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Journal of Hazardous Materials 50 (1996) 129–141

JOURNAL OF HAZARDOUS MATERIALS

Anoxic treatment of trifluralin-contaminated soil

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Received 28 November 1995; accepted 28 February 1996

Abstract

Amending anoxic soils with stoichiometric amounts of sodium acetate led to the complete transformation of trifluralin within the 45 day treatment period. Under these conditions, a maximum trifluralin transformation rate of 4.9 mg kg^{-1} of soil per day was estimated, which corresponded to a chemical half life of 11.9 days. Regression analyses indicated that the zero order rate model provided the best fit to the experimental data, suggesting that the trifluralin transformation rate is independent of concentration during acetate addition. Using radiolabeled trifluralin, it was determined that the principal contaminant transformation mechanisms were degradation and bound residue formation (i.e., irreversible adsorption). Volatilization and mineralization of trifluralin were found to be negligible over the 45 day treatment period. Using poisoned controls, it was determined that trifluralin transformation under acetate-amended conditions was biologically mediated.

Amending trifluralin contaminated soils with stoichiometric amounts of iron sulfide resulted in complete trifluralin transformation within 24 hours of treatment. A maximum trifluralin transformation rate of 380 mg kg^{-1} of soil per day was estimated for this system, which corresponded to a chemical half life of 4.4 h. The rates of trifluralin transformation followed the first-order kinetic model during iron sulfide addition. Using radiolabeled trifluralin, it was found that chemical degradation was the principal removal mechanism. Neither volatilization nor mineralization was found to be a significant contaminant removal mechanism during iron sulfide treatment. Poisoned controls indicated that trifluralin transformation was mediated primarily by an abiotic chemical reaction mechanism. Additional study is required to clarify the rate limiting steps so that full scale soil treatment systems may be properly designed.

Keywords: Anoxic; Soil treatment; Trifluralin

PII S0304-3894(96)01788-8

1. Introduction

The use of increasing quantities of the pesticide trifluralin for agricultural production has adversely impacted human health and the environment. Trifluralin (α,α,α-trifluoro-2,6-dinitro-*N*, *N*-di-n-propyl-*p*-toluidine) is widely used for selective weed control in cotton, soybean and sunflower production. The physical and chemical properties of trifluralin are given in Table 1. Public concern over trifluralin stems from the fact that it is a known human carcinogen which has been detected in several potable water supplies [1]. Improper application and storage of trifluralin have resulted in incidental spillage, leading to extensive soil contamination. Left untreated, these soils threaten both surface and subsurface drinking water supplies and may, in some cases, be classified as uncontrollable hazardous waste sites according to United States federal regulation [2].

Present disposal and treatment methods for trifluralin-contaminated soil consist of excavation with subsequent incineration or landfilling. Although incineration remains a technically feasible option for contaminated soil remediation, costs associated with soil excavation, transport, effluent gas monitoring and fuel have made this economically unattractive and unacceptable to the public. Landfilling is the least desirable option, as the contaminated soil retains its toxicity and has the potential of contaminating drinking water supplies. Factors contributing to the persistence of trifluralin in soil include the amount and type of soil organic matter, the activity and diversity of the microbial population, soil moisture content, pH, temperature and pesticide concentration [3].

It is now well known that the oxidation–reduction potential of soils has a significant impact on the persistence of certain pesticides [4–6]. Under aerobic conditions, the half life of trifluralin was reported to be over 900 days in an agricultural soil [7]. On the other hand, a chemical half life of less than 4 days has been reported in biologically active soils under anaerobic conditions [5]. The presence of the two nitro (i.e., NO_2) groups in trifluralin suggests that denitrifying conditions may encourage biotransformation. The availability of an appropriate electron donor to mediate the reduction of the nitro groups has been identified as a critical parameter for successful soil treatment [5].

The present study was designed to evaluate the effectiveness of two electron donors to mediate the anoxic transformation of trifluralin in nonsterile soil. The laboratory

Table 1
Physical and chemical properties of trifluralin [a]

Physical	Color form	Orange crystalline solid
Chemical	Molecular formula	$C_{13}H_{16}F_3N_3O_4$
	Molecular weight	335.3 g mol^{-1}
	Melting point	49.0°C
	Boiling point	140°C
	Solubility	24 mg l^{-1}
	Henry's law constant	1.9×10^{-3}
	Vapor pressure	1.99×10^{-4}
	K_{oc}	1.37×10^{4}
	K_{ow}	2.2×10^{5}

[a] Data from [15].

reactor systems were designed to simulate anoxic soil conditions. A chemical mass balance was conducted using radiolabeled trifluralin to estimate chemical transformation rates and mechanisms.

Sodium acetate was chosen as one of the electron donors because (1) acetate is a soluble organic substrate which can be easily utilized by a large range of anaerobic soil microorganisms, and (2) it is inexpensive. Iron sulfide, an insoluble iron species, was chosen as the other electron donor because (1) it occurs naturally in reduced soils and sediments, and (2) it has been reported that this chemical species may be involved in the heterogeneous catalysis of redox transformations of organic compounds [6].

The theoretical amount of each electron donor required to reduce trifluralin was estimated by calculating the number of moles of electrons necessary to transform one mole of trifluralin to its amino analogue (α,α,α-trifluoro-5-nitro-*N*,*N*-dipropyltoluene-3,4-diamine). The first step in this calculation was to estimate the average oxidation state of nitrogen in the parent compound relative to its reduced analogue.

The oxidation state of nitrogen in trifluralin (i.e., $R-NO_2$) is +5. The oxidation state of nitrogen in the reduced analogue (i.e., $R-NH_2$) state is −1. Thus, an effective transfer of six moles of electrons is necessary per mole of trifluralin reduced. To estimate the quantity of acetate required to generate these electrons, the following stoichiometric equation which describes the oxidation of acetate is used

$$\frac{1}{8}CH_3COO^- + \frac{3}{8}H_2O \rightarrow \frac{1}{8}CO_2 + \frac{1}{8}HCO_3^- + H^+ + e^- \quad (1)$$

According to Eq. (1), 1/8 mole of acetate must be oxidized to generate one mole of electrons. As six moles of electrons are required to transform one mole of trifluralin to its amino analogue, the minimum amount of acetate required to transform one mole of trifluralin is 6/8 or 0.75 mole. In other words, the reduction of one mole of trifluralin (335.3 g) theoretically requires 61.5 g (6/8 mole) of acetate.

In the case of iron sulfide, the oxidation of one mole of FeS generates one mole of electrons; see Eq. (2)

$$Fe^{2+} \rightarrow Fe^{3+} + e^- \quad (2)$$

Therefore, to reduce one mole of trifluralin to its amino analogue requires six moles (or 527.46 g) of iron sulfide.

2. Materials and methods

2.1. Standard soil

The soil used for this study was a benchmark sandy loam soil (Kidman series). The characteristics of the Kidman soil are provided in Table 2.

2.2. Microcosm construction and operation

To evaluate the effect of electron donor addition on the transformation of trifluralin in soil, four sets of nine microcosms were constructed for the acetate addition study and

Table 2
Characteristics of Kidman sandy loam soil [a]

Characteristic	Value
Physical properties	
Bulk density	1.5 g cm^{-3}
Moisture at 1/3 bar	12.4%
Chemical properties	
pH	7.9
CEC	10.1 meq per 100 g
Organic carbon	0.5%
Iron	9.0 ppm
Soil microbial plate counts	
Bacteria	6.7×10^6 g^{-1}
Fungi	1.9×10^4 g^{-1}
Soil classification	Typic haplustoll
Sand	52%
Silt	32%
Clay	16%
Texture	Sandy loam

[a] Analyzed by the Soil, Plant and Water Analysis Laboratory at Utah State University, 1991.

three sets of nine microcosms were prepared for the iron sulfide evaluation. (Note: the reason for using only three sets for the iron sulfide study was that the non-iron sulfide poisoned treatment was identical to the non-acetate-amended poisoned treatment.) At the initiation of the acetate-amendment study, 10 g each of uncontaminated Kidman soil (oven dry basis) was placed into each of the 36 microcosms (i.e., 125 ml Erlenmeyer flasks). Each microcosm was spiked with 250 μl of radiolabeled trifluralin (Sigma Chemical Co., St. Louis, MO) and nonradiolabeled trifluralin (Chem Service, Inc., West Chester, PA), resulting in a final trifluralin soil concentration of 100 ppm of which less than 0.1% was radioactive.

The first set of microcosms in the acetate study received 10 ml of double deionized water (DDW) (no acetate, nonpoisoned treatment) to bring the moisture content up to 80% of field capacity. The second set of microcosms in the acetate study received 10 ml of DDW containing a 2% solution of formaldehyde (no acetate, poisoned treatment). The third set received 10 ml of DDW containing 0.1 g of sodium acetate (acetate, nonpoisoned treatment) and the fourth set received 10 ml of DDW containing 0.1 g of sodium acetate and poisoned with a 2% solution of formaldehyde (acetate, poisoned treatment). The efficiency of microbial poisoning was confirmed by conducting a bacterial plate count test [8].

All microcosms were incubated in an anaerobic glove box (100% nitrogen atmosphere) which was maintained at a constant temperature of 20°C. The microcosms were sealed with rubber stoppers equipped with inlet and outlet ports to facilitate evaluation of the system headspace (Fig. 1). The microcosms were purged with nitrogen gas every

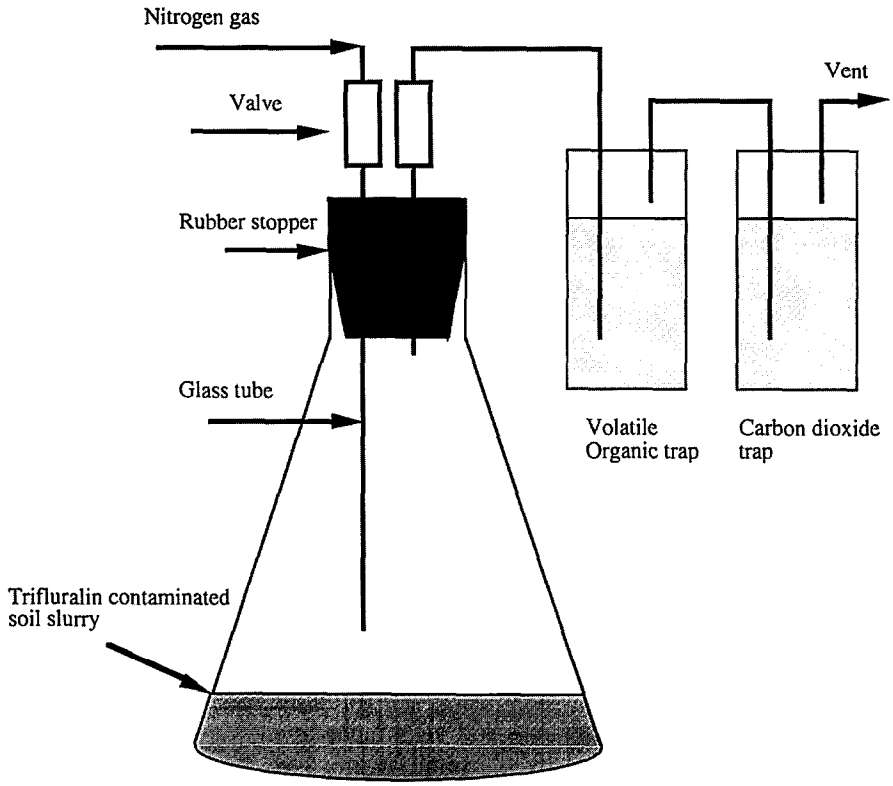

Fig. 1. Schematic representation of soil microcosm.

three days at a rate of 200 ml min^{-1} for 5 min. Evacuated gas from the microcosm headspace was directed to a series of gas impingers. The first set of impingers contained ethylene glycol, which was used to trap volatile organics from the effluent gas. The second set of impingers, which was used to capture radiolabeled carbon dioxide from the effluent gas, contained Ready Gel™ (50%), monoethanolamine (10%) and methanol (40%). The microcosms were extracted in triplicate on specific sampling days within the 45 day study period.

For iron sulfide treatment, each of the 27 microcosms received 50% by weight (i.e., 5 g) of iron(II) sulfide (Aldrich Chemical Co., Milwaukee, WI) per 10 g of soil. Before addition to soil, the iron sulfide was pulverized and passed through a 60 mesh sieve. Each microcosm was spiked with 250 μl of radiolabeled trifluralin and nonradiolabeled trifluralin. The final trifluralin soil concentration was 100 ppm, of which less than 0.1% was radioactive material.

The first set of microcosms in the iron sulfide study was designed to evaluate the extent of abiotic transformation of trifluralin as a result of iron addition. The poisoned microcosms were prepared by adding 10 ml of DDW containing 1000 mg l^{-1} of mercuric chloride (H_gCl_2) to the first set of microcosms. The second set of microcosms

received only iron sulfide and the third set received none. To determine the efficacy of microbial poisoning, a bacterial plate count was conducted [8].

2.3. Soil extraction

Soil from all microcosms was extracted with methanol using sonication applied for 2 min (EPA SW846, Method 3550). After extraction, the slurry was transferred into a 200 ml bottle and centrifuged at 3000 rpm for 10 min in a Beckman model J2-21 centrifuge. The supernatant was filtered through a 0.2 μm filter in preparation for analysis of residual trifluralin by high performance liquid chromatography (HPLC).

2.4. Quantitation

High performance liquid chromatography (Shimadzu model HPLC 10 AS) was used for the analysis of trifluralin. The mobile phase was acetonitrile:water (70:30, v/v). The system was equipped with a C_{18} column and the detector was operated at a wavelength of 200 nm. The HPLC was operated isocratically at a pressure of 300 kg m^{-2}. The mobile phase was pumped at a flow rate of 1 ml min^{-1}.

The samples were quantified by the method of external standards. The standard curve was plotted by taking the average of three trifluralin (purity 99%) responses obtained from a chemical standard (Chem Service, Inc., West Chester, PA). A stock standard solution in methanol containing 10000 mg l^{-1} of trifluralin was prepared by weighing 250 mg of trifluralin and bringing it to a 25 ml volume by methanol addition. A 50 mg l^{-1} stock solution was prepared by pipetting 0.5 ml from the 10000 mg l^{-1} stock solution in a 100 ml volumetric flask. Serial dilutions were then prepared from the 50 mg l^{-1} stock solution by taking equal portions (10 ml) of the standard and diluting them with an equal portion of methanol to obtain standards of 25, 12.5, 6.25, and 3.125 mg l^{-1} of trifluralin.

2.5. Bound residue

Soil bound residue was defined as the percentage of radiolabel which was not extractable with methanol. After methanol extraction, a subsample consisting of 2 g of soil was burnt in a biological oxidizer (R.J. Harvey, Inc., Model OX 600). The radiolabeled CO_2 evolved from combustion was trapped in a separate impinger containing Ready Gel and counted by liquid scintillation (Beckman Corp. Model LS 6000SE).

3. Results

In both the acetate and the iron sulfide amendment studies, microbial poisons appeared effective in reducing microbial activity. No colony-forming units (CFU) were detected in any of the poisoned controls. Using triplicate samples, the extraction efficiency of trifluralin from Kidman soil was found to be 93.8 ± 3.8%.

Fig. 2 illustrates the variation in trifluralin soil concentration over time for both the

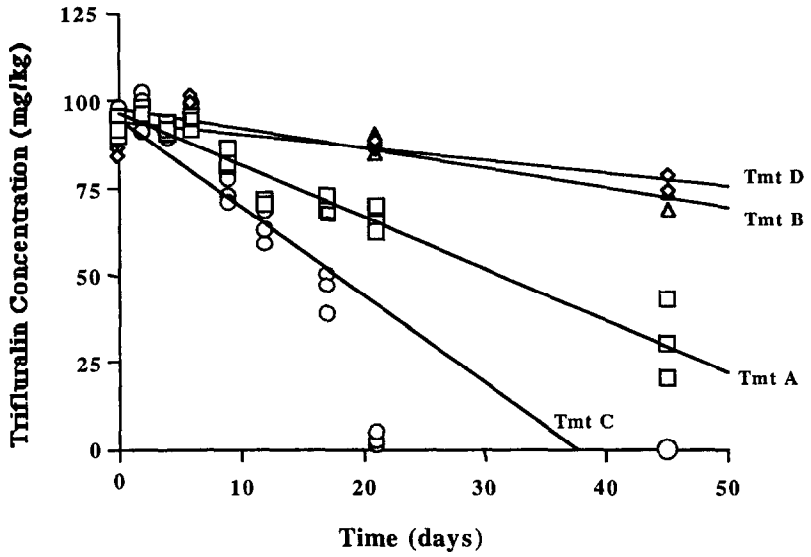

Fig. 2. Variation of trifluralin concentration with time. Tmt A, no acetate, nonpoisoned treatment; Tmt B, no acetate, poisoned treatment; Tmt C, acetate, nonpoisioned treatment; Tmt D, acetate, poisoned treatment.

poisoned and the nonpoisoned acetate treatments. Complete transformation of trifluralin was observed within the 45 days of treatment with the non-poisoned acetate addition. In contrast, in non-acetate-amended systems, approximately 65% of the initial trifluralin loading was removed over the same time period. Approximately 20% of the initial trifluralin loading was removed in the poison controls, suggesting that biological transformation was the principal removal mechanism under anoxic conditions.

Table 3
Zero order and first order reaction rate constants for the removal of trifluralin during acetate addition

Treatment	Order of reaction	Mean rate constant [a]	Lower 95% value of rate constant [a]	Upper 95% value of rate constant [a]	R^2
No acetate, nonpoisoned treatment	Zero	−1.48 [b]	−1.31	−1.65	0.93
	First	−0.026	−0.022	−0.030	0.89
No acetate, poisoned treatment	Zero	−0.36	−0.19	−0.54	0.58
	First	−0.0042	−0.0022	−0.0063	0.61
Sodium acetate, nonpoisoned treatment	Zero	−4.2	−3.6	−4.9	0.89
	First	−0.14	−0.09	−0.18	0.62
Sodium acetate, poisoned treatment	Zero	−0.56	−0.46	−0.66	0.92
	First	−0.0070	−0.0058	−0.0081	0.92

[a] Unit for zero order reaction rate constant is mg kg^{-1} per day; unit for first order reaction rate constant is day^{-1}.
[b] Values are rounded to the nearest significant digit.

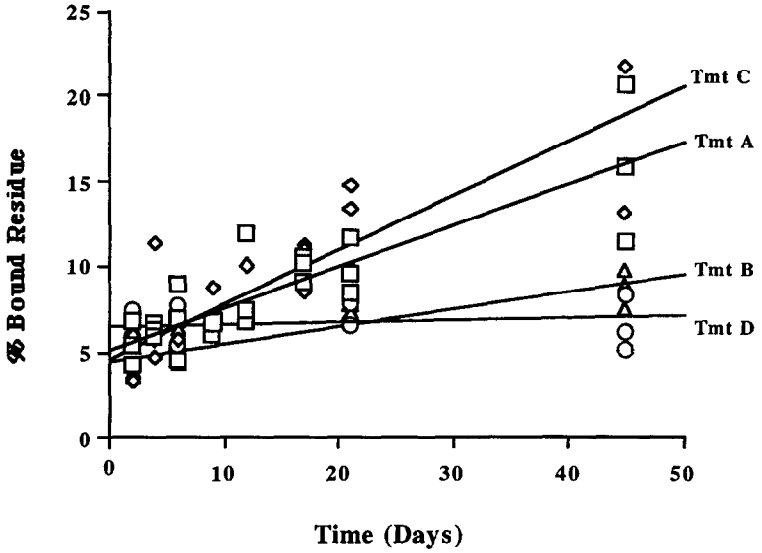

Fig. 3. Variation of bound residue formation during acetate treatment. Tmt A, no acetate, nonpoisoned treatment; Tmt B, no acetate, poisoned treatment; Tmt C, acetate, nonpoisoned treatment; Tmt D, acetate, poisoned treatment).

Regression analyses were used to determine the best fit kinetic model which described the trifluralin removal data. Table 3 lists the rate constants for zero order and first order reactions together with the 95% confidence limits for the reaction rate constants. The coefficient of determination (R^2) obtained from regression analysis indicated that a zero order reaction provided the better fit for all treatments. The implication of this analysis is that the concentration of trifluralin was not limiting its removal. An upper 95% confidence limit zero order removal rate of 4.9 mg kg^{-1} per day was estimated for the non-poisoned acetate-amended system. This removal rate corresponds to a mean trifluralin half life of 11.9 days.

The effect of acetate addition on the rate and extent of bound residue formation is depicted in Fig. 3. The nonpoisoned acetate-amended system showed the most significant increase in bound residue formation relative to the other treatments. The extent of bound residue formation increased from approximately 5% to 18% over the 45 day study period. Moreover, the insignificant change in bound residue formation in the poisoned systems suggested that bound residue formation was microbially mediated.

The influence of iron sulfide addition on trifluralin transformation in the poisoned and nonpoisoned treatments is shown in Fig. 4. Complete trifluralin removal was observed within 24 h with iron sulfide addition. The zero order and first order reaction rate constants with their 95% confidence limits are presented in Table 4. The coefficient of determination (R^2) indicated that the first order model gives the better fit to the trifluralin removal data.

These data suggested that the rate of trifluralin transformation during iron sulfide treatment was a function of the trifluralin concentration. An upper 95% confidence limit

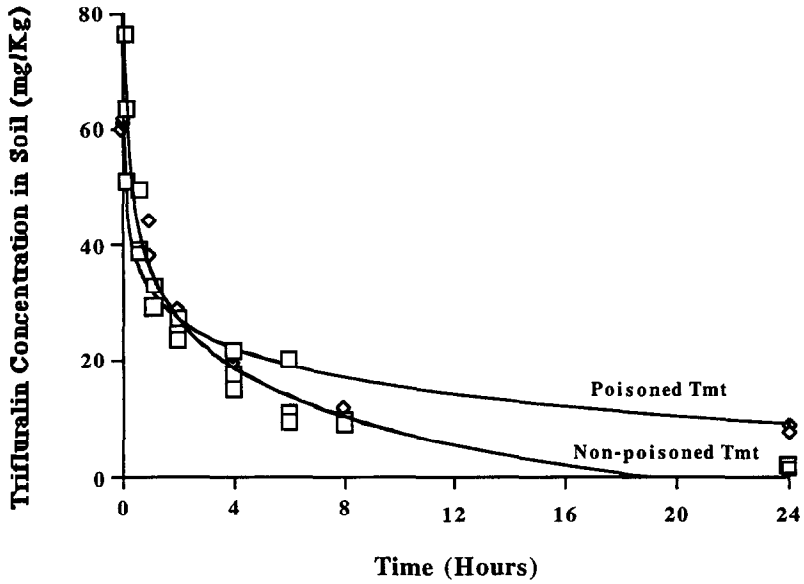

Fig. 4. Variation of trifluralin concentration with time during iron sulfide addition.

for the nonpoisoned first-order reaction rate constant of 3.8 h^{-1} was estimated, which corresponded to a chemical half life of 4.4 h. The maximum trifluralin transformation rate under these conditions was 380 mg kg^{-1} soil per day. In the poisoned systems, an upper 95% confidence limit reaction rate constant of 2.3 h^{-1} was estimated which corresponded to a chemical half life of 7.2 h. The similarity in chemical half lives between the nonpoisoned and poisoned systems suggested that trifluralin transformation under iron sulfide additions was predominantly chemical in nature.

A comparison of the residual trifluralin and radioactivity levels indicated that the amount of radioactivity was significantly greater than the trifluralin recovered, suggesting that trifluralin was transformed to nonvolatile chemical intermediates. No attempt was made to identify or quantify the chemical intermediates. Fig. 5 shows a plot of ^{14}C recovery from the methanol extracts in the iron sulfide study. Approximately 79% of the

Table 4
Zero order and first order reaction rate constants for the removal of trifluralin during iron sulfide addition

Treatment	Order of reaction	Mean rate constant [a]	Lower 95% value of rate constant [a]	Upper 95% value of rate constant [a]	R^2
Nonpoisoned iron sulfide	Zero	−47	−23	−71	0.45
	First	−3.2	−2.7	−3.8	0.88
Poisoned iron sulfide	Zero	−37	−18	−57	0.51
	First	−1.7	−1.2	−2.3	0.73

[a] Unit for zero order reaction rate constant is mg kg^{-1} h^{-1}; unit for first order reaction rate constant is h^{-1}.
[b] Values are rounded to the nearest significant digit.

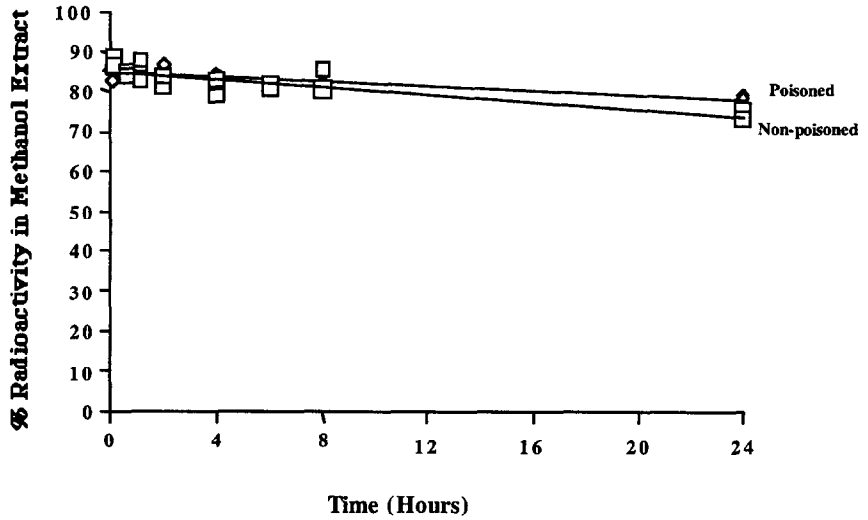

Fig. 5. Percent recovery of radioactivity in methanol extract (iron sulfide study).

initial radioactivity was recovered from the methanol extracts in the nonpoisoned treatment, whereas 85% of the initial radioactivity was recovered in the poisoned treatments.

The behavior of the soil bound residue in the nonpoisoned and the poisoned treatments is shown in Fig. 6. After 24 h, approximately 22% of the initial radioactivity

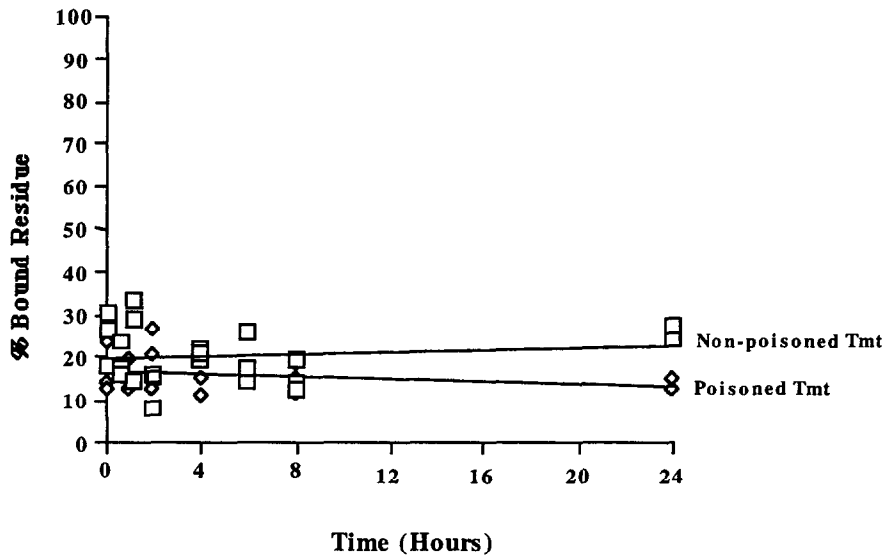

Fig. 6. Bound residue formation during iron sulfide addition.

was recovered in the form of soil bound residue in the nonpoisoned treatment and 15% was observed in the poisoned iron sulfide treatment. These observations suggested that microbial activity was responsible for bound residue formation.

4. Discussion

The present study demonstrated that trifluralin volatilization and mineralization were insignificant during anoxic soil treatment. The present data agree with earlier results reported by Willis et al. [5]. The major contaminant removal mechanisms were found to be degradation and bound residue formation. During acetate treatment, trifluralin removal was biologically mediated, with complete contaminant removal observed over the 45 day treatment period. The results of kinetic modeling efforts indicated that the trifluralin concentration did not limit its removal rate during acetate treatment.

Bound residue formation was found to be significant during the acetate treatment, with approximately 20% of the radiolabeled carbon associated with the soil matrix. Poisoned control experiments indicated that the bound residue formation was biologically mediated. Although bound residue formation has been found to be an effective contaminant transformation mechanism for soils under aerobic conditions [9,10], this is the first report of bound residue formation known to occur in an anoxic soil environment. More studies are required to evaluate the extent and potential reversibility of anoxic bound residue formation.

During iron sulfide addition, chemical degradation was the predominant removal mechanism. Kinetic modeling indicated that the trifluralin concentration controlled its removal rate. The upper 95% confidence limit on the rate constant was estimated to be 3.8 h^{-1} during iron sulfide treatment. This rate corresponded to a maximum contaminant removal rate of 26.3 mg kg^{-1} h^{-1} of trifluralin from soil.

From the iron sulfide treatment data, it is unclear whether the iron or the sulfide atom acts as the reductant during anoxic soil treatment. Previous work has indicated that hydrogen sulfide in solution was effective in reducing carbon tetrachloride [11], and insoluble iron(II) was reported to be an effective reductant of various chlorinated organic compounds [12]. Additional work is required to clarify the actual reducing species so that the trifluralin anoxic treatment system can be optimized.

More recent applications of iron(II) treatment of organic compounds have focused on the use of elemental iron(II) as the reducing species for halogenated organic compounds under aerobic conditions [13,14]. Although initial results have appeared promising, no complete chemical mass balance has ever been reported. It is unclear whether contaminant dehalogenation and/or chemical adsorption to iron oxide surfaces is the controlling removal mechanism.

The present study represents the first reported use (to the author's knowledge) of the electron donors acetate and iron sulfide to treat trifluralin-contaminated soils. The present results suggest that creation of anoxic soil conditions followed by electron donor amendment may be a practical way of accelerating trifluralin removal. Although enhanced transformation of trifluralin was observed under anoxic conditions, it is unknown whether or not the chemical intermediates produced are toxic. Further research

into the identity and toxicity of the chemical intermediates will be necessary to determine whether soil leachate collection and treatment systems are required.

5. Conclusions

Transformation of trifluralin under anoxic conditions was enhanced by the addition of a soluble organic electron donor, sodium acetate, and an insoluble inorganic electron donor, iron sulfide. Complete removal of trifluralin was observed over the 45 day treatment period. Within the same time period, 65% of trifluralin removal was achieved in the soil with no acetate amendment. In all treatments, trifluralin was found to be transformed to chemical intermediates that were nonvolatile. Mineralization of trifluralin was found to be insignificant during the 45 day study period. Soil bound residue was found to account for nearly 20% of the removed trifluralin in the nonpoisoned treatments.

Within 24 h, complete transformation was observed in contaminated soil treated with iron sulfide. The reaction appeared to be chemically and not biologically mediated, as similar transformation rates were observed in both nonpoisoned and poisoned microcosms. The mechanism of trifluralin removal by iron sulfide is unclear. Both the iron and the sulfur atom are sufficiently reduced to act as a reducing agent under anoxic soil conditions. More studies are necessary to clarify the chemical reaction mechanism.

Acknowledgements

The authors thank the Tennessee Valley Authority's National Fertilizer & Environmental Research Center for sponsoring this research program under contract number TV-85761V. The authors acknowledge the Electric Power Research Institute (EPRI) and Dr. Ishwar P. Muraka (Program Manager) for their support of this research program under Contract No. RP2879-9. The authors also thank Ms. Ivonne Cardona Harris for the preparation of this manuscript.

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