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Enantiomerization Of Current-use Chiral Pesticides in Soil

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ENANTIOMERIZATION OF CURRENT-USE CHIRAL PESTICIDES IN SOIL

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ABSTRACT

Pesticides are widely used around the world because, in part, they increase food production, decrease the spread of disease via insects, and protect buildings from damage due to these pests. Chiral pesticides, pesticides which contain molecules that can have at least two stereoisomers, make up about 25% of all pesticides. In order to decrease the mass of pesticides applied to the environment, only the bioactive enantiomer could be marketed as a chiral switch formula; however, if the enantiopure pesticide undergoes enantiomerization in the environment, it would defeat the purpose of marketing such a formula. The goal of this thesis is to elucidate if two chiral pesticides, metalaxyl and malathion, undergo enantiomerization in soil.

Soil was collected at Lake Hartwell near Anderson, SC. The soil was characterized for metal oxide content, trace metals, particle size distribution, pH, and organic carbon. Then, the process of enantiomerization was observed under the following conditions for metalaxyl: acid-unsterilized, lime-unsterilized, acid-sterilized, and lime-sterilized. For malathion, enantiomerization was observed under the following conditions: acid-unsterilized, lime-unsterilized at ambient temperatures, and lime-unsterilized at 10°C. Chiral analysis was performed to determine if enantiomerization took place; achiral analysis was performed to determine mass balance.

Racemic metalaxyl was found to have no statistically significant change in enantiomeric fraction (EF) over two weeks in any of the treatments listed above, which is consistent with previous research. Metalaxyl-M, the chiral switch formula composed of 97% of the R-enantiomer, showed statistically significant differences in both of the

unsterilized treatments, which may be due to the small presence of the (+)-enantiomer, allowing any variation in EF to magnify error and cause a statistically significant difference. There was no evidence of degradation for either formulation over two weeks.

The R-enantiomer for malathion demonstrated a statistically significant change in EF on day three in acid-unsterilized soil while the S-enantiomer and racemic mixture did not. There was also evidence of degradation occurring over three days. For the lime-unsterilized treatments in a 10°C environment, statistically significant differences in EF were found in all three incubations over three days. For the lime-unsterilized treatments at ambient temperature, there was a statistically significant change in EF for R-malathion but not for S-malathion. There was evidence of degradation for all incubations in both 10°C and ambient temperatures; however, degradation was much slower for the incubations in the 10°C environment. These observations support the hypothesis that metalaxyl will not undergo enantiomerization in the environment while malathion will.

DEDICATION

This thesis is dedicated to my parents, Jerome and Manuela Rollerson. Thank you for your never ending love and support throughout the years.

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I would like to thank my advisor, Dr. Cindy Lee, for all of her help and support over these past two years. I am not only grateful for her scientific insights, but also for the many comments and suggestions for my writing over the years. I would also like to acknowledge my committee members, Dr. Elizabeth Carraway and Dr. Lindsay Shuller-Nickles for their valuable insight on the soil analysis. I am also grateful to the EE&ES department for their support.

I would like to give a very special thanks to Rodney Campbell and the rest of Mineral Labs, Inc. for providing the soil analysis free of charge. This research would have not been possible if it were not for your generosity, thank you.

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CHAPTER ONE

INTRODUCTION

Overview

Pesticides are widely used around the world because, in part, they increase food production, decrease the spread of disease via insects, and protect buildings from damage due to pests. Chiral pesticides, which contain molecules that can have at least two stereoisomers, make up about 25% of all pesticides (Williams 1996). However, there is a concern about the effects of pesticides on non-target organisms. For example, the LD_{50} of metalaxyl in rats is 669 mg/kg, and metalaxyl has been shown to cause cellular enlargement in the livers of rats fed 62.5 mg/kg of 90 days (PMEP 1993). The LD_{50} of malathion in rats is between 5400 and 5700 mg/kg. Malathion is also very toxic to bees, beneficial insects, and aquatic invertebrates (Gervais 2009). A decrease in the amount of pesticides applied to the environment may decrease the potential of negative effects to non-target organisms. One way to decrease the mass is to manufacture only the effective enantiomer of chiral pesticides.

Enantiomers are structures that are mirror images and non-superimposable. They have the same structure, therefore, have the same chemical and physical properties. However, they will behave differently in the presence of asymmetrical solids and enzymes. They are denoted with an R or S configuration, which indicates the placement of the functional groups on the chiral center. In addition, a (+) or (-) indicates the direction in which a plane of polarized light will rotate due to the enantiomers. The R-

and S- and (+) and (-) labels are not related. When one enantiomer is converted to its mirror image, it is said to undergo enantiomerization or racemization.

Chiral pesticides are usually sold as a racemic mixture, which contains an equal mass of both enantiomers. Usually only one enantiomer is effective for the target organism, but one, both, or neither enantiomer may have negative effects on non-target organisms. For example, R-(+)-malathion is more toxic to bees and earthworms than S-(-)-malathion (Sun 2012). Manufacturers could market only the most effective enantiomer of the pesticide, known as a "chiral switch" formula. However, if the pesticide undergoes rapid enantiomerization in the environment, it defeats the purpose of selling the single enantiomer formula. In addition, the degradation rate for each enantiomer needs to be taken into consideration.

Previous research has shown that pesticides with a hydrogen on the chiral carbon, that is an acidic hydrogen, undergo rapid enantiomerization, both in protic liquids and pure solids (e.g., Li et al. 2010 and Hall 2012). Those pesticides which do not have an acidic hydrogen undergo more limited enantiomerization (Li et al. 2010). This project aims to study the enantiomerization of chiral pesticides with one hydrogen per chiral carbon in a well-characterized soil.

Based on previous research, two current-use chiral pesticides were investigated to understand whether they undergo enantiomerization in soil (Hall 2012). Metalaxyl is a systemic phenylamide fungicide. It is available as a racemic mixture, as well as a chiral switch formula, metalaxyl-M, which is made of approximately 97% of the bioactive R-enantiomer (Hall 2012). Malathion is an organophosphate insecticide used on crops and

in some lice treatments. It is composed of two enantiomers, R-(+), which is the bioactive enantiomer, and S-(-), the inactive enantiomer; however, it is only sold as a racemic mixture.

Literature Review

Buser and Müller (1997) explored the mechanism for the enantiomerization of phenoxyalkanoic acid herbicides 2-(4-chloro-2-methylphenoxy)propionic acid (MCPP) and 2-(2,4-dichlorophenoxy)propionic acid (DCPP) using soil and deuterated water (D_2O) under laboratory conditions. They tracked the movement of the deuterium on the chiral carbon using the deuterated water and tandem mass spectrometry. They hypothesized that the pesticides either formed a carbanion intermediate or an enoic acid intermediate (Figure 1.1). A carbanion is a molecule that has a negatively charged carbon atom, and an enoic acid indicates a molecule that possesses an alkene and a carboxylic acid group. They concluded from the H – D transfer that enantiomerization occurred via a carbanion intermediate.

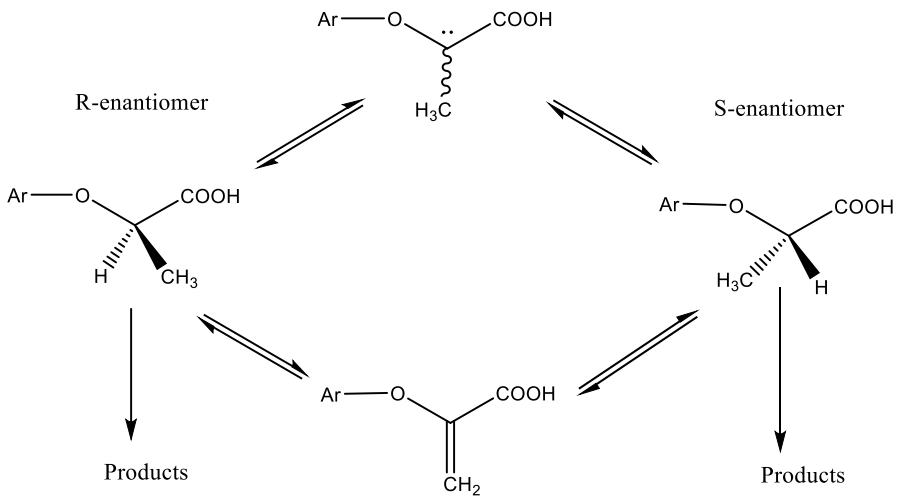


Figure 1.1. Two hypothetical pathways for the enantiomerization of MCPP and DCPP. The top pathway is the carbanion intermediate. The bottom pathway is the enoic acid intermediate (Adapted from Buser and Müller, 1997).

In addition, Buser et al. (2002) studied the chiral stability and enantioselective degradation of metalaxyl in a sandy loam soil (pH=7) over the course of three months. They studied this phenomena by incubating racemic metalaxyl along with the pure enantiomers of metalaxyl. They found that for the enantiopure incubations, there was negligible formation of the other enantiomer over the course of the incubation. For the racemic incubations, they found that the R enantiomer degraded more rapidly than the S enantiomer. However in a later study, Buerge et al. (2003) found that enantioselective degradation was pH dependent, with the R enantiomer dissipating faster in high pH soils

and the S enantiomer dissipating faster in low pH soils. It should be noted that Buerge et al. (2003) did not conduct any enantiopure incubations.

Li et al. (2010) studied various types of pesticides and how they behaved in organic solvents and water. The pesticides included those with a phosphorus and carbon chiral center and those with and without a hydrogen on the chiral carbon (Figure 1.2). In addition, they studied the effects that pH and temperature had on the rate of enantiomerization. They found that those pesticides which did not have an acidic hydrogen on the chiral carbon were stable because the enantiomers did not undergo conversion in organic solvents and water. However, pesticides with an acidic hydrogen, such as malathion, phentoate, and fenpropathrin, were found to undergo enantiomerization in protic solvents such as methanol and ethanol, and deionized water. Moreover, the rate of enantiomerization for these pesticides was pH dependent; enantiomerization took place more rapidly at a higher pH (7.0) than at a lower pH (5.8). However, no enantiomerization took place in non-protic solvents such as hexane and acetone. Moreover, they expanded on the mechanism of the hydrogen removal found in the study by Buser and Müller (1997). They concluded that the ability of a pesticide to undergo enantiomerization depended on the acidity of the hydrogen on the chiral carbon. The acidity of the hydrogen is determined by the amount of carbanion stabilizing groups (groups that are electron withdrawing, such as ketones, esters, and cyano groups) on the chiral carbon.

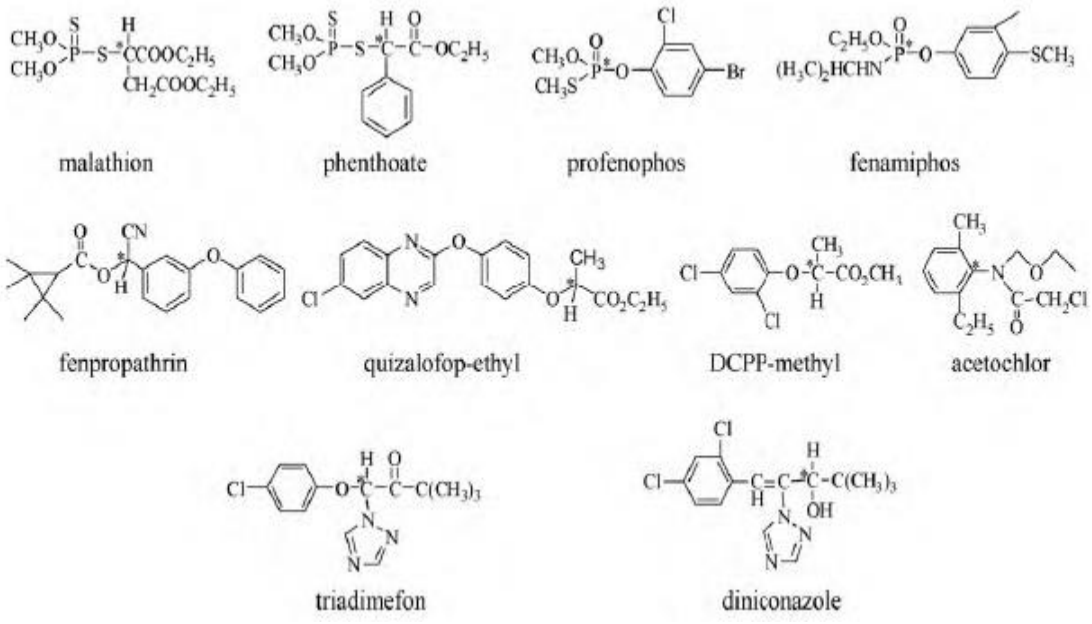


Figure 1.2. Structures of the chiral pesticides studied in Li et al. (2007). The chiral center is denoted with an *.

Li et al. (2007) studied the chiral stability of phenthoate in soil. They used racemic phenthoate and collected only the bioactive (+) enantiomer of phenthoate through a separation procedure using high pressure liquid chromatography (HPLC). They incubated the racemate and (+) enantiomer in a garden soil (alkaline sandy loam; pH=8.2) and an agricultural soil (acidic light clay loam; pH=5.4) with sterile and nonsterile treatments over the course of 13 days. In the case of the racemic incubation, the enantiomeric ratios, which is the ratio of one enantiomer with respect to the other, decreased more in the alkaline soil than the acidic soils However, the decrease was due to degradation, not enantiomerization. It should be noted that enantiomeric ratios (ER) are not the preferred method of determining chiral stability because the ER is undefined

when only one enantiomer is present and can present problems when determining the environmental fate (Eish and Wells, 2008). Therefore analysis using enantiomeric fractions (EF) is the preferred method and is used in later literature. For the (+) – phenthoate incubation, Li et al. (2007) observed no conversion of the enantiomer in acidic soil; but they did observe conversion in the alkaline soil, in both the sterile and non – sterile experiments. They noted that there was higher conversion of (+) – phenthoate in the sterilized soils; thus they concluded that microorganisms may inhibit conversion. In addition, they observed the same degradation and enantiomerization effects in pure water at the same pH values used in the soil incubations. Their conclusion was that enantiomerization of phenthoate may be due to the presence of water in the soil, not the soil itself. In addition, they concluded that the reaction is pH dependent.

Li et al. (2009) also researched the chiral stability of fenpropathrin in soils. The soils were the same soils used in the phenthoate experiment (Li et al., 2007), and the experimental conditions were also nearly the same. They prepared the bioactive S-enantiomer of fenpropathrin via separation and collection on the HPLC. The experiment took place over 55 days. They found that there was no conversion in the acidic soils, but significant conversion in the alkaline soil (both sterile and nonsterile), which was the same observation for phenthoate. They saw the same phenomenon in methanol combined with buffer solutions. They concluded that, like phenthoate, conversion of the S – fenpropathrin enantiomer is chemically induced and only happens under alkaline conditions.

Sun et al. (2011) investigated the enantiomerization of malathion in environmental soil and water samples. They collected soils from five different agricultural sites in China, all varying in pH (4.8 – 8.1), soil texture, and organic carbon content. In addition, they collected five water samples from different channels in Beijing; these varied in conductivity, microorganism count and pH (about 6 – 8.5). They found that in the incubation of R – (+) malathion in one of the higher pH soils (pH=8.1), the enantiomeric fraction (EF), which is the fraction of the (+)-enantiomer present in the soil, decreased from 1.0 to 0.76 in the span of one hour, 0.50 at one day, and finally 0.29 at seven days (with both enantiomers degrading to their minimum observed concentrations). In a lower pH soil (pH=6.9), the rate of enantiomerization was slower, reaching an EF of 0.58 in seven days. In the incubation of the S – (-) enantiomer, the EF reached 0.5 in the span of six hours in the pH=8.1 soil and 0.42 in seven days in the pH=6.9 soil. In one of the soil with pH=5.0, there was no interconversion of either enantiomer observed over the course of 15 days. In three of the water samples (pH=8.24, 7.8, and 6.01) spiked with R – (+) malathion, they observed that the EF reached 0.5 in the span of 3 – 24 hours; in addition, they had similar results with water spiked with the S – (-) malathion.

Hall (2012) investigated the role of pure minerals with chiral surfaces in the enantiomerization of malathion and metalaxyl. The minerals included calcite, bentonite, kaolinite, and montmorillonite. When individual malathion enantiomers were incubated in an aqueous solution with no solids, she found that both enantiomers transformed towards the racemic mixture over the span of 13 days, which was consistent with the findings of Li et al. (2010) and Sun et al. (2011) in environmental water samples.

However, when the malathion enantiomers came into contact with the sorbents listed above, the transformation required less than two hours. With racemic metalaxyl and its chiral switch formula, metalaxyl-M, the EF increased after 11 days in water, indicating the formation of the S-enantiomer. In the samples containing the pure minerals, she saw no statistically significant changes in the EF of racemic metalaxyl after 24 hrs. However, she did observe an increase in EF for metalaxyl-M in the presence of bentonite and montmorillonite. She stated the observed increase could be due to either enantioselective sorption of the R-enantiomer or the small percentage of the S-enantiomer in metalaxyl-M causing an error in quantitation (Hall 2012). The reason for the lack of conversion of metalaxyl may be the electron-donating alkyl group on the chiral carbon preventing the formation of the carbanion and loss of the hydrogen.

CHAPTER TWO

RESEARCH OBJECTIVES

The goal of this work was to determine the enantiomeric behavior of the chiral pesticides metalaxyl and malathion in an acid soil and a soil treated with lime. Another goal was to determine the suitability of an accelerated solvent extractor (ASE) for enantiomer analysis. My hypotheses for this project were as follows.

2.1. Although metalaxyl has an acidic methane hydrogen, the electron-donating alkyl group on the chiral carbon will prevent racemic metalaxyl and its chiral switch formula, metalaxyl-M, from undergoing any significant enantiomerization in soil.

2.2. Since malathion has an acidic methane hydrogen and electron withdrawing groups on the chiral carbon, the individual malathion enantiomers will undergo enantiomerization in limed soil, but not in acid soil. Since both enantiomers undergo conversion, I expect the EF of racemic malathion to stay fairly consistent.

2.3. I expect degradation for both pesticides to increase in the lime-treated soil, since previous literature found that higher pH increases the rate of degradation.

CHAPTER THREE

METHODS AND MATERIALS

Metalaxyl in Soil Experiments

Racemic metalaxyl and metalaxyl-M (Pestanal™, analytical grade) were obtained from Sigma-Aldrich. Acetone (reagent grade), methanol (LC/MS grade), hexane (HPLC grade), isopropyl alcohol (HPLC grade), and sodium sulfate (10-60 mesh) were obtained from Fisher Chemical. Acetonitrile (HPLC grade) was obtained from EMD Millipore Corps (Billerica, MA). Silica sand was obtained from Wedron Silica Company (Wedron, IL). Deionized distilled water (DDI) was used for all experiments. Soil was collected near Rich Laboratory (See Figure A-1 for map), which is located on Lake Hartwell in Anderson County, South Carolina. For complete soil analysis, see Table 4.1.

Soil Incubation Experiments

The experimental setup was adapted from Buser et al. (2002), which had a duration for metalaxyl and metalaxyl – M incubation of 60 d; other studies had incubations up to 120 d. However, in the Buser study and other studies, the goal was not only to study chiral stability, but also observe enantioselective degradation; therefore, the incubations took place over several months. Since this project aimed to elucidate only enantiomerization, the experiments were ended at 14 d, since metalaxyl did not show any statistically significant change in EF over 14 d.

Soil was air dried in a fume hood then sieved using a No. 18 sieve (1 mm) to remove any rocks and large debris. Incubation experiments were run in triplicate in

Mason jars, with 60 grams of soil placed in each jar. Ten mL of the 25 mg/L standard of racemic metalaxyl and metalaxyl-M were added to the soil and allowed to evaporate for a final concentration of 4.2 mg/kg. Then, 13 mL of water were added to the soil to give a final moisture content of ~18%. The jars were covered with aluminum foil to protect them from light and opened periodically to add water to retain the 18% moisture content and stir the soil. The vapor pressure of metalaxyl is $5.62X10^{-6}$ mm Hg at 25°C, and the Henry's Law constant is $3.0X10^{-9}$ atm-m^3/mole (Tomlin 1997); therefore volatilization is not expected to change the concentration of metalaxyl in soil. In a second treatment, between 70 and 75 mg of hydrated lime was added to the soil in order to raise the pH from 5.3 (see soil characterization information below) to approximately 6.8, which was determined by adding 5 mL of DDI water to 5 g of soil and checking the pH after 10 minutes (McLean 1982). Two additional treatments included sterilizing the untreated (acid) and limed soils. The soil and jars were sterilized in an autoclave at 120°C for 20 min each day for two days. These treatments are summarized in Table 3.1.

Table 3.1: Metalaxyl Incubation Treatments

Experiment	Treatment	
Racemic metalaxyl	Acid unsterilized	Acid sterilized
Racemic metalaxyl	Lime unsterilized	Lime sterilized
Metalaxyl-M	Acid unsterilized	Acid sterilized
Metalaxy-M	Lime unsterilized	Lime sterilized

Ten g (dry weight) of spiked soil was removed from the jars and placed in 50 mL glass centrifuge tubes at the following time points:

- 0 hr
- 2 hr
- 1 d
- 3 d
- 7 d
- 14 d

Samples were kept in the freezer at -15°C until extraction and analysis.

Extraction and Cleanup

Extraction was done using a Dionex Accelerated Solvent Extractor 200 (ASE 200). The conditions used were adapted from Gan et al (1999). Samples (10 g) were placed in 33 mL stainless steel cells, to which 15 g of sodium sulfate were added, and the remaining space filled with silica sand; both ends were capped with glass wool. The extracting solvent was methanol, the oven temperature was set at 100°C, and the pressure was set at 1500 psi. There was a 5 min heating time followed by a 5 min static time, which was repeated for two cycles, finishing with a 90 s purge with nitrogen. Then, to remove any remaining water, the extract was run through a column with 5 g anhydrous sodium sulfate conditioned with 2 mL of methanol.

Chiral Metalaxyl Analysis

The chiral analysis to determine if enantiomerization took place was adapted from Hall (2012). The analysis was performed on a Dionex UltiMate 3000 high performance liquid chromatograph (HPLC) with a Dionex UltiMate 3000 Variable Wavelength UV-vis detector equipped with Chromeleon software. The column used was a 4.6 mm x 250 mm Chiralcel® OJ® packed with cellulose tris – (4 – methylbenzoate) coated on a 10 μm silica gel substrate (Chiral Technologies, West Chester, PA), which was suitable to separate the metalaxyl enantiomers. The mobile phase was 90:10 hexane:isopropyl alcohol at a flow rate of 0.9 mL/min, with a column temperature of 25°C and a sampler temperature of 10°C. The injection volume was 100 μL, and the metalaxyl enantiomers were analyzed at a wavelength of 210 nm. For the samples extracted from soil, the acquisition time was started at 8.5 mins to eliminate the large peaks that eluted at the beginning of the run and obtain a better image of the analyte peaks. For the racemic metalaxyl standards, S-(+)-metalaxyl eluted first at 11 min and R-(-)-metalaxyl at about 15 min (Figure 3.1a). The metalaxyl-M standard confirmed the elution of the R-(-)-enantiomer at about 15 min (Figure 3.1b).

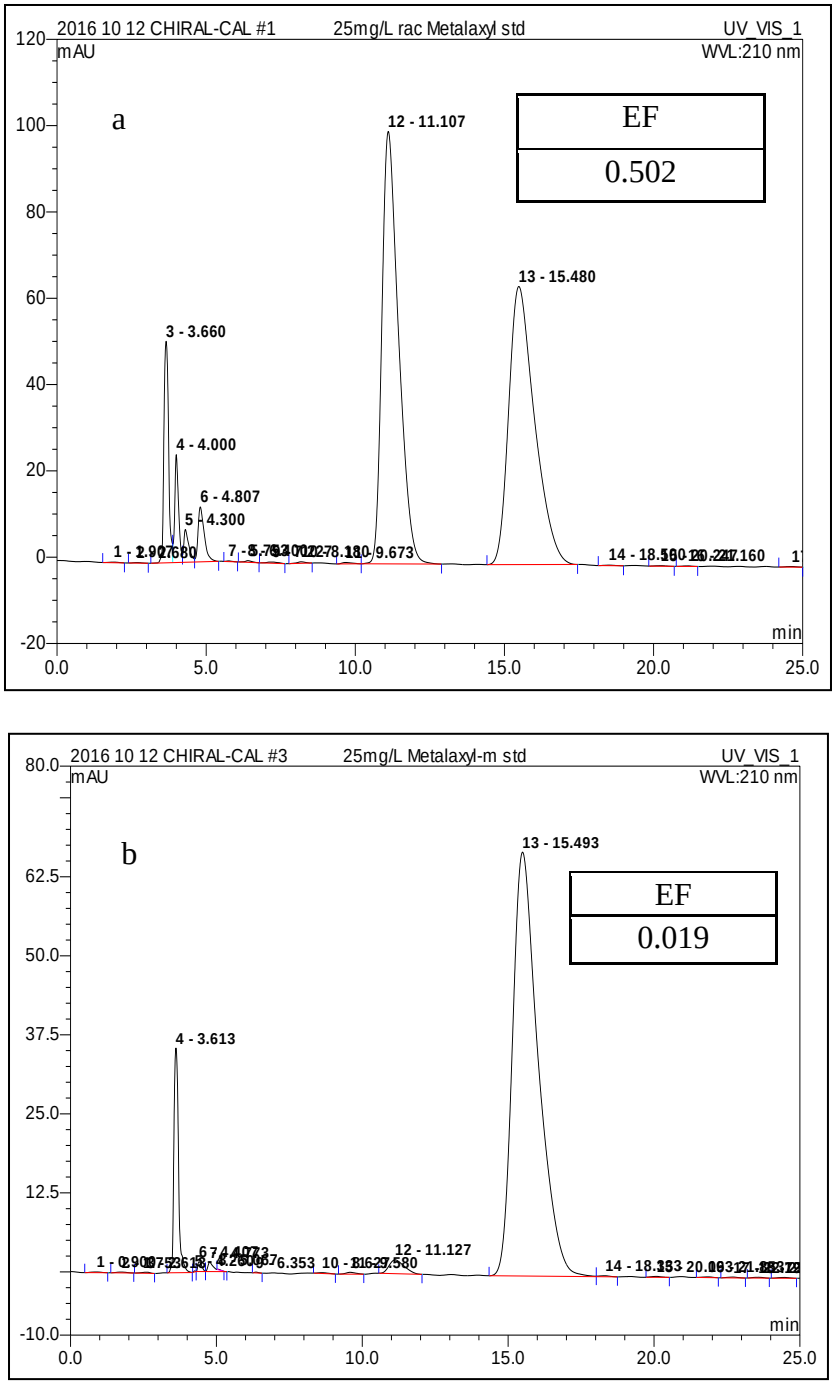


Figure 3.1. Chromatogram of (a) 25 mg/L standard of racemic metalaxyl and (b) 25 mg/L standard of metalaxyl-M, with S-(+)-metalaxyl eluting at 11 minutes and R-(-)-metalaxyl eluting at 15.5 minutes.

In the samples extracted from soil, however, the S enantiomer eluted between approximately 12.5 and 13 min and the R enantiomer eluted between approximately 19.5 and 20 min (Figure A-2).

The enantiomeric fraction (EF) was calculated using the following equation:

$$EF = \frac{S\ -(+)}{R-(-)+S-(+)} \qquad 3.1$$

where R-(-) and S-(+) are the peak areas of the metalaxyl enantiomers. An EF>0.5 indicates a greater concentration of the S-(+)-enantiomer, and an EF<0.5 indicates a greater concentration of the R-(-)-enantiomer. The EF of the racemic metalaxyl standards was 0.50 ± 0.002 (n=3), and the EF of the metalaxyl-M standards was 0.022 ± 0.003 (n=3). An extraction of unspiked soil found no trace of metalaxyl (Figure A-3). After the dehydration step with the $NaSO_4$ column, half of the metalaxyl containing solution was placed in a 100 mL evaporating flask and put on a rotary evaporator in a 65°C water bath and evaporated to dryness and subsequently reconstituted in 2 mL of hexane. Finally, the sample in hexane was passed through a 0.45 μm PFTE syringe filter into a 2 mL autosampler vial and analyzed.

Achiral Metalaxyl Analysis

Achiral analysis to determine recovery and mass balance was adapted from Hall (2012). Analysis was performed on a Dionex UltiMate 3000 HPLC with a UV spectrophotometer equipped with Chromeleon software. The column used was a Zorbax

SB – C18 rapid resolution column, 3.5 μm pore size, 4.6 x 100 mm (Agilent Technologies, Wilmington, DE). The mobile phase started as a 50:50 isocratic phase of acetonitrile and DDI water. However, due to a large pressure increase in the column, I changed the mobile phase to an isocratic phase of 70:30 acetonitrile and DDI water. The flow rate was 1 mL/min, with a 50 μL injection, and a column temperature of 25°C. Absorbance was measured at 210 nm, with metalaxyl eluting at about 2 min (Figure A-4). For the sample analysis, the acquisition time was started at 1.25 min to eliminate large peaks that eluted at the beginning of the run. Sample preparation was the same as that of the chiral analysis explained above, with the exception that the sample was reconstituted in a mixture of 1 mL acetonitrile and 1 mL of DDI water. Finally, the sample in acetonitrile/water was passed through a 0.45 μm PFTE syringe filter into a 2 mL autosampler vial and analyzed. Recoveries for the metalaxyl extractions, determined by spiking and extracting 10 g of soil, were 96.9% ± 11.8% (n=3).

Calibration Standards

Standards of 3.125, 6.25, 12.5, 25, and 50 mg/L of metalaxyl were made in acetone. A 25 mg/L standard of metalaxyl-M was made and checked alongside 25 mg/L racemic metalaxyl as a quality control measure. The limit of detection (LOD) was 0.037 mg/L for racemic metalaxyl. The fit (R^2) of the calibration curve was 0.993. Since racemic metalaxyl and metalaxyl-M are the same in the achiral sense, I assumed the LOD and fit would be the same for metalaxyl-M.

Malathion in Soil Experiments

Malathion (Pestanal™, analytical grade) was obtained from Sigma – Aldrich. Acetone (reagent grade), ethyl acetate (HPLC grade), hexane (HPLC grade), isopropyl alcohol (HPLC grade), and sodium sulfate (10 – 60 mesh) were obtained from Fisher Chemical. Acetonitrile (HPLC grade) was obtained from EMD Millipore Corps (Billerica, MA). Silica sand was obtained from Wedron Silica Company (Wedron, IL). Deionized distilled water (DDI) was used for all experiments. The same soil collected for the metalaxyl experiments was used for the malathion experiments. For complete soil analysis, see Table 4.1.

Enantiomer Separation and Collection

Since malathion is not sold as a chiral switch formula, I made a 1 mg/mL standard of the racemic malathion, separated, and collected the enantiomers at the UV outlet on the HPLC. The HPLC conditions are provided below in the Chiral Analysis section. Purities for each enantiomer were >99%, determined through chiral analysis (Figure A-5). The final concentration of each enantiomer was 26 mg/L, determined through achiral analysis.

Soil Incubation Experiments

Soil was air dried in a fume hood then sieved using a No. 18 sieve (1 mm) to remove any rocks and large debris. Incubation experiments were run in triplicate in Mason jars, with 50 g of soil placed in each jar. Ten mL of the 25 mg/L standard of racemic malathion and each of the separated enantiomers (26 mg/L) were added to the

soil for a final concentration of about 5 mg/kg. Then, 11 mL of water were added to the soil to give a final moisture content of ~18%. The jars were covered with aluminum foil to protect them from light.

The vapor pressure of malathion is 3.97 x 10^{-5} mm Hg at 30°C (MacBean 2010), and the Henry's Law constant is 4.89 x 10^{-9} atm-m^3/mole (Fendinger and Glotfelty 1990); therefore, volatilization is not expected to change the concentration of malathion in the soil. In a second treatment, between 70 and 75 mg of hydrated lime was added to the soil in order to raise the pH from 5.3 (see soil characterization information below) to approximately 7.1, which was determined by adding 5 mL of DDI water to 5 g of soil and checking the pH after 10 minutes (McLean 1982). To determine if there was any difference in the rate of enantiomerization due to temperature, one set of jars was placed in a 10°C refrigerator and the other set was placed on the counter at ambient temperatures. The treatments are summarized in Table 3.2.

Table 3.2: Malathion Incubation Treatments

Experiment	Treatment	
R-(+)-malathion	Acid	-
R-(+)-malathion	Lime ambient	Lime 10°C
S-(-)-malathion	Acid	-
S-(-)-malathion	Lime ambient	Lime 10°C
Racemic malathion	Acid	-
Racemic malathion	-	Lime 10°C

Note: all treatments are unsterilized.

The jars were opened periodically to add water and stir the soil. Ten g of sample was removed from the jars at the following time points:

- 0 hrs
- 1 hr
- 2 hrs
- 1 d
- 3 d

Samples were kept in the freezer at -15°C until extraction and analysis.

Extraction and Cleanup

Extraction was done using a Dionex Accelerated Solvent Extractor 200 (ASE 200). The conditions used were adapted from Gan et al (1999). The extracting solvent was ethyl acetate, the oven temperature was set at 65°C, and the pressure was set at 1500 psi. There was a 5 min heating time followed by a 10 min static time, which was repeated for two cycles, and finished with a 90 s purge with nitrogen. Then, to remove any remaining water, the extract was run through a column with 5 g anhydrous sodium sulfate conditioned with 2 mL of ethyl acetate.

Chiral Malathion Analysis

Chiral analysis to determine if enantiomerization took place was adapted from Hall (2012). The analysis was performed on a Dionex UltiMate 3000 with a Dionex UltiMate 3000 Variable Wavelength UV-vis detector equipped with Chromeleon

software. The column used was a 4.6 mm x 250 mm Chiralcel® OJ® packed with cellulose tris – (4 – methylbenzoate) coated on a 10 μm silica gel substrate (Chiral Technologies, West Chester, PA), which was able to separate enantiomers. The mobile phase was 90:10 hexane:isopropyl alcohol at a flow rate of 0.9 mL/min, with a column temperature of 20°C and a sampler temperature of 10°C. The injection volume was 100 μL, and the malathion enantiomers were analyzed at a wavelength of 210 nm. For the malathion standards, R-(+)-malathion eluted first at 14 minutes and S-(-)-malathion at about 19 minutes (Figure 3.2).

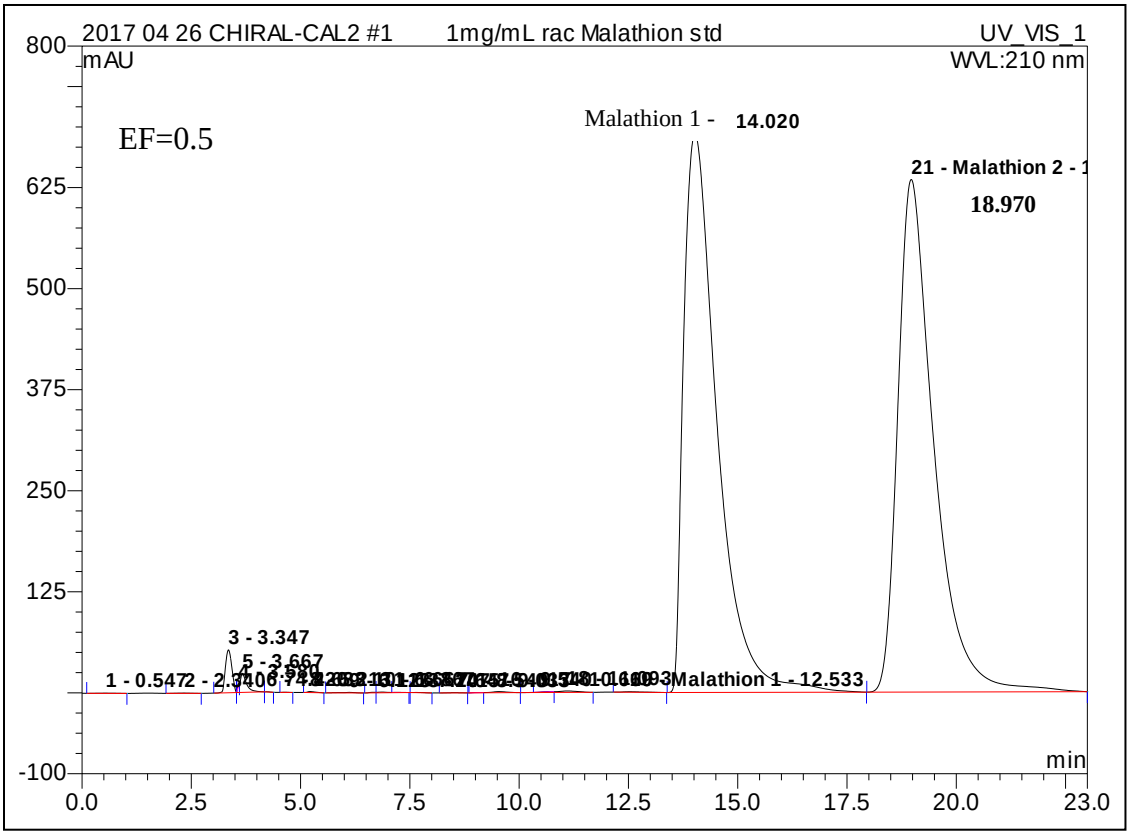


Figure 3.2. Chiral chromatogram of 1 mg/mL of racemic malathion, with R-(+)-malathion (malathion 1) eluting at 14 minutes and S-(-)-malathion (malathion 2) eluting at 19 minutes.

In the samples extracted from soil, however, the S enantiomer eluted between approximately 12.5 and 13 minutes and the R enantiomer eluted between approximately 17.5 and 18 minutes (Figure A-6). The acquisition time was started at 8 min to eliminate large peaks that eluted at the beginning of the run. The enantiomeric fraction (EF) for malathion was calculated using the following equation:

$$EF = \frac{R-(+)}{R-(+)+S-(-)} \qquad 3.2$$

where R-(+) and S-(-) are the peak areas of the malathion enantiomers. An EF>0.5 indicates a greater concentration of the R-(+)-enantiomer, and an EF<0.5 indicates a greater concentration of the S-(-)-enantiomer. The EF of the racemic malathion standards was 0.50 ± .0012 (n=3). An extraction of unspiked soil found no trace of malathion (Figure A-3). After the dehydration step, half of the malathion containing solution was placed in a 100 mL evaporating flask and put on a rotary evaporator in a 65°C water bath and evaporated to dryness and subsequently reconstituted in 2 mL of hexane. Finally, the sample containing hexane was passed through a 0.45 μm PFTE syringe filter into a 2 mL autosampler vial and analyzed.

Achiral Malathion Analysis

Achiral analysis to determine recovery and mass balance was adapted from Hall (2012). The analysis was performed on a Dionex UltiMate 3000 HPLC with a Dionex UltiMate 3000 Variable Wavelength UV-vis detector equipped with Chromeleon software. The column used was a Zorbax SB – C18 rapid resolution column, 3.5 μm pore size, 4.6 x 100 mm (Agilent Technologies, Wilmington, DE). The mobile phase consisted of an isocratic phase of 70:30 acetonitrile (ACN) and DDI water with a flow rate of 1 mL/min, 50 μL injection, and a column temperature of 25°C. Absorbance was measured at 210 nm, with malathion eluting at about 2.5 minutes (Figure A-7). The acquisition time was started at 2 mins to eliminate large peaks that eluted at the beginning of the run. After the dehydration step, half of the malathion containing solution was placed in a 100 mL evaporating flask and put on a rotary evaporator in a 65°C water bath and evaporated

to dryness and subsequently reconstituted in a mixture of 1 mL acetonitrile and 1 mL of DDI water. Finally, the ACN:water mixture was passed through a 0.45 μm PFTE syringe filter into a 2 mL autosampler vial and analyzed. Recoveries for the malathion extraction, determined by spiking 10 g of soil, was 84.9% ± 6.9% (n=3).

Calibration Standards

Standards of 2.5, 5, 12.5, 25, and 50 mg/L of malathion were made in acetone. The fit (R^2) was 0.998 and the limit of detection (LOD) was 0.043 mg/L.

Statistical Analysis

For both pesticides, statistical analysis was performed to determine if the changes in the enantiomeric fraction were significant. This was done by using single factor ANOVA in Microsoft Excel, using $\alpha = 0.05$. For treatments that indicated a statistically significant difference with Excel, I used SAS® (v. 9.4, SAS Institute Inc., Cary, NC, USA) to determine which time points were significantly different.

CHAPTER FOUR

RESULTS AND DISCUSSION

Soil Analysis

The soil used for this work was collected near Rich Laboratory, which is on Lake Hartwell in Anderson County, SC. Mineral Labs, Inc. (Salyersville, KY) characterized the soil by determining its metal oxide analysis (determine via atomic absorption /inductively-coupled plasma/X-ray fluorescence), particle size distribution (by pipette), pH, and other properties. The results are summarized in the following tables (Table 4.1 and 4.2).

Table 4.1: Metal Oxide Analysis (Mineral Labs, Inc.)	
Metal Oxide Analysis	% Wt.
silicon dioxide (SiO_2)	61.65
aluminum dioxide (Al_2O_3)	20.58
titanium dioxide (TiO_2)	1.61
iron oxide (Fe_2O_3)	9.20
calcium oxide (CaO)	0.37
magnesium oxide (MgO)	1.20
potassium oxide (K_2O)	1.37
sodium oxide (Na_2O)	0.24
sulfur trioxide (SO_3)	0.14
phosphorus pentoxide (P_2O_5)	0.15
strontium oxide (SrO)	0.01
barium oxide (BaO)	0.07
manganese oxide (MnO)	0.05
undetermined	3.36

Table 4.2: Other Soil Properties (Mineral Labs, Inc.)	
Other Analyses	Value
pH	5.3
Sand (%)	13.3
Silt (%)	6.67
Clay (%)	80
Organic Carbon (%)*	0.3

*Measured at Rich Laboratory using loss on ignition method

A full table listing all the analyses can be found in Figure B-1. For the particle size analysis, the clay content was reported as 80%, which is typical in this area of the country (E. Carraway, personal communication, 2017). It should be noted that the clay value represents particle size, not mineralogy. The high clay content contributed to the high SiO_2 and Al_2O_3 content in the soil (Table 4.1). For the trace analysis, the metals that had a higher concentration than expected were chromium, copper, lead, manganese, nickel, phosphorus, selenium, and zinc. The values for these metals were above the 90^{th} percentile compared to the metals found in sediments collected from streams around the Clemson/Anderson, SC, area (Jones 2010). This might be due to the fact that stream sediments have had opportunity for extraction of metals by the stream water for some time whereas the soil has not (E. Carraway, personal communication, 2017). None of the previous literature that studied enantiomerization discussed metal oxide content of the soils used (e.g., Buser et al. 2002, Sun et al. 2011). Compared to soils used in other

studies, this soil was lower in organic carbon content and much higher in clay content. The pH of the soil was within the range of pH values of soils used in previous literature.

Metalaxyl in Soil Experiments

Experimental Setup and Extraction Method Development

For the lime experiments, I added between 70 and 75 mg of lime to the soil. Calculations determining the amount of lime added are shown in Appendix B. The initial pH of the untreated soil was 5.3. The soil pH after lime was added was approximately 6.9.

Several approaches were taken while developing an extraction and cleanup method for the metalaxyl experiments. The first approach was adapted from Buser et al. (2002), which was by hand. This included adding 10 mL of methanol to the centrifuge tube, briefly agitating the sample using a vortex mixer, placing the tubes on a wrist action shaker for 10 minutes, sonicating for 15 minutes, and centrifuging the sample at 2000 rpm for 15 minutes; the cycle was repeated two times. Finally, the supernatants were combined, evaporated and reconstituted in the mobile phase solvents. However, this process took several hours to extract only a few samples and the recovery was low (34%); therefore, a method was developed for an ASE extraction. Gan et al. (1999) developed an ASE method to extract alachlor and atrazine from soil. They compared recoveries using methanol, 1:1 dichloromethane (DCM):acetone, and hexane as extracting solvents; they found 1:1 DCM:acetone had the best recovery. Therefore, this was the solvent chosen to observe if enantiomerization took place. As a comparison, I

tested 1:1 hexane:acetone and methanol as well. The 1:1 DCM:acetone gave the best recovery. The recovery for 1:1 DCM:acetone was 95%, versus 46% for 1:1 hexane:acetone. However, dichloromethane is a carcinogen. To reduce the use of chlorinated solvents, I used methanol as an extracting solvent. Despite methanol being a protic solvent, metalaxyl is not expected to undergo enantiomerization in the presence of methanol (Li et al. 2010).

Chiral Stability of Metalaxyl and Metalaxyl-M in Soils

Figures 4.1 and 4.2 below display the EFs of metalaxyl and metalaxyl-M at different time points for all treatments. Results from a preliminary incubation are shown in Figures B-2 and B-3. All samples were extracted via ASE using methanol. An EF of 0.5 indicates equal parts of each enantiomer. The racemic metalaxyl showed no statistically significant changes in EF over time in any of the treatments (Figure 4.1). The metalaxyl-M showed a statistically significant difference in the acid-unsterilized and lime-unsterilized treatments ($p<0.05$) (Figure 4.2). Time points with statistically significant differences are labeled with different letters. The SAS outputs can be found in Figures B-4 and B-5. Since metalaxyl-M is composed of 97% of the bioactive R-enantiomer, the EF for metalaxyl-M will be low. In addition, since the y-axis will have smaller numbers, the standard deviation will appear larger. Hall (2012) observed that in the presence of pure minerals such as calcite, there was not a statistically significant change in the EF for racemic metalaxyl. Conversely, she observed a statistically significant change in the EF of metalaxyl-M in the presence of bentonite and montmorillonite. I should note she did not measure the pH of her system.

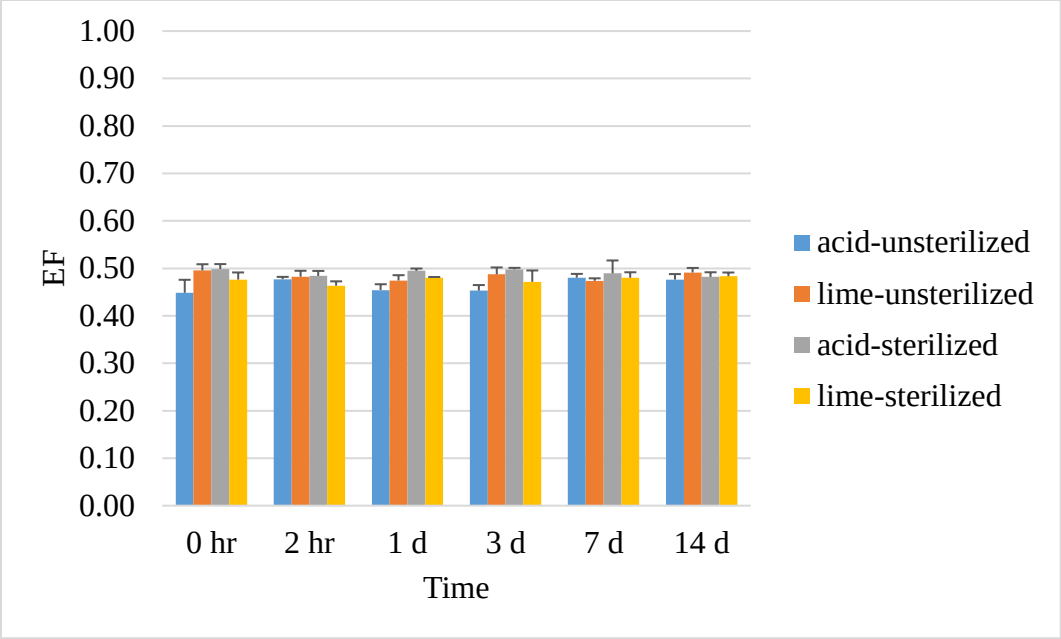


Figure 4.1. The EF of racemic metalaxyl in all soil treatments. The error bars represent one standard deviation (n=3).

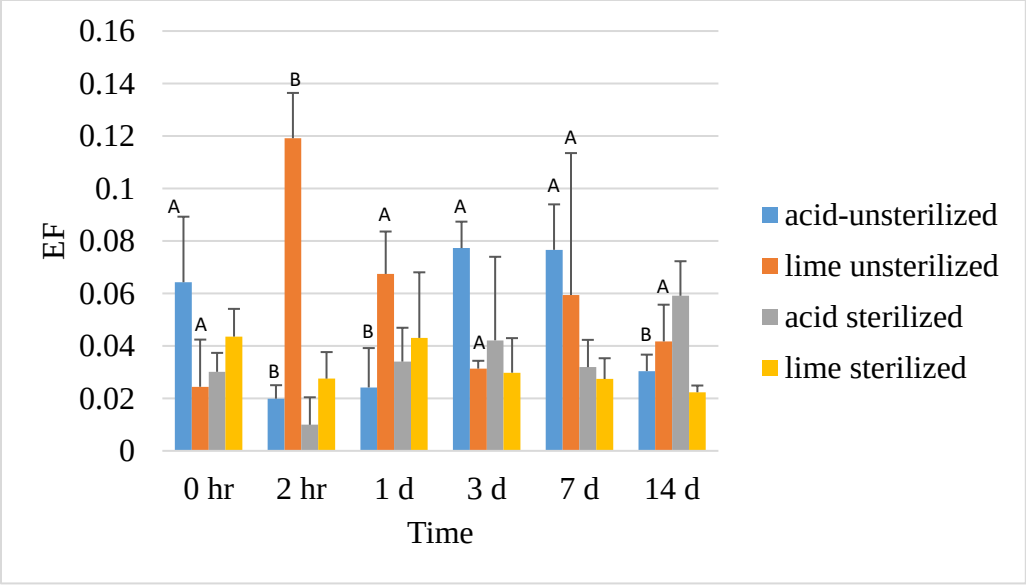


Figure 4.2. The EF of metalaxyl-M in all soil treatments. Error bars represent one standard deviation (n=3). Time points with statistically significant differences in EF are indicated with different letters.

Mass Balance of Metalaxyl

Figures 4.3 and 4.4 below displays the mass balance as the ratio of concentration at the time point to the spiked concentration for both racemic metalaxyl and metalaxyl-M, respectively, in acid-unsterilized soil. The error bars represent one standard deviation (n=3). There was no evidence of degradation for either formulation.

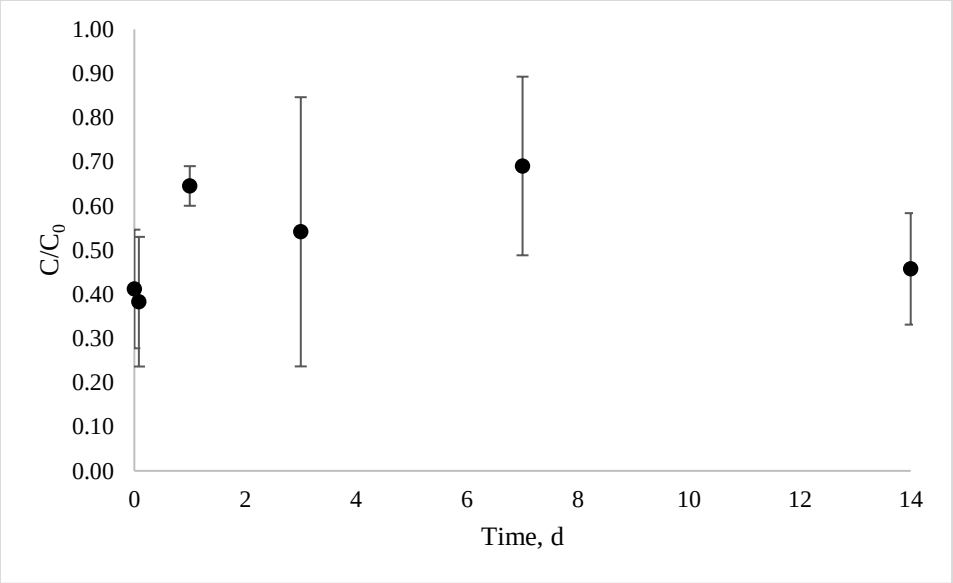


Figure 4.3. The change in concentration over time for racemic metalaxyl in acid-unsterilized soil. Error bars represent one standard deviation (n=3).

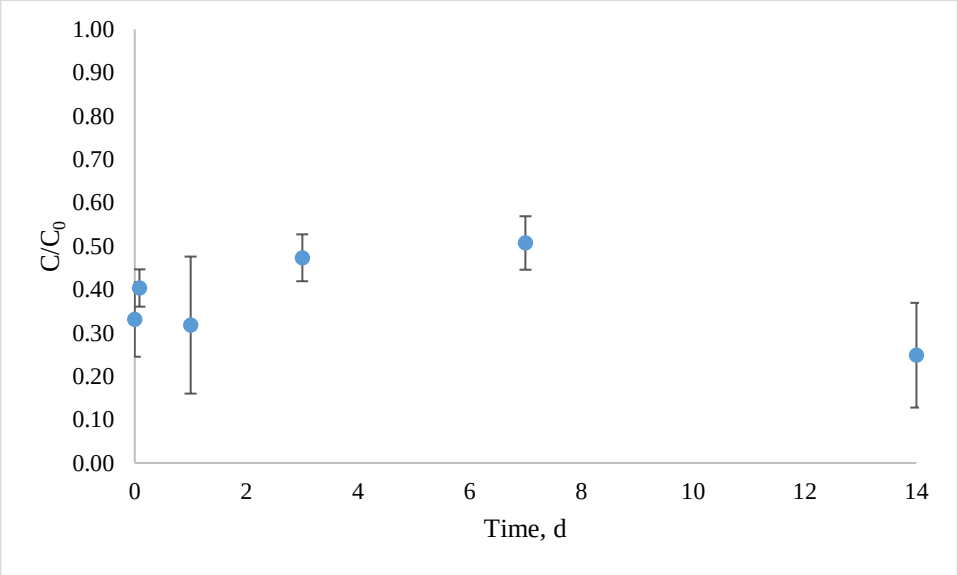


Figure 4.4. The change in concentration over time for metalaxyl-M in acid-unsterilized soil. Error bars represent one standard deviation (n=3).

The change in concentration over time in the lime-unsterilized soil for both racemic metalaxyl and metalaxyl-M are shown in Figures 4.5 and 4.6, respectively. The error bars represent standard deviation (n=3). There was no evidence of degradation for either formulation of metalaxyl in the lime-unsterilized soil.

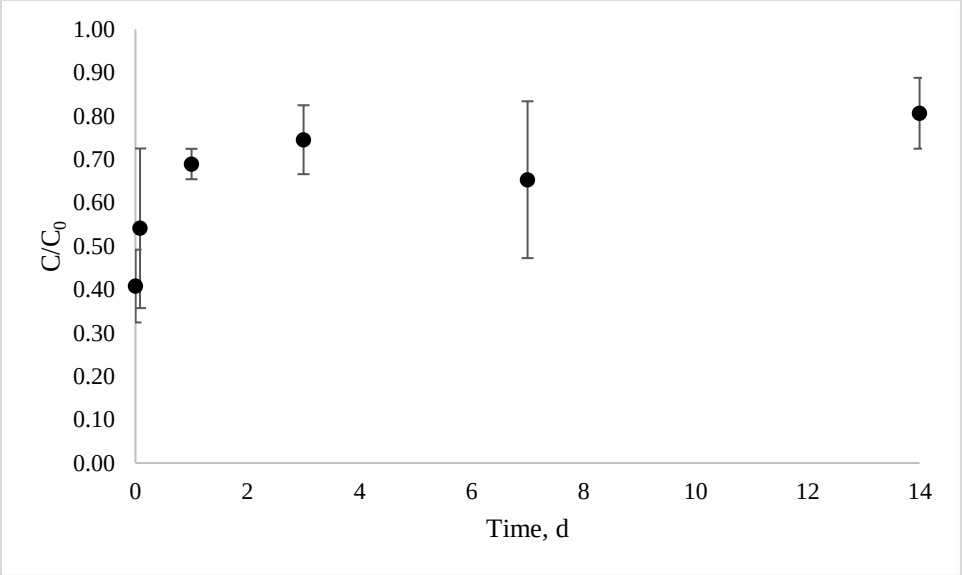


Figure 4.5. The change in concentration over time for racemic metalaxyl in lime-unsterilized soil. Error bars represent standard deviation (n=3).

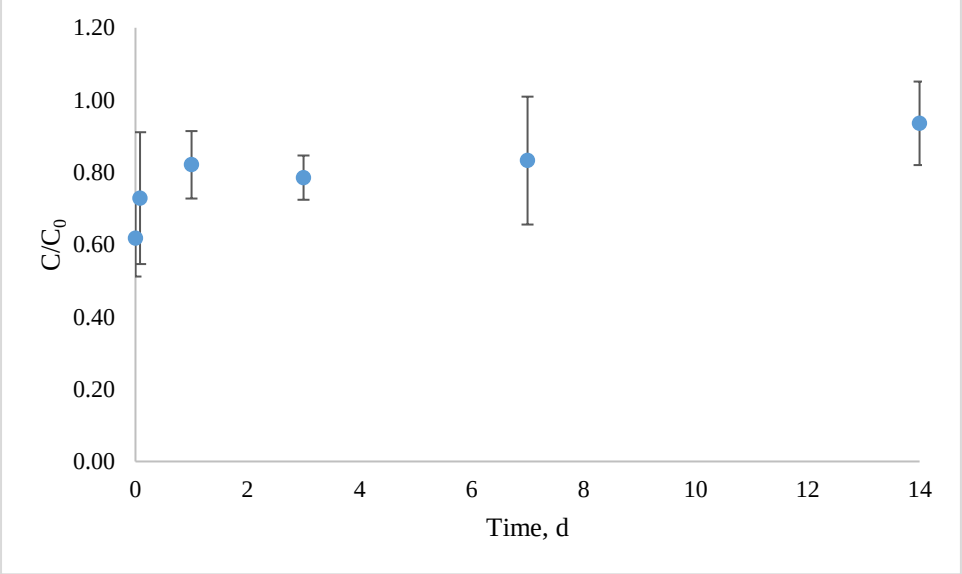


Figure 4.6. The change in concentration over time for metalaxyl-M in lime-unsterilized soil. Error bars represent one standard deviation (n=3).

Figures 4.7 and 4.8 show the change in concentration in acid-sterilized soil for both racemic metalaxyl and metalaxyl-M, respectively. The error bars represent one standard deviation (n=3). As with the acid-unsterilized experiments, there was no evidence of degradation.

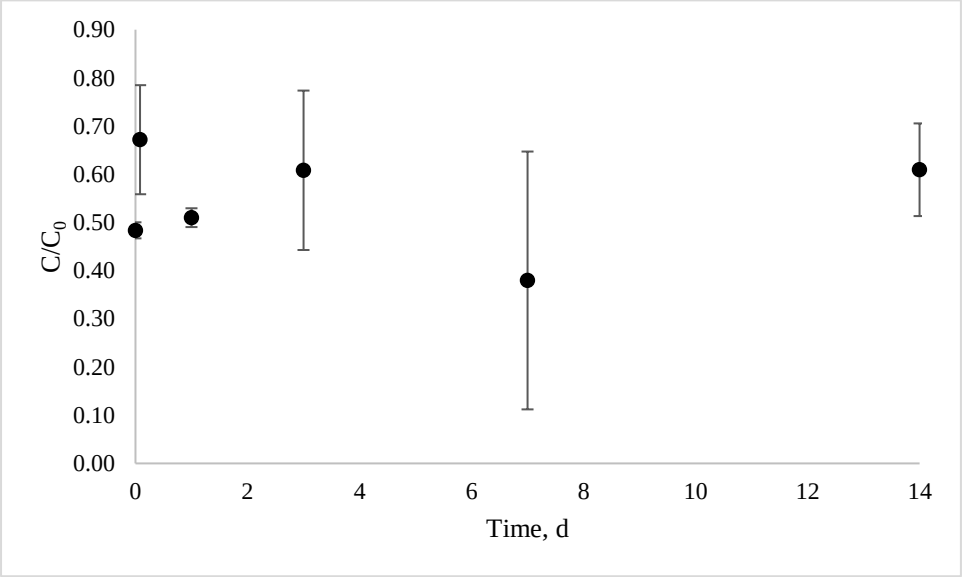


Figure 4.7. The change in concentration over time for racemic metalaxyl in acid-sterilized soil. Error bars represent one standard deviation (n=3).

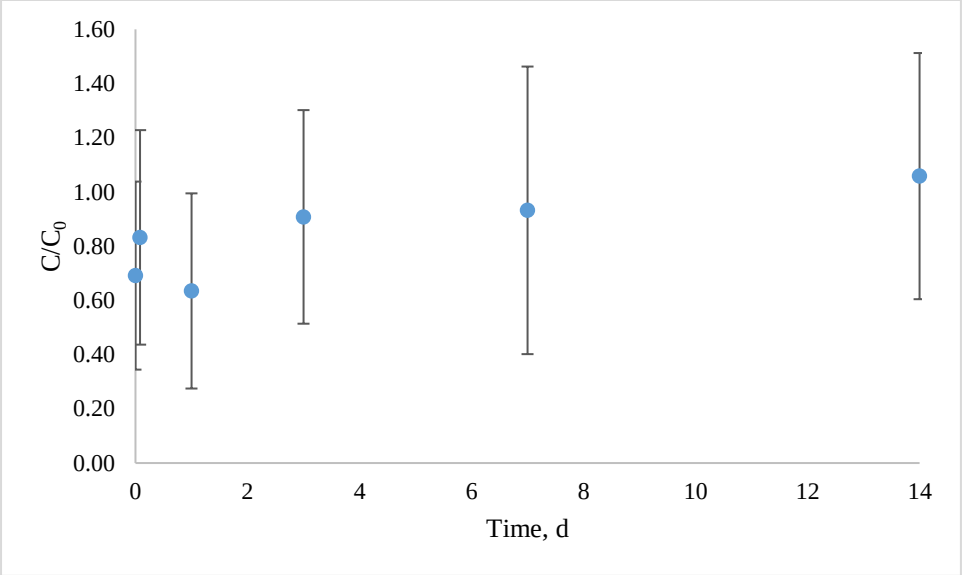


Figure 4.8. The change in concentration over time for metalaxyl-M in acid-sterilized soil. Error bars represent one standard deviation (n=3).

Figure 4.9 and 4.10 display the change in concentration in lime-sterilized soil for both racemic metalaxyl and metalaxyl-M, respectively. The error bars represent one standard deviation (n=3). As with the lime-unsterilized experiments, there was no evidence of degradation.

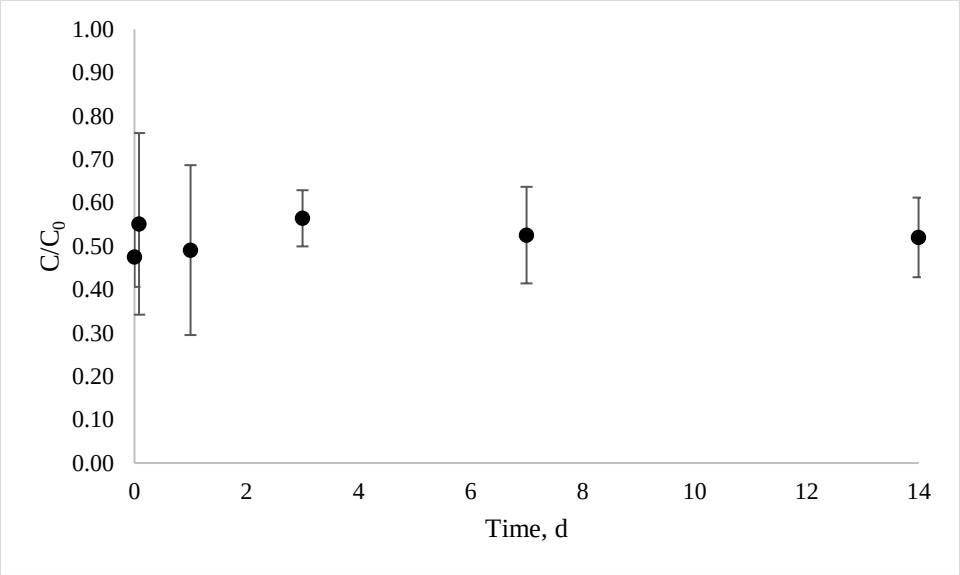


Figure 4.9. The change in concentration over time for racemic metalaxyl in lime-sterilized soil. Error bars represent one standard deviation (n=3).

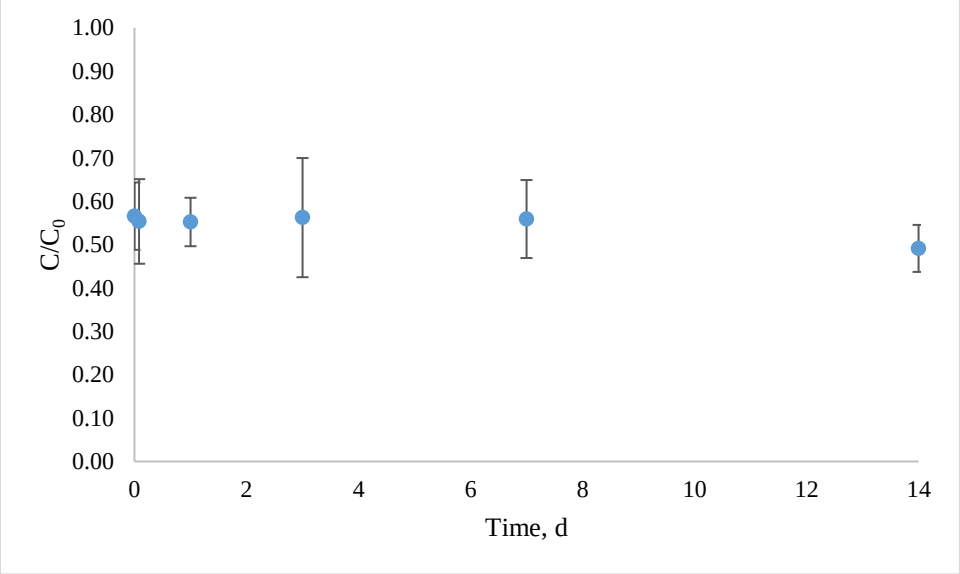


Figure 4.10. The change in concentration over time for metalaxyl-M in lime-sterilized soil. Error bars represent one standard deviation (n=3).

Discussion of Metalaxyl in Soil Experiments

Racemic metalaxyl behaved as expected in all soils. There was not a statistically significant difference among any of the time points in any of the treatments (α=0.05). Buser et al. (2002) and Hall (2012) reached the same conclusion, although I will note that the Buser study did not perform any statistical analysis. An interesting observation was that the EF for the samples extracted from the soil was consistently below 0.5, even though the standard was 0.5. The discrepancy may be due to an issue resolving the S-(+)-enantiomer. From the chromatogram of the samples extracted from soil (Figure A-2), there was constant high background that was not present in the standard chromatogram (Figure 3), which could be due either from an interference from methanol or soil organic matter. Another interesting observation was that the EF for the racemic mixture stayed below 0.5 after 14 d incubation in both the unsterilized and sterilized treatments for limed soil, because the R enantiomer degraded faster than the S enantiomer in high pH soils in previous studies (Monkeidje et al. 2003 and Buerge et al. 2003). In the Buerge et al. (2003) study, they collected soils from different parts of Germany with varying pH values and physical properties. They observed that the R enantiomer degraded faster in all high pH soils. To further determine whether it was truly pH that affected the change in enantioselectivity, they selected a soil and added acid to one incubation and base to another incubation. They observed that in the acid-treated soil, the S enantiomer preferentially degraded, while in the base-treated soil, R preferentially degraded. Their results demonstrated that enantioselective degradation is pH dependent. Therefore, with

the R enantiomer degrading faster than the S enantiomer, I would have expected the EF to slightly increase by the 14 d point.

I was surprised to see that there was a statistically significant difference in EF for the metalaxyl-M in the acid-unsterilized and lime-unsterilized soils, and the differences did not occur chronologically (e.g. 2 hr, 1 d, and 14 d for acid-unsterilized soil). As stated above, there were issues resolving the S-(+)-enantiomer in the chiral analysis. Furthermore, since the S-(+)-enantiomer is present in such a small amount (≈3%), any changes in the area of the peak will cause a higher standard deviation and possibly cause a statistically significant difference.

Furthermore, enantioselective degradation should not have played a role in the difference in EF. Buerge et al. (2003) found that the S-(+)-enantiomer preferentially degraded in acidic soil, and the R-(-)-enantiomer preferentially degraded in alkaline soil. However, there was not a statistically significant difference in the 14 d samples in the lime-unsterilized soils; therefore, it is unlikely degradation of the R-enantiomer occurred after 2 hrs. Also, the degradation of S-(+)-metalaxyl was slow in acidic soils. The ER in acidic soil (Monkeidje 2003) was greater than 1 after about 20 d, with ER being defined as %R/%S, so it is likely that degradation did not play a significant role in the difference. Buser et al. (2002) incubated the separated enantiomers in a basic soil (pH=7.0) and found there was a negligible formation of the opposite enantiomer (<1%) after 28 d for the R enantiomer and 60 d for the S enantiomer, but the conversion was insignificant compared to the degradation of both enantiomers. Hall (2012) also observed a statistically significant change in EF for metalaxyl-M after 24 h for bentonite,

montmorillonite, kaolinite, and calcite but was unsure whether it was due to enantioselective sorption or enantiomerization.

For the mass balance data, I would expect the concentration for both racemic metalaxyl and metalaxyl-M to remain nearly constant over two weeks. Monkiedje et al. (2003) calculated a half-life of 18 d in a high pH soil and 38 d in a low pH soil for racemic metalaxyl and a half-life of 17 d and 38 d for metalaxyl-M in a high pH and low pH soil, respectively. Both Buerge et al. (2003) and Monkiedje et al. (2003) observed slight decreases in mass by the 14 day time point for both high and low pH soils. From Figures 4.3-4.10 above, I observed that the mass of metalaxyl was nearly constant after two weeks. Therefore, I concluded that metalaxyl does not significantly degrade in high or low pH soil over the course of two weeks.

Malathion in Soil Experiments

Experimental Setup and Extraction Method Development

The experimental setup was adapted from Sun et al. (2011). I chose a 3 d incubation because Hall (2012) observed complete enantiomerization in 2 hrs in aqueous solution, and Sun et al. (2011) observed enantiomerization between a few hours and 7 days, depending on the pH of the soil.

For limed experiments, between 70 and 75 mg of lime were added to the soil in order to obtain a more neutral pH. The final soil pH was approximately 7.1.

The same ASE method was used for malathion that was used for metalaxyl, with the exception that ethyl acetate was used instead of methanol because malathion has been

found to undergo enantiomerization in protic solvents such as methanol (Li et al. 2010). I started with the same ASE conditions as metalaxyl, which was a 100°C oven temperature and 1500 psi pressure. There was a 5 min heat up time, followed by a 5 min static time, which was repeated for two cycles. Finally, the method ended with a 90 s purge with nitrogen. The method described above worked well with S-(-)-malathion. There was limited conversion of the S-enantiomer (EF≈0.04, which indicates very little presence of the R-enantiomer), therefore, ASE with ethyl acetate was a practical method for enantiomer analysis for malathion (Figure 4.11).

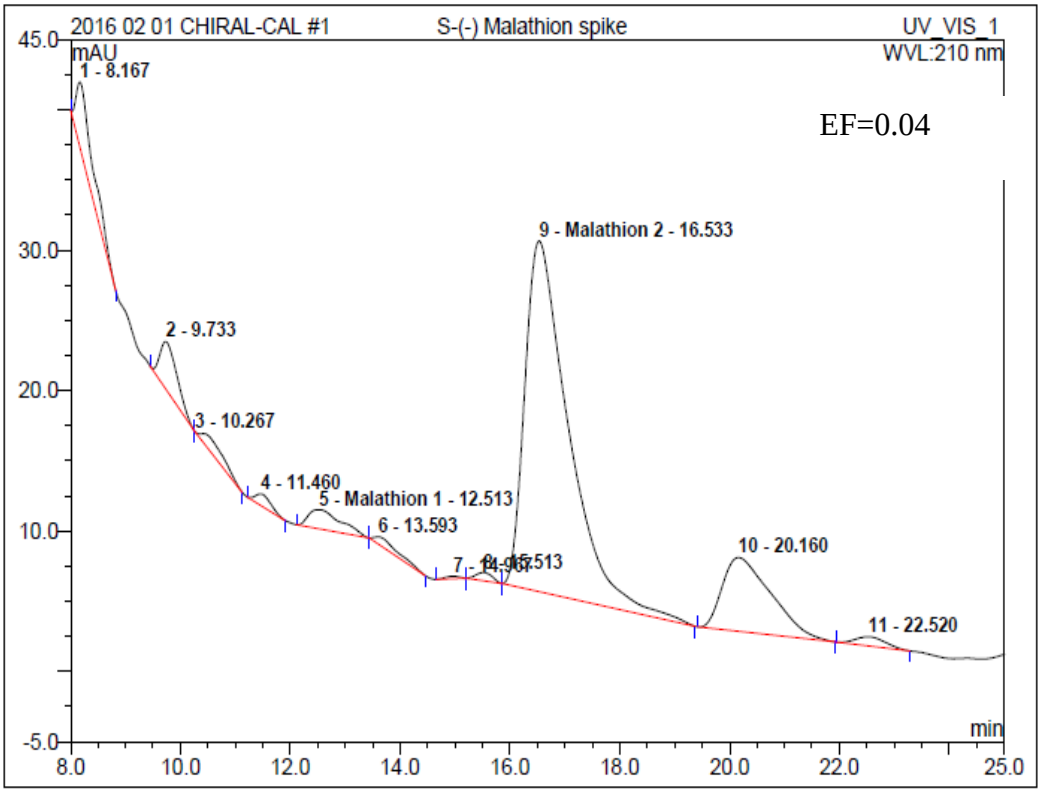
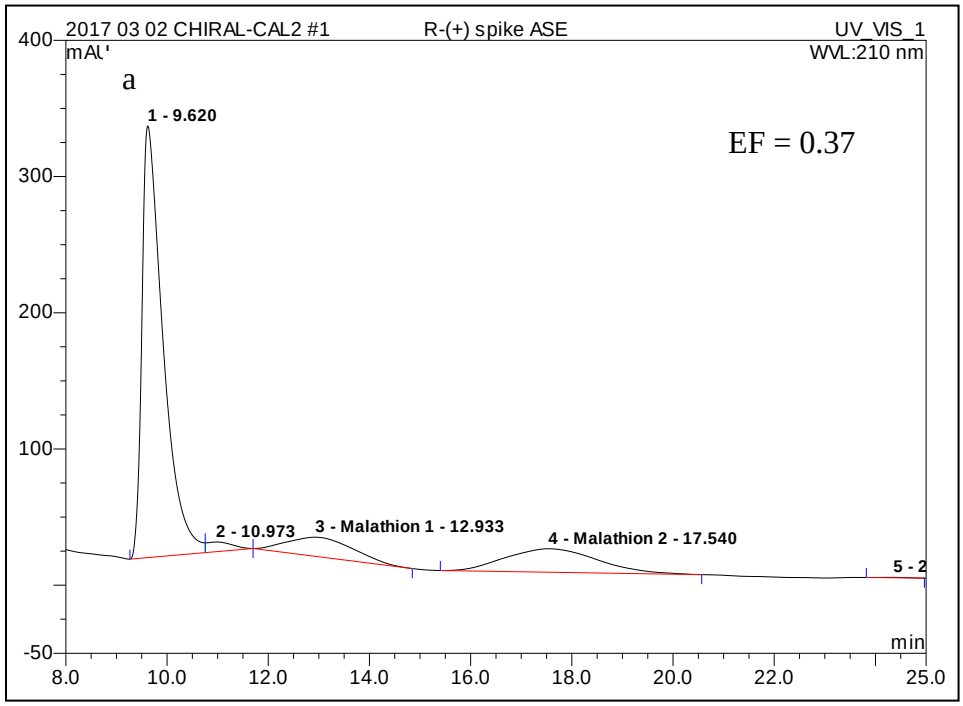


Figure 4.11. Chromatogram of S-(-)-malathion spike (malathion 2) extracted with ASE at 100°C.

However, when I extracted and analyzed the acid-unsterilized soil spiked with the R-enantiomer via chiral analysis, there was a large presence of the S-enantiomer. The presence of the S-enantiomer was unexpected because Sun et al. (2012) saw no conversion of either malathion enantiomer in acidic soil. Two separate dry soil samples were spiked with the R-enantiomer and one was extracted via ASE and one was extracted by hand for comparison. The results are shown in Figures 4.12a and 4.12b below.



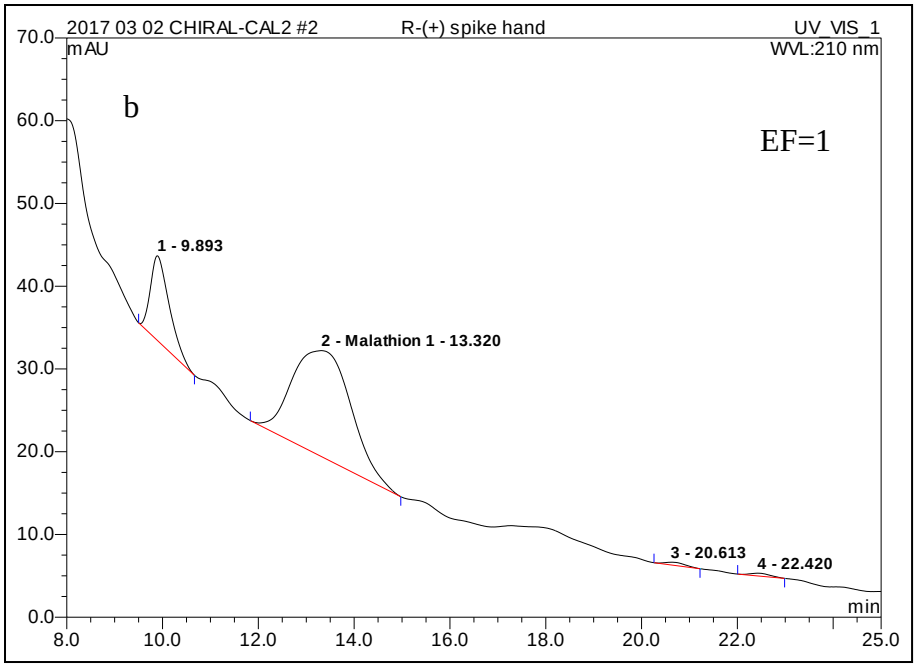


Figure 4.12. Chromatograms of R-(+)-malathion (malathion 1) spikes extracted using (a) the ASE method at 100°C and (b) a hand extraction method.

Since both extracts were evaporated in a water bath set at 65°C, the ASE method was modified to an oven temperature at 65°C. With a lower oven temperature, I had to extend the static times and increase the number of cycles (C. Sober, Personal Communication, 2017). The final method was a 65°C oven temperature, 1500 psi, 5 min heating, 10 min static (repeated for two cycles), finishing with a 90 s purge with nitrogen. The method above resulted in no conversion of R with a recovery of 91.6% (Figure 4.19).

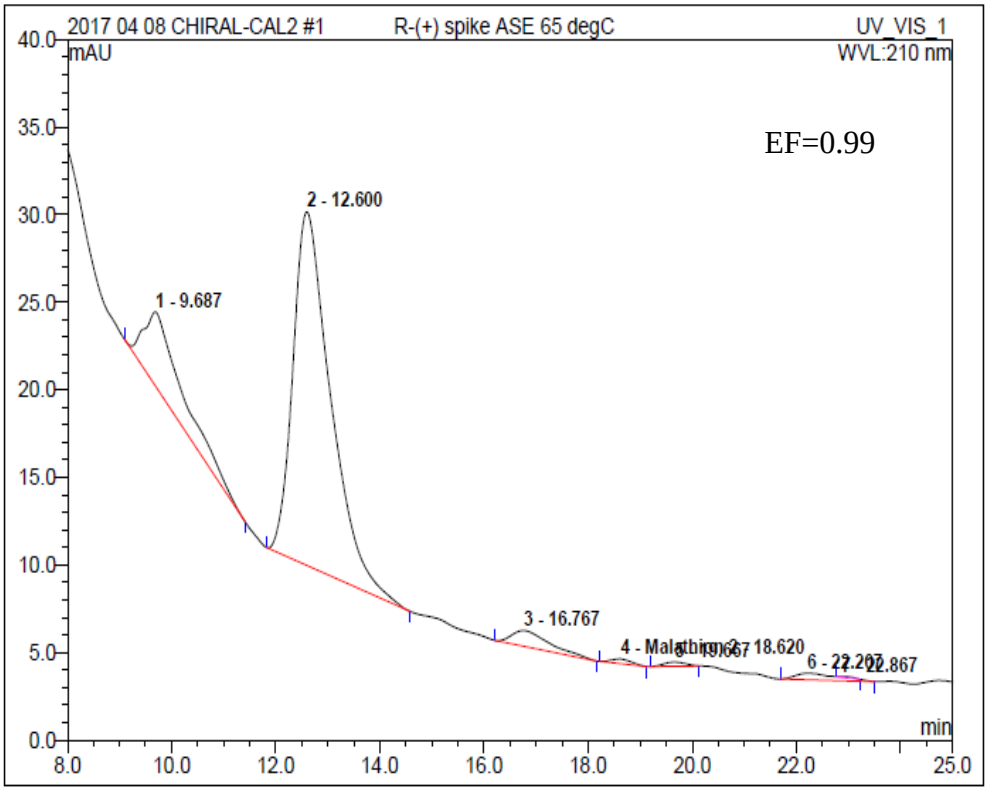


Figure 4.13. Chromatogram of R-(+)-malathion spike with the ASE oven set at 65°C.

Chiral Stability of Malathion in Soil

Figure 4.14 represents the change in EF of R-(+)-malathion in all soil treatments over time. An EF of 0.5 indicates a racemic mixture, an EF>0.5 indicates a higher concentration of the R enantiomer, and an EF<0.5 indicates a higher concentration of the S-(-)- enantiomer. Malathion was not detected in the R-(+) experiment at day 3 in the acid-unsterilized treatment (Figure B-6). In addition, there is no EF for day 3 in the lime-unsterlized incubation at ambient temperature because no R-(+)-malathion was detected, although there was a distinct peak for the S-(-)-enantiomer (Figure B-7). Statistical analysis in Excel found a significant difference in the R-(+)-malathion incubation for all

soil treatments (p value<0.05). SAS identified statistically significant differences for the time points as shown in Figure 4.14. In summary, 1 d was statistically different in the acid-unsterilized treatment, 3 d was statistically different in the lime-unsterlized soil at 10°C, and 1 d and 3 d were different in the lime-unsterilized soil at ambient temperature. The SAS outputs can be found in Figures B-8-B-10.

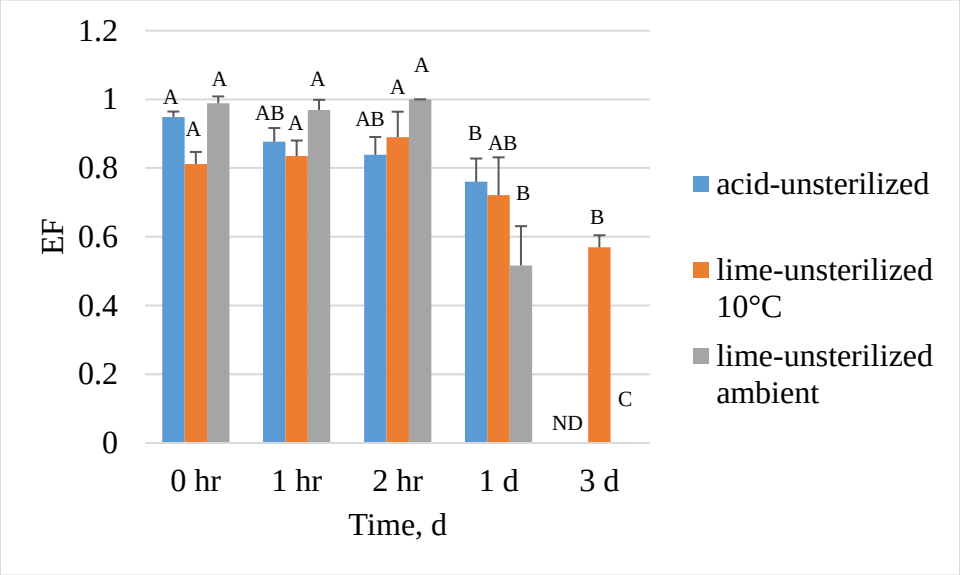


Figure 4.14. The EF of R-(+)-malathion in all soil treatments. Error bars represent one standard deviation (n=3). Different letters indicate a statistically significant difference. ND indicates that malathion was below detection limits.

Figure 4.15 represents the change in EF for S-(-)-malathion in all soil treatments. The EF for the S-(-)-enantiomer incubation is small because there is little presence of the R-(+)-enantiomer in the samples (see Eqn. 3.2). In addition, due to the small EF, the error bars will appear to be larger than the EF for the R-(+)-enantiomer. There are no bars for

the 1 d and 3 d points for the acid-unsterilized soil and for the 3 d point in the lime-unsterilized soil at 10°C because no R-malathion eluted (Figures B-11 and B-12). For S-(-)-malathion, there was a statistically significant difference found at 3 d for the lime-unsterlized soil incubated at 10°C. There were no statistically significant differences found in the acid-unsterilized soil or the lime-unsterilized soil at ambient temperatures. The SAS output can be found in Figure B-13.

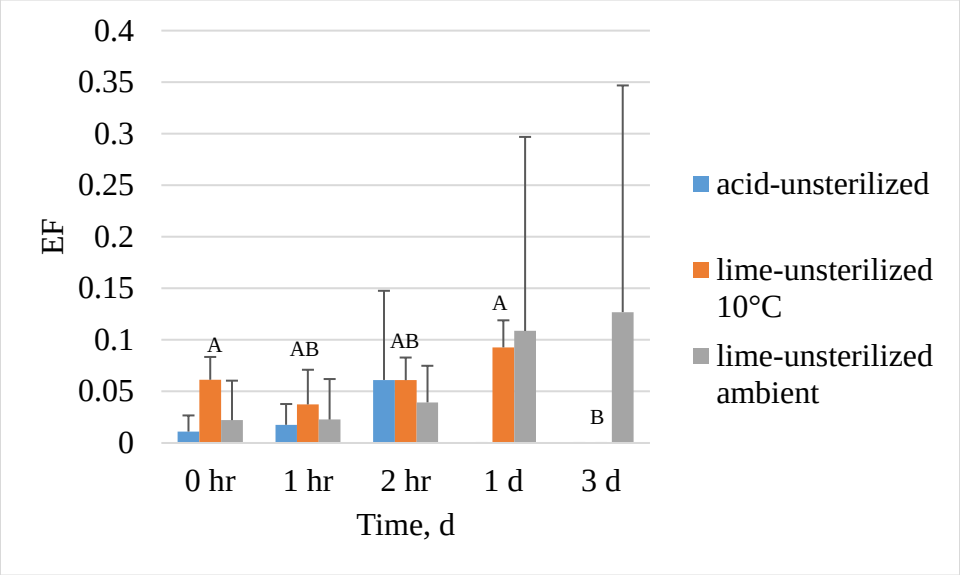


Figure 4.15. The EF of S-(-) malathion over time in all soil treamtents. Error bars represent one standard deviations (n=3).

Figure 4.16 represents the EF change for racemic malathion in all soil treatments. For racemic malathion, there was a statistically significant difference between samples collected at 3 d in the lime-unsterilized soil incubated at 10°C. There were no significant differences found in the acid-unsterlized soil. The SAS output can be found in Figure B-14. I did not perform an incubation of the racemic mixture at room temperature because

with the separate enantiomer incubations the EF analysis was more clear and the degradation was slower than the enantiomers incubated at room temperature. Therefore, I performed the racemic malathion incubation only at 10^0C.

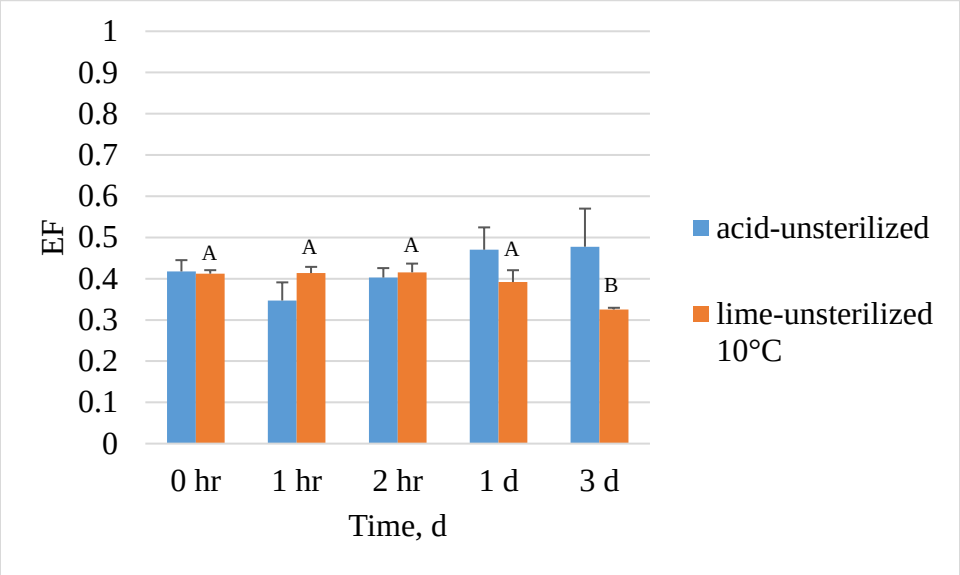


Figure 4.16. The EF of racemic malathion over time in all soil treamtents. Error bars represent one standard deviations (n=3).

Mass Balance of Malathion

Figures 4.17-4.19 display the mass balance as a change in concentration for R-(+)-malathion, S-(-)-malathion, and racemic malathion in acid-unsterilized soil at ambient temperature, respectively. The error bars represent one standard deviation (n=3).

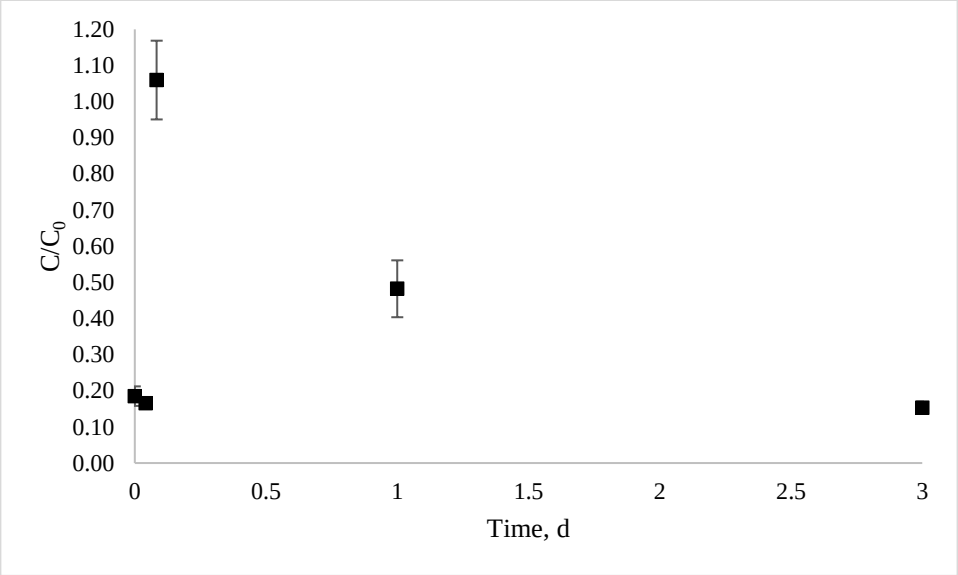


Figure 4.17. The change in concentration over time for R-malathion incubated in acid-unsterlized soil. Error bars represent one standard deviation (n=3).

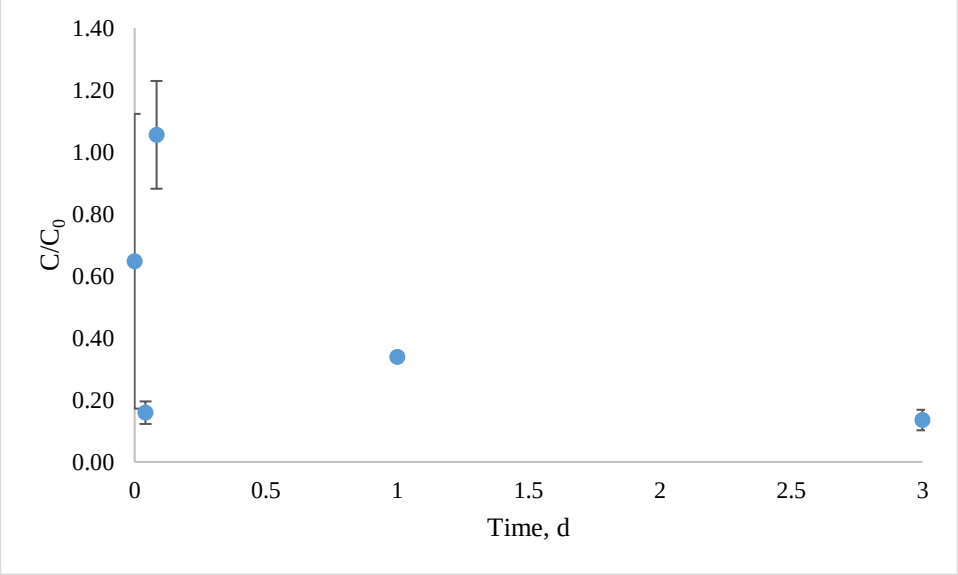


Figure 4.18. The change in concentration over time for S-malathion incubated in acid-unsterlized soil. Error bars represent one standard deviation (n=3).

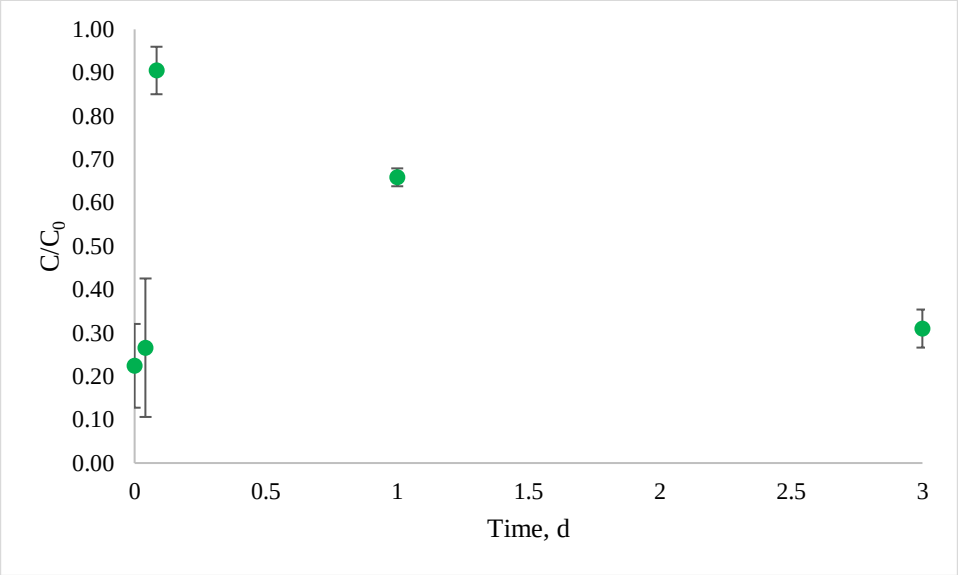


Figure 4.19. The change in concentration over time for racemic malathion incubated in acid-unsterlized soil. Error bars represent one standard deviation (n=3).

Figure 4.20-4.22 shows the mass balance displayed as concentration for R-(+)-malathion, S-(-)-malathion, and racemic malathion in lime-unsterilized soil at 10^0C, respectively. The error bars represent one standard deviation (n=3).

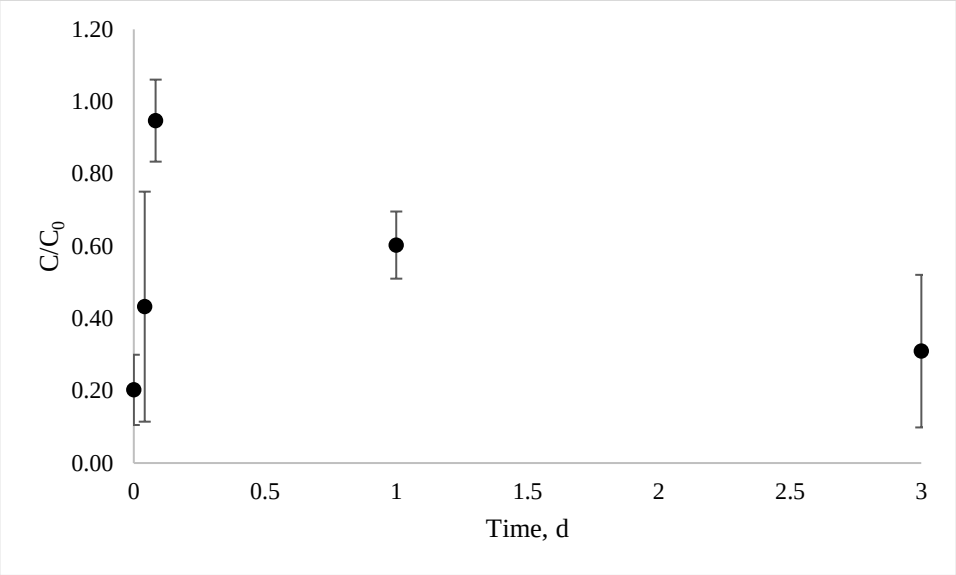


Figure 4.20. The change in concentration of R malathion in lime-unsterilized soil incubated at 10°C. Error bars represent one standard deviation (n=3).

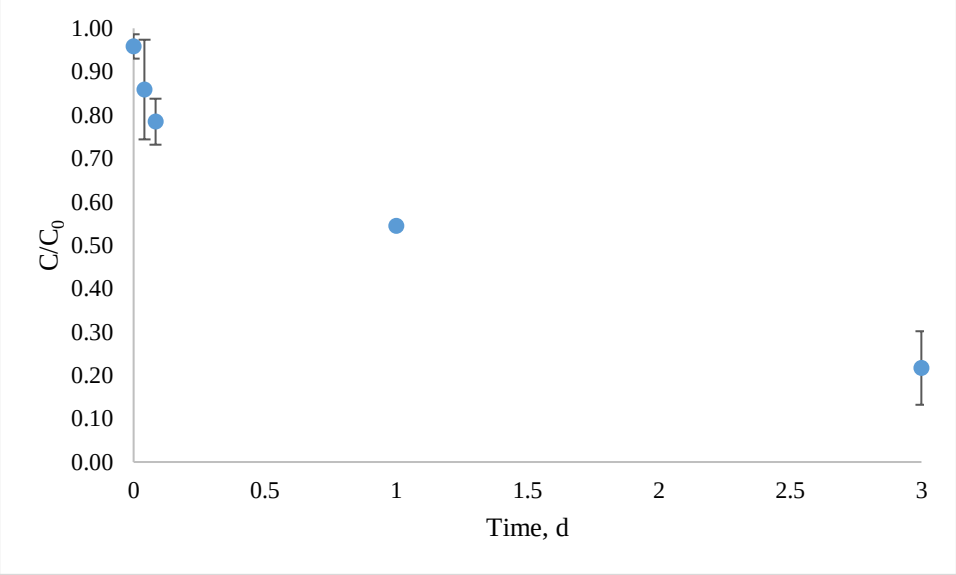


Figure 4.21. The change in concentration of S malathion in lime-unsterilized soil incubated at 10°C. Error bars represent one standard deviation (n=3).

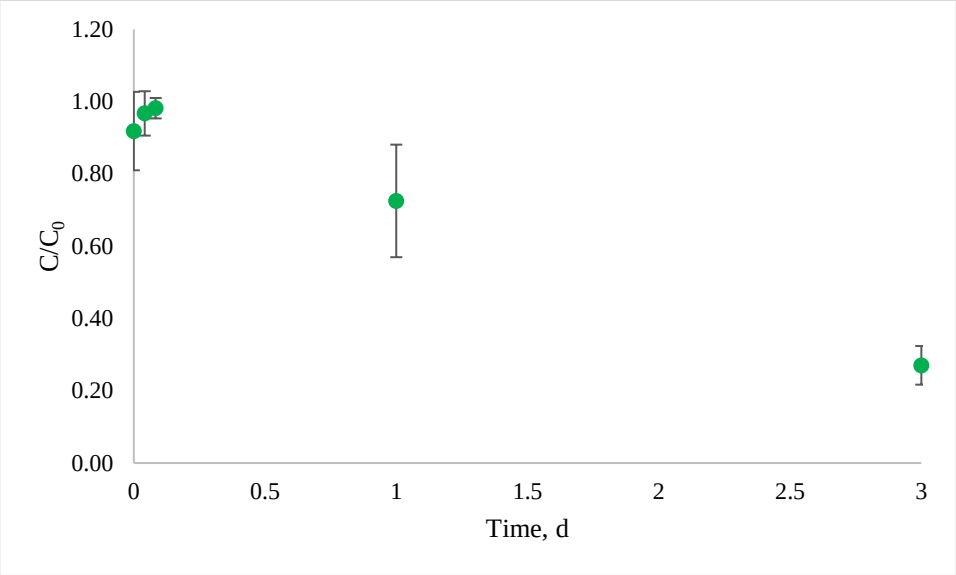


Figure 4.22. The change in concnentration of racemic malathion in lime-unsterilized soil incubated at 10°C. Error bars represent one standard deviation (n=3).

Figures 4.23 and 4.24 indicate the change in concentration for R-(+)-malathion and S-(-)-malathion in lime-unsterilized soil incubated at ambient temperature, respectively. The error bars represent one standard deviation (n=3).

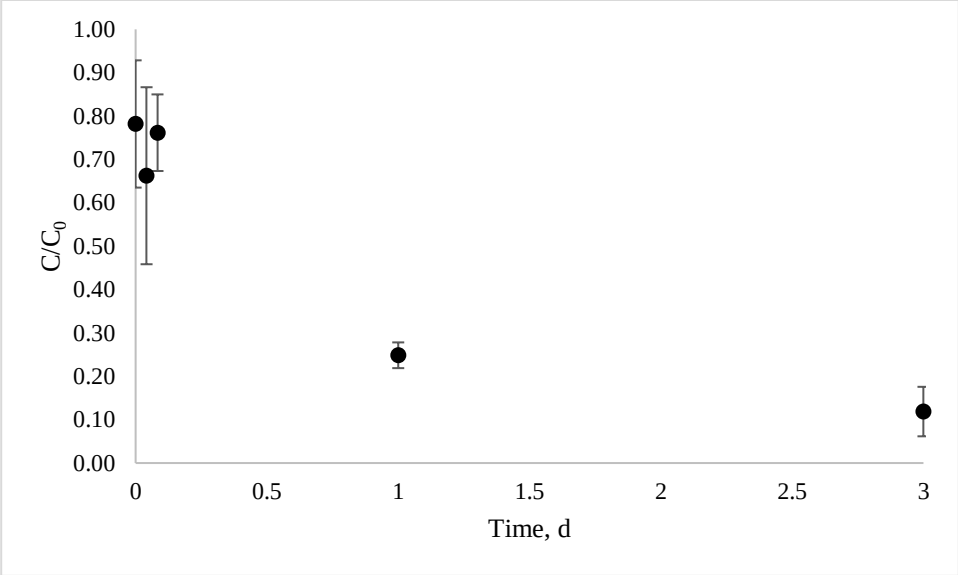


Figure 4.23. The change in concentration of R-malathion in lime-unsterilized soil placed incubated at ambient temperature. Error bars represent one standard deviation (n=3).

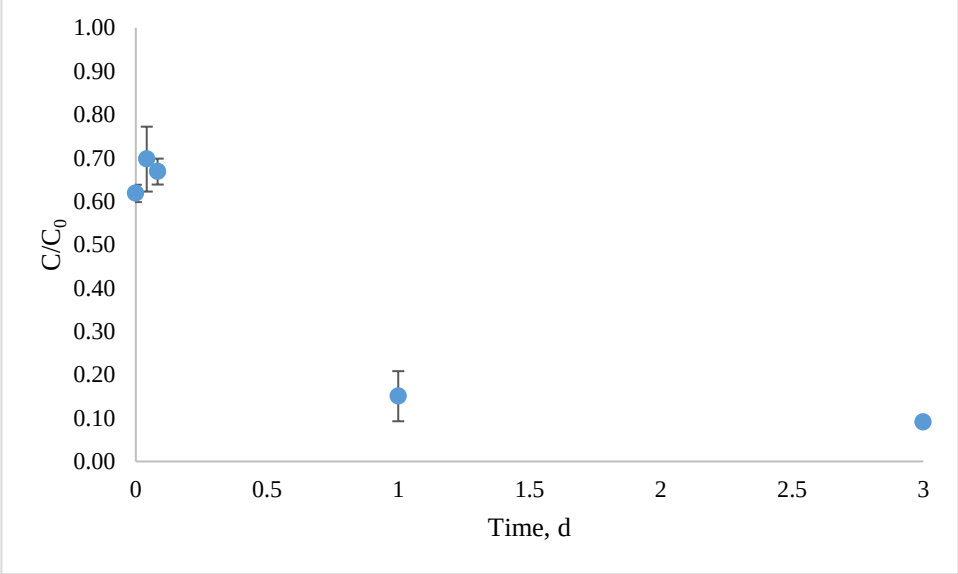


Figure 4.24. The change in concentration of S-malathion in lime-unsterilized soil placed at ambient temperature. Error bars represent one standard deviation (n=3).

Discussion of Malathion in Soil Experiments

My hypothesis was that the separated enantiomers would not undergo enantiomerization in acidic soils and rapid enantiomerization in limed soils, as Sun et al. (2011) observed. However, both chiral chromatograms for the acid-unsterilized soil showed an appearance of the opposite enantiomer. Surprisingly, the ANOVA analysis by Excel (and confirmed by the SAS ANOVA) showed a statistically significant difference for the experiments with the R-(+)-malathion in the acid-unsterilized soil. The EF continuously decreased for R-malathion, indicating an increase in the presence of the S enantiomer. In addition, the EF increased in the S-malathion incubation, indicating an increase in the presence of the R enantiomer. Sun et al. (2011) saw no conversion of either enantiomer in a pH 5.0 soil; however, my results in the acid soil (5.3) were markedly different. In addition, Sun et al. (2011) found the half life of R-malathion to be 2.42 d in a pH 5.0 soil, however, neither enantiomer was above the detection level in my study at day three. The lack of malathion could be due to degradation because the soil was not sterilized. For the racemic incubation, the EF was consistently below 0.5, even though the standard was 0.5. The lower EF could be due to interferences from organic matter or ethyl acetate. As with metalaxyl, there was an issue resolving the first peak, resulting in an EF below 0.5 for all racemic samples.

For the limed soil, I expected the EF for both enantiomers to approach 0.5 (an equal concentration of both enantiomers) after 3 d at ambient temperature. In a pH 6.9 soil, Sun et al. (2012) noted that the EF for the incubation of S-malathion reached a maximum of 0.42 after seven days. Hall (2012) saw conversion in two hours in the

presence of all minerals studied. I expected the process of enantiomerization to be slower at 10°C. For the R-enantiomer, there was a slight decrease in the rate of enantiomerization. For example, there was an EF of 0.72 after 1 d in the experiments at 10^{o}C, while the EF was 0.52 after 1 d in the experiments at room temperature. However, I could not compare the 3 d samples because no R-malathion was detected at 3 d for the incubation at ambient temperature. For the S-malathion incubations, I observed the EF steadily increasing in the ambient experiments while the EF in the 10^{0}C experiments varied, with an EF of 0 on 3 d (indicating no presence of R-malathion). In addition, the EFs were higher for the colder temperature experiments than for the room temperature experiments. I would have expected the EFs to be higher in the room temperature incubations because the higher temperature would allow for faster conversion of R-malathion to S-malathion. As with the acid experiments, the EF was below 0.5 for the lime experiments, indicating a smaller presence of the R-enantiomer. The statistically significant decrease in EF at day three is not the same result that was observed by Sun et al. (2011). For a pH 7.2 soil, they determined the half life for each enantiomer to be similar (1.4 d for R-malathion; 1.36 for S-malathion), so I would anticipate the EF at day 3 to be consistent with the other time points; however, this was not the case.

For the achiral data, malathion behaved almost as expected for all treatments. For the acid-unsterilized soil, I observed an increase in concentration after 2 hrs, then a continuous decrease thereafter (Figures 4.28-4.30). The low concentrations at the beginning may be due to the fact that the 0 hr and 1 hr samples were refrigerated before analysis, and I did not allow sufficient time for those samples to reach room temperature.

Sun et al. (2011) found that for separate enantiomers incubated in a pH 5.0 soil, the half-lives were 2.42 d for R-malathion and 2.94 d for S-malathion. In addition, the half-life of malathion in an acidic soil was reported as about seven days (Newhart 2006), so the low concentrations after three days of all three experiments is interesting.

For the lime experiments, malathion degraded as expected. The 10°C experiments showed slower degradation than those at room temperature (Figures 4.31-4.32 and Figures 4.34-4.35, respectively). There was still some variability in the concentrations in the first two hours, due to not allowing the samples reach room temperature before analysis. Sun et al. (2011) observed that the separated enantiomers degraded quickly in higher pH soils. In their pH 6.9 soil, the half-lives of the R and S enantiomers of malathion were 1.1 d and 0.76 d, respectively. This explains the low concentrations of the separated enantiomers after one day of incubation; in addition this would also explain why S-malathion approaches a concentration of 0 mg/kg faster the R-malathion.. In comparison, the concentrations of malathion during the 10^0C experiments were about double after one day of incubation, which indicated that the low temperature of the incubations decreased the degradation.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

I investigated enantiomerization of two current-use pesticides, metalaxyl and malathion, in soils under both acid and alkaline conditions. Racemic metalaxyl displayed no statistically significant differences in EF in acid-unsterilized, acid-sterilized, lime-unsterilized, or lime-sterilized soils over 14 days. Previous studies had observed similar results with pure minerals and soils. My study provides more evidence that the hydrogen on the chiral carbon of metalaxyl is not removed easily. Likely the electron-donating $-CH_3$ group influences the acidity of the hydrogen. Metalaxyl-M, the chiral switch formulation composed of about 97% of the bioactive R-enantiomer, displayed some statistically significant differences, but the EF remained mainly below 0.15, which indicated that the R-enantiomer maintained its dominance. Since the S-enantiomer is present in such small quantities in the chiral switch formulation, any variability in the concentration could lead to a larger standard deviation and less certain EF values. I would expect some degradation in the unsterilized treatments and lime treatments; however, the concentrations varied too much to obtain an accurate profile over two weeks.

The individual malathion enantiomers showed interesting behavior in both acid and lime soils. Previous studies indicated that there would be little conversion in an acid soil; however, I observed the presence of the opposite enantiomer in both soils during chiral analysis. The presence of the opposite enantiomer, however, was small compared to the enantiomer that had been spiked in the incubation. For both the room temperature

and 10^0C limed soils, there was evidence of enantiomerization over three days, although the process was slower at the lower temperatures for the R-enantiomer. The enantiomerization of S-malathion was more evident in the room temperature than the cold temperature experiment, where the EF showed more uncertainty with time. The degradation profiles of all experiments behaved as expected in the lime-unsterilized experiments. For the acid-unsterilized experiments, the separated enantiomers and racemic degraded quickly in acidic soils, whereas other literature cited a slower degradation rate in acidic soils. Malathion degraded quickly in the high pH soil, which is similar for the ambient experiment; the degradation rate was slower at 10°C than at room temperature.

The results presented above indicates that enantiomerization is likely controlled by the behavior of a hydrogen and other functional groups on the chiral carbon. The hydrogen on the chiral carbon of metalaxyl is not easily removed due to the electron-donating alkyl group on the chiral carbon. Therefore, a chiral switch formula for those pesticides which have an electron donating group along with a hydrogen on the chiral carbon may be possible. Conversely, the hydrogen on the chiral carbon of malathion could be removed under acidic and alkaline conditions due to the electron-withdrawing groups on the chiral carbon. Since malathion enantiomers were not chirally stable in any of the soil treatments, a chiral switch formula for malathion and other pesticides that have a similar molecular structure to malathion would not be useful.

Recommendations

For future research, extraction via ASE shows potential for future enantiomeric analysis for both pesticides. Throughout the experiment, there were issues with resolving the first enantiomer peak for both pesticides; therefore, for the racemic incubations, the EF values were consistently below 0.5 (smaller presence of the first eluting enantiomer) even though the standard chromatogram showed an EF of 0.5. Therefore, a better ASE and subsequent cleanup method should be developed to resolve the first peak in the chiral chromatogram and improve recovery. For both pesticides, I completely air-dried the soil before extracting it. For future high pH experiments, I recommend that the incubations be placed in a 10°C environment. Not only does the cooler conditions slow the rate of degradation, making observations of degradation behavior easier, but it can also be beneficial when studying enantiomerization. Furthermore, if a high pH soil (pH≈8.0) is to be used in the future, conversion of malathion will happen very quickly (about 12 hrs according to Sun et al. (2011)), so completely air drying the soil before extraction would hinder enantiomer analysis. Therefore, an improved cleanup method would be beneficial. As stated above, R-malathion is not stable at 100°C in the ASE oven; it would be interesting to determine what the temperature threshold for the conversion. This would allow future researchers to increase the temperature of the ASE oven and possibly shorten the extraction time and improve recovery. If a higher temperature does not work, I recommend either using a two solvent system, such as ethyl acetate:acetonitrile, or using only acetonitrile. Another recommendation is to obtain soils from different places in the United States to investigate whether the same behavior is exhibited by different

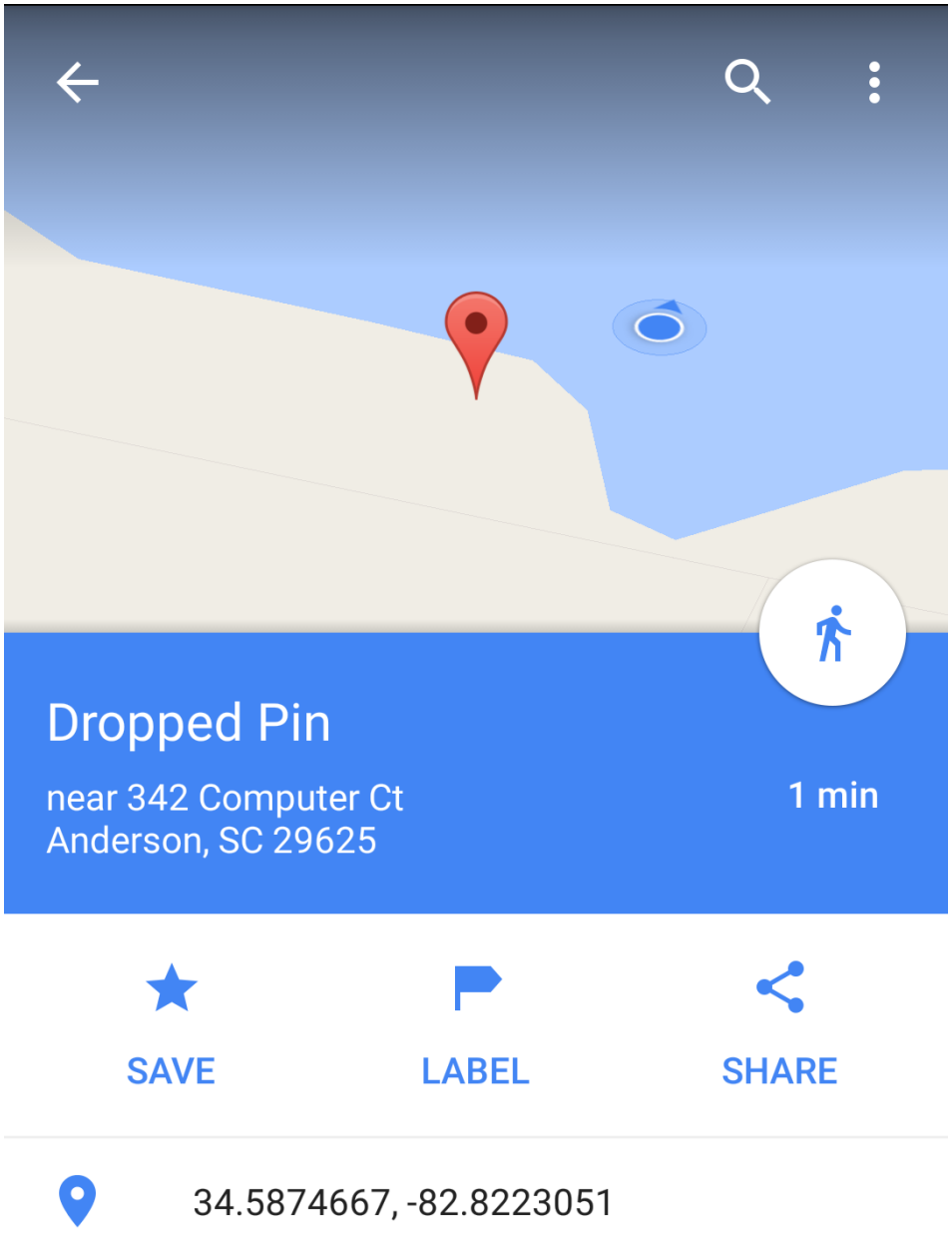
soils. Different pesticides should be investigated to determine their behavior in the high clay soil that is present in South Carolina. It is worth performing molecular modeling to determine the mechanism for enantiomerization. Modeling would allow parameters to be changed without the considerable amount of work for laboratory studies.

APPENDICES

Appendix A

Supplementary Data for Chapter III

a



b

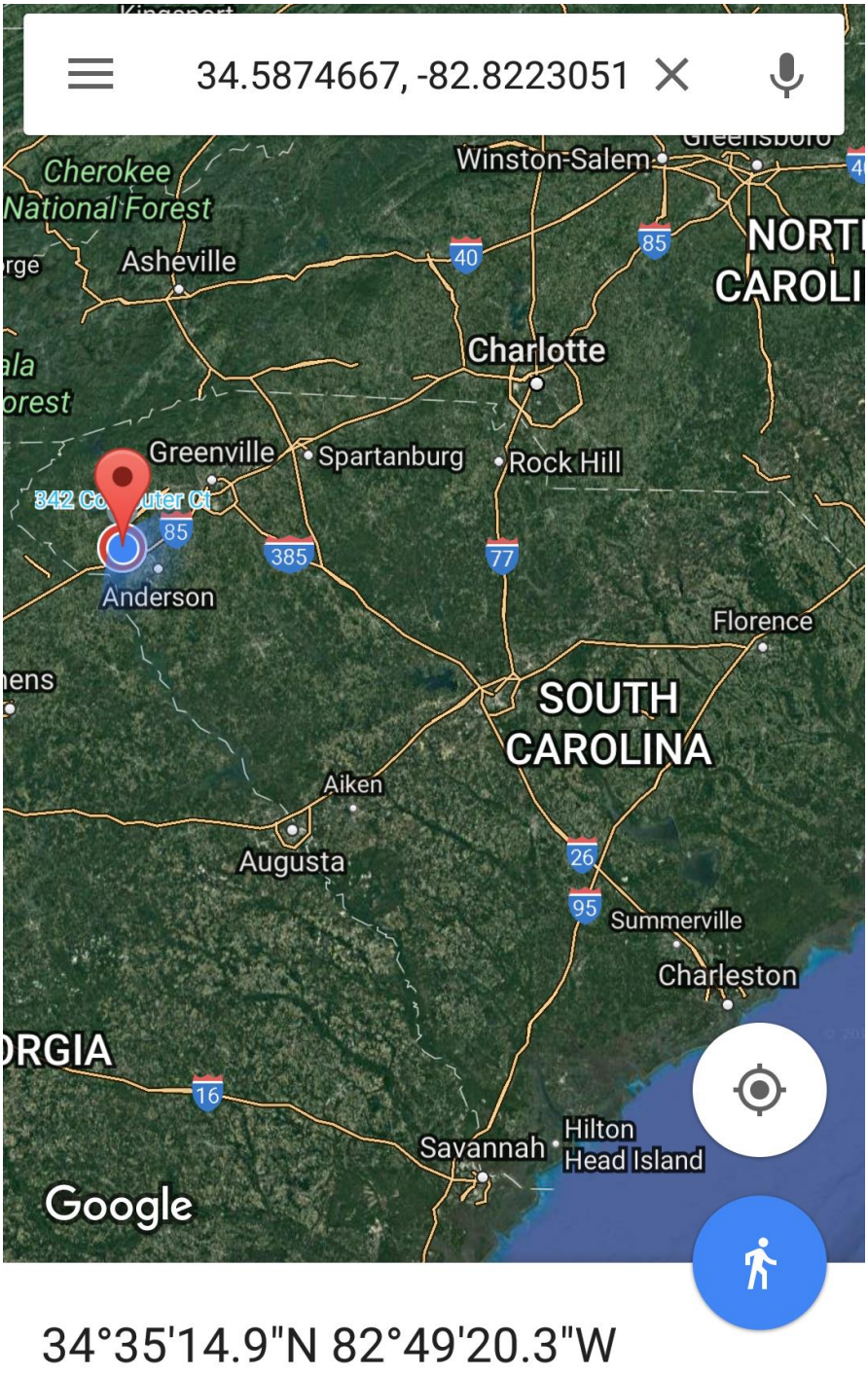


Figure A-1: Map of soil collection area (a) GPS pin drop and (b) SC map.

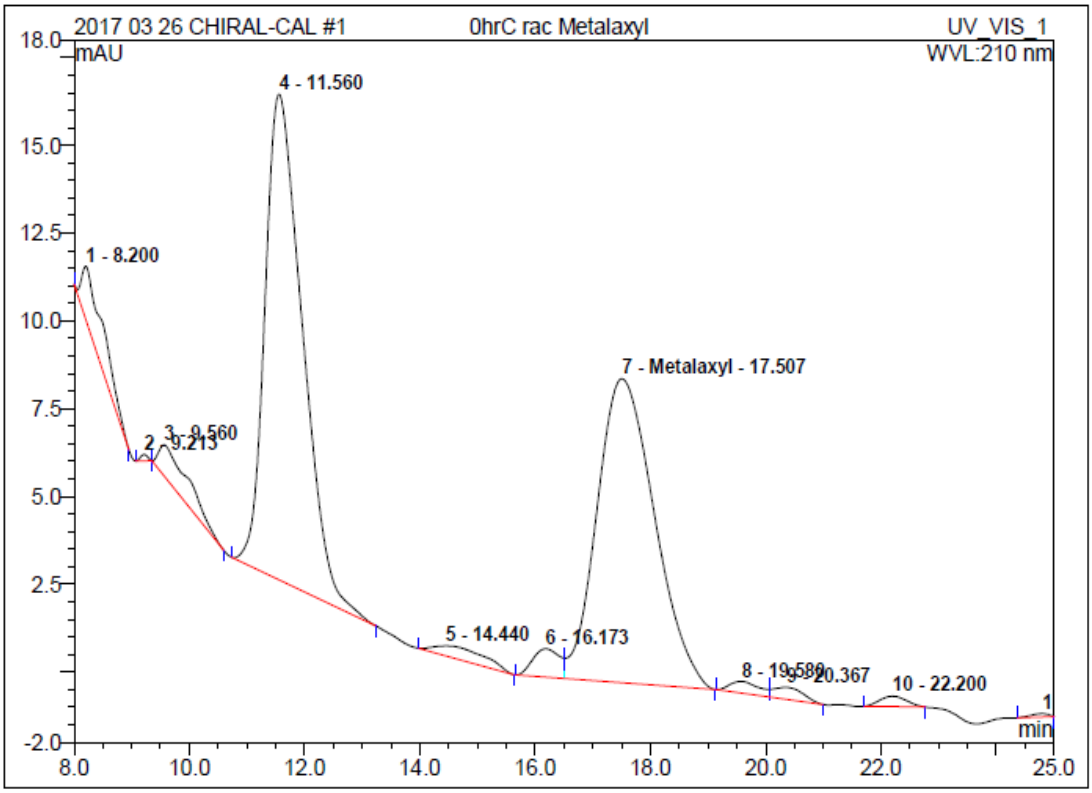


Figure A-2: Chiral chromatogram showing the elution of S-(+)-metalaxyl at 11.6 min and R-(-)-metalaxyl at 17.5 min.

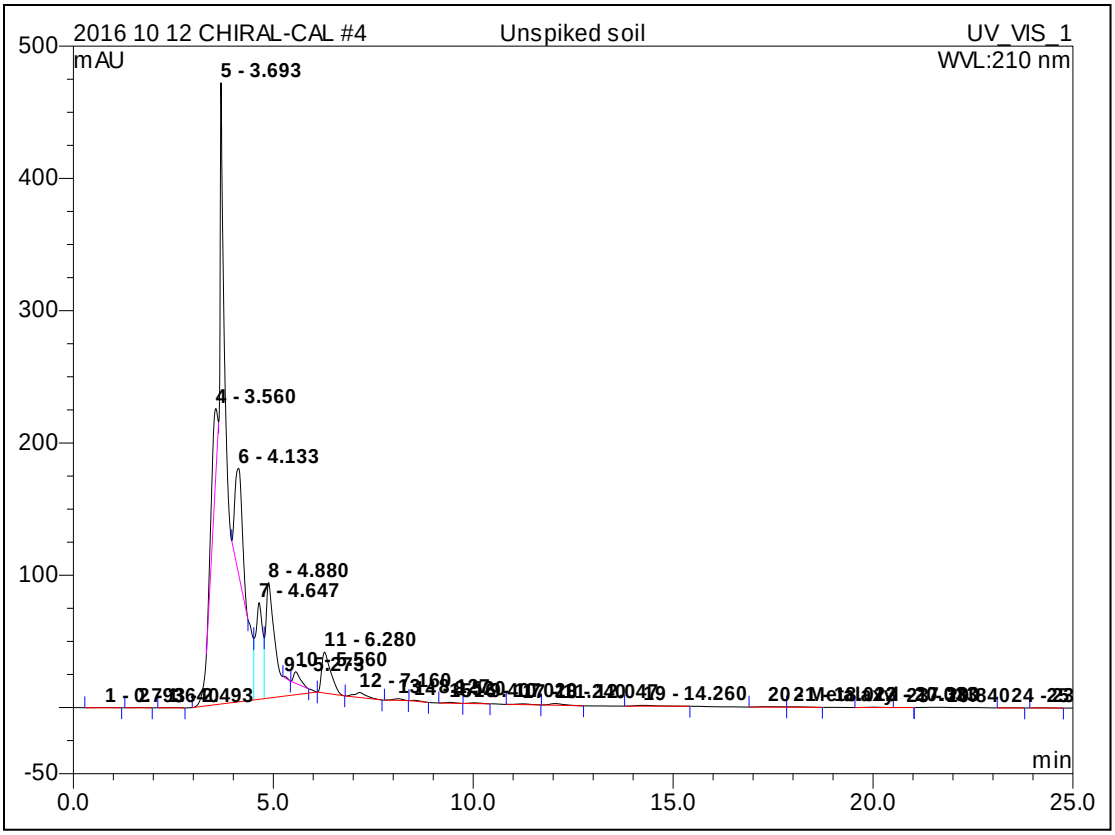


Figure A-3. Chromatogram showing an unspiked soil with no metalaxyl or malathion present.

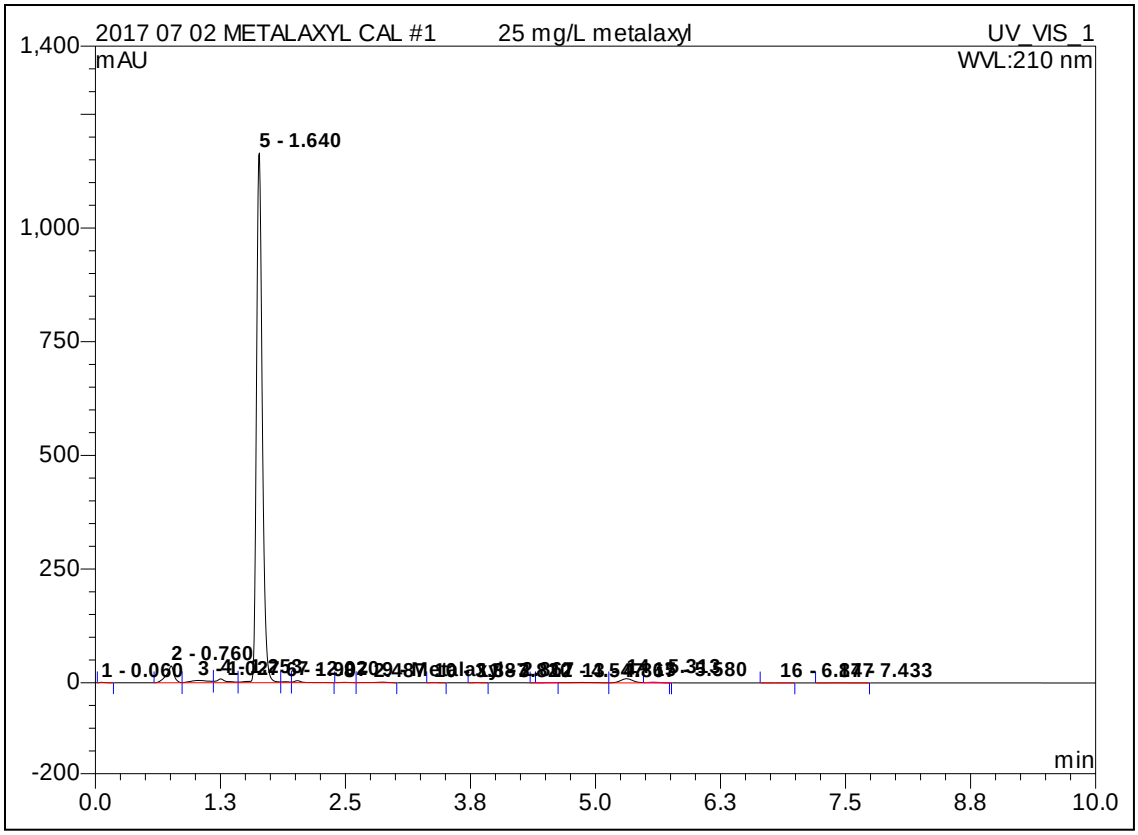


Figure A-4: Achiral chromatogram showing the elution of metalaxyl at 1.6 min.

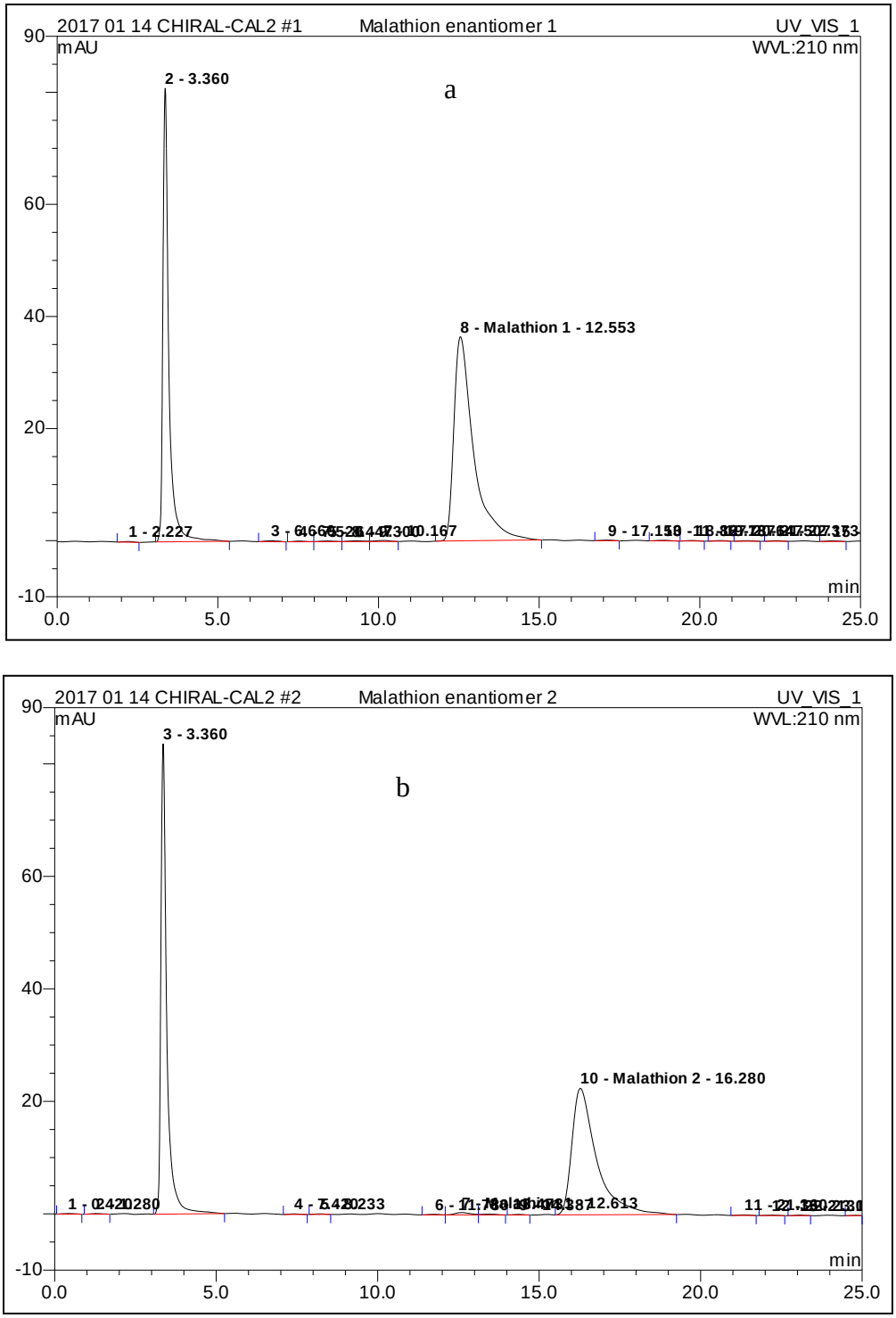


Figure A-5: Chiral chromatogram showing the elution of (a) R-(+)-malathion and (b) S-(-)-malathion.

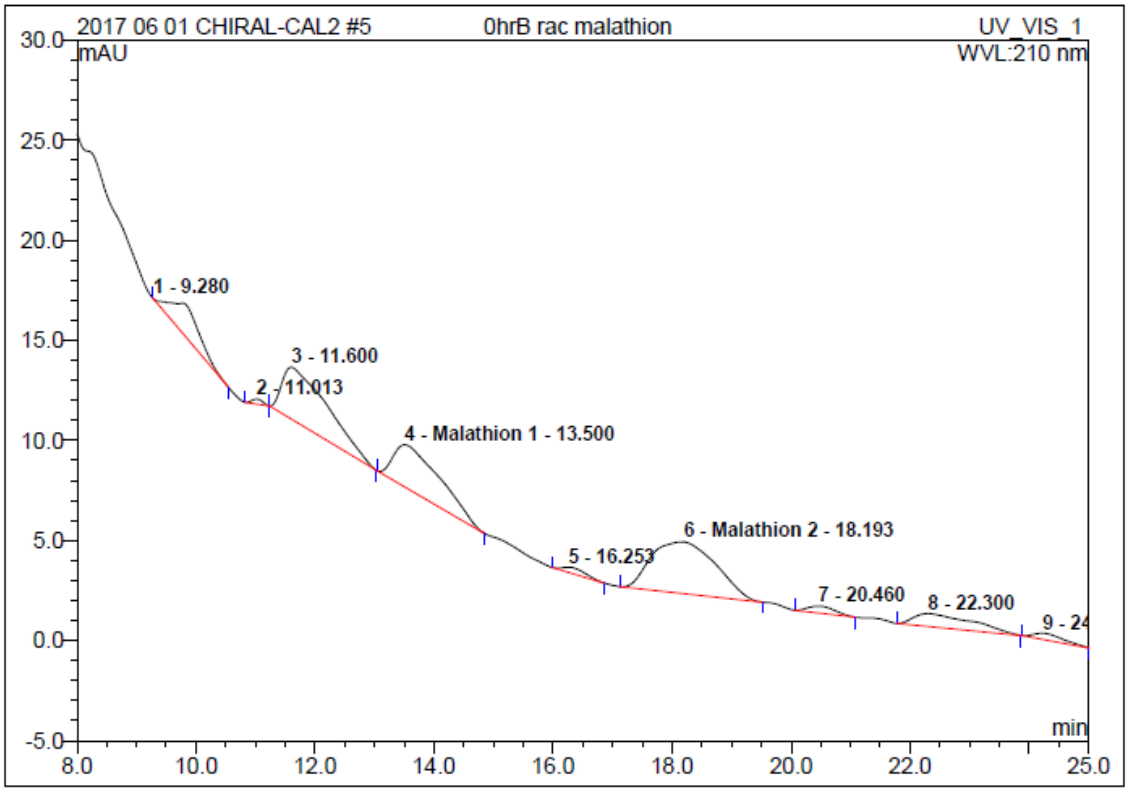


Figure A-6. Chiral chromatogram showing the elution of R-(+)-malathion (malathion 1) and S-(-)-malathion (malathion 2).

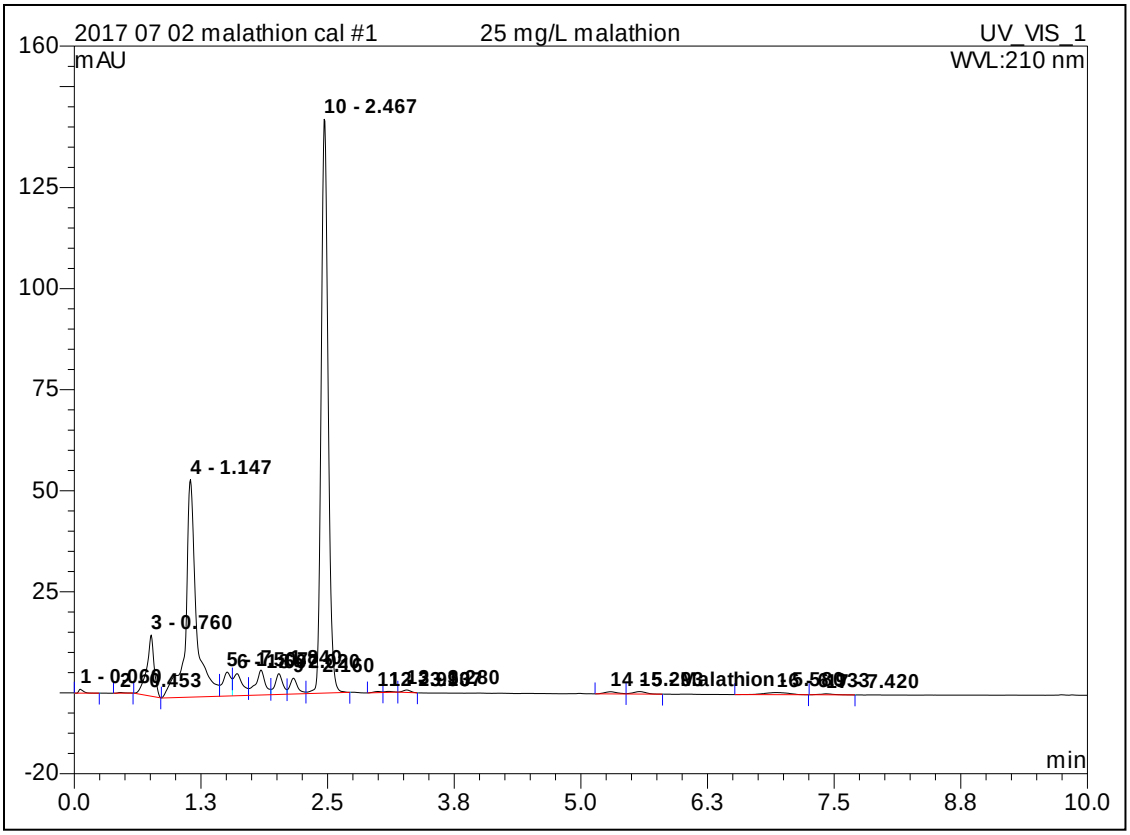


Figure A-7. Achiral chromatogram showing the elution of malathion at 2.5 min.

Appendix B

Supplementary Material for Chapter IV

MINERAL LABS INC.
Box 549
Salyersville, Kentucky 41465
Phone (606) 349-6145

Certificate of Analysis

COMPANY REQUESTING ANALYSIS:	Date Analyzed:	5/1/2017
Clemson University	Lab No.	17011643
	Sampled By/Type:	Customer

Sample ID: Mail In: Rollerson Soil 1: 5-30-2016

PROXIMATE ANALYSIS	As Received	Dry Basis
% Moisture (D3302/D3173)	3.22	
% Ash (D3174)	87.04	89.94
% Volatile (D3175)	xxxxx	xxxxx
% Fixed Carbon (Calculated)	xxxxx	xxxxx
B.T.U (D5865/D5864)	xxxxx	xxxxx
M.A.F.B.T.U. (Calculated)	xxxxx	
% Sulfur (D4239)	xxxxx	xxxxx
SO_2 lbs./mm Btu	xxxxx	
Ash lbs./mm Btu	xxxxx	

ULTIMATE ANALYSIS (ASTM D5373)	As Received	Dry Basis
Moisture	xxxxx	
Carbon	xxxxx	xxxxx
Hydrogen	xxxxx	xxxxx
Nitrogen	xxxxx	xxxxx
Sulfur	xxxxx	xxxxx
Ash	xxxxx	xxxxx
Oxygen (diff.)	xxxxx	xxxxx

SULFUR FORMS (ASTM D2492)	As Received	Dry Basis
% Pyritic Sulfur	xxxxx	xxxxx
% Sulfate Sulfur	xxxxx	xxxxx
% Organic Sulfur	xxxxx	xxxxx
% Total Sulfur	xxxxx	xxxxx

MINERAL ANALYSIS (ASTM D4326)		% Wt. Ignited Basis
Silicon dioxide	SiO_2	61.65
Aluminum oxide	Al_2O_3	20.58
Titanium dioxide	TiO_2	1.61
Iron oxide	Fe_2O_3	9.20
Calcium oxide	CaO	0.37
Magnesium oxide	MgO	1.20
Potassium oxide	K_2O	1.37
Sodium oxide	Na_2O	0.24
Sulfur trioxide	SO_3	0.14
Phosphorus pentoxide	P_2O_5	0.15
Strontium oxide	SrO	0.01
Barium oxide	BaO	0.07
Manganese oxide	MnO	0.05
Undetermined		3.36

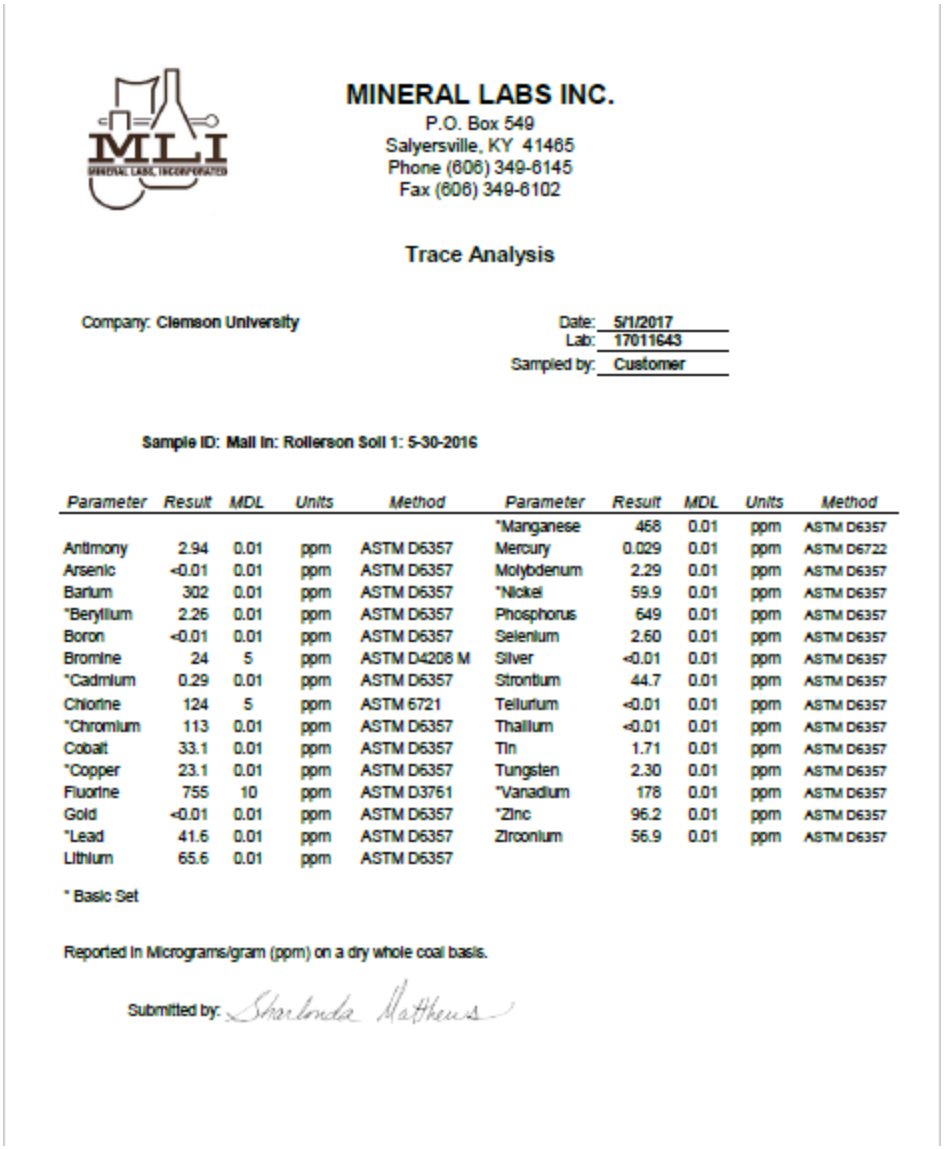
FUSION TEMPERATURE OF ASH (D1857)	Reducing (°F)	Oxidizing (°F)
Initial Temp.	xxxxx	xxxxx
Softening Temp. H=W	xxxxx	xxxxx
Hemispherical Temp. H=1/2 W	xxxxx	xxxxx
Fluid Temp	xxxxx	xxxxx
T-250 Temp. of Ash	2610	

Base/Acid Ratio	0.1477
Fouling Factor	0.0351
Slagging Factor	xxxxx

Arsenic ppm (ASTM D6357)	<0.01
Chlorine ppm (ASTM 6721)	124
Mercury ppm (ASTM D6722)	0.029
Sand	13.3%
Silt	6.67%
Clay	80.0%
pH	5.3%
Grindability Index (D409)	xxxxx

WATER SOLUBLE ALKALIES (Reported in %)	
CaO	xxxxx
K_2O	xxxxx
Na_2O	xxxxx

Submitted By: Shantinda Matthews



MINERAL LABS INC.
P.O. Box 549
Salyersville, KY 41465
Phone (606) 349-6145
Fax (606) 349-6102

Trace Analysis

Company: **Clemson University**

Date: **5/1/2017**
Lab: **17011643**
Sampled by: **Customer**

Sample ID: Mail In: Rollerson Soil 1: 5-30-2016

Parameter	Result	MDL	Units	Method	Parameter	Result	MDL	Units	Method
					*Manganese	468	0.01	ppm	ASTM D6357
Antimony	2.94	0.01	ppm	ASTM D6357	Mercury	0.029	0.01	ppm	ASTM D6722
Arsenic	<0.01	0.01	ppm	ASTM D6357	Molybdenum	2.29	0.01	ppm	ASTM D6357
Barium	302	0.01	ppm	ASTM D6357	*Nickel	59.9	0.01	ppm	ASTM D6357
*Beryllium	2.26	0.01	ppm	ASTM D6357	Phosphorus	649	0.01	ppm	ASTM D6357
Boron	<0.01	0.01	ppm	ASTM D6357	Selenium	2.60	0.01	ppm	ASTM D6357
Bromine	24	5	ppm	ASTM D4208 M	Silver	<0.01	0.01	ppm	ASTM D6357
*Cadmium	0.29	0.01	ppm	ASTM D6357	Strontium	44.7	0.01	ppm	ASTM D6357
Chlorine	124	5	ppm	ASTM 6721	Tellurium	<0.01	0.01	ppm	ASTM D6357
*Chromium	113	0.01	ppm	ASTM D6357	Thallium	<0.01	0.01	ppm	ASTM D6357
Cobalt	33.1	0.01	ppm	ASTM D6357	Tin	1.71	0.01	ppm	ASTM D6357
*Copper	23.1	0.01	ppm	ASTM D6357	Tungsten	2.30	0.01	ppm	ASTM D6357
Fluorine	755	10	ppm	ASTM D3761	*Vanadium	178	0.01	ppm	ASTM D6357
Gold	<0.01	0.01	ppm	ASTM D6357	*Zinc	96.2	0.01	ppm	ASTM D6357
*Lead	41.6	0.01	ppm	ASTM D6357	Zirconium	56.9	0.01	ppm	ASTM D6357
Lithium	65.6	0.01	ppm	ASTM D6357					

* Basic Set

Reported in Micrograms/gram (ppm) on a dry whole coal basis.

Submitted by: Sharlonda Matthews

Figure B-1: Full soil analysis performed by Mineral Labs, Inc. (Salyersville, KY).

For the lime experiments, I added 3.5 lbs of lime per 70 sq. ft. of soil (per instructions on the bag). Based on that guideline, I made the following assumptions:

One acre of soil is equal to 43560 sq. ft. of soil. The approximate mass of one acre of soil is 2,000,000 lbs (H. Liu, personal communication, 2016). Also 50 g soil=0.110231 lb soil.

$$\frac{x\ sq.ft.}{0.110231 lb\ soil} = \frac{43560\ sq\ ft}{2{,}000{,}000\ lb}$$

X = 0.00240 sq. ft.

$$\frac{3.5\ lb\ lime}{70\ sq.ft.} = \frac{x}{.00240\ sq.ft.}$$

X = 1.20E-4 lb lime

1.20E-4 lb of lime is equal to 0.0544 g of lime, which is equal to 54 mg of lime.

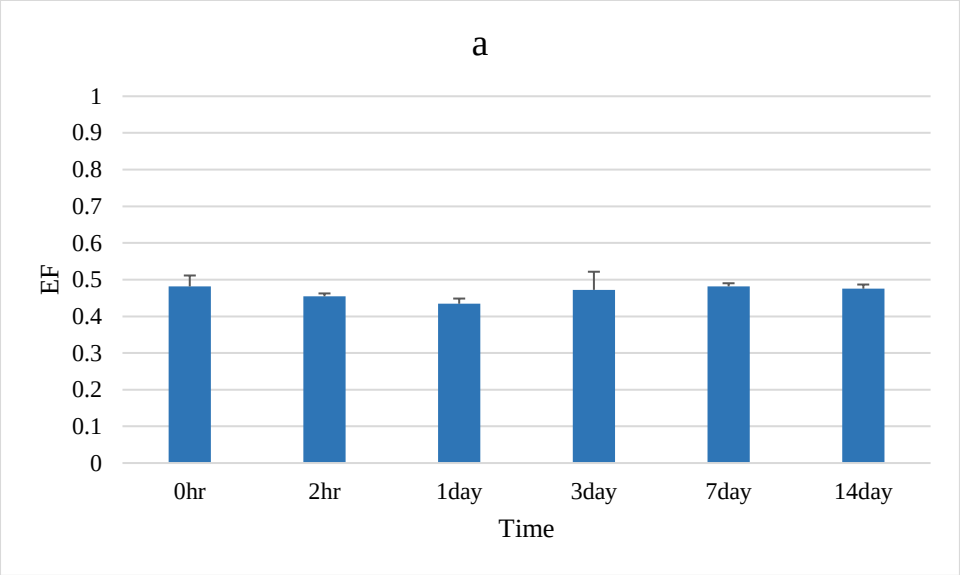


Figure B-2. Enantiomeric fractions (EF) of racemic metalaxyl in acid-unsterilized soil extracted with 1:1 DCM:acetone. The error bars represent the standard deviation (n=3). There was not a statistically significant difference between any of the time points.

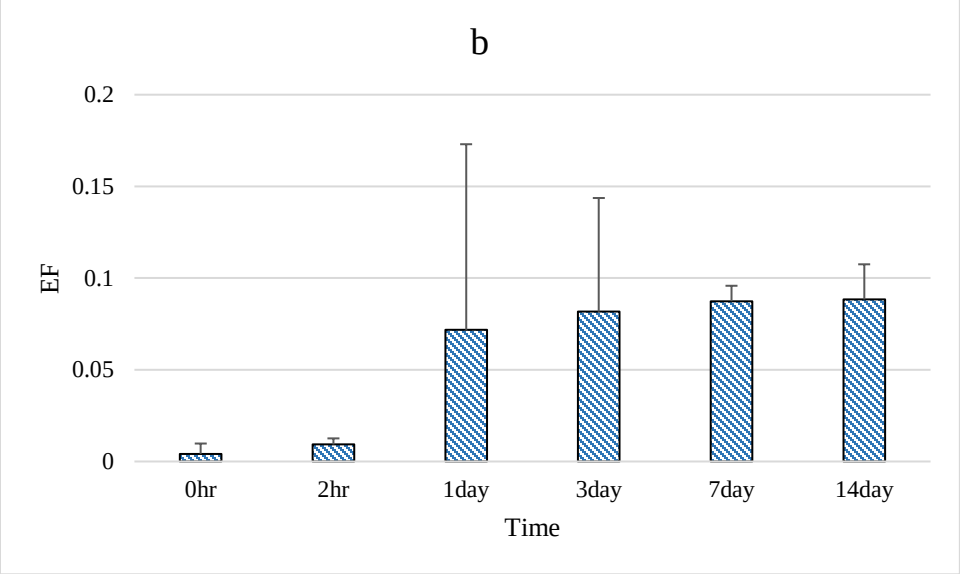


Figure B-3. Enantiomeric fractions (EF) of metalaxyl-M in acid-unsterilized soil extracted with 1:1 DCM:acetone. The EF for metalaxyl-M will be small because there is little presence of the S-(+)-enantiomer (Equation 1). The error bars represent the standard

deviation (n=3). There was not a statistically significant difference between any of the time points.

For the preliminary incubation above, the 0hr, 2 hr, and 1 d racemic metalaxyl time points were extracted via ASE. However, the ASE sensor malfunctioned during the extraction of the 1 d metalaxyl-M samples; therefore, I had to extract the remaining samples by hand. This caused the standard deviation for the 1 d metalaxyl-M sample to increase and, surprisingly, caused the EF to increase as well. Despite using two different extraction techniques and having an apparently increased EF for metalaxyl-M, there was not a statistically significant difference between any of the time points for either formulation of metalaxyl. However, DCM is a carcinogen and a chlorinated solvent, of which labs are trying to reduce its use. Therefore, my final batch of incubations used methanol as the extracting solvent.

The GLM Procedure

t Tests (LSD) for EF

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	12
Error Mean Square	0.000219
Critical Value of t	2.17881
Least Significant Difference	0.0264

Means with the same letter are not significantly different.

t Grouping	Mean	N	Time
A	0.07728	3	3day
A			
A	0.07661	3	7day
A			
A	0.06427	3	0hr
B	0.03040	3	14day
B			
B	0.02417	3	1day
B			
B	0.01989	3	2hr

Figure B-4: SAS output showing the differences in metalaxyl-M in acid-unsterilized soil

Metalaxyl-M EF
CHANTAL ROLLERSON

The GLM Procedure

Tukey's Studentized Range (HSD) Test for EF

Note: This test controls the Type I experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	9
Error Mean Square	0.000498
Critical Value of Studentized Range	5.02344

Comparisons significant at the 0.05 level are indicated by ***.

Time Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
2hr - 1day	0.05172	-0.02062	0.12406	
2hr - 7day	0.05975	-0.01950	0.13899	
2hr - 3day	0.08780	0.01546	0.16014	***
2hr - 14day	0.09234	0.02000	0.16468	***
2hr - 0hr	0.09473	0.01548	0.17397	***
1day - 2hr	-0.05172	-0.12406	0.02062	
1day - 7day	0.00803	-0.06431	0.08037	
1day - 3day	0.03608	-0.02862	0.10078	
1day - 14day	0.04062	-0.02409	0.10532	
1day - 0hr	0.04301	-0.02934	0.11535	
7day - 2hr	-0.05975	-0.13899	0.01950	
7day - 1day	-0.00803	-0.08037	0.06431	
7day - 3day	0.02805	-0.04429	0.10039	

2hr - 1day	0.05172	-0.02062	0.12406	
2hr - 7day	0.05975	-0.01950	0.13899	
2hr - 3day	0.08780	0.01546	0.16014	***
2hr - 14day	0.09234	0.02000	0.16468	***
2hr - 0hr	0.09473	0.01548	0.17397	***
1day - 2hr	-0.05172	-0.12406	0.02062	
1day - 7day	0.00803	-0.06431	0.08037	
1day - 3day	0.03608	-0.02862	0.10078	
1day - 14day	0.04062	-0.02409	0.10532	
1day - 0hr	0.04301	-0.02934	0.11535	
7day - 2hr	-0.05975	-0.13899	0.01950	
7day - 1day	-0.00803	-0.08037	0.06431	
7day - 3day	0.02805	-0.04429	0.10039	
7day - 14day	0.03259	-0.03975	0.10493	
7day - 0hr	0.03498	-0.04427	0.11422	
3day - 2hr	-0.08780	-0.16014	-0.01546	***
3day - 1day	-0.03608	-0.10078	0.02862	
3day - 7day	-0.02805	-0.10039	0.04429	
3day - 14day	0.00454	-0.06017	0.06924	
3day - 0hr	0.00692	-0.06542	0.07926	
14day - 2hr	-0.09234	-0.16468	-0.02000	***
14day - 1day	-0.04062	-0.10532	0.02409	
14day - 7day	-0.03259	-0.10493	0.03975	
14day - 3day	-0.00454	-0.06924	0.06017	
14day - 0hr	0.00239	-0.06995	0.07473	
0hr - 2hr	-0.09473	-0.17397	-0.01548	***

Figure B-5: SAS output showing the differences in metalaxyl-M in lime-unsterilized soil. Note: The chart lists every single sample point due to unequal sample sizes. For 0 hr and 2 hr, n=2, due to contamination in the samples, causing no metalaxyl to elute in the chiral chromatogram.

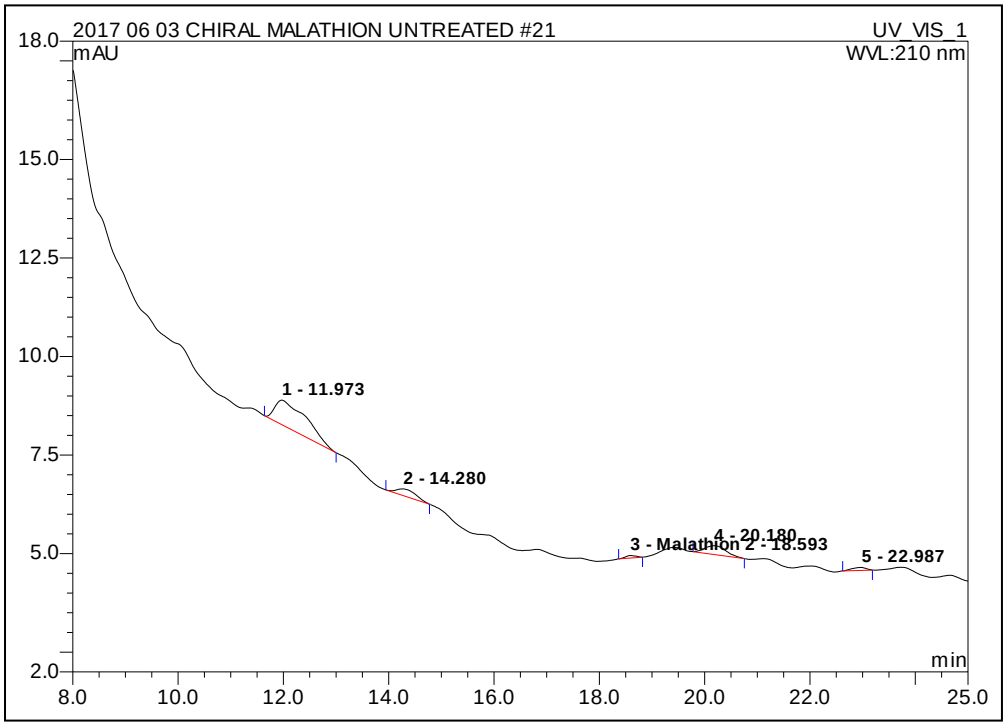


Figure B-6. Chromatogram of 3 d A R-(+)-malathion in acid-unsterlized soil showing no elution of either malathion enantiomer. 3 d B and C also showed no elution of either enantiomer.

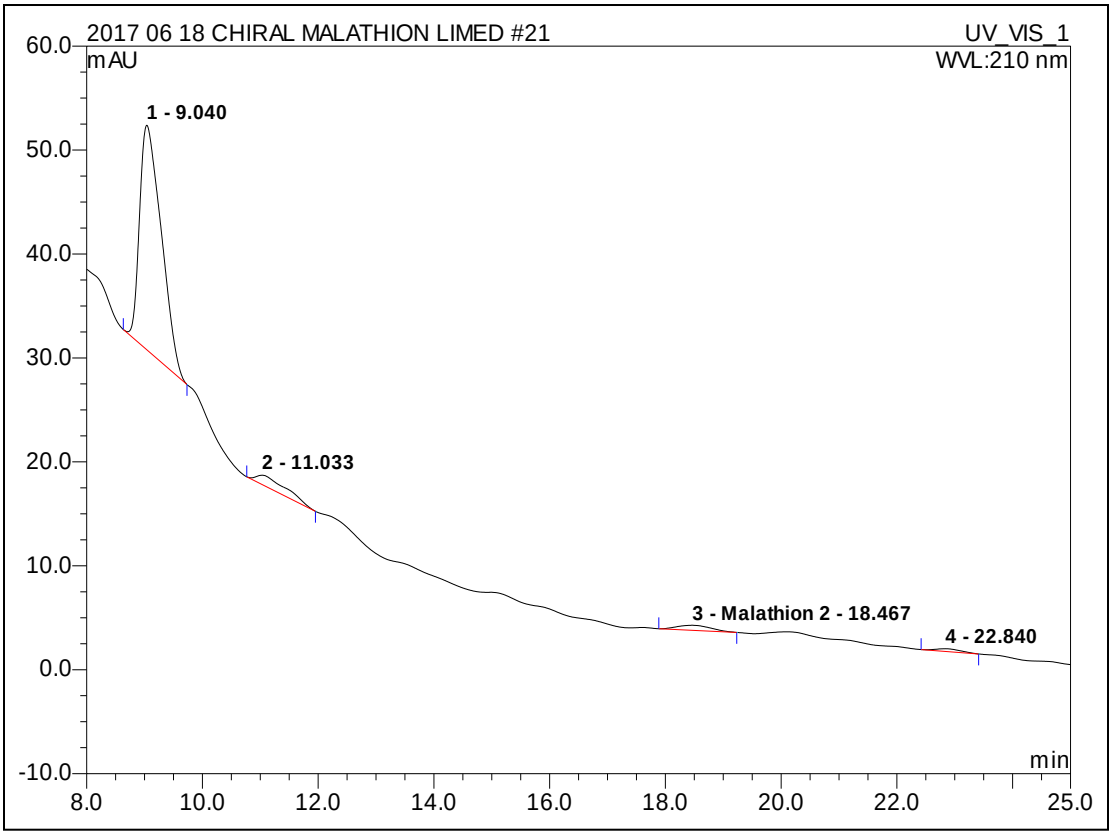


Figure B-7. Chromatogram displaying no elution of R-malathion in lime-unsterilized soil incubated at ambient temperature.

R Malathion EF
CHANTAL ROLLERSON

Sunday, July 2, 2(

The GLM Procedure

Tukey's Studentized Range (HSD) Test for EF

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	8
Error Mean Square	0.002282
Critical Value of Studentized Range	4.52877
Minimum Significant Difference	0.1249

Means with the same letter are not significantly different.

Tukey Grouping		Mean	N	Time
	A	0.94864	3	0hr
	A			
B	A	0.87666	3	1hr
B	A			
B	A	0.83827	3	2hr
B				
B		0.76014	3	1day

Figure B-8: SAS output showing the differences in R-malathion in acid-unsterilized soil

R Malathion lime fridge EF
CHANTAL ROLLERSON

Sunday, July 2, 2

The GLM Procedure

Tukey's Studentized Range (HSD) Test for EF

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.004429
Critical Value of Studentized Range	4.65425
Minimum Significant Difference	0.1788

Means with the same letter are not significantly different.

Tukey Grouping		Mean	N	Time
	A	0.89008	3	2hr
	A			
	A	0.83447	3	1hr
	A			
	A	0.79314	3	0hr
	A			
B	A	0.72110	3	1day
B				
B		0.54903	3	3day

Figure B-9: SAS output showing the differences in R-malathion in lime-unsterilized soil incubated at 10°C.

R Malathion lime amb EF
CHANTAL ROLLERSON

Tuesday, July 11, 2017 0

The GLM Procedure

Tukey's Studentized Range (HSD) Test for EF

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.002885
Critical Value of Studentized Range	4.65425
Minimum Significant Difference	0.1443

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Time
A	1.00000	3	2hr
A			
A	0.98837	3	0hr
A			
A	0.96907	3	1hr
B	0.51633	3	1day
C	0.00000	3	3day

Figure B-10: SAS output showing the differences in R-malathion in lime-unsterilized soil incubated at ambient temperature.

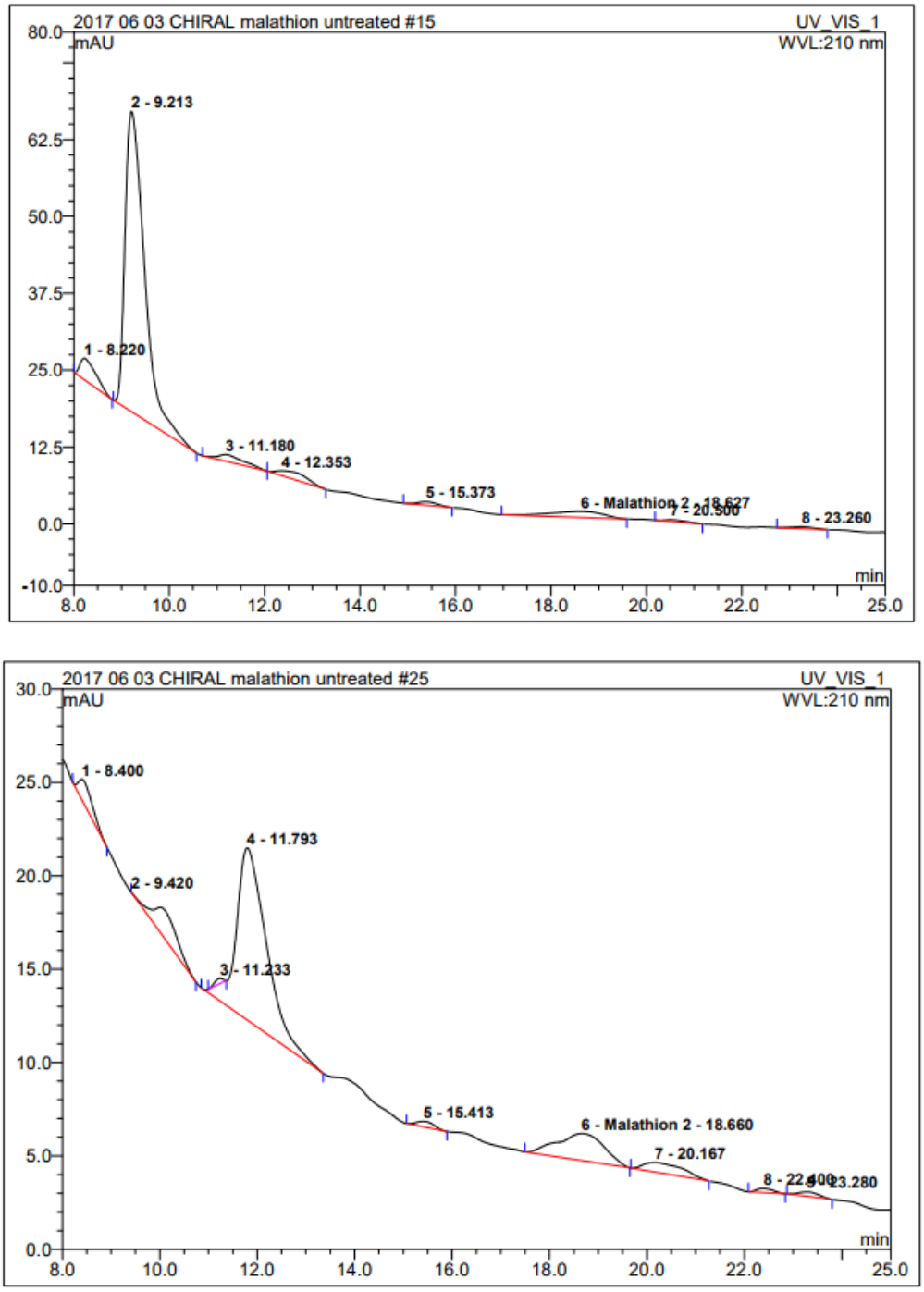


Figure B-11. Chromatograms of (a) 1 d A and (b) 3 d A of the S enantiomer incubation in acid-unsterilized soil displaying no elution of the R-(+)-enantiomer.

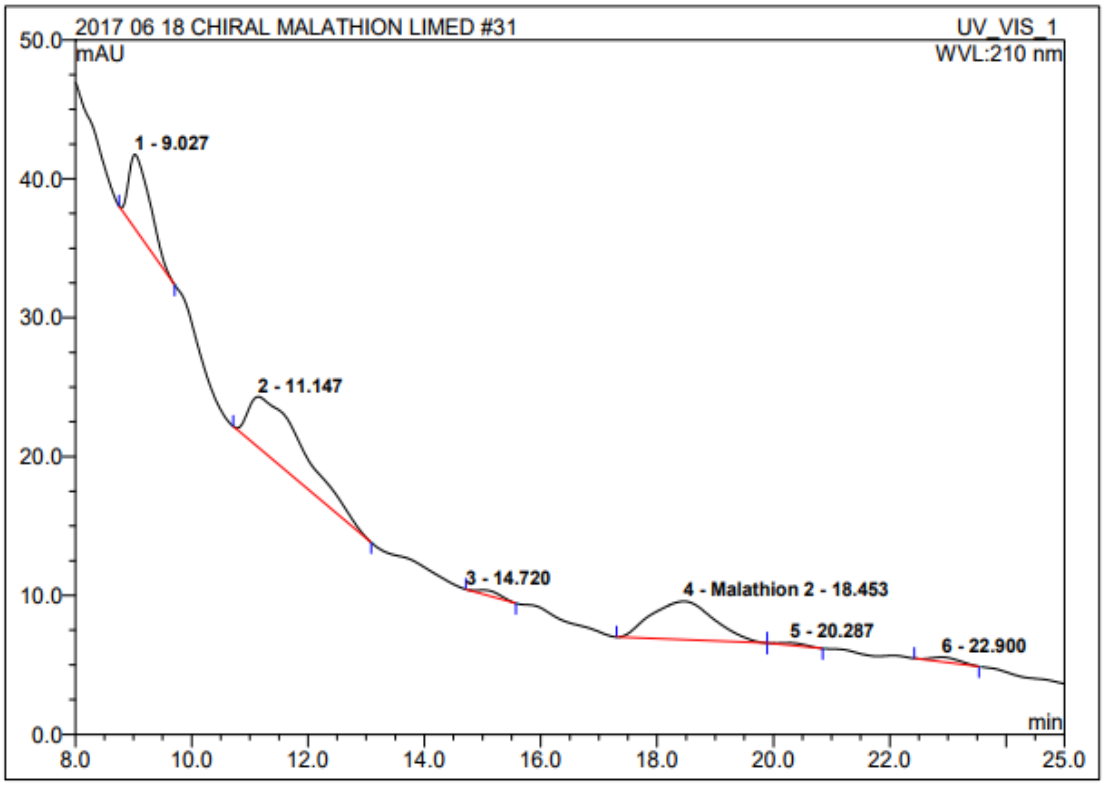


Figure B-12. Chromatogram of 3 d A S-(-)-malathion in the lime-unsterilized soil incubated at 10°C showing no elution of the R enantiomer.

S Malathion lime fridge EF Sunday, July 2, 2017
CHANTAL ROLLERSON

The GLM Procedure

Tukey's Studentized Range (HSD) Test for EF

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.000558
Critical Value of Studentized Range	4.65425
Minimum Significant Difference	0.0635

Means with the same letter are not significantly different.

Tukey Grouping		Mean	N	Time
	A	0.09271	3	1day
	A			
B	A	0.06132	3	0hr
B	A			
B	A	0.06094	3	2hr
B	A			
B	A	0.03737	3	1hr
B				
B		0.00000	3	3day

Figure B-13: SAS output showing the differences in S-malathion in lime-unsterilized soil incubated at 10°C.

Rac Malathion lime fridge EF Sunday, July 2, 201
CHANTAL ROLLERSON

The GLM Procedure

Tukey's Studentized Range (HSD) Test for EF

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.000406
Critical Value of Studentized Range	4.65425
Minimum Significant Difference	0.0541

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	Time
A	0.41535	3	2hr
A			
A	0.41354	3	1hr
A			
A	0.41216	3	0hr
A			
A	0.39186	3	1day
B	0.32527	3	3day

Figure B-14: SAS output showing the differences in racemic malathion in lime-unsterilized soil incubated at 10°C.

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