

Solvent extraction followed by ultraviolet detection for investigation of tetramethylthiuram disulfide at soil-water interface

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ABSTRACT: Tetramethylthiuram disulfide is a widely used pesticide and a soil treatment agent in the agricultural sector. Consequently, its detection in the environment and investigation of the distribution between the aqueous and soil phases would be of highly importance. In this study, the detection of tetramethylthiuram disulfide in CHCl_3 was successfully achieved through absorbance measurements at 280 nm, which resulted in linear calibration curves. This detection method was extended for the determination of the partition coefficient (K_D) of tetramethylthiuram disulfide between CHCl_3 and H_2O phases at ambient temperature. The partition coefficient was subsequently used to determine the apparent partition coefficient (K_D) of tetramethylthiuram disulfide between sieved soil of diameter < 2.0 mm used for agricultural practices and water where by the quantification of tetramethylthiuram disulfide in water after adsorption equilibrium with soil was achieved through a solvent extraction step of tetramethylthiuram disulfide-contaminated water into CHCl_3 . The K_D determined for an initial tetramethylthiuram disulfide concentration ranging from 3.00 ppm to 10.00 ppm showed a decreasing trend, which leveled off at high concentrations, indicating the strong irreversible adsorption behavior of tetramethylthiuram disulfide on soil at low concentrations. The proposed detection method would have the potential to determine the extent of adsorption of tetramethylthiuram disulfide in soil or its levels in water when tetramethylthiuram disulfide is introduced to soil systems for agricultural practices.

Key words: Pesticides, adsorption, partition coefficient, analytical methods, environmental monitoring

INTRODUCTION

Tetramethylthiuram disulfide (Thiram) belongs to the dithiocarbamate family of pesticides, which is registered as a general purpose pesticide by the U.S. Environmental Protection Agency (EPA) and classified as toxicity class III. Formulations of thiram are available as dust, flowable or wettable powder, water dispersible granules and water suspensions (Kidd and James, 1991). Thiram is used as a fungicide to protect harvested crops and prevent crop damage in the field, and as a seed protectant to prevent various fungal diseases (Han *et al.*, 2003; Sharma *et al.*, 2003; Bradley *et al.*, 2007). It is also used in rubber industry as an accelerator and a vulcanization agent (Ceresera *et al.*, 2001; Grosicka *et al.*, 2005). It is used by humans in the treatment of scabies and as a bactericide for skin (Ceresera *et al.*, 2001). Ingestion and inhalation of thiram is slightly toxic while dermal adsorption is

moderately toxic. Thiram breaks down in the human body to give carbon disulfide which causes some damage to the liver. Ecologically it is nontoxic to birds (high LC_{50}), but highly toxic to fish as LC_{50} values for most of the fish species are below 5 ppm (Fernandez *et al.*, 1996). Thiram is slightly soluble in water (30 ppm at 25°C), and consequently, it is immobile in soils high in clay and organic matter content. The half-life of thiram in the soil (15 days) is shorter compared to that of many pesticides (Aulakh *et al.*, 2005).

Thiram, being a toxic substance for aquatic life, should be detected with an acceptable accuracy. Among many methods available for the detection of thiram and related substances, gas chromatography, high performance liquid chromatography, thin layer densitometry, Fourier transform infrared spectroscopy, capillary electrophoresis and voltammetry have been successful for a variety of sample matrices (Procopio *et al.*, 1988; Sharma *et al.*, 2003; Priyantha and

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Weliwegamage, 2005 and 2008). Special reagents have been used in many of these techniques to improve selectivity and/or sensitivity. For instance, thin layer densitometry of copper complexes of dithiocarbamates provides a simple and rapid means of their detection (Onuska, 1974). Extraction of thiram with cyclohexane-ethyl acetate followed by HPLC with UV detection allows the detection at 0.1 - 0.2 ppm level (Ekroth *et al.*, 1998). Detection limits have been further enhanced to 3.8 ppt (parts per trillion) through a postcolumn reaction detector that employs UV photolysis with o-phthalaldehyde-2-mercaptoethanol followed by fluorometric determination (Miles and Moye, 1988). In a different study, conversion of thiram to a selenium dithiocarbamate complex, followed by extraction into chloroform and measuring at 430 nm is able to detect at ppb levels (Malik and Rao, 1990). Improvement in detection limits has also been achieved by incorporating a solid phase microextraction step (Aulakh *et al.*, 2005).

Sorption equilibrium of a chemical species at the soil/water interface plays a major role in aquatic chemistry and environmental science (Spark *et al.*, 2002; Kang *et al.*, 2002; Boivin *et al.*, 2005; Sheng *et al.*, 2005). When toxic substances, such as pesticides, are involved in such equilibrium, sorption characteristics should be known with high accuracy as the molecules that are not bound to soil particles directly reach the environment which may ultimately cause a strong impact on humans (Fava *et al.*, 2005).

This problem would be alarming if the levels of a pesticide in the environment were greater than the tolerable limits. Consequently, there has been much attention on the adsorption behavior of organic contaminants on natural particles (Rytwo *et al.*, 2002; Schmidt *et al.*, 2004; Tsai and Lai, 2006; Changwen *et al.*, 2006).

Additionally, many model studies for adsorption of organic substances with clay-based materials have been reported (Pu *et al.*, 2003; Akçay, 2004; Kaya and Ören, 2005). Nevertheless, due to inhomogeneity of soil, which results in unpredictable surface reactivity and irreproducible measurements, designing sorption models for pesticides at the soil/water interface has become a challenge despite the information obtained from such processes being of great importance. In the present study, a reliable, economical detection method for thiram present in soil suspensions was employed based on a solvent extraction step followed by ultra-

violet (UV) detection. Sorption studies were also conducted for commercially available bentonite clay for comparison. The results were subsequently used for investigation of the validity of adsorption models.

MATERIALS AND METHODS

Instrumentation

A UV-Visible scanning spectrophotometer (Shimadzu 160 UV-Visible) was used to record absorption spectra of thiram in both aqueous and CHCl_3 media. The optimum wavelength for the detection of thiram was determined, and used throughout the study. The metals present in soil samples were determined using an Atomic Absorption Spectrophotometer (Buck Scientific, Model 200-A).

Materials

Three agricultural fields representing different geographical areas of Sri Lanka were selected to obtain soil samples. Each sample was collected (August, 2006) from the upper 25 cm. of the surface horizon following removal of surface vegetation. Samples were mixed well, air dried for 24 h. and sieved to remove solid particles of diameter over 2 mm in order to make the soil matrix homogeneous. Bentonite, a commercial soil used for comparison studies, was purchased from Sigma-Aldrich.

A commercial sample of thiram [Tetramethylthiuram disulfide, $(\text{H}_3\text{C})_2\text{NCS}_2\text{S}_2\text{CN}(\text{CH}_3)_2$, 80 % w/w] was purchased from the local market. Tetramethylthiuram disulfide (97 %), which was purchased from Sigma-Aldrich, was recrystallized, melting point (Reported 155.5 °C) was checked, and used for the standardization of commercial samples. All solutions were prepared in either distilled water or double distilled CHCl_3 . Standard solutions of metals ions (Cu, Fe, Zn, Na and K) for atomic absorption measurements were prepared using analytical grade reagents in deionized water. Soil samples were digested using HF and aqua regia for absorption measurements.

Methods

Optimization of experimental parameters and determination of analytical characteristics are important in accurate detection of any substance. High solubility of thiram in CHCl_3 and its immiscibility with water would provide sufficient conditions for the selection of CHCl_3 as the solvent of choice. The absorbance of thiram solutions in CHCl_3 of

concentrations varying from 1 to 10 ppm was measured in triplicate at 280 nm, and the corresponding calibration curve was constructed. Aqueous solutions of thiram of the same concentration range as stated above were allowed to reach partition equilibrium with CHCl_3 , after which, aliquots of the CHCl_3 layer were withdrawn and the adsorption of each solution was measured in triplicate at 280 nm. Homogenized soil samples (30 g) and aqueous solutions of thiram (60 cm^3) of different concentrations (1 to 10 ppm) were stirred for 15 min. Three replicate experiments were performed for each concentration. Each sample was allowed to stand for 2 h. to reach equilibrium. An aliquot of 20 cm^3 of the supernatant of each solution was withdrawn and shaken with 20 cm^3 of chloroform in a separating funnel. The mixture was allowed to separate, after which, the chloroform layer was withdrawn, and its absorbance measured at 280 nm. This same procedure was used for bentonite. Calibration curves were used for the determination of the concentration of thiram in the chloroform layer. Average apparent distribution coefficient for each concentration for each soil type and for bentonite was then determined.

RESULTS AND DISCUSSION

The calibration curve for the direct UV determination of thiram in CHCl_3 at 280 nm, constructed using average absorbance values, is shown in Fig. 1. The minimum detection limit (MDL), linear dynamic range (LDR) and sensitivity were 0.170 ppm, 1 ppm to 10 ppm, and 0.086/ppm, respectively. The linear regression equation and the correlation coefficient of the calibration curve drawn within the LDR are $y = 0.037x + 0.075$ and $R^2 = 0.9753$, respectively. The partition coefficient of thiram between CHCl_3 and water, determined by measuring the levels of thiram in the CHCl_3 phase after partitioning with the aqueous phase containing thiram, resulted in an average value of 5.86 ± 0.54 over 15 replicates within the initial concentration range of 1 ppm to 10 ppm in water. The minimum detection limit obtained in this method was comparable to that of the more sophisticated methods such as high performance liquid chromatography that employs sensitive detectors (Fernandez *et al.*, 1996; Ekroth *et al.*, 1998). The simplicity of the detection method developed is an added attraction for quantification of thiram. Other advantages of using CHCl_3 as the medium are that aqueous solutions of low

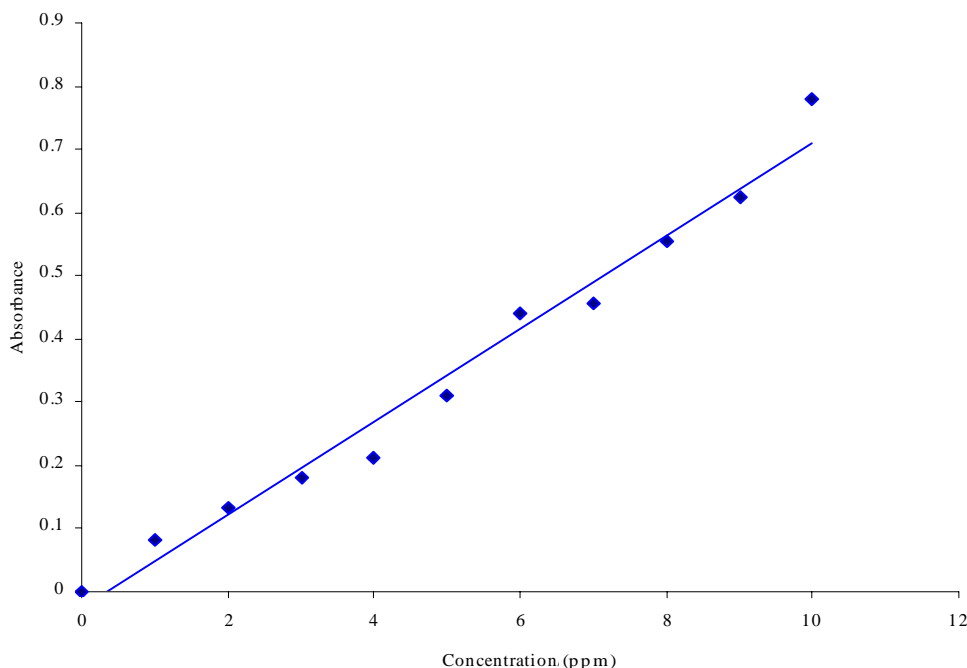


Fig. 1: Calibration curve of the UV detection method of thiram in CHCl_3

Investigation of thiram at soil/water interface

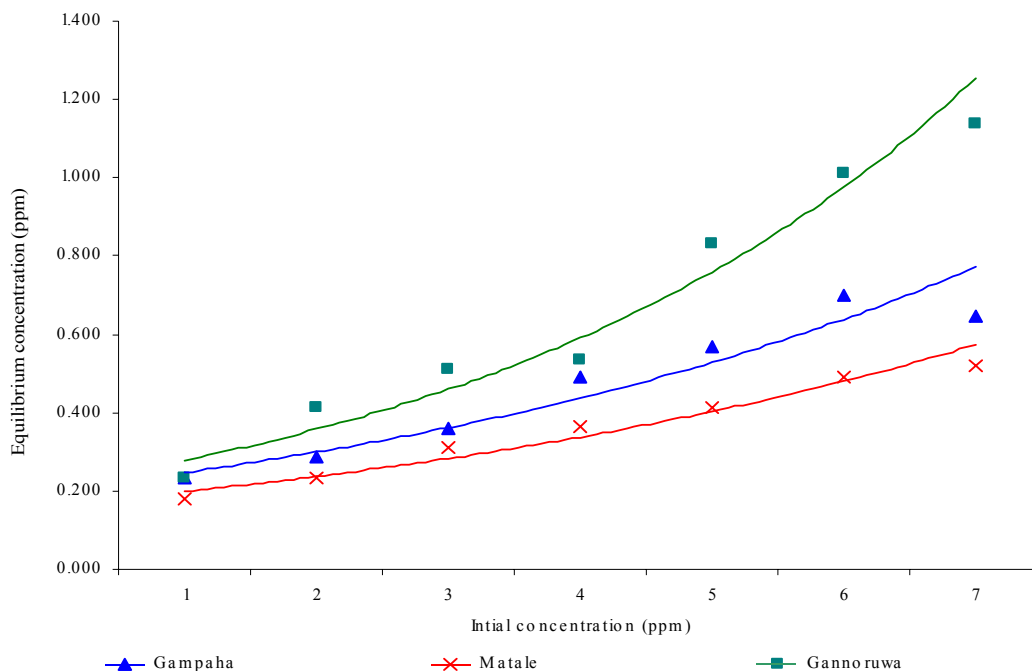


Fig. 2: Thiram concentrations of the water phase after establishment of partition equilibrium of aqueous thiram solutions of different initial concentrations. The standard addition method was used to determine concentrations below the minimum detection limit

concentrations of thiram can be preconcentrated by extracting into CHCl_3 as it shows a higher solubility in CHCl_3 , and that thiram, when present in turbid solutions which cause erroneous results in the UV detection due to scattering, can be extracted into CHCl_3 to produce clear solutions. This is of an utmost advantage for the present study to determine thiram in water in contact with soil particles. Determination of thiram in other organic solvents has also been attempted due to similar reasons (Hernandez Olmos *et al.*, 2000).

As thiram is introduced to soil for many reasons, sorption equilibrium of thiram at the soil/water interface would be important to predict the levels of thiram in the environment. The levels would highly depend on the nature of the soil in addition to other conditions such as weather patterns and temperature. The equilibrium concentrations of thiram in the aqueous phase, as determined through solvent extraction into CHCl_3 when aqueous thiram solutions of different initial concentrations varying from 1.0 ppm to 10.0 ppm are partitioned with soil (particle of $d < 2$ mm), are shown in Fig. 2. However, no detectable levels of thiram were found in the aqueous phase at initial concentrations

below 3.0 ppm. This indicated that adsorption was complete and irreversible when the added amount is less than the capacity of soil. Based on this concentration limit beyond which detectable levels of thiram were obtained in the solution phase, the capacity of soil for thiram was determined to be greater than 6 mg/kg. The capacity would be more for charged adsorbates due to the charge of soil particles (Rytwo *et al.*, 2004).

All three soil types, obtained from different geographical areas of Sri Lanka representing the wet zone (Gannoruwa and Gampaha) and the intermediate wet zone (Matale), showed an increasing trend when the initial concentration was increased. Similar increasing trend was also observed for the thiram content in the soil phase, which was determined by taking the difference between the initial and equilibrium thiram concentrations in the aqueous phase (Fig. 3). Thiram was preferably adsorbed to clay particles and organic matter in soil, and not on sand. Consequently, thiram concentrations were higher in the aqueous phase when partitioned with more sandy soil. Although differences among the three soil types were not

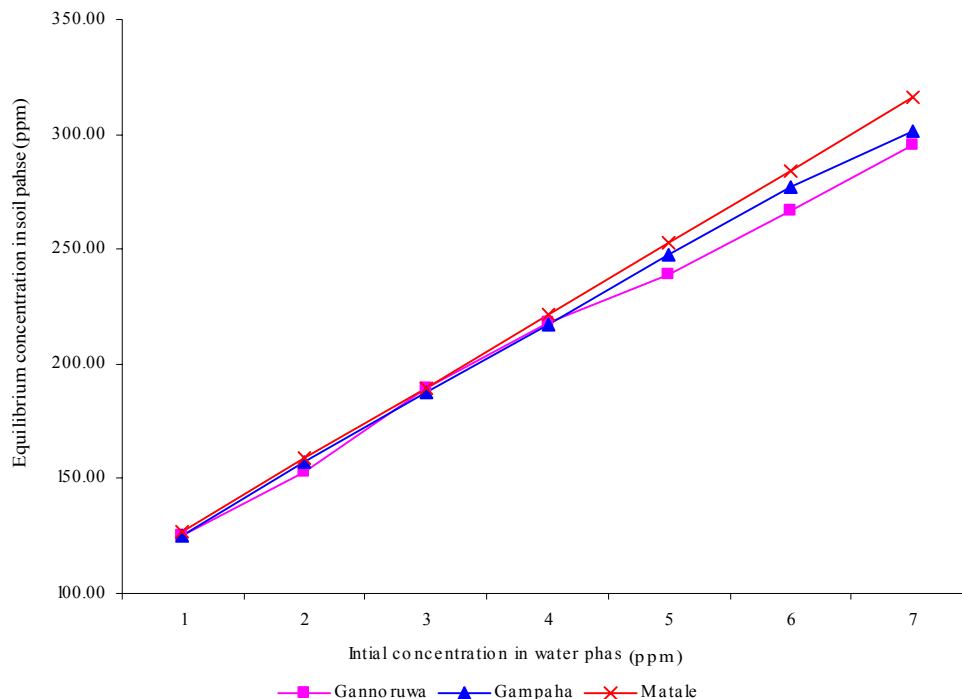


Fig. 3: Average thiram concentrations of the soil phase after establishment of partition equilibrium of aqueous thiram solutions of different initial concentrations

Table 1: Cations present in soil, in ppm, obtained from different areas as determined by atomic absorption spectroscopy

| Cations | Gannoruwa | Gampaha | Matale |
|---------|--------------------|--------------------|--------------------|
| Fe | 1.69×10^5 | 7.24×10^4 | 1.92×10^5 |
| Cu | < 10.0 | < 10.0 | < 10.0 |
| Zn | < 8.00 | < 8.00 | < 8.00 |
| Na | 3.66×10^4 | 2.34×10^4 | 3.30×10^4 |
| K | 5.46×10^3 | 5.52×10^3 | 5.58×10^3 |

significant, the small differences observed could be attributed to different cations present in soil (Table 1). Adsorption of thiram on soil containing higher concentrations of cations (e.g., soil from Matale) would be higher due to complex formation ability of thiram.

The reason for not observing a significant difference among the three soil types may be due to the presence of relatively high levels Cu (II) in all soil types, which readily form a complex with thiram (Zhao *et al.*, 2003). The ratio of the concentration of thiram in the soil to that in the aqueous phase; *i.e.*, the apparent partition coefficient (K_D') changed with the initial thiram concentration (Fig. 4), indicating that the rate of

increase in the thiram concentration in the aqueous phase and that in the soil phase was not proportional to each other. Further, as there was no detectable thiram in the aqueous phase below 3.0 ppm thiram, the K_D' values at low concentrations would be much higher. This trend was apparent for all three soil types. Although change in K_D' with concentration seemed incorrect when the fundamental aspects of equilibrium systems were concerned, it can be argued that true dynamic equilibrium had not been established at smaller initial concentrations, probably up to 5.0 ppm due to the availability of vacant adsorption sites. Hence, irreversible transfer of thiram molecules from the aqueous phase to the solid phase was probably predominant at such concentrations. Additionally, diffusion of thiram molecules through cavities and pinholes of the soil matrix would probably occur when soil is exposed to thiram solution. However, electrostatic attractions are not possible as thiram is not ionic.

Due to the factors stated above, at low concentrations, the rate of adsorption should be greater than rate of desorption. Upon increasing concentration, thiram molecules would probably be adsorbed on the surface of soil particles and the dynamic equilibrium is

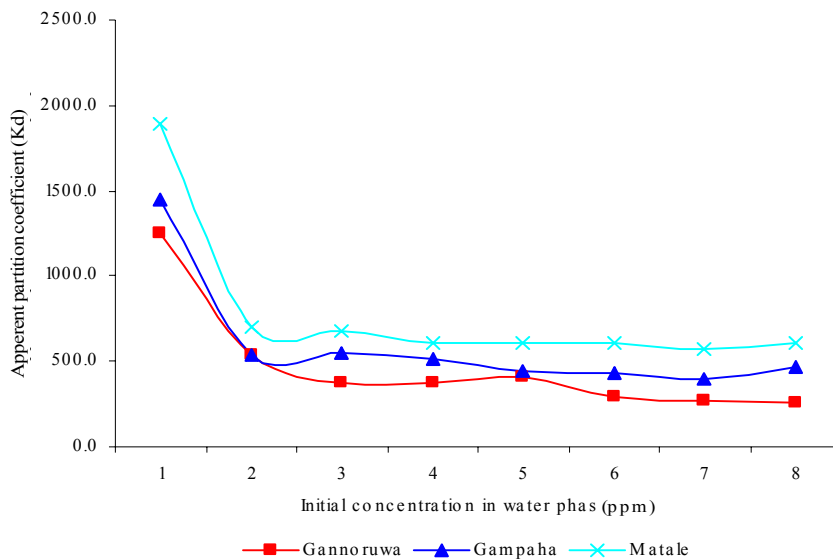


Fig. 4: Variation of apparent partition coefficient (K_D') with initial concentration

established, as indicated by the plateau of Fig. 4 after 5.0 ppm thiram concentration. Therefore, the average K_D' value obtained beyond this concentration can be considered as the accurate K_D' representing the distribution of thiram at the soil/water interface. The K_D' value of thiram at the bentonite/water interface was higher probably due to the clay nature of the adsorbent. The average K_D' values obtained for three soil types and for bentonite are given in Table 2.

As the soil used in this investigation was inhomogeneous in nature, the applicability of the Langmuir isotherm is questionable. Consequently, the validity of the Freundlich isotherm, which is often used to investigate adsorption from liquid solutions, was checked in the present study (Fig. 5).

The correlation coefficient of the least squares lines obtained for the three soil types and the corresponding empirical constants in the isotherm together with associated errors are given in Table 3.

These constants are statistically not different from each other, indicating that the extent of adsorption of thiram on the selected soil types in Sri Lanka did not have a significant variation.

CONCLUSION

The simple detection method developed for thiram present in aqueous medium provides excellent analytical characteristics; a minimum detection limit of

Table 2: Average apparent partition coefficient values (K_D') for distribution of thiram at the soil/water interface

| Soil type | Average K_D' |
|-----------|----------------|
| Gannoruwa | 357 |
| Gampaha | 478 |
| Matale | 629 |
| Bentonite | 5193 |

Table 3: Empirical constants and correlation coefficient of the Freundlich isotherm

| Soil type | Correlation coefficient | Empirical constants | |
|-----------|-------------------------|---------------------|------|
| | | n | k |
| Gannoruwa | 0.9982 | 1.08 | 4.69 |
| Gampaha | 0.9995 | 1.04 | 4.59 |
| Matale | 0.9997 | 1.03 | 4.58 |

0.170 ppm based on the signal-to-noise ratio of 3, which is comparable with sophisticated detection methods such as those employed in chromatography, linear dynamic range from 1.00 ppm to 10.0 ppm, and a sensitivity of 0.0860/ppm. The average partition coefficient of thiram (K_D') between water and $CHCl_3$ phases was determined to be 5.86 ± 0.54 . The apparent partition coefficient (K_D') of thiram between soil, obtained from agricultural fields, and water phases was in the range of 357-629, which increased rapidly at low concentrations. Strong irreversible adsorption of thiram on soil was thus demonstrated. The extent of adsorption on agricultural soil was however lower than that on bentonite, a commercial clay, indicating that

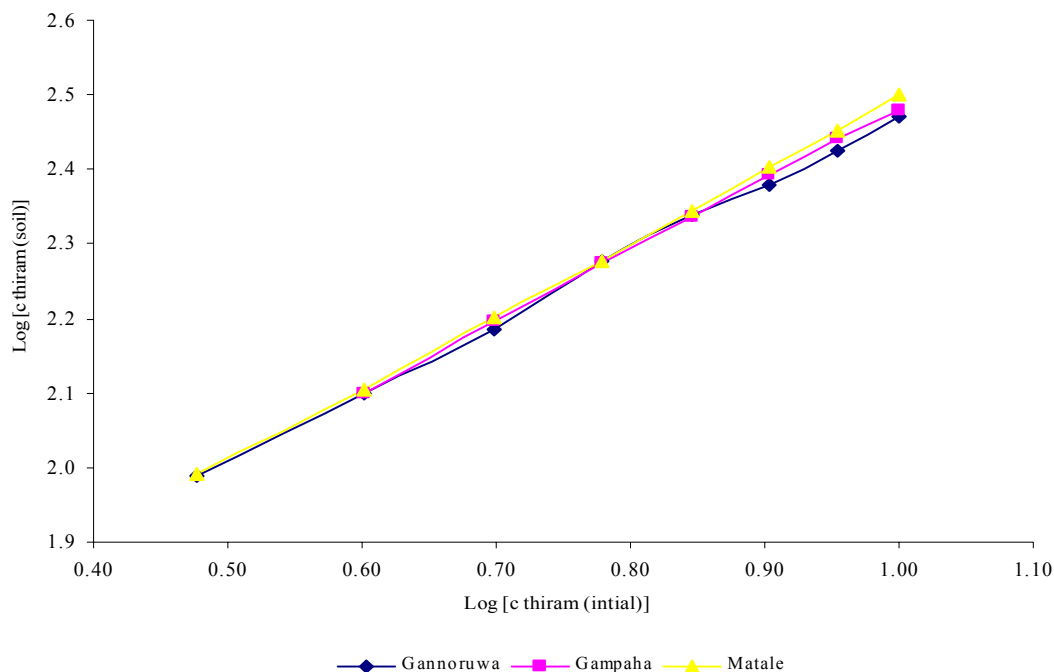


Fig.5: Adsorption analysis for the Freundlich isotherm

thiram was more strongly adsorbed to clay-based materials. Further, there were no major differences among the three soil types with regard to the extent of adsorption of thiram on soil.

Such studies would easily be extended to determine the amount of thiram adsorbed to soil or present in water when thiram is introduced to soil systems for agricultural practices. Adsorption of thiram on soil also indicates the possibility of bioaccumulation through the food chain, if the agricultural run-off water is contaminated with thiram.

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