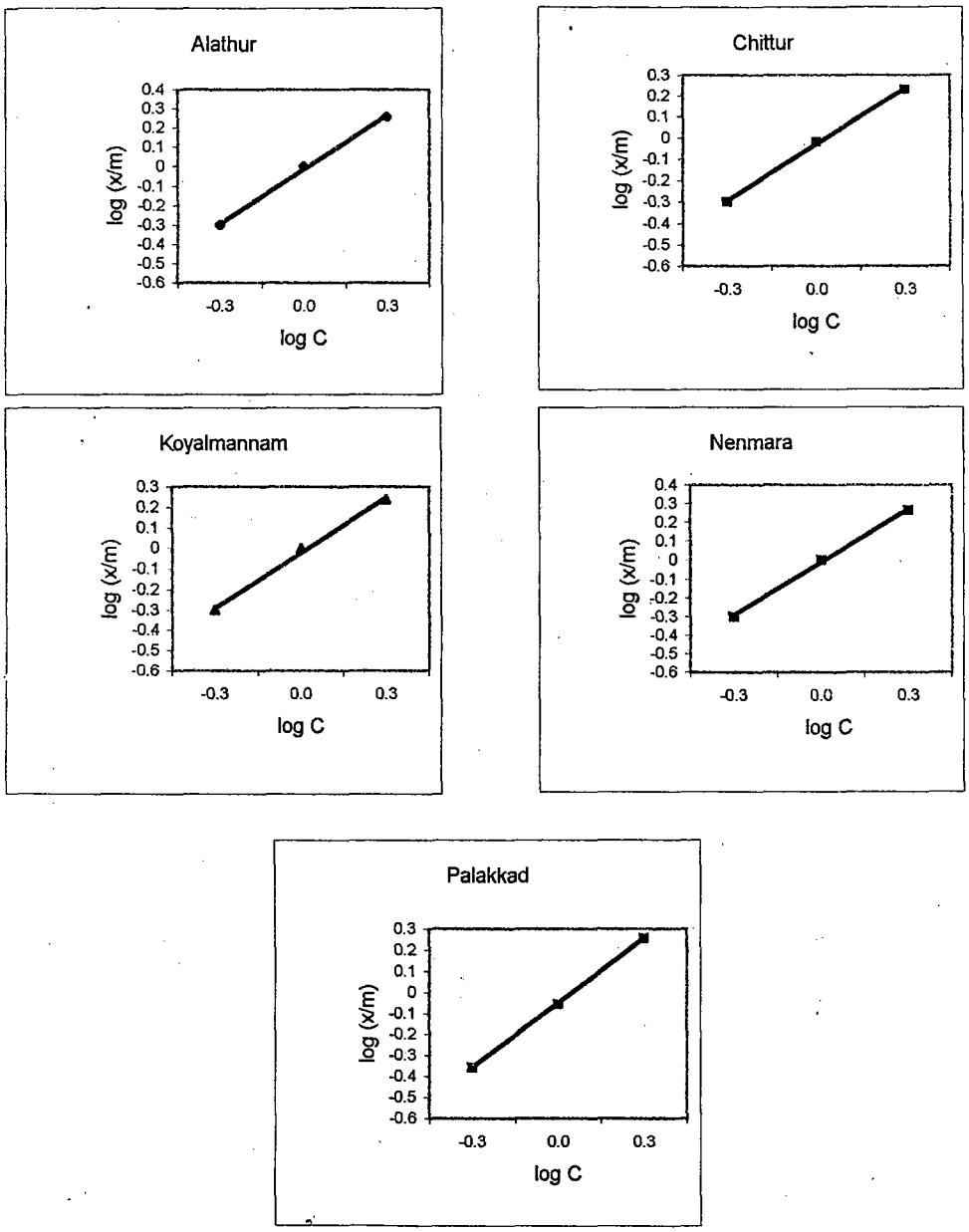
Table 14. Adsorption of 2,4-D ($\mu g\ g^{-1}$) by the soils of Palakkad region charged at different levels at different period of equilibration

Quantity of 2,4-D added ($\mu g\ g^{-1}$)	Soil	Quantity of 2,4-D adsorbed by the soil ($\mu g\ g^{-1}$) at		
		2 hrs	4 hrs	6 hrs
0.50	Alathur	0.350	0.500	0.500
	Chittur	0.500	0.500	0.500
	Koyalmannam	0.370	0.500	0.500
	Nenmara	0.500	0.500	0.330
	Palakkad	0.290	0.440	0.260
1.00	Alathur	0.900	0.840	1.000
	Chittur	0.700	0.950	1.000
	Koyalmannam	0.620	0.960	1.000
	Nenmara	0.840	1.000	1.000
	Palakkad	0.960	0.880	1.000
2.00	Alathur	1.260	1.660	1.810
	Chittur	1.460	1.690	1.660
	Koyalmannam	1.570	1.350	1.720
	Nemmara	1.820	1.830	1.700
	Palakkad	1.450	1.800	1.640

hours (0.44 to 0.50 μg g^{-1}). Quantity of 2,4-D adsorbed by soil samples at six hours was less than that at two and four hours in Nenmara and Palakkad samples, the values being 0.33 and 0.26 μg g^{-1} respectively. In the other three samples (Alathur, Chittur and Koyalmannam), the quantity of 2,4-D adsorbed at six hours was same as that at four hours.

When the level of 2,4-D application was 1.00 μg g^{-1}, quantity of 2,4-D adsorbed at two hours was varying from 0.62 to 0.96 μg g^{-1}. Palakkad soil registered the highest value of 0.96 μg g^{-1} and the lowest value was recorded by Koyalmannam sample. At four hours, Alathur and Palakkad samples recorded lower adsorption compared to that of two hours and the values being 0.84 and 0.88 μg g^{-1} respectively. The other three samples recorded higher values in the range of 0.95 to 1.00 μg g^{-1} at four hours. At six hours, all the soil samples adsorbed the entire quantity of 2,4-D added to the soil.

At 2.00 μg g^{-1} level of application of 2,4-D, the variation in the quantity adsorbed at two hours by the five samples was in the range of 1.26 to 1.82 μg g^{-1}. At longer period of equilibration (4 hours) only one soil sample (Koyalmannam) recorded lower values compared to that of two hours. At the end of an equilibration period of six hours, only two samples recorded higher adsorption of 2,4-D compared to that of two and four hours. These soil samples were from Alathur, Koyalmannam. The other samples recorded values in the range of 1.64 to 1.72 μg g^{-1}.



x/m - Quantity of 2,4-D adsorbed by unit mass of soil $\mu g\ g^{-1}$

C - Equilibrium concentration, $\mu g\ ml^{-1}$

Fig.13 Freundlich isotherm for adsorption of 2,4-D in the soils of Palakkad region

In general maximum quantity was adsorbed by the soil 4 hours after application at the lowest level (0.50 μg g^{-1}) while as the level increased adsorption increased with time.

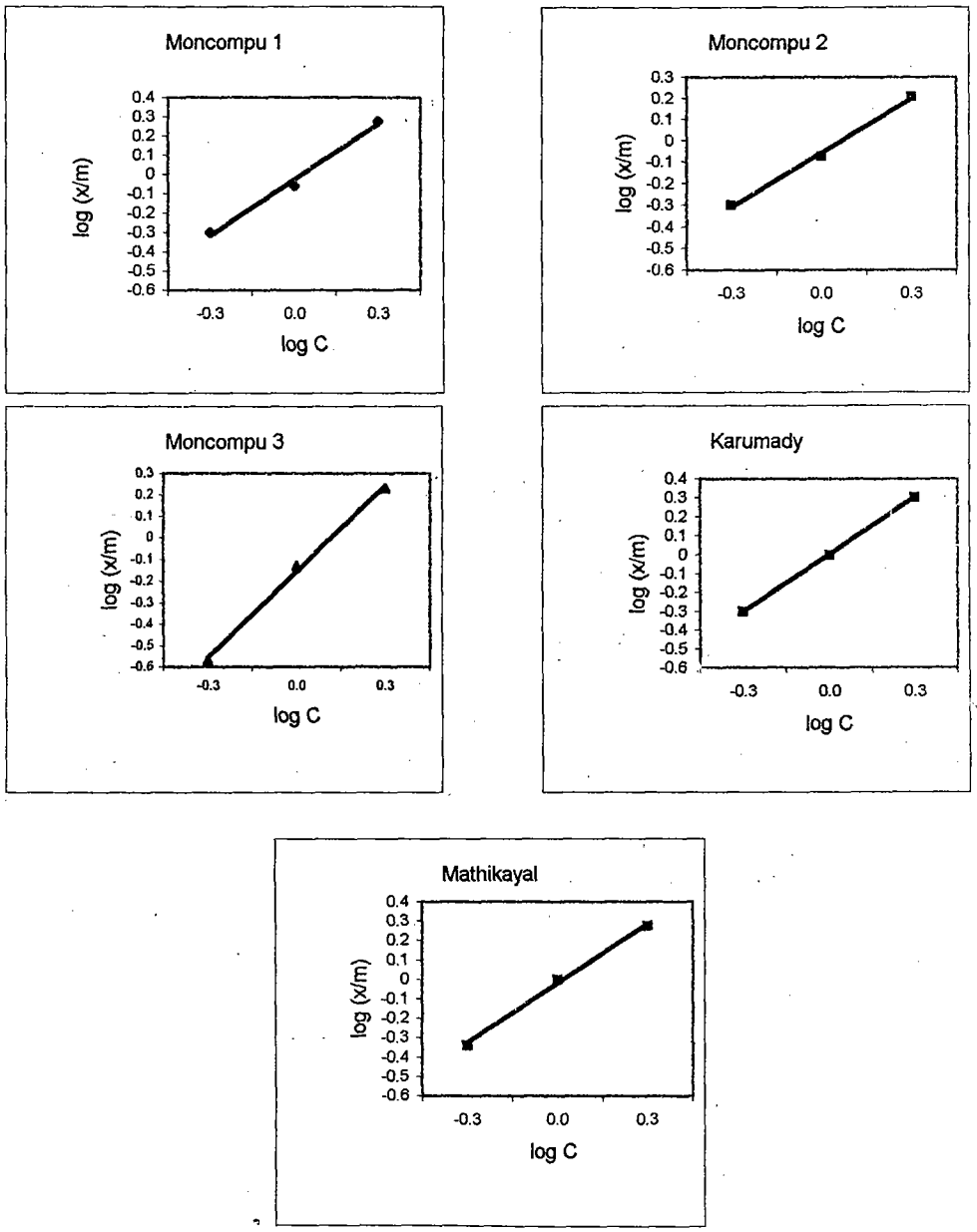
Adsorption isotherms were drawn by plotting the logarithm to base 10 of the equilibrium concentration of 2,4-D on the X-axis and the quantity of 2,4-D adsorbed per unit mass of soil on the Y-axis. Only one equilibration was selected for calculating the degree of adsorption of 2,4-D by the soils. The equilibration period at which maximum K (Freundlich isotherm constant K) and R^2 (regression coefficient) were obtained, was selected for each soil of the region and the respective isotherms are presented in Fig. 13. The figure indicated that 2,4-D adsorption in the soils under study followed Freundlich isotherm. The K values (degree of adsorption of 2,4-D) of the soils ranged from 3.62 (Koyalmannam) to 4.61 (Palakkad). The selected equilibration period and Freundlich isotherm constants, K and n for each soil of the region are presented in Table 17.

4.4.2 Soils of kuttanad region

The pattern of 2,4-D adsorption by the soil samples of kuttanad at 0.50 μg g^{-1} level of application was similar to that of kole soils (Table 15). Variation between soils was in the range of 0.20 to 0.50, 0.15 to 0.50 and 0.15 to 0.49 μg g^{-1} for 2, 4 and 6 hours respectively.

Table 15. Adsorption of 2, 4-D (μg g^{-1}) by the soils of kuttanad region charged at different levels at different period of equilibration

Quantity of 2,4-D added (μg g^{-1})	Soil	Quantity of 2,4-D adsorbed by the soil (μg g^{-1}) at		
		2 hrs	4 hrs	6 hrs
0.50	Moncompu 1	0.500	0.460	0.300
	Moncompu 2	0.500	0.150	0.150
	Moncompu 3	0.200	0.270	0.490
	Karumady	0.500	0.500	0.500
	Mathikayal	0.460	0.430	0.150
1.00	Moncompu 1	0.870	1.000	1.000
	Moncompu 2	0.840	0.870	0.950
	Moncompu 3	0.690	0.740	0.900
	Karumady	1.000	1.000	1.000
	Mathikayal	0.280	1.000	1.000
2.00	Moncompu 1	1.890	1.670	1.780
	Moncompu 2	1.600	1.760	1.770
	Moncompu 3	1.410	1.700	1.700
	Karumady	1.400	2.000	2.000
	Mathikayal	1.570	1.880	1.990



x/m - Quantity of 2,4-D adsorbed by unit mass of soil $\mu g\ g^{-1}$

C - Equilibrium concentration, $\mu g\ ml^{-1}$

Fig.14 Freundlich isotherm for adsorption of 2,4-D in the soils of kuttanad region

When the kuttanad soils were treated with 1.00 μg g^{-1} 2,4-D the reverse trend was observed i.e. quantity of 2,4-D adsorbed at six hours was greater than that of two and four hours. There was wide variation between the soils i.e., from 0.28 to 1.00 μg g^{-1} at 2 hours. At four and six hours the variation became narrow and the range in values were 0.74 to 1.00 and 0.90 to 1.00 μg g^{-1} respectively.

When 2,4-D was applied to kuttanad soils at a concentration of 2.00μg g^{-1}, quantity adsorbed increased with period of equilibration. A variation in the range of 1.40 to 1.89 μg g^{-1} was noticed at two hours. Variation at four and six hours were in the range of 1.67 to 2.00 μg g^{-1} and 1.70 to 2.00 μg g^{-1} respectively.

Adsorption isotherms for the different soils in this region at varying periods of equilibration were prepared. As mentioned in section 4.4.1. the period of equilibration was selected for calculating the degree of adsorption. Freundlich isotherm for each soil at the selected equilibration period is depicted in Fig.14. The K values (strength of adsorption of 2,4-D) of the soils ranged from 3.65 (Moncompu 2) to 9.02 (Moncompu 3). The selected equilibration period and Freundlich isotherm constants, K and n for each soil of the region are presented in Table 17.

4.4.3 Soils of kole region

At 0.50 μg g^{-1} level of application, the variation in the quantity adsorbed was in the range of 0.22 to 0.50 μg g^{-1} (Table 16). Anthikkad sample adsorbed lesser quantity (0.22 μg g^{-1}) at two hours. In this sample, adsorption increased

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Table 16. Adsorption of 2,4-D (μg g^{-1}) by the soils of kole region charged at different levels at different period of equilibration

Quantity of 2,4-D added (μg g^{-1})	Soil	Quantity of 2,4-D adsorbed by the soil (μg g^{-1}) at		
		2 hrs	4 hrs	6 hrs
0.50	Anthikkad	0.220	0.380	0.420
	Cherpu	0.440	0.370	0.420
	Kattoor	0.350	0.350	0.170
	Manalur	0.340	0.490	0.400
	Venkitengu	0.490	0.140	0.310
	Pidikkaparambu	0.500	0.500	0.310
1.00	Anthikkad	0.870	0.830	0.990
	Cherpu	0.800	0.820	0.950
	Kattoor	0.680	0.780	0.950
	Manalur	0.600	0.880	0.900
	Venkitengu	0.870	0.750	0.900
	Pidikkaparambu	0.560	1.000	0.960
2.00	Anthikkad	1.700	1.750	1.620
	Cherpu	1.740	1.570	1.820
	Kattoor	1.760	1.900	1.470
	Manalur	1.640	1.640	1.700
	Venkitengu	1.500	1.720	1.630
	Pidikkaparambu	1.670	1.900	1.790

with time (0.38 μg g^{-1} at 4 hours and 0.42 μg g^{-1} at 6 hours) while in the other soil samples, no definite trend was observed. The quantity adsorbed by the other soil samples varied from 0.14 to 0.50 μg g^{-1} at four hours and from 0.17 to 0.42 μg g^{-1} at six hours.

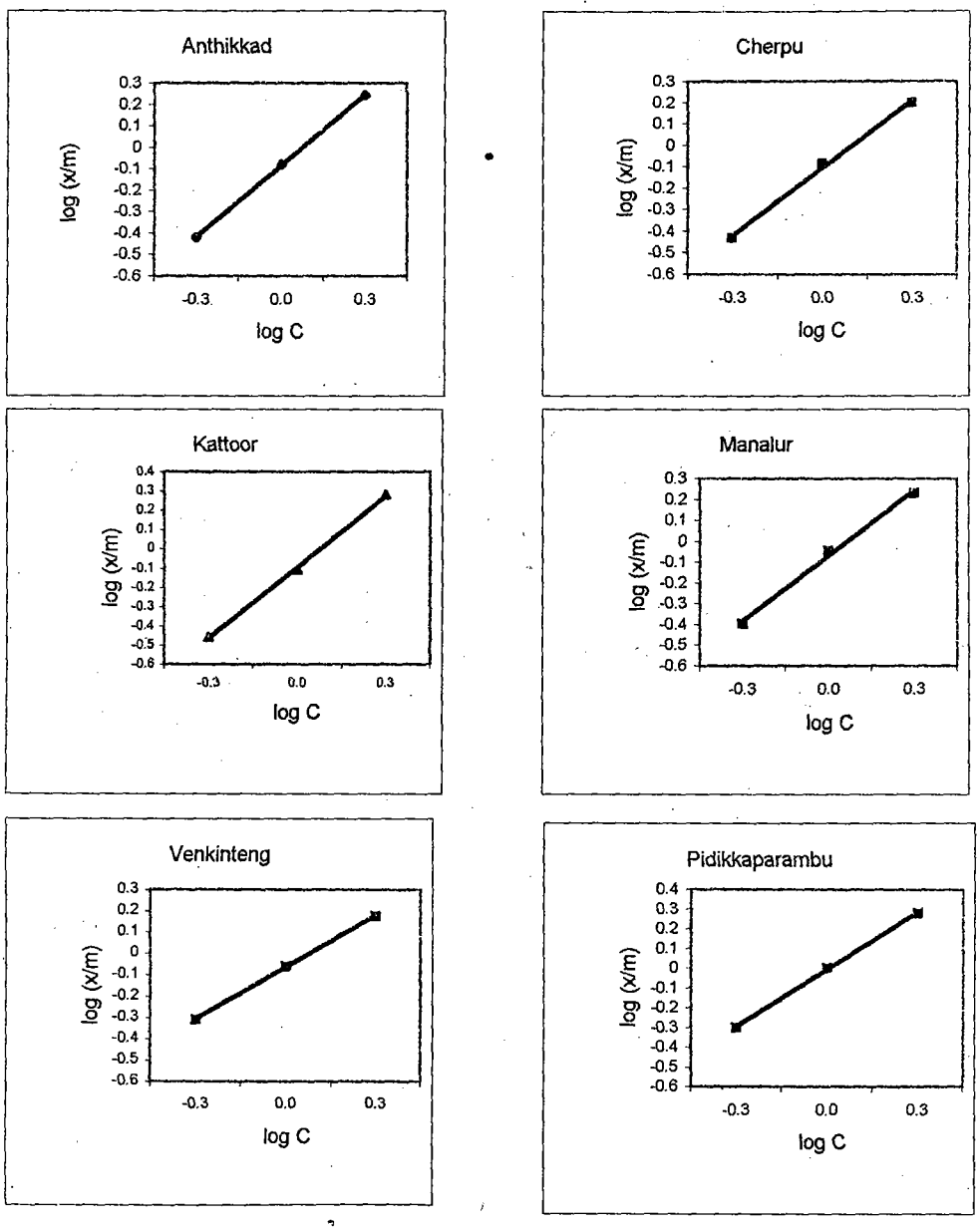
The degree of adsorption in all the soils increased with the level of application. At 1.00 μg g^{-1} level of fortification the quantity of 2,4-D adsorbed by the six soil samples varied from 0.56 to 0.87, 0.75 to 1.00 and 0.90 to 0.99 μg g^{-1} at 2, 4 and 6 hours respectively.

At the highest level of 2, 4-D application soils of kole region adsorbed 2,4-D in the range of 1.50 to 1.76 μg g^{-1}, 1.57 to 1.90 μg g^{-1} and 1.47 to 1.82 μg g^{-1} respectively for 2, 4 and 6 hours.

Adsorption isotherms for the 6 representative soils of the region at the selected equilibration period are presented in Fig. 15. The K values (strength of adsorption of 2,4-D) of the soils ranged from 3.55 (Venkitengu) to 6.75 (Kattoor).

The selected equilibration period and Freundlich isotherm constants, K and the exponent, n for each soil of the region are presented in Table 17.

Results of the adsorption study gave an indication that soils of the kole and kuttanad regions have greater capacity for the adsorption of 2,4-D. Soils of Palakkad region were poor in the adsorption of 2,4-D.



x/m - Quantity of 2,4-D adsorbed by unit mass of soil $\mu g\ g^{-1}$

C - Equilibrium concentration, $\mu g\ ml^{-1}$

Fig.15 Freundlich isotherm for adsorption of 2,4-D in the soils of kole region

Table 17. Freundlich isotherm constants and coefficients of determination (R^2) for adsorption of 2,4-D in the major rice soils of Kerala.

Rice growing region	Soil	Equilibration period selected (hrs)	K	n	R^2
Palakkad	Alathur	4	3.74	0.28	1.00
	Chittur	4	3.64	0.26	1.00
	Koyalmannam	6	3.62	0.27	1.00
	Nenmara	4	3.77	0.28	1.00
	Palakkad	4	4.61	0.31	1.00
kuttanad	Moncompu 1	2	4.03	0.29	0.99
	Moncompu 3	2	3.64	0.25	0.99
	Moncompu 3	4	9.02	0.40	1.00
	Karumady	4	4.00	0.30	1.00
	Mathikayal	4	4.28	0.31	1.00
kole	Anthikad	4	5.61	0.33	1.00
	Cherpu	4	5.43	0.31	1.00
	Kattoor	4	6.75	0.37	1.00
	Manalur	6	5.00	0.31	1.00
	Venkitengu	2	3.55	0.24	1.00
	Pidikkaparambu	4	3.86	0.29	1.00

4.5 Leaching and movement of 2,4-D in the soil columns

The study was conducted in 16 soil columns (five columns from each of Palakkad and kuttanad rice growing tracts and six columns from kole area) and the treatments consisted of three levels of 2,4-D viz., 1.00, 2.00 and 4.00 kg ha^{-1}. The results of the study conducted in different soils are presented below.

4.5.1 Leaching and movement of 2,4-D in the soils of Palakkad region

When the soil columns were treated with 2,4-D at 1.00 kg ha^{-1} concentration, Chittur and Koyalmannam soils had lower quantity of residues in the 0-10 cm layer (Table 18). These columns recorded residues, levels of 0.20 and 0.17 μg g^{-1} respectively while the other three soils contained higher quantity of 2,4-D residues which varied from 0.27 to 0.36 μg g^{-1}. At 10-20 cm depth, Alathur and Chittur soils recorded no residues. Residue content at this depth in Nenmara soil column was 0.07 μg g^{-1}. Koyalmannam and Palakkad soils had greater quantity of residue at 10-20 cm depth.

Regarding the movement of 2,4-D to the lower layers (> 20 cm depth), it was found that no detectable quantity is present in the leachate collected below the soil column (below the 20 cm layer).

The rate of movement of water through the soil columns was measured in ml min^{-1}. Among the five soil columns in Palakkad region, the sample collected from Palakkad area had shown greater rate of movement of water, (0.37 ml min^{-1}) and the lowest rate was shown by Alathur soil (0.04 ml min^{-1}) Chittur,

Table 18. Residues of 2,4-D ($\mu g\ g^{-1}$)in the soil columns of Palakkad region at various depths and at different rates of application

Quantity of 2,4-D applied to the soil column ($kg\ ha^{-1}$)	Soil	Residues of 2,4-D ($\mu g\ g^{-1}$) at depths		Residues of 2,4-D in the leachate ($\mu g\ ml^{-1}$)	Percolation rate ($ml\ min^{-1}$)
		0-10 cm	10-20cm		
1.00	Alathur	0.27	ND	ND	0.04
	Chittur	0.20	ND	ND	0.11
	Koyalmannam	0.17	0.12	ND	0.09
	Nemmara	0.36	0.07	ND	0.05
	Palakkad	0.31	0.15	ND	0.37
2.00	Alathur	0.43	0.08	0.07	0.08
	Chittur	0.25	0.36	0.05	0.23
	Koyalmannam	0.31	0.16	0.03	0.19
	Nemmara	0.60	0.09	ND	0.11
	Palakkad	0.60	0.21	ND	2.44
4.00	Alathur	0.45	0.35	0.09	0.08
	Chittur	1.02	ND	0.09	0.11
	Koyalmannam	1.26	0.23	0.10	1.60
	Nemmara	1.24	0.57	0.10	1.04
	Palakkad	0.51	0.25	0.10	1.27

ND – Not Detected

Koyalmannam and Nenmara samples recorded values of 0.11, 0.09 and 0.05 ml min^{-1} respectively.

When the soil columns were treated with 2,4-D at 2.00 kg ha^{-1} level, Nenmara and Palakkad samples had greater content of residues in the 0-10 cm layer. The lowest concentration was obtained in Chittur (0.25 μg g^{-1}) and Koyalmannam recorded a value of 0.31 μg g^{-1}. Similar results were obtained at 0.50 μg g^{-1} level also. In the next soil layer i.e. at 10-20 cm depth, Alathur and Nenmara recorded lower values viz., 0.08 and 0.09 μg g^{-1} respectively while Chittur, Koyalmannam and Palakkad samples had residue levels of 0.36, 0.16 and 0.21 μg g^{-1} respectively. Nenmara and Palakkad samples had no detectable level of residues in the leachate. In Alathur, Chittur and Koyalmannam, quantities of 2,4-D residues obtained in the leachate were 0.07, 0.05 and 0.03 μg ml^{-1} respectively.

While comparing the percolation rate in the different soil samples of Palakkad area, it was found that percolation rate was higher in the soil columns used for applying the treatment viz., 2.00 kg ha^{-1}. As the soil columns were taken from the field using PVC tubes certain degree of compaction would have occurred in soil columns used for the study. The soil columns used for the application of 2,4-D at 1.00 kg ha^{-1} level varied in the percolation rate in the range of 0.08 to 2.44 ml min^{-1}. Higher values were recorded by Palakkad soil and the lowest value was recorded by Alathur soil.

At 4.00 kg ha^{-1} level of application, Chittur, Koyalmannam and Nenmara samples had higher levels of residues in the 0-10 cm soil layer and the corresponding values were 1.02, 1.26 and 1.24 μg g^{-1} respectively. Alathur and Palakkad samples had lower residue levels (0.45 and 0.51 μg g^{-1} respectively). Linear increase in residue content at 0-10 cm depth with levels of fortification was obvious in the soil columns of Chittur, Koyalmannam and Nenmara.

At 10-20 cm soil layer, Nenmara soil column recorded higher residues (0.57 μg g^{-1}) compared to other soil columns. Chittur soil recorded nondetectable level of 2,4-D. Alathur, Koyalmannam and Palakkad columns recorded 2,4-D residues of 0.35, 0.23 and 0.25 μg g^{-1} respectively.

Quantities of 2,4-D residues found in the leachate in the soil columns at 4.00 kg ha^{-1} level of application were comparatively higher than that of the lower levels of application. The values varied from 0.09 to 0.10 μg ml^{-1} among the five soil columns. Higher levels of residues in the leachate were noticed in Koyalmannam, Palakkad and Nenmara samples.

Percolation rate of water in the soil columns under study (at 4.00) was in the range of 0.08 to 1.60 ml min^{-1}. Higher rate of percolation was noticed in Koyalmannam, Palakkad and Nenmara (1.60, 1.27 and 1.04 ml min^{-1}) respectively.

4.5.2 Leaching and movement of 2,4-D in the soils of kuttanad region

Five soil columns collected from kuttanad area consisted of three from Moncompu (which represented karappadam), one from Karumady (which represented kari lands) and one from Mathikayal (kayal lands). Quantity of 2,4-D residues retained by each soil layer (0-10 and 10-20 cm) were estimated at varying rates of 2,4-D application (1.00, 2.00 and 4.00 kg ha^{-1}). The degree of residue accumulation in the leachate and the rate of movement of water through each soil column were also estimated. The results are presented in Table 19.

At 1.00 kg ha^{-1}, the quantity of 2,4-D residues retained in the first soil layer (0-10 cm) showed a variation ranging from 0.26 to 0.40 μg g^{-1} between soils. Moncompu-1 registered the highest value of 0.40 and the lowest value of 0.26 was shown by Moncompu-3. Karumady and kayal columns registered 0.30 and 0.33 μg g^{-1} 2,4-D respectively in 0-10 cm layer.

In the 10-20 cm layer, soils differed widely in the content of 2,4-D residues. Soil columns of Karumady recorded no detectable level of 2,4-D in the 10-20 cm layer where as the other soils recorded values in the range of 0.11 to 0.17 μg g^{-1}. Moncompu-1 registered lowest value (0.11 μg g^{-1}) while Mathikayal represented the highest value (0.17 μg g^{-1}). Residues of 2,4-D were not detected in the leachate of any soil. Percolation rate was lowest in the soil columns of Karumady (0.03 ml min^{-1}) while the soil column of Mathikayal recorded higher rate of movement of water (2.82 ml min^{-1}). The average percolation rate of the five soil columns was 1.30 ml min^{-1}.

Table 19. Residues of 2,4-D ($\mu g\ g^{-1}$) in the soil columns of kuttanad region at various depths and at different rates of application

Quantity of 2,4-D applied to the soil column ($kg\ ha^{-1}$)	Soil	Residues of 2,4-D recovered ($\mu g\ g^{-1}$) at depths		Residues of 2,4-D in the leachate ($\mu g\ ml^{-1}$)	Percolation rate ($ml\ min^{-1}$)
		0-10 cm	10-20cm		
1.00	Moncompu 1	0.40	0.11	ND	2.00
	Moncompu 2	0.33	0.16	ND	0.22
	Moncompu 3	0.26	0.12	ND	1.41
	Karumady	0.30	ND	ND	0.03
	Mathikayal	0.33	0.17	ND	2.82
2.00	Moncompu 1	0.50	0.21	0.05	0.25
	Moncompu 2	0.50	0.20	0.05	0.25
	Moncompu 3	0.56	0.32	ND	1.00
	Karumady	0.78	ND	ND	0.01
	Mathikayal	0.64	0.38	0.04	1.92
4.00	Moncompu 1	0.78	0.31	0.15	2.00
	Moncompu 2	0.79	0.39	0.10	2.00
	Moncompu 3	0.92	0.51	0.05	0.67
	Karumady	1.00	ND	0.00	0.01
	Mathikayal	1.00	0.30	0.11	1.27

ND – Not Detected

At 2.00 kg ha^{-1} level of 2,4-D application, quantity of 2,4-D residues present in the soil columns at 0-10 cm depth was in the range of 0.50 to 0.78 $\mu g\ g^{-1}$. Moncompu-1, 2 and 3 recorded more or less similar values (0.50, 0.50 and 0.56 $\mu g\ g^{-1}$ respectively), while Karumady and Mathikayal had 0.78 and 0.64 $\mu g\ g^{-1}$ respectively. In the soil columns of Karumady, no residue was found in the 10-20 cm layer. The other four soil columns recorded residues ranging from 0.20 to 0.38 $\mu g\ g^{-1}$ of which Moncompu-2 showed the lowest value (0.20 $\mu g\ g^{-1}$) and the highest value (0.38 $\mu g\ g^{-1}$) was for Mathikayal.

2,4-D residues present in the leachate was very low, the average of the five columns being 0.03 $\mu g\ ml^{-1}$. Moncompu-3 and Karumady soils did not have detectable levels of 2,4-D residues in the leachate. Moncompu-1, 2 and Mathikayal had residues of 0.05, 0.05 and 0.04 $\mu g\ ml^{-1}$ respectively in the leachate.

At 4.00 kg ha^{-1} level of 2,4-D application Moncompu-1, 2 and 3 had residues of 2,4-D in 0-10 cm soil layer to a concentration of 0.78, 0.79 and 0.92 $\mu g\ g^{-1}$ respectively. Both Karumady and Mathikayal retained 1.00 $\mu g\ g^{-1}$ 2,4-D residue in the 0-10 cm soil layer. In the 10-20 cm soil layer, soil column of Karumady did not register 2,4-D residues to the detectable level Mathikayal had 0.30 $\mu g\ g^{-1}$ and Moncompu-1, 2 and 3 had 0.31, 0.39 and 0.51 $\mu g\ g^{-1}$ 2,4-D residues respectively in this soil layer.

In the leachate also, Karumady soil did not register any 2,4-D residue. The other columns viz., Moncompu-1, 2 and 3 and Mathikayal recorded 2,4-D residues to the extent of 0.15, 0.10, 0.05 and 0.11 μg ml^{-1} in the leachate.

Rate of movement of water through the soil columns was in the range of 0.01 to 2.00 ml min^{-1}. Soil column of Karumady registered 0.01 ml min^{-1} and Moncompu-1 and 2 registered 2.00 ml min^{-1}. Moncompu-3 and Mathikayal registered 0.67 and 1.27 ml min^{-1}. The average rate of movement of water in the five soil columns was 1.18 ml min^{-1}.

A strong relationship between the rate of movement of water through the soil column and the quantity of 2,4-D in the leachate was observed in the soil columns of kuttanad.

4.5.3 Leaching and movement of 2,4-D in the soils of kole region

Soil columns collected from Anthikkad, Cherpu, Kattoor, Manalur, Venkitengu and Pidikkaparambu were charged with different levels of 2,4-D viz., 1.00, 2.00 and 4.00 kg ha^{-1} and the residues recovered at various depths (0-10, 10-20 and >20 cm) are presented in Table 20.

At 1.00 kg ha^{-1} level of application, the quantity of residues retained in the 0-10 cm layer was in the range of 0.10 to 0.35 μg g^{-1}. The highest value of 0.35 was recorded by Pidikkaparambu and the lowest value of 0.10 was recorded by Cherpu. At 10-20 cm depth Manalur (0.00) and Kattoor (0.01 μg g^{-1}) registered lower residues. Residues of 2,4-D retained by Anthikkad, Cherpu, Venkitengu

Table 20. Residues of 2,4-D ($\mu g\ g^{-1}$) in the soil columns of kole region at various depths and at different rates of application

Quantity of 2,4-D applied to the soil column ($kg\ ha^{-1}$)	Soil	Residues of 2,4-D recovered ($\mu g\ g^{-1}$) at depths		Residues of 2,4-D In the leachate ($\mu g\ ml^{-1}$)	Percolation rate ($ml\ min^{-1}$)
		0-10 cm	10-20cm		
1.00	Anthikkad	0.31	0.13	ND	3.33
	Cherpu	0.10	0.18	ND	0.75
	Kattoor	0.29	0.01	ND	1.08
	Manalur	0.16	ND	ND	0.01
	Venkitengu	0.27	0.09	ND	0.15
	Pidikkaparambu	0.35	0.10	ND	0.20
2.00	Anthikkad	0.40	0.35	0.20	20.00
	Cherpu	0.42	0.31	0.14	20.80
	Kattoor	0.56	0.26	0.02	1.30
	Manalur	0.79	0.19	ND	0.07
	Venkitengu	0.79	ND	ND	0.02
	Pidikkaparambu	0.55	0.23	ND	0.25
4.00	Anthikkad	1.09	0.67	0.19	2.41
	Cherpu	1.44	0.36	0.20	5.88
	Kattoor	1.01	0.30	0.08	1.00
	Manalur	0.61	0.15	0.05	0.15
	Venkitengu	0.45	0.05	0.23	2.20
	Pidikkaparâmbu	1.30	0.42	0.15	3.00

ND – Not Detected

and Pidikkaparambu were 0.13, 0.18, 0.09 and 0.10 μg g^{-1} respectively. None of the soil columns recorded residues in the leachate (>20cm depth). Percolation rate was very low in the Manalur column (0.01 ml min^{-1}) and the other columns had a variation in the rate of movement of water in the range of 0.15 to 3.33 ml min^{-1}. Anthikkad column had higher percolation rate (3.33 ml min^{-1}).

At higher level of fortification viz., 2.00 kg ha^{-1}, soil columns of Manalur and Venkitengu recorded higher residues (0.79 μg g^{-1}) in the 0-10 cm layer. Anthikkad column had the lowest content of residues in the 0-10 cm layer (0.40 μg g^{-1}). Cherpu, Kattoor and Pidikkaparambu recorded residues of 0.42, 0.56 and 0.55 μg g^{-1} respectively in the 0-10 cm soil layer. When the residues were estimated in the 10-20 cm layer, it was found that Anthikkad and Cherpu had higher levels of residues in 10-20 cm layer. The same soils recorded higher levels of residues in the same layer at 1.00 kg ha^{-1} level of application also. Venkitengu soil column recorded no residues in the 10-20 cm layer. Kattoor, Manalur and Pidikkaparambu recorded residues to the extent of 0.26, 0.19 and 0.23 μg g^{-1} respectively.

In the leachate collected from Manalur, Venkitengu and Pidikkaparambu soil columns no residues of 2,4-D was observed. These columns had lower percolation rate (0.07, 0.02 and 0.25 ml min^{-1} respectively). Anthikkad, Cherpu and Kattoor recorded 0.29, 0.14 and 0.02 μg ml^{-1} respectively in the leachate. The corresponding percolation rate of water in these columns were 20.00, 20.80 and 1.30 ml min^{-1}.

The data on recovery of 2,4-D in different soil layers at 4.00 kg ha^{-1} level of application indicated that soil columns differ widely in their retention capacity and leaching behavior of 2,4-D. At 0-10 cm depth, Cherpu soil recorded higher residues (1.44 μg g^{-1}). Anthikkad, Kattoor and Pidikkaparambu soil columns also behaved similar to Cherpu soil in the retention of 2,4-D and the quantities retained by these columns in the 0-10 cm layer were 1.09, 1.01 and 1.30 respectively. Soil columns of Manalur and Venkitengu registered 2,4-D residues to the tune of 0.61 and 0.45 μg g^{-1} respectively. Anthikkad (0.67 μg g^{-1}), Cherpu (0.36 μg g^{-1}), Pidikkaparambu (0.42 μg g^{-1}) and Kattoor (0.30 μg g^{-1}) soil columns retained higher 2,4-D residues in 10-20 cm layer also. Manalur and Venkitengu had 2,4-D residues of 0.15 and 0.05 μg g^{-1} respectively in the 10-20 cm layer.

While studying the quantity of 2,4-D residues in the leachate (> 20 cm depth) it was found that Manalur (0.05 μg ml^{-1}) and Kattoor (0.08 μg ml^{-1}) had lower values, while Anthikkad, Cherpu, Venkitengu and Pidikkaparambu had 2,4-D residues of 0.19, 0.20, 0.23 and 0.15 μg ml^{-1} respectively.

When the rate of movement of water through the soil columns was studied it was found that Cherpu had the highest rate of percolation (5.88 ml min^{-1}) followed by Pidikkaparambu (3.00 ml min^{-1}). Anthikkad and Venkitengu had percolation rates of 2.41 and 2.20 ml min^{-1} respectively. Kattoor and Manalur had lower percolation rates (1.00 and 0.15 ml min^{-1} respectively).

The results indicated the direct relationship between percolation rate and leaching losses of 2,4-D from soil.

4.6 Persistence of 2,4-D in soil and rice plant under field condition

The study was programmed to assess the persistence of 2,4-D in wet land paddy and the extent of contamination of 2,4-D residues in rice plant, grain and straw. A representative paddy field of Trichur district (Plate 1) was selected and the experiment was laid out with five levels of 2,4-D. Residues of 2,4-D in the soil and crop produces at different periods were estimated.

4.6.1 Persistence of 2,4-D residues in the rice soil

The persistence of 2,4-D in the treated plots at different sampling intervals are presented in table 21. There was significant increase in the soil residue levels by increasing the level of application of the herbicide. Maximum quantity of 2,4-D was recorded on the same day of application for all treatments except T_3 which recorded highest value at 3 DAS. The residue levels at 0 DAS in the treatments T_2, T_4 and T_5 were 0.062, 0.439 and 0.957 $\mu g\ g^{-1}$ respectively. The treatment T_2 recorded 2,4-D residues to levels of 0.046, 0.039, 0.041 and 0.028 respectively on 1^{st}, 3^{rd}, 6^{th} and 9^{th} days after spraying. At the recommended level of 1.0 kg ha^{-1}, 2,4-D residues in soil at 1^{st}, 3^{rd}, 6^{th} and 9^{th} days after spraying were 0.125, 0.195, 0.012 and 0.086 respectively. At 2.00 kg ha^{-1}, the residue levels estimated in the soil samples were 0.264, 0.376, 0.157 and 0.153 $\mu g\ g^{-1}$ respectively for 1^{st}, 3^{rd}, 6^{th} and 9^{th} days after spraying. At 4.00 kg ha^{-1}, soil

Plate I. The experimental field

Table 21. Residues of 2,4-D in soil and rice plant parts at different stages and at different rates of application

Dosage of 2,4-D application (kg ha^{-1})	2,4-D residues in soil (µg g^{-1}) at different stages (DAS)*								2,4-D residues in plant (µg g^{-1}) at different stages		
	0	1	3	6	9	15	30	60	30 DAS	At harvest	
										Grain	Straw
T_1 - 0.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
T_2 - 0.5	0.062 (0.749)	0.046 (0.739)	0.039 (0.734)	0.041 (0.735)	0.028 (0.727	ND	ND	ND	ND	ND	ND
T_3 - 1.0	0.178 (0.822)	0.125 (0.790)	0.195 (0.831)	0.102 (0.775)	0.086 (0.765)	ND	ND	ND	ND	ND	ND
T_4 - 2.0	0.439 (0.966)	0.264 (0.873)	0.376 (0.933)	0.157 (0.810)	0.153 (0.806)	ND	0.0001	ND	0.0004	0.00015	ND
T_5 - 4.0	0.957 (1.192)	0.710 (1.086)	0.446 (0.969)	0.326 (0.908)	0.202 (0.836)	0.021	0.0011	ND	0.0007	0.00017	0.0025
CD (0.05)	0.175	0.160	0.101	0.124	0.072	+	+	+	+	+	+

Figures in parentheses indicate sqrt(x+0.5) transformed values

+ Not analysed statistically

*DAS – Days after spraying

*ND Not Detected

residues of 2,4-D were in the order 0.710, 0.446, 0.326 and 0.202 μg g^{-1} on the 1st, 3rd, 6th and 9th days respectively. Only the highest rate of application viz., 4.0 kg ha^{-1} resulted in residues detectable by colorimetric procedure at 15 days (0.021 μg g^{-1}). Levels of 2,4-D residues in soil were trace at 30 DAS (0.0001 and 0.0011 μg g^{-1}) even for the higher rates of application, of 2.00 and 4.00 kg ha^{-1} and were not detectable at 60 DAS. As the dose increased, the rate of degradation was found to be faster during the initial period.

4.6.2 2,4-D residues in the rice plant, straw and grain

When the whole rice plants were analysed for 2,4-D residues at 30 DAS stage, the residues were detected only at higher doses, the values being 0.0004 and 0.0007 μg g^{-1} for 2.00 and 4.00 kg a.i. ha^{-1} respectively (Table 21). The maximum levels recorded for rice grain and straw were 0.00017 and 0.0025μg g^{-1} respectively (T_5). In T_4 no residues were detected in the straw. The quantity of 2,4-D in rice straw was very much higher compared to grain (0.0025 μg g^{-1}) for the treatment T_5. This was much higher than the values recorded at PI stage also.

4.6.3 Effect of herbicides on weed control

Statistical analysis of the data on the weed count and dry matter production of weeds at 60 DAS did not show significant difference between treatments (Table 22). Even though there were a large number of grasses and sedges in the experimental plots difference between treatments was not significant on their dry matter content.

Table 22. Species wise count (no. m^{-2}) and total dry matter production (g m^{-2}) of weeds at 60 DAS in the experimental field

Dosage of 2,4-D application (kg ha^{-1})	*Cyperus* sp. (no. m^{-2})	*Fimbristylis miliaceae* (no. m^{-2})	*Oryza rufipgon* (no. m^{-2})	*Sacciolepis interrupts* (no. m^{-2})	*Isachne* sp. (no. m^{-2})	*Cynodon dactylon* (no. m^{-2})	Total (no. m^{-2})	Weed dry matter (g m^{-2})
T_1 - 0.0	2.50 (1.34)	8.75 (2.74)	0.25 (0.84)	2.75 (1.68)	0.75 (1.00)	0.00 (0.71)	15.00 (14.25)	9.25
T_2 - 0.5	1.50 (1.12)	2.50 (1.63)	0.00 (0.71)	1.00 (1.14)	0.00 (0.71)	0.00 (0.71)	6.25 (5.00)	13.15
T_3 - 1.0	0.00 (0.71)	6.50 (2.43)	0.25 (0.84)	0.00 (0.71)	1.5 (1.32)	0.50 (0.93)	7.50 (8.75)	5.48
T_4 - 2.0	0.00 (0.71)	1.00 (1.06)	0.25 (0.84)	1.00 (1.06)	1.25 (1.23)	0.00 (0.71)	2.75 (3.50)	11.23
T_5 - 4.0	1.00 (1.14)	6.00 (2.30)	0.00 (0.71)	1.25 (1.22)	0.50 (0.97)	0.50 (0.93)	9.25 (5.89)	8.63
CD(0.05)	NS	NS	NS	NS	NS	NS	NS	NS
CV (%)	74.53	27.81	27.59	34.84	29.05	36.21	43.72	109.08

Values in parenthesis indicate sqrt(x+0.5) transformed values

Table 23. Yield attributes and yield of paddy at various rates of 2,4 -D application

Dosage of 2,4-D application (kg ha^{-1})	Population of rice plants (no. m^{-2})	Total no. of tillers (no. m^{-2})	Productive tillers (no. m^{-2})	Height of plants (cm)	Grain yield (kg m^{-2})	Straw yield (kg m^{-2})
T_1 - 0.0	483.00	1565.70	808.38	65.78	0.495	1.813
T_2 - 0.5	383.25	757.18	787.20	57.75	0.452	1.875
T_3 - 1.0	398.25	953.20	902.30	59.55	0.520	1.875
T_4 - 2.0	382.75	957.50	875.38	63.88	0.570	1.875
T_5 - 4.0	469.50	959.70	1121.55	57.75	0.505	1.750
CD (0.05)	NS	NS	NS	NS	NS	NS
CV (%)	19.28	36.57	18.19	7.14	12.68	27.44

Data on the grain and yield attributes viz. height of the plant, number of total and productive tillers and yield were not significantly different between treatments (Table 23).

4.6.4 Effect of 2,4-D on soil microbial population

Influence of 2,4-D on the population of major soil microorganisms viz., bacteria and fungi was also studied in the field experiment. From the observations on the total number of bacteria per 10^{-6} g soil and fungal colonies per 10^{-4} g soil, changes in population of micro flora with 2,4-D levels (percentage increase/decrease over control) was worked out.

By taking the population in the control plot as 100 (normal) corresponding values in the treatment plots were calculated. Graphs were plotted using these values on the Y- axis and days after spraying on the X-axis (Fig.24).

The data on the total count of bacteria and fungi in soil at 3, 6, 15 and 30 days after herbicide application are given in Table 24. The herbicide had a negative influence on the population of soil bacteria. At 3 DAS, bacterial population has been reduced to a tune of –58.18 to –87.45 per cent. Increasing the levels of application of 2,4-D resulted in greater reduction of bacterial growth at 3rd and 6th DAS. In the plot where 4 kg 2,4-D per hectare was applied, bacterial population had been reduced to a tune of 89.50 per cent at 6 DAS. In the other three treatments (T_2, T_3 and T_4) the percentage reduction values were – 61.90, -73.50 and –84.20 respectively. The percentage reduction of bacterial

Table 24. Effect of 2,4-D on bacterial and fungal population in soil

Rate of 2,4-D application (kg ha^{-1})	Bacterial population (x 10^6 g^{-1})				Fungal population (x 10^4 g^{-4})			
	3 DAS	6 DAS	15 DAS	30 DAS	3 DAS	6 DAS	15 DAS	30 DAS
T_1 - 0.0	550 (100)	496 (100)	385 (100)	18.5 (100)	7.0 (100)	2.7 (100)	4.0 (100)	48.5 (100)
T_2 - 0.5	230 (-58.18)	189 (-61.90)	98 (-74.60)	28.5 (+54.05)	7.5 (+7.1)	12.0 (+344.44)	4.0 (0.00)	45.0 (-7.22)
T_3 - 1.0	104 (-81.10)	131 (-73.50)	187 (-51.40)	35.0 (+89.19)	9.0 (+28.6)	12.3 (+355.60)	5.0 (+25.00)	56.0 (+15.46)
T_4 - 2.0	82 (-85.10)	78 (-84.20)	87 (-77.40)	30.0 (+62.16)	6.0 (-14.29)	13.0 (+381.48)	6.5 (+62.50)	56.5 (+16.49)
T_5 - 4.0	69 (-87.45)	52 (-89.50)	59 (-85.0)	20.5 (+10.81)	5.5 (-21.43)	19.3 (+614.81)	20.5 (+412.50)	63.0 (+29.90)

Values given in parentheses indicate the % reduction / increase over control

* DAS – Days after spraying

population over control for T_5, at 15 days after spraying was 85.00 per cent and in the other treatments viz., T_2, T_3 and T_4 the corresponding values were –74.60, –51.40 and –77.40 per cent respectively. At 30 DAS there was an increase in the bacterial population at all levels of application of the herbicide. Percentage increase in bacterial count over control for the treatments T_1, T_2, T_3 and T_4 were 54.05, 89.19, 62.16 and 10.81 respectively indicative of the complete dissipation of the herbicide residues from soil.

The observation on the total number of fungal colonies showed a reverse trend of bacterial population for most of the treatments and intervals except at the lower doses of 2,4-D i.e. at 0.5 kg ha^{-1}. With an increase in the level of application of 2,4-D, there was a corresponding improvement in the growth of fungi. Even though a decline in the fungal population was observed at 3 DAS, a considerable increase in their number was seen at 6 DAS at higher doses. The percentage change in the population of fungi over control at this stage ranged from + 344% to + 615% depending on the rate of the application. This variation was reduced as the time advanced and the corresponding change was between -7.22 and + 29.9% at 30 DAS. Attainment of nearly normal level of fungi and bacterial population by 30 DAS could be attributed to the complete loss of residual effect of 2,4-D by various mechanisms.

Discussion

5. DISCUSSION

Four separate experiments were conducted at the College of Horticulture, Vellanikkara to assess the fate of 2,4-D in rice plant and the major rice soils of the state. The results obtained are presented in the previous chapter and the salient findings under each experiment are discussed in sections 5.3 to 5.6. Discussion with regard to physico-chemical characteristics of the soils and standardization of procedure for the estimation of 2,4-D are given in sections 5.1 and 5.2 respectively.

5.1. Physico-chemical characteristics of soils

All the soil samples were collected from representative rice growing tracts of the state. The samples were characterised by a high clay content which ranged from 24.60 to 74.00 %. Based on sand, silt and clay, the five samples of Palakkad and Pidikkaparambu of kole region could be classified under sandy clay. Soils of kuttanad and five samples of kole region were of clayey texture. Except Chittur soil, all the samples were highly acidic in reaction. Comparatively higher pH in Chittur samples could be attributed to low rainfall of the region and high base status of the soil (KAU, 1989). Clay content of Palakkad soils were lower than that of kole (except Pidikkaparambu) and kuttanad. The Palakkad soils are relatively old and are formed as a result of weathering of gneissic rocks. Kole and kuttanad soils are reclaimed lake beds. Due to higher clay and organic carbon content in kole (except Pidikkaparambu) and kuttanad soils cation exchange capacities were higher compared to soils of Palakkad

region. Anion exchange capacities were also higher in the soils of these two regions, as they had higher sesquioxide content. Sand content was very high in Pidikkaparambu sample and hence silica content also showed the same trend. Thus in most of the characteristics, kole and kuttanad soils showed resemblance, which reflected their similarity in origin. Both are alluvial belts formed of sediments. Similar observations in respect of physicochemical characteristics and morphology of kole and kuttanad soils has been reported by Johnkutty and Venugopal (1993)

From the data on physico chemical analysis of the sixteen soil samples of the three rice growing regions of Kerala, it would appear that soils of a particular rice growing tract differ in many soil characteristics, even if they showed resemblances in some of their properties. Therefore,the soil samples collected from a particular region could not be treated as a homogeneous group . Each soil was taken as an entity and ranking for each property was done in descending order. Bar diagrams were prepared for the major characteristics of all the soils under study (Fig.16a,16b and 16c) so as to make clear the above aspect.

It could be noticed from the above mentioned figures that differences in the organic mater content within a particular region was much more pronounced than any other soil property. Extreme variations in the organic carbon content among the soils of kuttanad region reflected the differences in their origin. Moncompu 1,2 and 3 are coming under the soil type, karappadam which are river borne alluvial soils. Mathikayal belongs to kayal soils and are reclaimed lake

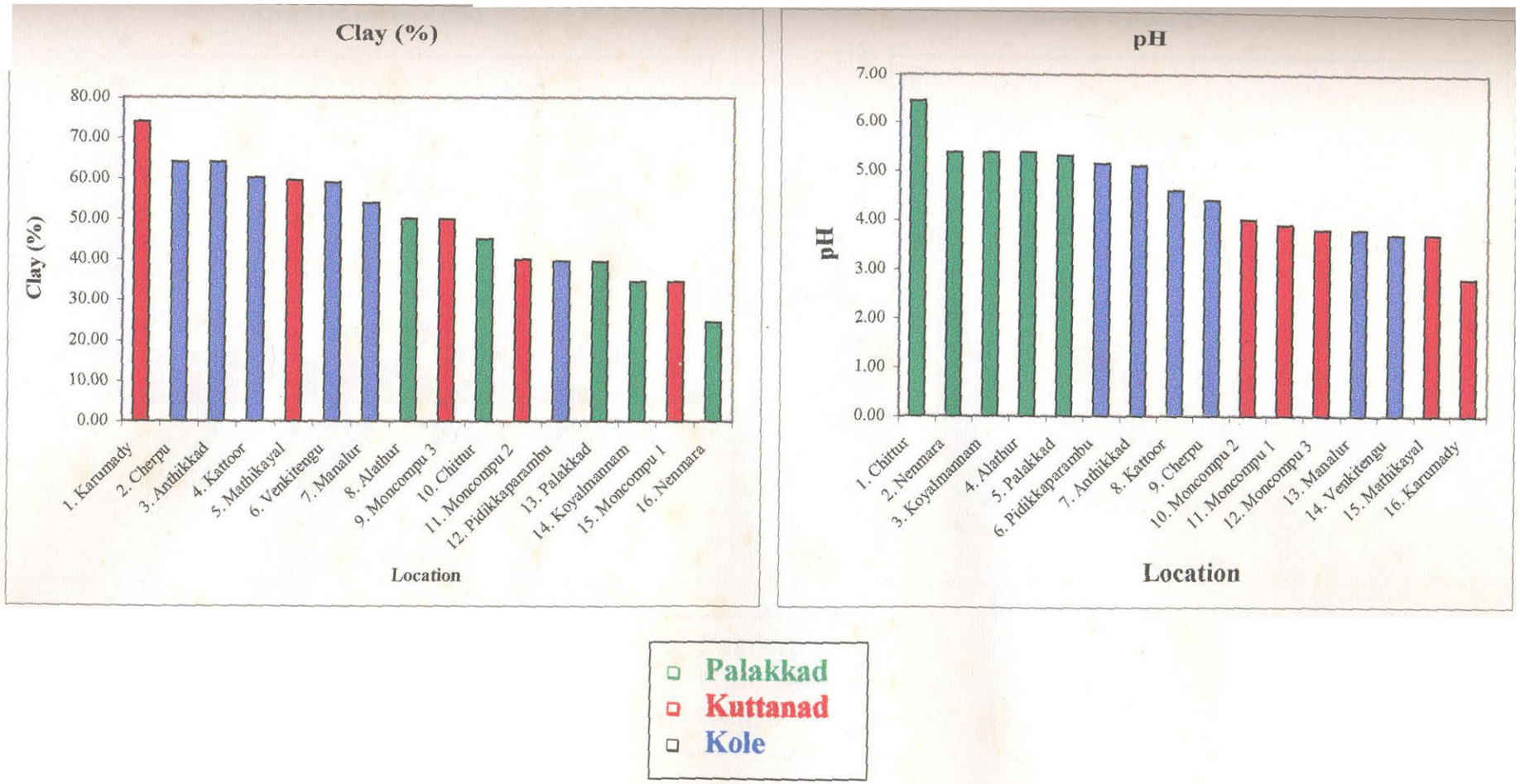


Fig. 16 a. Physico-chemical characteristics of the major rice soils of Kerala

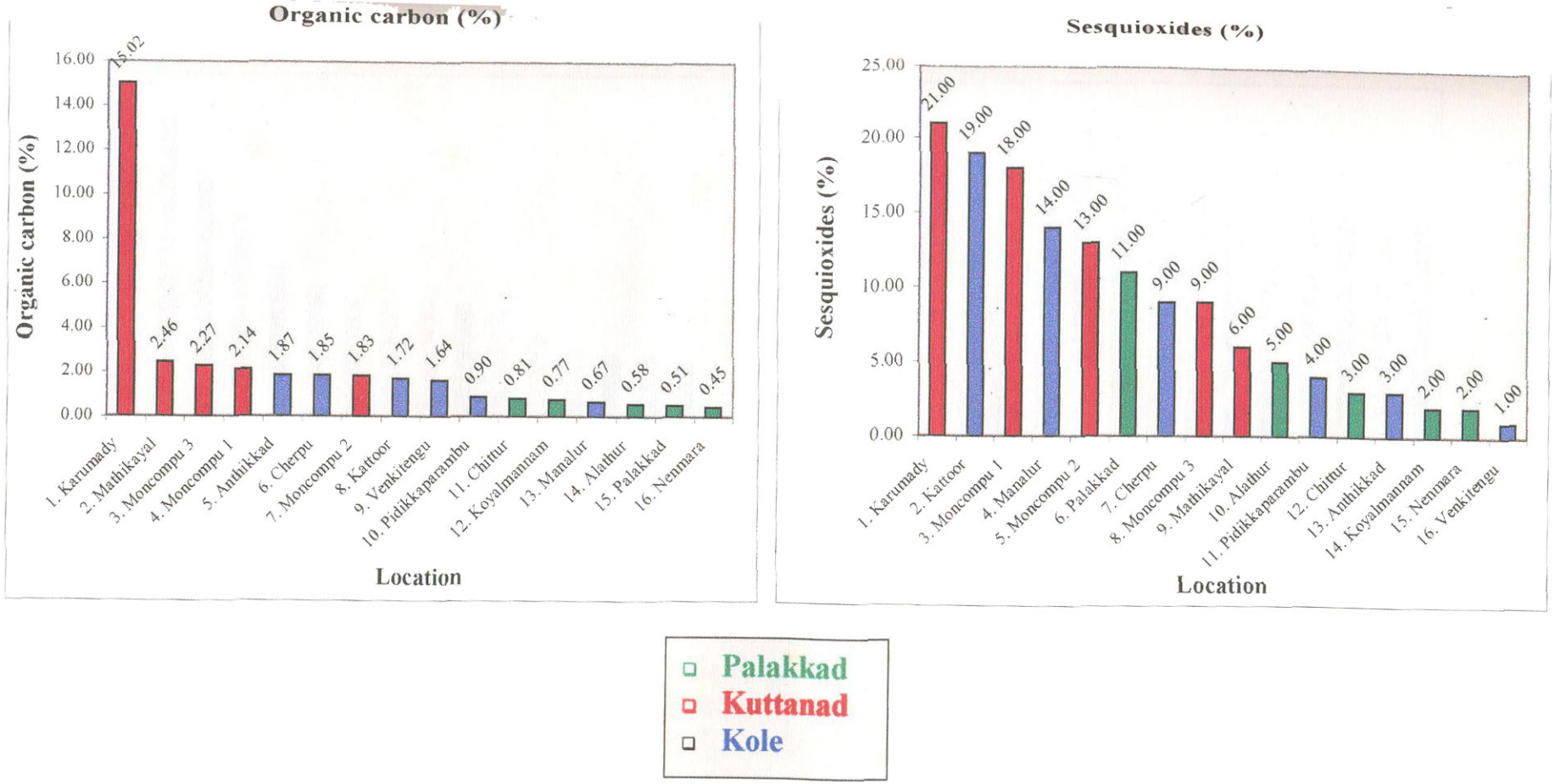


Fig. 16 b. Physico-chemical characteristics of the major rice soils of Kerala (contd.)

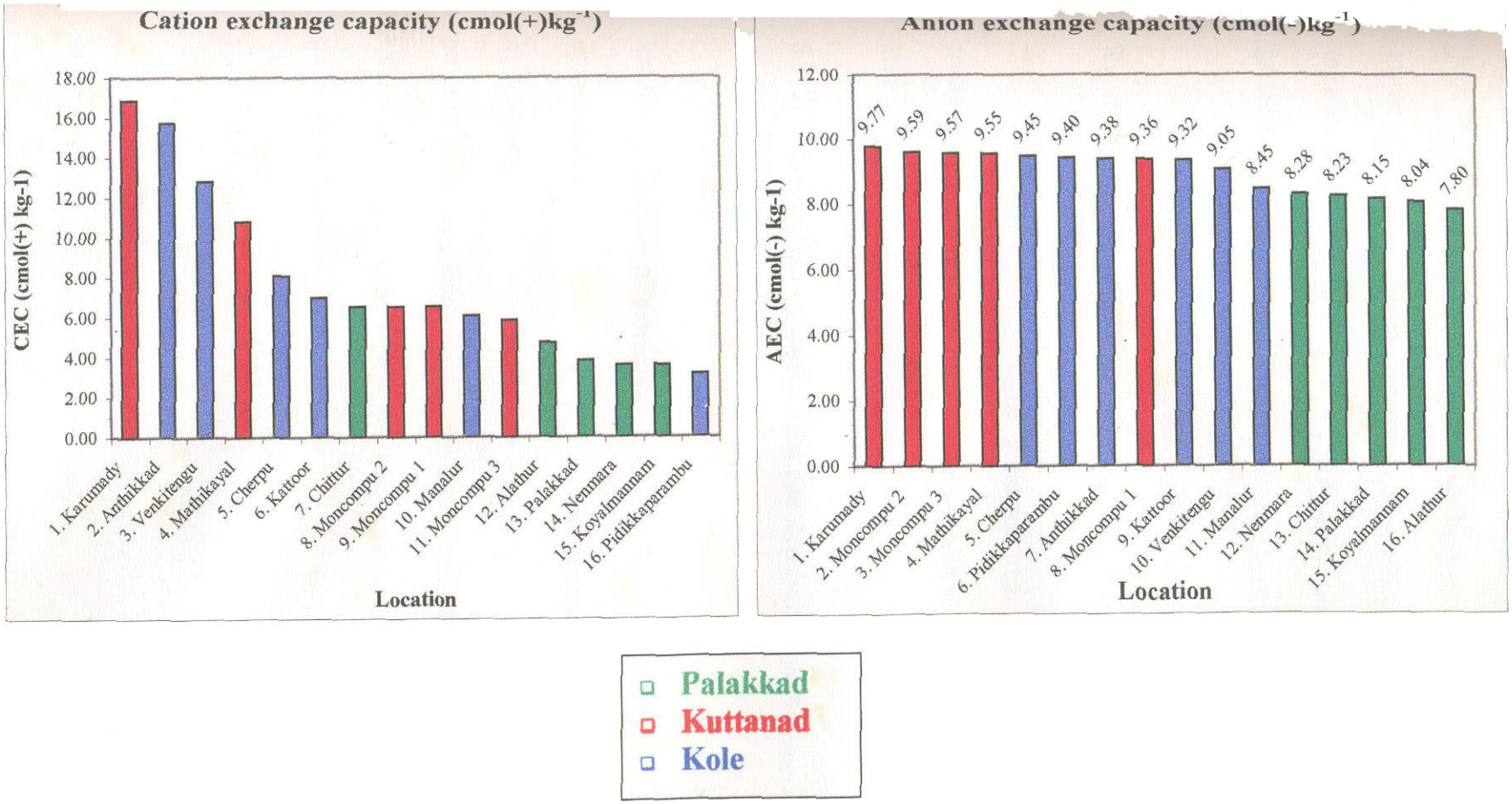


Fig. 16 c. Physico-chemical characteristics of the major rice soils of Kerala (contd.)

beds. Both karappadam and kayal lands have medium to high amount of organic matter. Karumady sample represents kari soil which exhibits characteristics of once submerged forest area. Top soil layer of this area is very often underlain by partially decomposed fibrous plant residues. (KAU,1984).

The other soil characteristics showing wide variations within the region are (i) sesquioxide content and (ii) cation exchange capacity

The above observations indicated that each soil sample should be considered separately while studying the behaviour of 2,4-D in the major rice soils of Kerala.

5.2 Standardisation of method for the determination of residues of 2,4-D in soil samples

Levels of herbicide may be very low in soil and its estimation involves lengthy procedures of extraction and clean up. The type of extraction and clean up procedures are decided by the instrumental technique with which the residue is proposed to be estimated. In the present study, selection of estimation procedure, standardization of extraction method, selection of clean up and validation of method for 2,4-D residue estimation are considered separately and summarized below.

5.2.1 Selection of estimation procedure

The observations made by Freed (1948) indicated clearly that in the aromatic acids only halogen derivatives of aryl oxy acetic acids react with chromotropic acid to give colour and the results of analysis conducted by

Marquardt and Luce (1955) also showed that the colour with chromotropic acid is sufficiently characteristic for the determination of 2,4-D. Keeping the above views in mind, the soil samples were taken from areas with no history of 2,4-D application, fortified with 2,4-D at 1.00ppm (1.00 μg per gram) level and proceeded for the standardization of extraction method.

5.2.2 Standardisation of extraction method

Soil samples collected from kole areas of Thrissur district were pooled and a representative portion was taken for the study.

Extractant proposed for colorimetric estimation of 2,4-D in milk, grain or seed by Marquardt and Luce (1951 and 1955) could not be directly employed for the estimation of 2,4-D residues in soil. In the initial step of extraction of 2,4-D from milk, the above scientists used ethylene glycol, sodium hydroxide and ether. These reagents were used to remove fat from milk. In the next step they used concentrated hydrochloric acid for separating proteins and phosphotungstic acid for precipitating soluble proteins. For extraction of 2,4-D from grain and seed, the above scientists proposed shaking the sample with the extractant viz., chloroform: ether: acetic acid (500:500:10) followed by extraction with the reagents in the following order: water → sodium hydroxide → chloroform → HCl → ether followed by NaOH → chloroform → concentrated HCl → phosphotungstic acid solution. Minute quantity of 2,4-D picked up by the plant and translocated to the grain could be estimated by this extraction procedure. In the case of soil, such a long extraction method is not required as fat, protein and

coloring materials do not form major components of soil. The analytical scheme illustrated by the above scientists actually provided a basic outline for the determination of residues of 2,4-D by colorimetry.

Many organic solvents and combinations of organic solvents and water have been proposed by earlier workers (Henkel, 1966; Purkayastha, 1974; Renberg, 1974; Khan, 1975; Smith, 1978). Extraction of phenoxys from soil is dependent on soil type and the presence of microorganisms etc. (QueeHee and Sutherland, 1981). As 2,4-D is a strongly polar herbicide, an extraction with a solvent of high polarity is required. Phenoxy alkanoic acids are anionic compounds and free acids are generally extracted from acidified media (Hammerstrand, 1976). The above factors were considered during standardization process.

A combination of seven extractants (organic and inorganic compounds of high polarity and mixture of organic solvents in acidic media) and two soil: extractant ratios were compared for efficiency of extraction of 2,4-D residues from soil.

After shaking the soil with the extractants for a period of 30 min., the extracts were filtered out. It was observed that the filtrate obtained from sodium hydroxide extraction (both concentrations) was deep yellow in colour which indicated that some compounds other than 2,4-D are also extracted from soil by sodium hydroxide. Among the other five extractants acetonitrile: distilled water: glacial acid (80: 20: 2.5) and chloroform: ether: acetic acid produced less intense

colour in the filtrate compared to that of the other three extractants viz., acetone, benzene and acetonitrile. This itself showed the superiority of the above two extractants in the estimation of residue. But conclusions could be arrived only after cleanup and recovery values calculated from absorbance readings noted on Spectronic 20 spectrophotometer.

5.2.3 Selection of clean up procedure

Cleanup is required to remove interfering substances and concentration of the residue. Even clear, colourless extracts can have large amounts of contaminants (Hammerstrand, 1976). Usually the clean up consists of partitioning of extract between a polar and non polar system. So in most of the earlier methods (Marquardt and Luce, 1951 and 1955; Renberg, 1974) organic solvent extraction was followed by alkaline hydrolysis → ether extraction → acidification → ether extraction. It has been reported that concentrated base is more efficient than concentrated acid for the hydrolysis of conjugates present in the matrix. The previous workers used *1N* sodium hydroxide for hydrolysis of conjugates and diethyl ether for partitioning. Concentrated hydrochloric acid was used for acidification and again diethyl ether was employed for partitioning. In the present study, the soil extracts were taken in separating funnels and 100 ml of *1N* NaOH followed by, 15 ml concentrated HCl and 50 ml diethyl ether was added and diethyl ether portion was separated. The next step consisted of adding 50 ml diethyl ether to the aqueous portion and the diethyl ether portion was separated and the ether extracts were pooled.

Literature on the recovery of 2,4-D from aqueous solution clearly mentioned that consistent recoveries of at least 99 % are obtained with diethyl ether even if the pH was adjusted by HCl or H_2SO_4 instead of phosphatic buffer (Bayer and Lump, 1973). 2,4-D is a weak acid with pKa 2.73 (Nelson and Faust,1969) and the free acid can be partitioned at pH below the pKa of free acid. Addition of concentrated HCl (15 ml) after alkaline hydrolysis served the above purpose.

During the next step, buffered extraction solution was added to remove acidic materials other than 2,4-D from the ether solution which was suggested by the previous workers. This was followed by acidification with 1 ml concentrated HCl and extraction two times each with 10 ml carbon tetrachloride. This procedure as mentioned above gave good recovery of 2,4-D at varying concentrations tried (Marquardt and Luce, 1951). The organic solvents and other chemicals employed for clean up of 2,4-D by the earlier workers were found satisfactory for estimation of 2,4-D acid from soil in this study also. However, the volume of solvent and the number of extractions required were quite large because the earlier procedure was meant for the estimation of 2 4-D from milk, grain and seed. For clean up of soil extracts of 2,4-D residues, no convenient method was available in the literature. Ideally the partitioning technique should utilize the smallest aqueous volume, minimum number of extraction steps and minimum volume of solvent. Partitioning value of >0.90 % are necessary before >95% of a pesticide can be extracted from water in fewer number of extractions and the minimum number of extractions should be five

(QueeHee and Sutherland,1981). Therefore, in the present study, an attempt was made to reduce the number of extractions and solvent volume as much as possible by suitably combining the procedures adopted by different workers (Marquardt and Luce, 1951 and 1955; Smith, 1978) without compromising the recovery of 2,4-D from the soil samples.

Among the seven extractants, both concentrations of NaOH (1 % and 4 % NaOH) produced much more recovery than the level of fortification of 2,4-D which pointed out that even after the clean up employed as in the case of other extractants, co extractives could not be separated and these two extractants were eliminated from the final selection. Among the other five extractants, acetonitrile: distilled water: glacial acetic acid (80: 20: 2.5) recovered about 94.00 % of the applied 2,4-D at a soil: solvent ratio of 1:4. This ratio was superior to 1:2 as it recovered 8.00 % more herbicide from soil.

5.2.4 Validation of the method

The method developed for estimation of 2,4-D residues from soil samples was validated by studying the recovery of 2,4-D from the soils collected from five representative locations, each of the three rice growing areas at varying levels of fortification. Fig.17 illustrates the recovery of 2,4-D averaged over the different levels of fortification (0.50,1.00 and 2.00 $\mu g\ g^{-1}$). Out of the 15 samples, 9 samples showed more than 80% recovery of the applied 2,4-D and only two samples had less than 60% recovery which indicated the suitability of the method to estimate 2,4-D residues from rice soils of Kerala. The recovery percentage

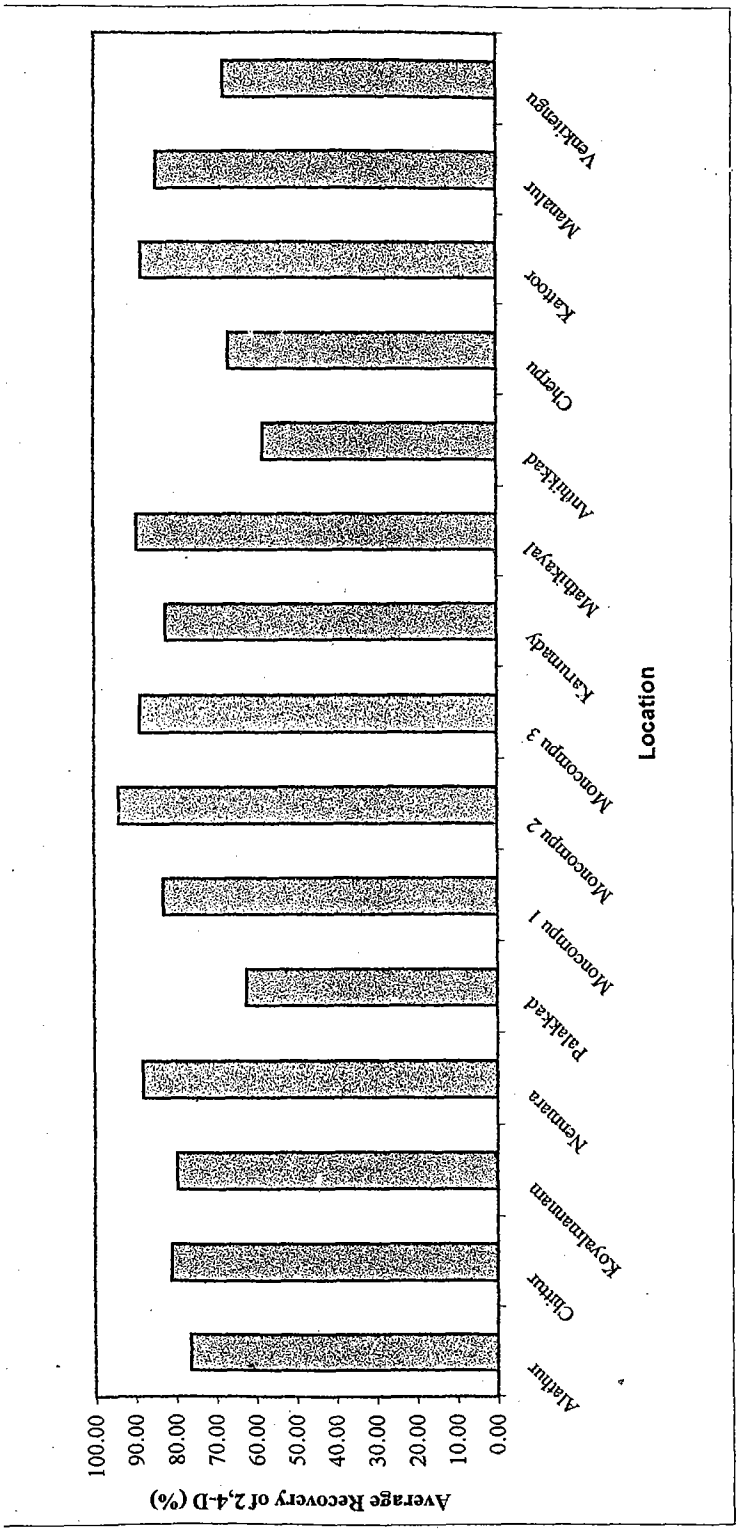


Fig 17. Average recovery of 2,4-D (%) in the soil samples collected from different locations

was more at 0.50 μg g^{-1} level of fortification compared to those at 1.00 to 2.00 μg g^{-1} levels (Fig.18). This could be explained in the following way.

2,4-D adsorption on soil components usually follow Fredundlich isotherm of the form $x/m = KC^n$, where x is the mass of 2,4-D adsorbed, m is the mass of soil, C is the equilibrium concentration of 2,4-D being adsorbed and K and n are constants. The constant, K indicates the strength of adsorption. When the equation is expressed in logarithimic form, a linear relationship of log (x/m) = log K + n log C was obtained. Normally within a reasonable range of pesticide concentration, the relationship between log (x/m) and log C is linear with n being constant (Giles *et al.*, 1960). Concentration effects on adsorption of 2,4-D were studied intensively by Watson *et al.* (1973). According to them anions were bound onto goethite at low concentrations so that carboxylate groups were weakly bound to positive sites with the hydrophobic aromatic ends directed towards the solution. At high concentrations, the anion orientation was reversed with the aromatic ring binding via π- π interaction (interaction between mobile electrons in the benzene ring of the adsorbent molecules and the adsorbent surface result in considerable molecular adsorption by Van der Waal's forces) with the first adsorbed layer and the surface reverting to its hydrophilic nature.

As the pH of the soils under study ranged from 2.8 to 6.40, both molecular and anionic forms of 2,4-D might be present in the soil (pKa=2.73) and the above mentioned concentration factor would have played a key role in the adsorption process.

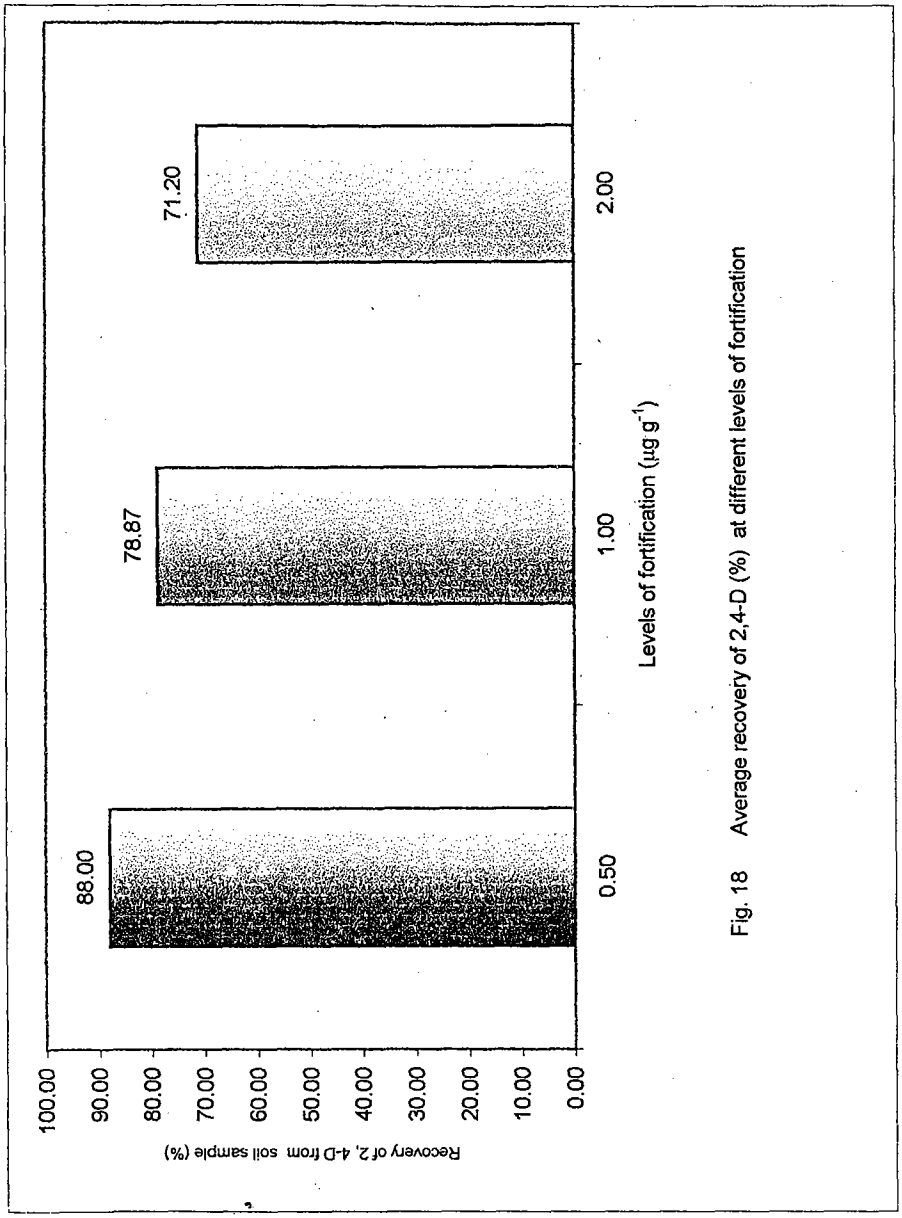


Fig. 18 Average recovery of 2,4-D (%) at different levels of fortification

2,4-D molecules are weakly bound to the soil at low concentration and are likely to be released into water (Harris and Warren, 1964). Higher recovery of 2,4-D from the soils under study at 0.50 μg g^{-1} level of fortification could be sufficiently interpreted based on the differences in the orientation of aromatic ring and side chain of the 2,4-D molecule.

Figure 18 also showed that the recoveries were 79.00 and 71.00 % for 1.00 and 2.00 μg g^{-1} levels of fortification respectively and it is concluded that the method is applicable for the determination of 2,4-D residues in soil samples of the laboratory experiments.

5.3 Studies on persistence and degradation of 2,4-D in the major rice soils of Kerala

Although 2,4-D is a post emergence herbicide, a large portion of the chemical applied on the foliage falls on the soil and are retained by soil particles. The period of time for which the herbicide remains in the active form is a factor determining its effectiveness and safety in weed control. A sound knowledge in this aspect helps to ascertain the long term effect of herbicide application on the soil and water environments.

Several mechanisms are responsible for the loss of phenoxy alkanoic acid herbicide residues from soil. Precipitation, evaporation, crop uptake etc. are the mechanisms of physical loss of herbicide from soil. They do not actually degrade the chemical. Photochemical, chemical and biochemical mechanisms are responsible for the degradation of herbicide molecules. Data generated so far on

2,4-D degradation pointed out that there is little breakdown of phenoxyacids in the absence of microbial activity and chemical degradation is very little (Rao, 1992). Photochemical degradation also would not be expected to be a major mechanism for the loss of phenoxy alkanoic acids from the soil, as these are applied on the foliage and herbicides reaching the soil surface will be partially protected from the sun's radiation by the crop canopy. Volatilisation is important in the case of ester formulation as it has higher vapour pressure. In the case of water soluble formulation of 2,4-D, run off and leaching will be the major pathways of dissipation.

Many studies have been conducted on the soil persistence of 2,4-D (De Rose and New man, 1947; Brown and Mitchell, 1948; Newman *et al.*, 1952; Burger *et al.*, 1962; Norris, 1966; Altom and Stritzke, 1973; Foster and Mckercher, 1973; Plumb *et al.*, 1977; Smith and Aubin, 1991; Sankaran *et al.*, 1993 and Cox, 1999). There is wide controversy on the persistence of 2,4-D in soils. Although the normal period of 2,4-D persistence was 2 to 4 weeks, detoxification varied from 14 to 94 days depending on the soil. Cox (1999) reported that persistence of 2,4-D is variable with half lives varying from 2 to 297 days.

Relatively little is known regarding the persistence of 2,4-D in soils of Kerala. The present investigation has provided some insight into the persistence of 2,4-D in the major rice soils of Kerala.

A detailed analysis of the magnitude of persistence of 2,4-D in the rice soils of Kerala was conducted by testing the data (Fig. 4 to 12) for correspondence to the first order kinetic equation of the form $C= C_0 e^{-kt}$ where C is the concentration of herbicide ($\mu g\ g^{-1}$) remaining at time t (days); C_0 is the initial concentration of the herbicide ($\mu g\ g^{-1}$) and k is the degradation rate constant.

Coefficients of determination obtained for the use of first order rate equation for describing the degradation of 2,4-D indicated that the degradation of 2,4-D in the soils of the three major rice growing regions followed first order kinetics and therefore it was possible to workout half life of the herbicide in these soils using the equation $t_{1/2}= 0.693/K$. Several scientists had reported that breakdown rate approximate first order kinetics below 10 ppm (Smith, 1978, Walker and Smith, 1979; Smith and Hayden, 1980 Mc Call *et al.,* 1981 Smith and Hayden, 1981; Sattar, 1982; Ou, 1984) whereas at higher concentrations upto about 500 ppm, degradation is biphasic with slow initial breakdown followed by an increased breakdown rate (Fournier *et al.*, 1981; Ou *et al.*, 1978; Payne and Fultz, 1947 and Parker and Doxtader, 1983). The data which gave significant R^2 value (>0.90 only) only were considered for calculating the half lives of the soils under study and the average half life over the three levels of fortification of 2,4-D was worked out and presented in Fig.19.

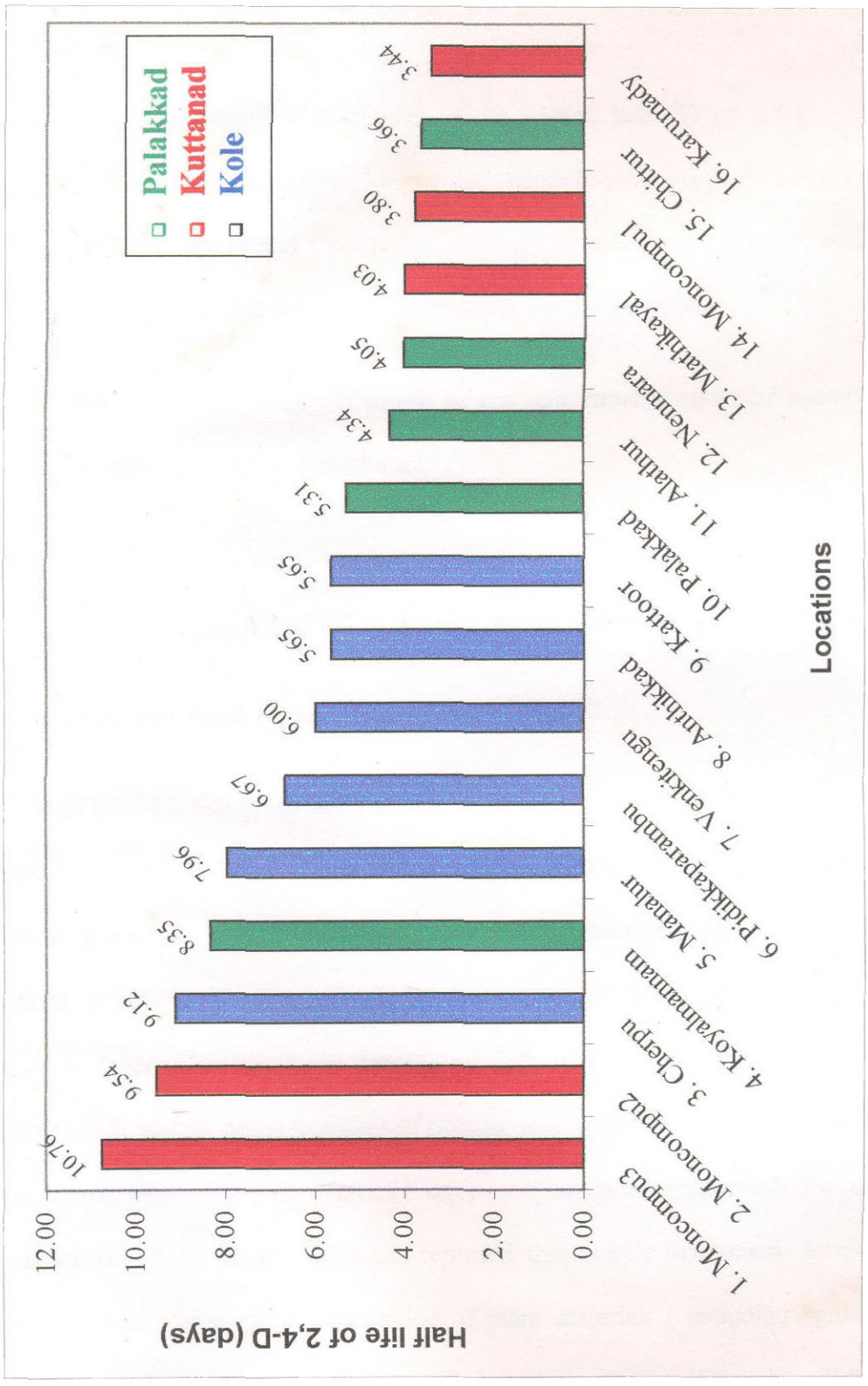


Fig. 19 Half life of 2,4-D (days) in the major rice soils of Kerala

5.3.1 Half life of 2,4-D in the major rice soils of Kerala

5.3.1.1 Soils of Palakkad region

Among the different soil samples of the region, half life of 2,4-D was lowest (Fig. 19) in Chittur sample (3.66 days). Higher values were recorded by Koyalmannam (9.01 days) and Palakkad (5.31days) samples. Possible reasons for the rapid degrdation of 2,4-D in Chittur sample are the following.

i) Due to near neutral pH (6.44) of the soil, more number of bacteria may be involved in degradation.

ii) Higher organic carbon content (0.81 %) in this soil compared to other soils of the region would have favoured the growth of soil microflora.

5.3.1.2 Soils of kuttanad region

Half life of 2,4-D in the soils of this region (Fig 19) varied from 3.44 days (Karumady) to 10.76 days (Moncompu 3). Extremely high organic carbon content of Karumady sample might have resulted in better multiplication of soil microflora especially of soil fungi. It has been reported that fungi belonging to the genera *Aspergillus niger* are capable of degrading 2,4-D (Rao, 1992). In addition to this, partial decomposition of organic matter present in the Karumady sample would have favoured microbial enzymatic activities responsible for the degradation of 2,4-D. several scientists reported that simple monomers formed during the initial stages of decomposition of plant materials (including lignin) are highly reactive than the stable humified organic matter (Stevenson,1985; Hedges,1988; Hatcher and Spiker,1988; Benoit and Barriuso,1997).

5.3.1.3 Soils of kole region

The degree of variation in half life between soil samples of this region was lower (Fig. 19) than that of Palakkad and kuttanad regions. This could be attributed to the homogeneity of samples in physicochemical properties especially in their clay content (39.60 to 40.00 %), while the clay content range from 24.60 to 50.10 % and 34.60 to 74.00 % for Palakkad and kuttanad regions respectively. Clay organic matter complex is the seat of microbial activity and the clay content would have played a key role in the availability of herbicide for degradation reactions.

5.3.2 Relationship between persistence of 2,4-D and soil characteristics

Correlation coefficients were worked out between soil properties and half life of 2,4-D in the major rice soils of Kerala and are given in Table 25. It was noticed that none of the soil properties had significant relationship with half life of 2,4-D. This could be due to the greater influence of some other factors on the rate of degradation of 2,4-D. Since kuttanad and kole soils are under submerged condition for major part of the year, the total number of microflora and the degree of humification of organic matter would be highly varying and these two factors would have significant effects on the degradation process.

As mentioned in section 5.1 the soils vary widely between the regions and within a particular region even though there are some similarities. In the

Table 25 Coefficients of correlation (r) between soil characteristics and half life of 2,4-D

Soil characteristics	Coefficients of correlation (r) with half life of 2,4-D
Sand %	0.146
Silt %	-0.109
Clay %	-0.095
pH	-0.248
Organic carbon %	-0.318
Cation exchange capacity cmol (+) kg^{-1}	-0.216
Anion exchange capacity cmol (-) kg^{-1}	-0.227
Sesquioxides %	-0.041
Silica %	-0.327
Maximum water holding capacity %	-0.028

degradation pattern of 2,4-D also the same trend was observed. Therefore, grouping of soils based on half life of 2,4-D was not possible.

The bar diagram (Fig 19) shows the rank order according to the half life values. Among the sixteen samples, the lowest value of 3.44 was recorded by Karumady sample of kuttanad and the highest value of 10.76 was also recorded by the soil sample of the same region (Moncompu 3). Wide variations in the organic matter and clay content between these two soils were not sufficient to give significant R^2 values with half life of 2,4-D .One of the explanations for the same could be the variations in the number and type of microflora present in the soils. Since the degradation of 2,4-D is mediated by microorganisms, this factor would have exerted greater influence than the soil properties.

From the above observations it could be concluded that the determination of soil properties alone is not sufficient to make predictions on the persistence of 2,4-D in the rice soils of Kerala.

5.4 Adsorption of 2,4-D by the soils

The importance of adsorption in herbicidal action is that it determines the availability of herbicide in the soil for plant uptake, the soil particles, its persistence in soil and also its movement to lower soil layers and to ground water. The degree of adsorption of a pesticide depends upon the nature and properties of the chemical itself, the kind and amount of adsorbents in the soil and the physico-chemical environment existing in the soil. There are conflicting reports

in the literature regarding the reversible nature of adsorption (Khan, 1974). Complete adsorption of a pesticide on organic surfaces (Nearpass, 1965), partial adsorption (Hance, 1967) as well as nearly complete irreversibility (Coffee and Warren, 1969) have been observed.

In the present study an attempt was been made to study the adsorption of 2,4-D in the 16 soil samples collected from major paddy growing areas of the state at three different levels of fortification (0.50, 1.00 and 2.00 $\mu g\ g^{-1}$) and at three periods of equilibration. At all the three levels of application (0.50, 1.00 and 2.00 $\mu g\ g^{-1}$ soil) major quantity of 2,4-D was adsorbed by the soils at two hours of equilibration. In general, the magnitude of adsorption increased at four hours of equilibration compared to two hours in the soils at different fortified levels of 2,4-D.

From the results obtained on the adsorption of 2,4-D in the major rice soils of Kerala, it was also found that the degree of adsorption was dependent on concentration of herbicide and period of equilibration. Higher degree of adsorption at higher concentration was a quite common phenomenon in the adsorption studies of many pesticides (Mandal and Adhikari, 1995; Bastin, 1996) and the same phenomenon was observed in the present study also. Reasons for the same has already been explained in section 5.2.

5.4.1.1 Soils of Palakkad region

The Freundlich isotherm constant K showed a variation in the range of 3.64 to 4.61 between soil samples of this region (Table 17). Comparatively lower pH and organic matter content of the soils of this region (Fig. 16a and 16b) would have reduced the adsorption of 2,4-D by these soils.

The highest value of 4.61 was recorded by Palakkad sample and this could be resulted from the higher sesquioxide content of this sample.

5.4.1.2 Soils of kuttanad region

The K values in the soil samples of this region ranged from 3.64 to 9.02 (Table17). Even though Karumady sample had a higher organic carbon content, the same trend was not reflected in the strength of adsorption of 2,4-D by the soil. This could be explained in the following manner.

Organic matter present in the soil consists of different components like humic acid, fulvic acid, humin etc. and their proportion varies depending on the degree of decomposition of organic matter. QueeHee and Sutherland (1981) reported that humic acid strongly adsorbed 2,4-D and the 'isosteric' heat of adsorption of 2,4-D for the humic acid / clay complex was approximately –2.00 to +2.00 k cal mol^{-1} while the corresponding values were –1.20 to –0.30 k cal mol^{-1} for fulvic acid. Humic acid is formed at later stages of decomposition of organic matter, whereas fulvic acid is a product formed during the early stages of decomposition. Lower k values observed in Karumady sample could be

attributed to lower degree of humification. These soils are always under submerged condition and they are underlain by blocks of wood and organic matter of varying stages of decomposition.

5.4.1.3 Soils of kole region

The range in K values between samples was 3.55 (Venkitengu) to 6.75 (Kattoor) (Table 17). As in the case of Palakkad region the major difference between the two soils was in their sesquioxide content. Venkitengu sample had very low sesquioxide content (1.0 %) while the Kattoor sample recorded a high value (19.00 %) for the same.

The results suggested that sesquioxide content of the soil would have influenced the degradation of 2,4- D.

5.4.2 Relationship between soil properties and adsorption of 2,4-D

Correlations were worked out between soil characteristics and adsorption of 2,4-D (Table 26). Lack of significance in the R^2 values indicated that the wide variations in the soil properties would have exerted varying influence on the degree of adsorption of 2,4-D. It seems that adsorption of 2,4-D is a function of combination soil properties, rather than that of a specific soil characteristic.

Figure 20 illustrates the rank order of each soil of the three rice soils of Kerala, with respect to their capacity to adsorb 2,4-D. Moncompu 3 of kuttanad was ranked as 1 and Venkitengu of kole region was the last in the rank order.

Table 26 Coefficients of correlation (r) between soil characteristics and degree of adsorption (Freundlich isotherm constant, K) of 2,4-D

Soil characteristics	Coefficients of correlation (r) with degree of adsorption of 2,4-D
Sand %	-0.151
Silt %	-0.157
Clay %	0.288
pH	-0.212
Organic carbon %	-0.028
Cation exchange capacity cmol (+) kg^{-1}	0.025
Anion exchange capacity cmol (-) kg^{-1}	0.350
Sesquioxides %	0.251
Silica %	0.029
Maximum water holding capacity %	0.341

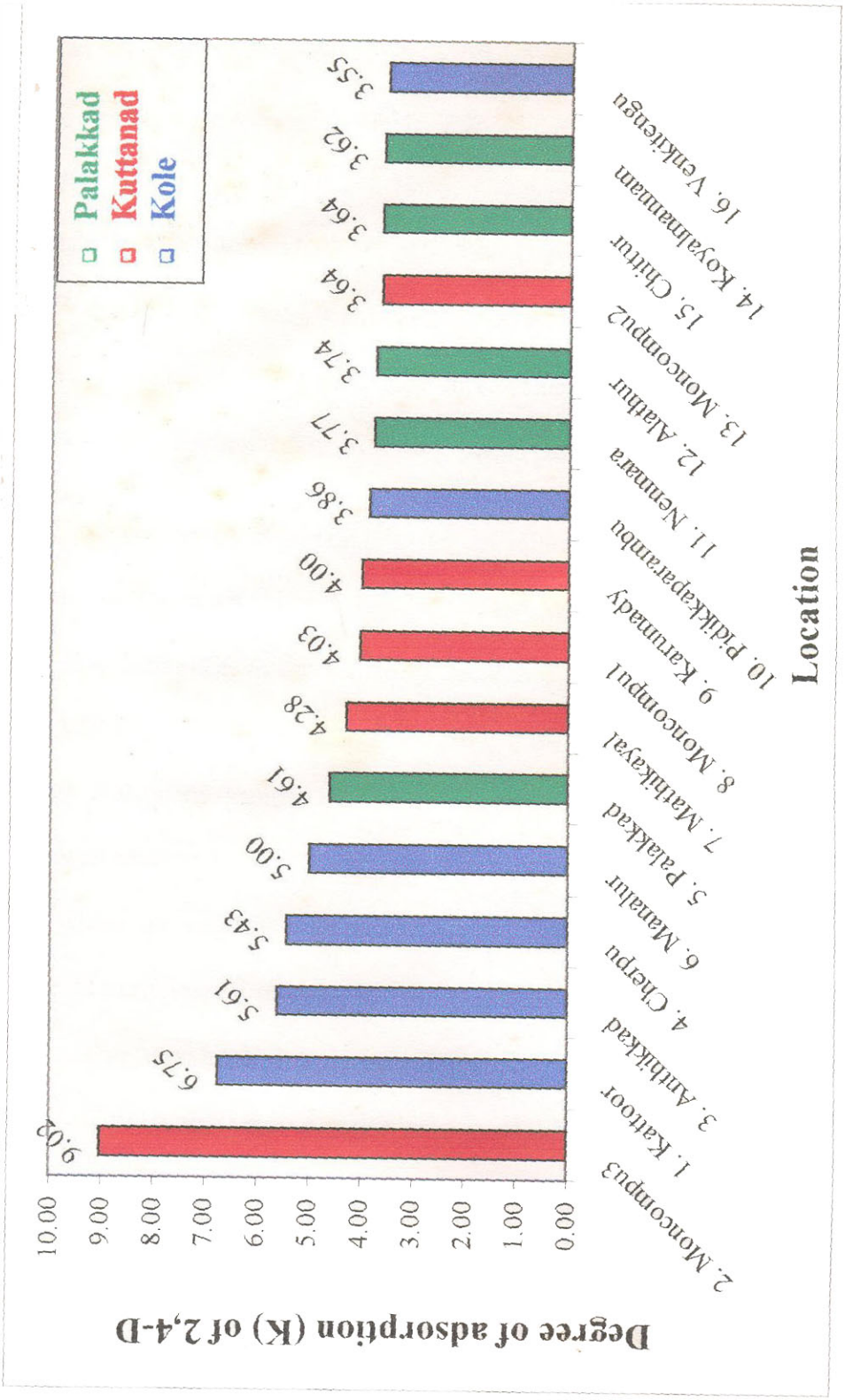


Fig. 20 Degree of adsorption (K) of 2,4-D in the major rice soils of Kerala

Although organic matter has been implicated in the sorption of 2,4-D in soils as reported by previous workers (Sherburns and Freed, 1954; Sheets *et al.*, 1962; Dubey and Freeman, 1964; Talbert and Fletchell, 1965; Hance, 1965; Nearpass, 1965; Obein *et al.*, 1966; Upchurch *et al.*, 1966; Hamaker and Thompson, 1972; Karickhoff *et al.*; 1979; Grover *et al.*, 1985; Mc Grath, 1994 and Baskaran *et al.*,1996) the magnitude of its influence was not considerable in the study. There was no difference in K values between soils of low and high organic matter contents. The following points may be considered in this regard.

Since hydrogen bonding plays an important role in the adsorption of organic molecules (Kohl and Taylor, 1961; Mortland and Meggitt, 1966), it is likely that differences in the degree of decomposition of organic matter will make much variations in the number of reactive groups available for hydrogen bonding. Though Karumady sample had very high organic carbon content no better K value was observed. This is because the soil sample is a representative of kari lands where the organic matter is in varying stages of decomposition. As the degree of humification of organic matter decreases, a corresponding reduction in the K value was implied in the pesticide adsorption (Hamakar and Thomson, 1972). This factor takes a major role on 2,4-D adsorption under low pH as prevailing in Karumady merely because of the presence of the herbicide in molecular form and the adsorption through hydrogen bonding.

The findings of the studies on adsorption of 2,4-D in the major rice soils of Kerala can be summarized in the following way.

1) Adsorption of 2,4-D in the major rice soil of Kerala followed Freundlich isotherm i.e magnitude of adsorption increased with concentration.

2) Prediction of degree of adsorption of 2,4-D in the rice soils of Kerala is a complex process, as it is decided by a large number of factors.

5.5 Leaching and movement of 2,4-D in soil

Movement of herbicide within the soil profile is influenced by many factors such as chemical nature of herbicide, the adsorptive capacity of soil and the amount of water available for downward movement through the soil. So the same factors of adsorption work in reverse for leaching (Coffee and Warren, 1969). As early as 1964 it has been reported that percolating water appeared to be the principal means of movement of water soluble compounds (Hartley, 1964). Sodium salt of 2,4-D is the most common herbicide in paddy fields and it is highly soluble in water (4.5 g per 100 ml at 25^0C). In Kerala rice is grown mostly under submerged condition and percolation of water is a continuous process in these soils. Report of Lavy *et al.* (1973) had shown that 2,4-D was leached down as deeply as 90 cm. If so, there is a potential danger of surface and ground water contamination with this herbicide. In order to understand the leaching behaviour of 2,4-D in rice soils of Kerala, column leaching study was conducted by taking soil columns from paddy fields of the 16 representative locations, 2,4-D was applied on the top of the soil columns and water was added to pass through the soil columns of 20 cm length. Leachate (50ml) was collected in a beaker kept below the soil column. Columns were cut at 10 cm and 20 cm

length, and residue content in the 0-10 cm and 10-20 cm layers and in the leachate was estimated by colorimetry.

5.5.1 Leaching pattern of 2,4-D in the soils of Kerala

5.5.1.1 Soils of Palakkad region

In all the five soil samples quantity of 2,4-D retained in 0-10 cm and 10-20 cm soil layers and in the leachate increased with concentration. Twenty three (Alathur) percent to seventy two percent (Nenmara) of the applied 2,4-D retained in the 0-10 cm layer depending on concentration and type of soil under study (Fig 21). Retention of 2,4-D in the 10-20 cm layer also varied (ND to 36.00 percent) widely between soil types and concentration. No residue was found in the leachate at the lowest level of application. Differences in soil characteristics were not sufficient to explain the variations in the retention of 2,4-D in the soil layers at varying depths. One of the reasons for the above could be attributed to the degradation of chemical by microorganisms. Since 2,4-D is degraded rapidly, the time during which water is allowed to percolate through the column may be sufficient for degradation of the chemical. Percolation rate varied from 0.04 to 2.44 ml min^{-1} which showed that the time taken to collect the 50 ml leachate varied from 20.49 min to 20.83 hours. The Palakkad soil column used for applying the treatment Viz. 1.0 kg 2,4-D ha^{-1} recorded a percolation rate of 2.44 ml min^{-1}. It registered the highest percolation rate as well as higher recovery of 2,4-D in both 0-10 cm and 10-20 cm soil layers. The Alathur sample which registered the lowest percolation rate (0.04 ml min^{-1}) recovered low quantity of 2,4-D from the soil layers.

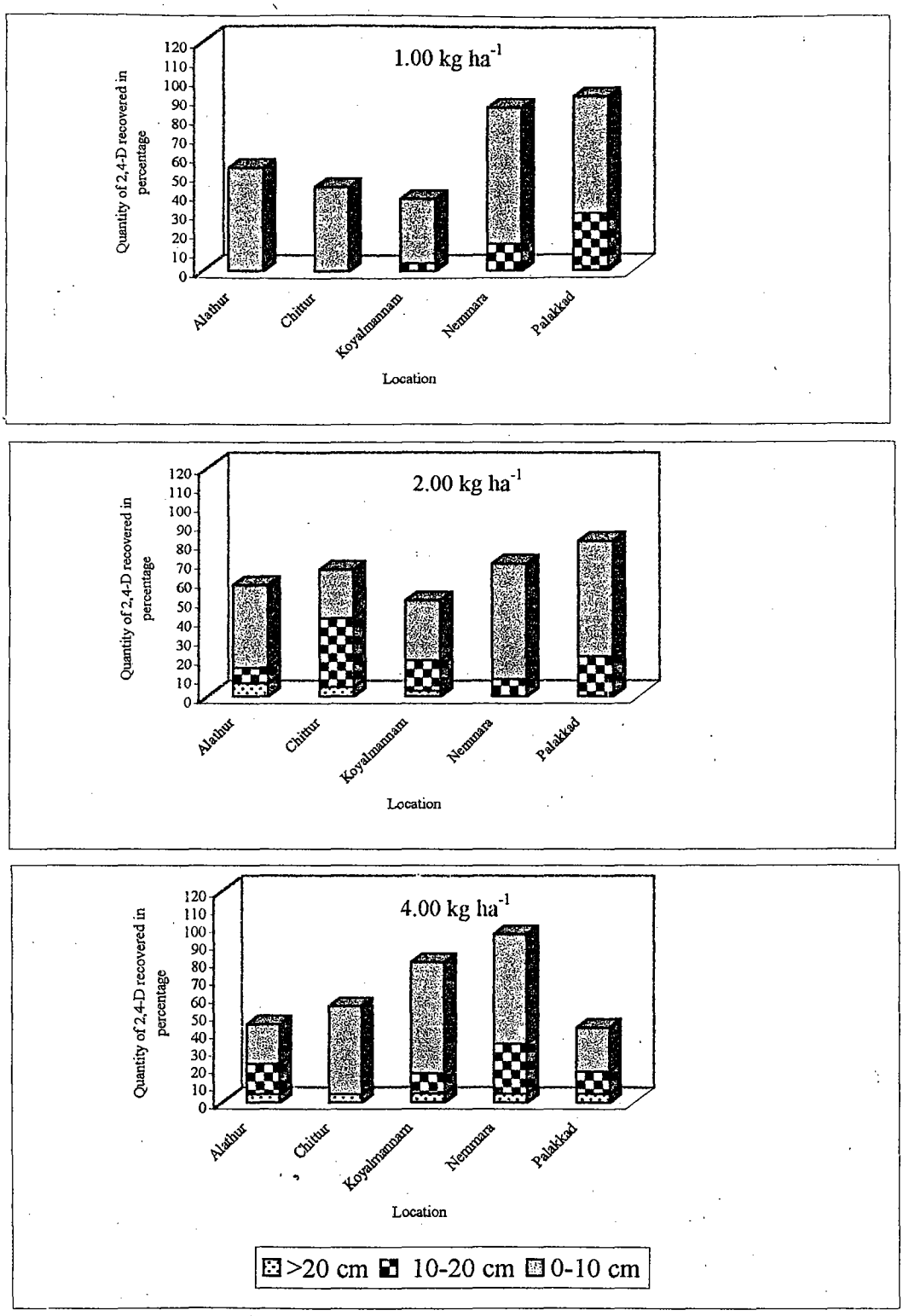


Fig.21 Depth wise distribution of 2,4-D residues in Palakkad soils at different rates of application (kg ha^{-1})

It could be inferred that in a compacted soil, leaching of 2,4-D is restricted not only by the effect of rate of movement of water but also due to the degradation of chemicals by micro organisms. The extent of leaching of 2,4-D through the soil columns of this region was comparatively lower when compared to the soils of kuttanad and kole regions. A maximum of 5.00 to 7.00 percent of the applied 2,4-D may reach upto 20 cm depth depending upon the rate of movement of water through the soil.

5.5.1.2 Soils of kuttanad region

Among the different soil columns of this region, Karumady sample registered no 2,4-D residue in the 10-20 cm layer and leachate (Fig 22). As discussed in the case of Palakkad soils, microbiology of the soils has played a key role in the movement of 2,4-D through the soil columns of this region. Karumady sample registered an extremely high organic matter content and hence microbial degradation of 2,4-D would have taken place at a faster rate. Laboratory studies on the persistence of 2,4-D in the soils of this region (section 5.3.1.2) had shown that rate of degradation of 2,4-D in the soils of this region is very high with a half life of 3.44 days. The findings made it very clear that, leaching pattern of 2,4-D is influenced by three major factors viz., soil type, rate of percolation of water and number of microflora present in the soil. The leaching pattern of 2,4-D in the soils of kuttanad region is similar to that of Palakkad region i.e only 5.00 to 7.00 percentage of the applied herbicide may reach upto a soil depth of 20 cm.

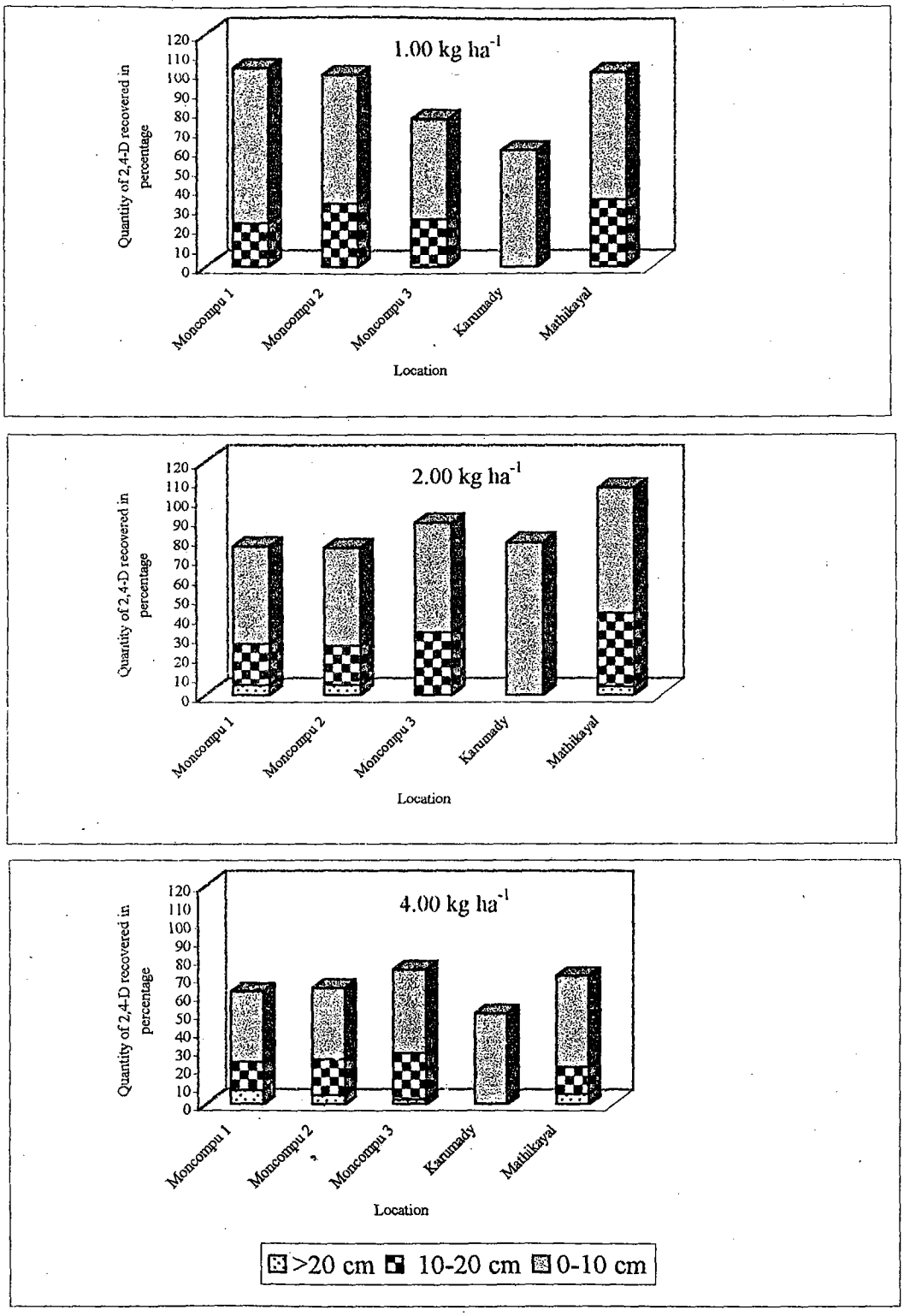


Fig.22 Depth wise distribution of 2,4-D residues in kuttanad soils at different rates of application (kg ha^{-1})

5.5.1.3 Soils of kole region

While comparing the retention 2,4-D in the 0-10 cm and 10-20 cm soil layers of kole region, it was found that 20.00 to 79.00 per cent of the applied 2,4-D remained in the upper layer (Fig. 23) and this variation is attributed to differences in rate of application, soil type and rate of movement of water through the soil. In the lower layers (10-20 cm) no residues was found in the Manalur and Venkitengu columns due to the extremely lower rate of movement of water (0.01 ml min^{-1}). Among the soil columns studied, maximum recovery of 2,4-D in the 10-20 cm layer was registered by Anthikkad and Cherpu columns (60.00 to 70.00 %). Water movement through the columns was at a very high rate especially in the columns used for applying 2,4-D at the rate of 1.00 kg ha^{-1} which resulted in almost 100.00 per cent recovery of the applied 2,4-D from different layers.

From the column leaching studies of the soil columns of this region it was found that even up to 20 per cent of the applied 2,4-D may leach beyond depth of 20 cm if the rate of movement of water through the soil column is high.

5.5.2 Effect of rate of movement of water through the soil columns on the leaching pattern of 2,4-D in the rice soils

In the present column leaching study the rate of percolation of water through the column varied from 0.01 ml min^{-1} to 20.80 ml min^{-1}. So the time taken to collect 50ml leachate differed widely from 2.4 min to 83.33 hours,

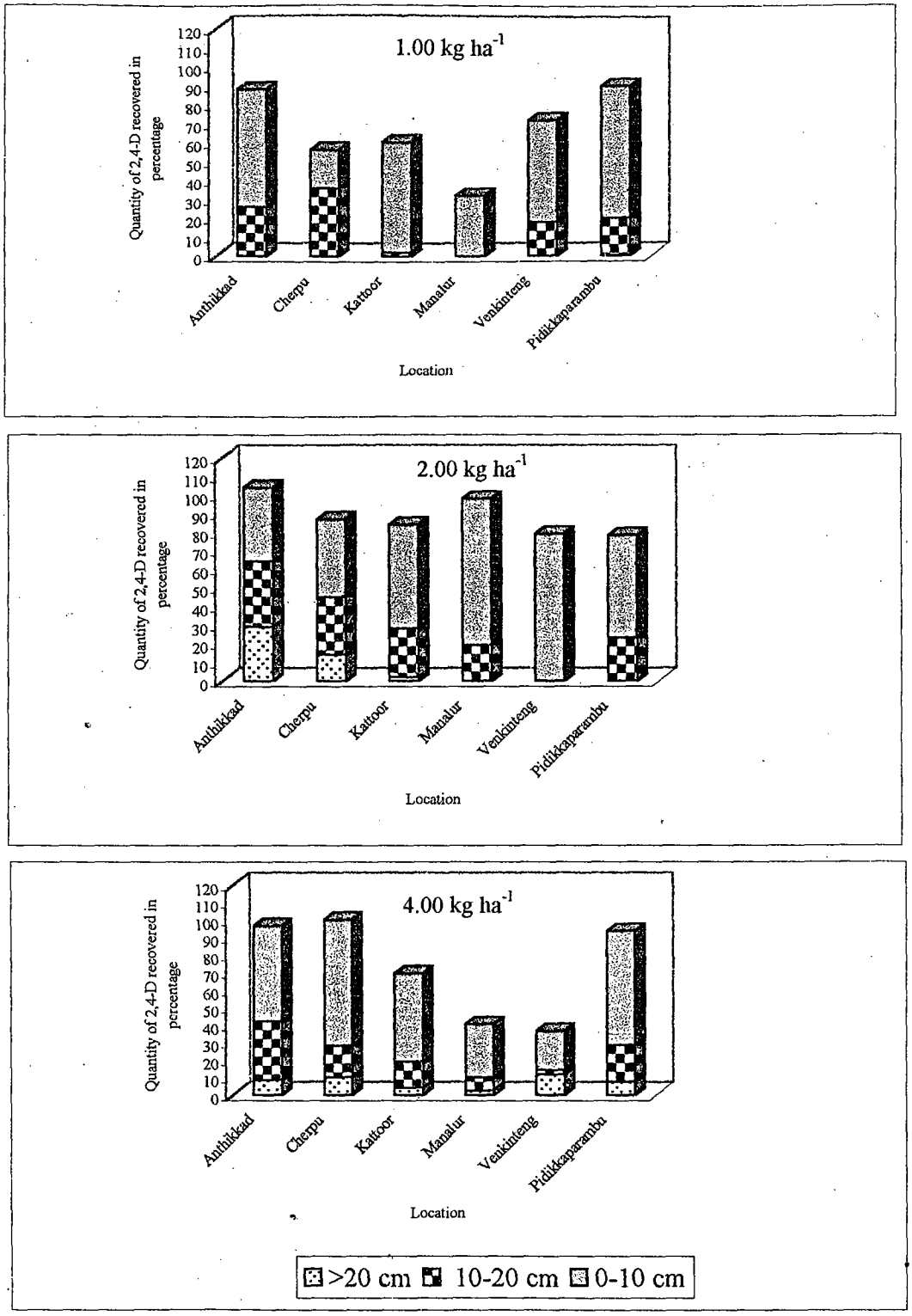


Fig.23 Depth wise distribution of 2,4-D residues in kole soils at different rates of application (kg ha^{-1})

during which degradation of 2,4-D also could have taken place. Degradation is mainly brought about by microorganisms and occurred at relatively rapid rates both under aerobic and anaerobic conditions even when it leaches to a depth of 90 cm (Lavy *et al.*, 1973). This is one of the reasons for the differences in the residue content in different layers of the soil columns taken for the study.

One of the factors responsible for restricting the movement of water through the soil is field compaction .It is influenced by soil properties as well as external pressure on the soil. In the present leaching study, soil columns within the same location differed in their percolation rate e.g. Anthikkad column, which registered maximum concentration of 2,4-D in the leachate, had three different rates of percolation when they were taken from three sites (3.33, 20.00 3and 2.41 ml min^{-1} in the columns used for the treatments viz;0.50, 1.00 and 2.00 respectively). This is due to the difference in the degree of compaction that occurred while taking soil columns of smaller dimensions, directly from the field. The force applied on the PVC tubes would not be the same and the variations in the rate of movement of water through the soil column is mainly attributed to this factor.

The relationship between percolation rate and concentration of 2,4-D in the leachate (Table 27) was highly significant (0.494* and 0.771**) at both the higher levels of applications (2.00 and 4.00 kg ha^{-1}).

Based on the above findings the following conclusions could be drawn from column leaching studies.

Table 27. Coefficients of correlation (r) between soil characteristics and quantity of 2,4-D residues in the leachate (>20 cm depth)

Levels of 2,4-D (kg ha^{-1})	Sand %	Silt %	Clay %	pH	Organic Carbon %	Cation exchange capacity cmol(+) kg^{-1}	Anion exchange capacity cmol(-) kg^{-1}	Sesqui oxides %	Silica %	Maximum water holding capacity, %	Percolation rate (ml min^{-1})
2.00	-0.216	-0.089	0.286	0.042	-0.095	0.564*	0.130	-0.325	-0.238	0.104	0.494*
4.00	0.046	-0.108	0.006	0.132	0.429	0.137	0.149	-0.476*	-0.133	0.034	0.771**

1. Leaching pattern of 2,4-D in rice soils of Kerala is influenced by the rate of percolation of water through the soil .

2. At the present recommended rate of 1.00 kg ha^{-1}, the risk of ground water contamination with 2,4-D residues is negligible.

3. Maximum residue limit of 2,4-D for drinking water is 0.1 µg l^{-1} (0.0001 µg per milliliter) and hence 2,4-D application at rates higher than 1.00 kg ha^{1}(particularly in areas where movement of water through the soil column is greater than 0.01ml min^{-1})should be restricted..

5.6 Persistence of 2,4-D residue in soil and rice plant under field condition

Most of the residue studies on 2,4-D conducted so far in India and elsewhere pertained mainly to the persistence of 2,4-D in soils. Information on the dissipation of 2,4-D from rice plant is limited. The present study was taken up to monitor the dissipation of 2,4-D from soil under field condition, accumulation of 2,4-D in rice plant and contamination of grain and straw with its residues. The effect of 2,4-D residues on soil microbial population was also investigated.

A farmer's field at Pidikkaparambu in the kole area of Thrissur district was selected for conducting the trial .The treatments consisted of 5 levels of 2,4-D (0.5, 1.0, 2.0 and 4.0 kg ha^{-1}) with 4 replications. Effect of 2,4-D on crop and weeds, persistence of 2,4-D in soil and rice and the effect of 2,4-D on soil microflora in the paddy field are discussed in sections 5.6.1 to 5.6.3.

5.6.1 Effect of 2,4-D on crop and weeds in the field

Within a week after spraying the herbicide, characteristic phytotoxicity symptoms appeared on the rice plant in the plots where 2,4-D was applied. The degree of phytotoxicity increased with rate of application (Plate II). Severe swelling and splitting of the basal nodes (PlateIII) and drying up of lower leaves was noticed on the rice plant at the highest rate of application of 2,4-D (4.00 $kgha^{-1}$). It could be inferred that the selectivity of 2,4- D decreases at higher rate of application. Similar results were reported by Rao (1992) The resistance of graminaceous species to lower concentrations of 2,4-D is due to the capability to metabolize the chemical to non herbicidal compounds by hydroxylation and conjugation.

Conjugation of 2,4-D with glucose molecules is a detoxification reaction (Weintrub et *al*;1952), while conjugation with aminoacids in rice plant will not completely detoxify the herbicide. Aminoacid conjugates of 2,4-D possessed herbicidal properties(Feung et al;1977).The most active compounds are less polar aminoacid conjugates of leucine, isoleucine, valine, alanine and methionine. Rice plant contain these aminoacids in appropriate proportions (Sikkha *et al.*, 1993) and they can form complexes with 2,4-D which may be toxic to the rice plant. This could be one of the reasons for the occurrence of phytotoxicity on rice plant at higher rates of application of 2,4-D.

Chlorophenoxy acetic acids are auxin type herbicides. The mechanism of action of 2,4-D is at gene level (Chen *et al.*, 1973). It was proposed that the

4.0 kg ha^{-1} **0.0 kg ha^{-1}**

0.5 kg ha^{-1}

1.0 kg ha^{-1}

2.0 kg ha^{-1}

4.0 kg ha^{-1}

Plate II. Phytotoxicity in rice by 2,4-D application

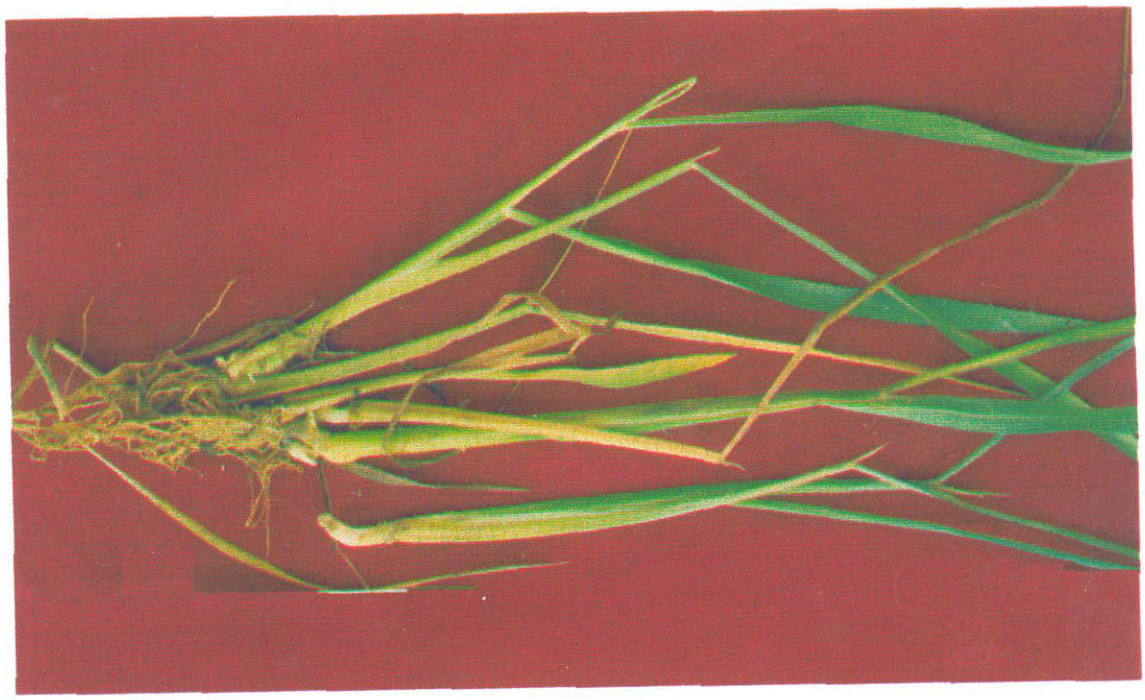


Plate III. Phytotoxicity of 2,4-D at 4.0 kg ha^{-1}

selective phytotoxicity of auxin like herbicides is based on differential alteration of RNA species, change in the number of DNA sites available for transcription and interference with protein synthesis.

Though phytotoxicity was better visualized in the plots which received higher concentration of 2,4-D, grain or straw yields was not affected significantly. This is attributed to the inherent capacity of rice plant to detoxify 2,4-D by hydroxylation and conjugation with plant constituents. Presence of greater amounts of hydroxylases in resistant species has been reported by many scientists (Weintrub *et al.,* 1952).

No broad leaved weeds were completely controlled by 2,4-D application. Only the sedges and grasses (*Cyperus sp., Fimbristylis miliaceae , Oryza rufipogon, Sacciolepis interrupta, Isachne sp.and Cynodon dactylon*) were observed at 60 days after spraying the herbicide. There was not much difference in the weed population between the different levels of 2,4-D. However, in the hand weeded plot, significantly higher number of sedges were noticed, though there was no difference in the total dry weight of weeds. Maximum dry weight of weeds was obtained in the plots where 2,4-D was applied at 0.50 kg ha^{-1}. The findings of the field study confirmed the efficacy of 2,4-D in controlling the broad leaved weeds in paddy at the present recommended rate of 1.0 kg ha^{-1}.

5.6.2 Persistence of 2,4-D in the rice soil and plant at varying rates of application

Under the field condition 2,4-D persisted in soil is less than 30 days. Microbial degradation is the major path way of 2,4-D degradation (Alexander and Aleem, 1961). Both bacteria and fungi are involved in the degradation. This is due to the presence of adaptive enzymes present in the microbial cells. Microbes possessing those enzymes (mainly oxidases) can utilize carbon atoms of 2,4-D molecules as their energy source and they proliferate at a faster rate. Degradation rate also increases with higher number of 2,4-D resistant micro biota present in the soil. This could be the sound reason for the higher dissipation rate noted at 2.00 and 4.0 kg ha^{-1}, under field situation.

The pH of soil at Pidikkaprambu was 5.15, which would have favoured microbial activity. Higher rate of detoxification of 2,4-D at pH 5.30 has been reported (Corbin Upchurch, 1967). The microorganisms involved in herbicide detoxification includes bacteria, fungi, algae, moulds etc. of which bacteria predominates (Rao,1992). Since both bacteria and fungi predominates under the above field condition, degradation of 2,4-D at higher rate is possible.

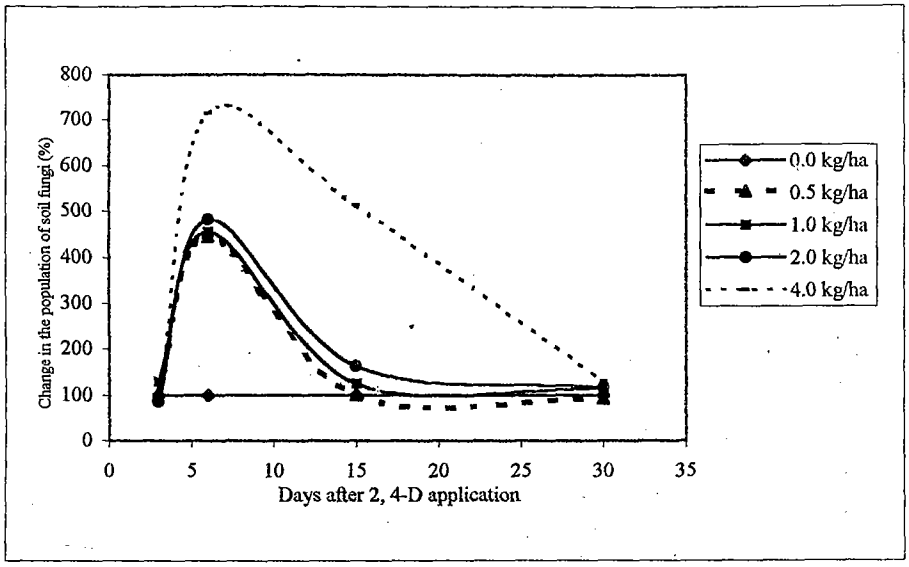
Residues of 2,4-D estimated in the rice plant was very low even when applied at 4.00 kg ha^{-1} (0.40 and 0.70 ppb at 2.00 and 4.00 $kgha^{-1}$ respectively). In the rice grain and straw samples the residues were very much lower than the maximum residue limits. It is very clear that little translocation of 2,4-D to the grain occurred even at higher rates of application of 2,4-D.

5.6.3 Effect of 2,4-D on soil microbial population

As discussed in the section 5.6.2, the major degradation pathway of 2,4-D is microbiological. In order to see the changes in soil micoflora consequent to the application of 2,4-D under field condition, soil plate dilution study was conducted in the samples taken from different treatments. Population of both bacteria and fungi changed considerably with rate of application and time after spraying. Greater change was observed at 6 days after spraying. Relationship between change in microbial population over varying rates of application of 2,4-D and at varying periods is presented in (Fig.24)

Varying trends in the population of soil bacteria and fungi were observed in the plots where 2,4-D was applied. The herbicide had a negative effect on the population of soil bacteria while its influence on fungi was positive (Plate IV). Maximum count of fungi and minimum count of bacteria coincided at one stage i.e. at 6 days after spraying. Explanation for this phenomenon is given under.

(i) The major fungal species noticed in the soil was *Aspergillus niger*. This can hydroxylate 2,4-D at 2, 4 or 2 and 4 positions to yield the corresponding hydroxy phenoxy acetic acids (Rao, 1992). Since this organism is adapted to the herbicide, proliferation rate will be larger. (ii) In the field experiment, rice was grown under submerged condition and hence denitrifying bacteria may predominate which transform nitrate to nitrite or nitrogen for their oxygen requirement. The reduction in the bacterial count consequent to 2,4-D application could be explained on the following basis.



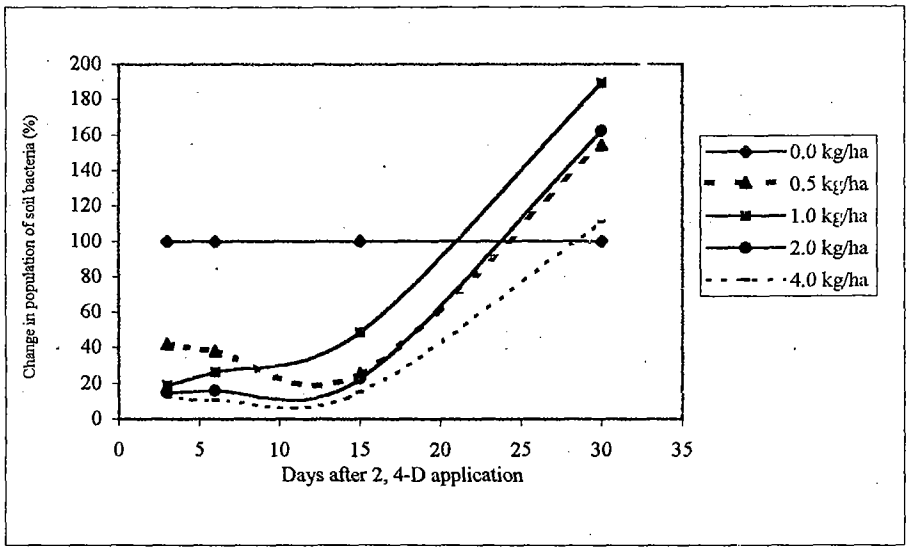


Fig. 24 Changes in the population of soil microorganisms (%) over varying periods and varying rates of application of 2, 4-D in rice field

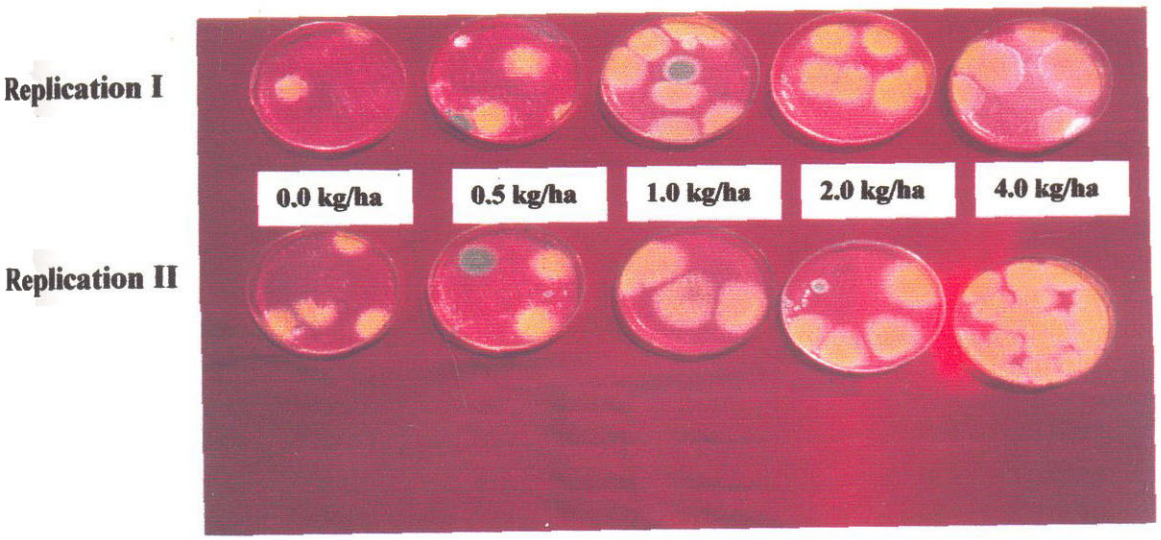


Plate IV. Effect of 2,4-D on the growth of soil fungi

At high concentration of 2,4-D, it could inhibit denitrifiers even though this is a herbicide of low antimicrobial activity and it has been observed that the relative toxicity of 2,4-D to three species was reduced under aerobic condition (Audus,1960). This has a practical advantage that herbicide reducing the number of denitrifying bacteria would provide an additional bonus to weed control.

Favourable influence on *Aspergillus* noticed in the study has an important bearing on disease control, i.e. presence of larger number of *Aspergillus sp.* can reduce the number of disease causing fungal species of the rice field.

Although there was reduction in the bacterial population with increase in the rate of 2,4-D application, their total population in all the treatments was higher than that of fungi. It means that both bacteria and fungi have taken part in the degradation of 2,4-D.

In general, an equilibrium between the population of bacteria and fungi prevails in soil. If the population of one organism increases, definitely there will be a decline in the total number of some other organisms. Since the major fungal genera observed in the plots were of *Aspergillus niger*, their population increases at a faster rate due to greater ability for degrading the chemical. This would have resulted in suppression of bacterial growth. Thus the inhibitory effect on bacteria was not attributed to the effect of 2,4-D alone.

Over all, the results of the study indicated the key role of microorganisms in the degradation of 2,4-D in the rice soils of Kerala. With regard to the safety of

present recommended rate of 2,4-D application in the paddy fields, the study made it very clear that there is no chance for toxic accumulation of 2,4-D residues in grain, straw or in the soil and water environments of the wetland paddy of the state.

Environmental issues may arise if the recommendations are not strictly followed.

Fate of 2,4-D in the major rice soils of Kerala

From the discussion made so far it could be inferred that the fate of 2,4-D in rice soils is primarily governed by physical processes such as leaching and run off, chemical processes like adsorption and microbial processes. Therefore, the persistence of 2,4-D residues in soil is a function of physicochemical characteristics of the soil, rate of movement of water through the soil and the total number and type of micro flora present in the soil. The present investigation on the behaviour of 2,4-D in the major rice soils of Kerala indicated that its halflife is variable from 3.44 days (karumady) to 10.76 days (Moncompu 3). Both Karumady and Moncompu 2 belongs to the rice growing region, kuttanad. Extreme variations in the half life of 2,4-D within a particular rice growing region could not be explained with a single soil characteristic. Though the soils of a particular region resembled in some of the characteristics, wide variations were also noticed in certain properties (Fig.16a,16b and 16c). This could be one of the reasons for the differences in their capacity to degrade 2,4-D. More over, degradation of 2,4-D is mainly micro biological and the total number of micro

flora and the type of micro flora responsible for 2,4-D degradation may vary considerably and cannot be predicted with great certainty.

Degree of adsorption of 2,4-D in the rice soils showed a variation in the range of 3.55 (Venkitengu) to 9.02 (Moncompu 3). Differences were noticed in the degree of adsorption of 2,4-D among the soils of a particular region which was also not influenced by soil properties alone. Regarding the movement of 2,4-D to lower soil layers, it was found that at the present recommended rate of 2,4-D application viz.1.00 $kgha^{-1}$, in the rice soils of Kerala, risk of ground water contamination is negligible. However, at higher rates of application it has a potential for polluting ground water, especially when the rate of movement of water through the soil column is greater than 0.01 ml $min.^{-1}$

Under field condition, persistence of 2,4-D residues in soil was less than 2 weeks and the crop produce was not contaminated with the herbicide at levels higher than the maximum residue limits. The effect of 2,4-D on soil micro flora was also temporary (1 month) and therefore it could be concluded that the present recommendation of 2,4-D (1.00 kg ha^{-1}) for weed control in rice is safe to soil environment, human beings and animals.

Summary

6. SUMMARY AND CONCLUSIONS

An attempt was made to study the behaviour of 2,4-D residues in the soils of major rice growing areas of the state during 1997-2001 at College of Horticulture, Vellanikkara. Major objectives of the study were to work out the magnitude of persistence, degradation and movement of 2,4-D in the soils of major rice growing areas of the state viz., Palakkad, kuttanad and kole lands and also to monitor the extent of contamination of soil and crop produce with residues of this herbicide. Three laboratory experiments were conducted to understand the fate of 2,4-D residues in these rice soils. A field experiment was also conducted to estimate the rate of dissipation of 2,4-D residues from the rice ecosystem. Persistence of 2,4-D in rice soil and plant under field condition and the effect of the herbicide on soil micro flora were also investigated. Major findings of the study are summarized below.

1. A colorimetric procedure was standardized for the estimation of 2,4-D residues in soil. Shaking soil sample with acetonitrile: distilled water: glacial acetic acid (80: 20: 2.5) in the ratio of 1: 4 for a period of 30 min. gave good recovery of 2,4-D from the soils irrespective of the soil type.

2. Persistence of 2,4-D in the soils of three major rice growing regions of Kerala was estimated at 0.50, 1.00 and 2.00 $\mu g\ g^{-1}$ levels of fortification. The results showed that 2,4-D residues persisted in soil

for less than 60 days irrespective of the soil type and level of fortification.

3. Degradation of 2,4-D in the rice soils under investigation followed first order rate equation. Half life of the herbicide varied from 3.44 to 10.76 days. Lowest half life was recorded by karumady sample of kuttanad and the highest value by Moncompu 3 of the same region.

4. Adsorption of 2,4-D in the major rice soils of the state at different periods of equilibration and at different levels of application was estimated. Adsorption of 2,4-D by the soils under study followed Freundlich isotherm $x/m = KC^{n}$. Adsorption of 2,4-D in the soil types studied did not vary considerably.

5. Magnitude of adsorption of 2,4-D by the soils increased with concentration of 2,4-D in solution at all the periods of equilibration.

6. A column leaching study was conducted with the sixteen soil samples at three rates of application (1.0, 2.0 and 4.0 kg ha^{-1}) of 2,4-D. Magnitude of downward movement of 2,4-D residue in the soil was dependent on rate of application, soil type and percolation rate.

7. The experiment conducted in the rice field of kole region showed that 2,4-D residues did not persist in soil beyond 30 days after application even when the rate of application was 4.0 kg ha^{-1}

8. 2,4-D residues were not detected in rice plant when applied at 0.50 and 1.0 kg ha^{-1}. At higher rates of application viz., 2.0 and 4.0 kg ha^{-1} 2,4-D content of the plant was at parts per billion levels.

9. Residues of 2,4-D in the rice grain and straw were below the maximum residue limits at all the levels of application.

10. Population of micro flora in rice soil varied with time after application of 2,4-D. Though a negative influence of 2,4-D on soil bacteria was observed in the early period, their population was restored by 30 days after spraying and this period coincided with the persistence of 2,4-D in the wetland paddy under investigation.

11. At the present recommended rate of 1.00 kg ha^{-1}, application of 2,4-D has no ill effect on rice and soil environment.

References

7. REFERENCES

Adams, R.S. and Li, P. 1971. Soil properties influencing sorption of lindane. *Proc. Soil Sci. Soc. Am.* **35**: 78-81

Agnihotri, N.P. 2000. Pesticide consumption in agriculture in India - an update. *Pestic. Res. J.* **12**:150-155

Albanis, T.A. 1992. Herbicide losses in runoff from the agricultural area of Thessatoniki in Thermaikos Gulf, N. Greece. *Sci. Tot. Environ.* **114**: 59-71

Alexander, M. and Aleem, M.I.H. 1961. Effect of chemical structure on microbial decomposition of stomatic herbicides. *J. Agric. Fd Chem.* **9**: 44-47

Altom, J.D. and Stritzke, J. F. 1973. Degradation of dicamba, picloram and four phenoxy herbicides in soils. *Weed Sci.* **21**: 556-560

Aly, O.M. and Faust, S.D. 1964. Studies on the fate of 2,4-D and ester derivatives in natural surface waters. *J. Agric. Fd Chem.* **12**: 541-546

Anon. 1999. *Final Report of ICAR Adhoc Project on Evaluation of Kuttanad Ecosystem for Possible Contamination by Pesticides, Herbicides and Toxic Heavy Metals.* Regional Agricultural Research Station, Kumarakam, Kottayam, Kerala, India, 39p.

Audus, L.J. 1951. The biological detoxication of hormone herbicides in soil. *Pl. Soil* **3**: 170-192

Audus, L.J. 1960. Microbiological breakdown of herbicides in soils. *Herbicides* (ed. Audus, L.J.). Blackwell, Oxford, pp. 1-19

Audus, L.J. 1964. Herbicide behaviour in the soil: II. Interaction with soil micro-organisms. *The Physiology and Biochemistry of Herbicides* (ed. Audus, L.J.). Academic Press, London, pp. 163-206

Bach, M.K. 1961. Metabolites of 2,4-dichlorophenoxy acids from bean stems. *Pl. Physiol.* **36**: 558-585

Balajee, S. and Mahadevan, A. 1993. Biodegradation of 2,4-Dichlorophenoxyacetic acid in soil by *Azotobacter chroococcum*. *Toxicol. environ. Chem.* **39**: 169-172

Balinova, A.M. and Mondesky, M. 1999. Pesticide contamination of ground and surface water in Bulgaian Danube plain. *J. environ. Sci. Health* **34**: 33-46

Baskaran, S., Bolan N.S., Rahman, A. and Tillman, R.W. 1996. Effects of exogenous carbon on the sorption and movement of atrazine and 2,4-D by soils. *Aust. J. Soil Res.* **34**: 609-622

Bastin, B. 1996. Fate of carbofuran applied to the soil basin of black pepper. Ph.D. thesis, Kerala Agricultural University, Thrissur, Kerala, India. 96p.

Bayer, D.E. and Lump, J.M. 1973. *Pesticide Formulations.* Marcel Dekker, New York. 387p.

BCPC. 1979. *The Pesticide Manual.* Sixth edition. British Crop Protection Council Survey and Royal Society of Chemistry, Cambridge, U.K. 1341p.

Bear, F. 1964. *Chemistry of the soil.* Second edition. Oxford & IBH, Publishing Co., New Delhi. 515p.

Benoit, P. and Barriuso, E. 1997. Fate of ^{14}C ring labelled 2,4-D, 2,4-dichlorophenol and 4-chlorophenol during straw composting. *Biol. Fertil. Soils* **25**: 53-59

*Bhan, V.M. 1978. Weed control in drilled rice. *Proceedings of the National Symposium on Increasing Rice Yield in Kharif* 1978. Central Rice Research Institute, Cuttack, India, pp. 201-206

Bhan, V.M. and Misra, J.S. 2001. Herbicides in relation to food security and environment in India. *Pestic. Inf.* **2**: 28-33

Bhardwaj, S.S. and Singh, K. 1991. Adsorption of 2,4-D by some soils. *J. Res.* **28**: 378-383

Binkley, R. W. and Oaks, T.R. 1974. Photochemical reactions of alkyl 2,4-dichloro-phenoxyacetates. *Chemosphere* **1**: 3-4

Bolan, N.S. and Baskaran, S. 1996. Biodegradation of 2,4-D herbicide as affected by its adsorption-desorption behaviour and microbial activity of soils. *Aust. J. Soil Res.* **34**: 1041-1053

Bollag, J.M., Liu, S.Y. and Minard, R. D. 1980. Cross-coupling of phenolic humus constituents and 2,4-Dichlorophenol. *Soil Sci. Soc. Am. J.* **44**: 52-56

Boval, B. and Smith, J.M. 1973. Photodecomposition of 2,4-dichlorophenoxyacetic acid. *Chem. Engg. Sci.* **28**: 1661-1665

Bovey, R.W. 1980. *The Science of 2,4,5-T and Associated Phenoxy Herbicides.* John Wiley & Sons, New York. 462p.

Brown, J.W. and Mitchell, J.W. 1948. Inactivation of 2,4-dichlorophenoxyacetic acid in soil as affected by soil moisture, temperature, addition of manure and autoclaving. *Bot. Gaz.* **109**: 323-325

Burger, K., Macrae, I.C. and Alexander, M. 1962. Decomposition of phenoxyalkyl carboxylic acids. *Proc. Soil Sci. Soc. Am.* **26**: 243-246

Carter, A.D. 2000. Herbicide movement in soils: Principles, pathways and processes. *Weed Res.* **40**: 113-122

Cheah-VanBol, K., Kirkwood, R.C., Lum-Keng, Y., Cheah, U.B. and Lum, K.Y. 1997. Adsorption, desorption and mobility of four commonly used pesticides in Malaysian agricultural soils. *Pestic. Sci.* **50**: 53-63

Chen, L.G., Ali, A., Fletcher, R.A., Switzer, C.M. and Stephenson, G.R. 1973. Effects of auxin like herbicides on nucleohistones in cucumber and wheat roots. *Weed Sci.* **21**: 181-184

Chhokkar, R.S. 2001. Development and use of herbicides. *Pestic. Inf.* **2**: 25-27

Chopra, S.L. and Kanwar, J.S. 1976. *Analytical Agricultural Chemistry.* Kalyani Publishers, New Delhi. 518p

Coffee, D.L. and Warren, G.E. 1969. Inactivation of herbicides by activated carbon and other adsorbents. *Weed Sci.* **17**: 16-19

Cohen, S.Z., Creeger, S.M., Cassel, R.F. and Enfield, C.G. 1984. Treatment and disposal of pesticide wastes. *ACS Symp. Ser.* 259 (eds. Krueger, R.F. and Seiber, J.N.) American Chemical Society, Washington D.C. pp.294-325

Corbin, F.T. and Upchurch, R.P. 1967. Influence of pH on detoxification of herbicides in soil. *Weeds* **15**: 370-377

Covalier, T.C., Lavy, T.L. and Mattice, J.D. 1991. Persistence of selected herbicides in ground water samples. *Groundwater* **29**: 225-231

Cox, C. 1999. 2,4-D: Exposure. *J. Pestic. Reform* **19**: 14-19

Croll, B.T. 1995. Pesticides in surface and groundwaters. *J. Inst. Water Environ. Mgmt* **5**: 389-395

Crosby, D.G. and Tutass, H.O. 1966. Photodecomposition of 2,4-Dichlorophenoxyacetic acid. *J. Agric. Fd Chem.* **14**: 596-599

Cullimore, D.R. 1981. The enumeration of 2,4-D degraders in Saskatchewan soils. *Weed Sci.* **29**: 440-443

Deka, S.K. and Gogoi, A.K. 1993. Studies on the persistence of butachlor in soil and residues in straw and grain of rice (*Oryza sativa* L.). *Proceedings of International Symposium on Integrated Weed Management for Sustainable Agriculture Vol. II.* Indian Society of Weed Science, Hisar, India, pp. 96-98

DeRose, H.R. and Newman, A.S. 1947. The comparison of the persistence of certain plant growth regulators when applied to soil. *Proc. Soil Sci. Am.* **12**: 222-226

Doherty, P.J. and Warren, G.F. 1969. The adsorption of four herbicides by different types of organic matter and a bentonite clay. *Weed Res.* **9**: 20-26

Dubey, H.D. and Freeman, J.F. 1964. Influence of soil properties and microbial activity on the phytotoxicity of linuron and diphenamid. *Soil Sci.* **97**: 334-340

FCN. 1982. *The Pesticide Chemical News Guide.* Food Chemical News Inc, UK, 544p.

Felding, G. 1995. Leaching of phenoxy alkanoic acid herbicides from farm land. *Sci. Total Environ.* **168**: 11-18

Feung, C.S., Hamilton, R.H. and Mumma, R.O. 1977. Metabolism of 2,4-dichlorophenoxy acetic acid: II. Herbicidal properties of aminoacid conjugates. *J. Agric. Fd Chem.* **25**: 898-900

Foster R.K. and Mckercher, R.B. 1973. Laboratory incubation studies of chlorphenoxyacetic acids in chernozemic soils. *Soil Biol. Biochem.* **5**: 333-337

Fournier, J.C. 1980. Enumeration of the soil microorganisms able to degrade 2,4-D by metabolism or co-metabolism. *Chemosphere* **9**: 169-174

Fournier, J.C., Codaccioni, P., Soulas, G. and Repiquet, C. 1981. Soil adaptation to 2,4-D degradation in relation to the application rates and the metabolic behaviour of the degrading microflora. *Chemosphere* **10**: 977-984

Freed, V.H. 1948. Qualitative reaction for 2,4-Dichlorophenoxyacetic acid. *Science* **107**: 98

Freed, V.H. and Montgomery, M.L. 1963. The metabolism of herbicides by plants and soils. *Res. Rev.* **3**: 1-18

Fryer, J.D. and Kirkland, K. 1970. Field experiments to investigate long term effects of repeated applications of MCPA, tri-allate, simazine and linuron: Report after 6 years. *Weed Res.* **10**: 133-158

Gahlot, R. and Narula, N. 1996. Degradation of 2,4-Dichlorophenoxy acetic acid by resistant strains of *Azotobacter chroococcum. Indian J. Microbiol.* **36**: 141-143

Gahukar, R.T. 1999. *Agromedical Guide of Synthetic Pesticides.* Agri-Horticultural Publishing House, Nagpur. 487p.

Giles, G.H., McEvan, T.H., Nakhwa, N.S. and Smith, A.E. 1960. Studies on adsorption. Part II: A system of classification of solution adsorption isotherm and its use in diagnosis of adsorption mechanism and in the measurement of specific areas of solids. *J. Chem. Soc.* 3973-3993

Grover, R. 1973. The adsorption behaviour of acid and ester forms of 2,4-D on soils. *Weed Res.* **13**: 51-58

Grover, R. and Smith, A.E. 1974. Adsorption studies with the acid and dimethylamine forms of 2,4-D and dicampa. *Can. J. Soil Sci.* **54**: 179-186

Grover, R., Cessna, A.J. and Kerr, L.A. 1985. Procedure for the determination of 2,4-D and dicamba in inhalation, dermal handwash and urine samples from spray applicators. *J. environ. Sci. Health* **820**: 113-128

Grover, R., Waite, D.T., Cessna, A.J., Nicholaichuck, W., Irwin, D.G., Kerr, L.A. and Best, K. 1997. Magnitude and persistence of herbicide residues in farm dugouts and ponds in the Canadian prairies. *Environ. Toxicol. Chem.* **16**: 638-643

Hamaker, J.W. and Thompson, J.W. 1972. Adsorption. (ed. Goring, C.A.I. and Hamaker, J.W.) *Organic Chemicals in the Soil Environment*, Marcel Dekker, NewYork. pp. 49-144

Hammarstrand, K. 1976. *Gas Chromatographic Analysis of Pesticides.* Varian Associates, 611 Hansenway, Palo Alto, CA 94303. 90p.

Hamner, C.L. and Tukey, H.B. 1944. The herbicidal action of 2,4-Dichlorophenoxyacetic acid and 2, 4-5-trichlorophenoxyacetic acid on bindweed. *Science* **100**: 154-155

Han, S.O. and New P.B. 1994. Effect of water availability on degradation of 2,4-dichlorophenoxy acetic acid (2,4-D) by soil micro organisms. *Soil Biol. Biochem.* **26**: 1689 – 1697

Hance, R.J. 1965. The adsorption of urea and some of its derivatives by a variety of soils. *Weed Res.* **5**: 98-107

Hance, R.J. 1967. The speed of attainment of sorption equilibria in some systems invoving herbicides. *Weed Res.* **7**: 29-36

Harris, C.I. and Warren, G.F. 1964. Adsorption and desorption of herbicides by soil. *Weeds* **12**: 120-126

Harris, R.C. and Skinner, A.C. 1992. Controlling diffuse pollution of groundwater from agriculture and industry. *J. Inst. Water Environ. Mgmt* **6**: 569-575

*Hartley, G.S. 1964. The physiology and biochemistry of herbicides. *Herbicides.* (ed. Audus, L.J.), Academic Press, New York, pp. 111-161

Hatcher, P.G. and Spiker, E.C. 1988. Selective degradation of plant biomolecules. *Humic Substances and Their Role in the Environment.* (eds. Frimmel, F.H. and Christman, R.F.). John Wiley and Sons, New York. pp. 59-74

Hedges, J.I. 1988. Polymerization of humic substances in natural environments. *Humic Substances and Their Role in the Environment.* (eds. Frimmel, F.H. and Christman, R.F.) John Wiley and Sons, New York, pp. 45-58

*Henkel, H.G. 1966. *Ein Beitrag Zur Gas Chromatographic von Phenoxyalkancarbonsauren and anderen herbicidwirksamen sauren. J. Chromatogr.* **22**: 446

Hesse, P.R. 1972. *A Textbook of Soil Chemical Analysis.* Chemical Publishing Co., Inc New York. 520p.

Horner, J., Quee-Hee, S.S. and Sutherland, R.G. 1974. Esterification of (2,4-dichlorophenoxy) acetic acid – a quantitative comparison of esterification techniques. *Anal Chem.* **46**: 110-112

Hurle, K 1993. The problem of surface water pollution with herbicides. *Proceedings of International Symposium on Integrated Weed Management for Sustainable Agriculture Vol. II* 1993. Indian Society of Weed Science, Hisar, India, pp. 121-128

Jackson, M.L. 1958. *Soil Chemical Analysis.* Indian Reprint 1967. Prentice Hall of India Pvt. Ltd., New Delhi. 498p.

John, P.S. and Sadanandan, N. 1995. Effect of joint application of 2,4-D and urea on weed control in rice. *J. trop. Agric.* **33**: 40-42

Johnkutty, S. and Venugopal, V.K. 1993. *Kole Lands of Kerala.* Kerala Agricultural University, Thrissur. 68p.

*Jorgensen, P., Felding, G., Mogensen, B.B. and Splicid, N.H. 1995. Leaching of phenoxy acids in clay and sand soils. *Proceedings of Twelfth Danish Plant Protection Conference, 1995.* Geoteknisk Institute, Denmark, pp.71-87

Karickhoff, S.W., Brown, O.S. and Scott, T.A. 1979. Sorption of hydrophobic pollutants on natural sediments and soils. *Water Res.* **13**: 241

KAU. 1984. *NARP Status Report of Southern Region.* Kerala Agricultural University, Thrissur, Kerala, India. 113p.

KAU. 1989a. *NARP Status Report of the Special Zone of Problem areas.* Kerala Agricultural University, Thrissur, Kerala, India. 120p.

KAU. 1989b. *NARP Status Report of the Central Zone.* Kerala Agricultural University, Thrissur, Kerala, India. 143p.

KAU. 1993. *Package of Practices Recommendations Crops '93.* Kerala Agricultural University, Vellanikkara, Trichur, Kerala, India. 253p.

KAU. 1996. *Package of Practices Recommendations Crops '96.* Directorate of Extension, Kerala Agricultural University, Trichur, Kerala, India. 267p.

Kearney, P.C. and Kaufman, D.D. 1972. Microbial degradation of some chlorinated pesticides. *Degradation of Synthetic Organic Molecules in*

the Biosphere. (eds. Kearney, P.C. and Kaufman, D.D). National Academy of Sciences, Washington, pp. 166-189

Khan, S.U. 1974. Determination of diquat and paraquat residues in soil by gas chromatography *J. Agric. Fd Chem.* **22**: 863-865

Khan, S.U. 1975. Chemical derivatization of herbicide residues for gas liquid chromatographic analysis. *Res. Rev.* **59**: 21-50

Kirby, C. 1980. *The Hormone Weed Killers – A Short History of Their Discovery and Development.* British Crop Protection Council, Lavenham Press Ltd. Lavenham, UK. 55p.

Kirkland, K. 1967. Inactivation of MCPA in soil. *Weed Res.* **7**: 364-367

Kohl, R.A. and Taylor, S.A. 1961. Hydrogen bonding between the carbonyl group and Wyoming bentonite. *Soil Sci.* **91**: 223-227

Kunc, F. and Rybatova, J. 1983. Mineralization of carbon atoms of ^{14}C-2,4-D side chain and degradation ability of bacteria in soil. *Soil Biol. Biochem.* **15**: 141-144

Kuwatsuka, S. and Miwa, N. 1989. Change in population of 2,4-D degraders in the processes of 2,4-D degradation in soils under upland and flooded conditions. *Soil Sci. Pl. Nutr.* **35**: 535-543

Lavy, T.L., Dewell, R.A., Beard, C.R., Mattice, J.D. and Skulman, B.W. 1996. *Environmental Implication of Pesticides in Rice Production.* Research Series. No.453. Arkanas Agricultural Experiment Station, USA. 69p.

Lavy, T.l., Roeth, F.W. and Fenster, R. 1973. Degradation of 2,4-D and atrazine at three soil depths in the field. *J. environ. Qual.* **2**: 132-137

Leela, D. 1986. Studies on crop tolerance to oxyfluorfen for selection of companion crop and bioassay for persistence study. *Pestology* **2**:11-15

Lindstrom, F.T., Boersma, L. and Gardiner, H. 1968. 2,4-D diffusion in standard soils. A mathematical theory. *Soil Sci.* **106**:107-113

Loos, M.A. 1975. Phenoxy alkanoic acids. *Herbicides*: *Chemistry, Degradation and Mode of Action. Vol. I.* (eds. Kearney, P.C. and Kaufman, D.D.), Second edition. Marcel Dekker, New York. 500p.

Loos, M.A., Schlosser, I.F. and Mapham, W.R. 1979. Phenoxy herbicide degradation in soils Quantitation studies on 2,4-D and MCPA degrading microbial populations. *Soil Biol. Biochem.* **11**: 377-385

Luckwill, L.C. and Lloyd-Jones, C.P. 1960a. Metabolism of plant growth regulators: I. 2,4-dichlorophenoxy acetic acid in leaves of red and black carrot. *Ann. appl. Biol.* **48**: 613-625

Luckwill, L.C. and Lloyd-Jones, C.P. 1960b. Metabolism of plant growth regulators. II. Decarboxylation of 2,4-dichlorophenoxy acetic acid in leaves of apple and strawberry. *Ann. appl. Biol.* **48**: 626-636

Majka, J.T., Cheng, H.H. and Muzik, T.J. 1982. Dissipation of massive quantities of 2,4-D and 2,4,5-T n-butyl esters in field mini lysimeters. *J. environ. Qual.* **11**: 645-649

*Maltseva, O., McGowan, C., Fulthrope, R. and Oriel, P. 1996. Degradation of 2,4-dichlorophenoxy acetic acid by haloalkaliphilic bacteria. *Microbiology- Reading* **142**: 1115-1122

Mandal, A.K. and Adhikari, M. 1995. Role of organic matter and sesquioxides in the adsorption of pesticides on soils. *J. Indian Soc. Soil Sci.* **43**: 561-567

Marquardt, R.P. and Luce, E.N. 1951. Determination of small amounts of 2,4-D in milk *Anal. Chem.* **23**: 1484

Marquardt, R.P. and Luce E.N. 1955. Determination of 2,4-D in grain and seed. *J. Agric. Fd Chem.* **3**: 51-53

Marquardt, R.P. and Luce, E.N. 1961. A new basic procedure for determining phenoxy acid herbicides in agricultural products. *J. Agric. Fd Chem.* **9**: 266-270

McCall, P.J., Vrona, S.A. and Kelley, S.S. 1981. Fate of uniformity carbon-14 ring labeled 2,4,5-trichlorophenoxyacetic acid and 2,4-Dichlorophenoxyacetic acid. *J. Agric. Fd Chem.* **29**: 100-107

McGrath, D. 1994. Anote on the adsorption characteristics of organic pollutants in Irish soils. *Irish J. Agric. Fd Res.* **35**: 55-61

Minard, R.D., Liu, S.Y. and Bollang, J.M. 1981. Oligomers and quinines from 2,4-Dichlorophenol. *J. Agric. Fd Chem.* **29**: 250-253

Miwa, N. and Kuwatsuka, S. 1990. Enrichment process of 2,4-D degraders in different soils under upland conditions. *Soil Sci. Pl. Nutr.* **36**: 261-266

Moreale, A. and VanBladel, R. 1980. Behaviour of 2,4-D in Belgian soils. *J. environ. Qual.* **9**: 627-633

Mortland, M.M. and Meggitt, W.F. 1966. Interaction of ethyl N,N-dipropylthiol carbamate (EPTC) with montmorillonite. *J. Agric. Fd Chem.* **14**: 126-129

Nearpass, D.C. 1965. Effects of soil acidity on the adsorption, penetration and persistence of simazine. *Weeds* **13**: 341-346

Nelson, H.S. and Faust, S.D. 1969. Acid dissociation constants of selected aquatic herbicides. *Environ. Sci. Technol.* **3**: 1186-1188

Newmann, A.S., Thomas, J.R. and Walker R.L. 1952. Disappearance of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid from soil. *Proc. Soil Sci. Soc. Am.* **16**: 21-24

Norris, L.A. 1966. Degradation of 2,4-D and 2,4,5-T in forest litter. *J. For.* **64**: 475-476

NRCWS. 1987. *Consolidated Report for 1982-83 to 1986-89 of All India Co-ordinated Research Programme on Weed Control.* National Research Centre for Weed Science, Adhartal, Jabalpur. 17p.

Obien, S.R., Suehis, R.H. and Younge, O.R. 1966. The effects of soil factors on phytotoxicity of neburon to oats. *Weeds* **14**: 105-109

*Ooi, G.G. and Lo, N.P. 1992. Toxicity of herbicides to Malaysian rice field fish. *Proceedings of Third International Conference on Plant Production in the Tropics* 1990. Malaysian Plant Protection Society, Genting Highlands, Kuala Lumpur, Malaysia, pp.110-118

Ou, L.T. 1984. 2,4-D degradation and 2,4-D degrading microorganisms in soils. *Soil Sci.* **137**: 100-107

Ou, L.T., Rothwell D.F., Wheeler, W.B. and Davidson, J.M. 1978. The effects of high 2,4-D concentrations of degradation and carbon dioxide evolution in soils. *J. environ. Qual.* 7: 241-246

*Pak, K.I. and Pak, N.A. 1975. Results of field trials of herbicides in rice crop. *Khimiya v-Selskom-Khozyaistve* **13**: 39-41

Parker, L.W. and Doxtader, K.G. 1982. Kinetics of microbial decomposition of 2,4-D in soil: Effects of herbicide concentration. *J. environ. Qual.* **11**: 679-684

Parker, L.W. and Doxtader, K.G. 1983. Kinetics of microbial degradation of 2,4-D in soil: Effects of temperature and moisture. *J. environ. Qual.* **12**: 553-558

Payne, M.G. and Fults, J.L. 1947. Some effects of untraviolet light on 2,4-D and related compounds. *Science* **106**: 37-39

Plumb, T.R., Norris, L.A. and Montgomery, M.L. 1977. Persistence of 2,4-D and 2,4,5-T in Chaparral soil and vegetation. *Bull. environ. Contam. Toxicol.* **17**: 1-8

Pokorny, R. 1941. Some chlorophenoxyacetic acids. *J. Am. Chem. Soc.* **63**: 1768

Purkayastha, R. 1974. Simultaneous determination of 2,4-Dichlorophenoxyacetic acid, 2,4,5–trichlorophenoxyacetic acid and 2-methoxy 3, 6-dichlorobenzonicacid in soil and water by gas chromatography with electron capture detector. *J. Agric. Fd Chem.* **22**: 453-458

QueHee, S.S. and Sutherland, R.G. 1981. *The Phenoxy Alkalonic Herbicides.* CRC Press, Inc., Boca Raton, Florida. 321p.

Rangaswami, R. 1988. *Diseases of Crop Plants in India.* Prentice Hall of India, Pvt. Ltd., New Delhi. 469 p.

Rao, V.S. 1992. *Principles of Weed Science.* Oxford &IBH Publishing Co. Pvt. Ltd., New Delhi. 540 p.

Reghupathy, A. and Dhamu, K.P. 1990. *Statistics Workbook for Insecticide Toxicology* Suriya Desktop Publishers, Coimbatore. 165p.

Renberg, L. 1974. Ion exchange technique for the determination of chlorinated phenols and phenoxy acids in organic tissue, soil and water. *Anal Chem.* **46**: 459-461

*Repiquet, C. and Fournier, J.C. 1977. *Etude en laboratoire de la degradation du 2,4-D et del`isoproturon en presence d`autres produits de treatment. Comptes desjournees d`etudes sur les herbicides. Columa* 801-810

Rocco-Estrella, M., Brusseau, M.L., Maicer, R.S., Pepper, I.L., Wierenga, P.J. and Miller, R.M. 1993. Biodegradation, sorption and transport of 2,4-dichlorophenoxyacetic acid in saturated and unsaturated soils. *Appl. environ. Microbiol.* **59**: 4266-4273

Sankaran, S., Jayakumar, R. and Kempachetty, N. 1993. *Herbicide Residues.* Gandhi Book House, Coimbatore. 251 p.

Sattar, M.A. 1982. Persistence of 4-chloro-2-methylphenoxyacetic acid in soil. *Bull. environ. Contam. Toxicol.* **28**: 348-352

Sharpee, K.W. 1973. Microbial degradation of phenoxy herbicides in culture, soil and aquatic ecosystems. *Diss. Abstr. Int.* **34**: 104

Sheets, T.J., Grafts, A.S. and Drever, H.R. 1962. Influence of soil properties on the phytotoxicities of the S-triazine herbicides. *J. Agric. Fd Chem.* **10**: 458-462

Sherburne, H.R. and Freed, V.H. 1954. Adsorption of 3 (P-chloropheyl)-1, 1-dimethyl urea as a function of soil constituents. *J. Agric. Fd Chem.* **2**: 939

Sikka, K.C., Lodha, M.L. and Mehta, S.L. 1993. Nutritional quality of cereal and grain legume proteins. *Recent Advances in Plant Biochemistry* (eds. Mehta, S.L., Lodha, M.L. and Sane, P.V.). Indian Council of Agricultural Research, New Delhi, pp. 302-340

Smith A.E. 1978. Relative persistence of di and trichlorophenoxyalkanoic acid herbicides in Saskatchewan soils. *Weed Res.* **18**: 275-279

Smith, A.E. 1979. Soil persistence experiments with ^{14}C-2,4-D in herbicidal mixtures, and field persistence studies with tri-allate and trifluralin both singly and combined. *Weed Res.* **19**: 165-170

Smith, A.E. 1980. Persistence studies with ^{14}C-2,4-D in soils previously treated with herbicides and pesticides. *Weed Res.* **20**: 355-359

Smith, A.E. 1982. Soil persistence studies with ^{14}C MCPA in combination with other herbicides and pesticides. *Weed Res.* **22**: 137-142

Smith, A.E. 1985. Identification of 2,4-dichloroanisole and 2,4-dichlorophenol as soil degradation products of ring-labelled ^{14}C-2,4-D. *Bull. environ. Contam. Toxicol.* **34**: 150-157

Smith, A.E. 1989. Degradation, fate and persistence of phenoxyalkanoic acid herbicides in soil. *Rev. Weed Sci.* **4**: 1-24

Smith, A.E. and Aubin, A.J. 1991. Transformation of [^{14}C] 2,4-dichlorophenol in Saskatehewan soil. *J. Agric. Fd Chem.* **39**: 801-804

Smith, A.E. and Bridges, D.C. 1996. Movement of certain herbicides following application to simulated golf course greens and fairways. *Crop Sci.* **36**: 1439-1445

Smith, A.E. and Hayden, B.J. 1980. Hydrolysis of MCPA esters and the persistence of MCPA in Saskatchewan soils. *Bull. environ. Contam. Toxocol.* **25**: 369-373

Smith, A.E. and Hayden, B.J. 1981. Relative persistence of MCPA, MCPB and mecoprop in Saskatchewan soils, and the identification of MCPA in MCPB- treated soils. *Weed Res.* **21**: 179-183

Smith, A.E. and Muir, D.C.G. 1980. Determination of extractable and non extractable radioactivity from prairie soils treated with carboxyl and ring labeled [^{14}C] 2,4-D. *Weed Res.* **20**: 123-129

Soderquist, C.J. and Crosby, D.G. 1975. Dissipation of 4-chloro-2-methyl-phenoxyacetic acid (MCPA) in a rice field. *Pestic. Sci.* **6**: 17-33

*Sokolov, M.S., Knyr, L.L., Strekozov, E.P., Agarkor, V.D., Chubenko, A.P. and Kryzhko BA. 1974. The behaviour of herbicides in a rice irrigation system. *Agrokhimiya* **11**: 95-106

Soulas, G., Chaussod, R. and Verguet, A. 1984. Chloroform fumigation technique as a means of determining the size of specialized soil microbial populations: Application of pesticide-degrading microorganisms. *Soil Biol. Biochem.* **16**: 497-501

Stevenson, F.J. 1985. Geochemistry of soil humic substances. *Humic Substances in Soil, Sediment and Water: Geochemistry, Isolation and Characterisation.* (eds. Aitken, G.R.., McKnight, D. and Wershaw, R.L.). John Wiley, New York, pp. 13-52

Stewart, D.K.R. and Gaul, S.O. 1977. Persistence of 2,4-D, 2,4,5-T and dicamba in a dykeland soil. *Bull. environ. Contam. Toxicol.* **18**: 210-218

Stott, D.E. Martin, J.P., Focht, D.D. and Haider, K. 1983. Biogradation, stabilization in humus and incorporation into soils biomass of 2,4-D and chlorocatechol carbons. *Soil Sci. Soc. Am. J.* **47**: 66-70

Talbert, R.E. and Fletchall, O.H. 1965. The adsorption of some S-triazines in soils. *Weeds* **13**: 46-62

*Tejada, A.W., Varca, L.M., Columpang, SMF., Ocampo, P.P., Medina, MJB., Bajet, C.M., Paningbatan, E.B., Medina, J.R., Justo V.P., Habito, C.L., Mortinez, M.R., Magallona, E.D. and Pingali, P.L. 1995. Assessment of environmental impact of pesticides in paddy rice production. *Impact of Pesticides on Farmer Health and Rice Environment.* (ed. Roger, P.A.). Kluwer Academic Publishers, Netherlands. 248p.

*Torstensson, N.T.L. 1975. Degradation of 2,4-D and MCPA in soils of low pH. *Enivironmental Quality and Safety.* (eds. Coulston, F. and Korte, F.) Suppl. Vol. III George Thieme, Struttgart, pp. 267-265

Totstensson, N.T.L., Stark, J. and Goransson, B. 1975. The effect of repeated applications of 2,4-D and MCPA on their breakdown in soil. *Weed Res.* **15**: 159-164

Upchurch, R.P., Selman, F.L., Mason, D.D. and Kamprath, E.J. 1966. The correlation of herbicidal activity with soil and climatic factors. *Weeds* **14**: 42-49

USEPA 1988. Pesticides and Toxic substances. *Guidance for reregistration of pesticide products containing 2,4-dichlorophenoxyacetic acid (2,4-D) as the active ingredient,* Sept. 1988, U.S. Environmental Protection Agency,Washington D.C. 18 p.

Vee, R.H., Inskeep, W.P. and Caper, A.K. 1996. Soil depth and temperature effects on microbial degradation of 2,4-D. *J. environ. Qual.* **25**: 5-12

Vettorazzi, G. 1979. *International Regulatory Aspects for Pesticide Chemicals* Vol. II CRC Press, Boca Raton. 141 p.

Voos, G. and Groffman, P.M. 1997. Relationships between microbial biomass and dissipation of 2,4-D and dicamba in soil. *Biol. Fertility Soils* **24**: 106-110

Walker, A. and Smith A.E. 1979. Persistence of 2,4,5-T in a heavy clay soil. *Pestic. Sci.* **10**: 151-157

Watson, J.R., Posner, A.M. and Quirk, J.P. 1973. Adsorption of 2,4-D on to goethite. *J. Soil. Sci.* **24**: 503

171949

Weintrub, R.L., Brown, J.W., Fields, M. and Rohan, J. 1952. Metabolism of 2,4-dichlorophenoxyacetic acid: I. $^{14}CO_2$ production by bean plants treated with labeled 2,4-Dichlorphenoxy acetic acid. *Pl. Physiol.* **27**: 293-301

Wilcox, M., Moreland, D.E. and Klingman, G.C. 1963. Aryl hydroxylation of phenoxy aliphatic acids by excised roots. *Pl. Physiol.* **16**: 565-571

Willems, H.P.L. Lewis, K.J., Dyson, J.S. and Lewis, F.J. 1996. Mineralization of 2,4-D and atrazine in the unsaturated zone of a sandy loam soil. *Soil Biol. Biochem.* **28**: 989-996

Wilson, R.G. and Cheng, H.H. 1978. Breakdown and movement of 2,4-D in the soil under field conditions. *Weed Sci.* **24**: 461-466

WSSA. 1983. *Herbicide Handbook.* Fifth edition. Weed Science Society of America, Illinois. 515p.

Zepp, R.G., Wolf, N.L., Gordon, J.A. and Baughman, R. 1975. Dynamics of 2,4-D esters in surface waters: hydrolysis, photolysis and vaporization. *Environ. Sci. Technol.* **9**: 1144-1150

*Zimmerman, P.W. and Wilcoxin, F.L. 1935. Several chemical growth substances which cause initiation of roots and other responses in plants. *Contrib. Boyce Thompson Inst.* **7**: 209-229

* Originals not seen

ASSESSMENT OF 2,4-D RESIDUES IN THE MAJOR RICE SOILS OF KERALA

By

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ABSTRACT OF THE THESIS

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ABSTRACT

An attempt was made to assess the behaviour of 2,4-D in the three major rice soils of Kerala viz., Palakkad, kuttanad and kole lands. Three laboratory experiments were conducted in sixteen soil samples (five each from Palakkad and kuttanad and six from kole lands) collected from the major rice growing regions, so as to understand the persistence, degradation, adsorption and leaching pattern of 2,4-D in these samples. A field experiment was also conducted in kole area of Thrissur district (farmer's field at pidikkaparambu) for estimating the dissipation pattern of 2,4-D from soil and plant under field condition.

In order to estimate 2,4-D residues in soil samples of the laboratory experiments, a colorimetric procedure was standardized. The standardization process consisted of selection of estimation procedure, standardization of extractants and soil extractant ratios and clean up method. Among the different extractants tried, acetonitrile: distilled water: glacial acetic acid (80:20:2.5) in the soil extractant ratio of 1:4 was the best on in terms of recovery of applied 2,4-D from soil. Clean up of the exctract with chemicals in sequence: NaOH (1*N*), concentrated HCl, diethyl ether, sodium hydrogen phosphate buffer, concentrated HCl and carbon tetrachloride was efficient to remove the co-extractives. Heating the residue with chromotropic acid in concentrated sulphuric acid at 135 °C for 20 min. was employed to develop the colour. Absorbance of the coloured extract was measured

at 565 mμ in a Spectronic 20 spectrophotometer. Eighty to ninety percent of the applied 2,4-D was recovered from most of the soils under study.

Persistence of 2,4-D in the soil samples was studied by fortifying the samples with 2,4-D at 0.50, 1.00 and 2.00 μg g^{-1} and incubating them under submerged condition for varying periods. Residues of 2,4-D in the soil samples were estimated at 0, 1, 3, 6, 9, 15, 30 and 60 days after incubation. Half life of 2,4-D in the soil samples was worked out by fitting first order kinetic equation of the form $C = C_o e^{-kt}$. A variation of 3.44 to 10.76 days was noticed in the half life of 2,4-D in the major rice soils of Kerala. Lowest half life was recorded by Karumudy sample of the kuttanad region and the highest by Moncompu 3 of the same region. Extremely high organic carbon content in combination with partial decomposition of organic matter in the karumady soil would have favoured the microbial growth and enzymatic reactions and enhanced the rate of degradation in this soil.

Adsorption of 2,4-D in the three rice soils of Kerala was studied at three different levels of equilibration (2,4 and 6 h) and three levels of 2,4-D (0.5,1.0 and 2.0 μg g^{-1} soil). The results indicated that degree of adsorption increased with concentration of 2,4-D in the soil solution i.e. adsorption of 2,4-D in the rice soils of Kerala followed Freundlich isotherm of the form $x/m = KC^n$. A comparison of the strength of adsorption of 2,4-D in the soil samples was made by using the isotherm constant K. It indicated that there was not much variation in the adsorptive capacity of soils with high and low organic matter contents. This could be attributed to the difference in the degree of humification of organic matter in the soils studied.

Organic matter present in karumady area is under varying stags of decomposition and due to the lower degree of humification, the adsorption of 2,4-D on the clay organic matter complex would not have taken place to a great magnitude.

Leaching and movement of 2,4-D in the rice soils was studied by applying 2,4-D to the top of soil columns collected in PVC tubes, directly from the field. The treatments consisted of 2,4-D @ 1.0, 2.0 and 4.0 kg ha^{-1}. 2,4-D residues retained in 0-10 cm, 10-20 cm and in the leachate (>20cm) were estimated. The result showed that major part of the 2,4-D (more than 50% of the applied 2,4-D) remained in the 0-10 cm depth and less than 36 per cent remained in the 10-20 cm soil layer. Ten per cent of the applied 2,4-D leached up to a depth of > 20 cm in some soils of kole region (Cherpu and Anthikkad) which had higher rates of percolation of water. However, no 2,4-D residue was available in the leachate of any soil at the lowest level of application of herbicide i.e.1.0 kg ha^{-1}. 2,4-D residues in drinking water at concentrations greater than 0.0001 $\mu g\ l^{-1}$ is considered to be toxic to human beings and animals. The findings of the study emphasizes the need for restricting the 2,4-D application to 1.0 kg ha^{-1}, particularly in the sandy soils of Kerala.

Studies on the dissipation of 2,4-D from soil and rice plant under field condition consisted of five treatments viz., 2,4-D @ 0.0, 0.5, 1.0, 2.0 and 4.0 kg ha^{-1}. At the present recommended level of 1.0 kg ha^{-1} 2,4-D residues persisted in paddy field for less than 30 days. Residues of 2,4-D in the soil were not detectable at 60 days even if the rate of application was increased to 4.0 kg ha^{-1}.

Effect of 2,4-D on microbial population was studied by soil plate dilution method. The total number of fungal colonies in the soil samples were higher in the treatments which received higher quantities of 2,4-D, while the bacterial population was inhibited at higher rates of 2,4-D application. Major fungal species noticed on the study was *Aspergillus niger*. The results of the study revealed the key role of fungi in the degradation of 2,4-D in the paddy field under investigation.

2,4-D residues in the grain and straw samples were very much lower than the maximum residue limits. The findings of the present investigation made it clear that the present recommendation of 2,4-D at the rate of 1.0 kg ha^{-1} for weed control in rice does not cause any adverse effect in the soil or crop produce.