Journal of Hazardous Materials 279 (2014) 125–132

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Effect of copper ions on the degradation of thiram in aqueous solution: Identification of degradation products by HPLC–MS/MS

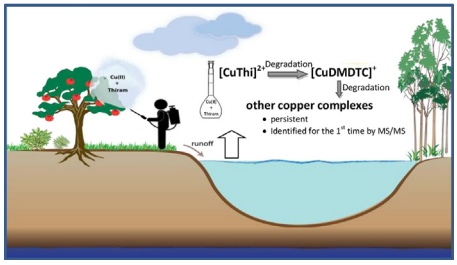
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HIGHLIGHTS

- Cu(II) in excess accelerates the degradation of thiram in aqueous solutions.
- The $[CuThi]^{2+}$ complex degrades into $[(DMDTC)Cu]^{+}$ which readily decomposes.
- New degradation products of $[CuThi]^{2+}$ were identified for the 1st time by HPLC–MS^n.
- Some degradation products are quite persistent, at least during 2 months.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 28 March 2014
Received in revised form 28 May 2014
Accepted 23 June 2014
Available online 30 June 2014

Keywords:
Thiram degradation
Copper ions
Dimethyldithiocarbamate
Aqueous solutions
UV–vis spectrophotometry
HPLC-UV–MS^n

ABSTRACT

The aim of this work was to examine the effect of Cu(II) on the degradation of thiram (Thi) in aqueous solutions, since the literature focused on this effect is scarce and copper based fungicides can be applied together with thiram or during the same season to agricultural crops. The effect of Cu(II) on the degradation of thiram was followed by both UV–vis and HPLC–MS/MS. When thiram is dissolved in pure water its degradation occurs very slowly, being negligible during the first 7 days. However, the presence of Cu(II) has a strong influence on the thiram degradation in aqueous solutions along time. In the presence of an excess of Cu(II), a $[CuThi]^{2+}$ complex is initially formed which degrades into a complex formed between the dimethyldithiocarbamate anion (DMDTC) and Cu(II) ion, $[Cu(DMDTC)]^{+}$. This complex further degrades leading to other copper complexes which were identified for the first time, by MS^n. The results obtained in the present work also demonstrated that a redox reaction involving DMDTC anions and Cu(II) ions gives rise to the formation of a Thi–Cu(I) complex. Finally, some of the complexes resulting from the degradation of $[CuThi]^{2+}$ are quite persistent in solution for long periods of time (>1 month).

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1. Introduction

Pesticides are intensively used in agriculture and much effort is devoted to control and reduce possible damaging effects on the environment, such as contamination of soil and leaching to ground and surface waters, with the possible contamination of aquatic

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http://dx.doi.org/10.1016/j.jhazmat.2014.06.051
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organisms, and, ultimately, contamination of water and food consumed by human beings, with the consequent toxic effects. The fate of a pesticide is determined by processes that affect mobility, such as sorption or volatilization, and those that affect persistence, including photo-, chemical and microbial degradation. According to the literature, the degradation products of some pesticides may be more toxic and persistent, representing a higher environmental risk than the parent compounds [1,2]. To understand the fate of a pesticide in soil and water systems an accurate knowledge of its environmental behavior is essential.

Thiram, tetramethylthiuramdisulfide, is a dithiocarbamate compound that has been used as a contact fungicide with preventive action, worldwide applied not only in agriculture, but also in rubber industry as an accelerator and vulcanization agent [3,4]. In Portugal, thiram was considered the second most popular contact fungicide of the dithiocarbamate group, after manconzeb. Dithiocarbamates contributed with ~12% of the total sales of fungicides, followed by the copper-based fungicides (~10%) [5].

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

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

Thus, the aim of this work was to examine the effect of copper ions on the degradation of thiram in aqueous solutions. The effect of copper ions was studied during 1 or 2 months, following the UV–vis spectral changes of different thiram–Cu(II) mixtures. The identification of complexes formed over time was also studied by HPLC–MS/MS.

2. Experimental

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). Thiram (Thi, 97%) and acetonitrile (HPLC grade) were obtained from Aldrich and LabScan, respectively. Sodium dimethyldithiocarbamate solution (DMDTC, purum, ~ 40% in H_2O) and cupric perchlorate hexahydrate were purchased from Fluka. Cupric acetate, used in the solutions preparation for MS analysis, was from May and Baker LTD. Aqueous thiram stock solutions 20 mg L^{-1} were prepared by previous dissolution of thiram in acetonitrile followed by dilution with water (percentage of acetonitrile in the final solution always lower than 1%). Stock solutions of 1000 mg L^{-1} Cu(II) and 0.5 g L^{-1} DMDTC were prepared, from the reagents, in ultra-pure water. Thiram standard solutions 2.0 mg L^{-1} with increasing copper contents were prepared by dilution of both 20 mg L^{-1} thiram and 1000 mg L^{-1} Cu(II) stock solutions, obtaining the following Thi:Cu(II) molar ratios: 1:3, 1:10, 1:25 and 1:50. 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) molar ratios: 1:3, 1:10 and 1:25.

2.2. UV–vis spectrophotometry

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

2.3. Identification of degradation products by HPLC–MS^n

The HPLC system consisted of a variable loop Accela auto sampler (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 using a phenomenex C_{18} column (150 mm × 4.60 mm, 5 μm, 110 Å). The separation of the compounds was carried out with a mobile phase of acetonitrile:water (60:40, v/v) with 0.1% HCOOH at a flow rate of 0.7 mL min^{-1}, at 25 °C. The injection volume in the HPLC system was 20 μL. Single online detection was carried out in PDA detector, at 270 nm, and UV spectra in the range of 200–600 nm were also recorded for relevant chromatographic peaks. 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 was 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. Collision-induced dissociation (CID)-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 and 40 (arbitrary units), using helium as collision gas. The data acquisition was carried out by using Xcalibur® data system (ThermoFinnigan, San Jose, CA, USA).

3. Results and discussion

3.1. Evaluation of thiram stability in aqueous solution

To evaluate thiram stability in aqueous solution, UV–vis spectra and pH values of a 2.0 mg L^{-1} thiram aqueous solution were monitored during 1 month (Fig. S1A and S1B, respectively of the Supplementary data). The spectrum of a fresh thiram solution exhibits two absorption maxima, at 220 nm and at 272 nm, showing no significant changes up to the 7th day (Fig. S1A, curve a). For longer periods, the absorbance maximum at 272 nm begins to decrease and a new maximum appears at 207 nm (Fig. S1A, curve c). From the 11th day onwards, a sharp rise of the pH of the solution is observed (Fig. S1B).

The degradation of thiram into DMDTC due to the cleavage of the disulphide bond is frequently reported in the literature as being the first step of thiram degradation in environmental matrices [3,15] and it has been also referred its occurrence in solutions of thiram in milli-Q water [12]. However, the UV spectra changes observed in the present study cannot be explained only by the formation of DMDTC. In fact, the UV spectrum of the fresh DMDTC solution (Fig. S2) exhibited two absorption maxima: one at 254 and the other at 280 nm. It is possible to see a significant decrease of the bands at the end of the 1st day, coincident with the appearance of a new band at

ca. 207 nm, previously observed for thiram degradation (Fig. S1A). After 2 days, the degradation of DMDTC is almost complete (Fig. S2, curve d). It is interesting to notice that the absorption maxima at 250 and 280 nm, characteristic of DMDTC, do not appear in the spectra of thiram solutions after several aging times, suggesting that, although being a degradation product of thiram, DMDTC is only an intermediate which undergoes further degradation, in agreement with its aqueous solution behavior.

The results obtained for thiram aqueous solutions and described above are in agreement with those obtained by Gupta et al. [12,13] who followed the degradation of thiram in aqueous solution by HPLC-UV and HPLC–MS. These authors observed that the degradation of thiram, at pH 5.5, was quite slow. The authors did not detect any other products in solution, besides thiram, until 7 days and, only after 11 days DMDTC and other degradation products were detected. In the present work, as the aqueous solution is not buffered, degradation of thiram is initially very slow because the pH is low. However, after 11 days, the pH of the solution increases and the increase of pH increases the degradation rate of thiram, as previously observed by Gupta et al. [12]. The effect of pH on the rate of thiram hydrolysis can be explained assuming that the first step of the hydrolysis of thiram in water involves the attack of OH^- to the SS bond, as reported for other organic compounds with disulfide bonds [16], giving rise to the formation of DMDTC (product I) and dimethyldithiocarbamoylsulfenic acid (product II), as shown in Scheme 1.

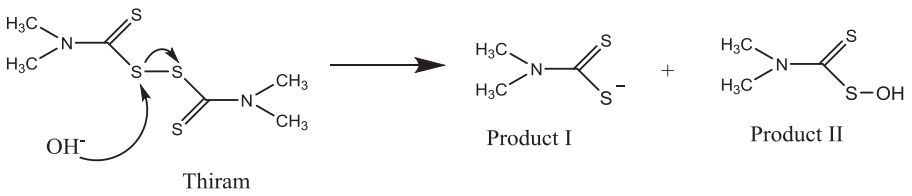
These two degradation products were identified by Gupta et al. [13] as being the first which are formed during the degradation of thiram in water buffered at pH 8.0. However, the same authors did not detect the presence of the dimethyldithiocarbamoylsulfenic acid during the degradation of thiram at pH 5.5. The increase of pH, which starts after the 11th day, may be attributed to the fact that the degradation of the intermediate DMDTC can give rise to the consumption of H^+ (according to the degradation Scheme S1 of the Supplementary data). Indeed, an increase of pH was also observed during the degradation of DMDTC in water, but this increase was fast (2 days), in agreement with the fast degradation of DMDTC and it was of the same order of magnitude (~0.6 units) as the increase of pH observed between the 11th and 17th days in the solutions of thiram. The possibility of contribution of other reactions to the higher increase of pH observed for longer aging times cannot be excluded.

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

3.2. UV spectral evidence of changes in the composition of Thi:Cu(II) solutions along time

In natural waters the concentrations of thiram will certainly be quite low and it is expected that the concentration of Cu(II) will be higher. Cu(II) concentrations in natural waters can be in the order of 1–100 $\mu g\,L^{-1}$ [17], while pesticides' concentrations are in the order of $ng\,L^{-1}$ to a few $\mu g\,L^{-1}$ [18,19], being 0.1 $\mu g\,L^{-1}$ the maximum allowed concentration in natural waters for each individual pesticide or 0.5 $\mu g\,L^{-1}$ for total pesticides [20]. Thus, in order to study the degradation of thiram in the presence of an excess of Cu(II), thiram solutions were prepared, with Thi–Cu(II) molar ratios 1:3, 1:10, 1:25 and 1:50, and initial concentrations of thiram 2 $mg\,L^{-1}$, 0.2 $mg\,L^{-1}$ and 11 $\mu g\,L^{-1}$. The evolution of the UV–vis spectra over time for the solutions containing 2 $mg\,L^{-1}$ of thiram is shown in Fig. 1. After mixing thiram with Cu(II), the spectrum of thiram gives rise to spectra dominated by two absorption maxima at 260 nm and 420 nm, respectively. Over time, the maximum at 420 nm decreases and gives rise to a new maximum at 385 nm. The formation of the species responsible for the absorption at 420 nm and the conversion to those responsible for the absorption at 385 nm occur faster when the excess of copper is higher. Thus, after 24 h of equilibration the absorbance of the maximum at 420 nm is higher in the solutions with the higher content of Cu(II), i.e. the absorbance at 420 nm increases as the Thi:Cu(II) ratio decreases from 1:3 to 1:25 (1:3 < 1:10 < 1:25). The solution Thi:Cu 1:50 already shows a decrease of the maximum at 420 nm and the appearance of the new maximum at 385 nm. For the solution with the lowest content of Cu(II), the solution with Thi:Cu ratio 1:3, after 7 days the absorption maximum at 420 nm is still present and the maximum at 385 nm is not observed. For longer periods, the replacement of the maximum at 420 nm is also observed for this solution, so that, at the end of the 14th day, only the absorption maxima at 385 and 260 nm are present in the UV–vis spectra of all the solutions (Fig. 1, 14th day). One hypothesis to explain the reported observations is the following: in the presence of an excess of Cu(II) a Thi:Cu(II) complex is formed. The formation of a Thi:Cu(II) complex 1:1 with a stability constant of $\log \beta = 5.38$ is documented in the literature [21]. This complex degrades over time, being converted into the species responsible for the absorption at 385 nm. Weissmahr et al. [22], reported a spectrum identical to those of solutions Thi:Cu after 14 days, with maxima at 260 nm and 385 nm, for an aqueous solution of DMDTC:Cu 1:100 and they attributed it to a 1:1 DMTC:Cu complex, i.e. $[Cu(DMDTC)]^+$. However, the authors have not reported any insight into the identification of the complex formed.

In order to clarify whether the degradation of thiram in the presence of an excess of copper gives rise to the $[Cu(DMDTC)]^+$ complex, some experiences were performed with solutions containing 2.0 $mg\,L^{-1}$ DMDTC and copper ion at the following DMDTC:Cu(II) molar ratios of 1:3, 1:5, 1:10 and 1:25. Fig. 2 shows the UV–vis spectra of those solutions over time. For a 1:3 DMDTC:Cu ratio, the spectrum registered immediately after solution preparation shows the absorption maxima at 262, 303 and 450 nm, but a shoulder at 385 nm is already observed; for longer periods of time, the maximum at 450 nm decreases, and at the end of the 28th day, only the absorption maxima at 385 and 260 nm are present in the UV–vis spectra (Fig. 2, 28th day). The UV–vis spectra for the 1:10 and 1:25 DMDTC:Cu(II) mixtures show only the absorption maxima at 385 and 262 nm, even immediately after solution preparation (Fig. 2, time 0 h). The results obtained for the 1:3 ratio suggest that a $[Cu(DMDTC)]^+$ complex, characterized by absorption maxima at 262, 303 and 450 nm, is first formed in the solution. This complex degrades along time giving rise to a spectrum characterized by absorption maxima at 385 and 262 nm, as the spectra of aged solutions of Thi:Cu (Fig. 1, 14 and 30 days). The degradation of the $[Cu(DMDTC)]^+$ complex is very fast in the presence of higher excess of Cu (1:10 and 1:25 ratios), reason why the absorption maximum of the DMDTC:Cu complex at 450 nm is not observed in solutions with 1:10 and 1:25 ratios, even when the spectra are recorded immediately after preparation. It also interesting to notice that in the presence of a high excess of copper ions, 1:25 DMDTC:Cu, the complexes responsible for the absorption at 260 and 385 nm are immediately formed and more than 80% of their initial absorption still remains after 28 days, suggesting a high persistence of these



Scheme 1. Proposed mechanism for thiram hydrolysis in alkaline medium.

complexes (as can also be seen in Fig. S3 and Table S1 of the Supplementary data). 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 1:25 (Fig. S4, Supplementary data). Both solutions exhibit two absorption maxima at 262 and 385 nm. The absorbances of the DMDTC:Cu solution are kept approximately constant during 2 months after the solution preparation and they are similar to the absorbances of the Thi:Cu solution after the same time. These results suggest that, in the presence of an excess of Cu, thiram and DMDTC give rise to the same products which absorb at 260 and 385 nm and which are quite persistent in solution.

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

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

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

3.3. Identification of the complexes by HPLC–MS/MS

HPLC–MS analysis of Thi:Cu(II) complex, in the LC–MS conditions showed that this complex elutes at a retention time of ~4.7 min, prior to thiram (~4.8 min) and the ion observed in the MS spectrum in positive mode has an m/z value of 303. Since the Thi:Cu solution was prepared by mixing thiram and Cu(II), it was expected that the complex formed in solution would be $[CuThi]^{2+}$, whose m/z value is 151.5. However this ion is absent in the MS spectra. Thus, the presence of the ion at m/z 303, was identified as $[CuThi]^{+}$ indicating that the reduction of Cu(II) may have occurred during the ESI ionization process. This reduction behavior has already been described in literature for Cu(II) pyridil chelates [23], Cu(II)-resveratrol complexes [24] and dinuclear Cu(II) complexes of isomeric bis-(3-acetylacetonate)benzene ligands [25]. According to the literature, this process can be due to one charge transfer between the solvent and the metal complex in the gas phase. The MS^n fragmentation pattern of the molecular ion at m/z 303 at retention time ~4.7 min is shown in Scheme 2.

Thus, the MS^2 of compound $[CuThi]^{2+}$, gives the product ions at m/z 260, 227, 202, 184, 151 and 88. The product ion at m/z 260 results from the loss of $-CH_2NCH_3$ (−43 Da), the product ion at

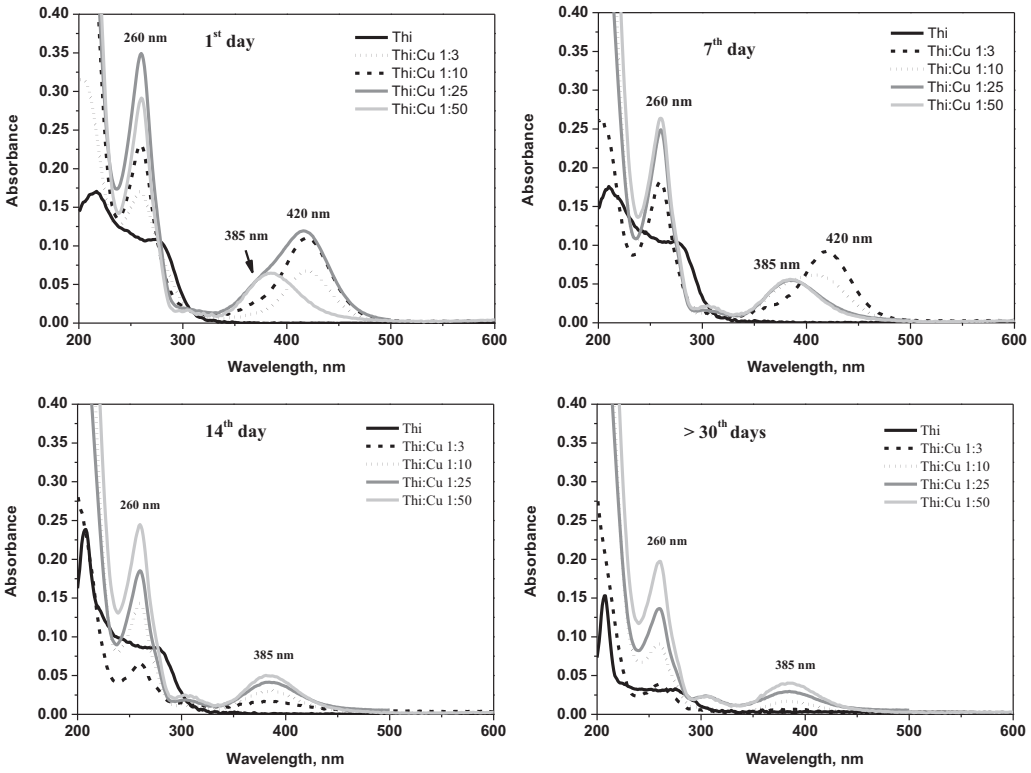


Fig. 1. UV–vis spectra of a 2.0 mg L^{-1} thiram aqueous solution (—) containing molar ratios of Thi:Cu: (- - -) 1:3, (···) 1:10, (▬) 1:25 and (▬) 1:50, and recorded 1, 7, 14 and 34 days after preparation.

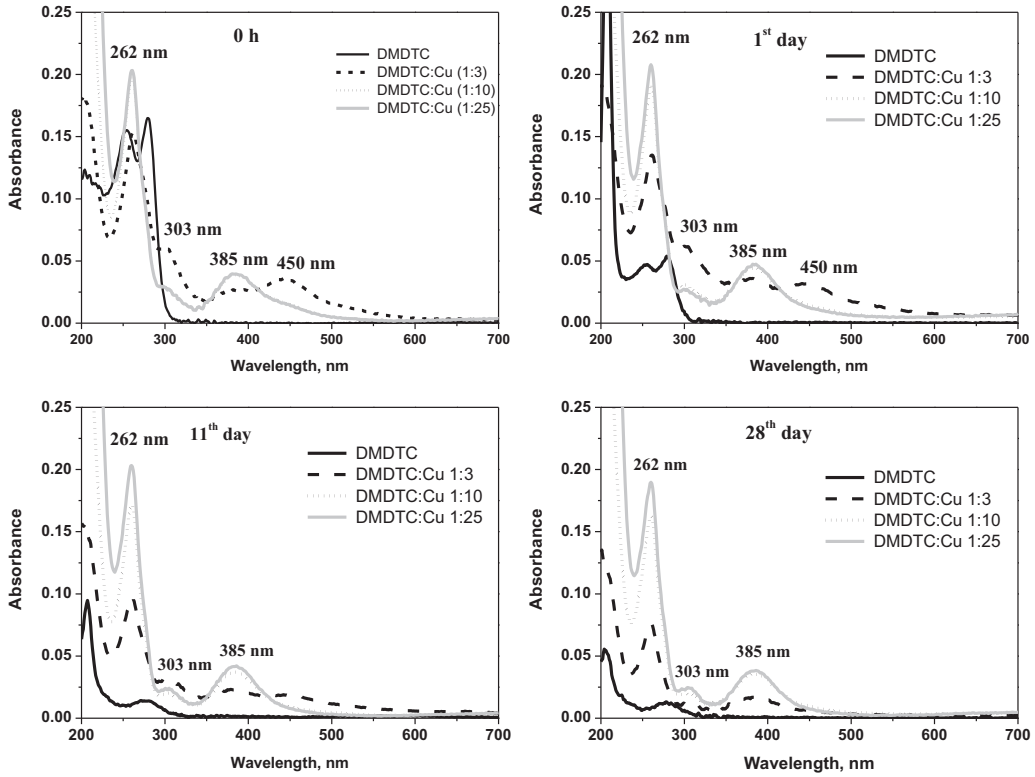
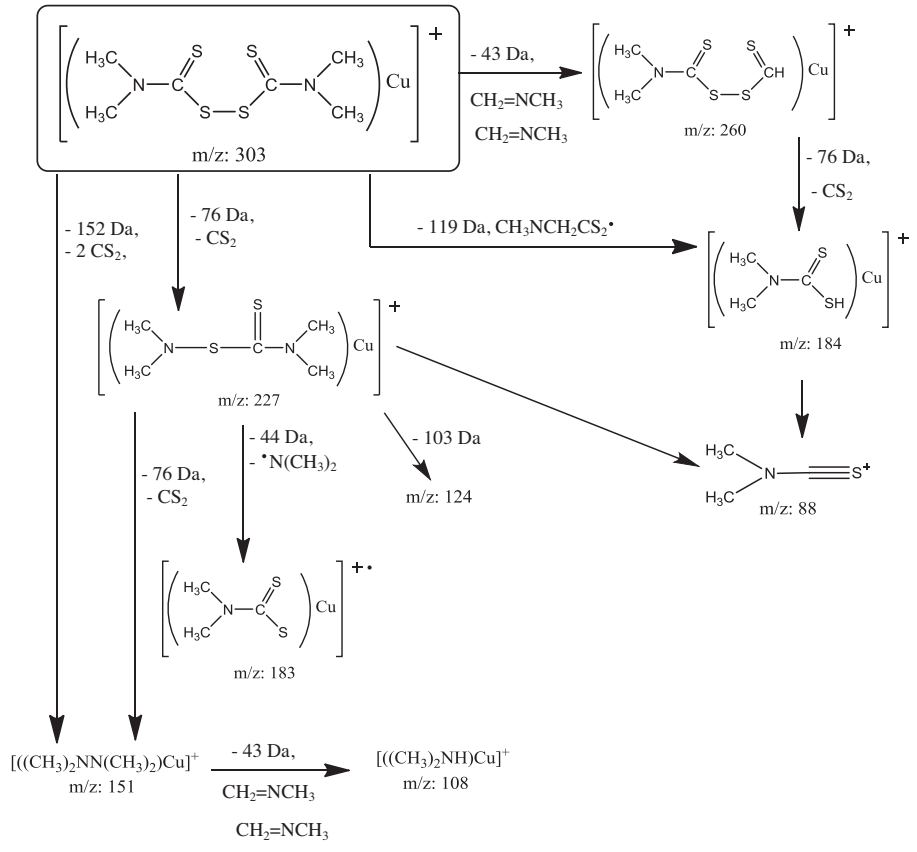


Fig. 2. UV–vis spectra of a 2.0 mg L^{-1} DMDTC aqueous solution (—) containing molar ratios of DMDTC:Cu(II): (- - -) 1:3, (···) 1:10, (▬) 1:25 and recorded at 0, 1, 11 and 28 days after preparation.



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

m/z 227 results from the loss of carbon disulphide (−76 Da, —CS_2), the product ion at m/z 151 may correspond to a copper complex with *N*,*N*,*N*′,*N*′-tetramethylhydrazine formed from the original compound (m/z 303) by the loss of two carbon disulphide molecules (−152 Da) or from the product ion at m/z 227 by the loss of carbon disulphide (−76 Da, —CS_2) and the product ion at m/z 88, identified as *N*,*N*-dimethylthioformamide, was detected not only in the MS^2 of the Thi:Cu(II) compound (m/z 303) but also in the MS^3 of the product ion at m/z 227 and in the MS^3 of the product ion at m/z 184.

To identify the products that can be formed during the degradation of $[CuThi]^{2+}$, a 1:50 Thi:Cu(II) solution after 7 h and 10 days was analyzed by HPLC-UV–MS^n. Following UV–vis data and to confirm that the same degradation products are formed in a DMDTC solution in the presence of copper ions, a 1:25 DMDTC:Cu(II) solution was also analyzed by HPLC-UV–MS^n. Fig. 3 shows the HPLC–UV chromatograms at 270 nm of both 1:50 Thi:Cu and 1:25 DMDTC:Cu solutions after 7 h and 10 days. The HPLC-UV chromatograms of the solutions revealed the occurrence of five peaks. However, based on the MS fragmentation profiles it was possible to identify four compounds (compounds **I**, **II**, **III**, **IV**) in the first two poorly resolved peaks. The other three peaks correspond to three perfectly resolved compounds (compounds **V**, **VI** and **VII**). In order to identify the structure of these compounds, HPLC–MS^n analysis was performed. Table 1 summarizes the number of compounds obtained at each retention time (R_t), including $[CuThi]^{2+}$, identified by HPLC–MS^n, as well as the mass of each molecular ion, the product ions obtained by MS^n and the proposed structure based in each fragmentation pattern.

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

Compound **VII**, corresponding to the molecular ion at m/z 303 at retention time ∼8.6 min was identified as a Cu(I) complex, i.e. $[CuThi]^+$ formed in solution during Thi:Cu(II) degradation. The $[CuThi]^+$ complex shows the same MS^n profile as for $[CuThi]^{2+}$ complex (Table 1). The results described in the above section suggest that the $[CuThi]^{2+}$ complex gives rise to absorption at 420 nm in aqueous solution and the solutions which gave rise to the chromatograms in Fig. 3A did not exhibit that band. The band at 420 nm was observed in the 1:50 Thi:Cu(II) solution only for very short time after preparation (e.g. 1 h), giving rise to the band at 385 nm as the time increases. Analyzing the TIC chromatogram of the ion at m/z 303 for the solution ∼1 h after preparation (Fig. S6, Section S4 of the Supplementary data), it is clear the presence of two different compounds at m/z value of 303 which appear at ∼4.7 and ∼8.6 min assigned, respectively, to $[CuThi]^{2+}$ and $[CuThi]^+$ (compound **VII**). Thus, these results confirm that we are in the presence of two complexes of thiram, one with Cu(II), which is formed first in solution and which gives rise to absorbance at 420 nm and appears at lower retention time (∼4.7 min) in the HPLC–MS chromatogram, and the other with Cu(I) which is formed in the aged solution and appears at a retention time of ∼8.6 min. The $[CuThi]^+$ complex (compound **VII**) was also detected during the degradation of the DMDTC:Cu solutions (Fig. 3) suggesting its formation in solution through a redox reaction between Cu(II) and DMDTC, i.e. DMDTC is oxidized to thiram and Cu(II) is reduced to Cu(I). The dimerization of DMDTC with simultaneous reduction of Fe(III) into Fe(II) has been reported by Bergendorff and Hansson [26] for $Fe(DMDTC)_3$ solutions. These authors did not observe this behavior in $Cu(DMDTC)_2$ solutions, but in those solutions Cu(II) was not in excess relatively to DMDTC, while in the present work a large excess of Cu(II) was used. Besides, the dimerization (oxidation) of other dithiocarbamates by reduction of Cu(II) into Cu(I) has been reported by Macías et al. [27].

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

Compounds **I**, **II**, **III** were tentatively identified as copper complexes with DMDTC degradation products, while product **IV** contains DMDTC and C_2H_3N as ligands. Beyond the typical fragments CS_2 (76 Da) and amino groups (−45 or −44 Da) which also appear in the thiram fragmentation pattern (Scheme S1, Supplementary data), it is also possible to observe other fragments such as the loss of 41 Da, in the MS^n fragmentation patterns of compounds **I**, **II**, **III** and **IV**. The ligand C_2H_3N (41 Da), present in the complex **IV** and also in complexes **I**, **II** and **III**, was tentatively identified as methyl isonitrile (CH_3NC). It is known that isonitriles form stable Cu(I) complexes [29–31]. The MS^n fragmentation patterns of compounds **I** (m/z 145) and **II** (m/z 190) are shown in Scheme S3 of the Supplementary data. The molecular ion at m/z 204 (compound **III**) was also assigned to a Cu(I) complex with DMDTC degradation products; however, the fragmentation data shown in Table 1 suggest the presence of oxygen since a loss of water molecule was detected. Scheme S4 (Supplementary data) shows the structure proposed for compound **III** (m/z 204) based on the MS^n fragmentation pattern. One of the ligands in the compound **III** is tentatively identified as *N*-methylformamide (CH_3NHCHO), which can be formed by oxidation of DMDTC or degradation of oxidized thiram. Finally, the molecular ion at m/z 224 (compound **IV**) was also assigned to copper (I) complex formed with DMDTC and a DMDTC degradation product (Scheme S5, Supplementary data).

It is noteworthy that, despite the presence of DMDTC as ligand in the compound **IV**, the $[Cu(DMDTC)]^+$ complex (product ion at m/z 183) was not identified in these solutions with higher excess of copper ions. However, when a solution with lower Thi:Cu molar ratio, i.e. 1:3, was analyzed 10 days after preparation, the presence of the product ion at m/z 183 was detected by direct MS/MS. These results suggest that the $[Cu(DMDTC)]^+$ complex (m/z 183) is an intermediate which degrades into other degradation products (complexes), and that this degradation is faster for high Cu:DMDTC ratios as mentioned in section 3.2. This was confirmed by HPLC–MS/MS and direct MS/MS analysis of a 1:3 DMDTC:Cu solution after 4 h, 1, 4 and 12 days. Four hours after the solution preparation the molecular ion at m/z 183 was present but its intensity decreases along time and new molecular ions are detected after 4 days or more. Thus, in the presence of an excess of copper ions (II) a 1:1 complex is formed, $[Cu(DMDTC)]^+$, and along time other copper complexes are formed with DMDTC degradation products. The identification of the $[Cu(DMDTC)]^+$ complex was confirmed by the MS^n fragmentation profile (Scheme S6, Supplementary data). It is worth to notice that the existence of two thiram complexes, with m/z value 303 but which appear at two different retention times were also observed in these solutions with molar ratios 1:3. Since the products formed depend on the Thi:Cu or DMDTC:Cu molar ratio and the equilibrium time of the solutions before analysis, a summary of various copper complexes identified in several solutions of different molar ratios analyzed is presented in Table 2.

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

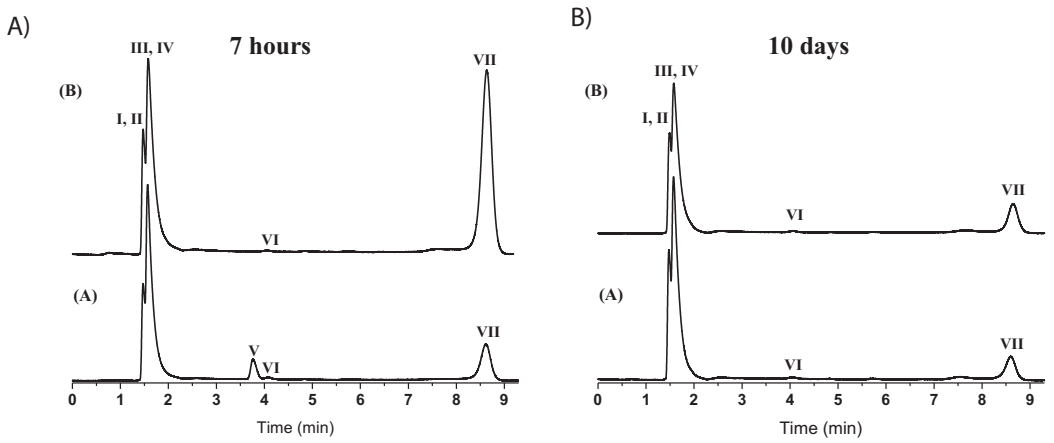


Fig. 3. HPLC–UV chromatograms of (A) 1:50 Thi:Cu and (B) 1:25 DMDTC:Cu solutions 7 h and 10 day after preparation, obtained with a mobile phase of acetonitrile:water 60:40 (v/v) flowing at 0.7 mL min^{-1} and detection at 270 nm.

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

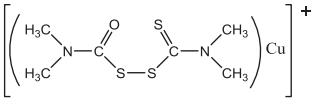
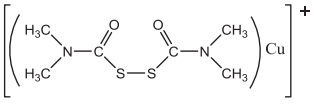
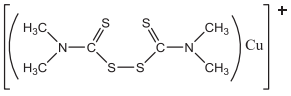
R_t (min)	Compound	$[M]^+$ *m/z*	MS^2 *m/z*	MS^3 *m/z*	MS^4 *m/z*	Proposed structures
1.5	**I**	145	104 (100)			$[(CH_3NC)Cu]^+$
	II	190	**145** (100)	104		$[(CH_3NC)_2((CH_3)NH)Cu]^+$
1.6	**III**	204	**163** (100) **145** (5)	**148**, 122 104	122, 104	$[(CH_3NC)_2(CH_3NHCHO)Cu]^+$ $[(CH_3)_2NCS_2((CH_3)NC)Cu]^+$
	IV	224	**183** (100)	147, 142, 88		
3.8	**V**	287	269 (10) **227** (100) 182 (10) 151 (25) 124 (5) 88 (5)	**182**, **151**, 124	106	
4.1	**VI**	271	**253** (100) 239 (20) 211 (5) 88 (80)	235, 211		
4.7	$[CuThi]^{2+}$	303	**260** (15) **227** (30) **202** (40) **184** (100) **151** (20) 88 (25)	202, **184** 183, **151**, 124, 88 **184** 88 108	88 108 88	
8.6	**VII**	303	**260** (15) **227** (30) **202** (40) **184** (100) **151** (20) 88 (25)	202, **184** 183, **151**, 124, 88 **184** 88 108	88 108 88	

Table 2
Byproducts detected by HPLC–MS and MS^n; na – not analyzed.

Solution	Molar ratio	Compounds		
		7 h	4 days	10 days
Thi:Cu(II)	1:3	**$[ThiCu]^{2+}$ Thiram**	**$[CuThi]^{2+}$ Thiram VI, VII**	**$[Cu(DMDTC)]^+$ 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	**$[Cu(DMDTC)]^+$ I, IV, VII**	**$[Cu(DMDTC)]^+$ (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**

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

Acknowledgments

O. Filipe wishes to acknowledge the PhD grant (SFRH/BD/39551/2007) from the Portuguese Science and Technology Foundation (FCT). S. A. O. Santos wishes to thank FCT and POPH/FSE for the postdoctoral grant (SFRH/BPD/84226/2012).

This work was supported by European Funds through COMPETE and by National Funds through the FCT within project PEst-C/MAR/LA0017/2013.

The authors also thank the financial support provided to CERNAS (PEst-OE/AGR/UI0681/2014), CICECO (PEst-C/CTM/LA0011/2013) and QOPNA (PEst-C/QUI/UI0062/2013) by the FCT.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhazmat.2014.06.051.

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