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Short communication

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

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Abstract

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

Keywords: Thiram; Solid-phase extraction; Humic substances; Aqueous samples; UV–vis spectrophotometry

1. Introduction

Thiram is a dithiocarbamate compound that acts in breath activity of fungi inhibiting spore's germination (Fig. 1). It is one of the contact fungicides most widely used to prevent crop damages, either in the field or during its transport and storage [1,2]. In Portugal, its sales have increased significantly, according to the reports available (2001–2003) [3], and it is used to protect seeds, fruits, vegetables and ornamental plants from a variety of fungi (*Botrytis* spp., *Anthracnosis*, *Fusarium* and *Manilia*) [2]. In addition, it is also used as a repellent to protect the fruit and ornamental trees from damages caused by rabbits and rodents [2].

The progressive increase in the application of chemicals for agriculture has lead to a rise in concern for the problem of soil contamination and for the risk of contaminants leaching to ground and surface waters. Adsorption studies are very important and useful to provide essential information about mobility of chemicals and their distribution in soil, water and air. The information supplied by this kind of studies can be used, for example, in the prediction or estimation of the availability of a chemical for degradation, transformation and bioavailability, leaching through the soil profile, volatility from soil and run-off from land surfaces into natural waters.

However, in spite of the intensive use of thiram worldwide, physicochemical parameters for its adsorption onto soils have not been evaluated, as highlighted by Stathi et al. [4]. Studies of its adsorption on lignin [5] and on alumina and clay [4] have been reported, but there are no studies about its adsorption on humic substances, which constitute 60–70% of the organic matter in soils [6] and which have been reported to interact with several other pesticides, influencing their fate in the environ-

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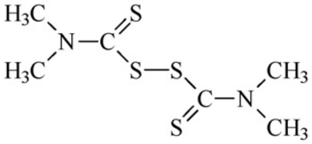

Fig. 1. Structure of the fungicide thiram ($C_6H_{12}N_2S_4$, tetramethylthiuram disulfide).

ment [7–9]. Adsorption studies of organic contaminants on soil and on humic substances are usually performed by mixing and equilibrating a known amount of soil or solid humic substances with a known volume of pesticide standard solutions in 0.01 M $CaCl_2$ [8–12]. The amount of pesticide adsorbed is then calculated from the difference between the initial and equilibrium pesticide concentrations in solution. The solutions equilibrated with soil or humic substances do contain high concentrations of UV–vis absorbent organic matter which can interfere in the analytical determination of the pesticide. Thus, for studying the adsorption of thiram either on soils or on humic substances, a clean-up procedure is required to remove these substances from solution before thiram analysis by any UV–vis spectrophotometric method or by HPLC–UV. Solid-phase extraction (SPE) has already been used for clean-up of the aqueous solutions of other pesticides after equilibration with soils [13,14].

SPE with C_{18} stationary phase has been also applied by other authors [15–17] to the extraction and preconcentration of thiram from aqueous standard solutions. Good recoveries were obtained using dichloromethane/methanol [15] or acetonitrile [16] as eluents. However, very few applications to aqueous solutions containing humic substances, such as natural waters, have been published [16,17], and the results obtained were contradictory. Garcia et al. [17] did successfully apply a C_{18} cartridge to the extraction/concentration of thiram from river water. On the other hand, Tovar and Santos-Delgado [16] did apply the same stationary phase as adsorbent of several carbamate pesticides (carbaryl, propoxur, propham, methiocarb and thiram) from river water, and the same authors observed that thiram was the only pesticide that was not recovered. As far as we know there are no published results concerning the applicability of C_{18}-SPE cartridge as a clean-up procedure of thiram in aqueous solutions with high concentrations of humic matter.

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

2. Experimental

2.1. Chemicals and reagents

All chemicals used were of analytical grade. Thiram (97%) was purchased from Aldrich and commercial humic acids (ash 10–15%, M_r 600–1000) were supplied by Fluka. Methanol and chloroform (HPLC grade) were obtained from Riedel-de Haen and LabScan, respectively. An aqueous 0.01 M $CaCl_2$ solution was prepared from $CaCl_2$ anhydrous (Fluka, p.a.). Ultra pure water for aqueous solutions was obtained with a Milli-Q water purification system (Millipore).

A stock solution of 15 mg/L of thiram in 0.01 M $CaCl_2$ was used to prepare spiked aqueous humic and soil extracts.

A stock solution of 25 mg/L thiram in $CHCl_3$ was used to prepare standard solutions of thiram for calibration of absorbance measurements.

2.2. Soil

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

2.3. Apparatus

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

2.4. Preparation of aqueous humic acid and soil extracts

Soil suspensions were prepared using a ratio of 5 g of soil to 25 mL of 0.01 M $CaCl_2$, in a pyrex centrifuge tube, while humic substances suspensions were prepared using a ratio of 25 mg of humic acids to 5 mL of solution. These ratios are in agreement with those used in the literature concerning adsorption studies of other pesticides onto soils or humic substances [7–9]. Both suspensions were centrifuged during 30 min at 4000 rpm and the supernatants were filtered through a 0.2 μm filter. These filtered extracts were used as samples. Spiked samples were prepared by addition of a known volume of the 15 mg/L thiram stock solution (0.01 M $CaCl_2$).

2.5. SPE procedure

Commercial Supelclean envi-18 cartridges (Supelco) of 500 mg, 75 Å pore diameter and 56 μm particle size were set up in a 12-place manifold from Phenomenex in order to perform the SPE experiments. Before use, all the cartridges were washed with 3 mL of $CHCl_3$, the solvent used for thiram elution.

Before sample application, the cartridges were preconditioned with 6 mL of methanol, 6 mL of Milli-Q water and 6 mL of 0.01 M $CaCl_2$ (the sample matrix).

Next, an aliquot of 2 mL of the spiked extracts was percolated through the cartridge at a flow rate of 2 mL/min under vacuum 6 kPa. Before elution, the SPE cartridge was rinsed with 3 mL of Milli-Q water in order to remove any residual sample and subsequently dried under nitrogen, during 30 min. Thiram was then eluted with 3 mL of $CHCl_3$, which was collected in a graduated tube, and its concentration was determined by spectrophotometry at 283 nm against $CHCl_3$.

Non-spiked soil or humic extracts were used as blanks and a control sample of thiram in 0.01 M $CaCl_2$ aqueous solution (not