Universidade de Aveiro Departamento de Química
Ano 2012

OLGA MANUELA SIMÃO FILIPE

TIRAME NO AMBIENTE: EFEITO DE SUBSTÂNCIAS HÚMICAS E IÕES Cu(II)

THIRAM IN THE ENVIRONMENT: EFFECT OF HUMIC SUBSTANCES AND Cu(II) IONS

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Tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Química, realizada sob a orientação científica da Doutora Eduarda Santos, Professora Auxiliar do Departamento de Química da Universidade de Aveiro e co-orientação da Doutora Maria Manuel Vidal, Professora Adjunta do Departamento de Ciências Exatas da Escola Superior Agrária de Coimbra.

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palavras-chave

tirame, adsorção-desadsorção, solo, substâncias húmicas, iões cobre, águas naturais, fotodegração

resumo

Em Portugal, o tirame é um dos fungicidas mais utilizados, cujas vendas aumentaram significativamente nos últimos anos, sendo também um dos fungicidas mais utilizados em todo o mundo. No entanto, em comparação com outros pesticidas, existe falta de informação na literatura sobre o seu comportamento em sistemas ambientais, nomeadamente, no que diz respeito à sua degradação no solo ou em águas e produtos a que dá origem. Neste contexto, o objetivo deste trabalho foi estudar a influência das substâncias húmicas e iões cobre no comportamento e destino do tirame no meio ambiente. Foram realizados vários estudos para analisar o comportamento do tirame em solos com diferentes conteúdos de matéria orgânica e de iões cobre, e em águas naturais, estudando como as substâncias húmicas, os iões cobre e a luz solar podem afetar a sua degradação. Os estudos de adsorção-desadsorção do tirame nos solos revelaram que a matéria orgânica do solo e o conteúdo de cobre afetavam os processos de adsorção-desadsorção do tirame, influenciando a sua lixiviação e persistência no solo. De facto, verificou-se que o teor de cobre do solo tinha um efeito bastante marcante no processo de adsorção-desadsorção do tirame. Verificou-se a ocorrência de reações entre o tirame e os iões cobre, cuja extensão durante os estudos de adsorção pode ser fortemente dependente do teor de cobre do solo e da concentração inicial de tirame em solução. Assim, a escolha do tempo de equilíbrio em estudos de adsorção e a determinação das isotérmicas de adsorção ao solo torna-se uma tarefa difícil. Além disso, os complexos formados com o cobre existente no solo são persistentes, não sendo facilmente lixiviados para as águas subterrâneas. Conclui-se que os iões cobre(II) podem contribuir para a imobilização do tirame no solo e o aumento da persistência dos seus resíduos ligados ao cobre. A partir de estudos de recuperação do tirame em águas naturais verificou-se a ocorrência de uma rápida degradação do tirame, devido provavelmente aos iões metálicos, nomeadamente, iões cobre. Verificou-se que dependendo da razão tirame:Cu podiam ocorrer dois processos: (i) complexação entre o tirame e o cobre, quando não há excesso de iões cobre, sendo o complexo formado mais persistente que o tirame; (ii) ou, quando há um grande excesso de iões cobre, a degradação do tirame e a estabilização dos produtos de degradação por complexação, podendo formar-se complexos que permanecem sem alteração em solução durante pelo menos dois meses. No geral, foi possível, pela primeira vez, identificar alguns dos complexos de cobre formados ao longo do tempo. Por fim, estudou-se a cinética de fotodegradação do tirame em solução aquosa sob a ação da luz solar e identificaram-se, pela primeira vez, três fotoprodutos. Verificou-se um aumento da velocidade de fotodegradação do tirame na presença de substâncias húmicas. Assim, podemos concluir que a matéria orgânica, os iões cobre(II) e a luz solar têm um efeito importante no comportamento do tirame no meio ambiente. Contudo, os iões cobre têm um efeito mais marcante na degradação e persistência dos produtos que são formados.

keywords

thiram, adsortion-desorption, soil, humic substances, copper ions, natural waters, photodegradation

abstract

In Portugal thiram is one of the most used fungicides, whose sales have increased significantly along the years and it is also one of the most used fungicides all over the world. However, comparing to other pesticides, there is a lack of information in the literature about the behaviour of thiram in environmental systems, namely, in what concerns its degradation in soil or in water systems and products formed.

In this context, the aim of this work was to assess the influence of humic substances and copper ions on the behaviour and fate of thiram in the environment. Different studies were performed to assess the behaviour of thiram in soils, with different organic amendments and with different copper contents, and in natural water, studding how humic substances, copper ions and sunlight can affect its degradation.

Adsorption-desorption studies of thiram onto soil revealed that organic mater and soil copper contents affect thiram adsorption-desorption processes, influencing its leachability and persistence in soil. In fact, soil copper content has a marked effect on the sorption process of thiram onto soil. Reactions between thiram and copper ions occur and the extent of their occurrence during adsorption studies may be strongly dependent on the soil copper content and on the initial thiram concentration in solution making the choice of the equilibration time for batch sorption studies and adsorption isotherms determination a difficult task. The complexes formed with copper in soil are persistent, and they are not easily leached from soil to groundwater. Thus, we can conclude that copper may contribute to the immobilization of thiram in soil.

Thiram recovery from natural waters showed rapid thiram degradation in environmental matrices, probably due to metal ions, namely copper. Depending on the Thi:Cu ratio two processes can occur: (i) when there is no excess of copper ions there will be the complexation between thiram and copper ions and the complex formed is more persistence than thiram; (ii) in the presence of a large excess of copper ions, which is the more probable situation in natural waters, thiram degradation can occur and then the degradation products are stabilized by complexation, forming complexes which can persist in aqueous solutions for at least two months. It was possible, by the first time, to identify some copper complexes formed.

The kinetics of the photodegradation of thiram in aqueous solutions, under sunlight, was studied and three photodegradation products were identified for the first time. Besides, it was shown that humic substances enhance the thiram photodegradation rate.

Thus, we can conclude from this work that the organic matter, copper ions and sunlight have important effects on the behaviour and fate of thiram in the environment. However it should be highlighted that copper ions have an extremely marked effect on the degradation of thiram and persistence of products which are formed.

List of contents

1 Introduction ---------- 1

1.1 Background ---------- 1

1.2 Ditiocarbamate fungicides ---------- 4

1.3 Thiram characteristics and applications ---------- 8

1.4 Determination of thiram ---------- 11

1.5 Thiram in environmental matrices ---------- 12
1.5.1 Adsorption-desorption of thiram ---------- 13
1.5.2 Persistence of thiram ---------- 16
1.5.3 Photochemical degradation ---------- 17

1.6 Research objectives ---------- 19

1.7 References ---------- 23

2 A solid-phase extraction procedure for the clean-up of thiram from aqueous solutions containing high concentrations of humic substances ---------- 31

2.1 Introduction ---------- 32

2.2 Experimental ---------- 33
2.2.1 Chemicals ---------- 33
2.2.2 Soil ---------- 34
2.2.3 Apparatus ---------- 34
2.2.4 Preparation of aqueous humic acid and soil extracts ---------- 34
2.2.5 SPE clean up procedure ---------- 34

2.3 Results and discussion ---------- 35
2.3.1 Measurement of thiram by UV spectrophotometry ---------- 35
2.3.2 Testing of the C18–SPE procedure with solutions containing only humic acids or thiram ---------- 36
2.3.3 SPE as a clean-up step of thiram from aqueous solutions equilibrated with humic acid or soil samples ---------- 38

2.4 Conclusions ---------- 39

2.5 References ---------- 40

3 Modelling adsorption isotherm data: effect of fitting methods ---------- 43

3.1 Introduction ---------- 44

3.2 Isotherm Models ---------- 45
3.2.1 Freundlich isotherm ---------- 45
3.2.2 Langmuir isotherm model ---------- 46
3.2.3 Brunauer–Emmett–Teller (BET) isotherm ---------- 47

3.3 Experimental ---------- 49
3.3.1 Chemicals ---------- 49
3.3.2 Adsorption studies ---------- 49
3.3.3 SPE clean up procedure ---------- 50
3.3.4 HPLC-UV analysis of thiram ---------- 50
3.3.5 Error functions: statistical tools for choosing the best isotherm ---------- 51
3.3.6 Data analysis ---------- 53

3.4 Results and discussion ---------- 53
3.4.1 Measurement of thiram by HPLC-UV ---------- 53
3.4.2 Modelling the adsorption isotherms ---------- 54
3.4.3 Error estimation using statistical tools ---------- 61

3.5 Conclusions ---------- 63

3.6 References ---------- 65

4 Adsorption-desorption behaviour of thiram onto humic acid ---------- 69

4.1 Introduction ---------- 70

4.2 Experimental ---------- 71
4.2.1 Chemicals ---------- 71
4.2.2 Adsorption studies ---------- 72
4.2.3 Desorption studies ---------- 73
4.2.4 Kinetic study ---------- 73
4.2.5 Calculation of distribution of thiram ---------- 73
4.2.6 Isotherms models ---------- 75
4.2.7 Statistical analysis ---------- 75

4.3 Results and discussion ---------- 75
4.3.1 Kinetic studies ---------- 75
4.3.2 Adsorption isotherms ---------- 76
4.3.3 Desorption isotherms ---------- 79

4.4 Conclusions ---------- 83

4.5 References ---------- 84

5 Effect of long term organic amendments on adsorption-desorption of thiram onto a luvisol soil derived from loess ---------- 87

5.1 Introduction ---------- 88

5.2 Experimental ---------- 89
5.2.1 Chemicals ---------- 89
5.2.2 Soils ---------- 89
5.2.3 Kinetic Study ---------- 90
5.2.4 Batch adsorption/desorption studies ---------- 90
5.2.5 EDTA extraction of copper ions from COM soil ---------- 91
5.2.6 Calculation of thiram distribution between aqueous phase and soil ---------- 91
5.2.7 Models for the adsorption isotherms ---------- 92
5.2.8 Statistical analysis ---------- 92

5.3 Results and discussion ---------- 93
5.3.1 Kinetic studies ---------- 93
5.3.2 Adsorption studies ---------- 94
5.3.3 Desorption studies ---------- 100
5.3.4 Effect of copper ions on hysteresis ---------- 103

5.4 Conclusions ---------- 106

5.5 References ---------- 107

6 Influence of soil copper content on the kinetics of thiram adsorption and on thiram leachability from soils ---------- 111

6.1 Introduction ---------- 112

6.2 Kinetic equations ---------- 112
6.2.1 The pseudo first-order equation (Lagergren's equation) ---------- 113
6.2.2 The pseudo second-order equation ---------- 113
6.2.3 The Elovich equation ---------- 113
6.2.4 The intraparticle diffusion model ---------- 114

6.3 Experimental ---------- 114
6.3.1 Chemicals ---------- 114
6.3.2 Soil ---------- 115
6.3.3 Batch adsorption-desorption studies of thiram ---------- 115
6.3.4 Study of adsorption kinetics ---------- 116
6.3.5 Adsorption of copper ions onto soil ---------- 117
6.3.6 Analysis of metal ions in the soil organic extracts ---------- 117
6.3.7 Calculation of thiram distribution between aqueous phase and soil ---------- 118
6.3.8 Models for the adsorption isotherms ---------- 118
6.3.9 Statistical analysis ---------- 119

6.4 Results and discussion ---------- 119
6.4.1 The influence of ageing period on thiram desorption percentage ---------- 119
6.4.2 Influence of copper content in soil and initial concentration of thiram in solution on the adsorption kinetics of thiram. ---------- 124
6.4.3 Isotherms at different contact times ---------- 130

6.5 Conclusions ---------- 132

6.6 References ---------- 134

7 Influence of fulvic acids and copper ions on thiram determination in water ---------- 139

7.1 Introduction ---------- 140

7.2 Experimental ---------- 141
7.2.1 Chemicals ---------- 141
7.2.2 C_{18}-SPE-HPLC-UV procedure ---------- 142
7.2.3 Samples ---------- 142

7.3 Results and Discussion ---------- 143
7.3.1 Thiram pre-concentration on C_{18}-SPE cartridge: effect of flow rate ---------- 143
7.3.2 Application of SPE-HPLC-UV procedure to river water samples ---------- 144
7.3.3 Effect of copper ions on thiram recoveries by C_{18}-SPE ---------- 149
7.3.4 Effect of river Fulvic Acid and Cu(II) on thiram recoveries by C_{18}-SPE ---------- 152

7.4 Conclusions ---------- 155

7.5 References ---------- 157

8 Effect of copper ions on the degradation and persistence of thiram in aqueous solutions ---------- 161

8.1 Introduction ---------- 162

8.2 Experimental ---------- 163
8.2.1 Chemicals and solutions ---------- 163
8.2.2 Apparatus ---------- 163
8.2.3 C_{18}-SPE-HPLC-UV procedure ---------- 164
8.2.4 Identification of degradation products by HPLC-MS/MS ---------- 164

8.3 Results and Discussion ---------- 164
8.3.1 Evaluation of thiram stability in aqueous solution ---------- 164
8.3.2 Changes in the composition of thiram:copper solutions along time ---------- 166
8.3.3 Copper influence on thiram persistence in aquatic environment ---------- 173
8.3.4 Identification of the complexes by HPLC-MS/MS ---------- 175

8.4 Conclusions ---------- 184

8.5 References ---------- 185

9 Photodegradation of the fungicide thiram in aqueous solutions. Kinetic studies and identification of the photodegradation products by HPLC-MS/MS ---------- 187

9.1 Introduction ---------- 188

9.2 Experimental ---------- 189

9.2.1 Reagents and solutions ---------- 189
9.2.2 Water samples ---------- 189
9.2.3 Irradiation apparatus ---------- 190
9.2.4 Photodegradation of Thi-P and Thi-F ---------- 190
9.2.5 Photodegradation of Thi-P in the presence of natural fulvic and commercial humic acids --- 191
9.2.6 Identification of photodegradation products by HPLC-MS/MS ---------- 191
9.2.7 Statistical analysis ---------- 191

9.3 Results and discussion ---------- 193

9.3.1 Photodegradation of thiram (Thi-P) and thiram formulation (Thi-F) in aqueous solution ---- 193
9.3.2 Effect of humic substances in the photodegradation of Thi-P ---------- 195
9.3.3 Identification of the photoproducts of the direct degradation of thiram ---------- 198

9.4 Conclusions ---------- 205

9.5 References ---------- 207

10 Final conclusions ---------- 209

11 Supplementary information ---------- 215

11.1 DIRECTIVE 2003/81/CE ---------- 215

11.2 Summary of the principal methods used for determination of thiram in several matrices----- 221

Figure’s index

Figure 1.1 – Mean values of percentage sales in Portugal between 2002 and 2010 of (A) pesticides by type of pest and (B) fungicides by active substance 5

Figure 1.2 – Mean values of percentage sales of thiram, ziram and mancozeb, as the main used dithiocarbamates in Portugal between 2002 and 2010. 7

Figure 1.3 – Schematic representation of the potential fates of the pesticides in environment and some of the most important agents responsible of transfer and degradation phenomena. 13

Figure 2.1 - (A) UV-Vis spectra of 24.2 mg L-1 thiram solution in $CHCl_3$; (B) Calibration curve of thiram in $CHCl_3$ 35

Figure 2.2 – UV-Vis spectra of non-spiked aqueous extracts of humic acids before (a) and after (b) application to the C_{18} cartridge (absorbances were measured after adequate dilution of the solutions and were then multiplied by the dilution factor). 37

Figure 2.3 – UV-Vis spectra of $CHCl_3$ eluates of the C_{18}-SPE cartridges after application of: (A) humic acid extract non-spiked and spiked with thiram ([Thi]=7.6 mg L^{-1}); (B) soil extract non-spiked and spiked with thiram ([Thi]=10.4 mg/L). 38

Figure 3.1 – Thiram calibration curve and HPLC chromatograms of thiram standard solutions: (a) 0.56 mg L^{-1} (b) 1.10 mg L^{-1} (c) 2.23 mg L^{-1} (d) 3.34 mg L^{-1} (e) 4.56 mg L^{-1}. 54

Figure 3.2 - Adsorption isotherm of thiram onto commercial HA. Linearized forms of the Freundlich, Langmuir and BET isotherms adjusted to the experimental values of Y and X by the least-squares method.. 55

Figure 3.3 - Adsorption isotherm of thiram onto COM soil. Linearized forms of the Freundlich, Langmuir and BET isotherms adjusted to the experimental values of Y and X by the least-squares method.. 56

Figure 3.4 – Adsorption isotherm modelling of thiram onto both commercial HA and COM soil using linear and non-linear regression analysis. 59

Figure 4.1 - Effect of equilibrium time on the % of adsorption of Thi-P onto humic acids. 76

Figure 4.2 – Adsorption isotherm of both Thi-P and Thi-F onto HA and respective K_D values 77

Figure 4.3 – Adsorption-desorption isotherms of Thi-P and Thi-F onto commercial humic acids 81

Figure 5.1 – Percentage of thiram adsorbed of onto COM soil as a function of the contact time. $C_0 = 20.4$ (± 1.0) mg L^{-1}. 93

Figure 5.2 – Thiram adsorption isotherms onto a luvisol soil submitted to different controlled amendments: experimental data (open symbols) and line (------) corresponding to the fitted BET model. Desorption data are represented by black symbols. 94

Figure 5.3 – Experimental adsorption data of thiram onto FYM soil fitted to both BET (a) and Freundlich model (b) and (c).C_{eq} range: a) and b) 0 to 21 mg L^{-1}; c) 0 to 12 mg L^{-1}... 96

Figure 5.4 - Plots of (a) K_D and (b) K_{OC} values obtained for each soil versus thiram initial concentration 98

Figure 5.5 – Percentage of thiram desorbed (a) in each individual desorption cycle and after all desorption cycles and (b) in a unique desorption step with CH_3CN. Desorption values represent the % of the amount initially adsorbed. $[Thi]_0 = 22$ mg L^{-1}. 101

Figure 5.6 – Adsorption-desorption isotherms of thiram onto (A) $COM_{original}$ soil and (B) $COM_{extracted}$ soil. 103

Figure 5.7 – Experimental distribution coefficients (K_D) data for adsorption and desorption of thiram from $COM_{original}$ soil (●) and $COM_{extracted}$ soil (○)........ 104

Figure 5.8 – Hysteresis indices (HI) for thiram onto original COM soil (●) and extracted COM soil (○). 105

Figure 6.1 – HPLC-UV chromatograms for the acetonitrile extracts obtained for different ageing periods: a) 0, b) 1, c) 2, d) 3, e) 4, f) 8 and g) 18 days; 120

Figure 6.2 – Peak area profile of free thiram and copper complex. 120

Figure 6.3 – (A) Percentage of free thiram desorbed with acetonitrile and (B) area of the copper complex in the HPLC chromatograms of the acetonitrile extracts as a function of the equilibration time for two initial aqueous thiram concentrations, $C_0 = 6.83$ mg L^{-1} (☐) and $C_0 = 18.6$ mg L^{-1} (▨). Desorption values represent the % of the amount initially adsorbed. 123

Figure 6.4 – Percentage of thiram adsorbed onto COM soil as a function of the contact time 124

Figure 6.5 - Percentage of thiram adsorbed onto COM soil fortified with copper (COM_Cu_{80}) as a function of the contact time. 125

Figure 6.6 – Experimental kinetic adsorption data of thiram onto soil fitted to (▬) pseudo 2nd order, (▬) Elovich and (▬) intraparticle diffusion equations. 128

Figure 6.7 – (A) Adsorption isotherm of thiram onto $COM_{original}$ soil. (B) Percentage of thiram desorbed with CH_3CN as a function of initial thiram concentration. Desorption values represent the % of the amount initially adsorbed. 130

Figure 6.8 – (A) Adsorption isotherm of thiram onto COM soil fortified with Cu (500 mg kg^{-1}). Equilibration time of 15 h (B) Linear plot of the complex area *vs.* thiram concentration initially adsorbed 132

Figure 7.1 – HPLC-UV chromatogram of 1 L of river water sample after C_{18}-SPE procedure. 144

Figure 7.2 – HPLC-UV chromatograms of the natural water sample spiked with 11.2 µg L^{-1} thiram and equilibrated during 0 and 24 h, following SPE procedure. 146

Figure 7.3 – HPLC-UV chromatogram of the natural water sample spiked with 2.8 µg L^{-1} thiram and equilibrated during 24 h, following SPE procedure. 147

Figure 7.4 – Chromatograms of the river water samples collected in March of 2007: (a) $(River+Thi)_{24}$, (b) $[(River+EDTA)_{24}+Thi]_{24}$ and (c) $[(River+thi)_{24}+EDTA]_{24}$ samples. [EDTA] = 10^{-3} mol L^{-1}, [Thi] = 11.2 µg L^{-1} (The samples were submitted to the SPE-HPLC-UV analytical procedure). 148

Figure 7.5 - HPLC-UV chromatograms of a standard solution of thiram 2.4 mg L^{-1} (a) in the absence of Cu(II) and (b-d) in the presence of Cu(II) (16 mg L^{-1}) at different times of equilibration: b) t = 20 min, c) t = 2 h and d) t = 8 h. 150

Figure 7.6 – HPLC-UV chromatograms of thiram standard solutions, in the absence and in the presence of Cu(II), after equilibration for 24 h and SPE treatment. 151

Figure 7.7 – HPLC-UV chromatogram of yellow coloured complex Thi-Cu(II) retained in the C_{18} cartridge after elution with $CHCl_3$, drying under N_2 atmosphere and re-dissolution of the residue in acetonitrile. 152

Figure 7.8 – Chromatograms obtained in the analysis of aqueous solutions: (a) $(FA+Thi)_{24}$, (b) $[(FA+Cu^{2+})_{24}+Thi]_{24}$ and (c) $[((FA+Cu^{2+})_{24}+Thi)+EDTA]_{24}$, and UV-Vis spectrum of the peaks. [FA] = 2 mg L^{-1}, [Cu] = 0.01 mg L^{-1}, [EDTA] = 10^{-5} mol L^{-1}, [Thi] = 11.2 µg L^{-1}. 153

Figure 8.1 - (A) UV-Vis spectra of a 2.0 mg L^{-1} thiram aqueous solution registered during *ca.* a month: (⬛) time 0 to 5 days, (🟥) 7, (🟩) 11, (🟦) 19 and (🟪) 34 days; (B) pH values of a 2.0 mg L^{-1} thiram aqueous solution along solution aging time. 165

Figure 8.2 – UV-Vis spectra of a 2.0 mg L^{-1} thiram solution in the presence of copper ion registered at 1, 7, 14 and 34 days after thi:Cu(II) mixing, (⬛) thiram, (🟥) thi:Cu 1:0.3 and (🟦) thi:Cu 1:1 ratio (UV-vis spectra of 3rd and 4th graphs were obtained after filtration). 167

Figure 8.3 – UV-Vis spectra of a 2.0 mg L^{-1} thiram solution in the presence of copper ion registered at 1, 7, 14 and 34 days after thi:Cu(II) mixing; (⬛) thiram, (🟥) thi:Cu 1:3, (🟩) thi:Cu 1:10, (🟦) thi:Cu 1:25 and (🟪) thi:Cu 1:50 ratio. 168

Figure 8.4 – UV-Vis spectra of a 2.0 mg L^{-1} DMDTC solution registered with time: (a) time 0, (b), 3 hours, (c) 1, (d) 2 to 28 days. 169

Figure 8.5 – UV-Vis spectra of a 2.0 mg L^{-1} DMDTC solution in the presence of copper ion registered at 0, 1, 11 and 28 days after DMDTC:Cu(II) solutions preparation. (▬) DMDTC, (▬) DMDTC:Cu 1:0.3 and (▬) DMDTC:Cu 1:1 ratio. 170

Figure 8.6 – UV-Vis spectra of a 2.0 mg L^{-1} DMDTC solution in the presence of copper ions registered at 0, 1, 11 and 28 days after solutions preparation.(▬) DMDTC, (▬)DMDTC:Cu 1:3, (▬)DMDTC:Cu 1:10, (▬) DMDTC:Cu 1:25 ratio...................... 171

Figure 8.7 - UV-Vis spectra of (A) 2 mg L^{-1} DMDTC solution and (B) DMDTC:Cu 1:25 solution registered at time zero (▬) and 28th day after solutions preparation (▬). 172

Figure 8.8 - UV-Vis spectra of a DMDTC and Thi solution in the presence of copper ions registered at different days after solutions preparation. [DMDTC] = [Thi] = 2 mg L^{-1}; (▬) Thi:Cu 1:50, (▬) DMDTC:Cu 1:25. 173

Figure 8.9 – ESI-MS spectra of a (A) 6 h Thi:Cu 1:3 solution, (B) 10 days Thi:Cu 1:3 solution and (C) DMDTC:Cu 1:25 solution.. 177

Figure 8.10 - UV-Vis spectra of a DMDTC and Thi solution in the presence of copper ions registered at different days after solutions preparation. [DMDTC] = [Thi] = 2 mg L^{-1}.... 178

Figure 9.1 – Spectral Irradiance of the 1500 W arc xenon lamp when using an outdoor UV filter, as given by the manufacturer (Solarbox 1500, Co.fo.me.gra, Italy). The spectrum is referred to a total irradiance of 550 W m^{-2} between 290-800 nm, the conditions used during the irradiation experiments... 190

Figure 9.2 – Direct photodegradation curves of 2 mg L^{-1} thiram (Thi-P and Thi-F) in aqueous solutions: black circles are the experimental data and the lines are the graphic representation of the first order equation (equation 9.2) fitted to the mean values of C *vs.* time. Open circles are the dark controls.. 194

Figure 9.3 - Photodegradation curves of 2 mg L^{-1} thiram in the presence and absence of 10 mg L^{-1} natural FA and commercial HA; circles are the experimental data and the lines are the graphic representation of the first order equation (equation 9.2) fitted to the mean values of C vs. time. 195

Figure 9.4 - Photodegradation of 2 mg L^{-1} thiram in milli-Q water, in the presence of FA and in natural river water. (•) Control samples of thiram in natural river water. Symbols represent the mean values of replicates of the kinetic studies for each condition. The curves were obtained by non-linear regression of equation (9.2) to the mean values of *C vs.* time 197

Figure 9.5 – (A) HPLC-UV at 230 nm chromatograms of a 10 mgL^{-1} thiram solution after direct photolysis during 120 min and (B) HPLC-PDA at 270 nm chromatogram of the same irradiated solution after pre-concentration, obtained using the HPLC-PDA- MS/MS instrument.. 199

Figure 9.6 – Plot of the area of peak I and peak IV along photodegradation time with an initial thiram concentration of C_0 = 10 mg L^{-1}. 204

Table's index

Table 1.1. Pesticides classification according the type of pest.. 2

Table 1.2. Pesticide classification according to their mode of action. 3

Table 1.3. Pesticides classification according to their chemical group. 3

Table 1.4. Dithiocarbamates used as fungicides.. 6

Table 1.5. List of thiram uses (adapted from European commission, Thiram 6507/VI/99, 2003)... 8

Table 1.6. Thiram characteristics. 10

Table 1.7. Adsorption-desorption studies of thiram onto different adsorbents. 14

Table 1.8. Identification of the major products of thiram degradation............................... 18

Table 2.1. Thiram recoveries (R) and respective standard deviations, for the control aqueous solution containing only thiram in 0.01 mol L^{-1} $CaCl_2$, after applied to a C_{18} SPE cartridge: Thi_0 – immediately after preparation and Thi_{24} – after 24 h of shaking. 37

Table 2.2. SPE recoveries (R) of thiram and respective standard deviations for both humic acid and soil extracts. C_{ad} - concentration of thiram added to the extract.......................... 39

Table 3.1. Mathematical error functions to predict the best fit isotherm. 51

Table 3.2. Linear and non-linear equations to which experimental data were adjusted following the three mathematical models: Freundlich, Langmuir and Brunauer-Emmett-Teller isotherms……………………………………………………………………57

Table 3.3. Isotherm parameters obtained for thiram adsorption onto HA and COM soil using the linear and non- linear method……………………………………………..….60

Table 4.1. K_D values for the adsorption of various pesticides onto humic acids................ 77

Table 4.2. Adsorption equilibrium data of Thi-P and Thi-F fittings to Linear, Freundlich, Langmuir and BET isotherm models^a. 79

Table 4.3. Experimental data of adsorption-desorption isotherms of both Thi-P and Thi-F onto humic acids. 80

Table 4.4. First desorption equilibrium data of Thi-P and Thi-F fitting to Linear, Freundlich, Langmuir and BET isotherm models; fitting parameters and their respective errors for a 95% confidence interval. 82

Table 4.5. Hysteresis indices (HI) for both Thi-P and Thi-F onto humic acids. 83

Table 5.1. Fitting parameters for thiram adsorption equilibrium data to Freundlich and BET isotherms and respective errors within a 95% confidence interval. 95

Table 5.2. Distribution coefficients (K_D) and organic carbon normalized distribution coefficients (K_{OC}) of thiram adsorption onto COM, SLU, FYM and MIN soils obtained at three initial concentration levels. 99

Table 5.3. Experimental K_D data for desorption of thiram from soils and calculated adsorption K_D values for the same thiram equilibrium concentrate................................ 100

Table 5.4. Total thiram recovery obtained from two different desorption procedures. 102

Table 5.5. Fitting parameters for thiram adsorption-desorption data to BET isotherm and respective errors within a 95% confidence interval 103

Table 6.1.. Kinetic parameters for the adsorption of thiram onto COM and COM_Cu$_{80}$ soils... 126

Table 6.2. Initial adsorption factor (R_i) and kinetic behaviour based on intraparticle diffusion model. 129

Table 6.3. Initial adsorption factor (R_i) and kinetic behaviour based on intraparticle diffusion model for thiram adsorption onto both COM and COM_Cu$_{80}$ soils. 129

Table 7.1. Thiram recoveries (R) from 1 L of thiram standard solution after C$_{18}$-SPE procedure at different flow rates* 143

Table 7.2. Thiram recoveries (R) from 1 L of river water sample spiked with thiram*... 145

Table 7.3. Effect of EDTA (1 mM) in thiram recoveries (R) from 1 L of river water sample spiked with thiram and submitted to SPE* 147

Table 7.4.Thiram recovery (R) from 1 L standard solutions in the presence and absence of Cu(II) after C$_{18}$-SPE procedure*. 150

Table 7.5. Thiram recoveries (R) from standard solutions ([FA] = 2 mg L^{-1}, [Cu^{2+}] =0.01 mg L^{-1}, EDTA= 10^{-3} mol L^{-1})*... 154

Table 8.1. Percentage of decrease (%Abs$_d$) in the absorbance intensity of the bands at 260 and 385 nm after 28 days for DMDTC:Cu(II) 1:3, 1:10 and 1:25 ratios. 172

Table 8.2. Thiram recovery of 1 L of 11.0 μg L^{-1} thiram solution in the presence of an excess of copper (Thi:Cu ratio 1:25).. 174

Table 8.3. Thi:Cu (1:3) and DMDTC:Cu (1:25) byproducts identified and corresponding HPLC-MS/MS and MS^n fragmentation profiles 175

Table 9.1. Kinetic parameters for the photodegradation of Thi-P and Thi-F in aqueous solutions. 194

Table 9.2. Kinetic parameters for the photodegradation of 2 mg L^{-1} thiram in presence of humic substances in aqueous solutions, obtained by non-linear regression of equation (9.2) to the mean values of C *vs.* time (95% confidence intervals are presented). The number of replicates used to calculate the mean values of *C* for each irradiation time is indicated between parentheses. 196

Table 9.3. Thiran and photodegradadtion products identified and corresponding LC-MS/MS and MS^n fragmentation profiles. 200

Table 11.1. Determination of thiram in various matrices. 221

Abbreviations

$\% Ads$	percentage of thiram adsorbed onto adsorbent
$\% Des$	percentage desorbed
ANOVA	two-way analysis of variance
ARE	average relative error
BET	Brunauer–Emmett–Teller isotherms
C_0	thiram initial aqueous phase concentration (mg L^{-1})
C_{eq}	equilibrium concentration in solution
C_{s}	solubility of the adsorbate or the saturation concentration for adsorbate in solution
C_{eq}^{Des}	thiram equilibrium concentration in solution after desorption (mg L^{-1})
df	degrees of freedom
DMDTC	Dimethyldithiocarbamate anion
DOC	dissolved organic carbon
EDTA	Ethylenediamine tetraacetic acid
EPA	Environmental Protection Agency
FA	fulvic acids
FAAS	flame atomic adsorption spectrophotometry
HA	humic acids
HI	hysteresis index
HPLC	High-performance liquid chromatography
K	empirical constant in BET isotherm
K_1	rate constant of pseudo-first order sorption (h^{-1}).
K_2	rate constant of pseudo second-order adsorption (g μg^{-1} min^{-1}).
K_{D}	distribution coefficient (L g^{-1})
K_{F}	Freundlich constant
k_i	intraparticle diffusion rate constant (μg $(g\ h)^{-1/2}$)
K_{L}	Langmuir constant
K_L^*	equilibrium constant of adsorption for upper layers in BET isotherm
K_{OC}	organic carbon partition coefficients.
K_{S}	equilibrium constant of adsorption for the first layer in BET isotherm
LOD	limit of detection
LSD	least significant difference
m_{ads}	mass of thiram adsorbed during the adsorption experiment
m_{des}	amount of thiram desorbed (mg)
MPSD	marquardt's percent standard deviation
m_{r}	amount of thiram in the residual solution after the adsorption experiment (mg)
MS	Mass spectrometry
N	Freundlich parameter
n	number of points
OC	organic carbon content
OECD	Organisation for Economic Co-operation and Development
$\overline{Q}$	mean of the Q_{exp}
Q	amount adsorbed by unit mass of the adsorbent
Q^{Ads}	solid phase thiram concentrations (mg g^{-1}) after adsorption

Q^{Des}	solid phase thiram concentrations (mg g^{-1}) after a single desorption cycle experiment
Q_{est}	amount adsorbed by unit mass adsorbent estimated from the model
Q_{exp}	amount adsorbed by unit mass of adsorbent obtained experimentally
Q_{max}	maximum adsorption (mg g^{-1})
Q_{ref}	adsorbed amount at time t_{ref}
Q_t	sorption capacities at equilibrium and at time t
R^2	coefficient of determination
R^2_{Adj}	R^2 adjusted
RMS	residual mean square
SAE	sum of the absolute errors
SD	standard deviation
SPE	solid-phase extraction
SSE	sum of the squares of the errors
$S_{y/x}$	standard deviation of y-residuals
$t_{df(0.05)}$	t-Student value for *df* degrees of freedom
UV	Ultraviolet
UV/Vis	Ultraviolet-visible spectroscopy
V_0	aqueous phase volume in contact with the adsorbent during adsorption experiments
V_r	residual volume of the supernatant that could not be removed prior to desorption and which was gravimetrically determined.

Greek letters

χ^2	Chi-square
σ	standard deviation

Chapter 1

Introduction

1.1 Background

At the begining of 1940, organic compounds obtained from plant extracts and inorganic compounds containing heavy metals were the most widely used pesticides. During the 1940s decade, the use of pesticides has increased significantly, due to the introduction of synthetic chemicals and generalization of their use. Among the different applications of pesticides, the main and most important application is in agriculture in order to increase the productivity, quality and quantity of the foodstuffs, allowing to mantain yields and so contributing to the economic viability of agriculture. Most are complex compounds that can be released directly and/or indirectly to the surrounding environment what may be of some environmental concern, because of their production quantities, toxicity, persistence, tendency to bioaccumulate and of their potentially harmful metabolites. Today, the public demands more and more a safe environment, free from chemicals and pesticides. Consequently, many efforts have been done in order to protect environment and safely manage hazardous substances, resulting in the worldwide decision to registrate the new chemicals before their commercial use, as stated on the Regulation (EC) Nº 1107/2009 of the European Parliament and of Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC (Information available at http://europa.eu/legislation_summaries/food_safety/plant_health_checks/sa0016_en.htm; last accessed on 20th November 2012).

Besides, understanding the sources, fate, behaviour and effects of these chemicals in the environment becomes essential for society to accept risks of adverse ecological or human health effects inherent to their use. So, the composition of pesticides, transport processes and migration in and between the various environments must be completely understood, especially when factors, such as, pesticide properties, soil properties, hydraulic loading on the soil and crop management practices, are determinant on contamination of air, water and soil systems.

On the other hand, the chemistry of pesticides has been constantly growing, with new effects in both environmental and public health to be detected, demanding the development of new analytical methodologies, allowing the detection of small amounts in different matrices and the identification of their metabolites. Finally, the regulatory agencies are more and more demanding, providing more complex guidelines, requiring more tests and restrictions on the use of newer chemicals as well as of the oldest. Consequently, the research in this field has intensified greatly due to the need for consistent and meaningful environmental data and their respective interpretation.

According to the U.S. Environmental Protection Agency (EPA) (information available at http://www.epa.gov/pesticides/about/index.htm; last accessed on 20th November 2012) "*A pesticide is any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest*" (Racke, 2003, Estéves et al., 2008). It is a generic term which covers a wide range of biologically active substances. There are various ways by which pesticides can be classified: based on their target (the pests that they control) (Table 1.1), according to their mode of action (the way they control the pest) (Table 1.2) or chemical group (Tables 1.3).

Table 1.1. Pesticides classification according the type of pest.

Pesticide type	**Pest/function**
Algicides	Control algae in water systems (lakes, canals, swimming pools)
Antifouling agents	Kill or repel organisms that attach to underwater surfaces, such as boat bottoms.
Antimicrobials	Microorganisms (such as bacteria and viruses).
Bactericides (disinfectants and sanitizers)	Kill or inactivate disease-producing microorganisms on inanimate objects
Fumigants	Produce gas or vapor intended to destroy pests in buildings or soil.
Fungicides	Fungi (including blights, mildews, molds, and rusts).
Herbicides	Weeds and other plants that grow where they are not wanted
Insecticides	Insects and other arthropods
Miticides (or acaricides)	Mites that feed on plants and animals.
Microbial pesticides	Microorganisms that kill, inhibit, or out compete pests, including insects or other microorganisms.

Table 1.2. Pesticides classification according the type of pest (continuation).

Pesticide type	Pest/function
Molluscicides	Snails and slugs.
Nematicides	Nematodes (microscopic, worm-like organisms that feed on plant roots).
Pheromones	Biochemicals used to disrupt the mating behaviour of insects.
Plant growth regulator	Control the crop growth processes.
Repellents	Repel pests, including insects and birds.
Rodenticides	Control mice and other rodents as rats.
Wood preservative	Wood-destroying organisms.

Table 1.3. Pesticide classification according to their mode of action.

Pesticide type	Description of action mode
Contact poison	The pesticide enters the body of pests via their epidermis upon contact and causes death
Fumigation	The pesticide in gas form enters the body of pests via their respiration system and causes death.
Stomach poison	The pesticide enters the body of pests via their mouthpart and digestive system and causes death
Systemic action	Pesticides consumed by a host organism will stay in its body fluids. Pests feeding on the body fluids of the host organism will then be killed by poisoning**.**

Table 1.4. Pesticides classification according to their chemical group.

Pesticide type	Chemical group	
Fungicides	Benzimidazoles Dicarboximides Carbamates Phenylamides	Diazols and triazols Dithiocarbamates Copper based fungicides Sulfur-based fungicides
Herbicides	Amides Nitrophenols Chlorophenoxys Dipyridyls	Carbamates Uracils Triazines Ureas
Insecticides and acaricides	Carbamates Organochlorines	Organophosphates Pyrethroids
Nematodicides	Carbamates Organophosphates	
Rodenticides	Organochlorines Organophosphates	Pirimidinaminas Ureas

1.2 Ditiocarbamate fungicides

Fungicides are chemical compounds extensively used in industry (suppression of mildews that attack painted surfaces; control of slime in paper pulps), in medicine (control human fungal diseases) and agriculture for a number of purposes. It is known that fungi may seriously damage plants, either during growth or after harvesting, resulting in a decrease of production and/or in the product quality. Thus, fungicides are extensively used in agriculture, where they are very effective against fungi without harming the crop. Their use includes protection of seed grains during storage, shipment and germination; protection of mature crops, berries, seedling, flowers and grasses in the fields or during their storage and shipment.

According to 2002-2010 reports of Direção-Geral de Agricultura e Desenvolvimento Rural, the Portuguese Official Agency of Agriculture, (DGADR), the fungicide group represents, in Portugal, about 75-80% of pesticide market sales (Figure 1.1A) (Vieira, 2004, 2005a, 2005b, 2005c, 2006, 2007, 2008; Abreu et al., 2009; Abreu and Mourão, 2010, Abreu, 2011). From the different chemical groups of fungicides, the inorganic-based fungicides are the largest contributor to fungicides sales with ~86% (corresponding ~80% to the sulphur-based fungicides and ~6% to the copper-based fungicides), while, within the organic group, dithiocarbamates are the major group, contributing with ~10% of the total fungicide sales (Figure 1.1B).

According to the literature (Thorn and Ludwing, 1962), dithiocarbamates have a great effect on biological systems, due to their metal binding capacity or ability to interact with compounds containing sulfhydryl groups. Although they were first used in medicine for the control of dermatophytes and in chronic alcoholism correction (Hald and Jacobsen, 1948), dithiocarbamates have also been extensively employed in biochemical investigation as inhibitors of enzymes and mainly in the agriculture field. The mechanism of action associated with the pesticide activity of the dithiocarbamates is the inhibition of metal-dependant and sulfhydryl enzyme systems in fungi, bacteria, plants, and insects, as well as mammals (Miller, 1982).

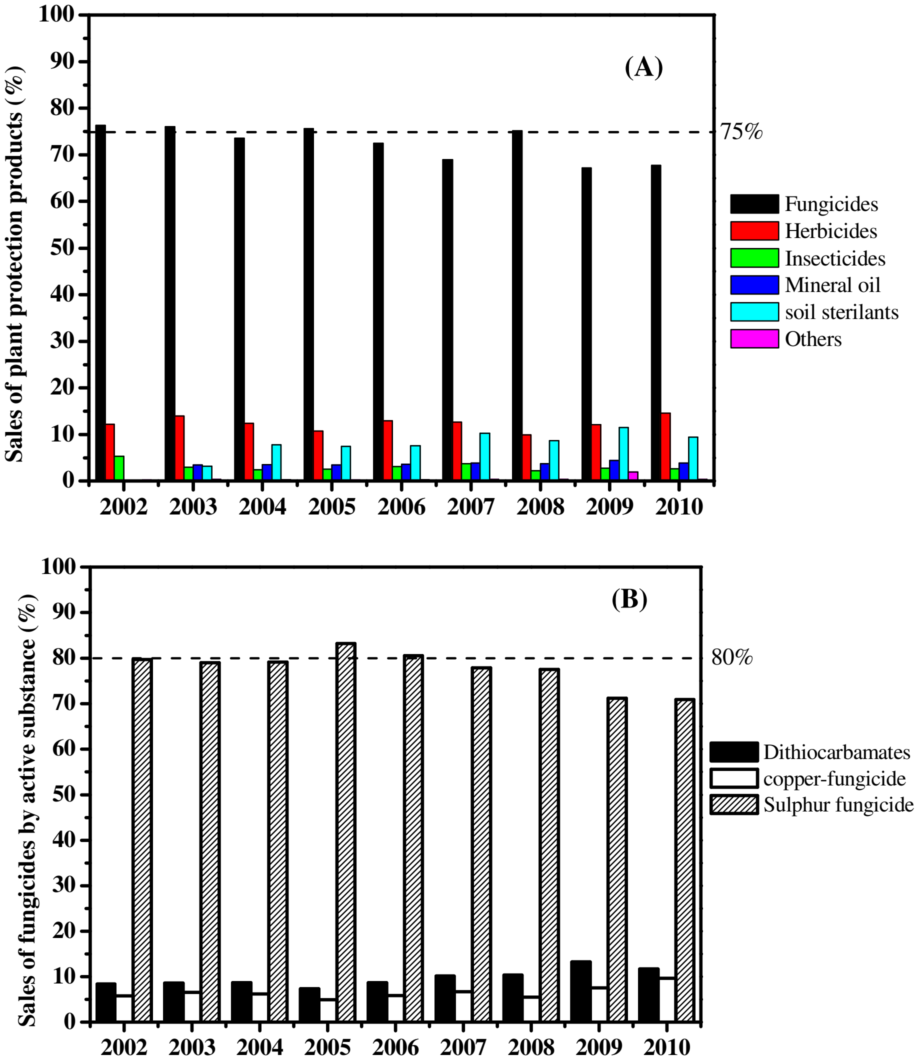
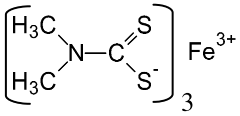
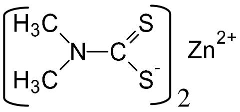
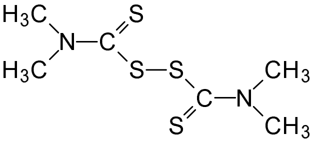
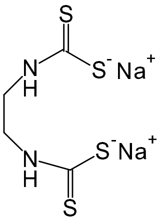
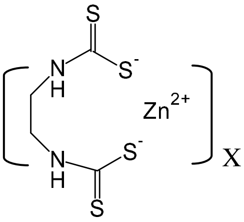
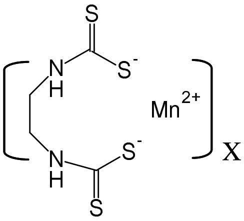
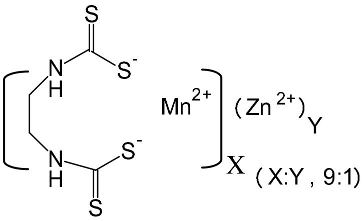


Figure 1.1 – Mean values of percentage sales in Portugal between 2002 and 2010 of **(A)** pesticides by type of pest and **(B)** fungicides by active substance

Among the various dithiocarbamates available, very few have acceptance as commercial fungicides (Table 1.4). Among them, those derived from dialkylamines, only the iron **(1)** and zinc **(2)** salts of dimethyldithiocarbamic acid and tetramethylthiuram disulfide **(3)**, known as **ferbam**, **ziram** and **thiram**, respectively, are widely used. Other compounds, such as disodium ethyenebisdithiocarbamate, known as **nabam (4),** and its zinc and manganese polymeric salts, named **zineb (5)**, **maneb (6)** and **mancozeb (7)** are also used as fungicides Thorn and Ludwing (1962) make an extensive review of the main characteristics of the dithiocarbamates and their activity in the different fields of application.

Table 1.5. Dithiocarbamates used as fungicides

Chemical	**Structure**
DMDTC's= dimethyldithiocarbamates	
Ferbam **(1)**	
Ziram **(2)**	
Thiram **(3)**	
EBDC's=ethylene-(bis)-dithiocarbamates	
Nabam **(4)**	
Zineb **(5)**	
Maneb **(6)**	
Manconzeb **(7)**	

According to the 2002-2010 available reports (Vieira, 2004, 2005a, 2005b, 2005c, 2006, 2007, 2008; Abreu et al., 2009; Abreu and Mourão, 2010, Abreu, 2011) among the various dithiocarbamates used in agriculture, the mancozeb, thiram and ziram are the fungicides most sold in Portugal (Figure 1.2).

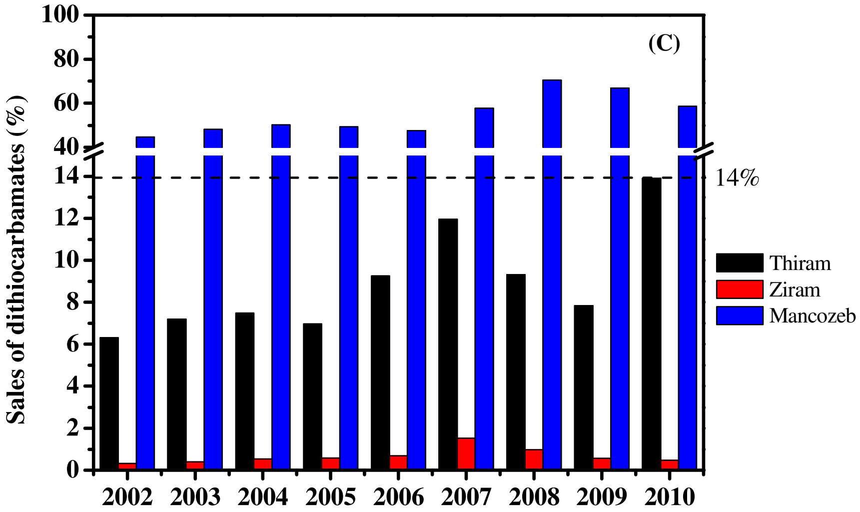


Figure 1.2 – Mean values of percentage sales of thiram, ziram and mancozeb, as the main used dithiocarbamates in Portugal between 2002 and 2010.

During this period, thiram selling rate registered a significant increase until 2007, with a slight decrease in 2008 and 2009 that follows the downward trend of the fungicide sales between 2008 and 2009. With mancozeb as only exception, pesticides in general showed a fall of 27% between 2007 and 2009, probably because of unfavourable climatic and agronomic conditions. In 2010, sales of thiram have increased significantly again, showing that thiram still occupies a relevant position in the sales of fungicides in Portugal.

According to information supplied by DGADR at the moment there is no information about distribution of pesticide sales by the various regions of Portugal. However, they are working on the implementation of the Regulation (EC) 1185/2009 of the European Parliament and of the Council of 25 November 2009 concerning statistics on pesticides (Information available at http://eur-lex.europa.eu/en/index.htm; last accessed on 20th November 2012) in order to have statistics about the use of pesticides based in both crops and country regions.

Thus, thiram is a dithiocarbamate compound registered in Annex I of the Directive 91/414/CEE, regarding the placing of plant protection products on the market since 2003

(Chapter 11, Supplementary information, section 11.1) that was replaced by the EC Directive 1107/2009 (Information available at http://dgadr.pt/default.aspx; http://ec.europa.eu/food/plant/protection/evaluation/stat_active_subs_3010_en.xls; last accessed on 20th November 2012). In April of 2012 the list of the plant protection products authorized for sale in Portugal was updated by the DGADR and thiram continues to be part of that list (Information available at http://madrp.dgadr.pt/; last accessed on 20th November 2012).

1.3 Thiram characteristics and applications

Thiram or tetra-methyl-thiuram disulfide is a dithiocarbamate compound that has been used as a contact fungicide with preventive action. It is a compound that acts in breath activity of fungi inhibiting spore's germination. It is one of the contact fungicides most widely used to prevent crop damages, either in the field or during its transport and storage (Roberts and Hutson, 1999; Sharma et al., 2003). It is used to protect seeds, fruits, vegetables and ornamental plants from a variety of fungi (such as, *Botrytis* spp., *Anthracnosis*, *Fusarium* and *Manilia*) in lettuce, spinach, tomatoes, strawberry, peach, apple and pear. In addition, it is also used as a repellent to protect the fruit and ornamental trees from damages caused by rabbits and rodents (Sharma et al., 2003). Table 1.5 shows a list of thiram uses, adapted from the review report for the active substance thiram finalised in the Standing Committee on the Food Chain and Animal Health at its meeting on 4th July 2003 in view of the inclusion of thiram in Annex I of Directive 91/414/EEC (European commission, Thiram 6507/VI/99, 2003).

Table 1.6. List of thiram uses (adapted from European commission, Thiram 6507/VI/99, 2003).

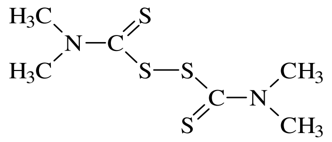
Crop	Application	Pests or group of pests controlled	Country
Apple	Outdoor or field; use foliar.	*Venturia inaequalis* (Scab) *Monilia* *Gloeosporium* *Gloeodes pomigena* *Schizothirium pomi*	All Europe
Almond	Outdoor or field; use foliar.	*Monilia spp.* *Coryneum* *Taphrina*	South-Europe

Table 1.7. List of thiram uses (adapted from European commission, Thiram 6507/VI/99, 2003) (continuation).

Crop	Application	Pests or group of pests controlled	Country
Pear	Outdoor or field; use foliar.	*Venturia pirina* (Scab) *Stemphylium vesicarium* *Monilia* *Gloeosporium* *Gloeodes pomigena* *Schizothirium pomi*	All Europe
Peach	Outdoor or field; use foliar.	*Monilia spp* *Fusicladium.* *Taphrina deformans* *Coryneum beijerinckii (Stigmina carpophila)* *Fusicoccum amygdali*	All Europe
Wine grapes	Outdoor or field; use foliar.	*Botrytis* *Colletotrichum*	All Europe
Strawberry (indoor & outdoor)	Outdoor or field use glasshouse application; use foliar.	*Botrytis* *Colletorichum* *Mycosphaerella*	All Europe
Ornamentals (indoor & outdoor)	Outdoor or field use glasshouse application;use foliar.	*Botrytis*	All Europe
Ornamentals bulbs	Outdoor or field; use foliar.	*Sclerotinia*	All Europe
Seed	Outdoor or field; use foliar.	*Bird repellent*	All Europe
Beans	Outdoor or field; use foliar.	soil / seed borne fungi	All Europe
Celery	Outdoor or field; use foliar.	soil / seed borne fungi	All Europe
Cucumber	Outdoor or field; use foliar.	soil / seed borne fungi	All Europe
Grass	Outdoor or field; use foliar.	soil / seed borne fungi	All Europe
Lettuce	Outdoor or field; use foliar.	soil / seed borne fungi	All Europe
Maize	Outdoor or field; use foliar.	soil / seed borne fungi animal repellency	All Europe
Wheat	Outdoor or field; usefoliar	soil / seed borne fungi animal repellency	All Europe

Thiram is also used in industry as accelerator and antioxidant in rubber production and more recently, used as precursor of films with semiconducting and luminescent properties (Ivanov et al., 2007). Thiram is available as dust, flowable, wettable powder, water dispersible granules, and water suspension formulations, and in mixtures with other fungicides. Table 1.6 shows the main characteristics of thiram (information available in http://www.eu-footprint.org/, last accessed on 20th November 2012).

Table 1.8. Thiram characteristics.

Chemical name	Thiram
Chemical structure	
IUPAC name	dimethylcarbamothioylsulfanyl N,N-dimethylcarbamodithioate tetramethylthiuram disulfide
Commercial name (in Portugal)	Pomarsol Ultra D (Bayer CropScience)
CAS	137-26-8
Physical state	White to yellow crystalline powder
Molecular mass ($g\ mol^{-1}$)	240.4
Melting point	146 ºC
Log K_{ow}	1.73
Vapor pression	2.3 mPa at 25 ºC
Solubility in water	30 mg L^{-1} (25 ºC); 16.5 mg L^{-1} (20ºC)
Solubility in other solvents	**Soluble**: acetone (69.7 g L^{-1} 25°C), chloroform (205 g L^{-1} 25°C) and most organic solvents. **Slightly soluble:** carbon disulfide, diethyl ether and ethanol (<10 g L^{-1} 25°C). **Insoluble**: dilute alkali, gasoline and aliphatic hydrocarbons
pKa	8.19 at 25 ºC (weak acid)
Soil degradation DT50(a) (days)	**DT50** typical**:** 15.2; **DT50** laboratory at 20ºC: 4.6; **DT50** field: 15
Aqueous photolysis(b) (days)	**DT50:** 0.4 at pH 7
GUS leaching potential índex(c)	0.02 (Low leachability)

(a) DT50 is the time required for the pesticide concentration under defined conditions to decline to 50% of the amount at application. Usually DT50 is in a field or laboratory soil sample. However, three DT50 parameters are given. Typically data is derived from laboratory studies, but when the substance is persistent in soil under laboratory conditions, field studies may be carried out. 'Typical values' quoted are those given in the general literature and are often a mean of all studies field and laboratory. This is the value normally used in the regulatory modelling studies and is for aerobic conditions.

(b) is the rate of chemical decomposition in the aquatic environment induced by light or other radiant energy expressed as a DT50.

(c) GUS index (Groundwater Ubiquity Score) is a very simple indicator of a chemical potential for leaching into groundwater. It is based on the environmental fate properties of the chemical and takes no account of environmental conditions. It is not a substitute for modelling and risk assessment studies. It is calculated from the soil degradation rate (DT50) and the Organic-carbon sorption constant (Koc) where: GUS = log(DT50) x (4 - log (Koc))

If GUS > 2.8 = pesticide likely to leach; If GUS < 1.8 = pesticide unlikely to leach; If GUS 1.8 - 2.8 = leaching potential is marginal

It is worth to notice that, despite the fact that Table 1.6 includes some information relatively to soil degradation and aqueous photolysis, we did not find information about the experiments which gave rise to these data. The data refer only to the desapearence of thiram and there is no information about degradation products. Besides, these processes may be strongly influenced by the soil characteristics or the composition of natural waters and the information about these subjects is very scarce, as discussed later in this chapter.

1.4 Determination of thiram

The most recognized method for dithiocarbamate determination, namely thiram, is based on hot mineral acid decomposition of these compounds to amine and carbon disulfide (CS_2) and subsequent analysis of the CS_2 released (Thorn and Ludwig, 1962; Cullen, 1964). The analysis of CS_2 can be carried out spectrophotometrically or directly by gas chromatography (Thorn and Ludwig, 1962; Cullen, 1964; AOAC official methods, 1997a; Kesari et al., 1998; Caldas et al., 2001; Royer et al., 2001; Vryzas et al., 2002; Caldas et al., 2004; Qin et al., 2010; EPA-Method 630; EPA-Method 630.1). In brief, spectrophotometric analysis of the CS_2 released consisted on a few colorimetric reactions involving the gas complexation with suitable reagents. The most popular method is based on CS_2 absorption in an ethanolic solution, forming, in the presence of diethanolamine, a yellow complex with copper (II) that absorbs at 435 nm (Thorn and Ludwig, 1962; Cullen, 1964; Caldas et al., 2001; Caldas et al., 2004; EPA-Method 630). As alternative, the determination of thiram in water, vegetables and wheat grains may be carried out after the CS_2 absorption in an ethanolic sodium hydroxide solution, forming a xanthate that, in presence of potassium iodine and leuco crystal violet, results in a product that absorbs at 595 nm (Kesari et al., 1998) or, as suggested by the AOAC official method (1997a), for the determination of thiram in formulations, the xanthate can be determined after neutralization, by titration with a standard solution of I_2.

The methods referred above have the inconvenient of determining the total level of dithiocarbamates without identifying or quantifying the compounds individually, so that the value of the maximum residue level is usually expressed in terms of CS_2. Despite this limitation, this is the method currently used in Portugal to determine the content of dithiocarbamates in foods, with either spectrophotometric detection or analysis by gas chromatography with MS detection, according to the European standard EN 12396-1:1998 and EN 12396-2:1998 (information available at http://www.cen.eu/cen/Sectors/TechnicalCommitteesWorkshops/CENTechnicalCommittees/Pages/Standards.aspx?param=6256&title=CEN/TC%20275, last accessed on 20th November 2012). However, with the increase of the thiram sales, the risks associated to the use of this fungicide should be properly evaluated not only in foods but also in other natural resources such as soil and water and more specific and selective analytical methods are needed to cover, quantify and confirm thiram residues in various matrices. Analytical techniques, such as, spectrophotometry, chromatography, voltammetry, capillary electrophoresis, have been used in the analysis of thiram in water, soils, food and biological fluids. Although some review works have been published about quantitative analysis of some dithiocarbamates in various matrices (Thorn and Ludwing, 1962; Malik and Faubel, 1999; Szolar, 2007; Crnogorac and Schwack, 2009) as far as we know, only one work has been published about thiram (Sharma et al., 2003). The information about the methods for determination of thiram in different matrices, retrieved from works published in the last years is compiled in Chapter 11, Supplementary information (section 11.2).

1.5 Thiram in environmental matrices

Comparing to other pesticides, there is a lack of information in the literature about the environmental impact of the worldwide use of thiram in agriculture. Pesticides fate in the environment is ruled by *transformation* processes, which can include pesticide molecule breakdown by chemical, photochemical or biological degradation, which affect the persistence, or by *transfer processes*, such as adsorption/desorption, runoff, volatilisation and leaching, affecting the pesticide mobility (Andreu and Picó, 2004; Gavrilescu, 2005). Both types of physicochemical processes are influenced by the properties of the pesticide, soil environment and site conditions like weather, local flora and application method (Figure 1.3).

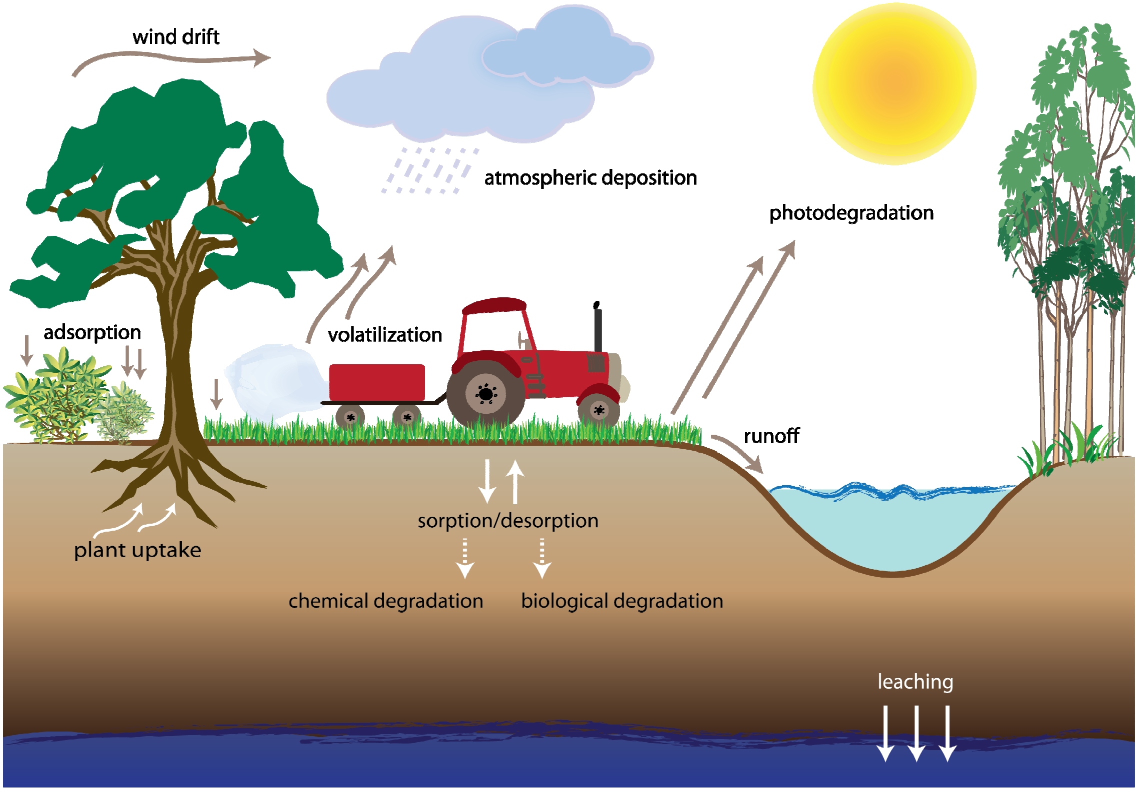


Figure 1.3 – Schematic representation of the potential fates of the pesticides in environment and some of the most important agents responsible of transfer and degradation phenomena.

1.5.1 Adsorption-desorption of thiram

The study of pesticides sorption onto soils is a commonly used method to evaluate mobility of pesticides in soil, providing essential information about the pesticide distribution between soil and water systems (Senesi, 1992; Aboul-Kassim et al., 2001a). Even though, comparing to other pesticides, the adsorption studies of thiram onto soils or soil components are very scarce and the physicochemical parameters have not been yet properly evaluated (Stathi et al., 2006). During the last three decades, only three studies have been reported about adsorption of thiram onto soils from three distinct geographical points, one in Mediterranean (Valverde-Garcia et al., 1988) and, more recently, others in India (Sharma et al., 2011) and in Chile (Copaja et al., 2012). On the other hand, there are some published studies about adsorption of thiram onto specific adsorbents, such as lignins (Rupp and Zuman, 1992), SiO_2 particles (Stathi et al., 2006), activated carbon (Gonzalez-Pradas et al., 1987, Zahoor, 2010), wastes resulting from a coal mine (Misirli at al., 2004), sepiolite (Gonzalez-Pradas et al., 1987) or activated bentonite (Gonzalez-Pradas et al., 1988). More recently, Stathi *et al.* (2007) have studied in detail the mechanism of thiram interaction with natural humic acids (HA), either in aqueous solution or immobilized onto SiO_2 particles. The role of carboxylate groups of the humic macromolecules on the interaction with thiram was also studied using two syntectic systems with mono- or di-carboxylic moieties covalently immobilized SiO_2 (SiO_2-COOH or SiO_2-$(COOH)_2$).

In most of the published works, the adsorption data obtained for thiram are adjusted to the most common mathematical models, Freundlich and Langmuir isotherms, obtaining the respective empirical parameters by regression methods and using them to describe and predict the adsorption phenomena of thiram onto the several matrices. Table 1.7 shows a summary of the sorption studies of thiram published in literature in the last 25 years.

Table 1.9. Adsorption-desorption studies of thiram onto different adsorbents.

Adsorbent	Observations	Reference
Mediterranean soils (Almeria, Spain)	**Batch conditions:** 1:8.3 soil:solution ratio, 24 h equilibration at 30 ºC; equilibrium concentrations lower than 12 mg L^{-1}. **Isotherm:** Freundlich isotherm (linearised form); K_F values in the range 4.8 – 13.7 and correlated with the organic matter content;	Valverde-Garcia et al., 1988
Indian soils	**Batch conditions:** 2 g soil, 24 h equilibration; equilibrium concentrations 24-72 mg L^{-1}. **Isotherm:** Freundlich isotherm (linearised form); K_F values in the range 5.3 – 7.7. **K_D values:** 4.4, 4.0, and 4.7 for soil I, soil II, and soil III, respectively; According to the Groundwater Ubiquity Score (GUS) score, which is a model that relates half life and adsorption in soil (K_{oc}), thiram is a non-leacher pesticide (GUS = 1.8).	Sharma et al., 2011
Chilen soils	**Batch conditions:** 1:10 soil:solution ratio, 24 h equilibration; equilibrium concentrations 0-1000 mg L^{-1}. **Isotherm:** Freundlich isotherm (linearised form); K_F values in the range 5.3 – 12 for natural soils; K_F values in the range 8.0 – 20 for incubated soils with biosolids; . **K_D values:** 0.2-3 L g^{-1} (calculated as the average between all the points of the curve).	Copaja et al., 2012
Lignin	**Batch conditions:** pH 7; 1:50 adsorbent:solution ratio, 4h equilibration; equilibrium concentrations lower than 2 mg L^{-1}; desorption time between 15 min and 18 h. **K_D values**: 0.15 – 0.20 L g^{-1};	Rupp and Zuman, 1992
Activated carbon and sepiolite	**Isotherm:** L type, Langmuir isotherm (linearised form)	Gonzalez-Pradas et al., 1987
Activated bentonite	**Isotherm:** L type, Freundlich isotherm (linearised form); K_F range 230-2010, depending the temperature.	Gonzalez-Pradas et al., 1988

Table 1.10. Adsorption-desorption studies of thiram onto different adsorbents (continuation).

Adsorbent	Observations	Reference
Activated carbon	**Batch conditions:** 0.01:250 adsorbent:solution ratio, 220 min of equilibration at 20, 30, 40 and 50 ºC; equilibrium concentrations lower than 15 mg L^{-1}. **Isotherm:** Langmuir and Freundlich isotherms (linearised forms); Q_{max} = 169.5^{-1}, K_L = 2.68 L mg^{-1}, K_F = 0.0087 and N = 0.838	Zahoor, 2010
Waste resulting from a coal mine	**Batch conditions:** 1:25 adsorbent:solution ratio, 70 min equilibration; equilibrium concentrations lower than 15 mg L^{-1}. **Isothem:**_H type, (the adsorbent has high affinity to thiram); Langmuir and Freundlich isotherms (linearised forms) K_D **value:** 4.4 L g^{-1} (C_{eq}= 2.3 mg L^{-1})	Misirli at al., 2004
Dissolved humic acids	**Batch conditions:** 3:100 (w/w) HA:pesticide, 20 min of equilibration; equilibrium concentrations lower than 10 mg L^{-1}. **Isotherm:** Langmuir, K_L = 0.4	Stathi *et al.* 2007
Humic acids immobilized SiO_2	**Batch conditions:** 1:6.7 adsobent:solution ratio, 20 min of equilibration; equilibrium concentrations lower than 10 mg L^{-1}. **Isotherm:** Langmuir, K_L = 0.7	Stathi *et al.* 2007
Mono- or di-carboxylic moieties covalently immobilized SiO_2	**Batch conditions:** 1:6.7 adsobent:solution ratio, 20 min of equilibration; equilibrium concentrations lower than 10 mg L^{-1}. **Isotherm**: Langmuir, K_L = 0.2	Stathi *et al.* 2007

In the published works concerning the adsorption of thiram onto soil, the linearized form of the Freundlich equation was fitted to the data by linear regression analysis. However, this approach may be criticised, since "*the conversion of non-linear isotherm equations to linear forms implicitly alters their error structure and may also violate the error variance and normality assumptions of least square method*" (Ho, 2004). Thus, the calculated parameters may be incorrect. Besides, the equilibration time used in these studies was 24 hours but kinetic studies are not shown.

Recent works have shown that the copper content in soil can influence the adsorption of other pesticides onto soils (Arias et al., 2006; Dousset et al., 2007; Liu et al., 2007; Pateiro-Moure et al., 2007). However, despite the well known affinity of thiram for copper, there are no studies about the influence of the soil copper content on thiram adsorption. Thus, we can conclude that more studies are needed about the factors which can influence the sorption of thiram onto soils.

1.5.2 Persistence of thiram

In what concerns the study of the persistence of thiram in the environment, the information in the literature, when the present work was started, was practically inexistent and continues to be scarce. Usually the persistence of a compound is evaluated by the half-life time, $t_{1/2}$, defined as the time required for one-half of the original amount of the compound to react/break down. Pesticides can be divided into three categories based on the respective soil half-life times: *nonpersistent* pesticides with a typical soil half-life of less than 30 days, *moderately persistent* pesticides with a typical soil half-life of 30 to 100 days, or *persistent* pesticides with a typical soil half-life of more than 100 days.

Very recently, Gupta et al. (2012a) conducted a study to evaluate the persistence of thiram in water and soil under controlled conditions and on two plants, namely, tomato and radish, in field conditions. According to the authors, the decay of thiram in water depends upon the nature of medium and environmental conditions. So, studies conducted in water at different experimental conditions revealed that thiram persistence decreases as pH, temperature and organic matter content increased. Among the three variables, it is the organic matter which has a more pronounced effect. For a pesticide:humic acid ratio of 1:1 at pH 8 and 30 ºC the half-life of thiram was 0.5 d, while in the absence of humic acids the half life was 2.4 d. It must be noticed, however, that the concentration of thiram used in these studies is not clearly indicated, but from Figure 1 of the paper it seems that a concentration as high as 50 mg L^{-1} has been used, which is higher than thiram solubility in water, and the percentage of organic solvent (from the stock solution) in the final solutions is not referred. In the three different types of soils studied, with pH 5.1 to 8.1, the half-life of thiram was in the range 7.7 to 4.6 days. The relationship observed between thiram decay and pH was explained by the authors as a consequence of the faster kinetics in alkaline medium of thiram cleavage into DMDTC, besides for higher pH values the persistence of DMDTC, more toxic than thiram, increased significantly (Weissmahr and Sedlak, 2000). Moreover, other authors (Sherif et al., 2011) observed that the half life of thiram in soil (52% clay, 47% silt, 1% sand, 0.18% total N, 0.36% O.C and pH 7.8.) was 10 days. The influence of copper on the thiram persistence has not been evaluated.

1.5.3 Photochemical degradation

Photodegradation is one of the transformation processes that can affect the behaviour of organic pollutants mainly in surface waters exposed to the sunlight. In fact, many studies are reported in the literature about photodegradation of organic pollutants and how the natural organic and inorganic major components of natural waters can influence their degradation (Guerard et al., 2009, Mao et al., 2011). In a water system, a pesticide can undergo direct photochemical transformation which involves direct capture of photons by the target molecule, or indirect photochemical transformation involving the energy captured and carried by another molecule present in the natural water – a photosensitizer.

In spite of the wide thiram application, and in comparison to other pesticides (Burrows et al. 2002), the information about photodegradation of thiram in aquatic matrices is scarce (Thomas, 2001; Harino and Langston, 2009). Most of the studies published in the literature refer to the photodegradtion of thiram in the presence of synthetic catalysts or photosensitizers, such as TiO_2 (Haque and Muneer, 2005; Thakare and Bhave, 2005; Kaneco et al., 2009) or rose Bengal (Crack and Morsyidi, 1992), and are focused on wastes treatment. Samanidou et al. (1988), have studied the degradation of thiram in natural waters, at a concentration of 48 mg L^{-1} (2×10^{-4} mol L^{-1}), which is higher than thiram aqueous solubility (probably some organic solvent was added with the spike). The authors observed the complete degradation of thiram in 24 h under UV irradiation (> 290 nm) and after 7 days of exposure to the sunlight. Furthermore, Samanidou et al. (1988) have observed differences in degradation rate of thiram depending on the source of natural waters. Thus, under sunlight, the rate of degradation of thiram followed the order river water > lake water > seawater, while, under UV irradiation, the degradation followed the order river water ≈ seawater > lake water. These observations have stressed the importance of the matrix components on degradation phenomena. Still, the authors referred the occurrence of two non identified photodegradation products. As far as we know there are no other studies about photodegradation of thiram by sunlight in natural waters or in aqueous solutions containing natural photosensitizers such as humic substances.

Thus, more studies are needed in order to evaluate the influence of components of natural waters on the photodegradtion of thiram in water, as well as to identify the photodegradation products which can be formed. The photodegradation pathways identified in other studies of the photocatalytic or chemical degradation of thiram may be useful for future work concerning the identification of photoproducts formed in natural waters. Thus, relevant information obtained so far may be found summarized in Table 1.8.

Table 1.11. Identification of the major products of thiram degradation.

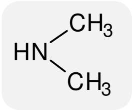
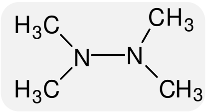
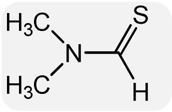
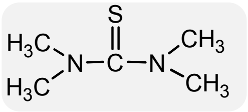
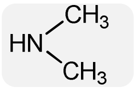
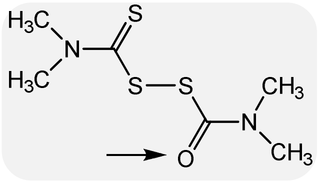
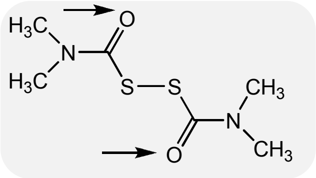
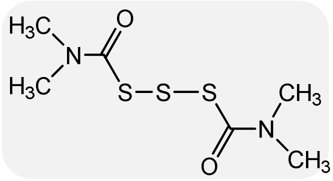
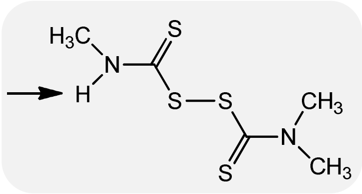
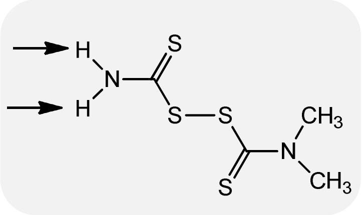
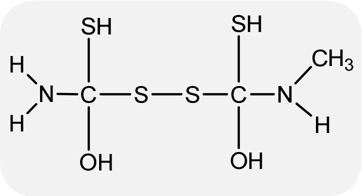
Authors	**Degradation products**	
Crack and Morsyidi, 1992 **Solution:** ethanolic solutions of thiram (4 g L^{-1}) and Rose Bengal (as photosensitizer) **Photodegradation:** UV light (presence/absence O$_2$) Visible photosensitized oxidation **Technique:** GC-MS	**CS_2**	Carbon disulfide
		Dimethylamine
		Tetramethylhydrazine
		*N,N*-dimethylthioformamide
		Tetramethylthiourea
Kodoma et al., 1999 **Solution:** aqueous solutions of thiram (0.72 mg L^{-1}) **Chemical degradation:** sodium hypochlorite (0-100 mg L^{-1} free chlorine) **Technique:** LC-MS and ionic chromatography (dimethylamine identification)		Dimethylamine
		*N,N*-dimethylcarbamoyl-*N,N*-dimethylthiocarbamoyldisulphide
		Bis(dimethylcarbamoyl) disulphide
		Bis(dimethylcarbamoyl) trisulphide

Table 1.12. Identification of the major products of thiram degradation (continuation).

Authors	**Degradation products**	
Haque and Muneer, 2005 **Solution:** aqueous thiram solutions (120 mg L^{-1}) **Photocatalytic degradation:** UV light in the presence of TiO_2 (1 g L^{-1}) **Technique:** GC-MS	  	

1.6 Research objectives

The overall goal of this work was to achieve a better understanding of thiram in environmental matrices, namely in soil and natural waters, and the role of organic matter and copper ions in its behaviour and fate.

The specific objectives of this study were:

a) To study the relevance of organic matter on the adsorption-desorption of thiram onto soils. For this, adsorption-desorption studies were performed using humic substances and samples of a luvisol soil submitted to different long-term organic amendments.

b) To study the effect of soil copper content on the adsorption-desorption of thiram onto soil.

c) To study the influence of humic substances and copper ions on the analytical determination of thiram, for future studies of the influence of these parameters on the thiram behaviour in water

d) To study the persistence of thiram in aqueous solutions and identify the byproducts by mass spectrometry.
e) To study the photodegradation of thiram under simulated sunlight in aqueous solutions and identify the photodegradation products by mass spectrometry.
f) To compare the behaviour of pure thiram with a commercial formulation

Chapters of the present thesis have been written as papers for international peer-reviewed journals. All these papers have been reworked, so that they are presented in a consistent style and format in this thesis. In addition, introductions and some experimental sections have been modified to eliminate repetition.

The thesis includes 10 chapters; in the 1st chapter the state of the art concerning the knowledge about the behaviour of thiram in soils and natural waters is presented; chapters 2,3,4,5 and 6 are focused on the various adsorption-desorption studies of thiram in different conditions that were performed; chapters 7,8 and 9 refer to the behaviour of thiram in aqueous solutions and natural waters evaluating the effect of organic matter, copper ions and sunlight in its determination, degradation and persistence; and, finally, chapter 10 summarizes the main conclusions of this work.

Thus, *Chapter 1* is a review focused on the behaviour and fate of thiram in environmental matrices. This chapter is part of a review paper being prepared to be published as

> **Filipe, O.M.S.,** Vidal, M.M.**,** Santos E.B.H. “Thiram: analytical methods and environmental behaviour – a review”. *In preparation*

Chapter 2 describes the development of a solid phase extraction procedure (SPE) with a C_{18} column for clean-up of thiram from aqueous solutions equilibrated with commercial humic acids or soils. This chapter was published as

> **Filipe, O.M.S.,** Vidal, M.M., Duarte, A.C., Santos E.B.H. 2007. A solid-phase extraction procedure for the clean-up of thiram from aqueous solutions containing high concentrations of humic substances. *Talanta*, 72, 1235-1238.

Chapter 3 presents a comparison between linear and non-linear regression analysis applied to the fitting of Langmuir, Freundlich and Brunauer-Emmett-Teller isotherms to experimental adsorption data. Thiram adsorption data onto commercial humic acids (HA) and a soil sample amended with organic compost (COM soil) are presented and used to estimate accuracy of the mathematical model chosen. This chapter was submitted as

> **Filipe, O.M.S.,** Vidal, M.M., Santos, E.B.H. Modelling adsorption isotherm data: effect of fitting methods. Submitted to the *J. Agric. Food Chem.* in October 2012.

Chapter 4 presents the studies of the adsorption-desorption behaviour of thiram onto solid humic substances. Batch adsorption-desorption experiments were also made using commercial formulations containing thiram in order to compare the adsorption phenomena when using thiram in its pure form or in a commercial formulation. This chapter was published as

> **Filipe, O.M.S.**, Vidal, M.M., Duarte, A.C., Santos, E.B.H. 2009. Adsorption-Desorption behavior of thiram onto humic acid. *J. Agric. Food Chem.,* 57, 4906-4912.

Chapter 5 describes the influence of different organic amendments on the adsorption-desorption of thiram onto a luvisol soil derived from loess when compared to mineral fertilizer. This chapter was published as

> **Filipe, O.M.S.,** Vidal, M.M., Duarte, A.C., Scherer, H.W., Schneider, R.J., Santos, E.B.H. 2010. Effect of long term organic amendments on adsorption–desorption of thiram onto a luvisol soil derived from loess. *Chemosphere*. 80, 293-300.

Chapter 6 investigates the influence of copper ions on the adsorption and desorption of thiram from soil in order to clarify the occurrence of reactions with copper ions which can affect the persistence and mobility of thiram in soil. This chapter was published as

> **Filipe, O.M.S.,** Costa, C.A.E, Vidal, M. M., Santos E.B.H. 2013. Influence of soil copper content on the kinetics of thiram adsorption and on thiram leachability from soils. *Chemosphere, 90, 432-440.*

Chapter 7 presents a study on the use of a C_{18}-SPE procedure combined with HPLC-UV for thiram analysis in river water. The influence of aquatic fulvic acids and copper ions on thiram recoveries was studied. This chapter was published as

> **Filipe, O.M.S.,** Vidal, M.M., Duarte, A.C., Santos, E.B.H. 2008. Influence of fulvic acids and copper ions on thiram determination in water" *J. Agric. Food Chem.*, 56 (16), 7347–7354

Chapter 8 investigates the persistence of thiram in aqueous solution along time, in the absence and presence of copper ions. HPLC-MS/MS was used to identify the compounds in solution. This chapter is being prepared to be published as

> **Filipe, O.M.S.,** Santos, S.A.O., Domingues, M.R.M., Vidal, M. M., Silvestre A.J.D., C.P. Neto, Santos, E.B.H. 2012. Effect of copper ions in the degradation and persistence of thiram in environmental matrices. *In preparation*

Chapter 9 studies the degradation of thiram under simulated sunlight radiation in aqueous solutions, and the influence of humic substances is evaluated. Kinetics studies were performed and the photodegradation of pure thiram was compared with that of the commercial formulation of thiram. Photodegradation products were identified by HPLC-MS/MS and a photodegradation pathway mechanism is proposed. This chapter was accepted for publication as

> **Filipe, O.M.S.,** Santos, S.A.O., Domingues, M.R.M., Vidal, M. M., Silvestre A.J.D., C.P. Neto, E.B.H. Santos. Photodegradation of the fungicide thiram in aqueous solutions. Kinetic studies and identification of the photodegradation products by HPLC-MS/MS. *Chemosphere*. Under revision.

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Chapter 2

A solid-phase extraction procedure for the clean-up of thiram from aqueous solutions containing high concentrations of humic substances[1]

A simple solid-phase extraction (SPE) procedure with octadecyl bonded phase silica (C_{18}) was developed for clean-up of the fungicide thiram from aqueous solutions containing high concentrations of humic substances, for future studies of thiram adsorption onto solid humic substances or soils. Suspensions of humic acids and soil, in aqueous 0.01 mol L^{-1} $CaCl_2$ solution, were prepared and used as samples. These extracts were spiked with thiram and immediately applied to a C_{18}-SPE cartridge. Thiram was eluted with chloroform and its concentration measured by spectrophotometry at 283 nm. Non-spiked aqueous extracts (blanks) and a control sample of thiram in 0.01 mol L^{-1} $CaCl_2$ aqueous solution were also prepared and submitted to the same SPE procedure. The results show that humic substances are extensively retained by the C_{18} cartridge but are not eluted with $CHCl_3$. Recoveries of 100–104% were obtained for thiram in the presence of humic substances. The SPE procedure described in this work is an efficient clean-up step to remove the interference of humic substances absorbance and to be coupled to any spectrophotometric or HPLC–UV method, usually used for thiram analysis in food extracts.

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[1] Adapted from: **Olga M. S. Filipe,** M.M. Vidal, A.C. Duarte, E. Santos (2007) "A solid-phase extraction procedure for the clean-up of thiram from aqueous solutions containing high concentrations of humic substances" *Talanta*, **72**, 1235-1238.

2.1 Introduction

The progressive increase in the application of chemicals for agriculture has lead to a rise in concern for the problem of soil contamination and for the risk of contaminants leaching to ground and surface waters. Adsorption studies are very important and useful to provide essential information about mobility of chemicals and their distribution in soil, water and air. The information supplied by this kind of studies can be used, for example, in the prediction or estimation of the availability of a chemical for degradation, transformation and bioavailability, leaching through the soil profile, volatility from soil and run-off from land surfaces into natural waters. However, in spite of the intensive use of thiram worldwide, physicochemical parameters for its adsorption onto soils have not been evaluated, as highlighted by Stathi et al. (2006). Studies of its adsorption on lignin (Rupp and Zuman, 1992), specific adsorbents (e.g., waste resulting from a coal mine, SiO_2 particles, actived carbon or sepiolite) (Gonzalez-Pradas et al., 1987; Misirli at al., 2004; Rupp et al., 1992; Stathi et al., 2006) and soils (Valverde-Garcia et al., 1988) have been reported, but there are no studies about its adsorption on humic substances, which constitute 60–70% of the organic matter in soils (Aiken, 1985) and which have been reported to interact with several other pesticides, influencing their fate in the environment (Celi et al., 1996; Ferreira et al., 2002; Liu et al., 2002; Prosen et al., 2007; Iglesias et al., 2009; Kovaios et al., 2011). Adsorption studies of organic contaminants on soil and on humic substances are usually performed by mixing and equilibrating a known amount of soil or solid humic substances with a known volume of pesticide standard solutions in 0.01 mol L^{-1} $CaCl_2$ (OECD, 2000; Ferreira et al., 2002; Liu et al., 2002; Wauchoupe et al., 2002; Arias et al., 2005, Kovaios et al., 2011). The amount of pesticide adsorbed is then calculated from the difference between the initial and equilibrium pesticide concentrations in solution. The solutions equilibrated with soil or humic substances do contain high concentrations of UV–Vis absorbent organic matter which can interfere in the analytical determination of the pesticide. Thus, for studying the adsorption of thiram either on soils or on humic substances, a clean-up procedure is required to remove these substances from solution before thiram analysis by any UV–Vis spectrophotometric method or by HPLC–UV.

Solid-phase extraction (SPE) has already been used for clean-up of the aqueous solutions of other pesticides after equilibration with soils (Konda et al., 2002; Mahommed and Baugh, 2003, Ramezania et al., 2009). SPE with C_{18} stationary phase has been also applied by other authors (Tovar and Santos Delgado, 1995; Garcia et al, 1996; Blasco et al., 2004) to the extraction and preconcentration of thiram from aqueous standard solutions. Good recoveries were obtained using dichloromethane/methanol (Blasco et al., 2004) or acetonitrile (Tovar and Santos Delgado, 1995) as eluents. However, very few applications to aqueous solutions containing humic substances, such as natural waters, have been published (Tovar and Santos Delgado, 1995; Garcia et al, 1996), and the results obtained were contradictory. Garcia et al. (1996) did successfully apply a C_{18} cartridge to the extraction/concentration of thiram from river water. On the other hand, Tovar and Santos-Delgado (1995) did apply the same stationary phase as adsorbent of several carbamate pesticides (carbaryl, propoxur, propham, methiocarb and thiram) from river water, and the same authors observed that thiram was the only pesticide that was not recovered. As far as we know there are no published results concerning the applicability of C_{18}-SPE cartridge as a clean-up procedure of thiram in aqueous solutions with high concentrations of humic matter.

The main goal of this work was to test a C_{18}–SPE procedure for clean-up of thiram from aqueous solutions equilibrated with commercial humic acids or soils, for future studies of thiram adsorption.

2.2 Experimental

2.2.1 Chemicals

All chemicals used were of analytical grade. Thiram (97%) was purchased from Aldrich and commercial humic acids (ash 10–15%, *M*r 600–1000) were supplied by Fluka. Methanol and chloroform (HPLC grade) were obtained from Riedel-de Haen and LabScan, respectively. An aqueous 0.01 mol L^{-1} $CaCl_2$ solution was prepared from $CaCl_2$ anhydrous (Fluka, p.a.). Ultra pure water for aqueous solutions was obtained with a Milli-Q water purification system (Millipore). A stock solution of 15 mg L^{-1} of thiram in 0.01 mol L^{-1} $CaCl_2$ was used to prepare spiked aqueous humic and soil extracts. A stock solution of 25 mg L^{-1} thiram in $CHCl_3$ was used to prepare standard solutions of thiram for calibration of absorbance measurements.

2.2.2 Soil

The soil sample used in this work was kindly provided by Prof. H.W. Scherer from the Agrikulturchemisches Institute of the University of Bonn (Germany) and it was collected from the top soil layer (0–30 cm) of a field situated in Meckenheim (15 km southwest of Bonn). It is a luvisol derived from loess (sand 6.9%, silt 77.1%, clay 16%), which has been amended with farmyard manure since 1959.

2.2.3 Apparatus

The absorbance measurements were performed with an UV–Vis Shimadzu spectrophotometer, in a 1.00 cm cell.

2.2.4 Preparation of aqueous humic acid and soil extracts

Soil suspensions were prepared using a ratio of 5 g of soil to 25 mL of 0.01 mol L^{-1} $CaCl_2$, in a pyrex centrifuge tube, while humic substances suspensions were prepared using a ratio of 25 mg of humic acids to 5 mL of solution. These ratios are in agreement with those used in the literature concerning adsorption studies of other pesticides onto soils or humic substances (Celi et al., 1996; Ferreira et al., 2002; Liu et al., 2002; Kovaios et al. 2011). Both suspensions were centrifuged during 30 min at 4000 rpm and the supernatants were filtered through a 0.2 μm filter. These filtered extracts were used as samples. Spiked samples were prepared by addition of a known volume of the 15 mg L^{-1} thiram stock solution (0.01 mol L^{-1} $CaCl_2$).

2.2.5 SPE clean up procedure

Commercial Supelclean envi-18 cartridges (Supelco) of 500 mg, 75 Å pore diameter and 56 μm particle size were set up in a 12-place manifold from Phenomenex in order to perform the SPE experiments. Before use, all the cartridges were washed with 3 mL of $CHCl_3$, the solvent used for thiram elution. Before sample application, the cartridges were preconditioned with 6 mL of methanol, 6 mL of Milli-Q water and 6 mL of 0.01 mol L^{-1} $CaCl_2$ (the sample matrix). Next, an aliquot of 2 mL of the spiked extracts was percolated through the cartridge at a flow rate of 2 mL min^{-1} under vacuum 6 kPa. Before elution, the SPE cartridge was rinsed with 3 mL of Milli-Q water in order to remove any residual sample and subsequently dried under nitrogen, during 30 min. Thiram was then eluted with 3 mL of $CHCl_3$, which was collected in a graduated tube, and its concentration was determined by spectrophotometry at 283 nm against $CHCl_3$. Non-spiked soil or humic extracts were used as blanks and a control sample of thiram in 0.01 mol L^{-1} $CaCl_2$ aqueous solution (not equilibrated with soil or humic acids) was also prepared. Both the blanks and the control sample were submitted to the same SPE procedure.

2.3 Results and discussion

2.3.1 Measurement of thiram by UV spectrophotometry

Thiram quantification in the $CHCl_3$ eluates from the SPE cartridges was performed by absorbance measurements at 283 nm (Figure 1.1A). Calibration curves were obtained with standard solutions in $CHCl_3$ with concentrations in the range of 0.5–25 mg L^{-1} (Figure 2.1B).

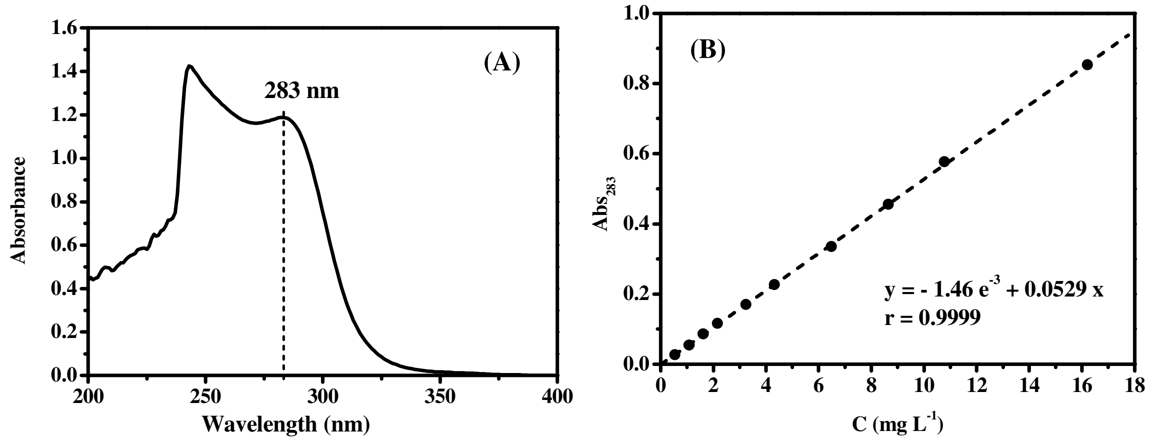

Figure 2.1 - (A) UV-Vis spectra of 24.2 mg L-1 thiram solution in $CHCl_3$; (B) Calibration curve of thiram in $CHCl_3$

The limit of detection (LOD) was calculated from each calibration curve as

$$LOD = 3S_{y/x}/m \qquad (2.1)$$

where m is the slope of the linear regression and $S_{y/x}$ is the statistical parameter which estimates the random errors in the y axis (signal) (Miller and Miller, 2005),

$$S_{y/x} = \sqrt{\frac{\sum_i \left(y_i - \hat{y}_i \right)^2}{n-2}} \qquad (2.2)$$

where $y_i - \hat{y}_i$ are the y-residuals, the $\hat{y}_i$ values are the y-values calculated from the fitted equation and corresponding to the individual x-values used in the calibration, while *n* is the number of points. A mean value of 0.33 mg L^{-1} (S.D. = 0.14 mg L^{-1}, $n = 6$) was obtained for LOD, which is of the same order of magnitude as those obtained by other authors in the determination of thiram using UV–Vis spectrophotometry (Tunceli et al., 2001; Sharma et al., 2004) and HPLC–MS (Barrek et al., 2003).

2.3.2 Testing of the C18–SPE procedure with solutions containing only humic acids or thiram

The aqueous phase from suspensions of humic acids in 0.01 mol L^{-1} $CaCl_2$, after a dilution of 1:100, still has an absorbance of about 0.125 at 283 nm, which would correspond to an absorbance of about 12.5 in the original solution, as shown in Figure 2.2a. Such high absorbance would interfere in thiram analysis by any UV–Vis spectrophotometric method or by HPLC–UV. The SPE procedure was applied to these non-spiked aqueous extracts of humic acids (blanks), and their retention was evaluated by measurement of the absorbance of the aqueous solution applied to the cartridge and of the solution collected at the outlet during the sample application and washing with water, before elution. Taking into account the dilution factor (volume applied/volume collected), the retention of organic matter absorbing at 283 nm was of about 75%. Figure 2.2b shows the high decrease of absorbance of the solutions after percolation through the SPE cartridge.

Chloroform was used as eluent since it is a very good solvent for thiram. Such is the reason why chloroform is the most used solvent for extraction of thiram from fruits and vegetables (Cassella et al., 2000; Sharma et al., 2004). But it is also required that the eluent does not elute the humic substances retained by the C_{18} cartridge. Experimental results obtained with the blanks showed that humic acids were not eluted by chloroform. The absorbance of the blank eluates was measured at 283 nm and its mean value is 0.015, which is less than 0.2% of the absorbances of the aqueous extracts before clean-up.

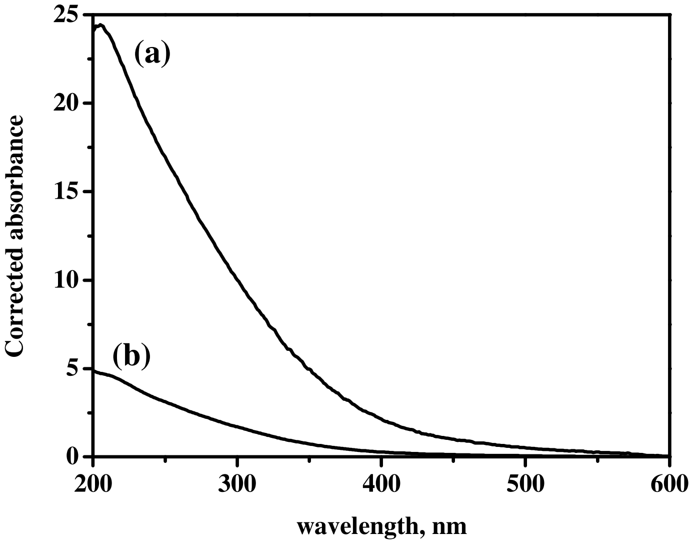

Figure 2.2 – UV-Vis spectra of non-spiked aqueous extracts of humic acids before **(a)** and after **(b)** application to the C_{18} cartridge (absorbances were measured after adequate dilution of the solutions and were then multiplied by the dilution factor).

The C_{18} cartridge capacity and the overall procedure were also tested for the thiram extraction, using a control aqueous solution containing only thiram in 0.01 mol L^{-1} $CaCl_2$. As in adsorption studies thiram solutions are shaken during 24 h with soil or humic substances, the control solution was applied to the SPE column, immediately after preparation (Thi_0), and after 24 h of shaking (Thi_{24}). Table 2.1 shows that the SPE procedure allows to completely recover thiram from these standard solutions (Thi_0 and Thi_{24}).

Table 2.1. Thiram recoveries (R) and respective standard deviations, for the control aqueous solution containing only thiram in 0.01 mol L^{-1} $CaCl_2$, after applied to a C_{18} SPE cartridge: Thi_0 – immediately after preparation and Thi_{24} – after 24 h of shaking.

Samples	**[Thiram] (mg L^{-1})**	**R (%)**
Thi_0	2.1-10.4	103 ± 6 (n=7)
Thi_{24}	3.6-14.8	97 ± 6 (n=7)

The comparison of the results for Thi_0 and Thi_{24} allows concluding that there are no losses of thiram by degradation, adsorption on the flaskwalls, or during centrifugation and filtration steps. Still it allowed establishing that a chloroform volume of 3 mL is enough for the complete elution of thiram.

2.3.3 SPE as a clean-up step of thiram from aqueous solutions equilibrated with humic acid or soil samples

The SPE procedure was then applied to spiked extracts in order to evaluate if thiram was completely recovered in the presence of humic matter. The concentration of thiram added to the filtered extracts is in agreement with those usually used in adsorption studies of other pesticides, usually in the range of 0.25–100 mg L^{-1} (Celi et al., 1996; Ferreira et al., 2002; Konda et al., 2002; Liu et al., 2002; Mahommed and Baugh, 2003; Arias et al., 2005; Kovaios et al., 2011). Thus, a spike of thiram was done to the filtered soil and humic extracts, in order to obtain thiram concentrations of 2.1 and 10.4 mg L^{-1} in the soil extracts and 7.6 mg L^{-1} in the humic acids extracts. Non-spiked extracts were also submitted to the SPE procedure for calculation of recoveries. The values found for the non-spiked extracts were lower than the LOD (<0.33 mg L^{-1} in the cartridge eluates). Figure 2.3 shows the UV–Vis spectra of the $CHCl_3$ eluates obtained from the SPE treatment of spiked and non-spiked extracts. Thiram recoveries of 100–104% were obtained, as shown in Table 2.2.

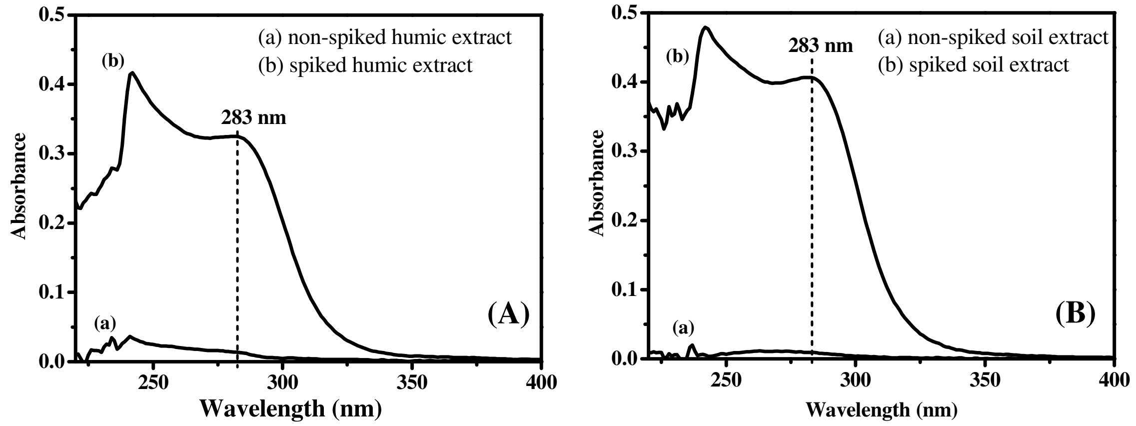

Figure 2.3 – UV-Vis spectra of $CHCl_3$ eluates of the C_{18}-SPE cartridges after application of: **(A)** humic acid extract non-spiked and spiked with thiram ([Thi]=7.6 mg L^{-1}); **(B)** soil extract non-spiked and spiked with thiram ([Thi]=10.4 mg/L).

Table 2.2. SPE recoveries (R) of thiram and respective standard deviations for both humic acid and soil extracts. C_{ad} - concentration of thiram added to the extract

Samples	C_{ad} **(mg L^{-1})**	**R (%)**
Aqueous HA extract	7.6	103 ± 5 (n=3)
Aqueous soil extract	2.1	100 ± 3 (n=3)
	10.4	104 ± 3 (n=3)

2.4 Conclusions

In the present work, the efficiency of a C_{18}–SPE procedure for isolation and clean-up of thiram from aqueous samples with high concentrations of humic substances was evaluated. The application of C_{18} cartridges to aqueous soil and humic extracts non-spiked and spiked with thiram has shown that:

(i) humic substances are extensively retained by the C_{18} cartridge but are not eluted with $CHCl_3$;

(ii) thiram is retained by the C_{18} cartridge and eluted with $CHCl_3$, in the presence of natural organic matter, with recoveries not significantly different from 100%.

Thus, we can conclude that the SPE procedure described in this work can be applied as a clean-up step which removes the interference of humic matter absorbance in the analysis of thiram by any spectrophotometric method. Several spectrophotometric and HPLC–UV methods published in the literature were developed and applied for thiram analysis in food extracts, which do not contain humic matter (Sharma et al., 2003; Sharma et al., 2004). Coupling of those methods to this clean-up procedure will allow their application to aqueous solutions containing high concentrations of humic matter, such as those used in adsorption studies. A solvent change from $CHCl_3$ to a more adequate solvent for the analytical method, if required, can be done by evaporating $CHCl_3$ to dryness and dissolving the residue in the other solvent.

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Chapter 3

Modelling adsorption isotherm data: effect of fitting methods[2]

This paper carries out a comparative and critical study of the procedures used to fit different adsorption models to experimental adsorption data and to evaluate the goodness of the fitting. The experimental data used for this study were obtained for the adsorption of the fungicide thiram onto both commercial humic acids (HA) and a luvisol soil amended with compost (COM soil). The Freundlich, Langmuir and Brunauer–Emmett–Teller (BET) isotherms were fitted to the adsorption data by linear regression analysis using the least squares method and by non-linear regression analysis. Five linearized forms of adsorption isotherms, which included the Freundlich equation, two forms of the Langmuir equation and two of the BET equation, and three non-linear isotherm equations, were discussed in this work. In order to determine the best fitting model, seven error functions that are usually used to estimate the deviations between experimental and estimated data were tested. In addition, another error function, the adjusted R^2 (R^2_{Adj}), was included in this study to assess its suitability as a tool to evaluate isotherm model fitness. Depending on the procedure used to fit a given isotherm model to the data, different isotherm parameters for thiram adsorption onto both HA and COM soil were obtained. The results show that the non-linear method is the best way to obtain the isotherm which best fits to the data.

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[2] Adapted from: Filipe O.M.S., Vidal M. M., Santos E.B.H. Modelling adsorption isotherm data: effect of fitting methods. *Submitted J Food Agric. Chem.* (October 2012)

3.1 Introduction

Pesticide adsorption studies have been reported as an important tool to evaluate pesticide/soil affinity. Information about a large number of pesticides and their tendency to adsorb onto soil or soil components is available in the literature. In general, information provided by studies of the adsorption phenomena is used to predict pesticide run-off into natural waters, evaluating the substance retention from the aqueous media to a solid phase, or mobility in the opposite direction, at a constant temperature. Usually, adsorption phenomena are studied by batch equilibration, putting well known quantities of the solid and an aqueous solution of the pesticide into contact, at a constant temperature, until equilibrium is attained. The adsorption isotherm is the plot of concentration adsorbed onto the solid phase versus the concentration that remains in solution, after a certain contact time (Tan, 1998; Aboul-Kassim and Simoneit, 2001a; Aboul-Kassim and Simoneit, 2001b). Throughout the years, a wide range of equilibrium isotherm models have been used to describe the data generated by pesticide adsorption studies, contributing to the enlightenment on pesticide behaviour and fate throughout the environment. Freundlich and Langmuir isotherms are among the most commonly used equations to describe pesticide adsorption phenomena onto soil (Tan, 1998; Aboul-Kassim and Simoneit, 2001a; Foo and Hameed, 2010). Literature shows that, due to their non-linear nature, these equations are used after mathematical linearization in the majority of the adsorption studies (Tan, 1998; Aboul-Kassim and Simoneit, 2001b). After the mathematic transformation, the linearized equations are then fitted to the experimental data by linear regression using the method of least squares, in order to determine the parameters of the models, which are calculated from the slope and the Y-intercept. The coefficient of determination of the fitting is usually used to decide which is the model that best fits the data. However, as referred by Ho (2004), "*the conversion of non-linear isotherm equations to linear forms implicitly alters their error structure and may also violate the error variance and normality assumptions of least squares method*" and depending on the way as the isotherm equations are linearized, the error distribution changes either the worse or the better, distorting the isotherms parameters. So, depending on the way of fitting the adsorption models, as linearized or non-linearized equations, different adsorption parameters are obtained and, so far, such differences have not been properly discussed in the literature.

Information about the way of choosing the adsorption isotherm model which best fits the data and about the fitting method is scarce in the literature. During these last six years, however, some studies have been emerging in the literature about problems associated with the isotherm equation linearization methods (Kumar, 2006, 2007; Kumar and Sivanesan, 2006a, 2006b, 2008; Kumar et al., 2008a, 2008b; Ncibi, 2008; Subramanya and Das, 2009; Chowdhury et al., 2011). Moreover, since 2005, Kumar *et al.* (Kumar, 2006, 2007; Kumar and Sivanesan, 2006a, 2006b, 2008; Kumar et al., 2008a, 2008b) have been suggesting that isotherm data should be adjusted to Freundlich and Langmuir original equations by non-linear regression analysis, which, according to this author, gives rise to rather better values for the isotherm parameters. However, despite this statement, it is still very common to find in the literature adsorption studies in different solid matrixes which continue to use the linearized forms of the isotherms (Larsbo et al., 2009; Gamiz et al., 2010; Alonso et al., 2011; Smaranda et al., 2011; Hiller et al., 2012; Marin-Benito et al., 2012; Tang et al., 2012; Wu et al., 2012) maybe due to their simplicity. In the present work, a comparative study between linear and non-linear regression analysis applied to the Langmuir, Freundlich and Brunauer-Emmett-Teller (BET) isotherms is carried out. Experimental data were obtained in studies about Thiram, a fungicide widely used in our country, Portugal (Information available at http://www.dgadr.pt/default.aspx; last accessed on October 22nd 2012). Thiram adsorption data on commercial humic acids (HA) and on a soil sample amended with organic compost (COM soil) are presented and used to estimate accuracy of the mathematical model chosen to adjust experimental data.

3.2 Isotherm Models

3.2.1 Freundlich isotherm

The Freundlich model is an empirical adsorption model that has been widely used in adsorption studies and is expressed as (Tan, 1998; Aboul-Kassim and Simoneit, 2001a; Foo and Hameed, 2010)

$$Q = K_F \, C_{eq}^{N} \qquad (3.1)$$

where K_F and N are constants determined by fitting the equation to the experimental data of Q and C_{eq}. K_F ((mg g^{-1})(dm^3 g)^N), defined as Freundlich constant, has been considered as indicative of the adsorption strength (Aboul-Kassim and Simoneit, 2001a) or related to adsorption capacity (Subramanya and Das, 2009; Chowdhury et al., 2011). The N constant has been described as the degree of linearity (for $N = 1$ the adsorption is linear) (Tan, 1998; Aboul-Kassim and Simoneit, 2001a; Aboul-Kassim and Simoneit, 2001b) and has been considered indicative of the heterogeneity of the adsorbent (Crini et al., 2007; Subramanya and Das, 2009; Foo and Hameed, 2010; Chowdhury et al., 2011). Thus, the Freundlich isotherm is widely applied to describe the adsorption onto heterogeneous materials, containing sites with different binding energies on the adsorbent surface. So, the amount adsorbed is the sum of adsorption on all sites (each having a specific binding energy), with the stronger binding sites being occupied first and the adsorption energy exponentially decreasing until the completion of the adsorption process. The closer the value of N is to zero, more heterogeneous is the system

The linearized form of the equation (3.1) can be obtained by taking logarithms on both sides:

$$\log Q = \log K_F + N \log C_{eq} \qquad (3.2)$$

When this form of the equation is used, K_F and N are determined by fitting equation (3.2) to the experimental data of log Q *vs.* log C_{eq}.

3.2.2 Langmuir isotherm model

Langmuir adsorption isotherm is another model widely used to describe the adsorption of many compounds on various solid surfaces. It has been originally developed to describe gas–solid-phase adsorption onto activated carbon (Langmuir, 1916). This model assumes that the adsorbent is homogeneous and sorption takes place at specific sites which are all identical and energetically equivalent. It assumes a monolayer formation with no lateral interaction between the adsorbed molecules (the forces of interaction between sorbed molecules are negligible) (Tan, 1998; Kassim and Simoneit, 2001a; Foo and Hameed, 2010). Graphically, it is characterized by a plateau, an equilibrium saturation where, once each site is occupied by an adsorbate molecule, no further adsorption can takes place. The Langmuir model is described as

$$Q = \frac{Q_{\max} K_L \, C_{eq}}{1 + K_L C_{eq}} \qquad (3.3)$$

where K_L is the adsorption equilibrium constant, also known as Langmuir constant, and $Q_{\max}$ is the maximum adsorption capacity (mg g^{-1}). According to the literature the Langmuir isotherm can be linearized in at least four different manners (Foo and Hameed, 2010). In the present work we used only the two equations which, according to the literature, are the linearized forms most used (Foo and Hameed, 2010). The Langmuir-I is obtained by the ratio between C_{eq}/Q and is expressed as

$$\frac{C_{eq}}{Q} = \frac{1}{Q_{\max} K_L} + \frac{1}{Q_{\max}} \times C_{eq} \qquad (3.4)$$

and the Langmuir type II is the inverse of equation 3.3 and is expressed as

$$\frac{1}{Q} = \frac{1}{Q_{\max}} + \frac{1}{Q_{\max} K_L} \times \frac{1}{C_{eq}} \qquad (3.5)$$

3.2.3 Brunauer–Emmett–Teller (BET) isotherm

The BET isotherm was originally developed for gas phase adsorption (Tan, 1998) and has the form:

$$Q = \frac{Q_{\max} K \dfrac{P}{P^S}}{(1 - \dfrac{P}{P^S})\left[1 + (K-1)\dfrac{P}{P^S}\right]} \qquad (3.6)$$

Where P is the partial pressure of the adsorbate and P^S is the saturation pressure of the adsorbate. Brunauer et al. (1938) assumed that Q tends to infinite as P tends to P^S, i.e. the adsorbate will condensate on the surface of the adsorbent when P attains P^S. As P^S resembles the maximum possible concentration of the adsorbate in the gas phase, a number of researchers, when applying BET equation to liquid phase adsorption, have used C_S, the solubility of the adsorbate, or saturation concentration of the adsorbate in the liquid phase, instead of P^S, and C_{eq} was used instead of P (Ebadi et al., 2009). Thus, equation 3.6 gives rise to the equation 3.7.

$$Q = \frac{Q_{\max} K \, C_{eq}}{(C_S - C_{eq})\left[1 + (K-1)\dfrac{C_{eq}}{C_S}\right]} \qquad (3.7)$$

where $Q_{\max}$ (µg g^{-1}) corresponds to the monolayer adsorption capacity i.e., maximum of adsorption of the 1st layer, C_S is the saturation concentration for adsorbate in solution (mg L^{-1}), and K is an empirical constant.

Recently, Ebadi et al. (2009) have claimed that a more correct form of the BET equation for liquid adsorption is:

$$Q = \frac{Q_{\max} K_S C_{eq}}{(1 - K_L^* C_{eq})(1 - K_L^* C_{eq} + K_S C_{eq})} \qquad (3.8)$$

where, K_S is the equilibrium constant of adsorption for the first layer and K_L^* is the equilibrium constant of adsorption for upper layers. This equation is equivalent to equation 3.7 making $C_S = \frac{1}{K_L^*}$ and $K = \frac{K_S}{K_L^*}$.

The linearised form of the BET equation is expressed as,

$$\frac{C_{eq}}{Q \times \left(C_S - C_{eq}\right)} = \frac{1}{KQ_{\max}} + \frac{K-1}{Q_{\max} K} \times \frac{C_{eq}}{C_S} \qquad (3.9)$$

what means that the C_s value has to be assumed as a constant. Assuming C_S as saturation concentration, then, according to the EXTOXNET data base (information available at http://extoxnet.orst.edu/pips/thiram.htm; last accessed on 20th November 2012) C_S for thiram is 30 mg L^{-1} at 25ºC and 16.5 mg L^{-1} at 20 ºC in FOOTPRINT data base (information available at http://www.eufootprint.org; last accessed on 20th November 2012). In this work we linearised the BET equation using the two values of C_s that appears in the literature, 30 and 16.5 mg L^{-1}.

Concerning to the non-linear regression analysis, the BET equation 3.7 can be re-arranged to the equation 3.10 in order to facilitate the curve fitting (Hinz, 2001)

$$Q = \frac{K_1\, C_{eq}}{(1 + K_2 C_{eq}) \times (1 - K_3 C_{eq})} \qquad (3.10)$$

where, $K_1 = \frac{Q_{\max} K}{C_S}$, $K_2 = \frac{K-1}{C_S}$ and $K_3 = \frac{1}{C_S}$.

3.3 Experimental

3.3.1 Chemicals

All chemicals were of analytical grade. Thiram (97%) was purchased from Aldrich and commercial humic acids (Ash 10-15%, Mr 600-1000) were supplied by Fluka. Methanol and acetonitrile (HPLC grade) were obtained from Riedel-de Haen and LabScan, respectively. An aqueous 0.01 mol L^{-1} $CaCl_2$ solution was prepared from $CaCl_2$ anhydrous (Fluka, p.a.). Ultra pure water for aqueous solutions was obtained with a Milli-Q water purification system (Millipore).

Standard stock solutions of thiram (~20 mg L^{-1}) were prepared by previous dissolution of the solid in acetonitrile (1% of the final solution volume) followed by dilution with 0.01 mol L^{-1} $CaCl_2$ aqueous solution. Further dilutions were made in 0.01 mol L^{-1} $CaCl_2$. A stock solution of 100 mg L^{-1} thiram in acetonitrile was used to prepare more diluted standard aqueous solutions of thiram for the HPLC-UV method calibration.

3.3.2 Adsorption studies

Batch adsorption experiments were carried out according to the standard batch equilibrium technique, described on the OECD guideline (2000). Adsorption studies were performed using portions of both grounded commercial humic acids (30 mg) (Filipe et al., 2009) and grounded soil samples (1.2 g) (Filipe et al., 2010). Each portion was accurately weighed into 10 mL Pyrex centrifuge tubes and mixed with 6 ml of thiram standard solution with the concentration within the ranges 2-28 and 2-35 mg L^{-1} for HA and soil, respectively. A 0.01 mol L^{-1} $CaCl_2$ aqueous solution was used as solvent to prepare the standard solutions of thiram in order to enhance phase separation and to simulate natural soil solution ionic strength. The tubes were capped and shaken at 100 rpm and 21 ± 1 ºC (Heidolph Reax shaker), during a 15 h period, after which they were centrifuged at 4000 rpm for 30 min. Finally, the supernatant was filtered using a 0.2 μm filter, cleaned-up using the SPE method previously described by Filipe *et al.* (2008, 2009) and analysed by HPLC-UV at 270 nm. The amount of thiram adsorbed by unit mass of the adsorbent obtained for the studied equilibration time (Q; mg g^{-1} or μg L^{-1} for HA or soil, respectively) was calculated from the initial thiram aqueous phase concentration (C_0; mg L^{-1}) and the thiram equilibrium concentration after adsorption (C_{eq}; mg L^{-1}),

$$Q = \frac{(C_0 - C_{eq}) \times V_0}{m} \qquad (3.11)$$

where V_0 (L or mL for HA or soil, respectively) is the aqueous phase volume in contact with the adsorbent (HA or soil) during adsorption experiments and m (g) is the adsorbent mass used in each essay. Thiram initial concentration, C_0, was obtained from control samples performed as described above except with no use of adsorbent at all (only thiram, without HA or soil). Non simultaneous triplicate essays were made for each initial concentration, as well as blanks (essays without thiram) while control samples (only thiram) have been performed in every sample batch.

3.3.3 SPE clean up procedure

The SPE clean up procedure was adapted from one described previously by Filipe *et al.* (2007). The SPE cartridges (500 mg commercial supelclean, Supelco) were set up in a 12-place manifold from Phenomenex and pre-conditioned with 6 mL of methanol, 6 mL of Milli-Q water and 6 mL of 0.01 mol L^{-1} $CaCl_2$ aqueous solution (to match the sample matrix). Finally, an aliquot of 5 mL of the $CaCl_2$ supernatant was percolated through the cartridge at a flow rate of 2 mL min^{-1} under vacuum 6 kPa. Before elution, the SPE cartridge was rinsed with 3 mL of milli-Q water in order to remove any residual sample and subsequently dried under nitrogen, during 30 min. Thiram was then eluted with 5 mL of acetonitrile and collected in a 5 mL volumetric flask, and the respective thiram concentration was determined by HPLC-UV at 270 nm.

3.3.4 HPLC-UV analysis of thiram

Thiram was determined by a Jasco HPLC apparatus equipped with a PU-980 Pump, a detector UV-Vis Barspec operating at 270 nm, a phenomenex C18 column (150x4.60 mm, 5 μm, 110 Å) and a Rheodyne injector with a 20 μL loop. The mobile phase was acetonitrile:water 70:30 (v/v) flowing at 0.7 mL/min, previously filtered by a membrane filter 0.2 μm NL16 (Schleicher & Schuell).

3.3.5 Error functions: statistical tools for choosing the best isotherm

In order to evaluate the goodness of the fit of the isotherm to the experimental data, statistical tools are required. Usually, in most of the adsorption studies, the coefficient of determination (R^2) is the error function widely used to predict the optimum isotherm model (Bermúdez-Couso et al., 2011a, 2011b, 2012; Liang, et al, 2011). The best fitting isotherm is selected based on the magnitude of the R^2, i.e., the R^2 value closest to the unity. However, in recent years some authors have been aware of the need of several error functions to predict which isotherm model is the most adequate. According to the literature (Ncibi, 2008; Foo and Hameed, 2010; Chowdhury et al., 2011) the most commonly used error functions are presented in Table 3.1.

Table 3.1. Mathematical error functions to predict the best fit isotherm.

Error function	Equation*
SSE **sum of the squares of the errors**	$SSE = \sum_{i=1}^{n} (Q_{est} - Q_{\exp})_i^2$
SAE **sum of the absolute errors**	$SAE = \sum_{i=1}^{n} \left\| Q_{est} - Q_{\exp} \right\|_i$
ARE **average relative error**	$ARE = \frac{100}{n} \sum_{i=1}^{n} \left\| \frac{Q_{\exp} - Q_{est}}{Q_{\exp}} \right\|_i$
σ **percent standard deviation**	$\sigma = 100\sqrt{\frac{\sum (Q_{\exp} - Q_{est})^2}{N-1}}$
MPSD **marquardt's percent standard deviation**	$MPSP = 100\sqrt{\frac{1}{n-p} \sum_{i=1}^{n} \left[\frac{Q_{\exp} - Q_{est}}{Q_{\exp}} \right]_i^2}$
χ^2 **Chi-square**	$\chi^2 = \sum_{i=1}^{n} \frac{(Q_{\exp} - Q_{est})^2}{Q_{est}}$
R^2 **coefficient of determination**	$R^2 = 1 - \frac{\sum_{i=1}^{n} (Q_{\exp} - Q_{est})^2}{\sum_{i=1}^{n} (Q_{\exp} - \overline{Q_{\exp}})^2}$

*where $Q_{\exp}$ is the amount of thiram adsorbed by unit mass of HA or soil obtained experimentally, Q_{est} is the amount of thiram adsorbed by unit mass of HA or soil estimated from the model, $\overline{Q}$ is the mean of the $Q_{\exp}$ values, n is the number of data points and p is the number of adjustable parameters in the model.

In the present study, besides the error functions presented in Table 3.1, we decided to employ another function, the R^2 adjusted (R^2_{Adj}), to investigate the best-fitting isotherm. The R^2_{Adj} evaluates the goodness of fitting taking into account the number of adjustable parameters of each model, as the MPSD function, and is defined as (Miller and Miller, 2005)

$$R^2_{Adj} = 1 - \left(\frac{\frac{SS_{residual}}{n-1}}{\frac{SS_{total}}{n-p}} \right) \qquad (3.12)$$

where, $SS_{residual} = \sum (Q_{\exp} - Q_{est})^2$, $SS_{total} = \sum (Q_{\exp} - \overline{Q})^2$; Q_{exp} is the amount of thiram adsorbed by unit mass of humic acids obtained experimentally, Q_{est} is the amount of thiram adsorbed by unit mass of humic acids estimated from the model, $\overline{Q}$ is the mean of the Q_{exp} values, n is the number of data points and p is the number of adjustable parameters in the model. R^2_{Adj} can take on any value less than or equal to 1, with a value closer to 1 indicating a better fit. According to the literature (http://www.graphpad.com; last accessed on 20th November 2012), "*Negative values can occur when the model contains terms that do not help to predict the response. Note that* R^2 *is not really the square of anything. If* $SS_{residual}$ *is larger than* SS_{total}, R^2 *will be negative. While it is surprising to see something called "squared" have a negative value, it is not impossible (since* R^2 *is not actually the square of R).* R^2 *will be negative when the best-fit curve fits the data even worse than does a horizontal line*". Normally, error functions are used to minimize the error distribution between the experimental data and predicted isotherms. Thus, the best fitting isotherm was selected based on the error distribution that will be minimized either by minimizing the error function or by maximizing the error function depending on the error function type.

However, when the authors use the linearized forms of the isotherm equations, the goodness of the fit is usually evaluated on the basis of the determination coefficient of the linear regression, i.e., the linear determination coefficient, r^2 (Alonso et al., 2011; Smaranda, et al., 2011; Hiller et al., 2012; Tang et al., 2012), which is the square of the Pearson correlation coefficient, calculated according to equation (3.13)

$$r = \frac{\sum_i \left[\left(x_i - \bar{x}\right)\left(y_i - \bar{y}\right)\right]}{\sqrt{\sum_i \left(x_i - \bar{x}\right)^2 \sum_i \left(y_i - \bar{y}\right)^2}} \quad (3.13)$$

This determination coefficient evaluates whether there is a linear relation between the experimental values of Y and X, which are calculated from the experimental Q and C_{eq} values. For example, in the case of the Freundlich isotherm, y_{i} are the values of "*log* Q_{exp}", and x_{i} are the values of "*log* C_{eq}".

3.3.6 Data analysis

Linear and non-linear regression analysis was performed using the program GraphPadPrism5® (Trial version; http://www.graphpad.com; last accessed on 20th November 2012). Several non simultaneous replicates of adsorption batch equilibration studies were performed and all the individual values of Q_{exp} and C_{eq} were used for the fittings.

3.4 Results and discussion

3.4.1 Measurement of thiram by HPLC-UV

Thiram quantification was performed by HPLC with UV detection at 270 nm. Calibration curves were obtained with thiram standard solutions with concentrations within the range 0.5-4.5 mg L^{-1}, showing correlation coefficients higher than 0.999 (Figure 3.1). The LOD was calculated from each calibration curve as described previously in Chapter 2. A mean value of 0.088 mg L^{-1} (SD=0.034 mg L^{-1}, n=24) was obtained for the LOD. Relative standard deviation for replicate injections was lower than 5% (same sample).

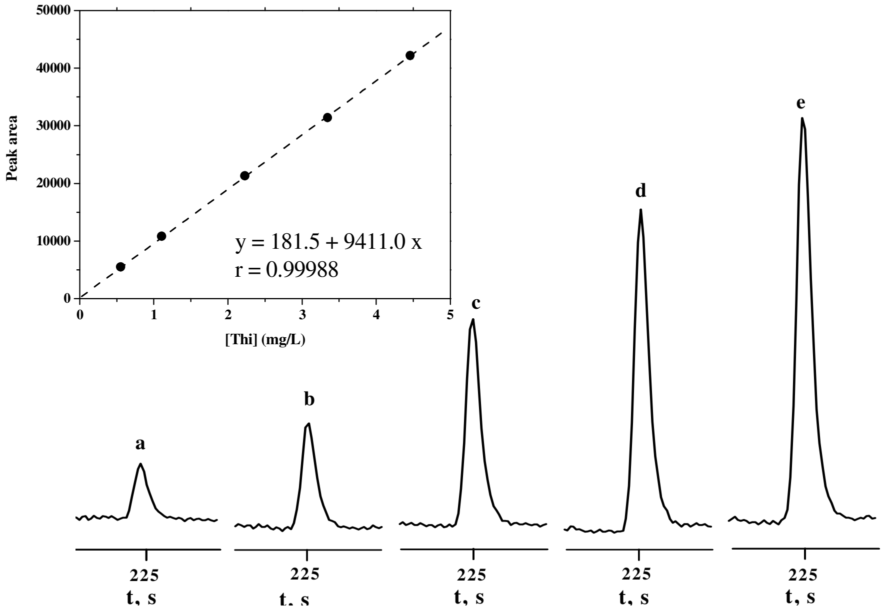

Figure 3.1 – Thiram calibration curve and HPLC chromatograms of thiram standard solutions: (a) 0.56 mg L^{-1} (b) 1.10 mg L^{-1} (c) 2.23 mg L^{-1} (d) 3.34 mg L^{-1} (e) 4.56 mg L^{-1}.

3.4.2 Modelling the adsorption isotherms

The thiram experimental adsorption isotherms, obtained according to the experimental conditions previously described, are shown as dark symbols in Figure 3.2 HA and 3.3 COM soil. Linear regression analysis, based on the least-squares method was used to fit the linearized forms of the Langmuir, Freundlich and BET isotherms to the data (see Table 3.2 for a summary of the used equations). As already mentioned, this method is widely used in the literature on batch adsorption studies of different compounds not only onto soil but also onto various adsorbents. The linearized forms of the isotherms adjusted to the experimental values of Y and X of each of those linear equations, are shown in Figure 3.2 and 3.3, together with the experimental data. The linear correlation coefficients obtained (r^2) are also shown in Figure 3.2 and 3.3.

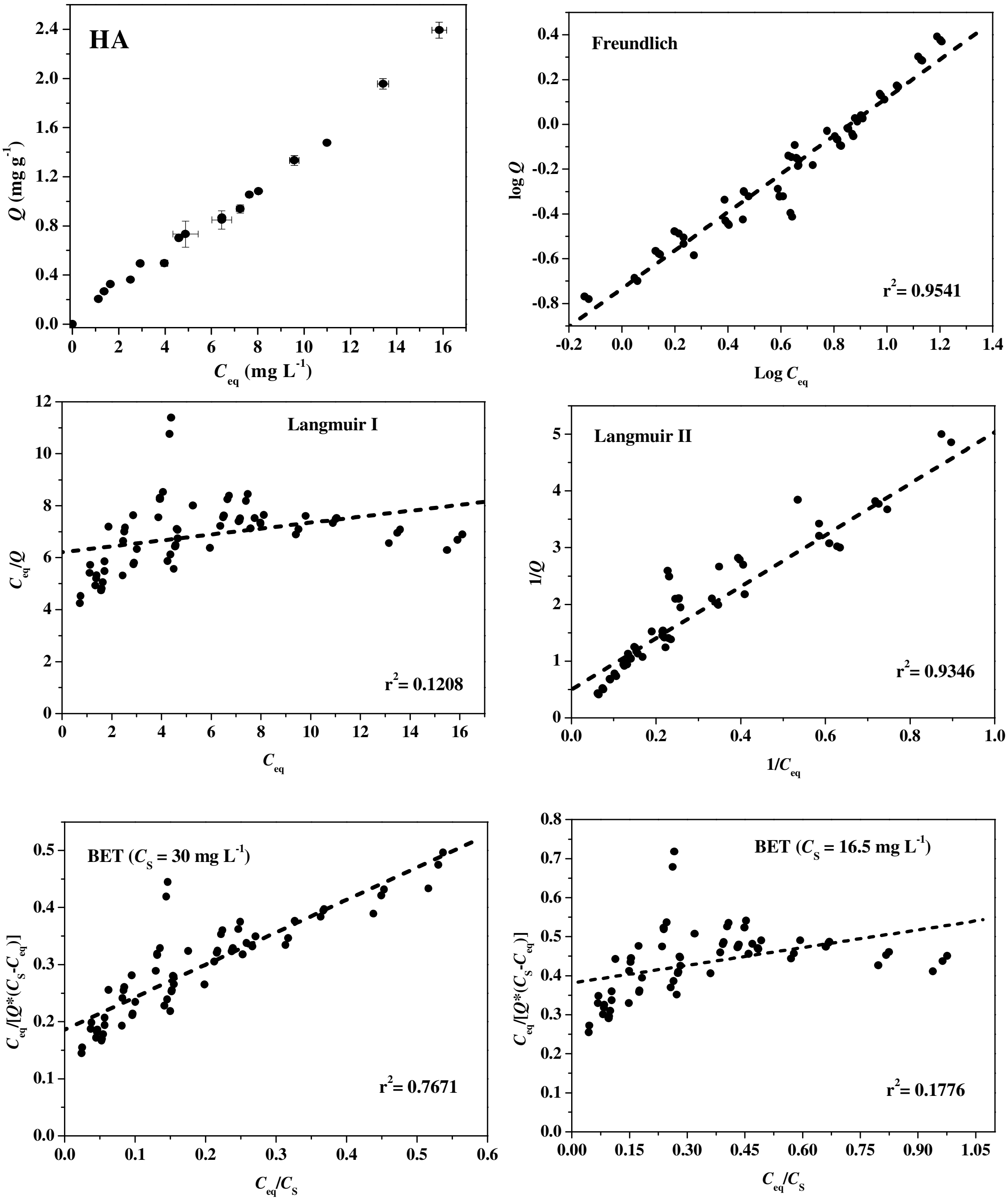

Figure 3.2 - Adsorption isotherm of thiram onto commercial HA. Linearized forms of the Freundlich, Langmuir and BET isotherms adjusted to the experimental values of Y and X by the least-squares method.

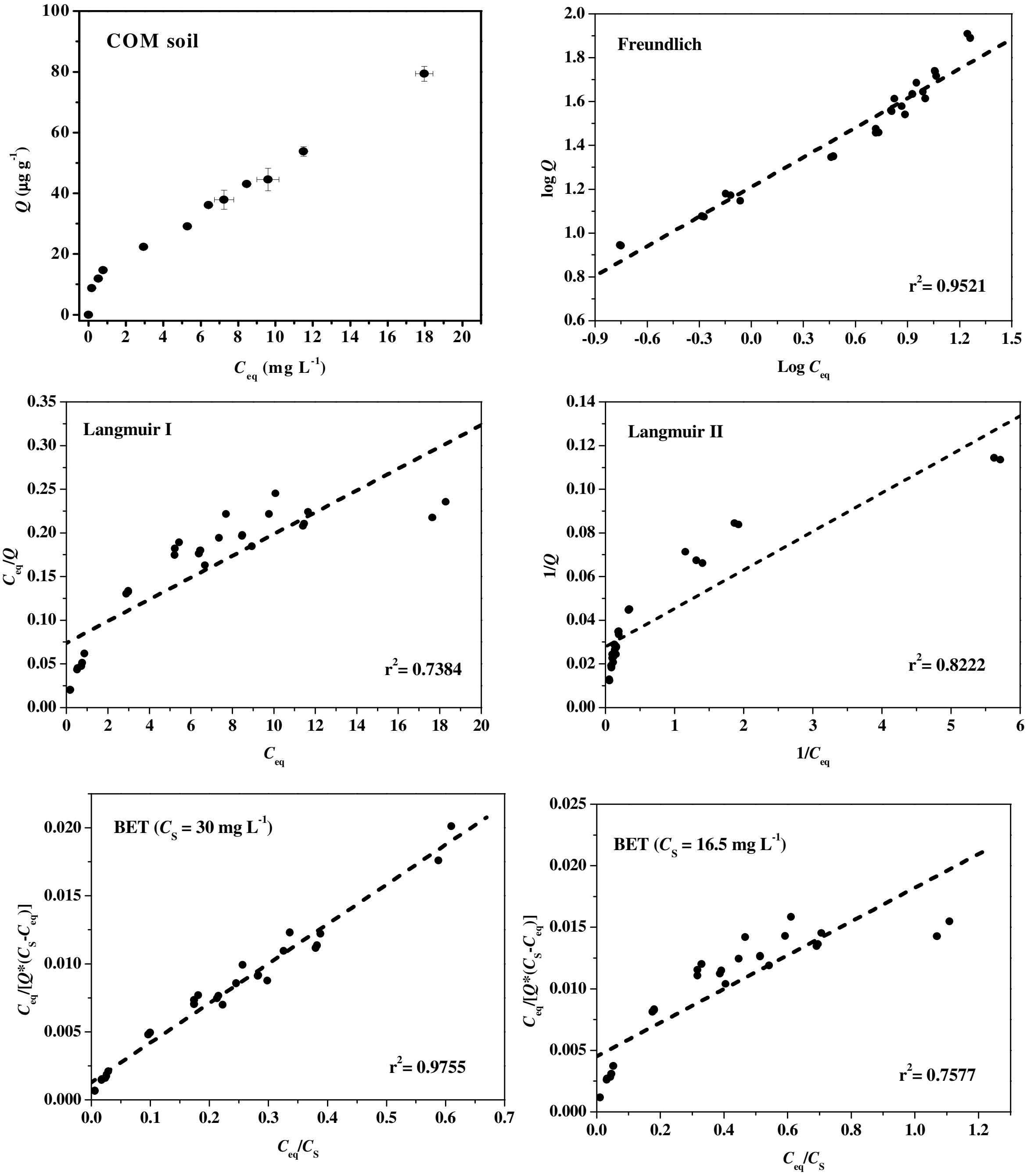

Figure 3.3 - Adsorption isotherm of thiram onto COM soil. Linearized forms of the Freundlich, Langmuir and BET isotherms adjusted to the experimental values of Y and X by the least-squares method.

Table 3.2. Linear and non-linear equations to which experimental data were adjusted following the three mathematical models: Freundlich, Langmuir and Brunauer-Emmett-Teller isotherms.

Isotherm	**Non-Linear equation***	**Linear equation*** $(y = a + bx)$	**Plot**	**Isotherm parameters**
Freundlich	$Q = K_F\, C_{eq}^{N}$	$\log Q = \log K_F + N \log C_{eq}$	Log Q vs. log C_{eq}	$K_{\mathrm{F}} = 10^{\mathrm{a}}$ and $N = \mathrm{b}$
Langmuir	$Q = \dfrac{Q_{\max} K_L\, C_{eq}}{1 + K_L C_{eq}}$	$\dfrac{C_{eq}}{Q} = \dfrac{1}{Q_{\max} K_L} + \dfrac{1}{Q_{\max}} \times C_{eq}$ (Lang I) $\dfrac{1}{Q} = \dfrac{1}{Q_{\max}} + \dfrac{1}{Q_{\max} K_L} \times \dfrac{1}{C_{eq}}$ (Lang. II)	$\dfrac{C_{eq}}{Q}$ vs. C_{eq} $\dfrac{1}{Q}$ vs. $\dfrac{1}{C_{eq}}$	$Q_{\max} = \dfrac{1}{b}$ and $K_L = \dfrac{1}{Q_{\max} \times a}$ $Q_{\max} = \dfrac{1}{a}$ and $K_L = \dfrac{1}{Q_{\max} \times b}$
BET	$Q = \dfrac{Q_{\max} K\, C_{eq}}{(C_S - C_{eq})\left[1 + (K-1)\dfrac{C_{eq}}{C_S}\right]}$	$\dfrac{C_{eq}}{Q \times (C_S - C_{eq})} = \dfrac{1}{K Q_{\max}} + \dfrac{K-1}{Q_{\max} K} \times \dfrac{C_{eq}}{C_S}$	$\dfrac{C_{eq}}{Q \times (C_S - C_{eq})}$ vs. $\dfrac{C_{eq}}{C_S}$	$K = \dfrac{1}{Q_{\max} \times a}$ and $Q_{\max} = \dfrac{1}{a + b}$

* where K_{F} and K_{L} are the Freundlich and Langmuir constants, respectively, which are a measure of sorption capacity, N reflects the degree of linearity, $Q_{\max}$ is the maximum adsorption (mg g^{-1}), which corresponds to the maximum of adsorption of the first layer in the BET isotherm model, C_{S} is the saturation concentration for adsorbate in solution (mg L^{-1}), and K is an empirical constant.

As above referred, in many studies, the goodness of the fit is evaluated on the basis of the value of r^2. According to this criterion, the Langmuir isotherm would be discarded as a model for adsorption of thiram onto both commercial HA and COM soil if the Langmuir I linearized equation was used to fit the data, but it could be considered as a reasonable model if the linearized Langmuir II equation was used, since a $r^2 = 0.9346$ ($p<0.0001$) was obtained for commercial HA. In many papers in the literature, the linear equation of the Freundlich isotherm (equation 3.2) is fitted to the experimental data, and whenever an r^2 close to the unit is obtained, the adsorption data are considered to be adequately modelled by the Freundlich isotherm, even without comparison to other isotherms (Crini et al., 2007; Hiller et al., 2012; Marín-Benito et al., 2012; Wu et al., 2012). Some authors have considered an $r^2 > 0.92$ high enough to conclude that the data were adequately modelled by the Freundlich isotherm (Hiller et al., 2012), and only some of them have also analysed the significance of the linear correlation (p value lower than 0.05) (Marín-Benito et al., 2012). If a similar approach was applied to our data of the adsorption of thiram onto commercial HA or COM soil, one would conclude that the data were well fitted by the Freundlich isotherm since r^2 values are higher than 0.95 and $p<0.001$ (cf. Figure 3.2 and 3.3). Besides, the comparison of the values of r^2 for the linear regression of the linearized forms of different types of isotherms would allow us to conclude that this is the isotherm which best fits the data of adsorption onto commercial HA.

In the case of the BET isotherm the use of a linearized equation requires the previous knowledge of C_S and, as can be seen in Figure 3.2, the use of the two different limit values found in the literature for thiram aqueous solubility give rise to a drastic change of the quality of the fitting (r^2=0.7671 for C_S=30 mg L^{-1} and r^2=0.1776 for C_S=16.5 mg L^{-1}). For the BET isotherm a non-linear curve fitting does not require the knowledge of C_S since this is one of the adjusted parameters.

The non-linearized equations of the adsorption isotherms above referred (equations 3.1, 3.3 and 3.10) were then fitted to the data by non-linear regression and the curves obtained are presented in Figure 3.4. Using the isotherm parameters calculated from the intercept and the slope of the linearized equations of the same isotherms, fitted by linear regression analysis, the values of Q_{est} for each C_{eq} were calculated and were also plotted in Figure 3.4.

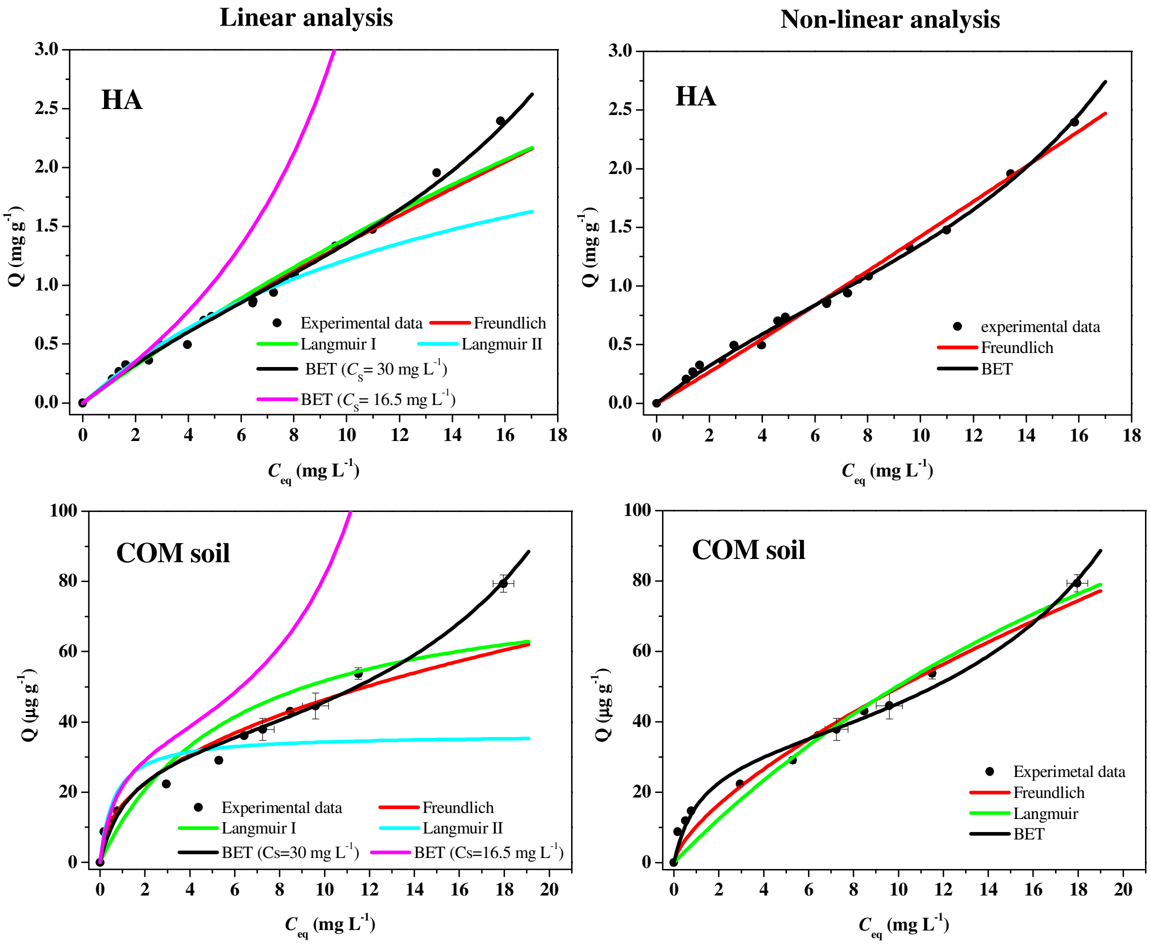

Figure 3.4 – Adsorption isotherm modelling of thiram onto both commercial HA and COM soil using linear and non-linear regression analysis.

The calculated isotherm parameters for adsorption of thiram onto both commercial HA and COM soil using the linear and non-linear regression analysis are shown in Table 3.3. From Table 3.3 we can see that the estimates of the isotherm parameters depend on the method of regression analysis.

Table 3.3. Isotherm parameters obtained for thiram adsorption onto HA and COM soil using the linear and non- linear method.

Isotherm	**Linear method**		**Non-linear method**	
	Commercial HA	**COM soil**	**Commercial HA**	**COM soil**
Freundlich	$K_F = 0.185 \pm 0.007$ $N = 0.85 \pm 0.02$	$K_F = 16.2 \pm 0.60$ $N = 0.45 \pm 0.02$	$K_F = 0.129 \pm 0.008$ $N = 1.04 \pm 0.025$	$K_F = 10.5 \pm 1.01$ $N = 0.67 \pm 0.04$
Langmuir I	$K_L = 0.018 \pm 0.006$ L mg^{-1} $Q_{max} = 8.77 \pm 2.93$ mg g^{-1}	$K_L = 0.168 \pm 0.03$ L mg^{-1} $Q_{max} = 80.2 \pm 9.36$ µg g^{-1}	Ambiguous fit	$K_L = 0.035 \pm 0.012$ L mg^{-1} $Q_{max} = 194 \pm 48.6$ µg g^{-1}
Langmuir II	$K_L = 0.109 \pm 0.014$ L mg^{-1} $Q_{max} = 2.01 \pm 0.26$ mg g^{-1}	$K_L = 1.58 \pm 0.21$ L mg^{-1} $Q_{max} = 36.4 \pm 3.48$ µg g^{-1}		
BET	**C_S = 30 mg L^{-1}** $Q_{max} = 1.32 \pm 0.07$ mg g^{-1} $K = 4.06 \pm 0.29$ **C_S = 16.5 mg L^{-1}** $Q_{max} = 1.88 \pm 0.13$ mg g^{-1} $K = 1.40 \pm 0.15$	$Q_{max} = 32.9 \pm 1.01$ µg g^{-1} $K = 23.4 \pm 4.42$ $Q_{max} = 54.9 \pm 5.10$ µg g^{-1} $K = 4.05 \pm 0.76$	$C_S = 28.7 \pm 1.95$ mg L^{-1} $Q_{max} = 1.32 \pm 0.35$ mg g^{-1} $K = 3.71 \pm 0.92$	$C_S = 29.7 \pm 1.25$ mg L^{-1} $Q_{max} = 32.6 \pm 10.6$ µg g^{-1} $K = 23.9 \pm 6.08$

3.4.3 Error estimation using statistical tools

In order to determine which is the isotherm, among those studied, that best fits to the data, we used seven well known and usually applied error functions (SSE, SAE, ARE, **σ,** MPSP, χ^2, R^2) and another function, R^2 adjusted. Table 3.4 and 3.5 show the results for commercial HA and COM soil, respectively.

Table 3.4. Isotherm error deviation data related to the adsorption of thiram onto commercial HA.

	Error Isotherm	SSE	SAE	ARE	σ	MPSD	χ^2	R^2	R^2_{Adj}
Linear method	**Freundlich**	1.245	5.703	11.08	13.63	16.37	**14.38**	0.9414	0.9405
	Langmuir I	46.39	46.07	82.89	83.21	84.76	413.5	-1.184	-1.217
	Langmuir II	6.636	11.37	15.88	31.47	22.04	26.23	0.6876	0.6829
	BET (Cs=30 mgL^{-1})	**0.4641**	**4.115**	**10.62**	**8.323**	**15.76**	14.42	**0.9782**	**0.9778**
	BET (Cs=16.5 mgL^{-1})	8972	203.0	159.6	1157	500.0	-210.0	-421.4	-427.8
Non-linear method	**Freundlich**	0.5566	4.986	14.27	9.115	19.95	20.42	0.9738	0.9734
	Langmuir	-	-	-	-	-	-	-	-
	BET	**0.4111**	**4.006**	**10.81**	**7.833**	**15.84**	**15.34**	**0.9800**	**0.9806**

Table 3.5. Isotherm error deviation data related to the adsorption of thiram onto COM soil

	Error Isotherm	SSE	SAE	ARE	σ	MPSD	χ^2	R^2	R^2_{Adj}
Linear method	**Freundlich**	1.12 e-3	0.1144	**10.54**	0.6329	**13.34**	0.0226	0.8970	0.8932
	Langmuir I	0.0419	0.9521	95.25	0.3867	100.5	70.023	-2.843	-2.986
	Langmuir II	6.11 e-3	0.2772	24.99	1.477	30.09	0.1809	0.4391	0.4183
	BET (Cs=30 mgL^{-1})	**3.51e-04**	**0.0868**	0.203	**0.3540**	18.64	**0.0203**	**0.9678**	**0.9666**
	BET (Cs=16.5mg L^{-1})	1.214	2.425	149.5	20.82	279.9	-1.095	-110.5	-114.6
Non-linear method	**Freundlich**	**5.09e-04**	0.0972	19.38	**0.4263**	25.55	0.0433	0.9533	0.9515
	Langmuir	8.49e-04	0.1238	23.47	0.5508	38.57	0.2018	0.9220	0.9191
	BET	1.20e-03	**0.1105**	**13.14**	0.6558	**19.86**	**0.0365**	**0.9679**	**0.9654**

According to the values of the error functions presented in Table 3.3, the BET model is the most suitable model to describe the adsorption of thiram onto commercial HA. In fact, the highest R^2 and R^2_{Adj} and the lowest SSE, SAE, ARE, σ and MPSP were found when modelling the experimental equilibrium data using the BET equation estimated both by linear and non-linear regression analysis. As referred above, when the linearized forms of the isotherm equations are used and the goodness of the fit is evaluated on the basis of the linear determination coefficient, r^2, as made by several authors (Larsbo, et al., 2009; Gamiz, B et al., 2010; Alonso, et al., 2011; Smaranda, et al., 2011 ; Hiller, et al., 2012; Marín-Benito, et al., 2012 ; Tang, et al., 2012; Wu, et al., 2012), one can conclude that the Freundlich isotherm is the one which best fits the data of adsorption onto commercial HA. However, using the parameters K_F and N estimated by linear regression, the values of Q_{est} were calculated and plotted versus C_{eq}. The adjustment of the curve to the experimental data (Figure 3.4) does not reflect the r^2 value obtained for the linearized Freundlich isotherm, $r^2 = 0.9541$. The r^2 value is higher for the linear form of the Freundlich isotherm than for the linear form of the BET isotherm with C_S=30 mg L^{-1}, but the error functions for the corresponding Q_{est} *vs.* C_{eq} curves show that the data are better fitted by the BET isotherm (Table 3.4). Thus, we can conclude that, when linear methods are used, the Q_{est} *vs.* C_{eq} should be plotted and additional error functions should be taken into account, besides the usually used linear coefficient of determination r^2.

Moreover, it seems that, in that specific case, by using non-linear regression analysis there are no problems with transformation of non-linear BET isotherm equation to linear form, since the C_S values obtained, 28.7 ± 1.95 mg L^{-1} for HA and 29.7 ± 1.25 mg L^{-1} for COM soil are very similar to the value of 30 mg L^{-1} reported in the literature. The disagreement observed by Ebadi *et al.* (2009) between estimated and experimental C_S values was probably due to the fact that the experimental equilibrium concentrations data used to fit the BET equation were much lower than the solubility of the compounds (Ebadi et al., 2009; Filipe et al., 2010).

For adsorption of thiram onto COM soil (Table 3.5), the results obtained by using non-linear regression analysis were similar to those obtained for adsorption onto commercial HA, i.e., the best fitting model was the BET isotherm, with the R^2 and R^2_{Adj} values closest to the unity and the other error functions with the lowest values. For the curves calculated with the parameters obtained by linear regression analysis, the results presented in Table 3.5 show that among the eight investigated statistical tools, only the ARE and MPSD functions do not agree with the fact that the BET equation is the most appropriate isotherm to fit the experimental adsorption data.

In general, the results obtained show us that more statistical functions are valid for non-linear than linear analysis. The same behaviour was also observed by Subramanya et al. (2009) and Ncibi (2008). Such tendency could be explained by the difference between the equilibrium data estimated using linear and non-linear regression analysis. In fact, the linearization of a non-linear expression alters the experimental error, producing, consequently, an inherent error estimation problem which limits the validity of the studied statistical tools. The linear regression method assumes that the vertical scatter of points around the line follows a Gaussian distribution, and the error distribution is constant at every value of X. However, that is basically impossible since that assumption applies to the Q_{exp} values and in the linearized forms of the isotherms the Y and the X values plotted for regression analysis are not Q_{exp} and C_{eq}. So, the transformation of a non-linear equation to a linear form may change its error distribution. In fact, the linear regression analysis is not a suitable method to get isotherm parameters. Instead, it is better to fit the non-linear equations of the isotherms, by non-linear regression analysis, since the Y values (Q) have a uniform error distribution for the whole range of experimental adsorption data, Q_{exp} and C_{eq}.

Relatively to the new statistical tool used in this work, R^2 adjusted, the results obtained agree with the other functions. However, we would like to highlight that the R^2_{Adj} takes into account the residual degrees of freedom of each model, being very important when isotherms with a different number of inner parameters are compared. So, we recommend the use of R^2_{Adj} instead of the coefficient of determination R^2.

3.5 Conclusions

Adsorption of pesticides onto different systems, namely onto soil or soil components such as humic substances, is usually estimated by using the linearization of the isotherm models due probably to the simplicity in these estimation. The present work highlights the variation in isotherm parameters which occurs when different procedures, namely linear and non-linear regression analysis, are used to fit the same isotherm model. The results presented in this work advice against linearization, which may lead to incorrect results and erroneous conclusions about the best fitting model. So, the use of non-linear regression analysis in sorption studies is, as far as practicable, the better way to find the isotherm parameters and to find the best model that describes the experimental adsorption data. In case of application of the linearized equations of the models, the results must be considered as approximate, and the adsorption parameters determined by linear regression analysis should be used to estimate the non-linear curve, Q vs. C_{eq}, whose adjustment to the experimental data must be evaluated.

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Chapter 4

Adsorption-desorption behaviour of thiram onto humic acid[3]

The adsorption/desorption behaviour of pure thiram (Thi-P) and formulated thiram (Thi-F) onto commercial humic acids (HA) was studied using a batch equilibration procedure. Results of adsorption kinetic experiments showed that thiram adsorption is a fast process since 85 % of the equilibrium concentration is reached within two hours. Experimental K_D values between 0.110 to 0.210 L g^{-1} were obtained for the adsorption of both Thi-P and Thi-F onto HA, suggesting that thiram is strongly sorbed by humic acids. In general, for both Thi-P and Thi-F, the lower the initial thiram concentration, the stronger is its adsorption (higher K_D and percentage adsorption values). The adsorption isotherms were found to match the BET model. The results show that thiram adsorption onto condensed humic acids can not be explained only in terms of specific interactions, such as those identified in studies of adsorption of thiram with humic acids in solution. The comparison of sorption and desorption results allowed the observation of hysteresis phenomena. Desorption $K_{D(des)}$ values were consistently higher than those for adsorption at the same equilibrium concentration. Hysteresis was lower for the formulated thiram suggesting that adsorption is more reversible in the presence of the formulation components turning the pesticide more susceptible to be leached.

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[3] Adapted from: **Filipe, O.M.S.**, Vidal, M.M., Duarte, A.C., Santos, E.B.H., 2009. Adsorption-Desorption behaviour of thiram onto humic acid. *J. Agric. Food Chem.,* 57, 4906-4912.

4.1 Introduction

With the intensive application of chemicals in agriculture, the contamination of soils, ground waters and surface waters has become a subject of environmental concern. As the fate of chemicals and the potential risk of contamination of aquatic systems depend on the distribution of contaminants between the aqueous and the solid phases, it is important to understand the interaction mechanisms with soil, by establishing the influence of each individual component of the soil system.

Humic substances are one of the major and important components of soil organic matter; they are the most ubiquitous natural component in the environment accounting for 50-80% of the carbon in soil, natural water and sediments. Due to their characteristics, such as polyfunctionality, polydispersive nature and polyelectrolytic character, combined with their content of hydrophobic active sites, such as aliphatic side chains and aromatic lignin derived moieties, those substances can interact with both metal ions and organic contaminants by different modes (Senesi, 1992: Senesi and Miano, 1995).

Although thiram is one of the most used fungicides in Portugal (Information available at http://dgadr.pt/default.aspx; last accessed on 20th November 2012) and all over the world (Sharma et al., 2003), few data are available about the sorption behaviour of thiram onto soils or onto some specific adsorbents. The literature reports adsorption studies of thiram onto soils of Almeria (Spain), lignins and specific adsorbents (e.g. waste resulting from a coal mine or SiO_2 particles) (Valverde-Garcia et al., 1988; Rupp and Zuman, 1992; Misirli et al, 2004; Stathi et al., 2006). Recently, Stathi *et al.* (2007) have studied the mechanism of thiram interaction with natural humic acids (HA), either in aqueous solution or immobilized onto SiO_2 particles, and emphasized the role of carboxylate groups of the humic macromolecules on the interaction. That type of specific interaction was properly modelled by Langmuir sorption isotherms (see supplementary material from reference Stathi *et al.* (2007)).

However, either in the condensed form or in the soil system, humic substances may form aggregates providing an organophilic medium for sorption of hydrophobic molecules from water (Pignatello, 1998). It is recognized, in the literature, that soil organic matter acts as a partition medium for non-ionic organic contaminants (Chiou and Kile, 1998). Linear sorption isotherms would be expected if partition was the only interaction mechanism, but deviations from linearity have been observed by several authors (Spurlock and Biggar, 1994; Pignatello, 1998; Chiou and Kile, 1998; Chiou et al., 2000). Several hypotheses have been forwarded to explain the non-linear solute sorption observed at equilibrium concentrations under the contaminant solubility level (Chiou and Kile, 1998). Those deviations are higher for more polar organic contaminants (Chiou and Kile, 1998) and one possible cause for these deviations is the existence of specific interactions with functional groups of the organic matter (Spurlock and Biggar, 1994). Stathi *et al.* (2007) emphasized the role of specific interactions on thiram adsorption, but, as referred above, these authors have studied the interactions of thiram with humic substances in solution or immobilized at the surface of silica and other mechanisms of interaction may be present when condensed humic substances, isolated or in the soil system, are considered (Pignatello, 1998).

Thus, the sorption-desorption behaviour of thiram onto solid humic substances is investigated using the standard batch equilibration procedure, that has been applied for studying the sorption of many other organic contaminants onto humic substances (Benoit et al., 1996; Piccolo et al., 1996; Ferreira et al., 2002; Liu et al., 2002; Wang et al., 2005). Different models for fitting adsorption-desorption isotherms were compared. Results are compared with other similar studies involving humic substances and other than thiram organic contaminants. Batch adsorption-desorption experiments were also made using commercial formulations containing thiram in order to compare the adsorption phenomena when using thiram in its pure form (Thi-P) or in a commercial formulation (Thi-F).

4.2 Experimental

4.2.1 Chemicals

All chemicals were of analytical grade. Pure thiram, Thi-P, (pure substance, 97%) was purchased from Aldrich and commercial humic acids (Ash 10-15%, Mr 600-1000) were supplied by Fluka. A commercial formulation of thiram, Thi-F was obtained from Bayer (Pomarsol ultra D, 80% thiram active substance). Methanol and acetonitrile (HPLC grade) were obtained from Riedel-de Haen and LabScan, respectively. An aqueous 0.01

mol L^{-1} $CaCl_2$ solution was prepared from $CaCl_2$ anhydrous (Fluka, p.a.). Ultra pure water for aqueous solutions was obtained with a Milli-Q water purification system (Millipore).

Standard stock solutions of Thi-P and Thi-F (~20 mg L^{-1}) were prepared by previous dissolution of the solids in acetonitrile, followed by dilution with 0.01 mol L^{-1} $CaCl_2$ aqueous solution (percentage of acetonitrile in the final solution was always under 1%). Further dilutions were made in 0.01 mol L^{-1} $CaCl_2$. Both stock solutions were prepared just before application to solid humic acids. A stock solution of 100 mg L^{-1} thiram in acetonitrile was used to prepare more diluted standard aqueous solutions of thiram for the HPLC-UV method calibration.

4.2.2 Adsorption studies

Each batch adsorption experiment was carried out using the standard batch equilibration technique, performed according to an OECD guideline (2000). A 0.01 mol L^{-1} solution of $CaCl_2$ was used as aqueous phase, making the phase separation easier and simulating the ionic strength of a soil solution. Portions of commercial HA of 30 mg each, previously grounded, were taken into 10 mL Pyrex centrifuge tubes. Then, 6 ml of thiram solutions (Thi-P or Thi-F), within the concentration range of 2-28 mg L^{-1}, were added. The tubes were stoppered and shaken on an end-over-end shaker (Heidolph Reax) at 100 rpm, during 15 h at 21 ± 1 ºC. The time needed for reaching equilibrium was based on the study of the adsorption kinetics, described in kinetic section (4.2.4). After reaching equilibrium, suspensions were centrifuged at 4000 rpm for 30 min, the supernatant was filtered using a 0.2 µm filter and cleaned-up using SPE as described in Chapter 3, section 3.3.3. The concentration of thiram in the supernatant was determined by HPLC-UV at 270 nm as described in Chapter 3, section 3.3.4. The difference of pesticide concentration between the initial and the final equilibrium solutions was assumed to be due to the adsorption, and the amount of thiram adsorbed was then calculated. In order to test reproducibility at least two batch experiments have been done in different occasions. In each batch experiment, triplicates have been done for each initial concentration and for the blank (without thiram). Control samples (only thiram, without HA) have been performed for every sample batch.

4.2.3 Desorption studies

Desorption studies were also carried out in triplicate and were performed immediately after adsorption equilibrium. After the adsorption period and centrifugation of the aqueous suspensions, the supernatant was decanted; the residual supernatant that could not be removed was determined by gravimetry. Thiram concentration in this residual solution was considered to be the same as that measured in the bulk supernatant. A 6 mL of fresh 0.01 mol L^{-1} $CaCl_2$ solution (without thiram) was added to the centrifuge tubes. The tubes were shaken for another 15 h, centrifuged at 4000 rpm for 30 min and the supernatants were collected and submitted to the same procedure as described in the adsorption experiment. The $CaCl_2$ desorption cycle was repeated once more. At last, a desorption cycle using 5 mL of methanol instead of $CaCl_2$ 0.01 mol L^{-1} was also performed. All experiments were done at 21 ± 1ºC. In order to confirm that thiram does not degrade during all the adsorption-desorption process, thiram solutions 4.6 and 22.9 mg L^{-1}, without HA, were shaken during four days at the same temperature of the adsorption/desorption studies, and the recoveries of thiram obtained were always higher than 94 %.

4.2.4 Kinetic study

Aliquots of 6 ml of approximately 3 or 12 mg L^{-1} Thi-P solution in 0.01 mol L^{-1} $CaCl_2$ were added to 30 mg of commercial humic acids in the pyrex centrifuge tubes and shaken at 100 rpm, during 0, 0.5, 1, 2, 4, 8, 12, 20, 24 and 36 h. For each equilibration time, triplicate assays were conducted and processed as described above for adsorption studies.

4.2.5 Calculation of distribution of thiram

The amount of thiram adsorbed by unit mass of humic acids during the equilibration time (Q; mg g^{-1}) was calculated from the difference between initial aqueous phase concentration (C_0; mg L^{-1}) and the thiram equilibrium concentration after adsorption (C_{eq}; mg L^{-1}),

$$Q = \frac{(C_0 - C_{eq}) \times V_0}{m_{HA}} \qquad (4.1)$$

where V_0 (L) is the initial volume of the aqueous phase in contact with HA during the adsorption experiments and m_{HA} (g) is the mass of humic acids used in each essay. C_0 was obtained from the control sample (only thiram, without HA).

The percentage of thiram adsorbed onto HA was calculated according to the equation

$$\%Ads = \frac{(C_0 - C_{eq})}{C_0} \times 100 \qquad (4.2)$$

The distribution coefficient (K_D; L g^{-1}) was calculated as the ratio of the adsorbed concentration of thiram onto HA and its equilibrium concentration after adsorption,

$$K_D = \frac{Q}{C_{eq}} \qquad (4.3)$$

The amount of thiram desorbed (m_{des}; mg) after the first desorption cycle was calculated using the following equation:

$$m_{des} = C_{eq}^{Des} \times (V_0 + V_r) - m_r \qquad (4.4)$$

where, C_{eq}^{Des} (mg L^{-1}) is the thiram equilibrium concentration in solution after desorption, and m_{r} (mg) is the amount of thiram in the residual solution after the adsorption experiment, calculated from:

$$m_r = C_{eq} \times V_r \qquad (4.5)$$

where V_{r} is the residual volume of the supernatant that could not be removed prior to desorption and which was gravimetrically determined.

The percentage of thiram desorbed from HA was calculated according to the equation

$$\%Des = \frac{m_{des}}{m_{ads}} \times 100 \qquad (4.6)$$

where m_{ads} is the mass of thiram adsorbed onto HA during the adsorption experiment.

The adsorption-desorption hysteresis was quantified using the Hysteresis Index (HI) (Huang et al., 1998a; Huang et al., 1998b),

$$HI = \left. \frac{Q^{Des} - Q^{Ads}}{Q^{Ads}} \right|_{Ceq} \qquad (4.7)$$

where, Q^{Ads} and Q^{Des} refer to the solid phase thiram concentrations (mg g^{-1}) after adsorption and after a single desorption cycle experiment, respectively, corresponding to a predetermined aqueous solute concentration C_{eq}.

4.2.6 Isotherms models

The adsorption and desorption data were fitted to the Linear isotherm (equation 4.8) by linear regression analysis

Linear equation $$Q = K_D\ C_{eq} \tag{4.8}$$

where K_{D}, the distribution coefficient, is assumed as constant.

The experimental data were also fitted to the Freundlich, Langmuir and Brunauer-Emmett-Teller (BET) isotherms by non-linear regression analysis, as described in Chapter 3, section 3.2.

4.2.7 Statistical analysis

The experimental adsorption data were fitted to linear isotherm by linear regression analysis, using the least square method, and to Freundlich, Langmuir and BET isotherms using the non-linear regression analysis. It was used the linear and non-linear regression analysis from the program GraphPadPrism5® (Trial version; http://www.graphpad.com; last accessed July 15th 2012). According to the results obtained in Chapter 3 we decide to use R^2 adjusted (R^2_{Adj}) to evaluate the goodness of fitting, which taking into account the residual degrees of freedom of each model, instead of the coefficient of determination R^2. The program GraphPadPrism5® was also used for the paired t-test, which was used to compare the HI data from Thi-P and Thi-F, being the level of significance indicated by the p value.

4.3 Results and discussion

4.3.1 Kinetic studies

Figure 4.1 shows the adsorption percentages of thiram onto solid HA (*% Ads*, calculated according to equation 4.2) for different equilibration times, for thiram initial concentrations of 3.3 mg L^{-1} and 12 mg L^{-1}.

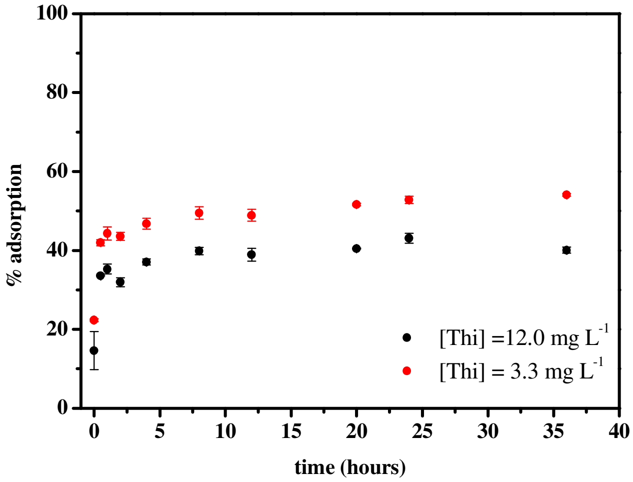

Figure 4.1 - Effect of equilibrium time on the % of adsorption of Thi-P onto humic acids.

According to these results, thiram is quickly adsorbed during the first two hours, since, for both initial concentrations of thiram, 85 % of the equilibrium extent adsorption is reached within this contact time. The equilibrium state was considered attained after 15 h of equilibration since the variations in adsorbed percentages did not change more than 5%, when the equilibration time was increased from 12 h until 35 h (Monkiedje and Spiteller, 2002). Based on such results, further studies for the establishment of both adsorption and desorption thiram behaviour were conducted for 15 h of equilibrium time (overnight equilibration). In previous studies, other authors observed a similar kinetic behaviour for the adsorption of atrazine and some of its metabolites onto soil (Abate et al., 2004).

4.3.2 Adsorption isotherms

Adsorption isotherms for both Thi-P and Thi-F onto commercial HA are presented in Figure 4.2. Also in the same Figure 4.2, the distribution coefficients (K_D; L g^{-1}) are plotted against thiram equilibrium concentration. Those K_D values are within the range 0.110 – 0.210 L g^{-1}, decreasing with the increase of thiram concentration in solution. Those values are quite comparable to the K_D values for thiram adsorption onto natural lignin (0.15 – 0.20 L g^{-1}, as calculated from Figure 3.4 in reference Rupp and Zuman, 1992). Besides, K_D values for Thi-P are not significantly different from those for Thi-F (p=0.63). The experimental K_D values obtained in this work, compared to values for the adsorption of other organic contaminants onto other humic acid samples (Table 4.1) suggest that thiram is strongly sorbed by humic acids.

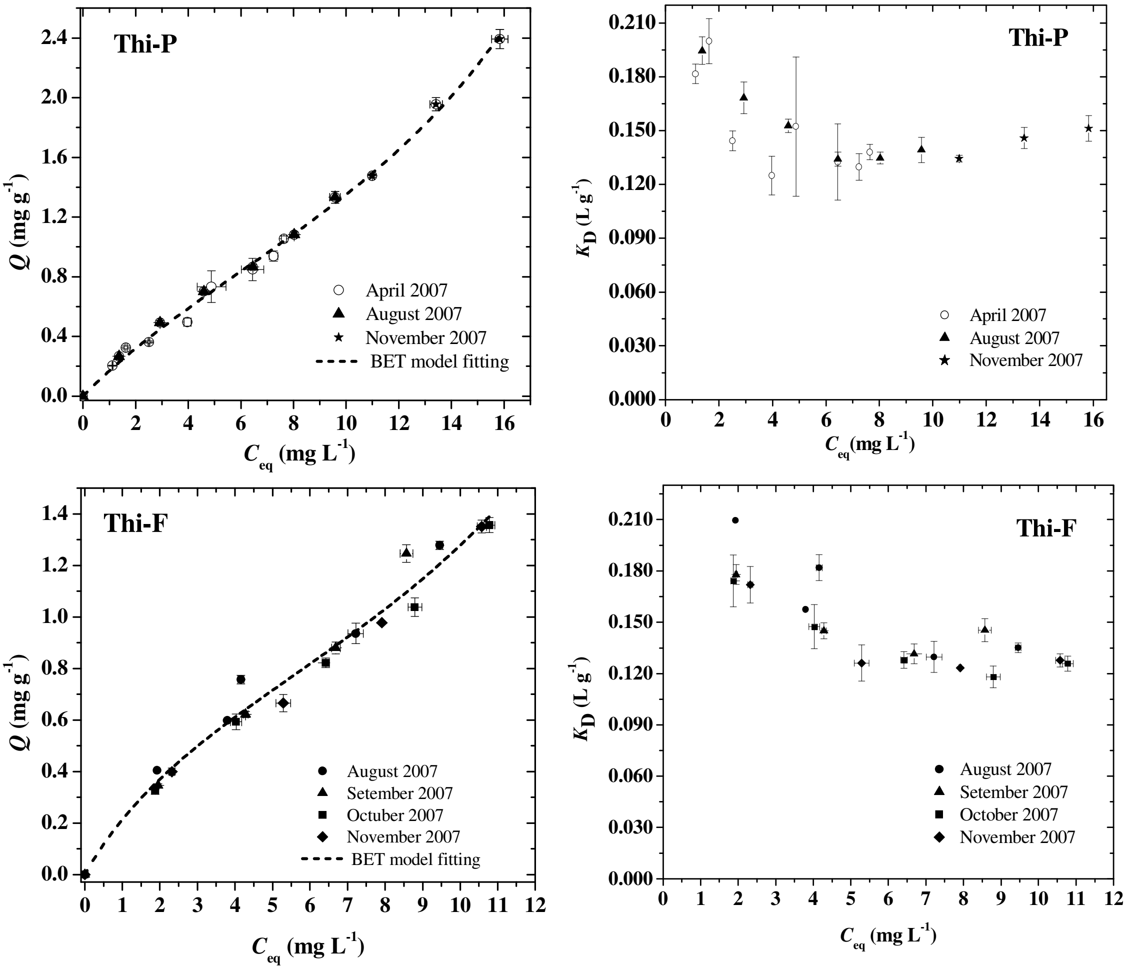

Figure 4.2 – Adsorption isotherm of both Thi-P and Thi-F onto HA and respective K_D values

Table 4.1. K_D values for the adsorption of various pesticides onto humic acids.

Pesticide	Adsorbent	K_D (L g^{-1})	Water Solubility (mg L^{-1})	Ref
2,4-dichlorophenoxyacetic acid	Soil humic acid	0.080	900 (25 °C)	Benoit et al., 1996
Glyphosate	Soil humic acid	0.006-0.057	Very soluble	Piccolo et al., 1996
Imazaquin	Soil humic acid	0.088-0.922	60 (25ºC)	Ferreira et al., 2002
Imidacloprid	Soil humic acid	0.005	510 (20ºC)	Liu et al., 2002
2,4,6-Trichlorophenol	Soil and peat humic acid	0.100-0.200	negligible	Wang et al., 2005
Thiram	**Commercial humic acid**	**0.110-0.210**	**30 (21 ºC)**	**this work**

As referred, K_D values are not constant, decreasing as the equilibrium concentration of thiram increases up to approximately 6 mg L^{-1}, remaining approximately constant for higher concentrations. This behaviour is confirmed by the values of the percentage of adsorption, which follow the same trend, varying between 52 and 35%. This deviation from linearity for low thiram concentrations suggests that adsorption can not be explained only by a partition mechanism (Chiou et al., 2000). Several types of isotherm equations have been used for fitting the experimental adsorption data of pesticides onto soils and humic substances. The Linear, Langmuir, and Freundlich isotherm equations are the most frequently applied (Benoit et al., 1996; Piccolo et al., 1996; Ferreira et al., 2002; Liu et al., 2002; Monkiedje abd Spiteller, 2002; Abate et al., 2004; Cruz-Guzmán et al., 2004; Gupta and Gajbhiye, 2004; Morillo et al., 2004; Wang et al., 2005; He et al., 2006). According to Hinz (2001), in order to choose the isotherm equation to fit a given adsorption data set, it is useful to begin by identifying the class and subgroup of the isotherm, according to the Giles classification. Following the qualitative approach recommended by Hinz (2001) it was concluded that the sorption isotherm of thiram onto humic acids is an 'L3' type (class L, subgroup 3). 'L' type isotherms are characterized by an initial decrease of K_D as the solution concentration increases and the subgroup 3 is characterized by the existence of a plateau followed by an inflection point in the Q *versus* C_{eq} plot. Thus, the Brunauer-Emmett-Teller model (BET model), which describes multi-layer Langmuir adsorption and is classified as an L3 isotherm, was also tested to fit the experimental sorption data of thiram onto HA. Thus, in the present work, the four isotherm equations have been tested to fit the data. The parameters for each isotherm equation were determined by non-linear curve fitting and are presented in Table 4.2, considering adsorption data for both pure (Thi-P) and formulated thiram (Thi-F). "Ambiguous" is a GraphPad term to describe a fit that doesn't really nail down the values of all the parameters and when many combinations of parameter values lead to curves that fit equally well (Trial version; http://www.graphpad.com; last access 15 July 2012). According to R^2 adjusted values, the BET equation is the most suitable to model the adsorption of thiram onto commercial humic acids, discarding clearly the Langmuir isotherm. Such a result highlights that the sorption of thiram onto condensed humic substances can not be explained only in terms of a specific interaction with the carboxyl groups as proposed by Stathi *et al.* (2007) for the interaction with dissolved humic acids or humic acids immobilized on the surface of silica particles, since that would give rise to a Langmuir isotherm (see supplementary material from Stathi *et al.* (2007)).

Also the Linear model is rejected on the basis of R^2 adjusted values. Several authors attributed the non-linearity of adsorption isotherms to a dual mode adsorption mechanism (Chiou and Kile, 1998; Huang et al., 1998). In the present case, as the adsorption data are described by a BET isotherm, the mechanism may include specific adsorption with a limited number of adsorption sites, and the occurrence of pesticide-pesticide interactions after saturation of those sites (multilayer adsorption). As referred above, specific interactions between thiram and carboxyl groups of humic substances have been identified by others (Stathi et al., 2007) and may be responsible for the concave downward shape of the sorption isotherm at low concentration, while the upward shape of the isotherm for high concentrations can be due to pesticide-pesticide interactions (multilayer adsorption). A similar behaviour was observed for the adsorption of thifluzamide onto soil, and the upward nature of the isotherm for higher concentrations was also attributed to pesticide-pesticide interactions (Gupta and Gajbhiye, 2004).

Table 4.2. Adsorption equilibrium data of Thi-P and Thi-F fittings to Linear, Freundlich, Langmuir and BET isotherm models[a].

	Thi-P	**Thi-F**
Linear Isotherm	$K_D = 0.141 \pm 0.004$ L g^{-1} $R^2_{Adj} = 0.987$	$K_D = 0.117 \pm 0.005$ L g^{-1} $R^2_{Adj} = 0.963$
Freundlich Isotherm	$K_F = 0.130 \pm 0.011$ $N = 1.04 \pm 0.04$ $R^2_{Adj} = 0.988$	$K_F = 0.199 \pm 0.019$ $N = 0.80 \pm 0.05$ $R^2_{Adj} = 0.968$
Langmuir Isotherm	Ambiguous fit	$K_L = 0.037 \pm 0.013$ L mg^{-1} $Q_{max} = 4.63 \pm 1.26$ mg g^{-1} $R^2_{Adj} = 0.962$
BET Isotherm	$C_S = 28.2 \pm 1.83$ mg L^{-1} $Q_{max} = 1.27 \pm 0.351$ mg g^{-1} $K = 3.99 \pm 1.01$ $R^2_{Adj} = 0.992$	$C_S = 22.7 \pm 4.13$ mg L^{-1} $Q_{max} = 0.839 \pm 0.467$ mg g^{-1} $K = 6.93 \pm 3.35$ $R^2_{Adj} = 0.970$

[a] fitting parameters and their respective errors for a 95% confidence interval are presented.

4.3.3 Desorption isotherms

The extent of desorption of both pure and formulated thiram was determined using two consecutive desorption cycles of 15 h in aqueous solution 0.01 mol L^{-1} $CaCl_2$ and a desorption cycle of 15 h in methanol. *% Des* and $K_{D(des)}$ results for each desorption cycle are presented in Table 4.3.

Table 4.3. Experimental data of adsorption-desorption isotherms of both Thi-P and Thi-F onto humic acids.

	C_i (mg L^{-1})	**% Ads**	**Desorption**					
			1st cycle[a]		**2nd cycle[a]**		**3rd cycle[b]**	**Total (%)***
			(% Des)*	**K_D (L g^{-1})**	**(% Des)***	**K_D (L g^{-1})**	**(% Des)***	
Thi-P	2.155	47.8 ± 0.8	19.9 ± 1.8	0.626 ± 0.050	<LOD	<LOD	nd	-
	2.708	49.4 ± 1.0	30.9 ± 0.6	0.399 ± 0.016	<LOD	<LOD	25.5 ± 0.9	56.4 ± 0.8
	4.309	41.9 ± 0.9	33.2 ± 1.0	0.339 ± 0.011	<LOD	<LOD	nd	-
	5.417	45.9 ± 1.3	34.5 ± 1.7	0.337 ± 0.030	12.9 ± 0.0	0.729 ± 0.035	21.0 ± 2.7	68.4 ± 2.6
	6.464	38.5 ± 2.0	38.4 ± 0.4	0.271 ± 0.007	10.9 ± 1.9	0.810 ± 0.170	nd	-
	8.125	43.5 ± 0.6	36.4 ± 2.4	0.312 ± 0.029	13.8 ± 1.0	0.651 ± 0.065	22.4 ± 0.7	72.6 ± 2.8
	8.619	43.4 ± 6.3	35.2 ± 0.0	0.299 ± 0.000	10.0 ± 0.0	0.901 ± 0.000	nd	-
	10.77	40.2 ± 4.0	38.2 ± 1.1	0.269 ± 0.014	12.2 ± 0.2	0.705 ± 0.005	23.7 ± 1.5	74.1 ± 2.4
	10.83	40.4 ± 0.7	36.8 ± 0.6	0.296 ± 0.011	13.1 ± 1.3	0.678 ± 0.072	22.3 ± 2.4	72.2 ± 3.9
	12.93	40.9 ± 0.7	35.6 ± 1.1	0.302 ± 0.013	12.7 ± 0.3	0.720 ± 0.039	28.5 ± 1.6	76.8 ± 1.2
	13.54	40.7 ± 0.6	36.5 ± 0.9	0.291 ± 0.007	15.1 ± 0.4	0.579 ± 0.005	24.4 ± 2.5	76.0 ± 2.6
	16.25	41.1 ± 1.3	36.3 ± 0.8	0.313 ± 0.010	14.8 ± 1.3	0.583 ± 0.015	25.8 ± 0.1	76.9 ± 1.1
Thi-F	3.512	46.6 ± 2.1	32.8 ± 1.9	0.365 ± 0.027	<LOD	<LOD	32.9 ± 6.1	65.7 ± 5.2
	4.332	46.4 ± 1.6	34.9 ± 1.7	0.327 ± 0.016	15.8 ± 1.4	0.559 ± 0.077	21.0 ± 1.8	71.7 ± 4.8
	7.023	42.6 ± 2.1	36.9 ± 0.9	0.297 ± 0.015	14.6 ± 3.2	0.628 ± 0.003	24.4 ± 0.0	75.9 ± 0.5
	8.665	39.0 ± 2.3	39.9 ± 1.3	0.250 ± 0.011	17.2 ± 1.5	0.437 ± 0.057	28.3 ± 0.1	85.5 ± 2.8
	10.54	39.1 ± 0.9	39.1 ± 1.1	0.270 ± 0.010	18.4 ± 1.5	0.476 ± 0.065	22.0 ± 6.2	79.6 ± 6.8
	13.00	39.1 ± 0.3	37.1 ± 1.4	0.273 ± 0.08	16.2 ± 1.1	0.472 ± 0.081	27.7 ± 4.2	81.0 ± 6.3
	14.05	37.4 ± 1.3	39.2 ± 0.2	0.261 ± 0.007	17.6 ± 0.6	0.442 ± 0.032	27.6 ± 1.1	84.4 ± 1.8
	17.33	39.0 ± 0.7	36.6 ± 2.6	0.280 ± 0.044	16.3 ± 0.7	0.526 ± 0.066	29.1 ± 1.4	82.1 ± 4.6
	17.56	38.6 ± 0.8	37.1 ± 1.0	0.294 ± 0.016	17.8 ± 1.3	0.475 ± 0.039	26.9 ± 2.0	81.8 ± 2.2

*Desorption value represent % of the amount initially adsorbed (LOD – limit of detection, nd-not determined); (a) 0.01 mol L^{-1} $CaCl_2$; (b) methanol

The results show that the percentage of thiram that is desorbed (% Des) decreases as the initial thiram concentration decreases, suggesting, once more, a stronger adsorption for low concentrations (in agreement with the results obtained in the adsorption studies). However, the adsorption-desorption isotherms for both Thi-P and Thi-F, which are compared in Figure 4.3, show that desorption behaviour is deviated from that corresponding to the adsorption isotherm, which indicates that thiram sorption onto HA was not completely reversible. The desorption K_D values after each desorption cycle were consistently higher than those for adsorption at the same equilibrium concentrations, which strongly suggests the irreversibility of the thiram adsorption onto HA, i.e., hysteresis phenomena. The experimental desorption data of the first cycle were fitted to Linear, Freundlich, Langmuir, and BET isotherms, and the results are shown in Table 4.4.

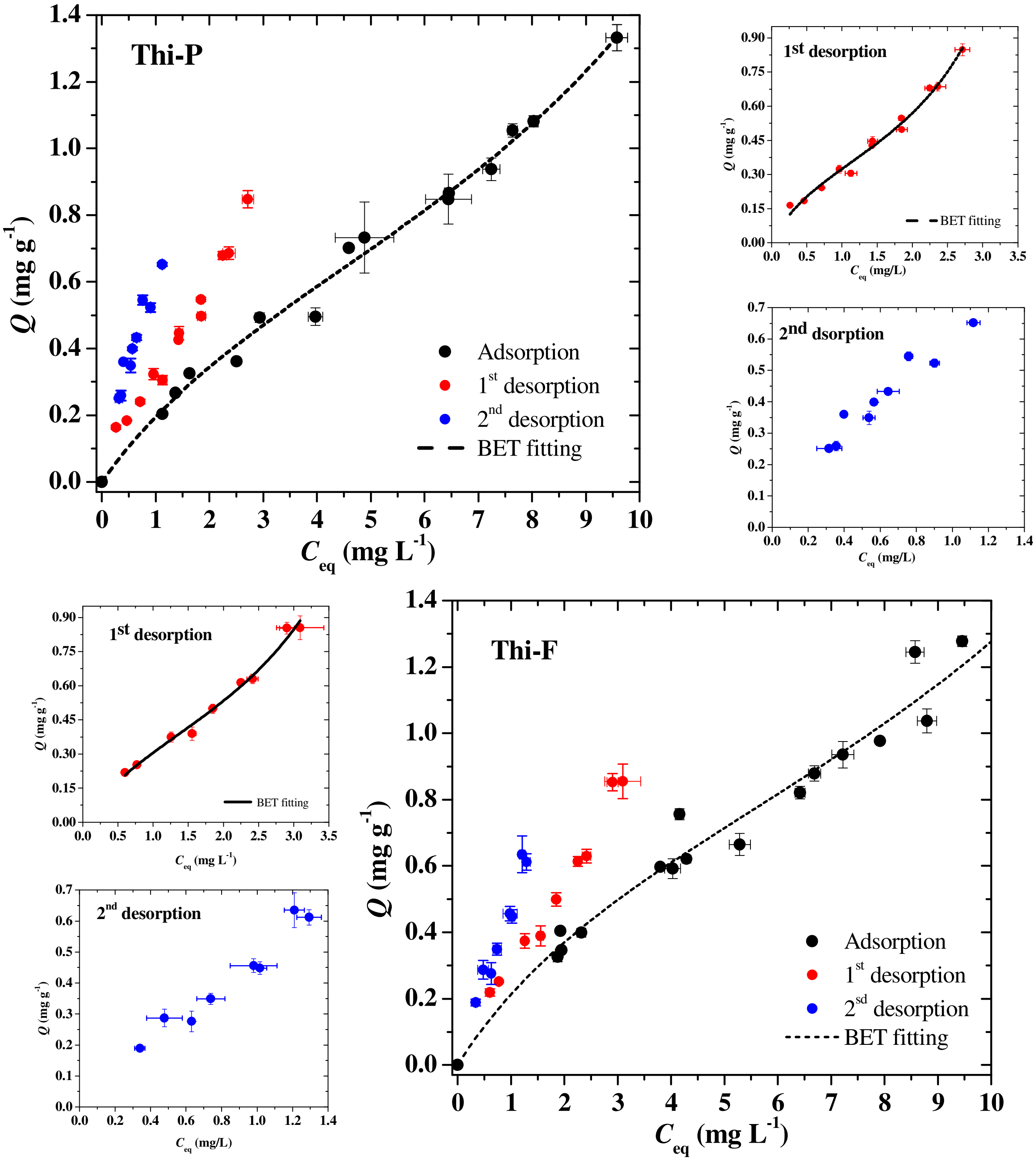

Figure 4.3 – Adsorption-desorption isotherms of Thi-P and Thi-F onto commercial humic acids

Table 4.4. First desorption equilibrium data of Thi-P and Thi-F fitting to Linear, Freundlich, Langmuir and BET isotherm models; fitting parameters and their respective errors for a 95% confidence interval.

	Thi-P	**Thi-F**
Linear Isotherm	$K_D = 0.274 \pm 0.013$ L g^{-1} $R^2_{Adj} = 0.974$	$K_D = 0.263 \pm 0.015$ L g^{-1} $R^2_{Adj} = 0.976$
Freundlich Isotherm	$K_F = 0.316 \pm 0.016$ $N = 0.923 \pm 0.067$ $R^2_{Adj} = 0.966$	$K_F = 0.287 \pm 0.018$ $N = 0.962 \pm 0.070$ $R^2_{Adj} = 0.973$
Langmuir Isotherm	Ambiguous fit	Ambiguous fit
BET Isotherm	$C_S = 4.48 \pm 0.31$ mg L^{-1} $Q_{max} = 0.366 \pm 0.162$ mg g^{-1} $K = 7.70 \pm 2.91$ $R^2_{Adj} = 0.983$	$C_S = 5.51 \pm 0.63$ mg L^{-1} $Q_{max} = 0.440 \pm 0.258$ mg g^{-1} $K = 5.91 \pm 3.06$ $R^2_{Adj} = 0.984$

The results obtained point out that the Langmuir model should be discarded and indicate the BET equation as the best fitting method for the first desorption cycle. The irreversibility of adsorption onto soils and humic acids has been attributed by several authors to micropore deformation at high concentration levels due to penetration of adsorbate molecules and entrapment when the solution concentration is abruptly decreased during desorption (Lu and Pignatello, 2002; Braida et al., 2003; Oren and Chefetz, 2005). Hysteresis due to a capillary phase separation mechanism (Miyahara et al., 1994; Dural and Chen, 1997) may also occur in humic acid mesopores.

For comparing the irreversibility of adsorption-desorption for both Thi-P and Thi-F, the HI indices at 21ºC and at some equilibrium concentrations were calculated using equation (4.7) (Table 4.5). For each C_{eq} value, the values of Q_{ads} and Q_{des} used in equation (4.7) were calculated by application of the isotherm equation which best fitted the adsorption and the first desorption data, i.e., the BET equation.

Table 4.5. Hysteresis indices (HI) for both Thi-P and Thi-F onto humic acids.

C_{eq} (mg L^{-1})	**HI**	
	Thi-P	**Thi-F**
1.0	0.927	0.434
1.2	0.847	0.415
1.5	0.781	0.407
1.8	0.767	0.421
2.0	0.785	0.441
2.2	0.824	0.471
2.5	0.928	0.535

HI values for Thi-P are consistently higher than for Thi-F. Applying the paired t-test to the HI data it was concluded that hysteresis was significantly higher for Thi-P than for Thi-F ($p<0.0001$), i.e. desorption of thiram is more facilitated in its formulation form than in its pure form. These results suggest that thiram formulation components have an influence on the desorption process of thiram from humic acids, turning the pesticide more susceptible to be leached. Such conclusion highlights the need for performing adsorption-desorption studies into soils using commercial formulations of the pesticides and not only the active ingredient.

4.4 Conclusions

The present work assesses the behaviour of pure thiram (Thi-P) and formulated thiram (Thi-F) onto commercial humic acids (HA). Experimental K_D values suggesting that thiram is strongly sorbed by humic acids. Adsorption of thiram onto commercial HA was found to conform to BET isotherm, indicating a multilayer adsorption and adsorbate-adsorbate interactions after the saturation of the surface layer. The comparison of sorption and desorption results indicated a hysteresis phenomena. Desorption K_D values were consistently higher than those for adsorption at the same equilibrium concentration. Additionally, hysteresis was lower for the formulated thiram suggesting that adsorption is more reversible in the presence of the formulation components turning the pesticide more susceptible to be leached.

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Chapter 5

Effect of long term organic amendments on adsorption-desorption of thiram onto a luvisol soil derived from loess[4]

The objective of this work was to assess the influence of soil organic amendments on the sorption properties of the fungicide thiram. The organic amendments studied were organic household compost (COM), sewage sludge from municipal water treatment facilities (SLU) and farmyard manure (FYM), which were compared to mineral fertilizer application (MIN). Sorption–desorption experiments were performed using the batch method and the results indicated that the adsorption isotherms were non-linear and were found to conform to the Brunauer-Emmett-Teller (BET) model, suggesting multilayer adsorption and adsorbate-adsorbate interactions after the saturation of the surface layer. In general, distribution coefficient values, K_D, are dependent on, but not proportional to, the initial concentration of thiram. For a fixed thiram initial concentration, a significant correlation ($r^2>0.851$; $p<0.001$) between K_D values and the soil organic carbon content (OC) was observed. The highest value of K_D was observed for the soil amended with compost, which is the one with the highest organic carbon content. K_D values were divided by the soil organic carbon contents in order to obtain organic carbon partition coefficients K_{OC}. Comparing K_{OC} means from 3 (initial concentrations) x 4 (soil organic matter compositions) x 3 (replicates) factorial ANOVA allow us to conclude that there is a significant but not proportional influence of the initial concentration of thiram on those values, but changes in the soil organic matter composition, associated to different soil amendments, have no significant influence on adsorption of thiram. To evaluate the reversibility of thiram adsorption, two consecutive desorption cycles were performed with $CaCl_2$ 0.01 mol L^{-1}. The desorption K_D values were consistently higher (approximately twice) than those for adsorption at the same equilibrium concentrations for all soil samples supporting the existence of hysteresis in the adsorption-desorption behaviour of thiram. Despite the fact that the adsorption K_D values were proportionally increased with increasing total organic carbon content, this was not the case for the desorption K_D values.

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[4] Adapted from: **Filipe, O.M.S.**, Vidal, M.M., Duarte, A.C., Scherer, H.W., Schneider, R.J., A.C., Santos, E.B.H., 2010. Effect of long term organic amendments on adsorption–desorption of thiram onto a luvisol soil derived from loess. Chemosphere 80, 293-300.

5.1 Introduction

Pesticide fate in the environment can be ruled by *transformation* processes, which can include pesticide molecule breakdown by chemical, photochemical or biological degradation, or by *transfer processes*, such as adsorption/desorption, runoff, volatilisation and leaching. Among transfer processes, sorption is a key process that extensively controls the behaviour of pesticides in soil, determining its distribution between soil and water systems (Senesi, 1992; Aboul-Kassim et al., 2001). In a previous work, we have reported a strong interaction between thiram and humic acids, suggesting the relevance of the soil organic matter on thiram sorption onto soils (Filipe et al., 2009). Other published works have reported adsorption studies of thiram onto lignins (Rupp and Zuman, 1992), specific adsorbents (e.g., waste resulting from a coal mine, SiO_2 particles, actived carbon or sepiolite) (Gonzalez-Pradas et al., 1987; Misirli at al., 2004; Stathi et al., 2006) and soils (Valverde-Garcia et al., 1988). However, so far, and to the best of our knowledge, there are no studies reported in the literature about the effects of organic amendments on thiram sorption onto soil. The application of organic wastes, such as municipal solid waste compost, sewage sludge, and farmyard manure, to agricultural soils is a common practice nowadays, being an economically attractive solution to waste disposal, with benefits in terms of improvement of soil properties and fertility (Tejada et al. 2007). However, if it is true that organic amendments improve soil fertility by enhancing organic matter levels, maintaining soil health, nutrition, fertility and crop productivity, it is also true that some of the amendments, such as sewage sludge, are prone to introduce several potentially toxic metals and organic contaminants in soils. Recently, an increasing interest has been focused on the effects of these organic amendments on pesticides behaviour in soils (Briceño et al., 2007) and it has been demonstrated that organic amendments can significantly change pesticide sorption behaviour onto the soil (Dolaptsoglou et al., 2007; El-Aswad, 2007; Ghosh et al., 2009; Majumdar et al., 2007).

In the present work, we investigate the influence of different organic amendments on the adsorption of thiram onto a luvisol soil derived from loess when compared to mineral fertilizer. The studied organic amendments were municipal solid waste compost, sewage sludge from municipal water treatment facilities and farmyard manure.

5.2 Experimental

5.2.1 Chemicals

All chemicals were of analytical grade. Thiram (pure substance, 97%) was purchased from Aldrich, methanol and acetonitrile (HPLC grade) were obtained from Riedel-de Haen and LabScan, respectively. An aqueous 0.01 mol L^{-1} $CaCl_2$ solution was prepared from anhydrous $CaCl_2$ (Fluka, p.a.). Ultra pure water for aqueous solutions was obtained with a Milli-Q water purification system (Millipore).

Standard stock solutions of thiram (~35 mg L^{-1}) were prepared by previous dissolution of the solid in acetonitrile, followed by dilution with 0.01 mol L^{-1} $CaCl_2$ aqueous solution (percentage of acetonitrile in the final solution was always lower than 1%). Further dilutions were made in 0.01 mol L^{-1} $CaCl_2$. The stock solution and the diluted ones were prepared just before application to soil samples. A stock solution of 100 mg L^{-1} thiram in acetonitrile was used to prepare more diluted standard aqueous solutions of thiram for the HPLC-UV method calibration.

5.2.2 Soils

Soils used in this study were collected on an agricultural field systematically submitted to controlled fertilization since 1962 (Scherer et al., 2002). It is a luvisol soil derived from loess (17.8 % clay, 76.3 % silt, 5.9 % sand) that has followed a cereal-root crop sequence. The field was divided in to plots which were treated with different organic amendments and with a regular mineral fertilizer (MIN), this one acting as the control fertilization. The organic fertilizers were: organic household compost (COM, 58 t ha^{-1}), sewage sludge from municipal water treatment facilities (SLU, 14.9 t ha^{-1}) and farmyard manure (FYM, 9 t ha^{-1}). These amounts, given on a dry weight basis, were applied every second year until 1997, then the amounts were changed to 90 t ha^{-1} for COM and 10 t ha^{-1} for SLU once in 3 years. The amendments were distributed in a completely randomized block design. A total of 12 samples were collected for the present work, corresponding to 3 replicates (3 different plots) for each of the four soil amendment treatments. Soil samples were taken from the topsoil layer (0-30 cm) of each plot. They were air-dried, ground, passed through a 2 mm mesh size sieve, and stored in plastic containers until further use.

Some properties of the soils were determined by Lima et al. (2009). The total organic carbon contents (TOC) are 2.8, 2.0, 1.5 and 1.2 % for COM, SLU, FYM and MIN soils, respectively.

5.2.3 Kinetic Study

Aliquots of 6 mL of approximately 20 mg L^{-1} Thi solution in 0.01 mol L^{-1} $CaCl_2$ were added to 1.2 g of soil in the pyrex centrifuge tubes and shaken at 100 rpm, during 0, 1, 3, 5, 8, 10, 12, 15, 20 and 24 h. For each equilibration time, duplicate assays were conducted and processed as described for the adsorption studies.

5.2.4 Batch adsorption/desorption studies

Batch adsorption studies were performed using a soil:solution ratio of 1:5, according to OECD guideline (OECD, 2000). Portions of 1.2 g of soil were taken into 10 mL Pyrex centrifuge tubes and mixed with 6 mL of a thiram solution prepared in 0.01 M $CaCl_2$. The concentration of thiram used was in the range 3-35 mg L^{-1}. The centrifuge tubes were shaken on an end-over-end shaker (Heidolph Reax) at 100 rpm, during 15 h at 21 ± 1 ºC to achieve equilibrium. Next, the suspensions were centrifuged at 4000 rpm for 30 min and the supernatant was filtered by 0.22 μm filters (Millex-GV, PVDF membrane) and cleaned-up using a SPE procedure described in Chapter 3 (section 3.3.3). The thiram concentration in the supernatant was then determined by HPLC-UV, with detection at 270 nm following a procedure described in Chapter 3 (section 3.3.4). For each batch experiment, a blank (without thiram) and a control sample (only thiram, without soil) have been performed.

Desorption studies were performed immediately after adsorption equilibrium. After the adsorption period and centrifugation of the aqueous suspensions, the supernatant was decanted; the residual supernatant that could not be removed was determined by gravimetry. Thiram concentration in this residual solution was considered to be the same as that measured in the bulk supernatant. Three sets of desorption experiments were done: (i) for the soil samples equilibrated with the initial concentration of thiram of 22 mg L^{-1} a 6 mL portion of fresh 0.01 mol L^{-1} $CaCl_2$ solution (without thiram) was added to the centrifuge tubes; the tubes were shaken for another 15 h, centrifuged at 4000 rpm for 30 min and the supernatants were collected and submitted to the same clean-up and analysis procedure as described in the adsorption experiment; the $CaCl_2$ desorption cycle was repeated once more followed by two desorption cycles using 5 mL of methanol (4 h) and 5 mL of acetonitrile (48 h); (ii) for the soil samples equilibrated during 15 h with the initial concentration of thiram of 22 mg L^{-1}, a desorption cycle with 5 mL of acetonitrile (24 h) was performed; (iii) for both $COM_{original}$ and $COM_{extracted}$ soil samples a $CaCl_2$ desorption cycle was performed, for all range of thiram initial concentrations, adding 6 ml of fresh 0.01 mol L^{-1} $CaCl_2$ to the centrifuge tubes; the tubes were shaken for another 15 h, centrifuged at 4000 rpm for 30 min and the supernatants were collected and submitted to the clean-up procedure as described previously. After the desorption period, the organic extracts were immediately centrifuged, filtered and analysed by HPLC-UV without any previous clean-up procedure. All experiments were done at 21 ± 1 ºC.

5.2.5 EDTA extraction of copper ions from COM soil

Approximately 10 g of COM soil sample was equilibrated with 70 mL of a 0.05 mol L^{-1} EDTA during 24 h at room temperature. Soil-EDTA suspension was centrifuged and filtered. The residue was washed with deionised water to remove the EDTA solution and centrifuged; the supernatant was discarded and soil residue dried during 24 h at 40ºC. The soils was grounded and stored in a desiccator prior to use. The extracts were kept at 4ºC until copper content measurement by flame atomic absorption spectrophotometry. Total Cu content in the original COM soil sample ($COM_{original}$) was 39 mg kg^{-1} and about 58% of the copper content was removed by EDTA extraction ($COM_{extracted}$).

5.2.6 Calculation of thiram distribution between aqueous phase and soil

The amount of thiram adsorbed by unit mass of soil during the equilibration time (Q; μg g^{-1}) was calculated from the difference between initial aqueous phase concentration (C_0; mg L^{-1}) and the thiram equilibrium concentration after adsorption (C_{eq}; mg L^{-1}),

$$Q = \frac{(C_0 - C_{eq}) \times V_0}{m_{soil}} \times 1000 \qquad (5.1)$$

where V_0 is the initial volume (L) of the aqueous phase in contact with soil during the adsorption experiments and m_{soil} (g) is the soil mass used in each essay. C_0 was obtained from the control sample.

The distribution coefficient (K_D; mL g^{-1}) was calculated as the ratio of the adsorbed concentration of thiram onto soil and its equilibrium concentration in solution,

$$K_D = \frac{Q}{C_{eq}} \qquad (5.2)$$

The distribution coefficients K_D are often reported as normalised to the content of organic carbon (%OC), and represented as organic carbon partition coefficients K_{OC}

$$K_{OC} = \frac{K_D}{\%OC} \times 100 \qquad (5.3)$$

The amount of thiram desorbed (m_{des}; mg) after each desorption cycle was calculated using the following equation:

$$m_{des} = \left(C_{eq}^{Des} \times \left(V_0 + V_r\right)\right) - m_r \qquad (5.4)$$

where, C_{eq}^{Des} (mg L^{-1}) is the thiram equilibrium concentration in solution after desorption, and m_r (mg) is the amount of thiram in the residual solution after the adsorption experiment, calculated from $m_r = C_{eq} \times V_r$, where V_r (L) is the residual volume of the supernatant that could not be removed prior to desorption and which was gravimetrically determined.

The percentage of thiram desorbed from soil was calculated according to the equation

$$\%Des = \frac{m_{des}}{m_{ads}} \times 100 \qquad (5.5)$$

where m_{ads} is the mass of thiram adsorbed onto soil during the adsorption experiment.

5.2.7 Models for the adsorption isotherms

The adsorption data were fitted to the Freundlich and Brunauer-Emmett-Teller (BET) isotherms as described in Chapter 3, section 3.2.

5.2.8 Statistical analysis

The experimental adsorption data were fitted to Freundlich and BET isotherms using the non-linear regression analysis from the program GraphPadPrism5® (Trial version; http://www.graphpad.com; last accessed on November 20th 2012). R^2 adjusted (R^2_{Adj}), which takes into account the degrees of freedom of each model, was used to assess the suitability of the fitting process and is well described in Chapter 3. Linear regression analysis, relating K_D or K_F values with soil organic carbon contents, was performed using also GraphPadPrism5® software. The value of the square of correlation coefficient (r^2) is presented in each case, as well as the level of significance of the correlation which is indicated by the p value. The two-way analysis of variance (ANOVA), applied to the K_D and K_{OC} values for adsorption, and the total desorption percentages, were performed using the SPSS software package, Version 15. To identify where differences between each factor lie, the Least Significant Difference (LSD) was calculated through the application of equation:

$$LSD = t_{df(0.05)}\sqrt{\frac{2\times RMS}{n\times P}} \qquad (5.6)$$

where *RMS* is the residual mean square, *n* is the number of replicates, *P* is the number of levels of each factor and $t_{df(0.05)}$ is the t-Student value for *df* degrees of freedom of the residual mean square. Unpaired and paired-t tests were also performed using GraphPadPrism5® software.

5.3 Results and discussion

5.3.1 Kinetic studies

Figure 5.1 shows the adsorption percentages of thiram ~20 mg L^{-1} onto COM soil for different equilibration times.

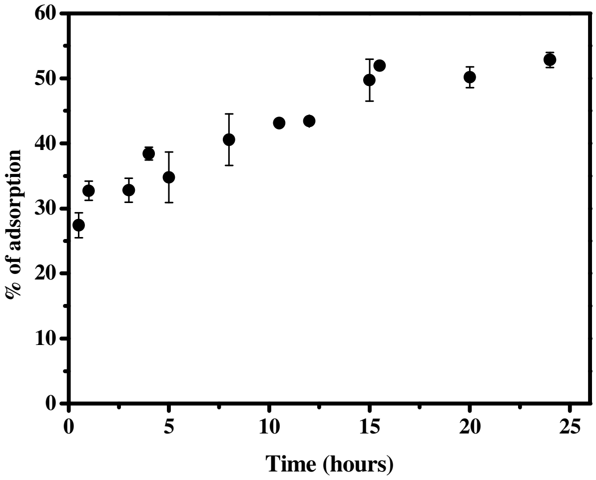

Figure 5.1 – Percentage of thiram adsorbed of onto COM soil as a function of the contact time. C_0 = 20.4 (± 1.0) mg L^{-1}.

According to these results, thiram is quickly adsorbed during the first hour (about 65% of the equilibrium concentration is reached within this contact time). An equilibration time of 15 h was chosen for determination of adsorption isotherms because the change of adsorption percentage between 15 and 24 h of equilibration was less than 5% (difference not significant, p=0.10).

5.3.2 Adsorption studies

Thiram adsorption isotherms onto luvisol soil samples submitted to different types of fertilization are shown in Figure 5.2.

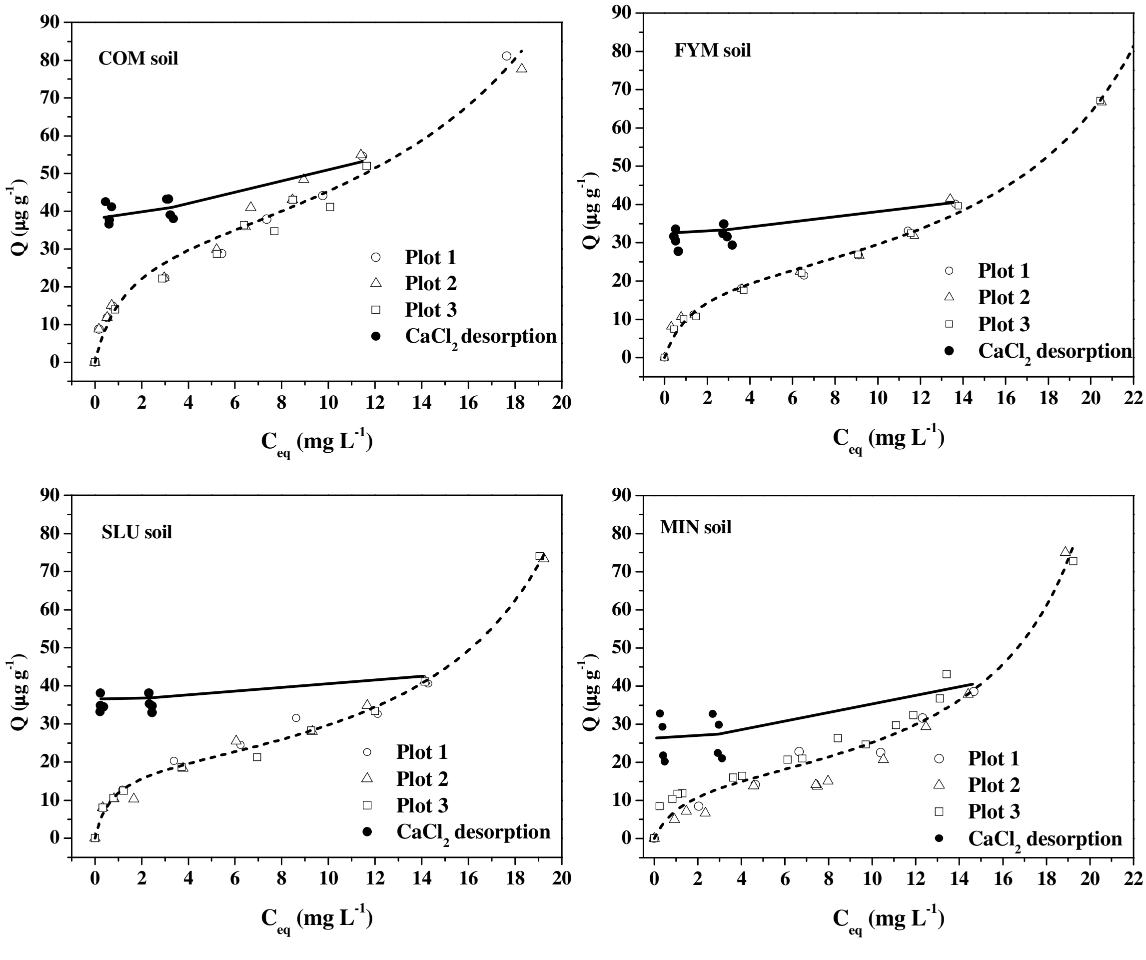

Figure 5.2 – Thiram adsorption isotherms onto a luvisol soil submitted to different controlled amendments: experimental data (open symbols) and line (------) corresponding to the fitted BET model. Desorption data are represented by black symbols.

These experimental isotherms exhibit an initial concave shape followed by an inflexion point after which they become convex, approaching an asymptotic value. This shape is typical of a BET isotherm (Ebadi et al. 2009), which assumes multilayer adsorption. Then, the BET isotherm was fitted to the data and compared to the fitting by the Freundlich isotherm, which is much more frequently used to fit experimental data for the adsorption of pesticides onto soils. Besides, the Freundlich isotherm has also been used by Valverde-Garcia et al. (1988) to fit adsorption data for thiram onto eight Mediterranean soils typical of Almeria (East-Andalucía, Spain). Parameters for each isotherm equation were determined by non-linear curve fitting of adsorption data for every soil (Table 5.1).

Table 5.1. Fitting parameters for thiram adsorption equilibrium data to Freundlich and BET isotherms and respective errors within a 95% confidence interval.

Soil	Freundlich Isotherm	BET Isotherm
COM	$K_F = 10.5 \pm 1.0$ $N = 0.67 \pm 0.04$ $R^2_{Adj} = 0.951$	$C_S = 29.7 \pm 1.2$ mg L^{-1} $Q_{max} = 32.6 \pm 10.6$ µg g^{-1} $K = 23.9 \pm 6.1$ $R^2_{Adj} = 0.955$
SLU	$K_F = 4.94 \pm 1.25$ $N = 0.85 \pm 0.10$ $R^2_{Adj} = 0.871$	$C_S = 25.7 \pm 0.33$ mg L^{-1} $Q_{max} = 18.9 \pm 4.9$ µg g^{-1} $K = 37.2 \pm 7.2$ $R^2_{Adj} = 0.989$
FYM	$K_F = 4.90 \pm 0.84$ $N = 0.83 \pm 0.07$ $R^2_{Adj} = 0.928$	$C_S = 29.6 \pm 0.6$ mg L^{-1} $Q_{max} = 21.2 \pm 4.8$ µg g^{-1} $K = 23.1 \pm 4.1$ $R^2_{Adj} = 0.990$
MIN	$K_F = 1.25 \pm 0.41$ $N = 1.34 \pm 0.13$ $R^2_{Adj} = 0.855$	$C_S = 24.2 \pm 0.5$ mg L^{-1} $Q_{max} = 15.9 \pm 9.0$ µg g^{-1} $K = 18.3 \pm 8.0$ $R^2_{Adj} = 0.946$

Analysis of values presented in Table 5.1, particularly R^2_{Adj} values, suggests BET model to best fit the experimental adsorption data for soil samples (see Figure 5.2). This isotherm shape has also been found by Sánchez et al. (2003) for the adsorption of an organophosphorus insecticide, methiathion, onto sewage sludge and it implies the occurrence of multilayer adsorption and adsorbate-adsorbate interactions after the saturation of the surface layer. The same type of adsorption isotherm was also observed in a previous study of thiram adsorption onto humic acids (Filipe et al., 2009). Gonzalez-

Pradas et al. (1987) did also observe a similar isotherm for the adsorption of thiram onto activated carbon, with an asymptotic increase after a plateau, indicating multilayer adsorption. Since Valverde-Garcia et al. (1988) fitted their experimental adsorption data of thiram onto Almeria soils using the linearised Freundlich equation, as an example, we showed in Figure 5.3 the experimental adsorption data of thiram onto FYM soil fitted to both BET and Freundlich model (Figure 5.3a and 5.3b).

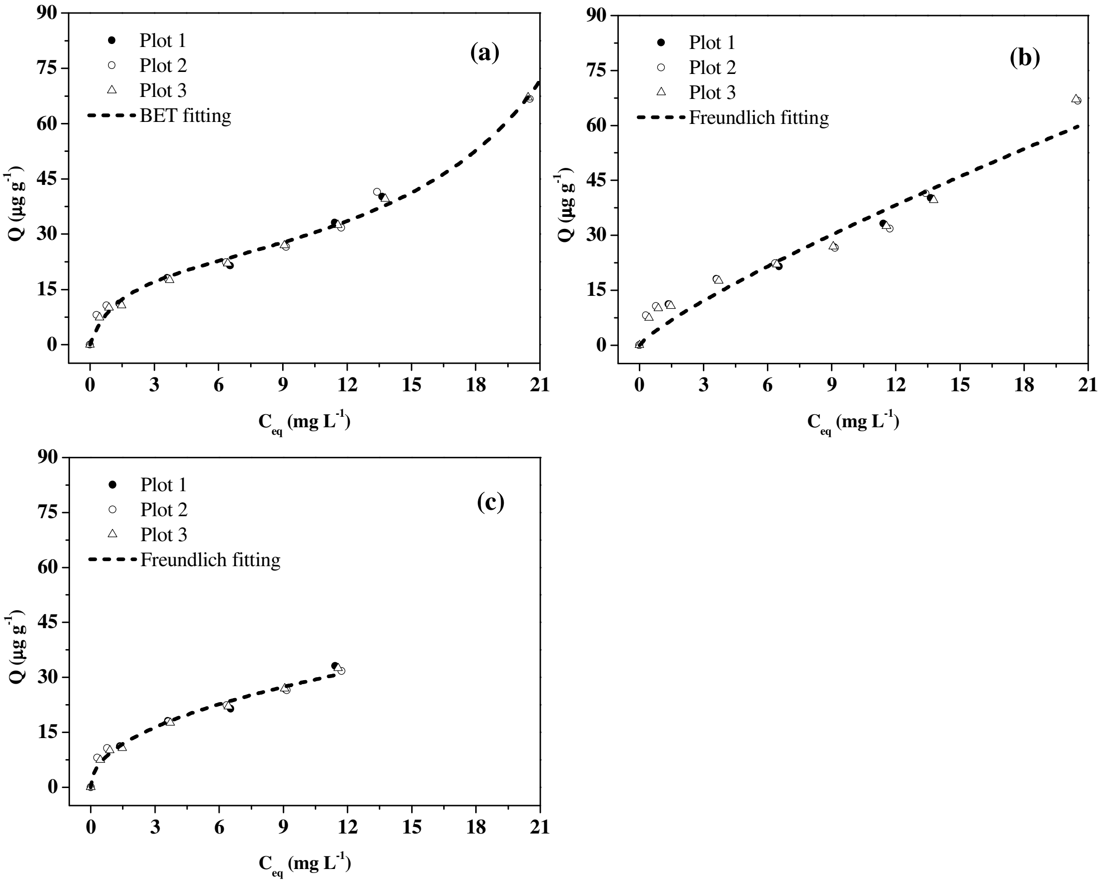

Figure 5.3 – Experimental adsorption data of thiram onto FYM soil fitted to both BET (a) and Freundlich model (b) and (c).C_{eq} range: a) and b) 0 to 21 mg L^{-1}; c) 0 to 12 mg L^{-1}.

It seems clearly that, for the thiram equilibrium concentration range studied (0.3 - 21 mg L^{-1}) the BET isotherm is the best model to describe adsorption of thiram onto studied soils instead of the usual Freundlich isotherm used by Valverde-Garcia et al. (1988). However, the data obtained by these authors correspond to equilibrium concentrations lower than 12 mg L^{-1}, while our data go up to an equilibrium concentration of 21 mg L^{-1}. Removing our data for equilibrium concentrations higher than 12 mg L^{-1}, a better fitting of the Freundlich equation is obtained (Figure 5.3c) showing that this isotherm fits well the data for low equilibrium concentrations. The asymptotic increase of adsorbed thiram for concentrations higher than 12 mg L^{-1}, which suggests the occurrence of multilayer adsorption, can be fitted only by the BET model which assumes two types of interactions responsible for the adsorption: interactions with soil adsorption sites, followed by adsorbate-adsorbate interactions after saturation of the adsorbent surface layer. The K_F values obtained by Valverde-Garcia et al. (1988) for thiram adsorption onto Almeria soils were in the range 4.8 – 13.7 and were correlated with the organic matter content. Fitting the Freundlich equation to our experimental data in the same range of thiram equilibrium concentration, i.e., discarding the data corresponding to $C_{eq} > 12$ mg L^{-1}, the K_F values obtained (7.3- 13.7) are in the same range as those obtained by Valverde-Garcia et al. (1988). Besides, a significant correlation ($r^2 = 0.946$; $p = 0.0274$) between these K_F values and the organic carbon content of the soil samples was obtained:

$$K_F = 3.92(\pm 0.66) \times \mathrm{OC} + 3.2(\pm 1.3)$$

However, the above mentioned values of K_F are lower than 108, which is the value reported in FOOTPRINT pesticide database (Information available at http:/www.eu-footprint.org; last access March 30, 2010). That can be due to the fact that K_F values depend on the soil properties and on the experimental conditions, namely, on the soil:solution ratio used in the adsorption studies (Cox et al., 1998). Considering the experimental data fitting to BET model, it is worth to notice that the saturation concentration for adsorbate in solution, i.e., the C_s average value obtained (27.3 ± 2.8 mg L^{-1}) is not far from those reported in the literature: 30 mg L^{-1} at 25 ºC in EXTOXNET data base (information available at http://extoxnet.orst.edu/pips/thiram.htm; last accessed on 20th November 2012) and 16.5 mg L^{-1} at 20 ºC in FOOTPRINT data base (Information available at http:/www.eu-footprint.org; last accessed on 20th November 2012). Slight differences between our results and those from literature can occur due to differences in the solution matrix, such as the content of some organic matter solubilised from the soil. According to Ebati et al. (2009) C_S is the reverse of K_L and does not correspond to the adsorbate solubility. The authors have fitted the BET equation to the adsorption data of several organic compounds from aqueous solution onto different types of adsorbent such as activated carbon, or perfluorooctyl aluminas and carbonized bark, and they have shown that the values of C_S obtained were quite different from the solubility data published in the literature. That difference was particularly high for the most soluble compounds, being the equilibrium concentrations of the experimental data used to fit the BET equation much lower than the solubility of those compounds. Thiram is much less soluble than those compounds (phenol and methyl *tert*-butyl ether) and, in the present work, the upper limit of the range of equilibrium concentrations used approaches thiram solubility. That may be the reason why such a big difference between C_S and the solubility values for thiram reported in the literature was not observed.

In what concerns Q_{max} values, which correspond to the saturation of the first adsorption layer, it is not possible to establish any significant correlation between those values and organic carbon content due to the errors associated to the fitting and their propagation during calculations. The uncertainties associated to the values of Q_{max} are high and probably mask the effects of fertilizations. Thus, we decided to compare the values of K_D and K_{OC}. Plots (Figure 5.4) of K_D and K_{OC} values obtained for each soil versus thiram initial concentration (for all the range of initial concentrations used) show that those values decrease as the initial concentration of thiram increases up to 12-15 mg L^{-1} (equilibrium concentration 6-9 mg L^{-1}), remaining approximately constant for higher concentrations.

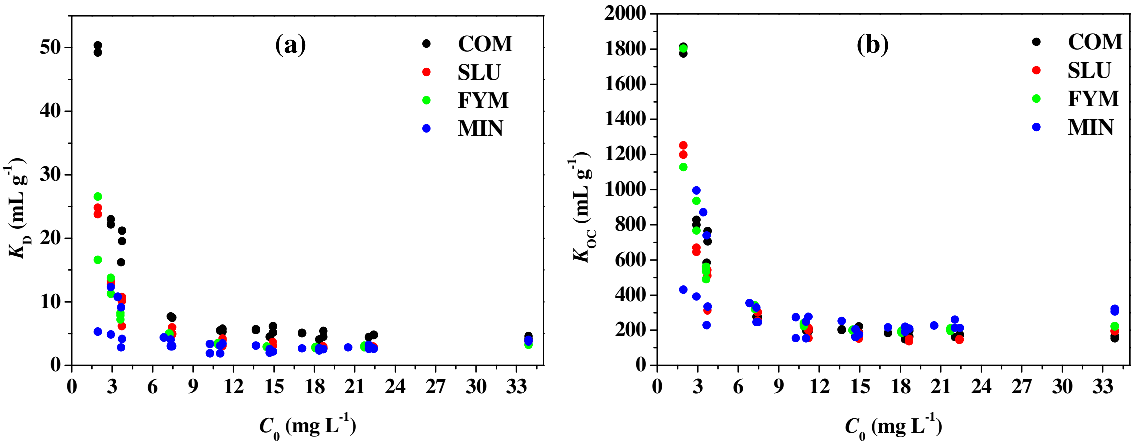

Figure 5.4 - Plots of **(a)** K_D and **(b)** K_{OC} values obtained for each soil versus thiram initial concentration

Values of K_D and K_{OC} for three different levels of the initial thiram concentration are shown in Table 5.2. For each of the concentration levels a significant correlation ($r^2>0.815$; $p<0.001$) between K_D values and the soil organic carbon content was observed. Both K_D and K_F are a measure of the extent of adsorption and it is worth to notice that this trend observed for K_D values is similar to the one above mentioned for the K_F values.

Comparing the calculated K_{OC} means from the 3 (initial concentrations) x 4 (soil organic matter compositions) x 3 (replicates) factorial ANOVA allow us to conclude that there is a significant, but not proportional, influence of the initial thiram concentration (in agreement with the above mentioned trend observed in the plots of K_{OC} versus thiram concentration), but K_{OC} values are not influenced by the organic amendments applied to the soils. It is worth to notice that K_{OC} values are K_D values normalized to the soil OC content, thus it is expected that they do not change with soil OC content. Any changes of K_{OC} values between soils submitted to different amendments, if they existed, should be attributed to different characteristics of soil organic matter and not to its quantity. ANOVA shows that K_{OC} values do not change with soil amendment, thus, the characteristics of soil organic matter, which have been shown to change with soil amendment (Lima et al., 2009), do not have a significant influence on thiram adsorption. However, as above shown, the concentration of organic matter has a significant influence on thiram adsorption, which increases with soil OC content. That effect of the soil organic carbon content is in agreement with results reported for the adsorption of other pesticides onto soil (Krishna et al., 2008; Shareef et al., 2008).

Table 5.2. Distribution coefficients (K_D) and organic carbon normalized distribution coefficients (K_{OC}) of thiram adsorption onto COM, SLU, FYM and MIN soils obtained at three initial concentration levels.

C_0 (mg L^{-1})	**K_D (mL g^{-1})**					**K_{OC} (mL g^{-1} of OC)**				
	MIN	**FYM**	**SLU**	**COM**	**Overall mean**	**MIN**	**FYM**	**SLU**	**COM**	**Overall mean**
3.69 ± 0.05	4.12 2.82 9.15	8.25 7.87 7.22	10.7 6.19 10.1	19.5 21.2 16.2	10.28	333 228 739	560 534 490	541 312 510	704 764 584	525
14.8 ± 0.20	2.16 1.97 2.54	2.93 2.89 2.97	3.66 3.02 3.06	5.15 6.14 4.51	3.42	175 159 205	199 197 202	184 152 154	186 221 163	183
22.2 ± 0.30	2.62 2.63 3.21	2.94 3.09 2.87	2.84 2.92 2.91	4.75 4.81 4.46	3.34	212 212 259	200 210 195	143 147 147	171 173 161	186
Overall mean	3.47	4.56	5.04	9.64		280	310	254	347	
amendment Factor	Significant effect (p<0.001) LSD = 1.4					No significant effect (p=0.190)				
Initial conc. Factor	Significant effect (p<0.001) LSD = 1.2					Significant effect (p<0.001) LSD = 89				

5.3.3 Desorption studies

Valverde-Garcia et al. (1988) did not assess the reversibility of the adsorption of thiram onto the soils studied. Thus, in the present work, to evaluate the reversibility of thiram adsorption, two consecutive desorption cycles were performed with $CaCl_2$ 0.01 mol L^{-1} (aqueous desorption) using the second highest initial thiram concentration (ca. 22 mg L^{-1}). If the adsorption was completely reversible, the data (Q, C_{eq}), obtained after equilibration of the soil with fresh solution of $CaCl_2$ 0.01 mol L^{-1} should fall on the adsorption isotherm curve. Those points are represented in Figure 5.2 (black symbols) and show that, for the same concentration of thiram in solution, the concentration of thiram adsorbed which is in equilibrium with it is higher for the desorption cycle, indicating the occurrence of hysteresis phenomena. In Table 5.3 the K_D values obtained for the 1st desorption cycle are presented together with the adsorption K_D values, calculated for the same equilibrium concentration C_{eq}.

Table 5.3. Experimental K_D data for desorption of thiram from soils and calculated adsorption K_D values for the same thiram equilibrium concentrate

Soil	C_{eq} (mg L^{-1})	**K_D (mL g^{-1})**	
		Adsorption	**1st desorption**
COM	***Plot 1***		
	3.36	8.22	11.3
	3.16	8.53	13.7
	Plot 2		
	3.23	8.42	12.1
	3.07	8.68	14.0
SLU	***Plot 1***		
	2.45	6.81	13.4
	2.31	7.08	16.5
	Plot 2		
	2.45	6.81	14.2
	2.33	7.04	15.1
FYM	***Plot 1***		
	3.18	5.50	9.23
	2.94	5.76	10.8
	Plot 2		
	2.78	5.94	12.6
	2.74	5.99	11.8
MIN	***Plot 1***		
	3.12	4.27	6.74
	2.92	4.43	7.67
	Plot 2		
	2.97	4.39	10.0
	2.69	4.63	12.1

For each C_{eq} value, the value of Q was calculated by application of the BET equation (Chapter 3, section 3.2.3, equation 3.7) with the parameters from Table 5.1, followed by application of equation 5.2 to obtain K_D values for adsorption of thiram onto soils. The desorption K_D values were consistently higher than those for adsorption at the same equilibrium concentrations for all soil amendments. These results confirm the hysteresis also shown in Figure 5.2. It is interesting to notice that the K_D values for desorption, presented in Table 5.3, are not so strongly correlated with soil OC as the K_D values for adsorption, presented in the same Table.

Immediately after the aqueous desorption cycle, thiram remaining in the soil was desorbed with organic solvents: 4 h in methanol and 48 h in acetonitrile. Figure 5.5a shows the total thiram recovery after all desorption cycles, and the recoveries for each individual cycle. Another set of experiments were performed in which CH_3CN was used to desorb thiram from soil immediately after adsorption, and the recovery percentages obtained are shown in Figure 5.5b.

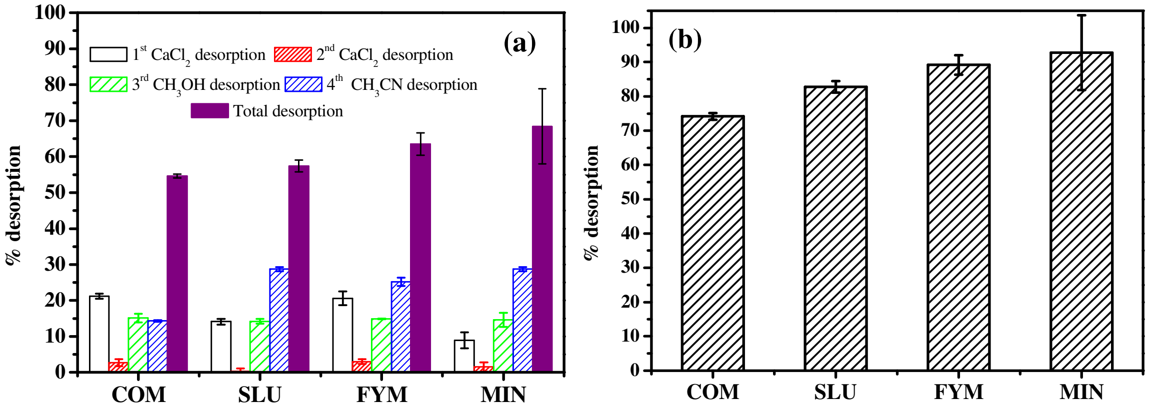

Figure 5.5 – Percentage of thiram desorbed **(a)** in each individual desorption cycle and after all desorption cycles and **(b)** in a unique desorption step with CH_3CN. Desorption values represent the % of the amount initially adsorbed. $[Thi]_0 = 22$ mg L^{-1}.

Comparing the total recovery means from the 4 (soil amendments) × 2 (desorption procedures) × 2 (replicates) factorial ANOVA allow us to conclude that there is a significant influence of the soil amendment ($p=0.0016$) as well as an extremely significant influence of the desorption procedure ($p<0.001$) on thiram recovery. The recovery of thiram is higher when the acetonitrile is applied immediately after adsorption. Besides, it was observed that when acetonitrile was applied at the end of the sequence of desorption cycles, i.e. 4 days after the adsorption, it presented a yellow colour and a new peak was observed in the chromatograms. That peak appears at the same retention time of one peak which was previously attributed to a Cu complex formed in thiram-copper solutions (Filipe et al, 2008). The yellow colour and the new peak were absent (MIN, FYM, SLU) or weak (COM) when acetonitrile was applied immediately after adsorption. These results suggest that thiram reacts along time, perhaps with metal ions in soil. In fact, as we can see in Table 5.4, thiram recovery from COM soil was significantly lower than those obtained from FYM and MIN soils.

Table 5.4. Total thiram recovery obtained from two different desorption procedures.

Soil	**Thiram recovery (%)**		
	Sequential desorption	**One step CH_3CN desorption**	**Overal l mean**
COM	55.0 54.3	74.9 73.5	64.4
SLU	56.2 58.5	81.8 84.0	70.1
FYM	65.7 61.3	91.2 87.2	76.4
MIN	75.8 61.0	100.5 85.1	80.6
Overall mean	61.0	84.8	
Amendment factor	Significant effect (p=0.016) LSD = 6.5		
Desorpotion procedure factor	Significant effect (p<0.001)		

The tendency of recoveries is in agreement with the results for total Cu contents of these soils, since COM is the soil with the highest copper content (Santos et al., 2010). According to FOOTPRINT database, thiram degradation half-life (DT_{50}) in soil is 4,6 days (in lab conditions at 20ºC). It is possible that, after the 4 days of sequential desorption, some thiram has been degraded to dimethyldithiocarbamate anion (DMDTC), which forms a complex with copper ions, probably the complex corresponding to the above mentioned new peak which occurs in the HPLC chromatograms. In order investigate the effect of copper on thiram adsorption and desorption, its transformation and its byproducts persistence in soil along time some experiences were performed.

5.3.4 Effect of copper ions on hysteresis

Batch adorption-desorption studies of thiram onto a soil submitted to the EDTA extraction ($\mathrm{COM_{extracted}}$) and with the original COM soil were performed. Original COM soil has a copper content of 39 mg $\mathrm{kg^{-1}}$ and after EDTA extraction about 58% of the initial copper content was removed. Figure 5.6 shows the adsorption-desorption isotherms obtained. BET equation (solid line) fits both adsorption and desorption isotherms and the corresponding fitting parameters are listed in Table 5.5.

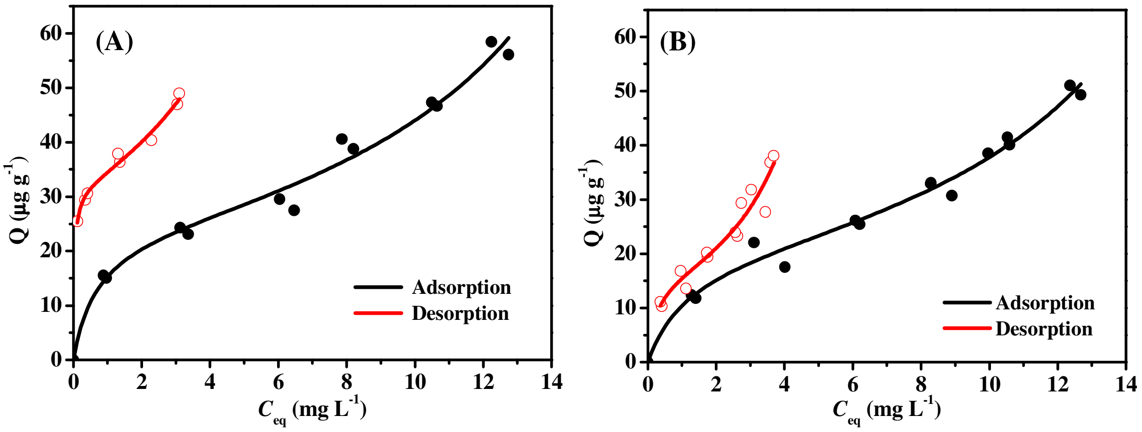

Figure 5.6 – Adsorption-desorption isotherms of thiram onto **(A)** $\mathrm{COM_{original}}$ soil and **(B)** $\mathrm{COM_{extracted}}$ soil.

Table 5.5. Fitting parameters for thiram adsorption-desorption data to BET isotherm and respective errors within a 95% confidence interval

Sample	Adsorption Isotherm	Desorption Isotherm
$\mathrm{COM_{original}}$	$C_S = 21.2 \pm 1.2$ mg $\mathrm{L^{-1}}$ $Q_{max} = 24.1 \pm 13.1$ µg $\mathrm{g^{-1}}$ K = 31.1 ± 13.0 $R^2_{Adj} = 0.981$	$C_S = 8.93 \pm 0.70$ mg $\mathrm{L^{-1}}$ $Q_{max} = 31.5 \pm 13.8$ µg $\mathrm{g^{-1}}$ K = 273 ± 88 $R^2_{Adj} = 0.985$
$\mathrm{COM_{extracted}}$	$C_S = 20.9 \pm 1.2$ mg $\mathrm{L^{-1}}$ $Q_{max} = 20.9 \pm 9.9$ µg $\mathrm{g^{-1}}$ K = 17.8 ± 6.63 $R^2_{Adj} = 0.984$	$C_S = 6.03 \pm 0.56$ mg $\mathrm{L^{-1}}$ $Q_{max} = 14.5 \pm 16.2$ µg $\mathrm{g^{-1}}$ K = 33.8 ± 28.7 $R^2_{Adj} = 0.930$

In general, the adsorption of thiram onto soil with less copper content is slightly lower than in the original soil, although statistically there is no significative effect of copper ions considering all thiram concentration range (p=0.2911). In Figure 5.7, experimental distribution coefficients (K_D; mL g^{-1}) obtained for both adsorption and 1st desorption cycle are plotted against thiram initial concentration.

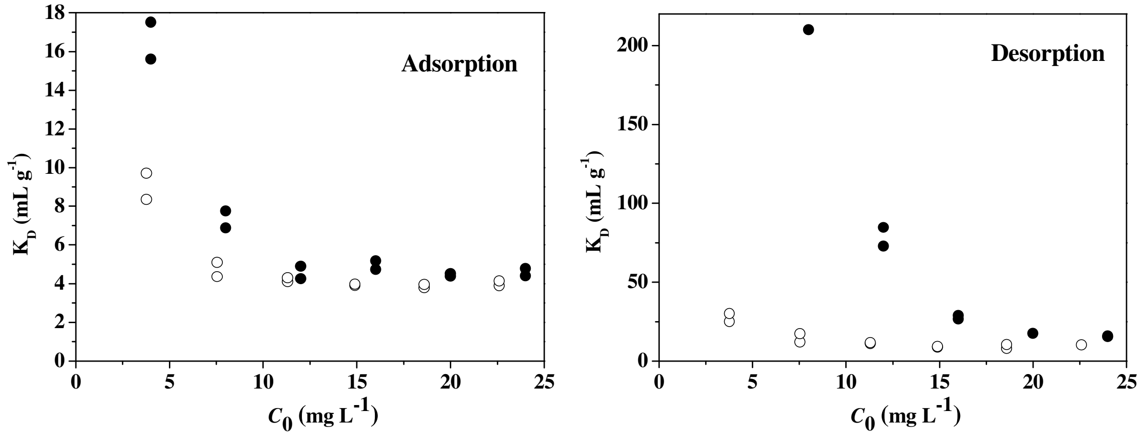

Figure 5.7 – Experimental distribution coefficients (K_D) data for adsorption and desorption of thiram from $COM_{original}$ soil (●) and $COM_{extracted}$ soil (○).

Adsorption K_D values are within the range 17.5 – 4.3 mL g^{-1} for original COM soil and 9.7 – 3.8 mL g^{-1} for extracted COM soil decreasing with the increase of thiram concentration in solution. K_D values for adsorption of thiram onto original COM soil are not significantly different from those for extracted COM soil ($p>0.1$), however, it is worth to notice that for the lowest initial thiram concentration the K_D values for original COM soil are twice the values for extracted COM soil, showing the strong effect of the presence of copper ions on the adsorption of thiram onto soil and how important is the ratio Thi:Cu in the adsorption of thiram onto soils, since the influence of the Cu soil content is more notorious for the lowest concentration of thiram, where the Cu:Thi ratio is higher.

In what concerns the desorption of thiram from these soils, K_D values (Figure 5.7) are within the range 210 – 15 mL g^{-1} for original COM soil and 25 – 8 mL g^{-1} for extracted COM soil. So, desorption K_D values are consistently higher than those for adsorption for both original and extracted COM soils, which confirms the hysteresis phenomenon also shown by the plots in Figure 5.6. The irreversibility of thiram adsorption in the presence of copper ions was also compared calculating the hysteresis index (HI). The adsorption-desorption hysteresis was quantified using the Hysteresis Index (HI) (Huang et al., 1998a; Huang et al., 1998b),

$$HI = \left. \frac{Q^{Des} - Q^{Ads}}{Q^{Ads}} \right|_{Ceq} \qquad (5.7)$$

where, Q^{Ads} and Q^{Des} refer to the solid phase thiram concentrations (mg g^{-1}) after adsorption and after a single desorption cycle experiment, respectively, corresponding to a predetermined aqueous solute concentration C_{eq}. HI values for each soil studied were calculated, for the thiram equilibrium concentration range 1.0 – 3.0 mg L^{-1}, using equation 5.7 and the BET adsorption and desorption parameters listed in Table 5.5, and the results are plotted in Figure 5.8.

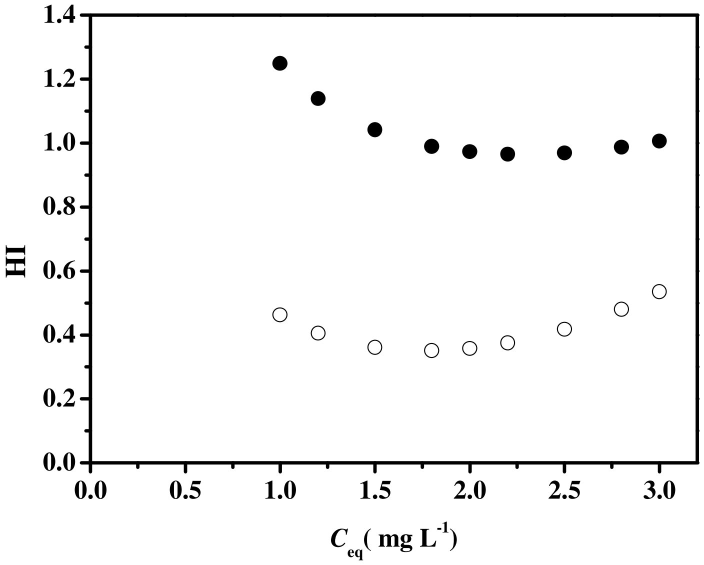

Figure 5.8 – Hysteresis indices (HI) for thiram onto original COM soil (●) and extracted COM soil (○).

HI values for thiram in original COM soil are consistently higher (twice) than for thiram in extracted COM soil. Applying the paired t-test to the HI data, it was concluded that hysteresis was significantly higher in the soil with higher copper content ($p<0.0001$), i.e., desorption of thiram is more hindered in soils with higher copper content. These results suggest a decrease of the leachability of the fungicide with the increase of the copper content of the soils.

5.4 Conclusions

The present work assesses the effect of different soil amendments on the adsorption-desorption behaviour of thiram in a luvisol soil. Adsorption of thiram onto soil samples was found to conform to BET isotherm, indicating a multilayer adsorption and adsorbate-adsorbate interactions after the saturation of the surface layer. This study revealed that organic amendments greatly affect thiram adsorption-desorption processes. The magnitude of adsorption, characterized by the K_D values, was found to be in the order: COM>SLU>FYM>MIN, which is consistent with the organic carbon content of the different soils samples. Desorption studies with $CaCl_2$ 0.01 mol L^{-1} revealed the existence of an hysteresis phenomenon. Additionally, the comparison of thiram recoveries with organic solvents applied immediately after adsorption or after the aqueous desorption suggests that thiram reacts in soil along time, what can influence their leachibility and persistence in soil. Adsorption-desorption experiments performed with one of the soil samples in its original form and after metals extraction with EDTA showed that those reactions involve metal cations in soil, most probably copper ions.

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Chapter 6

Influence of soil copper content on the kinetics of thiram adsorption and on thiram leachability from soils[5]

This work aimed to assess the influence of soil copper content on the sorption processes of thiram, a fungicide widely used in agriculture, most of the times together with copper. Two different types of studies were performed: (1) desorption studies of thiram with acetonitrile after batch adsorption equilibration, and ageing of the wet soil for a variable period of time; (2) kinetic studies of thiram adsorption performed using the soil in its original form and after fortification with copper ions. In the desorption studies, with the increase of the ageing time, a decrease of the thiram peak and a simultaneous increase of a new peak, assigned to a copper complex, were observed in the chromatograms. This new peak increases sharply until an ageing period of about 4 d and then this area is maintained approximately constant until 18 d, the maximum ageing period studied. These results indicate that thiram reacts with copper ions along time giving rise to the formation of relatively persistent copper complexes in soil. Desorption studies with $CaCl_2$ 0.01 mol L^{-1} solution showed that this complex is not extracted. Thus, it is not easily leached to ground and surface waters and copper may contribute to thiram immobilization in soil. The kinetic studies of thiram adsorption were performed in both soils and for two initial thiram concentrations (~7 and 20 mg L^{-1}). For the soil fortified with copper the percentage of adsorbed thiram is higher than observed for the original soil at the same initial concentrations and equilibration times and 100% of adsorption is attained in 15 h or 48 h, depending on the thiram initial concentration. Four kinetic equations, the pseudo first- and second-order equations, the Elovich and the intraparticle diffusion equations were selected to fit the kinetic data of the adsorption process of thiram onto both original and fortified soil. The best model to describe the kinetics of thiram adsorption onto the original soil is the intraparticle diffusion model. For the soil fortified with copper ions we verified that for the highest initial thiram concentration, the best model is also the intraparticle diffusion model, however, for the lower initial thiram concentration the best model is the pseudo second-order kinetic equation, suggesting that, for a high Cu:Thi ratio, a chemical reaction of thiram with copper ions on the soil surface can occur, and it may be the rate controlling step. Since the kinetics of adsorption depends on both soil copper content and the initial thiram concentration in solution, i.e. depends on Cu:Thi ratio, it is difficult to choose a fixed batch equilibration time for adsorption studies of thiram.

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[5] Adapted from: O. M. S. Filipe, Carina A.E Costa, M. M. Vidal, E. B. H. Santos. 2013. Influence of soil copper content on the kinetics of thiram adsorption and on thiram leachability from soils. *Chemosphere,* 90, 432-440.

6.1 Introduction

In a previous work, (Filipe et al., 2009) we have reported a strong interaction between thiram and commercial humic acids, suggesting the relevance of the organic matter content on thiram sorption onto soils. That effect of soil organic matter on thiram sorption and desorption has been confirmed on a later study about the effect of organic amendments on thiram sorption onto a luvisol soil (Filipe et al., 2010), but the results obtained in this second study suggested that metals ions, namely copper ions, could have also a relevant effect on thiram desorption. Although many studies have been published on the adsorption of pesticides and heavy metals independently, little attention has been given to the phenomena which take place when both are present together. As far as we know, the first studies on the possible influence of copper ions on the adsorption and mobility of organic pesticides in soils were focused on glyphosate (Morillo et al., 1997, 2000, 2002). More recently, some studies about the effect of soil copper ions on other pesticides adsorption and degradation have emerged in the literature (Arias et al., 2006; Dousset et al., 2007; Liu et al., 2007; Pateiro-Moure et al., 2007). However, to our knowledge, no studies have been reported on the effects of heavy metals, namely copper ions, on thiram sorption and desorption onto/from soil. Copper ions are very important since several copper-based fungicides have been extensively used for more than 100 years and many thousands of tons are consumed annually all over the world to prevent plant diseases (Borkow and Gabbay, 2005). Besides, the supply of organic amendments, namely sewage sludge and municipal waste compost to agricultural land, which is an usual and economically attractive practice nowadays, can represent an important source of contamination with this metal (Santos et al., 2010). Thus, in the present work, a systematic study of the influence of copper ions on the adsorption and desorption of thiram from soil was performed in order to clarify the occurrence of reactions with copper ions which can affect the persistence and mobility of thiram.

6.2 Kinetic equations

Thiram adsorption kinetics onto soils was analysed using pseudo first-order, pseudo second-order, Elovich and intraparticle diffusion equations.

6.2.1 The pseudo first-order equation (Lagergren's equation)

The Lagergren's equation was the first kinetic equation presented to describe the adsorption in solid–liquid systems based on the sorption capacity of solids and is used when the rate of occupation of binding sites is proportional to the number of unoccupied sites on the sorbent (Ho, 2004; Lagergren, 1898). It is assumed that one molecule of adsorbate is adsorbed onto one sorption site on the adsorbent surface and is generally expressed as follows

$$\frac{dQ_t}{dt} = k_1(Q_e - Q_t) \qquad (6.1)$$

where, Q_e and Q_t are the sorption capacities at equilibrium and at time t, respectively (μg g^{-1}) and k_1 is the rate constant of pseudo-first order sorption (h^{-1}). After integration and applying boundary conditions t = 0 to t = t and $Q_t = 0$ to $Q_t = Q_t$, the integrated form of equation 6.1 becomes:

$$Q_t = Q_e(1 - e^{-k_1 t}) \qquad (6.2)$$

6.2.2 The pseudo second-order equation

The most commonly applied form of the pseudo second-order equation is expressed as (Ho and MacKay, 2000, Ho, 2006):

$$\frac{dQ_t}{dt} = k_2(Q_e - Q_t)^2 \qquad (6.3)$$

where k_2 is the rate constant of pseudo second-order adsorption (g μg^{-1} min^{-1}). For the boundary conditions t = 0 to t = t and $Q_t = 0$ to $Q_t = Q_t$, the integrated form of equation 6.3 becomes:

$$Q_t = \frac{Q_e^2 k_2 t}{1 + k_2 Q_e t} \qquad (6.4)$$

6.2.3 The Elovich equation

The base of this equation is that a continuous and specific range of site reactivities are hypothesized. So, no physicochemical model is associated to the Elovich equation, i.e. it is an empiric equation as is generally expressed as:

$$\frac{dQ_t}{dt} = Ae^{(-BQ_t)} \qquad (6.5)$$

where A is the initial adsorption rate (µg g^{-1} min^{-1}) and, B is the desorption constant (g µg^{-1}) during any one experiment. Given that $Q_t = Q_t$ at t = t and $Q_t = 0$ at t = 0, the integrated form of equation 6.5 is

$$Q_t = \left(\frac{1}{B}\right)\ln(t - t_0) - \left(\frac{1}{B}\right)\ln t_0 \qquad (6.6)$$

where $t_0 = \dfrac{1}{AB}$.

6.2.4 The intraparticle diffusion model

The intraparticle diffusion model is originated from Fick's second law and is expressed as

$$Q_t = k_i t^{1/2} + C \qquad (6.7)$$

where k_i is the intraparticle diffusion rate constant (µg (g h)$^{-1/2}$) that can be obtained for the slope of the plot Q_t *vs.* t$^{1/2}$ and C is a constant (µg g^{-1}).

According to the literature (Mezenner and Bensmaili, 2009; Wu et al., 2009) if intraparticle diffusion is involved in the adsorption process the plot Q_t $_{vs.}$ t$^{1/2}$ results in a straight line whose slope is the intraparticle diffusion rate constant k_i. However three situations can occur: (i) the straight line pass through the origin, indicating that intraparticle diffusion is the rate controlling step; (ii) multi-linearity in the Q_t vs. t$^{1/2}$ plot indicating that adsorption occurs in various steps, i.e. instantaneous adsorption occurs in the first step, due to external surface adsorption; the second step is the gradual adsorption where intraparticle is controlled and the third step is the final equilibrium where the solute moves slowly causing a slow adsorption rate; and (iii) the straight line don't pass through the origin, i.e. there is a intercept (C), indicating that the intraparticle diffusion is not the only rate-limiting step, i.e., the process is very complex with more than one mechanism limiting the rate of adsorption.

6.3 Experimental

6.3.1 Chemicals

All chemicals were of analytical grade. Thiram (pure substance, 97%) was purchased from Aldrich; HNO_3 (65%) was obtained from Riedel-de Haen; methanol and acetonitrile

(HPLC grade) were obtained from Riedel-de Haen and LabScan, respectively. An aqueous 0.01 mol L^{-1} $CaCl_2$ solution was prepared from anhydrous $CaCl_2$ (Fluka, p.a.). Ultra pure water for aqueous solutions was obtained with a Milli-Q water purification system (Millipore). Standard solutions of thiram were prepared by previous dissolution of the solid in acetonitrile, followed by dilution with 0.01 mol L^{-1} $CaCl_2$ aqueous solution (percentage of acetonitrile in the final solution was always lower than 1%). Thiram solutions were prepared just before application to soil samples. A stock solution of 100 mg L^{-1} thiram in acetonitrile was used to prepare more diluted standard aqueous solutions of thiram for the HPLC-UV method calibration. Cu, Zn, Pb, Cd and Cr atomic spectroscopy standards 1000 mg L^{-1} were from BDH Chemicals, Lta.

6.3.2 Soil

Soil used in this study was kindly supplied by Professor Scherer of the Institute of Agricultural Chemistry, University of Bonn and was collected on an agricultural field systematically submitted to controlled fertilization since 1962 (Scherer et al., 2002). It is a luvisol soil derived from loess (17.8 % clay, 76.3 % silt, 5.9 % sand) that follows a cereal-root crop sequence. The field was divided into plots which were treated with different organic amendments and with a regular mineral fertilizer. The fertilizer was distributed in a completely randomized block design. For this study we selected two different and aleatory soil plots that were treated with 58 t ha^{-1} of compost from organic household waste (designated as COM soil). Soil samples (1000 g) were taken from the topsoil layer (0-30 cm) of each plot. They were air-dried, ground, sieved through a 2 mm mesh size, and stored in a plastic container until further use. Some characteristics of COM soil are pH = 7.1, TOC = 2.8 %, OM = 5.4 %, $[Cu]_{total}$ = 39 mg Kg^{-1}, $[Pb]_{total}$ = 100 mg Kg^{-1}; $[Zn]_{total}$ = 177 mg Kg^{-1} (Santos et al, 2010), $[Cr]_{total}$ = 35 mg Kg^{-1}, $[Ni]_{total}$ = 23 mg Kg^{-1} (Costa, 2009).

6.3.3 Batch adsorption-desorption studies of thiram

Although three different sets of adsorption–desorption experiments were performed (Experiment 1, Experiment 2 and Experiment 3), the general conditions for all of them follow a procedure described previously (Filipe et al., 2010). Briefly, 1.2 g of soil was mixed with 6 mL of a 0.01 M $CaCl_2$ solution with a known initial thiram concentration in a

Pyrex centrifuge tube. For each batch experiment, a blank (without thiram) and a control sample (only thiram, without soil) have been performed. The centrifuge tubes were shaken on an end-over-end shaker at 100 rpm. Next, suspensions were centrifuged at 4000 rpm for 30 min and the supernatant was filtered by a syringe filter unity (0.2 mm, Millex-GV) and cleaned up using an SPE procedure described in Chapter 3 (section 3.3.3). The thiram concentration in the supernatant was then determined by HPLC-UV, with detection at 270 nm following a procedure described in Chapter 3 (section 3.3.4). The residual supernatant that could not be removed was determined by gravimetry and thiram concentration in this residual solution was considered to be the same as that measured in the bulk supernatant. Thiram was desorbed from the residual wet soil with 5 mL of acetonitrile and shaking during 24 h. After the desorption cycle the extracts were centrifuged at 4000 rpm for 30 min, filtered by a syringe filter unit (0.2 mm, Millex-GV) and submitted to thiram analysis by HPLC–UV (Filipe et al., 2008). All experiments were done at 21 ± 1 ºC. Experiments using 6 mL of $CaCl_2$ 0.01 M for desorption of thiram were also performed. The procedure was the same but the aqueous extract was previously cleaned up, as previously described, before analysis by HPLC–UV.

The specific conditions for the different sets of experiments are as follows; **Experiment 1:** batch adsorption equilibration for 15 h, with a single initial thiram concentration (20.0 mg L^{-1}), followed by an ageing period of 0, 1, 2, 3, 4, 8 and 18 days before desorption; **Experiment 2:** batch adsorption equilibration for different time periods with two initial thiram concentrations (6.83 and 18.6 mg L^{-1}), followed by immediate desorption; **Experiment 3:** batch adsorption equilibration during 4, 15 and 48 h with thiram concentrations in the range 3-25 mg L^{-1}, followed by immediate desorption.

6.3.4 Study of adsorption kinetics

Aliquots of 6 mL of approximately 7 or 20 mg L^{-1} Thi solution in 0.01 mol L^{-1} $CaCl_2$ were added to 1.2 g of COM soil and COM soil after fortification with copper (described in section 6.3.5) in the Pyrex centrifuge tubes and shaken at 100 rpm, during 0, 1, 3, 5, 8, 10, 12, 15, 20, 24 and 48 h. For each equilibration time, duplicate assays and a control sample (only thiram) were conducted and processed. The centrifuge tubes were shaken on an end-over-end shaker at 100 rpm. Next, suspensions were centrifuged at 4000 rpm for 30 min and the supernatant was filtered by a syring filter unit (0.2 μm, Millex-GV) and cleaned up using an SPE procedure described in Chapter 3 (section 3.3.3) and elsewhere (Filipe et al., 2010). The thiram concentration in the supernatant was determined by HPLC-UV with detection at 270 nm as also described in Chapter 3 (section 3.3.4) and elsewhere (Filipe et al., 2008).

6.3.5 Adsorption of copper ions onto soil

Each soil plot sample (10 g) was equilibrated with 100 mL of a 50 mg L^{-1} or 4 mg L^{-1} $Cu(NO_3)_2.5H_2O$ on an end-over-end shaker at 60 rpm, during 24 h at room temperature. The suspensions were centrifuged during 8 min at 6500 rpm and were filtered by 0.45 µm membrane filters (Millipore), in order to measure copper content by flame atomic adsorption spectrophotometry (FAAS). The amount of Cu adsorbed in the soil was calculated as the difference between that initially added and that remaining in solution after equilibration. The residue was washed and stirred during 5 min with deionised water to remove any excess of copper solution, and, after separation by centrifugation during 10 min at 6500 rpm, the soil was dried during 12 h at 40 ºC. The soil samples were grounded and stored in a desiccator for at least 1 month prior to use in thiram adsorption studies. The soil copper content is an estimative based on the original copper content of COM soil (39 mg kg^{-1}) plus the quantity of copper adsorbed. Thus, the soil plots treated with copper are referred here as COM_Cu_{500} or COM_Cu_{80}, depending on the copper concentration in soil, 500 mg kg^{-1} and 80 mg kg^{-1} respectively. A control sample, without soil, was also performed.

6.3.6 Analysis of metal ions in the soil organic extracts

All measurements were performed by flame atomic absorption spectroscopy (FAAS), using a Perkin–Elmer AAnalyst 100 absorption spectrophotometer with an air–acetylene flame. Detection of Cu, Zn, Pb, Cd and Cr was made at 324.8, 213.9, 283.3, 228.8 and 357.9 nm, respectively. The relative standard deviations of all measured absorbances were always below 5% (n = 5). Calibrations were performed using mixed standard calibration solutions of Cu and Zn, Cr and Cd, and Pb, which were prepared by dilution of commercial standard solutions, for atomic spectroscopy, in nitric acid 0.4%. The acetonitrile of the organic extracts from soil was evaporated and the residue was redissolved in 0.4% HNO_3 solution.

6.3.7 Calculation of thiram distribution between aqueous phase and soil

The amount of thiram adsorbed by unit mass of soil during the equilibration time (Q; µg.g^{-1}) was calculated from the difference between initial aqueous phase concentration (C_0; mg L^{-1}) and the thiram equilibrium concentration after adsorption (C_{eq}; mg.L^{-1}),

$$Q = \frac{(C_0 - C_{eq}) \times V_0}{m_{soil}} \times 1000 \qquad (6.8)$$

where V_0 is the initial volume (L) of the aqueous phase in contact with soil during the adsorption experiments and m_{soil} (g) is the soil mass used in each assay. C_0 was obtained from the control sample.

The percentage of thiram adsorbed onto soil was calculated according to the equation

$$\% Ads = \frac{(C_0 - C_{eq})}{C_0} \times 100 \qquad (6.9)$$

The amount of thiram desorbed (m_{des}; mg) was calculated using the following equation:

$$m_{des} = \left(C_{eq}^{Des} \times \left(V_0 + V_r\right)\right) - m_r \qquad (6.10)$$

where, C_{eq}^{Des} (mg.L^{-1}) is the thiram equilibrium concentration in solution after desorption, and m_{r} (mg) is the amount of thiram in the residual solution after the adsorption experiment, calculated as $m_r = C_{eq} \times V_r$, where V_{r} is the residual volume of the supernatant that could not be removed prior to desorption and which was gravimetrically determined.

The percentage of thiram desorbed from soil was calculated according to the equation

$$\% Des = \frac{m_{des}}{m_{ads}} \times 100 \qquad (6.11)$$

where m_{ads} is the mass of thiram adsorbed onto soil during the adsorption experiment.

6.3.8 Models for the adsorption isotherms

The adsorption data were fitted to the Langmuir and Brunauer-Emmett-Teller (BET) isotherms as described in Chapter 3, section 3.2.

6.3.9 Statistical analysis

Experimental kinetic adsorption data were fitted to the equation models by non-linear regression analysis using the program GraphPadPrism5® (Trial version; http://www.graphpad.com; last accessed on 20th November 2012), except for the intraparticle diffusion model to which the experimental data were fitted by linear regression analysis. R^2 adjusted (R^2_{Adj}), which takes into account the residual degrees of freedom of each model, was used to assess the suitability of the fitting process and is well defined in Chapter 3 (section 3.3.5). The two-way analysis of variance (ANOVA) was performed using also the program GraphPadPrism5® (Trial version; http://www.graphpad.com; last accessed on July 15th 2012). The limit of detection (LOD, mg L^{-1}) of each metal was calculated according to the equation 2.1 defined in Chapter 2. LOD's of 0.076, 0.014, 0.476, 0.098 and 0.018 mg L^{-1} were obtained for Cu, Zn, Pb, Cd and Cr, respectively.

6.4 Results and discussion

6.4.1 The influence of ageing period on thiram desorption percentage

Figure 6.1 shows the HPLC chromatograms for the acetonitrile extracts obtained after different ageing periods.

As we can see in the chromatograms, with the increase of the ageing time, there is a decrease of the thiram peak and a simultaneous increase of a new peak. The area of this new peak increases markedly until an ageing period of about 4 days, after which it is maintained approximately constant from 4 until 18 days, the maximum ageing period studied (Figure 6.2). A slight increase (~5%) occurs from 4 to 8 days and then a slight decrease (~13%) occurs from 8 to 18 days.

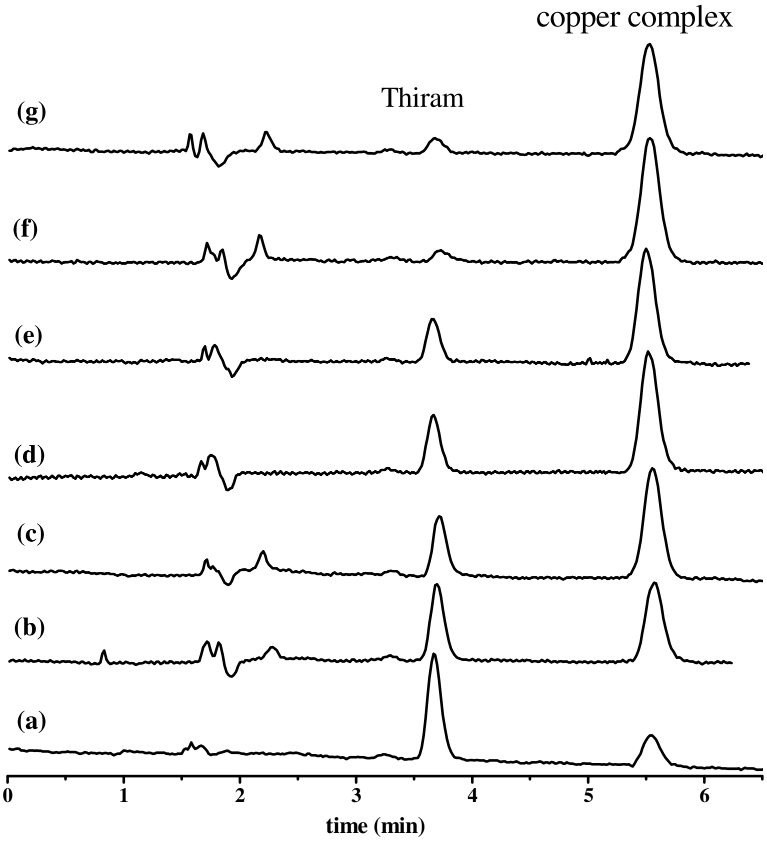

Figure 6.1 – HPLC-UV chromatograms for the acetonitrile extracts obtained for different ageing periods: a) 0, b) 1, c) 2, d) 3, e) 4, f) 8 and g) 18 days;

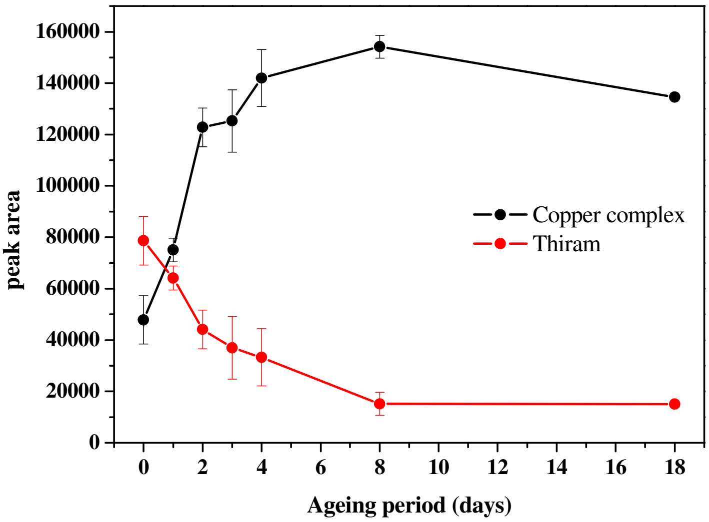

Figure 6.2 – Peak area profile of free thiram and copper complex.

This new peak was already assigned previously to a copper complex (Thi:Cu or a complex with a degradation product of thiram, the dimethyldithiocarbamate – DMTDC:Cu) (Filipe et al., 2008, 2010). This attribution was confirmed in the present work by the analysis of metal ions in the organic extracts; the contents of the analysed metal ions in the acetonitrile extract of the original COM soil were lower than the LOD; however, after thiram adsorption (initial concentration approximately 20 mg L^{-1}) and an ageing period of 4 days, only copper increased to a concentration of about 3 mg L^{-1} in the acetonitrile extract, while the concentration of the other metal ions continued to be lower than the LOD. Desorption studies with $CaCl_2$ 0.01 M, immediately after adsorption with an equilibration time of 15 h, showed that only free thiram is desorbed and none copper complex is present in solution, while after an ageing period of 8 days, no free thiram or complex were detected in the extracts (results not shown). Taking into account that free thiram is practically inexistent after 8 days, as shown in Figure 6.2, the desorption studies with $CaCl_2$ 0.01 M show that the copper complex formed is not easily leached and that copper may contribute to thiram immobilization in soil.

According to the literature (Aboul-Kassim and Simoneit, 2001; Rudzinski and Plazinski, 2007; Plazinski et al., 2009; Boparai et al., 2011) when a solute in solution adsorbs on porous adsorbents the following consecutive steps can be involved: (i) film or surface diffusion where the adsorbate molecules are transported from the bulk solution to the external surface of the adsorbent solid phase particle across the boundary layer or surface film; (ii) intraparticle or pore diffusion, where adsorbate molecules move into the interior of adsorbent particles, and (iii) adsorption of the adsorbate molecules on the interior sites of the adsorbent by physical or chemical bindings. Usually the adsorption step is considered very quick, and so, as not limiting the overall adsorption rate (Boparai et al., 2011). However, the results presented above show that reactions with copper occur, thus the extent of their occurrence during adsorption studies may be strongly dependent on the copper content and availability in the soil and on the thiram concentration in solution (Cu:Thi ratio). If these reactions are unmeasurably slow during the kinetic adsorption studies due probably to the few available copper sites in the surface, a pseudo-equilibrium can be attained, due to a slow gradual diffusion of the solute into soil micropores, and the adsorption mechanism will not involve these reactions, but, in certain conditions, i.e. a large Cu:thi ratio, it is possible that the reaction with copper may occur in a measurable extent, being the rate limiting step of the adsorption, since the amount of available copper sites relatively to the solute molecules is higher.

In order to verify if these complexes are already involved in the initial adsorption step, a set of batch adsorption experiments onto original COM soil with different equilibration times were performed followed by a desorption cycle of 24 h with acetonitrile (Experiment 2). The desorption with acetonitrile immediately after the adsorption step will recover all the free thiram and indicate the presence of copper complex by the appearance of a chromatographic peak at about 6 min already attributed to a copper complex (Filipe et al., 2008, 2010). Desorption percentages obtained for free thiram are shown in Figure 6.3A. For both initial thiram concentrations studied, a desorption percentage of free thiram higher than 80% was obtained for an equilibration time of 4 and 8 h. However, for an equilibration time of 15 and 48 h thiram mean desorption percentages in the range 61–73% and 48–65% were obtained, respectively (Figure 6.3A). The comparison of the desorption percentages by a 2 (initial concentrations) x 3 (equilibration time: 8, 15 and 48 h) x 2 (replicates) factorial ANOVA allows us to conclude that there is an extremely significant influence of both the initial thiram concentration and equilibration time ($p<0.001$) on thiram desorption percentage. The areas of the complex peak in the chromatograms of the organic extracts (Fig. 6.3B) show that the complex is present even for a low batch equilibration time, but when this time increases to 48 h the peak area increases and, as thiram reacts with copper, the desorption percentage of free thiram decreases. These results show that, in fact, during the equilibration time in batch adsorption studies, there is not only an adsorption of thiram, which allows its recovery during the desorption step with acetonitrile, but also an irreversible chemical reaction of thiram which gives rise to the formation of a complex of copper ions with thiram, or with DMTDC, a product of thiram degradation (Kumarasamy and Raghu, 1976). The rate and extent of the reactions with copper may depend on the initial concentrations of thiram in solution and copper ions in soil, and may or not occur during batch adsorption studies of thiram onto soils, raising some difficulty in the choice of the equilibration time for those studies.

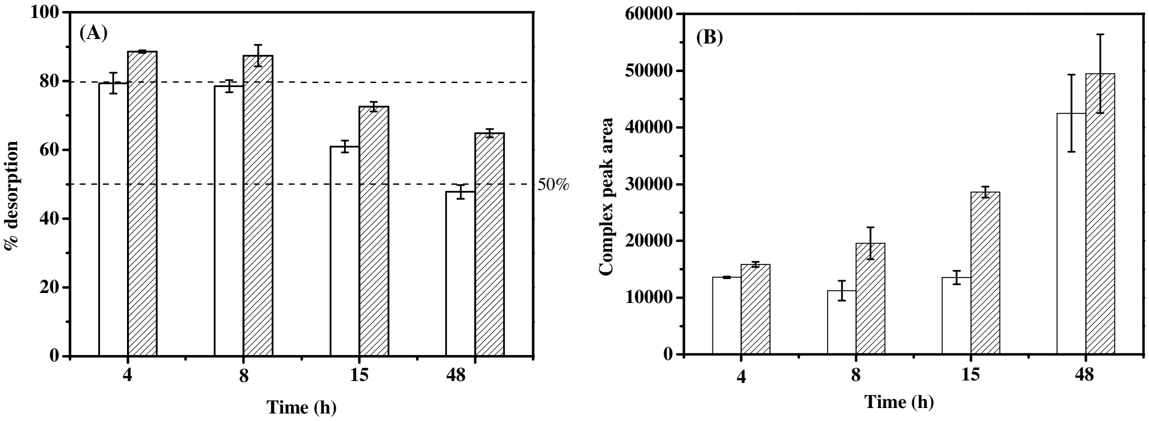

Figure 6.3 – **(A)** Percentage of free thiram desorbed with acetonitrile and **(B)** area of the copper complex in the HPLC chromatograms of the acetonitrile extracts as a function of the equilibration time for two initial aqueous thiram concentrations, $C_0 = 6.83$ mg L^{-1} (☐) and $C_0 = 18.6$ mg L^{-1} (▨). Desorption values represent the % of the amount initially adsorbed.

In the adsorption studies of thiram and other pesticides onto soil the equilibration time frequently used is 24 h, which is usually considered long enough to attain the equilibrium. In several works published in the literature this equilibration time is used without any kinetic previous studies (Arias et al., 2005; Konda et al., 2002; Larsbo et al., 2009; Rodríguez-Rubio et al., 2006) and in several others a kinetic study with only one initial pesticide concentration was performed (Ghosh and Singh, 2009; He et al., 2006; Lima et al. 2010; Vischetti et al., 2010). In our previous work (Chapter 5, section 5.3.1) an equilibration time of 15 h was used based on kinetic studies of the adsorption of thiram onto humic acids (Filipe et al., 2009) and on a study of the kinetics of adsorption of thiram (initial concentration ~20 mg L^{-1}) onto the COM soil sample. This study revealed that the change of adsorption percentage between 15 and 24 h of equilibration was less than 5% (difference not significant, $p = 0.10$), reason why 15 h was the equilibration time selected for determination of adsorption isotherms. However, the occurrence of the above mentioned reactions with copper in soil along time, rises the question of knowing how the kinetics of adsorption depends on the initial concentrations of thiram in solution and copper in soil. Thus, in the present work, the kinetics of adsorption was studied for the same soil, but using an initial concentration of thiram much lower (~7 mg L^{-1}) and for the same initial concentration of thiram (~20 mg L^{-1}) but using the COM soil after fortification with copper.

6.4.2 Influence of copper content in soil and initial concentration of thiram in solution on the adsorption kinetics of thiram.

Figure 6.4 compares the adsorption kinetics of thiram onto COM soil for an initial concentration of thiram of ~7 mg L^{-1} with the kinetics for an initial concentration of thiram of ~20 mg L^{-1}.

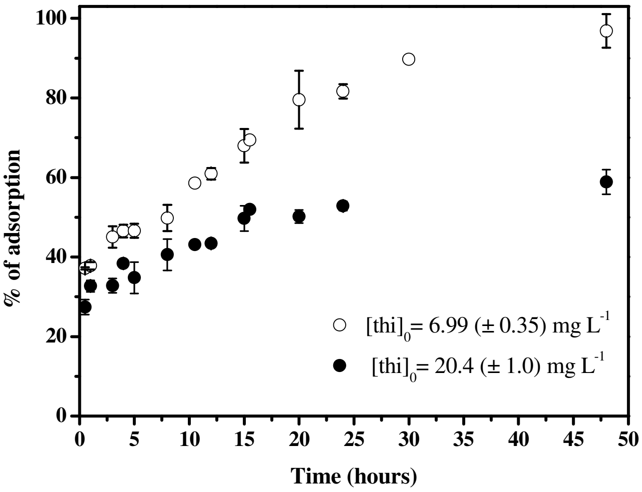

Figure 6.4 – Percentage of thiram adsorbed onto COM soil as a function of the contact time

It is possible to observe that there is a rapid adsorption followed by a slow increase of the percentage of adsorption with time. When the equilibration time changes from 15 to 48 h, the adsorption percentage increases from 50 ± 3% $(\bar{x} \pm \sigma,\ n = 6)$ to 59 ± 3% $(\bar{x} \pm \sigma,\ n = 5)$, when the initial concentration of thiram is ~20 mg L^{-1}, and from 68 ± 4% to 97 ± 4% $(\bar{x} \pm \sigma,\ n = 4)$, when the initial concentration is ~7 mg L^{-1}. Thus, a kinetic study performed with the initial thiram concentration of 20 mg L^{-1} and a maximum equilibration time of 24 h (the maximum time usually used) would suggest that equilibrium was attained after 15 h (the difference in the percentage adsorbed between 15 h and 24 h is less then 5%; difference not significant p=0.10). However, for the thiram initial concentration of ~7 mg L^{-1}, it is clear that equilibrium was not attained after 15 h. Thus the kinetic study with only one initial concentration may be not enough to choose the equilibration time for batch adsorption studies. In order to study the effect that soil copper ions could have during the equilibration of thiram in batch adsorption experiments, an adsorption kinetic study of thiram onto COM soil fortified with copper (COM_Cu$_{80}$, 80 mg kg^{-1} of copper) was performed using the same initial aqueous thiram concentrations as those of the study above described (Figure 6.5).

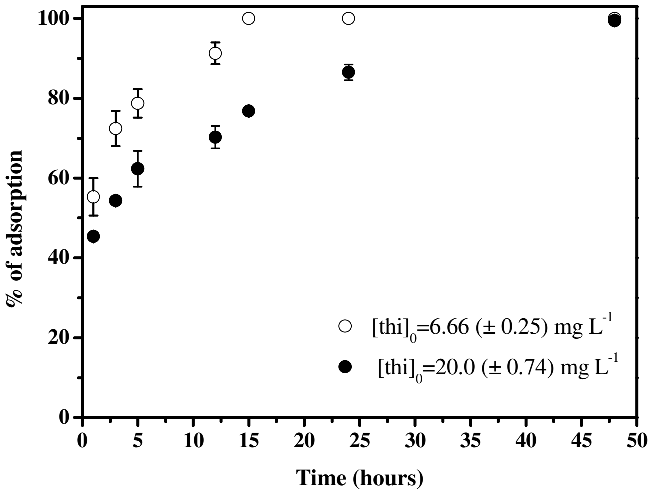

Figure 6.5 - Percentage of thiram adsorbed onto COM soil fortified with copper (COM_Cu_{80}) as a function of the contact time.

For the same initial concentrations and equilibration times, the percentage of adsorbed thiram is higher for COM soil fortified with copper, COM_Cu_{80}, than observed for the original COM soil. Besides, it is observed that 100% of adsorption is attained in 15 h or 48 h, depending on the thiram initial concentration. In fact, for a soil fortified with a great excess of copper (500 mg kg^{-1}) a 100% of adsorption was obtained for a contact time of 4 h and both initial thiram concentrations (results not shown).

Four different kinetic equations were used to fit the experimental kinetic data of thiram adsorption onto both original COM soil and COM soil fortified with copper. Since there is evidence that a chemical reaction occurs, as mentioned above in section 6.4.1, and, depending on the content of copper in soil and initial thiram concentration, the rate limiting step of the adsorption may or not involve the chemical reaction between copper ions and the molecules of thiram, we decided to use three kinetic equations that have usually been used as models for chemical reaction based adsorption (Kumar and Gaur, 2011; Qiu et al., 2009; Rudzinski and Plazinski, 2007). According to the literature (Rudzinski and Plazinski, 2006; Rudzinski and Plazinski, 2007; Plazinski, et al., 2009) the Lagergren empirical equation and the second-order equation, have been the most widely used rate equations for describing the kinetics of sorption controlled by a surface reaction. In addition, the Elovich equation, also considered an empirical equation, has been used in the description of chemical adsorption mechanisms in various heterogeneous systems, such as soil (Aharoni et al., 1991; Wu et al. 2009a). On the other hand, under certain conditions, these reactions between thiram and copper ions can be unmeasurably slow during the kinetic adsorption studies, and, consequently, the adsorption mechanism will not involve these reactions, and may be controlled by intraparticle diffusion, reason why the equation usually used to model intraparticle diffusion has also been considered in this work (Kumar and Gaur, 2011; Qiu et al., 2009; Wu et al., 2009b). Table 6.1 shows the adsorption kinetic parameters obtained by non-linear fitting of the experimental data to the pseudo 1st order, pseudo 2nd order and Elovich kinetic equations and by linear regression analysis of the intraparticle diffusion model. The adjustment of the several models to the experimental data can be seen in Figure 6.6. According to the R^2_{aj} values and the adjustment shown in Figure 6.6, the best model to describe the kinetics of thiram adsorption onto COM soil is the intraparticle diffusion model.

Table 6.1.. Kinetic parameters for the adsorption of thiram onto COM and COM_Cu$_{80}$ soils.

Soil	**COM soil**		**COM_Cu$_{80}$ soil**	
	$C_0 = 6.99 \pm 0.35$ mg L^{-1}	$C_0 = 20.4 \pm 1.0$ mg L^{-1}	$C_0 = 6.66 \pm 0.25$ mg L^{-1}	$C_0 = 20.0 \pm 0.7$ mg L^{-1}
$Q_{e,exp}$ (μg g^{-1})	34.1	63.6	34.5	103.1
Pseudo 1st order				
Q_e, (μg g^{-1})	28.0 ± 2.6	46.0 ± 2.8	31.4 ± 1.4	81.0 ± 7.0
k_1 (h^{-1})	0.18 ± 0.06	1.4 ± 0.6	0.64 ± 0.14	0.47 ± 0.18
R^2_{aj}	0.393	0.250	0.747	0.452
Pseudo 2st order				
Q_e, (μg g^{-1})	30.5 ± 2.9	51.4 ± 3.2	33.8 ± 0.9	89.7 ± 7.1
K_2 (g μg^{-1}h^{-1})× 10^{-2}	1.02 ± 0.50	2.25 ± 1.06	2.95 ± 0.55	0.69 ± 0.33
R^2_{aj}	0.586	0.522	0.935	0.705
Elovich equation				
A, (μg.g^{-1}.h^{-1})	39.3 ± 28.1	303 ± 243	423 ± 324	239 ± 155
B (g.μg^{-1})	0.20 ± 0.03	0.13 ± 0.02	0.24 ± 0.03	0.07 ± 0.01
R^2_{aj}	0.734	0.823	0.934	0.917
Intraparticle diffusion				
k_i, (h^{-1})	3.72 ± 0.25	5.94 ± 0.47	2.49 ± 0.58	9.28 ± 0.53
C (μg g^{-1})	8.98 ± 0.93	24.6 ± 1.7	19.8 ± 5.3	38.7 ± 2.0
R^2_{aj}	0.945	0.928	0.740	0.981

Comparing the intraparticle rate constant values, k_i, it is observed that the k_i value increases with the initial thiram concentration, as has been observed for the adsorption of other compounds onto porous adorbents controlled by intraparticle diffusion (Mezenner and Bensmaili, 2009; Smaranda et al., 2011). Relatively to the adsorption kinetics of thiram onto COM soil fortified with copper ions (COM_Cu$_{80}$ soil), and according to the R_{aj}^2 values presented in Table 6.1 and the graphics of Figure 6.6, we verified that for the highest initial thiram concentration, the best model is also the intraparticle diffusion model (R_{aj}^2 = 0.981). However, for the lower initial thiram concentration the best model is the pseudo second-order kinetic equation (R_{aj}^2 = 0.935) and the obtained Q_e value is very close to experimental Q value for 48 h of equilibration. This suggests that, for a high Cu:Thi ratio, the chemical reaction can occur during the time range of the adorption kinetic studies and it may be the rate controlling step. It is also worth to notice that in the cases for which the intraparticle diffusion model is the best model, the straight line adjusted to the experimental data of Q versus $t^{1/2}$ doesn't pass through the origin (intercepts C are in the range 9-39 $\mu g\ g^{-1}$, see Table 6.1), suggesting that adsorption involves intraparticle diffusion but this is not the only rate limiting mechanism.

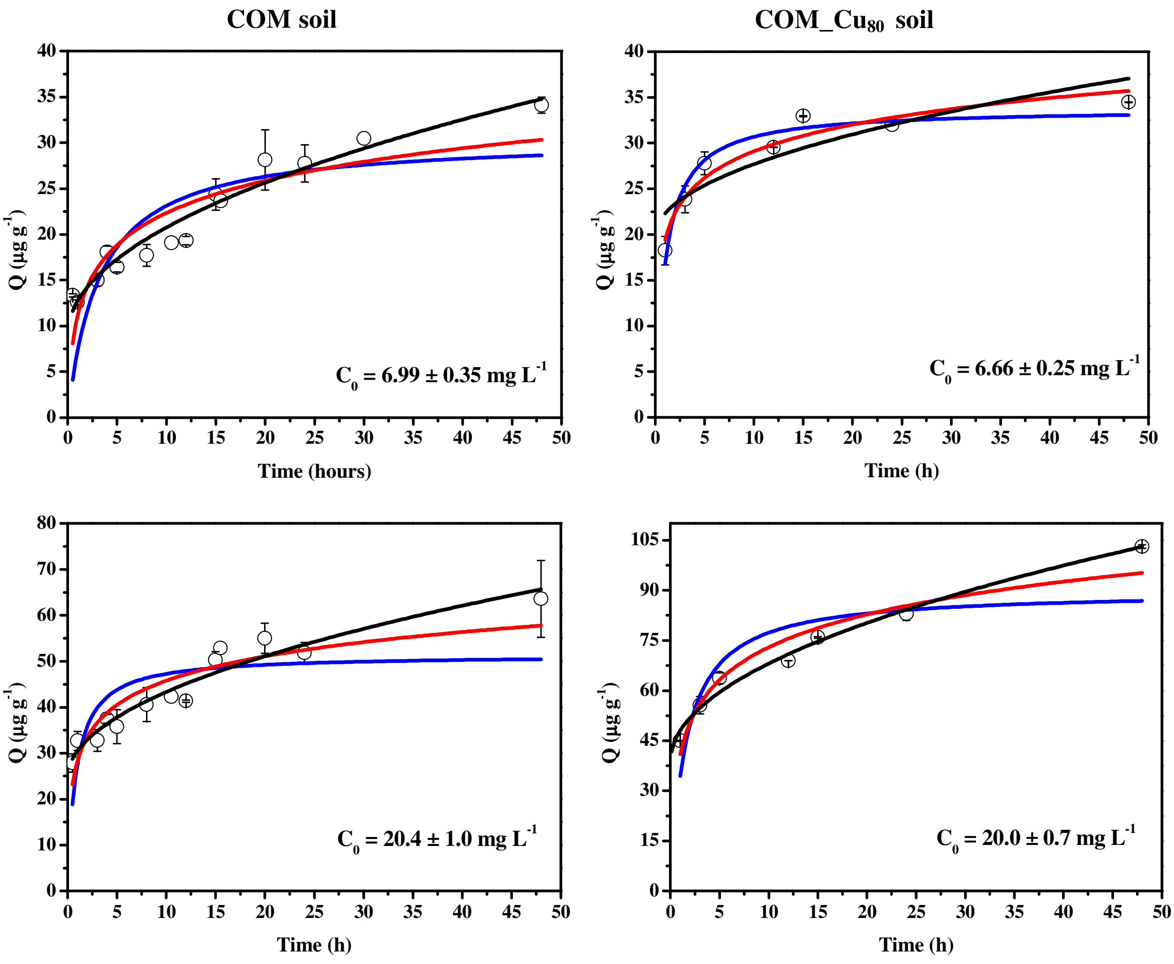

Figure 6.6 – Experimental kinetic adsorption data of thiram onto soil fitted to (▬) pseudo 2nd order, (▬) Elovich and (▬) intraparticle diffusion equations.

According to the literature (Wu et al., 2009b) this intercept (C) indicates the occurrence of a rapid adsorption within a short period of time. Wu et al. (2009b) investigated this initial adsorption using the intercept C to define an initial adsorption factor (R_i),

$$R_i = 1 - \left(\frac{C}{Q_{ref}} \right) \quad (6.12)$$

where C is the intercept of the Q_t vs. $t^{1/2}$ plot and Q_{ref} ($\mu g\ g^{-1}$) is the adsorbed amount at time t_{ref}; t_{ref} is the longest time in adsorption process (48 h in this case). According to these authors, R_i value can be divided into four zones, as presented in Table 6.2.

Table 6.2. Initial adsorption factor (R_i) and kinetic behaviour based on intraparticle diffusion model.

R_i	Initial adsorption behaviour
$R_i = 1$	No initial adsorption
$1 > R_i > 0.9$	Weakly initial adsorption
$0.9 > R_i > 0.5$	Intermediately initial adsorption
$0.5 > R_i > 0.1$	Strongly initial adsorption
$R_i < 0.1$	Approaching completely initial adsorption

R_i values between 0.57 and 0.74 were obtained suggesting that there is a considerably strong initial adsorption in a short time (Table 6.3). In the present work, the intercept (C value) increases with increasing copper soil content, indicating that the soil with high copper content has a large initial adsorption. These results suggest that some binding to copper ions which are distributed onto the outer surface or in very accessible sites of the soil particles may be involved in this initial adsorption and complexation may occur in some extension. This is in agreement with Figure 6.3B, related to desorption after batch adsorption of thiram to the COM soil, which shows that some complex is formed even after only 4 h of batch equilibration time.

Table 6.3. Initial adsorption factor (R_i) and kinetic behaviour based on intraparticle diffusion model for thiram adsorption onto both COM and COM_Cu$_{80}$ soils.

Soil	C_0 (mg L^{-1})	Ri	Initial adsorption behaviour
COM	6.99 ± 0.35 20.4 ± 1.0	0.74 0.57	Intermediately initial adsorption
COM_Cu$_{80}$	6.66 ± 0.25 20.0 ± 0.7	---- 0.63	------ Intermediately initial adsorption

All the results obtained in this work converge towards the same point, thiram adsorption process is widely dependent on the soil copper content and initial thiram concentration, which means that the ideal equilibration time that should be used in batch sorption studies of thiram onto soils can not be chosen based on a kinetic study with only one initial concentration or knowing copper concentration. Besides, the reactions with copper may involve thiram degradation into dimethyldithiocarbamate (DMDTC) which complex with copper (Kumarasamy and Raghu, 1976). Those reactions may be considered as degradation of thiram in soil and it is difficult to define the equilibration time for adsorption studies since degradation may begin before adsorption equilibrium is attained.

6.4.3 Isotherms at different contact times

In order to show how the adsorption equilibration time used in batch studies can influence the isotherm model that describes the experimental data of adsorption of thiram onto soil, we selected an equilibrium time of 4 and 48 h for batch adsorption of thiram onto original COM soil (Experment 3) (Figure 6.7). Simultaneously, a desorption cycle of 24 h with acetonitrile was performed after the adsorption period (Figure 6.7B).

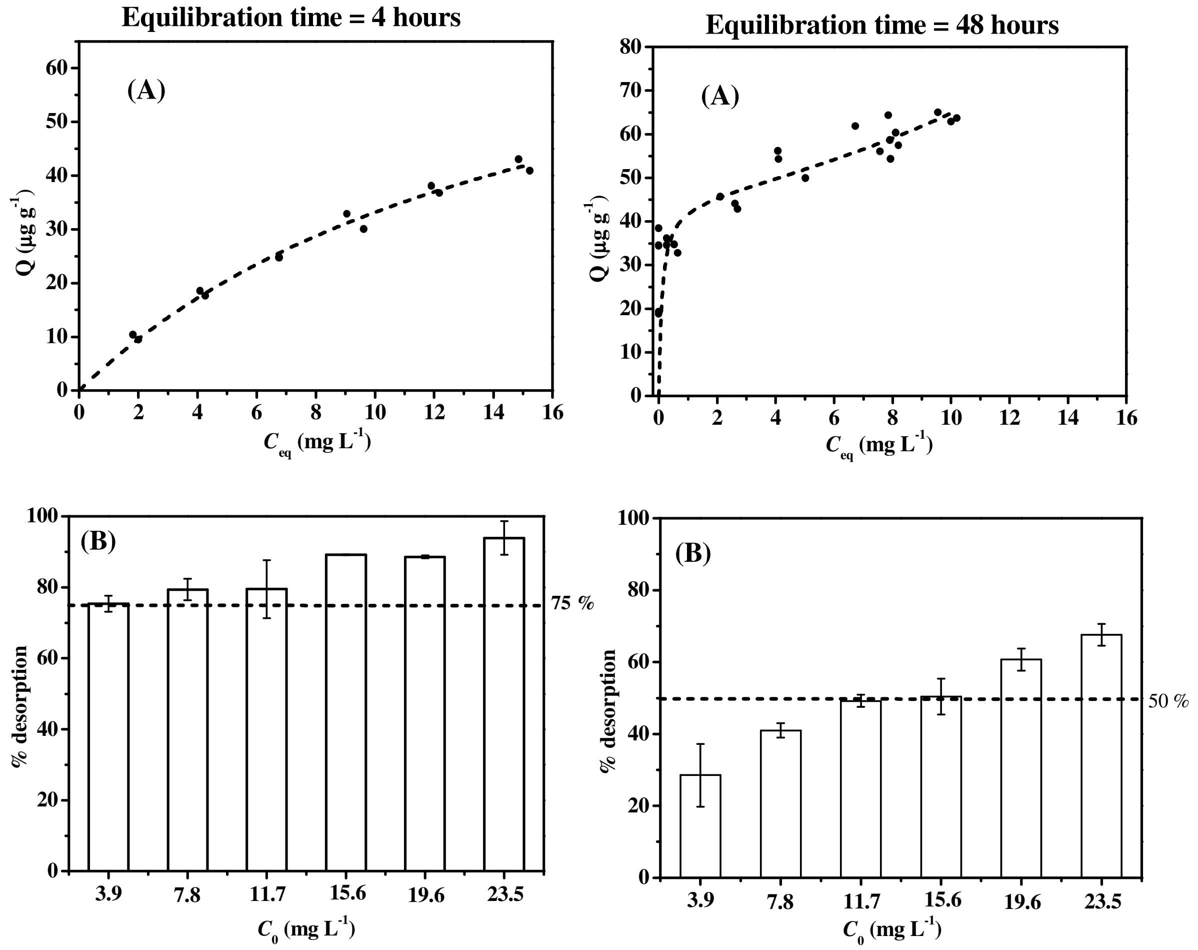

Figure 6.7 – **(A)** Adsorption isotherm of thiram onto $\mathrm{COM_{original}}$ soil. **(B)** Percentage of thiram desorbed with CH_3CN as a function of initial thiram concentration. Desorption values represent the % of the amount initially adsorbed.

With an adsorption equilibration time of 4 h, more than 75% of the free thiram is recovered for all initial concentration range, i.e. recoveries between 74 and 98 % were obtained. In 4 h of equilibration, thiram molecules are transported from the bulk solution to the external surface of sorbent where they are preferentially adsorbed, occurring the chemical reaction in low extent with the copper ions which are distributed onto the outer surface of the soil particles. So, considering all the initial concentration range, the percentage of thiram that complex with copper ions can be considered insignificant as compared with the amount of thiram that adsorbs onto soil. Thiram diffuses to the interior of the soil particles but does not have time to react with less accessible copper and is almost completly recovered during the desorption cycle. On the other hand, for an equilibration time of 48 hours less than 50% of the free thiram is recovered for initial concentrations up to 15.6 mgL^{-1}. For the two highest initial concentrations recoveries between 58 and 70 % were obtained. Considering all the initial concentration range, we can not ignore the percentage of thiram that complexes with copper ions. It is curious to notice that the sum of the chromatographic areas of thiram and complex gives always 100% of the initial area of total thiram, suggesting a conversion of free thiram into a complex of thiram or thiram degradation product. The adsorption isotherms obtained using these two different equilibration times are also represented in Figure 6.7. As we can see the isotherm model depends on the equilibration time during batch adsorption studies. For a contact time of 4 h, the isotherm is an L type (Langmuir) which assumes monolayer adsorption on a homogeneous surface with a finite number of adsorption sites. Once a site is filled, no further adsorption can take place at that site. Consequently, the surface will eventually reach saturation where the maximum adsorption ($Q_{\max}$) of the surface will be attained. The experimental data are well fitted by the Langmuir model (R^2_{aj} = 0.990) with a $Q_{\max}$ = 86.6 ± 7.7 μg g^{-1} and a K_L = 0.0621 ± 0.009 L mg^{-1}. In these 4 h there is only a physical surface adsorption (monolayer) while complexation occurs in low extent i.e. the reaction is not measurable at that time. For a contact time of 48 h the isotherm is H type (high affinity) which is indicative of strong adsorbent-adsorbate interactions such as inner-sphere complexes (Aboul-Kassim and Simoneit, 2001). The experimental data fits well the BET model (R^2_{aj} = 0.956), with a maximum adsorption of the 1st layer ($Q_{\max}$) of 44.4 ± 24.6 μg g^{-1}, a thiram saturation concentration in solution (C_S) of 31.3 ± 4.6 mg L^{-1} (very close of the theoretical value) and a constant K of 300 ± 123. Usually, BET isotherm describes a multilayer adsorption involving weak Van der Wall interactions, characteristic of a physical adsorption. However, in our case we can not explain that result based only on physical adsorption, since we have shown clearly the existence of a chemical reaction between thiram and copper ions which, depending on their initial concentrations, is or is not involved in the adsorption.

Considering a soil fortified with a great excess of copper, COM_Cu$_{500}$, in a batch adsorption study performed with an equilibration time of 15 h, we obtained a high-affinity adsorption isotherm (H type) (Figure 6.8). In this type of isotherm the molecules are completely adsorbed, or at least there is no measurable amount remaining in solution, being the initial part of the isotherm vertical (Giles et al, 1960). This behaviour is usually found for chemisorption.

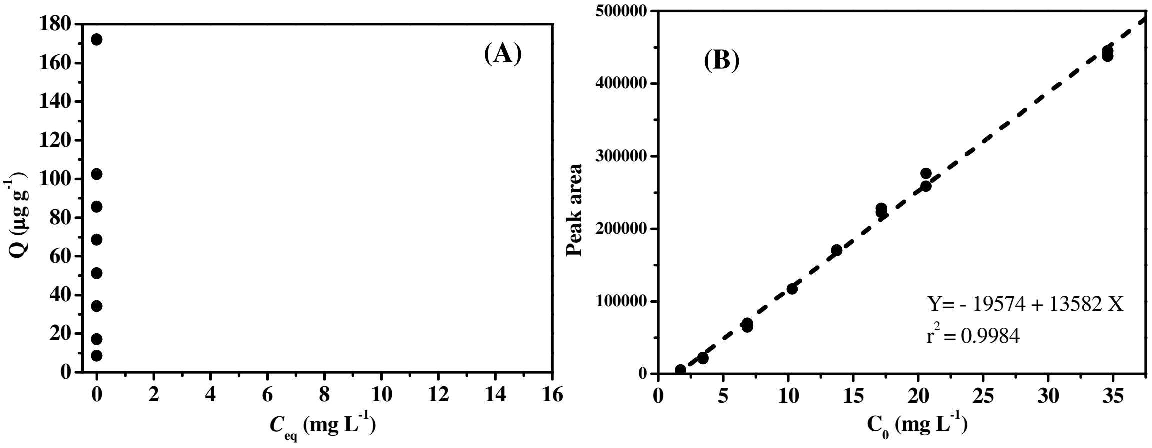

Figure 6.8 – **(A)** Adsorption isotherm of thiram onto COM soil fortified with Cu (500 mg kg^{-1}). Equilibration time of 15 h **(B)** Linear plot of the complex area *vs.* thiram concentration initially adsorbed

As we can see in Figure 6.8 the isotherm obtained for the adsorption of thiram onto COM_Cu$_{500}$ soil is a high affinity isotherm with a vertical line for the entire range of concentrations and not only for the lower concentrations as represented in Figure 6.7. A desorption cycle of 24 h with acetonitrile performed after adsorption period did not recover any thiram for all initial concentration range, instead we recovered the complex with copper ions, whose peak area is proportional to the initial thiram concentration (Figure 6.8B).This behaviour may be attributed to the excess of copper available.

6.5 Conclusions

This paper shows that the soil copper content, which may be increased by the use of copper based fungicides or by the application of certain organic amendments, has a marked effect on the sorption process of thiram onto soil. The experimental results showed that reactions between molecules of thiram and copper ions occur and the extent of their occurrence during adsorption studies may be strongly dependent on the soil copper content and on the initial thiram concentration in solution (Cu:Thi ratio). For a certain equilibration time, the percentage of thiram adsorption increases with the increase of Cu:thiram ratio. Kinetic studies performed with a soil amended with compost (COM soil) and the same soil after fortification with copper ions (COM_Cu_{80} soil) showed that there is an extremely significant influence of both the initial thiram concentration and the soil copper content on the kinetics of thiram sorption onto soil. Thus, the choice of the equilibration time for batch sorption studies and adsorption isotherms determination is a difficult task, namely because the reactions with copper may involve thiram degradation which may begin during batch adsorption studies not allowing to distinguish between adsorption and degradation.

So, the results obtained give rise the question how we must choose the equilibrium time when we performed sorption studies of thiram onto soils with different soil copper contents?

Our results have also shown that the complexes formed with copper in soil (Thiram:Cu or DMDTC:Cu) are persistent, at least until 18 days (the maximum ageing period studied), but they are not easily leached from soil to groundwater, since they are not extracted by $CaCl_2$ solution. Thus, copper may contribute to the immobilization of thiram in soil.

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Chapter 7

Influence of fulvic acids and copper ions on thiram determination in water[6]

The literature concerning the application of solid-phase extraction (SPE) to the concentration of thiram (bis(dimethyldithiocarbamoyl) disulfide) from natural waters is scarce, the available results being contradictory or with no analytical significance. To clarify these contradictory results, a C_{18}-SPE procedure combined with HPLC-UV was applied to thiram analysis in river water, and the influence of several factors on recoveries was studied. This procedure gave thiram recoveries of about 100% when applied to thiram standard solutions. However, when the same procedure was applied to river water samples spiked with thiram, the recoveries depended on the equilibration time after spiking. The influence of river fulvic acids (FAs) and Cu(II) on thiram recoveries from standard solutions was studied as a possible interference for such a result. In the presence of FA, thiram recoveries were always higher than 85%. In the presence of Cu(II), thiram recoveries decreased significantly, due to complexation, but the addition of an excess of EDTA before C_{18}-SPE eliminated that interference, and thiram was completely recovered. However, in river water samples the addition of EDTA had to be done before thiram spiking to obtain a recovery > 90%. Thiram standard solutions containing both river FA and Cu(II) showed a behaviour similar to the one observed in river water samples. On the basis of these results, the catalytic effect of Cu(II) on the degradation of thiram by FA, with formation of a Cu(II)-dimethyldithiocarbamate complex, was hypothesized.

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[6] Adapted from: **Filipe, O.M.S.,** Vidal, M.M., Duarte, A.C., Santos, E.B. 2008. Influence of fulvic acids and copper ions on thiram determination in water. J. Agric. Food Chem. 56, 7347–7354.

7.1 Introduction

Dithiocarbamates are a group of organosulfur compounds extensively used due to their wide range of applications in agriculture and industry (Malik and Faubel, 1999; Sharma et al., 2003, Szolar, 2007). Among them, thiram is one of the most used in Portugal as a fungicide (Information available at http://dgdr.pt; last accessed on 20th November 2012) and it is also one of the most largely applied worldwide (Szolar, 2007). According to the U.S. EPA (Environmental Protection Agency) "*thiram is expected to be sufficiently mobile and persistent in some cases to reach surface waters in concentrations high enough to impact aquatic life*" (Information available at http://www.epa.gov/oppsrrd1/reregistration/thiram/, document RED Fact Sheet; last accessed on 20th November 2012) To evaluate the risks of its intensive use, thiram determination in environmental matrixes is necessary, as well as the study of its reactions in the environment.

Frequently, dithiocarbamates are determined using methods based on their decomposition to carbon disulphide, which is then measured either by spectrophotometry or by chromatographic techniques coupled with different types of detection (EPA methods 603 and 603.1; Royer et al., 2001; Vryzas et al., 2002; Caldas et al, 2004). These methods are able to measure the total content of dithiocarbamates in samples but they fail to distinguish between the individual compounds. Thus, more selective analytical methods have been developed to allow discrimination and quantification of one or more dithiocarbamates (Gustafsson and Thomson, 1981; Weissmahr et al., 1998; Blasco et al., 2004; Nakazawa et al., 2004). Many analytical methods have been applied to thiram analysis in fruits and vegetables (Silva et al., 1999; Cassella et al, 2000; Fernandez et al., 1996; Ekroth et al., 1998; Malik et al., 1998; Sharma et al., 2003; Blasco et al., 2004; Sharma et al., 2004) but only a few studies have been published concerning thiram analysis in natural waters (Tovar and Santos-Delgado, 1995; Garcia et al, 1996; Tunçeli et al., 2001; Aulakh et al, 2005; Sasaki et al., 2006).

The low concentrations expected in natural waters require an enrichment step before the analysis and solid phase extraction (SPE), with C_{18} adsorbent, has been the most used procedure for extraction and enrichment of several non-polar pesticides from liquid samples (Liska, 2000; Pichon, 2000). However, its application to the enrichment of thiram from natural waters has been referred in very few published works and the results obtained were contradictory (Tovar and Santos-Delgado, 1995; Garcia et al, 1996). Garcia *et al* (1996) obtained a thiram recovery of 70% from 1 L of river water spiked with thiram (5 μg L^{-1}). On the other hand, Tovar and Santos-Delgado (1995), using also a C_{18}-SPE procedure, did not recover thiram from tap, natural and ground water samples spiked with thiram and other pesticides. The authors attributed this fact to the complexation of thiram by metals in natural waters, since they obtained recoveries of 50 to 90% when the same procedure was applied to a standard mixture of the same pesticides. More recently, Sasaki *et al.* (2006) used an on-line SPE method combined with LC/TOF-MS for analysis of 21 pesticides, including thiram, from river water and observed that thiram was the only pesticide with low recoveries (lower than 25 %). The authors assumed that these low values were due to the decomposition of thiram during the sample-preparation procedure.

The aim of this paper was to clarify the contradictory results of the literature concerning the recoveries of thiram during its analysis in natural waters, and contribute to gain a better insight on the reactions involving thiram in natural waters. For that, a C_{18}-SPE procedure combined with HPLC-UV was used for thiram analysis in river water, and the influence of aquatic fulvic acids and copper ions on thiram recoveries was studied. The metal ion chosen for this study was Cu(II), since it is usually applied in agriculture as inorganic fungicides, frequently in conjunction with, or during the same season as thiram is used. Besides, in Portugal, Cu(II) based fungicides are in third place among the most used fungicides while dithiocarbamates are in second (Information available at http://dgdr.pt; last access 15 July 2012).

7.2 Experimental

7.2.1 Chemicals

All chemicals were of analytical grade. Thiram (97%) was purchased from Aldrich, sodium dimethyldithiocarbamate solution (purum, ~40% in H_2O) was purchased from Fluka and Fulvic Acids (FA) were extracted from River Vouga water, collected at Carvoeiro (Aveiro, Portugal), by Santos and Duarte (1998). Methanol (HPLC grade) was obtained from Riedel-de Haen and acetonitrile (HPLC grade) was obtained from LabScan. Ultra-pure water was obtained using a Milli-Q water purification system (Millipore). Thiram standard solutions were prepared by dilution, with Milli-Q water, of a 100 mg L^{-1} stock solution of thiram prepared in acetonitrile. This stock solution was also used to spike water samples. Aqueous solutions of 0.05 mol L^{-1} EDTA and 1000 mg L^{-1} Cu(II) were prepared from disodium salt dihydrate (Merck, p.a.) and cupric perchlorate hexahydrate salt (Fluka, purum > 98%), respectively. Solutions of FA from River Vouga were prepared by dissolving, in Milli-Q water, 2 mg of FA isolated from the river water (final concentration was 2 mg L^{-1}, which is within the concentration range usually found in river waters (Thurman, 1985). The pH of these solutions was measured and it was about 6.

7.2.2 C_{18}-SPE-HPLC-UV procedure

Commercial Supelclean envi-18 cartridges (Supelco) of 500 mg, 75 Å pore diameter and 56 µm particle size were used and a 12-place manifold from Phenomenex was used to execute the SPE procedure. The SPE cartridge was pre-conditioned with 6 mL of methanol and 6 mL of deionised water. After loading 1 L of sample at 15-20 mL min^{-1}, by means of a vacuum pump, the cartridge was washed with 3 mL of ultra-pure water, and dried under N_2 during 30 min. Thiram was then eluted with 5 mL of acetonitrile and analysed by HPLC-UV at 270 nm as described previously in Chapter 3 (section 3.34). Thiram concentrations were calculated from the average of the peak areas of at least three injections.

7.2.3 Samples

Water samples from River Vouga (Aveiro, Portugal) were collected at Carvoeiro, near a water collection facility where the dissolved organic carbon (DOC) concentration is around 1 mg L^{-1} (Santos and Duarte, 1998). Samples were collected in June and October of 2006, and March and April of 2007, in 5 L glass bottles, previously washed with NaOH 1 M, distilled water and rinsed with river water immediately before sample collection. Samples were immediately filtered through a 0.45 µm filter (Gelman Sciences), stored at 4 ºC and analysed within a period of time as short as possible (less than 2 weeks).

Three types of aqueous solutions were also prepared: an aqueous solution containing only Cu (II) (0.01 mg L^{-1}), an aqueous solution containing only FA (2 mg L^{-1}) and aqueous solutions containing both FA (2 mg L^{-1}) and Cu (II) (0.01 mg L^{-1}). A copper concentration of 0.01 mg L^{-1} was chosen according to the mean values reported for copper concentration in River Vouga, at Carvoeiro (information available at htpp://snirh.pt; last acces at 15 July 2012).

All river water samples and synthetic aqueous solutions (as referred above) were spiked with the stock solution of thiram. Although this stock solution used to spike samples was prepared in acetonitrile, the final content of this solvent in the samples was always lower than 0.01%.

7.3 Results and Discussion

7.3.1 Thiram pre-concentration on C_{18}-SPE cartridge: effect of flow rate

The low levels of pesticides in waters require a high pre-concentration factor. Therefore a large volume of sample must be applied to the cartridge and pesticides must be recovered in a small volume of solvent. The SPE procedure described in section 7.2.2 was tested with 1 L of standard solution of thiram in milli-Q water and an elution volume of 5 ml of acetonitrile (pre-concentration factor of 200). This pre-concentration step would take about 3 hours using a flow rate of 5 mL min^{-1} as used by Garcia *et al.* (1996). In order to reduce the time of pre-concentration, we studied the effect of the flow rate on thiram recovery from standard solutions. Several experiments were done with the highest flow rate in order to be sure that thiram is completely recovered with those high flow rates which do decrease the time for pre-concentration. Some of the standard solutions were analysed immediately after preparation (Thi_0) while others were stored for 24 h in the dark (Thi_{24}), in order to evaluate the stability of thiram in solution and any losses by adsorption on the flask walls. Table 7.1 shows thiram recoveries obtained from those standard solutions pre-concentrated at different flow rates.

Table 7.1. Thiram recoveries (R) from 1 L of thiram standard solution after C_{18}-SPE procedure at different flow rates*

Samples	$[Thi]_{ad}$ ($\mu g\ L^{-1}$)	Flow rate (mL min^{-1})	R ± SD (%)
Thi_0	2.87	10	99.9
	11.0	20	99.6 ± 3.9 (n=2)
Thi_{24}	2.87	10	106.4
	11.2	20	102.3
	2.87	25	97.3 ± 2.9 (n=5)

*SD=standard deviation, n=number of experiments performed in each case.

As we can see, there are no losses of thiram either by changing flow rates up to 25 mL min^{-1} or by adsorption on the flask walls, with thiram recoveries not different from 100% for practical purposes, even for the lowest studied concentration, 2.9 μg L^{-1}. Thus, the results show that it is possible to obtain a pre-concentration factor of 200 using flow rates up to 25 mL min^{-1}, and concentrations as low as 2.9 μg L^{-1}. In this work, flow rates between 15 and 20 mL min^{-1} were used, representing a significant time improvement relatively to the procedure described in the literature by Garcia *et al.* (1996).

7.3.2 Application of SPE-HPLC-UV procedure to river water samples

The SPE-HPLC-UV procedure described previously was applied to the analysis of thiram in fortified river water samples. Firstly, aliquots of 1 L of filtered river water were submitted to C_{18}-SPE–HPLC-UV analytical procedure and the presence of thiram was not observed, as shown in Figure 7.1.

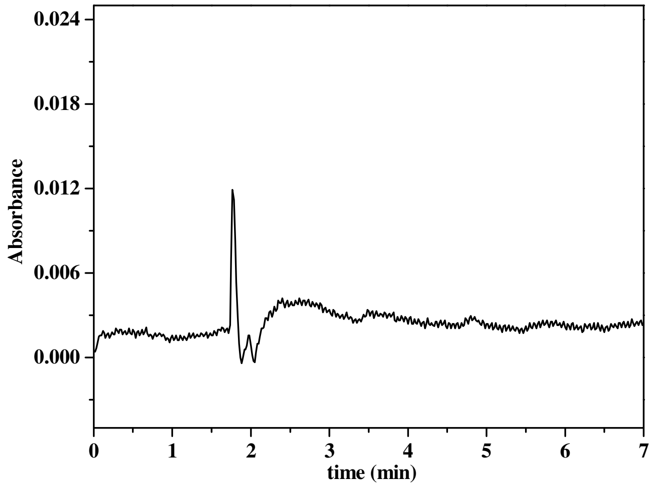

Figure 7.1 – HPLC-UV chromatogram of 1 L of river water sample after C_{18}-SPE procedure.

Then, river water samples were spiked with the thiram stock solution, obtaining concentrations of *ca.* 3 and 11 μg L^{-1}. Spiked river water samples were analysed both immediately after preparation ($River_0$) and after 24 h and 48 h periods of storage in the dark ($River_{24}$ and $River_{48}$). Table 7.2 shows thiram recoveries obtained from spiked river water samples, after different equilibration periods of time.

Table 7.2. Thiram recoveries ® from 1 L of river water sample spiked with thiram*

Sampling Date	**Monthly precipitation (mm)**	**Equilibration time (h)**	**[Thi] (µg L^{-1})**	**R ± SD (%)**
Jun 2006	88	0	2.82	77.6 ± 1.4 (n=3)
			11.3	79.6 ± 1.1 (n=2)
		24	2.82	< LOD (n=2)
			11.3	40.9 ± 2.4 (n=2)
Oct 2006	244	0	11.1	98.0 ± 1.3 (n=2)
		24	11.1	41.8 ± 0.8 (n=2)
Mar 2007	187	0	11.2	98.8
		24	11.2	61.3 ± 6.3 (n=6)
		48	11.2	25.0
Apr 2007	54	0	11.2	83.1
		24	11.2	20.1 ± 2.7 (n=3)
		48	11.2	< LOD (n=3)

*SD=standard deviation, n=number of experiments performed in each case.

Independently of the thiram initial concentration, water samples immediately analysed (for equilibration time equal to 0 h) showed thiram recoveries higher than 76 %. Such results are in agreement with those obtained by Garcia *et al.* for river water samples (R=70±8%, using C_{18}-cartridges, 1L of sample and 5 µg L^{-1} of thiram) (Garcia et al., 1996). On the other hand, thiram recoveries decreased significantly when aliquots of the same water samples were spiked with thiram and equilibrated for 24 or 48 h before analysis (Table 7.2). In fact, at low concentrations or longer equilibration times, thiram was not even detected. These results agree with those presented in the literature by Tovar and Delgado (1995) who did not recover thiram by C_{18}-SPE from fortified natural waters. These authors did not refer the equilibration time after sample spiking and attributed the recovery failure to complexation of thiram with metal ions in solution. This could be also suggested by the analysis of the chromatograms shown in Figure 7.2. After equilibration of thiram with the river water, the peak of thiram decreases, appearing a new peak at about 6 min. It was observed that the height of this new peak depends on the initial thiram concentration, equilibration time and dilution of river solutes by the river flow increase due to the rainfall (rainfall data can be found at http://snirh.pt).

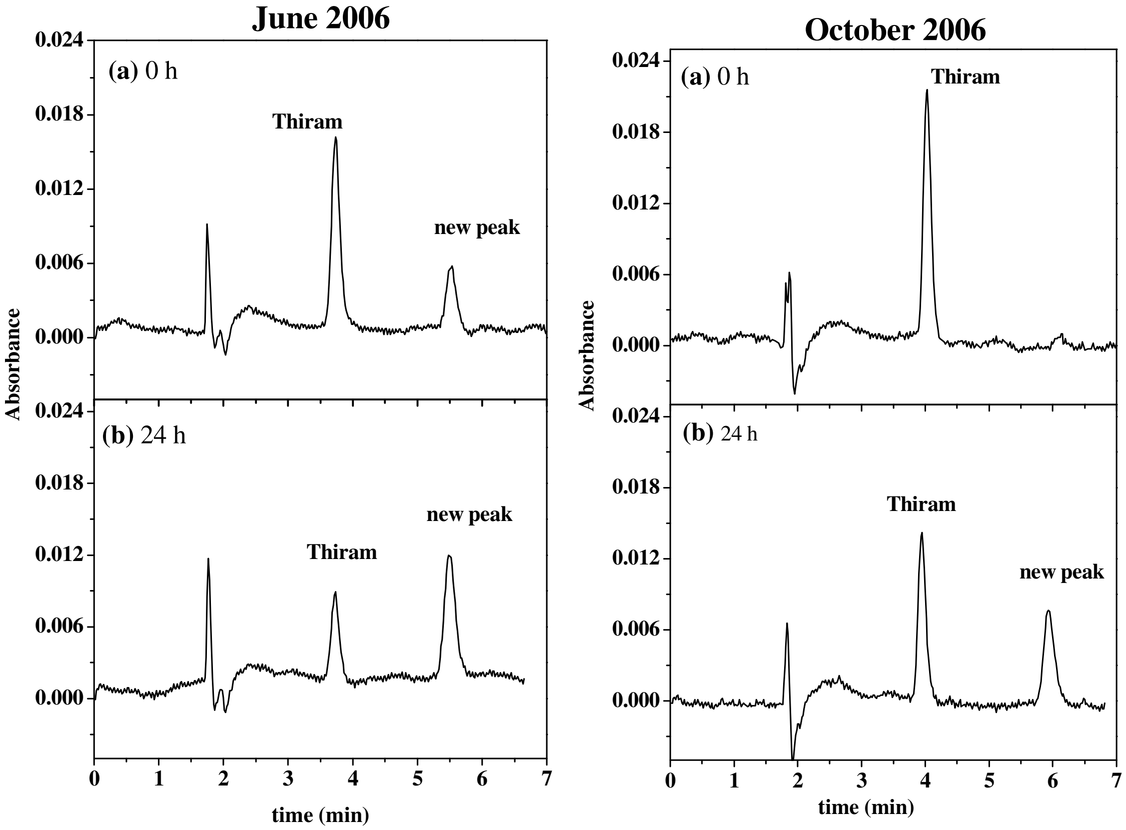

Figure 7.2 – HPLC-UV chromatograms of the natural water sample spiked with 11.2 μg L^{-1} thiram and equilibrated during 0 and 24 h, following SPE procedure.

For example, after 24 h of equilibration, for thiram concentrations of 2.8 μg L^{-1}, the peak of thiram disappears and only the new peak is visible (Figure 7.3) while for 11.2 μg L^{-1} thiram concentration, the peak of thiram decreases but its presence is still noteworthy (Figure 7.2). It should be highlighted that a small peak at 6 min is already present in the chromatogram of one of the samples showed in Figure 7.2 (June of 2006), even when it was analysed immediately after spiking. The same was observed for the sample collected in April of 2007 (chromatogram not shown). Those were the samples where the lowest recoveries for equilibration time zero were obtained (see Table 7.2). Such results are probably related to the fact that those samples were collected during a dry period of summer and spring seasons (see Table 7.2, monthly precipitation data). That may be associated to higher concentrations of river water solutes available to react with thiram.

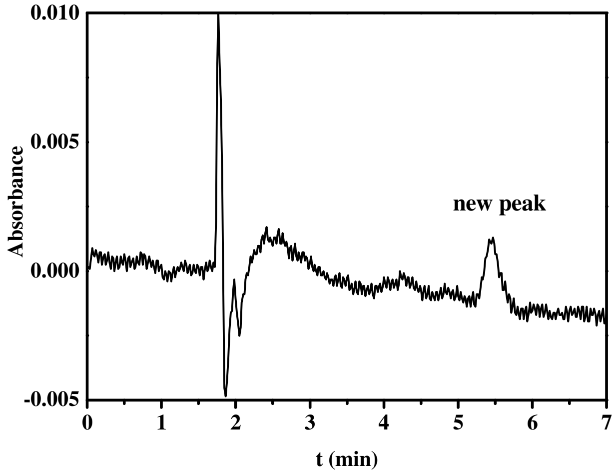

Figure 7.3 – HPLC-UV chromatogram of the natural water sample spiked with 2.8 μg L^{-1} thiram and equilibrated during 24 h, following SPE procedure.

Next, in this work, complexing properties of EDTA were explored as a means to overcome the interference of thiram complexation by metallic ions. Thus, an excess of EDTA (10^{-3} $molL^{-1}$) was added to the water samples in two different ways: **(i)** addition of EDTA to the river water sample and equilibration for 24 h before thiram spike, $[(River+EDTA)_{24}+Thi]_{24}$; **(ii)** addition of EDTA to the river water sample previously equilibrated during 24 h with thiram, $[(River+Thi)_{24}+EDTA]_{24}$. The samples were analysed by SPE-HPLC-UV. Thiram recovery results are shown in Table 7.3**,** while some examples of the obtained chromatograms are presented in Figure 7.4.

Table 7.3. Effect of EDTA (1 mM) in thiram recoveries (R) from 1 L of river water sample spiked with thiram and submitted to SPE*

Samples	Sampling Date	[Thi] (μg L^{-1})	R ± SD (%)
$[(River+EDTA)_{24}+Thi]_{24}$	Oct 2006	11.1	96.1 ± 5.0 (n=4)
	Mar 2007	11.2	95.6 ± 0.8 (n=2)
$[(River+Thi)_{24}+EDTA]_{24}$	Mar 2007	11.2	47.3 ± 4.4 (n=4)
	Apr 2007	11.2	<LOD (n=3)

*SD=standard deviation, n=number of experiments performed in each case.

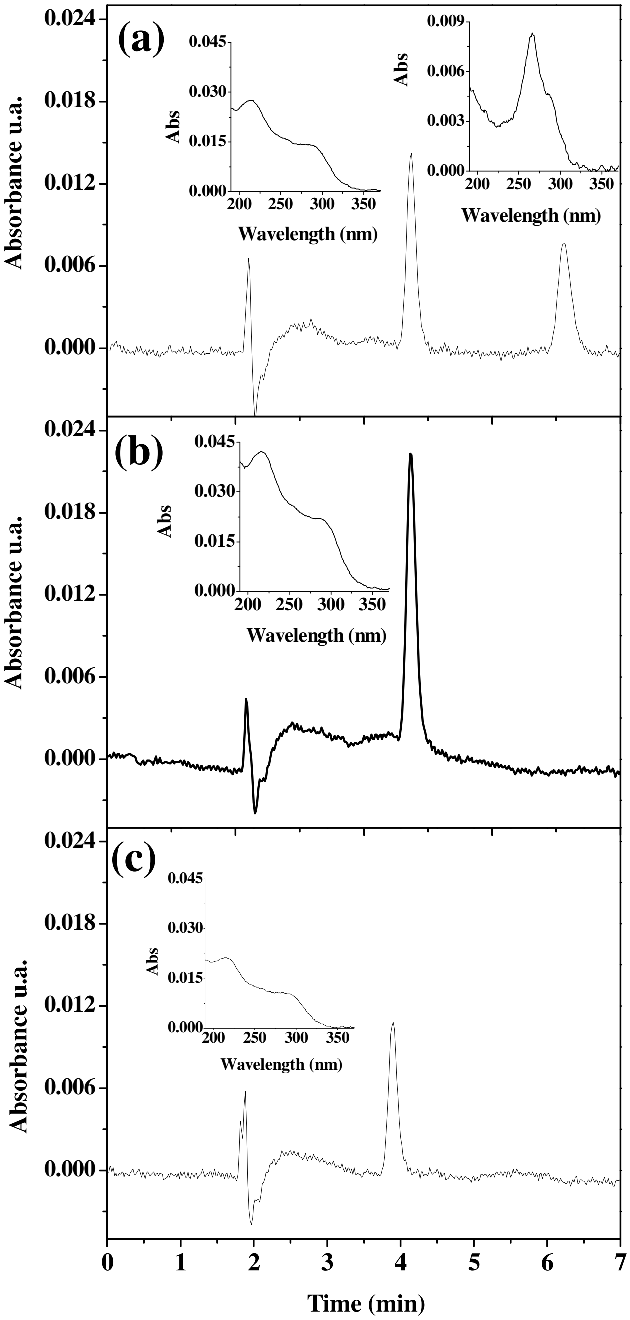

Figure 7.4 – Chromatograms of the river water samples collected in March of 2007: (a) $(\text{River+Thi})_{24}$, (b) $[(\text{River+EDTA})_{24}\text{+Thi}]_{24}$ and (c) $[(\text{River+thi})_{24}\text{+EDTA}]_{24}$ samples. [EDTA] = 10^{-3} mol L^{-1}, [Thi] = 11.2 μg L^{-1} (The samples were submitted to the SPE-HPLC-UV analytical procedure).

When EDTA was previously added to water samples, before thiram spike, $[(\text{River+EDTA})_{24}\text{+Thi}]_{24}$, thiram was completely recovered (>90%), while in the absence of EDTA, the recoveries were only *ca.* 42 and 61%, respectively (see Table 7.2, results for October of 2006 and March of 2007 after a 24 h equilibration period of time). This suggests that metal ions are somehow involved in reactions originating the low recoveries of thiram. However, when EDTA was added after a previous equilibration time between the river water and thiram, $[(\mathrm{River+Thi})_{24}+\mathrm{EDTA}]_{24}$, recovery levels were always lower than 51%. If the low recoveries were due to a simple complexation of thiram with metals ions, the addition of EDTA should overcome the interference even after previous equilibration of thiram with metals ions in river water. Thus, the results suggest that a different reaction involving metal ions is the cause of the low recoveries that was registered.

Figure 7.4 shows the chromatograms obtained for the sample collected in March of 2007, in the absence, $(\mathrm{River+Thi})_{24}$, and in the presence of EDTA, before $[(\mathrm{River+EDTA})_{24}+\mathrm{Thi}]_{24}$ and after a 24 h time period of equilibration between thiram and river water $[(\mathrm{River+Thi})_{24}+\mathrm{EDTA}]_{24}$. With the addition of EDTA the new peak at 6 min disappeared, suggesting that this peak corresponds to a complex which is destroyed by EDTA. However, when EDTA was added only after previous equilibration of thiram with the river water during 24 h, the new peak at 6 min disappeared, but the thiram peak did not recover the area expected for the concentration added to the sample. These results suggest that, besides its complexation with metallic ions, a partial degradation of thiram also occurs when it is equilibrated with the river water. To clarify the interferences for such low recoveries in river waters, the behaviour of thiram in aqueous solutions containing river FA, a metal ion or both was further studied. The metal ion focused on this study was Cu(II), since it is usually applied in agriculture as inorganic fungicides, frequently in conjunction with, or during the same season as thiram is used.

7.3.3 Effect of copper ions on thiram recoveries by C_{18}-SPE

Tovar and Santos Delgado (1995) suggested that thiram was not recovered from river water by C_{18}-SPE preconcentration due to its complexation with metal ions in the sample. Hence, we have decided to investigate the interference of metals to find a way to minimize it. Standard solutions containing 2.4 mg L^{-1} thiram and an excess of Cu(II) were analyzed by HPLC-UV without C_{18}-SPE preconcentration, while standard solutions containing 2.8 or 11.2 μg L-1 thiram and an excess of Cu(II) were analyzed by C_{18}-SPE-HPLC-UV. In both cases an excess of Cu (II) was guaranteed, according to data presented in Table 7.4 and Figure 7.5. The results in Table 7.4 show that thiram recoveries decrease significantly in the presence of Cu(II), depending on the Thi-Cu(II) ratio.

Table 7.4. Thiram recovery (R) from 1 L standard solutions in the presence and absence of Cu(II) after C_{18}-SPE procedure*.

Samples	**[Thi] (µg/L)**	**$[Cu^{2+}]_{ad}$ (mg/L)**	**Molar ratio Cu/Thi**	**R ± SD (%)**
$(Thi)_{24}$	2.82	-	-	97.4 ± 4.1 (n=2)
	11.2	-		96.2 ± 3.6 (n=5)
$(Thi + Cu^{2+})_{24}$	2.82	0.035	45	< LOD (n=3)
	11.2	0.15		17.5 ± 6.6 (n=6)
	11.1	0.01	3	55.5 ± 0.91 (n=3)

*SD is the standard deviation and n is the number of experiments performed in each case.

In the chromatograms of the solutions not submitted to SPE (Figure 7.5), the intensity of the thiram peak at ca. 4 min (peak 1) decreases as the Thi-Cu(II) equilibration time increases and a new peak, attributable to a Thi-Cu(II) complex, appears at about 6 min (peak 2) (Figure 7.5). After 8 h of equilibration, peak 1 disappears completely.

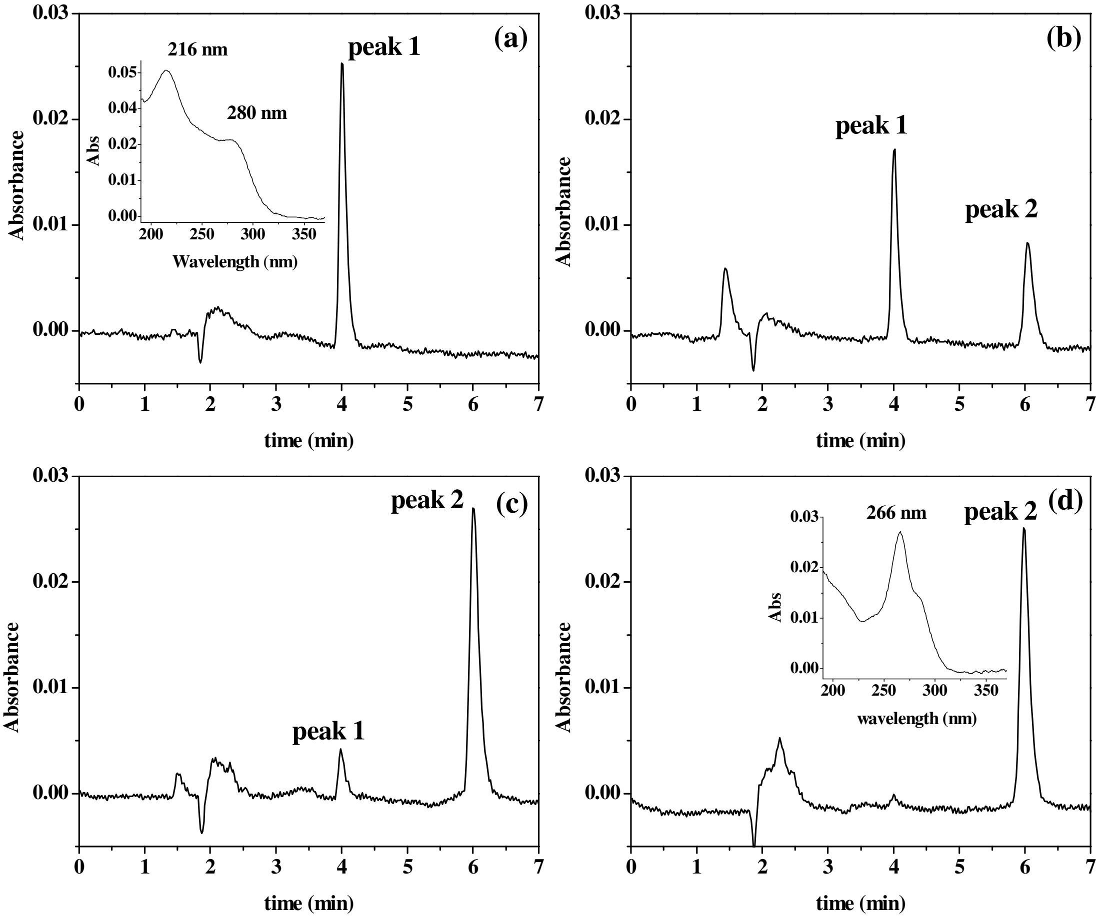

Figure 7.5 - HPLC-UV chromatograms of a standard solution of thiram 2.4 mg L^{-1} (a) in the absence of Cu(II) and (b-d) in the presence of Cu(II) (16 mg L^{-1}) at different times of equilibration: b) t = 20 min, c) t = 2 h and d) t = 8 h.

In the chromatograms of the solutions submitted to SPE (Figure 7.6), peak 1 decreases in intensity or even disappears after 24 h of equilibration with Cu(II) in the dark. The absence of peak 2 in these chromatograms (Figure 7.6) suggests that the Thi-Cu(II) complex is strongly retained in the C_{18} cartridge and is not eluted with acetonitrile. In fact, a yellow color, due to the complex, remains in the cartridge after elution. To elute the yellow-colored complex from the cartridge, $CHCl_3$ was tested as an eluent (5 mL). After $CHCl_3$ elution, the eluate was evaporated under a N_2 atmosphere until dryness, and the residue was redissolved in acetonitrile and analyzed by HPLC-UV (Figure 7.7). The chromatogram exhibited only one peak, with the retention time and the UV spectrum characteristic of peak 2 (cf. Figure 7.5). These results confirm that the preconcentration/extraction of thiram from aqueous solutions is strongly affected by the presence of metals, such us Cu(II), since Cu(II) forms a hydrophobic complex with thiram that is retained in the C18 cartridge and not eluted with acetonitrile.

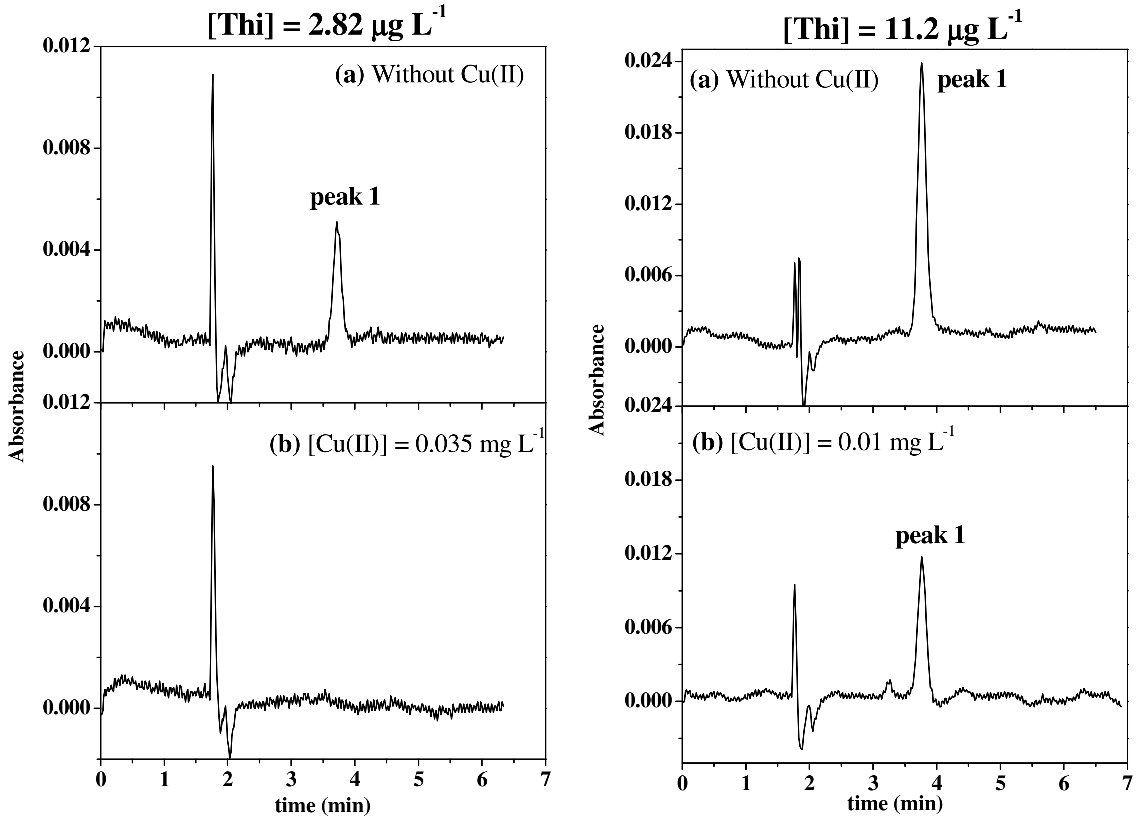

Figure 7.6 – HPLC-UV chromatograms of thiram standard solutions, in the absence and in the presence of Cu(II), after equilibration for 24 h and SPE treatment.

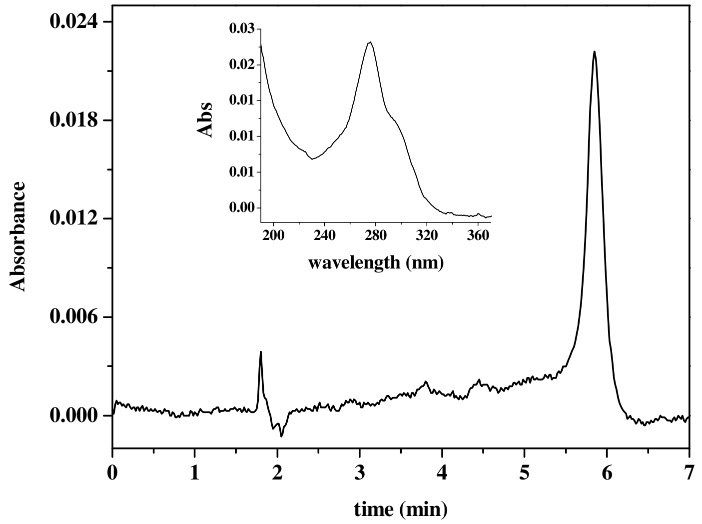

Figure 7.7 – HPLC-UV chromatogram of yellow coloured complex Thi-Cu(II) retained in the C_{18} cartridge after elution with $CHCl_3$, drying under N_2 atmosphere and re-dissolution of the residue in acetonitrile.

Still, the Thi-Cu(II) nature of peak 2 was also confirmed by using the complexing properties of EDTA. Solutions of thiram and Cu(II), 11.2 μg L^{-1} and 0.15 mg L^{-1}, respectively, previously equilibrated for 24 h, were treated with a solution of 10^{-5} mol L^{-1} EDTA and left for another 24 h to equilibrate ($[(Thi + Cu^{2+})_{24} + EDTA]_{24}$). The results show that EDTA eliminates the Cu(II) interference, allowing good recoveries of thiram (>83%). However, complexation with Cu(II) does not explain the behaviour observed in river water, since the addition of EDTA to river water equilibrated with thiram did not allow good recoveries.

7.3.4 Effect of river Fulvic Acid and Cu(II) on thiram recoveries by C_{18}-SPE

Aqueous solutions containing FA from River Vouga (2 mg L^{-1}) and Cu(II) (0.01 mg L^{-1}) in concentrations similar to those usually found in the rivers were prepared and stored in the dark during 24 h for equilibration before thiram spike, $[(FA+Cu^{2+})_{24}+Thi]_{24}$. Solutions containing only FA and spiked with thiram were also prepared and analysed by SPE-HPLC-UV for comparison. Figure 7.8 shows the HPLC chromatograms of the solutions spiked with thiram and the UV/Vis spectra of each chromatographic peak.

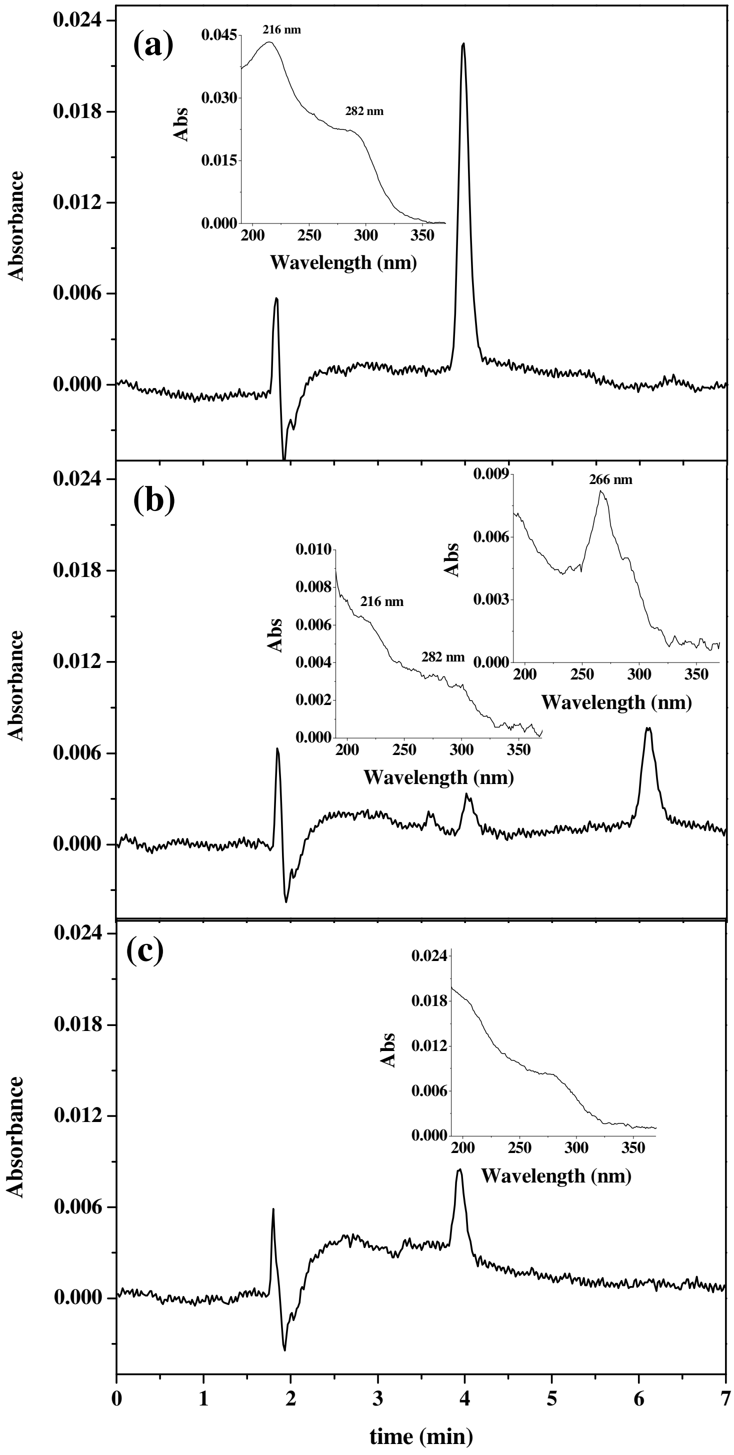

Figure 7.8 – Chromatograms obtained in the analysis of aqueous solutions: (a) $(FA+Thi)_{24}$, (b) $[(FA+Cu^{2+})_{24}+Thi]_{24}$ and (c) $[((FA+Cu^{2+})_{24}+Thi)+EDTA]_{24}$, and UV-Vis spectrum of the peaks. [FA] = 2 mg L^{-1}, [Cu] = 0.01 mg L^{-1}, [EDTA] = 10^{-5} mol L^{-1}, [Thi] = 11.2 μg L^{-1}.

As shown in Figure 7.8b, in the solutions containing both FA and Cu(II), there is a significant decrease of the thiram peak height (relatively to the sample without Cu(II),

Figure 7.8a) and the peak at about 6 min appears, as observed in spiked river water samples after 24 h of equilibration (Figure 7.6b). This additional peak at 6 min appears at the same retention time of peak 2 observed in the chromatograms of Thi-Cu(II) solutions without SPE treatment (Figure 7.5) and it could perfectly result from formation of a thiram/Cu complex. However, these samples were submitted to the SPE procedure and peak 2 disappeared from the chromatograms of Thi-Cu(II) solutions when they were submitted to SPE (Figure 7.6), since the complex was irreversibly retained by the C_{18} cartridge. When EDTA was added to the standard solutions containing both FA and Cu(II) (Figure 7.8c) the peak at *ca.* 6 min disappeared but the thiram peak height is lower than expected for the total concentration of thiram added to the solution. So, the addition of EDTA destroys that complex but since thiram is not totally recovered, it is clearly an indication of its irreversible degradation.

As shown in Table 7.5, the recovery of thiram in the presence of FA and Cu(II) is lower than the LOD, while recoveries in the presence of only FA, at the same concentrations, were approximately 90%. When EDTA was added to the aqueous solutions of FA and Cu(II), equilibrated with thiram, $[((FA+Cu)_{24}+Thi)_{24}+EDTA]_{24}$, only a small fraction of thiram was recovered, suggesting that it had been degraded. Once more, when EDTA is added before equilibration with thiram, $[((FA+Cu)_{24}+EDTA)_{24}+Thi]_{24}$, the result showed that EDTA eliminates the Cu (II) interference, allowing good recoveries of thiram. Such behaviour is similar to the one observed in the river water samples (Figure 7.4c) clearly indicating that the degradation of thiram only occurs when both FA and Cu(II) are present together in the same solution.

Table 7.5. Thiram recoveries (R) from standard solutions ([FA] = 2 mg L^{-1}, [Cu^{2+}] =0.01 mg L^{-1}, EDTA= 10^{-3} mol L^{-1})*

Samples	$[Thi]_{ad}$ (µg L^{-1})	R ± SD(%)
$(FA+Thi)_0$	11.3	92.8 ± 1.9 (n=2)
$(FA+Thi)_{24}$	11.2	89.8 ± 4.3 (n=5)
$[(FA+Cu)_{24}+Thi]_{24}$	11.2	<LOD (n=4)
$[((FA+Cu)_{24}+Thi)_{24}+EDTA]_{24}$	11.2	24.6 ± 2.6 (n=4)
$[((FA+Cu)_{24}+EDTA)_{24}+Thi]_{24}$	11.2	88.0 ± 3.5 (n=4)

*SD=standard deviation; n=number of experiments performed in each case.

These results support the hypothesis of thiram reduction by FA catalysed by Cu(II), in river waters. According to the literature (Victoriano, 2000; Victoriano, 2000a), thiram can be reduced by some reagents and scission of the S-S bond occurs, leading to the formation of dimethyldithiocarbamate anions (DMDTC, $(CH_3)_2NCSS^-$) which have chelating properties. It is also known that FA may act as electron donors (Xie ey al., 2005; Bauer et al., 2007). Therefore, when thiram is equilibrated with solutions of FA and Cu(II), the reduction of thiram by FA, catalysed by Cu(II) may occur, leading to the formation of DMDTC anions whose complex with Cu(II) gives rise to the additional peak at 6 min in the chromatograms. Solutions of DMDTC anions (11.2 μg L^{-1}) and Cu(II) (0.01 mg L^{-1}) were prepared and submitted to the SPE procedure. The HPLC-UV chromatograms of the solutions eluted from de cartridge (with acetonitrile) exhibited an intensive peak at 6 min, which was absent in the chromatograms of the solutions thiram/Cu after SPE treatment (Figure 7.6). This also supports the hypothesis of thiram degradation into DMDTC anions. According to Weissmahr and Sedlak (2000) the complexation of DMDTC anions with Cu(II) stabilizes them in solution increasing their persistence. Thus, these results highlight the need for monitoring not only thiram but also its degradation products when the risks of thiram contamination are evaluated. At this very moment studies are being made with the aim to better clarify the nature of this degradation and to identify the product.

7.4 Conclusions

In this work, we evaluated the effects of natural organic matter and metal ions on the determination of thiram in natural waters by a C_{18}-SPE-HPLC-UV method. River water samples were spiked with thiram, and recoveries higher than 76% were obtained, when samples were analyzed immediately after spiking. However, a significant decrease of the thiram recoveries was observed when river water samples were equilibrated with thiram, for 24 h or more, before the analysis. This suggests an interference of the natural components of river waters, such as organic matter and metals ions. In the present paper it has been shown that copper ions decrease considerably the recovery of thiram by SPE, but the addition of EDTA eliminates its interference and thiram is completely recovered. However, when EDTA was added to the river water previously equilibrated with thiram, the recoveries continued to be low, suggesting a partial degradation of thiram. Studies in standard aqueous solutions, containing both FA and copper ions at concentrations similar to those found in the river, showed a behaviour similar to the one observed in the river water suggesting that in the presence of both FA and Cu(II) thiram is reduced, leading probably to the formation of DMDTC anions, which then complex with copper ions in solution.

7.5 References

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Chapter 8

Effect of copper ions on the degradation and persistence of thiram in aqueous solutions[7]

The aim of this work was to examine the effect of copper ions on the degradation of thiram in aqueous solutions, since the literature focused on this effect is scarce or inexistent and copper based fungicides can be applied together or during the same season to agriculture crops, increasing the fungicidal activity. The effect of copper ions on the thiram degradation was followed by UV-Vis spectrophotometry and by HPLC-MS/MS. Thiram degradation in aqueous solutions at pH ~ 6 was monitored by UV-Vis during one month and, after a stability period of ~ 7 days, the decay follows a 1st order kinetics, corresponding to a total half life of 19-20 days. The presence of copper ions in solution has a strong influence on the thiram persistence along time. For thiram solutions 2 mg L^{-1} containing an excess of thiram, a complex is slowly formed and then it precipitates and leaves some free thiram in solution which degrades along time. In the presence of an excess of copper ions, a Thi:Cu complex 1:1 is formed as confirmed by HPLC-MS/MS analysis. This complex degrades along time at a rate which depends on the Thi:Cu ratio and on the initial concentrations. In the presence of a large excess of copper ions, the UV-Vis spectra analysis suggested that a copper complex 1:1 is formed with the anion dimethyl dithiocabamate (DMDTC), i.e., $[CuDMDTC]^+$, since the spectra of Thi-Cu solutions (1:50) after 1 day are identical to the spectra of DMDTC-Cu solutions (1:25). The spectra do not suffer any changes for a period as long as 56 days. The results suggest that, depending on Thi:Cu ratio, the persistence of thiram in solution is affected by copper ions and that copper ions stabilise the principal product of thiram degradation, the anion DMDTC. However, the presence of $[CuDMDTC]^+$ complex was not confirmed by HPLC-MS/MS analysis. In addition, MS/MS data confirmed the existence of other copper complexes in both DMDTC:Cu and Thi:Cu solutions with an excess of copper ions.

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[7] Adapted from: Filipe, O.M.S., Santos, S.A.O., Domingues, M.R.M., Vidal, M.M., Silvestre, A., Neto, C.P., Santos, E.B.H. 2012. Effect of copper ions on the degradation and persistence of thiram in aqueous solutions. *In preparation*.

8.1 Introduction

There are few studies on the degradation of thiram in waters. Very recently, Gupta et al. (2012) conducted a study to evaluate the persistence of thiram in water and soil under controlled conditions and on two plants, namely, tomato and radish, in field conditions. According to the authors, the decay of thiram (100 mg L^{-1}) depends upon the nature of medium and the environmental conditions. So, studies conducted in water at different temperature, pH and organic content revealed that thiram persistence decreases with the increase in all the three variables, and among the three variables, it is the organic matter which has a more pronounced effect. In both studies there is no reference to the possible effect of metal ions, namely copper ions.

In our previous work (Filipe et al., 2008), data about thiram recovery from natural waters showed rapid thiram degradation in environmental matrices. Thiram was well recovered from samples when analysed immediately after spike but scarcely recovered when analysed one or two days after. However, if EDTA was added previously to the thiram spike, thiram was always completely recovered, but that did not occur if EDTA was added after the thiram spike. These results suggested that metal ions, namely copper ions, were involved in thiram degradation.

Cu(II) based fungicides have been widely used for more than a century and many thousands of tons are consumed annually all over the world to prevent plant diseases (Borkow and Gabbay, 2005); in Portugal they are in third place among the most used fungicides as mentioned above. Because of its worldwide use, some considerations about copper effects on behaviour of some pesticides in environmental matrices have been object of attention (Dousser et al., 2007; Moure et al., 2007; Ting-feng et al., 2007; Liu et al., 2009).

Thus, the aim of this work was to examine the effect of copper ions on the degradation and persistence of thiram in aqueous solutions, since the literature focused on this effect is scarce or inexistent and Cu(II) based fungicides can be applied together or during the same season to agriculture crops, increasing the effectiveness of fungicidal activity. Moreover, the ability of dithiocarbamates to form very stable complexes with some transition metals is well documented (Thorn, 1962). The effect of copper ions was studied during a month following up the spectral changes in the range of 200 nm to 700 nm of different thiram-Cu(II) mixtures in which thiram concentration was kept constant and the copper content varied. The identification of different complexes formed along time was also studied by HPLC-MS/MS.

8.2 Experimental

8.2.1 Chemicals and solutions

All chemicals used were of analytical grade and ultra-pure water was obtained using a Milli-Q water purification system (Millipore). A 20 mg L^{-1} aqueous thiram stock solution (Thi) was prepared by previous dissolution of thiram (97%, Aldrich) in acetonitrile (HPLC grade, LabScan) followed by dilution with water (percentage of acetonitrile in the final solution always lower than 1%). Aqueous solution of 1000 mg L^{-1} Cu(II) was prepared from solid cupric perchlorate hexahydrate (> 98%, Fluka). Thiram standard solutions, 2.0 mg L^{-1} were prepared with increasing copper contents by dilution of both 20 mg L^{-1} thiram and 1000 mg L^{-1} Cu(II) standard solutions, obtaining the following Thi:Cu(II) ratios: 1:0, 1:0.3, 1:1, 1:3, 1:10, 1:25 and 1:50. A 0.5 g L^{-1} sodium dimethyldithiocarbamate stock solution (DMDTC) was prepared by dilution with water of commercial DMDTC solution (~ 40% in H_2O, Fluka). DMDTC standard solutions 2.0 mg L^{-1} with increasing copper contents were prepared by dilution of both 0.5 g L^{-1} DMDTC and 1000 mg L^{-1} Cu(II) standard solutions, obtaining the following DMDTC:Cu(II) ratios: 1:0, 1:0.3, 1:1, 1:3, 1:10 and 1:25. Thiram aqueous solutions 11 μg L^{-1} were also prepared in the presence and absence of Cu (II). Aqueous solution of EDTA 0.05 mol L^{-1} was prepared from the ethylenediaminetetraacetic acid disodium salt dihydrate (Merck).

8.2.2 Apparatus

UV-Vis spectra of 2.0 mg L^{-1} thiram standard solutions and 2.0 mg L^{-1} DMDTC standard solutions and respective mixtures with ion copper were recorded in a UV-Vis Shimadzu Spectrophotometer using a 1.00 cm cell. pH values of the solutions were measured using the pH-meter. Thiram was determined by HPLC-UV as described in Chapter 3 (section 3.3.3).

8.2.3 C_{18}-SPE-HPLC-UV procedure

The pre-concentration procedure used for 1L of thiram aqueous solutions 11 µg L^{-1} is described previously in Chapter 7, section 7.2.2.

8.2.4 Identification of degradation products by HPLC-MS/MS

The HPLC system consisted of a variable loop Accela autosampler (set at a temperature of 16 °C), an Accela 600 LC pump and an Accela 80 Hz PDA detector (Thermo Fisher Scientific, San Jose, Ca, USA). Analyses were carried out by using a Discovery® C18 (150×2.1 mm, 5 µm) column supplied by Supelco (Agilent Technologies, Waldbronn, Germany). The separation of the compounds was carried out with a mobile phase of acetonitrile:water (70:30, v/v) with 0.1% HCOOH at a flow rate of 0.2 ml min^{-1}, at 25ºC. The injection volume in the HPLC system was 15 µl. Single online detection was carried out in PDA detector, at 270 nm, and UV spectra in a range of 200-600 nm were also recorded.

The HPLC was coupled to a LCQ Fleet ion trap mass spectrometer (ThermoFinnigan, San Jose, CA, USA), equipped with an ESI source and operating in positive mode. The flow rate of nitrogen sheath and auxiliary gas were 40 and 5 (arbitrary units), respectively. The spray voltage was 5 kV and capillary temperature 300°C. The capillary and tune lens voltages were set at -28 V and -115 V, respectively. CID[8]-MS^n experiments were performed on mass-selected precursor ions in the range of *m/z* 100–1000. The isolation width of precursor ions was 1.0 mass unit. The scan time was equal to 100 ms and the collision energy was optimized between 15-40 (arbitrary units), using helium as collision gas. The data acquisition was carried out by using Xcalibur® data system (ThermoFinnigan, San Jose, CA, USA).

8.3 Results and Discussion

8.3.1 Evaluation of thiram stability in aqueous solution

To evaluate thiram stability in an aqueous solution, UV-Vis spectra and pH values of a 2.0 mg L^{-1} thiram aqueous solution were monitored during a month (Figures 8.1A and 8.1B, respectively).

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[8] Collision-induced dissociation (CID): fragmentation method for MS/MS

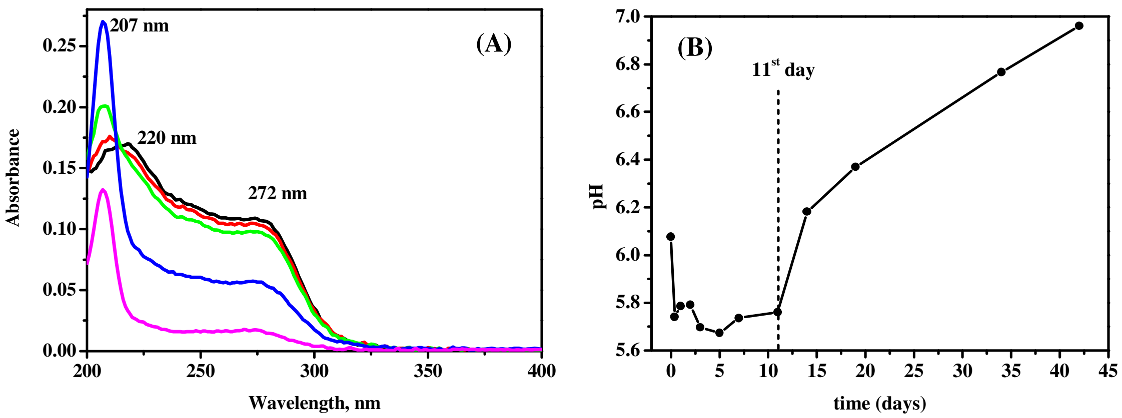
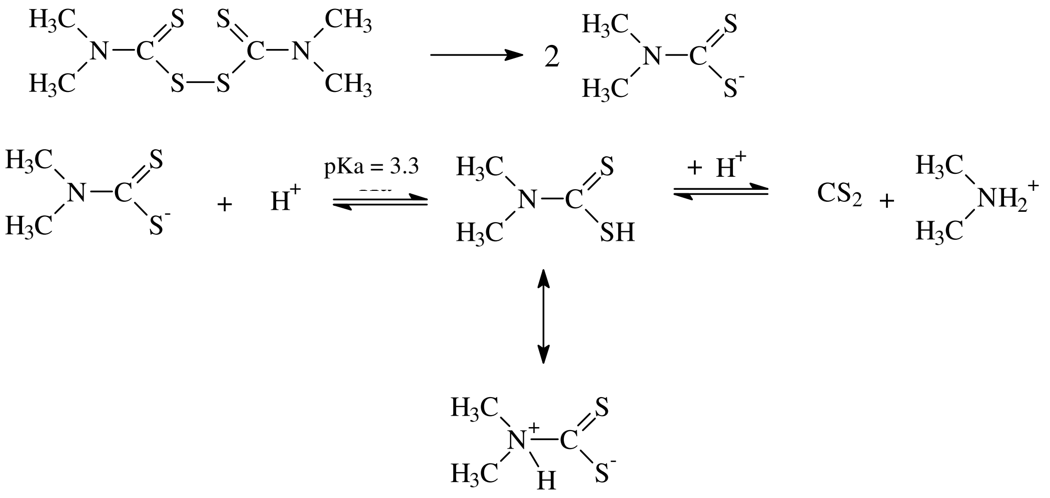


Figure 8.1 - (A) UV-Vis spectra of a 2.0 mg L^{-1} thiram aqueous solution registered during *ca.* a month: (▬) time 0 to 5 days, (▬) 7, (▬) 11, (▬) 19 and (▬) 34 days; (B) pH values of a 2.0 mg L^{-1} thiram aqueous solution along solution aging time.

As shown in Figure 8.1, the spectrum of a thiram fresh solution exhibits 2 peaks, one at 220 nm and other at 272 nm, showing no significant changes up to the 7th storage day (Figure 8.1A, curve a). For periods longer than one week, the absorbance at 272 nm begins to decrease and a new peak appears at 207 nm (Figure 8.1A, curve —). From the 11th day, there is a sharp rise of pH, suggesting a reaction with the H^+ consumption, in accordance to the reactions presented in Scheme 8.1 (Thorn and Ludwig, 1962).

Scheme 8.1



The observed changes suggest that, at room temperature and in the absence of light, thiram solutions prepared in milli-Q water, keep stable during the first 7 days of storage. From that day on, thiram degrades following a 1st order kinetics (R^2 = 0.9591 for the non-linear regression analysis). Using the data between 7 and 42 days a kinetic rate constant of 0.057 ± 0.006 d^{-1} and a half life of 12 days were obtained. Considering the initial period of thiram stability, a global half life time of 19 days was obtained for thiram in milli-Q water, meaning an improved stability with respect to thiram solutions prepared in natural waters (Filipe et al., 2008). According to this previous work, thiram recovery from spiked river samples decreases with the contact time between thiram and water. Significant decreases are observed when thiram is analysed one day after thiram spiking, though, nearly total recoveries were observed if EDTA was added previously to thiram spike, clearly indicating the metallic ion interference.

8.3.2 Changes in the composition of thiram:copper solutions along time

Figure 8.2 shows the modifications observed on the UV-Vis spectrum of a 2.0 mg L^{-1} thiram solution due to the copper ion presence in solutions with 1:0.3 and 1:1 Thi:Cu(II) ratios. The UV-Vis spectra, obtained on the 1st day of thiram and copper mixing, show two new bands, one at 420 nm, giving rise a yellowish colour of the solution, and other at 260 nm, with the simultaneous disappearance of the characteristic thiram band at 272 nm. The increase of the band at 420 nm with the increase of copper content in the mixture suggests that a reaction between thiram and copper ion (Figure 8.2, 1st day) occurs, while the enhancement of this band indicates the progress of the reaction up to the 7th day (Figure 8.2, 7th day). On the 14th day after the mixture preparation, an orange precipitate is formed that, settling down, gives rise to the elimination of the bands at 420 nm and 260 nm in the UV-Vis spectra of the supernatant solutions which exhibit the same spectral features of pure thiram solutions after the same ageing time (Figure 8.2, 14th day). At the end of one month, the precipitates were removed by filtration and redissolved in acetonitrile and both bands at 260 nm and 425 nm were observed. The solutions were also analysed by HPLC, being the chromatograms obtained characterized by a peak with the retention time characteristic of the Thi-Cu(II) complex and the absence of the thiram peak (see Chapter 7, section 7.3.3)

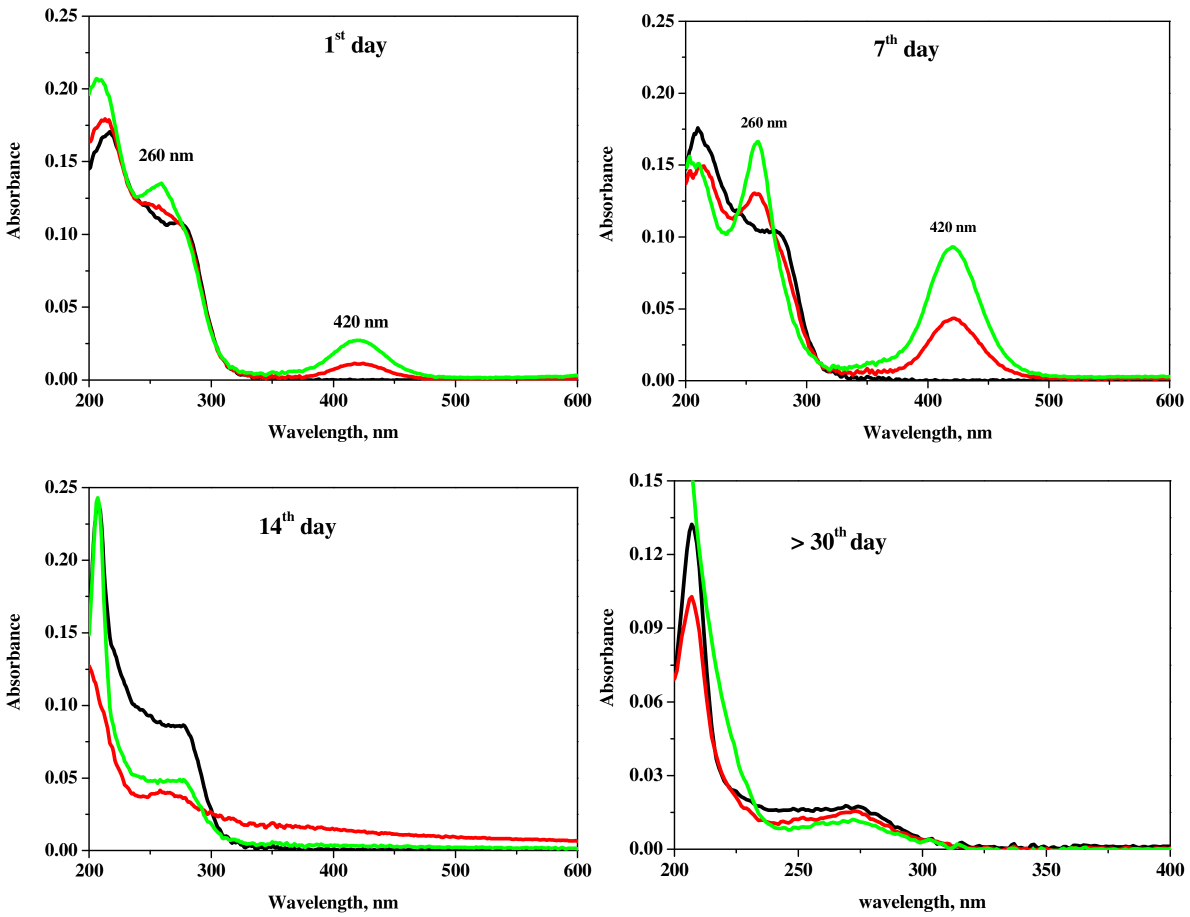


Figure 8.2 – UV-Vis spectra of a 2.0 mg L^{-1} thiram solution in the presence of copper ion registered at 1, 7, 14 and 34 days after thi:Cu(II) mixing, (⬛) thiram, (🟥) thi:Cu 1:0.3 and (🟩) thi:Cu 1:1 ratio (UV-vis spectra of 3th and 4th graphs were obtained after filtration).

In order to study the effect of higher contents of copper, thiram-Cu(II) mixtures of 1:3, 1:10, 1:25 and 1:50 Thi:Cu(II) ratios were prepared (Figure 8.3). The UV-Vis spectra, registered during the 1st day of mixing show the increase of the absorption band at 420 nm with increasing copper content. That band seems to reach a maximum intensity for the 1:25 ratio, exhibiting a shoulder for shorter wavelength, while for the 1:50 ratio, there is the replacement of the band at 420 nm by a new band at 385 nm. For longer periods of time, the replacement of the band at 420 nm is also observed for the other Thi:Cu ratios, so that, at the end of the 14th day, only the bands at 385 and 260 nm are present in the respective UV-Vis spectra (Figure 8.3, 14th day).

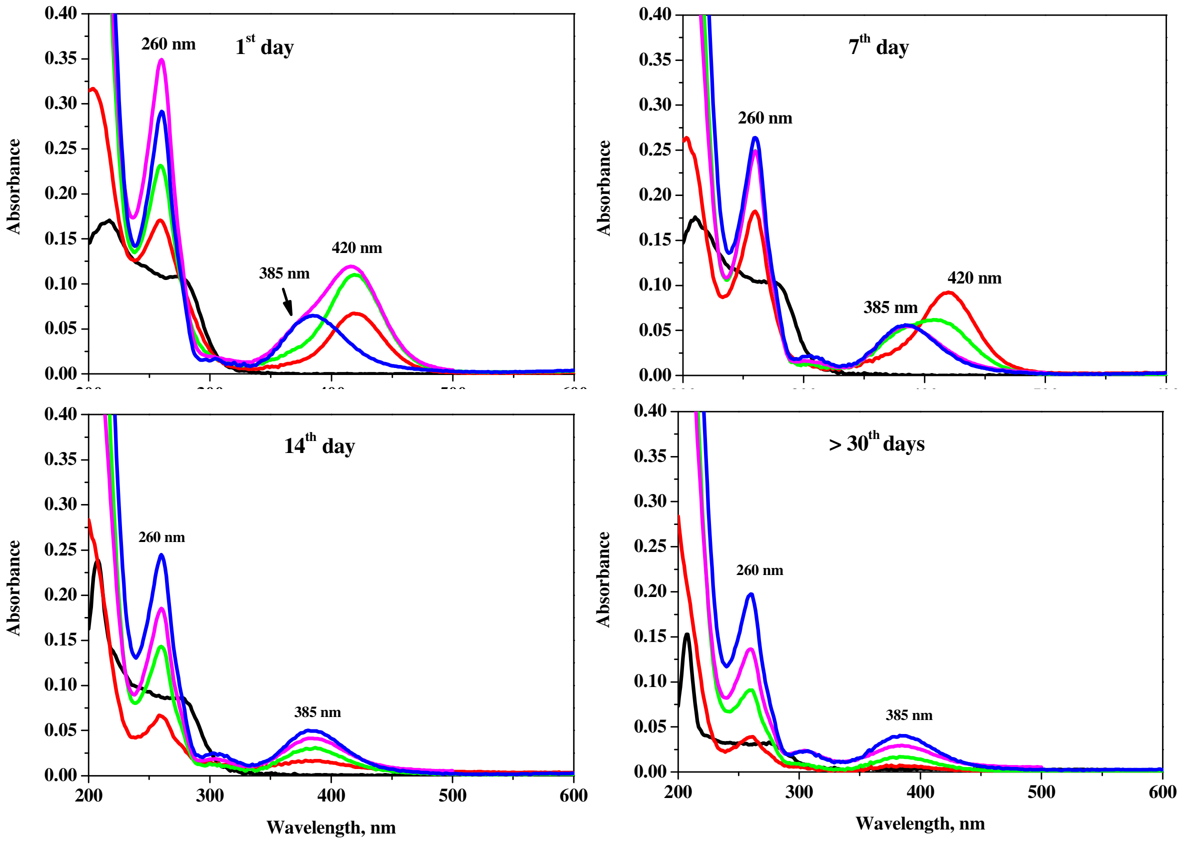


Figure 8.3 – UV-Vis spectra of a 2.0 mg L^{-1} thiram solution in the presence of copper ion registered at 1, 7, 14 and 34 days after thi:Cu(II) mixing; (▬) thiram, (▬) thi:Cu 1:3, (▬) thi:Cu 1:10, (▬) thi:Cu 1:25 and (▬) thi:Cu 1:50 ratio.

Therefore, in the presence of an excess of thiram (Thi:Cu ratios 1:0.3) or in an equimolar ratio (Thi:Cu ratio 1:1) a complex between thiram and copper ions, responsible for the band at 420 nm, is formed, precipitating at the end of some time. The fact that, for higher copper contents no precipitate was observed, suggests that the band at 385 nm corresponds to another complex, probably with some product of thiram degradation. Some experiences were then performed with dimethyldithiocarbamate anion (DMDTC) which, according to the literature (Roberts, T.R., Hutson D.H. 1999), is considered as the principal product of thiram degradation.

Figure 8.4 shows the spectra of the 2.0 mg L^{-1} DMDTC solution registered during one month. The spectrum of the solution after preparation exhibited 2 bands: one at 254 and the other at 280 nm, showing both a significant decrease at the end of the 1st day, coincident with the appearance of a band at *ca.* 207 nm, previously observed for thiram degradation (Figure 8.1A). Complete degradation is observed in 2 days (Figure 8.4, curve d).

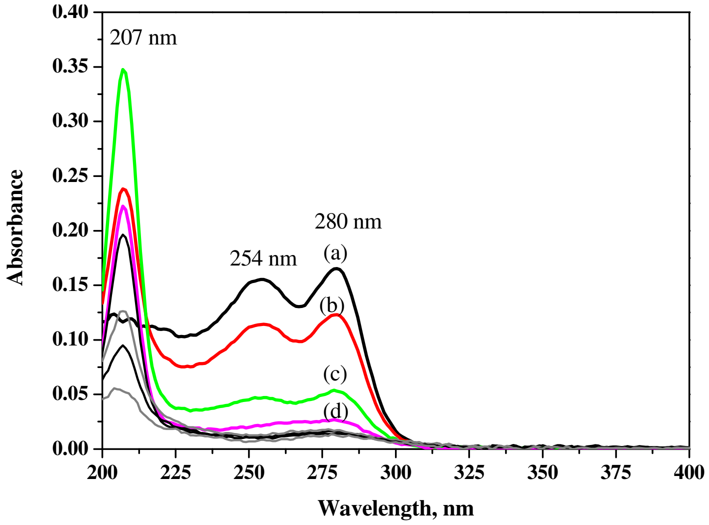


Figure 8.4 – UV-Vis spectra of a 2.0 mg L^{-1} DMDTC solution registered with time: (a) time 0, (b), 3 hours, (c) 1, (d) 2 to 28 days.

It is interesting to notice that, as can be seen in Figure 8.1A, the bands at 250 and 280 nm, characteristic of DMDTC do not appear in the spectra of thiram solutions after several ageing times, suggesting that, if DMDTC is a degradation product of thiram, it is only an intermediate which quickly degrades into smaller molecules, in agreement with its fast degradation observed in water. In order to clarify the possibility of the degradation of the thiram in the presence of an excess of copper and consequent complexation and stabilization of the degradation product by copper ions, some experiences were performed with solutions containing 2.0 mg L^{-1} DMDTC and copper ion at the following DMDTC:Cu molar ratios: 1:0.3; 1:1, 1:3, 1:5, 1:10 and 1:25. Figure 8.5 shows the changes of the UV-Vis spectra of a 2.0 mg L^{-1} DMDTC solution due to the presence of copper ions at 1:0.3 and 1:1 DMDTC:Cu(II) ratios.

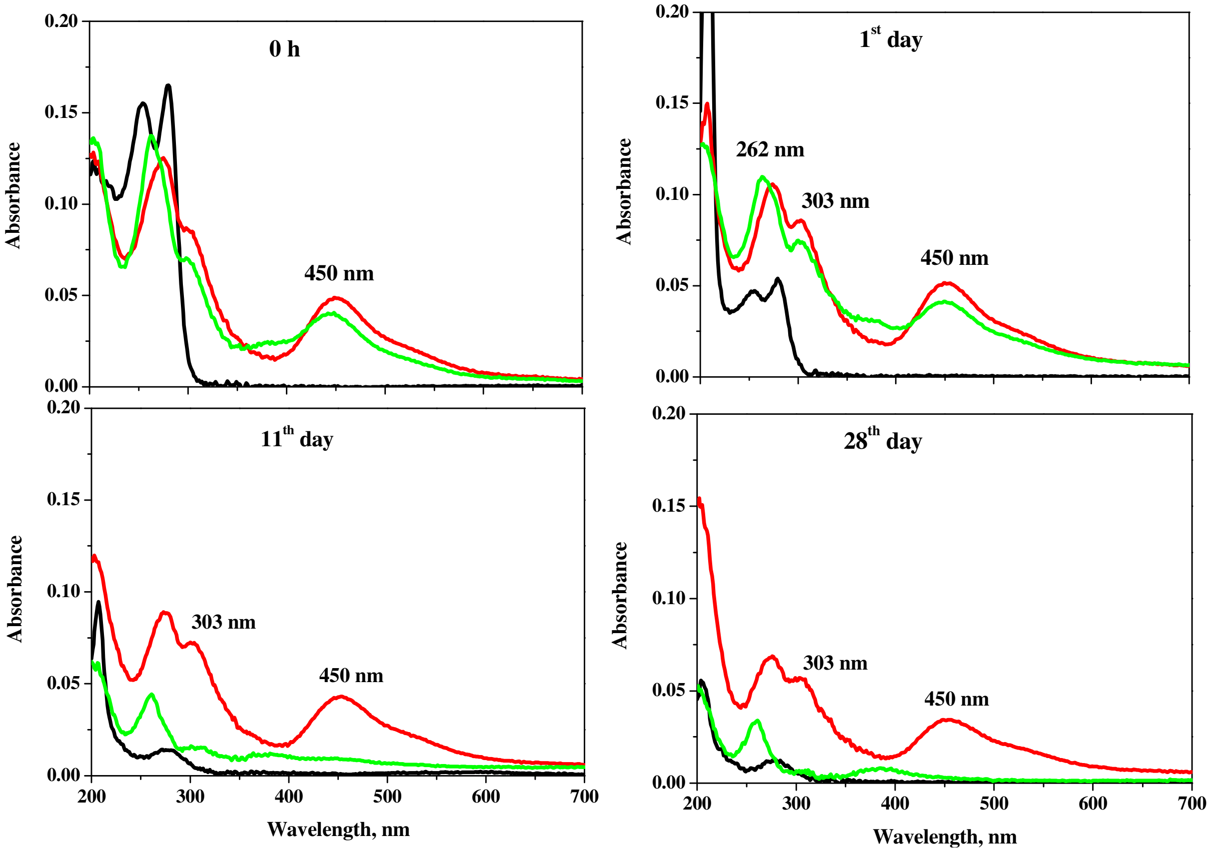


Figure 8.5 – UV-Vis spectra of a 2.0 mg L^{-1} DMDTC solution in the presence of copper ion registered at 0, 1, 11 and 28 days after DMDTC:Cu(II) solutions preparation. (▬) DMDTC, (▬) DMDTC:Cu 1:0.3 and (▬) DMDTC:Cu 1:1 ratio.

The UV-Vis spectra, obtained immediately after DMDTC:Cu(II) mixing (t=0 days), exhibit three new bands, one at 450 nm, giving rise to a yellowish colour, other at 303 and another at 275 nm, with the simultaneous disappearance of the characteristic DMDTC bands at 254 and 280 nm. In fact, in the presence of an excess of DMDTC, i.e. DMDTC:Cu(II) 1:0.3 ratio, these three bands remain for 28 days, moreover after the 28th day the absorbance of these three bands is still about 54-70% of the original absorbance (Figure 8.5, 28th day). This suggests that DMDTC is completely complexed with copper ions and that the complexes formed are much more stable than free DMDTC. However, a different behaviour is observed if there is an equilomar DMDTC:Cu(II) ratio; the spectra registered immediately after DMDTC:Cu(II) mixing (Figure 8.5, t = 0 day) show the same bands at 450 and 303 nm, but the band at 275 appears slightly shifted to 262 nm. In fact, at the end of 28 days the band at 450 nm completely disappears, the band at 303 nm decreases and a new band appears at 385 nm. In addition, after the 28th day, the absorbance of the band at 262 nm is still about 25% of the original absorbance (Figure 8.5, 28th day).

Experiences with higher copper contents were performed in order to investigate the Cu(II) complexes which are formed and their changes along time. Figure 8.6 shows the UV-Vis spectra for the mixtures of 1:3, 1:10 and 1:25 of DMDTC:Cu(II). The UV-Vis spectra, registered during one month for the DMDTC:Cu(II) 1:10 and 1:25 show only the bands at 385 and 262 nm, even at time zero (Figure 8.6, time 0 hours). For a DMDTC:Cu(II) 1:3 ratio and time zero it is visible, besides the bands at 262, 303 and 450nm, a new shoulder at 385 nm; for longer periods of time, the 450 nm band replacement is observed, so that, at the end of the 28th day, only the bands at 385 and 260 nm are present in the respective UV-Vis spectra (Figure 8.6, 28th day).

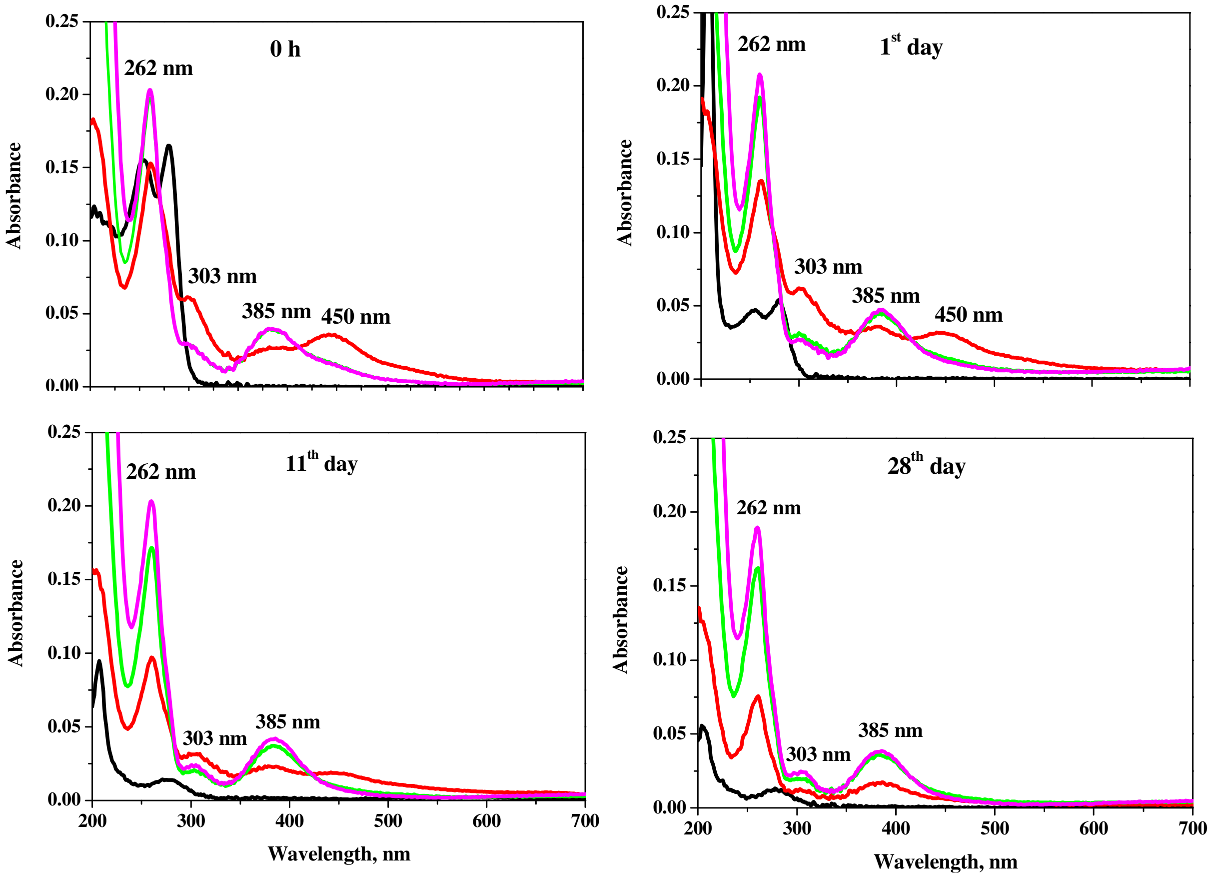


Figure 8.6 – UV-Vis spectra of a 2.0 mg L^{-1} DMDTC solution in the presence of copper ions registered at 0, 1, 11 and 28 days after solutions preparation.(⬛) DMDTC, (🟥)DMDTC:Cu 1:3, (🟩)DMDTC:Cu 1:10, (🟪) DMDTC:Cu 1:25 ratio.

Table 8.1 shows the percentage of absorbance decrease (% Abs_d) of the bands at 260 and 385 nm during 28 days for DMDTC:Cu(II) 1:3, 1:10 and 1:25 ratios.

Table 8.1. Percentage of decrease (%Abs$_d$) in the absorbance intensity of the bands at 260 and 385 nm after 28 days for DMDTC:Cu(II) 1:3, 1:10 and 1:25 ratios.

λ peak (nm)	**DMDTC:Cu**	**Absorbance**				**% Abs$_d$**
		0	**1st**	**11st**	**28th**	
260	**1:3**	0.152	0.135	0.097	0.075	51
	1:10	0.198	0191	0.171	0.163	18
	1:25	0.203	0.208	0.204	0.190	6.4
385	**1:3**	0.026	0.036	0.023	0.017	53
	1:10	0.038	0.044	0.037	0.035	20
	1:25	0.040	0.047	0.042	0.038	19

After 28 days the absorbance of both bands is still about 47-94% of the original absorbance, depending of the DMDTC:Cu(II) ratio. Thus, the results obtained allow us to conclude that in the presence of a high excess of copper ions, DMDTC:Cu 1:25, the complexes responsible for the absorption at 260 and 385 nm are immediately formed and more than 80% of their initial concentration still remains in solution after 28 days, suggesting a high persistence of these complexes (as we can also see in Figure 8.7).

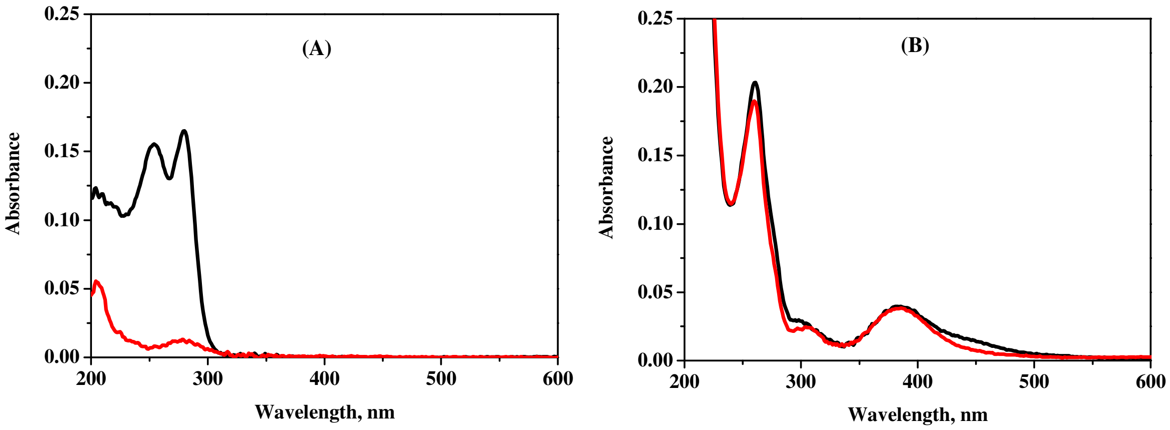


Figure 8.7 - UV-Vis spectra of **(A)** 2 mg L^{-1} DMDTC solution and **(B)** DMDTC:Cu 1:25 solution registered at time zero (▬) and 28th day after solutions preparation (▬).

Comparing the DMDTC-Cu spectra (Figure 8.6, 28th days) with the spectra previously obtained for Thi-Cu (Figure 8.3, > 30th days) we conclude that they are very similar for high copper ion ratios. At the end of approximately 30 days after the preparation of the solutions both spectra exhibit only two bands at 260 and 385 nm. As two molecules of DMDTC are equivalent to one thiram molecule in terms of electron-donor atoms, we compared the spectra of solutions containing Thi:Cu 1:50 with the spectra of solutions DMDTC:Cu 1 1:25 (Figure 8.8). All the spectra exhibit two bands at 262 and 385 nm whose absorbances are maintained approximately constant during 2 months of the solution preparation. Thus, we can conclude that in the presence of an excess of copper ions there is a degradation of thiram to DMDTC (or DMDTC degradation products) which form highly persistent complexes with copper ions. Weissmahr et al., (1998) obtained a spectrum identical to the one of Figure 8.7B for a aqueous solution of DMDTC:Cu 1:100 and they attributed it to a complex DMTC:Cu 1:1, i.e. $[CuDMDTC]^+$. However the authors did not perform any further structural characterization to identify the complex formed.

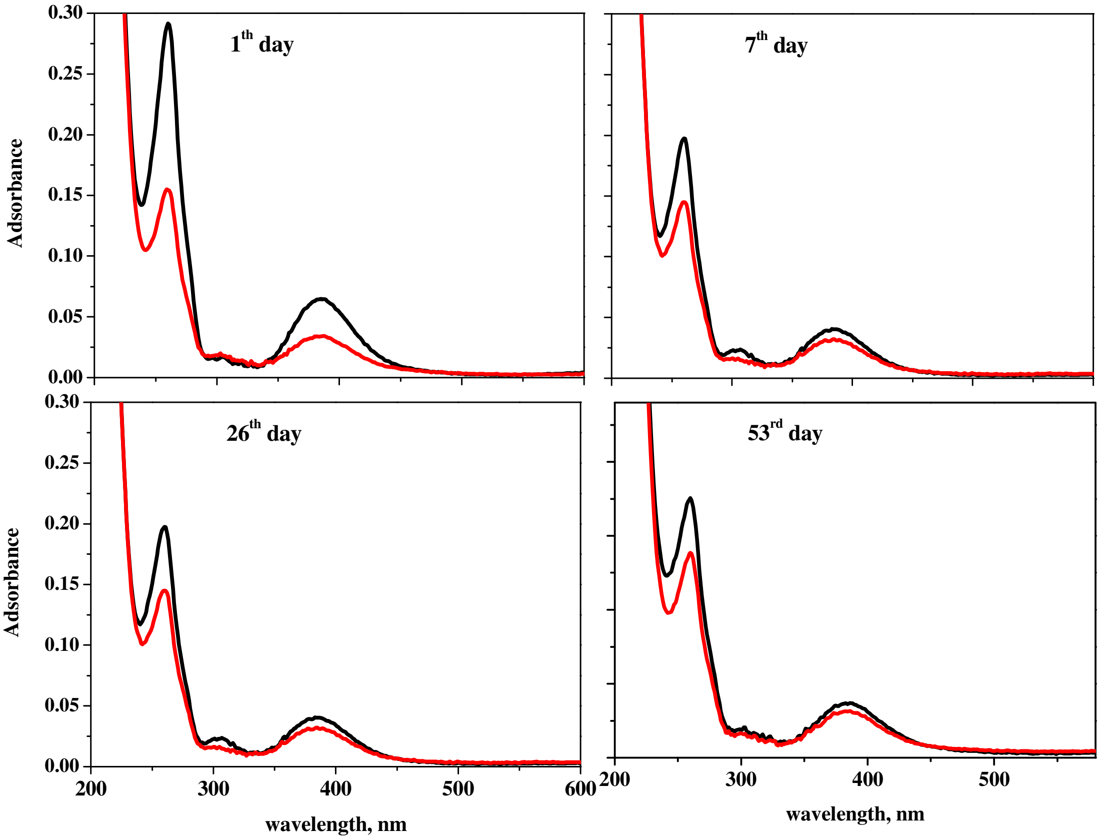


Figure 8.8 - UV-Vis spectra of a DMDTC and Thi solution in the presence of copper ions registered at different days after solutions preparation. [DMDTC] = [Thi] = 2 mg L^{-1}; (▬) Thi:Cu 1:50, (▬) DMDTC:Cu 1:25.

8.3.3 Copper influence on thiram persistence in aquatic environment

In order to clarify the attribution of the UV-Vis bands observed in the solutions of thiram:Cu and the contribution of copper to the degradation of thiram at more realistic concentrations for natural waters, several experiments with 1 L of 11 μg L^{-1} thiram solution in the presence of an excess of copper (Thi:Cu ratio 1:25) were prepared and left to equilibrate during one and five days. Thiram solutions without copper ions were also prepared for comparison. The effect of the addition of an excess of EDTA to the Thi:Cu solutions after 1 and 5 ageing days was also studied. After the equilibration time the samples were analysed by HPLC-UV after SPE pre-concentration and the percentage of thiram recovery was evaluated. According to the UV-Vis spectra showed previously in Figure 8.3, the greater the Cu:Thi ratio, the earlier the band at 420 nm "disappears" and, consequently, the band at 385 nm appears. For Thi:Cu 1:25 solutions, the band at 385 nm is evident at the 4th day. So, the ageing times 1 and 5 days were chosen since they correspond to the presence in the spectrum of just one of the bands, 420 or 385 nm, and those ageing times are shorter than the time needed for the beginning of thiram degradation when it is alone in solution. However, an equilibration time of 22 days was also performed. Thus, EDTA was added to the solutions after the ageing time and the solution was analysed by HPLC-UV after SPE pre-concentration. The recoveries of thiram are shown in Table 8.2.

Table 8.2. Thiram recovery of 1 L of 11.0 µg L^{-1} thiram solution in the presence of an excess of copper (Thi:Cu ratio 1:25)

Sample	Recovery (%)	Sample	Recovery (%)	Sample	Recovery (%)
$(Thi)_{1d}$	94.7 ± 1.5 (n=4)	$(Thi)_{5d}$	93.9 ± 0.14 (n=2)	$(Thi)_{22d}$	50.8 ± 1.0 (n=2)
$(Thi + Cu)_{1d}$	<LOD (n=3)	$(Thi + Cu)_{5d}$	<LOD	$(Thi + Cu)_{22d}$	<LOD (n=2)
$[(Thi + Cu)_{1} + EDTA]_{1d}$	91.3 ± 2.2 (n=2)	$[(Thi + Cu)_{5d} + EDTA]_{1d}$	59.8 ± 3.0 (n=2)	$[(Thi + Cu)_{22d} + EDTA]_{1d}$	<LOD (n=2)

From the results of Table 8.2 we can conclude that after 1 day of equilibration a complex Thi-Cu(II) is present in solution, since thiram is completely recovery (> 90%) after EDTA addition. When EDTA is added to Thi:Cu solutions after 5 days of equilibration, only approximately 60% of free thiram is recovered, which means that part of the thiram was degraded and the degradation product can complex with copper ions. These solutions were too diluted to obtain their UV-Vis spectra. The fact that some thiram has been recovered, means that the disappearance of the band at 420 nm was probably not completed, suggesting that the degradation of thiram is slower than observed in the solution of Thi:Cu containing 2 mg L^{-1} of thiram. Indeed, we made the same experiments described in Figure 8.3 with Thi:Cu 1:25 but using 0.2 mg L^{-1} of thiram instead of 2 g L^{-1}, and we observed that after 7 days a band at 385 nm was present but a shoulder at 420 nm was still observed. Thus, in conclusion, the band at 420 nm can be attributed to a complex of thiram with copper, 1:1 as will be confirmed by mass spectra in the following section. This complex degrades into other copper complex (or complexes) which gives raise to the band at 385 nm. The degradation rate of the complexes increases when the Thi:Cu ratio decreases and when the concentrations of the solutions are higher.

8.3.4 Identification of the complexes by HPLC-MS/MS

A Thi:Cu (1:3) solution in Milli-Q water was analysed 6 hours and 4 and 10 days after its preparation by HPLC-MS and HPLC-MS/MS. Simultaneously, a thiram solution without copper ions (2 mg L^{-1}) was used as control. Table 8.3 summarizes the number of compounds obtained at each retention time, identified by HPLC-MS, as well as the mass of each molecular ion and the product ions obtained by HPLC-MS/MS in further MS^n fragmentation. In some cases, it was necessary to confirm *m/z* peaks and their fragmentation patterns by direct injection in the MS/MS, since the solutions were too much diluted. Since, in the previous section, the results obtained allow us to conclude that in the presence of an excess of copper ions there is a degradation of thiram to DMDTC (or DMDTC degradation products) which complexes with copper ions, a DMDTC:Cu 1:25 solution was also prepared and the HPLC-MS analysis of the solution was performed (cf. Table 8.3).

Table 8.3. Thi:Cu (1:3) and DMDTC:Cu (1:25) byproducts identified and corresponding HPLC-MS/MS and MS^n fragmentation profiles

Solution	**Compound**	**Retention time (min)**	**[M]$^+$ *m/z***	**MS^2 *m/z***	**MS^3 *m/z***	**MS^4 *m/z***
control	**Thiram (1)**		241	**196**,152,120,88	120	
Thi:Cu (6 h)		**2.74**				
	Thiram (1)	**3.07**	241	**196**,152,120,88	152,120,88	
	2	**3.33**	303	260 (15) **227** (30) **202** (40) **184** (100) **151** (20) 88 (25)	n.d 183,**151**,124,88 **184** 88 108	108 88
	3	**4.68**	303	260 (15) **227** (30) **202** (40) **184** (100) **151** (20) 88 (25)	n.d 183,**151**,124,88 **184** 88 108	108 88

Table 8.4. Thi:Cu (1:3) and DMDTC:Cu (1:25) byproducts identified and corresponding HPLC-MS/MS and MS^n fragmentation profiles (continuation).

Solution	**Compound**	**Retention time (min)**	**$[M]^+$ *m/z***	**MS^2 *m/z***	**MS^3 *m/z***	**MS^4 *m/z***
Thi:Cu (4 d)	**4**	2.62	271	Weak signal		
	2	2.96	303	260 (10) **227** (30) **202** (40) **184** (100) **151** (15) 88 (25)	202,184 183,**151**,124 **184** 88 108	108 88
	3	4.40	303	260 (10) **227** (30) **202** (40) **184** (100) **151** (15) 88 (25)	202,184 183,**151**,124 **184** 88 108	108 88
Thi:Cu (10 d)	**4**	2.62	271	**253** (100) 239 (20) 88 (80)	235,211	
	2	2.96	303	259 (30) 227 (25) 184 (100)		
	5		315*	**274**	230,**212**	168
	6		204*	**187** (100) **163** (60)	**169** **148**,122	137,123 122,104
	7		190*	**145**	104	
	8		145*	104		
DMDTC:Cu	**8**	1.93	**145**	104		
	7		**190**	**145**	104	
	6	2.10	204	**163**	**148**,122	122,104
	4	2.62	271	**253**,144	235	
	2	2.96	303	262 (10) 227 (30) 202 (40) 184 (100) 151 (25) 88 (30)		

* direct injection in MS/MS for fragmentation; m/z in bold was subjected to MS^n analysis

As we can see in Table 8.3, it is possible to identify different compounds in the Thi:Cu 1:3 solution depending on the ageing period. For the solution analysed after 6 hours and 4 days there were no differences concerning the compounds identified (compounds **2** and **3**, m/z 303), since the fragmentation profiles are the same. However, it was possible to identify an additional ion at m/z 271 in the solution after 4 days but the signal was too weak to do its fragmentation. The abundance of this ion (*m/z* 271, compound **4**) intensifies after 10 days and the M^+ at m/z 303 (compound **2**) almost disappears; simultaneously other compounds with M^+ at *m/z* 315, 204, 190 and 145 (compounds **5**,**6**,**7** and **8**) were identified. Besides, comparing the HPLC-MS data of the DMDTC:Cu 1:25 solution with the HPLC-MS data of Thi:Cu 10 days solution, we obtained the same compounds, i.e. compounds **2**,**4**,**6**,**7** and **8**. The MS spectra of both solutions were also similar and quite different from the MS spectrum of the initial Thi:Cu solution (Figure 8.9).

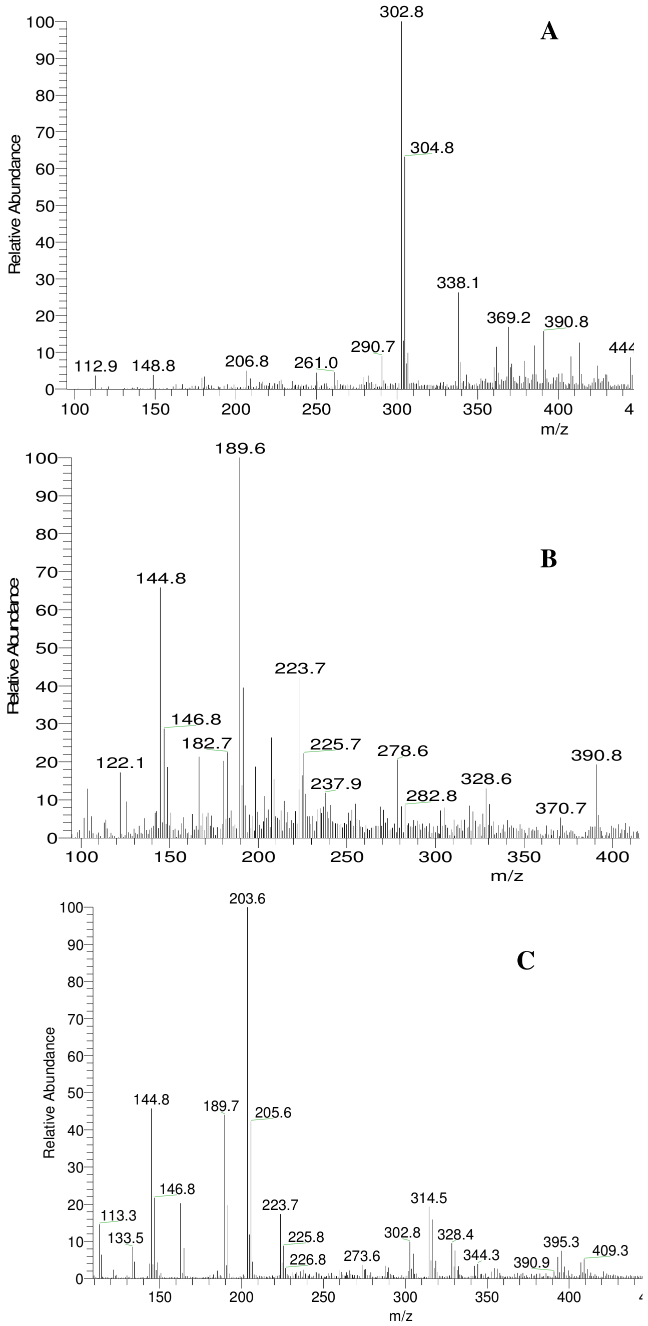


Figure 8.9 – ESI-MS spectra of a (**A**) 6 h Thi:Cu 1:3 solution, (**B**) 10 days Thi:Cu 1:3 solution and (**C**) DMDTC:Cu 1:25 solution.

These results are in agreement with the results obtained by Uv-Vis spetrophotometry in section 8.3.2. In fact, comparing the UV-Vis spectrum of a 10 days Thi:Cu 1:3 solution with the spectrum of the DMDTC:Cu (1:25) solution we can confirmed that they are similar (Figure 8.10). Since the MS spectra were done in the positive mode, the anion of DMDTC is not detected.

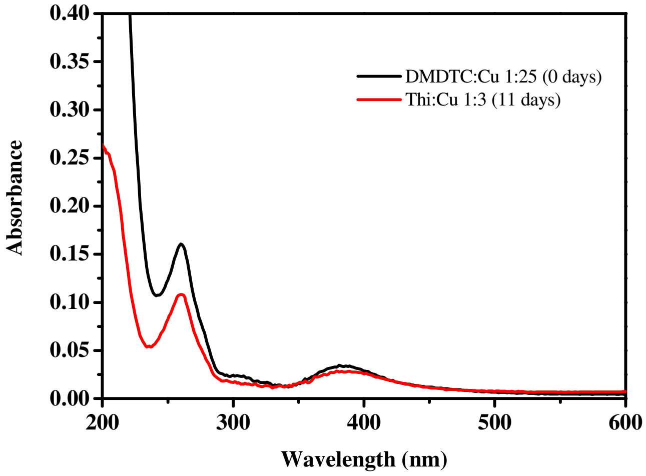
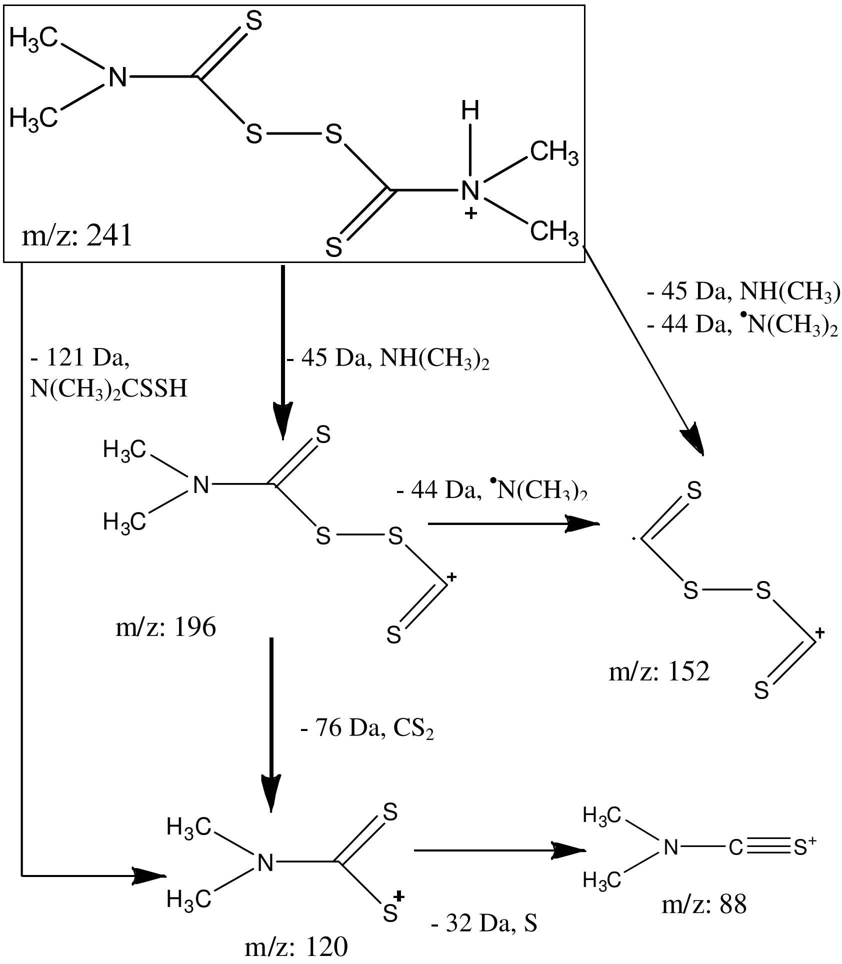


Figure 8.10 - UV-Vis spectra of a DMDTC and Thi solution in the presence of copper ions registered at different days after solutions preparation. [DMDTC] = [Thi] = 2 mg L^{-1}.

A tentative of identification of the several products formed in the Thi:Cu 1:3 solution along time was performed based in each MS^n fragmentation profile. After 6 hours, the aqueous Thi:Cu solution exhibits the presence of thiram (compound **1**, $[M+H]^+$ ion at m/z 241) confirmed by the HPLC-MS analysis of thiram aqueous solution (2 mg L^{-1}) used as control. The identification of thiram (**1**) was also confirmed by the MS^n fragmentation profile **(Scheme 8.2)**. Thus, MS^2 of $[M+H]^+$ ion at *m/z* 241 shows product ions at *m/z* 196, 152 and 120, corresponding, respectively, to the loss of the dimethylamine group (-45 Da, $HN(CH_3)_2$), the loss of the two dimethyamine groups (-45 Da, $HN(CH_3)_2$ and -44 Da, $^\bullet N(CH_3)_2$) and the loss of the dimethyldithiocarbamate (-121 Da, acid *N,N*-dimethyldithiocarbamic). In addition, the MS^3 of the product ion at *m/z* 196 gave a product ion at *m/z* 120, due to the loss of the carbon disulphide (-76 Da, $-CS_2$). The product ion at *m/z* 120, by the loss of a sulphur atom (-32 Da), gives rise the product ion at *m/z* 88 identified as *N,N*-dimethylthioformamide. These product ions at *m/z* 196, 120 and 88 have been also identified in mass spectra of thiram obtained by other authors using EI-MS[9] (Kodoma et al.1999) and [10]DESI-MS^2, Cajka et al., 2011).

Scheme 8.2

Proposed fragmentation pathways for Thiram (bis(dimethylthiocarbamoyl) disulfide) (**1**)



Compounds **2** and **3** (*m/z* 303) were identified as Thi:Cu(I) complexes, and appear at two different retention times in the HPLC-MS, as can be seen in Table 8.3. The MS^n fragmentation pattern is the same for both compounds **2** and **3**, suggesting that these two complexes can be isomers. It is worth to notice that although we are in the presence of a solution containing Cu(II), meaning that the complex formed in solution would be $[CuThi]^{2+}$, the molecular ion *m/z* 303 was identified as a Cu(I) complex, i.e. $[CuThi]^{+}$. This

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[9] EI-MS: Electron Impact Mass Spectrometry;

[10] DESI-MS^2: Desorption Electrospray Ionization Mass Spectrometry.

means that in some way, during the ionization process (ESI), Cu(II) ions can capture one electron being reduced to Cu(I):

$$Cu^{2+}\,(aq) + Thi\,(aq) \longrightarrow [CuThi]^{2+}\,(aq) \xrightarrow[\text{(ionization)}]{\text{ESI}} [CuThi]^{+}\,(g)$$

This reduction behaviour has already been described in literature for Cu(II) pyridil chelates (Gianelli et al. 2001), Cu(II)-resveratrol complexes (Tamboli et al., 2011) and dinuclear Cu(II) complexes of isomeric bis-(3-acetylacetonate)benzene ligands (Rancan et al., 2012). According to the literature this process can be due to one charge transfer between the solvent and the metal complex in the gas phase. It is worth highlighting that the electrospray ion source can be viewed as a particular electrolytic cell, in which electrolysis maintains the charge balance allowing the continuous production of charged droplets (Blades et al., 1991, Kebarle and Verkerk, 2010). The identification of the Thi:Cu(I) complex (compounds **2** and **3**) was confirmed by the MS^n fragmentation profile **(Scheme 8.3)**. Thus, MS^2 of the compounds **2** and **3** (*m/z* 303) gives the product ions at *m/z* 260, 227, 202, 184, 151 and 88:

product ion at *m/z* 260: corresponds to the loss of *N*-methylmethanimine (-43 Da, -CH_2NCH_3), and its MS^3 gives a product ion at *m/z* 184 attributed to the complex DMDTC-Cu(I), which is formed by loss of carbon disulphide (-76 Da, -CS_2);

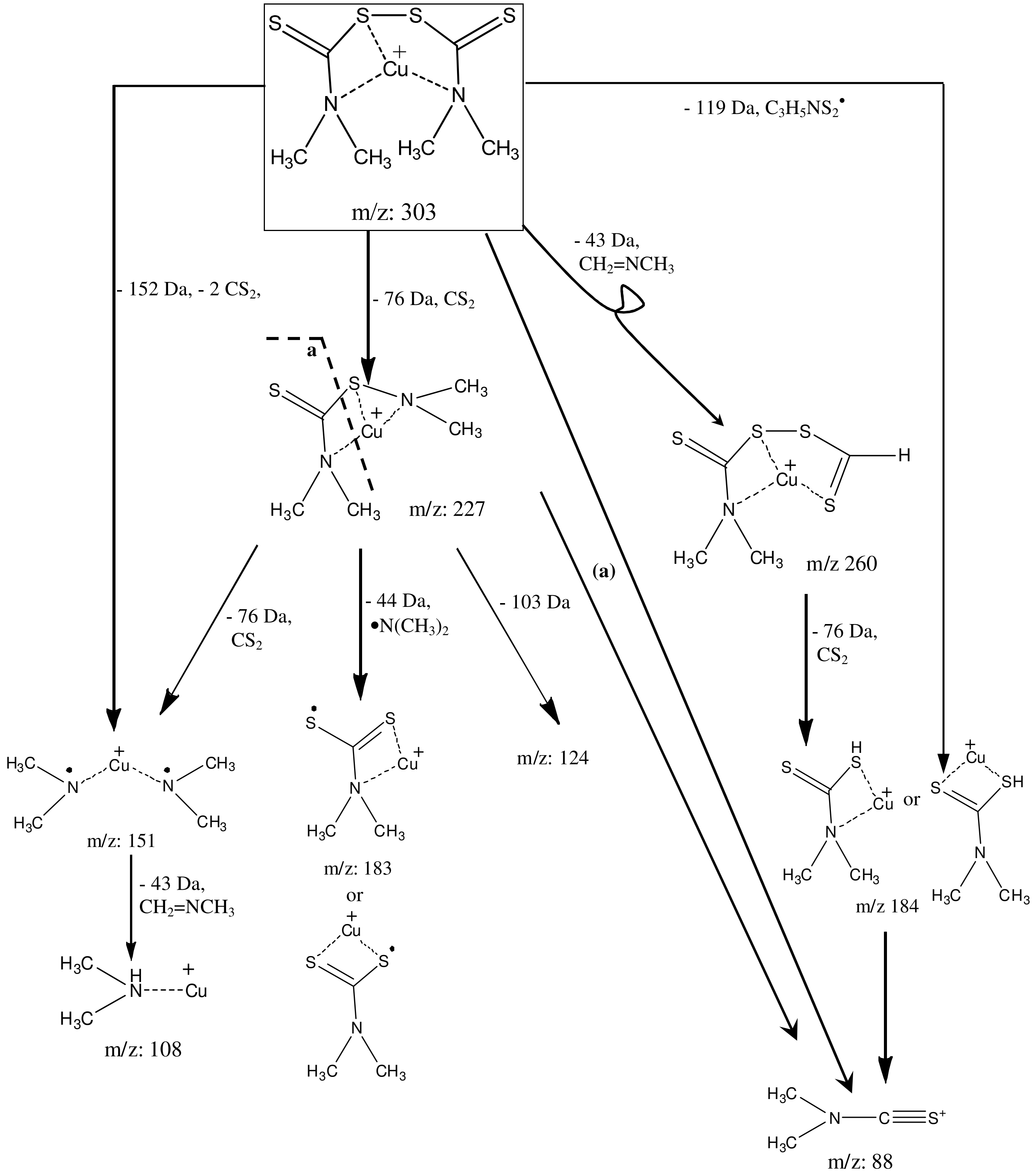
product ion at *m/z* 227: attributed to the loss of carbon disulphide (-76 Da, -CS_2) (**Scheme (8.4)**). Besides, the MS^3 spectrum of the product ion at *m/z* 227 gives rise to three product ions at *m/z* 183, 151 and 124, which may correspond, respectively, to the loss of dimethylamine (-44 Da, -$^{\bullet}N(CH_3)_2$), carbon disulphide (-76 Da, -CS_2) and to the loss of a neutral with 103 Da;

product ion at *m/z* 151: may correspond to a copper complex with 2 dimetilamine radicals formed from the original compounds **2** and **3** (*m/*z 303) by the loss of two molecules of carbon disulphide (-152 Da) and it is also observed in the MS^3 of the product ion at *m/z* 227.

the product ion at *m/z* 88: identified as *N,N*-dimethylthioformamide radical, was detected not only in the MS^2 of compounds **2** and **3** (*m/*z 303) but also in the MS^3 of the product ion at *m/z* 227 (fragmentation **(a)** of scheme **3**) and in the MS^3 of the product ion at *m/z* 184.

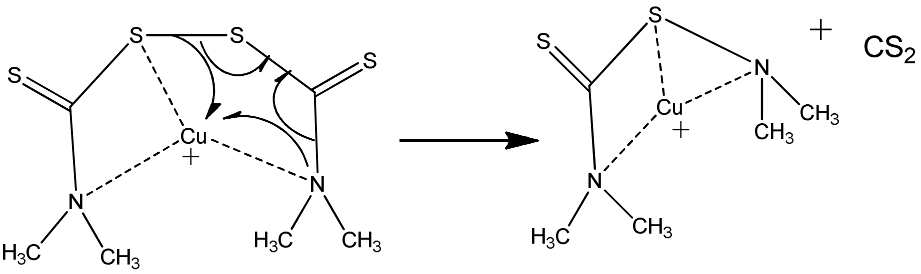
Scheme 8.3

Proposed fragmentation pathways for Thi:Cu(I) complex (**2, 3**)



Scheme 8.4

Propose mechanism for the loss of CS_2 from the M^+ ion at *m/z* 303



After 10 days, as we previously refereed the Thi:Cu complex (*m/z* 303) is almost absent and the most abundant molecular ions have *m/z* 271 (20%), 204 (20%), 190 (100%) and 145 (70%). Molecular ions at *m/z* 271 (very weak signal), 204 (100%), 190 (50%) and 145 (50%) were also detected in the HPLC-MS analysis of the DMDTC:Cu solution. These results are not in agreement with the degradation of Thi:Cu into a $[CuDMDTC]^+$ complex as suggested by UV-Vis analysis (section 8.2). Indeed, none of the molecular ions detected can be attributed to this complex. If, as occurred with the Thi:Cu complex, the Cu(II) ions capture one electron being reduced to Cu(I) during the ionization process (ESI), the DMDTC:Cu complex would be uncharged and not detected. However the MS/MS data confirm the existence of other copper complexes in the solution, since the molecular ions exhibit the typical copper isotope pattern.

The compound **4** (*m/z* 271), which was only detected in the solution of Thi:Cu after 4 days ageing and in the DMDTC:Cu solution but in a very small relative percentage, presents the MS^n fragmentation pattern shown in **Scheme 8.5** and may be assigned to a copper complex formed with a thiram molecule which was previously oxidised. It is worth to notice that this oxidation product of thiram was also detected during photodegradation of thiram aqueous solutions as will be shown in Chapter 9. The MS^2 of the molecular ion at *m/z* 271 gives the product ions at *m/z* 253, 239, 211 and 88:

product ion at *m/z* 253: corresponds to the loss of one molecule of water (-18 Da, H_2O), which MS^3 gives a product ion at *m/z* 235, due to the loss of one other molecule of water (-18 Da, H_2O) and a product ion at *m/z* 211 attributed to the loss of -42 Da;

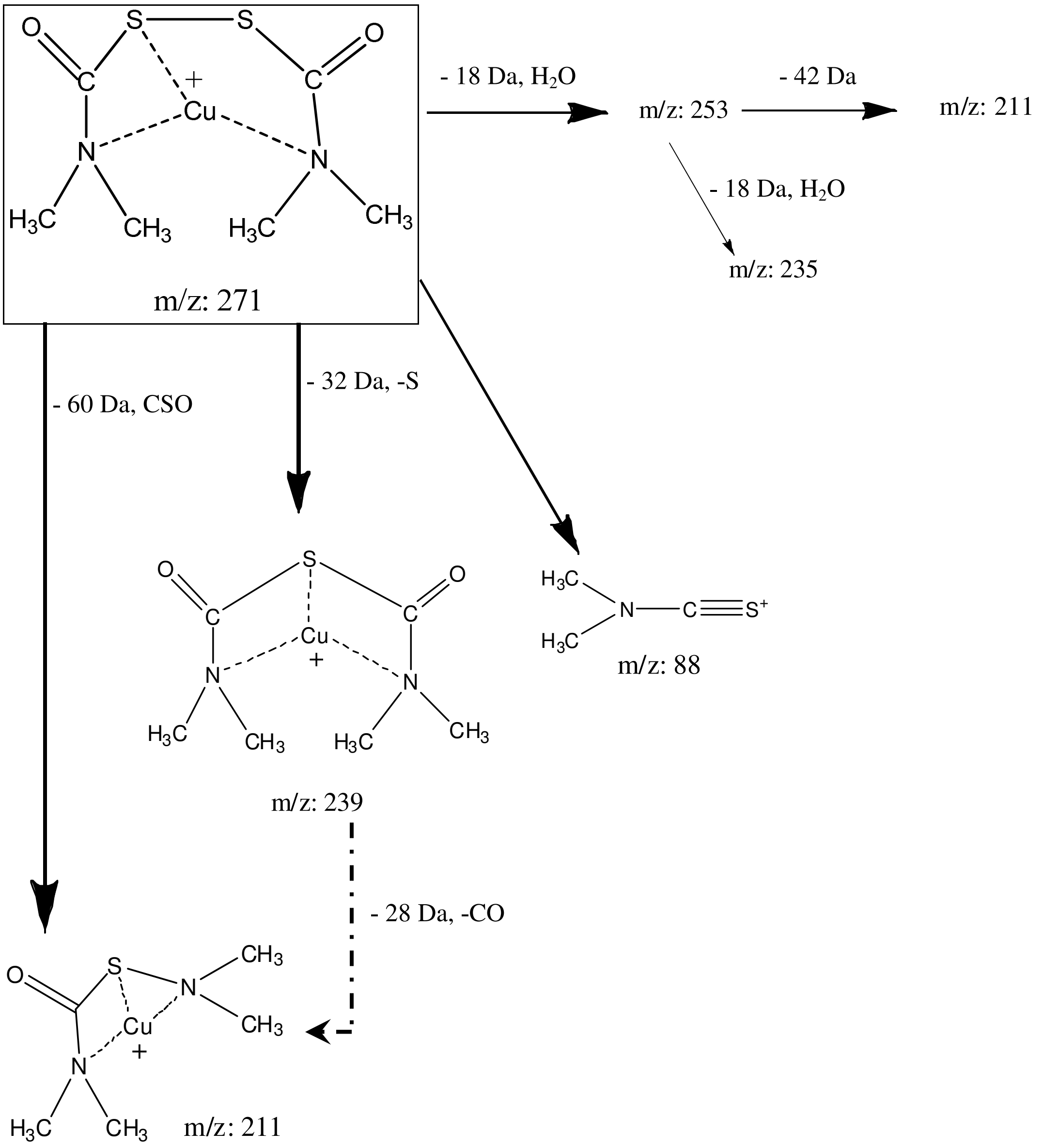
product ion at *m/z* 239: can be assigned to the loss of one sulphur (-32 Da);

product ion at *m/z* 211: can be attributed to the loss of carbonyl sulphide (-60 Da, OCS).

Further studies are under way in order to identify the major compounds at *m/z* 204, 190 and 145 that were observed in the MS spectrum, and to which the complex thiram:copper gives rise over time and which are also present in the DMDTC:Cu solution.

Scheme 8.5

Proposed fragmentation pathways for **Compound 5,** a copper complex formed with oxidised thiram



8.4 Conclusions

In the present work the effect of copper ions on the degradation and persistence of thiram in aqueous solutions was assessed. For thiram solutions 2 mg L^{-1} containing an excess of thiram, a copper complex with thiram is slowly formed, which precipitates after some time. The results obtained for solutions containing an excess of Cu(II), which is the situation most probable in natural waters, it was observed that a complex Thi:Cu 1:1 is formed, as confirmed by HPLC-MS/MS, and that complex degrades at a rate which depends on the ratio Thi:Cu and on the initial concentration of the solutions. For a 2 mg L^{-1} solution containing a Thi:Cu ratio of 1:3, this complex was still present in solution after 7 days and then started to degrade, but for a ratio 1:50, the complex was immediately degraded during the first day after preparation of the solution, giving rise to copper complexes which are quite persistent, since no changes were observed in their UV-Vis spectra during a period of time as long as 56 days. The UV-Vis spectra of Thi-Cu solutions (1:50) after 30 or 56 days are identical to the UV-Vis spectra of DMDTC-Cu solutions (1:25), suggesting that the degradation of the Thi:Cu complex gave rise to a very persistent DMDTC:Cu complex 1:1. However, the analysis of the degraded solutions of Thi:Cu solutions 1:3 by HPLC:MS revealed the presence of other degradation products which can be formed.

These results have important implications on the evaluation of risks associated to the presence of thiram in waters, because of the high persistence of the degradation products formed in the presence of an excess of copper ions.

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Chapter 9

Photodegradation of the fungicide thiram in aqueous solutions. Kinetic studies and identification of the photodegradation products by HPLC-MS/MS[11]

In this study, the relevance of photodegradation processes on the persistence of the fungicide thiram in waters was investigated. Different experiments were carried out, under simulated solar irradiation, using synthetic aqueous solutions of thiram and river water spiked with thiram. In all the experimental conditions studied, the photodegradation of thiram followed a pseudo-first order kinetics. The comparison of the kinetics of photodegradation of pure thiram (Thi-P) with one of its commercial formulations (Thi-F) suggested that the formulation components have a significant effect on the photodegradation of thiram. The photodegradation of thiram in aqueous solutions containing commercial humic acids (HA) or fulvic acids (FA) from river water was also studied. The results allowed us to conclude that FA and HA enhance the photodegradation rate of thiram, however the rate constant values for the degradation of thiram in the presence of HA and FA are similar. In addition, photodegradation of thiram in natural river water showed that there is a significant ($p<0.0001$) enhancement of the degradation rate constant of thiram photodegradation relatively to Milli-Q water, corresponding to a decrease of about 38% in its half-life time. The enhancement of the degradation rate in river water seems to be higher than that observed in the presence of fulvic acids, suggesting that beyond organic matter other natural river components can be influencing the photodegradation of thiram. HPLC-MS/MS was used to identify the products of the photodegradation of thiram in aqueous solution. Three compounds were identified and their structure was corroborated by the MS^n spectra fragmentation profile. The main photodegradation pathway of thiram involves the oxidation of C=S bonds to C=O. Detailed mechanism for the formation of the products from thiram photodegradation are proposed and discussed.

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[11] Adapted from: Filipe, O.M.S., Santos, S.A.O., Domingues, M.R.M., Vidal, M.M., Silvestre, A., Neto, C.P., Santos, E.B.H. 2012. Photodegradation of the fungicide thiram in aqueous solutions. Kinetic studies and identification of the photodegradation products by HPLC-MS/MS. *Chemosphere*. Under revision.

9.1 Introduction

The environmental impact of organic pollutants, such as pesticides, has raised a growing concern over the years, mainly due to the world wide application of intensive agriculture methods and to the development of the agrochemical industry. Thus, information about possible degradation pathways of pesticides in the environment is important in order to understand their transport and fate in surface and ground waters and to identify the degradation products to which they can give rise. Photodegradation is one of the factors that can affect the environmental behaviour and persistence of organic pollutants mainly in surface waters exposed to sunlight. In fact, many studies report the photodegradation of organic pollutants and how the natural organic and inorganic major components of natural waters can influence their degradation (Guerard et al., 2009, Mao et al., 2011). Once present in water systems the pesticides can undergo direct photochemical transformation when exposed to sunlight by direct photons absorption, or indirect photoreactions i.e., photo-transformations indirectly caused by the excitation of other chromophoric compounds present in natural waters.

In spite of the wide thiram application, and in comparison to other pesticides, there is a lack of information available in the literature about photodegradation of thiram in aquatic matrices (Thomas, 2001; Harino and Langston, 2009). According to the literature, thiram is degraded within 24 h by UV irradiation (> 290 nm) and within 7 days by sunlight, in natural waters (Samanidou et al., 1988). Furthermore, Samanidou et al (1988) observed that the characteristics of the water body affect the degradation rate of thiram, i.e., the degradation rate of thiram decreases in the order river>lake>sea water (Samanidou et al., 1988). These authors referred the occurrence of two non identified photodegradation products. As far as we know there are no other studies of photodegradation of thiram by sunlight in natural waters or in aqueous solutions containing natural photosensitizers, such as humic substances (HS). However, some works have been published about the TiO_2 photocatalytic degradation of thiram (Haque and Muneer, 2005; Thakare and Bhave, 2005; Kaneco et al., 2009). Haque and Muneer (2005) have identified three degradation products, but Kaneco et al. (2009) observed the formation of dimethylamine and monomethylamine and proposed the formation of intermediate products different from those identified by Haque and Muneer (2005).

Thus, the pathways of photodegradation of thiram in water and the influence of natural organic matter have not been studied and even the products formed by photocatalytic degradation in the presence of TiO_2 are not clarified. The main objective of the present work is to fulfil this lack of information about pathways and products of thiram photodegradation in natural waters. Thiram photodegradation in aqueous solutions was studied, pure thiram (Thi-P) was compared to its commercial formulation (Pomarsol Ultra D, Bayer) (Thi-F) and the influence of humic substances, major components of dissolved organic matter in natural waters, was evaluated. Then, thiram photodegradation in natural river water was also studied.

9.2 Experimental

9.2.1 Reagents and solutions

All chemicals used were of analytical grade. Pure thiram, Thi-P, (pure substance, 97%) was purchased from Aldrich and a commercial formulation of thiram, Thi-F, was obtained from Bayer (Pomarsol ultra D, 80% thiram active substance). Commercial humic acids (HA) were supplied by Sigma-Aldrich as sodium salt and fulvic acids (FA) were extracted from River Vouga water, collected at Carvoeiro (Aveiro, Portugal), by Santos and Duarte (1998). Acetonitrile (HPLC grade) was obtained from LabScan. Ultra pure water for aqueous solutions was obtained with a Milli-Q water purification system (Millipore). Standard stock solutions of both Thi-P and Thi-F (~40 mg L^{-1}) were prepared by previous dissolution of the solid in acetonitrile followed by dilution with Milli-Q water (maximum of 1% acetonitrile in the final solution). Stock solutions of 100 mg L^{-1} FA from River Vouga and commercial HA were prepared by dissolving the solid in Milli-Q water. The pH of these solutions was measured to be about 6.

9.2.2 Water samples

Water samples from River Vouga (Aveiro, Portugal) were collected at Carvoeiro, near a water collection facility where the dissolved organic carbon (DOC) concentration is around 1 mg L^{-1} (Santos and Duarte, 1998). The samples were collected on March 2012, in 5 L glass bottles, previously washed with 1 M NaOH and distilled water and rinsed with river water immediately before sample collection. The samples were immediately filtered through a 0.45 μm filter within a period of time as short as possible (less than 2 weeks).

9.2.3 Irradiation apparatus

Irradiations were carried out in 25 cm quartz tubes with an internal diameter of 1.5 cm. Samples were irradiated under simulated solar radiation using a Solarbox 1500 (Co.fo.me.gra, Italy) equipped with a 1500 W arc xenon lamp and outdoor filters that restrict the light transmission with wavelengths below 290 nm. During the experiments the irradiance was 55 W m^{-2} (290-400 nm). Lamp spectrum is presented in Figure 9.1. A multimeter (Co.fo.me.gra, Italy), equipped with a temperature sensor and a UV 290-400 nm large band sensor, was used in order to monitor the levels of temperature and irradiance, respectively. During the experiments the irradiance was 55 Wm^{-2} in the range 290-400 nm, corresponding to 550 W m^{-2} in the whole spectrum. The quartz tubes were suspended inside the chamber using a home-made metallic support which allows a homogeneous irradiation.

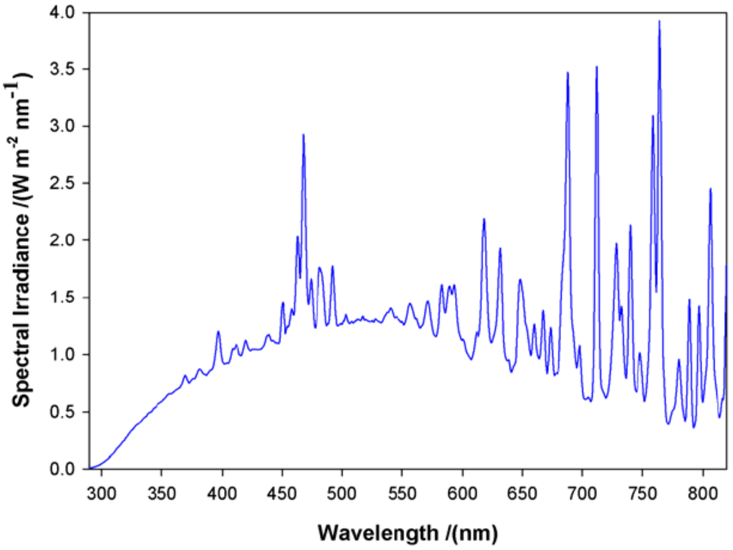


Figure 9.1 – Spectral Irradiance of the 1500 W arc xenon lamp when using an outdoor UV filter, as given by the manufacturer (Solarbox 1500, Co.fo.me.gra, Italy). The spectrum is referred to a total irradiance of 550 W m^{-2} between 290-800 nm, the conditions used during the irradiation experiments.

9.2.4 Photodegradation of Thi-P and Thi-F

Thiram samples (Thi-P and Thi-F) with different concentrations, 2 and 10 mg L^{-1} were prepared by diluting the stock solution of ~40 mg L^{-1}, in water Milli-Q. Sample aliquots of 20 mL were placed in the 50 mL quartz tubes and irradiated during different time periods, depending on thiram concentration. Simultaneously, dark controls were also prepared with the same thiram concentrations, inside tubes covered with several layers of aluminium foil and irradiated in the same conditions. The samples were always analysed within 2 hours by HPLC-UV at 270 nm, using a phenomenex C18 column (150x4.60 mm, 5 µm, 110 Å) and a mobile phase of acetonitrile:water 70:30 (v/v) flowing at 0.7 mL min^{-1}, previously filtered by a membrane filter 0.2 µm NL16 (Schleicher & Schuell). Details about apparatus, calibration and limits of detection (LOD) were described in Chapter 3.

Photodegradation of Thi-P was also performed in natural river water. Thus, 2 mg L^{-1} thiram solutions were prepared by diluting the stock solution in natural river water, instead of Milli-Q water. Next, sample aliquots of 20 mL were placed in the 50 mL quartz tubes and irradiated during 120 min at maximum.

9.2.5 Photodegradation of Thi-P in the presence of natural fulvic and commercial humic acids

Thiram solutions for photodegradation were prepared as described above with the exception that humic acids (HA) were added to achieve a final concentration of 10 mg L^{-1} and natural fulvic acids (FA) were added to achieve a final concentration of 10 or 24 mg L^{-1}.

9.2.6 Identification of photodegradation products by HPLC-MS/MS

The HPLC-MS/MS system used for the identification of the phototodegradation products is described in Chapter 8 (section 8.2.4.)

9.2.7 Statistical analysis

Experimental kinetic data were fitted by non-linear regression analysis using the program GraphPadPrism5® (Trial version; http://www.graphpad.com; last accessed on July 20th 2012). The same statistical program was used for the t-test, which was used to compare the mean values of k and $t_{1/2}$, for the different experimental conditions studied, after the pseudo-first order model be fitted to the data of each replicate of the kinetic study, with the level of significance being indicated by the p value. The two-way analysis of variance (ANOVA) applied to the photodegration of thiram in presence of FA and in natural river water, was performed using also the program GraphPadPrism5®. Besides the two geral approaches mentioned above to perform statistical comparisons between the same model parameters before and after changing the experimental conditions, there is one more statistical tool that was used to compare the thiram photodegradation in different experimental conditions (Christopoulos and Lew, 2000; www.graphpad.com/faq/view faq.cfm?faq=1765). This third tool uses all the experimental data generated in different experimental conditions. The method compares entire curves using the following approach:

i) Fit each data set to a model separately;

ii) Take the total the sum-of squares (SS) and the degrees of freedom (*df*) from the two fits.

iii) Sum the SS resulting from each fit to give a new "total" sum-of-squares value (SS_A). Similarly, sum the two degrees of freedom values from each fit to give a "total" degrees of freedom (df_A).

iv) Combine the two data sets (the data sets obtained with different experimental conditions) into one big data set on a new table and analyze the data using the same fitted equation.

v) Take the new sum-of squares and the degrees of freedom of this combined data set, SS_B and df_B, respectively.

vi) Calculate the following *F* ratio:

$$F = \frac{\frac{(SS_B - SS_A)}{(df_B - df_A)}}{\frac{SS_A}{df_A}} \qquad (9.1)$$

vii) Calculate the corresponding p value, using for example the Excel, i.e. **=FDIST(Fvalue, *df* of the numerator, *df* of the denominator)**.

A small p value (i.e., large *F* value) indicates that there is difference between the two experimental conditions treated curves overall, i.e, the two curves are different. Since this method compares the entire curve, it doesn't help us focus on which parameter(s) differ between the two different experimental conditions (unless, of course, you only fit one variable). It just tells us that the curves differ overall.

9.3 Results and discussion

9.3.1 Photodegradation of thiram (Thi-P) and thiram formulation (Thi-F) in aqueous solution

Most of studies about degradation of pesticides are focused on the active substance, being scarce the information in literature about its commercial formulations. Therefore, the consequences of the use of commercial formulations on the environment are unknown. In a previous work, Filipe et al. (2009) compared the adsorption-desorption behaviour of pure thiram (Thi-P) and one of its formulations (Thi-F) onto commercial humic acids. The authors concluded that thiram formulation components influence the desorption process of thiram from humic acids, turning the pesticide more susceptible to be leached. Recently, some studies using the commercial formulations of pesticides have emerged in the literature, and it has been shown that some coadjuvants in formulations may also influence the photodegradation of some pesticides (Malouki et al., 2009). Thus, the direct photodegradation of thiram in aqueous solution (~2 mg L^{-1}) was studied using both Thi-P and Thi-F. Figure 9.2 shows that thiram photodegradation follows a pseudo-first order kinetics for both Thi-P and Thi-F, as confirmed by the good fitting of the equation (9.2) to the data

$$C(t) = C_0 e^{-kt} \quad (9.2)$$

where C_0 is the initial concentration of thiram at time zero (mg L^{-1}) and k is the reaction rate constant (min^{-1}). Figure 9.2 also shows that there are no variations of thiram concentration along irradiation time for the dark controls, confirming that thiram degradation is induced by light and does not occur in the dark.

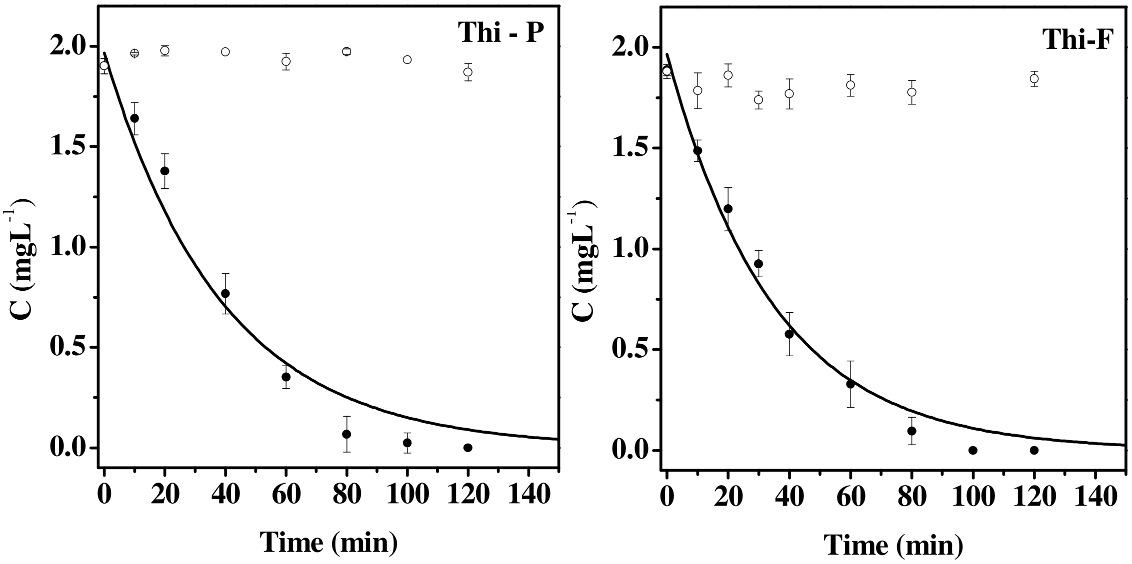


Figure 9.2 – Direct photodegradation curves of 2 mg L^{-1} thiram (Thi-P and Thi-F) in aqueous solutions: black circles are the experimental data and the lines are the graphic representation of the first order equation (equation 9.2) fitted to the mean values of C *vs.* time. Open circles are the dark controls.

Table 9.1 shows the values of the kinetic parameters obtained fitting curve (9.2) to the mean values of *C* vs. time.

The half-life time, $t_{1/2}$, in the case of pseudo-first order kinetic is expressed as

$$t_{1/2} = \frac{\ln 2}{k} \qquad (9.3)$$

The rate of the reaction is proportional to the rate constant; thus the larger the rate constant, the shorter the half-life.

Table 9.1. Kinetic parameters for the photodegradation of Thi-P and Thi-F in aqueous solutions.

[Thi]$_0$ (mgL^{-1})	Kinetic parameters	
	Thi-P	**Thi-F**
2.0	$k = 0.025 \pm 0.002$ min^{-1} $t_{1/2} = 27.9 \pm 2.0$ min n = 6 R^2 = 0.927	$k = 0.030 \pm 0.003$ min^{-1} $t_{1/2} = 23.6 \pm 2.4$ min n = 3 R^2 = 0.986
10.0	$k = 0.014 \pm 0.003$ min^{-1} $t_{1/2} = 48.3 \pm 9.4$ min n = 1 R^2 = 0.965	$k = 0.015\pm 0.001$ min^{-1} $t_{1/2} = 45.9 \pm 4.1$ min n = 3 R^2 = 0.985

In order to evaluate whether the kinetics of photodegradation was different for Thi-P and Thi-F, equation (9.2) was fitted to the data of each replicate of the kinetic study and the mean values of k and $t_{1/2}$ for thiram photodegradation were compared by the t-test. The results showed that *k* is significantly higher for Thi-F than for Thi-P (p=0.02) suggesting that the formulation components have a significant enhancement effect on the photodegradation of thiram.

9.3.2 Effect of humic substances in the photodegradation of Thi-P

Photodegradation of Thi-P was studied in the presence of natural FA and commercial HA. Dark controls, obtained in the same conditions were performed for all the irradiation experiments and we observed that there was no degradation of thiram during the irradiation period. The photodegradation of pure thiram in the presence of either FA or HA follows a pseudo-first order kinetics, as shown in Figure 9.3.

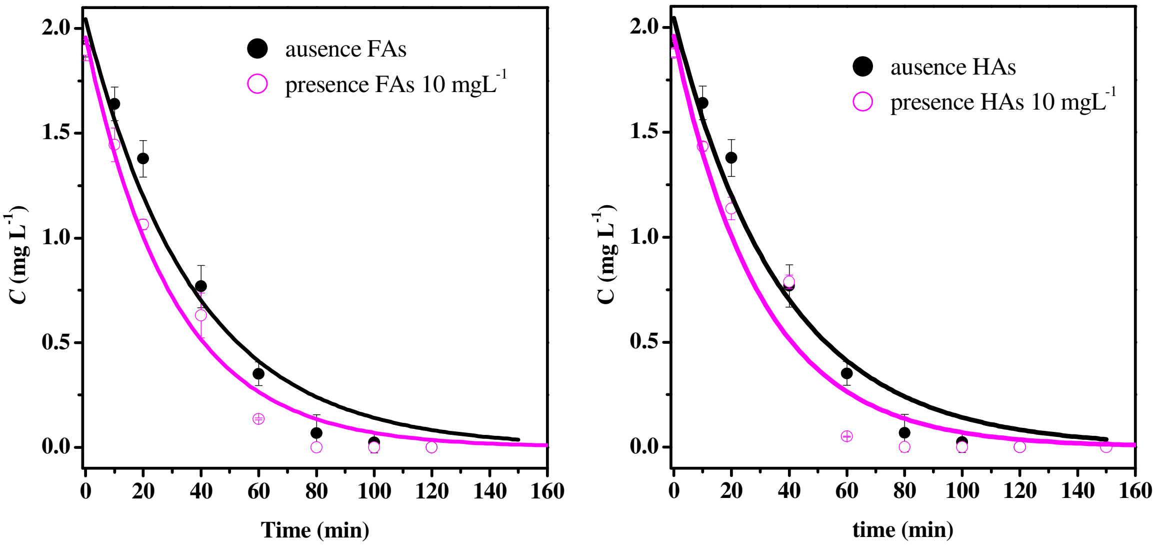


Figure 9.3 - Photodegradation curves of 2 mg L^{-1} thiram in the presence and absence of 10 mg L^{-1} natural FA and commercial HA; circles are the experimental data and the lines are the graphic representation of the first order equation (equation 9.2) fitted to the mean values of C vs. time.

Table 9.2 shows the kinetic parameters obtained by non-linear curve fitting of equation (9.2) to the mean values of *C* vs. time, for the photodegradation of thiram in the presence and absence of HS in aqueous solutions.

Table 9.2. Kinetic parameters for the photodegradation of 2 mg L^{-1} thiram in presence of humic substances in aqueous solutions, obtained by non-linear regression of equation (9.2) to the mean values of C *vs.* time (95% confidence intervals are presented). The number of replicates used to calculate the mean values of *C* for each irradiation time is indicated between parentheses.

Humic substances		**Kinetic parameters**
FA	**C = 10 mg L^{-1}**	k = 0.032 ± 0.003 min^{-1} $t_{1/2}$ = 21.5 ± 2.0 min R^2 = 0.980 (n=2)
	C = 24 mg L^{-1}	k = 0.035 ± 0.006 min^{-1} $t_{1/2}$ = 19.6 ± 3.6 min R^2 = 0.980 (n=1)
HA	**C = 10 mg L^{-1}**	k = 0.032 ± 0.008 min^{-1} $t_{1/2}$ = 21.8 ± 5.9 min R^2 = 0.9637 (n=1)
Absence	**----**	k = 0.025 ± 0.002 min^{-1} $t_{1/2}$ = 27.9 ± 2.1 min R^2 = 0.972 (n = 6)

In order to evaluate whether the presence of FA in solution had any effect on the kinetics of degradation of thiram, mean values of k and $t_{1/2}$ for thiram photodegradation in the presence and absence of FA were compared by the t-test. The results allowed to conclude that natural river FA have a significant enhancement effect on degradation ($p=0.002$ for k and $p=0.004$ for $t_{1/2}$). The comparison of the rate constant values (Table 9.2) for the degradation of thiram in the presence of HA and FA shows that the kinetics of degradation of thiram is similar in the presence of both samples of humic matter. Thus, these results do not point to an influence of the nature of humic matter, since natural river fulvic acids or commercial humic acids enhance photodegradation of thiram in the same way. However, as shown by the results of the t-test and as one can observe in Table 9.2, humic substances induce a slight increase (~25%) of the kinetic rate constant of photodegradation of thiram in aqueous solution. The role of humic substances on the photodegradation of other organic pollutants is well documented and two contradictory effects can be observed (Canonica and Laubscher 2008; Guerard et al., 2009). On one hand they can inhibit photodegradation due to inner filter effects (competition for photons) or binding to the contaminants (Rav-Acha and Redhun, 1992; Doll and Frimmel, 2003; Dimou et al., 2005). On the other hand, humic substances can act as photosensitizers enhancing photodegradation, since they are excited by solar light absorption giving rise to triplet excited states which can directly react with the contaminants or which can give rise to the sensitized formation of several reactive oxygen species, mainly singlet oxygen and hydroxyl radicals (Aguer et al., 1999). It is known that humic substances vary considerably in structural composition and distribution of chromophores which means that their role on photochemical reactions can change considerably with their origin and composition (Guerard et al., 2009). However, the results obtained in the present work do not point to a variation of the effects of humic substances with their origin and composition in the case of thiram.

In natural waters, besides humic matter, there are other components, such as nitrate, Fe(III) and bicarbonate ions, which can influence the photodegradation of contaminants (Espinoza et al., 2007). In order to verify the effect of the environmental matrices on the photodegradation of thiram, two non-simultaneous experiments were done using natural river water samples collected at the same site of river Vouga, where water samples had been collected for FA isolation. Figure 9.4 shows the photodegradation curve of thiram in natural river water sample, in comparison with the direct photodegradation in Milli-Q water and in an aqueous solution containing 10 mg L^{-1} of FA.

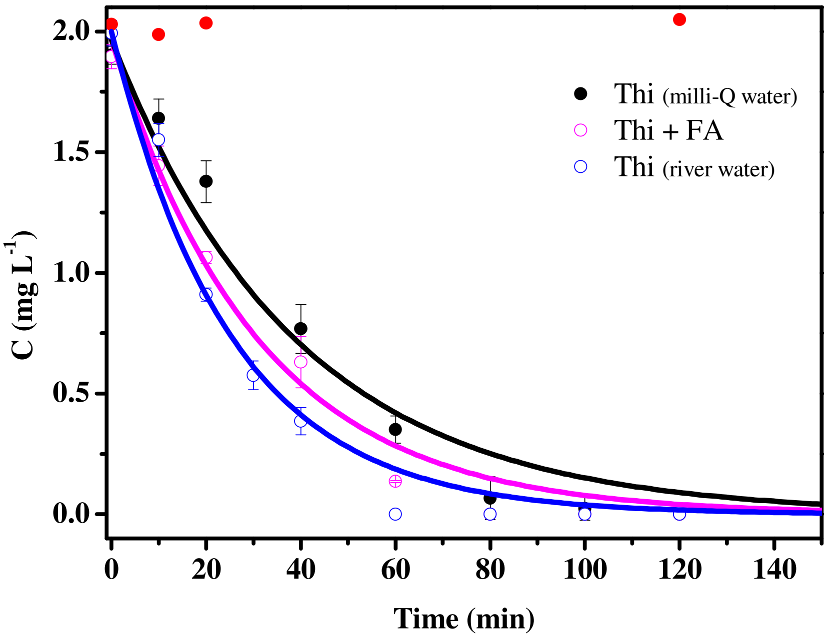


Figure 9.4 - Photodegradation of 2 mg L^{-1} thiram in milli-Q water, in the presence of FA and in natural river water. (•) Control samples of thiram in natural river water. Symbols represent the mean values of replicates of the kinetic studies for each condition. The curves were obtained by non-linear regression of equation (9.2) to the mean values of *C vs.* time

The photodegradation of thiram in river water also follows a pseudo-first order kinetics of degradation ($R^2 = 0.986$) with $k = 0.040 \pm 0.004$ min^{-1} and $t_{1/2} = 17.6 \pm 1.8$ min (95% confidence intervals are presented). Dark controls (●), obtained in the same conditions, suggested that there was no degradation of thiram due to thermal or hydrolytic processes. The t-test was applied to compare the means of the values of k and $t_{1/2}$ obtained in Milli-Q water (6 replicates) and in natural river water (2 replicates). The results allowed to conclude that there is a significant ($p<0.0001$) enhancement of the degradation rate constant of thiram photodegradation in the river water relatively to Milli-Q water, corresponding to a decrease of about 38% in thiram half-life. The enhancement of the degradation rate in river water seems to be higher than that observed in the presence of FA, suggesting that other components of natural river water besides FA, are influencing thiram photodegradation. However, the comparison of the mean values of k and $t_{1/2}$ in the presence of FA and in river water by the t-test does not allow to obtain clear conclusions about the significance of that difference ($p=0.056$ for k; $p = 0.046$ for $t_{1/2}$)). However, by applying the F ratio (equation 9.1) we obtained a very small p value ($p=0.0004$), which is indicative that the individual fits (FA and river water) are better than the pooled fit, i.e., the thiram photodegradation in the presence of FA and the photodegradation of thiram in natural river water resulted in a significant difference in the model parameters between the two data sets. On the other hand, the comparison of mean values of C/C_0 by two way ANOVA (8 times x 2 conditions: FA and river water x 2 replicates) indicates a strong interaction between factors, and thus the p values ($p<0.0003$) are difficult to interpret.

It must be noticed that the concentration of fulvic acids in the river is lower than 10 mg L^{-1}. More studies must be done in order to evaluate the influence of the concentration of humic substances and of other components of river water, besides humic substances, on thiram photodegradation.

9.3.3 Identification of the photoproducts of the direct degradation of thiram

The HPLC chromatograms of the thiram solutions with detection at several wavelengths revealed the occurrence of new peaks, attributable to photodegradation products. Then, we performed the irradiation of a more concentrated thiram solution (10 mg L^{-1}) in Milli-Q water, in order to better detect and identify the photodegradation products. One kinetic study was performed and it was observed that the photodegradation rate constant was lower (k=0.014±0.003) but the additional peaks observed were the same (same retention times) as those observed during photodegradation of the solution 2 mg L^{-1}. In order to iendtify the structure of these new photodegradation, HPLC-PDA-MS and MS_n analysis was performed. Figure 9.5A shows the HPLC-UV chromatogram with detection at 230 nm, of a ~10 mgL^{-1} thiram solution after direct photolysis during 120 min. To obtain the HPLC-PDA chromatogram shown in Figure 9.5B, 8 mL of each of 4 replicates of the solution of thiram irradiated for 120 minutes were combined, lyophilised and then redissolved in 600 µL of methanol. The final solution was injected in the HPLC-UV-MS instrument. In Figure 9.5A it is possible to see the thiram peak at ~3.8 min and two new peaks at 3.2 and 4.4 min. These two peaks can be attributed to photodegradation products of thiram. For the lowest thiram concentration these two peaks are only visible with detection at 207 nm (results not shown). However, in the HPLC-PDA chromatogram of Figure 9.5B it is possible to identify four peaks corresponding to thiram (compound **II**) and three different degradation products (compounds **I**, **III** and **IV**). In addition, the results obtained by in Figure 9.5B by HPLC-PDA-MS validate the attribution of the peaks at 3.3 and 4.5 min of the chromatogram of Figure 9.5A, to photoproducts of thiram.

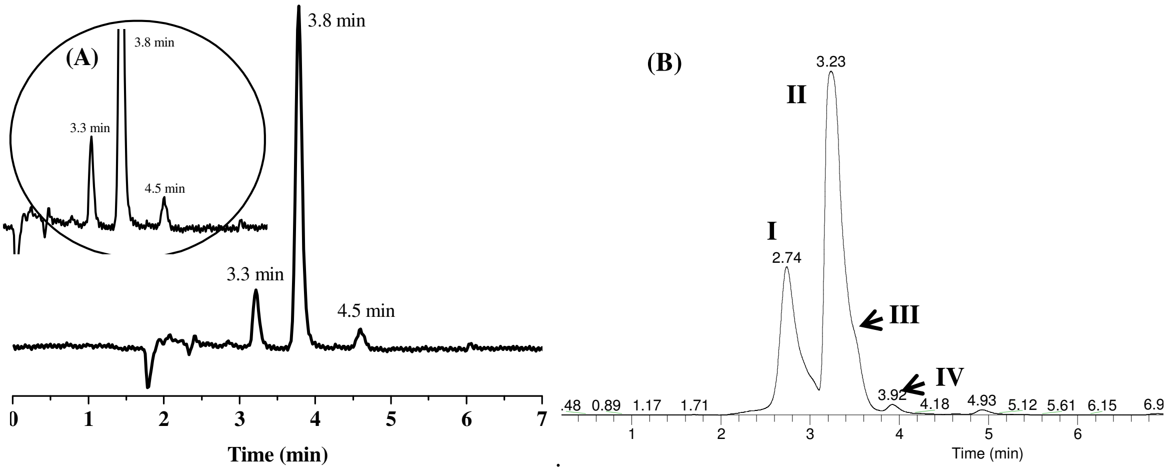


Figure 9.5 – (A) HPLC-UV at 230 nm chromatograms of a 10 mgL^{-1} thiram solution after direct photolysis during 120 min and **(B)** HPLC-PDA at 270 nm chromatogram of the same irradiated solution after pre-concentration, obtained using the HPLC-PDA- MS/MS instrument

The identification of the photodegradation products was performed by comparing the HPLC-MS spectra of the irradiated sample with spectra of the control sample, allowing the identification of the $[M+H]^+$ ions of the new photodegradation products, which were then analysed in detail by MS/MS and MS^n experiments as summarised in Table 9.3.

Table 9.3. Thiran and photodegradadtion products identified and corresponding LC-MS/MS and MS^n fragmentation profiles.

Compound	**Retention time (min)**	**Identification**	**$[M+H]^+$ *m/z***	**MS^2 *m/z***	**MS^3 *m/z***	**MS^4 *m/z***
I	2.74	*N,N*-dimethylcarbamoyl-*N,N*-dimethylthiocarbamoyl disulphide	225	**180**	152	88
II	3.23	**Thiram** (bis(dimethylthiocarbamoyl) disulfide)	241	**196** 152 120 88	152,120	
III	3.30	bis(dimethylcarbamoyl) disulphide	209	**164** 88 72	136, 88, 72	
IV	3.92		335	**271** 184	**256**, 88	224, 183

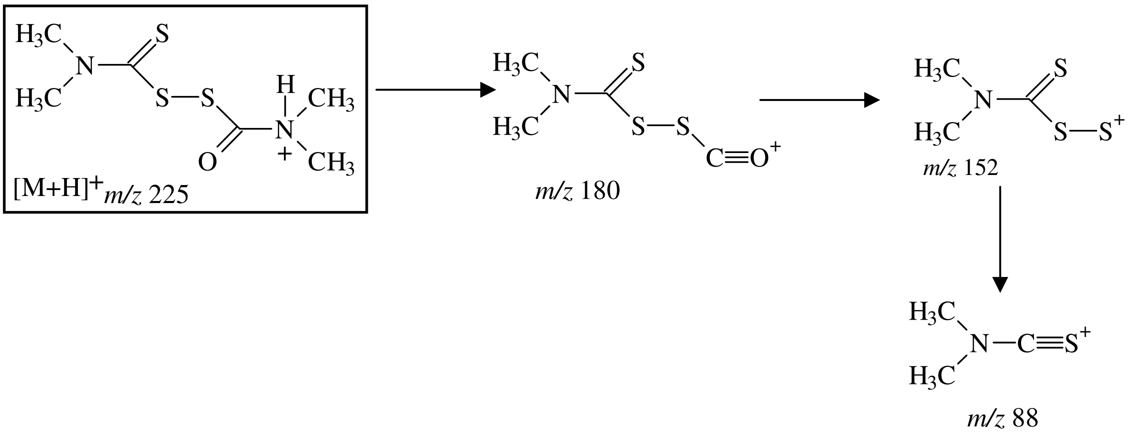
m/z in bold was subjected to MS^n analysis

The aqueous solution of thiram after 120 min of irradiation showed, the presence of thiram (**II**, $[M+H]^+$ ion at m/z 241) and the formation of three byproducts with $[M+H]^+$ ions at *m/z* 225 (**I**), *m/z* 209 (**III**) and *m/z* 335 (**IV**). Each $[M+H]^+$ ions were subjected to further LC-MS^n, in order to elucidate their structural features. As referred above, compound **II** was identified as thiram. The identification of thiram was confirmed by the MS^n spectra fragmentation profile and is described in Chapter 8, section 8.3.4.

Compound **I** was identified as *N,N*-dimethyl carbamoyl-*N,N*-dimethyl thiocarbamoyl disulphide formed by oxidation of C=S group to C=O. The identification of this compound was confirmed by the MS^n spectra fragmentation profile **(Scheme 9.1).** Thus, MS^2 of $[M+H]^+$ ion at *m/z* 225 gives a product ion at *m/z* 180, due to the loss of the dimethylamine group (-45 Da, $HN(CH_3)_2$). Furthermore, the MS^3 of the product ion at *m/z* 180 gave a product ion at *m/z* 152, due to the loss of the carbonyl group (-28 Da, -CO), which, by MS^4, generates a product ion at *m/z* 88 identified as *N,N*-dimethylthioformamide.

Scheme 9.1

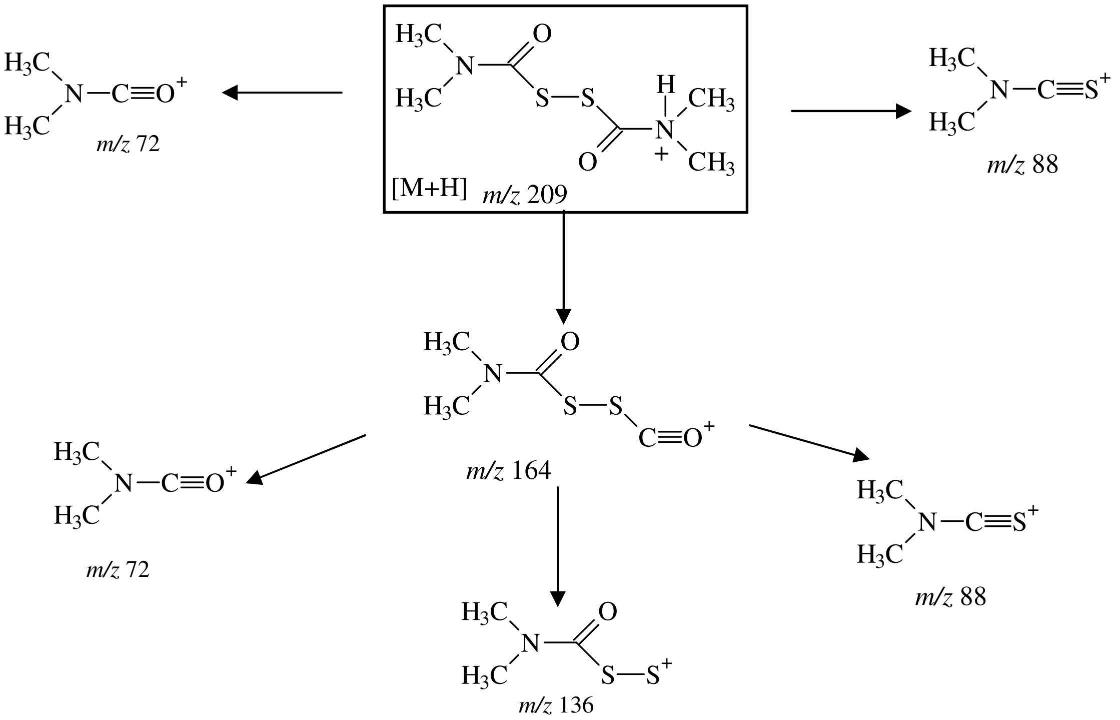
Proposed fragmentation pathways for *N,N*-dimethyl carbamoyl-*N,N*-dimethyl thiocarbamoyl disulphide (**I**)



Compound **III** was identified as bis(dimethylcarbamoyl) disulphide formed by oxidation of the two C=S groups to C=O. The identification of this compound was also corroborated by the MS^n spectra fragmentation profile (**Scheme 9.2**).

Scheme 9.2

Compound III: bis(dimethylcarbamoyl) disulphide

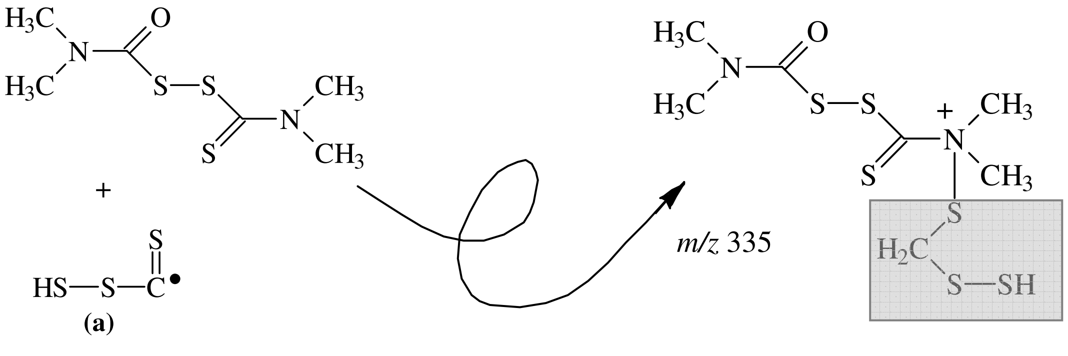


The MS^2 of $[M+H]^+$ ion at *m/z* 209 gives three product ions at *m/z* 164, 88 and 72. The product ion at *m/z* 164 corresponds to the loss of the dimethylamine group (-45 Da, $HN(CH_3)_2$) of the compound bis(dimethylcarbamoyl) disulphide (*m/z* 209); the MS^3 of the product ion at *m/z* 164 generates a product ion at *m/z* 136, formed by the loss of carbonyl group (-28 Da, -CO), and the product ions at *m/z* 72 and 88 identified as *N,N*-dimethylformamide and *N,N*-dimethylthioformamide, respectively.

Compound **IV** was tentatively assigned to a photodegradation derivative of compound **I** based on its fragmentation patterns observed by MS^n spectra. This adduct **(Scheme 9.3)** results from compound **I** linked to an extra $-SCH_2SSH$ moiety. The formation of this structure may involve the radical **(a)**. Although the mechanism leading to the formation of **IV** from **I** and radical **(a)**, is still unclear, the structure of this compound was corroborated by the MS^n spectra fragmentation profile **(Scheme 9.4).** The MS^2 of ion at *m/z* 335 gives one product ion at *m/z* 271 corresponding to the loss of S_2 (-64 Da, -S, -S); the MS^3 of the product ion at *m/z* 271 generates a product ion at *m/z* 256, due to the loss of the methyl group (-15 Da, $-^{\bullet}CH_3$), and the product ion at *m/z* 88 identified, previously, as *N,N*-dimethylthioformamide. Furthermore, the MS^3 of the product ion at *m/z* 256 generates a product ion at *m/z* 224, corresponding to the loss of one sulphur atom (-32 Da, $-^{\bullet}S$), and a product ion at *m/z* 183, corresponding to the simultaneous loss of *N*-methylmethanimine (-43 Da,$-CH_2{=}NCH_3$) and the two methyl groups (- 30 Da, $-2\,^{\bullet}CH_3$).

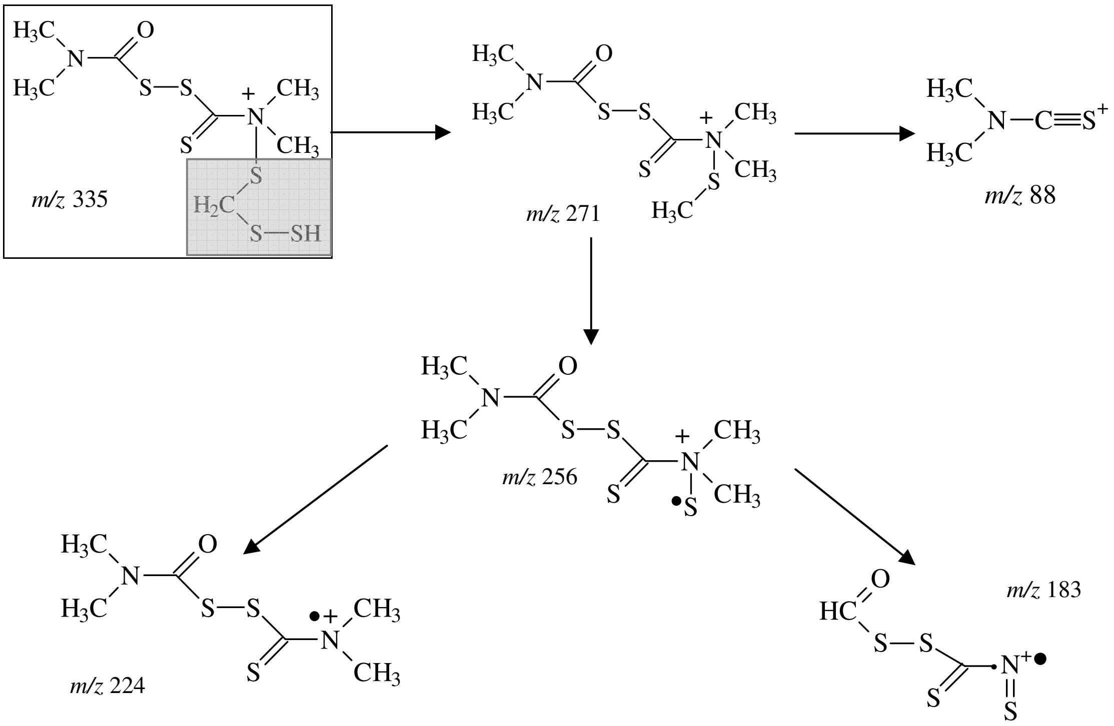
Scheme 9.3

Proposed **Compound IV** from of the photodegradation of thiram (**I**) bonded to a small structural moiety resulting from photodegradative oxidation of thiram



Scheme 9.4

Proposed fragmentation pathways for **Compound IV** a derivative of the photodegradation of thiram



Observing the profile of the area of the chromatographic peaks of the compounds **I** and **IV** along time (Figure 9.6) we can see that compound **IV** only appears after the formation of the compound I, which is consistent with formation of **IV** from **I** shown in Scheme 9.3. This compound **IV** is easily degraded when compared with the compound **I**, since at the end of 160 min of irradiation it is completely degraded.

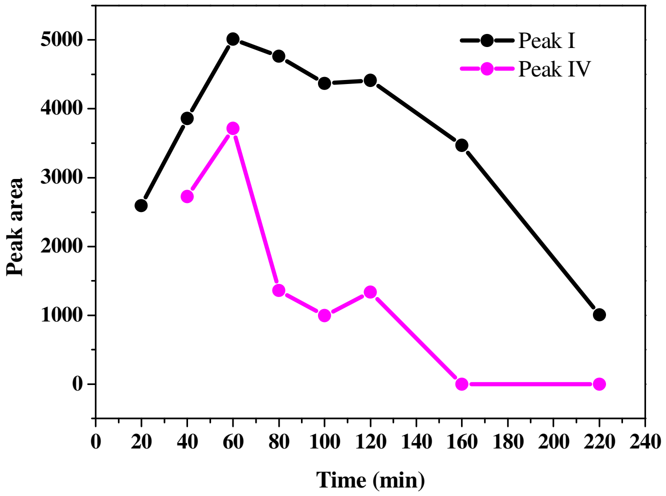
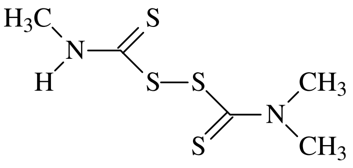
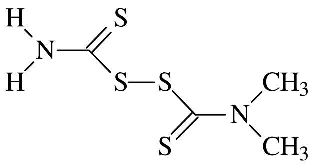


Figure 9.6 – Plot of the area of peak **I** and peak **IV** along photodegradation time with an initial thiram concentration of $C_0 = 10$ mg L^{-1}.

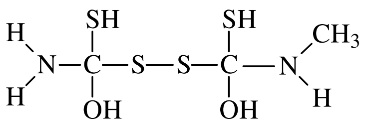
The photoproducts with $[M+H]^+$ ions at *m/z* 225, *N,N*-dimethylcarbamoyl-*N,N*-dimethylthiocarbamoyl disulphide (**I**) and *m/z* 209, bis(dimethylcarbamoyl) disulphide (**III**) have been identified by Kodoma et al (1999) when studying the chemical degradation of thiram in the presence of sodium hypochlorite. On the other hand, Kaneco et al (2009), on the basis of molecular orbital simulation of frontier electron density, proposed these same products as intermediate degradation products of the TiO_2 photocatalytic degradation of thiram. However, Haque and Muneer (2005) presented contradictory results about TiO_2 photocatalytic degradation of thiram, since they identified three compounds corresponding to the loss of the methyl groups in the amine group (*N*-dealkylation) and involvement of electron transfer reactions and reaction with hydroxyl radicals (compounds **1**,**2** and **3**).



(1)



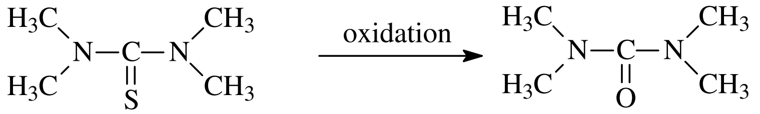
(2)



(3)

Crank and Mursyidi (1992) identified a variety of products, such as, carbon disulfide, tetramethylthiourea, *N,N*-dimethylthioformamide, tetramethylhydrazine, and dimethylamine by UV photolysis, photo-oxidation and visible photosensitized (rose Bengal) oxidation of thiram but in ethanolic solution. Very recently, Gupta et al. (2012)

studied the degradation of thiram (100 mg L^{-1}) in deionised water (pH 8.0 and 5.5), soils (pH 5.1 and 8.1) and plants (radish leaves, roots and tomato fruits) and identified a large number of metabolites at different time intervals of incubation. According to the authors the degradation products identified can be generated by different processes such as, hydrolysis, oxidation, *N*-dealkylation, *S*-methylation, sulfuration, desulfuration, cyclization and photodegradation, which indicate a complex degradation pathway of thiram. The two intermediate products, *N,N*-dimethylcarbamoyl-*N,N*-dimethylthiocarbamoyl disulphide and bis(dimethylcarbamoyl) disulphide, identified by Kodoma et al. (1999) as products of thiram oxidation by sodium hypochlorite and by Kaneco et al (2009) in the photocatalytic degradation of thiram, were also identified by Gupta et al. (2012). The authors identified these two products in aqueous solutions of thiram (pH 8.0 and 5.5) after an ageing period of 11 and 30 days. They also detected the presence of these two compounds at the end of the 11th day in the Sriganganagar soil (pH 8.1). In what concerns the photodegradation process, the authors only attributed the formation of the compound 1,1,3,3-tetramethyl-2-thiourea (compound **4**) to photodegradation of thiram. According to the authors the photodegradation of thiram gives rise to *N,N*-dimethyl thiocarbamoyl and dimethyl amine moieties which react to form the above mentioned product, which can be oxidized to the compound **5,** 1,1,3,3-tetramethylurea.



1,1,3,3-tetramethyl-2-thiourea

(4)

1,1,3,3-tetramethylurea

(5)

9.4 Conclusions

In this work the importance of photodegradation processes on the persistence of the fungicide thiram was assessed. First, it has been shown that thiram is susceptible to photodegradation, showing a half-life time of about 28 min. It was also observed that the commercial formulation components and humic substances (natural FA or commercial HA) slightly enhance the photodegradation of thiram in aqueous solution, decreasing its half-life time to 24 and 22 min, respectively. Nevertheless, the most significant enhancement of the degradation rate of thiram was observed when the thiram photodegradation was performed in natural river water (half-life time of about 18 min). Moreover, one of the main focuses of this work consisted on the identification of the photodegradation products of thiram in aqueous solutions, by mass spectrometry. Overall, it was possible to identify for the first time 3 photodegradation products. Thus, this work constitutes a valuable approach to the study of environmentally relevant photodegradation processes of thiram since the pathways of thiram photodegradation in the absence of synthetic catalysers, and the influence of natural organic matter on thiram photodegradation have not been studied before.

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Chapter 10

Final conclusions

Thiram is a dithiocarbamate compound widely used in Portugal as fungicide in agriculture. According to the Direção Geral de Agricultura e Desenvolvimento Rural, the Portuguese Official Agency of Agriculture, (DGADR), it is the second most popular contact fungicide among of the dithiocarbamates group and in the period 2002-2010, thiram selling rate showed a significant increase, mainly in the last year. However, in spite of the reported burst on thiram application, and in comparison to other pesticides, there was a lack of information on thiram behaviour in environmental matrices, namely, in soils and water systems.

The aim of this work was to achieve a better understanding of thiram behaviour in environmental matrices, namely, in soil and natural waters, and of the role of organic matter and copper ions in its behaviour and fate. For that, there was the need of optimizing some experimental details. First, a solid phase extraction (SPE) procedure for the clean up of the organic extracts from soil and for water samples pre-concentration was successfully developed and, second, a HPLC-UV method for the determination of thiram was optimized.

In this context, adsorption-desorption studies of thiram onto humic acids and onto soils with different organic amendments and copper contents were performed in order to investigate the role of each soil constituent in the sorption behaviour and fate of thiram. The studies revealed that thiram was strongly sorbed by humic acids and the results obtained indicated a multilayer adsorption and adsorbate-adsorbate interactions after the saturation of the surface layer. Adsorption-desorption studies of thiram onto a luvisol soil, submitted to different organic amendments, also converged to the same point, the import role of the organic matter on the sorption of thiram. However, besides of the importance of soil organic matter, this work showed a marked effect of the soil copper content on the adsorption-desorption of thiram onto soil. The experimental results put into evidence that reactions between molecules of thiram and copper ions occur along time and the extent of their occurrence during adsorption studies may be strongly dependent on the soil copper content and on the initial thiram concentration in solution (Thi:Cu ratio). For a certain equilibration time, the percentage of thiram adsorption increases with the increase of Cu:Thi ratio. Kinetic studies, performed with a soil with and without fortification with copper ions, showed that there was an extremely significant influence of both the initial thiram concentration and the soil copper content on the kinetics of thiram sorption onto soil. Thus, it was possible to conclude that the choice of the equilibration time for batch sorption studies and adsorption isotherms determination is a difficult task, mainly because the reactions with copper may involve thiram degradation into dimethyldithiocarbamate (DMDTC) that can complex with copper, which may begin during batch adsorption studies not allowing to distinguish between adsorption and degradation. Our results also show that the complexes formed with copper in soil (Thi:Cu or DMDTC:Cu) were persistent but not easily leached from the soil to groundwater. Thus, we can conclude that copper ions may contribute to the immobilization of thiram in soil, giving rise to persistent thiram bound residues.

For the study of the role of natural organic matter and copper ions in the persistence of thiram in natural waters, the effects of those components on the determination of thiram in natural waters by a C_{18}-SPE-HPLC-UV method were first studied. The present work showed that copper ions decreased considerably the recovery of thiram by SPE, but the addition of EDTA eliminated this interference. However, when EDTA was added to the river water samples previously equilibrated with thiram, the recoveries continued to be low, suggesting a partial degradation of thiram leading probably to the formation of DMDTC anions, which then complex with copper ions in solution. So, the effect of copper ions was studied in aqueous solutions containing different Thi:Cu ratios and followed by UV-Vis spectrofotometry and HPLC-MS/MS. The results obtained suggested that for an excess of thiram, a copper complex with thiram is slowly formed, which precipitates after some time. However, since most of natural waters have concentrations of Cu(II) of at least 0.01 mg L^{-1}, the most probable situation to be found in natural waters is that of an excess of Cu(II) relatively to thiram, even because thiram application in agriculture is frequently associated to the use also of copper fungicides. In the present work, for an excess of Cu(II) it was observed that a complex Thi:Cu 1:1 is formed, confirmed by HPLC-MS/MS, and the degradation rate of that complex depends on the ratio Thi:Cu and on the initial concentration of the solutions. The results obtained by UV-Vis suggested that a copper complex 1:1 is formed with the anion DMDTC, i.e., $[CuDMDTC]^+$, since the spectra of Thi-Cu solutions (1:50) after 1 day are identical to the spectra of DMDTC-Cu solutions (1:25). In fact, it was possible to conclude that these copper complexes are quite persistent, since no changes were observed in their UV-Vis spectra during a period of time of about 2 months. So, depending on the Thi:Cu ratio, the persistence of thiram in solution is affected by copper ions and copper ions stabilise the principal product of thiram degradation, the anion DMDTC. However, the presence of $[CuDMDTC]^+$ complex could not be confirmed by HPLC-MS/MS analysis. In addition, MS/MS data confirmed the existence of other copper complexes in both DMDTC:Cu and Thi:Cu solutions with an excess of copper ions. Some of the complexes formed were identified for the first time by HPLC-MS/MS. These results highlight the importance of evaluating the risks associated to the presence of thiram in waters, because of the high persistence of the degradation products formed in the presence of an excess of copper ions.

One of the important degradation pathways of pesticides is photodegradation induced by the sunlight. So, due to the lack of information about thiram photodegradation in aqueous solutions and the effect of natural organic matter, the photodegradation of thiram in aqueous solutions was also subject of study. In general, the thiram degradation follows a pseudo-first order kinetics and in the presence of humic substances (natural FA from river Vouga or commercial HA) its degradation rate was enhanced about 28% in comparison to the rate in the absence of humic substances, putting into evidence the role of humic substances as natural photosensitizers. However, when the thiram photodegradation was made in natural river water (Rio Vouga, Carvoeiro, Aveiro) there was a significative enhancement of the degradation rate of thiram (~65%), suggesting that other matrix components besides FA may be responsible for the enhancement of thiram photodegradation. Overall, it was possible to identify for the first time three photodegradation products.

Most of the studies about degradation of pesticides are focused on the active substance, being the information in literature about its commercial formulations scarce. Therefore, the consequences of the use of commercial formulations on the environment are unknown. In order to compare the behaviour of thiram in its pure form with one of its commercial formulations used in agriculture (Pomarsol Ultra D, 80% thiram from Bayer), adsorption-desorption studies onto commercial humic substances and photodegradation studies in aqueous solutions were performed. The results obtained allow us to conclude that thiram formulation components have an influence on the desorption process of thiram from humic acids, turning the pesticide more susceptible to be leached. Furthermore, the formulation components have a significant enhancement effect on the photodegradation of thiram in water.

Thus, this work constituted a valuable approach to the study of environmental relevance of thiram and contributed to fulfil some gaps identified in the existent literature about its behaviour and fate in the environment. In what concerns the soil systems it was possible to clarify the role of organic mater and, by the first time, the high importance of soil copper ions content on the sorption, persistence and mobility of thiram. Relatively to the water systems, some relevant conclusions were obtained mainly about the effect of copper ions on the degradation rate of thiram, which is increased, and on the persistence of the degradation products (copper complexes) formed, which remain unaltered in solution for at least 2 months. Also, by the first time, it was demonstrated that humic substances (natural FA or commercial HA) do increase the photodegradation rate of thiram in aqueous solution, and evidence for the possible sensitizing role of other components of natural waters was also obtained.

Finally, some thiram degradation products which can be formed in natural waters were identied for the first time

Future work should include the continuation of the thiram photodegradation experiments in order to study the effect of various scavengers on the degradation rate of thiram to clarify the thiram degradation pathways and the photosensitizing role of humic substances.

Soil incubation studies with both pure thiram and formulated thiram using soils with different copper contents and/or soils submitted to thiram application in the field should be made. With these studies it would be possible to better know the persistence of copper complexes formed and the role of formulation components on the persistence of these complexes. The identification of copper complexes formed in soil during the ageing periods, by HPLC-MS/MS, is also extremely important and must be done.

Furthermore, the toxicological properties of these complexes are also very important, since they are more persistent than the parent molecule. So, it would be very interesting to explore the effect of these complexes in some organisms.

Moreover, the know-how acquired with this work is a positive feature for the study of other dithiocarbamates in order to reach information about the role of organic matter and metal ions and, consequently, understand their behaviour and fate, namely, their mobility, persistence and degradation, in different environmental systems, and, consequently, identify their degradation products.

Chapter 11

Supplementary information

11.1 DIRECTIVE 2003/81/CE

COMMISSION DIRECTIVE 2003/81/CE of 5 de September 2003 amending Council Directive 91/414/EEC to include molinate, thiram and ziram as active substances.

11.2 Summary of the principal methods used for determination of thiram in several matrices

Table 11.1. Determination of thiram in various matrices.

Sample	Analytical method	Observations*	Ref
Formulations and wheat and rice grains	UV-Vis	**Extraction:** Chloroform **Linear:** up to 8 mg L^{-1} **Recovery:** 97-99 % (formulations) and 92-96 % (grain samples) for thiram concentrations between 10 and 150 µg	Verma et al., 1984
Synthetic mixtures, wheat grains,vegetables	UV-Vis	**Extraction:** Chloroform **Linear:** 0.44-13.25 mg L^{-1} **RSD:** 0.86% (n=10, 88.3 µg)	Malik et al., 1998
Polluted water, vegetables and wheat grains	UV-Vis	**Extraction:** Chloroform **Linear:** 0.02-0.20 mg L^{-1} **Recovery:** 93-99 %	Kesari and Gupta, 1998
Wheat grains and formulations	UV-Vis	**Extraction:** Chloroform **Linear:** 2-40 mg L^{-1}; **LOD:** 0.3 µg mL^{-1}.	Sharma et al. 2004
River waters	UV-Vis	**Pre-concentration:** *Saccharomyces cerevisiae* immobilized on sepiolite **Linear:** up to 20 mg L^{-1}: **LOD:** 0.161 mg L^{-1} **Recovery:** 96.1 % (n=5; 5-15 µg/100 mL)	Tunçeli et al., 2001
Formulations and soil samples	FIA-FTIR	**Extraction:** Chloroform **Linear:** 0.5-10 mg L^{-1}; **LOD:** 0.098 mg L^{-1} **Recovery:** 93-100 %	Cassela et al., 2000
Apples	HPLC-UV	**Extraction:** Chloroform **Clean up:** silica gel column **HPLC column:** Nucleosil RP-C_{18} (200x4mm, 5µm) **Mobile phase:** H_2O/CH_3CN (30:70, v:v), flow rate of 0.8 ml min^{-1} **UV detection:** 272 nm **Linear:** 0.02-1.4 mg L^{-1}; **LOD:** 0.01 mg L^{-1} **Recovery:** 88% (1 mg L^{-1})	Gustafsson and Thompson, 1981
Water	HPLC-UV	**Clean up:** minicolumn C_{18} (27x7mm; 70 µm) **HPLC column:** RP-ODS (250x4.6mm) **Mobile phase:** H_2O/CH_3CN 40:60 (v/v), flow rate of 1 ml min^{-1} **UV detection:** 270 nm **Recovery:** 87% (5 µg L^{-1})	Suzuki et al., 1993

*Limit of detection (LOD); Limit of quantification (LOQ); Relative standard deviation (RSD); Solid phase extraction (SPE); Solid phase micro extraction (SPME); Ultrasphere octadecylsilane reversed-phase column (RP-ODS); Cetytrimethylammonium bromide (CTAB); Matrix solid-phase dispersion (MSPD); Atmospheric pressure chemical ionization (APCI); Electrospray ionization (ESI);

Table 11.2. Determination of thiram in various matrices (continuation).

Sample	Analytical method	Observations*	Ref
Water	HPLC-UV	**Pre-concentration:** *SPE*- C_{18} **HPLC column:** Spherisorb ODS (200x4 mm; 5 µm) **Mobile phase:** H_2O/CH_3CN 58:42 (v/v), flow rate of 1.5 ml min^{-1} **UV detection:** 220 nm **Linear:** up to 2.3 g L^{-1}; **LOD:** 0.093 mg L^{-1} **RSD:** 0.48 % (1.3-10 mg L^{-1}, n=7) **Recovery:** ~50% in milli-Q water (2.0 µg L^{-1}) and not recovery in tap, natural and underground water samples	Tovar and Santos-Delgado, 1995
Water	HPLC-UV	**Pre-concentration:** *SPE*- C_{18} **HPLC column:** Spherisorb ODS-2 (100x4 mm; 3 µm) **Mobile phase:** 10mM CTAB aqueous solution buffered with phosphate (10 mM):acetonitrile 80:20 (v/v), flow rate of 1 mL min^{-1}; **UV detection:** 254 nm **Linear:** up to 30 mg L^{-1}; **LOD:** 25 µg L^{-1} **RSD:** 5 % (0.2 mg L^{-1}, n=5) **Recovery:** 70% (5 µg L^{-1})	Garcia et al., 1996
Fruits and vegetables	HPLC-UV	**Extraction:** Ethyl acetate/cyclo-hexane 10:30 (v/v) in an ultrasonic bath **HPLC column:** Apex II diol (150x4.6 mm; 3 µm) **Mobile phase:** (A) cyclohexane, (B) cyclohexane/2-propanol/methanol/10 µL of 25% ammonia per 250 mL of solution (80+15+5+0.01 v/v/v/v), and (C) 2-propanol; gradient program of 95% of A and 5% of B and after 18 min changed to 54% of A, 20% of B and 26% of C, flow rate of 0.65 mL min^{-1} **UV detection:** 225, 240, 283 and 330 nm **LOQ:** 0.1-0.2 mg kg^{-1} (0.1-1 mg kg^{-1}) **Recovery:** 40-92% ((0.1-1.3 mg kg^{-1})	Ekroth et al., 1998
Water	HPLC-UV	**Extraction/clean up:** SPE-active carbon **Linear:** 0.01-0.5 mg L^{-1}; **LOD:** 4.5 µg L^{-1} **RSD:** 4.9-5.3% (2.5 µg L^{-1}) **Recovery:** 89-94 % (2.5 µg L^{-1})	Kitami et al., 2002
Water	HPLC-UV	**Pre-concentration:** SPME **HPLC column:** C18 (250x4.6 mm; 5 µm) **Mobile phase:** H_2O/CH_3CN 30:70 (v/v), flow rate of 0.7 mL min^{-1}; **UV detection:** 254 nm **Linear:** 5-600 µg L^{-1}; **LOD:** 1 µg L^{-1} **RSD:** 2.6 % (20-40 µg L^{-1}, n=3) **Recovery:** 96-98% (20-40 µg L^{-1})	Aulakh et al., 2005

*Limit of detection (LOD); Limit of quantification (LOQ); Relative standard deviation (RSD); Solid phase extraction (SPE); Solid phase micro extraction (SPME); Ultrasphere octadecylsilane reversed-phase column (RP-ODS); Cetytrimethylammonium bromide (CTAB); Matrix solid-phase dispersion (MSPD); Atmospheric pressure chemical ionization (APCI); Electrospray ionization (ESI);

Table 11.3. Determination of thiram in various matrices (continuation).

Sample	Analytical method	Observations*	Ref
Formulations, Soil samples and soybean	HPLC-UV	**Extraction:** acetone and dichloromethane **HPLC column:** RP-C18 (RP-C18e Purosphere STAR and RP-Select B Lichrospher) (250x4 mm; 5 μm) **Mobile phase:** H_2O/CH_3OH 35:65 (v/v), flow rate of 0.75 mL min^{-1}; **UV detection:** 217 nm **Linear:** 2-200 mg L^{-1}; **LOD:** 0.2 mg L^{-1} **RSD:** 1.55 % (100 mg L^{-1}, n=6) **Recovery:** 86.9-99 % (50-100 mg L^{-1})	Walia et al., 2009
Water, soil, plants	HPLC-UV	**Extraction:** Ethyl acetatefor for water samples, acetonitrile for soil samples and dichloromethane for plants **HPLC column:** RP-C18 (250x4.6 mm; 5 μm) **Mobile phase:** H_2O/CH_3CN 30:70 (v/v), flow rate of 1 mL min^{-1}; **UV detection:** 223 nm **Linear:** 1-200 mg L^{-1} **Recovery:** 90 ± 5% for water and soil samples	Gupta et al., 2012a
Apples	HPLC-EC	**Extraction:** Chloroform **HPLC column:** Lichrosorb RP18 (100x4.6 mm, 5 μm) **Mobile phase:** 0.01 mol L^{-1} phosphate buffer pH 7.4/CH_3CN (55:45, v:v), flow rate of 2 ml min^{-1} **Detection:** graphite electrode modified with poly(tetrafluoroethylene) (Teflon) **Linear:** 1-10 mg L^{-1}; **LOD:** 0.14 mg L^{-1} **Recovery:** 97% (0.5 mg Kg^{-1})	Fernandez et al., 1996
Apples and grapes	HPLC-EC	**Extraction:** Chloroform **HPLC column:** Sperisorb ODS-2 (150x3.9 mm, 5 μm) **Mobile phase:** H_2O/methanol (60:40, v/v) containing ammonium acetate buffer (pH 5), flow rate of 0.9 ml min^{-1} **Detection:** glassy carbon electrode **Linear:** up to 10 mg L^{-1}; **LOD:** 0.7 mg L^{-1} **Recovery:** 79.3% (0.203 μg g^{-1}) for apples samples and 83.0% (0.180 μg g^{-1}) for grapes samples	Silva et al. 1999
Tap water and beetroot juice	HPLC-EC	**Extraction:** Acetonitrile (beetroot juice) **HPLC column:** Diaspher C16 (150x4.6 mm, 5 μm) **Mobile phase:** CH_3CN/0.1% aqueous solution of phosphoric (40:60, v/v), flow rate of 0.7 ml min^{-1} **Detection:** glassy carbon electrode **Linear:** 0.01-0.15 or 0.006-50 mg L^{-1} **LOD:** 3 or 0.6 μg L^{-1}; **RSD:** 2 % (10 μg L^{-1}, tap water, n=3); 4 % (20 μg L^{-1}, beetroot juice, n=3) **Recovery:** 98% (10 μg L^{-1}, tap water, n=3); 90 % (20 μg L^{-1}, beetroot juice, n=3)	Shapovalova et al., 2009

*Limit of detection (LOD); Limit of quantification (LOQ); Relative standard deviation (RSD); Solid phase extraction (SPE); Solid phase micro extraction (SPME); Ultrasphere octadecylsilane reversed-phase column (RP-ODS); Cetytrimethylammonium bromide (CTAB); Matrix solid-phase dispersion (MSPD); Atmospheric pressure chemical ionization (APCI); Electrospray ionization (ESI);

Table 11.4. Determination of thiram in various matrices (continuation).

Sample	Analytical method	Observations*	Ref
Fruits, cereals and vegetables	HPLC-APCI-MS	**Extraction:** *MSPD* → ENVICarb, CH_2Cl_2/CH_3OH (80:20, v/v); **HPLC column:** C8 Phenomenex (150x4.6mm, 5 μm) **Mobile phase:** water and methanol, whose gradient is: t = 0, 10% methanol, t = 7.5 min, 70% methanol, t = 12.5 min, 90% methanol and t = 15 min, 90% methanol, flow rate of 0.8 ml min^{-1} **MS Detection**: ionization source was positive polarity atmospheric pressure APCI **Linear:** 0.25-50 mg L^{-1}; **LOD:** 0.05 mg L^{-1} **Recovery:** 70-101% (n=5; 2.5 mg mL^{-1}); 65-104% (n=5; 25 mg mL^{-1}).	Blasco et al., 2004
River waters	HPLC-TOF-MS	**Pre-concentration:** *SPE* **HPLC column:** C18 Atlantis (150x4.6mm, 5 μm) **Mobile phase:** Water, acetonitrile and acid formic 2%, whose gradient is: t = 0 min, 95% water and 5% acid formic 2%, t = 2 min, 60% water, 30 acetonitrile and 5% acid formic 2%, t = 12 min, 95 acetonitrile and 5% acid formic 2% and t = 17 min, 95% water and 5% acid formic 2%, flow rate of 1 ml min^{-1} **MS Detection**: positive polarity atmospheric pressure ESI **Linear:** 1-500 μg L^{-1}; **LOD:** 0.18 μg L^{-1} **Recovery:** < 25 %	Sasaki et al., 2006
Olive oil	HPLC-MS	**Extraction:** Tetrahidrofurano **HPLC column:** C18 Chrompack (150x3 mm, 5 μm) **Mobile phase:** water (buffered at pH 4.5 with acetic acid and 10^{-3} mol L^{-1} amonium acetate) and methanol, whose gradient was 2 to 100% acetonitrile in 32min, flow rate of 0.4 ml min^{-1} **UV Detector:** 210 nm **MS Detection**: ionization source was positive polarity atmospheric pressure electrospray (ESI) **Linear:** 0.8-10 mg L^{-1}; **LOD:** 0.4 mg L^{-1} **Recovery:** not recovery	Barrek et al., 2003
Pears	MS	Direct analysis in real time (DART) combined with medium-high resolution/accurate mass time-of-flight mass spectrometry (TOF-MS) and high-resolution/accurate mass Orbitrap MS: calibration levels: 1 mgkg^{-1} Desorption electrospray ionization (DESI) combined with tandem-in-time mass spectro-metry (MS2): calibration levels: 0.1 mg kg^{-1}	Cajka et al., 2011

*Limit of detection (LOD); Limit of quantification (LOQ); Relative standard deviation (RSD); Solid phase extraction (SPE); Solid phase micro extraction (SPME); Ultrasphere octadecylsilane reversed-phase column (RP-ODS); Cetytrimethylammonium bromide (CTAB); Matrix solid-phase dispersion (MSPD); Atmospheric pressure chemical ionization (APCI); Electrospray ionization (ESI);

Table 11.5. Determination of thiram in various matrices (continuation).

Sample	Analytical method	Observations*	Ref
Water and soils	Adsortive cathodic stripping voltammetry	**Extraction:** chloroform (soil samples), SPE-C_{18} (water samples) **Electrode:** Dropping mercury electrode **Accumulation time:** 120 and 600 s at -0.2 V **Linear:** 0.1 – 5 µg L^{-1}; **LOD:** 0.3 and 0.03 µg L^{-1} for 120 and 600 s of accumulation time, respectively **Recovery:** 88-96% (1-5 µg L^{-1}, water samples); 89-94% (1-20 µg L^{-1}, soil samples)	Procopio et al., 1988
Strawberries	Linear sweep voltammetry,	**Extraction:** chloroform **Electrode:** graphite-poly (tetrafluoroethy-lene) composite electrodes **Accumulation time:** 300 s at 0 V **Linear:** 0.05 – 2.4 mg L^{-1}; **LOD:** 13.1 µg L^{-1} **Recovery:** 97% (1.17 mg L^{-1}, n=5)	Fernandez et al., 1995
Synthetic samples	Voltammetry, Carbon electrode	**Electrode:** carbon paste electrode modified with iron (II) and cobalt(II) phthalocyanines **Recovery:** 97 % (10 µM, n=6)	Shaidarova et al., 2001
Water leachates from soil, bean seed samples	Ampereometric	**Extraction**: chloroform **Electrode:** Glassy carbon electrode **Linear:** 6.7-24 µg L^{-1}; **LOD**: 2.4 µg L^{-1} **Recovery:** 47 % (bean seed samples)	Priyantha and Weliwegamage, 2005
Synthetic samples	Polarography	**Electrode:** **Linear:** 2.4-240 µg L^{-1}; **LOD:** 0.5 µg L^{-1} **RSD:** 1.2% (n = 10); **Recovery:** 83.8% - 91.4%.	Wang et al., 2008
Formulations, grains residues (wheat and rice), aqueous samples	Differential pulse polarography	**Extraction:** chloroform **Electrode:** Dropping mercury electrode **Linear:** 0.08 - 4 mg L^{-1}; **RSD:** 1.2%; **Recovery:** 97% - 99% (Formulations); 86% - 91% (Wheat); 84% - 94% (Rice); 93% - 99% (Water).	Sharma et al., 2011
Wheat grains	Capillary electrophoresis	**Extraction:** chloroform **LOD:** 0.5 mg L^{-1}; **Recovery:** 98.8%	Malik and Faubel, 2000.
Sintethic samples	ELISA	**Linear:** 0.02-40 mg L^{-1} **LOD:** 30 µg L^{-1}.	Gueguen et al. 2000
Lettuces	ELISA	**Extraction:** acetone/water (50:50 v/v) **LOD:** 5 or 40 µg L^{-1}, depending of the imunoassays used (microwell plates or tube based assay) **Recovery:** 80-120 % (1.7-27 mg L^{-1})	Queffelec et al., 2001

*Limit of detection (LOD); Limit of quantification (LOQ); Relative standard deviation (RSD); Solid phase extraction (SPE); Solid phase micro extraction (SPME); Ultrasphere octadecylsilane reversed-phase column (RP-ODS); Cetytrimethylammonium bromide (CTAB); Matrix solid-phase dispersion (MSPD); Atmospheric pressure chemical ionization (APCI); Electrospray ionization (ESI);

Table 11.6. Determination of thiram in various matrices (continuation).

Sample	Analytical method	Observations*	Ref
Honeybees	Chemiluminescence -ELISA	**Extraction:** liquid-liquid or SPE with graphitized carbon; **Linear:** 0.009-15 mg L^{-1}; **LOD:** 17 $\mu g L^{-1}$ (liq-liq extract) and 19 $\mu g L^{-1}$ (SPE extraction) **Recovery:** 60 % (75 $\mu g L^{-1}$, liq-liq extract); 72 % (75 $\mu g\ L^{-1}$, SPE extraction).	Girotti et al., 2008
Natural waters	Flow-injection chemiluminesce detection (FI-CL)	**Pre-concentratio:** *SPE*- C_{18} **Linear:** 7.5 –2500 $\mu g\ L^{-1}$; **LOD:** 7.5 $\mu g\ L^{-1}$; **RSD:** 2.5% (n=10, 500 $\mu g\ L^{-1}$ thiram) **Recovery:** 99-104% (25-100 $\mu g\ L^{-1}$)	Waseem et al, 2010
Tomatoes	High-performance thin layer chromatographic (HP-TLC)	**Extraction:** mechanical vibration with acetone-dichloromethane 1:1 (v/v); **TLC:** glass-backed silica gel 60F (254) HPTLC plates. **Eluent:** hexane/acetone (60:404,v/v) **Linear:** 10-300 ng; **LOD:** 30 ng **RSD:** 4.6-22 % (0.2-5 mg Kg^{-1}, n=3) **Recovery:** 67 – 98 % (RSD =0.13-22.06%)	Fan et al., 2007

*Limit of detection (LOD); Limit of quantification (LOQ); Relative standard deviation (RSD); Solid phase extraction (SPE); Solid phase micro extraction (SPME); Ultrasphere octadecylsilane reversed-phase column (RP-ODS); Cetytrimethylammonium bromide (CTAB); Matrix solid-phase dispersion (MSPD); Atmospheric pressure chemical ionization (APCI); Electrospray ionization (ESI);

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