ADSORPTION OF DDT
BY
SOILS AND BIOLOGICAL MATERIALS

Thesis for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
YOUNG-OH SHIN
1970

This is to certify that the

thesis entitled

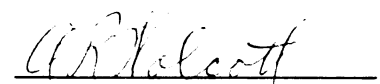
ADSORPTION OF DDT BY SOILS
AND BIOLOGICAL MATERIALS

presented by

Young-Oh Shin

has been accepted towards fulfillment
of the requirements for

Ph. D. degree in Soil Science



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Date Feb. 19, 1970

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ABSTRACT

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The very great persistence and mobility of DDT in the environment has been amply documented. However, very few attempts have been made to deal quantitatively with some of the basic interactions of DDT with mineral and organic constituents and ecological systems in soils and sediments.

In the present study, a special partitioning function was developed to facilitate estimation of the distribution coefficient(Kd). Theoretical consideration was given to relationships between the distribution coefficient and constants of classical adsorption isotherms under the conditions of equilibrium partitioning employed.

A gas-liquid chromatograph was employed for the analysis of DDT. Appropriate extraction and concentration procedures were developed to estimate DDT in the ambient aqueous matrix at concentrations of 0.01 to 1.00 ppb. Linear adsorption isotherms were obtained over this range.

A sequence of proximate fractional extractions was used to prepare a series of soil derivatives which could be interpreted in terms of ecological and structural relation-

ships in the microfabric of the soil.

The equilibrium distribution ratios(Kd) of solid phase to aqueous phase DDT for sandy loam, clay and muck soils were 1,300, 13,700 and 106,300, respectively.

Extraction of mineral soils with ether and alcohol increased their sorptive capacity. This result was attributed to increased wetability and a probable increase in surface area due to aggregate disruption.

Additional increases in sorptive capacity were associated with removal of materials soluble in hot water and/or 2 % HCl. These treatments may have resulted in further increase in surface area due to aggregate breakdown. However, both quantitative and qualitative differences in residual organic matter and its relation to soil minerals were also indicated.

Treatment with H_2O_2 drastically reduced sorptive capacity of all soils, both in total and per unit residual carbon.

Alfalfa and fungus tissues were similar to muck soil in their capacity to adsorb DDT.

The biological materials and soil preparations used in this study appear to have promise as model systems for studying ecological interactions of pesticides in soils.

ADSORPTION OF DDT
BY
SOILS AND BIOLOGICAL MATERIALS

BY

Young-Oh Shin

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirement
for the degree of

DOCTOR OF PHILIOSOPHY

Department of Crop and Soil Sciences

1970

To my K

ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to Dr. A. R. Wolcott for his understanding and help throughout this investigation and in preparation of the manuscript.

He would also like to extend his thanks to Drs. R. L. Cook, M. M. Mortland, J. L. Lockwood, M. J. Zabik and D. P. Penner for their interest in this work.

The work upon which this thesis is based was supported, in part, by the United States Public Health Service(Grant CC 00246) and by the United States Food and Drug Administration(Grant ROI FD 00223-05). Use of the Michigan State University computing facilities was made possible through support, in part, from the National Science Foundation.

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INTRODUCTION

It has been estimated that 5 million lives were saved from malaria between the advent of use of DDT(1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane) during World War II and 1953, and at least 100 million illnesses prevented. In our technical society, hundreds of people still fall prey to mosquito-born encephalitis for which there is no cure or specific treatment. The only current method of preventing this dreaded disease is to use chemicals to control mosquitoes. Insect-born diseases, such as yellow fever, typhus, African sleeping sickness, and bubonic plague still cause world-wide sickness, and death. Chemicals can also be hazardous if misused. They can kill or harm forms of life other than the ones we are trying to destroy. They can cause temporary undesirable ecological changes in the environment(Shaw, 1966).

It has been reported that not a single stream sampled from the Western States was free of pesticides(Manigold, 1969), the most frequent pesticide observed being DDT(Brown and Nishioka, 1967). In view of the toxicity to both fish and warm-blooded animals of some pesticides, serious consideration has been given to their removal when their presence is known or suspected in a source of potable water(Robeck, 1965; Sigworth, 1965). DDT has been found in most soil samples collected in the Eastern and Southern States ranging from

0.10 to 12.8 ppm in concentration(Seal et al., 1967; Plant Pest Control Division, 1968). DDT was found in all wildlife at Pesidio, Texas(Culley and Applegate, 1967). Brain levels of DDT and its metabolites were not significantly different between house sparrow nestlings from treated zones and those from untreated zones in the Miami area(Lehner et al., 1967).

Many concerned investigators are measuring the level of pesticides in biological materials, especially in food products and human diets(Marth, 1965; Durgan et al., 1967; Lindgren et al., 1968). DDT and its metabolites are the pesticides most frequently detected. They occurred in 76 percent of the total diet composite samples and in 84 percent of the meat, fish and poultry category samples examined by Cummings(1966). In an earlier study, DDT and its metabolites were found in every meal tested(Armstrong and Quinby, 1965).

As a matter of course these pesticides enter the human body. Although the organochlorine compounds are very insoluble in water, DDT and dieldrin(1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo,exo-5,8-dimethanonaphthalene) have been observed in blood during exposure episodes at concentrations larger than 1,000 ppm for DDT and larger than 0.5 ppm for dieldrin. Evidence to date indicates that levels of DDT in human blood in excess of 0.15 ppm are indicative of either: (a) recent exposure over and above that normally assimilated from the environment or (b) the mobilization of fat deposits associated with a loss in total body weight (Schafer, 1968). A survey shows that the mean concentration

of total DDT-type compounds in the whole blood of 44 persons with no known occupational exposure was 0.013 ppm(Robinson and Hunter, 1966).

All the data compiled so far suggest that DDT is a current constituent of human fat in the general population of the world. The average storage in fat for the general population of the United States, with ordinary dietary habits, with no occupational contact with DDT, and with little or no environmental exposure to the insecticide, was 4.9 ppm for DDT and 6.1 ppm for DDE(1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene). Persons who had had moderate occupational exposure within a year stored DDT at an average concentration of 14.0 ppm and DDE at 19.0 ppm. One worker with extensive occupational exposure showed 648 ppm of DDT and 434 ppm of DDE in his body fat(Durham, 1965). The average storage of DDT in Canada and most of the European countries was slightly less than it was in the United States. However, people in Hungary stored at least as much as the Americans and people in Israel showed much higher concentration of DDT in body fat(Hoffman et al., 1964; Wasserman et al., 1965; Hunter et al., 1963; Read and McKinley, 1961; Wasserman et al., 1967).

Hoffman et al.(1964) reported that there was no evidence of progressing storage of DDT in the general population since 1951. He further stated that the known metabolism of DDT supported the idea that with low chronic intake of DDT, equilibrium was soon established in which excretion is equal to intake. The effect of DDT and its metabolites stored in

human body is not yet clearly understood.

Analysis of the basic processes involved in the effectiveness, deposition, degradation and persistence of pesticides must be developed prior to and concurrently with their use, if the presence of harmful residues in the human environment is to be avoided(Ebeling, 1963).

The research, which this thesis is based on, has been a part of a broad area of pesticide investigation to determine their distribution and possible side effects in the environment. The specific objective of the present research was to develop experimental methods and rationale for studying, quantitatively, the interactions of DDT with ecologically significant components of soil organic matter.

LITERATURE REVIEW

Properties of DDT and Some Reactions

First synthesized and described by Zeidler in 1879, the insecticidal properties of DDT were discovered by Paul Müller in 1939, who then received the Nobel Prize for that discovery(Miskus, 1964). DDT has a melting point of 108.5-109.0 C(Haller et al., 1945). It is very soluble in a number

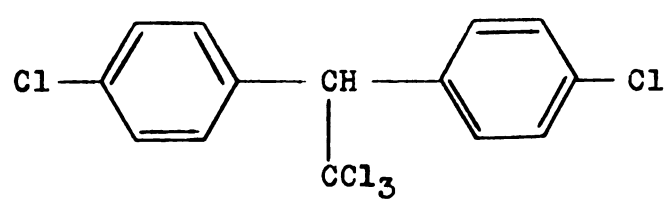

Figure 1. Chemical structure of DDT: 1,1,1-trichloro-2,2-bis(p-chlorophenyl)-ethane. M.W. 354.5 $C_{14}H_9Cl_5$

of organic solvents. Its solubility in water, however, has claimed considerable discussion among investigators. DDT is practically insoluble in water, having great affinity for

the air-water interface which facilitates a high codistillation rate with water(Acree, 1963). Bowman et al.(1960) reported the solubility of DDT in water as 1.2 ppb or less at 25 C. Other workers found that the solubility was about 1.7 ppb(Biggar et al., 1967). According to data compiled by Gunther et al.(1968), it appears that the most reliable solubility of DDT in water is about 1.2 ppb at 25 C as reported by Bowman et al(1960). It is clear that, even when dissolved in a good solvent, a small quantity of DDT will partition into water(Voerman, 1969).

DDT is chemically a very stable compound. It is resistant to mild reducing agents, and resistant to oxidation. But DDT is decomposed by boiling with anhydrous zinc chloride in glacial acetic acid solution. Reduction also occurs when treated in boiling alcoholic solution with zinc and concentrated hydrochloric acid(Forrest et al., 1946). Anhydrous ferric and aluminum chlorides, iron, iron oxides, and certain mineral materials have been found to act catalytically to eliminate hydrogen chloride from DDT(Fleck and Haller, 1944). An experiment showed that benzene hexachloride had a pronounced deleterious effect on the thermal stability of DDT in admixture. In all probability the hexachloride preparations contained minute traces of iron or other catalyzing materials which elicited this response(Gunther, 1947). In a vineyard soil where a large amount of copper residues was built up, about half of the total DDT applied over a period of 6 years and two thirds of that applied over 12

years were not recovered from the soil(Taschenberg et al., 1961). Under field conditions the highest total decrease in DDT residues was shown by those sprays containing aluminum stearate(Gunther et al., 1946). An interesting phenomenon was that under absolutely dry soil conditions the adsorbed DDT was catalytically decomposed to an ethylene derivative, the soil used having a high iron content(Hadaway, 1951). Conversion of DDT was observed in the presence of reduced porphyrins(Miskus et al., 1965) and dechlorination of DDT was incurred by exposure to reduced Fe(II) cytochrome oxidase(Wedmeyer, 1966).

It has been shown that ultraviolet light catalyzes the decomposition of DDT(Fleck, 1949; Chisholm et al., 1949). Therefore the toxicity of DDT deposits may be reduced by exposure to sunlight or during storage. In the field, minimum tillage after thorough mixing of the insecticide throughout the soil profile and extremely high rates of application has been reported to contribute to persistence of DDT in soil(Nash and Woolson, 1967; Lichtenstein and Schulz, 1961).

Although the majority of the organic pesticides appear to be subject to microbial degradation, DDT is apparently highly resistant(Martin, 1963; Kallman and Andrews, 1963; Miskus et al., 1965; Chacko et al., 1966; Barker et al., 1965; Stenersen, 1965; Wedmeyer. 1966; Guenzi and Beard, 1967; Duffy and Wong, 1967; and Johnson et al., 1967). Reductive dechlorination to DDD does occur in anaerobic

systems. Further biological alteration appears to be extremely slow.

Insecticidal preparation containing DDT was first brought to the attention of the USDA in October, 1942, by the Geigy Co., Inc., New York(Haller et al., 1945). United States production of DDT in 1965 was 140 million pounds(The Pesticide Review, USDA, 1968). World yearly production is in the order of 10^{11} grams, only five orders of magnitude less than the amount of carbon fixed annually by plants into organic matter(Risebrough, 1969).

Adsorption of DDT by Soils

Soils with higher clay and silt content generally were more adsorptive than those with less clay and silt content (Bowman et al., 1965; Berck, 1953). It was, however, found that the adsorptive capacities, expressed as the distribution coefficient(Kd), per unit area of adsorbent were the same order when the sand and silt material fractions were compared(Kay and Elrick, 1967). In moist soil adsorption of DDT is proportional to the organic matter content of the soil(Harris, 1964; Lichtenstein, 1959; Lichtenstein and Schulz, 1959; Woodwell, 1961; Fleming and Maines, 1953).

DDT applied to soil has moved very slightly in 2 decades of weathering in an open field in Southern Mississippi. Neither DDT nor its analogues or degradation products were detected below a depth of 12 inches under the originally

treated soil. The most distant movement of DDT detected was about 100 feet downgrade on the surface. This movement was undoubtedly caused by sheet erosion of soil particles carrying adsorbed DDT molecules. The area examined had received more than 100 feet of rain during 20 years(Smith, 1968). The report is consistent with those of other workers. Most of the DDT had accumulated in soil horizons corresponding to plow and cultivation depths. In general, the results indicate that DDT residues do not penetrate vertically downward below the plow and cultivated depths(Chisholm et al., 1950; Ginsburg and Read, 1954; Ginsburg, 1955; Lichtenstein, 1957; Lichtenstein, 1958).

The general absence of major contamination of ditch sediments after extensive application of DDT(Trautman et al., 1968), no detectable residues in water sampled after treatment of soil(Fahey et al., 1968), and little horizontal movement of the residues in bog soils(Deubert and Zuckermann, 1969) all suggest that DDT is not highly mobile in runoff waters(Harris, 1969).

However, it is recognized that a major mechanism for entry of highly insoluble hydrocarbons into surface waters is through erosion movement of soil particles on which the pesticides are adsorbed(Barthel et al., 1966). The particles most likely to move are those of colloidal dimension which are most readily dispersed and most stable in suspension. There is evidence that these are also most active in adsorption. In a recent study of runoff movement, Epstein

and Grant(1968) found generally higher concentrations of DDT in the runoff suspension than in the settled sludge. If their data for the suspension had been calculated on the basis of dry weight of suspended solids, as their sludge data were, a very much greater adsorption by suspended particles than by settled materials would have appeared.

It is well established that DDT and its metabolites are concentrated in bottom sediments of lakes and streams and to an even greater extent in suspended particulate matter(Berck, 1953; Keith and Hunt, 1966). Chlorinated hydrocarbons in raw water samples from Lake Erie were not detected by the usual liquid-liquid extraction procedures, but they were found in microparticulates(<.15 micron) which were separated by centrifugation(Pfister et al, 1966). Density gradient fractionation of the microparticulates indicated that lindane was associated principally with inorganic particles, whereas aldrin and endrin were associated with less dense organic fractions, detritus and microorganisms. There was evidence that DDT and its metabolites were also present but specific association with particles of different density was not clearly expressed.

Thus, its very low solubility in water and its sorption by soil materials may immobilize DDT and restrict its movement on landscapes and under management programs where erosion is minimized. On the other hand, the probability that it is preferentially adsorbed by microparticulate fractions which are readily suspended is a mechanism for its

concentration in runoff waters and bottom sediments.

A preferential association with small particles would be expected simply because of the large sorptive surface which they expose. However, the nature of these colloidal surfaces is also important. Because of the non-polar nature of DDT, it is likely that it will interact more strongly with organic than with mineral surfaces(Kunze, 1966). Wershaw et al.(1969) observed a 40 to 100-fold increase in solubility of DDT in aqueous solution when 0.5 % soil humic acid was added in the form of the sodium salt.

Adsorption of DDT by Soil Microbes

Several investigators have reported extensive uptake of DDT and other chlorinated hydrocarbons by microorganisms from culture media and from soils(Chacko and Lockwood, 1967; Ko, 1967). Under appropriate anaerobic conditions, uptake is accompanied by metabolic conversion of DDT to DDD*(Chacko et al., 1966; Guenzi and Beard, 1967; Guenzi and Beard, 1968; Johnson et al., 1967; Kallman and Andrews, 1963; Ko and Lockwood, 1968a).

However, metabolic activity is not necessary for uptake, since absorption is similar for living and heat-killed cells or mycelia. The phenomenon appears to be a non-specific property of fungi, actinomycetes and bacteria.

Ko and Lockwood(1968b) used a differential particle size technique to recover fungal and actinomycete mycelia

* DDD(1,1-dichloro-2,2-bis(p-chlorophenyl)ethane)

after exposure to Conover loam soil to which DDT, dieldrin and PCNB* had been added. The tissues absorbed the chemicals from the soil. After 48 hours, concentrations in recovered moist mycelia were 2 to 8 times greater than in the ambient soil. These and other experiments involving addition of mycelium to H_2O_2 treated soil led the authors to conclude that microbial tissues may be more effective than other kinds of organic matter in soils in retention of these compounds against leaching. They noted that differential concentration of resistant pesticides in the soil microflora might represent a first step in the cycling of these chemicals along food chains in the soil environment.

Adsorption of DDT by Plant Materials

Crafts and Foy(1962) have presented a review on the chemical and physical nature of plant surfaces in relation to the use of pesticides and in relation to their residues.

Ware et al(1968) reported that DDT and related degradation products in alfalfa fields in Arizona were found in the following order of decreasing concentration: wax, root, epidermis, top 1/4 inch of soil, upper 6 inches of soil, whole root, root cortex, leaves, leaves plus stems, and stems. The residues, however, were acquired directly from drift of agricultural insecticides and indirectly from wind blown contaminated soil, rather than by translocation through the roots.

* PCNB(pentachloronitrobenzene)

However, the absorption and concentration by plant roots of chlorinated hydrocarbons directly from soil has been shown for several crop species(Lichtenstein, 1959).

Extensive surveys in California suggest that DDT residues are more transitory in upland soils and vegetation than in poorly drained areas with marsh vegetation(Keith and Hunt, 1966). A recent study by Odum et al.(1969) in a marsh on Long Island indicates that decomposing plant detritus represents an environment in which resistant hydrocarbons can accumulate and persist for many years. They determined the concentration of these compounds in standing vegetation, in larger litter fragments and in particle size fractions of detritus in a marsh which had been sprayed regularly for 15 years with DDT for mosquito control. Maximum concentrations (up to 50 ppm) were found in detritus fractions of intermediate particle size(250 to 1,000 microns).

These concentrations were 20 to 50 times greater than in the living plants and 3 to 5 times greater than in the coarse litter. Detritus particles of this size are associated with decomposition stages where microbial activity is intense. They are also ingested by many deposit and filter feeding organisms. Retention and accumulation of DDT residues in plant detritus was credited in this study for disappearance of the fiddler crab from this marsh.

Adsorption Mechanisms

Soils and clay minerals: Much of what is known regarding interactions between pesticides and natural soils is based on indirect evidence from studies in which various factors relating to the pesticide, the soil or the environment have been correlated and weighted in terms of their effects on bioactivity, persistence or leachability of the pesticide. Edwards(1966) summed up these factors as follows: (1) Primary factors are the chemical structure of the pesticide and its intrinsic stability. (2) Secondary factors relate to soil type. The most important single soil characteristic is its organic matter content, although clay content and soil structure are also important. (3) Tertiary factors are temperature and soil moisture. (4) Quaternary factors include formulation and concentration of the pesticide, soil mineral composition, soil acidity and plant cover.

It is recognized that these general relationships derive from the collective action of numerous microfactors which influence adsorption of organic molecules by soil colloids(Bailey and White, 1964). A great deal of information regarding fundamental mechanisms of adsorption has been obtained in recent years through application of thermodynamic and spectrophotometric methods and concepts in studies with model compounds. Most studies with pesticides have used highly purified and well characterized clay minerals(mont-

morillonite, kaolinite, vermiculite) as models for the mineral component of soil colloids(Bailey et al., 1968; Kunze, 1966; Mortland, 1968; Weidhaas et al., 1961).

Adsorption isotherms and infrared spectra have been widely used in such studies. Supporting principles for interpretation of data obtained by these methods are drawn from an extensive literature based on studies with organic compounds other than pesticides and with a variey of adsorbents(Jordan, 1949; Cowan and White, 1958; Grim, 1968; Farmer and Mortland, 1965, 1966; Tensmeyer et al., 1960; Greenland, 1965a).

From the studies with model silicates it is established that adsorption is a complex function of properties related to the adsorbate, the adsorbent and the interfacial environment. Important characteristics of the adsorbed molecule are its solubility, degree of acidic or basic dissociation, resonance and tautomeric susceptibility of the essential molecule, and the nature and distribution of substituent peripheral groups (-Cl, $-NH_2$, $-NO_2$, $-CH_3$, $-C_3H_7$, etc.). Important features of the adsorbing colloid include surface area, sign and density of electrical charge, and polarizing and coordinating capabilities associated with intrinsic structure or with adsorbed water or exchangeable ions.

The most energetic forces leading to adsorption are chemical. These involve electrovalent bonds between charged colloid surfaces and organic ions which arise by dissociation, protonation or charge transfer(Rooney and Pink, 1962). Polyvalent mineral ions may be involved as salt bridges.

Physical forces provide the weakest sorptive attraction. These are commonly referred to as van der Waals' forces. They include gravitational and electrostatic forces which come into play when adsorbate and adsorbent approach each other closely. Electrostatic orientations arise through interaction of dipoles in which charge separation exists or is induced by close proximity.

Adsorptive forces of intermediate strength are represented by coordinate-covalent bonding(ion-dipole interaction), bridging by water molecules and H-bonding. Water itself competes strongly for these sites. As a result, the degree to which numerous pesticides are adsorbed by soil and their bioactivity are strongly influenced by soil moisture content (Bailey and White, 1964; Ko and Lockwood, 1968b; Mortland and Meggitt, 1966).

The extent of chemical adsorption will be strongly influenced by pH, since hydrogen ion concentration determines the state of dissociation of acidic, basic or amphoteric molecules and the total charge of natural soil colloids. With basic compounds, the pH of the colloid surface is critical, whereas with acidic compounds it is the pH of the ambient solution. With some clay minerals, such as montmorillonite, the surface pH may be 3 to 4 pH units less than that of the bulk solution. Chemical attraction for basic compounds will be negligible and adsorption due mainly to physical forces when surface acidity is more than 2 pH units larger than the pKa of the compound. With acidic compounds,

repulsive forces between negative charges on the colloid and the anion will result in negative adsorption when the pH of the ambient solution is more than 1 to 1.5 units larger than the pKa of the acid(Bailey et al., 1968).

Adsorption of neutral or weakly polar compounds is influenced to a much lesser extent by pH, although there is a general tendency for adsorption of most classes of pesticides to increase with decreasing pH by reason of enhanced hydrogen bonding. Polarizing effects of either high or low pH would be expected to promote physical adsorption by electrostatic attraction between dipoles. However, increasing polarity also enhances forces leading to dispersion, dissociation or chemical alteration of the compound, the adsorbent, or both(Masterton et al., 1969; Wershaw et al., 1969; Scott and Vinogradov, 1969; Mortland and Raman, 1967).

If we start with a colloidal surface with a given density of charge and other active sites, the extent of adsorption will be a function of the total surface accessibly exposed for interaction with the adsorbate(Bailey and White, 1964; Kunze, 1966). In soils, the extent to which a pesticide is exposed to adsorptive surfaces will be determined, in part, by the extent to which it is physically mixed with the soil during application or subsequent tillage or the extent to which it moves from the point of application through the aqueous or gaseous phase. On the other hand, the total colloidal interface with soil air or water will be determined by such factors as particle size, aggregate size,

aggregate porosity, and the presence of clay minerals with expansible interlayers. In the case of compounds which present themselves principally in the aqueous phase, adsorption will also be influenced by the wettability of colloidal surfaces.

The effectiveness of pesticides in the field is influenced by structure(degree of aggregation of soil particles). The materials used in pesticide formulations as solvents, emulsifying agents or surfactants also influence the activity and mobility of the pesticides(Bailey and White, 1964; Edwards, 1966). Solvent effects and surface tension effects could influence significantly the extent to which a pesticide is exposed to sorptive surfaces within the soil.

Temperature is an important factor affecting adsorption, both in the field and in experimental systems(Bailey and White, 1964). Adsorption processes are exothermic, desorption processes are endothermic. The tendency for adsorption to increase with decreasing temperature and decrease with increasing temperature is generally reinforced by the inverse effects on solubility and vapor pressure. By definition, adsorption isotherms are determined at constant temperature.

Soil organic matter: Highly purified and well-characterized clay minerals have been very useful as model adsorbents in studying sorptive mechanisms. The clay minerals that have been used are important components of the colloidal fractions of many natural soils. However, through action of soil

forming processes soil mineral colloids have already reacted extensively with organic compounds released or formed during humification of biological materials(Bremner, 1965, 1967; Felbeck, 1965; Greenland, 1965a, 1965b; Kononova, 1966).

The colloidal content of upland soils is determined by their content of mineral colloids contributed directly or by weathering from parent materials. Even in highly organic soils, mineral constituents are structurally incorporated and contribute to the stability of humic colloids(Schnitzer and Desjardins, 1965). Nevertheless, regardless of soil type, active soil colloids frequently behave as though their surfaces were organic rather than mineral.

In zonally related soils, organic matter content and mineral colloid content tend to be highly correlated with each other, as well as with important soil properties, such as structure, water-holding capacity, cation-exchange, pH buffering and the capacity to interact with pesticides (Bailey and White, 1964; Chesters et al., 1957; Helling et al., 1964; Oades, 1967). In the expression of many of these properties, organic matter is frequently judged to be more influential than mineral constituents(Acton et al., 1963b; Chesters et al., 1957; Deshpande et al., 1968; Edwards. 1966; Ginsburg, 1955; Ginsburg and Read, 1954; Griffiths, 1965; Kay and Elrick, 1967; Lambert, 1967; Mehta et al., 1960).

Soil organic matter is understood to be a heterogeneous and dynamic mixture of carboniferous materials at varying stages of humification, from products of recent synthesis

by plants, animals or microorganisms to an extensively altered, amorphous end product, humus(Felbeck, 1965; Flaig, 1960; Greenland, 1965b; Kononova, 1966).

a. Nonhumified detritus

Among the relatively non-humified organic materials are structural fragments of plants or insects which can be separated from the soil by densimetric flotation, elutriation or mild sonication(Greenland, 1965a, 1965b; Edwards and Bremner, 1967a; Oades, 1967; Pfister et al., 1966). Such materials are characterized by low external surface and large internal surface. They may account for as much as 50 percent of the organic matter in upper surface horizons of some soils, but are normally less than 10 or 15 percent, depending upon vegetation and on seasonal, climatic and management factors which influence the nature and activity of faunal and microbial populations responsible for decomposition (Kononova, 1966).

As has been noted, earlier, decomposing detritus and the associated animals and microorganisms may be particularly effective in absorbing and concentrating pesticides.

Organic materials left after removal of floatable detritus appear to be intimately associated principally with clay-size mineral colloids($< 2 \mu$). The association is sufficiently energetic that very little organic matter is separated from the mineral colloids by ultrasonic dispersion(Greenland, 1965a; Edwards and Bremner, 1967a, 1967b). Sand and silt-size minerals which are separated by sedimentation after ultrasonic

dispersion are probably coated with organic materials, also. However, the quantities of organic matter which can be associated with these non-colloidal mineral fractions is limited by their low surface area.

b. Lipoid materials

Organic coatings which form on sand particles are frequently hydrophobic and give rise to the phenomenon of water repellency in sandy soils(Bond, 1968; Butler et al., 1964). A similar "waterproofing" effect is observed when alkylamines with more than six carbon atoms are adsorbed by clay minerals(Greenland, 1965b). Most soils contain hydrophobic materials which may be classified as lipids or pseudolipids by reason of their solubility in alcohol and/or nonpolar solvents(Stevenson, 1966). These include paraffins (C_{23} to C_{33} normal alkanes), normal primary alcohols(C_{36} to C_{52}) and acids(C_{12} to C_{30}), porphyrins, steroids, terpenoids, carotenoids, as well as pigments of insect or fungal origin (Butler et al., 1964; Kumada and Hurst, 1967).

Lipoid materials in soils appear to be products of plant, animal or microbial synthesis which are difficultly biodegradable. They tend to accumulate in highly acid soils or in soils which are either drouthy or poorly drained, where their decomposition relative to other substances is further retarded. For this reason, they must be considered relict substrates rather than products of humification.

Significant portions of the lipoid materials in soil are directly extractable with lipid solvents without acid

pretreatment(Stevenson, 1966). This is evidence that they are deposited on peripheral surfaces and are not extensively complexed with humified organo-mineral colloids. Their resistance to decomposition may derive, in part, from their deposition on soil surfaces which are frequently dry and, at such times, unfavorable for microbial activity, e.g. sand grains and surfaces of large pores. Their tendency to accumulate at such sites may be due to their deposition from the leading edges of water films during cycles of wetting and drying. The presence of hydrophobic coatings on the surfaces of large pores and structural peds is recognized as one probable mechanism for stabilization of soil aggregates (Griffiths, 1965; Martin et al., 1955; McCalla, 1950; Quirk and Panabokke 1962).

Hydrophobic organic materials in soils would have special affinity for nonpolar pesticides. Lipid solubility and alternate wetting and drying would tend to isolate such pesticides on infrequently wetted surfaces when they would be immobilized and protected against degradation. Such probable interactions have not been investigated.

Lipid materials normally comprise 1 to 6 percent of the soil organic carbon. The balance of the organic matter which has been altered beyond the point where plant or insect structures can be recognized is a heterogeneous mixture which has been studied most extensively in terms of differential solubility or dispersibility in alkali and acids(Felbeck, 1965; Greenland, 1965b; Kononova, 1966; Mortensen, 1965;

Stevenson, 1965).

c. Humic acid

The fractions most usually differentiated are "humin" (insoluble in alkali), "fulvic acid"(soluble in alkali and not precipitated by acids at pH 1 to 3) and "humic acid" (soluble in alkali, precipitated by acids). The humic acid fraction has received the most attention because its solubility at alkaline pH and its insolubility in acids, alcohol, ether, facilitate its extensive purification (Burges et al., 1964).

In spite of extensive study, the chemical nature of humic acids is imperfectly understood. On hydrolysis with acids or alkali, amino acids, amino sugars, sugars, uronic acids, nucleic acid derivatives and aromatic derivatives of lignin are released(Acton et al., 1963a; Anderson, 1967; Bremner, 1965, 1967; Dormaar, 1967a, 1967b; Mehta et al., 1961; Stevenson and Mendez, 1966). Although up to 50 percent of the nitrogen in humic acids may be released as amino acids on hydrolysis, humic acids have none of the properties of true proteins, nor of any biologically synthesized polymer, except perhaps lignin(Steelink, 1964). Reductive degradation or alkaline fusion methods indicate that the essential structural units are aromatic degradation products of lignin and of flavonoid pigments(Burges et al., 1964; Coffin and DeLong, 1960; Stevenson and Mendez, 1966).

The essential chemical nature of humic acids is best understood in terms of model polymerization reactions which

lead to similar dark, amorphous, chemically and biologically resistant products. The most useful parallels can be drawn with the synthetic humic acids formed by oxidative condensation of polyphenols in the presence of ammonia or amino acids and with the melanoidins formed in the "browning reactions" between amino acids and fission products of sugars. Out of such studies have evolved numerous views of the skeletal structure of humic acids. In general, all envision a three-dimensional, randomly condensed network of aromatic or heterocyclic rings and/or polycycles held together by various bridging structures. Probable bridging structures include direct C-C linkages, ether linkages, amide nitrogen and carbon chains of varying length and degree of unsaturation (Bremner, 1965, 1967; Felbeck, 1965; Flaig, 1960, 1964; Hurst and Burges, 1967).

Amino acids, amino sugars and nitrogen bases may be directly incorporated into the skeletal structure through amino groups which serve also as bridging structures. They are strongly held. Extended hydrolysis or oxidative degradation are required to release them from soil humic acids (Anderson, 1967; Savage and Stevenson, 1961; Stevenson, 1965a, 1965b).

Amino acids and uronic acids may also be bound by ester linkages or salt bridging through polyvalent cations. Sugars may be held by glycosidic linkages or they may be H-bonded, held by van der Waals' forces or trapped sterically in the aromatic network, mechanisms which may also be involved

in retention of other species, both organic and inorganic (Hurst and Burges, 1967; Mehta et al., 1960). However, the great difficulty encountered in removing silicates and iron and aluminum from soil humic acid preparations suggests that these are involved as binding structures in the intimate structural framework of the humic acids themselves(Edwards and Bremner, 1967a, 1967b; Greenland, 1965b; Schnitzer and Desjardins, 1965).

Functional group analysis of soil humic acids reveals surface groupings which would be expected of a predominantly aromatic structure derived from degradation products of lignin or flavonoid pigments: carboxyl, carbonyl and phenolic hydroxyl(Dubach et al., 1964; Leenheer and Moe, 1969; Schnitzer and Skinner, 1968). Infrared spectra of humic acids also provide evidence for these groupings, although such spectra are frequently lacking in detail because of background absorption which increases with degree of humification (Burges et al., 1964; Ziechmann, 1964).

In composting plant materials, advancing humification is accompanied by decreasing alcoholic -OH and increasing content of carboxyl and carbonyl groups(Flaig, 1960, 1964; Hurst and Burges, 1967). In soil environments, the increase in carboxyl groups may be obscured by reaction with iron or aluminum oxides or with polyvalent cations associated with silicate minerals, unless these mineral constituents are first removed by treatment with hydrofluoric acid(Leenheer and Moe, 1969; Schnitzer and Desjardins, 1965) and suitable

desalting procedures(Schnitzer and Skinner, 1968).

An increase in methoxyl groups during early stages of humification is evidence for release of guaiacyl and/or syringyl groups. Demethylation may or may not occur before these lignin units are condensed into humic acid structures. A decrease in methoxyl groups is characteristic of advancing humification in aerobic composts(Flaig, 1960, 1964; Hurst and Burges, 1967). In poorly drained situations where organic soils develop, humification sequences going from peats to mucks are accompanied by an increase in methoxyl content, probably because aerobic fungi responsible for demethylation are absent(Schnitzer and Desjardins, 1966).

Increases in phenolic -OH due to hydrolysis of methyl or phenyl ethers and decreases in phenolic -OH due to oxidation are frequently not observed(Leenheer and Moe, 1969; Schnitzer and Desjardins, 1966). Nor is there direct evidence for the quinoid carbonyl which would be expected from oxidation of phenols(Dubach et al., 1964; Hurst and Burges, 1967). This is not surprising, since free radical intermediates of phenol oxidation tend to regain aromaticity by forming addition products which are at the same oxidation level as the quinone which might otherwise result but are more stable (Flaig, 1960, 1964; Mortland and Wolcott, 1965; Scheffer and Ulrich, 1960). Addition of water leads to regeneration of phenolic -OH. Addition of other aromatic units or nitrogen compounds leads to synthesis and growth of the humic acid molecule.

Polymerization by free radical mechanisms may be autocatalytic, as in the Maillard browning reactions at acid pH or in the oxidative condensations of phenols at alkaline pH. In nature, condensations of lignin degradation products are probably catalyzed principally by phenoloxidases, although mineral catalysts including reducible ions(Fe III, Cu II) and silica are also effective(Scheffer and Ulrich, 1960; Ziechmann and Pawelke, 1959).

Stable free radicals are formed by charge transfer mechanisms when aromatic compounds are adsorbed by silica-alumina cracking catalysts(Rooney and Pink, 1962). Free radical mechanisms are probably involved, also, in stabilizing complexes between aromatic molecules and transition metals when these are formed on clay mineral surfaces(Farmer and Mortland, 1966; Doner and Mortland, 1969). Extremely stable π-coordination complexes of benzene, toluene, xylene and chlorobenzene with copper are formed by adsorption on clay minerals such as montmorillonite in which charge originates in the octahedral layer. The infrared spectra of the adsorbed complexes of benzene with Cu I or Cu II are identical, which implies that free radical hybrid structures are involved. These copper complexes are not stabilized by adsorption on clays such as saponite or vermiculite in which charge is situated principally in the tetrahedral layer. This failure to stabilize has been ascribed to steric crowding and more stable hydration properties associated with high surface charge density. However, the greater stability

of metallo-organic complexes on hectorite and montmorillonite may be understood, also, in terms of more stable free-radical character due to resonance afforded by delocalizing of charge in the octahedral layer. In clays from natural sources, a high unpaired electron spin concentration has been found in montmorillonite and illite but not in vermiculite(Friedlander et al., 1963).

In keeping with the free radical mechanisms which appear to be involved in synthesis of humic acids, it is found that humic acids themselves have a high concentration of unpaired electrons(Friedlander et al., 1963; Theng and Posner, 1967; Steelink and Tollin, 1967). Steelink(1964) has observed an increase in spin concentration with increase in degree of humification in the sequence: native lignin(3 to 5 x 10^{16} spins per gram), fulvic acid(3 x 10^{17} spins per gram), humic acid(0.3 to 1.4 x 10^{18} spins per gram). The spin concentration of humic acids increased with extensive acid hydrolysis and with chemical oxidation. Chemical reduction tended to lower spin concentrations only slightly. Thus, it may be expected that free radical content of organic materials in soils will increase with increasing degree of oxidation and with increasing resistance to chemical or biological degradation. A number of authors have noted that free radical mechanisms may be important in the interactions of pesticides with soil organic fractions(Farmer and Mortland, 1966; Friedlander et al., 1963; Steelink and Tollin, 1967).

Synthesis and increase in molecular weight of humic

acids is dependent upon a continuing supply of appropriately oxidized products of hydrolysis or metabolism released by the animal and microbial populations which decompose plant materials. However, the humic acids themselves are also subject to continuing attack by extracellular enzymes which hydrolyze or oxidize surface structures, leading to ring cleavage and degradation. A dynamic situation exists, therefore, at the surface of a humic acid molecule, with synthesis and degradation going on simultaneously(Hurst and Burges, 1967).

Qualitatively, the same functional groups(carboxyl, carbonyl, phenolic -OH) appear on the surface of the humic acid during condensation and synthesis as are exposed by ring cleavage and degradation(Flaig, 1960, 1964). Thus, the central humic acid molecule presents always an active surface for interaction with mineral and organic species as they appear in the immediate environment through mineral weathering or enzymatic activity.

For these reasons, humic acids are heterogeneous both as to composition and molecular size. Ultracentrifugation and gel filtration studies show that humic acid preparations are polydisperse. The range of molecular weights in a given sample will vary with source and method of preparation. Reported values run from 5,000 to 100,000 or more(Greenland, 1965b; Hurst and Burges, 1967).

d. Fulvic acids

The fulvic acid fraction is a more heterogeneous

mixture. Its principal non-dialyzable components appear to be mixtures of polysaccharides and colored phenolic materials (Greenland, 1965b).

The phenolic substances in the fulvic acid fraction are essentially similar to humic acids except smaller and more polar. In size they range from dialyzable, low molecular weight materials which are soluble in water, alcohol or organic solvents(Keefer et al., 1966; Kononova, 1966; Stevenson, 1967; Wright and Schnitzer, 1960) to large molecules in the humic acid range with molecular weights up to 9,000(Hurst and Burges, 1967). Their greater polarity derives from a higher surface carboxyl content than in humic acids and a higher degree of oxidation(Schnitzer and Gupta, 1964; Wright and Schnitzer, 1960).

The yield of fulvic acid and, accordingly, the ratio of fulvic to humic acids are increased by extraction procedures which reduce ash content. The fulvic/humic acid ratio increases with advancing humification. These observations have led to the proposal that phenolic residues in the fulvic fraction are products of humic acid degradation which have been stabilized in the soil by interaction with minerals (Schnitzer, 1967). The degree of stabilization is evidenced by radiocarbon measurements which indicated a mean residence time for carbon of 375 years in a Podzol B horizon(Tamm and Ostlund, 1960) and 630 years for the fulvic acid fraction of a Chernozemic plow soil(Paul et al., 1960).

In the latter study, the apparent residence time for

carbon in the fulvic acid fraction was about half that for the humin(1,240 years) and humic acid fraction(1,308 years). This reflects the more dynamic nature of fulvic acid materials. An important implication is that stable antique carbon in this fraction is diluted by modern carbon in the form of rapidly cycling non-humified or partially humified materials.

Carbohydrates of recent synthesis are an important diluent. In Sackatchewan soils associated with the one used in the above carbon dating study, 15 to 25 percent of the total soil carbohydrate appears in the fulvic acid fraction (Acton et al., 1963a). With appropriate isolation procedures, up to 30 percent of the carbon in the fulvic fraction can be recovered in the form of high molecular weight, water-soluble polysaccharides(Greenland, 1965b). These appear to be products of microbial synthesis since, in addition to uronic acids and sugars normally found in plants, hydrolysis releases hexosamines and a number of sugars which are not usual plant constituents(Mehta et al., 1961). They exist in a dynamic state of degradation and resynthesis. The quantity of extractable soil polysaccharide increases over a period of weeks after addition of plant materials and then declines as added primary substrates are depleted(Acton et al., 1963b; Oades, 1967). When ^{14}C-labelled substrates are used, the labelling of individual sugars follows the fluctuations in quantity of polysaccharide(Keefer and Mortensen, 1963).

Extractable polysaccharides represent only a portion

(perhaps 30 to 60 percent) of the carbohydrate in the fulvic fraction, and usually 10 percent of the total hydrolyzable carbohydrate in the soil. Since no more than 10 to 20 percent of total soil carbon can be accounted for as carbohydrates, the extractable polysaccharides represent no more than 1 or 2 percent of the total organic material in the soil(Acton et al., 1963a; Gupta, 1967; Mehta et al., 1961).

Nevertheless, from the standpoint of investigations into the microstructure and ecology of soil organic matter, soil polysaccharides and extracts in which they appear are extremely important.

It is clear that the polysaccharides come from sites of microbial activity in the soil. Considerable quantities of proteinaceous materials which are intimately associated with the polysaccharides are evidently products of microbial degradation and synthesis, also(Bremner, 1967; Gupta, 1967; Mortensen, 1960; Roulet et al., 1963). At least a portion of the carbohydrates in fulvic acid are associated with phenolic materials, some of which are recent products of decomposition of lignin and flavonoid pigments(Hurst et al., 1967; Keefer et al., 1966; Kononova, 1966; Mehta et al., 1961). Low molecular weight materials representative of most classes of organic compounds are also found and are considered to be products of current metabolic activity.

Thus, the relatively great apparent age of fulvic acid preparations must be ascribed to humic acid fragments whose bonding to larger humic complexes has been loosened through

action of exoenzymes, chelating agents and acids produced by a closely associated microflora(Hurst and Burges, 1967; Mehta et al., 1961; Stevenson, 1967).

It is recognized that colonization of solid surfaces in soils by microorganisms is a random matter, depending upon the nature of substrates which appear by chance in a particular microenvironment(Kononova, 1966). The physiological types of organisms and their period of occupancy of a given site will vary over time. The metabolic activities of different organisms will have different effects on the organo-mineral colloids within reach of their influence. As a result, the colloids in any soil will be characterized by a wide range of bonding energies.

This wide range of bonding energies is evidenced by the fact that the quantity of organic matter which can be extracted from a soil varies with the ionic strength or chelating properties of the extracting agent. Neverthless, when different extracted fractions of the same soil are subjected to hydrolysis, a similar spectrum of monomeric products is obtained(Bremner, 1965, 1967; Hurst and Burges, 1967; Kononova, 1966; Mehta et al., 1961). The quantities of organic matter which can be extracted increase markedly during early stages of decomposition of added plant materials and then decline as these primary substrates are used up.

These relationships indicate that materials coextracted by a given reagent come from sites in the soil which have been similarly influenced by metabolic processes. The

extractable polysaccharides are identifiable products of microbial activity and provide a direct index of microbial activity at these sites.

The total quantity of polysaccharides is highest in soils containing large amounts of non-humified or partially humified detritus(Acton et al., 1963a; Mehta et al., 1961; Oades, 1967). The composition of soil polysaccharides changes in a characteristic way as energy in these primary substrates is utilized. This is because constituent hexose and pentose sugars are subject to wider fluctuations than are uronic acid components. The ratio of sugars to uronic acids increases as total polysaccharide increases over a period of several weeks after substrate addition. After reaching a peak, decreasing polysaccharide content is accompanied by decreasing ratio of sugars to uronic acids(Gupta, 1967; Keefer et al., 1963). A similar dilution of sugars by uronic acids is a striking feature of polysaccharide synthesis in natural soils(Lowe, 1967).

Within the extractable soil polysaccharides themselves there is a non-uniform distribution of uronic acids and this is related to the extractability of the polysaccharides and of associated organic and mineral materials. The polysaccharides from a given soil can be partially separated by electrophoresis into several groups of polymers which are distinctly different in uronic acid content but contain a similar spectrum of sugars(Mortensen, 1960; Roulet et al., 1963; Thomas et al., 1967). Those of highest uronic acid

content appear in the humic acid fraction, from which, however, they are readily extracted with dilute acids (Barker et al., 1965; Gupta and Sowden, 1964; Roulet et al., 1963).

The effectiveness of several polysaccharide extractants that have been used increases in the order: hot water $\simeq$ dilute phosphate buffer < sodium pyrophosphate < dilute alkali < dilute acids. Increasing yield of polysaccharides, however, is accompanied by increasing amounts of contaminating minerals and other organic materials(Bernier, 1958; Gupta, 1967). This supports the view that coextracted soil materials come from sites where colloidal complex structures have been similarly weakened by microbial activity. The quantity and uronic acid content of soil polysaccharides has been shown to be related to the progress of decomposition of plant residues. Thus, it would appear that reagents which result in differential extraction of polysaccharides may be used to make ecological inferences regarding the stage of detritus decomposition and the level of microbial activity at sites of origin of polysaccharides and coextracted materials in the microfabric of the soil. No systematic basis for such inferences has been developed, however.

e. Humin

The traditional "humin" fraction includes carbonized residues and humic acids which, by reason of high molecular weight or complexing with clay minerals in resistant aggregates, are not dispersed in alkali(Edwards and Bremner,

1967: Greenland, 1965b; Hurst and Burges, 1967; Kononova, 1966). Non-humified materials may also appear in the humin fraction. They may be a major component unless flotation or other pretreatments are employed to reduce their contribution(Edwards and Bremner, 1967a; Greenland, 1965a, 1965b; Oades, 1967).

f. Other fractionation schemes

Proximate fractionation schemes borrowed from plant and feed chemistry have been used to study gross changes in composition of plant materials during decomposition(Flaig, 1960, 1964; Mortensen and Himes, 1964; Stevenson, 1965). One such scheme involves sequential extraction of the same sample with a series of reagents to remove fractions analogous to several classes of plant constituents:

Ether(fats, waxes, oils)

Alcohol(resins)

Hot water(water-soluble polysaccharides)

2 % HCl(hemicelluloses)

Cold 80 % H_2SO_4(cellulose)

Residue("protein plus lignin-humus")

Such schemes have been used extensively in studying decomposition of plant materials in forest litter and in composts apart from soil. They have been considered inappropriate for studies of decomposition in soils because 80 to 90 percent of the soil organic matter is humified normally beyond the point of useful analogy with biological materials (Kononova, 1966; Mortensen and Himes, 1964). Also, as has

been noted in earlier sections, a given reagent coextracts from soils many substances other than the type compounds implied by analogy to plant constituents. These impurities include representatives of most important classes of compounds, as well as mineral matter containing Fe, Al, Si, and frequently other polyvalent cations and S and P(Anderson, 1967; Dubach et al., 1964; Bremner, 1965, 1967; Felbeck, 1965; Gupta, 1967; Keefer et al., 1966; Mortensen, 1960; Roulet et al., 1963; Schnitzer and Desjardins, 1965).

Major advances have been made in the last 20 years in methods for separating specific organic fractions or groups of compounds from their coextracted organic and mineral contaminants. At the same time, it has become evident that the "contaminants", in many cases, originate in the same organo-mineral complex as that from which the isolates of interest come. The fact that they appear together in a given extract with certain solvation properties indicates that they were bound together or to non-extracted soil residues by similar mechanisms or strengths of binding.

The principal natural agents which lead to dissolution and weakening of bonds in soil minerals, organic polymers, and organo-mineral complexes are exoenzymes, acids and chelating agents produced by plants, animals and microorganisms(Barshad, 1964; Skujins, 1967). As noted earlier, the extractability of soil materials appears to be a function of the intensity and recency of metabolic activity integrated over random sites throughout the microfabric of the soil.

This concept provides a basis for interpreting chemical data obtained by fractional extraction procedures in terms of ecological and structural relationships in the soil.

In the proximate fractionation cited above, for example, ether and alcohol will remove, as type substances, lipoid materials that are not strongly bound to or embedded in complex colloidal structures. Coextracted materials will include "free" amino acids, peptides, sugars, mineral salts and other monomeric or low molecular weight compounds with similar peripheral relationships to soil colloids.

Materials extracted by hot water and 2 % HCl may be taken as specific indicators of microbial activity. It may be assumed that these two reagents in sequence will remove practically all of the microbial polysaccharides, since it has been shown that cellulose is the principal polysaccharide left after 2 % HCl extraction of soils or humic acids (Gupta and Sowden, 1964; Gupta, 1967; Mortensen and Himes, 1964).

Hot water-extracted materials are more likely to come from sites of current microbial activity. Materials extracted with 2 % HCl have a higher uronic acid content and are more likely to come from sites of declining microbial activity. Nitrogenous materials which are coextracted with microbial polysaccharides are clearly of microbial origin, also. By inference, coextracted minerals and phenolic compounds include products of degradation of soil minerals and humic acids. These would have been released by dissolution or

chelation of structural or bridging cations by products of heightened metabolic activity.

Cellulose is present, but as a minor constituent, in organic materials which remain after 2 % HCl extraction. When extracted with cuprammonium hydroxide(Schweitzer's reagent), cellulose can be recovered as an amorphous product. Thus, it is partially degraded and is probably present, together with partially altered lignin, in the form of slowly decomposing fragments of woody tissue(Daji, 1934; Gupta and Sowden, 1964).

Cellulose is hydrolyzed by acids under conditions which are not destructive of natural proteins. This was the basis for earlier assumptions that the residue after H_2SO_4 hydrolysis was a mixture or complex of proteins and lignin-derived humic acids. This view is no longer accepted (Mortensen and Himes, 1964; Stevenson, 1967). In most soils, this residue comprises a major portion of the organic matter and is now considered to be extensively humified. Only a portion of the nitrogen it contains can be assigned to amino acids, and these are not likely present as proteins but as part of the complexed structure of humic acids and humin.

Thus, organic materials which are not extracted by 2 % HCl are of low energy value and can support only restricted types and levels of microbial activity. In ecological terms, these microbial populations would represent an "autochthonous" microflora(Alexander, 1961). By contrast, materials extracted by hot water and 2 % HCl reflect a much higher

activity and a dynamic relation to levels of primary substrates, characteristic of "zymogenous" populations.

Model Systems for Study of Pesticide-Organic Interactions

Soil organic matter is a mixture of organic materials at all stages of biological decomposition and humification. When we consider effects of organic matter on soil properties in the field, we must include in the organic fraction the soil animals and microorganisms responsible for its biological transformations.

With reference to pesticide-soil interactions, an increased understanding of ecological relationships is a more urgent need than is a detailed understanding of sorption mechanisms. In either case, the investigator must resort to the use of biological materials, organic compounds or soil preparations as models for important components of the total system.

These model materials must be selected with reference to what is known about the chemical, physical and biological properties of soil organic fractions. Some of the essential properties have been outlined in previous sections.

A fundamental approach to study of clay-induced organic-organic interactions involves the use of clay minerals saturated with organic cations as model adsorbents. Such systems lend themselves to use of infrared absorption to identify bonding mechanisms. Using this technique Farmer and

Mortland(1965) observed that absorption of ethylamine by ethylammonium montmorillonite involved the formation of dimeric ethylamine-ethylammonium, hydrogen bonded through a common proton to the clay. Mortland(1968) concluded that sorption of EPTC(ethyl N,N-Di-n-propylthiocarbamate) also involved a hydrogen bond between the carbonyl of EPTC and the protonated N of pyridinium. Serratosa(1968) found that halogenated benzenes were absorbed by moving into the vacant spaces produced as pyridinium ions were displaced from a parallel to a vertical orientation with the clay surface.

Soil humic acids do not reveal much detail in their infrared spectra because of high background absorption. However, Sullivan and Felbeck(1968) found that absorption peaks in the 3.0 to 3.4 micron and the 5.8 to 7.2 micron regions were much more sharply defined in an alcohol-soluble fraction of humic acid than in the parent humic acid fraction. Changes in absorption in these regions indicated that the complex formed by reaction with s-triazines involved salt linkages and/or H-bonding between amino groups of the triazines and carboxyl, carbonyl and phenolic hydroxyl groups of the humic acids.

Traditionally, the alcohol-soluble humic acid fraction has been called "hymatomelanic acid"(Stevenson, 1965). Because of the greater definition of its infrared spectrum, this fraction may provide a useful model for humic acids, as well as for their degradation products which appear in fulvic acids and among the materials extracted with hot water,

buffers, dilute acids and other reagents.

In the above study, atrazine* was also reacted with benzoic acid and with salicylic acid as simple model compounds with carboxylated aromatic and phenolic structures such as are considered to be elements of the humic acid molecule. Infrared spectra of the reaction products closely resembled those for the triazine-hymatomelanic acid complexes and supported the inference that both carboxyl and phenolic hydroxyl groups were involved in the complexation reactions.

This use of simple model compounds with well characterized spectral absorbance patterns is fundamental to the study of bonding mechanisms. No attempt has been made to develop this approach systematically to elucidate bonding mechanisms between pesticides and soil organic materials.

Humic acids from soils or from lignite are frequently used in sorption studies since they represent a characteristic form of soil organic matter which can be readily isolated from the bulk of the mineral fraction and from plant detritus and the heterogeneous mixture of organic materials in the fulvic acid fraction(Dunigan, 1967; Porter and Beard, 1968; Wershaw et al., 1969).

In an ecological context, humic acids represent the least biologically active organic matter in the soil.

In a recent study of atrazine adsorption, Dunigan(1967) used a number of biological materials to represent the range of primary substrates which might appear in the soil in association with plant or insect remains. The materials fell

* atrazine(2-chloro-6-ethylamino-4-isopropylamino-1,3,5-triazine)

into two groups: Relatively small amounts(15 to 65 μg/gm) of atrazine were adsorbed by readily decomposed materials, such as corn starch, amylopectin, albumin, nucleic acid, cellulose and chitin; two lignin preparations and three humic acids adsorbed 350 to 480 μg/gm. Quinizarin(1,4-dihydroxyanthraquinone), which was used as a model compound for humic acid, adsorbed 250 μg/gm. The sorptive capacities of charcoal had not been satisfied after sorbing 560 ppm.

In the above study, the proximate fractionation scheme outlined in the previous section was used to prepare model soil systems sequentially depleted of materials extracted with ether, alcohol, hot water and 2 % HCl. The reported results did not lead to clear conclusions regarding effects of these pretreatments on atrazine sorption. The approach has promise, however, and was used in the work reported in this thesis.

Plant detritus, soil insects and microorganisms all are components of the organic fraction of natural soils. They are in themselves relevant model systems for sorption studies. Studies of Chacko, Lockwood and Ko in which sorption of chlorinated hydrocarbons by soil microorganisms was studied were cited earlier.

MATERIALS AND METHODS

Materials

Three surface soils from Michigan and five fractional preparations of each soil were used in this study. Soil properties and methods employed by Chodan(1967) in preparing the derivative soil fractions used in the present study are shown in Table 1 and Table 2.

For the study of adsorption of DDT by microorganism Rhizoctonia solani(Kühn) was used. This organism was kindly furnished by Dr. J. L. Lockwood of the Botany and Plant Pathology Department of Michigan State University.

Alfalfa(Medicago sativa L.) plant tissues(stem plus leaves) were used for the study of adsorption of DDT by plant materials. The sample was obtained from the Michigan State University Experimental Farm, East Lansing.

Preparation of Materials

Soils and their derivatives

Air-dried surface soils, Montcalm sandy loam, Sims clay and Houghton muck, were ground to pass an 80-mesh sieve. Soil pH in water and in N KCl were determined by glass electrode (Peech, 1965). Organic matter content was determined from ignition

Table 1. Characteristics of soils

Soil	pH		% Organic matter	% Total N	% Organic carbon	C/N	% Sand	% Silt	% Clay
	H_2O	KCl							
Montcalm sandy loam	4.58	4.18	2.2	0.08	1.2	15.0	63.3	19.0	17.7
Sims clay	6.05	5.50	8.7	0.37	3.9	10.5	24.6	29.6	45.8
Houghton muck	5.50	5.00	81.0	3.27	39.9	12.2	-	-	-

Table 2. Fractional preparations of soils

Fraction No.	Derivation	Type component removed *	% Carbon	CEC/100 gm soil pH 5.5	pH 7.0	pH 8.0
Montcalm sandy loam						
1	Whole soil	-	1.20		5.0	
2	Ether extraction	Fats, waxes, oils	1.15		4.4	
3	Alcohol extraction	Resins	1.24		4.4	
4	Hot water extraction	Polysaccharides	1.43		4.4	
5	Hydrolysis (2 % HCl)	Hemicelluloses	0.82		3.6	
6	H_2O_2 digestion	Organic matter	0.62		3.0	
Sims clay						
1	Whole soil	-	3.95		29.9	
2	Ether extraction	Fats, waxes, oils	3.36		27.0	
3	Alcohol extraction	Resins	2.58		27.2	
4	Hot water extraction	Polysaccharides	3.79		28.8	
5	Hydrolysis (2 % HCl)	Hemicelluloses	2.84		17.0	
6	H_2O_2 digestion	Organic matter	2.09		(44.7)	
Houghton muck						
1	Whole soil	-	39.91	188	214	231
2	Ether extraction	Fats, waxes, oils	34.39	175	205	213
3	Alcohol extraction	Resins	37.97	169	204	215
4	Hot water extraction	Polysaccharides	38.17	187	229	229
5	Hydrolysis (2 % HCl)	Hemicelluloses	49.16	193	234	245
6	H_2O_2 digestion	Organic matter	9.96	-	87	-

* Stevenson, 1965a; Waksman and Stevens, 1930.

loss at 550 C(Rather, 1917). Total nitrogen content was obtained through the semi-micro Kjeldahl method(Bremner, 1965a). Organic carbon content was determined with a LECO High Induction Carbon Analyzer. Particle size analysis was obtained through the method of Bouyoucos(1951). Cation exchange capacity was determined using a modified conductometric method of Mortland and Mellor(1954). Soils were saturated with Ba^{2+} in $\underline{N}$ $Ba(OAc)_2$ and titrated continuously with 0.25 $\underline{N}$ $Ba(OH)_2$ to stable equilibrium at pH 7.0. After equilibration soils were leached with unbuffered $BaCl_2$ to remove displaced cations and then with water to remove chloride. Adsorbed Ba^{2+} was estimated conductometrically by displacement with $MgSO_4$ as described by Mortland and Mellor. In the case of H_2O_2-treated soils, insoluble oxidation products gave rise to two breaks in the titration curves, so that no reliable estimate of cation exchange capacities could be made. The higher value was considered as the best estimate. Proximate fractional preparations of the soils were obtained through procedures described by Stevenson (1965). The H_2O_2-treated soil fraction was obtained by the method of Robinson(1927).

Fungal mycelia

The fungus, Rhizoctonia solani, was grown in glucose-potato broth. The broth was prepared as following: 22 grams of dehydrated commercial potatoes were added to 1,000 ml of distilled water, cooked for 15 minutes, and then filtered in

a Buchner funnel through a combined filter of cheesecloth, cotton and filter paper. Suction was applied. Twenty grams of glucose was added to the filtrate and sterilized in an autoclave for 30 minutes. A roux bottle containing about 300 ml of broth medium and a small amount of fungal mycelium was placed in a growth chamber at 25 C until a mat of mycelia was spread over the surface of the medium. The bottle was then taken from the growth chamber and stored in a refrigerator at 10 C for future use. Experiments were conducted with macerated mycelia. The mat of fungi grown was taken out of the roux bottle and macerated with a variable speed blender (Virtis "45", Research Equipment, Gardiner, New York). The fragmented mycelia were collected on a 40-mesh sieve after passing through a 28-mesh sieve, then washed with distilled water. Experiments were conducted with fresh and with autoclaved mycelia. Autoclaving was done before blending and sieving.

Alfalfa tissues

Second growth alfalfa was collected well before bloom from plots with no known history of exposure to DDT on the University Experimental Farm. Stems plus leaves were dried in an oven at 105 C, then ground in a Wiley mill. A portion of the ground material was collected after wet-sieving in distilled water. Other portions of the dry ground material were autoclaved in distilled water, then wet-sieved as in the case of the unautoclaved tissues to collect the particle

size fraction between 28-mesh and 40-mesh.

Preparation of DDT solution

One ppb DDT in aqueous solution was used throughout this study. The solution was prepared by adding 1,000 ng of DDT dissolved in 1 μl acetone into 1,000 ml of distilled water.

Experimental Procedure

Experiments were designed to permit estimation of distribution coefficients(Kd) by graphical and least squares methods. Soil-to-solution ratio was varied by varying the quantity of soil or tissue added to a constant volume containing, initially, 1 ppb DDT. Equilibrium concentrations were determined at five incremented soil-to-solution ratios for each adsorbent. Equilibrium concentrations were corrected for those found in the supernatant of a parallel sample equilibrated in distilled water.

An accurately weighed sample of the adsorbent was put into a 1,000 ml Erlenmeyer flask and 500 ml of 1 ppb DDT aqueous solution was added. The Erlenmeyer flask was tightly stoppered with a rubber stopper wrapped in aluminum foil. Then the flask was placed on a reciprocating shaker for 24 hours to obtain equilibrium partitioning of DDT between the sample and aqueous solution at a controlled room temperature of 25 C. Sedimentation was allowed to take place for 3 days

before the supernatant solution was carefully poured off for analysis. With the fungal mycelia and alfalfa tissues sedimentation was unnecessary. The mycelia and plant tissues were separated from the supernatant solution by passing the equilibrium suspension through a stainless steel net with opening less than 80-mesh. The adsorbent tissues were completely removed, since fragments less than 40-mesh had been eliminated by wet-sieving during sample preparation.

Four hundred ml of the supernatant solution was taken for extraction. It was extracted with 3 successive 100 ml aliquots of hexane-isopropyl alcohol mixture(3:1) in a 1,000 ml separatory funnel. It was shaken 1 minute at each extraction. The hexane-isopropyl alcohol extracts were combined together into the separatory funnel and washed with 4 successive 150 ml aliquots of distilled water to eliminate isopropyl alcohol from the hexane-isopropyl extract. This time, however, shaking was done for 30 seconds for each washing. The washed hexane extract was then transferred into a 300 ml Erlenmeyer flask and the wall of the separatory funnel was flushed with 3 successive 10 to 15 ml aliquots of hexane, giving a final volume of about 260 ml. Finally the hexane extract was transferred into a Kuderna-Danish concentrator. The capacity of the concentrator flask was 1,000 ml. Concentration of the hexane extract was performed in a water bath until no condensation was observed on the upper ring of the condenser. Hexane trapped above the two lower rings came down, washing the sides of the concentrator flask into

the concentrator tube at the bottom. After wiping away water carefully from the joint and bottom of the concentrator flask, the concentrator tube was removed from the flask and placed under an infrared lamp. Evaporation was allowed to take place until the total volume of the hexane extract reached less than 0.5 ml. After the volume was brought back to exactly 0.5 ml, the tube was promptly stoppered with a ground glass stopper, awaiting injection into the gas chromatograph to determine the content of DDT. An 800-fold concentration of DDT was effected by this procedure so that supernatant concentrations from 0.01 to 1.00 ppb could be estimated quantitatively.

DDT in the hexane extract was identified by elution time and quantitatively measured by peak area. A Beckman GC-5 Model Gas Chromatograph, with an electron capture detector and a 1.83 m, 3 mm I. D., glass column packed with 2 % DC-11 on 60/80 mesh Gaschrom Q, was employed in this study. Helium was used as the carrier gas. Operating parameters were as follows: helium discharge flow rate, 70 ml, column flow rate, 40 ml per minute; column oven temperature, 210 C; column inlet temperature, 230 C; detector line temperature, 250 C; detector oven temperature, 275 C. The detector was operated at the maximum polarizing voltage response and maximum CO_2 response.

A Beckman Model 1005 Linear Ten-Inch Laboratory Potentiometric Recorder was used. With the operational parameters noted above, retention time for DDT was 2.5 minutes and the

maximum linear response range was about 500-fold. The standard calibration curve was obtained by plotting known amounts of DDT against calculated peak area on full logarithmic scale graph paper(McNair and Bonelli, 1969). In order to check possible contamination during the analytical procedures a DDT-free water blank was concurrently run with other samples in every experiment. Triplicate injections were made of each sample.

Analytical grade o,p-DDT(99.9 %) was supplied for these studies through the courtesy of Dr. H. M. LeBaron, Geigy Agricultural Chemicals, New York. Distilled water of appropriate purity was obtained from the building supply in the Biochemistry Building(laboratory of Dr. N. C. Leeling). No confounding contaminants were found in hexane extracts of aqueous supernatants so no column clean-up procedures were employed.

Traces of DDT were found in soils and their derivative fractions. The quantity of indigenous DDT decreased in successive proximate fractions. Correction was made for DDT already present by including a sample blank to which no DDT was added in every experiment.

The difference between the amount of DDT in the equilibrium supernatant, less the appropriate sample blank, and that in the standard 1 ppb matrix solution was assumed to be the quantity adsorbed by the sample.

Precision of Analysis

It was not possible to estimate accurately the overall reproducibility of the analytical procedures throughout the experiment because there were continuous changes in the operating parameters of the instrument, which resulted in different instrumental response from day to day. However, six experimental systems were normally compared in a given experimental period. This normally required 4 to 8 hours of sample injection. The coefficient of variability(CV) could be estimated. The CV in the standard solution analysis was about 8.6 %. CV was inversely proportional to the sample concentration.

Evaluation of Kd

The two most frequently used equations for describing adsorption phenomena in aqueous solution are the empirical Freundlich adsorption equation and the Langmuir adsorption equation.

In the present study, the distribution coefficient(Kd) was used as a quantitative index for comparing sorptive capacities of soils, soil fractions and fungal or plant tissues. Kd is related to constants in both classical equations, as well as to more recently proposed indexes for evaluating sorptive capacities of soils for pesticides. These relationships are presented here to justify the use of

Kd and to develop the special form of the partitioning function which was used to evaluate Kd.

The Freundlich adsorption equation is written:

$$x/M = Kf\ Ceq^{1/n} \qquad (1)$$

where x is the amount of adsorbate adsorbed by an amount of adsorbent, M; Kf and n are constants which are characteristic for a given system; Ceq is the equilibrium solution concentration(Laidler, 1965). Conformity with the Freundlich equation was found for all organic herbicides, with a few exceptions, which were studied by Bailey et al.(1968), using montmorillonite as the adsorbent. None of the compounds showed conformity with the Langmuir adsorption equation.

Similar results showing conformity with the Freundlich equation in the adsorption of many other herbicides by natural soils have been reported(Kunze, 1966). However, Nearpass(1969) obtained a close approximation to the Langmuir adsorption isotherm in a study of adsorption of amitrol(3-amino-1,2,4-triazole) by a Michigan muck soil.

A common expression of the Langmuir adsorption equation is as follows:

$$x/M = \frac{Xm\ Kl\ Ceq}{1 + Kl\ Ceq} \qquad (2)$$

where x is the amount of adsorbate adsorbed by an amount of adsorbent, M; Xm is the adsorption capacity of the adsorbent;

Kl is a constant which is characteristic for the system; Ceq is the equilibrium solution concentration(Shoemaker and Garland, 1962).

Lambert(1967) proposed to utilize parachor as a measure of functional relationships between adsorption in soil and chemical structure of organic substances. Nevertheless, he had to restrict the functional relationship to uncharged organic chemicals, because parachor is purely a physical function.

Later Lambert(1968) proposed another function as an index of soil adsorption equilibria. In this report the chosen index, omega(Ω) is nothing but a manipulation of the distribution coefficient(Kd). Omega is obtained, according to Lambert, by using an arbitrarily chosen standard indicator organic comound and also an arbitrarily chosen standard soil. A Ripperdan soil(1 % organic matter content based on organic carbon analysis) was set as the standard soil while planovin* (Shell trademark) herbicide analogs, monuron** analogs and several Shell trademark insecticides were employed to serve as the standard chemicals. Lambert has developed this conceptual model for adsorption in soil of organic compounds on the basic assumption that soil organic matter is not only the most representative index of soil adsorptive capacity, a truly characteristic sorption index, but also that the active fraction of soil organic matter in its sorptive characteristics is independent of soil origin or type.

A simple partition function has been used by several

* planovin(4-(methylsulfonyl)-2,6-dinitro N,N-dipropylaniline)
** monuron(N'-(4-chlorophenyl)-N,N-dimethylurea)

investigators to describe pesticide adsorption equilibria in soil(Talbert and Fletchall, 1965; Kay and Elrick, 1967; Kunze, 1966; Lambert, 1968). It is an appropriate function for very small equilibrium solution concentrations. The distribution coefficient(Kd) is defined:

$$Kd = \frac{x/M}{Y/V} \qquad (3)$$

where x is the amount of adsorbate adsorbed by an amount of adsorbent, M; Y is the amount of chemical in the solution; and V is the volume of solvent.

If Kd is evaluated at equilibrium, then Equation(3) may be rewritten as;

$$Kd = \frac{x/M}{Ceq} \qquad (4)$$

or

$$x/M = Kd\ Ceq \qquad (5)$$

where Ceq is the equilibrium solution concentration(Y/V). Comparison of Equation(5) with Equation(1) immediately leads to the conclusion that if n, a constant in the Freundlich adsorption equation, is 1, then Kf and Kd are exactly the same entity. This is what actually has been observed in most pesticide investigations. At the range of very low equilibrium solution concentrations, the adsorption

isotherms invariably exhibit a linear response with a specific degree of slope which is characteristic for a given system. In order for the adsorption isotherms to be a straight line on plotting, the necessary and sufficient condition is that n be 1. Thus, the distribution coefficient (Kd), at equilibrium, has its own merits for being considered a universal constant, as well as other equilibrium constants.

It may be noted that Kd or Kf are related to Kl and Xm in the Langmuir adsorption equation in the following way: If the equilibrium solution concentration, Ceq, is sufficiently small, then Equation(2) can be written as;

$$x/M \simeq \frac{Xm\ Kl\ Ceq}{1}, \quad (Kl\ Ceq << 1) \qquad (6)$$

or

$$\frac{x/M}{Ceq} \simeq Xm\ Kl$$

therefore,

$$Kd \simeq Xm\ Kl \qquad (7)$$

This relationship should hold true at very low equilibrium solution concentrations where a linear relationship can be obtained in the adsorption isotherm.

Equation(3) is rearranged for purposes of graphical

plotting or least squares analysis to obtain Kd from experimental data. At equilibrium

$$Kd = \frac{x/M}{Yeq/V} \qquad (8)$$

or

$$Kd = (x/Yeq)\ (V/M)$$

If the total amount of DDT added into the system is Yt, then

$$Kd = \left(\frac{Yt - Yeq}{Yeq}\right)\ (V/M) \qquad (9)$$

because $Yt = x + Yeq$, and $x = Yt - Yeq$.

Equation(10) is derived from Equation(9):

$$Yt/Yeq = Kd\ (M/V) + 1 \qquad (10)$$

Consequently if Yt/Yeq is plotted against (M/V), then Kd is the slope of the regression line. The regression line intercepts the vertical ordinate at $Yt/Yeq = 1$ when $M/V = 0$, in other words when no adsorbent is present.

RESULTS AND DISCUSSION

Adsorption of DDT by Soils and Derivative Fractions

The experimentally determined values used for least squares estimation of Kd by Equation 10(p. 58), together with the calculated regression lines for the three soils and their derivative fractions, are presented graphically in Figures 2, 3 and 4.

Deviations from linear regression were minimal for Fractions 1, 2 and 6. Deviation from regression increased with increasing sorptive capacity in the other fractions. This is due to the fact that the precision of recovery and measurement of DDT decreased with decreasing supernatant solution concentration at equilibrium(Yeq). The procedures used for extraction and concentration of DDT were approaching their limit of useful reproducibility at a concentration of 0.01 ppb DDT in the supernatant.

Nevertheless, least squares solutions for all fractions were highly significant($P < 0.01$). Statistical parameters are given in Appendix I.

The slopes of the regression lines are, by definition, the distribution coefficients(Kd) which are presented in Table 3. These data clearly demonstrate the commonly accepted fact that soil organic matter is the most important single factor affecting adsorption of non-polar organic pesticides

(Edwards, 1966; Lambert, 1968; Kunze, 1966). Houghton muck and its derivatives, with the highest organic matter content, comprise the greatest Kd's, while Montcalm sandy loam and its derivatives, with lowest organic matter content, have the smallest Kd's. Sims clay and its derivatives are intermediate.

However, it is apparent that successive fractional extractions altered the nature and extent of sorptive surfaces to produce striking differences in Kd among derivative fractions of each soil. The relation of these changes to changes in organic carbon content is also shown in Table 3.

Carbon contents in Table 3 were transcribed from Table 2. It is obvious that changes in carbon content cannot be understood simply in terms of removal of specific organic components as is assumed in the proximate fractionation scheme that was used(Waksman and Stevens, 1930; Stevenson, 1965). It this were true, it would be expected that organic carbon content would decrease progressively from Fraction 1 to Fraction 6. Instead, marked increases in residual carbon content occurred in some fractions, notably after hot water extraction in the two mineral soils and after 2 % HCl extraction in Houghton muck.

As has been extensively documented in the literature review, mineral and organic materials appear in any fractional extract of soils. An increase in carbon content from one fraction to the next in Table 3 must be ascribed to the fact that the extracting reagent removed more mineral matter than

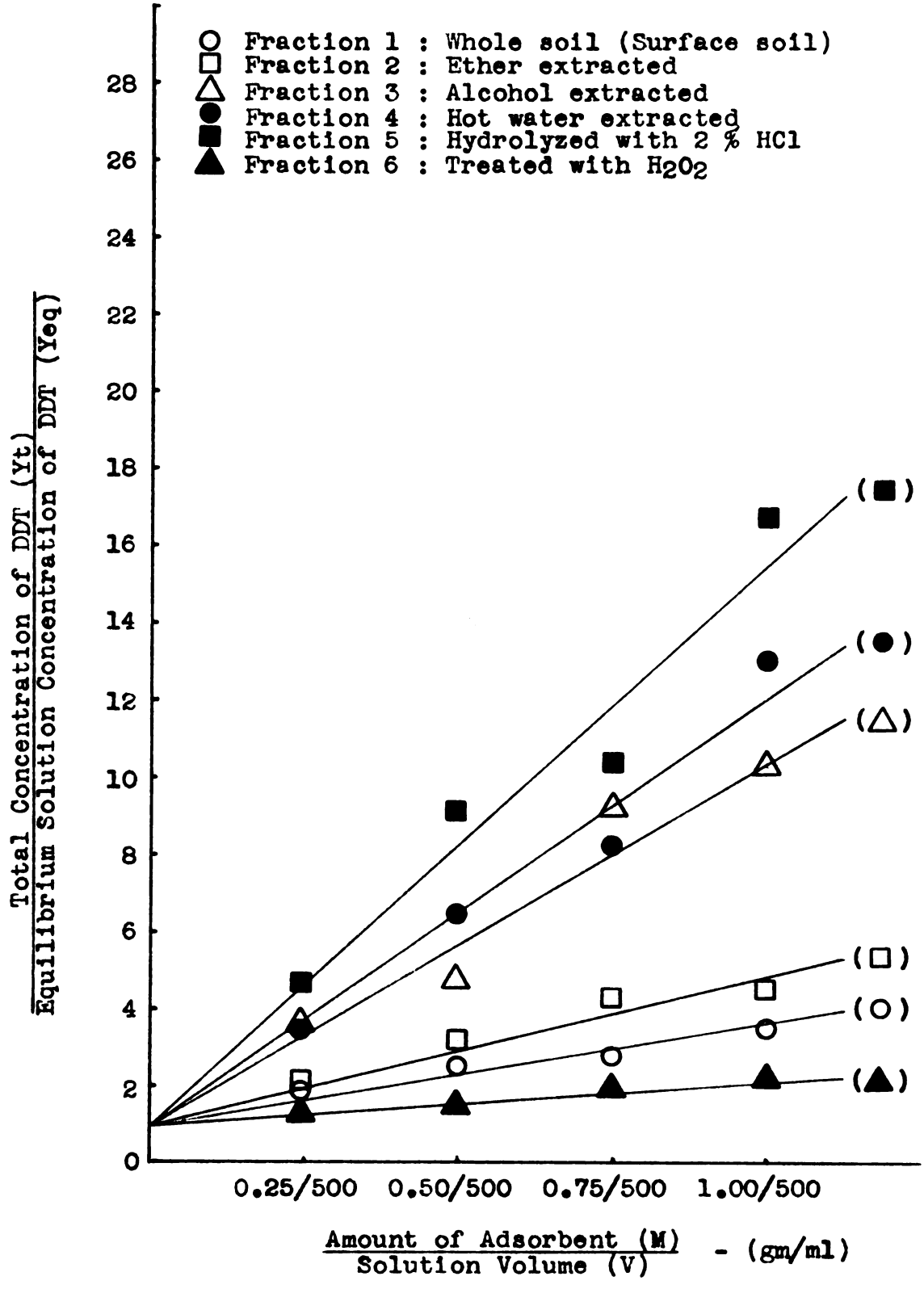

Figure 2. Adsorption of DDT by Montcalm sandy loam

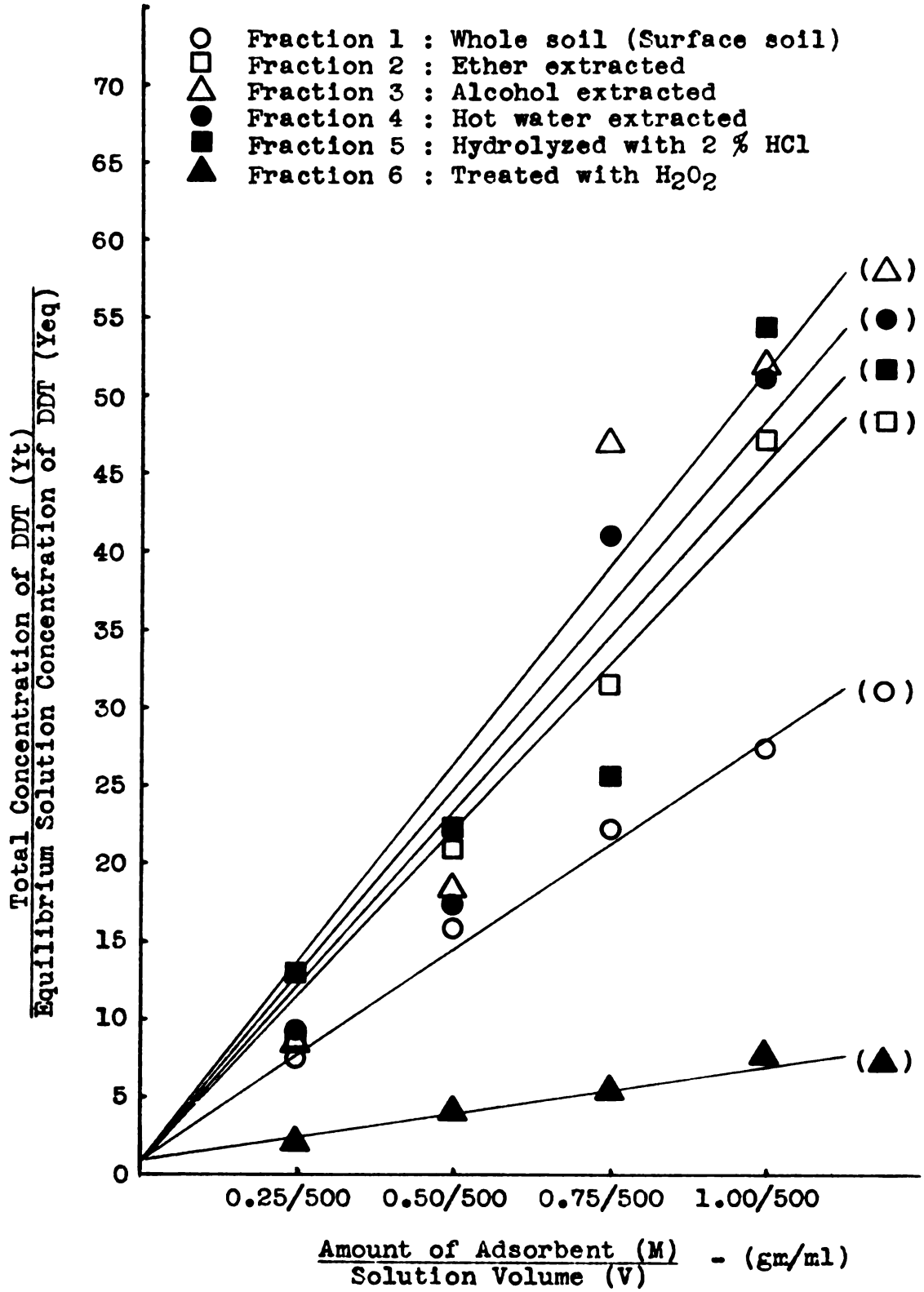

Figure 3. Adsorption of DDT by Sims clay

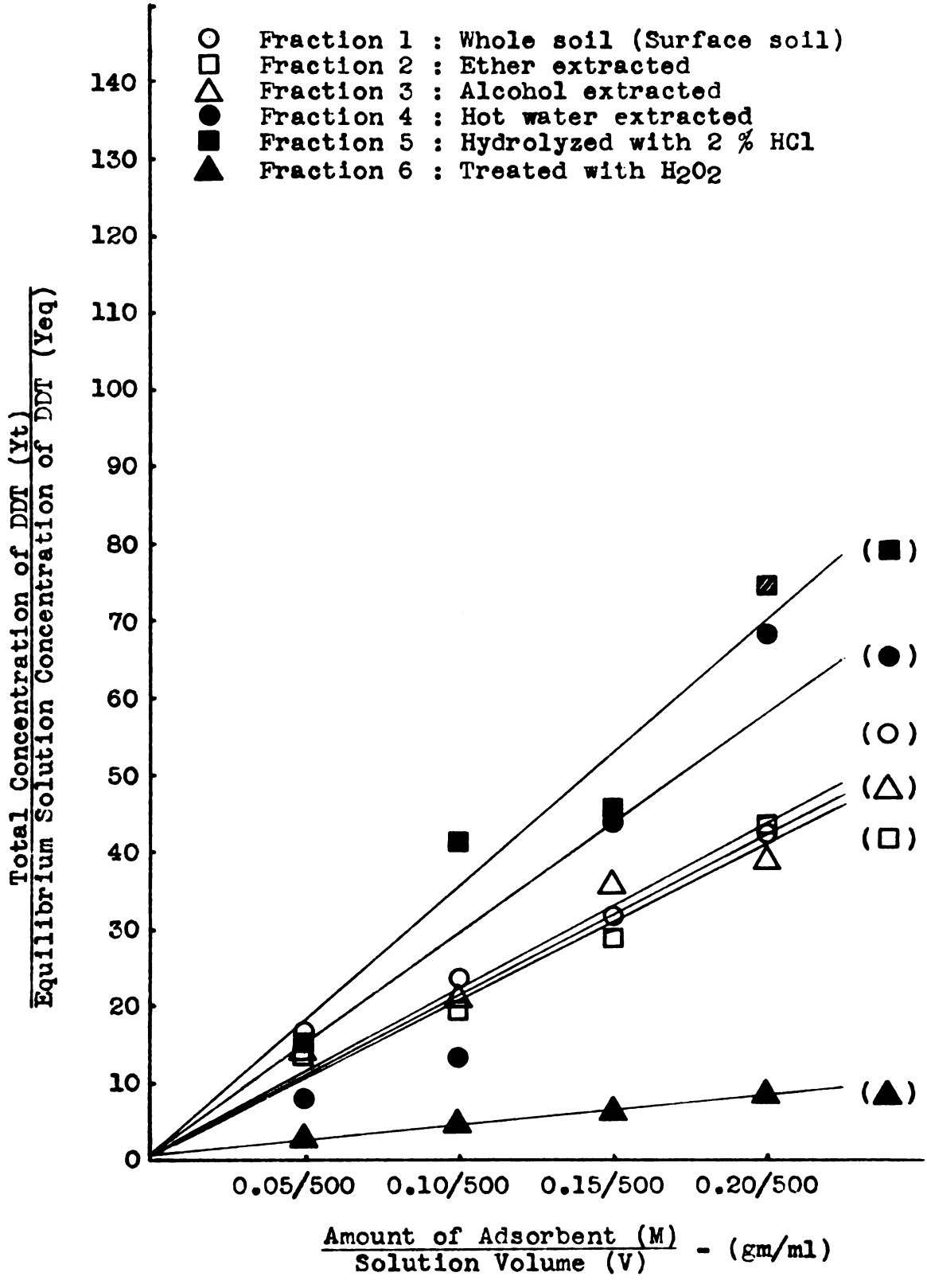

Figure 4. Adsorption of DDT by Houghton muck

Table 3. Distribution coefficients(Kd) of soils and derivative fractions and their relation to carbon content.

Fraction No.	1	2	3	4	5	6
Montcalm sandy loam						
Kd(x 10^{-2})	12.86	19.39	47.73	55.74	73.83	5.57
% Carbon	1.20	1.15	1.24	1.43	0.82	0.62
Kd/% Carbon (x 10^{-2})	10.75	16.80	38.43	38.90	90.48	9.03
Sims clay						
Kd(x 10^{-2})	137.09	214.43	255.64	214.17	226.83	31.05
% Carbon	3.95	3.36	2.58	3.79	2.84	2.09
Kd/% Carbon (x 10^{-2})	34.75	63.76	98.93	56.43	79.84	14.84
Houghton muck						
Kd(x 10^{-2})	1063.37	1001.77	1022.47	1428.17	1737.67	179.50
% Carbon	39.91	34.39	37.97	38.17	49.16	9.96
Kd/% Carbon (x 10^{-2})	26.64	29.13	26.93	37.41	35.35	17.95

Note: Within soils, underscored values are not different at P(0.05). Within fractions, all values for soils are different at P(< 0.01)(Steel and Torrie, 1960).

organic.

The Kd's in Table 3 have been recalculated per unit percent carbon. It is apparent that differences in sorptive capacity were not due simply to differences in carbon content. Qualitative differences in the nature of organic materials remaining in each fraction were also involved.

No attempt was made to characterize either the extracted or the residual materials, beyond determining carbon content and cation exchange capacity of the latter(Table 2).

The decrease in CEC in Fraction 5 of the two mineral soils is consistent with removal of materials high in carboxyl content, i.e., microbial polyuronides which are analogous to plant hemicelluloses. It is known that 2 % HCl removes these rather completely(Gupta and Sowden, 1964; Gupta, 1967; Mortensen and Himes, 1964).

In the muck soil, extraction with 2 % HCl resulted in increased CEC, principally in sites active at pH 8.0. This is consistent with exposure of phenolic -OH groups in humic acids(Broadbent and Bradford, 1952).

Obviously, additional information regarding the nature of materials removed and functional groups exposed at each step of fractionation would be helpful in interpreting these data. However, some useful inferences can be made if it is assumed (1) that mineral and organic materials which are coextracted by a given reagent were attached to the main matrix of soil by similar bonding mechanisms or energies and (2) that products of metabolism are mainly responsible for

weakening these bonds in soils as they exist in the field.

In the proximate fractionation used here, it is assumed that materials removed by hot water and by 2 % HCl came largely from sites of heightened metabolic activity or from sites within reach of metabolic influence. Their removal would leave largely humic acids which are very resistent biologically and chemically.

On the other hand, lipoid materials removed by ether and alcohol are not very strongly complexed with soil colloids and probably originate in peripheral surface deposits. Their removal in this study substantially increased DDT sorption by the two mineral soils(Table 3). Dunigan(1967) observed a similar increase in atrazine sorption by several soils after ether extraction and attributed it to increased wetability. A similar effect did not occur with Houghton muck in this study.

The presence of hydrophobic coatings on the surfaces of large pores and structural peds is recognized as a probable mechanism for stabilization of soil aggregates(Griffiths, 1965; Martin et al., 1955; McCalla, 1950; Quirk and Panabokke, 1962). The soils in this study were ground to pass an 80-mesh sieve, so that aggregation would have been extensively destroyed. Nevertheless, there were undoubtedly aggregates which could be broken down still further. Removal of lipids by ether and alcohol extraction could have promoted this breakdown to increase total surface area and DDT sorption in the two mineral soils.

Polysaccharides have been widely studied as aggregating agents and shown to be effective when added directly or produced in quantity in the soil(Acton et al., 1963b; Chesters et al., 1957; Geoghegan and Brian, 1946; Greenland, 1965b; Martin, 1946; Martin et al., 1955; Whistler and Kirby, 1956). The normal base level polysaccharides that are present from season to season do not appear to contribute significantly to stable aggregation(Mehta et al., 1960; Oades, 1967). Nevertheless, removal of polysaccharides by hot water and by 2 % HCl may have contributed to aggregate breakdown and increasing surface area and sorption of DDT in Fractions 4 and 5 of the sandy loam and the muck.

Removal of mineral constituents(iron and aluminum oxides, amorphous silicates, bridging polyvalent cations) would also contribute to disruption of aggregates and increased surface area(Arca and Weed, 1966; Deshpande et al., 1968; Mackenzie, 1954; Lutz, 1936; Peterson, 1946; Saini et al., 1966). Increased carbon contents in Table 3 suggest that major removals of mineral matter occurred with hot water extraction in the two mineral soils and with 2 % HCl in the muck. Associated changes in Kd(Table 3) or Kd per unit percent carbon do not support any simple interpretation of the interrelationship, however.

Decreasing particle size and increasing surface area may have been an important factor giving rise to the general tendency for sorptive capacity to increase in going from Fraction 1 to Fraction 5. However, data in Table 3 suggest

that the sorptive capacity of residual organic matter increased more rapidly than in the muck. Thus there appears to have been an inductive effect of mineral colloids on organic materials intimately complexed with them.

The proportion of residual organic matter remaining in the form of humic acids would have increased with each successive extraction. The free radical nature of humic acids and of reactions leading to their synthesis are well established(Flaig, 1960, 1964; Steelink, 1964; Theng and Posner, 1967). It has been shown recently that neutral aromatic compounds are adsorbed by π -bonding to copper on montmorillonite to form what appears to be a free-radical complex(Doner and Mortland, 1969). It is, perhaps, useful to speculate that the inductive effect of clay noted above may have been through stabilization of free radical structures with an affinity for DDT.

Inductive effects of soil minerals on the quantity and nature of soil organic matter are generally recognized phenomena in the field(Barshad, 1964; Colom and Wolcott, 1967; Dormaar, 1967c; Schnitzer and Desjardins, 1965).

The quantity and chemical and physical nature of soil organic matter is also influenced by such factors as vegetation, topography, climate, management, season of the year and time over which these factors have acted(Barratt, 1967; Babel, 1967; Chahal and Wagner, 1965; Walker and Adams, 1959; Walker et al., 1959; Wang et al., 1967).

Accordingly, the content and nature of functional

groups in soil organic matter are not independent of the genetic history of the soil(Leenheer and Moe, 1969; Theng and Posner, 1967).

It has been proposed recently that soil organic matter is not only the most representative index of adsorption of pesticides by soils but that also the "active fraction" of soil organic matter is independent of soil origin or type (Lambert, 1968). The data in Table 3 suggest that this is an over-generalization. In particular they raise the question as to what is an "active fraction".

The activity of soil organic materials was greatly reduced by H_2O_2 treatment in Fraction 6. Much of the residual organic matter in this fraction was probably in the form of artifacts, although some unaltered soil organic materials may have been protected by strong binding to clays.

Adsorption of DDT as Influenced by Mixing Soils

Incorporation of Houghton muck increased the adsorptive capacity of Montcalm sandy loam, measured by Kd. It appears that a linear relationship could be obtained up to about 5 percent of total organic carbon content in the mixture with mineral soil(Figure 5; Appendix II).

Figure 5 shows that incorporation of Houghton muck also increased the adsorptive capacity of Sims clay. But in this instance the relationship between the adsorptive capacity and total organic carbon content was not linear. There was

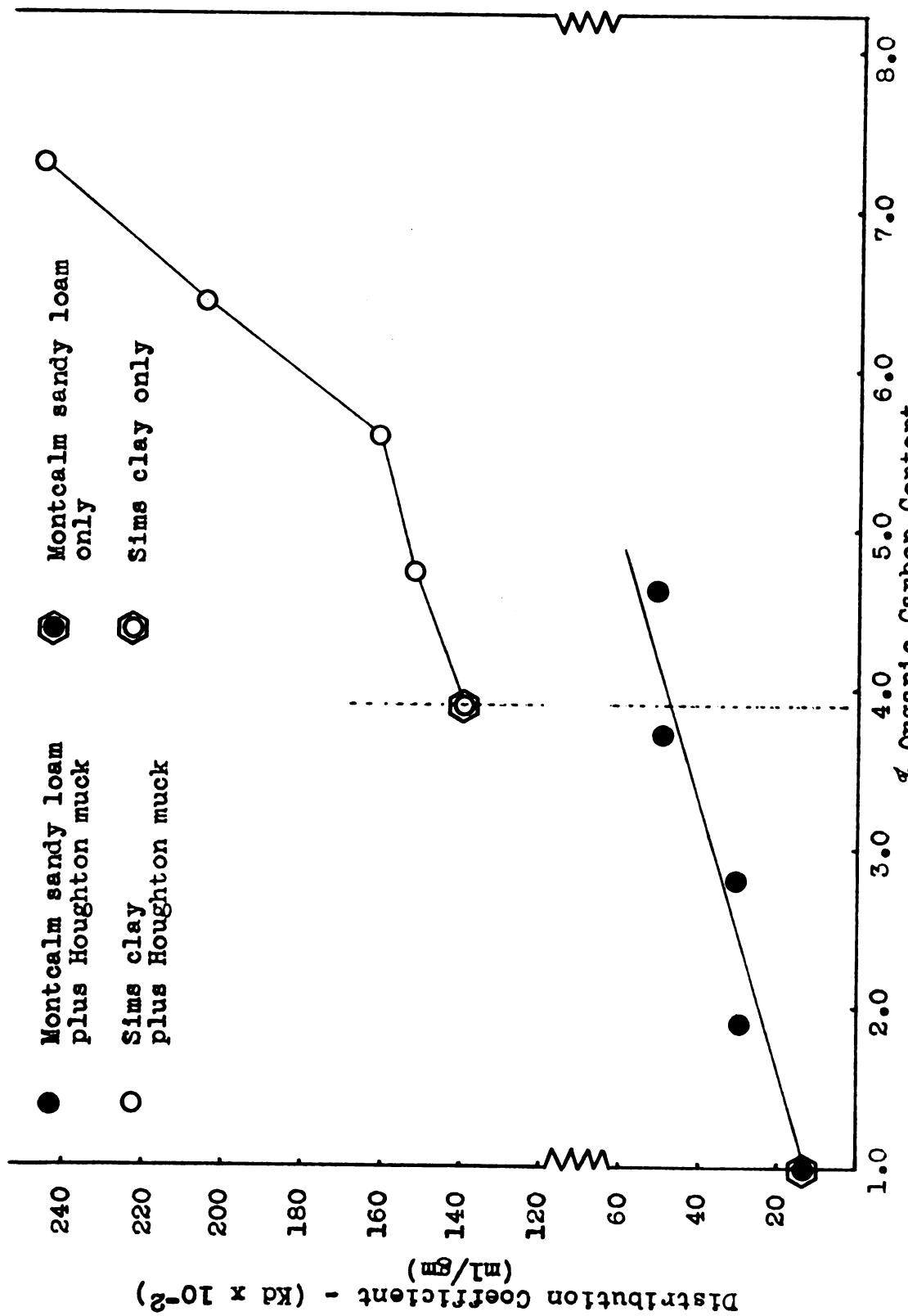

Figure 5. Effects of Houghton muck on the adsorption of DDT by Montcalm sandy loam and Sims clay

a gap in adsorptive capacity between muck-amended Montcalm sandy loam and unamended Sims clay at about 4 percent organic carbon content.

Similar trend is shown in Figure 6 when Sims clay was mixed with Montcalm sandy loam. It seems that at low organic matter content, the interaction of the clay fraction and organic matter of one soil with these fractions of another soil was so vigorous that there was a reduction in the available total surface area and in the number of functional

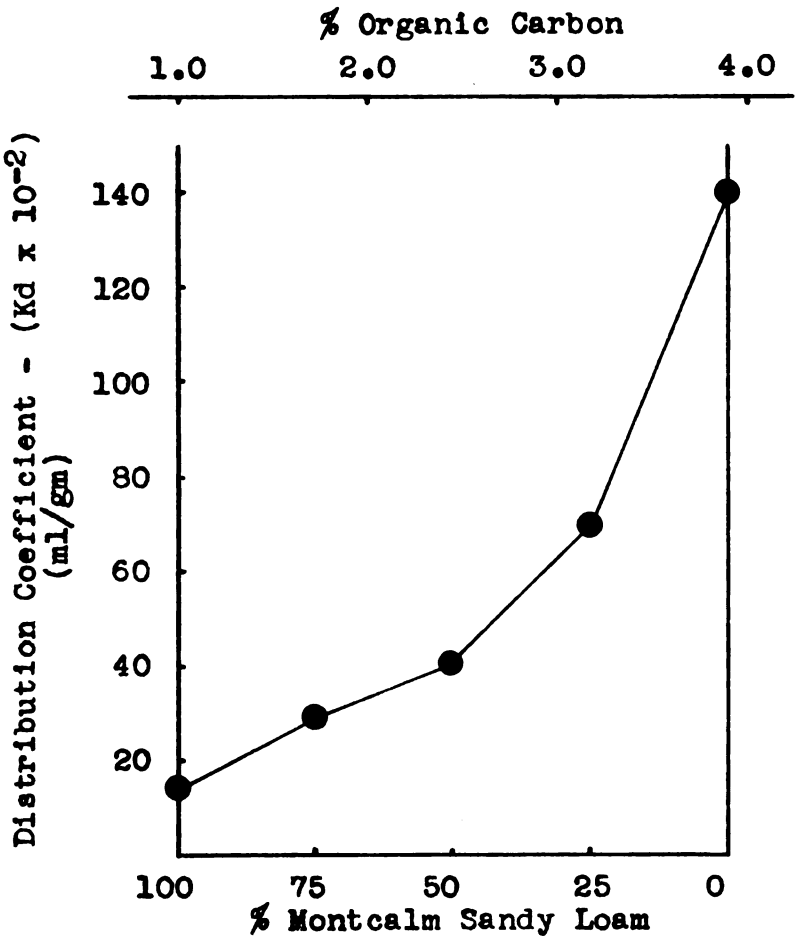

Figure 6. Mixing Sims clay with Montcalm sandy loam and adsorption of DDT

groups responsible for the adsorption of DDT.

A number of physical forces contributes to adsorption, but collectively they are called van der Waals' forces. There are at least four types of forces which contribute to the van der Waals' forces: (1) orientation energy, (2) induction energy, (3) dispersion energy(instantaneous dipole), and (4) a large and repulsive energy intimately related to the Pauli exclusion principle(Companion, 1964). Orientation energy, induction energy and dispersion energy depend on the polarizability of the molecule(Murrell et al., 1965).

It has been shown that the electrokinetic properties of clay and organic colloids are modified when they are mixed and complex formation occurs. In general, there is a marked reduction in the electronegative charge of the two colloids (DeSilva et al., 1964; Somasundaran et al., 1966). This may explain, in part, the results obtained when two soils were mixed in the present study. In accordance with the results from this study it has been reported that the mobility of dalapon(2,2-dichloropropionic acid) was decreased by adding manure and increased by adding sand to several Iowa soils (Holstun and Loomis, 1956).

Lichtenstein et al.(1968) reported that treatment of soil with carbon powder could in some cases reduce the amount of insecticidal residues in crops to such an extent that a farmer could use a soil of abnormally high insecticidal content. Carbon has been known for a long time to be an active adsorbent. Early studies on adsorption phenomena were

conducted mainly by using carbon(Davis, 1907). In fact carbon treatment, along with coagulation and oxidation, is a recommended method to remove pesticides from potable water sources(Sigworth, 1965; Robeck, 1965). However, a farmer must consider the possibility of excessive adsorption of fertilizer elements, especially trace nutrient elements, by carbon when carbon is practically incorporated into his soil. Organic soil, either muck or peat, may be an economical substitute for carbon.

Adsorption of DDT by Rhizoctonia solani

The accumulation of DDT by microorganisms, reported by Chacko and Lockwood(1967), appeared to be an adsorption phenomenon. Later it was indicated that the ability to accumulate chlorinated hydrocarbon pesticides may be a generally nonspecific property of cells of actinomycetes, bacteria and fungi(Ko and Lockwood, 1968b). This is a characteristic of weak physical adsorption. Therefore, it was postulated that the accumulation of DDT by fungi could be described by a simple distribution coefficient(Kd). The experimental results are plotted in Figure 7. The least squares values for Kd are listed in Table 4 with methods of two different preparations. Statistical parameters are given in Appendix III.

The results indicate that it is an adsorption phenomenon. It is interesting to see that dead cells adsorb more DDT

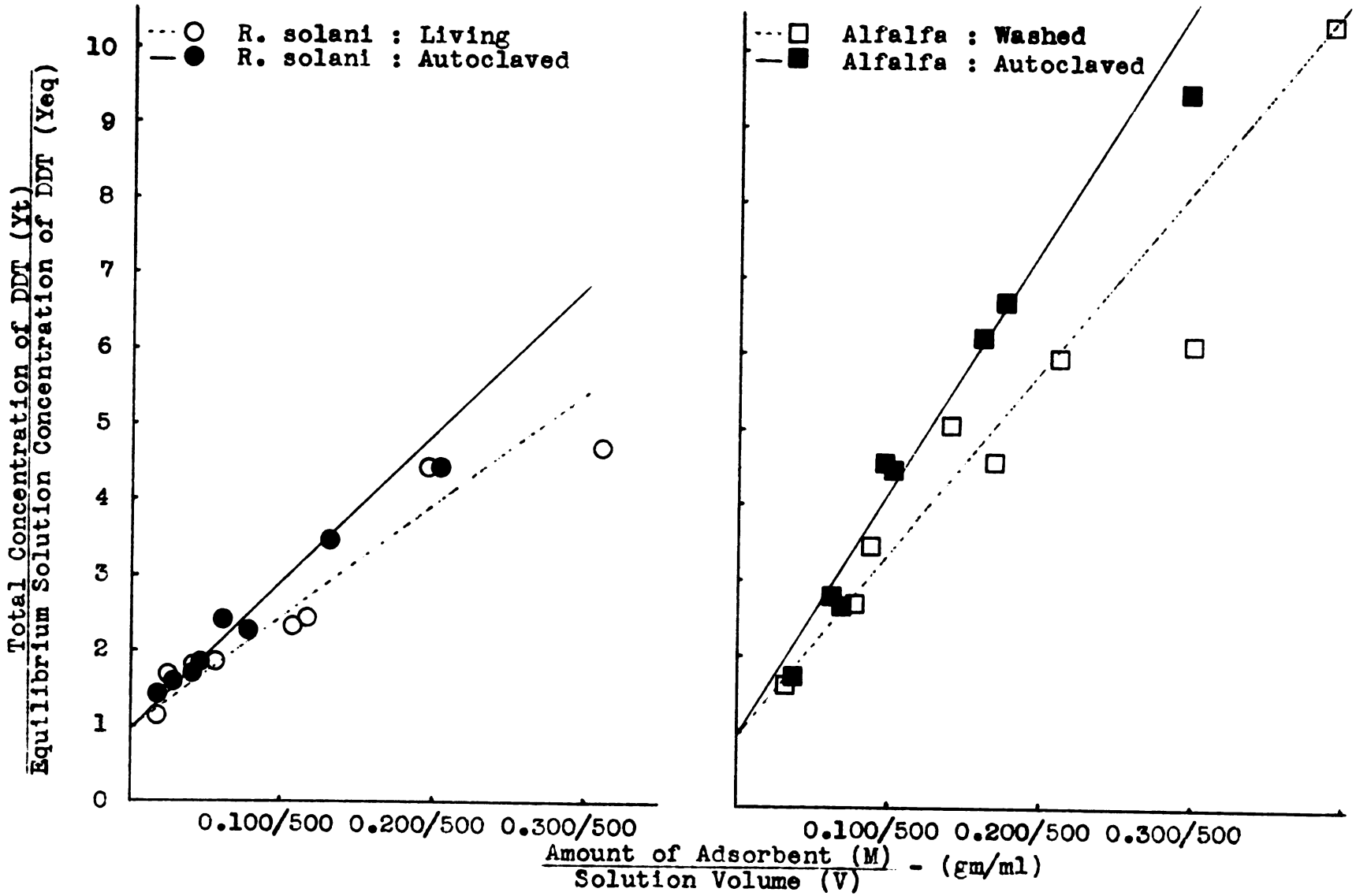

Figure 7. Adsorption of DDT by Rhizoctonia solani cells and alfalfa tissues

Table 4. Adsorption of DDT by *Rhizoctonia solani* and alfalfa tissues

	Treatment	Kd x 10-2 Dry weight	Kd x 10-2 Wet weight
Rhizoctonia solani	Living	903.24	66.66
	Autoclaved	1073.20	87.68
Alfalfa tissues, stem plus leaves	Washed	1047.83	109.72
	Autoclaved	1358.73	149.64

than living cells do. The fungal mycelia used in this study can not be considered as intact cells which are protected wholly by unbroken cell walls, because maceration of fungal mycelia was performed before adsorption of DDT was allowed. Therefore, adsorption is not only by the external surface of the cell wall but also to some extent by the internal surface of the cell wall. The cytoplasmic inclusions were largely washed out by wet-sieving and washing with water from the broken cells.

Autoclaving mycelia in water might have removed polysaccharides which are primary cell wall components(Stanier et al., 1957). However, autoclaving not only removes polysaccharides from the cell walls but causes, conceivably, many other physical and chemical changes in the remaining portion of fungal mycelia. Thus, it is not reasonable to explain the increased adsorptive capacity of autoclaved mycelia compared with that of living mycelia only as the result of removing polysaccharides from the fungal mycelia. The effect of autoclaving on the structure of the cell and adsorption of DDT calls for further investigation.

Adsorption of DDT by Alfalfa Tissues

The study with alfalfa tissues indicates that the uptake of DDT by these materials is also an adsorption phenomenon(Figure 7; Table 4; Appendix III).

During sample preparation, alfalfa tissues may have

lost many cell constituents through wet-sieving. Cell walls were certainly disrupted by drying and cell constituents were washed out during wet-sieving. Consequently, the adsorption of DDT is not only by the external surface of cell walls but also by their internal surfaces.

Autoclaving in water removes not only polysaccharides from alfalfa tissues but remove also other substances, such as fats, oils, waxes, proteins, and various pigments which are cell wall components(Robbins et al., 1965). The increased adsorptive capacity of autoclaved alfalfa compared with that of alfalfa tissues washed with cold water cannot be explained simply. It is evident that physical and chemical properties of the tissues are greatly changed during the autoclaving.

Ecological Implications

In Figure 8, calculated isotherms for dry matter in alfalfa and fungal tissues are projected for comparison with those for the untreated surface soils(Fraction 1). The sorptive capacities of these tissues were closely similar to that for Houghton muck. They were very much greater than for the two mineral soils. These differences readily explain the observations of Ko and Lockwood(1968b) who found that fungus mycelium, living or dead, contained 18 times the concentration of DDT (dry weight basis) as the ambient loam soil after 48 hours' contact.

The ecological implications of these relationships are

extremely important. The concentration of a resistant pesticide such as DDT in plant residues and microbial tissues places it at a focal point for entry into detritus food chains. This is also a focal point for its entry into humification sequences where it may be incorporated into resistant complexes and retained until released again by a flush of microbial activity at some unpredictable later date. The tendency for sorptive capacity to increase in soil residues from each successive fractional extraction is evidence that end products of humification may have an even greater affinity for DDT than unhumified plant or microbial tissues.

In the proximate fractionation sequence used in this study, it may be assumed that hot water and 2 % HCl extraction would have removed materials closely associated with what would have been sites of metabolic activity at the time the soil samples were taken. These materials would have remained after alcohol extraction in Fraction 3. This was the fraction in Sims clay which was most active in DDT sorption.

However, in neither of the two mineral soils were these biologically active fractions fully accessible for adsorption of DDT until ether and alcohol solubles had been removed. Under the conditions of equilibrium partitioning employed in this study, this result must be attributed to water proofing and structural occlusion of sorptive surfaces by peripheral lipoid deposits.

In the field, repeated cycles of wetting and drying

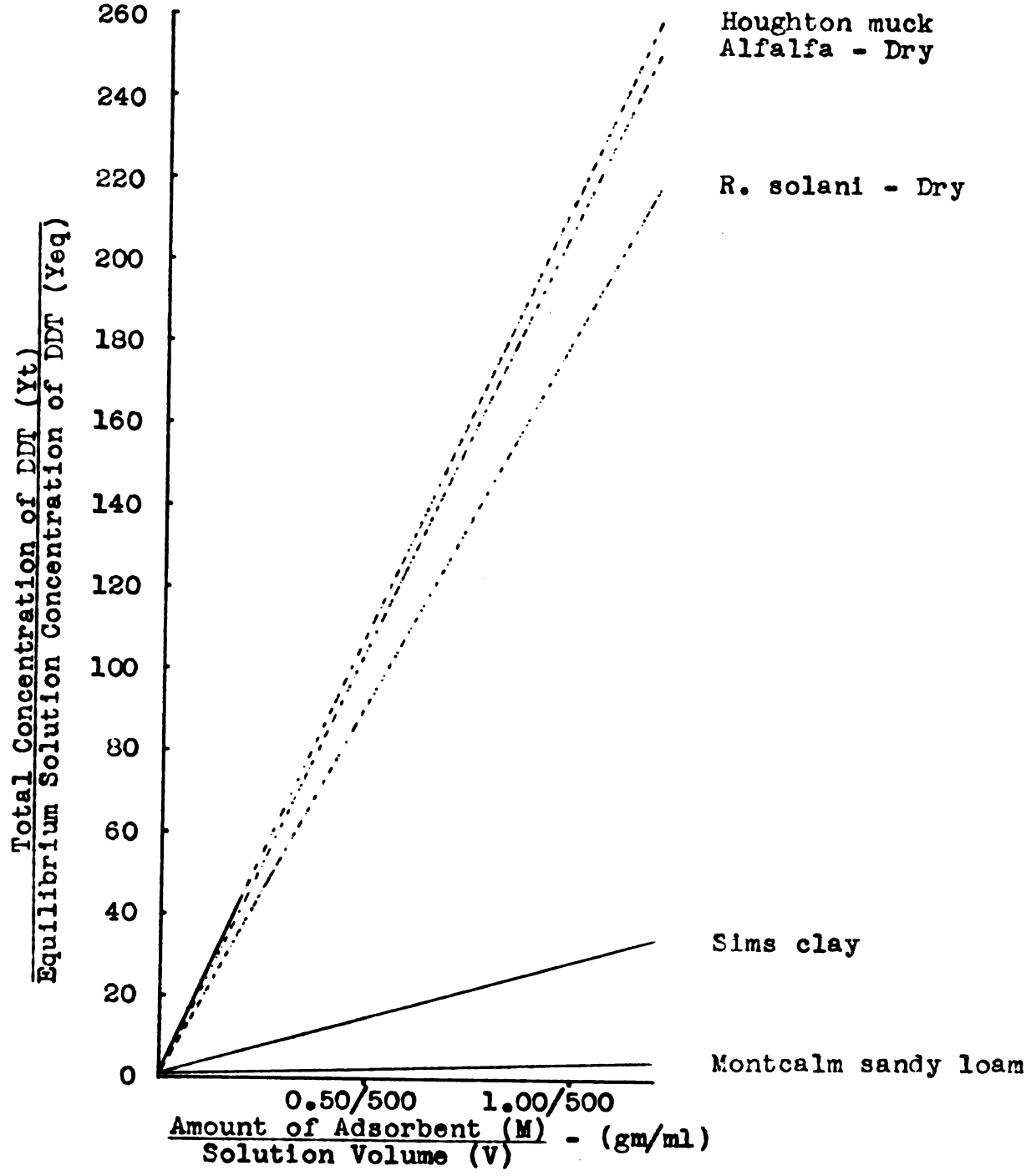

Figure 8. Comparative plot for Kd's of surface soils of Montcalm sandy loam, Sims clay, Houghton muck, R. solani(living), and raw alfalfa tissues(stems plus leaves)

would tend to concentrate DDT in these peripheral lipoid deposits because of its lipid solubility(Acree et al., 1963: Forrest et al., 1946). DDT and lipids tend to concentrate at the air-water interface. Their deposition on solid surfaces in the soil is analogous to the ring in the bath tub. They tend to be isolated on surfaces which are frequently dry and unfavorable for microbial activity. This and the protection afforded by the hydrophobic lipids themselves may be an important factor in retention of DDT in soils.

DDT was present in the soil materials used in this study. A detectable DDT peak was present in the distilled water supernatants used as sample blanks. This peak in the sample blank declined with each successive fractional extraction. No attempt was made to quantitate the peaks nor to extract DDT from any of the soil preparations.

SUMMARY AND CONCLUSIONS

The very great persistence and mobility of DDT in the environment has been amply documented. However, very few attempts have been made to deal quantitatively with some of the basic interactions of DDT with mineral and organic constituents and ecological systems in soils and sediments which contribute to persistence and mobility.

The extremely low solubility of DDT in water has been a discouraging factor. The lack of a theoretical basis for placing DDT interactions into an ecological context in soils and aquatic systems is another.

In the present study, appropriate extraction and concentration procedures were developed for following changes in DDT concentration in aqueous systems over the range of 0.01 to 1.0 ppb. Linear adsorption isotherms were obtained over this range. A special distribution partitioning was developed to facilitate least squares estimation of the distribution coefficient(Kd). Theoretical consideration was given to relationships between the distribution coefficient and constants of the Freundlich and Langmuir adsorption isotherm equations.

A sequence of proximate fractional extractions was used to prepare a series of soil derivatives which could be interpreted in terms of ecological and structural

relationships in the microfabric of the soil.

Under the conditions of equilibrium partitioning employed, the sorptive capacity of two mineral soils(a sandy loam and a clay) was restricted by hydrophobic properties and/or structural effects of lipoid materials extractable with ether and alcohol. It was recognized that the relationship of DDT to these materials in the field might be very different. Cycles of wetting and drying might result in concentration and isolation of DDT in lipid deposits, thereby contributing to its persistence.

Hot water-extractable and 2 % HCl-extractable materials were assumed to come from sites associated with metabolic activity. These fractions were characterized by sorptive capacities greater than in the whole soil.

Sorptive capacity of the sandy loam and a muck soil reached a maximum after 2 % HCl extraction. Residual organic materials would have been predominantly humic acid in nature. It was speculated that humic acids may have an affinity for aromatic molecules because of their free radical character enhanced by an inductive effect of clay minerals on organic materials intimately complexed with them.

Soil materials used had been ground to pass an 80-mesh sieve. Nevertheless, it was recognized that removal of organic materials and minerals in each successive fractional extraction could have resulted in disruption of aggregates. The resulting increase in surface area would have promoted also the observed tendency for sorptive capacity to increase

in successive derivative soil fractions.

When the three soils were mixed in varying proportions, the relationship between sorptive capacity and carbon content of the mixtures was non-linear. Similarly, no consistent relationship existed between sorptive capacity and carbon content of derivative soil fractions. Sorption of DDT was affected by both quantitative and qualitative differences in organic matter content as well as by its relation to soil minerals.

Alfalfa and fungus tissues were similar to untreated muck in their capacity to absorb DDT. Sorption of DDT by plant and microbial tissues represent a focal point for entry into detritus food chains and for entry into humification sequences leading to resistant complexes with strong affinity for DDT.

The biological materials and soil preparations used in this study appear to have promise as model systems for studying ecological interactions of pesticides in soils. Greater detail is needed in characterizing the materials removed and the surface properties of derivative fractions obtained at each step of proximate fractionation. Extension to field situations is also needed, with particular reference to effects of season and wetting cycles.

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APPENDICES

APPENDIX I

Experimental ratios and least squares solutions for Kd in soils and derivative fractions.

$$Yt/Yeq = Kd\ (M/V) + 1$$

Soil/Solu. (M/V:gm/ml)	Yt/Yeq of fractions 1	2	3	4	5	6
Montcalm sandy loam						
1.00/500	3.51	4.48	10.31	13.06	16.71	2.13
.75/500	2.81	4.27	9.15	8.20	10.37	1.87
.50/500	2.49	3.14	4.67	6.44	9.09	1.48
.25/500	1.84	2.08	3.57	3.49	4.62	1.27
Kd x 10^{-2}	12.86	19.39	47.73	55.74	73.83	5.57
s_{Kd} x 10^{-2}	0.66	1.23	3.19	3.32	4.37	0.18
Signi. level	<.0005	.001	.001	<.0005	<.0005	<.0005
Sims clay						
1.00/500	27.46	47.17	51.99	51.25	54.41	7.58
.75/500	22.23	31.42	46.86	40.95	25.06	5.28
.50/500	15.85	20.07	18.29	17.41	22.23	4.13
.25/500	7.40	8.65	8.34	9.09	12.96	2.15
Kd x 10^{-2}	137.09	214.43	255.64	214.17	226.83	31.05
s_{Kd} x 10^{-2}	3.57	11.31	26.21	20.38	27.20	1.40
Signi. level	<.0005	<.0005	.002	.001	.004	<.0005
Houghton muck						
.20/500	42.58	43.05	38.78	68.10	74.40	8.35
.15/500	31.55	28.76	35.55	43.78	45.60	6.14
.10/500	23.67	19.35	20.81	13.32	41.20	4.67
.05/500	16.70	13.35	13.55	8.07	14.50	2.69
Kd x 10^{-2}	1063.37	1001.77	1022.47	1428.17	1737.67	179.50
s_{Kd} x 10^{-2}	58.09	44.04	57.42	214.82	113.80	3.44
Signi. level	<.0005	<.0005	<.0005	.007	.001	<.0005

APPENDIX II

Experimental ratios, distribution coefficients and organic carbon contents of soil mixtures.

Soil/Solution (gm/ml)	Yt/Yeq	Kd x 10-2 (ml/gm)	% Organic carbon
Sims clay(Sc) and Montcalm sandy loam(Msl) mixture			
(Sc 1.00+ Msl 0.00)/500	28.88	139.4	3.90
(Sc .75+ Msl .25)/500	14.84	69.2	3.17
(Sc .50+ Msl .50)/500	9.03	40.1	2.45
(Sc .25+ Msl .75)/500	6.69	28.4	1.73
(Sc .00+ Msl 1.00)/500	3.68	13.4	1.00
Montcalm sandy loam(Msl) incorporated with Houghton muck(Hm)			
(Msl 1.00+ Hm 0.00)/500	3.68	13.4	1.00
(Msl .98+ Hm .02)/500	6.98	29.9	1.91
(Msl .96+ Hm .04)/500	7.20	31.0	2.82
(Msl .94+ Hm .06)/500	10.97	49.9	3.73
(Msl .92+ Hm .08)/500	11.22	51.1	4.64
Sims clay(Sc) incorporated with Houghton muck(Hm)			
(Sc 1.00+ Hm 0.00)/500	28.88	139.4	3.90
(Sc .98+ Hm .02)/500	31.48	152.4	4.75
(Sc .96+ Hm .04)/500	33.35	161.6	5.60
(Sc .94+ Hm .06)/500	42.05	205.3	6.45
(Sc .92+ Hm .08)/500	50.19	246.0	7.31

APPENDIX III

Experimental ratios and least squares solutions for Kd in fungus and alfalfa tissues.

$$Yt/Yeq = Kd\ (M/V) + 1$$

Adsorbate/Solution (gm/ml)	Yt/Yeq	Adsorbate/Solution (gm/ml)	Yt/Yeq
Rhizoctonia solani			
Living cells		Autoclaved cells	
0.312/500	4.69	0.205/500	4.42
.197/500	4.41	.132/500	3.46
.117/500	2.42	.079/500	2.27
.108/500	2.33	.062/500	2.40
.057/500	1.85	.046/500	1.85
.041/500	1.78	.042/500	1.72
.026/500	1.66	.029/500	1.63
.019/500	1.12	.020/500	1.43
$Kd \times 10^{-2}$			
Wet	66.66		87.68
Dry	903.24		1073.20
$s_{Kd} \times 10^{-2}$	4.72		2.98
Signi. level	< .0005		< .0005
Alfalfa tissues			
Washed tissues		Autoclaved tissues	
0.392/500	10.32	0.298/500	9.42
.301/500	6.08	.178/500	6.64
.212/500	5.85	.161/500	6.21
.170/500	4.55	.104/500	4.46
.141/500	5.04	.099/500	4.50
.089/500	3.41	.071/500	2.68
.078/500	2.68	.064/500	2.73
.034/500	1.60	.038/500	1.72
$Kd \times 10^{-2}$			
Wet	109.72		149.64
Dry	1047.83		1358.73
$s_{Kd} \times 10^{-2}$	6.39		5.14
Signi. level	< .0005		< .0005