

**FATE OF 2,4-D AS AFFECTED BY SOIL PROPERTIES AND
LANDSCAPE POSITION**

BY

NURUN NAHAR

**A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba
in partial fulfilment of the requirements for the Degree**

of

Masters of Science

**Department of Soil Science
University of Manitoba
Winnipeg, Manitoba, CANADA**

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ABSTRACT

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The objectives of this study was to determine the spatial variability of soil properties and 2,4-dichlorophenoxyacetic acid (2,4-D) sorption and mineralization within an agricultural field near Miami, Manitoba. The data were also used to determine the effect of soil properties on 2,4-D sorption and mineralization in soil.

A total of 96 soil cores (at a depth of 0-15 cm) were collected from a conventionally-tilled field and segregated into three different landscape position (upper-slope, middle-slope and lower-slope) by LandMapR software program (MacMillan and Pettapiece 2000). Soils were characterized for soil organic carbon (SOC) content, soil pH, soil texture and carbonate content. Batch equilibrium technique was used to determine soil-water partitioning coefficients (K_d) of 2,4-D. Soil microcosms were used to monitor 2,4-D mineralization.

Both soil organic carbon content (SOC) and 2,4-D sorption by soil significantly increased from upper-slope to middle-slope to lower-slope positions. Herbicide sorption was adequately predicted by SOC content, however, the prediction of K_d improved significantly when both SOC content and pH were taken into account when predicting herbicide sorption by linear regression. The level of 2,4-D sorption per unit organic carbon also varied among landscape positions due to differences in soil pH and/or organic matter quality.

First-order mineralization rate constants varied from 0.03 to 0.15 day⁻¹, corresponding to calculated mineralization half-lives of 24 and 4 days, respectively. Total mineralization and the mineralization rate constant of 2,4-D decreased from upper-slope to middle-slope to lower-slope positions. Total mineralization was negatively correlated to both SOC and K_d . The prediction of total 2,4-D mineralization and the mineralization rate constant was optimized by a linear regression model that contained SOC and soil pH.

Variability of soil properties within soil-landscapes affects herbicide sorption and degradation by soil. Therefore, the spatial variability of soil properties in different landscape position and their effects on 2,4-D sorption and mineralization must be taken into consideration in order to accurately predict the fate of 2,4-D in agricultural fields. The result of this study suggest that some weed management strategies such as the application of soil-residual herbicides may require some adjustments to account for the effects of SOC content and soil pH in different landscape position of the agricultural field to achieve improved weed control or/and reduced risk of herbicide off-site movement. Therefore, this study provides important information to the scientific community as well as producers to established a science-based management practices of herbicide application to achieve effective weed control while minimizing off-site movement of 2,4-D.

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1. INTRODUCTION

1
2 Pesticides are chemicals used in agriculture for the control of weeds, diseases, and insects,
3 thereby improving crop yield and quality. According to WWF (1999), 50 million Kg of
4 pesticides are being applied annually in Canada. Approximately 43 million Kg of
5 pesticides are being used in agriculture, whereby herbicides (chemicals used to control
6 weeds) account for approximately 77%. Huskes and Levsen (1997) calculated that, in some
7 cases, less than 3% of land applied herbicides actually reaches the target pest, such that
8 potentially a large portion may be transported from treated agricultural areas into the
9 broader environment endangering air, soil, and water quality as well as human health. So,
10 understanding the fate of herbicides in agricultural land is essential to ensure good
11 agricultural production as well as limiting off-site movement and potential environmental
12 contamination.

13 There are approximately 600 active ingredients and 6,000 formulated products registered
14 for use in Canada (WWF 1999). 2,4-Dichlorophenoxyacetic acid (2,4-D) is an active
15 ingredient in several herbicide formulations that are extensively used. In 1990, more than
16 3.8 million Kg of 2,4-D was used in the western Canada for the post-emergent control of
17 broadleaf weeds, particularly in cereal crops (Waite et al. 2002). 2,4-D is also
18 recommended for controlling the herbicide-tolerant canola volunteers (Simard and Legere
19 2002). Due to its widespread use and its low sorption potential in soil, 2,4-D has been
20 detected in both surface and ground waters throughout the North America (Goodrich et al.
21 1991; Waite et al. 1992; Rawn et al. 1999a; Cessna and Elliot 2004). In addition, the
22 persistence of 2,4-D residues in the soil may result in injury to sensitive crops, such as

1 sunflower, lentil, canola, and buckwheat (Wall 1996). Thus, knowledge of the fate of 2,4-D
2 in agricultural soils is important for both agronomic reasons and environmental protection.
3 In addition, reduction of environmental contamination is crucial as exposure of this
4 phenoxy herbicide may cause cancers in humans, especially non-Hodgkin's lymphoma
5 (Hoar et al. 1990; Duchnowicz et al. 2002). Therefore, the focus of this study is on the fate
6 of 2,4-D in soil following land application.

7 Sorption is the main process to predict herbicide fate, because this process determines the
8 persistence, leaching, and bioavailability of herbicides in soil (Bollage and Liu 1990;
9 Picton and Farenhorst 2004). Studies (Reddy and Gambrell 1987; Hermosin and Cornejo
10 1991) have suggested that soil organic carbon content is the single most important soil
11 characteristics influencing 2,4-D sorption. Other soil properties such as soil pH and clay
12 content also affect the amount of 2,4-D sorption by soil (Green and Karickhoff 1990).

13 Mineralization directly influences 2,4-D fate in soil by transforming (Cheng and Koskinen
14 1986). Soil microorganisms are responsible for the mineralization of 2,4-D in soil (Wu and
15 Nofziger 1999). So, the rate of 2,4-D mineralization largely depends on the presence of
16 competent microorganisms, the bioavailability of the compound and favourable
17 environmental conditions for microbial activity (Parker and Doxtader 1983; Ou 1984). The
18 rates of 2,4-D mineralization can vary considerably between different soil types,
19 particularly among those with different textural classes and soil organic matter contents
20 (Hassink 1994; Reimer 2004).

1 There is evidence that an increased sorption of 2,4-D in soil may decrease the availability
2 of the herbicide for mineralization by soil microbes (Moshier and Penner 1978; Ogram et
3 al. 1985). Ogram et al. (1985) showed that the 2,4-D degradation was limited by sorption
4 because the herbicide moved deep into the soil organic matter matrix making the herbicide
5 unavailable to breakdown by microorganisms. However, the effect of sorption on 2,4-D
6 biodegradation is not universal. Bolan et al. (1996) explained that soils with greater soil
7 organic matter content may have greater microbial activity therefore, greater 2,4-D
8 biodegradation rates.

9 Soil properties (e.g. soil organic carbon content, pH, texture, carbonate content) vary
10 largely depending on the landscape position of the field (Farenhorst et al. 2001). For
11 instance, soils at lower slope position tend to have greater soil organic matter content than
12 soils in upper or middle slope positions (Mallawatantri and Mulla 1992; Khakural et al.
13 1994). The variability of soil properties across landscape are due to the influence of
14 topography (Gerrard 1981), irregularities in parent material deposition (Goderya 1998) and
15 agricultural management practices (Gregorich and Anderson 1985; Lobb et al. 2003).
16 Cultivation of fields, especially in hilly landscapes, increases the spatial variability of soil
17 properties as soil loss occurs in the convex upper slope positions and soil accumulation
18 occurs in the concave lower slope positions due to tillage practices (Gregorich and
19 Anderson 1985; Arshad et al. 1990). In addition to tillage erosion, wind and water erosion
20 may increase spatial distribution of soil within agricultural fields (Lobb et al. 2003).
21 Therefore, persistence and mobility of 2,4-D may vary in field soils as sorption and
22 mineralization processes respond to the heterogeneity nature of soil properties as affected
23 by landscape position (Strebe and Talbert 2001; Farenhorst et al. 2003). The spatial

1 variability of soil properties in different landscape position must be taken into
2 consideration in order to accurately predict the fate of 2,4-D in agricultural fields.

3 In the past, soil properties (Walker et al.1968), soil erosion rates (Daniels et al. 1985),
4 fertilizer rate (Beckie et al. 1997; Solohub et al. 1999), and agricultural productivity (Stone
5 et al.1985) have been studied with respect to different landscape position. Spatial variation
6 of soil properties in different landscape position has been examined recently to explain the
7 variation of atrazine (Novak et al. 1997), imazethapyr (Oliviera et al. 1999) and
8 metolachlor (Wood et al. 1987) sorption. Limited studies (Farenhorst et al. 2003) focused
9 on the spatial variability of 2,4-D sorption within agricultural landscape. To this point, no
10 study has been reported on 2,4-D mineralization as affected by landscape position. An
11 understanding of the sorption and mineralization of 2,4-D across landscape position would
12 provide important information to develop a science based management practices to
13 minimise the off-site movement of 2,4-D. Therefore, the objective of this study was to
14 investigate the fate of 2,4-D as affected by soil properties and landscape position.

2. LITERATURE REVIEW

2.1 Fate of Pesticides in the Soil Environment

The fate of a pesticide in the environment is governed by sorption, transformation, transport processes, and the interaction of these processes. Sorption, however, is the most important process affecting pesticide fate (Koskinen and Harper 1990) since it regulates the rates and magnitude of transformation and transport processes (Clapp et al. 2001). Each of these processes will be discussed in the following sections.

2.1.1 Pesticide Sorption

Sorption is defined as the interaction between pesticides and soil constituents (e.g. mineral particles, organic matter) (Cheng 1990). Specifically, sorption refers to the ability of the soil to hold a pesticide or to prevent the molecule from moving within or outside of the soil matrix (Koskinen and Harper 1990). The sorption process determines whether a pesticide will persist or not, as well as whether or not it will be transported and become a pollutant (Wauchope et al. 2002). The extent and amount of pesticide sorption by soil is determined by the physical and chemical properties of the soil, as well as the chemical nature of the pesticide (Stevenson 1972).

Sorption of pesticides by soil occurs due to adsorption and absorption. Adsorption is considered a reversible process comprised of attraction of a chemical to the soil particle surface and retention of the chemical on the surface for a time that depends on the affinity of the chemical for the surface (Koskinen and Harper 1990). Various mechanisms explain the adsorption process (Stevenson 1972; Koskinen and Harper 1990; Senesi 1992),

1 including London van der Waals forces and hydrogen bonding. London van der Waals
2 forces consist of weak, short range dipolar or induced dipolar attractions that operate
3 through physical adsorption in all adsorbent-adsorbate reactions (Koskinen and Harper
4 1990; Senesi 1992). These forces occur frequently when pesticides, particularly those that
5 are non-ionic, come into contact with humic acid molecules (Senesi 1992). Since these
6 forces are additive, their contribution increases with the area of contact of interacting
7 molecules (Koskinen and Harper 1990; Senesi 1992). Hydrogen bonds can be formed when
8 oxygen and hydroxyl-containing functional groups are present in both soil organic matter
9 and the pesticide molecules (Senesi 1992). Hydrogen bonds are stronger dipole-dipole
10 interactions than van der Waals interactions (Koskinen and Harper 1990). Hydrogen bonds
11 are of great importance to the sorption of non-ionic pesticides by soil. Hydrogen bonds can
12 vary from weak bonding (2 to 4 kJ/mol) to strong bonding (60 kJ/mol) (Koskinen and
13 Harper 1990). In addition, absorption occurs due to hydrophobic sorption or partitioning
14 whereby pesticides move out of the soil water into the hydrophobic active sites of humic
15 substances (Senesi 1992). Hydrophobic sorption is considered to be an important binding
16 mechanism for nonpolar pesticides such as DDT, but also for more polar compound
17 (Senesi 1992).

18 Soil organic matter (SOM), is considered to be the most important factor controlling the
19 sorption of many pesticides (Wolfe et al. 1990; Benoit et al. 1999). Organic components in
20 soil include decomposed organic substances (humic substances), decomposing plant and
21 animal residues, and living soil organisms. Humic substances can be further classified into
22 humic acids, fulvic acids, and humin fraction (Collins et al. 1997). Of the three humic
23 substances, the humic acid fractions have the greatest effect on the capacity of soil to sorb

1 pesticides (Clapp et al. 2001; Senesi et al. 2001). The ability of humic acids to bind with
2 pesticides is mainly due to a high content of oxygen-containing functional groups,
3 including COOH, phenolic-, aliphatic- and enolic-OH, and C=O structures of various types
4 (Stevenson 1972). Shea (1989) described that sorption onto SOM is greatest for non-
5 ionizable, hydrophobic pesticides and least for weakly acidic, highly water-soluble
6 pesticides, but all pesticides have some affinity for organic matter.

7 Pesticide sorption in soil may cause a decrease in the biological activity of a pesticide and
8 its rate of biological degradation. However, it could enhance abiotic degradation due to
9 catalyzing hydrolysis (Stevenson 1994). Since sorption reduces the availability of
10 herbicides for plant uptake, it reduces the ability of soil-residual herbicides to control
11 weeds (Upchurch and Mason 1962). Therefore, greater soil organic matter content requires
12 an increase in the application rate of some herbicides (e.g. trifluralin) to ensure equivalent
13 efficacy of the herbicide (Crop Protection Guide 2000).

14 The most important inorganic solids for sorption are clay minerals (Koskinen and Harper
15 1990). Clay minerals can play a significant role in pesticide sorption particularly for ionic
16 pesticides. The 1:1 type of clay minerals, such as kaolinite, possess low surface area and
17 low negative charge, and have a lower capacity for sorption than 2:1 type clay minerals,
18 such as montmorillonite. For example, the surface area of montmorillonite is
19 approximately $800 \text{ m}^2\text{g}^{-1}$ and kaolinite is $20 \text{ m}^2\text{g}^{-1}$ (Brady and Weil 1999), therefore
20 montmorillonite is more reactive to cationic or ionizable weakly basic pesticides. The
21 inorganic hydroxyl groups are the most reactive functional groups on clay minerals
22 (Koskinen and Harper 1990). Sorption of pesticides can also take place in the interlayer

1 spaces of clay minerals (Glass 1987). Clays may have pH-dependent charges, and therefore
2 their ion exchange capacities can change with pH. The effect of soil pH on pesticide
3 sorption is also influenced by the chemical nature of the pesticide, because soil pH
4 regulates the chemical dissociation or protonation of weakly-acidic and weakly-basic
5 pesticide molecules. Depending on soil pH, clay minerals can repel the pesticides or form
6 complexes with the pesticides, thus affecting sorption (McConnell and Hossner 1985). In
7 addition to clay minerals, the presence of iron oxides is also known to influence pesticide
8 sorption (Reddy and Gambrell 1987; Madsen et al. 2000).

9 **2.1.2 Pesticide Transport**

10 Once pesticides are applied to soil, pesticides may be transported into surface and
11 groundwater. Surface run-off and soil erosion are the main driving forces for surface water
12 contamination, while leaching is responsible for transporting pesticides into groundwater.
13 Pesticide transportation plays a critical role in determining the fate of pesticides in the
14 environment. The following discussion reviews these transportation processes.

15 **2.1.2.1 Pesticide Surface run-off and Erosion** The off-site movement of pesticides
16 via surface runoff has been considered as one of the key process for surface water
17 contamination (Sarmah et al. 2004). Many factors such as pesticide properties, soil
18 properties, pesticide application rate, application technique, and application timing (i.e.
19 rainfall events following application) influence pesticide runoff losses (Zhang et al. 1997).
20 It is evident that the greatest pesticide runoff occurs with the first significant rainfall or
21 irrigation event following pesticide application (Wauchope 1978; Gouy et al. 1999). The
22 amount of pesticides lost by surface runoff is influenced by management practices

1 (Wauchope 1978). For example, pesticides that are incorporated into the soil surface are
2 less prone to transport by surface runoff than those that are surface-applied. Conservation
3 tillage practices that increase the amount of crop residues on the soil surface can reduce
4 risks of runoff and erosion, thereby reducing the risk of pesticide movement.

5 Even though loss of pesticides through surface runoff is a concern, the loss of pesticides
6 through surface runoff is generally small, i.e. on an average about 2% of the initial amount
7 applied (Leonard 1990; Wauchope et al. 1995). Moreover, Waite et al. (1992) conducted
8 studies in a small prairie watershed in Saskatchewan and demonstrated that pesticide
9 concentrations in surface waters (pond water) were lower than these found in ground water.
10 For the southern prairies, Nicholaichuk and Grover (1983) estimated that snowmelt
11 accounts for over 90% of the total amount of surface runoff occurring each year. Snowmelt
12 could contain significant amounts of pesticide residues.

13 **2.1.2.2 Pesticide Leaching** Leaching is the downward movement of pesticides with
14 water in soil. Pesticide leaching is influenced by the physical and chemical characteristics
15 of pesticides, method and time of application, and environmental conditions (Sarmah et al.
16 2004). The most important physical properties that affect pesticide leaching are the
17 pesticides sorption coefficient and water solubility (White and Kookana 1998). Greater
18 pesticide sorption by soil results in less leaching. Soils high in organic matter have less
19 pesticide leaching (Chesters et al. 1989) because of the strong sorption of pesticides by
20 organic matter. Soil pH also influences leaching by influencing pesticide sorption in soil
21 (Kookana et al. 1998). Pesticides with low water solubilities and strong sorption are not
22 readily leachable through the soil profile even if they are relatively persistent (Elliot et al.

1 2000). Boesten and van der Linden (1991) described that leaching of pesticide is very
2 sensitive to both sorption distribution coefficient per unit organic matter (K_{om}) and the
3 transformation rate. They found that changes in K_{om} or the transformation rate by a factor
4 of 2, changed the pesticide leached fraction by factor of 10.

5 Pesticide leaching is dependent on the time between pesticide application and rainfall
6 events (Flury 1996). The longer the time between pesticide application and a major rainfall
7 event, the less pesticide leaching will occur. This is related to an increasing amount of
8 pesticide being degraded, and a progressively greater sorption of pesticide residues onto
9 soil constituents. Repeated application of pesticides during the growing season maintains
10 high concentrations of pesticides in soil and therefore could result in high pesticide
11 leaching rates (Gold et al. 1988). Similarly, Rao and Davidson (1979) found that when the
12 concentration of pesticides in soil increased, the pesticide biodegradation rates decreased as
13 well as the likelihood that pesticide molecules sorbed onto the soil. As a result, the risk of
14 pesticide leaching increased.

15 Application time influences pesticide persistence. For example, Nicholaichuk and Grover
16 (1983) found that fall applications of herbicides resulted in noticeable increases in off-site
17 losses of 2,4-D because the rate of microbiological degradation slowed down in cooler
18 weather. Off course, the degree of pesticide leaching depends on the rate at which other
19 removal processes occur (Boesten and van der Linden 1991). If a pesticide is significantly
20 lost through other processes (such as volatilization), there may be little pesticide available
21 for leaching.

1 Tillage practices may impact pesticide leaching. Under long-term zero tillage, increased
2 infiltration resulted from reduced soil disturbance and the development of stable and
3 continuous macropores (Elliot and Efetha 1999). Conservation tilled soils preserve
4 preferential flow pathways such as earthworm burrows and root channels and therefore
5 lead to preferential flow of pesticides into the lower soil profile (Flury 1996). Roulier and
6 Jarvis (2003) observed rapid pesticide leaching through macropores in fine-textured hilltop
7 soils but less pesticide leaching due to absence of macropores in organic-matter-rich
8 depressional soils.

9 **2.1.2.3 Pesticide Volatilization** Volatilization of pesticides from soil is dependent on the
10 inherent vapour pressure of the pesticide, soil characteristics, climatic factors, as well as
11 land management and pesticide usage factors (Taylor and Spencer 1990). Volatilization
12 increases with increasing soil water contents (Walker and Bond 1977) and with increasing
13 soil temperatures (Wienhold et al. 1993). Pesticide volatilization rates are greatest with
14 surface applications of pesticide to moist soils or plant surfaces. Volatilization is greatly
15 reduced by the incorporation of pesticides into the soil (Taylor and Spencer 1990).

16 **2.1.3 Pesticide Transformation**

17 Pesticide transformation or degradation in soils occurs by three processes: photochemical,
18 chemical (abiotic), and microbial (biotic) (Guth 1981).

19 Photodegradation occurs when a pesticide molecule is irradiated by sunlight (Topp et al.
20 1997). Light can penetrate up to 1 cm depth in soils, depending on the soil texture and
21 compaction. Solar radiation can therefore only contribute towards the degradation of

1 pesticides present in the top 1 cm soil surface layer (Mansour and Korte 1986).
2 Photodegradation can occur by direct or indirect absorption of light (Choudhry and
3 Webster 1985). In direct photolysis, the pesticide absorbs UV-visible light energy,
4 therefore pesticide transformation occurs. In indirect photolysis, light energy is absorbed
5 by other constituents in soil, such as clay minerals and humic acids (Torrents et al. 1997).
6 When exposed to sunlight, both clay minerals and humic substances produce atomic
7 oxygen and hydroxyl radicals (Mansour et al. 1989), which, in turn, facilitate pesticide
8 transformation. Konstantinou et al. (2001) found that photodegradation in soil generally
9 increases with increasing soil organic matter content as it increases the formation of
10 reactive intermediates such as singlet oxygen or hydroxyl radicals.

11 Chemical degradation occurs when a pesticide molecule is chemically unstable in the
12 condition of its environment (Topp et al. 1997). Chemical processes, particularly
13 hydrolysis, are responsible for the degradation of many pesticides in soils (Wolfe et al.
14 1990). Hydrolysis is the cleavage by water of an intramolecular bond in the pesticide
15 molecule and formation of a new bond involving the oxygen atom of water (Wolfe et al.
16 1989). The hydrolysis reactions are commonly catalyzed by the presence of hydrogen or
17 hydroxide ions, and are therefore strongly pH dependent. Soil pH greatly influences
18 hydrolysis process because the aqueous solubility of some herbicide is pH-dependent (acid-
19 hydrolysed or base-hydrolysed) (Sarmah and Sabadie 2002). Sarmah and Sabadie (2002)
20 found that the sulfonylurea group of herbicides (weak acids) hydrolyzes rapidly in water at
21 acidic pH, but sulfonylurea remained fairly stable in neutral solutions. Other factors that
22 influence the hydrolysis process include soil organic matter (Stevenson 1982) and clay

1 content because these soil constituents increase the surface area available for enhancing
2 hydrolytic conversion (Yaron 1978).

3 Biodegradation is the transformation of pesticides by living microorganisms (Topp et al.
4 1997), and, for many pesticides, biodegradation is the most important process of
5 transformation (Bollag and Liu 1990). The rate of pesticide biodegradation depends on the
6 presence of pesticide degrading microorganisms, the bioavailability of the pesticide to
7 these microorganisms, and the occurrence of favourable environmental conditions for
8 microbial activity (Gonod et al. 2003). For example, environmental factors that affect the
9 activity of microorganisms are soil moisture, temperature, O₂ and nutrients, therefore these
10 factors also influence the rate and amount of pesticide biodegradation (Topp et al. 1997).
11 The bioavailability of pesticides to microorganisms plays an important role in the rate of
12 biodegradation of pesticides in soil. Pesticides may not be available to microorganisms due
13 to pesticide sorption by soil colloids (Scow 1993). Biodegradation of a pesticide may
14 involve one or a combination of transformation mechanisms and result in the construction
15 of various end products (Bollag and Liu 1990). Two types of main processes of microbial
16 degradation can be distinguished, metabolism and cometabolism. Metabolism refers to the
17 process, where the microbes can use the pesticide as a substrate for growth such that
18 transformation is completed for means of obtaining energy. This is a common way of
19 pesticide degradation in soil and it could lead to complete pesticide mineralization, i.e. the
20 transformation of a pesticide molecule to CO₂, water and inorganic ions (Topp et al. 1997).
21 In this process, little or no degradation occurs on exposure to the pesticides, but after a lag
22 phase, microbes adapt to use the added chemical as a source of carbon and energy. From an
23 environmental point of view, the complete metabolism of a pesticide is desired for

1 mitigating environmental contamination (Bollag and Liu 1990). Cometabolism is the
2 process by which the pesticide is not utilized for microbial nutritional benefit or energy, but
3 result in the formation of breakdown products that could be degraded further. Many
4 pesticides are incidentally transformed in soil by this mechanism (Felsot and Shelton
5 1993). In cometabolism, decomposition starts as soon as the pesticides reach the soil due to
6 catalysis by enzymes already present in the microbial population. Cometabolism generally
7 does not result in an extensive degradation of a certain substrate, but it is possible that
8 different microorganisms can transform a molecule by sequential cometabolic attacks or
9 that cometabolic products of one organism can be used by another as a growth substrate
10 (Bollag and Liu 1990).

11 Most pesticides require many different microbes before they are mineralized. Smith and
12 Paul (1990) showed that soils with high levels of microbial activity provide greater
13 pesticide degradation rates. However, Sparling et al. (1998) reported that knowledge of the
14 specific microbial biomass capable of degrading the specific pesticide was more important
15 than information on the total soil microbial biomass. Allan (1989) showed that previous
16 exposure to a certain pesticide enhances the biodegradation process of this or chemically-
17 related pesticides because of the adaptation of specific microbes to the chemical(s).
18 Additions of organic matter and fertilizer have been shown to increase pesticide
19 degradation, but only when they can encourage the growth of microorganisms that can
20 degrade the pesticides (Shea 1989).

2.2 The Spatial Variability of Soil Properties within Landscape

1
2 Soil formation involves additions and losses to soil, as well as translocation and
3 transformation of material within the soil (Greenland and Hayes 1981). Spatial variability
4 in soil-landscapes can be classified as intrinsic or extrinsic variability (Rao and Wagnet
5 1985). Intrinsic variability is due to natural variations of soil due to soil formation
6 processes, while extrinsic variability is the result of soil management practices (i.e. tillage,
7 fertilizer application techniques, irrigation practices etc.). Intrinsic variability of soil
8 properties across slope positions is caused by geological, pedological processes, and
9 irregularities in parent material deposition (Goderya 1998). Landscape morphology has a
10 strong influence on the nature and distribution of soil properties (Gerrard 1981; Gregorich
11 and Anderson 1985). For example, topography significantly effects the spatial distribution
12 of soil moisture, temperature, and organic matter in landscapes (Burt and Butcher 1985;
13 Moore et al. 1993), with resulting changes in soil properties in different landscape positions
14 (Pennock and de Jong 1990). Pennock et al. (1987) found that A- horizon depth and depth
15 to carbonates increased from shoulders to backslopes to footslopes landscape positions.

16 It has been demonstrated that the spatial variability of soil properties in agricultural fields is
17 more affected by extrinsic variability than intrinsic variability (Beckett and Webster 1971;
18 Dahiya et al. 1984). In particular, the redistribution of large quantities of soil occurs within
19 the landscape due to tillage practices. Tillage increases the spatial variability of soil
20 properties due to redistribution of soil from upper to lower slope positions (Gregorich et al.
21 1985). Tillage erosion is the main cause of the loss of organic- rich topsoil from the
22 divergent upper slopes to lower slope positions (Lindstrom et al. 1992; Lobb et al. 1995,

1 1999). Tillage also accelerates wind and water erosion due to the loosening of soil by
2 tillage erosion (Lobb et al. 2003). Wind and water erosion may increase spatial variability
3 in soil characteristics within agricultural fields (Lobb et al. 2003). Moulin et al. (1994)
4 studied the spatial distribution of soil properties, erosion, and crop yield along a transect in
5 a cultivated field and found that the spatial distribution of soil properties and crop yield had
6 been influenced mainly by tillage erosion. Other researchers have also observed that
7 erosion induced thin soils with low productivity in the upper convex slopes whereas
8 enhanced deposition of eroded sediments resulted in deeper soils in the lower slopes with
9 greater fertility (Miller et al. 1988; Fiez et al. 1994).

10 Soil characteristics such as SOC content, soil pH and clay content are the primary factors
11 that affect pesticide fate (Jury 1986; Hermosin and Cornejo 1991; Novak et al. 1997;
12 Farenhorst et al. 2001). Carbonate content has also been found to significantly affect the
13 sorption of pesticide (Gaultier et al. 2005). Therefore, it is important to review the
14 variability in these soil characteristics across landscape positions to further understand the
15 spatial variability of pesticide fate within agricultural fields.

16 **2.2.1 Soil Organic Carbon Content (SOC)**

17 SOC content regulates many processes through its influence on soil structural stability,
18 water holding capacity, nutrient bioavailability, buffering capacity, and soil biodiversity
19 (Bajracharya et al. 1997). Cultivation of agricultural landscapes by tillage mainly causes
20 the redistribution of SOC within the field (Arshad et al. 1990; Campbell et al. 1995; Elliot
21 and Efetha 1999). Researchers found that SOC was greater in the lower landscape position
22 than in the upper landscape position in agricultural fields (Mallawatantri and Mulla 1992;

1 Novak 1999; Farenhorst et al. 2001). Cultivation also greatly alters the amounts and quality
2 of SOC within agricultural landscapes. Mermut et al. (1983) observed a 41% reduction of
3 SOC content in cultivated land that had been cultivated for approximately 70 years
4 compared to grassland in Saskatchewan due to tillage erosion. Many studies have reported
5 greater amounts of soil organic carbon content under reduced or zero-tillage compared to
6 soil under conventional tillage (Havlin et al. 1990; Novak 1999). Intensive tillage results in
7 less crop residues on the soil surface, and also accelerates losses of organic rich topsoil by
8 wind and water erosion (Bowman et al. 1990; Arshad et al. 1990). Bowman et al. (1990)
9 studied a Colorado sandy loam soil that had been cultivated for 60 years and found that
10 total SOC in the surface layer (0-15 cm) had decreased by 62% and the labile organic
11 carbon fraction had declined by 72%, when compared to an adjacent virgin rangeland site.
12 Arshad et al. (1990) determined the effect of tillage on soil organic matter quality and
13 found that zero-tilled soil had a greater carbon content, nitrogen, carbohydrates, amino
14 acids, aliphatic carbon and had less aromatic carbon than the conventionally tilled soil.

15 **2.2.2 Soil pH**

16 The variation of soil pH at different landscape position could be attributed to different
17 cultivation and management practices such as non uniform N fertilizer application,
18 irrigation and tillage operations (Sarmah et al. 2004). Brubaker et al. (1993) found that pH
19 increased from upslope to downslope and with depth, which was attributed to calcareous
20 parent materials. They have also reported that the use of anhydrous ammonia fertilizer
21 contributed to a lower pH in the upper surface soils (30 cm). However, Mallawatantri and
22 Mulla (1992) reported that soil's pH decreased significantly from upper (7.12) to lower

1 (5.42) landscape position. The neutral pH in the upper slope position was possibly due to
2 exposure of subsoil material that was less acidic than the surface soil. The acidic pH in the
3 lower slope position resulted from long term fertilizer application (Mallawatantri and
4 Mulla 1992).

5 **2.2.3 Clay Content**

6 The influence of soil management on soil texture, such as clay content was found less
7 pronounced than other soil properties such as pH, and SOC content (Becket and Webster
8 1971). However, high erosion rates in the conventional soil management system remove
9 top soil along with organic matter and could exposed subsoils that are rich in clay content
10 (Reganold 1988). Mallawatantri and Mulla (1992) found that the clay content at the
11 midslope position was significantly higher than the bottom slope position, indicating that
12 subsoils have been exposed due to erosion. Campbell et al. (1996) reported a better positive
13 relationship between clay and SOM contents in no-tillage than in conventional tillage soils
14 at three sites in western Canada. In contrast, Paustian et al. (1997) found no relationship
15 between texture and the effects of tillage on SOM contents whereby data was collected
16 from twenty-seven long-term tillage trials.

17 **2.2.4 Carbonate Content**

18 The distributions of carbonates in soils are largely influenced by climate (Doner and Lynn
19 1989). For example, an increased rainfall will increase the leaching of carbonates from the
20 upper soil layers to the lower layers. Carbonates are relatively soluble in water and can be
21 easily redistributed within the soil either vertically or laterally depending on slope

1 (FitzPatrick 1980; Doner and Lynn 1989). Malo and Worcester (1975) observed a ring of
2 shallow carbonates at a footslope location adjacent to a depression. Moulin et al. (1994)
3 reported carbonate-rich surface soils due to upward water flux from “rego rings” close to
4 recharge depressions. Due to intensive tillage, higher amount of calcium carbonate were
5 also present in the knoll positions where subsoil material had been exposed by tillage
6 erosion. Simmons et al. (1989) observed that upper slope soils are often eroded and carbon
7 rich with relatively thinner A horizons (Fiez et al. 1994a), whereas footslope soils have
8 thicker A horizons and deeper carbonates. Depth to carbonates is high at locations where
9 net downward infiltration is high and sustained, e.g., in footslope and toeslope positions.
10 However, local hydrologic conditions may result in high carbonate content in convergent
11 landscape positions, since depth to carbonates is also a function of water table dynamics
12 (Richardson et al. 1992).

13 **2.3 Environmental Concerns**

14 Pesticide residues have been found in the earth’s atmosphere with greater pesticide
15 concentrations in areas where they are applied (Pimentel and Levitan 1986). Wetlands near
16 cultivated fields are at risk for pesticide contamination due to pesticide use in agricultural
17 fields (Donald et al. 1999). A range of pesticides have been also detected in farm dugouts
18 in western Canada (Cessna and Elliott 2004). In addition, the contamination of
19 groundwater by pesticide is a major concern because our society largely depends on ground
20 water for drinking and household use, and in addition, groundwater is being used for
21 agricultural and recreational purposes. The Environmental Protection Agency (EPA, USA)
22 listed 46 pesticides as confirmed groundwater contaminants in many different states. The

1 presence of pesticides in the wells was directly attributed to their agricultural use (Williams
2 et al. 1988). Agriculture Canada tested 1300 farm wells across Ontario in 1991-92 and
3 found that 8% of these tested wells had detectable levels of pesticides (Rudolph and Gross
4 1993). Similarly, Miller et al. (1995) found residues of MCPA, bromoxynil and diclofop in
5 Alberta groundwater, where the concentrations of bromoxynil and diclofop exceeded the
6 levels of Canadian drinking water guidelines. Groundwater contamination is a long-term
7 concern because pesticide degradation rates are very slow in groundwater due to cold water
8 temperatures, low microbial activities and low oxygen contents. DDT has been detected in
9 groundwater many years after its last use (Wade et al. 1998).

10 2,4-D was detected in 52 of 805 raw and treated drinking water samples collected from
11 municipal and private supplies in six Canadian provinces from 1971 to 1986 (Health
12 Canada 1991). The maximum concentration found was $29 \mu\text{g L}^{-1}$. Seventy-eight of 447
13 samples of surface waters in Canadian agricultural areas surveyed from 1981 to 1985 had
14 detectable concentrations of 2,4-D which ranged from 0.01 to $0.7 \mu\text{g L}^{-1}$ (Frank and Logan
15 1988). Currie and Williamson (1995) examined several southern Manitoba river waters and
16 found that 2,4-D was the most frequently detected herbicide. However, only 0.1% of the
17 samples exceeded ($> 4 \mu\text{g L}^{-1}$) aquatic water quality guideline (Currie and Williamson
18 1995). 2,4-D has also been detected in 32 to 67% of air samples taken near Regina,
19 Saskatchewan depending on growing season (Waite et al. 1995). Average 2,4-D deposition
20 over the growing season (May-August) was $93.3 \mu\text{g m}^{-2}$. The maximum amounts of 2,4-D
21 in precipitation and air coincides with local application use (Waite et al. 1995; Rawn et al.
22 1999). Rawn et al. (1999) collected rainfall over four years (1993-1996) from southern

1 Manitoba watershed area and reported 0.0033 to 0.43 $\mu\text{g L}^{-1}$ of 2,4-D yearly in rainfall
2 water they tested. 2,4-D was the most ubiquitous herbicide in Alberta rainfall and the
3 concentration of 2,4-D in Alberta rainfall frequently exceeded the Canadian Irrigation
4 Water Quality Guidelines (Hill et al. 2001). Hill et al. (2001) detected notable levels of 2,4-
5 D in the Alberta atmosphere as well. 2,4-D is a widely found contaminant in air, rainfall,
6 surface waters and groundwater; therefore my research will focus on 2,4-D.

7 2.4 2,4-Dichlorophenoxyacetic Acid (2,4-D)

8 The herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) (Figure 1) was first registered in
9 Canada in 1946. This phenoxy herbicide is still extensively used for controlling broadleaf
10 weed in agriculture and pasture, as well as for controlling aquatic vegetation. 2,4-D mimics
11 the effect of auxins, it overstimulates plant growth and causes death of plants (Vencill
12 2002). The predominant use of 2,4-D in Canada is in cereal crop production, where it is
13 typically tank mixed with other herbicides such as, dicamba, MCPA, triclopyr, picloram
14 and mecoprop (AAFC 1994). Recently, 2,4-D and 2,4-D tank mixes have been used as pre-
15 seed burnoff treatments to eliminate glyphosate-tolerant canola volunteers (Simard and
16 Legere 2002).

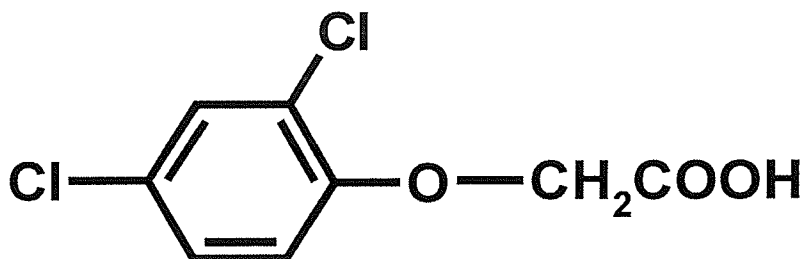


Figure 2.1 2,4-D molecule

1 The fate of 2,4-D in soil is governed by its physico-chemical properties (Table 2.1). Due to
 2 its relatively high water solubility and low sorption capacity in soil particles, the potential
 3 of 2,4-D runoff and leaching exists. As a result, 2,4-D has been detected in both surface
 4 and ground water throughout North America (Goodrich et al. 1991; Waite et al. 1992;
 5 Rawn et al. 1999a).

6 Table 2.1 The physico- chemical properties of 2,4-D (Ahrens 1994)

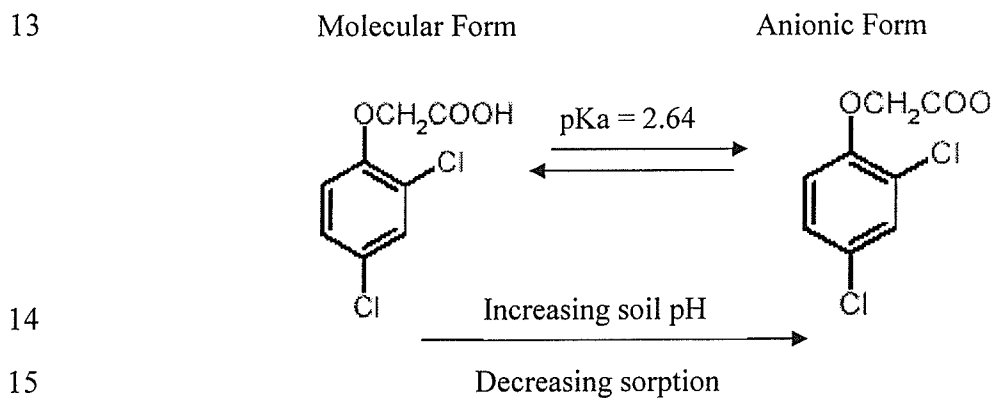
Molecular weight	Water Solubility (mg L ⁻¹)	Vapor pressure (mm Hg)	pK _a	Soil half-life (Days)	K _{ow} (a)
221.04	900	1.4 x 10 ⁻⁷ at 25° C	2.8	10	2.81 (b)

7 (a) is the 2,4-D octanol-water partitioning coefficient
 8 (b) is from the British Crop Protection Council (1987)
 9

10 **2.4.1 2,4-D Sorption**

11 Soil organic carbon content is the single most important soil characteristic influencing 2,4-
 12 D sorption in soils, such that there is a strong positive correlation between soil organic
 13 carbon content and the amount of 2,4-D sorption by soil (Grover 1977; Reddy and
 14 Gambrell 1987; Hermosin and Cornejo 1991; Johnson et al. 1995a; Wu et al. 2000). Other
 15 soil properties, such as soil pH and clay content also affect 2,4-D sorption (Reddy and
 16 Gambrell 1987; Green and Karickhoff 1990; Hermosin and Cornejo 1991). Hermosin and
 17 Cornejo (1991) reported that high soil organic matter content favoured the sorption of 2,4-
 18 D, while high pH and high clay content decreased 2,4-D sorption. 2,4-D is a carboxylic
 19 acid herbicide, with a pKa of 2.5- 2.7 (Wauchope et al. 1992). It becomes predominantly
 20 anionic when the pH of the soil is above 3.5 (Vasudevan and Cooper 2004) (Figure 2).

1 Sorption decreases as soil pH increases due to the lower adsorption potential of the
2 dissociated anionic form compared with the undissociated molecular form (Nicholls and
3 Evans 1991). At lower pH ($\text{pH} < 3$), the undissociated form of 2,4-D predominates and
4 sorption occurs through hydrophobic interactions, whereas at higher soil pH, little bonding
5 with soil occurs because of repulsion of the anionic 2,4-D molecule by the negatively
6 charged soil (Wu et al. 2000). Low pH can cause hydrolysis of clays, transforming Al^{3+} and
7 Fe^{3+} ions to hydroxides, which is responsible for observed increases in 2,4-D adsorption at
8 low pH (Koskinen and Harper 1990). Hermosin and Cornejo (1991) found that 2,4-D
9 sorption decreased as soil pH increased from 6.3 to 8.3. When soil pH is near the pKa
10 value, the 2,4-D sorption by soil is sensitive to changes in soil pH, however, above soil pH
11 of 5, 2,4-D sorption per unit organic carbon (K_{oc}) is only slightly affected by pH (Wu et al.
12 2000). Sannino et al. (1997) found no sorption of 2,4-D onto clay minerals at neutral pH.



16 Figure 2.2 The dissociation of 2,4-D with rising pH

17

18 The presence of free iron oxides is also known to influence 2,4-D sorption (Reddy and
19 Gambrell 1987; Hermosin and Cornejo 1991; Madsen et al. 2000). Hermosin and Cornejo

1 (1991) found that in soils with low free iron content (<5%), 2,4-D was not strongly
2 correlated with soil organic matter content, but sorption increased with increasing sand
3 content, free iron contents, clay content and calcium content. However, for soils with
4 greater iron content (>5%), soil organic matter had a large influence on 2,4-D sorption.
5 Other factors such as soil phosphate and exchangeable aluminum content are additional
6 properties that play a significant role in 2,4-D sorption (Vasudevan and Cooper 2004).
7 Vasudevan and Cooper (2004) studied 2,4-D sorption in North Carolina soil and found that
8 2,4-D sorption was lower in the presence of phosphate, due to competition between
9 phosphate and 2,4-D for surface sites. 2,4-D sorption was greater in the presence of
10 exchangeable aluminum as 2,4-D may form surface complexes with exchangeable
11 aluminum in the soil (Vasudevan and Cooper 2004).

12 **2.4.2 2,4-D Degradation**

13 Johnson et al. (1995 *a, b*) reported that 2,4-D degradation rates in soils remained relatively
14 constant with and without sunlight, suggesting that photodegradation is not an important
15 process for 2,4-D degradation. Soil microorganisms are the principal factor that causes 2,4-
16 D degradation in soils (Loos 1975; Wu and Nofziger 1999). 2,4-D biodegradation is
17 typically done either by specific degraders using the compound as a C source or by
18 populations acting by co-metabolism (Soulas 1993; Robertson and Alexander 1994). Under
19 conditions in which 2,4-D is degraded co-metabolically, the availability of nutrients from
20 sources such as fertilizers and soil organic matter may control biodegradation (Gonod et al.
21 2003). A number of soil fungi and bacteria have been isolated that have the ability to
22 degrade 2,4-D in soil environments (Bollag et al. 1968; Donnely et al. 1993). Some

1 bacterial species capable of degrading 2,4-D in the soil environment are *Pseudomonas*,
2 *Rhizobium* and *Arthrobacter* (Loos et al. 1967; Bollag et al. 1968). Donnely et al. (1993)
3 found that mycorrhizal and nonmycorrhizal fungi can degrade 2,4-D, primarily by
4 incorporating the carbon from the herbicide into tissue. *Phanerochaete chrysosporium* was
5 the best 2,4-D degrading mycorrhizal fungi (Donnely et al. 1993).

6 2,4-D mineralization rates were rapid following herbicide application and then decreased
7 over time to a constant mineralization rate (Willems et al. 1996). Maximum mineralization
8 rates are usually obtained within the first 20 days of incubation and may coincide with
9 maximum amounts of readily-mineralizable 2,4-D metabolites in soil (Willems et al. 1996).
10 The final very slow mineralization rate may reflect the mineralization of more persistent
11 metabolites, or the mineralization of bound 2,4-D residues in microbial biomass and
12 organic matter (Soulas 1993). Barriuso et al. (1997) found 45-56 % of the initial 2,4-D
13 radioactivity as bound residues at the end of a 250 day incubation study. Ogram et al.
14 (1985) found that 30% of 2,4-D was not mineralized as a result of C incorporation into
15 microbial biomass. They also found that sorption limits 2,4-D degradation as the herbicide
16 moved inside the soil matrix and therefore was protected from degradation. Benoit et al.
17 (1999) argued that the protection of chemicals from microbial attack due to sorption can
18 not be generalized because the effects of sorption greatly depends on the nature of
19 chemicals and sorbent. Mineralization of sorbed 2,4-D was higher in humic acid than in
20 fresh or partially humified organic matter like wood or straw, because 2,4-D desorbs more
21 easily from an acidic, polar sorbent like humic acid than from more aromatic or
22 hydrophobic sorbents like lignin or straw (Benoit et al. 1999). Furthermore, they showed an
23 increase of 2,4-D mineralization rates onto nondecomposed or decomposed straw relative

1 to direct applications of 2,4-D onto soil because straw contained easily available carbon
2 and active microbes which enhanced mineralization rate.

3 Studies on 2,4-D mineralization have shown an initial lag phase (Audus 1960; Veeh et al.
4 1996) which is a period of adaptation in which the enzymes needed for decomposition of
5 2,4-D and its metabolites are synthesized from closely related enzymes. An initial lag
6 phase often occurs in soils that had no prior exposure to 2,4-D. Soils never treated with 2,4-
7 D contain a low but significant amount of microbes capable of degrading 2,4-D (Ou 1984;
8 Shaw and Burns 1998). Repeated applications of 2,4-D have been shown to enhance the
9 number of these microbes and the rate of herbicide degradation (Biederbeck et al. 1987).
10 Robertson and Alexander (1994) found that 2,4-D mineralization began with a lag phase
11 when first applied to soil, while mineralization was faster and without a lag phase
12 following the second application.

13 2,4-D mineralization was found to be poorly correlated with SOC content in surface soils
14 (Veeh et al. 1996; Voos and Groffman 1997). Many researchers observed that pesticide
15 degradation rates decrease with soil depth and they suggested that the lower degradation
16 rate in subsurface soil is due to decreasing soil temperature, SOC content, and microbial
17 populations with depth (Moorman and Harper 1989; Pothuluri et al. 1990; Adams and
18 Thurman 1991). Willems et al. (1996) reported that 2,4-D mineralization rates were four
19 times greater at depths between 1 and 1.5 m than at the soil surface layer. They explained
20 that a considerable amount of readily available SOC exists in the surface layer, which
21 microbes might prefer to use in preference to 2,4-D.

1 2,4-D is readily degraded by soil microorganisms under favourable moisture and
2 temperature regimes. Rates of 2,4-D mineralization were found to be greater in the spring
3 and fall than in the summer and winter, reflecting the greater moisture availability during
4 the spring and fall (Entry and Emmingham 1996). Han and New (1994) have shown that
5 2,4-D degradation is greatest at moisture contents close to field capacity, and 2,4-D
6 degrades less rapidly at moisture contents greater than saturation (Lavy et al. 1973). Parker
7 and Doxtader (1983) reported that the optimum temperature for 2,4-D degradation is about
8 27° C. A major drop in 2,4-D mineralization rates occurred below 7° C, whereas
9 mineralization rates increased twice as temperature increased from 10 to 20° C (Willems et
10 al. 1996). Ou (1984) found that the degradation rate of 2,4-D was reduced at 35° C
11 compared with 25° C. In Manitoba, where mean annual temperature of the soil is 3 to 5° C,
12 (Environment Canada 2005) an increased persistence of 2,4-D can be expected due to
13 decreased mineralization rate at lower temperature.

14 There is some evidence that fertilizer applications influences 2,4-D degradation rates in
15 soils. Duah-Yentumi et al. (1982) found that 2,4-D degradation was enhanced in soils with
16 800 mg P/Kg. Whereas Entry et al. (1993) found that high concentrations of N (250 Kg N
17 ha⁻¹) addition suppressed 2,4-D mineralization in pasture soils. High concentration of
18 nitrogen also inhibited 2,4-D mineralization by fungi in vitro (Donnelly et al. 1993).
19 Similarly, Picton and Farenhorst (2004) found total 2,4-D mineralization and rates were
20 negatively correlated to NO₃⁻ content in Manitoba soils.

1 **2.4.3 Variation of 2,4-D Sorption and Mineralization within Landscape**

2 Farenhorst et al. (2001, 2003) observed variations in 2,4-D sorption in agricultural soils due
3 to the heterogeneous nature of soil properties across the slope positions. This spatial
4 variability of soil properties within agricultural fields occurs due to the redistribution of
5 topsoil from upper to lower slope positions by tillage, wind and water erosion (Gregorich et
6 al. 1985; Lobb et al. 2003).

7 The spatial distribution of pesticide sorption within landscape has only been studied for a
8 few pesticides. Novak et al. (1997) examined spatial variability of atrazine sorption at the
9 field scale in an Iowa agricultural landscape and found that atrazine sorption increased
10 from shoulders < level < foot and back slopes < depressional areas. The strength of atrazine
11 sorption in different landscape positions was positively correlated to the variations in SOC,
12 pH, and clay content at landscape positions. Similarly, Mallawatantri and Mulla (1992)
13 studied the effect of soil properties on metribuzin, diuron, 2,4-D and triallate sorption at
14 different landscape positions. They observed significant decreases in herbicide sorption at
15 top-slope positions relative to bottom-slope positions, reflecting the increase of SOC from
16 top to bottom slope position. Oliveira et al. (1999) studied the spatial variability of the
17 imazethapyr sorption in Minnesota soil and they found that imazethapyr sorption was
18 predominantly influenced by soil pH, but under acidic conditions, SOC content did have
19 some effect. Oliveira et al. (1999) reported that landscape can be separated into two
20 potential management areas (areas with soil pH > 6.25 and $K_d < 1.5$ and areas with soil pH
21 < 6.25 and $K_d > 1.5$), which allowed them to identify the areas in the field where
22 imazethapyr sorption would be minimal and where there is a greater potential for leaching.

1 Therefore, the importance the effect of landscape position on the sorption of pesticides has
2 been well established.

3 The spatial variability of pesticide degradation has received less attention. Several
4 researchers studied the relation between microbial biomass and 2,4-D degradation and they
5 reported that 2,4-D mineralization was poorly correlated with microbial biomass (Entry
6 and Emmingham 1996; Willems et al. 1996). However, Veeh et al. (1996) found a positive
7 relation between 2,4-D degradation and bacterial number. Although there is evidence of
8 spatial variation of microbial population in different landscape position (Zak et al. 1994;
9 Broughton and Gross 2000; Sveshnikova et al. 2001), little is known regarding the
10 variation in pesticide degradation across the landscape. Walker et al. (2002) studied the
11 variation in isoproturon degradation rates at the field level and reported that half-life varied
12 from 6 to 30 days. Walker et al. (2001) suggested that the variability in the isoproturon
13 degradation was related to microbial biomass and diversity, which appeared to be
14 influenced by pH at the field scale. To this point, no research has been conducted to
15 characterise the spatial variability of soil properties within agricultural fields that affects
16 2,4-D degradation in soil. The rate of 2,4-D mineralization has been recognized as an
17 important factor to include in risk assessments for pesticide leaching (Rao et al. 1985;
18 Boesten 2000). Therefore, knowledge of spatial variability of 2,4-D sorption and
19 mineralization is important to minimize environmental and agronomic concerns associated
20 with agricultural use.

1 **3. 2,4-D SORPTION AS AFFECTED BY LANDSCAPE POSITION IN**
2 **A CONVENTIONALLY TILLED AGRICULTURAL FIELD**

3 **3.1 Abstract**

4 This study examined the spatial distribution of soil properties in a topographically diverse
5 landscape to explore their impact on 2,4-D sorption in a conventionally tilled site near
6 Deerwood, Manitoba. In total, 96 soil samples (0-15 cm) were collected using a grid
7 spacing of 50 m in a 25-ha area (500 m length × 500 m width). 2,4-D sorption was
8 determined by the batch equilibrium method and then correlated with four soil properties
9 (SOC content, soil pH, soil texture, and total carbonate content) as well as landscape
10 position (upper, middle, and lower slope position) as classified by LandMapR software.

11 The soil water partitioning coefficient (K_d), a measure of herbicide sorption per unit soil,
12 increased significantly in the order of upper < middle < lower slope positions. Within each
13 of the slope positions, K_d increased significantly with increasing SOC content, and with
14 decreasing soil pH. The amount of 2,4-D sorbed per unit organic carbon (K_{oc}) also
15 increased significantly in the order of middle > upper > lower slope positions probably due
16 to differences in soil pH, clay content and organic matter quality among slope positions.

17 Predicting K_d with soil properties using multiple linear regression analysis revealed that
18 SOC content as an independent variable was able to predict sorption adequately for the
19 whole field ($R^2 = 0.59$). In terms of upper, middle and lower slope positions, SOC content
20 also predicted sorption adequately with $R^2 = 0.41$, 0.51 , and 0.47 , respectively. The
21 prediction of K_d improved significantly when SOC content and pH were simultaneously

1 taken into account either for the whole field ($R^2 = 0.78$) or for within slope positions ($R^2 =$
2 0.74, 0.84, and 0.73 for upper, middle and lower slope positions respectively). Adding soil
3 texture or carbonate content did not improve the K_d prediction significantly. Soil pH was
4 moderately able to predict K_{oc} as a single variable, both for the whole field ($R^2 = 0.68$) and
5 for within slope positions ($R^2 = 0.44, 0.75, \text{ and } 0.44$ for upper, middle and lower slope
6 positions respectively). Adding SOC content, carbonate content and/or soil texture did not
7 improve the K_{oc} prediction considerably.

8 **3.2 Introduction**

9 On the Canadian Prairies, agriculture is a major industry where most of the cultivated land
10 is treated with pesticides (Waite et al. 2002). The increasing dependency on pesticides for
11 crop production has resulted in environmental contamination (Mass et al. 1995; Miller et
12 al. 1995a; Waite et al. 1995; Rawn et al. 1999b) with potential adverse risks to human
13 health (Lampman 1995; Duchnowicz et al. 2002). This has raised considerable concern to
14 the government, the agricultural community, and environmental interest groups which, in
15 turn, have intensified the investigation of cause-and-effect issues of pesticide
16 contamination from agricultural uses.

17 The herbicide 2,4-D [2,4-(dichlorophenoxy) acetic acid] is the most widely-used herbicide
18 in Canada for the post-emergent control of broadleaf weeds (Waite et al. 2002). Although
19 this herbicide is mostly applied to the foliage, a large amount is deposited onto the soil
20 during application, or is washed off from foliar surfaces by rain and precipitation. The
21 herbicide 2,4-D is mobile in the soil (Parker and Doxtader 1982) due to low sorptive

1 capacity and it has been found in surface and ground water (Waite et al. 1992; Currie and
2 Williamson 1995; Johnson et al. 1995; Miller et al. 1995*b*; Rawn et al. 1999*a*). Because of
3 2,4-D mobility, it is important to have a good understanding of the behavior of the
4 herbicide in agricultural soils for minimizing the adverse environmental impact of the
5 herbicide.

6 Sorption is the main process used to predict herbicide fate, because this process determines
7 the persistence, leaching, and bio-availability of herbicides in soil (Bollage and Liu 1990).
8 For example, sorption can decrease or minimize pesticide availability for biodegradation
9 and transport (Koskinen and Harper 1990; Greer and Shelton 1992; Clapp et al. 2001).

10 A common parameter that is used for expressing sorption is the soil–water partitioning
11 coefficient, K_d (Wagnet and Rao 1985; Coquet and Barriuso 2002; Wauchope et al. 2002),
12 which is the ratio of pesticide sorbed onto soil to pesticide remaining in solution. As such,
13 K_d is an indication of the sorption capacity of a given chemical by a specific soil. K_d is
14 commonly determined using the batch or slurry method (Wauchope et al. 2002).

15 SOC content is the dominant factor in determining pesticide sorption by soil, thus
16 variability in K_d across a wide range of soils can generally be reduced by normalising it to
17 the SOC content (Hamaker and Thompson 1972). This gives the organic carbon sorption
18 partition coefficient, K_{oc} , which is universally used as a measure of the relative mobility of
19 pesticides in soils (Koskinen and Harper 1990; Coquet and Barriuso 2002). Gerstl (2000)
20 stated that variability in K_{oc} is generally due to experimental errors or a variation in the
21 nature of the soil organic matter. Also other scientists (Coquet and Barriuso 2002) reported

1 that differences in K_{oc} values across soils are due to variations in the nature of the soil
2 organic matter.

3 Soil properties vary both spatially and temporally within soil-landscapes because of soil
4 formation processes, as well as agricultural management practices such as tillage (Goderya
5 1998; Oliveira Jr. et al. 1999). For example, soils at the lower slope positions tend to have
6 greater soil organic matter content than soils in the upper or middle slope positions
7 (Mallawatantri and Mulla 1992; Khakural et al. 1994; Farenhorst et al. 2001). Differences
8 in soil properties amongst slope positions across the landscape may affect herbicide
9 persistence and mobility, as sorption depends on soil properties (Farenhorst et al. 2003).

10 Understanding the spatial variability of herbicide sorption in a topographically diverse
11 landscape is important to reduce off-site movement of herbicides. The aim of this research
12 was to quantify the spatial distribution of soil properties in a conventionally tilled soil, as
13 well as to assess the impact of soil properties on 2,4-D sorption.

14 **3.3 Materials and Methods**

15 **3.3.1 Study Site**

16 The study was conducted in a sandy loam (soil) agricultural field near Deerwood, Miami
17 (NE-6-5-7W), about 150 km south-west of Winnipeg, Manitoba, Canada. According to the
18 Soil Landscape of Canada system, the field is in polygon 460072, its dominant slope
19 gradient ranges from 4-9%, its dominant local surface form is dissected (70%), and its
20 subdominant local surface form is ridged (30%). The soil landscape is characteristic of a

1 broad region of undulating-to-hummocky glacial till landscapes in western Canada
2 (Clayton et al. 1977). This study area represents 10% of Manitoba soil-landscape (Glenn
3 Lelyk 2005 person. Comm.). The soil is characterized as a Dark Grey Chernozem (Soil
4 Classification Working Group 1998), developed on shale and calcareous glacial till. Upper
5 and middle slope positions have well drained soils which belong to the Dezwood loam
6 series (Orthic Dark Grey Chernozem), whereas lower and depressional positions have
7 imperfectly drained soils which belong to the Zaplin soil series (Gleyed Dark Grey
8 Chernozem). Since 1928, the study area has been continuously planted to a wheat-oilseed
9 crop rotation with conventional tillage by deep tilling in the fall and disking and harrowing
10 in the spring. For the past 40 years, 2,4-D has been used in addition to other pesticides.

11 **3.3.2 Soil Sampling and Analyses**

12 The field was surveyed using a Trimble AgGPS 214 global positioning system (GPS) at a
13 spacing of 5 m in hummocky portions (covering two-third of the field) and a spacing of 10
14 m in the leveled portions of the field. The Trimble AgGPS 214 is accurate to 1 cm.
15 Georeferenced points surveyed by GPS were used to develop digital elevation model
16 (DEM) of the study site. Elevation of the field ranged from 440 to 460 m above the sea
17 level.

18 Soil samples (0-15 cm) were collected using a grid spacing of 50 m in a 25-ha area (500 m
19 length \times 500 m width) (Figure 3.1). Thus, a total of 100 samples were collected from a 10
20 by 10 grid across the landscape. The grid points were surveyed by Total Station (Sokkia set
21 4110), using GPS reference points. The Sokkia 4110 Total Station is accurate to 1 mm.
22 Using the GPS and Total Station output, sample points were classified according to their

1 slope position by LandMapR software program (MacMillan and Pettapiece 2000). Samples
2 were delineated into one of three different Landform Element Complexes (LECs): upper,
3 middle, and lower slope positions. Out of 100 samples, three samples went missing and one
4 sample was discarded since that sampling point was an isolated sample in a depressional
5 area. According to the LandMapR, 12 samples were in the upper slope, 55 samples were in
6 the middle slope, and remaining 29 samples were in the lower slope positions, comprising
7 13, 57, and 30% of the total 96 sampling points.

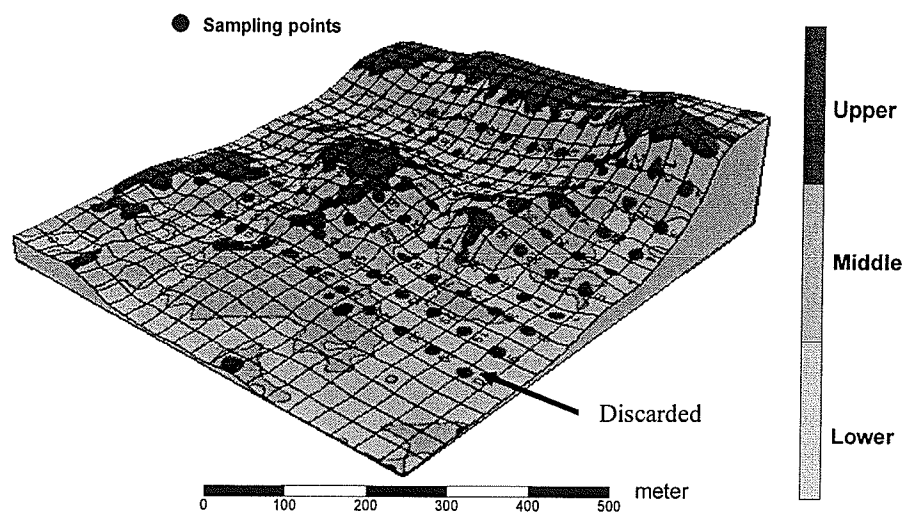


Figure 3.1 Study site segmented by LandMapR

8

9 Soil samples were air-dried and sieved (<2 mm) prior to quantifying soil properties (i.e.,
10 soil particle size or texture, soil pH, SOC content and carbonate content). Soil texture was
11 measured using the hydrometer method (Gee and Bauder 1986). Soil pH was determined
12 using 20 ml of 0.01M CaCl₂ and 10 g air-dried soil (McKeague 1978). For the SOC content
13 analyses, inorganic carbon was removed by digestion with 6N HCl (Tiessen et al. 1983),
14 and organic carbon content was determined by dry combustion of 0.12 g oven-dried soil
15 using a Leco model CHN 600 C and N determinator (Nelson and Sommers 1982). Total

1 carbonate content was determined using a volumetric calcimeter that measured evolved
2 carbon dioxide upon addition of 6N HCl-FeCl₂ to a soil sample (Loeppert and Suarez
3 1996).

4 **3.3.3 Batch Equilibrium Experiments**

5 Batch equilibrium technique was used to determine K_d and K_{oc} . The herbicide 2,4-D stock
6 solution were prepared in 0.01 M CaCl₂ by mixing ¹⁴C-ring labeled 2,4-D (99%
7 radiochemical purity; sp. act. 250 μ Ci ; Sigma Aldrich Chemical Company, St. Louis, MO)
8 with analytical-grade 2,4-D (95% chemical purity, Sigma Chemical Co., St. Louis, MO) to
9 have an initial concentration of 1 μ g ml⁻¹ active ingredient and 16.7 Bq ml⁻¹ radioactivity.
10 10 ml of herbicide solution was added to 5 g of sieved and air-dried soil in 50 ml Teflon
11 centrifuge tubes. Tubes were then shaken in a rotary shaker for 24 hours at room
12 temperature, in a dark place to reach equilibrium. Previous batch experiments have shown
13 that 2,4-D sorption on soil occurs rapidly, with most of the herbicide being removed from
14 solution within the first few hours, and that a 24 hour equilibrium period is sufficient to
15 characterize the second phase of slow sorption (Sannino et al. 1997; Wauchope et al.
16 2002). Preliminary testing showed that 2,4-D did not adsorb onto the Teflon centrifuge
17 tubes (Stephens 2003).

18 After 24 hours, the soil slurries were centrifuged at 10 000 RPM for 10 minutes. To
19 measure the concentration of herbicides remaining in solution, 1 ml supernatant was
20 subsampled in duplicate. A Liquid Scintillation Counter (LSC) (LS 7500 Beckman
21 Instruments, Fullerton, CA) was used to measure the amount of ¹⁴C 2,4-D in equilibrium

1 solutions by adding 5.0 ml of ScintiSafe 30% scintillation cocktail (Fisher, Fairlawn NJ) to
2 subsampled (1.0 ml) supernatant.

3 The amount of herbicide sorbed onto soil was determined by the difference between the
4 initial and equilibrium herbicide concentrations. The soil-water partitioning coefficient
5 (sorption coefficient), K_d (ml g^{-1}) was calculated as follows:

$$6 \quad K_d = C_s / C_e \quad (3.1)$$

7 where C_s = the amount of 2,4-D sorbed by the soil at equilibrium ($\mu\text{g g}^{-1}$), and C_e = the
8 concentration of 2,4-D in the solution at equilibrium ($\mu\text{g ml}^{-1}$).

9 The amount of 2,4-D sorption per unit soil organic carbon, i.e. the organic carbon
10 partitioning coefficient, K_{oc} (ml g^{-1}) was calculated as:

$$11 \quad K_{oc} = \left(K_d / \% \text{ soil organic carbon content} \right) \times 100 \quad (3.2)$$

12 Higher K_d values indicate greater sorption per unit soil (all soil properties combined),
13 relative to smaller K_d values, and higher K_{oc} values indicate greater sorption per unit
14 organic carbon, relative to smaller K_{oc} values.

15 3.3.4 Data Interpretation and Statistical Methods

16 The Landform Element Complexes classifications which are calculated by a landform
17 segmentation model are based on differences in water distribution and soil development
18 (MacMillan and Pettapiece 2000). The program was run through Surfer version 8.0

1 (Golden Software, Boulder Co. 1998). Surfer gridding and contouring with the “Kriging
2 method” was used to produce the 3D data maps.

3 Principal component analysis (PCA), which is a multivariate statistical analysis technique,
4 was used to identify general relations among the variates and how they cluster. PCA
5 explain large proportions of the variance from correlated data and gives a picture
6 containing most of the information of the dataset in a leading few principal axes. PCA have
7 been developed under the assumption that the variables are normally distributed (Legendre
8 and Legendre 1998). Variables considered in this study were reasonably unskewed except
9 total carbonate content. Therefore, a square root transformation of total carbonate content
10 was performed to normalize its distribution. PCA was calculated using a correlation matrix
11 rather than the covariance matrix to eliminate the effects of different measurement units.
12 PCA was performed with version 5 of SYN-TAX 2000 (Podani 2000).

13 All statistical analyses were performed using SAS version 8.01 (SAS Institute Inc. 2000).
14 The Tukey’s multiple mean comparison tests were used to compare the effects of slope
15 position on herbicide sorption. Data were also analyzed to determine the strengths of
16 correlations between soil properties (i.e. SOC content, soil pH, carbonate content, and soil
17 clay, silt, and sand content) and 2,4-D sorption (i.e. K_d and K_{oc}) using the Pearson pairwise
18 correlation coefficient method.

19 Stepwise multiple linear regression analysis was carried out to determine which
20 combinations of soil properties have the greatest effect on herbicide sorption, and
21 eventually they were used for predicting K_d and K_{oc} . The use of R^2 values from multiple

1 regressions for obtaining a predictive equation is complicated because adding additional
2 variables will always increase the R^2 of the fit. Including many variables also increases the
3 instability and the complexity of the models. Therefore in addition to R^2 , the Mallows's C_p
4 statistic was also used for selecting the best model to predict K_d and K_{oc} . Mallows's C_p
5 attempt to balance maximizing the fit while trying to protect against overfitting. C_p was
6 first proposed by Mallows (1973) and is defined as follows:

$$7 \quad C_p = \frac{RSS_p}{\sigma^2} + 2p - n \quad (3.3)$$

8 where, n = the number of observations,
9 p = the number of variables in the regression,
10 RSS_p = the residual sum of squares using p variables, and
11 σ^2 = an independent estimate of the error.

12 The residual variance from the full model is used as the estimate of σ . If the model is
13 optimal, C_p will be approximately equal to the number of variables added in the regression
14 (p).

15 **3.4 Results**

16 **3.4.1 Soil Properties and 2,4-D Sorption Across the Landscape**

17 **3.4.1.1 The Variability of Soil Properties and 2,4-D Sorption** SOC content varied
18 across the landscape from 0.5 to 4.6% with an average value of $2.0\% \pm 0.7$ (standard
19 deviation) (Figure 3.2a). The soil surface pH ranged from 5.3 to 7.7, with a mean of $7.1 \pm$
20 0.6 (Figure 3.2b). The field, therefore, ranged from slightly acidic to slightly alkaline. The

1 average percent of total carbonates was $2.5\% \pm 3.8$ varying from 0.0 to 23.9% (Figure
2 3.2c). Clay content ranged from 6 to 21%, with an average of $13.3\% \pm 3.1$ (Figure 3.2d).
3 Sand content across the landscape ranged from 57 to 84% with an average value of 71.1%
4 ± 4.7 and silt content varied from 8 to 25% with an average value of $15.5\% \pm 3.9$. Soils
5 texture ranged from sandy clay loam to sandy loam. K_d had an average value of $2.7 \text{ ml g}^{-1} \pm$
6 0.9 and ranged from 0.5 to 5.6 ml g^{-1} (Figure 3.2e). K_{oc} , ranged from 122 to 153 ml g^{-1}
7 (Figure 3.2f), with an average value of 143 ml g^{-1} .

8 Total carbonate content was the most variable soil property, whereas soil pH was the least
9 variable (Appendix- I). The coefficient of variation (c.v.) for total carbonate content and
10 soil pH was 1.48 and 0.09 respectively. The variability of the SOC content (c.v. = 0.38)
11 was slightly higher than the clay content (c.v. = 0.23). For sorption parameters, K_{oc} was
12 less variable (c.v. = 0.25) than K_d (c.v. = 0.33), which was expected as K_{oc} is known to
13 reduce the variability associated with K_d .

14

15

16

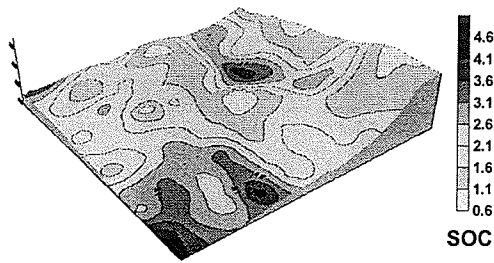


Figure 3.2a The spatial distribution of soil organic carbon content (%).

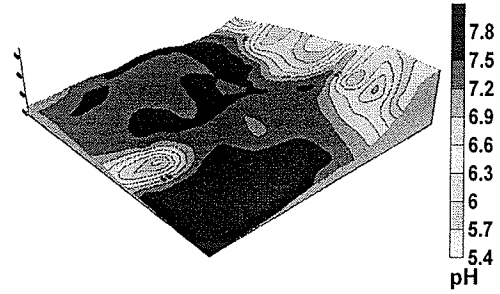


Figure 3.2b The spatial distribution of soil pH.

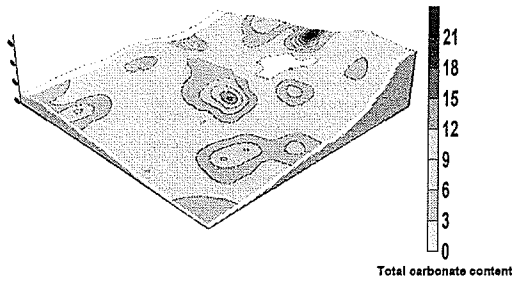


Figure 3.2c The spatial distribution of total carbonate content (%).

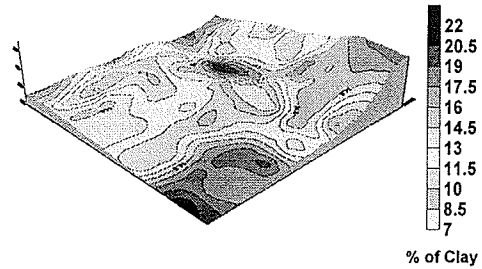


Figure 3.2d The spatial distribution of clay content (%).

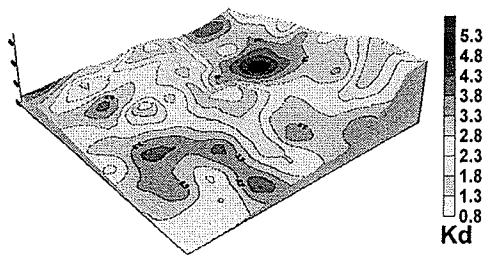


Figure 3.2e The spatial distribution of 2,4-D's K_d (ml g^{-1}).

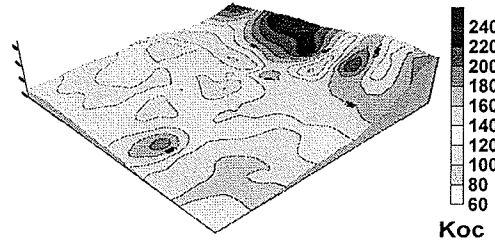


Figure 3.2f The spatial distribution of 2,4-D's K_{oc} (ml g^{-1}).

1 **3.4.1.2 Principal Component Analysis** Figure 3.3 displays the results of the principal
 2 component analysis, where SOC content, soil pH, clay content and K_d were heavily
 3 weighted on the first axes. Overall, the first principal axes accounted for 33.3% of the total
 4 variance. The second axes accounted for 23.8% of the total variance, which was weighted
 5 by the carbonate content, soil pH, K_d and K_{oc} (Figure 3.3). These two principal components
 6 together explained 57% of total variation of data and the remaining 43% may be explained
 7 by other variables or factors which were not measured in this experiment.

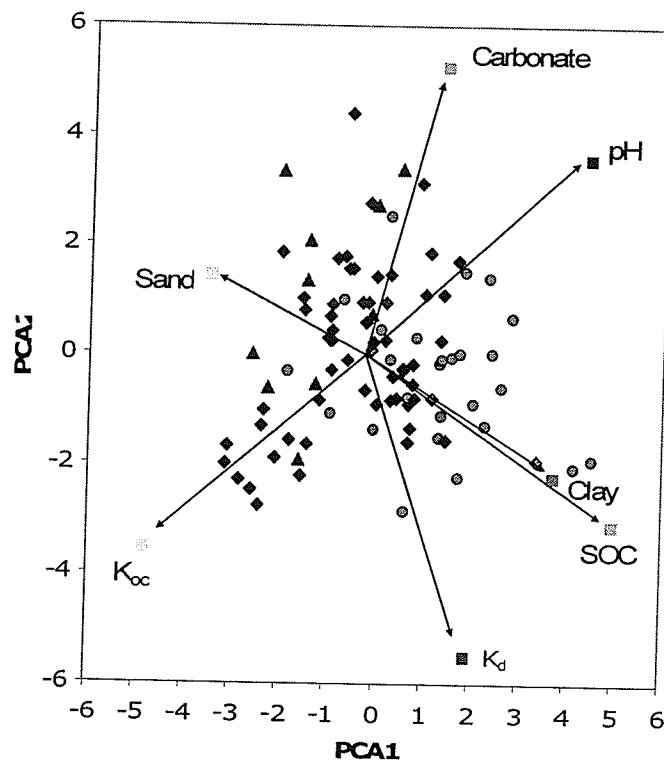


Figure 3.3 The first two principal components showing the variables with major contribution. Variable identifications are, SOC= soil organic carbon content, K_d = soil-water partition coefficient, K_{oc} = organic carbon partition coefficient. ▲= upper slope samples; ■ = middle slope samples; ● = lower slope samples.

1 Lines pointing in the same direction correspond to variables which are positively
2 correlated. This is the case for SOC and clay content, K_d and SOC content as well as soil
3 pH and carbonate content (Figure 3.3). Lines pointing in opposite direction correspond to
4 negatively correlated variables, e.g. clay and sand content as well as K_{oc} and soil pH
5 (Figure 3.3). Based on the cluster of samples, one can infer which variables are accounting
6 for which sample clusters. For example, soils that were rich in SOC content and clay
7 content were clustered in the lower slope position, whereas upper and middle slope
8 positions were characterized by soils that were rich in carbonate and sand content,
9 respectively (Figure 3.3). Soils with greater SOC contents had greater K_d values, whereas
10 acidic soils had greater K_{oc} values, which is expected because 2,4-D is a weakly-acidic
11 molecule.

12 The orientation of the principal component is also very important for grouping soil
13 properties and sorption coefficients. For example, the first horizontal component can
14 separate sandy soils from clay soils. On the other hand, the second component allows for
15 discrimination between soils with the highest SOC and clay content, and K_d those with the
16 sand and carbonate content and K_{oc} .

17 **3.4.1.3 Correlations Between Soil Properties and 2,4-D Sorption for the Whole Field**

18 Total carbonate content had a very weak negative correlation ($r = -0.23$, $p < 0.05$, Table
19 3.1) with SOC content. Soil pH was positively correlated ($r = 0.39$, $p < 0.001$, Table 3.1)
20 with total carbonate content but the correlation was weak, despite the fact that the alkaline
21 character of carbonates act as pH buffers in soil. Soil pH was weakly positively correlated

1 (r = 0.36, p < 0.001) with SOC content, but clay content was moderately associated (r =
 2 0.43, p < 0.001) with SOC content.

3 Table 3.1 Pearson correlation coefficients among soil properties and 2,4-D sorption for all
 4 sampling points (n = 96).

	SOC (%)	Soil pH	Carbonate (%)	Clay (%)	Silt (%)	Sand (%)	K _d (ml g ⁻¹)	K _{oc} (ml g ⁻¹)
SOC (%)	1.00							
Soil pH	0.36 ***	1.00						
Carbonate (%)	-0.23 *	0.39 ***	1.00					
Clay (%)	0.43 ***	-	-	1.00				
Silt (%)	-	-	-	-	1.00			
Sand (%)	-0.31 **	-	-	-0.55 ***	-0.76 ***	1.00		
K _d (ml g ⁻¹)	0.77 ***	-	-0.51 ***	0.24 **	-	-	1.00	
K _{oc} (ml g ⁻¹)	-0.41 ***	-0.82 ***	-0.43 ***	-	-	-	0.21*	1.00

5 *, **, and *** denote P < 0.05, P < 0.01, and P < 0.001 level of significance, respectively.

6 SOC content was an important soil property influencing the sorption of 2,4-D by soil, since
 7 a strong positive correlation (r = 0.77, p < 0.001, Figure 3.4) between SOC content and K_d
 8 was observed. Soil pH was expected to have an effect on 2,4-D sorption since 2,4-D is a
 9 weakly acidic herbicide. However, there was no significant correlation found between soil
 10 pH and K_d (Figure 3.5). K_d values were very weakly positively correlated to clay content (r
 11 = 0.24, p < 0.01, Figure 3.6). The herbicide's K_d had a moderate negative correlation (r = -
 12 0.51, p < 0.001, Figure 3.7) with total carbonate content which indicated that the soil
 13 constituents sorbed less herbicide in the presence of more carbonate content in the soil.

14

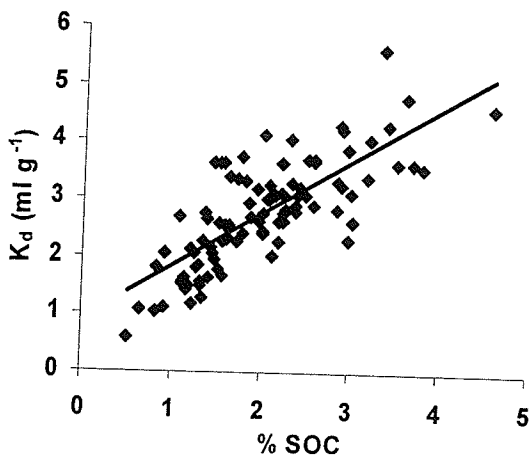


Figure 3.4 Relationship between soil organic carbon content (%SOC) and the sorption partition coefficient (K_d) of 2,4-D.

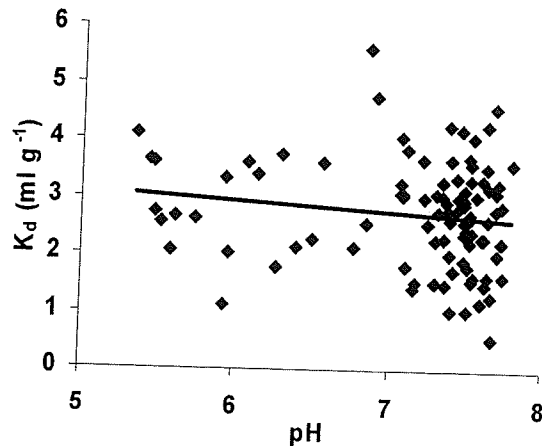


Figure 3.5 Relationship between pH and soil-water partition coefficient (K_d) of 2,4-D.

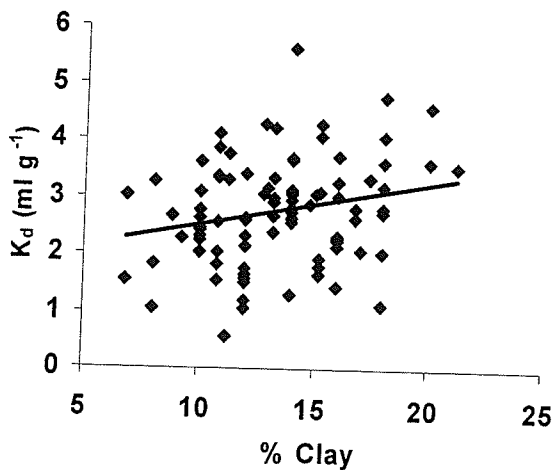


Figure 3.6 Relationship between clay content and soil-water partition coefficient (K_d) of 2,4-D

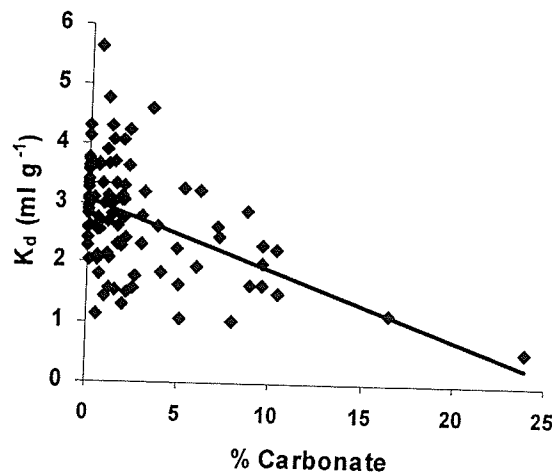


Figure 3.7 Relationship between carbonate content and soil-water partition coefficient (K_d) of 2,4-D

1 The herbicide's K_{oc} , was weakly negatively correlated ($r = -0.41$, $p < 0.001$, Table 3.1) to
2 SOC content (Figure 3.8). This indicated that soils with high SOC contents sorbed less 2,4-
3 D per unit of organic carbon, relative to soils with low organic carbon contents. K_{oc} was
4 very weakly positively correlated with K_d ($r = 0.21$, $p < 0.05$) however, the spatial
5 distribution of K_{oc} (Figure 3.2f) and K_d (Figure 3.2e) were not the same. K_{oc} was very
6 strongly negatively correlated with pH ($r = -0.82$, $p < 0.001$, Figure 3.9), and was weakly
7 correlated with carbonate content ($r = -0.43$, $p < 0.001$, Figure 3.10). There was no
8 significant correlation found between K_{oc} and clay content (Figure 3.11).

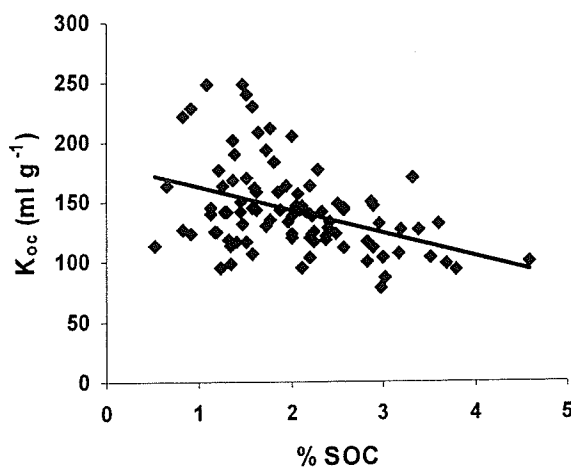


Figure 3.8 Relationship between soil organic carbon content (% SOC) and the organic carbon partition coefficient (K_{oc}) of 2,4-D.

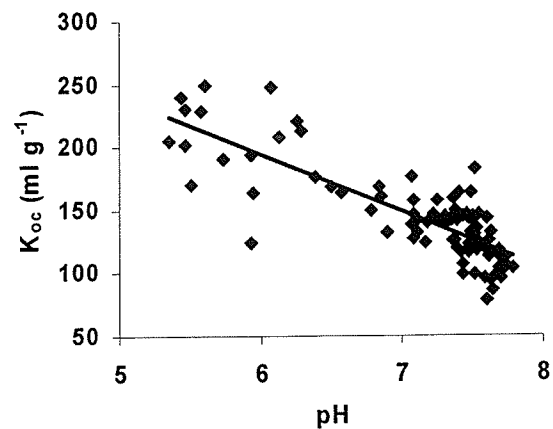


Figure 3.9 Relationship between soil pH and the organic carbon partition coefficient (K_{oc}) of 2,4-D.

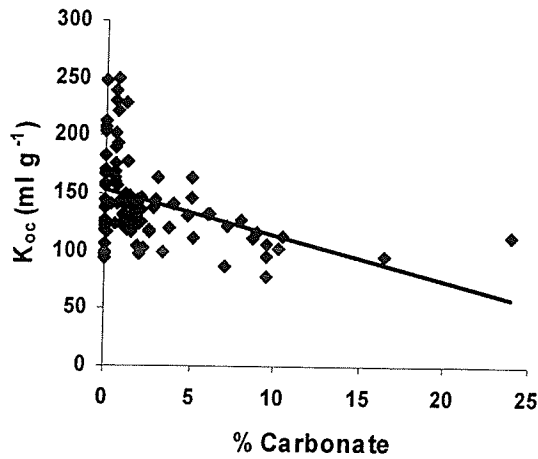


Figure 3.10 Relationship between % carbonate content and the organic carbon partition coefficient (K_{oc}) of 2,4-D.

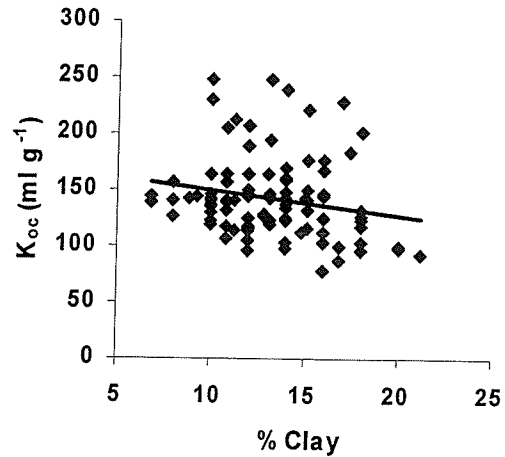


Figure 3.11 Relationship between % clay content and the organic carbon partition coefficient (K_{oc}) of 2,4-D.

1 **3.4.1.4 Predicting 2,4-D Sorption with the Soil Properties for the Whole Field**

2 Stepwise multiple linear regression analysis showed that SOC content, as a sole variable,
3 produced adequate results in predicting K_d ($R^2 = 0.59$, Table 3.2).

4 Table 3.2 Regression equations describing dependency of sorption partitioning coefficient
5 (K_d and K_{oc}) on soil organic carbon content (SOC), pH, clay content and carbonate
6 content.

	Regression equation	R^2	C(p)	Pr > F
K_d	$K_d = 0.92 + 0.92 \text{ SOC}$	0.59	107.33	0.0001
	$K_d = 5.16 - 0.65 \text{ pH} + 1.11 \text{ SOC}$	0.78	19.61	0.0001
	$K_d = 5.90 - 0.05 \text{ Clay} - 0.68 \text{ pH} + 1.22 \text{ SOC}$	0.80	9.53	0.0001
	$K_d = 5.24 - 0.05 \text{ Clay} - 0.57 \text{ pH} + 1.13 \text{ SOC} - 0.03 \text{ Carbonate}$	0.81	4.57	0.0001
K_{oc}	$K_{oc} = 468.93 - 45.63 \text{ pH}$	0.68	18.69	0.0001
	$K_{oc} = 490.29 - 1.81 \text{ Clay} - 45.24 \text{ pH}$	0.70	15.17	0.0001
	$K_{oc} = 426.73 - 35.82 \text{ pH} - 11.04 \text{ SOC} - 1.10 \text{ Carbonate}$	0.73	3.89	0.0001

7 Soil pH, alone was not able to predict K_d . However, when pH was included in regression
8 models with SOC content, the prediction of K_d improved significantly ($R^2 = 0.78$, Table

1 3.2). Similarly, when total carbonate content ($R^2 = 0.79$) or clay content ($R^2 = 0.80$, Table
2 3.2), or both ($R^2 = 0.81$, Table 3.2) were included in addition to SOC content and soil pH in
3 the model, the prediction value improved slightly. Combining clay content, pH, SOC
4 content and carbonate content (all measured soil properties combined, $R^2 = 0.81$, Table 3.2)
5 was as good as using only two or three variables. So, the least complex and optimal
6 prediction of 2,4-D sorption was achieved by a regression model containing SOC content
7 and soil pH (Figure 3.12). In addition, by using polynomial and cross product interaction,
8 the final prediction of 2,4-D sorption revealed that soil pH and SOC content has an
9 interaction effect on K_d ($K_d = -3.09 + 0.52 \text{ pH} + 5.98 \text{ SOC} - 0.67 \text{ pH} \cdot \text{SOC} - 0.04$
10 Carbonate, $R^2 = 0.86$, $p < 0.0001$).

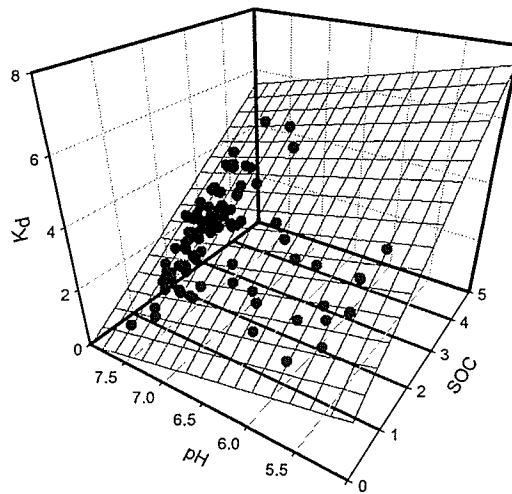


Figure 3.12 Graphical representation of K_d , SOC content and soil pH

11

12 Soil pH was found to be the most important factor predicting K_{oc} ($R^2 = 0.68$, $p < 0.0001$),
13 i.e. pH alone predicted 68% of the variation of K_{oc} . Including clay content in addition to

1 soil pH did not improve K_{oc} prediction significantly ($R^2 = 0.70$, $p < 0.0001$). A K_{oc} model
2 containing soil pH, SOC content, and carbonate content predicted 73% variability of K_{oc}
3 (Table 3.2).

4 **3.4.2 Soil Properties and 2,4-D Sorption in Relation to Landscape Position**

5 **3.4.2.1 The Variability of Soil Properties and 2,4-D Sorption** SOC content increased
6 significantly from upper ($1.1\% \pm 0.30$) to middle ($1.7\% \pm 0.51$) to lower slope positions
7 ($2.7\% \pm 0.73$) (Figure 3.13a). Kleiss (1970) reported a similar trend where the amount of
8 SOC content increased from upper slope (0.9%) to lower slope (3.5%) positions. Other
9 researchers (Miller et al. 1988; Mallawatantri and Mulla 1992; Novak 1999) also reported
10 similar spatial distribution of SOC content.

11 Similar to SOC content, soil pH also increased significantly from upper (6.8 ± 0.7) to
12 middle (7.0 ± 0.7) to lower slope position (7.4 ± 0.2) (Figure 3.13b). The distribution of
13 total carbonate content was heavily skewed (histogram not shown) with many samples
14 having little or no carbonates (Figure 3.2c). Total carbonate content in the upper slope
15 ($4.66\% \pm 6.13$) was higher than in the middle ($2.66\% \pm 4.39$) and lower ($2.49\% \pm 3.05$)
16 slope positions but the differences were not statistically significant (Figure 3.13c). The clay
17 content of the lower slope position ($14.7\% \pm 3.2$) was significantly higher than the middle
18 slope position ($12.5\% \pm 2.7$), however, no significant difference in clay content was
19 observed between the upper slope position ($13.2\% \pm 3.1$) and either lower or middle slope
20 positions (Figure 3.13d). Similar to SOC content, K_d increased significantly from upper
21 ($1.7 \text{ ml g}^{-1} \pm 0.5$) to middle ($2.6 \text{ ml g}^{-1} \pm 0.8$) to lower ($3.3 \text{ ml g}^{-1} \pm 0.8$) (Figure 3.13e)
22 slope positions. Soil organic partitioning coefficient (K_{oc}) in the lower slope ($122 \text{ ml g}^{-1} \pm$

- 1 24.2) had significantly lower K_{oc} values relative to upper ($150 \text{ ml g}^{-1} \pm 36.7$) and middle
- 2 ($153 \text{ ml g}^{-1} \pm 37.5$) slope positions (Figure 3.13f).

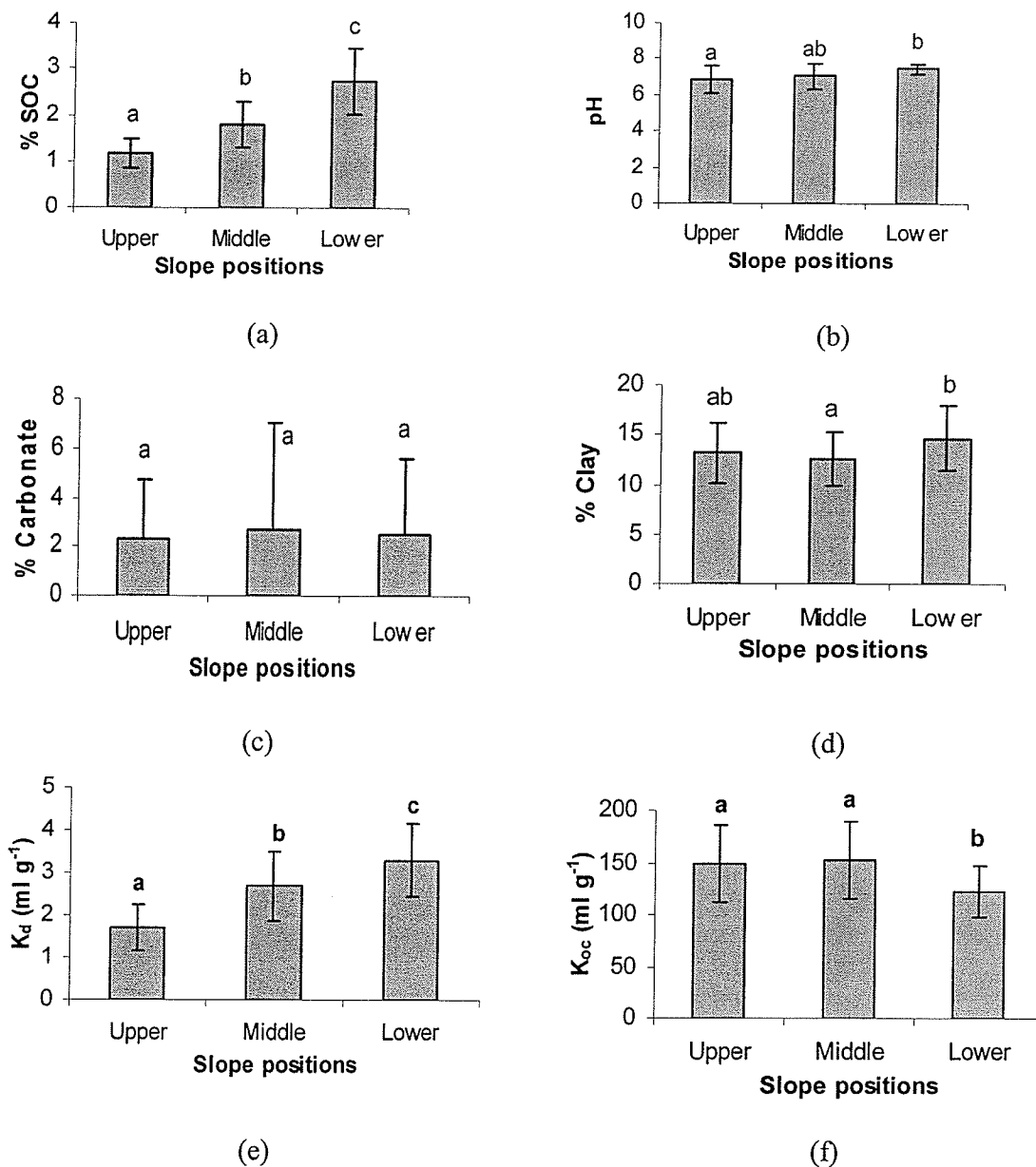


Figure 3.13 The distribution of soil properties and sorption coefficients: (a) Soil organic carbon content (% SOC); (b) Soil pH; (c) Carbonate content (%); (d) Clay content (%); (e) Soil-water partition coefficient ($K_d \text{ ml g}^{-1}$); (f) Organic carbon partition coefficient of ($K_{oc} \text{ ml g}^{-1}$) at different slope positions (upper, middle and lower). Sample size (n) was 12 for upper, 55 for middle and 29 for lower slope positions. Error bars represent standard deviation.

1 The variability of SOC content (c.v. = 0.2) and clay content (c.v. = 0.2) was similar for
2 upper, middle and lower slope positions (Appendix - I). Considering three different slope
3 positions, soil pH was the least variable soil property with the coefficient of variation
4 ranging from 0.04 to 0.11 and the smallest variation was found in the lower slope position
5 (Appendix - I). Variability of carbonate content in the middle (c.v. = 1.6) slope position
6 was slightly higher than the upper (c.v. = 1.1) and lower (c.v. = 1.2) slope positions. K_{oc}
7 was less variable than K_d regardless of slope positions (Appendix - I). Both K_d and K_{oc}
8 were less variable in the lower slope position when compared with the upper and middle
9 slope position (Appendix - I).

10 **3.4.2.2 Correlations Between Soil Properties and 2,4-D Sorption in Different**

11 **Landscape Position** Soil pH was positively correlated with total carbonates in the middle
12 ($r = 0.41$, $p < 0.01$, Table 3.3), and lower ($r = 0.37$, $p < 0.05$, Table 3.3) slope positions. In
13 the middle slope position, the soil pH ($r = 0.27$, $p < 0.05$, Table 3.3) and total carbonates (r
14 $= -0.29$, $p < 0.05$, Table 3.3) were weakly correlated to SOC content.

15 K_d was moderately positively correlated with SOC content in upper, middle and lower
16 slope positions with $r = 0.64$ ($p < 0.05$), $r = 0.71$ ($p < 0.001$) and $r = 0.69$ ($p < 0.001$),
17 respectively (Figure 3.14). In the middle slope, K_d was weakly negatively correlated to pH
18 ($r = -0.36$, $p < 0.01$, Figure 3.15) and moderately negatively correlated with total carbonate
19 content ($r = -0.58$, $p < 0.001$, Figure 3.16). Similarly, in the lower slope position, K_d was
20 moderately negatively correlated with soil pH ($r = -0.45$, $p < 0.05$, Figure 3.15) and with
21 total carbonate content ($r = -0.52$, $p < 0.01$, Figure 3.16). There was no significant
22 correlation between K_d and clay content for any of the slope positions (Figure 3.17).

1 Table 3.3 Pearson correlation coefficients among soil properties and 2,4-D sorption as
 2 affected by landscape position.

	SOC (%)	pH	Carbonate (%)	Clay (%)	Silt (%)	Sand (%)	K_d (ml g ⁻¹)	K_{oc} (ml g ⁻¹)
Upper (n = 12)								
pH	-	1.00						
Carbonate (%)		0.66 *	1.00					
Clay (%)	-	-0.68 *	-	1.00				
Silt (%)	-	-	-	-	1.00			
Sand (%)		-	-	-0.74 **	-0.74 **	1.00		
K_d (ml g ⁻¹)	0.64 *	-	-	-	-	-	1.00	
K_{oc} (ml g ⁻¹)	-	-0.66 *	-0.34 *	-	-	-	-	1.00
Middle (n = 55)								
pH	0.27 *	1.00						
Carbonate (%)	-0.29 *	0.41 **	1.00					
Clay (%)	-	-	-	1.00				
Silt (%)	-	-	-	-0.26 *	1.00			
Sand (%)	-	-	-	-0.40 **	-0.78 ***	1.00		
K_d (ml g ⁻¹)	0.71 ***	-0.36 **	-0.58 ***	-	-	-	1.00	
K_{oc} (ml g ⁻¹)	-	-0.87 ***	-0.48 ***	-	-	-	0.48 ***	1.00
Lower (n = 29)								
pH	-	1.00						
Carbonate (%)	-	0.37 *	1.00					
Clay (%)	0.65 ***	-	-	1.00				
Silt (%)	-	-	-	-	1.00			
Sand (%)	-0.47 *	-0.46 *	-	-0.65 ***	-0.80 ***	1.00		
K_d (ml g ⁻¹)	0.69 ***	-0.45 *	-0.52 **	-	-	-	1.00	
K_{oc} (ml g ⁻¹)	-0.41 *	-0.67 ***	-0.39 *	-0.56 ***	-0.38 *	0.62 ***	0.36 *	1.00

3 * , ** , and *** denote P < 0.05, P < 0.01, and P < 0.001 level of significance, respectively.

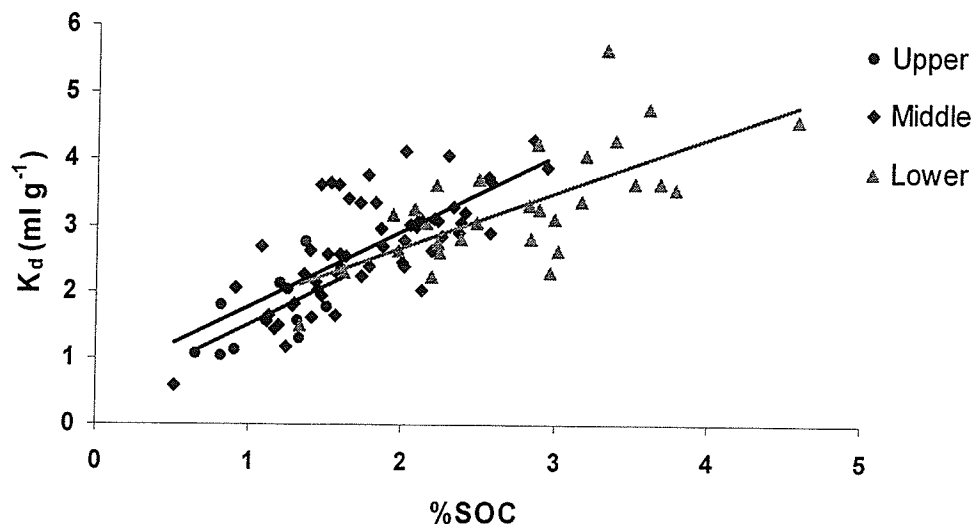


Figure 3.14 Relationship between soil organic carbon (SOC) content and soil-water partition coefficient (K_d) of 2,4-D as affected by landscape position. Upper slope ($K_d = 0.37 + 1.15\%SOC$; $r = 0.64$, $P < 0.05$), middle slope ($K_d = 0.66 + 1.13\%SOC$; $r = 0.71$, $P < 0.001$) and lower slope ($K_d = 1.06 + 0.82\%SOC$; $r = 0.69$, $P < 0.001$).

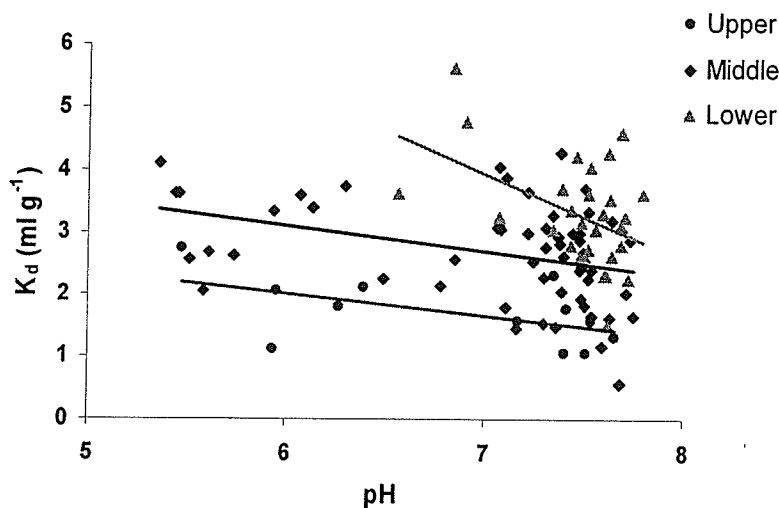


Figure 3.15 Relationship between soil pH and soil-water partition coefficient (K_d) of 2,4-D as affected by landscape position. Upper slope ($K_d = 4.14 - 0.36\text{pH}$; $r = -0.52$, *ns*), middle slope ($K_d = 5.58 - 0.41\text{pH}$; $r = -0.36$, $P < 0.01$) and lower slope ($K_d = 13.58 - 1.37\text{pH}$; $r = -0.45$, $P < 0.05$).

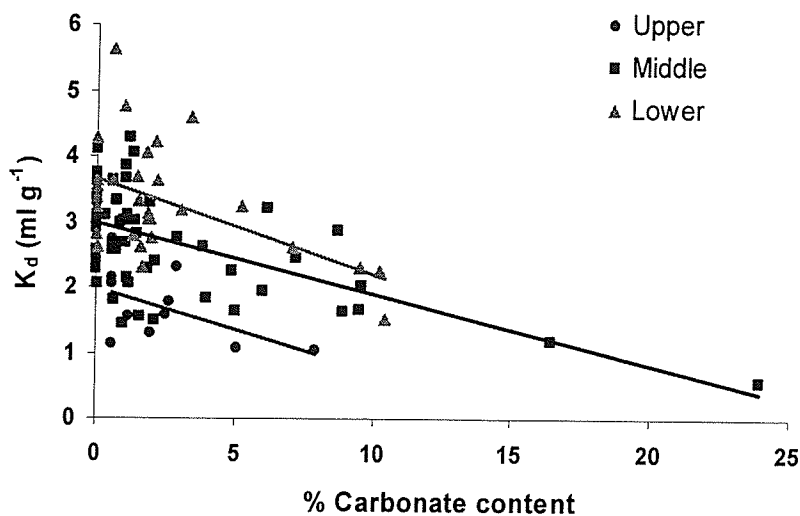


Figure 3.16 Relationship between carbonate content and soil-water partition coefficient (K_d) of 2,4-D as affected by landscape position. Upper slope ($K_d = 1.99 - 0.13\% \text{Carbonate}$; $r = -0.54$, *ns*), middle slope ($K_d = 2.98 - 0.11\% \text{Carbonate}$; $r = -0.58$, $P < 0.001$) and lower slope ($K_d = 3.67 - 0.15\% \text{Carbonate}$; $r = -0.52$, $P < 0.01$).

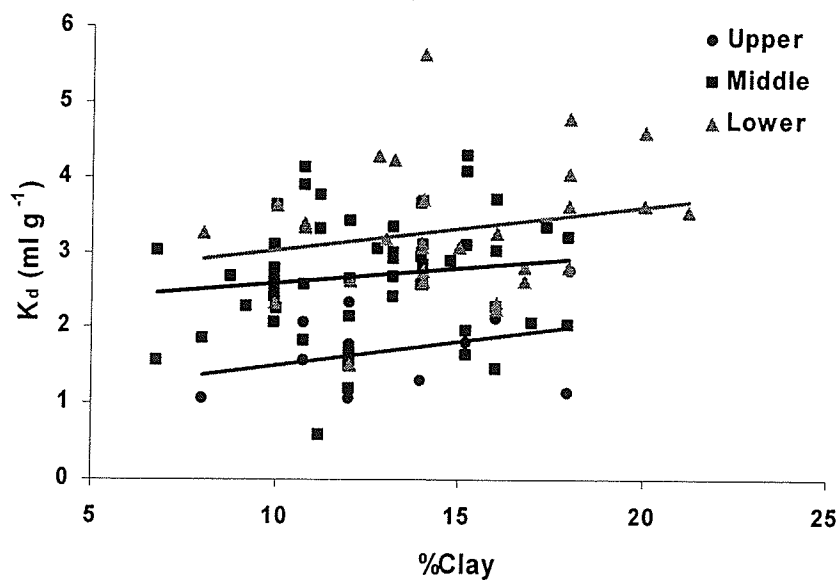


Figure 3.17 Relationship between clay content and soil-water partition coefficient (K_d) of 2,4-D as affected by landscape position. Upper slope ($K_d = 0.89 + 0.06\% \text{Clay}$; $r = 0.35$, *ns*), middle slope ($K_d = 2.19 + 0.04\% \text{Clay}$; $r = 0.13$, *ns*) and lower slope ($K_d = 2.44 + 0.06\% \text{Clay}$; $r = 0.22$, *ns*).

1 In the lower slope position, K_{oc} was significantly negatively correlated with SOC content (r
2 = -0.41, $p < 0.05$, Figure 3.18). Although the 2,4-D sorption per unit organic carbon was
3 dependent on the amount of SOC content in soils of the lower slope position, there was no
4 significant relation between K_{oc} and SOC content in the upper or middle slope positions
5 (Figure 3.18). K_{oc} was moderately negatively correlated with soil pH in the upper ($r = -$
6 0.66, $p < 0.05$) and lower ($r = -0.67$, $p < 0.001$) slope positions (Figure 3.19) and strongly
7 negatively correlated in the middle slope position ($r = -0.87$, $p < 0.001$, Figure 3.19). There
8 was a weak negative correlation between K_{oc} and carbonate content in the upper ($r = -0.34$,
9 $p < 0.05$) and lower ($r = -0.39$, $p < 0.05$) slope positions (Figure 3.20) and a moderate
10 negative correlation ($r = -0.48$, $p < 0.001$, Figure 3.20) in the middle slope position. There
11 was no significant correlation found between K_{oc} and clay content in the upper or middle
12 slope positions, but K_{oc} and clay were moderately negatively correlated in the lower slope
13 position ($r = -0.56$, $p < 0.001$, Figure 3.21).

14

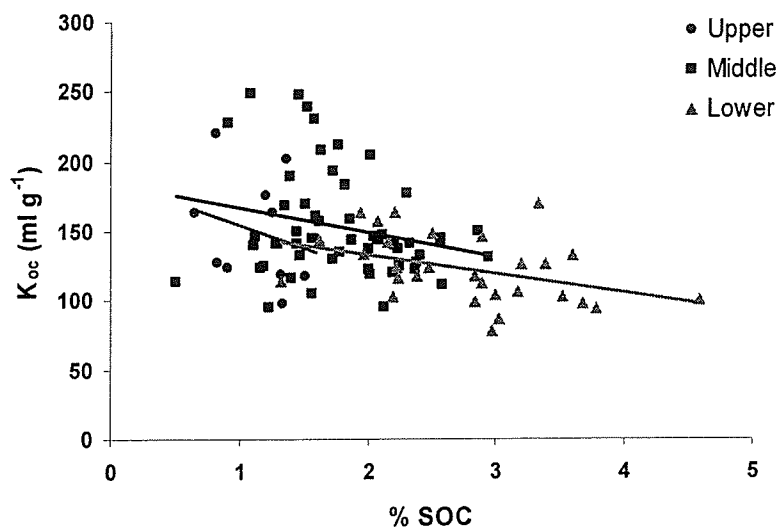


Figure 3.18 Relationship between soil organic carbon (SOC) content and the organic carbon partition coefficient (K_{oc}) of 2,4-D as affected by landscape position. Upper slope ($K_{oc} = 186.96 - 32.17\%SOC$; $r = -0.26$, *ns*), middle slope ($K_{oc} = 184.54 - 17.80\%SOC$; $r = -0.24$, *ns*) and lower slope ($K_{oc} = 160.17 - 13.68\%SOC$; $r = -0.41$, $P < 0.05$).

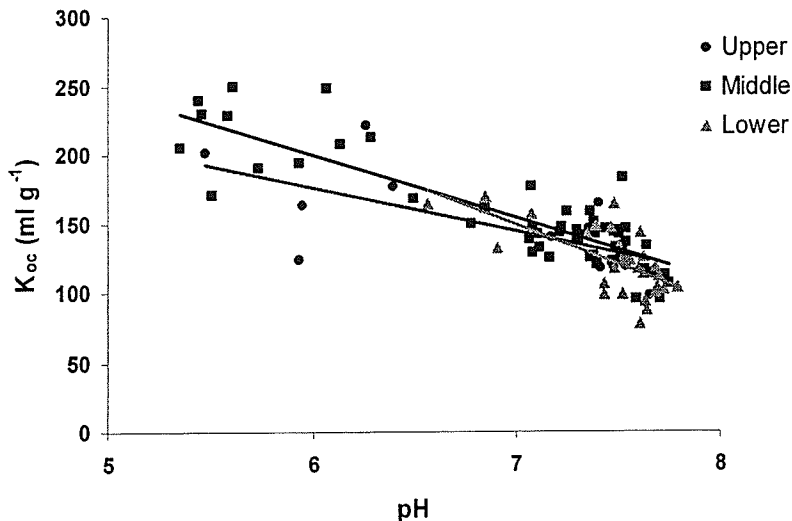


Figure 3.19..Relationship between soil pH and the organic carbon partition coefficient (K_{oc}) of 2,4-D as affected by landscape position. Upper slope ($K_{oc} = 364 - 31.33\text{pH}$; $r = -0.66$, $P < 0.05$), middle slope ($K_{oc} = 477.26 - 46.17\text{pH}$; $r = -0.87$, $P < 0.001$) and lower slope ($K_{oc} = 547.79 - 56.93\text{pH}$; $r = -0.67$, $P < 0.001$).

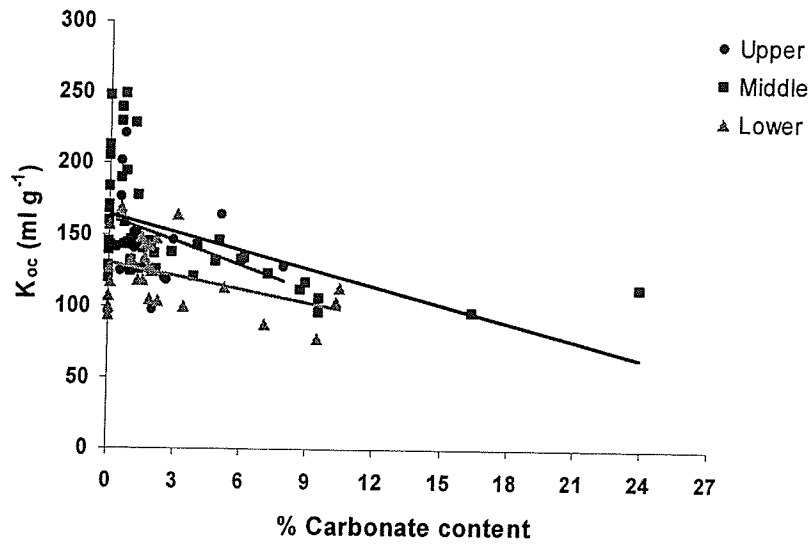


Figure 3.20 Relationship between carbonate content and the organic carbon partition coefficient (K_{oc}) of 2,4-D as affected by landscape position. Upper slope ($K_{oc} = 162.28 - 5.55\% \text{Carbonate}$; $r = -0.34$, $P < 0.05$), middle slope ($K_{oc} = 163.69 - 4.15\% \text{Carbonate}$; $r = -0.48$, $P < 0.001$) and lower slope ($K_{oc} = 130.2 - 3.10\% \text{Carbonate}$; $r = -0.39$, $P < 0.05$).

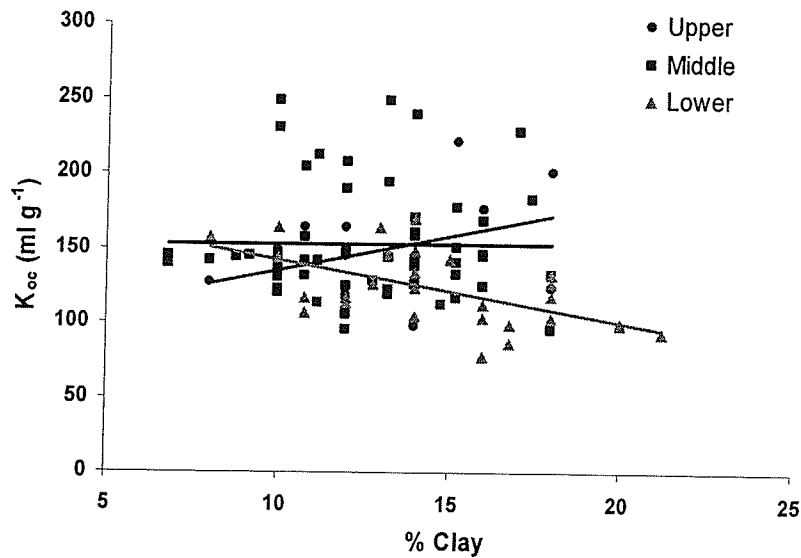


Figure 3.21 Relationship between clay content and the organic carbon partition coefficient (K_{oc}) of 2,4-D as affected by landscape position. Upper slope ($K_{oc} = 89.15 + 4.58\% \text{Clay}$; $r = 0.38$, ns), middle slope ($K_{oc} = 153 - 0.03\% \text{Clay}$; ns) and lower slope ($K_{oc} = 183.04 - 4.12\% \text{Clay}$; $r = -0.56$, $P < 0.001$).

1

1 The coefficient of correlations between K_d and K_{oc} were 0.48 ($p < 0.001$) and 0.36 ($p <$
2 0.05) for middle and lower slope positions, respectively. The upper slope also had a
3 positive correlation between K_d and K_{oc} , but it was not statistically significant ($r = 0.55$, $p =$
4 0.06). The variation of correlation between K_d and K_{oc} in the different slope positions could
5 be the result of the differences in soil organic matter quality or soil pH in different slope
6 positions.

7 **3.4.2.3 Predicting 2,4-D Sorption with the Soil Properties in Different Landscape**
8 **Position** The percent of SOC content was found to be the most dominant factor affecting
9 the sorption of 2,4-D onto soil regardless of landscape positions, but, the strength of
10 prediction of K_d by SOC content varied among landscape positions (Table 3.4). In the
11 upper slope position, SOC content predicted K_d with $R^2 = 0.41$ ($p < 0.024$, $C_p = 13.3$,
12 Table 3.4), in the middle slope position with $R^2 = 0.51$ ($p < 0.0001$, $C_p = 110.0$, Table 3.4),
13 and in the lower slope position with $R^2 = 0.47$ ($p < 0.0001$, $C_p = 29.9$, Table 3.4).

14 Regardless of slope position, K_d could not be adequately predicted using soil pH alone.
15 Combining pH with SOC content significantly improved K_d prediction for all slope
16 positions. Specifically, in the upper slope position, pH and SOC content combined
17 predicted K_d with a R^2 value of 0.74 ($p < 0.0025$, $C_p = 3.5$, Table 3.4), while the middle
18 slope position had $R^2 = 0.84$ ($p < 0.0001$, $C_p = 4.5$, Table 3.4), and the lower slope position
19 had $R^2 = 0.73$ ($p < 0.0001$, $C_p = 5.0$, Table 3.4).

20
21

1 Table 3.4 Regression equations for different slope positions (upper, middle and lower)
 2 describing dependency of the sorption partitioning co-efficient (K_d and K_{oc}) on soil
 3 organic carbon content (SOC), pH, clay content and carbonate content.

Slope positions	Regression equation	R^2	C(p)	Pr > F
Upper (n=12)				
K_d	$K_d = 0.37 + 1.15 \text{ SOC}$	0.41	13.30	0.024
	$K_d = 2.97 - 0.39 \text{ pH} + 1.23 \text{ SOC}$	0.74	3.53	0.002
K_{oc}	$K_{oc} = 363.99 - 31.33 \text{ pH}$	0.44	4.41	0.018
Middle (n=55)				
K_d	$K_d = 0.66 + 1.13 \text{ SOC}$	0.51	110.01	0.0001
	$K_d = 4.99 - 0.68 \text{ pH} + 1.39 \text{ SOC}$	0.84	4.46	0.0001
	$K_d = 4.66 - 0.60 \text{ pH} + 1.30 \text{ SOC} - 0.02 \text{ Carbonate}$	0.85	2.83	0.0001
K_{oc}	$K_{oc} = 477.26 - 46.17 \text{ pH}$	0.75	4.39	0.0001
	$K_{oc} = 456.95 - 42.78 \text{ pH} - 1.33 \text{ Carbonate}$	0.77	1.94	0.0001
Lower (n=29)				
K_d	$K_d = 1.06 + 0.82 \text{ SOC}$	0.47	29.86	0.0001
	$K_d = 12.48 - 1.55 \text{ pH} + 0.86 \text{ SOC}$	0.73	4.97	0.0001
	$K_d = 11.04 - 1.31 \text{ pH} + 0.80 \text{ SOC} - 0.06 \text{ Carbonate}$	0.76	3.82	0.0001
K_{oc}	$K_{oc} = 547.79 - 56.93 \text{ pH}$	0.44	13.62	0.0001
	$K_{oc} = 563.10 - 54.54 \text{ pH} - 12.03 \text{ SOC}$	0.57	6.64	0.0001
	$K_{oc} = 499.82 - 44.23 \text{ pH} - 14.82 \text{ SOC} - 2.43 \text{ Carbonate}$	0.65	3.56	0.0001

4 Adding other soil properties such as clay content and/or total carbonate content did not
 5 improve the prediction ability of K_d for the upper slope position. However, adding total

1 carbonate content to soil pH and SOC content did improve the predictability very slightly
2 for middle ($R^2 = 0.85$, $C_p = 2.8$, Table 3.4) and lower ($R^2 = 0.76$, $C_p = 3.81$, Table 3.4)
3 slope positions. In summary, the least complex regression model for predicting K_d was
4 achieved by using only pH and SOC content, which explained 74%, 84% and 73%
5 variability of 2,4-D sorption by soil in the upper, middle and lower slope positions,
6 respectively.

7 Soil pH was the single most significant factor affecting K_{oc} , regardless of slope positions,
8 but the prediction strength was different among slope positions. Soil pH accounted for 44%
9 of the variability of 2,4-D sorption to organic carbon, for both the upper and lower slope
10 positions, but it accounted for 75% of the variability for the middle slope position.

11 In the upper slope position, the best model for predicting K_{oc} contained only soil pH ($R^2 =$
12 0.44 , $C_p = 4.41$, $p < 0.02$). In the middle slope position, a combination of both soil pH and
13 carbonate content provided the best regression model for predicting K_{oc} ($R^2 = 0.77$, $C_p =$
14 1.94 , $p < 0.0001$). The best prediction of K_{oc} in the lower slope position was achieved by a
15 regression model containing soil pH, SOC content, and carbonate content ($R^2 = 0.65$, $C_p =$
16 3.56 , $p < 0.0001$).

17

3.5 Discussion

18 SOC content increased from upper to middle to lower slope positions. In other studies,
19 differences in amounts of SOC content among landscape positions were largely attributed
20 to the redistribution of topsoil by tillage, wind and water erosion (Verity and Anderson

1 1990; Mulla 1993; Moulin et al. 1994). For this study site (Deerwood), the increase in SOC
2 content from the upper to the lower slope position was probably due to the movement of
3 organic rich top soil from the upper slope by tillage erosion (Lindstrom et al. 1992; Lobb et
4 al. 1999) and water erosion (Shelton et al. 2000). Wind erosion may also play a part in
5 enhancing organic matter content in down slope positions (Roberts and Bettany 1985). The
6 loss of SOC content via tillage erosion has been shown to accelerate wind and water
7 erosion in several agricultural fields (Kachanoski et al. 1992; Lobb et al. 1995). In addition,
8 tillage decreases soil organic matter by disturbing aggregates and exposing soil organic
9 matter to microbial decomposition (Dexter 1988).

10 The large variation in soil carbonate content across the landscape may be due to differences
11 in soil moisture, as the distribution of carbonates in soils within a toposequence is
12 controlled mainly by soil moisture (Landi et al. 2004). Upper slopes are drier as a result of
13 water loss due to runoff whereas lower slopes are wetter because of runoff. A study by
14 Richardson et al. (1992) explained that the accumulation of carbonate in both footslope and
15 shoulder complex landscape position may be related to the upward movement of
16 carbonates from a shallow water table or lateral flow of water carrying dissolved
17 carbonates from the upper slope.

18 The K_d values obtained in this study confirmed the K_d values obtained in a previous study
19 (Gaultier et al. 2005) that used the same study site. However, the 2,4-D K_d values obtained
20 were higher compared to those obtained by Mallawatantri and Mulla (1992), Farenhorst et
21 al. (2001) and Stephens et al. (2002) for soils with a similar SOC content. Soils with a

1 similar SOC content may have different K_d values as a result of differences in soil organic
2 matter composition, soil pH, soil texture, or other soil properties.

3 SOC content was the single best predictor of the sorption of 2,4-D to soil regardless of
4 slope position. This is in agreement with other researchers (Mallawatantri and Mulla 1992;
5 Farenhorst et al. 2001), who also indicated that SOC content is the main predictor of the K_d
6 of 2,4-D. Various other researchers have also concluded that SOC content is the main
7 determinant in the sorption of other pesticides by soil, including imidacloprid (Cox et al.
8 1998), atrazine (Novak et al. 1997), metribuzin, diuron, and triallate (Mallawatantri and
9 Mulla 1992). In this study, prediction strengths of 2,4-D sorption by SOC content varied in
10 different landscape positions possibly due to differences in pH at different slope positions.
11 Combining SOC content and soil pH together had a very strong effect on sorption
12 prediction by linear regression, regardless of slope position. Similar results were found by
13 polynomial regression and cross product interaction, which also showed pH and SOC
14 interaction effects when predicting 2,4-D sorption. The acid dissociation constant of 2,4-D
15 is 2.64; therefore with increasing soil pH the anionic form of 2,4-D increases relative to the
16 molecular form, as a result sorption decreases with increasing pH.

17 In this study, soil pH alone was not sufficient for predicting K_d either on a whole field basis
18 or by slope position. Mallawatantri and Mulla (1992) reported that soil pH ranging from
19 6.1 to 7.3 had no influence on 2,4-D sorption. Similarly, Dubus et al. (2001) concluded that
20 2,4-D sorption in a range of soils was also not statistically related to soil pH. In contrast,
21 Johnson et al. (1995) showed that 2,4-D sorption was lower at pH 7 than at pH 5. Dyson et

1 al. (2002) also found that soil pH can have a strong influence on mesotrione sorption in
2 soil, which is also a weak acid pesticide.

3 Total carbonate content was found to be the third most important soil property affects the
4 sorption of 2,4-D. However, it is also possible that the association between carbonate
5 content and herbicide sorption was an indirect effect of soil pH as soil pH and carbonate
6 are positively correlated.

7 Various researchers (Khan 1973; Reddy and Gambrell 1987; Mallawatantri and Mulla
8 1992; Johnson et al. 1995; Cheah et al.1997; Farenhorst et al. 2001) observed that soil
9 organic matter is an important factor in the sorption of 2,4-D by soil and sediment.
10 However, Schwarzenbach and Westall (1981) reported that SOC content is a predominant
11 sorbent of pesticides when total organic carbon is higher than 0.1%. When SOC content is
12 below 0.1%, interactions with mineral surfaces may be more important (Pignatello 1989).
13 As the SOC content of this study site ranged from 0.5% to 4.5%, the effect of mineral
14 surfaces on 2,4-D sorption was less important. Therefore, the K_d value was not correlated
15 to clay content when the upper, middle and lower slope positions were considered
16 separately (Table 3.3). However, a positive correlation between K_d and clay content was
17 found when all data was combined (the whole field). This was unexpected, as soil pH was
18 slightly neutral to alkaline (soil pH 5.3 to 7.8) in this study, and the anionic form (soil pH >
19 pKa) of 2,4-D is repelled by negatively-charged clay minerals (Weber et al. 1965). The
20 positive correlation may be the indirect consequence of the sorption of 2,4-D onto organo-
21 clay complexes (Benoit et al. 1996). 2,4-D is adsorbed by organo-clays at different rates
22 depending on the clay mineral type (Hermosin and Cornejo 1992), such that the herbicide

1 sorption capacity differs for soils according to soil clay mineralogy (Hermosin and Cornejo
2 1991). A study by Farenhorst et al. (2001) also showed that K_d was significantly positively
3 correlated with clay content in a zero tillage field in Manitoba. However, another
4 researcher (Grover 1973) determined that 2,4-D was not correlated with clay content in
5 Canadian prairie soils. Effects of clay on sorption not only depend on the amount of clay
6 but also the clay types, and the nature of the exchange cations on the clay surfaces
7 (Stevenson 1994).

8 The average K_{oc} values obtained in this study were higher than those obtained in Georgia
9 by Rao and Davidson (1979), where they reported 2,4-D K_{oc} values of 72 ml g^{-1} for soil
10 with a similar texture to the soils in this study. Similarly, Cheah et al. (1997) reported an
11 average 2,4-D K_{oc} value of $44 \pm 53 \text{ ml g}^{-1}$ for a sandy loam soil in Malaysia, which is lower
12 than the value obtained in this study. Higher average K_{oc} values obtained in this study for
13 similar textured soils (sandy loam) indicated that the sorption of 2,4-D per unit organic
14 carbon was quite strong.

15 A very strong negative correlation between K_{oc} and soil pH was observed in this study. If
16 pH increases, 2,4-D is more present in anionic form and more soil organic matter is
17 dissociated, therefore less sorption occurs per unit organic carbon. The herbicide's K_{oc} had
18 a weak negative correlation to SOC content suggesting that soil organic matter quality may
19 have influenced 2,4-D sorption. Organic matter has different components that have
20 different degrees of decomposition and reactivity. Specific component(s) are responsible
21 for herbicide sorption. Clapp et al. (2001) and Senesi et al. (2001) observed that the humic
22 acid fractions of soil organic matter have the greatest effect on sorption. For the Deerwood

1 site, results showed that dilute alkali (0.5 N NaOH) extracted humic acid are significantly
2 responsible for herbicide sorption (Saiyed and Farenhorst, unpublished data).

3 There was a negative correlation between total carbonates and K_{oc} when considering data
4 from the whole field or from different slope positions (Table 3.1, and 3.3). Soil carbonates
5 may have interacted with soil organic matter decreasing 2,4-D sorption per unit organic
6 carbon. In this study, it seems that the sorption of 2,4-D anions was impeded by the
7 carbonates. In contrast, Tunesi et al. (1999) showed that sorption occurred between
8 carbonate minerals and anions when they examined the role of soil carbonates in anionic
9 heavy metal sorption.

10 **3.6 Conclusions**

11 2,4-D sorption significantly varied with landscape position mainly because of variation in
12 SOC content. The highest average soil-water partitioning coefficient (K_d : 3.31 ml g⁻¹) was
13 observed in the lower slope areas, which had the highest levels of SOC content (2.76%).
14 On the other hand, the lowest K_d (1.71 ml g⁻¹) was observed in the upper slope positions
15 where the lowest SOC content (1.16%) was observed. Although 2,4-D sorption was
16 adequately predicted by SOC content, the level of herbicides sorption onto soil organic
17 matter varied among slope positions due to differences in soil pH. In addition, differences
18 in soil organic matter quality among slope positions may have also contributed to different
19 K_d levels among landscape positions.

1 **4. EFFECTS OF LANDSCAPE VARIABILITY ON THE RATE AND**
2 **MAXIMUM 2,4-D MINERALIZATION**

3 **4.1 Abstract**

4 This study quantified the variability of soil properties and 2,4-D mineralization in a
5 conventionally tilled site near Deerwood, Manitoba as affected by landscape position. One
6 hundred soil samples (0-15 cm) were collected at regular intervals in a 500 m x 500 m grid
7 and sample points were classified into upper, middle and lower slope positions with the use
8 of LandMapR software. Soil samples were analyzed for soil texture, soil pH, soil organic
9 carbon content (SOC), carbonate content as well as for the mineralization of 2,4-D by soil
10 in soil microcosms. Results indicated that total 2,4-D mineralization ranged from 20 to
11 42%, at 130 days (the end of the experiment). Half-lives of 2,4-D mineralization varied
12 from 4 to 27 days, corresponding to first-order mineralization rate constants of 0.15 to 0.03
13 day⁻¹.

14 The prediction of total 2,4-D mineralization and the mineralization rate constant was
15 optimized by a regression model that contained SOC content and soil pH when the whole
16 field was considered. Optimal prediction of total mineralization in the upper, middle and
17 lower slope position contained carbonate content, sand content, and soil pH, respectively,
18 in addition to SOC content. For predicting the mineralization rate constant in the upper
19 slope, the best model contained pH and SOC content, whereas in the middle slope the best
20 model contained sand, pH and SOC and in the lower slope the best model contained silt,
21 pH and SOC.

1 Total mineralization and mineralization rate constant of 2,4-D decreased significantly in the
2 order of upper = middle < lower slope positions. Greater persistence of 2,4-D in the lower
3 slope position (half-life 13 days) compared to upper (7 days) and middle (9 days) slope
4 position was particularly due to greater SOC content in the lower slope position. Higher SOC
5 content in the lower slope position probably decreased 2,4-D mineralization and increased 2,4-
6 D persistence because of increased 2,4-D sorption and a decreasing availability of 2,4-D
7 molecules to soil microorganisms.

8 Greater 2,4-D persistence in soil could mean greater risks for 2,4-D off-site movement, as
9 well as greater risks of 2,4-D injury of sensitive crops. However, pesticide off-site
10 movement is influenced by many other factors such as the availability of 2,4-D residues to
11 be moved by water, as well as climatic and hydrological factors. It is shown in this study
12 that locations with greater 2,4-D persistence in the landscape, also have greater 2,4-D
13 sorption by soil. Thus, in these locations, 2,4-D in soil may not be available for uptake by
14 plants or for movement by water. Additional studies are required to determine more
15 comprehensively the agronomic or environmental sensitive zones in Manitoba soil
16 landscapes.

17 **4.2 Introduction**

18 Biodegradation is the primary process of 2,4-D transformation in soils (Bollag and Liu
19 1990). The organisms responsible for 2,4-D biodegradation are diverse in their taxonomic
20 group, therefore 2,4-D biodegradation occurs under a wide range of environmental
21 conditions. 2,4-D can degrade very quickly as it can either be cometabolized or used as a
22 carbon and energy source by soil microorganisms (Fournier 1980). Smith and Aubin

1 (1991) have shown that half-life of 2,4-D could be as short as one day. Despite the short
2 half-life of 2,4-D, several studies reported that the 2,4-D is mobile in soil because of its
3 small sorption potential by soil and has been detected in surface and ground waters (Waite
4 et al. 1992; Currie and Williamson 1995; Johnson et al. 1995; Miller et al. 1995a).

5 Persistence and mobility of 2,4-D may vary in the agricultural field due to heterogeneous
6 nature of soil properties across the landscape positions (Farenhorst et al. 2003). Soil
7 properties such as soil organic matter content vary spatially within landscapes as a result of
8 soil formation processes, as well as due to irregularities in parent material deposition
9 (Mallawatantri and Mulla 1992; Khakural et al. 1994; Goderya 1998), and erosion by
10 tillage practices, wind and water (Arshad et al. 1990; Bowman et al. 1990). The spatial
11 variability of soil properties across landscape position in agricultural fields may affect
12 microbial populations and activities, which, in turn, can affect 2,4-D degradation. Accurate
13 assessment of the degradation kinetics of organic contaminants in soils is critical to
14 pesticide fate and transport models. Boesten and van der Linden (1991) investigated the
15 effects of degradation rate on predicted pesticide leaching and persistence and they
16 reported that changing the degradation rate by a factor of two changed the fraction of
17 pesticide leached by about a factor of ten.

18 An increased sorption of pesticides by soil may decrease the availability of the pesticide for
19 mineralization by the soil microbes (Moshier and Penner 1978). Ogram et al. (1985)
20 showed that the 2,4-D degradation was limited by sorption since the herbicide moved deep
21 into the soil organic matter matrix making the herbicide unavailable to breakdown by
22 microorganisms. In another study, degradation rates were the lowest in fine textured soils

1 containing the highest organic matter content (Ogram et al. 1985). However, the effect of
2 sorption on 2,4-D biodegradation is not universal. Bolan and Baskaran (1996) explained
3 that soils with greater soil organic matter content may also have greater microbial activity
4 and therefore greater 2,4-D biodegradation rates. In addition to pesticide bioavailability, the
5 nature of microbial community, as well as environmental conditions which affect the
6 microbial community, are important factors that influence biodegradation rates.

7 The relation between microbial biomass or activity and 2,4-D degradation has been studied
8 for decades and the results of the studies varied widely. For example, Willems et al. (1996)
9 found that 2,4-D mineralization was poorly correlated with microbial biomass. Similarly,
10 Entry and Emmingham (1996) found poor correlation between fungal and bacterial
11 biomass and 2,4-D degradation when considering a range of surface soils. However, Veeh
12 et al. (1996) found a positive relation between 2,4-D degradation and bacterial numbers.

13 There are few studies on differences in microbial communities across landscape positions.
14 For example, Zak et al. (1994) found that microbial biomass trends to vary topographically.
15 Broughton and Gross (2000) found more productive and active microbial communities in
16 soils at downslope positions compared to soils in up and midslope positions. Sveshnikova
17 et al. (2001) found that the number of bacteria in up and downslope positions was higher
18 than the midslope positions in forest soils. However, none of these studies considered the
19 effect of variations in microbial communities on pesticide persistence.

20 The rates of 2,4-D degradation can vary considerably with different soil types and different
21 soil organic matter contents (Ogram et al. 1985; Benoit et al. 1999). To this point, little is

1 known concerning variation of pesticide degradation across landscape. Therefore, the
2 objective of this study was to quantify the spatial variability of soil properties, microbial
3 communities and 2,4-D sorption and mineralization across a soil-landscape near Miami,
4 Manitoba, Canada.

5 **4.3 Materials and Methods**

6 **4.3.1 Study Site: As described in Chapter 3.3.1**

7 **4.3.2 Soil Sampling and Analyses: As described in Chapter 3.3.2**

8 **4.3.3 Microcosm Experiments**

9 **4.3.3.1 Analytical Methods** The herbicide stock solutions for experiments were prepared
10 in 0.01 M CaCl₂ by mixing ¹⁴C-ring labeled 2,4-D (99% radiochemical purity; sp. act. 250
11 μCi; Sigma Aldrich Chemical Company, St. Louis, MO) with analytical-grade 2,4-D (95%
12 chemical purity, Sigma Chemical Co., St. Louis, MO). The amounts of radioactivity in
13 herbicide solutions and samples from experiments were determined by Liquid Scintillation
14 Counting (LSC) (LS 7500 Beckman Instruments, Fullerton, CA) using 10 ml of 30%
15 Scintisafe scintillation cocktail (Fisher Scientific, Fairlawn, NJ).

16 **4.3.3.2 Herbicide Mineralization** Herbicide mineralization rates were determined in
17 microcosms (4 replicates) consisting of sealed 500 mL mason jars. Field moist soils were
18 sieved (< 2 mm) and added (25 g, weight on an oven-dry basis) into a 50 ml flint jar inside
19 each microcosm (Figure 4.1). The water content of the soil was then adjusted to 80% of its
20 water holding capacity and soils were pre-incubated at 20° C for 21 days to stimulate soil
21 microbial activity. To represent the field situation following herbicide application, a 0.5
22 mL water solution containing 0.834 μg analytical-grade 2,4-D and 8,333 Bq of ¹⁴C-ring

- 1 labeled 2,4-D was applied onto the soil surface in each microcosm at recommended field
2 application rates (0.28-0.71 L/ac from Crop protection Guide 2002).

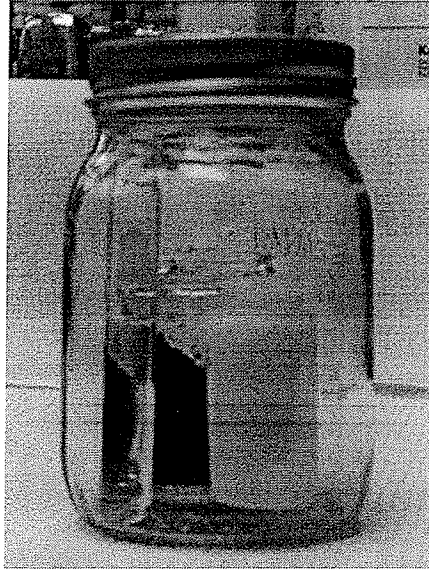


Figure 4.1 Microcosm

- 3 Soils were then incubated at 20° C and the herbicide mineralization was quantified by
4 trapping the evolved $^{14}\text{C-CO}_2$ in a 15 mL scintillation vial containing 5 mL 0.5 M NaOH.
5 The NaOH traps were replaced every alternate day for the first 30 days, twice a week for
6 the next 24 days, once a week for next 20 days and once in two weeks for the last 56 days
7 for a total of 130 days after 2,4-D application. Each microcosm also included a vial with 5
8 mL acidified water (pH~3) to maintain a humid environment. The water was acidified to
9 prevent CO_2 trapment and microbial growth.
- 10 Mineralization rate constants were calculated in SigmaPlot 2000 (SPSS Inc.) based on the
11 assumption that 2,4-D mineralization rates were first-order:

12
$$Mt = M_T (1 - e^{-kt}) \quad (4.1)$$

1 where M_t = 2,4-D mineralization at time t (days), expressed as a percentage of the initially
2 applied radioactivity (%); M_T = total 2,4-D mineralized at time infinity, expressed as a
3 percentage of the initially applied radioactivity (%); k = first-order mineralization rate
4 constant (day^{-1}); and t = time (day).

5 The half-life of 2,4-D is here defined as the time it takes for 50% of the mineralizable
6 fraction to be mineralized. The following equation was used:

$$7 \quad t_{1/2} = 0.693/k \quad (4.2)$$

8 where $t_{1/2}$ is half-life (days) and k is the first-order mineralization rate constant (day^{-1})

9 **4.3.4 Microbial Community Composition**

10 Ten soil samples along a transect (elevation ranged from 416 to 432 m above the sea level)
11 were chosen from the same field to examine variations in the size and diversity of the soil
12 microbial communities.

13 Soil samples were analyzed for microbial community biomass and composition using
14 phospholipids fatty acids (PLFA) derived from each soil sample (White and Ringelberg
15 1998). Total lipids were extracted from soil samples (equivalent to 8 g of dry soil) using
16 the procedure described by Macnaughton et al. (1997). Phospholipid fatty acids were
17 separated by silicic acid chromatography and derivitizing the PLFAs in an alkaline system
18 to form fatty acid methyl esters (FAMES). Fatty acid methyl esters (FAMES) were
19 separated and quantified by gas chromatography (Varian star 3400 CX). The column was
20 set initially at 150 °C. Thereafter, the temperature raised to 185 °C at a rate of 1.5 °C min^{-1}

1 and further 325 °C at a rate of 25 °C min⁻¹ and held for one minute. Thus, total run time was
2 30 minutes. The injector and detector were maintained at 270 and 290 °C respectively.
3 Individual FAMES were identified by comparison with an external standard (Bacterial acid
4 methyl ester standard, Sigma). Fatty acid terminology utilizes 'A:BωC' where A indicates
5 the total number of carbon atoms and B, the number of unsaturated carbon and ωC
6 indicates the number of carbon atoms between the aliphatic end of the molecule and the
7 first unsaturated bond.

8 **4.3.5 Statistical Methods:**

9 Principal component analysis (PCA) was used to identify general relations among the
10 variates and how they cluster. PCA was calculated using a correlation matrix rather than
11 the covariance matrix to eliminate the effects of different measurement units and was
12 performed with version 5 of SYN-TAX 2000 (Podani 2000).

13 Further statistical analyses were performed using SAS version 8.01 (SAS Institute Inc.
14 2000). The Tukey's multiple comparison tests were used to compare the effects of slope
15 position on herbicide mineralization. Data were also analyzed to determine the strengths of
16 correlations between soil properties (i.e. SOC content, soil pH, carbonate content, and soil
17 clay, silt, and sand content) and 2,4-D mineralization parameters (i.e. mineralization rate
18 constant k, total mineralization ExpM_T and half-life) using the Pearson pairwise correlation
19 coefficient method.

20 Stepwise multiple linear regression analysis was carried out to determine which
21 combinations of soil properties have the greatest affect on herbicide mineralization, and

1 eventually they were used for predicting k , ExpM_T and half-life. The use of R^2 values from
2 multiple regressions for obtaining a predictive equation is complicated because adding
3 additional variables will always increase the R^2 of the fit. Including many variables also
4 increases the instability and the complexity of the models. Therefore in addition to R^2 , the
5 Mallow's C_p statistic was also used for selecting the best model to predict k , ExpM_T and
6 half-life. Mallow's C_p attempt to balance maximizing the fit while trying to protect against
7 overfitting.

8 **4.4 Results**

9 **4.4.1 Soil Properties and 2,4-D Mineralization Across the Landscape**

10 The variability of soil properties i.e. SOC content, surface pH, soil texture and total
11 carbonate content across the landscape has been described in chapter 3.4.1.1. Therefore, the
12 following sections describe the variability of 2,4-D mineralization across the landscape.

1 **4.4.1.1 The Variability of 2,4-D Mineralization** For each soil sample, the first-order
 2 model (Equation 4.1) had an excellent fit with the calculated $^{14}\text{C-CO}_2$ mineralization data
 3 with coefficients of determination (r^2 values) ranging from 0.94 to 1.0 with an average
 4 value of 0.97 (Appendx-II). The rate constant, k , varied across the landscape from 0.03
 5 day $^{-1}$ to 0.15 day $^{-1}$, with an average value of 0.08 ± 0.03 day $^{-1}$ (standard deviation)
 6 (Figures 4.2a). Total 2,4-D mineralization at 130 days following herbicide application
 7 ranged from 20 to 42% with an average value of $35 \pm 4\%$ (Figure 4.2b). Half-life varied
 8 from 4 to 27 days across the landscape with an average value of 10 days (Figure 4.2c).

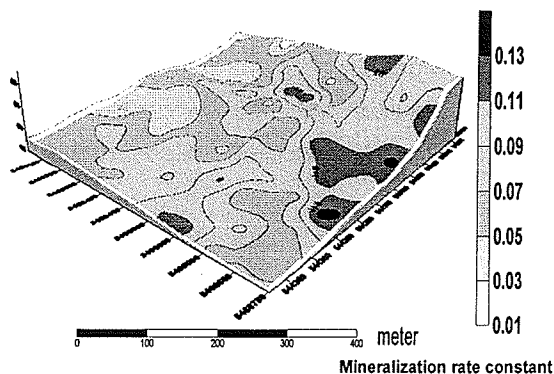


Figure 4.2a The spatial distribution of the mineralization rate constant (day $^{-1}$).

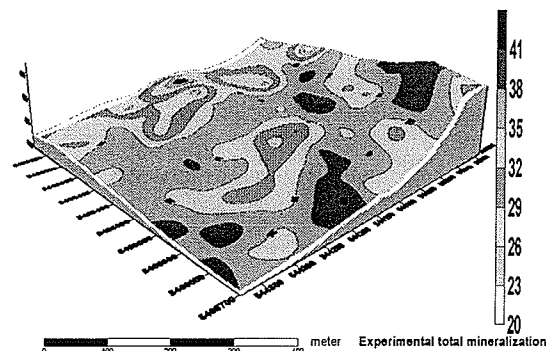


Figure 4.2b The spatial distribution of total mineralization at 130 days as % of initially applied 2,4-D.

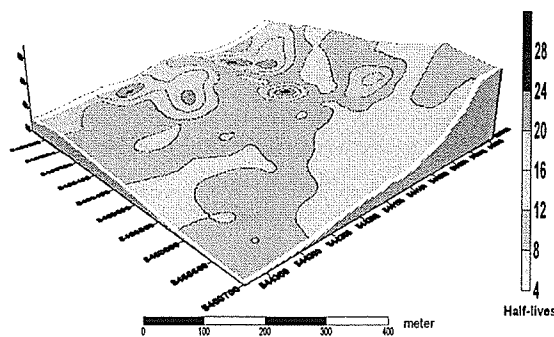


Figure 4.2c The spatial distribution of half-lives (days).

1 A representative mineralization curve is given in Figure 4.3. In general, there was no lag
 2 phase in the 2,4-D mineralization (Appendix-III), which could suggest that mineralization
 3 was due to cometabolism, the process by which a pesticide is not utilized for microbial
 4 nutritional benefit or energy (Topp et al. 1997).

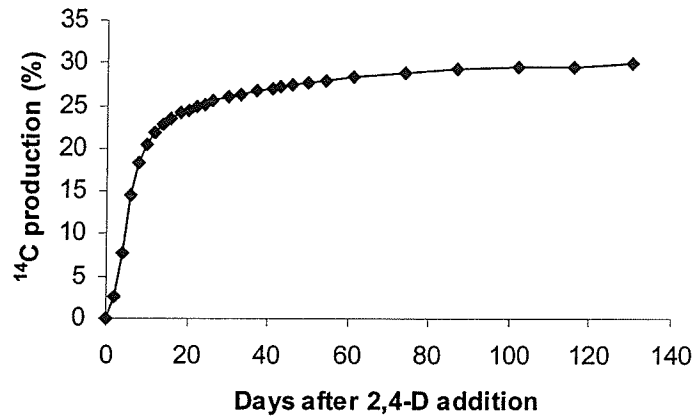


Figure 4.3 2,4-D mineralization (measured as percent of ^{14}C production).

5 Good positive correlation ($r = 0.65$, $p < 0.001$, Figure 4.4) was observed between k and
 6 ExpM_T . However, ExpM_T and 2,4-D half-lives were moderately negatively correlated ($r = -$
 7 0.53 , $p < 0.001$, Figure 4.5).

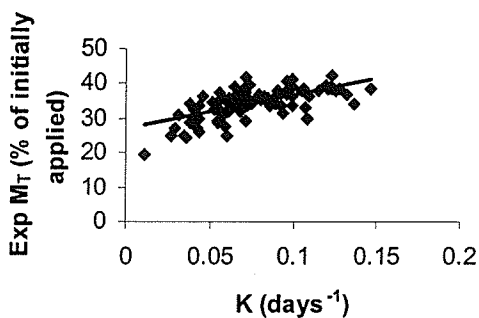


Figure 4.4 Relationship between total mineralization at 130 days (ExpM_T) and mineralization rate constant (k) of 2,4-D.

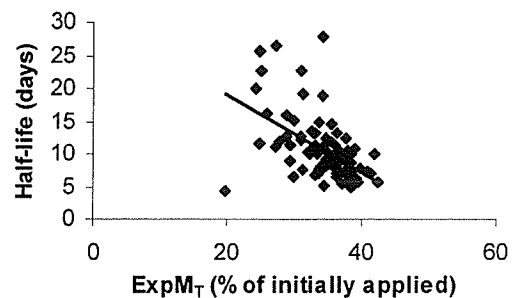


Figure 4.5 Relationship between total mineralization (ExpM_T) and half-lives of 2,4-D.

1 **4.4.1.2 Principal Component Analysis** The first principal axes accounted for 28% of
 2 the total variance. Soil organic carbon (SOC) content, pH and clay content were heavily
 3 weighted on the first axes (Figure 4.6). The second principal axes accounted for 26% of the
 4 initial variance and it was weighted by carbonate content, k and ExpM_T . Therefore, the first
 5 two principal components together explained 54% of the total variability and the remaining
 6 46% may be explained by other variables or factors, including those that were not
 7 measured in this experiment.

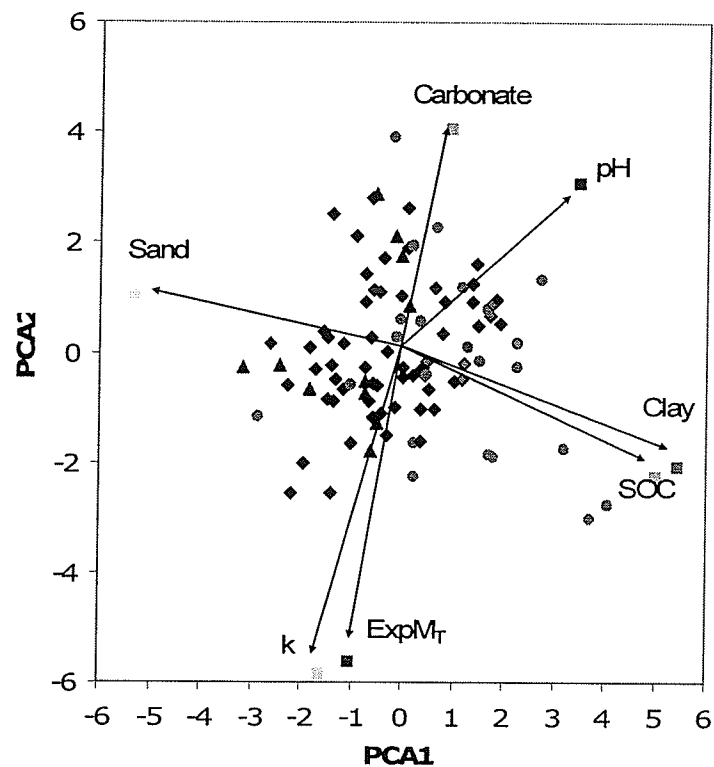


Figure 4.6 The first two principal components showing the variables with major contribution. Variable identifications are, SOC= soil organic carbon content, k = mineralization rate constant, ExpM_T = total mineralization. ▲= upper slope samples; ■ = middle slope samples; ● = lower slope samples.

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1 As explained in section 3.4.1.2, lines pointing in the same direction correspond to variables
 2 which are positively correlated. This is the case for k and ExpM_T as well SOC content and
 3 clay (Figure 4.6). Lines pointing in opposite direction correspond to negatively correlated
 4 variables, e.g. clay and sand content (Figure 4.6). Soil samples from the lower slope
 5 positions were clustered to one side where SOC content and clay content were high.
 6 Whereas, soil samples from either the upper or middle slope positions demonstrated no
 7 distinct clustering, but their soils generally had higher carbonate and sand contents (Figure
 8 4.6).

9 **4.4.1.3 Correlations Between Soil Properties and 2,4-D Mineralization for the Whole**

10 **Field** The following discussion focuses mainly on variables that are significantly
 11 correlated (Table 4.1).

12 Table 4.1 Pearson correlation coefficients between all soil properties (soil organic carbon
 13 content (SOC), pH, carbonate content, clay, silt, and sand content) and mineralization
 14 parameters (mineralization rate constant k, total mineralization ExpM_T and half-life)
 15 for all sampling points (n = 96).

	SOC (%)	Soil pH	Carbonate (%)	Clay (%)	Silt (%)	Sand (%)	k (day ⁻¹)	ExpM_T (%)	Half-life (days)
k (day ⁻¹)	-0.72 ***	-0.46 ***	-	-0.32 ***	-	0.35 ***	1.00		
ExpM_T (%)	-0.76 ***	-	0.29 **	-	-	0.34 ***	0.65 ***	1.00	
Half-life (days)	0.54 ***	0.26 *	-	0.33 **	-	-0.25 *	ND	-0.53 ***	1.00

16 *, **, and *** denote $P < 0.05$, $P < 0.01$, and $P < 0.001$ level of significance, respectively.

17 ND = half-life was calculated from k, therefore correlation is not determined.

18

- 1 SOC content was the most important factor affecting 2,4-D mineralization (Table 4.1) and
- 2 had a strong negative correlation with k ($r = -0.72$, $p < 0.001$, Figure 4.7a) and $\text{Exp}M_T$ ($r =$
- 3 -0.76 , $p < 0.001$, Figure 4.7b). 2,4-D half-lives were adequately positively correlated with
- 4 SOC content ($r = 0.54$, $p < 0.001$, Figure 4.7c).

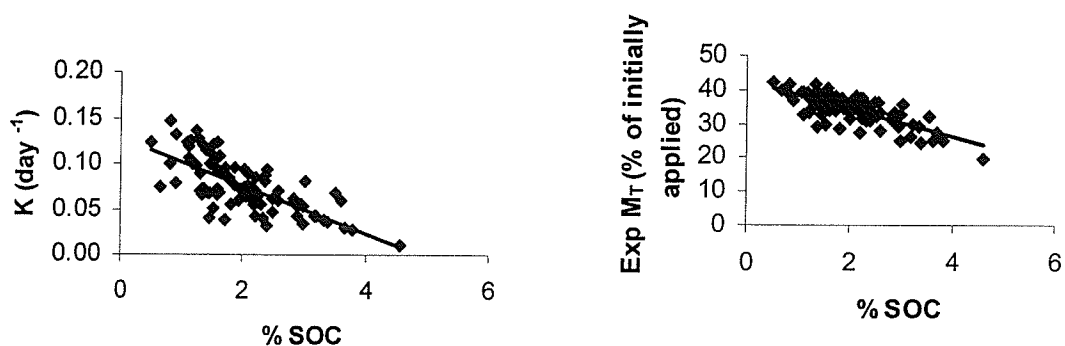


Figure 4.7a Relationship between soil organic carbon content (SOC) and mineralization rate constant (k) of 2,4-D.

Figure 4.7b Relationship between soil organic carbon content (SOC) and total mineralization ($\text{Exp}M_T$) of 2,4-D.

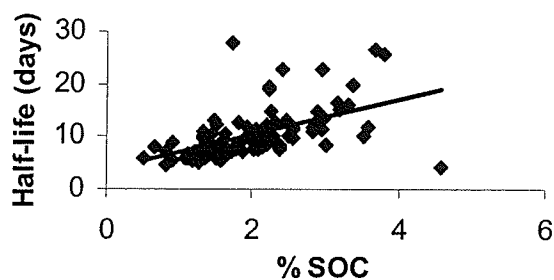


Figure 4.7c Relationship between soil organic carbon content (SOC) and half-lives of 2,4-D.

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- 1 Similar to SOC content, soil pH had a negative correlation with k ($r = -0.46$, $p < 0.001$,
- 2 Figure 4.8a). $\text{Exp}M_T$ also appeared to have a negative association with pH, but the
- 3 correlation coefficient was not statistically significant (Figure 4.8b). 2,4-D half-lives
- 4 were weakly positively correlated ($r = 0.26$, $p < 0.05$, Figure 4.8c) with surface pH.

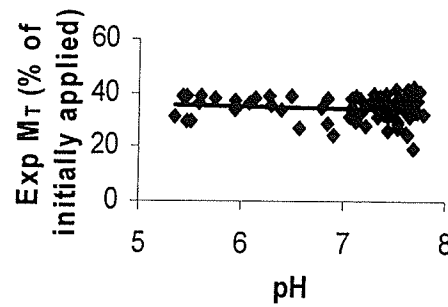
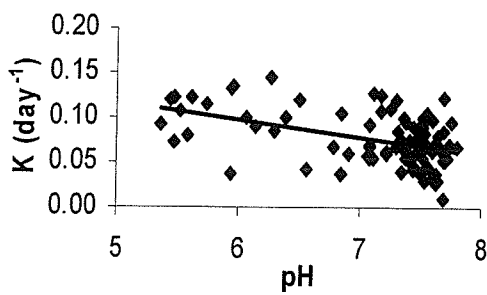


Figure 4.8a Relationship between pH and mineralization rate constant (k) of 2,4-D.

Figure 4.8b Relationship between pH and total mineralization ($\text{Exp}M_T$) of 2,4-D.

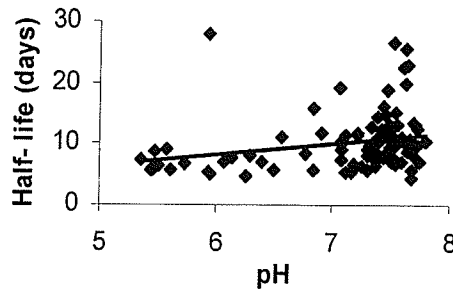


Figure 4.8c Relationship between pH and half-lives of 2,4-D.

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- 1 Total carbonate content had a weak positive correlation ($r = 0.29$, $p < 0.01$, Figure 4.9a)
- 2 with ExpM_T although, a negative correlation was expected because pH and carbonate
- 3 content was positively correlated ($r = 0.39$, $p < 0.001$) as discussed in chapter 3.4.1.3. Total
- 4 carbonate content either with k or 2,4-D half-life did not show any significant correlation
- 5 (Figures 4.9 b, c).

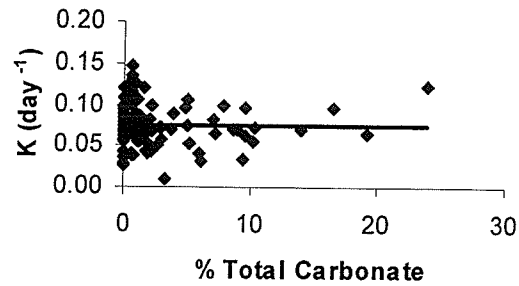
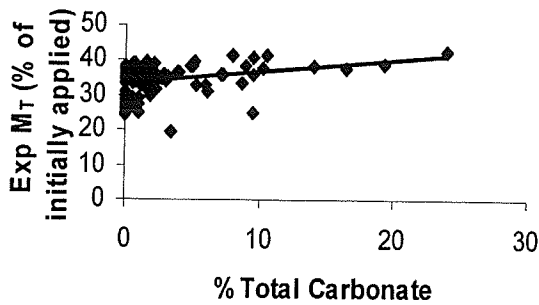


Figure 4.9a Relationship between % total carbonate content and total mineralization (ExpM_T) of 2,4-D.

Figure 4.9b Relationship between % total carbonate content and mineralization rate constant (k) of 2,4-D.

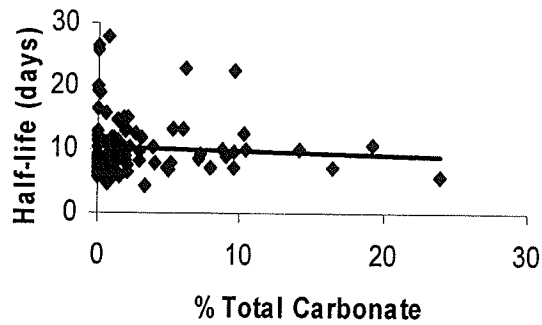


Figure 4.9c Relationship between % total carbonate content and half-lives of 2,4-D.

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- 1 Clay content had a weak negative correlation with k ($r = -0.32$, $p < 0.001$, Figure 4.10a).
- 2 $\text{Exp}M_T$ appeared to have a negative correlation with clay content but the correlation was
- 3 not statistically significant (Figure 4.10b). 2,4-D half-life was weakly positively correlated
- 4 with clay content ($r = 0.33$, $p < 0.01$, Figure 4.10c).

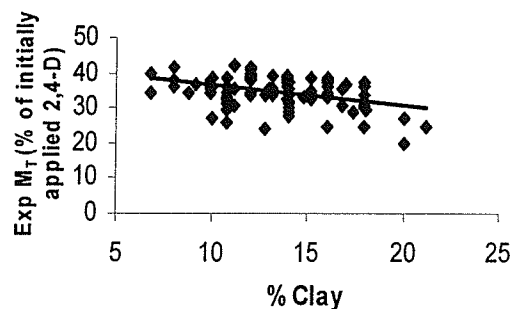
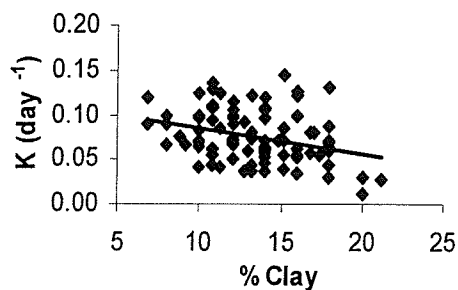


Figure 4.10a Relationship between % clay and mineralization rate constant (k) of 2,4-D.

Figure 4.10b Relationship between % clay and total mineralization of 2,4-D.

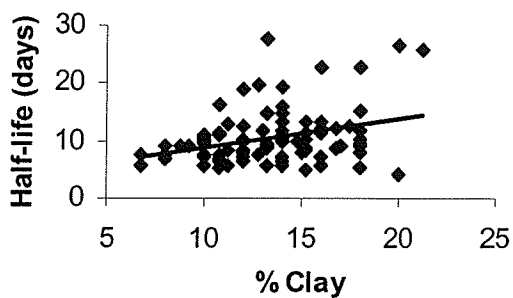


Figure 4.10c Relationship between % clay and half-lives of 2,4-D.

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1 Sand content was weakly positively correlated with both k ($r = 0.35$, $p < 0.001$, Figure
2 4.11a) and $\text{Exp}M_T$ ($r = 0.34$, $p < 0.001$, Figure 4.11b). The 2,4-D half-lives were weakly
3 negatively correlated with sand content ($r = -0.25$, $p < 0.05$, Figure 4.11c). The correlations
4 between silt content and any of the three mineralization parameters were not significant
5 (Data not shown).

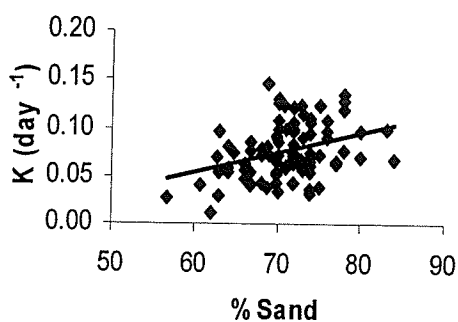


Figure 4.11a Relationship between % sand and mineralization rate constant (k) of 2,4-D.

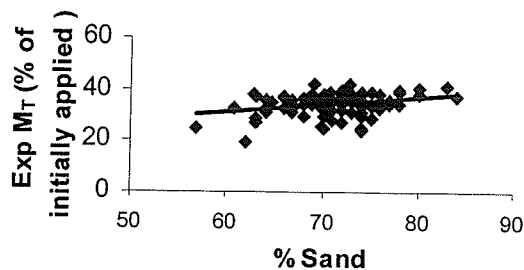


Figure 4.11b Relationship between % sand and total mineralization of 2,4-D.

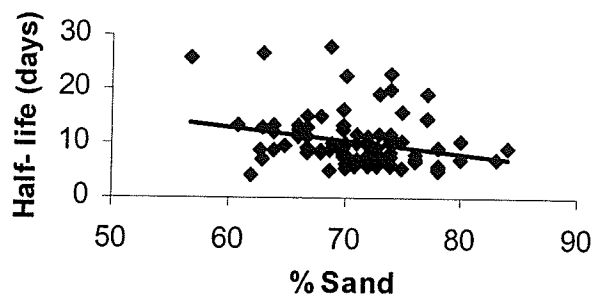


Figure 4.11c Relationship between % sand and half-lives of 2,4-D.

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1 **4.4.1.4 Predicting 2,4-D Mineralization with the Soil Properties for the Whole Field**

2 SOC content was the single most important factor in predicting ExpM_T ($R^2 = 0.58$, Table
 3 4.2). The prediction improved somewhat when soil pH ($R^2 = 0.63$) or both soil pH and sand
 4 content ($R^2 = 0.64$) were added (in addition to SOC content) in the regression model (Table
 5 4.2).

6 Table 4.2 Best regression equations describing dependency of total mineralization
 7 (ExpM_T) on soil organic carbon content (SOC), pH, clay content and carbonate
 8 content combining all sampling points ($n = 96$).

Regression equation	R^2	C(p)	Pr > F
$\text{ExpM}_T = 42.89 - 4.18 \text{ SOC}$	0.58	14.85	0.0001
$\text{ExpM}_T = 32.93 + 1.52 \text{ pH} - 4.64 \text{ SOC}$	0.63	4.58	0.0001
$\text{ExpM}_T = 24.92 + 0.11 \text{ Sand} + 1.52 \text{ pH} - 4.44 \text{ SOC}$	0.64	3.30	0.0001

9 Similarly, k was predicted adequately by SOC content alone ($R^2 = 0.52$, Table 4.3). The
 10 prediction of k was somewhat improved, when soil pH ($R^2 = 0.56$, Table 4.3), or both soil
 11 pH and sand content ($R^2 = 0.58$, Table 4.3) were included (in addition to SOC content) in
 12 the regression model.

13 Table 4.3 Best regression equations describing dependency of mineralization rate constant
 14 (k) on soil organic carbon content (SOC), pH, clay content and carbonate content
 15 combining all sampling points ($n = 96$).

Regression equation	R^2	C(p)	Pr > F
$k = 0.13 - 0.03 \text{ SOC}$	0.52	13.63	0.0001
$k = 0.19 - 0.01 \text{ pH} - 0.02 \text{ SOC}$	0.56	5.14	0.0001
$k = 0.13 + 0.0008 \text{ Sand} - 0.01 \text{ pH} - 0.02 \text{ SOC}$	0.58	2.99	0.0001

1 2,4-D half-life was only weakly predicted by SOC content ($R^2 = 0.29$, Table 4.4). Adding
2 other soil properties in addition to SOC content did not improve the prediction strength.

3 Table 4.4 Best regression equations describing dependency of 2,4-D half-lives on soil
4 organic carbon content (SOC), pH, clay content and carbonate content combining all
5 sampling points ($n = 96$).

Regression equation	R^2	C(p)	Pr > F
Half-life = $3.71 + 3.31 \text{ SOC}$	0.29	0.34	0.0001

6 4.4.2 Soil Properties and 2,4-D Mineralization in Relation to Landscape Position

7 4.4.2.1 The Variability of 2,4-D Mineralization The rate constant, k , was significantly
8 slower in the lower slope ($0.05 \text{ day}^{-1} \pm 0.02$) compared to the middle ($0.08 \text{ day}^{-1} \pm 0.03$)
9 and upper ($0.09 \text{ day}^{-1} \pm 0.03$) slope positions (Figure 4.12). ExpM_T was significantly
10 lower in the lower slope ($31.6 \% \pm 5.0$) compared to the middle ($35.7 \% \pm 3.0$) and upper
11 ($36.1 \% \pm 3.4$) slope positions (Figure 4.13). As expected, the calculated total
12 mineralization at time infinity (Figure 4.14) followed the same pattern as ExpM_T . 2,4-D
13 half-lives increased significantly from the upper slope position ($8 \text{ days} \pm 2$) to middle (9
14 $\text{days} \pm 4$) and lower ($13 \text{ days} \pm 5$) slope positions, respectively (Figure 4.15).

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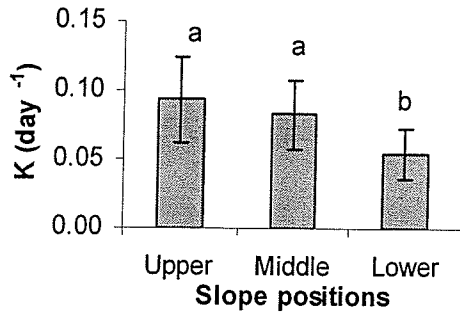


Figure 4.12 Mineralization rate constants ($k \text{ day}^{-1}$) at different slope position. Sample size (n) is 12 for upper, 55 for middle and 29 for lower slope positions. Error bars represent standard deviation.

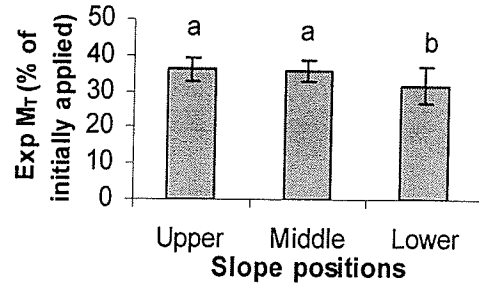


Figure 4.13 Experimental total 2,4-D mineralization (% $\text{Exp } M_T$) at different slope position. Sample size (n) is 12 for upper, 55 for middle and 29 for lower slope positions. Error bars represent standard deviation.

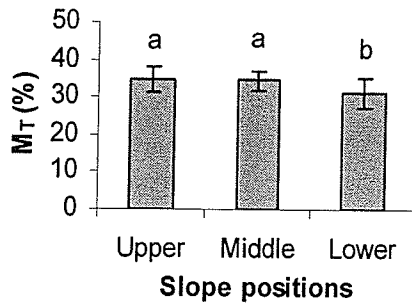


Figure 4.14 Total mineralization at time infinity (% M_T) at different slope position. Sample size (n) is 12 for upper, 55 for middle and 29 for lower slope positions. Error bars represent standard deviation.

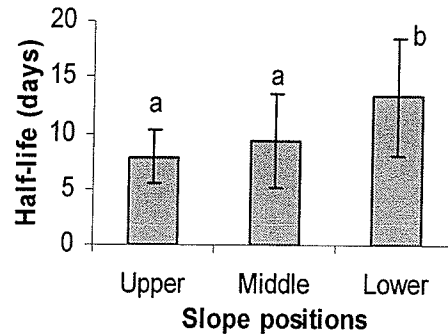


Figure 4.15 Half-lives (days) at different slope position. Sample size (n) is 12 for upper, 55 for middle and 29 for lower slope positions. Error bars represent standard deviation.

1 **4.4.2.2 Correlations Between Soil Properties and 2,4-D Mineralization in Different**
 2 **Landscape Position** A good positive correlation was observed between k and ExpM_T in
 3 the middle slope position ($r = 0.64$, $p < 0.001$, Table 4.5) and a strong positive correlation
 4 ($r = 0.75$, $p < 0.001$, Table 4.5) was found between k and ExpM_T in the lower slope
 5 position. In the upper slope position, k and ExpM_T appeared to be very weakly positively
 6 correlated but the correlation was not statistically significant (Table 4.5).

7
 8 Table 4.5 Pearson correlation coefficients among soil properties (SOC, pH, total
 9 carbonate, clay, silt, and sand) and mineralization parameters (mineralization rate
 10 constant, k , and total mineralization, ExpM_T) as affected by landscape position.
 11

	SOC (%)	Soil pH	Carbonate (%)	Clay (%)	Silt (%)	Sand (%)	k (day^{-1})	ExpM_T (%)	Half-li (days)
Upper (n=12)									
k (day^{-1})	-	-0.57 *	-	-	-	-	1.00		
Exp M_T (%)	-0.60 *		0.64 *	-	-	-		1.00	
Half-life (days)	0.59 *	-	-	-	-	-	ND	-	1.00
Middle (n=55)									
k (day^{-1})	-0.65 ***	-0.37 **	-	-0.28 *	-	0.27 *	1.00		
Exp M_T (%)	-0.66 ***	-	0.32 *	-0.27 *	-	-0.29 *	0.64 ***	1.00	1.00
Half-life (days)	0.44 ***	-	-	0.31 *	-	-	ND	-0.52 ***	1.00
Lower (n= 29)									
k (day^{-1})	-0.68 ***	-	-	-	-	0.42 *	1.00		
Exp M_T (%)	-0.81 ***	-	-	-0.42 *	-	-	0.75 ***	1.00	
Half-life (days)	-	-	-	-	-	-	ND	-0.39 *	1.00

12 *, **, and *** denote $P < 0.05$, $P < 0.01$, and $P < 0.001$ level of significance, respectively.
 13 ND = half-life was calculated from k , therefore correlation is not determined.

14

1 The rate constant, k , was moderately negatively correlated with SOC content in the middle
 2 ($r = -0.65$, $p < 0.001$) and lower ($r = -0.68$, $p < 0.001$) slope positions, but no significant
 3 correlation was found between k and SOC content in the upper slope position (Figure
 4 4.16). $\text{Exp}M_T$ was moderately negatively correlated with SOC content in the upper ($r = -$
 5 0.60 , $p < 0.05$) and middle ($r = -0.66$, $p < 0.05$) slope positions (Figure 4.17). In the lower
 6 slope position, $\text{Exp}M_T$ was strongly negatively correlated with SOC content ($r = -0.81$, $p <$
 7 0.001 , Figure 4.17). Half-life was moderately positively correlated with SOC content in the
 8 upper and middle positions, but no significant correlation was found between half-life and
 9 SOC content in the lower slope position (Figure 4.18).

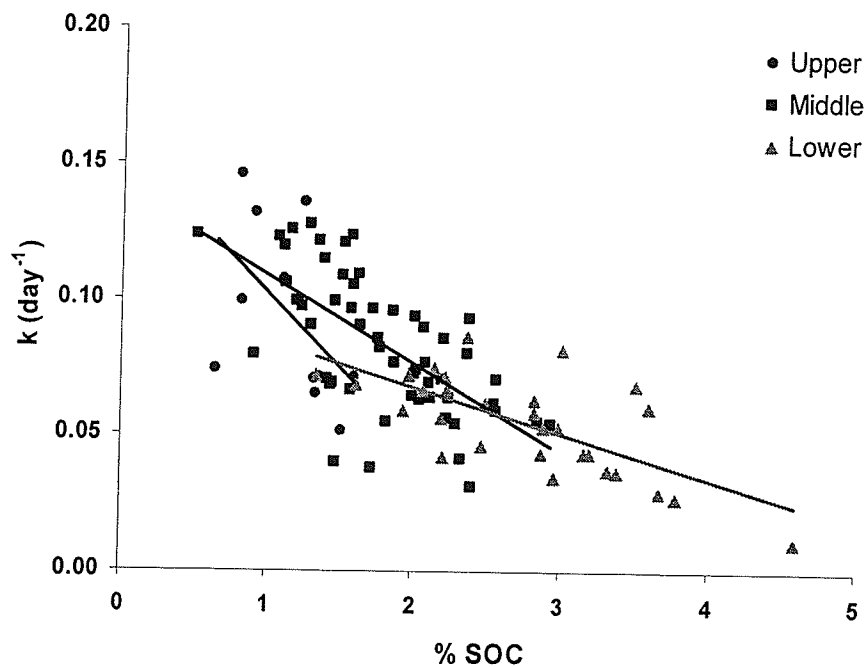


Figure 4.16 Relationship between soil organic carbon content (SOC) and mineralization rate constant (k) of 2,4-D as affected by landscape position. Upper slope ($k = 0.16 - 0.05\% \text{SOC}$; $r = 0.52$, ns), middle slope ($k = 0.14 - 0.03\% \text{SOC}$; $r = -0.65$, $P < 0.001$) and lower slope ($k = 0.10 - 0.02\% \text{SOC}$; $r = -0.68$, $P < 0.001$).

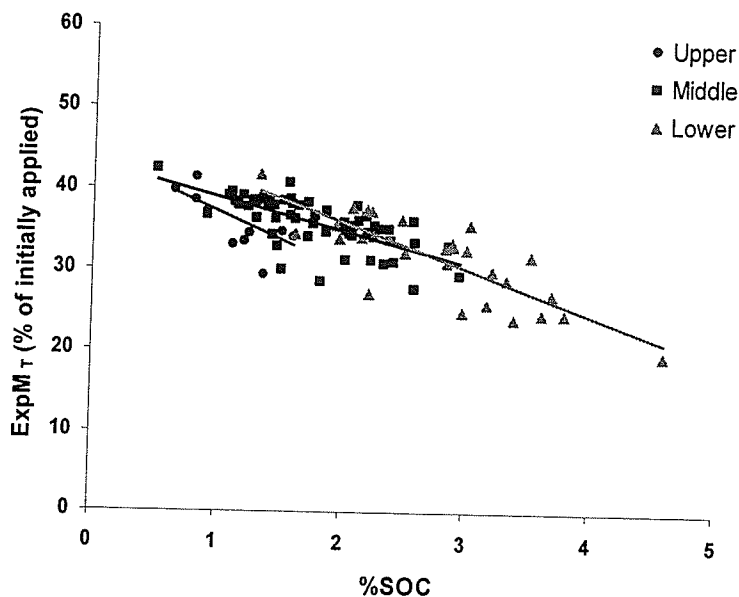


Figure 4.17 Relationship between soil organic carbon content (SOC) and total mineralization ($ExpM_T$) of 2,4-D as affected by landscape position. Upper slope ($ExpM_T = 44.22 - 7.03\%SOC$; $r = -0.60$, $P < 0.05$), middle slope ($ExpM_T = 42.89 - 4.01\%SOC$; $r = -0.66$, $P < 0.001$) and lower slope ($ExpM_T = 47.13 - 5.63\%SOC$; $r = -0.81$, $P < 0.001$).

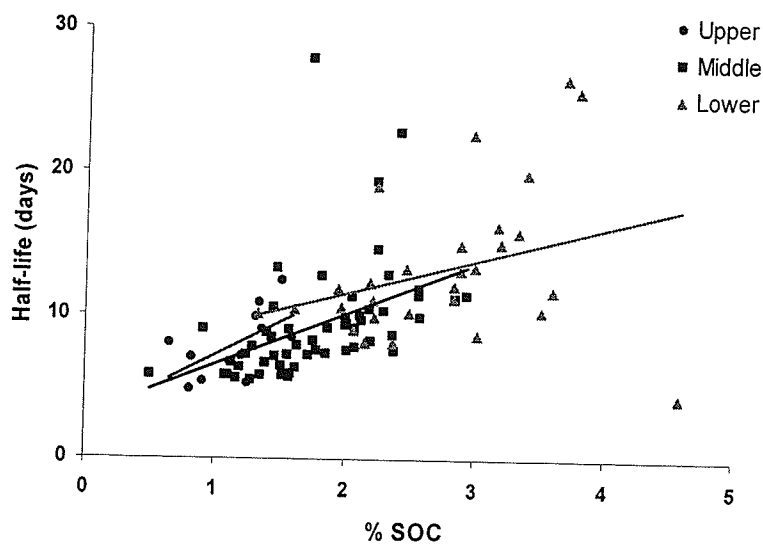


Figure 4.18 Relationship between soil organic carbon content (SOC) and half-lives of 2,4-D as affected by landscape position. Upper slope (Half-life = $2.40 + 4.72\%SOC$; $r = 0.59$, $P < 0.05$), middle slope (Half-life = $2.96 + 3.56\%SOC$; $r = 0.44$, $P < 0.001$) and lower slope (Half-life = $6.95 + 2.30\%SOC$; $r = 0.32$, ns).

1 It was observed that k and soil pH were moderately negatively correlated in the upper slope
2 position ($r = -0.57$, $p < 0.05$, Figure 4.19), weakly negatively correlated in the middle slope
3 position ($r = -0.37$, $p < 0.01$, Figure 4.19), but no significant correlation was found between
4 k and soil pH in the lower slope position (Figure 4.19). In contrast, ExpM_T and half-life
5 had no significant correlation with soil pH in any of the slope positions (Figures 4.20,
6 4.21).

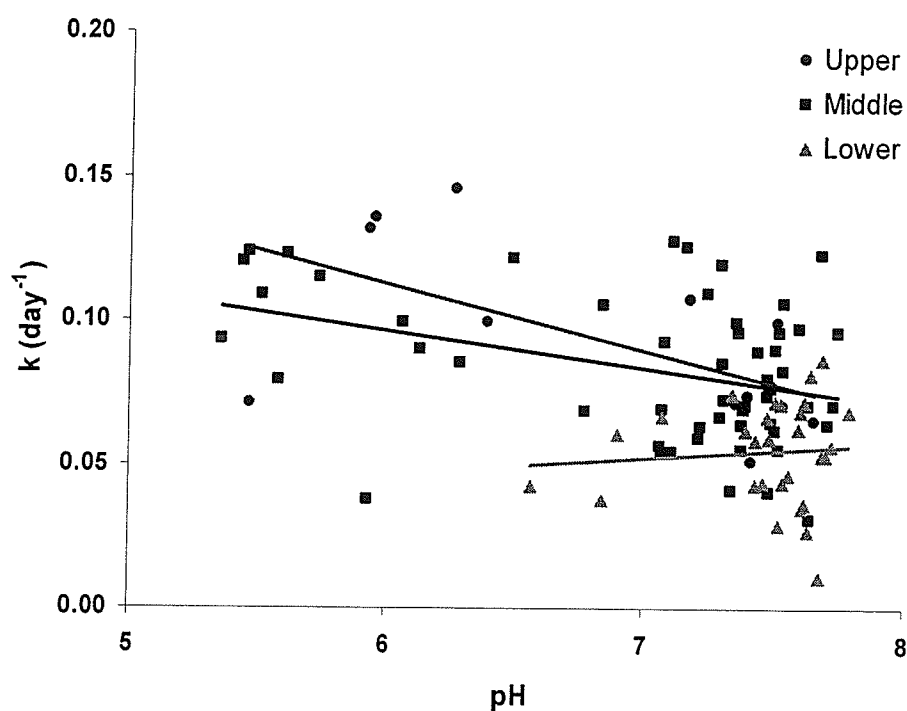


Figure 4.19 Relationship between soil pH and mineralization rate constant (k) of 2,4-D as affected by landscape position. Upper slope ($k = 0.25 - 0.02 \text{ pH}$; $r = -0.57$, $P < 0.05$), middle slope ($k = 0.17 - 0.01 \text{ pH}$; $r = -0.37$, $P < 0.01$) and lower slope ($k = 0.01 + 0.01 \text{ pH}$; $r = 0.09$, *ns*).

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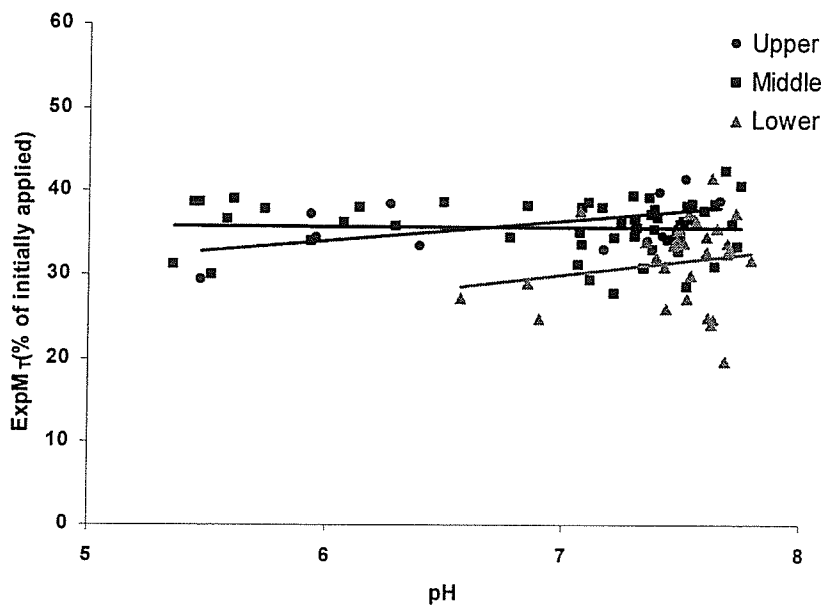


Figure 4.20 Relationship between soil pH and total mineralization (ExpM_T) of 2,4-D as affected by landscape position. Upper slope ($\text{ExpM}_T = 19.54 + 2.42 \text{ pH}$; $r = 0.54$, *ns*), middle slope ($\text{ExpM}_T = 36.13 - 0.06 \text{ pH}$; $r = -0.014$, *ns*) and lower slope ($\text{ExpM}_T = 6.99 + 3.29 \text{ pH}$; $r = 0.18$, *ns*).

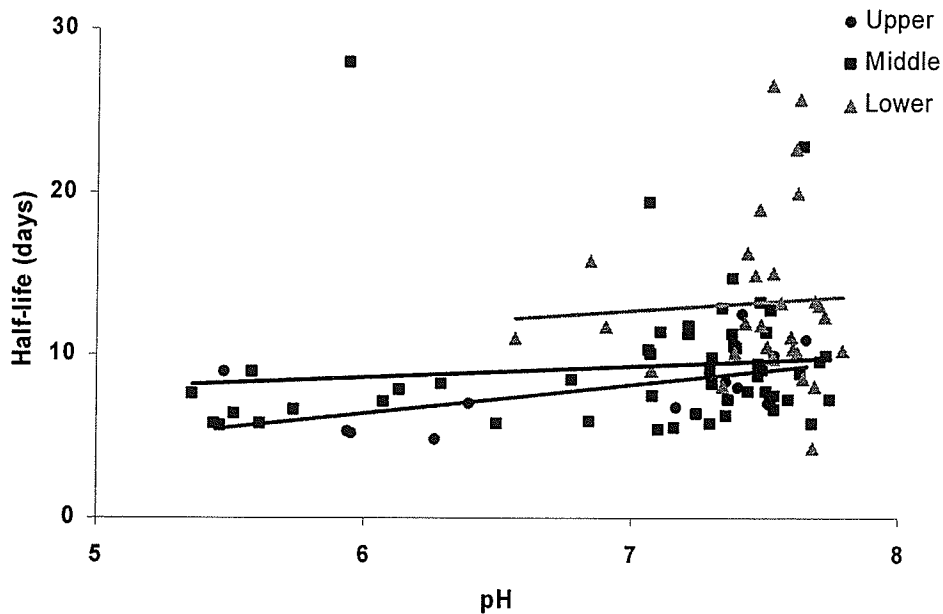


Figure 4.21 Relationship between soil pH and half-lives of 2,4-D as affected by landscape position. Upper slope ($\text{Half-life} = -3.71 + 1.69 \text{ pH}$; $r = 0.56$, *ns*), middle slope ($\text{Half-life} = 4.44 + 0.70 \text{ pH}$; $r = 0.12$, *ns*) and lower slope ($\text{Half-life} = 5.10 + 1.10 \text{ pH}$; $r = 0.06$, *ns*).

1 Total mineralization, $ExpM_T$ had a moderate and weak positive correlation with carbonate
2 content in the upper ($r = 0.64$, $p < 0.05$, Figure 4.22) and middle ($r = 0.32$, $p < 0.05$, Figure
3 4.22) slope positions. However, in the lower slope position, there was no significant
4 correlation observed between $ExpM_T$ and carbonate content (Figure 4.22). There were no
5 significant correlations found between k and carbonate content (Figure 4.23) and between
6 half-life and carbonate content in any of the slope positions (Figure 4.24).

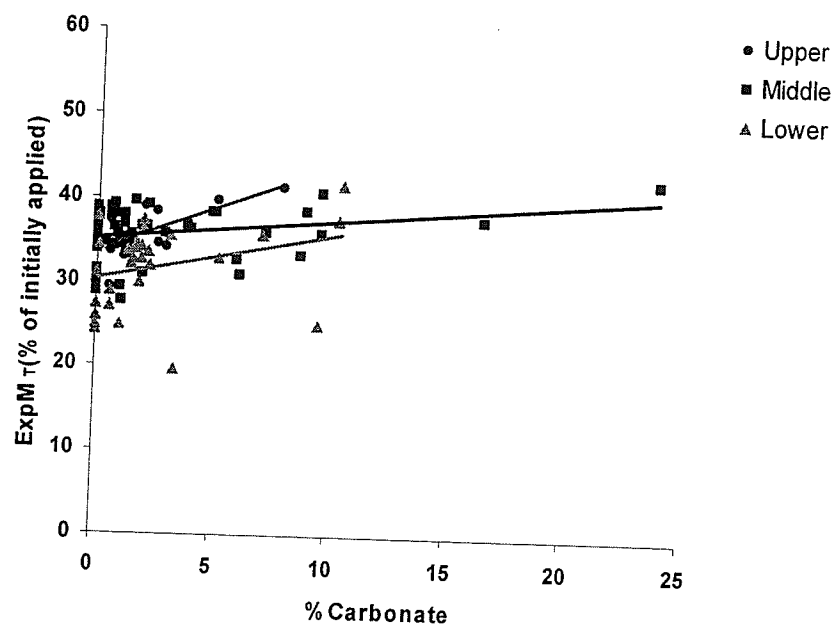


Figure 4.22 Relationship between carbonate content and total mineralization ($ExpM_T$) of 2,4-D as affected by landscape position. Upper slope ($ExpM_T = 33.84 + 0.98$ %Carbonate; $r = 0.64$, $P < 0.05$), middle slope ($ExpM_T = 35.01 + 0.23$ %Carbonate; $r = 0.32$, $P < 0.05$) and lower slope ($ExpM_T = 30.26 + 0.55$ %Carbonate; $r = 0.33$, *ns*).

7

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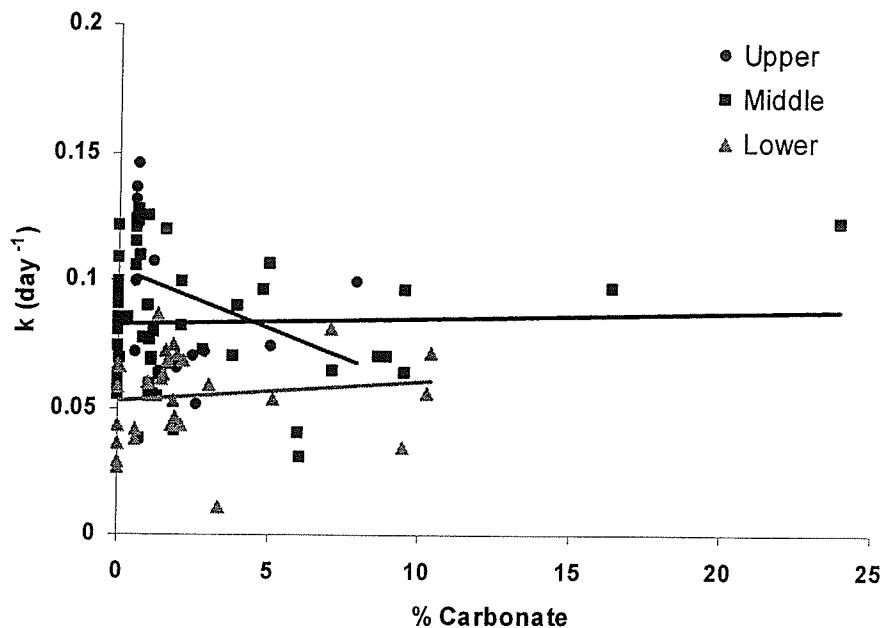


Figure 4.23 Relationship between carbonate content and mineralization rate constant (k) of 2,4-D as affected by landscape position. Upper slope ($k = 0.10 - 0.004$ %Carbonate; $r = -0.33$, *ns*), middle slope ($k = 0.08 + 0.0002$ %Carbonate; $r = 0.04$, *ns*) and lower slope ($k = 0.05 + 0.0007$ %Carbonate; $r = 0.13$, *ns*).

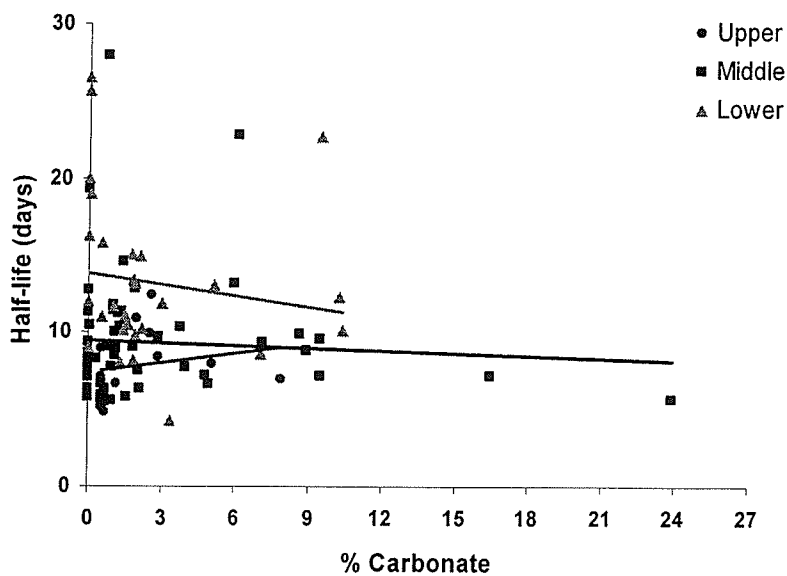


Figure 4.24 Relationship between carbonate content and half-lives of 2,4-D as affected by landscape position. Upper slope (Half-life = $7.40 + 0.21$ %Carbonate; $r = 0.20$, *ns*), middle slope (Half-life = $9.48 - 0.05$ %Carbonate; $r = 0.06$, *ns*) and lower slope (Half-life = $13.89 - 0.24$ %Carbonate; $r = 0.14$, *ns*).

1 In the upper slope position, neither k , nor ExpM_T had any significant correlation with
2 percent clay, silt or sand content (Table 4.5). Similar trend was also observed for half-life
3 (Table 4.5). The rate constant, k , had a weakly significant negative correlation with % clay
4 ($r = -0.28$, $p < 0.05$, Figure 4.25) in the middle slope position, whereas half-life had a weak
5 positive correlation with % clay in the middle slope position ($r = 0.31$, $p < 0.05$, Figure
6 4.27). However, neither k nor half-life had any significant correlation with % clay in the
7 lower slope position. ExpM_T had weak negative correlation with % clay ($r = -0.27$, $p <$
8 0.05 , Figure 4.26) in the middle slope position and a moderate negative correlation ($r = -$
9 0.42 , $p < 0.05$, Figure 4.26) in the lower slope position. The rate constant, k , had weakly
10 positive correlation with % sand in both middle ($r = 0.27$, $p < 0.05$) and lower ($r = 0.42$, $p <$
11 0.05) slope positions (Table 4.5). On the other hand, ExpM_T had a negative correlation ($r =$
12 -0.29 , $p < 0.05$) with sand content in the middle slope position. No significant correlation
13 was found between mineralization parameters and silt content in any slope positions (Table
14 4.5).

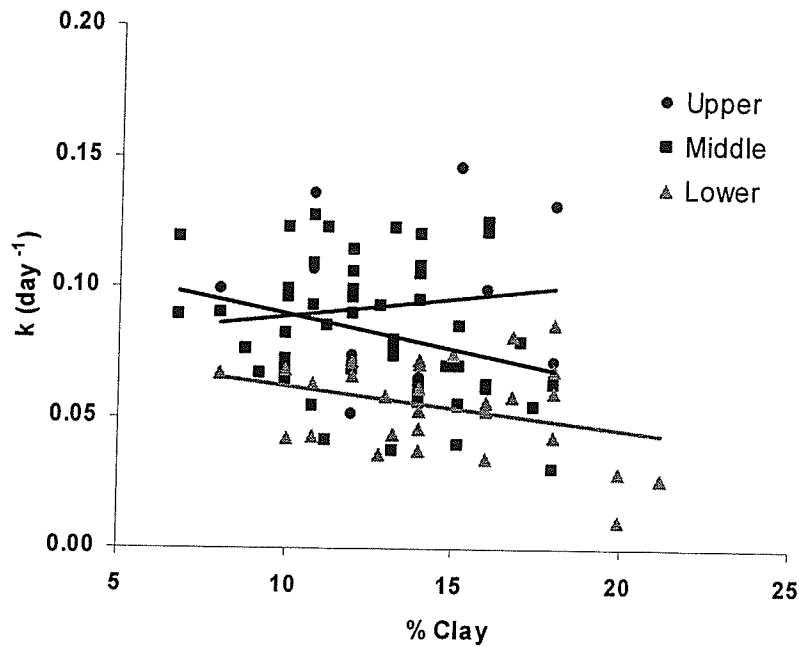


Figure 4.25 Relationship between clay content and mineralization rate constant (k) of 2,4-D as affected by landscape position. Upper slope ($k = 0.08 + 0.001\%Clay$; $r = 0.13$, ns), middle slope ($k = 0.12 - 0.002 \%Clay$; $r = -0.28$, $P < 0.05$) and lower slope ($k = 0.08 - 0.001\%Clay$; $r = 0.30$, ns).

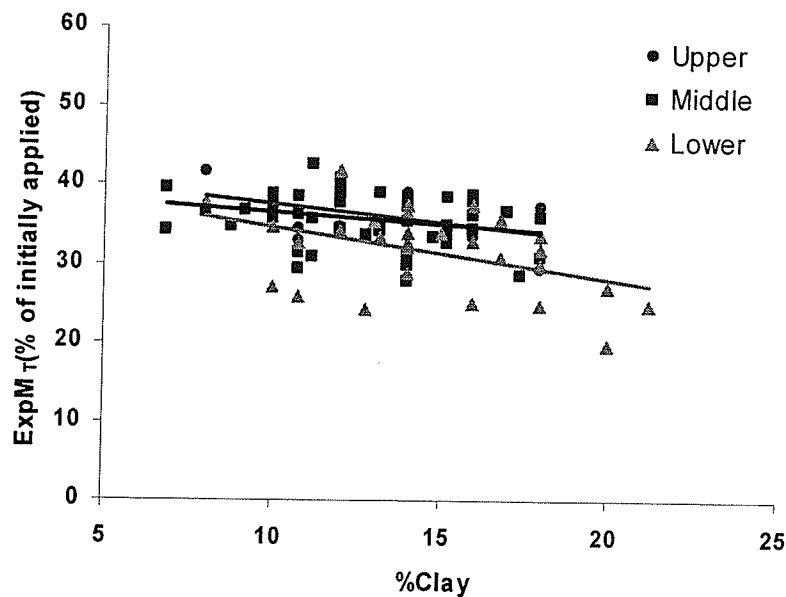


Figure 4.26 Relationship between clay content and total mineralization ($ExpM_T$) of 2,4-D as affected by landscape position. Upper slope ($ExpM_T = 42.01 - 0.45\%Clay$; $r = -0.39$, ns), middle slope ($ExpM_T = 39.5 - 0.30\%Clay$; $r = -0.27$, $P < 0.05$) and lower slope ($ExpM_T = 41.12 - 0.65\%Clay$; $r = -0.42$, $P < 0.05$).

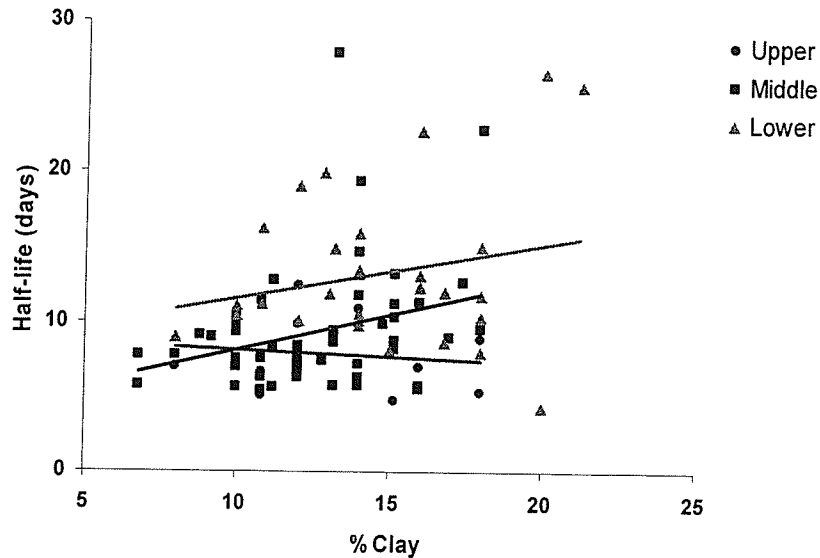


Figure 4.27 Relationship between clay content and half-lives of 2,4-D as affected by landscape position. Upper slope (Half-life = $8.92 - 0.08\%Clay$; $r = 0.10$, *ns*), middle slope (Half-life = $3.43 + 0.47\%Clay$; $r = 0.31$, $P < 0.05$) and lower slope (Half-life = $8.05 + 0.36\%Clay$; $r = 0.22$, *ns*).

1 **4.4.2.3 Predicting 2,4-D Mineralization with the Soil Properties in Different**
 2 **Landscape Position** SOC content was the most important factor determining $ExpM_T$ of
 3 2,4-D regardless of landscape positions (Table 4.6). However, the strength of prediction by
 4 SOC content varied significantly amongst the landscape position. In the upper slope
 5 position, SOC content predicted $ExpM_T$ with $R^2 = 0.36$ ($p < 0.04$, $C_p = 13.97$, Table 4.6),
 6 however, as the slope position changes to middle and lower, the coefficient between SOC
 7 content and $ExpM_T$ increased to $R^2 = 0.44$ and $R^2 = 0.65$, respectively (Table 4.6).
 8 Therefore, only 36% variability of total mineralization could be explained by SOC content
 9 in the upper slope position whereas, in the middle and lower slope positions, 44% and 65%
 10 variability in $ExpM_T$ could be explained by SOC content.

11

1 Table 4.6 Best regression equations for different slope positions (upper, middle and lower)
 2 describing dependency of the total mineralization ($ExpM_T$) on soil organic carbon
 3 content (SOC), pH, clay content and carbonate content.

Landscape positions	Regression equation	R^2	C(p)	Pr > F
Upper (n=12)	$ExpM_T = 44.22 - 7.03 \text{ SOC}$	0.36	13.97	0.04
	$ExpM_T = 43.57 - 7.92 \text{ SOC} + 0.36 \text{ Carbonate}$	0.76	2.37	0.001
Middle (n=55)	$ExpM_T = 42.88 - 4.01 \text{ SOC}$	0.44	12.21	0.0001
	$ExpM_T = 27.26 + 0.22 \text{ Sand} - 4.02 \text{ SOC}$	0.53	4.26	0.0001
	$ExpM_T = 22.10 + 0.22 \text{ Sand} + 0.79 \text{ pH} - 4.31 \text{ SOC}$	0.56	2.86	0.0001
Lower (n=29)	$ExpM_T = 47.13 - 5.63 \text{ SOC}$	0.65	3.32	0.0001
	$ExpM_T = 14.33 + 4.44 \text{ pH} - 5.76 \text{ SOC}$	0.71	0.28	0.0001

4

5 Regardless of slope position, the prediction was improved when one or two other soil
 6 properties were included along with SOC content. The natures of the soil properties were
 7 somewhat different for each slope position. In the upper slope position, when carbonate
 8 content was included with SOC content, the regression model best predicted $ExpM_T$ with a
 9 $R^2 = 0.76$ ($p < 0.001$, $C_p = 2.37$, Table 4.6). Whereas, in the middle slope position, the
 10 prediction was optimal when sand content was added to the model ($R^2 = 0.53$, $p < 0.0001$,
 11 $C_p = 4.26$, Table 4.6), while the addition of pH to both SOC content and sand content in
 12 the model additionally improved the prediction, but not greatly ($R^2 = 0.56$, $p < 0.0001$, C_p
 13 $= 2.86$, Table 4.6). In the lower slope position, the prediction was strongly improved when
 14 soil pH was included in the model with SOC content ($R^2 = 0.71$, $p < 0.0001$, $C_p = 0.28$,
 15 Table 4.6).

16 When soil properties were used to predict k for the middle and lower slope positions, SOC
 17 content was the most important contributing parameter (Table 4.7), with a $R^2 = 0.42$ and

1 0.46 respectively ($p < 0.0001$, Table 4.7). Adding sand content ($R^2 = 0.49$, $p < 0.0001$) or
 2 both sand content and pH ($R^2 = 0.53$, $p < 0.0001$) in the model, improved the prediction
 3 level for the middle slope position. In the lower slope, the prediction improved when silt
 4 content ($R^2 = 0.52$, $p < 0.0001$) or both silt content and pH ($R^2 = 0.57$, $p < 0.0001$, Table
 5 4.7) were included in the model in addition to SOC content. In contrast, in the upper slope
 6 position, soil pH had greatest effect on k as a single variable ($R^2 = 0.32$, $p < 0.05$, $C_p =$
 7 3.24, Table 4.7), but adding SOC content improved the model prediction ($R^2 = 0.56$, $p <$
 8 0.02, $C_p = 1.37$, Table 4.7). Half-life was weakly determined by SOC content in the upper
 9 and middle slope positions (Table 4.8) with a $R^2 = 0.35$ and 0.19 respectively. Adding soil
 10 pH ($R^2 = 0.61$, $p < 0.01$) in the upper slope position and clay ($R^2 = 0.24$, $p < 0.0007$) in the
 11 middle slope position (in addition to SOC content) improved the prediction strength.

12 Table 4.7 Best regression equations for different slope positions (upper, middle and lower)
 13 describing dependency of the mineralization rate constant (k) on soil organic carbon
 14 content (SOC), pH, clay content and carbonate content.

Landscape positions	Regression equation	R^2	$C(p)$	$Pr > F$
Upper (n=12)				
	$k = 0.25 - 0.02 \text{ pH}$	0.32	3.24	0.05
	$k = 0.29 - 0.02 \text{ pH} - 0.05 \text{ SOC}$	0.56	1.37	0.02
Middle (n=55)				
	$k = 0.14 - 0.03 \text{ SOC}$	0.42	10.68	0.0001
	$k = 0.02 + 0.001 \text{ Sand} - 0.03 \text{ SOC}$	0.49	4.58	0.0001
	$k = 0.07 + 0.001 \text{ Sand} - 0.01 \text{ pH} - 0.03 \text{ SOC}$	0.53	2.54	0.0001
Lower (n=29)				
	$k = 0.10 - 0.02 \text{ SOC}$	0.46	4.99	0.0001
	$k = 0.11 - 0.001 \text{ Silt} - 0.02 \text{ SOC}$	0.52	3.80	0.0001
	$k = 0.01 - 0.001 \text{ Silt} + 0.01 \text{ pH} - 0.02 \text{ SOC}$	0.57	2.98	0.0001

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1 Table 4.8 Best regression equations for different slope positions (upper, middle and lower)
 2 describing dependency of the half-lives on soil organic carbon content (SOC), pH,
 3 clay content and carbonate content.

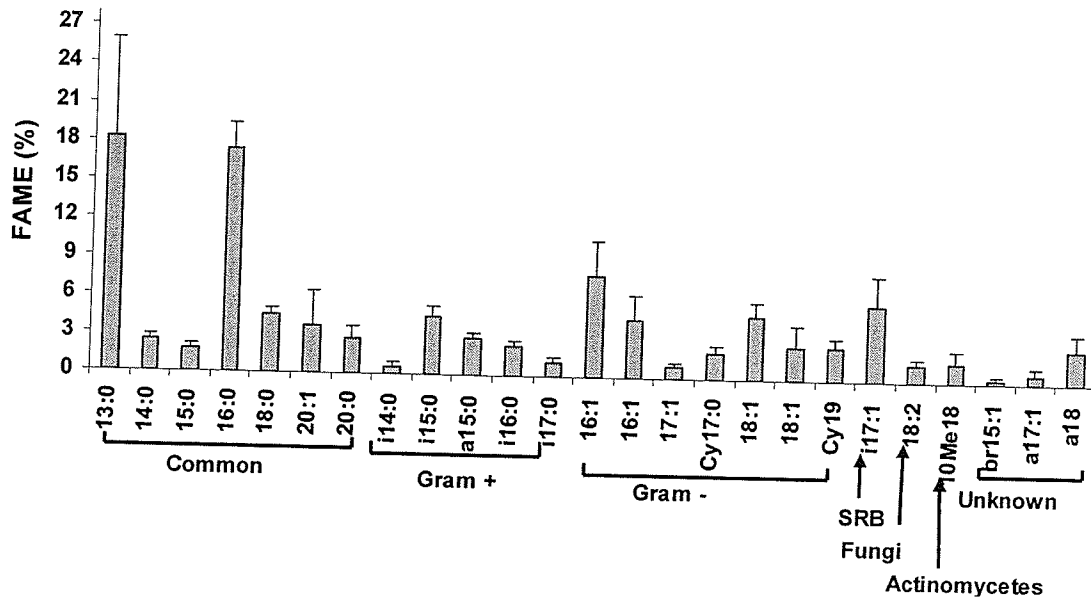
Landscape positions	Regression equation	R ²	C(p)	Pr > F
Upper (n=12)	Half-life = 2.39 + 4.72 SOC	0.35	5.42	0.041
	Half-life = -7.90 + 1.56 pH + 4.41 SOC	0.61	2.01	0.013
Middle (n=55)	Half-life = 2.96 + 3.56 SOC	0.19	1.73	0.0008
	Half-life = -0.87 + 0.35 Clay + 3.19 SOC	0.24	0.32	0.0007
Lower (n=29)	--*			

4 --* = not significant

5 4.4.3 Fatty Acid Methyl Ester (FAME) and 2,4-D Mineralization Across the 6 Landscape

7 The diversity of FAME was large across the transect. Twenty-five FAMEs were separated
 8 into their representative taxonomic groups (gram positive bacteria, gram negative bacteria,
 9 sulfate reducing bacteria, fungi, actinomycetes; Figure 4.28).

10 There was no particular trend observed along the transect in the distribution of different
 11 microbial community (gram positive bacteria, gram negative bacteria, fungal, sulfate
 12 reducing bacteria, actinomycetes) or number of fatty acids extracted (Figures 4.29 and
 13 4.30).



Microbial groups

1
2 Figure 4.28 Average percent of fatty acid methyl esters (FAME) and their taxonomic
3 groups. (The prefixes 'i' and 'a' refer to iso and anti-iso methyl branching, 'Cy' indicates a
4 cyclic group).
5

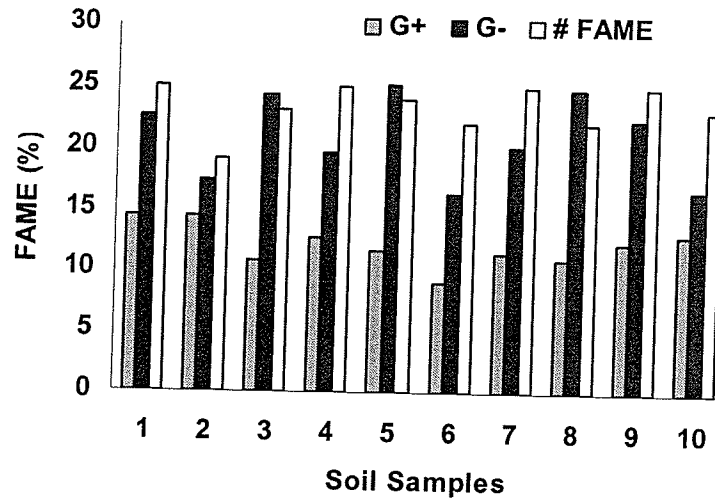


Figure 4.29 Variability of the number of different fatty acid methyl esters (FAME), and the amount of FAME associated with Gram positive (G+) and Gram negative (G-) bacteria.

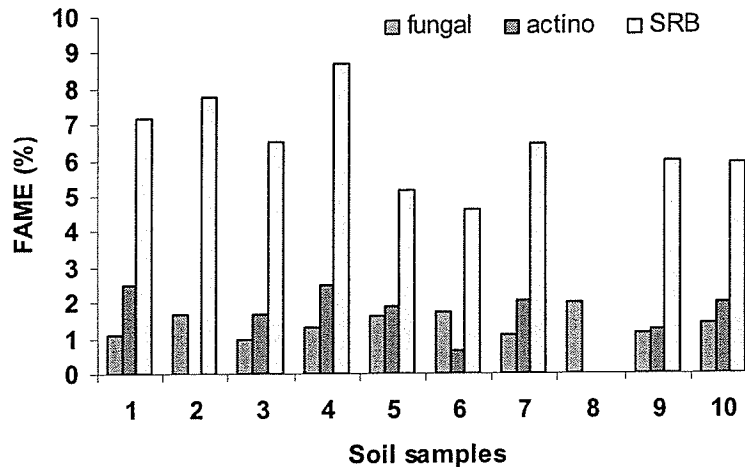


Figure 4.30 Variability of fungal, actinomyces, and sulfate reducing bacteria (SRB) in ten soil samples. In sample # 2, no actinomyces were found whereas in sample # 8 both actinomyces and SRB were not noticed.

1 There was no correlation between any of the microbial groups and either the k or ExpM_T
 2 (Table 4.9). However, the extent of 2,4-D mineralization in soil was strongly negatively
 3 correlated to 2,4-D sorption by soil ($r = -0.82$, Figure 4.31), suggesting that the extent of
 4 2,4-D mineralization in soil was more influenced by variations in the strength of 2,4-D
 5 sorption than by variations in microbial biomass.

6 Table 4.9 Pearson pairwise correlation coefficients amongst dominant microbial groups,
 7 soil properties, and 2,4-D sorption and mineralization along the transect ($n = 10$).

	Soil properties					Mineralization	
	SOC	Soil pH	Carbonate	Clay	Sand	k	ExpM_T
Biomass (total FAME)	-	-	0.73 **	-	-	-	-
Gram +	-	-0.61 *	-	-	-0.67 *	-	-
Gram -	-	-	-	-	-	-	-
18:2 (Fungal)	-	-	0.64 *	-	-	-	-
i17:1 (SRB)	-	-	-0.89 ***	-	-	-	-
10 Me18 (Actinomycetes)	-	-	-	-	-	-	-

8 *, **, and *** denote $P < 0.05$, $P < 0.01$, and $P < 0.001$ level of significance, respectively.

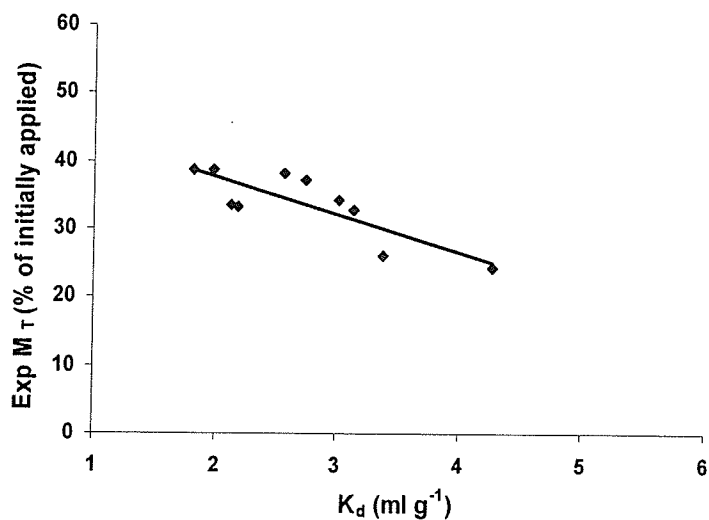


Figure 4.31 Relationship between 2,4-D sorption (K_d) and total mineralization ($ExpM_T$) across the transect.

1 Total FAME was positively correlated with SOC content ($r = 0.35$, Figure 4.32a), soil pH
 2 ($r = 0.41$, Figure 4.32b), clay content ($r = 0.54$, Figure 4.32c). However, the correlation was
 3 not statistically significant. Total FAME was also positively correlated with total carbonate
 4 content ($r = 0.73$, Figure 4.32d). Although the correlation between total FAME and total
 5 carbonate content was high, most of the data points, except one, concentrated in one end of
 6 the regression line (Figure 4.32d) therefore, it would be wise not to conclude that total
 7 carbon content was a strong predictor of total FAME. Total FAME was weakly negatively
 8 correlated with $ExpM_T$ ($r = -0.34$, Figure 4.32 e) and with rate constant, k ($r = -0.45$, Figure
 9 4.32 f), although they were not statistically significant.

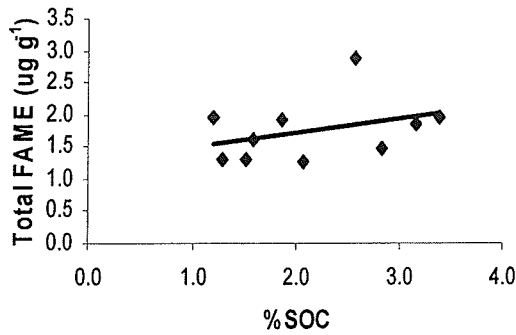


Figure 4.32a Relationship between total FAME and soil organic carbon content.

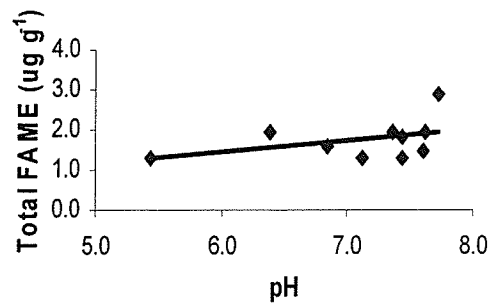


Figure 4.32b Relationship between total FAME and soil pH.

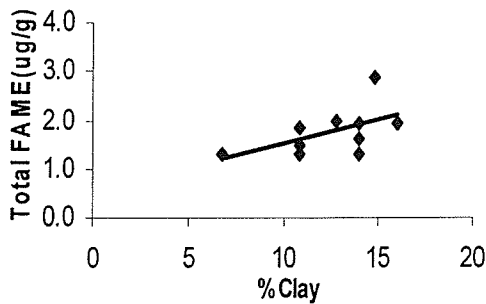


Figure 4.32c Relationship between total FAME and clay content.

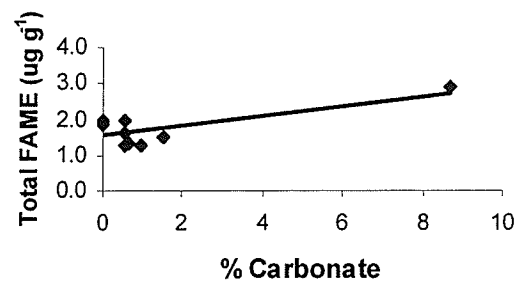


Figure 4.32d Relationship between total FAME and carbonate content.

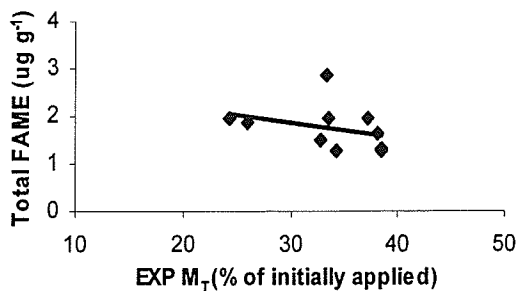


Figure 4.32e Relationship between total FAME and total mineralization

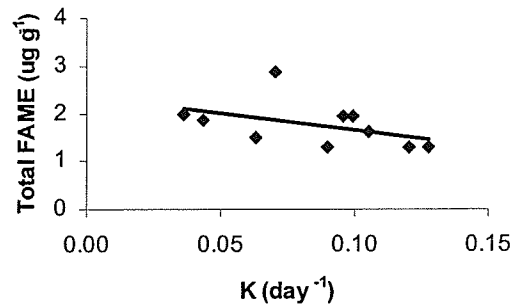


Figure 4.32f Relationship between total FAME and mineralization rate constant.

1 4.4.4 Relation Between Sorption and Mineralization for the Whole Field

- 2 Since the transect revealed that 2,4-D sorption by soil had a strong influence on 2,4-D
- 3 mineralization, the relation between sorption coefficients (K_d and K_{oc}) and mineralization

1 parameters (k , ExpM_T and half-life) was also examined for the whole field (Table 4.10). k
 2 ($r = -0.51$, $p < 0.001$, Figure 4.33a) and ExpM_T ($r = -0.65$, $p < 0.001$, Figure 4.34a) had an
 3 adequate negative correlation with K_d , suggesting that 2,4-D mineralization decreased as
 4 2,4-D sorption by soil increased. Similarly, 2,4-D half-life and K_d were also weakly
 5 positively correlated ($r = 0.37$, $p < 0.001$, Figure 4.35a). However, k was moderately
 6 positively correlated with K_{oc} ($r = 0.41$, $p < 0.001$, Figure 4.33b) and ExpM_T was weakly
 7 positively correlated with K_{oc} ($r = 0.2$, $p < 0.05$, Figure 4.34b), suggesting that 2,4-D
 8 mineralization was greater in soils that sorbed more 2,4-D per unit soil organic matter.
 9 Thus, 2,4-D half-life and K_{oc} were weakly, negatively correlated ($r = -0.29$, $p < 0.01$, Figure
 10 4.35b).

11 Table 4.10 Pearson correlation coefficients among 2,4-D sorption coefficients and
 12 mineralization parameters for all sampling points ($n = 96$).

	K_d (ml g^{-1})	K_{oc} (ml g^{-1})	k (day^{-1})	ExpM_T (%)	Half-life (days)
K_d (ml g^{-1})	1.00				
K_{oc} (ml g^{-1})	0.21*	1.00			
k (day^{-1})	-0.51***	0.41***	1.00		
ExpM_T (%)	-0.65***	0.2*	0.65***	1.00	
Half-life (days)	0.37***	-0.29**	-0.74***	-0.53***	1.00

14 *, **, and *** denote $P < 0.05$, $P < 0.01$, and $P < 0.001$ level of significance, respectively.

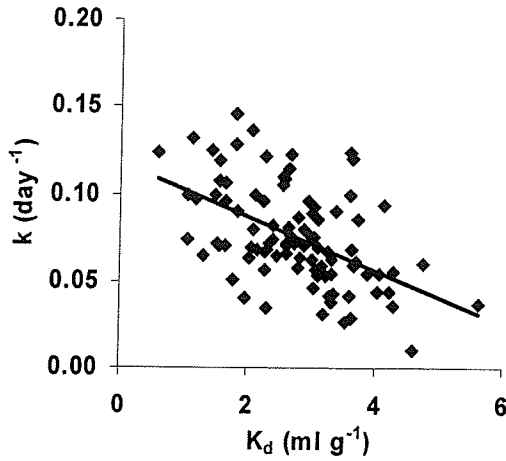


Figure 4.33a Relationship between mineralization rate constant (k) and sorption distribution coefficient (K_d) of 2,4-D.

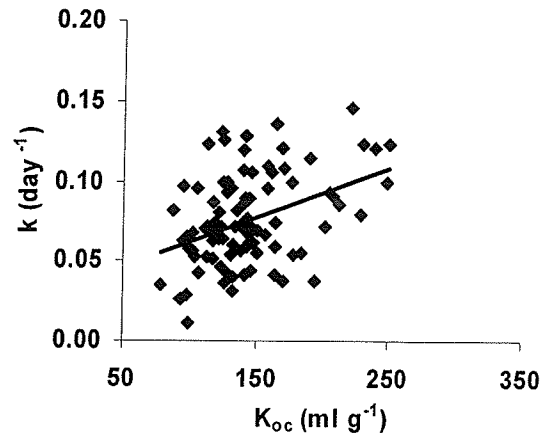


Figure 4.33b Relationship between mineralization rate constant (k) and sorption distribution coefficient per unit organic carbon (K_{oc}) of 2,4-D.

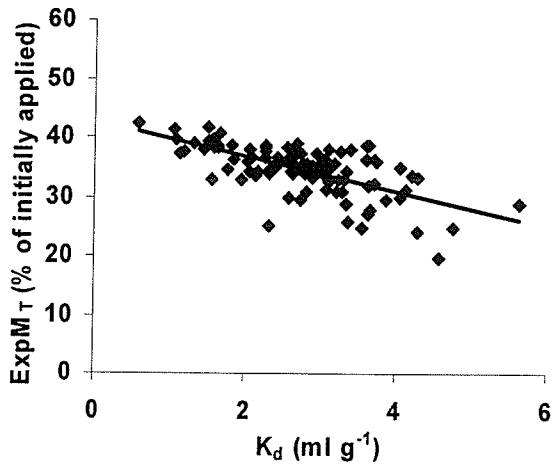


Figure 4.34a Relationship between total mineralization ($ExpM_T$) and sorption distribution coefficient (K_d) of 2,4-D.

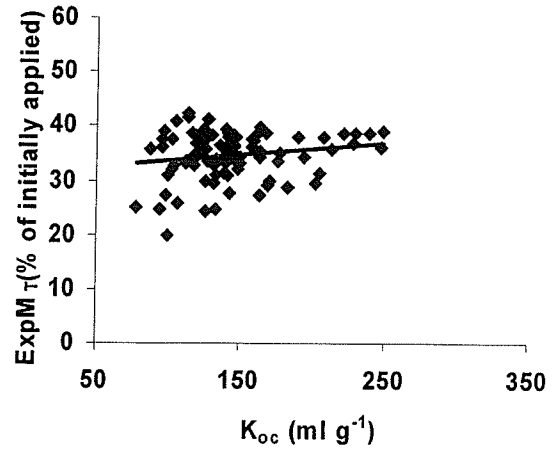


Figure 4.34b Relationship between total mineralization ($ExpM_T$) and sorption distribution coefficient per unit organic carbon (K_{oc}) of 2,4-D.

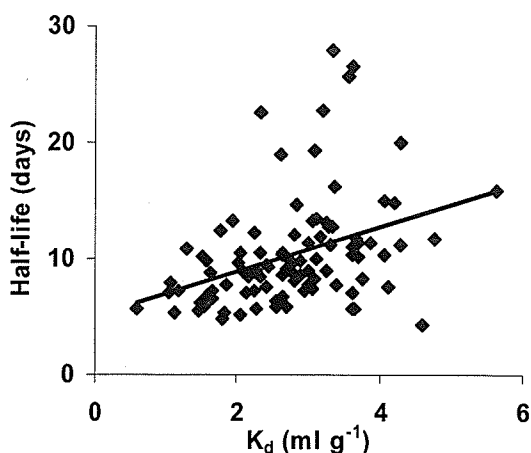


Figure 4.35a Relationship between half-life and sorption distribution coefficient (K_d) of 2,4-D.

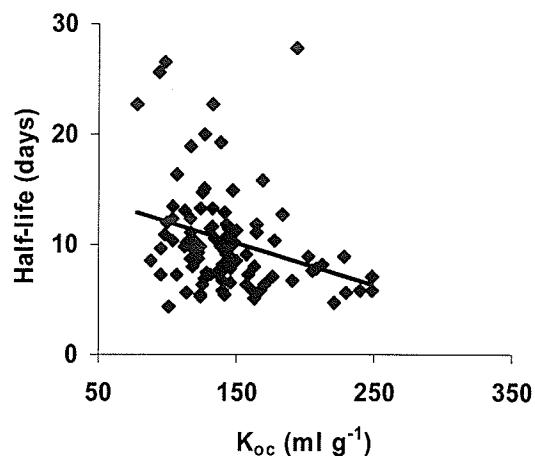


Figure 4.35b Relationship between half-life and sorption distribution coefficient per unit organic carbon (K_{oc}) of 2,4-D.

- 1 Both k and half-life were weakly predicted by K_d with a R^2 value of 0.26 and 0.14
 2 respectively (Table 4.11). ExpM_T was moderately predicted by K_d ($R^2 = 0.42$, $p < 0.0001$,
 3 Table 4.11). Rate constant, k and half-life were very weakly predicted by K_{oc} with a R^2
 4 value of 0.16 and 0.09 but prediction of ExpM_T with K_{oc} was not significant (Table 4.12).

5 Table 4.11 Best regression equations describing dependency of mineralization rate
 6 constant (k), total mineralization (ExpM_T), and half-life on sorption distribution
 7 coefficient (K_d) combining all sampling points ($n = 96$).

Regression equation	R^2	C(p)	Pr > F
$k = 0.12 - 0.02 K_d$	0.26	2.0	0.0001
$\text{ExpM}_T = 42.67 - 2.96 K_d$	0.42	2.0	0.0001
$\text{Half-life} = 5.09 + 1.91 K_d$	0.14	2.0	0.0002

8

9 Table 4.12 Best regression equations describing dependency of mineralization rate
 10 constant (k), total mineralization (ExpM_T), and half-life on sorption coefficient per
 11 unit organic carbon (K_{oc}) combining all sampling points ($n = 96$).

Regression equation	R^2	C(p)	Pr > F
$k = 0.03 + 0.0003 K_{oc}$	0.16	2.0	0.0001
$\text{ExpM}_T = --$	--*	--	--
$\text{Half-life} = 15.84 - 0.04 K_{oc}$	0.09	2.0	0.0036

12 *-- = not significant

1 **4.4.5 Relationship between Sorption and Mineralization for Individual Slope**
2 **Position**

3 ExpM_T had a negative correlation with K_d in all slope positions (Table 4.13). The
4 relationship between ExpM_T and K_d was stronger in the upper slope position ($r = -0.84$, $p <$
5 0.001 , Figure 4.36a), than in the middle ($r = -0.57$, $p < 0.001$, Figure 4.36a) and lower ($r = -$
6 0.63 , $p < 0.001$, Figure 4.36a) slope positions. For the middle and lower slope positions, k
7 was also negatively correlated with K_d ($r = -0.38$, $p < 0.01$, and $r = -0.54$, $p < 0.01$, for middle
8 and lower, respectively) (Figure 4.36b), but in the upper slope position, k was not
9 significantly correlated to K_d (Figure 4.36b). There was no significant correlation found
10 between half-life and K_d in the upper and lower slope positions but in the middle slope
11 position, half-life was weakly positively correlated with K_d ($r = 0.33$, $p > 0.05$, Figure 4.36
12 c)

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1 Table 4.13 Pearson correlation coefficients among 2,4-D sorption coefficients and
 2 mineralization parameters as affected by soil landscape position.

3

	K_d (ml g ⁻¹)	K_{oc} (ml g ⁻¹)	k (day ⁻¹)	ExpM _T (%)	Half-life (days)
Upper (n = 12)					
K_d (ml g ⁻¹)	1.00				
K_{oc} (ml g ⁻¹)	-	1.00			
k (day ⁻¹)	-	-	1.00		
ExpM _T (%)	-0.84 ***	-	-	1.00	
Half-life (days)	-	-	ND	-	1.00
Middle (n = 55)					
K_d (ml g ⁻¹)	1.00				
K_{oc} (ml g ⁻¹)	0.48 ***	1.00			
k (day ⁻¹)	-0.38 **	0.27 *	1.00		
ExpM _T (%)	-0.57 ***	-	0.64 ***	1.00	
Half-life (days)	0.33 *	-	ND	-0.52 ***	1.00
Lower (n = 29)					
K_d (ml g ⁻¹)	1.00				
K_{oc} (ml g ⁻¹)	0.36 *	1.00			
k (day ⁻¹)	-0.54 **	-	1.00		
ExpM _T (%)	-0.63 ***	-	0.75 ***	1.00	
Half-life (days)	-	-	ND	-0.39 *	1.00

4 *, **, and *** denote P < 0.05, P < 0.01, and P < 0.001 level of significance, respectively.

5 ND = half-life was calculated from k, therefore correlation is not determined.

6

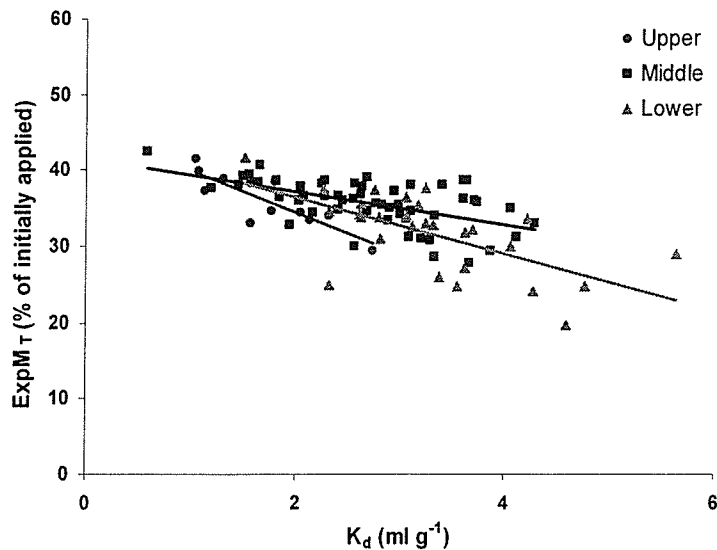


Figure 4.36a Relationship between total mineralization (ExpM_T) and sorption distribution coefficient (K_d) of 2,4-D as affected by landscape position. Upper slope ($\text{ExpM}_T = 45.46 - 5.50 K_d$; $r = -0.84$, $P < 0.001$), middle slope ($\text{ExpM}_T = 41.59 - 2.19 K_d$; $r = -0.57$, $P < 0.001$) and lower slope ($\text{ExpM}_T = 43.80 - 3.69 K_d$; $r = -0.63$, $P < 0.001$).

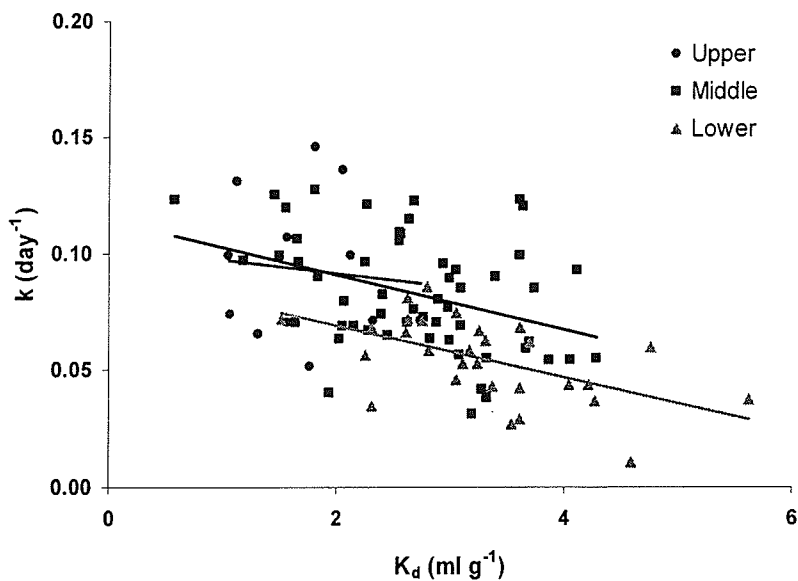


Figure 4.36b Relationship between mineralization rate constant (k) and sorption distribution coefficient (K_d) of 2,4-D as affected by landscape position. Upper slope ($k = 0.10 - 0.01K_d$; $r = -0.10$, *ns*), middle slope ($k = 0.11 - 0.01K_d$; $r = -0.38$, $P < 0.01$) and lower slope ($k = 0.09 - 0.01K_d$; $r = -0.54$, $P < 0.01$).

1

2

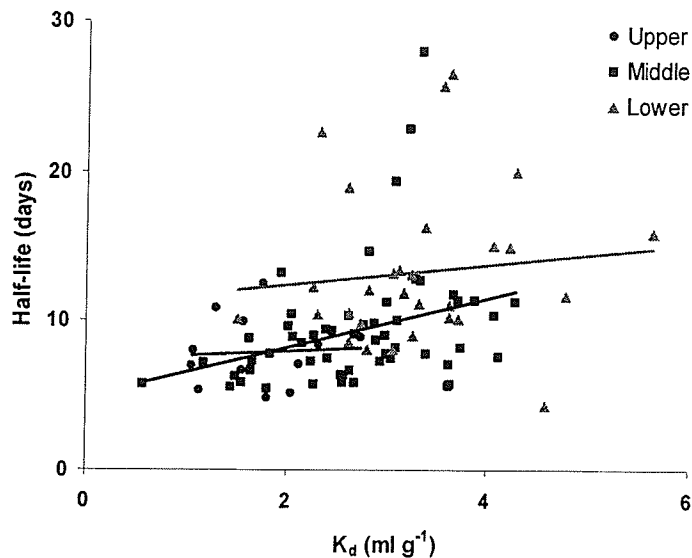


Figure 4.36c Relationship between half-life and sorption distribution coefficient (K_d) of 2,4-D as affected by landscape position. Upper slope (Half-life = $7.41 + 0.27K_d$; $r = 0.06$, *ns*), middle slope (Half-life = $4.85 + 1.67K_d$; $r = 0.33$, $P < 0.05$) and lower slope (Half-life = $11.05 + 0.68K_d$; $r = 0.11$, *ns*).

1

2 $ExpM_T$ and half-life was not associated with K_{oc} in any slope positions (Figure 4.37a, and
 3 Figure 4.37c respectively). Similarly, k was also not associated with K_{oc} in the upper and
 4 lower slope positions (Figure 4.37b). However, in the middle slope position, k was weakly
 5 positively correlated with K_{oc} ($r = 0.27$, $p < 0.05$, Figure 4.37b).

6

7

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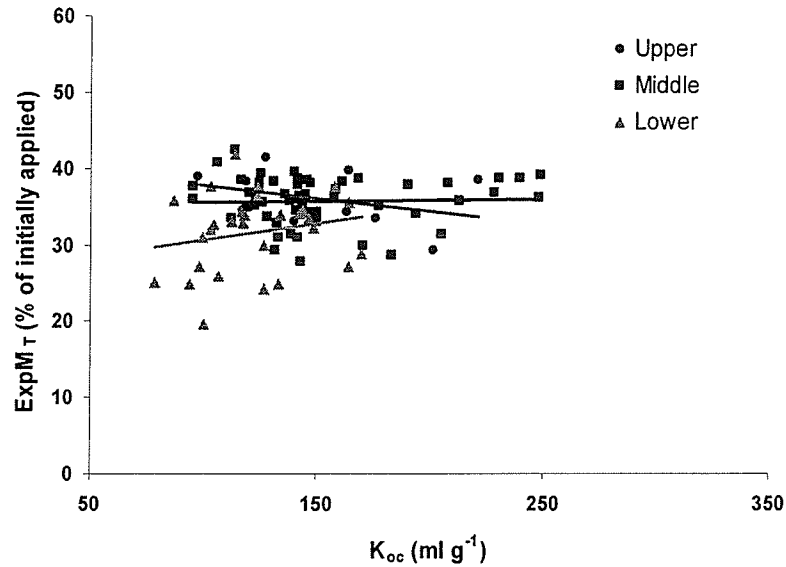


Figure 4.37a Relationship between total mineralization (ExpM_T) and sorption distribution coefficient per unit organic carbon (K_{oc}) of 2,4-D as affected by landscape position. Upper slope ($\text{ExpM}_T = 41.04 - 0.03K_{oc}$; $r = -0.35$, *ns*), middle slope ($\text{ExpM}_T = 35.36 + 0.002K_{oc}$; $r = 0.03$, *ns*) and lower slope ($\text{ExpM}_T = 26.35 + 0.04K_{oc}$; $r = 0.21$, *ns*).

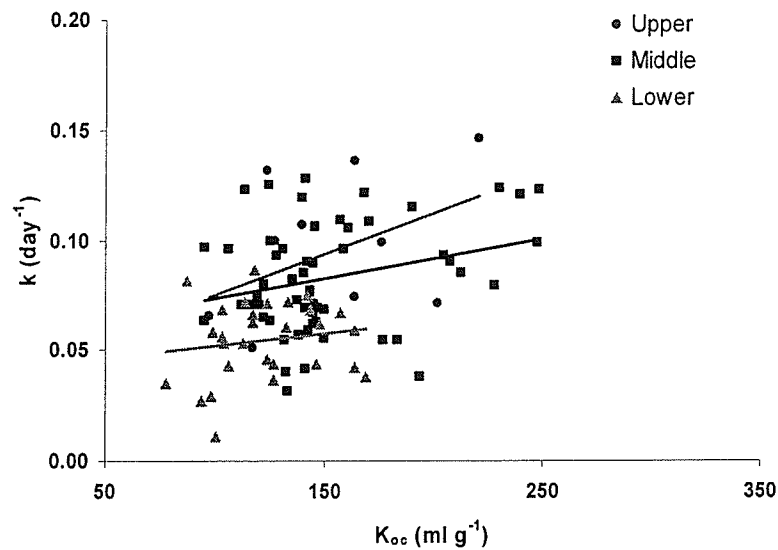


Figure 4.37b Relationship between mineralization rate constant (k) and sorption distribution coefficient per unit organic carbon (K_{oc}) of 2,4-D as affected by landscape position. Upper slope ($k = 0.04 + 0.0004K_{oc}$; $r = 0.43$, *ns*), middle slope ($k = 0.06 + 0.0002K_{oc}$; $r = 0.27$, $P < 0.05$) and lower slope ($k = 0.04 + 0.0001K_{oc}$; $r = 0.15$, *ns*).

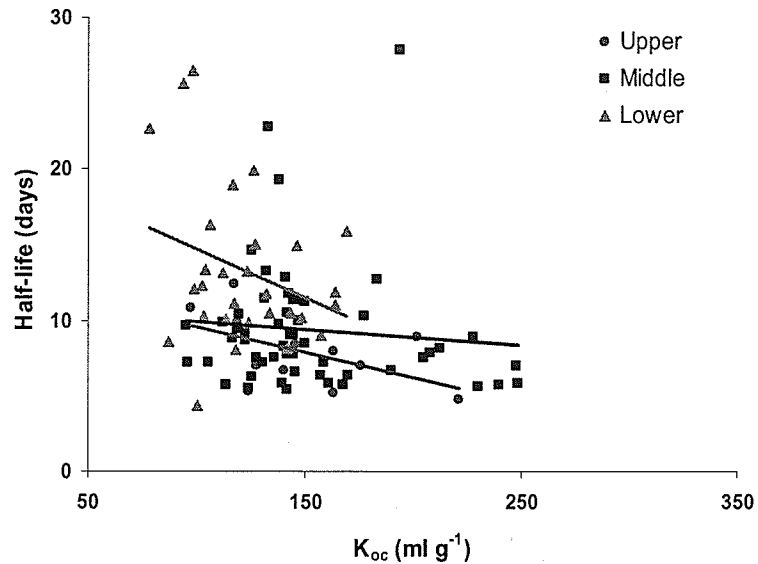


Figure 4.37c Relationship between half-life and sorption distribution coefficient per unit organic carbon (K_{oc}) of 2,4-D as affected by landscape position. Upper slope (Half-life = $12.85 - 0.03K_{oc}$; $r = -0.52$, *ns*), middle slope (Half-life = $10.92 - 0.01K_{oc}$; $r = -0.09$, *ns*) and lower slope (Half-life = $21.02 - 0.06K_{oc}$; $r = -0.29$, *ns*).

1

2 $ExpM_T$ was predicted well by K_d , but the strength of prediction varied significantly among

3 landscape positions (Table 4.14). In the upper slope position, $ExpM_T$ was predicted well by

4 K_d ($R^2 = 0.72$, $p < 0.0005$, Table 4.14), whereas prediction was weak in the middle ($R^2 =$

5 0.33 , $p < 0.0001$, Table 4.14) and lower ($R^2 = 0.39$, $p < 0.0003$, Table 4.14) slope positions.

6 The prediction of k by K_d was not significant in the upper slope position but, k was weakly

7 predicted by K_d in middle ($R^2 = 0.15$, $p < 0.0034$, Table 4.14) and lower ($R^2 = 0.28$, p

8 < 0.0033 , Table 4.14) slope positions. In the upper and lower slope positions half-life could

9 not be predicted by K_d but, in the middle slope position it was predicted very weakly by K_d

10 ($R^2 = 0.11$, $p < 0.0155$, Table 4.14). Predictions of k , $ExpM_T$ and half-life by K_{oc} were not

11 significant for any slope positions and therefore data has not shown.

12

1 Table 4.14 Best regression equations for different slope positions (upper, middle and
 2 lower) describing dependency of the mineralization rate constant (k), total
 3 mineralization (ExpM_T), and half-life with sorption distribution co-efficient (K_d).
 4

Landscape positions	Regression equation	R ²	C(p)	Pr > F
Upper (n=12)				
	k = --	--*	--	--
	ExpM _T = 45.47 - 5.5 K _d	0.72	2.0	0.0005
	Half-life = --	--	--	--
Middle (n=55)				
	k = 0.12 - 0.01 K _d	0.15	2.0	0.0034
	ExpM _T = 41.58 - 2.18 K _d	0.33	2.0	0.0001
	Half-life = 4.86 + 1.66 K _d	0.11	2.0	0.0155
Lower (n=29)				
	k = 0.09 - 0.01 K _d	0.28	2.0	0.0033
	ExpM _T = 43.81 - 3.69 K _d	0.39	2.0	0.0003
	Half-life = --	--	--	--

5 *-- = not significant
 6

7 4.5 Discussion

8 In this study there was a remarkable difference in the 2,4-D half-lives across the field.
 9 Lower slope position (13 days) was particularly different than upper (7 days) and middle (9
 10 days) slope position. Higher 2,4-D persistence in the lower slope position was particularly
 11 due to greater SOC contents. The spatial variability of 2,4-D half-lives in soil-landscapes
 12 should be considered in pesticide fate modelling to more accurately predict the risk of
 13 pesticide leaching from agricultural landscapes.

14 The spatial variability of soil characteristics in soil-landscapes results from soil formation
 15 processes (Goderya 1998) as well as erosional and depositional processes due to land use

1 and management practices (Gregorich et al. 1985). The variability in 2,4-D half-lives
2 across the landscape would be influenced by the intensity of tillage or by topographic
3 variables (i.e. elevation, slope gradient, horizontal or vertical curvature etc.).

4 Regardless of the location in the landscape (upper, middle or lower slopes), SOC content
5 was the single best predictor of total 2,4-D mineralization and 2,4-D mineralization rates.
6 In general, 2,4-D mineralization was slower and less complete in soils with greater SOC
7 contents. This was due to strong sorption of the herbicide by soil organic carbon causing
8 the herbicide to be less bioavailable to microorganisms, consequently less degradation of
9 2,4-D occurred. This agrees with other studies (Ogram et al. 1985; Scow 1993). In contrast,
10 Veeh et al. (1996) found that the degradation rates of 2,4-D were positively correlated to
11 soil organic carbon content because of the greater microbial activity in soils with greater
12 soil organic carbon content.

13 Although 2,4-D mineralization was negatively correlated with SOC contents, soils that
14 sorbed more 2,4-D per unit soil organic matter showed generally greater total 2,4-D
15 mineralization and 2,4-D mineralization rates than soils that sorbed less 2,4-D per unit soil
16 organic matter. The similar findings were also reported by Picton and Farenhorst (2004)
17 that 2,4-D mineralization rates were greater in soils that sorbed more 2,4-D per unit organic
18 carbon. This may be due to high desorption of 2,4-D as well as organic matter quality
19 which makes the herbicide available for microbial degradation. For example, sorption is
20 lower in fresh or less decomposed organic matter, which results in higher degradation.
21 However, in this study correlations between K_{oc} and the mineralization parameters were
22 weak.

1 In this study, increasing sand contents increased total mineralization and mineralization
2 rates since sand has less specific surface area than any other soil particles. In general, less
3 specific surface area means less reactive site, hence weak sorption of herbicide in the sandy
4 soil. Therefore, more herbicide was available for mineralization due to weak sorption. The
5 significance of sand on 2,4-D degradation was also reported in a study by Ou (1984),
6 where the number of 2,4-D degrading organisms were initially much lower in sandy soil
7 than in clay loam but the number of 2,4-D degrading organisms increased rapidly in the
8 sandy soil when the herbicide was added. In contrast, Thompson et al. (1984) determined
9 the persistence of 2,4-D applied at recommended rates in agricultural soils in Canada and
10 found that half-life for sandy clay loam soil was 7 days whereas for sandy soils was 23
11 days, presumably due to differences in soil pH (i.e. pH for sandy clay loam and sand were
12 6.4 and 5.2, respectively). Similarly, Reimer (2004) observed 2,4-D mineralization rates
13 were significantly greater in clay loam soils relative to sandy soils because the clay loam
14 had a greater microbial population capable of mineralizing 2,4-D.

15 The size of the soil microbial biomass offers a general measure of soil organism activity.
16 Changes in the size of the microbial biomass considered to be an indicator of long-term
17 changes in soil organic matter content (Carter 1986). Even though soil organic matter
18 content varied by slope positions and the size of the microbial biomass varied within the
19 field, the contribution of landscape position to variations in the size of the microbial
20 biomass was not significant in this study. Ross et al. (1984) reported that soil enzyme
21 activities are more related to the variations of the quality and quantity of soil organic matter
22 than on the size of the microbial biomass. Sparling and Ross (1993) stated that biomass
23 carbon is an indicator of the dynamics of organic matter quality and quantity. However,

1 Beyer (1995) concluded that the active microbial biomass may not necessarily reflect a
2 typical soil organic matter content or composition. This study indicates a poor correlation
3 between microbial biomass and soil organic carbon content. The relation between
4 microbial biomass and specific soil organic matter, like bioavailable organic carbon, was
5 not examined.

6 Studies have shown that PLFA profiles are influenced by management factors such as
7 tillage (Calderón et al. 2000; Calderón et al. 2001). Tillage induced erosion leading to
8 changes in soil properties of surface soils in the landscapes examined in this study.
9 However, in this study spatial variability due to tillage or other erosion process did not
10 show any clear effects on microbial community composition across the landscape.
11 Broughton and Gross (2000) reported no significant changes in the soil microbial
12 community composition along a topographic gradient in a grassland in Michigan. This was
13 also supported by McCulley and Burke (2004) where they reported no significant
14 differences in total microbial biomass between uplands and lowlands at the landscape-
15 scale.

16 Bossio et al. (1998) concluded that soil type has a strong effect on soil microbial
17 community structure. Grayston et al. (2004) demonstrated that lipid characteristics of
18 bacteria, in particular Gram -ve, were highly positively correlated with high pH and
19 negatively correlated with high soil organic matter. Grayston et al. (2001) also found that
20 fungi become more abundant as soil pH decreases and the soil organic matter increases. In
21 contrast, McCulley and Burke (2004) reported that fungal biomass does not increase across
22 the gradient. In this study, the FAME analysis was carried out only with 10 samples,

1 therefore, further investigation into microbial distribution and concentration using more
2 samples would improve the confidence level in explaining the influence of microbial
3 activity on 2,4-D mineralization.

4 Despite the higher amount of SOC in the lower slope positions, relatively low 2,4-D
5 mineralization was observed at that slope position. One explanation may be that the
6 presence of relatively large amounts of readily available soil organic carbon in the lower
7 slope positions would have been used by microbes in preference to 2,4-D. Addition of 2,4-
8 D in samples from the upper slope area resulted in higher 2,4-D mineralization rates. Since
9 the upper slope contained comparatively lower amounts of soil organic carbon, 2,4-D may
10 have been readily utilized by the soil microorganisms as a carbon source. Alternatively,
11 total mineralization of 2,4-D in the lower slope position may have been reduced due to
12 increased 2,4-D sorption by soil, lower bioavailability of 2,4-D and its degradation
13 products to microorganisms. Dec et al. (1990) found that 2,4-dichlorophenol, the first
14 intermediate products of 2,4-D degradation was incorporated into humic substances,
15 making it less susceptible to further degradation. Sorption of pesticides to organic matter
16 resulted in lower degradation rates and increased half-lives because they are not as
17 accessible to microbial attack in the bound state (Smith and Muir 1980; Stott et al. 1983;
18 Bolan and Baskaran 1996). The sorption of organic substrates decreases their availability to
19 microorganisms (Ogram et al 1985; Benoit et al. 1996). Ogram et al. (1985) showed that
20 sorbed 2,4-D is less mineralized than 2,4-D in soil water. However, in soils with an organic
21 carbon content of more than 12%, the rate of both sorption and degradation of 2,4-D
22 increases due to an increased biological activity in these soils and a decreased 2,4-D-
23 induced inhibitory effect on microbial activity (Bolan and Baskaran 1996).

4.6 Summary and Conclusions

1
2 First-order mineralization rate constants for upper, middle, and lower slope positions were
3 0.09, 0.08, 0.05, respectively, whereas mineralization of initially applied 2,4-D at those
4 slope positions were 36, 35 and 31% respectively at 130 days following herbicide
5 application. Greater half-lives in the lower slope position (13 days) compared to upper (7
6 days) and middle (9 days) slope position was particularly due to greater SOC content in the
7 lower slope position. Since 2,4-D is degraded by co-metabolism, the greater amount of soil
8 organic carbon in the lower slope position will have greater microbial activity and was
9 expected to cause an increase in total 2,4-D mineralization comparing with upper and
10 middle slope positions. However, higher SOC content in the lower slope position probably
11 decreased 2,4-D mineralization because of increased 2,4-D sorption and a decreasing
12 availability of 2,4-D molecules to soil microorganisms.

13 This study has demonstrated clear implications of 2,4-D applications in agricultural fields.
14 Greater half-lives in the lower landscape position means greater 2,4-D persistence in that
15 location which will result in greater risks of 2,4-D off-site movement as well as greater
16 risks of 2,4-D injury of sensitive crops. However, 2,4-D residues in soil may not be
17 necessarily available for uptake by plants or for movement of water, particularly if 2,4-D is
18 strongly sorbed in soil. This study concludes that the extent of 2,4-D mineralization in soil
19 was more influenced by the strength of 2,4-D sorption by soil than by the size and
20 composition of the soil microbial communities.

5. GENERAL DISCUSSION

1

2 This study provides information on the spatial distribution of the 2,4-D sorption and
3 mineralization across landscape positions. This information enables farmers to better
4 understand differences across agricultural landscapes as well as differences in the risk of
5 crop injury by herbicide residues in soil. In addition, information on herbicide fate as
6 affected by landscape position is useful for assessments on the risk of herbicide off-site
7 movement.

8 For understanding the spatial variability of soil properties and herbicide behaviour within
9 the landscape, 96 sampling points were classified into one of three Landform Element
10 Complexes: upper, middle and lower slope positions. Results indicated that SOC content
11 significantly increased with decreasing slope position, with soils in upper slope positions
12 containing on average only 1.1% SOC but soils in lower slope positions containing on
13 average 2.7% SOC. The agricultural field had been under conventional tillage for decades,
14 suggesting that these differences in SOC content across landscape positions were probably
15 due to the movement of organic rich top soil from the upper to lower slope positions by
16 tillage, wind and water erosion (Lindstrom et al. 1992; Lobb et al. 1999). A study
17 conducted in the same experimental field concluded that tillage erosion was the most
18 important factor in transporting significant amounts of surface soil from upper to lower
19 landscape position (Li et al. 2004). Li et al. (2004) observed that the ranges of soil loss in
20 the field are from 16.1 to 26.8 ton ha⁻¹ yr⁻¹ for tillage erosion and from 10.3 to 12.2 ton ha⁻¹
21 yr⁻¹ for water erosion, respectively.

1 Regardless of the slope position, SOC content was the most important factor in predicting
2 2,4-D sorption by soil. This is in agreement with the previous findings in which SOC
3 content was the primary factor influencing 2,4-D sorption in surface soil (Mallawatantri
4 and Mulla 1992; Johnson et al. 1995; Farenhorst et al. 2003). Soils in the lower slope
5 position had a greater SOC content and therefore a greater capacity to retain 2,4-D than
6 soils in upper slope positions. Specifically, the average K_d value of 2,4-D in soils of the
7 lower slope position (3.3 ml g^{-1}) was two times greater than the average K_d value at the
8 upper landscape position (1.7 ml g^{-1}). Boesten and van der Linden (1991) demonstrated
9 that an increase of K_d by a factor of two, increases the leaching risk by a factor of ten.
10 Although laboratory results in this study suggested that soils in the upper slope positions
11 are potentially more vulnerable to herbicide leaching, the actual leaching of 2,4-D to depth
12 would depend on the soil-hydrological conditions in the field. The rate of herbicide
13 leaching in a given position in the field depends on K_d but also on water infiltration rate
14 and ground water depth. Shallow groundwater depth in low-lying flat areas is more
15 vulnerable to herbicide groundwater contamination than deep groundwater under sloping
16 areas or hills. Barbash and Resek (1996) observed high pesticide leaching in areas where
17 soil is highly permeable, infiltration rate is rapid, and water table is shallow. Koterba et al.
18 (1993) also detected pesticides more frequent in groundwater under a well-drained soil than
19 in groundwater under poorly drained soil.

20 The variability in 2,4-D sorption across the landscape was best explained by differences in
21 soil organic matter contents across slope positions. However, when both soil pH and SOC
22 content were considered in prediction models, 2,4-D sorption could be better predicted (r^2
23 = 78% for the whole field, and r^2 ranged from 73 to 84% in upper, middle and lower slope

1 positions). Because 2,4-D is a weakly-acidic herbicide (its acid dissociation constant, $pK_a =$
2 2.64), an increase in soil pH increases the anionic form of 2,4-D relative to molecular form.
3 Soil pH ranged from 5.3 to 7.8 in this study. An increasing soil pH, increases the portion of
4 2,4-D in anionic form (relative to the portion of 2,4-D in molecular form), therefore
5 herbicide sorption decreased with increasing soil pH. Soil organic matter quality may also
6 have influence on 2,4-D sorption. Previous studies have shown that the humic acid
7 fractions of soil organic matter have the greatest effect on sorption (Clapp et al. 2001,
8 Senesi et al. 2001). In fact, the net negative charged soil constituents such as humic
9 substances may repel 2,4-D when in anionic form. As such, due to differences in soil pH
10 among landscape positions, soils in the lower slope position had on average a smaller K_{oc}
11 values (122 ml g^{-1}) than soils in the upper (149.7 ml g^{-1}) and middle (152.6 ml g^{-1}) slope
12 positions. Soil pH alone was able to predict K_{oc} moderately, both for the whole field ($R^2 =$
13 0.68) and within slope positions ($R^2 = 0.44, 0.75, \text{ and } 0.44$ for upper, middle and lower
14 slope positions, respectively). SOC and Soil pH are more readily determined than K_d and
15 K_{oc} and therefore can be used to get first hand information on the influence of soil input
16 parameter as well as in screening and management models for predicting herbicide
17 behaviour in soil.

18 Regardless of slope position, the total amount of 2,4-D mineralized and the mineralization
19 rate constants of 2,4-D were negatively correlated with SOC content. The total amount of
20 2,4-D mineralized was less in the lower slope position (31%) compared to the upper (36%)
21 and middle (35%) slope positions. Mineralization rate was significantly slower in the lower
22 slope (0.05 day^{-1}) relative to upper (0.09 day^{-1}) and middle (0.08 day^{-1}) slope positions.

1 Based on the information provided in the literature, the differences in 2,4-D mineralization
2 among slope positions can be explained by several factors:

3 1) The greater mineralization rates in upper slope positions could result from the fact that
4 2,4-D was used as an energy and carbon source because this slope position contained small
5 amounts of functional soil organic carbon. For example, Gonod et al. (2003) concluded that
6 the spatial distribution of easily decomposable organic carbon controls the spatial
7 distribution of 2,4-D mineralization potential. Willems et al. (1996) also reported that
8 microbes might not prefer to use 2,4-D when a considerable amount of readily available
9 SOC exists.

10 2) Soil in lower slope positions contained relatively more SOC content (1.3 to 4.6%) than
11 soils in upper slope positions (0.6 to 1.6%). The variation of SOC content is important
12 since high levels of organic matter have been shown to reduce the rate of 2,4-D
13 mineralization in soils (Greer and Shelton 1992). When 2,4-D sorbed to soil organic matter
14 in soils, it is not readily available for microbial degradation (Ogram et al. 1985). Sorption
15 onto soil organic matter may provide a temporary protection from microbial attack,
16 especially if herbicide desorption rates from soil are slow. However, Bolan and Baskaran
17 (1996) found that soils with higher SOC contents had a greater sorption capacity for
18 herbicides but also had greater soil microbial activities, therefore herbicide biodegradation
19 was greater in these soils relative to soils with lesser SOC contents.

20 3) The mineralization of 2,4-D in the lower slope position may have been reduced due to
21 the lower bioavailability of 2,4-D degradation products in these soils, compared to soils in

1 the upper and middle slope positions. Dec et al. (1990) found that the incorporation of 2,4-
2 dichlorophenol (the first intermediate product of 2,4-D degradation) into humic substances,
3 reduced further degradation. Specifically, they found that 46 to 80% of the initially applied
4 2,4-D remained soil-bound to the humic materials, for example by the formation of ether
5 linkages between humic materials and metabolites. If the metabolite is actually
6 incorporated into the core structure of the humic acid (Stevenson 1994), the residence time
7 of the metabolite-derived carbon will be decades to centuries. Many other pesticide-binding
8 studies have indicated that soil-bound herbicides are associated mainly with soil organic
9 matter (Katan et al. 1976; McCall et al. 1981). McCall et al. (1981) reported that 20 to 35%
10 of the initially applied 2,4-D remained soil-bound in association with soil organic matter.

11 4) Differences in the size and composition of the soil microbial populations across
12 landscape positions could have influenced 2,4-D degradation. In other studies, the
13 mineralization of 2,4-D was positively correlated with the size of microbial biomass and
14 soil organic matter content or with the numbers of total bacteria in soil and organic carbon
15 content (Veeh et al. 1996; Voos and Groffman 1997). McCall et al. (1981) suggested that
16 variations in the decomposition rates of chemicals among soils are mainly due to the basic
17 ability of a given soil microbial community to degrade a specific chemical. Entry et al.
18 (1995) found that variations in the composition of the microbial community, which may or
19 may not be related to its biomass, is a key regulator of herbicide degradation.

20 In applying the four factors to the study results, this study provides evidence that sorption
21 is the most likely reason of differences in mineralization rate in different landscape
22 positions. The negative correlation between sorption and mineralization of this study

1 suggest that the sorption of 2,4-D decreases its availability to microbial degradation. Scow
2 (1993) acknowledges that sorption can be a predominant factor influencing the rate at
3 which pesticides are degraded in soil, since it governs the availability of pesticides in soil
4 solution. Ogram et al. (1985) reported that sorbed 2,4-D might have been located
5 sufficiently deep within the soil organic matter matrix that the bacteria were unable to
6 attack it. However, the effect of sorption on 2,4-D degradation in soil can not be
7 generalized as 2,4-D sorption onto soil organic matter may promote 2,4-D biodegradation
8 in soil (Benoit et al. 1999; Picton and Farenhorst 2003). This study also provided evidence
9 that the size of the microbial biomass varied in the field, but the contribution of landscape
10 position to the variations in the size of the microbial biomass was not a large factor in this
11 study. Microbial composition and biomass was not significantly correlated to 2,4-D
12 mineralization, suggesting that mineralization was more influenced by the extent of 2,4-D
13 sorption by soil. Entry et al. (1994) also found no correlation between active or total
14 microbial biomass with 2,4-D degradation. However, the microbial composition and
15 biomass measurements in my study were done for only 10 (representative) soil samples,
16 and further investigations using more samples are warranted to confirm the current results.
17 The relationship between microbial biomass and specific soil organic matter, like
18 bioavailable organic carbon was also not examined in this study.

19 This study has a range of limitations. It was conducted in the laboratory under controlled
20 environmental conditions rather than in the field. Under field conditions, soils would have
21 been subjected to a wide range of soil moisture and temperature regimes which might
22 affect 2,4-D sorption. Generally, the sorption of 2,4-D decreases with the increases of soil
23 moisture (Koskinen and Harper 1990). In my study, the lower slope position of the field

1 contained higher moisture content compared to the upper and middle slope positions which
2 would reduce herbicide sorption. Wauchope et al. (2002) considered that laboratory batch
3 experiment probably varies from the true average K_d value in a field of the same soil by a
4 factor of up to two.

5 In the field, soil moisture contents and temperatures would play a significant role in
6 determining the activity of soil microbial populations and therefore herbicide
7 mineralization rates. In addition, the persistence of herbicides in the field is generally
8 shorter than that measured under controlled laboratory conditions because other fate
9 processes occur in the field, including pesticide transport by volatilization, surface runoff
10 and leaching.

11 This mineralization study measured the amount of 2,4-D mineralized to CO_2 therefore the
12 $^{14}CO_2$ production in this study does not indicate the rate at which 2,4-D metabolites are
13 being formed. However, the leaching potential of herbicides to groundwater will also
14 depend on the formation and characteristics of their metabolites because metabolites have a
15 different mobility in soil than parent molecules. In addition, the use of degradation rate
16 constants or half-lives from surface soils are not adequate for describing degradation rates
17 throughout the entire soil profile. In order to predict 2,4-D fate in the soil environment,
18 knowledge of how 2,4-D degradation rates varies within the soil profile is an important
19 consideration (Gaultier et al. 2005).

20

6. SUMMARY AND CONCLUSIONS

1
2 This study examined the spatial variability of soil properties and 2,4-D sorption and
3 mineralization in a conventionally-tilled field near Miami, Manitoba, Canada. Soil samples
4 (96 samples in total) were collected from the field and classified as upper (12 samples),
5 middle (55 samples), and lower (29 samples) landscape positions using LandMapR
6 software (MacMillan and Pettapiece 2000). Soil properties (SOC content, soil pH, texture
7 and carbonate content) and 2,4-D sorption and mineralization in soil were determined for
8 each sample. Batch equilibrium experiments were used to measure 2,4-D sorption in soil
9 and soil microcosms were used to monitor 2,4-D mineralization.

10 SOC content increased significantly from upper to lower landscape positions. Similarly,
11 2,4-D sorption also increased significantly from upper to lower landscape positions,
12 presumably due to the variation in SOC content across the field. SOC content alone could
13 be used to predict sorption adequately, however, the prediction of herbicide sorption by soil
14 (K_d) improved significantly when both SOC content and soil pH were taken into account in
15 the linear prediction model. The strength of 2,4-D sorption onto soil organic matter (K_{oc})
16 decreased significantly from upper to lower landscape positions likely due to variation of
17 soil pH across the landscape or differences in soil organic matter quality. Greater sorption
18 in soils would result in a lesser risk of 2,4-D transport by leaching, runoff or volatilization.

19 Between 20 to 42% of the initially applied 2,4-D was mineralized at 130 days following
20 herbicide application. Total 2,4-D mineralization decreased significantly from the upper to
21 lower landscape positions, while mineralization half-lives increased significantly from the

1 upper to lower landscape positions. Herbicide mineralization was negatively correlated
2 with SOC content and K_d . Therefore, sorption of 2,4-D to organic matter may be
3 responsible for reduced degradation and greater half-lives in the lower slope position since
4 sorption decreases herbicide availability to microorganisms. Greater 2,4-D persistence in
5 soil may result in greater risk of 2,4-D off-site movement, although herbicide off-site
6 movement is influenced by the availability of 2,4-D residues to be moved by volatilization,
7 leaching, runoff, soil and wind erosion as well as climatic and hydrological factors.
8 Depending on whether 2,4-D is strongly or weakly sorbed, these pathways may or may not
9 be significant. The lower slope positions with greater 2,4-D persistence also had greater
10 2,4-D sorption by soil, thus in these locations, 2,4-D is less likely to move dissolved in
11 water and is also less likely to volatilize in the atmosphere. Additional research is required
12 to more precisely determine the environmental sensitive zones in Manitoba soil landscapes.

13 Overall, soil organic matter was the most important soil property influencing both 2,4-D
14 sorption and mineralization. This study suggested that the type of soil organic matter may
15 have influenced 2,4-D sorption by soil. The chemical composition of soil organic matter
16 varies as a result of the degree of organic matter decomposition, the type of parent material
17 as well as environmental factors. Therefore, further study is needed to examine the
18 interactions between 2,4-D and various components of soil organic matter for quantifying
19 the fate of 2,4-D in agricultural landscape. In addition, further studies on 2,4-D sorption
20 can be carried out considering other soil properties (i.e. the nature of clay, cation exchange
21 capacity, iron oxides etc.).

1 In this study, no significant correlation was found between total 2,4-D mineralization and
2 microbial biomass, suggesting that mineralization was influenced more by the extent of
3 2,4-D sorption by soil. Further study needs to take place concerning the potential effects of
4 microbial biomass and community composition on 2,4-D mineralization since only ten
5 samples were studied.

6 This study focussed on 2,4-D mineralization and did not identify 2,4-D degradation
7 products. Therefore, further study is also needed to investigate the presence of herbicide
8 metabolites. It would be valuable to identify initial degradation of the herbicide molecule
9 and subsequent metabolite mineralization in order to create a timetable of degradation
10 events as well as to determine whether the fractions left in the soil have any agronomic and
11 environmental concerns.

12 Results from laboratory studies may not be applicable to field environment due to wide
13 variation of environmental factors. Therefore, additional research is also needed to validate
14 the laboratory findings under field conditions. It should also be investigated whether the
15 regression equations derived to predict sorption and degradation in the Miami field are site-
16 specific or also applicable to other agricultural fields. In addition, this study was limited to
17 2,4-D, other herbicides should be considered in future studies. Finally, this study was
18 limited to 0-15 cm depth and additional studies are required to understand 2,4-D sorption
19 and degradation through the entire soil profile.

20

7. CONTRIBUTION TO KNOWLEDGE

1
2 This is the first study that used the Landform Element Complexes classification system in
3 classifying different landscape positions and examined the spatial variation of 2,4-D
4 mineralization as affected by soil properties and landscape position. The result of this study
5 revealed that 2,4-D mineralization varied significantly from upper to lower slope positions
6 and total mineralization can be predicted well with SOC content and soil pH. This has not
7 been observed in any other previous 2,4-D mineralization studies. This study also revealed
8 that sorption is likely the reason for differences in mineralization rate in different landscape
9 positions. Based on this study, when relatively mobile herbicides such as 2,4-D are applied
10 on sensitive landscapes, site-specific landscape soil properties (i.e. SOC content and soil
11 pH) should be considered to minimize agronomic and environmental concerns from off-site
12 movement of 2,4-D.

8. REFERENCES

- 1
- 2 **AAFC 1994.** 2,4-D re-evaluation update and label improvement program. Publication no.
3 C94-08. Agriculture and Agri-Food Canada.
- 4 **Adams, C.D. and Thurman, E.M. 1991.** Formation and transport of deethylatrazine in the
5 soil and vadose zone. *J. Environ. Qual.* **20**:540-547.
- 6 **Ahrens, W.H. (ed.) 1994.** *Herbicide Handbook*. 7th ed. Weed Science Society of America,
7 Champaign, Illinois. 352pp.
- 8 **Allan, E.S. 1989.** Degradation, fate and persistence of phenoxyalkanoic acid herbicides in
9 soil. *Rev. Weed Sci.* **4**:1-24.
- 10 **Arshad, M.A., Schnitzer, M., Angers, D.A. and Ripmeester, J.A. 1990.** Effects of till vs
11 no-till on the quality of soil organic matter. *Soil Biol. Biochem.* **22**:595-599.
- 12 **Audus, L.J. 1960.** Herbicides and the soil. p. 1-19. *In* E.K. Woodford and G.R. Sagar (ed)
13 Blackwell, Oxford.
- 14 **Bajracharya, R.M., Lal, R. and Kimble, J.M. 1997.** Soil organic distribution in
15 aggregates and primary particle fractions as influenced by erosion phases and landscape
16 position. Pages 353-357. *In* Layle et al. (Eds). *Soil Processes and the carbon cycle* CRC
17 Press. Boca Raton Florida.
- 18 **Barbash, J.E. and Resek, E.A. 1996.** Pesticides in ground water: distribution, trends, and
19 governing factors: Chelsea, Michigan, Ann Arbor Press. *Pesticides in the Hydrologic*
20 *System* series **2**:590pp.
- 21 **Barriuso, E., Houot, S. and Serra-Wittling, C. 1997.** Influence of compost addition to
22 soil on the behaviour of herbicides. *Pestic. Sci.* **49**:65-75.
- 23 **Beckett, P.H.T. and Webster, R. 1971.** 'Soil Variability - A Review'. *Soils and Fertilisers*
24 **34(1)**:p15.
- 25 **Beckie, H.J., Moulin, A.P. and Pennock, D.J. 1997.** Strategies for variable rate nitrogen
26 fertilization in hummocky terrain. *Can. J. Soil Sci.* **77**:589-595.
- 27 **Benoit, P., Barriuso, E. and Soulas, G. 1999.** Degradation of 2,4-D, 2,4-Dichlorophenol,
28 and 4-Chlorophenol in soil after sorption on humified and nonhumified organic matter. *J.*
29 *Environ. Qual.* **28**:1127-1135.
- 30 **Benoit, P., Barriuso, E., Houot, S. and Calvet, R. 1996** Influence of the nature of soil
31 organic matter on the sorption-desorption of 4-chlorophenol, 2,4-dichlorophenol and the
32 herbicide 2,4-dichlorophenoxyacetic acid. *European J. Soil Sci.* **47**:567-578.

- 1 **Beyer, L. 1995.** Soil microbial biomass and organic matter composition in soils under
2 cultivation. *Biol. Fertil. Soils.* **19:**197–202.
- 3 **Biederbeck, V.O., Campbell, C.A. and Smith, E.A. 1987.** Effects of long-term 2,4-D
4 field applications on soil biochemical processes. *J. Environ. Qual.* **16:**257- 262.
- 5 **Boesten, J.J.T.I. 2000.** Modeller subjectivity in estimating pesticide parameters for
6 leaching models using the same laboratory dataset. *Agr. Water Manage.* **44:**389-409.
- 7 **Boesten, J.J.T.I. and van der Linden, A.M.A. 1991.** Modeling the influence of sorption
8 and transformation of pesticide leaching and persistence. *J. Environ. Qual.* **20:**425-435.
- 9 **Bolan, N.S. and Baskaran, S. 1996.** Biodegradation of 2,4-D herbicide as affected by its
10 adsorption-desorption behavior and microbial activity of soils. *Aust. J. Soil Res.* **34:**1041-
11 1053.
- 12 **Bollag, J.M. and Liu, S.Y. 1990.** Biological Transformation Processes of Pesticides. In
13 Cheng: *Pesticides in the Soil Environment: Processes, Impacts, and Modeling.* Soil Science
14 Society of America. Madison, Wisconsin, USA. p169-211.
- 15 **Bollag, J.M., Helling, C.S. and Alexander, M. 1968.** 2,4-D metabolism: Enzymatic
16 hydroxylation of chlorinated phenols. *J. Agric. Food Chem.* **16:**826-828.
- 17 **Bossio, D.A., Scow, K.M., Gunapala, N. and Graham, K.J. 1998.** Determinants of soil
18 microbial communities: effects of agricultural management, season, and soil type on
19 phospholipid fatty acid profiles. *Microb. Ecol.* **36:**1-12.
- 20 **Bowman, R.A., Reeder, J.D. and Lober, R.W. 1990.** Changes in soil properties in a
21 central plains rangeland soil after 3, 20, and 60 years of cultivation. *Soil Sci.* **150:**851-857.
- 22 **Brady, N.C. and Weil, R.R. 1999.** The nature and properties of soils. Prentice-Hall Inc.,
23 Upper Saddle River, NJ, 881 pp. *Can. J. Soil Sci.* **75:**211-218.
- 24 **British Crop Production Council. 1987.** The Pesticide Manual. A World Compendium,
25 Worthing, C.R. and Walker, B.S. (Eds.). Lavenham Press Limited. Lavenham, Suffolk.
26 1081pp.
- 27 **Broughton, L.C. and Gross, K.L. 2000.** Patterns of diversity in plant and soil microbial
28 communities along a productivity gradient in a Michigan old-field. *Oecologia* **125:** 420-
29 427.
- 30 **Brubaker, S.C., Jones, A.J., Lewis, D.T. and Frank, K. 1993.** Soil properties associated
31 with landscape position. *Soil Sci. Soc. Am. J.* **57:**235-239.
- 32 **Burt, T.P. and Butcher, D.P. 1985.** Topographic controls of soil moisture distributions.
33 *Soil Sci.* **36:**469-486.

- 1 **Calderón, F., Jackson, L.E., Scow, K.M. and Rolston, D.E. 2000.** Microbial responses to
2 simulated tillage in cultivated and uncultivated soils. *Soil Biol. Biochem.* **32**:1547–1559.
- 3 **Calderón, F.J., Jackson, L.E., Scow, K.M. and Rolston, D.E. 2001.** Short-term dynamics
4 of nitrogen, microbial activity, and phospholipid fatty acids after tillage. *Soil Sci. Soc. Am.*
5 *J.* **65**:118-126.
- 6 **Campbell, C.A., McConkey, B.G., Zentner, R.P., Dyck, F.B., Selles, F. and Curtin, D.**
7 **1995.** Carbon sequestration in a Brown Chernozem as affected by tillage and rotation. *Can.*
8 *J. Soil Sci.* **75**:449–458.
- 9 **Campbell, C.A., McConkey, B.G., Zentner, R.P., Selles, F. and Curtin, D. 1996.** Long-
10 term effects of tillage and crop rotations on soil organic C and total N in a clay soil in
11 southwestern Saskatchewan. *Can. J. Soil Sci.* **76**:395–401.
- 12 **Carter, M.R. 1986.** Microbial biomass as an index for tillage induced changes in soil
13 biological properties. *J. Soil Till. Res.* **7**:29-40.
- 14 **Cessna, A.J. and Elliott, J.A. 2004.** Seasonal variation of herbicide concentrations in
15 prairie farm dugouts. *J. Environ. Qual.* **33**:302-315.
- 16 **Cheah, U., Kirkwood, R.C. and Lum, K. 1997.** Adsorption, desorption and mobility of
17 four commonly used pesticides in Malaysian agricultural soils. *Pest. Sci.* **50**:53- 63.
- 18 **Cheng, H.H. 1990.** Pesticides in the soil environment-an overview. Pages 1-5 *In.* H.H.
19 Cheng, (Ed.). *Pesticides in the soil environment: processes, impacts and modeling.* Soil
20 Science Society of America. Madison, WI.
- 21 **Cheng, H.H. and Koskinen, W.C. 1986.** Processes and factors affecting transport of
22 pesticides to groundwater, p12-14. In W. Y. Garner, R.C.Honeycutt, and H. N. Nigg (ed),
23 *Evaluation of pesticides in groundwater.* American Chemical Society Symposium Series
24 no. 315. American Chemical Society, Washington, D.C.
- 25 **Chesters, G., Simsiman, G.V., Lavy, J., Alhajjar, B.J., Fatulla, R.N. and Harkin, J.M.**
26 **1989.** Environmental fate of alachlor and metolachlor. *Reviews of Environ. Contam.*
27 *Toxicol.* **110**:1-73.
- 28 **Choudhry, G.G. and Webster, G.R.B. 1985.** Protocol guidelines for the investigations of
29 photochemical fate of pesticides in water, air, and soils. *Residue Rev.* **96**:79-136.
- 30 **Clapp, C.E., Hayes, M.H.B. and Mingelgrin, U. 2001.** Measurements of sorption-
31 desorption and isotherm analysis.p.205-240. *In* Clapp *et al.* (eds). *Humic substances and*
32 *chemical contaminants.* Soil Science Society of America. Madison, WI.
- 33 **Clayton, J.S., Ehrlich, W.A., Cann, D.B., Day, J.H. and Marshall, I.B. 1977.** Soils of
34 Canada. Vol. 1. Soil Rep. Res. Branch, Canada Department of Agric., Ottawa, ON, 243 pp.

- 1 **Collins, H.P., Paul, E.A., Paustian, K. and Elliott, E.T. 1997.** Characterization of Soil
2 Organic Carbon Relative to Its Stability and Turnover. In *Soil Organic Matter in Temperate*
3 *Agroecosystems: Long-term experiments in North America.* CRC Press, Inc. p51-72.
- 4 **Coquet, Y. and Barriuso. 2002.** Spatial variability of pesticide adsorption within the
5 topsoil of a small agricultural catchment. *Agronomie.* **22**:389-398.
- 6 **Cox, L., Koskinen, W.C., and Yen P.Y. 1998.** Influence of soil properties on sorption-
7 desorption of imidacloprid. *J. Environ. Sci. Health B.* **33**:123-134.
- 8 **Crop Protection Guide. 2000.** Saskatchewan Agriculture and Food: Saskatoon, SK, 330
9 pp.
- 10 **Crowe, A. and Milburn, P. 1995.** Prediction of pesticide leaching on a watershed basis:
11 methodology and application *Water Qual. Res. J. Canada* **30(3)**:365-381.
- 12 **Currie, R.S. and Williamson, D.A. 1995.** An assessment of pesticide residues in surface
13 waters of Manitoba, Canada. Manitoba Environment Rep. No. 95-08. Manitoba
14 Environment, Winnipeg.
- 15 **Dahiya, I.S., Kersebaum, K.C. and Richter, J. 1984.** Spatial variability of some nutrients
16 constituents of an Alfisol from loess I. Classical statistical analysis. *Zeitschrift für*
17 *Pflanzenernährung und Bodenkunde.* **147**:695-703.
- 18 **Daniels, R.B., Gilliam, J.W., Cassel, D.K. and Nelson, L.A. 1985.** Soil erosion class and
19 landscape position in the North Carolina Piedmont. *Soil Sci. Soc. Am. J.* **49**:991-995.
- 20 **Dec, J., Shuttleworth, K.L. and Bollag, J.-M. 1990.** Microbial release of 2,4-
21 Dichlorophenol bound to humic acid or incorporated during humification. *J. Environ. Qual.*
22 **19**:546-551.
- 23 **Dexter, A.R. 1988.** Advances in characterization of soil structure. *Soil Till. Res.* **11**:199-
24 238.
- 25 **Donald, D.B., Syrgiannis, J., Hunter, F. and Weiss, G. 1999.** Agricultural pesticides
26 threaten the ecological integrity of northern prairie wetlands. *Sci. Total Environ.* **231**:173-
27 181.
- 28 **Doner, H.E. and Lynn, W.C. 1989.** Carbonate, halide, sulfate, and sulfide minerals. Pages
29 279-330. Dixon, J.B. and Weed, S.B. (Eds.). *In Minerals in Soil Environment.* 2nd edition.
30 Soil Science Society of America. Madison, WI.
- 31 **Donnelly, P.K., Entry, J.A. and Crawford, D.L. 1993.** Degradation of atrazine and 2,4-
32 dichlorophenoxyacetic acid by mycorrhizal fungi at three nitrogen concentrations in vitro.
33 *Appl. Environ. Microbiol.* **59**:2642-2647.

- 1 **Duah-Yentumi, S and Kuwatsuk, S. 1982.** Microbial degradation of benthocarb, MCPA
2 and 2,4-D herbicides in perfused soils amended with organic matter and chemical
3 fertilizers. *Soil Sci. Plant Nutr.* **28**:19-26.
- 4 **Dubus, I.G., Barriuso, E. and Calvet, R. 2001:** Sorption of weak organic acids in soils:
5 clofencet, 2,4-D and salicylic acid. *Chemosphere* **45**:767-774.
- 6 **Duchnowicz, P., Koter, M. and Duda, W. 2002.** Damage of erythrocyte by phenoxyacetic
7 herbicides and their metabolites. *Pest. Biochem. and Physiol.* **74**:1-7.
- 8 **Dyson, J.S., Beulke, S., Brown, C.D. and Lane, M.C.G. 2002.** Adsorption and
9 degradation of the weak acid mesotrione in soil and environmental fate implications. *J*
10 *Environ. Qual.* **31**:613-618.
- 11 **Elliot, J.A. and Efetha, A.A. 1999.** Influence of tillage and cropping system on soil
12 organic matter, structure, and infiltration in a rolling landscape. *Can. J. Soil Sci.* **79**:457-
13 463.
- 14 **Elliot, J.A., Cessna, A.J., Nicholaichuk, W. and Tollefson, L.C. 2000.** Leaching rates
15 and preferential flow of selected herbicides through tilled and untilled soil. *J. Environ.*
16 *Qual.* **29**:1650-1656.
- 17 **Entry, J.A. and Emmingham, W.H. 1995.** The influence of dairy manure on atrazine and
18 2,4-dichlorophenoxyacetic acid mineralization in pasture soils. *Can. J. Soil Sci.* **75**:379-
19 383.
- 20 **Entry, J.A. and Emmingham, W.H. 1996.** Influence of vegetation on microbial
21 degradation of atrazine and 2,4-dichlorophenoxyacetic acid in riparian soils. *Can. J. Soil*
22 *Sci.* **76**:101-106.
- 23 **Entry, J.A., K.G. Mattson, and W.H. Emmingham. 1993.** The influence of nitrogen on
24 atrazine and 2,4-dichlorophenoxyacetic acid mineralization in grassland soils. *Biol. Fertil.*
25 *Soils.* **16**:179-182.
- 26 **Environment** **Canada.**
27 http://www.climate.weatheroffice.ec.gc.ca/climate_normals/index_e.html (accessed on
28 August 9, 2005).
- 29 **Farenhorst A., Muc, D., Monreal, C. and Florinski, I. 2001.** Sorption of herbicides in
30 relation to soil variability and landscape position. *J. Environ. Sci. Health. B* **36**:379-387.
- 31 **Farenhorst, A., Florinski, I., Monreal C. and Muc, D. 2003.** Evaluating the use of
32 digital terrain modeling for quantifying the spatial variability of 2,4-D sorption within
33 agricultural landscapes. *Can. J. Soil Sci.* **83**:557-564.
- 34 **Felsot, A.S. and Shelton, D.R. 1993.** Enhanced biodegradation of soil pesticides:
35 Interactions between physicochemical processes and microbial ecology. p. 227-251 *In*

- 1 Sorption and Degradation of Pesticides and Organic Chemicals in Soils. SSSA Spec. Publ.
2 32. ASA and SSSA, Madison, WI.
- 3 **Fiez, T.E., Miller, B.C. and Pan, W.L. 1994.** Winter wheat yield and grain protein across
4 varied landscape positions. *Agron. J.* **86**:1026-1032.
- 5 **Fitzpatrick, E.A. 1980.** Soils their formation, classification, and distribution. Longman
6 Inc. New York. 353 pp.
- 7 **Flury, M. 1996.** Experimental evidence of transport of pesticides through field soils- a
8 review. *J. Environ. Qual.* **25**:25-45.
- 9 **Fournier, J.C. 1980.** Enumeration of the soil micro-organisms able to degrade 2,4-D by
10 metabolism or co-metabolism. *Chemosphere* **9**:169-174.
- 11 **Frank, R. and Logan, L. 1988.** Pesticide and industrial chemical residues at the mouth of
12 the Grand, Saugeen and Thames rivers, Ontario, Canada, 1981-85: *Archives of Environ.*
13 *Contam. Toxicol.* **17**:741-754.
- 14 **Gaultier, J., Farenhorst, A. and Crow, G. 2005.** Spatial variability of soil properties and
15 2,4-D sorption in a hummocky field as affected by landscape position and soil depth. *Can.*
16 *J. Soil Sci.* (in press).
- 17 **Gee G.W. and Bauder, J.W. 1986.** Particle-size analysis. *In* *Methods of soil analysis-part*
18 *1. Physical and Mineralogical Methods*; A. Klute, Ed.; ASA/SSSA: Madison, WI. 383-412 .
- 19 **Gerrard, A.J. 1981.** Soils and Landforms. An Integration of Geomorphology and
20 Pedology. George Allen and Unwin, London, 219 p.
- 21 **Gerstl, Z. 2000.** An update on the Koc concept in regard to regional scale management.
22 *Crop Prot.* **19**:643-648.
- 23 **Glass, R.L. 1987.** Adsorption of glyphosate by soils and clay minerals. *J. Agric. Food*
24 *Chem.* **35**:497-500.
- 25 **Goderya, F. S. 1998.** Field scale variations in soil properties for spatially variable control:
26 A review. *J. Soil Contam.* **7(2)**: 243-264.
- 27 **Gold, A.J., Morton, T.G., Sullivan, W.M. and McClory, J. 1988.** Leaching of 2,4-D and
28 dicamba from home lawns. *Water Air Soil Pollution.* **37**:121-129.
- 29 **Golden Software. 1998.** SurferTM software version 8.0. Boulder, Co.
- 30 **Gonod, L.V., Chenu, C. and Soulas, G. 2003.** Spatial variability of 2,4-
31 dichlorophenoxyacetic acid (2,4-D) mineralisation potential at a millimetre scale in soil.
32 *Soil Biol. Biochem.* **35**:373-382.

- 1 **Goodrich, J.A., Lykins, B.W. Jr. and Clark, R.M. 1991.** Drinking water from
2 agriculturally contaminated groundwater. *J. Environ. Qual.* **20**:707-717.
- 3 **Gouy, V., Dur, J-C., Calvet, R., Belamie, R. and Chaplain, V. 1999.** Influence of
4 adsorption-desorption phenomena on pesticide run-off from soil using simulated rainfall.
5 *Pestic. Sci.* **55**:175-182.
- 6 **Grayston, S.J., Gri.th, G.S., Mawdsley, J.L., Campbell, C.D. and Bardgett, R.D. 2001.**
7 Accounting for variability in soil microbial. Communities of temperate upland grassland
8 ecosystems. *Soil Biol. Biochem.* **33**:533-551.
- 9 **Grayston, S.J., Campbell, C.D., Bardgett, R.D., Mawdsley, J.L., Clegg, C.D., Ritz, K.,**
10 **Griffiths, B.S., Rodwell, J.S., Edwards, S.J., Davies, W.J., Elston, D.J. and Millard, P.**
11 **2004.** Assessing shifts in microbial community structure across a range of grasslands of
12 differing management intensity using CLPP, PLFA and community DNA techniques.
13 *Appl. Soil Ecol.***25**:63-84.
- 14 **Green, R.E. and Karickhoff, S.W. 1990.** Sorption estimates for modeling. Pages 79- 101.
15 *In.* H.H. Cheng, (Ed). *Pesticides in the soil environment: processes, impacts and modeling.*
16 *Soil Science Society of America.* Madison, WI.
- 17 **Greenland, D.J. and Hayes, M.H.B. 1981.** Soil processes. Pages 1-35. *In.* D.J. Greenland,
18 and M.H.B Hayes (Eds.). *The chemistry of soil processes.* John Wiley and Sons, Ltd. New
19 York.
- 20 **Greer, L.E. and Shelton, D.R. 1992.** Effect of inoculant strain and organic matter content
21 on kinetics of 2,4-dichlorophenoxyacetic acid degradation in soil. *Appl. Environ.*
22 *Microbiol.* **58(5)**:1459-1465.
- 23 **Gregorich, E.G. and Anderson, D.W. 1985.** Effects of cultivation and erosion on soil of
24 four toposequences in the Canadian Prairies. *Geoderma* **36**:343-354.
- 25 **Grover, R. 1973.** The adsorptive behaviour of acid and ester forms of 2,4-D on soils.
26 *Weed Res.-* **13**:51-58
- 27 **Grover, R. 1977.** Mobility of dicamba, picloram and 2,4-D in soil columns. *Weed Sci.*
28 **25**:159-162.
- 29 **Guth, J.A. 1981.** Experimental approaches to studying the fate of pesticides in soil. *In*
30 "Progress in pesticide biochemistry". (Eds DH Hutson, TRC Roberts) pp.85-114. (John
31 Wiley and Sons Ltd: New York).
- 32 **Hamaker, J.W. and Thompson, J.M. 1972.** Adsorption, p. 49-144. *In* Hamaker, J.W. and
33 Goring, C.A.I. (Eds.) *Organic chemicals in the soil environment*, volume 1. Marcel Dekker
34 Inc, New York.

- 1 **Han, S.K. and New, P.B. 1994.** Effect of water availability on degradation of 2,4-
2 dichlorophenoxyacetic acid (2,4-D) by soil microorganisms. *Soil Biol. Biochem.*
3 **26(12):1689-1697.**
- 4 **Hassink, J. 1994.** Effect of soil texture on the size of the microbial biomass and on the
5 amount of C and N mineralized per unit of microbial biomass in Dutch grassland soils. *Soil*
6 *Biol. Biochem.* **26:1573-1581.**
- 7 **Havlin, J.L., Kissle, D.E., Maddux, L.D., Claassen, M.M. and Lang, J.H. 1990.** Crop
8 rotation and tillage effects on soil organic carbon and nitrogen. *Soil Sci. Soc. Am. J.*
9 **54:448-452.**
- 10 **Health Canada. 1991.** 2,4-Dichlorophenoxyacetic Acid. Available at [http://www.hc-](http://www.hc-sc.gc.ca/hecs-sesc/water/pdf/dwg/dichloro.pdf)
11 [sc.gc.ca/hecs-sesc/water/pdf/dwg/dichloro.pdf](http://www.hc-sc.gc.ca/hecs-sesc/water/pdf/dwg/dichloro.pdf) (accessed on September 16, 2005).
- 12 **Hermosin, M.C. and Cornejo, J. 1991.** Soil adsorption of 2,4-D as affected by the clay
13 mineralogy. *Toxic. Environ. Chem.* **31-32:69-77.**
- 14 **Hermosin, M.C. and Cornejo, J. 1992.** Removing 2,4-D from water by organoclays.
15 *Chemosphere* **24:1493-1503.**
- 16 **Hermosin, M.C. and Cornejo, J. 1993** Binding mechanism of 2,4-dichlorophenoxy-acetic
17 acid by organoclays. *J. Environ. Qual.* **22:325-331.**
- 18 **Hill, B.D., Harker, K.N., Hasselback, P., Moyer, J.R., Inaba, D.J. and Byers, S.D.**
19 **2001.** 'Phenoxy' herbicides in Alberta rainfall as affected by location, season, and weather
20 patterns. Final Technical Report, Alberta Agricultural Research Institute.
- 21 **Hoar, S.K., Zahm, S., Weisenburger, D.D. and Babbitt, P.A. 1990.** A case-control study
22 of Non-Hodgkin's Lymphoma and the herbicide 2,4-dichlorophenoxyacetic acid(2,4-D) in
23 Eastern Nebraska. *Epidemiol.* **1: 349-356.**
- 24 **Honeycutt, C.W., Heil, R.D. and Cole, C.V. 1990.** Climatic and topographic relations of
25 three Great Plains soils II: Carbon, nitrogen, and phosphorus. *Soil Sci. Soc. Am. J.* **54:476-**
26 **483.**
- 27 **Huskes, R. and Levsen, K. 1997.** Pesticides in rain. *Chemosphere* **35(12):3013-3024.**
- 28 **Johnson, W.G., Lavy, T.L. and Gbur, E.E. 1995.** Sorption, mobility and degradation of
29 Triclopyr and 2,4-D on four soils. *Weed Sci.* **43:678-684.**
- 30 **Jury, W.A. 1986.** Chemical movement through soil. p. 135-158. in S.C. Hern and S.M.
31 Melancon (eds.) *Vadose zone modeling of organic pollutants*, Lewis Pub. Inc., Chelsea, MI.
- 32 **Kachanoski, R.G., Gregorich, E.G. and Protz, R. 1992.** Quantification of soil loss in
33 complex topography. In R.G. Kachanoski *et al* (Eds.) *Management of farm field variability*
34 *SWEEP/TED Report*. University of Guelph, ON. 156 pp.

- 1 **Katan, J., Greenberger, A., Alon, H. and Grinstein, A. 1976.** Solar heating by
2 polyethylene mulching for the control of diseases caused by soil-borne pathogens.
3 *Phytopathology* **66**:683-688.
- 4 **Khakural, B.R., Robert, P.C. and Koskinen, W.C. 1994.** Runoff and leaching of
5 alachlor under conventional and soil-specific management. *Soil Use and Manage.***10**:158-
6 164.
- 7 **Khan, S.U. 1973.** Equilibrium and kinetic studies of the adsorption of 2,4-D and picloram
8 on humic acid. *Can. J. Soil Sci.* **53**:429-434.
- 9 **Kleiss, H.J. 1970.** Hillslope sedimentation and soil formation in northeastern Iowa. *Soil*
10 *Sci. Soc. Am. Proc.* **34**:287-290.
- 11 **Konstantinou, I.K., Zarkadis, A.K. and Albanis, T.A. 2001.** Photodegradation of
12 selected herbicides in various natural waters and soils under environmental conditions. *J.*
13 *Environ. Qual.* **30**:121-130.
- 14 **Kookana, R.S., Baskaran, S. and Naidu, R. 1998.** Pesticide fate and behaviour in
15 Australian soils in relation to contamination and management of soil and water: a review.
16 *Aust. J. Soil Res.* **36**:715-764.
- 17 **Koskinen, W.C. and Harper, S.S. 1990.** The retention process: mechanisms. Pages 51-77.
18 *In*. H.H. Cheng, (Ed.). *Pesticides in the soil environment: processes, impacts and modeling.*
19 *Soil Science Society of America. Madison, WI*
- 20 **Koterba, M.T., Banks, W.S.L. and Shedlock, R.J. 1993.** Pesticides in shallow
21 groundwater in the Delmarva Peninsula. *J. Environ. Qual.* **22**:500-518.
- 22 **Lampman, W. 1995.** Susceptibility of groundwater to pesticide and nitrate contamination
23 in predisposed areas of southwestern Ontario. *Water Qual. Res. J. Can.* **30**:443-468.
- 24 **Landi, A., Mermut, A.R. and Anderson, D.W. 2004.** Carbon distribution in a hummocky
25 landscape from Saskatchewan, Canada. *Soil Sci. Soc. Am. J.* **68**(1):175-184.
- 26 **Lavy, T.L., Roeth, F. W. and Fenster, C.R. 1973.** Degradation of 2,4-D and atrazine at
27 three soil depths in the field. *J. Environ. Qual.* **2**:132-137.
- 28 **Legendre, P. and Legendre, L. 1998.** Numerical ecology. 2nd English edition. Elsevier,
29 Amsterdam. 853 pages.
- 30 **Lelyk, G. 2005.** Personal communication.
- 31 **Leonard, R.A. 1990.** Movement of pesticides into surface waters, In "Pesticides in the soil
32 environment: processes, impacts and modelling". (Ed. HH Cheng) pp.303-349. (SSSA:
33 Madison, WI).

- 1 **Li, S., Lobb, D.A. and Lindstrom, M.J. 2004.** Tillage translocation measurement and
2 tillage erosion modeling in southwest Manitoba, Canada, *In: G. Huang, L. S. Pereira (ed.),*
3 *Land and water management decision tools and practices Vol. II, 829-839, Beijing, China.*
- 4 **Lindstrom, M.J., Nelson, W.W. and Schumacher, T.E. 1992.** Quantifying tillage erosion
5 rates due to mouldboard plowing. *J. Soil Till. Res. 24:243-255.*
- 6 **Lobb, D.A. and Kachanoski, R.G. 1999.** Modelling tillage erosion on the topographically
7 complex landscapes of southwestern Ontario. *Soil Till. Res. 51:261-277.*
- 8 **Lobb, D.A., Kachanoski, R.G. and Miller, M.H. 1995.** Tillage translocation and tillage
9 erosion on shoulder slope landscape positions measured using ¹³⁷Cs as a tracer. *Can. J.*
10 *Soil Sci. 75:211-218.*
- 11 **Lobb, D.A., Kachanoski, R.G. and Miller, M.H. 1999.** Tillage translocation and tillage
12 erosion in the complex upland landscapes of southwestern Ontario. *Soil Till. Res. 51:189-*
13 *209.*
- 14 **Lobb, D.A., Lindstrom, M.J. and Schumache, T.E. 2003.** Soil erosion processes and
15 their interactions: implications for environmental indicators. OECD Expert Meeting on
16 *Soil Erosion and Soil Biodiversity Indicators. 25-28 March, 2003, Rome, Italy.*
- 17 **Loeppert, R.H. and Suarez, D.L. 1996.** Carbonate and gypsum *In Methods of Soil*
18 *Analysis Part 3: Chemical Methods. Soil Science Society of America/ American Society of*
19 *Agronomy; J.M. Bartels, ed.; Madison, WI. Chapter 15.,437-474 pp.*
- 20 **Loos, M.A. 1975.** Phenoxyalkanoic acids, p. 1-128. *In P.C. Kearney and D.D. Kaufman*
21 *(ed.), Herbicides: chemistry, degradation and mode of action. Marcel Dekker, Inc., New*
22 *York.*
- 23 **Loos, M.A., Bollag, J.M. and Alexander, M. 1967.** Phenoxyacetate herbicide
24 detoxication by bacterial enzymes. *J. Agric. Food Chem. 15:858-860.*
- 25 **MacMillan, R.A. and Pettapiece, W.W. 2000.** Alberta Landforms: Quantitative
26 morphometric descriptions and classification of typical Alberta landforms. Technical
27 Bulletin No. 2000-2E. Research Branch, Agriculture and Agri-Food Canada, Semiarid
28 Prairie Agricultural Research Centre, Swift Current, SK. 118 pp.
- 29 **MacNaughton, S.J., Jenkins, T.L., Wimpee, M.H., Cormier, M.R. and White, D.C.**
30 **1997.** Rapid extraction of lipid biomarkers from pure cultures and environmental samples
31 using pressurized accelerated hot solvent extraction. *J. Microb. Methods 31:19-27*
- 32 **McCall, P.J., Vrona, S.A. and Kelly, S.S. 1981.** Fate of uniformly carbon-14 ring labeled
33 2,4,5-trichlorophenoxy-acetic acid and 2,4-dichlorophenoxyacetic acid. *J. Agric. Food*
34 *Chem. 29:100-107.*

- 1 **Madsen, L., Lindhardt, B., Rosenberg, P., Clausen, L. and Fabricius, I. 2000.** Pesticide
2 sorption by low organic carbon sediments: a screening for seven herbicides. *J. Environ.*
3 *Qual.* **29**:1488-1500.
- 4 **Mallawatantri, A.P. and Mulla, D.J. 1992.** Herbicide adsorption and organic carbon
5 contents on adjacent low-input versus conventional farms. *J. Environ. Qual.* **21**:546-551.
- 6 **Mallows, C. 1973.** Some Comments on *Cp.* *Technometrics* **15**:661-675.
- 7 **Malo, D.D. and Worcester, B.K. 1975.** Soil fertility and crop responses at selected
8 landscape positions. *Agron. J.* **67**:397-401.
- 9 **Mansour, M. and Korte, F. 1986.** Abiotic degradation pathways of selected xenobiotic
10 compounds in the environment. In "Studies on environmental science, 29, Chemistry for
11 protection of the environment". (Eds L Pawlowski, G Alaerts) pp. 257. (Elsevier:
12 Amsterdam).
- 13 **Mansour, M., Feicht, E. and Meallier, P. 1989.** Improvement of the photostability of
14 selected substances in aqueous medium. *Toxicol. Environ. Chem.* **139**:20-21.
- 15 **Mass, R.P., Kucken, D.J., Patch, S.C., Peek, B.T. and Van Engelen, D.L. 1995.**
16 Pesticides in eastern North Carolina rural supplies wells: land use factors and persistence.
17 *J. Environ. Qual.* **24**:426-431.
- 18 **McConnell, J.S. and Hossner, L.R. 1985.** pH-dependent adsorption isotherms of
19 glyphosate. *J. Agric. Food Chem.* **33**:1075-1078.
- 20 **McCulley, R.L. and Burke, I.C. 2004.** Microbial community composition across the
21 Great Plains: landscape versus regional variability. *Soil Sci. Soc. Am. J.* **68(1)**:106-115.
- 22 **McKeague, J.A. 1978.** Manual on soil sampling and methods of analysis. Second edition,
23 Soil Research Institute, Agriculture Canada, Ottawa, Ont. 212 pp.
- 24 **Mermut, A.R., Action, D.F. and Eilers, W.E. 1983.** Estimation of soil erosion and
25 deposition by a landscape analysis technique on clay soils in southern Saskatchewan. *Can.*
26 *J. Soil Sci.* **63**:727-739.
- 27 **Miller, J.J., Foroud, N., Hill, B.D. and Lindwall, C.W. 1995b.** Herbicides in surface
28 runoff and groundwater under surface irrigation in southern Alberta. *Can. J. Soil Sci.*
29 **75**:145-148.
- 30 **Miller, J.J., Hill, B.D., Chang, C. and Lindwall, C.W. 1995a.** Residue detections in soil
31 and shallow groundwater after long-term herbicide applications in Southern Alberta. *Can.*
32 *J. Soil Sci.* **75**:349-356.
- 33 **Miller, M.P., Singer, M.J. and Nielsen, D.R. 1988.** Spatial variability of wheat yield and
34 soil properties on complex hills. *Soil Sci. Soc. Am. J.* **52**:1133-1141.

- 1 **Moore, I.D., Gessler, P.E., Nielson, G.A. and Peterson, G.A. 1993.** Soil attribute
2 prediction using terrain analysis. *Soil Sci. Soc. Am. J.* **57**:443-452.
- 3 **Moorman, T.B. and Harper, S.S. 1989.** Transformation and mineralization of metribuzin
4 in surface and subsurface horizons of a Mississippi Delta soil. *J. Environ. Qual.* **18**:302-
5 306.
- 6 **Moshier, L.J. and Penner, D. 1978.** Factors influencing microbial degradation of ¹⁴C-
7 glyphosate to ¹⁴CO₂ in soil. *Weed Sci.* **26**:686-691.
- 8 **Moulin A., Anderson, D.W. and Mellinger, M. 1994.** Spatial variability of wheat yield,
9 soil properties and erosion in hummocky terrain. *Can. J. Soil Sci.* **74**:219-228.
- 10 **Mulla, D.J. 1993.** In: *Soil-Specific Crop Management: Proceedings of a Workshop on*
11 *Research and Development Issues.* p15-26. ASA, Madison, USA.
- 12 **Nelson, D.E. and Sommers, L.E. 1982.** Total carbon, organic carbon, and organic matter.
13 Pages 539-577 *In* A.L. Page *et al.* (Eds.). *Methods of Soil Analysis, Part 2: Chemical and*
14 *Microbiological Properties.* ASA, Madison.
- 15 **Nicholaichuk, W. and Grover, R. 1983.** Loss of fall-applied 2,4-D in spring runoff from a
16 small agricultural watershed. *J. Environ. Qual.* **12**:412-414.
- 17 **Nicholls, P.H. and Evans, A.A. 1991.** Sorption of ionisable compounds by field soils. Part
18 1: Acids. *Pestic. Sci.* **33**:319-330.
- 19 **Novak, J.M. 1999.** Soil factors influencing atrazine sorption: implications on fate.
20 *Environ. Toxic. Chemistry* **18**:1663-1667.
- 21 **Novak, J.M., Moorman, T.B. and Cambardella, C.A. 1997.** Atrazine sorption at the
22 field scale in relation to soils and landscape position. *J. Environ. Qual.* **26**:1271-1277.
- 23 **Ogram, A.V., Jessup, R.E., Ou, L.T. and Rao, P.S.C. 1985.** Effects of sorption on
24 biological degradation rates of 2,4-dichlorophenoxyacetic acid in soils. *Appl. Environ.*
25 *Microbiol.* **49**:585-587.
- 26 **Oliveira Jr., R.S., Koskinen, W.C., Ferreira, F.A. Khakural, B. R., Mulla, D.J. and**
27 **Robert, P.J. 1999.** Spatial variability of imazethapyr sorption in soil. *Weed Sci.* **47**:243-
28 248.
- 29 **Ou, L-T. 1984.** 2,4-D degradation and 2,4-D degrading microorganisms in soils. *Soil Sci.*
30 **137**:100-107. Pages 15-49. *In* E.A. Paul *et al.* (Eds.). *Soil Organic Matter in Temperature*
31 *Agroecosystems Long-Term Experiments in North America.* CRC Press. New York.
- 32 **Parker, L.W. and Doxtader, K.G. 1983.** Kinetics of the microbial degradation of 2,4-D in
33 soil: effects of temperature and moisture. *J. Environ. Qual.* **12**:553-557.

- 1 **Parker, L.W. and Doxtader, K.G. 1982.** Kinetics of microbial decomposition of 2,4-D in
2 soil: effects of herbicide concentration. *J. Environ. Qual.* **11**:679-684.
- 3 **Paustian, K., Collins H.P. and Paul, E. A. 1997.** Management controls on soil carbon In:
4 Paul, E.A., Paustian, K., Elliott, E.A., Cole, C.V. (Eds.). *Soil organic matter in temperate*
5 *agroecosystems: Long-term experiments in North America*, CRC Press, Boca Raton, pp.
6 15-49.
- 7 **Pennock, D.J. and de Jong, E. 1990.** Regional and catenary variations in properties of
8 Borolls of Southern Saskatchewan, Canada. *Soil Sci. Am. J.* **54**:1697-1701.
- 9 **Pennock, D.J., Zebarth, B.J. and De Jong, E. 1987.** Landform classification and soil
10 distribution in hummocky terrain, Saskatchewan, Canada. *Geoderma* **40**:297-315.
- 11 **Peterson, G.E. 1967.** The discovery and development of 2,4-D. *Agricultural History.*
12 **41**:243-253.
- 13 **Picton, P. and Farenhorst, A. 2004.** Factors influencing 2,4-D sorption and
14 mineralization in soil. *J. Environ. Sci. Health B.* **39**(3):367-379.
- 15 **Pignatello, J.J. 1989.** Sorption dynamics of organic compounds in soils and sediments.
16 Pages 45-80. In B.L. Sawhney and K. Brown (Eds.). *Reactions and movement of organic*
17 *chemicals in soil.* SSSA Special Publication. 22. Soil Science Society of America and
18 American Society of Agronomy. Madison, WI.
- 19 **Pimentel, D. and Levitan, L. 1986.** Pesticides: amounts applied and amounts reaching
20 pests. *BioScience* **36**:86-91.
- 21 **Podani, J. 2000.** SYN-TAX 2000 - Computer Programs for Data Analysis in Ecology and
22 Systematics, User's manual.
- 23 **Pothuluri, J.V., Moorman, T.B., Obenhuber, D.C. and Wauchope, R.D. 1990.** Aerobic
24 and anaerobic degradation of alachlor in samples from a surface-to-groundwater profile. *J.*
25 *Environ. Qual.* **19**:525-530.
- 26 **Rao, P.S.C. and Davidson, J.M. 1979.** Adsorption and movement of selected pesticides at
27 high concentrations in soils. *Water Res.* **13**:375-380.
- 28 **Rao, P.S.C. and Wagenet, R. J. 1985.** Spatial variability of pesticides in field studies:
29 methods of data analysis and consequences. *Weed Sci.* **33**:18-34.
- 30 **Rao, P.S.C., Hornsby, A.G. and Jessup, J.E. 1985.** Indices for ranking the potential for
31 pesticide contamination of groundwater. *Soil Crop Sci. Soc. Florida Proc.* **44**:1-8.
- 32 **Rawn, F.K. D, Halldorson, T. H.J., Lawson, B.D., and Muir, D.C.G. 1999b.** A multi-
33 year study of four herbicides in air and precipitation of a small prairie watershed. *J.*
34 *Environ. Qual.* **28**:898-906.

- 1 **Rawn, F.K.D., Halldorson, T.H.J., Turner, W.N., Woychuk, R.N., Zakrevsky, J. and**
2 **Muir, D.C.G. 1999a.** A multi-year study of four herbicides in surface water of a small
3 prairie watershed. *J. Environ. Qual.* **28**:906-917.
- 4 **Reddy, K.S. and Gambrell, R.P. 1987.** Factors affecting the adsorption of 2,4-D and
5 methyl parathion in soils and sediment. *Agric. Ecosys. Environ.* **18**:231-241.
- 6 **Reganold, J.P. 1988.** Comparison of soil properties as influenced by organic and
7 conventional farming systems. *Am. J. Alter. Agric.* **3**:144-155.
- 8 **Reimer, M. 2004.** The effect of hog manure and municipal biosolids on the mineralization
9 and sorption of pesticides in soil. M.Sc. thesis, Department of Soil Science, University of
10 Manitoba, Winnipeg, Canada. 179p.
- 11 **Richardson, J.L., Wilding, L.P. and Daniels, R.B. 1992.** Recharge and discharge of
12 ground water in aquic conditions illustrated with flowent analysis. *Geoderma* **53**:65-78.
- 13 **Roberts, T.L. and Bettany, J.R. 1985.** The influence of topography on the nature and
14 distribution of soil sulfur across a narrow environmental gradient. *Can. J. Soil Sci.* **65**:419-
15 434.
- 16 **Robertson, B.K. and Alexander, M. 1994.** Growth-linked and cometabolic
17 biodegradation: possible reason for occurrence or absence of accelerated pesticide
18 biodegradation. *Pestic. Sci.* **41**:311-318.
- 19 **Ross, D.J., Orchard, V.A. and Rhoades, D.A. 1984.** Temporal fluctuations in
20 biochemical properties of soils under pasture. I. Respiratory activity and microbial
21 biomass. *Aust. J. Soil Res.* **22**:303-317.
- 22 **Roulier, S. and Jarvis, N. 2003.** Modeling macropore flow effects on pesticide leaching:
23 inverse parameter estimation using microlysimeters. *J. Environ. Qual.* **32**:2341-2353.
- 24 **Rudolph, D. and Goss, M. 1993.** Ontario Farm Groundwater Quality Survey, Report
25 prepared for Agriculture Canada. ISBN 0-662-20879-X. Available at
26 http://res2.agr.ca/initiatives/manurenet/env_prog/gp/download/ofgqs_93.pdf. (accessed on
27 August 13, 2005).
- 28 **Sannino, F., Violante, A. and Gianfreda, L. 1997.** Adsorption-desorption of 2,4-D by
29 hydroxy aluminium montmorillonite complexes. *Pestic.Sci.* **51**:429-435.
- 30 **Sarmah, A.K. and Sabadie, J. 2002.** Hydrolysis of sulfonylurea herbicides in soils and
31 aqueous solutions: a review. *J. Agric. Food Chem.* **50**:6253-6265.
- 32 **Sarmah, A.K., Muller, K. and Ahmad, R. 2004.** Fate and behaviour of pesticides in the
33 agroecosystem-a review with a New Zealand perspective. *Aust. J. Soil Res.* **42**:125-154.
- 34 **SAS Institute, Inc. 2000.** SAS user's guide: Statistics, Version 8.01 Ed. SAS Institute Inc.
35 Cary, NC.

- 1 **Schwarzenbach, R.P. and Westall, J. 1981.** Transport of nonpolar organic compounds
2 from surface waters to groundwater. Laboratory sorption studies. *Environ. Sci. Technol.*
3 **15:1360-1367.**
- 4 **Scow, K.M. 1993.** Effect of sorption-desorption and diffusion processes on the kinetics of
5 biodegradation of organic chemicals in soil. *In* "Sorption and degradation of pesticides and
6 organic chemicals in soil". *Soil Sci.Soc.Am. Special Edition No.32*, p.73-115.
- 7 **Senesi, N. 1992.** Binding mechanisms of pesticides to soil humic substances. *Sci. Total*
8 *Environ.* **123/124:63-76.**
- 9 **Senesi, N., Loffredo, E., D'Orazio, V., Brunetti, G., Miano, T.M. and La Cava, P.**
10 **2001.** Adsorption of Pesticides by Humic Acids from Organic Amendments and Soils. *In*
11 Clapp et al.: *Humic Substances and Chemical Contaminants.* Soil Science Society of
12 America. Madison, Wisconsin, USA. p129-153.
- 13 **Shaw, L.J. and Burns, R.G. 1998.** Biodegradation of 2,4-D in a noncontaminated
14 grassland soil profile. *J. Environ. Qual.* **27:1464-1471.**
- 15 **Shea, P.J. 1989.** Role of humified organic matter in herbicide adsorption. *Weed Technol.*
16 **3:190-197.**
- 17 **Shelton, I.J., Wall, G.J., Cossette, J.M., Eilers, R.G., Grant, B.A., King, D.J.,**
18 **Padbury, G.A., Rees, H.W., Tajek, J. and van Vliet, L.J.P. 2000.** Risk of water erosion.
19 *In: Environmental health of Canadian agroecosystems.* T. McRae, Smith, S., Gregorich,
20 L.J. (eds). AAFC. Ottawa. pp. 59-67.
- 21 **Simard, M.J. and Legere, A. 2002.** Control of volunteer canola with auxinic herbicides:
22 Does cold hardening or plant size matter? *Canadian Weed Science Society, Proceedings of*
23 *the National Meeting 2002*, pp. 166-167.
- 24 **Simmons, F.W., Casel, D.K. and Daniels, R.B. 1989.** Landscape and soil property effects
25 on corn grain yield response to tillage. *Soil Sci. Soc. Am. J.* **53:534-539.**
- 26 **Smith, A.E. and Aubin, A.J. 1991.** Metabolites of [¹⁴C]-2,4-D in Saskatchewan soils. *J.*
27 *Agri. Food Chem.* **39:2019-2021.**
- 28 **Smith, A.E. and Muir, D.C.G. 1980.** Determination of extractable and non-extractable
29 radioactivity from prairie soils treated with carboxyl-labeled and ring-labeled ¹⁴C 2,4-D.
30 *Weed Res.* **20:123**
- 31 **Smith, J.L. and Paul, E.A. 1990.** The significance of soil microbial biomass estimations.
32 *In: Bollag J-M, Stotzky G (eds) Soil biochemistry, vol 6.* Marcel Dekker, NewYork, pp
33 357-396.
- 34 **Soil Classification Working Group. 1998.** The Canadian System of Soil Classification.
35 *Agric. and Agri-Food Can. Publ.* 1646 (Revised). 187 pp.

- 1 **Solohub, M.P., Pennock, D.J. and Walley, F.L. 1999.** Mineral nitrogen variability across
2 and within landscape positions. p. 85-90. In: Soil and Crops Workshop Proceedings,
3 Saskatoon, Sk, Division of Extension, Univ. of Saskatchewan, Saskatoon, Sk. Canada.
- 4 **Soulas, G. 1993.** Evidence for the existence of different physiological groups in the
5 microbial community responsible for 2,4-D mineralization in soil. *Soil Biol. Biochem.*
6 **25:443-449.**
- 7 **Sparling, G., Dragten, R., Aislable, J. and Fraser, R. 1998.** Atrazine mineralization in
8 New Zealand topsoils and subsoils: Influence of edaphic factors and numbers of atrazine-
9 degrading microbes. *Aust. J. Soil Res.* **36:557-570.**
- 10 **Sparling, G.P. and Ross, D.J. 1993.** Biochemical methods to estimate soil microbial
11 biomass: current development and applications. Pages 21-37 in K. Mulungoy and R.
12 Mercks, Eds. *Soil organic matter dynamics and sustainability of tropical agriculture.* John
13 Wiley and Sons, Chichester, UK.
- 14 **Stephens, K.D. 2003.** The spatial variability of soil properties and 2,4-D sorption and
15 desorption in two calcareous prairie landscapes. M.Sc. thesis, Department of Soil Science,
16 University of Manitoba, Winnipeg, Canada. 187p.
- 17 **Stephens, K.D., Farenhorst, A. and Fuller, L.G. 2002.** Effect of soil sterilization by
18 mercuric chloride on the sorption of herbicides by soil. *J. Environ. Sci. Health. B* **37:559-**
19 **569.**
- 20 **Stevenson, F.J. 1972.** Organic matter reactions involving herbicides in soil. *J. Environ.*
21 *Qual.* **1:333-343.**
- 22 **Stevenson, F.J. 1982.** "Humus chemistry: genesis, composition, reactions". (Wiley-
23 Interscience: New York).
- 24 **Stevenson, F.J. 1994.** Organic matter reactions involving pesticides in soil. In "Humus
25 chemistry: genesis, composition, reactions". 2nd edn, pp.453-471 (John Wiley and Sons
26 Inc.: New York).
- 27 **Stone, J.R., Gilliam, J.W., Cassel, D.K., Daniels, R.B., Nelson, L.A. and Kleiss, H.J.**
28 **1985.** Effect of erosion and landscape position on the productivity of Piedmont soils. *Soil*
29 *Sci. Soc. Am. J.* **49:987-991.**
- 30 **Stott, D.E., Martin, J.P., Focht, D.D. and Haider, K. 1983.** Biodegradation, stabilization
31 in humus, and incorporation into soil biomass of 2,4-D and chlorocatechol carbons. *Soil*
32 *Sci. Soc. Am. J.* **47:66.**
- 33 **Strebe, T.A. and Talbert, R.E. 2001.** Sorption and mobility of flumetsulam in several
34 soils. *Weed Sci.* **49(6):806-813.**
- 35 **Sveshnikova, A.A., Polyanskaya, L.M. and Lukin, S.M. 2001.** The effect of tillage and
36 mesorelief on the structure of soil microbial cenoses. *Microbiology* **70:484-491.**

- 1 **Taylor, A.W. and Spencer, W.F. 1990.** Volatilization and vapour transport processes. In
2 "Pesticide in the soil environment: processes, impacts and modelling". (Ed. HH Cheng)
3 pp.213-269. (SSSA:Madison, WI).
- 4 **Thompson, D.G., Stephenson, G.R., Solomon, K.R. and Skepasts, A.V. 1984.**
5 Persistence of (2,4-Dichlorophenoxy) acetic acid and 2-(2, 4-Dichlorophenoxy)propionic
6 acid in agricultural and forest soils of northern and southern Ontario. *J. Agric. Food Chem.*
7 **32:578-581.**
- 8 **Tiessen, H., Roberts, T.L. and Stewart, J.W. 1983.** Carbonate analysis in soils and
9 minerals by acid digestion and two end-point titration. *Comm. Soil Sci. Plant Anal.*
10 **14:161-166.**
- 11 **Topp, E., Vallaey, T. and Soulas, G. 1997.** Pesticides: microbial degradation and effects
12 on microorganisms. Pages 547-575. *in* J.D. van Elsas et al. (Eds.) *Modern Soil*
13 *Microbiology.* Marcel Dekker, New York, NY, U.S.A.
- 14 **Torrents, A., Jayasundera, S. and Schmidt, W.J. 1997.** Influence of the polarity of
15 organic matter on the sorption of acetamide pesticides. *J. Agric. Food Chem.* **45:3320-**
16 **3325.**
- 17 **Tunesi, S., Poggi, V. and Gessa, C. 1999.** Phosphate adsorption and precipitation in
18 calcareous soils: the role of calcium ions in solution and carbonate minerals. *Nutrient*
19 *Cycling in Agroeco.* **53:219-227.**
- 20 **Upchurch, R.P. and Mason, D.D. 1962.** The influence of soil organic matter on the
21 phytotoxicity of herbicides. *Weeds* **10:9-14.**
- 22 **Vasudevan, D. and Cooper, E.M. 2004.** 2,4-D sorption in iron oxide-rich soils: role of
23 soil phosphate and exchangeable Al. *Environ. Sci. Technol.* **38:163-170.**
- 24 **Veeh, R.H., Inskeep, W.P. and Camper, A.K. 1996.** Soil depth and temperature effects
25 on microbial degradation of 2,4-D. *J. Environ. Qual.* **25:5-12.**
- 26 **Vencill, W.K. Ed. *Herbicide Handbook.* 8th Edition. 2002.** Weed Science Society of
27 America, Lawrence, KS, U.S.A.
- 28 **Verity, G.E. and Anderson, D.W. 1990.** Soil erosion effects on soil quality and yield.
29 *Can. J. Soil Sci.* **70:471-484.**
- 30 **Voos, G. and Groffman, P.M. 1997.** Relationships between microbial biomass and
31 dissipation of 2,4-D and dicamba in soil. *Biol. Fertil. Soils.* **24:106-110.**
- 32 **Wade, H. F., York, A. C., Morey, E., Padmore, J. M. and Rudo, K. M. 1998.** The
33 impact of pesticide use on groundwater in North Carolina. *J. Environ. Qual.* **27:1018-1026.**
- 34 **Wagenet, R.J. and Rao, P.S.C. 1985.** Basic concepts of modeling pesticide fate in the
35 crop root zone. *Weed Sci.* **33(2): 25-32.**

- 1 **Waite D.T., Grover, R. and Westcott. N.D. 1992.** Pesticides in groundwater, surface
2 water and spring runoff in a small Saskatchewan watershed. *Environ. Toxicol. Chem.*
3 **11:741-748.**
- 4 **Waite, D.T., Cessna, A.J., Grover, R., Kerr, L.A. and Snihura, A.D. 2002.**
5 Environmental concentrations of agricultural herbicides: 2,4-D and triallate. *J. Environ.*
6 **Qual. 31:129-144.**
- 7 **Waite, D.T., Grover, R., Westcott, N.D., Irvine, D.G., Kerr, L.A. and Sommerstad, H.**
8 **1995.** Atmospheric deposition of pesticides in a small southern Saskatchewan watershed.
9 *Environ. Toxicol. Chem.* **14:1171-1175.**
- 10 **Walker, A. and Bond, W. 1977.** Persistence of the herbicide AC92, 553, N-(1
11 ethylpropyl)-2,6 dinitro-3,4-xylidine in soils. *Pestic. Sci.* **8:359-365.**
- 12 **Walker, A., Bromilow, R.H., Nicholls, P.H., Evans, A.A. and Smith, V.J.R. 2002.**
13 Spatial variability in the degradation rates of isoproturon and chlorotoluron in a clay soil.
14 *Weed Res.* **42:39-44.**
- 15 **Walker, A., Jurado-Exposito, M., Bending, G.D. and Smith, V.J.R. 2001.** Spatial
16 variability in the degradation rate of isoproturon in soil. *Environ. Pollut.* **111:407-415.**
- 17 **Walker, P.H., Hall, F.F. and Protz R. 1968.** Relation between landform parameters and
18 soil properties. *Soil Sci. Soc. Am. Proc.* **32: 101-104.**
- 19 **Wall, D.A. 1996.** Effect of sublethal dosages of 2,4-D on annual broadleaf crops. *Can. J.*
20 *Plant Sci.* **76:179-185.**
- 21 **Wauchope, R.D. 1978.** The pesticide content of surface water draining from agricultural
22 fields – a review. *J. Environ. Qual.* **7:459-472.**
- 23 **Wauchope, R.D., Buttler, T.M., Hornsby, A.G., Beckers, P.W.M.A. and Burt, J.P.**
24 **1992.** The SCS/ARS/CES Pesticide properties database for environmental decision-
25 Making. *Rev. Environ. Contam. Tox.* **123:1-157.**
- 26 **Wauchope, R.D., Graney, R.L., Cryer, S.A., Eadsforth, C. and Klein, A.W. 1995.**
27 Pesticide runoff: Methods and interpretation of field studies. *Pure and Applied Chem.*
28 **67:2089-2108.**
- 29 **Wauchope, R.D., Yeh, S., Linders, B.H.J., Kloskowski, R., Tanaka, K., Rubin, B.,**
30 **Katayama, A., Kördel, W., Gerstl, Z., Lane, M. and Unsworth, J.B. 2002.** Pesticide soil
31 sorption parameters: theory, measurement, uses, limitations, and reliability. *Pest Manage.*
32 *Sci.* **58:419-445.**
- 33 **Weber, J.B., Perry, P.W. and Upchurch, R.P 1965.** The influence of temperature and
34 time on the adsorption of paraquat, diquat, 2,4-D and prometone by clays, charcoal, and
35 an anion-exchange resin. *Proc. Soil Sci. Soc. Am. J.* **29:678-688.**

- 1 **White, D.C. and Ringelberg, D.B. 1998.** Signature Lipid Biomarker Analysis. *In:*
2 Burlage, R., Atlas, R., Stahl, D., Geesey, G., Sayler, G. (Eds.), *Techniques in Microbial*
3 *Ecology*. New York Oxford, Oxford University Press, pp 255-272.
- 4 **White, R.E. and Kookana, R.S. 1998.** Measuring nutrient and pesticide movement in
5 soils: benefits for catchment management. *Aus. J. Exper. Ag.* **38**:725-743.
- 6 **Wienhold, B.J., Sadeghi, A.M. and Gish, T.J. 1993.** Effect of starch encapsulation and
7 temperature on volatilization of atrazine and alachlor. *J. Environ. Qual.* **22**:162-166.
- 8 **Willems, H.P.L., Lewis, K.J., Dyson, J.S. and Lewis, F.J. 1996.** Mineralization of 2,4-D
9 and atrazine in the unsaturated zone of a sandy loam soil. *Soil Biol. Biochem.* **28**:989-996.
- 10 **Williams D.T., LeBel, G.L. and Junkins, E. 1988.** Organohalogen residues in human
11 adipose autopsy samples from six Ontario municipalities. *J. Asso. Off Anal. Chem.*
12 **71**(2):410-414.
- 13 **Wolfe, N.L., Metwally, M.E. and Mofteh, A.E. 1989.** Hydrolytic transformations of
14 organic chemicals in the environment. In "Reactions and movement of organic chemicals
15 in soils" (Eds. BL Sawhney, K Brown) pp. 229-242. (SSSA:Madison, WI).
- 16 **Wolfe, N.L., Mingelgrin, U. and Miller, G.C. 1990.** Abiotic Transformations in Water,
17 Sediments, and Soil. In Cheng: *Pesticides in the Soil Environment: Processes, Impacts, and*
18 *Modeling*. Soil Science Society of America. Madison, Wisconsin, USA. p103-168.
- 19 **Wood, L.S., Scott, H.D., Marx, D.B. and Lavy, T.L. 1987.** Variability in sorption
20 coefficients of metolachlor on a Captina silt loam. *J. Environ. Qual.* **16**:251-256.
- 21 **Wu, J. and Nofziger, D.L. 1999.** Incorporating temperature effects on pesticide
22 degradation into a management model. *J. Environ. Qual.* **28**:92-100.
- 23 **Wu, Q., Blume, H.-P., Rexilius, L. Fölschow, M. and Schleuss, U. 2000.** Sorption of
24 atrazine, 2,4-D, nitrobenzene and pentachlorophenol by urban and industrial wastes.
25 *European J. Soil Sci.* **51**:335-344.
- 26 **WWF. 1999.** The problems with pesticides in Canada. A briefing book for
27 parliamentarians. World Wildlife Fund Canada, April 1999.
28 (<http://wwf.ca/satellite/prip/resources/briefing-book.pdf>, accessed on October 10, 2005)
- 29 **Yaron, B. 1978.** Some aspects of surface interactions of clays with organophosphorus
30 pesticides. *Soil Sci.* **125**:210-216.
- 31 **Zak, J.C., Willig, M.R., Moorhead, D.L. and Wildman, H.G. 1994.** Functional diversity
32 of microbial communities: a quantitative approach. *Soil Biol. Biochem.* **26**:1109-1115.
- 33 **Zhang, X.C., Norton, L.D. and Hickman, M. 1997.** Rain pattern and soil moisture
34 content effects on atrazine and metolachlor losses in runoff. *J. Environ. Qual.* **26**:1539-
35 1547.

9. APPENDICES

Appendix-I. Soil Properties and 2,4-D Sorption Table

Table 1. Variability of soil properties and 2,4-D sorption in soil as affected by slope position.

Slope position	Soil properties				2,4-D sorption	
	SOC (%)	pH	Clay (%)	Carbonate (%)	K _d (ml g ⁻¹)	K _{oc} (ml g ⁻¹)
Upper (n=12)						
Mean ± SD	1.1 ± 0.3 ^{a*}	6.8 ± 0.7 ^a	13.2 ± 3.1 ^{ab}	2.2 ± 2.4 ^a	1.7 ± 0.5 ^a	149.7 ± 36.7 ^a
c.v. [#]	0.26	0.11	0.23	1.1	0.3	0.24
Minimum	0.6	5.5	8	0.6	1.0	97.6
Maximum	1.6	7.6	18	7.9	2.7	220.9
Middle (n=55)						
Mean ± SD	1.8 ± 0.5 ^b	7.0 ± 0.7 ^{ab}	12.5 ± 2.7 ^a	2.6 ± 4.3 ^a	2.6 ± 0.8 ^b	152.6 ± 37.5 ^a
c.v.	0.28	0.1	0.21	1.6	0.3	0.24
Minimum	0.5	5.3	6.8	0	0.6	95.5
Maximum	2.9	7.7	18	23.9	4.3	248.7
Lower (n=29)						
Mean ± SD	2.7 ± 0.7 ^c	7.4 ± 0.2 ^b	14.7 ± 3.3 ^b	2.4 ± 3.0 ^a	3.3 ± 0.8 ^c	122.4 ± 24.2 ^b
c.v.	0.26	0.04	0.22	1.2	0.26	0.19
Minimum	1.3	6.6	8	0	1.5	77.8
Maximum	4.6	7.8	21.2	10.4	5.6	169.3
Combined (n=96)						
Mean ± SD	2.0 ± 0.7	7.1 ± 0.6	13.3 ± 3.1	2.5 ± 3.8	2.7 ± 0.9	143.1 ± 36.3
c.v.	0.38	0.09	0.23	1.48	0.33	0.25
Minimum	0.5	5.3	6.8	0.0	0.5	77.9
Maximum	4.6	7.8	21.2	23.9	5.6	248.7

[#] c.v = coefficient of variation.

* Means followed by the same letter in each column and treatment is not significantly different (P=0.05) according to Tukey's multiple mean comparison test.

1 **Appendix-II. 2,4-D Mineralization Table**

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Table 2. First-order mineralization rate constants (k), half-lives ($\frac{1}{2}$ -lives), total 2,4-D mineralized at time infinity (M_T), and coefficients of determination of the mineralization model (r^2) as determined by fitting the evolved $^{14}\text{C-CO}_2$ in each of the five surface soils to the equation: $M_t = M_T(1 - e^{-kt})$, where t = time [days]. Experimental values for total 2,4-D mineralization (Exp- M_T) are displayed for comparison with predicted values.

Slope position	k [day ⁻¹]	$\frac{1}{2}$ -lives [day]	M_T [%]	r^2	Exp- M_T [%]
Upper (n=12)	0.09±0.03 * a**	7.87±2.37* a**	34.58±3.58* a**	0.97±0.01*	36.07±3.48* a**
Middle (n=55)	0.08±0.03 a	9.34±4.14 a	34.41±2.80 a	0.97±0.01	35.70±3.08 a
Lower (n=29)	0.05±0.02 b	13.28±5.22 b	31.48±4.02 b	0.97±0.01	31.62±5.06 b
All combined (n=96)	0.08±0.03	10.34±4.73	33.55±3.55	0.97±0.01	34.51±4.25

3 *Mean of four replicates followed by standard deviation.

4 **Means followed by same letters are not significantly different at $P < 0.05$ (One-way ANOVA
5 followed by Tukey's studentized range test).

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1 Appendix- III. 2,4-D Mineralization Graphs

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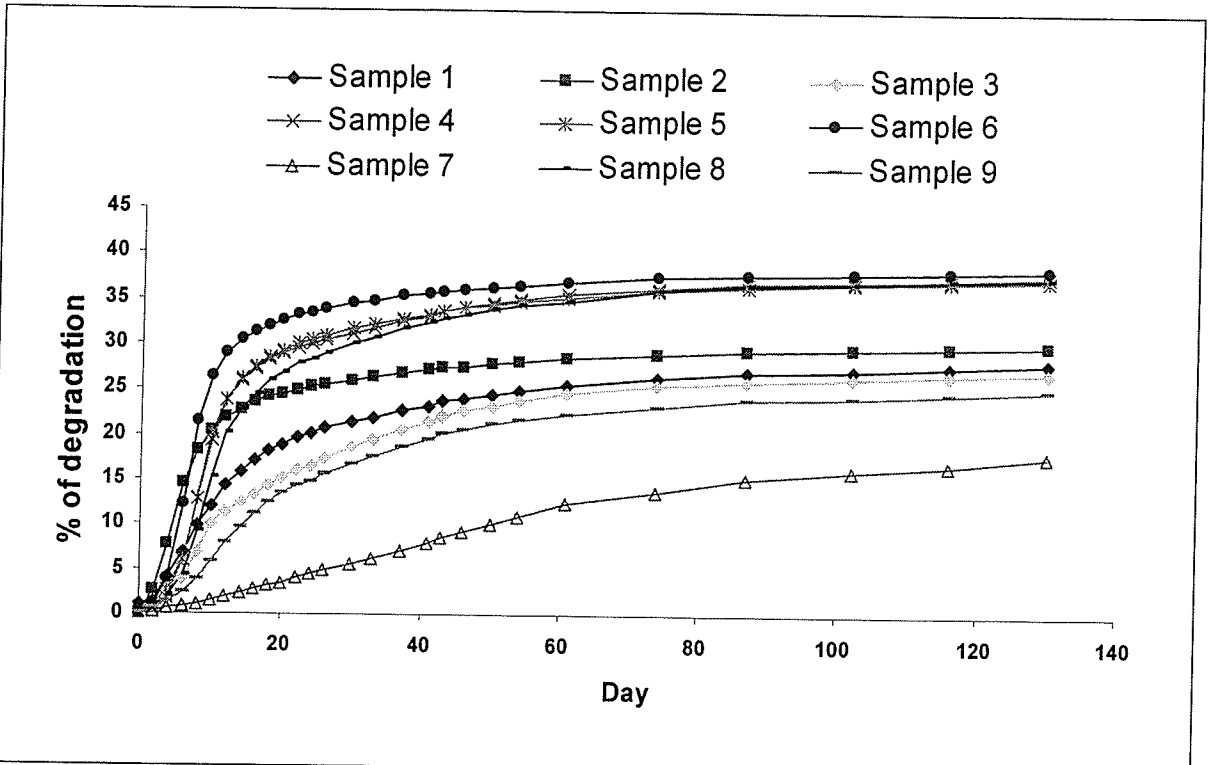


Figure 1 2,4-D mineralization (measured as %¹⁴C production) in 1st transect.

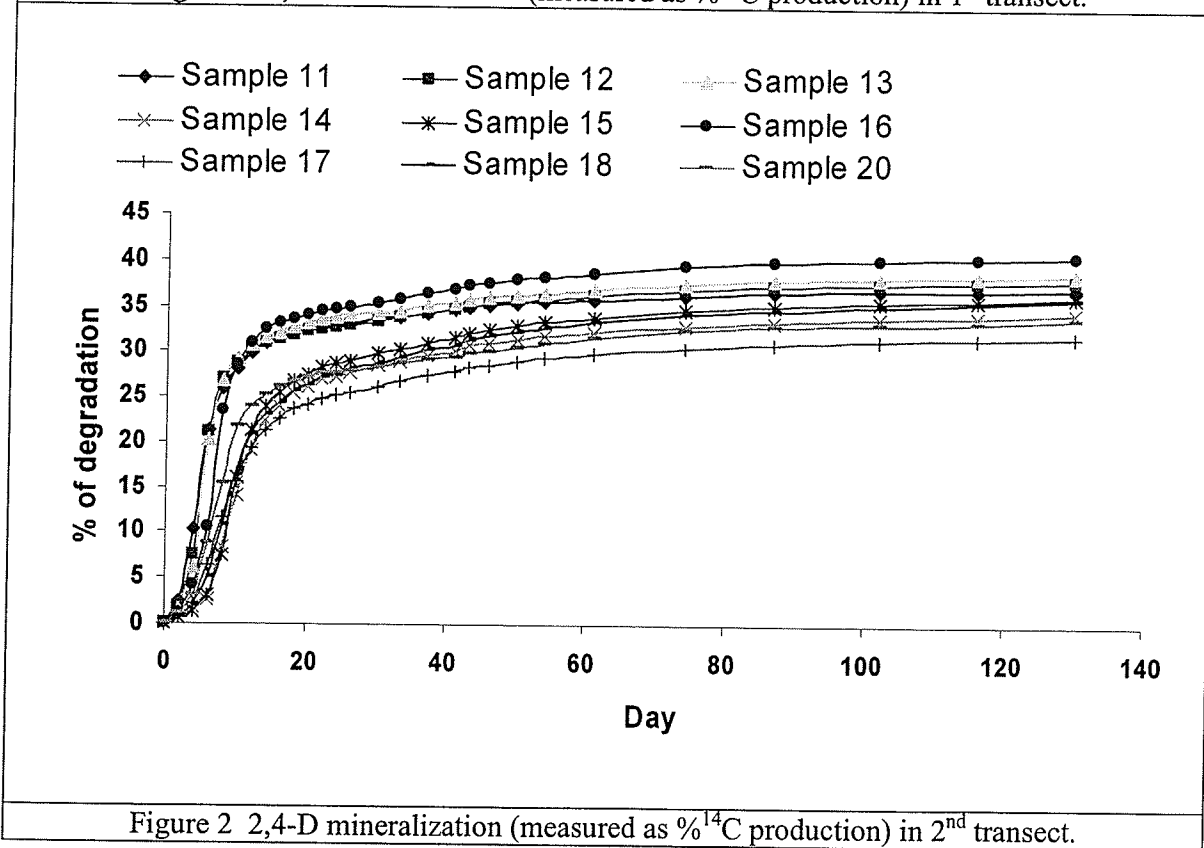


Figure 2 2,4-D mineralization (measured as %¹⁴C production) in 2nd transect.

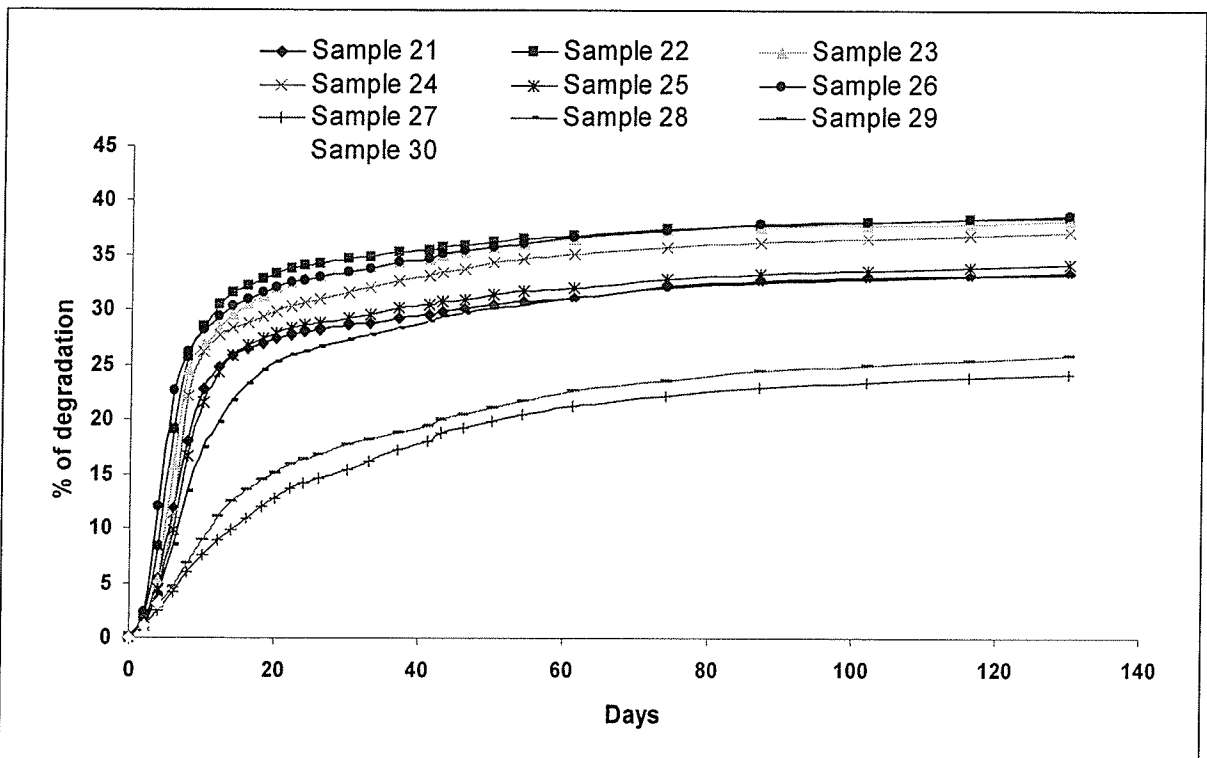


Figure 3 2,4-D mineralization (measured as %¹⁴C production) in 3rd transect.

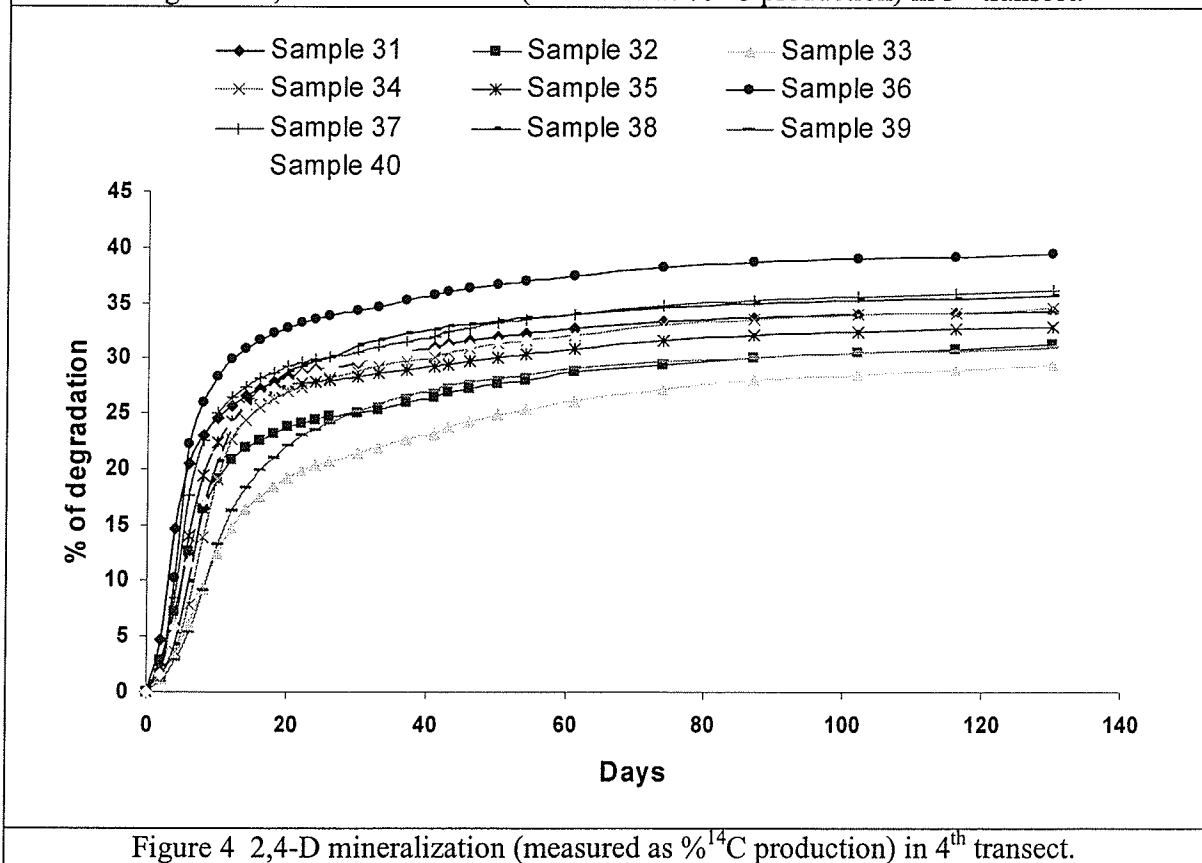


Figure 4 2,4-D mineralization (measured as %¹⁴C production) in 4th transect.

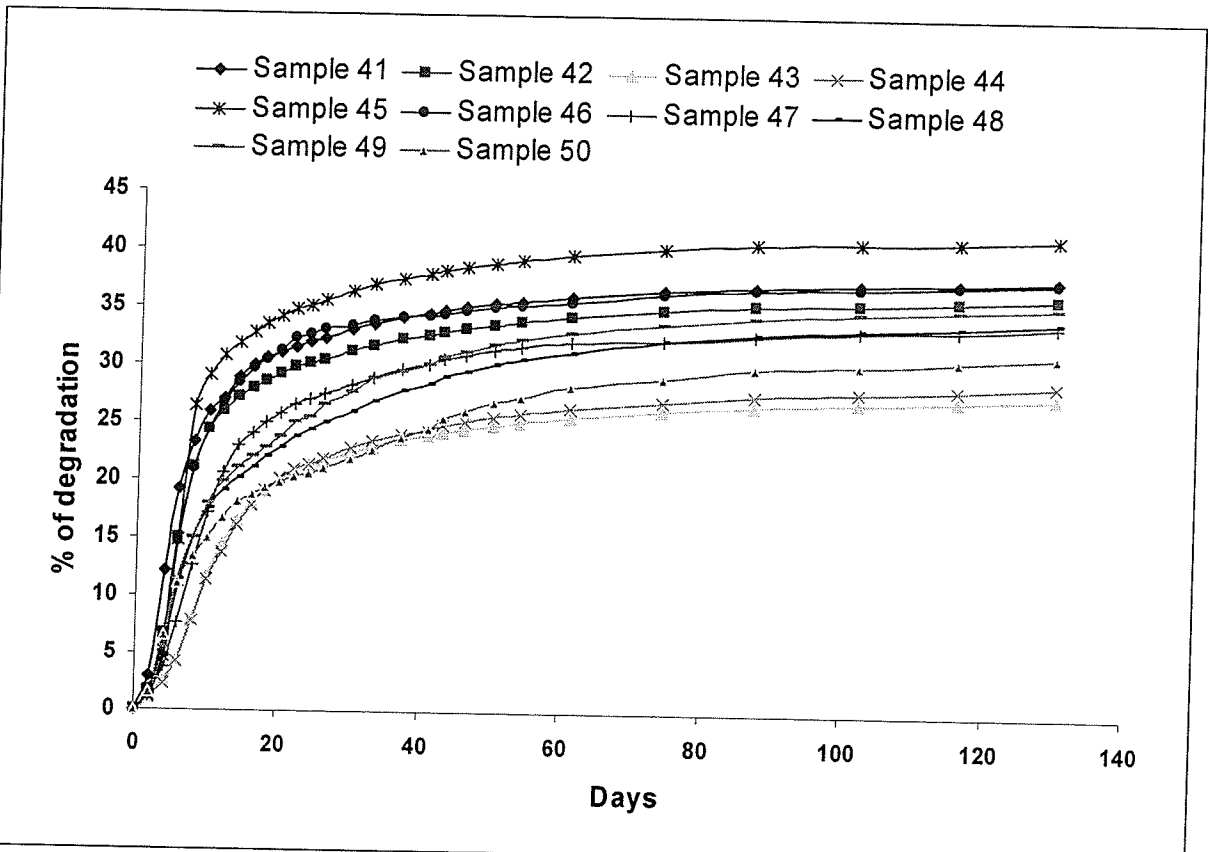


Figure 5 2,4-D mineralization (measured as %¹⁴C production) in 5th transect.

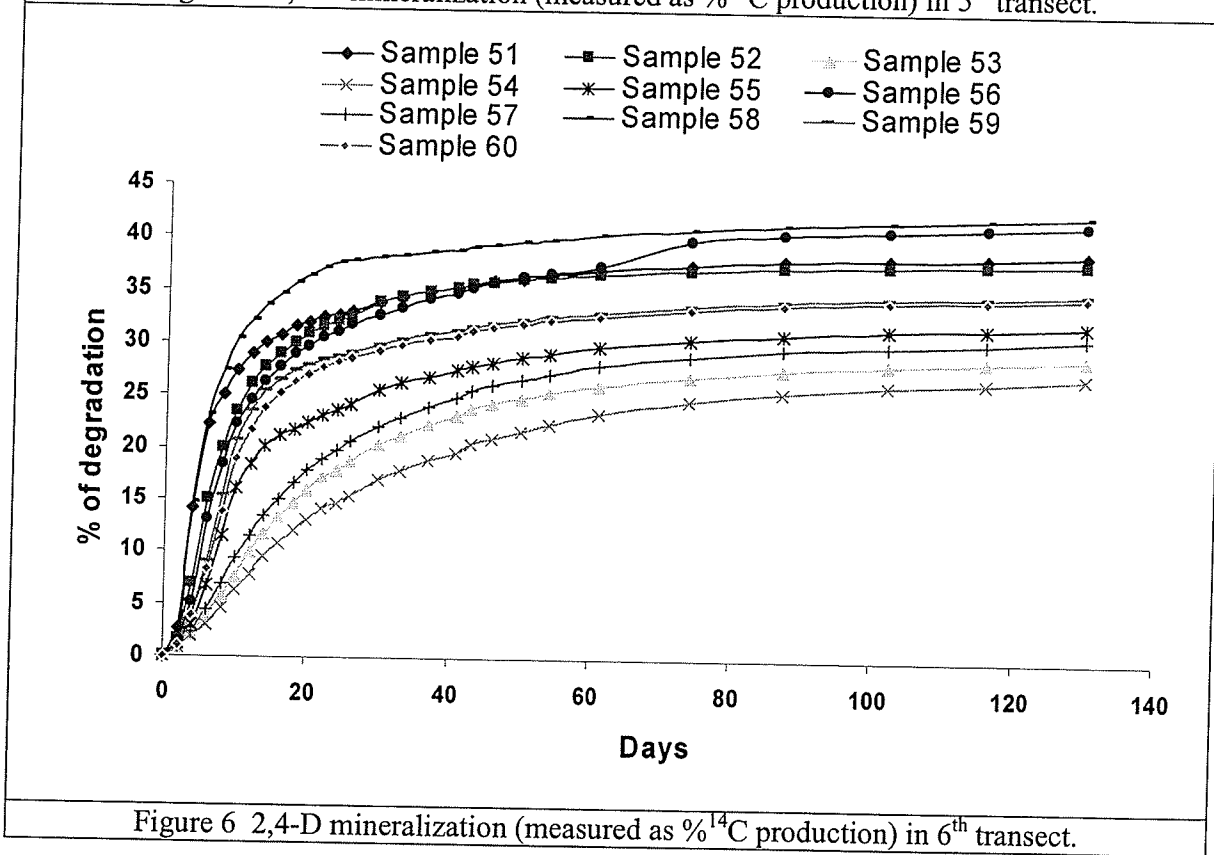


Figure 6 2,4-D mineralization (measured as %¹⁴C production) in 6th transect.

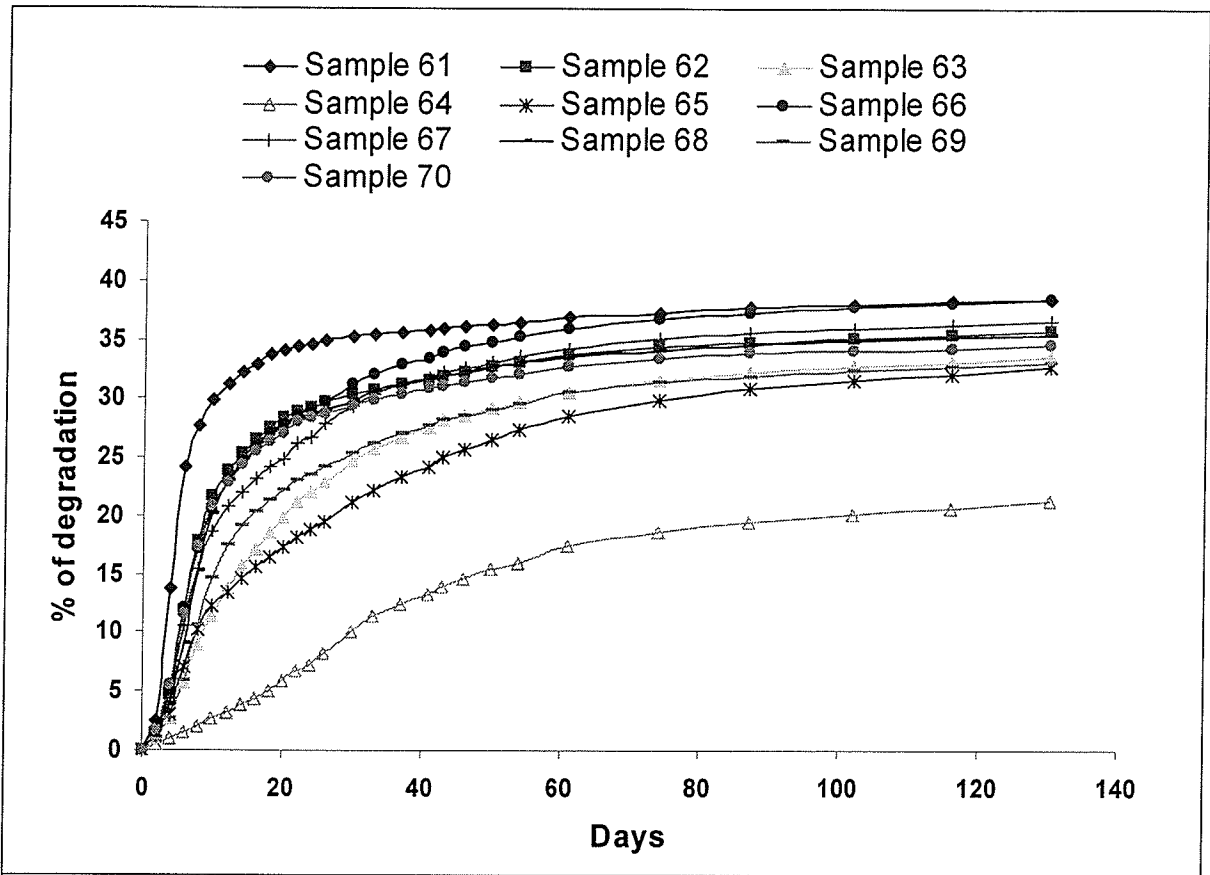


Figure 7 2,4-D mineralization (measured as %¹⁴C production) in 7th transect.

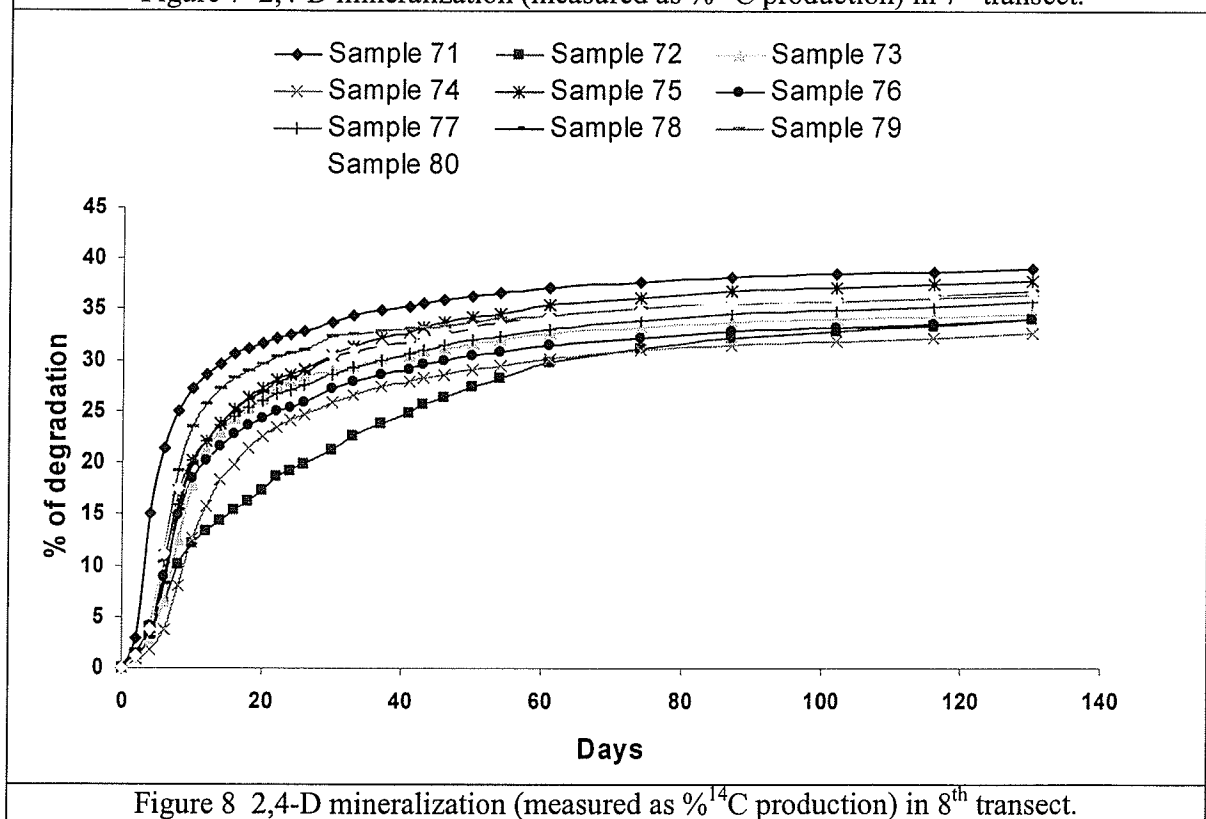


Figure 8 2,4-D mineralization (measured as %¹⁴C production) in 8th transect.

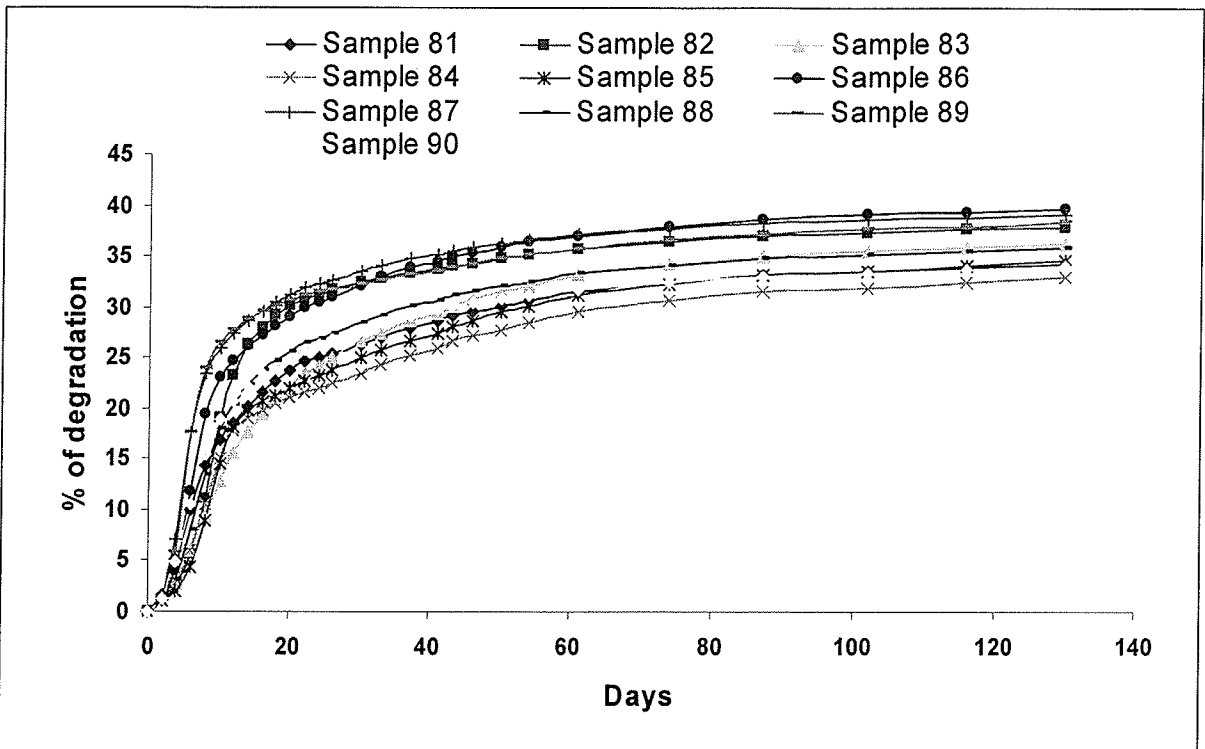


Figure 9 2,4-D mineralization (measured as %¹⁴C production) in 9th transect.

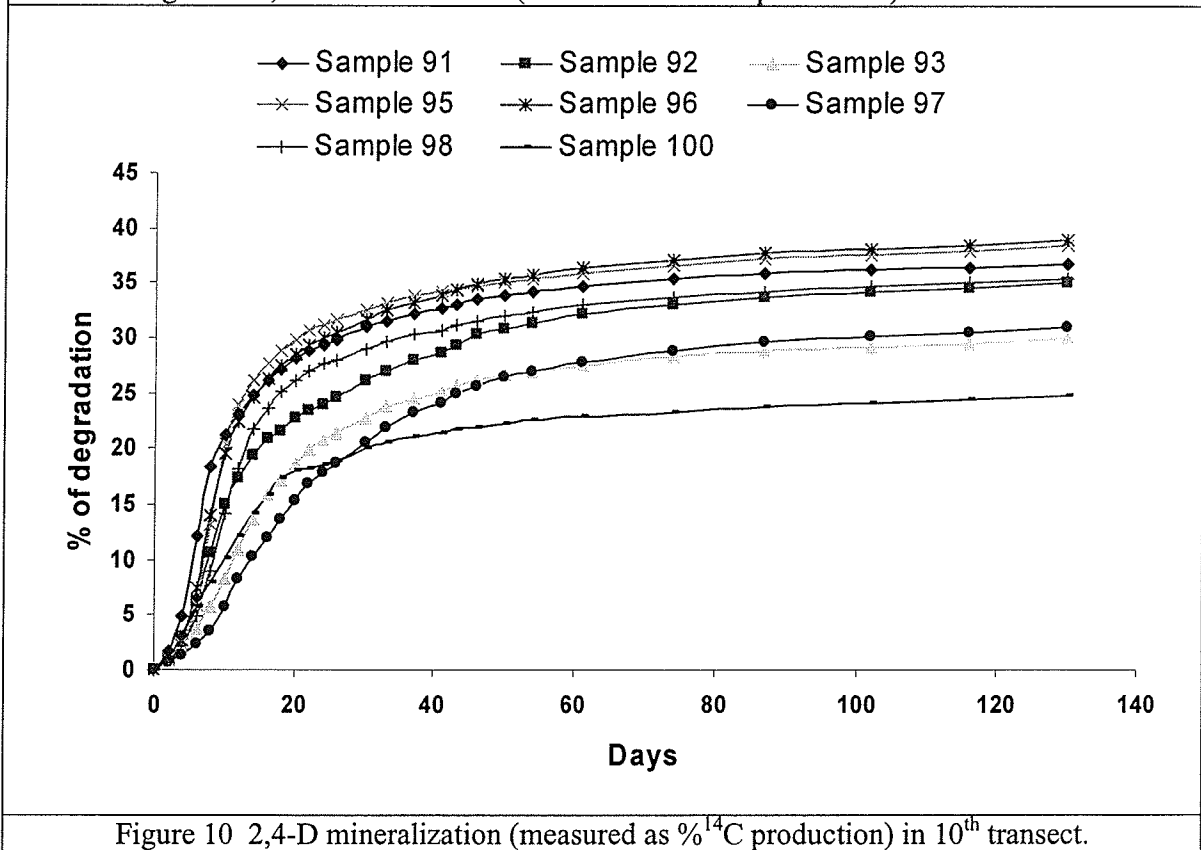


Figure 10 2,4-D mineralization (measured as %¹⁴C production) in 10th transect.

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