Persistence, Fate, and Metabolism of [^{14}C]Metalaxyl in Typical Indian Soils

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The biodegradation of ring-labeled [^{14}C]metalaxyl in six Indian soils was examined. The total recovery of radioactivity from soil was 100 ± 6% of the applied radioactivity. Volatile organics and $^{14}CO_2$ were detected at lower levels. This suggests that neither mineralization nor volatilization is a major route of metalaxyl dissipation. The most rapid degradation of metalaxyl was observed in Bannimantap soil, in which the half-life of metalaxyl was 36 days. An inverse relationship was found when half-lives were plotted against microbial biomass and soil clay content. However, soil total organic carbon did not correlate with metalaxyl persistence. Five metabolites detected by thin-layer chromatography were more polar than metalaxyl.

Keywords: *Metalaxyl; degradation; metabolism; distribution; soil*

INTRODUCTION

Metalaxyl [*N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl)-alanine methyl ester] is used to control diseases caused by various fungi, such as late blight of potato caused by *Phytophthora infestans*, downy mildew and white rust of mustard and rapeseed caused by *Peronospora parasitica* and *Albugo candida*, downy mildew of pearl millet caused by *Sclerospora graminicola*, seed rot of pea caused by *Fusarium solani* and *Pythium ultimum*, and root and crown rot of capsicum caused by *Phytophthora capsici* (*1*−*6*). It is a stable compound, resistant to a wide range of pH, temperature, and light (*7*). These properties lead to its easy use in agriculture. Due to its broad spectrum of activities, metalaxyl is registered for use in many countries worldwide, including the United States, Europe, Australia, and India, on a variety of fruit and vegetable crops. Upon spray or soil drench metalaxyl is exposed to various biotic and abiotic factors, which may affect its activity. There are few studies on its photodegradation (*8, 9*), enhanced biodegradation (*10*), and plant metabolism using cell suspension culture, etc. (*11*). However, a holistic approach toward ascertaining the overall distribution pattern of metalaxyl in soil has not been made so far, especially not with Indian soils. This investigation examines the contributions of physicochemical and microbial processes on metalaxyl mineralization and volatilization and quantitates extractable and bound metalaxyl residues from six diverse Indian soils. In addition, the degradation kinetics of metalaxyl in soil and the isolation of metabolites of metalaxyl in these soils will be presented.

MATERIALS AND METHODS

Chemicals. Metalaxyl, analytical standard grade (99%), was obtained from Riedel-de Haen, Seelze, Germany, and [*phenyl*-U-^{14}C]metalaxyl with a specific activity of 1.37 MBq/mg was obtained from Novartis Crop Protection AG, Basel, Switzerland. The radiochemical purity was 97.2% as shown by HPLC.

Soil. Six soils originating from India, representing different physicochemical properties, were included in the present study. The soil collection areas are shown in Figure 1. Two batches of each soil sample were collected from a depth of 0−5 cm. One batch, shipped immediately by air freight to Germany to maintain its original moisture status, is designated fresh soil. The other batch, shipped by surface after air-drying, is designated stored soil. It took nearly 4 months to reach Germany. Different conditions, especially the moisture status during transportation, were expected to affect the biological activity of the soils. Prior to the laboratory experiments, the soils were sieved to a maximum particle size of <2 mm. The physical−chemical properties are shown in Table 1. None of the soils investigated had been treated with metalaxyl in the previous 5 years.

Application of Metalaxyl in Soil. Ring-labeled [^{14}C]-metalaxyl (80 μg of active ingredient/100 g of soil on a dry weight basis, which is equivalent to 109.6 kBq of radioactivity) was mixed into the soils with a recommended commercial use rate of 600 g of active ingredient/ha, assuming a soil depth of 5 cm and a soil density of 1.5 g/cm^3. The moisture content was adjusted to 60% of maximum water holding capacity. To avoid any effects of the solvents upon the biological activity of the soils, the calculated volumes of the application solution (1 mL of a solution of metalaxyl in methanol) were initially dispensed onto portions of ∼25 g of dry soil in porcelain dishes. The treated subsamples of soils were thoroughly mixed with a spatula until the solvent was completely evaporated (∼10 min) and afterward added to the total mass of the corresponding soils (1200 and 1800 g for fresh and stored soils, respectively). Subsequently, the gross mass of each soil was mixed in a tumbling mixer for 1 h. This was followed by aliquotation. Batches of 100 g of soil each (relative to dry soil) were incubated in the dark in Erlenmeyer flasks under controlled temperature (20 ± 1 °C). The moisture content in each flask was gravimetrically checked every 2 weeks. The flasks were fitted with trap attachments to absorb volatile substances, CO_2 [soda lime (NaOH in the form of self-indicating granules)], and organic volatiles [PU foam (polyurethane foam in solid state)]. Each experiment was carried out in duplicate.

Soil Biological Property. The microbiological activity in soils was determined with and without the addition of chemicals at the beginning and end of the experiment with the help of a method described by Joergensen (*12*). The application of

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Figure 1. Illustration map of India showing soil collection areas.

the active ingredient and the subsequent incubation for the soil samples used for the determination of microbial biomass (C_{mic}) were performed similarly as described under Application of Metalaxyl in Soil.

Recovery, Extraction, and Quantitative Determination of Radioactivity. The actual amount of radioactivity applied was determined by combustion of four aliquots (1 g each) of the soil immediately after the treatment in an oxidizer (Biological Oxidizer OX 500, R. J. Harvey Instrument Corp.), and the released $^{14}CO_2$ was measured by liquid scintillation counting (LSC). The distribution of the test substance was found to be homogeneous. This result served as a reference value for the total mass value. Prior to the opening of an incubation vessel, volatile compounds possibly present in the headspace of the vessel were purged into the trap attachment by means of a gentle stream of air (20 min). Soil samples were exhaustively extracted three times at room temperature on a mechanical shaker in a centrifuge beaker for 1 h each. The first extraction was done with 100 mL of methanol. The second and third extractions were made with 100 mL of methanol/water (80:20) followed by ultrasonic vibration (Branson Sonifier) for 5 min. After each extraction step, the extracts were separated from soil solids by centrifugation (Beckman J2-HS, Beckman) for 20 min (9600*g*) and pooled. The extract was analyzed for radioactivity content by LSC (Beckman LS 6500) using Rotiscint Eco plus (Zinsser Analytic) and for metabolites by thin-layer chromatography (TLC). The PU foam of each trap was extracted twice with 30 mL of ethyl acetate. For the determination of $^{14}CO_2$, sodalime (10 g) of the trap was dissolved with 18% HCl (60 mL) in a suitable apparatus. The liberated $^{14}CO_2$ was absorbed by a series of three vials containing 15 mL of ice-cooled cocktail of Oxysolve C-400 (Zinsser Analytic), and the radioactivity was subsequently measured by LSC. The soil-bound residue was freeze-dried, homogenized in a mortar, and radioassayed by combustion followed by LSC.

Quantitative Determination of Metalaxyl and Its Metabolites in Soil by Chromatographic Methods. The degradation of metalaxyl and the formation of its metabolites were monitored by using TLC. The radioactive components in the extracts were separated and quantified by TLC using silica gel plates on aluminum sheets (silica gel 60 F_{254}, Merck, Darmstadt, Germany) and a mixed solvent system of ethyl acetate, propanol-2, and water (65:23:12). A 1 mL aliquot was directly taken from the pooled extract and spotted on the plates as bands by using an automatic plate spotter (Linomat IV, Camag). The distribution of the radioactive zones on the TLC plates was measured by using a Bio-Imaging Analyzer (BAS 1000, Fuji Co.). Radioactive regions on the measured tracks were quantified with the software package TINA (Raytest).

Selected samples were also subjected to high-performance liquid chromatography (HPLC) to confirm the results obtained by TLC. After reduction of the volume to 3 mL on a rotary evaporator, the soil extracts were analyzed by HPLC using a Shimadzu LC 10 AT equipped with a Raytest Ramona 2000 and a UV detector operated at 205 nm. A Macherey-Nagel (250/8/4) C_{18} column was used. For the first 15 min, the mobile phase (1 mL/min) was composed of 10% acetonitrile and 90% water. For the next 5 min, the acetonitrile content was increased to 90%.

The separation and identification of metalaxyl and its acid metabolite were also confirmed by HPLC-MS/MS. The operating parameters were as follows: HPLC; Phenomenex Nucleosil 3 C_{18} 100A (150 mm × 2 mm) column, mobile phase; solvent A water (0.1% HCOOH) and solvent B acetonitrile (0.1% HCOOH) with a gradient system (0−5 min 95% A and 5% B and 5−35 min 80% A and 20% B), flow 250 μL/min. Fifty microliters of sample were injected in methanol/water (1:1) with 1% HCOOH (0.5 μL of crude soil extract was diluted with 50 μL of injection solvent). MS: TSQ 7000 (Finnigan MAT, Bremen, Germany) equipped with an ESI and APCI source. For ESI, the ionization voltage of 5 kV and transfer capillary temperature of 220 °C were set. For APCI, a vaporizer temperature of 450 °C and a transfer capillary temperature of 230 °C were used. The ionization current and detector voltage were set to 5 μA and 1.3 kV, respectively. For MRM scans, a dwell time of 400 ms and a scanning time of 0.5 s were applied.

Preparation of Comparison Compound: Metalaxyl Acid Metabolite [*N*-(2,6-Dimethylphenyl)-*N*-(methoxyacetyl)alanine]. Metalaxyl (100 mg) was added to 5 mL of 2 N KOH, and the mixture was refluxed (110−115 °C) for 1 h and then cooled to ambient temperature. Seven milliliters of 2 N HCl were added to the reaction mixture, wich was kept in a freezer overnight. The resulting white crystals were collected and washed with ice-cold H_2O.

RESULTS AND DISCUSSION

Radioactivity Distribution. On the basis of the LSC data a radioactivity balance was established for each vessel in stored and fresh soils at each sampling interval (Tables 2 and 3). The total recovery of radioactivity was 100 ± 6% of the applied radioactivity. The total amount of extractable radioactivity declined while the percentage of nonextracted radioactivity increased simultaneously. Volatile organics and $^{14}CO_2$ were not detected in higher amounts in either stored or fresh soil at any sampling interval, which indicates that neither mineralization nor volatilization is a major route of metalaxyl dissipation in the case of the studied soils. The evolution of a low level of $^{14}CO_2$ (0.4−1.5%) from the ring-labeled metalaxyl suggests that the aromatic moiety of metalaxyl was not degraded. A much higher $^{14}CO_2$ evolution rate (2.1−11.3%) may occur in the enhanced biodegradation of metalaxyl (*10*).

Soil Biological Property. The microbial activity of the test soil is particularly an important factor to study the degradation and metabolism of agrochemicals in soils (*13*−*15*). The microbial biomasses of the stored and fresh soils used in this study are shown in Table 4. In all soils, a small decline in microbial biomass was observed at the end of the experiment. The same trend was also observed in the control soil without metalaxyl application. This may be attributed to the influence of the incubation period and incubation conditions rather than to the effect of metalaxyl. During the experimental period, the microbial populations in these soil samples were not supplied with exogenous food or energy. With the passage of time, a similar phenomenon of decreasing microbial biomass in soil was also demonstrated with different pesticides (*16*). However, there are many studies reporting the favorable effect of pesticides on the growth and activities of microorganisms in soil (*17*−

Table 1. Physical−Chemical Characteristics of Test Soils

soil	Bannimantap	Canning	CFTRI farm	Coochbehar	Mohanpur	Purulia
texture (%)						
clay	11.7	42.0	16.4	15.5	42.5	19.4
silt	13.6	15.5	18.2	12.5	34.0	12.4
sand	74.7	42.5	65.4	72.0	23.5	68.2
pH (H_2O)	7.4	6.7	6.9	6.9	8.2	5.8
org C (g/kg)	14.0	6.3	41.0	12.4	7.6	5.3
total N (g/kg)	1.3	1.4	2.3	2.1	0.9	0.8
available N (mg/kg)	nd[a]	13.7	nd	113.7	37.2	31.4
available P_2O_5 (mg/kg)	nd	2.7	nd	10.0	10.9	9.0
total Fe (%)	1.6	2.6	1.5	1.5	2.2	1.3
free Fe_2O_3 (%)	0.1	0.3	0.1	0.1	0.1	0.2
CEC (mol/kg)	11.9	9.2	20.3	10.0	11.0	7.4
exch Ca (mol/kg)	12.0	7.6	19.5	4.2	7.5	2.5
exch Mg (mol/kg)	2.7	1.3	3.2	1.7	1.2	0.7
max water holding capacity (%)	37.3	45.2	44.9	41.5	38.3	26.8
origin	Mysore, Karnataka, India	Canning, WB, India	Mysore, Karnataka, India	Coochbehar, WB, India	Mohanpur, WB, India	Purulia, WB, India

[a] nd, not determined.

Table 2. Distribution of Radioactivity in Different Soils (Stored) after Application of [^{14}C]Metalaxyl at Periodical Intervals

soil/day	intact metalaxyl remaining (%) in extractable radioactivity	recovered radioactivity as % of applied dose	% recovered radioactivity			
			org extract	soil-bound residue	$^{14}CO_2$	org volatiles
Canning						
0	93.9	101.6	93.9	7.7	<0.01	<0.01
1	90.1	101.2	91.8	9.4	<0.01	<0.01
3	90.0	104.9	94.7	10.2	<0.01	<0.01
7	75.2	102.1	83.8	18.3	<0.01	<0.01
14	66.5	99.7	78.9	20.8	<0.01	<0.01
30	61.7	102.5	80.8	21.5	0.2	<0.01
60	48.3	101.7	78.9	22.3	0.5	<0.01
90	42.3	101.6	78.1	22.8	0.7	<0.01
120	40.3	106.4	72.2	32.7	1.5	<0.01
Coochbehar						
0	101.2	104.9	101.5	3.4	<0.01	<0.01
1	99.8	103.3	98.2	5.1	<0.01	<0.01
3	97.1	105.6	97.3	8.3	<0.01	<0.01
7	94.7	104.9	94.8	9.8	0.3	<0.01
14	94.8	106.3	95.6	10.7	<0.01	<0.01
30	88.9	99.8	88.8	11.0	<0.01	<0.01
60	87.5	100.4	88.3	12.1	<0.01	<0.01
90	86.2	99.4	87.2	12.2	<0.01	<0.01
120	83.3	96.5	83.7	12.7	0.1	<0.01
Mohanpur						
0	88.1	97.0	87.9	9.1	<0.01	<0.01
1	88.6	98.1	88.6	9.5	<0.01	<0.01
3	90.0	100.3	90.6	9.7	<0.01	<0.01
7	87.7	99.7	88.5	11.2	<0.01	<0.01
14	84.7	99.3	88.0	11.2	0.1	<0.01
30	81.0	104.6	86.9	17.5	0.2	<0.01
60	71.3	101.6	81.5	19.9	0.2	<0.01
90	60.9	104.5	80.5	23.7	0.3	<0.01
120	57.9	101.9	77.2	24.3	0.4	<0.01
Purulia						
0	98.2	101.6	98.2	3.4	<0.01	<0.01
1	98.4	101.7	98.0	3.7	<0.01	<0.01
3	100.3	102.2	97.4	4.8	<0.01	<0.01
7	95.9	103.8	95.8	7.9	0.1	<0.01
14	89.3	100.2	88.9	11.3	<0.01	<0.01
30	86.2	100.7	87.2	13.5	<0.01	<0.01
60	83.0	97.4	84.9	12.5	<0.01	<0.01
90	81.7	95.3	82.5	12.7	0.1	<0.01
120	79.2	97.1	81.1	15.9	0.1	<0.01

20). Therefore, the present experiment clearly shows the limitations of soil degradation studies under standardized laboratory conditions using microbially depleted soils due to drying and storage. Therefore, it may be unrealistic to extrapolate laboratory results to the natural environment.

Bound Residues. In both stored and fresh soils, the amount of bound residues gradually increases with time. Interestingly, a higher bound residue quantity was found under fresh than under stored conditions of the same soil (Tables 2 and 3). This might be explained through microbial activity in soil. More microbial activity means more microbial exudates, which generate a cementing effect on the pesticides with soil matrix by rendering their biounavailable. Microbes are also responsible for the conversion of parent organic com-

Table 3. Distribution of Radioactivity in Different Soils (Fresh) after Application of [14C]metalaxyl at Periodical Intervals

soil/day	intact metalaxyl remaining (%) in extractable radioativity	recovered radioactivity as % of applied dose	% recovered radioactivity			
			org extract	soil-bound residue	$^{14}CO_2$	org volatiles
Bannimantap						
0	96.4	99.4	95.6	3.8	<0.01	<0.01
7	84.6	98.0	91.7	6.3	<0.01	<0.01
14	74.2	97.3	87.9	9.0	0.4	<0.01
30	55.8	100.3	83.1	16.5	0.7	<0.01
60	25.8	99.0	68.0	30.2	0.8	<0.01
90	18.9	99.4	66.0	32.4	1.0	<0.01
CFTRI Farm						
0	98.6	101.9	98.0	3.9	<0.01	<0.01
7	88.7	99.8	88.7	10.9	0.2	<0.01
14	78.7	95.9	80.5	15.1	0.3	<0.01
30	75.8	99.1	79.6	19.1	0.4	<0.01
60	58.9	96.9	64.4	32.0	0.5	<0.01
90	56.3	98.7	63.0	35.0	0.7	<0.01
Coochbehar						
0	97.7	102.8	97.5	5.3	<0.01	<0.01
7	94.8	102.9	95.4	7.4	0.1	<0.01
14	84.9	103.1	94.7	8.1	0.3	<0.01
30	79.7	103.6	94.4	8.8	0.4	<0.01
75	78.7	99.6	86.9	12.2	0.5	<0.01
90	77.0	101.1	85.8	14.7	0.6	<0.01
Mohanpur						
0	87.5	97.7	87.1	10.6	<0.01	<0.01
7	73.3	100.8	82.5	18.2	0.1	<0.01
14	49.9	99.2	76.1	23.0	0.1	<0.01
30	41.3	97.5	73.9	23.5	0.1	<0.01
75	39.8	94.0	61.5	32.1	0.4	<0.01
90	21.4	96.6	49.5	46.5	0.6	<0.01
Purulia						
0	95.8	101.8	95.8	6.0	<0.01	<0.01
7	91.0	102.1	93.0	8.8	0.3	<0.01
14	85.1	101.8	92.5	8.9	0.4	<0.01
30	79.8	99.0	88.6	10.0	0.4	<0.01
75	65.5	97.0	73.8	23.0	0.2	<0.01
90	57.5	101.8	73.7	27.8	0.3	<0.01

Table 4. Microbial Properties of Soils Investigated

soil	microbial biomass (mg of C_{mic}/kg of soil)	
	start	end
	Stored Soil	
Canning	288	241
Coochbehar	104	83
Mohanpur	252	210
Purulia	96	71
	Fresh Soil	
Bannimantap	1364	1330
CFTRI Farm	1031	992
Coochbehar	634	590
Mohanpur	1316	1250
Purulia	978	920

Table 5. Regression Equations, Correlation Coefficients, and Calculated Half-Lives for Degradation Kinetics of Metalaxyl in Different Soils

soil	regression eq	r^2	cald $t_{1/2}$ (days)
	Fresh Soil		
Bannimantap	$Y = 1.87 - 0.0083X$	0.9845	36
CFTRI farm	$Y = 1.88 - 0.0026X$	0.9297	116
Coochbehar	$Y = 1.74 - 0.001X$	0.7339	301
Mohanpur	$Y = 1.64 - 0.0052X$	0.8149	58
Purulia	$Y = 1.75 - 0.0022X$	0.9842	137
	Stored Soil		
Canning	$Y = 1.83 - 0.0031X$	0.8980	97
Coochbehar	$Y = 1.86 - 0.0006X$	0.8539	502
Mohanpur	$Y = 1.84 - 0.0017X$	0.9871	177
Purulia	$Y = 1.89 - 0.0008X$	0.8372	376

pounds into reactive intermediates, which cannot be extracted with normal organic solvents (*21*). However, bound residues may also be generated by adsorption. Metalaxyl seemed to be preferentially adsorbed on soil mineral surfaces (*22*). A xenobiotic substance may also undergo chemical reactions with humic acid precursors, lignin, and polysaccharides present in soil and thus be incorporated into the polymeric structure without being degraded (*23*−*26*). The spaces between the sheets of clay minerals can also incorporate pesticides, as well as natural products derived from them (*27*). However, as far as we know, there is not any knowledge about the nature and characterization of metalaxyl bound residue. This should be investigated.

Dissipation of Metalaxyl. Intact metalaxyl residues remaining in extractable radioactivity in stored and fresh soils are separately presented in Tables 2 and 3. Log residues of intact metalaxyl were plotted against time, and the half-lives were calculated by means of the formula $t_{1/2} = \ln 2/K$. The straight-line regression equations with correlation coefficient values and the half-lives of different soils under different conditions are presented in Table 5. In general, greater persistence of metalaxyl was found in stored soil than in fresh soil. However, in stored Canning soil metalaxyl persistence was comparatively less (calculated $t_{1/2} = 97$ days). This may be attributed to the presence of a microbial population, although it was low (241 mg of C_{mic}/kg of soil, Table 4), which might have a high potential to degrade metalaxyl. The aerobic soil metabolism half-life of metalaxyl was determined to be ∼40 days (*28*). However, there was no evidence of metalaxyl biodegradation in sandy loam soil even after 10 weeks (*29*). A half-life in the range of 69−159 days was observed in a soil degradation study of metalaxyl (*30*). In the present study, different calculated half-lives in different soils

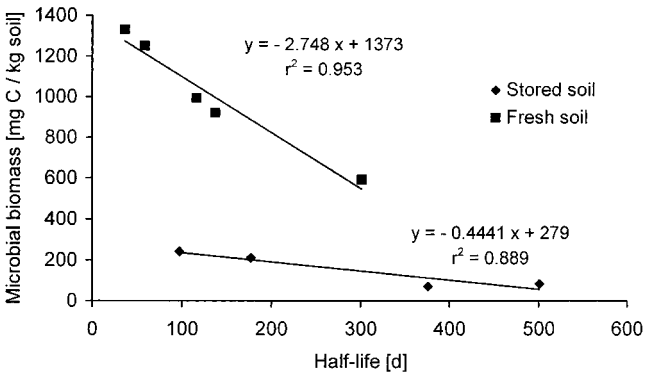

Figure 2. Correlation between microbial biomass and half-life of metalaxyl in stored and fresh soils.

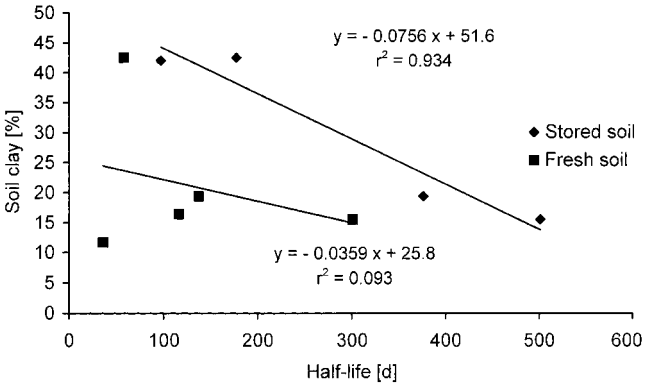

Figure 3. Correlation between soil clay content and half-life of metalaxyl in stored and fresh soils.

under different conditions highlight the differences in the degradation rates, which can be attributed to the differences in physicochemical properties of soils, the presence of microbial population in varying degrees, variability in activity among particular components of a microbial population taking part in the degradation of metalaxyl, and the high potential of specific soils to degrade metalaxyl. Although the present study does not encounter the identification of specific microbial strains being responsible for the degradation of metalaxyl, it needs to be investigated.

In any of the soils, an initial fast degradation and a decrease at the later stage were observed. This might be a reflection of the higher microbial biomass at the beginning and the low biomass at the end of the experiment. Moreover, the selected soils seem to be extremely sensitive to microbial characters.

Correlation between Soil Properties and Metalaxyl Persistence. An attempt was made to correlate the half-life values (Table 5) of metalaxyl in stored and fresh soils and the soil properties (Tables 1 and 4). An inverse relationship (Figure 2**)** was found when half-lives were plotted against C_{mic} in fresh ($r^2 = 0.9532$) and stored ($r^2 = 0.8893$) soils, suggesting a possible microbial degradation of metalaxyl in soils. From former experiments (*10*, *31*, *32*) it is a well-known fact that a wide range of fungi, bacteria, and actinomycetes seem to take part in the degradion process of metalaxyl. A correlation was also found between the soil clay content and the half-lives of metalaxyl in stored soils ($r^2 = 0.9344$; Figure 3). Metalaxyl seems to be preferentially adsorbed on soil mineral surfaces (*22*, *33*). A possibility of the trapping of metalaxyl in the intralattice structure of clay components may not be ruled out, either (*27*). Thus, a higher clay content may lead to a higher amount of

Table 6. Metabolites Generated in Soils (Fresh) at Periodical Intervals (Percent)[a]

soil/day	metab 1 $R_f = 0.70$	metab 2 $R_f = 0.36$	metab 3 $R_f = 0.31$	metab 4 $R_f = 0.29$	metab 5 $R_f = 0.10$
Bannimantap					
0					
7		6.7		0.4	
14		12.7		1.0	
30		24.2		1.7	
60		38.2		2.4	
90		44.5		2.6	
CFTRI farm					
0				-	
7		0.7		0.2	
14		1.9		0.4	
30		2.3		0.4	
60		3.8		0.6	
90		4.6		0.4	
Coochbehar					
0					
7		0.8			
14		3.9			
30		2.3		1.4	
75		3.4		1.4	
90		4.9		2.2	
Mohanpur					
0					
7		9.2			
14		25.0			
30		23.6			
75		22.1			
90		26.2			
Purulia					
0					
7		0.8			0.5
14		3.4			1.4
30		3.2		0.6	
75	7.5				
90	9.6	0.7	1.5	1.5	0.6

[a] Values are presented in percent of extracted radioactivity.

bound residues of the chemical. As in the present study, the half-life of metalaxyl was calculated on the basis of its extractable form; an inverse relationship was found between the soil clay content and the half-life values. However, the effect of the clay content in the dissipation of metalaxyl is not evidenced in fresh soil ($r^2 = 0.0929$). Fresh soils contained a high amount of C_{mic}. So it may be suggested that, in the absence of sufficient microbial population, soil clay plays a vital part in attenuating metalaxyl in soil; otherwise, microbes are responsible for the metalaxyl dissipation. Soil organic carbon did not correlate with metalaxyl dissipation in the present study.

Metabolism. In the course of the experiments, few radioactive metabolites were detected along with the unchanged metalaxyl. In almost all soils, except for stored Coochbehar soil, two metabolites were found (metabolite 2, $R_f = 0.36$, and metabolite 4, $R_f = 0.29$; Tables 6and **7**), which were generated at various degrees at different intervals. However, in stored Coochbehar soil no metabolites were detected. This confirms the higher persistence of metalaxyl in stored Coochbehar soil, as has also been observed in the kinetic study of metalaxyl dissipation. In fresh Purulia soil three metabolites ($R_f = 0.70$, 0.31, and 0.10) were also detected. All of the metabolites are more polar than metalaxyl ($R_f = 0.78$). In most soils the main metabolite found was metabolite 2, and an increasing trend in its formation was noticeable during the experiment. In Bannimantap soil the formation of this metabolite was maximum, 44.5% of total extractable radioactivity after 90 days of incubation. However, in fresh Purulia soil the main

Table 7. Metabolites Generated in Soils (Stored) at Periodical Intervals (Percent)[a]

soil/day	metab 2 $R_f = 0.36$	metab 4 $R_f - 0.29$
Canning		
0		
1	0.9	
3	4.3	
7	9.2	0.2
14	14.1	0.3
30	22.6	0.9
60	37.1	1.7
90	44.0	1.5
120	44.3	1.8
Coochbehar	no metabolite detected	
Mohanpur		
0		
1		
3		
7	1.1	
14	2.4	0.2
30	5.4	0.5
60	11.4	0.6
90	23.1	1.0
120	23.8	1.0
Purulia		
0		
1		
3		
7		
14		
30	0.5	0.8
60	0.7	0.2
90	0.8	0.3
120	0.9	0.6

[a] Values are presented in percent of extracted radioactivity.

metabolite was metabolite 1 ($R_f = 0.70$), which accounts for 9.6% of total extractable radioactivity after 90 days of incubation. During the initial periods of incubation, it was not observed but appeared after 75 days of incubation.

The accumulation of metabolites combined with low levels of $^{14}CO_2$ evolution provides good evidence for a degradation process involving the transformation rather than the mineralization of metalaxyl. The attempt was made to identify the metabolites spectroscopically. Metabolite 2 was identified as metalaxyl acid metabolite. MS data were in good agreement with those of the synthesized one. The daughter ion spectra of protonated metalaxyl and its acid metabolite and their fragmentation scheme from the Bannimantap 90 day soil sample are presented in Figures 4 and 5. The fragmentation of metalaxyl, as well as its acid metabolite, preferentially yields fragment ions, which keep the right part of the molecule intact and lead to the same *m*/*z* values (248, 220, and 192). Additional fragments result from secondary fragmentation pathways: 160 = 192 − 32 (−MeOH), 148 = 220 − 72 (elimination of the right part as ketene, $-C_3H_4O_2$). The fragment ion with *m*/*z* 206 (parent *m*/*z* 266) is probably due to a rearrangement of the original molecule. The amounts of other metabolites were so small that no spectral data could be obtained. In former reports (*10*, *28*) the acid metabolite of metalaxyl was found to be the main metabolite in the soil study.

Conclusion. Our study highlights the high potential of specific Indian soils to degrade metalaxyl. A significant effect of microbial biomass on metalaxyl transformation and formation of bound residues is noticed. The major metabolite is the metalaxyl acid. Metalaxyl and its acid metabolite have a tendency to migrate to deeper soil horizons with a potential to contaminate ground water, particularly in soils with low organic matter and clay content (*34*). It has been noticed that, on average, Indian soils contain low amounts of organic matter, and metalaxyl is extensively used in India on a variety of fruit and vegetable crops due to its broad spectrum activity. Therefore, precautions should be taken for the continuous application of metalaxyl to crops. If use of metalaxyl is greatly increased, the risk of occurrence in ground water must be assessed, as by monitoring studies in the most vulnerable areas in main use regions.

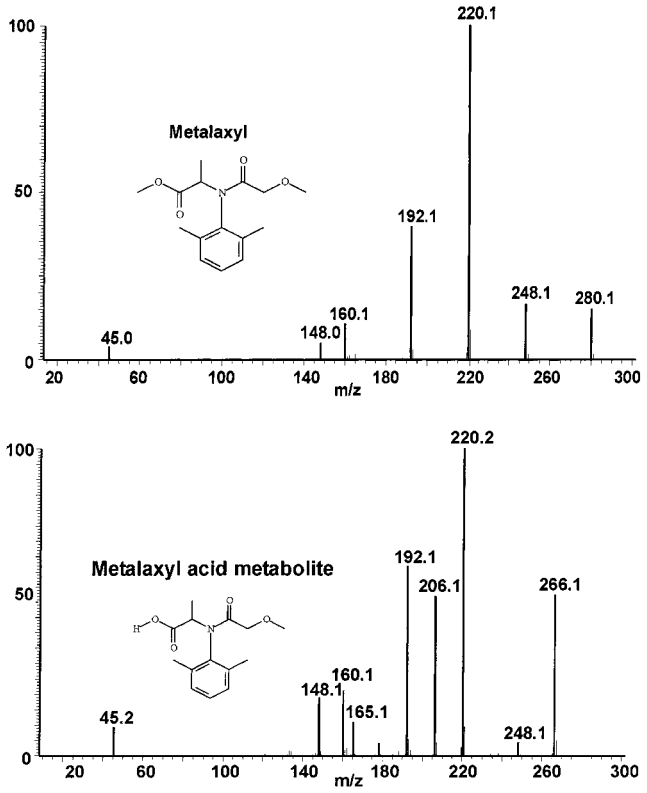

Figure 4. Daughter ion spectra of protonated metalaxyl (280) and protonated metalaxyl acid metabolite (266) at 20 eV collision energy and 1.5 mbar collision cell pressure from Bannimantap 90 day soil sample.

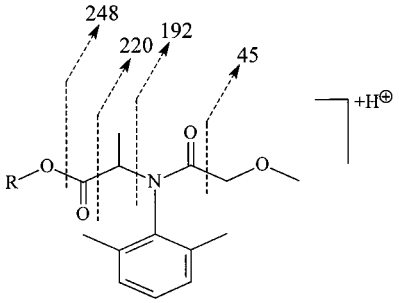

Figure 5. Primary fragmentation pathways of metalaxyl (R = Me) and its acid metabolite (R = H).

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