

# BEHAVIOR OF THE FUNGICIDE METALAXYL IN SOME BRAZILIAN SANDY AND CLAYEY SOILS

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In this study high pressure liquid chromatography (HPLC) was used to measure the adsorption coefficients ( $K_d$ ) and degradation of metalaxyl added to four different Brazilian soils at concentrations of 0.003 and 0.03 g Kg<sup>-1</sup>. Previous *in vitro* studies have shown that soil microorganisms can degrade N-(2,6-dimethylphenyl)-N-(2-methoxyacetyl)-alanine methyl ester (metalaxyl), a fungicide used in agriculture to control diseases caused by fungi from the order *Peronosporales*. The results showed that metalaxyl was not degraded in autoclaved soils during the time-course of the experiments. The HPLC results indicated that at both concentrations the  $K_d$  values were highest and more metalaxyl was degraded in clayey soils than in the other types of investigated soils. It appears that the behavior of metalaxyl in soils differs depending on the application rate, soil characteristics and level of microbial activity.

**KEY-WORDS:** METALAXYL; SOILS – DEGRADATION; FUNGICIDE.

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## 1 INTRODUCTION

Methyl *N*-(2,6-dimethylphenyl)-*N*-(2-methoxyacetyl)-DL-alaninate (metalaxyl) is an important anilide group fungicide used in the control of *Pythium* and *Phytophthora* (ARX, 1987). Metalaxyl is an ionic pesticide (solubility in water 8.4 g L<sup>-1</sup> at 22°C), which is stable at both neutral and acidic pH values and binds to soil primarily by electrostatic mechanisms involving both mineral and organic matter (SUKOP & COOGER, 1992). Its half-life in soil is variable and the influence of soil type, soil microflora and application history has already been demonstrated (BAILEY & COFFEY 1985, 1986).

Metalaxyl is recommended for crops such as apples, citrus fruits, grapes, potatoes and tomatoes (GELMINI, 1991). It has been reported that metalaxyl may be degraded by microorganisms (MUSUMECI & RUEGG, 1986; ZHENG, *et al.*, 1989; ANAN'EVA *et al.*, 1997; TYKVA *et al.*, 1999; SOUDAMINI *et al.*, 1999).

Adsorption and mobility of pesticides in the environment have been directly correlated to rates of degradation. KOOKANA, DI & AYLMOORE (1995) and PENG *et al.* (1995) have suggested that these phenomena may influence the availability of metalaxyl to soil microorganisms and that it is adsorbed onto clay particles and organic matter and has low mobility under these conditions (SUKOP & COGGER, 1992; SHARMA & AWASTHI, 1997).

Pesticide persistence is dependent on several factors, including the characteristics of the molecule, environmental conditions, soil characteristics, adsorption and desorption, degradation processes and the number of pesticide applications in the soil.

Repeated applications of pesticides are sometimes required during crop rotation and these may promote an increase in soil microorganisms able to attack a particular pesticide, a process known as enhanced degradation (RACKE & COATS, 1990).

Few studies on metalaxyl degradation, its transport in soils and plants have been performed in tropical soils and information on the behavior of this fungicide is limited (MUSUMECI & RUEGG, 1986; OSTIZ & MUSUMECI, 1989; PAPINI & ANDREA, 2000). The aim of this study was to gather information on metalaxyl degradation by studying the behavior of this fungicide in four Brazilian tropical soils.

## 2 MATERIALS AND METHODS

### 2.1 SOIL COLLECTION SITES AND SAMPLING PROCEDURE

The laboratory tests were conducted on soil samples from each of four Brazilian sites, three sites being in the state of São Paulo near the towns of Aguaí (22° 03' 45" S; 46° 56' 15" W), Estiva Gerbi (22° 18' 45" S; 46° 56' 15" W) and Jaguariúna (22° 41' 15" S; 46° 56' 15" W) and one near the town of Petrolina (9° 26' 15" S; 40° 33' 45" W) in the state of Pernambuco. At each site 30 random samples were collected at depths of 0-15 cm surface layer, sieved through a 2 mm mesh and stored at 4°C under moisture conditions similar to those in the field, soil moisture being determined gravimetrically.

The soils were classified as Typical Haplorthox (Aguaí), Quartzipsammentic Haplorthox (Estiva Gerbi), Typical Haplorthox (Jaguariúna) and Typical Quartzipsamment (Petrolina) according to the United States Department of Agriculture (USDA) classification. The fungicide methyl *N*-(2,6-dimethylphenyl)-*N*-(2-methoxyacetyl)-DL-alaninate (metalaxyl) was applied to the Estiva Gerbi site in January 1998, the Aguaí site in January 1999 and the Petrolina site in April 1999, while no fungicide was applied to the Jaguariúna site. Aguaí, Estiva Gerbi and Petrolina soils samples presents historical of repeated applications of fungicide. The Jaguariuna soil samples represents a forest soil control, without application historical of metalaxyl.

## 2.2 METALAXYL SUPPLEMENTATION EXPERIMENTS

A recovery experiment was carried out by supplementing soil samples with metalaxyl based on the protocol of DROBY & COFFEY (1991). For each of the four soils, two sets of three 200 g (dry weight) replicates of soil were placed in individual 200 mL flasks and supplemented with metalaxyl using an aqueous solution of a commercial formulation of metalaxyl (Apron® 70SD, Novartis), one set of flasks being supplemented with metalaxyl at 0.003 g kg<sup>-1</sup> and the other at 0.03 g kg<sup>-1</sup>.

All the soils were thoroughly mixed and the moisture content adjusted to 75% water holding capacity and the flasks incubated for 32 days at 28 to 30°C (IBAMA, 1990). After 0, 2, 4, 7, 14, 21, 28 and 32 days of incubation, 20 g sub-samples were removed from each flask and the metalaxyl extracted with ethyl acetate according to the methodology described in section 2.4.

A previous experiment was set up in a similar way except that the flasks were autoclaved at 140 kPa for 60 min and allowed to cool before addition of metalaxyl and, after metalaxyl supplementation the rates of recovery were determined in the samples. The incubation period was of 24 hours. After this time, the extraction procedures of metalaxyl were performed.

## 2.3 METALAXYL ADSORPTION MEASUREMENTS

For each of the four soils, 1 g (dry weight) soil samples were placed in stoppered Teflon centrifuge tubes and different concentrations of metalaxyl added with 5 mL of 0.01 M CaCl<sub>2</sub> solution containing an appropriate concentration of the commercial metalaxyl fungicide Apron® 70SD such that the final metalaxyl concentration in the tubes would be 0.001, 0.003, 0.005, 0.01, 0.020 or 0.03 g L<sup>-1</sup>.

The tubes were shaken for 48 h by inversion using an overhead shaker at 30 revs min<sup>-1</sup>, after which the suspension was centrifuged at 2500 revs min<sup>-1</sup> for 10 minutes and the metalaxyl extracted from the supernatant with ethyl acetate which was then filtered through a 0.45 µm Millipore filter and the concentration analyzed as described in section 2.4.

The amount of metalaxyl retained in each soil was estimated from the decrease in the metalaxyl concentration as detected by HPLC by calculating the adsorption coefficient (K<sub>d</sub>) using the relationship:  $k_d = (1 - c/co)/(c/co) \times L/S$ , where S is the mass of soil in grams, L the volume (5 mL) of 0.01 M calcium chloride solution and c/co the relationship between the area of the concentration peak of the sample (c) and the initial concentration (co) of the solution added minus the concentration of the standard in g kg<sup>-1</sup> (ÁLVAREZ-BENEDI, CARTON & FERNANDEZ, 1998)

## 2.4 SOIL EXTRACTION METHODOLOGY AND HIGH PERFORMANCE LIQUID CHROMATOGRAPH (HPLC)

Metalaxyl was extracted from each soil sample by placing 20 g (dry weight) of the soil in a 200 mL flask, with 100 mL of ethyl acetate, shaking for 4 h at 250 revs min<sup>-1</sup>, filtering the mixture through Whatman No. 1 filter paper, and concentrating the pooled ethyl acetate extract under vacuum in a rotary evaporator at 35-40 °C, the dry soil extract being dissolved in 3 mL of 70:30 (v/v) acetonitrile/water which was then filtered using a 0.45 µm Millipore filter (GETZIN, COGGER & BRISTOW, 1989).

The metalaxyl concentration of the extracts was determined at 210 nm using a Shimadzu model LC10AD HPLC equipped with a 4.6 mm x 25 cm x 5 µm Bondesil C<sub>18</sub> column and a SPD M6A ultraviolet detector, 70:30 (v/v) acetonitrile/water was used as the mobile phase at a flow rate of 1 mL min<sup>-1</sup> and a sample volume of 50 µL (KOOKANA, DI & AYLMOORE, 1995).

The fungicide was identified by comparison of retention time with metalaxyl standards and the final concentration expressed as grams.Kg<sup>-1</sup> soil dry weight. The metalaxyl recovery rates were calculated by linear regression procedures from analysis of standard solutions versus peak area of chromatograms.

Analytical standard metalaxyl (99.6%) was obtained from Novartis® (Basle – Switzerland) and a 0.1 g L<sup>-1</sup> standard solution prepared in 70:30 (v/v) acetonitrile/water and used to obtain working solutions

containing 0.001, 0.003, 0.005, 0.01, 0.020 and 0.04 g L<sup>-1</sup> metalaxyl for use in the HPLC analysis.

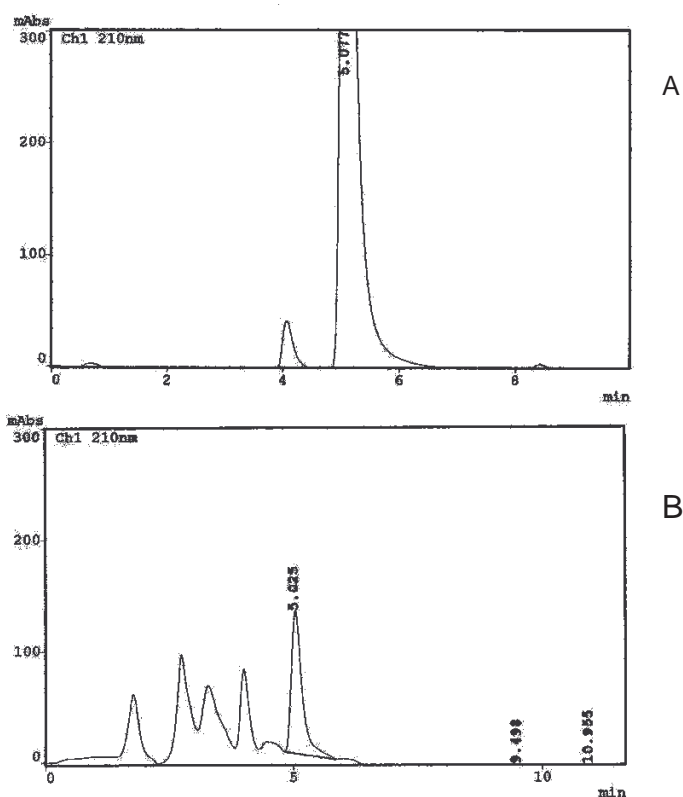
## 2.5 STATISTICAL ANALYSIS

The results at each sampling period and from over the total incubation period were compared using analysis of variance (ANOVA), with treatment as the independent variable. For the effects studies, results were reported as percentages of the control. When treatment responses differed significantly from controls ( $p < 0.05$ ), multiple comparisons were made using paired-t test procedure (DUNNETT, 1955). Effect levels for significant responses were based on the nominal test.

## 3 RESULTS AND DISCUSSION

The methodology described by DROBY and COFFEY (1991) was efficient in recovering metalaxyl from the Brazilian tropical sandy, sandy loam and clay soils investigated. Under the experimental conditions, the ultraviolet spectra of metalaxyl showed a maximum absorption at 210 nm and a HPLC retention time of 5.00 minutes (Figure 1). Recovery test of metalaxyl from the four soils analyzed ranged from 89.3 to 97.5%.

**FIGURE 1 - HIGH PERFORMANCE LIQUID CHROMATOGRAPH (HPLC) RETENTION TIME OF A STANDARD SOLUTION CONTAINING 0.02 g L<sup>-1</sup> OF METALAXYL (A) AND OF METALAXYL EXTRACTED AFTER 32 DAYS INCUBATION FROM PETROLINA SOIL SUPPLEMENTED WITH 0.03 g Kg<sup>-1</sup> OF METALAXYL (B)**



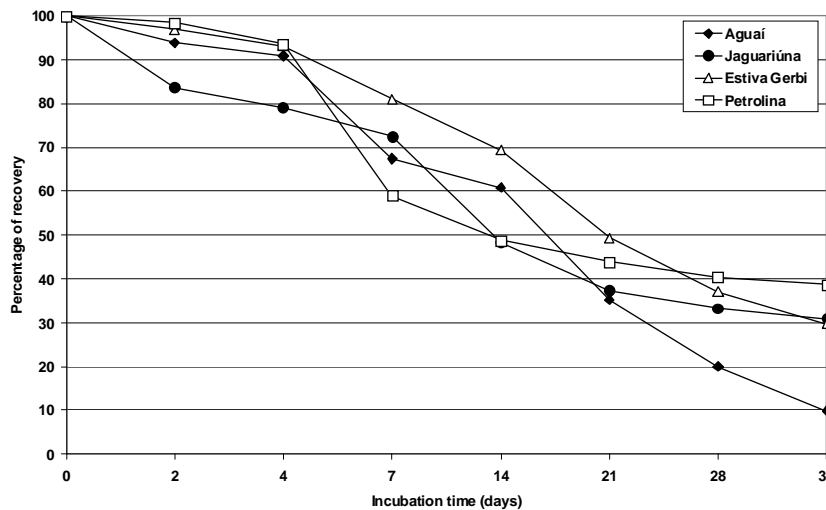
The percentage of metalaxyl remaining in the four non-autoclaved soils during 32 days incubation is shown in Figures 2 and 3, from which it can be seen that there was a reduction in metalaxyl between 55 to 90% during 32 days of incubation.

For non-autoclaved soils supplemented with 0.003 g kg<sup>-1</sup> of metalaxyl the degradation rates in the clayey Aguai soil was about 90%. Only about 60–70% appeared to be degraded in the Jaguariúna, Estiva Gerbi and Petrolina soil samples and there was no significant difference (ANOVA: F = 0.824; p < 0.05; n=3).

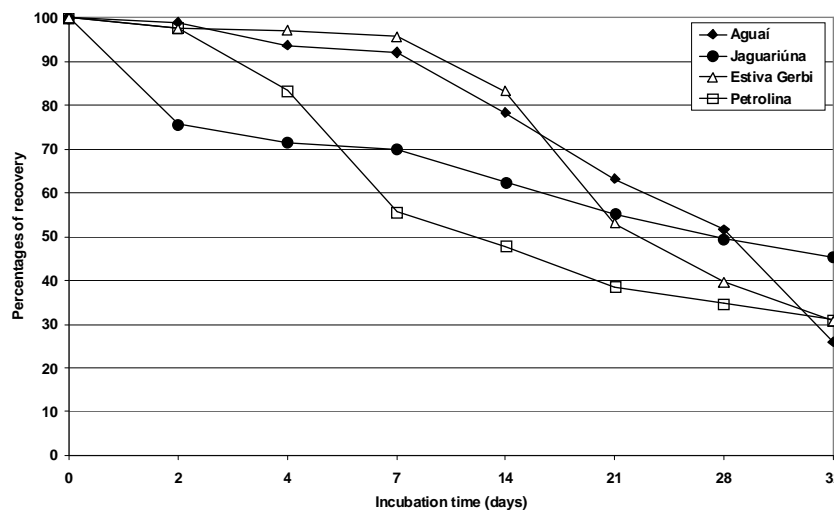
At 0.03 g kg<sup>-1</sup> of metalaxyl the degradation rate was 70–75% in all the soils except for the Jaguariúna soil in which metalaxyl degradation was about 55%. The dissipation rates to clayey metalaxyl-treated soil samples were significantly higher than others soils (p > 0.05).

ANOVA test on the experimental data demonstrated a strong positive correlation between the amount of fungicide dissipated with the initial rates of metalaxyl supplemented and also with the clay content of the soil (p ≥ 0.05). These results suggest that metalaxyl degradation is dependent on several factors and support the view of SHELTON & PARKIN (1991) that sorption of a solute to a soil depends not only on the compound itself but also on the characteristics of the soil.

**FIGURE 2 - METALAXYL RECOVERY OVER A PERIOD OF 32 DAYS FROM SOME BRAZILIAN SOILS SUPPLEMENTED WITH 0.003 g Kg<sup>-1</sup> OF METALAXYL**

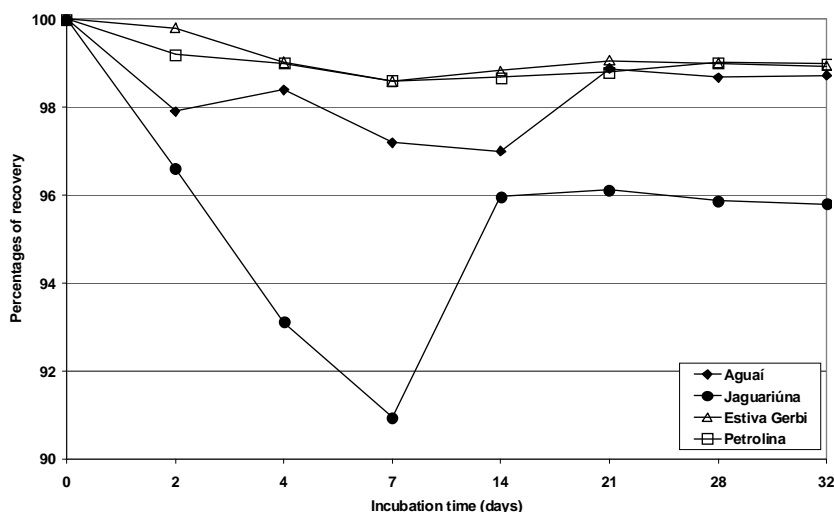


**FIGURE 3 - METALAXYL RECOVERY OVER A PERIOD OF 32 DAYS FROM SOME BRAZILIAN SOILS SUPPLEMENTED WITH 0.03 g Kg<sup>-1</sup> OF METALAXYL**



In the autoclaved soils there was no significant metalaxyl degradation (Figure 4), indicating that this fungicide was not broken down by chemical mechanisms under the conditions used in these experiments, which suggests that biodegradation is a very important pathway for the removal of metalaxyl from soils.

**FIGURE 4 - RECOVERY OF METALAXYL AT 3  $\mu\text{m A.I. g}^{-1}$  METALAXYL, UNTIL 32 DAYS OF SOILS STERILISED INCUBATION**



The metalaxyl adsorption coefficients ( $K_d$ ) were lower for the sandy Estiva Gerbi and Petrolina soils as compared to the clayey Aguaí and Jaguariúna soils (Tabela 1), demonstrating an association between the presence of clay particles and adsorption of metalaxyl.

**TABLE 1 - METALAXYL ADSORPTION COEFFICIENT ( $K_d$ ) FOR THE DIFFERENT SOIL SAMPLES**

Soil	Adsorption coefficients ( $K_d$ )*				
	Metalaxyl concentration ( $\text{g L}^{-1}$ )				
	0.003	0.005	0.01	0.015	0.03
Aguaí	$10.94 \pm 0.92$	$10.02 \pm 0.98$	$9.03 \pm 0.25$	$8.32 \pm 0.34$	$7.90 \pm 0.30$
Estiva Gerbi	$3.00 \pm 0.24$	$1.77 \pm 0.20$	$0.85 \pm 0.07$	$0.81 \pm 0.09$	$0.69 \pm 0.02$
Jaguariúna	$14.65 \pm 1.06$	$16.19 \pm 1.34$	$24.30 \pm 0.97$	$33.54 \pm 1.19$	$41.11 \pm 1.45$
Petrolina	$0.27 \pm 0.03$	$0.28 \pm 0.04$	$0.30 \pm 0.03$	$1.15 \pm 0.11$	$2.00 \pm 0.15$

\* Mean of the values for three samples of the same soil. Coefficient of variation = 1.02%

The high solubility of metalaxyl in water described by TOMLIN (1995) and the  $K_d$  values obtained in present study suggest that metalaxyl molecules have a high affinity for clay particles. Such a phenomenon was also observed by SHAROM & EDGINGTON (1986) and FOMSGAARD (1995) who demonstrated that the mobility of metalaxyl is variable and depends on rainfall and the clay and organic matter contents of soils. SHARMA & AWASTHI (1997) have stated that organic matter is the most important factor in metalaxyl mobility in soils. PETROVIC *et al.* (1998) showed that soils with alkaline pH could favor hydrolysis of metalaxyl, but this was not a factor in this study because all the soils were acidic.

It has been suggested by several authors (SPESSOTO & MONTEIRO, 1995; MONTEIRO, SPESSOTO & LEÃO, 1996, SILVA, *et al.*, 1999) that the lack of information on the risks associated with the inappropriate implementation, best-use practices and downstream contamination from the movement of pesticides could be a huge problem for understanding the fate of pesticides in Brazilian soils.

The present results seem to suggest that sandy soils may be not able to retain metalaxyl for extended periods of time and that this fungicide could easily leach into groundwater ecosystems thus contaminating important water supplies, indicating that precautions should be taken when metalaxyl is continuously applied to crops. This is extremely important and in accordance with the findings presented by DI & AYLMOORE (1997) who studied potential groundwater contamination and detected traces of metalaxyl at depths of up to 300 cm in a sandy soil profile.

#### 4 CONCLUSION

This work highlights the possibility that leaching of metalaxyl from soil to groundwater could potentially occur in the Petrolina region of Pernambuco where this fungicide is continuously applied to sandy vineyard soils with low organic matter content.

#### **COMPORTAMENTO DO FUNGICIDA METALAXIL EM SOLOS BRASILEIROS ARENOSOS E ARGILOSOS**

Utilizou-se a cromatografia a líquido de alta eficiência (CLAE) para quantificar o coeficiente de adsorção (Kd) e a degradação do metalaxil suplementado em quatro diferentes solos brasileiros nas concentrações de 0,003 e 0,03 g Kg<sup>-1</sup>. Os resultados mostraram que o metalaxil não foi degradado em solos autoclavados durante o período de avaliação. Estudos preliminares "in vitro" têm demonstrado que microrganismos do solo podem degradar N-(2,6-dimetilfenil)-N-(2-metoxiacetil)-alanina metil éster (metalaxil), fungicida usado na agricultura para o controle de doenças causadas por fungos da ordem dos *Peronosporales*. Os resultados da avaliação por CLAE revelaram (em ambas as concentrações testadas do fungicida) altos valores de Kd e maior degradação de metalaxil nos solos argilosos que nos demais tipos de solos investigados. Esses dados evidenciaram que o comportamento do metalaxil em solos varia conforme as taxas de aplicação, características do solo e atividade microbiana.

**PALAVRAS-CHAVE:** METALAXIL; SOLOS-DEGRADAÇÃO; FUNGICIDA.

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