Development of a new analytical method for the study of atrazine sorption on soils

by

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A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfilment of the Requirements
for the Degree of
Master of Science

Department of Soil Science
University of Manitoba
Winnipeg, Manitoba

ISBN 0-612-16345-8

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DEVELOPMENT OF A NEW ANALYTICAL METHOD FOR THE STUDY OF ATRAZINE SORPTION ON SOILS

BY

ANCA-MARIA TUGULEA

A Thesis/Practicum submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

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Abstract

Atrazine, a herbicide used widely for weed control in corn, has a relatively high persistence and the capacity to leach through soil to the water table. It is considered to be a pesticide of concern and its concentration in natural waters is closely regulated. Atrazine residues in soils may undergo extensive speciation which will decisively influence their environmental fate and their extractability. In the analytical methods used for atrazine in natural waters and soils, the extraction step is the most time consuming and the most expensive, involving the use of large quantities of organic solvents. The reported values for the concentration of atrazine in soils are highly dependent on the analytical method used, particularly on the extraction procedure. The accurate description of transport and biological phenomena affecting atrazine in soils, essential for environmental risk assessment and eventual remediation, depends on analytical data. Analytical methods using a water extraction step seem to be the most useful approach to the quantification of atrazine in soils, because data correlate directly with the bioavailability and the mobility of residues. Solid phase microextraction (SPME) is a solvent-free extraction method which has been previously used in the analysis of organic compounds in aqueous samples. The method that we are presenting, a direct water-phase extraction from soil slurries using an SPME device, does not use any organic solvent and has no separation steps. It is thus less likely to perturb the already complicated soil-water system and appears to be the simplest approach to study

equilibrium processes in atrazine-soil-water systems. Soil slurry analysis by SPME-GC was expected to be more complicated than analysis of spiked water or the headspace over water because soil slurries contain dissolved organic matter and soil particles which could interact with the analysis leading to shortening of the fibre life and generation of less reproducible analytical results. We have developed a new method for the direct SPME of atrazine in soil slurries followed by cleaning of the fibre, enabling extraction of more than 15 soil samples with good reproducibility. Data from the new SPME-GC method correlate well with the data obtained by using a previously published microfiltration-HPLC method, suggesting that by both methods the freely dissolved atrazine is being measured. Thus a new simple, clean, solventless extraction technique, easily compatible with capillary GC, has been added to the arsenal of methods available for the analysis of less volatile compounds from soils and water bodies with a high content of particulate and humic materials. A correlation has been pursued between atrazine sorption data on two different soils and the organic matter content of the soils. Due to the complex nature of the interaction between the moderately hydrophobic organic contaminant and soils with a high organic carbon content more experiments will be necessary to establish a relevant correlation.

1. Introduction

1.1 Agricultural and ecotoxicological relevance of pesticide soil sorption phenomena. Linking analytical extraction behaviour and environmental behaviour of pesticide residues.

Pesticide behaviour in soils has become a matter of concern in recent years because many of the commonly used products have been shown to contaminate surface waters and ground water through phenomena such as migration through soil or run-off from treated soil.

Sorption has been shown to retard the transport of organic contaminants through soils out of the agriculturally effective zone and into ground water or to enhance transport of organic contaminants by run-off, and it has become evident that organic contaminants tend to be sorbed by the SOM (soil organic matter), WSSOM (water soluble soil organic matter), DOM (dissolved organic matter), and POM (particulate organic matter), in fact by all forms of humic substances found in soils, sediments, and natural waters.

The importance of the sorption of organic contaminants (not restricted to pesticides) to humic materials from both an agricultural and an environmental point of view is evident: sorption modifies all expected transport properties of the sorbed material. Sorption on SOM retards solute transport to the ground water, but sorption on WSSOM can lead to unexpected contamination by runoff or lateral transport, or even preferential

flow phenomena in soils. As a consequence, many researchers believe that the sorption of organic contaminants to humic substances should be accounted for in any attempt to set risk criteria for crop rotation, ground and surface water contamination (Chesters et al., 1989), or sediment toxicity (Di Toro et al, 1991; Ankeley et al., 1994). At the same time, sorption of organic compounds to humic materials is expected to significantly affect procedures used in soil and ground water remediation (Krishnayya et al., 1994).

All the reasons cited above have promoted, in recent years, extensive efforts to understand the mechanism by which organic contaminants are associated with humic substances, to analyze the different fractions of contaminant and to estimate the relevance of each fraction in the risk assessment of pesticide residues in the presence of humic materials in natural waters, soils, and sediments. A very important step in these studies is the development of analytical methods for pesticide residues which can be directly related to a well defined fraction of the pesticide in the soil matrix.

Analytical methods for pesticide residues in soils (as well as analytical methods for air or water samples) have been traditionally designed to recover all the pesticide molecules present in the matrix, regardless of the form in which they occur in the sample, or the "fraction" of the sample they are bound to (most environmental samples and specifically soils are heterogenous in nature). For a long period of time the ultimate goal of analytical chemists (upheld by regulatory requirements) has been to attain "100 %" recovery of the contaminant. More and more efficient solvents (or solvent systems) have been used and improved extraction technologies (microwave extraction, sonication, accelerated solvent extraction, supercritical fluid extraction) have been designed to attain

this goal. For real samples in which the contaminant has undergone an "aging" process in the matrix, the degree of success has been limited. Sorption to DOM has been demonstrated to lead to the underestimation of the real total concentration of the contaminant, as determined by traditional extraction procedures (Maguire, 1994) and, although it is largely believed that the associated organic compounds are not bioavailable, they may become bioavailable under certain circumstances (e.g., if changes of the soil moisture content, soil solution pH or ionic strength occur).

In contrast, researchers concerned with the environmental fate of organic contaminants have worked hard to demonstrate that bioavailability (Alexander, 1995) and transport phenomena (Pignatello et al., 1996) are associated with certain "species" of contaminant molecules, more or less "tightly" bound to specific fractions of the humic materials, and are not directly proportional with the " total concentrations" of the analyte in the sample. The necessity to develop analytical extraction methods which specifically target a certain "species" of "loosely" or "tightly" bound molecules, and which can, in turn, be associated with the "real" concentrations available for environmental processes has become more and more evident.

1.2. Why atrazine?

Atrazine is a herbicide that has been used in large quantities all over the world for over 30 years mainly in corn crops; it is still in widespread use.

Atrazine was developed by researchers at Geigy Chemical Company, Basel, Switzerland, beginning in 1952 (Knuesli, 1970). Atrazine was patented in Switzerland in 1958 and registered for commercial use in the United States in 1959 (Solomon et al., 1996).

Solomon et al. (1996) report that 36,000 t of atrazine were used in the USA in 1993 and 584 t of atrazine were used in Ontario in 1993. Shirtliffe (1994) has reported a decline in atrazine use in Canada, due to better management practices, from 2,200 t in 1988 to 1,100 t in 1993, parallel with a decline in the application dose from 2.5 kg a.i./ha in 1988 to 1.6 kg a.i./ha in 1993. In spite of a significant decline, the quantities of atrazine used each year remain important for Canada and even more so for the USA.

Atrazine is a moderately hydrophilic substance able to migrate through soil to the water table and has been found to have a moderate to high persistence in the environment (Howard, 1991). These properties make atrazine a subject of concern from both agricultural and environmental points of view. Owing to its relatively long life and capacity to leach through the soil to the water table, and to suspicions of toxic effects at low dosage (atrazine is included in the EPA list of possible carcinogens), atrazine is considered a pesticide of concern and its concentration in water is strongly regulated (CCME, 1989; Trotter et al., 1990).

Atrazine produces a reversible inhibition of photosynthesis. Having a moderate K_{ow} value, it does not bioaccumulate appreciably and is subject to abiotic and biotic breakdown. Some of the metabolites are phytotoxic, but much less so than the parent compound. Although atrazine has never been an issue with respect to human health, the

existence of widespread residues, which has been amply documented (Howard, 1991, Solomon et al., 1996) has raised concerns related to potential effects on aquatic organisms and terrestrial plants or ecosystems in their entirety. The phytotoxicity of atrazine to green algae has been documented by many authors (Hersh and Crumpton, 1992; Brown and Lean, 1995) and selected green algae species are used as biotests for atrazine (Radetski et al., 1994). The impact of repeated atrazine applications at 100 μg/L on the plankton communities in aquatic enclosures has been studied by Hamilton et al.(1988) in Lake St. George, Ontario, Canada. They found a distinct shift in the taxonomic composition of the affected communities which persisted for at least 77 days, inducing changes in other compartments of the aquatic ecosystem. Adverse effects on the fish population at environmentally relevant concentrations (below 20 μg/L) have been reported and are believed to be both indirect, related to changes in the diet, and direct, due to morphological effects (Kettle et al., 1987). Fischer-Scherl et al. (1991) have found alterations of the different components of the renal corpuscles and renal tubules as well as an increase in cells with mitotic figures in renal haematopoietic interstitium in the rainbow trout under low chronic exposure to atrazine (5, 10, 20, 40 μg/L for 28 days). Reviews of toxicological and ecotoxicological data have been published (Eisler, 1989; Trotter et al., 1990; Sumner, 1994; Purdy, 1994). A comprehensive risk-assessment study for atrazine has been recently published by Solomon et al. (1996).

The fate of atrazine in soils and waters has been extensively described, but many earlier studies of its interaction with soils lack a theoretical approach, making it difficult to extrapolate and to use results for the development of models with predictive capacity.

In the last ten years, studies have been designed to relate atrazine fate to soil properties or soil component properties and extensive information about atrazine is available. Its physico-chemical properties (Howard, 1991; Eisler, 1989; Trotter et al., 1990) as well as the main degradation pathways and metabolites have been well studied. Thorough studies of atrazine behaviour in the presence of clays (Laird et al., 1992), isolated organic matter components, or whole soil (Wang, 1989; Wang et al., 1992) for podzolic soils under different ionic strength, pH, and humic substances concentration have been performed giving results which can be used in the interpretation of results in the current study.

In the current project, a fast, less expensive, and convenient method has been developed, which will be useful in theoretical studies and real life monitoring of atrazine in soil slurries, for both agricultural and environmental purposes. The method is designed to measure the concentration of water extractable atrazine, the atrazine fraction "truly" dissolved in soil solution. This water extractable fraction of atrazine has been shown to correlate very well with the "plant available" fraction of atrazine in soil (Pestemer et al., 1984). The new analytical method has been used for a preliminary study of atrazine behaviour in a chernozemic soil from Manitoba, and a soil of tropical origin. The results of the study will be useful to complete the image of atrazine fate in soils with different physico-chemical properties and will provide data with the potential to be used to build a model with strong predictive capabilities.

1.3. Challenges in atrazine analysis in connection with sorption studies

Pesticide residues in soils may undergo extensive speciation which will influence their toxicology and environmental fate. The speciation will be affected by the physico-chemical properties of both the contaminant and the humic substance fractions, and by selected environmental factors. When the pesticide is introduced to the soil, equilibrium processes between the pesticide dissolved in water and soil organic matter result in a speciation of the pesticide in the soil system: a fraction is free in solution, another fraction is bound to the solid phase and a third one is bound to the dissolved organic matter ("the third phase"). In the last two cases, residues can be "loosely" bound or "tightly" bound to the organic matter. The "loosely" bound residues are often termed "reversibly bound" residues and the "tightly" bound are termed "irreversibly bound" residues.

The degree of extractability of the pesticide residue is highly dependent on the extraction procedure, and extraction conditions. Numerous methods of analysis using Soxhlet extraction, solid phase extraction (SPE), supercritical fluid extraction (SFE), microfiltration, and gas chromatography (GC) with specific detectors, gas chromatography coupled with mass spectrometry (GC-MS), high pressure liquid chromatography (HPLC), and enzyme-linked immunoassays (ELISA) are described in the literature for the recovery and quantification of the dissolved and reversibly bound phases (reviewed in chapter 3). In all these methods the extraction and clean-up step are relatively elaborate, time consuming, and solvent intensive. The irreversibly bound fraction is generally calculated

as a material balance loss and a number of theories have been proposed to explain the process or processes accounting for this phenomenon.

The reported values for the concentrations of pesticide residues in soils are dependent on the analytical method used for analysis, particularly the extraction procedure. Notions such as "total residues", "dissolved pesticide", "reversibly bound pesticide", and "irreversibly bound residues" are largely operationally defined terms, but they also have a real meaning conceptually. Because of the fundamental differences, especially in the extraction step of the analysis procedures, it is very difficult to compare data obtained by different authors and to relate the concentration data to processes of agricultural or environmental significance (e.g. present or future bioavailability of the compound, leaching, long range transport). Accurate description of transport and biological phenomena affecting pesticides in soils, which is of paramount importance for environmental risk assessment, agrotechnical decisions, and eventual remediation, depends on such analytical data. Only if the measured species is well defined, can the results of the analytical procedure be adequately used for predicting the fate of the contaminant, its mobility, and its toxicological and ecotoxicological relevance.

Analytical methods using a water extraction step seem to be the most useful approach to the study of pesticide speciation in soils, because data can be more directly correlated with the bioavailability and the mobility of pesticide residues. Attempts have been made to identify the atrazine bioavailable fraction to the water (or "simulated soil solution"- 0.01 M $CaCl_2$ solution) extractable fraction of atrazine. A very good correlation of the atrazine concentration of the water extract, as determined by a gas

chromatographic method using a specific NP detector, with the bioavailable concentration of atrazine as determined by biotests using sensitive species (Brassica spp.) has been established for a number of different soils (Pestemer et al., 1984).

A number of techniques have been used in the past few years for the concentration and clean-up of the water extract. An increased trend of using solid-phase extraction (SPE) based methods to replace the conventional liquid-liquid extraction for the analysis of pesticide residues in water samples and soil water extracts is evident from the literature and was documented by Sherma (1993). SPE methods have many advantages such as simplification of the extraction procedure with the option of full automatization, minimization of organic solvent consumption, and combination of the concentration and clean-up steps of the analytical procedure. SPE methods are especially easy to use in conjunction with HPLC analysis methods. SPE methods do not completely eliminate the use of organic solvents and are still relatively expensive.

Another category of methods which can be used in conjunction with water extraction to measure only the freely dissolved pesticide residues are the immuno-assay based methods (Del Valle and Nelson, 1994; Stearman and Adams, 1992). Characterized by very high specificity and sensitivity the immuno-assay based methods have been mainly developed as fast and inexpensive screening tools for environmental monitoring (Sherma, 1993). The "cross-reactivity"of anti-pesticide antibodies with naturally occurring compounds and the difficulties in developing multiresidue methods have limited to a certain extent the development of immuno-assay based analytical methods.

Gamble and Khan (1990) have proposed a method, combining "off line" and "on line" microfiltration and HPLC which allows the simultaneous determination of the dissolved atrazine, the reversibly sorbed atrazine and, by calculation, the "irreversibly" sorbed atrazine (described by the authors as a "material balance loss"). The method is remarkable in its simplicity and the comprehensive description of the pesticide-soil system it provides. This method has been chosen to compare with the new SPME (solid phase microextraction) method we developed because there appears to be a theoretical basis (reverse-phase chromatographic theory) to support the idea that the atrazine species measured by "off-line" microfiltration-HPLC is the "truly dissolved" atrazine (see also 2.3.).

The method presented in the current work, a direct water-phase extraction from soil slurries using an SPME device is based on a solvent free extraction method for the isolation of organic compounds from aqueous samples (Belardi et al., 1989). The technique uses a fused silica optical fibre coated with a material similar to a chromatographic stationary phase (polydimethylsiloxane or polyacrylate) to extract the organic analyte from an aqueous phase (Arthur et al., 1992; Louch et al., 1992) or from the headspace above an aqueous sample (Zhang and Pawliszyn, 1993). This new method does not use any organic solvent and has no separation steps (filtration, centrifugation, or chromatographic clean-up). It is thus less likely to perturb the already complicated soil-water system and appears to be the simplest approach to study equilibrium processes in soil-water systems. The method offers good linearity over a large concentration range, ppb range sensitivity and good reproducibility (see section 2.6).

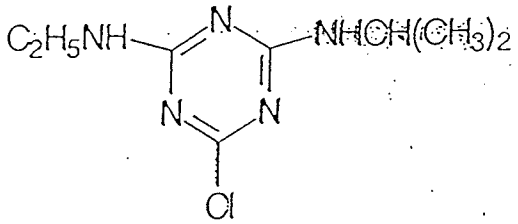
In view of previous success in the use of SPME in conjunction with a GC-EC (capillary gas chromatography using an electron capture detector) analytical procedure for pesticide analysis of water samples in our laboratory (Webster et al., 1994), the technique was examined for the direct extraction of atrazine residues. However, although numerous studies have been conducted on the SPME of pesticide residues in spiked water, headspace over water phases or solids (including soils) and real water samples have been reported, no study has been published yet (to our knowledge) regarding the direct analysis of soil slurries. The development of a new SPME-based method for the direct analysis in soil slurries and its application to the study of atrazine sorption on two soils with different origins and characteristics is presented in this thesis.

2. Literature review

2.1. Chemical and physical properties of atrazine (Howard, 1991; Eisler, 1989)

Chemical name: 2-Chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine

Structure:



CAS Registry Number: 1912-24-9

Molecular Formula: $C_8H_{14}ClN_5$

Melting Point: 171-174 °C

Molecular Weight: 215.68

Dissociation Constants: pKa = 1.7 at 21 °C

Log Octanol/Water Partition Coefficient: 2.75

Water Solubility: 30 mg/L at 20^0.

Solubility in:

- n-pentane: 360 mg/L at 27^0
- petroleum ether: 12,000 mg/L at 27^0
- methanol: 18,000 mg/L at 27^0
- ethyl acetate: 28,000 mg/L at 27^0

- chloroform: 52,000 mg/L at 27^0

- dimethylsulfoxide: 183,000 mg/L at 27^0

Vapour Pressure: 2.78×10^{-7} mm Hg at 20^0 (3.7×10^{-5} Pa)

Henry's Law Constant: 2.63×10^{-9} $atm\text{-}m^3/mol$ (2.66×10^{-4} Pa) (approximate - calculated from water solubility and vapour pressure)

Atrazine is a moderately hydrophobic organic contaminant with a very weak basic character.

2.2. Pesticide sorption to soils

The necessity to predict the fate of organic contaminants in the environment with as much accuracy as possible has promoted, in the last fifteen years, extensive efforts to understand the mechanism by which organic contaminants are associated with different fractions of humic materials in natural waters, soils, and sediments.

Two different research strategies have been applied to the study of the mechanisms by which organic contaminants interact with humic substances: a thermodynamic research strategy, based on the study of sorption-desorption isotherms, sometimes accompanied by kinetic-diffusion studies, and structural investigation strategies at the molecular level relying on modern spectroscopic techniques: nuclear magnetic resonance (NMR), infra-red spectroscopy (IR), electron spin resonance (ESR), and fluorescence methods.

2.2.1. Mechanisms of pesticide sorption on soil components

Multiple mechanisms (covalent bonding, cation exchange, sorption by weak bonding) have been described for the association of low molecular mass organic substances to polymeric humic substances.

The possibility of formation of covalent bonds between humic substances or humic substance precursors and small organic molecules of diverse structures (phenols, aromatic amines, polychlorinated benzenes), leading to the inclusion of the small organic molecule into the primary structure of the humic polymer has been shown by many researchers (Martin and Haider, 1980; Liu et al.,1985; Bollag et al., 1988; Bartha et al., 1982; Ononye et al., 1994; Choudhry et al., 1986). By covalently binding into the humic molecule, the organic contaminants have very little chance of becoming bioavailable again under environmentally relevant conditions (cleavage of covalent bonds would be required). Bollag has proposed to use the laccase mediated reactions, responsible for covalent bonding inclusion of contaminants into humic polymers, as a bioremediation method, especially useful for chlorinated and alkylated phenols (e.g., 2,4-dichlorophenol; *p*-cresol (Bollag et al., 1988). Not enough data are available to estimate the degree to which covalent bonding occurs in natural systems, or the significance of covalent binding as a detoxification mechanism in natural ecosystems.

Organic contaminants possessing a positive ionic charge can interact with humic substances by cation exchange mechanisms. Diquat, paraquat, chlordimeform, and amitrol, have been shown to form complexes which precipitate from aqueous solution with

humic and fulvic acids. The comparative study of the IR spectra suggests ionic bonding between the humic polymer molecule and the charged organic contaminant (Maqueda et al., 1989). Cation exchange was proven to be the most important mechanism for triclopyr sorption, the hydrogen form of humic acid adsorbing more triclopyr than the Ca form (Pusino et al., 1994). It is interesting to observe that 90% of the triclopyr was recovered by extraction was extracted from the soil organic fraction. It appears that the interaction between the clay and the humic acids reduces the surface available for pesticide sorption on the clay component. The humic polymers are bound on clay surfaces occupying most of the sites which could be implicated in pesticide sorption. The studies of Murphy et al. (1994) show that the extent of the clay surface coverage is a function of the conformation of the humic polymers, which, in turn is determined by the soil solution parameters such as pH, ionic strength.

The overwhelming majority of organic contaminants are uncharged, more or less hydrophobic molecules, and it is to be expected that they will be sorbed to the humic fractions by weak bonding (van der Waals, hydrogen bonding or hydrophobic effects). Sorption is a generic term used for the uptake of a solute without reference to a specific mechanism.

Two fundamentally different theories of low molecular weight hydrophobic organic molecules sorption to humic substances have been developed so far: the partitioning theory and the adsorption theory.

2.2.2. Sorption of organic compounds to SOM (soil organic matter)

2.2.2.1. The partitioning theory

On the basis of evidence such as linear adsorption isotherms, no adsorption-desorption hysteresis, low heat released by bonding, and absence of competition among organic chemicals for sorption, the phase partitioning theory was developed at the end of the 1970's, mainly by the work of Karickhoff and coworkers (Karickhoff et al, 1979), with a decisive subsequent theoretical development by Chiou and coworkers (Chiou et al, 1986; Chiou et al., 1987; Chin et al., 1991). The development of the model was based on an analogy of the partition of the hydrophobic organic molecules between water and humic materials with the octanol-water partition of hydrophobic organics.

The humic material is treated as a homogenous liquid organic phase, a "microscopic organic environment" (Chiou et al., 1986). The extent of the partition is expected to depend on the properties of the solute and on the properties of the humic polymer. The solute-solvent interactions are only non-specific, non-localized interactions. An "association constant", K_d, analogous to the octanol-water partition coefficient, K_{ow}, is defined to quantitatively describe the sorption process.

The relevant solute properties are considered to be its water solubility and its "compatibility with the organic phase", usually described as hydrophobicity. The more hydrophobic the solute, the more important its partitioning into the organic phase is expected to be. The relevant properties of the humic materials according to the partitioning theory are their molecular weight and the source of the humic material, which

ultimately determines the structure of the humic polymers. Humic acids, having a higher molecular mass than fulvic acids, are expected according to the partitioning theory to be more suitable for partitioning, and humic material with a higher degree of aromaticity is expected to be also a better "solvent" for hydrophobic organic solutes (Chiou et al., 1986). No interference is expected between two different pollutants in the same system, no saturation limit under environmentally relevant conditions and no hysteresis should be found between adsorption and desorption isotherms.

This model has been succesfully used to model sorption of hydrophobic organics in numerous studies: Carter and Suffett (1982), Horzempa and Di Toro (1983); Gschwend and Wu (1985), Chiou et al. (1986), Chiou et al. (1987).

Carter and Suffett (1982), Horzempa and Di Toro, (1983) report an apparent negative correlation between the concentration of humic substances and the "association constant" which can not be explained under the assumptions of the classic partitioning theory. Suggested explanations vary from a simultaneous increase in concentration of the DOM fraction leading to experimental artifacts (Gschwend and Wu,1985) to conformational changes in the polymer structure (see 2.2.2.3. The adsorption theory).

Baker et al. (1991) advanced the hypothesis that the poor correlation observed between PCBs (polychlorinated biphenyls) K_d values and the OC (organic carbon) fraction of the particulate matter in surface waters may be due to sampling artifacts related to DOM concentration (due to the impossibility to separate effectively the water phase and the solid phase, a "third phase", a colloidal phase being allways present in the water phase and acting as a sorbent) , but also, more importantly, to the fact that highly hydrophobic

PCB congeners may not attain equilibrium in partitioning, which is consistent with the diffusivity model proposed by Gschwend and coworkers (Wu and Gschwend, 1988), a kinetic approach of the partition theory. One parameter, D_{eff}, the effective intraparticle diffusivity (cm^2/s) is used in this improved model to predict the sorption kinetics based on the properties of the solute (diffusivity in solution, hydrophobicity) and the properties of the natural sorbent (organic content, particle size, porosity).

The phase partitioning theory enjoys widespread popularity, due especially to its capacity to generate relatively simple models with good predictive capabilities for a large range of compounds and environmental conditions. It has been used as a theoretical base to establish Sediment Quality Criteria (Di Toro et al, 1991) and to evaluate the bioavailability of pesticides (Ankeley et al., 1994).

Probably the most important limitation of the partitioning model comes from the fact that it offers no details on how the interaction between the organic matter and the chemical occurs at a molecular level. The qualitative, structural part of the phenomena has been deliberately neglected in an attempt to offer an easy to apply, quantitative, predictive tool for environmental specialists.

2.2.2.2. Wershaw's humus model

Wershaw's humus model (Wershaw, 1993) can be considered an attempt towards a molecular model of the partition theory. The central idea in Wershaw's model for humic substances is that amphiphilic molecules originating from the degradation of plant

materials and lignin-carbohydrate complexes interact with mineral grains of soil and form membrane-like coatings of the organic materials on the mineral grains. These coatings constitute the humus in soil. The hydrophobic interior of humic aggregates will be "liquid-like". The exterior face of the aggregates will be hydrophilic and highly charged.

In this model the phase partitioning can be explained by the partitioning of the organic pollutant into the hydrophobic, "liquid - like" interior phase of the aggregates coatings.

Wershaw's theory is intuitively attractive but it brings little improvement to the most critical points of the partition theory. It is difficult to imagine a highly hydrophobic compound partitioning without any thermodynamic barriers through a highly hydrophilic, highly charged surface. Moreover, the model offers no explanation for the documented correlation between partition coefficients and pH, ionic strength, metal ion concentrations and humic substance concentration or for phenomena such as adsorption-desorption hysteresis, saturation and competition reported by many authors.

2.2.2.3. The adsorption theory

The adsorption theory has not enjoyed such a large popularity because of the difficulties related to its use for quantitative predictions, but the accumulation of experimental data seems to lead to the conclusion that the adsorption theory is better placed to accurately explain the sorption process at the molecular level and finally generate

a more coherent model, mainly by accounting for the influence of humic substance structure on quantitative aspects of organic contaminants sorption.

The interaction of organic contaminants with humic substances is considered in the adsorption theory as being a surface process in which the organic compound associates with the organic polymer by weakly bonding to specific families of functional groups. The bonding forces can be ionic attraction, hydrogen bonding, van der Waals forces, or hydrophobic exclusion. This theory assumes that there are only a limited number of active sites, so phenomena such as saturation and competition are expected to exist, even if they are not always observed because of the very low water solubilities of the pollutants, because of low concentration under environmentally relevant conditions, or because the pollutants may have different binding sites. The "hysteresis" between adsorption and desorption curves is explained by "retarded intraparticle diffusion" and has been found to be well correlated with the labile surface site coverage (Gamble et al., 1994).

A complex conformation of humic molecules is assumed. The conformation of humic substances is strongly correlated with pH, ionic strength. metal ion concentrations and humic substance concentrations which explains the influence of those parameters on organic compound sorption by humic materials (Wang, 1989; Li et al.,1992)

One of the central ideas of the use by Gamble of stoichiometrical calculations in complex geochemical systems having humic substances as main components is the use of "inner variables" which characterize the system at a molecular level (e.g., the mole fraction of sorption sites and the concentration of ionized carboxyl groups) instead of "outer variables" which are structurally non-specific and become irrelevant for the

process outside the system in which they have been measured (e.g., pH and total molarity of the pesticide added), (Gamble et al., 1994). This idea is very appealing because it allows the variability in the structure and the properties of humic substances to be accounted for, which has been increasingly proven to be relevant to sorption processes. To date, the model for atrazine based on this hypothesis has given good predictive results when applied to whole soils and compared with laboratory tests and field experiments (Gamble et al., 1994).

The most important argument in favour of the adsorption theory is that there seems to be no controversy between researchers studying adsorption-desorption of pesticides on humic materials about the existence of a "material balance loss". They all report a significant adsorption-desorption "hysteresis" correlated with the residence time of the compound in soil, also referred to as "aging" of the residues (Blumhorst and Weber,1994; McCall and Agin, 1985; Senesi et al.,1994; Gennari et al.,1994).

The correlation reported by many authors between the extent of pesticide sorption and pH, which appears to be independent of pesticide structure (Wang, 1989; Li et al., 1992; Weber et al.,1993; Gennari et al.,1994) is also consistent with the idea that pH influences adsorption of organic hydrophobic pollutants indirectly by modifying the conformation of humic substances, one of the consequences of the adsorption theory. This type of correlation can not be explained under the hypothesis of the partitioning theory.

The possibility of competition between organic contaminants for sorption sites is predicted by the adsorption theory but contradicts the basic principle of the partitioning theory. Murphy et al. (1994) report that competitive behaviour between carbazole and

anthracene was observed in their experiments (the concentration of the sorbent, peat humic acid, was 100 times greater, on a molar carbon basis, than the highest combined concentration of carbazole and anthracene).

The formation of weak bonds between the organic contaminant molecules and specific sites on the humic polymers is the foundation of the adsorption theory. By contrast, in the partitioning theory the humic material is seen as an organic phase with homogenous solvating properties. Madhun et al. (1986) using a gel-filtration method, demonstrated the formation of complexes between some herbicides (bromacil, chlortoluron, simazine, glyphosate, diquat) and the humic materials by the appearance of a new peak attributed to the complex and a trough in the position where the pesticide peak was expected, in the elution diagram. From the elution diagram it was also evident that each herbicide had different affinities for different molecular weight fractions of humic substances, separated from WSSOM by the gel-filtration method, which is consistent with the idea of specific bonding sites being more frequent in some humic fractions than in others.

2.2.2.4. New approaches to the study of organic contaminant sorption on soils

For many organic pollutants, it has been demonstrated that the mineral component of soil has much lower sorption capacity than the organic matter component (Pusino et al, 1994; Chester et al., 1989, Gennari et al., 1994), but at the same time, experimental data

suggest that the interaction between humic substances and clay particles influences the conformation of the humic acids and, consequently, their sorptive properties (Pusino et al., 1994).

Murphy et al. (1990), have studied the sorption of carbazole, dibenzothiophene and anthracene on two different mineral materials (haematite and kaolinite) coated under controlled laboratory conditions with standardized humic substances. They observed different sorptive properties for different mineral material coated with a given humic acid, which suggests that the orientation and the configuration of the humic substance on the mineral may affect the surface area of the organic phase and the accessibility of the hydrophobic domains implicated in the sorption of organic pollutants. In a subsequent study Murphy et al. (1994) studied the sorption of the same three organic pollutants on mineral-associated peat humic acid produced under various pH and electrolyte composition conditions. The configuration of the humic acid in solution was influenced by pH and ionic strength and the adsorption of humic acid on haematite and kaolinite also varied with the ionic strength and electrolyte cation, suggesting that the configuration of the humic acids in solution affected their structure on the mineral surface. The humic acid was found to occupy at low ionic strength twice the mineral surface that it covered at high ionic strength. The sorption of the organic contaminants was found to be more important at low ionic strength, indicating that the structure of the humic acid was relevant for the sorptive properties of the humic-mineral complexes.

Young and Weber-jr. (1995) bring an interesting approach to the controversy between adsorption and partitioning models. They have studied phenanthrene sorption on

three natural soil materials having organic carbon fractions subjected to varying degrees of diagenetic alteration (varying degrees of transformation of the starting materials, leading to humic substances with a variable degree of aromaticity). The authors observe that the experimental data cannot be explained using a purely partitioning or adsorptive model for the sorption of hydrophobic organics. They propose a model in which the sorption process occurs in a combination of amorphous, condensed and microcrystalline regions of humic substances, the importance of the microcrystalline regions increasing in older fractions, which have been subjected to more extensive diagenetic alterations. This new approach may be the ground of reconciliation between classic partitioning and adsorption theories, leading to a new, more comprehensive and more flexible model.

2.3. Atrazine sorption on soils

Atrazine persistence and its residual effect in soil depend to a great extent on atrazine sorption on soil components. Both from the theoretical and the practical point of view it is interesting to know which components of the soil determine sorption intensity and which are the most relevant environmental parameters of the sorption process. Numerous studies have been dedicated to the study of atrazine sorption on various soil types, under different climatic conditions. Due to the complexity of the soil matrix and to the high number of variables in the soil ecosystem relevant correlations have been difficult to establish and most of the scientific research regarding interactions between atrazine and soils has provided empirical or semiempirical data, characteristic only for that

particular system in which they have been measured and which are not appropriate to be used for predicting atrazine behaviour in other soils, under different climatic conditions. The fate of atrazine in soils and waters has been extensively described, but many earlier studies of its interaction with soils lack a theoretical approach, which makes it difficult to extrapolate and to use these results for the development of models with predictive capacity.

As an illustrative example, in a very thorough field study of atrazine sorption on 15 different soils, in three fertilization variants (including mineral fertilization and fertilization with manure) over an extended period of time (Ghinea, 1988) it was possible to establish a correlation between the cation exchange capacity of soils and atrazine sorption capacity, but it was not possible to establish a correlation between the humus content of the soils and atrazine sorption capacity. These findings contradict previous results of the same authors which show an increase in the atrazine sorption capacity of soils by increasing their organic matter content (by amendment with manure). The 15 soils included in the study have a wide range of clay compositions, clay contents, soil pH values and other soil characteristics which makes data correlation practically impossible. An interesting observation of this study refers to the fact that soil organic matter has a limited capacity to decrease atrazine concentration in soil solution by sorption: at high atrazine dosages (over 50 kg a.i./ha) this capacity is exceeded, even after amendment with manure.

In most soils under a variety of natural conditions atrazine is sorbed on both organic and inorganic soil constituents. The relative importance of organic versus inorganic constituents in sorption phenomena depends on the amount, distribution, and

properties of these constituents and the chemical properties of the pesticide (Pusino et al, 1994; Chester et al., 1989, Gennari et al., 1994).

Of the various inorganic soil constituents smectites have the greatest potential for adsorption of pesticides due to their large surface area and abundance in agricultural soils. Atrazine adsorption on smectites has been studied by Laird et al., 1992. Atrazine adsorption on 14 reference and soil smectites ranged from 0 to 100%, depending on the surface properties of the smectites. The correlation of the adsorption data with the surface charge density (SCD) and surface area (SA) suggest that low-charge-density siloxane surfaces have a greater affinity for atrazine than high-charge-density siloxane surfaces which in turn suggests that atrazine is dominantly adsorbed on smectite clays in a neutral form at least for the pH range for which the study has been conducted (pH = 4.75-6.45). These sorption studies have been conducted on pure clay samples, in which the clay surface is entirely available for atrazine sorption. In real soils the clay surface is covered with the humic components, forming clay-humic complexes and, especially in soils with high organic matter content, the soil organic matter is considered to play a more important role in atrazine sorption than the clay component.

A model for atrazine sorption on whole soils has been proposed by Wang et al., 1992 which approximates atrazine binding capacity of the soil with two terms: a strongly pH dependent term describing the humic components binding capacity and a weakly pH dependent term which appears to describe the clay sorption behaviour.

In soils with an appreciable organic matter content, atrazine sorption is associated mainly with the organic fraction of the soil. Atrazine mobility in soil columns has been

found to have a strong inverse correlation with the organic matter content of the soil (Weber, 1993). Soil size fractionation has been used by Barriuso and Koskinen (1996) to study atrazine sorption on soil components and bound residue formation. They concluded that nonhumified organic matter localized in size fractions coarser than 50 μm has the largest capacity to form atrazine bound residues. Bound residues were mainly localized in humified fractions especially in those associated with the clay size fraction 0.2 to 2 μm. These were also the most stable residues.

Many researchers have suggested, based on theoretical assumptions and laboratory studies, that the pH and the ionic strength conditions are essential in determining the extent of atrazine sorption on the organic component of soils (Wang, 1989; Li et al., 1992). Liu et al., 1995 have studied the influence of ammonia fertilizer application on atrazine adsorption-desorption characteristics under field conditions. The ammonia treatments induced changes in the pH of the soil solution (increases of 2-3 pH units), consequently increasing dissolved organic matter (DOM) concentration and decreasing atrazine sorption by 50 %. The authors concluded that ammonia-induced changes in atrazine sorption/desorption characteristics have the potential to increase atrazine movement through soil. It is difficult to conclude though if the low sorption/high desorption is due to a decreased binding capacity of the soil organic matter (SOM) with increasing pH (predicted by Wang, 1989) or to a higher mobilization of atrazine in the water phase due to an increase in DOM concentration. The dissolved atrazine being a rather poorly defined notion in this study, it is difficult to separate the effects of those two parameters

and to predict the influence of ammonia treatment on atrazine bioavailability: the atrazine residues may be present in the water phase bound to the WSSOM but not free in solution.

Recently, a rigorous theoretical approach to atrazine sorption on soil organic matter has been developed, based on the idea that chemically complex organic matter of soil can be treated as any complex mixture of chemical compounds and described by appropriate variables which reflect the actual molecular properties of the mixture (Gamble et al., 1994). The central idea of the Gamble theory of stoichiometrical calculations for processes involving humic substances is the use of "inner variables" which characterize the system at a molecular level (e.g., the mole fraction of sorption sites and the concentration of ionized carboxyl groups) instead of "outer variables" which are structurally non-specific and become irrelevant for the process outside the system in which they have been measured (e.g., pH and total molarity of the pesticide added). This idea is appealing because it allows the variability in the structure and the properties of humic substances to be accounted for, both of which have been increasingly proven to be relevant to sorption processes.

A highly significant observation is that although the structure and many of the properties of humic substances vary considerably from site to site, some chemical and physical properties are highly conserved among a variety of humic acid samples. One of the most important properties in this category is that they act as polyanions. That is why their reactions with metallic cations (Gamble et al., 1980) and their titration curves (Gamble, 1972) with bases have been considered so relevant in describing their properties (Gamble and Langford, 1988). To date, the model for atrazine sorption based on the

correlation with "inner variables" derived by this theory has given good predictive results when applied to whole soils and compared with laboratory tests and field experiments (Gamble et al., 1994).

Gamble and Khan (1988) calculated the fraction of the total number of carboxylic groups implicated in atrazine adsorption and the total binding capacity of fulvic acids for atrazine, using sorption data and titration curves for the humic fraction. In a very detailed study, Wang et al. (1989) found relevant correlations between pH, ionic strength, humic and fulvic acids concentration in solution and the binding capacity of humic substances and whole soils for atrazine. The inverse correlation between the ionic strength or the pH of the solution and the adsorption capacity was also found by Li et al. (1992) for lindane and is explained by conformational equilibria creating more hydrophobic sites at low values of pH due to more limited dissociation of the carboxyl groups. The inverse correlation between humic and fulvic acid concentration and atrazine or lindane binding capacity is explained by conformational equilibria associated with sorption equilibria between low molecular weight fulvic acids and high molecular weight humic acid molecules, a higher concentration leading to higher association between humic molecules and lowering the fraction of binding sites available for the pesticide molecule (Wang et al., 1989). Humic acids have a less marked correlation between adsorption capacity and concentration than fulvic acids. The overall higher adsorption capacity of humic acids compared to the adsorption capacity of fulvic acids is considered to be due to the more complex structure of humic acids which allows for the formation of more binding sites through conformational changes.

An important success of this model is its capacity to correlate well two different processes occurring simultaneously in atrazine solution, in the presence of humic substances: adsorption and chemical hydrolysis. Gamble and Kahn (1988; 1990) measured the apparent hydrolysis rates for the reaction of atrazine in solution and correlated them with pH, binding site coverage, and fulvic and humic acid titration curves. Atrazine hydrolysis is acid catalyzed by the unionized carboxyl groups of humic and fulvic acids and the concentration of atrazine in solution is determined by both sorption and chemical reaction until a steady state is reached. The reaction rates could not be correlated with pH, but were correlated with the degree of ionization of carboxylic groups at different pH values. This enabled the authors to explain the relatively lower catalytic activity of humic acids compared to fulvic acids based on the fact that humic acids generally have fewer weakly acidic carboxylic groups per unit mass than fulvic acids (Gamble and Khan, 1988).

Another interesting observation is related to the fact that a very much lower binding capacity was found for lindane than for atrazine (Li, 1993), which is consistent with the idea that lindane is only bound by hydrophobic exclusion forces and humic substances being very hydrophilic media have a very limited number of hydrophobic binding sites whereas hydrogen bonding and even charge transfer complexation have been suggested as being implicated in atrazine sorption.

Martin-Neto et al. (1994) used multiple spectrometric methods to investigate the mechanism of atrazine binding to humic acid. Their studies, conducted under environmentally relevant conditions, using UV, FTIR, and ESR spectrometry, provide

evidence for hydrogen-bonding and proton-transfer interaction mechanisms, especially at pH < 4. No charge transfer reaction or significant chemical bond formation was observed. This study confirmed, on different soil and humic materials samples, by spectrometric techniques, the results of Wang et al. (1989).

Finally, all experiments conducted with atrazine and lindane showed an important hysteresis effect. This effect was explained by Gamble et al. (1994) as being a "retarded intraparticle effect" and was found to be well correlated with labile surface site coverage. This model is somewhat similar to the Gschwend model for intraparticle diffusion (Gschwend and Wu, 1985), but assumes that retardation occurs by adsorption on the interior surfaces of the particles, not by repeated partitioning between the mobile and immobile phases in the particle.

The definition of the "material balance loss" or "bound residue" fraction as a product of "retarded diffusion" seems to be consistent with evidence from other experiments which suggest a physico-chemical mechanism of formation for this atrazine fraction. Blumhorst and Weber (1994) have found an identical "nonextractable" residue fraction in two different soils in both the nonsterile as well as the sterile variant. Khan (1995) reports a super critical fluid extraction method for atrazine from a contaminated soil 9 years old with 89.2 % recovery of the bound fraction.

To develop a consistent model which will correctly evaluate the contribution of different atrazine residue fractions to biological, chemical and transport processes in the soil environment it is essential to have an important data base including data on atrazine sorption to soils with different physico-chemical characteristics. But to develop such a

data base the analysis methods used for data generation must be well correlated with certain fractions of the pesticide in soil. If a comprehensive analysis of atrazine content in a contaminated soil is conducted (for example by using ^{14}C labelled atrazine), five categories of residues can be defined: atrazine dissolved in soil solution, atrazine reversibly bound to the dissolved humic substances in soil solution ("the third phase"), atrazine irreversibly bound to the dissolved humic substances, atrazine reversibly bound to soil components and atrazine irreversibly bound to soil components. The reversibility of the sorption process is relative, and highly dependent on extraction conditions due to thermodynamic and kinetic limitations of the extraction process. In addition to the competition for the pesticide between the extractant and the aqueous phase, other equilibrium processes occur between the pesticide dissolved in water and soil organic matter. The extraction procedure in most cases affects the equilibrium in soil solution, by removing most of the dissolved pesticide from the water phase. By exhausting the water phase, fresh pesticide is brought into solution, either from the pesticide fraction sorbed to the DOM or from the pesticide fraction sorbed to the SOM. One of the most important aspects in developing a new method of analysis for such systems is related to defining which pesticide fraction is measured by that specific method.

2.4. Analytical methods for "total extractable" atrazine in soil

Numerous methods of analysis, using Soxhlet extraction (Stearman and Adams, 1992), solid phase extraction (SPE) (Font et al., 1993; Barcelo et al., 1993, Del Valle and

Nelson, 1994; Crespo et al., 1994) , supercritical fluid extraction (SFE) (Del Valle and Nelson, 1994; Khan, 1995,), microfiltration (Gamble and Khan, 1990), and thin layer chromatography (TLC)(Blumhorst and Weber, 1994; Sorensen et al., 1995), gas chromatography (GC) with specific detectors (Sanchez-Brunete et al., 1994), gas chromatography coupled with mass spectrometry (GC-MS) (Crespo et al., 1994; Sanchez-Brunete et al., 1994) and high pressure liquid chromatography (HPLC) (Barcelo et al., 1993), are described in the literature for the analysis of "total extractable" atrazine residues. The ultimate goal of such procedures is to extract all the atrazine present in the soil samples. In all these methods the extraction and clean-up step are relatively elaborate, time consuming and solvent intensive.

The three important steps in the chemical analysis of pesticide residues from soils are extraction, cleanup of the extract, and determination of the amount of analyte present.

2.4.1. Extraction procedure

Residue extraction, the first step in a usually long, multi-stage procedure, is as important as the subsequent analysis, because residues that are not extracted can not to be detected and quantified.

A variety of solvents have been used for atrazine extraction from soils. In a critical review, Mattson et al., 1970, quote six solvents of very different polarities used for atrazine extraction: chloroform, carbon tetrachloride, dichloromethane, dioxane, methanol and 8 M urea solution. They recognized a definite trend to move from the less polar

solvents used in earlier studies to more polar, water-miscible solvents and that solvent-water mixtures appear to give the best results. Their predictions were confirmed by another review by Dao et al., 1983, who found methanol to be the most commonly used solvent for the extraction of atrazine from soils.

Different procedures for carrying out the extraction have also been developed. Agitation of the soil and solvent for various periods of time at various temperatures (most frequent at room temperature or at solvent reflux) and Soxhlet extractions have been the classic procedures used. New methods such as ultrasonic extraction, microwave extraction or SFE (supercritical fluid extraction) have been introduced more recently with the hope of overcoming difficulties in the extraction of "aged" residues and to improve the overall recoveries by mobilizing the most recalcitrant residue fractions (the "bound residues"). Mattson et al. (1970) compare the performances of different extraction techniques and conclude that: "No one "best" method for extracting soils can be selected from literature". They also reported that most extraction and analysis methods have acceptable recoveries (ranging from 80 %-110 %) for atrazine added to the soil matrix immediately before extraction, but much lower recoveries for field samples.

They conducted a study to compare a number of extraction methods, using different solvents (chloroform, 10 % water-methanol, methanol, 10 % water-chloroform, 10 % water-acetonitrile) and extraction procedures (two consecutive 24 h Soxhlet extractions, refluxing for one h, and standing overnight (18 h) at room temperature, followed by half hour mechanical shaking) with respect of recoveries of atrazine residues from two weathered soils at two residue concentration levels (0.08 and 1.9 ppm).

The results are summarized in Table 1 (Mattson et al., 1970):

Table 1. Comparison of extraction procedures for weathered atrazine residues in soil

Solvent	Extraction Conditions	Time[a](h)	Residue concentration (ppm)	Atrazine detected (ppm)
Chloroform	Soxhlet	I 24	0.08	0.05
		II 24		0.00
		T 48		0.05
		I 24	1.9	0.70
		II 24		0.37
		T 48		1.07
10% Water-methanol	Soxhlet	I 24	0.08	0.05
		II 24		0.00
		T 48		0.05
		I 24	1.9	1.95
		II 48		0.05
		T 48		2.00
Methanol	Reflux	1	0.08	0.03
		1	1.9	1.09
10% Water-methanol	Reflux	1	0.08	0.05
		1	1.9	0.80
10% Water-chloroform	Reflux	1	0.08	0.06
		1	1.9	1.55
10% Water-acetonitrile	Reflux	1	0.08	0.07
		1	1.9	1.95
Methanol	Room temp.	18[b]	0.08	0.06
		18[b]	1.9	0.60

[a] I = first 24-hour extraction, II = second 24-hour extraction of the same sample, and T = total for both I and II.
[b] 18 hours standing at room temperature followed by ½-hour mechanical shaking.

The authors concluded that the preferable procedure was the water-acetonitrile one hour reflux procedure which gave the same results as the 24-h Soxhlet extraction. It is less time consuming and requires less complicated apparatus. Based on this choice of the extraction method Mattson et al. (1970) report a detailed analytical method for atrazine in soils which is reproduced here as an example of analysis of total atrazine residues in soil.

2.4.2. Typical analytical method for total atrazine residues in soil (Mattson et al., 1970):

The soil sample is mixed thoroughly and a 100 g subsample is refluxed for one hour with 300 mL of 10 % water-acetonitrile (v/v). The mixture is cooled, the soil allowed to settle, and a 60 mL aliquot of the filtrate (the equivalent of 20 g of soil) is transferred to a 500 mL separatory funnel, diluted with 300 mL of water and ca. 20 mL of a saturated solution of sodium sulphate. The resultant aqueous solution is extracted twice with 25 mL of methylene chloride. The methylene chloride solution is filtered through a 2.5 cm pad of anhydrous sodium sulphate (about 70 g) and collected into a 250 mL Erlenmeyer flask. The sodium sulphate is washed with 25 mL of methylene chloride into the Erlenmeyer flask. The solvent is evaporated to dryness in a flash evaporator (bath temperature 40 ^{0}C).

The cleanup of the extract is performed on an alumina column. A 22 mm o.d. chromatographic column, equipped with a perforated support, is filled with 12.5 g of

Woelm Aluminum Oxide, Basic (Alupharm Chemical Co, New Orleans, LA), adjusted to activity V, as described on the package. The sample is transferred to the column with two 5 mL portions of carbon tetrachloride. The flask is rinsed with 65 mL of carbon tetrachloride and this solvent added to the cleanup column. The eluate is discarded.

The cleanup column is eluted with 5 % ethyl ether in methylene chloride. This eluate, which contains the atrazine, is collected in a 250 mL Erlenmeyer flask. The solvent is evaporated to dryness using a flash evaporator. The residues are quantitatively transferred into vials with successive 1 to 2 mL potions of methylene chloride. The solvent is evaporated in the vials at 40 ^{0}C with a gentle stream of air.

The residues in the vials are then diluted with 0.2-2 mL of benzene (depending on the expected concentration range for residues) and 5 μL of the benzene solution is injected into the gas chromatograph.

2.4.3. Challenges in performing and comparing results of total residue methods for atrazine in soil samples

The classical method presented in the previous paragraph used no less than 5 organic solvents, some of them highly toxic or inflammable and banned from the laboratory practice in our times (benzene, carbon tetrachloride, ethyl ether). A total of approximately 500 mL of organic solvent is used to extract 100 g of soil for each individual analysis. The manipulation and disposal of such organic solvent volumes has become a more and more expensive problem. Liquid-liquid partitioning and column

chromatography are used for clean-up and three concentration steps are involved in the process. Numerous manipulations require a very skilled operator and provide opportunity for errors.

All the aspects quoted above are part of a very time consuming, expensive, even frustrating procedure. Highly skilled personnel and very good laboratory organization are required to attain adequate reproducibility of the results in the same laboratory. Reproducibility between laboratories becomes an almost impossible task. Small variations in the laboratory practice or the quality of the materials used result in important variations of the recoveries.

Analysts all over the world have attempted to answer these problems by evaluating the recovery for each particular method used and reported. Unfortunately, it has become clear that freshly spiked samples using the exact same matrix do not behave the same way as weathered field samples (see 2.3.1); therefore, extraction of spiked samples is not very useful in accounting for recovery from "field aged" materials.

2.4.4. New methods for the analysis of total atrazine residues in soils

The progress of analytical methods for total atrazine residues in soils has tried to address the most important practical problems of the classic liquid-liquid partitioning methods described in the above paragraph: the simplification of the extraction and cleanup methodology and the reduction of the volume of organic solvents required. In an attempt

to extract more and more of the residue present in "aged" samples, to bring the recovery closer to the ideal 100 %, many new extraction procedures have been developed.

Liquid-liquid partitioning methods are labour intensive, which is both expensive and conducive to low precision. Automation of the extraction procedure has been one of the proposed solutions. Koskinen et al. (1991) describe a computerized robotic laboratory system for the extraction of atrazine and alachlor from soils. The method uses a 16 h extraction with a methanol/water mixture (4:1), separation of the supernatant by centrifugation, methanol evaporation at 50^{0}, solid phase extraction using a C_{18} cartridge, final elution of atrazine from the cartridge with 1 mL of methanol, and injection into a capillary GC system. The sample throughput increased by a factor of three over the manual procedure and the precision of the extraction procedure increased as well. The organic solvent quantities used decreased as compared with liquid-liquid partitioning methods.

Other authors preferred to use in their studies more sensitive and specific detection methods which do not require extensive clean-up and concentration of the extracts. Sorensen et al. (1995) analyzed the extractable fraction by refluxing the soil three times with an undefined combination of methanol-water, separating the extracts by thin layer chromatography (TLC) and detecting the atrazine by scintillation counting (^{14}C atrazine was used in the study). Blumhorst and Weber (1994) used a four hour Soxhlet extraction with a methanol-water mixture directly followed by TLC and UV detection to define the extractable atrazine concentrations in different soils. Barriuso and Koskinenen (1996) extracted different soil size fractions with simulated soil solution (0.01 M $CaCl_2$) by

mechanical shaking for 24 hours and water phase separation by centrifugation. The centrifugation conditions were selected to result in "negligible loss of fine soil particles in the supernatant". Each isolated size fraction was then exhaustively extracted with methanol. ^{14}C labelled atrazine was used in the study and detection was achieved by scintillation counting. The authors reported that radioactivity was negligible in the methanol extracts after six successive extractions.

The development of more robust and sensitive gas-chromatographic systems has diminished the necessity of extensive clean-up and concentration steps. Many researchers now use simplified extraction procedures; e.g., Lafrance et al. (1992) extracted the atrazine from soil with ethyl acetate by sonication and filtered the extract through a 0.45 μm filter, and analyzed the solution by gas chromatography. They related the atrazine concentrations obtained by this method to its effect on soil microbial activity, considering the ethyl acetate extractable fraction of atrazine bioavailable for the microorganisms. Method # G121.0 for simazine, atrazine, metribuzen, prometryne and cyanazine in soil (Alberta Manual, 1991) uses three succesive extractions with methylene chloride using a cell disrupting sonicator, separation of the supernatant by centrifugation, evaporation of the solvent at ca. 35 ^{0} on a flash evaporator, further evaporation under a nitrogen stream, solubilization of the residue in 2 mL of hexane and injection into a capillary GC system.

Khan (1995) describes a supercritical fluid extraction (SFE) method for "aged" residues of atrazine in soil characterized by very high recoveries of total atrazine residues.

These are just a few examples of the numerous methods used by different laboratories to analyze the "total atrazine" residues. It can be said that practically every

laboratory uses one particular method and every regulatory agency recommends a different method for "total atrazine" analysis. None of these "total" extraction methods offers any theoretical basis for correlation between the analytical data and environmentally relevant concentrations. Comparing the environmental relevance of analytical data resulting from such different extraction procedures appears to be a challenging enterprise.

2.5. Analytical methods for "truly dissolved" atrazine

One of the most important aspects in developing a new method of analysis for a soil-water-pesticide system is related to defining which pesticide fraction is measured by that specific method. Only if the measured species is well defined can the results of the analytical procedure be adequately used to predict the fate of the contaminant, its mobility, and its toxicological and ecotoxicological relevance.

Defining the pesticide species measured by different analytical procedures is a very challenging matter because the boundary between solution and solid phase is often operationally defined; the 0.45 μm boundary has no fundamental physical meaning (Fig.2.1).

Many analytical methods fail to extract the whole amount of contaminant present in the water phase (the "nonextractable" fraction associated with the DOM) and underestimate the total amount of contaminant (Maguire et al., 1994) available for transport in the water phase (by leaching or run-off). Theoretical estimations of the

concentration of contaminants based on their partitioning constants between water and soil organic matter (K_d or K_{oc}) on the other hand tend to overestimate the "bioavailable" concentration of the contaminant in the water phase, because they disregard the fraction of the contaminant which is sorbed to the dissolved organic matter (Servos at al., 1992).

Methods based on water extraction from soil (more specifically on extraction with simulated soil solution, 0.01 M $CaCl_2$) have been demonstrated (Pestemer et al., 1984) to analyze the freely dissolved atrazine in soil solution, considered to be the fraction bioavailable to plants. Comparing the results of a water extraction method for atrazine with the results of biotests using an atrazine-sensitive plant species (Brassica spp.) the authors of this study reported similar results obtained by the two methods.

One of the clearest trends in the past few years has been the increased use of solid-phase extraction (solid-matrix partition), using columns, cartridges and disks filled with solid extractants to replace the conventional liquid-liquid extraction for the analysis of pesticide residues in water samples. In solid-phase extraction (SPE) methods the water sample is passed through a short bed of packing material which may contain functional groups of different polarity (C_8, C_{18} bonded silica phase, graphitized carbon black, Amberlite XAD resins). The analyte is retained on the packing material. Impurities can be washed from the solid phase with water or another appropriate solvent which does not elute the analyte. In the final step the analyte is then eluted from the sorbent with a small volume of an appropriate organic solvent. This technique allows for simultaneous concentration and clean-up of the sample.

Membrane extraction disks (e.g., Empore disks) are based on the same SPE principle. Their main advantage over SPE cartridges and columns is the higher flow rate, because of the lack of channelling and the faster mass transfer due to smaller particle size.

SPE methods have many advantages: they avoid the emulsion formation which plagues most liquid-liquid partitioning methods, they minimize organic solvent consumption, they reduce the necessity for concentration steps and, in many cases, they reduce the need for cleanup of the sample extracts. They can be easily converted into fully automated on-line systems coupled with HPLC systems. Such systems are referred to as "precolumn technology" and show additional advantages : lower detection limits due to the analysis of the whole eluate instead of just an aliquot of it, no loss by evaporation, and markedly reduced chances for contamination. SPE methods have been used to analyse the water extractable (and, consequently, bioavailable) atrazine in soils (Tugulea, 1992) or the atrazine fraction extractable with methanol-water (4:1) (Del Valle and Nelson, 1994; Koskinen et al., 1991).

Another category of methods which could be expected to measure only the freely dissolved atrazine are the immuno-assay based methods. ELISA (enzyme-linked immuno-sorbent assay) tests for atrazine and other *s*-triazines based on monoclonal and polyclonal antibodies have been developed. Based on the high specificity of the antigen-antibody reaction between anti-atrazine antibodies and the atrazine in the sample and the high sensitivity of the enzymatic reaction catalyzed by the enzyme bound to the anti-atrazine antibodies, the ELISA methods offer a convenient screening method for atrazine in water samples, foods (Wittman and Hock, 1993) and soils (Tugulea, 1992). Because of the high

specificity of the method, no clean-up is necessary and because of the high sensitivity of the method no concentration step is required. Extraction with simulated soil solution and gravitational filtration was the only sample preparation required to analyse atrazine concentrations in soils as low as 0.1 ppb (Tugulea, 1992). Nevertheless, many researchers use very radical extraction methods (solvent extraction, sonication, SFE (Del Valle and Nelson, 1994), vortex mixing, or Soxhlet extraction (Stearman and Adams, 1992) to prepare the extract subsequently used for ELISA analysis in an attempt to use immuno-assay based methods for atrazine as "total" extraction methods.

A new approach to the systematic separation and quantitation of the truly dissolved pesticide in a solution containing humic substances is the use of alkyl-diol silica (ADS) restricted access (RA) precolumns (Onnerfjord et al., 1995). These precolumns have been specially designed for biomedical applications. The RA stationary phases are based on non- adsorptive size exclusion of macromolecules (e.g., proteins). The authors have tried to use these method as a clean-up and preconcentration step with limited success, but the method may prove to be much more useful in the future for discrimination between the dissolved and the sorbed atrazine phase.

Other extraction methods have been used to define the "water extractable", dissolved atrazine fractions, but the one that seems to have the most solid theoretical support is the separation by reverse-phase chromatography of dissolved atrazine and atrazine bound to DOM. The moderately hydrophobic atrazine is expected to be retained on the C_{18} column by hydrophobic interaction and subsequently eluted by using an appropriate solvent and detected by the detector of the HPLC system; whereas, the polar

complexes formed by atrazine with fulvic acids will not be retained by the chromatographic column and therefore will not remain on the column to be detected after elution.

"Off-line" microfiltration through a 0.45 μm filter separates the "soil solution" from the particulate phase and allows the filtrate to be directly injected into the reverse-phase chromatographic system. The UV detector will detect and quantify only the atrazine retained by the stationary phase (the "truly dissolved atrazine"). The eluent, a mixture of water and methanol, is expected to disrupt the equilibrium between the dissolved atrazine and the atrazine sorbed to the DOM only to a small extent. Microfiltration-HPLC thus measures only the truly dissolved atrazine in solution (Gamble and Khan, 1990).

In developing a new method for the analysis of atrazine concentration in soil slurries it was important to compare data obtained by the new method to data obtained by an established method. To better understand the significance of the results of the SPME based analysis method in terms of identifying the fraction of the residue which is practically recovered by this particular extraction method, it was decided to use the "off line" microfiltration-HPLC method to compare the newly developed SPME method for "truly" dissolved atrazine in soil slurries.

2.6. SPME

Solid phase microextraction (SPME) is an extraction method developed by Pawliszyn and coworkers at the University of Waterloo (Belardi and Pawliszyn, 1989). It is a new microscaled approach to the well established method of solid phase extraction (SPE) which employs chemically modified fused silica fibres as solid phase extractors. The unique feature of these fibres, due to their small size is the ability to place them directly into the water sample and then, after the extraction process has occurred, directly into the injector port of the capillary gas-chromatograph where the analyte is thermally desorbed and analysed.

The method is based on the tendency of more hydrophobic analytes from the aqueous phase to partition into the organic coating of the fused silica fibre, leading to the concentration of the analyte in the coating material. Unlike SPE, SPME is not an exhaustive extraction technique. It is based on the equilibrium between the concentration of the compound in the organic layer (the fibre coating) and the concentration of the compound in the aqueous medium, related to the partition coefficient of the analyte. This adds a new advantage to the method: the analysis is independent of the sample size (under appropriate conditions) which eliminates a possible source of error and promises the feasibilities of direct sampling in natural systems.

2.6.1. Theory

The principle behind SPME is the partitioning of analytes between the matrix of the sample and the liquid polymeric coating of the fibre. The amount of analyte sorbed by the coating at equilibrium is directly related to its concentration in the sample matrix (Zhang et al.,1994):

$$n = \frac{K_{fs}\, V_f\, C_0\, V_s}{K_{fs} V_f + V_s} \qquad (1)$$

where: n = the mass of an analyte absorbed by the coating

V_f = the volume of the coating

V_s = the volume of the sample

K_{fs} = the partition coefficient of the analyte between the coating and the sample matrix

C_0 = the initial concentration of the analyte in the sample

Equation 1 describes a linear relationship between the amount of analyte absorbed by the fibre coating and the initial concentration of the analyte in a sample. The coatings used in SPME have strong affinities for organic compounds, K_{fs} values for targeted analytes are quite large, which leads to high concentrating effects and improves the sensitivity of the analytical method.

In most cases, however, the extraction capacity of the coating is not large enough to exhaustively extract the analyte from the matrix. In these cases, SPME is an equilibrium sampling method which, through proper calibration, can be used for accurate determination of the concentration of target analytes in a sample matrix.

If V_s is very large with respect to the volume of the coating ($V_s \gg K_{fs}\ V_f$) , the amount of analyte extracted by the fibre coating

$$n = K_{fs}\ V_f\ C_0 \tag{2}$$

is not related to the sample volume.

The sensitivity of the method is related to the partition coefficient of the analyte. For poly(dimethylsiloxane), a nonpolar coating material, the partition coefficient has been approximated by the octanol-water partition coefficient, although the actual K values seem to be slightly higher (Arthur et al., 1992) . The limits of detection can be estimated by rearranging equation 1 to (Potter and Pawliszyn, 1994):

$$C_0 = \frac{n\ (K_{fs}\ V_f + V_s)}{K_{fs}\ V_f\ V_s} \tag{3}$$

where

C_0 = the predicted LOD

V_{fs} = the stationary phase volume

V_s = the volume of solution

n = the LOD of the detector

K_{fs} = the partition coefficient

If the partition coefficient (K_{fs}) is too small, limited or no concentration in the fibre coating will occur, resulting in poor sensitivity. The extraction will have a thermodynamic limitation and the solution will be to find a more appropriate fibre coating material for that particular analyte. If the coating has a higher ability to sorb the analyte than the matrix does, it is only a matter of time for a substantial amount of analyte to be extracted by the fibre coating, and kinetics plays an important role in the extraction procedure.

The extraction time is controlled by the mass transfer of the analytes from the sample matrix to the coating. In direct SPME sampling, the mass transfer rate is determined by the diffusion of analytes in the coating and the diffusion of the analyte through the water phase to the surface of the coating.

Mathematical models have been developed to describe the diffusion of the analyte through the matrix and through the water phase (Louch et al., 1992). When the mass transfer rate is determined by the diffusion of the analyte in the coating (if the sample matrix is perfectly agitated), for most analytes equilibrium is achieved in less than 1 min (Zhang et al., 1994), because the coating is very thin (between 7 and 100 μm). In practice, this limit can only be achieved for gaseous samples because of the large values of the diffusion coefficients in the gaseous phase. For aqueous samples vigorous agitation methods (sonication, magnetic stirring) are used to shorten extraction times.

In many cases it is not necessary to reach complete equilibrium to achieve reproducible sampling, if consistent extraction times are used . A number of studies have

shown reproducibilities of peak areas of 1-6 %, even in the absence of total equilibrium.

Typical extraction times are in the order of minutes to tens of minutes as determined by the diffusion of the hydrophobic analyte through a thin static aqueous layer adjacent to the fibre (Berg, 1993).

Headspace SPME can be used to extract organic compounds from virtually any matrix as long as target compounds can be released from the matrix into the headspace. Zhang and Pawliszyn (1993) describe the use of headspace SPME to analyze both volatile and semivolatile compounds from a range of matrices: water, sand, soil, clay, waste water, and sludge.

For volatile compounds, the release of analytes into the headspace occurs without problem because analytes tend to vaporize once they are dissociated from their matrix. For semivolatile compounds, the low volatility and relatively large molecular size may impede the mass transfer from the matrix to the headspace, resulting in a long extraction time.

One of the most efficient ways to overcome the kinetic limitation is to heat the sample to higher temperatures, which increases the vapour pressure of analytes, provides the necessary energy for analytes to be dissociated from the matrix, and accelerates the mass transport of analytes.

Headspace SPME involves mass transfer processes between three phases: the coating material, the sample headspace and the sample matrix, according to the affinity of the analyte for each of the phases. The difference between the chemical potentials of the analyte in the three phases is the driving force of the headspace extraction procedure.

There are two partitioning processes involved: between the matrix and the headspace and between the headspace and the coating. For aqueous samples, the headspace/water partition coefficient (K_{hs}) is directly related to the Henry's law constant of the analyte, which in turn is determined by its volatility and hydrophobicity.

Although SPME is mainly used as an equilibrium extraction technique, it has the ability to perform exhaustive extraction. If the coating/matrix partition coefficient, K_{fs}, is very large ($K_{fs}\ V_f \gg V_s$), the amount of analyte absorbed by the coating is $n = C_0\ V_s$, and exhaustive extraction is achieved. In some cases this is an unwanted effect because it can perturb the system to be analyzed (it can change the equilibrium in the matrix). Extensive extraction can be avoided by changing to a less efficient extracting material or using a smaller volume of coating, sacrificing some of the sensitivity of the process.

To enable the extraction and analysis of different groups of organic compounds a variety of sorbents have been tested as SPME stationary phases. The principle of chemical similarity between the solvent and the solute applies very well in this case. The oldest and best studied coating, the nonpolar poly(dimethylsiloxane)-PDMS, retains a large number of nonpolar organic compounds and the more polar coatings such as carbowax and polyacrylate extract well the polar organics such as chlorinated phenols and nitrophenols (Buchholz and Pawliszyn, 1994). Derivatization procedures can also be used to reduce the polarity of such analytes and simultaneously improve their chromatographic properties (Buchholz and Pawliszyn, 1994; Hay et al., 1996).

Thermal desorption of analytes from an SPME coating is generally very effective. Desorption at higher temperatures is due to the fact that the coating/gas partition

coefficients decrease with temperature and the extracting material is no longer able to retain the analyte over a certain temperature. The constant flow of carrier gas within a GC injector also facilitates the desorption process. Current desorption temperatures range from 150 ^{0} to 300 ^{0} . Typical desorption times are in the order of 1-5 min. For compounds with high molecular weights or high affinity for the coating material, carry-over may become a problem and can be detected and corrected by conducting blank runs in between the analytical runs. Compounds with high boiling points tend to show more carry-over, but the boiling point is not the only determining factor in the desorption process.

Due to kinetic limitations, the thickness of the fibre coating is an important factor in determining desorption time. Carry-over also increases with coating thickness (Buchholtz and Pawliszyn, 1994).

Long desorption times compared with a classical solvent injection have raised concern about broadening of the peaks in the chromatogram and the first SPME studies recommended cryofocusing as a necessary procedure to be used in conjunction with SPME (Arthur et al., 1992). But many studies have demonstrated that the usual focusing at the front of the capillary GC column is sufficient to give very sharp peaks for most analytes.

The adjustment of pH in the sample and the use of salt addition, individually or combined, have been studied as means to improve the extraction on the fibre coating and consequently the sensitivity of the analytical method for some polar analytes. The pH adjustment is used for acidic analytes, in an attempt to hinder ionization and enhance the concentration of the neutral form of the analyte, the form which partitions into the non-

polar fibre coating. The saturation of the sample with salt is done in an attempt to "salt out" the analyte, to decrease the solubility of the analyte in the water phase and to promote the partitioning of the analyte into the organic coating of the fibre. With a pH of 2 in a saturated salt solution an improvement in sensitivity by a factor of 2-17 has been demonstrated for a series of phenols, methylphenols, chlorophenols and nitrophenols. It is interesting to point out that the extraction time had to be also increased, due probably to a slower diffusion through the saturated salt solution (Buchholz and Pawliszyn, 1994).

The mass transfer from water to headspace can be speeded up by constantly stirring the water sample to generate a continuously fresh surface.

Water addition (10%) to clay samples helped release analytes to headspace from this very recalcitrant matrix (Zhang and Pawliszyn, 1993). Water addition has been previously reported to improve the recovery of atrazine residues from soils by solvent extraction as well (Matteson et al., 1970). Water acts probably on the hydrophillic humic polymers, inducing changes in conformation which promote the release of the atrazine molecules from their sorption sites.

2.6.2. SPME methods for pesticide analysis

Pesticide residues in water and soil samples have been analyzed before by researchers using variations of the SPME method developed by Pawliszyn and coworkers (Belardi and Pawliszyn, 1989).

In a comprehensive report investigating the various operating parameters and the overall performance of the new SPME technique by Geelen (1993), the extraction of 14 compounds from clean water solutions was described, including the pesticides trifluralin and atrazine. Although static sorption was used (no stirring of the solution) the study provides useful information for the overall optimization of the SPME procedure. Geelen (1993) reports that a very long extraction time is needed for atrazine to attain equilibrium, but, for extraction times longer than 10 min, variations of the amount of analyte extracted with extraction time are very slow. Good reproducibility of the results and linearity of the calibration curve for atrazine in pure water solutions are also reported by Geelen (1993).

Shirey et al. (1994) reported the analysis of chlorinated phenols, including the pesticides pentachlorophenol and dinoseb, from pure water, using a polyacrylate fibre and a pretreatment of the sample by pH adjustment to pH = 2 and saturation with sodium chloride. All components were extracted and detected at 20 ppb concentration. The same authors report the analysis of a mixture of organochlorinated pesticides and their metabolites using a PDMS fibre for extraction and an EC detector for quantitation at concentrations as low as 25 ppt. Shirey (1994) uses a similar analysis for real water.

Eisert and Levsen (1995) and Eisert et al.(1994) reported the analysis of organophosphorous insecticides, triazine herbicides and 2,6-dinitroaniline herbicides from ground well water and spiked river water by sampling with a polyacrylate fibre. They also noted the effect of sodium chloride addition which increases the extraction efficiency of the polar compounds. Very low limits of detection are reported for SPME-GC with an NP specific detector and the authors found the method of appropriate sensitivity to be used

to verify the maximum admissible level for pesticides in drinking water set by the European Union.

Popp et al. (1995) reported the analysis of a mixture of organochlorinated insecticides from a soil percolate, using a 7μm PDMS fibre. Popp et al. (1994) used the 100 μm PDMS fibre to extract HCH isomers from soil percolates as part of a HCH isomers mobility study. Before extraction the soil percolates were filtered through 0.45 μm filters to separate the particulate matter.

A more original approach to SPME has been proposed by Wan et al., 1994. They extracted methyl-parathion and lindane from pure water using a pencil lead rod instead of the coated optical fibre. The extracting material had good extractive properties, but also a relatively high background and could only be effectively used at desorption temperatures under 210°.

In our laboratory, SPME-based methods have been succesfully developed and used for the analysis of the herbicides metolachlor, atrazine and diclofopmethyl in water (Webster at al., 1994), the herbicide metolachlor (Graham et al., 1995; Ng et al., 1996; Webster et al., 1994) in runoff and tile drainage water, for the analysis of chlorophenols and acid herbicides in soil and water (Hay et al., 1996) and for the analysis of the insecticide lindane in water and soil samples (Anderson et al., 1995). The SPME-GC-EC method for metolachlor analysis in runoff and tile drainage water has been succesfully compared with an SPE-GC-MS method and an ELISA-based procedure (Webster et al., 1995; Gaynor et al., 1996).

2.6.3. SPME-based methods used in soil sample analysis. Limitations.

SPME had been previously used as an extraction method for a variety of analytes in different aqueous phases and solid matrices, but was generally believed to be impossible to use in such a "dirty" environment as a soil slurry.

Analysis of soil samples had been reported, but such methods used either the "head-space" technique (Zhang and Pawliszyn, 1993), based on the sampling of the gas phase above the soil sample, or a preliminary solution separation by centrifugation of the water phase (Bengtsson and Berglof, 1995) before the actual SPME procedure.

The use of head-space SPME methods is limited by the air-water partition coefficient (Henry's law constant) value of the contaminant and the water content of the sample. For moderately hydrophobic organic contaminants with a limited volatility, such as atrazine, this approach could lead to considerably less sensitive analytical methods. Moreover, some soil matrices have been found to be more recalcitrant in releasing the analytes (extraction from clay matrices has been found to be more difficult than extraction from sandy matrices (Zhang and Pawliszyn, 1993). The authors increased the extraction by adding 10 % water to the clay sample. In subsequent work Zhang and Pawliszyn (1995) described an improved SPME device with internal cooling which permitted the heating of the sample (to increase the transfer from the matrix to the headspace) while simultaneously cooling the fibre coating (to avoid the desorption of the analyte from the fibre). Such a procedure is less effective in quantifying total contaminant residues in the

soil than supercritical fluid extraction (SFE) for example and is more difficult to relate to the contaminant fractions with environmental significance.

Bengtsson and Berglof (1995) have proposed a method using separation of the soil solution by centrifugation prior to the SPME procedure. This method has been reported to extract 5 different pesticides from a representative set of 19 soils selected from 72 initial agricultural soils. Recoveries ranging from 2% to 72 % are reported by the authors, depending on the soil matrix and the pesticide extracted. The analytical method used in conjunction with SPME in this case was near infrared spectrometry (NIR).This method is more difficult to perform, not taking full advantage of the possibilities of SPME to simplify analytical work.

In view of previous success in the use of SPME in conjunction with a GC-EC analytical procedure for pesticide analysis of water samples in our laboratory (Webster et al., 1994), the technique was examined for the direct sampling of atrazine residues from soil slurries.

3. Direct analysis of atrazine residues in soil slurries by solid-phase microextraction/ capillary column gas chromatography method.

I. Development of the analytical method

3.1. Introduction

Pesticide behaviour in soils has become a matter of concern in recent years because many of the commonly used products have been shown to contaminate surface waters and ground water through phenomena such as migration through soil or run-off from treated soil.

Atrazine is a herbicide that has been used in large quantities all over the world, particularly in corn crops. Because of its relatively long life and capacity to leach through the soil to the water table (Howard, 1991), atrazine is considered to be a pesticide of concern and its concentration in natural waters is closely regulated (CCME, 1989;Trotter et al., 1990). Many analytical procedures have been developed for atrazine in natural waters, soils, and soil slurries. In almost all of these methods, the extraction step is the most time consuming and the most expensive, involving the use of large quantities of organic solvents (Mattson et al., 1970; Smith and Walker, 1989; Obrador et

al., 1993; Sanchez-Brunete et al., 1994; Leavitt et al., 1991; Rouchard et al., 1994; Wang et al., 1995).

Solid phase microextraction is a solvent-free extraction method used, for instance, for the analysis of organic compounds in aqueous samples (Belardi and Pawliszyn, 1989). The technique uses a fused silica optical fibre coated with a material similar to a chromatographic stationary phase (polydimethylsiloxane or polyacrylate) to extract the organic analyte from an aqueous phase (Arthur et al., 1992; Berg, 1993) or from the headspace above an aqueous sample (Zhang and Pawliszyn, 1993). The SPME extraction is non-exhaustive, based on a process of equilibrium partitioning. In view of previous successes in the use of SPME in conjunction with a GC-ECD analytical procedure for pesticide analysis of water samples in our laboratory (Webster et al., 1994), the technique was examined for the direct extraction of atrazine residues from soil slurries.

SPME had been previously used in the analysis of a variety of analytes in different aqueous phases, but was generally believed to be impossible to use in such a "dirty" environment as a soil slurry. Analysis of soil samples had been reported, but all such methods used the "head-space" technique (Zhang and Pawliszyn, 1993), based on the sampling of the air phase above the soil sample. For moderately hydrophobic compounds with limited volatility such as atrazine, this approach could lead to a considerable loss of sensitivity of the method.

Important difficulties were expected to appear in the direct sampling of soil slurries with such a delicate device as a coated optical fibre. Coextracted compounds from the slurry were expected to interfere with the analysis and the mechanical impact of soil

particles with the small volume of the coating was expected to damage the coating ("exfoliation" of the coating material was expected) and shorten the life of the fibre. A schematic representation of the direct interaction of soil particles with the fibre coating is presented in Fig. 3.1. The figure also enables comparison with the simpler case of headspace sampling, I which no interaction between soil particles from the slurry and the fibre coating occurs.

A simple, new method for the direct analysis of atrazine in soil slurries, with no interference from coextracted materials from the soil is presented. The analytical method is completed with a suggested fibre cleaning procedure which helps maintain the performance of the fibre over at least 15 soil slurry extractions.

3.2. Materials and Methods

3.2.1. Chemicals and Instruments

Atrazine Pestanal® standard (98 %) (Riedel de Haen AG-D 3016 Seelze 1, Germany) was used.

HPLC-grade water (Acusolv-Anachemia, Rouses Point NY 12979, USA) was used to prepare the standard atrazine solution, the soil slurry samples and as the cleaning agent for the fibre in the ultrasonic bath.

SPME fibres with 100 μm thick polydimethylsiloxane coating (PDMS) (Supelco No. 5-7300 from Sigma-Aldrich Canada Ltd.; 1300 Aimco Blvd., Mississauga, ON, Canada L4W 9Z9) for manual extraction were used throughout the experiment.

One 20 μm PDMS fibre for manual extraction was also used in the experiment.

A Supelco No 5-7330 fibre-holder for manual extraction from Sigma-Aldrich Canada Ltd. was used to perform the extractions.

A Stereomaster model 12-593-IT optical microscope (40 X magnification) (Fisher Scientific Ltd, NJ 07410, USA) was used to monitor the aspect of the fibre coating surface during the experiment. Photos were made using a Minolta X-700 camera mounted on the optical microscope, using both incident and transmitted light and a 30 s exposure time.

A Zeiss Photo II photomicroscope (Carl Zeiss Yena Comp. Ltd., Germany) was used for 75 X magnification photographs. Both transmitted and reflected light were used and the exposure time was 100 s. Reflected light was achieved using an external fiberoptic source with a gooseneck optic cable and a focusing lens. Photomicrographs were taken using Kodak T Max 100 film and the microscopic automatic camera system. The photomicrographic work was done in the Zoology Department laboratories, University of Manitoba.

An Ultrasonic Cleaner model No. BP-1 (Electromation Components Corp., Farmingdale, L.I., NY, USA) was used to perform the cleaning of the fibre surface in between extractions.

3.2.2 Atrazine standard solution

Atrazine (2.67 mg, 98%) was dissolved in 100 mL distilled water (HPLC grade) with stirring for 48 h and occasional heating (50^0-60^0). Atrazine concentration in the standard stock solution was 1.21 x 10^{-4} mol/L (26.17 ppm).

3.2.3. Soil characteristics

The soil used in this experiment was Miniota sand having the following characteristics:

Table 2. Miniota soil characteristics.

Soil Property	**Organic matter**	**Sand**	**Silt**	**Clay**	**pH**	**CEC (mequiv./ 100g)**
Miniota soil	2%	84%	7%	9%	6.21	9.32

Soil analysis was performed in the Manitoba Soil Survey laboratories, according to the internal manual of soil analysis methods (Haluschak ed., 1986).

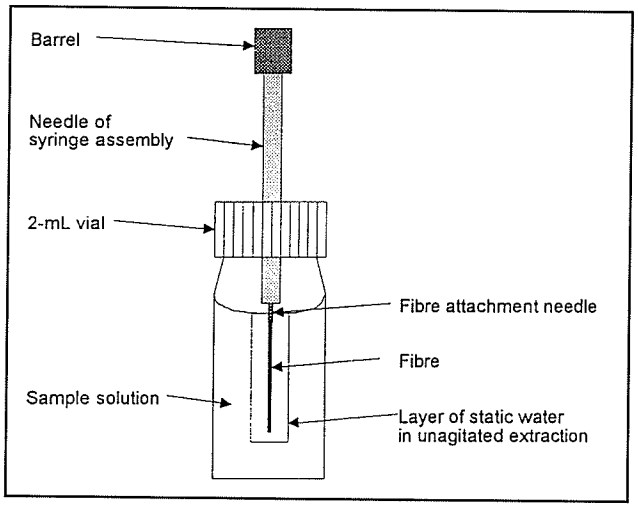
3.2.4. Soil slurry samples

Miniota sand (0.5 g, previously sieved through a 300 μm sieve) was weighed into specially constructed 30 mL round bottom vials equipped with septum screw caps, and, to each of the vials, a total of 25 mL distilled water (HPLC grade) and atrazine standard stock solution (respectively 2 mL, 3 mL, 5 mL, 10 mL, 15 mL solution, in HPLC grade water) was added. The soil slurry was stirred for 48 h before measurements were made and stirred at 20° during the course of the experiment. Soil concentration for all soil slurry samples used was 20 g/L.

Soil slurry samples were spiked with atrazine in quantities corresponding to total concentrations ranging between 1.05 ppm and 26.2 ppm and, after a minimum 48 h equilibration period, were analyzed by the above procedure.

3.2.5. Extraction

SPME extraction was performed using a Supelco No. 5-7300 manual 100 μm polydimethylsiloxane solid-phase microextraction fibre assembly. The extraction was performed by piercing the septum of the 30 mL vials containing the soil slurry samples with



the septum-piercing needle and extending the fibre directly into the magnetically stirred soil suspension. The SPME assembly was clamped in place above and resting on the vial cap. The extraction process was allowed to continue for 30 min, then the fibre was retracted into the septum-piercing needle and the needle was withdrawn from the septum. A magnetic stirrer at high speed was used to ensure effective mixing during the extraction.

3.2.6. Injection into the GC system

To determine the optimum depth of penetration of the fibre in the GC injection port, the needle/fibre assembly was compared with a normal 10 μL GC syringe. The SPME fibre assembly was clamped upright over the GC injection port and the GC septum was pierced using the septum-piercing needle. The fibre was extended into the injection port with the purge off and desorbed at 220 ^{0} for 10 min.

Blank runs of the extraction-analysis procedure were performed from time to time between the analytical runs to check for "memory" effects.

3.2.7. Analysis

A Hewlett-Packard 5890 GC system equipped with an EC detector and operating in the splitless mode was used. The gas-chromatograph was equipped with a 30 m J&W Scientific DB-5, 0.32 mm i.d., 1.0 μm film thickness column, (Chromatographic Specialties Inc., Brockville, ON, Canada). The chromatographic conditions were as

follows: injector temperature, 220 ^{0}; column, 100 ^{0} (10 min), 10 ^{0}/min to 250 ^{0} (5 min); flow-rates: helium carrier, 1.0 mL/min; argon-methane 5% make-up gas, 60 mL/min.

The quantification of the analyte was done based on external calibration using dilutions of the standard atrazine solution in HPLC grade water. Calibration curves were constructed for each fibre.

3.2.8. Cleaning of the fibre

The physical appearance of the fibre was monitored by direct observation under an optical microscope Stereomaster model 12-593-IT (40x magnification). Pictures of the fibre were made using a camera mounted on the optical microscope.

Ultrasonic cleaning of the fibre was performed, combined with a gentle mechanical cleaning of the fibre with a camel hair brush. After each injection the fibre was immersed for 15 min in an ultrasonic bath filled with HPLC grade water, then gently brushed and immersed for another 10 min in the ultrasonic bath. The cleaned fibre was than desorbed in the injection port of the GC during a blank run.

3.3. Results and Discussion.

The first atrazine analysis was performed in the summer of 1994 when only the 100 μm poly(dimethylsiloxane) coating fibres (100 μm PDMS) and the 20 μm PDMS fibres were well tested and commercially available. Atrazine is not a very hydrophobic organic

analyte and its partition coefficient, proportional to its octanol-water partition coefficient (K_{ow} = 512), is not high. The amount of analyte which partitioned into the non-polar PDMS fibre coating was sufficient for the purposes of this study. The partition coefficient into the extracting material is one of the limiting factors for the sensitivity of this analytical method.

The newer 85 μm polyacrylate coating fibre, now commercially available from Supelco, is probably more appropriate for atrazine extraction, based on the principle of similarity of structure between solvent and solute. The polyacrylate fibre has also some quoted disadvantages, e.g. higher bleeding, leading to a higher background of the chromatogram. Thus, the choice of the 100μm PDMS fibre was a reasonable choice at the time this study was designed.

The 20 μm PDMS fibre, a fibre recommended by the manufacturer for pesticide analysis, was also tested during the work. The principle behind switching to a thinner coating was that a thinner coating would favour a quicker desorption of the analyte from the coating. The 20 μm PDMS fibre was expected to conserve the favourable extractive properties of the 100 μm PDMS fibre, but allow a more rapid desorption, with a reduced "memory" effect or carry-over from one injection to the next. An unexpected effect was experienced: the 20 μm PDMS fibre had an inverse extraction selectivity for atrazine and co-extractives from the soil matrix. In the case of the 100 μm fibre, the atrazine was extracted in a high proportion and soil components were only marginally coextracted by the fibre coating; in the case of the 20 μm fibre coating, the apparent co-extractives were extracted in higher proportion than atrazine. The 20 μm PDMS fibre has since been

replaced by the manufacturer with a new 30 μm PDMS fibre (Supelco No. 57 308), recommended for pesticide analysis. The new 30μm PDMS fibre has been introduced recently and was not tested in this experiment.

The 100 μm PDMS fibre was chosen for this study based on satisfactory extraction capacity, good selectivity for atrazine compared with coextractants from the soil matrix, very good stablity, low bleed, and good reproducibility of the extraction capacity among fibres.

The extraction time was set at 30 minutes (Geelen, 1993). Geelen determined the adsorption/time curve for atrazine and reported that a long equilibration time was necessary. Using extraction at equilibrium is impractical for analytical purposes, but, having plotted the characteristic equilibration curve, an extraction time could be chosen which allowed for good reproducibility in the analysis, if extraction time were consistent.

Stirring is very important in all SPME extractions, because the partition between the matrix and the fibre coating is diffusion limited. According to mathematical models which describe the diffusion of the analyte through the matrix and through the water phase (Louch et al., 1992), if the sample matrix is perfectly agitated, for most analytes equilibrium is achieved in $<$ 1 min (Zhang et al., 1994), because the coating is very thin. The determining factor in the extraction time is the diffusion through the aqueous matrix. In these cases vigorous agitation methods (sonication, magnetic stirring) are used to shorten extraction times.

In the case of soil slurries, vigorous stirring is doubly important because of the necessity to maintain an equilibrated soil-water system and a homogenous soil solution concentration.

A magnetic stirrer at high speed was used. It was expected that, because whole soil was used in the sorption experiments, "exfoliation" of the fibre coating by the coarse soil particles would occur at high stirring speeds. Following observation of the fibre surface under an optical microscope, such an effect was ruled out (Fig. 3.2). It is clear from Fig. 3.2. that exfoliation did not occur , rather deposition of small amounts of soil particles on the fibre surface.

If static extraction is used and the extraction time is kept constant at 30 minutes, the sensitivity of the analytical method is considerably less.

The maximum desorption temperature in the GC injection port recommended by the technical specification for the 100 μm PDMS fibre is 220 ^{0}. Because of the high melting point of atrazine (171^0-174 ^{0}) the maximum recommended temperature was used.

Desorption time was set at 10 minutes by a set of trial and error experiments. This is a long desorption time, but atrazine desorption appeared somewhat difficult from the fibre coating and shorter desorption times would have led to the creation of "memory" effects. Other authors (Geelen, 1993) have reported similar desorption times for atrazine.

In spite of the unusually long desorption time (necessary to avoid atrazine carry over) no cryofocussing is necessary to obtain sharp peaks in the GC analysis (Fig. 3.3). During the desorption of the analyte in the injection port (similar to a solvent injection) the septum purge of the GC system was off, to prevent the loss of the analyte. Some

authors report using a shorter purge- off period, after which the purge is turned on, while the desorption of the fibre continues. This procedure implies a small loss in sensitivity (10 %-20 %). A purge-off time of 10 min and of 2 min were tried with similar results. The focusing of the analytes in the first portion of the capillary column was efficient enough to preserve an excellent shape of the chromatographic peaks in both cases, therefore a 10 minutes purge-off time was used throughout the project. Complete desorption was achieved, as shown by clean blank runs between the analytical runs.

A linear relationship between the amount of analyte sorbed by the fibre coating and the initial concentration of the analyte in a sample is expected (see equation 1, Chapter 2). Because of the extreme simplicity of the analytical procedure, it was possible to run a calibration curve using standard solutions of atrazine in HPLC-grade water for each fibre, which allowed avoidance of any errors due to manufacturing differences between fibres. In the range of concentrations used in the study, the method showed good linearity (Fig.3.4).

The reproducibility of the analytical results for pure water extraction was checked for several atrazine concentrations and showed to be < 95 % in accordance with data presented by other authors for SPME water extractions (Geelen, 1993; Eisert and Levsen, 1995).

During the extraction of the analyte, the coating of the fibre was subjected to mechanical impact from soil particles in the stirred soil slurry. Soil particles were deposited on the surface of the fibre coating and the fibre lost about 10-20 % of its sorption ability after each use. The physical appearance of the fibre was monitored by

direct observation under an optical microscope (40x magnification) and documented by pictures of the clean fibre as well as the fibre used in soil slurry extractions (75 x magnification; Fig. 3.2.) To the authors' knowledge, this was the first time that the fibre coating surface has been studied in such detail, using microscopy techniques. It was observed that the variations in the extraction capacity of the fibre coating were due to variations in the coverage of the surface of the coating caused by soil particle deposition and, eventually, resuspension from the coating surface. Contrary to the general belief, no loss of the organic coating could be seen and the soil particles appeared to be loosely bound to the surface of the coating. These observations were valuable, because they showed that the decline in the extraction capacity of the fibre was not due to an irreversible destruction of the coating material. It appeared therefore possible to clean the surface of the coating between extraction procedures and to maintain constant the extraction efficiency of the fibre.

Ultrasonic cleaning in HPLC grade water for 25 min, combined with a gentle mechanical cleaning of the fibre with a small camel hair brush was successfully used. After each extraction-desorption cycle, the fibre was cleaned, then desorbed in the GC injector port for 10 min at 220 ^{0}, during a blank run. Using this procedure it was possible to maintain constant the performance of the fibre for at least 10 injections when sampling from soil slurries containing a 20 g/L concentration of suspended soil. In the absence of the cleaning procedure the performance of the fibre was continuously declining (Fig. 3.5).

No bleeding of the fibre was observed during pure water extractions. After a number of extractions, a peak appeared at a very long retention time in the chromatogram (27 min), attributed to the loss of some components of the glue used to attach the fibre to the syringe needle (R. Belardi, Supelco, personal communication).

When sampling from pure water solutions, the life of the fibre was virtually unlimited. The most likely end to its use was from mechanical accident. When sampling from real water samples (run-off water, natural surface water) with low organic matter content and low solid particle content the fibre life was limited to 25-30 extractions (Graham et al., 1996). For the direct sampling of soil slurries, if the cleaning procedure was used in between the analytical runs, the fibre could be used with good results up to 10 samplings. Compared with traditional solvent extraction methods and solid phase extraction (SPE) methods this was still a very cost effective procedure. In the absence of a cleaning step between samplings, the performance of the fibre decreased quickly.

The new SPME method, previously demonstrated to be useful for extracting a wide range of analytes from relatively clean aqueous solutions, was successfully tried in our laboratory for the direct extraction of atrazine residues from soil slurries. This adds a simple, clean, solventless extraction technique, easily compatible with capillary GC, to the arsenal of methods available for the analysis of less volatile compounds from soils and water bodies with a high content of humic and other particulate and dissolved materials.

A remarkable observation is that no relevant interference of coextracted soil components was observed, even at the lowest atrazine concentration (1.05 ppm). Very good resolution of the peaks was obtained for all samples. Experiments reported in this

paper did not approach the LOD of the method. The method was, however, linear over the range of concentrations studied.

As was previously reported (Geelen, 1993) for the sampling of atrazine from pure water, for the extraction time used (30 min) the partitioning of the analyte (atrazine) between the organic phase (the fibre coating) and water did not reach equilibrium, but was very near equilibrium. Little variation was found with small variations in the extraction time.

The new method for atrazine residue analysis in soil slurries, based on SPME sampling and capillary GC analysis, has important practical advantages for analytical laboratory practice related to the simplicity of the extraction method, the short analysis time and the zero solvent consumption. The new method has also the potential of being an ideal tool for the study of the sorption-desorption of atrazine on soils and for direct field sampling, a potential for which it will be developed by subsequent studies.

3.4. Figures

Fig. 3.1. Schematic showing soil particle interaction with the fibre coating in direct SPME sampling and headspace sampling of soil slurries.

Fig. 3.2. Photo: clean and used fibre by optical microscopy (75X magnification)

Fig. 3.3. Chromatogram of atrazine in soil slurry (1.05 ppm)

Fig. 3.4. Standard curve atrazine by SPME

Fig. 3.5. Stability of the fibre extraction capacity. Mechanical cleaning performance

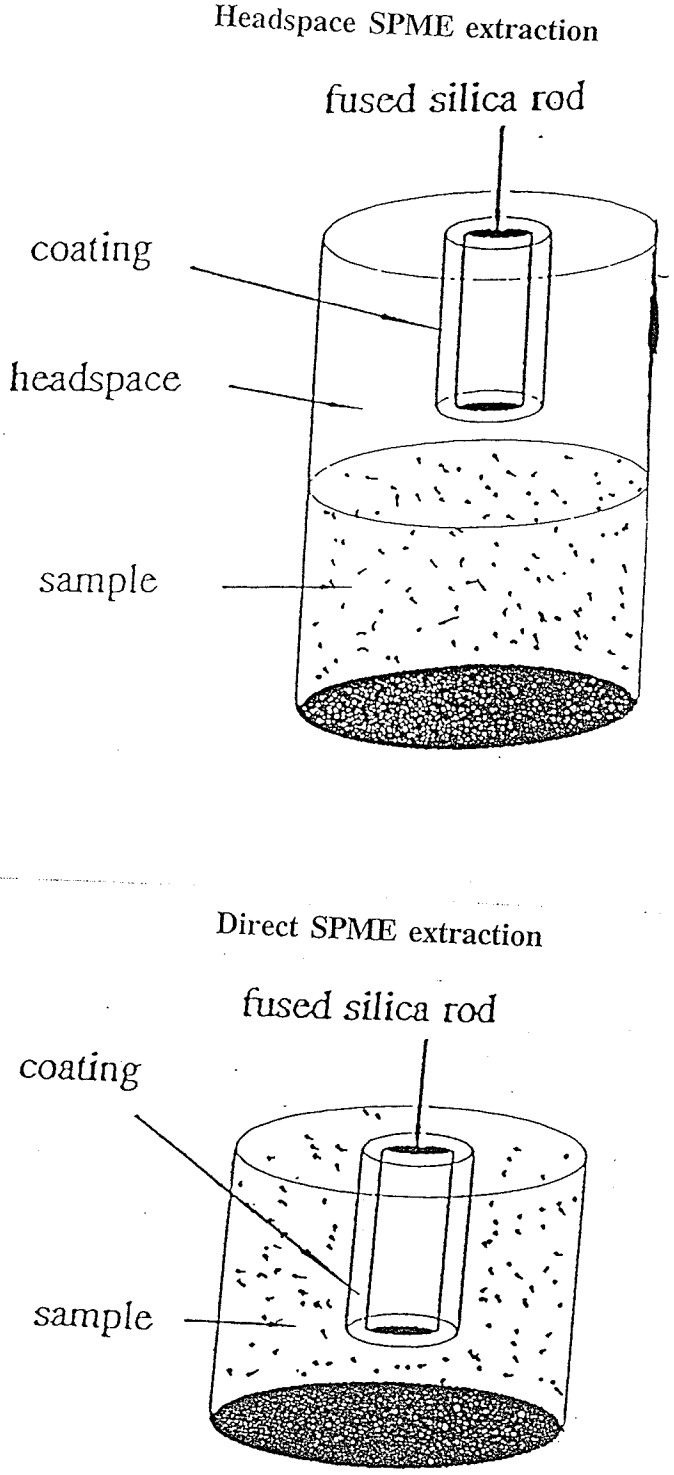


Fig. 3.1. Schematic showing soil particle interaction with the fibre coating in direct SPME sampling and headspace sampling of soil slurries.

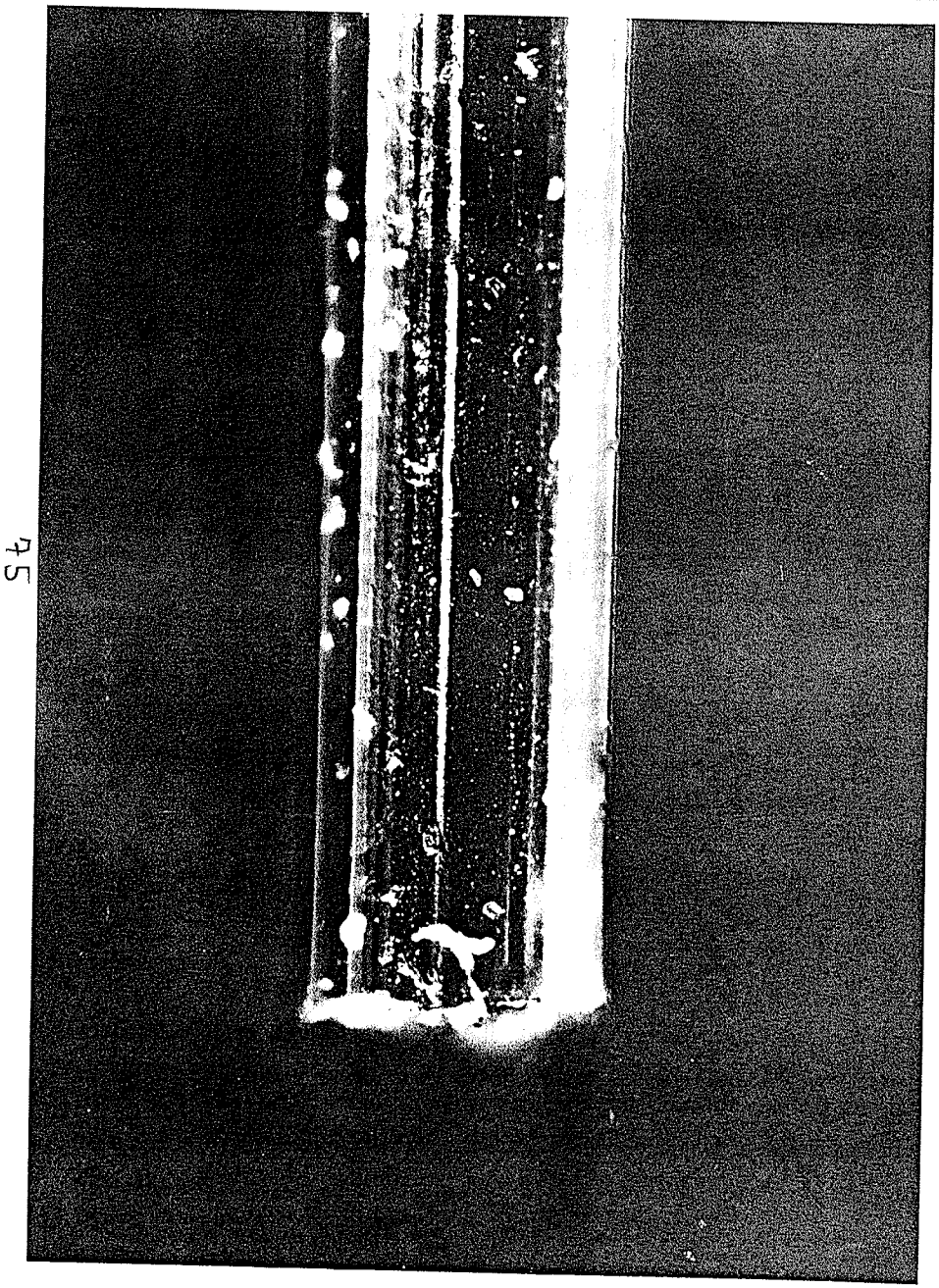
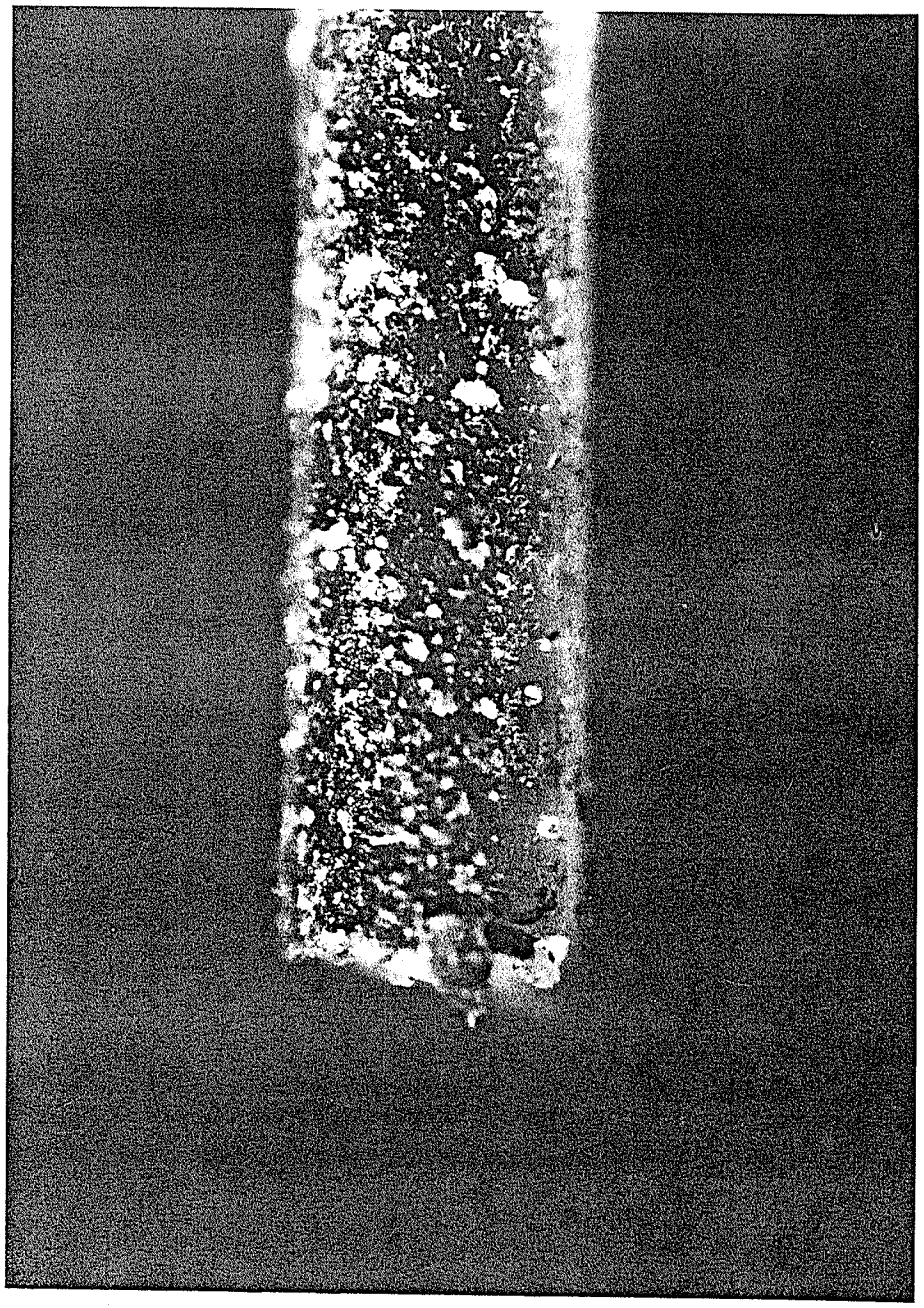


Fig. 3.2. New Fibre
(75 x)



"Dirty" Fibre
(75 x)

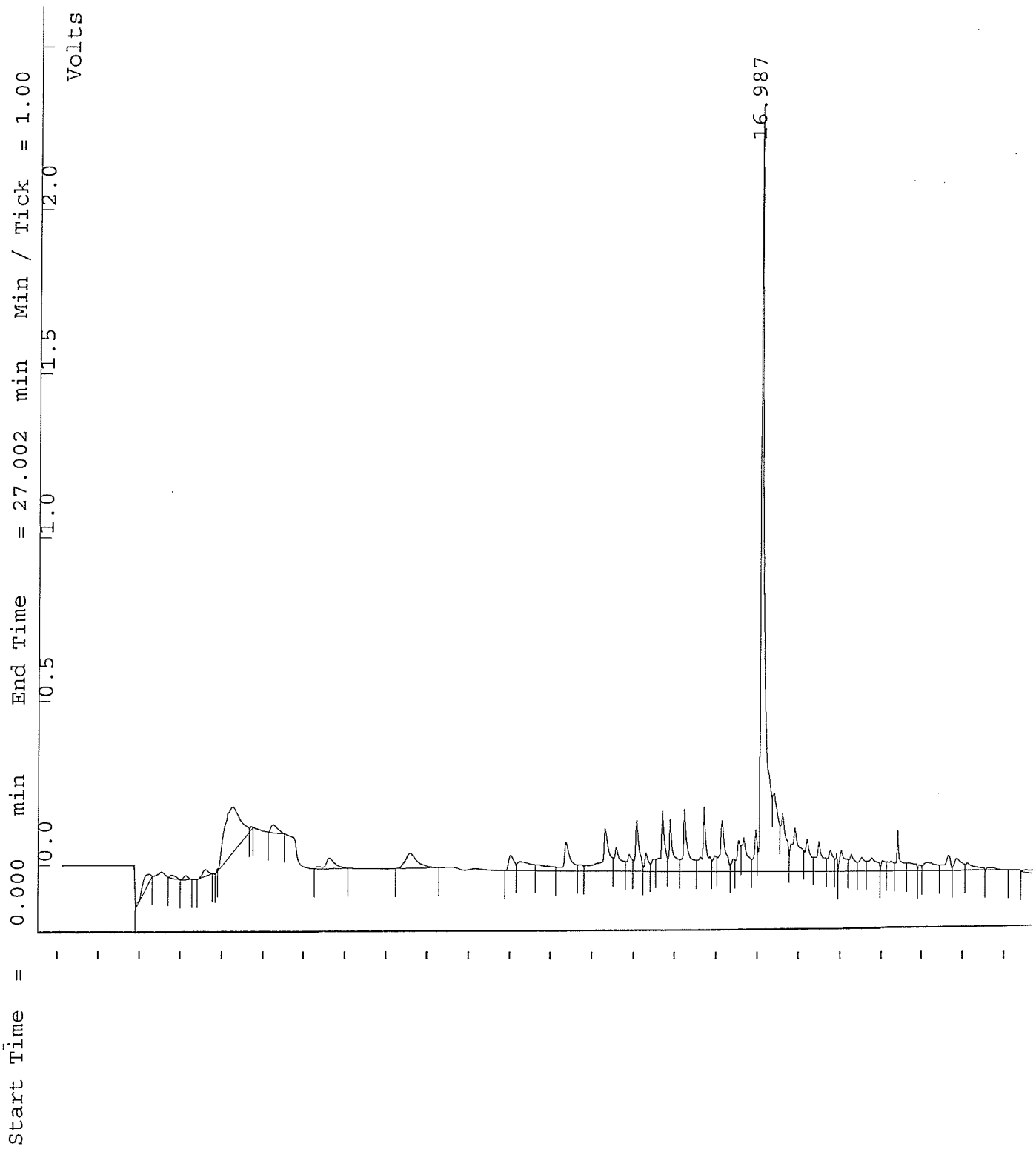


Fig.3.3.Chromatogram of atrazine in soil slurry (1.05 ppm)

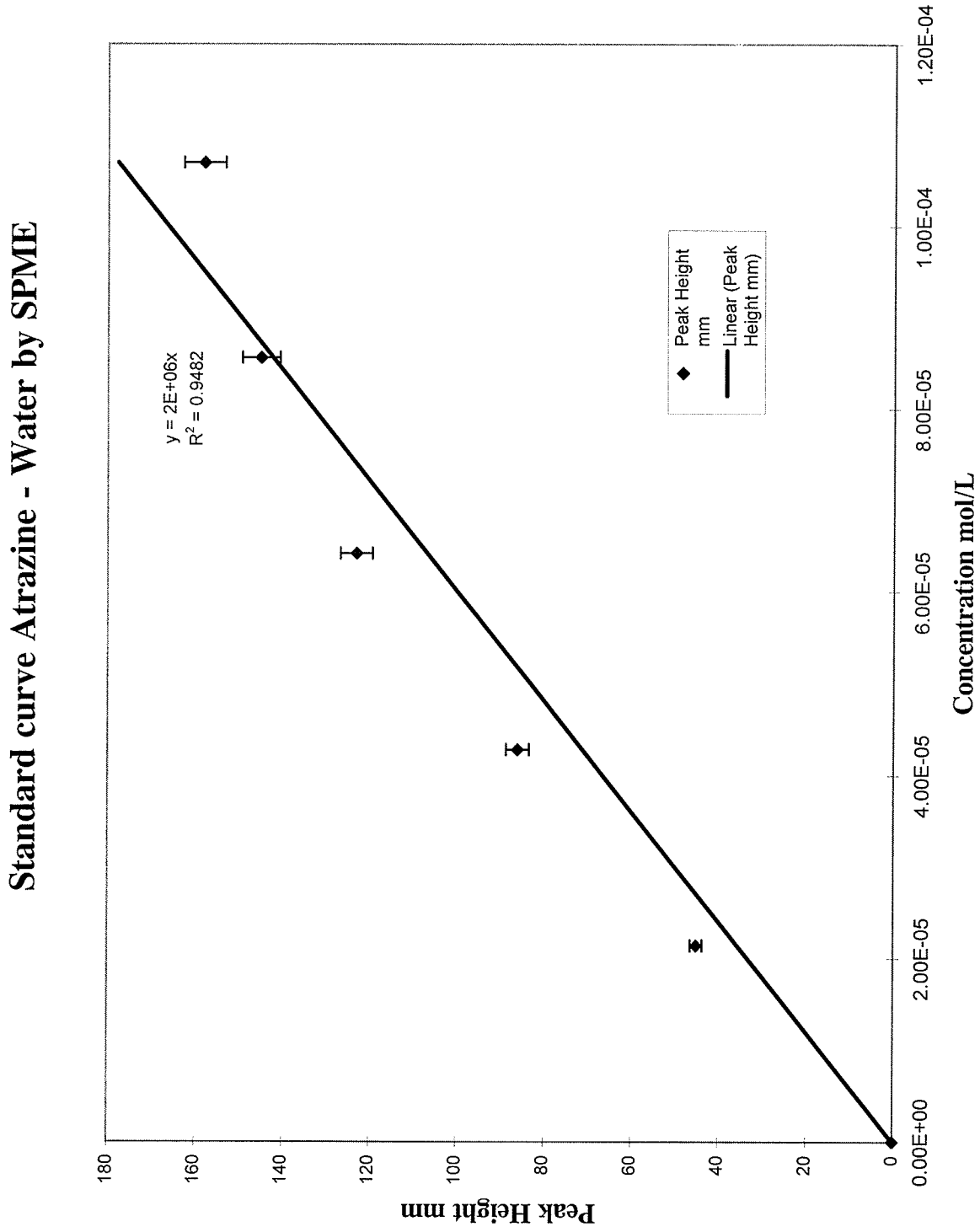


Fig. 3.4. Standard curve atrazine in water by SPME-GC.

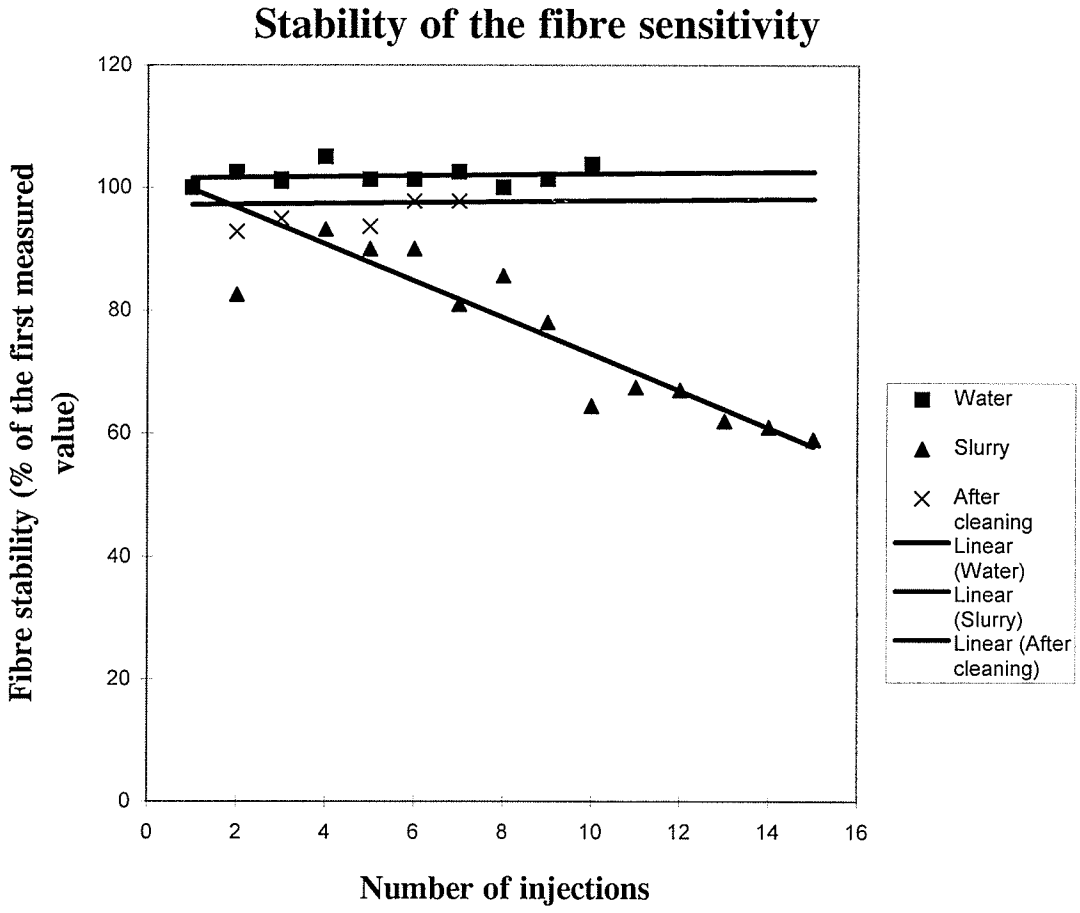


Fig. 3.5. Stability of the fibre extraction capacity.

4. Direct analysis of atrazine residues in soil slurries by solid-phase microextraction/ capillary column gas chromatography method.

II. Study of the fibre coating-soil particles interaction. Development of a chemical cleaning method for the SPME fibre.

4.1. Introduction

Pesticide residues in water and soil samples had been analyzed before by researchers using variations of the SPME method developed by Pawliszyn and coworkers at the University of Waterloo, but direct sampling from such "dirty" matrices as soil slurries had been considered to be impossible.

Soil slurry analysis by direct SPME-GC was expected to be more complicated than analysis of pure spiked water or headspace over water because soil slurries contained soil particles which could interact with the fibre, altering the surface and the volume of the coating, with possible impact on the extraction properties of the fibre.

Important difficulties were expected to appear in the direct sampling of soil slurries with such a delicate device as a coated optical fibre. Coextracted compounds from the slurry were expected to interfere with the analysis. "Exfoliation" of the fibre surface was

expected to be a contributing factor in shortening the fibre life and in generating less reproducible results over the life-time of the fibre.

Many organic molecules with low molecular mass occur naturally in soils and some were expected to be extracted by the fibre at the same time as the analyte, atrazine. In the first part of this study it was shown that for the 100 μm PDMS fibre and the Miniota sand, this was not the case. The background of the chromatogram is quite low and does not interfere with atrazine analysis (see Fig.3.2). Soils of different origins, with different physical and chemical characteristics, may contain different low molecular mass compounds able to interfere with atrazine GC analysis. For this reason a soil with different origin and characteristics than the soil used in the first study was chosen for this study. The reproducibility of the analytical results for the extraction of atrazine from pure water was shown to be < 95 %, in accord with data presented by other authors (Geelen, 1993; Eisert and Levsen, 1995). Small differences between fibres were accommodated by calibrating each fibre. The life-time of the fibre was virtually unlimited. The most likely end to its use was by mechanical accident.

Important limitations in the reproducibility of the extraction were generated in earlier work by the high content of solid particles in the soil slurry (20 g/L). When direct extraction from a soil slurry is performed, the extraction capacity of the fibre declines with each sampling (see Fig.3.5.).

Two possible mechanisms were considered to be responsible for the continuous decline in the fibre extraction capacity: the reduction of the coating volume by mechanical "erosion" or the reduction of the transfer surface area by plastic impact of the soil

particles with the surface of the coating and deposition of the particles on the fibre coating surface.

If the "erosion" mechanism was confirmed the "degradation" of the fibre would have been an irreversible process and direct SPME from soil slurries would have been shown to be not possible, as many researchers had predicted.

If the decline in extraction capacity was due to a "coating" effect by deposition of soil particles on the fibre surface, the process would have been not necessarily irreversible and a technique for "cleaning" the surface of the fibre coating could be designed.

By using optical microscopy and scanning electron microscopy (SEM) methods, the impact of the soil particles with the fibre coating was studied in more detail. The "degradation" of the extraction capacity of the fibre has been found to be due not to an "exfoliation" of the coating, but to coating of the transfer surface for the analyte with many layers of particles deposited from the solution (very likely by electrostatic attraction: D. S. Gamble; private communication). The process was shown to be not fundamentally irreversible and it led to the development of a "cleaning" procedure for the fibre which would maintain the extraction performance of the fibre consistent for an adequate number of samplings.

An ultrasonic-mechanical cleaning procedure had been developed before, based on the first observations under an optical microscope (see section 3.2.6). Based on more detailed observations by SEM, a new chemical cleaning procedure was designed.

Two chemical cleaning agents which were expected to have good properties for dissolving and dispersing humic compounds were selected for this experiment. It was

expected that destroying the organic coating of the soil particles would reduce their capacity to adhere to the fibre surface.

A diluted solution of sodium hydroxide (0.01 M) and a solution of 5 % sodium pyrophosphate (well known for its property to dissolve humic compounds) were tried as cleaning agents. An ultrasonic bath was used to perform the cleaning of the fibre. The results of the cleaning procedures were monitored by SEM. The extraction capacity of the fibres was determined for 15 successive sampling and cleaning cycles.

4.2 Materials and Methods

4.2.1. Chemicals and Instruments

Atrazine Pestanal® standard (98 %) from Riedel de Haen AG-D 3016 Seelze 1, Germany, was used to prepare the standard stock solution.

HPLC-grade water (Acusolv-Anachemia, Rouses Point NY 12979, USA) was used to prepare the standard atrazine solution, the soil slurry samples and the solutions used as cleaning agents for the fibre in the ultrasonic bath.

Sodium hydroxide pellets and sodium pyrophosphate reagent grade from Fisher Scientific Company, NJ 07410, USA were used to prepare the cleaning solutions.

SPME fibres 100 μm polydimethylsiloxane (PDMS) (Supelco No. 5-7300) from Sigma-Aldrich Canada Ltd., 1300 Amico Blvd., Mississauga, ON, Canada, L4W 9Z9 were used for manual extraction throughout the experiment.

A Supelco No 57330 fibre-holder for manual extraction from Sigma-Aldrich Canada Ltd., 1300 Amico Blvd., Mississauga, Ontario, Canada, L4W 9Z9 was used to perform the extractions.

A Stereomaster model 12-593-IT optical microscope (40 X magnification) (Fisher Scientific Ltd, NJ 07410, USA) was used to monitor the aspect of the fibre coating surface during the experiment. Photos were made using a Minolta X-700 camera mounted on the optical microscope, using both incident and transmitted light and a 30 s exposure time.

A scanning secondary electron microscope (SEM) Cambridge Instr. 120 at 150 X and 2000 X magnification was used to monitor the aspect of the fibre coating surface during the experiment. The SEM study was conducted at the Geological Sciences Department of the University of Manitoba.

An Ultrasonic Cleaner model No. BP-1 from Electromation Components Corp., Farmingdale, L.I., NY, was used to perform the cleaning of the fibre surface in between extractions.

4.2.2 Atrazine standard solution

Atrazine (2.67 mg, 98%) was dissolved in distilled water (HPLC grade) with stirring and occasional heating (50^0-60^0) for 48 h. Atrazine concentration in the standard stock solution was 1.08 x 10^{-4} mol/L (23.13 ppm).

4.2.3. Soil characteristics

The soil used in this experiment was collected from the Caribbean island nation of Dominica and had the following characteristics:

Table 3. Fifi Road soil characteristics.

Soil Property	Organic matter	Sand	Silt	Clay	pH	CEC (mequiv./100g)
Fifi Road soil	3.65%	80.5%	7.3 %	12.2 %	4.52	21.37

Soil analysis was performed in the Manitoba Soil Survey laboratories, according to the internal manual for soil analysis methods (Haluschak ed., 1986).

4.2.4. Soil slurry samples

Fifi road soil # 1 (0.5 g, previously sieved through a 300 um sieve) was weighed into specially constructed 30 mL round bottom vials equipped with septum screw caps, and, to each of the vials, 25 mL distilled water (HPLC grade) and atrazine standard stock solution (respectively 2 mL, 3 mL, 5 mL, 10 mL, 15 mL solution, in HPLC grade water) were added. The soil slurry was stirred for 48 h before the measurements were made and

kept stirred, at 20 ° during the course of the experiment. Soil concentration for all soil slurry samples used was 20 g/L.

4.2.5. Extraction and Analysis

SPME was performed using a Supelco No. 5-7300 manual 100 mm polydimethylsiloxane solid-phase microextraction fibre assembly. Extraction time used was 30 min. The desorption time in the GC injection port was 10 min, with the purge off.

A Hewlett-Packard 5890 GC system operating in the splitless mode, equipped with a 30 m J&W Scientific DB-5, 0.32 mm i.d., 1.0 μm column and an EC detector was used. Conditions: injector: 220 ^{0}; column, 100 ^{0} (10 min), 10 ^{0}/min to 250 ^{0} (5 min); flow-rates: helium carrier, 1.0 mL/min; argon-methane 5% make-up gas, 60 mL/min.

Soil slurry samples (20 g soil/L) spiked with atrazine in quantities corresponding to total concentrations ranging between 4 x 10^{-6} to 1 x 10^{-4} mol/L were analyzed by the above procedure after 2 weeks' equilibration time.

4.2.6 Preparation of the cleaning solutions

A 0.01 M solution of sodium hydroxide (pH = 12) was prepared by dissolving 0.2 g of NaOH, reagent grade, in 500 mL HPLC-grade water.

A solution of 5% (w/w) sodium pyrophosphate (pH approx. 9.5) was prepared by dissolving 25 g of sodium pyrophosphate (reagent grade) in 500 mL of HPLC-grade water.

4.2.7. Chemical cleaning procedure

The cleaning of the fibre was performed by immersing the fibre for 25 minutes in the cleaning solution, using an ultrasonic laboratory cleaning instrument. After each cleaning the fibre was desorbed in the injection port of the gas chromatograph for 10 minutes at the desorption temperature of 220 ^{0}, during a blank run.

4.2.8. Monitoring of the fibre

The physical appearance of the fibre surface was monitored by direct observation under an optical microscope (75 X magnification) and by scanning electron microscopy (SEM) (150 X and 2000 X magnification).

Observation under an optical microscope is nondestructive: the fibre was extended using the fibreholder and brought into the optical field for direct observation and photography.

For observation using the scanning electron microscope, the fibre was cut from the supporting metallic rod, glued to a special sample holder using an electron conducting carbon paint and coated with a very thin gold layer (less than 100 Å thin).

4.3. Results and Discussion

The Fifi road soil used for slurry preparation in this experiment was a tropical soil of volcanic origin, with different characteristics than the one used for the experiments described in Chapter 3.

The use of a soil with different origin and characteristics than in the first series of experiments was motivated by the necessity to confirm the initial finding that coextractants would not interfere with atrazine extraction from the slurry of either soil.

The experimental results showed no interference of coextracted compounds from the soil with atrazine analysis (using an EC detector) (Fig. 4.1).

The interaction of soil particles in suspension with the coating of the fibre leads to a continuing reduction in the extraction ability of the fibre (Fig. 4.2).The physical appearance of the fibre was monitored by direct observation and photography under an optical microscope (75 X magnification). The photographs (see Fig. 3.2) show that the fibre coating volume is not diminished by an "exfoliation" process due to the mechanical impact of soil particles under high stirring. A secondary "coating" of soil particles is deposited on the fibre coating, apparently completely covering the surface of the extractive material. It was difficult to obtain a well focused picture of the fibre under the optical microscope, even in a very fine tuned optical photographic system, because of limitations due to the cylindrical shape of the fibre and the shallow depth of field of the optical microscope and to difficulties related to the positioning of the relevant region of the fibre in the microscopic observation field without causing mechanical damage to the fibre. The

observations under an optical microscope were valuable because they gave a "real"image of the fibre (no pre-treatment of the fibre surface is required for optical observation, the fibre retains its transparency, its "glass-like" appearance) and especially because observations under an optical microscope do not require the destruction of the fibre. An optical microscope was used to monitor fibre surface in its dynamic evolution throughout the experiment.

Through the use of scanning electron microscopy (SEM) (150 X and 2000 X magnification) the changes in the appearance of the surface of the coating during extraction were easy to follow. The technique also allowed for a much more detailed observation. Unfortunately, due to the necessity to gold-coat the object under observation, the method is destructive for the fibre and, consequently, expensive. Only a limited number of fibre "samples" could be "sacrificed" in a carefully planned experiment to point out the most relevant steps in the modification of the fibre coating surface by soil particle deposition and to guide the cleaning experiments. Photos of the fibres were taken at relatively low magnification (150 X) to give a more comprehensive image of the fibre and make the whole process of "coating" by the soil particles easer to understand. Photos at higher magnification (800 X and 2000 X) were taken to enable the study of the changes of the coating surface during the process in more detail. Higher magnification was tried (3000 X), but no significant information was produced and the quality of the photographs was declining.

Photos of the new fibre (Fig. 4.3; 4.4.) showed an almost perfectly cylindrical fibre coating, with a smooth outer surface. Some solid, spheroid particles adhered to the

surface of the fibre. It was not possible to distinguish if these irregularities were dust particles or grains of the siloxane material from which the coating itself had been made and it was not considered to be relevant for our work. The tip of the fibre was rough, resulting from an imperfect cutting in the manufacturing process and was the most sensitive region to the interaction with soil particles as shown by the SEM observations (Fig. 4.5).

A fibre which was only used for one extraction from a soil slurry (Fig. 4.6) showed a different picture: the aspect of the coating surface itself was not changed, but a good part of the surface was covered with soil particles adhering on the coating surface. At higher magnification, the soil particles seemed to adhere to the surface by some kind of fibrilar structures (Fig. 4.7.) derived from their own surface material, which suggested that if this material is of organic nature and if an appropriate chemical agent can be found to dissolve this material, the soil particles could be expected to loosen from the fibre coating surface and allow fibre to be cleaned.

Further observation of fibres used in 15 successive extractions from soil slurry (Fig. 4.8- 4.11) showed that particles are continually depositing on the surface, forming a new "coating" which seems compact at the first approach. More detailed observation revealed that the structure of the "coating" was not compact, but granular, consisting of successive layers of soil particles. The fibre in fig. 4.8 and 4.9 was used for extractions in Miniota soil slurry. Fig. 4.10 and 4.11 show photographs of a fibre used for 15 samplings in the Fifi Road soil slurry. The aspect of the soil particle "coating" was

similar for both soils, suggesting that the deposition process was also similar in its essential characteristics and that a similar cleaning process may be applicable.

In conclusion, the variations in the extraction capacity of the fibre coating appear to have been due to variations of the surface of the coating caused by soil particle deposition and, eventually, resuspension from the coating surface. No loss of the organic coating could be seen and the soil particles present appeared to be loosely bound to the surface. Many layers of soil particles were deposited on the fibre coating surface during a number of extractions, building a secondary "coating" which appeared compact.

Based on these observations, the chemical cleaning agents were selected for their expected properties of dissolving and dispersing humic compounds. Sodium hydroxide solution, as well as sodium pyrophosphate solution are largely used for the isolation of humic substances. It was hoped that by dissolving the organic coating of the soil particles the particles would lose the capacity to adhere to each other and to the fibre surface and the soil particle "coating" which covers the fibre and diminishes the transfer surface would be removed.

The use of sodium hydroxide solution (0.01 M) as a cleaning agent in an ultrasonic bath was not successful. The high alkalinity of the solution (pH = 12) appeared to affect the surface of the coating, continually increasing its extraction ability for atrazine (Fig. 4.12.). A possible explanation for this effect might be a surface hydrolysis process of the siloxane coating under highly alkaline conditions, changing the polarity of the coating and making it more suitable for atrazine sorption. At the same time, the sodium hydroxide solution did not seem to have a very important dispersing effect on the soil particle

"coating", as proven by SEM observations (Fig. 4.13; 4.14). The fibre showed in the SEM photos had been used for 15 extractions from soil slurry. An ultrasonic-sodium hydroxide cleaning was performed on the fibre after each soil extraction-desorption. The particles of the "coating" appear to be smaller after the sodium hydroxide treatment and the "coating" less compact, but it still covered the whole surface of the fibre and was multi-layered. The much lower dispersing effect of sodium hydroxide solution on humic compounds compared with sodium pyrophosphate solution may be due to a much lower ionic strength of the sodium hydroxide solution used in this experiment (0.01 M NaOH compared to a 0.23 M sodium pyrophosphate solution). A higher sodium hydroxide solution concentration was not considered for experiments because it was assumed to be even more aggressive to the siloxane surface of the fibre coating.

A suggestion for further study might be the use of one initial treatment of the fibre with sodium hydroxide solution to improve its extraction capacity for atrazine and, consequently, the sensitivity of the analytical method.

Ultrasonic cleaning in a saturated $Na_2P_2O_7$ solution for 25 min proved to be very efficient in dispersing the soil particles from the surface of the coating, as proven by analytical measurements results (4.15) and by SEM observations (Figs. 4.16 and 4.17). The cleaned fibre was then desorbed in the GC injection port for 10 min. The temperature and the time used to desorb the cleaned fibre was identical to the desorption temperature and time used in the analytical procedure (220 ^{0}) in order to avoid any contamination which might occur during the cleaning process. The SEM photos show the dramatic effect of this cleaning procedure on the secondary soil particles "coating". The fibre showed in

the SEM photographs had been used for 15 extractions from soil slurry. After each slurry extraction-desorption the cleaning procedure was performed. After 15 samplings, most of the surface was free of soil particle deposit5, and, where such deposit was still present, it appeared to be thin, consisting of a single layer of soil particles. The first cleaning seemed to slightly increase the extraction capacity for atrazine of the fibre, an effect which was not observed after further cleaning steps. The extraction capacity for atrazine of the fibre remained unchanged during the experiment. The effect of the initial cleaning procedure might be due to removal of dust particles from the fibre or of other particles accumulated on the surface of the fibre during manufacturing and storage. An initial cleaning, before the use of the fibre for extraction, is therefore recommended. The ultrasonic cleaning using a sodium pyrophosphate solution enabled maintenance of constant performance of the fibre for at least 15 samplings from soil slurries. It was more effective than the ultrasonic-mechanical cleaning described in Chapter 3 and it involved less risk for the fibre, which is mechanically a sensitive device.

The design of an effective cleaning procedure completes the development of a reliable direct analysis method for atrazine in soil slurry and encourages the development of analysis methods from other "dirty" matrixes, traditionally considered inaccessible by such an apparently delicate technique as SPME. Appropriate cleaning procedures would have to be designed according to the properties of the matrix.

SPME followed by GC separation with electron capture (EC) detection and using the chemical cleaning procedure appears to be a very promising method (simple, solvent

less, inexpensive) for the study of atrazine behaviour in soil-water systems, a possibility that will be developed in subsequent studies.

4.4. Figures

Fig. 4.1. Chromatogram of atrazine in soil slurry (6.5 ppm).

Fig. 4.2. Fibre extraction performance: decline of extraction performance with the number of extractions

Fig. 4.3. SEM Photo: clean fibre-150 X magnification

Fig. 4.4. SEM Photo: clean fibre-2000 X magnification

Fig. 4.5. SEM Photo: fibre tip after one soil slurry extraction-150X magnification

Fig. 4.6. SEM Photo: fibre after one soil slurry extraction-150 X magnification

Fig. 4.7. SEM Photo: fibre after one soil slurry extraction-2000 X magnification

Fig. 4.8. SEM Photo: fibre after 15 soil slurry extractions (Miniota soil)-150 X magnification

Fig. 4.9. SEM Photo: fibre after 15 soil slurry extractions (Miniota soil)-2000 X magnification

Fig. 4.10. SEM Photo: fibre after 15 soil slurry extractions (Fifi Road soil)-150 X magnification

Fig. 4.11. SEM Photo: fibre after 15 soil slurry extractions (Fifi Road soil)-2000 X magnification

Fig. 4.12. Fibre stability: 0.01 M NaOH effect.

Fig. 4.13. SEM Photo: NaOH treatment effect-150 X magnification

Fig. 4.14. SEM Photo: NaOH treatment effect-2000 X magnification

Fig. 4.15. Fibre stability: 5 % $Na_2P_2O_7$ cleaning performance

Fig. 4.16. SEM Photo: $Na_2P_2O_7$ treatment effect-150 X magnification

Fig. 4.17. SEM Photo: $Na_2P_2O_7$ treatment effect-2000 X magnification

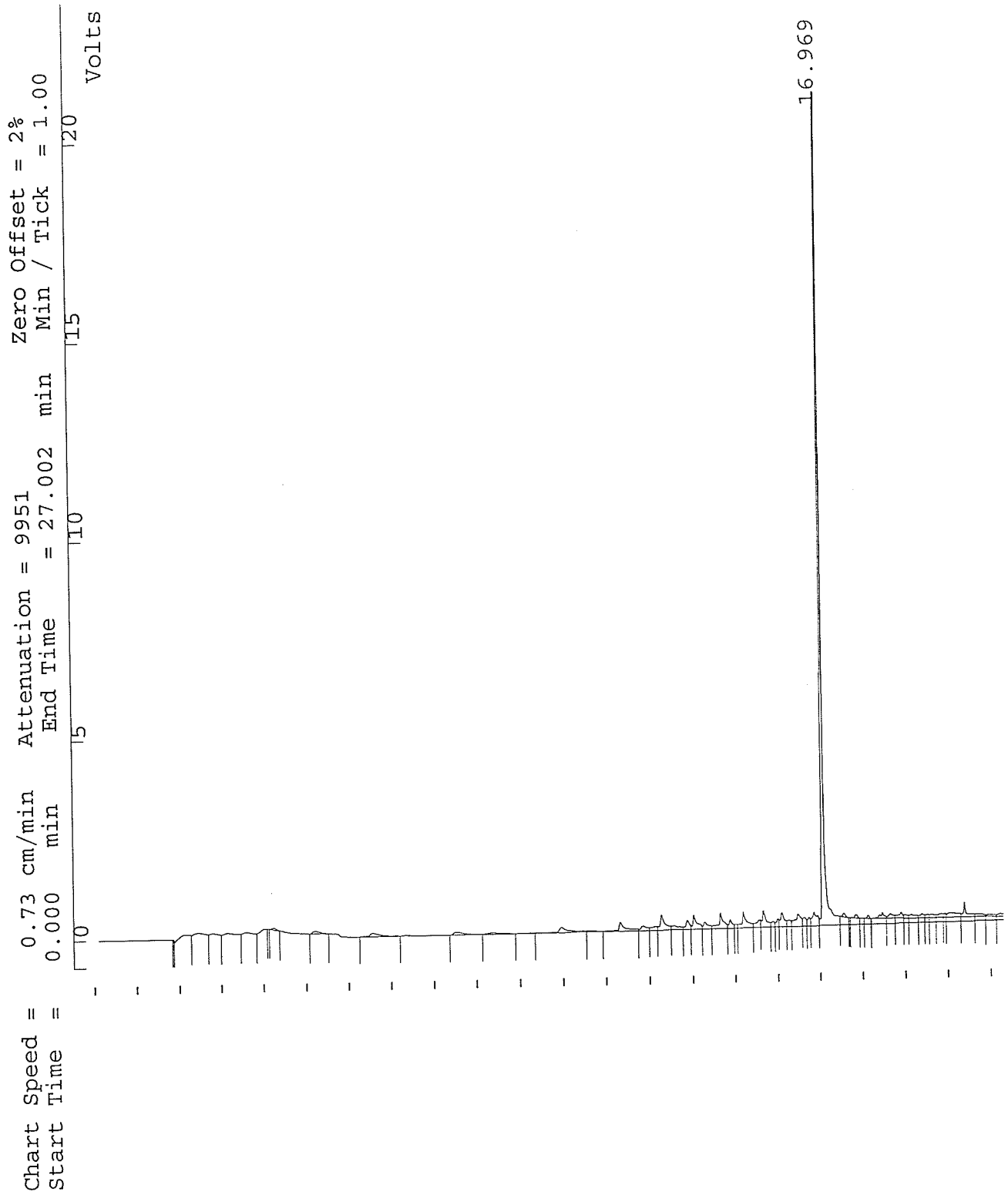


Fig.4.1. Chromatogram of atrazine in soil slurry (6.5 ppm).

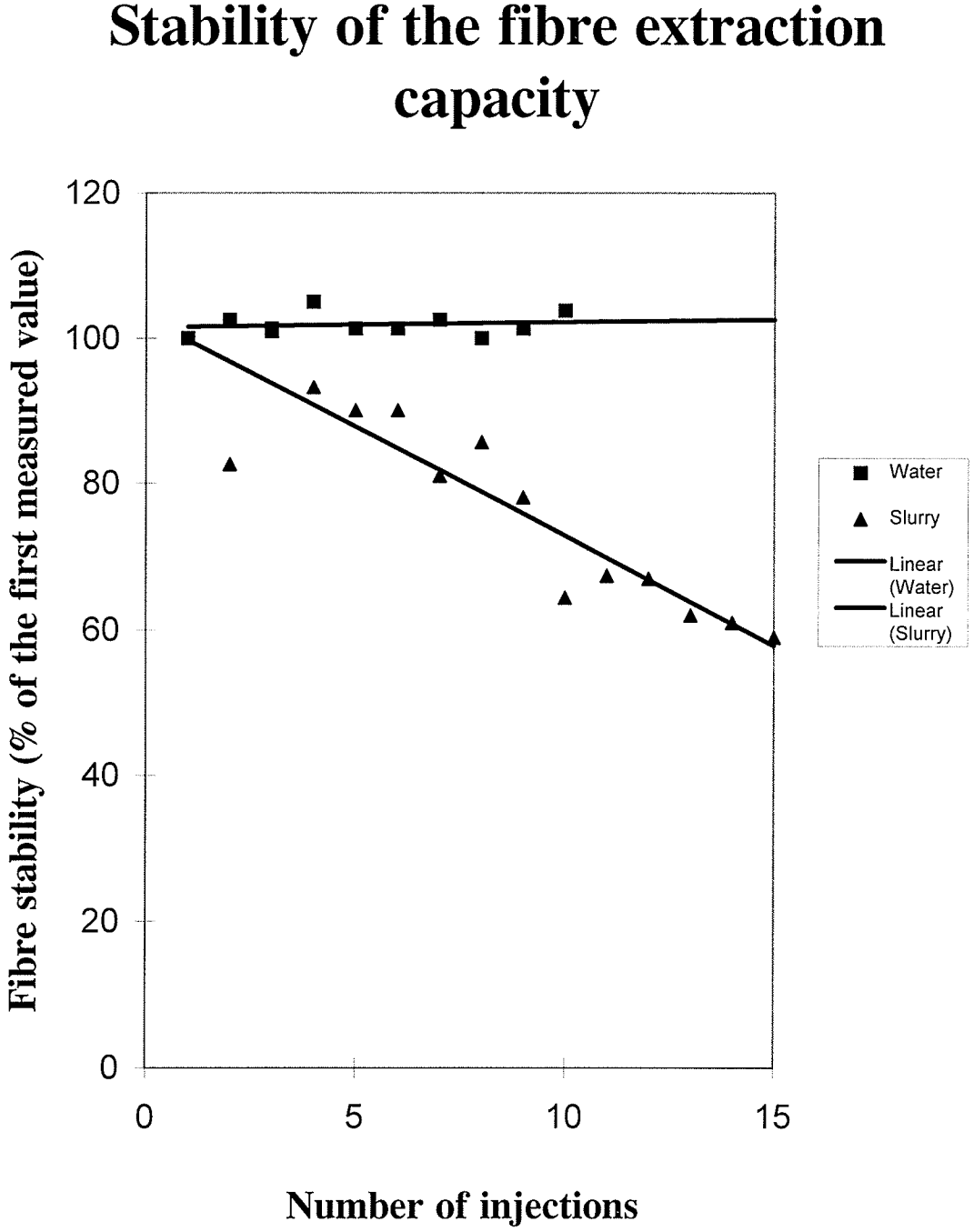


Fig. 4.2. Fibre extraction performance: decline of extraction performance with the number of extractions.

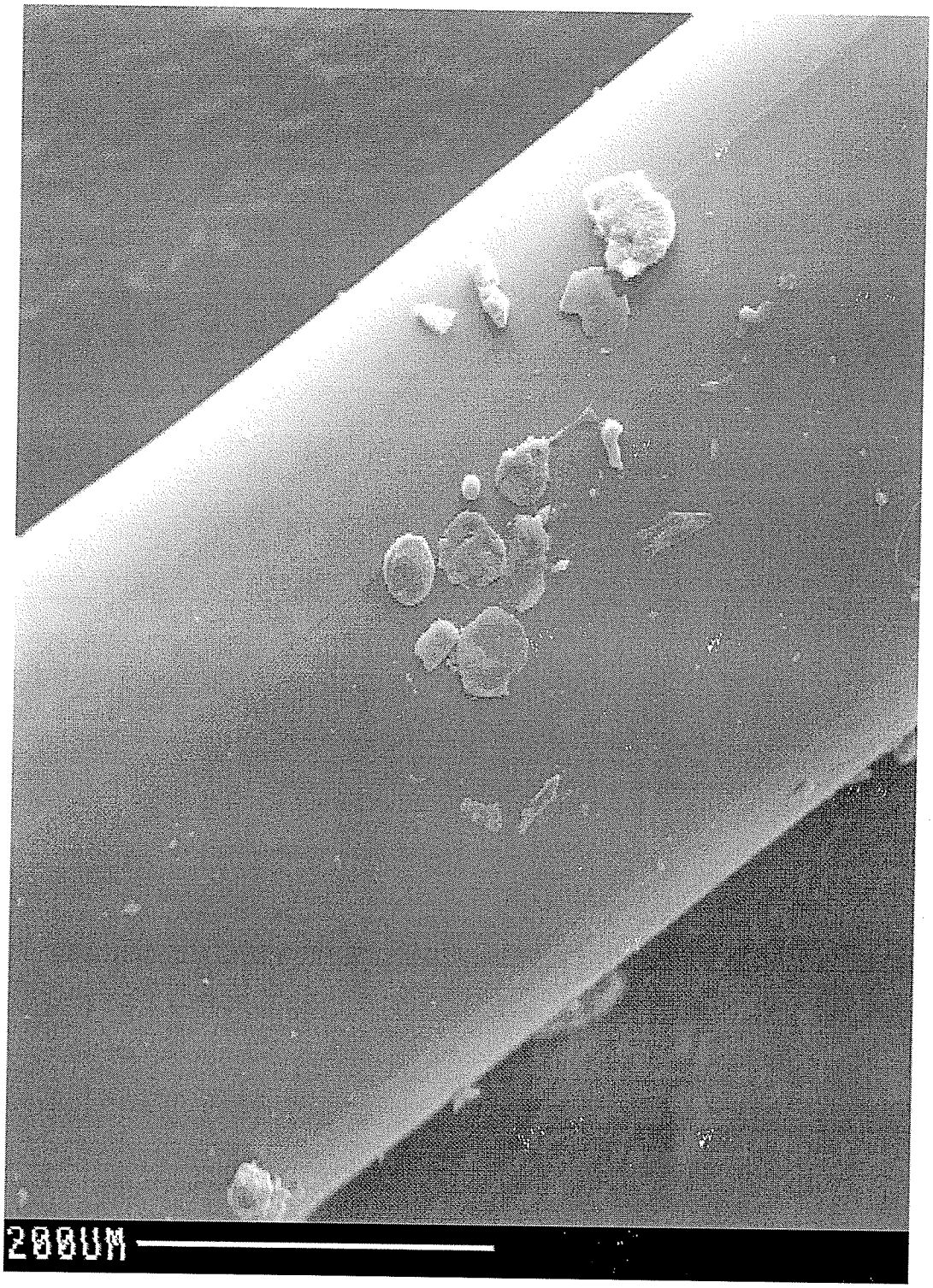


Fig. 4.3. SEM Photo: clean-fibre-150 X magnification.

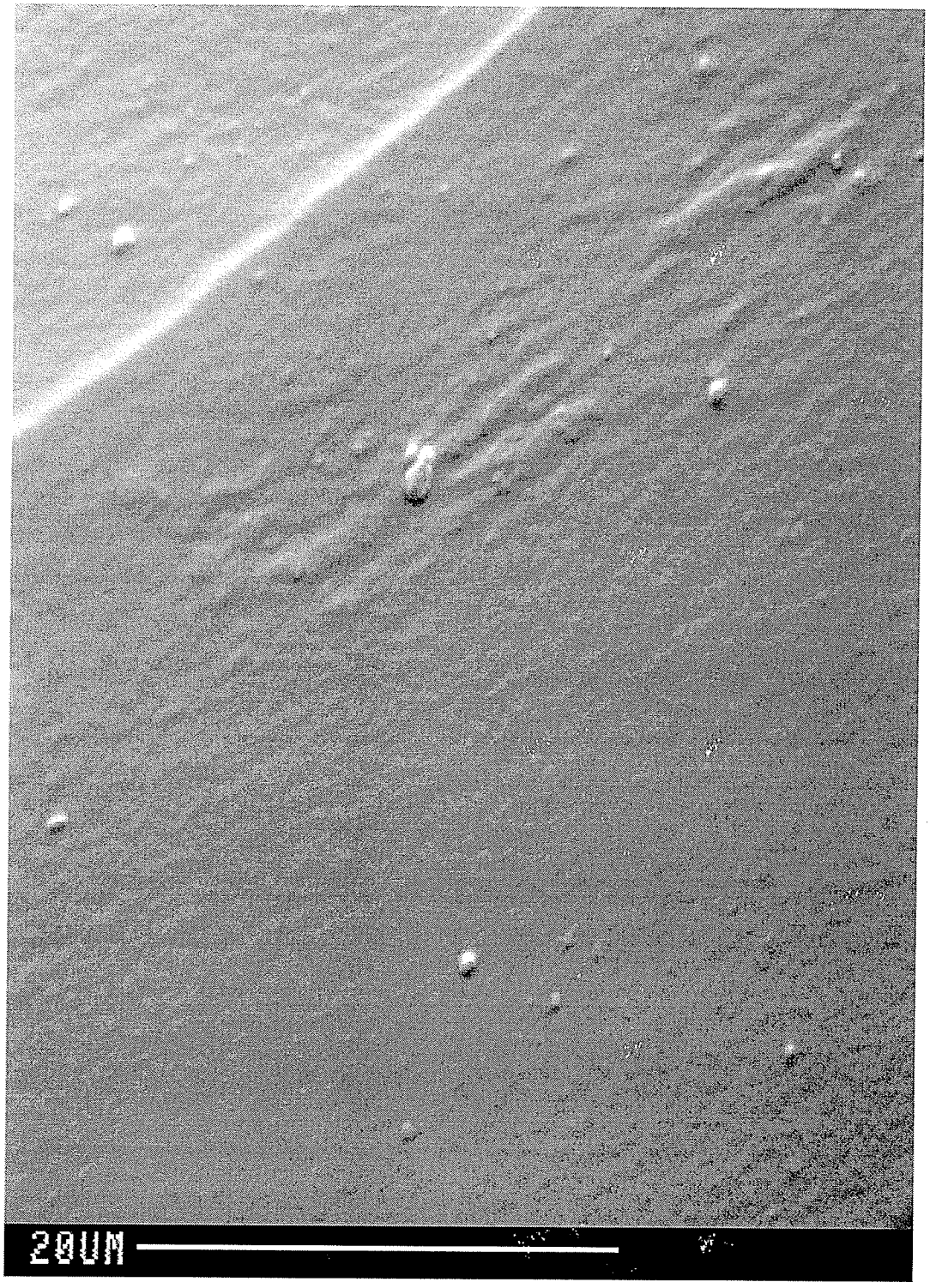


Fig. 4.4. SEM Photo: clean fibre-2000 X magnification.

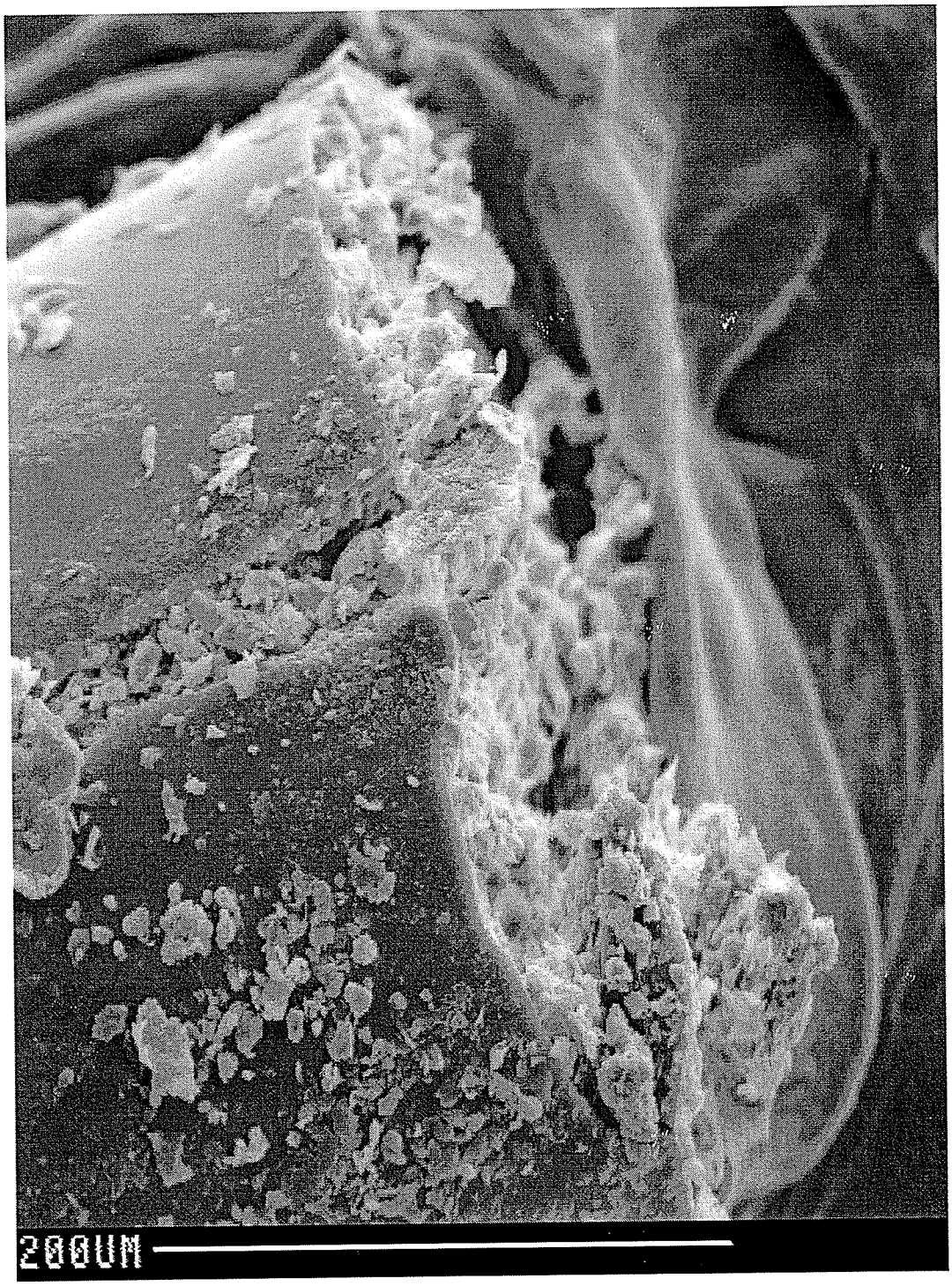


Fig. 4.5. SEM Photo: fibre tip after one soil slurry extraction- 150 X magnification.

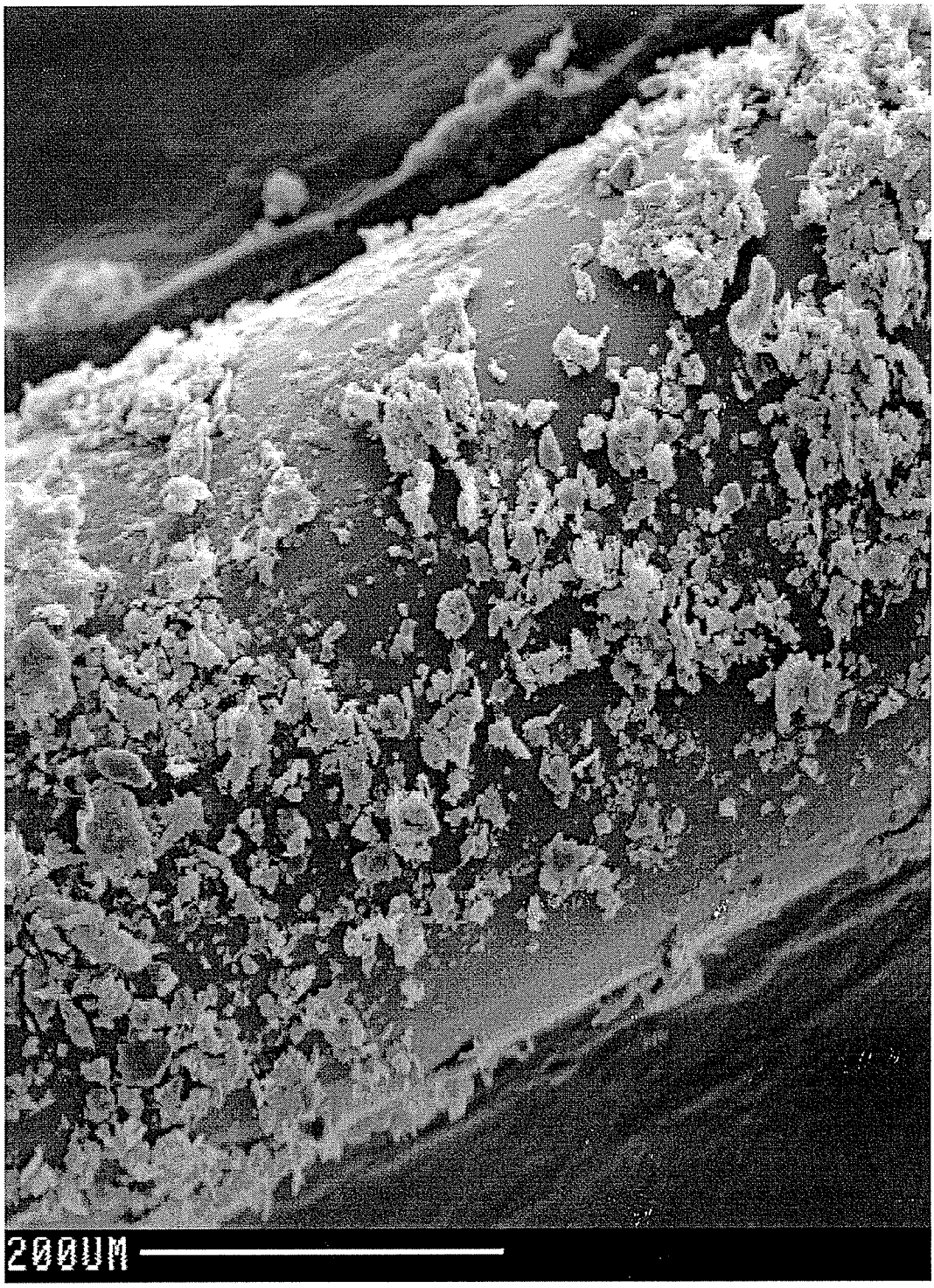


Fig. 4.6. SEM Photo: fibre after one soil slurry extraction-150 X magnification

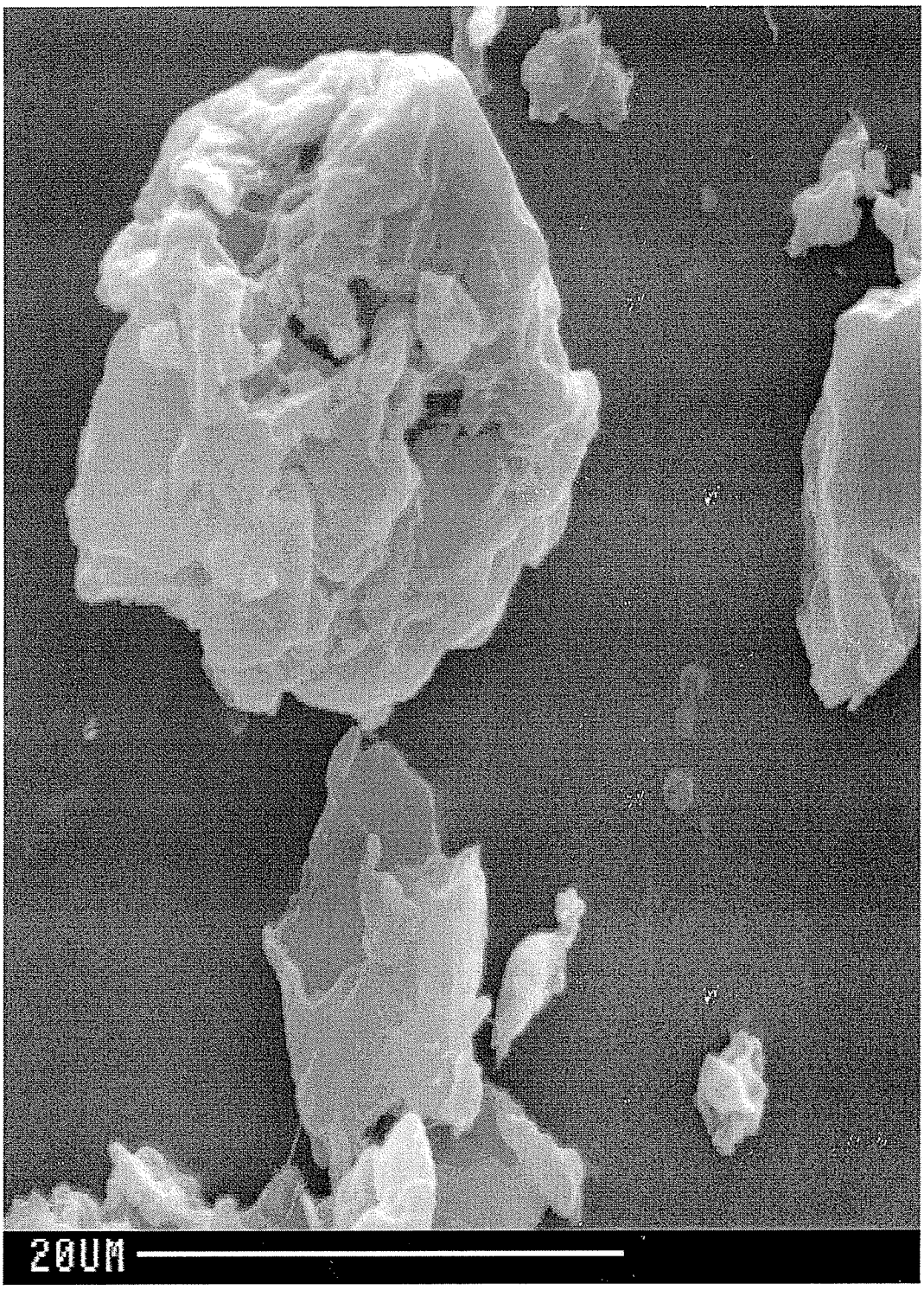


Fig. 4.7. SEM Photo: fibre after one soil slurry extraction-2000 X magnification.

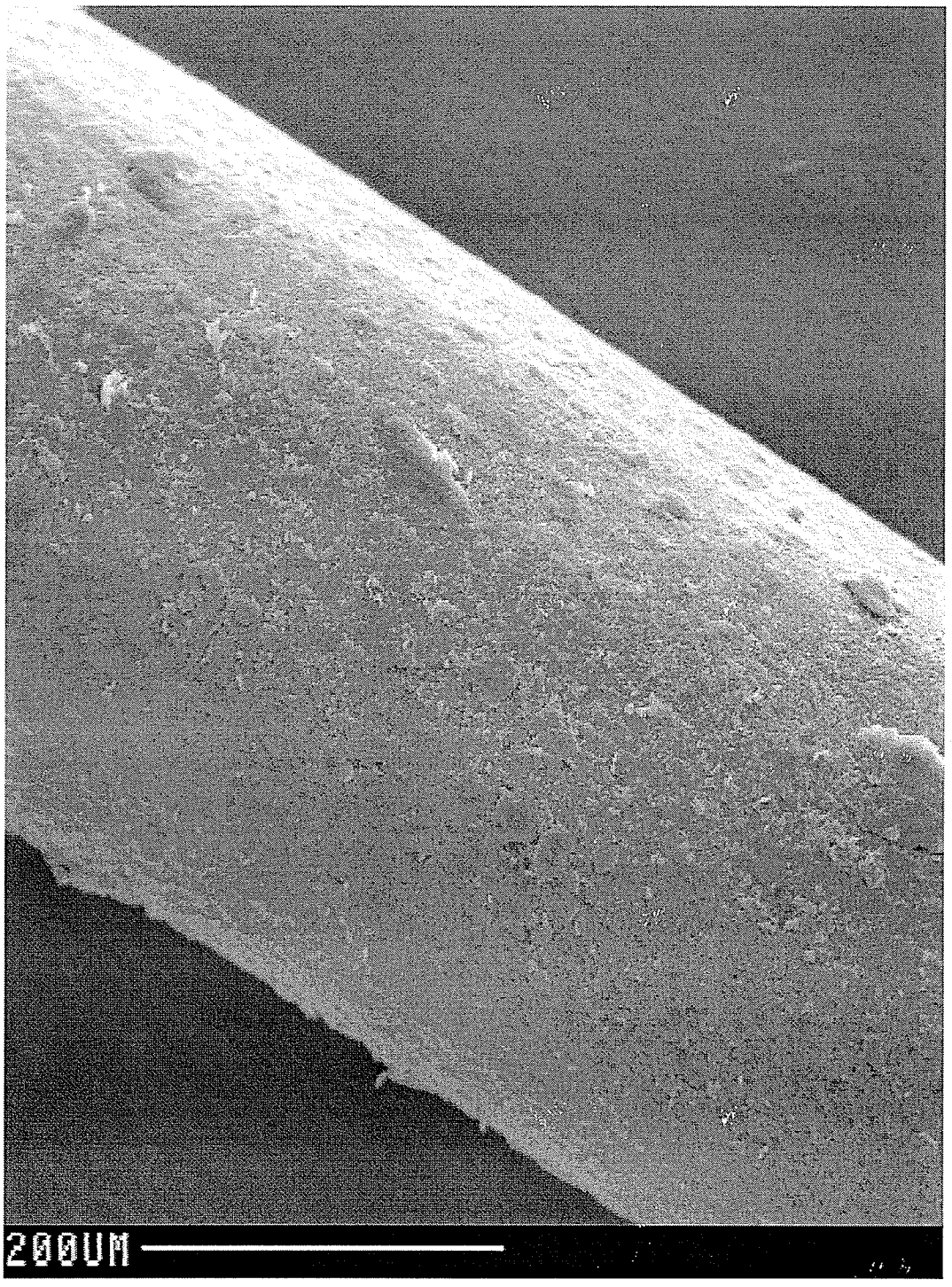


Fig. 4.8. SEM Photo: fibre after 15 soil slurry extractions (Miniota soil)-150 X magnification.

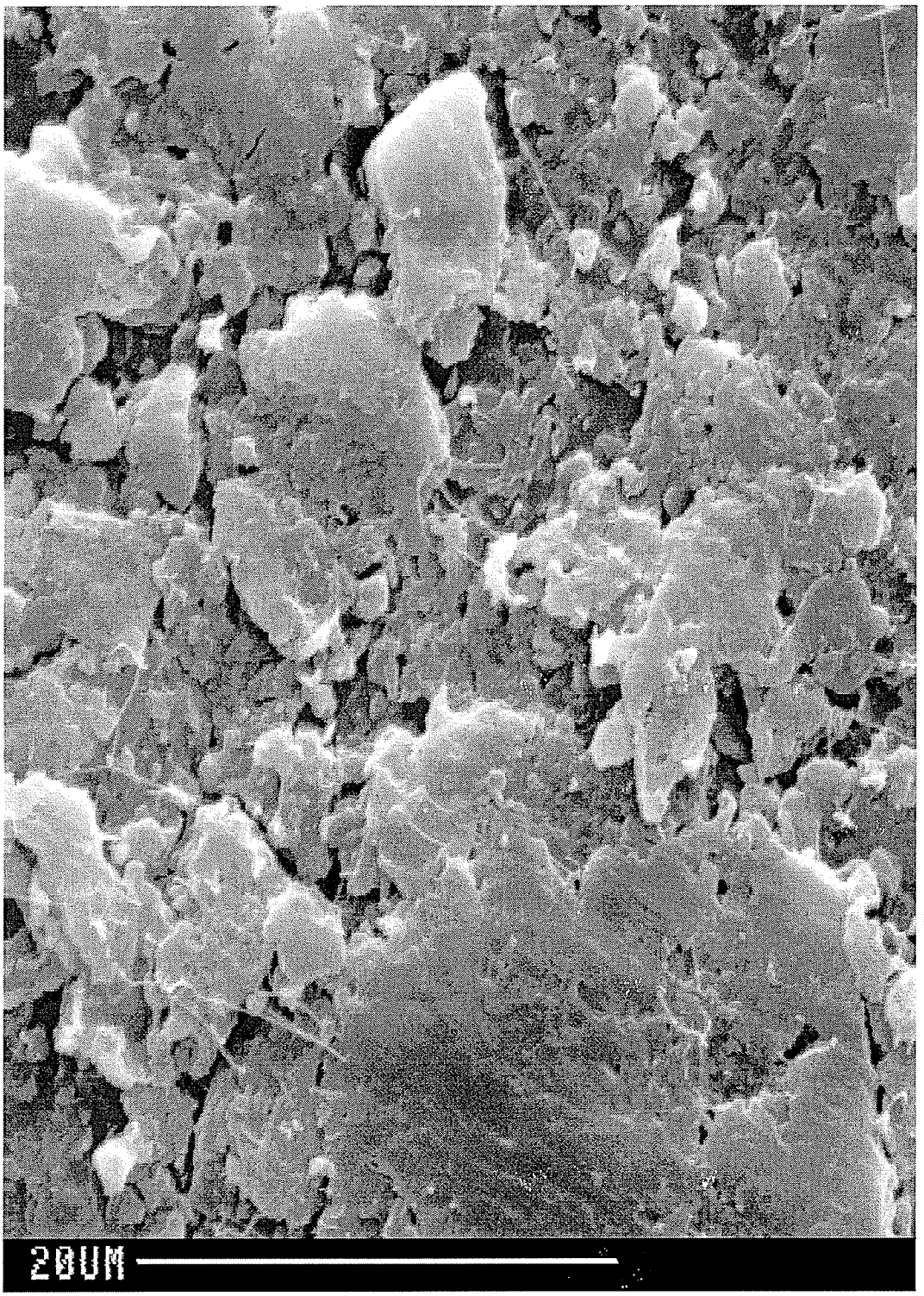


Fig. 4.9. SEM Photo: fibre after 15 soil slurry extractions (Miniota soil)-2000 X magnification.

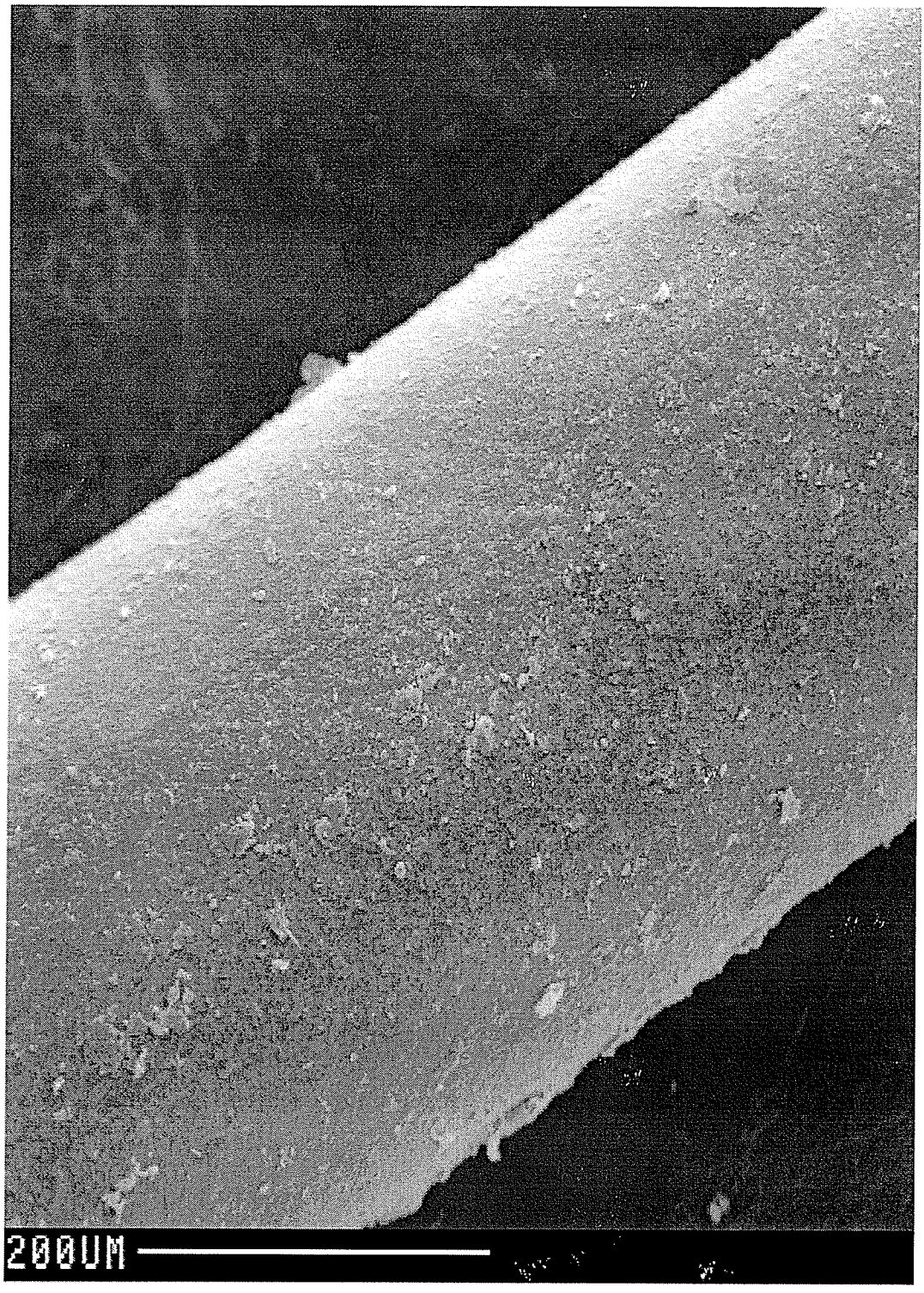


Fig. 4.10. SEM Photo: fibre after 15 soil slurry extractions (Fifi Road soil)-150 X magnification.

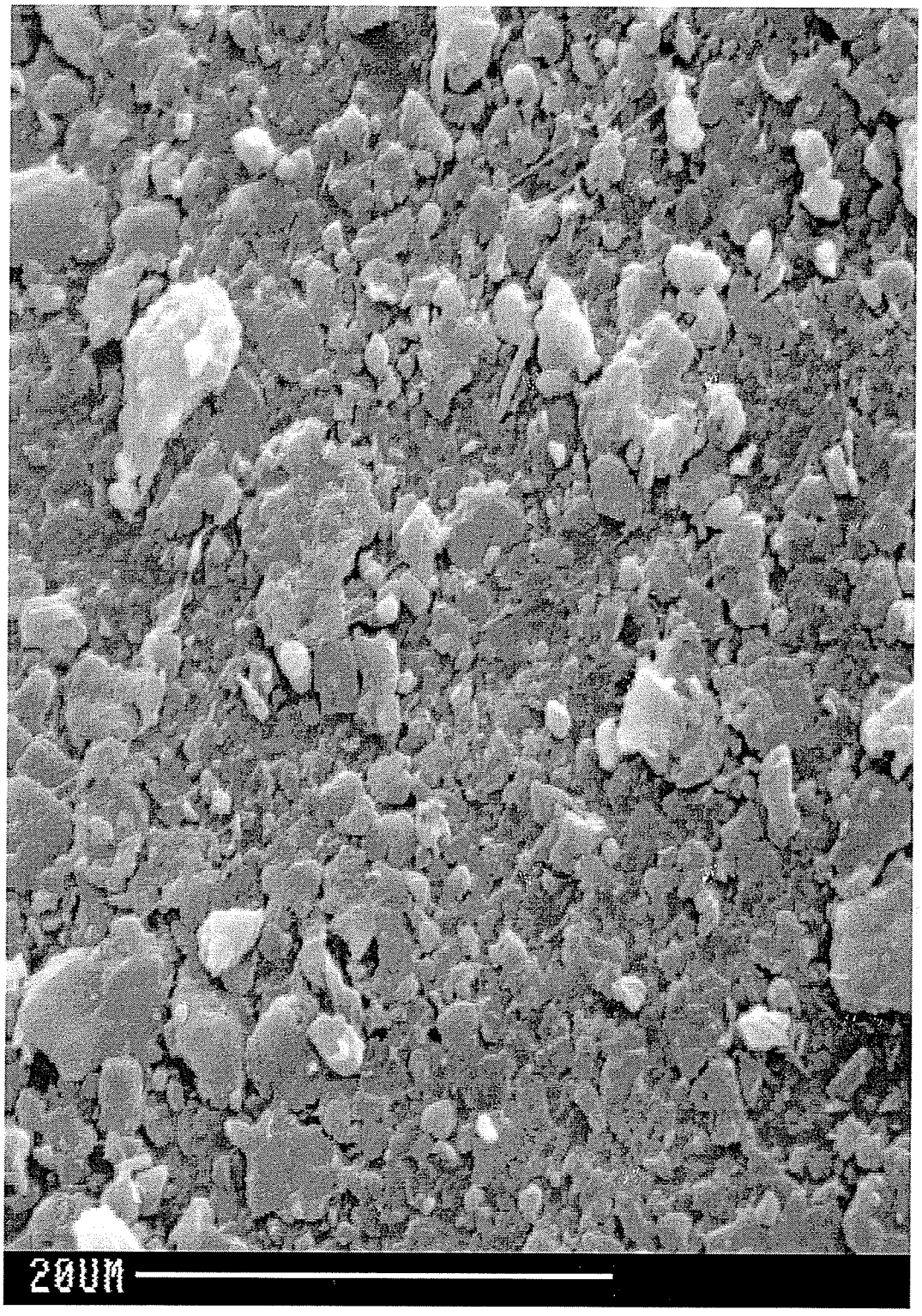


Fig. 4.11. SEM Photo: fibre after 15 soil slurry extractions (Fifi Road soil)-2000 X magnification.

Stability of the fibre extraction capacity

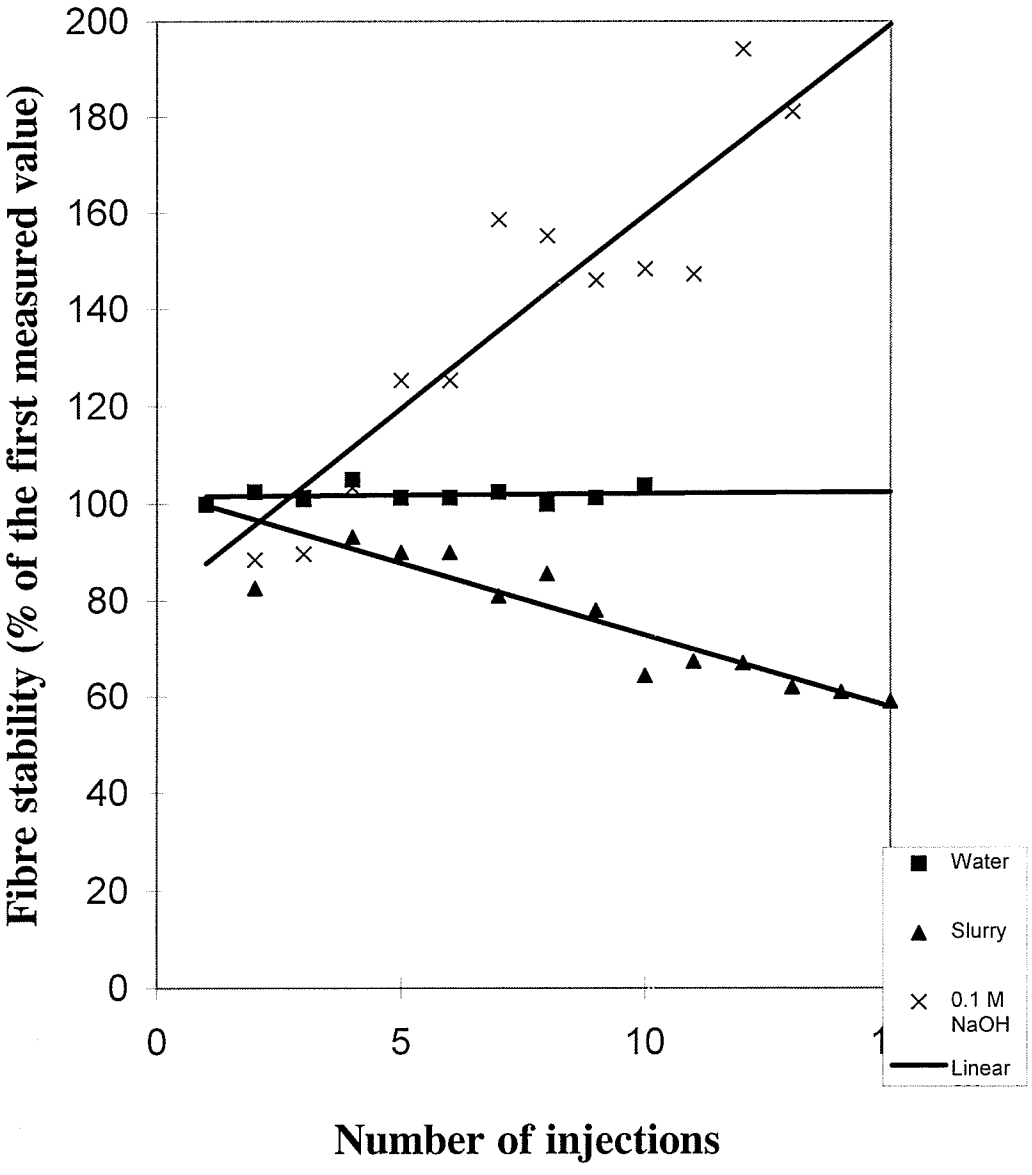


Fig. 4.12. Fibre stability: 0.01 M NaOH effect.

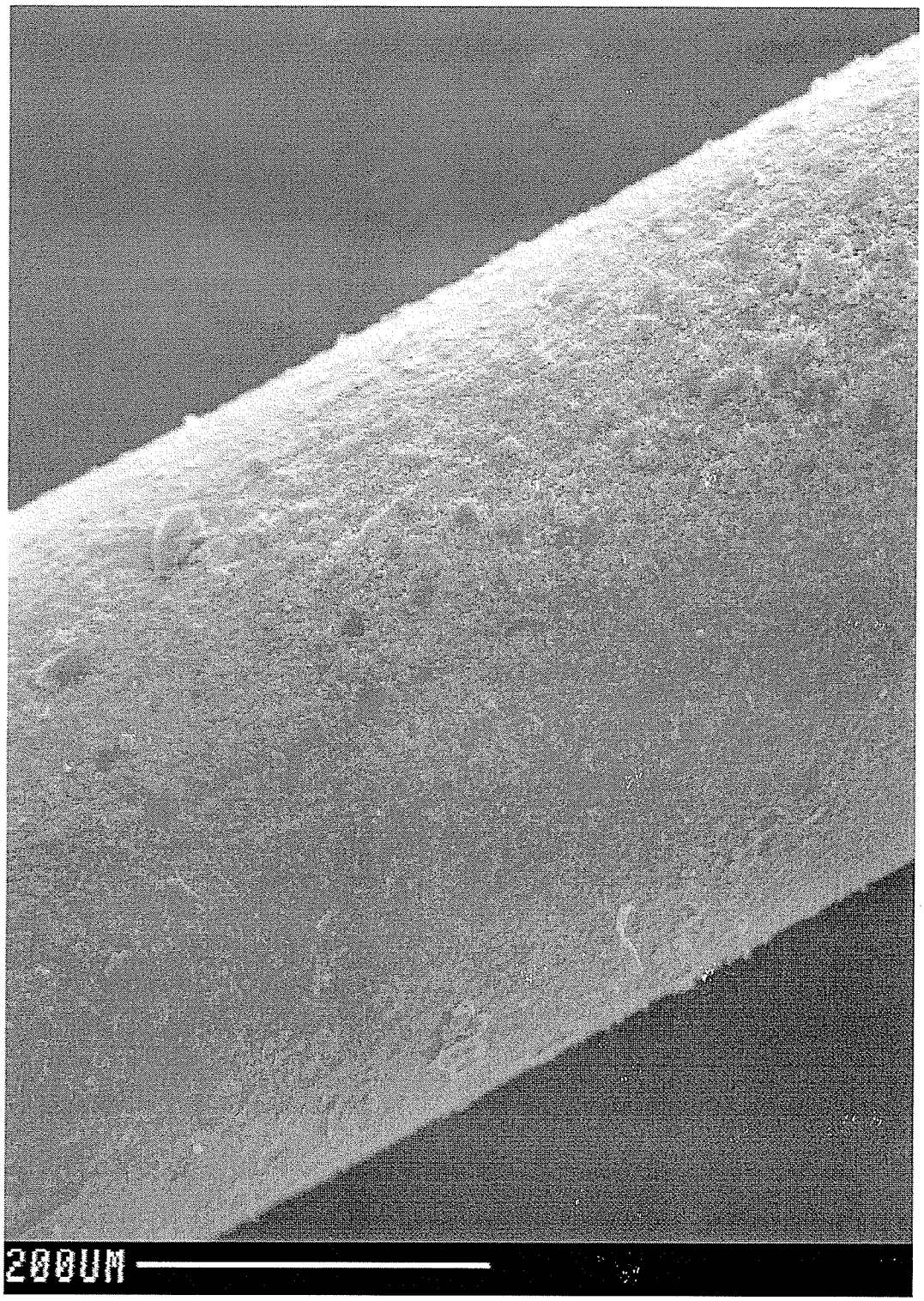


Fig. 4.13. SEM Photo: NaOH treatment effect-150 X magnification.

Fig. 4.14. SEM Photo: NaOH treatment effect-2000 X magnification.

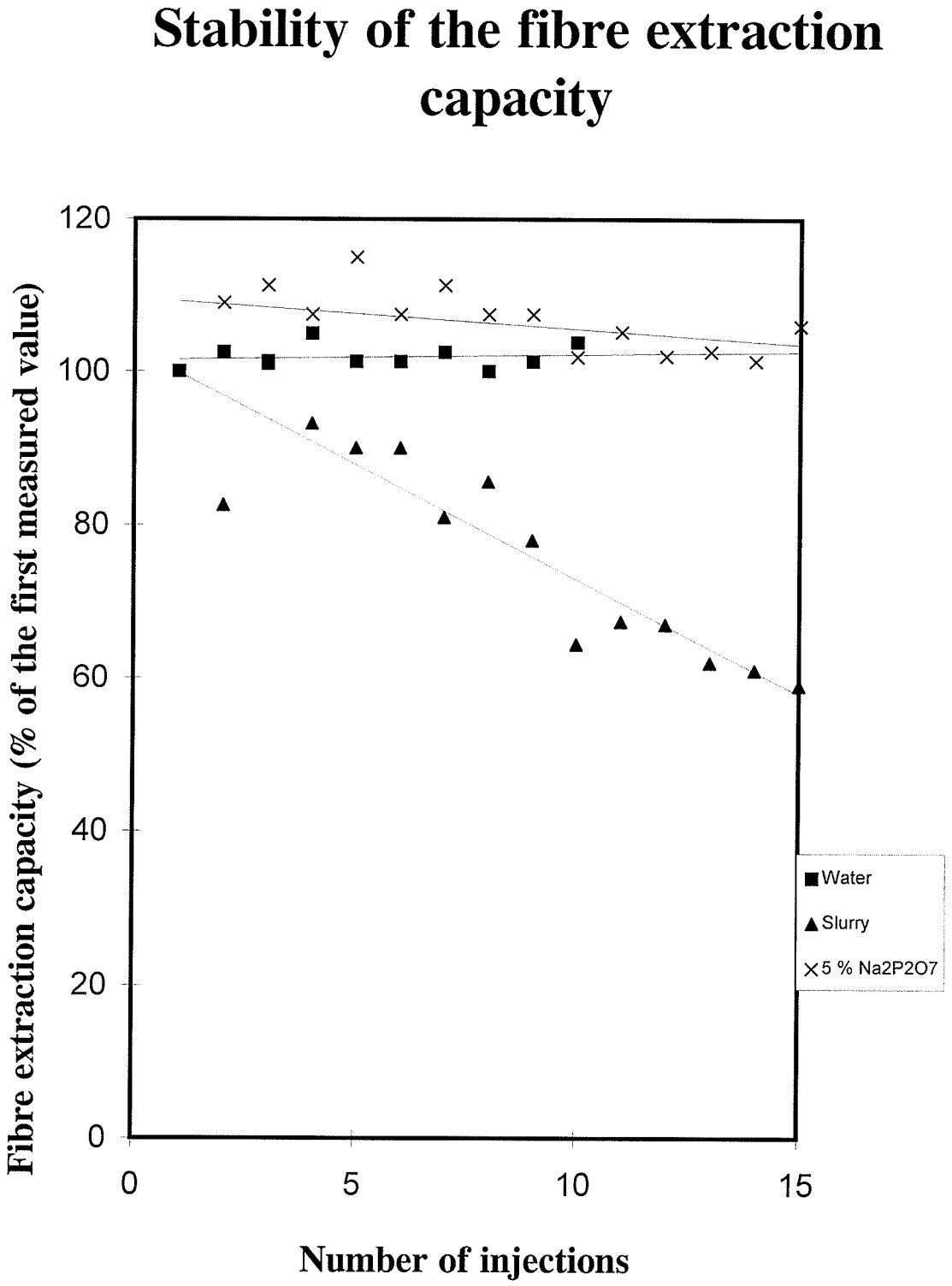


Fig. 4.15. Fibre stability: 5% $Na_2P_2O_7$ cleaning performance.

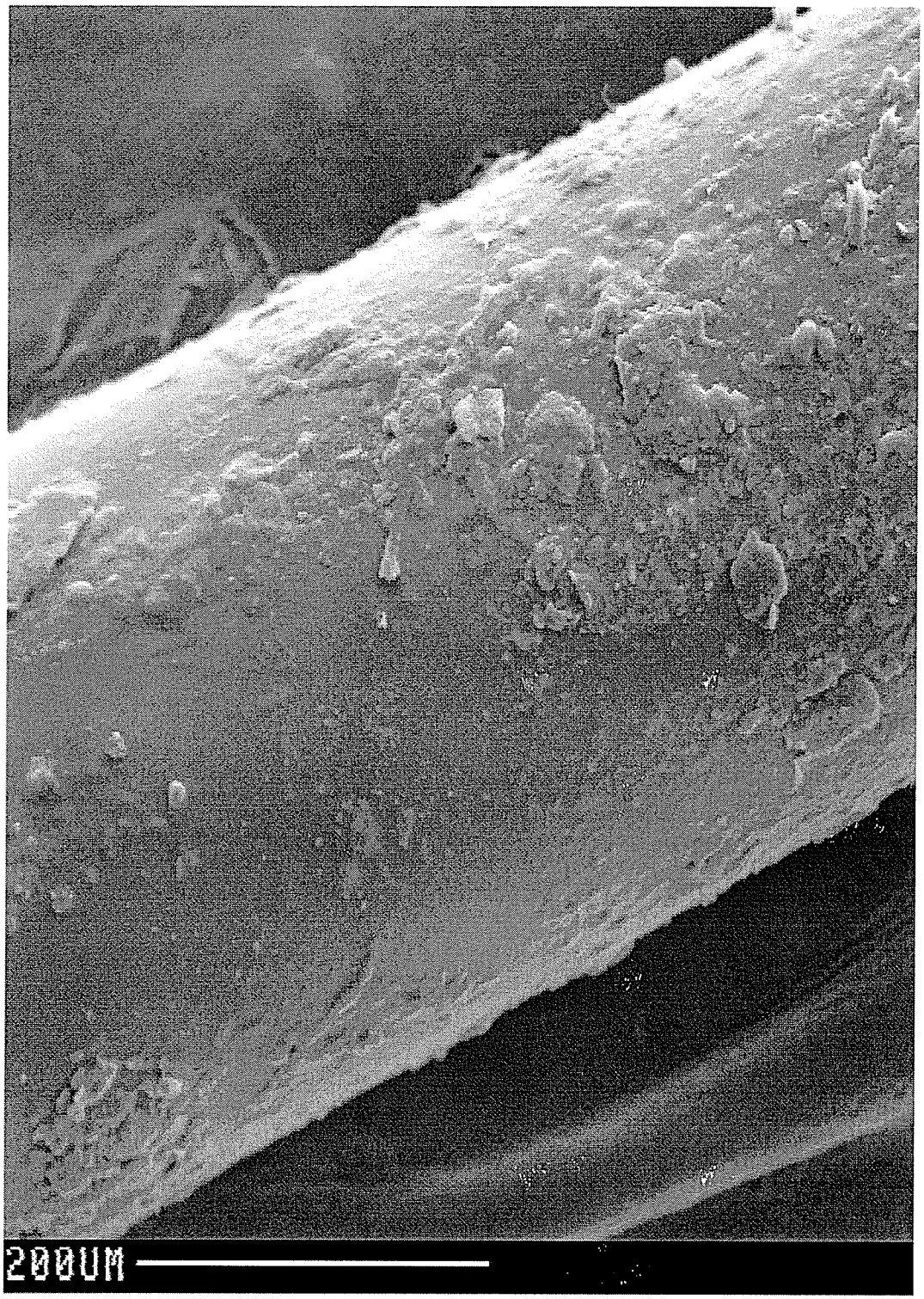


Fig. 4.16. SEM Photo: $Na_2P_2O_7$ treatment effect-150 X magnification.

Fig. 4.17. SEM Photo: $Na_2P_2O_7$ treatment effect-2000 X magnification.

5. Use of a new SPME-based method in the study of the soil sorption of the herbicide atrazine

5.1. Introduction

Transport and biological phenomena affecting pesticide residues in soils depend on interactions between the organic contaminant and soil organic matter. Pesticide residues in soils undergo speciation which influence their environmental fate. The speciation will be affected by the physico-chemical properties of both the contaminant and the humic substance fractions, and by selected environmental factors. The reported values for the concentrations of the pesticide residues in soils are highly dependent on the analytical method used for analysis, particularly on the extraction procedure. Notions such as "total residues", "dissolved pesticide", "reversibly bound pesticide", "irreversibly bound residues" are largely operationally defined terms. The accurate description of transport and biological phenomena affecting pesticides in soils, which is of paramount importance for environmental risk assessment and eventual remediation, depends on such analytical data. Analytical methods using a direct water phase extraction step appear to be the most useful approach to the study of pesticide speciation in soils, because data are easier to correlate with the bioavailability and the mobility of pesticide residues.

The herbicide atrazine is a moderately hydrophobic organic contaminant with moderate to high persistence in the environment. It is known to form a range of

intermediates (most of them non-phytotoxic or less phytotoxic than the parent compound) and associations with soil system components.

A new method for atrazine analysis by direct water-phase extraction from soil slurries using an SPME (solid phase microextraction) device is presented in this study. The method is based on the direct partitioning of the analyte (atrazine) between the water phase of the soil slurry and the organic coating of the fibre. In parallel other equilibria are going on in the soil suspension between the atrazine free in solution, the atrazine sorbed to the soil organic matter (SOM), and the atrazine sorbed to the dissolved organic matter (DOM) sometimes termed the water soluble soil organic matter (WSSOM). SPME had been previously used as an extraction method for a variety of analytes in different aqueous phases (Belardi and Pawliszyn, 1989; Arthur et al., 1992; Geelen, 1993), but was generally believed to be impossible to use in such a "dirty" environment as a soil slurry. Analysis of soil samples has been reported, but such methods use either the "head-space" technique (Zhang and Pawliszyn, 1993), based on sampling of the air phase above the soil sample, or a preliminary solution separation by centrifugation (Bengtsson and Berglof, 1995).

Headspace SPME involves mass transfer processes between three phases: the coating material, the sample headspace and the sample matrix, according to the affinity of the analyte for each of the phases. The difference between the chemical potentials of the analyte in the three phases is the driving force of the headspace extraction procedure. There are two partitioning processes involved: between the matrix and the headspace and between the headspace and the coating. For aqueous samples, the headspace/water

partition coefficient (K_{hs}) is limited by the air-water partition coefficient (Henry's law constant) value of the contaminant and the water content of the sample matrix (soil). For moderately hydrophobic organic contaminants with a limited volatility, such as atrazine (water solubility = 30 mg/L at 25 ^{0}; vapour pressure = 2.78 x 10 $^{-7}$ mm Hg at 20 ^{0}; Henry's law constant approx. 2.63 x 10^{-9} atm m^3/mol), the analyte will partition in small proportion into the head-space of the sample, leading to a poor sensitivity of the head-space SPME based analytical methods.

Bengtsson and Berglof (1995) have proposed a method using a separation of the soil solution by centrifugation prior to the SPME procedure. From a practical point of view this method is more difficult to perform, requires more expensive instrumentation and does not take full advantage of the possibilities of SPME to simplify analytical work. From a theoretical point of view, the centrifugation step interferes with equilibrium processes in soil solution, introducing experimental artifacts into an already complicated system. Depending on centrifugation conditions a different proportion of the dissolved organic matter fraction (DOM), the so called "unsettling phase" or "third phase" will be added to the soil organic matter fraction (SOM) or discarded with the supernatant.

The method for atrazine analysis presented here is a direct water-phase extraction from soil slurries using an SPME device. The new method does not use any organic solvent and has no separation steps (filtration, centrifugation, or chromatographic clean-up). It is thus less likely to perturb the already complicated soil-water system and appears to be the simplest approach to study equilibrium processes in soil-water systems.The experimental results obtained on two soils of different origins (a Manitoba soil and a

tropical soil of volcanic origin) show no interference of coextracted compounds from the soil with the quantification of atrazine (using an EC detector) (see chromatograms in Chapters 3 and 4).

A chemical-ultrasonic procedure was successfully used to maintain the extraction capacity of the fibre coating consistent during sampling (described in Chapter 4). Experimental results show that the performance of the fibre can be maintained relatively constant for at least 10-15 subsequent samplings from soil slurry, making this method reliable and cost effective for studies of pesticide sorption on soils, studies which require numerous analyses of the pesticide concentration in the water phase.

Only if the measured species is well defined can the results of the analytical procedure be used for predicting the fate of the contaminant, its mobility, and its toxicological and ecotoxicological relevance. In an attempt to define the atrazine fraction measured by the new SPME method and to evaluate the performance of the new method, a comparison between the sorption isotherms of atrazine on soils acquired by SPME-GC to the sorption isotherms of atrazine on the same soils, measured by an established technique, microfiltration-HPLC, (Gamble and Khan, 1990) was conducted.

A correlation between the sorption data measured by our new method and soil characteristics was pursued, in an attempt to make the data more relevant for the understanding of the mechanisms of interaction between the moderately hydrophobic organic contaminant and soils with a high content of humic substances.

5.2. Materials and methods

5.2.1. Chemicals and Instruments

Atrazine Pestanal® standard (98 %) (Riedel de Haen AG-D 3016 Seelze 1, Germany) was used to prepare the standard stock solutions.

HPLC-grade water (Acusolv-Anachemia, Rouses Point N Y 12979, USA) was used to prepare the standard atrazine solution, the soil slurry samples and the sodium pyrophosphate solution used as the cleaning agent for the fibre in the ultrasonic bath.

HPLC-grade methanol (Acusolv-Anachemia, Rouses Point N Y 12979, USA) was used to prepare the eluent for HPLC analysis.

Sodium pyrophosphate (reagent grade) from Fisher Scientific Company, New Jersey 07410 has been used to prepare the cleaning solution.

SPME 100 μm polydimethylsiloxane (PDMS) fibres (Supelco No. 5-7300) from Sigma-Aldrich Canada Ltd., 1300 Amico Blvd., Mississauga, Ontario, Canada, L4W 9Z9 for manual extraction were used throughout the experiment.

A Supelco No 57330 fibre-holder for manual extraction from Sigma-Aldrich Canada Ltd., 1300 Amico Blvd., Mississauga, Ontario, Canada, L4W 9Z9 was used to perform the extractions.

A Hewlett-Packard 5890 GC system equipped with an EC detector (Hewlett-Packard Canada Ltd.) was used to perform the gas-chromatographic analyses.

A 30 m J&W Scientific DB-5, 0.32 mm i.d.,1.0 μm column, (Chromatographic Specialties Inc., Brockville, Ontario, Canada) was used to separate the analyte from eventual soil coextractants.

An Ultrasonic Cleaner model No. BP-1 from Electromation Components Corp., Farmingdale, L.I., N Y, was used to perform the cleaning of the fibre surface in between extractions.

Disposable tuberculin, Luer tip, syringes and Cameo 3N syringe filters, Nylon, 3 mm, 0.45 μm where used for "off-line" soil slurry filtration before HPLC analysis.

An HPLC system consisting of a Waters 6000A pump from Millipore-Waters Canada, a Rph - BioSil-ODS 10 column from BioRad, and a Waters 440 UV fixed wavelength detector (λ = 254 nm) was used for soil slurry filtrate analysis.

5.2.2 Preparation of the atrazine standard solutions

Atrazine (98%) was dissolved in distilled water (HPLC grade) with stirring and occasional heating (50^0-60^0) for 48 h. Atrazine concentrations in the standard stock solutions used throughout the experiments were as follows:1.21 x 10^{-4} mol/L; 1.08 x 10^{-4} mol/L and 0.78 x 10^{-4} mol/L.

5.2.3. Preparation of the HPLC solvent

The eluting solvent for HPLC (35:65 water/methanol) was prepared by combining 350 mL of HPLC-grade water and 650 mL of HPLC-grade methanol for the HPLC analysis of atrazine from the soil slurry filtrate. The solution was stirred for 24 h, then allowed to rest for another 24 h period, to avoid gas bubbles in the mobile phase which can interfere with HPLC analysis.

5.2.4. Preparation of the sodium pyrophosphate cleaning solution

The sodium pyrophosphate cleaning solution was prepared by dissolving 25 g of sodium pyrophosphate (reagent grade) in 500 mL HPLC-grade water with slight heating.

5.2.5. Soil slurry samples

Soil samples (0.5 g, previously sieved) were weighed into specially constructed 30 mL round bottom vials equipped with septum screw caps, and to each of the vials, 25 mL distilled water (HPLC grade) and atrazine standard solution (respectively 2 mL, 3 mL, 5 mL, 10 mL, 15 mL, in HPLC grade water) were added. The soil slurry was stirred for two weeks before the measurement were made and kept stirred, at constant temperature

(20 °) during the course of the experiment. Soil concentration for all soil slurry samples used was 20 g/L.

5.2.6. Soil characteristics

One Manitoba soil (Miniota sand) and one tropical soil (Fifi Road soil) were used for sorption experiments.

Some relevant characteristics of the soils were determined: particle size distribution, organic carbon content , pH, and CEC.

Table 4. Soils characteristics.

Soil Property	Organic matter	Sand	Silt	Clay	pH	CEC (mequiv./ 100g)
Miniota soil	2%	84%	7%	9%	6.21	9.32
Fifi Road soil	3.65%	80.5%	7.3%	12.2%	4.52	21.37

Soil analysis was performed in the Manitoba Soil Survey laboratories, according to the internal manual for soil analysis methods (Haluschak ed., 1986).

5.2.7. SPME-GC Analysis

SPME extraction was performed using a Supelco No. 5-7300 manual 100 mm polydimethylsiloxane solid-phase microextraction fibre assembly. The extraction was performed by piercing the septum of the 25 mL vials containing the soil slurry samples with the septum-piercing needle and extending the fibre directly into the magnetically stirred soil solution. The SPME assembly was clamped in place above and resting on the vial cap. After 30 min, the fibre was retracted into the septum-piercing needle and the needle was withdrawn from the septum.

The syringe needle was used to pierce the septum of the GC injection port. The fibre was extended into the splitless injector. The desorption time used was 10 min. The purge remained off for the whole desorption time. The desorption temperature used was 220^0.

A Hewlett-Packard 5890 GC system equipped with an EC detector and operating in the splitless mode was used. A 30 m J&W Scientific DB-5, 0.32 mm i.d., 1.0 mm column, (Chromatographic Specialties Inc., Brockville, Ontario, Canada) was used. The chromatographic conditions were as follows: injector temperature, 220^0; column, 100^0 for 10 min., 10^0/min. to 250^0, hold 5 min; flow-rates: helium carrier, 1.0 mL/min; argon-methane 5% make-up gas, 60 mL/min.

Soil slurry samples were spiked with atrazine in quantities corresponding to total concentrations ranging between 0.67 ppm and 26.2 ppm were analyzed by the above procedure and concentrations calculated based on a standard curve constructed using

dilutions of the standard atrazine solution in HPLC grade water. A calibration curve was constructed for each fibre, to account for any possible manufacturing differences. The sorbed atrazine concentrations were determined by mass balance with the determined concentrations of free atrazine in solution to build sorption isotherms for atrazine on soil particles.

5.2.8. Chemical-ultrasonic cleaning of the fibre

The cleaning of the fibre was performed by immersing the fibre for 25 minutes in the cleaning solution (5 % sodium pyrophosphate in HPLC-grade water), using an ultrasonic laboratory cleaning instrument. After each cleaning the fibre was desorbed in the injection port of the gas chromatograph for 10 minutes at the desorption temperature of 220 ^{0}, during a blank run.

5.2.9. Microfiltration-HPLC analysis

Immediately after an SPME injection, a small aliquot ($\sim$ 100 μL) of the soil suspension was filtered and injected into the HPLC system.

Filtration was performed manually, using disposable tuberculin, Luer tip, syringes and Cameo 3N syringe filters, Nylon, 3 mm, 0.45 μm.

Analyses were performed on a HPLC system consisting of a Waters 6000A pump from Millipore-Waters Corp, a Rph - BioSil-ODS 10 column from BioRad and a Waters

440 UV fixed wavelength detector (λ = 254 nm). The eluent used was water-methanol (35:65) at flow rate of 1 mL/min, under a pressure of 1200 psi.

The analyses were considered to be essentially simultaneous. The atrazine concentrations determined by HPLC analysis were calculated using a multiple point calibration curve based on the measurement of aqueous standard solutions.

5.3. Results and Discussion

5.3.1. Challenges in the direct SPME of atrazine from a soil-water system

A theoretical problem related to the use of the SPME method in the study of pesticide sorption-desorption processes on soils is related to the degree in which the sorption of the analyte to the fibre coating affects the soil solution equilibrium. An estimation of this influence can be made, assuming the extraction process is an equilibrium process and the partition coefficient of atrazine between water and the organic coating of the fibre is equal to the octanol-water partition coefficient, using the equation which defines the number of moles of analyte extracted by the fibre in a SPME procedure (Zhang et al., 1994):

$$n = K V c_o$$

if $K = K_{ow}$ atrazine = 562

V = the volume of the coating = 6.34×10^{-7} L, for the 100 μm fibre

$c_o = 4.8 \times 10^{-6}$ mol/L

then the calculated concentration after one sampling will be:

$c_o' = 4.732 \times 10^{-6}$ mol/L,

reflecting a decrease in concentration of 0.17 %, which is below the sensitivity limit of the method.

In view of these theoretical estimations the sorption of the analyte to the fibre coating was not expected to observably deplete in atrazine the soil solution even after several extractions from the most diluted soil slurry. Repeated sampling with the SPME device was not expected to have any significant impact on the equilibrium between the dissolved atrazine and the atrazine sorbed on soil components.

During the experiments, no decrease in concentration was measured even after sampling 10 times the same standard water solution in the range of concentrations 10^{-4} - 10^{-6} mol/L. These experimental results, and associated theoretical considerations support the idea that SPME does not deplete observably the soil solution in the range of concentrations considered and, consequently, does not disturb the equilibrium of the atrazine-water-soil system. This is due to the limited hydrophobicity of atrazine, described by a relatively low octanol-water partition coefficient. For highly hydrophobic analytes (PCBs; PAH) repeated samplings have been found to deplete the solution. In the case of atrazine it is a good argument in favour of using the PDMS fibre for soil sorption studies:

the use of a more extraction-efficient coating may improve the sensitivity of the analytical method, but may also disturb the equilibrium of pesticide-water-soil system under study.

5.3.2. Comparison between the new SPME-GC method and an established microfiltration-HPLC method for dissolved atrazine in soil-water systems

The necessity to compare the new SPME analysis method with an already established method arises from both a theoretical and a practical point of view.

Theoretical problems of sampling from soil slurries are related to the properties of the water-soil-pesticide system. In addition to the competition for the pesticide between the coating of the fibre and the aqueous phase, other equilibrium processes are going on between the pesticide dissolved in water and soil organic matter: a fraction of the pesticide is free in solution, another fraction is bound by the solid phase and a third one is bound to the dissolved organic matter ("the third phase") (Fig. 5.1). One of the most important aspects in developing a new method of analysis for such systems is related to defining which pesticide fraction is measured by that specific method. Defining the pesticide species measured by different analytical procedures is a very delicate matter because the boundary between solution and solid phase are only operationally defined, the 0.45 μm boundary has no fundamental physical meaning.

Other extraction methods have been used to define the "water extractable", dissolved atrazine fractions, but the one that seems to have the most solid theoretical

support is the separation of dissolved atrazine and atrazine bound to DOM by reverse-phase chromatography. The "off-line" microfiltration-HPLC method thus measures only the truly dissolved atrazine in solution (Gamble and Khan, 1990). According to the principle of reverse phase chromatography separation, the moderately hydrophobic atrazine is expected to be retained on the C_{18} column by hydrophobic interaction and consequently detected by the detector of the HPLC system, whereas the polar complexes formed by atrazine with fulvic acids will not be retained by the chromatographic column and will not be detected. The detector will detect and quantify only the atrazine retained by the stationary phase (the "truly dissolved atrazine").

By "off-line" microfiltration-HPLC only the truly dissolved atrazine in solution is measured. The SPME-GC method is also expected to measure only the dissolved atrazine because it does not measurably influence the equilibrium between dissolved and sorbed atrazine in the soil slurry. The comparison between the sorption isotherms of atrazine on soils acquired by SPME-GC to the sorption isotherms of atrazine on the same soils, measured by microfiltration-HPLC can confirm this assumption.

From a practical point of view, the comparison with an already established method enables us to evaluate the performance of the new SPME-GC analytical procedure.

The SPME-GC method for atrazine analysis in soil slurries is linear over the range of concentrations studied (10^{-6}-10^{-4} mol/L) (Fig. 5.2). A remarkable observation is that no relevant interference of coextracted soil components was observed in any of the soil slurry samples used, even at the lowest atrazine concentration (using an EC detector) (see Figs. 3.3 and 4.1). Very sharp peaks were obtained for all samples. The experiments

reported in this paper did not approach the LOD of the method. As it was previously reported (Geelen, 1993) for the sampling of atrazine from pure water for the extraction time used (30 min) the partitioning of the analyte (atrazine) between the organic phase (the fibre coating) and water does not reach equilibrium, but is very near equilibrium. Little variation has been found with small variations in the extraction time (no measurable variation has been found for a 30 min $\pm$ 5 min time).

Atrazine concentrations determined by the SPME-GC-EC method have been correlated with atrazine concentrations of the same soil slurry samples determined by the "off-line" microfiltration-HPLC method. The calibration curves for HPLC analysis, using standard solutions of atrazine in HPLC-grade water, in the range of concentrations measured were found to be linear (Fig. 5.3). The correlations have been made for both soils investigated (the Miniota sand and the Fifi Road soil) (Figs. 5.5 and 5.7). For the Miniota soil three series of experiments were conducted, in an attempt to obtain more detailed sorption data (instrument limitations allowed only 5 soil slurries to be maintained under constant temperature conditions and stirring in the same time) (Figs. 5.6 and 5.7). The data obtained by the SPME-GC method correlate very well with the data from the microfiltration-HPLC measurements for both soils under investigation (Figs. 5.4 and 5.5). The atrazine species measured by SPME-GC and microfiltration-HPLC appears therefore to be the same: the truly dissolved atrazine in the soil solution. This result is important because it provides a theoretical basis for the use of data provided by the SPME-GC analysis method in the ecological risk assessments for atrazine residues in soils.

5.3.3. Correlation of atrazine sorption data with soil characteristics

The soils used in this study have a relatively high organic matter content and both have a low clay content. In this conditions, the organic component of soil is expected to play the major role in atrazine sorption (Pusino et al, 1994; Chester et al., 1989, Gennari et al., 1994).

The sorption curves for atrazine on the soils under study are presented in Fig. 5.8. It appears from the graphs that the soil with the highest sorption capacity is the Fifi Road soil (3.65 % OM) compared with the Miniota soil (2 % OM). The sorption capacity correlates with the organic matter content as it was expected There is also a marked difference in soil pH between the two soils: the Fifi Road soil has a pH of 4.52 (in water), compared with a pH value of 6.72 (in water) for the Miniota soil. Other authors (Wang, 1989; Li , 1993) reported a decrease in atrazine sorption capacity with an increase in the pH value of the soil. This pH effect is explained by the authors by changes in humic substances configuration as a function of carboxylic groups dissociation, changes affecting the number of available specific sorption sites for atrazine in the humic polymeric molecules. The inverse variation of the soil sorption capacity with soil pH value could account in part for the higher sorption on the almost neutral Miniota soil compared to the acidic tropical soil (Fifi Road soil) under study.

The marked difference between the Miniota soil and the Fifi Road soil may also be due to a difference in composition and characteristics of the soil organic matter. Many authors report differences in atrazine sorption capacities related to differences in the

structure and properties of different organic matter fractions (Wang, 1989; Li et al., 1992; Barriuso et al., 1992). Differences in starting materials and degree of alteration between two soils developed under very different climatic conditions are very likely to lead to relevant differences in the structure and properties of the soil organic matter and may in part explain the difference in atrazine sorption capacity for the two soils under study.

The experimental results in this study suggest that no simple correlation between the organic matter content of soils and their atrazine sorption capacity can be made. More experimental work is necessary to distinguish between the effects of pH, soil organic matter content and soil organic matter structure on atrazine soil sorption capacity. A more detailed characterization of organic matter composition and structure and its relation with atrazine sorption is needed to provide results that can be used for predicting the extent of atrazine sorption on a well characterized soil, to develop a model with predictive capacity.

5.3.4. Final considerations

The new SPME method, previously demonstrated to be useful for extracting a wide range of analytes from relatively clean aqueous solutions, was successfully tried in our laboratory for the direct extraction of atrazine residues from soil slurries. This adds a simple, clean, solventless extraction technique, easily compatible with capillary GC, to the arsenal of methods available for the analysis of less volatile compounds from soils and water bodies with a high content of humic and other particulate and dissolved materials.

The new method for atrazine residues analysis in soil slurries, based on direct SPME sampling and capillary GC analysis has important advantages related to the simplicity of the extraction method, the short analysis time and the zero solvent consumption and, consequently, the low cost per analysis. Because it is based on a direct water-phase extraction from the soil slurries using the SPME device this new method appears to be the simplest approach to study equilibrium processes in soil-water systems. The new method does not use any organic solvent and has no separation steps (filtration, centrifugation, chromatographic clean-up) and it is therefore less likely to perturb the already complicated pesticide-soil-water system. The results of the new SPME-GC method correlate very well with the results of the "off-line" microfiltration-HPLC method, suggesting that the new method measures the "truly" dissolved atrazine, which makes the method very valuable for ecotoxicological risk assessment studies.

5.4.Figures

Fig. 5.1. Atrazine speciation in soil slurry.

Fig. 5.2. Standard curve atrazine by SPME.

Fig. 5.3. Standard curve atrazine by HPLC.

Fig. 5.4. Sorption isothermes for atrazine on Fifi Road Soil by SPME-GC and microfiltration-HPLC.

Fig. 5.5. SPME-GC versus microfiltration-HPLC measurements for the Fifi Road soil.

Fig. 5.6. Sorption isothermes for atrazine on Miniota Soil by SPME-GC and microfiltration-HPLC.

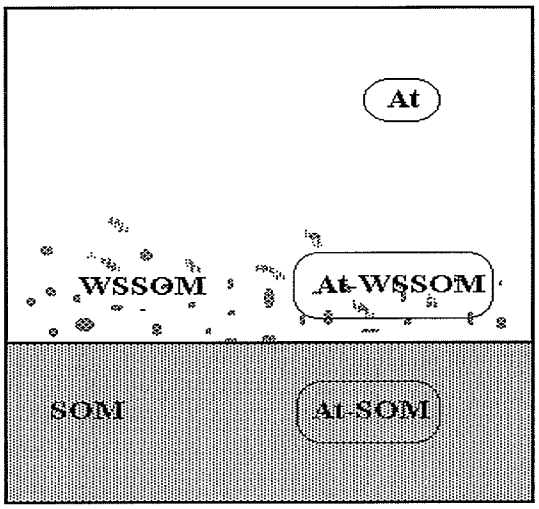
Fig. 5.7. SPME-GC versus microfiltration-HPLC measurements for the Miniota soil.

Fig. 5.8. Sorption isothermes for atrazine on two soils with different organic matter content.

Atrazine speciation: what are we measuring?

-dissolved atrazine

-dissolved atrazine + atrazine bound to WSSOM



$$At + WSSOM \rightleftharpoons At\text{-}WSSOM$$

$$At + SOM \rightleftharpoons At\text{-}SOM$$

Fig. 5.1. Atrazine speciation in soil slurry.

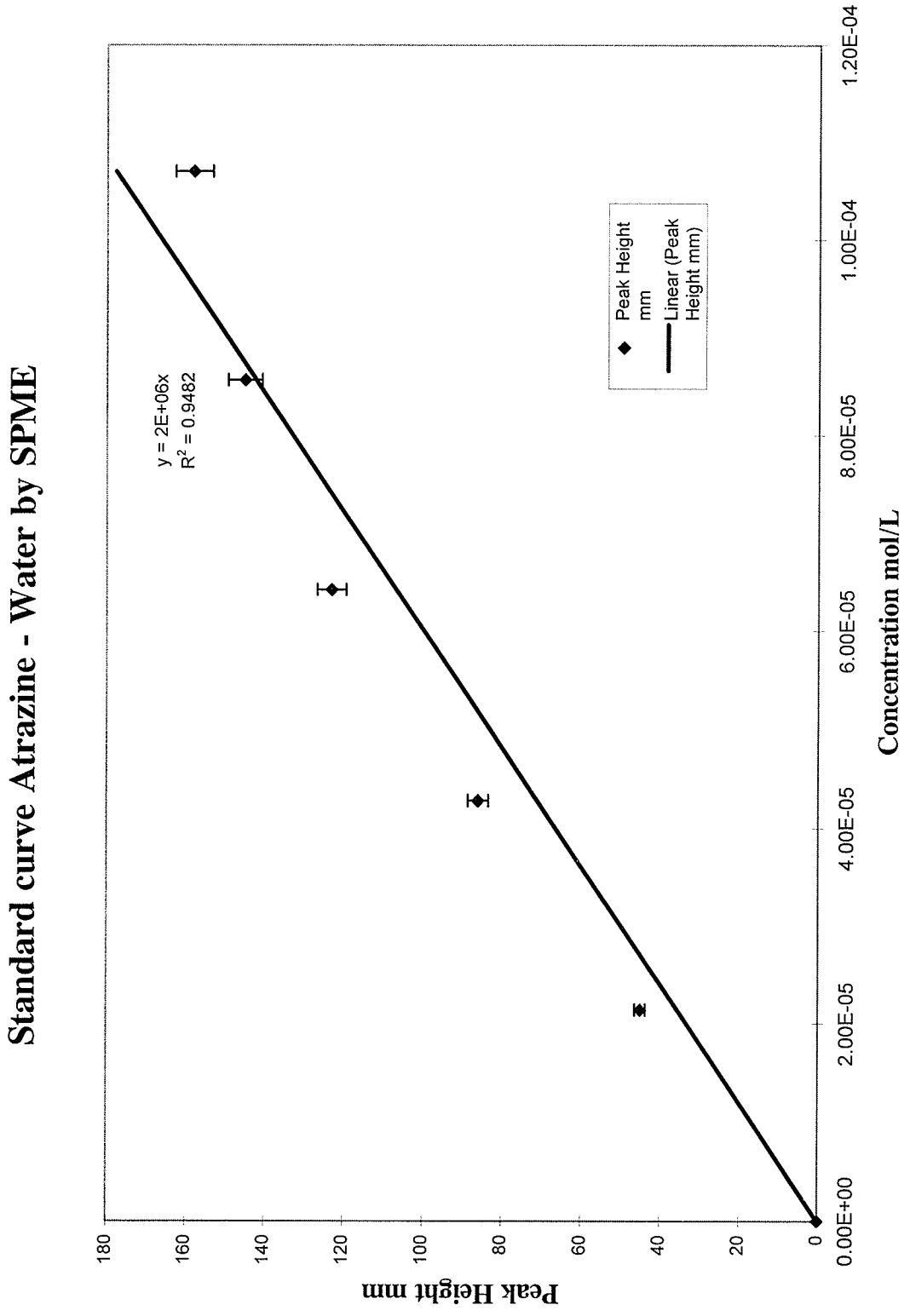


Fig. 5.2. Standard curve atrazine in water by SPME.

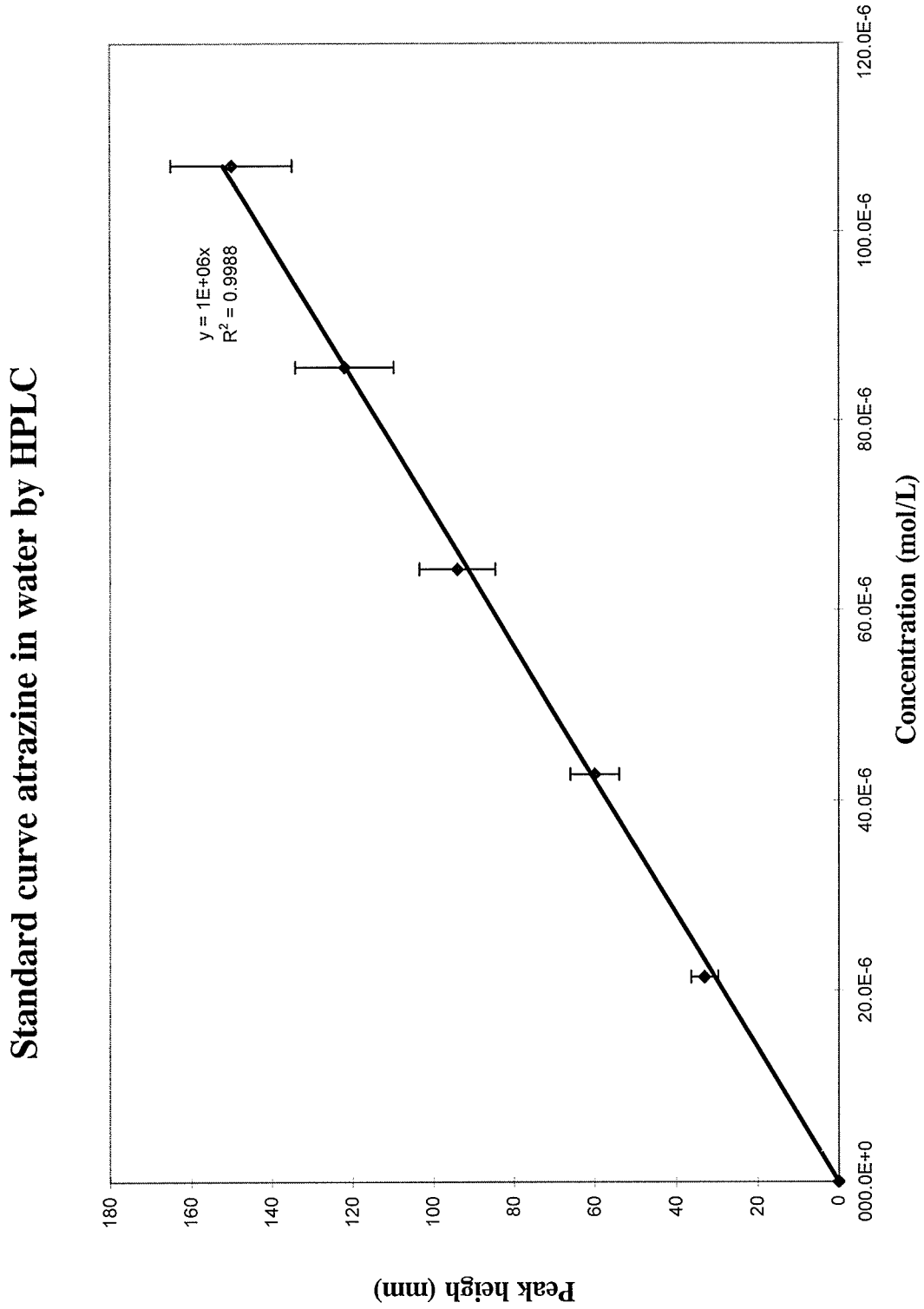


Fig. 5.3. Standard curve atrazine in water by HPLC.

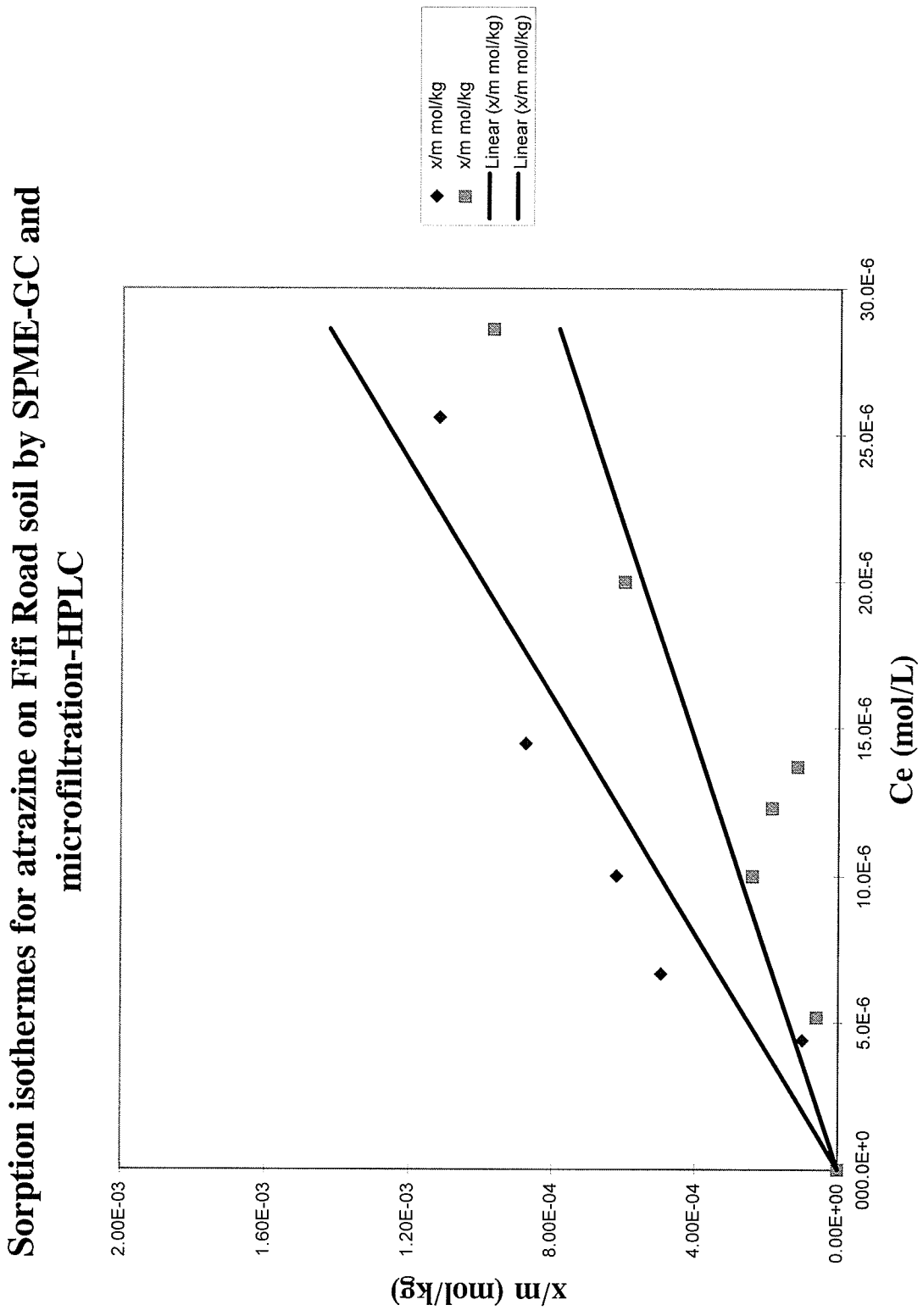


Fig. 5.4. Sorption isothermes for atrazine on Fifi Road soil.

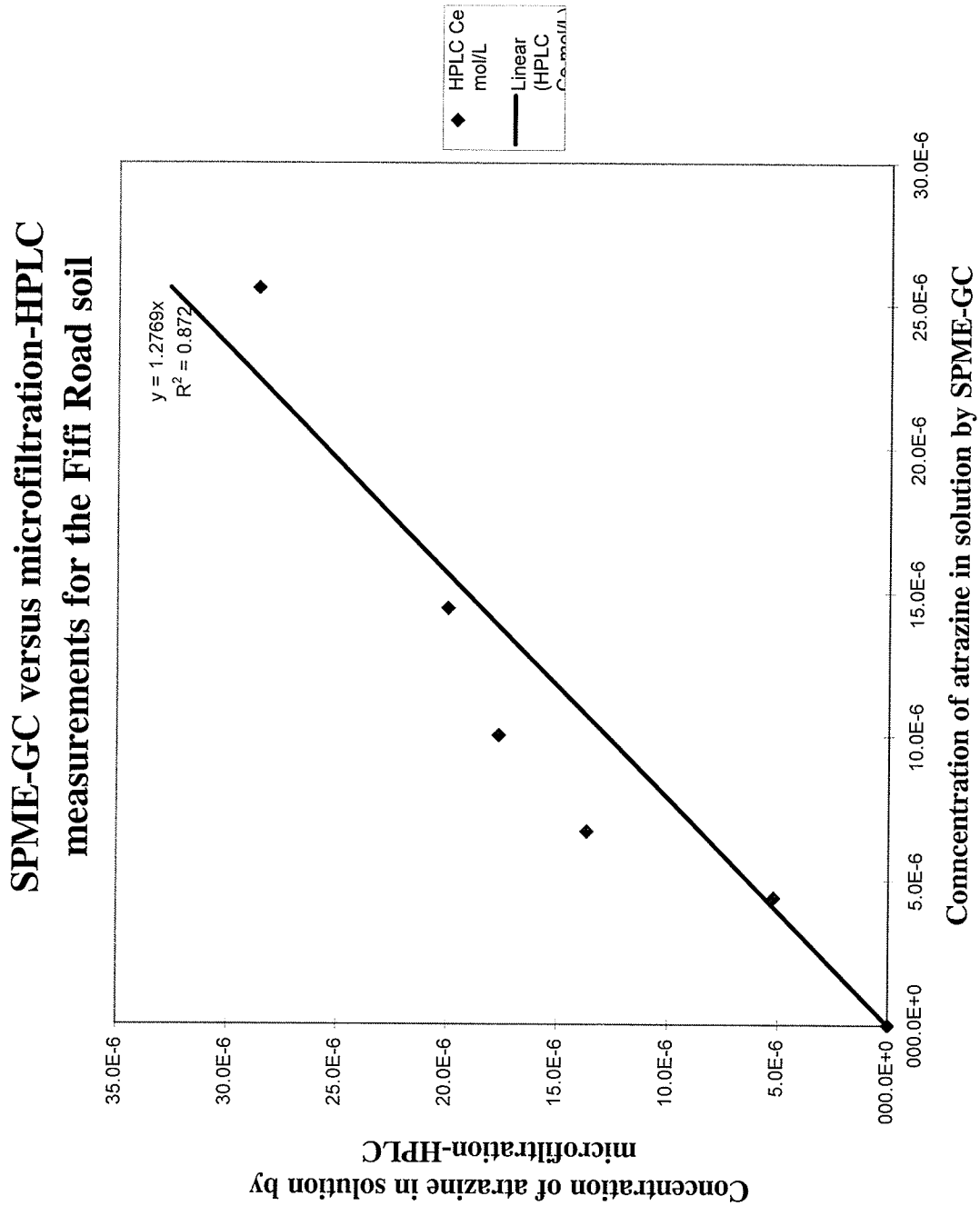


Fig. 5.5. SPME-GC versus microfiltration-HPLC mesurements for the Fifi Road soil.

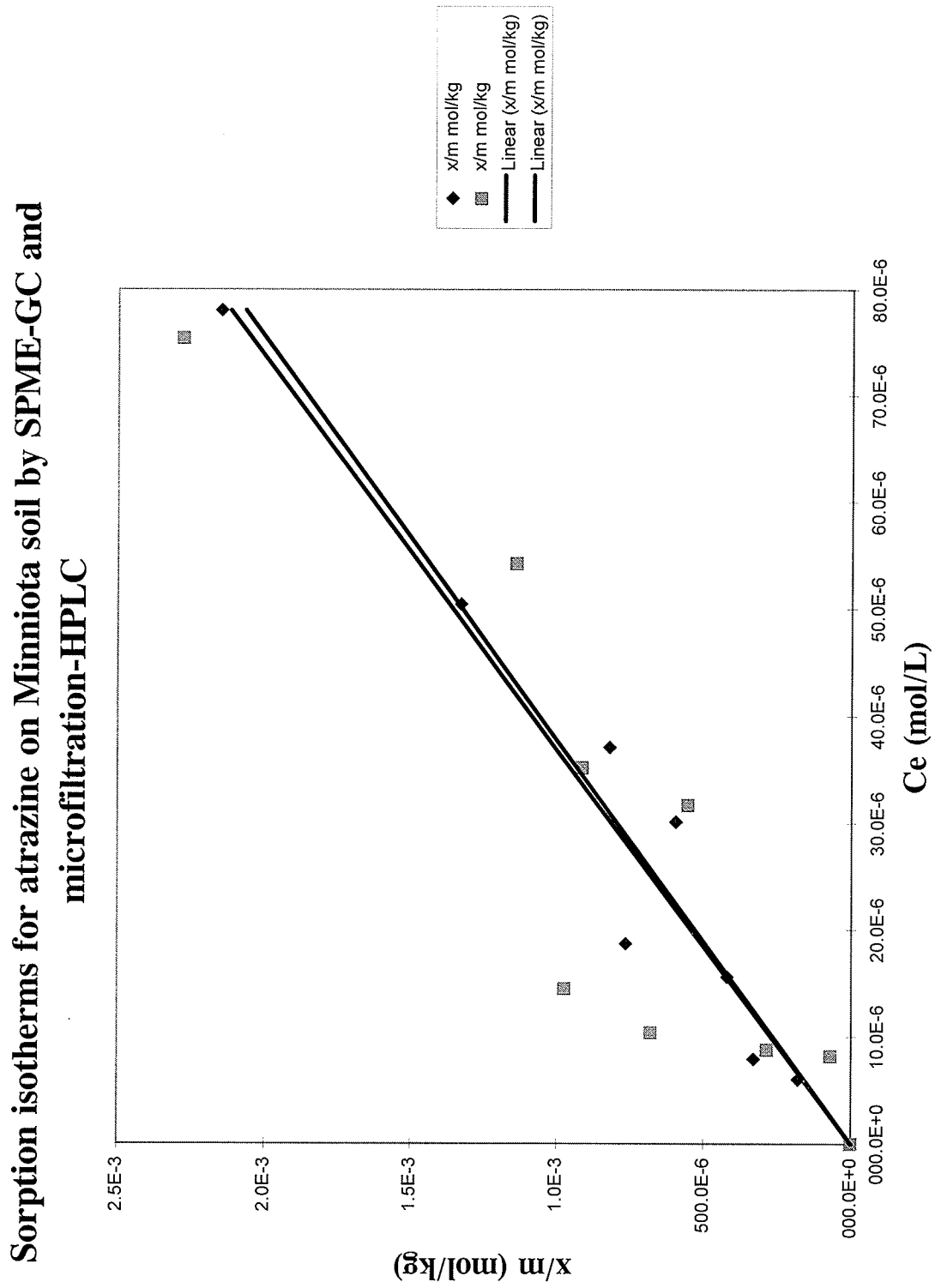


Fig. 5.6. Sorption isothermes for atrazine on Miniota Soil.

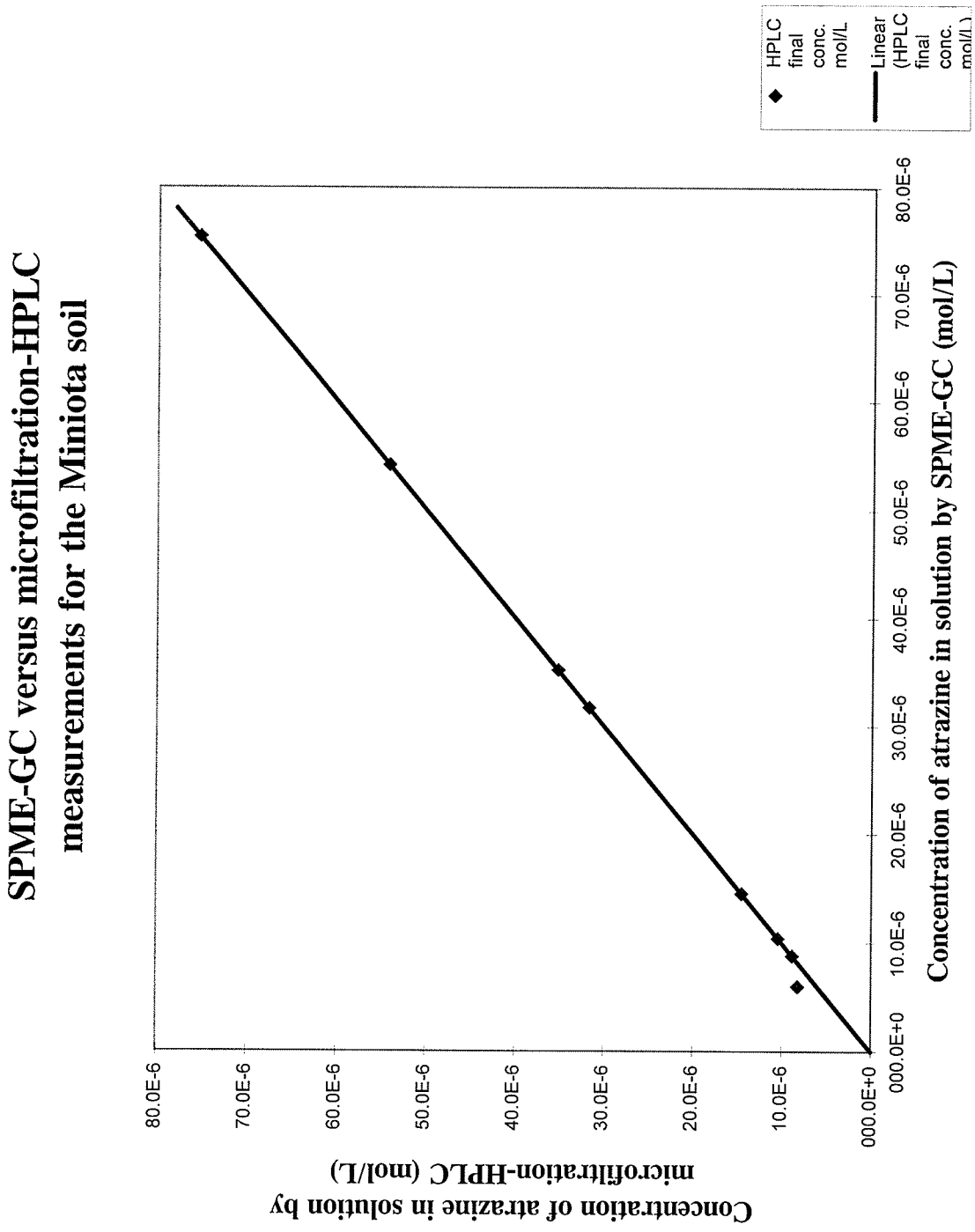


Fig. 5.7. SPME-GC versus microfiltration-HPLC mesurements for the Miniota Soil.

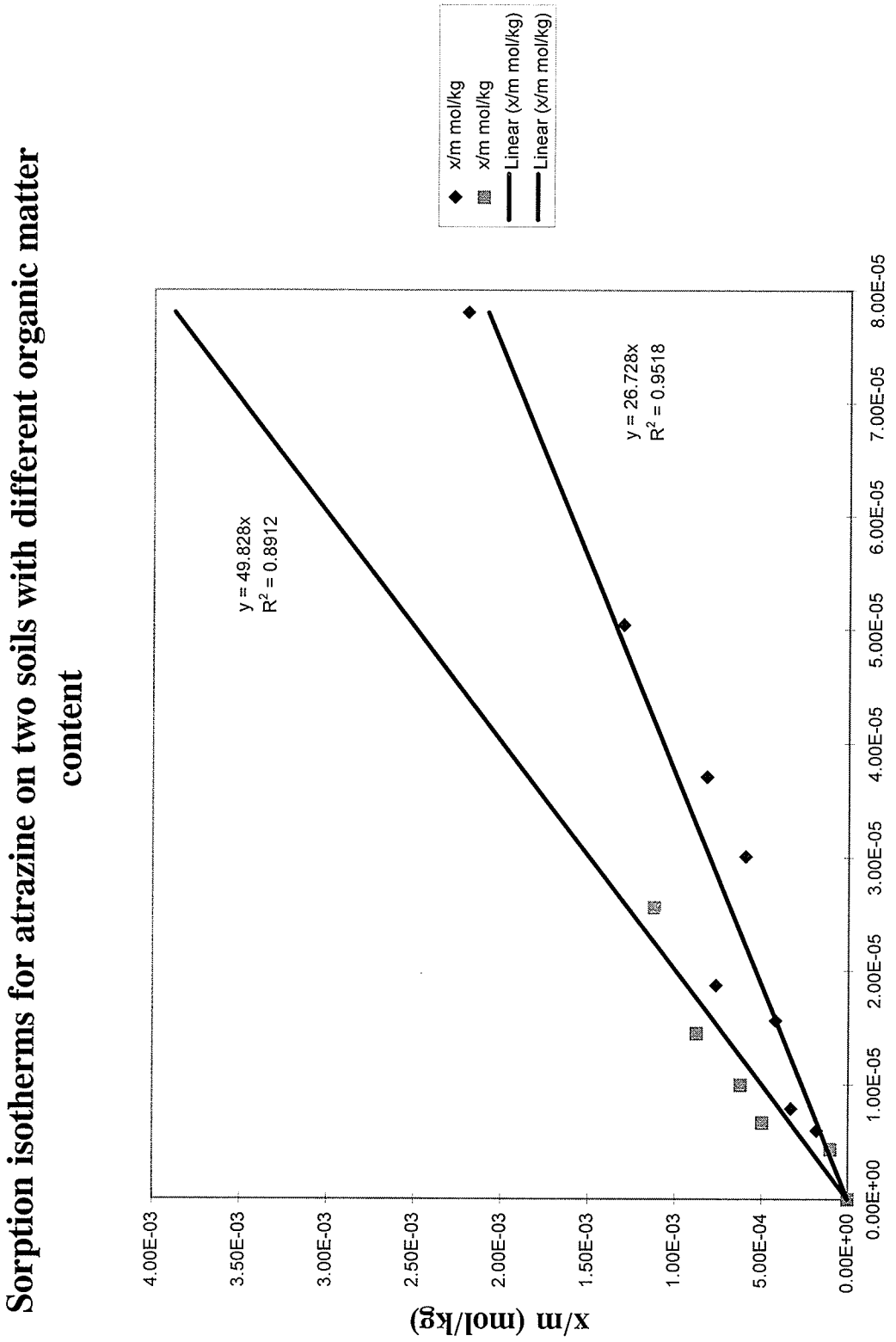


Fig. 5.8. Sorption isothermes for atrazine on two soils with different OM content.

6. Conclusions

The new SPME method, previously demonstrated to be useful for extracting a wide range of analytes from relatively clean aqueous solutions, was successfully used in this study for the direct extraction of atrazine residues from soil slurries. The development of a new, highly effective, technique for the cleaning of SPME fibres makes the method easy to perform, very reliable and highly cost effective. The method enabled more than 15 soil extractions without significant variation in the extraction capacity of the fibre.

Soil slurry samples spiked with atrazine in quantities corresponding to total concentrations ranging between 0.7 ppm and 26 ppm were analyzed by the procedure presented in the thesis after 2 weeks equilibration time. The experimental results showed no interference of coextracted compounds from the soil with atrazine analysis (using an EC detector). Very good resolution of the peaks was obtained for all samples. The method was linear over the range of concentrations studied (10^{-6}-10^{-4} mol/L).

Only if the measured species is well defined, can the results of the analytical procedure be adequately used for predicting the fate of the contaminant, its mobility and its toxicological and ecotoxicological relevance. By "off-line" microfiltration-HPLC only the truly dissolved atrazine in solution is measured (Gamble and Khan, 1990). The SPME-GC method is also expected to measure only the dissolved atrazine. The comparison between atrazine concentrations measured by SPME-GC and the same concentrations measured by microfiltration-HPLC has confirmed this assumption and has enabled evaluation of the performance of the new SPME-GC method. The data from the

measurements by the SPME-GC method correlate well with the data from the microfiltration-HPLC measurements suggesting that by both methods the freely dissolved atrazine alone is measured.

The new method, a direct water-phase extraction from soil slurries with high humic substances content using a solid phase microextraction (SPME) device, does not use any organic solvent and has no separation steps (filtration, centrifugation, chromatographic clean-up). It is thus less likely to perturb the already complicated pesticide-soil-water system and appears to be the simplest approach to study equilibrium processes in soil-water systems. In view of theoretical estimations and experimental results, sorption to the fibre coating is not expected to deplete observably the soil solution or to have any important impact on the equilibrium between the dissolved atrazine and the atrazine sorbed on soil components..

The interaction of soil particles in suspension with the coating of the fibre leads to a continuing reduction in the extraction ability of the fibre. The physical appearance of the fibre surface during a series of extraction procedures was monitored by direct observation under an optical microscope and by scanning electron microscopy (SEM). The variations in the extraction capacity of the fibre coating were due to variations of the surface of the fibre coating caused by soil particle deposition and, eventually, resuspension from the coating surface. No loss of the organic coating could be seen and the soil particles present appeared to be loosely bound to the surface. A mechanical-ultrasonic cleaning, using HPLC-grade water as cleaning agent and two chemical-ultrasonic cleaning procedures using 0.1 M sodium hydroxide solution and, respectively, 5 % sodium pyrophosphate

solution as cleaning agents were tried. The best results were obtained with the ultrasonic cleaning in the sodium pyrophosphate solution, as shown by extraction performance and confirmed by the SEM observations. Ultrasonic cleaning in a saturated $Na_2P_2O_7$ solution for 25 min enabled maintenance of a constant extraction capacity of the fibre for at least 15 extractions from soil slurries.

A correlation between the sorption data measured by our new method and soil characteristics has been pursued in an attempt to make the data more relevant for the understanding of the mechanisms of interaction between the moderately hydrophobic organic contaminant (atrazine) and soils with a high content of humic substances. Experiments have been conducted on two different soils: a sandy chernozemic soil from Manitoba and a soil of volcanic origin from the nation island of Dominica. Both soils had a high organic matter content. No simple correlation was found between the organic matter content of the soils and their atrazine sorption characteristics. Other factors, mainly soil pH and the structure of soil organic matter (the degree of humification) may play an important role in influencing the process of atrazine sorption on soil components. Further study is necessary to detailed the influence of these factors in determining atrazine sorption capacity of soils with a high organic matter content.

This study adds a simple, clean, solventless extraction technique, easily compatible with capillary GC, to the arsenal of methods available for the analysis of less volatile compounds from soils and water bodies with a high content of humic and other particulate and dissolved materials.

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