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Influence of Fulvic Acids and Copper Ions on Thiram Determination in Water

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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 behavior 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.

KEYWORDS: Thiram; solid-phase extraction; humic substances; metals; river water; HPLC−UV

INTRODUCTION

Dithiocarbamates are a group of organosulfur compounds extensively used due to their wide range of applications in agriculture and industry (*1–3*). Among them, thiram (bis-(dimethyldithiocarbamoyl) disulfide), a dithiocarbamate fungicide, is one of the most used fungicides in Portugal (*4*), and it is also one of the most largely applied worldwide (*1*). 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" (*5*). 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 disulfide, which is then measured either by spectrophotometry or by chromatographic techniques coupled with different types of detection (*6–10*). 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 (*11–14*). Many analytical methods have been applied to thiram analysis in fruits and vegetables (*2, 11, 15–20*), but only a few studies have been published concerning thiram analysis in natural waters (*21–25*).

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 concentration of several nonpolar pesticides from liquid samples (*26, 27*). However, its application to thiram analysis in natural waters has been referred to in very few published works, and the results obtained are contradictory (*22, 23*). Garcia et al. (*23*) obtained a thiram recovery of 70% from 1 L of river water spiked with thiram (5 μg L^{-1}). On the other hand, Tovar et al. (*22*), also using a C_{18}-SPE procedure, did not recover thiram from tap water, natural water, and groundwater samples spiked with thiram and other pesticides. The authors attributed this fact to the complexation of thiram by metal ions in natural waters, since they obtained recoveries of 50−90% when the same procedure was applied

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Table 1. Thiram Recoveries (R) from 1 L of River Water Sample Spiked with Thiram[a]

sampling date	monthly precipitation (mm)	equilibration time (h)	[Thi] (μg L^{-1})	$R \pm$ SD (%)
June 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)
October 2006	244	0	11.1	98.0 ± 1.3 (n = 2)
		24	11.1	41.8 ± 0.8 (n = 2)
March 2007	187	0	11.2	98.8
		24	11.2	61.3 ± 6.3 (n = 6)
		48	11.2	25.0
April 2007	54	0	11.2	83.1
		24	11.2	20.1 ± 2.7 (n = 3)
		48	11.2	<LOD (n = 3)

[a] SD = standard deviation, and n = number of experiments performed in each case.

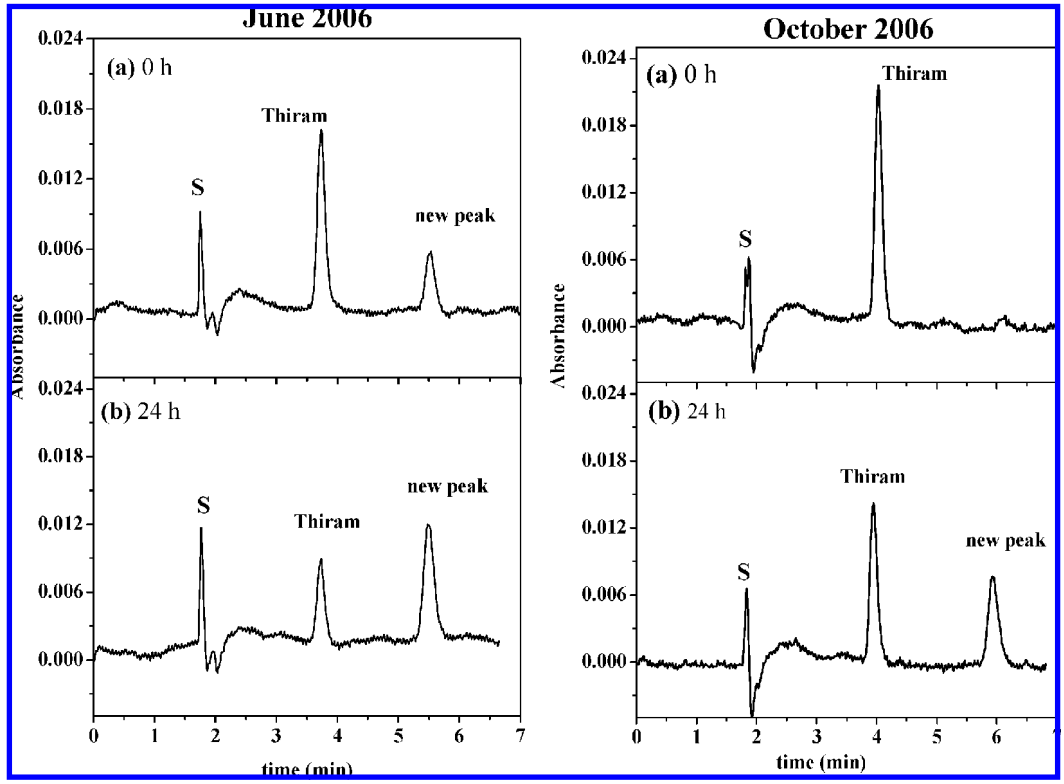

Figure 1. HPLC−UV chromatograms of the natural water sample spiked with 11.2 μg L^{-1} thiram and equilibrated for 0 and 24 h, following the SPE procedure. In all panels, the peak marked "S" is associated with the solvent.

to a standard mixture of the same pesticides. More recently, Sasaki et al. (*25*) used an online 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 study was to clarify the contradictory results found in the literature concerning the recoveries of thiram during its analysis in natural waters and to gain better insight into 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 fungicide, frequently in conjunction with thiram 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 (*4*).

Table 2. Effect of EDTA (1 mM) on Thiram Recoveries (R) from 1 L of River Water Sample Spiked with Thiram and Submitted to SPE[a]

sample	sampling date	[Thi] (μg L^{-1})	$R \pm$ SD (%)
[(river + EDTA)$_{24}$ + Thi]$_{24}$	October 2006	11.1	96.1 ± 5.0 (n = 4)
	March 2007	11.2	95.6 ± 0.8 (n = 2)
[(river + Thi)$_{24}$ + EDTA]$_{24}$	March 2007	11.2	47.3 ± 4.4 (n = 4)
	April 2007	11.2	<LOD (n = 3)

[a] SD = standard deviation, and n = number of experiments performed in each case.

EXPERIMENTAL PROCEDURES

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 (FAs) were extracted from River Vouga water, collected at Carvoeiro (Aveiro, Portugal), by Santos and Duarte (*28*). Methanol (HPLC grade) was obtained from Riedel-de Haen, and acetonitrile (HPLC grade) was obtained from LabScan. Ultrapure 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

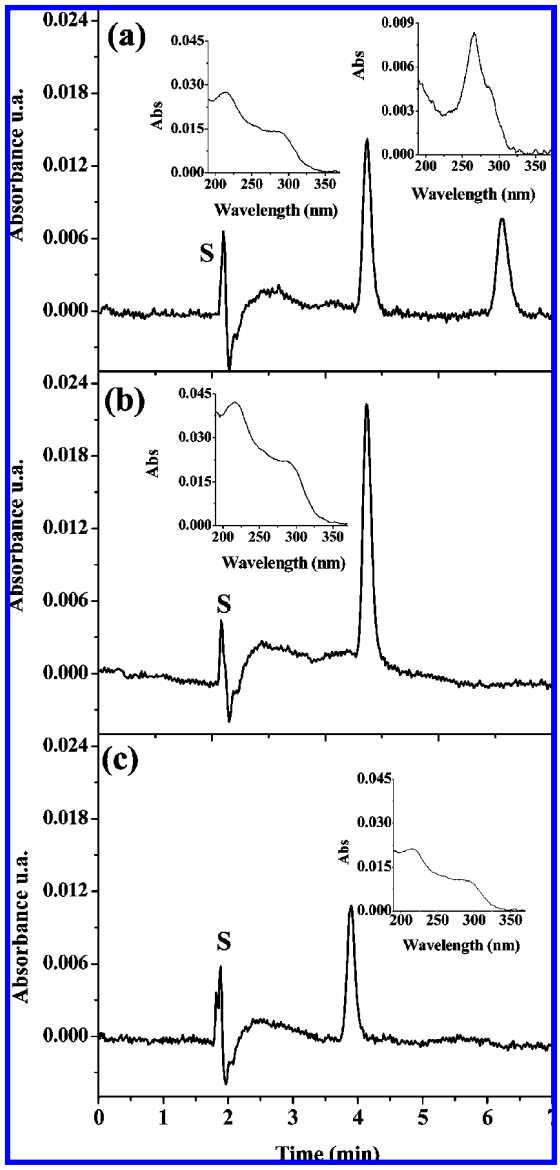

Figure 2. Chromatograms of the river water samples collected in March 2007: (**a**) (river + Thi)$_{24}$, (**b**) [(river + EDTA)$_{24}$ + Thi]$_{24}$, (**c**) [(river + Thi)$_{24}$ + EDTA]$_{24}$. [EDTA] = 10^{-3} mol L^{-1}, and [Thi] = 11.2 μg L^{-1}. (The samples were submitted to the SPE−HPLC−UV analytical procedure).

of thiram prepared in acetonitrile. This stock solution was also used to spike the 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 (the final concentration was 2 mg L^{-1}, which is within the concentration range usually found in river waters (*29*)). The pH of these solutions was measured to be about 6.

Apparatus. Commercial Supelclean envi-18 cartridges (Supelco) of 500 mg mass, 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. Thiram was determined by HPLC−UV using a Jasco apparatus equipped with a PU-980 pump, a UV−vis Banspec detector operating at 270 nm, a Phenomenex C$_{18}$ column (150 × 4.60 mm, 5 μm, 110 A), and a 20 μL loop. The UV−vis spectra were obtained in a UV−vis Shimadzu spectrophotometer, using a 1.00 cm cell.

C$_{18}$-SPE−HPLC−UV Procedure. The SPE cartridge was preconditioned with 6 mL of methanol and 6 mL of deionized water. After 1 L of sample was loaded at 15−20 mL min^{-1}, by means of a vacuum pump, the cartridge was washed with 3 mL of ultrapure water and dried under N$_2$ for 30 min. Thiram was then eluted with 5 mL of acetonitrile and analyzed by HPLC−UV at 270 nm. The mobile phase was 70:30 (v/v) acetonitrile/water, previously filtered through a membrane filter, 0.2 μm NL16 (Schleicher & Schuell), and flowing at 0.7 mL min^{-1}. Thiram concentrations were calculated from the average of the peak areas of at least three injections.

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} (*28*). The samples were collected on June 23 and Oct 17 of 2006, as well as on March 2 and April 22 of 2007, 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 (Gelman Sciences), stored at 4 °C, and analyzed 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 (*30*).

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

RESULTS AND DISCUSSION

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. The limit of detection (LOD) was calculated from each calibration curve as $a + 3s_{y/x}$, where a is the interception of the regression line and $s_{y/x}$ is the residual standard deviation. A mean value of 0.088 mg L^{-1} (SD = 0.034 mg L^{-1}, n = 24) was obtained for the LOD, which, considering the applied concentration factor of 200, means an LOD in the original sample of 0.44 μg L^{-1}. The relative standard deviation for replicate injections was lower than 5% (same sample).

Effect of Flow Rate in the C$_{18}$-SPE Thiram Preconcentration from Standard Solutions. To optimize the time of preconcentration, the effect of the flow rate on the water sample loading into the SPE cartridge was assessed for thiram standard solutions of two distinct concentrations: 2.9 and 11 μg L^{-1}. Losses of thiram were not observed by increasing the flow rate to 25 mL min^{-1}. Some of the standard solutions were analyzed immediately after preparation (Thi$_0$), while others were stored for 24 h in the dark (Thi$_{24}$), to evaluate both the stability of thiram in solution and any losses by adsorption on the flask walls. The results showed that thiram recoveries were not different from 100% for practical purposes even for the lowest studied concentration, 2.9 μg L^{-1}, showing that flow rates up to 25 mL min^{-1} allowed enough time for thiram−adsorbent interaction. In this work, flow rates between 15 and 20 mL min^{-1} were used, representing a significant time improvement related to the procedure described in the literature by Garcia et al. (*23*).

Application of the SPE−HPLC−UV Procedure to River Water Samples. The SPE−HPLC−UV procedure was applied to the analysis of thiram in the river water samples, and the chromatograms detected no presence of thiram. Then the river water samples were spiked with the thiram stock solution, obtaining concentrations of ca. 3 and 11 μg L^{-1}. Spiked river

Table 3. Thiram Recovery (R) from 1 L Standard Solutions in the Presence and Absence of Cu(II) after the C_{18}-SPE Procedure[a]

sample	[Thi] (μg L^{-1})	[Cu^{2+}] (mg L^{-1})	Cu:Thi molar ratio	$R \pm$ 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 ($n = 3$)

[a] SD = standard deviation, and n = number of experiments performed in each case.

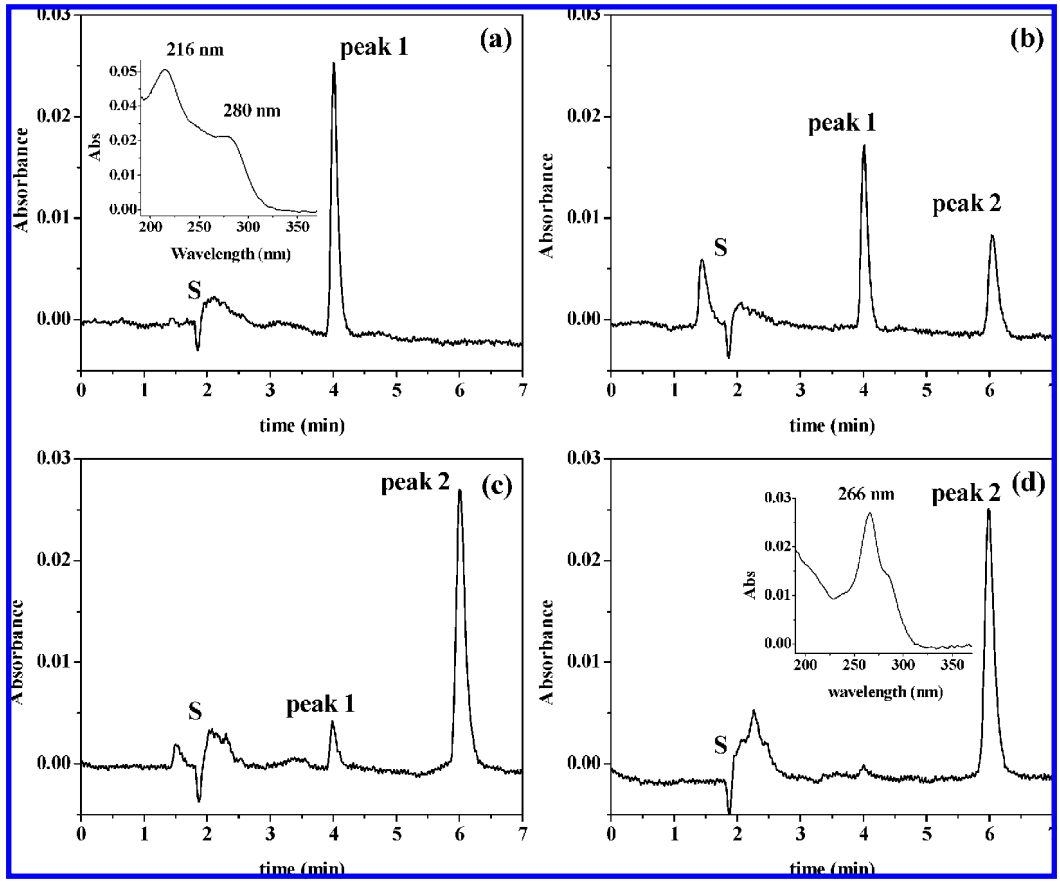

Figure 3. HPLC−UV chromatograms of a standard solution of thiram (2.4 mg L^{-1}) (**a**) in the absence of Cu(II) and (**b**−**d**) in the presence of Cu(II) (16 mg L^{-1}) at different times of equilibration: (**b**) $t = 20$ min, (**c**) $t = 2$ h, (**d**) $t = 8$ h.

water samples were analyzed both immediately after preparation ($river_0$) and after 24 and 48 h periods of storage in the dark ($river_{24}$ and $river_{48}$). **Table 1** shows thiram recoveries obtained from spiked river water samples after different equilibration periods of time. Independently of the thiram initial concentration, water samples immediately analyzed (for an 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 ($R = 70 \pm 8\%$, using C_{18} cartridges, 1 L of sample, and 5 μg L^{-1} of thiram) (*23*). 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 1**). 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 et al., who did not recover thiram by C_{18}-SPE from fortified natural waters (*22*). These authors did not refer to the equilibration time after sample spiking and attributed the recovery failure to complexation of thiram with metal ions in solution. This could also be suggested by the analysis of the chromatograms shown in **Figure 1**. After equilibration of thiram with the river water, the peak of thiram decreases, a new peak appearing 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 in ref *30*). For example, after 24 h of equilibration, for a thiram concentration of 2.8 μg L^{-1}, the peak of thiram disappears and only the new peak is visible (chromatogram not shown) while, for an 11.2 μg L^{-1} thiram concentration, the peak of thiram decreases but its presence is still noteworthy (**Figure 1**). It should be highlighted that a small peak at 6 min is already present in the chromatogram of one of the samples shown in **Figure 1** (June 2006), even when it was analyzed immediately after spiking. The same was observed for the sample collected in April 2007 (chromatogram not shown). Those were the samples where the lowest recoveries for equilibration time zero were obtained (see **Table 1**). Such results are probably related to the fact that those samples were collected during a dry period of the summer and spring seasons (see **Table 1**, monthly precipitation data). That may be associated with higher concentrations of river water solutes available to react with thiram.

Next, in this work, the complexing properties of EDTA were explored as a means to overcome the interference of thiram

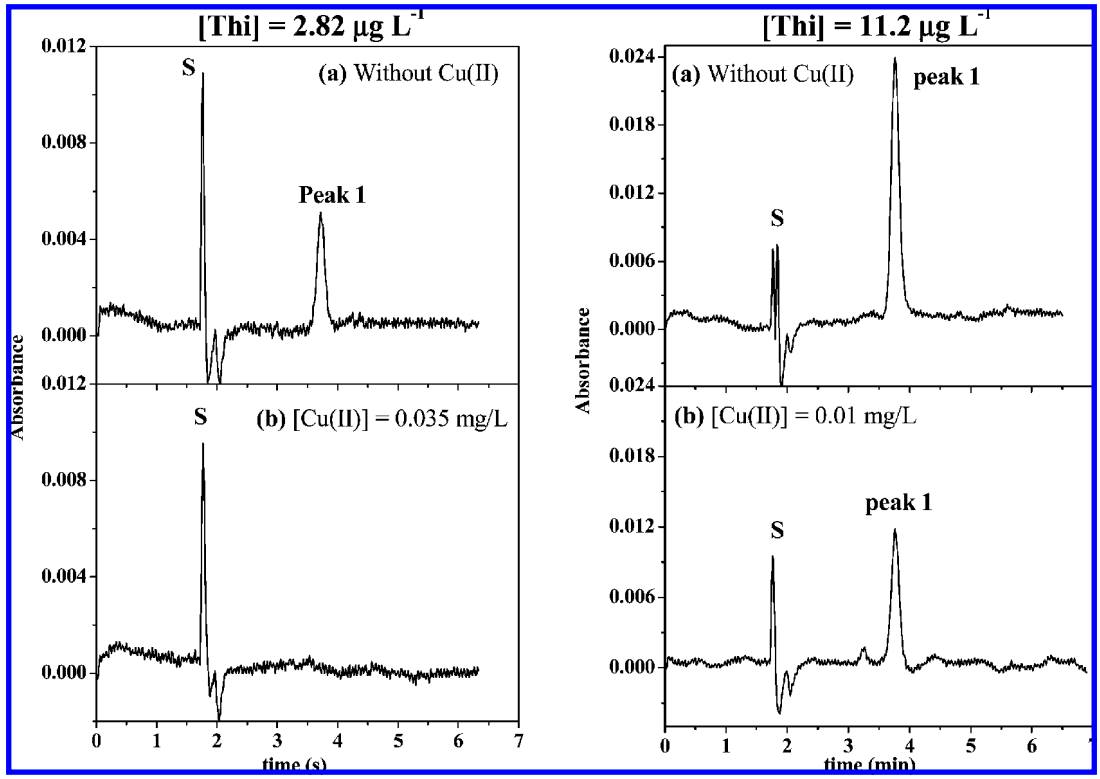

Figure 4. HPLC−UV chromatograms of thiram standard solutions in the absence and in the presence of Cu(II) after equilibration for 24 h and SPE treatment.

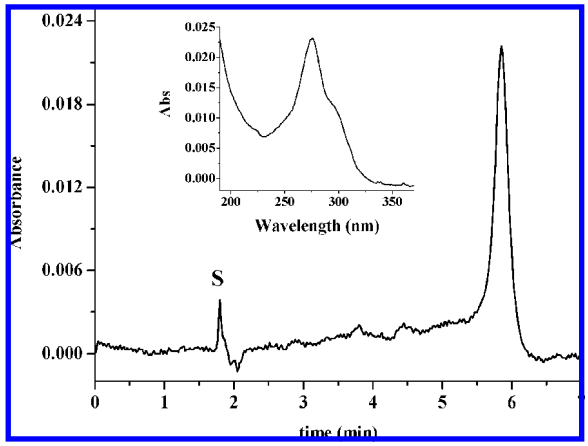

Figure 5. HPLC−-UV chromatogram of the yellow-colored complex Cu(II)−thiram retained in the C_{18} cartridge after elution with $CHCl_3$, drying under a N_2 atmosphere, and redissolution of the residue in acetonitrile.

complexation by metallic ions. Thus, an excess of EDTA (10^{-3} mol L^{-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, $[(\text{river} + \text{EDTA})_{24} + \text{Thi}]_{24}$; (ii) addition of EDTA to the river water sample previously equilibrated for 24 h with thiram, $[(\text{river} + \text{Thi})_{24} + \text{EDTA}]_{24}$. The samples were analyzed by SPE−HPLC−UV. Thiram recovery results are shown in **Table 2**, while some examples of the obtained chromatograms are presented in **Figure 2**.

When EDTA was previously added to the water samples, before thiram spike, $[(\text{river} + \text{EDTA})_{24} + \text{Thi}]_{24}$, thiram recoveries were higher than 90%, while in the absence of EDTA, the recoveries were only ca. 42% and 61%, respectively (see **Table 1**, results for October 2006 and March 2007 after a 24 h equilibration period of time). This suggests that metal ions are somehow involved in the reactions giving the low recoveries of thiram. However, when EDTA was added after a previous equilibration time between the river water and thiram, $[(\text{river} + \text{Thi})_{24} + \text{EDTA}]_{24}$, 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, as shown in the following section of this paper. Thus, the results suggest that a different reaction involving metal ions is the cause of the low recoveries that were registered.

Figure 2 shows the chromatograms obtained for the sample collected in March 2007, in the absence of EDTA, $(\text{river} + \text{Thi})_{24}$, and in the presence of EDTA, before, $[(\text{river} + \text{EDTA})_{24} + \text{Thi}]_{24}$, and after, $[(\text{river} + \text{Thi})_{24} + \text{EDTA}]_{24}$, a 24 h time period of equilibration between thiram and river water. 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 behavior of thiram in aqueous solutions containing river FA, a metal ion, or both was further studied. The metal ion focused on in this study was Cu(II), since it is usually applied in agriculture as inorganic fungicides, frequently in conjunction with thiram or during the same season as thiram is used.

Effect of Copper Ions on Thiram Recoveries by C_{18}-SPE. Tovar et al. (*22*) suggested that thiram was not recovered from river water by C_{18}-SPE preconcentration due to its complexation

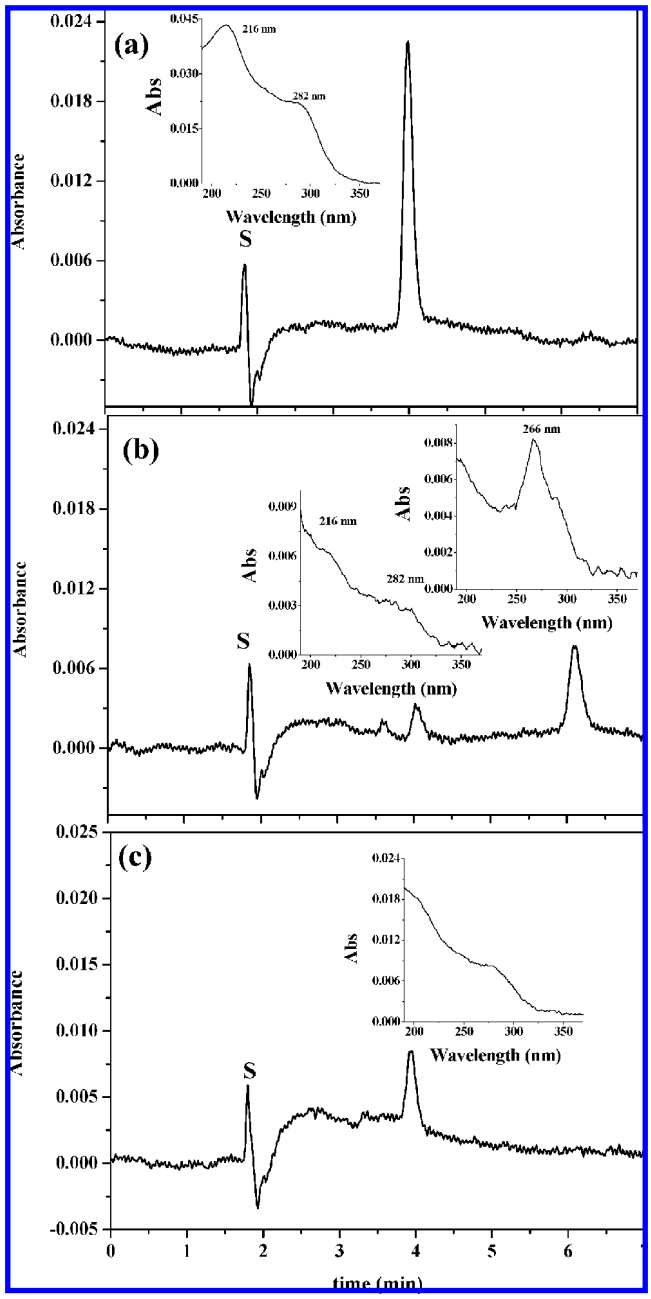

Figure 6. Chromatograms obtained in the analysis of aqueous solutions [(**a**) $(FA + Thi)_{24}$, (**b**) $[(FA + Cu^{2+})_{24} + Thi]_{24}$, and (**c**) $[((FA + Cu^{2+})_{24} + Thi) + EDTA]_{24}$] and UV−vis spectrum of the peaks. [FA] = 2 mg L^{-1}, [Cu] = 0.01 mg L^{-1}, [EDTA] = 10^{-5} mol L^{-1}, and [Thi] = 11.2 μg L^{-1}.

Table 4. Thiram Recoveries (R) from Standard Solutions ([FA] = 2 mg L^{-1}, $[Cu^{2+}]$ = 0.01 mg L^{-1}, EDTA = 10^{-3} mol L^{-1}))[a]

sample	[Thi] (μg L^{-1})	$R \pm$ 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 ($n = 4$)

[a] SD = standard deviation, and n = number of experiments performed in each case.

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 3** and **Figure 3**. In the chromatograms of the solutions not submitted to SPE (**Figure 3**), the intensity of the thiram peak at ca. 4 min (peak 1) decreases as the Cu(II)−thiram equilibration time increases and a new peak, attributable to a Cu(II)−thiram complex, appears at about 6 min (peak 2) (**Figure 3**). After 8 h of equilibration, peak 1 disappears completely. In the chromatograms of the solutions submitted to SPE (**Figure 4**), 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 4**) suggests that the Cu(II)−thiram 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 5**). The chromatogram exhibited only one peak, with the retention time and the UV spectrum characteristic of peak 2 (cf. **Figure 3**). 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 C_{18} cartridge and not eluted with acetonitrile. The results in **Table 3** show that thiram recoveries decrease significantly in the presence of Cu(II), depending on the Cu(II):thiram ratio.

Still, the thiram−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 behavior observed in river water, since the addition of EDTA to river water equilibrated with thiram did not allow good recoveries.

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 for 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 analyzed by SPE−HPLC−UV for comparison. **Figure 6** shows the HPLC chromatograms of the solutions spiked with thiram and the UV−vis spectra of each chromatographic peak. As shown in **Figure 6b**, in the solutions containing both FA and Cu(II), there is a significant decrease of the thiram peak height (relative to the sample without Cu(II), **Figure 6d**) and the peak at about 6 min appears, as observed in spiked river water samples after 24 h of equilibration (**Figure 4b**). This additional peak at 6 min appears at the same retention time of peak 2 observed in the chromatograms of thiram/Cu(II) solutions without SPE treatment (**Figure 3**), and it could perfectly result from formation of a thiram−Cu(II) complex. However, these samples were submitted to the SPE procedure, and peak 2 disappeared from the chromatograms of thiram/Cu(II) solutions when they were submitted to SPE (**Figure 4**), 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 6c**), 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. Therefore, 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 4**, 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 was 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 behavior is similar to that observed in the river water samples (**Figure 2c**), clearly indicating that the degradation of thiram only occurs when both FA and Cu(II) are present together in the same solution. These results support the hypothesis of thiram reduction by FA catalyzed by Cu(II) in river waters. According to the literature (*31, 32*), 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 FAs may act as electron donors (*33, 34*). Therefore, when thiram is equilibrated with solutions of FA and Cu(II), the reduction of thiram by FA, catalyzed 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 the cartridge (with acetonitrile) exhibited an intensive peak at 6 min, which was absent in the chromatograms of the thiram/Cu(II) solutions after SPE treatment in **Figure 4**. This also supports the hypothesis of thiram degradation into DMDTC anions. According to Weissmahr and Sedlak (*35*), 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 done with the aim to better clarify the nature of this degradation and to identify the product.

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