Laboratory Analysis of 2,4-D and Dicamba Residues in Soil

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Soil residues of 2,4-D (2,4-dichlorophenoxyacetic acid) and dicamba (2-methoxy-3,6-dichlorobenzoic acid) were extracted using a solid-phase extraction technique and quantified using high-performance liquid chromatography (HPLC) during 80-day field and laboratory experiments. Five soil materials collected within a heterogeneous landscape were used during the laboratory microcosm study; four of these were represented in the accompanying field study. Five samples per material were created during the course of each study to evaluate extraction efficiency and the HPLC limit of detection. Average percent recovery of 2,4-D during the laboratory study ranged from 50 to 77% and during the field study ranged from 25 to 67%. Average percent recovery of dicamba during the laboratory study ranged from 79 to 114% and during the field study ranged from 65 to 137%. There was a negative relationship between organic matter content and extraction efficiency.

INTRODUCTION

Traditionally, the analysis of 2,4-D and dicamba residues has been performed using gas chromatography (GC). The GC technique has adequate selectivity and sensitivity; however, 2,4-D must be derivatized prior to analysis. Derivatization has certain disadvantages, for example, the time required for derivative formation and the chance of error, which is increased due to added sample manipulation. Also, there is the risk of an irreproducible yield in the derivatization reaction as well as the formation of interfering by-products (Sandmann et al., 1988). For phenoxy herbicide analysis, high-performance liquid chromatography (HPLC) may be more suitable than GC analysis since it allows for the separation of a large number of compounds without derivatization (Roseboom and Greve, 1983).

Established methods of dicamba analysis involve its extraction from acidic aqueous media with diethyl ether followed by derivatization and GC analysis. However, these procedures are time-consuming and require expensive pesticide-grade solvents (Arjmand et al., 1988). Analysis by HPLC also may be more suitable than GC analysis because of its inherent simplicity, speed, and sensitivity (Connick and Simoneaux, 1982).

As part of a larger study of herbicide dissipation in a heterogeneous landscape which included a laboratory microcosm study and a field plot experiment, we developed a modified method for herbicide extraction from soil. The original method (Arjmand et al., 1988) was developed for the extraction of dicamba from water samples. Our objective was to evaluate the efficiency and sensitivity of this method for herbicide analysis in a wide range of soil conditions, in both field and laboratory experiments.

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METHODS AND MATERIALS

Soils, Herbicides, and Herbicide Concentrations. Five different soil materials representing five different components of a heterogeneous landscape were used during the herbicide extraction and quantification procedures. Included were soils collected from a freshwater wetland (WTL), an undisturbed hardwood forest (HDW), a home lawn (SOD), and a cornfield (CORN) and aquifer material (AQ). The soils were collected at the University of Rhode Island Peckham Farm, in Kingston, RI. The soils used are classified as Enfield silt loam (CORN), Merrimac sandy loam (HDW and SOD), and Walpole sandy loam (WTL) Tables 1 and 2.

The soil materials for the laboratory experiment were collected in bulk from the top 25 cm of the soil profile in each site except the AQ. Prior to sample collection, decomposed and nondecomposed organic material was removed from the soil surface. The AQ material was collected 60 cm below the water table and 160 cm below the soil surface from an open excavation at a location between the HDW and WTL sites. The SOD, HDW, and CORN soils were passed through a 2-mm wire mesh screen to remove nonsoil materials. The WTL soil and AQ material were too moist to pass through the screen.

For the field study, 1-m² plots were established at the CORN, HDW, SOD, and WTL sites immediately adjacent to where soil was collected for the laboratory microcosms. Soil samples were collected from the field plots at 0–5-cm depth. In both studies, herbicide residues were extracted and analyzed at 5, 10, 20, 40, and 80 days following herbicide application. The herbicide applied was a commercially available mixture: Trimec Classic (PBI-Gordon Corp.). Trimec Classic (TC) contains 25.93% 2,4-D (dimethylamine salt), 13.85% MCPP [2-(2-methyl-4-chlorophenoxy)propionic acid] (dimethylamine salt), and 2.76% dicamba (dimethylamine salt) as active ingredients.

At each sampling date during both the field and laboratory studies, one untreated soil sample from each soil type was used as a spiked control. This produced a cumulative total of four or five spiked samples over the course of each 80-day experiment for each soil material. Each of the spiked control samples was used to determine herbicide extraction efficiency on that sampling date and for the estimation of herbicide recovery and method sensitivity presented here.

For the laboratory incubation study, soil samples (100 g oven dry weight, ODW) were incubated in 300-mL beakers. The spiked control samples were spiked with 1 mL of 1% TC solution on days 5, 10, 20, and 40 and with 0.1 mL on day 80. This yielded herbicide concentrations of 24.38 μg of 2,4-D (g of soil)$^{-1}$ (ODW) and 2.52 μg of dicamba (g of soil)$^{-1}$ (ODW) in

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Table 1. Soil Characteristics

material	pH[a]	% moisture by mass at field capacity[b]	% organic matter[c]
AQ	4.98	19.12[d]	<0.5[e]
CORN	5.61	19.2	3.5[f]
HDW	4.57	25.8	8.8[f]
SOD	5.94	24.2	2.0[g]
WTL	3.51	172.1	14.5[f]

[a] 20 g of soil/20 mL of deionized water. [b] Klute (1986). [c] Site averages. The percent organic matter in the soils used during the laboratory microcosm study was slightly lower than these figures because they were sampled from 0 to 25 cm, and those materials used during the field study were slightly higher because they were sampled from 0 to 5 cm. [d] Percent moisture by weight; AQ material was too unconsolidated to use pressure membrane technique. [e] University of Rhode Island Soil Testing Laboratory. [f] Groffman et al. (1991). [g] Gold et al. (1988).

Table 2. Soil Classification[a]

CORN	Enfield slit loam (coarse-silty over sandy or sandy-skeletal, mixed, mesic Typic Dystrochrepts)
HDW	Merrimac sandy loam (sandy, mixed, mesic Typic Dystrochrepts)
SOD	Merrimac sandy loam
WTL	Walpole sandy loam (sandy, mixed, mesic Typic Endoaquepts)

[a] Wright and Sautter (1979).

the control samples amended with 1 mL of 1% TC solution. Herbicide concentrations in the control samples spiked with 0.1 mL of 1% TC solution were 2.44 μg of 2,4-D (g of soil)$^{-1}$ (ODW) and 0.25 μg of dicamba (g of soil)$^{-1}$ (ODW). Each spiked control sample was covered with parafilm and shaken by hand for 2 min to ensure complete mixing of the herbicide (Parker and Doxtader, 1982).

During the field study, 30-g control samples were spiked with 0.3 mL of 1% TC solution on days 5 and 10, 0.1 mL on day 20, and 0.05 mL on days 40 and 80. The samples were then covered and shaken by hand for 2 min to ensure complete mixing of the herbicides. The moisture levels of each soil differed at the time of application, and therefore the actual concentration of amendment varied slightly between soils. The 0.3-mL amendment of 1% TC solution corresponded to concentrations of 28.68 μg of 2,4-D (g of soil)$^{-1}$ (ODW) and 2.97 μg of dicamba (g of soil)$^{-1}$ (ODW) if added to 30 g of soil at 15% moisture. The 0.1-mL amendment corresponded to concentrations of 9.56 μg of 2,4-D (g of soil)$^{-1}$ and 0.99 μg of dicamba (g of soil)$^{-1}$ if added to 30 g of soil at 15% moisture. The 0.05-mL amendment corresponded to concentrations of 4.78 μg of 2,4-D (g of soil)$^{-1}$ and 0.49 μg of dicamba (g of soil)$^{-1}$ if added to a soil at 15% moisture.

Extraction of Herbicide Residues. Thirty grams of soil from each sample was weighed into 250-mL centrifuge bottles. To these were added 50 mL of deionized (DI) water and 50 mL of HPLC grade methanol. The samples were then placed on an orbital-action shaker for 1 h. Following shaking, samples were clarified either by filtration (days 5, 10, 20, and 40 of the laboratory incubation) or by centrifugation (day 80 of the laboratory study and all samples from the field experiment).

Samples clarified by filtration were allowed to settle for 2 h following shaking. The supernatant was then decanted and filtered using a Büchner funnel fitted with Whatman No. 42 filter paper. Samples clarified by centrifugation were centrifuged at 1000–2300g for 20–60 min depending on the sample material (the WTL and HDW soils had a high organic matter content and required longer and faster centrifugation to settle the majority of soil particles). The clarified methanol/water solutions were decanted into microanalysis filtration flasks and vacuum filtered through 2.7-μm glass-fiber prefilter and 0.45-μm nylon membrane filters. Dicamba and 2,4-D were isolated from the filtered solutions using 3-mL amino bonded solid-phase extraction columns (SPE) (J. T. Baker Chemical Co.). The columns were preconditioned by rinsing with 1 M acetic acid at a vacuum suction of 69.0–103.5 kPa without letting

Table 3. Recovery (Percent) of 2,4-D from Spiked Soils during Laboratory Experiment[a]

material	day 5	day 10	day 20	day 40	day 80	mean[b]
AQ	70.4	75.6	68.1	73.7	60.2	69.7 ± 6.0a
CORN	60.9	69.4	69.3	73.0	75.4	69.9 ± 5.5a
HDW	52.7	65.3	63.3	66.9	53.4	60.3 ± 6.8ab
SOD	74.5	93.0	74.4	67.4	75.3	76.9 ± 9.6a
WTL	31.0	NS[c]	84.3	54.5	29.8	49.9 ± 25.6b

[a] Amounts spiked were 24.4 μg g^{-1} on days, 5, 10, 20, and 40 and 2.4 μg g^{-1} on day 80. [b] Mean recovery during 80-day laboratory study ± standard deviation. Values followed by the same letter are not significantly different at the $p = 0.05$ level. [c] NS, no sample.

the column bed go dry. The columns were rinsed with DI water, and the sample was then introduced at a rate of 5–7 mL min^{-1}, again without allowing the column bed to go dry. The entire sample was passed through the SPE columns, and the columns were then dried by passing air through them for 30 min using the vacuum source. The columns were then rinsed with 2 mL of methanol, dried for 20 min, and eluted with four 0.5-mL aliquots of 0.1 M K$_2$HPO$_4$. The eluate was then brought to a final volume of 2.0 mL and analyzed for 2,4-D and dicamba content by HPLC analysis.

An LCD/Milton Roy CM4000 HPLC equipped with a variable-wavelength UV detector and a metal column (25 cm × 4.6 mm i.d.) which contained a 5 μm C$_{18}$ bonded phase (Spherisorb OD-S2; LDC/Milton Roy) preceded by a 2-cm guard column (Arjmand et al., 1988) was used to quantify herbicide residues. The mobile phase was a 1:1 methanol/acidified DI water mix (10 mL of concentrated acetic acid brought to 1 L volume with DI water) at a flow rate of 1.0 mL min^{-1}. The UV detector was set at 236 nm (Connick and Simoneaux, 1982). A 20-μL aliquot of the 2-mL sample eluted from the SPE column was injected for each sample. Analytical standards (Fisher Chemical Co.) were dissolved in 0.1 M K$_2$HPO$_4$ and injected at various concentrations, the range of concentrations being dependent upon the amount of herbicide predicted to have been dissipated by that date.

Statistical Analysis. The percent 2,4-D and dicamba recovery data that were generated during the laboratory incubation and field studies were analyzed using the CoStat statistical package (Cohort Software, 1990). Because the results represent proportions of the total amount of herbicide applied, the data were mathematically transformed prior to statistical analysis (Neter et al., 1985). Generally, the data were transformed using the equation 2 arcsin ($\sqrt{y}$); however, for the dicamba data, some of which had values >1, the equation 2 arcsin ($\sqrt{0.5y}$) was employed. The residuals for the data sets were plotted as time sequence and frequency plots to determine if the data met the assumptions of the analysis of variance (AOV) model. Differences in recovery efficiency for each herbicide between soil types were determined for both the laboratory and field experiments using the CoStat AOV procedure and Duncan's multiple comparison of means. Individual t tests were conducted comparing recovery efficiencies of 2,4-D and dicamba within soil types to determine if there were significant differences in the recovery efficiencies of the two compounds.

RESULTS AND DISCUSSION

Percent Herbicide Recovery. Mean percent recovery for 2,4-D during the laboratory study ranged from a high of 76.9 ± 9.6% for the SOD soil to a low of 49.9 ± 25.6% for the WTL soil (Table 3). Percent recovery of 2,4-D from the AQ, CORN, SOD, and HDW soils was not significantly different at the $p = 0.05$ level. The percent recovery of 2,4-D for the WTL soil was significantly less than for the other soils at the $p = 0.05$ level.

The mean levels of recovery of dicamba for soils during the laboratory study ranged from a high of 114.0

Table 4. Recovery (Percent) of Dicamba from Spiked Soils during Laboratory Experiment[a]

material	day 5	10	20	40	80	mean[b]
AQ	79.2	84.2	86.5	101.0	70.0	84.0 ± 11.3a
CORN	148.0	84.3	80.0	144.0	NS[c]	114.0 ± 36.9a
HDW	65.9	97.7	77.1	87.0	NS	81.9 ± 13.6a
SOD	86.5	109.0	86.8	83.4	NS	91.4 ± 11.8a
WTL	55.3	NS	147.0	99.1	15.7	79.2 ± 56.5a

[a] Amounts spiked were 2.5 μg g^{-1} on days 5, 10, 20, and 40 and 0.25 μg g^{-1} on day 80. [b] Mean recovery during 80-day laboratory study ± standard deviation. Values followed by the same letter are not significantly different at the $p = 0.05$ level. [c] NS, no sample.

Table 5. Recovery (Percent) of 2,4-D from Spiked Soils during Field Experiment[a]

material	day 5	10	20	40	80	mean[b]
CORN	59.0	64.4	71.4	73.5	NS[c]	67.1 ± 6.7a
HDW	43.6	49.6	36.4	40.4	36.7	41.3 ± 5.5b
SOD	60.3	61.7	66.5	58.4	NS	61.7 ± 3.5a
WTL	21.9	27.5	27.5	21.3	27.8	25.2 ± 3.3c

[a] Amounts spiked were about 28.7 μg g^{-1} on days 5 and 10, 9.6 μg g^{-1} on day 20, and 4.8 μg g^{-1} on days 40 and 80. Slight variations between soils were due to the differences in moisture content on the sampling dates. [b] Mean recovery during 80-day field study ± standard deviation. Values followed by the same letter are not significantly different at the $p = 0.05$ level. [c] NS, no sample.

Table 6. Recovery (Percent) of Dicamba from Spiked Soils during Field Experiment[a]

| material	day 5	10	20	40	80	mean[b]
CORN	130.0	108.0	174.0	NS[c]	NS	137.0 ± 33.3a
HDW	55.3	65.5	73.4	NS	69.5	67.4 ± 7.5b
SOD	63.7	72.7	86.1	83.2	NS	76.4 ± 10.3b
WTL	51.4	59.3	NS	76.8	75.0	65.6 ± 12.3b

[a] Amounts spiked were about 3 μg g^{-1} on days 5 and 10, 1 μg g^{-1} on day 20, and 0.5 μg g^{-1} on days 40 and 80. Slight variations between soils were due to the differences in moisture content on the sampling dates. [b] Mean recovery during 80-day field study ± standard deviation. Values followed by the same letter are not significantly different at the $p = 0.05$ level. [c] NS, no sample.

± 36.9% for the CORN soil to a low of 79.2 ± 56.5% for the WTL soil (Table 4). There were no significant differences in the mean level of dicamba recovery between any of the soil types.

The average recovery of 2,4-D from soils during the field experiment ranged from a high of 67.1 ± 6.7% for the CORN soil to 25.2 ± 3.3% for the WTL soil (Table 5). Mean recoveries of 2,4-D for the CORN and SOD soils were significantly greater than for the HDW and WTL soils at the $p = 0.05$ level.

Dicamba recovery during the field experiment ranged from a high of 137 ± 33.3% for the CORN soil to a low of 65.6 ± 12.3 for the WTL soil (Table 6). The recovery of dicamba from the CORN soil was significantly different from that recovered from the three other soils at the $p = 0.05$ level.

Figures 1–6 present chromatograms for a blank (0.1 M K$_2$HPO$_4$) injection and spiked control samples from each soil type on day 20 of the laboratory study. Herbicide concentrations in the spiked samples on day 20 of the laboratory microcosm study were 24.38 μg of 2,4-D and 2.52 μg of dicamba per gram of soil (ODW). When viewing Figures 2–6, one should keep in mind that the peak indicated between dicamba and 2,4-D on
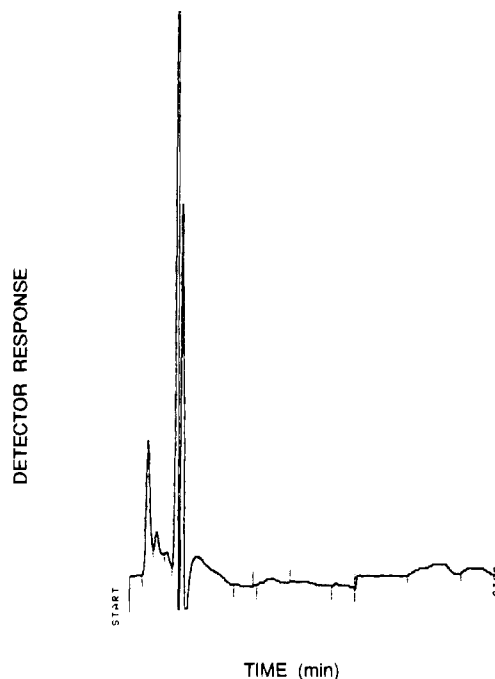
**Figure 1.** Detector response during blank (0.1 M K$_2$HPO$_4$) injection.
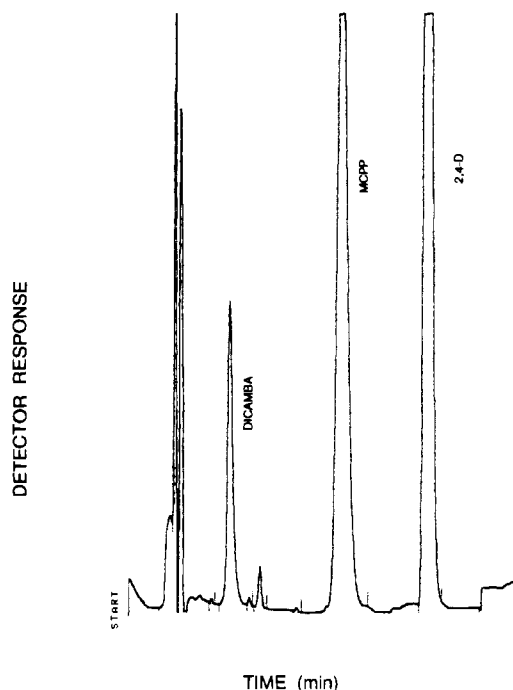
Figure 2. Detector response to spiked control: aquifer material.

the chromatograms depicts detector response to MCPP that was introduced during the previous sample injection. The overlap of chromatograms was calculated since we were not interested in quantifying MCPP in the soil samples and its peak did not interfere with that of either dicamba or 2,4-D.

For each set of percent recovery data, the WTL and HDW soils ranked lowest and next to lowest in recovery efficiency, though the differences were not always statistically significant. Much of the difference in herbicide recovery between the WTL and HDW soils and the other soils was most likely due to the greater organic matter content of the WTL and HDW soils, especially for 2,4-D (Figure 7). The levels of soil organic matter

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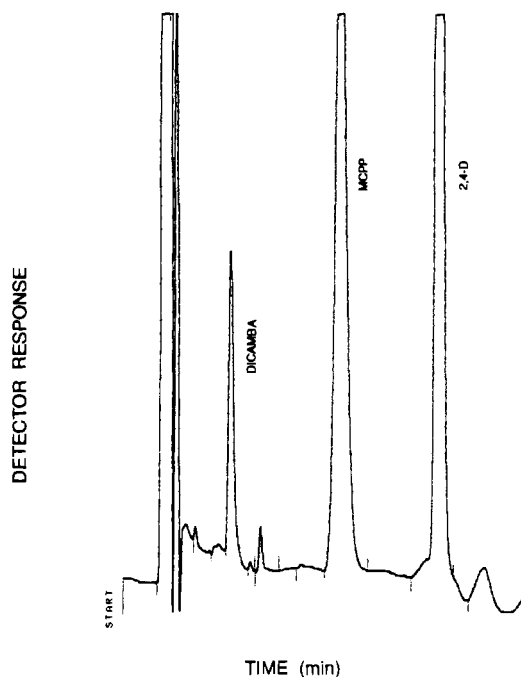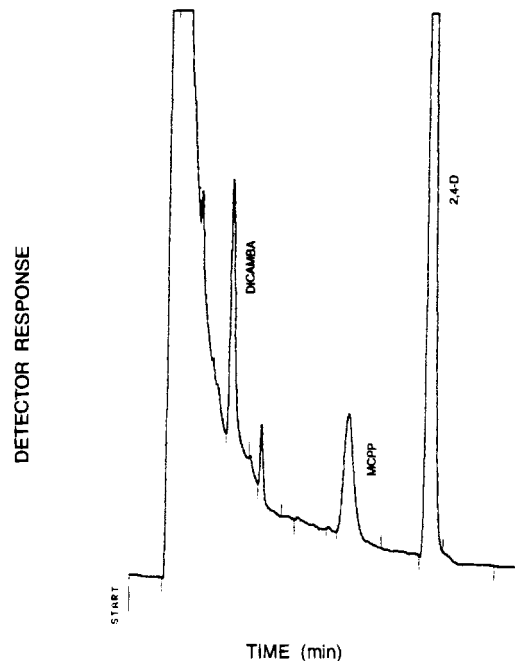

Figure 3. Detector response to spiked control: CORN soil.
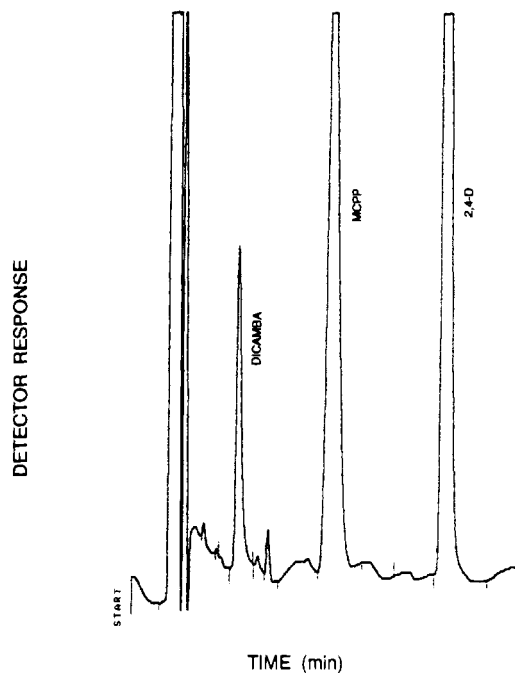
Figure 5. Detector response to spiked control: SOD soil.
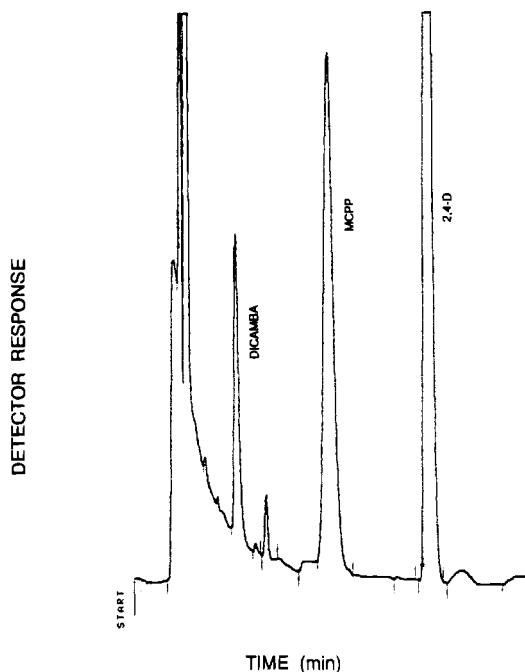
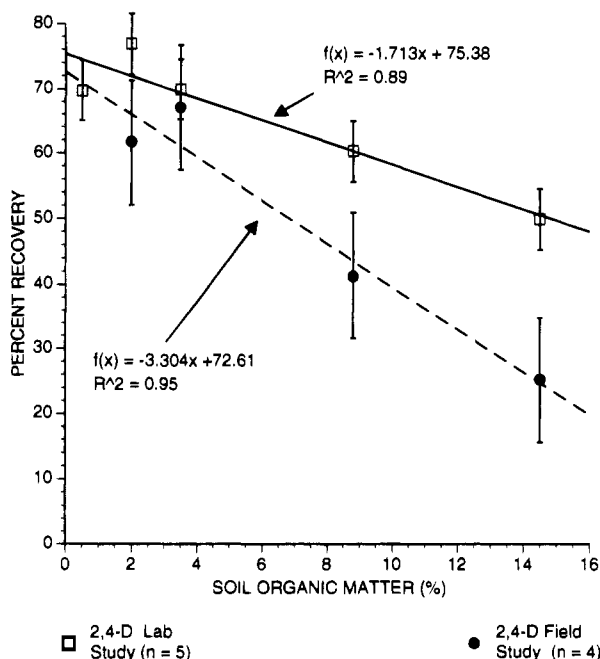**Figure 4.** Detector response to spiked control: HDW soil.

Figure 6. Detector response to spiked control: WTL soil.

(SOM) depicted in Figure 1 and Table 1 are site means supplied by various sources (Gold et al., 1988; Groffman et al., 1991) and will differ slightly between the materials used during the laboratory and field experiments. The SOM contents of the materials used during the laboratory microcosm experiment (except the AQ material) were probably less because they were collected at 0–25 cm. The materials used during the field study most likely had a slightly greater SOM content than the depicted values because they were sampled at 0–5 cm.

The greater organic matter content of the WTL and HDW soils may have affected extraction efficiency in two ways: (1) by physically hampering the initial extraction procedure by clogging filters and (2) through adsorption of the herbicides to organic soil particles. The extraction of herbicides from the WTL and HDW soils

typically took 25–100% longer than extraction from the other three soils, especially when filtration alone was used to clarify the samples. During the preliminary Büchner funnel filtration, the filter paper would often become coated by fine particles requiring a gentle scraping of the filter paper with a plastic spatula before filtration could continue. Even with the use of centrifugation, the microanalysis unit filters became clogged by the minute particles in the decanted fluid and required scraping. Though the spatula was carefully rinsed into the filter apparatus after each scraping, a small amount of herbicide may have been lost during these manipulations.

Dicamba and 2,4-D are not considered to be strongly adsorbed by soils because of their weak acidic nature. However, the adsorption of these compounds is known to increase with increasing soil organic carbon content

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$f(x) = -1.713x + 75.38$
$R^2 = 0.89$

$f(x) = -3.304x + 72.61$
$R^2 = 0.95$

□ 2,4-D Lab
Study (n = 5)

● 2,4-D Field
Study (n = 4)

Figure 7. Mean percent recovery of 2,4-D during 80-day laboratory and field studies and percent soil organic matter (bars indicate ± standard error).

and decreasing pH (Ogram et al., 1985; Murray and Hall, 1989). The WTL and HDW soils had the highest organic matter content and the lowest pH of the soils used, and therefore it is reasonable to assume that if adsorption was a factor in extraction efficiency, these soils would be the most indicative of that.

Percentage recovery was higher for dicamba than for 2,4-D in both the laboratory and field experiments. This difference was statistically significant ($p < 0.05$) for the CORN and HDW soils in the laboratory study and for the HDW, SOD, and WTL soils in the field experiment. These differences may result from a greater extraction efficiency of the water/methanol mix in removing dicamba from soil, or 2,4-D may be more strongly adsorbed onto the SPE columns and therefore eluted with less efficiency than dicamba. Though a greater percentage of dicamba was extracted as compared to 2,4-D, the variability of these results was also greater. This may be due to the lower amounts of dicamba used to spike the soils—almost an order of magnitude less on each sampling date.

Detector and Method Sensitivity. The lowest quantifiable limit of the HPLC system, with the UV detector set at 236 nm, was 1.0 μg mL^{-1} for both dicamba and 2,4-D. Standard curves developed for the two herbicides near the lower limit of detection (Figures 8 and 9) typically had coefficients of regression (r) of ≥ 0.998 for dicamba and 0.999 for 2,4-D. The 1.0 μg mL^{-1} detection limit corresponds to approximately 0.1 μg of herbicide (g of soil)$^{-1}$ (ODW) when a 2-mL injectable sample is obtained from 30 g of extracted soil (15% moisture content), assuming 80% extraction efficiency. Lower limits of detection were achieved, to approximately 0.5 μg mL^{-1}, but this level was not consistent.

Conclusions. A method to quantify 2,4-D and dicamba residues in soils from different components of a heterogeneous landscape has been described. This method, unlike the commonly used GC methodology, requires no derivatization or use of hazardous chemicals. Centrifugation of the soil/methanol/water mixture

$f(x) = 0.127x + 0.085$
$R^2 = 0.999$
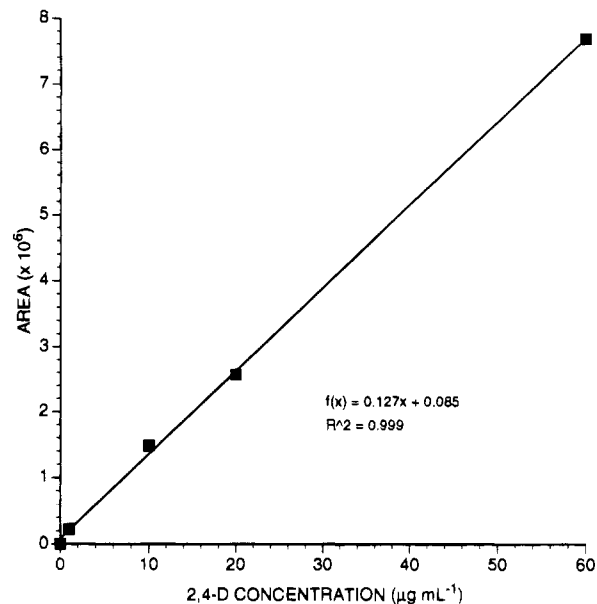
Figure 8. 2,4-D standard curve.

$f(x) = 1.13x - 0.141$
$R^2 = 0.996$
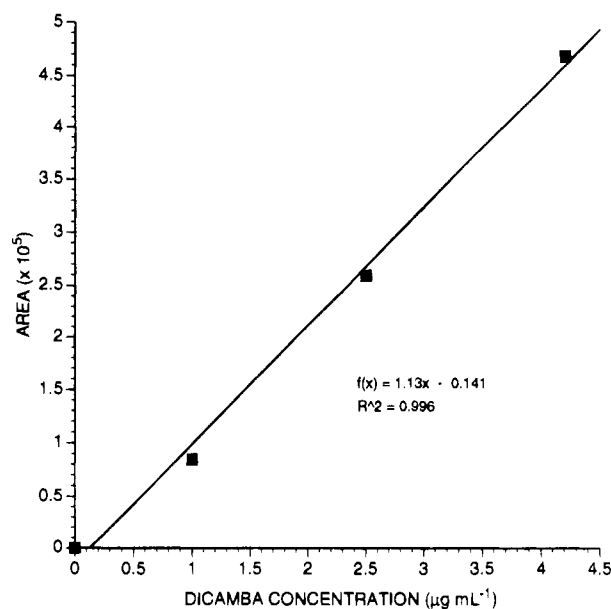
Figure 9. Dicamba standard curve.

after shaking to clarify samples rather than filtration is advised. Though centrifugation did not increase extraction efficiency, several hours of extraction time were saved per sampling date by centrifuging the sample mixtures.

The method proved to be reliable in the extraction and HPLC analysis of 2,4-D and dicamba from mineral soils at levels above 0.2 μg g^{-1}. Modifications in the extraction methodology must be made for it to be applied to organic soils at these and lower levels and possibly to mineral soils at lower levels. The variability in percent recovery was greater for dicamba than for 2,4-D during both the field and laboratory studies, regardless of soil type. Given the heterogeneity of the soil environment and the low levels of compound that we were using, this type of variability is reasonable and should be expected by other investigators. Herbicide extraction from the HDW and WTL soils, which had high organic matter contents, was less efficient and more time-consuming than for the other soils and AQ material. Longer centrifuge times for organic samples, a method to

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precipitate the organic soil without affecting herbicide analysis, or filtering through a series of decreasing filter pore sizes may reduce clogging problems during filtration. Improved extraction of herbicide from organic soils might also be accomplished by changing the methanol/water ratio in the extracting solution or using different or additional solvents.

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