

The University of Manitoba

PHYSICOCHEMICAL STUDIES OF THE MECHANISM OF COMPLEXATION AND  
RELEASE OF 4-CHLOROANILINE FROM SOIL AND SOIL HUMIC ACIDS

by

Brian L. Worobey

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Submitted to the Faculty of Graduate Studies in Partial  
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## ABSTRACT

Research was conducted to develop a method to release and quantitate bound or tightly complexed 4-chloroaniline from soil humic acids. The nature of the humic acid adsorbent was studied to elucidate possible sites and mechanisms of its interaction with 4-CA. Mild extraction techniques were used to isolate the humic acid from soil and its structure was characterized using infrared spectrophotometry (IR) and  $^{13}\text{C}$ -Fourier transform-nuclear magnetic resonance ( $^{13}\text{C}$ -FT-NMR or  $^{13}\text{C}$ -NMR). Finally an attempt was made to explain the mechanism of binding of 4-CA to soil humic acids.

Pesticide-derived 4-chloroaniline residues are immobilized in soil by interaction with the soil matrix and its components, especially humic acids and clays. Tightly complexed 4-chloroaniline was released from treated soil humic acids by pyrolysis techniques; however, recoveries were low and nonreproducible precluding the use of this procedure as an analytical method. An evaluation of several alternative methods of release resulted in development of an analytically acceptable method based upon alkaline hydrolysis and quantitation of 4-chloroaniline as its N-heptafluorobutryl (HFB) derivative by electron capture-gas-liquid chromatography (EC-GLC). A lower detectable limit of 75 fg ( $75 \times 10^{-15}$  g) was possible. Alkaline hydrolysis released 80% of the applied radioactivity; with 46% of the applied compound quantitated as 4-CA-HFB. "Nonhydrolyzable" 4-chloroaniline residue in soil humic acids is probably an artifact of the hydrolysis procedure in short term

experiments, and not the result of indigenous reactions of 4-chloroaniline with soil humic substances.

Different methods of extracting humic acids from soil were found to yield preparations of varying physicochemical characteristics. A mild extraction procedure ( $\text{Na}_4\text{P}_2\text{O}_7$ -pH 7 precipitation with acetone) yielded the least aromatic and most aliphatic humic acid, whereas, increasing harshness of extraction resulted in humic acids of increasing aromaticity. Analysis of these humic acid structures by a novel technique,  $^{13}\text{C}$ -FT-NMR, supported IR evidence that aliphatic and alicyclic substances are important components of indigenous humic acid.

Fulvic acid spectra appeared to be less aromatic cf. humic acid from the same soil. Among several models and precursors of humic acid investigated, only cellulose yielded a precipitate via the classical (NaOH) extraction procedure. Its spectral characteristics were (IR,  $^{13}\text{C}$ -NMR) most similar to a fulvic acid isolated by the same procedure. A  $\alpha$ 1-4,  $\alpha$ 1-6-glucan exhibited a  $^{13}\text{C}$ -NMR spectrum very similar to a cellulose component  $^{13}\text{C}$ -NMR spectrum. Hemicellulosic compounds may, therefore, be essential components of fulvic acids, and, possibly, humic acids.

Soils of varying organic matter (OM) concentrations and clay (C) concentrations were reacted with 4-chloroaniline, and OM concentration evidently controlled the amount complexed. The quantity of the tightly complexed fraction released by pyrolysis was independent of clay concentration but dependent on the concentration of OM.

The micellar nature of these humic acids in solution was implied by the observation that three molecular weight ranges of humic acid,



prepared by ultrafiltration, exhibited similar IR spectra.

Aliphatic benzoic acid-type structures may form micelles in solution and so explain the physicochemical properties of HA which characterize its ability to complex 4-chloroaniline, xenobiotics, and various other soil components.

The results of these studies have provided some insight into the mechanism(s) of 4-chloroaniline interaction(s) with soil and especially soil humic substances, culminating in a de novo (from basic principles) theory of complexation. According to this theory 4-chloroaniline and possibly other xenobiotics interact with the soil matrix via three prominent mechanisms mediated by interaction of 4-CA with soil humic acid micelles:

- (1) "loosely" complexed and physically sorbed organic solvent-extractable surface complexes;
- (2) "tightly" complexed micellar inclusion products (physically and/or chemically sorbed) released by hydrolysis; and
- (3) "irreversibly" complexed residues due to hydrolytic action or long term biological incorporation.

The relative importance of each kind of complexation will depend on the physicochemical characteristics of the soil and xenobiotic involved. For 4-chloroaniline, mechanism (2) appears to be predominant.

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To my wife

CAROL

Her sacrifice and faith  
made this endeavor a reality.

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## I INTRODUCTION

Mankind has become progressively more dependent upon the use of pesticides for the control and eradication of crop pests (plants, animals, insects, fungi, bacteria, nematodes, etc.). In the United States alone, the sales of just one class of pesticides, herbicides, has been estimated to be 492 million lbs. in 1975 (Rawis, 1975).

Increased pesticide usage in the past thirty years or so, has led to concern for their environmental fate and toxicity as contaminants. Knowledge of soil-pesticide interaction is crucial to an understanding of their potential or present toxicological hazards. Soil and sediment (dissolved and undissolved forms) are the environmental "sink" for all xenobiotics.

Of special concern are the bioactivity of the pesticides and their persistence or recalcitrance. The finding that fulvic acids and humic acids can interact with substantial amounts of hydrophobic dialkyl phthalates and solubilize them in water suggests that pesticides and other xenobiotics with low water solubilities may also be complexed and solubilized by this mechanism (inclusion); Ogner and Schnitzer, 1970a,b; Ballard, 1971, Kirkland, 1973.

Several toxicological hazards have been described for chlorinated anilines. Substituted anilines arising from degradation of pesticides are acetylated to form acylamides (Tweedy et al., 1970; Kearney and Plimmer, 1972). Hoffman and Allen (1960) observed that N-acetyl derivatives of 3,4-dichloroaniline (3,4-DCA) were phyto-

toxic. Other compounds formed by the condensation of 4-CA and 3,4-DCA are chloroazobenzenes, anilinobenzenes and azoxybenzenes, all of which have been reported to be carcinogenic (Neish, 1964; Hall, 1977) and toxic to mammals (Bartha and Pramer, 1967; Weisburger and Weisburger, 1966) and microbes (Corke and Thompson, 1970). Many of the parent pesticides and/or their degradation products persist in the soil, so that environmental contamination must be a concern. Concern has been expressed for the long range effects of "poly-halogenated" soil organic matter on vital soil, sediment, or dissolved organic matter processes and conditions. Continued buildup of bound residues may affect physical and biochemical characteristics such as water-holding capacity, soil structure, soil organisms, and the processes of nitrification, ammonification, and cellulose decomposition. Once mechanisms of binding are clear, an assessment of potential release would be possible. For the present, attempts to elucidate the bound residue problem have centered around methods for analysis of bound compounds which resist extraction by the techniques normally used.

Changes in cultural practices may liberate bound residues, re-introducing them into the soil solution and thereby allowing subsequent uptake and translocation into the economic portions of plants, for example, in rice and soybeans (Nashed and Ilmicki, 1970; Still, 1968; Tweedy, et al., 1970), cotton (Smith and Sheets, 1967), rape, barley and rye (Guess, 1974). All these examples apply to substituted chloroanilines derived from several pesticides,

especially phenylurea, phenylcarbamate and anilide herbicides.

A soil bound residue has been defined as the chemically unidentified pesticide residue or its degradation product remaining in fulvic acid, humic acid, and humin soil fractions after exhaustive sequential extraction with nonpolar and polar solvents (Kaufman et al, 1976). The precise definition of a bound residue has yet to be wholly agreed upon. The complexity of the systems involved has frustrated research in this area. Analysis of soil bound residues is of paramount importance because of the potential accumulation of these residues in soil organic matter. Knowledge of the various interactions between xenobiotics and soil components may be gained by isolating those fractions most important in complexation realizing that a combination of fractions (i.e. fractions interacting with each other) may be especially important in the binding of residues.

The work described in this study involves 4-chloroaniline (4-CA), a common and abundant degradation product of many pesticides which is bound rapidly and tenaciously to soil components, especially humic substances (Hsu and Bartha, 1974a, 1974b, 1976; Worobey, 1977; Moreale and Van Bladel, 1976). The research presented herein describes experiments conducted to release and quantitate 4-CA bound to soil humic acids. The nature of this binding was investigated in relation to indigenous humic acid structure and the physicochemical properties of humic acids.

The objectives of the studies reported herein may be divided into the following three main areas:

1. To investigate methodology for release of soil (humic acid) bound 4-CA, residues, including so-called "nonhydrolyzable" residues;
2. To determine the nature of the humic acid substrate regarding the nature of its interaction with 4-CA;
3. To elucidate the mechanisms of complexation or "binding" of 4-CA by soil humic acids.



## II LITERATURE REVIEW

### A Soil Organic Matter Chemistry

#### A.1 Introduction

The organic matter in soils includes a complete spectrum of materials ranging from relatively undecomposed plant litter to the various decomposition and excretion products derived from plants, animals and microorganisms. Soil organic matter may be broadly classified into non-humic and humic (major organic component, usually) substances. The former consists of the recognizable remains of soil flora and fauna and the latter are formed by biological and nonbiological degradative and synthetic chemical reactions. Soil organic matter, which generally represents less than five percent of dry weight of mineral soils, is very resistant to further degradation and can be intimately associated with the mineral components of the soil. The general group of compounds included under the term "humic matter" or "humic substances" can be broken down into several subgroups, namely, humic and fulvic acid, humatomelanic acid and humin (Schnitzer and Khan, 1972).

They serve as a storehouse of chemical elements essential for plant growth, especially carbon and nitrogen, and, to a lesser extent, of phosphorus, sulfur, calcium, iron, manganese and other trace minerals. Humic substances have profound effects on soil structure, drainage, aeration, water and buffering capacity and physiological functions of plants (Linehan, 1977; De Haan, 1977;

Rotini, 1977; Hernando et al., 1977; Nopamornbodi, 1977). Although humic compounds are relatively resistant to both chemical and biological attack, they too are degraded into the simplest molecules ( $\text{CO}_2$ ,  $\text{H}_2\text{O}$ ,  $\text{NH}_3$ ) as a continuation of steps in the carbon and nitrogen cycles.

## A.2 Genesis of humic substances

An understanding of the chemistry of humic substances and of the role they play in soil, requires some insight into the processes by which they are formed. Several hypotheses have been proposed to explain the formation of humic substances in soil (Felbeck, 1971).

### a) The plant alteration hypothesis.

According to this view, the nature of the original plant compound strongly influences the nature of the humic substances (i.e., the carbohydrate, lignin, cellulose, lipid, protein, etc. of plants). The higher molecular weight humin then represents the first stages of humification followed by subsequent microbial attack to degrade the humin to humic acid, then fulvic acid, and finally mineralization (very slowly, 2-3% per year) to  $\text{CO}_2$  and  $\text{H}_2\text{O}$ .

### b) The chemical polymerization hypothesis.

Plant material is degraded microbially to small molecules, which are absorbed and utilized by the soil microbes as sources of carbon and energy. Microbes synthesize various products, usually phenols

and amino acids, which are excreted into the surrounding medium, where chemical oxidation and polymerization to humic substances take place. According to this hypothesis, the nature of the original plant tissue has no effect on the kind of humic substances finally produced.

c) The cell autolysis hypothesis.

Humification is thought to be the product of the autolysis of dead plant and microbial cells. The product is a heterogeneous substance formed by random condensation and polymerization of cellular debris such as sugars, amino acids, phenols, and other aromatic compounds, which are converted to free radicals by autolytic enzymes released on the death of the cell.

d) The microbial synthesis hypothesis.

As in the chemical polymerization hypothesis, microbes initially utilize the dead plant tissue only as a source of carbon and energy. However, microbes then synthesize various high molecular weight humic-like compounds intracellularly and these compounds are only released to the soil when the microbes die and their cells are lysed. According to this view, the higher molecular weight compounds represent the first stages of humification followed by extracellular microbial degradation to humic acid, fulvic acid, simple organic compounds, and, finally, to product of mineralization.

e) Chemical models of humic substances.

Several simple chemical monomers have been shown to yield humic-like compounds upon enzymatic or chemical oxidative polymerization (Schnitzer and Khan, 1972; Gieseking, 1975).

Hydroquinone, catechol, substituted benzene and hydroxybenzene carboxylic acids, etc., yield humic-like polymers upon alkaline or enzymatic (phenolase, tyrosinase) oxidation. It was demonstrated that the enzymatic oxidation of phenols (such as catechol, chlorogenic acid, and caffeic acid) in the presence of amino acids, peptides, or proteins yielded "humic acid" very similar to those from soil, and that the most rapid polymerization occurred at pH 7 (Swaby and Ladd, 1962; Ladd and Butter, 1966). Haider et al. (1965) oxidized phenolic compounds with phenolases in the presence of amino acids and peptides, and showed that the binding of nitrogen occurred at pH values above 6.5 and that only those phenols reacted which had no methylated hydroxyl groups. Synthetic polymers resembling humic acids have also been synthesized by heating solutions of glucose and glycine or methylglyoxal and glycine. Hayes (1960) showed that, when appropriate ratios of reactants were chosen and the polymerization process controlled, the resulting synthetic humic acids had physicochemical properties similar to humic acid isolated from a muck soil. Finally, the nonenzymatic browning reaction (Maillard reaction) of carbohydrates with amines also yields humic-like compounds (Gieseking, 1975).

These hypotheses all represent possible pathways in humic matter formation. Selection of one theory over the others is impossible, since the actual processes probably involve a combination of two or more mechanisms.

### A.3 Postulated structures of humic substances

Numerous attempts have been made to devise a structural formula representative of humic acids, but none were entirely satisfactory. The widely quoted model of Fuchs, Fig. 1, is untenable for several reasons (Kononova, 1966, Swain, 1963). The data obtained by x-ray diffraction excluded polycondensed systems containing more than a few rings. A polycondensed system failed to account for conformational changes implied by viscosity studies (Mukerjee and Lahiri, 1958).

Contemporary investigators favor a structural model consisting of micelles of polymeric nature, the basic structure of which is an aromatic ring of the di- or trihydroxyphenol-type bridged by -O-, -CH<sub>2</sub>-, -NH-, -N=, -S- and other groups, and containing both free OH groups and quinone linkages. Dragunov's structure (Fig. 2) most nearly meets the above requirements. However, a fully acceptable model would also need to account for the occurrence of significant amounts of aromatic -COOH groups, some arranged in positions such that cyclic anhydrides can be formed by various chemical treatments. The presence of 5-membered ring structures and condensed elements containing two or three rings is also a distinct possibility.

Felbeck (1965a, 1965b) on the basis of his organic matter

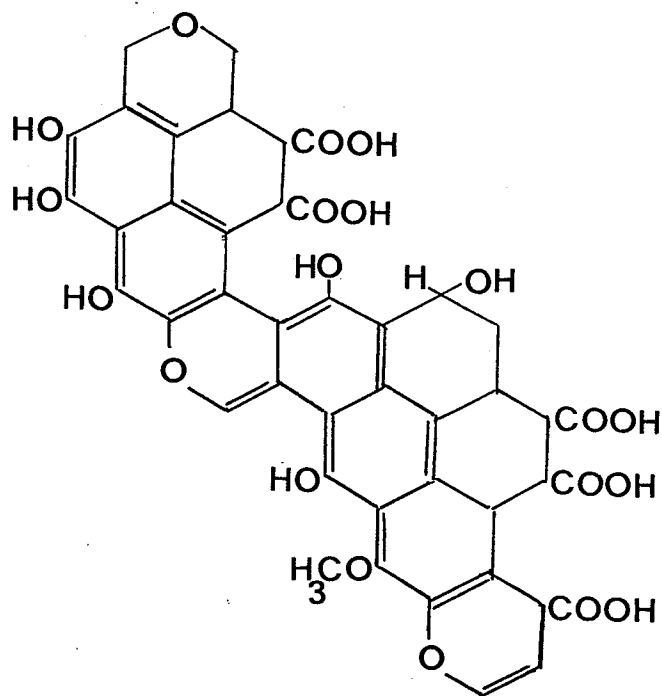


FIGURE 1. Structure of humic acid according to Fuchs (Kononova, 1966; Swain, 1963).

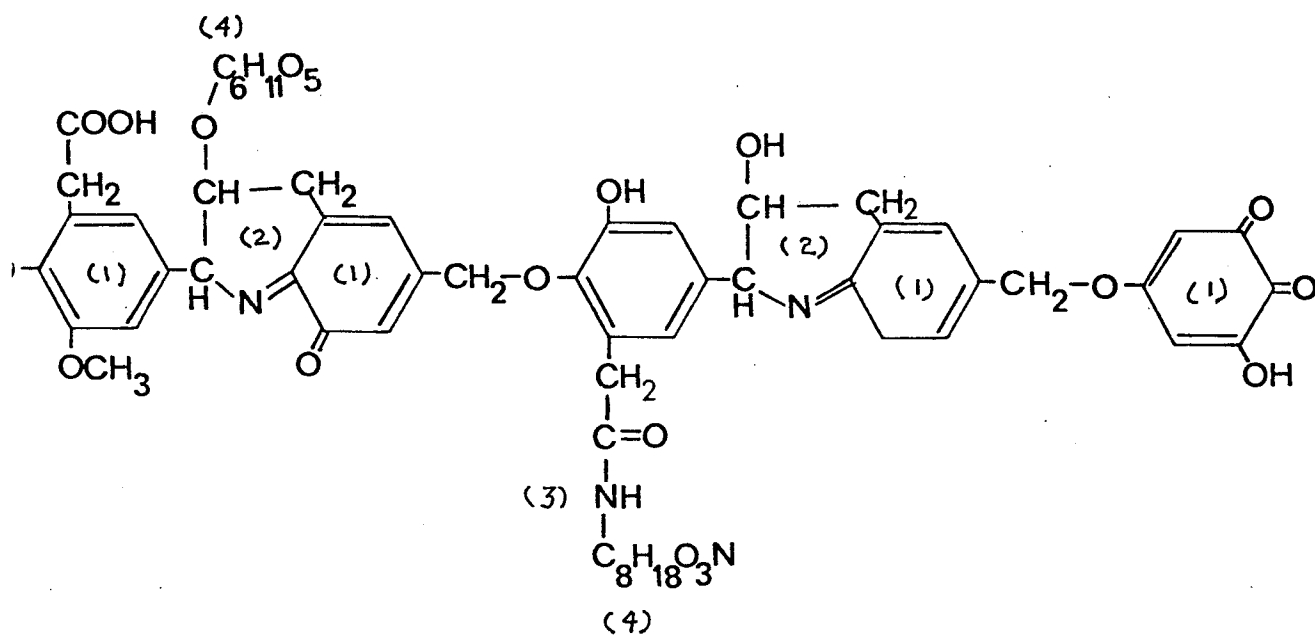
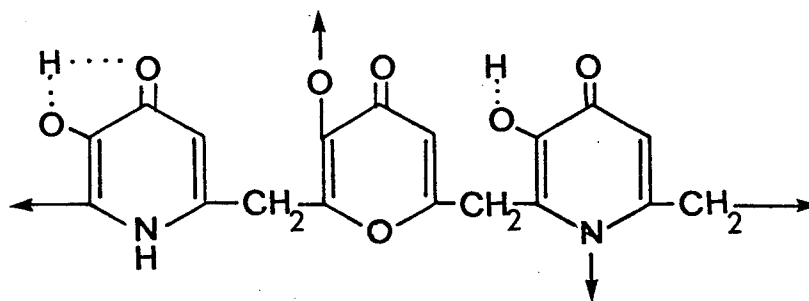


FIGURE 2. Structure of humic acid according to Dragunov (Kononova, 1966). (1) Aromatic ring of the di- and tri-hydroxybenzene type, part of which has the double linkage of a quinone group. (2) Nitrogen in cyclic forms. (3) Nitrogen in peripheral chains. (4) Carbohydrate residues.

hydrogenation studies, proposed a humic structure in which 4-pyrones (as illustrated below) act as a central structural unit for phenolic



and amino acid side chains. However, this hypothesis lacks credibility since the molecule does not contain COOH and OH groups which can be readily methylated. Haworth and his coworkers (Cheshire *et al.*, 1967) suggested that humic acid possesses a chemically resistant polycyclic aromatic core to which polysaccharides, proteins, and phenolic acids were attached. These side chains are linked to each other. Such a molecule would have the ill-defined structure typical of humic substances, and the central core, which would contain the stable semiquinone radicals, should be resistant to microbial attack.

Stevenson (1972a,b) summarizes the structure for humic acid (Fig. 3). As can be seen, this humic acid model contains a high density of reactive functional groups (COOH, phenolic OH, and quinone C=O). While the humic acids in a given soil will vary in composition, most molecules would be expected to contain the same basic units and the same types of functional groups indicated by the model structure. Most recently Schnitzer and coworkers (1972) have



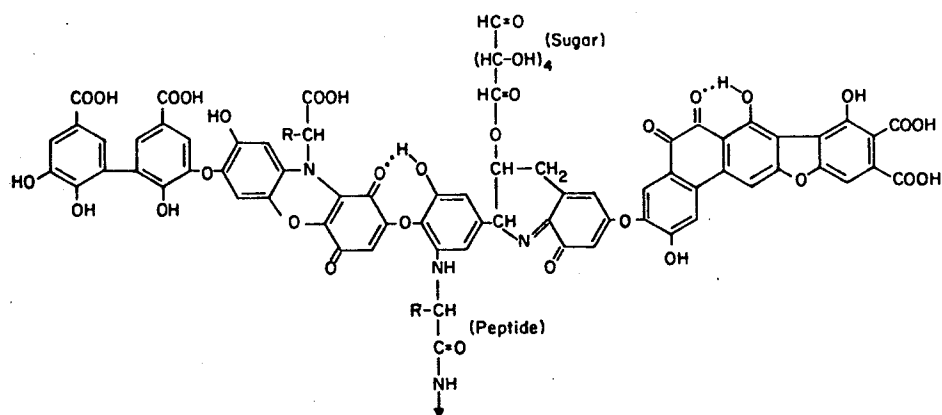


FIGURE 3. Type structure of humic acid according to Stevenson (1972).

proposed a new model based upon hydrogen-bonded monomers of variously substituted benzenecarboxylic, phenolic and hydroxy-benzene carboxylic acid compounds. This last model is favored by the author and will be discussed in a latter section in some detail.

The reactive nature of humic substances is due to their usually high content of oxygen-containing functional groups, including carboxyl (-COOH), phenolic (PhOH), aliphatic (-OH), and enolic-hydroxyl (-C=C-OH), and carbonyl (-C=O) structures of various types. Carboxyl and phenolic-OH groups are the main oxygen-bearing groups. Amino (-NH<sub>2</sub>), heterocyclic amino(-NH-), imino (-C=N-), and sulfhydryl (-SH) groups may also be present (Schnitzer and Khan, 1972; Gieseking 1975).

#### A.4 Extraction and Fractionation of humic substances

Humic substances consist of a series of highly acidic, yellow-to black-colored polymeric molecules. They are normally recovered by extraction of soil with alkali (usually 0.5N NaOH), although mild reagents, such as sodium pyrophosphate (Bremner and Lees, 1949) have also been used. Humic substances are classically fractionated into three distinct portions based upon solubility characteristics (Fig. 4): (1) humic acid (soluble in alkali, insoluble at pH 1); (2) fulvic acid (soluble in both alkali and in acid); (3) humins (insoluble both in alkali and in acid); and (4) hymatomelanic acid (soluble in ethanol) (Gieseking 1975). The current view is that the various humic fractions represent a complex mixture of polymeric substances

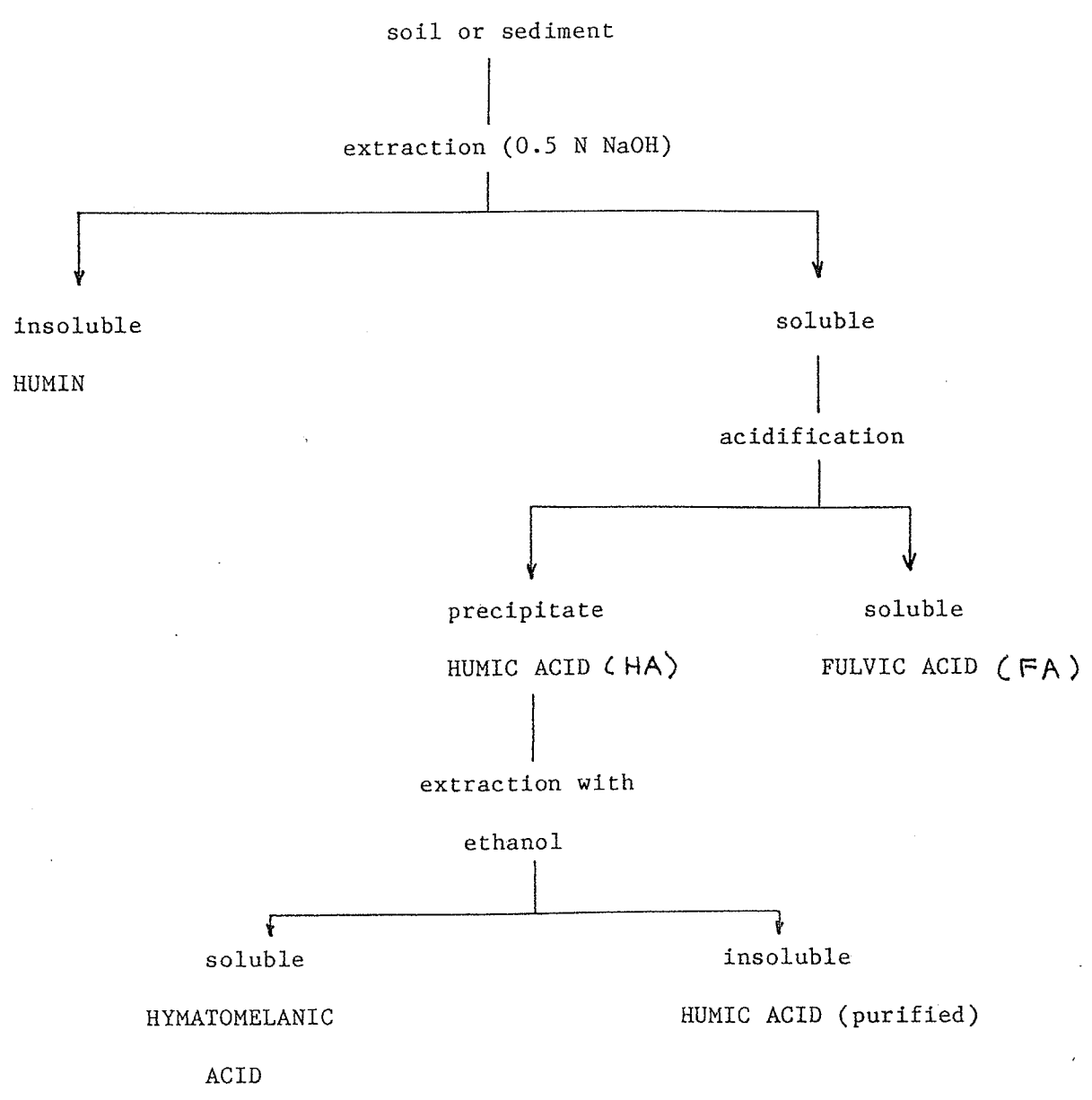


FIGURE 4. Classical fractionation procedure for humic substances.

having molecular weights ranging from under 1,000 to over 300,000 (Stevenson and Butler, 1969; Stevenson, 1972a,b).

Most workers have concentrated on the "humic" and "fulvic" acid fractions. The classical technique has been criticized by several workers such as Swift and Posner (1972) who found that auto-oxidation readily occurs, with resulting increases in oxygen content and reduction in molecular weight. Ceccanti et al. (1978) points out that degradation is pronounced with use of strong alkali, becoming part of the material extracted. Also, it is well known in organic chemistry that strong base easily hydrolyzes polysaccharides and proteins, and can even, under some circumstances, lead to polymerization and aromatization of polysaccharides, (Forsskahl, et al., 1976). Swift and Posner (1977) reported the effects of various extractants on the isolation of humic acids from humified plant materials and soils. Cation-exchange capacity of the extracts were in the following order:  $\text{Na}_4\text{P}_2\text{O}_7$  > cold NaOH > hot NaOH;  $\text{Na}_4\text{P}_2\text{O}_7$  extracts contained more highly oxidized organic matter (greater  $\text{CO}_2\text{H}$  content) cf. NaOH extracted organic matter. Kononova and Alexandrova (1973) found that the use of NaOH to extract humifying plant material resulted in the in-vitro (artificial) formation of humic-like substances due to oxidation and condensation reactions of tannins, phenols, precursors to lignin and several amino acids. Yields using sodium pyrophosphate, as an extractant, vary widely; Gascho and Stevenson (1968) found that 0.02M  $\text{Na}_4\text{P}_2\text{O}_7$  extracted almost as much humic matter from a Brunizem soil as 0.5N NaOH, but

Butler and Ladd (1969) and Posner (1966) obtained much lower yields using 0.1M  $\text{Na}_4\text{P}_2\text{O}_7$  from extraction of various red-brown soils. These workers found that  $\text{Na}_4\text{P}_2\text{O}_7$  extracted a lower molecular weight and higher oxygen content fraction than NaOH. However, the  $\text{Na}_4\text{P}_2\text{O}_7$  extractant, when adjusted to pH 7, is a very gentle technique compared with 0.5N NaOH, and the danger of alteration is widely accepted as being greatly reduced. Also lower concentrations of Fe, Al and Si have been reported for  $\text{Na}_4\text{P}_2\text{O}_7$  than for NaOH extracts (Posner, 1966). The action of pyrophosphate as an extractant has been proposed to involve competition with organic matter for metals binding organic matter to soil particles. Many workers, when dealing with soil organic characterization, use both techniques.

Other methods that have been used include hot sodium bicarbonate (Posner, 1966), ethylenediamine and pyridine (which results in good yields but causes enrichment of N), sulpholone, DMSO, and DMF, organic chelating agents such as EDTA and Dowex A-1 (Hayes et al., 1975), 0.5 N HCl (Martin, 1976) and dispersion of organic colloids by ultrasonification (Leenheer and Moe, 1969). Dormaar et al. (1970) reported ash contents of humic acids as high as 68.1% using  $\text{Na}_4\text{P}_2\text{O}_7$  or NaOH extractants; filtration through a 0.45  $\mu$  Millipore filter produced almost no change in the ash content. Purification is usually accomplished by washing the extracted material with a fairly strong HCl or HCl/HF solution to displace or dissolve impurities. Tan (1976) found that infrared absorption bands due to

fine clays and silica gel disappeared on washing with HCl/HF. Gascho and Stevenson (1968) found that pretreating soil with 0.3N HF, followed by dialysis of the extract against 0.3N HF, was sufficient to yield low ash humic acids, but several workers including Schnitzer and Khan (1972) claim that HF alone denatures the humic substance. Even HCl alone can lead to some modification of constituents (Olsson et al., 1977). Another method which has been investigated is the use of ultracentrifugation to remove fine clay particles. These facts were considered in the development of the extraction methods used in this thesis.

#### A.5 Characterization of humic and fulvic acids

##### A.5.1 Infrared Spectrophotometric analysis of humic substances

In the characterization of humic material by infrared spectroscopy, different interpretations are sometimes possible because of uncertainties in functional group designations; Schnitzer (1972) assigns the  $1630\text{ cm}^{-1}$  to three different components. Tan (1977b) asserts that humic material extracted by pyrophosphate gives a more highly resolved spectrum than that extracted by NaOH, allowing for a definite distinction between soil groups. However, assignment of key absorption bands to various impurities has not been uncommon, particularly with contamination by silicate clays which absorb strongly in the range  $970\text{-}1100\text{ cm}^{-1}$ . IR spectra also vary according to the nature of the most abundant metal cations present (Tan, 1978).

The most common method used in the preparation of samples is the pressed KBr discs. MacCarthy and Mark (1975) state that this could easily lead to conformational or even structural deformation and thus took the infrared spectrum of a "wet" humic acid by saturating the sample with deuterium oxide. They noted substantial changes from 1600 to 1700  $\text{cm}^{-1}$  and 2900 to 3500  $\text{cm}^{-1}$ , both of which are used extensively in the characterization of humic materials. They found no evidence for aromatic absorption in the 3000-3100  $\text{cm}^{-1}$  range but they attached no importance to it.

MacCarthy et al. (1975) used a Fourier Transform-IR to analyze a humic acid. The C-H stretching absorption was made more prominent, and the 1600-1700  $\text{cm}^{-1}$  region was much more resolved. On this basis, they suggested that bands at 1610 and 1510  $\text{cm}^{-1}$ , formerly applied to the stretching of aromatic systems, were more likely due to carboxylate groups.

Generally one must be careful not to assign the absorptions of humic substances with too much certainty. Resolution is often poor and the heterogeneity of their structure results frequently in broad, monotonous spectra due to overlapping of functional group absorptions.

#### A.5.2 $^1\text{H}$ -NMR versus $^{13}\text{C}$ -FT-NMR analysis of humic and fulvic acids

Generally the  $^{13}\text{C}$  spectral range may be divided as follows (Levy and Nelson, 1972; Villa et al., 1978a): 10-60 ppm aliphatic carbon; 60-100 carbohydrate; 100-160 aromatic, olefinic and

heteroaromatic; 160-200 carboxyl, esters, amides; and 200-210 ketones and aldehydes. Low signal to noise ratios (S/N) are common in humic spectra due to multiple causes of line broadening. In complex substances such as soil humic substances, one has to consider another complication. The molecules may consist of rapid and mobile regions in which only long transversal relaxation times,  $T_2$ , predominate to be observable as broad line widths in a high resolution spectrum. Consequently, caution is required when deriving concentrations of carbon classes from intensities of the signals or in deducing from the absence of a signal the essence of a specific structure (Villa et al., 1978a).

(1)  $^1\text{H}$ -NMR of humic and fulvic acids

(i) Humic acids

Spectra of humic acids extracted at different pH values showed an increase in aromatic protons as pH decreased from 14.0 to 4.5, however, aliphatic protons were always most abundant at any pH (65 to 81%,  $^1\text{H}$ -FT-NMR, Lenz et al., 1977). Thus it would appear that decreasing pH, as well as increasing pH, may cause aromatization in agreement with the results of this thesis, as well as, results of other studies to be discussed.

Other  $^1\text{H}$ -NMR studies reported 19% aromatic protons (Ruggiero et al., 1979); fractions of aromatic protons were 0.16 to 0.43 depending on soil type (Wilson et al., 1978) indicating a polycyclic or heavily substituted monocyclic structure. Also, 32% of the aliphatic protons



or 27% of the total protons were bound to carbon, to oxygen as in carbohydrates, or to other polyhydroxyl or polyether compounds.

(ii) Fulvic acids

Schnitzer and Skinner (1968) found no aromatic protons in the spectra of soil fulvic acid fractions, indicating that either the aromatic nuclei were fully substituted or that no aromaticity is present in the indigenous fulvic acid. Broad signals at 3-4 ppm (due to methylenes or methyls linked to oxygen and or nitrogen), weak signals at 1.0-1.3 ppm (aliphatics), and at 7.4-8.2, very weak aromatic signals were reported recently for fulvic acid (Ruggiero et al., 1978).

The following researchers reported the lack of aromatic protons for fulvic and humic acids: Neyroun and Schnitzer, 1974; Barton and Schnitzer, 1963; Haworth, 1971; Lakotos and Meisel, 1977; Ogner and Gronneberg, 1977. Ruggiero et al., (1979) reported a fulvic acid sample contained only 20% aromatic protons. Strong aliphatic signals and weak aromatic proton shifts appear typical of fulvic acids (Stuermer and Payne, 1976) and even a preponderance of polymethylenes has been reported (Grant, 1977).

(2)  $^{13}\text{C}$ -NMR spectra of soil humic and fulvic acids

Table 1 summarizes the research reported on  $^{13}\text{C}$ -NMR spectra of soil and sediment humic and fulvic acids.

It is evident that aromatic structures (110-160 ppm range) are

Table 1.  $^{13}\text{C}$ -NMR Spectra of Soil Humic and Fulvic Acids

SAMPLE	ppm chemical shifts (S max)	REFERENCE
fulvic acid	(0.5N HCl) 38,75,115,135,170,180	Villa <u>et al.</u> , 1978a
humic acid	(0.5N NaOH) 28,38,55,75,104,135,180	
- 10-100 range showed the strongest absorption indicative of carbohydrates, aliphatic amino acids and side chains, 135 then 170-180 next in intensity.		
fulvic acid	[NaOH + acid $\rightarrow$ filtrate FA] almost no aromatic carbon (110-160), broad resonances at 60-100 (singly bonded carbon) and 170-185 (carboxyl ester, amide)	Stuermer and Payne, 1976
humic acid	most intense peak 29.7, spectrum generally supported presence of aliphatic $\text{C}_4\text{-C}_{14}$ ( $\text{C}_9$ most prominent) dicarboxylic acids	Ogner, 1973 Ogner, 1979
humic acids	strong shift at 29, integration of a methylated sample showed 21% aromatic -C, 35% aliphatic-C, 3% -C=O, 13% $\text{CH}_3\text{-O}$ and 28% other-C, probably aliphatic -C bound to oxygen as in carbohydrates or amides of peptides	Ogner and Gronneberg, 1977
fulvic and humic acids	no aromatic signals, intense broad signal at 60-90, 71 and 101 peak for fulvic acid only indicative of ROH or ROR as in carbohydrates	Wilson and Goh, 1977b
Ah* humic acid	$\text{Na}_4\text{P}_2\text{O}_7^*$ NaOH	11-46,60,71 24,29.5,55.6,68.8,110.3,129.8,168-179
Bh humic acid	$\text{Na}_4\text{P}_2\text{O}_7$ NaOH	61.8,64-80 (71.7 max.), 164-179 (174.6 max.) 23.2,71.2,157.7,160.6,163,169.5-176 (175.5 max.)

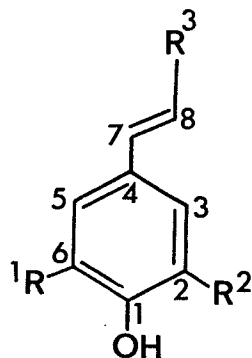
\* thus chemical shifts vary with soil horizon (Ah,Bh) and soil extractant used to isolate humic acid ( $\text{Na}_4\text{P}_2\text{O}_7$ , NaOH).

organic matter	organic solvent extracts, exhibit up to 30% poly-methylene carbon of the total O.M. (almost all OM extracted)	Grant, 1977
oxidized soil	$\text{Et}_2\text{O}$ extract, very strong signals at 0-40 and very minor one at 125-130	Wilson and Goh, 1977a
Humic and fulvic acids	[(HA) NaOH, (FA) HCl] intense 28 signal, 38,59,75, 92-109,125-135,170-180; spectra very similar for HA and FA and for 2 HA from different soils	Villa <u>et al.</u> , 1976
humic and fulvic acids	[NaOH $\rightarrow$ acid] (HA) 67% of carbon was aromatic and carboxyl carbon and fraction of aromatic protons was 0.16 to 0.43 depending on soil type, FA and HA spectra very similar	Wilson <u>et al.</u> , 1978
(HA)	184-169 (177 max, strong), 143-106 (127 max),84-65 (70 max), 39.7,54,35,27,26,20	
(FA)	185-171 (181 max), 144-104 (128 max), 89-63 (74 max), 52,34,29 (52-29 strong)	
soil fungal melanins and plant lignins	more dissimilar <u>cf.</u> humic or fulvic acid spectra	Ogner and Gronnerberg, 1977 Villa <u>et al.</u> , 1978a, 1978b
cellulose polymethmethacrylate	showed resonance in regions of humic acids studied by CP-MAS	Miknis <u>et al.</u> , 1979b
fulvic and humic acid	[NaOH $\rightarrow$ acid filtrate FA] (FA)33-37, 42-57, 65-72, 72-95, 85-90, humic acids 110-120, 175-185 HA spectra same as FA	

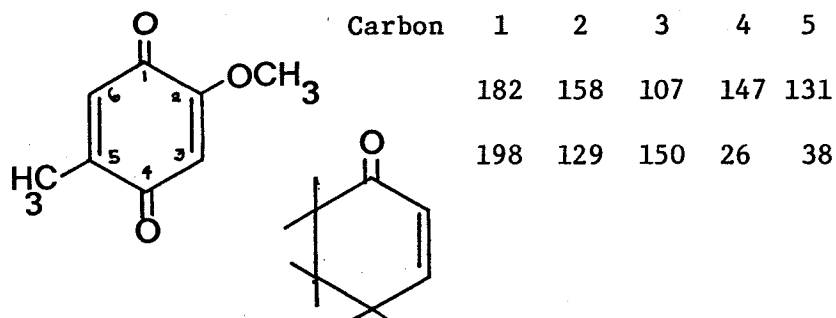
present in the humic acids but assignments of chemical shifts must be interpreted in terms of the extractant and/or precipitant used for their isolation. Milder extractants appear to yield spectra indicating much lower aromaticity (Wilson and Goh, 1977b). The possible presence of polymethyl groups in humic substances identified by  $^{13}\text{C}$ -NMR is well documented; Ogner (1973, 1979), Grant (1977), Miknis et al. (1979b). Miknis et al. (1979b) found many similarities in the spectra of cellulose and polymethyl<sup>yl</sup>methylacrylate when compared to humic acid spectra. Often HA and FA appear almost identical indicating that their basic structure or "core" structures are the same [Miknis et al. (1979b), Wilson et al. (1978), Villa et al., 1978a)].

The importance of interpretation of the 110-160 ppm range is emphasized again as an attempt is made to reach some generalized conclusion regarding humic acid structure.

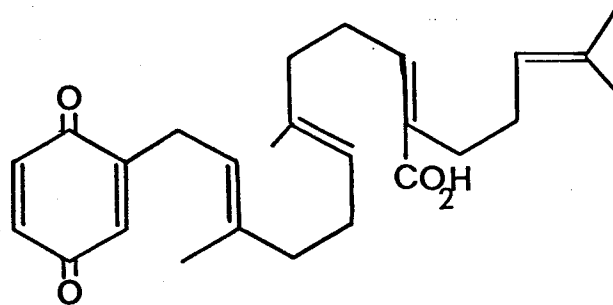
Chemical shifts of n- and branched-alkanes, mono- and polycyclics, mixed cyclic systems (aromatic and alicyclic),  $\text{CH}_2$  bridges, and partially unsaturated rings all give rise to signals in the range of 40-45 ppm (Zilm et al., 1979) in  $^{13}\text{C}$ -NMR spectra of coals. Brunow and Lemmetyinen (1978) studied increasingly substituted 4-propenylphenolates and phenols using  $^{13}\text{C}$ -NMR, in relation to lignin spectra. They reported that as the ring becomes substituted with methoxyl groups, for example, the ring carbon shifts from ca. 116 to 146 ppm and olefinic carbon resonances were at 145 ( $\text{C}_7$ ) and 114 ppm ( $\text{C}_8$ ).



Hofle (1976) showed that benzoquinones (2,5-substituted) and 2-cyclohexenone gave the following  $^{13}\text{C}$  NMR resonances:



and for quinones substituted with unsaturated aliphatic acids (isolated from brown-algae, Kusumi *et al.*, 1979) the following  $^{13}\text{C}$ -NMR assignments were made:



$\text{CH}_3$  = 16-25 ppm;  $\text{CH}_2$  = 26-40, olefins = 130-150 singlets and 118-145 doublets; benzoquinone  $\text{C}=\text{O}$ -188 ppm and carboxyl carbon = 173.5 ppm.

### A.5.3 Pyrolysis-gas-liquid chromatography/mass spectrometry (Py-GLC-MS)

Py-GLC-MS analysis of several soils and their humic fractions have revealed a preponderance of polysaccharide-like compounds indicating that they are more important than had been presumed (esp. fulvic and humin fractions, but also the humic acid fraction, SaizJimenez et al., 1978). Martin (1977) reported as much as 33.5% of the MS-total ion intensity of fulvic acids was due to polysaccharide.

Py-GLC-MS spectra of soil and sedimentary fulvic acids exhibited a low concentration of phenols or benzenecarboxylic acids (<5% of total ion intensity, Saiz-Jimenez et al., 1978) which was unexpected based on the hydrogen-bonded model proposed by Schnitzer and Khan (1972). This low percentage of substituted aromatics for fulvic acid agrees with the findings of Anderson and Russell (1976). Tan (1976), however, found that pyrolysates of humic and fulvic acids yielded mostly a compound believed to be benzoic acid and o-hydroxybenzoic acid. Aromatic and polysaccharide structures were prevalent in humic and fulvic acid spectra obtained by pyrolysis-GC-MS (Wershaw and Bohner, 1969). Other reports indicate a preponderance of: (a) in humic acid-phenols, polysaccharide, protein and minor lignin peaks, (b) in humins and fulvic acid - more prominent polysaccharide peaks; (c) whole soil - most prominent peaks were due to polysaccharides, (pyrolysis - MS, Haider et al., 1977; Bracewell et al., 1976). This agrees with the most recent work of Martin et al., (1977, 1979) and Saiz-Jimenez et al., (1979). The latter authors reported also the major importance of polysaccharides (Py-GLC-MS) in humatomelanic acid

spectra.

Using Py-GC, Martin (1975a,b) reported that humic and fulvic acids differed not in the presence or absence of some compounds but in their amounts. Pyrolysis followed by GLC-MS of methylated fulvic acid (extracted with water and ultrasound) showed the presence of n-C<sub>16</sub> and C<sub>18</sub> fatty acid methyl esters; di, tri, tetra and pentabenzene-carboxylic acids and esters; and succinic acid (Skinner and Schnitzer, 1975).

(GC-MS)

Fungal melanin spectra<sup>^</sup> showed some similarities to soil humic acid spectra (Meuzelaar et al., 1977; Nagar et al., 1975). Composting straw humic acids showed decreasing lignin signals with increasing incubation time (Meuzelaar et al., 1977) in agreement with lack of lignin peaks in soil and humic spectra.

Some caution must be exercised in interpretation of these spectra since various anomalies may occur. For example, the mineral matrix can catalyze reactions (Kimber and Searle, 1970a, 1970b, Russel et al., 1974) and produce greater yields of cyclized and branched products in the C<sub>5</sub> to C<sub>9</sub> range from lipids (fats, waxes and hydrocarbons, Simmonds et al., 1969). In addition, alkene peaks are typical of lipids and not carbohydrates or protein (Simmonds et al., 1969) as listed by most authors herein.

Formation of aromatic compounds (toluene, benzene) via pyrolysis of unsaturated alkenes (aliphatic and/or alicyclic) and cellulose (benzene, aromatic acids) is well documented (Edmunds and Johnstone, 1965; Smith and Howard, 1937). Substituted benzene structures are

produced in high yields from pyrolysis of substituted polyvinyl-diene (Irwin, 1979).

(B) Humic-like Substances in Plants and the Humification Process

Humic acid-like precipitates may be prepared from decomposing plant residues such as roots (Zhigunor and Simakov, 1977) by using the classical procedure for isolating humic acid from soils, namely, 0.5N NaOH with acid precipitation. As root decomposition increased heterocyclic nitrogen increased in the humic acids isolated, the main contributors to the humic-like material were hemicelluloses and proteins. When  $^3\text{H}$ -triforine, (1,1'-piperazine-1,4-diyl-di-[N-2,2,2-trichloroethyl) foramide]), was incorporated into field grown barley (Rouchaud et al., 1979), most of the label in the straw of the mature plants was bound (88%) with hemicelluloses containing 58% followed by cellulose at 13%, acidic methanol at 8%, lignin (6%) and 3% forming part of a complex extractable with a neutral detergent. The importance of hemicelluloses and cellulose (71% of label bound) as precursors to humic acids (re. IR +  $^{13}\text{C}$ -NMR spectra, this thesis) and their humic acid behavior in binding is evident.

Visser (1964) studied the progressive humification of Papyrus plants. Humification was accompanied by increases in the phenolic character of the molecules and in the substitution of aliphatic side-chains on the phenyl rings, while the hydrophilic character of the molecule decreased over the period of humification. Infrared spectra of humic acids extracted before decomposition (fresh plants), and



after 20 months incubation, were virtually identical. These humic-like substances were prepared via the classical humic procedure with a pretreatment of exhaustive soxhlet extraction using petroleum ether, chloroform, and finally ether to remove lipids, glucosides, alkaloids etc. IR spectra of plant wheat tops humic-like substances were very similar to the IR spectra of soil humic acids (Swift and Posner, 1977).

Newly formed humic acids have been envisaged as having a relatively high aliphatic component, much of which is in the form of side chains associated with a dominantly aromatic core (Kimber and Searle, 1970b). Increased humification was associated with increases in fulvic acids and decreases in humic acid concentrations; as the amount of humic acid in the soil decreased, a larger proportion of the humic acid was combined with clay (Cheng, 1979). Recently formed humic material was found by McGill and Paul (1976) to be associated with mineral colloids. Even colloidal particles in natural aqueous systems are coated with humic compounds which bind metal ions (Davis and Leckie, 1978). Anderson (1979) reported that up to 50% of humus in Canadian soils was associated with clays.

An analysis of humic acids by Schnitzer (1976) revealed the following composition; 24% aliphatics in humic acid cf. 22% in fulvic acid, 20 and 30% phenolics, and 32 and 23% benzenecarboxylics respectively.

Extraction with 0.5N NaOH of partly decayed leaf and stem plant litter yielded materials very similar to soil humic acids (Given and

Dickenson, 1975). Acid hydrolysis of these humic-like products yielded a mixture of carbohydrates, especially xylose and uronic acids. Sauerbeck and Fuhr (1968) extracted fulvic and humic acids from undecomposed barley straw and rape shoots using the classical 0.5N NaOH solubilization followed by fractionation based on acid precipitation.

Kononova and Alexandrova (1973) also extracted humic acids from humifying leaves and roots of clover. IR spectra of fulvic, humic and humin fractions were almost identical to the same fractions from a soil. Soil humic acids showed greater concentrations of aromatic nuclei and carboxyl groups, a higher degree of condensation, carboxyl content, and more uniform structure.

Humification of raw plants (clover and wheat) showed little change in aromaticity in the humic acid-like products (Swift and Posner, 1977). The conclusion was reached that humic acid-like materials from fresh plant debris must be formed rapidly and be complex. It is also evident, however, that some components of fresh plant material are incorporated into humic acid molecular structure.

Pectin may be considered part of the complex hemicellulose group of plant natural products (Hellendorn, 1978). Pectin is released from protopectin by treatment with mild acid or alkali (Campbell and Palmer, 1978) and so would also be solubilized as part of the base soluble organic matter of soil via the classical humic extraction scheme. Such pectin is composed mainly of galacturonic residues with branches of typical hemicellulose saccharides (arabinose, galactose,

xylose).

Approximately 35% of wheat straw is composed of hemicellulose (Robertson, 1978). The involvement of hemicellulose in the formation of humic acids is thus possible; especially in prairie mineral soils where cereal crops are grown extensively and much of the straw remains on the soil.

The extraction of wood with 4% NaOH followed by the addition of excess acetic acid to the alkaline filtrate produced a precipitate which Williams and Bevenue (1956) termed hemicellulose. This is an analogous procedure to the classical humic acid preparation scheme from soil.

#### B.1 Soil carbohydrates - especially cellulose and hemicellulose

The term hemicellulose was first applied by Schultze (1892) to a product which he obtained from cereals with dilute alkali treatment and precipitation of the extract by acid. The product was also easily hydrolyzed to give a mixture of sugars.

Gupta and Sowden (1967) found 0.3 - 1.9% of the organic matter of several soils was present as cellulose. Holocellulose in peat soils accounted for 25-35% of the total organic matter. Hydrolysis of the cellulose yielded 65-82% glucose and up to 9% of four other sugars, xylose, arabinose, galactose, and a trace of mannose. Sediments are also high in cellulose since 2-39% of algae cell mass is cellulosic and settles to the bottom of lakes (Rho and Litsky, 1979).

On the average, ca. 10% of soil organic matter is carbohydrate

(glucose, galacturonic and glucuronic acids, xylose, arabinose, galactose in decreasing order of abundance in a Scottish arable soil) Cheshire (1977); 20-40% of soil glucose may be in cellulose (Gupta and Sowoden, 1964) while soils rich in plant remains have been found to be rich also in arabinose, xylose and glucose (Oades, 1972). Arabinose and xylose may account for 18-50% of the total soil sugars depending on soil type.

Arabinose and xylose polysaccharides persist and accumulate more readily than other polysaccharides (Cheshire, 1977; Murayama et al., 1979). Soil polysaccharide is very stable (clay bound; Lynch and Cotnoir, 1956), not in solution in the soil (Cheshire, 1977), polydispersed (<4000->200,000 M.Wt.) (Ogsten, 1968; Swincer et al., 1968), and binds metal cations (Martin et al., 1966). 0.2M NaOH extracted 42% of the total polysaccharides from a sandy loam soil (Cheshire, 1977). Anderson et al. (1977) isolated appreciable amounts of acidic polysaccharides some resembling pectic acid. Pectins are abundant in plant leaf structures and are produced by cellulolytic soil bacteria which form a variety of bacterial products such as pectin-like slime which is thought to play an important role in the formation of humics (Donath and Lissner, 1972). Wheat leaves contain cellulose (30%), hemicellulose (11%), and pectin (22%) (Burstrom, 1958) cf. rye straw containing 21% hemicellulose and 39% cellulose; after 3 years of decomposition, 20 and 28 % respectively remains (Waksman and Reuzer, 1932).

Hemicelluloses generally contain pectin (galacturonic acid),

xylose, arabinose, galactose and cellulose (Gray and William, 1971; Shorey and Martin, 1930; Waksman and Reuzer, 1932). A podzol A horizon soil was analyzed by Anderson et al., (1977) and reported to contain 23.5% galactose and 7.5% uronic acid in the ash-free fulvic acid isolated from it. Total carbohydrate content was 66.1% (excluding uronic acid). Clark and Tan (1969) reported that the humatomelanic acid soil fraction was a naturally occurring ester compound of humic substances bonded by ester linkages to polysaccharides. Carbohydrates in other soil humic fractions ranged between 60-70% humin, 2-23% humic acid, and 17-35% fulvic acid - a red brown earth, for example, contained 61, 11.5 and 28% carbohydrates respectively (Oades and Swincer, 1968). Dormaar (1967) reported for a chernozem soil, 43% carbohydrates for the fulvic acid fraction; 7% in humic acid and 50% in humin.

Cheshire (1977) has studied soil polysaccharide interactions with humic acids. He reported that humic acid may be polyphenolic in character (characterized by its sorption by PVP) and so tanning reactions occur with protein, cellulose, and pectin. At low pH virtually no release of polysaccharides from soil was evident, but base treatment (0.2M NaOH) released 42% (69% using 0.2M NaOH and sonification).

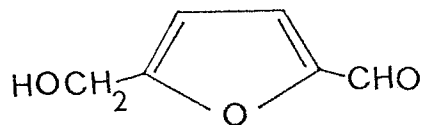
Total monosaccharides were determined in prairie soils and found to account for 24% of the total organic carbon; pentoses were more abundant cf. hexoses (Folsom et al., 1978).

#### B.1.1 Action of acid and base on carbohydrates

Plant carbohydrates are important precursors to soil humic substances. The purpose of this section is to illustrate how these carbohydrates are possibly affected by acid or base treatments used to isolate and fractionate humic substances.

Gupta and Sowden (1967) fortified soil with [ $^{14}\text{C}$ ] glucose and upon extraction with 72% acid<sup>(aq.)</sup> could recover only 55% of the glucose; the same acid treatment of glucose above-yielded only 48% glucose demonstrating the profound degradation of glucose by acid, even in soil.

In slightly acidic (pH 6.5) solution, glucose is largely degraded to I, 5-(hydroxymethyl)-2-furaldehyde (Feather and Harris, 1973;

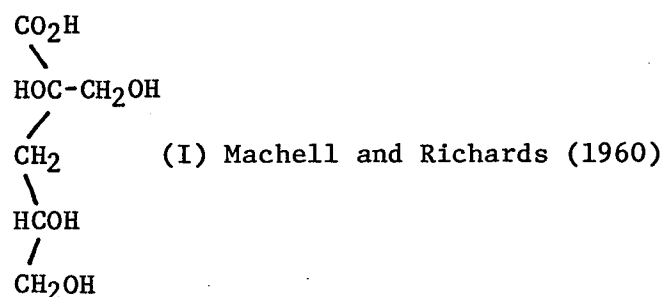


I

Ollson et al., 1977, 1978); Popoff and Theander (1970, 1972, 1976) reported the formation of aromatic compounds from treatment of d-gluconic acid, d-xylose and d-galacturonic acid (separately) in slightly acidic (pH 3.5) conditions. Products identified included: dihydroxybenzoic acids, dihydroxybenzenes, heterocyclic chromones, reductic acid, 2-furoic acid and 2-furaldehyde (furfural). Although yields were low (3.5% or less) the yields may be much greater in the presence of clays etc. Several aromatic products were produced from the slightly acidic treatment of glucose or fructose (Popoff and Theander, 1976). Products identified included: 5-(hydroxymethyl)-2-

furaldehyde, 1-(2-furyl)-2-hydroxyethanone, levulinic acid, formic acid and traces of di- and tri-hydroxybenzenes, as well as, di-hydroxybenzoic acids.

Solutions of glucose or xylose in 0.63M NaOH resulted in degradation to a large number of phenols and pentenones (e.g., pyrocatechol, methylbenzene diols, dihydroxybenzaldehydes and variously substituted hydroxy- and hydroxy-methyl acetophenones, Forskahl et al, 1976). Treatment of uronides and pentosans, found in soil, with 12% HCl produces 12-35% furfural (Shorey and Martin, 1930; Waksman and Reuzer, 1932); cereal straw hemicelluloses contain mostly pentosans (and some resistant uronides) accounting for up to 28% of total organic carbon in soil (Waksman and Reuzen, 1932). Alkaline degradation (0.05 N NaOH, 25°C) of partially substituted glucose containing reducing sugar and cellulose gave high yield of d-glucoisosaccharinic acid (I) (also formic acid, glycollonic acid and  $\beta$ -OH- $\gamma$ -butyrolactone)



Thus, the use of acid and/or base to isolate and extract humic substances from soil may cause formation of aromatic structures from the indigenous carbohydrates, and so artificially increase the contribution of aromatic structures in humic substances.

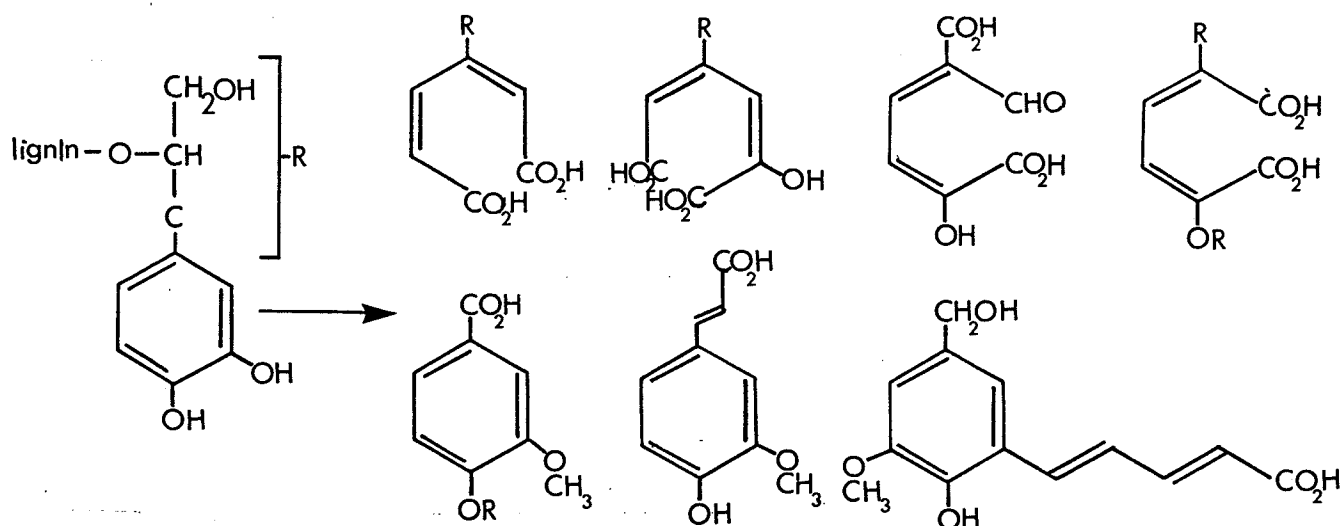
## B.2 Fatty acids and other soil lipids

The importance of aliphatic structures in humic substances may be related to such in situ processes as ring opening reactions of lignin, carbohydrates and other aromatic or alicyclic natural products. Aliphatic acids are also present in the soil from important natural sources such as plant suberin and cutin.

The surface activity of alkali humates suggests that the humic acid molecule must contain hydrophilic as well as hydrophobic parts. These parts probably contain polar functional groups (OH, CO<sub>2</sub>H, NH<sub>2</sub>, etc.) and nonpolar hydrocarbon groups (CH<sub>3</sub>, CH<sub>2</sub> etc.) (Tschapek and Wasowski, 1976). The hydrophobicity of humic acid particles may be due to substances like waxes coating the humic acid particles or the presence of nonpolar groups in humic acid. Since ethanol, benzene, or a mixture of these does not change the surface tension or hydrophobicity, the latter proposition is the preferred explanation (Tschapek and Wasowski, 1976).

Lignin is readily decomposed by soil fungi and recently several bacteria have been shown to be important decomposers also (Crawford, 1979). Typical decomposition reactions include ring cleavage, and oxidation products:





Such lipid-like structures could thus be part of the soil organic matter structure. The significant contribution of bacterial lipids in sediments (10-20 mg/g sediment) and of yeast or fungi lipid content may be as high as 50% by weight (Perry *et al.*, 1979). The main long chain fatty acids found were C<sub>16</sub> and C<sub>18</sub> monounsaturated (C<sub>16</sub>) and polyunsaturated (C<sub>18</sub>) acids and dicarboxylic acids from vegetation (Mendez and Ogner, 1979).

Cutin and suberin are dominant lipids in plant leaf membrane tissue, especially. Cutin is readily broken down by soil fungi and bacteria to free fatty acids (Heinen and De Vries, 1966) with C<sub>16</sub> and C<sub>18</sub> fatty acids predominating. Cutin is insoluble in organic solvents, resistant to strong mineral acids, but readily attacked by alcoholic alkali solution (Baker *et al.*, 1964), and leaf membrane tissue is composed of cutin (70%) and of fatty acids (15%). Pectin and cellulose are also found with cutin, the pectin containing arabinose, galactose and galacturonic acid (Huelin, 1959).

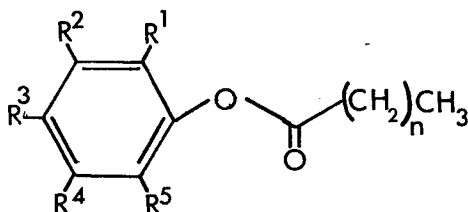
Cutin acids were highly stable as shown by their occurrence in fossil sediments (Eglinton et al., 1968), river shale (McIntosh, 1968) and brown coal (Hunnerman and Eglinton, 1972). Algae contain C<sub>16</sub> and C<sub>18.2</sub> as the most abundant fatty acids and wheat C<sub>16</sub> and C<sub>18.3</sub> (Hitchcock, and Nichols, 1971).

As much as 20% (organic soils) or 5% (mineral soils) of soil humus may occur in the form of lipids (paraffin hydrocarbons, phospholipids, fats, waxes, fatty acids, and terpenoids); Stevenson (1966). This does not include physically and covalently bound lipid forms which may raise the % lipid contribution considerably.

Oxidative CuO-NaOH treatment of chernozem soil humic acid yielded considerable amounts of aliphatic carbon predominated by C<sub>16</sub> and C<sub>18</sub> fatty acids (Schnitzer and Vendetti, 1975).

Stuermer and Harvey (1978) subjected marine fulvic acid to a reductive sequence of reactions to transform polyfunctional molecules to hydrocarbons. The products of the scheme indicated that significant (3% of fulvic acid carbon) amounts of lipids were incorporated into the humic substance structure. Seawater fulvic acid is highly aliphatic and of low aromaticity as indicated by the high H/C ratio of 1.7 and by <sup>13</sup>C and <sup>1</sup>H-NMR spectra (Stuermer and Harvey, 1974; Stuermer and Payne, 1976). They also found even-numbered n-alkanes C<sub>12</sub>-C<sub>30</sub> with C<sub>18</sub> predominant, as well as alkylbenzene of varying alkyl chain lengths, C<sub>9</sub>-C<sub>12</sub>. Their reduction method did not reduce aromatic rings, only esters, acids, aldehydes, ketones and olefins.

Tightly bound lipids (Schnitzer and Neyround, 1975; Serra and Felbeck, Jr., 1972) must be released by more energetic extraction methods. Thus 5% of fatty acids in HA were termed "loosely bound" and 95% "tightly bound" cf. 25% and 75% for FA (Schnitzer and Neyround, 1975). Since phenolic acids are also released by the more energetic extraction (saponification) at a ratio of 0.76 (HA) and 1.90 (FA) for phenolic acids to fatty acids, respectively, Schnitzer and Neyround (1975) concluded that, in humic substances, fatty acids react with phenolic-OH groups to form hydrolyzable esters of the type shown below:



Thus, they stated that the small portions of fatty acids loosely-held are possibly physically adsorbed on the humic surfaces, and in internal voids; however most of the fatty acids form esters with phenolic humic "building blocks." Aliphatic-alicyclic structures may thus constitute an important contribution to the "backbone" structure of humic substances, but because of the varying yields of aromatic components reported by various instrumental and isolation techniques a conclusive assignment to humic structures is tenuous.

### (C) Humic and Fulvic Acid Physicochemical Properties

It is well known that humic acid fractions form molecular aggregates in solution (Wershaw and Pinckney, 1977) and the size of

these aggregates as determined by x-ray scattering is a function of pH (Wershaw and Pinckney, 1973). Wershaw and Pinckney (1977) also showed that the size of aggregates in solutions can be changed by oxidation with air, especially basic aqueous solutions (Meikle et al., 1976; Slawinski and Slawinski, 1975a, 1975b).

The radius of gyration of a particle, which is defined as the root mean square distance of the electrons in the particle from the center of charge, is a useful general parameter of comparative molecular or particle size (Wershaw and Pinckney, 1977). In a system in which all the scattering of particles are of equal size, the Guinier plot (logarithm of scattered intensity of x-rays versus scattering angle<sup>2</sup> X 10<sup>4</sup>) will be a straight line. In a polydisperse system, the Guinier plot, which is actually a summation of many Guinier plots, is curvilinear-concave up.

A Guinier plot of a polydisperse system of particles of uniform electron density will yield the range of particle sizes present in the system. Changes in aggregation sizes were thought to be the result of changes in hydrogen bonding between humic particles - intermolecular hydrogen bonding, which would cause aggregation of the molecules into larger particles and intramolecular resulting in molecules assuming more compact configuration. In aqueous base solutions (Na salt) at pH 4.7, a polydispersed system was indicated (1.36-2.06 nm). Near pH 9 the solution was essentially monodisperse with particles of radius of gyration of 1.3 nm. Bubbling of O<sub>2</sub> into this system reduced the radius of the particles to 1.1 nm. The

absence of evidence for larger particles in pH 9 solution indicates that there is less aggregation of the particles in basic solutions, probably due to a lack of intermolecular hydrogen bonding between acid (esp. carboxylic) groups in basic solutions. Any inhibition of hydrogen bonding intermolecularly may also reduce intramolecularly hydrogen bonded particles, and thus decrease their compactness.

Wershaw and Pinckney (1977) conclude by stating that humic acid interactions with other components are surface-adsorption mediated or incorporated into the humic acid aggregate. Humic acid molecules form molecular aggregates in solution that appear to be held together by hydrogen bonds and other weak chemical bonds.

Electron microscopic and x-ray analysis of isolated soil fulvic (Schnitzer and Kodoma, 1975; Kodoma and Schnitzer, 1967, respectively) indicates that the carbon skeleton of fulvic acid consists of a broken network of poorly condensed aromatic rings with appreciable numbers of disordered aliphatic chains or alicyclic structures around the edges of aromatic layers. Thus, a fulvic acid has a relatively "open" structure which allows for the presence of a large number of voids. Schnitzer and Khan (1972) proposed that fulvic acid consists of phenolic and benzene carboxylic acids joined by hydrogen bonding and Van der Waal's forces to form a stable polymeric structure. Such a structure would be expected to be sensitive to changes in pH, salt concentrations and valence of cations. Finally, they conclude that such a structure, punctured by voids or holes of different dimensions, could trap or fix organic molecules.

Electron micrographs of fulvic acid (Schnitzer, and Kodoma, 1975) prepared at pH 2.5 showed the presence of elongated, irregularly shaped aggregates ( $20 \times 10^3 \text{ \AA}$  in length) perforated by voids  $200\text{--}300 \text{ \AA}$  in diameter. The smallest particles were spheroids,  $15\text{--}20 \text{ \AA}$  dia., which tended to form aggregates ranging from  $200\text{--}300 \text{ \AA}$ . At the natural pH of fulvic acid, pH 3.5, a sponge-like structure,  $100\text{--}300 \text{ \AA}$  thick, perforated by voids  $200\text{--}1,000 \text{ \AA}$  was evident. Particles  $20\text{--}30 \text{ \AA}$  in diameter were observable - probably corresponding to molecules [Mn = 951 (Kodoma and Schnitzer, 1967)] rather than aggregates. At higher pH, fulvic acid is highly dispersed and one observes flat, sheet-like, very thin lamellae perforated by voids,  $200\text{--}2000 \text{ \AA}$  in diameter.

Humic acid molecular weight is very close to that of fulvic acid ca. 1300 (Visser and Mendel, 1971) cf. 951 (Kodoma and Schnitzer, 1967) (both determined "dry") and their only difference chemically is a greater number of carboxyl groups in fulvic acids which may permit one to infer a similar remaining structure for humic acids. In regard to the dispersed fulvic acid (FA) micrograph (Schnitzer and Kodoma, 1975), Camier and Siemon (1978a,b) reported similar conclusion for x-ray diffraction results on brown coal. Humus material is genetically related to brown coal (Camier and Siemon, 1978a,b). Scheele (1937) had determined a molecular weight of 26,000 for brown coals, this being almost identical to an average found by various authors for humic acids reported by Visser & Mendel, (1971). Later workers (Agde et. al., 1942) found a m.wt. of 1200-1600 for brown



coals and concluded as did Visser et al. (1971), that Scheele measured micelles and there must be ca. 20 molecules per micelle [cf. 25 molecules, Visser et al., (1971)].

Camier et al., (1979) most recently described the physical structure of brown coal. A humic acid fraction isolated as a dark brown suspension did not settle out after standing for 6 months. The slurry was centrifuged at high speed and the sedimented material showed the presence of closely sized particles. They concluded that the persistent appearance of the same size groups in all tests at different pH (NaOH extracting solution) and with different coal types suggested that some fundamental unit was present. Microscopic examination revealed cylindrical rods about 0.9  $\mu\text{m}$  in dia. and 26.8  $\mu\text{m}$  long were common. Larger particle sizes which could have been accretions of these basic units and a number of agglomerations were noted. The rods were arranged side by side, close packed in bundles. It appeared that the alkali "peels" these rods from the coal mass and they subsequently agglomerate in solution, doubling in volume at each coalescence. The rods were rich in carbon and hydrogen cf. the original coal. Such rods were thought to be derived from plant cell remains or precipitates formed from the HA groundmass and represent a further step in the coalification process. Their model of coal structure envisages a gel of HA molecules swollen by waters incorporating particulates. These include rods and detrital matter like pollen, cell remains, etc. held together within the HA macrostructure. They conclude that brown coal can be regarded as a gel of HA

which incorporates larger particles bound by nonregenerable bonds. Thus such particles may correspond to the sources of aromatic constituents in soil HA.

Cartz and Hirsch (1960) described coals of carbon content less than 85% (Victorian brown coals = 67% carbon) as having randomly oriented lamellae interconnected by cross-links in all directions. This appears similar to the dispersed lamellae structure identified for fulvic acids by Schnitzer and Kodoma (1975). Such lamellar, micellar and amorphous structures would surely behave as a permeable matrix capable of complexing organic molecules as inclusion complexes. Such an included molecule would be permanently trapped unless the structure were deformed or perturbed, facilitating release and hence extraction. Pollack et al., (1971) also, found a humic acid unit diameter of ca. 15 Å agreeing well with Schnitzer and Kodoma (1975) for fulvic acid (15-20 Å).

Shapiro (1957) reported that water soluble yellow organic acids were aliphatic, unsaturated, polyhydroxy, dicarboxylic acids with an approximate m wt of 456. No evidence was found for aromaticity. Thus, fulvic acids isolated using base (Schnitzer and Kodoma, 1975) may be the result of polymerization of aromatized acids described above or as indicated before derived from breakdown of HA (Meikle et al., 1976) by the action of base.

The smallest humic acid particle detected by Wershaw et al., (1977a) using x-ray scattering techniques was 0.7 nm (radius of gyration) or 1500 m wt.



Wershaw and Pinckney (1971) found that at increasing pH the concentration of smaller particles (or smaller aggregates) of humic acid (as a  $\text{Na}^+$  salt) increased. Only at pH 7 was a solution in water described as monodisperse. Even at pH 11.5 some large particles were present in a humic acid fraction (separated on Sephadex G-50) (Wershaw and Pinckney, 1973a; 1973b). Wershaw et al., (1970) studied small angle x-ray scattering of sodium humate fractions and reported radii of gyration of 494 A and 159 A for a curvilinear Guinier plot. They concluded that this fraction was made up either of molecules of two different sizes or of "zoned" molecules of a single size.

Rajalakshmi et al. (1959) studied the viscosity of sodium humate solutions and determined that the results obtained were characteristic of the behavior of a flexible (linear, Ramchandran et al., 1973) polyelectrolyte (ca. 80% dissociated) and suggested that the sodium humate molecule or aggregate must have many single bonds in its structure so as to permit the conformational changes they observed.

The molecular weights of humic acids (Mukerjee and Lahiri, 1956) lie in the range from 300 to 1200. In solution, a large number of units may be linked together by hydrogen bonding forming a polymer chain-like structure; thus, molecular weights determined via humic acid solutions give rise to much higher weights (26,000 or greater) (Mukerjee and Lahiri, 1956). Thus a coiled chain-like polymer could exist (Mukerjee and Lahiri, 1956) which would easily provide voids suitable for permeation of organic molecules with subsequent

entrapment as inclusion complexes (micelle : intermicellar, as well as, in intramicellar capillaries).

### C.1 Colloidal chemical properties of humic substances

The colloidal physicochemical properties of humic substances have recently been reviewed (Van Dijk, 1971; Giesecking, 1975). It became generally accepted that for many organic substances the sol state represents principally an equilibrium state reached spontaneously by dissolution of the dry colloid in an appropriate solvent (Van Dijk, 1971). The particles may be coiled molecular chains forming a three dimensional cross-linked matrix, and may be present as more-or-less swollen, loosely built structures. An electric charge on the particles generally originates from ionization of acid or base groups regularly distributed throughout the particles. This ionization often causes an extra expansion of the particles as a result of neutral repulsion of equally charged groups and usually promotes dissolution. For these colloids, flocculation can often simply be explained by a repression of ionization.

Studies on the colloidal properties of humic substances have mostly been performed on humic acids (extracted from peat or mineral soils), since fulvic acids generally have a particle size below the colloidal range. Colloid chemical investigation of humic acids have been performed mostly on sols or suspensions and comprise molecular weight determinations and fractionation by partial flocculation, chromatography, and electrophoresis (Schnitzer and Khan, 1972;

Kononova, 1966; Van Dijk, 1971). In general, these studies showed that humic acids represent polydisperse systems with a wide range of molecular weights varying from 1000 to more than 100,000 and are amorphous, disk or flat ellipsoid shaped particles having a more or less open, spongy structure (Van Dijk, 1971; Giesking, 1975; Dudas and Pawluk, 1969/1970).

Humic acids may be considered as solutions of macro ions or negatively charged hydrophilic colloids and usually exhibit properties generally attributed to this type of colloid. The coagulation of humic acids was found to be most sensitive to trivalent metal ions and decreased in the following order:  $\text{Al}^{3+} > \text{Fe}^{3+} > \text{Cu}^{2+} > \text{Zn}^{2+} > \text{Ni}^{2+} > \text{Co}^{2+} > \text{Mn}^{2+}$  (Khan, 1969). The lower molecular weight fractions complex 2-6 times more metals than higher molecular weight fractions, per unit weight (Rashid and Leonard, 1973). The coagulation of humic acids depends on the pH and the ionic strength of the solution. In the absence of salts, virtually complete peptization occurs at pH 3.0, an increase in ionic strength raises the pH of peptization to 4.5-5.0. Peptization usually occurs at a somewhat higher pH than coagulation, possibly because of association of humic acid particles by hydrogen bonding (Schnitzer and Khan, 1972).

### C.2 Free radicals in humic substances

A unique feature of soil humic substances is the relatively high content of stable free radicals, probably of the semiquinone type

(Steelink and Telkin, 1967; Wilson and Weber, 1977). These free radicals may be of considerable importance in explaining the effect of humic substances on plant respiration and growth, biological electron transport processes in soil, catalytic polymerizations, and the formation of complexes with such substances as transition metal ions, small organic molecules, and oxides (Stevenson, 1972). Hsu and Bartha (1974a), however, could find no evidence for the involvement of soil free radicals in interactions with 3,4-dichloroaniline. Their importance will be illustrated later in regard to  $^{13}\text{C}$ -NMR metal induced relaxation mechanisms.

#### D. Interaction of Xenobiotics with Soil Organic Matter

The adsorption of pesticides (esp. herbicides) by humic substances is a key factor influencing the behavior of herbicides in soil. Numerous examples of a direct effect of humic content on the bioactivity and persistence of these compounds can be found in the reviews of Bailey and White, (1970) Hayes (1970), Stevenson (1972), Upchurch (1966), Wolcott (1970) and Weber (1972). All the evidence indicates that the higher the organic matter content in a soil the higher the rate of herbicide application necessary to maintain a given level of herbicidal activity, since complexation phenomena render some of the herbicide biologically unavailable and therefore inactive and relatively persistent.

Three major types of complexing or interacting surfaces are

normally available to the pesticide: organic (humus), inorganic (clay, and iron, manganese and aluminum oxides), and organic-inorganic complexes. Generally, pesticides have a greater affinity for the organic than for the mineral surfaces. In some soils (peats) the organic fraction is the only surface of significance. When the humus content of the soil exceeds about 8 percent, only the organic surfaces will normally be available for interaction (Stevenson, 1972b). Below this value, the clay will become increasingly important. The character of the organic and inorganic colloids of the soil, and their interactions, determine the nature, availability, and total area of the potentially reactive surfaces (Saltzman et al., 1972). For soils having similar textures and organic levels, the sorptive capacity for herbicides follows the order: montmorillonite > illite > kaolinite. Stevenson (1972b) has shown diagrammatically some of the possible interactions of organic (humic and fulvic acid) with inorganic (clay) fractions of soil based on experimental evidence of other researchers, especially pertaining to soil organic and inorganic fractions interacting with metal cations (Fig. 5). The various mechanisms proposed for the adsorption of pesticides, especially those which are reversibly adsorbed (extractable), include hydrogen-bonding, van der Waals forces, hydrophobic bonding, and ion-exchange, while nonextractable (irreversibly adsorbed) include charge-transfer complexation, inclusion phenomena, and, possibly, covalent bonding, the predominant form of which varies with the physicochemical properties of the pesticide and the amount of organic

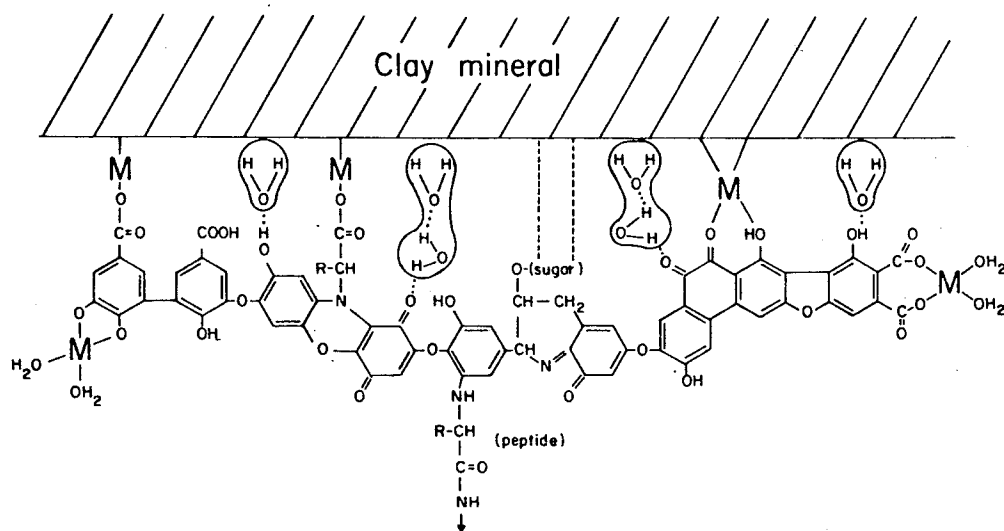


FIGURE 5. Interaction of soil organic and inorganic fractions (Stevenson, 1972b).

matter and inorganic matter in the soil. These have all been reviewed in detail (Bailey and White, 1970; Schnitzer and Khan, 1972; White and Mortland, 1970) except inclusion which will be discussed later.

Czuchajowska (1977) reported that linuron [3,(3,4-dichlorophenyl)-1-methoxyl-1-methylurea] adsorbed to whole soil via hydrogen bonding. The existence of hydrogen bonding was confirmed by the shifts in specific absorption maxima in the IR spectra of the soil-linuron system.

Strzelec (1977) reported that soil treated with atrazine showed decreasing degradation with increasing soil ion exchange capacity. In soils with high organic matter content, larger amounts of the herbicide were apparently bound by the organic colloids, which bind the sorbed ions less closely than the mineral colloids.

Terce and Calvet (1978) studied the sorption of several herbicides by clays. Montmorillonite had, generally, a greater adsorption capacity cf. kaolinite and illite. They concluded that their results strongly indicated the adsorption was probably mainly due to surface impurities such as Al or Fe oxyhydroxides. Bonding mechanisms discussed include: ionic binding via protonation at low soil pH or hydration water at clay surfaces; polar and non-polar forces via aluminum in allitic soils, in which the surface aluminum cations can interact with organic molecules through coordination bonds, and, finally, hydrogen bonding via water molecules on the clay surface or surface hydroxyl groups of the clays. Trisubstituted phenylureas

(chlortoluron, isoproturon and metoxuron) were not adsorbed at all, at any pH.

Hankawa (1974) showed that aldrin and dieldrin, two hydrophobic insecticides, were retained at higher concentrations in a soil containing 15.2% humus cf. one at 1.9%. This might suggest that these nonpolar, hydrophobic, lipophilic pesticides are retained by an inclusion phenomenon, especially once methylation of HA releases what were nonextractable residues (Schnitzer, 1971; Ogner and Schnitzer, 1970a, 1970b, 1971; Schnitzer and Ogner, 1970; Khan and Schnitzer, 1971a, 1971b, 1972a; Schnitzer and Ortiz de Serra, 1973; Kodoma and Schnitzer, 1967).

Khan (1974) reported that the interaction of HA with chlorinated phenoxyacetic and benzoic acids appeared to be complexed by a mechanism that involves adsorption on large surfaces provided by the HA in addition to firmer retention in internal spaces of its structure. Six per cent of the applied compounds (2,4-D and picrolam) were bound to the humic acids used. The HA bound residues were methylated and reextracted with hexane; ca. 60% of the retained herbicide was extracted with hexane after methylation. Considerable amounts of hydrophobic organic compounds such as alkanes, fatty acids, adipates and dialkyl phthalates were also extracted from methylated HA and FA (Matsuda and Schnitzer, 1971; Khan and Schnitzer, 1972a, 1971a, 1971b; Schnitzer and Neyroun, 1975; Ogner and Schnitzer, 1970a, 1970b; Schnitzer and Ogner, 1970).

Schnitzer sees the fulvic acid as a hydrophilic hydrogen bonded



structure of variously substituted hydroxybenzene and/or benzene-carboxylic units. Such a structure could adsorb hydrophobic compounds. Larger amounts of the monomers could be released only upon exhaustive methylation indicating the high stability of such a structure. Schnitzer's fulvic acid model contains monomers cross-linked by hydrogen-bonding such that "holes" exist with internal spaces like a molecular sieve ("sponge"). Organic and inorganic compounds are envisaged as being surface adsorbed or retained more firmly in the internal spaces, and held until the superstructure is broken as by methylation (Kirkland, 1973). Electron microscopic examination of humic acid revealed the presence of small spherical particles having "spongy" appearances (Dudas and Pawluck, 1969/1970).

It was suggested that fulvic acid (Khan and Schnitzer, 1971b) and humic acids (Chipalkatti et al., 1954) are made up of phenolic and benzene carboxylic acids joined by hydrogen bonds to form a molecular sieve-type polymeric structure of considerable stability on which hydrophobic organic compounds would adsorb. The structure appeared to be weakened by methylation which is known to reduce hydrogen bonding between monomers or units, if micellar, and thus permit the release of compounds that are adsorbed. The type of structure envisaged is shown below (Fig. 6). One of the characteristics of the structure is that it contains voids or holes of different dimensions which can trap or fix organic molecules. Unpublished support (Schnitzer) for such a mechanism comes from the same author's data

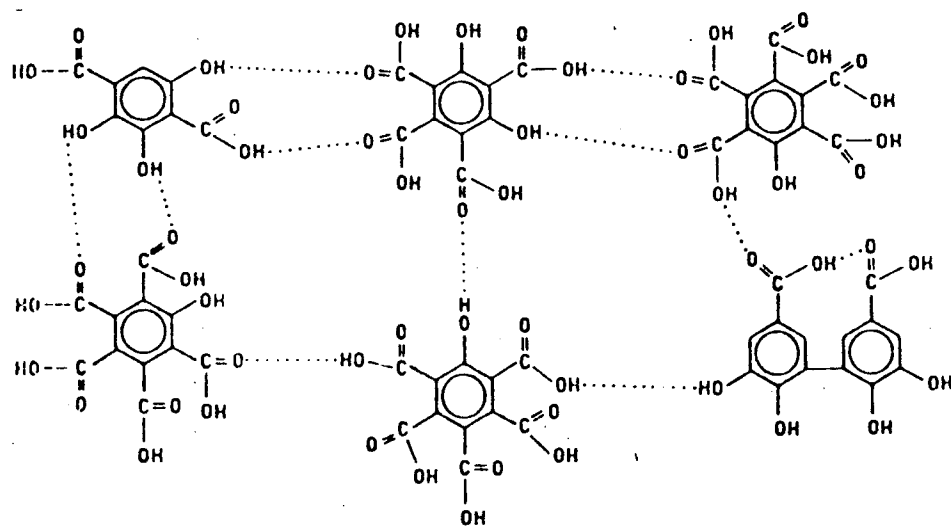


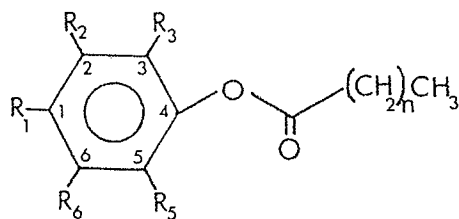
Figure 6. Structure of FA as proposed by Schnitzer (1976).

indicating that considerably more DDT was extracted from methylated humic substances.

Schnitzer and Neyround (1975) studied the extractability of bound alkanes and fatty acids in humic substances. Several methods were investigated for HA and FA complexes. Hydrolysis with water at elevated temperature (170°C for 3h) and methylation were the most effective treatments (extraction after pretreatments using hexane and EtOAc) for extraction of alkanes from HA. Ultrasonic dispersion and water hydrolysis were most effective with FA. By far the most efficient pretreatment for extraction of fatty acids from HA and FA (saponification) was 2N NaOH at 170° for 3h. Approximately 10% of the weight used were fatty acids and 60-80% of this were C<sub>16</sub> - C<sub>18</sub> carbon units. On the basis of the less effective pretreatment, ca. 50% was described as loosely held and 50% tightly bound for HA, and 25/75% for FA. Although the HA and FA were obtained from soil samples 3000 miles apart fatty acid composition and % extracted were virtually identical.

Methylation pretreatment failed to increase the amount of fatty acids extracted over other methods. The reason for this was given as follows: (1) the fatty acids were firmly sorbed (via H-bonding and Van der Waal's forces) on external surfaces and in internal voids of HA and FA's and (2) the fatty acids were bound by ester linkings to hydroxyl groups of phenolic acids which were thought to be the building blocks of HA & FA (Khan and Schnizer, 1972b; Ogner and Schnitzer, 1971; Schnitzer and Ortiz de Serra, 1973). Support of the

second theory was strengthened by the identification after base hydrolysis of a number of phenolic acids; the structure envisaged is shown below;  $R_1-R_6 = CO_2H, OH, OCH_3$  or  $H$ .



Hydrophilic humic substances, HA + FA, can combine by absorption, adsorption, inclusion and combinations, thereof, with organic compounds to form stable bound complexes that are recalcitrant to all but the most severe extraction conditions. The proposed structure is quite loose or open in accord with the results of x-ray analysis, which indicate a broken network of poorly condensed aromatic rings for FA (Kodoma and Schnitzer, 1967). Such a model was based on monomers isolated from nondegradative studies.

Turski and Steinbuch (1971), and Sullivan and Feldbeck (1968) studied interaction of triazines with HA and found them to influence the IR absorption of the HA carboxyl groups indicating the reactivity of amino groups of triazine herbicides. They concluded that these amino groups are fundamental to the formation of complexes, HA-herbicide, and that the site of binding also depends on the inner

structure of the HA. Turski and Steinbrich (1971) also showed that these were perturbations in the aliphatic region of the IR spectrum of triazine \* HA complexes (esp. re. HA from chernozem soils).

Farmer and Ahlrichs (1969) found that phenylureas were not adsorbed in the interlayer space of montmorillonite so that such clay adsorption must be attributed to an interaction of the carbonyl of the urea moiety with exchangeable cations on external surfaces and edge aluminum groups. Parathion however, was shown to be interlamellarly sorbed by a montmorillonite clay (Cheung et al., 1979). Such adsorption would be small (Bartha, 1971) and difficult to detect by IR. By analogy, chloroanilines may interact via the lone pair of electrons on the nitrogen and account for loosely adsorbed chloroanilines. Infrared spectra of bound [phenylurea, Shuie and Wang (1977)] humic acid - pesticide complexes indicated degradation products (probably the corresponding substituted anilines) containing NH or NH<sub>2</sub> probably combined with carbonyl groups of the humic acids through hydrogen bonding. Ionic bonding with NH of the degradation product or parent pesticide and COO<sup>-</sup> groups (carboxylate anion) was also indicated. Thus, they concluded chemisorption was indicated as a mechanism of binding.

Mueller-Wegener (1977a, 1977b) reported that several triazine pesticides -ametryne, atrazine, and prometryne- were bound to soil humic acids by electron-donor-acceptor complexes, the lone pair of electrons on the heterocyclic nitrogens being accepted by the humic acid via a positive charge arising as a result of chelation with

polyvalent metal cations. Usually greater complexing by clays is related directly to higher valency of the predominant metal cation (Khan, 1974b; Hance, 1969). Charge-transfer complexes are molecular complexes; the distance between the interacting charged centres being within  $2-4\text{\AA}$  in all cases (Porath and Larson, 1978).

It is known that humic substances enter into charge-transfer complexation reactions (Wershaw et al., 1977). Polynitro groups, phenols, and quinones of the humic acid (as well as bridged metal cations mentioned above) may also act as electron acceptors. Adsorption by charge-transfer complexation is characterized by the donor molecule (usually the adsorbate) donating an unshared electron pair (n-donor) or a pair of electrons in a  $\pi$  orbital such as an aromatic system ( $\pi$ -donor) to an acceptor molecule. Donor sites for this mechanism may be stereochemically limited so that this behavior is that of an acceptor.

Charge-transfer (C-T) complexation would be favored by highly delocalized ionic organic adsorbates. Adsorption with ionic adsorbates may be almost irreversible even against elution with dilute base (Thurman et al., 1978). Thermodynamic parameters for C-T complexes are typically negative (for eg.,  $\Delta G^\circ = -1$ ,  $\Delta H^\circ = -3$  kcal mole<sup>-1</sup>, and  $\Delta S^\circ = -10$  cal deg<sup>-1</sup> mole<sup>-1</sup>; Lotli and Roberts, 1979).

#### D.1 Relationships Between OM and clay to Adsorption of Pesticides

Wahid and Sethunathans (1979) found that the sorption of hexa-

chlorocyclohexane (HCH) isomers by twelve different soils was correlated most strongly to soil organic matter (OM) content. A plot of % HCH sorption versus % soil organic matter exhibited two linear branches. The increase in sorption of HCH with unit increase in OM within a range of 3.4-24% was ca. 41 times over that in the range of 0 - 3.4 % OM. The change in slope at an OM level of 3.4% could be due to organic masking, i.e. for OM levels of <2% sorption is governed by OM and inorganic components. The second curve, >2% OM, OM covers inorganic adsorbing surfaces, masking them so that a correlation with inorganic constituents at high OM levels is not manifested. Similar results were reported for the pesticide parathion by the same authors (Wahid and Sethunathan, 1979). Using a soil containing 1.6% organic matter and 26% clay, Chiou et al., (1979) studied the sorption of nonionic pesticides (PCB's, hexachlorocyclohexanes, chloropropanes and chloroethanes, DDT, parathion and 1,2-dichlorobenzene). They determined that the uptake of neutral chemicals by soils was consistent with the hypothesis of solute partitioning to the organic content of the soil. The sorption of nonionic pesticides is governed by a partitioning process involving the soil organic matter. Sorption isotherms were linear even at high concentrations and conformance to linearity at high concentrations is indicative of a partitioning effect and not physical adsorption (Chiou et al., 1979, Giles et al., 1960).

A further analysis of Newland's (1969) results, regarding sorption of  $\gamma$ -hexachlorocyclohexane by several soils, revealed that

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numbering



if one calculates the ratio of % organic matter to % clay, the ratio reflects the fact that, as the ratio increases, % adsorbed increases.

Soil properties were significantly related to 4-CA adsorption (Moreale and Van Bladel, 1976). Multiple regression analysis indicated that organic matter plus available water (or clay) correlated better ( $R^2 = 0.94$ ) than the simple linear relationship with organic matter (OM) alone and the contribution of OM was calculated as 77%.

Wahid and Sethunathan (1978) correlated 13 physicochemical characteristics of 12 soils and found that organic matter content was the most important single factor involved in parathion sorption. Figure 7 shows that a critical OM concentration (2.5%) may control the sorption of pesticides; above 2.5% the clay is masked and OM sorption predominates. In high organic matter soils, sorption was almost irreversible. Soils high in clay and cations showed almost as much sorption of parathion whether the soils were oxidized or nonoxidized.

Similar results were reported for several organophosphorus and carbamate insecticides in soil by Felsot and Dahm (1979). All  $\Delta G$ 's were negative (-1.6 to -4.6 kcal/mole) illustrating that adsorption was an exergonic process. The rapid equilibration they found was consistent with a physical type of adsorption mechanism (Freundlich, 1926; Hayward and Trapness, 1964). Also, physical adsorption does not require any activation energy and occurs as fast as the adsorbate reached the adsorbent surface. Physical adsorption is also indicated by examination of the free-energy changes calculated for adsorption. All  $\Delta G$ 's were much lower than those associated with

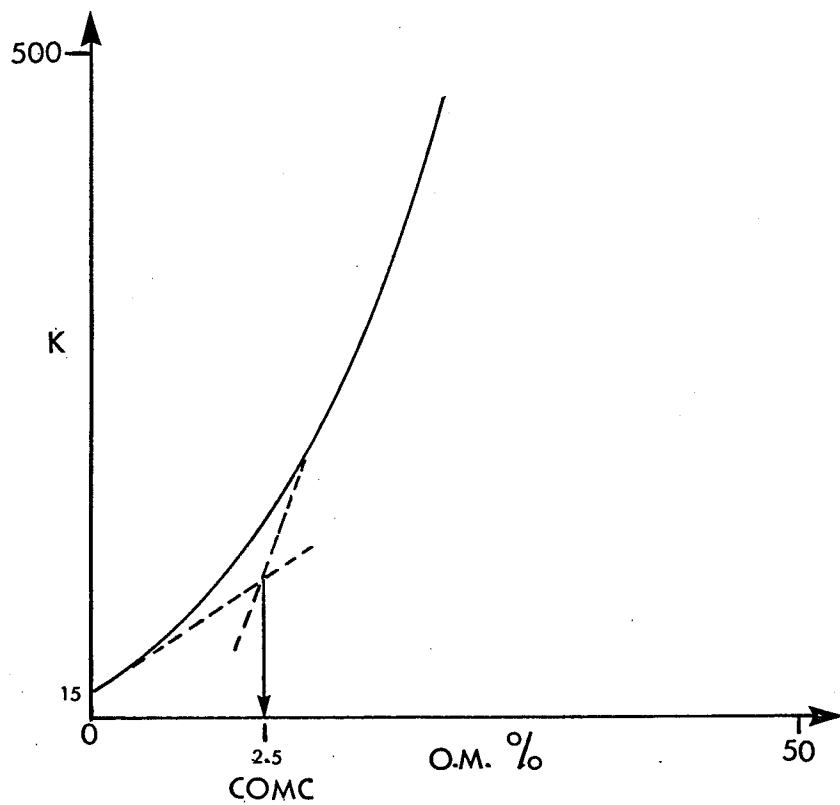


Figure 7. Critical organic matter concentration, based on the results of Wahid and Sethunathan (1978), parathion sorption (Freundlich  $K$ ) vs. % OM.

chemisorption (Hamaker and Thompson, (1972)). The authors concluded that insecticide adsorption in soils was largely due to van der Waal's forces or hydrophobic bonding.

#### D.2 Hysteresis effects

Non-coincidence of adsorption and desorption isotherms is called hysteresis, and it indicates that a different range of processes are involved in adsorption than in desorption (Figure 8). This effect was observed in the above study (Wahid and Sethunathan, 1978) as well as elsewhere (Yuan, 1964; Saltzman et al., 1972; Bowman and Sans, 1977). Thus, although adsorption was generally reversible, desorption was not always complete. Giles et al., (1974) stated that there was an energy of activation for desorption equivalent to the heat of adsorption. Thus, the thermodynamic parameters governing desorption would be quantitatively different from those affecting adsorption, i.e.  $\Delta H$  value would vary due to hysteresis effect.

Wahid and Sethunathan (1979) studied the sorption and desorption of nonionic hexachlorocyclohexane isomers ( $\alpha, \beta, \gamma$ ) on 12 soils. Organic matter content of the soil was the most important factor regarding the extent of sorption and desorption, i.e., hysteresis was demonstrated in that, as [OM] increased, sorption increased but desorption decreased.

Van Bladel and Moreale (1977) reported a hysteresis effect for the desorption of 4-CA from several soils; as organic matter content

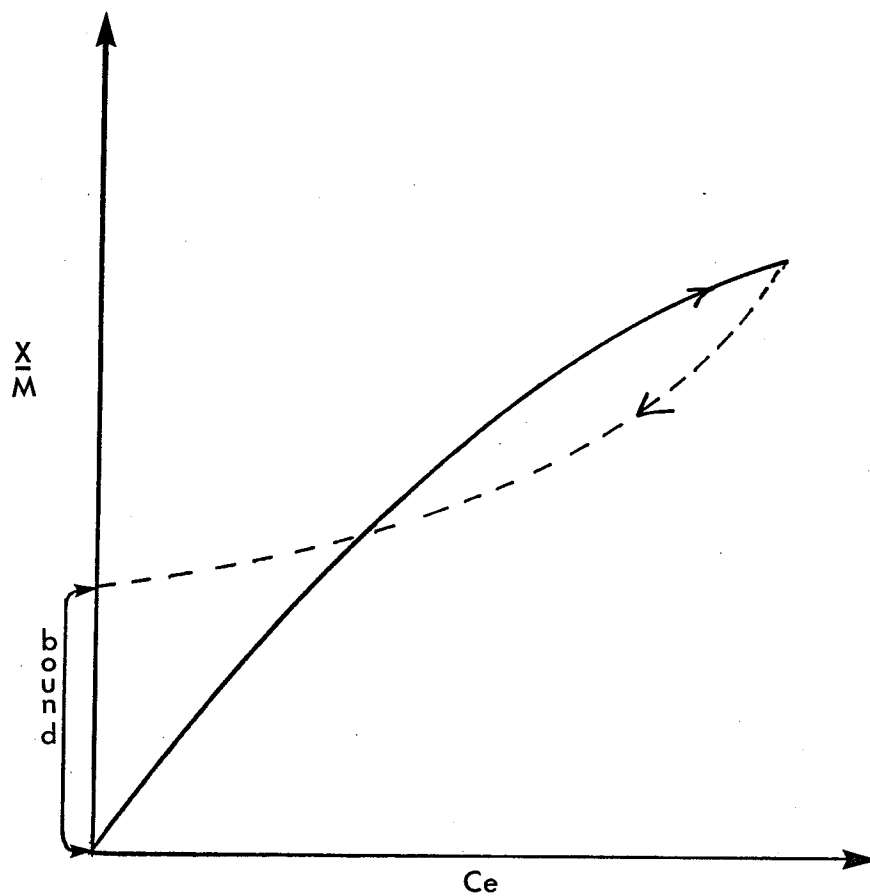


Figure 8. Hysteresis illustrated by sorption-desorption curves, ( $x/m$  = quantity of compound per unit weight of adsorbent versus  $C_e$  = equilibrium concentration of compound).

increased the desorbability decreased. Thus, hysteresis is related to desorbability of xenobiotics; nondesorbable residues being termed bound.

### D.3 Bound pesticide residues

What is a bound residue? Like many new terms, the definition or interpretation of a bound residue has varied to a very large degree depending upon the extraction techniques used. Concern for bound residues has varied all the way from preoccupation with characterization of the elusive unextractable few percent of a radiolabelled chemical, to disbelief that an unextractable product should be of any concern whatsoever. Responsible science, however, dictates that we know at least something about the unextractable entity so that biological availability, toxicology and hence, acceptability may be evaluated.

The 1975, ACS-Vail Conference on "Bound" and Conjugated Pesticide Residues" defines a soil bound residue as "that unextractable and chemically unidentifiable pesticide residue remaining in fulvic acid, humic acid, and humin fractions after exhaustive sequential extraction with nonpolar organic and polar solvents." An analogous definition could be proposed for unextractable pesticide residues in plants, as that residue remaining with lignin. A definition based on a more nearly universal methodology has the advantage of providing a standard point of reference from which to evaluate more objectively

individual chemicals and groups of chemicals.

The U.S. Environmental Protection Agency (Federal Regist. 40 (123 ) 26802, 1975) has also described a significant quantity of bound residue as being "any amount of unextractable residue, greater than 10% expressed as the parent compound, remaining one year after a single treatment."

Conjugation is a metabolic process whereby endogenous as well as exogeneous chemicals are converted to polar components for the purpose of facilitating their removal from the site(s) of continuing metabolic processes. Conjugates are defined as water soluble residues and bound residues are those which are unextractable (i.e. by exhaustive polar and apolar solvent extractants). Conjugate type reactions which have been observed include:

1. reactions with carbohydrates (glycoside formation)
2. reactions with amino acids
3. sulfur reactions (sulfate conjugation)
4. reactions involving alkylation and acetylation.

Fractionation of soil organic matter is often used in studies to investigate binding of residues to soil (Fig. 5).

The great difficulty with all fractionation procedures, however, is that they either separate out products which are not definite chemical entities or form artifacts which do not have the properties of the original material. Nevertheless, the various fractionation procedures can prove useful for investigation and characterization of bound residues by comparing and contrasting physicochemical charac-

teristics.

Concern for the identity or nature of the bound residue of intact pesticide or first or second generation degradation products or their conjugates, which are adsorbed (physical, chemical), incorporated (chemical synthetic reactions), or entrapped (inclusion complexes, guest host interactions, clathrates) in the soil or plant matrix, involves elucidation of these mechanisms so that an evaluation of their release (and the toxic effects) at some future date may be known or predicted. In soil, concern has also been expressed for the long range effects of the polyhalogenation (through incorporation of halogenated pesticides) of soil organic matter on vital soil processes and conditions.

The major questions one must be concerned with when investigating methods of releasing bound residues are:

1. How can we fractionate the soil matrix with the least possible alteration so that the residue which is bound or non-extractable is released in its indigenous state and not altered by the action of the fractionation forming a new compound which is non-extractable.

2. Do we know enough about the structure of these fractions to evaluate extraction methodologies and predict the recalcitrance of a bound residue?

3. Can we be certain that the extraction method chosen for the bound residue is not responsible for the creation of further non-extractable residues?

It is the purpose, then, of this review to describe all known studies involving development of methods to: 1) extract bound soil residues, 2) characterize these residues, and 3) elucidate the mechanism of their binding. The complexity of the problem has required the use of radioactive tracer techniques to study the above objectives.

Regarding the methodology, it is not enough to speak of unextractable radioactivity ( $^{14}\text{C}$ ) since we wish to identify the nature of the compound containing the radioactivity ( $^{14}\text{C}$ ). Is it the parent pesticide or a primary or secondary metabolite? Or is the radioactivity "bound" because the parent pesticide has been completely metabolized to an exogeneous precursor of proteins and fatty acids? For instance, dalapon is metabolized to pyruvate and alanine (Kearney et al., 1974; Beall, 1964); TCA is metabolized to serine (3); the 2,4-dichlorophenolic portion of 2,4-D goes through a series of reactions yielding succinic acid (Kaufman, 1974). Such products would not be of toxicological concern.

It is of the utmost importance to note the format of experiments investigating bound residues, because the nature of bound radioactivity (i.e., the mechanism of binding) may vary according to the period of time after which characterization is attempted. This will be discussed in detail with regards to bound radioactivity in organic matter fractions, clays, soil fungi and bacterial cells, including fractions thereof.



#### D.4 Physicochemical effects of the binding of xenobiotics to soil

##### 1. Effect of wetting and drying of soil or humic acids on the extractability of bound residues

Hill (1976) found that reextraction of air-dried and/or freeze-dried soils (previously extracted as moist or wet soils), containing bound [ $^{14}\text{C}$ ]pirimicarb and/or metabolites, with the same solvents used to extract free or unbound radioactivity, released a further 63-78% radioactivity from two different soils. He postulated that the drying step may have released radioactivity bound to soil surfaces, or the pesticide and/or degradation products could accumulate inside microbial cells. Thus, these cells might not all be ruptured without an extraction from the dried state.

Richey et al. (1977) found similar effects of drying on extractability of bound aldicarb from soil. Bartha (1971) reported reduced enzymatic activity in air-dried soil compared to soil which had been stored moist for the same length of time. It was expected that metabolism in the air-dried sample would be less rapid. However, they found the reverse situation. They concluded that this may be a result of greater oxygen supply in the air-dried soil which stimulated bacterial activity. A similar effect on rate of increased  $\text{CO}_2$  production of air dried soil has been reported elsewhere (Hesse, 1971); these workers found that if a soil is dried and rewetted the rate of conversion of its organic matter to  $\text{CO}_2$  is much

greater than if the soil had not been dried. This effect was ascribed to release of available organic matter by the drying/re-wetting process and a resulting increase of microbial growth. This would be expected to stimulate more rapid breakdown of aldicarb. After 54 days the air dried soil was wetted again and a marked increase of  $^{14}\text{CO}_2$  was noted at 61 days (ca. 4%).

Lyophilization of acid precipitated humic acids, entire extraction solutions, or the fulvic acid solution is commonly practised to remove all traces of water from these preparations by as gentle a method as possible. Several reports caution the researcher on the use of this technique.

Bowman (1975) found that the use of freeze-dried clays to study adsorption of fensulfothion resulted in increased adsorption; conditioning the freeze-dried clay to 100% relative humidity prior to adsorption decreased fensulfothion adsorption. Moreover, freeze-dried clay structure at the macro and micro levels is altered (see references in Bowman, 1975). It has been shown (Burns et al. 1973) that freeze drying of Ca humates decreases the ability of humates to swell in water and this can increase steric sieving effects for paraquat. Non-dried  $\text{H}^+$  humic acid (Grice and Hayes, 1972) has a slightly lower adsorptive capacity for paraquat than a similar material which was freeze-dried. Other structural changes and physical effects of freeze-drying of humic acids have been reviewed by Van Dijk (1971).

The importance of water in the soil matrix and its effect on organic residue recoveries has been demonstrated (Saha et al., 1969a;

Saha et al., 1969b), especially when added just prior to extraction. Dieldrin treated soils were compared in terms of wet vs. dry original moisture conditions followed by incubation for several months; dieldrin residues from wet incubated soils were much more difficult to extract. Yaron and Saltzman (1972) showed the importance and effect of water in adsorption processes as adsorption to soil was greatest from a hexane solution of parathion, since the polar solute could compete more effectively for adsorption sites cf. water as a solvent. In addition, Suess et al. (1972) have shown that, in general, the lower the water solubility, the higher the amount adsorbed.

It is not surprising then that a compound such as 4-chloro-aniline (4-CA) with its low water solubility and reactive nitrogen electron pair, is strongly bound to soil humic substances.

## 2. Effects of organic matter content of soil

Several authors (Meikle et al., 1976; Hill, 1976; Helling and Krivonak, 1978a, 1978b; Richey et al., 1977; Fuhr et al., 1977; Hankawa, 1974; Golab et al., 1979; Bartha, 1971; Bollag et al., 1978; Harvey and Pease, 1973) have fractionated the soil containing aerobic-bound residues into fulvic acid, humic acid, and humin using the classical procedure. Interestingly, for all the pesticides investigated and reported herein, only aldicarb and methomyl did not follow the trend of the fulvic acid fraction containing more radioactivity than the humic acid fractions. In the case of aldicarb and

methomyl, the reverse was true. This may be due to aldicarb and methomyl stereochemical conformations precluding diffusion into micropores of the soil organic matter or from differences in the extraction procedure used to isolate them such as, length of extraction, temperature, and whether or not the extractant was degassed to diminish alkaline oxidation of lower molecular weight fulvic acids. Even when the binding to each fraction was reported as specific radioactivity (Meikle et al., 1976), in lieu of percentage of total radioactivity, the pattern clearly followed the order: fulvic acid (2162 dpm/mg), humic acid (1904) and humin (481). Variations for humin fractions are due to intrinsic variations in the amount of clays, silicates, oxides or sesquioxides, already bound to them - this varying with the nature of each soil's inorganic constituents. These findings support a genetic relationship of fulvic acid → humic acid → humin. Golab et al. (1979) found lower recoveries of soil bound [<sup>14</sup>C]trifluralin with Dowex A-1 resin than with aqueous base - this contrasts with Meikle et al. (1976) who reported the reverse for [<sup>14</sup>C]ditalimfos.

Removal of organic matter with oxidizing agents may or may not reduce sorptive capacity. Organic matter is incompletely removed, for example, even by exhaustive treatment with H<sub>2</sub>O<sub>2</sub>. Thus, one must be cautious about assuming that sorption effects observed after oxidation is due to the clay-sized minerals alone. Residual organic matter is related to clay content so the extent to which clay surfaces or organic matter surfaces contribute to adsorption is not

additive (Woolcott, 1970).

### 3. Effects of extractants

Generally, it may be concluded that strong aqueous acid or base solutions are the most efficient extractants for isolation of large amounts of soil-bound pesticides (the majority of this being the parent compound). At first glance, it may appear that methylation of the organic matter followed by organic solvent extraction might yield high recoveries of radioactivity and of the expected compound; inevitably, however, good extraction conditions were more favorably met by using strong base or acid. Ultrasound did not significantly improve extractions of bound residues in the studies reported herein (Hill, 1976; Helling and Krivonak, 1978b; Fuhr *et al.*, 1977). The use of enzymes or microbial cultures may hold some promise for release of bound residues; however, one is then confronted with cleanup problems, especially for polar pesticides and/or their metabolites and at lower levels of contamination this problem is attenuated further.

Hsu and Bartha (1974b) reported that the more aggressive extractant NaOH *cf.*  $\text{Na}_4\text{P}_2\text{O}_7$  yielded HA which bound more [ $^{14}\text{C}$ ]4-CA: under aerobic incubation conditions 78% (*cf.* 57%) was bound at the 5 ppm treatment level. Similar results were reported for anaerobic binding of dinitroaniline herbicides to soil (Helling and Krivonak, 1978a). NaOH extracted soil FA, and HA contained 14 and 40% bound  $^{14}\text{C}$  *cf.* 1.7% and 26% for a  $\text{Na}_4\text{P}_2\text{O}_7$  extract of

the same soil. Thus, binding to the fulvic acid (FA) cf. humic acid (HA) fractions, from a  $\text{Na}_4\text{P}_2\text{O}_7$  extractant, was greatly reduced (1:3 NaOH cf. 1:15 FA:HA for  $\text{Na}_4\text{P}_2\text{O}_7$ ).

Hill (1976) found an interesting variation in extraction of bound pirimicarb. Virtually all of this (40-25% of applied) was extracted using 0.1 NaOH, or 0.1 M  $\text{Na}_4\text{P}_2\text{O}_7$ , 60-80% with 0.5 M  $\text{H}_2\text{SO}_4$ , and only 33-70% to 1.0 M HCl.

The use of premethylation of humic substances followed by the increased extractability of organic compounds (including pesticides) will be discussed in detail in the discussion section.

### 3.1 Alkaline hydrolysis

Aqueous sodium hydroxide increases the dispersivity and solubility of humic substances by disrupting hydrogen bonds of the fixed metal cations. Degradation of soil organic matter using basic solutions is well documented (Miekle et al., 1976; Bremner, 1965; Danneberg, 1973; De Serra and Schnitzer, 1972; Kaminsky and Muller, 1978). Oxidation, polymerization, condensation and hydrolysis readily occur during humus extraction and preparation, especially when alkaline solvents are applied (Sauerbeck and Fuhr, 1968). Striking differences were reported for the physicochemical properties of soil humic acids extracted with NaOH cf.  $\text{Na}_4\text{P}_2\text{O}_7$  followed by NaOH (Vila et al., 1974; Martin, 1974).

Meikle et al. (1976) found that hot aqueous alkali extraction of soil caused degradation of high molecular weight humic acid to lower

molecular weight components which tended to fractionate as fulvic acids. Caution is required because alkaline hydrolysis of parent pesticides to substituted degradation products (eg. phenylureas → substituted anilines) may also occur. Swift and Posner (1972) reported that alkaline extraction of soil resulted in decrease in the molecular weight of the HA fraction. An increase in CEC of the fulvic acid fraction indicated, along with increased O<sub>2</sub> consumption in alkali, a reason for the increased acidity (COOH) function of fulvic acid fractions. Thus autooxidation in alkaline solution is of considerable importance in altering the indigenous structure of humus substances. Theng et al. (1967) found that extraction of soil using aqueous base yielded HA fractions with higher C-H contents and lower [COOH] and [C=O, ketone] groups, indicating decomposition reactions. Finally, Sauerbeck and Fuhr (1968) concluded from their studies, on alkali extraction and fractionation of humus, that oxidation, polymerization, condensation, and hydrolysis readily occurred. The use of NaOH and HCl in isolating humic acids accelerates the formation and humification of these acids in vitro (Viser, 1964).

### 3.2 Acid hydrolysis

Acid hydrolysis or extraction also leads to perturbation in the structure in the humus fractions (Kosaka et al., 1961). Riffaldi and Schnitzer (1973) reported that 6 M HCl treatment produced a decreased H content, increases in % C and free radicals leading to possible

increases in degree of condensation or aromaticity in the humic acids. Up to 46% of the original weights of HA were lost upon acid hydrolysis. Once treatment of humic acid has been shown to result in decreased methoxyl content, higher molecular weight fractions after hydrolysis, lower carboxyl group content, and higher carbon content (Kosaka et al., 1961). Polymerization reactions occur due to acid hydrolysis (6N HCl) of humic acid and increased aromaticity (Rashid, 1972; Farmer and Morrison, 1960). After acid hydrolysis of lignite humic acid, a molecular condensation was indicated (Martin, 1975a).

Interestingly, the fact that similar results were obtained for chloroaniline extractions by acid or base extraction from soil and humic acids (Hsu and Bartha, 1976) agrees with studies where acid or base extracted the same amount of soil FA (Martin, 1976).

### 3.3 Nonhydrolyzable residues

The nonhydrolyzable residues described by Hsu and Bartha (1976) may be no more than inclusion products entrapped by condensation of degraded humic polymers (or aggregates), polymerized and unextractable chloroanilines via alkaline oxidation (as per Holland and Saunders, 1968), reaction products of the aniline with humic monomers, or aggregates mediated by alkaline or acid hydrolysis, and/or catalytic effects due to inorganic constituents. Martin (1976) expressed the same concern for the altered humic substances (due to extractants HCl or NaOH) from which other organic substances (pesticides non-humic substances) might be coextracted and incorporated



among the humic molecules.

Adams and Perry (1973) studied the effect of pH on the incorporation of amino acids into HA extracted from soil and reported that under alkaline conditions, humic acids may react with amino nitrogen. Since by definition, the isolation of HA involves treatment of the soil with alkali, it is to be expected that reactions of this type could take place during humic acid extraction and alkaline hydrolysis.

It appears most difficult to separate reactions taking place during incubation of a substrate with humic substances in situ, from those occurring during the extraction procedure. Kinetics and thermodynamic studies described in the following sections may help to explain this further.

The existence of nonhydrolyzable covalently bound chloroanilines, and probably other xenobiotics, in soil over the short term is minimal (i.e., in situ reactions). Most of the nonhydrolyzable chloroanilines probably arise from in vitro reactions mediated by the acid or base extractant. Over the long term covalently bonded chloroanilines may bind via in situ biochemical reactions of soil microflora, microfauna and surface photochemical reactions. Forty percent of the lignin-bound [ $^{14}\text{C}$ ]3,4-DCA was released using HCl, HBr,  $\text{HClO}_4$ , or dioxane in HCl (9:1) but products isolated were not stable and could not be stabilized (Still et al., 1976).

The hydrobromic acid hydrolysis yielded a fraction extractable into hexane and containing an adduct of 3,4-DCA and furfural,

probably produced via the strong hydrolytic conditions from interaction of 3,4-DCA with carbohydrate derived furfural.

In the presence of sulphuric acid and manganese dioxide, aniline condenses (through a radical mechanism) yielding colored dyes and quinone via oxidation and hydrolysis (Fieser and Fieser, 1961). Phenazines and phenoxazones may also form from o-aminophenols (Scott, 1965 and references therein). Thus such products may account for nonhydrolyzable forms of 4-CA in soil, via acid or base hydrolysis oxidation and catalysis in the presence of inorganic soil components, especially clays and oxides of iron and aluminum.

Bollag et al. (1978) found virtually the same amount of nonhydrolyzable [<sup>14</sup>C]4-CA in sterile and nonsterile soil, 12.5 and 8.8% of the applied radioactivity respectively, after a six week incubation. In addition, there were only slight differences in the percentage of nonhydrolyzable residues for four substituted chloroanilines (7.9-17.9% for sterile and nonsterile soil) or for 3,4-DCA over a period of 190 days incubation (Hsu and Bartha, 1976).

Again this is indicative of in-vitro covalently formed nonhydrolyzable residues; the extent to which they are formed being largely independent of structure and completely independent of biochemically mediated reactions (i.e., in short term experiments).

Still et al. (1976) isolated a dimer of 3,4-DCA from treated rice plants via acid hydrolysis and identified it as an adduct of 3,4-DCA and furfural, N-(3,4-dichlorophenyl)furfurylidimine. Furfural is known to be a common acid degradation product of carbohydrates

which subsequently would react via a Maillard reaction followed by Amadorri rearrangement (Feather and Harris, 1973).

Different soils were treated (Riley et al., 1976) with paraquat at several levels of treatment. "Loosely" bound paraquat was extracted (saturated  $\text{NH}_4\text{Cl}$ ) and "tightly" bound paraquat released by 18N  $\text{H}_2\text{SO}_4$  hydrolysis into aqueous solution intact. Non-hydrolyzable residues of paraquat accounted for 0-37% averaging 14%. All soils, except a muck soil (100%OM), contained nonhydrolyzable residues in the range of 10-20% (sand, loamy sand, loam - 1,2,13% clay and 0.5, 1.5 and 2.8% OM; muck soil = 37% nonhydrolyzable residues).

Nine to eighteen percent of all soil-nitrogen resists acid hydrolysis by 6N HCl (Freney, 1968; Sowden et al., 1976). Freney and Miller (1970) reported that a clay mineral reduced the solubility of organic nitrogen compounds in the presence of boiling acid. This reduction in solubility did not appear to be due to humin formation or to adsorption of nitrogen compounds on the amorphous residues produced from acid treatment of montmorillonite. Their results suggested a clay mineral protection theory for nonhydrolyzable nitrogen. Several organic compounds were tested, and of the applied dose, 4-10% remained nonhydrolyzable. It was postulated that the nitrogen compounds were trapped in the interlamellar spaces of the clay and the greater the basicity of the predominant cations, the greater the percentage varied.

Olsson et al. (1977, 1978) showed that in even slightly acidic

aqueous solution (pH 4.5) glucose and glycine or glucose and methylamine reacted to form heterocyclic nitrogen containing compounds (30% yield). Such reactions involving soil carbohydrates and 4-CA could account for the nonhydrolyzable forms of 4-CA.

#### 4. Effects of soil bacteria and fungi

It is probably safe to conclude that when pesticides are incubated with pure cultures of soil fungi or bacteria, considerable incorporation will occur. Hill (1979) found 50-60% of [ $^{14}\text{C}$ ] theraol associated with a soil fungi within 2 days. Only a small percentage of the  $^{14}\text{C}$  was extractable; bacteria were poorer than fungi at incorporating  $^{14}\text{C}$ .

After 4 weeks incubation of azobenzene and phenol with soil bacterial cultures, 90-100% was incorporated (Kosinkiewicz, 1977). The same author reported 80% incorporation for aniline and o-chloroaniline and ca. 50% for m or p-chloroanilines. Similar findings for other organics have been reported for fungal incorporation (Martin et al., 1967; Bailey and Njundikye-Desseaux, 1975; Mathur and Morley, 1975).

Fuhr et al. (1977) found that upon incubation of [ $^{14}\text{C}$ ] methabenzthiazuron in fungal (soil fungi) cultures for 5 weeks, 36-46% of the  $^{14}\text{C}$  was associated with the cells. From 8-31% was extractable using organic solvent and NaOH extracted 90% or more of the  $^{14}\text{C}$ .

Wolf and Martin (1976) studied incorporation of [ $^{14}\text{C}$ ] 2,4-D

and chlorprophan by soil fungal culture. Approximately 12% of the activity for 2,4-D was found in the humic-like polymers and 4-16%  $^{14}\text{C}$  was recovered in the mycelia. Three to twenty-four percent of the activity from chlorprophan was found in the humic-like polymers and 35%  $^{14}\text{C}$  in the mycelia.

Thus, pesticides or their degradation products may be linked into bacterial and fungal melanins or phenolic polymers present in the cell wall; however, this would be of importance in the long term only where the substance was not too strongly bound or included such that it was still accessible to microbes for assimilation.

Research by McLaren and Skujins (1971) has shown that the  $^{14}\text{C}$  label from simple substances (such as glycine and glucose) applied to soil is incorporated into "humic substances" of bacterial origin. Another report by Harvey and Pease (1973) reported incorporation of  $^{14}\text{C}$  label from methomyl into the humic acid fraction of the soil organic matter. Other studies with [ $^{14}\text{C}$ ]methabenzthiazuron (Fuhr et al., 1977) and [ $^{14}\text{C}$ ]parathion (Fuhremann et al., 1977) incubated with microbial cells, showed only small amounts of incorporated radioactivity.

Binding of pesticides by microbes via incorporation into cell walls does not appear to be a useful explanation for recalcitrant residues. Several studies have shown that such incorporated residues were removed easily with organic solvents (Katan and Lichenstein, 1977; Fuhr et al., 1977).

## 5. Effects of sterilization

Sterilization of soil has several effects upon the binding of pesticide to soil:

1. Steam sterilization may affect trace metal catalysis (Kaufman et al., 1968).
2. Equal binding of 4-CA to sterile vs. nonsterile soils (cf. and Bollag et al., 1978 and Hsu and Bartha, 1974b for 3,4-DCA) at 40 days or 6 weeks has been recorded.
3. Higher volatilization of residual pesticides has been reported (Freundlich, 1926) for steam sterilized soils.
4. Decreased binding for parathion was observed by Katan et al. (1976) - 58% binding in sterile vs. 84% nonsterile was reported. Similar results were reported for pirimicarb (Hill, 1976).

The effects of sterilization appear to be variable at a first glance; however, binding would be less in sterile soil for those parent compounds which required microbial degradation for significant binding - i.e., parathion to aminoparathion and pirimicarb to amino and hydroxyl pirimicarb, etc.

Lichenstein et al. (1977) has reported, on the other hand, that the binding of several organophosphate pesticides including fonofos was not dependent upon microbial activity. Kaufman et al., (1968) reported a comparative study of soil sterilization methods and their effects on amitrole degradation. Amitrole degradation occurred in both potassium azide-treated and ethylene oxide-sterilized soils but

not in autoclaved soils. Potassium azide gave nearly complete inhibition of microbial activity at the concentrations used; no organisms were isolated from the ethylene oxide sterilized or autoclaved soils at the conclusion of the experiment. Reinoculated steam sterilized soil failed to show any degradation of amitrole.

Hsu and Bartha (1974a) found 80% 4-CA "bound" to sterilized whole soil or isolated humic acids in these studies and nonsterilized humic acid contained 88% of the applied dose as bound or non-extractable by conventional methodology. The incubation time in these studies was 24 h or less. Bollag et al., (1978) pointed out that during 24 h, little biological transformation could be expected (for substituted CA) and that initial binding takes place physico-chemically without the active participation of soil microorganisms. The same authors reported no differences in the amount of 4-CA bound after 6 weeks in sterile or nonsterile soil (41% vs. 39% of radioactivity determined as 4-CA); and cf. 49% bound after only 24 h incubation).

Also of note is the fact that, in the study of Bollag et al. (1978) the so-called "nonhydrolyzable" residues were virtually the same for sterile and nonsterile soil (12.5% of 8.8% respectively after 6 weeks incubation). Nonhydrolyzable residues for 3,4-DCA (190 days incubation) varied by only ca. 0.8% (Hsu and Bartha, 1976); this may reflect an in-vitro origin rather than a steady state condition.

#### 6. Effect of aerobic versus anaerobic conditions of incubation

Hill (1976) observed that for flooded soils treated with pirimicarb, more than half of the  $^{14}\text{C}$  released by 1.0M HCl (75% removed) was present as [ $^{14}\text{C}$ ]carbonate. Flooded soils contained 14% of the  $^{14}\text{C}$  ring labelled pirimicarb while aerobically incubated soils contained 40%. Katan et al. (1976) reported that under flooded conditions (anaerobic), the binding of parathion was twice (35 and 65% of applied  $^{14}\text{C}$  within 2 weeks) that in aerobic (but moist) soils. Parathion was reduced to aminoparathion (Lichenstein et al., 1977) under flooded conditions, accounting for the increased binding of radioactivity. Butralin binding (Helling and Krivonak, 1978a) in whole soil was 3 and 13% for aerobic vs. anaerobic conditions. Interestingly, the oxygen status affected the amount bound to humus fractions in the following manner - for aerobic conditions, the amount bound to each fraction was: FA = 51%, HA = 7%, humin = 42%, compared with anaerobic conditions: 14, 40 and 46% respectively. Binding in flooded conditions was probably as the triamine [as per trifluralin, (Golab et al., 1979)].

Griffin (1977) reported for a diamine pesticide (USB 3153) 22%  $^{14}\text{C}$  bound aerobically cf. 46% anaerobically after 199 days. Further, studies attempting to determine the nature of the bound radioactivity clearly showed the existence of metabolic products.

CNP (2,4,6-trichlorophenyl 4'-nitrophenyl ether) was rapidly degraded in soils under flooded conditions producing its amino analog (Kuwatsuka et al., 1978). Many strains of isolated microorganisms reduced the nitro group of CNP, however, the mechanism of



the reduction occurring in flooded soils was found to be a chemical reduction with ferrous ion produced from ferric ion by microbes in the soil.

Thus oxygen status of the soil is seen to be an important consideration regarding degradation or alteration of parent compound and/or metabolites to reduced forms capable of extensive binding to soil organic matter. Although microbial metabolism is an attractive general explanation for this oxygen demand, Bollag et al. (1978) reported that no biological activity was required for binding of 4-CA to soil as reported by Hsu and Bartha (1976).

#### 7. Effect of temperature

Temperature would be expected to have an indirect effect on binding in that increased temperature would increase degradative reactions to products that are more bindable. Also microbial incorporation would be enhanced as would rates of all reactions and movements within the soil matrix. For [ $^{14}\text{C}$ ]parathion a loam soil (Katan et al., 1976) binding at 6°C accounted for 2.3% of the applied  $^{14}\text{C}$  cf. 34.8% bound  $^{14}\text{C}$  at 27°C. Twice as much  $^{14}\text{C}$  (51.5 vs. 97.8%) was extractable at 6°C cf. the 27°C incubation. Thus, a lower temperature favored less binding as would be expected for an endothermic reaction. These effects will be discussed in a subsequent section describing mechanisms and thermodynamic considerations.

Bailey & White (1964) suggested that temperature affects adsorp-

tion by altering the pesticide solubility such that higher temperatures result in desorption. Since adsorption processes are exothermic and desorption endothermic, one would expect a temperature increase to reduce adsorption with a corresponding increase in solute solubility, however, there are exceptions. Freed et al. (1962) reported an increase in EPTC adsorption as the temperature rose. The effect of temperature on adsorption may be different for clay cf. organic matter (Sullivan and Felbeck, 1968) and accounts, in part, for these apparent anomalies. The adsorption of triazine by humic acids increases with increasing temperature (McGlamery and Slife, 1966) and reports of enhanced pesticide adsorption in organic soils may be due to temperature induced changes in the structure and porosity of the organic matter (Guenzi and Beard, 1970; Hayes et al., 1968). Exchange reactions, such as those exhibited by the bipyridylium herbicides, tend to be independent of temperature fluctuations (Weber et al., 1965; Harris and Warren, 1964) although Faust and Farins (1969) observed an increase in adsorption of diquat with a rise in temperature.

Fuhr and Mittelstadt (1979) reported on the effect of temperature and extractability of methabenzthiazuron, isocarbamid, and metamitron from soil. Extractability of the radioactivity decreased with increasing temperature (0° to 20°C). For methabenzthiazuron, the fraction of non-extractable radioactivity doubled from 16% at 0°C to 34% at 20°C, for isocarbamid-less than 22% at all temperatures and for metamitron 32 to 54% of the radioactivity was nonextractable.

Extractable metamitron was ca. 60% of the applied radioactivity at 0 and 10°C but only 9% at 20°C, isocarbamid was 89% (0°C) and 61% (20°C), and methabenzthiazuron ca. 60-70% at all temperatures.

#### 8. Effect of applied xenobiotic concentration

The usual effect reported for concentration is as follows: as applied concentration decreases (e.g., 10 to 1 ppm) recalcitrance to extraction increases as does the total amount bound (Riley et al., 1976; Lichenstein et al., 1977).

However, in terms of absolute amount of bound residues, i.e., ppm vs. %, more is bound at higher concentrations (Katan et al., 1976). This indicates that at lower concentrations only a portion of the binding sites in a soil are saturated. Similar results have been reported for other pesticides and metabolite or degradation products (Hsu and Bartha, 1974a; Bartha, 1971). Evidence that saturation of binding sites may occur is provided by Hsu and Bartha (1974b) who found 78% [<sup>14</sup>C]4-CA bound to humic acid at 5 ppm but only 31% at 500 ppm after aerobic incubation.

The reader is referred to one of several texts explaining the apparent vs. real recalcitrance to desorption of organic compounds from adsorbents at low concentrations (Hayward and Trapwell, 1964; Vponec et al., 1974; Gregg and Sirg, 1967).

Briefly, the explanation involves the available number of adsorbent sites relative to solute (adsorbate) concentration. Consequently, as the concentration of solute increases the numbers of

adsorbent sites decrease resulting in less adsorption or the strength of sorption increases as the fraction of adsorbate covered decreases. The fractional area covered is directly proportional to the pesticide concentration, especially for surface adsorption of ionic or polar pesticides.

The reader is referred to an excellent review (Hamaker and Thompson, 1972) of factors affecting adsorption of pesticides in soil for a comparison with those just described in terms of bound residues.

#### 9. Effects of time

Fuhr (1976) reported an increased percentage of binding for methabenzthiazuron as post-treatment period increased. Such increased binding with time is consistently reported (Schiavon et al., 1977; Golab et al., 1979; Wheeler et al., 1979; Katan et al., 1976; Richey et al., 1977; Helling and Krivonak, 1978 and references therein).

In a sterile soil (Hsu and Bartha, 1974a), 4-CA was almost completely (>80%) bound within 24 h post-treatment. Hamaker et al. (1966) found that the adsorption of picloram increased over a period of 23 days with no indication of having reached a final steady state. Soil bound 3,4-DCA varied between cf. 45 and 33% of the applied dose over a period of 190 days (Hsu and Bartha, 1976).

#### E. Binding of Chloroanilines to Soil

Many of today's pesticides contain substituted chloroaniline

moieties which are released into the environment by metabolic or degradative processes. A number of investigations to date on bound residues of chloroanilines (especially 3-chloro, 4-chloro, and 3,4-dichloro) commonly derived from phenylurea herbicides, in soil have concluded that humic acids are the major pool for these residues (Hsu and Bartha, 1976; *ibid*, 1974a, 1974b, Chisaka and Kearney, 1970). For example, 73% of the soil applied herbicide propanil degraded to 3,4-dichloroaniline (3,4-DCA), the majority of which was bound to humic substances (Chisaka and Kearney, 1970). In addition, up to 90% of the 3,4-dichloroaniline released during the biodegradation of several other phenylamide herbicides became bound to humic substances (Hsu and Bartha, 1974b). Chloroanilines seem especially prone to form bound residues (Chisaka and Kearney, 1970; Bartha, 1971). The importance of the  $\text{NH}_2$  group has been emphasized by several researchers (Bandal *et al.*, 1976; Ward and Upchurch, 1965, Golab *et al.*, 1979; Kuwatsuka *et al.*, 1978; Griffins, 1977; Fuhremann *et al.*, 1977).

Viswanathan *et al.* (1978) reported that 3,4-DCA was bound to whole soil (at the 1 ppm level), to the extent of ca. 88% after one or two years aging.

Suess and Eben (1975) treated whole soil at the 1-5 ppm level with the  $^{14}\text{C}$ -labeled compounds, aniline. $\text{H}_2\text{SO}_4$ , 4-CA and 3,4-DCA; for 24 hours. For three soils studied, averages of 38, 72, and 33% of the applied  $^{14}\text{C}$  was bound. After 8 weeks incubation,  $^{14}\text{CO}_2$  evolution was 18.8, 8.8, and 1.7% respectively.

Duel et al. (1977) could recover only <50% 3,4-dichloroaniline from laboratory treated soils, with widely varying recoveries. Chisaka and Kearney (1970) reported studies on degradation of propanil and fate of its 3,4-dichloroaniline (3,4-DCA) metabolite. Approximately 30%-40% of the applied propanil degraded to 3,4-DCA within 15 days soil incubation. Recoveries of 3,4-DCA from different soils ranged from 60-80% at 200 ppm treatment level to less than 50% at the 20 ppm level. Bound 3,4-DCA residues were also studied with various extractants investigated for their release (Cheng, 1978). Organic solvents, HCl or NaOH (aqueous) were not effective, nor was alkaline hydrolysis. However, acid hydrolysis was very effective in removing bound 3,4-DCA from aged soil [90%  $^{14}\text{C}$  was released], most of which was 3,4-DCA as determined by TLC- $R_f$ ; only one TLC was done to confirm the nature of the extracted radioactivity. Cheng (1978) postulated that the 3,4-DCA protonates at very low pH so that both the hydrolytic power of boiling acid and protonation of 3,4-DCA effected the release from binding sites (more will be said on this later). TCAB (3,3', 4,4'-tetrachloroazobenzene) was a prevalent metabolite formed from condensation of 3,4-DCA (e.g. 20% at 850 ppm cf. 1% at 85 ppm for the same soil). 3,4-DCA rate of formation from propanil (and persistence of the 3,4-DCA) was slowest and longest with soils highest in sand and lowest in organic matter content. Bartha (1971) studied the fate of herbicide-derived chloroanilines in treated soil. Incubation studies revealed that for 4-CA and 3,4-DCA 78 and 73% of the applied  $^{14}\text{C}$  was found at 5 ppm, and 54 and 53%

at 500 ppm treatment levels.  $^{14}\text{CO}_2$  from 4-CA mineralization was < 2.5% in 14 days and volatile [ $^{14}\text{C}$ ]4-CA was 0.1% of the applied  $^{14}\text{C}$  at the 5 ppm level. From a 500 ppm propanil treatment, 54% of the applied  $^{14}\text{C}$  remained as soil bound residues. Forty percent of the soil bound aromatic moiety could be recovered as unchanged 3,4-DCA following caustic digestion and no  $^{14}\text{C}$  remained in the alkali or residue after steam distillation. For 4-CA, high concentrations favored the formation of solvent-extractable metabolites; low ones, soil-bound residues. Other organic solvents,  $\text{H}_2\text{O}$ , 10% NaCl solution, and 0.1 M HCl failed to extract any substantial portion of the bound chloroanilines. Bartha (1971) concluded that immobilized chloroanilines were "chemically" bound to humic substances, and physical adsorption was, at most, a secondary mechanism.

Hsu and Bartha (1974a) studied the interaction of chloroanilines with soil organic matter. Whole soil and isolated humic acids were treated with 3,4-DCA and 4-CA. Unfortunately, Hsu and Bartha precipitated the humic acid without prior extraction of the solution for unbound chloroanilines; consequently, residues determined in the precipitated humic acid may reflect included complexes of chloroanilines. In addition, the precipitated humic acid was redissolved in 0.5N NaOH and reprecipitated; this may have hydrolyzed covalently bound residues or altered the humic acid structure ( $1^\circ$ ,  $2^\circ$ ,  $3^\circ$ ,  $4^\circ$ ) resulting in recalcitrant residues via inclusion complexes. Alkaline or acid hydrolysis and fractionation was performed on a weathered

soil previously treated with [ $^{14}\text{C}$ ]propanil. Alkaline hydrolysis released 35.8% of the bound  $^{14}\text{C}$  as 3,4-DCA and acid-hydrolysis released 44.9%; 55.1% and 56.2%  $^{14}\text{C}$  respectively remained in bound forms as follows: alkaline treated soil - humin = 7.5%, humic acid = 20.0%, fulvic acid = 27.6%; acid treated soil - humin = 37.4%, and 18.8% in the extracted alkaline solution (cf. 27.6% in alkaline soln. for the former. Material balances for both hydrolytic treatments were ca. 100%).

Hsu and Bartha (1976) also suggested a routine method for analysis of "hydrolyzable" bound soil chloroaniline residues based on alkaline hydrolysis. However, no indication as to sensitivity, resolution, accuracy or precision is given for their results.

Recently, Bollag et al. (1978) reported their results on the study of adsorption and transformation of four substituted [ $^{14}\text{C}$ ] anilines in soil. After 40 days incubation of 4-CA with whole soil (5 ppm level), ca. 7.5% of the applied radioactivity was trapped as  $^{14}\text{CO}_2$ . 4-CA was bound to sterile and nonsterile soil to virtually the same degree as reported for 3,4-DCA by Hsu and Bartha (1974a) ca. 70-80%; however, various alkyl-substituted anilines showed considerably greater binding in nonsterile soil measured as volatile radioactivity from the soil surface trapped in acidic media; for 4-CA this amounted to 3.5% of the applied radioactivity. Hsu and Bartha (1974a) concluded that biological activity was not required for binding. For sterile (autoclaved) vs. nonsterile soil after 6 week incubation - 16.6%  $^{14}\text{C}$  vs. 6.6% was soxhlet



extractable, of which, ca. 40% in each case had an  $R_f$  value equal to that of the 4-CA standard. 71.0% vs. 72.4% was bound and 0% vs. 7.5% trapped as  $^{14}\text{CO}_2$ . It should be noted here that Hsu and Bartha (1974a) used steam sterilization and metabolic inhibitors for their studies since steam may affect trace metal catalysis in soil (Kaufman et al. 1968); no significant differences in the results were found. After 24 hours incubation, 49% of the applied radioactivity (5 ppm) was bound to whole soil, (soil pH 7.5, organic matter = 3.4), cf. Hsu and Bartha (1974a) who found ca. 80% bound  $^{14}\text{C}$  to a sterilized soil under the same conditions (soil pH 5.8, organic matter 6.0%). Bollag et al. (1978) used methanol soxhlet extraction cf. acetone/benzene shaking extraction by Hsu and Bartha (1974a).

The decreased binding of 4-CA in a 24 hour period reported by Bollag et al. (1978) may be due to one or both of the following reasons: (a) ca. 1/2 the soil organic matter content = ca. 1/2 the amount of binding, and (b) pH is neutral for this study, whereas for Hsu and Bartha's a pH of 5.8 would favor ion-exchange, coordination and charge-transfer complexes via protonation of the amino group.

In order to obtain better characterization of the bound residue, Bollag et al. (1978), attempted to extract the soil again by Bleidner distillation, which simultaneously uses alkaline hydrolysis and steam distillation. They used sterile and nonsterile soil which was incubated with [ $^{14}\text{C}$ ]4-CA for a 6 week period and extracted by soxhlet using methanol. They reported that 56.8 versus 28.8%  $^{14}\text{C}$  was ex-

tracted from the steam distillate into isooctane; 34.6 versus 58.7%  $^{14}\text{C}$  remained nondistillable in alkali, and 8.8 versus 12.5%  $^{14}\text{C}$  was left in soil humin fraction. For the 24 h experiments nearly all the bound radioactivity could be solubilized and most of this extracted into isooctane (most of which had an  $R_f$  equal to that of the 4-CA standard). Hsu and Bartha (1974a) found that ca. one-half of the bound [ $^{14}\text{C}$ ] propanil or 3,4-DCA, was liberated by acid or base hydrolysis, from weathered soil. They concluded that since they were able to release more radiolabelled materials with the Bleidner method, that it appears that the differences of hydrolyzable compounds depends on the method applied. In fact, Hsu and Bartha (1974a) released 93.6% (47.5% nonextractable from alkali) of the radiolabel from [ $^{14}\text{C}$ ] propanil of which 35.8% was 3,4-DCA (using alkaline hydrolysis followed by steam distillation). Bollag et al. (1978) using the Bleidner method released 87.5% (58.7% nonextractable from alkali) of the radiolabel from 4-CA, of which an unreported amount was parent 4-CA itself (6 week incubation); a 1 day incubation resulted in "nearly all" radioactive label being solubilized and extractable into isooctane. All of this  $^{14}\text{C}$  had an  $R_f$  value coinciding with the 4-CA standard.

The Bollag et al. (1978) conclusions regarding Hsu and Bartha's work cf. their own appear to be inaccurate. The methods applied to extract bound chloroanilines are similar, rather than dissimilar, and, in fact, the most obvious differences are in the nature of the soil, length of weathering, activation of the soil and the different

chloroanilines studied.

Higher volatilization (Bollag et al., 1978) from sterilized soil may be due to the alteration of soil physicochemical properties during autoclaving or to eliminated biological activity which is in part responsible for the transformation or binding of the anilines in soil. However, volatilization or sterilization accounted for 25% or less of the applied radioactivity for all anilines studied.

Suess et al. (1974) reported that the adsorption capacity of several aniline derivatives had no relation to clay and organic matter content of three differing soils. Approximately 72% 4-CA, 32% 3,4-DCA, and 43% aniline.H<sub>2</sub>SO<sub>4</sub> were nonextractable from the same three soils.

Binding of trifluralin to soil has been recently reported by Wheeler et al. (1979). As much as 10% of the <sup>14</sup>C was bound after 10 days at 10 ppm level, 30% at 35 days, and 72% after 63 days. At the 1000 ppm level, ca. 30% was bound at 63 days and only ca. 5% at 20,000 ppm. The dealkylated metabolite was 57% bound in 4 h to a sterilized soil. This agrees in analogy to Katan and Lichensteins' (1977) work with the bound radioactivity from [<sup>14</sup>C] parathion being due to aminoparathion. Other trifluralin dealkylated, nitro reduced metabolites were thought to be responsible for the bound radioactivity.

Burge and Gross (1972) studied the fate of aniline, 3-CA and 3,4-DCA in soil at 52, 60 and 74 ppm treatment levels respectively. The incubation period was not indicated. Recoveries of free anilines

were surprisingly high for a simple shaking using EtOH (95%); 58-92% (aniline), 57-77% (3-CA) and 89-101% (3,4-DCA) extracted.

Shiue and Wang (1977) found that, for the phenylurea herbicides monuron and diuron, applied to several whole soils (incubated for 24 h), 12.7 to 52.5% and 54.6 to 79.5% of the herbicide was still bound after exhaustive extraction.

Van Bladel and Moreale (1977) investigated various physico-chemical aspects of soil responsible for adsorption of 4-CA at the 80 ppm treatment level. At a soil/solution ratio of 1:1 the organic colloid contributed to the total adsorption variation by 85%, while at a 1:10 ratio, the percentage was 62%. Results also revealed the existence of the phenomena of hysteresis which increased with the rising percentage of organic material in the soil. They concluded that the stability of the complexes formed was responsible for the irreversibility of the adsorption processes observed.

Suess et al. (1978) reported the degradation of aniline, 4-CA and 3,4-DCA in various soils at the 1 ppm level over a period of 10-16 weeks. Aniline was mineralized during 10 weeks from 16.2 to 26.3%; 4-CA from 12.3 to 17.2% and 3,4-DCA 3.9 to 11.9%. Extractable  $^{14}\text{C}$  was 0.8-5.2%; 1.0-3.7% and 2.5-5.8% respectively, while bound radioactivity was 57.3-67.4%, 70.3-84.1% and 80.6-95.8% respectively. Various solvents could not extract this bound radioactivity, however, the extractants used and the amounts extracted were not described.

Again, Fuchsbichlen et al. (1978) reported their studies concerning the degradation of [ $^{14}\text{C}$ ] 2-chloro, 3-chloro, 4-chloro,

and 3,4-dichloroaniline in two different soils over a period of 16 weeks (at 30°C). Treatments were at the 100 ppm level. Extractable  $^{14}\text{C}$  for most chloroanilines varied between 5-10% of the applied  $^{14}\text{C}$  and between 50-80% was bound. 4-CA gave about 6% extractable and 70% bound radioactivity for both soils. For the soil higher in % OM (9.2% versus 1.3%) 4-CA exhibited 5.1% extractable  $^{14}\text{C}$  and 73.3%  $^{14}\text{C}$  bound.

A pyrolytic method for release of lignin bound 3-CA and 3,4-DCA from plants was developed recently by Balba et al. (1979). Plant-bound residues were pyrolyzed in a Dohrman combustion furnace modified to use a stainless steel combustion tube in place of a quartz one. Optimum conditions were established and the volatile products were swept through the tube (in a stream of He) and trapped in a dry-ice acetone cooled "U"-tube plus a secondary 25% HCl-CH<sub>3</sub>OH trap. The aniline was extracted from the basified trap solutions and derivatized to its trifluoroacetate derivative for quantitation by EC-GLC.

For 3-CA, 92% of the original radioactivity was released and trapped in the pyrolysate - 41% (of 92%) of which was extracted from alkali and 38.0% identified as 3-CA-TFA; for 3,4-DCA, 84% of the  $^{14}\text{C}$  was trapped in the pyrolysate - 52.5% of which was extractable from base and 50.2% identified as 3,4-DCA-TFA. Recoveries from lignin-bound residues which were acid hydrolyzed prior to pyrolysis yielded recoveries ca. 50% lower than the above.

Still et al. (1976) studied the extraction of bound 3,4-DCA in

rice roots. Strong oxidative digestion oxidized products to  $^{14}\text{CO}_2$ . Acid digestion solubilized 40%  $^{14}\text{C}$ . Dioxane-HCl removed most of the bound  $^{14}\text{C}$  but isolated products were not stable and could not be stabilized. The use of other lignin solubilization reagents also led to the formation of unstable products.

How should bound residues be measured? The Vail ACS-Bound Residue conference (Kearney, 1976) concluded that the classical method recommended by the AIBS Committee (American Institute of Biological Sciences - Environmental Chemistry, Task Group 1974 Meeting) which subsequently appeared in the Environmental Protection Agency's "Guidelines for Registering Pesticides in the U.S." (Federal Register 40 [123]: 26802-26928, June 25, 1975) be followed until a better method(s) is developed and verified. It involves extraction of organic matter from soils with caustic alkali and further subdivision of the extract by partial precipitation with mineral acids for separation of humic (precipitation) from fulvic acids (acid soluble). This is the classical fractionation scheme for organic matter which was first proposed over 200 years ago.

Although it is simple and conducive to routine analytical techniques, it suffers the disadvantage of employing rather strong reagents to separate the humic and fulvic acid fractions. Consequently there has been criticism as to the integrity of the extracted components. These alkali oxidative conditions may also alter the bound pesticide moiety to the point where it no longer

resembles what is initially bound in the undisturbed soil organic matrix. It was concluded that methodology needs considerable future attention. It is the opinion of the author that attention will only be fruitfully directed through a more lucid and fundamental understanding of the nature of bound residues. Therefore, mechanisms of binding have been evaluated in terms of coordinating knowledge of the structure of humic substances and thermodynamics of binding studies from related fields to propose a new model to explain the phenomena of recalcitrant residues in soil organic matter.

#### E.1 Chloroaniline binding mechanisms

Hsu and Bartha (1974b) speculated that the hydrolyzable 3,4-DCA was presumably bound as an anil or anilino-quinone to the aldehyde and quinone groups of the humic and fulvic acids; the remaining 3,4-DCA was believed to be integrated into the core structure of the soil organic matter and to be held by ether bonds or a heterocyclic ring system that the amino group of the 3,4-DCA served to close. Such heterocyclic bonds would resist acid or alkaline hydrolysis.

A time-course study (Hsu and Bartha, 1974b) of the binding of [ $^{14}\text{C}$ ]4-CA (5 ppm) to sterile soil showed that within 27 h, binding was near completion at ca. 80% binding. Similar kinetics were observed using humic acid although experimental techniques used in their work may be somewhat open to criticism; e.g., precipitation of treated HA solutions prior to extraction of unbound  $^{14}\text{C}$ ); although the authors state "solubility and diffusion did not appear to be

limiting factors so that the curve is regarded to approximate the actual reaction rate of 4-CA with the soil organic matter." They found that biological activity was not required for binding of 3,4-DCA to humic material. Binding of 3,4-DCA to isolated humic and fulvic acids was similar to that experienced with whole soil. The use of more aggressive extractants such as NaOH increased binding cf. neutral pyrophosphate extractant; e.g., humic acid - NaOH yielded 77.7% <sup>14</sup>C bound cf. 57.3% using Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> at the 5 ppm treatment level and 30.7% vs. 16.3% at the 500 ppm level. Increasing concentrations of 3,4-DCA resulted in increased binding of 3,4-DCA to purified humic acid and soil, but the proportion of added DCA bound to soil or humic acid gradually decreased with higher concentrations. "Binding" to whole soil was more extensive than to a corresponding amount of purified humic acid, especially at high DCA concentrations; however, exhaustive solvent extraction (acetone and benzene) removed little of the 3,4-DCA bound to humic acid, but removed a large portion of 3,4-DCA bound to whole soil. After the solvent wash, whole soil retained much less 3,4-DCA than the corresponding amount of humic acid. Hsu and Bartha (1974b, 1976) conclude, therefore, that in whole soil a dual physical-chemical mechanism is active, i.e., physical - extractable, bound to inorganic components cf. chemical nonextractable, bound to humus acids. The nature of their treatment of humic acids, however, raises questions about the reliability of this statement (i.e., precipitation of treated HA solutions prior to extraction of unbound 3,4-DCA).



Less than 6.0% of the 3,4-DCA that remained attached to soil after exhaustive solvent extraction was released into solution after 36 h shaking with a large volume of water. Thus, these residues were no longer subject to equilibrium distribution or they were irreversibly bound under ambient conditions.

Studies were also reported regarding elucidation of the types of bonding between 3,4-DCA and humic acid. Free radical inhibition of binding at various concentrations was very low indicating at most a minor role for free radicals in binding mechanisms.

Using model humic acid monomers, only reaction products with aldehydes and quinones were obtained with 4-CA; phenol and carboxylic acids failed to react with 4-CA even when inorganic clay particles were added as possible catalysts. Hsu and Bartha (1974b) postulate that these model experiments elucidate the reactions likely responsible for hydrolysis-susceptible aniline binding. Hydrolysis-resistant model anil-compounds were not obtained; however, an explanation of such attachment would involve heterocyclic bonding with the N of the aniline and components of the humic acid. Finally, they conclude that, regarding analytical methodology for bound residues, there are those which are hydrolyzable (35-44%, base vs. acid) and those which are nonhydrolyzable for which no methodology exists. It is this last statement which appears to be open to question, since the authors have not considered the fact that the nature of the hydrolytic extraction step could be, and probably is, responsible for formation of an unknown amount of so-called nonhydrolyzable

or recalcitrant residues.

Hydrolysis alters the structure of the humic substances profoundly so it is not unreasonable that released anilines are trapped as the structure of the humic substances is altered, or that they may be initially included and prevented from release by dramatic changes in the 1°, 2°, 3° and 4° structure of the humic substances, thereby precluding their solvent extraction. Alkaline hydrolysis would also facilitate alkaline oxidation of the anilines; autooxidation products must also be considered as shown by several researchers (Holland and Saunders, 1968; Wood et al., 1977; Ward and Gretzen, 1970).

Hsu and Bartha (1979a) reported the mineralization of chloroaniline-humus complexes in soil and in cultures of soil fungi. Whole soil containing bound residues showed only 2.9%  $^{14}\text{CO}_2$  of total radioactivity released in 3 weeks near optimum hot and humid conditions for  $\text{CO}_2$  production. However, these actions failed to consider  $^{14}\text{CO}_2 \rightarrow \text{Na}_2^{14}\text{CO}_3$  formation. From, Hsu and Bartha's (1974a) projections of time course studies for  $^{14}\text{CO}_2$  production, complete mineralization would occur within 2-4 years from the soil studied. One soil fungi, Aspergillus versicolor, contained 25.9%  $^{14}\text{C}$  from a [ $^{14}\text{C}$ ]3,4-DCA-humus complex incubation. Penicillium frequentans released only 0.5% of the  $^{14}\text{C}$  cf. 1.3% for A. versicolor. Mathur and Paul (1967a, 1967b) also studied P. frequentans to utilize humic acids and release bound methoxychlor (Mathur and Morley, 1975). Hsu and Bartha (1976) using A. versicolor

(a "humus" degrading soil fungus) to release bound residues, reported that so-called "nonhydrolyzable" 3,4-DCA residues were not inherently resistant to biodegradation. They thought that a gradual shift of physical adsorbed 3,4-DCA and of hydrolyzable 3,4-DCA to the nonhydrolyzable form occurred, since during a time-course experiment nonhydrolyzable residues increased slightly while hydrolyzable residues decreased, yet A. vesicolor utilized either ( $[^{14}\text{C}]3,4\text{-DCA} \rightarrow ^{14}\text{CO}_2$ ) equally well. Further, in regard to site or nature of binding, they suggested that the resistance of the residue to solvent extraction and ion exchange combined with a sensitivity to acid as well as alkaline hydrolysis strongly suggested a covalent binding of the nitrogen of chloroanilines to, for example, the carbon of a carboxyl group, or to a quinonoidal ring of the humic compounds. However, these results with soil fungi suggest also that the anilines may be inclusion complexes, and as the fungi utilize or break the complexes down, the  $[^{14}\text{C}]$ chloroanilines are released, as they would be by hydrolytic breakdown followed by base catalyzed reaction products to so-called, inaccurately in this author's opinion, "nonhydrolyzable" residues.

Experiments using model humic compounds and chloroanilines resulted in heterocyclic reaction products of the phenoxazine and phenazine type. Hsu and Bartha (1976) cite references regarding similar nonhydrolyzable attachment for ammonia to model humic compounds; however, the use of aromatic humic precursors is premature since humic acid structure is still open to conjecture. Is it

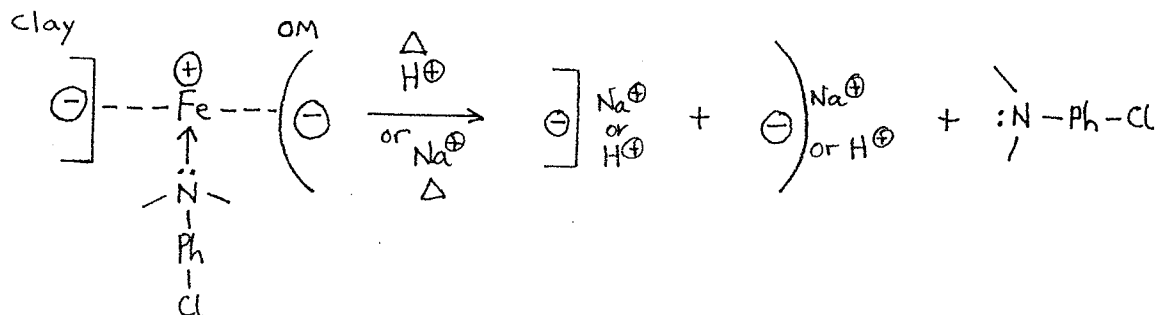
basically aromatic or aliphatic in its indigenous or natural state? Also the analogy between chloroanilines and ammonia in terms of reactivity is not the same. The lone pair of nitrogen electrons of 4-chloroaniline are extensively delocalized by the phenyl ring and the 4-Cl substitution and, therefore, not as readily available for overlap of orbitals in formation of a covalent bond.

Recently, Fletcher and Kaufman (1979), reported the metabolism of 2-, 3-, and 4-chloroaniline by isolated cultures of the soil fungus Fusarium oxysporum. Ortho-hydroxychloroanilines were isolated after 6 to 11 days of incubation; 1.2%, 0.7%, and 4.1% of 2-, 3-, and 4-CA were isolated as metabolites (% varied with inoculum age and size and different incubation periods), mostly as o-OH-chloroanilines.

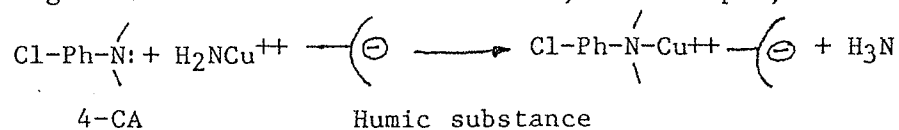
Ortho-aminophenols are relatively unstable molecules forming quinonoid structures which are very reactive in condensation and polymerization reactions with the organic matter in soils. Such a reaction could account for some bound radioactivity when radioactive parent compounds are used as tracers in evaluating extraction of bound residues. Even the above percentage may, however, be artificially high due to the use of pure cultures.

Moreale and Van Bladel (1980) stated that charge-transfer complexes between 4-chloroaniline (4-CA) and montmorillonite were involved in the high stability of 4-CA-clay complexes, especially when  $Fe^{3+}$  was the predominant cation (almost complete irreversible desorption, i.e. hysteresis). Such a model may very well apply to the other anionic substrates in soil - the humic substances. These were

very high in Fe content in the present studies (2.3%) so that sorption may be characterized by charge-transfer complexes which may be broken and desorption effected only by using more drastic (high energy input) methods such as acid or base hydrolysis as follows:



Ligand exchange or the complex formation combines ion exchange and coordination chemistry. Very strong interactions are involved with, for example, metal ions providing a strong driving force for ligand sorption (Helfferich, 1961). Such an interaction may be envisaged for 4-CA in humic substances, for example,



Complex formation is a highly specific interaction, much more so than physical adsorption or ion exchange. Protonated species ( $\text{Cl-Ph-NH}^{3+}$ ) would not be expected to be ligand bound. Release or exchange of complex such as that shown above may be affected by high  $\text{OH}^-$  or  $\text{Cl}^-$  concentrations and application of heat.

## E.2 Model reactions involving ammonia and amino acids with soil

This section describes some of the known reactions of other nitrogenous compounds within the soil. Possibly some insight pertaining to 4-CA interactions, by analogy, may be forthcoming. It has been shown that N-phenoxyamino acids can be formed readily by reaction of amino acids with phenols (Flaig, 1966). It has also been shown that the amino acid will not be hydrolyzed from such structures until they have been oxidized to the corresponding quinonimine (Ladd and Butler, 1966). When base oxidation is carried out in the presence of air this oxidation proceeds rapidly (Piper and Posner, 1968). Piper and Posner (1972) postulate that some of the amino acids bound into structures such as these, may account for some of the nonhydrolyzable nitrogen that has been reported in HA (Bremner, 1955). Total hydrolyzable-N equals about 45% of the applied dose (Piper and Posner, 1972). This may apply to 4-CA in soil or humic acids.

Allison (1973) doubted that much of the nitrogen in soil humus is the result of ammonia-lignin reactions, for if it were, one must conclude that such reactions can proceed under conditions of extremely low concentrations of ammonia and in acid soils. At present there is no evidence for this. In fact, several researchers (Mattson, 1943; Broadbent et al., 1960; Nyborg, 1969) stress the fact that this reaction is with ammonia and not ammonium ion, and hence can occur only at higher pH values. The concentration of ammonia normally formed in soil biologically would not be high enough to

raise the pH appreciably and thus allow the reaction to proceed. Sohn and Peech (1958) determined the total ammonia fixation by a number of inorganic and organic soils. Acid soils high in organic matter fixed the largest amounts of ammonia and the fixation capacity was increased by heating. At least 50% of the fixed ammonia could be attributed to its reaction with organic matter. Nommik (1965) and Mortland (1958) found that about 50% of the combined ammonia in a soil could be released by treatment with strong alkali. This is about the same amount of 4-CA released by alkaline hydrolysis from HA (i.e. 44%) in the studies to be discussed in the experimental section of this thesis.

The importance of considering the effects of alkaline extractions on the nature of the extractable compounds is shown by the work of Mortland and Woolcott (1965). Their extensive studies with model compounds indicate that the principal chemical reactions to be expected under natural conditions between ammonia and organic soil components involve the oxygen-containing carboxyl, carbonyl, enolic, phenolic, or quinonic hydroxyl groups, or unsaturated carbon sites. In acid soils, the concentration of reactive groups is low as is the ammonia fixation capacity; fixation that does occur is attributable to the slow production of oxidized groups. As the pH is raised and aeration improved, the structures become more polarized and reactive and the number of reactive groups is markedly increased, especially at pH values above seven. Fixation also increases with ammonia concentration and temperature.

When the pH, of the treated humic acid solutions (containing various amino acids), was maintained below pH 7.0, Schnitzer et al. (1974) found that humic acids per se did not interact chemically with the amino acids. Had a major binding mechanism involved chemical interactions between phenols or quinones and amino acids, one would have expected significant increases in reaction rates when the reaction mixtures were subjected to the high temperature of autoclaving; however, this was not observed.

Adams and Perry (1973) reported that humic acids (NaOH extracted from soil using NaOH) reacted with  $\alpha$ -amino nitrogen under alkaline conditions, and since isolation of the humic acid involves treatment of the soil with alkali, it is to be expected that reactions of the type recorded could take place during humic acid extraction. Possibly 4-CA would react in this way during extraction of HA from treated soil or during base hydrolysis of treated soil or humic acids thus producing a portion of nonhydrolyzable 4-CA, amounting to about 10% of the applied 4-CA.



## III EXPERIMENTAL

A Studies on the Release of Bound 4-CA from Soil Humic Acids and Whole SoilA.1 Introduction

Pesticides have the capacity to complex "tightly" and abundantly with soil humic acids. Concern has been expressed for the inability to analyze for bound (hydrolyzable or tightly complexed and nonhydrolyzable) residues; to date, the soil burden has been difficult to determine. Research was conducted to develop an analytical method to release and quantitate bound 4-chloroaniline (a common pesticide degradation product) from soil humic acids. Humic acids isolated from a Manitoba soil (with no known previous history of pesticide treatment) were treated with [ $^{14}\text{C}$ ]4-CA and the unextractable 4-CA\*HA complex was used to study methods of release and quantitation of the bound 4-CA. NOTE: humic acid and fulvic acid are synonymous with HA and FA respectively.

A.2 Materials and MethodsA.2.1 Reagents

- (a) Solvents - all pesticide grade - Caledon Co.
- (b) Heptafluorobutyric anhydride - Aldrich Chemical Co.
- (c) [ $^{12}\text{C}$ ]4-chloroaniline (4-CA) - Aldrich Chemical Co.; recrystallized three times from hexane, mp = 71-72°C.
- (d) [ $^{14}\text{C}$ ]-UL-4-chloroaniline.HCl - California Bionuclear Corp. 11.2 mCi/mM - aqueous solution basified and extracted

with benzene to yield the free base. TLC confirmation was based on  $R_f$  determined by autoradiography and iodine vapour reaction.

- (e) N-heptafluorobutyryl- $[^{12}\text{C}]$ 4-chloroaniline - primary standard synthesized by addition of excess heptafluorobutyric anhydride to 4-CA in benzene; recrystallization from hexane/methanol, mp = 93-94°C, spectral confirmation  $[M^+] = 323$ .
- (f) "Omnifluor" - New England Nuclear Corp. - 4 g/L toluene (Fisher Scintanalyzed) and 10 mL per vial was used for liquid scintillation counting of nonaqueous samples.
- (g) "Aquasol-2" - New England Nuclear Corp. for aqueous samples, 5 mL per sample.
- (h) Soil - Brandon, Carol clay-loam (or Black Chernozem), first 30 cm sampled from farm with no previous (known) history of pesticide treatment. Analysis: 9.3% organic matter, 30% sand, 38% silt, 32% clay, 0.6%  $\text{CaCO}_3$ , and pH = 5.7 Cation analysis: (meq/100 g)  $\text{Ca}^{2+} = 30.3$ ,  $\text{Mg}^{2+} = 7.0$ ,  $\text{K}^+ = 0.1$ ,  $\text{Na}^+ = \text{trace}$ ,  $\text{H}^+ = 2.8$ ; total = 40.2 meq/100 g.
- (i) Boron trifluoride methanol - 10-14% (W/V) Aldrich Chemical Co.
- (j) Diazomethane - generated from N-methyl-N'-nitro-N-nitrosoguanidine (Aldrich Chemical Co.) (Caution - potential carcinogen, mutagen, irritant) according to the method of Stanley (1966).
- (k) "Carbosorb," "Permafluor" - Packard Instrument Co., Inc.

### A.2.2 Apparatus:

- (a) Gas Liquid Chromatograph (GLC) - Varian Model 2440, equipped with a tritium foil electron capture (EC) detector. Operating conditions: temperatures ( $^{\circ}\text{C}$ ) - injector = 180, column = 150, detector = 210; nitrogen carrier gas flow rates = 45 ml/min.
- (b) GLC Column - pyrex 1.7 m x 4 mm id., packed with 3% OV-17 (V/W) on 120-130 mesh Anakrom A/B.
- (c) Mass Spectrometer - Finnigan 1015 with direct inlet probe accessory.
- (d) Pyrolyzer - Chemical Data Systems Corp. 18580A pyroprobe - solids analyzer, with macro quartz, micro platinum probes and a macro platinum probe especially designed for use with the instrument. Operating conditions are given in the tables following.
- (e) Radioassay - Searle Model 6880 Mark III liquid scintillation spectrometer (counter - LSC) was used to assay radioactivity with a low level activity program ( $E^2/B$ ) for dpm computation.

Radioactivity in solid residues were determined as  $^{14}\text{CO}_2$  by combustion in a Packard-Tricarb Model 306 Biological Oxidizer, followed by liquid scintillation counting of the trapped combustate (Carbosorb 5 mL; Permafluor 10 mL than 98%). Combustion of  $^{14}\text{C}$  standards assayed at greater than 98%.

- (f) Ultrasonic apparatus - Artek Systems Corp. Sonic Dismembrator with titanium microtip.

### A.2.3 Methodology

#### (a) Isolation of humic acids from soil

Figure 9 outlines details of the isolation procedure. The soil was washed with 1.0N HCl to remove carbonates followed by distilled water (ca. pH 5) to remove excess HCl. Humus substances were extracted with 0.5 N NaOH under nitrogen after purging the solution with nitrogen. Centrifuging removed the insoluble (humins) material and acidification of the supernatant with HCl caused flocculation of the humic acid fraction, + the fulvic acid fraction remaining in solution. All procedures were performed with nitrogen purged systems. The humic acid precipitate was recovered by centrifuging and was washed thoroughly with distilled water to remove excess HCl (0.1 N AgNO<sub>3</sub> test for Cl<sup>-</sup>).

Humic acid recovered by this method accounted for 16% of the total organic matter (determined as total organic carbon). Approximately 50% of the isolated humic acid was accounted for as inorganic components, i.e., 50% ash, determined at 600°C for 3 h in a muffle furnace.

#### (b) Preparation and hydrolyses of 4-chloroaniline "bound" humic acid samples

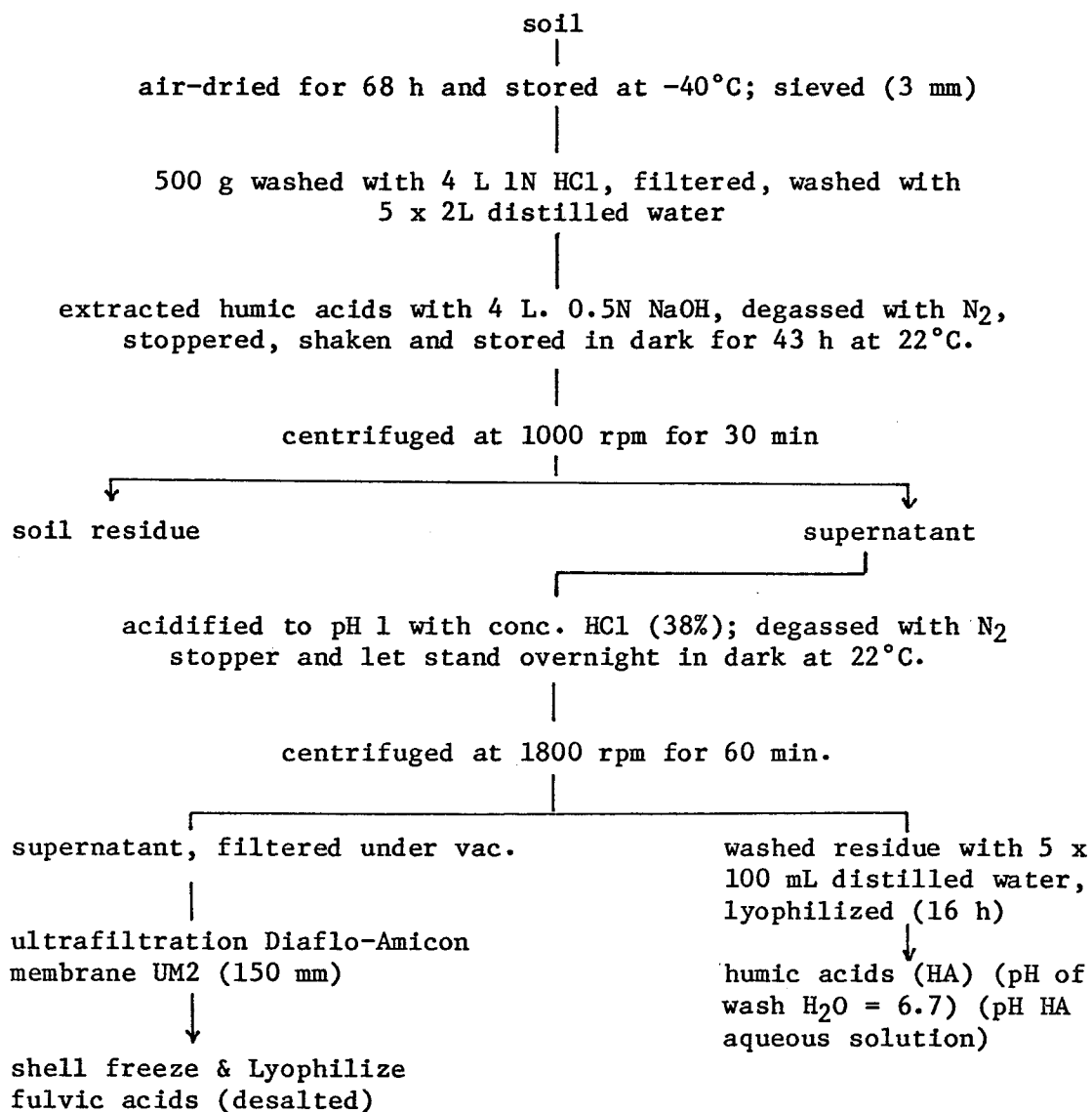


Figure 9. Isolation of humic and fulvic acids from a Manitoba Carrol clay-loam soil.

Humic acids (HA) were treated (at the 477 ppm level with 4-chloroaniline) as indicated in Figure 10. The humic acids were added to distilled water and the solution incubated for 5 days (for reaction equilibrium), after which the solid HA was filtered off. After removal of extractable (free) radioactivity ( $^{14}\text{C}$ ) the residue was hydrolyzed under reflux for 2h with base or acid and the Rachig ring column washed with distilled water into the hydrolyzate. The residues were recovered and termed "nonhydrolyzable" residues and the filtrate  $^{14}\text{C}$  (extracted using  $\text{C}_6\text{H}_6$  prior to precipitation of the HA for base hydrolysis) after hydrolysis termed the "hydrolyzable" fraction. Specific activities were determined by oxidation of dried residues to  $^{14}\text{CO}_2$  in a Packard Biological Oxidizer, as follows: nonhydrolyzed HA (neutral) sp. ac. = 1124 dpm/mg humic acid; base hydrolyzed HA (neutral) sp. ac. = 104 dpm/mg.; specific activity based on 4-CA = 2340 dpm/mg.

(c) Whole soil treatments

Fifty grams (dry wt.; Brandon Carroll clay-loam) of soil was placed in a 250 mL erlenmeyer flask and treated at the 500 ppm level with [ $^{12}\text{C}$  +  $^{14}\text{C}$ ]4-CA (in 95% EtOH) and enough distilled water to bring soil to 80% of field capacity added dropwise, with shaking. The flask was stoppered, shaken thoroughly and let stand at room temperature in the

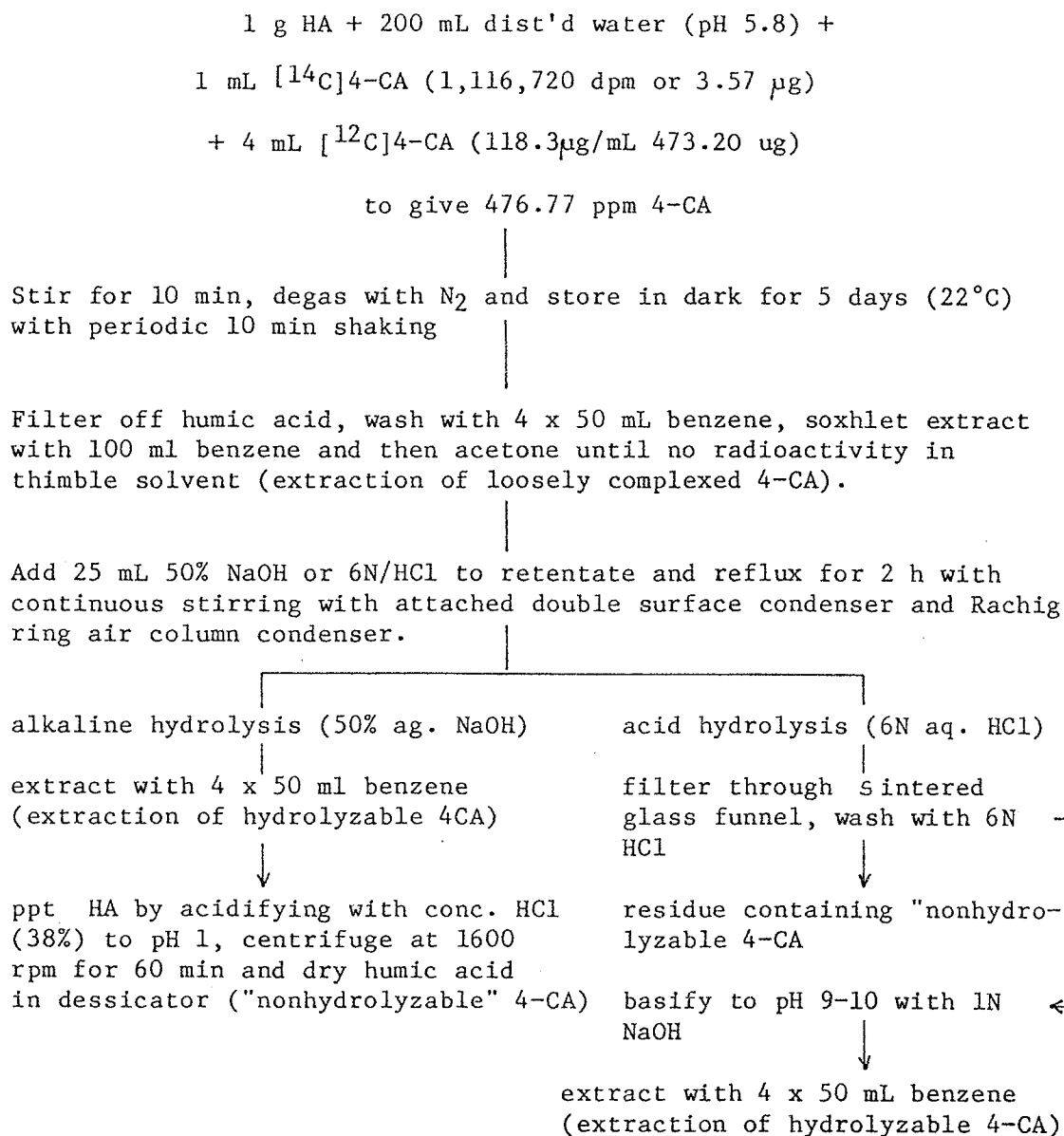


Figure 10. Treatment of humic acids and hydrolyses.

dark. Aeration was carried out the first day for 3 x 1 min intervals.

The flasks were unstoppered and left to air dry in fume hood until completely dry, and stoppered until analysis.

(d) Other extraction methods for nonextractable "bound" 4-CA\*HA complex (Figure 11)

(1) Boron trifluoride-methanol (10-14%), heptafluorobutyric anhydride (20% in acetone), N,N-Dimethylformamide.

BF<sub>3</sub>.MeOH and HFBA were investigated as HA derivatization reagents, i.e., derivatization of HA carboxyl groups and C- and O-heptafluorobutyrylation respectively, to release and/or solubilize complexed 4-CA. DMF was investigated for its solvent properties and therefore, solubilization of bound 4-CA. A sample of humic acid containing nonhydrolyzed "bound" radioactivity was refluxed for 1h with each solvent. After cooling, the solution was filtered through a sintered glass funnel and the HA washed thoroughly with the same solvent used for extraction. The filtered residue was collected and dried in a desiccator under vacuum.

(2) Ultrasonic (microprobe) extraction with (i) distilled water pH 9.2 and (ii) N,N- dimethylformamide. 10 mL of solvent in a 15 mL glass - stoppered centrifuge tube plus ca. 20 mg nonhydrolyzed HA bound 4-CA complex was used with the micro probe for 30 minutes at maximum power and an ice bath



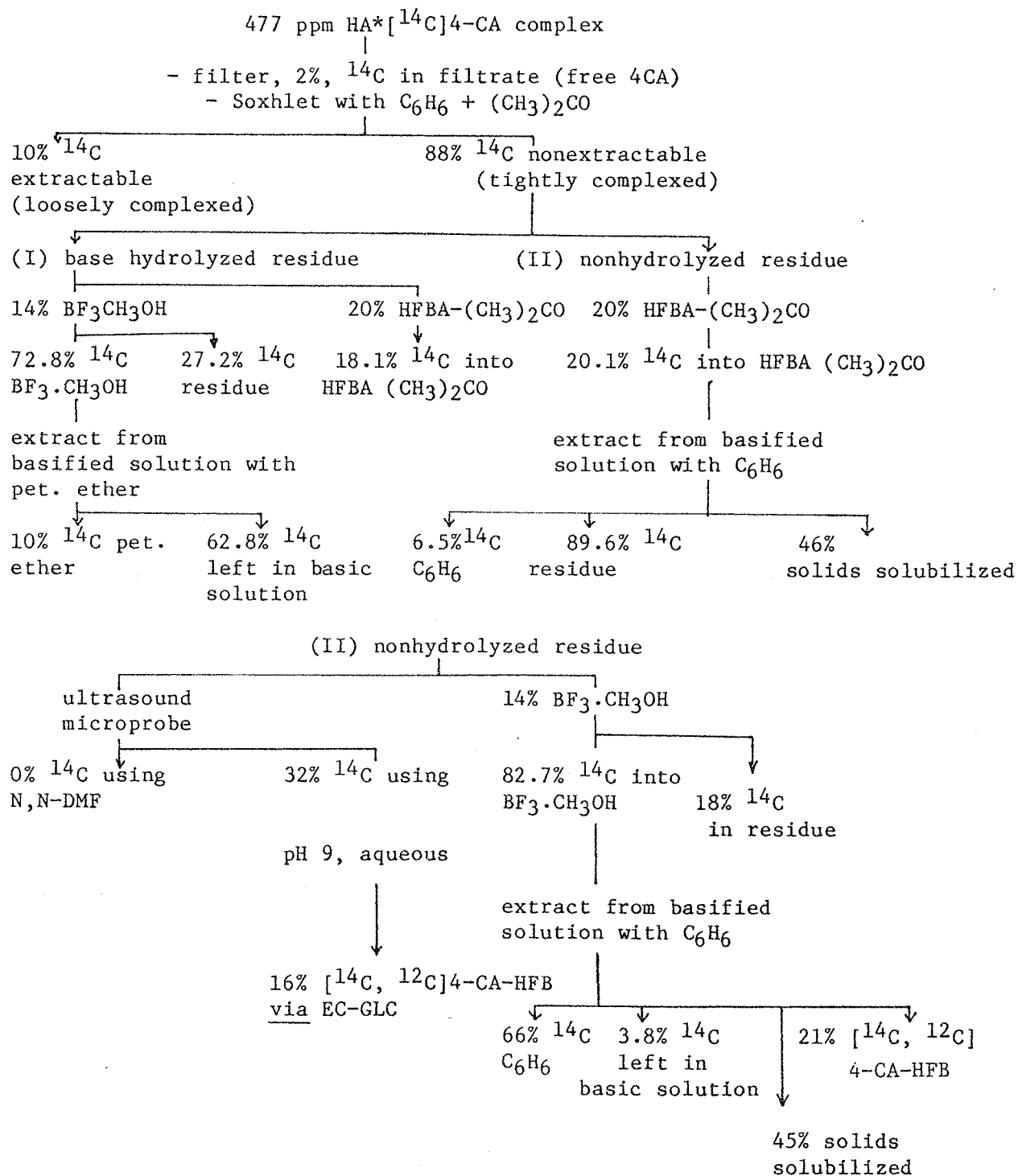


Figure 11. BF<sub>3</sub>CH<sub>2</sub>OH, HFBA-(CH<sub>3</sub>)<sub>2</sub>CO, and Ultrasound procedures and results.

surrounding the centrifuge tube to dissipate heat. After sonication, the extract was adjusted to pH 12 and extracted with 3 x 5 mL hexane. Aliquots of the hexane extract were analyzed by LSC.

(3) Diazomethane treatment (for HA and whole soil, Figure 10)

followed by: [i] hexane extraction and methanol extraction  
[ii] a nonmethylated soil sample refluxed with 20% distilled water in methanol.

Diazomethane treatment was used to investigate the release of bound 4-CA via methylation of the humic acid carboxyl groups and thus destroy the tertiary structure and perhaps release 4-CA (if present as an inclusion complex).

Freshly prepared diazomethane in diethyl ether was added to HA (477 ppm containing nonextractable bound residues) or whole soil (477 ppm, containing extractable plus nonextractable 4-CA) until the yellow color remained for at least 1 h. The excess diazomethane was destroyed with acetic acid and the ether evaporated off in the fume hood. For extraction [i], 4 mL of hexane was added, shaken briefly, and filtered through a 13 mm lining (0.5  $\mu$ m) filter adapter and the entire filtrate counted. The retentate was reextracted with methanol and the filtrate counted.

The second [ii] extraction was performed with fresh soil (nonmethylated) and refluxed for 30 min, filtered as above and made up to 50 mL with methanol. Aliquots were counted in the LSC.

(e) Pyrolysis, Cleanup and Analysis of Pyrolyzates

Pyrolysis was investigated in detail, as it was anticipated that it would release more tightly complexed residues intact than previously available methods. Studies with filament-type probes were begun by varying the temperature of the probe filament in increments of 100° from 100° to 900°C.

Type of Probe

(1) micro platinum - dried unground or ground (agate mortar and pestle) samples of HA were weighed into a 1 mL volumetric flask (ca. 50 mg) diluted to 1 mL with N,N-dimethylformamide and shaken thoroughly at least 3 days prior to usage. Prior to use the solution was shaken thoroughly again to suspend any undissolved HA. Using a 50  $\mu$ L syringe, 2 x 10  $\mu$ L (ca. 1 mg) was withdrawn and applied carefully to Pt ribbon probe, placed in an inert atmosphere ( $N_2$ ) and N,N-DMF flashed off at 75°C with a 2s interval.

(2) macro quartz - dried unground or ground samples were weighed into a quartz tube (ca. 10 mg) containing a small pledget of quartz wool at one end. Quartz wool was placed into the open end after weighing and the quartz tube was inserted carefully into the Pt coil probe.

(3) macro platinum - a stainless steel probe was designed with a boat opening tooled out at one end. This was lined

with platinum foil and the dried and unground or ground samples weighted into it (ca. 80 mg).

(4) macro stainless steel - as above without Pt foil covering.

The pyroprobe was inserted into the inlet of the quartz lined pyrolyzer interface (Figure 12) and the collection tube was inserted into the outlet with the "U"-tube immersed in acetone - dry ice cooling mixture. The end of the collection tube was immersed in 5 mL 25% methanolic HCl contained in a 25 mL glass stoppered separatory funnel. Pyrolysis conditions are given in Table 6. The collection tube was removed from the pyrolyzer interface and washed with 10 mL 25% methanolic HCl into the separatory funnel. The condensate was concentrated to ca. 5 mL under N<sub>2</sub> on a 60% water bath, 10 mL 5N NaOH was added and the condensate was extracted with 4 x 10 mL benzene.

(f) Quantitation of Released 4-CA

To enhance the electron capturing properties and hence the EC detector sensitivity to 4-CA, a heptafluorobutyryl derivative was prepared. Heptafluorobutyric anhydride (0.5 mL) was added to the final extract (benzene) and allowed to stand at room temperature for 10 min in a glass stoppered 100 mL round bottom flask. The benzene solution was concentrated to 1 mL

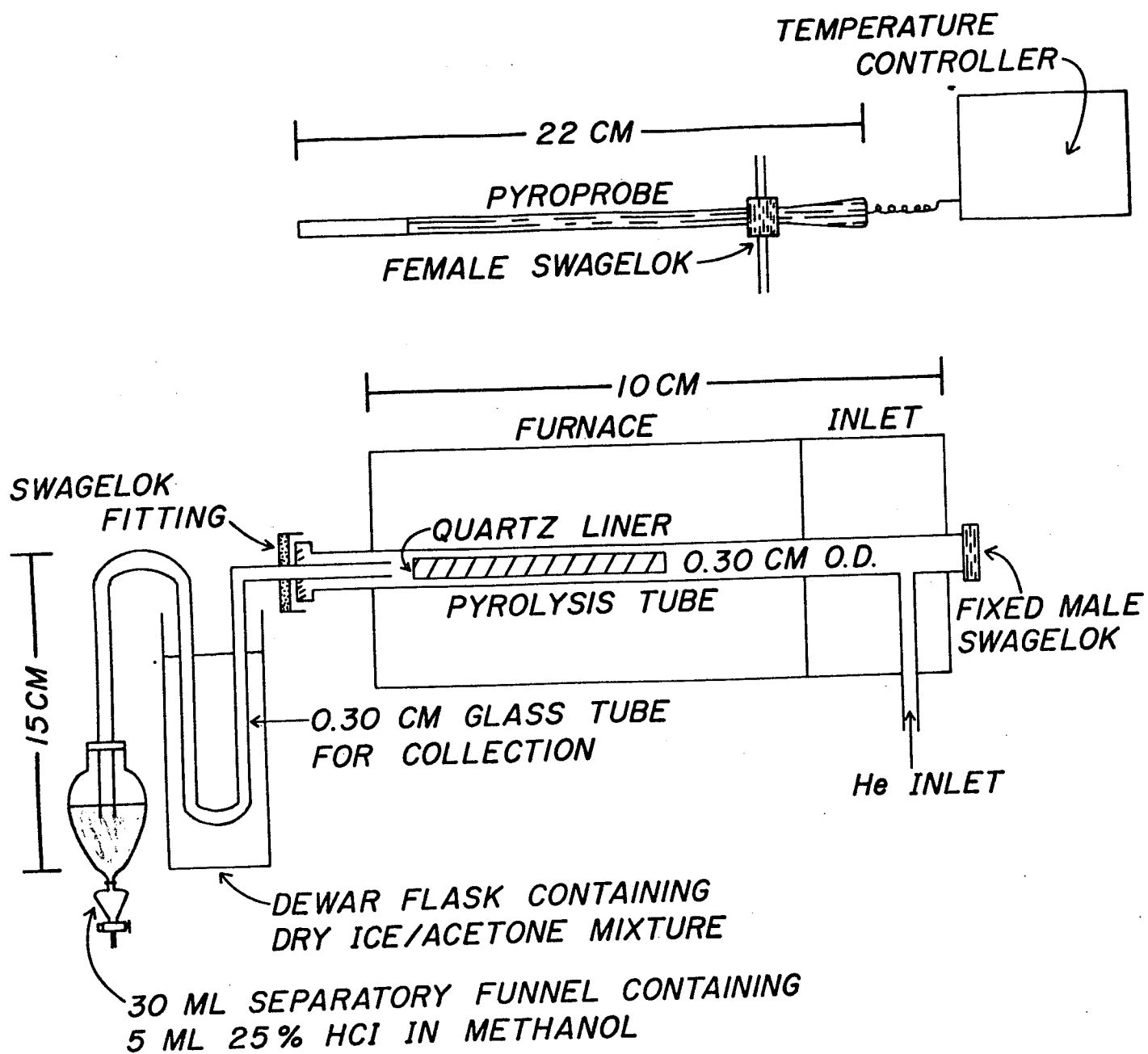


Figure 12. Apparatus used for pyrolysis of tightly complexed 4-CA.

Leaf blank to correct  
numbering

in a water bath (50°C) under a low flow of nitrogen. Hexane was added to the desired volume and an aliquot of the sample injected. Depending on the nature of the humic acid and the pyrolysis conditions employed (esp. temperature) the heptafluorobutyryl derivative was quantitated by EC-GLC analysis or, if the chromatogram was unsatisfactory, further purified by addition of 0.5-1.0 mL of hexane-washed distilled water, and aliquots injected from the hexane layer.

External standard method of quantitation was performed by comparing sample peak heights with those of a 4-CA-heptafluorobutyryl (authentic) standard solution peak height, within the dynamic linear range of the detector. The structure of the derivatized standard was confirmed by mass spectrometry.

### A.3 Results and Discussion

#### A.3.1 Hydrolysis Methodology

Figure 13 shows that acid or base hydrolysis of "bound" 4-CA in HA released 38 and 46% of the original  $^{14}\text{C}$  as 4-CA determined as its heptafluorobutyryl derivative using EC-GLC. A control HA was free of interferences at the  $R_T$  for 4-CA-HFB. Ten percent of the applied [ $^{14}\text{C}$ ]4-CA was extractable ("loosely-complexed") into benzene/acetone and 88% of the radioactivity was nonextractable or bound ("tightly-complexed"). Two per cent of the radioactivity was found to be in the original filtrate obtained after

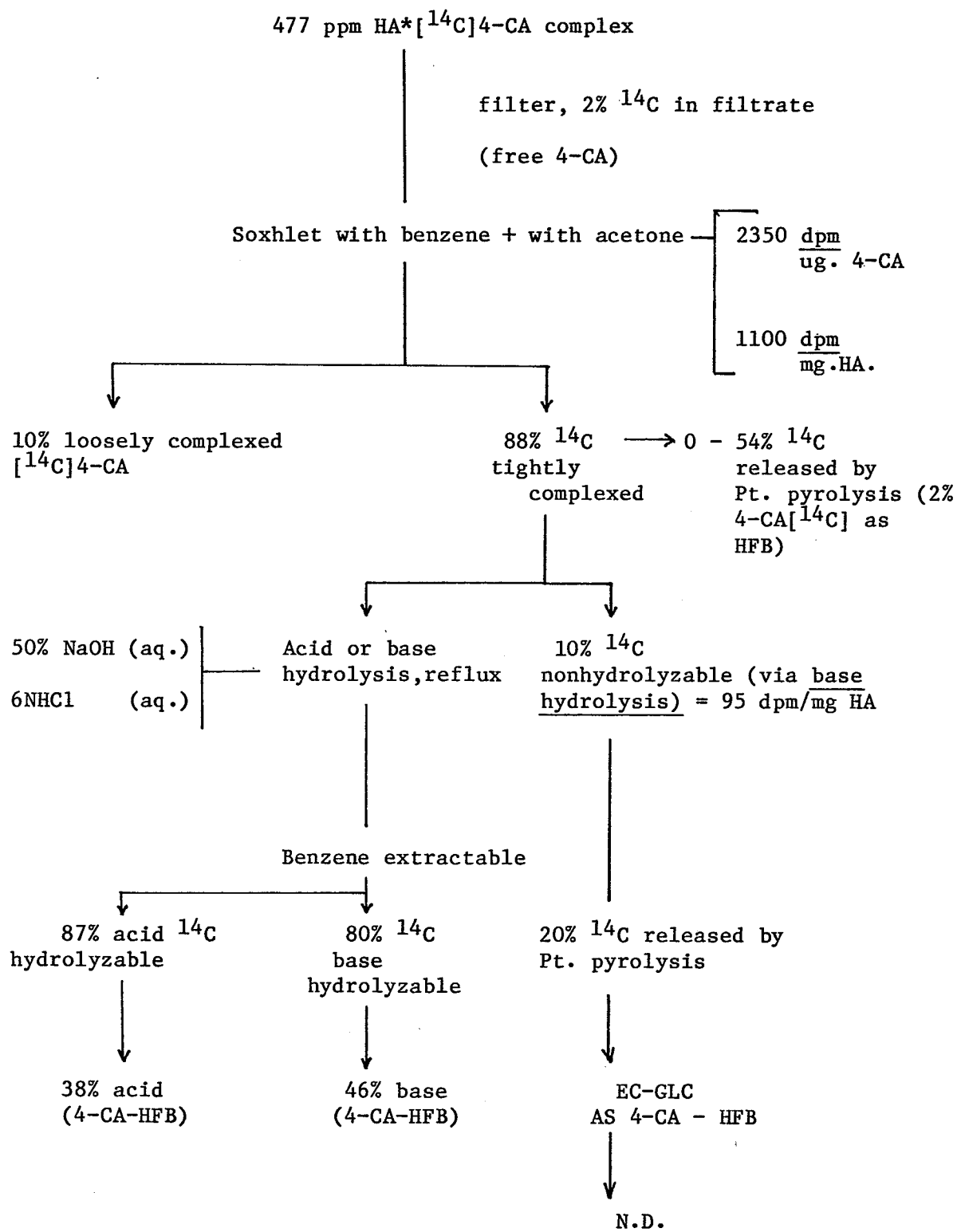


Figure 13. Hydrolysis procedure and results for HA\*<sup>14</sup>C]4-CA complexes (tightly complexed).



incubation; this was termed "free" or nonadsorbed 4-CA. Similar results were reported by Hsu and Bartha (1974b). They reported 35.8% for 3,4-DCA from propanil treated (500 ppm) soil following 50% NaOH hydrolysis and 44.9% from 70% H<sub>2</sub>SO<sub>4</sub> hydrolysis. Fuchsbichler et al. (1978) treated two soils (9.2% OM and 1.3% OM) with 2-CA, 3-CA, 4-CA, and 3,4-DCA; extractable residues accounted for 3.4 and 18.8, 5.0 and 8.6, 5.1 and 7.4, and 6.1 and 6.2% of the applied <sup>14</sup>C, respectively. Alkaline hydrolysis was applied (3 h reflux) to release nonextractable 3-CA and 3,4-DCA residues; this hydrolysis released 35% (of 75% bound) and 25% (of 51% bound) for 3-CA, 53.5% (of 88.9) and 30.7% (of 67.7) released for 3,4-DCA; all released <sup>14</sup>C was identified as the intact chloroaniline. Again these results are of similar magnitudes for basic hydrolytic release of substituted chloroanilines from treated soil or soil HA, i.e., 31 to 54% depending on the CA analog, and for whole soil, the amount of organic matter.

Cheng (1978) reported that alkaline hydrolysis was not an efficient treatment in solubilizing 3,4-DCA; however, he found that acid hydrolysis (6 N HCl) was most effective in removing soil-bound 3,4-DCA. Possibly this contrast to Hsu and Bartha's work lies in soil differences, especially the organic matter content of the soil studied. Cheng (1978) proposed that acid treatment was effective in release of

3,4-DCA due to its protonation at low pH; however, Moreale & Van Bladel (1976), and Van Bladel and Moreale (1977) have shown that at low pH, or as pH decreases, adsorption of 4-CA increases.

Sutherland (1974) reported that rice plants, grown in [ $^{14}\text{C}$ ]propanil treated soil contained nonextractable  $^{14}\text{C}$  residues as follows: hemicellulose - 8.7%, lignin - 24.4%, cellulose - 2%; alkaline hydrolysis released 48.4, 63.2, and 47.3%  $^{14}\text{C}$  as 3,4-DCA respectively. Since such natural products are precursors to HA in soil, it is interesting that similar percentages of 3,4-DCA were released by alkaline hydrolysis from soil. Either such products could be part of HA and/or the mechanism of complexation are similar. Again, Still *et al.* (1976) found ca. 40% of bound  $^{14}\text{C}$ , from insoluble 3,4-DCA treated rice plant root complexes, were released with strong acid (HCl), which is analagous to acid hydrolysis of 4-CA\*HA releasing 38% [ $^{14}\text{C}$ ]4-CA in this study (Fig. 11).

#### A.3.2 The nature of nonhydrolyzable residues

Still *et al.* (1976) attempted to solubilize lignin bound [ $^{14}\text{C}$ ]3,4-DCA using HCl, HBr,  $\text{HClO}_4$ ; ca. 40%  $^{14}\text{C}$  was released, but products isolated were not stable. Dioxane in HCl (9:1) released most of the  $^{14}\text{C}$  but the isolated products were again labile. Hydrolysis products

were generally unstable. A similar explanation may apply to the release of radioactivity by reagents used herein, portions of which were shown to be compounds other than 4-CA. Although Hsu and Bartha (1974b) have quantitated the released "bound" 3,4-DCA derived from propanil treated soil (Bartha, 1968) by FID-GLC neither publication indicates the sensitivity or reproducibility attained with this method. Fuchsbichler et al. (1978) used GLC to quantitate 3-CA and 3,4-DCA released from hydrolytic treatments of soil but no details are given regarding the quantitative aspects.

Bollag et al. (1978) studied release of bound substituted anilines from treated soil and for [ $^{14}\text{C}$ ]4-CA, found that alkaline hydrolysis released 40.3%  $^{14}\text{C}$  into isooctane from aqueous base for autoclaved soil and 20.8%  $^{14}\text{C}$  from nonautoclaved soil. Similar amounts of  $^{14}\text{C}$  were left in soil as nonhydrolyzable residues following alkaline hydrolysis; i.e., 7.5% [ $^{14}\text{C}$ ] propanil about 1 year after treatment (Hsu and Bartha, 1974a); 12.5% 4-CA, 10.6% 4Cl-o-toluidine, 17.8% 4-isopropylaniline, 16.5% 2,6-dimethylaniline 6 weeks after treatment (Bollag et al., 1978); and 31 and 0% 3-CA and 25 and 76% 3,4-DCA 6 weeks after treatment of soil containing 4.2 and 1.3% organic matter respectively (Fuchsbichler et al., 1978). Since similar amounts (10.6-17.8%) of "nonhydrolyzable" residues exist for different compounds, covalent binding of these

nonhydrolyzable residues appear unlikely (and 7.2-9.4% this study, Table 2).

Further, ca. 10-20% (varies with soil pH) of several  $^{14}\text{C}$  labelled amino acids were nonhydrolyzable from treated soil following acid hydrolysis (Adams and Perry, 1973). There was an indication, in addition, that the nonhydrolyzable portions of the total  $^{14}\text{C}$  incorporated increased with an increase in pH. They concluded that under alkaline conditions, humic acids reacted with the  $\alpha$ -amino nitrogen. Thus, extraction of the HA with alkali could produce such reaction products which are nonhydrolyzable. Another study by Perry and Adams (1971), reported 11.3% nonhydrolyzable (with acid) of glycylglycine in HA.

Prolonged 6N HCl hydrolysis or strong base hydrolysis, as well as mild acid and base conditioning can lead to possible increases in the degree of condensation, aromaticity, polymerization, oxidation or other structural alterations in the HA as well as providing an environment favorable to in-vitro reactions (Riffaldi and Schnitzer, 1973; Bremner, 1954; Kosaka et al., 1961; Bremner and Lees, 1949; DeSerra and Schnitzer, 1972; Tinsley and Salam, 1961; Sauerbeck and Fuhr, 1968; Posner and Creeth, 1972; Rashid, 1972; Farmer and Morrison, 1960).

Acid precipitation or hydrolysis of HA\*4-CA complexes may

Table 2. Analysis of 4-chloroaniline - humic acid complexes.

Substrate	Extractable <sup>14</sup> C (C <sub>6</sub> H <sub>6</sub> )		% <sup>14</sup> C		Supernatant after nonhydrolyzable HA precipitated	Nonhydrolyzable HA-4CA complex	
	Shaking	Soxhlet	Hydrolyzable C <sub>6</sub> H <sub>6</sub> base	extractable acid		base	acid
triplicate analysis							
HA * 4-CA complex	2.9	7.8	82.5 <sup>a</sup>	87.0 <sup>b</sup>	2.4	9.4	7.2
control, no HA	99.1	-	0.59	-	0	-	-

a,b = 70.5 & 79.5% <sup>14</sup>C in EC-GLC solution respectively.

cause untoward reactions with aniline. Quinones are the end product of aniline in sulphuric acid solution with manganese dioxide (Fieser and Fieser, 1961). Other products may be dimeric phenazines and phenoxazones (Scott, 1965). This again indicates the potential of acid hydrolysis to facilitate the conversion of aniline and probably substituted anilines, in the presence of soil inorganic constituents (oxides), to nonextractable residues, i.e., nonhydrolyzable components [via polymerization of quinones or interaction of the initially formed free radical (Fieser and Fieser, 1961) with HA components.]

Schnitzer et al. (1974) reported regarding reactions of HA and amino acids, that humic acids per se did not interact with amino acids but the major reaction was with microorganisms and oxidative degradation leading to the formation of ammonium ion.

In addition, tightly complexed nonhydrolyzable (bound) 4-CA does not appear to be related to microbial activity, at least within a few months of incubation. I found 88% 4-CA tightly complexed by nonsterilized HA. Hsu and Bartha (1974b) reported 80% 4-CA tightly complexed to soil or HA whether sterilized or nonsterilized and Bollag et al. (1978) reported 49% 4-CA tightly complexed to nonsterilized soil. Differences appear to be reflected in percentage organic matter in the soil used or based on equivalent

amount of soil, i.e., 8.4, 6.0 and 3.4% organic matter respectively. Bollag et al. (1978) found no differences in the amount of 4-CA tightly complexed after 6 weeks incubation whether sterile or nonsterile soil was used. Also 9-12% of the applied radioactivity appears to be nonhydrolyzable (Hsu and Bartha, 1974b; Bollag et al., 1978 and this study) whether sterilization was used or not, and even after more than 6 months incubation (Hsu and Bartha, 1976). Finally, little difference exists in the percentage of nonhydrolyzable residues formed for variously substituted chloroanilines (7.9-17.9) in sterile or nonsterile soils (Bollag et al., 1978, Hsu and Bartha, 1974b, 1976). Such phenomena may again indicate that so-called nonhydrolyzable fraction is an artifact or the result of alkaline hydrolysis and not endogenous covalent bonding as proposed by Hsu and Bartha (1976). Thus aerobic hydrolysis at high temperatures with base for several hours may well be expected to produce an amount of radioactivity (10-20%) which is "nonhydrolyzable" because of the reactions (between xenobiotic and HA and occurring in-vitro inter and intramolecularly within the HA during the hydrolytic extraction procedure).

A.4 Other methods for release of tightly complexed and nonhydrolyzable 4-CA\*HA

#### A.4.1 BF<sub>3</sub> methanol extraction

Attempts to release tightly complexed 4-CA nonhydrolyzed and base hydrolyzed residues yielded large amounts of extracted <sup>14</sup>C (Fig. 9), 82.7 and 77.2% respectively, and 18 and 27.2% <sup>14</sup>C remained in the residues respectively; material balances were therefore, approximately 100%. Although a high percentage was extracted into benzene (66.4%) from a basified solution for the nonhydrolyzed HA, only 21% of this was detected as intact 4-CA (as its HFB derivative). A most interesting result from the base hydrolyzed HA shows that 72.8% of the <sup>14</sup>C was solubilized by the BF<sub>3</sub> methanol but only 10% was extractable from base. When this result is compared with that for nonhydrolyzed HA (82.7% <sup>14</sup>C extracted), 66% was extracted into benzene from base and only 3.8% was left in the alkaline solution whereas 68.2% remained in the basic solution for the former (the hydrolyzed HA). Apparently the action of hydrolysis altered the 4-CA (alkaline oxidation) to base soluble products and/or the HA structure was altered such that methylation of its carboxyl groups increases its solubility in BF<sub>3</sub> methanol but the alteration in structure (1°, 2°, 3°, 4°) prevent release of 4-CA. The fact that 45% of the nonhydrolyzed residue was solubilized might support a mechanism of carboxyl methylation followed by the breaking



of intermolecular hydrogen-bonding and hence solubilization of the residue.

#### A.4.2 20% Heptafluorobutyric anhydride in acetone extraction

Figure 11 shows that the method was not useful for extraction of 4-CA as evidenced by the fact that only 20% of the  $^{14}\text{C}$  was solubilized by this reagent for either hydrolyzed or nonhydrolyzed HA\*4-CA complexes. It is of interest that for the nonhydrolyzed HA\*4-CA complex, 46% of the solids were solubilized and ca. 90% of the  $^{14}\text{C}$  was still present in the solids after extraction. The effect of solubilization using HFBA may be analogous to that described for  $\text{BF}_3$ .methanol, that is, O-heptafluorobutyrylation.

The portion of HA solubilized (almost half) was not the fraction containing  $[\text{}^{14}\text{C}]4\text{-CA}$ . HFBA is most often used to derivatize substituted amines and sometimes phenols. However, mainly N-heptafluorobutyrylation of N-containing HA compounds would result so that components, which may not be associated with 4-CA binding would be solubilized, resulting in high  $^{14}\text{C}$  in the residue.

#### A.4.3. Diazomethane methylation and extraction

Figure 14 shows that methanol polar and apolar (hexane  $^{14}\text{C}$

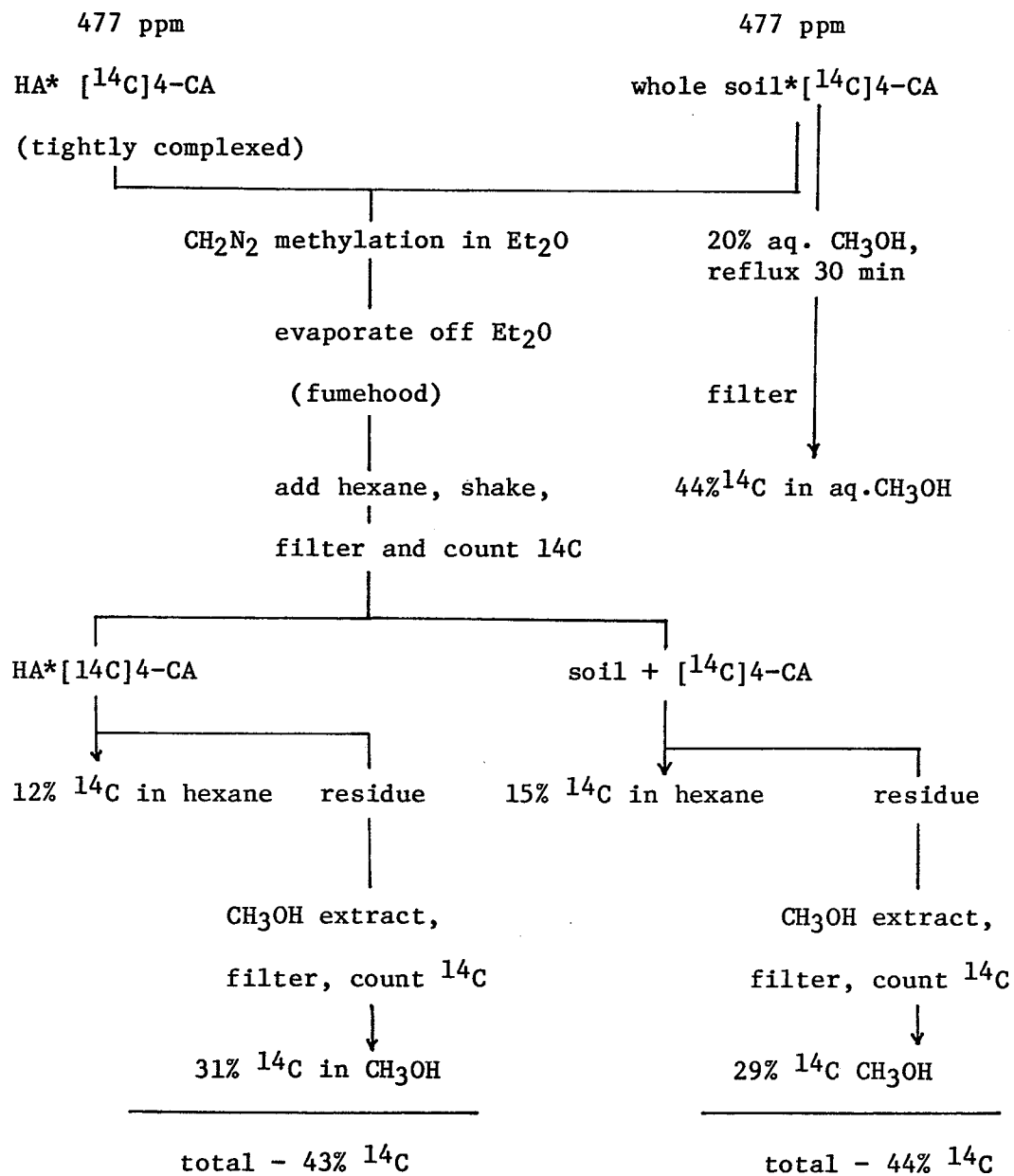


Figure 14. Soil\*[<sup>14</sup>C]4-CA versus HA\*[<sup>14</sup>C]4-CA complexes, extractability studies.

extracted from "bound" HA-4CA and complexed soil 4-CA) accounted for 12/31 and 15/29% of the original radioactivity respectively or for both ca. 43%  $^{14}\text{C}$  extracted in total. Extraction of methylated vs. nonmethylated soil using 20% aq. MeOH under reflux, released the same amount 44% of the  $^{14}\text{C}$ . Since the hydrolysis procedure is safer ( $\text{CH}_2\text{N}_2$  is explosive and carcinogenic) and released more total  $^{14}\text{C}$  (Table 3) and as much of the released  $^{14}\text{C}$  was determined as 4-CA as was released in total radioactivity from methylated HA (44%), this method is suggested at present to be the one of choice for estimation of recalcitrant 4-CA and possibly other xenobiotics in soil. These results for soil and HA are similar to data reported by Hsu and Bartha (1974a), i.e., 68% DCA is extractable (free) from whole soil at 500 ppm cf. 12% for HA. In the current study 44 versus 10% was extractable.

#### A.4.4 Ultrasonic extraction

Figure 11 showed that use of ultrasound to supply energy for release of 4-CA from the nonhydrolyzed 4-CA\*HA complex was partially successful with a pH 9 aqueous solution but only 15% was detected as 4-CA-HFB.

### A.5 Pyrolysis Methodology

#### A.5.1 Pyrolysis of the base hydrolyzed HA-4-CA complex

Table 3. Selected Pyrolysis Results.

Sample Description	Probe Temp.	Probe Type	% <sup>14</sup> C hexane Solution	% <sup>14</sup> C Carbosorb	% <sup>14</sup> C pyrolyzate 25% HCl/CH <sub>3</sub> OH	% <sup>14</sup> C <sub>6</sub> H <sub>6</sub> extract k	% <sup>14</sup> C residue
Brandon clay loam/ <sup>a</sup>							
HA	900	macro quartz			21.2		71.3
Base hydrolyzed	550	macro quartz	6.7	20.1 <sup>f</sup>	24.3		58.0
Specific activity =	550	micro platinum			20.0		
95 dpm/mg HA	400	macro stainless steel					39.3
nonhydrolyzed HA <sup>b</sup>	900	macro quartz			26.0		
Specific activity =	200	macro quartz			27.6		57.5
1120 dpm/mg HA	550	micro platinum	5.5	11.1 <sup>i</sup>	53.2 <sup>g</sup>	4.6	j
	550	micro platinum	N.D.	7.1 <sup>i</sup>	41.4 <sup>h</sup>		j
	400	macro stainless steel			0.95		59.7
	400	macro platinum				4.5	74.7
HFB-HA*4-CA Complex <sup>c</sup>	400	macro platinum				4.4	79.7
BF <sub>3</sub> .2CH <sub>3</sub> OH-HA*4-CA <sup>d</sup> complex	200	macro quartz			50.0		50.0
Whole soil - Brandon clay loam <sup>e</sup>	400	macro platinum				6.5	78.5

a,b,c,d = [<sup>14</sup>C, <sup>12</sup>C] 4-CA complex with extractable portion (benzene/acetone) removed; 477 ppm treatment level.

e = 500 ppm treated and aged.

f = total <sup>14</sup>C from sample using only carbosorb train for trapping.

g = 10 second pyrolysis interval.

h = 20 m second pyrolysis interval.

i = % <sup>14</sup>C in Carbosorb trap located after the separatory funnel (containing 25% HCl in CH<sub>3</sub>OH).

j = impossible to determine residual <sup>14</sup>C.

k = before derivatization.

For the macroquartz (ca. 5-10 mg sample size) and microplatinum (ca. 0.5-1 mg sample size) probes, the amount of radioactivity ( $^{14}\text{C}$ ) released was similar, viz., 21.2 and 20.0% respectively from base hydrolyzed HA at 550°C (optimum temperature for  $^{14}\text{C}$  release), (Table 3). Five and 8% respectively of the  $^{14}\text{C}$  in the pyrolyzate was present in the solution used for quantitation by EC-GLC. Quantitation of the 4-CA (as its heptafluorobutyryl (HFB) derivative) did not yield reproducible results and accountability varied according to the particle size of the HA. HA ground with an agate mortar and pestle resulted in 18% higher accountability. EC-GLC chromatograms were characterized by a plethora of peaks making quantitation difficult. No 4-CA-HFB was detected.

There was a greater variability in the  $^{14}\text{C}$  remaining in residues when the macroquartz probe was used, as observed by Balba (1977) with lignin bound chloroaniline. The macro stainless steel probe gave reproducible release of  $^{14}\text{C}$  (39.6, 39.1% O.M. residues) but very low radioactivity in the EC-GLC solution (hexane) (ca. 2%) and no 4-CA-HFB was detected. Pyrolysis of the original [ $^{14}\text{C}$ ]4-CA led to 20% of the  $^{14}\text{C}$  being trapped in the Carbosorb solution indicating thermolysis of [ $^{14}\text{C}$ ]4-CA to  $^{14}\text{CO}_2$ . When the hexane solutions were cleaned up by extraction with distilled water which had previously been washed with hexane,

virtually all the  $^{14}\text{C}$  stayed in the water indicating the presence of polar, hydrophilic pyrolytic thermolysis products.

#### A.5.2 Pyrolysis of the nonhydrolyzed HA\*4-CA complex

For the nonhydrolyzed HA\*4CA complex pyrolyzed using the micro quartz probe (Table 3), only 27.1%  $\pm$  0.5%  $^{14}\text{C}$  was collected in the cold trap, although most of this  $^{14}\text{C}$  was released at only 200°C cf. 550°C for the hydrolyzed HA-4CA complex. Material balances were excellent for nonhydrolyzed samples: 26% trapped + 66% remaining in the residue + 8% trapped as  $^{14}\text{CO}_2$  = 100%. More  $^{14}\text{C}$  was released using the micro platinum probe compared with the macro quartz probe, viz., 41.4% (900°C) or 53% (550°C) vs. 26.8% (900°C) respectively. Both released the same amount of  $^{14}\text{C}$  trapped in Carbosorb, ca. 8%. Use of ultrashort pyrolysis intervals, 20 ms cf. 10s to prevent secondary reactions resulted in zero  $^{14}\text{C}$  being released when using the micro platinum probe. For the macroquartz probe, 47% + 7%  $^{14}\text{C}$  was released and collected in the trap; however, of this released  $^{14}\text{C}$ , only 5.5% was in the hexane solution; 4CA-HFB was not detected. Incorporation of a 60% benzene/hexane wash cleanup prior to derivatization, as used by Balba et al. (1979) for their lignin-chloroaniline pyrolysis studies, resulted in 0% radioactivity in the solution from

which quantitation was determined by EC-GLC.

Pyrolysis using the macro stainless steel probe (40-50 mg) released only 1%  $^{14}\text{C}$  that was trapped after 15 minutes at ca. 400°C, 60%  $^{14}\text{C}$  remained in the residue and no 4-CA-HFB was detected. Counting the benzene extract of the basified pyrolyzate from the macroplatinum probe revealed that 4.5% + 0.1%  $^{14}\text{C}$  was present, and the remaining residue contained ca. 74%  $^{14}\text{C}$ . 5.5%  $^{14}\text{C}$  (microplatinum probe) was present in the EC-GLC solution. This represented all of the benzene extractable  $^{14}\text{C}$  (4.6% microplatinum probe) indicating that losses due to derivatization were negligible.

#### A.5.3 Other pyrolysis results

Pyrolysis of heptafluorobutyrylated nonhydrolyzed HA\*4-CA complexes using the macroplatinum probe gave similar results to nonHFB-HA; i.e., 4.4%  $^{14}\text{C}$  was trapped and ca. 79%  $^{14}\text{C}$  was left in the residue.

Pyrolysis of whole soil (477 ppm [ $^{14}\text{C}$ ] 4-CA treated, aged 9 months) using the macro-platinum probe (400°C for 30 min) resulted in only 6.5% of the original  $^{14}\text{C}$  being extractable into benzene from the basified trapped pyrolyzate. About 21%  $^{14}\text{C}$  in total was released.

Pyrolyzed control HA gave variable results and often showed the presence of an interfering EC-GLC peak at the same

retention time ( $R_T$ ) as 4-CA-HFB. Pyrolysis of 4-CA resulted in zero recoveries, probably due to thermolysis of 4-CA to unidentified degradation products. Controls were run for  $^{14}\text{C}$ -4CA through the entire procedure, from the extraction from 25% HCl in methanol trapping solution to derivatization, and recoveries averaged 98%.

#### A.6 Methodology Conclusions

The low recoveries, reported herein, of 4-CA complexed with HA may be due both to the thermolysis of 4-CA and the effects of pyrolysis on the HA. Chemiko (1978) and Kimber and Searle (1970a) isolated humic acids from soils using various extraction techniques. They reported that the activation energy and kinetic parameters of thermal destruction reactions (pyrolysis) of humic acids depended on the method of extraction.

It has been shown that pyrolysis of humic acid under nitrogen at  $700^\circ\text{C}$  results in aromatic groups remaining virtually intact while considerable breakdown of non-aromatic groups and substituents occurs (Kimber and Searle, 1970a; Wolf and Rosie, 1967; Farre-Rise and Guiochon, 1968; Doz et al., 1965). In addition, coextracted inorganic soil components might be expected to catalyze the following reactions: C-C fission, oxidation (auto) and ether



formation (Kimber and Searle, 1970b; Martin, 1975b).

Covalently linked macromolecules yield simpler pyrograms because of inter- and intramolecular bonding (Danielson et al., 1978) and dimers (Still et al., 1980), whereas, micellar aggregates may yield a plethora of peaks, as was observed in this study.

As HA particle size decreased, the position of a differential thermal gravimetric 420° peak shifted to a lower temperature (Levesque and Dinell, 1978). In this study I reported up to 18% greater accountability by decreasing particle size variations in pyrolysis conditions and their effects on pyrolysate characteristics of biopolymers have been discussed (Meuzelaar, 1978).

Khan and Hamilton (1979) recently studied the pyrolytic release of "bound" prometryn residues from a treated organic soil using a method similar to Balba et al. (1979). Khan and Hamilton (1979) use the term "high temperature distillation" in lieu of pyrolysis, indicating that the prometryn was not covalently bonded. Recoveries of bound residues from treated (12.4 ppm) soil were 88.8 to 62.1% (0-150 days) of the released <sup>14</sup>C determined mainly as the parent compound. Recoveries of prometryn or hydroxypropazine (a metabolite) by pyrolysis were 70 - 82%. Recovery of hydroxypropazine from treated soil by pyrolysis was considerably lower cf. prometryn; 80% vs.

53%. Thus, the more polar metabolite was much more recalcitrant to pyrolytic release (less volatile, therefore, removed less rapidly from the pyrolysis tube resulting in greater thermal decomposition). The authors cautioned that the data reported for residues of hydroxypropazine should only be regarded as qualitative. Moreale and Van Bladel (1979) showed that adsorption of 4-CA increased with increasing temperature while Weber et al. (1965) found that prometryn adsorption decreased at higher temperatures.

Soils high in clay content (e.g. the mineral soil used in this study) may give rise to predominantly clay and clay-humic organic matter complexation which is recalcitrant to pyrolytic release; complexes remain intact, even at high pyrolysis temperatures (900°C).

Pyrolysis temperatures of up to 900°C, in this study, failed to release more  $^{14}\text{C}$  than at 200°C from non-hydrolyzed 4-CA\* HA complexes. No additional  $^{14}\text{C}$  was released when the sample fired at 200°C was refired at 900°C. Again, unstable products must be forming in the pyrolysis chamber which are not swept out and/or are removed but contain no intact 4-CA (as evidenced also by the zero recovery of 4-CA standard from the pyrolyzer).

Haider (1979) attempted to release 4-CA from soil by pyrolysis in a mass spectrometer and recovered mainly

chlorobenzenes.

In conclusion, the pyrolysis techniques investigated herein were not as efficient cf. hydrolysis for release of nonextractable 4-CA\*HA or 4-CA\*soil bound residues. If the 4-CA were not covalently bound but trapped in a free state, the low recoveries observed could be explained by thermolysis of 4-CA itself. Attempts to recover 4-CA alone from the pyrolyzer led to zero recovery of 4-CA. The pyrolytic rearrangements of arylamines has been reported by Bode et al., 1973.

The abundance of clays, oxides and metal cations present in 4-CA\*HA may facilitate the formation of pyrolytic degradation products. whereas, in the case of lignin bound residues, few of these components would be present.

Balba (1979) was able to release as much intact 3-CA or 3,4-DCA from lignin bound residues by the same pyrolysis techniques employed herein and the nature of the bound residue was identified by the same authors (Still et al., 1979) to be a predominately covalent linked with the  $\alpha$ -carbon of coniferyl alcohol, (as would be expected via biosynthetic incorporation by plants). Their chloroanilines were released at 550°C. 4-CA bound to HA was released at 200. °C indicating that 4-CA is retained in a tightly complexed manner by a mechanism other than covalent bonding.

The pyrolysis results and discussion from the current study have been presented in detail because the method may be useful for other pesticides or xenobiotics generally, especially when the concentration of organic matter is low (<3%) and/or clays play a dominant role in the complexing of the compound. Knowledge regarding the influence of varying pyrolysis parameters may thus facilitate attempts to release other compounds which are tightly complexed.

Figure 15 summarizes results pertaining to extractability of tightly complexed 4-CA\*HA residues. Hydrolysis with base or acid appears to be the best procedure to release large amounts of 4-CA from soil HA (90%) with approximately 40-50% analyzable as intact 4-CA. The above figures seem generally comparable to other substituted chloroanilines, as reported herein.

The quantitative method reported in this study is very sensitive. A 75 fg ( $75 \times 10^{-15}$ g) injection of 4-CA, as its heptafluorobutyryl derivative, into an EC-GLC produced a standing current decrease equivalent to a 10% full-scale recorder deflection at an attenuation of 1 and a signal/noise ratio of 3/1.

Hydrolysis and quantitation techniques were reproducible and free from coextracted interferences; high sensitivity may permit one to dilute out interferences from other substrates or if more elaborate cleanups are required or

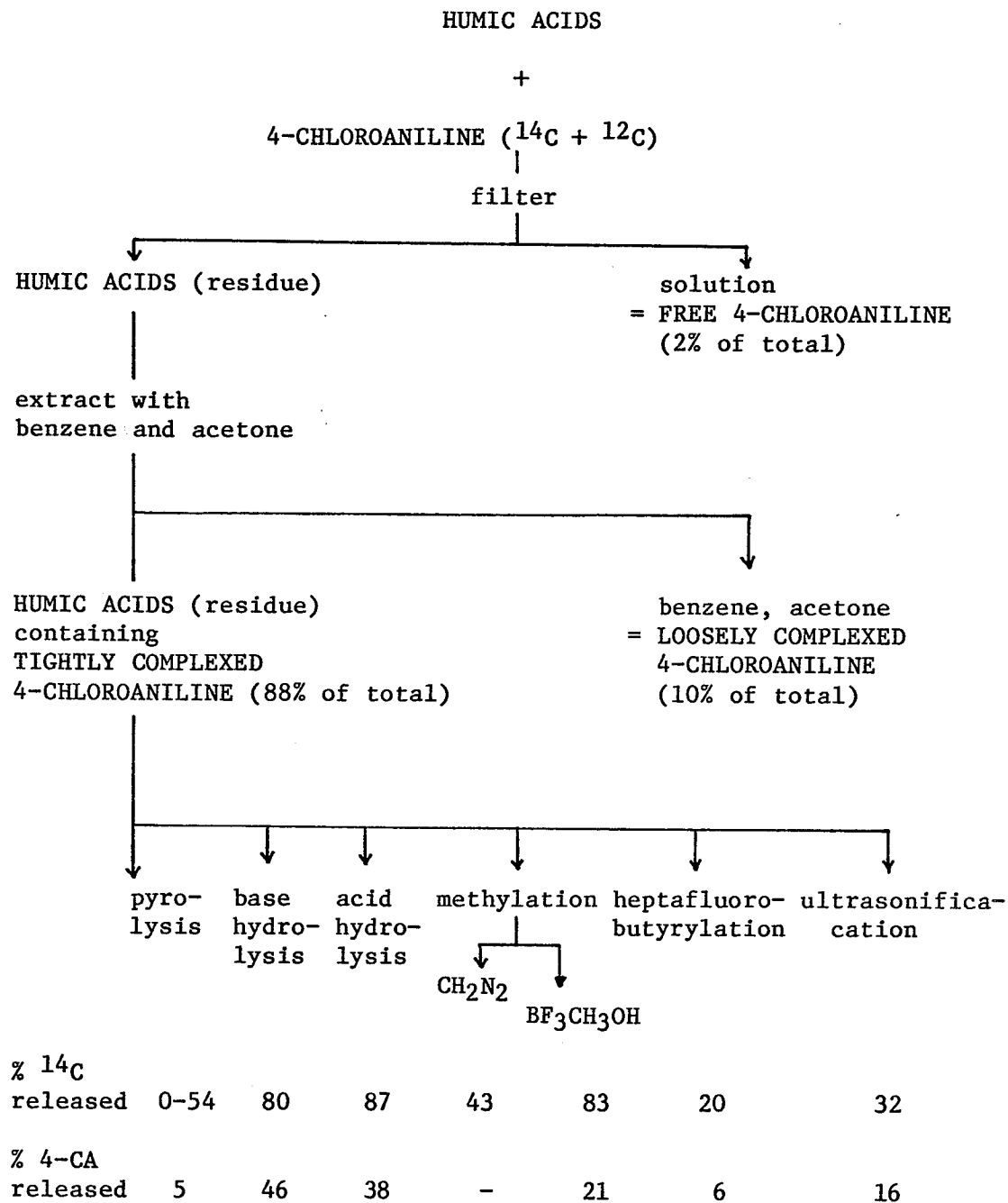


Figure 15. Extractability of tightly complexed 4-Chloroaniline from treated soil humic acids.

extensive subsampling employed such sensitivity will facilitate, if not make possible, quantitation of very low concentrations. Material balances using hydrolysis were excellent; 94-100%  $^{14}\text{C}$  was accounted for.

Figure 15 shows operational definitions of 4-CA interaction complexes with soil HA. The same scheme may be applicable to whole soil, as well as other xenobiotics.

The nature of the tightly complexed residue is not known; however, section (D) of this thesis presents in detail a theory (de novo) based on inclusion phenomena to explain the "bound" residue problem in soils.

## B. Studies on the Binding of 4-CA to Three Different Soils and its Release by Pyrolysis

### B.1 Introduction

The purpose of this study was to investigate whether the low recoveries of 4-CA via pyrolysis were related to the nature of the soil used in these studies. Since Khan and Hamilton (1980) reported high recoveries of prometryn from soil via pyrolysis and had used an organic soil with a very high [OM] it was deemed prudent to determine if [OM] of the soil substrate influenced the recovery of 4-CA using pyrolysis. In addition, an evaluation could be made of the relative

amounts of loosely complexed/tightly complexed 4-CA in soil of varying OM concentrations. NOTE: humic acid and fulvic acid are synonymous with HA and FA respectively.

## B.2 Materials and Methods

### B.2.1 Reagents

- (a) Solvents - all pesticide grade - Caledon Co.
- (b) [ $^{12}\text{C}$ ]4-chloroaniline - Aldrich Chemical Co., recrystallized three times from hexane, mp = 71-72°C.
- (c) [ $^{14}\text{C}$ ]4-chloroaniline - California Bionuclear Corp., purity confirmed by TLC and autoradiography.
- (d) "Omnifluor" - New England Nuclear Corp., scintillator in scintanalyzed toluene (Fisher Co.)
- (e) "Aquasol-2" - New England Nuclear Corp., scintillator for aqueous samples.
- (f) Soils - Piney organic peat, Brandon Carroll Clay-loam, Soignies silt-loam, Table 4 lists several physicochemical properties of these soils. All soils were air-dried and ground so as to pass through a 3mm sieve. The physicochemical properties of these soils were determined as follows: textural classification by the "standard pipette" method (Toogood and Peters, 1953), pH at a 1:1 (w/v) ratio in 0.1 M  $\text{CaCl}_2$  (Peech, 1965), % organic carbon by oxidation and titration (Walkley and Black, 1934), cation analysis (i)  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$ ,  $\text{K}^+$  by extraction into ammonium acetate and quantitation using atomic absorption spectrophotometry, and

Table 4. Physicochemical properties of the soils used in this study.

SOIL	%	%	%	%	%	pH	H+	meg/100 g. exchangeable				C.E.C.	%	OM
								Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>	K <sup>+</sup>			
	sand	silt	clay	carbon	matter							Ash	Abs	=550
organic														
(Piney)	0.0	0.0	0.0	43.2	74.5	5.8	23.6	135.2	28.4	0.5	0.9	200	18.2	0.43
Carrol														
clay-loam														
(Brandon)	38	30	32	5.4	9.3	5.7	2.8	27.2	6.5	0.3	1.5	36.5	86.2	0.47
silt-loam														
(Soignies)	15	72	13	8.7	15.0	3.2	-	1.4	0.2	0.1	0.4	28.3	85.5	0.17



(ii)  $H^+$  by extraction with  $BaCl_2$ /triethanolamine (Peech et al., 1962), C.E.C. as per the method of Chapman (1965), % ash by gravimetric analysis (400°C overnight in a muffle furnace), degree of humification of soil via 0.025 M  $Na_4P_2O_7$  and absorption at 550 $\mu$  (Schnitzer and Desjardins, 1965).

### B.2.2 Apparatus

- (a) Radioassay - Searle Model 6880 Mark III liquid scintillation spectrometer was used to assay radioactivity with a low level activity program ( $E^2/B$ ) for dpm computation. Radioactivity in solid residues were determined as  $^{14}CO_2$  by combustion in a Packard-Tricarb Model 306 Biological Oxidizer, followed by liquid scintillation counting of the trapped combustate (Carbosorb 5 mL, Permafluor 10 mL). Combustion of  $^{14}C$  standards assayed at greater than 98%.
- (b) Pyrolyzer - Chemical Data Systems Corp. 18580A pyroprobe-solids analyzer with macro quartz probe and a macro platinum probe. Probe temperatures, macro quartz (maq.) = 900°C, macroplatinum (map) = 450°C; pyrolysis time, maq = 20s, map = 15 min, flow rate, maq = map = 200 mL min<sup>-1</sup> helium, sample size = 40-50 mg.

### B.2.3 Methodology

#### (a) Treatment of soils with [ $^{14}\text{C}$ ] + [ $^{12}\text{C}$ ]4-CA.

One hundred grams of each soil was adjusted to 80% of its water holding capacity with distilled water, shaken thoroughly and allowed to equilibrate for one hour. Soils were then treated with  $0.031\ \mu\text{Ci}$  [ $^{14}\text{C}$ ]4-CA per gram of soil plus sufficient [ $^{12}\text{C}$ ]4-CA to result in final dosages of 5 and 500 ppm ([ $^{14}\text{C}$ ] + [ $^{12}\text{C}$ ]4-CA was added dropwise in a total volume of 5 mL 95% ethanol). The soils were shaken thoroughly and left standing in stoppered erlenmeyer flasks for 4 h, after which they were opened and air-dried in a fumehood for 40 h.

#### (b) Extraction of unbound residues

Soils were extracted with 5 x 50 mL acetone, filtered (through no. 41 Whatman paper) under vacuum. The soil was redried in the fumehood and 2 g subsamples re-extracted under reflux (15 min) using 20 mL methanol/water (1:1) and then reextracted by shaking (1 min each time) with 2 x 15 mL methanol/water (1:1) without refluxing. The dried soil was used for pyrolysis and combustion analysis, all extracts were counted and controls for each soil included for the entire procedure. All analyses were performed in duplicate.

#### (c) Pyrolysis and release of bound 4-CA radioactivity.

Figure 12 shows the apparatus used for pyrolysis. Released radioactivity was trapped in the "u"-tube and 5 mL 25% HCl in methanol contained in a 30 mL separatory funnel. The pyrolysate was washed out of the "u"-tube into the separatory funnel directly using 10 mL 25% HCl/methanol. This solution was basified with 10 mL 5N NaOH (to pH 13) and extracted using 4 x 10 mL of benzene. The benzene extract and the basic solution (adjusted to pH 6.0 with conc. HCl) were counted in a liquid scintillation spectrometer.

### B.3 Results and Discussion

Figures 16 and 17 and Tables 5-7 show the evidence upon which the following conclusions may be formulated in regard to the binding and release of 4-CA's from soil influenced by (1) the concentration of applied 4-CA; (2) varying soil properties such as organic matter and clay, (3) the influence of two different pyrolysis probes.

#### B.3.1 The influence of 4-CA concentration applied on binding and release

For all soils studied, binding (% of applied dose) was always greatest at 5 ppm cf. 500 ppm treatment levels. This suggests that a fixed number of binding sites are available per unit of adsorbent for complexation of 4-CA and that these sites may be saturated at some concentration less than 500 ppm or that the system was disequibrated. Usually a greater percentage of bound radioactivity was released by pyrolysis at the 500 ppm level. This may be due to the

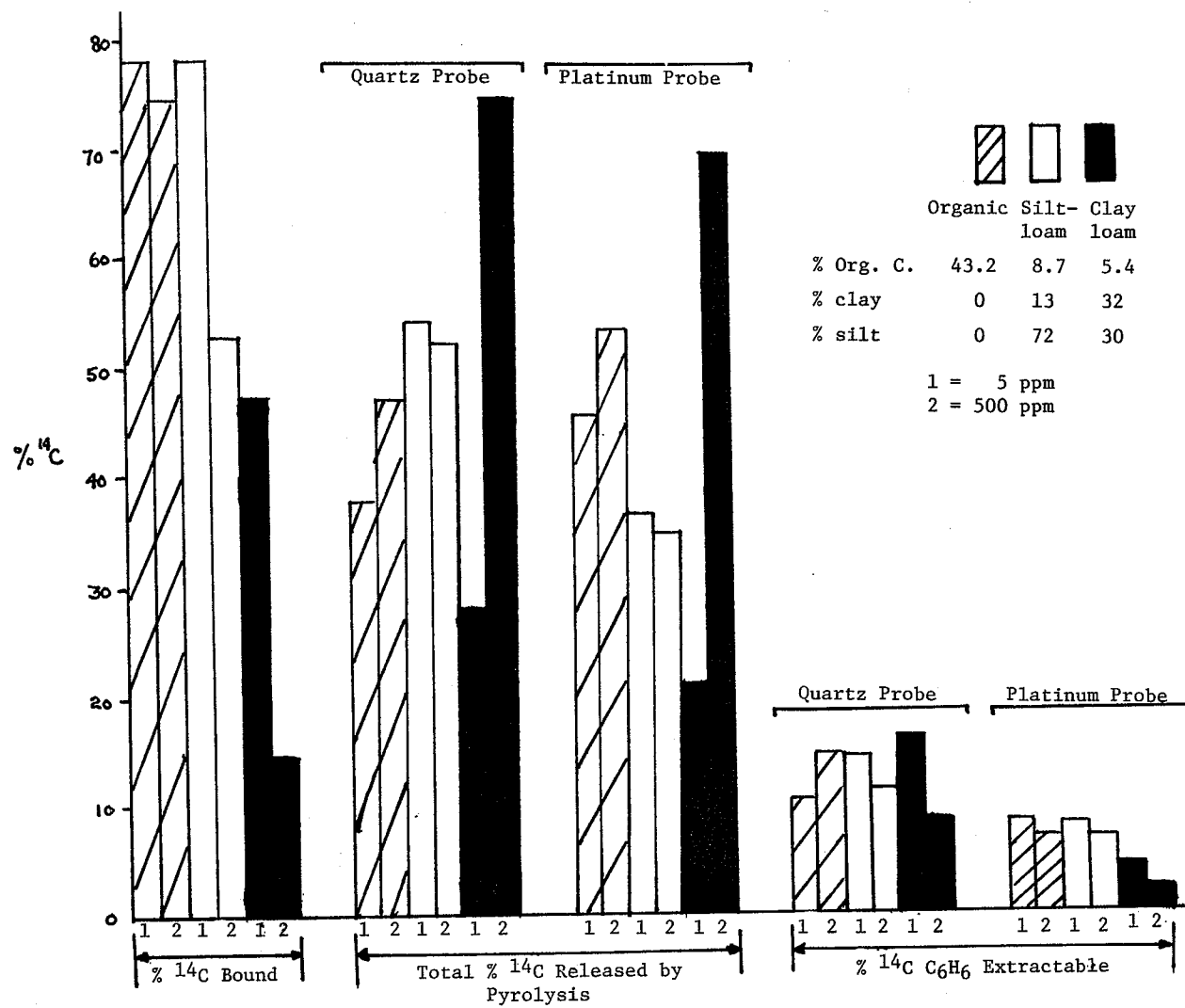


Figure 16. Binding, pyrolytic release, and extractable [<sup>14</sup>C]4-CA from three different soils.

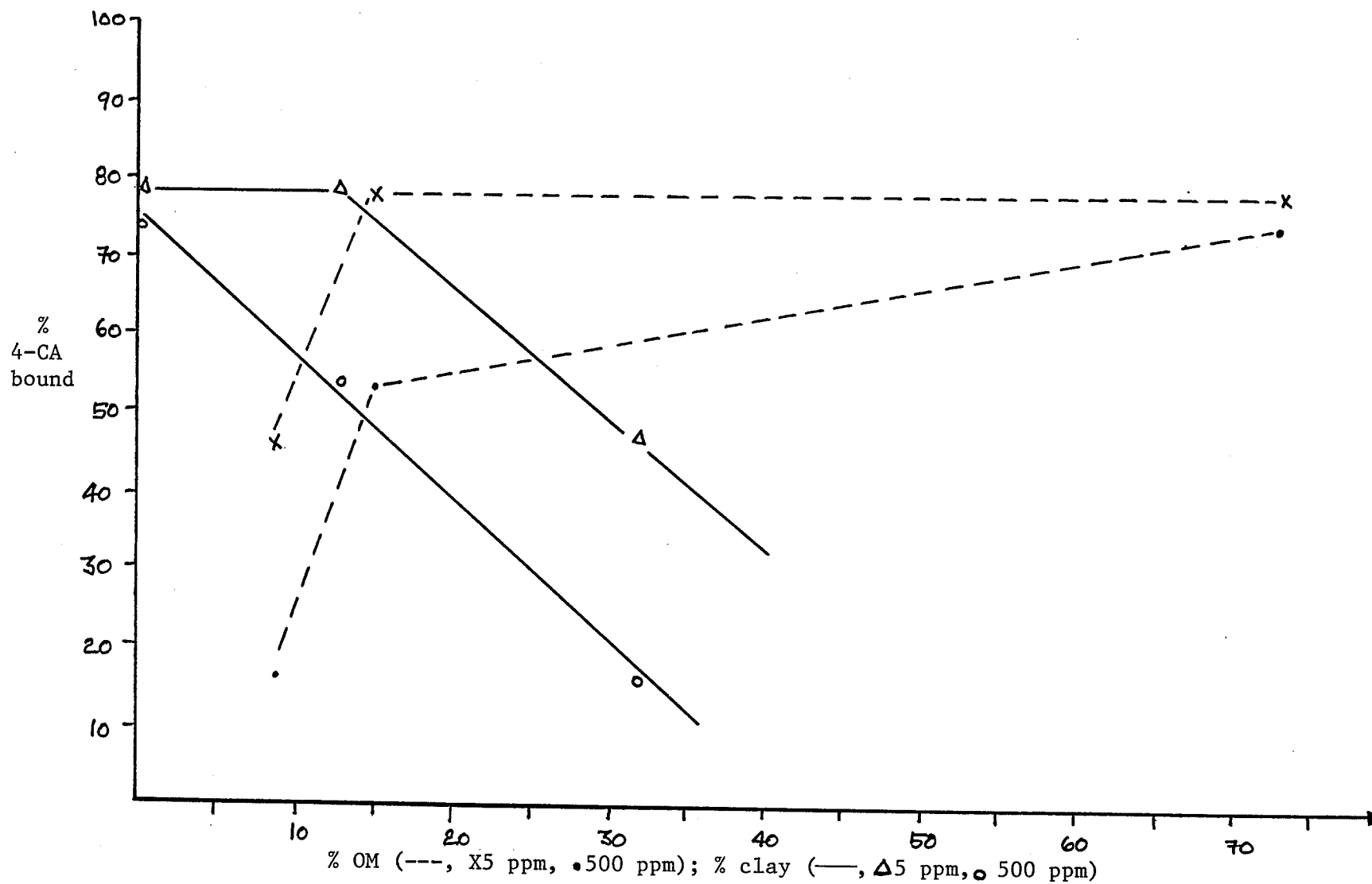


Figure 17. Effect of varying OM and clay concentrations on % 4-CA bound.

Table 5. Extractability of [<sup>14</sup>C]4-CA from several soils.\*

SOIL	PPM	TOTAL DPM x 10 <sup>6</sup>		TOTAL EXTRACTED	BOUND IN RESIDUE	% BOUND IN RESIDUE
		ACETONE EXTRACT	WATER METHANOL			
ORGANIC	5	0.428	1.11	1.58	5.26	76.9
			0.038			
	500	0.861	0.966	1.88	4.95	72.5
			0.056			
SILT-LOAM	5	0.421	1.04	1.53	5.31	77.6
			0.071			
	500	1.70	1.58	3.28	3.56	52.1
			0.095			
CLAY-LOAM	5	1.41	2.17	3.70	3.14	45.9
			0.116			
	500	4.46	1.36	5.85	0.987	14.4
			0.036			

\* original radioactivity applied =

6.84 X 10<sup>6</sup> dpm per 100 g soil

TABLE 6. Pyrolytic results for bound [<sup>14</sup>C]4-CA complexes in various soils\*

Soil	PPM	dpm HCl/CH <sub>3</sub> OH		dpm C <sub>6</sub> H <sub>6</sub>		% <sup>14</sup> C Released Quartz Platinum		% <sup>14</sup> C C <sub>6</sub> H <sub>6</sub> Extractable		Total dpm used	
		Quartz	Platinum	Quartz	Platinum	HCl/CH <sub>3</sub> OH	Quartz	Platinum	Quartz	Platinum	Quartz
ORGANIC	5	240	450	86	93	36	43	12	9	671	1054
	500	240	540	86	83	43	51	15	8	556	1062
SLIT-	5	1290	990	372	269	53	35	15	10	2450	2797
LOAM	500	780	810	197	217	50	34	13	9	1568	2399
CLAY-	5	300	380	176	103	28	22	16	7	1070	1479
LOAM	500	100	240	10	10	73	67	4	3	245	358

\* Actual dpm used - determined from combustion of a known soil air-dried weight.

Table. 7 Summary of binding of 4-CA to three soils of varying OM and clay contents

Soil	PPM	% <sup>14</sup> C Extracted	% <sup>14</sup> C Bound	% of <sup>14</sup> C Bound OM		% <sup>14</sup> C of Bound and Released in C <sub>6</sub> H <sub>6</sub>		% OM	% Clay	pH	% ASH
				Qz	Pt	Qz	Pt				
Organic	5	22	78	36	43	12	9	73	0	5.8	18
	500	26	74	43	51	15	8				
Silt - Loam	5	22	78	58	35	15	10	15	13	3.2	86
	500	47	53	50	34	13	9				
Clay - Loam	5	54	46	28	22	16	7	9	32	5.7	86
	500	85	15	73	67	4	3				



occurrence of less strongly bound 4-CA in the form of multi-layered or included sorbed molecules at the 500 ppm level. The percentage  $^{14}\text{C}$  extractable into benzene from a basified pyrolyzate, however, was similar for all soils at both concentrations with slightly more  $^{14}\text{C}$  extractable from 5 ppm pyrolyzates. Differences were too small to draw definite conclusions.

### B.3.2 The influence of varying concentrations of soil organic matter and clay

Figures 16 and 17 show that the percentage of binding increased with increasing [OM] and decreasing [clay], especially at the 500 ppm treatment level. At 5 ppm 78%  $^{14}\text{C}$  was bound by the organic and silt-loam soils. This may be because enough OM is available for interaction, whereas for the clay-loam soil much of the OM interacts with the high [clay] thus reducing the amount of OM available for interaction with 4-CA directly. This may also explain why the organic soil bound about the same fraction of  $^{14}\text{C}$  at 5 and 500 ppm (i.e. 78 and 74% respectively), while the other two soils exhibited a considerably less binding<sup>(%)</sup> at 500 ppm than at 5 ppm. That is more "free" OM is available to interact with [ $^{14}\text{C}$ ]4-CA in the organic soil. This illustrates the importance of soil OM in the binding of 4-CA to soil. Clay seems to have little direct effect on binding at the range of OM it is combined with in this study. This is

demonstrated by the fact that the OM versus % bound and the OM/clay ratio versus % bound curves exhibit the same kind of curve, so that, consideration of clay does not alter the shape of the curve or in fact the position of the curves on the X axis (Figure 17).

### B.3.3 The influence of pyrolysis and different pyrolysis probes on release of bound 4-CA residues

Pyrolysis released between 22% and 73% of the bound radioactivity depending on the type of probe and soil involved (Table 11). For the organic soils, more bound radioactivity was released at either concentration when the platinum probe was used; the silt-loam and clay-loam released greater radioactivity when the quartz probe was used. Possibly this is because of the less humified OM of the organic soil was more reactive on the quartz surface, thus reacting with the 4-CA to yield thermally stable products which are nonvolatile or trapped within the OM matrix. Balba (1977) obtained lower recoveries of 3-CA and 3,4-DCA complexed to plant lignin-cellulose when using a quartz probe and increased recoveries with the platinum probe. The genetic relationship of plant lignin-cellulose to soil OM has been well documented.

In conclusion the results of this study show the dominant role of soil OM in binding 4-CA and the fact that between 9 - 15% OM, OM has a greater effect than clay on the extent of binding. For example, a 15% OM soil bound as high a percentage of 4-CA as a 73% OM soil, even though part of the binding capacity of the former was lowered by interaction with 13% clay. Even at 9% OM and 32% clay a high percentage of binding occurs; *vi .*, 46% at 5 ppm, so that the clay has been masked by the OM but enough remains for considerable binding of 4-CA. This masking effect is demonstrated by the fact that, as the OM content of the soil decreased with a concomitant increase in clay, the % 4-CA bound decreased at the 500 ppm level. However, at 5 ppm the organic and silt-loam soil binding was identical (78% of applied dose) showing that, at the low concentration level of 4-CA applied, the OM is available for binding as if no clay were present; but at 500 ppm it is evident that the number of sites has in fact, decreased because of clay interaction with the OM and insufficient sites are available for binding (78% at 5 ppm cf. 53% at 500 ppm). This effect is magnified with the clay-loam soil (9% OM - 32% clay) where the total amount bound decreased to 46 and 15% at 5 and 500 ppm due to masking of OM activity by a greater clay content. Figure 14 shows that as clay concentration increases and OM concentration decreases, total amount of 4-CA bound decreases, thus supporting the above conclusion.

Since the amount of radioactivity released by pyrolysis and extractable into benzene (4-CA) for all soils was very similar (ca. 10%) this observation might be taken to be indicative of a binding mechanism related to organic matter inclusion phenomena. For geographically different soils of varying OM contents, OM\*4-CA interactions would be expected to vary such that greater differences in release result. From these results it appears that OM concentration is not important for release (except below a limiting concentration).

Low recoveries of benzene extractable radioactivity from the pyrolysate may be due to inclusion phenomena also, whereby application of heat results in thermolysis of 4-CA itself rather than of a 4-CA-OM covalent bond.

## C. Mechanism of Binding as Related to the Nature of Humic Acid

### Structure

#### C.1 Introduction

The purpose of the current studies which follow was to investigate the physicochemical properties of indigenous humic acid binding sites. Because the nature of the extractant may affect the state of indigenous humic substances, several procedures were

developed and applied to a Manitoba soil. These extractions included the classical method (NaOH) and two milder methods using  $\text{Na}_4\text{P}_2\text{O}_7$  at pH 7.0 and the use of several different precipitation reagents. Model precursors of humic substances were investigated to ascertain their possible contribution to humic structure. IR and  $^{13}\text{C}$ -NMR spectra, and other physicochemical parameters of humic and fulvic acids and humic and fulvic-like substances were determined. A discussion of these findings correlated with studies from the literature resulted in novel conclusions regarding the structure of humic substances and their mechanisms of binding. NOTE: humic acid and fulvic acid are synonymous with HA and FA respectively.

## C.2 Materials and Methods

### C.2.1 Reagents

- (a) Solvents - distilled water; Fisher reagent grade acetone;  $\text{D}_2\text{O}$ , NaOD, Merck Sharp and Dome (Montreal), HCl (38%) Baker Analyzed.
- (b) Soil - Orthic black chernozem, Carroll clay-loam (Brandon), air-dried and ground to 3 mm, see Table 8 for physicochemical properties. Transition metal cation analysis: a sample of HA was predigested with conc.

HNO<sub>3</sub> for 4h and HClO<sub>4</sub> added (1:2 HNO<sub>3</sub>/HClO<sub>4</sub>) for a 1h digestion, filter, bring to a standard volume with deionized-distilled water and quantitate by atomic absorption spectrophotometry.

- (c) Chemicals - all reagent grade (Fisher), NaOH, Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>·10H<sub>2</sub>O, palmitic acid, glucose, galacturonic acid, cellulose, sucrose and cellulose esters, monoacetate, triacetate, and propionate.

#### D.2.2 Apparatus

- (a) Ultrafiltration - Amicon Diaflo membrane ultrafiltration unit (2L cap.), with stirred cell and purified argon for a positive pressure.
- (b) Centrifugation - (i) low speed (2000 rpm) IEC Universal Model; (ii) high speed (13,000 rpm) IEC-B-20A Model.
- (c) Infrared spectra - recorded on a Perkin-Elmer 567 Model, KBr macro pellets used (400 mg KBr plus 0.5 mg sample), fast scan.
- (d) Filtering - Whatman GF/A glass fiber paper and Spectrapore No. 2 (m.wt. <10,000 excluded).
- (e) <sup>13</sup>C-NMR - <sup>13</sup>C-NMR spectra were obtained on a Bruker WS 900S multinuclear NMR spectrometer operating at 22.63 MHz (35 C). Free induction decays were digitized, accumulated and a Fourier transform performed by computer. D<sub>2</sub>O lock and full proton

decoupling were used during an accumulation of 80,000 - 100,000 scans using 90 degree pulse and total pulse delay and acquisition time of 1.589s, pulse width = 14  $\mu$ sec., internal standard deuterio-dioxane (67.86 ppm), chemical shifts reported in ppm, resolution = +10Hz(ca. 5 ppm).

(f) Lyophilization - Virtis freeze drier.

### C.2.3 Methodology

(a) Extraction procedures for humic acids

(a.1) NaOH + acid precipitation (I) 0.5 Kg. of soil was extracted with 4 L of 0.5 M NaOH (aq.) solution purged with N<sub>2</sub> in a brown glass bottle by shaking on a reciprocating shaker for 6 h, and allowed to stand overnight prior to filtration. All samples were centrifuged in 250 mL polycarbonate bottles at high speed (13,000 rpm for 30 min) to remove suspended colloidal material - especially clays - prior to precipitation of humic acids. Plant material (detritus) was removed by filtering through glassfiber paper under vacuum. The filtrate was slowly acidified with conc. HCl and constant stirring until the pH was lowered to 1-2 (Baker Calorplast sticks 0-2.5 pH range). The total solution was centrifuged (250 mL glass bottles) at 1500 rpm to sediment out the humic acid precipitate (I). Supernatants were decanted and the bottles containing the humic acid pellets frozen (-40° C) until further work could be performed.

Ultrafiltration - due to uncontrollable interaction between gels (Sephadex) and humus, the ultramembrane filtration technique is

considered preferable for desalting and molecular size fractionation of humus (Gijessing, 1973). Desalting, concentration and molecular fractionation were performed using ultrafiltration membranes and an Amicon Diaflo apparatus.

The pellets were dissolved in weak alkali (0.2N, NaOH, pH 10-11 to dispense the humic acid. The solution was ultrafiltered (15 cm UM2, <1000 m. wt. excluded) in a continuous - discontinuous mode continuous stirred cell until the pH of the filtrate reached 6.5 and the initial volume (2L) was concentrated to ca. 500 mL. The retentate was shell frozen in a 2L flask using a Buchi rotoevaporator and a dry ice-acetone bath and lyophilized. The humic acids were stored in a desiccator under vacuum.

(a.2) Fulvic acid preparation Fulvic acid was prepared from a separate soil sample using 0.5N NaOH as described above, except that the alkali-acid soluble supernatant obtained after the humic acid centrifugation was used (see Fig. 1).

(a.3) Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> + acid precipitation (II). The procedure for this extraction is the same as for (I) except that the 4L of aqueous 0.02M Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> was neutralized to pH 7 using conc. HCl.

(a.4) Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> + acetone precipitation (III). Prior to precipitation, the procedure is identical to that used for preparation (II). Precipitation was accomplished by adding acetone to the solutions at



a ratio of 1:1 and centrifuging at high speed in polypropylene bottles. This precipitate did not have to be dispersed using base for the ultrafiltration as it was the sodium salt and dissolved freely in distilled water. Part of the retentate obtained after ultrafiltration was refiltered through another membrane, XM-100A (< 100,000 m.wt. excluded). The retentate was lyophilized and the filtrate was refiltered through XM-50 (< 50,000 m.wt. excluded) and the retentate and filtrate, after evaporation under N<sub>2</sub> to reduce the volume were lyophilized. Three desalted fractions were thus obtained, more than 100,000, 50,000-100,000, and less than 50,000 m.wt.

Figures 18, 19, and 20 illustrate the isolation procedures for the three humic acids prepared, I, II, and III. A second fraction was precipitated from humic acid III supernatant by further acetone dilutions at 5:1, this is termed III<sub>2</sub> fraction.

(a.5) Low ash humic acid. Prepared from a Red River clay soil by the method of Povoledo and Pitze (1979), involving HF-HCl treatment of the soil and ultrafiltration of a Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> solution followed by acid precipitation of the humic acid.

(b) Physicochemical characteristics of isolated humic acids

Physical and chemical properties of preparations were determined by various techniques, visual observations of color, size and shape of I, II and III; yields; ash contents (except III - less than 50,000

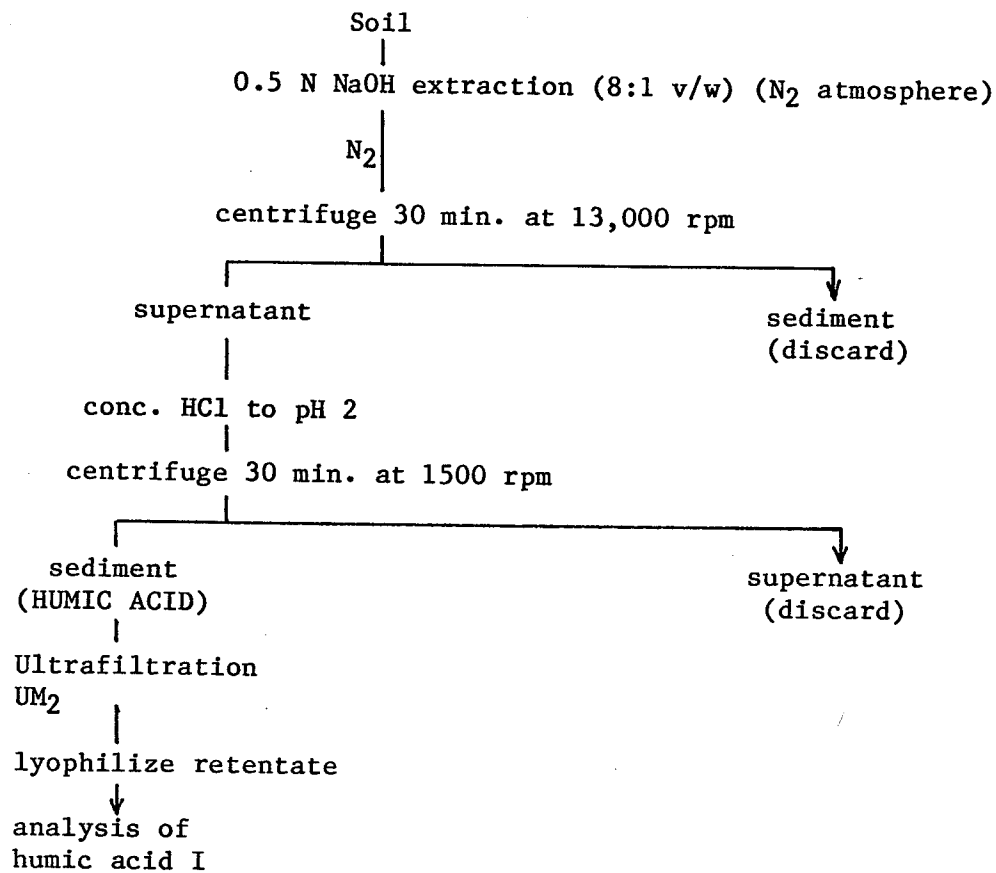


Figure 18. Preparation of humic acid by classical NaOH soil extraction + acid precipitation (I)

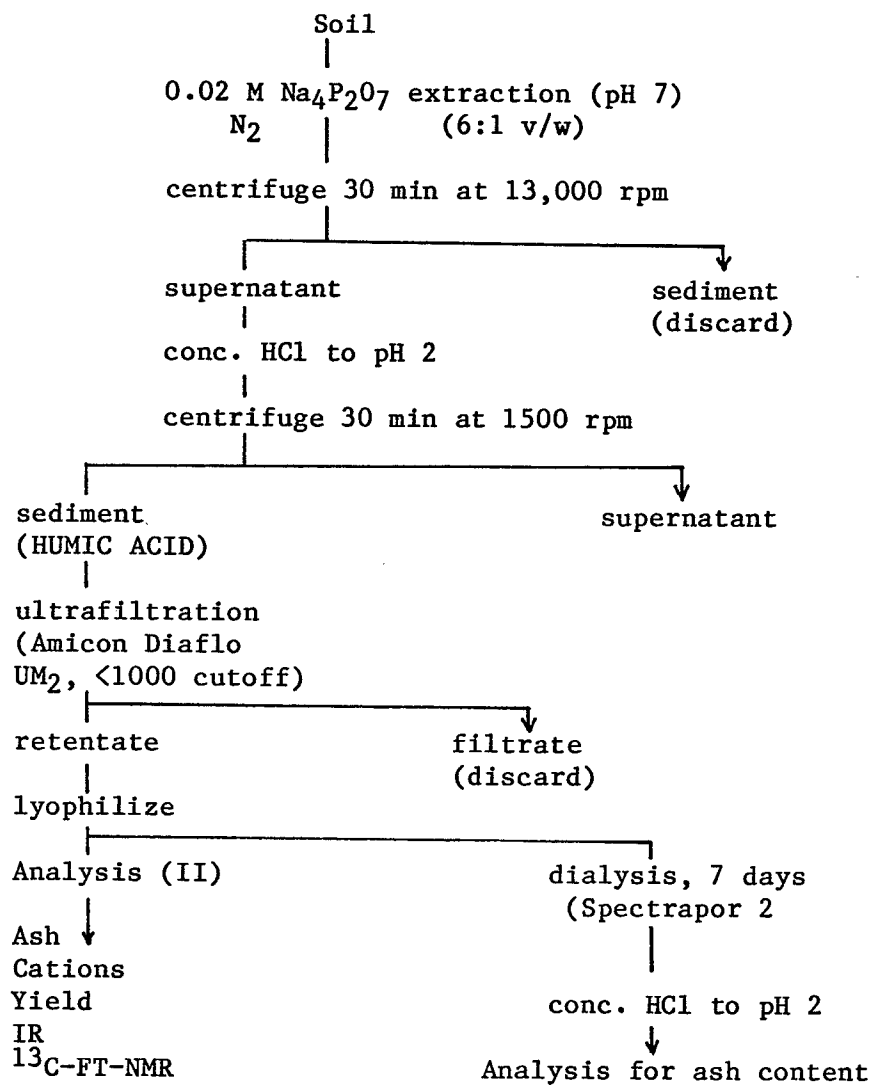


Figure 19.  $\text{Na}_4\text{P}_2\text{O}_7$  soil extraction + acid precipitation (II)

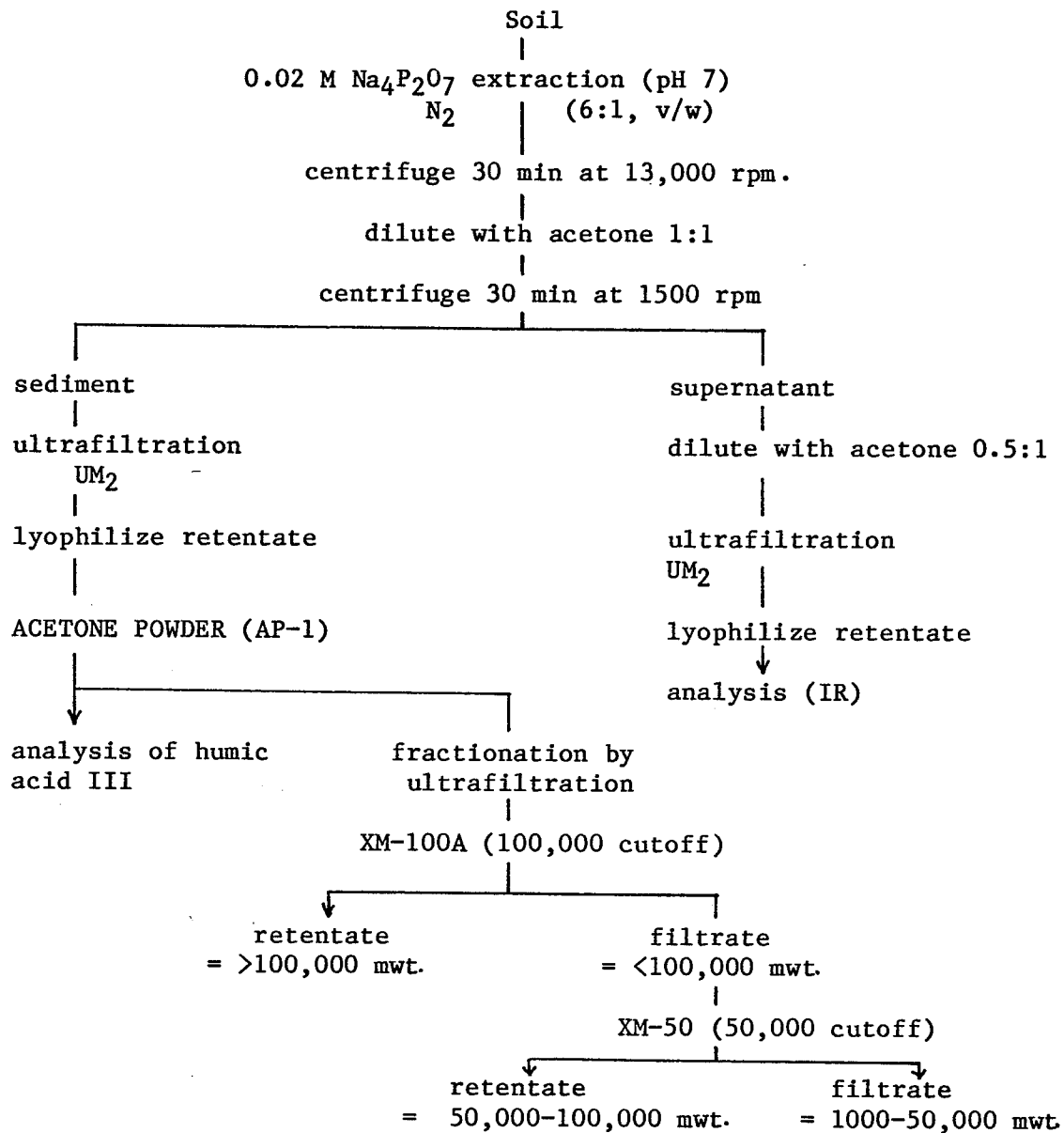


Figure 20. Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> soil extraction + acetone precipitation (III)

m,wt. fraction because of low yield) were determined by combustion in ceramic crucibles at 700° C (4h) in a muffle furnace, cation assays were performed on nitric and perchloric acid digests by atomic absorption spectrometry.

Tables 8 and 9 list these physicochemical characteristics. IR and  $^{13}\text{C}$ -NMR spectra were obtained on the three humic acids isolated as their sodium salts, one as its free acid, as well as spectra of fulvic acid and a HA precursor.

#### (c) Infrared Spectrophotometric procedures

KBr macro pellets were prepared using standard procedures. On scale spectra were obtained by reducing the concentration of sample and/or using a reference beam attenuator. All IR absorptions are reported in  $\text{cm}^{-1}$ .

#### (d) $^{13}\text{C}$ -FT-NMR Spectrometric procedures

Solution spectra were obtained by dissolution (dispersion) of the humic acid in 4N NaOD/D<sub>2</sub>O at a concentration of 5% W/W (2mL final volume). If suspended material was evident, it was removed by filtration through a Swinney disc filter holder (13mm) containing a 0.5  $\mu\text{m}$  (FH) filter plus a prefilter.

The sample was placed in a 10 mm NMR tube, degassed with N<sub>2</sub>, capped, and sealed with parafilm.

Repetitive intense rf pulses excited the  $^{13}\text{C}$  spin system, and

the resulting NMR transient relaxation responses, or free induction decays were digitized; 80,000 - 100,000 scans were accumulated by a time averaging computer. A fourier transform analysis yields a computerized average of transient spectra. All chemical shifts are reported as ppm between 0-200. Reduced ash content procedure; in an effort to reduce the ash content of humic acid II, 1.0 g of it was dissolved in 200 mL distilled water (Fig. 17) and dialyzed against distilled water for one week. A portion of the retentate (pH 6.5) was evaporated under nitrogen, dried in a vacuum desiccator and ash content redetermined as described previously. A humic acid of <0.1% ash (IV) (Red River clay soil) was supplied and prepared by the method of Povoledo and Pitze (1979) for  $^{13}\text{C}$ -NMR analysis.

(e) Model Humic precursors and methodology

Several actual or model humic acid precursors such as: palmitic acid, galacturonic acid, glucose, sucrose, cellulose and the following cellulose esters: acetate, triacetate, and propionate, were studied. Concentrations of ca. 1g/10 mL of 0.5N NaOH were prepared in capped vials which were shaken periodically over a period of 10 h. The solution was filtered (glass sintered funnel) under vacuum and the filtrate acidified with conc. HCl to pH 1-2. After 5 days standing in a capped vial, the solution was filtered (sintered glass funnel) and washed with distilled water and the retained precipitate

dried in a vacuum desiccator.

Galacturonic acid, glucose, sucrose and cellulose were also reacted with palmitic acid (0.4g/0.8g per 10 mL) and carried through the procedure described above to note if any precipitate formed upon acidification (characterized by an IR spectrum different from the sample without palmitic acid). IR and  $^{13}\text{C}$ -NMR spectra were obtained on the dried precipitate.

### C.3 Results and Discussion

#### C.3.1 Extraction of Humic Acids

The ratio of 1:1, acetone/aqueous extract, used for preparation of humic acid III was found to be the least amount of acetone necessary for precipitation to occur. The supernatant from humic acid II did not yield any visible precipitate upon addition of acetone (1:1) and conversely, humic acid III supernatant did not yield a precipitate upon acidification to pH 1-2; therefore, the precipitates may be taken in each case to represent humic acids and not just a fraction thereof.

A second acetone fraction from HA-III ( $\text{III}_2$ ) was found to precipitate out at an acetone/supernatant ratio of 5:1.

An interesting phenomenon occurred with the acetone powders (III). Fine, long light-brown-to-white crystals in the supernatants were observed upon standing. Several samples were collected, dried,

and ground; IR analysis indicated that they were some form of pyrophosphate since their spectra were similar to the spectrum of  $\text{Na}_4\text{P}_2\text{O}_7$  partially oven-dried (Fig. 21). The supernatants were especially rich in this regard, with several crops of crystals filtered out over a period of several days.

During ultrafiltration dark deposits formed on the membranes. The HA-III in particular, left a thin film of gelatinous material on the Amicon XM-100A membrane (>100,000 molecular weight cutoff). This soft flexible film, washed off easily in a sheet.

The  $\text{Na}_4\text{P}_2\text{O}_7$ -extracted humic acids were high in ash content (Table 8) and are in agreement with those reported by Biederbec and Paul (1972). Using an Orthic Chernozemic soil they obtained sodium humate containing 37% ash before demineralization (which included the use of 2.0 N HF and 0.01 M HCl) and 12% ash after. Part of the reason why the ash contents were so high is that the samples were in the Na+ salt form. The acetone powders had never been subjected to an acid treatment, and the acid precipitated fractions were redissolved in a weak NaOH solution prior to being placed in the ultrafiltration unit.

The use of Spectrapor 2 (Fig. 19) to lower the ash content of HA-II resulted in only a reduction of 8.6% ash, 50.6% ash before dialysis compared with 42% ash after (Table 8). This is not a dramatic decrease considering that the dialyzed HA was in the free acid form as well. The inorganic components must therefore be bound very tightly such that high speed centrifugation (13,000 rpm),



crystals pptd.  
from HA-III<sub>2</sub>  
supernatant after  
ultrafiltration

crystals pptd.  
from HA-III<sub>2</sub>  
supernatant  
(whole) on standing

Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>·10H<sub>2</sub>O  
partially dried

HA-I ash

HA-II ash

Ha-III ash

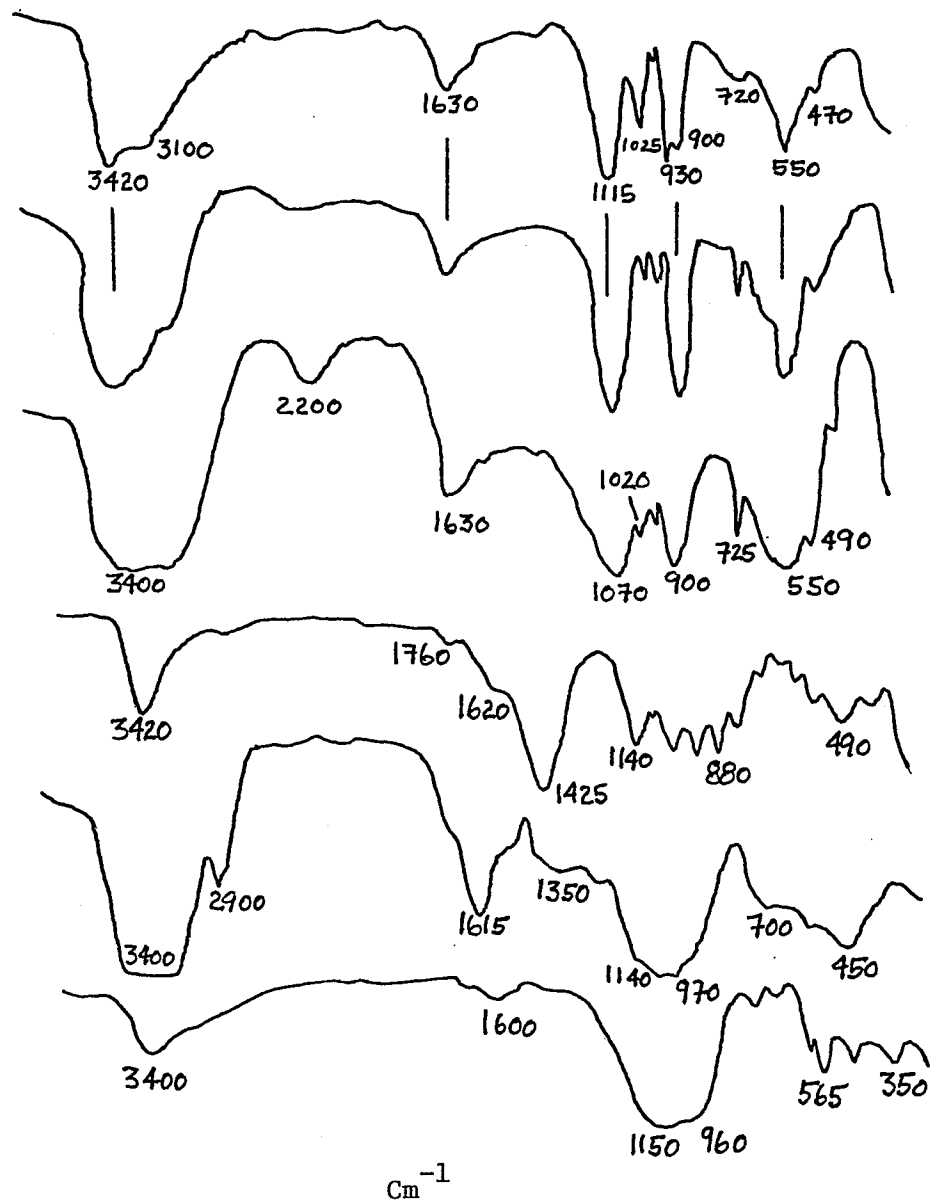


Figure 21. IR spectra of HA-III<sub>2</sub> and HA-I, II and III ashes.

ultrafiltration (< 1000 m. wt. excluded) and dialysis (< 10,000 m. wt. excluded) would not lower the ash content to less than 42% for HA-II.

Table 8 shows the amount of each HA extracted by the three procedures plus the yields for the ultrafiltered HA-III sample. The greatest yield (ash-free weight) was obtained using  $\text{Na}_4\text{P}_2\text{O}_7$  as extractant with acetone precipitation followed by NaOH-acid precipitation and lastly  $\text{Na}_4\text{P}_2\text{O}_7$ -acid precipitation. This is somewhat unexpected in that the NaOH (HA-I) extractant usually gives the greatest yield. The yield of HA-III (considering ash content subtracted) is considerably greater than the first two extractants. Thus the yield obtained for HA-III preparation may be the result of a greater extraction efficiency for HA using the acetone precipitation, or the acetone powder produced represents a portion of organic matter which is not the same as HA but may be a mixture of precursor natural products. Conversion of HA-II from its salt form to the free acid lowered the ash content by only 8.6% (50.6 and 42% respectively).

#### C.4 Characterization of Humic and Fulvic Acids

##### C.4.1 IR Spectra of Humic and Fulvic Acids

IR spectra were run on several of the ashes (Fig. 21) to compare them with the IR spectra of the humic materials (Figs. 22 and 23). The only significant correlation between the pyrophosphate extracted acid and acetone precipitated humic materials and their corresponding

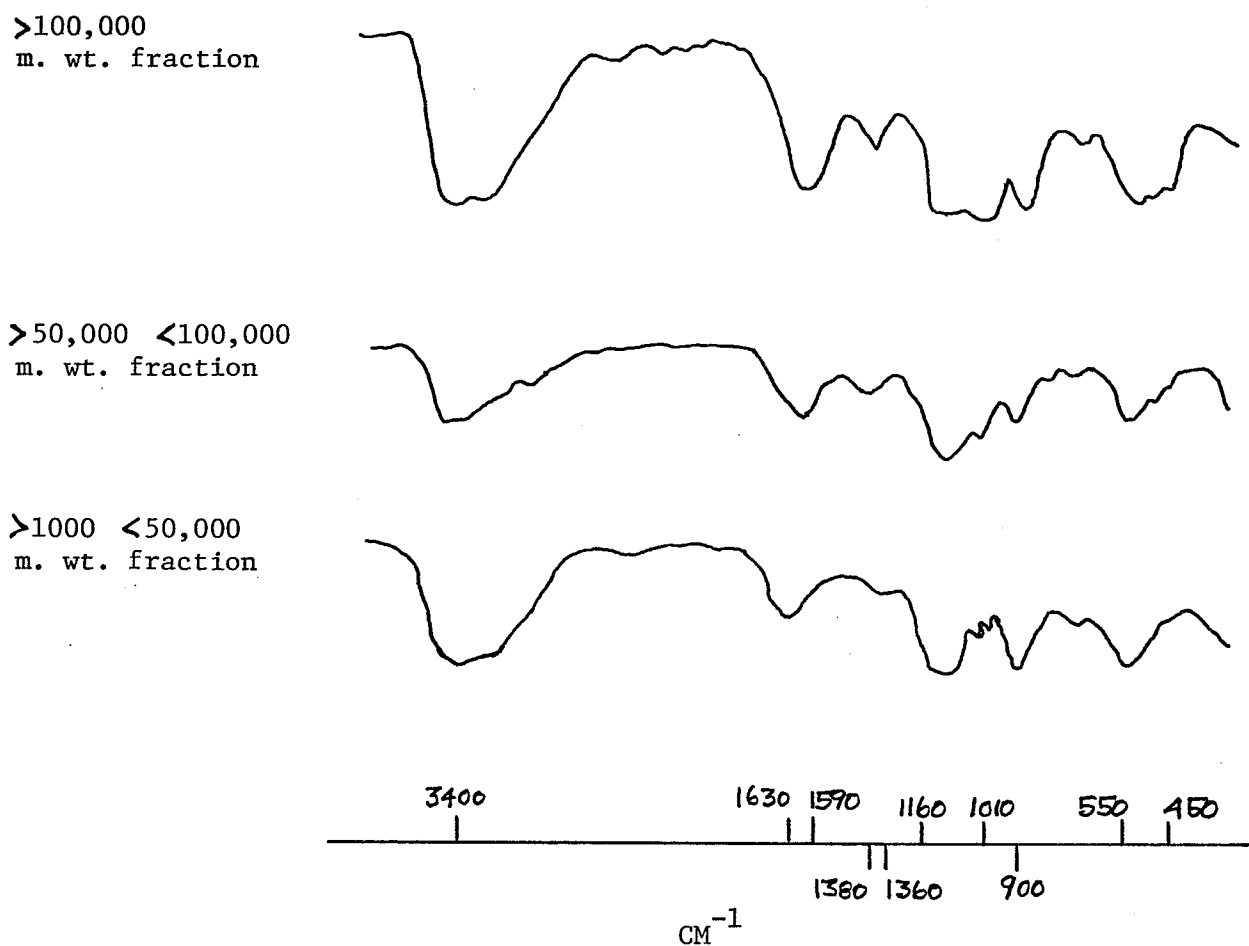


Figure 22. IR spectra of ultrafiltered HA-II, three molecular weight ranges ( $\text{Na}^+$  salts).

Acetone ppt (III)

Na+ Salt

Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>

Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>

Acid ppt.

Na+ salt (II)

Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>

Acid ppt. (II)

free acid

NaOH acid ppt.

Na+ Salt (I)

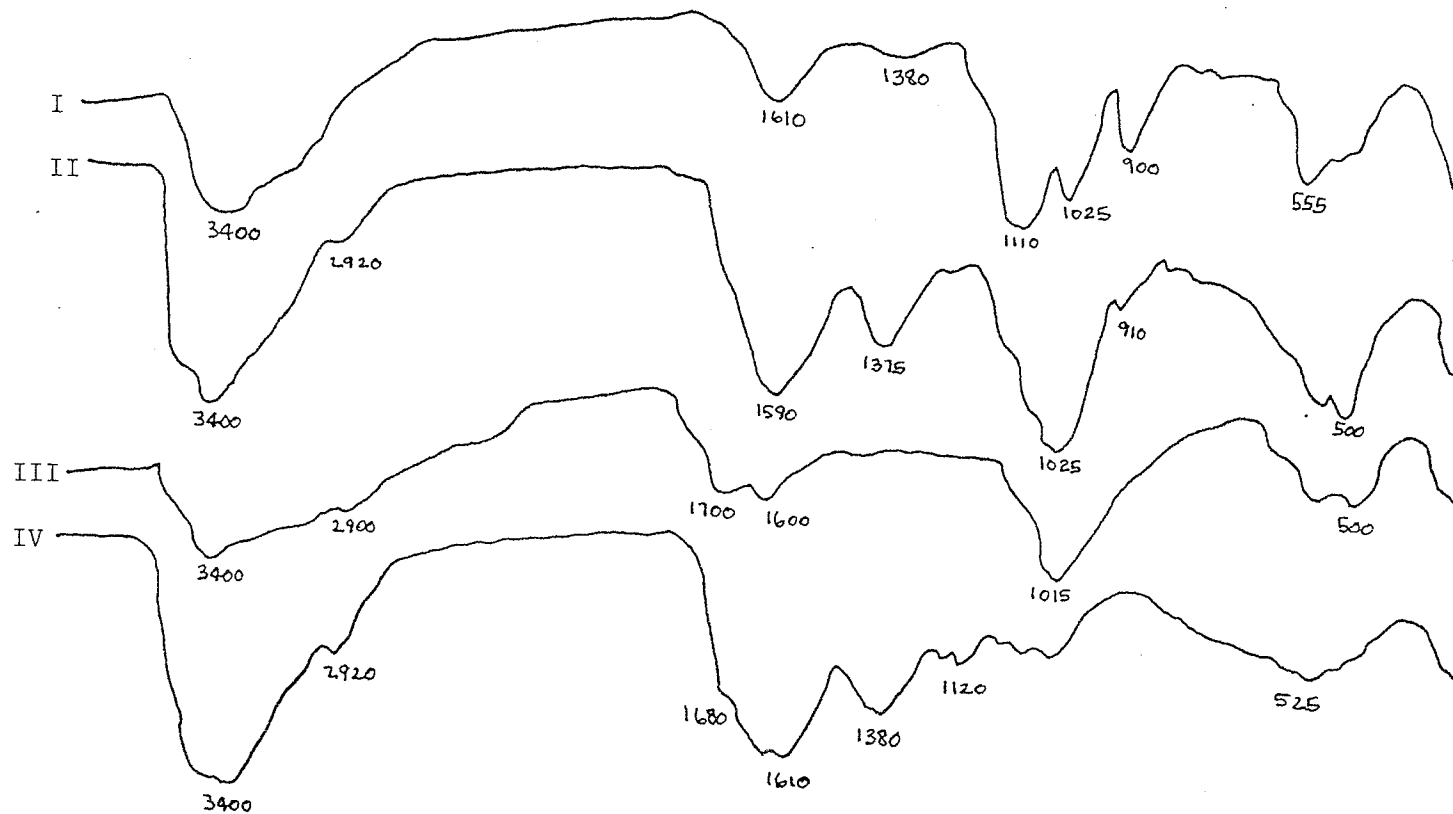


Figure 23. IR spectra of humic acids from various extractants, ( $\text{CM}^{-1}$ ).

Table 8. Yield and ash content of fractions extracted by various techniques (as the salt form).

Treatment	Yield (g/500 g soil)	Ash Content (%)	Ash-free Yield
0.5 M NaOH + acid ppt. (I)	1.1	15.3	0.93
0.02 M Na <sub>4</sub> P <sub>2</sub> O <sub>7</sub> +acid ppt. (II)	1.16	50.6	0.57
III entire	6.60	77.7	1.47
III <sub>2</sub> entire	1.4	50.2	0.70
III more than 100,000 m wt	3.5	71.7	0.99
III less than 100,000 m wt, and more than 50,000 m wt	0.4	-	-
III less than 50,000 m wt, and more than 1,000 m wt	1.3	86.3	0.18
0.02 M Na <sub>4</sub> P <sub>2</sub> O <sub>7</sub> + acid ppt. (II) (acid form, after dialysis using Spectrapor 2 tubing)	-	42	-

ashes, was in the intensive absorption centred around  $1000\text{ cm}^{-1}$ . For the HA-III fraction, there was a much higher correlation between the IR spectrum of the crystals which precipitated out of the supernatant HA-III<sub>2</sub> (Fig. 21) than with the ash IR spectrum (Fig. 21). This indicates that IR spectra of the ashes above do not yield an accurate indication of the IR absorption for the the inorganic fraction of humic substances. Ashing would lead to crystalline changes in the inorganic fraction, esp. the clay sized fraction.

(a) NaOH extracted acid precipitated HA-IR spectrum interpretation

The absorption at  $3400\text{ cm}^{-1}$  has conventionally been assigned to hydrogen bonded OH groups (Schnitzer 1965), including those of COOH groups. However, it has recently been demonstrated that this absorption is due to  $\text{H}_2\text{O}$ , probably introduced during a grinding process of the KBr (MacCarthy and Mark Jr., 1975; MacCarthy et al., 1975). Other absorptions are the  $2920\text{ cm}^{-1}$  shoulder (aliphatic C-H stretch; Giesecking, 1975; MacCarthy et al. 1975);  $1680\text{ cm}^{-1}$  peak (carboxylic acids, aldehydes, ketones; Giesecking 1975),  $1620\text{ cm}^{-1}$  often assigned to aromatic C-C and/or conjugated carboxyl, (Theng et al., 1967),  $1580\text{ cm}^{-1}$  and  $1360\text{ cm}^{-1}$  as carboxyl ion; (Giesecking 1975), and  $1020\text{ cm}^{-1}$  Si-O stretch, (Khan 1969 and Tan 1977b). Montmorillonite gives very strong absorption at 1020-1120; strong absorption at 468-523; and moderate absorption at 800-920  $\text{cm}^{-1}$  (Farmer and Russell, 1964). Montmorillonite is the dominant clay in the soil used for isolation of these humic acids and hence would perturb the humic acid spectra.

(b)  $\text{Na}_4\text{P}_2\text{O}_7$ -extracted humic material - IR spectra

In comparison, all the  $\text{Na}_4\text{P}_2\text{O}_7$ -extracted humic material, except HA-II free acid, had major absorption peaks centered at ca. 1040 and  $900\text{ cm}^{-1}$ , which is most likely due to residual phosphate (Figs. 22 and 23). These are the two main bands that are in common with those of crystals that crystallized out of the acetone powder supernatants. However, this area displays the main variations between the acetone powder subfractions; hence, analytical techniques

other than IR spectroscopy will have to be conducted in future work to determine actual differences due to humic substance content. The main difference between the acid and acetone powder precipitation methods for the pyrophosphate extraction was the appearance of a fairly sharp peak at  $1025\text{ cm}^{-1}$  as contrasted to much broader, more complex pattern of absorption for the acetone powder method. All the IR were initially run on samples in the humate or sodium salt form, and so to compare to the free acid form, an alkaline solution of  $\text{Na}_4\text{P}_2\text{O}_7$ -extracted acid precipitated humate-II (Fig. 23) was reprecipitated with HCl (Fig. 23). The main difference was in the appearance of a strong peak at  $1700\text{ cm}^{-1}$  and the disappearance of the  $1375\text{ cm}^{-1}$  peak. The main IR spectra absorptions are listed in Table 9.

Fractionation of III into three molecular weight ranges yielded spectra (Fig. 22) which were very similar, providing evidence for the micellar/aggregate nature of humic substances which will be discussed in detail in the last section of this thesis. The shift in the absorption maxima from  $1590\text{ cm}^{-1}$  and  $1380\text{ cm}^{-1}$  to  $1630\text{ cm}^{-1}$  and  $1360\text{ cm}^{-1}$  with decreasing molecular weight may reflect a greater concentration of carboxylate anion groups (Dyer, 1965) and/or more inter and intramolecularly bonded carboxylate anion groups for lower m.wt. fractions. Assignments of absorption maxima in the  $1030 - 1200\text{ cm}^{-1}$  are possibly related to esters or ether functional groups (Simon and Clerc, 1967), however, pyrophosphate absorption in the same area precludes accurate assignments.



Table 9. Major IR absorptions for isolated humic acids, I,II,III and III<sub>2</sub>.  
s = strong sh = shoulder m = medium w = weak

I	II (Na+salt)	II(acid)	III (entire)	III(>100,000)	III(>50,000,<100,000)	III(<250,000)	III <sub>2</sub>
3400s 2920sh	3400s 2900sh	3400s	3400s	3400s 2920sh	3400s 2910sh	3400s	3400s
		2900sh 2320sh 1700sh m				2360w	2900sh 2360w 1700sh
1680sh						1630s	1630s
1620sh			1610m				
		1600m			1600s		
1580s	1590s		1380w	1590s 1380m	1370m		
1360s	1375m					1360w	1350w
	1230						
1220m				1170s		1080s	1080s
					1120s		1150s
1030m			1110s				1110sh
	1025s		1025s		1030m	1030m	
	910m	1025s		1010s		985w 910s	900s
			900s 725w	900s	900s 730w		
						720w	710m
			555s				
				550s	550s		530s
525m						510m	
	500s 460s	500m 450m 415sh	510sh 460sh	510sh 450sh 415s	480sh 420sh		

The infrared spectra (Figure 23) allow one to assign absorption peaks to structural features; however, in the case of heterogeneous substances, such as humic acids, overlapping and masking of discrete absorption peaks yields spectra with broad absorption bands perturbed in this case by inorganic coextractives. General qualitative information is thus available, but resolution is poor.

Figure 23 shows the IR spectrum of each HA extracted, including the free acid and  $\text{Na}^+$  salt for the 0.02M  $\text{Na}_4\text{P}_2\text{O}_7$ -acid precipitated HA.

The first observation is the spectra contain different absorption maxima;  $1690 \pm 10 \text{ cm}^{-1}$  can be assigned (Dyer, 1965) to aromatic acids (very strong absorption) and from the comparison of the spectra it appears that aromaticity is decreasing from bottom to top (Fig. 23) or from most harsh to least harsh extraction method. The conversion of the  $\text{Na}_4\text{P}_2\text{O}_7$ -acid HA (II) to its free acid (III) shows the expected increase of absorption at  $1700 \text{ cm}^{-1}$ (s) due to  $\text{COOH}$  cf.  $\text{COO}^-$  at  $1600$  (s);  $1025\text{--}1100 \text{ cm}^{-1}$  and  $1360\text{--}1380$  (vs) is indicative of C-O of polysaccharides (Boyd et al., 1979) and is most obvious in the least harsh methods ( $\text{Na}_4\text{P}_2\text{O}_7$  acid ppt and acetone powder);  $1610$  may be assigned to C=C of aromatic rings (Williams and Fleming, 1966; Schnitzer, 1971) and from Fig. 23 this absorption clearly decreases ( $\text{Na}^+$  salt) from IV to I, further substantiating the greater aromaticity in humic acids prepared with harsher extractants. The peak at ca.  $1600 \text{ cm}^{-1}$  for HA-II is not very different in intensity and may reflect the effect of the acid

used for precipitation, i.e. increasing aromatization of carbohydrates as discussed in the literature review (section B.1.1). The  $900\text{ cm}^{-1}$  (s) may be alternatively assigned to olefinic C-H groups (Williams and Fleming, 1966) and again is strongest in the least harsh extraction method -  $\text{Na}_4\text{P}_2\text{O}_7$  acetone powder HA, however,  $\text{Na}_4\text{P}_2\text{O}_7$  absorbs strongly at  $910$  or  $920\text{ cm}^{-1}$ .

From the above observations it may be reasonable to conclude that IR evidence supports the conclusion that the nature of the extraction procedure is important; that the mildest extraction yields the most resolved spectra; that evidence for aromaticity decreases as milder extractions are used, and that olefinic and carbohydrate ether absorption is greater for the less harsh extraction,  $0.02\text{M Na}_4\text{P}_2\text{O}_7$  (pH7).

Because of the high ash content of  $\text{Na}_4\text{P}_2\text{O}_7$  in acetone powder preparations, no other specific conclusions will be made at this time concerning the fractionated acetone powder; some absorption differences do exist and for this reason it was included in Fig. 23 for comparison with the other two methods. Fig. 25 shows a spectrum of an ultrafiltered fulvic acid ( $\leq 1000$  mol.wt. excluded). Its spectrum is most similar to that of the  $\text{Na}_4\text{P}_2\text{O}_7$  acid precipitated-free acid and the acetone powder (Fig. 25) humic acids. The differences in absorption maxima at  $1150$  and  $1200\text{ cm}^{-1}$  for the FA spectrum cf.  $1025 - 1110\text{ cm}^{-1}$  for HA II or III may be due to the presence of higher aliphatic esters (ca.  $1185\text{ cm}^{-1}$ ) in the FA (Simon and Clerc, 1967).

This is of interest because FA and HA are thought to be related in terms of being parts of the sequential steps involved in humification. Therefore, the milder  $\text{Na}_4\text{P}_2\text{O}_7$  extractant spectra show that humic acids exhibit several similarities in structure to FA and that some spectral differences exist possibly because of other natural products and inorganic compounds associated with a basic type of structure.

Table 10 lists the concentrations (ppm) of the transition metal ion contents of the humic and fulvic acids used in this study. The highest iron concentration was found in the HA-II preparation at 2.78% followed by HA-III (1.06%) and HA-I (0.47%). HA-III had the greatest manganese concentration at 848 ppm and HA-I the highest copper content at 1315 ppm. Zinc concentrations were similar for each of the three main humic acids used in this study (120, 147 and 114 ppm). The low-ash HA contained the lowest levels of all metals except copper in which it had a greater concentration than even HA-III, 114 compared with 105 ppm respectively. The FA-I was also very low in metal ion content except for iron at 533 ppm.

Since paramagnetic metal ions (transition metals) can cause broadening of  $^{13}\text{C}$ -NMR peaks it is important to note that shape and resolution of the  $^{13}\text{C}$ -NMR spectra which follow will be influenced in all cases by these metals.

#### C.4.2 $^{13}\text{C}$ -NMR spectra of humic and fulvic acids

$^{13}\text{C}$ -NMR spectra were obtained for the humic acids I, II, III

Table 10. Transition metal ion contents of humic and fulvic acids studied

Sample	PPM*			
	Fe	Mn	Cu	Zn
HA-I	0.47%	22	1315	120
HA-II	2.78%	175	275	147
HA-III	1.06%	848	105	114
HA-low ash	68	ND	114	20
FA-I	533	4	52	33

\* unless otherwise indicated

and a NaOH extracted HA acid precipitated and exhaustively de-ashed (HA-IV). (Fig. 24). The resulting peaks of maximum absorption are listed in Table 11.

$^1\text{H-NMR}$  spectra of humic and fulvic acids indicate the presence of  $\text{CH}_3(\text{CH}_2)_n$  and various environments of  $\text{CH}_2(\text{CO})$ ,  $\text{CH}_2\text{-NH}$ , carbohydrate  $\text{H-C-O}$ , and usually to a lesser extent some aromatic groups. Especially of note is the recurring importance of methylene or polymethylene structures,  $(\text{CH}_2)_n$  (Barton and Schnitzer, 1963, Schnitzer and Skinner, 1968; and Ishiwatari, 1973). Polymethylene chains would be expected to have long term stability.

$^{13}\text{C-NMR}$  spectra confirm the above conclusion as methylene-carbon is shown again to be prominent, and polymaleic acid is suggested as a possible structure  $(\text{HO}_2\text{C-CH=CH-CO}_2\text{H})_n$  (Anderson and Russell, 1976). Carbohydrate is also especially prominent in most studies reported. As with the  $^1\text{H-NMR}$ ,  $^{13}\text{C-NMR}$  spectra show a aromatic component that is directly related to the nature of the extractant and the soil horizon or type. The findings reported herein confirm this. Figure 24 shows a composite of HA spectra for comparison.

Aromatic/olefinic absorption in the 100-160 ppm region increases (I>II>III) as the harshness of the extraction procedure increases, i.e., when NaOH and HCl are used. This corroborates the IR evidence, which illustrated the same effect.

A humic acid, prepared from a Red River Clay soil, with a very low ash content (<0.1% ash) yielded a spectrum (Fig. 24) not much

Table 11. Major  $^{13}\text{C}$  NMR spectral absorptions for isolated humic acids I, II, III and IV

Humic acid	NMR solvent	Chemical shifts (ppm)						
0.5N NaOH-acid, I	4N NaOD/D <sub>2</sub> O	30.1(s)	39.0(s)	57.5(s)	119.7(w)	124.8(w)	129.9(s)	174.4(s)
0.5N NaOH-acid, I	D <sub>2</sub> O	30.5(s)	39.0(s)	57.1(s)	116.3(s)	130.5(s)	135.2(s)	174.3(s)
0.02M Na <sub>4</sub> P <sub>2</sub> O <sub>7</sub> -acid, II	D <sub>2</sub> O	44.4(v. broad)	102.5(v.weak)	106.0(broad)	132.1(broad)			
0.02M Na <sub>4</sub> P <sub>2</sub> O <sub>7</sub> -acetone, III	D <sub>2</sub> O	45.1(s)	46.3(s)	118.1(w)	149(broad)		173(m)	
0.1M Na <sub>4</sub> P <sub>2</sub> O <sub>7</sub> -acid IV	0.1N NaOD/D <sub>2</sub> O	29.1(w),		59.3(w)	96.0(w)	174.3(w)	185.5(w)	

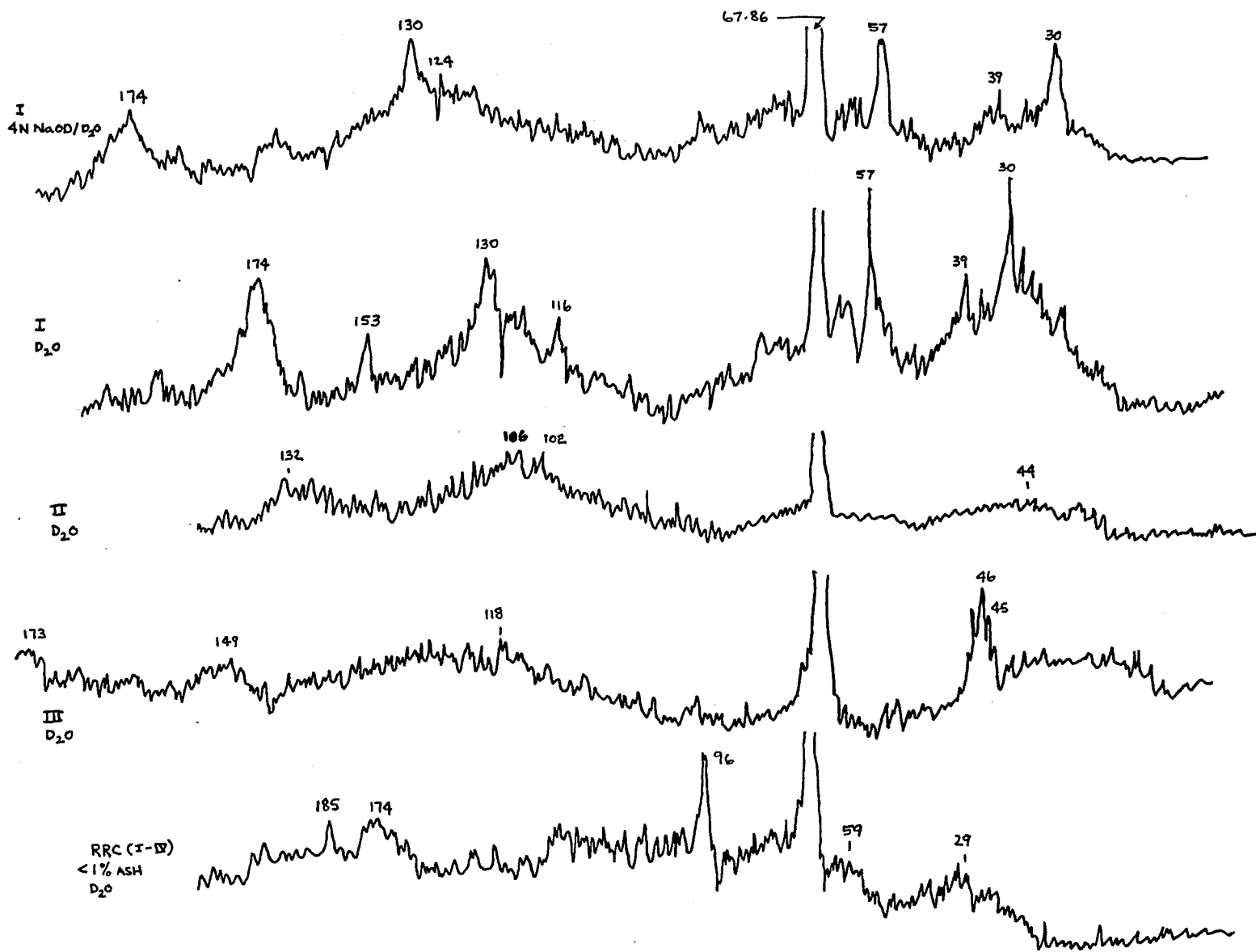


Figure 24. Composite of HA  $^{13}\text{C}$  NMR spectra



different in terms of broad and low intensity absorption when cf. higher ash content humic acids. The spectrum was most similar to that for fulvic acid (Fig. 25) and cation analysis showed these two humic substances to have the lowest concentration of  $\text{Cu}^{2+}$  and  $\text{Fe}^{3+}$  (Table 9). Although the concentrations were much lower than the other humic acids (I to III) they were still high enough to cause profound broadening (see discussion re paramagnetic ion induced relaxation). Concentrations as low as 6 ppm of  $\text{Cu}^{2+}$  in a glycylglycine sample caused absorptions to almost disappear (Bovey, 1972).  $\text{Cu}^{2+}$  concentrations were 52 and 114 ppm for the fulvic acid and de-ashed humic acid respectively.

There were only slight differences between the spectra of I in  $\text{D}_2\text{O}$  solution cf. that in a 4 N NaOD solution (Fig. 24), indicating the lack of observable ( $^{13}\text{C}$ -NMR) alteration in the HA during radiation (ca. 48h). Any alkaline degradation would have already occurred during the preparation of I. In addition, the compound being investigated by NMR is not in true solution, it will give a much more broadened pattern of absorption than if it were in true solution (Abraham and Loftus, 1978).

For the humate extracted by 0.5 N NaOH, the peaks at 30.05, 38.96 and 57.52 ppm fall in the range of aliphatic carbon absorption. According to Abraham and Loftus, possible configurations for 30.05 ppm would be  $\text{R-CH}_2\text{-R}$  or  $\text{R}_3\text{C-R}$ , while the bands at 38.96 and 57.52 ppm could reflect  $\text{R}_2\text{CH-R}$ . The major peaks at 116.26 and 130.46 ppm could be due either to olefinic or aromatic carbons. Early

workers in  $^{13}\text{C}$ -NMR have previously assigned the olefinic carbons to a fairly narrow absorption between 120 and 155 ppm, as opposed to a much broader aromatic region (Levy and Nelson 1972). Work done on humic substances with  $^{13}\text{C}$ -NMR has generally assigned the area 110 - 160 ppm to aromatic C (Sposito et al., 1978, Wilson and Goh, 1977a). However, a more recent interpretation has broadened the area of absorption of olefinic carbons, such that they cover a wider band than aromatic systems (Abrahms and Loftus 1978). Thus, any absorption in the area of 110 - 160 ppm for a complex biopolymer such as humic matter, should be regarded as belonging to possibly either aromatic or olefinic carbons. Indeed, several studies on humic substances have attributed a portion of this region to olefinic groups (Wilson and Goh, 1977b; Vila et al. 1976), but it seems that no previous workers have realized that olefinic groups have the potential to absorb throughout the aromatic region. However, Ogner (1979) believes that he has identified positively two C-H aromatic carbons at 130 and 128 ppm. This he supports by the observation that upon loss of proton decoupling the peaks split into doublets. Thus, in soil humic matter there are some  $^{13}\text{C}$ -NMR absorptions that might be assigned to aromatic carbons, as opposed to olefinic carbons. However, it has been noted that sometimes there is no absorption in this area (Wilson and Goh, 1977a) for some humic acids. The peak at 174.33 ppm is in close agreement with the value of 174 ppm obtained by Wilson and Goh (1977a), which they attribute to carboxylic groups, esters, and amides, but primarily to carboxylate entities.

The  $\text{Na}_4\text{P}_2\text{O}_7$  extracted acid precipitated humic material (II) gave a  $^{13}\text{C}$  NMR spectrum with a very high background noise level and very broad peaks (Fig. 24). This can be attributed to the high ash content, which include paramagnetic ions, principally Fe. There is a corresponding loss of information, with only three main absorption bands, that of 44.5 ppm (aliphatic), 106.5 ppm (olefinic or aromatic) and 132.12 (olefinic or aromatic).

The HA-III spectrum (Fig. 24) suffered from the same defect, but sharp split peaks appeared at 40-47 ppm. Zilm et. al. (1979) noted that six membered aliphatic rings absorb strongly in a narrow 39 - 44 ppm range. It is interesting to note that the  $\text{Na}_4\text{P}_2\text{O}_7$  extracted acid precipitated humic substance absorbs partially in this region as well.

#### C.4.3 IR and $^{13}\text{C}$ - NMR spectra of model or precursor humic substances.

Only two samples showed a visible precipitate upon acidification of a basic extraction, cellulose and cellulose plus palmitic acid. Palmitic acid was included because of the identified occurrence of n- $\text{C}_{16}$  fatty acids in humic acid (discussed previously). Fine colloidal white precipitates formed with a very slight amount of precipitation visible from cellulose acetate precursor; all other samples yielded no visible precipitate upon acidification and standing. Yield of precipitate for cellulose precursor was ca. 100 mg/50 g acid washed chromatography cellulose; yields would have been

higher for unwashed cellulose. Microcrystalline cellulose did not yield a filterable precipitate (slight cloudiness in the acidified solution).

Cellulose contains hemicelluloses (as discussed previously) which are alkali soluble and precipitable with acid. Polysaccharides of special prominence in hemicelluloses (HC) are arabinogalactans, arabinoxylans and glucans. Acid treatment can degrade such compounds readily to furfurals. Glucuronic and galacturonic (associated with HC) acids are degraded by 12% HCl to xylose and arabinose respectively; both of these are subsequently degraded to furfural in the presence of acid (Shorey and Martin, 1930). IR spectra (Fig. 25) showed the similarity of the cellulose precipitate to that of humic and fulvic acid, esp. fulvic acid, prepared by the same classical isolation procedures. The palmitic acid and cellulose ppt IR spectrum was identical to that of cellulose precipitate alone. The evidence is consistent with cellulose being an integral part of the innate structure of humic substances as well as a precursor. Precursor designation is obvious or to be expected because of the preponderance of cellulose and hence hemicellulose in plants; their occurrence in humifying plant material and in soil will be outlined latter.

$^{13}\text{C}$  - FT-NMR spectra (Table 12 and Fig. 26) revealed some similarity between the cellulose precipitate spectrum compared to the fulvic acid spectrum and a hemicellulose component - a 1 $\rightarrow$ 4, 1 $\rightarrow$ 6 glucan (Jennings and Smith, 1973) was quite similar to that of the cellulose precipitate.

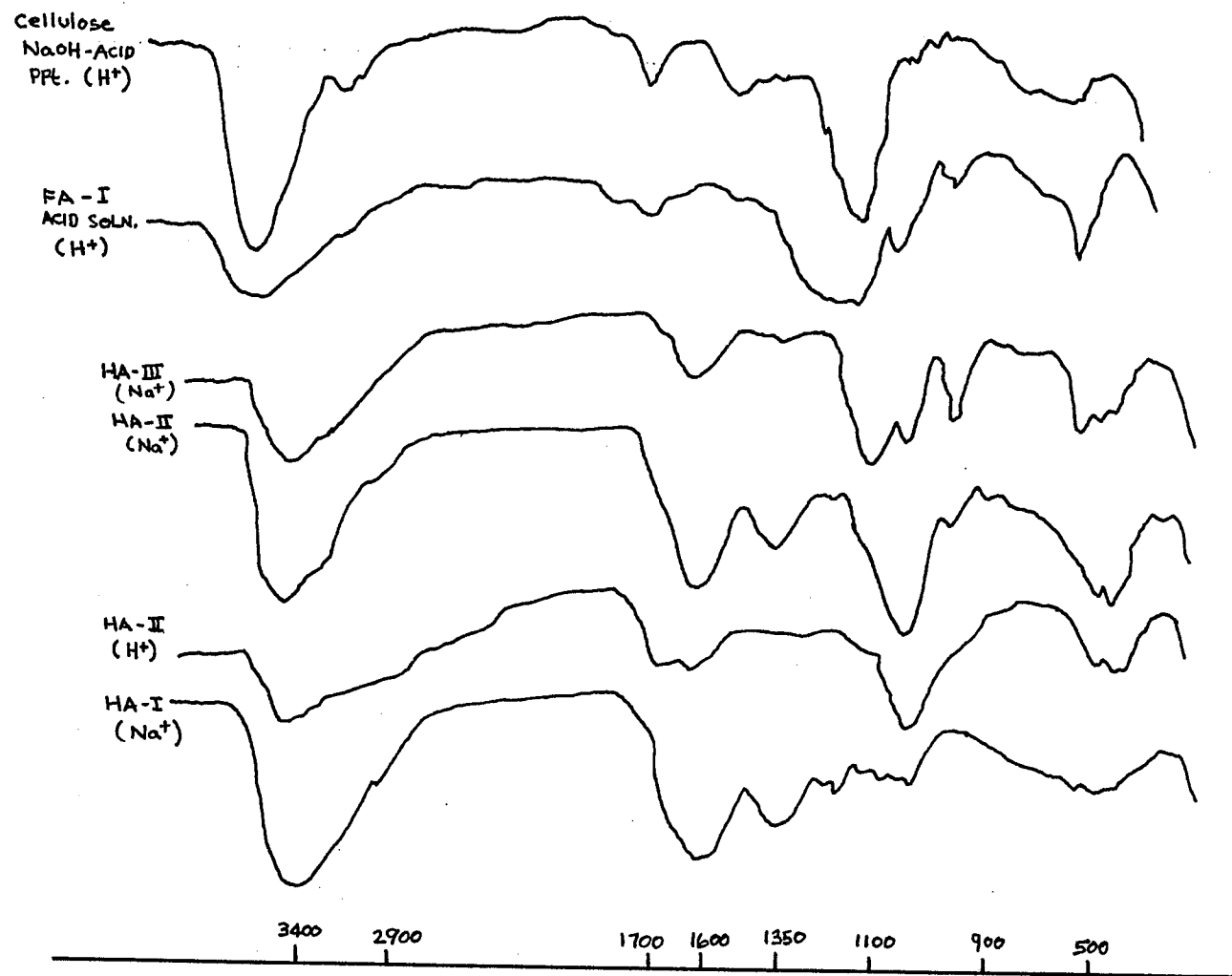
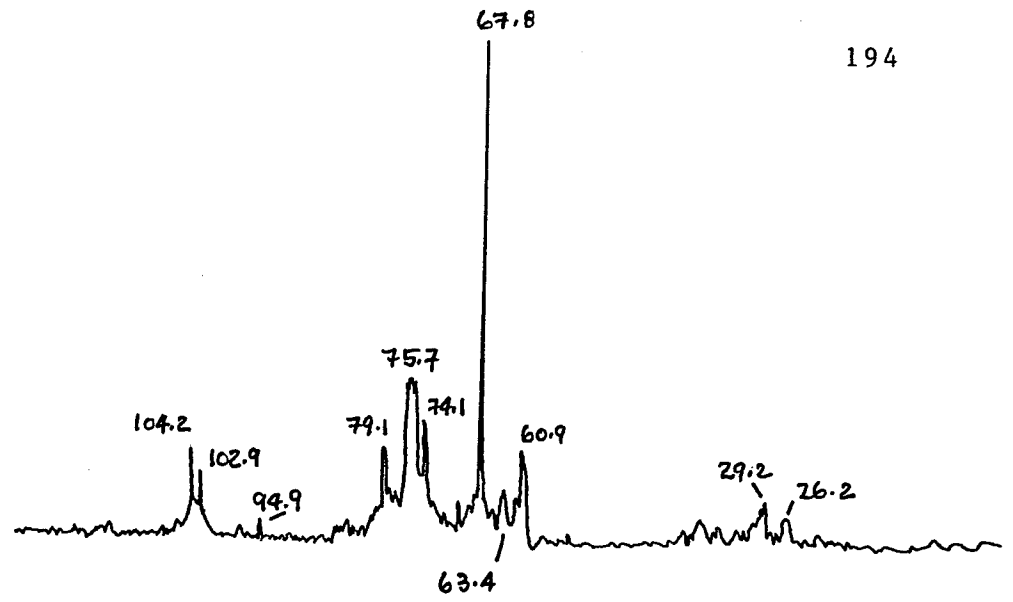
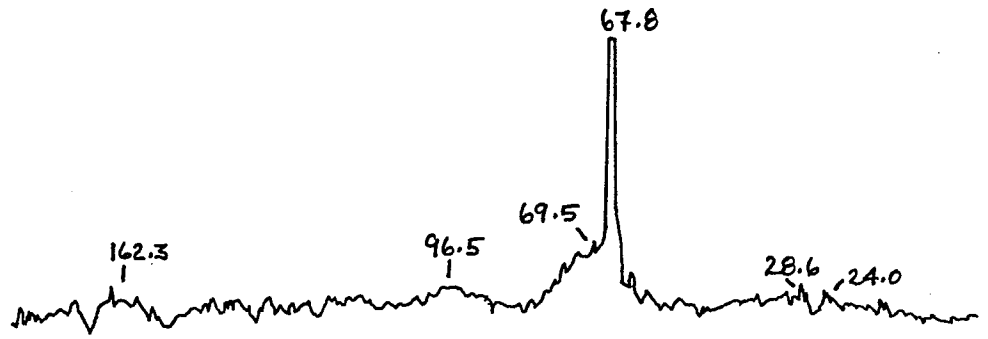


Figure 25. Composite IR spectra of humic, fulvic acids and a cellulose component isolated in these studies.

cellulose component  
(NaOH - H<sup>+</sup>)



fulvic acid  
(NaOH - H<sup>+</sup>)



1 → 4, 1 → 6  
glucan

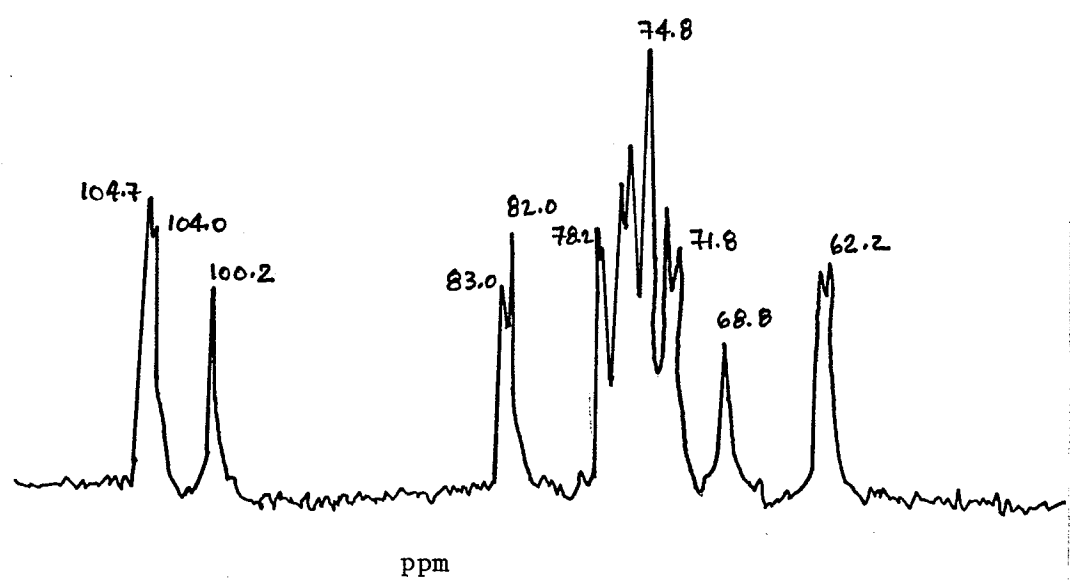


Figure 26. <sup>13</sup>C-NMR spectra of a FA, a cellulose component and a glucan, nit. std. = d<sub>6</sub>-dioxane (67.8 ppm) all in NaOD/D<sub>2</sub>O.

Table 12. Fulvic acid and cellulose extract  $^{13}\text{C}$ -NMR chemical shifts.

Fulvic acid	range	Cellulose extract	range
24.4 (w)	22.4-25.7	26.2(w)	25.1-26.5
28.6(w)	20.7-30.8	29.2(w)	28.1-30.6
62.0(w)	60.4-63.5	60.9(m)	60.2-62.0
		63.4(m)	
		66.9(w)	
69.5(m)	63.5-76.1	69.4(w)	62.7-64.5
		74.0(s)	
		75.7(s)	
		79.1(s)	
96.5(w)	92.2-101.2	94.9(w)	72.0-81.6
		102.9(m)	
162.3(w)	158.0-181.1	104.2(s)	102.1-106.4

Schnitzer and Neyroun (1975) stated that small portions of fatty acids are loosely-held and may possibly be physically adsorbed on the humic surfaces, and in internal voids, however most of the fatty acids form esters with phenolic humic "building blocks." Aliphatic-alicyclic structures may thus constitute an important contribution to the "backbone" structure of humic substances, but because of the varying yields of aromatic components reported by various instrumental and isolation techniques a conclusive assignment to humic structures is tenuous. Figure 27 gives a generalized structure based on this discussion which prepared the stage for the final discussion of mechanisms of 4-CA binding to soil and soil humic substances.

D. Physicochemical Mechanisms of 4-CA, and Other Xenobiotics Binding to and Release from Soil - a de novo Theory

D.O. Introduction

The following section will describe various physiochemical factors which affect 4-CA interaction with soil, especially soil organic matter, and which when presented as an overview results in a unifying theory to explain the mechanism(s) responsible for the binding of 4-CA and possibly other xenobiotics to soil.

NOTE: humic acid and fulvic acid are synonymous with HA and FA.



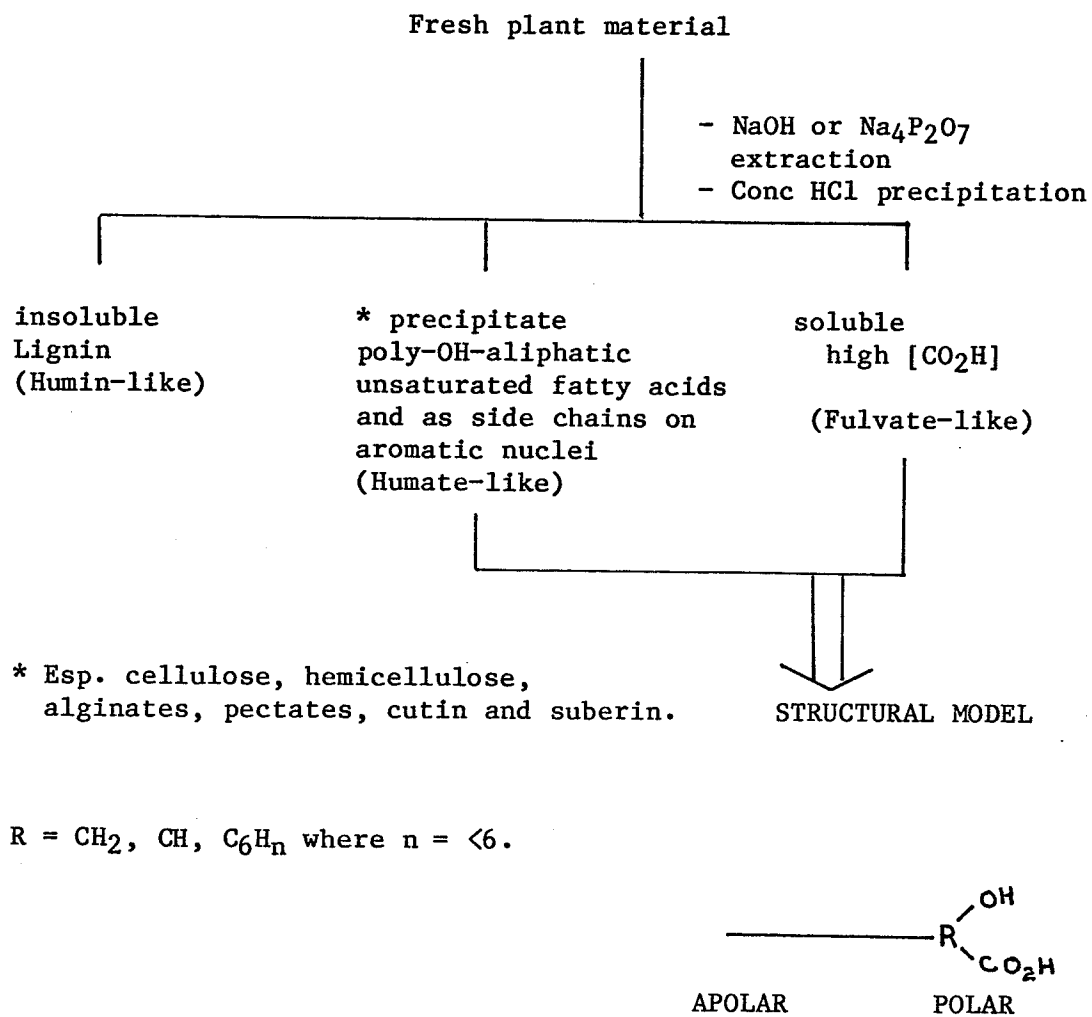


Figure 27. Derivation of a proposed humic monomer model.

The support for this theory comes from data in the literature as well as evidence described in the experimental section of this thesis.

A flow diagram (Fig. 28) shows the areas of experimental investigation leading to a theory of 4-CA (and perhaps other xenobiotics and their degradation products) binding to soil, especially humic and fulvic acid components.

The main physicochemical experimental results supporting this theory fall into six major areas as follows:

1. thermodynamics of binding phenomena
2. sorption isotherms
3. diffusion results
4. kinetic results
5. inclusion phenomena
6. porous media and micelles

#### D.1 Thermodynamic considerations

Khan and Mazurkewich (1974) reported the isosteric heat of adsorption for linuron on humic acid,  $\Delta H$ , to be -0.17 to -0.75 kcal/mole, this magnitude being consistent with physical adsorption. Such adsorption probably involved coordination with humic acid through cations. Khan (1974a) reported that relatively low values of the isosteric heat of adsorption for 2,4-D from aqueous solution on a fulvic acid-clay complex indicated a physical type of adsorption. Values calculated were always negative and varied between -1.2 to -0.3 kcal/mole with increasing surface coverage. For humic acid the

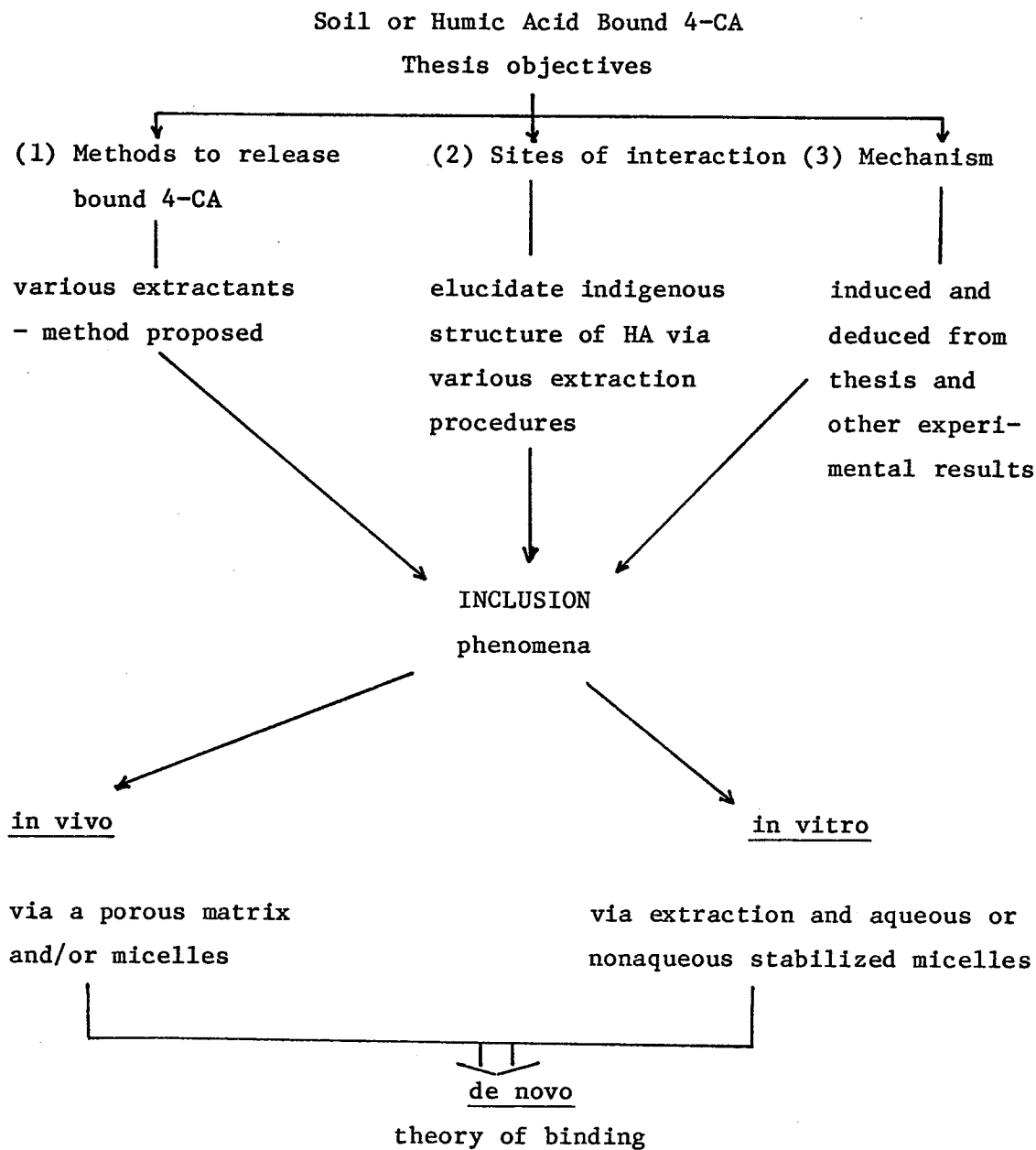


FIGURE 28. Flow diagram of thesis experimental results leading to a de novo theory of 4-CA binding to soil and soil humic acids.

values varied from -2.0 to +2.0 kcal/mole (Haque and Sexton, 1968).

Adsorption processes are entropically driven and a gain in entropy stems from dehydration of the adsorbant surface and adsorbed species. Enthalpy of reformation is endothermic (Norde and Lyklema, 1979). Harvey (1974) illustrated an important insight into amount of adsorption vs. energy of adsorption. For a series of dinitroaniline herbicides isopropalin (2,6-dinitro-N,N-dipropylcumidine) was adsorbed to the greatest extent, but was among the least strongly adsorbed (-7.5 kcal/mole,  $\Delta H_{\text{a}}^{\circ}$  heats of adsorption; the more negative the number the greater the energy required to return adsorbed herbicides to solution).

Equilibrium concentrations and heats of adsorption values were not related. Equilibrium concentrations are primarily dependent upon the extent of adsorption as determined by the number of adsorption sites available in the soil, while  $\Delta H_{\text{a}}^{\circ}$  is primarily dependent upon the mean strength of adsorption at these sites. The equilibrium concentrations describe the quantity of herbicide available to plants directly from the soil solution.  $\Delta H^{\circ}$  values would describe the reversibility of the adsorption process and the relative availability of adsorbed herbicides.

Mills and Biggar (1969b) reported an increase in adsorption of  $\gamma$ -hexachlorocyclohexane with an increase in temperature, especially for organic matter cf. clay. In a study by Van Bladel and Moreale (1974) fenuron and monuron adsorption on montmorillonite clays containing various exchangeable cations was reported. The latent

heat of solution ( $\Delta H_s$ ) followed the same pattern as for enthalpy changes,  $\Delta H$ . The more positive the heat of solution (the organic compound does not readily dissolve), the more likely the herbicide or degradation product will escape the solution phase towards the surface. Such results are consistent with the view that the more endothermic the heat of solution of the compound, the more its adsorption.

Adsorption isotherms for aniline on montmorillonite showed that when sodium or iron was the saturating cation, the sorption process was exothermic (negative  $\Delta H^\circ$ ), when aluminum was the dominant cation an endothermic process was indicated (Moreale and Van Bladel, 1980). Moreale and Van Bladel (1979b) reported positive  $\Delta H^\circ$  values for the interactions of aniline and 4-CA with two soils. The soil with a higher pH and lower  $[Al^{3+}]$ , as exchangeable, (pH 6.6, 0.03 me/100g cf. pH 3.4, 4.6 me/100g) had a less positive  $H^\circ$  value, +1.92-6.32 (4-CA) and 3.28-6.69 (aniline) kcal mole<sup>-1</sup>. Ablov et al., (1965) showed that, with montmorillonite, aniline sorption was greatest with transition metal (polyvalent)-clays cf. monovalent-clay and the heat of reaction indicated covalent binding.

Free energy changes that occur when a chemical is adsorbed can be used as a measure of the extent or driving force of the process. The greater the absolute magnitude of the G values, the greater the extent to which adsorption will take place. A large extent of adsorption does not, however, indicate that the compound adsorbed is tightly bound.

To fully characterize an adsorptive process it is necessary to know the following:

- (i) the amount of chemical adsorbed (x or q)
- (ii) strength (enthalpy) with which the molecule is held by the surface ( $\Delta H$ )
- (iii) degree of order (entropy) attained during the adsorption reaction ( $\Delta S$ )

For clays and several families of pesticides, adsorption obeys the Freundlich equation but not the Langmuir equation (Bailey et al., 1968), therefore, multilayer adsorption may be indicated, and  $\Delta G$  was consistently positive (see below).

<u>Family</u>	<u>Avg. <math>\Delta G</math> kcal/mole</u>	
	<u>H mont.</u>	<u>Na mont.</u>
s-triazine	4.1	2.5
aniline	3.2	2.2
carbarnates, anilines, amides	2.5	2.7
substituted ureas	2.2	2.7
acids	2.7	-

The Gibbs free-energy equation ( $\Delta G = \Delta H - T\Delta S$ ) permits us to describe equilibrium and spontaneous phenomena in relation to the manifestations of pesticide\*HA interactions. If  $\Delta G$  is negative the process is spontaneous. The greater the amount of heat ( $\Delta H$ ) given off in a reaction or process, the greater will be its tendency to take place, since  $\Delta G$  is made more negative. The greater the

magnitude of  $\Delta S$  the greater is the tendency for the process to occur.

At equilibrium,  $dG$  must be zero for all possible interactions. This implies that the chemical potential of a given species must be the same everywhere in the system, otherwise, there would be further reaction, or migration of material from regions of higher to regions of lower activity.

Haque and Sexton (1968) studied the absorption of 2,4-D by various adsorbents including humic acid. Activation energy for most surfaces was almost negligible and physical adsorption was indicated by all experimental results.

$\Delta E$ , energy of activation, for humic acid was ca. 1.6 kcal/mole, entropy of activation -72.0 e.s.u. and heat of activation -3.7 to +2.5 kcal/mole depending on concentration of 2,4-D absorbed. The data did not give a linear Langmuir isotherm but did for the Freundlich isotherm (see Appendices, Definitions).

The x intercept (K) of a Freundlich isotherm plot, is a measure of the extent of adsorption analogous to the equilibrium constant of a reaction, and can be related to free energy,  $\Delta G = -RT \ln K$  (Haque and Sexton, 1968). They reported almost no change in K with increasing temperature. Heat of adsorption change for  $\gamma$ -HCH (lindane) on soil from an aqueous solution was reported to be +2.6 kcal/mole and  $\Delta S$  to be +8.8 cal/mole.deg (25°C), (Mills and Biggar, 1969a). Yaron and Saltzman (1972) found that parathion was adsorbed by soils from aqueous solution without any appreciable thermic effect, therefore,

they concluded that only weak adsorptive forces were implicated.

Khan (1974a) reported a decrease in  $K$  with increasing temperature for a 2,4-D, fulvic acid-clay system, a more negative  $\Delta G$  and less negative  $\Delta H$  (-1.2 to -0.3 kcal/mole with increasing surface coverage), indicative of weak physical sorptive forces.

Recently, Moreale and Van Bladel (1979b) reported on the interactions between soil and 4-CA residues. A direct estimate of the strength of adsorption can be obtained from the effect of temperature on equilibrium adsorption. Previous studies (Hau and Bartha, 1974a, 1974b; Moreale and Van Bladel, 1976) concerning chloroanilines have shown that organic matter and solvent take a prominent part in the equilibrium relationships in the medium. Studies on the thermodynamics of organic residues adsorbed on the surface of soil colloids are relatively scarce (Mills and Biggar, 1969a; 1969b; Yamane and Green, 1972; Yaron and Saltzman, 1972; Biggar and Cheng, 1973). The application of thermodynamic methods for calculating the sorption parameters not only represents a reference method for any comparative study on pesticide adsorption properties on one or several adsorbents, but is also useful for a better understanding of the retention mechanisms.

Moreale and Van Bladel's (1979b) purpose was to characterize the adsorption of aniline and 4-CA on soil organic matter. Thermodynamic analysis was used to determine the deactivation extent of the aniline derivatives and to examine the respective contribution and magnitude of the various phenomena in the overall energetic processes. Two



soils were used; Soignies (8.52% OM, 0.5% clay) and a Heverlee soil (2.49% OM, 11.8% clay).

Adsorption isotherms were determined (adsorption  $\mu\text{g/g}$  vs. equilibrium concentration  $\mu\text{g/mL}$ ) for the two soils at three temperatures each. Adsorbed amounts increased with increasing temperature and 4-CA was adsorbed more than aniline in both soils. Isotherm patterns were "L" shaped (Giles et. al., 1960). Such a curvilinear response suggests that the number of available soil sites for adsorption becomes a limiting factor (Lafleur, 1973).

An increase in temperature always promotes interactions between soil and aniline residues, but to a greater extent in the Soignies soil. Exergonic values for the standard free energy change indicate a greater affinity of Soignies soil colloids ( $-3.8 \text{ kcal mole}^{-1}$  at  $25^\circ\text{C}$ ). It was shown that aniline analogues adsorb to a greater extent as water solubility decreases (decreasing solubility aniline  $>$  4-CA  $>$  3,4-DCA). Alternately, as hydrophobicity increases, the escaping tendency from water solvation is greater, as measured by a greater chemical potential ( $\mu_c$ ) in solution, and so more adsorption is observed, without considering other limiting or consequential factors (such as hydrophobic bonding, steric considerations of substituents, or electronic charges in the ring which varies directly with the nature and position of the substituent).

$K'$  is the thermodynamic equilibrium constant for adsorption expressed per unit of soil organic matter or  $K' = K_0 \times 100\%$  organic matter. This allows a more accurate description of the influence of

the nature of soil organic surfaces on their reactivity, since the nature of soil organic matter governs organic solute-soil interactions (Stevenson, 1972). Thus, Soignies soil organic matter had a greater reactivity per unit of organic matter ( $K'$  ratio, i.e.  $K'_{\text{soil-1}}/K'_{\text{soil-2}} > 1$ ) for aniline and 4-CA. Also, as the temperature increased over the range 4-40°C the affinity of Soignies soil organic surfaces for adsorbates increased, especially for 4-CA. If the extent of aniline and 4-CA adsorption depends mainly on the ratio of hydrophobic to hydrophilic surfaces of the organic matter, the greater affinity per unit weight of Soignies organic matter for aniline derivatives could be explained by the more hydrophobic nature of the Soignies soil surfaces. Such a situation would facilitate the liberation of adsorption sites occupied by water molecules with increasing temperature, and since 4-CA has a greater chemical activity it can compete efficiently with water molecules for adsorption sites. It follows that the net increase in the entropy change calculated ( $34.5 \text{ cal mole}^{-1}\text{K}^{-1}$ ) can be considered as being mainly due to a lesser binding of water molecules adjacent to the soil organic surfaces; this must, conclude Moreale and Van Bladel (1979b), contribute favorably to the adsorption process.

If covalent bonding was involved in the formation of recalcitrant, bound, residues within time for equilibration (10-16h) then we would expect heats of formation (exothermic, negative  $\Delta H^\circ$ ) to be negative. Approximately  $-60 \text{ kcal mole}^{-1}$  would be required for a C-N bond to form with soil organic matter components; since only a

value of approximately +6 kcal/mole was determined for the interaction of 4-CA with soil covalent bond formation is precluded (Moreale and Van Bladel, 1979b).

#### D.2 Sorption isotherms

Other studies have been reported which are analogous to Moreale and Van Bladel's (1959b) two phase sorption isotherm (Fig. 29i). It must be stated at the beginning of this section, that the interpretation given to a curvilinear Langmuir isotherm in these studies is based upon inductive reasoning. That is, physical chemical information is not yet available to allow interpretation of a two-slope Langmuir isotherm as being indicative of anything but (a) the results do not obey the Langmuir isotherm and therefore, the explanations inherent in its derivation are not applicable, and (b) the interaction of reacting species is not one to one, that is, adsorbent to adsorbate. Consequently the interpretation which follows may be open to criticism but this does not preclude the conclusions which are consistent with the available facts.

Martin and Al-Bahrani (1977) studies competitive sorption of several solutes (in water) on charcoal and found that the Langmuir isotherm was still linear. They concluded that for a non-porous (homogeneous) adsorbent, such as carbon, a monolayer represents the maximum amount adsorbable. A two stage Langmuir isotherm was obtained by Wershaw et al., (1969) for the interaction of 2,4,5-T with humic acids in aqueous solution and for  $\gamma$ -hexachlorocyclohexane in whole soil (Newland, 1969). A single stage Langmuir sorption

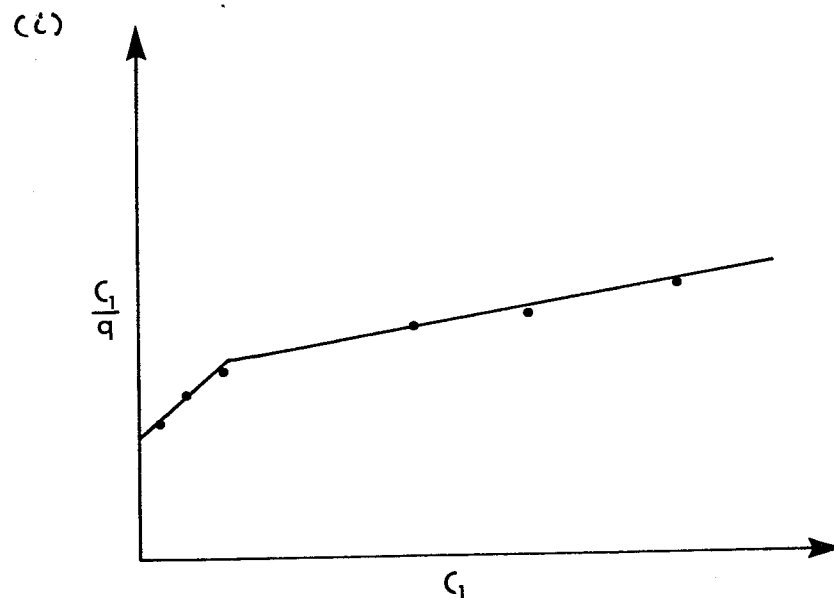
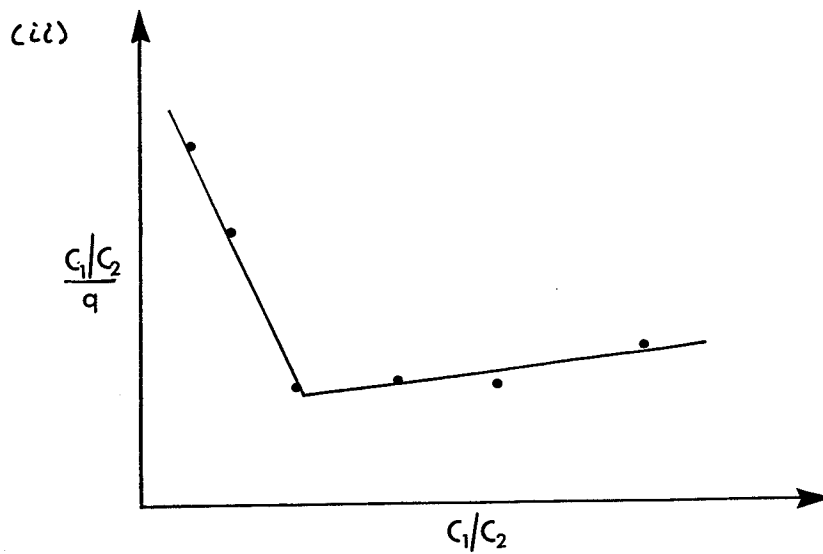


FIGURE 29. Langmuir isotherms with and without correction for ion exchange phenomena ([i] no consideration of desorbed species), ([ii] consideration of competitive sorption), [i] as per Moreale and Van Bladel (1979b) and [ii] data from [i] replotted as per the method of Harter and Baker (1977);  $C_1$ ,  $C_2$  = equilibrium concentrations of the two species in solution  $q$  = amount of adsorbate adsorbed per unit weight of adsorbent.

isotherm compared with a two stage may be due to differences in soil adsorbents (or sorbents) or differences in the solubility, reactivity and molecular volume of the adsorbate (or sorbate).

Ion exchange resins sorb solutes in conformity to Langmuir and Freundlich adsorption isotherms (Helfferich, 1962); i.e., the dependence of sorption on the solution concentrations is usually well described by a Langmuir or Freundlich isotherm when the amount of solute in the sorbent increases and the distribution coefficient decreases with increasing concentration. Since temperature dependence for ion-exchange phenomena is small and clays usually conform to the classical isotherm whereas organic matter does not, we can probably eliminate most direct clay interaction and ion exchange phenomena for 4-CA in Moreale & Van Bladel's study (1979b).

Adsorption phenomena of organic compounds on soil colloids are usually exothermic. In Moreale and Van Bladel's study (1979b) the overall adsorption process was energy (heat) consuming (positive  $\Delta H^\circ$  values). The heat involved in the reaction results from several energetic components so that endothermic phenomena such as lateral interactions (repulsion) between sorbed molecules, as well as the desorption of solvent molecules from the surface of the adsorbent contribute to the overall interaction energy. "Each of these energetic components can be prominent as shown by Haque and Sexton (1968), who pointed out that solvent desorption was mainly responsible for the positive values measured for the heat of

adsorption of 2,4-D on humic matter. Similarly, an increase in the mutual repulsion of sorbed molecules also explains a positive heat of adsorption (Kuo and Lotse, 1974)", (Moreale and Van Bladel, 1979b).

Deviation from linearity may indicate that at least two sorption sites mechanisms exist. Linear transforms of the Langmuir equation permitted Moreale and Van Bladel (1979b) to construct isotherms at three temperatures (Fig. 29(i)). The isotherm for the 4-CA-Soignies soil exhibited a definite change in slope. Adsorption of 4-CA may occur preferentially on higher energetic (or more available) sites; the lower energy (or less available) sites being filled at the final stages of adsorption.

Recently, Cloos et al., (1979) have shown that the extent of adsorption of aniline and 4-CA (from  $\text{CHCl}_3$  or  $\text{CH}_3\text{OH}$ ) to montmorillonite depended on the nature of the exchange cation. Complexes (broad IR absorption due to loss aromaticity) were identified, characterized by an intense charge-transfer (ionic forms of the electron donor or adsorbed species and acceptor or saturating cation) band in the infrared region and by the formation of radical cations, evidenced by ESR spectroscopy. Adsorption mechanisms proposed by Cloos et al., (1979) were dominated by hydrogen-bonding and could represent the initial adsorption curve described earlier (Moreale and Van Bladel, 1979).

Moreale and Van Bladel (1979b) showed conclusively that adsorbed or recalcitrant 4-CA residues were most prevalent in soils with high organic matter content. The same authors (Moreale and Van Bladel, 1976) reported further that multiple regression analysis indicated

that organic matter plus available water (or clay) correlated with sorption better than the simple linear relationship with organic matter alone. The resulting equations accounted for most of the variability in aniline or 4-CA adsorbed ( $R^2 = 0.92$  &  $0.94$  respectively). The contribution of organic matter to the adsorption was calculated as 79 & 77% for aniline and 4-CA. No other combinations of variables significantly improved the relationship studied.

The two slope Langmuir isotherm of Moreale & Van Bladel (1979b) and Worobey (1977) for 4-CA interactions in soil may be indicative at phase I of physical adsorption followed by phase II influx into the porous HA followed by simultaneous or subsequent interactions. This concept would be analagous to Hamaker and Thompsons' (1972) description of chemisorption for pesticides in soil ("the phenomena of chemisorption can be interpreted as a rapid physical adsorption followed by a slow establishment of the stronger chemisorption bond") except the second phase in the 4-CA isotherms may be the result of an inclusion phenomena. Such isotherms for 4-CA did, in fact, go through the origin thus indicating physical adsorption.

Fuchsbichler and Suess (1978) studied the desorption of 4-CA sorbed to 8 different agricultural soils. Exchangability was investigated as a means of desorbing 4-CA. 4-CA (free) and 3,4-DCA were capable of displacing more sorbed 4-CA from soil cf. monolinuron, atrazine, pyrazon, citric acid, 4-aminobenzoic acid, potassium, aluminum, and calcium chloride as exchangers. The longer 4-CA resided in the soil, the more resistant it was to being exchanged with 3,4-DCA; for example, after 16 weeks only ca. 2% exchange was

possible (however, water alone extracted the same amount). Seventy percent [ $^{14}\text{C}$ ]4-CA was adsorbed to a soil of which 32.6% was exchangeable with [ $^{14}\text{C}$ ]4-CA; 17.6% was extractable into water. This indicates that a portion of sorbed 4-CA is weakly held, ca. 20-30% (as per this thesis study, Hsu and Bartha, 1974b; Fuchsichler and Suess, 1978) possibly via surface complexes. Fuhr et al., (1977) also found that a 25 ppm [ $^{12}\text{C}$ ]methabenzthiazuron solution would displace 59.0% of the [ $^{14}\text{C}$ ]radiolabelled methabenthiazuron from treated soil (10 ppm). Extraction with 0.1N ammonium oxalate, a solution normally used to determine the exchange capacity of soil, also removed 59%  $^{14}\text{C}$ . A further 20% could be removed using acetone/ $\text{CHCl}_3/\text{H}_2\text{O}$  and 12% with 0.1N NaOH. A first extraction with 0.1N NaOH released 72%  $^{14}\text{C}$ . These results support the idea of inclusion products - readily exchangeable in the first instance and a lesser amount nonexchangeably included.

Such results may also support the explanation of the nature of the two slope Langmuir curve of (Fig. 29(i)) curve I corresponding to physically sorbed exchangeable 4-CA and curve II corresponding to 4-CA included complexes (4-CA could have penetrated the porous organic matter so that water was excluded). The second curve may also represent micellar (solvent stabilized) inclusion complexes.

A similar two slope Langmuir curve was obtained by Worobey (1977) for sorption of 4-CA to suspended and dissolved organic matter in pond water. To determine if such a curvilinear response could be due



to competitive sorptive phenomena (for e.g., with counter ions on the OM) a modified form of the Langmuir equation was used and isotherms plotted (Harter and Baber, 1977). Fig. 29 shows that a curvilinear response (two slope curve) still prevailed for 4-CA in soil (Moreale and Van Bladel, 1979b data replotted) and for 4-CA in pond water (Worobey, 1977 data replotted) a similar curve resulted indicating that competitive sorption was not involved.

### D.3 Diffusion controlled mechanisms

Transport by diffusion is slow relative to mass transfer by soil-water movement, but may be exceedingly important over short distances. It is the only means of pesticide movement within the soil profile under static conditions (Bode et al., 1973).

Lafleur (1979) reported that metribuzin (triazine) adsorption rates (amount sorbed/amount applied) were independent of time when applied to inorganic substrates (clays, sand, alumina). Adsorption was wholly independent and desorption marginally dependent in some cases. Metribuzin sorption by substrates containing organic matter increased with time; equilibrium was not reached within  $10^4$  min (ca. 6 days), but extrapolation suggested it would be reached before  $10^5$  min. Equilibrium was near completion within 1000 min ( $P_s/P_o = 0.26$  cf. 0.30 for  $10^4$  min). This agrees well with the 4-CA results reported by Hsu and Bartha (1974a), i.e. 80% completion in 27 hrs (1620 min). Lafleur, (1979) concluded that adsorption by inorganic substrates seemed to be restricted to easily accessible surface sites, and internal surfaces had a negligible effect on sorption rate.

The equilibrated state is then perturbed (extracted) and the main thrust in the system is toward desorption or desorption-adsorption readjustments. Ultimately, within  $10^4 - 10^5$  min., the free (driving) energy of the desorption system becomes dissipated as equilibrium is approached. Thus, early desorption responses tend to fluctuate and be somewhat unpredictable, but are resolved with time.

Metribuzin adsorption by peat or soil organic matter seemed to be a multistage process involving (1) movement (Brownian, mechanical) of the herbicide to the organic matter - solution interface (2) adsorption at accessible external organic matter sites, and (3) gradual invasion of restricted inner surfaces by a dynamic adsorption-desorption movement - replenishment sequence, as the system adjusts to a minimum free energy equilibrium condition. During desorption the same mechanisms would operate in reverse to release metribuzin to the external solution if the extractant did not change the 1°, 2°, 3° and 4° structure of the adsorbent. The above mechanism may help explain the results of Moreale and Van Bladel (1979b).

If low water solubility enhances diffusions then one would expect dinitroaniline herbicides to behave similarly to 4-CA. The water solubility of substituted dinitroaniline is usually <1 ppm and for a silty loam soil (4.0% organic matter) Harvey (1974) found heats of adsorption for 12 dinitroanilines were -6.9 to -17.0 kcal/mole. This indicates an exothermic interaction and may be due to adsorption on soil colloids (not via charged complexes since most pKa values are <0), especially via dipole or induced dipole interactions; since soil - water ratios used were so high (25:1, 50:1, 100:1) diffusion controlled movement in such a solid matrix would be secondary (unless the compounds were especially volatile and, therefore, vapor phase diffusion may be prominent). Dinitroaniline vapor pressures are ca.  $10^{-4}$  -  $10^{-8}$  mm Hg. Thus, Helling (1976) indicates that, for

trifluralin, diffusion may be a more important mode of transport than mass flow because of its  $10^{-4}$  mm Hg vapor pressure.

Scott and Phillips (1972) noted that trifluralin diffusion increased as soil water content decreased (in contrast to other compounds). They attributed this to substantial vapor phase movement. This contrasts with the results of Bode et al., (1973) who state that for trifluralin diffusion in soil, maximum diffusion occurred at ca. 10% soil moisture content. At low bulk density maximum diffusion was shifted to higher moisture contents and was ca. 2.5 times larger than values from soil at high bulk density. When the air-filled fraction of the soil volume was reduced below 40% v/v by either compression or addition of moisture, diffusion of trifluralin began to decrease.

Self-diffusion is defined (Nye, 1966) as the mechanism by which an ion (organic or inorganic or neutral species) exchanges with its own isotope ; the environment is otherwise unchanged. Bulk diffusion involves diffusion of an ion (etc.) in bulk, accompanied by, and exchanging for, other ion species. Further self-diffusion is dependent on: (1) numbers of ions in free solutions, the diffuse double layer, and the solid 'sorbed' states; (2) the electrochemical mobility of the species in the various states; (3) moisture content; and (4) conformational 'impedence' factors, depending on the size, distribution, and continuity of the pores.

Scott and Phillips (1972) concluded that as soil moisture content increased, the self-diffusion coefficient (rate of diffusion) of

several herbicides increased. For example, the diffusion coefficient for chlorophropham increased seven times over a moisture range of 17-25%. This increase in diffusion with an increase in water content was thought to be due to a combination of the geometry effects of the diffusion pathlength and of the adsorption-desorption properties of the herbicide-soil complex. The geometry of the diffusion pathway for each herbicide depends on the nature of the pore space formed by the particles in the soil and on the moisture content. In a saturated soil the diffusion pathlengths are shorter than in an unsaturated soil, and the shorter the pathlength the greater the expected rate of diffusion. Also, in a saturated soil the amount of water competing with the herbicide for adsorption sites is greater than when the soil is unsaturated. Thus, a greater amount of herbicide may be in the solution phase which would result in an increase in the rate of diffusion because of (1) there would be a higher concentration gradient, (2) the herbicide would be expected to diffuse faster in the solution phase than in the adsorbed phase. In an unsaturated soil the water is contained in the smaller pores and the geometry of the water pathway is less continuous. The exact way the pore geometry and moisture content influence the diffusion depends on the solubility of the herbicides in water and their volatility which govern the relative amounts moving in the solution and vapor phases.

Lavy (1970) found that diffusion rates for several triazines in soil were positively correlated with total surface area of the soil.

Increasing soil moisture contents, soil pH, and soil temperature resulted in greater diffusion rates.

Lafleur (1979) studied the sorption of several pesticides in soils and models thereof. He concluded that sorption by inorganic substrates appeared to be restricted to easily accessible surface sites. Internal surfaces had a negligible effect on sorption rate, if they are present. Pesticide sorption by peat or soil organic matter seemed to be a multistage process involving (A) movement (Browman, mechanical) of the pesticide to the organic matter-solution interface, (B) sorption at accessible external organic matter sites and (C) gradual permeation of inner surfaces by a dynamic diffusive adsorption-desorption sequence, as the system attempts to approach a minimum free energy equilibrium condition.

Kuo and Lotse (1974) studied phosphate adsorption and desorption from lake sediments. The rate of phosphate adsorption was initially rapid and decreased with time. The initial rapid adsorption was probably a high energy adsorption associated with low surface saturation. The slow reaction was assumed to be a result of increased negative charge, increased interaction energy and decreased adsorption energy (Kuo and Lotse, 1973). The phosphate adsorption increased with temperature indicating that phosphate adsorption by sediments was an endothermic reaction. Kuo and Lotse (1974) concluded that phosphate adsorption in sediment was a diffusion controlled process.

Leenheer and Ahlrichs, (1971) point out that hydrophobic neutral

(as well as acids or bases) organic solutes can sorb on sediment through Van der Waal's forces (physical adsorption); hydrophilic neutral solutes should not be significantly sorbed on any natural sediment. As water solubility of the solute decreases sorption increases, because diffusion rates decrease (Scott and Phillips, 1972).

#### D.4 Kinetics

Rao et al., (1979), described both adsorption and desorption of pesticides in soil as two stage processes. Adsorption on one group of sites was assumed to be instantaneous, while the rate of adsorption on the second group of sites followed either non-linear reversible kinetics.

In applying the adsorption desorption relationships to model chemical transport in soil, the current trend is to assume that chemical transfer follows either a kinetic or an equilibrium model, but not both. Cameron and Klute (1977) presented a view in which they assumed that "adsorption" of a specific chemical by a soil may be represented by a combination of kinetic and equilibrium models.

The reasoning behind such an approach involved making the transition from a chemical reaction at a single location within a soil pore, for example (microscopic viewpoint), to the average observed results for a very large number of pores (macroscopic viewpoint). A chemical moving through the ensemble of pores may react at different rates with different constituents, organic and

inorganic. The solute may react rapidly or slowly, depending upon the variability in the arrangement of molecules of that constituent (e.g, functional groups of organic matter, edge, interlayer, and interlattice sites on clay minerals).

Microscopic nonuniformities will influence the rate at which the solute concentration is renewed at various reaction sites. Under these conditions it is possible for a site-specific instantaneous reaction to appear kinetic because of a limited rate of supply of chemical through solution to the site. In order to simplify the complex nature of the soil pore reactions, one could assume that there are two types of soil reaction sites: (1) those which appear to adsorb or react rapidly with a chemical (surface reactions, for example), inducing an instantaneous equilibrium, and (2) those which appear to adsorb the solute more slowly, indicating a kinetic reaction.

Sites where the reaction rate is limited by diffusion are assumed to fall into the kinetic category. Cameron and Klute (1977) concluded that the exact microscopic location of the two types of reaction sites was speculative. Thus, on a macroscopic level, reactions of a given compound with soil could be described by kinetic and equilibrium models. The equilibrium component results in instantaneous adsorption and causes a retardation in the breakthrough curve ([atrazine] versus time) of the compound. The kinetic component governs the rate of climb of the breakthrough.

Most recently, Karickhoff (1979) described similar results for



sorption of hydrophobic pollutants in natural sediments (aromatic polycyclics). When sediment suspensions were mixed with aqueous solutions of a pollutant, the approach to equilibrium was resolved into two line segments. All components displayed kinetic factoring, whereby the aqueous phase concentration decreased rapidly (5-15 min), then approached equilibrium at a much reduced rate.

(1) A rapid component - 50% of sorptive capacity requiring a few minutes for equilibrium with the solution phase of the pollutant.

(2) A much slower component (requiring days or weeks for equilibrium).

The slower process was again viewed as diffusive transfer to sorption sites that are inaccessible directly to the bulk solution. Diffusive movement on a particle surface or to the particle interior (i.e., into components or in particle pores) should be mediated by sorptive steps, and thus increased sorption should result in increased independence to diffusive transfer.

Two distinct rates of sorption for several nonionic chlorinated pesticides (DDT, heptachlor, and dieldrin) on clay were also reported (Huang and Liao, 1970). In non-expandable clays, adsorption was completely instantaneous cf. expandible clays where adsorption was gradual and the reaction was divided into two distinct stages i.e., (1) the instantaneous sorption on the clay surface, and (2) the gradual diffusion of additional pesticide into the clays interlamellar spaces. Adsorptive capacities of the clay minerals were not correlated with their ion-exchange capacities or their

specific surface areas. It also appeared that only nonrigid molecules were sorbed interlamellarly; dieldrin was sterically hindered.

A series of hexachlorocyclohexane (HCH) isomers ( $\alpha$ ,  $\beta$ ,  $\gamma$ ) were allowed to interact with an organic matter-inorganic matter matrix (soil) cf. the inorganic matrix alone (Wahid and Sethunathan, 1979). The steric requirements for interlamellar sorption were again demonstrated;  $\gamma$ -HCH was sorbed on clay and iron oxides of the soil,  $\beta$ -HCH sorbed only by iron oxides, and  $\alpha$ -HCH was sorbed on neither. Sorption of all three isomers by the whole soil (12 different soils) was similar, indicating lack of steric hindrance for organic matter interactions. Above 3.4% organic matter (OM) the OM masks the inorganic components such that all sorptive phenomena are due to the organic components.

Interlayer adsorption of atrazine into the silicate spaces of montmorillonite has recently been ascertained by x-ray diffraction (Hernandez-Crespo and Rodriguez-Pascual, 1978). The presence of parathion in the interlayer regions of a montmorillonite clay was demonstrated recently by Cheung et al., (1979). The included parathion was nonexchangeable with metal cations and its persistence in soil as well as resistance to microbiological degradation was attributed to interlamellar inclusion. Interlamellar sorption has been demonstrated to occur for various amines in montmorillonite (Vansant and Uytterhoven, 1973), as positively charged ions or neutral amines (Shoval and Yariv, 1979).

The binding of aniline with montmorillonite clays containing transition metal (polyvalent) cations is much stronger cf. monovalent cations, with the bonding strength approaching that of covalent forces (Ablov, et. al., 1965). Smith (1936), reacted organic bases and their salts with montmorillonite and presented evidence that the reaction was one of ion exchange.

The affinity of  $\alpha$ -amino acids for clays (at their Ip) was more extensive when the clay was saturated with divalent cations cf. monovalent cations; the mechanisms was shown to be one of ion exchange, formation of salts, and/or coordination complexes with metal cations or the incorporation into the clay combined with polycondensation (Siffert and Kessaissia, 1978).

Finally, as discussed previously, Moreale and Van Bladel (1979b) reported a slower rate of reaction for 4-CA (lower rate constant) in a soil with higher [OM]. This may possibly support the view or hypothesis that adsorption of 4-CA in soil containing sufficient OM is more a diffusion-controlled process within the porous organic matter adsorbent.

#### D.5 Inclusion phenomena

##### (a) Introduction

Boiling acid (6N HCl), was used by Cheng (1968) to release bound 3,4-DCA, and is known to alter the structure of humic acids (Riffaldi and Schnitzer, 1973), possibly releasing included 3,4-DCA. Hsu and Bartha (1974a) found that biological activity was not required for the binding of 3, 4-DCA to whole soil, since sterilized soil bound

virtually as much 3,4-DCA as nonsterilized. This further supports an inclusion phenomena being responsible for the majority of bound 3,4-DCA or, in fact, substituted chloroanilines or pesticides generally.

Thermal analysis (combustion) of reduced [ $^{14}\text{C}$ ]triazines as bound residues (Helling and Krivomak, 1978a) suggested that chemically, stabilizing reactions between phenolic hydroxyls and free aromatic amino groups of a metabolite were easiest to postulate. It seemed unlikely from the thermal profile data that bound  $\text{C}^{14}$  had become part of a highly condensed "nucleus" of soil organic matter since this oxidizes mainly in a higher region, 450-550°C cf. 300-350° required for the anaerobic release of most [ $^{14}\text{C}$ ]butralin bound residues. Other possibilities for the bound dinitroanilines may involve interaction with the interlayered organic matter within the expanding clay mineral lattices; such organic matter becomes thermally more stable (Schnitzer and Khan, 1972) and again suggests the feasibility of inclusion complexes.

Other evidence or considerations of inclusion phenomena for substituted chloroanilines in lignin have been expressed. Chin et al., (1964) concluded that the bound portion of an acetamide degradation product in rice, 3,4-DCA, was a lignin complex. The mechanism of combination was thought to resemble an inclusion process. The unusual stability of the lignin-residue complex and its related molecular size suggest that the residues were deeply trapped within the lignin polymer. Still et al., (1980) studied binding of 3-CA and

3,4-DCA in rice lignin and suggested that residues may bind because of being trapped inside or included in the cage of the lignin molecule. Although, in-vitro enzymatic studies indicated covalent binding of the anilines to a lignin precursor monomer (coniferyl alcohol), isolated Bjorkman lignin (with bound residues) seemed to contain some included anilines. Still et al., (1980) thought they had proven covalent bonding of chloroanilines to coniferyl alcohol (monomeric lignin precursor) at the  $\alpha$ -carbon. It must be emphasized, however, that such evidence was not obtained for chloroaniline bound residues from plants (only from copolymers of 3-CA formed during polymerization of coniferyl alcohol in a peroxide enzymatic model system).

Wood et al., (1977) showed that 4-iodoaniline forms an inclusion complex with cyclohexaamylose [ $\alpha$ -cyclodextrin, the smallest member of the family of Schardinger dextrans, cyclic oligosaccharides consisting of 6 or more  $\alpha$ -(1,4)-linked D-glucofuranose units]. By analogy, this may be evidence for 4-CA inclusion within soil polysaccharides. Polysaccharides are considered to be one of the most abundant organic components of soil [10-20% and up to 45%, (Flaig et al., 1975)].

Thermodynamic parameters for cycloamylose complexes at 25°C for a number of organic substrates were (Helfferich, 1961, 1962):  $\Delta G^\circ = -3.1$  to  $-4.6$  kcal/mole;  $\Delta H^\circ = -1$  to  $+6.6$  kcal/mole;  $\Delta S^\circ = 8$  to  $-9.6$  eu.

Associations of hydrocarbons in water have been explained in terms of hydrophobic bonding (Schreraga, 1979). Thus, the thermo-

dynamic parameters for formation of hydrophobic bonds have opposite signs from those for mixing hydrocarbon and water, viz.,  $\Delta H^\circ > 0$ ,  $\Delta S^\circ > 0$  and  $\Delta G^\circ < 0$ . For example, hydrophobic bond formation between like amino acid residues are as follows:  $\Delta G^\circ = -0.3$  to  $-1.5$  kcal/mole,  $\Delta H^\circ = 0.4$  to  $1.8$  kcal/mole,  $\Delta S^\circ = 2.1$  to  $11.1$  eu. If we consider hydrophobic-type bonding or hydrophobic interaction of 4-CA to hydrophobic areas of humic acid or fulvic acid the thermodynamic values determined by Moreale and Van Bladel (1979b) are of similar magnitude as those above,  $\Delta G^\circ = -3.8$ ,  $\Delta H^\circ = 6.34$ , and  $\Delta S^\circ = 34.5$ ; the larger  $\Delta S$  value indicating a high tendency for the interaction to occur.

Roques and Bastick (1979) reported some structural characteristics of microporous carbons based on adsorption and diffusion data. A slit-like pore system was envisaged between graphite planes (7 to  $8\text{\AA}$  wide with constrictions of Ca. 5Å). Isosteric heats of adsorption for  $O_2$ ,  $N_2$ , and  $CO_2$  were, 11.3, 20.9 and 32.6 KJ/mole. Sibernagel et al., (1979) concluded (from ESR & NMR data) that 2,2,6,6-tetramethyl-4-piperidinol-1-oxyl, a nitroxide spin label compound (Tempol), was included in the matrix of a coal sample as substrate, and that Tempol molecules were rapidly absorbed on the surface of the coal. Subsequent loss of Tempol from the liquid phase was associated with diffusion of the molecules into the coal matrix. Other compounds were also shown to be trapped as inclusion complexes i.e., benzene, pyridine, 2-fluoropyridine and  $\alpha$ -fluorophenol. Larger

molecules and weaker bases were less readily included, e.g., hexafluoropropane diamine.

Isotherms for aniline (Hansen et al., 1949) on various graphites, carbon blacks and sugar charcoal were similar to that found by Moreale and Van Bladel (1979) on soil. This again shows the analogy of humic substance structures to that of coals may be valid.

#### D.6 Humic substances as micelles and micellization as a binding mechanism

It is possible that humic acids may form spherical or more likely linear ionic micelles which permit the penetration of lipophilic organic compounds into the lipophilic areas of the overlapping humic acid chains.

Shinoda et al. (1963) suggested four possible modes for incorporation of material into polyoxyethylene micelles: (a) inclusion into the micellar core; (b) penetration into the micellar core; (c) adsorption onto the surface of the micellar core; and (d) incorporation into the polyoxyethylene groups of the micelle. Tanaka et al. (1979) described the photodegradation of monuron in aqueous solutions mixed with nonionic surfactants (all surfactant solutions were at concentrations in excess of critical micelle concentration). Among other observations, reductive dehalogenation was a major reaction in oxygenated aqueous media. Such results indicated that the photochemical reactions occurred in the organic phase of the micelles of the surfactant rather than in the aqueous phase of the solvent.

Suggested mechanisms of monuron incorporation into micelles is shown in Fig. 30.

By analogy such a micelle formed by nonionic (polar) surfactants may serve as a model for micellar units of HA and FA. The lipophilic chains (see Schnitzer and Neyround, 1975 and results of this thesis) would form a micelle from monomers of long chain fatty acids with absorption, penetration, inclusion, and adsorption of the pesticide occurring within the micelle between micelles, i.e. inter versus intramolecular inclusion (micellization).

Surfactants generally contain a long hydrophobic chain, and a polar or ionic group (hydrophilic part), and are capable of forming high molecular weight aggregates, or micelles, in dilute solution. Such a substance, with additional but analogous physicochemical characteristics, would resemble the structures of HA and FA. Interaction between the micelle and a substrate may greatly inhibit further reactions (Fendler and Fendler, 1970). Water molecules may be entrapped by a micelle (Fendler and Fendler, 1970 and references therein), and so may other molecules. When the surfactant concentration exceeds the CMC (critical micelle concentration) the shape of the spherical micelle undergoes gradual changes. It elongates to assume cylindrical or lamellar structures (Fendler and Fendler, 1975). This lamellar structure has been reported for high and low rank coals.

Humic substances behave in many ways as surfactants or detergents (structure, foaming). Detergents have been used in several fields to



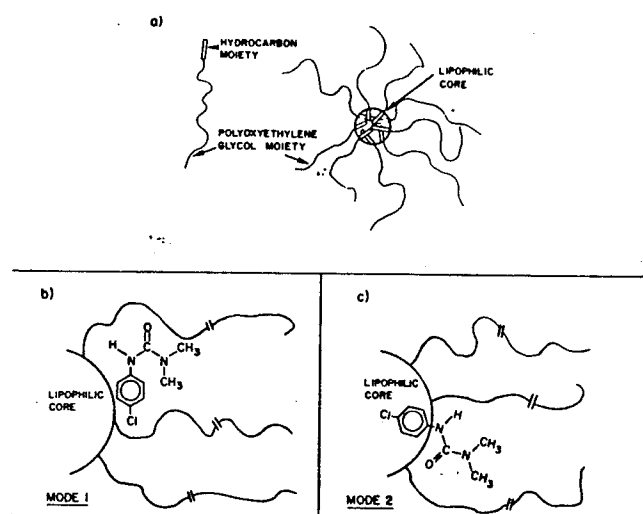


Figure 30. Schematic diagram for the illustration of two possible modes of monuron incorporation into nonionic surfactant micelles: (a) nonionic surfactant micelle, (b) adsorption onto the surface of the lipophilic core, (c) penetration into the lipophilic core (Tanaka et al., 1979)

enhance the solubility of organic compounds. Solubilization (by a surfactant) is closely related to micellization since little or no solubility increase is observed until the CMC of the surfactant is reached. Thus, the seemingly paradoxical binding of highly apolar pesticides ( $\gamma$ -HCH, p,p'-DDT etc.) may be viewed as a solubilization of them by HA and FA (esp. FA) thus accounting for their recalcitrance to extraction ( $\gamma$ -HCH, inclusion) translocation and degradation in anaerobic environments. Fulvic acids especially have been implicated in the movement of pesticides through the soil matrix (Schnitzer and Khan, 1977). Since changes in the temperature or counter ion and the addition of electrolytes are known to alter the CMC, the micellar weight, and the aggregation number of the surfactant, as well as the solubility properties of the solubilizate, it is clear that treatment such as hot aqueous sodium hydroxide would release any included organic components, as would other extractants.

The solubilizate may be entrapped in the polar core of the micelle, be oriented radially in the micelle with its polar group buried (deep penetration) or near the surface (shallow penetration), or be adsorbed on the micelle surface (Fendler and Fendler, 1970).

Since solubilization is a dynamic equilibrium process, resistance to extraction may increase with time post-treatment. Details of incorporation of several organic compounds (ethyl benzene, naphthalene, azobenzene, benzoic acid, N,N-dimethylaniline, etc. into surfactant micelles may be found in the review by Fendler & Fendler, (1970)).

Fendler and Fendler (1970) state: "the decrease in the free energy of

the system which results from the preferential self-association of the hydrophobic hydrocarbon chains of monomeric detergent molecules is the primary reason for the formation of molecular aggregates". The free energy of micelle formation has been found to be more dependent on entropy than on enthalpy factors.

The charged head groups and the relatively small counter ions of the ionic micelle (Fig. 31) are located in a compact region, known as the Stern layer, which extends from the core to within a few angstroms of the sheer surface of the micelle (Fendler and Fendler, 1975). The compactness of the Stern Layer is responsible for the reduction of the net charge on the micelle. Most of the counterions are, however, located outside the sheer surface in the Gouy-Chapman electrical double layer where they are completely dissociated from the charged aggregate and are able to exchange with ions in the bulk solution. The amount of free counterions in the bulk solvent is expressed as the fraction of charge. The affinity of the micelle for the counterion is entropy controlled, however, entropy might not be the dominant factor for all counterions.

When the surfactant concentration markedly exceeds the CMC, the shape of the spherical or ellipsoidal micelle undergoes gradual changes; it elongates to assume cylindrical or lamellar structures or rod-shaped micelles whose length increases with increasing surfactant concentrations.

The results of various studies are consistent with the general principle that non-polar organic molecules are solubilized in the

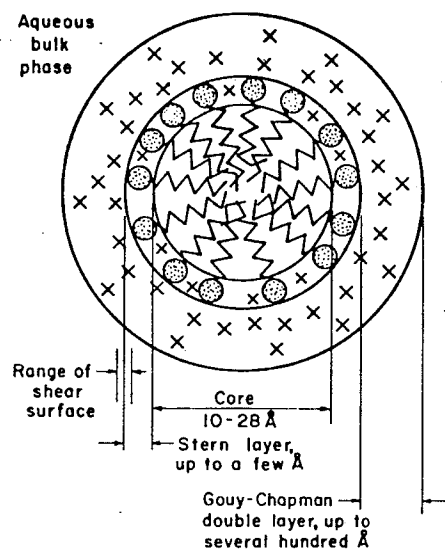
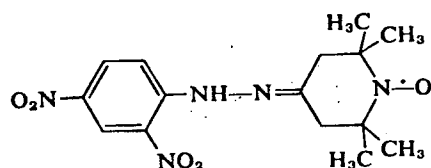


Figure 31. A two-dimensional schematic representation of the regions of a spherical ionic micelle. The counterions (x), the head groups ( $\odot$ ), and the hydrocarbon chains ( $\sim$ ) are schematically indicated to denote their relative location but not their number, distribution, or configuration (Fendler and Fendler, 1975).

hydrocarbon core while polar and ionic ones are incorporated in the Stern layer.

The stable free radicals of the 2,4-dinitro phenylhydrazone of 2,2,6,6-tetramethyl-4-piperidone nitrogen oxide (I) have been shown to be associated strongly with surfactant (5% sodium dodecylsulfate) micelles (Fendler and Fendler, 1970).



(I)

$^{19}\text{F}$ -NMR studies of  $\text{CF}_3(\text{CH}_2)_{11}\text{OSO}_3\text{Na}$  micellar solutions in the presence of urea, acetone, and acetamide suggested that these organic solubilizates were incorporated into the micelles.

Bloom and McBride (1979) stated that in soils the organic matrix is composed of polymers bound together with at least two types of bonds - the metal ion bridges and the intraparticle bonds. The metal ion bridges are not stable at high pH in alkali metal salt solutions while the intra-particle bonds cannot be broken by mild acid or base treatments. Several types of investigation have shown that HA particles are spherical with diameters of 80-100 Å (Flaig *et al.*, 1975). With an increase in pH, these particles retain their shape and do not unfold as would be expected for linear polymers. Rather,

they behave more like strongly cross-linked polymers.

With increasing pH molecular aggregation (inter and intramolecularly complexed) is disrupted.

Wershaw et. al. (1969) studied the interaction of p,p<sup>1</sup>-DDT and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) with humic acids. Because of the ability of sodium humates to behave as moderately strong surfactants and so lower water surface tension, these researchers investigated the possibility that sodium humate might solubilize low solubility (aq.) pesticides. Solubilization of DDT was much greater (40 X) using sodium humate solutions (0.5%) and addition of 0.1N NaCl did not change the amount solubilized significantly. NaCl was added in the belief that it would provide additional counterions for neutralizing the surface charges of the sodium humate micelle. Lack of any profound effect indicated that the solubilization of DDT was not due to coulombic attraction of DDT to the highly charged surface of the micelle.

Piorrer et. al. (1972) found that DDT associated with iron-organic colloids in water and that it was concentrated in the colloids by a factor of  $16 \times 10^3$ . Fulvic acid fractions of soil extracts increase the solubility of phthalate esters and, the effect decreased with decreasing molecular weight of the phthalate ester (Matsuda and Schnitzer, 1971). Similar effects have recently been demonstrated for cholesterol and a PCB isomer in dissolved organic matter (Hassett and Anderson 1979); GPC of bound complexes showed a high molecular weight fraction of organic matter was responsible for

binding - probably in aggregates or micelles.

Characterization of soil humic acids by polyacrylamide disc electrophoresis (Castagnola et al., 1978, 1979) revealed two high molecular weight fractions which were found to be formed of aggregates - not covalently bonded but stabilized by hydrogen and ionic bonds. Fulvic acids also are thought to aggregate in solution (Wershaw et al., 1977) and have particle volumes as large as  $1600 \text{ \AA}^3$  at pH 2 and of rod-shape with a length of  $40 \text{ \AA}$  and  $7.1 \text{ \AA}$  width (Chen and Schnitzer, 1976).

Visser (1964) and Piret et al., (1960) have shown that sodium humate appreciably lowers the surface tension of water. Although sodium humate is not as strong a surfactant as sodium lauryl sulfate (which is one of the strongest anionic hydrocarbon surfactants), it is moderately strong (Wershaw et al., 1969).

Soil humic and fulvic acids were separated into three molecular weight (GPC) ranges of  $>12,000$ ,  $>3500 - <12,000$ , and  $<3500$ . IR spectra were identical for each fraction with only resolution and intensity increasing as molecular weight decreased (Tan, 1977). The instability of humic acid aggregates was recently demonstrated by Povoledo and Pitze (1979); successive continuous-discontinuous ultrafiltration, through a 1000 molecular weight cutoff, resulted in continuous exclusion and permeation of organic components. The phenomena of solubilization of aromatic compounds by adsorption at the micelle-water interface can be understood on the basis of thermodynamic arguments. If in the pure micellar solution some water

molecules penetrate into that hydrocarbon part of the micelle which is close to the polar heads, it is certainly favorable from an energetic point of view that 4-CA can be substituted for this penetrating water because of the high polarizability of the aromatic ring and the bonding abilities of the substituent groups.

Thermodynamic parameters, for protein assemblage (TMV, DeWitt et al, 1978), are similar to those for a proposed micellization of humic substances in the 4-CA-soil studies of Moreale and Van Bladel (1979); i.e.  $\Delta G^{5^\circ\text{C}} = -4.6 \text{ kcal mole}^{-1}$ ,  $\Delta H = 30 \text{ kcal mole}^{-1}$ , and  $\Delta S = 124 \text{ cal mole}^{-1} \cdot \text{K}^{-1}$  cf.  $-3.24^\circ\text{C}$ , 6.3, and 35 respectively; of  $-6.5$ ,  $3.6$ , and  $33.9$  for a nonionic surfactants micellization (Corkill et al, 1964). High positive  $\Delta S$  results from release of water from the subunit (monomer) surface upon association. For the very high  $\Delta H$  and  $\Delta S$  (positive) values for the protein aggregation it was concluded that no specific intersubunit interactions through hydrogen-bonds and/or salt bridges were required for the association process. The lower values for the soil system may then reflect the prominence of such interactions. The driving force for association is the large entropy increase, which is counteracted by the positive  $\Delta H$ .

When n-alcohols and n-alkanes are added to solutions of sodium dodecanonate there is a decrease in the CMC (Vikingstad, 1980). By analogy, micellar humic substances containing included or intercalated 4-CA would be stabilized by attempts to extract the 4-CA using for example methanol or hexane - two solvents conventionally



used to extract 4-CA and pesticide residues from soil (Still et al, 1976). In addition, the micellar molecular weight of aqueous sodium dodecylsulfate solutions increase from 12,000 to 30,000 upon addition of 0.3 M NaCl and 18,000 to 41,000 with 0.5 M NaCl (Mysels and Princen, 1959, Huisman, 1964). Thus, increasing ionic strength increases aggregation, further inhibiting release of trapped residues; this may explain, too, why acid or base treatment must include refluxing to breakup these aggregates.

Surface activity (lowering of interfacial tension) of soil and peat organic matter is attributed to the humic substances and occurs mainly after peptization (especially humic acids), Tschapek et al., (1978a, 1978b). They reported that the surface activity in general was inherent to the asymmetrical amphiphilic organic molecules of medium molecular weight (<1000). The organic matter and its main component, humic acid, may comprise the colloidal aqueous solutions for which the following equilibrium is characteristic: molecules  $\leftrightarrow$  micelles. Surface activity of organic matter occurs to a marked degree only after peptization, so that, surface active substances in soil are in a colloidal state (micelles) before peptization and pass into the molecular or subunit state upon peptization (Tschapek and Wasowski, 1976).

A further explanation of recalcitrance to extraction of 4-CA residues in soil would be the fact that micelles adsorb to clays or other mineral surfaces (Somasundaran, 1979). Increasing ionic strength induces an increase in adsorption of the surfactant.

Adsorption isotherms (adsorption density versus  $C_e$ ) were curvilinear; similar to Fig. 29. Somasundaran (1979) explained the fast step as surface sorption on the clay and the slow step being the result of slow dissolution of aluminum species from the clay during prolonged contact with water and concomitant exposure of new binding sites.

The micelle behavior and micelle structure of humic substances has been stated previously, (Stevenson, 1972; Povoledo and Pitze, 1979; Given and Dickinson, 1975; Degens and Mopper, 1976). Micelle weights for organic or mineral soil humic acids were 4400 at pH 5 and decreased to 3000 at pH 6.5, (Given and Dickinson, 1975). At higher pH the micelles disaggregated and became unmeasurable, so that the true molecular weight was quite low. Micelle weights increased on standing and upon adding various polyvalent transition metal cations ( $\text{Cu}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Co}^{2+}$ ,  $\text{Mg}^{2+}$ ).

Typical CMCs are in the range of  $10^{-6}$  M for anionic detergents (Reynolds, 1979; Tanford, 1973). Micelle formation occurs in aqueous and nonaqueous media; this has been studied in detail by Kitahar and Kon-on (1975 and references therein). CMC is decreased by organic and inorganic additive and is accompanied by an increase in N (aggregation number), Fendler and Fendler (1975).

The formation of micelles disrupts the structure of water with a resultant gain in entropy and heat content. The overall enthalpy change (monomer micelle) is positive. Typical  $\Delta H^\circ$  values for formation of some amphiphilic micelles are -5 to +5 kcal mole<sup>-1</sup> (Birdi, 1975). Increasing temperature causes a decrease in the CMC

and the molecular weights (or aggregation number) of the micelles ( $M_w$ ) may range between 100 to > 1000 with each micelle typically an association of 10-100 monomers (Tanford, 1973). Increasing the concentration of the amphiphile well above the CMC usually results in an increase in the micellar size (Fendler and Fendler, 1975).

Thus, by this adsorption, the system can lower its energy in comparison with the case of an even distribution of aromatic molecules within the micelle, and the energy difference may more than compensate the associated decrease in entropy.

The binding of benzoic acid by surfactants was found to be governed by the Langmuir isotherm (Donbrow and Rhodes, 1966, 1972; Rhodes and Donbrow, 1965). The most probable orientation of the benzoic acid molecule was concluded to be that in which the hydrophobic benzene ring was enclosed in the hydrocarbon core with the hydrophilic carboxyl group protruding into the polar layer.

The surface activity of humic acid micelles was found to be independent of pH (5-9) at concentrations >2% (Tschapek and Wasowski; 1976); thus the high stability of the micelles to pH demonstrates the importance of refluxing to supply enough energy to the micelle system to result in their disruption and hence release of micellar included 4-CA. Many solutes can partition and bind to aqueous and monaqueous micellels in a highly specific manner (Fendler and Fendler, 1975; Armstrong and McNeely, 1979); organic solutes can bind to the surface and the interior of surfactant micelles. Addition of an organic solvent, benzene, to an aqueous solution of an anionic surfactant

caused a reduction in the CMC (Fendler and Fendler, 1975); such a situation may enhance the recalcitrance of soil residues sorbed by organic-humic micelles during solvent extractions.

Micelles once formed (and containing included 4-CA) would, as we have seen, be stabilized by the solvent used for extraction; this is shown diagrammatically below, as well as the eventual release via acid or base hydrolysis (Figure 32). Figure 33 illustrates proposed humic monomers.

It has been shown (Entel, 1955) that copper salts of carboxylic acids (in this case polycarboxylic acids of coals) can be decarboxylated at a significantly lower temperature than the free acids. Thus, if the soil or humic acid has a high copper concentration and is heated as in an acid or base reflux then decarboxylation will be facilitated and aid in effecting the release of trapped residues.

Fig. 7 showed that a COMC (critical organic matter concentration) may exist for sorption of pesticides (parathion in this case); ca. 2% in this case. Other experiments including those herein have shown 8% to be a COMC for 4-CA sorption; however, soils containing less than 8% OM were not investigated. Consequently, the 2% figure of Wahid and Sethumathan (1978) may be an accurate lower limit for COMC. Sodium dodecanoate in aqueous solution has a CMC of ca. (Fendler and Fendler, 1975); a similar structure for HA may be envisaged in which part of the monomer interacts with clay, thus raising the CMC to ca. 2% in analogy to the COMC of Fig. 7.

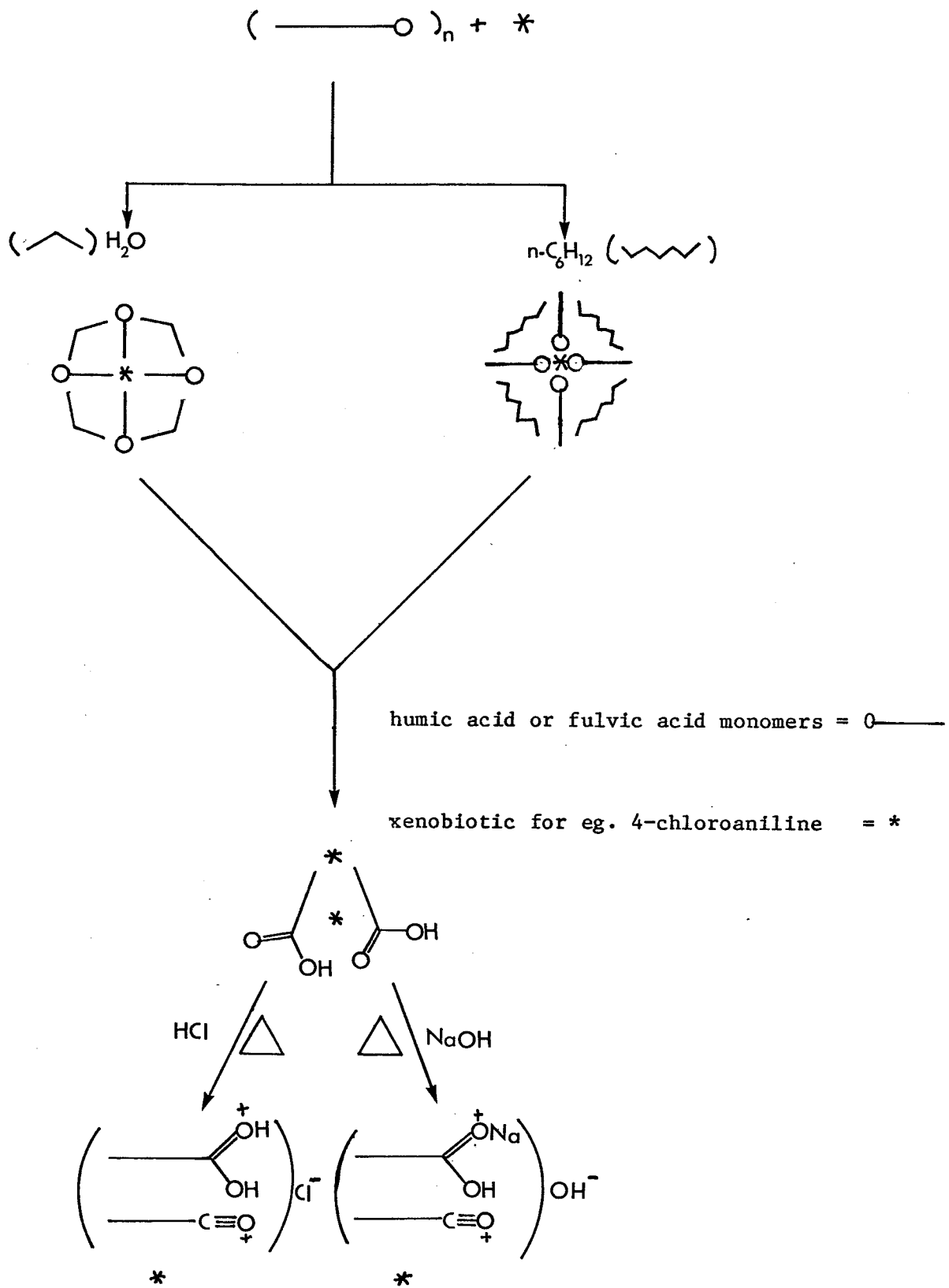


Figure 32. Solvent stabilization of micelles and hydrolytic effects

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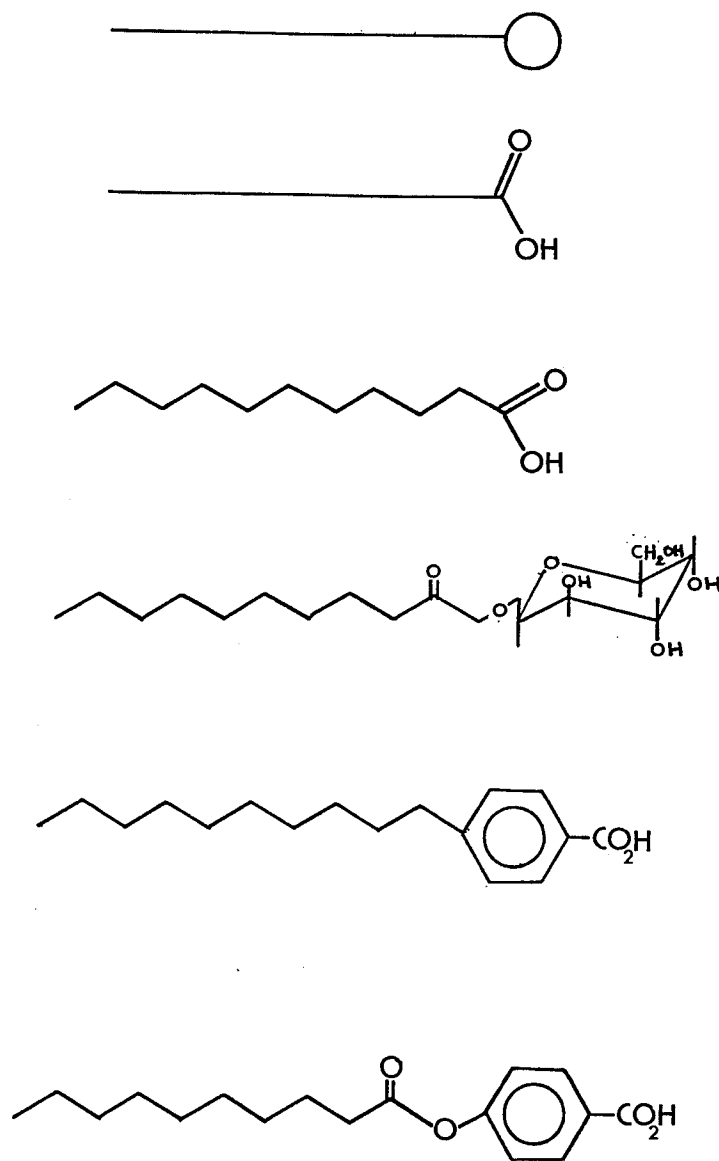


FIGURE 33. Proposed humic or fulvic acid monomers.

#### D.6.1 Mechanisms of alkaline hydrolytic release of bound 4-CA

The mechanism of alkaline hydrolysis on HA may help explain the mechanism involved in the release of HA bound 4-CA during base hydrolysis. Camier and Siemon (1978a) recently described mechanisms of alkaline digestion of brown coal and its humic acids. At high pH (14) most of the coal structure appeared to be disrupted. The structures of alkali-soluble and insoluble fractions of brown coals are apparently very similar (Agde et al., 1942) and thought to be micellar colloids (Edwards, 1953) consisting of humic acid molecules surrounded by water molecules. The water molecules are bound by van der Waal's forces and hydrogen bonding emanating from the hydrophilic hydroxyl and carboxyl groups on the surface of the humic acid molecules.

As ions ( $\text{Na}^+$ ,  $\text{OH}^-$ ,  $\text{H}^+$  etc.) enter the micellar matrix, more functional groups are ionized and thus solvated, leading to hydrophilic areas in the micelle. The entry of the hydrated sodium and hydroxyl ions into the matrix physically swells the coal (Dryden, 1963; Pampuch, 1953). Disruption of the matrix may be caused by mutual repulsion of electrical double layers formed by sodium ions at particle (micelle) surfaces overcoming the van der Waal's and hydrogen bonding forces. The introduced water may also help the double layer to expand. Photomicrographs have been taken (Camien, 1975) showing large rod-shaped particles being "peeled" from larger coal lumps by the action of alkali; probably this process corresponds

to bond disruption and ions diffuse along the pores. Coal particles dispersed in high pH solutions would be stabilized by electrical-double layer repulsion.

The sodium ions become strongly bound to the humic acids (Durie, 1961b; Coal Res. CSIRO, 1961); removal of such chemisorbed sodium required washing with 0.5 N HCl (Durie, 1961a; 1963). Whether or not coal particles were completely separated from each other or "dissolved" would be dependent upon the steric orientation of the sodium-affected sites (Camier and Siemon, 1978). It is the chemisorption of sodium ions at high pH (>12) in the matrix which is responsible for disruption of the structure. Such a mechanism may also occur with strong acid ( $H^+$ ) which would create compact double layers not sufficient, though, to dissolve or disperse the humic acids, nevertheless, the matrix "opens" sufficiently for extraction of trapped organic molecules. Acid treatment also causes other changes such as hydrolysis resulting in drastic conformational changes. Such dissociation from aggregated complexes might be envisaged as was shown for erythrocrucorin (Chiancone *et al.*, 1972) a protein which aggregates but dissociates at high pH. Such reactions may be analogous to release of 4-CA from the HA\*4-CA complex.

#### D.7 De novo theory of 4-CA and xenobiotic interaction with soil.

The interaction of 4-CA and other xenobiotics with soil depends upon the physical and chemical properties of the soil and the 4-CA or xenobiotic applied.



Three types of interactions are envisaged as generally applicable to all xenobiotics including 4-CA,

- (1) "loosely" complexed - surface extractable residues
- (2) "tightly" complexed - bound inclusion residues which are hydrolyzable
- (3) "irreversibly" complexed - bound due to the action of hydrolysis in (2) or via long term biological incorporation.

Thus, a multi-interaction process [especially (1) and (2)] is envisaged whose mechanism is described by one of the following interactions:

(A) in-vivo (soil interactions)

- with soil containing bound water acting as a porous matrix in which diffusive movement into the matrix occurs after surface sorption.

(B) in-vitro (analytical solvent interactions)

- with addition of solvent to soil or soil HA, micellization is stabilized by the solvent precluding isolation, except for surface adsorbed residues.

Diagrammatically:

(A)



(B)

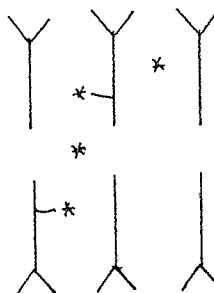


Figure 34 illustrates a conceptualized model of 4-CA (or other pesticides and their degradation products) interacting with soil organic and inorganic components by the three mechanisms discussed.

In conclusion, binding of 4-CA (and other pesticides, and their degradation products) to soil components is a complex phenomenon. Only by fractionating these components and evaluating their individual behavior can one separate out those components with the most significant effects; the research to date has shown that humic and fulvic acids play a dominant role in binding of organic compounds. It is envisaged that binding may occur as the result of micellization of fulvic acid monomers, especially, and humic monomers, if such differentiation is valid, held together by H-bonding, hydrophobic bonding, metal cations and stabilized by solvent, iron and aluminum oxide and clays, so that 4-CA may permeate

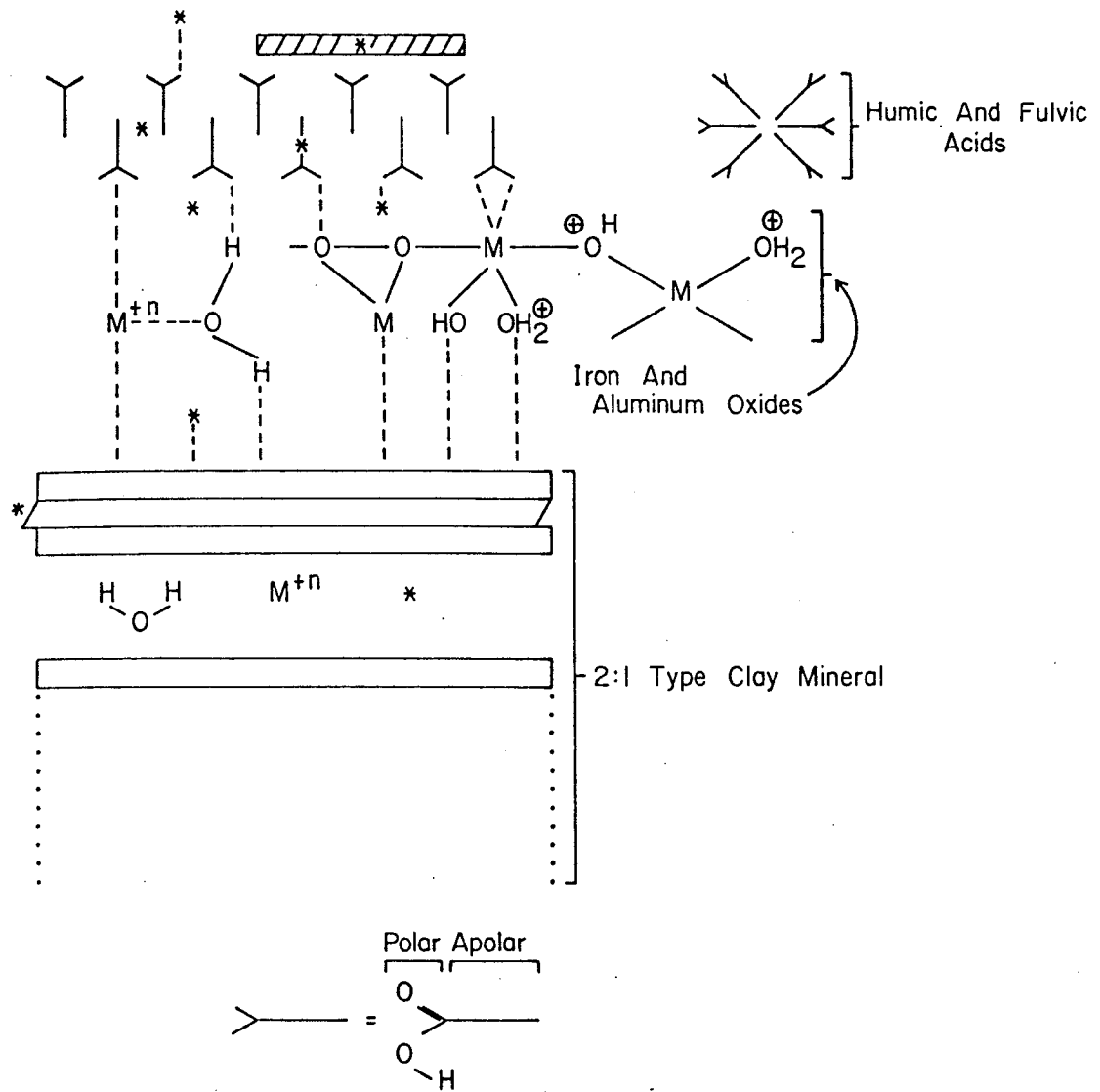


FIGURE 34. Proposed mechanisms of 4-CA (and other xenobiotics) interaction with soil.

\* = Pesticide Or Degradation Products

▨ = Other Natural Products

this matrix becoming recalcitrant to extraction because of (a) strong physical interactions with the matrix, (b) alteration of all structural matrix levels (1°, 2°, 3°, 4°) due to in situ and/or in vitro changes (micellization) leading to inclusion complexes. The theory is not meant to be a definitive explanation of bound residues; however, thermodynamic as well as other physicochemical evidence supports its plausability. The utility of such a proposition is to help correlate the vast and sometimes contradictory information pertaining to humic chemistry and bound residues, and to promote or stimulate the study of such problems via physicochemical approaches, and to lead the way to analytical processes to release intact "bound" residues.

## IV SUMMARY

Pesticides have the capacity to complex "tightly" and abundantly with soil humic acids. Concern has been expressed for the inability to analyze for these residues such that the soil burden may be determined. Research was conducted to develop an analytical method to release and quantitate "tightly" complexed 4-chloroaniline (4-CA) residues (a common type of pesticide degradation product) from soil humic acids and to elucidate the nature of the site of complexation such that an evaluation may be made of the conditions under which release might occur under held conditions. Results from these investigations will enable an appraisal to be made of the effect of "tightly" complexed pesticide residues on soil physicochemical, biochemical, and physiological processes, on soil microorganisms, and on the persistence of these residues and conditions under which they might be released. Evaluation of potential mammalian toxicity would thus be made possible.

Humic acids were isolated from a Manitoba agricultural soil having no previous history of pesticide treatment. The isolated humic acids were then treated with a mixture of [ $^{14}\text{C}$ ] and [ $^{12}\text{C}$ ] 4-chloroaniline at the 500 ppm level and at the required specific radioactivity. After incubation, "free" (remaining in solution) 4-CA was filtered off and the residue subsequently extracted using organic solvents. This extractable portion was termed the "loosely" complexed fraction, and the remaining humic acid

complex (HA\*CA), the "tightly" complexed fraction.

Anaerobic pyrolysis was intensively investigated as an analytical technique to release "tightly" complexed 4-CA since it has proven useful in release of 3-chloroaniline and 3,4-dichloroaniline from plant lignins (Balba et al., 1979). Although pyrolysis conditions yielded appreciable amounts of radioactivity (up to 54%), only a few percent of the released radioactivity was identified (GLC) as intact 4-CA. It is speculated that the 4-CA released may react with coextracted soil colloids to yield unstable products, or that it is present in the HA as an inclusion complex such that the applied heat results in the thermolysis of 4-CA.

Of the various methods investigated acid or base hydrolysis was the most efficient in terms of total radioactivity (90%) and intact 4-CA (38 and 46% respectively) released. Radioactivity remaining in the HA after hydrolysis (ca. 10%) is believed to be bound by the action of the hydrolytic conditions on 4-CA and HA. Structural alterations in the HA may result in unextractable, nonhydrolyzable inclusion complexes rather than being evidence of nonhydrolyzable 4-CA covalently bonded as heterocyclic structures and integrated into the structure of HA (as proposed by Hsu and Bartha, 1976). Studies of 4-CA binding to soils of various OM and clay contents clearly demonstrated the dominant role of organic matter in binding 4-CA and the apparent physical-type interaction as evidenced by the equal percentages of 4-CA released by pyrolysis.

Before isolating HA for studies directed towards elucidation of

the site of complexation, it was necessary to investigate milder extraction methods for HA. The classical method and other modifications were considered to be too harsh, i.e., capable of significantly altering the natural structure of humic acids.

Three types of extractions were selected for evaluation yielding three HA preparations with different physicochemical characteristics; (i) 0.5 M NaOH extraction and acid precipitation (Classical Method); (ii) 0.02 M  $\text{Na}_4\text{P}_2\text{O}_7$  at pH 7 and acid precipitation, and (iii) 0.02 M  $\text{Na}_4\text{P}_2\text{O}_7$  at 7 followed by precipitation with acetone.

After isolating the HAs, it was necessary to examine them in terms of their differences and similarities. Structural information was obtained using infrared (IR) and  $^{13}\text{C}$ -FT-NMR spectrometry. IR and  $^{13}\text{C}$ -NMR evidence show that the nature of the extraction procedure is important, that the mildest extraction yields the most resolved spectra, that evidence for aromaticity decreases as milder extractions are used; and that olefinic and carbohydrate absorption is greater the less harsh the extraction procedure. 0.02 M  $\text{Na}_4\text{P}_2\text{O}_7$  (pH 7) yields a less aromatic HA than the classical NaOH method.

These structural studies show that humic acid extracted and precipitated using milder conditions are aliphatic in nature. A milder HA isolation procedure such as 0.02M  $\text{Na}_4\text{P}_2\text{O}_7$ -acetone may facilitate the determination of 4-CA sites of interaction in a relatively unaltered HA. Structural information from these studies on HA and HA precursors indicates the importance of aliphatic

carboxylic acids.

Finally, a detailed theory of sites and mechanism of 4-CA complexation has been presented based on experimental results.

The most prevalent sites in the "short" term were proposed to be:

- (a) "loosely" complexed by surface interactions-extractable by non-polar or polar solvents;
  - (b) "tightly" complexed by inclusion (especially micellar-HAs and HA-clay or clay complexes) phenomena while the "long" term other forms of incorporation may be involved via plant, fungal and bacteria incorporation into cell wall biopolymers.
- Also, autooxidized 4-CA products accounts for some unextractable [ $^{14}\text{C}$ ]-4-CA or unidentifiable 4-CA.

Thus, a hydrolytic method has been developed for extraction and quantitation of "tightly" complexed 4-CA from HA which may be applicable to other "tightly" complexed pollutants; a highly sensitive method was developed for the quantitation of 4-CA at femtogram levels; a mild method of extraction for HA is described (acetone precipitation of pH 7- $\text{Na}_4\text{P}_2\text{O}_7$  HA); the importance of OM and clay in binding 4-CA and its release has been demonstrated; evidence is presented which shows lack of aromaticity in HA and FA structure in contrast to presently held concepts; a relationship between a cellulose component as a HA precursor was demonstrated; and a de novo theory of complexation based on inclusion of 4-CA in a porous humic substance matrix and micellization of HA and FA



components has been proposed to describe mechanisms of their interaction with pollutants and perhaps help direct fruitful approaches to further understanding of the ways these pollutants interact with and are subsequently released in soil.

In conclusion, the interaction of 4-CA and pesticides, toxicants, pollutants and their degradation products is envisaged as a multi-interaction process with inclusion complexes being predominantly responsible for recalcitrant residues and affecting release or mineralization of significant portions of these compounds. The potential for the existence of large amounts of included residues in their unaltered form clearly emphasizes the need for further research pertaining to soil organic matter and the potential release of complexed toxicants, pesticides and pollutants. The results of this research may permit an evaluation of the soil burden of "tightly" complexed pollutants such that recommendations may be made regarding further use of xenobiotics.

V APPENDICES(A) Contributions to original research

1. Development of a new method to analyze for "bound" 4-CA and probably other xenobiotics.
2. The refutation of a currently popular hypothesis proposed to explain hydrolyzable and nonhydrolyzable "binding" of 4-CA to soil organic matter.
3. Evidence to show the importance of soil organic matter from a wide range of soils in the binding and release (pyrolytic) of 4-CA.
4. Insight into the usefulness and limitations of the pyrolytic method in releasing "bound" 4-CA and other xenobiotics.
5. Further evidence showing that the nature of the extractant influences the structure of the humic acids isolated.
6. A relationship between a cellulose component as a precursor humic substances has been demonstrated.
7. Evidence for the micellar nature of humic acids.
8. The application and interpretation of a new instrumental technique,  $^{13}\text{C}$ -FT-NMR, to elucidate the structure of humic acids.
9. An analysis of problems and limitations of  $^{13}\text{C}$ -NMR when used to obtain spectra of high m.wt. or micellar heterogeneous solutions.

10. Development of a de-novo theory to help explain the nature of soil "bound" 4-CA and other xenobiotics. The theory is based on the experimental results of the author, as well as an extensive correlation of diverse experimental data reported by other researchers.

(B) Consequences of this research; elucidation, evaluation, amelioration of:

- (1) soil burden of bound residues
- (2) potential release of them
- (3) more accurate evaluation of efficacy, target specification, translocation into crops, decreased yield.
- (4) effects of such an accumulation on soil physiochemical processes.
- (5) effects on soil microflora and fauna.
- (6) effects on soil humus physiological functions.
- (7) toxicity concerns evaluated with more accuracy and credibility to the interpretation.
- (8) elucidation of importance of extraction mechanism in releasing recalcitrant residues in soil.
- (9) direct fruitful approaches to further mechanistic, release and physiochemical studies.

(c) Definitions

Adsorption equations:

Freundlich equation;

$$\frac{X}{M} = K C^{1/n}$$

in which:

$\frac{X}{M}$  = quantity of compound or ions adsorbed per unit weight of adsorbent

$C_0$  = equilibrium concentration of the adsorbing compound once adsorption has been established

K & n are constants, which may be derived from the experimental adsorption data.

Langmuir equation;

$$\frac{X}{M} = \frac{KbC_0}{1+KbC_0}$$

in which:

$\frac{X}{M}$  = amount adsorbed per unit weight of adsorbent

$C_0$  = equilibrium concentration of the adsorbing compound once adsorption has been established

K = constant related to the bonding energy

b = adsorption maximum or the amount adsorbed when the adsorbent is completely saturated

Adsorption: When two immiscible phases are brought into contact, it is nearly always found that the concentration of one phase is greater at the interface than in its bulk. This tendency for accumulation to take place at a surface is called adsorption.

Adsorption takes place with a decrease in surface free energy,  $\Delta G$  and entropy ( $\Delta S$ , degrees of freedom lost). ( $\Delta G = \Delta H - T\Delta S$ ) at a

constant temperature  $\Delta H$  is also negative. Adsorption is in general exothermic (no case of endothermic adsorption is definitely known (Hayward and Trapwell, 1964)).

Physical adsorption: The electron cloud of the adsorbate interacts as a whole with the adsorbent, and it becomes polarized; e.g., weak forces of inter- and intramolecular bonding are present, especially van der Waal's forces.

Chemical adsorption: Electron transfer and sharing of electrons and formation of a new molecular orbital occurs; e.g., strong forces of ionic or covalent bonding are present.

#### Physical vs Chemical Adsorption

There are certain differences in the properties of the two kinds of adsorption, which can be used as experimental criteria for deciding the adsorption type. The best being the magnitude of the heat of adsorption. Heats of physical adsorption should be in the area of heats of liquefaction; e.g., heats of chemisorption of carbon monoxide and hydrogen are greater than 20 and 15 kcal/mole respectively, whereas heats of physical adsorption are less than 6 and 2 kcal/mole.

Physical adsorption tends to occur only at temperatures near or below the boiling point of the adsorbate at the pressure considered. Chemisorption, however, can take place at temperatures far above the boiling point. Physical adsorption, unlike chemisorption, requires no

activation energy. Physical adsorption should be exceedingly rapid at any temperature, and take place as fast as the adsorbate reaches the surface. This action does not, however, preclude chemisorption.

It is important to note, however, that if an adsorbent is porous, or possesses fine capillaries, penetration of adsorbate to the interior may be a rather slow process. Finally, chemisorption tends to be monomolecular cf. multilayered physical sorption.

Absorption: Penetration of particles of one phase deep into the other phase, resembling dissolution to some extent. (Adsorption may occur within the penetrated surface). In cases where absorption is combined with adsorption, the process involved is most often called SORPTION and is generally applied to processes in which the spatial localization of the captured particles is not well-defined. Adsorption and sorption are often treated as synonyms (Ponec and Cerry, 1974).

Adsorption vs. Absorption: Absorption by solids is divided into three types:

1. diffusion and adsorption along fine capillaries (non-activated)
2. diffusion along crystal grain boundaries, (activated)
3. penetration of a gas between the atoms of a crystal to form a true solution

With carbon and clay adsorbents, capillary effects and sometimes solution tend to outweigh crystal boundary effects.

All phenomena normally classed as absorption, proceed slowly except at elevated temperatures, because either an activation energy or a slow nonactivated diffusion is involved. Thus an absorption may be thought to be a slow chemisorption on a free surface (unsaturated) or vice versa.

However, absorptions have certain characteristics which permit them to be distinguished from adsorption.

1. True solution in a solid is usually endothermic and is distinguished by this from adsorption which is normally exothermic.
2. For adsorption the heat of adsorption varies with the adsorbed amount.
3. Certain diffusions obeying Fick's law are non-activated. The ratio of these is proportional to  $T^{1/2}$ , whereas slow adsorptions are activated and are exponentially dependent on temperature.
4. If adsorption is associated with formation of a compound with the solid, very large amounts of adsorbate will be used, and measurement of the surface area would show these to be far in excess of the quantity which could possibly be adsorbed.

Coordination compound (complex compound): A compound formed by the union of a metal ion or molecule called a ligand or complexing agent.

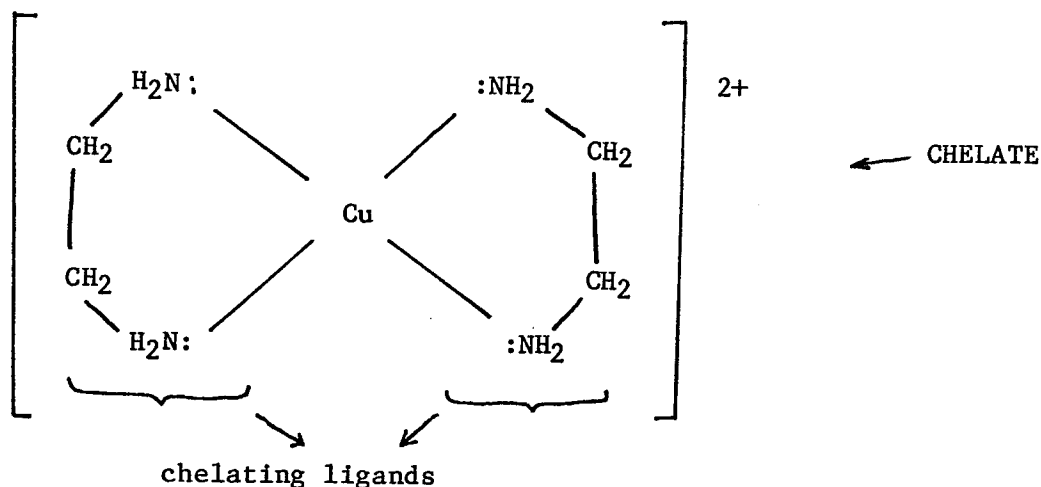
The ligand may be either ionic or nonionic. The total number of bonds linking the metal to the ligand is called the coordination number. All ligands have electron pairs on the coordinating atom, e.g., nitrogen, that can be either donated to or shared with the metal ions. The metal ion acts as a Lewis acid (electron acceptor) and the ligand as a Lewis base (electron donor). The bonding is neither covalent nor electrostatic, but intermediate between the two types.

Sequestration: When an ion is prevented from exhibiting its usual properties due to close combination with an added material, e.g., organic chelates.

Chelate: A type of coordination compound in which a central metal ion is attached by coordinate links to 2 or more nonmetal atoms in the same molecule, called ligands. Heterocyclic rings are formed with the central (metal) atom as part of each ring (Hawley, 1977).

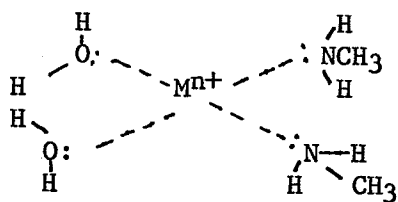
Chelating Ligand: A chemical entity containing two or more functional groups so arranged that they can simultaneously occupy positions in the first coordination sphere of the same metal ion (Orgel, 1969).



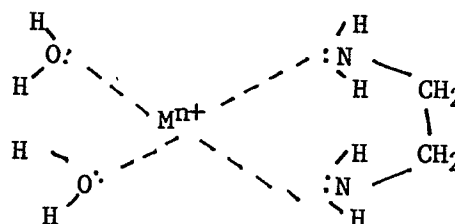


Metal chelate versus metal complex compounds: When a metal ion is coordinated simultaneously to more than one donor group of an organic complexing agent, called an organic "ligand," the resulting compound contains a heterocyclic ring which includes the metal ion, and is called a metal chelate compound.

A typical aqueous metal complex and chelate are shown below:



a diaquobismethylamine  
(metal complex)



a diaquoethylenediamine  
(metal chelate)

If the amino donor groups have the same affinity for the metal ion in both the complex and the chelate compound, the distinction between the two would be a formal one; however, when the metal chelate meets certain requirements, the chelate will differ markedly from the complex in stability, and in other ways (Martell, 1971).

Amphiphilic: Signifies that a molecules has a tendency to become

adsorbed on a hydrophilic or hydrophobic surface. Amphiphilic species such as detergent ions, lipids, soaps and certain polar compounds dissolve in water to form simple molecularly dispersed systems only at small [concentrations] below what is termed the CMC (critical micelle concentration). Micelles are agglomerates of these amphiphilic compounds in modest number on the order of 100 or less in which the solution structure seeks to minimize the hydrophobic/hydrophilic interactions by forming an inner core in an aqueous medium with an interface between them.

Colloid: Is a system consisting of a homogeneous continuous phase in which kinetic units (e.g., small solid particles, macromolecules, etc.) are dispersed. There are three classes of colloids; hydrophobic, hydrophilic and association.

Micelle: Any water soluble aggregate spontaneously and reversibly formed from amphiphilic molecules or ions. When amphiphilic molecules are dissolved in water they can achieve segregation of their hydrophobic portions from the solvent by self-aggregation; the aggregated products are called micelles (Tanford, 1973).

Clathrate compound: An inclusion complex in which molecules of one substance are completely enclosed within the other.

Inclusion complex (adduct): An unbonded association of two molecules, in which a molecule of one component is either wholly or partly locked within the other.

## (D) Common and chemical names.

<u>common name</u>	<u>chemical name</u>
amitrole	3-amino-1,2,4-triazole
antor	2-chloro-N-(2',6'-diethyl-phenyl)-N-methyl (ethylcarboxylate) acetamide
aldicarb	2-methyl-2-(methylthio)propionaldehyde-0- (methylcarbonyl) oxime
atrazine	2-chloro-4(ethylamino)-6-(isopropylamino)- 1,3,5-triazine
bentazon	3-isopropyl-2,1,3-benzo-thiadiazinone(4)-2, 2-dioxide
benzoylprop-ethyl	ethyl(+2-(N-(3,4-dichlorophenyl)benza- mide) propionate
bifenox	5-(2,4-dichlorophenoxy)-2-nitrobenzoate
butralin	4-(1,1-dimethylethyl)-N-(1-methylpropyl)-2, 6-dinitro-benzeneamine
chlorpropham	isopropyl N-(3-chlorophenyl)carbamate
croneton	2-ethylthiomethylphenyl-1-N-methyl carbamate
2,4-D	2,4-dichlorophenoxyacetic acid
dalapon	2,2-dichloropropionic acid
<u>p,p</u> <sup>1</sup> -DDT	1,1-bis-( <u>p</u> -chlorophenyl)-2,2,2-trichloro- ethane

<u>common name</u>	<u>chemical name</u>
dichlofop-methyl	methyl 2-(4-(2,4-dichlorophenoxy)phenoxy) propionate
dieldrin	1,2,3,4,10,10-hexachloro- <u>exo</u> -6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4- <u>endo-exo</u> -5,8-dimethanonaphthalene
diquat	1,1'-dihydropyrido-(1,2- <u>a</u> :2',1'- <u>c</u> ) pyrazidinium ion
ditalinifos	0,0-diethyl-phthalimido-1-phosphonothioate
EPTC	<u>S</u> -ethyl dipropylthiocarbamate
fensulfothion	0,0-diethyl-0-[ <u>p</u> -methylsulfinyl]phenyl] phosphorothioate
flamprop-methyl	methyl(+)-2-( <u>N</u> -(3-chloro-4-fluorophenyl)benzamido) propionate
fonofos	<u>O</u> -ethyl- <u>S</u> -phenyl ethylphosphonodithioate
isocarbamid	<u>N</u> -(2-methylpropyl)-2-oxo-1-imidazolidine carboxamide
lindane	$\gamma$ -isomer of 1,2,3,4,5,6-hexachlorocyclohexane
metamitron	4-amino-3-methyl-6-phenyl-1,2,4-triazin-5( <u>4H</u> )-one
methabenzthiazuron	<u>N,N'</u> -dimethyl- <u>N</u> -(2-benzthiazolyl)-urea, or 3-(2-benzothiazoyl)-1,3-dimethylurea

<u>common name</u>	<u>chemical name</u>
methazole	2-(3,4-dichlorophenyl)-4-methyl-1,2,4-oxadiazolidine-3,5-dione
methonyl	<u>S</u> -methyl- <u>N</u> -[(methylcarbomoyl)oxy]thioacetimidate
methoxychlor	1,1,1-trichloro-2,2-bis( <u>p</u> -methoxyphenyl)ethane
paraquat	1,1'-dimethyl-4,4'-bipyridylium ion
parathion	<u>O</u> , <u>O</u> -diethyl- <u>o</u> - <u>p</u> -nitrophenylphosphorothioate
primicarb	2-dimethylamino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate
prometryn	2-methylthio-4,6-bis(isopropylamino)- <u>S</u> -triazine
propanil	3',4'-dichloropropionanilide
TCA	trichloroacetic acid
TCAB	3,3',4,4'-tetrachloroazobenzene
trifluralin	<u>α</u> , <u>α</u> , <u>α</u> -trifluoro-2,6-dinitro- <u>N</u> , <u>N</u> -dipropyl- <u>p</u> -toluidine
USB 3153	<u>N</u> <sup>3</sup> , <u>N</u> <sup>3</sup> -di- <u>n</u> -propyl 2,4-dinitro-6-trifluoro methyl- <u>m</u> -phenylenediamine
DMSO	dimethylsulphoxide
DMF	<u>N</u> , <u>N</u> -dimethylformamide
PVP	polyvinylpyrrolidone

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