Sample preparation for pesticide analysis in water and sediments: A case study of the Okavango Delta, Botswana

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Lesego Cecilia Mmualefe

supervised by

Professor Nelson Torto

Dedication

To my parents,

For your unwavering support

Acknowledgements

Prof. Nelson Torto: Thank you for your guidance and patience through the years as my mentor. I will always remember your optimism amidst the most desperate situations. This work would not have been accomplished without you.

Dr. Philippa Huntsman-Mapila: The sampling trips to the Okavango Delta wouldn't have been smooth without you. I hope we can make one more trip one day!

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Ron Majors: Thank you for not only making my internship at Agilent Technologies possible, but also for exposing me to the applications of sample preparation in the industry.

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To all my family and friends: Thank you for being there!

And to God be the Glory!

Abstract

This thesis presents a first ever extensive analysis of pesticides in water and sediments from the Okavango Delta, Botswana, employing green sample preparation techniques that require small volumes of organic solvents hence generating negligible volumes of organic solvent waste.

Pesticides were extracted and pre-concentrated from water by solid phase extraction (SPE) and headspace solid phase microextraction (HS-SPME) while supercritical fluid extraction (SFE) and pressurized fluid extraction (PFE) were employed for sediments. Subsequent analysis was carried out on a gas chromatograph with electron capture detection and analytes were unequivocally confirmed by high resolution mass spectrometric detection.

Hexachlorobenzene (HCB), trans-chlordane, 4,4′-DDD and 4,4′-DDE were detected after optimized HS-SPME in several water samples from the lower Delta at concentrations ranging from 2.4 to 61.4 µg L^{-1} that are much higher than the 0.1 µg L^{-1} maximum limit of individual organochlorine pesticides in drinking water set by the European Community Directive. The same samples were cleaned with ISOLUTE C_{18} SPE sorbent with an optimal acetone/n-hexane (1:1 v/v) mixture for the elution of analytes. No pesticides were detected after SPE clean-up and pre-concentration. HCB, aldrin and 4, 4’-DDT were identified in sediments after SFE at concentration

ranges of 1.1 - 30.3, 0.5 – 15.2 and 1.4 – 55.4 µg/g, respectively. There was an increase of pesticides concentrations in the direction of water flow from the Panhandle (point of entry) to the lower delta. DDE, fatty acids and phthalates were detected after PFE with optimized extraction solvent and temperature.

The presence of DDT metabolites in the water and sediments from the Okavango Delta confirm historical exposure to the pesticide. However their cumulative concentration increase in the water-flow direction calls for further investigation of point sources for the long-term preservation of the Delta. The green sample preparation techniques and low toxicity solvents employed in this thesis are thus recommended for routine environmental monitoring exercises.

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List of Abbreviations

µg	microgram
µS	microsiemens
AquaRAP	Aquatic Rapid Assessment Programme
DDD	dichlorodiphenyldichloroethane
DDE	dichlorodiphenylethylene
DDT	dichlorodiphenyltrichloroethane
DI	direct immersion
DO	dissolved oxygen
EPA	Environmental Protection Agency
g	gram
GC-ECD	gas chromatography - electron capture detection
GC-ToF-MS	gas chromatography – time of flight – mass spectrometry
HCB	hexachlorobenzene
HPLC	high performance liquid chromatography
HS-SPME	headspace solid phase microextraction
L	liters
LLE	liquid-liquid extraction
LOD	limit of detection
LPME	liquid phase microextraction
mg	milligram
MS/MS	tandem mass spectrometry
NaCl	sodium chloride
NIH	National Institute of Health
NIST	National Institute of Standards and Technology

OCPs	organochlorine pesticides
OKACOM	The Permanent Okavango River Basin Water Commission
ORB	Okavango Delta Basin
PA	polyacrylate
PCBs	polychlorinated biphenyls
PCDD/Fs	polychlorinated dioxins and dibenzofurans
PDMS	polydimethylsiloxane
PDMS/DVB	polydimethylsiloxane/divinylbenzene
PFE	pressurized fluid extraction
POPs	persistent organic pollutants
PTFE	polytetrafluoroethylene
RSD	relative standard deviation
SBSE	stir bar sorptive extraction
SFE	supercritical fluid extraction
SPE	solid phase extraction
SPME	solid phase microextraction
TDA	trans-boundary diagnostic analysis
α-BHC	alpha-benzenehexachloride
β-BHC	beta- benzenehexachloride
γ-BHC	gamma- benzenehexachloride

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Chapter 1

1 Background

The Okavango Delta is one of the world's largest pristine wetlands famous for its fauna and flora and whose resources are important for the livelihood of the human communities living on its fringes (Kgathi et al., 2006). It is situated in the north-west Botswana, and covers an area of approximately 12 000 km^2 of which 6 000 km^2 is permanent swamp and another 6 000 km^2 is seasonal floodplains (McCarthy et al., 2003). Although commonly referred to as a delta, the Okavango Delta is actually a landlocked alluvial fan formed on a depression approximately 1 million years ago (Gumbricht et al., 2001).

Figure 1.1 shows the Okavango Delta as part of the Okavango River Basin (ORB) that is shared by three Southern Africa nations, namely Angola, Botswana and Namibia.

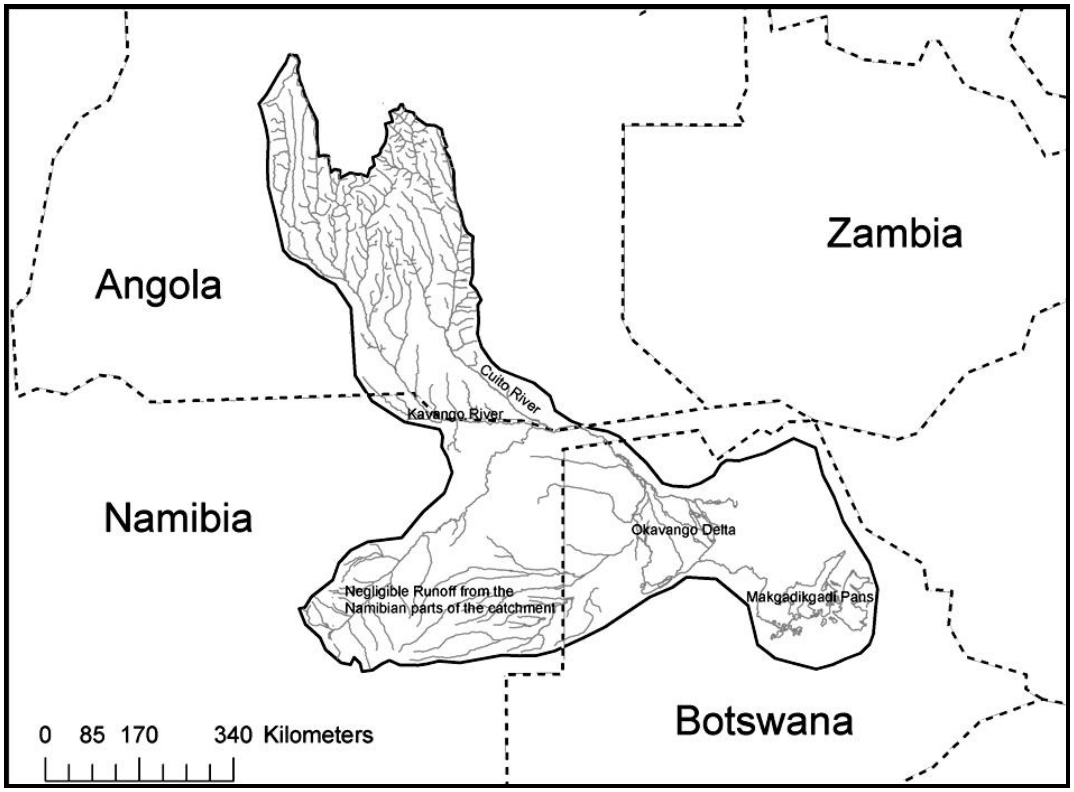


Figure 1.1: The Okavango River Basin showing the Cuito and Kavango Rivers in southern Angola traversing through northern Namibia before draining into the Okavango Delta in Botswana (Bauer-Gottwein et al., 2007).

The Cuito and Kavango Rivers in Angola and Namibia, respectively, combine to form the Okavango River, which discharges an average of 10.1 x 10^9 m^3 of water into the wetland annually (McCarthy et al., 2000). Rainfall in the Angolan highlands falls from December to March but because of the very low topographic gradient (1:3400), movement of the flood wave across the fan takes four to five months to traverse the 250 km distance from Mohembo at the apex of the panhandle to Maun at the southern end of the Delta (Ellery and McCarthy, 1994). Approximately 95 % of the water brought into the delta by the annual flood and rainfall is lost mainly to evapo-transpiration while only about 3 % leaves the Delta via the Boteti River at its

southern end as surface outflow and a further 2 % leaves as groundwater flow (McCarthy and Ellery, 1998).

Organochlorine pesticides such as dichlorodiphenyltrichloroethane (DDT) and endosulfan have been employed in the area surrounding the Okavango Delta in attempts to control the malarial and trypanosome vectors, from as early as the 1940's until the late 1990's when deltamethrin (a less persistent pyrethroid), was introduced (Allsopp, 2002; Mabaso et al., 2004). It is expected that the POPs employed decades ago should still be present in the ecosystem and hence information regarding their levels and impacts is essential for the sustenance of the system.

Other threats to the water quality and ecosystem of the ORB arise from consequences of human activities such as unregulated tourism, harvesting of fish and river reed in Botswana and Namibia, water abstraction for power generation in Angola as well as commercial agriculture along the floodplains in Namibia (Ashton and Neal, 2003; Porto and Clover, 2003; UNDP, 2002). It is expected that the pressure on the ORB and its natural resources would increase in the future mainly due to demographic changes, governmental development initiatives as well as the threat of climate desiccation due to global warming thus posing serious challenges to environmental and livelihood sustainability in the region (Kgathi et al., 2006). Finding effective responses to these challenges is hampered by a host of constraints among which is a general lack of adequate scientific databases on critical socio-economic and environmental issues (Totolo and Chanda, 2003).

In 1994, the three riparian countries signed the Okacom Agreement which commits the member states to manage the ORB as a single entity. The signatory countries established the Permanent Okavango River Basin Water Commission (OKACOM) which is a regional, high level committee whose objective is to act as a technical advisor to the governments of the three riparian states on matters relating to conservation, development and utilisation of resources of common interest to the riparian countries. The Commission ensures the water resources of the ORB are managed in appropriate and sustainable ways and to foster cooperation and coordination between the member states. The role of OKACOM is to anticipate or reduce unintended impacts that could occur within the ORB due to uncoordinated development. OKACOM has the legal responsibility to

- Determine the long term conservation and safe economic yield of the river basin.
- Estimate reasonable demand of the basin's resources from the riparian human communities.
- Set criteria for conservation, equitable and sustainable utilisation of resources
- Develop measures for the alleviation of short-term difficulties such as temporary droughts
- Conduct investigations related to water infrastructure
- Recommend pollution preventative measures (OKACOM, 2009).

As part of its mandate, OKACOM carried out a Trans-boundary Diagnostic Analysis (TDA) in 1999 to enable formulation of a sustainable resource management in the

ORB (Ashton and Neal, 2003; UNDP, 2002). The pre-TDA report stated that inorganic and organic toxic constituents of water in the Okavango Delta were present at low concentrations and represented mostly natural background accumulations. However it pointed out the lack of environmental quality data especially in the upper catchments in Angola and Namibia. Other Integrated Management Initiatives that have pointed out the need for an environmental quality study in the ORB include the Okavango Delta Management Plan (ODMP) and The Every River has its People (ERP) (Rothert, 2000).

Inorganic constituents of water from the Okavango Delta have been investigated (Cronberg et al., 1996; Sawula and Martins, 1991; Sawula, 2004). A number of studies have also been carried out on the ecotoxicological effects of endosulfan on aquatic invertebrates (Douthwaite, 1982; Fox and Matthiessen, 1982; Russell-Smith and Ruckert, 1981). Mbongwe and colleagues (2003) reported concentrations of DDT and its metabolites in water and biota. However no extensive investigation of pesticide levels has been carried out in the Delta.

1.1 Environmental analysis

During the past few decades, increased public awareness on the health risks associated with environmental contaminants has stimulated interest in environmental research and monitoring for toxic contaminants in air, water, sediments as well as soils.

For example, persistent organic pollutants (POPs) are chemical substances that persist in the environment, resisting biological, chemical and photolytic degradation. They bioaccumulate through the food web, and exert carcinogenic and reproductive consequences in animals and human beings (Di Bella et al., 2006; Warren et al., 2003). Because of their persistence, POPs can be transported through long distances via water, air and migrating animals to places such as the Arctic and Antarctic where they have never been used (Miniero et al., 2008; Walstrom, 2003). Their high lipophilicity allows them to accumulate in the fatty tissues of living organisms resulting in bioaccumulation up the trophic levels. Thus in aquatic environments, they display strong affinity for suspended particles, sediments and biota where they can reach concentrations higher than in the water (Abballe et al., 2008).

The Stockholm Convention on POPs came into force in May 2004 and aims to protect human and environmental health from the effects of exposure to specific POPs by restricting the use and production or banning these chemicals through a

unified global and multilateral effort. The initial group of POPs specified by the convention includes different classes of compounds such as organochlorine pesticides (OCPs), polychlorinated dibenzodioxins and dibenzofurans (PCDD/Fs) as well as polychlorinated biphenyls (PCBs) (Bouwman, 2004).

Despite the development of highly sophisticated and sensitive analytical instruments for quantification, the simple approach of “dilute and shoot” has not been applicable to environmental samples. Usually, an extraction step is required to isolate and enrich analytes of interest (that are often at trace levels) from complex matrices. Classical extraction techniques such as liquid-liquid extraction (LLE) and Soxhlet extraction often require large volumes of organic solvents (thus creating environmental and occupational hazards), are laborious and often provide little selectivity (Hyotylainen and Riekkola, 2007). Solvents are often released into the atmosphere during the volume reduction step following extraction by these techniques (Sporring et al., 2005). To address this issue, the Montreal protocol treaty called for a reduction of halogenated organic solvent consumption (Noble, 1993). As a result, the drive in analytical chemistry is to develop new sample preparation techniques that employ lower volumes of organic solvents, are faster and cost effective as compared to conventional techniques.

Sample preparation is an analytical step that involves isolation and enrichment of analytes from a sample matrix. The extraction of analytes from sample matrices can be considered as a 3-step simplified process;

i. Removal of analytes from the matrix.

ii. The solvation of analytes in the extraction medium.

iii. Mass transfer of solubilised analytes into the bulk solution.

Step (iii) is critically affected by agitation, turbulent flow or the diffusivity of the extraction medium which can be affected by temperature and pressure. Steps (i) and (ii) depend on the molecular interactions of the analyte and extraction medium (Kane et al., 1993).

Several sample preparation techniques are available that offer different degrees of selectivity, speed of operation and convenience. Modern techniques of sample preparation address the need for the reduction of organic solvent consumption, miniaturization, automation and ultimately on-site, in situ or in-vivo sampling (Wells and Lloyd, 2002). These extraction approaches are usually easy to carry out and call for optimization of several parameters to enhance the performance of the overall analysis. The key to rational choice of a sample preparation technique for a particular matrix is based on an understanding of the fundamental principles governing the kinetics of mass transfer within the extraction system (Pawliszyn, 2003).

1.2 Objectives

1. To investigate green sample preparation techniques for the analysis of pesticides in water and sediments. The techniques should be those that;

a. employ low volumes of solvent

b. are not carcinogenic

c. easily biodegradable

d. involve minimum handling yet permitting high throughput analysis

2. To establish levels of pesticides in the water and sediments along the direction of flow in the Okavango Delta.

Chapter 2

This chapter reviews the fundamental theories of SPE, SPME, SFE and PLE, their advantages and limitations as well as discusses their applications in environmental monitoring.

2 Sample preparation techniques for the extraction of pesticides in water and sediments

2.1 Sample preparation techniques for the determination of pesticides in water

Water, a key constituent of ecosystems, is a recipient of a variety of xenobiotics such as pesticides and industrial chemicals by way of direct discharges from point sources or contaminated storm water run-off (Mayon et al., 2006). Organochlorine pesticides (OCPs), in particular, have a potential to give rise to serious ecological effects in freshwater environments and as a result, regulatory bodies such as the European Community have set the maximum concentrations of individual OCPs in drinking water at 0.1 µg L^{-1} and the total amount of pesticides at 0.5 µg L^{-1} (European-Community, 1998). Maximum individual concentrations for aldrin, dieldrin and heptachlor epoxide have even been set lower at 0.03 µg L^{-1} (Beceiro-Gonzalez et al., 2007). There is therefore, a need for highly sensitive analytical methods involving

sample preparation techniques with high pre-concentration capacities for monitoring environmental pollutants especially in portable water.

Sample preparation is often considered to be a critical step in the analytical procedure because it not only helps to achieve the low detection limits set by regulatory authorities by cleaning up the sample matrix but also acts to pre-concentrate analytes of interest from a dilute sample matrix to a level of detection by an instrument of choice (Fontanals et al., 2007). Several sample preparation techniques can be employed for the extraction and concentration of contaminants in water e.g. liquid-liquid extraction (LLE), solid phase extraction (SPE), solid phase microextraction (SPME), stir-bar sorptive extraction (SBSE), liquid-phase microextraction (LPME).

Liquid-liquid extraction (LLE) is a technique traditionally employed for the extraction of pesticides in aqueous samples mainly because of its simple protocol and no need for special instrumentation (Baltussen et al., 1997). However its major limitation is the use of large volumes of organic solvents in order to achieve high extraction efficiencies (Moreno et al., 2007; Shimelis et al., 2007). Subsequent evaporation of solvent for enrichment purposes can cause loss of volatile analytes and the organic solvent into the atmosphere (Baltussen et al., 1997; Namiesnik et al., 1990). However, modern microextraction techniques such as solid phase extraction (SPE), solid phase microextraction (SPME), require minimal handling and consumption of organic solvents as well as offer high selectivity and enrichment factors (Hyotylainen and Riekkola, 2008).

SPE and SPME were employed as sample preparation techniques for water samples in this thesis for reasons that;

- They utilize much lower volumes of organic solvent (~6 ml required for SPE compared to ~100 ml for LLE while SPME is a completely solvent-free technique).
- High pesticide recoveries can be obtained by optimization of the relevant parameters for each technique. In SPE, the sorbent type, washing and elution solvents can be tuned in order to obtain high recoveries (see sections 3.2.2 and 4.1.2) while parameters such as fiber type, extraction temperature, ionic strength, stirring and extraction time can be optimized (see sections 3.2.5 and 4.1.5).
- They allow high pre-concentration factors hence are suitable for trace level analysis.

The principles behind SPE and SPME, their advantages and limitations are discussed in sections 2.1.1 and 2.1.2, respectively.

2.1.1 Solid phase extraction

Solid phase extraction (SPE) is a sample preparation technique based on principles similar to those of HPLC and is employed for the selective sorption of analytes of interest from liquid matrices. This transfer of analytes from the liquid sample matrix to the solid sorbent is influenced by the selection of appropriate conditions of three major components; the liquid matrix, the sorbent and the analyte. It has several advantages over its predecessor, liquid-liquid extraction (LLE), in that it;

i. Requires less organic solvent than LLE.

ii. Provides higher selectivity due to the wide range of sorbent chemistries available.

iii. Does not involve the formation of emulsions as in LLE.

iv. Yields cleaner extracts.

v. Provides higher and more reproducible recoveries.

vi. Achieves higher sample throughput.

vii. Is easily automated.

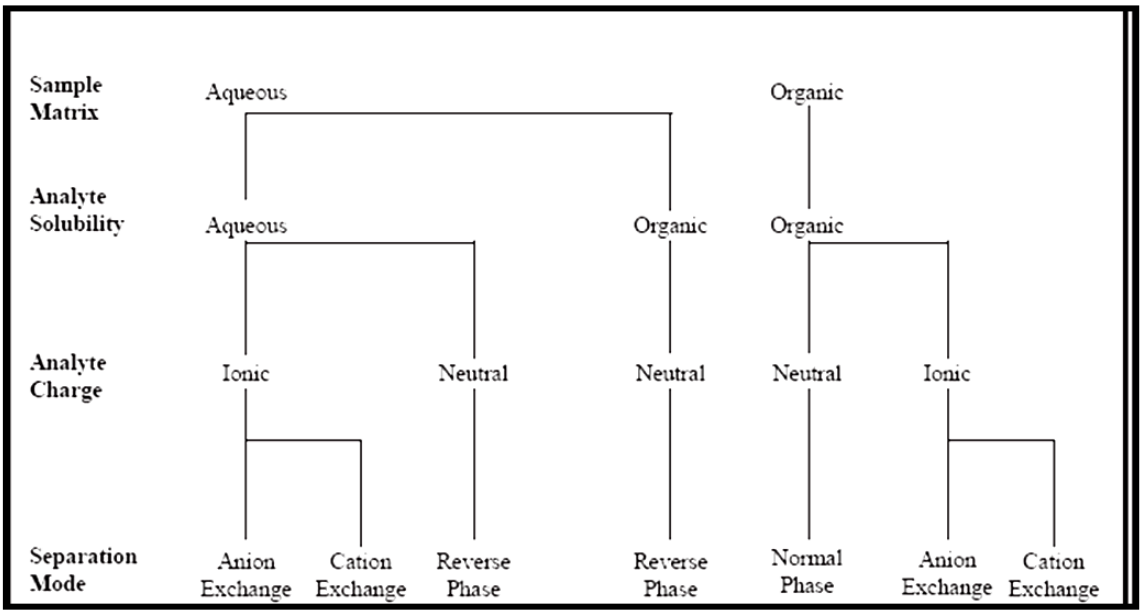
When the technique was newly introduced, short columns were hand-packed with normal-phase materials such as silica or Fluorisil. They were used mainly for the retention of interferents in the clean-up of pesticide residues from environmental samples. The introduction of disposable pre-packed SPE cartridges had a major impact on the applications of SPE on analytes contained in liquid matrices (Liska,

1993). The SPE cartridge introduced important features such as standardization, greater reproducibility and a wider range of phases to choose from. Although the cartridges are single-use and disposable, the overall cost of SPE is lower than the cost of solvents and the manpower needed for traditional LLE (Smith, 2003).

Several factors influence the efficiency of the SPE process but the two most important are retention and capacity. Retention of analytes on the sorbent should be maximum during the loading and washing steps but minimal during the elution step. To understand these, knowledge of the hydrophobic, polar and ionogenic properties of both the solute and sorbent are required. The most common retention mechanisms in SPE are based on van der Waals forces (non-polar interactions), hydrogen bonding, dipole-dipole forces (polar interactions) and cation-anion interactions (ionic interactions). As a result, separation on SPE sorbents can be referred to as reverse phase, normal phase or ion exchange.

Reverse phase separation involves a polar or moderately polar sample matrix such as water (mobile phase) and a non-polar sorbent. The analyte of interest is usually mid- to non-polar such as pesticides, retention of the organic analytes onto the sorbent is mainly due to the attractive forces between the carbon-hydrogen bonds in the analyte and the functional groups on the sorbent surface (van der Waals forces). A non-polar solvent is then employed to disrupt the interaction between the analyte and sorbent. Materials based on carbon, silica and polymers have been employed as reverse phase SPE sorbents. Carbon-based media consist of graphitic, non-porous

carbon with a high affinity for organic polar and non-polar matrices. Retention of analyte is based primarily on the structure of the analyte rather than on the interactions of functional groups on the analyte with the sorbent surface. Polymeric sorbents are based on styrene/divinylbenzene materials. These sorbents are used for the retention of hydrophobic compounds that have some hydrophilic functionality, especially aromatic compounds. A guide for choosing an appropriate sorbent is given in Scheme 2-1.



Scheme 2-1: SPE method development guide

Analytes are normally eluted with mid- and non-polar solvents since the polymeric material is stable in almost all matrices. Silica materials can be coated or bonded

with hydrophilic polymers. The pores of the polymer allow small hydrophobic organic compounds such as drugs to reach the silica surface while large molecules such as proteins are prevented from reaching the silica surface and can then be washed off the sorbent (Zwir-Ferenc and Biziuk, 2006).

Normal phase involves a polar analyte, a mid- to non-polar matrix (eg acetone, chlorinated solvents and hexane) and a polar sorbent. Retention of an analyte under normal phase conditions is primarily through interactions between polar functional groups of the analyte and polar groups on the sorbent surface via hydrogen bonding and π-π interactions. The elution solvent should be one that is more polar than the sample matrix. Bonded normal phase silica sorbents have short alkyl chain with polar functional groups (free hydroxyl groups) bonded to the surface. This causes them to be more hydrophilic compared to bonded reverse phase silica sorbents, hence they are used to adsorb polar compounds from non-polar matrices.

Ion exchange SPE is used for compounds that are charged when in solution. Anionic compounds can be isolated on an aliphatic quaternary amine that is bonded to the silica surface. Cationic compounds are isolated on aliphatic sulfonic groups bonded to the sorbent surface. The retention mechanism is mainly through electrostatic attraction of the charged functional group in the analyte to the charged one on the sorbent surface. Thus the control of pH is essential in ion-exchange SPE to ensure that the analyte of interest is charged during the loading and washing steps to enable its retention on the sorbent. In addition, the analyst has to bear in mind that silica

based sorbents are only stable within the pH range of 2 to 8. The bonded phase can be hydrolysed and cleaved off the silica surface or the silica itself can dissolve at pH levels below 2 or above 8.

A typical SPE protocol involves four steps;

i. Conditioning – the sorbent is wetted with a suitable solvent to activate the functional groups on its surface. This step may be followed by an equilibration step whereby the wetting solvent is replaced by water.

ii. Loading – the sample is percolated through the sorbent.

iii. Washing – interfering components of the matrix are removed while taking care not to elute the analytes as well.

iv. Elution – analytes of interest are eluted with an appropriate solvent.

The choice of an eluting solvent is determined by the relationship of the eluotropic strength of adsorption on silica (ε°) and the polarity of the analyte. The high ε° of methanol (0.73) is the basis for its selection as an elution solvent for the removal of moderately polar to strongly polar analytes from polar adsorbents. Methanol is unique in its interactions with both non-polar and polar groups (Zwir-Ferenc and Biziuk, 2006).

The scope of SPE is very wide with notable applications in the clean-up of biological fluids for the extraction of drugs. The disposable cartridges reduce the handling of body fluids such as urine and blood and hence the biohazard to the operator is minimized. The second widespread application of SPE has been in environmental samples such as river water and sewage effluents where large quantities of very dilute samples have to be extracted (Smith, 2003). In the extraction of pesticides from water, SPE efficiency depends on factors such as the nature of the water sample (presence of particulate matter, presence of interfering compounds such as surfactants as well as the ionic strength) and sorbent treatment (Font et al., 1996).

2.1.2 Solid phase microextraction

Solid phase microextraction (SPME) is a technique that was introduced in the early 1990s by Lord and Pawliszyn (2000). Its applications increased after it became commercially available in 1993. SPME has been employed in the analysis of a wide variety of samples including environmental, food, biological, metallic and organometallic species.

The SPME device (Figure 2.1) is based on a fused – silica fiber, coated with a thin film polymeric stationary phase that is exposed to the sample.

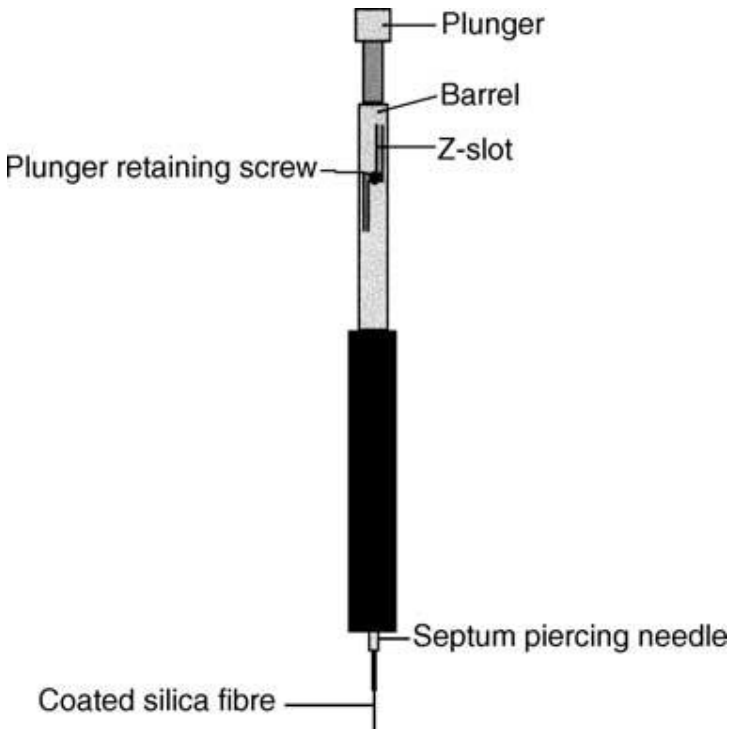


Figure 2.1: Design of the SPME device (King et al., 2003).

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The technique is based on the establishment of equilibrium between the analyte and a fused silica fiber coated with a polymer that can be liquid, solid or a combination of both. At equilibrium, a linear relationship exists between the number of moles of an analyte adsorbed on the fiber and the concentration of the analyte in the aqueous phase (Arthur and Pawliszyn, 1990; Lord and Pawliszyn, 2000). The relationship is represented by the equation;

$$n = \frac{K_{fs} V_f V_s C_o}{K_{fs} V_f + V_s} \quad (1)$$

Where;

n = number of moles of the analyte extracted by the fiber coating.

K_{fs} = the fiber coating/sample matrix distribution coefficient.

V_f = volume of the fiber coating.

V_s = the sample volume.

C_o = the initial concentration of a given analyte.

The analyte is then desorbed into a suitable separation and detection system, usually GC (Tena and Carrillo, 2007). The main advantages of this technique are the simplicity of operation, its solventless nature, analyte-matrix separation and pre-concentration. SPME can be employed for field, in situ or air sampling. The generally accepted limitations are the relatively poor reproducibility, lot-to-lot variations, lack of selectivity and tolerance to organic solvents and cost. In addition, the limited range of stationary phases that are commercially available restricts their use to a few compounds (Dietz et al., 2006).

The commercially available fiber coatings are polydimethylsiloxane (PDMS), divinylbenzene (DVB), polyacrylate (PA), carboxen (CAR – a cabon molecular sieve) and carbowax (CW – polyethylene glycol). The fibers are available in different coating combinations, blends or co-polymers, film thickness and fiber assemblies, thus widening the application fields.

Several variations of SPME are based on the geometry of the extraction phase such as coated fibers, vessels, stir bars, disks and coatings on the inside of tubes. The fiber design is the most convenient approach since analytes are easily desorbed from the fiber coating in the injection port of a GC (Musteata and Pawliszyn, 2007). Figure 2.2 illustrates the different configurations of SPME.

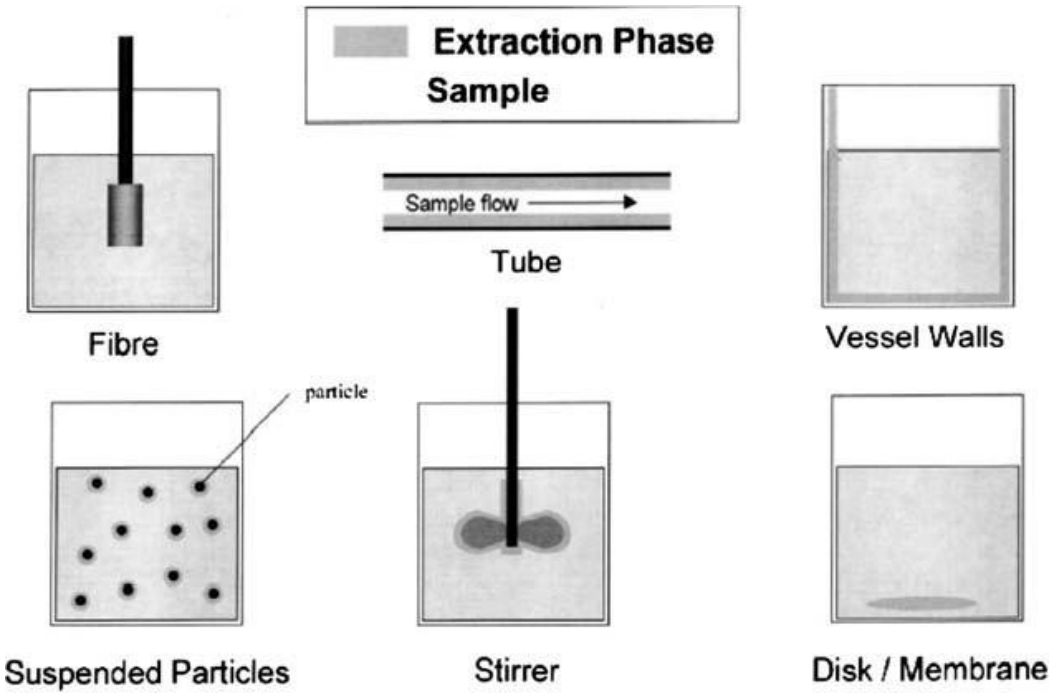


Figure 2.2: Configurations of solid phase microextraction (Ouyang and Pawliszyn, 2006)

SPME is performed by exposing the fiber coated polymer to a sample matrix or its headspace until equilibrium is reached between the analyte partitioned on the fiber coating and the analyte dissolved in the sample matrix. The concentration of the analyte extracted onto the fiber is proportional to its initial concentration in the sample matrix (Ouyang and Pawliszyn, 2006).

SPME can be performed in three modes; direct immersion, headspace and membrane protection modes. The different extraction modes are shown on Figure 2.3.

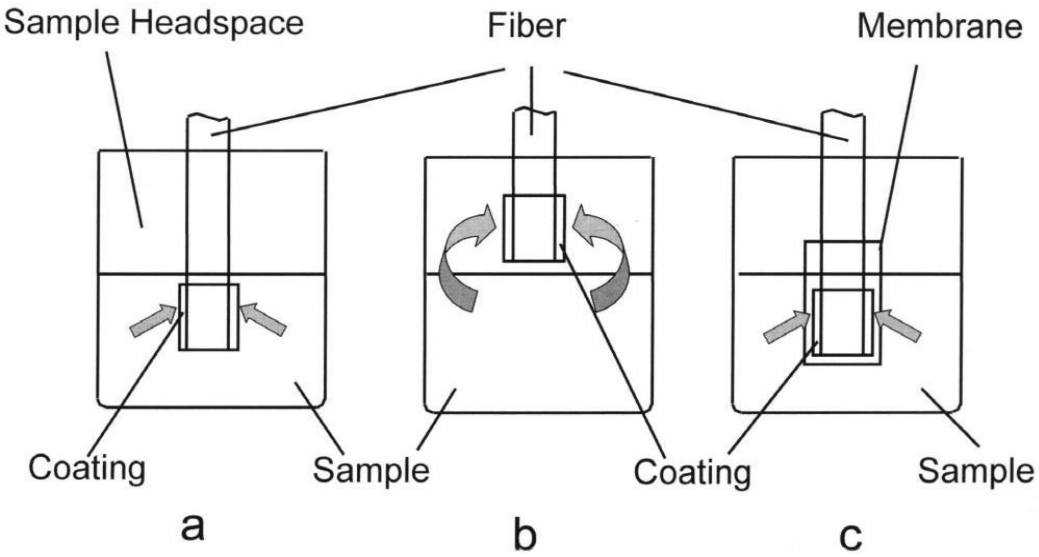


Figure 2.3: SPME modes (a) direct immersion; (b) headspace SPME; (c) membrane protected SPME (Lord and Pawliszyn, 2000).

In the direct immersion mode, the coated fiber is inserted directly into the sample matrix and analytes are transported to the fiber coating. Agitation may be employed to facilitate the transport of analytes from the bulk of the sample matrix to the vicinity of the fiber, resulting in rapid extraction. For aqueous matrices, agitation techniques such as fast sample flow, rapid fiber or vial movement, stirring or sonication may be employed. This is important to eliminate the formation of a “depletion zone” around the fiber as a result of fluid shielding and slow diffusion coefficients of analytes in liquid matrices. Agitation is not necessary for gaseous samples since the natural convection of air is sufficient for attainment of equilibrium in short periods of time (Baltussen et al., 1998; Chai and Pawliszyn, 1995).

In the headspace mode, analytes have to be present in the vapour phase before they can interact with the fiber coating which is suspended above the sample matrix. This mode protects the fiber from damage by high molecular and non-volatile species in the sample matrix such as humic acids and proteins. Headspace sampling also allows modification of the sample matrix parameters such as pH without affecting the fiber (Lambropoulou et al., 2007).

The choice of sampling mode has a significant impact on the extraction kinetics. For example, volatile analytes tend to be at a higher concentration in the headspace than in the liquid matrix and since diffusion rates in the gaseous phase are typically 4 orders of magnitude higher than in liquid media, equilibration times for volatiles are shorter in the headspace mode. Temperature and agitation also play an important

role in headspace extraction kinetics in the sense that higher temperatures and agitation rates favor transport of analytes from the liquid matrix into the headspace (Zhang and Pawliszyn, 1993).

In membrane protected SPME, the membrane functions to protect the fiber against damage when used for very dirty samples. A membrane made from an appropriate material may add some selectivity to the extraction process. The kinetics of membrane protected SPME are substantially slower than those of direct immersion since the analytes have to diffuse through the membrane before they can adsorb onto the coating. Zhang and co-workers (1996) covered a SPME fiber with a cellulose hollow membrane with a molecular weight cut-off of 18 000 Da and were able to extract PAHs from complex aqueous samples containing humic acids. Their investigations showed that mass transfer rates increased when using elevated extraction temperatures.

2.2 Sample preparation techniques for the determination of pesticides in sediments

In aquatic environments sediments, depending on their chemical composition and adsorption characteristics, have a high accumulation capacity for both inorganic and organic contaminants (Guevara-Riba et al., 2006). Organochlorine pesticides (OCPs) such as DDT are an important group of contaminants that have caused worldwide concern as toxic environmental contaminants (Zhao et al., 2007). Their hydrophobicity and persistence causes OCPs to be readily scavenged from the water through sorption onto suspended material and subsequently become part of sediment. However changes in environmental conditions may lead to the release of contaminants back into the aquatic system thus making sediments a possible source of exposure to aquatic organisms (Delistraty and Yokel, 2007).

The presence of contaminants at low concentrations as well as the chemical complexity of matrices such as soil, sediments and biota requires preliminary treatment and includes amongst other steps; extraction, clean-up and pre-concentration of the sample (Gabaldon et al., 2007; Santos and Galceran, 2002).

Several processes occur during the extraction of analytes from solids-containing matrices such as sediments. Pawliszyn (2003) described a model whereby an analyte is adsorbed on the pore surface of a matrix particle (impermeable but with a porous core) that is surrounded by an organic layer. The extraction process involves several steps outlined in Figure 2.4.

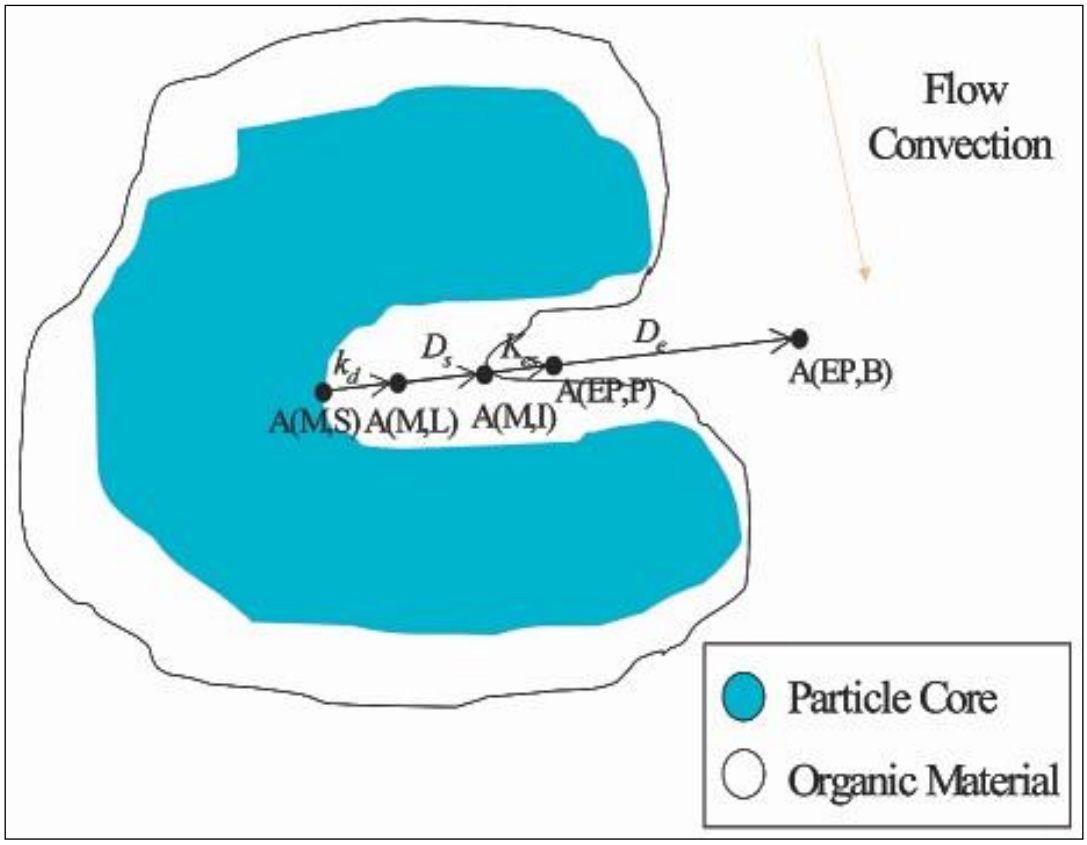


Figure 2.4: Processes involved in the interaction of heterogeneous samples containing porous solid particles. K_{es} is the extraction phase/sample matrix distribution constant. k_d is the dissociation rate constant of the analyte-matrix complex. D_s is the analyte diffusion in the sample matrix and D_e is the analyte diffusion in the extraction phase (Pawliszyn, 2003).

To extract the analyte from the surface of the matrix particle, the analyte must first be desorbed from the surface [A(M,S)]; it must then diffuse through the organic part of the matrix [A(M,L)] to reach the matrix/liquid interface [A(M,I)]. From the interface, the analyte must be solvated by the extraction phase [A(EP,P)] before being transported into the bulk of the extraction phase [A(EP,B)].

Soxhlet has been a standard technique and reference against which the performances of other newer techniques have been compared for the extraction of organic compounds from solid samples for more than a century (Sporring et al., 2005). It boasts of advantages such as low cost of the basic equipment, simple procedure that requires minimal training and is not matrix-dependent (de Castro and Garcia-Ayuso, 1998). However the large volumes of organic solvents (150 ml) and long extraction times (20 h or more) have led to the development of more environmentally friendly sample preparation techniques that are faster and employ less organic solvents (Hartonen et al., 1997; Schantz et al., 1998). Techniques such as microwave assisted extraction (MAE), sonication assisted extraction (SAE), supercritical fluid extraction (SFE), supercritical water extraction (SWE), pressurized fluid extraction (PFE) and matrix solid phase dispersion (MSPD) have in the past few decades been employed in place of Soxhlet for the extraction of organic compounds in sediments due to their shorter extraction times and lower solvent consumption (Hyotylainen and Riekkola, 2008).

In this thesis SFE and PFE were employed as sample preparation techniques for sediments because;

- They utilize much lower volumes of organic solvent (microlitre amounts to a few milliliters of organic solvent can be used as a modifier in SFE while less than 15 – 20 ml volumes are employed in PFE compared to 150 ml volumes employed in Soxhlet (Richter et al., 1997).

- Extraction times are much shorter for PFE (5 – 10 min) and SFE (30 - 60 min) compared to 2 – 24 h for Soxhlet (Bowadt and Hawthorne, 1995).
- High extraction efficiencies can be obtained through optimization of parameters such as temperature, pressure, organic solvent and extraction time for both techniques (Bowadt et al., 1995; Sanz-Landaluze et al., 2006).

The principles, advantages and limitations of SFE and PFE are discussed in sections 2.2.1 and 2.2.2, respectively.

2.2.1 Supercritical fluid extraction

This technique employs fluids in their supercritical states for the extraction of solid samples. Supercritical fluids behave like gases although they have the density of liquids and as a result, they have a high diffusivity, low viscosity, good penetration capability and adjustable density (Goncalves et al., 2006). In comparison to soxhlet extraction, supercritical fluid extraction (SFE) offers several advantages such as shorter extraction times, lower solvent consumption (hence environmentally friendly), suitability for thermally labile compounds and reduced working temperature (Brachet et al., 2000).

In SFE, a solid or semi-solid sample is placed in a pressure vessel and extracted with a re-circulated stream supercritical fluid which is well mixed with the sample matrix to allow analytes to transfer to the fluid. At the end of the extraction, the extract is collected in a vial or cartridge (Figure 2.5).

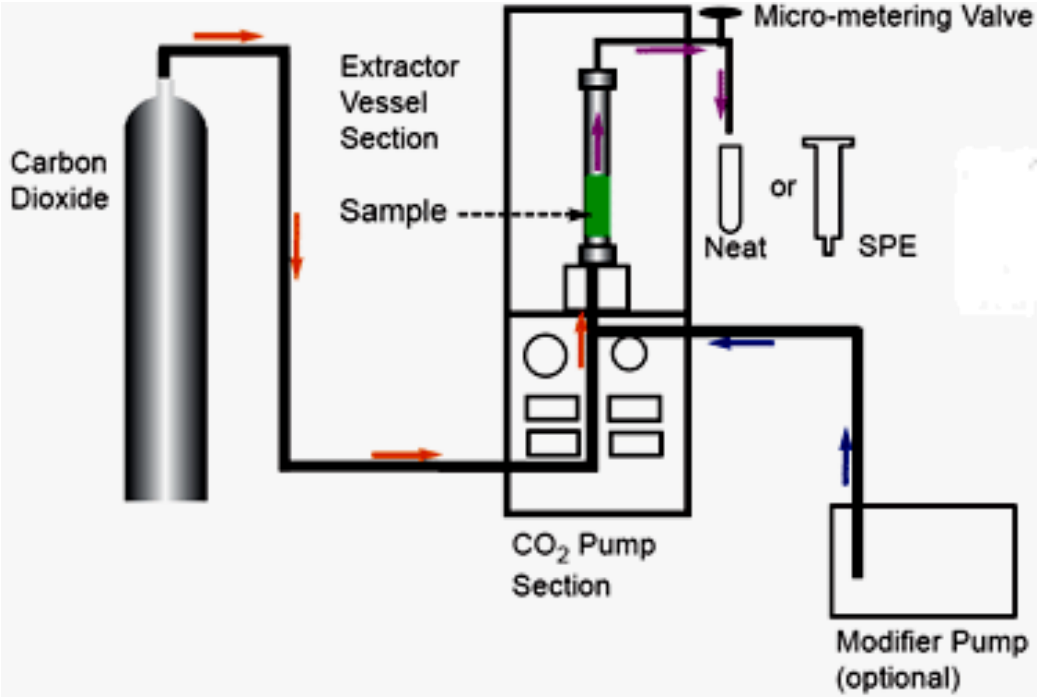


Figure 2.5: Diagram of a Spe-ed™ Prime SFE by Applied Separations Inc. (2008)

SFE involves five sequential steps;

1) Wetting of the matrix with supercritical fluid.

2) Partitioning of the analyte from the matrix into the supercritical fluid

3) Diffusion of analytes from the matrix.

4) Elution of the analyte from the extraction cell

5) Collection of the analytes.

Wetting of the sample with supercritical fluid is especially important when the sample matrix contains water. Partitioning of non-polar analytes from the matrix is a relatively fast process if supercritical carbon dioxide is employed. For analytes that are strongly bound to the matrix, a higher temperature or addition of an organic solvent is required. The second step depends on factors such as diffusion of the analyte between the matrix active sites and the ability of the supercritical fluid to displace the analyte from these sites. This initial desorption step is often the rate determining step in the SFE of most environmental samples (Bowadt and Hawthorne, 1995). All three steps contribute to the overall extraction efficiency (Hawthorne et al., 1993).

Several modeling studies of SFE have been carried out to explain the extraction process associated with the technique. Clifford and co-workers (Clifford et al., 1995) suggested a model to predict the kinetic features of dynamic SFE that have been attributed to effects of the matrix. Described in their model are three phases of the extraction process;

a) A slow initial extraction of analyte molecules from the matrix.

b) Transport of the analyte through the matrix caused by a concentration gradient. It is assumed that extraction initially occurs on the edges of the matrix resulting in the transport of the analyte into the bulk of the solvent. This erodes the analyte concentration at the edge of the matrix, thereby promoting extraction of analytes that are further inside the matrix. The rate of this process is high in dynamic extraction. For analytes with poor solubility in

the extracting solvent, the concentration at the edge of the matrix will remain higher than in the bulk solvent, hence less analyte proportions will be extracted in this phase.

c) A slow final phase that could be because all the analytes that are highly soluble in the extracting solvent or those that were concentrated on the edge of the matrix have been extracted and only the strongly bound analytes remain.

It has to be taken into consideration that the model assumes that a majority of analytes are adsorbed in the inner pores of the matrix while only a small proportion of analytes are distributed on a thin layer on the surface of the matrix. This is why the initial step in the model is slow - requiring an analyte concentration gradient to build up. In instances where a majority of analytes are on the surface of the matrix, the initial desorption is a fast process. The elution of the analyte from the extraction cell depends on the amount of fluid flow in relation to sample size as well as the solubility of the analyte in the supercritical fluid. Finally, the collection step is dependent on the restrictor type on the SFE instrument and the trapping arrangement employed (Langenfeld et al., 1995). The collection step is explained in more detail on pages 38-40.

Pressure and temperature are important in SFE since they define the density of the supercritical fluid. Figure 2.6 is a phase diagram of density, pressure and temperature for carbon dioxide. It shows that at a constant pressure, the density of

CO_2 decreases when the temperature is increased while an elevation of pressure at a given temperature results in an increase in fluid density.

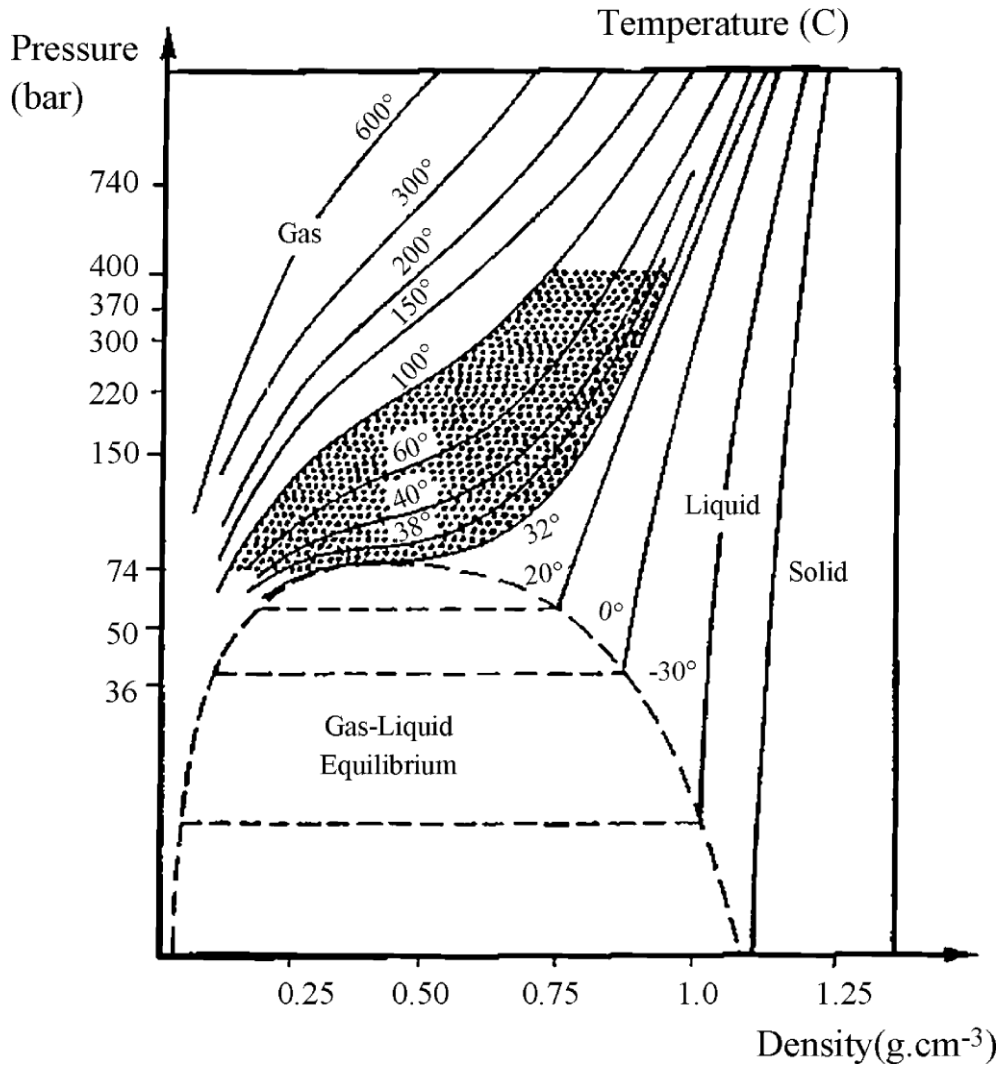


Figure 2.6: Pressure – density diagram for carbon dioxide. The shaded area corresponds to the commonly used experimental domain of supercritical phase extraction (Pourmortazavi and Hajimirsadeghi, 2007).

Maximum solubility of an analyte is achieved at the highest density for a given temperature and is affected by analyte volatility and the solvating capacity of the

supercritical fluid. This means that increasing the extraction temperature may significantly increase the solubility of compounds with high vapor pressures. For example, the solubility of anthracene is enhanced by a factor of 5 by raising pressure from 150 to 400 bar at a constant temperature of 50 °C. In contrast, raising the temperature from 50 to 200 °C at 150 bar increases the solubility of anthracene by a factor of 23 and by a factor of 48 at 400 bar (Miller and Hawthorne, 1995).

More than 90 % of SFE applications employ carbon dioxide (CO_2) as the solvent of choice mainly because of its lower supercritical temperature (31 °C) and pressure (74 bars). In addition, CO_2 is non-toxic, non-flammable or explosive, available at high purity and easily removed from the extract (Letisse et al., 2006). The pressure and temperature of the supercritical fluid can be varied so as to effect selectivity during extraction (Fidalgo-Used et al., 2007). In the supercritical state, the polarity of CO_2 is comparable to that of liquid n-pentane, hence its suitability for extracting hydrophobic compounds (Wang et al., 2003). N_2O was initially thought to be better suited for the extraction of polar compounds due to its dipole moment, but proved to be hazardous by causing explosions when used for samples having high organic matter content (Raynie, 1993).

Other supercritical fluids that have been employed in SFE are SF_6, freons, ethane, propane, ethylene, dimethyl ether and water. The high temperature (between 100 and 374 °C) and pressure (from 1 to 221 bar) required for supercritical water have limited its use in SFE due to the corrosive nature of water at these conditions. However subcritical water has shown to be effective for the extraction of several

classes of essential oils and environmental pollutants (Gámiz-Garcia and Luque de Castro, 2000).

Even though supercritical CO_2 is a poor solvent for the extraction of polar compounds, the addition of small quantities of polar modifiers (usually organic solvents such as methanol) have shown to improve the extraction of polar analytes when supercritical CO_2 is employed (Rial-Otero et al., 2007). Modifiers act to improve the desorption of analytes from the matrix and to increase the solubility of polar and medium-polar analytes in the supercritical fluid and their quantities in relation to the sample size are crucial to the extraction efficiencies (Friedrich et al., 1995; Janssen et al., 1989). For example, modifier concentrations of 1 % volume per mass are more effective compared to higher concentrations (10 % volume per mass) for environmental samples. This is because at higher modifier concentrations, there is co-extraction of analytes and matrix components or the modifier competes with the target analytes for sites on the sample matrix rather than increasing analyte solubility (Bowadt and Hawthorne, 1995). Thus the solubility of analytes in the supercritical fluid and the selectivity required should be the driving forces in the optimisation of modifier concentrations.

Modifiers can be introduced together with the extraction fluid using a modifier pump or added directly to the sample before extraction. The use of a modifier pump enables alteration of the modifier concentration during the extraction and is suitable for use during both static and dynamic extractions. While the direct addition of modifier is only practical for static extractions, it is the simplest way to evaluate

several modifiers and has demonstrated to yield reproducible recoveries (Dankers et al., 1993).

In the SFE of environmental samples, the presence of water may enhance or hinder the extraction process. Large amounts of water in samples can reduce the solubility of analytes in the extracting solvent or cause restrictor plugging. Thus removal of water by freeze drying prevents loss of volatile compounds compared to drying in the oven or at room temperature. Drying agents may be employed although they pose a risk of absorbing some of the analytes (Burford et al., 1993). However, complete removal of water from sediments has also been shown to reduce recoveries. Hence the presence of small quantities of water may enhance extraction efficiencies by swelling up the sample and occupying free adsorption sites (Bowadt and Hawthorne, 1995).

Sample size in SFE is dependent on the available quantity of sample, the concentration of analytes in the sample, the detection limit of the analytical method and the size of extraction cells available. If the sample size does not fill the extraction cell, an inert material (rinsed sand or sodium sulphate) may be added to fill up the void volume (Bowadt and Johansson, 1994; Hawthorne et al., 1993). Sample sizes <10 g are often employed in environmental analyses. After extraction, a sufficient volume of the supercritical fluid is essential for the success of the SFE process. A minimum of 4 - 5 cell volumes should be used to ensure that the extracted analytes are sufficiently swept out of the extraction cell (Bowadt and Hawthorne, 1995).

One of the advantages of SFE over Soxhlet is the shorter time of extraction (30 - 60 min compared to 2 - 24 h) and the resulting cleaner extracts especially from complex matrices such as soils and sediments (Bartle et al., 1992). While the strength of CO_2 can be tuned by altering the temperature, pressure and modifier type and proportion, selective extraction of compounds with similar polarities from complex environmental matrices has not been successful. However, class fractionation is possible, provided that the analytes are very different. When the selectivity of SFE with CO_2 is not possible, selective sorbents have been added to the extraction cell to minimise co-extraction of undesired matrix components. In their study, France and colleagues (1991) showed that Al_2O_3 (up to twice the sample weight) can be used to retain lipids from fats during the extraction of organochlorine pesticides.

Apart from the extraction process, efficient trapping of analytes is crucial to the success of SFE. Different approaches have been employed for off-line SFE, liquid solvent collection and solid phase trapping and each one is dependent on parameters such as the trap temperature, the nature of the analytes and modifier as well as flow rate. Liquid solvent collection is simple and has been used widely for environmental samples and two common approaches have been employed.

In the first approach, the end of the outlet valve restrictor is placed directly into the collection solvent contained in a test tube and the depressurised CO_2 - analyte mixture is deposited into the solvent. In the second approach, the CO_2 - analyte mixture is deposited in a glass transfer tube before contacting the solvent. The first approach has shown to give better recoveries for volatile compounds while the

second approach requires the use of glass wool to prevent loss of volatile compounds from the transfer tube (Bowadt and Hawthorne, 1995). Burford and co-workers (1992) tested the two different approaches and observed that depressurising the CO_2 - analyte mixture directly into methylene chloride yielded > 90 % recoveries for PAHs while expansion into a transfer tube yielded only 50 % under identical conditions.

Solvent collection efficiencies can also be affected by several factors such as the type and volume of solvent, flow rate and restrictor temperature. Choice of solvent depends on the solubility of analytes in it, its vaporizability and its compatibility with the analysis instrument following extraction. The volume of the collection solvent should be sufficient to dissolve the analytes as they are deposited into it. The restrictor temperature should be sufficient to vaporise the CO_2 and not degrade the analytes (Turner et al., 2002).

Solid phase trapping is usually performed by depressurising the CO_2 –analyte mixture prior to the trap and collecting analytes from the gas phase onto sorbents such as silica gel, Florisil or bonded phase packings or onto a cooled inert surface such as glass or stainless steel beads (Schantz and Chesler, 1986). Analytes are then eluted with liquid solvents for subsequent analysis. This trapping approach has an advantage over solvent collection by producing clean and concentrated extracts.

Selection of the trapping material should be one that yields high trapping efficiencies and provide opportunities to perform selective elution of analytes from complex matrices. Hartonen and colleagues (1997) compared the trapping efficiencies of Florisil and octadecylsilane (ODS) for chlorinated and brominated pollutants from sediment samples. Both materials gave recoveries > 95 % however at higher flow rates, ODS gave lower recoveries. Yang and co-workers (1995b) compared sorbent (XAD-2 resin) and solvent (methylene chloride) trapping after SFE of volatile hydrocarbons from soil and found that both configurations efficiently collected benzene, toluene, ethylbenzene and xylenes (BTEX) while Soxhlet extraction yielded much lower amounts of the volatile organics.

On-line collection of SFE extracts has also been reported in literature and eliminates analyte loss during the collection step. Most on-line SFE applications involve coupling SFE to a chromatographic system such as GC, SFC or LC. The major advantage of on-line SFE is to enhance collection efficiencies of highly volatile analytes. In the case of coupling SFE with GC, the SFE effluent is depressurised directly into the injection port. An obvious disadvantage of on-line collection is overloading of the chromatographic system in cases where the analyte concentration in the SFE effluent is high or if some of the matrix components such as fats are not compatible with the chromatographic system as in GC.

There are several parameters that need to be considered in SFE. Thus the optimization of SFE parameters such as modifier, pressure and temperature is

essential due to the diversity of sediment types and compositions, as well as their effects on pesticides adsorption (Goncalves et al., 2006).

2.2.2 Pressurized fluid extraction

Pressurized fluid extraction (PFE), also referred to as pressurized liquid extraction (PLE), pressurized solvent extraction (PSE) or accelerated solvent extraction (ASE), is a technique that was introduced by Dionex corporation in 1995 (Richter et al., 1996). The principle of the technique is based on using elevated temperatures (50 – 200 °C) and pressures (50-150 atm) to extract analytes from solid or semi-solid samples within short periods of time (5 – 15 min).

The increased temperatures can disrupt the strong solute-matrix interactions caused by van der Waals forces, hydrogen bonding and dipole interactions of the solute molecules and active sites on the matrix. When the solvent is in contact with the matrix, the thermal energy in the heated solvent assists to desorb analytes from the matrix by overcoming cohesive (solute-solute) and adhesive (solute-matrix) interactions. This decreases the activation energy required for the desorption process (Richter et al., 1996).

The elevated temperature also reduces the viscosity of the solvent thereby increasing the diffusion rates of both the solvent into the sample matrix and of the

analyte into the solvent. In addition to reducing the viscosity of the solvent, increased temperatures will reduce the surface tension of the solvent, analytes and matrix, thus allowing the solvent to better “wet” the sample matrix. This allows easy formation of solvent cavities around the analytes thus improving contact of the solvent with the analytes (Mockel et al., 1987).

For polar solvents such as water, increasing the temperature lowers the dielectric constant thus making it suitable for the extraction of less polar compounds (Turner et al., 2006). The dielectric constant of water at 25 °C is ~80, making it an extremely polar solvent. Increasing the temperature of water to 250 °C while applying sufficient pressure to maintain it in its liquid state reduces the dielectric constant to 27 which is midway between those of methanol ($\varepsilon = 33$) and ethanol ($\varepsilon = 24$) at 25 °C (Miller and Hawthorne, 1998). As a result, water at higher temperatures is more “miscible” or “soluble” in organic solvents.

Miller and Hawthorne (2000) demonstrated that increasing the temperature of water up to 200 °C (473 °K) enhanced the solubilities of organic solvents such as benzene, toluene, octane and isooctane in the water by factors ranging from 10 to 250. This becomes relevant in the extraction of analytes contained in wet matrices – the increased solubility of water in organic solvents caused by elevated temperatures facilitate the availability of water-sealed pores and the analytes contained therein. Consequently, the extraction efficiency and rate are enhanced with minimal volumes

(15 – 20 ml) of organic solvent compared to soxhlet extraction (150 ml) (Richter et al., 1997).

The high pressures employed in PFE maintain the solvent in its liquid state even at temperatures above its atmospheric boiling point. The high pressure increases the solvation power and speeds up the extraction kinetics of solvents by forcing solvent into the pores of the matrix that normally would not be in contact with solvent at atmospheric pressure. This helps solvate analytes trapped in matrix pores that have been “sealed” with water or air bubbles. The pressurized flow in PFE also assists to solubilize air bubbles surrounding analytes that are found on the surface of the matrix also (Richter et al., 1996).

The Dionex ASE® 200 system consists of a solvent delivery component controlled by an HPLC pump, nitrogen gas purge valve, a carousel for extraction cells and collection vials as well as a waste vial (Figure 2.7).

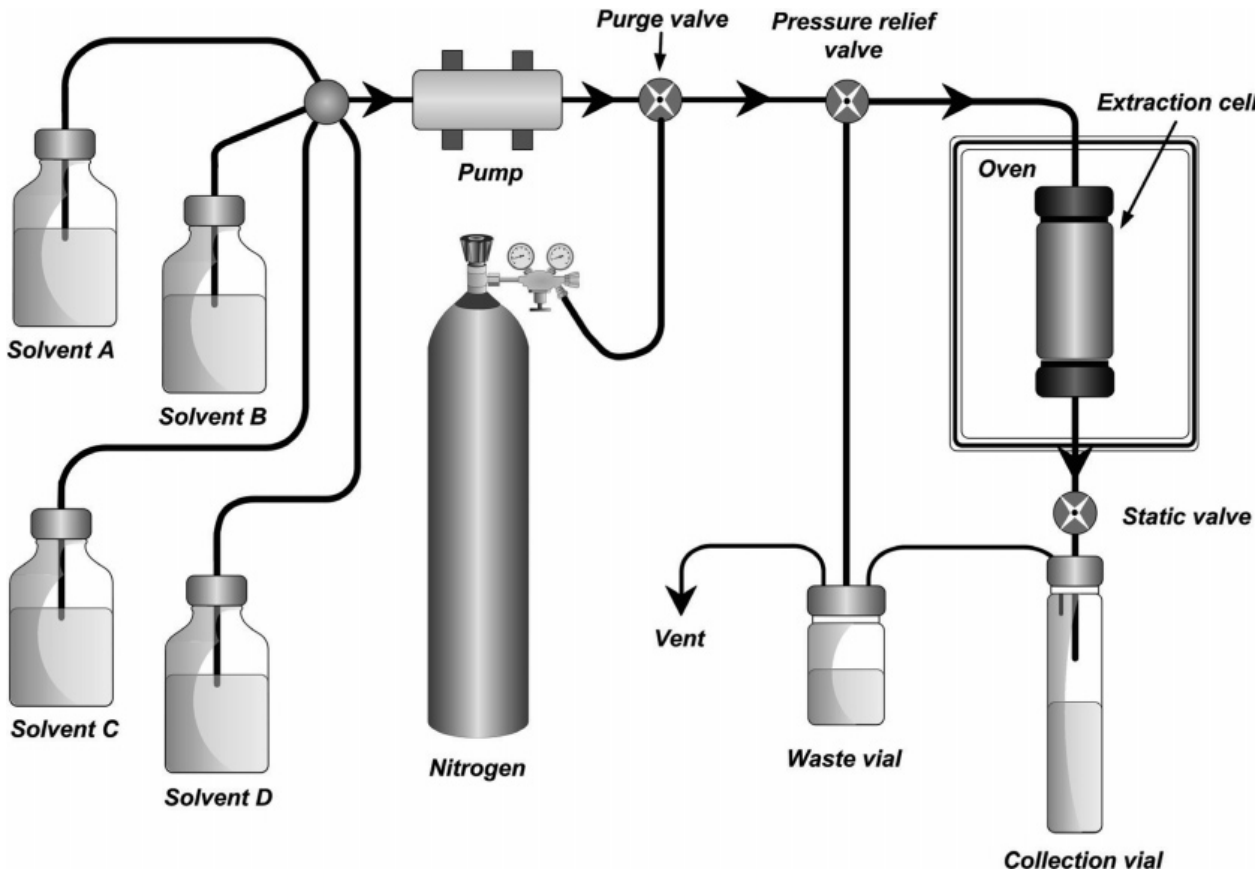


Figure 2.7: Diagram of a Dionex ASE® 200 pressurised fluid extraction system

Prior to extraction, a solid/ semi-solid sample is placed into a stainless steel extraction cell lined with a filter paper disk on the outlet end to prevent passage of solid matter from the cell into the collection vial. The extraction cell then placed onto a carousel and automatically drawn into the oven and filled with solvent. During extraction, the cell is heated, causing thermal expansion of the solvent and hence an increase of pressure inside the cell. The static and pressure relief valves function to regulate pressure inside the cell during static extraction by adding more solvent or opening the static valve to let solvent out of the extraction cell, whichever one is needed to maintain the desired pressure.

After static extraction, some of the solvent inside the extraction cell can be replaced by fresh solvent for a subsequent extraction cycle. This flush volume can vary from 5 to 150 % of the extraction cell. The introduction of fresh solvent increases the concentration gradient between the extraction solvent in the cell and the surface of the sample matrix resulting in improved mass transfer and consequently better extraction efficiency compared to a single cycle extraction (Richter et al., 1996). Finally, pressurized nitrogen purges the remaining solvent from the cell and lines to a collection vial. Parameters to optimize include

- Temperature (60 – 200 °C)
- Extraction time per cycle (5 – 10 min)
- Number of extraction cycles (1 – 5)
- Flush volume (50 – 80 %)
- Pressure (50 – 100 bar)

PFE has found applications in the environmental, food and pharmaceutical industries. Older applications of PFE frequently employed 100 % dichloromethane for the extraction of pesticides and PAHs (Zdrahal et al., 2000). However due to the carcinogenicity of dichloromethane, recent PFE applications have employed more environmentally friendly solvents such as n-heptane/acetone [1:1 v/v] at 100 °C and 3 x 10 min static extraction cycles for the extraction of DDT and its metabolites from aged contaminated soils (Hussen et al., 2006).

2.3 Scope of the thesis

This thesis presents ‘green’ sample preparation techniques employed in the profiling of water and sediments from the Okavango Delta, Botswana, for environmental contaminants. Solid phase extraction and solid phase microextraction were optimised for water samples while supercritical fluid extraction and pressurized fluid extraction were employed for sediment samples. Organochlorine pesticides were quantified on a gas chromatograph with electron capture detection (GC-ECD). The analytes were confirmed by gas chromatography- time-of-flight mass spectrometry.

Chapter 3

3 Experimental

3.1 Overview

The sampling of water and sediments, experimental procedures including optimizations of SPE, SPME, SFE and PFE techniques as well as GC-MS conditions are discussed in this chapter. SPE and SPME were employed for water samples while SFE and PFE were employed for sediments. The only parameter that was optimized during SPE was the eluting solvent while the fiber type, extraction mode, effects of temperature, ionic strength and stirring as well as the extraction time were optimized in SPME. Different settings of pressure, static extraction time and modifier types were investigated in the SFE of sediments. Extraction solvent type and temperature were optimized during PFE. Analytes were separated and quantified by GC-ECD and subsequently confirmed by high resolution GC-ToF-MS.

3.1.1 Standards, reagents and materials

α - BHC (97.9 %), β – BHC (98.0 %), γ – BHC (99.8 %), heptachlor (98.5 %) and methoxychlor (98 %) were obtained from Supelco (Bellafonte, PA, USA). Aldrin (98.1 %), trans - chlordane (98.4 %), 2, 4’ - DDD (99.7 %), 4, 4’ - DDD (98.9 %), 4, 4’ – DDE (99.5 %), 4, 4’ – DDT (99.6 %), dichlorvos (99.7 %), dieldrin (97.9 %), endrin (99.1 %), β – endosulfan (99.9 %) and HCB (99.6 %) were obtained from Riedel-de-Haën (Seelze, Germany). Stock solutions of each pesticide were prepared in

acetone at 100 mg L^{-1} concentrations (except for dichlorvos whose stock solution was 1000 mg L^{-1}) as well as intermediate standard solutions at 10 and 1 mg L^{-1} concentrations. For SPME work, a 1 – 10 mg L^{-1} working standard mixture was prepared containing 1 mg L^{-1} each of aldrin, α – BHC, γ – BHC, HCB and heptachlor, 2 mg L^{-1} each of 4,4' - DDE and dieldrin, 3 mg L^{-1} each of β – BHC, 4,4' - DDT and β – endosulfan, 4 mg L^{-1} each of 2,4' - DDD, 4,4' - DDD and endrin, 5 mg L^{-1} of chlordane as well as 10 mg L^{-1} of methoxychlor. The working standard mixture employed for SPE studies was similar to that employed for SPME work except that dichlorvos (50 mg L^{-1}) was used in place of methoxychlor due to lack of availability of methoxychlor at the time of study.

All solvents employed in the study were of HPLC grade. Acetone was obtained from Merck (Darmstadt, Germany), while ethyl acetate and n-heptane were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ultra pure water was obtained from a Milli-Q purification system by Millipore (Molsheim, France). Chem Tube Bond Elute Hydromatrix™ was from Varian Inc. (Palo Alto, Ca, USA) and glass beads (3 mm diameter) from Assistant (Sondheim, Germany).

Sodium chloride was obtained from Merck (Milan, Italy). HPLC/UV grade acetone, dichloromethane and n-hexane were obtained from Ultrafine Limited (London, England). Ultra high purity (UHP) water was generated from a Millipore Alpha-Q system supplied by Millipore (Molsheim, France). Silica SPME fibers (7 µm PDMS, 30 µm PDMS, 100 µm PDMS, 65 µm PDMS/DVB and 85 µm PA) and amber glass

screw cap vials (4 ml) for SPME with polytetra fluoroethylene (PTFE)/silicone septa (75 mm thick) were obtained from Supelco (Bellafonte, PA, USA). ISOLUTE C_{18} cartridges were obtained from International Sorbent Technology Ltd (Mid Glarmogan, UK).

3.1.2 Instrumentation

During water sampling, the conductivity, dissolved oxygen and pH were measured using Cond 330i/SET, Oxi 330i/SET and pH 340i/SET respectively, all manufactured by Wissenschaftlich – Technische Werkstätten (Weilheim, Germany).

Sediment samples were freeze-dried on a Modulyo 4K Freeze-dryer manufactured by Edwards High Vacuum (Crawley, England). A Speed SFE instrument manufactured by Applied Separations (Allentown, PA, USA) consisting of a Speed SFE pressure regulator, Speed SFE oven and a 5 ml extraction cell was employed for analyte extraction. A CO_2 (99.8 %) gas cylinder was connected to the pressure regulator.

For PFE of sediments, an ASE-200™ instrument by Dionex Corporation (Sunnyvale, CA, USA) was employed. Stainless steel Dionex extraction cells (22 ml) capped with PEEK seals and stainless steel frits were used together with glass collection vials (60 ml).

Initial experiments involving gas chromatographic separation and quantification of analytes after SPE, SPME and SFE were performed on an Autosystem XL gas chromatograph manufactured by Perkin Elmer (Norwalk, CT, USA) equipped with a split-splitless injector, a ^{63}Ni electron-capture detector (ECD). A Zebron ZB – 35 (35 % phenyl and 65 % dimethylsiloxane) fused silica capillary column 30 m x 0.25 mm x 0.25 µm (film thickness) manufactured by Phenomenex (Torrence, CA, USA) was employed in the separation of analytes. Ultra high purity (99.999 %) nitrogen gas was used as carrier gas at a column head pressure of 14 psi. The injector and detector temperatures were set to 250 and 300 °C, respectively. The temperature program is given on Table 3-1. The injection volume was 1 µl in the splitless mode.

Table 3-1 Temperature program for the separation of analytes on a ZB-35 column.

Ramp (°C/min)	**Temperature (°C)**	**Hold (min)**
-	50	1
40	200	2
4	240	1
4	300	5

Confirmation of analytes was carried out by transferring the ZB-35 column and oven temperature program employed in the Perkin Elmer GC-ECD system to a 6890N gas chromatograph equipped with a 7683B auto-sampler manufactured by Agilent

Technologies (Shanghai, China) coupled to a GCT Premier time-of-flight mass spectrometer manufactured by Waters (Manchester, England). Helium was used as a carrier gas at a rate of 1 ml/min. The injector, and transfer line temperatures were both maintained at 250 ºC while the detector was kept at 300 ºC. The electron impact (EI) source was operated at 70 eV and the mass spectra were acquired in the 50 – 500 *m/z* range. The solvent delay time was set to 3.8 min. A NIST/EPA/NIH version 2005 mass spectral library (Newfield NT, USA) was used for the identification of the separated pesticides.

PFE extracts were analyzed using a Varian 3800 GC connected to a Varian 1200 TripleQuad MS/MS system (Lake Forest, CA, USA). A Varian FactorFour (VF-5ms) column with dimensions 30 m x 0.25 mm x 0.25 µm was employed for separation. The GC was operated in the splitless mode with a helium carrier gas at a constant flow rate of 1.0 ml/min. The injection port program is given in Table 3-2.

Table 3-2 Temperature program on the injection port employed for the separation of pesticides on the Varian MS system.

Ramp (°C/min)	**Temperature (°C)**	**Hold (min)**
-	170	0.1
180	250	27.46

The oven temperature program is given in Table 3-3. The injection volume was 2 µl at all times.

Table 3-3 Temperature program for the separation of pesticides on the Varian FactorFour (VF-5ms) column.

Ramp (°C/min)	**Temperature (°C)**	**Hold (min)**
-	90	1
30	180	0.5
5	280	5
30	320	9.17

3.2 Water samples

3.2.1 Sampling

Water samples (100 ml) were collected in glass bottles between September 2005 and September 2008 from Chief's Island, Guma Lagoon, Lake Ngami, Maun, Mohembo, Samochima, Sepopa Water Swamp, Shakawe, Toteng and Xakanaxa in the delta (all sites are shown in Figure 3.1).

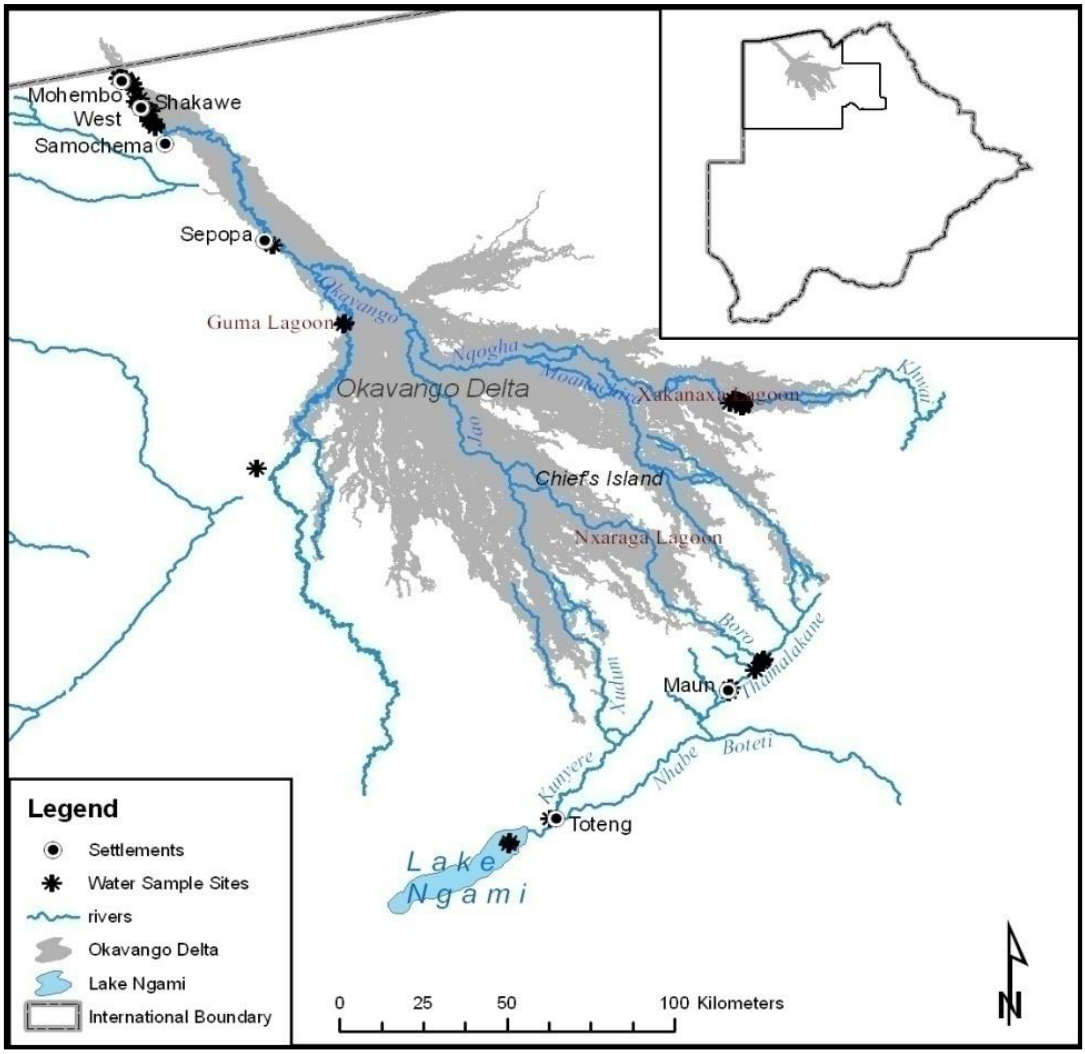


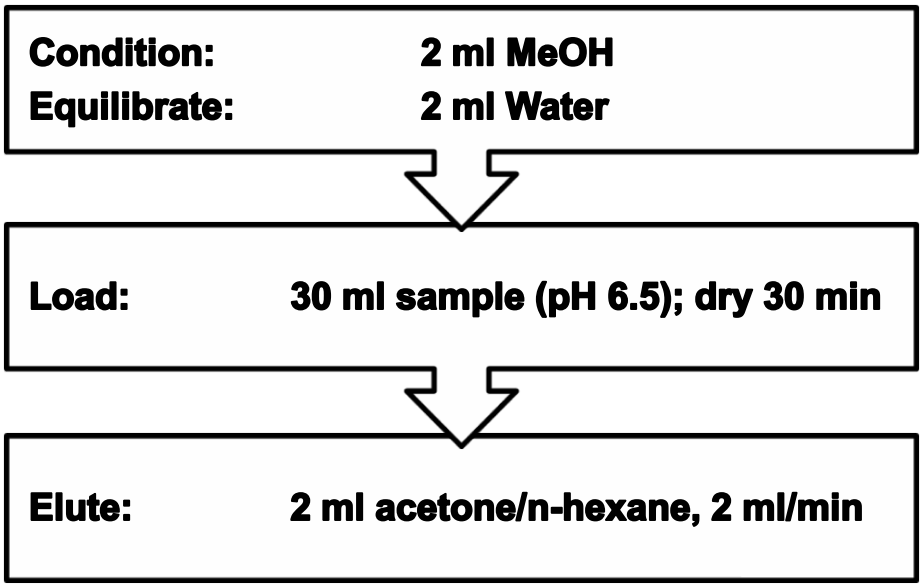
Figure 3.1: Map of the Okavango Delta showing water-sampling sites

The water conductivity, dissolved oxygen and pH were measured at each sampling point. Samples were acidified with 1 ml of nitric acid (1 M) and the bottles sealed immediately after sampling and stored in ice while in the field. Upon arrival at the laboratory the samples were filtered through 0.45 µm filter membranes to remove particulate matter and preserved in a cold room at 4 °C for a month prior to analysis.

3.2.2 SPE optimization

3.2.2.1 Optimization of the elution solvent

The pH of a 30 ml aliquot of ultra high purity water was adjusted to 6.5. The aliquot was spiked with 100 µl of the 1 – 50 mg L^{-1} standard mixture. The SPE procedure using ISOLUTE C_{18} sorbent is given in Scheme 3-1.



Scheme 3-1: SPE procedure employed for the water samples

Six elution solvents were investigated namely;

1. CH_2Cl_2 (100 %)
2. CH_2Cl_2/acetone (1:1 v/v)
3. CH_2Cl_2/MeOH (9:1)
4. CH_2Cl_2/MeOH (1:1 v/v)

5. Acetone/n-hexane (1:1 v/v)
6. $CHCl_3$/n-hexane (1:1 v/v)

The eluate was evaporated to complete dryness under a stream of N_2 gas. The analytes were reconstituted in 100 µl acetone/n-hexane (1:1 v/v). 1 µl was then injected into the GC-ECD and peak areas of each pesticide were compared to those of a 100 µl of standard mixture, which had been similarly dried and reconstituted in equal amounts of acetone and n-hexane. The elution solvent system that gave the highest recoveries for most pesticides was acetone / n-hexane (1:1 v/v) and hence was chosen for the analysis of water samples.

3.2.3 Evaluation of analytical parameters for SPE

The linearity of the SPE method was investigated by employing the optimized SPE conditions in the clean-up of aliquots of ultra pure water spiked with concentrations between (10 – 500) and (1000 – 50 000) µg L^{-1} of the standard mixture. Calibration concentrations ranged between 100 and 5 000 µg L^{-1} for SPE. The limits of determination of the SPE method (SPE – LDs) were defined as the lowest pesticides concentrations that could be determined from a sample employing SPE sample preparation procedure. They were calculated based on a signal-to-noise (S/N) ratio of 3:1 for individual peaks on the GC-ECD after SPE.

3.2.4 Quantification of water samples after SPE

Water samples (pH adjusted to 6.5) were cleaned and pre-concentrated by employing the optimized SPE. Pesticides were quantified by GC-ECD and confirmed by GC-ToF-MS.

3.2.5 SPME optimization

Before use, the fibers were conditioned for 2 h in the injection port of the GC-ECD according to the manufacturer's instructions while maintaining the GC oven and detector at 200 and 300 °C, respectively. A blank injection was performed to confirm removal of impurities from the GC system. Other blank experiments also carried out were those of fiber blanks, a blank of the fiber inserted into an empty vial, a vial containing 0.5 g NaCl and a vial containing 2 ml of ultra pure water. For all the optimization experiments, a 2 ml aliquot of ultra high purity water (adjusted to pH 6.5) was placed into a 4 ml vial and spiked with 20 µl of 1 - 10 mg L^{-1} pesticides standards mixture. Initially the extractions were carried out at 60 °C and the extraction time set to 30 min. Thermal desorption on the GC injection port was carried out at 250 °C for 5 min.

3.2.5.1 Fiber selection

The extraction efficiencies of the available fibers were evaluated by direct immersion of the fibers into spiked water aliquots as described in section 3.2.5. The peak areas of pesticides desorbed from each SPME fiber were compared. PDMS/DVB fiber gave the highest peak areas and hence it was chosen for further experiments.

3.2.5.2 Extraction mode

Both direct immersion and headspace extraction modes were evaluated employing PDMS/DVB fiber and the peak areas were compared. The headspace mode gave higher peak areas of the analytes.

3.2.5.3 Effect of temperature

The effect of temperature on the extraction efficiency was investigated at temperatures 40, 60 and 80 °C. Highest extraction efficiencies were obtained at a temperature of 80 °C.

3.2.5.4 Effect of ionic strength

The effect of ionic strength on the extraction efficiency was investigated by adding 10, 20, 30, 40 and 50 % of NaCl (w/v) to the spiked water. The vials were covered and swirled for 1 min to dissolve the salt before extraction. Addition of 10 % NaCl gave the highest extraction efficiencies.

3.2.5.5 Effect of stirring

The effect of agitation on the extraction efficiency was studied at a maximum speed of 300 rpm. A precaution was taken to modify the stirring bar by covering it with glass to prevent adsorption of pesticides onto the stirrer coating [Derouiche et al., 2007].

No positive effect was observed hence no stirring was employed for subsequent experiments.

3.2.5.6 Optimization of extraction time

The extraction profiles of pesticides at 80 °C were constructed for different times (15, 30, 45 and 60 min). The optimal time was 30 min.

3.2.6 Evaluation of analytical parameters for SPME

The linearity of the SPME method was determined with spiked concentrations between 0.1 and 100 ng L^{-1} and calibration concentrations ranged between 0.001 and 0.010 µg L^{-1}. The SPME method determination limits (SPME – LDs) were defined as the lowest pesticides concentrations that could be determined from a sample employing SPME sample preparation procedures. They were calculated based on a signal-to-noise (S/N) ratio of 3:1 for individual peaks on the GC-ECD after SPME.

3.2.7 Quantification of pesticides in water

The optimized HS-SPME conditions were applied to the sixty-one water samples (pH adjusted to 6.5) collected from the different sampling points in the Delta shown on

Figure 3.1. GC-ECD was employed for quantification while GC-ToF-MS was used for confirmation of analytes.

3.3 Sediments

3.3.1 Sampling

Samples were collected annually during the months of September or October from 2005 to 2008 from Chief's Island, Guma Lagoon, Lake Ngami, Maun, Mohembo, Samochema, Sepopa, Shakawe, Toteng and Xakanaxa in the Okavango Delta. Figure 3.2 shows a map of the sampling sites.

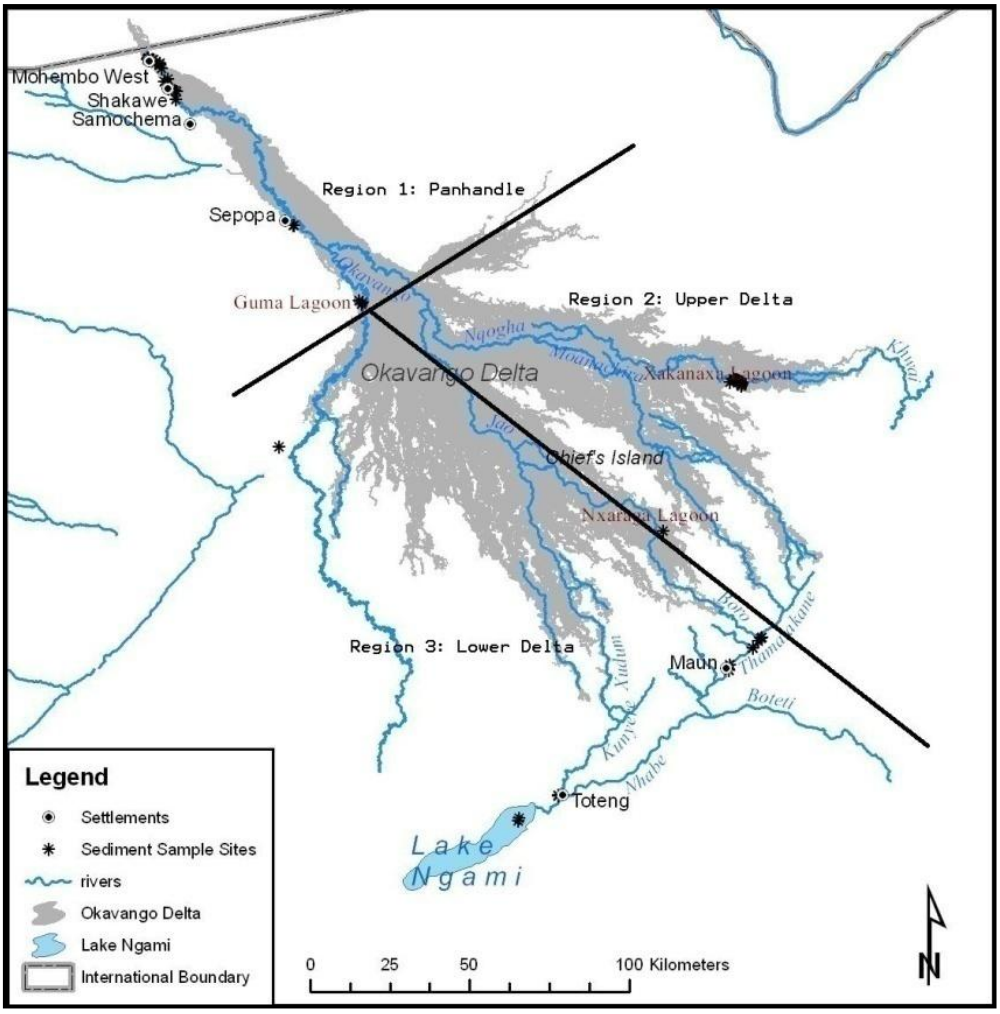


Figure 3.2: Map of the Okavango Delta showing sediment sampling sites. For ease of data analysis and data interpretation, samples were divided into the 3 regions indicated on the map.

Sediment samples (≈ 100 g) were collected using a sediment corer. The samples were wrapped in aluminium foil previously rinsed with acetone and dried under an extraction hood. The foil packages were then placed into labeled plastic bags with a tight seal and stored in ice while on the field. Upon arrival at the laboratory, the samples were preserved in a cold room at 4 °C prior to analysis within a month after sampling.

3.3.2 Sample preparation

Sediment samples were placed into 250 ml round - bottomed flasks, covered with aluminium foil and frozen in a deep-freezer set at -10 °C before being lyophilised overnight to remove water. The flasks were then removed and the sediment shaken to break lumps before being passed through a 100 µm sieve to remove rocks, roots and other debri. The sediment samples were wrapped in 10 x 10 cm pieces of acetone-rinsed aluminium foil, sealed in plastic bags and stored at 4 °C for a maximum of one week.

3.3.3 Testing of different SFE conditions

In order to reduce laborious investigations of effects of the individual parameters such as pressure, extraction time and modifier type and volume influencing recovery during SFE, three different combinations of parameters were evaluated. Oven and outlet valve temperatures were maintained at 60 and 100 °C, respectively. Glass wool (rinsed with acetone and dried) was placed at both ends of the extraction cell to prevent pieces of sediment being drawn into the capillaries of the instrument. The mass of sediment chosen for each extraction was 3 g as it left enough room for glass wool at both ends of the extraction cell. A sediment sample, dark in colour indicating high organic matter content was chosen as a ‘test’ sample and was employed to compare three SFE protocols.

Three different combinations of SFE parameters were investigated and are given in Table 1. For each setting of SFE parameters, three aliquots of an unspiked sample were extracted so as to obtain background pesticides concentrations that would be subtracted from those in the spiked sample. 1 000 µl of the 1 to 10 µg/ml standard mixture was employed for spiking each of the 3 g aliquots. In the first set of parameters, each of the 3 g aliquots was spiked, mixed and air-dried under a fume-hood prior to loading into the extraction cell. A modifier was added to each spiked aliquot for settings 2 and 3 as shown in Table 3-4. Due to the historical exposure of DDT to the Delta's sediments, it was assumed that DDT metabolites would be strongly bound to the matrix hence a need to employ long static times during the extraction process.

Table 3-4: Parameters of the 3 tested SFE settings

SFE Setting	Modifier & volume	Step (i)		Step (ii)		Step (iii)	
		Static	**Dynamic**	**Static**	**Dynamic**	**Static**	**Dynamic**
1	-	400 bar, 120 min	5 min	-	-	-	-
2	H_2O, 100 µl	200 bar, 60 min	5 min	400 bar, 60 min	5 min	-	-
3	Acetone, 50 µl	200 bar, 30 min	5 min	350 bar, 30 min	5 min	400 bar, 15 min	5 min

The extraction cell was then placed into the SFE oven that had been pre-set to the required conditions. The extracts were collected in 1 ml toluene contained in 10 cm x 1 cm test tubes with the tip of the outlet-valve needle just above the collection solvent. At the end of each collection the needle on the outlet valve was rinsed with 1 ml acetone. The extract in toluene was transferred to a 2 ml glass vial and evaporated completely. Analytes were reconstituted in 50 µl acetone and 50 µl n-hexane, mixing well after each addition and 1 µl injected into the gas chromatograph. Background pesticide concentrations in the un-spiked samples were subtracted from those in the spiked samples. The subsequent pesticide concentrations were compared to those in a 1 000 µl volume of standard mixture that had been similarly evaporated completely and reconstituted in 50 µl each of acetone and 50 µl n-hexane.

3.3.4 Quantification of pesticides in sediment samples after SFE

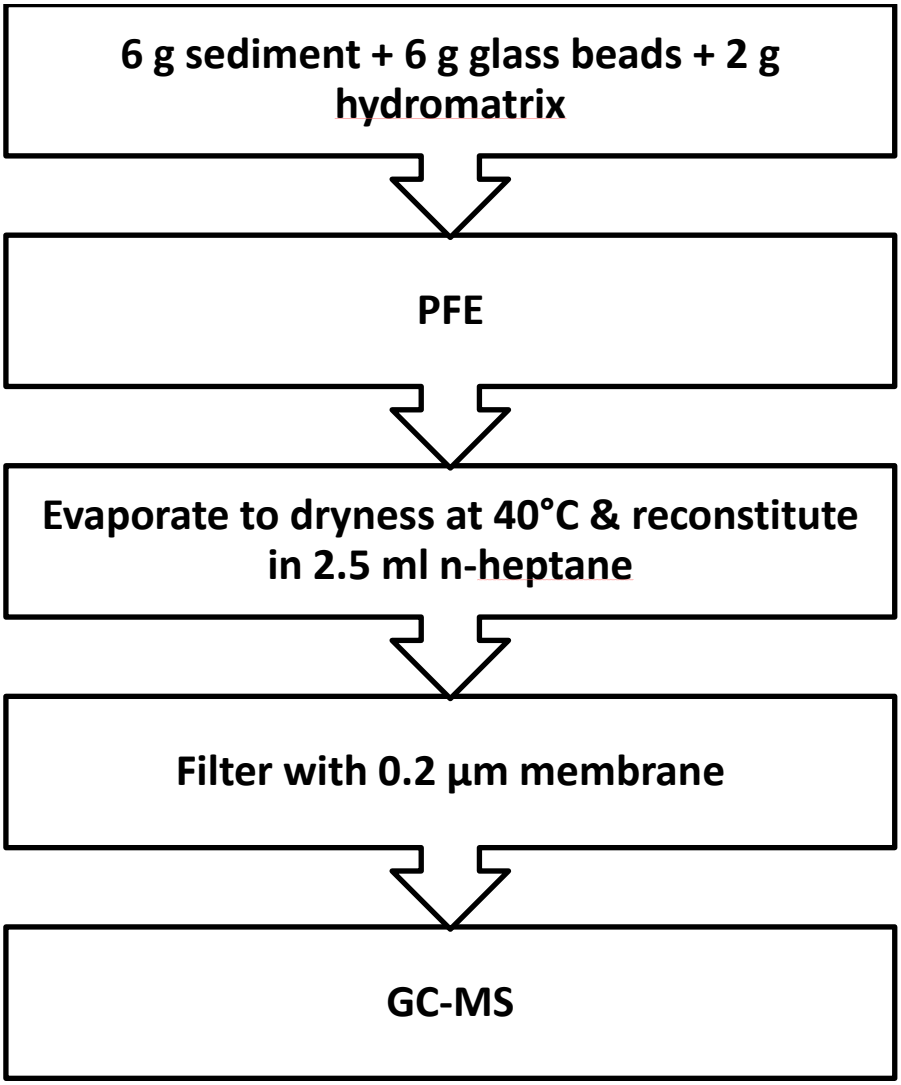
After optimization of SFE conditions, 3 g sediment samples were extracted employing optimal SFE conditions and the extracts analysed by GC-ECD. After each extraction, care was taken to clean the extraction cell thoroughly with water and soap before rinsing with acetone and n-hexane. The analyses were performed in triplicate.

3.3.5 Pressurized fluid extraction

As explained in section 2.2.2, the principle behind PFE is based on the use of elevated temperatures and pressures to extract analytes from sample matrices within short periods of time (5-10 min). In this work, the viability of PFE in the profiling or screening of sediments for pesticides was investigated. A sediment sample was obtained from the lower Delta (in Maun, the sampling location with the highest human population within the study area) and employed for screening by PFE, varying the extraction temperature and solvent.

3.3.5.1 Extraction procedure

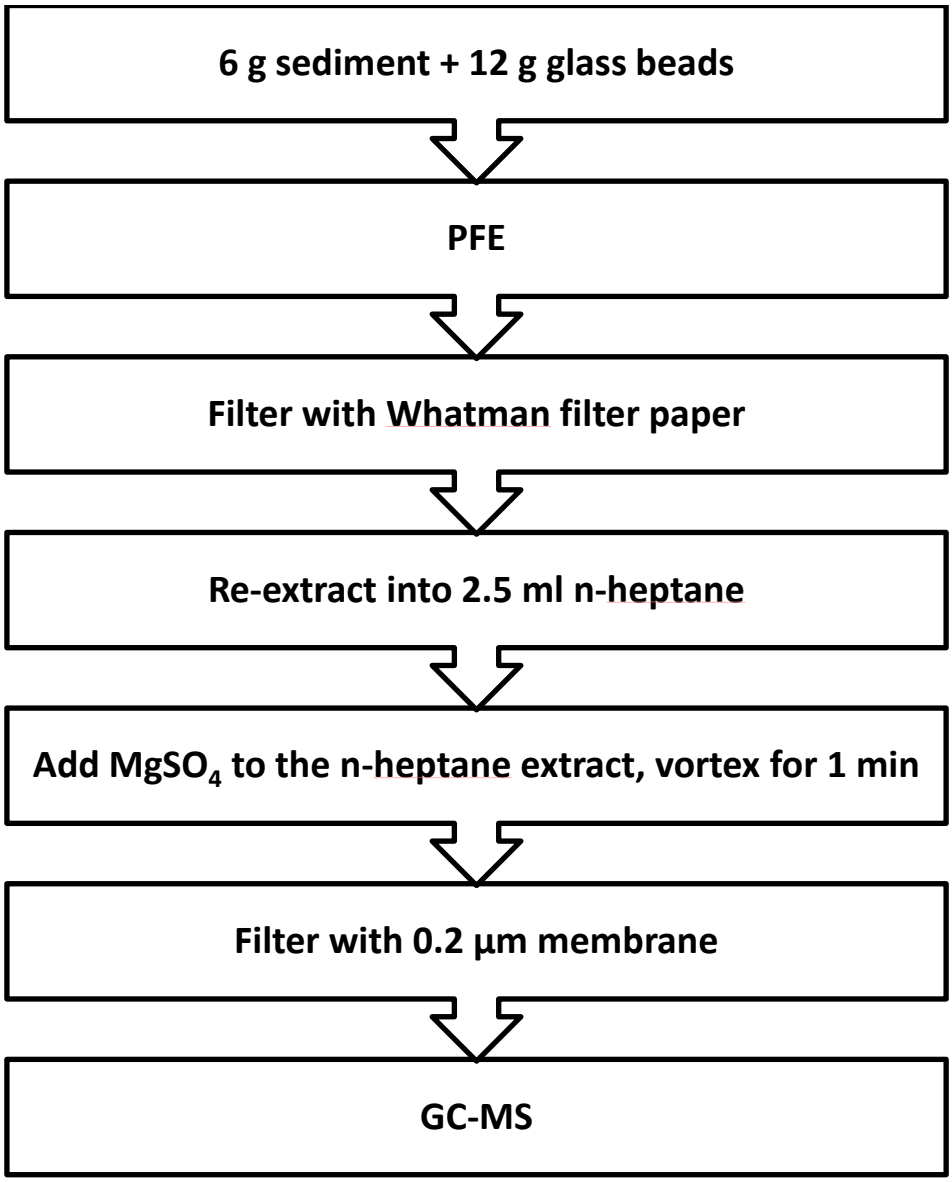
Three different solvents (ethyl acetate, n-heptane/acetone [1:1 v/v] and water) were investigated for their extraction capacity each at 50, 80 and 120 °C. A cellulose filter was placed at the outlet end of the extraction cell to prevent clogging of metal frits by sediment particles. The procedure for extracting sediment with pressurized ethyl acetate and n-heptane/acetone [1:1 v/v] is outlined in Scheme 3-2.



Scheme 3-2: Experimental procedure with pressurized ethyl acetate and n-heptane/acetone [1:1 v/v].

Extraction was carried out over 3 cycles at a pressure of 50 bar. Each cycle consisted of a static extraction period of 5 min, with a flush volume of 60 % in between the cycles (i.e. 60 % of the extraction solvent is replaced with fresh solvent). After the end of the last extraction cycle, the sample cell was purged with nitrogen gas for 60 s to completely remove and collect all extract. The hydromatrix was

omitted for extractions with water and instead, the mass of the glass beads was doubled to enable the mixture to fill the extraction vessel, as shown in Scheme 3-3.



Scheme 3-3: Experimental procedure for extraction with pressurized water

Extracts were re-extracted into n-heptane for compatibility of the extracts with the GC column coating. The more commonly used n-hexane was avoided due toits carcinogenicity.

Chapter 4

4 Results and discussions

This chapter presents data and observations made for the optimization of experimental parameters and analytical response characteristics of SPE and SPME as well as SFE and PFE employed for the sample preparation of water and sediments, respectively.

4.1 Water samples

This subsection presents results obtained for water samples employing SPE and SPME techniques. First to be discussed are the water quality parameters namely, conductivity, dissolved oxygen and pH that were measured during sampling as these give an indication of pollution levels in water bodies.

4.1.1 Water quality parameters

Water quality parameters were noted during sampling and for ease of data analysis, the different sampling points were classified according to distance from the reference point – Mohembo as shown in Table 4-1.

Table 4-1: Distances of sampling points with respect to the reference point - Mohembo.

Sampling village	Relative distance (km)
Mohembo	0.0 – 10.0
Shakawe	10.1 – 20.0
Samochema	20.1 – 30.0
Sepopa	60.1 - 70.0
Guma Lagoon	100.1 – 110.0
Xakanaxa	210.1 – 220.0
Maun	260.1 – 270.0
Lake Ngami	270.1 – 280.0

4.1.1.1 Conductivity

Mean electrical conductivities of the delta's water showed an increase from 30.9 µS cm^{-1} in samples at the entry point (0.0 km relative distance) to 101.0 µS cm^{-1} in Maun (260.1 – 270.0 km relative distance). Figure 4.1 shows that higher mean electrical conductivities were recorded at sampling points furthest from the delta water's point of entry (Mohembo).

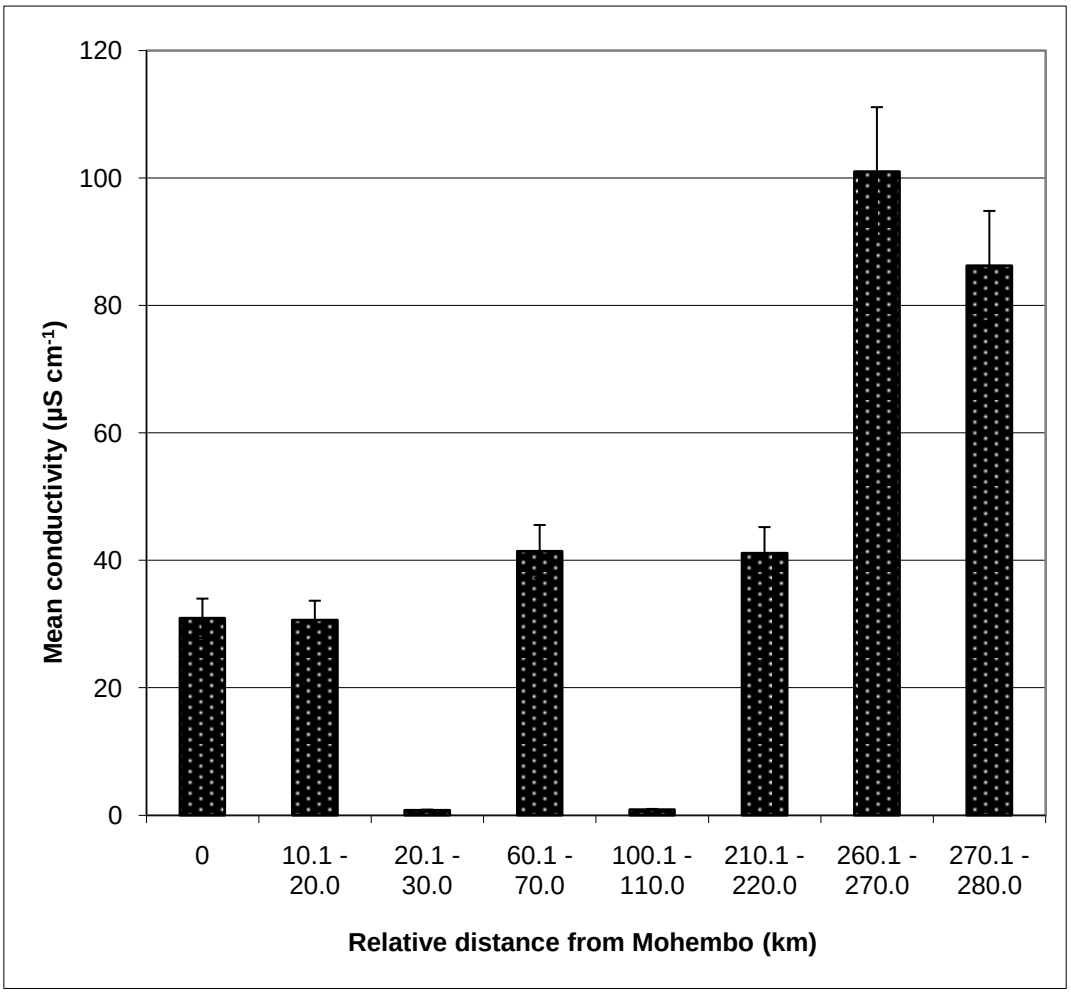


Figure 4.1: Average conductivity of the water samples in relation to distance from the point of entry (Mohembo). Error bars represent ±% RSD at n=3.

The higher conductivities downstream could be attributed to the flat floodplains that spread out the water in the alluvial fan, resulting in the loss of large volumes of water through evaporation-transpiration processes. These cause a concentration effect of dissolved salts in the lower Delta, thus increasing conductivity levels. Nevertheless, the conductivity levels of surface water in the lower Delta, though higher than those upstream, remain low. This is because considering that almost all of the water flowing into the Okavango Delta is eventually lost to evaporation or transpiration by plants resulting in depositions of around 300 000 tons of dissolved salts per year,

conductivities of the Delta's water would be expected to be extremely high (Zimmermann et al., 2006).. This is due to the accumulation of salts under islands (McCarthy et al., 2005; Zimmermann et al., 2006) and removal of the salts by a combination of seasonal flooding, uptake of solutes in peat (McCarthy et al., 1989) as well as groundwater leakage (McCarthy and Metcalfe, 1990). McCarthy and co-workers (1991) suggested that saline water produced by transpiration seeps into central open pans within the Delta where evaporation causes further concentration of salts. The dense brine slowly percolates downwards resulting in extremely high concentrations of dissolved salts in the groundwater. In their study, Bauer-Gottwein and colleagues (2007) demonstrated that a density-driven flow (precipitation of salts onto islands due to evaporation and transpiration) was an important salt removal process in the Okavango Delta.

4.1.1.2 Dissolved Oxygen

No particular trend of mean DO values with respect to distance from Mohembo was observed on the water samples as shown on Figure 4.2. This is to be expected given that DO levels are more of a function of habitat (i.e. DO is generally higher in channels and open water bodies (e.g. lagoons) while stagnant vegetation filled water bodies such as shallow pools are usually characterized by lower DO levels). A majority of sampling points had mean dissolved oxygen values between 2.7 and 5.5 mg L^{-1} that are higher than the minimum (2.4 mg L^{-1}) required by aquatic life (Koukal et al., 2004).

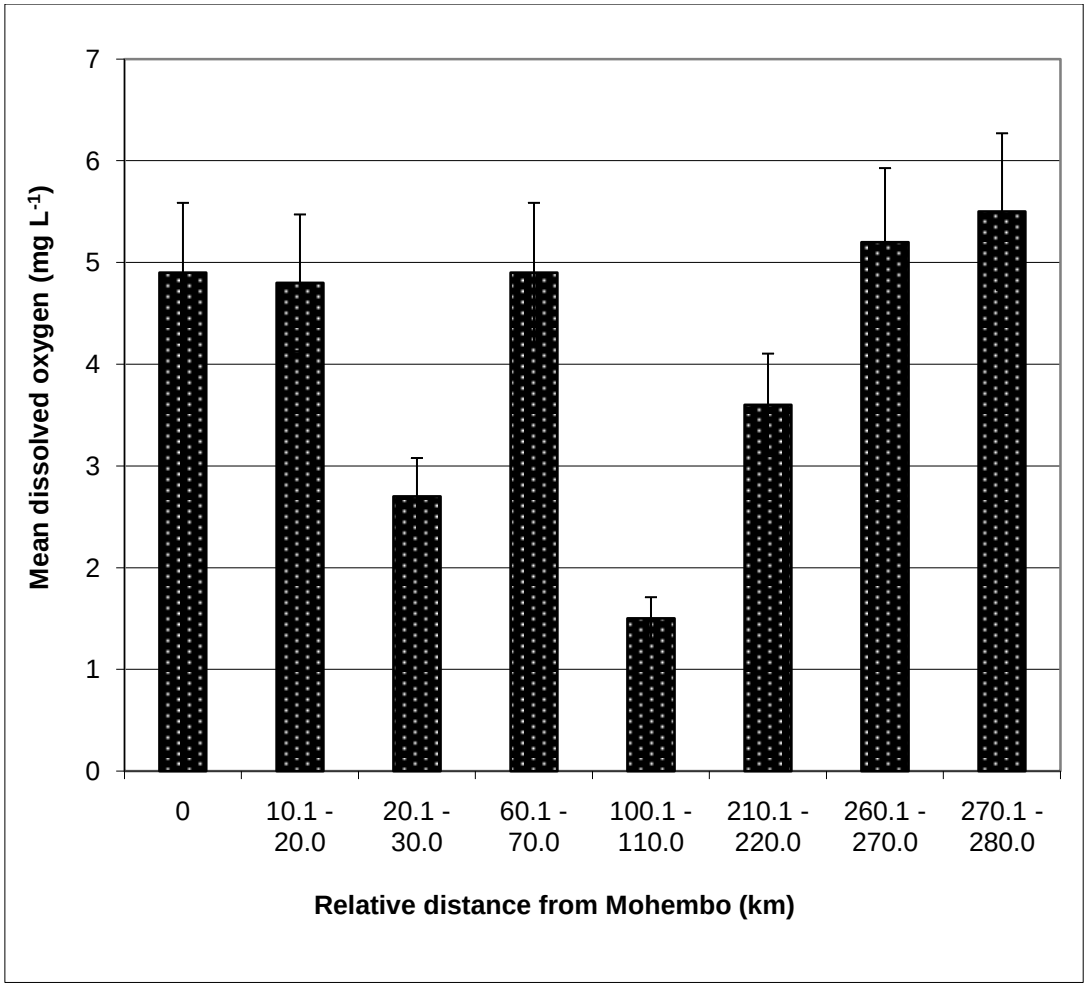


Figure 4.2: Mean dissolved oxygen values of the water samples collected in relation to distance from the point of entry. Error bars represent ±% RSD at n=3.

The obtained DO values are within the range observed by Masamba and Mazvimavi (2008) who reported drops in DO at the flood fronts in the thamalakane-Boteti

4.1.1.3 pH

Water at Xakanaxa (210.1 – 220.0 km relative distance) had the lowest mean pH (6.7) while water at Toteng and Lake Ngami (270.1 – 280.0 km relative distance) was the most alkaline (pH 9.0). Figure 4.3 shows that the pH of the water was

generally near neutral with all sampling points (except the furthest) having mean pH values between 6.7 and 8.3 that are within the recommended range (6.5 - 8.5) for portability (WHO, 1984).

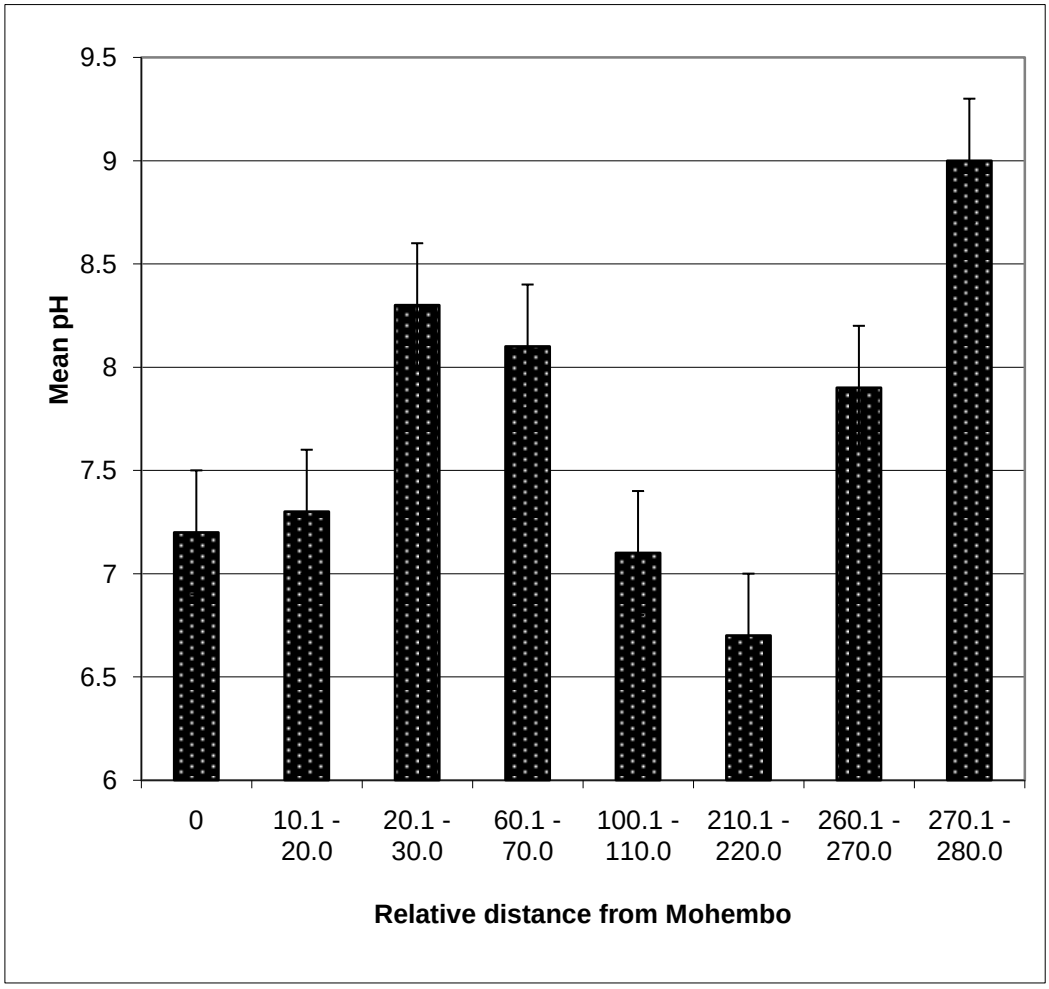


Figure 4.3: Mean pH of water samples in relation to distance from the point of entry. Error bars represent ±% RSD at n=3.

4.1.2 SPE optimization

4.1.2.1 Optimization of the elution solvent

The elution solvent was optimized during SPE on ISOLUTE C_{18} sorbent by comparing recoveries of pesticides after elution of 30 ml aliquots of ultra high purity water spiked with different solvent systems listed in section 3.2.2.1.

Figure 4.4 shows that the acetone/n-hexane (1:1 v/v) elution solvent (E) generally gave the highest recoveries for most pesticides. However, dichlorvos was the least recovered pesticide at 52.6 % recovery while 2, 4'-DDD had the highest recovery of 117.8 % in this solvent system.

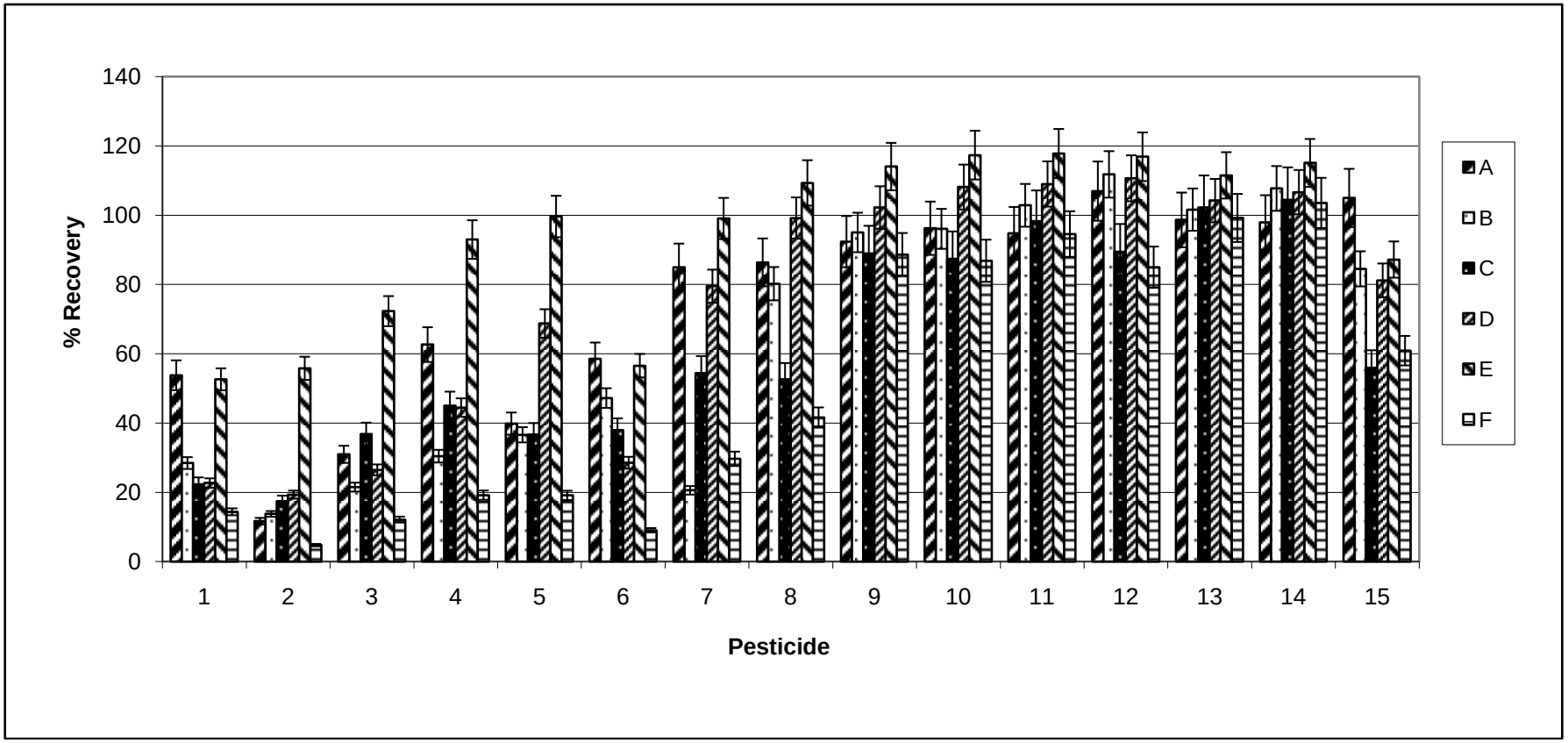


Figure 4.4: Recoveries of pesticides after elution with six different solvent systems namely: A [CH_2Cl_2]; B [CH_2Cl_2/MeOH] (9:1 v/v)]; C [CH_2Cl_2/MeOH (1:1 v/v)]; D [CH_2Cl_2/acetone (1:1 v/v)]; E [$CHCl_3$/n-hexane (1:1 v/v)] and F [acetone/n-hexane (1:1 v/v)]. Pesticides are as follows: 1 = Dichlorvos; 2 = Hexachlorobenzene (HCB); 3 = α-Benzenehexachloride (α-BHC); 4 = γ-Benzenehexachloride (γ-BHC); 5 = β- Benzenehexachloride (β-BHC); 6 = Heptachlor; 7 = Aldrin; 8 = Chlordane; 9 = 4, 4' – DDE; 10 = Dieldrin; 11 = 2, 4' – DDD; 12 = Endrin; 13 = 4, 4' – DDD; 14 = β-endosulfan; 15 = 4, 4' – DDT. Error bars represent ±% RSD at n=3.

The lower recovery of dichlorvos may be due to its polar character hence weak retention on the non-polar C_{18} sorbent as compared to the other pesticides. Acetone/n-hexane (1:1 v/v) mixture was chosen for further clean up of samples since it gave the highest extraction yields for most pesticides.

4.1.3 Evaluation of analytical parameters for SPE

Analytical parameters were obtained for SPE by the analysis of different spiked ultra pure water samples employing pesticides standards mixtures described in section 3.2.3. Linear relationships were obtained between peak areas and the analyte concentrations, with high correlation coefficients (≥ 0.9998). Table 4-2 shows that aldrin and α - BHC were the most recovered pesticides after SPE clean-up as evidenced by the lowest SPE - LDs of 31.0 and 510.0 µg L^{-1}, respectively. Precision was determined by reproducibility studies expressed by percent relative standard deviation (% RSD) of 3 spiked water aliquots and was less than 15 % for both methods.

Table 4-2: Analytical parameters obtained for SPE sample preparation method and subsequent analysis of pesticides by GC-ECD

Parameter	**SPE**
Linearity (µg L^{-1})	10 – 50 000
R^2	0.9980 – 0.9994
LDs (µg L^{-1})	31.0 – 510.0
% RSDs	5.3 – 15.0

4.1.4 Analysis of water samples after SPE

The optimized SPE conditions were applied to the clean-up of water samples. The general profile of water samples collected along the main channel of the delta – also known as the 'Panhandle' (Mohembo, Shakawe, Samochema, Sepopa and Guma Lagoon) – differed from that of samples collected downstream (Maun, Toteng and Lake Ngami) next to lodges and villages.

Compounds identified upstream were absent downstream probably due to degradation or change in pH from near-neutral upstream to alkaline downstream. Figure 4.5 shows a typical profile of the water samples upstream.

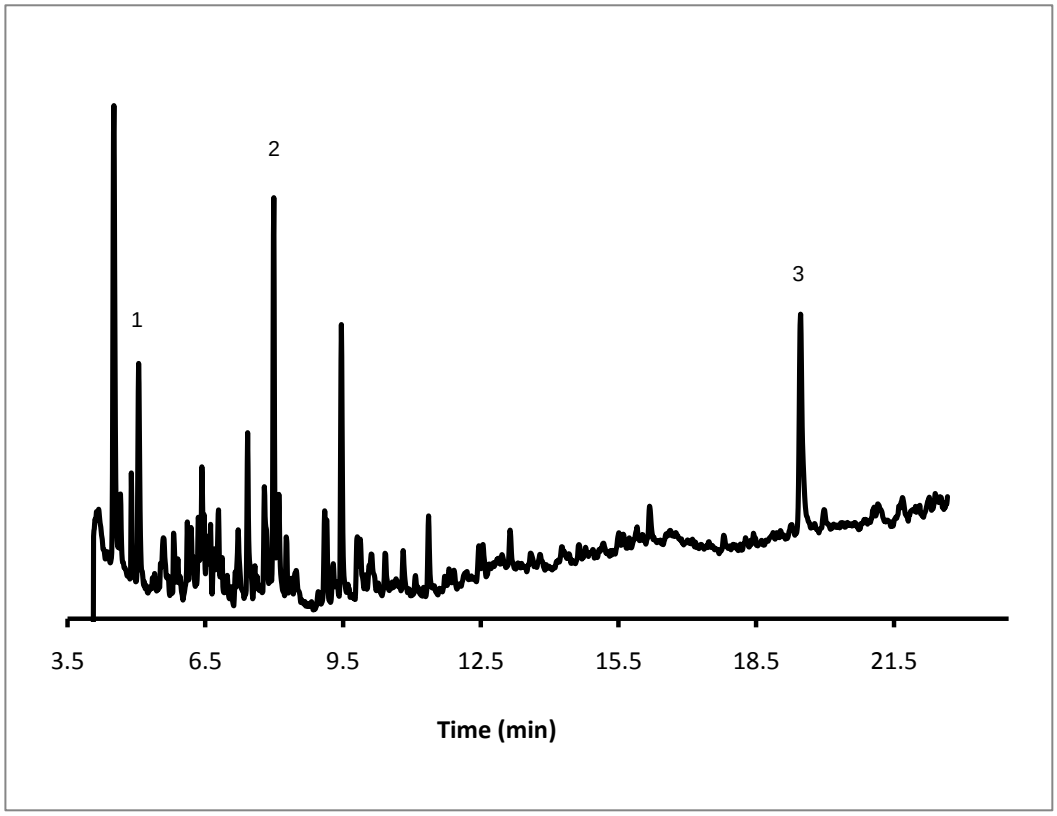


Figure 4.5: Chromatogram of a typical upstream water sample after SPE and GC-ECD showing (1) dodecamethylcyclohexasiloxane, (2) alpha-hexylcinnamaldehyde and (3) diethylhexylphthalate (DEHP). The rest of the peaks could not be identified with the help of the GC/MS NIST library since their spectra matched the suggested library ones by less than 80 %.

Dodecamethylcyclosiloxane is employed in a variety of industrial products including household and car care products as well as chemical formulations (EPA, 1992). DEHP is one of the main phthalates used as a plasticiser in the production of PVC and has been classified by the Environmental Protection Agency (EPA) as a possible human carcinogen substance (Kayali et al., 2006).

Water samples collected downstream next to lodges and villages showed the presence of hydrocarbons such as dodecane ($C_{12}H_{26}$) hexadecane ($C_{16}H_{34}$), octadecane ($C_{18}H_{38}$), eicosane ($C_{20}H_{42}$), and 1,1,3,3-tetramethyl-1,3-dioctadecyldisiloxane (Figure 4.6).

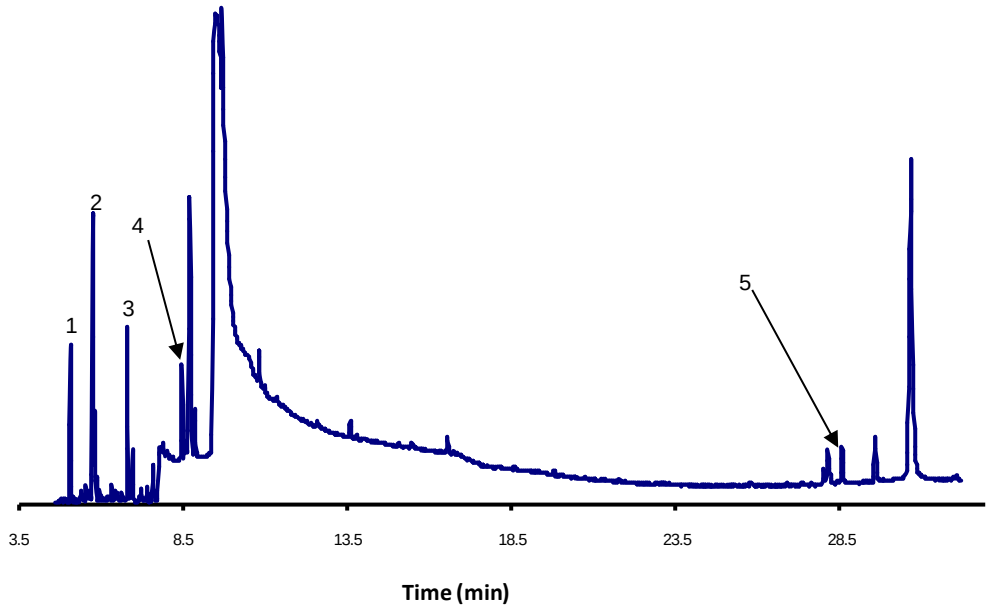


Figure 4.6: Chromatogram of a typical water sample from downstream showing (1) hexadecane, (2) octadecane, (3) eicosane, (4) dodecane and (5) 1,1,3,3-tetramethyl-1,3-dioctadecyldisiloxane. The rest of the peaks could not be identified with the help of the GC/MS NIST library since their spectra matched the suggested library ones by less than 80 %.

Hydrocarbons are naturally found in unpolluted environments as a result of biotransformation of plant materials but may also occur due to contamination from

petroleum spills of combustion processes (Perfumo et al., 2007). Low molecular weight hydrocarbons (n-C_{14} – n-C_{19}) are indicative of degradation of plant matter while high molecular hydrocarbons (>n-C_{20}) suggest possible petroleum contamination (Zaghden et al., 2005). Even though hydrocarbon standards were not available for quantification, the presence of eicosane - a high molecular weight hydrocarbon – in water samples collected in the vicinity of lodges and villages shows possible petroleum contamination of the delta's water due to point source pollution. Table 4-3 shows compounds detected in samples from the Panhandle and those from downstream after SPE clean-up.

Table 4-3: Compounds detected in the water samples after SPE and GC-ECD with subsequent confirmation by GC-ToF-MS.

Compound	Possible source	Region
Dodecamethylcyclohexasiloxane	- chemical formulations	Panhandle (upstream)
Diethylhexylphthalate	PVC products	
α-hexylcinnamaldehyde	- vegetation - wooden boats	
Hydrocarbons - dodecane	- plant materials - petroleum spills	Downstream
- hexadecane		
- octadecane		
- eicosane		
1,1,3,3-tetramethyl-1,3-dioctadecyldisiloxane	PVC products	

4.1.5 SPME optimization

The concentration of analyte extracted at equilibrium is proportional to the volume of the stationary phase and the partition coefficient. Hence the sensitivity and limit of detection of the method is dependent on the partition coefficient. Several parameters were optimized to enhance the partitioning of pesticides onto the SPME fiber.

4.1.5.1 Fiber selection

The most important factor affecting the magnitude of the partition coefficient is the affinity of the analyte to the fiber coating hence the relevance of optimization of the fiber type. Figure 4.7 shows results of extraction of 15 pesticides using 5 commercial SPME fibers, namely 7 µm PDMS, 30 µm PDMS, 100 µm PDMS, 65 µm PDMS/DVB and 85 µm PA. PDMS/DVB fiber gave the highest peak areas followed by 30 µm PDMS and PA fibers while 7 µm PDMS performed poorly for most pesticides.

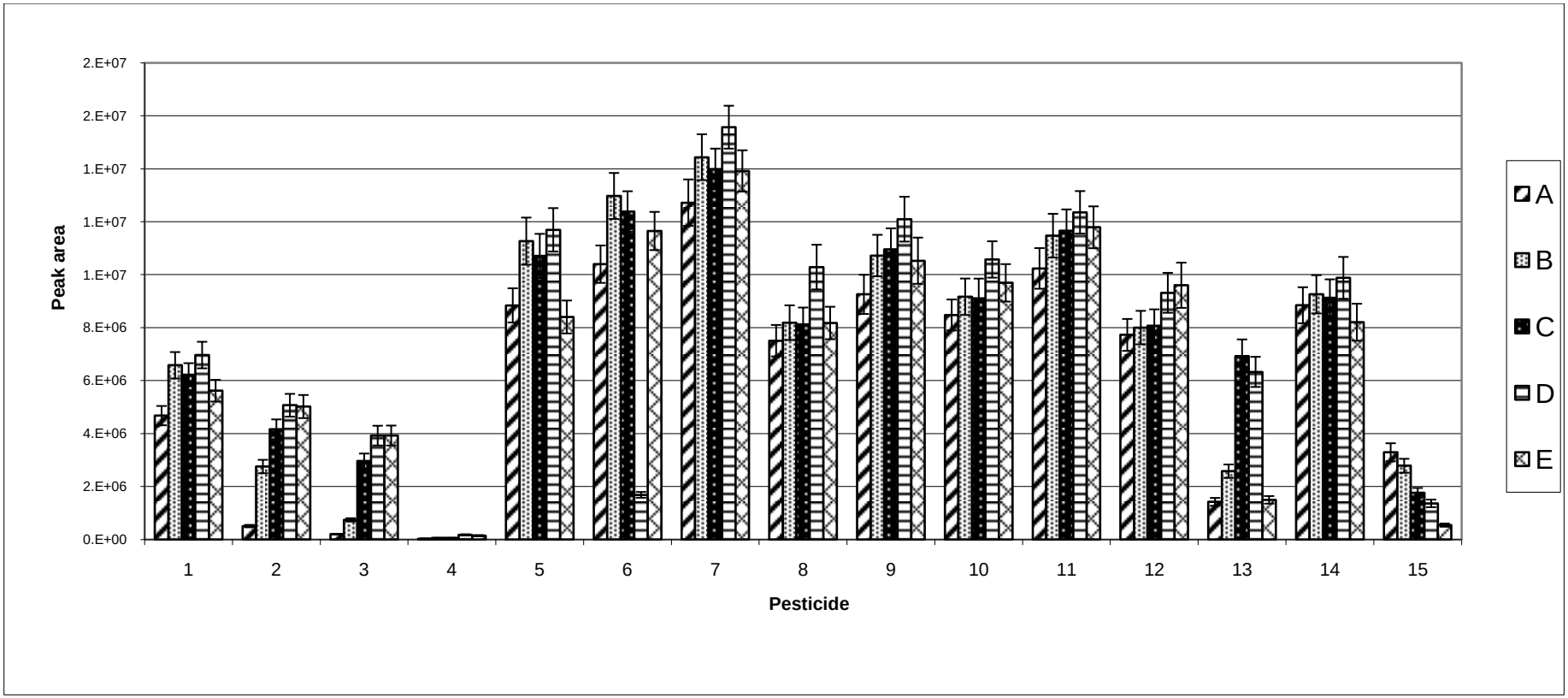


Figure 4.7: Comparison of extraction efficiencies of five SPME fibers: A = 7 µm PDMS; B = 30 µm PDMS; C = 100 µm PDMS; D = 65 µm PDMS/DVB; E = 85 µm PA. Pesticides are as follows: 1 = Hexachlorobenzene (HCB); 2 = α-Benzenehexachloride (α-BHC); 3 = γ- Benzenehexachloride (γ-BHC); 4 = β- Benzenehexachloride (β-BHC); 5 = Heptachlor; 6 = Aldrin; 7 = trans-chlordane; 8 = 4, 4' – DDE; 9 = Dieldrin; 10 = 2, 4' – DDD; 11 = Endrin; 12 = 4, 4' – DDD; 13 = β-Endosulfan; 14 = 4, 4' – DDT; 15 = Methoxychlor. Error bars represent ±% RSD at n=3.

Some pesticides such as HCB, α – BHC, β – BHC, γ – BHC, 4,4’ – DDE, endrin and 4,4’-DDD were extracted onto PA fiber (that is the most suitable for polar compounds) with efficiencies similar to PDMS/DVB fiber. Methoxychlor was the only pesticide that was extracted onto 7 µm PDMS with the highest efficiency. PDMS/DVB proved to be the best fiber coating for a majority of the pesticides in this study and hence was chosen for further experiments.

4.1.5.2 Extraction mode

As expected for semi-volatile and volatile compounds, HS-SPME was more sensitive than the DI mode (Figure 4.8) hence the HS mode was selected for the extraction of the target analytes. Moreover, HS sampling eliminates competition for adsorption sites on the fiber coating by non-volatile compounds present in the liquid sample (Palau et al., 2007).

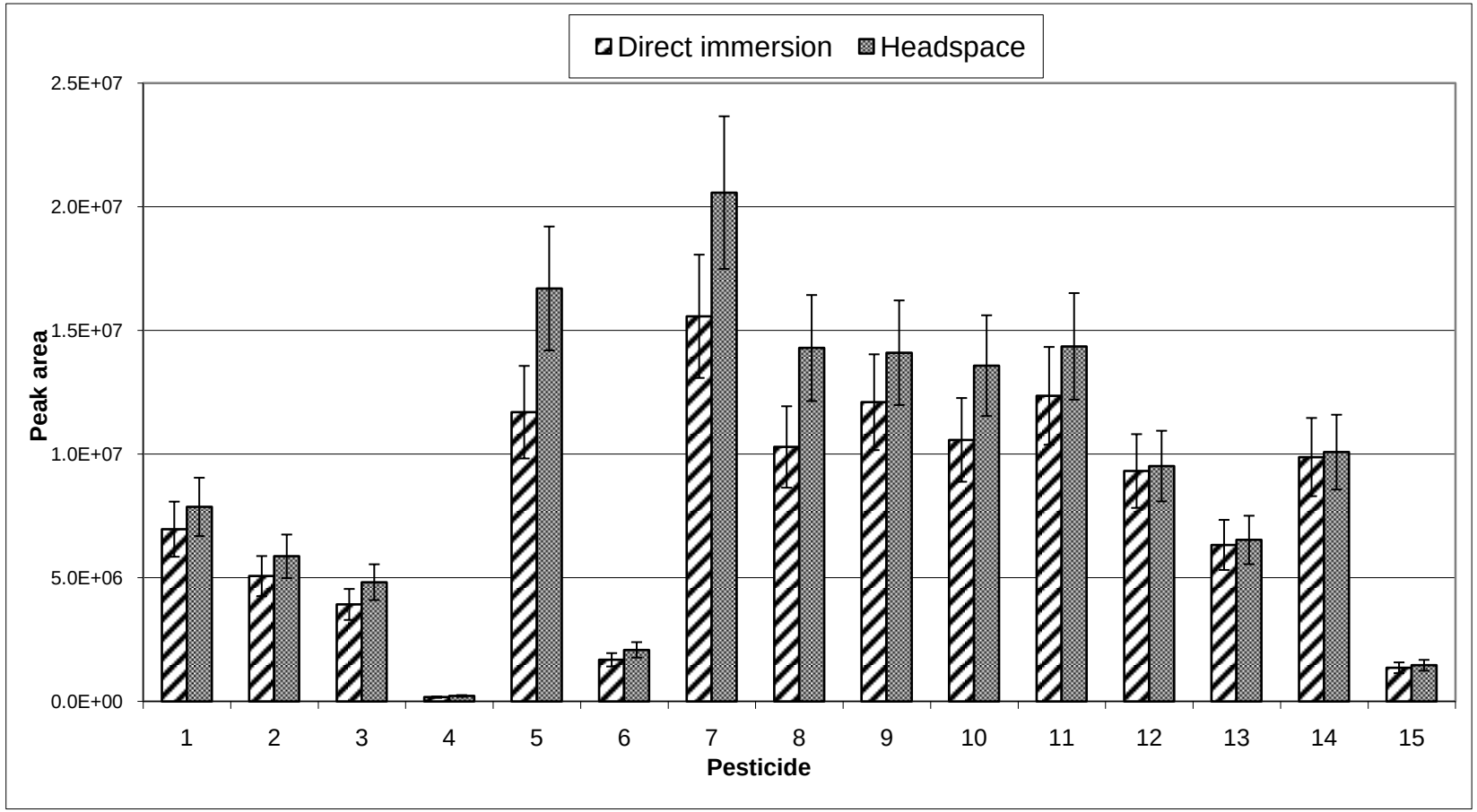


Figure 4.8: Comparison of extraction modes employing PDMS/DVB fiber. Pesticides are as follows: 1 = Hexachlorobenzene (HCB); 2 = α-Benzenehexachloride (α-BHC); 3 = γ- Benzenehexachloride (γ-BHC); 4 = β- Benzenehexachloride (β-BHC); 5 = Heptachlor; 6 = Aldrin; 7 = trans-chlordane; 8 = 4, 4' – DDE; 9 = Dieldrin; 10 = 2, 4' – DDD; 11 = Endrin; 12 = 4, 4' – DDD; 13 = β-Endosulfan; 14 = 4, 4' – DDT; 15 = Methoxychlor. Error bars represent ±% RSD at n=3.

4.1.5.3 Effect of temperature

The temperature was carefully monitored to ensure that it remained within ± 2 °C during extraction since large fluctuations might affect the rate of mass transfer and hence the equilibration time. This would in turn result in poor reproducibility and affect the overall precision of the method.

Figure 4.9 shows that an increase of temperature produced an improvement in the extraction efficiency for most pesticides. Higher temperatures increased the rate of transfer of pesticides to the fiber in the headspace hence 80 °C was selected as the optimum temperature for extraction.

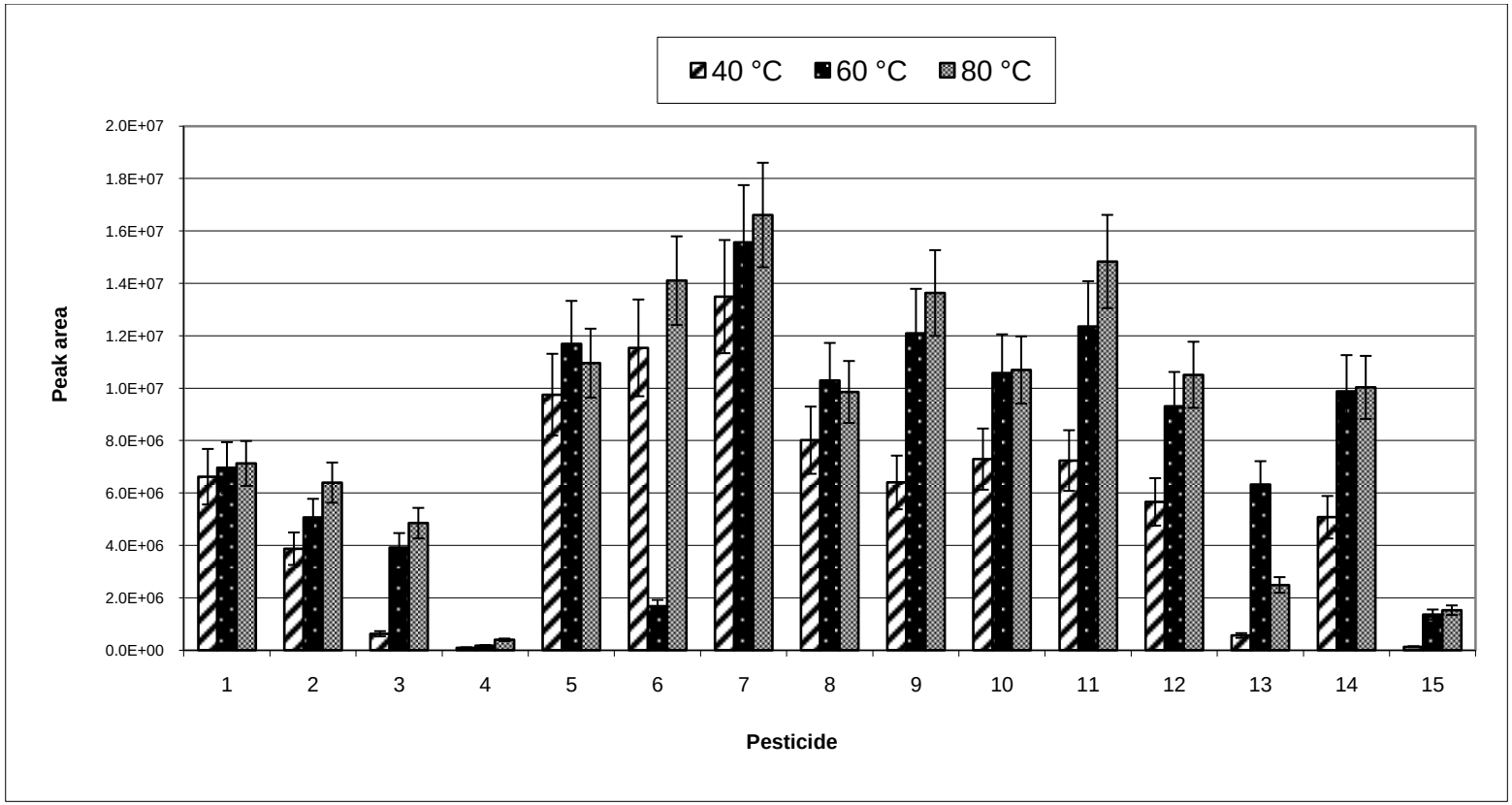


Figure 4.9: Effect of temperature on the extraction efficiencies of PDMS/DVB fiber. Pesticides are as follows: 1 = Hexachlorobenzene (HCB); 2 = α-Benzenehexachloride (α-BHC); 3 = γ- Benzenehexachloride (γ-BHC); 4 = β-Benzenehexachloride (β-BHC); 5 = Heptachlor; 6 = Aldrin; 7 = trans-chlordane; 8 = 4, 4' – DDE; 9 = Dieldrin; 10 = 2, 4' – DDD; 11 = Endrin; 12 = 4, 4' – DDD; 13 = β-Endosulfan; 14 = 4, 4' – DDT; 15 = Methoxychlor. Error bars represent ±% RSD at n=3.

4.1.5.4 Effect of ionic strength

The addition of 10 % (w/v) NaCl introduced a slight improvement in the extraction efficiency of a majority of pesticides except for β - endosulfan and methoxychlor whereby the addition of 10 % salt more than doubled the extraction efficiency of the fiber (Figure 4.10). Low recoveries for β – endosulfan have also been reported for HS-SPME by Lambropoulou and colleagues (2007). Increasing the salt content beyond 30 % (w/v) showed a decline of the extraction efficiency for all the pesticides. A salt content of 10 % was selected for further experiments.

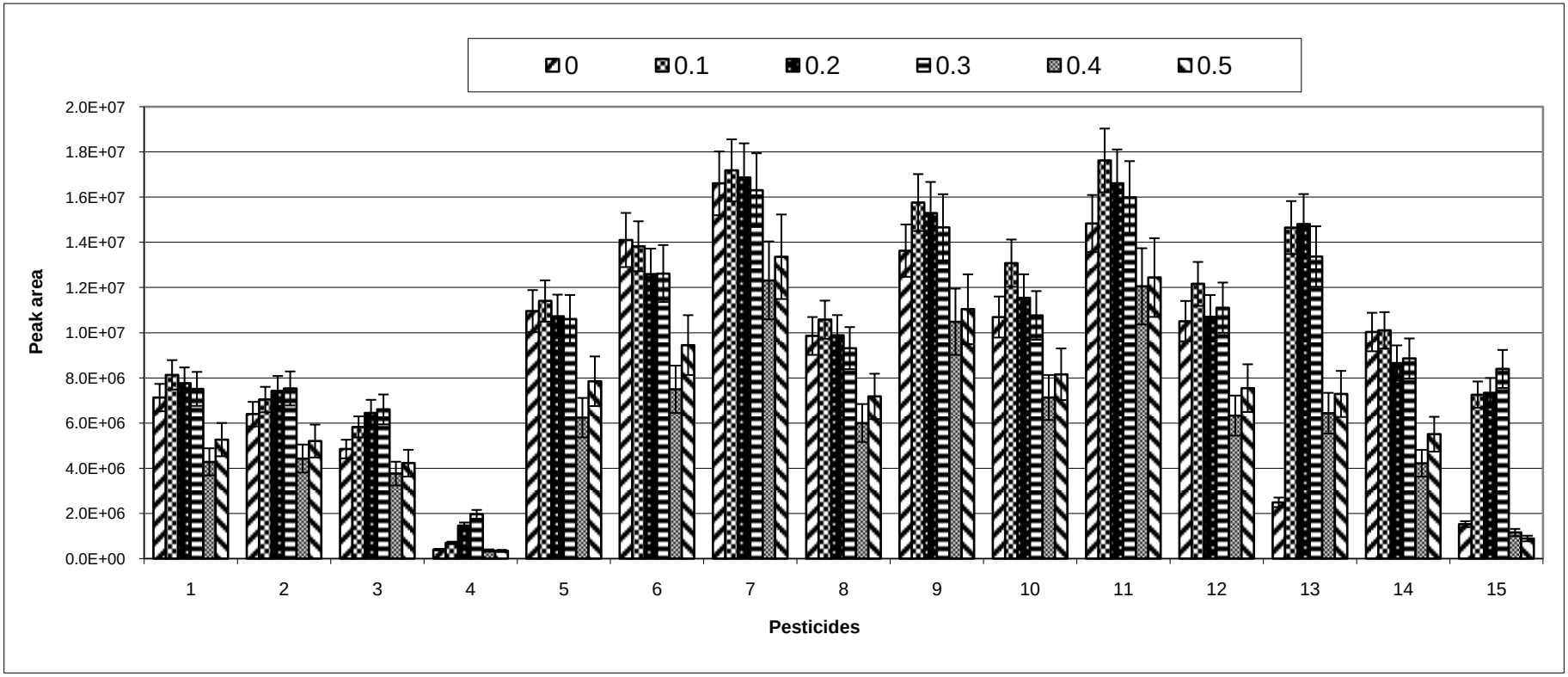


Figure 4.10: Effect of addition of salt (% NaCl w/v) on the extraction efficiencies of PDMS/DVB fiber: Pesticides are as follows: 1 = Hexachlorobenzene (HCB); 2 = α-Benzenehexachloride (α-BHC); 3 = γ- Benzenehexachloride (γ-BHC); 4 = β-Benzenehexachloride (β-BHC); 5 = Heptachlor; 6 = Aldrin; 7 = trans-chlordane; 8 = 4, 4' – DDE; 9 = Dieldrin; 10 = 2, 4' – DDD; 11 = Endrin; 12 = 4, 4' – DDD; 13 = β-Endosulfan; 14 = 4, 4' – DDT; 15 = Methoxychlor. Error bars represent ±% RSD at n=3.

4.1.5.5 Effect of stirring

Sample agitation affects the kinetics of chemical processes by reducing the time needed to attain equilibrium. Stirring resulted in a decrease of extraction efficiencies for a majority of pesticides (Figure 4.11) despite higher responses being reported with agitation by Llompart and colleagues (1998).

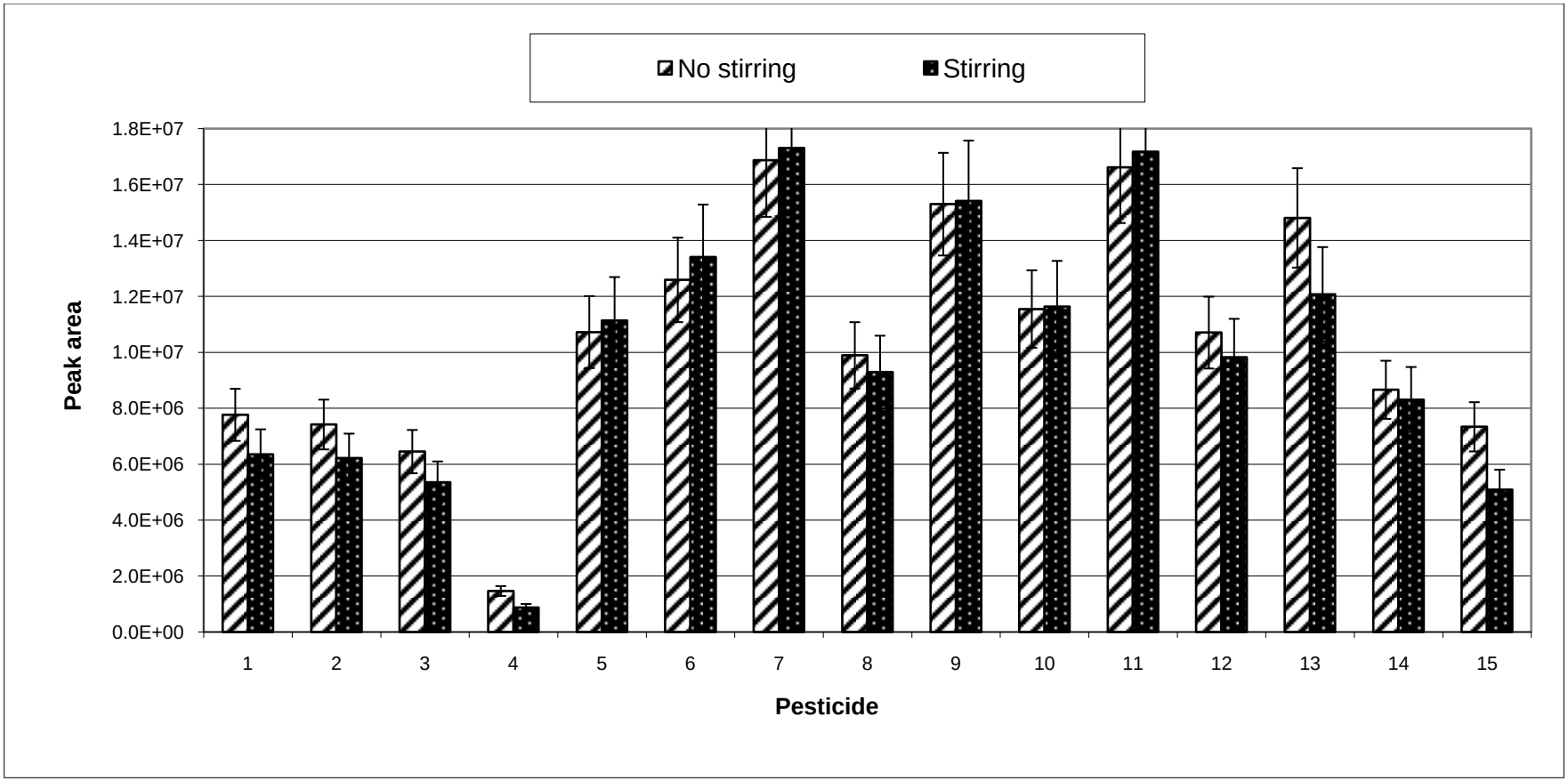


Figure 4.11: Effect of stirring on the extraction efficiencies of PDMS/DVB fiber: Pesticides are as follows: 1 = Hexachlorobenzene (HCB); 2 = α-Benzenehexachloride (α-BHC); 3 = γ- Benzenehexachloride (γ-BHC); 4 = β- Benzenehexachloride (β-BHC); 5 = Heptachlor; 6 = Aldrin; 7 = trans-chlordane; 8 = 4, 4' – DDE; 9 = Dieldrin; 10 = 2, 4' – DDD; 11 = Endrin; 12 = 4, 4' – DDD; 13 = β-Endosulfan; 14 = 4, 4' – DDT; 15 = Methoxychlor. Error bars represent ±% RSD at n=3.

4.1.5.6 Optimization of extraction time

The equilibration time was determined by constructing an extraction profile (Figure 4.12) in which the extraction time was plotted against peak area for each pesticide. Most pesticides reached equilibrium within 30 min hence this extraction time was employed for subsequent work.

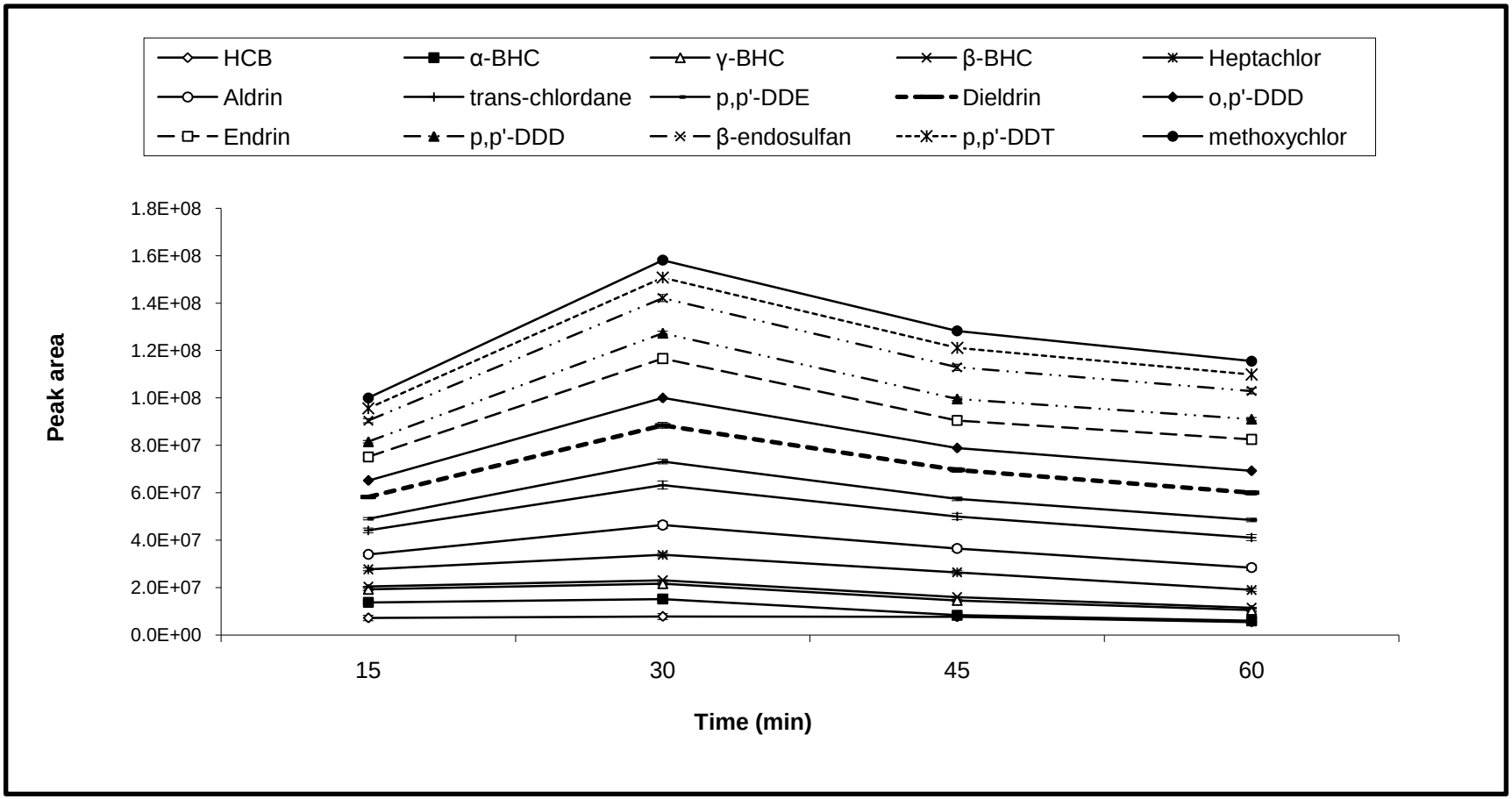


Figure 4.12: Optimization of extraction time on the extraction efficiencies of PDMS/DVB fiber. %RSDs varied from 5.2 to 14.0 (n=3).

4.1.6 Evaluation of analytical parameters for HS-SPME

Analytical parameters were obtained for HS-SPME by the analysis of different spiked ultra pure water samples employing pesticides standards mixtures described in section 3.2.6. Table 4-4 shows that linear relationships were obtained between peak areas and the analyte concentrations, with high correlation coefficients (≥ 0.9998). In HS-SPME the PDMS/DVB fiber was most sensitive to trans - chlordane as it had the lowest SPME - LD of 0.00051 µg L^{-1}. Precision was determined by reproducibility studies expressed by percent relative standard deviation (% RSD) of 3 spiked water aliquots and was less than 15 % for both methods

Table 4-4: Analytical parameters obtained for HS-SPME and subsequent analysis of pesticides by GC-ECD

Parameter	**HS-SPME**
Linearity (µg L^{-1})	0.0005 – 0.1000
R^2	0.9989 – 0.9998
LDs (µg L^{-1})	0.0005 – 0.0030
% RSDs	5.2 – 14.0

4.1.7 Analysis of water samples by HS-SPME/GC-ECD

The optimized SPME method was employed to determine pesticides in water samples and four pesticides namely HCB, trans-chlordane, 4,4'-DDE and 4,4'-DDD were detected by GC-ECD at concentrations ranging between 2.4 and 61.4 µg L^{-1} as shown in Table 4-5.

Table 4-5: Compounds detected in the water samples after HS-SPME and GC-ECD.

Pesticide	Concentration (µg L^{-1})	% RSD
HCB	61.4	7.9
Trans-chlordane	3.2	5.7
4,4'-DDE	5.3	9.4
4,4'-DDD	2.4	7.2
Isobutyl-4-octylester - phthalic acid	N.Q.	N.Q.
Dibutyl phthalate	N.Q.	N.Q.
DEHP	N.Q.	N.Q.

N.Q. - not quantified

Phthalates (commonly employed in the plastic industry) were also identified in the water samples but could not be quantified due to lack of pure standards. A chromatograph of a water sample after HS-SPME clean-up followed by GC-ECD is shown in Figure 4.13.

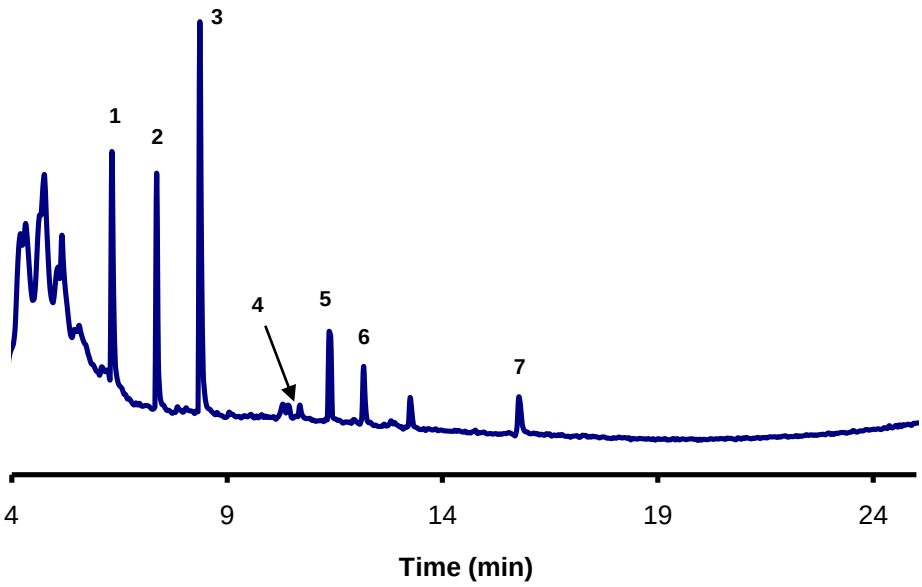


Figure 4.13: A chromatogram of a water sample showing (1) hexachlorobenzene [0.061 µg/ml]; (2) isobutyl-4-octylester phthalic acid; (3) dibutyl phthalate; (4) trans-chlordane [0.003 µg/ml]; (5) 4,4'-DDE [0.005 µg/ml]; (6) 4,4'-DDD [0.002 µg/ml] and (7) diethylhexylphalate after HS-SPME at 80 °C and 10 % NaCl. The fiber employed was PDMS/DVB. The rest of the peaks could not be identified with the help of the GC/MS NIST library since their spectra matched the suggested library ones by less than 80 %.

4.2 Sediment samples

Only 3 pesticides were quantitatively determined by the external standard method employing linear calibration curves with six concentration levels per compound. Correlation coefficients were all above 0.99. Detection limits ranged between 0.05 and 0.31 µg/g. A chromatogram (GC-ECD) showing the separation of a 15–component pesticide standard mixture is captured in Figure 4.14.

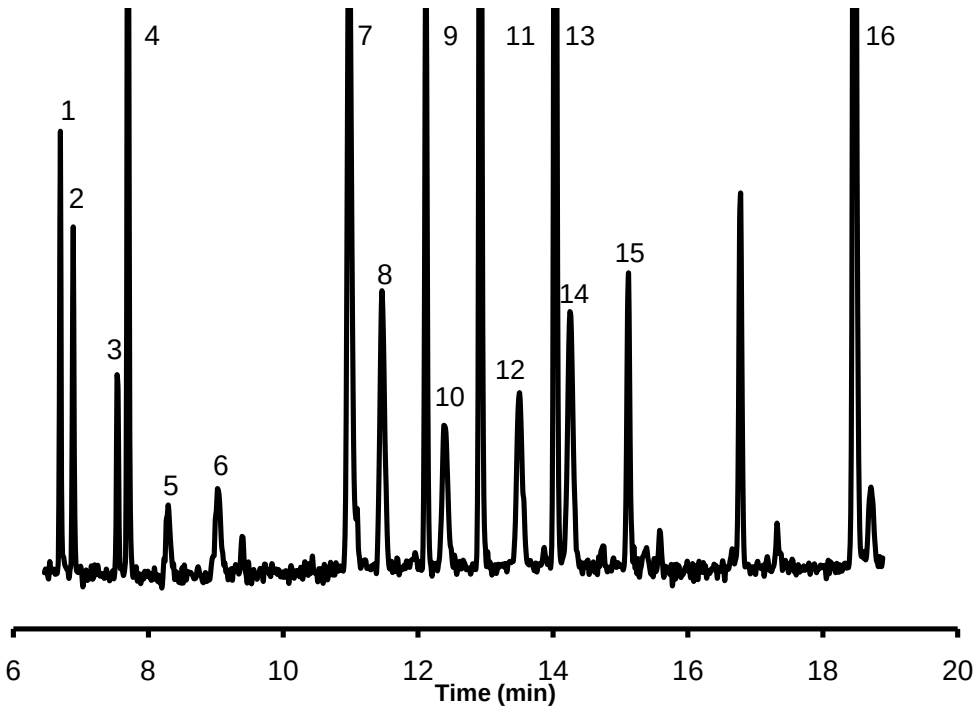


Figure 4.14: Chromatogram of pesticide standards mixture (1-50 µg/ml). 1 = Dichlorvos; 2 = Hexachlorobenzene (HCB); 3 = α-Benzenehexachloride (α-BHC); 4 = γ- Benzenehexachloride (γ-BHC); 5 = β- Benzenehexachloride (β-BHC); 6 = Heptachlor; 7 = Aldrin; 8 = trans-chlordane; 9 = 4, 4' – DDE; 10 = Dieldrin; 11 = 2, 4' – DDD; 12 = Endrin; 13 = 4, 4' – DDD; 14 = β-endosulfan; 15 = 4, 4' – DDT; 16 = methoxychlor. The chromatographic conditions are mentioned in section 3.1.2.

4.2.1 Testing of different SFE settings

CO_2 is a non-polar solvent and in the case when it is required to extract analytes such as pesticides that have a wide range of physico-chemical properties, its extraction efficiency is not satisfactory. The introduction of a modifier enhances analyte solubility, covering matrix active sites and inhibiting the desorbed analytes' re-adsorption into the matrix (Anitescu and Tavlarides, 2006; Hu et al., 2007). Evaluation of the different SFE settings showed that the use of modifier was essential. Figure 4.15 shows that without the addition of a modifier, pesticides recoveries were generally low, ranging from 5 to 50 % except for the 75 % recovery of 4, 4'-DDT.

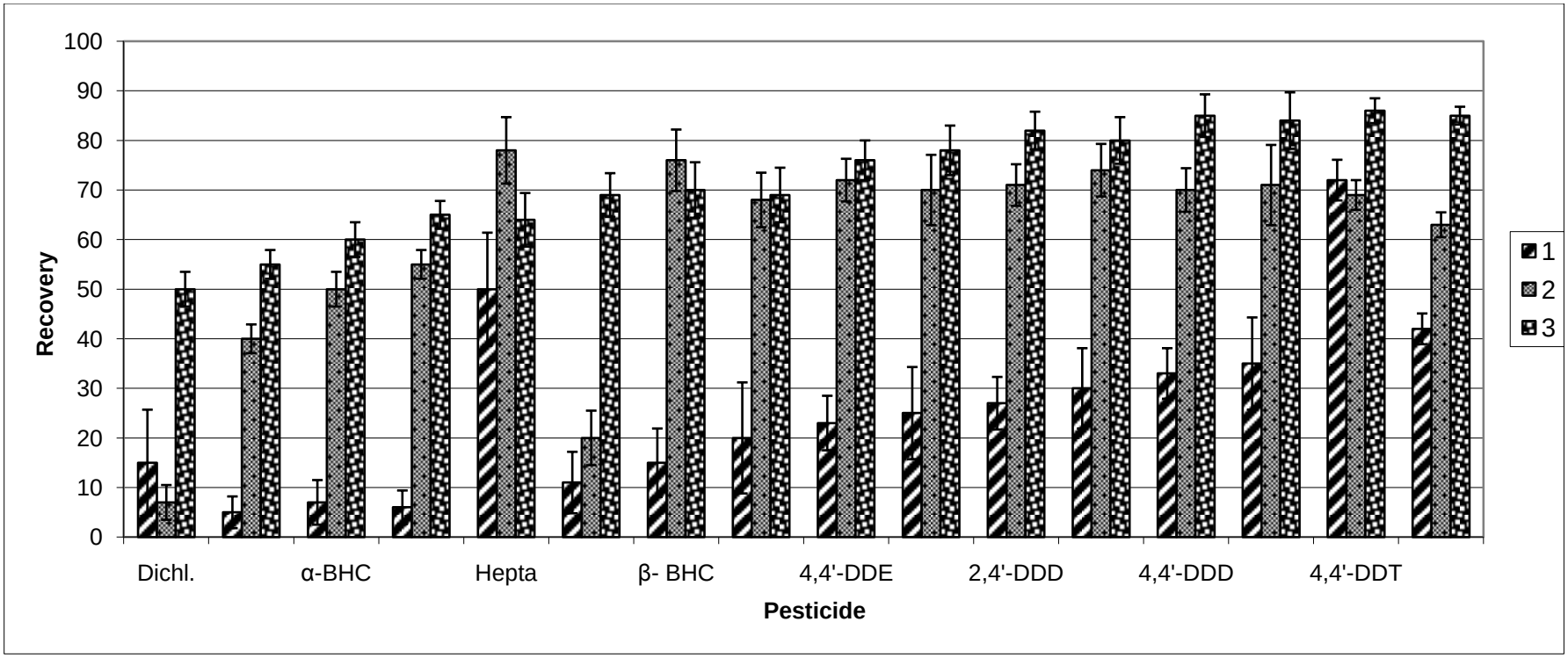


Figure 4.15: Recoveries of pesticides after SFE of a spiked sediment sample employing the 3 different settings mentioned in Table 3-4. Error bars represent ±% RSD at n=3.

The low recoveries indicated the insolubility of the pesticides in CO_2 at 400 bar while the conditions were suitable for the extraction of 4, 4'-DDT.

Water is suspected to favour swelling of the matrix thereby enhancing diffusion of the fluid inside the matrix (Nemoto et al., 1997). It was however observed that pesticide recoveries with a water modifier were generally lower than when acetone was employed except for heptachlor and β-BHC. SFE extracts in which acetone was employed as a modifier had an intense green colour probably due to co-extraction of organic matter in the sediment matrix by acetone.

Raising the extraction pressure at constant temperature leads to a higher fluid density that increases the solubility of analytes (Camel, 1998; Ghasemi et al., 2007). Ramping of pressure or fractional extraction was employed as suggested by Reverchon and De Marco (2006) resulting in recoveries ranging from 55 to 86 % with HCB being the least recovered and 4,-4'-DDT the most recovered pesticide. The recovery of HCB at 55 % was judged acceptable given that most pesticides had recoveries higher than 70 %. Thus the optimised SFE conditions that were employed for the extraction of pesticides in sediment samples were 50 μl of acetone, 200 bar (30 min static), 350 bar (30 min static) and 400 bar (15 min static) (Note 1 bar = 100 KPa). Dynamic extraction for 5 min was carried out after every static extraction.

4.2.2 Analysis of sediments after SFE

Sediment samples were extracted employing the SFE optimized conditions. Four pesticides, namely, aldrin, α-chlordene, HCB and 4,4'-DDT as well as hydrocarbons such as hexadecane, octadecane and 5-octadecene were tentatively identified by mass spectrometry as shown in Figure 4.16.

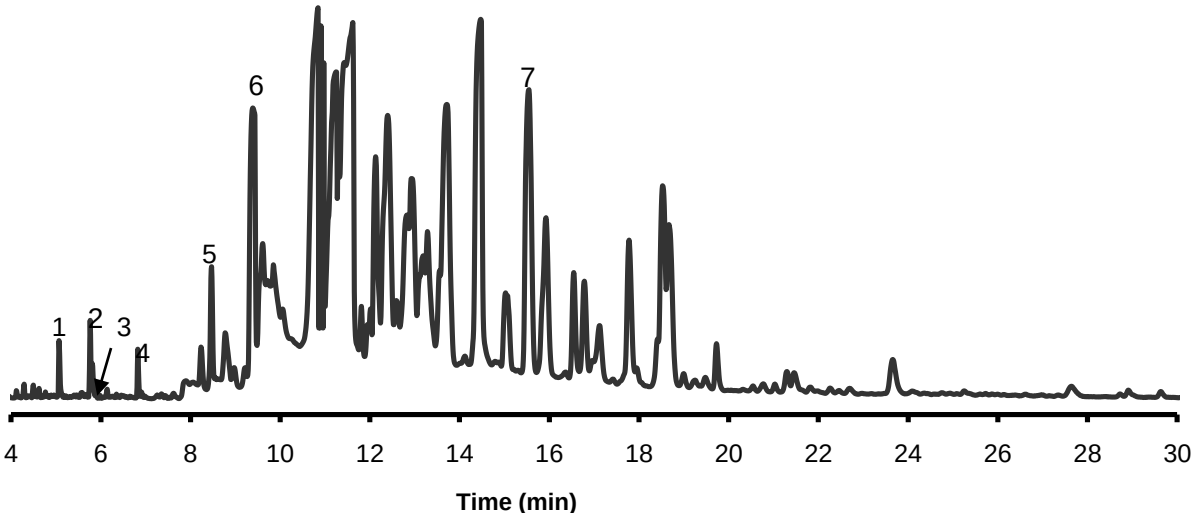


Figure 4.16: Chromatogram of a sediment sample after SFE/GC-ECD showing [1] hexadecane; [2] octadecane; [3] 5 - octadecene; [4] HCB; [5] α - chlordene; [6] aldrin and [7] 4,4' – DDT. The rest of the peaks could not be identified with the help of the GC/MS NIST library since their spectra matched the suggested library ones by less than 80 %. The chromatographic conditions are mentioned in section 3.1.2.

However only aldrin, HCB and 4,4'-DDT could be quantified due to lack of pure chemical standards for the other compounds. Concentrations of HCB, ranged from 1.1 to 30.3 μg/g while those of aldrin and 4,4'-DDT ranged from 0.5 to 15.2 and 1.4

to 55.4 µg/g, respectively. For simplicity of data analysis, the sampling area was divided into 3 regions (see Figure 3.2) and the mean concentration of each pesticide calculated for each region:

- Region 1 (the Panhandle) consisting of samples from Mohembo, Shakawe, Samochima, Sepopa and Guma Lagoon
- Region 2 (the upper delta) consisting of samples from Chief's Island and Xakanaxa
- Region 3 (the lower delta) consisting of samples from Lake Ngami, Maun and Toteng.

The distribution of pesticides in sediments from the 3 regions revealed an accumulation pattern as shown in Figure 4.17.

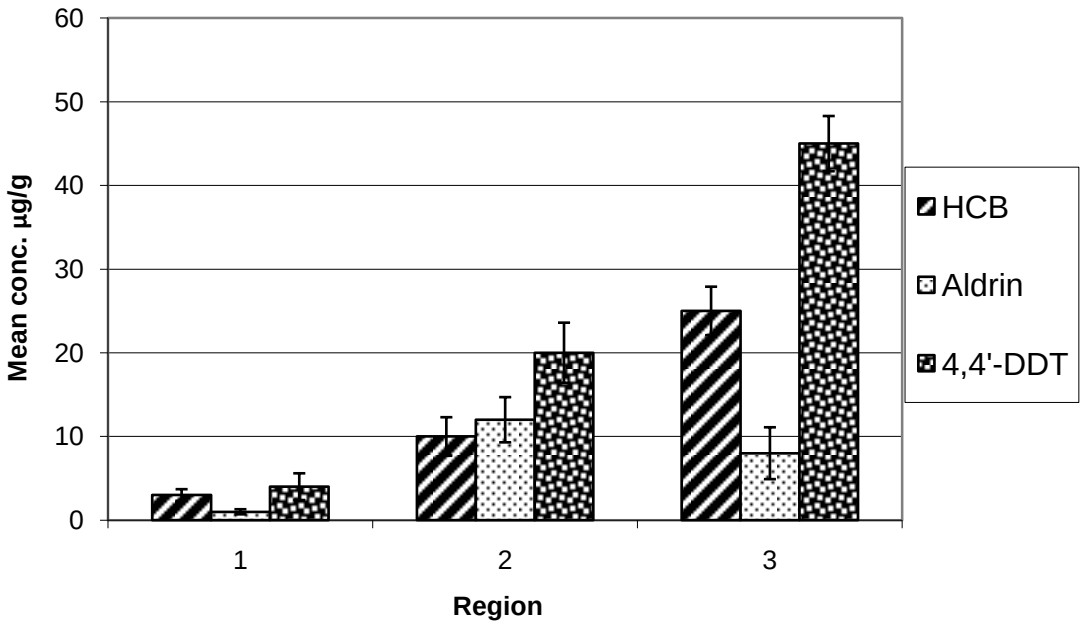


Figure 4.17: Mean concentrations of pesticides in sediment samples from the 3 regions of the Okavango Delta that were studied: (1) the Panhandle; (2) the upper Delta and (3) the Lower Delta. Error bars represent ±% RSD at n=3.

Sediments from Region 1 (the Panhandle) contained the lowest mean concentrations of pesticides ranging from 1 to 4 µg/g while sediments from Region 3 had the highest mean concentrations ranging from 8 to 45 µg/g. This trend may be due to the direction of flow of the water as well as the low topographic gradient of the Delta causing low flow rates (Andersson et al., 2003). The low flow rates allow partitioning of water insoluble components such as pesticides onto suspended matter that subsequently settles to the bottom of the river becoming part of sediment. Thus pesticides are more likely to be adsorbed onto organic-rich sediment as compared to the sandy sediment such as characteristic of the Panhandle as reported by Daka et al. (Daka et al., 2006).

HCB and aldrin are employed in agriculture as a dressing for seed grains due to their effectiveness against various pests and may be transported into rivers by water run-off. They are insoluble in water but readily adsorbed onto soils and sediments (Yuan et al., 2006). DDT has been employed for aerial spraying by the public health authorities in the study area from the 1940s until the late 1990s (Mabaso et al., 2004). However the presence of 4, 4'-DDT at elevated levels in sediments at the peripheries of the delta may be due to the fact that these areas act as final catchments for the water. Lake Ngami, for example, is at the receiving end of the delta and is not fed by any other water source. The sediments in these areas are rich in organic matter, capable of accumulating considerable amounts of pesticides by adsorption. Alternatively, there could be a subsistent input of pesticides employed on vegetable farming taking place on the peripheries of the delta. Similar observations were made by Sereda and Meinhardt (2005) in their study of water and sediments in KwaZulu-Natal, South Africa, whereby the most polluted areas were those where vegetable production occurred.

4.2.3 Pressurized fluid extraction

PFE was employed in this work as a screening technique on one sediment sample that had been collected from the lower Delta, where previous SFE analysis had shown concentrations of DDT metabolites being higher than in upstream sediments.

4.2.4 Optimization of extraction solvent and temperature

The ability to select an appropriate solvent for extraction is essential regardless of the technique employed and is often neglected in PFE in favour of optimization of instrumental parameters (Fitzpatrick et al., 2000). The extraction efficiencies of n-heptane/acetone [1:1 v/v], ethyl acetate and water were compared at 50, 80 and 120 °C. The organic solvent extracts (ethyl acetate and n-heptane/acetone) were relatively clean with a clear yellow colour while the water extracts were a muddy brown in colour. However the final water extracts were a clear brown after filtration and re-extraction with n-heptane. Figure 4.18 shows that amongst the three solvents, n-heptane/acetone extracted the highest amount of p,p'-DDE at the highest temperature setting, 120 °C.

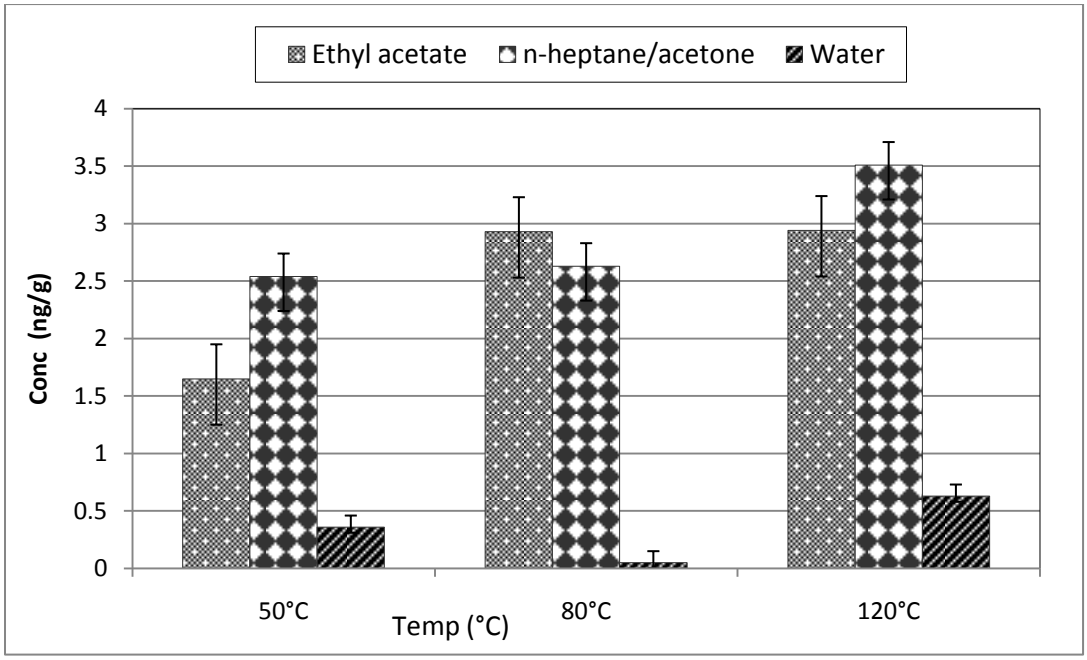


Figure 4.18: Concentration yields of p,p'-DDE from sediment after PFE with ethyl acetate, n-heptane/acetone 1:1 v/v and water at different temperatures. Error bars represent ±% RSD at n=3.

The satisfactory performance of the n-heptane/acetone system is in agreement with work reported by Hussen and colleagues (2007). In their comparison of n-heptane/acetone 1:1 v/v with n-heptane/ethyl acetate 1:1 v/v in the extraction of pesticides, the n-heptane/acetone gave higher yields. It has been reported that in the extraction of non-polar compounds from wet matrices, the use of a mixture of non-polar and polar or moderately polar solvents in proportions 1:1 v/v results in higher yields as compared to using a single non-polar solvent (Bjorklund et al., 2000). This may be because of the ability of the polar or moderately polar solvent (in this case acetone, ε° [on silica] = 0.53) to penetrate matrix pores that are sealed with aqueous films thereby causing a swelling of the matrix and consequently assisting the extraction of non-polar analytes by the non-polar solvent (in this case n-heptane, ε° =

0.01). This may also be the reason why ethyl acetate extracted p,p'-DDE appreciably well despite being moderately polar (ε° = 0.52). It was able to penetrate the aqueous barriers within the matrix pores and at the same time extract the non-polar analyte. Water performed badly under the conditions employed in the study. This may be due to the large differences in polarity between the water and p,p'-DDE as well as the conditions not being favourable to alter its polarity. The re-extraction of water extracts into n-heptane may have also affected the yield.

In their study, Fitzpatrick and colleagues (2000) assessed the influence of temperature on the recoveries of DDT, DDD and DDE from aged contaminated soils. They investigated temperatures from 80 to 200 °C in increments of 20 °C and concluded that temperature did not affect recoveries of DDT and its metabolites. Consequently, this thesis compares extraction yields at temperatures 50, 80 and 120 °C. Yields of p,p'-DDE were not affected by an increase in extraction temperature from 50 to 80 °C in n-heptane/acetone however extraction at 120 °C yielded 33 % more p,p'-DDE.

Ethyl acetate showed a 77 % increase in yield when temperature was raised from 50 to 80 °C but remained the same when the temperature was further increased from 80 to 120 °C. Extraction with water resulted in low yields for p,p'-DDE even at 120 °C. The drop in yield from 50 to 80 °C may be attributed to the inconsistency in volumes of the extract that were observed during the experiment or loss of analyte

during filtration of the muddy extract and / or re-extraction into n-heptane for analysis by GC-MS.

Sediments contain a wide variety of organic compounds derived from organic matter within the sediment and the overlaying water column. The organic matter consists of pigments, hydrocarbons, sterols, alcohols and fatty acids that are degradation products of terrestrial plants and microorganisms such as algae, bacteria and diatoms (Volkman et al., 1986). Fatty acids in particular, are less labile as compared to other components of organic matter and hence are often employed as biomarkers to determine sources of organic matter in sediments (Christodoulou et al., 2009). Long chain fatty acids ranging from $C_{16}H_{32}O_2$ to $C_{36}H_{52}O_{2,}$ indicating origins from leaf waxes of higher plants (MacAvoy et al., 2009) were present in extracts from the three solvents at 120 °C as shown on Figure 4.19.

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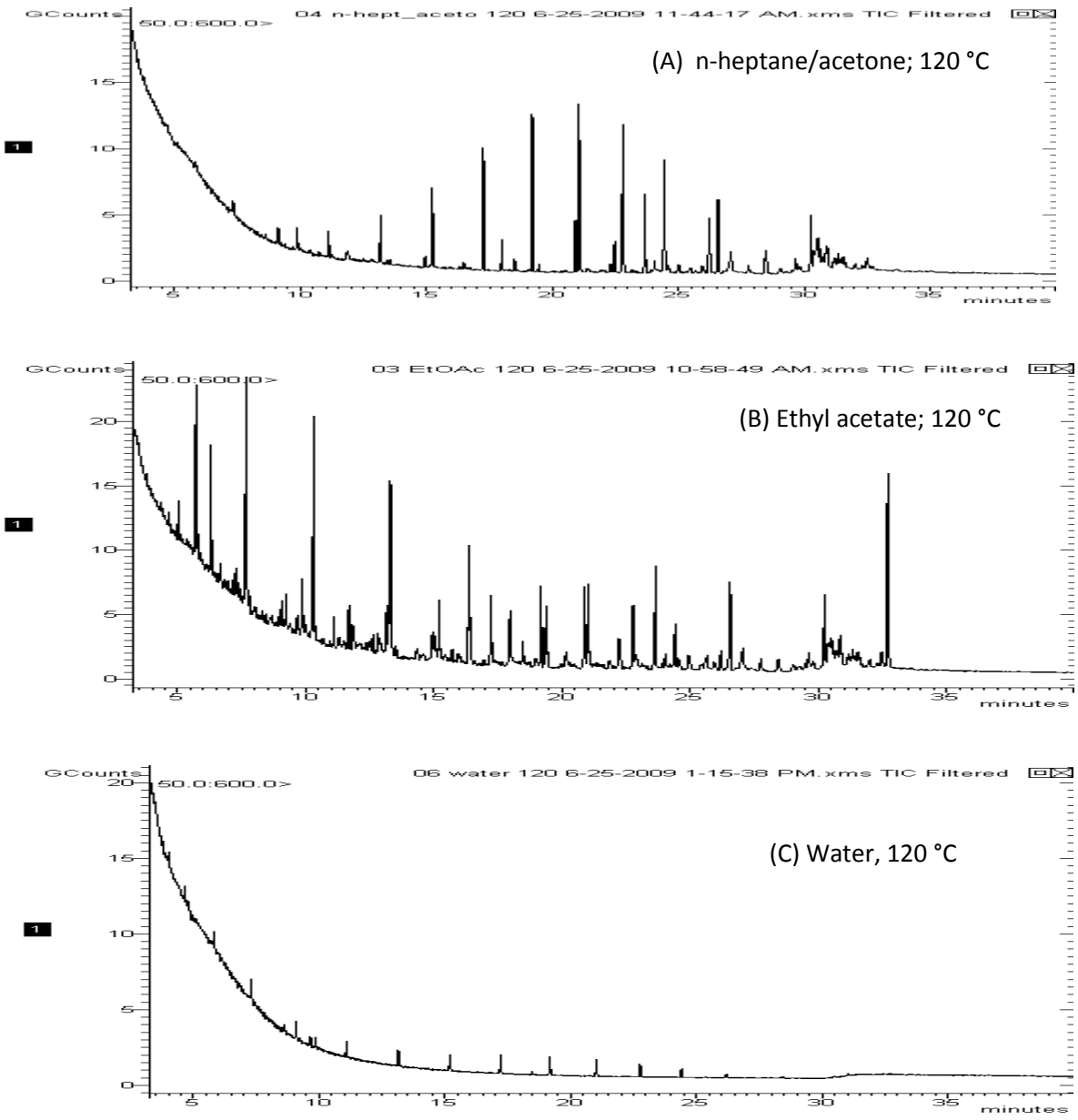

Figure 4.19: Chromatograms of a sediment sample after PFE with n-heptane/acetone 1:1 v/v (A); ethyl acetate (B) and water (C) at 120 °C, 50 bar, 3 x 5 min static extraction cycles. The rest of the peaks could not be identified with the help of the GC/MS NIST library since their spectra matched the suggested library ones by less than 80 %.

The n-heptane/acetone solvent system gave the highest yields of fatty acids while ethyl acetate extracted a greater number of compounds that could not be positively identified with match factors greater than 800. For tentative identification, the match factor must be greater than 850 out of a 1000. Compounds that were positively identified in the three extracts are given in Table 4-6.

Table 4-6: Compounds identified by GC-MS in the scan mode after PFE with three different solvent systems. All compounds had match factors greater than 800.

Number	Name of compound
1	$C_{16}H_{32}O_2$
2	$C_{18}H_{34}O_2$
3	Cyclic octaatomic sulfur
4	$C_{20}H_{36}O_2$
5	p,p'-DDE
6	$C_{22}H_{38}O_2$
7	Tetracosamethyl-cyclododecasiloxane
8	$C_{24}H_{40}O_2$
9	Triacontane
10	di-n-octyl-phthalate
11	$C_{26}H_{42}O_2$
12	$C_{28}H_{44}O_2$
13	Squalene
14	$C_{30}H_{46}O_2$
15	$C_{32}H_{48}O_2$
16	$C_{34}H_{50}O_2$
17	$C_{36}H_{52}O_2$
18	Diphenyl sulfone
19	Dibutyl phthalate

Water extracted mainly fatty acids and the chromatogram does not show the presence of other co-extracted compounds. It has to be noted, however, that polar

compounds could have been lost during the re-extraction into n-heptane for analysis by GC-MS. The low yield of p,p'-DDE by water implies that the conditions employed in the study (50-120 °C) were not sufficient to lower the dielectric constant of water to enable it to extract non-polar compounds. To be able to extract non-polar compounds such as DDE, it would likely be needed to use water at 250-300 °C, as shown by Hawthorne and colleagues (1994) when they quantitatively extracted polycyclic aromatic hydrocarbons (PAHs) from soil using subcritical water at 250 °C and 50 bar. In another study, Yang and co-workers (1995a) noted that quantitative extraction of polychlorinated biphenyls (PCBs) from reference soil and sediment samples with water occurred at 250 and 300 °C and 50 atm compared to lower temperatures of 50 and 100 °C.

The extraction temperature was further increased to 180 °C and the extract profiles compared for each solvent. The increase in temperature did not have noticeable effect on the sediment profiles as demonstrated by Figure 4.20, Figure 4.21 and Figure 4.22.

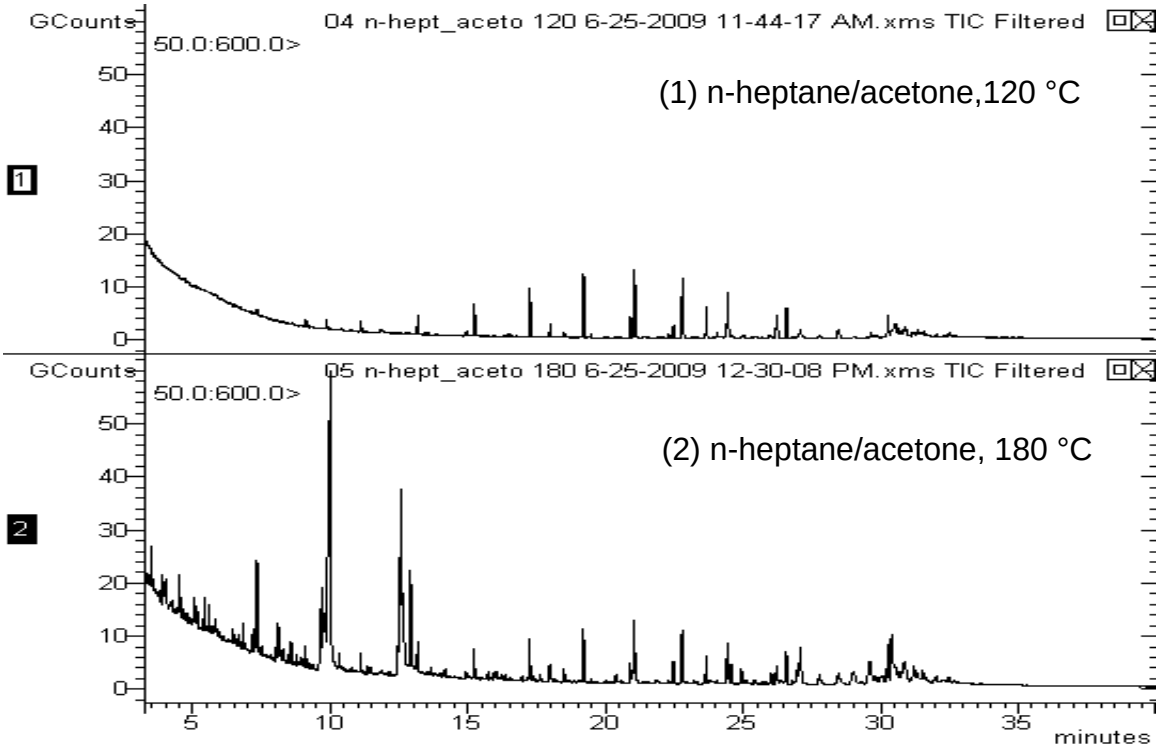

Figure 4.20: A comparison of sediment profiles after extraction with n-heptane/acetone at 120 and 180 °C. The rest of the peaks could not be identified with the help of the GC/MS NIST library since their spectra matched the suggested library ones by less than 80 %.

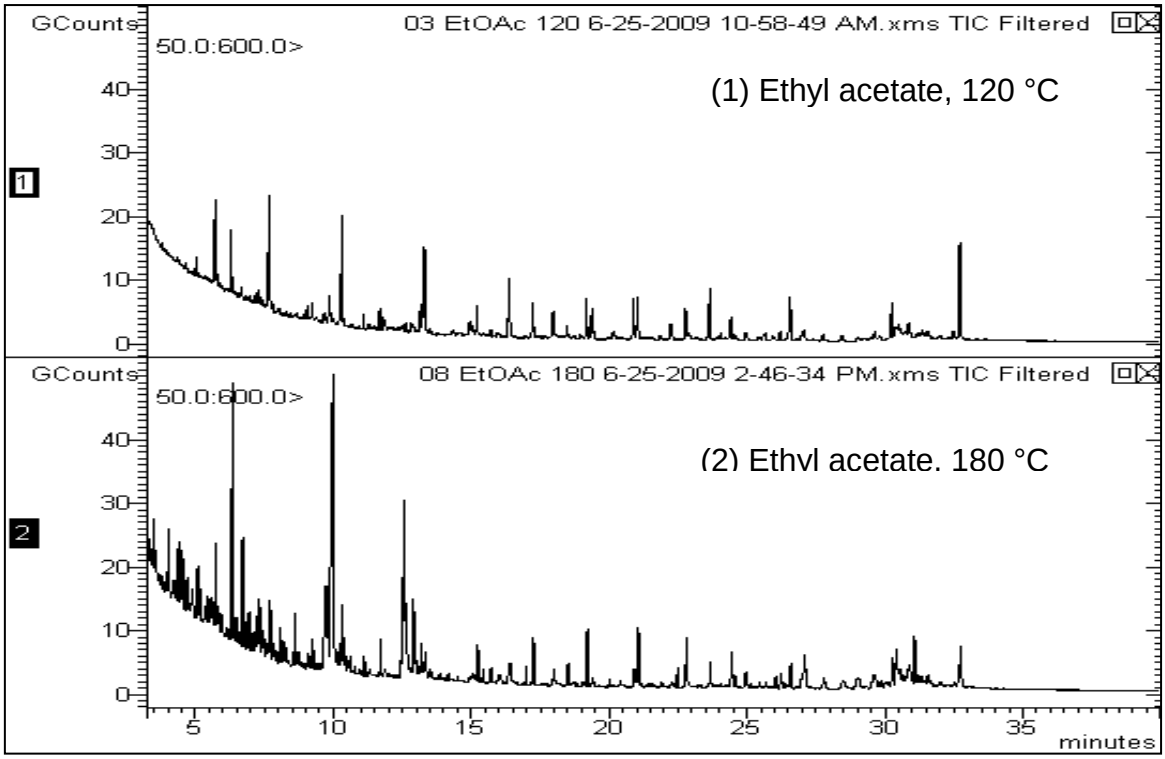

Figure 4.21: A comparison of sediment profiles after extraction with ethyl acetate at 120 and 180 °C. The rest of the peaks could not be identified with the help of the GC/MS NIST library since their spectra matched the suggested library ones by less than 80 %.

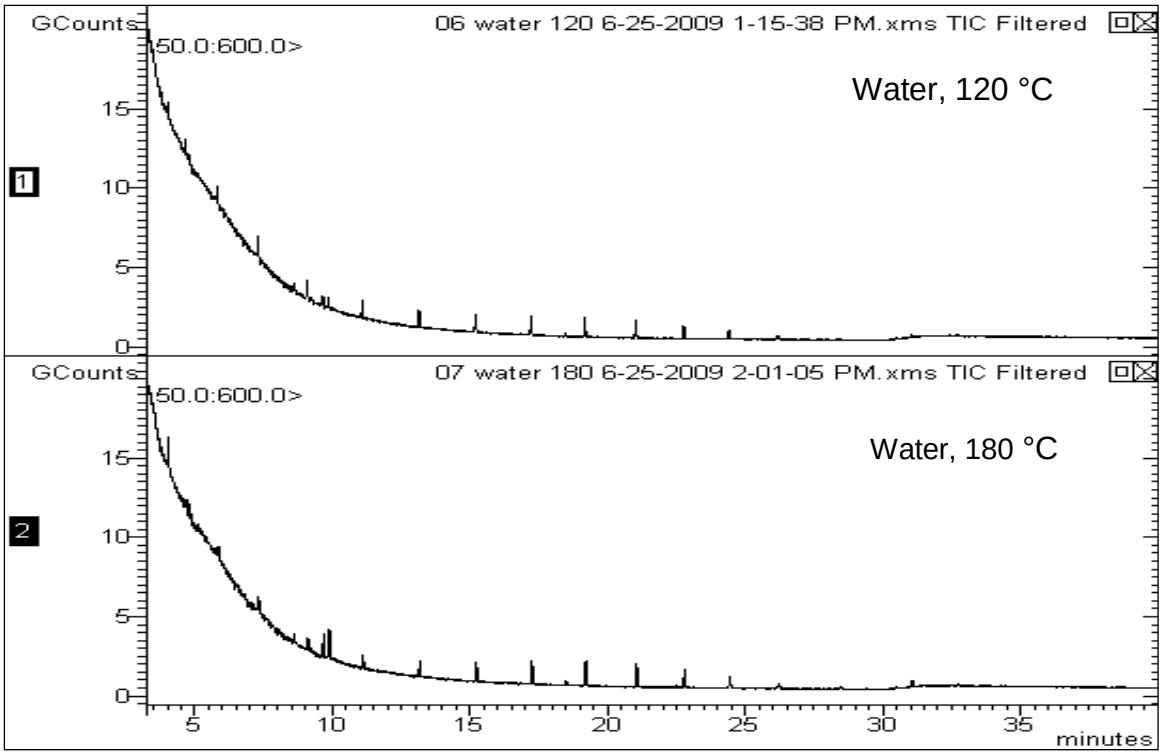

Figure 4.22: A comparison of sediment profiles after extraction with water at 120 and 180 °C. The rest of the peaks could not be identified with the help of the GC/MS NIST library since their spectra matched the suggested library ones by less than 80 %.

An increase in extraction temperature resulted in higher yields of phthalates and the early eluting compounds for sediment extracted with n-heptane/acetone and ethyl acetate. A raise in temperature from 120 to 180 °C did not have any effect on the extraction efficiency of water. However, regarding the water extracts, it should be noted that they were re-extracted into n-heptane, hence the sediment profiles after extraction with water reflect only compounds that distributed into the n-heptane. In an investigation of pressurized hot water extraction of pesticides from dust remaining from the production of seed-pellets, Eskilsson and colleagues (2004) reported no yield of non-polar compounds in water heated to 150 °C. This was attributed to the

low solubility of non-polar compounds in water and the risk of degradation of compounds of interest at high temperatures.

Chapter 5

5 Conclusions

Water samples were analysed for pesticides employing optimized HS-SPME and SPE extraction/ clean-up techniques with GC-ECD and confirmed by GC-ToF-MS. Satisfactory precision (less than 15 %) was obtained for both HS-SPME and SPE methods however HS-SPME exhibited a higher selectivity and sensitivity to pesticides with determination limits 3-fold lower than those for SPE. HCB, trans-chlordane, 4,4'-DDE and 4,4'-DDD were detected with the HS-SPME method while none were detected with the SPE method. Hence HS-SPME is recommended for environmental monitoring due to its high selectivity and high pre-concentration capacity.

SFE conditions were optimized for pesticides in sediments from the Okavango Delta. Lowest mean concentrations ranging from 1 to 4 µg/g were observed in sediments from the Panhandle while higher concentrations (8 to 45 µg/g) were observed in samples from the lower Delta. This indicated a cumulative effect of pesticides in the direction of water flow from the Panhandle to the lower Delta. The change of sediment type from sandy in the Panhandle to organic-rich clay in the lower delta may also have influenced the pesticide storage capacity of the sediments.

PFE was employed as a screening technique varying only the solvent and temperature (50 - 120 °C) for optimization. Organic solvents (n-heptane/acetone 1:1 v/v and ethyl acetate) gave higher yields of p,p'-DDE as compared to water. The n-heptane/acetone 1:1 v/v solvent system gave the highest extraction yield (2.5 ng/g) compared to ethyl acetate and water (1.65 and 0.36 ng/g, respectively) at the lowest temperature (50 °C). The extraction yield of p,p-DDE was also highest in the n-heptane/acetone 1:1 v/v solvent system at 120 °C (3.51 ng/g compared to 2.94 and 0.63 ng/g obtained from ethyl acetate and water, respectively) thus demonstrating its suitability as an extraction solvent for p,p'-DDE. The presence of p,p'-DDE in the sediment is an indication of old contamination of the sediment since DDE is the most stable metabolite of DDT. Profiles of all three solvent extracts also showed the presence of long chain fatty acids and phthalates which could not be quantified due to lack of pure standards. The extraction of phthalates by n-heptane/acetone and ethyl acetate was enhanced when the temperature was raised to 180 °C.

5.1 Further work

i. Validation of SFE and PFE methods with a reference sediment sample.
ii. Further investigation of PFE employing water at temperatures between 250 and 300 °C.
iii. Quantification of phthalates and hydrocarbons in the water in the water.
iv. Investigation of adsorption isotherms of sediments from the different areas of the Delta.

v. Investigation of distribution coefficients of pesticides between the different aquatic constituents.

vi. Scheduled regular monitoring of contaminants to enable timely remediation of the ecosystem.

vii. Exposure studies of pesticides on human beings and impacts on different animal and plant species dependent on the Delta's ecosystem.

viii. Identification of point sources for the pesticides.

Appendix

GPS coordinates of the sampling points

Sampling point	GPS coordinates
1a	S 20° 21' 34.6" EO 22° 56' 46.3"
1b	S 20° 21' 36.7" EO 22° 56' 44.9"
2a	S 20° 25' 25.5" EO 22° 50' 08.4"
2b	S 20° 25' 50.4" EO 22° 49' 57.8"
3a	S 18° 16' 36.4" EO 21° 47' 10.7"
3b	S 18° 16' 27.6" EO 21° 47' 16.2"
3c	S 18° 16' 23.4" EO 21° 47' 25.4"
3d	S 18° 16' 30.3" EO 21° 47' 23.9"
4a	S 18° 16' 36.0" EO 21° 48' 10.5"

4b	S 18° 16' 36.2" EO 21° 48' 18.7"
4c	S 18° 17' 14.4" EO 21° 48' 29.8"
5a	S 18° 17' 29.3" EO 21° 49' 21.0"
5b	S 18° 17' 19.6" EO 21° 49' 20.9"
6a	S 18° 18' 14.8" EO 21° 49' 41.3"
7a	S 18° 20' 02.0" EO 21° 50' 36.1"
8a	S 18° 20' 28.5" EO 21° 50' 01.9"
9a	S 18° 20' 50.2" EO 21° 50' 29.9"
10a	S 18° 21' 39.5" EO 21° 50' 53.5"
11a	S 18° 21' 54.2" EO 21° 51' 49.5"
12a	S 18° 22' 01.5" EO 21° 52' 13.7"

13a	S 18° 23' 27.2" EO 21° 52' 05.7"
14a	S 18° 23' 45.5" EO 21° 52' 04.1"
15a	S 18° 24' 18.5" EO 21° 53' 04.0"
16a	S 18° 24' 48.9" EO 21° 53' 05.9"
17a	S 18° 44' 46.2" EO 22° 11' 54.3"
18a	S 18° 44' 47.9" EO 22° 12' 03.3"
19a	S 20° 00' 18.7" EO 23° 25' 33.2"
20a	S 20° 00' 15.5" EO 23° 25' 33.8"
21a	S 19° 59' 23.8" EO 23° 25' 47.8"
22a	S 19° 59' 28.6" EO 23° 25' 42.1"
23a	S 19° 56' 29.7" EO 23° 29' 42.4"

24a	S 19° 56' 33.2" EO 23° 29' 41.1"
25a	S 19° 55' 17.1" EO 23° 30' 37.6"
26a	S 19° 54' 57.8" EO 23° 30' 56.7"
27a	S 19° 54' 42.2" EO 23° 31' 10.6"
28a	S 19° 54' 41.9" EO 23° 31' 10.8"
100	S 19° 11' 44.1" EO 23° 27' 44.8"
101	S 19° 11' 44.2" EO 23° 27' 44.3"
102	S 19° 11' 46.7" EO 23° 27' 44.7"
103	S 19° 11' 46.1" EO 23° 27' 42.7"
104	S 19° 11' 47.0" EO 23° 27' 43.2"
106	S 19° 11' 55.6" EO 23° 27' 37.0"

107	S 19° 11' 57.6" EO 23° 27' 42.7"
108	S 19° 11' 58.3" EO 23° 27' 41.5"
109	S 19° 11' 56.8" EO 23° 27' 40.3"
110	S 19° 11' 38.8" EO 23° 27' 46.9"
111	S 19° 11' 34.6" EO 23° 27' 42.3"
112	S 19° 11' 27.5" EO 23° 27' 40.5"
113	S 19° 11' 23.9" EO 23° 27' 45.2"
114	S 19° 11' 15.2" EO 23° 27' 39.1"
115	S 19° 11' 04.3" EO 23° 27' 14.8"
116	S 19° 11' 05.3" EO 23° 27' 11.8"
117	S 19° 10' 57.9" EO 23° 26' 57.8"

118	S 19° 11' 00.1" EO 23° 26' 42.0"
119	S 19° 11' 02.8" EO 23° 26' 35.2"
120	S 19° 11' 04.5" EO 23° 26' 34.3"
121	S 19° 10' 45.3" EO 23° 26' 19.0"
122	S 19° 11´ 21.1" EO 23° 25´ 42.7"

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