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# Effect of copper ions on the degradation of thiram in aqueous solution: identification of degradation products by HPLC-MS/MS

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3 **Highlights**

- 4 • Cu(II) in excess accelerates the degradation of thiram in aqueous solutions
- 5 • The  $[\text{CuThi}]^{2+}$  complex degrades into  $[(\text{DMDTC})\text{Cu}]^+$  which readily decomposes
- 6 • New degradation products of  $[\text{CuThi}]^{2+}$  were identified for the 1<sup>st</sup> time by HPLC-MS<sup>n</sup>
- 7 • Some degradation products are quite persistent, at least during two months

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13 **ABSTRACT**

14 The aim of this work was to examine the effect of Cu(II) on the degradation of thiram (Thi) in  
15 aqueous solutions, since the literature focused on this effect is scarce and copper based  
16 fungicides can be applied together with thiram or during the same season to agricultural crops.

17 The effect of Cu(II) on the degradation of thiram was followed by both UV-Vis and HPLC-  
18 MS/MS. When thiram is dissolved in pure water its degradation occurs very slowly, being  
19 negligible during the first 7 days. However, the presence of Cu(II) has a strong influence on the  
20 thiram degradation in aqueous solutions along time. In the presence of an excess of Cu(II), a  
21  $[\text{CuThi}]^{2+}$  complex is initially formed which degrades into a complex formed between the  
22 dimethyldithiocarbamate anion (DMDTC) and Cu(II) ion,  $[\text{Cu}(\text{DMDTC})]^+$ . This complex further  
23 degrades leading to other copper complexes which were identified for the first time, by MS<sup>n</sup>. The  
24 results obtained in the present work also demonstrated that a redox reaction involving DMDTC

25 anions and Cu(II) ions gives rise to the formation of a Thi-Cu(I) complex. Finally, some of the  
26 complexes resulting from the degradation of  $[\text{CuThi}]^{2+}$  are quite persistent in solution for long  
27 periods of time (> one month).

28

29 KEYWORDS. Thiram degradation; copper ions; dimethyldithiocarbamate; aqueous solutions;  
30 UV-Vis spectrophotometry; HPLC-UV-MS<sup>n</sup>

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## 34 **1 Introduction**

35 Pesticides are intensively used in agriculture and much effort is devoted to control and reduce  
36 possible damaging effects on the environment, such as contamination of soil and leaching to  
37 ground and surface waters, with the possible contamination of aquatic organisms, and,  
38 ultimately, contamination of water and food consumed by human beings, with the consequent  
39 toxic effects. The fate of a pesticide is determined by processes that affect mobility, such as  
40 sorption or volatilization, and those that affect persistence, including photo-, chemical and  
41 microbial degradation. According to the literature, the degradation products of some pesticides  
42 may be more toxic and persistent, representing a higher environmental risk than the parent  
43 compounds [1,2]. To understand the fate of a pesticide in soil and water systems an accurate  
44 knowledge of its environmental behavior is essential.

45 Thiram, tetramethylthiuramdisulfide, is a dithiocarbamate compound that has been used as a  
46 contact fungicide with preventive action, worldwide applied not only in agriculture, but also in  
47 rubber industry as an accelerator and vulcanization agent [3,4]. In Portugal, thiram was

48 considered the second most popular contact fungicide of the dithiocarbamate group, after  
49 manconzeb. Dithiocarbamates contributed with ~12% of the total sales of fungicides, followed  
50 by the copper-based fungicides (~10%) [5].

51 Because of the worldwide use of Cu(II) based fungicides, copper effects on the behavior of  
52 some organic pesticides in environmental matrices have been object of attention [6-10].  
53 However, the literature dealing with the effect of Cu(II) on the behavior of thiram in the  
54 environment is scarce [11], despite the fact that Cu(II) based fungicides are frequently applied in  
55 the same season and/or in the same crops as thiram, increasing the effectiveness of thiram  
56 fungicidal action. Recently, Gupta et al. [12,13] studied the persistence of thiram in water and  
57 soil, under controlled conditions. However, in both studies there is no reference to the possible  
58 effect of metal ions, namely copper ions.

59 In our previous work [14], data about thiram recovery from natural waters showed fast thiram  
60 degradation in environmental matrices. Thiram was completely recovered (>80%) from river  
61 water samples when analyzed immediately after spiking but scarcely recovered when analyzed  
62 after one or two days. Several thiram recovery experiences in the presence of EDTA suggested  
63 that metal ions, namely copper ions, were involved in thiram degradation. This mechanism might  
64 be environmentally relevant since, as referred above, copper based fungicides are often applied  
65 either in the same season or in the same crops as thiram.

66 Thus, the aim of this work was to examine the effect of copper ions on the degradation of  
67 thiram in aqueous solutions. The effect of copper ions was studied during one or two months,  
68 following the UV-Vis spectral changes of different thiram-Cu(II) mixtures. The identification of  
69 complexes formed over time was also studied by HPLC-MS/MS.

70

## 71 2 Experimental

### 72 2.1 Chemicals and solutions

73 All chemicals used were of analytical grade and ultra-pure water was obtained using a Milli-Q  
74 water purification system (Millipore). Thiram (Thi, 97%) and acetonitrile (HPLC grade) were  
75 obtained from Aldrich and LabScan, respectively. Sodium dimethyldithiocarbamate solution  
76 (DMDTC, purum, ~ 40% in H<sub>2</sub>O) and cupric perchlorate hexahydrate were purchased from  
77 Fluka. Cupric acetate, used in the solutions preparation for MS analysis, was from May and  
78 Baker LTD. Aqueous thiram stock solutions 20 mg L<sup>-1</sup> were prepared by previous dissolution of  
79 thiram in acetonitrile followed by dilution with water (percentage of acetonitrile in the final  
80 solution always lower than 1%). Stock solutions of 1000 mg L<sup>-1</sup> Cu(II) and 0.5 g L<sup>-1</sup> DMDTC  
81 were prepared, from the reagents, in ultrapure water. Thiram standard solutions 2.0 mg L<sup>-1</sup> with  
82 increasing copper contents were prepared by dilution of both 20 mg L<sup>-1</sup> thiram and 1000 mg L<sup>-1</sup>  
83 Cu(II) stock solutions, obtaining the following Thi:Cu(II) molar ratios: 1:3, 1:10, 1:25 and 1:50.  
84 DMDTC standard solutions 2.0 mg L<sup>-1</sup> with increasing copper contents were prepared by  
85 dilution of both 0.5 g L<sup>-1</sup> DMDTC and 1000 mg L<sup>-1</sup> Cu(II) standard solutions, obtaining the  
86 following DMDTC:Cu(II) molar ratios: 1:3, 1:10 and 1:25.

87

### 88 2.2 UV-Vis spectrophotometry

89 UV-Vis spectra of Thi and DMDTC standard solutions and respective mixtures with copper  
90 perchlorate were recorded against Milli-Q water in a UV-Vis Shimadzu Spectrophotometer using  
91 1.00 cm cells. The pH of the solutions was measured using a pH-meter Orion 720A, with a  
92 combined pH electrode Orion ROSS 8172BN.

93

### 94 **2.3 Identification of degradation products by HPLC-MS<sup>n</sup>**

95 The HPLC system consisted of a variable loop Accela auto sampler (set at a temperature of 16  
96 °C), an Accela 600 LC pump and an Accela 80 Hz PDA detector (Thermo Fisher Scientific, San  
97 Jose, Ca, USA). Analyses were carried out using a phenomenex C<sub>18</sub> column (150x4.60 mm, 5  
98 μm, 110 Å). The separation of the compounds was carried out with a mobile phase of  
99 acetonitrile:water (60:40, v/v) with 0.1% HCOOH at a flow rate of 0.7 ml min<sup>-1</sup>, at 25°C. The  
100 injection volume in the HPLC system was 20 μL. Single online detection was carried out in PDA  
101 detector, at 270 nm, and UV spectra in the range of 200-600 nm were also recorded for relevant  
102 chromatographic peaks. The HPLC was coupled to a LCQ Fleet ion trap mass spectrometer  
103 (ThermoFinnigan, San Jose, CA, USA), equipped with an ESI source and operating in positive  
104 mode. The flow rate of nitrogen sheath and auxiliary gas were 40 and 5 (arbitrary units),  
105 respectively. The spray voltage was 5 kV and capillary temperature 300°C. The capillary and  
106 tune lens voltages were set at -28 V and -115 V, respectively. Collision-induced dissociation  
107 (CID)-MS<sup>n</sup> experiments were performed on mass-selected precursor ions in the range of m/z  
108 100–1000. The isolation width of precursor ions was 1.0 mass unit. The scan time was equal to  
109 100 ms and the collision energy was optimized between 15-40 (arbitrary units), using helium as  
110 collision gas. The data acquisition was carried out by using Xcalibur® data system  
111 (ThermoFinnigan, San Jose, CA, USA).

112

## 113 **3 Results and Discussion**

### 114 **3.1 Evaluation of thiram stability in aqueous solution**

115 To evaluate thiram stability in aqueous solution, UV-Vis spectra and pH values of a 2.0 mg L<sup>-1</sup>  
116 thiram aqueous solution were monitored during one month (Figures S1A and S1B, respectively

117 of the Supplementary data). The spectrum of a fresh thiram solution exhibits two absorption  
118 maxima, at 220 nm and at 272 nm, showing no significant changes up to the 7<sup>th</sup> day (Figure S1A,  
119 curve a). For longer periods, the absorbance maximum at 272 nm begins to decrease and a new  
120 maximum appears at 207 nm (Figure S1A, curve c). From the 11<sup>th</sup> day onwards, a sharp rise of  
121 the pH of the solution is observed (Figure S1B).

122 The degradation of thiram into DMDTC due to the cleavage of the disulphide bond is  
123 frequently reported in the literature as being the first step of thiram degradation in environmental  
124 matrices [3,15] and it has been also referred its occurrence in solutions of thiram in milli-Q water  
125 [12]. However, the UV spectra changes observed in the present study can't be explained only by  
126 the formation of DMDTC. In fact, the UV spectrum of the fresh DMDTC solution (Figure S2)  
127 exhibited two absorption maxima: one at 254 and the other at 280 nm. It is possible to see a  
128 significant decrease of the bands at the end of the 1<sup>st</sup> day, coincident with the appearance of a  
129 new band at ca. 207 nm, previously observed for thiram degradation (Figure S1A). After 2 days,  
130 the degradation of DMDTC is almost complete (Figure S2, curve d). It is interesting to notice  
131 that the absorption maxima at 250 and 280 nm, characteristic of DMDTC, do not appear in the  
132 spectra of thiram solutions after several ageing times, suggesting that, although being a  
133 degradation product of thiram, DMDTC is only an intermediate which undergoes further  
134 degradation, in agreement with its aqueous solution behavior.

135 The results obtained for thiram aqueous solutions and described above are in agreement with  
136 those obtained by Gupta et al. [12,13] who followed the degradation of thiram in aqueous  
137 solution by HPLC-UV and HPLC-MS. These authors observed that the degradation of thiram, at  
138 pH 5.5, was quite slow. The authors did not detect any other products in solution, besides thiram,  
139 until 7 days and, only after 11 days DMDTC and other degradation products were detected. In

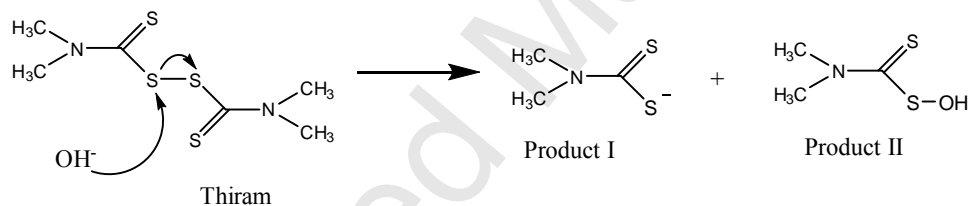


140 the present work, as the aqueous solution is not buffered, degradation of thiram is initially very  
 141 slow because the pH is low. However, after 11 days, the pH of the solution increases and the  
 142 increase of pH increases the degradation rate of thiram, as previously observed by Gupta et al.  
 143 [12]. The effect of pH on the rate of thiram hydrolysis can be explained assuming that the first  
 144 step of the hydrolysis of thiram in water involves the attack of  $\text{OH}^-$  to the SS bond, as reported  
 145 for other organic compounds with disulfide bonds [16], giving rise to the formation of DMDTC  
 146 (product I) and dimethyl dithiocarbamoylsulfenic acid (product II), as shown in Scheme 1.

147

148

149 **Scheme 1:** Proposed mechanism for thiram hydrolysis in alkaline medium



150

151 These two degradation products were identified by Gupta et al. [13] as being the first which are  
 152 formed during the degradation of thiram in water buffered at pH 8.0. However, the same authors  
 153 did not detect the presence of the dimethyl dithiocarbamoylsulfenic acid during the degradation  
 154 of thiram at pH 5.5. The increase of pH, which starts after the 11<sup>th</sup> day, may be attributed to the  
 155 fact that the degradation of the intermediate DMDTC can give rise to the consumption of  $\text{H}^+$   
 156 (according to the degradation Scheme S1 of the Supplementary data). Indeed, an increase of pH  
 157 was also observed during the degradation of DMDTC in water, but this increase was fast (2  
 158 days), in agreement with the fast degradation of DMDTC and it was of the same order of  
 159 magnitude ( $\sim 0.6$  units) as the increase of pH observed between the 11<sup>th</sup> and 17<sup>th</sup> days in the

160 solutions of thiram. The possibility of contribution of other reactions to the higher increase of pH  
161 observed for longer ageing times can't be excluded.

162 Thus, the results suggest that, at room temperature and in the absence of light, non-buffered  
163 thiram solutions prepared in milli-Q water remain stable during the first 7 days of storage. After  
164 that period, thiram degrades following a 1<sup>st</sup> order kinetics ( $R^2 = 0.9591$  for the non-linear  
165 regression analysis of absorbance at 272 nm vs. time). Using absorbance data between 7 and 42  
166 days a kinetic rate constant of  $0.057 \pm 0.006 \text{ d}^{-1}$  and a half life of 12 days were obtained.  
167 Considering the initial period of thiram stability, 7 days, followed by its first order decay, a  
168 global half life time of 19 days was obtained for thiram in milli-Q water, which corresponds to a  
169 higher stability than that reported in our previous work for thiram in natural waters [14]. As the  
170 results of that work suggested that metal ions, namely copper ions, were involved in thiram  
171 degradation, the stability of thiram in aqueous solutions containing Cu(II) was studied in more  
172 detail.

173

### 174 **3.2 UV Spectral evidence of changes in the composition of Thi:Cu(II) solutions along time**

175 In natural waters the concentrations of thiram will certainly be quite low and it is expected that  
176 the concentration of Cu(II) will be higher. Cu(II) concentrations in natural waters can be in the  
177 order of 1 to 100  $\mu\text{g L}^{-1}$  [17], while pesticides' concentrations are in the order of  $\text{ng L}^{-1}$  to a few  
178  $\mu\text{g L}^{-1}$  [18,19], being 0.1  $\mu\text{g L}^{-1}$  the maximum allowed concentration in natural waters for each  
179 individual pesticide or 0.5  $\mu\text{g L}^{-1}$  for total pesticides [20]. Thus, in order to study the degradation  
180 of thiram in the presence of an excess of Cu(II), thiram solutions were prepared, with Thi-Cu(II)  
181 molar ratios 1:3, 1:10, 1:25 and 1:50, and initial concentrations of thiram 2  $\text{mg L}^{-1}$ , 0.2  $\text{mg L}^{-1}$   
182 and 11  $\mu\text{g L}^{-1}$ . The evolution of the UV-Vis spectra over time for the solutions containing 2 mg

183  $L^{-1}$  of thiram is shown in Fig 1. After mixing thiram with Cu(II), the spectrum of thiram gives  
184 rise to spectra dominated by two absorption maxima at 260 nm and 420 nm, respectively. Over  
185 time, the maximum at 420 nm decreases and gives rise to a new maximum at 385 nm. The  
186 formation of the species responsible for the absorption at 420 nm and the conversion to those  
187 responsible for the absorption at 385 nm occur faster when the excess of copper is higher. Thus,  
188 after 24 h of equilibration the absorbance of the maximum at 420 nm is higher in the solutions  
189 with the higher content of Cu(II), i.e, the absorbance at 420 nm increases as the Thi:Cu(II) ratio  
190 decreases from 1:3 to 1:25 ( $1:3 < 1:10 < 1:25$ ). The solution Thi:Cu 1:50 already shows a decrease  
191 of the maximum at 420 nm and the appearance of the new maximum at 385 nm. For the solution  
192 with the lowest content of Cu(II), the solution with Thi:Cu ratio 1:3, after 7 days the absorption  
193 maximum at 420 nm is still present and the maximum at 385 nm is not observed. For longer  
194 periods, the replacement of the maximum at 420 nm is also observed for this solution, so that, at  
195 the end of the 14<sup>th</sup> day, only the absorption maxima at 385 and 260 nm are present in the UV-Vis  
196 spectra of all the solutions (Figure 1, 14<sup>th</sup> day). One hypothesis to explain the reported  
197 observations is the following: in the presence of an excess of Cu(II) a Thi:Cu(II) complex is  
198 formed. The formation of a Thi:Cu(II) complex 1:1 with a stability constant of  $\log \beta = 5.38$  is  
199 documented in the literature [21]. This complex degrades over time, being converted into the  
200 species responsible for the absorption at 385 nm. Weissmahr et al. [22], reported a spectrum  
201 identical to those of solutions Thi:Cu after 14 days, with maxima at 260 nm and 385 nm, for an  
202 aqueous solution of DMDTC:Cu 1:100 and they attributed it to a 1:1 DMTC:Cu complex, i.e.  
203  $[Cu(DMDTC)]^+$ . However, the authors have not reported any insight into the identification of the  
204 complex formed.

205 In order to clarify whether the degradation of thiram in the presence of an excess of copper  
206 gives rise to the  $[\text{Cu}(\text{DMDTC})]^+$  complex, some experiences were performed with solutions  
207 containing  $2.0 \text{ mg L}^{-1}$  DMDTC and copper ion at the following DMDTC:Cu(II) molar ratios of  
208 1:3, 1:5, 1:10 and 1:25. Figure 2 shows the UV-Vis spectra of those solutions over time. For a  
209 1:3 DMDTC:Cu ratio, the spectrum registered immediately after solution preparation shows the  
210 absorption maxima at 262, 303 and 450 nm, but a shoulder at 385 nm is already observed; for  
211 longer periods of time, the maximum at 450 nm decreases, and at the end of the 28th day, only  
212 the absorption maxima at 385 and 260 nm are present in the UV-Vis spectra (Figure 2, 28<sup>th</sup> day).  
213 The UV-Vis spectra for the 1:10 and 1:25 DMDTC:Cu(II) mixtures show only the absorption  
214 maxima at 385 and 262 nm, even immediately after solution preparation (Figure 2, time 0 hours).  
215 The results obtained for the 1:3 ratio suggest that a  $[\text{Cu}(\text{DMDTC})]^+$  complex, characterized by  
216 absorption maxima at 262, 303 and 450 nm, is first formed in the solution. This complex  
217 degrades along time giving rise to a spectrum characterized by absorption maxima at 385 and  
218 262 nm, as the spectra of aged solutions of Thi:Cu.(Figure 1, 14 and 30 days). The degradation  
219 of the  $[\text{Cu}(\text{DMDTC})]^+$  complex is very fast in the presence of higher excess of Cu (1:10 and 1:25  
220 ratios), reason why the absorption maximum of the DMDTC:Cu complex at 450 nm is not  
221 observed in solutions with 1:10 and 1:25 ratios, even when the spectra are recorded immediately  
222 after preparation. It also interesting to notice that in the presence of a high excess of copper ions,  
223 1:25 DMDTC:Cu, the complexes responsible for the absorption at 260 and 385 nm are  
224 immediately formed and more than 80% of their initial absorption still remains after 28 days,  
225 suggesting a high persistence of these complexes (as can also be seen in Figure S3 and Table S1  
226 of the Supplementary data). As two molecules of DMDTC are equivalent to one thiram molecule  
227 in terms of electron-donor atoms, we compared the spectra of solutions containing Thi:Cu 1:50

228 with the spectra of solutions DMDTC:Cu 1:25 (Figure S4, Supplementary data). Both solutions  
229 exhibit two absorption maxima at 262 and 385 nm. The absorbances of the DMDTC:Cu solution  
230 are kept approximately constant during 2 months after the solution preparation and they are  
231 similar to the absorbances of the Thi:Cu solution after the same time. These results suggest that,  
232 in the presence of an excess of Cu, thiram and DMDTC give rise to the same products which  
233 absorb at 260 and 385 nm and which are quite persistent in solution.

234 The variation of pH along the ageing time of Thi:Cu solutions is shown in Figure S5 of the  
235 Supplementary data. As the Cu:Thi ratio increases, the increase of pH is lower or even inexistent  
236 (for high contents of copper). This may be attributed to a decrease of the percentage of free  
237 thiram in the presence of higher concentrations of Cu(II), different degradation pathways for the  
238 Cu complexes and the free ligands (Thiram and DMDTC) and/or to the buffering capacity of the  
239 Cu salts in solution due to the formation of copper hydroxo complexes.

240 The influence of the initial concentration of thiram on the rate of occurrence of these  
241 processes is discussed in the Supplementary data, Section S3.

242 In order to identify the degradation products which are formed, some of the solutions were  
243 analyzed by HPLC-MS/MS.

244

### 245 3.3 Identification of the complexes by HPLC-MS/MS

246 HPLC-MS analysis of Thi:Cu(II) complex, in the LC-MS conditions showed that this complex  
247 elutes at a retention time of ~4.7 min, prior to thiram (~4.8 min) and the ion observed in the MS  
248 spectrum in positive mode has an  $m/z$  value of 303. Since the Thi:Cu solution was prepared by  
249 mixing thiram and Cu(II), it was expected that the complex formed in solution would be  
250  $[\text{CuThi}]^{2+}$ , whose  $m/z$  value is 151.5. However this ion is absent in the MS spectra. Thus, the

251 presence of the ion at  $m/z$  303, was identified as  $[\text{CuThi}]^+$  indicating that the reduction of Cu(II)  
 252 may have occurred during the ESI ionization process. This reduction behavior has already been  
 253 described in literature for Cu(II) pyridil chelates [23], Cu(II)-resveratrol complexes [24] and  
 254 dinuclear Cu(II) complexes of isomeric bis-(3-acetylacetonate)benzene ligands [25]. According  
 255 to the literature, this process can be due to one charge transfer between the solvent and the metal  
 256 complex in the gas phase. The  $\text{MS}^n$  fragmentation pattern of the molecular ion at  $m/z$  303 at  
 257 retention time  $\sim 4.7$  min is shown in **Scheme 2**.

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261 **Scheme 2:** Proposed fragmentation pathways of the ion at  $m/z$  303 for  $[\text{CuThi}]^{2+}$  complex

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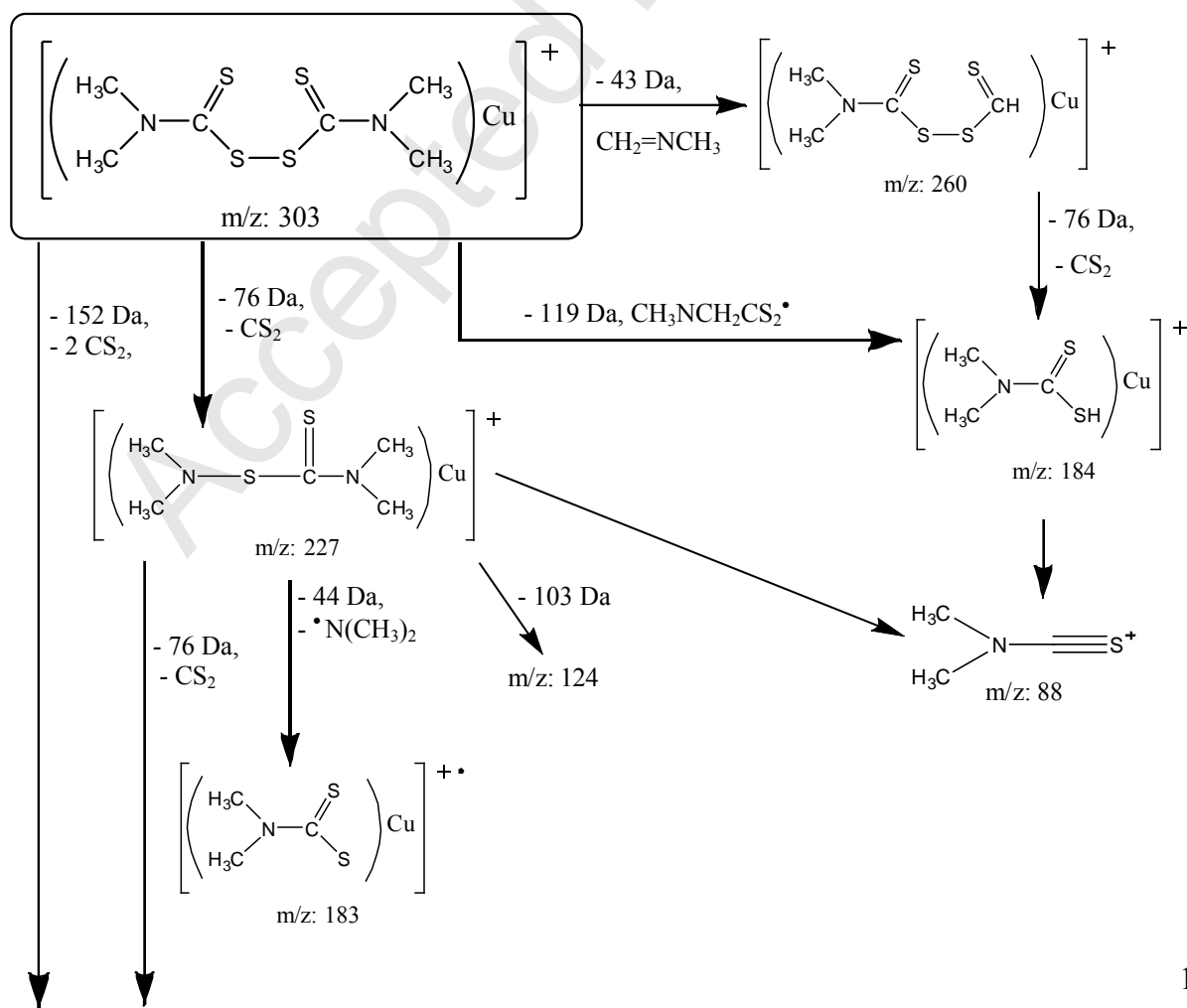
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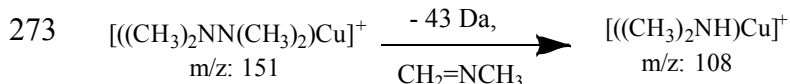
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275 Thus, the MS<sup>2</sup> of compound [CuThi]<sup>2+</sup>, gives the product ions at *m/z* 260, 227, 202, 184, 151  
 276 and 88. The product ion at *m/z* 260 results from the loss of -CH<sub>2</sub>NCH<sub>3</sub> (-43 Da), the product ion  
 277 at *m/z* 227 results from the loss of carbon disulphide (-76 Da, -CS<sub>2</sub>), the product ion at *m/z* 151  
 278 may correspond to a copper complex with *N,N,N',N'*-tetramethylhydrazine formed from the  
 279 original compound (*m/z* 303) by the loss of two carbon disulphide molecules (-152 Da) or from  
 280 the product ion at *m/z* 227 by the loss of carbon disulphide (-76 Da, -CS<sub>2</sub>) and the product ion at  
 281 *m/z* 88, identified as *N,N*-dimethylthioformamide, was detected not only in the MS<sup>2</sup> of the  
 282 Thi:Cu(II) compound (*m/z* 303) but also in the MS<sup>3</sup> of the product ion at *m/z* 227 and in the MS<sup>3</sup>  
 283 of the product ion at *m/z* 184.

284 To identify the products that can be formed during the degradation of [CuThi]<sup>2+</sup>, a 1:50  
 285 Thi:Cu(II) solution after 7 h and 10 days was analyzed by HPLC-UV-MS<sup>n</sup>. Following UV-Vis  
 286 data and to confirm that the same degradation products are formed in a DMDTC solution in the  
 287 presence of copper ions, a 1:25 DMDTC:Cu(II) solution was also analyzed by HPLC-UV-MS<sup>n</sup>.  
 288 Figure 3 shows the HPLC-UV chromatograms at 270 nm of both 1:50 Thi:Cu and 1:25  
 289 DMDTC:Cu solutions after 7 hours and 10 days. The HPLC-UV chromatograms of the solutions  
 290 revealed the occurrence of five peaks. However, based on the MS fragmentation profiles it was  
 291 possible to identify four compounds (compounds **I**, **II**, **III**, **IV**) in the first two poorly resolved  
 292 peaks. The other three peaks correspond to three perfectly resolved compounds (compounds **V**,  
 293 **VI** and **VII**). In order to identify the structure of these compounds, HPLC-MS<sup>n</sup> analysis was

294 performed. Table 1 summarizes the number of compounds obtained at each retention time ( $R_t$ ),  
295 including  $[\text{CuThi}]^{2+}$ , identified by HPLC- $\text{MS}^n$ , as well as the mass of each molecular ion, the  
296 product ions obtained by  $\text{MS}^n$  and the proposed structure based in each fragmentation pattern.

297 The HPLC chromatograms of both 1:50 Thi:Cu and 1:25 DMDTC:Cu (Figure 3) solutions are  
298 quite similar after 10 days with the compounds **I**, **II**, **III**, **IV**, **VI** and **VII** being detected in both  
299 cases. These results are in agreement with the similarity of the UV-Vis spectra of both solutions,  
300 as mentioned above and shown in Figure S4 of Supplementary data. Compound **V** is detected  
301 only in the 1:50 Thi:Cu solution after 7 h, suggesting that this compound can be an intermediate.  
302 A tentative identification of the products formed in the solutions was performed based on the  
303  $\text{MS}^n$  fragmentation profile of each chromatographic peak presented in Table 1.

304 Compound **VII**, corresponding to the molecular ion at  $m/z$  303 at retention time  $\sim 8.6$  min was  
305 identified as a Cu(I) complex, i.e.  $[\text{CuThi}]^+$  formed in solution during Thi:Cu(II) degradation.  
306 The  $[\text{CuThi}]^+$  complex shows the same  $\text{MS}^n$  profile as for  $[\text{CuThi}]^{2+}$  complex (Table 1). The  
307 results described in the above section suggest that the  $[\text{CuThi}]^{2+}$  complex gives rise to absorption  
308 at 420 nm in aqueous solution and the solutions which gave rise to the chromatograms in Figure  
309 3A did not exhibit that band. The band at 420 nm was observed in the 1:50 Thi:Cu(II) solution  
310 only for very short time after preparation, (e.g. 1 h), giving rise to the band at 385 nm as the time  
311 increases. Analyzing the TIC chromatogram of the ion at  $m/z$  303 for the solution  $\sim 1$ h after  
312 preparation (Figure S6, Section S4 of the Supplementary data), it is clear the presence of two  
313 different compounds at  $m/z$  value of 303 which appear at  $\sim 4.7$  and  $\sim 8.6$  min assigned,  
314 respectively, to  $[\text{CuThi}]^{2+}$  and  $[\text{CuThi}]^+$  (compound **VII**). Thus, these results confirm that we are  
315 in presence of two complexes of thiram, one with Cu(II), which is formed first in solution and  
316 which gives rise to absorbance at 420 nm and appears at lower retention time ( $\sim 4.7$  min) in the



317 HPLC-MS chromatogram, and the other with Cu(I) which is formed in the aged solution and  
318 appears at a retention time of ~8.6 min. The  $[\text{CuThi}]^+$  complex (compound **VII**) was also  
319 detected during the degradation of the DMDTC:Cu solutions (Figure 3) suggesting its formation  
320 in solution through a redox reaction between Cu(II) and DMDTC, i.e. DMDTC is oxidized to  
321 thiram and Cu(II) is reduced to Cu(I). The dimerization of DMDTC with simultaneous reduction  
322 of Fe(III) into Fe(II) has been reported by Bergendorff and Hansson [26] for  $\text{Fe}(\text{DMDTC})_3$   
323 solutions. These authors did not observe this behavior in  $\text{Cu}(\text{DMDTC})_2$  solutions, but in those  
324 solutions Cu(II) was not in excess relatively to DMDTC, while in the present work a large excess  
325 of Cu(II) was used. Besides, the dimerization (oxidation) of other dithiocarbamates by reduction  
326 of Cu(II) into Cu(I) has been reported by Macías et al. [27].

327 The  $\text{MS}^n$  fragmentation patterns of compound **V** ( $m/z$  283), detected only in the 1:50  
328 Thi:Cu(II) solution after 7 h of preparation, and compound **VI** ( $m/z$  271), detected in both  
329 solutions of Thi:Cu and DMDTC:Cu, are shown in Scheme S2 of the Supplementary data. These  
330 two compounds are oxidation products of Thi:Cu(II) complex resulting from oxidative  
331 desulfurization. It is worth to notice that these oxidation products of thiram were also detected  
332 during photodegradation of thiram in aqueous solutions [28].

333 Compounds **I**, **II**, **III** were tentatively identified as copper complexes with DMDTC  
334 degradation products, while product **IV** contains DMDTC and  $\text{C}_2\text{H}_3\text{N}$  as ligands. Beyond the  
335 typical fragments  $\text{CS}_2$  (76 Da) and amino groups (-45 or -44 Da) which also appear in the thiram  
336 fragmentation pattern (Scheme S1, Supplementary data), it is also possible to observe other  
337 fragments such as the loss of 41 Da, in the  $\text{MS}^n$  fragmentation patterns of compounds **I**, **II**, **III**  
338 and **IV**. The ligand  $\text{C}_2\text{H}_3\text{N}$  (41 Da), present in the complex **IV** and also in complexes **I**, **II** and  
339 **III**, was tentatively identified as methyl isonitrile ( $\text{CH}_3\text{NC}$ ). It is known that isonitriles form

340 stable Cu(I) complexes [29-31]. The MS<sup>n</sup> fragmentation patterns of compounds **I** (*m/z* 145) and  
341 **II** (*m/z* 190) are shown in Scheme S3 of the Supplementary data. The molecular ion at *m/z* 204  
342 (compound **III**) was also assigned to a Cu(I) complex with DMDTC degradation products;  
343 however, the fragmentation data shown in Table 1 suggest the presence of oxygen since a loss of  
344 water molecule was detected. Scheme S4 (Supplementary data) shows the structure proposed for  
345 compound **III** (*m/z* 204) based on the MS<sup>n</sup> fragmentation pattern. One of the ligands in the  
346 compound **III** is tentatively identified as *N*-methylformamide (CH<sub>3</sub>NHCHO), which can be  
347 formed by oxidation of DMDTC or degradation of oxidized thiram. Finally, the molecular ion at  
348 *m/z* 224 (compound **IV**) was also assigned to copper (I) complex formed with DMDTC and a  
349 DMDTC degradation product (Scheme S5, Supplementary data).

350 It is noteworthy that, despite the presence of DMDTC as ligand in the compound **IV**, the  
351 [Cu(DMDTC)]<sup>+</sup> complex (product ion at *m/z* 183) was not identified in these solutions with  
352 higher excess of copper ions. However, when a solution with lower Thi:Cu molar ratio, i.e., 1:3,  
353 was analyzed 10 days after preparation, the presence of the product ion at *m/z* 183 was detected  
354 by direct MS/MS. These results suggest that the [Cu(DMDTC)]<sup>+</sup> complex (*m/z* 183) is an  
355 intermediate which degrades into other degradation products (complexes), and that this  
356 degradation is faster for high Cu:DMDTC ratios as mentioned in section 3.2. This was confirmed  
357 by HPLC-MS/MS and direct MS/MS analysis of a 1:3 DMDTC:Cu solution after 4 hours, 1, 4  
358 and 12 days. Four hours after the solution preparation the molecular ion at *m/z* 183 was present  
359 but its intensity decreases along time and new molecular ions are detected after 4 days or more.  
360 Thus, in the presence of an excess of copper ions (II) a 1:1 complex is formed, [Cu(DMDTC)]<sup>+</sup>,  
361 and along time other copper complexes are formed with DMDTC degradation products. The  
362 identification of the [Cu(DMDTC)]<sup>+</sup> complex was confirmed by the MS<sup>n</sup> fragmentation profile

363 (Scheme S6, Supplementary data). It is worth to notice that the existence of two thiram  
364 complexes, with  $m/z$  value 303 but which appear at two different retention times were also  
365 observed in these solutions with molar ratios 1:3. Since the products formed depend on the  
366 Thi:Cu or DMDTC:Cu molar ratio and the equilibrium time of the solutions before analysis, a  
367 summary of various copper complexes identified in several solutions of different molar ratios  
368 analyzed is presented in Table 2.

369 Thus, in the present work, it was shown that copper ions have a strong influence on the  
370 degradation of thiram in aqueous solutions:

- 371 • In presence of an excess of  $\text{Cu}^{2+}$ , a  $[\text{CuThi}]^{2+}$  complex is initially formed and degrades to  
372  $[\text{Cu}(\text{DMDTC})]^+$ ;
- 373 • The complex  $[\text{Cu}(\text{DMDTC})]^+$  readily degrades leading to other copper complexes which  
374 are persistent in solution for long periods of time and which were identified for the first  
375 time, by  $\text{MS}^n$ .

376

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387 **5 References**

388 [1] V. Andreu, Y. Pico, Determination of pesticides and their degradation products in soil:  
389 critical review and comparison of methods, *Trends Anal. Chem.* 23 (2004) 772-788.

390 [2] M. Gavrilescu, Fate of Pesticides in the Environment and its Bioremediation, *Eng. Life Sci.*  
391 5 (2005) 497-526.

392 [3] T.R. Roberts, D.H. Hutson, *Metabolic Pathways of Agrochemicals. Part 2. Insecticides and*  
393 *Fungicides*, Royal Society of Chemistry, Cambridge, UK, 1999, pp. 1180–1185.

394 [4] V.K. Sharma, J.S. Aulakh, A.K. Malik, Thiram: degradation, applications and analytical  
395 methods, *J. Environ. Monit.* 5 (2003) 717–723.

396 [5] J.V. Abreu, *Vendas de produtos fitofarmacêuticos em Portugal em 2010*. Ministério da  
397 *Agricultura, do Desenvolvimento Rural e das Pescas. Direção-Geral de Agricultura e*  
398 *Desenvolvimento Rural. Direção de Serviços de Produtos Fitofarmacêuticos e Sanidade*  
399 *Vegetal. Lisboa, 2011. Information available at [http://www.dgv.min-](http://www.dgv.min-agricultura.pt/portal/page/portal/DGV)*  
400 *agricultura.pt/portal/page/portal/DGV*; last access 05<sup>th</sup> February 2014

401 [6] S. Dousset, A.R. Jacobson, J.B. Dessogne, N. Guichard, P.C. Baveye, F. Andreux,  
402 *Facilitated transport of diuron and glyphosate in high copper vineyard soils*, *Environ Sci.*  
403 *Technol.* 41 (2007) 8056-8061.

404 [7] M. Pateiro-Moure, C. Pérez-Novo, M. Arias-Estévez, E. López-Periago, E. Martínez-  
405 *Carballo, J. Simal-Gandara, Influence of copper on the absorption and desorption of*

- 406 paraquat, diquat, and difenzoquat in vineyard acid soils, *J. Agric. Food Chem.* 55 (2007)  
407 6219-6226.
- 408 [8] L. Ting-feng, S. Cheng, T. Na, H. Jun, Y. Shao-gui, C. Chuan-xiang, Effect of copper on the  
409 degradation of pesticides cypermethrin and cyhalothrin, *J. Environ. Sci.* 19 (2007) 1235–  
410 1238.
- 411 [9] T.F. Liu, C. Sun, N. Ta, J. Hong, S.G. Yang, C.X. Chen, Effect of copper on the degradation  
412 of pesticides cypermethrin and cyhalothrin, *J. Environ. Sci.* 19 (2007) 1235-1238.
- 413 [10] J. Liu, X. Lü, J. Xie, Y. Chu, C. Sun, Q. Wang, Absorption of lambda-cyhalothrin and  
414 cypermethrin on two typical Chinese soils as affected by copper, *Environm. Sci. Pollut.*  
415 *Res.* 16 (2009) 414-422.
- 416 [11] O.M.S. Filipe, C.A.E Costa, M. M. Vidal, E.B.H. Santos, Influence of soil copper content  
417 on the kinetics of thiram adsorption and on thiram leachability from soils, *Chemosphere* 90  
418 (2013) 432-440.
- 419 [12] B. Gupta, M. Rani, R. Kumar, Degradation of thiram in water, soil and plants: a study by  
420 high-performance liquid chromatography, *Biomed.Chromatogr.* 26 (2012) 69–75.
- 421 [13] B. Gupta, M. Rani, R. Kumar, P. Dureja, Identification of degradation products of thiram in  
422 water, soil and plants using LC-MS technique *J. Environ. Sci. Heal. B* 47 (2012) 823-831.
- 423 [14] O.M.S. Filipe, , M.M. Vidal, A.C. Duarte, E.B.H. Santos, Influence of fulvic acids and  
424 copper ions on thiram determination in water, *J. Agric. Food Chem.* 56 (2008) 7347–7354.

- 425 [15] Thiram - Food and Agriculture Organization of the United Nations. Information available in  
426 [http://www.fao.org/fileadmin/templates/agphome/documents/Pests\\_Pesticides/JMPR/Evalu](http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/JMPR/Evaluation96/thiram.pdf)  
427 [ation96/thiram.pdf](http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/JMPR/Evaluation96/thiram.pdf); last access 05<sup>th</sup> February 2014.
- 428 [16] S. Oae, Organic sulfur chemistry: Structure and mechanisms, Associate Editor, T. Joyce,  
429 CRC Press: Boca Raton, Florida, 1991
- 430 [17] Y. Iwasaki, S.J. Ormerod, Estimating safe concentrations of trace metals from inter-  
431 continental field data on river macroinvertebrates, *Environm. Poll.* 166 (2012) 182–186.
- 432 [18] E. Herrero-Hernández, M.S. Andrades, A. Álvarez-Martín, E. Pose-Juan, M.S. Rodríguez-  
433 Cruz, M.J. Sánchez-Martín, Occurrence of pesticides and some of their degradation  
434 products in waters in a Spanish wine region, *J. Hydrol.* 486 (2013) 234–245.
- 435 [19] P.V. Toan, Z. Sebesvari, M. Bläsing, I. Rosendahl, F.G. Renaud, Pesticide management and  
436 their residues in sediments and surface and drinking water in the Mekong Delta, Vietnam,  
437 *Sci.Total Environ.* 452–453 (2013) 28–39.
- 438 [20] Portuguese Decree-Law n° 306/2007 of 27<sup>th</sup> August, in *Diário da Republica n° 164 Série 1ª*  
439 *of 27/08/2007*, Imprensa Nacional Casa da Moeda, 2007.
- 440 [21] R. Kumar, Determination of stability constant for different metal ions using sandwich  
441 membrane method, Master thesis, Thapar University, Patiala; 2009.
- 442 [22] K.W. Weissmahr, C.L. Houghton, D.L. Sedlak, Analysis of the dithiocarbamates fungicides  
443 Ziram, Maneb, Zineb and the flotation agent Ethylxanthogenate by ion-pair reversed phase  
444 HPLC, *Anal.Chem.* 70 (1998) 4800-4804.

- 445 [23] L. Gianelli, V. Amendola, L. Fabbrizzi, P. Pallavicini, G. Mellerio, Investigation of  
446 reduction of Cu(II) complexes in positive-ion mode electrospray mass spectrometry, *Rapid*  
447 *Commun.Mass Spectrom.* 15 (2001) 2347-2353.
- 448 [24] V. Tamboli, A. Defant, I. Mancini, P. Tosi, A study of resveratrol-copper complexes by  
449 electrospray ionization mass spectrometry and density functional theory calculations, *Rapid*  
450 *Commun.Mass Spectrom.* 4 (2011) 526-532.
- 451 [25] M. Rancan, A. Dolmella, R. Seraglia, S. Orlandi, S. Quici, L. Sorace, D. Gatteschi,  
452 L.Armelao, DinuclearCu(II) Complexes of Isomeric Bis-(3-acetylacetonate)benzene  
453 ligands: synthesis, structure, and magnetic properties, *Inorg. Chem.* 51 (2012) 5409–5416.
- 454 [26] O. Bergendorff, C.Hansson, Spontaneous formation of thiuram disulfides in solutions of  
455 iron(III) dithiocarbamates, *J. Agric. Food Chem.* 50 (2002) 1092-1096.
- 456 [27] B. Macias, M.V. Villa, E. Chicote, S. Martín-Velasco, A., Castineiras, J. Borrás, Copper  
457 complexes with dithiocarbamates derived from natural occurring amino acids. Crystal and  
458 molecular structure of  $[\text{Cu}(\text{en})(\text{EtOH})(\text{H}_2\text{O})_3][\text{Cu}(\text{dtc-pro})_2]$ , *Polyhedron* 21 (2002) 1899-  
459 1904.
- 460 [28] O.M.S. Filipe, S.A.O. Santos, M.R.M. Domingues, M.M.B. Vidal, A.J.D. Silvestre, C.P.  
461 Neto, E.B.H. Santos, Photodegradation of the fungicide thiram in aqueous solutions.Kinetic  
462 studies and identification of the photodegradation products by HPLC-MS/MS,  
463 *Chemosphere* 91 (2013) 993-1001.
- 464 [29] A. Vogler, Coordinated Isonitriles. *In Isonitrile Chemistry*. Eddited by Ivan Ugi. *Organic*  
465 *Chemistry A series of Monography*, vol 20, New York, Chapter 10, 1971, pp 217-233.

466 [30] A.Bell, R.A. Walton, D.A. Edwards, M.A. Poulter, Cationic copper(I) isocyanide  
467 complexes,  $[\text{Cu}(\text{CNR})_4]^+$  ( $\text{R} = \text{CH}_3$ ,  $\text{C}(\text{CH}_3)_3$  and  $2,6\text{-(CH}_3)_2\text{C}_6\text{H}_3$ ): Preparations,  
468 spectroscopic properties and reactions with neutral ligands. A comparison of the vibrational  
469 spectra of  $[\text{Cu}(\text{CNCH}_3)_4]^+$ ,  $[\text{Cu}(\text{NCCH}_3)_4]^+$  and  $[\text{Cu}(\text{NCCD}_3)_4]^+$ , *Inorg. Chim. Acta*, 104  
470 (1985) 171–178.

471 [31] M. Benouazzane, S. Coco, P. Espinet, J. Barbera, Binuclear Mesogenic Copper(I)  
472 Isocyanide Complexes with an Unusual Inorganic Core Formed by Two Tetrahedra  
473 Sharing an Edge, *Inorg. Chem.* 41 (2002) 5754-5759.

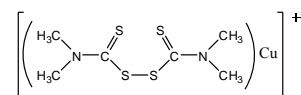
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475 **Table 1.** Compounds detected by HPLC-UV-MS in the 1:50 Thi:Cu(II) and 1:25  
 476 DMDTC:Cu(II) solutions and corresponding MS<sup>n</sup> fragmentation profiles. *m/z* in bold was  
 477 subjected to MS<sup>n</sup> analysis.

<b>R<sub>t</sub></b> <b>(min)</b>	<b>Compound</b>	<b>[M]<sup>+</sup></b> <b><i>m/z</i></b>	<b>MS<sup>2</sup></b> <b><i>m/z</i></b>	<b>MS<sup>3</sup></b> <b><i>m/z</i></b>	<b>MS<sup>4</sup></b> <b><i>m/z</i></b>	<b>Proposed structures</b>
1.5	I	145	104(100)			$[(\text{CH}_3\text{NC})_2\text{Cu}]^+$ $[(\text{CH}_3\text{NC})_2((\text{CH}_3)_2\text{NH})\text{Cu}]^+$ $[(\text{CH}_3\text{NC})_2(\text{CH}_3\text{NHCHO})\text{Cu}]^+$ $[((\text{CH}_3)_2\text{NCS}_2)(\text{CH}_3\text{NC})\text{Cu}]^+$



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483 **Table 2.** Byproducts detected by HPLC-MS and MS<sup>n</sup>; na – not analyzed.

Solution	Molar ratio	Compounds		
		7 h	4 d	10 d
Thi:Cu(II)	1:3	[ThiCu] <sup>2+</sup> Thiram	[CuThi] <sup>2+</sup> Thiram VI, VII	[Cu(DMDTC)] <sup>+</sup> I, II, III, IV, VI, VII,
	1:50	I, II, III, IV, V, VI, VII	na	I, II, III, IV, VI, VII
DMDTC:Cu(II)	1:3	[Cu(DMDTC)] <sup>+</sup> I, IV, VII	[Cu(DMDTC)] <sup>+</sup> (residual) I, II, III, IV, VII	I, II, III, IV, VII
	1:25	I, II, III, IV, VI, VII	na	I, II, III, IV, VI, VII

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486 **Figure captions**

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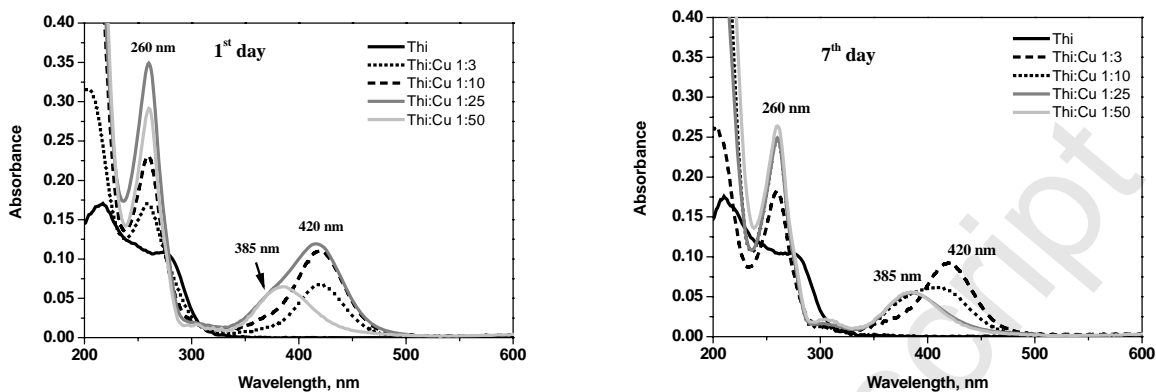
488 **Figure 1** – UV-Vis spectra of a 2.0 mg L<sup>-1</sup> thiram aqueous solution (—) containing molar ratios  
489 of Thi:Cu: (---) 1:3, (···) 1:10, (—) 1:25 and (—) 1:50, and recorded 1, 7, 14 and 34 days after  
490 preparation.

491 **Figure 2** – UV-Vis spectra of a 2.0 mg L<sup>-1</sup> DMDTC aqueous solution (—) containing molar  
492 ratios of DMDTC:Cu(II): (---) 1:3, (···) 1:10, (—) 1:25 and recorded at 0, 1, 11 and 28 days  
493 after preparation.

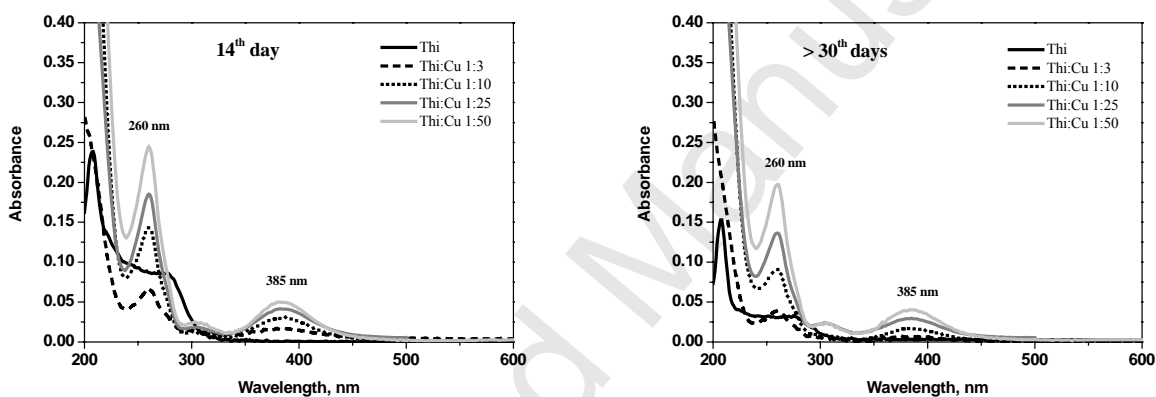
494 **Figure 3** – HPLC–UV chromatograms of **(A)** 1:50 Thi:Cu and **(B)** 1:25 DMDTC:Cu solutions 7  
495 hours and 10 day after preparation, obtained with a mobile phase of acetonitrile:water 60:40  
496 (v/v) flowing at 0.7 mL min<sup>-1</sup> and detection at 270 nm.

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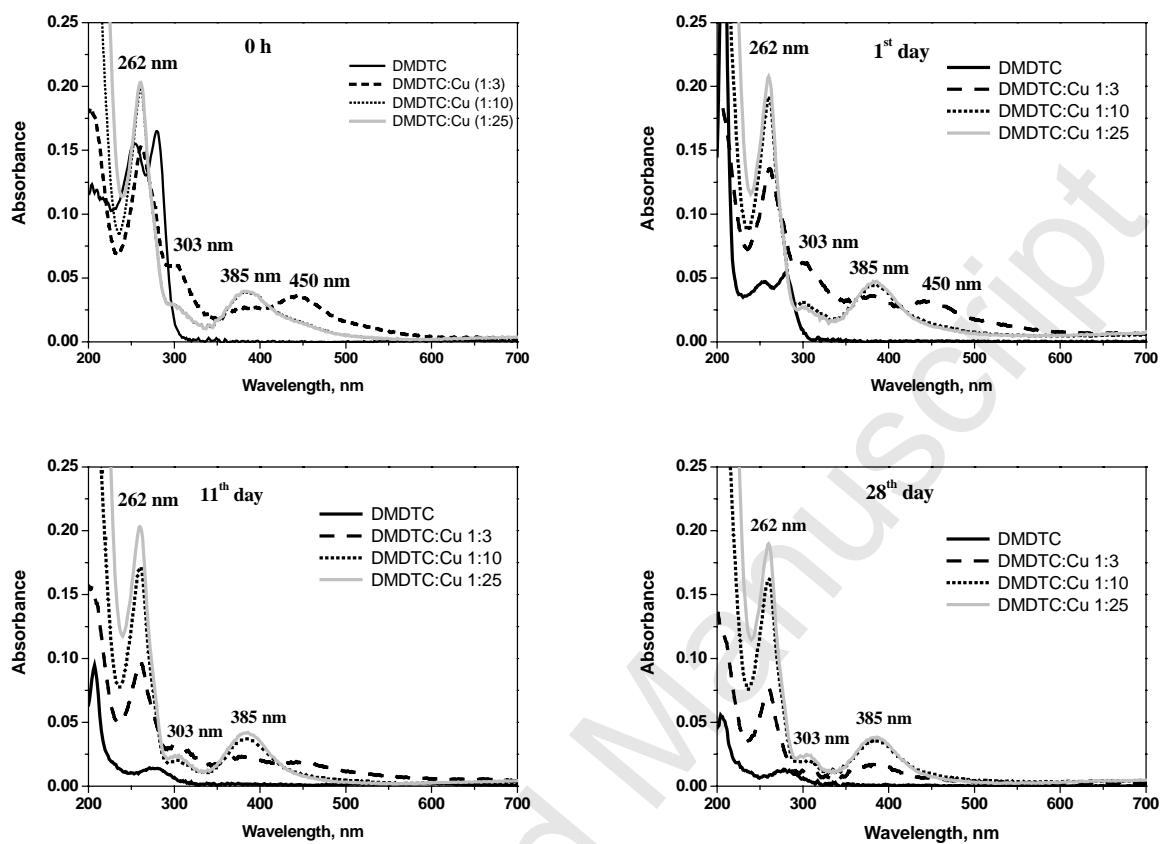


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500 **Figure 1** – UV-Vis spectra of a 2.0 mg L<sup>-1</sup> thiram aqueous solution (—) containing molar ratios  
 501 of Thi:Cu: (---) 1:3, (···)1:10, (— · —) 1:25 and (—) 1:50, and recorded 1, 7, 14 and 34 days after  
 502 preparation.

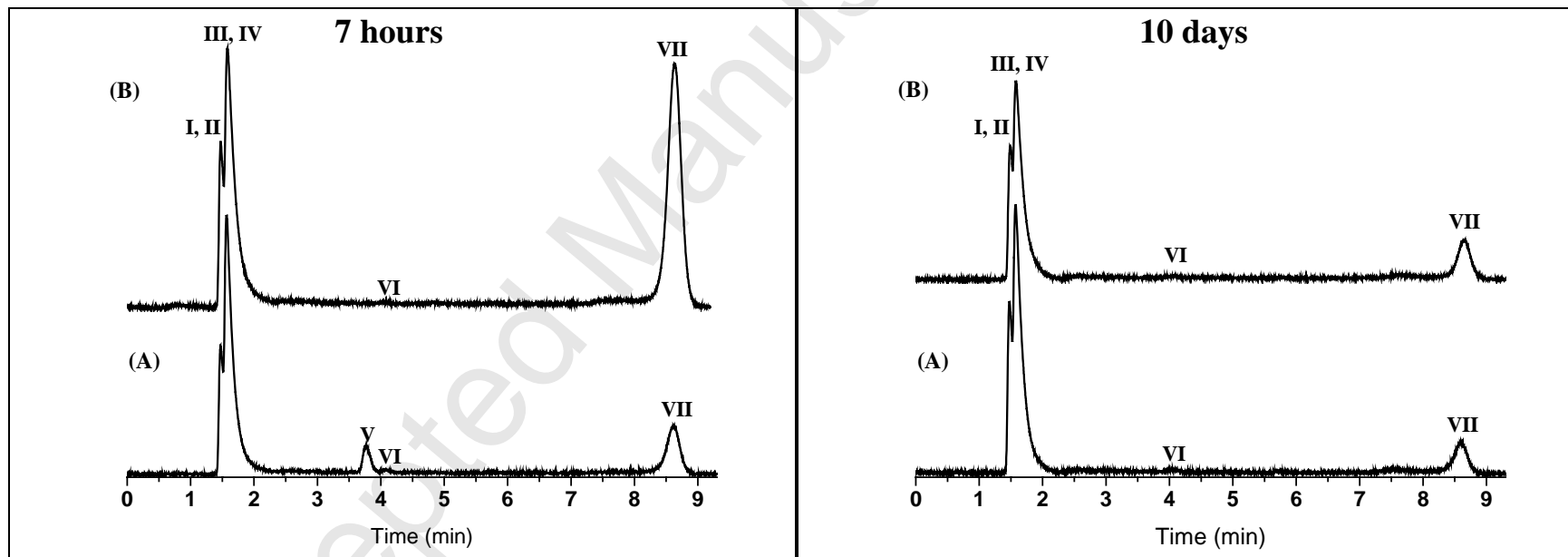


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505 **Figure 2** – UV-Vis spectra of a 2.0 mg L<sup>-1</sup> DMDTC aqueous solution (—) containing molar  
 506 ratios of DMDTC:Cu(II): (---) 1:3, (···) 1:10, (— · —) 1:25 and recorded at 0, 1, 11 and 28 days  
 507 after preparation.

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509

510 **Figure 3 – Figure 3** – HPLC–UV chromatograms of (A) 1:50 Thi:Cu and (B) 1:25 DMDTC:Cu solutions 7 hours and 10 day after  
511 preparation, obtained with a mobile phase of acetonitrile:water 60:40 (v/v) flowing at 0.7 mL min<sup>-1</sup> and detection at 270 nm.

512