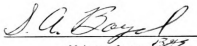




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MOBILITY OF SLUDGE-BORNE
3,3'-DICHLOOROBENZIDINE

By

Duck Young Chung

A THESIS

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

MASTER OF SCIENCE

Department of Crop and Soil Science

1987

ABSTRACT

MOBILITY OF SLUDGE-BORNE 3,3'-DICHLOROBENZIDINE (DCB)

By

Duck Young Chung

Fate of the carcinogen 3,3-dichlorobenzidine (DCB) in sludge-amended soil was evaluated. ^{14}C -labelled DCB was used as a tracer to examine mineralization and mobility of DCB present in sludge. DCB was shown to be persistent in soil and sludge-amended soil with <2% of the added [^{14}C]DCB evolved as $^{14}\text{CO}_2$ during a 182 day incubation. Mobility of DCB was evaluated by using soil columns (5 x 45 cm) that were irrigated with 2.54 cm water per day for 84 days. The total ^{14}C recovered in leachate water was from 0.16 to 0.063 percent of the total ^{14}C added to the top 15 cm of the soil column at time zero. The strong binding of DCB to soil constituents rendered it highly immobile despite being placed in a sandy soil which was irrigated with large amounts of water. However, some finite, albeit small, quantity was apparently leached through the soil profile at a relatively constant rate. The immobilization of DCB presumably results from the formation of humus bound DCB residues and from its low water solubility

To my parents and family

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Abbreviation:

L:leaves B:branch and stem R:root T:total

S:non sludge-amended soil SS:sludge-amended soil C:control

CHAPTER I

PRODUCTION AND CHARACTERISTICS OF DCB AND SEWAGE SLUDGE, AND BINDING OF AROMATIC AMINES TO SOIL AND HUMIC SUBSTANCES.

As a results of industrial discharges, sewage sludge generated at the Muskegon Wastewater Management System (MWMS) has become contaminated with the carcinogen 3,3'-dichlorobenzidine(DCB). The MWMS uses wastewater to irrigate over 2,025 ha of land in Muskegon County. Corn is the primary crop grown. Consideration is now being given to initiate a program of land application of sewage sludge generated at MWMS. Because MWMS sludge is contaminated with DCB, a potential hazard of this sludge management alternative is for DCB to be leached from sludge through the soil profile and into groundwater. The potential for movement is enhanced because (1) DCB is known to be persistent in agricultural soils, (2) the sandy soils at Muskegon have high infiltration rates and the capability to percolate large amounts of water and (3) these soils receive higher than normal quantities of water during the year. For these reasons, a study was initiated to evaluate the mobility of sludge-borne DCB in an experimental system designed to mimic conditions at the MWMS.

3,3'-dichlorobenzidine (3,3'-dichloro,4,4'-diaminobiphenyl), hereinafter referred to as DCB, is widely used for producing 95 tetrazodyes listed in the Column Index (1971); however, of these, only

five pigments are currently produced in the United States. DCB is suspected of being a human bladder carcinogen by the Occupational Health and Safety Administration because of its similarity in chemical structure to benzidine, a human bladder carcinogen (IARC, 1972a). It is of considerable commercial importance; DCB has been used as a starting material for the synthesis of diarylide yellow and orange azo pigments such as 5 chlorobenzidine-based pigments since the DCB plant went on stream in 1938. Pigments yellow 12, 13 and 83 (DCB-based) are not carcinogenic in rats and mice. Pigments yellow 12 and 13 differ structurally from the benzidine-based dyes for which there is positive evidence of carcinogenicity, which presumably results from metabolic breakdown to release the parent benzidine compound. In addition to a difference in solubility (pigments are insoluble, whereas the dyes are water-soluble), DCB is a benzidine derivative with chlorine substituents ortho to the amine. Figure 1 shows that a keto-enol tautomeric group adjacent to the azo linkage in the pigments may shield this linkage from enzymatic cleavage (Jones, 1980).

The DCB pigments are used in printing inks, rubber, plastics, paints and textiles. Total DCB production in the United States in 1977 was about 3.7 million pounds and approximately 1 million pounds of DCB were purchased from an outside suppliers (Ferber, 1978).

Some physical and chemical properties of DCB are shown Table 1. Chemically, DCB undergoes the reactions of the aromatic primary amines. The most important is the formation of the tetrazonium compound, which can be coupled with a variety of compounds for making

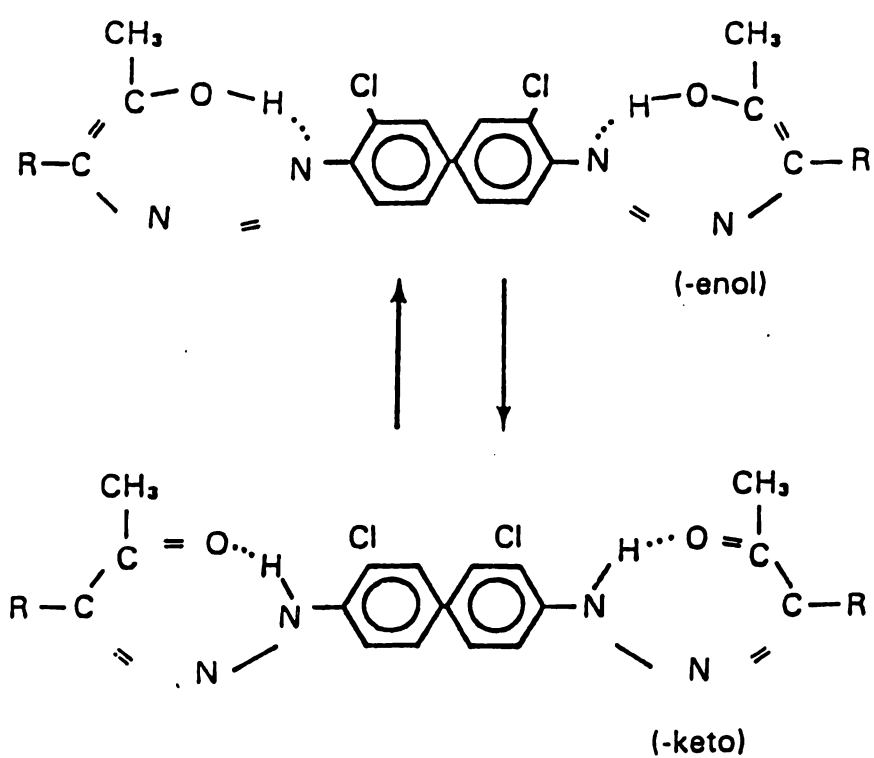


Figure 1. Proposed keto-enol tautomerism of dichlorobenzidine-based pigment, with presumed ring structure (EPA 560/ 3-78-003)

Table 1. Physical and Chemical Properties of

3,3'-Dichlorobenzidine (DCB)

Synonym : 2,2'-Dichloro, [1,1'-biphenyl]-4,4'-diamino

Chemical Formula : $C_{12}H_{10}Cl_2N_2$

Molecular Weight : 255.13 gram

Preparation from m-chloronitrobenzene

Needles from water or prism alcohol

Melting Point : 165.5 °C

Moderately soluble in alcohol

Readily soluble in ether

Insoluble in water

Soluble in ethanol and ethyl ether

pKa value : Approximately 4.4

Use : Manufacture of azo dyes

* Source : EPA 560/11-80-019 and Merck Index

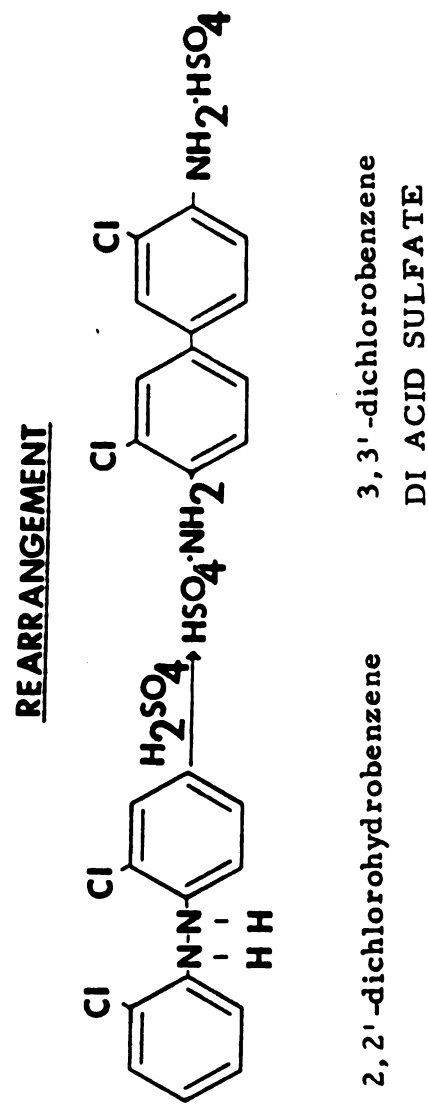
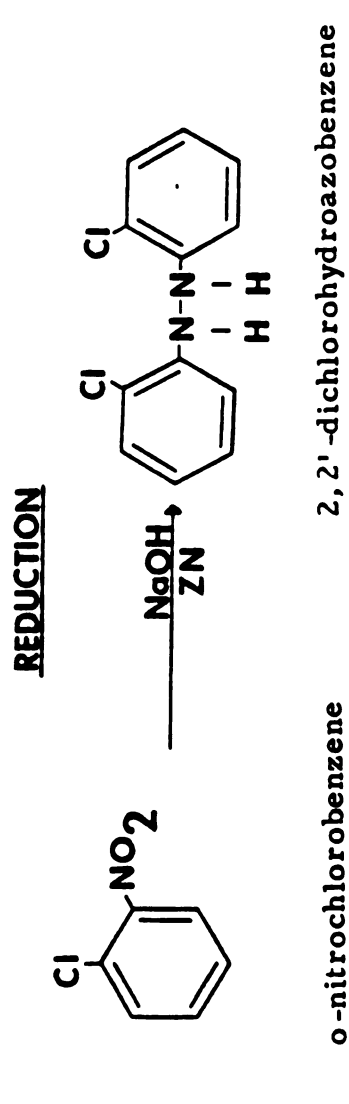
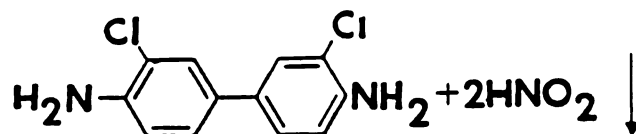
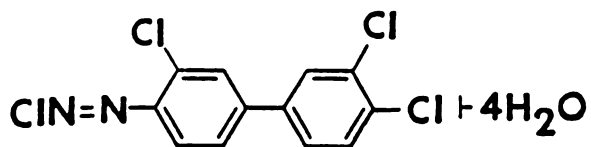


Figure 2. Synthesis of 3,3'-dichlorobenzidine DI ACID SULFATE
(Sikka, 1980)

TETRAZOTIZATION

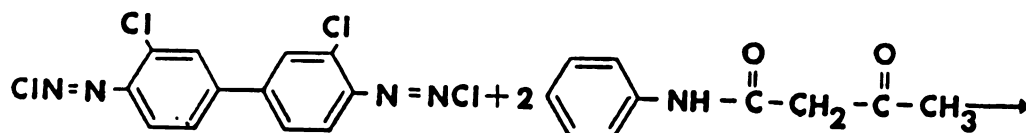


3,3'-dichlorobenzidine nitrous acid



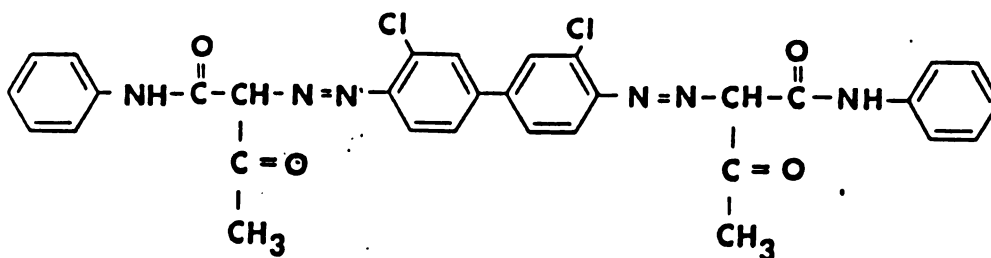
3,3'-dichlorobenzidine tetrazonium chloride

COUPLING



3,3'-dichlorobenzidine
tetrazonium chloride

acetoacetanilide



PIGMENT YELLOW 12

Figure 3. Synthesis of CI pigment Yellow 12 (EPA 600/3-78-003)

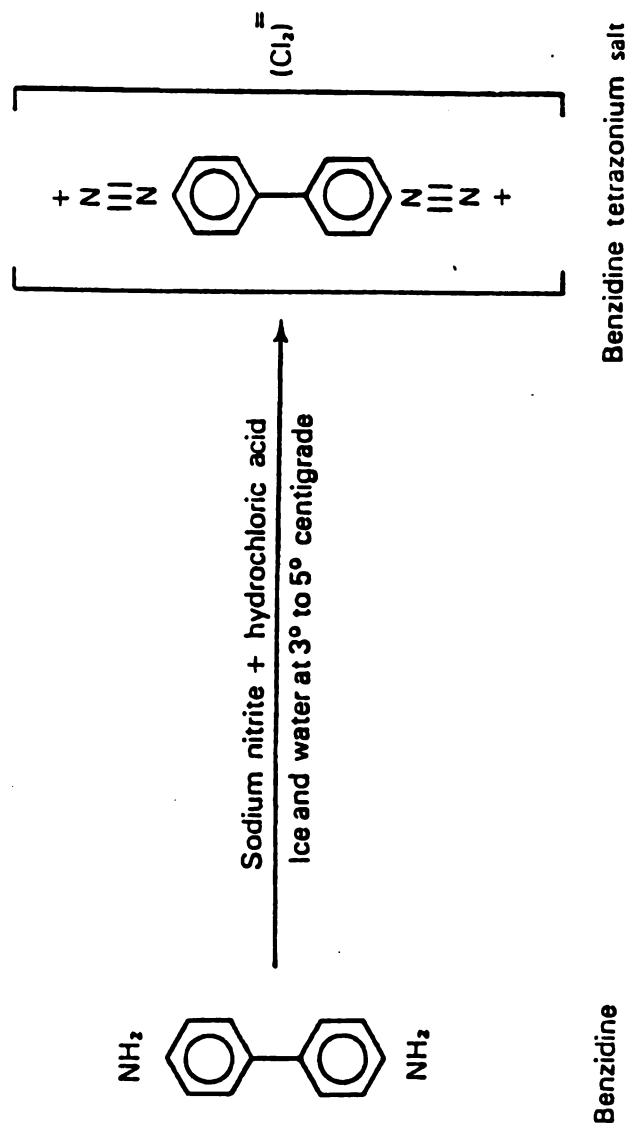


Figure 4. Tetratization of benzidine (Sikka et al., 1980)

dyes and pigments. In these reactions the chlorine atoms of DCB are passive and do not participate in the reaction. DCB is made by the alkaline reduction of ortho-nitrochlorobenzene to dichlorohydrazobenzene to DCB in the presence of hydrochloric or sulfuric acid (Lurie, 1964). The product is isolated as the dihydrochloride or as the acid sulfate. These reaction are shown in Figure 2.

DCB reacts with sodium nitrite in the presence of mineral acid (HCl or H_2SO_4) to form the tetrazonium salt. The salt is then coupled with various compounds, primarily acetoacetarilides, to form the azo pigments (USEPA, 1979a). An example of a coupling reaction is shown in Figure 3.

Production of dyes and pigments from dichlorobenzidine proceeds via tetrazotization to form the tetrazonium salt, which is followed by coupling of tetrazonium compound with a relative compound (e.g., aromatic hydroxy compounds or arylamines) to form a colored product (1). Tetrazotization is accomplished by reacting benzidine or dichlorobenzidine with nitrous acid (sodium nitrite in hydrochloric acid) in a water solution at 0 ~5 C. This produces the coupling agents, a tetrazonium hydrochloride (Fig.3).

The final step in the production of dye solution is the coupling of the tetrazonium salt with phenol, aromatic amines, or other reactive compounds (Fig.4). The coupled products have azo groups that are linked to sp² - hybridized carbon atoms; hence, they are azo dyes. In the coupling reaction the first diazo group of the tetrazonium salt of dichlorobenzidine couples readily to give the

diazomonoazo compound. The activity of the second diazonium group is diminished so that formation of unsymmetrical diazo dyes also can be accomplished (Lurie, 1964). By proper choice of coupling agents, the resulting diazo compound can be diazotized or used itself as a coupling component with another diazonium compound to give rise to bisazo, triazo, tetrakis-azo, or poly azo dyes (Lurie, 1964). A wide variety of dyes spanning the color spectrum is made possible by (1) a large number of possible coupling agents, (2) the choice of symmetrical or unsymmetrical coupling, and (3) the possibility of forming monoazo or polyazo derivatives.

An important source of environmental release of dichlorobenzidine is the wastewater from dye producer plants. Dichlorobenzidine is very rapidly degraded in aqueous solution by the action of natural or simulated sunlight (Sikka et al., 1978). Benzidine and 3-chlorobenzidine are intermediate in this process. In organic solvents the dechlorination reaction is considerably slower (Sikka, 1978). DCB absorbs extensively to a variety of aquatic sediments and becomes more tightly bound with time (Sikka, 1978). Dichlorobenzidine is more soluble in water and organic solvents than is DDT (a well known bioaccumulator). DCB-based dyes and pigments discharged into water from manufacturing facilities are believed to be chemically reduced back to the parent compound if either hydrogen sulfide or sulfur dioxide is present in the receiving waters. This was believed to be the case downstream of a dye plant on the Sumida River in Japan (Takemura et al., 1965). The DCB-based dyes and pigments discharged into Sumida River from two manufacturing sites were accumulated in the

soil profiles, and gradually back to the parent compound such as benzidine. Their degradation rates were slower than the degradation of other based dyes and pigments.

Hitz et al., (15) carried out laboratory studies on the adsorption of dyes to activated sludge. After 30 minutes, average adsorption of seven basic dyes ranged from 50 to 92 percent. No data were found on the distribution of dichlorobenzidine in air. The rate of DCB adsorption to all sediments studied by Gibson (1968) was initially very rapid. The end point of sorption was generally achieved within 24 hours (Gibson, 1968). Alkaline conditions may change the properties of the sediments because treatment of sediments with NaOH releases humic and fulvic acids from soil or sediments.

Metabolic conversion by aquatic microbial communities represents one route for the ultimate degradation and elimination of DCB residues. However, metabolites of aromatic amines may be more toxic than the parent compound, thereby compounding the potential hazard (IARC, 1972 a,b; IARC, 1973b; Jones, 1980). Appleton et al., (2) studied the degradation of DCB by aromatic microorganisms using samples of wastewater and activated sludges. Their findings indicate that DCB was not readily degraded by microorganisms. The progressive decrease in DCB is probably related to the increase of organic material. The action of sunlight on DCB leads to its degradation but not necessary to its detoxification since benzidine, a relatively photostable carcinogen, is one of the products (Gibson, 1968).

Boyd et al., (1984) studied the behavior of DCB in soil. They observed that an initial sharp decrease in the amount of NaOH-

extractable DCB was accompanied by a sharp increase in NaOH-extractable DCB of soil. This process indicated that movement of DCB into humic fraction was essentially complete after 108 days since NaOH-extractable DCB plus NaOH-extractable DCB remained constant in a Brookston clay loam. Mineralization of DCB was very slow (ca. 2% per year) in aerobic soil.

The formation of covalent linkages between DCB and soil humic components appeared to be the primary fate of DCB. The behavior of DCB in soil was consistent with that of structural analogues such as the chloaniline which has been studied with respect to their binding reaction and microbial transformation in soil (Bartha, 1971; Kearney and Plimmer, 1972; Kaufman et al., 1973; Hsu and Bartha, 1974a,b).

The fate of organic pollutant or xenobiotic chemicals in soil has become a major health concern in recent times due to the potential for surface and groundwater contamination and for crop uptake and movement in the food chain. Certain xenobiotic chemicals pose serious health hazards due to their toxic and carcinogenic properties. One major environmental fate of hazardous organic chemicals is immobilization at the soil surface by association with humic substances. It is important to understand the nature of the xenobiotic-humic substance complex, for only by understanding this association can we begin to put together a comprehensive soil pollution indices that would relate relevant xenobiotic physiological properties with bioavailability and hazard association data.

One important class of persistent xenobiotic compounds found in soil are the alkyl- and halogen- substituted anilines. Aromatic

amines serves as the basic structural matrix for many commonly used herbicides and pesticides and also used in the manufacture of plastic dyes and pigments. The appearance of aromatic amines in the soil environment may therefore be related to the intentional application of herbicides or the unintentional releases often associated with manufacturing process and waste disposal. Because the ring carbons are not rapidly mineralized in soil (Bartha, 1971), aromatic amines remain available for binding reactions to soil humic substances. This association with soil humus further stabilizes aromatic amines against microbial degradation. Even chlorocatechols (produced from degradation of 2,4-D) which are readily degradable in culture solutions are stabilized in soil against microbial degradation by attachment to soil humus (Statt et al., 1971). Many aromatic compounds are oxidized to catechols before the aromatic rings are cleaved (Dagley, 1971).

It has been well documented that aromatic amines, when added to soil, become strongly and irreversibly bound to soil humic materials (Saxena and Bartha, 1983). This binding is believed to result from the formation of covalent bonds between soil humus and the amine group of anilines. Aromatic amines such as DCB form covalent linkage to quinoidal sites of soil humus via nucleophilic addition of the amine group.

When anilines are applied to soil, they are apparently bound by both physical and chemical adsorption. It is also apparent that in soil a dual absorption mechanism for aromatic amines is active. For example, with dichloroaniline (DCA), a portion of the available DCA is

formly (chemically) bound to the soil organic matter (Saxena, 1983). The portion is loosely (physically) absorbed to both organic and inorganic soil particles. The latter portion can be extracted by organic solvents, the former portion can not. In whole soil the two binding mechanisms compete with each other with the result that at a given DCA concentration less DCA is bound firmly (chemically) to whole soil than to a corresponding amount of isolated humic acid. With time, a gradual shift occurs resulting in more chemically bound DCB. Increasing DCA concentration resulted the increased amounts of bound DCA, but the proportion of added DCA that become bound to soil or humic acid gradually decreased with higher concentrations (You and Bartha, 1982).

Removal of the soil organic matter not only reduces overall DCA-binding capacity of soil (Hsu and Bartha, 1976), but virtually eliminates the type of binding that is resistant to solvent extraction. The significance of bound residues as potential source of future contamination is essentially unknown.

Several examples can be described which illustrate that for aromatic amines in soil, binding processes are the prominent fate. For example, an investigation by Chisaka and Kearney (9) demonstrated that the herbicide 3',4'-dichloropropionanilide (propanil) is readily transformed by soil microorganisms to 3,4-dichloroaniline (DCA) in soil. They also observed that $^{14}\text{CO}_2$ evolution from ring labelled propanil was less than 3 % after 25 days of incubation in soil. Hsu and Bartha (17) found that up to 90 % of the 3,4-dichloroaniline released during biodegradation of phenylamide herbicides becomes

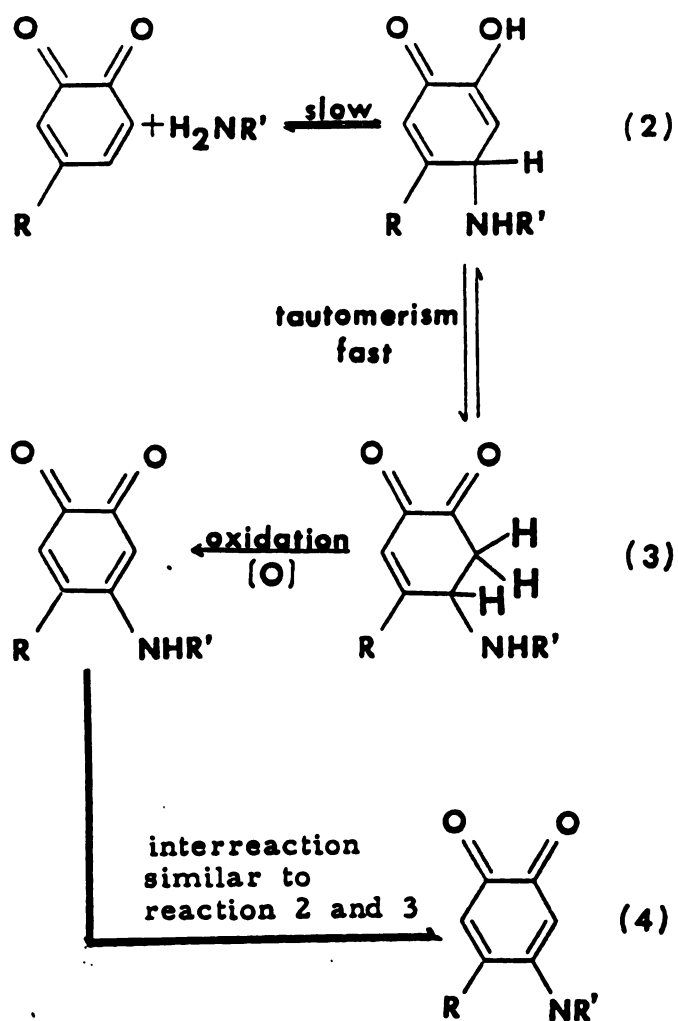
solvent-unextractable due to binding to soil organic matter. The difference in bond stabilities for hydrolyzable versus non hydrolyzable chloroaniline residue complexed with soil humic substances suggested to Hsu and Bartha (19) that two distinctly different covalent binding mechanisms were operative. Hsu and Bartha also demonstrated that with time physically adsorbed and hydrolyzable 3,4-dichloroaniline residues slowly shifted to the non-hydrolyzable form. Several investigators have studied the general phenomenon of aromatic amine binding to soil humic substances. It is generally thought that several different types of chemical or biochemical reactions are responsible for binding of aromatic amines to soil humic substances.

Hsu and Bartha (17,18) first proposed that imine formation to explain rapid binding of primary aromatic amines to soil humic substances (Fig,5). The reaction involving aromatic amines and the carbonyl group of aldehyde or ketones to produce imines is reversible reaction. The equilibrium can be shifted towards the two starting reactants if excess water is present. In soils, it is not clear where the equilibrium lies under normal conditions (Parris, 1980).

There is, however, another type of binding reaction of aromatic amines with soil humic substances that leads to the formation of covalently bound residues. Cranwell and Haworth (10) and later Hsu and Bartha (17,18) and Parris (23) proposed that primary or secondary (aromatic) amines might undergo a nucleophilic substitution reaction with a Michael acceptor. In this case the Michael acceptor would be a quinone-like residue which is attached to the soil humic matrix



Figure 5. Imine Formation Reaction (Stevenson , 1980)



R=quinone moiety in humate

Figure 6. Michael addition of aromatic amine to humus matrix, (Parris, 1980)

(Fig.6). This is followed by tautomerization and oxidation yielding aminoquinone (Reaction 2,3). The amino group may react further by essentially the same addition -tautomerization-oxidation sequence to form a variety of nitrogen heterocycles (Reaction 4). The nature of the resulting heterocycle will depend upon the reactive sites (R) in the humate juxtaposed to the aminoquinone. Progression along this sequence of reactions is expected to make the amine moiety more resistant to removal from the humate.

To date, three types of covalent aniline attachment to humus and to model humic materials have been proposed: Formation of imines, Michael addition adducts (Hsu and Bartha 1974a; Parris 1980), and enzymatic oxidative cross-coupling reactions (Bollag 1976). These studies have demonstrated that the above type of model reactions occurred with incorporation of substituted anilines, and have determined the chemical structures of the resulting products.

The comparison of hydrolysis and resistance of the 4-methylcatechol-3,4-dichloaniline (MC-DCA) adduct with that of Humic Acid-Dichloaniline (HA-DCA) complexes revealed substantial differences in behavior (You and Bartha, 1982). The MC-DCA adduct was completely resistant to alkaline hydrolysis (Fig.7), but it was subject to partial hydrolysis by acid. In addition, HA-DCA complexes were partially hydrolyzed both by alkaline and by acid, the alkali being somewhat more effective. In terms of biodegradation, the MC-DCA adduct was found to be much more recalcitrant than HA-DCA complexes. If the two attachment mechanisms were similar, one would expect the opposite result, since structurally analogous oligomers are generally more

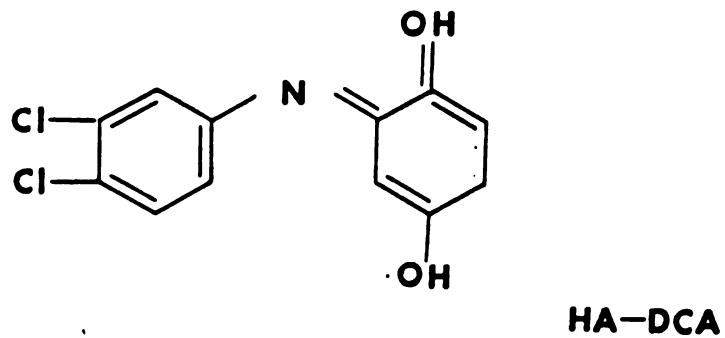
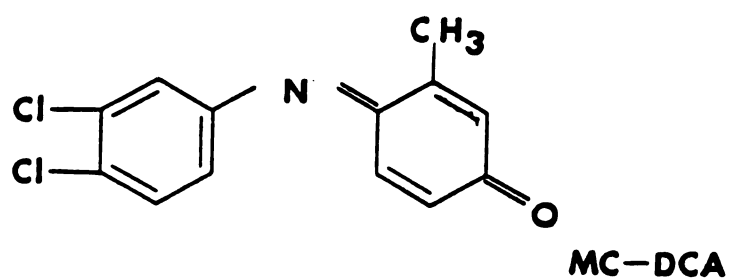


Figure 7. Chemical structure of MC-DCA (4-methylcatechol-dichloroaniline) and HA-DCA (Hydroquinone dichloroaniline). (You, I.S. 1982)

biodegradable than high molecular weight polymers (Albertsson and Banhida, 1980). $^{14}\text{CO}_2$ release from HA-DCA complexes was stimulated by the addition of aniline, butaniline failed to stimulate $^{14}\text{CO}_2$ release from the MC-DCA adduct. This finding also supports the conclusion that the bulk of DCA inhumic complexes is not likely to be bound in the same manner as in the MC-DCA adduct (You et al., 1982).

The imine formation and Michael addition mechanisms discussed thus far require, in principle, the presence of two reactants; an aromatic amine and the carbonyl and quinone moieties of the humic component. The next group of mechanism to be presented not only requires the presence of these two reactants but also a biocatalyst as well. In this process polyphenols are coupled together after being enzymatically oxidized to the aryloxy radical: This enzyme may also convert aromatic amines to radicals which then couple with soil humic materials. Alternatively, the aromatic amine may react chemically as described above with humic materials formed via oxidative coupling reactions. Oxidative coupling of polyphenols in soils is thought to be an important synthetic process leading to the formation of humic substances. The possible source of polyphenols for humus synthesis include lignin, microorganisms, uncombined phenols in plants, glycosides, and tannins. Of these, the next three have received attention: (1) Lignin, freed of its linkage with cellulose during decomposition of plant residues, is subjected to oxidative splitting with the formation of primary structural units (derivatives of phenylpropane), (2) The side chains of the lignin-building units are oxidized, demethylation occurs, and the resulting polyphenols are

converted to quinones by polyphenoloxidase enzymes. (3) Quinones arising from the lignin (as well as from other sources) reacts with N-containing compounds to form dark-colored polymers.

The results obtained by Bollag et al., (24) and Berry et al., (25) suggest that aromatic amine binding to soil humic components results from a chemical reaction which may be only indirectly controlled by the presence of oxidative coupling enzymes in soil. In this, oxidative coupling enzymes act to create reactive quinoidal site on the humic acid structure, and the amine reacts chemically via nucleophilic addition reactions to form the bound residues. Peroxidases and laccases are believed to be the responsible catalysts in the coupling processes.

Aromatic amines may also be sorbed physically by soil. Chemisorption may also occur if the amine moiety becomes protonated. For the higher pH, sorption increases with increasing surface area. The sorption of benzidine by "whole" soils and sediments has been known to be controlled primarily by the concentration of the ionized species. Sorption was highly correlated with pH, since pH controlled the ratio of neutral to ionized benzidine in the aqueous phase (Zierath et al., 1980). The potential effects of benzidine and related compounds can be greatly modified by sorption to soils and sediments. However, the resistance of the aromatic amine residues to solvent extraction and ion exchange combined with a sensitivity to acid as well as alkaline hydrolysis strongly suggest a covalent binding of the nitrogen atom of these compounds to the carbon of carbonyl group or to a quinoidal ring of the humic compounds.

As demonstrated with 3,4-dichloroaniline, it is very difficult to extract the anilines from soil by organic solvents or to obtain their release by acid and alkaline hydrolysis (Bartha, 1971; Chisaka and Kearney, 1970). It seems that anilines are bound to humus or soil organic matter rather than to clay particles (Hsu and Bartha, 1974). It has usually been found that CO formation from halogenated anilines occurs slowly (Bartha, 1971; Chisaka and Kearney, 1970), that ring cleavages of these compounds by microorganisms is not a very common reaction. The aeration by a continuous air stream and trapping in an acidic solution reveal that most anilines are bound to a lesser degree in autoclaved than in nonautoclaved soil (Bollag et al., 1983).

The absolute amount of DCA mineralized increased with increasing DCA concentrations, although the percentage mineralized decreased moderately (You and Bartha, 1983). At low concentration, the bulk of the aniline moiety becomes tightly bound to soil and cannot be extracted by the salt, sediments or organic solvents (Bartha, 1971; Chisaka and Kearney, 1970). Physical absorption to mineral soil particles competes with the chemical binding to humus, but not only the chemically bound position resists exhaustive solvent extraction.

In soil, DCB is immobilized rapidly by binding to humic materials (Boyd et al., 1983). Boyd et al., (7), observed that essentially 90 % of the DCB added to soil was irreversibly bound to soil humic fraction.

The progressive loss of solvent -extractable DCB, coupled with increasing extractability (of the bound residue) by alkali, was attributed to the formation of humus-bound DCB residues. Mineralization of DCB in soil was very low (2 % in 6 months). The formation of covalent bound DCB residues with soil humus is a natural detoxication process in soils. The bound residues are less mobile and less bioavailable, and therefore less likely to contaminate water or move into the food chain.

Berry and Boyd have studied ways to enhance the formation of DCB-bound residues in soil as a decontamination method. A single addition of ferulic and H_2O_2 to DCB-contaminated soil significantly decreased extractable radioactivity and thus dramatically decreased the level of free DCB in soil. A previous study by Berry and Boyd (1984) showed that phenolic compounds containing the three C-acrylic group (e.g., ferulic acid) were highly reactive with peroxidase enzymes, in contrast to other naturally occurring polyphenols that did not contain the acrylic group (e.g., vanillic acid). Addition of ferulic acid to a reaction mixture containing peroxidase and chloroaniline greatly enhanced (2000X) the rate of coupling of chloroaniline. A similar effect of ferulic acid on DCB binding in soil may occur. This procedure may be used in the future to enhance the covalent binding of DCB or similar pollutants and thereby decrease the potential for movement into ground water or the food chain.

The binding of xenobiotic chemicals to soil humic material is a phenomenon which warrants an intense research effort both from the standpoint of pure and applied science. It is important to understand

the physical nature of the binding process for only by understanding the nature of the xenobiotic-humic substance interaction can we be in a position to assess the contamination potential of soil and sludge-born xenobiotics. It is also important to gain a fundamental understanding of these binding of xenobiotic compounds in soils. enhancing the binding processes operative in soil which act to covalently couple xenobiotic and humic substances may offer great promise for detoxifying contaminated soils.

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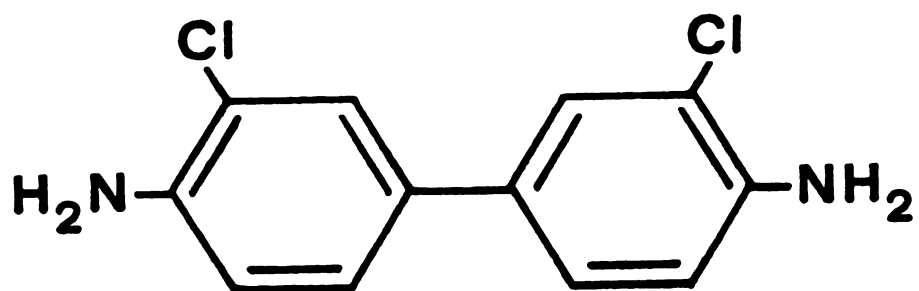
CHAPTER II

MOBILITY OF SLUDGE-BORNE 3,3'-DICHLOROBENZIDINE (DCB)

INTRODUCTION

3,3'-Dichlorobenzidine (3,3'-dichlo, 4,4'-diaminobiphenyl), hereafter referred to as DCB, is widely used as an intermediate in the production of azo pigments. With current work practice, effluents containing this chemical are discharged directly into receiving waters. Moreover, the discharge of DCB-pigment wastes constitutes an additional source of DCB contamination in the environment since free, unreacted DCB is reported to be present in these pigments. As a result of industrial discharges like this, sewage sludge generated at the Muskegon Wastewater Management System (MWMS) has become contaminated with the carcinogen 3,3'-dichlorobenzidine (Fig.1). The MWMS uses wastewater to irrigate over 2,025 ha of land in Muskegon County where corn is primary crop grown.

Consideration is now being given to initiate a program of land application of sewage sludges generated at the MWMS. Because agronomists have long reconized the value of sewage sludge in improving soil conditions and supplying nutrients for plant growth. Also, wastewaters that nomally are discharged into streams still contain valuable nutrients and water resources that could be used for agricultural production. However, applying sewage wastes to land as a method of advanced wastewater treatment is not without its problem. The MWMS sludge is contaminated with DCB, a potential hazard of this



3,3'-Dichlorobenzidine (DCB)

Figure 1. Chemical structure of 3,3'-dichlorobenzidine

sludge management alternative is for DCB to be leached from sludge through the soil profile and into groundwaters.

Contamination of natural waters by a soil-borne xenobiotic compound is a function of that compound's persistence and mobility in the soil. For persistence compounds such as DCB, mobility in the soil environment is specially important because the compound exists in soil for long periods of time thereby enhancing the potential movement and subsequent contamination of groundwaters. The potential for movement is enhanced because (1) DCB is known to be persistent in agricultural soils, (2) the sandy soils at Muskegon County have high infiltration rates and the capability to percolate large amounts of water, and (3) these soils receive higher than normal quantities of water during the year. For these reasons, a study was initiated to evaluate the mobility of sludge-born DCB in an experimental system designed to mimic conditions at the MWMS.

DCB is produced in the United States and in several foreign countries. Annual U.S. consumption of DCB reached 3.9 million pounds in 1978 (Jones, 1980). The primary use for DCB is the production of dyes and pigments. Although DCB can be used to produce 95 tetrazo dyes, only five pigments are currently used in the United States: Pigment Orange 13 and 14, and pigments. DCB is a demonstrated animal carcinogen and has been shown to give a positive test in the Ames Salmonella assay indicating that it possesses mutagenic properties (Jones, 1980). Furthermore, a positive correlation has been observed between exposure levels of benzidine (in work place) and the incidence of bladder cancer in humans (Jones, 1980). The DCB based pigment yellow 12 and 13 are not carcinogenic in rats and mice

(Jones,1980). The carcinogenic properties of other DCB-based pigments are currently unknown.

The prominent fate of aromatic amines (such as DCB) in soil generally appears to be a formation of stable covalent linkages with soil humic substances (Boyd et al., 1984; Chisaka and Kearny, 1970; Hsu and Bartha, 1974). Because aromatic amines are not readily mineralized in soil they remain available for binding (Chisaka and Kearny,1970; Hsu and Bartha,1974; Boyd et al.,1984). Generally, it has been observed that after entry into the soil environment, aromatic amines exhibit a sharp decrease in solvent-extractibility. They showed that for DCB, loss of solvent-extractibility. Their conclusion was that DCB had formed covalent linkages with the soil humic fraction.

The formation of bound (non-extractable) residues rendered DCB highly immobile (Boyd,1984), although some movement of DCB through the soil profile was observed. In general, bound residue would be expected to be less mobile and bioavailable than the unknown form.

Boyd et al., (1984) examined the fate of DCB in a Brookston clay loam soil. Mineralization of DCB was very slow (ca. 2 % per year) in aerobic soil. The behavior of DCB in soil was consistent with that of structural analogues such as the chloroanilins which have been studied with respect to their binding reactions and microbial transformation in soil (Bartha,1971; Kearny and Plimmer, 1972; Kaufman et al.,1973; Hsu and Bartha, 1974a; Parris,1980; and Saxna and Bartha,1983).

In the present study, the mobility of sludge-born DCB in sludge-amended soil was evaluated. Soil columns were constructed

using soil and DCB-contaminated sludge from the MWMS. Water was added at a high rate of 2.54 cm per day for 84 days to simulate the irrigation schedule at Muskegon. Mineralization of sludge-borne DCB in sludge amended soil was also examined.

EXPERIMENTAL

MATERIALS

A Rubicon sand (Entic Haplothod, sandy, mixed, frigid) obtained at the Muskegon Wastewater Management System (MWMS) Muskegon, Michigan was used. Soil was collected from the top 30cm of the soil profile, air dried and passed through a 2 mm sieve. The Michigan State University Soil Testing Laboratory carried out the soil analysis with respect to particle size analyses, organic matter content, pH and CEC. The Rubicon sand was found to contain the following percent of sand was 93.5 percent; percent organic matter was 2.54; pH was 7.7; and CEC was 6.6 me/100 gram.

Sewage sludge contaminated with DCB was collected at the MWMS. The sludge had originally been pumped from aeration cells and at the time of sampling was in the form of a thick paste. The sludge was spread out and allowed to air dry after which it was passed through a 2 mm sieve, and then stored at 4 °C. Dichlorobenzidine content of the sludge as 16 ug/g. Sludge was extracted with dichloromethane in a Soxhlet extractor and quantitated for DCB using HPLC by the method of Riggan and Howard (1979).

The 3,3'-dichlorobenzidine (3,3'-dichloro-4,4'-diaminobiphenyl) used in this study was purchased from Chem Service (West Chester, PA) as the free base and was used without further purification.

DCB (ring-UL- ^{14}C) was purchased from ICN Pharmaceuticals, Inc., a radiochemical purity of > 99% and a specific activity of approximately 35 nCi/mmol. The [^{14}C]DCB was dissolved in absolute ethanol and stored in the dark at 0 °C. This gave a stock [^{14}C]DCB solution with a specific activity of 0.023 uCi/ uL and a DCB concentration of 167 ug/mL.

SOIL INCUBATION

Mineralization of [^{14}C]DCB was assayed in soil amended with sludge at the rate of 0, 22.4, 112, and 224 Mg/ha (equivalent to 0, 10, 40 and 100 g sludge/kg soil). For each treatment, 50 g of air dry soil and the appropriate amount of sludge were placed in 250 mL Erlenmeyer flasks, and then moistened to approximately 60% field moisture capacity with 4.5 mL distilled water. each flask then received 40 uL of the [^{14}C]DCB stock solution (in ethanol) which was added dropwise from a 50 ul hamilton syringe while the soil was being stirred with spatula. In this fashion, 0.92 uCi of [^{14}C]DCB was added to each flask. To trap any $^{14}\text{CO}_2$ evolved, 1 mL of 0.1 N NaOH was placed in a 1.4 mL plastic vial attached to a glass rod which extended downward from a rubber stopper used to close the flasks. Tropaeolin 0 indicator dye was added to the aqueous NaOH solution to indicate when the NaOH had become saturated with $^{14}\text{CO}_2$. The flasks were opened every 7 days (or sooner if necessary), at which time the $^{14}\text{CO}_2$ traps

were collected and replaced with fresh aqueous NaOH. Flasks were incubated in triplicate in the dark at room temperature for 182 days.

Soil Extraction

Change in the solvent extraction recovery of [^{14}C] DCB over time from soil and sludge were evaluated. In the recovery study, 500 g batches of soil or sludge were placed into large-mouth amber glass jars. 0.5 milliliter of the [^{14}C]DCB stock solution was added to 47.5 mL distilled water and mixed. Two 0.5 mL samples were withdrawn and assayed for radioactivity. To each 500 g soil samples, 45 mL of the aqueous [^{14}C]DCB solution (60% field capacity) was added dropwise using a Pasteur pipet while continuously stirring the soil. The jars were closed and incubated for 28 days at room temperature. 35 g samples were taken after 0.04, 0.17, 1, 2, 5, 7, 10, 14, 21 and 28 days and stored frozen until analyzed. From each samples (35 g) duplicate 10 g subsamples were placed in 125 mL Erlenmeyer flasks along with 40 mL of a 60:40 (vol/vol) ethyl acetate/methanol solvent mixture, closed with aluminum foil-coated rubber stoppers (No.5), and allowed to shake for 22 hours on a rotary shaker at room temperature. After sedimentation, 5 mL of solvent was sampled and centrifuged (8000X g for 10 min.) from which 2 mL was used for liquid scintillation counting. All extractions were performed in duplicate. Figure 2 illustrates the amounts of solvent extractable radioactivity and (expressed as a percentage of the added radioactivity) was calculated on a soil or sludge air dry weight basis. Liquid-phase ^{14}C radioactivity was measured using a Beckman LS 8100 Liquid

Scintillation Counter. Samples were counted in Aquasol-2 (New England Nuclear) LSC cocktail.

SOIL COLUMNS

Glass columns with teflon fittings and sintered glass bottoms (Kontes) were used. The columns were 61 cm in length, had a 5.08 cm inner diameter, and were cut into 2.54 cm sections from the bottom up. The columns were taped together with plastic waterproof tape while compressing the ends. The columns were then filled with 30.5 cm of air dried soil and tapped repeatedly. The soil columns were then immersed in a large cylinder filled with distilled water, allowed to wet from the bottom up, and drained. This procedure was repeated twice before adding the top 15.2 cm of soil or sludge-amended soil which contained the [^{14}C]DCB. Two columns were prepared for each treatment. The three treatments were: 1 mL of [^{14}C]DCB added directly to 410 g soil with no sludge amendment, (2) 1 mL of [^{14}C]DCB added to 410 g soil amended with sludge at the rate of 11.2Mg/ha, (3) 1 mL of [^{14}C]DCB added to 4.1 g sludge, incubated for 14 days to allow equilibration on the added [^{14}C]DCB, then ^{14}C -sludge added to 410 g of air dried soil.

For treatment 1 and 2, the soil or sludge amended soil was thoroughly mixed and moistened with an aqueous solution (41 mL) containing the [^{14}C]DCB, incubated for 14 days in amber glass jars at room temperature to allow equilibration of the added [^{14}C]DCB, and then air dried. The air dried soil or sludge amended soil was then added to the top of the soil column. Total soil depth was 45.7 cm.

each soil column received 35,000 nCr of [^{14}C]DCB. Sludge was added at a rate of 10 g/kg. Total weight of soil in the lower 30.5 cm was 820 g; the upper (treated) 15.2 cm soil zone weighed 410 g. At this point (time zero), samples (4.1 g) of the [^{14}C]DCB-soil or sludge amended soils were taken and stored frozen for later ^{14}C analysis.

Distilled water (51.5 mL, equivalent to 2.54 cm) was added daily for 84 days. Leachate water (50 mL) was collected in 500 mL Erlenmeyer flasks (wrapped in foil) each week. A portion of this water (100 mL) was extracted using a C18-Pak (Millipore) and eluted with 2 mL of methanol into 10 mL scintillation cocktail. The pH of the leachate was always >7. Trail extraction using leachate water spiked with known quantities of [^{14}C]DCB gave > 90% recoveries.

At the end of the 84 day irrigation period the columns were disassembled into their 2.54 cm sections, air dried and weighed. Distribution of the ^{14}C with depth was determined by combusting triplicate 1.0 g samples for each 2.54 cm section and trapping the $^{14}\text{CO}_2$ produced. The total amount of ^{14}C added to each column was determined by combusting samples of ^{14}C -soil or sludge-amended soil taken at time zero.

^{14}C ANALYSIS

Radioactivity was measured with a Beckman LS8100 liquid scintillation counter using external quench correction. $^{14}\text{CO}_2$ samples from the soil incubation and extraction studies were counted in Aquasol-2 (New England Nuclear) LSC cocktail. Total soil ^{14}C for samples from the soil columns was determined using a OX200 Biological

Material Oxidizer (R.J. Harvy Instrument). The $^{14}\text{CO}_2$ was trapped and counted in $^{14}\text{Carbo-Sorb}$ (Research Product International Corp) LSC cocktail.

RESULTS AND DISCUSSION

Mineralization of [^{14}C]DCB was evaluated during a 182 day incubation in soil amended with sludge at rates of 0, 10, 50 and 100 g/kg. The total amount of [^{14}C]DCB recovered as $^{14}\text{CO}_2$ was less than 2% in all cases demonstrating the persistence of DCB in soil sludge-amended soil (Table 1). Mineralization of [^{14}C]DCB in the autoclaved controls was less by factor of at least 10X relative to the nonautoclaved treatments. Thus, a biological component appeared to be responsible for the slow release of $^{14}\text{CO}_2$ from [^{14}C]DCB. The effect of increasing sludge application rate was to increase the total amount of DCB mineralized. However, the percent of DCB converted to CO_2 decreased from 1.94% to 1.04% in going from a sludge rate of 0 to 100 g/kg. These differences are quite small and the important point is that DCB was highly resistant to mineralization in both soil and sludge-amended soil

This result is consistent with a previous study showing the persistence of DCB in a Brookston clay loam soil (Boyd et al., 1984). The ability to recover added DCB from soil and sludge as a function of incubation time was also evaluated (Fig. 2). Boyd et al., (1984) showed that the formation of soil bound (nonextractable) residues involving aromatic amines appears to occur rapidly. In this experiment, a rapid and dramatic loss of solvent extractable ^{14}C

Table 1. Mineralization of [^{14}C]DCB to $^{14}\text{CO}_2$ during 182 day incubation in sludge amended soil.

Sludge * rate	Total DCB ** added	Total DCB mineralized	[^{14}C]DCB as $^{14}\text{CO}_2$ ***	
			not autoclaved	autoclaved
g kg^{-1} .	ug			
0	6.7	0.13	1.94	0.10
10	14.7	0.25	1.70	0.08
50	46.7	0.60	1.28	0.08
100	86.7	0.90	1.04	0.08

* Equivalent to 0, 22.4, 112 and 224 Mg ha^{-1} .

** For each flask (50 g non-autoclaved soil).

*** Percent cumulative of added ^{14}C recovered as $^{14}\text{CO}_2$ after 182 day incubation.

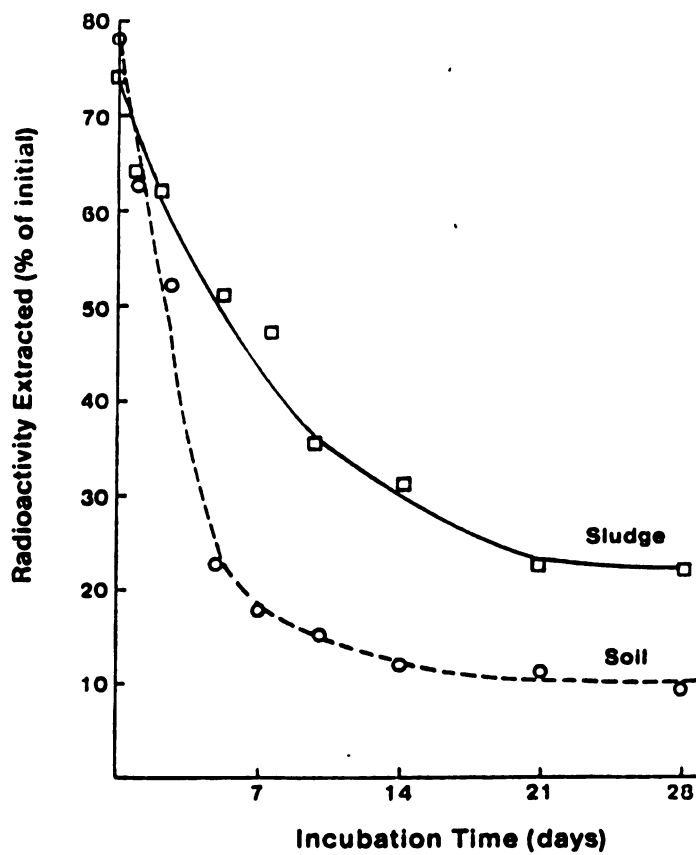


Figure 2. Solvent extractable radioactivity from [^{14}C] DCB-amended soil and sludge as a function of incubation time.

from soil was observed during the first week of incubation. In this study, a short term extraction experiment was conducted to examine in greater detail the loss of solvent-extractable radioactivity during the initial 4 weeks of incubation. The percent solvent-extractable and the corresponding incubation times (in parentheses) were 78 (1h), 73 (4h), 63 (1d), 52 (2d), 23 (5d), 18 (7d), 15 (10d), 12 (14d), 11 (21d) and 9 (28d). After 7 days incubation, approximately 18% of the added ^{14}C could be extracted in soil after extraction with ethyl acetate and methanol mixture. A more gradual decline in extractable ^{14}C occurred from day 7 to day 28 at which point approximately 10% of the added ^{14}C was extractable. A similar loss of solvent extractable ^{14}C from sludge, which was observed, although this loss was slower and less complete. The present solvent extractable radioactivity and the corresponding incubation times (in parentheses) were 74 (1h), 69 (4h), 64 (1d), 62 (2d), 51 (5d), 47 (7d), 35 (10d), 31 (14d), 23 (21d) and 22 (28d). After 7 days 47% of the added ^{14}C was extracted and at the end of the incubation (28 days) 22% was extracted. The greater recovery of added [^{14}C]DCB in sludge was probably due to fewer binding sites for the formation of humus bound DCB residues (Parris, 1980; Saxana and Bartha, 1983).

For aromatic amines in general, the time required for at least 50% of the residue to become bound is typically on the order of 1d to 1 wk (Hsu et al., Bollag and Voorman, 1981). Although some variance exists in these initial rates, well over 50% of the residues generally becomes nonextractable during the first several weeks of incubation.

Recoveries after only 1 h incubation were quite good (74 and 78% for soil and sludge) showing that the extraction procedure was effective. Total ^{14}C in soil and sludge was determined by combustion, remained constant over the 28 day incubation period (data not shown). A similar loss of solvent extractable DCB, coupled with increasing extractability by alkali, was observed previously for [^{14}C]DCB added to a Brookston clay loam soil (Boyd et al., 1984).

It has been well documented that aromatic amines, when added to soil, become strongly and irreversibly bound to soil humic materials. This binding results from the formation of covalent bonds between soil humus and the amine group of anilines. For example, an investigation by Chisaka and Kearny demonstrated that the herbicide 3',4'-dichloropropionanilid (propanil) is readily transformed to 3,4-chloroaniline (DCA) in soil. There is, however, another type of binding involving aromatic amines with soil humic substances that is resistant to hydrolysis and not readily exchangeable. Cranwell and Howorth (1971) proposed that primary and secondary aromatic amines might undergo a nucleophilic substitution with a Michael acceptor. In this case, the Michael acceptor would be a quinone-like residue which is attached to the soil humic matrix. The nucleophilic addition of a primary or a secondary aromatic amine to the quinone moiety of the soil humic substance matrix is a slow and reversible reaction which is followed by a rapid tautomeric step.

The formation of bound (nonextractable) DCB residues likely results from the nucleophilic addition of the amine group to quinoidal sites of soil humus (Hsu and Bartha, 1974a; Parris, 1980; Saxana and Bartha, 1983). By subsequent addition and oxidation

reactions the amine residue may become even more strongly bound to the humate structure (Parris, 1980). The formation of a covalent bond between DCB and soil organic matter strongly immobilizes DCB and, as a result, the residues are no longer solvent extractable, which accounts for the data in Figure 2.

Another immobilization mechanism operative in some soils is protonation of DCB adsorption of this cation by clays. However, since the first and second pKa's of DCB are less than 4 (Sikka et al., 1978), DCB should exist primarily as the free base in the Rubicon soil (pH 7.7).

Soil columns were used to directly assess leaching of [^{14}C]DCB in soil and sludge-amended soil. There are several features of the column experiments which merit attention. The columns used had 5.08 cm inner diameter and were 45.72 cm long. These dimensions were selected to minimize the "edge effect" and to provide sufficient soil column length to allow a natural soil moisture profile with depth.

The columns were irrigated with 2.54 cm of water per day which was selected to exceed the amount of water irrigated in most, if not all, wastewater irrigation system. This approach would, therefore, identify the maximum DCB losses by leaching. The top 15.24 cm of the columns were soil or sludge-amended soil which contained the [^{14}C]DCB. Radiolabeled [^{14}C]DCB was uniformly distributed in the upper 15.24 cm.

Distribution of ^{14}C with depth after 84 days of irrigation is illustrated in Figure 3. The vast majority of the ^{14}C -labeled remained in the top 15.2 cm of the soil column in the sludge-amended and non-sludge-amended soil. This attests to the

strong immobilization of DCB which showed little mobility despite being placed in a sandy soil subjected to intensive irrigation with 2.54 cm of water per day. The distribution profile of ^{14}C from 15 to 45 cm shows a finite and measurable quantity of radioactivity in each 2.4 cm section of the bottom 30 cm of the soil columns (Table 2). Radioactivity in the lower sections of the column tended to be higher in non amended soil with sludge. Thus, these appears to be a very "slow bleed" of DCB through the soil column. There does not appear to be a band of ^{14}C radioactivity moving through the column.

Of the two sludge treatments (i.e. treatment 2 and 3), adding the [^{14}C]DCB to sludge, then incorporating the spiked sludge into soil (treatment 3), resulted in less apparent DCB mobility. This may in fact be more representative of actual field conditions than treatment 2 where [^{14}C]DCB as added to sludge-amended soil

Radioactivity in leachate water collected from the soil columns was very low (Figure 4 and Table 2) and all or part of this could be due to ^{14}C impurity. However, when taken together, ^{14}C in the bottom 30 cm of the soil column plus ^{14}C in the leachate (Table 2) can not be accounted for as radiochemical impurity which was < 1%. The total amount of ^{14}C in the leachate was from 0.16 to 0.063% of the total ^{14}C added to the soil column (Table 2). Smaller amounts of total radioactivity leached through the soil receiving sludge amendment with treatment 3 showing the lowest ^{14}C mobility

Figure 4 show the cumulative ^{14}C -recovered in leachate water on a weekly basis for 12 weeks. These data show that after about two weeks the amount of ^{14}C leached per week remained relatively constant and showed no trend towards declining. These data suggest a "slow

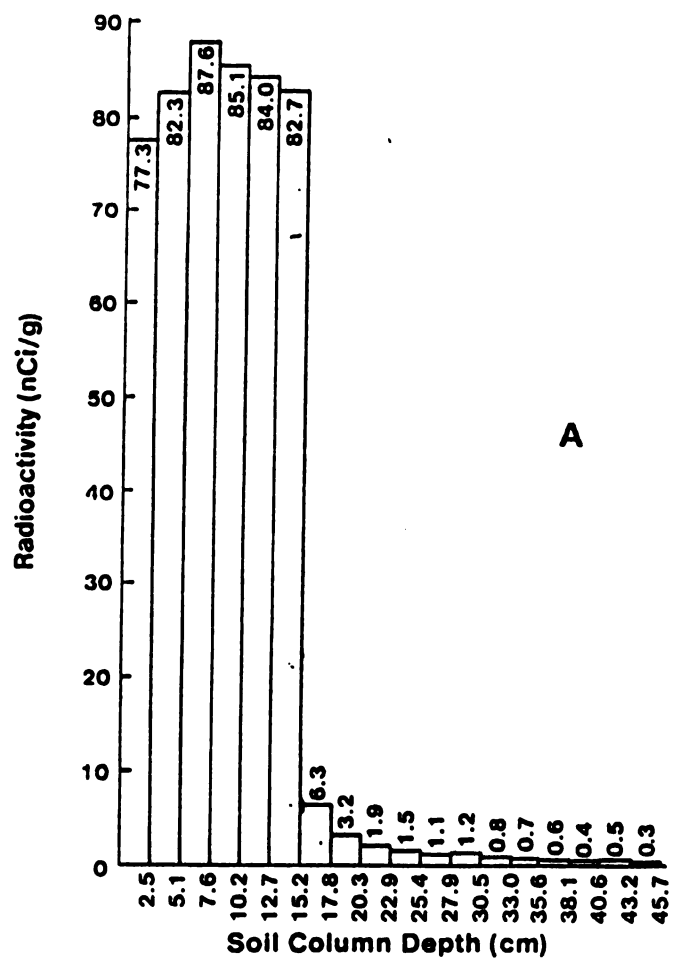


Figure 3a. Distribution of radioactivity with depth in [^{14}C]DCB-amended soil columns which were irrigated with 2.54cm of $\text{D-H}_2\text{O}$ per day for 84 days: ^{14}C -DCB plus soil.

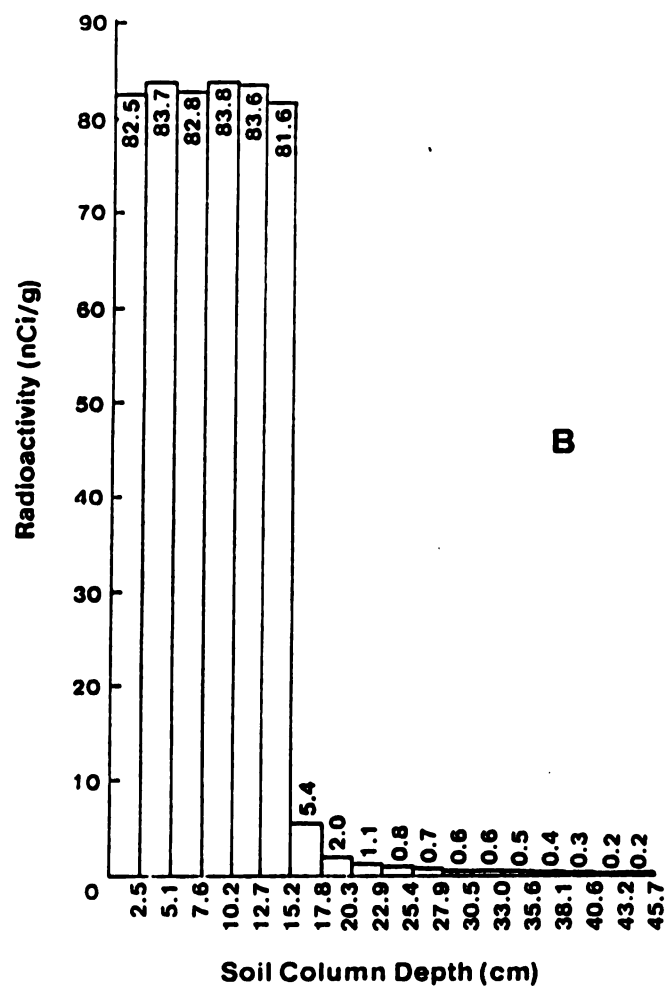


Figure 3b. Distribution of radioactivity with Depth in [^{14}C]DCB-amended soil columns which were irrigated with 2.54cm of $\text{D-H}_2\text{O}$ per day for 84 days: ^{14}C -DCB plus sludge-amended soil.

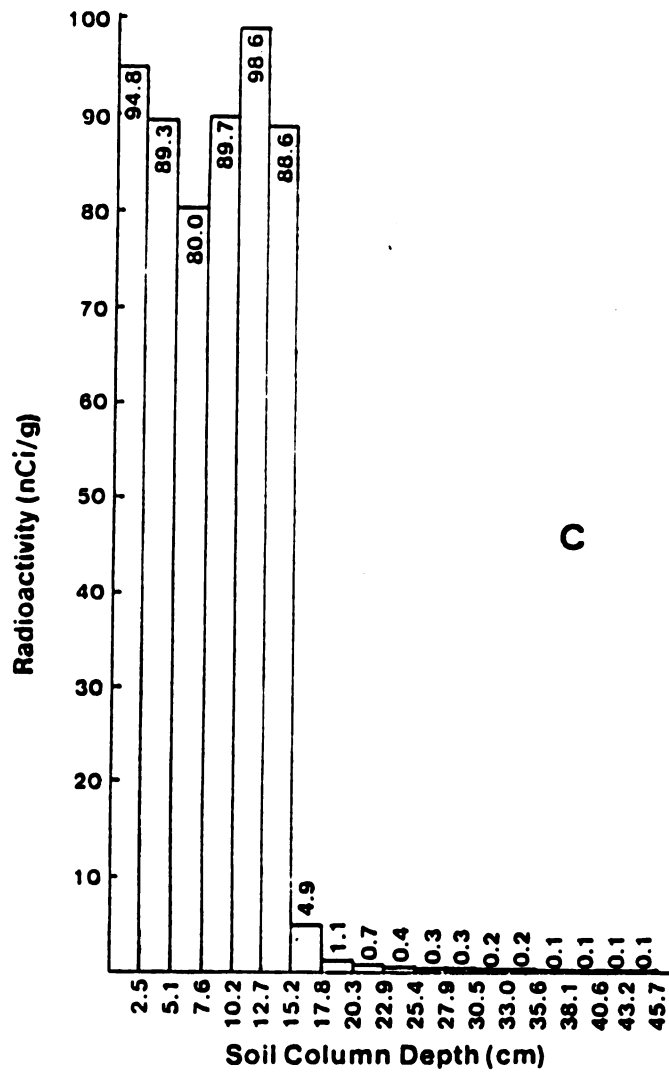


Figure 3c. Distribution of radioactivity with depth in [^{14}C]DCB-amended soil columns which were irrigated with 2.54cm of H_2O per day for 84 days: ^{14}C -DCB amended₁ sludge plus soil: Sludge rate was 10 g kg^{-1} .

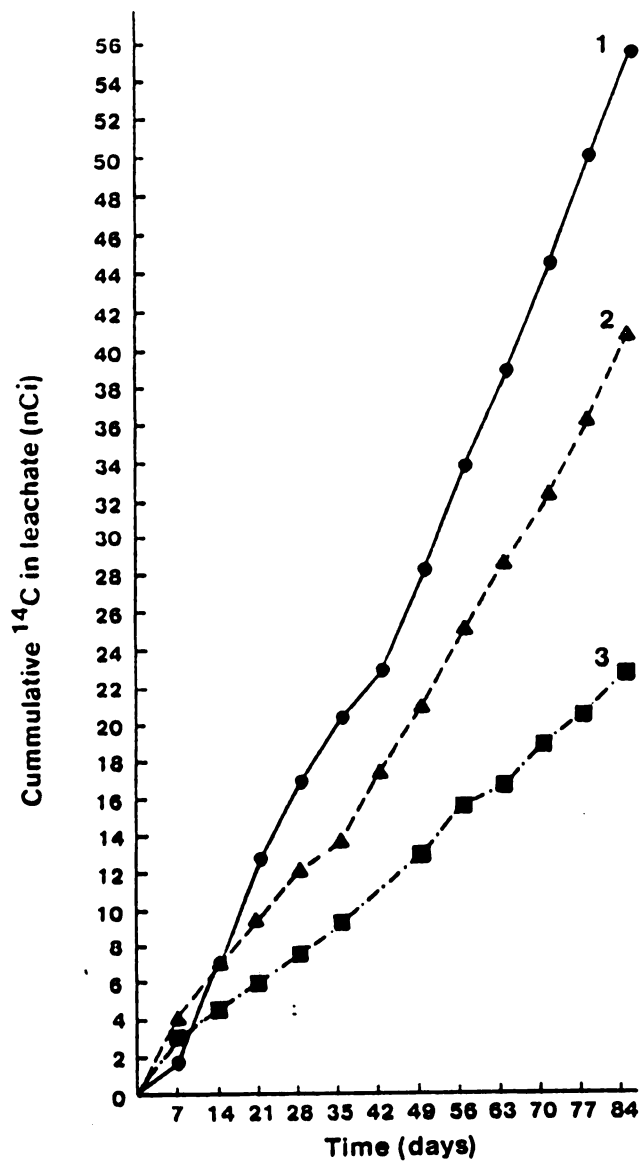


Figure 4. Cumulative ^{14}C in leachate water from soil columns: (1) ^{14}C -DCB plus soil, (2) ^{14}C -DCB plus sludge-amended soil, and (3) ^{14}C -DCB amended sludge plus soil. Sludge rate was 10g kg^{-1} .

Table 2. ^{14}C Distribution and recovery in soil columns and leachate.

Distribution	Column 1	2	3
Soil Sections *	- - - - - % of total - - - - -		
0 - 6	99.2	100.2	101.65
6 - 12	2.91	2.11	1.43
12 - 18	0.66	0.40	0.15
Leachate **	0.16	0.12	0.063
Total Recovery	102.9	102.8	103.3

* Each section was 2.54 cm (1 inch).

** Cumulative ^{14}C in leachate after 12 weeks.

bleed" of ^{14}C through the soil column. This movement, however small, may continue for some extended period of time.

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CHAPTER III

UPTAKE AND MOVEMENT OF 3,3'-DICHLOROBENZIDINE IN SOYBEAN PLANTS GROWN IN SLUDGE-AMENDED SOIL AND NON SLUDGE-AMENDED SOIL CONTAMINATED WITH [¹⁴C]DCB

INTRODUCTION

3,3'-dichlorobenzidine (3,3'-dichloro, 4,4'-diaminobiphenyl), hereafter, referred to as DCB, is widely used as an intermediate in the manufacture of azo pigments. It is of considerable commercial importance; total DCB consumption in the United States in 1978 was about 3.9 million pounds (Jones, 1980). Improper disposal of industrial wastes containing the carcinogen DCB has resulted in the contamination of soil, ground water near manufacturing sites. the appearance of DCB as an environmental pollution, in addition to its classification as carcinogen, demonstrates the importance of understanding the fate of DCB in natural environment.

With current work practices, effluents containing this chemical are discharged directly into receiving water. Moreover, the discharge of DCB-pigment wastes into receiving waters constitutes additional source of DCB is reported to be present in theses pigments. DCB is strongly carcinogenic to animals and regarded by the Occupation Health and Safety Administration (OSHA) as being carcinogenic to man. The discharge of DCB into aquatic environment is of great concern to human health because of possible exposure to the

chemical through drinking water supplies. Also, if DCB and its metabolites accumulate in the plants and food chains from contaminated waters are to be used as human food, that could pose a health hazard. The potential hazard of DCB may be compounded by its biological or non-biological conversion to compounds of even greater toxicity and/or persistence than the parent chemical. For instance, it is believed that in the case of carcinogenic response. Further, potential degradation products of DCB, such as benzidine, may constitute an even greater carcinogenic hazards.

Currently nothing is known about the environmental fate of DCB in a field planted crops. Therefore, this study was undertaken to assess the role of some of the processes that may determine the fate of DCB which is accumulated in soil and sludge-amended soil, leaching rate by irrigation, influence of DCB on field grown soybean plants. Particular attention was given to mobility of DCB and accumulation of DCB in soybean plant parts. [^{14}C]-labelled DCB was used to trace carbon through the various locations, including the final gaseous form as respiration.

Sludge may be defined as a semi-liquid waste waste having a suspended solid content of at least 2,500 ppm (i.e., 0.25 percent dry solids) which flows, can be pumped, and exhibit delayed settling characteristics in gravity settlers (Burd, 1968). Physically, sludge generally has a thin, early odor likened by some to that of crude oil. The chemical composition of sludge may vary considerably, depending on the number and types of industries in the community, the size and efficiency of the treatment plant, etc. Sludges are separated and pressed in a variety of ways to convert them into a

stable condition for ultimate disposal. They may be classified according to the stage of sewage treatment (e.g., primary, secondary, and tertiary) or the specific process by which they were produced (e.g., activated, chemically precipitated, digested, etc.). Municipal sludges commonly contain an inorganic mineral fraction and a combustible (i.e., organic matter) fraction. Water usually makes up 90 percent or more of the weight.

Sludge contains many chemical elements in both mineral and organic forms. Of the principal constituents, nitrogen (N), phosphorus (P), and organic carbons are the most important in promoting plant growth and good soil physical conditions. Potassium (K) is present in only small quantities. Sampling and testing of sludges to determine all the important constituents will be essential before considering their use on agricultural land. Sludges vary widely from community to community due to the source of wastewater and proportion of industrial wastes they contain. Data in Table 1 illustrate how a large number of elements may vary among treatment plants handling essentially domestic and industrial flows, mostly industrial flows. Values in Table 1 also illustrate the wide range in elemental concentration in the sludges within the same wastewater classification. This variability among plants which received similar types of raw wastewater is not uncommon. In addition, elemental concentration in the sludge from one particular treatment plant may vary considerably with time. Therefore, each sewage sludge treatment should be carefully sampled and analyzed before considering its use on land. Sludge contains appreciable amounts of N, P and small quantities of K which are available for plant growth. So sludges can

have considerable value. Research and experiment have shown that sludge can be beneficial both as a soil conditioner and as a low analysis fertilizer. Corn, soybean, lawn and forage grasses, legumes, and numerous ornamental annuls and perennials respond favorably. Some vegetables, conifres, and other species do less well with sludge fertilization because of lower nutrient requirements and greater sensitivity to metals and salts. Sludge also provide organic matter, which usually is beneficial as a soil conditioner. Organic matter helps to hold plant nutrients in the soil which are then capable of being released slowly during the growing season. Improved water-holding characteristics, structure, and tilth generally result from an increase in organic matter content, particularly for sandy soils low in organic matter. On fine-textured soils, the organic additions may result in improved infiltration capacities over time. while it can never be a major contributor to the overall fertilization requirements of crops grown in the field, it can provide important amounts of nitrogen and phosphorus in localized area. As a soil conditioner, sludges can promote desirable physical, chemical and biological conditions in the soil for plant growth.

While plant nutrients and organic matter are useful to plants and soils. Some of the metals and other constituents are potentially harmful if not managed correctly. Table 1 shows the varieties and wide range of elements found in sludge produced from treating domestic and industrial wastewater. In addition to these elements, other constituents may pose problems. Some concerns are; 1) Certain metals can produce toxicity on less tolerant plant species; 2) Additions of some potential toxic elements to soils may increase

Table 1. Elemental analysis of treatment plant sludge samples expressed as ranges on a dry weight basis(in mg/kg). **

Chemical element	Domestic wastewater	Industrial and domestic wastewater	Industrial wastewater
Phosphorus	2900 -19600	11000 -22800	12700 -38300
Calcium	4100 -120000	13200 -40000	32000 -128000
Magnesium	500 -5400	2700 -4300	3000 -7600
Potassium	400 -6000	700 -1700	1600 -4000
Sodium	200 -7000	500 -2300	800 -5400
Iron	2300 -12600	15320 -47600	64500 -225000
Aluminum	3800 -13400	3850 -12000	10800 -70000
Zinc	1000 -1800	800 -4600	3200 -14000
Copper	95 -7000	960 -2300	1640 -4700
Boron	50 -400	* *	* *

** : EPA analysis data - sludges collected from 4 plants for each type of wastewater received, 12 treatment plants in all

plant uptake of these elements and cause increased levels in the human food chain; 3) A few metals, cadmium, for example, can be translated to the vegetative or grain portion of the plant and thereby enter the food chain; 4) Organic contaminants like polychlorinated biphenyl (PCB) or pesticides can be present in sludges and might find a route into the food chain; 5) salts of many kinds occur in sludges and can be deleterious to seed germination or to growth of young plants.

Attention has been focused on utilizing sludge on crop land to determine the rates which will optimize crop production. This method uses sludges in a beneficial way and at rates will not result in (1) excessive loss of $\text{NO}_3\text{-N}$ to ground water; 2) high levels of P which can eventually leach to ground water; 3) building up toxic levels of metals in the soil; and 4) the bio-accumulation of elements in plants which may be harmful to man or animals. To prevent these concerns from becoming a problem, a number of factors must be considered in determining proper application rates.

The fate and biological transport of [^{14}C]DCB has been studied in an effort to predict the extent of their transport in the food web and their potential toxicity. Less is known of the effect of soil concentration of DCB on availability to plants, especially at background concentrations. plants vary in ability to accumulate specific elements although physical and chemical processes in the soil tend to be regulate the bioavailable fraction DCB in soil solution.

The difference in uptake potential have been attributed to physical variation in the development and intrusion of roots within

the soil and also to physiological differences such as not secretion or the affinity of the membrane transport processes for DCB. Plant age at sampling may affect total accumulation and changes in observed concentration ratios that may occur as a result of differences in rates of dry matter production at various stages of plant development. Table 2 shows the number of days required for a soybean plant to develop from one stage to the next. But less is known about root growth corresponding to the number of days after germination. In the great majority of species, seed germination begins with radicle (embryonic root) rather than epicotyl (shoot) protrusion through the seed coat (Berlyn, 1972). Root receive photosynthates and growth hormones from shoot and in return furnish water and essential minerals. Root and shoot growth are thus independent. During the first few days after wheat (*Triticum aestivum* L.) seeds germinate the radicle grows faster than the plumule; this relation causes the shoot-root (S:R) ratio by weight to be less than 1.0. Subsequently the plumule grows faster than the radicles and this increases the shoot-root ratio (Aung, 1974). Soybean development can be influenced by temperature , day length, variety, and other factors.

Consequently, there can be considerable variation in the number of days between stages. Temperature is the major factor influencing vegetative development. Low temperature retards, and high temperature enhances, seedling emergence and leaf development. The effect of temperature becomes less important after the fifth-node stage. A few node is produced on the main stem about every 3 days after the fifth stage.

Table 2 . Number of days required for a plant to develop from one stage to the next.*

Stage	Average number of days	Range in number of days *
Planting to VE	10	5-15
VE to VC	5	3-10
VC to V1	5	3-10
V1 to V2	5	3-10
V2 to V3	5	3-8
V3 to V4	5	3-8
V4 to V5	5	3-8
V5 to V6	3	2-5
Time interval between all vegetative stages after V5	5	2-5

* Data are from studies conducted in the United States. The range may differ from for cooler or more tropical climates.

The purpose of this study was to evaluate the uptake of soil-deposited DCB using a plant species and soil type indigenous to an area where DCB producing plants are located. Mobility of [^{14}C]DCB in soil depth was evaluated after plants were planted for a short term period.

MATERIAL AND METHODS

Pot experiment was conducted with soybean plant "Hardin" under controlled environmental conditions (20 °C day and 10 °C night). The pot which is polyvinyl chloride cylinders, having inside diameter of 7.6 and a wall thickness of 0.64 cm and containing 169 grams of sandy loam in this experiment were used to establish the layered soil containers was 2.5 cm. The containers were taped together with plastic water proof tape while compressing the end. One end was closed with cheese cloth to pass the irrigation water. The cores of this study were filled a Rubicon sandy loam obtained at Muskegon, Michigan. Soil was collected from the top 30 cm of soil profiles, air dried and passed through a 2 mm sieve. Particle size analysis, organic matter content, pH and CEC were determined by Michigan State University Soil Testing Laboratory; percent sand, silt and clay was 82.4, 4.2, 13.4; percent organic matter was 2.45; pH was 7.7; and CEC was 66 me/100g. Sewage sludge contaminated with DCB was collected at MWMS. The sludge had originally been pumped from aeration cells and at time of sampling was in the form of a thick paste. The sludge was spread out and allowed to air dry after it was passed through a 2 mm

sieve, and then stored at 4 °C. Dichlorobenzidine content of the sludge was 16 mg/ml.

3,3'-dichlorobenzidine, having a ring UL [^{14}C], was purchased from ICN Pharmaceuticals, Inc., having a radiochemical purity of > 99 % and a specific activity of approximately 35 nCi/mmol. The [^{14}C]DCB was dissolved in absolute ethanol and stored in the dark at 0 °C. This gave a stock [^{14}C] solution with a specific activity of 0.023 uCi [^{14}C] DCB/ul ethanol and a DCB concentration of 167/ml.

SOIL COLUMN

Polyvinyl chloride cylinders were used. The columns were then filled with 5.08 cm of soil from the bottom and tapped repeatedly. The soil columns were then immersed in a large cylinder filled with distilled water, allowed to wet from the bottom up, and drained. The procedure was repeated twice before adding the middle 2.54 cm of soil or sludge-amended soil which contained the [^{14}C]DCB, and top 2.54 cm of soil. One column was prepared for sludge rate of 11.2 Mg/ha. Sludge and soil were thoroughly mixed and moistened with and aqueous solutions of 15 ul [^{14}C]DCB and 19ml distilled water. These [^{14}C]DCB amended soil were incubated for 6 days in amber jars at room temperature to allow equilibration of the added [^{14}C]DCB, then added to the middle container for a soil depth of 2.54 cm. Total weight of soil in the column was 480 grams and total weight of soil or sludge - amended soil containing the [^{14}C]DCB in the middle layer 2.54 cm was 160 grams. There were four replications of each treatment.

Sterilized soybean seed "Hardin" was used in this experiment. Two soybean seeds were planted to each soil column directly at the depth of 1.0 cm in the growth chamber. All soybean plants were maintained in the growth chamber for 18 days. As irrigation treatment, 30 mls of distilled water was added to the soil column for 18 days. Leachate water was collected in 500 mls of beaker for 18 days. The soybean plants were harvested after 18 days, and sampling time corresponded to the second node stage. Sampled plants were separated to leaves, branches, stems and roots. Sampled plants were used for the following measurement; 1) Dry weight of each portion; 2) amount of radioactivity in each portion. The entire portion of leachate water was assayed for [^{14}C]DCB radioactivity.

After 18 days, the soil columns were disassembled into 2.54 cm section, air dried and weighed. Distribution of the [^{14}C]DCB radioactivity with depth was determined by combusting duplicate 1.0 gram samples from each 2.54 cm section and trapping the [$^{14}\text{CO}_2$] produced.

ANALYSES

All $^{14}\text{CO}_2$ -radioactivity was measured using a Beckman LS 8100 liquid scintillation counter. Total $^{14}\text{CO}_2$ for samples from soil columns and soybean plants were determined using a OX200 Biological Material Oxidizer (R. J. Harvey Instrument). The $^{14}\text{CO}_2$ was trapped and counted in Oxyflour- $^{14}\text{CO}_2$ LSC Cocktail.

RESULT AND DISCUSSIONS

Soil columns were used to directly assess leaching of [^{14}C]DCB in soil and sludge-amended soil. There are several features of the column experiments which merit attention. The columns used had a 2.54 cm and a 7.62 cm inner diameter. The soil columns were irrigated with 30 mls of distilled water per day which selected to provide enough water for soybean growth from seedling to the second node stage. This approach would, therefore, identify the maximum DCB losses by leaching. The second layer of the soil column was amended with 0 and 11.2 Mg/ha of sludge contaminated with DCB. Radiolabelled [^{14}C]DCB was uniformly distributed at soil of the second layer.

Distribution of [^{14}C]-radioactivity with depth after 18 days of irrigation is illustrated in Fig.1. The vast majority of [^{14}C]DCB remained in the second layer of the column in the sludge-amended and non sludge-amended soil. This attests to the strong immobilization of DCB which showed little mobility despite irrigation with 30 mls of distilled water per day. There was, however, a finite and measurable quantity of radiolabelled DCB in the first, third and fourth layer of the soil column. Radioactivity in the lower section of the column tended to be higher in soil that was not amended with sludge. Radioactivity in leachate water collected from the soil column was also measured (Table 3). The amount of [^{14}C] radioactivity in the leachate was < 0.001 %. The higher amount of radioactivity leached through the soil receiving no sludge amendment.

Uptake of [^{14}C]DCB by soybean roots and its translocation throughout the soybean plants were evaluated. The general concept is

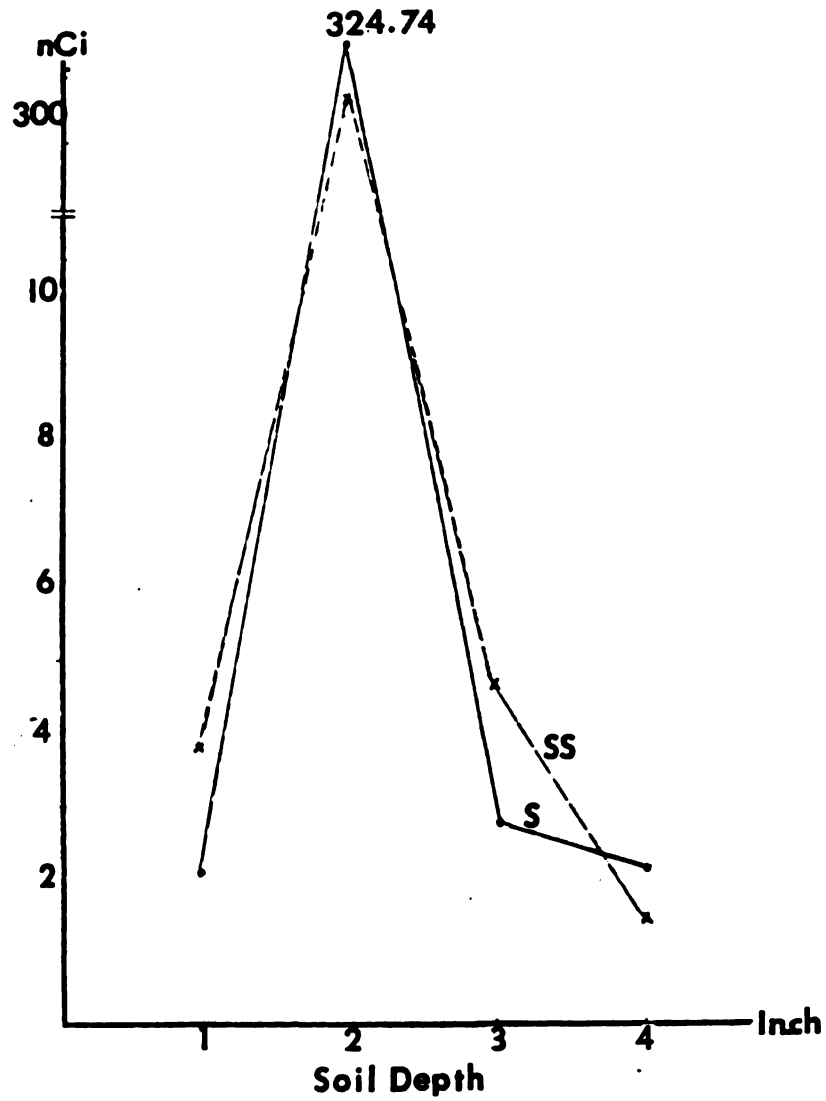


Figure 1. Distribution of radioactivity with depth in ^{14}C -DCB amended soil columns which were irrigated with 30 mls of distilled water per day for 18 days.

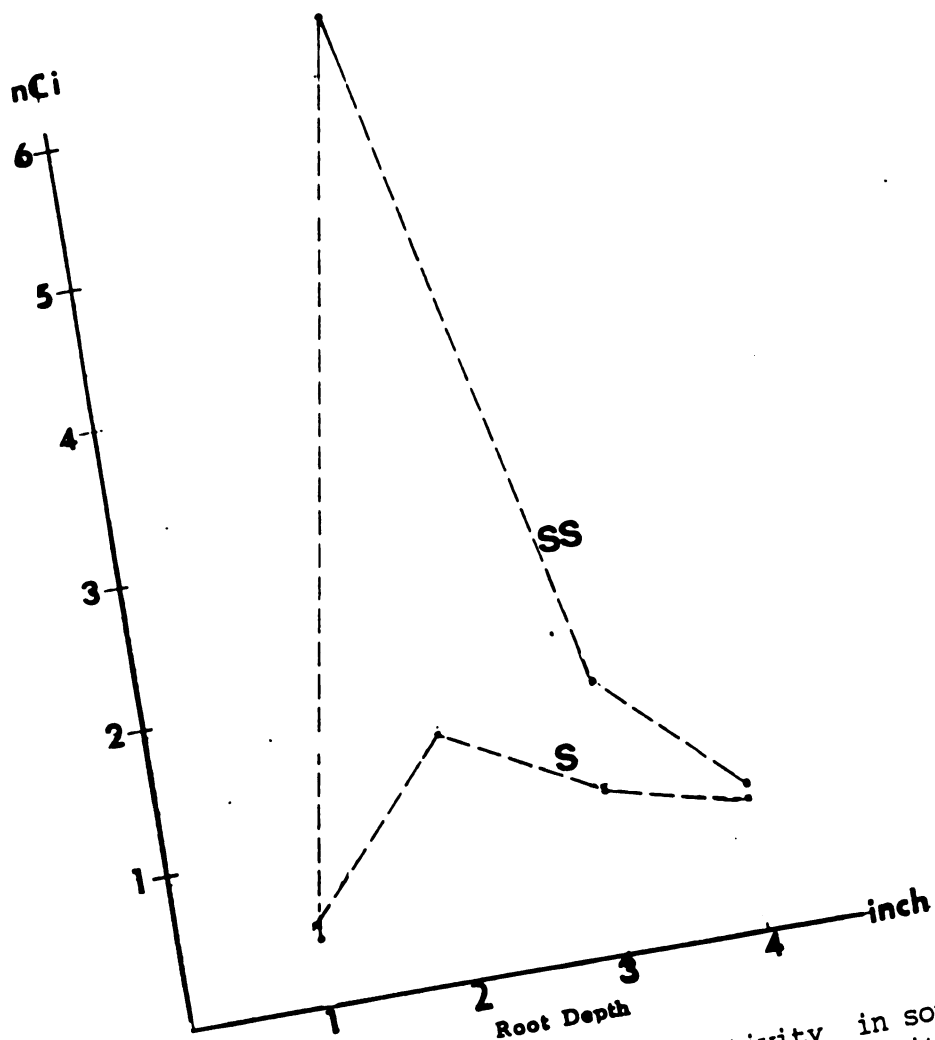


Figure 2. Distribution of radioactivity in soybean roots grown in ^{14}C -DCB contaminated soil with sludge rates of 0 and 11.2 Mg.ha^{-1} .

that certain ions are adsorbed strongly by soil and hence are not susceptible to rapid movement. The radioactivity transported by water can be absorbed directly by the surface roots.

Plants vary in ability to accumulate specific radioactives although physical and chemical processes in the soil solution. But less is known of the effect of soil concentrations of DCB on absorption to plants, especially at background concentration.

The relation between dry weight of roots and treatment is shown Table 4. Dry weights of roots decreased in [^{14}C]DCB amended soil as follows; 0.2937, 0.2934 and 0.4483.g/plant for sludge-amended soil, non sludge-amended soil and control, respectively. At each treatment, dry weight of roots decreased from the first layer to the fourth layer. The decrease being greater in DCB-amended soil than in control. The number of nodes were 3.8, 3.8 and 4.2 per plant in the order of sludge-amended soil, non sludge-amended soil and control. The length of roots ranged from 12 to 14.4 cm for all treatment. The S:R ratio for sludge-amended soil, non sludge-amended soil and control was 1.53, 3.59, and 1.72, respectively. I concluded that non sludge-amended soil contaminated with [^{14}C]DCB increased S:R ratio of soybean plants because of less immobilization of [^{14}C]DCB in non sludge-amended soil.

Absorption of [^{14}C]DCB in roots was 529.1 and 1028.26DPM in sludge-amended soil and non sludge-amended soil at rate of 0 and 11.2 Mg/ha, respectively. In each root layer, absorption of [^{14}C]DCB concentration remained in soil columns, but not correspond to the dry weight of root for each layer. In non sludge-amended soil, absorption of [^{14}C]DCB of the second layer was not stronger than in sludge-

Table 4. Dry weight of soybean plants grown in control and ^{14}C -DCB amended soils. ⁺

Treatment (Sludge Rate)	Root Depth			
	1st Layer	2nd Layer	3rd Layer	4th Layer
-Mg. ha ⁻¹	-	-	-	-
0	0.1046	0.0998	0.0543	0.0353
11.2	0.1275	0.0619	0.0340	0.0143
Control	0.1988	0.1216	0.0854	0.0514

+1.5ul of ^{14}C -DCB was added to each treatment except control. Total radioactivity added to each treatment was approximately 35000 nCr.

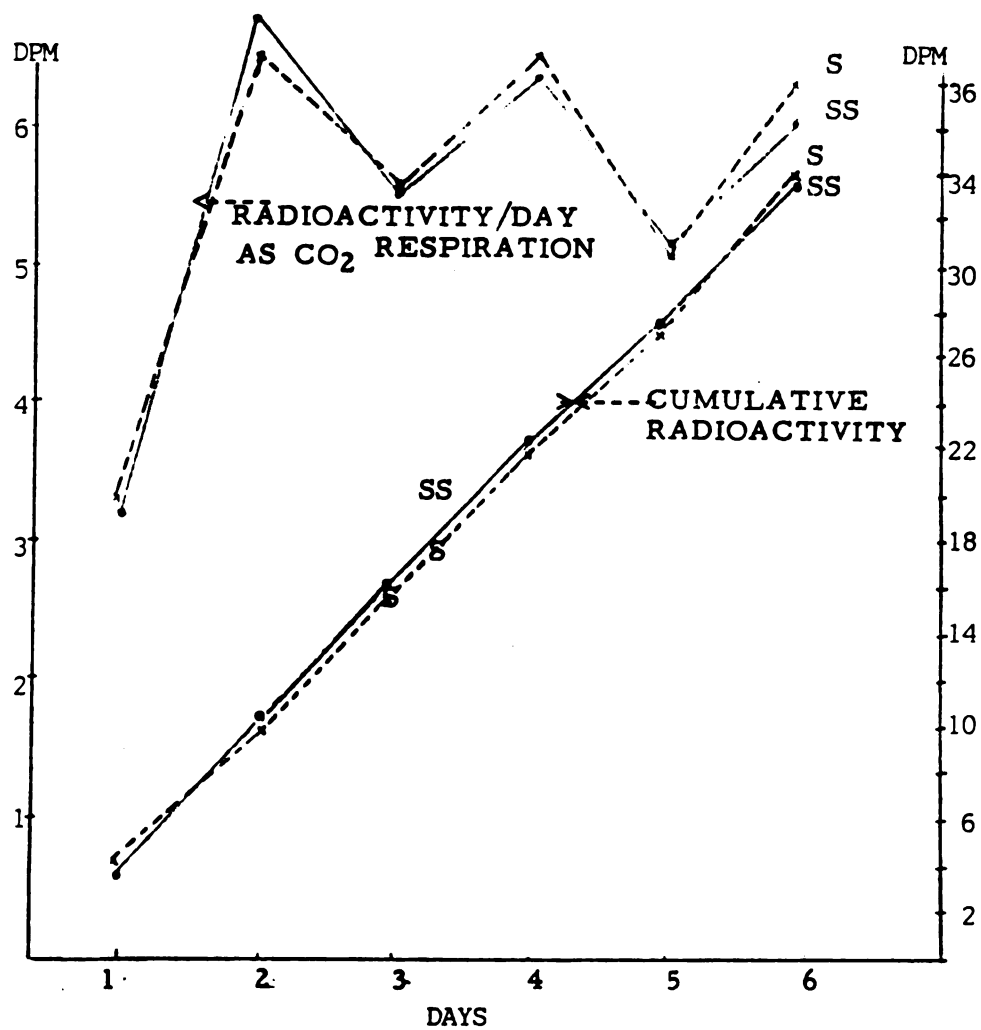


Figure 5. ¹⁴C-DCB recovered as CO₂ respiration for 6 days after 11 days.

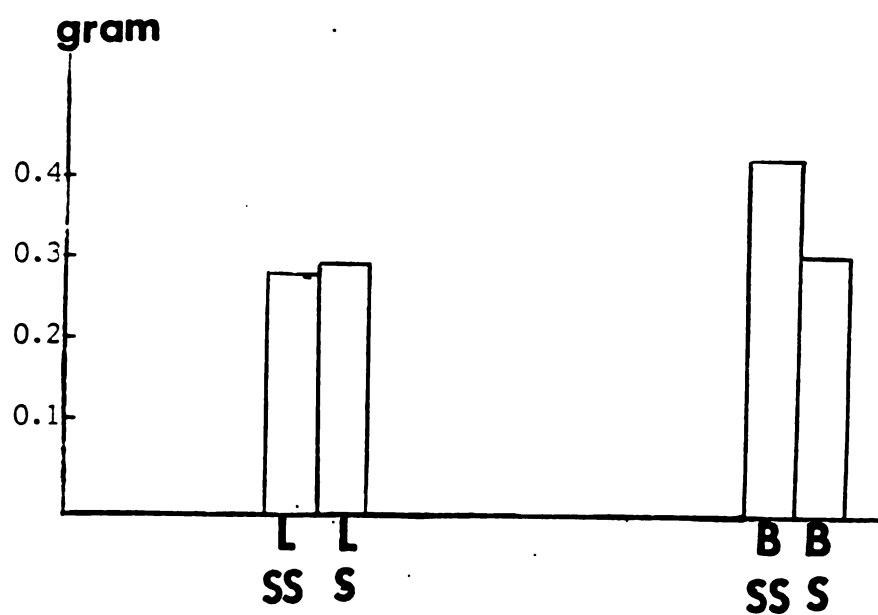


Figure 4. Comparison of dry weight in parts of soybean plants grown in ^{14}C -DCB contaminated soil with sludge rates of 0 and 11.2 Mg.ha^{-1} .

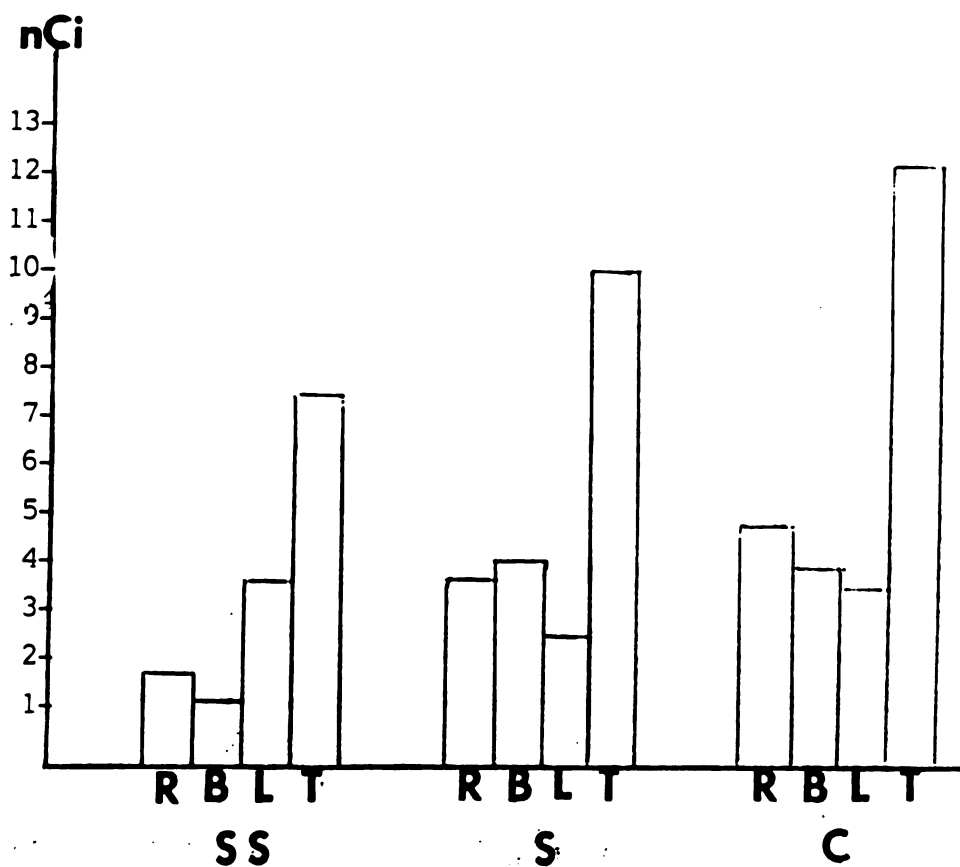


Figure 3. Uptake of ^{14}C -DCB by soybean plants grown in ^{14}C -DCB contaminated soil with sludge rates of 0 and 11.2 Mg.ha^{-1} .

amended soil. [^{14}C]DCB concentration of roots for 18 days growth of soybean plants was less in sludge-amended soil than in non sludge-amended soil indicating that there was some stronger adsorption of DCB to the sludge. Translocation of [^{14}C]DCB in plant parts, leaves and branches, was 231.25 and 310.95 DPM for sludge-amended soil and non sludge-amended soil. The higher uptake of [^{14}C]DCB occurred in non sludge-amended soil.

Total dry weight of soybean plants at each treatment were as follows; 0.7407, 1.091 and 1.2323 g/plant for sludge-amended soil, non sludge-amended soil and control, respectively. As we see in the above results, yields were significantly and negatively affected by the [^{14}C]DCB in the soil because dry weight of soybean plants grown with [^{14}C]DCB and sludge at rate of 11.2 Mg/ha were smaller than soybean plants with no sludge and no [^{14}C]DCB. And the concentration of [^{14}C]DCB of plant parts for the fourth node stage were decreased at the higher sludge application rate.

[^{14}C]radioactivity released as [$^{14}\text{CO}_2$] respiration for 6 days was observed. The range of [^{14}C]DCB released as respiration was 3.1 to 6.9DPM. this indicate that [^{14}C] radioactivity transported to the plant tissues can also be released to the atmosphere even though the amount of [^{14}C] radioactivity transported to the plant parts are very small. In this experiment, the portion of [^{14}C]radioactivity released as [$^{14}\text{CO}_2$] was <0.0001 %.

Total [^{14}C] radioactivity recovered from combustion was 93.49 and 82.55 % for in sludge-amended soil and non sludge-amended soil. If sludge contaminated with DCB is utilized for land application, it would be advisable to restrain from irrigation for some period time

to allow the immobilization of DCB to occur. Soil or sludge contaminated with DCB affects growth of soybean plants. If there is not a sludge-borne DCB, that will be less harmful. In most case, sludge application to land will be used as fertilizer depending on the contents of nutrients existed in sludge. DCB in the soil can move upward or leach downward depending on irrigation.

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