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The behavior of six herbicides in soil was studied under controlled laboratory conditions. Sorption of bromacil, chlorotoluron and diuron was assessed by the batch equilibration procedure over a broad range of aqueous phase sorbate concentration. Linear equilibrium sorption constants (K_p) were slightly higher at 4° C than at 25° C and many-fold higher in the organic soil than in loamy sand soil. By referencing sorption to soil organic carbon content (OC), this vast variation was reduced to less than 3-fold variation in K_{oc}. The isosteric heats of sorption were exothermic and low suggesting an entropy driven mechanism. Water solubilities (S) and octanol-water partition coefficients (K_{ow}) of the compounds were determined and used to derive equations for predicting K_{oc}. These equations are useful in estimating sorption of related pesticides and pollutants from knowing their K_{ow} and S.

Degradation of the three herbicides was followed by monitoring $^{14}CO_2$ evolution from soil. Temperature affected degradation more than did soil type or initial herbicide concentration. The degradation was tripled with a 10° rise in temperature from 25 to 35° C. The presence of herbicides did not affect the activity of soil microorganisms indicating that degradation was a co-metabolism process. Degradation fit first-order kinetics with no lag phase and with minimum half-lives of about 200 days for diuron and chlorotoluron and 2600 days for bromacil.

The binding of these three herbicides plus diquat, glyphosate and simazine with water-soluble soil organic materials (WSSOM) and soil humic acids (HA) was studied by gel filtration chromatography. The chromatographic method adapted by Hummel and Dreyer (1962) was very successful in proving the existence of binding. Noted differences in the elution behavior of herbicides were related to their physical and chemical properties and their interaction complexes.

Infrared (IR) spectroscopy showed the involvement of ion exchange reactions in diquat and H-bonding in diuron, chlorotoluron and bromacil binding with WSSOM and HA. Shifts in some characteristic IR absorption bands suggested the formation of charge transfer complexes in WSSOM and HA interactions with bromacil, chlorotoluron and diuron but not with diquat.

The results appear significant in evaluating pesticides and pollutants mobility and transport in soil and water environments.

Interaction of Selected Herbicides with Soil Constituents

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INTRODUCTION

Modern agriculture has become increasingly reliant on the use of pesticides. The use of these chemicals has been dictated by the growing worldwide demand for food and fiber. Pesticides are vitally important to increasing and protecting the quantity and quality of food commodities and in providing the society with better health and higher standard of living. However, the proliferation of chemical usage has elicited concern over the possible effect of these chemicals on the environment. The cause of this concern was the finding that many pesticides have become widespread environmental contaminants (Freed et al., 1977). Since pesticides will continue to be used in the production of food and fiber, more should be learned about their transport in the environment, the relationship of their physical-chemical properties to transport, their partitioning properties, and their persistence in the biosphere (Freed and Haque, 1975). A thorough knowledge and understanding of the soil processes affecting pesticides is imperative, if methods for controlling pesticide persistence and minimizing their undesirable effects are to be devised (Kaufman, 1974).

The herbicides selected for this study were chosen because they represent major herbicide groups and are widely used on agricultural crops. The structural formulae of the herbicides are shown in Fig. 1. The information presented below on the use and application rates of the herbicides was extracted from the Herbicide Handbook, WSSA (1983) except where indicated. Bromacil (5-bromo-3-sec-butyl-6-methyluracil) is a substituted uracil herbicide. It is a general non-selective weed killer in non-agricultural areas where bare ground is desired. At lower application rates (1.8 to 7.2 kg/ha) bromacil is used for selective control of annual and perennial weeds in certain deep-rooted crops such as pineapple, alfalfa and citrus. Both diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea] and chlorotoluron [3-(3-chloro-4-methylphenyl)-1,1-dimethylurea] are substituted urea herbicides. At low rates (0.7 to 7.2 kg/ha) diuron is used as a selective herbicide for the control of germinating broadleaf and grass weeds in crops such as cotton, grapes, apples, pears, and citrus. At higher rates (4.5 to 18 kg/ha) diuron is a general weed killer. Combinations of bromacil and diuron are used to control weeds in citrus and on non-cropland areas. Chlorotoluron is effective both as a residual soil-acting herbicide and as a contact foliar-applied against annual grass and many broad-leaved weeds of cereal crops. It is used at 1.5 to 3 kg/ha on winter cereals before or after emergence of crop (Farm Chemicals Handbook, 1979; Worthing, 1983). Diquat (1,1-ethylene-2, 2-bipyridylium ion) is completely soluble in water and rapidly absorbed by green plants. Diquat is used as a non-crop weed killer, a general aquatic herbicide and as a preharvest top killer or desiccant of seed crops. Glyphosate [N-(phosphonomethyl) glycine] is very soluble in water. It is foliar-applied herbicide, relatively non-selective, and very effective on deep-rooted perennial species and on annual and biennial species of grasses and broad-leaved weeds. Simazine [2-chloro-4,6-bis-(ethylamino)-s-triazine] is a member of the s-triazine group of herbicides. It is widely used as a selective herbicide for control of broadleaf and grass weeds in corn, established alfalfa, citrus, olives and deciduous fruits and nuts. Simazine is also used for selective control of algae and submerged weeds in ponds. At higher application rates (>4.4 kg/ha) simazine is used as a non-selective herbicide for vegetation control in non-agricultural areas.

The present extensive use of these herbicides has resulted in a great deal of interest in their fate and behavior in soil. A literature review pertaining to the subject matter covered is presented in the appropriate chapters of this treatise. Although diuron and diquat have been quite extensively studied, very little is known about the dynamics of chlorotoluron and glyphosate in the soil environment. The published data on all of these herbicides were determined using different methodologies and dissimilar soils.

The objectives of this study were:

(i) To assess sorption of bromacil, chlorotoluron and diuron by soils for concentrations approaching their aqueous solubilities.

(ii) To determine the value and reliability of the octanol-water partition coefficient and water solubility of herbicides in predicting sorption.

(iii) To establish the use of gel filtration chromatography for studying the binding of herbicides with water-soluble soil organic materials (WSSOM) and humic acids (HA) extracted from soil.

(iv) To characterize the binding mechanisms the herbicides undergo with WSSOM and HA using infrared spectroscopy.

(v) To assess the kinetics of degradation and estimate the half-lives of diuron, chlorotoluron and bromacil in soil.

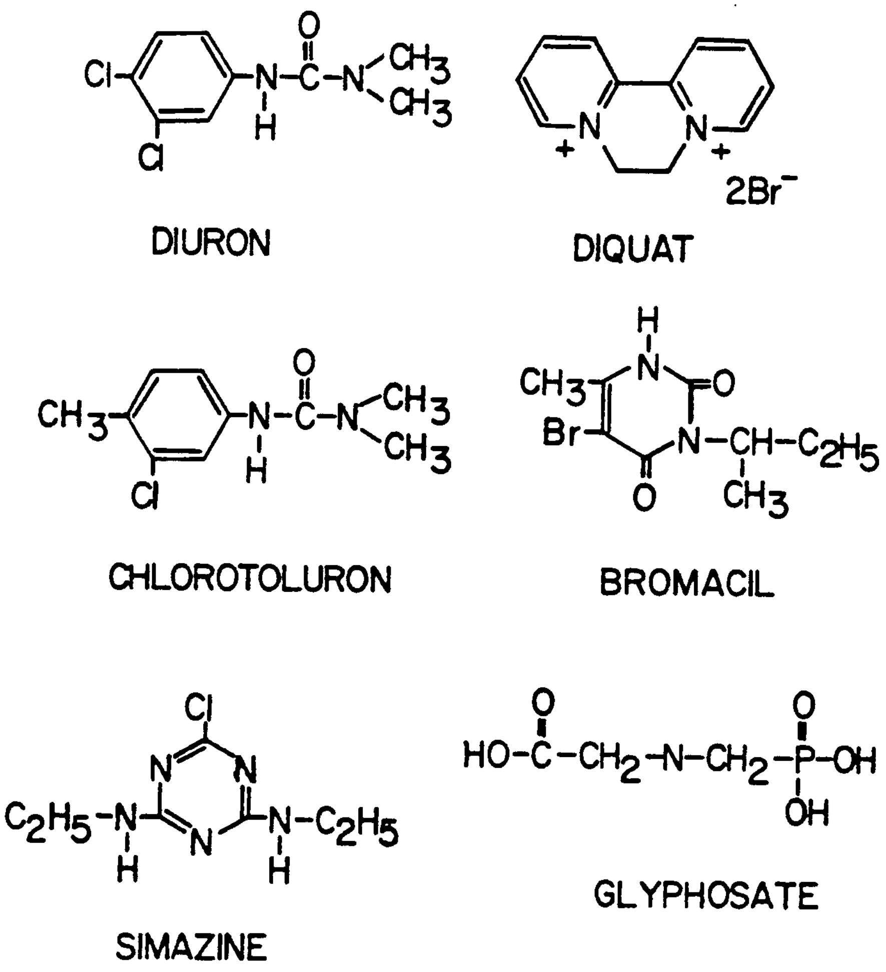

Figure 1. Structural formulae of the herbicides bromacil, diuron, chlorotoluron, diquat, glyphosate and simazine.

CHAPTER I

Sorption of Bromacil, Chlorotoluron, and Diuron by Soils[1]

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ABSTRACT

Sorption of bromacil (5-bromo-3-Sec-butyl-6-methyluracil), chlorotoluron [3-(3-chloro-4-methylphenyl)1,1-dimethylurea] and diuron [3-(3,4-dichlorophenyl)1,1-dimethylurea] by soils of greatly different organic carbon content from aqueous solution was studied. The sorptive capacity of the two soils was assessed by the batch equilibrium procedure over a broad range of aqueous phase herbicide concentrations at two temperatures, 4 and 25° C. Sorption isotherms exhibited good fit to both the Freundlich adsorption equation and the modified Freundlich equation ($n = 1$). Although the highly significant ($r \geqslant 0.992$) correlation coefficients from least-squares-fit appeared to reinforce a common misinterpretation of linear relationships, the sorption isotherms for nearly all the soil-herbicide combinations showed some dependence on concentration thereby demonstrating their nonlinear nature.

The sorption coefficients (K_p) of chlorotoluron were over 10 times higher than those of bromacil but less than one-half the corresponding K_p values of diuron. The K_p values were slightly higher at 4° C than at 25° C and 40-200-fold higher for the organic soil than for the loamy sand soil, hence reflecting sorption dependence on soil organic carbon. By referencing sorption to soil organic carbon, this vast variation in K_p was reduced to less than three-fold variation in K_{oc}. The isosteric heats (ΔH_s) of sorption as a function of amount of chemical sorbed were exothermic and relatively low suggesting the involvement of entropy driven low energy requiring mechanism. Experimental values of the water solubility (S) and octanol-water partition coefficients (K_{ow}) were determined at 4, 25, and 40° C, for the three compounds. Sorption constants (K_{oc}) estimated from the octanol-water partition coefficients were in better agreement with the measured K_{oc}'s, than their counterparts predicted from aqueous solubilities of the compounds. A fit of the experimentally determined K_{ow} and K_{oc} data for bromacil, chlorotoluron and diuron yielded a highly significant positive relationship ($r = 0.999$). A highly significant negative correlation ($r = -0.995$) was observed between water solubilities and K_{oc} values for these compounds. These relationships should be useful in predicting sorption (K_{oc}) of related (neutral) compounds from aqueous solution by soils when experimentally precise values are not required. The advantage of these correlations is that sorption of many organic pesticides and pollutants can be estimated from easily determined (available) sorbate parameters, namely, octanol-water partition coefficient (K_{ow}) or water solubility (S) of the chemical in question.

Additional Index Words: Aqueous solubility, octanol-water partition coefficient.

INTRODUCTION

The phenomenon of pesticide sorption in soil has been invoked by many investigators to explain differences in the biological activity of a pesticide in different soils. When a herbicide is applied to soil a major part of it comes in contact with soil colloids. Although sorption may occur from vapor, sorption from solution is the most significant in a soil-herbicide system. Sorption has the effect of reducing the concentration of chemical in solution. Consequently any concentration-dependent process is affected (Osgerby, 1973). In other words, sorption determines the chemical's ultimate fate in soil. Sorption is one of the major factors affecting the chemical persistence, biological activity and mobility in soil (Bailey et al., 1968; Haque and Freed, 1974; and Osgerby, 1973). This explains why this phenomenon has been given great attention as reflected in the large body of literature published in the last two decades (Banwart et al., 1982; Haque and Cashow, 1971; McCall et al., 1980; Hance, 1965b; Hamaker and Thompson, 1972; and Dao et al., 1982). These studies have been for the most part descriptive in nature, and as Wauchope and Koskinen (1983) pointed out, little new theory has been added in the last decade or so despite the enormous number of experiments conducted. This is due in most part to the variability and complexity of soils and the existence of several hundred different herbicide chemicals.

Equilibrium sorption is usually described by an equation known as isotherm. An isotherm is measured at constant temperature and represents a relation between the amount of chemical sorbed and the chemical left in the solution at equilibrium. Sorption data are usually expressed in the form of a constant such as the Freundlich adsorption constant (K_f) or the simple proportionality constant (K_p) which represent the extent of sorption. This constant is a characteristic of the particular soil-herbicide combination and is related to the free energy changes of sorption (Haque and Freed, 1974).

Two of the major factors known to influence sorption are soil properties and the molecular characteristics of the compound under consideration (Bailey and White, 1964). Although the effect of a number of soil properties including clay type and content, pH, and cation exchange capacity have been demonstrated (Bailey et al., 1968; Lie et al., 1970; Mustafa and Gramar, 1972), sorption of neutral organic pesticides has been found in many studies to depend primarily on the organic carbon content of the soil (Kozak and Weber, 1983; Nkedi-Kizza et al., 1983; Gerstl and Yaron, 1983; Grover, 1975; Hance, 1965; Peck et al., 1980; Hilton and Yuen, 1963; Hassett et al., 1980; Means et al., 1980). The minor role displayed by the mineral fraction is presumably due to its strong dipole interactions with the highly polar water, which precludes an effective association of neutral organic compounds with these mineral components of the soil (Chiou, 1981). Lambert (1968) discussed sorption equilibria in terms of interaction with soil organic carbon. This work along with that of Hamaker and Thompson (1972) has produced a sorption constant (K_{oc}) based on the soil organic carbon content instead of the total soil mass. The approach used in putting sorption on a uniform organic carbon generated a unique constant (K_{oc}) which appeared dependent on the properties of the compound being studied but was unaffected by the makeup of soil organic materials and was independent of soil origin or type (Lambert, 1968; Chiou et al., 1979; Osgerby, 1973; Hamaker and Thompson, 1972). The approach permitted estimation of sorption with respect to a single soil property, i.e., organic carbon content.

Numerous investigators have shown that sorption (K_{oc}) of neutral organic pesticides can be related systematically to a number of sorbate properties including water solubility, parachor, and organic-aqueous (e.g. octanol-water) partition coefficient. The parachor was the first sorbate parameter correlated with K_{oc} (Lambert, 1967). The rationale behind the use of parachor stems from its being an approximate measure of the molecular volume of the chemical. Hance (1969) modified Lambert's relationship in order to take into account the influence of solute-solvent hydrogen-bonding. These combined efforts marked the first step towards establishing a quantitative relationship between soil sorption and chemical structure. Lambert (1968) also suggested that the role of soil organic materials was similar to that of an organic solvent and that partitioning of a neutral organic compound between soil organics and water should correlate well with its partitioning between water and an organic solvent. Briggs (1973) had successfully derived a relationship relating sorption in four soils of 30 nonionic chemicals to the octanol-water partition coefficient (K_{ow}). The term partition coefficient expresses the equilibrium concentration ratio of an organic chemical partitioned between an organic liquid (e.g. octanol) and water (Chiou et al., 1977). More recently, a number of workers (Brown and Flagg, 1981; Felsot and Dahm, 1979; Karickoff et al., 1979) reported highly correlated semi-empirical equations associating sorption of many pesticides from water to the octanol-water partition coefficients. The significance of these relationships is that for certain organic compounds (nonionic herbicides) the distribution coefficients which described sorption equilibria are predictable from a simple liquid-liquid partitioning experiment. Water solubility of an organic solute can be regarded as partition of the chemical between itself and water (Briggs, 1981). Thus, one would anticipate a correlation between partition coefficient and water solubility (S). Chiou et al. (1977) have shown such a correlation between log K_{ow} and log S. Later, Chiou et al. (1979) reported a similar relationship between sorption (K_{oc}) of many organic chemicals and aqueous solubility of the sorbate. Empirical equations similar to the one developed by Chiou et al. (1979) were reported (Briggs, 1981; Mingelgrin and Gerst, 1983; Means et al., 1980; Karickoff et al, 1979; Felsot and Dahm, 1979) which permitted estimation of a nonionic compound's K_{oc} from its water solubility. The equations relating K_{oc} and aqueous solubility are sometimes modified by correcting for the crystal energy contributions of solid compounds (Briggs, 1981; Karickoff, 1981).

Sorption of herbicides from water onto soil is in most instances an exothermic process. A decrease in temperature is usually associated with an increase in sorption (Freed et al., 1977). In general, for neutral organic compounds, the isosteric heats of sorption (ΔH_s), which measure the strength of sorption are relatively low, and increased sorption is not associated with comparable increases in ΔH_s (Chiou et al., 1979). This one type of interaction has been considered to be an entropy driven process and refered to as hydrophobic or nonpolar sorption (Hamaker and Thompson, 1972; Hassett et al., 1980).

The objectives of this study were: (i) to examine the effect of soil organic carbon content on the sorption of bromacil, chlorotoluron, and diuron at concentrations approaching aqueous solubilities of those compounds; (ii) to study the dependence of sorption on temperature; and (iii) to evaluate the value and reliability of the octanol-water partition coefficient and water solubility parameters in predicting sorption of these hydrophobic herbicides.

MATERIALS AND METHODS

Two soil types differing greatly in organic carbon content were obtained from the upper (0-20 cm) layer of the soil profile from two locations in the state of Oregon. Adkins loamy sand (coarse loamy, mixed, mesic, Xerollic Camborthid) was collected from the dry region of eastern Oregon; whereas, Semiahmoo mucky peat (Euic, mesic, Typic Medisaprist) was collected from the wet area of western Oregon. The soil samples were air-dried, and ground to pass through a 60 mesh (0.246 mm) screen. Selected properties of the two soils are given in Table 1.

Analytical grade and 2-^{14}C-Carbonyl labeled bromacil and ^{14}C-labeled diuron were supplied by E. I. de Pont de Nemours and Co. The ^{14}C-labeled at the carbonyl carbon and analytical grade unlabeled chlorotoluron were provided by Ciba-Geigy Corp. Bromacil, diuron, and chlorotoluron were more than 99% analytically and radiochemically pure and their specific activities were 3.97, 4.22, and 30.6 μCi/mg, respectively. The purity of the analytical grade samples was confirmed with thin layer chromatography, whereas purity of the ^{14}C-labeled materials was determined with a Packard Model 7200, Radiochromatogram Scanner. Consequently, the ^{14}C-labeled and the analytical grade unlabeled herbicides were used without any further purification.

Herbicide sorption on the two soils was determined using the batch equilibrium procedure with a 1:5 soil to solution ratio. Solutions of ^{14}C-labeled and analytical grade unlabeled compounds were used to obtain desired herbicide concentrations. Aqueous solutions, containing herbicide concentrations of 2, 5, 20, 50, and 100 μ_M_ were prepared for all three herbicides. Additional concentrations were: six for bromacil at 500, 750, 1000, 2000, 2500, and 3000 (780 ppm) μ<u>M</u>; four for chlorotoluron at 150, 200, 250, and 300 (64 ppm) μ<u>M</u>; and three for diuron at 75, 125, and 150 (35 ppm) μ<u>M</u>. Concentrations were chosed such that the lowest level for each herbicide was below the usual recommended field rate of application and the upper level was slightly under the aqueous solubility limit (at 25° C) of the chemical. Five milliliters of herbicide solution were added to 1 g of soil in 50-mL Corex glass centrifuge tubes. The tubes were subsequently screw-capped with Teflon-coated caps and shaken on a mechanical shaker for 24 h at 25° C or at 4° C in the later case the shaker was placed in a cold room maintained at 4 ± 1° C. At the end of the 24 h equilibration period, the samples were centrifuged at 755 RCF for 30 min in a Sorvall superspeed RC2-B refrigerated centrifuge set at the appropriate temperature. Following centrifugation the clear supernatant was removed and a 1 mL aliquat was transfered into glass scintillation vials where 10 mL of Handifluor Liquid Scintillation Counting Fluid (Mallinckvodt, Inc.) were added. Equilibrium concentrations of the herbicides were determined by measuring the radioactivity in solution (Packard Model 3330 Tri-Carb Liquid Scintillation Spectrometer). Since the counting efficiency of the machine changes with color change and other factors, an internal ^{14}C-labeled standard was counted in each experiment to correct for any variation in counting efficiency. The amount of herbicide sorbed by soil was determined by subtracting the concentration of the compound after equilibration with the soil from the initial herbicide concentration. Triplicate samples were measured for each initial concentration; untreated soil samples were included as blanks.

The octanol-water partition coefficients (K_{ow}) of the three herbicides were determined following the method described by Chiou et al. (1977) with some modifications. Known quantities of ^{14}C-labeled compounds were dissolved in 5 mL of high purity n-octanol in a 50 mL, screw-capped Corex centrifuge tubes. When solution was achieved, 15 mL of distilled water was added to make the total volume in each tube 20 mL. The tubes were allowed to equilibrate by shaking on a mechanical shaker for 2.5 h. The phases were separated by centrifugation at 1085 RCF for 30 min. The octanol phase was removed and sampled for liquid scintillation counting. After complete removal of the water-octanol interface, the water phase was also sampled and analyzed with liquid scintillation spectrometer. The K_{ow} of each herbicide was calculated as follows:

$$K_{ow} = \frac{C_o}{C_w}$$

where C_o refers to the concentration of herbicide in the octanol phase and C_w refers to the concentration in the aqueous (water) phase at equilibrium. The octanol-water partition coefficients were determined in quadruplicate at 4, 25, and 40° C.

The aqueous solubilities of bromacil, diuron and chlorotoluron were determined in quadruplicate at 4, 25, and 40° C. The method described by Hurle and Freed (1972) which consisted of equilibrating a quantity of the compound with water and measuring the concentration at the end of the equilibration period was followed with few modifications. An excess of compound (^{14}C-labeled) was added to 20 mL of distilled water in screw-capped centrifuge tubes. The solutions were equilibrated for 24 h at the appropriate temperature as follows: in temperature controlled water-bath shaker set at 40° C; on a shaker at room temperature 25° C; and on a shaker at 4° C in a temperature controlled cold room. At the end of the equilibration period, the samples were centrifuged at 1085RCF for 1 h and aliquots were removed and analyzed by measuring the radioactivity in liquid scintillation spectrometer. The solubility of herbicide in water was therefore established by measuring the solute concentration in solution after equilibration. It should be noted that not only were equilibration and centrifugation conducted at the designated temperature but also the removal of aliquot samples from the tubes was performed at the same temperature.

RESULTS AND DISCUSSION

Sorption Isotherms

Equilibrium sorption is described graphically by a sorption isotherm, which is a plot of herbicide concentration in the sorbed phase versus its concentration in solution. Representative isotherms obtained using the method of least squares fitting of the sorption data are shown in Fig. 1. Sorption of the three herbicides was well expressed with the simple proportionality relationship:

$$C_s = K_p C_e \qquad (1)$$

as described by Hamaker and Thompson (1972), where C_s is the amount of herbicide sorbed per unit mass of soil in μmol/kg, C_e is the solution concentration after equilibration in μ<u>M</u>, and K_p is the linear partition (sorption) constant. The values of K_p, as determined from the slopes of the isotherms, along with the corresponding correlation coefficients (r) for each soil-herbicide combination are given in Table 2. All regression lines generated had a coefficient of determination (r^2) of 0.986 or higher, which indicated excellent fit of the data by this simple relationship, eq. (1). The fact that the correlation coefficients are all highly significant ($r \geqslant 0.944$) must be carefully interpreted, since the only safe conclusion one can make from this finding is that a linear trend <u>may</u> exist between C_s and C_e (McClave and Dietrich, 1979). These results do suggest such a linear trend. However, graphical representation of the data reveal that curvilinearity exists in all soil-herbicide combinations studied (Figs. 2-6). These curved sorption patterns were not surprising since high herbicide concentrations were included. Because soil is such a heterogeneous sorbent, nonlinear isotherms are usually obtained even when relatively very low herbicide concentrations were used (Wauchope and Koskinen, 1983). The upward bent in the isotherms of diuron and chlorotoluron on Adkins loamy sand at 4° C (Fig. 5 and 6) occurred when relatively very high solute (higher than water solubility) concentrations were equilibrated with the soil. For the peat soil, with its very high sorptive capacity, the amount of sorbate left in solution after equilibration was much less than the aqueous solubility (x-axis, Figs. 1 to 4), hence no abnormal curving observed even at 4° C. The sandy soil on the other hand sorbed only a small fraction of the total herbicide concentration applied, consequently, concentrations in excess of the solute water solubility remained in the equilibrium solution (x-axis, Figs. 5 and 6). This unusual upward deflection in the isotherms demonstrates the effect of temperature on the aqueous solubilities of organic solutes, and strongly suggests the presence of a crystalline phase in the equilibrium solution rather than increased sorption. Similar deviations (experimental errors) were noted by Karickoff et al. (1979) for the sorption of the hydrophobic compound pyrene on natural sediments.

The dependence of K_p, the sorption constant, on herbicide concentration can be assessed from equation (1). The calculated K_p ($K_p = C_s/C_e$) values, given in Table 3 for each herbicide concentration studied, show a rather consistent decrease in K_p as the concentration applied increased. These data further demonstrate the nonlinear nature of sorption and suggest that K_p may be considered constant for a narrow concentration range only. The data also show that the amount of herbicide sorbed by soil continued to increase with each increase in solute concentration but at a decreasing rate.

An examination of the data revealed that the results can be well described in terms of the empirical Freundlich equation, which may be written in the logarithmic form as follows:

$$\log C_s = \log K_f + n \log C_e \qquad (2)$$

where K_f and n are constants representing the intercept and the slope of the isotherm, respectively. It is important to note that Freundlich adsorption equation is equivalent to equation (1) when the exponent n is equal to one. Representative Freundlich isotherms obtained are shown in Fig. 7. Freundlich constants, K_f and n and the corresponding r values (Table 2) were determined using the method of least squares fit. The high correlation ($r \geqslant 0.993$) reflects the fit of the data to the Freundlich adsorption equation. The Freundlich constants K_f and n provide rough estimates of the sorbent capacity and the nature of sorption, respectively (Adamson, 1976: page 389). The n values were nearly constant (0.85 - 0.97) and all were less than one which again indicate the nonlinear nature of the isotherms (Hamaker and Thompson, 1972; Rao and Davidson, 1979). Isotherms where $n < 1$ were attributed to sorbents having higher affinity for the herbicide than the solvent, water in this case (Freed and Haque, 1973). The intercept, K_f, of Freundlich isotherm provides some indication regarding the extent of sorption and it is valuable for comparing sorption of different kinds of sorbates on various sorbents. It is important to emphasize, however, that K_f is a unit quantity estimated for a certain range of solute concentration (Bowman, 1982). Therefore, K_f should not be used before knowing the units of determination and the range of concentration. The K_f values of a given herbicide are similar in value to the linear sorption constants (K_p) of the respective soil and temperature (Table 2).

Both show clearly the effect of herbicide nature, soil properties and temperature on the process of sorption. The linear sorption constants are used here-in-below instead of Freundlich constants as the variation in sorption is expressed in one constant rather than two constants (K_f, n).

Sorption Dependence on Soil Organic Carbon

Inspection of the data in Table 2 shows that for a given chemical, the average sorption constant of the organic soil is manyfold that in the loamy sand soil. This great difference between the two soils is, of course, an expression of the high positive correlation between sorption and the soil organic carbon content that has been reported by many investigators (Felsot and Dahm, 1979; Khan et al., 1979; Gerstl and Yaron, 1983; Means et al., 1980; BanWart et al., 1982; Zierath et al., 1980; Karichoff et al., 1979; Hassett et al., 1980; Grover, 1975; Liu et al., 1970; Hilton and Yuen, 1963; Hance, 1965). Hamaker and Thompson (1972) introduced a relationship for calculating sorption constants based on soil organic carbon content instead of the total soil mass. This relationship is expressed by dividing the sorption coefficients, K_p, by their respective organic carbon (OC) contents:

$$K_{oc} = K_p/(\%\ OC/100) \qquad (3)$$

The K_{oc} values contained in Table 2 were calculated from equation (3). It is obvious that the K_{oc} values for a given herbicide are much less variable among the two soils than are the corresponding constants expressed on a total soil weight basis. In each case, a 40 to 200-fold variation in K_p was reduced to less than three-fold variation in K_{oc} by referencing sorption to organic carbon. These findings are in good agreement with the data reported by Karickoff (1981), Rao and Davidson (1979).

Thus putting sorption data on a uniform organic carbon basis has the effect of yielding nearly constant K_{oc} independent of soil origin or soil type (Lambert, 1968). Viewing sorption in this way incorporates two general assumptions. The first assumption is that all soil organic carbon is equally effective in sorbing herbicides, and the second, is that mineral components in the soil are not significantly active in the sorption process. The first assumption is not always correct (Mingelgrin and Gerstl, 1983) and as Hamaker and Thompson (1972) noted that variability in K_{oc} values from soil to soil may be due to variations in the composition of soil organic materials. In spite of the highly positive correlations with organic carbon content, contributions to the total sorption could come from soil mineral fractions. It has been shown by a number of workers (El-Madhun, 1979; Felsot and Dahm, 1979; Hilton and Yuen, 1963) that there is a significant sorption of many neutral compounds after soil organic carbon has been removed by wet oxidation or ignition. For all practical purposes, however, the two assumptions seem to hold fairly well for the sorption of nonionic organic pesticides from water by soils used in this study. Sorption coefficients (K_{oc}) normalized on organic carbon basis are better suited for comparison purposes than their counterparts (K_p). The K_{oc} values obtained in this study for the three herbicides compare well with the published data put on organic carbon basis Liu et al., 1970; Grover, 1975; Hance, 1976; Rhodes et al., 1970; McCall et al., 1980; Hance, 1965; Nkedi-Kizza et al., 1983; Majka and Lavy, 1977).

Effect of Temperature

The effect of temperature on the sorption of bromacil, chlorotoluron, and diuron on both soils studied is shown in Figs. 1 through 7 and in Table 2. An increase in temperature from 4° C to 25° C resulted in a decrease in K_{oc} values. The decreases however are rather small. Similar results were reported on the sorption of diuron, bromacil and related compounds (Haque and Coshow, 1971; Liu et al., 1970; Hurle and Freed, 1972; Peck et al., 1980). From the sorption data at 4 and 25° C the isosteric heat of adsorption, ΔH_s, was calculated using the expression described by Haque and Coshow (1971).

$$\Delta H_s = R\ (\partial \ln Ce/\partial\ 1/T)_{C_s} \qquad (4)$$

where C_e is the equilibrium concentration of the herbicide at a fixed amount of herbicide sorbed (C_s), T is the absolute temperature, and R is the gas constant. The ΔH_s values of chlorotoluron estimated at different C_s values were nearly constant ranging from -2.39 to -3.01 K Cal mol^{-1} for the two soils. For bromacil the values of ΔH_s became less negative with increasing the amount sorbed in both soils which agrees with the data reported by Haque and Coshow (1971). In the case of diuron, the isosteric heats of adsorption on sand were slightly higher than their counterparts on the peat. This was attributed to a stronger bonding by the sand (Freed and Haque, 1973). The isosteric heats of sorption calculated as a function of chemical sorbed (Table 5) are consistent with the generalization that sorption of herbicides onto soils is an exothermic (negative ΔH_s) process. The ΔH_s values are relatively small ranging from -1.58 K Cal mol^{-1} for bromacil to -2.93 K Cal mol^{-1} for diuron. By inference, therefore, one can speculate that the sorption mechanism for these herbicides is of the type which involve electrostatic or entropy-generating forces but not chemisorption.

Sorption Dependence on Sorbate Properties

Water solubilities (S) and octanol-water partition coefficients (K_{ow}) have recently been used to study sorption by soil of nonionic organic compounds from water (Briggs, 1981; Chiou et al., 1979; Karickoff, 1981). In this study, these two sorbate properties (S and K_{ow}) were experimentally determined at 4, 25 and 40° C (Table 4). The measured aqueous solubilities at 25° C of 775, 81, and 40 mg L^{-1} for bromacil, chlorotoluron, and diuron, respectively, are in good agreement with the data reported by Gunther et al. (1968) and the manufacturers i.e. 815 and 42 mg L^{-1} at 25° C for bromacil and diuron respectively and 70 mg L^{-1} for chlorotoluron at 20° C. The aqueous solubilities determined were used to calculate the heats of solution, ΔH_w for the herbicides using the following Clapeyron-type equation:

$$\ln S = \frac{-\Delta H_w}{R} \left(\frac{1}{T}\right) \tag{5}$$

where R is the gas constant, T is the absolute temperature in degrees Kelvin, and S is the water solubility (mol L^{-1}). The resulting ΔH_w values (Table 5) compare well with the results of Hurle and Freed (1972), and are consistent with the generalization that low water solubilities of organic compounds are usually associated with positive ΔH_w (Chiou, 1981). A comparison between the heats of solution, ΔH_w, and the heats of sorption ΔH_s reveals that ΔH_s is less negative than $-\Delta H_w$, that is $-\Delta H_w > \Delta H_s$ for all three herbicides. These sort of data together with low exothermic and nearly constant ΔH_s values over a wide range of solute concentration led Chiou et al. (1979) to suggest that sorption of neutral organic chemicals by soil organics is mainly a process of solute partitioning to the soil organic fraction. These investigators indicated that such results would not be compatible with an adsorption model. The results in Table 4 show that the octanol-water partition coefficients (K_{ow}) are less sensitive to temperature than the water solubilities (S). The change in K_{ow} values was inconsistent and fairly small, whereas the change in S was rather large and directly proportional to the increase in temperature, that is as temperature increases, S increases. The octanol-water partition coefficients determined in this study compare well with the published values of 257 and 479 for chlorotoluron and diuron, respectively (Briggs, 1981), and 70 to 80 for bromacil (Gerstl and Yaron, 1983).

As developed in previous sections, soil sorption coefficient estimation for neutral organic chemicals can be reduced to estimation of K_{oc} because of the high correlation between sorption and soil organic carbon content. In recent years, several attempts have been made to correlate sorption (K_{oc}) of these compounds with their aqueous solubilities or their octanol-water partition coefficients. Briggs (1981) and Karickoff (1981) have reviewed the theoretical basis, in thermodynamic terms, of the approaches used to predict K_{oc} for the hydrophobic organic compounds. These and other workers (Chiou et al., 1979; Felsot and Dahm, 1979; Means et al., 1980; Brown and Flagg, 1981; Mingelgrin and Gerstl, 1983) have reported significant relationships between K_{oc} and K_{ow} and between K_{oc} and S, that allow the estimation of a compound's K_{oc} value from the knowledge of its octanol-water partition coefficient (K_{ow}) or its water solubility (S). The range of predicted K_{oc} values obtained by using these relations to calculate K_{oc}s of the three herbicides are given in Table 5. Overall, the agreement between predicted and calculated K_{oc} values was good, generally agreeing within a factor of 3. This factor is comparable to the deviations in K_{oc} values for a given herbicide between the two soils (Table 2). When the octanol-water partition coefficients were used to predict K_{oc}, nearly all values obtained were in the range of the measured K_{oc}s. Water solubility on the other hand was not such a good predictor of K_{oc} as K_{ow}. This was indicated by the range of nearly one order of magnitude in estimated K_{oc}. It has been pointed out that the correlation of K_{oc} with S for solid compounds can be improved by including a term to account for the energy needed to break up the crystal structure (Briggs, 1981; Chiou, 1981; Karickoff, 1981). Since the octanol-water partition coefficient involves the distribution of solute monomers between an aqueous phase and a hydrophobic organic phase, it is not affected by the crystal energy contributions (Karickoff et al., 1979).

Linear least squares fitting of the experimentally determined K_{ow} and K_{oc} data for bromacil, chlorotoluron and diuron yielded the following relationship:

$$\log K_{oc} = 1.30 \log K_{ow} - 0.77 \qquad r = 0.999 \qquad (6)$$

whearas regression of the water solubilities (μmol/L) and K_{oc} gave the equation:

$$\log K_{oc} = -0.80 \log S + 4.41 \qquad r = -0.995 \qquad (7)$$

These correlations (equation 6 and 7) are similar to the equations used above to estimate K_{oc} from K_{ow} and S values. These highly significant correlations show the validity of such parameters (K_{ow} and S) to estimate sorption, K_{oc}. Hence, one might expect to estimate fairly accurately the K_{oc} values of other members of the substituted urea herbicides or related compounds from equation 6 based on the knowledge of K_{ow} alone. These relationships further demonstrate the similarity between soil sorption of neutral organic compounds from water and these chemicals partitioning between octanol and water (Chiou et al., 1979). Because the above correlations are generally valid only for hydrophobic (neutral) compounds, this type of sorption was refered to as hydrophobic sorption (Hassett et al., 1980) or as solute partitioning to the soil organic fractions (Chiou et al., 1979). Hydrophobic sorption was implied when a high degree of correlation exists between chemicals K_{ow} or S and sorption expressed on organic carbon basis (Hassett et al., 1981).

It is important to emphasize that although the three herbicides studied agree very well with K_{oc}-S relationships and with K_{oc}-K_{ow} predictions, the limits of validity of these relationships are not known. Evidence in the literature (Hassett et al., 1981) suggests that there is a limit beyond which these correlations may not hold and that this limit is not strictly a function of the compound's water solubility or its octanol-water distribution coefficient but is also a function of sorbent properties. This demonstrates the complexity of the sorption process and suggests that the actual K_{oc} may be estimated only within limits from K_{ow} and water solubility for members of diverse groups of neutral organic compounds on all sorbents. Experimentally determined sorption isotherms are therefore needed for a precise knowledge of a chemical's behavior in soil.

SUMMARY AND CONCLUSIONS

Sorption of three herbicides widely used in agriculture was studied at two temperatures on two soils of greatly varying organic carbon content. The results were well described by a simple proportionality relationship and Freundlich adsorption equation as indicated by the very highly significant correlation coefficients of all isotherms. Sorption constants of a given herbicide were many times higher on the peat than on the sand, reflecting the dependence of these chemicals sorption on soil organic carbon content. This wide variation was nearly eliminated when sorption constants were computed on organic carbon basis instead of total soil mass. Although sorption was higher at 4° C than at 25° C, the difference was only minor at this range. Thus sorption is little affected by temperature in the range common in temperate zone agricultural soils. Isosteric heats of sorption, ΔH_s calculated from sorption data at different temperatures were exothermic and relatively low suggesting the involvement of low energy (weak) mechanism.

The two sorbate parameters, aqueous solubility (S) and octanol-water partition coefficient (K_{ow}) were experimentally determined for each herbicide studied. Sorption constants estimated from these sorbate properties (S and K_{ow}) using relationships from the literature developed for similar compounds compared favorably with experimental values normalized for soil organic carbon (K_{oc}). It was shown that highly significant linear correlations exist between the log values of both K_{ow} and S and the corresponding sorption constants (K_{oc}). Thus by inference, it is concluded that such relationships may be useful in predicting sorption of neutral organic compounds on a given sorbent in instances when a reasonably accurate estimated value suffices in lieu of a costly determined value.

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Table 1. Selected properties of the two soils studied.

Soil	pH	O.M.	O.C.	Clay	Silt	Sand
		- - - - - - - - - -	%	- - - -	- - - -	- - - -
Semiahmoo Mucky Peat	5.4[a]	48[b] 37[c]	27.8[e] 21.5	16	65	19
Adkins Loamy Sand	7.3	0.7[d]	0.4	3	13	84

a: 1:2 soil to water ratio

b: ignition

c: hydrogen peroxide oxidation

d: Walkley-Black method

e: % OM x 0.58

Table 2. Linear sorption constants (K_p) and r values, K_{oc} values and Freundlich constants (K_f) and n and r values for the sorption of bromacil, chlorotoluron and diuron by the two soils.

Soil	Temp.	K_p[a]	r	K_{oc}[b]	K_f[c]	n	r
			Bromacil				
Peat	4	9.68	0.996	35	26.74	0.86	0.999
	25	9.23	0.997	33	21.31	0.89	0.999
Sand	4	0.26	0.995	63	0.52	0.91	0.999
	25	0.256	0.994	62	0.31	0.97	0.997
			Chlorotoluron				
Peat	4	103	0.997	370	158	0.85	0.999
	25	75	0.997	269	116	0.87	0.999
Sand	4	0.66	0.999	163	1.47	0.86	0.995
	25	0.5	0.99	122	1.08	0.87	0.999
			Diuron				
Peat	4	243	0.995	873	301	0.87	0.999
	25	185	0.992	665	244	0.87	0.996
Sand	4	1.28	0.998	315	2.48	0.9	0.993
	25	0.99	0.998	242	1.91	0.86	0.999

a = K_p the linear sorption constant

b = K_{oc} = K_p/fraction organic carbon

c = K_f Freundlich constant

Table 3. Sorption constants (K_p) of bromacil, chlorotoluron, and diuron calculated for each initial herbicide concentration studied at 25° C.

Bromacil		Chlorotoluron		Diuron	
Init. Conc. μM	K_p	Init. Conc. μM	K_p	Init. Conc. μM	K_p
2	21.0	2	158.5	2	383.3
5	19.1	5	142.5	5	286.5
20	19.2	20	133.9	20	337.8
50	21.6	50	118.6	50	326.1
100	15.0	100	93.3	75	219.1
500	13.2	150	88.2	100	214.9
750	11.6	200	83.9	125	191.9
1000	11.3	250	77.0	150	191.4
2000	10.0	300	75.1		
2500	9.2				
3000	9.3				
$\overline{X}$	14.6		107.9		268.9
S	4.8		31.1		74.4
C.V.	33%		29%		28%

Table 4. Octanol-water partition coefficient (K_{ow}) and water solubilities S, of bromacil, chlorotoluron and diuron measured at 4, 25, and 40° C.

Temperature °C	Bromacil	Chlorotoluron	Diuron
		K_{ow}	
4	69 ± 1	216 ± 1	490 ± 38
25	74 ± 2	219 ± 3	451 ± 25
40	80 ± 1	210 ± 6	423 ± 16
		S (mg L^{-1})	
4	626 ± 18	56 ± 6	20 ± 3
25	775 ± 8	81 ± 6	40 ± 1
40	1044 ± 35	99 ± 3	53 ± 1

Table 5. Determined or estimated parameters for the herbicides bromacil, chlorotoluron and diuron at 25° C.

Parameter	Bromacil	Chlorotoluron	Diuron
ΔH_s^a	-1.58	-2.59	-2.93
ΔH_w^a	2.32	2.73	4.86
K_{oc} determined	33-62	122-269	242-665
K_{oc} from K_{ow}	48-97	124-286	180-475
K_{oc} from S	50-665	212-2011	323-3069

[a] ΔH_s and ΔH_w are in K Cal mol^{-1}.

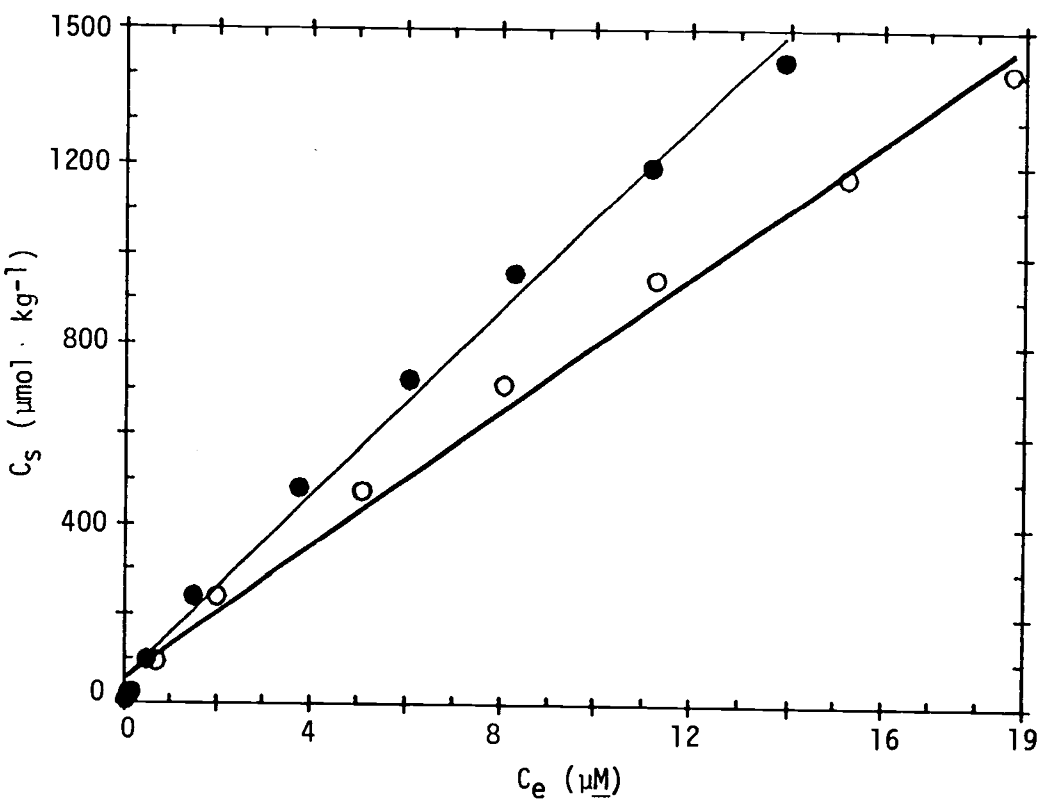

Figure 2. Fitted isotherms for the sorption of chlorotoluron by the peat soil at 4 (●) and 25°C (○).

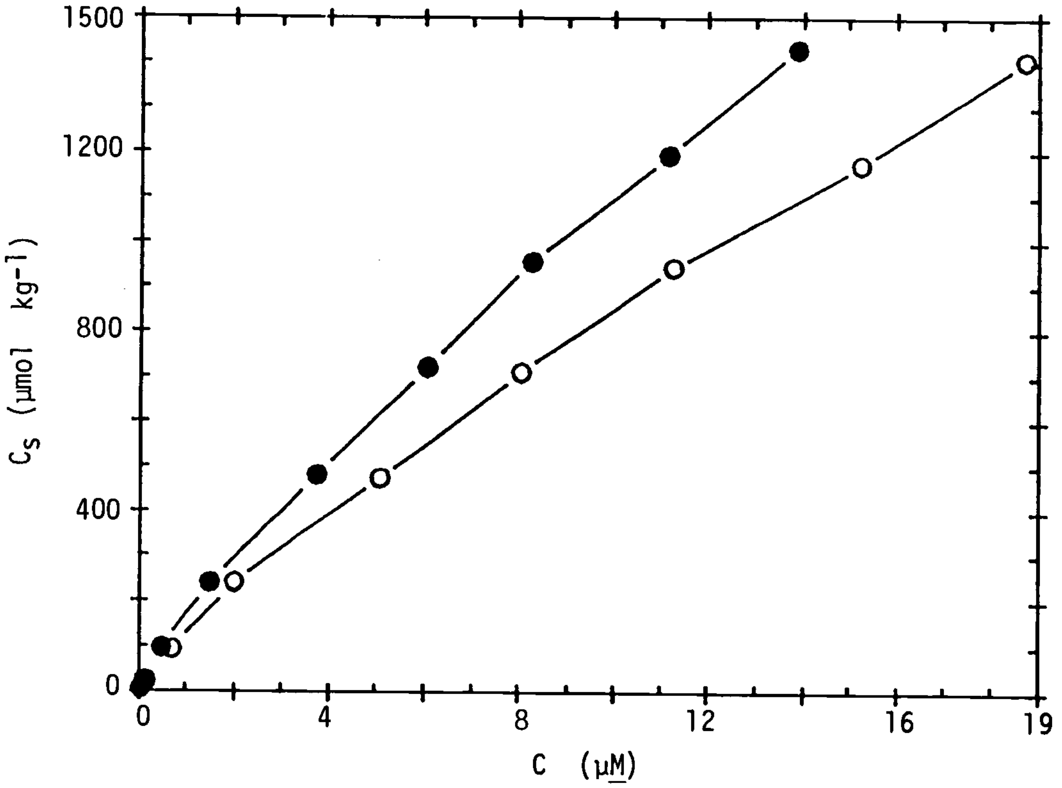

Figure 3. Representative isotherms for the sorption of chlorotoluron by the peat soil at 4 (●) and 25°C (○).

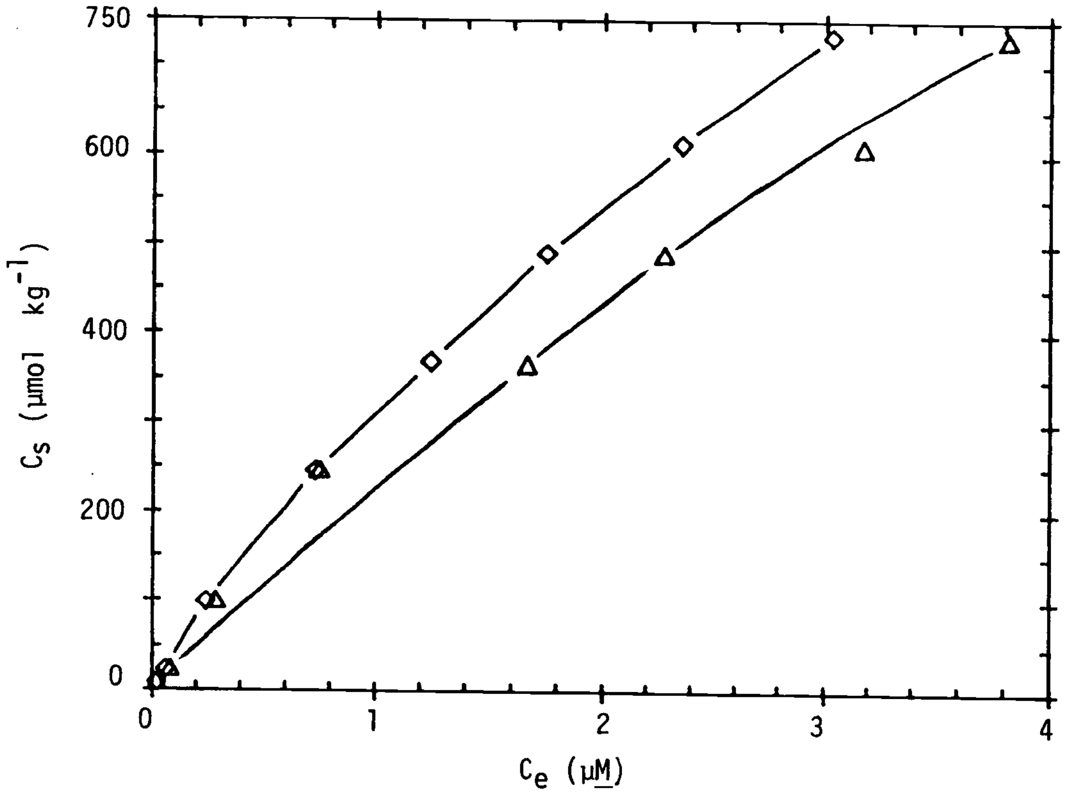

Figure 4. Representative isotherms for the sorption of diuron by the peat soil at 4 (◇) and 25°C (△).

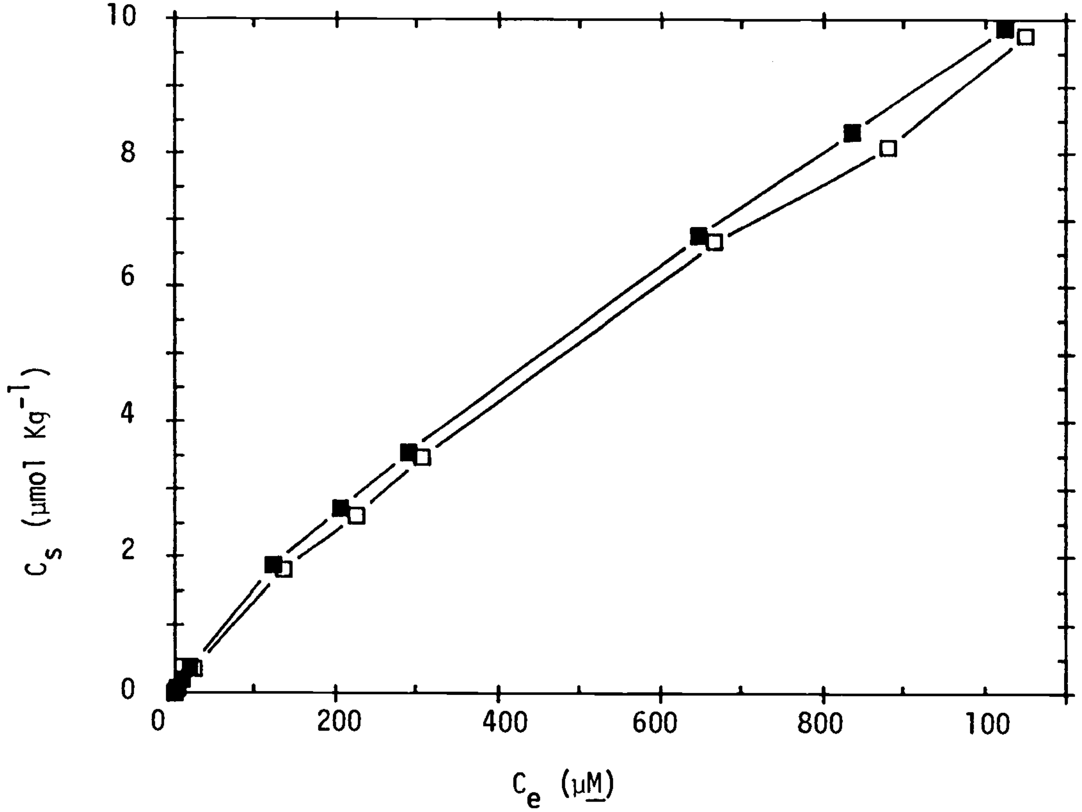

Figure 5. Representative isotherms for the sorption of bromacil by the peat soil at 4 (■) and 25° C (□).

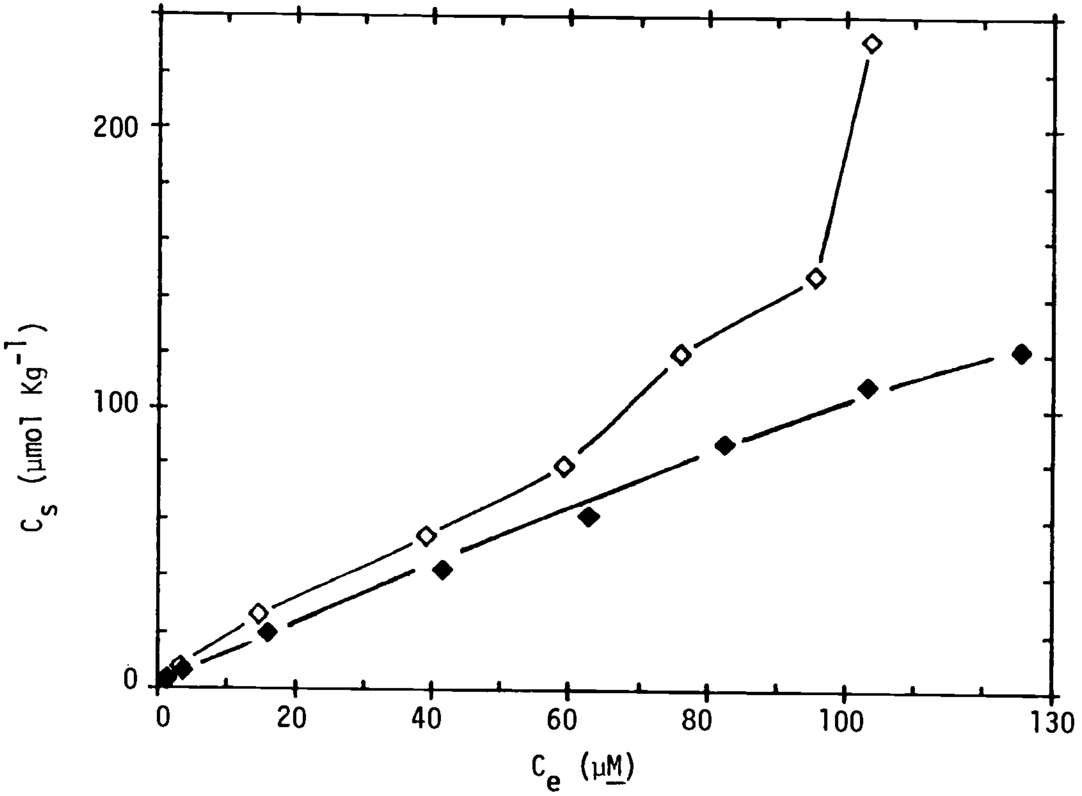

Figure 6. Sorption isotherms for diuron by the loamy sand soil at 4 (◇) and 25° C (◆).

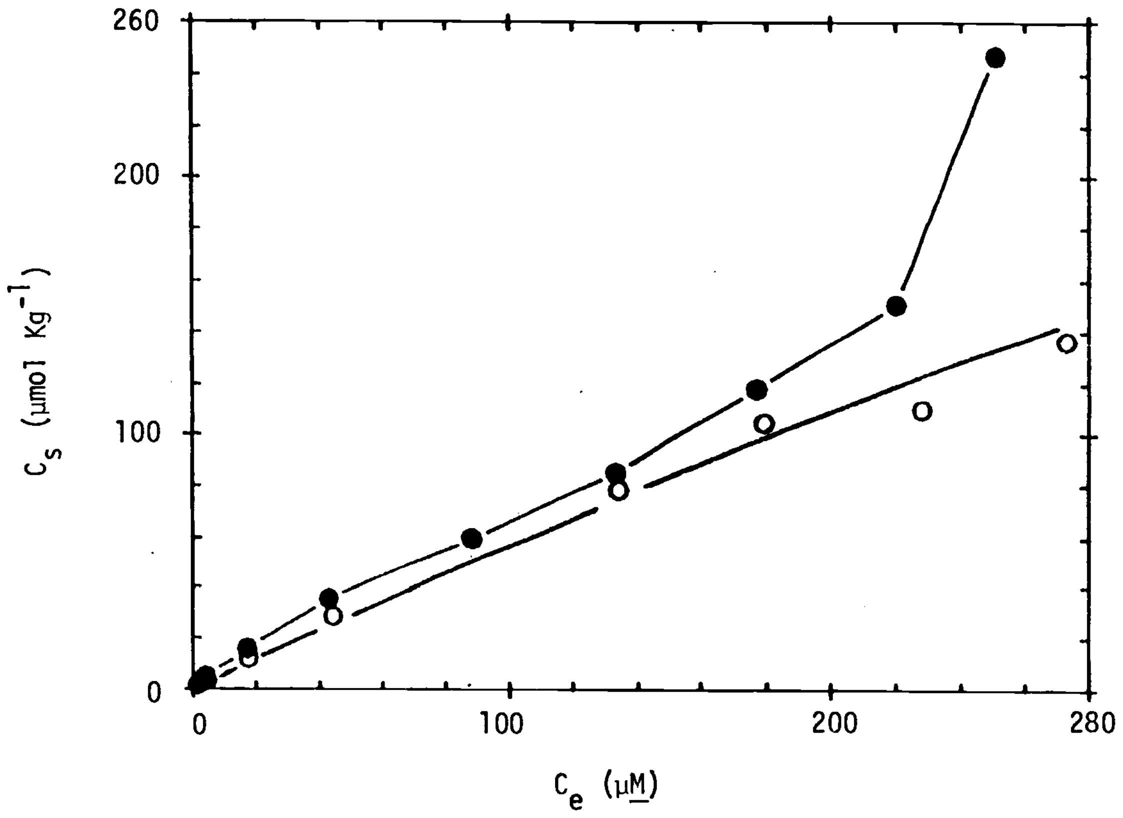

Figure 7. Sorption isotherms for chlorotoluron by the loamy sand soil at 4 (●) and 25° C (○).

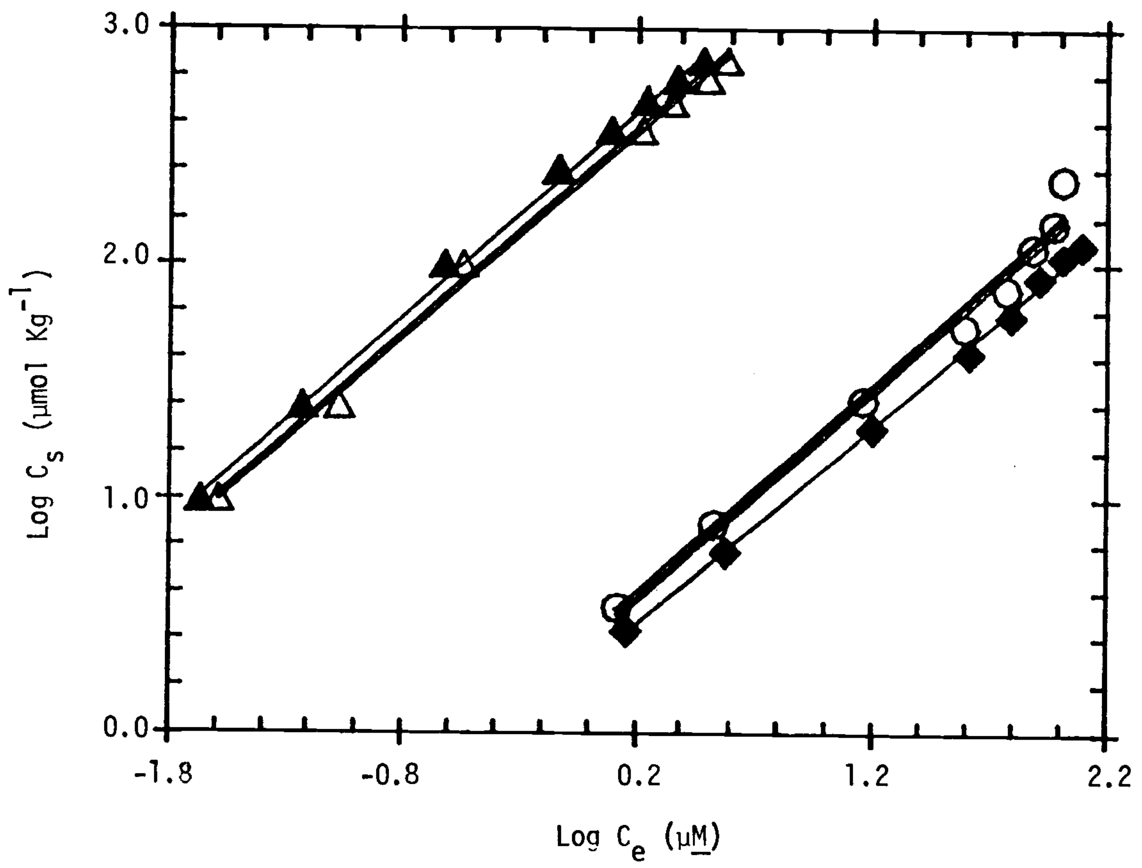

Figure 8. Feundlich isotherms for the sorption of diuron by soil at 4° C (▲, peat; o, sand) and at 25° C (△, peat; ◆, sand).

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CHAPTER II

Binding of Six Herbicides With Soil Humic Acid[1]

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ABSTRACT

The binding of bromacil (5-bromo-3-sec-butyl-6-methyluracil), chlorotoluron [3-(3-chloro-4-methylphenyl)1,1-dimethylurea], diuron [3-(3,4-dichlorophenyl)1,1-dimethylurea], diquat (1,1-ethylene-2,2-bipyridylium ion), glyphosate [N-(phosphonomethyl) glycine], and simazine (2-chloro-4,6-bis-ethylamino-s-triazine) with humic acid (HA) from soil was studied by means of gel filtration chromatography and infrared spectroscopy. The gel filtration procedure of Hummel and Dryer appears suitable for evaluating HA interactions with ionic and nonionic herbicides. The elution parameters (Kav) of herbicide-HA peaks were indifferent of HA Kav of 0.07 and the elution parameters of herbicide-HA troughs were approximately equal to the Kav's of the herbicides. Infrared spectroscopy was utilized to demonstrate the involvement of ion exchange mechanism in the binding of diquat by HA. Changes in the absorption bands of HA in the region 1720-1600 cm^{-1} after treatment with bromacil, diuron, and chlorotoluron showed that hydrogen bonding and possibly charge-transfer mechanisms were involved in HA binding with these herbicides. The results of this study could have important implications in the assessment of pesticide mobility and bioavailability in soil and water environments.

Additional Index Words: gel filtration, infrared spectroscopy, bromacil, diuron, chlorotoluron, diquat, glyphosate, simazine.

INTRODUCTION

The importance of soil organic materials with respect to pesticide sorption has been discussed in several reviews (Hayes, 1970; Khan, 1978; Stevenson, 1976; Burchill et al., 1980). Studies with simplified systems involving well-defined soil organic matter components such as humic acid or fulvic acid have evolved over the last decade (Khan, 1977; Burns et al., 1973b; Nearpass, 1976; Mueller-Wegener, 1977; Gaillardon et al., 1980; Senesi and Testini, 1980). Techniques used to study HA-herbicide interactions have included the slurry method (Gaillardon et al., 1980; Khan, 1974), gel filtration (Burns et al., 1973b; Grice and Hayes, 1970; Khan, 1973), and equilibrium dialysis (Gilmour and Coleman, 1971).

The slurry method, which has been widely used by investigators, can have a number of shortcomings in HA-herbicide binding studies. It has been pointed out (Hayes, 1970) that this technique cannot be satisfactorily applied to the study of binding by components which are not sedimented by centrifugation. The potential uses of gel filtration techniques as an alternative for the study of binding of triazines by soluble humic materials was outlined by Hayes (1970) and by Grice and Hayes (1970) who suggested that the gel filtration method described by Hummel and Dreyer (1962) would give a more quantitative evaluation of the interactions between HA and herbicides in general. This method was successfully employed by Khan (1973) in studying the interaction of bipyridylium herbicides with humic acid and fulvic acid using Sephadex G-10 and G-25 gels.

Infrared spectroscopy has been used to obtain information on the mechanism(s) of interaction between humic materials and herbicides. This technique is of considerable value in HA-herbicide interaction research since it yields a variety of bands diagnostic of specific molecular structures and functional groups. Infrared data provided clear evidence for the involvement of ion exchange mechanism in the binding of diquat and paraquat herbicides by humic acids (Khan, 1974; Burns et al., 1973). On the basis of infrared studies, Khan (1973) suggested that the bipyridylium herbicides, diquat and paraquat, form charge-transfer complexes with humic substances, a result that could not be confirmed by Burns et al. (1973). Infrared data provided support for the belief that binding of s-triazines by HA involves such mechanisms as ionic binding, H-bonding, Van der Waals forces and charge-transfer complexes (Sullivan and Felbeck, 1968; Senesi and Testini, 1980 and 1982). Results on the binding of several substituted urea herbicides by HA showed that besides the involvement of H-bonding, a prominent role is played by electron donor-acceptor processes which leads to the possible formation of charge-transfer complexes (Senesi and Testini, 1980 and 1983). In the study with infrared spectroscopy, Khan and Mazurkewich (1974) failed to show the coordination of linuron to cations on humic acid.

The objectives of this work were: (i) to examine the suitability of the Hummel and Dreyer gel filtration method for studying the binding of ionic and nonionic herbicides by humic acid extracted from soil, and (ii) to use infrared spectroscopy to characterize the binding mechanisms these herbicides form with humic acid.

MATERIALS AND METHODS

Humic acid was obtained from Dr. R. L. Malcolm, USGS, Denver, Colorado. It was extracted from Fairbanks soil, Fairbanks, Alaska. Malcolm (1976) described the methods of extraction and purification which involved centrifugation, pressure filtration, dialysis, resin exchange, and freeze drying steps. The HA was H-saturated, and contained on ash-free basis 57.13 % C, 34.45 % O, 3.74 % H, 3.94 % N, 0.39 % S, and 0.13 % P. The ash content was 0.38 %.

Six ^{14}C-labelled herbicides were used: bromacil, chlorotoluron, diuron, simazine, diquat, and glyphosate. Diquat and glyphosate are both dissociable and can exist in ionic forms; whereas bromacil, chlorotoluron, diuron, and simazine are nonionic and have limited solubility in water at 25° C ranging from 775 mg/L for the fairly soluble bromacil to 3.5 mg/L for the very low water soluble simazine. All chemicals used were >99 % analytically and radiochemically pure.

The Hummel and Dreyer (1962) gel filtration procedure was used to study the interactions between the herbicides and HA. This method was devised to detect interactions between macromolecules and substances of low molecular weight. It avoids the difficulties encountered in zonal gel filtration due to interaction of ionic and aromatic compounds of small molecular size with the Sephadex matrix. It also eliminates the problem of dissociation of the macromolecule-ligand complex in the chromatographic column. The Hummel and Dreyer method involves the adding of a small volume of HA-herbicide mixture to a column of Sephadex gel previously in equilibrium with a solution of the herbicide at the same concentration as in the mixture. When the column is eluted with the equilibration solution, the elution profile shows a leading peak which emerges from the column at the elution volume of HA, followed by a trough which emerges at the elution volume of the herbicide. The peak and the trough are usually separated by a short region in which the herbicide concentration returns to its base-line level. The appearance of a peak followed by a trough in the elution profile is used as the criterion of binding. The area of the trough provides a direct measure of the number of moles of herbicide bound by HA. Theoretically, the area of the peak should be equal to the area of the trough (Wood and Cooper, 1970).

The gel filtration study was carried out by preparing, in phosphate-buffer solution of pH 6.85 and ionic strength 0.05 M KCl, herbicide solutions of the following μmolar concentrations: bromacil, 0.966; chlorotoluron, 0.311; diuron, 1.01; diquat, 0.125; glyphosate, 1.01; and simazine, 0.109. A known amount of HA (3 mg) was dissolved in 3 mL phosphate-buffer-herbicide solution and applied to a 26 by 370 mm chromatographic column packed with fine mesh, Sephadex G-25 gel (exclusion limit, 5000), which has previously been equilibrated with the herbicide-buffer solution used to dissolve HA. The column was then eluted at 25 C with the same herbicide solution in the descending direction using a peristaltic pump driven by a stepping motor at a flow rate of 60 mL h^{-1}.

Uniform fractions (approx. 5 ml), were collected in a fraction collector. The effluent solution was continuously monitored by ISCO Model UA-2 Dual UV Analyzer set at 280 nm wavelength. Blue Dextran 2000, at a concentration of 2 mg mL^{-1} was used to determine the uniformity of packing and the void volume of the column.

Herbicide concentration in each fraction was determined by taking 0.5 mL aliquots, adding 3.5 mL of Handifluor liquid counting solution and measuring the radioactivity with a Packard Model 3330 Tri-Carb Liquid Scintillation Spectrometer. An internal standard was used regularly to evaluate the instrument counting efficiency throughout the course of study. The elution profile of HA was determined by passing 3 mg HA through the column. The concentration of HA in each fraction was assessed by measuring the absorbance (optical density) at 400 and 280 nm on a Beckman Model 25 UV-Visible spectrophotometer.

Infrared (IR) spectra were recorded on a Perkin-Elmer Model 457 Grating Infrared Spectrophotometer in the 4000 to 400 cm^{-1} wavenumber range using 1 mm thick (cell path) KBr pellets. The IR spectra were obtained for diquat, bromacil, chlorotoluron, and diuron at 1 m$\underline{M}$ concentration. Equal quantities of HA and herbicide: 7.2 mg diquat, 5.2 mg bromacil, 4.7 mg diuron, and 4.2 mg chlorotoluron were placed in Corex centrifuge tubes and dissolved in 20 mL distilled water. The tubes were shaken on a mechanical shaker for 15 hr and the excess moisture in the mixture was removed by freeze-drying. The KBr pellets were prepared by mixing 250 mg KBr with 1 mg of the freeze-dried HA-herbicide mixture. Recording conditions were: reference, air; slit, normal; and scan time, medium. A reference spectrum of polystyrene film (0.05 mm cell path) was used to calibrate the spectra.

RESULTS AND DISCUSSION

Gel filtration chromatography

Representative elution diagrams for the measurement of binding of herbicides by humic acid are given in Figs. 9, 10, and 11. The elution profile for diquat-HA system (Fig. 9a) showed the appearance of a peak at the elution volume of HA and a trough at the elution volume of diquat. The existence of a trough at the elution volume of diquat provided evidence for the binding between the herbicide and HA, as did the presence of excess diquat in the HA peak (Hummel and Dreyer, 1962). The attainment of equilibrium in the operation of the gel filtration method was indicated by the return of the base-line concentration of small molecule (herbicide) to its original level after the appearance of the trough in the elution diagram. This criterion was used to test the adequacy of the method performance (Hummel and Dreyer, 1962). The results obtained on the binding of herbicides by HA provided indication that equilibrium conditions were met satisfactorily in all systems. In Fig. 9b is shown the elution pattern of diquat-HA system superimposed on the elution profile of humic acid. This graphical presentation shows the coincidence of the herbicide-HA complex peak on the humic acid peak. The results on diquat-HA binding determined in this study were in essential agreement with the data reported by Khan (1973).

The elution profile for the measurement of diuron binding by HA is shown in Fig. 10. The trough in the diuron-HA system appeared at the elution volume of diuron and the peak appeared at the HA elution volume. This diagram represents a typical elution pattern for the binding of a small molecule with a macromolecule as described by Hummel and Dreyer (1962). The data for simazine, a triazine herbicide, interactions with HA (Fig. 11) indicate that the gel filtration method may be the technique of choice for measuring the complexation of these herbicides with humic materials. Similar results were obtained on the binding of bromacil, chlorotoluron, and glyphosate by HA. These data demonstrate that the Hummel and Dreyer gel filtration procedure was well suited for the study of binding ionic and nonionic herbicides by humic substances.

Since the primary purpose of the present study was to examine the utility of the gel filtration method to detect interactions between herbicides and HA, no attempt was made to determine quantitatively the amount of herbicide bound by HA. The procedure, nevertheless, provides a means of estimating extent of binding and gives data from which the number of moles bound by HA can be determined by graphical (Hummel and Dreyer, 1962) or mathematical procedures (Fairclough and Fruton, 1966).

Elution parameters (Kav) for the herbicides individually, and when in association with HA, are presented in Table 6. The Kav values reflect the characteristics of the chemical and only chemicals that have closely related properties can have similar Kav values. This was evident for the substituted urea herbicides, diuron and chlorotoluron, and for the ionic herbicides diquat and glyphosate. Elution parameters of herbicide-HA peaks were found to compare well with one another and with HA Kav of 0.07, indicating that herbicide-HA complexes have all appeared at the elution volume of HA. Comparison of the Kav values of herbicide-HA troughs with those of the herbicides shows that these values were very similar. These results are expected whenever the method is adequately suited for the system and carried out satisfactorily. The data in Table 6 reveal the occurrence of interactions between the Sephadex matrix and nonionic herbicides (Kav > 1). Evidently, the interactions had no effect on the herbicides binding, except the delay of the trough emergence from the column.

When the gel filtration procedure is carried out as originally described by Hummel and Dreyer, the area of the trough should be equal to the area of the leading ligand peak (ratio = 1). The ratios of trough areas to peak areas determined in this study were; 0.88, 1.28, 1.17, 0.97, 0.94, and 0.81 for bromacil, chlorotoluron, diuron, diquat, glyphosate, and simazine, respectively, all within experimental error from the theoretical value of 1.

Preliminary experiments with diuron, bromacil, and chlorotoluron, utilizing Sephadex G-10 and G-25 gels showed that studying the binding of these herbicides by humic materials was not possible by the zonal filtration technique. This was due to the dissociation of herbicide-HA complexes as they passed down the chromatographic column. These observations indicated that the herbicides were weakly bound by a reversible process to humic acid and suggested that the interactions were physical in nature and may involve such mechanisms as H-bonding, Van der Waals forces, and charge-transfer complexes (Wood and Cooper, 1970).

The Hummel and Dreyer (1962) gel filtration method can be used to study the simultaneous binding of two herbicides. This is possible when different analytical procedures are available for each herbicide.

Different assay methods are not, however, necessary if the two herbicides have elution volumes sufficiently different for their troughs to be completely separated (Fairclough and Fruton, 1966).

Infrared Spectroscopy

Infrared spectra of untreated humic acid and herbicide-HA complexes are given in Fig. 12. Interpretation of the IR spectra and band assignments were based on Bellamy (1975), Silverstein et al. (1981) and Stevenson (1982). All herbicide-HA spectra show a number of changes in functional group absorption bands when compared with the untreated humic acid spectrum, thus pointing distinctly to the binding of the herbicides by HA.

The IR data of untreated HA (Fig. 12a) show characteristic absorption bands of a typical HA preparation. The broad band around 3300 cm^{-1} was most likely due to N-H stretching and hydrogen bonded OH groups of COOH and others. The C=O stretching vibrations of carboxylic acids and ketones appeared at 1717 cm^{-1} in the IR spectrum. The absorption bands in the 1660-1600 cm^{-1} region were attributed to aromatic C=C vibrations and H-bonded C=O groups of quinones and ketones. The band near 1400 cm^{-1} was probably due to O-H deformation and C-O stretching of phenols and asymmetric stretching of COO^- ions. The absorption band near 1200 cm^{-1} was assigned to C-O stretching and O-H bending of COOH. Although detailed examination of IR spectra of a number of different humic acids has been made, it is still rather difficult to unequivocally assign all absorption bands to specific functional groups. This could probably be due to overlapping of many of the absorption bands (Burns et al., 1973) and interference caused by adsorbed moisture which produces bands in the 3300-3000 and 1720-1500 cm^{-1} regions (Stevenson 1982).

The main changes in all herbicide-HA spectra occured in 1720-1660 cm^{-1} region. These effects, the reduced absorption frequency at 1717 cm^{-1} and the enhanced intensity of the band in the 1660 to 1600 cm^{-1} region, can be explained in a number of ways with consideration of the chemical nature of the interacting herbicide.

For the positively charged herbicide diquat, the changes are. consistent with the conversion of HA carboxylic acids (COOH) to carboxylate ions (COO^-) upon the addition of diquat to HA. The absorption band near 1610 cm^{-1} in diquat-HA spectrum (Fig. 12b) indicate that carboxylate anions were produced when diquat was added to humic acid and provide clear evidence for the involvement of ion-exchange mechanism in this system. These results are consistent with those published by Khan (1974) and Burns et al. (1973a). Khan (1973) suggested the involvement of charge-transfer mechanism in diquat and paraquat interactions with HA, based on the shifting of the out-of-plane vibration bands of C-H groups to lower wavenumbers in the 900-600 cm^{-1} regions. Those shifts were not evident in this work nor in the study of Burns et al. (1973a).

For the remaining herbicides, it seems unlikely that the changes observed in the 1717 to 1600 cm^{-1} can be explained by the conversion of COOH to COO^- as in diquat, because these herbicides are nonionic and very weakly basic. These absorption patterns can however be attributed to any resonance effect that increases the C=O bond length and reduces the frequency of absorption. Intermolecular H-bonding, polar environment, delocalization of the π electrons of the C=O group could make the resonance effect to dominate, thus reducing the double bond character of the C to O bond and causes absorption at longer wavelength (shift absorption towards lower frequency). The herbicides bromacil, diuron, and chlorotoluron contain carbonyl, secondary and tertiary amine and amide groups which enable them to participate with HA in hydrogen bond formation and charge-transfer complexes. The appearance of strong absorption bands near 1650 cm^1, and the reduced absorption of the 1717 cm^{-1} band especially for diuron and chlorotoluron, provide clear indication for the formation of H-bonding and probably charge-transfer complexes between these herbicides and HA. The slight reduction of the absorption band near 1200 cm^{-1} particularly for diuron support the claim for H-bond formation. Similar results were reported for the substituted urea herbicides linuron, monuron, monolinuron, metobromuron, cycluron and fenuron (Senesi and Testini, 1980 and 1983).

In the herbicide-HA spectra, particularly those for chlorotoluron and bromacil, a number of unchanged absorption bands appeared in both the IR spectra of pure herbicides (Fig. 13) and the IR spectra of herbicide-HA complexes. The presence of these bands made the task of interpreting the spectra difficult because of the uncertainty of whether these bands emerged as a result of the interactions or were inherited from the parent herbicide molecules. The existence of these bands, on the other hand, strengthens the possibility that only weak, physical-type interactions were involved in the binding of these herbicides (bromacil, diuron and chlorotoluron) by HA (Senesi and Testini, 1980). It is interesting to note that although greatly reduced in intensity, the 1717 cm^{-1} band of HA has persisted in all herbicide-HA spectra. This indicates that in these complexes a considerable proportion of carboxylic acid (COOH) groups in humic acid remained inaccessible to the herbicide molecules.

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Table 6. Elution parameter (Kav) for the herbicide and herbicide-HA peaks and troughs determined on Sephadex G-25 gel.

Herbicide	Herbicide alone	Herbicide-HA peak	Herbicide trough
	- - - - - - - - Elution parameter, Kav[a]	- - - - - - - -	
Diuron	2.68	0.07	2.58
Chlorotoluron	2.65	0.06	2.67
Simazine	2.00	0.07	2.05
Bromacil	1.36	0.09	1.27
Glyphosate	0.72	0.07	0.72
Diquat	0.54	0.03	0.56

[a] $K_{av} = \frac{V_e - V_o}{V_t - V_o}$, where V_e is the elution volume; V_o is the void volume; and V_t is the total volume of the column.

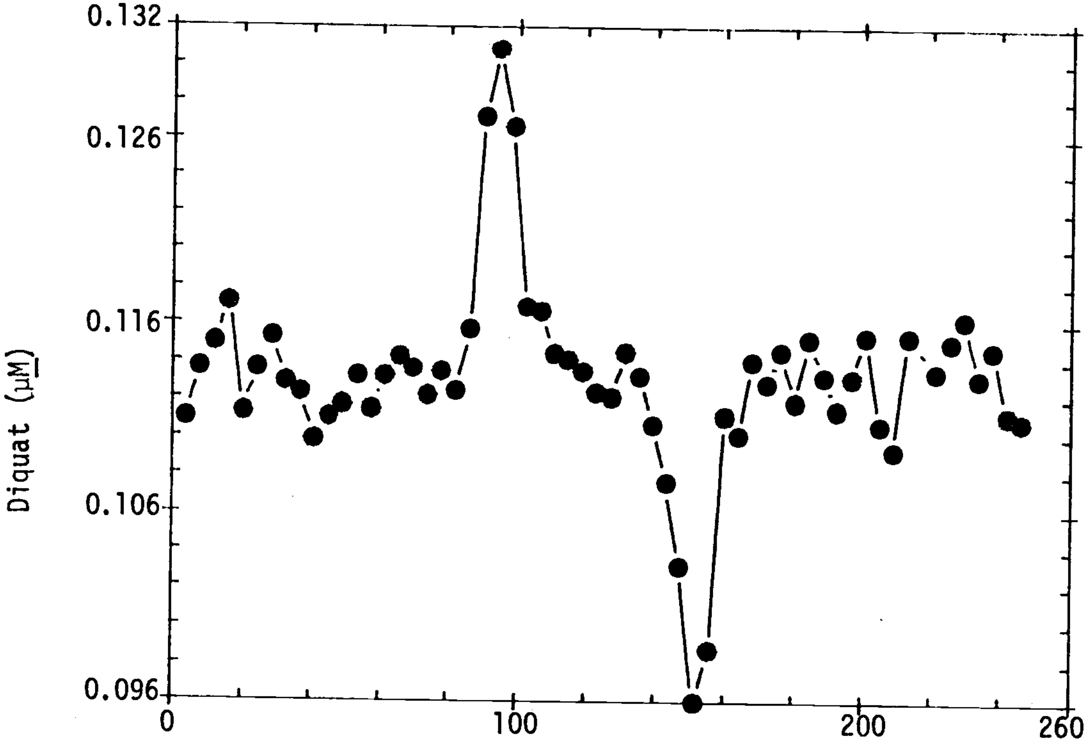

Figure 9a. Elution profile for the measurement of diquat binding by humic acid on Sephadex G-25 gel.

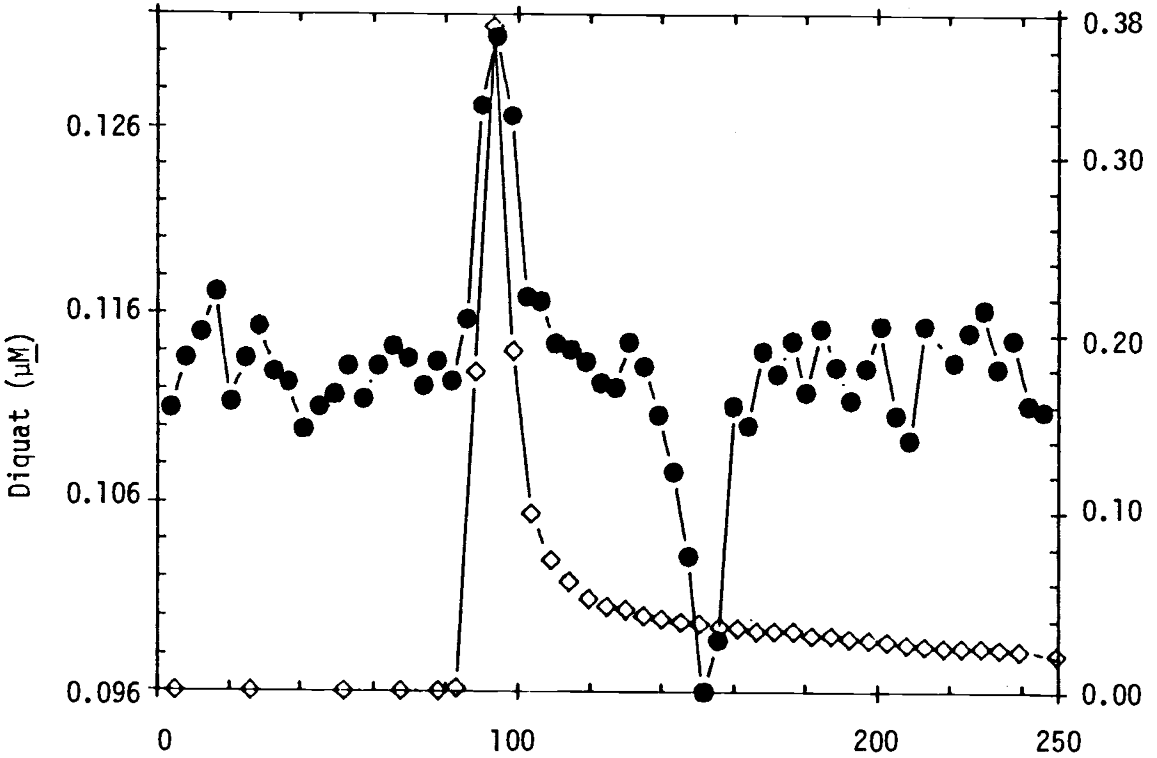

Figure 9b. Elution profile of diquat-HA binding (●) superimposed on the elution profile of humic acid (◇; right-Y-axis) on Sephadex G-25 gel.

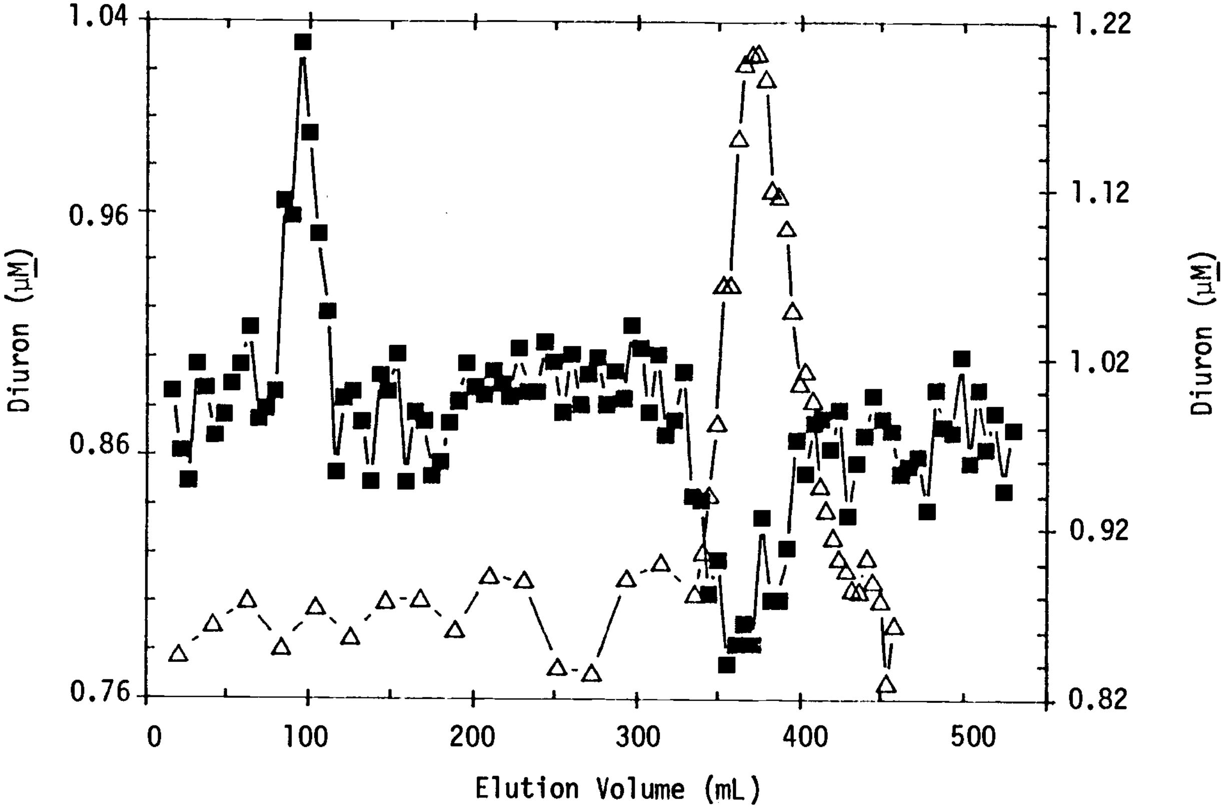

Figure 10. Elution profile of diuron-HA binding (■) superimposed on elution curve of diuron (△; right-Y-axis) on Sephadex G-25 gel.

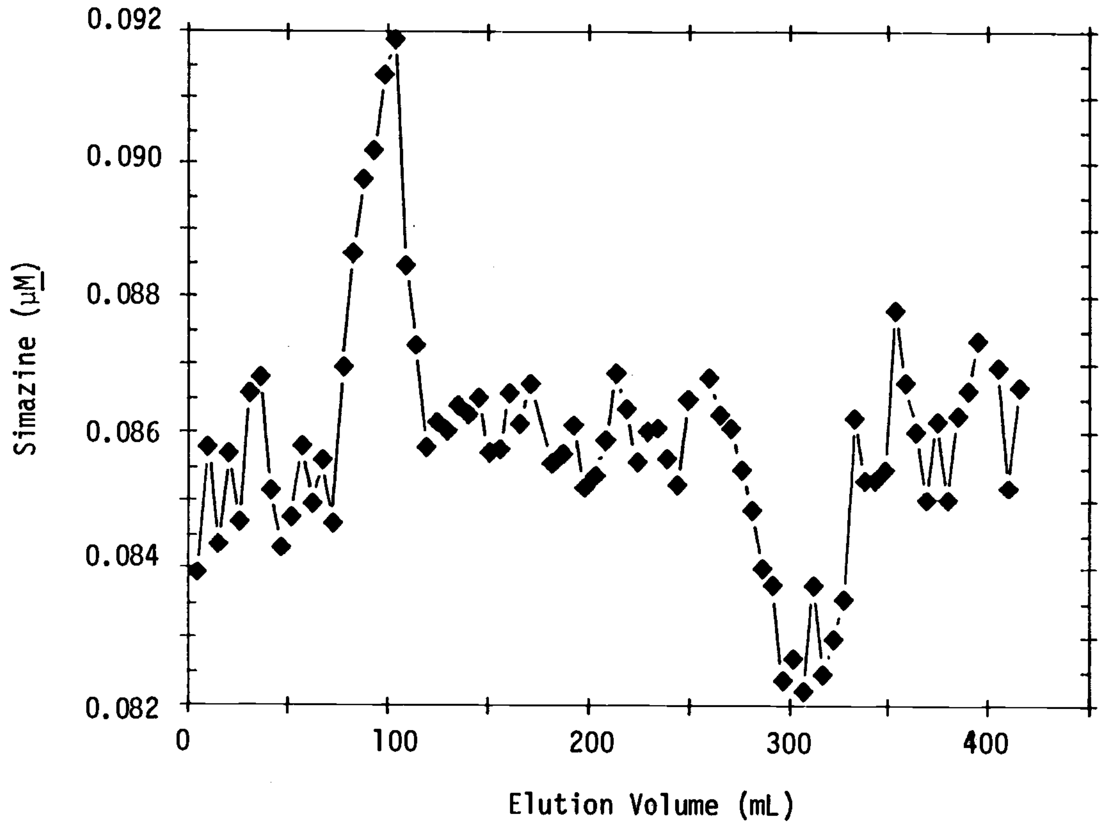

Figure 11. Elution profile for the measurement of simazine binding by humic acid on Sephadex G-25 gel.

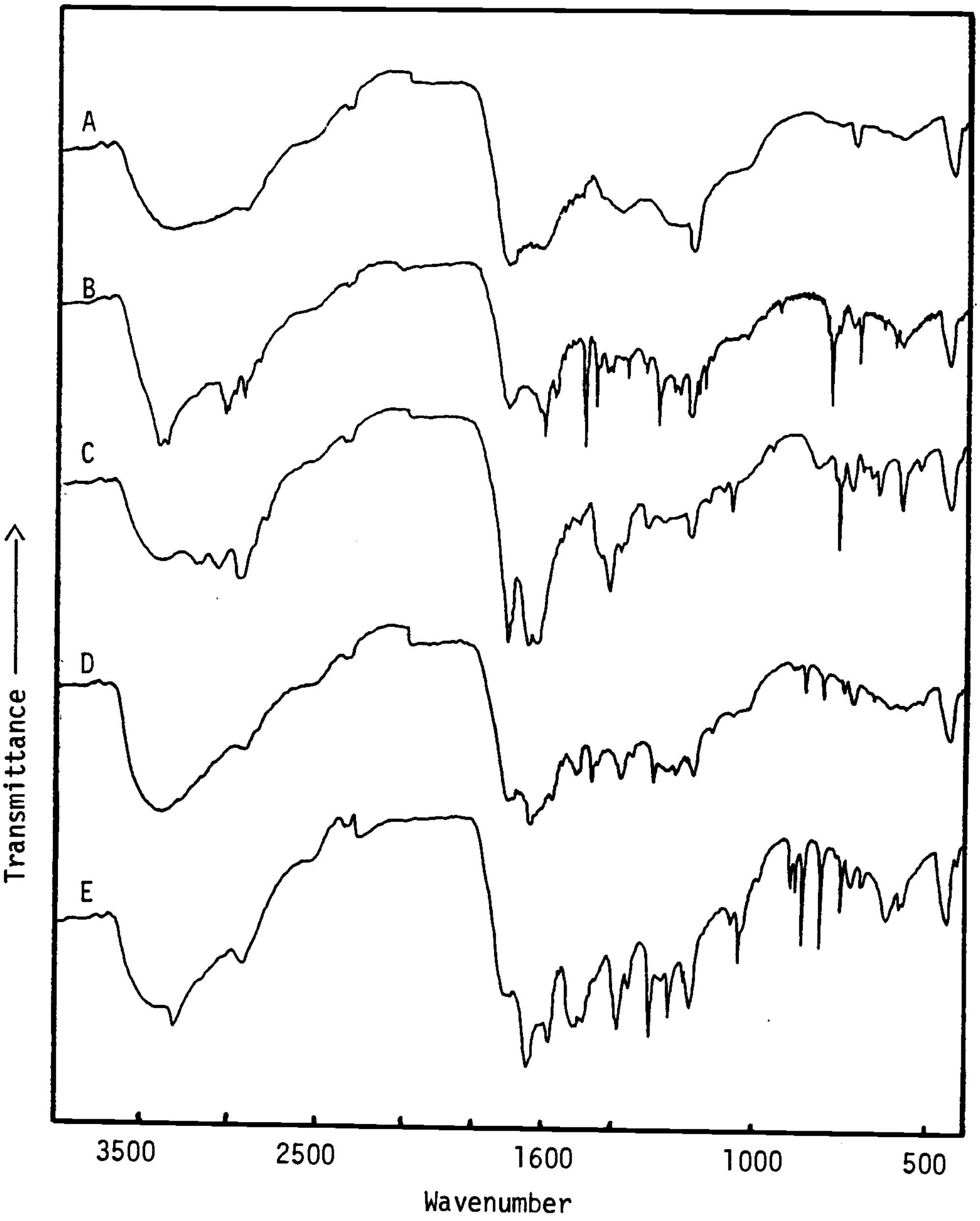

Figure 12. Infrared spectra of (A) humic acid (HA), (B) HA-diquat, (C) HA-bromacil, (D) HA-diuron, and (E) HA-chlorotoluron.

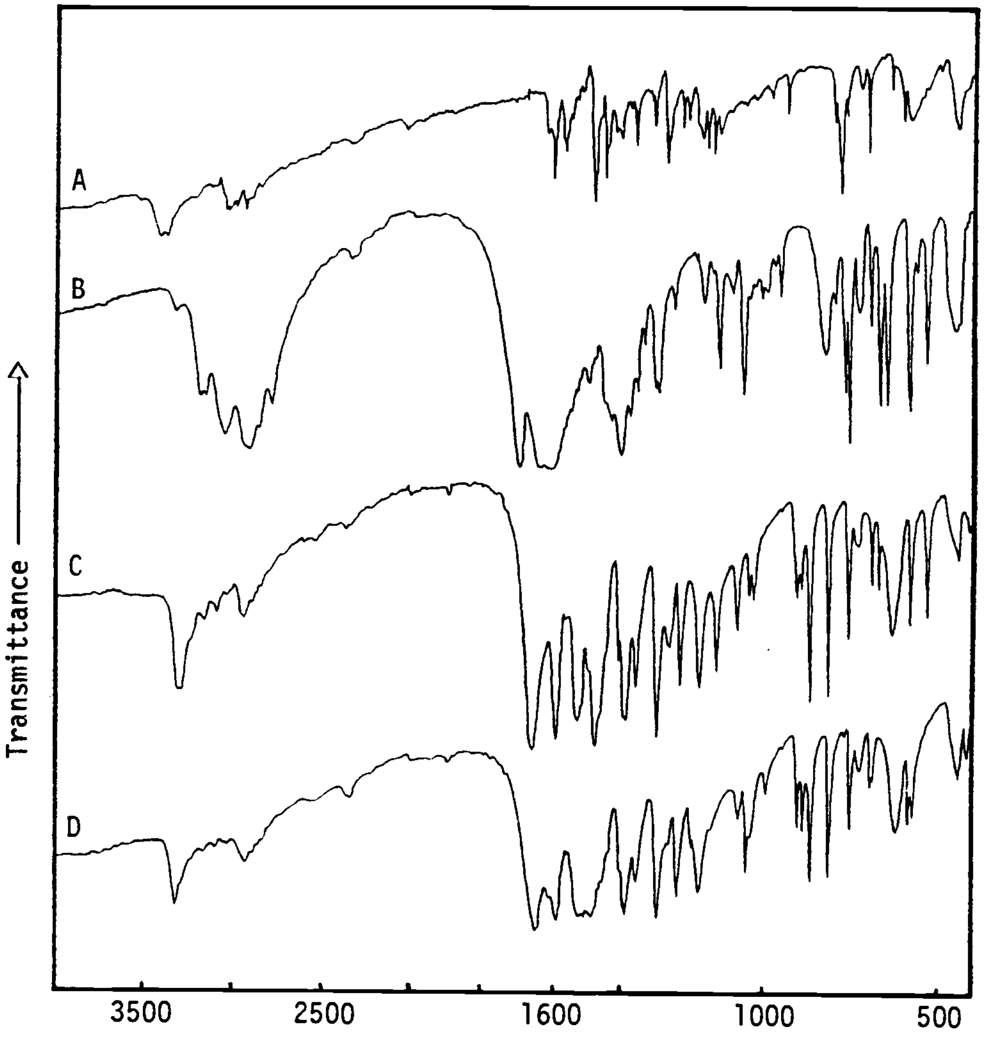

Figure 13. Infrared spectra of herbicides (A) diquat, (B) bromacil, (C) diuron, and (D) chlorotoluron.

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CHAPTER III

Association of Selected Herbicides With Water Soluble Organic Materials from Soil[1]

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ABSTRACT

The binding of six herbicides: diquat (1,1-ethylene-2,2-bipyridylium ion), glyphosate [N-(phosphonomethyl) glycine], simazine (2-chloro-4,6-bis-ethylamino-s-triazine), bromacil (5-bromo-3-sec-butyl-6-methyluracil), diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea], and chlorotoluron [3-(3-chloro-4-methylphenyl)-1,1-dimethylurea] by organic materials extracted with distilled water from an organic soil was studied by gel filtration chromatography. The gel filtration method revealed that most constituents of the water soluble soil organic materials (WSSOM) had molecular weights between 5000 and 700 daltons. Chromatographic procedures, as adapted by Hummel and Dreyer (1962) to detect interactions between small organic compounds and macromolecules, were very successful in proving the existence of binding between the herbicides and WSSOM. Observed differences in the elution curves reflected the characteristics of the interacting herbicides as well as their associations with WSSOM. The nonionic herbicides bromacil, diuron, chlorotoluron, and simazine showed comparable affinity for the three main fractions of WSSOM, whereas the herbicide glyphosate showed stronger tendency to complex with the most retarded fraction of WSSOM. The elution data reinforced the importance of adhering to the method requirements. Changes in infrared (IR) band position and intensity, particularly near 1640 cm^{-1} in the herbicides-WSSOM IR spectra, provided strong evidence for the involvement of an ion exchange mechanism in diquat and of hydrogen bonding in bromacil, diuron, and chlorotoluron interactions with WSSOM. Additionally observed shifts in some characteristic IR absorption bands suggested the formation of charge transfer complexes between bromacil, diuron, and chlorotoluron and WSSOM but not between diquat and water soluble soil organic materials. These results appear significant in assessing herbicides mobility and transport in soil and water, especially when water pollution is a matter of concern.

Additional Index Words: gel filtration, infrared spectroscopy, bromacil, diuron, chlorotoluron, diquat, glyphosate, simazine.

INTRODUCTION

Soil organic materials have been the subject of considerable interest in recent years because of their ability to interact with pesticides in soil and water. Compounds that reportedly interact with humic substances include s-triazines (Khan, 1978; Müller-Wegener, 1977; Gilmour and Coleman, 1971; Sullivan and Felbeck, 1968; Senesi and Testini, 1980 and 1982); bipyridilium ions (Burns et al., 1973a, 1973b; Khan, 1973, 1974); substituted ureas (Gaillardon et al., 1980; Khan and Mazurkewich, 1974; Senesi and Testni, 1980, 1983); and chlorinated hydrocarbons (Ballard, 1971; Choi and Chen, 1976; Pierce et al., 1974). According to Ballard (1971), the downward movement of the insecticide DDT in a forest soil is caused by water-soluble, humic-like substances. Ogner and Schnitzer (1970) reported that fulvic acid, a water-soluble humic component with relatively low molecular weight which occurs widely in soils and waters, can combine with lyophobic organic compounds such as alkanes and fatty acids to form stable water-soluble complexes. Pierce et al. (1974) suggested that suspended humic particulates in aqueous solutions may serve as transporting agents for chlorinated hydrocarbons through the water column and for accumulating them in sediments.

In view of the foregoing, water-soluble soil organic substances may have special functions with regard to herbicide mobilization and transformation, i.e. they may enhance the solubility of certain pesticides (Wershaw et al., 1969), may act as vehicles for the transport of pesticides in soils and natural water, and may function also in mediating and promoting the nonbiological degradation of many pesticides (Khan, 1978; Stevenson, 1982). Yet, precise characterization of the interactions occurring between humic substances particularly the water-soluble components and pesticides has remained quite difficult.

Various methods have been used to study the interaction of pesticides with soil and with isolated humic substances. The most common approach has been the slurry method which involves the bringing together of known amounts of solid and herbicide solution; then after equilibration, the solution concentration is measured and the decrease in the herbicide concentration is determined. This technique is not suitable in evaluating herbicide binding by soil organic substances, especially the water soluble components (Hayes, 1970), because it makes no distinction between the herbicide molecules that exist free in soil solution and those bound to water-soluble soil organic materials.

A gel chromatography technique based on the procedures of Hummel and Dreyer (1962) provides an appropriate alternative for determining the binding of small organic chemicals such as pesticides by water-soluble humic polymers. The procedure has been used by a few workers to study the binding of herbicides (diquat and paraquat) by humic substances (Burns et al., 1973b; Khan, 1973). More recently we utilized the Hummel and Dreyer method to study the binding of several ionic and nonionic herbicides by a soil humic acid (Chapter II).

Among the several spectrometric techniques that can be used to characterize the nature of the binding mechanism(s), infrared spectroscopy has been the most commonly chosen. This technique has shown the involvement of an ion exchange process in diquat, paraquat, chlordimeform and triazines (Burns et al., 1973a; Khan, 1973, 1974; Maqueda et al., 1983; Senesi and Testini, 1980; Sullivan and Felbeck, 1969); hydrogen bonding in triazines and substituted ureas (Senesi and Testini, 1980, 1983); and charge transfer complexes in triazines, substituted ureas, and bipyridilium herbicides binding by soil humic substances (Khan, 1973, 1974; Müller-Wegener, 1977; Senesi and Testini, 1980, 1982, 1983). We have obtained evidence for ion exchange reactions in diquat and H-bonding in bromacil, diuron and chlorotoluron association with humic acid (Chapter II).

The objectives of this study were: (i) to assess the interactions between herbicides and the water soluble organic materials extracted from soil; (ii) to establish the applicability of the Hummel and Dreyer gel filtration procedure as an alternative method in binding studies involving water soluble soil organic compounds; and (iii) to characterize the binding mechanisms the herbicides undergo when in association with dissolved organic substances utilizing infrared spectroscopy. This work is a parallel investigation to studies on reactions between these herbicides and larger molecular weight humic substances extracted from soil.

MATERIALS AND METHODS

Water soluble soil organic materials (WSSOM) were extracted from Semiahmoo mucky peat soil. The soil sample was collected from the surface layer (0-20 cm) from a farm near Salem, Oregon. The soil was chosen because of its high organic carbon content of 27.8%. The organic carbon content was determined from the weight loss upon ignition multiplied by 0.58. WSSOM was extracted by mixing a known quantity of soil in a measured volume of distilled water in Corex glass centrifuge tubes to give 1:5 soil to water ratio, and equilibrating on a mechanical shaker for 24 h at 25° C. Following centrifugation at 1085 RCF for 1 h, the yellow clear supernatant was removed and filtered through Carl Schleicher and Schuell filter paper No. 597. The carbon analysis was performed on an Oceanography International total carbon analyzer, model 0524, equipped with Lira IR detector model 303. The carbon content of WSSOM was 238 mg L^{-1}. The pH of this extract was 5.50. WSSOM was usually used immediately after preparation or stored at 3° C with no chemical additives. WSSOM was discarded if not used within 24 h from the time of preparation.

The herbicides selected for the study included the ionic compounds diquat and glyphosate, and the nonionic chemicals bromacil, diuron, chlorotoluron and simazine. These compounds were chosen because they represent major herbicide groups and are widely used on agricultural crops. The herbicides were ^{14}C-labelled and were >99% analytically and radiochemically pure.

The gel filtration procedure of Hummel and Dreyer (1962) was employed to descry any interaction between herbicide molecules and water soluble organic materials. This chromatographic method, which is analogous in principle to dialysis equilibrium, was devised to detect reversible interactions between macromolecules and substances of low molecular weight. It avoids the difficulties encountered in zonal gel filtration approach due to binding of ionic and aromatic compounds with the Sephadex gel. It also eliminates the problem of dissociation of macromolecule - ligand complex in the chromatographic column.

The gel filtration procedure was carried out by packing a 26 x 370 mm LKB 2137 chromatographic column with fine mesh, Sephadex G-25 gel (exclusion limit 5000). The column was equilibrated with phosphate-buffer solution of pH 6.85 and ionic strength 0.05M KCl. Blue Dextran 2000, at 2 g L^{-1} concentration was used to determine the uniformity of packing and the void volume of the column.

The elution profile of the water-soluble organic materials was determined by applying 20 mL of WSSOM to the column and eluting with the buffer at 25° C in the descending direction using a peristaltic pump driven by a motor at a flow rate of 60 mL h^{-1}. The emerging eluate was collected in 5 mL portions on a fraction collector (ISCO, model 328). The WSSOM concentration in each fraction was assessed by measuring the absorbance at 400 and 280 nm on a Beckman, model 25, UV-Visible Spectrophotometer.

Interactions between water-soluble organic materials and herbicides were determined by adding 20 mL of the following $\mu\underline{M}$ concentrations of WSSOM-herbicide solutions to the column: bromacil, 0.966; chlorotoluron, 0.311; diuron, 1.01; diquat, 0.125; glyphosate, 1.01; and simazine, 0.109. The chromatographic column was eluted with the phosphate buffer which had an identical herbicide concentration as in the WSSOM-herbicide solution introduced to the column. The column effluent was continuously monitored by ISCO model UA-2 dual UV analyzer set at 280 nm wavelength to follow the elution behavior of WSSOM-herbicide complex. The herbicide concentration in each fraction was determined by taking 0.5 mL aliquots and measuring the radioactivity in solution with Packard model 3330 Tri-Carb Liquid Scintillation Spectrometer after adding 3.5 mL of Handifluor counting fluid. An internal standard was used to evaluate the instrument counting efficiency regularly throughout the study period.

Infrared (IR) analysis was performed on a Perkin-Elmer model 457 grating infrared spectrophotometer in the 4000 to 250 cm^{-1} wavenumber range in KBr solvent matrix. Herbicide solutions of 1 m$\underline{M}$ concentration were prepared by dissolving 7.2 mg diquat, 5.2 mg bromacil, 4.7 mg diuron, and 4.2 mg chlorotoluron in 20 mL WSSOM in Corex centrifuge tubes. The tubes were securely capped and shaken on a mechanical shaker for 15 h before subjected to freeze-drying to remove the excess moisture. The pellets were prepared by mixing 250 mg KBr with 1 mg of the freeze-dried sample. The IR recording conditions were the following: reference, air; cell path, 1 mm; slit, normal; and scan time, medium. A polystyrene film with 0.05 mm cell path was regularly used to calibrate absorption band positions.

RESULTS AND DISCUSSION

Gel Filtration

The elution curves of the straw-colored, water-soluble soil organic materials determined on Sephadex G-25 and G-10 gels are shown in Fig. 14. The elution curve obtained on Sephadex G-25 shows a plateau with three peaks. Based on this elution behavior WSSOM may be divided into three main fractions with regard to molecular weight. Fraction 1, whose molecular weight is ⩾ 5000, was eluted with the void volume, whereas, the fraction whose components have intermediate molecular size (fraction 2) emerged in the middle of the elution curve. Fraction 3, which produced the strongest peak in the elution curve, emerged last from the column and corresponds to the fraction with the smallest molecular weight. Since, on Sephadex G-10, whose exclusion limit is 700, WSSOM eluted in a single sharp peak at the void volume with little tailing (Fig. 14), the molecular size of the majority of WSSOM components is most likely between 700 and 5000 daltons.

Humic substances generally comprise one-third to one-half of the dissolved organic carbon in surface waters (Malcolm, et al., 1981; Martin and Reuter, 1973; Thurman, et al., 1982). The molecular size of dissolved organic materials in water varies, due to such factors as sourceof material, method of determination, and aggregation of humic substances in solution (Thurman et al., 1982). Most values, however, are in the molecular weight range of 500 to 10000 daltons (Martin and Reuter, 1973; Oliver and Thurman, 1983; Thurman et al., 1982). The reported molecular weight range of aquatic fulvic acids is 500 to 2000 daltons (Malcolm et al., 1981; Thurman and Malcolm, 1983; Thurman et al., 1982). Other researchers have shown that fulvic acids from soil also are in that same range (Ogner and Schnitzer, 1970). The aquatic organic substances resemble soil fulvic acids by being water-soluble and of relatively low molecular weight (Baham and Sposito, 1983; Beck et al., 1974).

The elution behavior of bromacil-WSSOM, given in Fig. 15a, shows an ideal performance of the gel filtration method. The three distinct peaks coincided nicely with WSSOM peaks indicating that the three main fractions of WSSOM complexed with the herbicide. Fig. 15a also shows that more bromacil complexed with fraction three of WSSOM (low MW fraction) than with other components of WSSOM. In Fig. 15b, the elution curve of bromacil is shown superimposed on bromacil-WSSOM elution curve. The emergence of the herbicide-WSSOM trough at the elution volume of bromacil should be noted since it fulfills a criterion for the binding of the herbicide with WSSOM (Hummel and Dreyer, 1962).

The elution diagrams of the remaining nonionic herbicides diuron, chlorotoluron, and simazine complexes with the water-soluble soil organic substances are shown in Figs. 16, 17, and 18. Common features in the three curves are the multiple peaks coinciding with WSSOM peaks and the appearance of herbicide-WSSOM complex troughs at the same elution volumes as the herbicides. As in bromacil, these herbicides appear to form complexes with all three major components of WSSOM and the affinity of the herbicides for the three fractions seem very comparable. These patterns provide further evidence for the existence of interactions between herbicides and WSSOM and corroborate the reproducibility of the experiments as well as the adequacy of the procedure.

The elution curves of the herbicides glyphosate and diquat shown in Figs. 19 and 20, look rather different from the nonionic herbicides discussed above. Glyphosate-WSSOM complex shows a single sharp peak coincidental with fraction 3 peak of WSSOM and a trough appeared near the elution volume of glyphosate. This elution behavior suggests that glyphosate has a very high affinity for the most retarded (lowest MW) fraction of WSSOM. However, this strong tendency to associate with fraction 3 does not mean the lack of affinity for the other constituents of WSSOM since earlier results show that glyphosate interacts and forms complexes with humic acids from soil. The apparent lack of interaction between glyphosate and the relatively large-sized WSSOM components could be due to the low concentration of glyphosate. The sharp drop from the peak maxima to the trough minima in the elution curve (Fig. 19) was necessary since the elution volume of glyphosate is near where the trough has emerged.

Diquat-WSSOM elution diagram (Fig. 20) is different and shows nonideal behavior. The same pattern was obtained each time the elution experiment was repeated. Fig. 20a shows that WSSOM fractions 1 and 2 interacted with the herbicide as indicated by the coincidence of the peaks. The disappearance of a peak corresponding to fraction 3 complex with diquat was due to its occurrence in the herbicide zone (Fig. 20b) where the strong and sharp trough appeared. The presence of a second broad but rather sharp trough in the elution curve may be due to the occurrence of a second diquat deficient zone resulting from the complications brought about by the overlapping of fraction 3 plus diquat complex peak and the herbicide trough. Hence, this strange looking pattern is most likely due to the coincidence of fraction 3 complex peak with the herbicide trough and it may also be related to the existence of interaction between the gel matrix and diquat and the gel and WSSOM components, particularly those with low molecular weight. One of the principal requirements for a satisfactory performance of the gel filtration method is the separation of the complex peak from the trough by a distance in which the herbicide concentration returns to its original level (Fairclough and Fruton, 1966; Hummel and Dreyer, 1962; Wood and Cooper, 1970). Obviously this demand was not met in glyphosate or diquat association with WSSOM. The relevance of the Hummel and Dreyer (1962) method for studying the interaction of these two herbicides with humic materials from soil has been documented in an earlier study by these authors (Chapter II).

The potential role of WSSOM in pesticides mobility and transport in the soil and water environments has long been suggested (Ballard, 1971; Ogner and Schnitzer, 1970; Pierce et al., 1974; Wershaw et al., 1969) but little examined for lack of an adequate procedure. In this study, data have been presented in favor of the chromatographic method developed by Hummel and Dreyer (1962). The procedure is well suited and probably is the method of choice for the study of ionic and nonionic pesticide interactions with WSSOM as it was with soil humic substances when the method is adequately applied and its requisites are fulfilled.

Infrared Spectroscopy

Infrared spectra of untreated WSSOM, herbicides, and WSSOM-herbicide complexes were recorded to characterize the binding mechanisms occurring in WSSOM-herbicide complexes. The IR spectrum of the untreated WSSOM preparation displays broad and poorly resolved absorption bands reflecting the presence of many kinds of functional groups in differing chemical environments. Although the infrared data provide a wealth of information about the sample, many of the absorption bands can not be interpreted unequivocally (Silverstein et al., 1981; Dyer, 1965).

The infrared spectrum of WSSOM reproduced as pattern A in Fig. 21 shows absorption bands in the range 3700-3000 cm^{-1} (centered at 3400), assigned to intermolecular H-bonded O-H groups, and N-H and aromatic C-H stretching vibrations; near 2940 cm^{-1}, due to aliphatic C-H stretching; in the region 1800-1500 cm^{-1}, attributed to (i) C=O stretching, (ii) aromatic C=C stretching, (iii) H-bonded C-O groups of quinones and ketones, (iv) COO^- symmetric stretching, (v) N-H bending vibrations; near 1390 cm^{-1}, due to C-H and O-H bending, C-O stretching of alcohols and phenols, and COO^- asymmetric stretching; and in the 1200-1000 cm^{-1} region, assigned to O-H deformation and C-O stretching of COOH (Stevenson, 1982; Silverstein et al., 1981; Dyer, 1965).

The spectrum of WSSOM is similar to the spectra of humic and fulvic acids reported by Senesi and Testini (1980), Stevenson and Goh (1971), Sullivan and Felbeck (1968). Further, the spectrum closely resembles the IR pattern published by Baham and Sposito (1983) for the water-soluble organic fraction of Rialto sewage sludge. These authors demonstrated the occurrence of polypeptides, amino sugars, aliphatic acids and carbohydrates in their preparation, suggesting the presence of these substances in addition to humic materials in WSSOM.

The most significant differences between diquat-WSSOM spectrum

(Fig. 21) and the untreated WSSOM spectrum is the increased intensity of the broad band centered at 1640 cm^{-1}. As mentioned above, this absorption band is attributable to a number of functional groups, one of which is the carbonyl group of carboxylic acid (COOH). The presence of a rather weak shoulder at 1720 cm^{-1} provides further indication for the occurrence of free carboxylic acid in the WSSOM preparation (Dyer, 1965). The appearance of a fairly sharp band in the 1615 cm^{-1} region with the diminishing of the weak shoulder at 1720 cm^{-1} provides indication for the conversion of COOH to COO^- upon the addition of the herbicide suggesting the involvement of an ion exchange mechanism in diquat binding with WSSOM. These results are entirely consistent with those discussed in other sections and with data other researchers reported for diquat interactions with humic and fulvic acids (Burns et al., 1973a; Khan, 1974).

In this study, indication was found in the diquat-WSSOM spectrum for the formation of charge transfer complexes, suggesting that either this mechanism is not involved or that the absorption bands corresponding to these complexes were not well resolved to permit positive identification. Khan (1973, 1974) presented data for the formation of charge transfer complexes between diquat and the humic compounds; however, his results could not be confirmed by these workers nor by others (Burns, 1973a). The occurrence of a charge transfer mechanism in diquat-WSSOM seems highly possible since humic materials have been shown to possess charge transfer donor and acceptor properties (Lindqvist, 1982, 1983).

The IR spectra for the herbicides bromacil, diuron, and chlorotoluron interaction products with WSSOM (C and D Fig. 21) display several significant divergences from the spectra of the pure herbicides and untreated WSSOM. Like diquat-WSSOM spectrum, these spectra show changes in the broad absorption band centered near 1640 cm^{-1} (overlapping of C=O stretching, amide I band, and N-H bending, amide II band). Unlike diquat, however, these herbicides are nonionic and very weakly basic. Therefore, the observed sharpening and increase in intensity of the 1640 cm^{-1} band, particularly in diuron and bromacil, can not be explained by the conversion of COOH to COO^- as in diquat (Stevenson, 1972; Senesi and Testini, 1980, 1983). These effects are most likely due to the shifting towards lower wavenumbers of the O-H and N-H bending and C-O stretching frequencies of the N-H, O-H and C-O groups of WSSOM when involved in hydrogen bonding with the herbicides (Silverstein et al., 1981). Depending on the strength of the H-bonding, the frequency reduction ranges from less than 15 cm^{-1} to 50 cm^{-1} or more (Silverstein et al., 1981).

Hydrogen bonding can occur in any system that contains a proton donor group such as O-H and N-H, and a proton acceptor like oxygen, nitrogen, and halogens. The herbicides diuron, chlorotoluron and bromacil possess such chemical moieties as the carbonyl and amide groups which offer strong possibility for them to undergo H-bonding with WSSOM (Stevenson, 1982). The IR data, therefore, confirmed the kind of bonding expected from knowledge of the chemical properties of the herbicides.

Other changes in the IR spectra of the herbicides which support the formation of H-bonding include: the increased sharpness, especially in bromacil and chlorotoluron, of the absorption band near 1384 cm^{-1}, attributed to O-H bending and C-O stretching vibrations; and the observed alteration of bands particularly in chlorotoluron in the range 1200-1040 cm^{-1}, arising from C-O stretching and O-H bending. Hydrogen bond formation has been one of the suggested (Hayes, 1970; Stevenson, 1972; Weed and Weber, 1974) and experimentally demonstrated mechanisms for the binding of several triazine and substituted urea herbicides (Senesi and Testini, 1980, 1983; Sullivan and Felbeck, 1968).

Careful study of the nonionic herbicides-WSSOM spectra reveal some very interesting observations. The band at 1420 cm^{-1} in bromacil has been shifted to a new position (1384 cm^{-1}) and the 738 cm^{-1} band apparently moved to lower frequency (730 cm^{-1}), whereas the absorption band at 840 cm^{-1} has either disappeared or overlapped with the band at 770 cm^{-1} in bromacil-WSSOM. Similar shifts were observed in diuron-WSSOM absorption vibrations also; the 870 cm^{-1} shifted to 863 cm^{-1} and the 820 cm^{-1} appeared at 810 cm^{-1} in diuron-WSSOM complex spectrum. These shifts may be due to changes in the out-of-plane C-H vibration frequencies, suggesting the formation of charge transfer complexes between the herbicides and WSSOM components. Experimental evidence for the formation of charge-transfer complexes between a number of triazine and substituted urea herbicides and other chemicals has been reported (Maqueda et al., 1983; Senesi and Testini, 1980, 1982, 1983).

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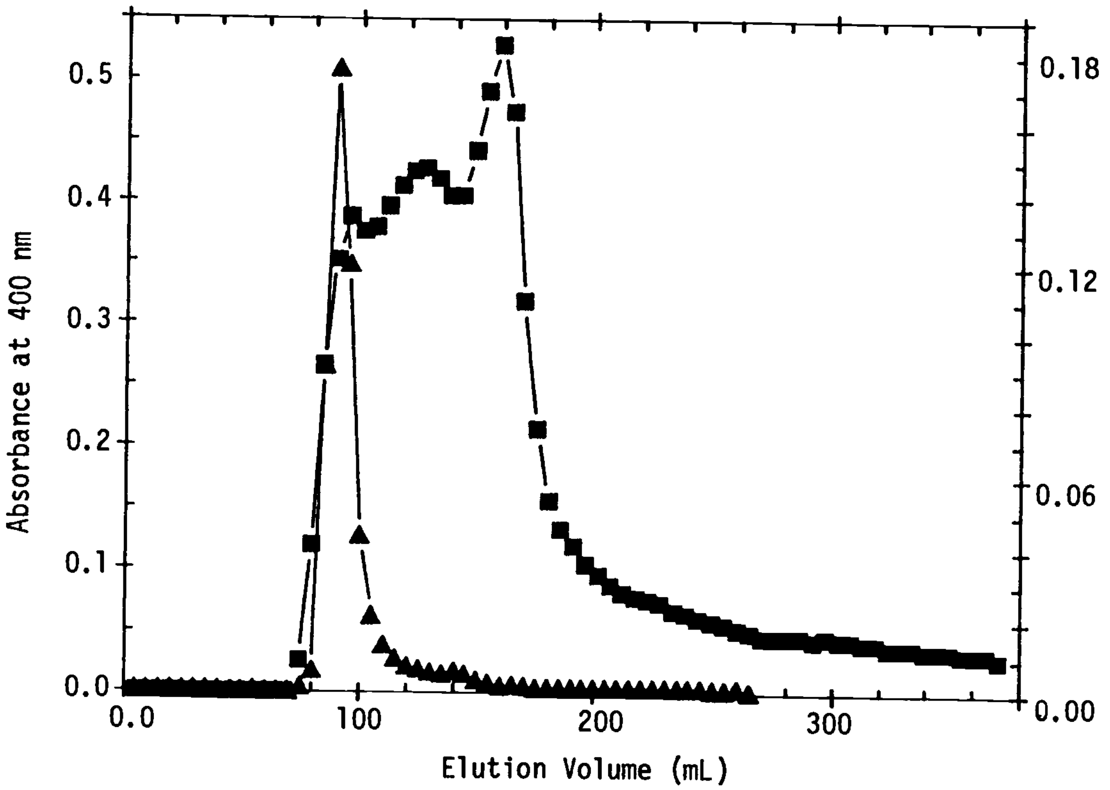

Figure 14. Elution behavior of water soluble soil organic materials (WSSOM) determined on Sephadex G-25 (■) and G-10 (▲, right-Y-axis) gels.

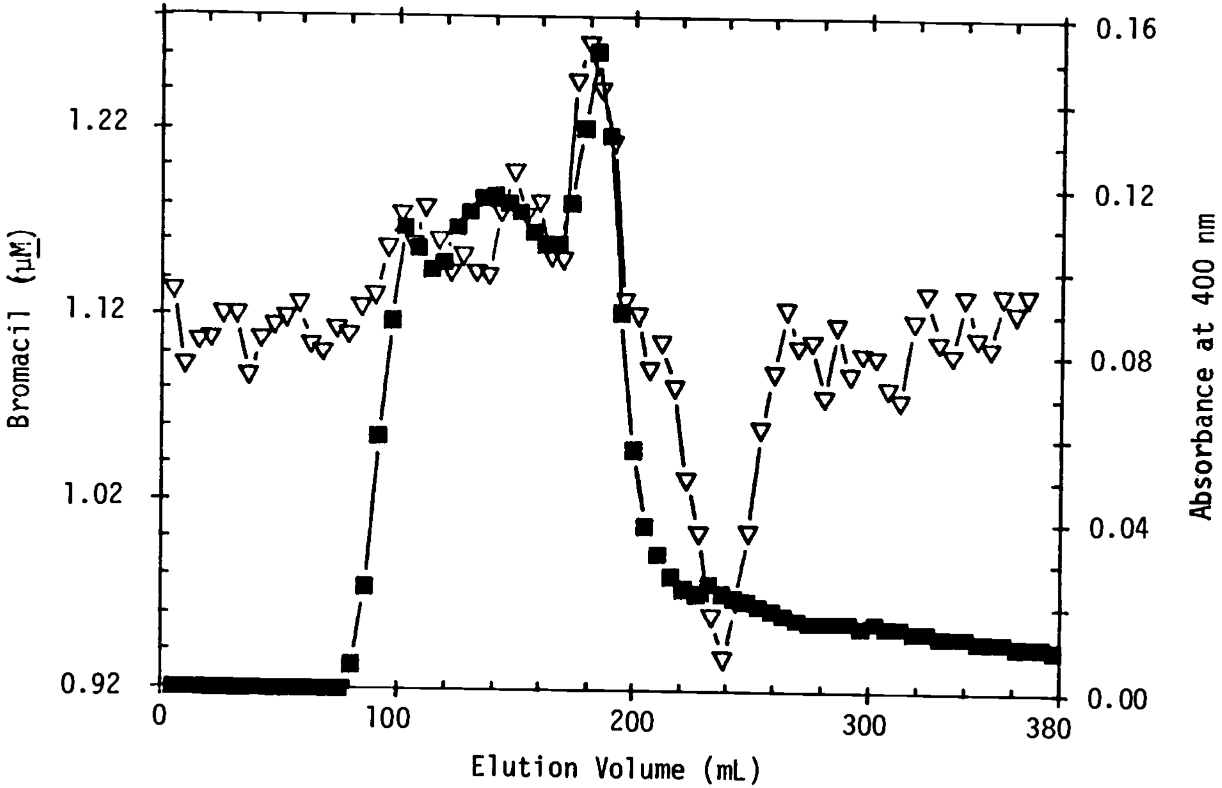

Figure 15a. Elution profile of WSSOM-bromacil (▽) superimposed on elution curve of WSSOM (■, right-Y-axis) determined on Sephadex G-25 gel.

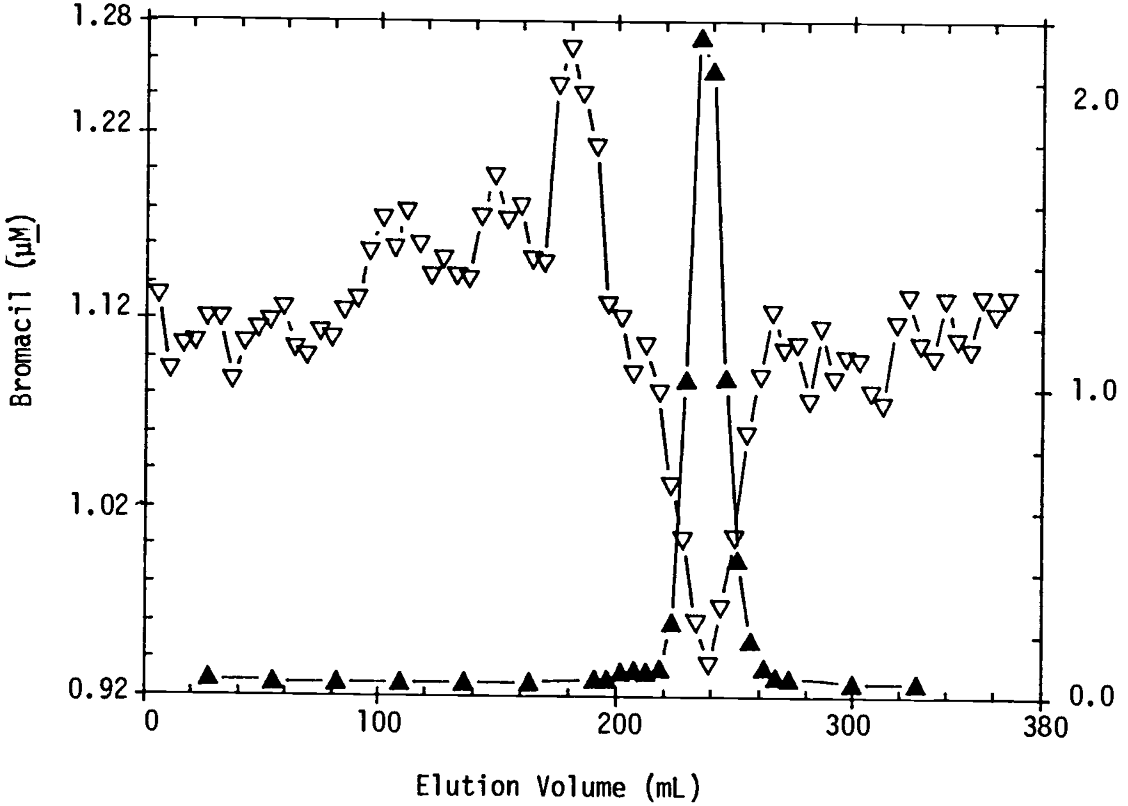

Figure 15b. Elution profile of WSSOM-bromacil (▽) superimposed on elution curve of bromacil (▲, right-Y-axis) determined on Sephadex G-25 gel.

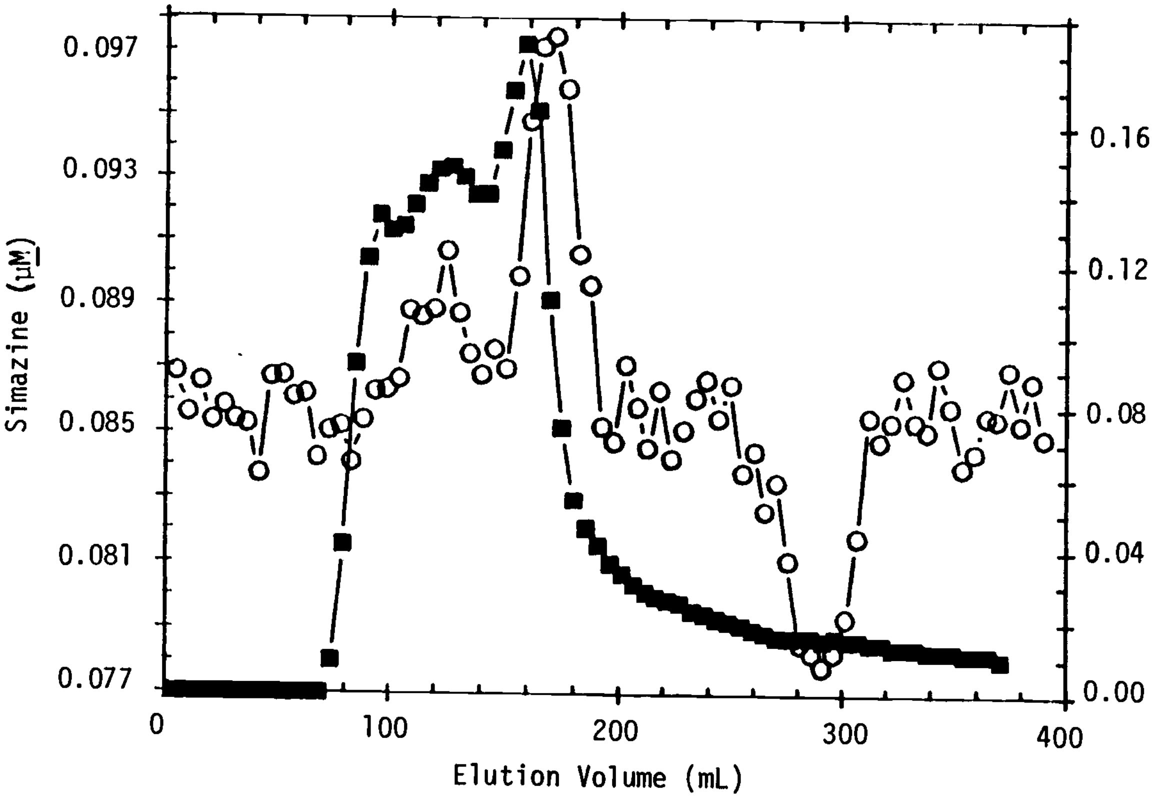

Figure 16. Elution profile of WSSOM-Simazine (○) superimposed on elution curve of WSSOM (■, right-Y-axis) determined on Sephadex G-25 gel.

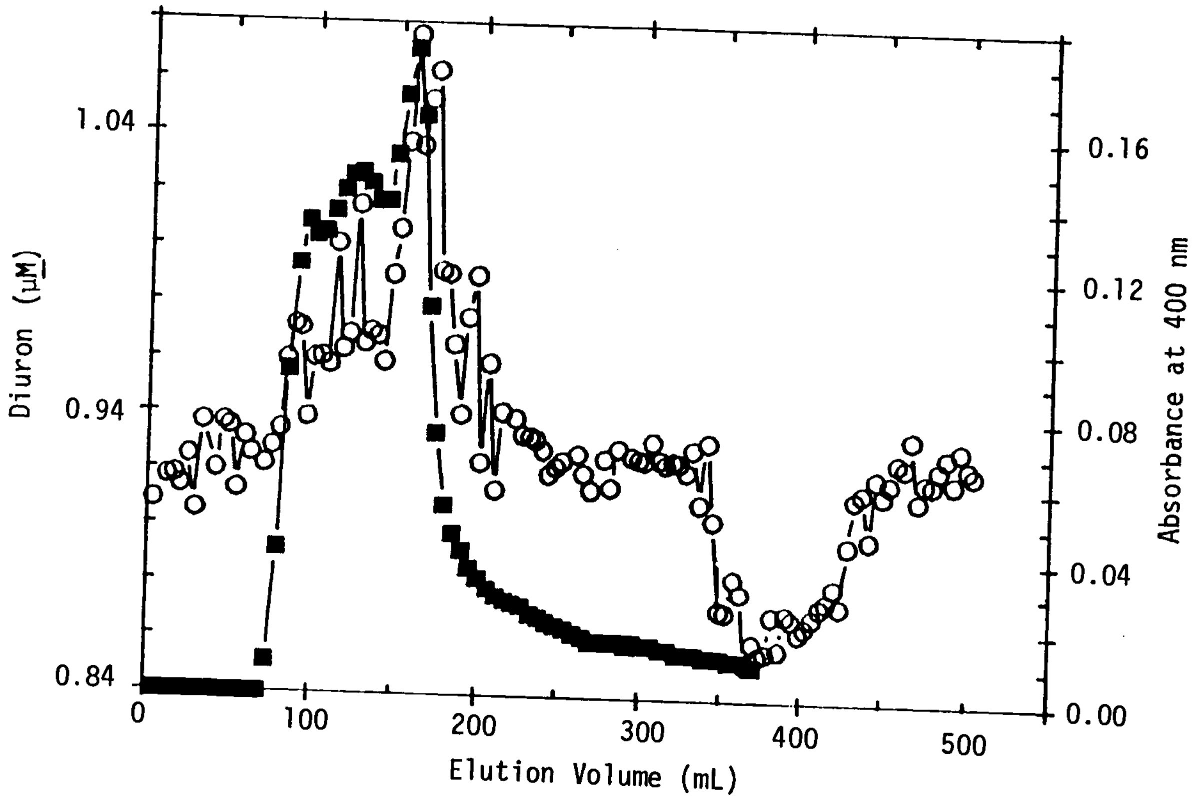

Figure 17. Elution profile of WSSOM-diuron (○) superimposed on elution curve of WSSOM (■, right-Y-axis) determined on Sephadex G-25 gel.

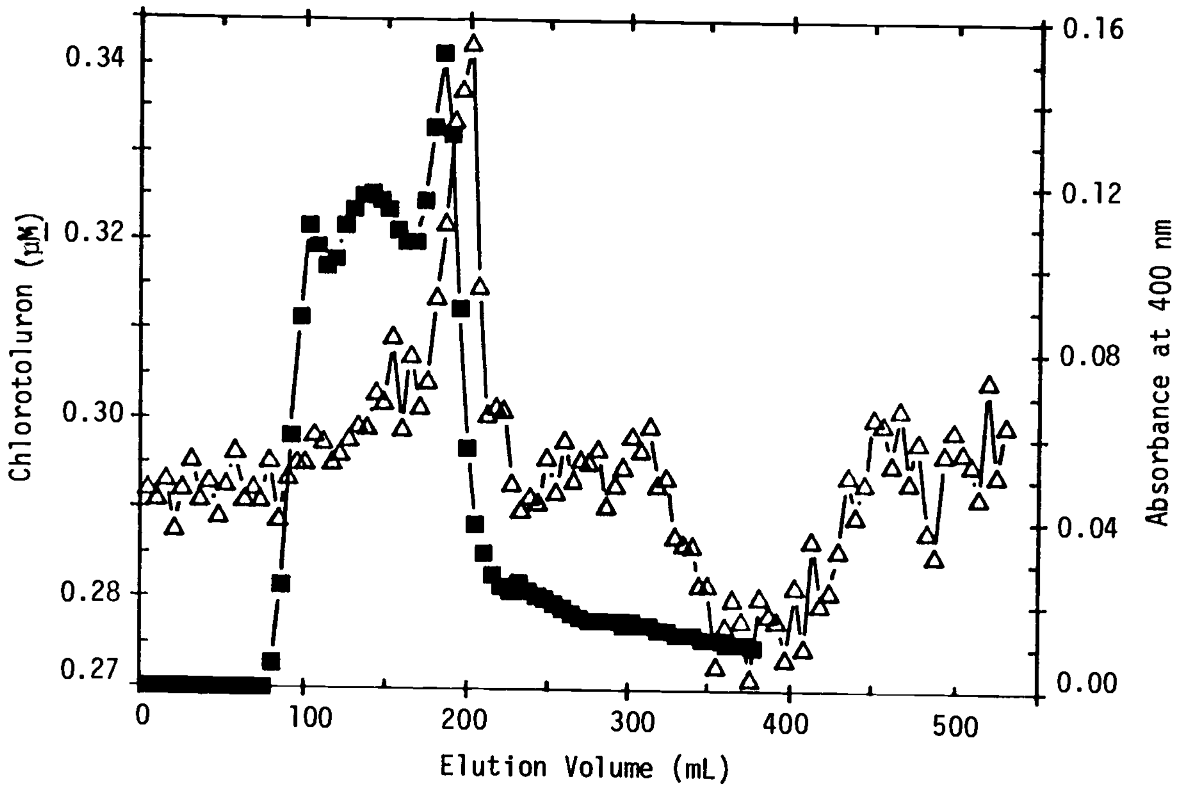

Figure 18. Elution profile of WSSOM-chlorotoluron (△) superimposed on elution curve of WSSOM (■, right-Y-axis) determined on Sephadex G-25 gel.

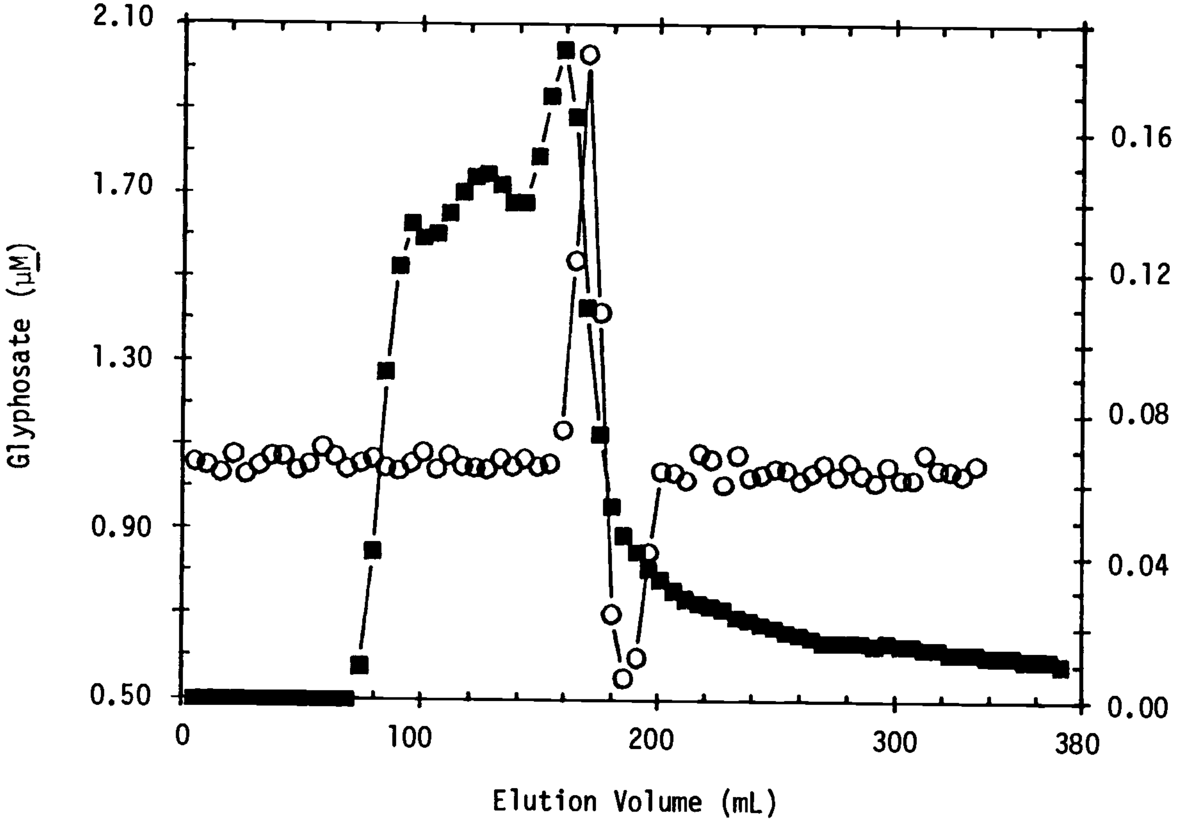

Figure 19. Elution profile of WSSOM-glyphosate (o) superimposed on elution curve of WSSOM (■, right-Y-axis) determined on Sephadex G-25 gel.

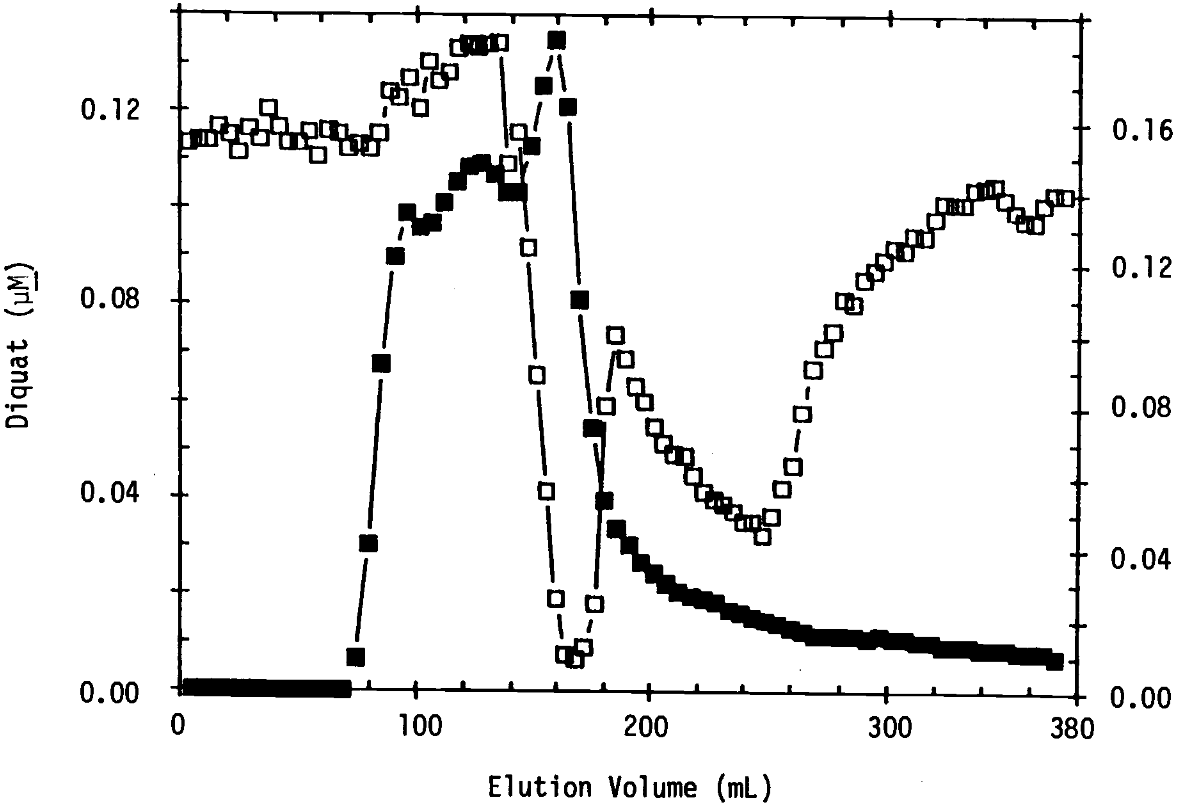

Figure 20a. Elution profile of WSSOM-diquat (□) superimposed on elution curve of WSSOM (■, right-Y-axis) determined on Sephadex G-25 gel.

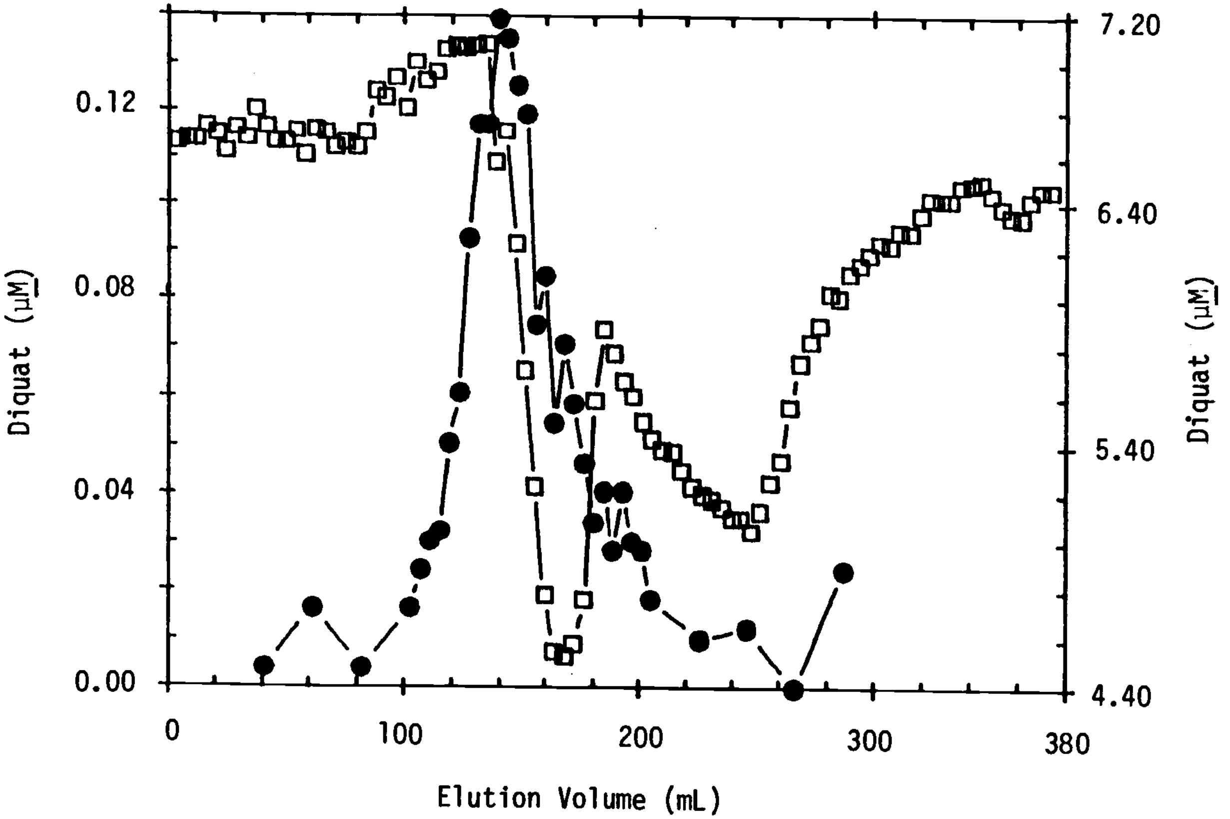

Figure 20b. Elution profile of WSSOM-diquat (□) superimposed on elution curve of diquat (●, right-Y-axis) determined on Sephadex G-25 gel.

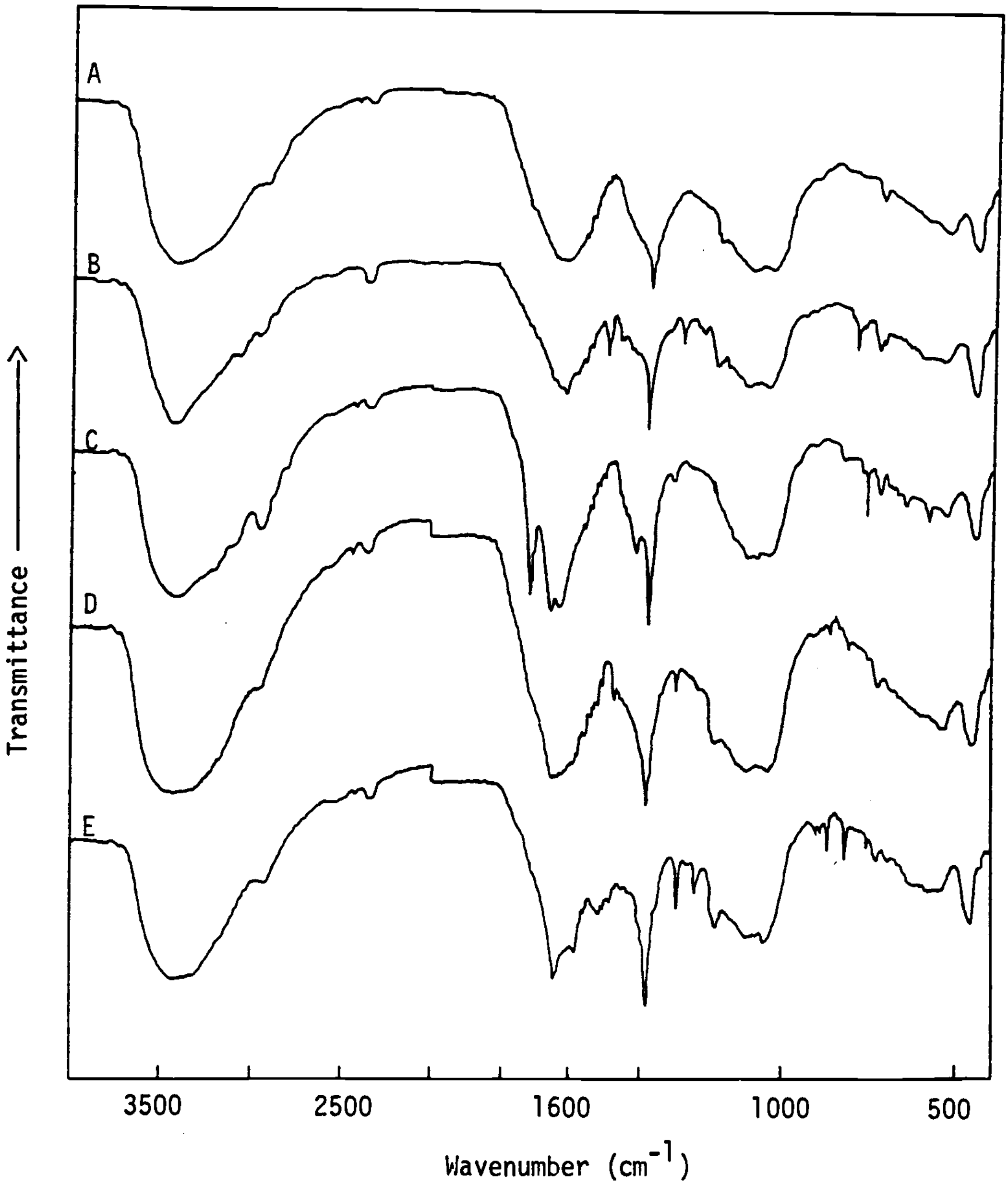

Figure 21. Infrared spectra of (A) WSSOM, (B) WSSOM-diquat, (C) WSSOM-bromacil, (D) WSSOM-diuron, and (E) WSSOM-chlorotoluron.

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CHAPTER IV

Degradation of Diuron, Chlorotoluron, and Bromacil by Soils at Three Temperatures[1]

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ABSTRACT

The rate of diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea], chlorotoluron [3-(3-chloro-4-methylphenyl)-1,1-dimethylurea], and bromacil (5-bromo-3-sec-butyl-6-methyluracil) degradation in two soils was measured at 25, 30, and 35° C. Degradation was assessed by monitoring $^{14}CO_2$ evolution from herbicide-treated soils. The degradation of the three herbicides was slow with no lag phase. Diuron and chlorotoluron break down was higher in Adkins loamy sand than in Semiahmoo mucky peat. Bromacil degradation was essentially unaffected by soil type. The affect of temperature on degradation was more pronounced than the effect of soil type or herbicide concentration. The rate of $^{14}CO_2$ production was approximately tripled by a 10-degree rise in temperature from 25 to 35° C. However the rate of total CO_2 evolution from soil only doubled. The presence of herbicides in the soil did not affect the activity of the microorganisms as measured by the total CO_2 production, indicating that the degradation was a co-metabolism process. Degradation of the three compounds followed a first-order rate law. The estimated half-lives ranged from 223 to 4101 days for diuron, 288 to 3397 days for chlorotoluron and 2596 to 46200 days for bromacil. Evaluation of the rate of degradation at three temperatures permitted calculation of the energy of activation from the Arrhenius equation.

Additional Index Words: First-order kinetics, half-life, $^{14}CO_2$ evolution, co-metabolism.

INTRODUCTION

Knowledge of decomposition of herbicides in soil is important to both agricultural management and environmental safety. The main processess leading to degradation of pesticides in soil are biological action, photochemical reaction and chemical reaction either operating independently or in combination. It may not be reasonable to expect a simple straight-forward behavior in degradation in so complex a milieu as the soil (Freed and Haque, 1973). In general, however, the dominant route for the loss of many herbicides in soil is microbial metabolism (Ashton, 1982; Freed and Haque, 1973).

Microbial involvement in decomposition of many pesticides including the substituted urea herbicides - of which diuron and chlorotoluron are examples - as well as bromacil has been shown by reduced degradation in sterilized soil (Hill et al., 1955; Wolf and Martin, 1974), evolution of $^{14}CO_2$ from soil treated with ^{14}C-labeled compounds (Gardiner et al., 1969; Gerstl and Yaron, 1983; Lopez and Kirckwood, 1974; McCormick and Holtbold, 1966); increased rate of loss upon the addition of an energy source such as glucose (McCormick and Holtbold, 1966; Murray et al., 1968; Wolf and Martin, 1974); and degradation of herbicides in isolated cultures of microorganisms (Attaway et al., 1982; Lopez and Kirckwood, 1974; Murray et al., 1968).

The rate of degradation of a herbicide in soil is influenced by various factors. Such factors include sorption, soil type, herbicide concentration, temperature, and soil moisture content. In general, conditions that promote microbial activity enhance the rate of loss, and those that inhibit the growth of microorganisms reduce the rate (Ashton, 1982).

The effect of temperature and soil moisture on the rate of degradation of herbicides has been studied by a number of investigators (Gerstl and Yaron, 1983; Majka and Lavy, 1977; Walker, 1976). Such studies have, however, usually been restricted to one soil; there are few cases in which the effect of soil type on degradation has been examined under similar experimental conditions. The effect of sorption on herbicides degradation has been the subject of reviews by Graham-Bryce, (1981), Hance, (1970), and Hurle and Walker, (1980).

Hill et al. (1955) proposed that degradation of the substituted urea herbicides in soil followed approximately a first-order rate law and presented limited data to show this for monuron and diuron. Burschel and Freed (1959) reasoned that since in soil, microorganisms are non-limiting or capable of becoming so and soil nutrients are normally present in enormous quantities compared with the pesticides, the rate-limiting component would be the herbicide concentration, hence first-order kinetics should be observed. Hance and Haynes (1981) argued that this proposition applies only to compounds that do not induce adaptation changes in the soil microbial population which seem to be a majority of soil applied herbicides. Actually very few kinetic studies have been reported and most of these studies did fit the first-order rate law permitting the calculation of the half-life and activation energy of degradation (Walker and Roberts, 1978; Wolf and Martin, 1974; Zimdahl et al. 1970). However, soil is such a complex biological and chemical medium that it is not surprising to find deviations from simple first-order kinetics. Such deviations have been shown for the degradation of linuron and metribuzin herbicides in soil (Hance and Haynes, 1981; Kempson-Jones and Hance, 1979). Because understanding the kinetics of herbicides degradation in soil is thus far from complete, one objective of the work presented here was to acquire more quantitative information on the kinetics of degradation and to estimate the half-lives of diuron, chlorotoluron and bromacil in soil. In particular little prior information is available on the fate of the herbicide chlorotoluron. The other objective of the study was to assess the influence of temperature and herbicide concentration on the rate of degradation of the three herbicides.

MATERIALS AND METHODS

The soil samples used in the incubation study were collected from the top 20 cm layer of the soil profile of two soil types in Oregon. These soils were Adkins loamy sand (coarse loamy, mixed, mesic, Xerollic Camborthid) and Semiahmoo mucky peat (Euic, mesic, Typic Medisaprist). Samples were air-dried and ground to pass through a 2 mm mesh seive. Selected properties of the two soils are shown in Table 1.

Carbonyl-^{14}C-labeled diuron and chlorotoluron and 2-^{14}C-Carbonyl labeled bromacil together with unlabeled analytical grade chemicals were used in the study. The herbicides diuron and bromacil were supplied by E. I. de Pont de Nemours and Co., whereas chlorotoluron was provided by Ciba-Geigy Corp. The chemicals were >99% radiochemically and analytically pure and had specific activities of 3.97, 4.22 and 30.6 μCi mg^{-1} for bromacil, diuron and chlorotoluron, respectively. Purity of the chemicals was determined by thin layer chromatography and scanning on a Packard 7200 Radiochromatogram.

Herbicide solutions were prepared in distilled acetone. Two-milliliters of the acetone solution were added drop-by-drop onto the surface of 10 g soil samples (water-free equivalent) contained in 50 mL flasks. These treatments were designed to give final herbicide concentrations of 0.005 and 0.1 μmole g^{-1} soil which correspond to approximately 1 and 20 ppm, respectively. After the acetone was evaporated, the soil was mixed thoroughly in the flasks and sufficient amount of water was added to bring the soil moisture to 50% by weight. The soil was then worked with a spatula to insure thorough mixing.

Immediately before incubation began all flasks were weighed, placed in a controlled temperature water bath, covered with a black polyethylene sheet to prevent photodecomposition, and connected to an assembly especially designed to measure CO_2 evolution from soil (Fig. 22). The incubation system was aerated with moist CO_2-free air at 60-80 mL min^{-1}. The purge gas was delivered from a compressed air cylinder. Evolved CO_2 from the flow-through system was absorbed in 50 mL of 0.5 N NaOH. The experiment was sampled by changing CO_2 trapping solution at intervals ranging from 1 to 7 days during the incubation period. Almost every time sampling was performed, weight adjustments were made and lost moisture was added.

Total CO_2 evolved from the soil was determined by precipitating the carbonate (Na_2CO_3) as $BaCO_3$ by adding 5 mL of 10% $BaCl_2$. Precipitates of $BaCO_3$ were filtered through preweighed Whatman glass microfibre filters and washed first with water and then with alcohol. The filter discs were dried with a heating lamp and reweighed to determine the weight of CO_2 produced by the soil. These filter discs were then placed in glass vials where 2 mL of Handifluor liquid scintillation counting solution (Mallinckrodt, Inc.) were added and the radioactivity was measured with a Packard model 3330 Tri-Carb Liquid Scintillation Spectrometer. An internal standard was used to correct for the instrument's counting efficiency. All treatments were duplicated and herbicide-free samples were included for each soil as controls.

RESULTS AND DISCUSSION

The rates of $^{14}CO_2$ production from herbicide-treated soils incubated at 25, 30, and 35° C are given in Table 7. The effect of temperature on the rate of degradation is evident with more rapid decomposition at the higher temperature. The rate approximately tripled with a 10-degree rise in temperature from 25 to 35° C.

The presence of herbicides apparently had no effect on the activity of soil microorganisms as measured by the total CO_2 evolved (Table 8). Production of CO_2 from the peat was twice that from the sand for all incubation combinations. Since the weight of herbicide-derived $^{14}CO_2$, would unlikely be detectable with this chemical method, these quantities of CO_2 may be considered to be derived almost entirely from soil organic materials decomposition. These differences in biological activity between the peat and the sand may be considered a likely factor in the degradation of herbicides. The data (Table 8) show that the rate of CO_2 production was doubled by a 10° C increase in temperature. This is in agreement with the data reported by McCormick and Holtbold (1966) and Wolfe and Martin (1974), and consistent with the generalization that an increase in temperature of 10° C within the tolerance range of the microorganisms results in a doubling of their activity (Atlas and Bartha, 1981).

The percentage $^{14}CO_2$ evolved from the herbicide-treated soil samples incubated at 25° C is shown in Figs. 23 and 24. These data show no sign of a lag phase in the degradation process. Similar results were reported by other investigators (Wolf and Martin, 1974; Zimdahl et al., 1970). This type of degradation is characteristic of the co-metabolic degradation process where the microorganisms do not proliferate on the decomposed compound because it does not serve as the energy and carbon source for the microorganisms. In such instances herbicides are metabolized as a result of general microbial activities which are controlled by the availability of nutrients and moisture and by the right temperature and pH (Matsumura, 1982). Microbial co-metabolism has been suggested for the degradation of many pesticides including diuron and bromacil (McCormick and Hiltbold, 1966; Lopez and Kirckwood, 1974; Sheets, 1964, Wolf and Martin, 1974).

The incubation data (Table 7) show that the degradation of bromacil was very low and essentially unaffected by the herbicide concentration and soil type. The rates of diuron and chlorotoluron degradation were similar, in that the rate of $^{14}CO_2$ evolution was a little higher in the sand than in the peat. In Adkins loamy sand the rate of loss of the two herbicides was increased as the initial concentration decreased but in the peat the rate was slightly increased as the initial concentration increased. This behavior is more likely due to the effect of sorption on the degradation process. From the results presented in Table 8, one expects more degradation in the peat because it has higher microbial activity than the sand. However, the sorptive capacity of the peat for the herbicides has been shown (Chapter I) to be much higher than the sandy soil. The present data seem to indicate that adsorption protected the herbicides from degradation by reducing the concentration in the soil solution. Hurle and Walker (1980) indicated that adsorption however, does not always protect a chemical from degradation, as was found in this study, nor does it always lead to increased rates of degradation.

Assuming that degradation of all three herbicides followed a first-order rate law, the incubation data were plotted as the concentration remaining in the soil on a logarithmic scale against time of incubation in days. The straight lines in Figs. 25, 26, and 27 are those of best fit obtained by regression analysis. The correlation coefficients of all herbicide treatments were highly significant ($r \geqslant -0.915$), in agreement with the first-order rate law and verifying that the assumption of first-order was valid (Bromberg, 1980). The first-order rate law has been used to interpret degradation of many pesticides in soil (Gerstl and Yaron, 1983; Hill et al., 1955; Walker, 1976; Walker and Roberts, 1978; Wolf and Martin, 1974; Zimdahl et al., 1970). The rate constant (K) which was derived from the slopes of fitted lines was used to calculate the half-life ($t_{\frac{1}{2}}$) using the expression:

$$t_{\frac{1}{2}} = \frac{0.693}{K} \qquad (8)$$

The half-life expresses the time it takes the concentration of the herbicide to fall to one-half its initial value. The half-lives of diuron, chlorotoluron, and bromacil calculated using equation (8) are given in Table 9. The half-life of diuron ranged from about 200 days to as high as 4100 days. Most of these values compare fairly well with those reported by other investigators (Laskowski et al., 1983; Freed and Haque, 1973). The estimated half-lives of chlorotoluron were slightly shorter than the corresponding values for diuron. This result was expected since chlorotoluron is less chlorinated than diuron, hence it is more susceptible to microbial attack (Matsumura, 1982). The half-lives of bromacil in soil determined in this study are considerably higher than most of the values observed in other soils (Wolf and Martin, 1974; Zimdahl et al., 1970; Leistra et al., 1975), but they compare favorably with some of the values reported by Gerstl and Yaron (1983). It should be noted that these half-life values were derived from the rate of $^{14}CO_2$ production from herbicide treated soils under controlled laboratory conditions. Freed and Haque (1973) pointed out that while the half-life is a useful parameter in estimating the loss of a pesticide from the soil, it does not tell when the concentration has been sufficiently reduced so as to have little biological activity. Since this study concerns itself only with the evolution of $^{14}CO_2$ from soil, it provides no information to whether altered but ^{14}C-containing compounds were produced or how much reduction in biological activity occurred during incubation nor how much of the ^{14}C had been metabolically bound in the soil. Martin and Stott (1981) reported that after one year of incubation the biological activity of bromacil was largely dissipated, but only 10 to 20% of ring carbon- 2 was released as CO_2. They argued that this is an indication that some of the partially altered or degraded structures are stabilized by incorporation into soil organic materials or by complexing with clays and metal ions. Therefore, the half-life value should be recognized as a useful first approximation but not as a precise figure.

The incubation data were used to obtain the activation energies of degradation using the Arrhenius equation:

$$K = A \exp(-E_a/RT) \qquad (9)$$

Where K is the first-order rate constant, T the absolute temperature, A the frequency factor, R the gas constant, and E_a the activation energy. The activation energies shown in Table 10 were derived from the slopes of least squares fit of the logarithm of the rate constant (K) against the reciprocal of the absolute temperature. The data followed the Arrhenius relationship as indicated by the highly significant ($r \geqslant -0.957$) correlation coefficients. The E_a values obtained in this study compare well with those reported in the literature for several herbicides including bromacil (Walker, 1976; Zimdahl et al., 1970). The activation energy is a measure of the amount of energy molecules must acquire in order to react (to form the activated complex). Based on the activation energy values, Zimdahl et al. (1970) postulated that bromacil degradation in soil would proceed by cleavage of the carbon-halogen bond, because it is the least-hindered bond and it has the lowest energy. They argued that the activation energy required to initiate degradation of a herbicide should be constant, dependent mainly on the nature of the bond involved and independent of the soil environment. There has been a limited number of studies in which the activation energies of degradation were determined to verify such an argument. The activation energy values obtained in this study seem to support this generalization however.

Table 7. Percentage $^{14}CO_2$ evolved per week from ^{14}C-diuron, ^{14}C-chlorotoluron, and ^{14}C-bromacil treated soil as affected by temperature and herbicide concentration.

	Peat		Sand	
Temperature	C_1^a	C_2^b	C_1	C_2
°C	% $^{14}CO_2$ Evolved Per Week			
		Diuron		
25	0.12	0.14	0.69	0.35
30	0.22	0.27	1.16	0.77
35	0.41	0.54	2.10	1.18
		Chlorotoluron		
25	0.21	0.25	0.14	0.38
30	0.40	0.47	0.56	0.91
35	0.78	0.82	1.56	1.40
		Bromacil		
25	0.09	0.08	0.01	0.03
30	0.08	0.11	0.04	0.05
35	0.06	0.19	0.11	0.08

[a] Initial herbicide concentration 0.005 μmol g^{-1}

[b] Initial herbicide concentration 0.1 μmol g^{-1}

Table 8. The rate per week of CO_2 Production from 10 g Soil at 25, 30, and 35° C.

Soil	Temp.	Diuron		Chlorotoluron		Bromacil		Control
		C_1^a	C_2^b	C_1	C_2	C_1	C_2	
	°C	mg CO_2 Week^{-1}						
Peat	25	13	12	12	12	14	13	14
	30	11	12	11	11	14	14	14
	35	23	22	23	19	18	16	18
Sand	25	6	6	5	6	5	5	6
	30	8	9	9	10	9	9	8
	35	12	12	11	14	12	11	10

[a] Initial herbicide concentration 0.005 μmol g^{-1}

[b] Initial herbicide concentration 0.1 μmol g^{-1}

Table 9. Half life ($t_{\frac{1}{2}}$) of diuron, chlorotoluron, and bromacil in peat and sandy soils as affected by temperature.

	Peat		Sand	
Temperature	C_1^a	C_2^b	C_1	C_2
°C	$t_{\frac{1}{2}}^c$, days			
	Diuron			
25	4101	3610	653	1378
30	2166	1805	420	596
35	1131	870	223	400
	Chlorotoluron			
25	2381	2050	3397	1251
30	1209	1031	804	472
35	617	591	288	370
	Bromacil			
25	5923	6417	46200	19250
30	7144	4029	10500	9493
35	8885	2596	4076	6417

[a] Initial herbicide concentration 0.005 μmol g^{-1}

[b] Initial herbicide concentration 0.1 μmol g^{-1}

[c] $t_{\frac{1}{2}} = \frac{0.693}{K}$ where K is the rate constant

Table 10. Activation energy (E_a) for degradation of diuron, chlorotoluron, and bromacil by soils.

Herbicide	Peat C_1^a	Peat C_2^b	Sand C_1	Sand C_2
	E_a^c (K Cal mol^{-1})			
Diuron	23.23	25.69	18.98	22.65
Chlorotoluron	24.49	22.48	44.63	22.40
Bromacil	-7.44	16.62	44.28	20.06

[a] Initial herbicide concentration 0.005 µmol g^{-1}

[b] Initial herbicide concentration 0.1 µmol g^{-1}

[c] E_a = Activation Energy = -(slope) (2.303) R

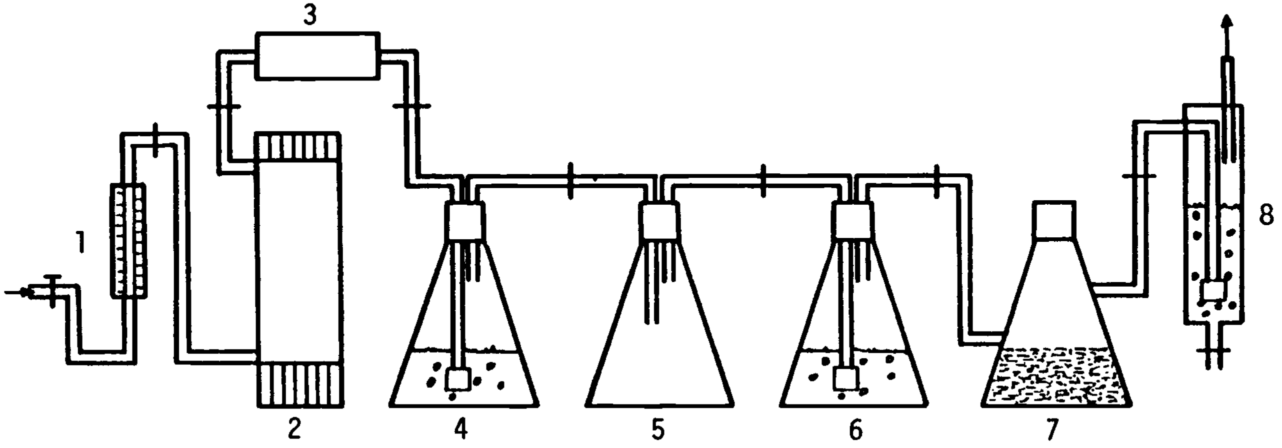

Figure 22. Flow-through soil incubation system used for monitoring degradation of herbicides. 1. air-flow meter, 2. drying tube filled with Drierite, 3. soda lime for removing CO_2 from air, 4. NaOH to absorb CO_2 from incoming air, 5. empty flask, 6. water to humidify incoming air, 7. soil metabolism flask and 8. NaOH for absorbing CO_2 evolved.

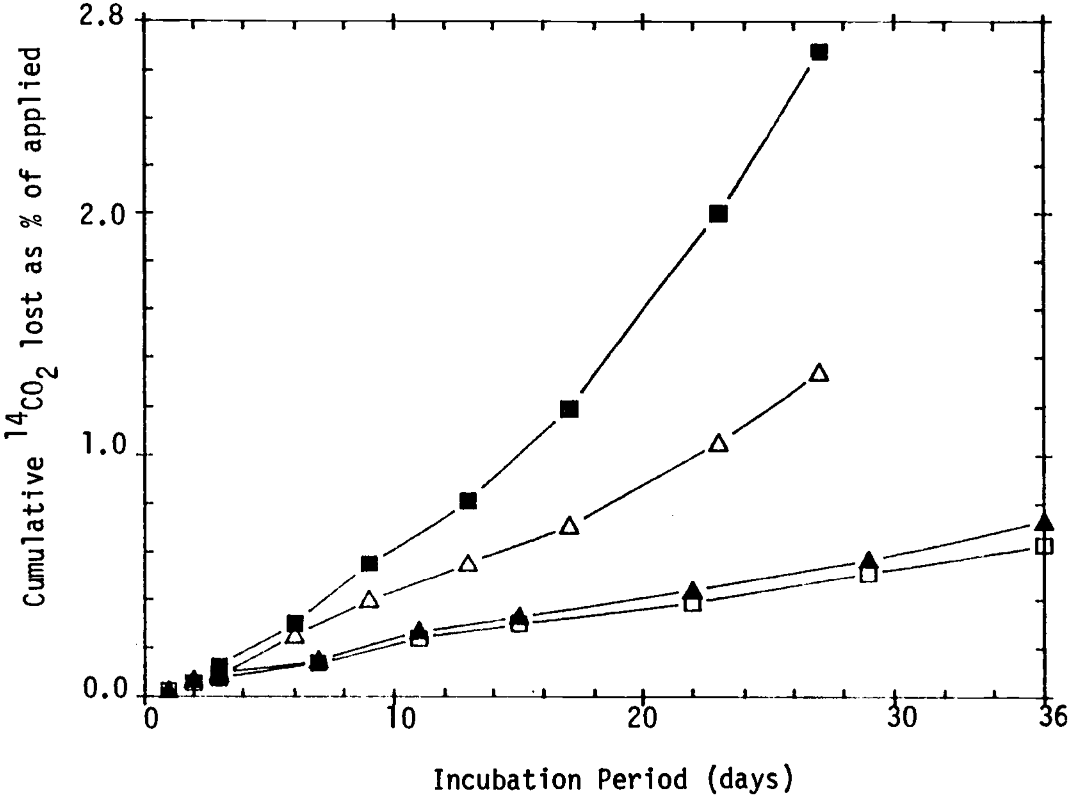

Figure 23. Cumulative percentage of $^{14}CO_2$ evolved from ^{14}C-diuron-treated soils incubated at 25 C. Herbicide concentration: 0.005 mol Kg^{-1} (■, sand; □, peat) and 0.10 mol Kg^{-1} (△, sand; ▲, peat).

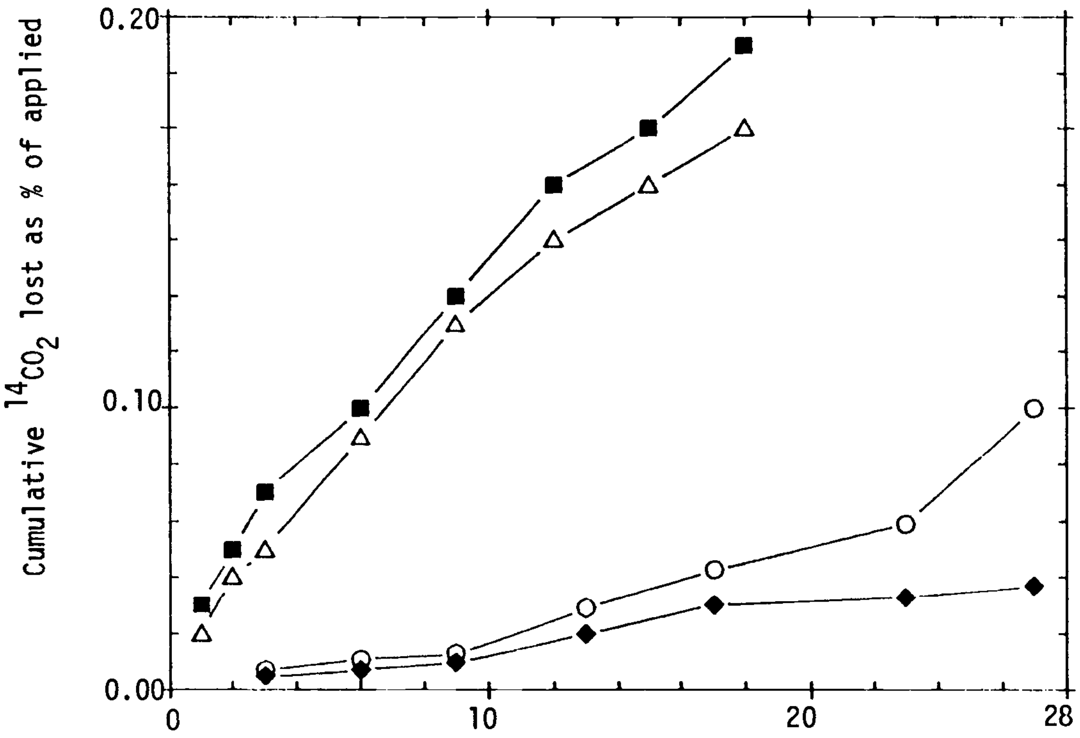

Figure 24. Cumulative percentage of $^{14}CO_2$ evolved from ^{14}C-bromacil-treated soils incubated at 25° C. Herbicide concentrations: 0.005 µmol Kg^{-1} (◆, sand; ■, peat) and 0.10 µmol Kg^{-1} (○, sand; △, peat).

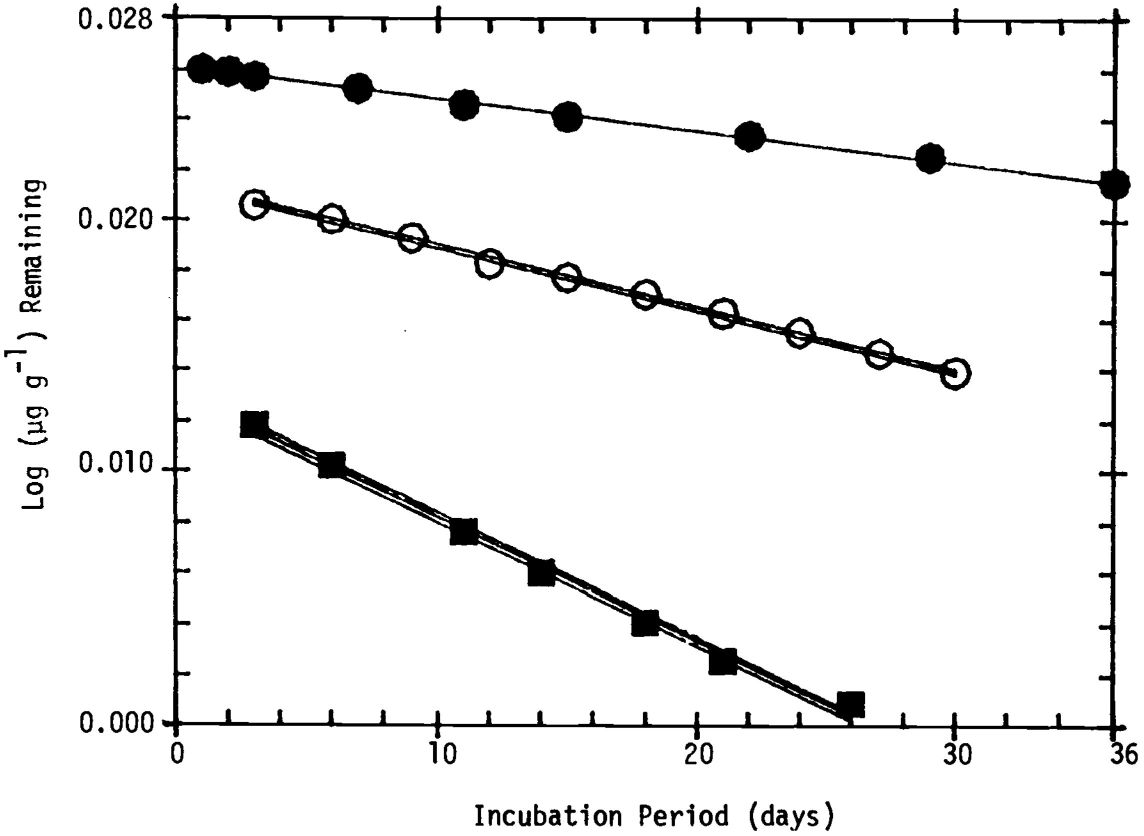

Figure 25. Effect of temperature on degradation of chlorotoluron (0.005 μmol g^{-1}) by the peat soil (●, 25° C; ○, 30° C; ■, 35°C).

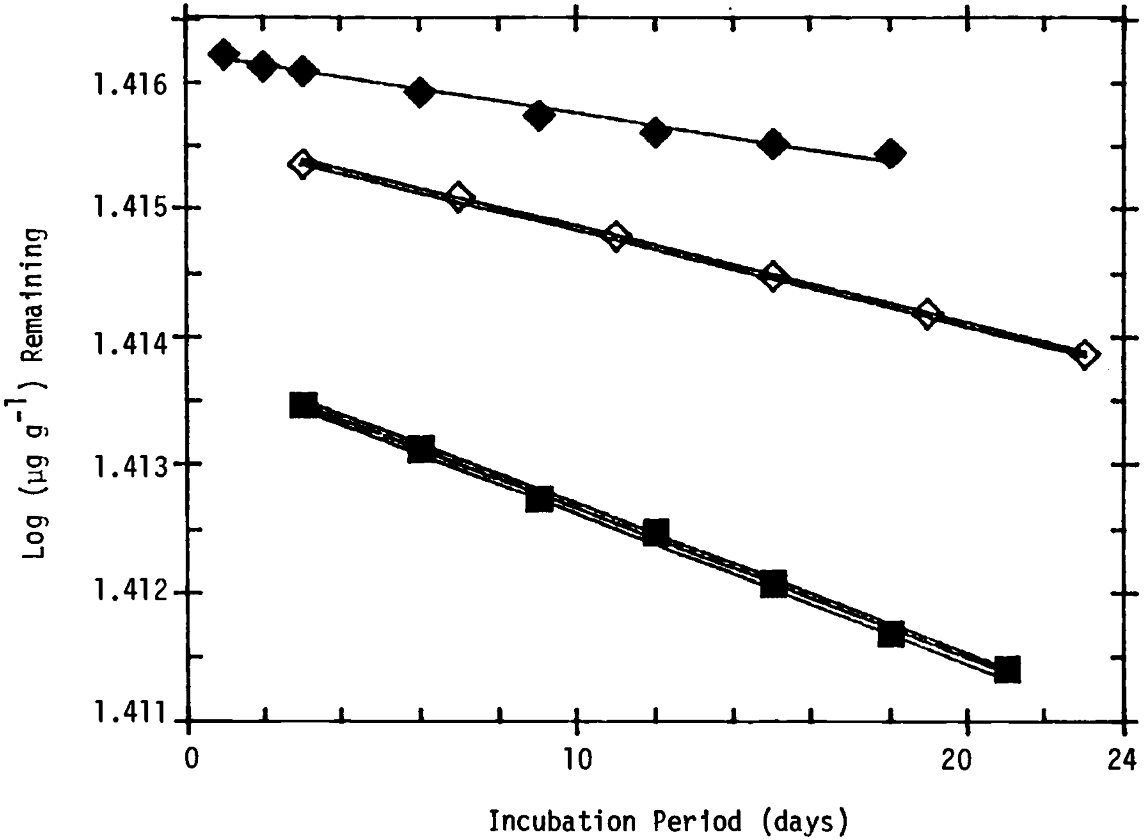

Figure 26. Effect of temperature on degradation of bromacil (0.10 µmol g^{-1}) by the peat soil (♦, 25° C; ◇, 30° C; ■, 35° C).

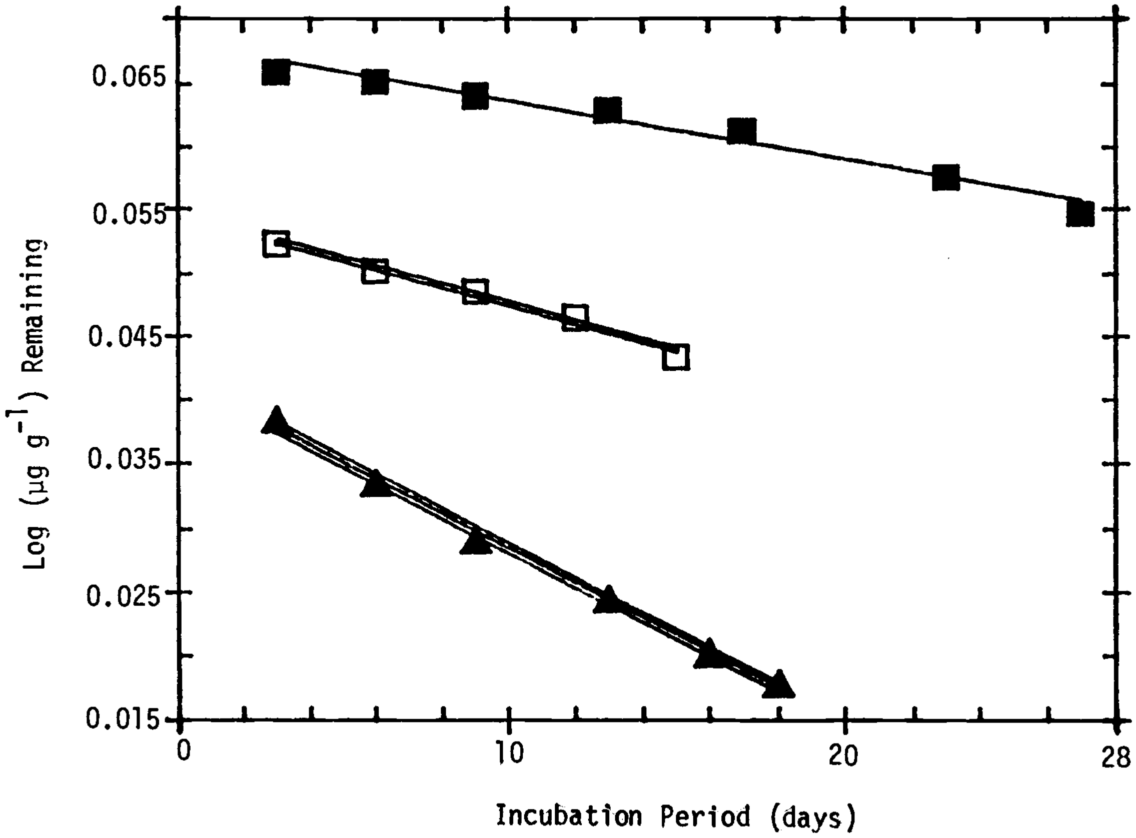

Figure 27. Effect of temperature on degradation of diuron (0.005 μmol g^{-1}) by loamy sand soil (■, 25° C; □, 30°C; ▲, 35°C).

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SUMMARY AND CONCLUSIONS

The behavior of six herbicides widely used in agriculture was studied in two soils: an organic soil, and a loamy sand soil. Sorption of diuron, chlorotoluron and bromacil from aqueous solution was evaluated over a broad range of herbicide concentrations. The effect of temperature on sorption was very small. The results showed the strong dependence of sorption on the organic carbon (OC) content of soil. The organic soil had much higher affinity for the herbicides than the sand, which contains very low OC content. Diuron was the most highly sorbed, followed by chlorotoluron and last was bromacil.

The sorbate parameters: octanol-water partition coefficient (K_{ow}) and water solubility (S) were found to be good estimators of sorption constant (K_{oc}) normalized for soil organic carbon content using emperical equations developed for other classes of pesticides and pollutants. It was shown that highly significant relationships exist between both K_{ow} and S of these herbicides and their corresponding K_{oc} values. It was concluded that such relationships are useful in predicting sorption of neutral organic herbicides as with other pesticides and pollutants. The importance of these relationships is the realization that sorption of such compounds by soil can be estimated from knowing sorbate parameters instead of an expensive experimental determination.

Sorption appeared to protect the herbicides from being degraded. The degradation rate of diuron and chlorotoluron was higher in the sand than in the peat but the rate of bromacil degradation was essentially unaffected by soil type. The herbicides degraded microbially with no lag phase apparently by a co-metabolism process. Breakdown of the herbicides was very slow in both soils. The influence of temperature on the rate of degradation was much more pronounced than the affect of soil type or herbicide concentration. The rate of $^{14}CO_2$ production from treated soils was approximately tripled by a 10-degree rise in temperature from 25 to 35°C. Degradation followed the first-order rate law with estimated half-lives ranged from 223 to 4101 days for diuron, 288 to 3397 days for chlorotoluron and 2596 to 46200 days for bromacil.

Infrared spectroscopy (IR) was used to show the involvement of ion exchange mechanism in the binding of diquat and H-bonding in the binding of diuron, chlorotoluron and bromacil by soil humic acids (HA) and water soluble soil organic materials (WSSOM). Noted changes in the IR absorption bands suggested the formation of charge transfer complexes between bromacil, diuron and chlorotoluron and HA and WSSOM but not between diquat and HA or WSSOM.

The gel filtration chromatography procedure adapted by Hummel and Dreyer (1962) for the study of binding of small organic ions by proteins and other macromolecules was used to show the binding of bromacil, diquat, diuron, chlorotoluron, glyphosate and simazine by HA and WSSOM. The study confirmed the suitability of the method for the study of ionic herbicides and extended its use to the nonionic herbicides binding by HA. The chromatographic procedure proved very successful in showing the existence of binding of the herbicides by WSSOM. The association of herbicides with WSSOM has not been studied before probably because of the lack of a suitable procedure. In this study experimental evidence has been provided for the suitability of the Hummel and Dreyer (1962) method and for the existence of association between herbicides and water-dissolved soil organic substances. These results are important for assessing the mobility and transport of herbicides and related compounds in soil and water.

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Weed Res. 10:18-26.

APPENDICES

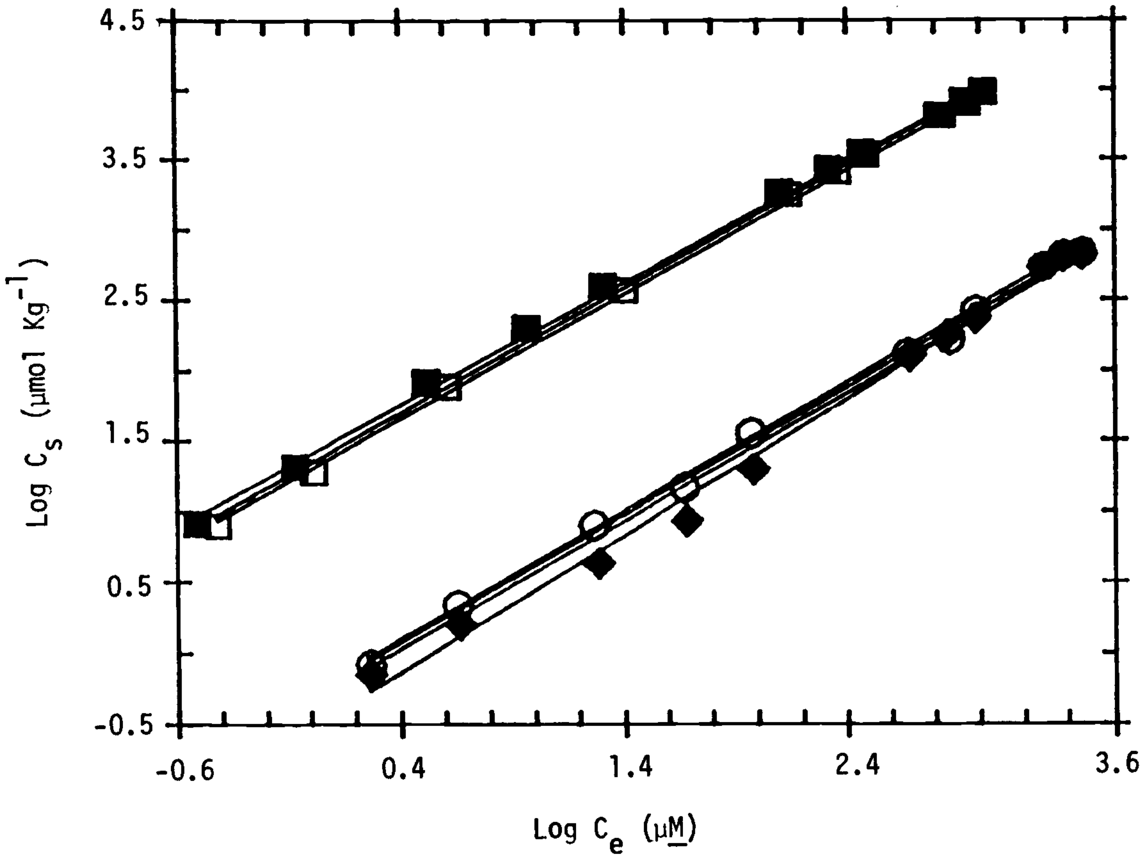
Appendix Table 1. First-order rate constant (K) for degradation of diuron, chlorotoluron, and bromacil by soils at 25, 30, and 35° C.

	Peat		Sand	
Temperature	C_1^a	C_2^b	C_1	C_2
°C	K^c (X 10^{-4}) day^{-1}			
	DIURON			
25	1.69	1.92	10.61	5.03
30	3.20	3.84	16.49	11.63
35	6.13	7.97	31.07	17.32
	CHLOROTOLURON			
25	2.91	3.38	2.04	5.54
30	5.73	6.72	8.62	14.67
35	11.23	11.73	24.09	18.73
	BROMACIL			
25	1.17	1.08	0.15	0.36
30	0.97	1.72	0.66	0.73
35	0.78	2.67	1.70	1.08

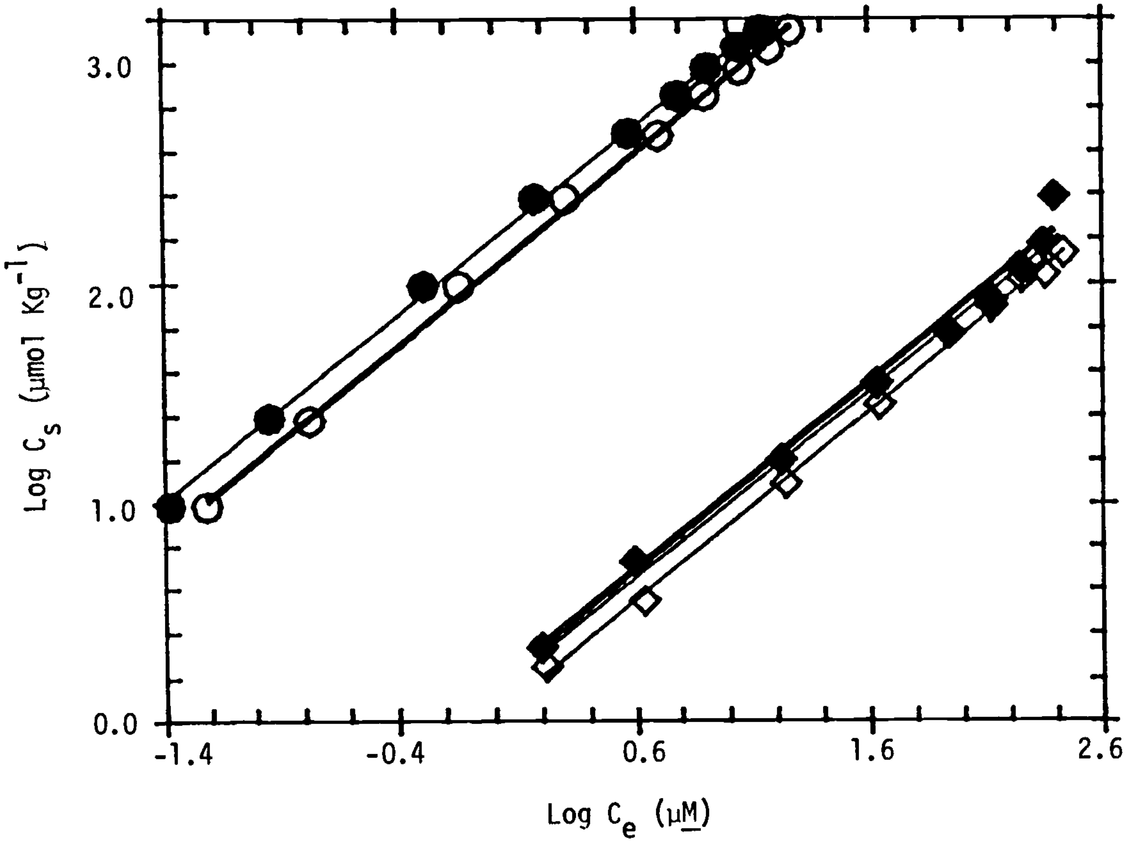
[a] Initial herbicide concentration 0.005 μmol g^{-1}

[b] Initial herbicide concentration 0.1 μmol g^{-1}

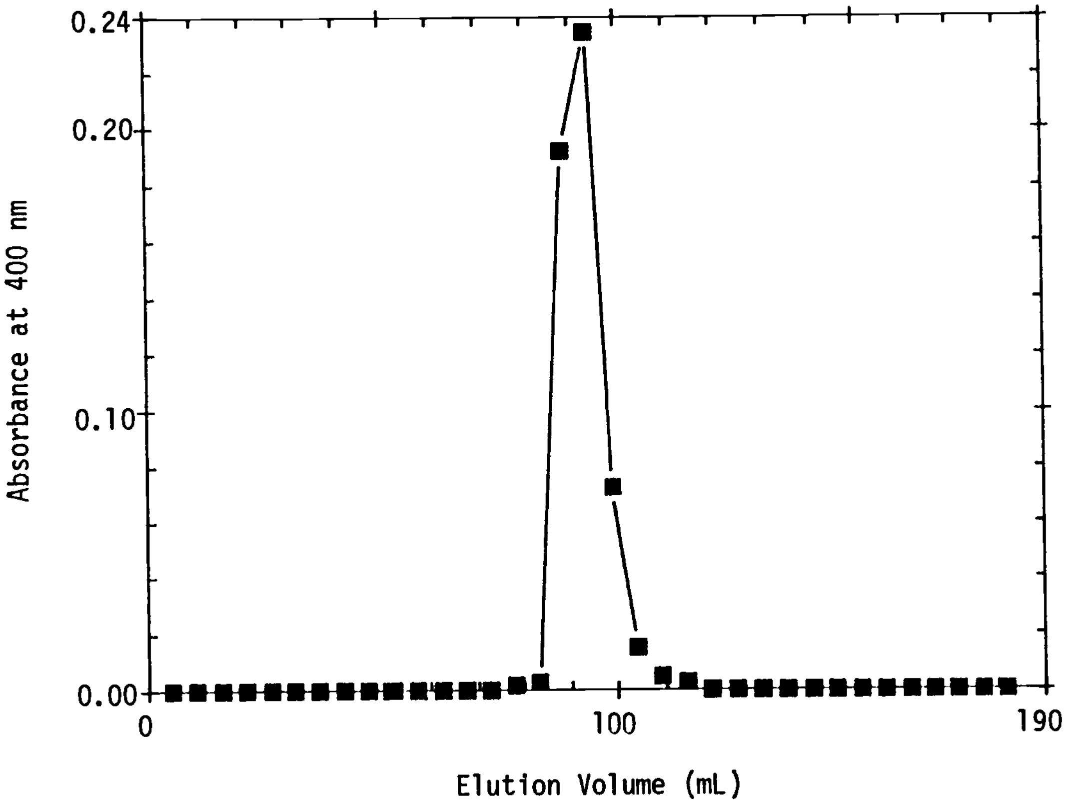
[c] K = Rate Constant = -(slope)(2.303)



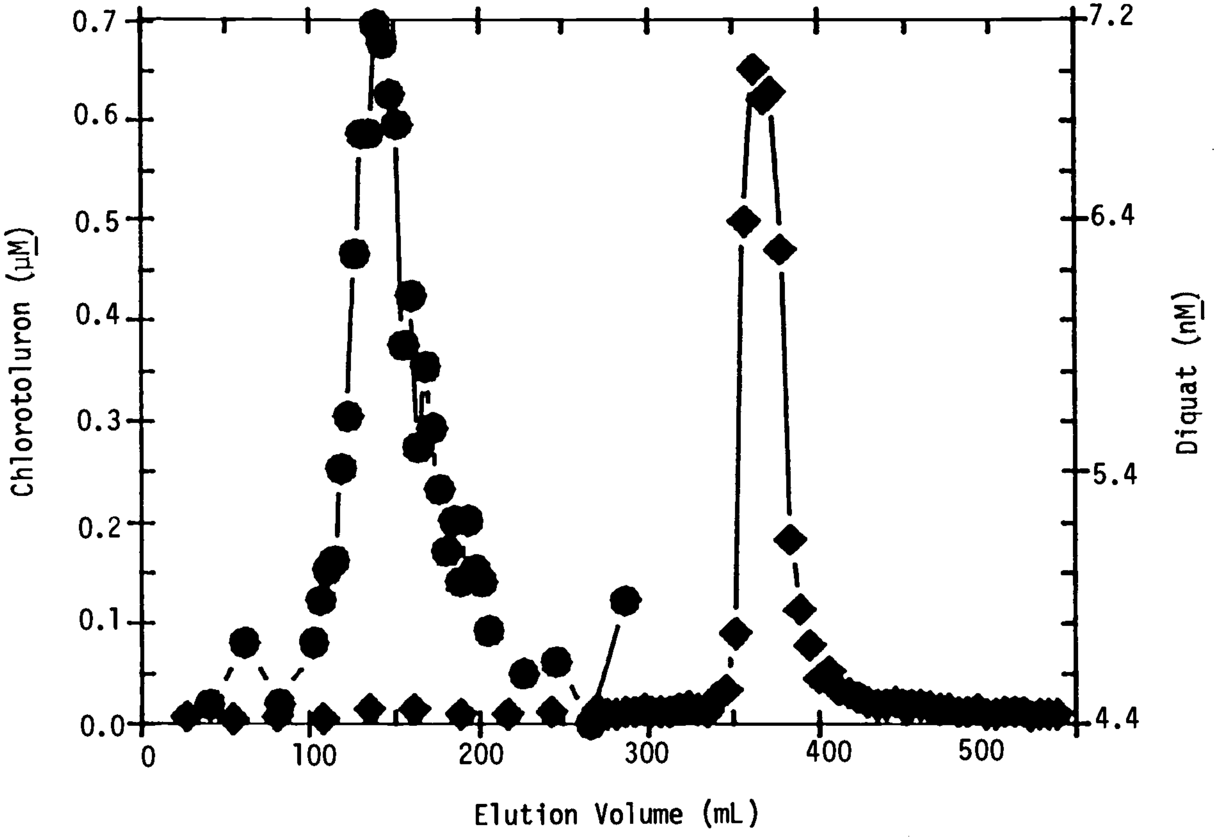
Appendix Figure 1. Freundlich isotherms for the sorption of bromacil by soil at 4° C (■, peat; ○, sand) and 25° C (□, peat; ◆, sand).



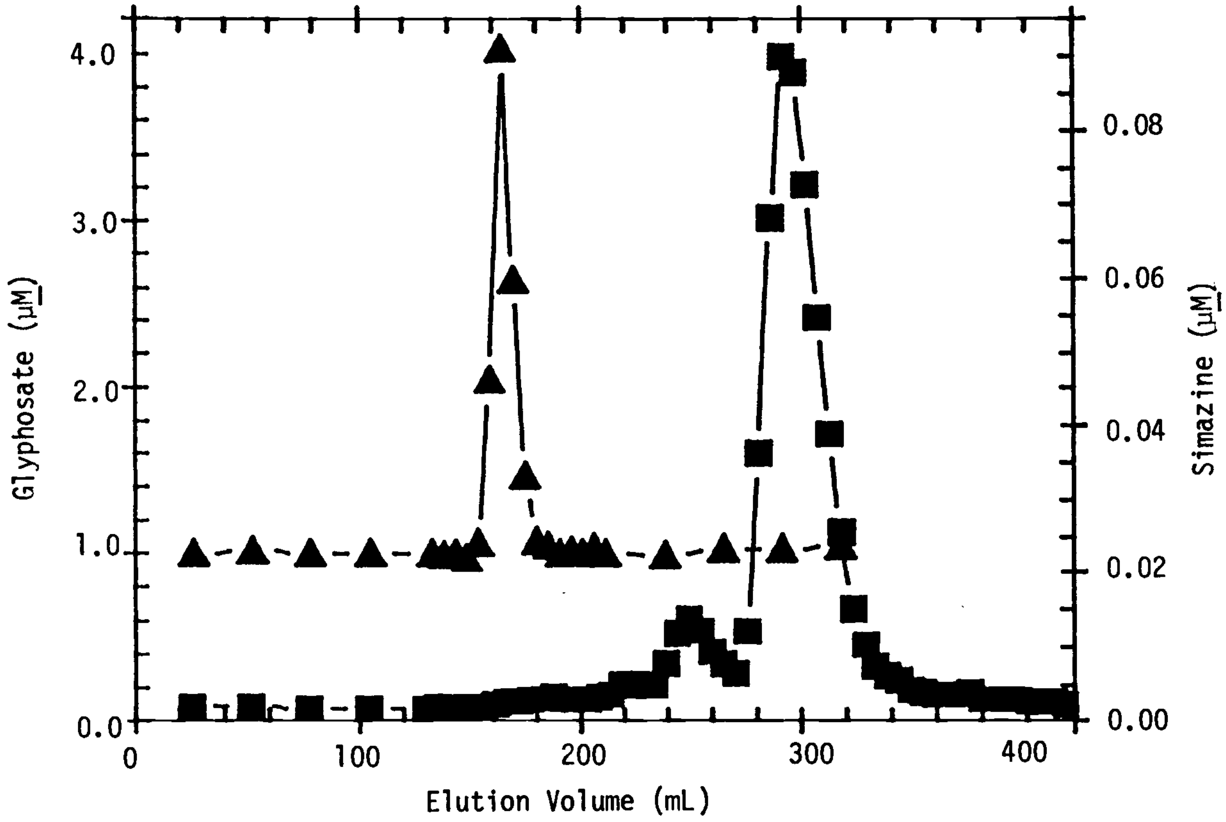
Appendix Figure 2. Freundlich isotherms for the sorption of chlorotoluron by soil at 4° C (●, peat; ◆, sand) and 25° C (○, peat; ◇, sand).



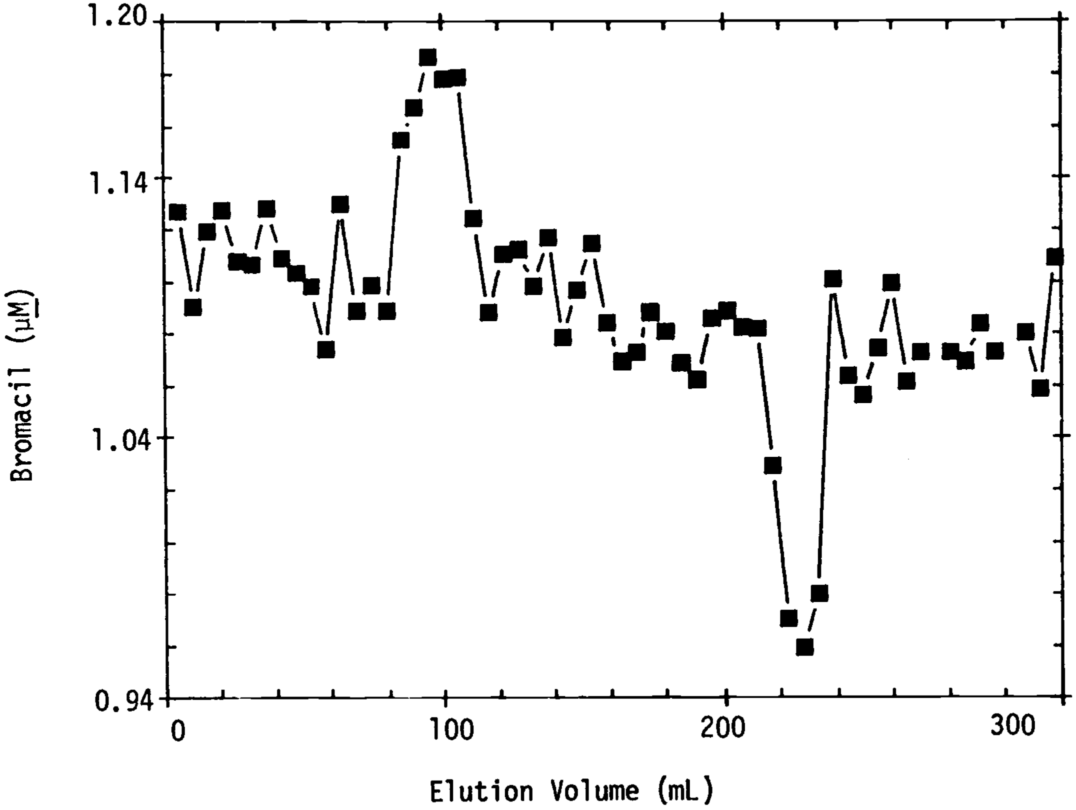
Appendix Figure 3. Elution profile of blue dextran 2000 on Sephadex G-25 gel for measurement of void volume of the column.



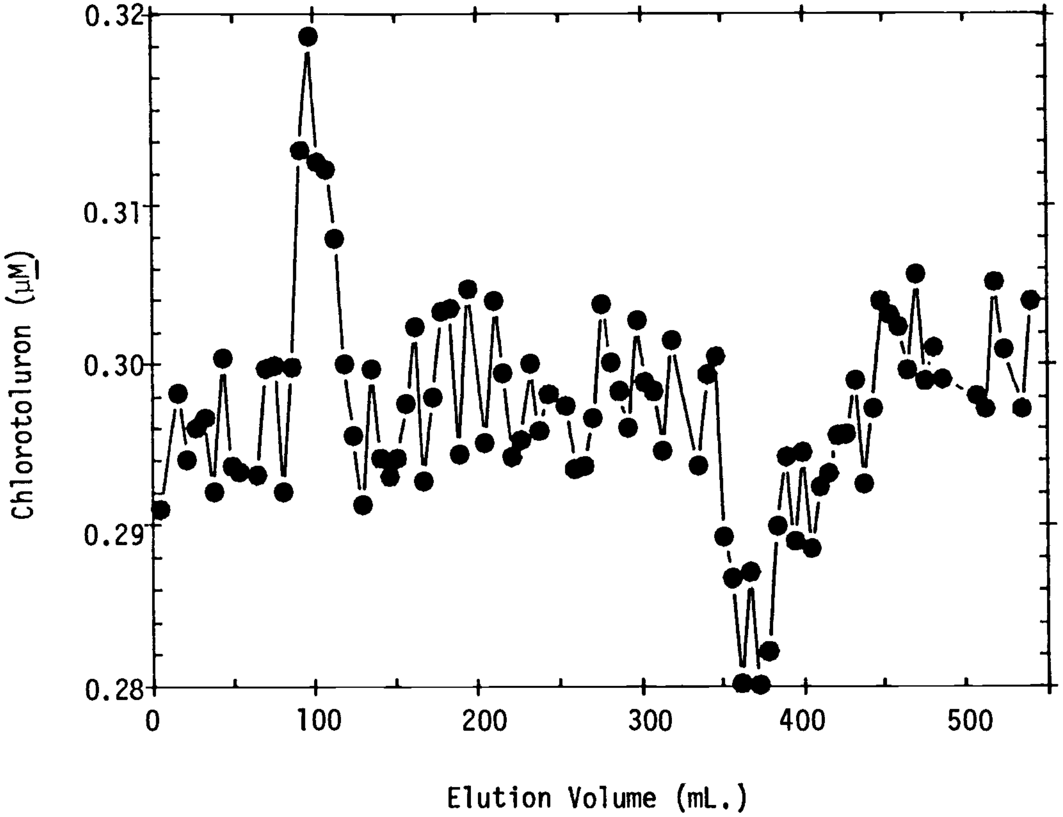
Appendix Figure 4. Elution profile of chlorotoluron (♦) and diquat (●, right-Y-axis) determined on Sephadex G-25 gel.



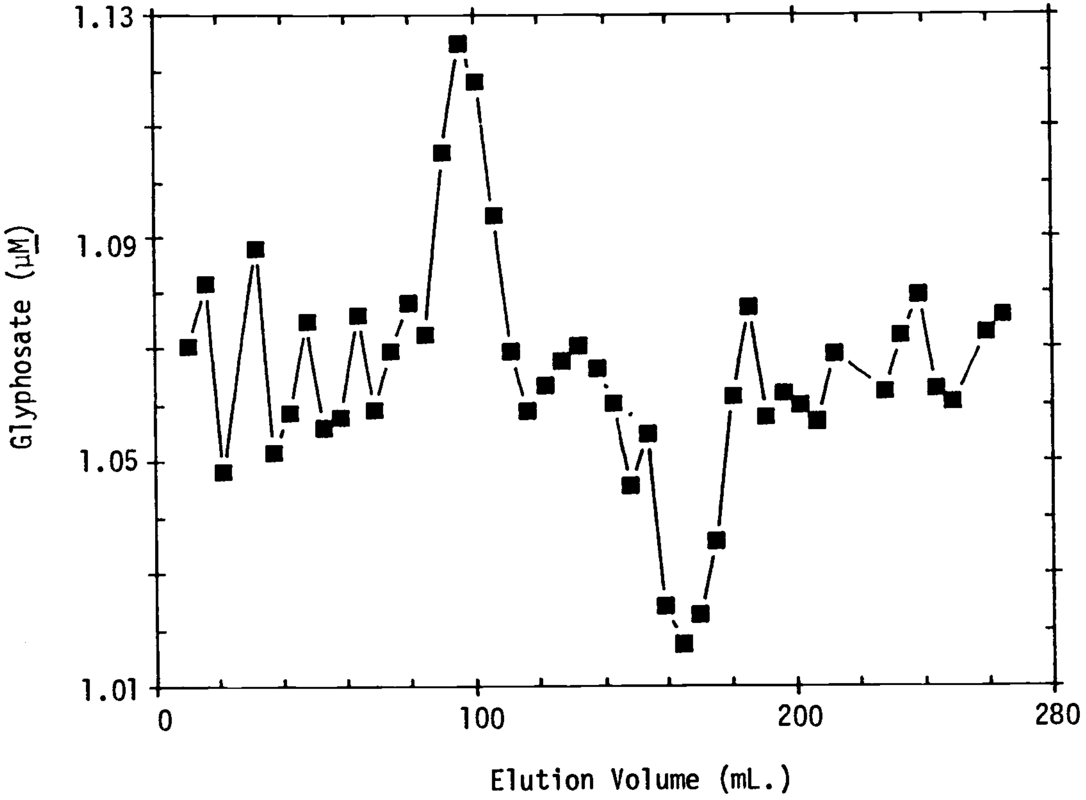
Appendix Figure 5. Elution profile of glyphosate (▲) and simazine (■, right-Y-axis) determined on Sephadex G-25 gel.



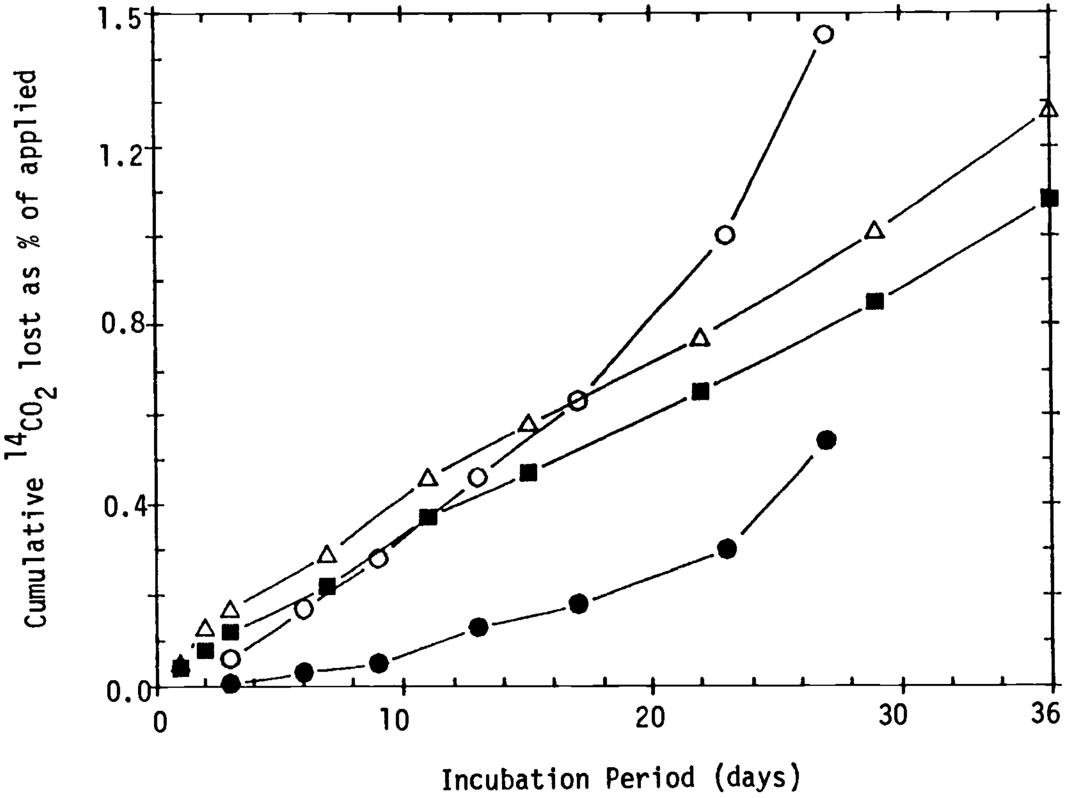
Appendix Figure 6. Elution profile for the measurement of bromacil binding by humic acid on Sephadex G-25 gel.



Appendix Figure 7. Elution profile for the measurement of chlorotoluron binding by humic acid on Sephadex G-25 gel.



Appendix Figure 8. Elution profile for the measurement of glyphosate binding by humic acid on Sephadex G-25 gel.



Appendix Figure 9. Cumulative percentage of $^{14}CO_2$ evolved from ^{14}C-chlorotoluron-treated soils incubated at 25° C. Herbicide concentrations: 0.005 µmol Kg^{-1} (●, sand; ■, peat) and 0.10 µmol Kg^{-1} (○, sand; ◇, peat).