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# Kinetics of the Microbial Degradation of 2,4-D and $^{14}$C-Labeled Paraquat in Two Types of Tropical Agricultural Soil

*[1]B.S. Ismail, [1]Mehdi Sameni and [2]M. Halimah*

[1]School of Environmental and Natural Resource Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM, Bangi, Selangor, Malaysia
[2]Malaysian Palm Oil Board, P.O. Box: 10620, 50720 Kuala Lumpur, Malaysia

**Abstract:** Degradation of agrochemicals in the soil plays an important role in the fate and transport of the contaminants in the environment. A study on the degradation of 2,4-D (2,4-Dichlorophenoxyacetic acid) and $^{14}$C-labeled paraquat (1,1-dimethyl-4,4-bipyridylium) in Malaysian clay and clay loam soils was carried out under laboratory conditions. The individual half-lifes were derived from the slope of the line of best fit, calculated by linear regression analysis of the logarithm of the concentration remaining against the time of incubation. The degradation rate of both herbicides at different temperature levels and moisture content was investigated. The degradation rate of 2,4-D compounds was strongly influenced by temperature and soil moisture content. In contrast, the half life of $^{14}$C-paraquat in both types of soils did not change significantly when temperature and soil moisture content increased.

**Key words:** Microbial degradation · 2,4-D · $^{14}$C-labeled paraquat · Tropical agricultural soil

## INTRODUCTION

Herbicides are essential components of modern agriculture in developed countries and their use is rapidly increasing in developing countries [1]. The research reported by Pingali and Gerpacio [2] showed that Malaysia used just 9 percent of Asia's total pesticide consumption but was the region's most intensive user of pesticides applying 23.42 kilograms of active ingredients per hectare of arable land. The records of the Department of statistics, Malaysia showed that the production of liquid herbicides in 2004, which amounted to 46.8 million litres, was the highest since 1997. The volume of this type of herbicide produced in 2006 was 44.2 million litres. In 2007, the production increased substantially by 32.2% to about 58.5 million litres [3].

The fate of pesticides in the soil is controlled by chemical, biological and physical dynamics of the matrix [4]. Concern about the environmental impact of pesticide use has prompted research into the environmental fate of the pesticides. Degradation studies in soils are essential for evaluation of the persistence of pesticides and their breakdown products [5]. Data on the rate of degradation are extremely important as they permit the prediction of the levels likely to remain in the soil and allow for the assessment of the potential risk associated with exposure.

Soil microorganisms, especially bacteria and fungi, have been reported as the most important degraders of agrochemicals and the environmental conditions that favor microbial development in the soil are the same as those that favor degradation of chemical compounds and these are temperature, moisture and aeration [6]. Soil moisture content directly influences soil oxygen content and microbial activity and therefore can influence pesticide persistence. For example, the half-life of alachlor increased from 23 days in surface soil (aerobic conditions) to >100 days in the vadose zone (anaerobic conditions) [7]. Temperature has also been shown to significantly affect degradation kinetics of organic contaminants in soils [8]. Walker and Zimdahl [9] studied the effects of temperature on the persistence of atrazine and metolachlor in three soil types: For temperature treatments that increased from 5 to 35°C, the corresponding half-lives decreased for atrazine by factors ranging from 7 to 9 and for metolachlor, from 6 to 8 days. Studies on degradation

**Corresponding Author:** B.S. Ismail, School of Environmental and Natural Resource Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM, Bangi, Selangor, Malaysia.
Tel: +60389214183, Fax: +60389252699,

of pesticides in tropical soils have also been conducted in the laboratory [10, 11]. The herbicides 2,4-D and paraquat are highlighted as the object of this study because they are two of the most used ricefield herbicides in the State of Perak, Malaysia. The primary goal of the present study was to investigate the persistence of ionic or ionizable herbicides in Malaysian rice field soils. To this end, paraquat and 2,4-D were selected as model compounds, which are commonly used in rice cultivation in Kerian, Perak. Paraquat is a polar organic compound, thus it is very rapidly adsorbed by soils and is quite immobile, while acidic 2,4-D is a phenoxy acid which is anionic herbicide that is bound onto soil particles by hydrogen bonding and is moderately adsorbed to soil. Paraquat has been used extensively as a pre- emergence herbicide for weed control in the Kerian ricefields for weed destruction before rice seeding in lieu of mechanical cultivation. There have been many authenticated cases of the detection of this cationic pesticide in water sources [12] and the compound is highly toxic if deliberately or accidentally ingested [13]. In contrast, 2,4-D is a dichlorophenoxy acid which acts as a selective systemic herbicide. The compound is widely used for the control of broad-leaved weeds in the Kerian ricefields and is a frequently detected contaminant of ground- and surface waters [14].

## MATERIALS AND METHODS

**Chemicals and Reagents:** All reagents used in the present study were of analytical grade. Methanol and hydrochloric acid were obtained from Merck and 2,4-D standard of 99.7% purity was purchased from the laboratories of Dr. Ehrenstofer (Germany). Radio-labeled [Methyl-$^{14}$C] paraquat was obtained from the Institute of Isotopes (Budapest) and non-labeled paraquat from Dr. Ehrenstorfer (Germany). The concentration of the radio-labeled paraquat was 100 μCi with a specific activity of 32.3 mCi mmol$^{-1}$. The radio purity of the supplied labeled compound was pre-checked by radio-TLC and found to be more than 95% pure. The $^{14}$C-paraquat was dissolved in distilled water in the laboratory and then labeled as RSS1. Calcium chloride ($CaCl_2$) was purchased from Merck.

**Soil Preparation and Physicochemical Analysis:** Soil samples, collected from a rice-growing area in the Kerian district, located in North West Perak, were air dried and sieved through a 2-mm sieve. The soil samples were classified as clay and clay loam. The organic carbon content and pH values in the clay loam and clay soils were 5.94 and 2.10% and 5.82 and 6.17, respectively. It was found that the clay soil had 22% more clay content than the clay loam soil. The clay loam soil had total carbon content of more than 64% compared to the clay soil. All soil data recorded are expressed on dry weight basis. The bulk density of the soils was also recorded. The results are shown in Table 1.

**Apparatus:** An Agilent High Performance Liquid Chromatograph (HPLC) 1100 Series fitted with a UV detector set at 214 nm was used to detect 2,4-D residues in the soil samples. The column used was a $C_8$-$NH_4$ (4.6-mm I.D x 250-mm length 5-μm particle size). The mobile phase was MeOH and buffer ($H_2O$ with + Potassium sodium 3.4 g/l + Hydrochloric acid in pH 2.3), were in the ratio of 30:70.

To extract $^{14}$C-paraquat residues in the soil samples, the Sample Oxidizer Model 307A (PerKin Elmer) and the TriCarb Liquid Scintillation Counter (LSC) Model TR 2550 AB used were purchased from Packard (Packard Instrument Co., Meriden, CT, USA). The reciprocating shaker bath was purchased from TungTec Instruments (Taiwan). The liquid scintillation cocktail PICOFLUORTM 15, was purchased from Packard.

**Preparation of the Standard Stock Solution:** One hundred μCi radio-labeled [methyl-$^{14}$C] paraquat in 0.5 ml distilled water, of specific activity 4,646.58 KBq/mg (32.3 mCi mmol$^{-1}$), was diluted with 9.5 ml distilled water and then labeled as RSS1. Then a working standard solution of 1,850 KBq/mg (RSS2) was prepared by pipetting 5 ml of the RSS1 into a 1- L volumetric flask and adding distilled water to give a final volume of 1,000 ml. The standard stock solution (1000 μg/ml) of 2,4-D was prepared in methanol and the dilutions required for obtaining a standard curve (0.05, 0.2, 0.5, 0.1, 2, 4 μg/ml) were prepared from the standard stock solution by serial dilutions.

Table 1: The physico-chemical properties of the clay loam and clay soils studied

| Soil Type | pH | Total C (%) | Coarse Sand (%) | Fine Sand (%) | Silt (%) | Clay (%) | Bulk Density (g/cm$^3$) |
|---|---|---|---|---|---|---|---|
| Clay loam | 5.82 | 5.94 | 3.13 | 31.04 | 26.76 | 39.07 | 1.29 |
| Clay | 6.17 | 2.10 | 8.2 | 13.03 | 28.51 | 50.26 | 1.24 |

**Herbicide Extraction Procedure:** To extract $^{14}$C-paraquat herbicide residues from the soil, triplicate soil samples (20 mg each) of the dried soil were packed in cellulose and combusted for 2 min in a Packard biological oxidizer (306 A, Perkin Elmer). The resulting $^{14}$C-paraquat was trapped in a scintillation vial containing Carbomax plus LSC cocktail. The samples were analysed in triplicate using the LSC and average values are recorded. The residue levels indicated were on dry weight basis.

For the 2,4-D soil samples the following procedure was carried out: to 5 g samples (in triplicate) was added 40 ml of methanol acidified to approximately pH 2 with acetic acid (85%) and sonicated for 1 h. Ten milliliters of the extract were pipetted out and transferred into a 10 ml vial. The extract was then filtered (Whatman grade 41) and dried under vacuum and redissolved with 1 ml of methanol prior to analysis using the HPLC [15].

**Degradation Study:** To establish the role of microorganisms on the degradation of the herbicides, the degradation rates of 2,4-D and $^{14}$C-paraquat were determined under greenhouse conditions on sterile and non-sterile clay and clay loam soil samples. For each soil type, 500 gm of the soil were autoclaved at 120°C at 15 psi for 30 min and left at room temperature overnight. The procedure was repeated three successive times in a 3 L autoclave. Deionized water used for the preparation of the stock herbicide solution, was also autoclaved in the same manner. The dose applied for clay and clay loam soil was based on the recommended application rates in Malaysia: 0.5-2.0 kg ha$^{-1}$ for 2,4-D and 0.6-1.0 kg ha$^{-1}$ for paraquat. An assumption that pesticide leaching does not occur to a depth of more than 10 cm was made in the calculation of the dose of the pesticides required in the study. The calculation was based on the highest recommended application rate. For the control soil samples were treated with sterile water. After spiking the soil with the aqueous solution of the herbicide at the appropriate concentration, 50-gm samples of the treated soil were kept in each of 10 polyethylene bags (15 cm by 20 cm). Immediately after spiking of the soil with the herbicides, extraction was carried out on triplicate samples (day 0). The remainder of the bags were then placed in the greenhouse. The final soil moisture level was maintained at 50% of the gravimetric water content by watering (with sterile deionized water) once every five days. One bag of each soil type was placed in a refrigerator at temperature below 0°C on Day 1, 3, 7, 10, 14, 21, 30, 45 and 60 days. After day 60 of incubation, samples were thawed and air-dried overnight, the concentration of 2,4-D and $^{14}$C-paraquat were determined by HPLC and LSC, respectively. Similar spiking and incubation was also carried out for non-autoclaved samples of both of the soil types. The tops of polyethylene bags were covered with a plastic polyethylene cover to minimize photodegradation and evaporative losses. All treatments were replicated three times.

For most pesticides, the rate of degradation is generally proportional to concentration so that the results can often be interpreted using first-order kinetics [16]. The metabolism rate constant (k) and half-life ($t_{1/2}$) of 2,4-D and $^{14}$C-paraquat were calculated using equation (1) derived from the law of first-order kinetics as follows:

$$\ln \frac{[C]_1}{[C]_2} = -K_1(t_1 - t_2) \quad (1)$$

Where: $[C]_1$ is initial concentration of herbicide (mg L$^{-1}$) in soil at time zero, $[C]_2$ the concentration of the herbicide (mg l$^{-1}$) in the soil at time t, $t_1$ and $t_2$ the incubation period in days at t = 0 and time, t respectively and k the herbicide metabolism rate constant (d$^{-1}$).

Thus a plot of the logarithm of concentration against time gives a straight line with the slope proportional to the rate constant. Writing $t_{1/2}$ as the time taken for 50% degradation or half- life, the equation (2) becomes;

$$K = \frac{0.693}{t_{1/2}} \quad (2)$$

The half-life concept is a valuable tool in comparing rates of herbicide degradation and because of the simplicity of first-order reaction kinetics, this rate law is widely used. The half-life in a first-order reaction is independent of initial concentration but this is not so for other orders of reaction. The use of this specific term should therefore be reserved for instances where only first-order kinetics apply [16, 17].

**Effects of Temperature and Moisture Content on the Half-Life of 2,4-D and $^{14}$C-paraquat:** Studies on the effect of temperature and moisture content on 2,4-D and $^{14}$C-paraquat dissipation in clay loam soil were carried out while keeping the samples incubated. Aqueous solutions of the herbicides corresponding to their maximum application rate to the soils (2 and 1 kg ha$^{-1}$ for 2,4-D and paraquat, respectively) was applied to the 3-kg fresh samples of clay loam soil. Control samples were similarly prepared with no herbicide treatment. After mixing, samples of 50-g each of the treated soil were kept in 54 polyethylene bags. The bags were divided into 3 groups and incubated at 30, 35 and 40°C. The final soil moisture

levels were maintained at about 50% field capacity by watering every five days after weighing. Immediately after treatment of the soil with herbicides, extraction was done on duplicate samples (day 0). The remainder of the bags (50 gm) at were kept at below 0°C from Day 1, 3, 7, 10, 14, 21, 30, 45 and 60 days.

After day 60 of incubation, samples were thawed and air-dried overnight, the concentration of 2,4-D and $^{14}$C-paraquat were determined by HPLC and LSC, respectively. The individual half-lives were derived from the slope of the line of best fit calculated by linear regression analysis of the logarithm of the concentration remaining against the time of incubation. The Arrheinus activation energy values were calculated from the slope of the graph of log K (degradation rate coefficients) against the 1/T in degrees Kelvin.

In a different set of experiments, the soil samples were divided into 3 groups with soil moisture levels of 25, 50 and 75% field capacity. All soil samples were kept at room temperature of 30°C. The bags were weighed every five days and when necessary, water was added to restore the initial moisture level. Immediately after treatment of the soil with herbicides, extraction was done on duplicate samples (day 0). The remainder of the bags (50 gm) at each soil moisture level were kept at below 0°C from Day 1, 3, 7, 10, 14, 21, 30, 45 and 60 days. After day 60 of incubation, samples were thawed and air-dried overnight, the distribution of 2,4-D and $^{14}$C-paraquat in each soil segment was analysed, as described previously.

## RESULTS AND DISCUSSION

**Degradation Study:** A laboratory study was conducted to investigate the persistence of 2,4-D and $^{14}$C-paraquat in two types of Malaysian soils. Fig. 1 and 2 are graphs of the logarithmic values of soil 2,4-D concentrations against time. These figures show linear relationship in sterilized ($r^2 = 0.82$) and non-sterilized ($r^2 = 0.88$) clay loam soil and sterilized ($r^2 = 0.90$) and non-sterilized ($r^2 = 0.91$) clay soil, respectively. Fig. 3 and 4 represent graphically the

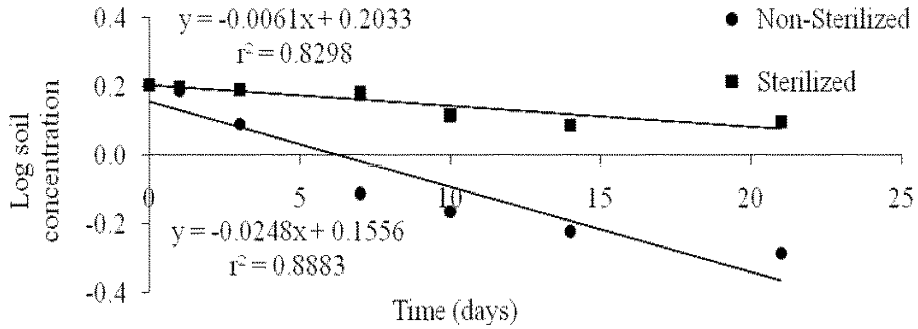


Fig. 1: Kinetics of 2,4-D degradation in sterilized and non-sterilized clay loam soil

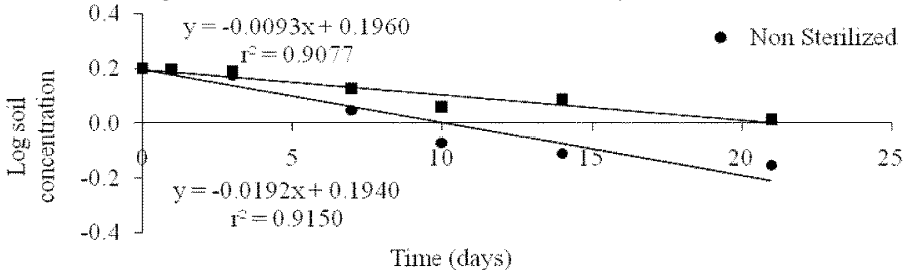


Fig. 2: Kinetics of 2,4-D degradation in sterilized and non-sterilized clay soil

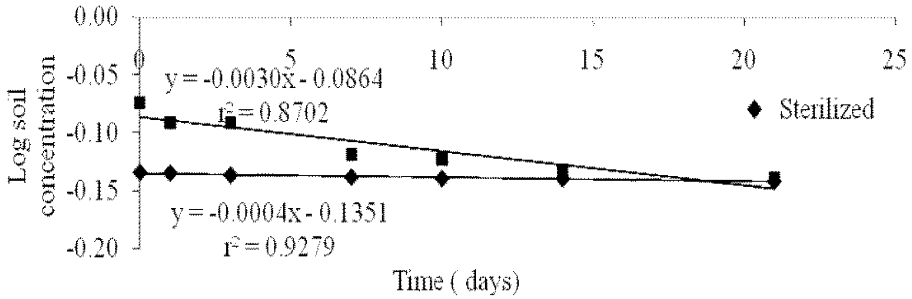


Fig. 3: Kinetics of $^{14}$C-paraquat degradation in sterilized and non sterilized clay soil

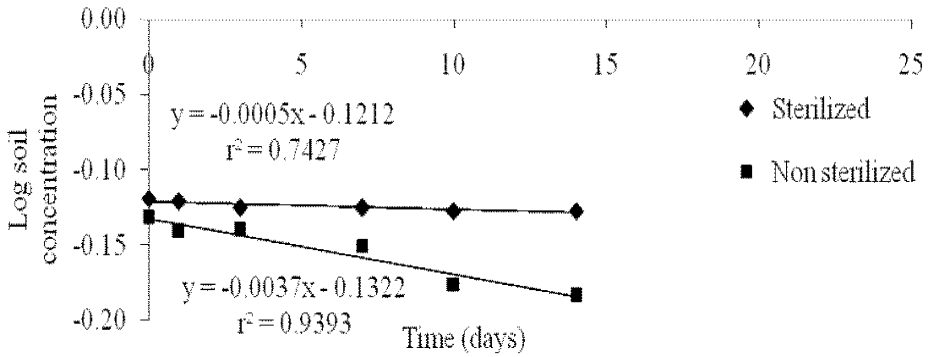


Fig. 4: Kinetics of $^{14}$C-paraquat degradation in sterilized and non-sterilized clay loam soil

Table 2: Degradation constant ($K_{deg}$), half life (days) of 2,4-D and $^{14}$C-paraquat and degradation coefficients ($r^2$) in two soil types

| Herbicide | Soil | $K_{deg}$ (days $^{-1}$) | Half life (days) | $r^2$ |
|---|---|---|---|---|
| 2,4-D | Non-sterilized clay loam | 0.0248 | 27.95 | 0.89 |
| | Sterilized clay loam | 0.0061 | 113.64 | 0.83 |
| | Non-sterilized clay | 0.0192 | 36.10 | 0.92 |
| | Sterilized clay | 0.0093 | 74.54 | 0.91 |
| Paraquat | Non-sterilized clay loam | 0.0037 | 187.35 | 0.93 |
| | Sterilized clay loam | 0.0005 | 1386.40 | 0.74 |
| | Non-sterilized clay | 0.0030 | 231.06 | 0.87 |
| | Sterilized clay | 0.0004 | 1733.00 | 0.92 |

logarithmic values of soil $^{14}$C-paraquat concentrations versus time and illustrate a linear relationship for sterilized ($r^2$ = 0.92) and non sterilized ($r^2$ = 0.87) clay soil and for sterilized ($r^2$ = 0.74) and non sterilized ($r^2$ = 0.93) clay loam soil, respectively.

The results of the half life studies of 2,4-D and $^{14}$C-paraquat in incubated soil samples are shown in Table 2. The half-life of 2,4-D in non-sterilized clay loam soil was 27.95 days while the half-life in the sterilized clay loam soil was 113.64 days. For the non-sterilized and sterilized clay soil, the half-life of 2,4-D was 36.10 and 74.54 days, respectively. The results of the half life studies of $^{14}$C-paraquat in incubated soil samples are also shown in Table 2. The half-life of $^{14}$C-paraquat in non-sterilized clay loam soil and non-sterilized clay soil was 187.35 and 231.06 days respectively. The half-life of paraquat was 1386.4 and 1733.00 days in the sterilized clay loam soil and clay soil, respectively (Table 2). It was observed that the half-life of 2,4-D in sterilized clay loam soil was longer than that in sterilized clay soil and consequently the rate constants for degradation of 2,4-D in sterilized clay loam soil were less than that of sterilized clay soil due to the higher organic matter content and the lower pH of the soil. The results reported from the 2,4-D study are in line with that of Cheah and Lum [10] where it was suggested that the rate of degradation of 2,4-D in higher organic carbon was more than in lower organic carbon. This indicates that the fate of 2,4-D in soil is critically affected by microbial degradation, an observation similar to that reported by Cheah and Lum [10]. Microbial degradation is considered to be the major route for the breakdown of 2,4-D in the soil. Most of the 2,4-D degrading microorganisms are aerobic. That is, they require oxygen and therefore they cannot function in anaerobic oxygen-starved water or sediments. Veeh *et al.* [18] reported that bacterial populations decreased significantly with increasing soil depth and were positively correlated to the rate of 2,4-D degradation. Soil organic matter has been shown by Oppong and Sagar [19] to have the highest cation exchange capacity of all the soil constituents.

The half-life of 2,4-D was inversely correlated to the degradation rate. The highest degradation rate of 2,4-D was observed in the non-sterilized clay loam soil, whereas the lowest was in the sterilized loam soil. Consequently, a longer half-life for 2,4-D was observed in the sterilized clay loam soil (Table 2). Increased adsorption of 2,4-D onto the sterilized clay loam soil can be expected to reduce the concentration of 2,4-D available in the soil solution. Conventional laboratory studies could not provide useful information on the degradation route and rate of paraquat in the soil because of its strong adsorption. Degradation of paraquat in soils was extremely slow.

Table 2 shows that the rate of $^{14}$C-paraquat degradation was higher in the non sterilized soils than in the sterilized soils, suggesting a slower rate of microbial degradation in the non-sterilized soil. The evidence obtained in the present study supports the concept that $^{14}$C-paraquat is not degraded rapidly. There was no significant decrease in herbicide levels in the sterilized or non-sterilized soil incubated for 60 days. The persistent nature of the paraquat has also been reported in many studies conducted previously under field and laboratory conditions. In certain soils paraquat is biologically inactive and is not available to plants or micro-organisms. When strongly bound to soil it has no phytotoxic effects and may persist indefinitely [20]. Paraquat is adsorbed (held) to a greater extent by soil with high cation exchange capacity; this increases with clay and organic matter content. The strong adsorption capacity, or maximum amount of paraquat that could be inactivated by a soil, was estimated to be several hundred times higher than the amount of paraquat that is normally applied during one year [21]. In one study, paraquat was applied to a sandy loam soil over six years at an annual rate of 4.48 kg/ha. Soil analysis after seven years revealed that essentially all of the applied paraquat was still present. A significant amount had penetrated to soil layers of 25-36 cm (probably due to a lower clay content), while most of the paraquat remained in the top most 5 cm [21]. Although paraquat is readily degraded by certain selected soil microorganisms when in a soil solution, its extremely strong adsorption to soil minerals and organic matter, accounting for its rapid biological deactivation, limits the rate at which degradation occurs. Alternative studies were therefore carried out to determine the route and rate of degradation of paraquat in soil.

**Effects of Temperature and Moisture Content on the Half-Life of 2,4-D and $^{14}$C-paraquat:** Changes of 2,4-D and $^{14}$C-paraquat levels in the non sterilized clay loam soil samples were detected during the incubation period. It was assumed that the degradation of these two herbicides at the different incubation conditions followed first-order kinetics. Table 3 shows the degradation of 2,4-D in the present study increased at higher temperature levels and a significant effect of temperature was observed. It is interesting to note that 2,4-D is a thermal degradable compound. The results reported from the 2,4-D study are in line with that of Veeh *et al.* [18] where it was suggested that the rate of degradation of 2,4-D at higher temperature was more than that at lower temperature levels. Consequently the half-life was longer at lower temperature compared to the half-life at higher temperature levels. Calculations on degradation (Table 3) and half-life showed that temperature had a significant influence on the degradation rates of 2,4-D in clay loam soil.

The apparent half-life of 2,4-D decreased from 46.21 to 31.50 days as the temperature increased from 30 to 40°C. Increasing the temperature by 10°C showed a significant decrease in the half-life of 2,4-D by approximately 45%. The degradation rate (k) was greater at 40°C (0.022/day) than at 30°C (0.015/day) with $r^2 > 0.9$ (Table 3). The results reported are in line with that of Johnson *et al.* [22] who found that degradation of 2,4-D was more rapid at 30°C than at 15°C in two surface soils.

Singh and Kulshrestha [23] reported that an increase of 10°C in temperature decreased the apparent half-life of pesticides by a factor of 2-3 times. Higher temperatures are also favourable for microbial growth and probably enhance both biological and non-biological activity of pesticide dissipation [24]. Faster degradation of pesticides at higher temperatures could also be attributed to volatility and photodecomposition of the molecules. Positive correlations between temperature and degradation rate have been obtained in previous studies [25].

Veeh *et al.* [18] reported 2,4-D acid degradation in two laboratory flask aerobic soil studies conducted at three temperature levels using soil taken from 0-12 inch and 3-4 foot depths. Half-lives in soils from 0-12 inches at 10 and 17°C ranged from seven to eleven days. At 24°C the half-lives were only 2 and 3 days in the two surface soils. In soils taken from a depth of 2 to 4 feet the half-lives dramatically increased. At 10°C, the half-lives were 593 and 1691 days (1.6 and 4.6 years). At 17 and 24°C, the half-lives ranged from 10 to 31 days. The authors demonstrated that the half-lives were strongly correlated with soil temperature. They also concluded that shorter half-lives were strongly correlated with higher soil organic carbon content and greater bacterial plate counts in the shallow soil layers. The rate of chemical reactions and most biological metabolic processes double for every 10°C increase in temperature.

Degradation of pesticides in soils is the result of a combination of chemical and biological events [26]. A first-order degradation model is usually used to simulate the variation of the residual mass of a chemical compound in a soil system after its application [27]. The first-order rate constant or half-life in the degradation model is dependent on soil temperature. This dependence can be

approximated by the Arrhenius equation [28]. The temperature dependence of the degradation rate can be described by determining the activation energy of the degradation. The activation energy is determined by the Arrhenius equation (3):

$$k = Ae^{-Ea/RT} \quad (3)$$

Where k is the rate constant, Eα is the activation energy (J $mol^{-1}$) of the reaction, R ($JK^{-1}$ $mol^{-1}$) is the gas constant, T is the absolute temperature (K) and A is an empirical constant ($h^{-1}$). Taking the natural ln of both sides of this equation (4) gives:

$$ln\, k = -\frac{E\alpha}{RT} + \ln A \quad (4)$$

In general, the rate of degradation of herbicides in soil is enhanced by increased temperature. In contrast, the paraquat herbicide is highly persistent in soil and in this study; temperature was slightly influential in the microbial decomposition of paraquat. Adsorption rapidly reduces the bioavailability of paraquat in the soil environment. Calculated degradation half-life showed that temperature had no significant influence on the degradation rates of $^{14}$C-paraquat in the non sterilized clay loam soil (Table 3).

Table 3 shows that degradation of $^{14}$C-paraquat did not change significantly when temperature increased from 30 to 40°C. Consequently, paraquat is not a thermal degradable compound. The degradation rate (k) and half life of the $^{14}$C-paraquat in the clay loam soil did not change when the temperature increased from 30 to 40°C (Table 3). It may have been inactivated nearly immediately when it contacted the soil, precluding buildup of active residues. Paraquat is so strongly absorbed to soils that it is extremely difficult to be extracted for analysis [29].

The activation energy, Eα, is generally stated in units of joules or calories per mole. Calculating the activation energy of a reaction can be important in predicting the rate of a reaction at any temperature. Table 4 shows the calculated activation energy values for 2,4-D are greater than the $^{14}$C-paraquat activation energy and indicates that 2,4-D is susceptible to a larger temperature effect on its degradation rate. The calculated Eá values were 30.22 and 0.86 kJ $mol^{-1}$ for the 2,4-D ($r^2$ = 0.98) and $^{14}$C-paraquat ($r^2$ = 0.98), respectively (Table 4).

Soil moisture often plays a role in the breakdown of herbicides. Herbicide degradation in the soil generally increases with increasing moisture up to field capacity; this probably reflects increased microbial activity. Soil water content directly influences soil oxygen content and microbial activity and therefore can influence pesticide persistence. For example, the half-life of alachlor increased from 23 days in the surface soil (aerobic conditions) to >100 days in the vadose zone (anaerobic conditions) [7].

In the present study of clay loam soil, the rate of 2,4-D degradation in 25% field water capacity was less than the rate of degradation in 50 and 75% field water capacity. Consequently the half-life of 2,4-D in clay loam soil in 75% of field water capacity was less than that in 25 and 50% of field water capacity. Fig. 5 shows that the degradation of 2,4-D was more rapid in soil with higher moisture content. The degradation of 2,4-D was more rapid at 75% field water capacity than at 25% field water capacity in clay loam soil (Fig. 5).

Willems *et al.* [30] reported that in an aerobic sandy loam soil from a depth of 10 to 20 inches, degradation increased slowly when field water capacity increased from 15 to 30%, but was much higher at 40% field water capacity, because of the favourable environment created for the soil bacteria, increased bacterial population and solute concentration and the availability of 2,4-D.

Table 3: Degradation rate coefficients (k) ($days^{-1}$), correlation coefficients (r) and half-life (days) of 2,4-D and $^{14}$C-paraquat at three different temperature levels in clay loam soil

| Herbicide | Temperature (°C) | Degradation rate coefficients (k) | Correlation coefficients ($r^2$) | Half- life |
|---|---|---|---|---|
| | 30 | 0.015 | 0.919 | 46.21 |
| 2,4-D | 35 | 0.019 | 0.944 | 36.48 |
| | 40 | 0.022 | 0.925 | 31.50 |
| $^{14}$C-paraquat | 30 | 0.002 | 0.946 | 346.6 |
| | 35 | 0.002 | 0.836 | 346.6 |
| | 40 | 0.002 | 0.770 | 346.6 |

Table 4: Activation energies for the clay loam soil degradation of 2,4-D and $^{14}$C-paraquat

| Herbicide | A | Eα (kJ/mol) | $r^2$ |
|---|---|---|---|
| 2,4-D | $2.4\times10^{3}$ | -30.22 | 0.98 |
| $^{14}$C-paraquat | $9.3\times10^{11}$ | -0.86 | 0.98 |

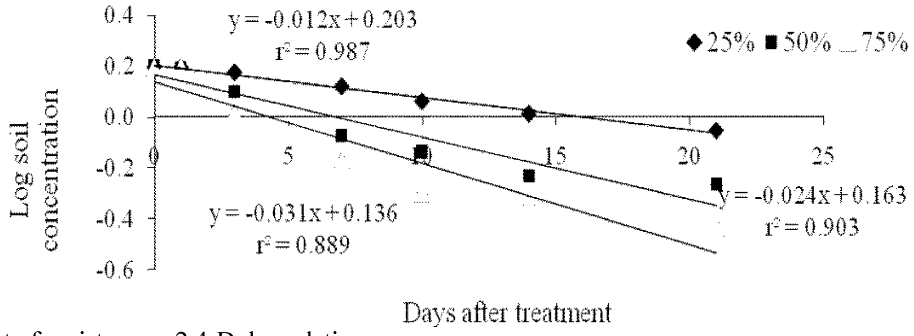


Fig. 5: Effect of moisture on 2,4-D degradation

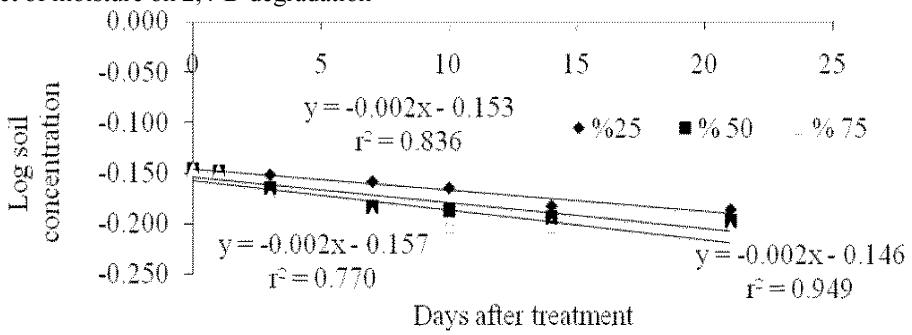


Fig. 6: Effect of moisture on $^{14}$C-paraquat degradation

Skidmore *et al.* [31] reported a similar observation, in which the half-life of permethrin was reduced from 23 to 19 days as the soil moisture content increased from 10.3 to 15.4%. A decrease in the half-life at higher soil moisture levels may be the result of weak adsorption of the pesticide molecules by the soil particles. At higher soil moisture levels, water molecules compete with the pesticide for adsorption sites on the soil colloids and increased pesticide concentration in the soil solution make them more readily available to soil microbes [31]. It was observed that degradation rate increased with increased soil moisture content for 2,4-D herbicides and this result is in agreement with the results of Walker and Blacklow [32] for atrazine and simazine and those of Bowmer [33] for atrazine.

Han and New [34] found that sandy loam soil containing 2,4-D degrading, single-celled bacteria, filamentous bacteria (actinomycetes) and fungi had the lowest degradation rates at a low water potential of -5.5 $MP_a$ (megapascals), with -0.1 $MP_a$ corresponding to soils at or below field capacity. An increase in the water potential resulted in increased rates of breakdown of up to an optimum at -0.1 $MP_a$ [30]. Dry soil conditions contribute to the inhibition of 2,4-D mineralization by restricting solute mobility, reducing the herbicide degrading activity of organisms and suppressing the 2,4-D degrading microorganism populations. Under dry conditions the addition of organic matter may enhance degradation by simulating the o-metabolizing fungal and actinomycete communities [34].

Fig. 6 shows the rate constant for degradation of $^{14}$C-paraquat in clay loam soil at 25, 50 and 75% field water capacity was similar. In the current study, the results were not significant in relation to the moisture effect on paraquat degradation. Consequently the half-life of $^{14}$C-paraquat in clay loam soil did not show change for 25, 50 and 75% field water capacity.

## CONCLUSION

Degradation of 2,4-D and $^{14}$C-paraquat in the laboratory was well described by first-order kinetics (single-rate dissipation sub-model), with the regression coefficient ($r^2$) being greater than 0.9. Calculated degradation half-lives showed that temperature and moisture content had a significant influence on degradation rates of the herbicide 2,4-D in the soil. The half-life of 2,4-D became longer with lower soil moisture content and temperature, due to the interference of these parameters with the functioning of soil microorganisms. There was no evidence of a moisture and temperature factor effect on $^{14}$C-paraquat degradation. The calculated activation energy values for 2,4-D were greater than those of the $^{14}$C-paraquat activation energy values and this

indicates that 2,4-D is subject to a greater effect of temperature on its degradation rate. Slower 2,4-D degradation in autoclaved soil indicated that microbial degradation was an important dissipation mechanism for 2,4-D in the soil. Although the observed degradation under sterile conditions was slower, chemical degradation also appears to be involved in 2,4-D dissipation in the soil. The very low degradation rate of $^{14}$C-paraquat in soil may lead to its accumulation in soil.

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