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MEMBRANE ASSISTED PASSIVE SAMPLER FOR AQUATIC ORGANIC CHEMICALS – characterization of environmental conditions and field performance

By

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A dissertation submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Science

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Declaration

I declare that this dissertation is my own, unaided work. It is being submitted for the Degree of Master of Science in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.

_____
(Signature of candidate)

_____day of_____2010

Abstract

Membrane assisted passive sampler (MAPS) is an informative, cost-effective and environmentally friendly approach for monitoring of ionisable organic compounds in water bodies. The sampler uses no organic solvent. By adjusting the pH of the acceptor phase, both acidic chlorophenols and basic triazine model compounds were extracted. The sampler was optimized under laboratory conditions followed by field applications on the same compounds. The optimised parameters were temperature of the water body, turbulence, protective cover, biofouling, matrix effects such as humic substances, degree of trapping in the acceptor phase and exposure time. It was found that the sampling kinetics of most of the tested analytes are dependent on temperature and on the hydrodynamic conditions. Also, a strong dependence of the sampling rates reduction on sample matrix and protective cover used was noted. The chemical uptake of both the acidic chlorophenols and basic triazine compounds into the passive sampler remained linear and integrative through out the exposure periods. The amounts quantified in the MAPS had relative standard deviations mostly between 10 % and 20 % (from repeat determinations) and did in no case exceed 30 %. The behaviour of the MAPS to monitor ionisable triazine compounds in dam water of the Hartebeespoort was compared to Chemcatcher and solid phase extraction technique with C_{18} sorbents of spot samples. Similarly, the behaviour of the MAPS to monitor ionisable chlorophenol compounds in wastewater of the Goudkoppies Wastewater Treatment Plant was compared to solid phase extraction technique. There were no triazine and chlorophenol compounds detected in any of the deployed passive samplers in the field applications. The same results were obtained in grab samples extracted with solid phase extraction under laboratory conditions. However, data from laboratory studies support the feasibility of MAPS to measure the freely dissolved fraction of ionisable organic chemicals in water. Using water from the Hartebeespoort dam spiked with 50 µg L^{-1} triazine, the detection limits of triazine compounds ranged from 11.38 to 61.86 µg L^{-1} for direct injection, 1.082 to 23.077 µg L^{-1} for MAPS, 0.892 to 5.769 µg L^{-1} for Chemcatcher and 1.482 to 7.410 µg L^{-1} for SPE. While using water from Goudkoppies Wastewater Treatment Plant spiked with 100 µg L^{-1} chlorophenols, the detection limits of the passive sampler were comparable with that of solid phase extraction and were around 1.5 µg L^{-1}. Estimation and interpretation of enrichment factors in the passive samplers and SPE were generally

comparable ranging from 46 to 295 for chlorophenol compounds. Also, for triazine compounds, the obtained enrichment factors in the passive samplers and SPE are generally comparable with the exception of enrichment factors of propazine, ametryn terbuthylazine, prometryn and terbutryn compounds which were higher for the MAPS ranging from 46 to 65.

Keywords: Water monitoring; passive sampling; MAPS; Environmental factors; Chlorophenols; Triazines; HPLC/UV

Dedication

This thesis is dedicated to my father, who taught me that the best kind of knowledge to have is that which is learned for its own sake and my mother, who taught me that even the largest task can be accomplished if it is done one step at a time. It is also dedicated to my wife who knowingly and unknowingly - led me to an understanding of some of the more subtle challenges to our ability to thrive.

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List of Abbreviations

% RSD	Percentage Relative Standard Deviation
AED	Atomic-Emission Detection
ANP	Aqueous Normal-Phase Chromatography
ATSDR	Agency for Toxic Substances and Disease Registry
ATZ	Atrazine
CAD	Charged Aerosol Detector
C_{18}	Octadecyl
CE	Capillary Electrophoresis
DACT	Diaminochloros - Triazine
DAD	Diode Array Detector
DDA	Didealkylatrazine
DEA	Deethylatrazine
DGTs	Diffusive Gradients in Thin films
DIA	Deisopropylatrazine
DMLS	Diffusive Multi-Layer Sampler
ECD	Electron-Capture Detection
ED	Electrochemical Detection
ELISA	Enzyme-Linked ImmunoSorbent Assay
ELSD	Evaporative Light Scattering Detector
EPA	Environmental Protection Agency
EU	European Union
FD	Fluorescence Detection
FID	Flame-Ionization detection
GC	Gas Chromatography
GWWTW	Goudkoppies Wastewater Treatment Works
HIC	Hydrophobic Interaction Chromatography
HILIC	Hydrophilic Interaction Chromatography
HPLC	High Performance Liquid Chromatography
IARC	International Agency for Research on Cancer

ID	Internal Diameter
IPW	Integrated Production of Wine
LC	Liquid Chromatography
LDPE	Low-Density Polyethylene
LOD	Limits of Detection
LSE	Liquid-Solid Extraction
MAPS	Membrane Assisted Passive Sampler
MESCO	Membrane Enclosed Sorptive Coating
MIPs	Molecularly Imprinted Polymers
MS	Mass Spectrometry
nd-SPME	Negligible Depletion Solid Phase Microextraction
NP- HPLC	Normal Phase HPLC
OCP	Organochlorine Pesticides
OD	Outer Diameter
ONP	Organic Normal Phase Chromatography
PAHs	Polycyclic Aromatic Hydrocarbons
PAS	Passive Sampler
PCBs	Polychlorinated Biphenyls
PDBS	Passive Diffusion Bag Samplers
PDMS	Poly Dimethylsiloxane
PE	Polyethylene
PES	Polyethersulphone
POCIS	Polar Organic Chemical Integrative Sampler
POPs	Persistent Organic Pollutants
PRC	Permeability Reference Compounds
PROP	Propazine
PTFE	Polytetrafluroethylene
RI	Refractive Index
RP-HPLC	Reversed phase HPLC
RPC	Reversed phase HPLC
SEC	Size-Exclusion Chromatography

SANCOLD	South African National Committee on Large Dams
SBSE	Stir Bar Sorptive Extraction
SIM	Simazine
SLM	Supported Liquid Membrane
SPATT	Solid-Phase Adsorption Toxin Tracking
SPE	Solid Phase Extraction
SPMDs	Semi-Permeable Membrane Devices
SPME	Solid-Phase Microextraction
TWA	Time Weighted Averaged
USEPA	US Environmental Protection Agency
UV-VIS	Ultraviolet-Visible
WBL	Water Boundary Layer

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~ Chapter One - Introduction ~

1.1 Background

Developments in the area of water quality assessment require that appropriate sampling tools are available to support current and future monitoring programs. The multitude of ionizable organic pollutants such as phenols and triazines, leads to an undesired and problematic exposure situation for aquatic organisms thus monitoring of the presence of these compounds in water bodies is paramount. Most aquatic monitoring programmes employed, rely on collecting discrete, grab, spot or bottle samples of water at a given time. Often, where pollutants are present at trace levels, large volumes of water need to be collected. The subsequent laboratory analysis of the sample provides only a snapshot of the levels of pollutants at the time of sampling. However, there are drawbacks to this approach in environments where contaminant concentrations vary over time, and episodic pollution events can be missed.

One solution to this problem is to increase the frequency of sampling or to install automatic sampling systems that can take numerous water samples over a given time period. This is costly and in many cases impractical, since a secure site and significant pre-treatment of water are required. Such systems are rarely used in widespread monitoring campaigns. Spot sampling sometimes yields different apparent concentrations of pollutants depending on the pre-treatment applied (e.g., filtering) and does not provide information on the truly dissolved, bioavailable fraction of the contaminants.

Another approach that yields information on biologically relevant concentrations of pollutants uses biota (Vrana et al., 2005). A number of test species can be used, depending on the water body being investigated. These organisms can be deployed for extended periods of time, during which they passively bioaccumulate pollutants in the surrounding water. Analysis of the tissues or lipid extracts of the test organism(s) can give an indication of the equilibrium level of waterborne contamination. A number of factors can influence the results such as metabolism,

depuration rates, excretion, stress, viability and condition of test organism. Furthermore, extraction of analytes from the tissue of animals prior to instrumental analysis is complex (Vrana et al., 2005).

Estimates of pollutant concentrations in water can also be made by measuring concentrations in benthic sediments and then using equilibrium distribution coefficients to derive levels of dissolved analytes. This approach is limited by the assumption of equilibrium between the sediments and the water column, and the potential effects of organic carbon quality differences among sediments or the formation of non-extractable, sediment-bound residues that are not accounted for in current equilibrium-partition models (Vrana et al., 2005).

In the last two decades, alternatives have been sought to overcome some of these difficulties (Vrana et al., 2005). Of these, passive sampling methods have shown much promise as tools for measuring aqueous concentrations of a wide range of priority pollutants. Passive samplers avoid many of the problems outlined above, since they collect the target analyte *in situ* and without affecting the bulk solution. Depending on sampler design, the mass of pollutant accumulated by a sampler should reflect either the concentration with which the device is at equilibrium or the time-weighted averaged (TWA) concentration to which the sampler was exposed.

The development and applications of passive samplers for water monitoring of various chemicals has undergone considerable increase. While the first passive sampler for water monitoring was reported in the 1980s, not much development and applications happened until the mid 1990s. Currently, passive samplers for water monitoring are seen as complementary to other standard active extraction methods. Lower detection limits can be achieved because they are deployed for longer periods.

In this work, a simple and very selective membrane assisted passive sampler (MAPS) that does not use organic solvents, based on a silicone hollow fibre membrane for extraction of ionisable organic compounds in water bodies, is reported. The potential for passive sampling of both acidic and basic compounds in MAPS is demonstrated. The sampler is inexpensive, selective

and uses no organic solvents. By changing the acceptor solution from basic to acidic conditions, the MAPS can be successfully used to extract basic organic compounds such as triazines. The influence of environmental factors such as temperature, sample matrix and hydrodynamics on enrichment factors and sampling rates has been investigated in order to calibrate the passive sampler for the measurement of TWA concentrations of ionizable organic pollutants. The selectivity, extraction efficiency and enrichment factor of the developed sampler has been compared to solid phase extraction technique and to commercially available Chemcatcher passive sampler.

1.2 Statement of the Problem

It is necessary to monitor ionizable organic chemicals in the aquatic environment to satisfy the requirements of legislative frameworks and directives, as many of these compounds can pose a threat to both human health and ecosystems. Currently, the method used for measuring chemical pollutants in water is spot (bottle/grab) sampling and laboratory analysis. This approach, among other advantages, provides manageable control over accuracy and precision of the results.

Emphasis on environmental monitoring technology including that of water bodies is now moving towards environmental friendly technologies especially those that use less organic solvents. These technologies are also called green extraction technologies. Previously developed passive samplers used large volumes of organic solvents either as receiving phase or during the subsequent re-extraction of the trapped analytes from the sampler before final analysis. New passive samplers however are designed to use less or no organic solvents. Further, they are designed to trap a variety of chemicals from water which is often done by changing the type of trapping media.

The MAPS is based on a silicone hollow fibre membrane for extraction of ionisable organic compounds in water bodies. The inside of the tube is filled with an aqueous solution at an appropriate pH. The tube is sealed at both ends and then immersed in a water sample. In order for the ionisable permeating compounds to be trapped in the aqueous receiving phase, the pH is adjusted such that the compounds are ionized and trapped. The major advantages are its simplicity, low cost and high selectivity, since only ionisable organic compounds are trapped. Additionally, the sampler uses no organic solvent. By adjusting the pH of the acceptor phase, it is possible to control the extraction process and whether the sampler is used in the kinetic or equilibrium regime. Since it is very selective, no further clean-up of the extract is required. It is non-mechanical, easy to deploy and require no maintenance and satisfy the requirements for green technology.

1.3 General and Specific Objectives

The general objective of this project is to characterize the effect of variable environmental conditions on the MAPS performance for monitoring of triazines and chlorophenols in water bodies and compare its performance with known extraction techniques.

The specific objectives of this project are:

- To demonstrate the potential of MAPS for passive sampling of phenols and triazines compounds in water bodies.

- To conduct calibration studies for variable environmental conditions (e.g. effects of temperature, sample matrix and hydrodynamics on sampler performance).

- To investigate the effect of humic substances on the sampler performance on the model compounds.

- To quantitatively investigate whether there is successive loss of the trapped analyte from the device through diffusion during complex environmental matrix exposure.

- To compare the selectivity, enrichment factor and extraction efficiency of the MAPS to the Chemcatcher sampler and Solid phase extraction techniques.

- To conduct a field study to test the sampler performance alongside spot sampling and commercially available passive sampler (Chemcatcher) and Solid Phase Extraction Techniques.

1.4 Hypothesis and Research Questions

1.4.1 Hypothesis

The silicone hollow fibre can be used as a passive field sampler for monitoring ionizable organic chemicals in water.

1.4.2 Research Questions

This research project seeks to answer the following research questions:

1. How does variable environmental conditions (e.g. temperature, water turbulence and biofouling) affect the uptake kinetics of the sampler?

2. How does enclosing the silicone hollow fibre into an enclosure, such as a stainless steel mesh affect the mass transfer of the compounds of interest?

3. Is there any successive loss of the trapped analyte from the device through diffusion during environmental exposure?

4. Do humic substances have an effect on the sampling rate of the sampler?

5. By changing the acceptor solution from basic to acidic, the MAPS can be used to extract basic compounds. Can this be demonstrated with basic compounds such as triazines?

6. How does the MAPS compare with Solid Phase Extraction and commercially available Chemcatcher techniques?

~ Chapter Two - Literature Review ~

2.1 General Theory of Passive Samplers

The trapping of chemicals in the passive sampling devices has been described as simple diffusion and partitioning between two compartments of the receiving phase and external environment separated by a diffusing-limiting membrane (Kingston et al., 2000; Stuer-Lauridsen, 2005; Vrana et al., 2006a). The dimensions of the sampling device and the materials it is made of determine the rate of chemical uptake into the receiving phase. Figure 2.1 illustrates most of the barriers to chemical uptake into the receiving phase. In this illustration, it is assumed that both external aqueous boundary layer and the membrane matrix control the uptake rates. The resistance to mass transfer for all steps or barriers are assumed to be additive. Therefore, a fractional reduction in the resistance of any barrier will results in some increase in chemical uptake rate.

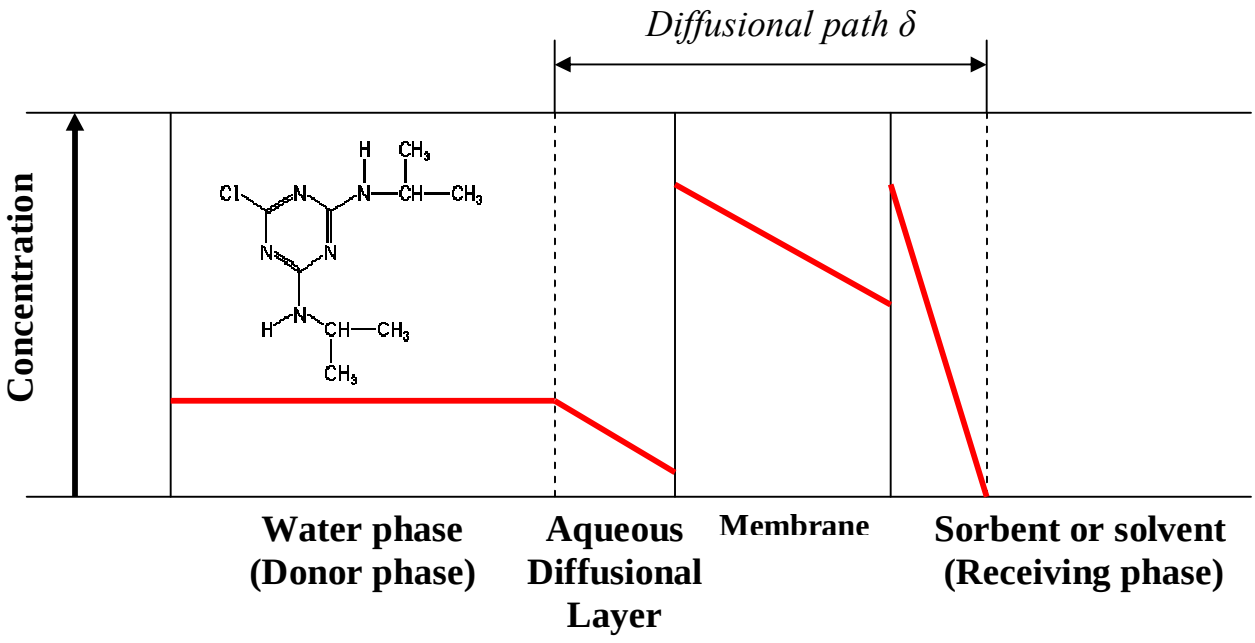


Figure 2.1 General representation of the barriers to chemical uptake into the sampler. The delta (δ) represents the effective diffusion path. The red line shows the chemical concentration gradient in the water through each barrier.

The exchange kinetics between the sampler and water can be described by a first-order one-compartment model for which graphic representation is shown in Figure 2.2A. Two main regimes (kinetic and equilibrium) which can be distinguished in the operation of a sampler during field deployment are presented in Figure 2.2 B

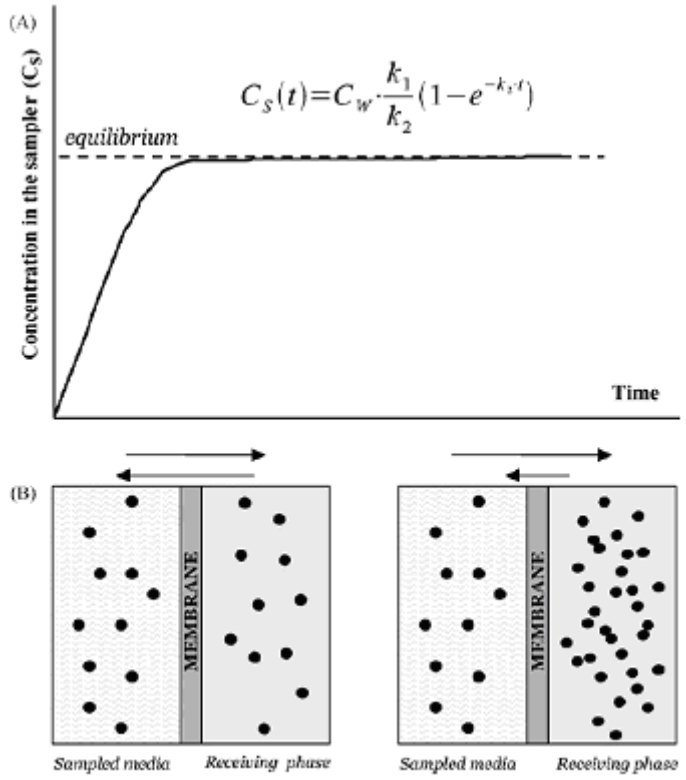


Figure 2.2 (A) Exchange kinetics between the sampler and the water. $C_S(t)$ is the concentration of the contaminant in the sampler as a function of time, t, C_W is the contaminant concentration in the aqueous environment, and k_1 and k_2 are the uptake rate and the offload rate constants, respectively. **(B)** Graphical presentation of equilibrium- and non-equilibrium passive sampler. This is indicated by the arrows above each regime and assumes equal volumes of the sample and receiving media.

Based on the concentration gradient of contaminants in the water and in the collection phase, contaminants can diffuse into passive sampling devices until an equilibrium is reached. Upon achieving equilibrium, further enrichment of contaminants within the sampler can no longer take place. The time span available until equilibrium is reached depends on the capacity of the collection phase for the contaminants of interest and the partition coefficient of the compounds into the receiving media. Thus, passive sampling devices can, for practical reasons, be divided

into equilibrium- and non-equilibrium samplers. In the case of equilibrium sampling, the deployment time is sufficiently long to permit the establishment of thermodynamic equilibrium between the water and the reference phase. Knowledge of reference phase-water partition coefficients allows for the calculation of the dissolved contaminant concentration. The use of equilibrium passive sampling devices has been recently reviewed (Mayer et al., 2003). The basic requirements for the equilibrium sampling approach are that stable concentrations are reached after a known response time. The sampler capacity is kept well below that of the sample to avoid depletion during extraction and the device response time needs to be shorter than the fluctuations in the pollutant concentration being measured. Equilibrium sampling devices based on solid-phase microextraction (SPME) (Pawliszyn, 1997) have been extensively used to measure dissolved concentrations of pollutants in different matrices (Kraaij et al., 2003; Mayer et al., 2000) and to estimate the bioaccumulation potential in effluents and surface waters (Verbruggen et al., 2000). Equilibrium samplers are characterized by a rapid achievement of equilibrium between contaminants in the water to be sampled and contaminants inside the passive sampler. One consequence of achieving equilibrium rapidly is that contaminants are also capable of diffusing back into the surrounding water, should aqueous concentrations of contaminants decline. Two other frequently used equilibrium samplers are water-filled polyethylene (PE) bags (PDBS, passive diffusion bag samplers) and the diffusive multi-layer sampler (DMLS). For both systems, equilibrium can be generally assumed to be reached within 7 days. Passive diffusion bag samplers have been employed to monitor volatile organic compounds in water (Kot-Wasik et al. 2007).

Non-equilibrium samplers are those that do not reach equilibrium with the surrounding water within the sampling period. These samplers are characterized by a high capacity for collecting the contaminants of interest. The high capacity ensures that contaminants can be enriched continuously throughout the sampling period. Based on the application of this type of passive samplers, average contaminant concentrations present in the water over the entire sampling period can be obtained. These concentrations are also referred to as time weighted average concentrations (TWA). Most passive samplers are being employed as non-equilibrium, i.e. time integrating samplers, for periods of 2 weeks to about 3 months. With kinetic sampling, it is assumed that the rate of mass transfer to the reference phase is proportional to the difference

in chemical activity of the contaminant between the water phase and the reference phase. When the proportionality constant or sampling rate is known, the TWA concentration of a pollutant in the water phase can be calculated. The advantage of kinetic or integrative sampling methods is that they sequester contaminants from episodic events commonly not detected with spot sampling, they can be used in situations of variable water concentrations, and they permit the measurement of ultra-trace, yet toxicologically relevant, contaminant concentrations over extended time periods (Vrana et al., 2005).

A range of integrative passive sampling devices has been developed and used in recent years. A comprehensive review of currently available passive sampling devices has been published (Namiesnik et al., 2005). Among the most widely used samplers are semi-permeable membrane devices (SPMDs) for hydrophobic organic pollutants (Huckins et al., 1993) and the diffusive gradients in thin films (DGTs) for metals and inorganic ions (Diviš et al., 2007; Zhang et al., 1998). Several novel passive sampling devices suitable for monitoring a range of non-polar and polar organic chemicals, including pesticides, pharmaceutical/veterinary drugs and other emerging pollutants of concern have recently been developed (Kingston et al., 2000; Alvarez et al., 2004; MacKenzie et al., 2004; Bopp et al., 2005; Vrana et al., 2006a). Among them, ceramic dosimeters (Bopp et al., 2005), MESCO (Vrana et al., 2006b) SPATT (MacKenzie et al., 2004), Chemcatcher (Kingston et al., 2000) and POCIS (Alvarez et al., 2004) are of greatest attention.

2.2 Sampler Design Considerations

2.2.1 Sampler Design

In order to have maximum sensitivity, a sampler design should have high A/L ratio where A is the area and L is the length of the active device. Tube type samplers are therefore less sensitive compared to badge type samplers. The latter have high A/L ratio and most passive samplers are therefore configured in the badge type. Whatever design is employed, passive samplers mostly

have a barrier between the sampled medium and the receiving phase. The barrier determines the rate at which analyte molecules are collected in the receiving phase. Some barriers have defined openings resulting into diffusion-based samplers. Others have the barrier in form of a non porous membrane, referred to as permeation-based samplers (Vrana et al., 2005). Some factors that influence the uptake rate are sampler design, physico-chemical properties of the analytes and environmental variables (e.g., water turbulence, temperature and biofouling) (Vrana et al., 2005).

2.2.2 Quality Control

It is very important that the concentration determined using the sampling devices reflect the true picture in the environmental media. Quality control procedures should address issues such as accuracy and precision of the results, contamination and loss of the trapped analytes. Stuer-Lauridsen (Stuer-Lauridsen, 2005) points out that when a passive sampler is retrieved, it must be inspected for signs of puncture, discolouring or any malfunctioning. When passive samplers are calibrated in the laboratory, it is generally easy to obtain good precision (< 5 %) between replicates. However, in real environmental media, it may be difficult to control certain parameters such as biofouling, turbulence and temperature. This lack of control results in high standard deviations between replicates and poor accuracy. The precision of some samplers has been reviewed recently (Stuer-Lauridsen, 2005). In this review it was noted that the average percentage relative standard deviations for aquatic passive samplers ranged from 10 % - 32 % (Stuer-Lauridsen, 2005). The physical chemical properties of the compounds as well as the materials used to construct the sampler may also influence the precision of the results similar to active sampling techniques. Others (Vrana et al., 2005; Booij and van Drooge, 2001; Verweij et al., 2004) have suggested the use of permeability reference compounds (PRCs) for quality control. Similar to internal standards, these reference compounds are added to the trapping media prior to the deployment of the sampler and correct for any changes in uptake rates due to environmental factors. The recovery in re-extracting the trapped compounds from the receiving phase needs also to be evaluated as part of the quality control. Recovery is determined by

spiking the compounds of interest to the receiving phase and then re-extracting them to check for the recovery (Verweij et al., 2004).

2.3 Types and Principles of Passive Samplers for Water Monitoring

2.3.1 Solvent Filled Dialysis Bag

In this case, a dialysis membrane made of regenerated cellulose in the form of a tube is filled with an organic solvent, typically hexane (Figure 2.3). This design was first introduced by Södergren (1987). It was the first passive sampler to be introduced for monitoring organic compounds in water bodies. The selectivity of the sampler is based on differences of the dissolution into the membrane and also on pore size. It was thought to mimic bioconcentration just like in fish and other invertebrates. The dialysis membrane has also a cut off that excludes large molecules, similar to biological membranes. It is very simple, cheap and there is a successive loss of the organic solvent from the device through diffusion during environmental exposure which is said to prevent biofouling on the surface of the sampler. However, the solvent-filled dialysis bag passive sampler has not gained much popularity because of this successive loss of the organic solvent from the device through diffusion during environmental exposure and because it results in lack of selectivity. The technique may be used to confirm bioaccumulation mechanisms, predict environmental hazards of bioavailable compounds, and to monitor lipophilic pollutants, especially in environments too severe for biological indicators to survive (Södergren, 1987).

2.3.2 Semi Permeable Membrane Devices (SPMDs)

The SPMDs is perhaps the most common passive sampler in use today. Its design and study was first published by Huckins et al. (1990). Since then, a number of studies have been performed on them and details can be found in review papers (Lu et al., 2002; Petty et al., 2000). SPMDs consist of lay flat tubing made of low-density polyethylene (LDPE) filled with

a high molecular weight lipid (Figure 2.4). Synthetic triolein is often used as the common lipid. The LDPE is non porous but has transient cavities with typical size of 1 nm (Vrana et al., 2005). The selectivity of the sampler is based on the size of the molecules and their ability to dissolve into the membrane. Large macromolecules, ionic compounds and polar compounds do not dissolve into the membrane.

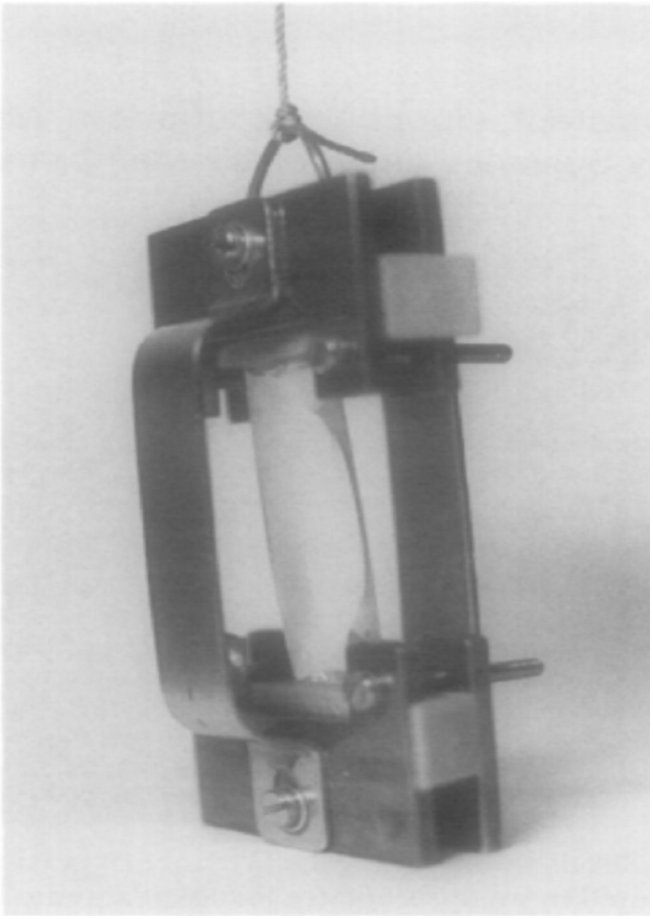


Figure 2.3 Solvent-filled dialysis membrane mounted in metal holders used for field investigations. The length of the sampler 8 cm (Södergren, 1987).

Compounds with Log K_{ow} > 3 are ideal for trapping in triolein (Petty et al., 2000; Huckins et al., 1996). The major shortcoming of SPMDs is the time needed to re-extract the trapped compounds from triolein and the use of large volumes of organic solvents. Microwave assisted

extraction has been proposed as a faster method to re-extract the trapped compounds (Yusá et al., 2005). Widely used method, commercially available, well-established passive sampler for monitoring hydrophobic semi-volatile organic compounds e.g. PAHs, PCBs and organotin compounds in air, water, sediments and wastewater.

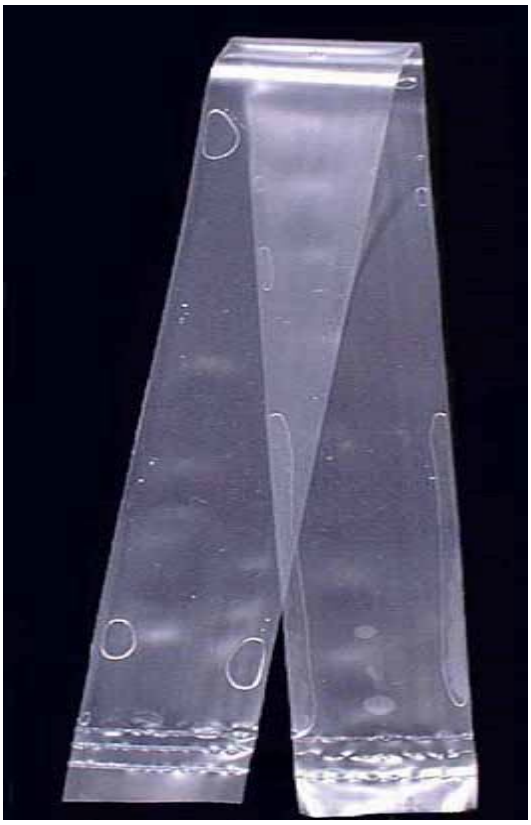


Figure 2.4 Picture of an examplary SPMD (Huckins et al., 1990). The lay-flat tubing made of low-density polyethylene (LDPE) filled with a high molecular weight lipid e.g. triolein

2.3.3 Ceramic Dosimeter

The sampler is made of a ceramic membrane (outer layer) and adsorbent material (Figure 2.5). A ceramic tube is the diffusion-limiting barrier, enclosing a receiving phase that consists of solid sorbent beads; the cap material is PTFE (an inner diameter of 1 cm). Filled ceramic tubes are fixed in stainless steel holders of 6 cm length; the receiving phase: Amberlite IRA-743; membrane thickness 0.15 cm; surface area (tube length: 5 cm; tube diameter: 1 cm) is 8.5 cm^2;

pore size–5 nm (Bopp et al., 2005). The contaminants accumulate by diffusing from the contact water through the membrane into the adsorbent bed. They accumulate with time, depending on the concentration gradient and the effective coefficient of mass transfer across the membrane. The sampler is used to monitor polycyclic aromatic hydrocarbons (PAHs), volatile aromatic compounds, volatile chlorinated hydrocarbons and alkylnaphtalenes in groundwater, rivers, lakes, wastewater sewers (Bopp et al., 2005).

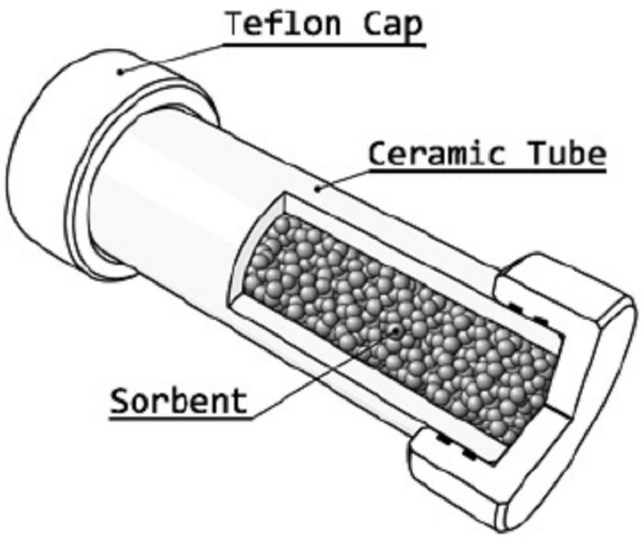


Figure 2.5 Design of ceramic dosimeter passive samplers for water environment analysis.

2.3.4 Chemcatcher (Passive Sampler using Empore Disk)

The chemcatcher's system uses a diffusion-limiting membrane and inside a commercially available solid-phase Empore disks as a receiving phase. A diffusion-limiting membrane and the solid-phase receiving material are supported and sealed in place by inert plastic housing (Figure 2.6). A number of designs are available with different combinations of receiving phase and a diffusion-limiting membrane. Accumulation rates and selectivity are regulated by the choice of both the diffusion-limiting membrane and the solid-phase receiving material.

In this case two separate prototype systems have been described, one suitable for the sampling non polar organic compounds with Log K_{ow} (partition coefficients) values greater than 4 and the other for polar species with Log K_{ow} values between 2 and 4. Both systems use the same receiving phase but different rate - limiting membranes. The use of well-known and commercially available receiving phase makes this sampler promising. Vrana et al., (2006a) reported to have calibrated the Chemcatcher passive sampler for monitoring of priority organic pollutants in water. Environmental factors such as turbulence and temperature were studied in a flow-through system under controlled conditions. PRCs were also used to correct for environmental factors. The results revealed that the absorption of test compounds on the sampler was similar to their desorption under the same exposure conditions. Therefore, in situ calibration of the sampler is possible using PRCs. The sampler has been calibrated for monitoring polar or nonpolar organics, some persistent organic pollutants (POPs) such as organochlorine pesticides, PCBs and polycyclic aromatic hydrocarbons (PAHs) in water (Alvarez et al., 2004).

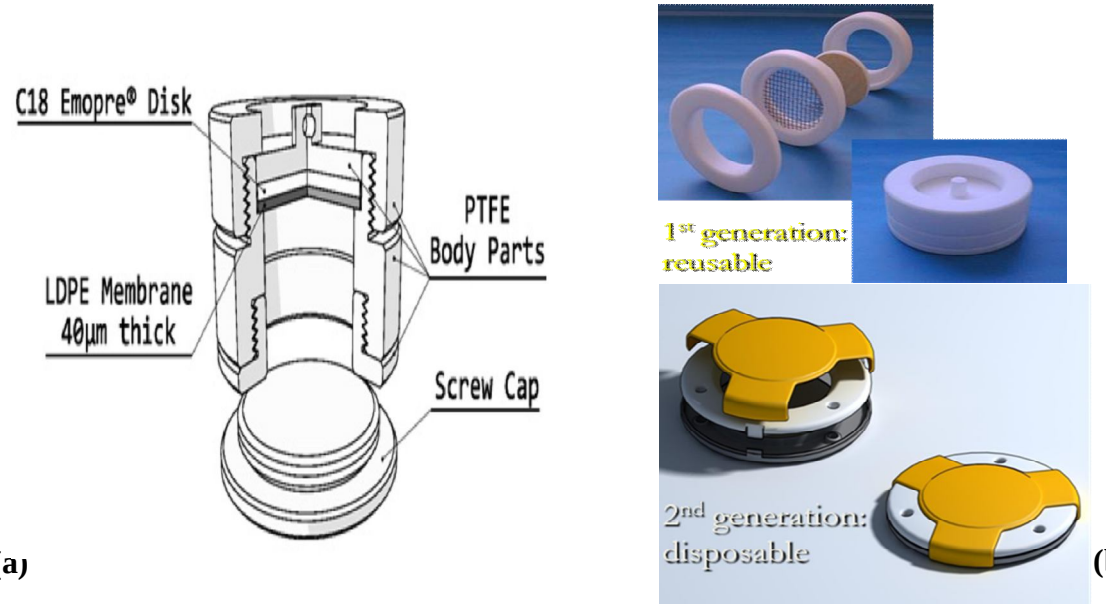


Figure 2.6 Views of the prototype Chemcatcher device, used during sampler development. The PTFE body parts (1st generation) support the receiving phase and the diffusion membrane and sealed them in place. The sampler is sealed by means of a screw cap for storage and transport; (2nd generations), The latest version of disposable Chemcatcher with housing made of inert plastic, containing a disk of solid sorbent and a disk of diffusion membrane (Kingston et al., 2000).

2.3.5 Diffusive Gradients in Thin Film (DGT) Sampler

The DGT was invented by William Davison and Hao Zhang in Lancaster (Zhang and Davison, 1995). The whole sampler is made from plastic and consists of three layers (Figure 2.7). The first one (a filter membrane) consist of a resin-impregnated gel layer (metal-binding layer). A plastic outer-sleeve is placed over the base in order to secure the layers, to maintain an even surface, and to inhibit water ingress into the resin-gel. The resin-layer is overlain by a diffusive layer of hydrogel and a filter. Ions have to diffuse through the filter and the diffusive layer to reach the resin layer. It is the establishment of a constant concentration gradient in the diffusive layer that forms the basis for quantitively measuring metal concentration in a solution without the need for separate calibration. The DGT sampler can be put directly to the sample and has low risk of sample contamination or preconcentration of the sample analytes and is simple in use. It is held by a rope or string when put in water. The sampler measures a wide range of concentrations. The maximum concentration that can be measured depends on the capacity of the resin (from 30 to 100 mg L^{-1} depending on the metal).

The DGT was tested for integrative sampling of trace metals (Cd, Ca, Mg, Zn and Mn) in natural waters (fresh water, deep sea water, rivers, lakes and estuaries). (Peters et al., 2003; Li et al., 2005). It has also been calibrated and tested for (1) in situ measurements; (2) monitoring (time averaged concentrations); (3) speciation (labile inorganic and/or organic species); (4) bioavailability (effective concentration); (5) fluxes in sediments and soils; (6) kinetic and thermodynamic constants and (7) high spatial resolution measurements (sub-mm) (Kot-Wasik et al., 2007).

2.3.6 Membrane Enclosed Sorptive Coating (MESCO)

This device consists of a stir bar coated with poly (dimethylsiloxane) (PDMS) enclosed in a dialysis membrane bag (Figure 2.8) (Vrana et al., 2001). It combines the advantages of passive sampling approach with solventless preconcentration of organic solutes from aqueous matrices and subsequent desorption of the sequested analytes on line with capillary gas chromatography.

It avoids clean up of extracts required for other samplers and whole extract is injected. Injection of the entire extract makes it quite sensitive despite the small surface area and volume of the sampler. The stir bar used as receiving phase is similar to the one used in stir bar sorptive extraction (SBSE) technique (Baltussen et al., 1999).



Figure 2.7 Photo (by A. Künzelmann, UFZ) of an exemplary DGT sampler made from plastic as an outer-sleeve placed over the base in order to secure the three layers and maintain an even surface, and to inhibit water ingress into the resin-gel (Zhang and Davison, 1995).

The passive sampler was tested for integrative sampling of hydrophobic persistent organic pollutants in the laboratory. Linear uptake rates of all test compounds were observed with one week exposure period (Vrana et al., 2001). Turbulence was evaluated as well as in situ calibration to account for its effects on uptake kinetics. Desorption of chemicals from the sampler was found to be similar to the absorption of the compounds onto sampler under same exposure conditions. This allows for in situ calibration using PRCs. The MESCO has recently been calibrated and tested for field performance for persistent organic pollutants such as pesticides, PCBs, PAHs and selected POPs in water (Vrana et al., 2006b).

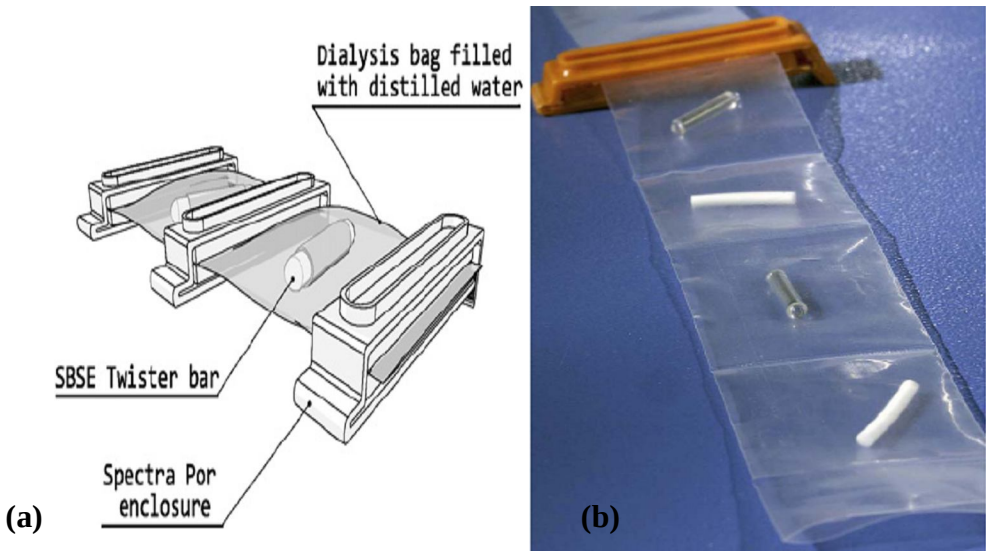


Figure 2.8 (a) Schematic diagram of the MESCO passive sampling device. A Gerstel-Twister bar used for SBSE is enclosed in a dialysis membrane bag made from regenerated cellulose. The dialysis membrane bag is filled with 3 mL of bi-distilled water and sealed at each end with Spectra Por enclosures. **(b)** Photo (by A. Künzelmann, UFZ) of an exemplary MESCO strip with four heat sealed segments. The lay-flat LDPE membrane encloses silicone rod pieces (from Goodfellow) and PDMS-coated Twister bars (from Gerstel), respectively (Paschke et al., 2006).

2.3.7 Negligible Depletion Solid Phase Microextraction (nd-SPME)

The nd-SPME uses a polymer coating of an optical silica fiber just like in traditional SPME. However, in this case, the fibre is exposed in the headspace above the sample or directly in the sample without any stirring. In the extraction there is equilibrium between the bound and free fraction of analyte, the depletion of the free fraction is negligible and the binding matrix does not affect the process (Heringa and Hermens, 2003) and nd-SPME takes advantage of the SPME technique of using little organic solvents, simplicity and precision (Arthur and Pawliszyn, 1990). The specific application to measure the free fraction by nd-SPME was introduced by Heringa and Hermens (2003). nd-SPME has the disadvantage of offering only small amounts of the sample for analysis that may lead to detection limit problems.

2.3.8 Polar Organic Chemical Integrative Sampler (POCIS)

Alvarez et al. (2004) has described the POCIS. It consists of a solid receiving phase material enclosed in microporous polyethersulphone diffusion - limiting membrane (Figure 2.9). Unlike other samplers with only one type of solid receiving material, this contains a mixture of three solid phase sorbents (Isolute ENV, polystyrene divinyl benzene and Ambersorb 1500 carbon) dispersed on S-X_3 biobeads. The sampler device is manufactured by Environmental Sampling Technology (USA) in a patent agreement with the U.S. Geological Survey. It is marketed under the name of AQUASENSE-P.

The main driving force in the operation of POCIS is the difference in concentrations. This enables sampling of chemicals by diffusion of the substances through a membrane. Contaminated water penetrates the membrane and infiltrates the sequestration polymers which sorb and immobilize water-soluble compounds. Chemicals are thereby cumulatively collected in the sorption medium and the method is less sensitive to variations in the water since sampling continues over a long period of time. Moreover, the sampler uses minimal organic solvents and the extracts are analyzed with no further clean up. The only drawback of POCIS is that for accurate concentrations to be calculated, calibration studies need to be conducted on the analytes of interest.

The sampler has been used to monitor pesticides, phenoxyacids, pharmaceuticals, sulphur drugs antibiotics, tetracycline-antibiotics, methamphetamine, MDMA (Ecstasy) estradiols and estrone, estriol, alkylphenols benzophenone, caffeine, antibacterial and antifungal agents, organic waster water originated contaminants in wastewater effluents, fresh and salt water (Alvarez et al., 2005; Alvarez et al., 2004).

2.3.9 Other Passive Samplers

Other passive samplers for monitoring organic compounds in water bodies have been reported. Vrana et al. (2005) and Stuer-Luarisdsen (2005) have highlighted these in their reviews. These

include the active carbon filled acrylic polymer sampler (Zhang and Handy, 1989), carbon filled silicone sampler (Peterson et al., 1995), silicone sampler with or without resin (DiGiano et al., 1989), trimethyl pentane passive sampler that uses a polymer tube field with isooctane as receiving phase (Zabik et al., 1992; Booij et al., 2002) and others.

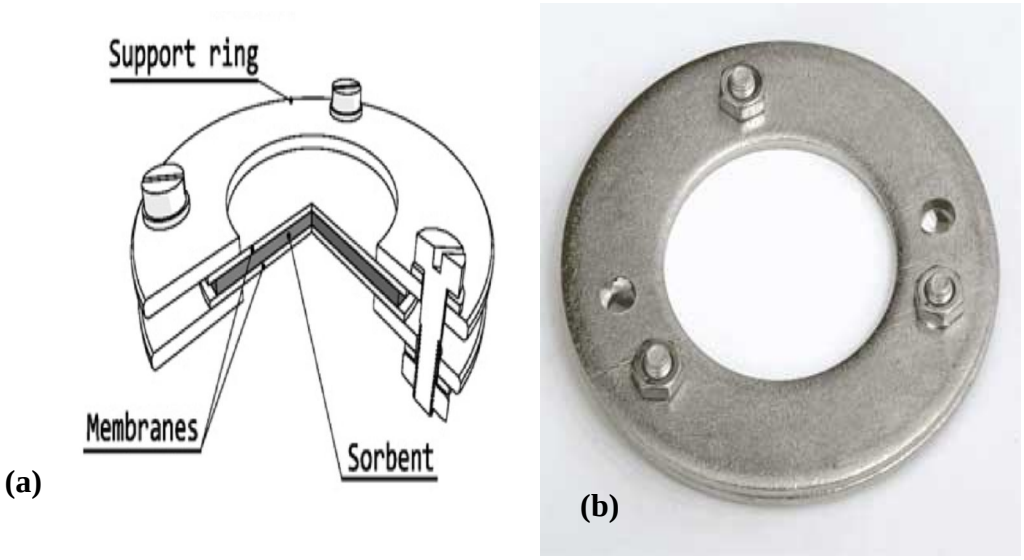


Figure 2.9 (a) Schematic diagram of the POCIS passive sampling device consisting of a solid receiving phase material enclosed in microporous polyethersulphone diffusion -limiting membrane and **(b)** Photo of an exemplary POCIS (Alvarez et al., 2004).

2.4 Calibration of Passive Samplers Used in Liquid Media

The time-weighted average concentration can be determined only when the equilibrium concentration of the analyte in the receiving phase is not achieved. Under these conditions, the time-weighted average concentration of an analyte can be calculated using the following equation:

$$C_W = \frac{M_S - M_0}{R_S t} \quad (1)$$

where C_W is the concentration of the analyte in water during exposure time, M_S the amount of the analyte accumulated in a sampler after exposure, M_0 the amount of the analyte in a sampler before exposure, t the exposure time and R_S is the sampling rate of the analyte under given environmental conditions.

The sampling rate is characteristic for the individual analyte of interest and can be estimated using the following equation (Vrana et al., 2006a):

$$R_S = k_o A = k_e K_{DW} V_D \quad (2)$$

where k_o is the overall mass transfer coefficient, A the surface area of a membrane, k_e the overall exchange rate constant, K_{DW} the receiving phase/water partitioning coefficient and V_D is the volume of the receiving phase.

As described above, the sampling rate of the analyte is dependent on the overall mass transfer coefficient k_o (molecular diffusivity in each layer divided by the respective thicknesses of layers) and the partitioning coefficient between the receiving phase K_{DW} and the membrane surface area A (Huckins et al., 2000). High R_S values are required, because analytes in the environment have low concentration levels. Therefore, increasing the exchange area between the water and the passive sampler or limiting the resistance to mass transfer through individual layers of the sampler is recommended. Improving sampling rates has been addressed using a variety of sampler design (Huckins et al., 2000).

It is very important to define which layer is limiting the sampling rate of the analyte of interest. Generally, four layers can be distinguished: the receiving phase, the membrane, possible biotic contamination of the membrane and the aqueous boundary layer. The resistance to mass transfer for all these layers is an additive value and does not depend one on another (Booij et al., 2002). Any layer contributing more than 50 % of the total resistance is considered uptake rate limiting. Taking into consideration the SPMD sampler and the relatively low water flow, the uptake rate of the analyte of interest is dependent on the membrane (when Log $K_{ow} < 4.5$) and on the aqueous boundary layer (when Log $K_{ow} > 4.5$). In case of samplers with a thick

biofilm layer and analytes having Log $K_{ow} \approx 6.0$, the biofilm can have the greatest impact on the sampling rate of the analyte (Huckins et al., 2002). Environmental conditions, such as water flow or biotic contamination of the membrane, influence the thickness of the aqueous boundary layer and the uptake rate of the analyte under investigation. It should be noted that the maximum sampling rate can be obtained for samplers in which the limiting barrier is the aqueous boundary layer (Paschke et al., 2006).

Therefore, if the purpose of the study is to determine ambient water concentration, it is required to calibrate the sampler before its application to field measurements. This involves an estimation of the impact of surrounding conditions on the performance of the sampler and a determination of sampling rates. In order to calibrate the passive sampler, it is necessary to construct an appropriate exposure system in the laboratory. The possibility of establishing both constant temperature and water flow in the calibration system should be ensured. To maintain and monitor constant water concentration is the most important factor in performing a calibration experiment. Generally, samplers are exposed to continuous flow through the exposure system with a constant flow rate (Vrana et al., 2006a). Another approach, called rapid serial batch extraction, was suggested recently (Paschke et al., 2006). Samplers were placed in bottles (1 L) filled with aqueous standard solution of the analytes. The content of the bottles was agitated constantly by means of rotator (10 rpm). To obtain constant water concentration, every 48 h concentrations of the analytes in the aqueous phase were determined and the standard solution replaced with the fresh one. In comparison with flow through exposure system, this calibration method is much easier to conduct in a typical laboratory. However, changing water every 48 h, or as some authors suggest, every 24 h is time consuming. In addition, the authors note the fact that analytical results received in this way require confirmation; therefore, the sampler should be recalibrated by means of flow through exposure system. However, taking into account the lack of calibration data, this approach can be useful for obtaining initial results (Paschke et al., 2006).

Performing calibration in the laboratory is not complicated, though it is a time-consuming procedure. Additionally, it is impossible to model the wide range of environmental exposure conditions. Therefore, the use of performance reference compounds (PRC) was suggested.

PRC are analytically non interfering organic compounds that are added to the receiving phase of the sampler before its exposure. The amount of PRC is determined in the sampler after exposure and compared with the appropriate calibration data. Assuming that isotropic exchange kinetics apply and that PRC's sampler-water partition coefficients are known, measurement of PRC dissipation rate constants during sampler field exposures and laboratory calibration studies permits the calculation of an exposure adjustment factor (Huckins et al., 2002). PRC allow the assessment of whether the analyte under investigation is in the integrative uptake phase or at the equilibrium. For example, if the PRC (Log K_{ow} = 5) were released from the sampler, this means that all analytes of the same or lower hydrophobicity reached a sorptive equilibrium state and the concentration of these analytes should be calculated using the sampler-water partitioning coefficient. On the other hand, if the reference compound (Log K_{ow} = 6) was retained, then all analytes of this hydrophobicity are still in the integrative uptake phase (Booij et al., 2002). According to the theory, the uptake of an analyte into the passive sampler is linear and integrative approximately until the concentration factor of the sampler reaches half-life (Eq. (3)):

$$\frac{m_D(t_{50})}{V_D/C_W} = \frac{m_D(\infty)}{2} = \frac{K_{DW}}{2} \qquad (3)$$

where m_D is the mass of analyte in the receiving phase and t_{50} is the time required to accumulate 50 % of the equilibrium concentration of the analyte. Under these conditions, the linear model for determining the time-weighted average concentration of the analyte under investigation can be applied (Huckins et al., 2002). In case the concentration of analytes in the sampler during exposure increases linearly, estimation of t_{50} requires the knowledge of analyte uptake rate for given environmental conditions (Huckins et al., 2000):

$$t_{50} = \frac{\ln 2\, K_{DW}\, V_D}{R_S} \qquad (4)$$

As previously indicated, it is difficult to exactly predict the environmental conditions existing at the sampling site over the exposure period and therefore PRC were also suggested in order

to estimate the t_{50} value. If the isotropic exchange kinetics law for the uptake of the analytes is fulfilled, then the t_{50} value corresponds to the release of PRC from the sampler (t_{50} is the time required for the concentration of PRC to decrease by 50 % in the receiving phase of the sampler) (Eq. (5)) (Huckins et al., 2000):

$$t_{50} = \frac{\text{In } 2}{k_e} \qquad (5)$$

Nowadays, PRC are successfully applied in such types of passive samplers as SPMD, MESCO and Chemcatcher. However, their application in the samplers assigned for more polar analytes (for example: POCIS samplers) is still under investigation. Parallel application of the two types of passive samplers-POCIS and mini-SPMD (containing PRC) was recently suggested (Alvarez et al., 2007).

2.5 Novelty and Theory of the Developed Passive Sampler

The theory of extraction for the MAPS is similar to the one developed for the supported liquid membrane (SLM) extraction technique (Jönsson et al., 1993; Jönsson and Mathiasson, 1999). In brief, in order for the chlorophenols to dissolve into the silicone membrane from the sample, they have to be non-ionized at the sample pH. The compounds then diffuse through the membrane into the acceptor phase.

Once in the acceptor phase, they are ionized and trapped (Figure 2.10a). The concentration of non-ionized phenols in the acceptor phase is thus kept at zero. This maintains a concentration gradient between the two phases; the donor and acceptor phases. In this way the concentration of the compounds in the acceptor solution can be increased much higher than in the original sample without experiencing a plateau or maximum and is limited by the sample volume and/or the extraction time. This also gives selective enrichment since only compounds that are ionized at the pH of the acceptor phase are enriched (Vrana et al., 2001).

Compounds that are ionized at the pH of the sample solution do not dissolve into the membrane since they are too polar. Larger molecules have slow diffusion in the membrane and are excluded. From the developed theory (Jönsson et al., 1993; Jönsson and Mathiasson, 1999), an acidic compound (like chlorophenols) needs an acceptor pH that is 3.3 units above its pK_a value for it to be completely trapped and the sample pH must be 2 units below its pK_a for the compounds to dissolve into the membrane and basic compounds (like triazines) needs an acceptor pH that is 3.3 units below its pK_a value for it to be completely trapped and the sample pH must be 2 units above its pK_a for the compounds to dissolve into the membrane (Table 2.1). Therefore it is possible to determine the best trapping conditions for the acceptor phase in the sampler from the pK_a of the ionisable organic compound. The same principles can be use to trap basic organic chemicals in water as illustrated in Figure 2.10b.

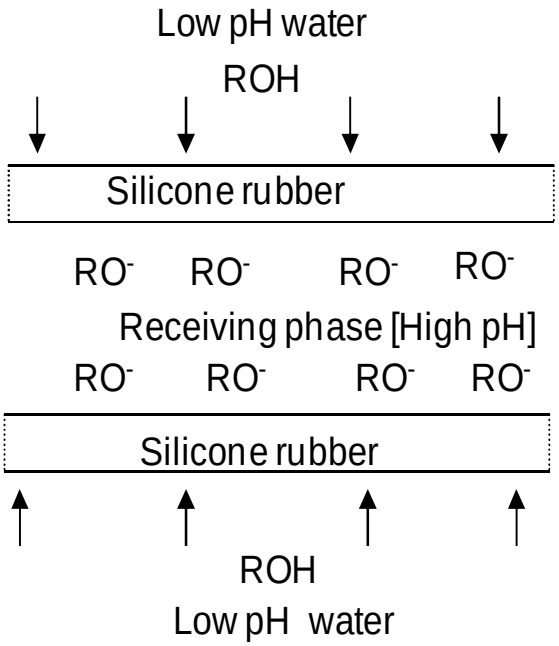


Figure 2.10 (a) Illustration of the principles of extraction of acidic organic chemicals from water in a developed passive sampler. Receiving phases is filled with a basic buffer solution where target analytes are ionised and are irreversibly trapped.

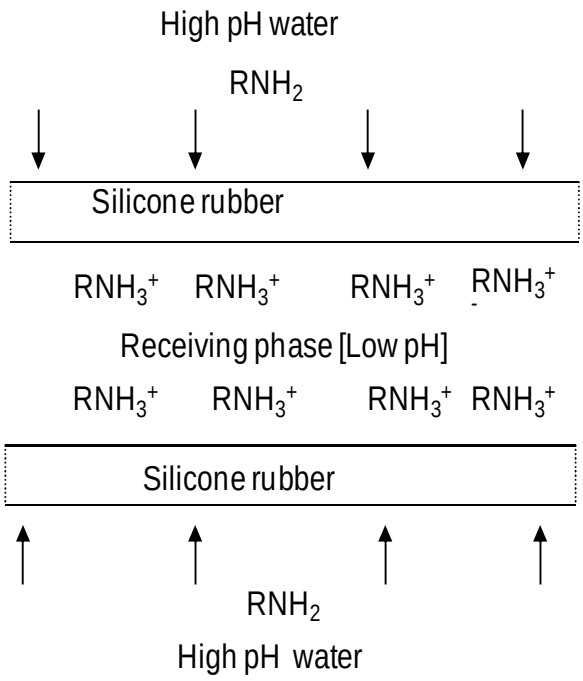
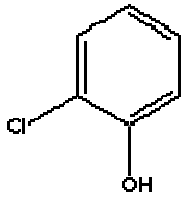
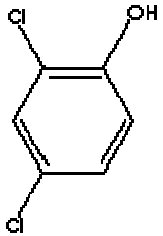
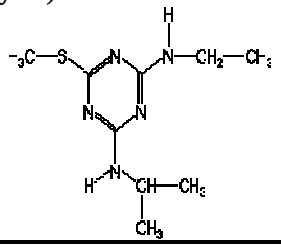
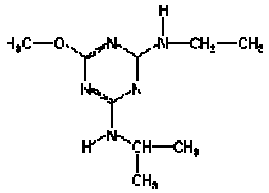
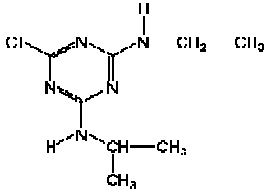
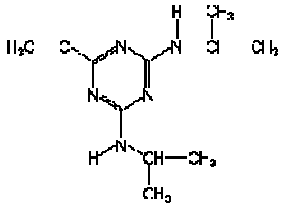
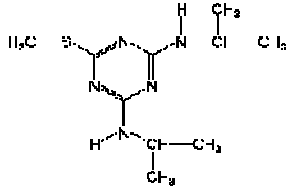
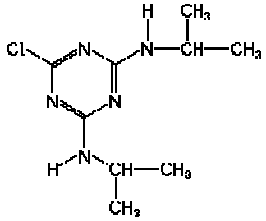
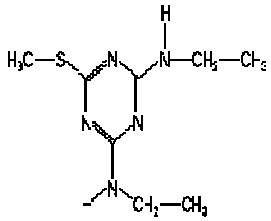
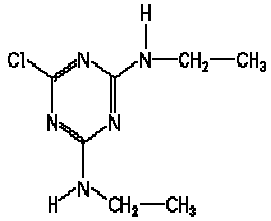
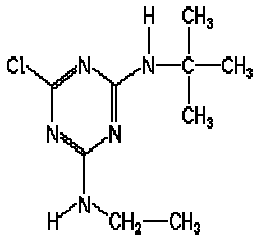


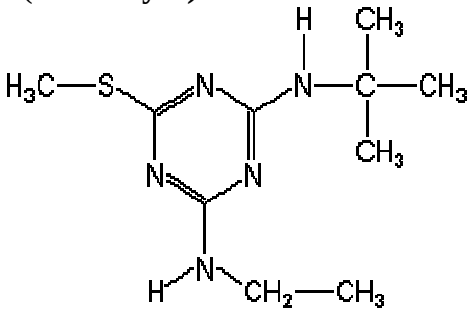
Figure 2.10 (b) Illustration of the principles of extraction of basic organic chemicals from water in a developed passive sampler. Receiving phase is filled with an acidic solution where target analytes are ionised and are irreversibly trapped.

Table 2.1 The physical parameters of the model compounds and ideal sample and acceptor pHs

Name and structure of chemical	pK_a	Sample pH (ideal)	Acceptor pH (ideal)
2-Chlorophenol 	8.55	8.55 - 2.00 = 6.55	8.55 + 3.30 = 11.85
4-Chlorophenol 	9.43	9.43 - 2.00 = 7.43	9.43 + 3.30 = 12.73
2,4-Dichlorophenol 	7.60	7.60 - 2.00 = 5.60	7.60 + 3.30 =10.90
2-ethylamino-4-isopropylamino-6-methyl-thio-*s*-triazine (**Ametryne**) 	4.10	4.10 + 2.00 = 6.10	4.10 - 3.30 = 0.80

Name and structure of chemical	pK_a	Sample pH (ideal)	Acceptor pH (ideal)
Atratone 	4.20	4.20 + 2.00 = 6.20	4.20 - 3.30 = 0.90
Atrazine  2,4-bis(isopropylamino)-6-methyoxy-*s*-triazine (**Prometone**)	1.70	1.70 + 2.00 = 3.70	1.70 - 3.30 < 0.00
 2,4-bis(isopropylamino)-6-(methylthio)-*s*-triazine (**Prometryne**)	4.20	4.20 + 2.00 = 6.20	4.20 - 3.30 = 0.90
	4.05	4.05 + 2.00 = 6.05	4.05 - 3.30 = 0.75

Name and structure of chemical	pK_a	Sample pH (ideal)	Acceptor pH (ideal)
2-chloro-4,6-bis[isoprpylamino-s-triazine (**Propazine**) 	1.85	1.85 + 2.00 = 3.85	1.85 - 3.30 < 0.00
Simetryne 	4.00	4.00 + 2.00 = 6.00	4.00 – 3.30 = 0.7
Simazine 	1.90	1.90 + 2.00 = 3.90	1.90 - 3.30 < 0.00
Terbutylazine 	1.94	1.94 + 2.00 = 3.94	1.94 - 3.30 < 0.00

Name and structure of chemical	pK_a	Sample pH (ideal)	Acceptor pH (ideal)
2-(tert-Butylamino)-4-(ethylamino)-6-(methylthio)-s-triazine (**Terbutryne*)*** 	4.40	4.40 + 2.00 = 6.40	4.40 - 3.30 = 1.10

Two important parameters that are often measured in the liquid membrane extraction techniques are the extraction efficiency (E), and enrichment factor, E_e. The extraction efficiency is defined as the fraction of analyte in the extracted sample that is found in the acceptor phase and is given by the equation below (Jönsson et al., 1993; Jönsson and Mathiasson, 1999). It is also a measure of mass transfer between the donor and acceptor phase and is constant under specified extraction conditions.

$$E = C_A V_A / C_D V_D \quad (6)$$

Where C_A is the concentration in the collected acceptor fraction and C_D is the concentration in the extracted sample. V_A is the collected acceptor volume while V_D is the volume of the sample that has been extracted. The enrichment factor is a ratio of concentration found in the acceptor phase to that in the original sample. This determines the detection limit of the method. It is given by the equation below (Jönsson et al., 1993; Jönsson and Mathiasson, 1999).

$$E_e = C_A / C_D \quad (7)$$

Both the extraction efficiency and the enrichment factor are constant under specified extraction conditions. The mass transfer of analytes from the donor into the receiving phase of the sampler includes several diffusion and interfacial steps across all barriers. The amount of chemical accumulated with constant chemical concentration is given by the following equation (Vrana et al., 2006a):

$$M_S(t) = M_0 + (C_W K_{SW} V_S - M_0) \{1-\exp(-k_{ov} A\alpha / K_{SW} V_S) t\} \quad (8)$$

where M_S is the mass of analyte in the receiving phase (acceptor phase), M_0 is the amount of analyte in the sampler at the start of exposure, C_W is the water concentration of the analyte during deployment period, K_{SW} is the receiving phase-water distribution coefficient, A is the membrane surface area, α is the pore membrane area as fraction of total membrane area

(membrane porosity) k_{ov} is the overall mass-transfer coefficient, V_S is the volume of the receiving phase (acceptor phase) and t is the exposure time. At the start of the exposure, the chemical uptake is linear and the exponential term is very small (< < 1) or $M_S/V_S C_W < < K_{SW}$. Equation 8 then reduces to (Vrana et al., 2006a):

$$M_S(t) = M_0 + C_W k_{ov} A \alpha t \quad (9)$$

Equation 9 can be simplified to equation 10 which is used to measure the sampling rate or rate of accumulation, R_S in practical applications (Vrana et al., 2006a):

$$M_S(t) = M_0 + C_W R_S t \quad (10)$$

Where $R_S = k_{ov} A \alpha = K_{SW} V_S k_e$ (11)

k_e is the overall exchange rate constant given by:

$$k_e = k_{ov} A \alpha / K_{SW} V_S \quad (12)$$

The sampling rates can also be calculated by combining equations 6 and 10 which results into equation 13:

$$C_A(t)/C_W = M_0/C_W V_A + 1/V_A (R_S t) \quad (13)$$

$$E_e = M_0/C_W V_A + 1/V_A (R_S t) \quad (14)$$

The slope from a plot of extraction time variation against the obtained enrichment factor, E_e is equal to:

$$\text{Slope} = 1/V_A (R_S) \quad (15)$$

2.6 Commercialisation and Applications of Passive Samplers

Commercialization of the passive sampler is very important since it reflects acceptance by the wider community. This means that potential users know where to buy the sampler with standard dimensions. It then becomes easy to compare the results and also to use the same sampling rates for the same compounds. This reduces the time needed to calibrate the sampler in the laboratory before deployment. Table 2.2 shows some of the commercial suppliers of passive samplers. Very few passive samplers for aquatic ecosystems have been commercialized. However, many of them have patent protection. A number of samplers for aquatic ecosystem are just being developed or validated. Research in developing samplers for water bodies is on the increase (Vrana et al., 2005) and in the near future many new samplers will be commercially available. On the other hand, many air passive samplers are commercially available.

Table 2.2 Some of the commercial suppliers of passive samplers for water monitoring

Name of sampler	Type	Supplier	Refs
SPMDs	Water	Environmental Science Technologies Inc. (USA)	(http://est-lab.com, accessed on 8 July 2008)
SPMDs	Water	Exposimeter (Tavelsjo, Sweden)	(Goarlay et al., 2005)
POCIS	Water	Environmental Science Technologies Inc. (USA)	(http://est-lab.com, accessed on 8 July 2008)

Detailed applications of the passive samplers for aquatic ecosystem monitoring have been reviewed by Vrana et al. (2005) and Stuer-Laurisden (2005). About 76 % of the applications have used SPMDs (Goarlay et al., 2005). This is not surprising since many other passive samplers are new having just been developed or calibrated (Richardson et al., 2002; Vrana et al., 2006a). Various compounds have been monitored by passive samplers especially using SPMDs ranging from pesticides and biocides, organochlorines and organohalogens, aromatic and alkylated aromatic compounds (Stuer-Lauridsen, 2005).

A review on the background and application of the SPMDs in aquatic ecosystems has been reported (Lu et al., 2002). Verweij et al., (2004), assessed the bioavailable PAH, PCB and OCP concentrations in fresh water sites in and around the city of Amsterdam using SPMDs. The study also compared the PAH concentrations in SPMDs to those found in sediments and caged carp. A significant correlation was observed between biliary PAH metabolite levels in fish and aqueous concentrations estimated with SPMDs. For quality control and assurance, SPMDs were spiked with standard solutions of PAHs, PCBs and OCPs in hexane. The recovery in this case was between 92 % and 110 % for PAHs, between 105 % and 115 % for PCBs and between 74 % and 82 % for non-polar pesticides. The SPMDs were also used to assess the level of pollution in Lithuania (Sabaliunas and Södergren, 1997). Predictions of aqueous concentrations were done in the linear region for eight pesticides of different classes (organochlorines, synthetic pyrethroids, dinitroanilines and amides) over 20 days exposure. Description of the design and application of passive samplers for water environment monitoring is summarised shown in Appendix A1.

2.7 Environmental Factors Affecting Passive Sampler Performance

2.7.1 Turbulence

Turbulence of the environmental media is one factor which is difficult to control and may affect the amount of compounds trapped in the receiving phase and therefore the quality of the results. The extent to which turbulence affects uptake kinetics depends on factors such as sampler material, hydrophobicity of the compound and environmental flow rates. For membrane based passive samplers, permeation of compounds through the membrane is seen as the rate-limiting step and is more pronounced for polar compounds (membrane/permeation controlled samplers). On the other hand, for non-polar compounds, diffusion through the unstirred layer and sampler controls the mass transfer (donor/diffusional controlled) (Mergesa et al., 2001). Once turbulence occurs in the environmental media, the unstirred layer becomes thin and therefore enhances the uptake of non polar compounds by the sampler.

Depending on the extent of the turbulence, at very high flow rates, poor dissolution of polar compounds into the membrane can result in decreased uptake rates. PRCs are one way to account for turbulence during sampling (Booij and van Drooge, 2001; Verweij et al., 2004; Vrana et al., 2005). Another way is to enclose the sampler in a container that reduces the effects of turbulence, a technique commonly used in air sampling (Wilford et al., 2004).

2.7.2 Biofouling

In active sampling, it is well known that matrix interferences can influence the accuracy and precision of the extraction process. However in passive sampling, the main problem is bacteria or other various flora and fauna growing on the surface of the sampler during deployment. This process is called biofouling (Huckins et al., 1990; Vrana et al., 2005). The biofilm formed can affect the sampler in terms of accuracy and precision since these are randomly formed and increase the overall mass transfer resistance. Biofilms can also block the membrane pores in the diffusion-limiting membrane. Richardson et al. (2002) studied the effect of biofouling on the uptake of trace organic contaminants by semi-permeable devices (SPMDs). The results showed that uptake of contaminants by SPMDs were severely reduced by as much as 50 % under fouling conditions compared to unfouled controls. One solution is once more adding PRCs prior to deployment (Booij and van Drooge, 2001; Booij et al., 2002; Verweij et al., 2004). Some organic filled dialysis samplers have been reported to reduce biofouling by slowly seeping out the organic liquid through the membrane (Södergren, 1987). Proper choice of the sampler design may also help to reduce biofouling (Petty et al., 2004).

2.7.3 Temperature

From a kinetic point of view, it is quite clear that temperature of the environmental media can influence the uptake rates in a sampler. A study on the effect of water temperature over a range of 4 °C to 20 °C has been reported by Kingston et al. (2000). In this study, one sampler consisted of a polysulfone limiting membrane while the other consisted of polyethylene. Both of these samplers used the same 47 mm C_{18} Empore disk as receiving phase. In both cases, an increase in water temperature led to an increase in sampling rate. The effects of temperature on

sampling rates have been observed in SPMDs (Yusá et al., 2005) and in membrane enclosed sorptive coating (MESCO) sampler (Vrana et al., 2001). For practical purposes, it is therefore necessary to determine the effects of temperature in the laboratory for each compound of interest and also measure the temperature during field deployment.

2.8 Alternative Techniques to Passive Samplers

2.8.1 Active Sampling

Active sample preparation techniques such as liquid-liquid extraction (Dean, 1998), solid phase extraction (Poole and Wilson, 2000), solid phase microextraction (SPME) (Arthur and Pawliszyn, 1990; Beltran et al., 2000) are still the major competitors to passive sampler, especially for monitoring compounds in aquatic ecosystems. These extraction techniques are well developed and known to provide very good accuracy and precision (typically below 5 %). Since extraction is performed in the laboratory, environmental factors that affect passive samplers are easily controlled. Quality assurance procedures of these techniques are also well known. On the other hand, quality assurance procedures for passive sampler especially for monitoring aquatic ecosystems are still being developed. Sample preparation is generally known to consume 60 % percent of the environmental monitoring process and most errors are introduced during this step. Using passive samplers therefore makes much more sense since sampling and sample preparation is combined in one step. This advantage is especially true for the passive samplers whose extracts do not need any further clean up. Passive samplers can easily be deployed over large area since they need no pumps and are much cheaper. In the near future, once quality assurance procedures become well known, passive samplers should become a more attractive alternative to common active sampling and sample preparation techniques.

2.8.2 Biomonitoring Organisms

Biomonitoring organisms have been used as passive samplers and have the major advantage that they reflect the true impact of the condition of the environment (Gorecki and Namiesnik, 2002). They do not need any deployment and preconcentrate the compounds through bioconcentration. However, they have some limitations too in their use as biological indicator passive samplers. Biomonitoring organisms cannot survive in certain environmental conditions and age, size, sex and physical condition might affect the uptake rates of compound (Phillips, 1980). The organisms should be abundant and for long term monitoring should be less mobile in the environment (Gorecki and Namiesnik, 2002). The trapped compounds also need to be re-extracted and often require additional clean-up step. A number of biomonitoring organisms have been reported and compared with SPMDs with good correlation observed in most cases (Goarlay et al., 2005; Richardson et al., 2001).

2.9 Environmental Concerns of Chlorophenols and Triazines

Potentially toxic ionisable organic compounds such as chlorophenols and triazines enter the aquatic environment from direct point and non-point or diffuse sources. At direct point sources, discharges enter a water source at a single point, for example discharges of domestic sewages and industrial effluents. At non-point or diffuse sources toxic ionisable organic compounds enter surface and underground water through runoff from urban and industrial area, leachates from domestic and solid wastes disposal sites and mining operations. It is therefore necessary to monitor these ionisable organic compounds to satisfy the requirements of legislative frameworks and directives.

2.9.1 Chlorophenols

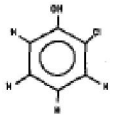
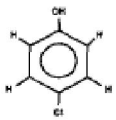
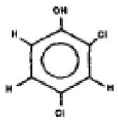
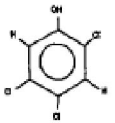
Chlorophenols are of environmental interest because of their widespread distribution in freshwater habitats. Chlorophenols with at least two chlorines either have been used directly as pesticides or converted into pesticides. Also, chlorophenols, especially 4-chlorophenol, have

been used as antiseptics. In addition to being produced commercially, small amounts of some chlorophenols, especially the mono- and dichlorophenols, may be produced when wastewater or drinking water is disinfected with chlorine, if certain contaminants are present in the raw water. They are also produced during the bleaching of wood pulp with chlorine when paper is being produced.

The chlorinated phenols consist of a group of nineteen different isomers which include mono, di, tri, tetra, and one pentachlorophenol. All these compounds are toxic to aquatic species, but in varying degrees with pentachlorophenol being the most toxic (Buikema et al., 1979). Structurally, chlorophenols fit the requirements for receptor-producing respiratory uncoupling. However, uncoupling oxidative phosphorylation is the most important mode of toxic action for chlorophenols with two or more chlorine substituents, while non-specific polar narcosis is the proposed mechanism in the case of mono substituted phenols (Exon, 1984). Exposure to high levels of chlorophenols can cause damage to the liver and immune system.

Because of their toxicity to organisms (IARC, 1999) and their capacity to affect the taste and the odour of water, a number of phenols have been included in the lists of priority pollutants of the US Environmental Protection Agency (EPA) (Environmental Protection Agency, 1995, 1984a, 1984b) and the European Union (EU). The EU decision 2455/ 2001/EC set a maximum phenolic concentration of 0.5 $\mu g\ L^{-1}$ in drinking water, while individual concentrations should not exceed 0.1 $\mu g\ L^{-1}$. It recommends that drinking water contain no more than 0.04 mg L^{-1} of 2-chlorophenol for a lifetime exposure for an adult, and 0.05 mg L^{-1} for a 1-day, 10-day, or longer exposure for a child (ATSDR, 1999). For 2, 4-dichlorophenol, the EPA recommends that drinking water contain no more than 0.03 mg L^{-1} for a 1-day, 10-day, or longer exposure for a child (ATSDR, 1999). Information regarding the chemical identity of chlorophenols is shown in Table 2.3 while that regarding the physical and chemical properties of the chlorophenols is shown in Table 2.4

Table 2.3 Chemical Identity of Chlorophenol Compounds

Characteristic	2-Chlorophenol	4-Chlorophenol	2,4-Dichlorophenol	2,4,5-Trichlorophenol
Synonym(s)	2-CP, 2 chloro-1-hydroxy-benzene, 2 hydroxy-chlorobenzene, *o*-chlorophenol, phenol	4-CP, 4-chloro-1-hydroxy-benzene, 4 hydroxy-chlorobenzene, *p*-chlorophenol, phenol	2,4-DCP, 2,4-dichlorohydroxybenzene	2,4,5-TCP
Registered trade name(s)	Sept-Kleen, Pine-O Disinfect	No data	No data	Preventol I, Collunosol
Chemical formula	C_6H_5ClO	C_6H_5ClO	$C_6H_4Cl_2O$	$C_6H_3Cl_3O$
Chemical structure				
Identification numbers:				
CAS registry	95-57-8	106-48-9	120-83-2	95-95-4
NIOSH RTECS	SK2625000	SK2800000	SR8575000	SN1400000
EPA hazardous waste	UO48	No data	UO51	DO41, FO27
OHM/TADS	8100049	81400051	7217235	8200170
DOT/UN/NA/IMCO shipping	UN 2020 liquid, IMO 6.1, UN 2021 solid	UN 2020 liquid, IMO 6.1, UN 2021 solid	UN 2020 solid, IMO 6.1	UN 2020, NA 2020, IMO 6.1
HSDB	1415	1414	1139	4067
NCI	No data	No data	C55346	C61187

[a]Data from HSDB 1994

CAS = Chemical Abstracts Service; DOT/UN/NA/IMCO = Department of Transportation/United Nations/North America/International Maritime Dangerous Goods Code; EPA = Environmental Protection Agency; HSDB = Hazardous Substances Data Bank; NCI = National Cancer Institute; NIOSH = National Institute for Occupational Safety and Health; OHM/TADS = Oil and Hazardous Materials/Technical Assistance Data System; RTECS = Registry of Toxic Effects of Chemical Substances

Table 2.4 Physical and Chemical Properties of Chlorophenol Compounds

Property	2-Chlorophenol	4-Chlorophenol	2,4-Dichlorophenol	2,4,5-Trichlorophenol
Molecular weight	128.56	128.56	163.00	197.46
Color	Light amber	White to pink crystals	White	Gray
Physical state	Liquid	Solid	Solid	Solid
Melting point	9.3°C	43.2–43.7°C	45°C	67°C
Boiling point	174.9°C at 760 mmHg	220°C	210°C	235°C
Density	1.2634	1.2238 at 78°C/4°C	1.383 at 60°C/25°C	1.678 at 25°C/4°C
Odor	Unpleasant, medicinal odor	Medicinal odor	Strong medicinal odor	Strong phenolic odor
Odor threshold:				
Water at 30°C[b]	0.33 µg/L	0.33 µg/L	0.35 µg/L	11 µg/L
Air[c]	0.0189 mg/m^3	0.0189 mg/m^3	1.40 mg/m^3	No data
Solubility:				
Water at 25°C[d]	20,000 ppm	27,000 ppm	4,500 ppm	948 ppm
Organic solvent(s)	Acetone, alcohol, benzene	Alcohol, glycerol, ether, chloroform, fixed and volatile oils, benzene	Ethyl alcohol, carbon tetrachloride, ethyl ether, benzene, chloroform	Acetone, benzene, carbon tetrachloride, ether, denatured alcohol, methanol, liquid petrolatum, toluene
Other solvent(s)	Sodium hydroxide			
pK_a[d]	8.49	8.85	7.68	7.43
Partition coefficients:				
Log K_{ow}[d]	2.17	2.4	3.2	3.72
Log K_{oc}[d]	1.25–3.7	1.2–2.7	2.42–3.98	2.55–3.98
Vapor pressure at 25°C (liquid)[d]	0.99 mmHg	0.23 mmHg	0.14 mmHg	0.05 mmHg
(solid)[d]	0.99 mmHg	0.15 mmHg	0.09 mmHg	0.02 mmHg
Henry's law constant at 25°C[d]	6.8×10^{-6} atm-m^3/mol	9.2×10^{-7} atm-m^3/mol	4.3×10^{-6} atm-m^3/mol	5.1×10^{-6} atm-m^3/mol
Autoignition temperature	No data	No data	No data	No data
Flashpoint	64°C	121°C	114°C	No data
Flammability limits	No data	No data	No data	No data
Conversion factors (ppm to mg/m^3)	2.2	1.1	1.1	No data
Explosive limits	No data	No data	No data	No data

[a]Data from HSDB 1994 except where indicated
[b]From Hoak 1957
[c]From Ruth 1986
[d]From Shiu et al. 1994

Table 2.4 Physical and Chemical Properties of Chlorophenol Compounds (*continued*)

Property	2,4,6-Trichlorophenol	2,3,4,5-Tetrachlorophenol	2,3,4,6-Tetrachlorophenol	2,3,5,6-Tetrachlorophenol
Molecular weight	197.45	231.89	231.89	231.89
Color	Yellow	No data	Light brown	No data
Physical state	Solid	Solid	Solid	Solid
Melting point	69°C	116–117°C	70°C	115°C
Boiling point	246°C	Sublimes	64°C	No data
Density	1.4901	No data	1.83 at 25°C/4°C	No data
Odor	Strong phenolic odor	No data	Strong odor	No data
Odor threshold:				
Water at 30°C[b]	100 µg/L	No data	915 µg/L	No data
Air[c]	No data	No data	No data	No data
Solubility:				
Water at 25°C[d]	434 ppm	166 ppm	183 ppm	100 ppm
Organic Solvent(s)	Acetone, benzene, carbon tetrachloride, diacetone alcohol, methanol, stoddard solvent, touene, turpentine, ether	Alcohol	Acetone, alcohol, benzene, chloroform, ligroin	Benzene
Other Solvent(s)	Hot acetic acid		Hot acetic acid, sodium hydroxide	
pK_a[d]	7.42	6.96	5.38	5.48
Partition coefficients:				
Log K_{ow}[d]	3.69	4.8	4.45	4.9
Log K_{oc}[d]	1.94–3.34	2.9–4.14	3.2–4.21	No data
Vapor pressure at 25°C (liquid)[d]	0.03 mmHg	0.0059 mmHg	0.0059 mmHg	0.0059 mmHg
(solid)[d]	0.0094 mmHg	0.0008 mmHg	0.0021 mmHg	0.0008 mmHg
Henry's law constant at 25°C[d]	5.7×10^{-6} atm-m^3/mol	1.3×10^{-6} atm-m^3/mol	3.6×10^{-6} atm-m^3/mol	2.2×10^{-6} atm-m^3/mol
Autoignition temperature	No data	No data	No data	No data
Flashpoint	No data	No data	No data	No data
Flammability limits	No data	No data	No data	No data
Conversion factors (ppm to mg/m^3)	No data	No data	No data	No data
Explosive limits	No data	No data	No data	No data

Transport, partitioning and fate of chlorophenols

The environmental fate and transport of chlorophenols are controlled by their physical and chemical properties and environmental conditions. All chlorophenols are solids at room temperature except 2-chlorophenol, which is a liquid. In general, as the number of chlorine molecules increase, there is a reduction in vapor pressure, an increase in boiling point, and a reduction in water solubility (Solomon et al., 1994). Therefore, increasing chlorination increases the tendency of the chlorophenols to partition into sediments and lipids and to bioconcentrate.

The higher vapor pressures of the monochlorophenols suggest that among the chlorophenols, these compounds are most likely to be found in air. The vapor pressures of the chlorophenols suggest that the compounds will not partition from the vapor phase to the particulate phase (Eisenreich et al., 1981). That 2,4-dichlorophenol and other chlorophenols do not partition into the particulate phase is supported by the identification of 2,4-dichlorophenol, 2,4,5-triphenol, 2,4,6-triphenol, and 2,3,4,6-tetraphenol in rain but not on rain filters (Leuenberger et al., 1985). This study indicates that gas scavenging rather than particle scavenging is the more important process for removing chlorophenols from the air (Leuenberger et al., 1985).

The rate of chemical evaporation from an aqueous solution largely depends on a chemical's vapor pressure and water solubility (Henry's law constant). Among the chlorophenols discussed in this dissertation, 2-chlorophenol has the highest vapor pressure and, therefore, is most likely to evaporate from water (Krijgsheld and van der Gen, 1986). In laboratory studies, evaporation half-lives of 2-chlorophenol and 4-chlorophenol from water 0.38 cm deep were 1.35-1.6 hours and 12.8-17.4 hours, respectively (Chiou et al., 1980). Since the evaporation rate is inversely related to the depth of water, extrapolation of these data indicates that-2-chlorophenol evaporation in water 1 meter deep would require approximately 15 days. The amount of volatilization of 2-chlorophenol from fine sandy soil (0.087 % organic carbon), applied in spiked municipal waste water, was too small to be directly measured (Piwoni et al., 1986).

Volatilization of 2,4-dichlorophenol from water is expected to be slow and, therefore, not a major removal process from surface waters. Using the Henry's law constant, a half-life of 14.8 days was calculated for evaporation from a model river 1 meter deep with a current of 1 meter/second and a wind velocity of 3 meters/second, neglecting adsorption to sediment (Thomas, 1982). The biological treatment of wastewater containing 2,4-dichlorophenol has shown that none of the chemical is removed by stripping (Stover and Kincannon, 1983). Volatilization from near-surface soil is also not expected to be a significant removal process.

The Henry's law constants for 2,4,5-trichlorophenol of 0.0039 and 0.0043 for 2,4,6-trichlorophenol are similar to 0.0033 for 2,4-dichlorophenol. Therefore, the volatilization of these trichlorophenols should be similar to that of 2,4-dichlorophenol. In 2-hour laboratory studies, the volatilization rates of 2,4,6-trichltophenol from water and three soil types were determined by Kilzer et al. (1979). These rates, expressed as the percentage of applied compound per milliliter of water evaporated from humus, loam, sand, and water, were 0.15,0.73, 1.05, and 1.4 %, respectively, in the first hour after the addition of 50 μg L^{-1} 2,4,6-trichlorophenol. Similar rates were reported during the second hour. In wind tunnel experiments, Sugiura et al. (1984) estimated a half-life of 48 hours for loss of 2,4,6-trichlorophenol from water through volatilization. An estimated 58 % of 2,4,6-trichlorophenol in a nutrient solution in which tomatoes were grown was lost to the air (from photolysis and/or volatilization) over a period of 30 days (Fragiadakis et al., 1981).

Experimental studies examining the volatilization of tetrachlorophenols have not been studies much. Based on lower Henry's law constants and a greater potential to exist as the dissociated compound in the environment, tetrachlorophenols would be less likely to volatilize from water and soil than the lower chlorinated chlorophenols.

In addition to vapor pressure and solubility, pK_a and Log K_{ow} (octanol water partition coefficients) are other important properties which determine the transport and partitioning of chemicals. The lower chlorophenols have higher pK_a values (7.42-8.49). Therefore, in natural waters these compounds will exist primarily as the undissociated compounds, and adsorption to sediments at a pH of not more than one unit greater than the pK_a can be predicted based on

the organic content of the sediments and the octanol/water partition coefficient (Schellenberg et al., 1984). In contrast, the pK_a values of the tetrachlorophenols are lower (5.48-6.96) so that at ambient pH values these compounds are present predominantly in the ionized form, and the adsorption to sediments will also be dependant on the ionic strength of the water (Schellenberg et al., 1984).

In general, a chemical will preferentially partition into organic matter if its Log K_{ow} is > 1 (Scow et al., 1982). Log K_{ows} for the chlorophenols are all > 2 (see Table 3-2); therefore, the chlorophenols will all tend to partition into sediments. Despite this prediction, a modelling study completed by Yoshida et al. (1987) suggests that most of the 2,4,6-trichlorophenol released to surface waters would remain in the water rather than absorb to sediments. They estimated that in a river receiving daily inputs of the compounds, 72 % would be in the water and 28 % in the sediment. In a deep, otherwise unpolluted lake, 84 % would be in the water and 16 % in the sediment. Laboratory sorption experiments with natural sediments containing up to 10 % organic matter have been conducted using 2-chlorophenol and 2,4-dichlorophenol (Isaacson and Frink, 1984). Sediment sorption capacity was extensive (up to 0.3 $mmolg^{-1}$), and up to 90 % of the adsorption was irreversible.

Chlorophenols are capable of binding to soil organic matter via covalent bond formation, resulting from biologically or chemically catalyzed reactions. In a batch sorption experiment, the binding of 4-chlorophenol to soil requires oxygen and soil bioactivity, indicating a biologically mediated oxidative coupling reaction. The addition of hydrogen peroxide, which may be an oxygen source, caused a 4.4 fold increase in 4-chlorophenol binding (Bhandari et al., 1996).

As the number of chlorines on phenols increases, sorption of chlorophenols to organic material in soil increases. For example, at two sawmills in Finland where chlorophenol wood preservative (primarily 2,3,4,6-tetrachlorophenol) was used, soil was contaminated to a depth of 80 to 100 cm to the same extent as at the surface (Vale et al., 1984). As soil depth increased, the concentration of dichlorophenols increased. The investigators attributed this observation to a greater transport of dichlorophenols through the soil and to the relatively increased

degradation of the higher chlorinated phenols. An experimental study that examined the movement of 2-chlorophenol and 2,4,5-trichlorophenol through two soil types (organic carbon 2.1 mg C g^{-1} soil or 1.5 mg C g^{-1} soil) found that the relative velocity of the chlorophenol through soil into water was 3.5-4 times greater for 2-chlorophenol compared to 2,4,5-trichlorophenol (Kjeldsen et al., 1990). The chlorophenols moved slowest in the soil with the greatest organic carbon content.

Chlorophenol groundwater contamination will occur if sufficient quantities of the chemical are present to exceed the sorption capacity of the vadose zone saturated soils (Scow et al., 1982). Contamination is most likely in soils with low organic carbon content or high pH. Once in groundwater, sorption of chlorophenols by the solid aquifer matrix may be estimated based on Log K_{ow} and organic carbon content, provided that the organic carbon content exceeds 0.1 % and the aquifer pH is not sufficiently high for significant dissociation to occur (Schellenberg et al., 1984; Schwarzenbach and Westall, 1985). In a natural gradient tracer test conducted within an unconsolidated aquifer, sorption was not an important factor, compared to dispersion and degradation, in the attenuation of 4-chlorophenol concentrations (Sutton and Barker, 1985). The authors attributed this finding to the low organic carbon content of the aquifer sand unit, which prevented significant hydrophobic sorption.

The bioaccumulation potential of 2-chlorophenol, 4-chlorophenol, 2,4-dichlorophenol, 2,4,5-trichlorophenol, 2,4,6-trichlorophenol, and 2,3,4,6-tetrachlorophenol was reviewed by Loehr and Krishnamoorthy (1988). Based on bioconcentration values and log octanol/water partition coefficients, they concluded that all chlorophenols studied had the potential for accumulation in aquatic organisms. Logs of bioconcentration factors ranged from 0.81-2.33 for 2-chlorophenol, 1.79-3.28 for 2,4,5-trichlorophenol, and 1.95- 2.3 for 2,3,4,6-tetrachlorophenol. Values of bioconcentration factors for 4-chlorophenol, 2,4-dichlorophenol, and 2,4,6-trichlorophenol were predicted mathematically (Veith et al., 1980).

Research on biomagnification of chemical residues within the aquatic food chain indicates that the potential for residue accumulation by fish through food chains is relatively insignificant (< 10 %) for most compounds when compared to the tissue residues resulting from the

bioconcentration process (i.e., direct uptake from water) (Barrows et al., 1980). This data suggest that only those chemicals that are relatively persistent in fish tissues appear to have any potential for significant transfer through food chains (Barrows et al., 1980). A very short tissue half-life of < l day was measured after bluegill sunfish exposure to 2-chlorophenol was terminated (Veith et al., 1980). Therefore, due to their relatively low bioconcentration factors (< 1,000) and short biological half-lives (< 7 days), monochlorophenols will probably not biomagnify within aquatic food chains (Barrows et al., 1980). Data regarding the biomagnification of the higher chlorophenols were not located.

Isensee and Jones (1971) studied the uptake of 2,4-dichlorophenol from solution and soil by oats and soybeans. The compound was taken up by the plants, with the concentrations decreasing as the plants matured. At maturity, 2,4-dichlorophenol in oat seeds was below detection (< 0.001 µg g^{-1}) and in soybeans was 0.003 µg g^{-1}. Data regarding the uptake of other chlorophenols by plants were not located.

The bioaccumulation of 2,3,4,6-tetrachlorophenol was examined in earthworms *(Lumbricus rubellus* and *Aporrectodea caliginosa tuberculata)* at a sawmill that had been closed 28 years before sampling (Haimi et al., 1992). At a distance of 5 meters from the dipping basin, 2,3,4,6-tetrachlorophenol concentrations were 430 and 1,980 µg g^{-1} fat in *Lumbricuss* and *Aporrectodea,* respectively, while soil concentrations were 336 μg^{-1} dry soil. The difference between the two species was attributed to greater ingestion of contaminated soil by *Aporrectodea.* Additional data regarding bioaccumulation of chlorophenols in terrestrial organisms was not identified. It is not known whether 2,3,4,6-tetrachlorophenol biomagnifies up the terrestrial food chain. Based on physical properties (i.e., greatest log octanol water partition coefficient), the tetrachlorophenols, rather than lower chlorinated phenols, would have the greatest potential to biomagnify.

2.9.2 Triazines

Triazine is the chemical species of six-membered heterocyclic ring compound with three nitrogens replacing carbon-hydrogen units in the benzene ring structure. The names of the

three isomers indicate which of the carbon-hydrogen units on the benzene ring position of the molecule have been replaced by nitrogens, called 1,2,3-triazine, 1,2,4-triazine, and 1,3,5-triazine respectively. Symmetrical 1,3,5-triazine is the common. Triazines are prepared from 2-azidocyclopropene through thermal rearrangement (1,2,3-triazine), from 1,2-dicarbonyl compound with amidrazone by condensation reaction (1,2,4-triazine) and from cyanic acid amide by trimerization (1,3,5-triazine). Pyridine is the aromatic nitrogen heterocycle compound having only one nitrogen, and diazines are with 2 nitrogen atoms and tetrazines are with 4 nitrogen atoms on the benzene ring system. Triazines are weak base. Triazines have much weaker resonance energy than benzene, so nucleophilic substitution is preferred than electrophilic substitution (Wauchope *et al.,* 1992). Triazines are basic structure of herbicides; examples are amitole, atrazine, cyanazine, simazine, trietazine. Large volume of triazines are used in the manufacture of resin modifiers such as melamine and benzoguanamine. Melamine (1,3,5-Triazine-2,4,6-triamine) is reacted with formaldehyde to from a very durable thermoset resin. Benzoguanamine (2,4-Diamino-6-phenyl-1,3,5-triazine) is used to increase thermoset properties of alkyd, acrylic and formaldehyde resins. Triazines are also useful as chromophore groups in colorants and Chlorine attached in Triazine compounds undergo nucleophilic substitution reactions well with hydroxyl groups in cellulose fibres. Some triazine family compounds are used in pharmaceutical industry as coupling agent for the synthesis of peptide in solid phase as well as solution and as side chain of antibiotics. Triazine compounds are used in formulating bactericide and fungicide. They are used as preservatives in oil field applications. They are used as disinfectant, industrial deodorant and biocide in water treatment.

Most widely, triazine compounds are often used as the basis for various herbicides such as cyanuric chloride (2,4,6-trichloro-1,3,5-triazine). Since their introduction, triazine herbicides have been widely used in agriculture as selective herbicides in many parts of the world for control of broadleaf and grassy weeds in many agricultural crops (Kolpin et al., 1998). Crops typically receiving triazine applications are (1) fruits such as apples (orchard weed control), peaches (orchard weed control), blueberries (orchard weed control); (2) grains such as Sweet corn and field corn; (3) vegetables such as soya beans, tomatoes, asparagus and potatoes.

The maximum contaminant levels for atrazine and simazine are 3 and 2 μgL^{-1}, respectively, as set by the U.S. Environmental Protection Agency (USEPA) (EEC No 1.229/11-29, 1980). These maximum contaminant levels are based on running annual averages of quarterly finished (treated) samples. While atrazine (ATZ) is classified by the USEPA as "not a likely human carcinogen", there are concerns about ATZ regarding its potentially adverse developmental and reproductive effects (EEC No 1.229/11-29, 1980). Triazine herbicides biodegrade in the environment to various degradates (Burnside et al., 1963; Tomlin, 2003). Dealkylation of ATZ, simazine (SIM), and propazine (PROP) results in deethylatrazine (DEA) and/or deisopropylatrazine (DIA). Further, dealkylation results in didealkylatrazine (DDA), also known as diaminochloros- triazine (or DACT). While other dechlorinated degradates are formed (e.g., hydroxyatrazine), it is the chlorinated parent and degradates (i.e., ATZ, SIM, PROP, DEA, DIA, and DDA) that have been implicated by the USEPA as exhibiting the potential for disrupting the endocrine systems of mammals, including humans (EEC No 1.229/11-29, 1980). Specific endocrine-disruption effects cited by the USEPA are attenuation of the luteinizing hormone serge, altered pregnancy maintenance, effects on pubertal development, and alteration of the oestrous cycle.

While pre-emergence herbicides belonging to the triazine group (active ingredients like simazine, atrazine, terbuthylazine) have been banned from agricultural use in several countries in Europe and US, due to environmental concerns, primarily contamination of water sources, this raises questions about the continued use of triazine herbicides in Integrated Production of Wine (IPW) in South Africa. Two triazine compounds/actives are registered for use in vineyards in South Africa, namely simazine and terbuthylazine (Ehrig et al., 1991). The following facts are considered:

1. The banning of triazines in the EU was precipitated mainly by the contamination of water sources by atrazine, which has a much higher solubility in water (33 mg $L^{-}1$ water at pH 7 and 22 °C) than either simazine (6.2 mg L^{-1} water at pH 7 and 20 °C) or terbuthylazine (8.5 mg L^{-1} water at pH 7 and 20 °C) (Burnside et al., 1961). Atrazine is not registered for use in South African vineyards.

2. The use of all pre-emergence herbicides, including simazine and terbuthylazine, is restricted under the IPW Guidelines to application on the ridges (planting row) and on the advice of an expert. Under dry land conditions (no irrigation, very little summer rainfall) it is occasionally applied in the work row, but due to the lack of water, leaching is not a factor. It is used only for certain problematic weeds that cannot be controlled by post-emergence herbicides or it is used at very low rates in combination with glyphosate for post-emergence weed control as an anti-resistance strategy. When used for post-emergence weed control in conjunction with glyphosate, it binds with the organic material (weeds) and is broken down rapidly, further reducing any risk of run-off and contamination of water sources.

3. Herbicides are applied in vineyards in spring when soil and general temperatures are getting higher. The high soil temperatures (26 – 34 °C) and exposure to high levels of UV light due to the long periods of sunshine combine to effect rapid breakdown of the active ingredients (simazine & terbuthylazine). Soil temperatures and UV light levels are considerably higher under South African summer conditions than during summers in Europe.

4. Simazine and terbuthylazine adsorbs strongly in soils with a high organic content. In Europe soils tend to have a relatively high organic content, which resulted in higher doses of triazine herbicides being used. South African soils are generally poor in organic content and due to the low levels of adsorption, lower dose rates are used (maximum 4-5 L/ha).

5. South Africa is a dry country; the water table is very deep in most places and summer rainfall is low to negligible in the areas where wine grapes are cultivated. Herbicides are applied at the beginning of and during the growing season, which means that there is a very low to negligible risk of run-off. There are also very few open ponds or perennial rivers in vine growing areas – most ponds and rivers are seasonal. Due to the depth of the water table, the low rainfall during summer and the low solubility of simazine and terbuthylazine in water, the risk of contamination of water sources due to run-off and/or leaching is very low to negligible (Dawson e al., 1968).

6. IPW recommends winter cover cropping, mainly for weed control, and the resultant mulch further reduces the risk of run-off after herbicide applications in spring and summer.

7. Soils in South Africa exhibit a very high rate of microbial breakdown of these herbicides.

8. It is important to note that the use of triazine herbicides in South African vineyards and orchards has decreased by approximately 40-50 % over the last five years, mainly because of minimum tillage and cover cropping practices. Many soils are also too alkaline (high pH), resulting in phytotoxicity risks or rapid breakdown of the products. The use of triazine herbicides is also not recommended in soils with less than 10 % clay, which further reduces any risk of leaching into water courses.

However, these herbicides and their degradation products are highly persistent and because of their importance for environmental concerns, their presence aquatic environments like rivers should be monitored. The concentration of triazines in aquatic environments like rivers is often found to be close to lower detection limits of most elaborate analytical procedures and thus methods of determination of these herbicides have to cope with low levels.

Transport, partitioning and fate of triazines

The fate and transport processes of a toxic chemical such as triazine herbicide, in aquatic environments are significantly influenced by both the physico-chemical properties of triazines and the conditions of the aquatic environment (i.e., water depth, temperature, pH, intensity and spectrum of solar radiation, wind speed, microorganism concentration, and suspended solids concentration). Physico-chemical properties shown in Table 2.5 below indicate that soluble and mobile herbicides, such as Atrazine, Propazine and Simazine are transported primarily in the dissolved phase hence their aquatic fate is strongly influenced by their moderate solubilities and their persistence.

Table 2.5 The physico-chemical properties of the triazine herbicides (Wauchope *et al.*, 1992)

Herbicide	Water solubility (mg L^{-1})	Log P_{ow}	Soil sorption coef (K_{oc} ml g^{-1})	pK_a	Vapor Pressure (mPa)
Atrazine	33	2.20	100	1.70	0.040
Propazine	5	2.50	154	1.70	0.004
Simazine	6.2	3.00	138	1.60	0.001

The compounds are not volatile and loses to the atmosphere is therefore likely to be minimal. Herbicides with K_{oc} values less than about 500 mL g^{-1} tend to be transported primarily in the dissolved phase where as K_{oc} values greater than 1000 mL g^{-1} are transported primarily on suspended-sediments particles.

Once herbicides are in surface water, they degrade much slower than in soil because surface water contains much less organic matter and fewer micro-organisms to degrade the herbicides. The main routes of removal of herbicides from water are photo-enhanced hydrolysis to 2-hydroxy derivatives, adsorption onto sediments and degradation by micro-organisms. However adsorption is believed to be rapid and reversible.

Atrazine and propazine degrade slowly to deethyatriazine by deethylation. Simazine and atrazine degrade to deisopropylatrazine by dealkylation. The degradation products are more soluble and mobile than the parent compound. The removal of an ethyl side-chain from atrazine relative to the removal of an isopropyl side-chain is preferred.

2.10 Analytical Methods used for Measuring environmental triazines and chlorophenols samples

2.10.1 Introduction

Instrumental analysis of underivatized chlorophenols and triazines has typically been carried out by high performance liquid chromatography (HPLC) or, less frequently, by capillary

electrophoresis (CE) using ultraviolet-visible (UV-Vis), fluorescence detection (FD), electrochemical detection (ED) or mass spectrometry (MS) detection. Chlorophenols and triazines are highly polar with relatively low vapour pressure, which makes them difficult to measure directly using gas chromatography. To prevent adsorption problems and to improve peak shapes, they are usually converted to less polar derivatives before analysis (Hedges and Ertel, 1982; Renberg and Lindström, 1981). Analysis is then carried out by gas chromatography (GC), after derivatization to the corresponding acetyl derivatives, in combination with various detectors including flame-ionization detection (FID), electron-capture detection (ECD), atomic-emission detection (AED) and selected ion monitoring mass spectrometry (MS). The purpose of this section is to describe the analytical method that was used for separating, and/or measuring chlorophenols and triazine pesticides.

2.10.2 HPLC Instrumentation

High-performance liquid chromatography (or high-pressure liquid chromatography, HPLC) is a chromatographic technique that can separate a mixture of compounds, and is used in analytical chemistry to identify, quantify and purify the individual components of the mixture. An HPLC system consists of a reservoir of mobile phase, pumping unit, sample-injection unit, separation unit, detection unit, and data-processing unit (Figure 2.11). Each of these units is essential for performing the analysis.

Pumping unit

The pump provides a steady high pressure without pulsation and can be programmed to vary the composition of mobile phase during the course of the separation. Conventional, analytical HPLC pumps are the most common type, but semi-micro and a preparative pumps are also used depending on the range of the eluent flow rate required. The pump is selected to suit the purpose of the analysis.

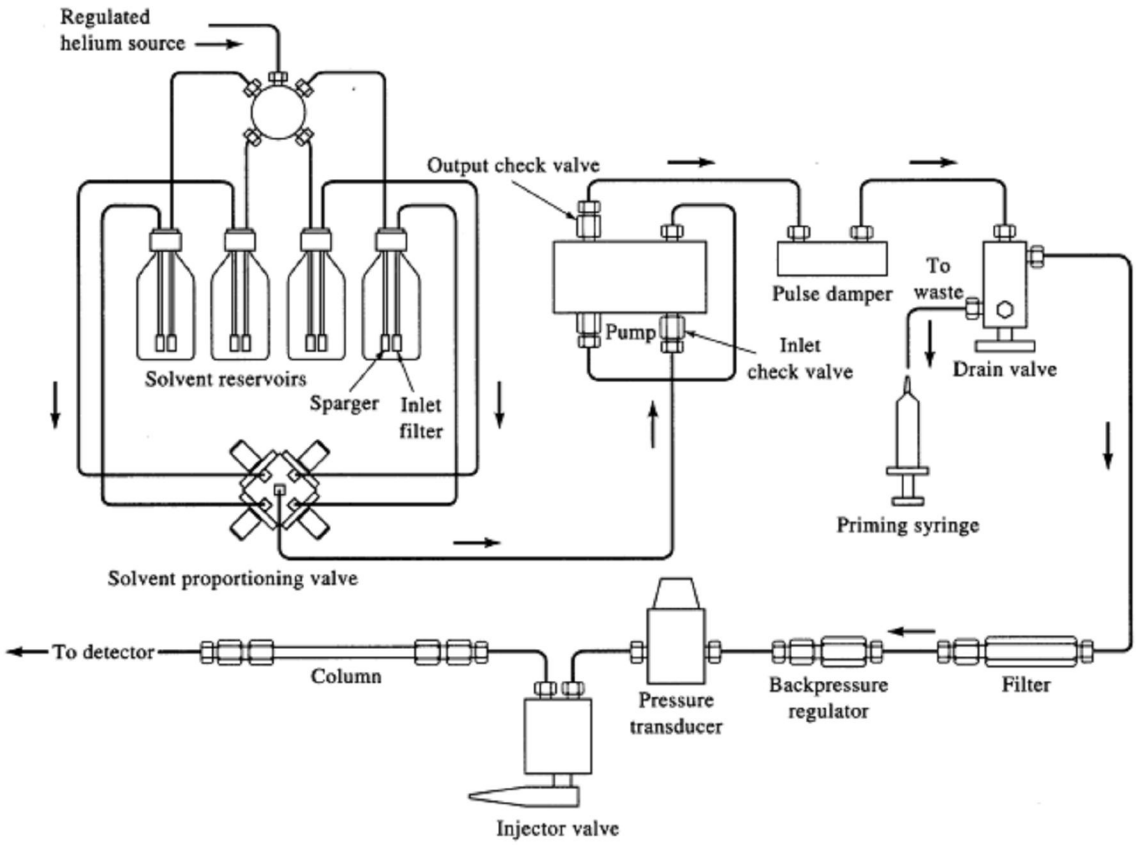


Figure 2.11 Schematic representation of the HPLC

Isocratic flow and gradient elution

A separation in which the mobile phase composition remains constant throughout the procedure is termed isocratic (meaning constant composition). Analyses were first performed using isocratic separations in which the eluent composition remains unchanged during the analysis. This technique is adequate for simple separations. When a sample contains many components, such as a sample for amino-acid analysis is analyzed, it is very difficult to separate all of the components effectively using only one eluent. A separation in which the mobile phase composition is changed during the separation process is described as a gradient elution (Olson et al., 1994). One example is a gradient starting at 10 % methanol and ending at 90 % methanol after 20 minutes. The two components of the mobile phase are typically termed "A" and "B"; A is the "weak" solvent which allows the solute to elute only slowly, while B is

the "strong" solvent which rapidly elutes the solutes from the column. Solvent A is often water, while B is an organic solvent miscible with water, such as acetonitrile, methanol, THF, or isopropanol.

A gradient analysis allows the composition of the eluent to be changed during the analysis. This often indicates that the concentration gradient is to be generated in a linear manner. However, if the eluent composition is changed in a stepwise fashion, this is called a step gradient**.**

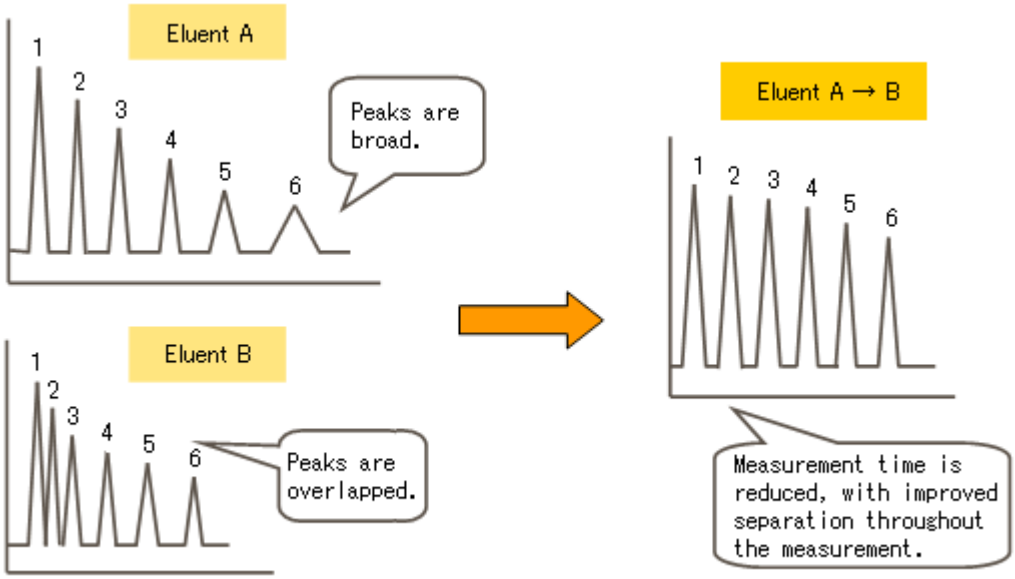


Figure 2.12 Merits of gradient analysis

Figure 2.12 illustrates the merits of gradient analysis. If eluent A is used, the components eluted earlier are clearly separated, but the components eluted later show broad peaks or may not elute from the column. In contrast, if eluent B is used, the former are insufficiently separated, while the latter show sharp peaks. In this case, a gradient analysis in which the eluent composition is changed from the A to B during the analysis can be used to improve the separation over time.

In isocratic elution, peak width increases with retention time linearly according to the equation for *N*, the number of theoretical plates. This leads to the disadvantage that late-eluting peaks get very flat and broad. Their shape and width may keep them from being recognized as peaks.

Gradient elution decreases the retention of the later-eluting components so that they elute faster, giving narrower (and taller) peaks for most components. This also improves the peak shape for tailed peaks, as the increasing concentration of the organic eluent pushes the tailing part of a peak forward. This also increases the peak height (the peak looks "sharper"), which is important in trace analysis. The gradient program may include sudden "step" increases in the percentage of the organic component, or different slopes at different times - all according to the desire for optimum separation in minimum time.

In isocratic elution, the selectivity does not change if the column dimensions (length and inner diameter) change - that is, the peaks elute in the same order. In gradient elution, the elution order may change as the dimensions or flow rate change. The driving force in reversed phase chromatography originates in the high order of the water structure. The role of the organic component of the mobile phase is to reduce this high order and thus reduce the retarding strength of the aqueous component.

Sample-injection unit

The function of the injector is to place the sample into the high-pressure flow in as narrow volume as possible so that the sample enters the column as a homogeneous, low-volume plug. To minimize spreading of the injected volume during transport to the column, the shortest possible length of tubing should be used from the injector to the column. When an injection is started, an air actuator rotates the valve: solvent goes directly to the column; and the injector needle is connected to the syringe (Figure 2.13). The air pressure lifts the needle and the vial is moved into position beneath the needle. Then, the needle is lowered to the vial.

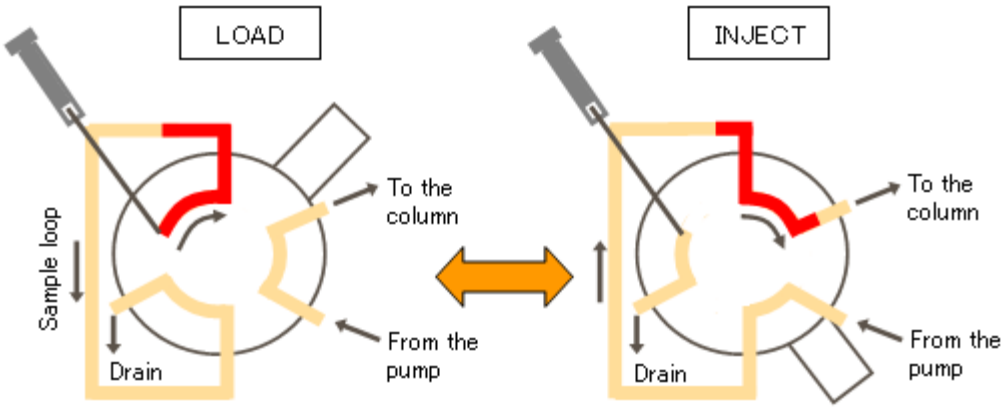


Figure 2.13 Flow path of the manual injector

The sample is drawn up into a sample loop by the syringe, metered by a stepper motor. The needle is raised a second time to allow the vial to move away. Then, the needle is lowered a second time and the air actuator reverses the valve, reconnecting the sample loop to the solvent flow. The entire sample is flushed out of the injector, reaching the column as an undiluted plug. Finally, the syringe stepper-motor moves the syringe plunger to the end of the syringe sending the remaining solvent to the waste.

Separation unit

The column is one of the most important components of the HPLC chromatograph because the separation of the sample components is achieved when those components pass through the column (Figure 2.14). The column is made out of stainless steel tubes with a diameter of 3 to 5 mm and a length ranging from 10 to 30 cm. Normally, columns are filled with silica gel because its particle shape, surface properties, and pore structure help to get a good separation. Silica is wetted by nearly every potential mobile phase, is inert to most compounds and has a high surface activity which can be modified easily with water and other agents. Silica can be used to separate a wide variety of chemical compounds, and its chromatographic behaviour is generally predictable and reproducible.



Figure 2.14 Picture of an HPLC column

The sample to be analyzed is introduced in small volume to the stream of mobile phase. The analyte's motion through the column is slowed by specific chemical or physical interactions with the stationary phase as it traverses the length of the column. How much the analyte is slowed depends on the nature of the analyte and on the compositions of the stationary and mobile phases. The time at which a specific analyte elutes (comes out of the end of the column) is called the retention time; the retention time under particular conditions is considered a reasonably unique identifying characteristic of a given analyte. The use of smaller particle size column packing (which creates higher backpressure) increases the linear velocity giving the components less time to diffuse within the column, leading to improved resolution in the resulting chromatogram. Common solvents used include any miscible combination of water or various organic liquids (the most common are methanol and acetonitrile). Water may contain buffers or salts to assist in the separation of the analyte components, or compounds such as trifluoroacetic acid which acts as an ion pairing agent.

Detection unit

The components eluted from the column are detected, and the detection data are converted into an electrical signal. The detector is selected to suit the sample. Major types of detector include:

1. UV detector: The light source is a D 2 lamp. This detector is used mainly to detect components having an absorption wavelength of 400 nm or less in the ultraviolet region.
2. UV-VIS detector: A D 2 lamp and a W lamp are used as the light source. This detector is effective in the detection of coloring components such as dyes and stains because of coverage of the visible light region.
3. Diode array detector (DAD): Data on the spectrum from the ultraviolet to visible light range is also collected.
4. Fluorescence detector (FD): Fluorescent substances can be detected specifically with high sensitivity.
5. Differential refractive index (RI) detector: Change in the refractive index is detected. Components absorbing no ultraviolet light can also be detected despite low sensitivity.
6. Conductivity detector: Mainly inorganic ions are detected by monitoring the conductivity.

The electrochemical detector (ECD), evaporative light scattering detector (ELSD), Corona® Charged Aerosol Detector (CAD), and others are also used. In addition, the LC-MS system, in which the components separated by HPLC are further analyzed using a mass spectrometer, is becoming widely used because of its high sensitivity and the possibility of specific detection.

The detector which was used in this work is ultra-violet absorption (Figure 2.15). Most triazines exhibit absorption maxima in aqueous solutions around 220 to 225 and/or 255 nm, while their hydroxyl derivatives absorb at lower wavelengths (around 215 nm). Three examples of UV detectors are fixed wavelength, which measures at one wavelength, usually 254 nm, variable wavelength which measures at one wavelength at a time, but can detect over a wide range of wavelengths and diode-array which measures a spectrum of wavelengths simultaneously (Carabias-Matinez et al., 2005). The diode array has an advantage in that it is used for further identification. The UV spectrum of any compound is unique though

compounds of the same family tend to have similar spectrum. These can be differentiated on the basis of retention time. The Beer-Lambert Law (Eq. 16) gives a quantitative relationship between the light absorbed as it passes through the cell containing sample and the concentration of the analyte (http://kerouac.pharm.uky.edu, assessed 24 August 2009).

$$A = \varepsilon bc \qquad (16)$$

where A is the absorbance and has no units, since $A = log_{10} P_0 / P$, ε is the molar absorbtivity with units of l mol L^{-1} cm^{-1}, b is the path length of the sample - that is, the path length of the cuvette in which the sample is contained in cm, c is the concentration of the compound in solution, expressed in mol L^{-1}.

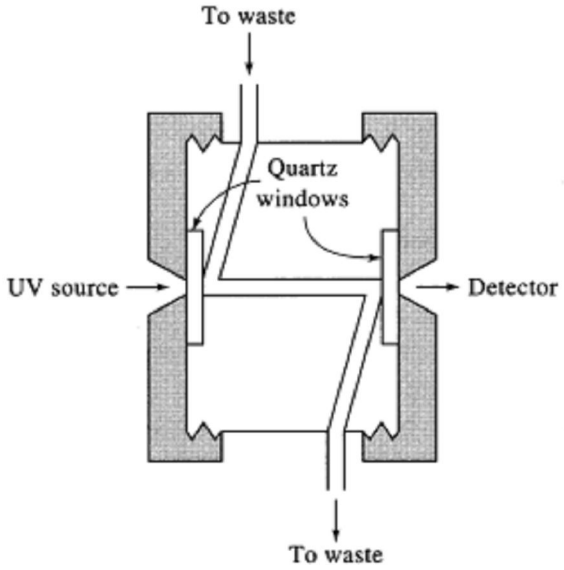


Figure 2.15 Schematic representation of UV detector.

Data-processing unit

The concentration of each detected component is calculated from the area or height of the corresponding peak, and reported. Although previously, easy-to-use integrators were mainly used, systems in which a PC performs both the operation of the units and the analysis of the results have recently played a central role.

2.10.3 Types of Liquid Chromatography

Partition chromatography

Partition chromatography was the first kind of chromatography that chemists developed. The partition coefficient principle has been applied in paper chromatography, thin layer chromatography, gas phase and liquid-liquid applications. Partition chromatography uses a retained solvent, on the surface or within the grains or fibres of an "inert" solid supporting matrix as with paper chromatography; or takes advantage of some additional coulombic and/or hydrogen donor interaction with the solid support. Molecules equilibrate (partition) between a liquid stationary phase and the eluent. Known as Hydrophilic Interaction Chromatography (HILIC) in HPLC, this method separates analytes based on polar differences (Carabias-Matinez et al., 2005). HILIC most often uses a bonded polar stationary phase and a non-polar, water miscible, mobile phase. Partition HPLC has been used historically on unbonded silica or alumina supports. Each works effectively for separating analytes by relative polar differences, however, HILIC has the advantage of separating acidic, basic and neutral solutes in a single chromatogram.

The polar analytes diffuse into a stationary water layer associated with the polar stationary phase and are thus retained. Retention strengths increase with increased analyte polarity, and the interaction between the polar analyte and the polar stationary phase (relative to the mobile phase) increases the elution time. The interaction strength depends on the functional groups in the analyte molecule which promote partitioning but can also include coulombic (electrostatic) interaction and hydrogen donor capability. Use of more polar solvents in the mobile phase will decrease the retention time of the analytes, whereas more hydrophobic solvents tend to increase retention times.

Partition and NP-HPLC had fallen out of favor in the 1970s with the development of reversed-phase HPLC because of a lack of reproducibility of retention times as water or protic organic solvents changed the hydration state of the silica or alumina chromatographic media. Recently

it has become useful again with the development of HILIC bonded phases which improve reproducibility.

Normal-phase chromatography

Also known as normal-phase HPLC (NP-HPLC), or adsorption chromatography, this method separates analytes based on adsorption to a stationary surface chemistry and by polarity. It was one of the first kinds of HPLC that chemists developed. NP-HPLC uses a polar stationary phase and a non-polar, non-aqueous mobile phase, and works effectively for separating analytes readily soluble in non-polar solvents. The analyte associates with and is retained by the polar stationary phase. Adsorption strengths increase with increased analyte polarity, and the interaction between the polar analyte and the polar stationary phase (relative to the mobile phase) increases the elution time. The interaction strength depends not only on the functional groups in the analyte molecule, but also on steric factors. The effect of sterics on interaction strength allows this method to resolve (separate) structural isomers.

The use of more polar solvents in the mobile phase will decrease the retention time of the analytes, whereas more hydrophobic solvents tend to increase retention times. Very polar solvents in a mixture tend to deactivate the stationary phase by creating a stationary bound water layer on the stationary phase surface (Carabias-Matinez et al., 2005). This behavior is somewhat peculiar to normal phase because it is most purely an adsorptive mechanism (the interactions are with a hard surface rather than a soft layer on a surface).

NP-HPLC fell out of favor in the 1970s with the development of reversed-phase HPLC because of a lack of reproducibility of retention times as water or protic organic solvents changed the hydration state of the silica or alumina chromatographic media. Recently it has become useful again with the development of HILIC bonded phases which improve reproducibility.

Displacement chromatography

The basic principle of displacement chromatography is: A molecule with a high affinity for the chromatography matrix (the displacer) will compete effectively for binding sites, and thus displace all molecules with lesser affinities (Carabias-Matinez et al., 2005). There are distinct differences between displacement and elution chromatography. In elution mode, substances typically emerge from a column in narrow, Gaussian peaks. Wide separation of peaks, preferably to baseline, is desired in order to achieve maximum purification. The speed at which any component of a mixture travels down the column in elution mode depends on many factors. But for two substances to travel at different speeds, and thereby be resolved, there must be substantial differences in some interaction between the biomolecules and the chromatography matrix. Operating parameters are adjusted to maximize the effect of this difference. In many cases, baseline separation of the peaks can be achieved only with gradient elution and low column loadings. Thus, two drawbacks to elution mode chromatography, especially at the preparative scale, are operational complexity, due to gradient solvent pumping, and low throughput, due to low column loadings. Displacement chromatography has advantages over elution chromatography in that components are resolved into consecutive zones of pure substances rather than “peaks”. Because the process takes advantage of the nonlinearity of the isotherms, a larger column feed can be separated on a given column with the purified components recovered at significantly higher concentrations.

Reversed-phase chromatography (RPC)

Reversed phase HPLC (RP-HPLC or RPC) has a non-polar stationary phase and an aqueous, moderately polar mobile phase. One common stationary phase is silica which has been treated with RMe_2SiCl, where R is a straight chain alkyl group such as $C_{18}H_{37}$ or C_8H_{17}. With these stationary phases, retention time is longer for molecules which are more non-polar, while polar molecules elute more readily. An investigator can increase retention time by adding more water to the mobile phase; thereby making the affinity of the hydrophobic analyte for the

hydrophobic stationary phase stronger relative to the now more hydrophilic mobile phase (Carabias-Matinez et al., 2005). Similarly, an investigator can decrease retention time by adding more organic solvent to the eluent. RPC is so commonly used that it is often incorrectly referred to as "HPLC" without further specification. The pharmaceutical industry regularly employs RPC to qualify drugs before their release.

RPC operates on the principle of hydrophobic forces, which originate from the high symmetry in the dipolar water structure and play the most important role in all processes in life science. RPC is allowing the measurement of these interactive forces (Olson et al., 1994). The binding of the analyte to the stationary phase is proportional to the contact surface area around the non-polar segment of the analyte molecule upon association with the ligand in the aqueous eluent. This solvophobic effect is dominated by the force of water for "cavity-reduction" around the analyte and the C_{18}-chain versus the complex of both. The energy released in this process is proportional to the surface tension of the eluent (water: 7.3×10^{-6} Jcm^{-2}, methanol: 2.2×10^{-6} Jcm^{-2}) and to the hydrophobic surface of the analyte and the ligand respectively. The retention can be decreased by adding a less polar solvent (methanol, acetonitrile) into the mobile phase to reduce the surface tension of water (Carabias-Matinez et al., 2005). Gradient elution uses this effect by automatically reducing the polarity and the surface tension of the aqueous mobile phase during the course of the analysis.

Structural properties of the analyte molecule play an important role in its retention characteristics. In general, an analyte with a larger hydrophobic surface area (C-H, C-C, and generally non-polar atomic bonds, such as S-S and others) results in a longer retention time because it increases the molecule's non-polar surface area, which is non-interacting with the water structure. On the other hand, polar groups, such as -OH, $-NH_2$, COO^- or $-NH_3^+$ reduce retention as they are well integrated into water (Olson et al., 1994). Very large molecules, however, can result in an incomplete interaction between the large analyte surface and the ligand's alkyl chains and can have problems entering the pores of the stationary phase.

Retention time increases with hydrophobic (non-polar) surface area. Branched chain compounds elute more rapidly than their corresponding linear isomers because the overall surface area is decreased. Similarly organic compounds with single C-C-bonds elute later than

those with a C=C or C-C-triple bond, as the double or triple bond is shorter than a single C-C-bond.

Aside from mobile phase surface tension (organizational strength in eluent structure), other mobile phase modifiers can affect analyte retention. For example, the addition of inorganic salts causes a moderate linear increase in the surface tension of aqueous solutions (ca. 1.5×10^{-7} Jcm^{-2} per Mol for NaCl, 2.5×10^{-7} Jcm^{-2} per Mol for $(NH_4)_2SO_4$), and because the entropy of the analyte-solvent interface is controlled by surface tension, the addition of salts tend to increase the retention time (Olson et al., 1994).. This technique is used for mild separation and recovery of proteins and protection of their biological activity in protein analysis (hydrophobic interaction chromatography, HIC).

Another important component is the influence of the pH since this can change the hydrophobicity of the analyte. For this reason most methods use a buffering agent, such as sodium phosphate, to control the pH. The buffers serve multiple purposes: they control pH, neutralize the charge on any residual exposed silica on the stationary phase and act as ion pairing agents to neutralize charge on the analyte. Ammonium formate is commonly added in mass spectrometry to improve detection of certain analytes by the formation of ammonium adducts. A volatile organic acid such as acetic acid, or most commonly formic acid, is often added to the mobile phase if mass spectrometry is used to analyze the column eluent. Trifluoroacetic acid is used infrequently in mass spectrometry applications due to its persistence in the detector and solvent delivery system, but can be effective in improving retention of analytes such as carboxylic acids in applications utilizing other detectors, as it is one of the strongest organic acids. The effects of acids and buffers vary by application but generally improve the chromatography.

Reversed phase columns are quite difficult to damage compared with normal silica columns; however, many reversed phase columns consist of alkyl derivatized silica particles and should never be used with aqueous bases as these will destroy the underlying silica particle. They can be used with aqueous acid, but the column should not be exposed to the acid for too long, as it can corrode the metal parts of the HPLC equipment. RP-HPLC columns should be flushed with clean solvent after use to remove residual acids or buffers, and stored in an appropriate

composition of solvent. The metal content of HPLC columns must be kept low if the best possible ability to separate substances is to be retained. A good test for the metal content of a column is to inject a sample which is a mixture of 2, 2' - and 4, 4' - bipyridine. Because the 2, 2' - bipy can chelate the metal, the shape of the peak for the 2, 2' - bipy will be distorted (tailed) when metal ions are present on the surface of the silica (Carabias-Matinez et al., 2005).

Size-exclusion chromatography

Size-exclusion chromatography (SEC), also known as gel permeation chromatography or gel filtration chromatography separates particles on the basis of size (Figure 2.16). It is generally a low resolution chromatography and thus it is often reserved for the final, "polishing" step of purification. It is also useful for determining the tertiary structure and quaternary structure of purified proteins. SEC is used primarily for the analysis of large molecules such as proteins or polymers. SEC works by trapping these smaller molecules in the pores of a particle. The larger molecules simply pass by the pores as they are too large to enter the pores. Larger molecules therefore flow through the column quicker than smaller molecules, that is, the smaller the molecule, the longer the retention time.

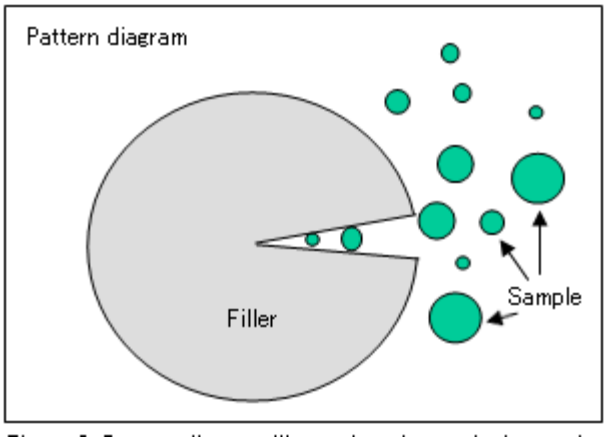


Figure 2.16 Pattern diagram illustrating size-exclusion mode

This technique is widely used for the molecular weight determination of polysaccharides. SEC is the official technique (suggested by European pharmacopeia) for the molecular weight comparison of different commercially available low-molecular weight heparins.

Ion-exchange chromatography

In ion-exchange chromatography, retention is based on the attraction between solute ions and charged sites bound to the stationary phase. Ions of the same charge are excluded. Types of ion exchangers include:

1. Polystyrene resins. These allow cross linkage which increases the stability of the chain. Higher cross linkage reduces swerving, which increases the equilibration time and ultimately improves selectivity.
2. Cellulose and dextran ion exchangers (gels). These possess larger pore sizes and low charge densities making them suitable for protein separation.
3. Controlled-pore glass or porous silica

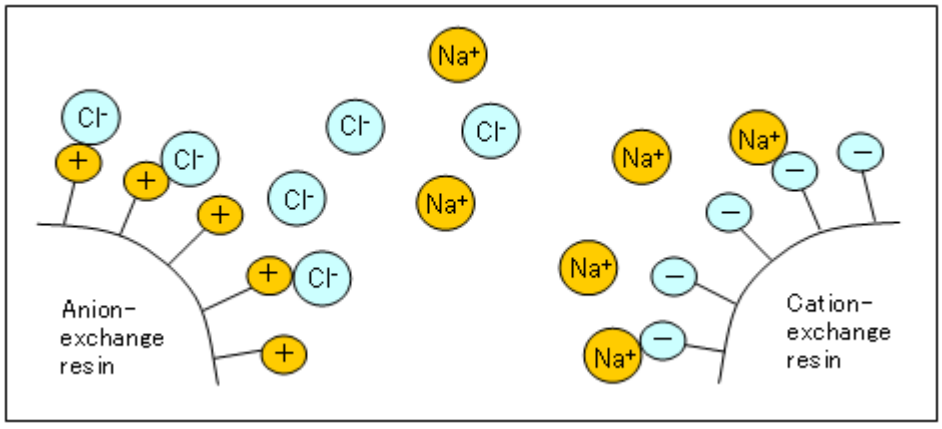


Figure 2.17 Pattern diagram illustrating ion exchange mode

In general, ion exchangers favor the binding of ions of higher charge and smaller radius (Figure 2.17). An increase in counter ion (with respect to the functional groups in resins)

concentration reduces the retention time. An increase in pH reduces the retention time in cation exchange while a decrease in pH reduces the retention time in anion exchange. This form of chromatography is widely used in the following applications: water purification, preconcentration of trace components, ligand-exchange chromatography, ion-exchange chromatography of proteins, high-pH anion-exchange chromatography of carbohydrates and oligosaccharides, and others.

Bioaffinity chromatography

This chromatographic process relies on the property of biologically active substances to form stable, specific, and reversible complexes. The formation of these complexes involves the participation of common molecular forces such as the Van der Waals interaction, electrostatic interaction, dipole-dipole interaction, hydrophobic interaction, and the hydrogen bond. An efficient, biospecific bond is formed by a simultaneous and concerted action of several of these forces in the complementary binding sites.

Aqueous normal-phase chromatography

Aqueous normal-phase chromatography (ANP) is a chromatographic technique which encompasses the mobile phase region between reversed-phase chromatography (RP) and organic normal phase chromatography (ONP). This technique is used to achieve unique selectivity for hydrophilic compounds, showing normal

2.10.4 Calibration and Quantification

Few methods of chemical analysis are truly specific to a particular analyte. It is often found that the analyte of interest must be separated from the myriad of individual compounds that

may be present in a sample. As well as providing the analytical scientist with methods of separation, chromatographic techniques can also provide methods of analysis or quantification.

Chromatography involves a sample (or sample extract) being dissolved in a mobile phase (which may be a gas, a liquid or a supercritical fluid). The mobile phase is then forced through an immobile, immiscible stationary phase. The phases are chosen such that components of the sample have differing solubilities in each phase (Olson et al., 1994). A component which is quite soluble in the stationary phase will take longer to travel through it than a component which is not very soluble in the stationary phase but very soluble in the mobile phase. As a result of these differences in mobilities, sample components will become separated from each other as they travel through the stationary phase.

Distribution of analytes between phases

The distribution of analytes between phases can often be described quite simply. An analyte is in equilibrium between the two phases;

$$A_{mobile} \rightleftharpoons A_{stationary}$$

The equilibrium constant, *K*, is termed the partition coefficient; defined as the molar concentration of analyte in the stationary phase divided by the molar concentration of the analyte in the mobile phase.

The time between sample injection and an analyte peak reaching a detector at the end of the column is termed the retention time (t_R). Each analyte in a sample will have a different retention time. The time taken for the mobile phase to pass through the column is called t_M.

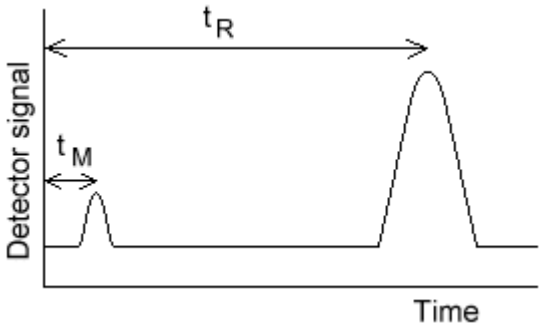


Figure 2.18 Two well resolved peaks in a chromatogram

A term called the retention factor, *k'*, is often used to describe the migration rate of an analyte on a column. You may also find it called the capacity factor. The retention factor for analyte *A* is defined as;

$$k'_A = t_R - t_M / t_M \qquad (17)$$

t_R and t_M are easily obtained from a chromatogram. When an analytes retention factor is less than one, elution is so fast that accurate determination of the retention time is very difficult. High retention factors (greater than 20) mean that elution takes a very long time (Dorsey and Cooper, 1994). Ideally, the retention factor for an analyte is between one and five.

We define a quantity called the selectivity factor, a, which describes the separation of two species (*A* and *B*) on the column;

$$a = k'_B / k'_A$$

When calculating the selectivity factor, species *A* elutes faster than species *B*. The selectivity factor is always greater than one.

Band broadening and column efficiency

To obtain optimal separations, sharp, symmetrical chromatographic peaks must be obtained. This means that band broadening must be limited. It is also beneficial to measure the efficiency of the column.

The theoretical plate model of chromatography

The plate model supposes that the chromatographic column is contains a large number of separate layers, called theoretical plates. Separate equilibrations of the sample between the stationary and mobile phase occur in these "plates". The analyte moves down the column by transfer of equilibrated mobile phase from one plate to the next.

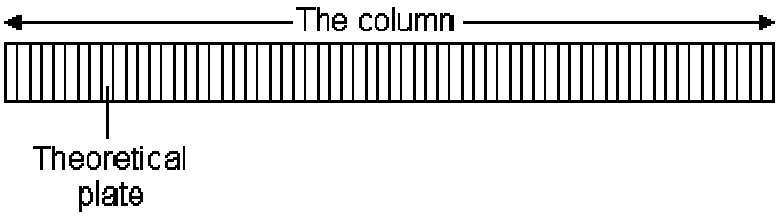


Figure 2.19 The plate model of a chromatographic column

It is important to remember that the plates do not really exist; they are a figment of the imagination that helps us understand the processes at work in the column (Dorsey and Cooper, 1994). They also serve as a way of measuring column efficiency, either by stating the number of theoretical plates in a column, *N* (the more plates the better), or by stating the plate height; the Height Equivalent to a Theoretical Plate (the smaller the better).

If the length of the column is *L*, then the HETP is

HETP = L / N

The number of theoretical plates that a real column possesses can be found by examining a chromatographic peak after elution;

$$N = \frac{5.55\ t_R^{\ 2}}{w_{1/2}^{\ 2}}$$

where $w_{1/2}$ is the peak width at half-height. As can be seen from this equation, columns behave as if they have different numbers of plates for different solutes in a mixture.

The rate theory of chromatography

A more realistic description of the processes at work inside a column takes account of the time taken for the solute to equilibrate between the stationary and mobile phase (unlike the plate model, which assumes that equilibration is infinitely fast). The resulting band shape of a chromatographic peak is therefore affected by the rate of elution. It is also affected by the different paths available to solute molecules as they travel between particles of stationary phase. If we consider the various mechanisms which contribute to band broadening, we arrive at the Van Deemter equation for plate height;

$$\text{HETP} = A + B / u + C u \qquad (18)$$

where u is the average velocity of the mobile phase. *A, B,* and *C* are factors which contribute to band broadening.

A - Eddy diffusion

The mobile phase moves through the column which is packed with stationary phase. Solute molecules will take different paths through the stationary phase at random. This will cause broadening of the solute band, because different paths are of different lengths.

B - Longitudinal diffusion

The concentration of analyte is less at the edges of the band than at the centre. Analyte diffuses out from the centre to the edges. This causes band broadening. If the velocity of the mobile phase is high then the analyte spends less time on the column, which decreases the effects of longitudinal diffusion.

C - Resistance to mass transfer

The analyte takes a certain amount of time to equilibrate between the stationary and mobile phase. If the velocity of the mobile phase is high, and the analyte has a strong affinity for the stationary phase, then the analyte in the mobile phase will move ahead of the analyte in the

stationary phase. The band of analyte is broadened. The higher the velocity of mobile phase, the worse the broadening becomes.

Van Deemter plots

The Van Deemter equation in chromatography relates the variance per unit length of a separation column to the linear mobile phase velocity by considering physical, kinetic, and thermodynamic properties of a separation (Dorsey and Cooper, 1994). These properties include pathways within the column, diffusion (axial and longitudinal), and mass transfer kinetics between stationary and mobile phases. In liquid chromatography, the mobile phase velocity is taken as the exit velocity, that is, the ratio of the flow rate in ml/second to the cross-sectional area of the 'column-exit flow path.' For a packed column, the cross-sectional area of the column exit flow path is usually taken as 0.6 times the cross-sectional area of the column. Alternatively, the linear velocity can be taken as the ratio of the column length to the dead time. If the mobile phase is a gas, then the pressure correction must be applied. The variance per unit length of the column is taken as the ratio of the column length to the column efficiency in theoretical plates. The Van Deemter equation is a hyperbolic function that predicts that there is an optimum velocity at which there will be the minimum variance per unit column length and, thence, a maximum efficiency. The Van Deemter equation was the result of the first application of rate theory to the chromatography elution process.

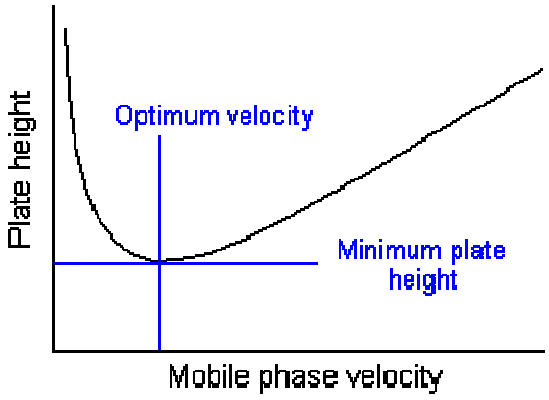


Figure 2.20 A plot of plate height vs. average linear velocity of mobile phase

Van Deemter equation

$$H = A + \frac{B}{u} + C \cdot u \qquad (19)$$

where A = Eddy-diffusion

B = Longitudinal diffusion

C = mass transfer kinetics of the analyte between mobile and stationary phase

u = Linear Velocity.

A is equal to the multiple paths taken by the chemical compound, in open tubular capillaries this term will be zero as there are no multiple paths. The multiple paths occur in packed columns where several routes exist through the column packing, which results in band spreading.

B/*u* is equal to the longitudinal diffusion of the particles of the compound.

Cu is equal to the equilibration point. In a column, there is an interaction between the mobile and stationary phases, *Cu* accounts for this.

A minimum value for *H* can be found by differentiating:

$$U = \sqrt{\frac{B}{C}} \qquad (20)$$

Resolution

Although the selectivity factor, a, describes the separation of band centres, it does not take into account peak widths. Another measure of how well species have been separated is provided by measurement of the resolution. The resolution of two species, *A* and *B*, is defined as

$$R = \frac{2[(t_R)_B - (t_R)_A]}{W_A + W_B} \quad (21)$$

where W_A is Gaussian curve width of solute *A* and W_B is the Gaussian curve width of solute *B*. Baseline resolution is achieved when $R = 1.5$

It is useful to relate the resolution to the number of plates in the column, the selectivity factor and the retention factors of the two solutes;

$$R = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{1 + k'_B}{k'_B}\right) \quad (22)$$

To obtain high resolution, the three terms must be maximised. An increase in *N*, the number of theoretical plates, by lengthening the column leads to an increase in retention time and increased band broadening - which may not be desirable. Instead, to increase the number of plates, the height equivalent to a theoretical plate can be reduced by reducing the size of the stationary phase particles.

It is often found that by controlling the capacity factor, *k'*, separations can be greatly improved. This can be achieved by changing the temperature (in Gas Chromatography) or the composition of the mobile phase (in Liquid Chromatography).

The selectivity factor, a, can also be manipulated to improve separations. When α is close to unity, optimising *k'* and increasing *N* is not sufficient to give good separation in a reasonable time (Olson et al., 1994). In these cases, *k'* is optimised first, and then α is increased by one of the following procedures:

1. Changing mobile phase composition
2. Changing column temperature
3. Changing composition of stationary phase
4. Using special chemical effects (such as incorporating a species which complexes with one of the solutes into the stationary phase)

2.10.5 Applications

Analytical tools for the detection of pesticides in environmental samples are well studied and have improved markedly, making it possible to report parts per billion and even parts per trillion residue levels. In the past, the environmental monitoring of pesticides has been largely confined to the investigation of polar, persistent compounds, analyzed by GC-based techniques. New developments in monitoring pesticides in water have been thoroughly investigated by DiGiano et al. (1989), who elaborated a sensitive method based on Liquid-Solid Extraction (LSE) and HPLC-UV and HPLC-ESI-MS quantitation (Reemtsma, 2001).

HPLC, especially when coupled with mass spectrometry, represents a very valuable analytical tool for the determination of modern pesticides and their degradation products, most of which are polar, low volatile, and/or thermo-labile compounds, and, therefore, not amenable to GC analysis. To achieve high sensitivity and selectivity, the combination of HPLC with tandem mass spectrometry has been shown very successful (Reemtsma, 2001).

An overview of the extraction and chromatographic techniques employed for the environmental water analysis of pesticides using LC-tandem mass spectrometry is presented in Table 2.6. A variety of SPE sorbents (e.g., modified silica-based materials, polymeric phases, carbon-based materials, etc.) have been used for efficient extraction. An interesting overview on SPE sorbents has been carried out by Hogenboom et al. (2001). The appearance of a typical product-ion mass spectrum obtained for atrazine, cyanazine, tertbutylazine, and simazine by HPLC-ESI-MS/MS in the PI mode and corresponding suggested structures is shown in Figure 2.21.

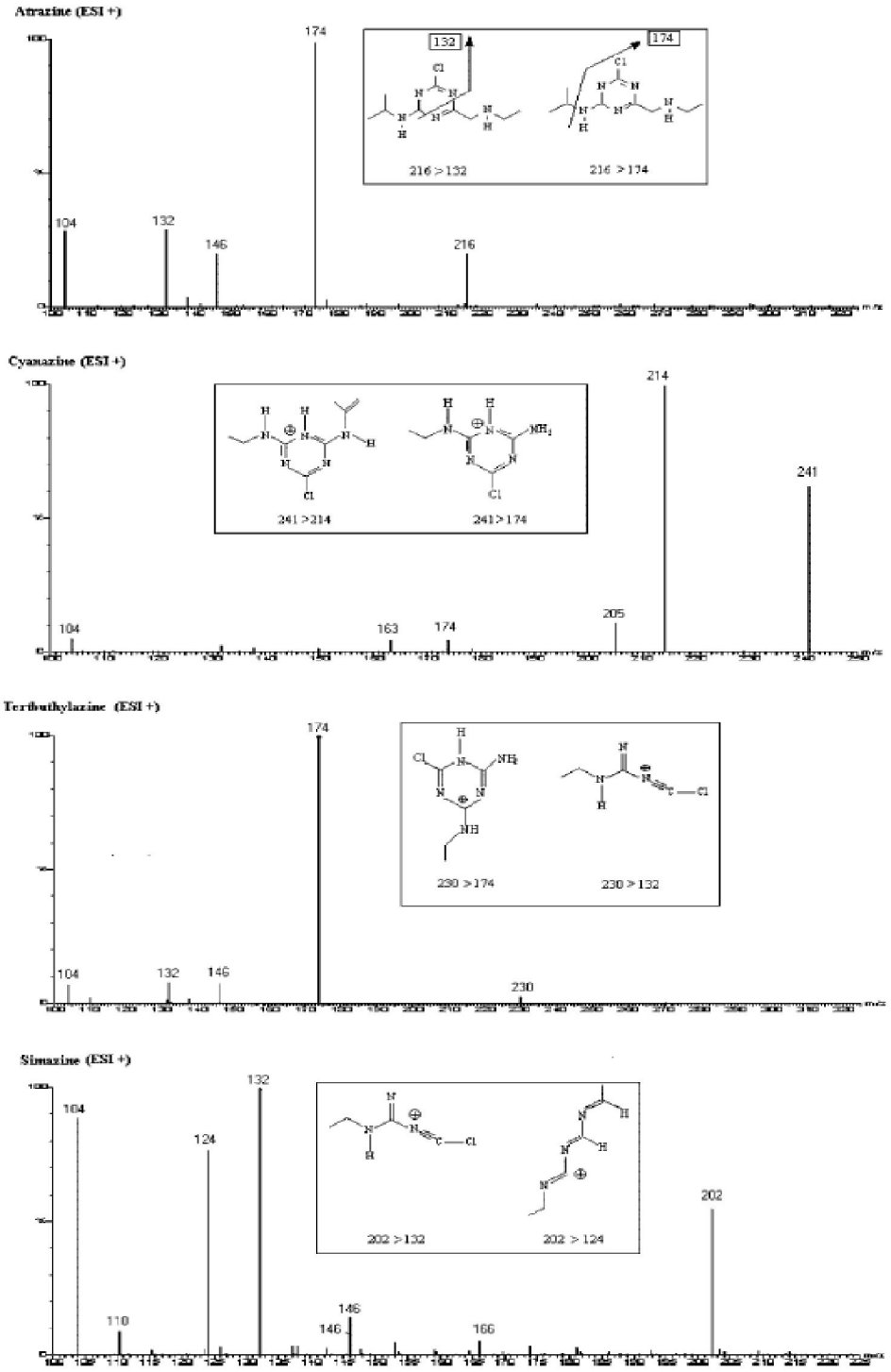


Figure 2.21 Product-ion mass spectra obtained for atrazine, cyanazine, tertbutylazine, and simazine by LC-ESI-MS/MS in the PI mode and corresponding suggested structures. (Reproduced from (Borba da Cunha et al., 2004), with permission from Springer-Verlag GmbH, copyright 2004)

Table 2.6 Overview of Methods Reported for the Water Analysis of Pesticides Using LC (Kuster et al., 2006)

Pesticides	Sample	Clean-up			LC	
		Volume	Method	Cartridge	Chrom.col.	Mob.phase
14 pesticides	spiked tap and filtered river water	4 mL	on-line SPE	C18 / PLRP-s (10 x 2 mm)	-	Water-Methanol
ESA of 4 pesticides	ground water	100 mL	SPE	Varian ENV	YMC ODS-AQ (150 x 4.6 mm, 5 µm)	Water (0.1% acetic acid)-ACN (0.1% acetic acid)
9 pesticides	drinking water	3 mL	on-line SPME	PDMS/DVB, PA, CW/TPR	YMC/3-4-5 C-8 (50 x 4 mm, 3 µm)	Water (0.1% formic acid)-ACN
17 pesticides	spiked tap water	1 L	SPE	graphitised carbon black	Alltima C18 (250 x 4.6 mm, 5 µm)	Water (for acidic pesticides 0.1% acetic acid)-Methanol
5 pesticides + TPs	water from photolysis experiment reservoir	10 mL	on-line SPE	PLRP-s (10 x 2 mm)	LC-18-DB (20 x 4.6 mm, 5 µm)	10mM aqueous ammonium acetate (pH 4.5)-Methanol
12 pesticides	ground and river water	1, 10 L	LLE, SPE	LLE: DCM+ 1g C18 bonded silica / SPE: Carbopack B	Hypersil ODS 2 (250 x 4.6 mm, 5 µm)	Water-ACN
8 polar and acidic pesticides	tap and river water	25, 50 mL	on-line SPE	In-line: PLRP-s, Hysphere-1	Hypersil BDS C18 (4 x 4 mm, 5 µm)	10mM aqueous ammonium formate (pH 3)-Methanol
7 polar organophosphorus pesticides	spiked surface water	1 mL	direct injection	-	Phenomenex Aqua C18 (150 x 4.6 mm, 5 µm)	Water (5% MeOH, 0.1% acetic acid)-MeOH (10% Water, 0.1% acetic acid)
35 pesticides + TPs	ground and river water	1.3 mL	on-line SPE	C18 (10 x 2 mm)	ABZ+ (100 x 2 mm, 5 µm)	Water (0.01% acetic acid)- ACN (0.01% acetic acid)
5 acidic pesticides	spiked drinking water	0.25-2 mL	direct injection	-	LC-LC: C18/ ABZ+ (100 x 2 mm, 5 µm)	25% up to 65% ACN in water (0.1% formic acid)
1 pesticide	ground and surface water	9 mL	on-line SPE	PLRP-s	Inertsil ODS-2 (250 x 4.6 mm, 5 µm)	Water -Methanol
6 pesticides	surface water	500 mL	SPE	Oasis HLB	Inertsil ODS-2 (250 x 4.6 mm, 5 µm)	Water (0.1% formic acid)-ACN (0.1% formic acid)

Pesticides	Sample	Clean-up			LC	
		Volume	Method	Cartridge	Chrom.col.	Mob.phase
11 pesticides	drinking and river water	10 mL	on-line SPE	Oasis HLB	Chromolith SpeedROD RP-18e (50 x 4.6 mm)	Water (3% MeOH,1mM ammonium acetate)-MeOH (3% Water,1mM ammonium acetate)
4 pesticides + ESA and OA TPs	ground and surface water	50 mL	SPE	Varian C-18	Zorbax SB C-8 (50 x 2.1 mm, 5 µm)	Water (0.1% acetic acid)-MeOH (0.1% acetic acid)
53 pesticides	rainwater	500 mL	SPE	Oasis HLB	Hypersil BDS C18 (250 x 2.1 mm, 5 µm)	5mM aqueous ammonium acetate (1% MeOH, 0.1% formic acid)-MeOH (10% 5mM aqueous ammonium acetate, 0.1% formic acid) for acidic pesticides: Water (10% MeOH, 0.1% acetic acid)-MeOH (0.1% acetic acid) ,
8 pesticides (including TPs)	surface and drinking water	500 mL	SPE	graphitised carbon black	HyPURITY Elite C-18 (250 x 4.6 mm, 5µm)	isocratic water-ACN (45:55 v/v), 0.3mM tetrabutylammonium
12 pesticides	drinking, drainage and river water	2, 1 L	SPE	Carbograph-1	Alltima C18 (250 x 4.6 mm, 5 µm)	Water (1mM formic acid)-Methanol:ACN, 30:70 (1mM formic acid)
TPs of propoxur	drinking, rain, river and seawater	100 mL	LLE	-	Phenomenex ODS (150 x 3.2 mm, 5 µm)	Water:Methanol
47 pesticides + TPs	ground and surface water	2 mL	on-line SPE	PRP-1 (10 x 2 mm)	Kromasil c18 (125 x 2.0 mm. 5 µm)	Water (0.01% acetic acid)-ACN (0.01% acetic acid)
6 pesticides	spiked tap, mineral and groundwater	30 mL	on-line SPE	Hysphere Resin GP (10 x 2 mm)	Kromasil C8 (200 x 2.1 mm, 5 µm)	20 mM HFBA in 100mM formic acid/ammonium formate (pH3)-ACN
15 pesticides + TPs	surface and waste water	1 L	SPE	Oasis HLB	Nucleodur C18 Gravity (125 x 2 mm, 3 µm) / Gromsil C18 (150 x 2 mm, 3 µm)	Water (0.1% formic acid)-Methanol (0.1% formic acid) for the acidic pesticides 0.6% formic acid
TPs of 4 triazines	spiked surface water	50 µL	direct injection	-	X-Terra C18 (250 x 2.1 mm, 5 µm)	Water (0.01% acetic acid)-Methanol (0.01% acetic acid)
20 pesticides	drinking water	20 mL	on-line SPE	Hysphere GP / PLRP-s (10 x 2 mm)	Purospher STAR RP-18 (125 x 2 mm, 5 µm)	Water-ACN

Pesticides	Sample	Clean-up			LC	
		Volume	Method	Cartridge	Chrom.col.	Mob.phase
8 pesticides + TPs	surface water	100 mL	SPE	C18	X-Terra (250 x 4.6 mm, 5 µm)	Water-ACN
Rotenone	surface water	50 mL	SPE	C18	-	-
3 pesticides	ground and surface water	4.3 mL	on-line SPE	Oasis HLB (20 x 2.1 mm)	Discovery C18 (50 x 2.0 mm, 5 µm)	5mM aqueous ammonium acetate/acetic acid (pH 4.8)-ACN
18 pesticides	surface water	18 mL	on-line SPE	Oasis HLB (20 x 2.1 mm)	Nucleodur C18 Gravity (125 x 2 mm, 5 µm) / Gromsil ODS 3 CP (125 x 2 mm, 3 µm)	Water, 20mM formic acid (pH 2.7)-Methanol, 20mM formic acid (pH 2.7); for acidic pesticides 120mM formic acid (pH 2.3)

~ Chapter Three – Research Methodology ~

3.1 Introduction

The purpose of this chapter is to expound details on chemicals, equipments and instruments and procedures used in pursuit of the research objectives outlined in chapter one.

3.2 Experimental

3.2.1Chemicals and Calibration Standards

The following chlorophenols standards were used: 4-chlorophenol (> 99 %), 2-chlorophenol (> 98 %) and 2, 4-dichlorophenol (> 99 %) from Merck-Schuchardt (Darmstadt, Germany). Other chemicals that were used are trisodium phosphate (99 %), proanalysis sulphuric acid (99 %), nitric acid (55 %) and HPLC grade methanol, acetone and acetonitrile all from Merck-Schuchardt (Darmstadt, Germany); Triazine pesticide mixture reference chemicals (500 µg/ml) (2-ethylamino-4-isopropylamino-6-methylthio-s-triazine, atraton, atrazine, 2-methoxy-4.6-bis [isopropylamino]-s-triazine, 2.4-bis [isopropylamino]-6-methylthio-s-triazine, 2-chloro-4.6-bis [isoprpylamino]-s-triazine, simetryn, simazine, terbuthylazine, 2-t-butylamino-4-ethylamino-6-methylthio-s-triazine) from Chemical Service (West Chester, USA). Triazine herbicides (2-ethylamino-4-isopropylamino-6-methylthio-s-triazine, atraton, atrazine, 2-methoxy-4.6-bis [isopropylamino]-s-triazine, 2.4-bis [isopropylamino]-6-methylthio-s-triazine, 2-chloro-4.6-bis [isoprpylamino]-s-triazine, simetryn, simazine, terbuthylazine, 2-t-butylamino-4-ethylamino-6-methylthio-s-triazine) in acetone, were purchased from Sigma Aldrich (Darmstadt, Germany).

Stock solutions of chlorophenol pesticides were prepared in methanol at 1000 mg L^{-1} while stock solutions of triazine herbicides were prepared in acetone at 1000 mg L^{-1}. These were

stored in the refrigerator at 4 ^{o}C. Fresh stock solutions were prepared after every three months. Calibration standards were prepared by diluting the stock solutions with deionized water.

3.2.2 Hollow Fibres

Silicone hollow fibre membranes used for the optimization process were from Technical Products Inc. (Georgia, USA). Their dimensions are summarized in Table 3.1. The membranes were bought as long tubes and were cut to appropriate lengths (48 cm x 0.1575 cm I.D x 0.2413 cm O.D giving a volume of ~1000 μL).

Table 3.1 Summary of the dimensions of silicon hollow fibre used in this study.

Type	I.D (cm)	O.D (cm)	Thickness (cm)	Length (cm)	Volume (cm^3)	Surface Area (cm^2)
Smallest	0.06	0.12	0.03	58.0	0.19	21.7
Medium	0.16	0.24	0.04	23.5	0.46	17.8
Biggest	0.48	0.95	0.24	7.8	1.40	23.3

3.2.3 Chromatographic Conditions

The chlorophenols pesticides and triazine herbicides were separated with a mobile phase composition of 60 % water and 40 % acetonitrile at a flow rate of 1.0 mL min^{-1}. The UV detector was set at 280 nm for chlorophenols (Chimuka et al., 2008) and 220 nm for triazine pesticides (Bjarnason et al., 1999). A C_{18} column with dimensions 5 μm x 4.6 mm x 25 cm was used (Supelco, Bellefonte, PA, USA). An SRI (LA, California, USA) 210 HPLC system with a UV detector (VUV-24) and peak simple chromatographic software (version 3.29) and quantification was done using an external calibration curve. The calibration curve was made from the standards solution ranging from 1 to 7 mgL^{-1}. The mobile phase was degassed offline and filtered before use. The injection volume was 20 μL.

Quantification and quality assurance

Quantification of the extracts for chlorophenols and triazines compounds was performed by external calibration curve that was linear in concentration range of 0.5 to 2.0 mgL^{-1}. Typical calibration curves and standard chromatograms for triazine and chlorophenol compounds are shown in Figures 3.1 and 3.2 below.

A number of steps were taken to ensure quality of the results obtained in any experimental part. This included repeating experiments two or more times. Certified reference standards as discussed earlier were also used as part of quality assurances.

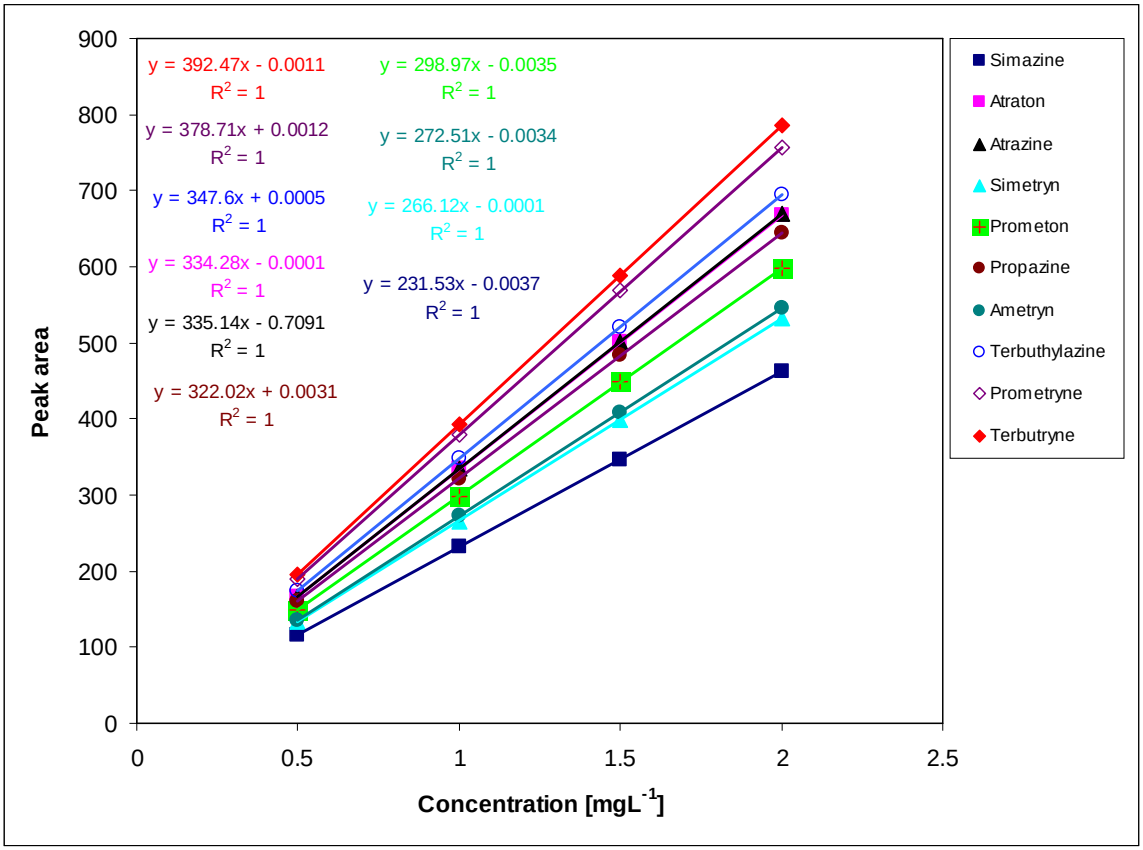


Figure 3.1a A typical calibration curve of triazines.

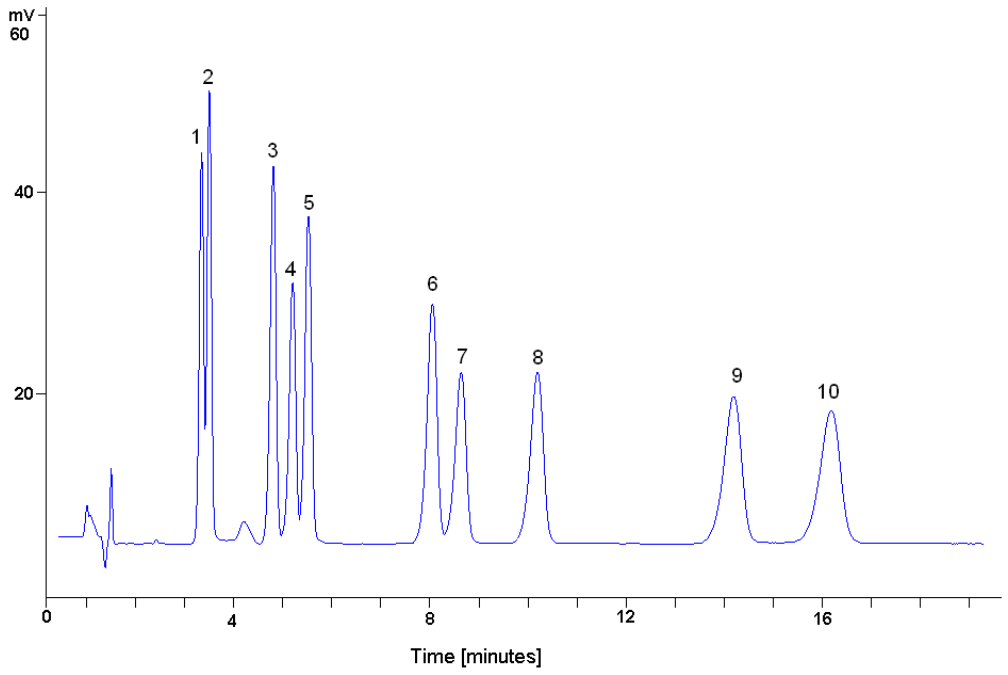


Figure 3.1b A typical chromatogram of 1.0 mgL^{-1} triazines standard injection where Simazine (1), Atrazine (3), Simetryn (4), Prometon (5), Propazine (6), Ametryn (7), Terbuthylazine (8), Prometryne (9) and Terbutryne (10) using HPLC – UV, with a mobile phase composition of 60 % water and 40 % acetonitrile at a flow rate of 1.0 mL min^{-1}, UV detector was set at 220 nm and a C_{18} column with dimensions 5 µm x 4.6 mm x 25 cm.

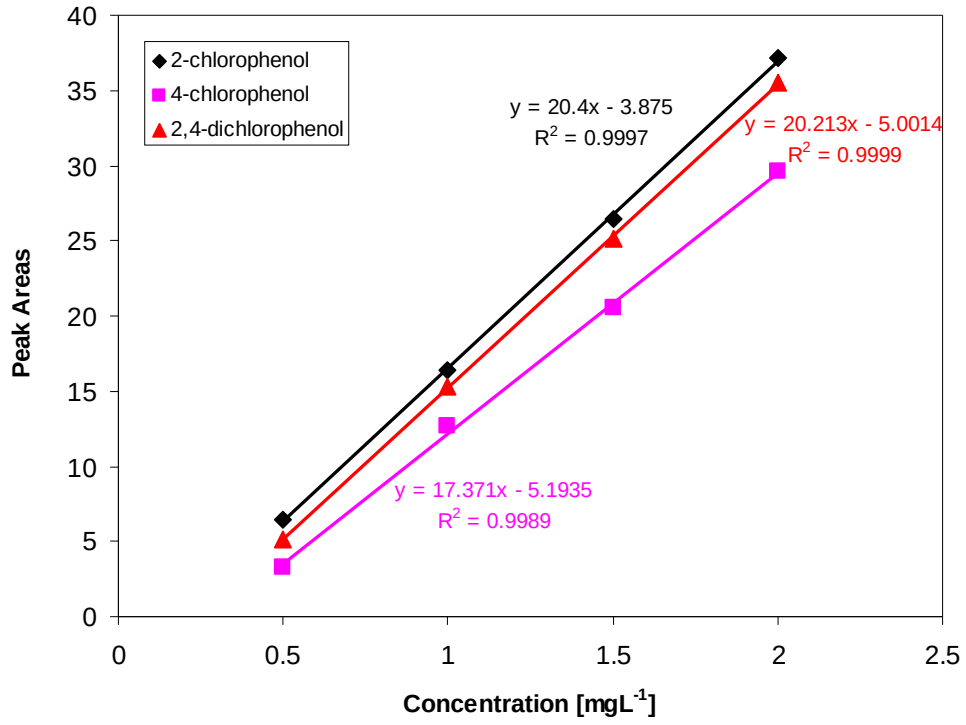


Figure 3.2a Typical calibration curve of chlorophenols

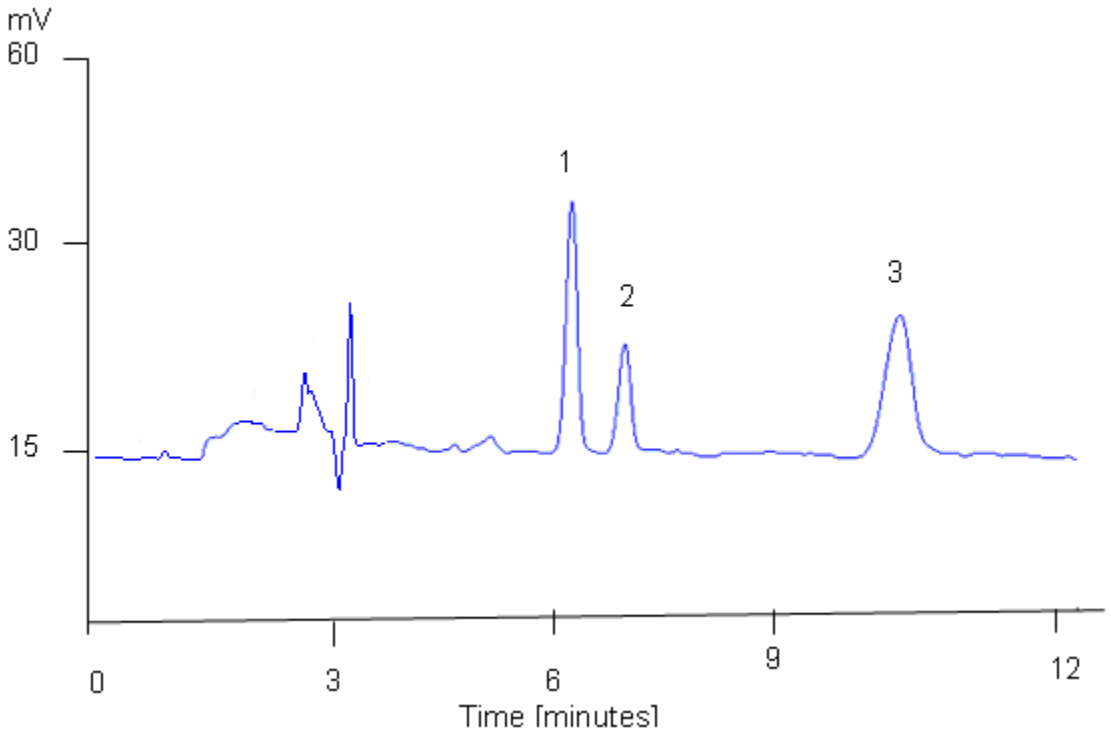


Figure 3.2b A typical chromatogram of a 1.0 mgL^{-1} chlorophenols standard injection where (1) = 2 - chlorophenol, (2) = 4- chlorophenol and (3) = 2, 4 – dichlorophenol

3.3 Calibration Procedure

3.3.1 Preparation of the Hollow Fibre Membranes and Extraction Procedure

The hollow fibre silicone membrane, previously soaked in deionised water, was filled with acceptor buffer using a 1000 µL micropipette. The basic acceptor solution for trapping chlorophenols was a 0. 5M solution of trisodium phosphate and the acidic acceptor for trapping triazines was 0.5 M solution of nitric acid. The hollow fibre ends were tightened together and made in the form of a loop about 3 cm in diameter (Figure 3.3). The outside was rinsed with deionized water thoroughly to remove any buffer spills and then immersed in an appropriate sample vessel and left hanging for an appropriate time.

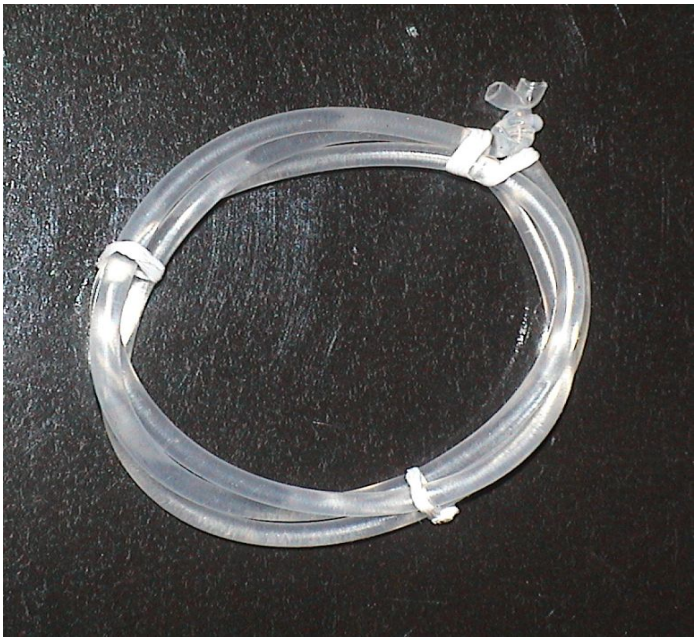


Figure 3.3 Photo of the silicone hollow fibre membrane with its ends tightened together and made in a form of a loop about 3 cm in diameter.

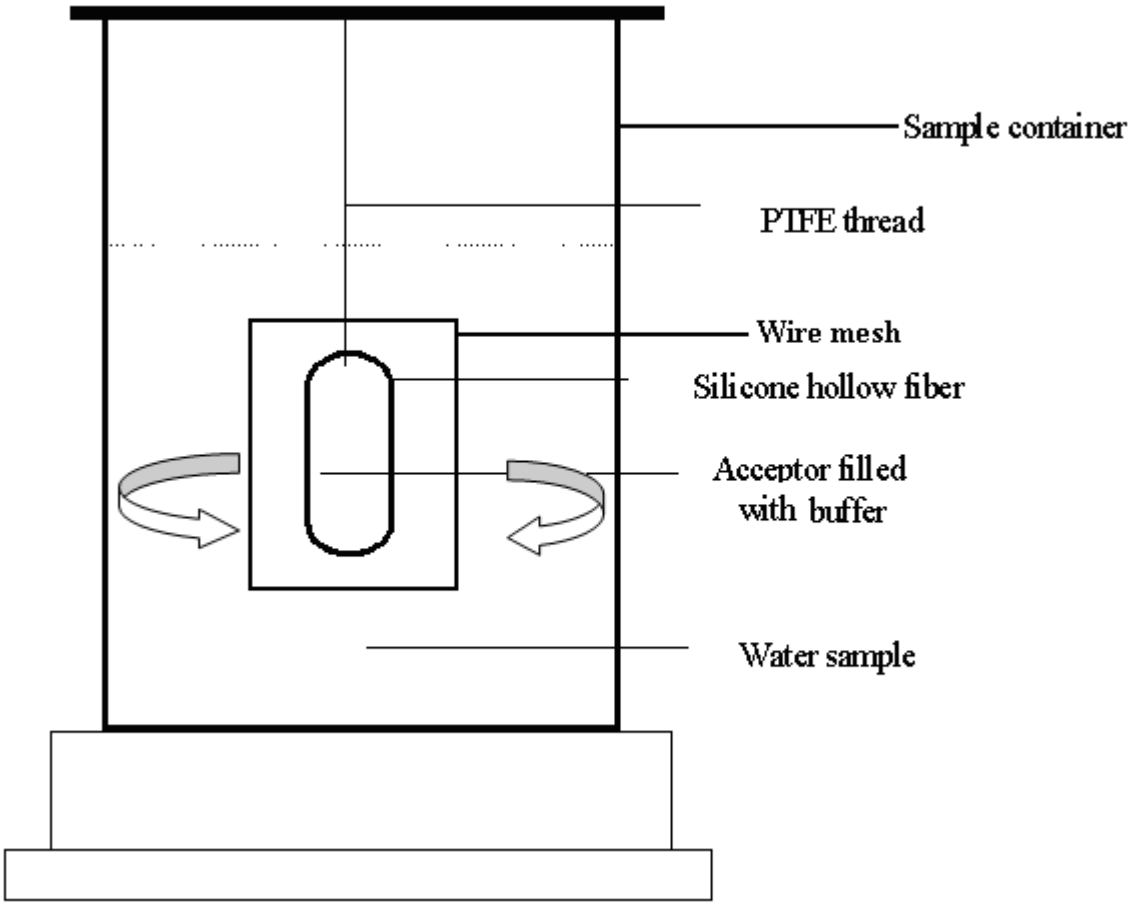


Figure 3.4 Schematic experimental set-up of membrane assisted passive sample extraction system.

Thereafter, it was taken out of the sample vessel, the outside flushed with deionised water and its contents transferred into a 4 mL vial. The buffer solution of the acceptor solution was adjusted by adding an appropriate amount of neutralising acid and/or base. The extracts were either analyzed immediately or stored in the refrigerator at 4 °C.

Figure 3.4 shows a depiction of the experimental set-up of membrane assisted passive sampler extraction. Un-spiked deionised water samples were also extracted to check for any target compounds as a quality measure.

3.3.2 Triazine Optimisation Experiments for Variable Environmental Conditions

Effect of temperature on the MAPS performance

In these influence-of-temperature experiments, up to 9 passive samplers three for each temperature were exposed in appropriate temperature-controlled systems. These systems were devised to allow calibration of the sampling devices to be compared at three different temperatures (4, 16 and 40 ºC). Deionized water containing 100 µg L^{-1} of a mixture of spiked chlorophenols was used as sample solution. Three sample vessels containing 3 L of this sample solution were placed (1) in refrigerator held at 4 ^{o}C, (2) in water bath held at 16 ^{o}C and the other three in (3) water bath heated and held at 40 ^{o}C. Before putting the MAPS, the samples were allowed to equilibrate for at least two hours at appropriate temperature. Passive exposure period was for 72 hours. After extraction, the extracts were treated in the same way as described in the extraction procedure.

Effect of hydrodynamics on the MAPS' uptake kinetics of triazines

Effect of hydrodynamics was studied by comparison of the sampling rates under static and under stirred conditions in deionised water spiked with 50 µg L^{-1} mixtures of the triazines were extracted for 7 days. The sample volume used was 2 L. The experiment was repeated at least

three times. The revolutions per minute were set at 220 rpm. After extraction, the extracts were treated in the same way as described in the extraction procedure.

Effect of biofouling layer on the MAPS' uptake kinetics

Deionised water samples

Deionised water containing 100 µg L^{-1} of a mixture of spiked 2-chlorophenol, 4-chlorophenol and 2,4-chlorophenol was used as sample solution. Three sample vessels containing 3 L of sample solution were each extracted using passive sampler technique under laboratory conditions.

River water samples

River water samples for optimization of the extraction procedure were used without any manipulation, except pH measurements. River water samples were taken at a depth of 10 cm from a stream near Super Sport Park, Centurion, Gauteng Province, South Africa, in glass containers. The containers were thoroughly washed with soap, soaked in methanol and then rinsed with deionized water before use. River water samples were spiked with 100 µg L^{-1} of 2-chlorophenol, 4-chlorophenol and 2,4-chlorophenol mixture and extracted using passive sampler technique under laboratory conditions except for the blank.

Wastewater samples

Wastewater samples were collected from the settling tanks after chlorination point of Goudkoppies WasteWater Treatment Works (GWWTW) west of Johannesburg. The GWWTW treats both house hold and industrial wastewater. The collected wastewater samples were spiked with 100 µg L^{-1} of 2-chlorophenol, 4-chlorophenol and 2,4-chlorophenol mixtures and extracted using passive sampler technique under laboratory conditions. Some of the wastewater was spiked with 50 µg L^{-1} and extracted with solid phase extraction technique. Wastewater samples that were extracted with solid phase extraction were filtered first and its

pH adjusted to 4.5 with drops of 1 M nitric acid. Results of the determined concentrations of any chlorophenols in the blank and spiked were compared.

3.3.3 Effects Humic Substances on Sampler Performance

Uptake rates experiments

To investigate the effect of humic substances on the uptake rates of both acidic and basic compounds in the MAPS, deionised water samples containing 20 mg L^{-1} of humic substances and 100 μg L^{-1} of chlorophenols mixture and that containing 10 mg L^{-1} of humic substances and 50 μg L^{-1} of triazines mixture, were used. The accumulated concentrations obtained, for both acidic and basic compounds, were compared with those found in deionised water containing 100 μg L^{-1} of chlorophenols mixture and 50 μg L^{-1} of triazines mixture only.

Degree of trapping experiments

To study whether compounds trapped in the acceptor phase can diffuse back, a series of experiments were performed for both acidic and basic compounds. The first series included spiking the 0.5 M phosphate buffer approximately 1.0 mg L^{-1} of each chlorophenols. This was filled into the hollow fibre as before. The hollow fibres were then deployed in (1) deionized water, (2) river water and (3) deionized water spiked with 20 mg L^{-1} of humic substances samples. The extraction was performed for three days. After which the contents of the acceptor solution were analysed to check for any loss of chlorophenols from the acceptor solution. As control, part of the spiked solutions was kept in the refrigerator at $4^{o}C$ during the entire period of extraction. This was analyzed at the same time as the acceptor solution from the hollow fibres.

The same study was done for basic compounds by spiking 0.5 M nitric acid with approximately 1.0 mg L^{-1} of triazine compounds and a total of 12 hollow fibres deployed in deionized water spiked with 20 mg L^{-1} of humic substances as sample only. The extraction was

performed for seven days, drawing out 3 hollow fibres at a time on day 3, 5 and 7 respectively. The contents of these acceptor solutions were analysed to check for any loss of triazines from the acceptor solution.

3.3.4 Optimization of MAPS' Extraction Parameters for Triazine Compounds

Extraction time

In order to assess the influence of exposure time on the uptake of the basic ionisable compounds in the sampler, silicone hallow fibre membranes of a given size (0.1575 cm i.d x 48 cm length) were exposed for 2, 3, 5 and 7 days. A sample volume of 3 L was used and each extraction was repeated thrice. Hollow fibres were filled with 0.5 M nitric acid and extraction was performed with deionized water, spiked with the 0.05 mg L^{-1} mixture of the triazines as sample.

Effect of stainless steal protective cover

Deionised water containing 50 µg L^{-1} of a mixture of triazines (2-ethylamino-4-isopropylamino-6-methylthio-s-triazine, atratone, atrazine, 2-methoxy-4,6-bis [isopropylamino]-s-triazine, 2.4-bis [isopropylamino]-6-methylthio-s-triazine, 2-chloro-4,6-bis [isoprpylamino]-s-triazine, simetryn, simazine, terbuthylazine, 2-t-butylamino-4-ethylamino-6-methylthio-s-triazine) was used as sample solution. Three sample vessels containing 3 L of the sample solution were each extracted using passive samplers enclosed in iron protective cover and the other three were extracted enclosed in a stainless steel protective cover (9 cm O.D x 11 cm length) with a pore size of 2 mm x 5 mm shown in Figure 3.5. The samplers were filled with 0.5 M nitric acid as acceptor solutions. The extraction was performed for 7 days.

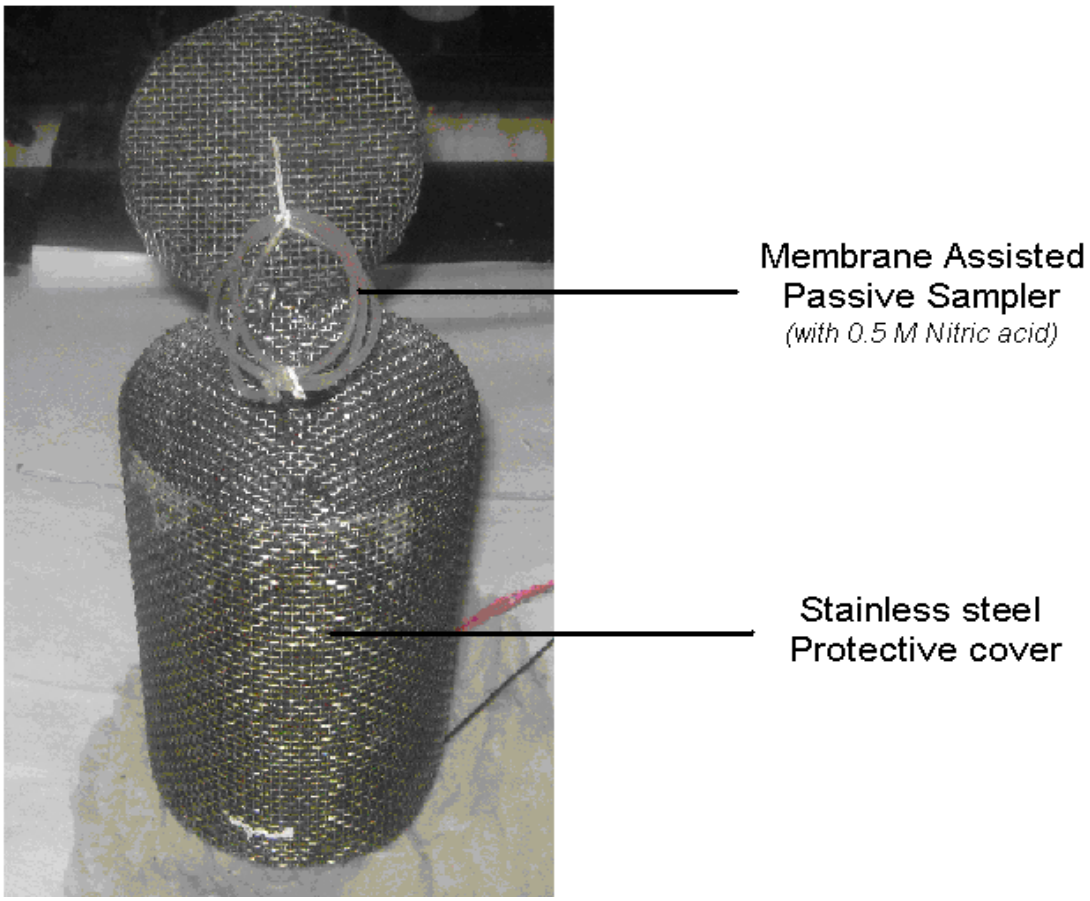


Figure 3.5 MAPS enclosed in a stainless steel protective cover (9 cm O.D x 11 cm length) with a pore size of 2 mm x 5 mm.

3.4 Field Performance of the MAPS in Comparison to the Polar Chemcatcher and Solid Phase Extraction Technique

3.4.1 Study Areas

For the evaluation of the MAPS under field conditions, two sites, the Hartebeespoort Dam and the Goudkoppies Wastewater Treatment Works were chosen. For assessing the field performance of the passive sampling device for triazines (basic compounds), the MAPS were deployed in the Hartebeespoort dam and for chlorophenols (acidic compounds), the MAPS were deployed in Goudkoppies Wastewater Treatment Works.

Hartebeespoort Dam

The Hartebeespoort Dam is situated on the Crocodile River, about 16 km southwest of the town of Brits and 37 km due east of Pretoria (SANCOLD, 1978) and in the Highveld region of northern South Africa, 250 km south of the tropic of Capricorn (Figure 3.6) (Hely-Hutchinson and Schumann, 1997). The five catchment basins of the dam are, from west to east, the Magalies/Skeerpoort, the Crocodile, the Jukskei, the Hennops and the Swartspruit basin (van Reit, 1987). The Crocodile River is the most intensive irrigation system in South Africa with numerous points and diffuse sources of domestic and industrial pollution (Heath and Claassen, 1999).

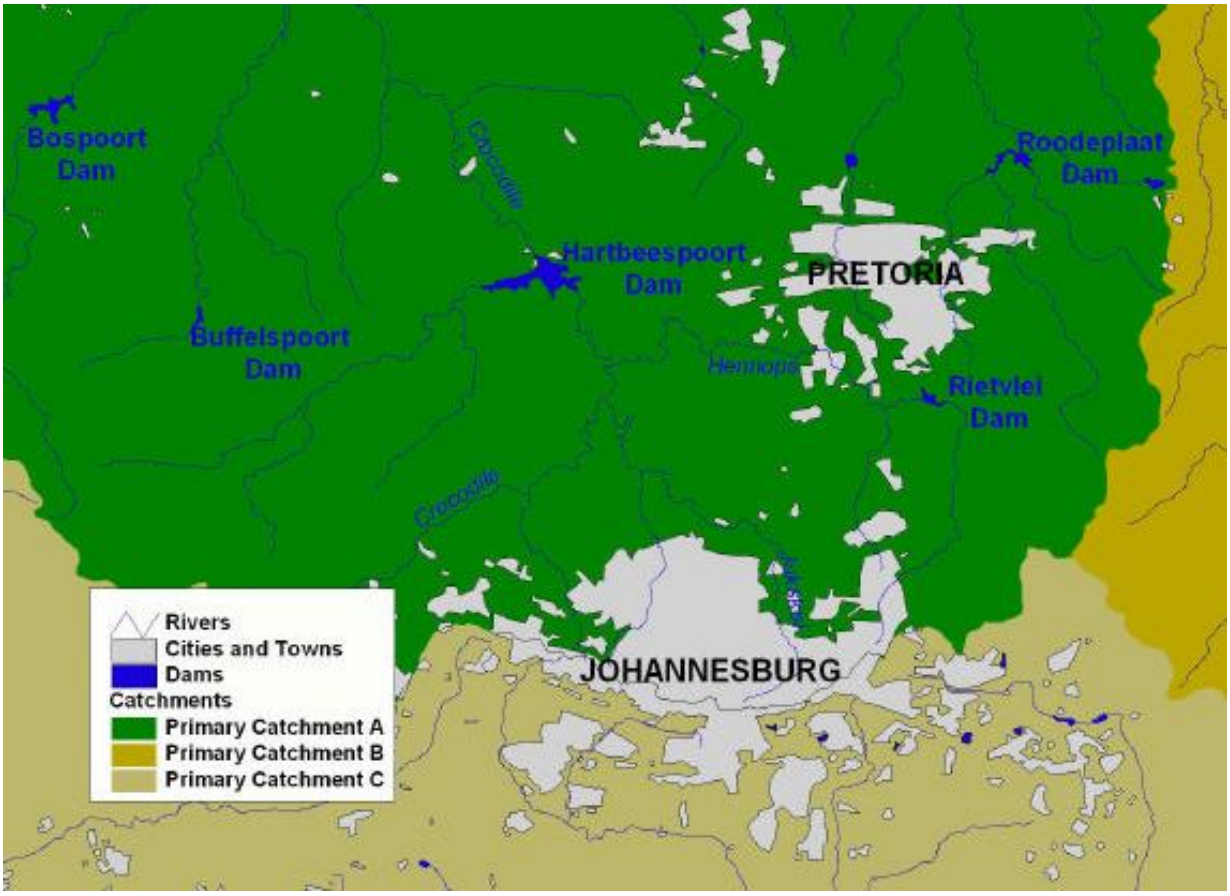


Figure 3.6 A map showing the catchment areas, rivers and urban/industrial areas of the Hartebeespoort Dam.

The Hartebeespoort Dam was built in 1923 downstream of the confluence of the Crocodile River and the Magalies River, and was raised in 1971 with 2.12 m. The dam has a total storage capacity of 185.49 x 10^6 m^3 and a catchment area of 412 km^2 (Rossouw, 1992). Rainfall is highly seasonal and occurs mainly between October and March. Land usage in the Hartebeespoort Dam catchment can be divided into two categories, namely rural and urban. The commercial, residential and industrial areas that are associated with the northern suburbs of Johannesburg and other smaller towns on the Witwatersrand make up the urban land use, while the area is used for natural reserves and agriculture (National Institute for Water Research, 1985). The rivers that flow into the Hartebeespoort Dam are carrying an ever-increasing volume of wastewater form a rapidly growing industrial and urban complex. Aucamp et al. (1987) and van Riet (1987) stated that the water of the Hartebeespoort Dam is becoming unsuitable for agriculture, development and recreation. The upper reaches of the Crocodile River drains the Johannesburg Northern suburbs and its Hennops tributary drains Kempton Park, Tembisa, Midrand and Centurion. The Magalies River drains the town of Magaliesburg and Swartspruit drains the town of Hartebeespoort (Sutton and Oliveira, 1987).
Other catchment areas include towns like Clayville, Olifantsfontein, Alexandra and a part of Atteridgeville and Saulsille (Rossouw, 1992). Due to the intense urbanization of this catchment it has the potential to decrease the water quality of the natural resources as a result of the dumping of effluents and solid-waste, mines, industrial activities, etc. There are also the sewerage treatment plants of Johannesburg, Midrand, Kempton Park, Centurion, Olifantsfontein, Randfontein, Krugersdorp and Roordepoort in the catchment area.

Industrial dumping sites include AEK (Pelindaba and Valindaba), AECI-Modderfontein and the Kelvin power station. There is also the potential contamination of storm water runoff from industrial areas like Clayville, Isando and Eastleigh as well as residential areas like Tembisa, Alexandra, Atteridgeville, etc. The biggest influence on the water quality of the Hartebeespoort Dam is from the Modderfontein stream that is upstream forming the confluence with the Jukskei River and thus the Crocodile River (Rossouw, 1992).



Figure 3.7 The Hartebeespoort Dam (A photograph taken by Nyoni H in September 2009)

Goudkoppies Wastewater Treatment Works

Goudkoppies Works, (Johannesburg Water (Pty) Limited) Devland in Marshalltown, treats both household and industrial wastewater from the City Centre and the south-eastern areas of Johannesburg before being discharged to a stream that eventually flows into Klip river. The works was commissioned in 1978 and consists of a new head of works with screening, degritting, primary sedimentation, raw sludge thickening / acid fermentation, flow balancing, activated sludge incorporating the five stage Phoredox process, final clarification, chlorination, waste sludge thickening, digestion, dewatering and solar drying of sludge. The treatment process is shown in Figure 3.8

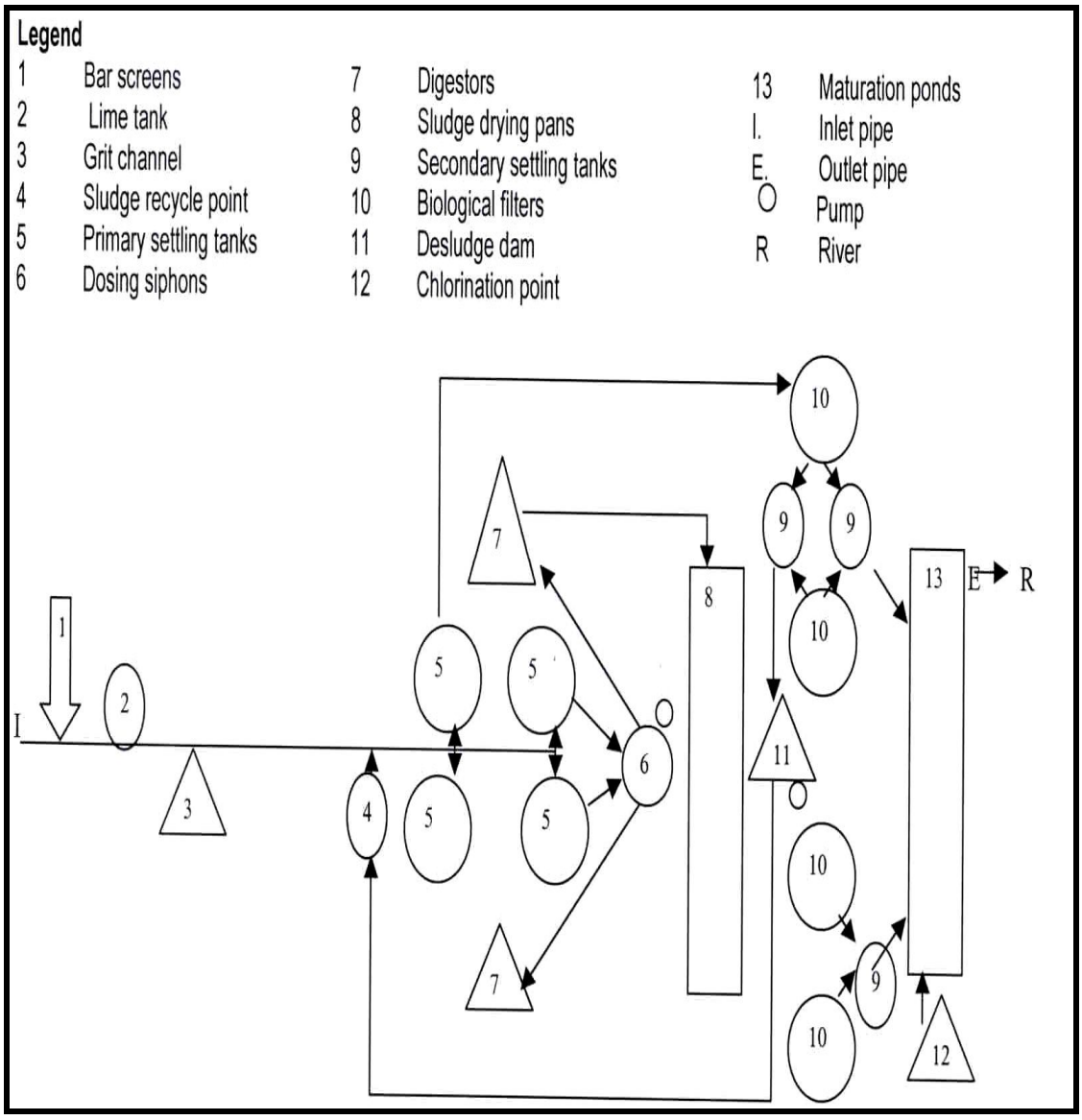


Figure 3.8 Sketch map of wastewater treatment works (GWWTW). Arrows indicate the direction of the flow of wastewater. The samplers were deployed at the secondary settling tanks (point 9) and after the chlorination point (point 12).

Waste activated sludge is thickened in dissolved air flotation units and mixed with raw, thickened sludge and thickened sludge produced at Bushkoppie Works. The mixture is anaerobically digested and thereafter dewatered on linear screen/belt press units and solar dried on drying beds. The dried sludge is ultimately disposed of on privately owned farmland.

3.4.2 Preparation, Deployment and Extraction of Analytes from Chemcatcher Passive Samplers

Preparation of the sampler

The Chemcatcher passive sampling device was used along side the MAPS in field studies for monitoring triazines. The Chemcatcher sampling device consists of a polycarbonate body containing a C_{18} Empore® disk as a receiving phase. A 40 µm thick polyethersulphone disk (47 mm diameter) of diffusion-limiting membrane is placed on the top of the receiving phase. The polycarbonate body parts supported both the receiving phase and the diffusion-limiting membrane and sealed them in place. The sampler was sealed by a cap for storage prior to use.

Before use, the C_{18} Empore® disks were conditioned by soaking in methanol HPLC-grade) obtained from Merck (Darmstadt, Germany) for 20 min until translucent and then stored in methanol until required. The conditioned disks were placed in the Chemcatchers body. A polyethersulphone (PES) membrane which was pre-cleaned for 24 h in methanol and thoroughly washed in deionised water was put on top of the C_{18} Empore® disk. Any air bubbles were removed from the space between the C_{18} Empore® disk and the PES membrane by gently pressing the top surface of the membrane using a paper tissue. The sampler was then assembled, closed using the transport lid and subsequently filled with deionised water in transport bottles until exposure to ensure that the C_{18} Empore® disk do not dry between conditioning and deployment of the device. Procedural blanks were stored non-exposed throughout the whole study period. Nine Chemcatcher devices were prepared and deployed for 7 days alongside MAPS for triazine compounds.

Deployment of the Chemcatcher passive samplers

The samplers were enclosed in stainless steel protective cages and these were dipped approximately 15 cm below the water surface. Three sites were equipped with both MAPS and Chemcatcher devices and in all these sites grab water samples were collected in order to assess the variability of the analyte uptake using SPE technique. The collected water samples were

also extracted using both passive sampling devices under laboratory. Some of the collected water samples were spiked with 50 μg L^{-1} triazine mixture and extracted with both passive sampling devices and the solid phase extraction technique. Field blanks were exposed to the air during deployment and retrieval of samplers to account for potential airborne pollution. After exposure, the passive samplers were filled with stream water from the respective site, closed and stored in dark transport bottles.

Extraction of analytes from Chemcatcher passive samplers

After exposure the sampler was carefully disassembled and the compounds were extracted from the C_{18} Empore® disk using a two-step extraction procedure with acetone. The PES membrane was carefully removed from the top of the C_{18} Empore® disk using forceps and put in a 25 mL extraction glass vial with a screw cap. The tips of the forceps were rinsed with acetone before use. The PES membrane in the vial was rinsed with 5 mL acetone to remove any target compounds on its surface.

The C_{18} Empore® disk was placed to a separate 25 mL extraction vial. The acetone from the extraction glass vial with PES membrane was transferred to the vial containing the C_{18} Empore® disk using a Pasteur pipette. The C_{18} Empore® disk was soaked in 5 mL acetone in an ultrasonic bath for 5 mins. The extract was filtered through a sodium sulphate drying cartridge to a 15 mL glass tube and the extraction repeated with 5 mL portion of acetone for 5 min in the ultrasonic bath. The second portion of the extract was filtered through the same drying cartridge as in the previous extraction step into the same glass tube. The extracts were reduced to approximately 4 mL under nitrogen.

3.4.3 Solid Phase Extraction

Triazine compounds

The collected water samples from the Hartebeespoort Dam were used as sample solutions. The sample solutions were divided into two parts. One part was spiked with 50 μg L^{-1} triazine

mixture and extracted with the solid phase extraction technique and the other part was extracted unspiked. The SPE column used was a C_{18} (Supelco, Park Bellefonte, USA) packed with 500 mg of the sorbent in a 6 mL polypropylene syringe barrel. Percolation of all solutions and solvents through the column was carried out using a vacuum manifold. For preconditioning 3 mL acetonitrile was used, followed by rinsing with 3 mL of deionized water for equilibration. Sample solutions were percolated at about 15 mL min^{-1}. The column was then flushed with 3 mL of water. For elution of the sample, 3 mL (2 x 1.5 mL) acetonitrile was used. The extracts were introduced into the separation system without any further treatment and results of the determined concentrations from the SPE were compared to those found in MAPS and Chemcatcher samplers of the same water samples extracted under laboratory conditions.

Chlorophenol compounds

Deionised water containing 50 µg L^{-1} of a mixture of 2-chlorophenol, 4-chlorophenol and 2,4-chlorophenol and wastewater samples collected from the settling tanks after chlorination point of Goudkoppies Waste Water Treatment Works (GWWTW) spiked with 50 ug L^{-1} of similar compounds were used as sample solutions. Similar C_{18} solid phase extraction cartridges packed with 500 mg sorbent was used (Supelco, Park Bellefonte, USA) and percolation of sample solutions and solvents through the column was also carried out using a vacuum manifold. For preconditioning 6 mL of methanol was used followed by equilibrating with 6 mL of deionized water. Water samples (500 mL) adjusted to pH 4.5 with 0.5M nitric acid was passed at flow rate of 5 mL min^{-1}. The cartridge was washed with 3 mL methanol-water (5:95 v/v) and eluted was with 2 x 2 mL methanol. The extracts were introduced into the separation system without any further treatment and results of the determined concentrations from the SPE were compared to those found in MAPS of the same water samples extracted under laboratory conditions.

3.4.4 Field Deployment of MAPS Devices

Chlorophenols

To demonstrate the potential of MAPS for monitoring chlorophenols, the passive sampling device was applied at Goudkoppies wastewater treatment works (GWWTW). The field parameters such as pH and conductivity were measured before deployment. Passive samplers were deployed in the settling tanks and also after chlorination. Three parallel passive samplers were deployed at each site. The passive samplers in the settling tanks were tied to a beam rotating at 50 revolutions per hour. Wastewater samples were also collected by grab samples from the two sites. The collected wastewater samples were also extracted using both the passive sampler under laboratory conditions and with the solid phase extraction technique. Some of the wastewater was spiked with 50 μg L^{-1} chlorophenols and extracted with both the passive sampler and the solid phase extraction technique.

Triazines

The MAPS devices enclosed in stainless steel protective cages were dipped approximately 15 cm below the water surface. The MAPS devices for monitoring triazines were deployed along side Chemcatcher devices and in all these sites grab water samples were collected in order to assess the variability of the analyte uptake using SPE technique. The collected water samples were also extracted using both passive sampling devices under laboratory. Some of the collected water samples was spiked with 50 μg L^{-1} triazine mixture and extracted with both passive sampling devices and the solid phase extraction technique. Field blanks were exposed to the air during deployment and retrieval of samplers to account for potential airborne pollution. After exposure, the passive samplers were filled with stream water from the respective site, closed and stored in dark transport bottles.

~ Chapter Four– Results and Discussion~

4.1 Introduction

In this chapter, experimental results are presented and discussed. Results on characterization of the effect of variable environmental conditions on the MAPS performance for monitoring of triazines and phenols in water bodies are presented. Field study results to test the sampler performance alongside spot sampling (SPE) and commercially available passive sampler (Chemcatcher) are also presented and discussed.

4.2 Effects of Variable Environmental Conditions on MAPS' Performance

4.2.1 Effect of Temperature

The relationship between sampling rates of three chlorophenolic compounds and temperature was compared at three temperatures (4, 16 and 40 °C). In general, the sampling rate increased with the increasing exposure temperature. The typical dependence of sampling rate on temperature is shown in Figure 4.1.

The influence of temperature on mass transfer in the MAPS is similar to the one developed for the SLM extraction technique (Chimuka et al., 1999; Michel et al., 2009; Chimuka et al., 2009). Theoretically, the influence of temperature can be summarised by equation 23.

$$D = \frac{K_{\beta}T}{6\pi\alpha\eta} \qquad (23)$$

where D is the diffusion coefficient of the analyte η is the viscosity of solvent, K_{β} is the Boltzmann distribution coefficient, T is the temperature in Kelvins, α is the radium of the molecule.

Since diffusion coefficient (D) is directly proportional to temperature, the latter influence on mass transfer is supposed to be obvious. However, in real application of the supported liquid membrane extraction, the influence of temperature on the mass transfer was not as straight forward as equation 20 suggests. Other factors such as the configuration of the module (Michel et al., 2009; Chimuka et al., 2009), type of mass transfer controlling the extraction process (Chimuka et al., 2009), whether the sample is stirred or not have been observed to play a role too (Michel et al., 2009). In a study of the influence of temperature on mass transfer in a flat sheet module with triazole fungicides as model compounds (Chimuka et al., 2009), it was observed that the diffusion coefficient increased with increase in temperature but with both donor and acceptor phases flowing. However, when the experiments were performed under same different temperatures and with a stagnant acceptor phase no noticeable increase in the extraction efficiency. The extraction process was also controlled by stripping of the analyte from the membrane into the bulk of the acceptor solution (Chimuka et al., 2009). The same study was also performed with a hollow fibre module (Michel et al., 2009). The results obtained in this case indicated that diffusion coefficient increased with temperature (Michel et al., 2009) and that the amount accumulated in the acceptor phase did also increase with temperature (Michel et al., 2009). These two studies clearly explain the influence of the module and experimental design on how temperature affects the mass transfer in a membrane extraction process.

Vrana et al. (2005) demonstrated that for the four polycyclic aromatic hydrocarbons with Log K_{OW} range from 4.0 to 5.1, the apparent receiving phase-water distribution coefficient K_{DW} was not significantly affected by temperature within the range from 6 to 18 °C. Thus, the temperature was expected to affect mainly the magnitude of the kinetic component of the sampling rate (k_e which is referred to as the overall exchange rate constant; equation 24).

$$K_{DW} = \frac{R_S}{k_e V_D} \qquad (24)$$

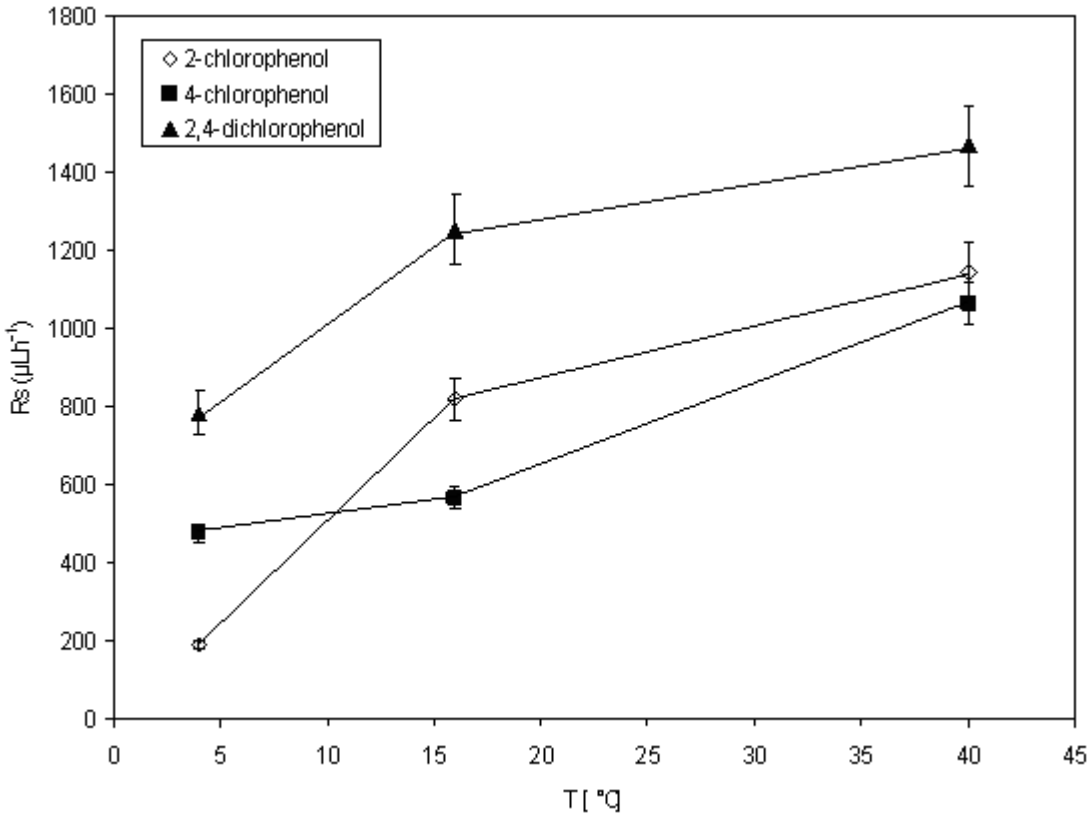


Figure 4.1 Effect of temperature on the analyte sampling rates R_S. The data represents nine exposures, of three chlorophenolic compounds, performed at various temperatures (4, 16 and 40ºC) under static conditions.

Typically, it is quite clear that increased temperature of the environmental media can enhance mass transfer of compounds in all media. Thus for the three chlorophenols namely 2-chlorophenol, 4-chlorophenol and 2, 4-dichlorophenol with Log K_{OW} 2.18, 2.39 and 2.96 respectively, the temperature dependence of the sampling rate R_S can then be described by the Arrhenius-type equation:

$$\ln R_S = \ln A - \frac{\Delta E_a}{RT} \qquad (25)$$

where R is the universal gas constant (kJ mol^{-1} K^{-1}), A is the pre-exponential factor expressing the maximum sampling rate at infinite temperature, T is the absolute temperature (K) and ΔE_a is the activation energy (kJ mol^{-1}).

Values of ΔE_a were obtained by plotting the natural logarithm of R_S against the reciprocal value of absolute temperature ($1/T$) (Figure 4.2). The intercept gives the value of ln A. The activation energy ΔE_a can be calculated by multiplying the slope of the regression line ($\Delta E_a/R$) by R.

Table 4.1 Summary of the calibration data used to determine the sampling parameters and observe how they are affected by temperature.

	2-Chlorophenol			**4- Chlorophenol**			**2,4- Chlorophenol**		
T [º C]	**4**	**16**	**40**	**4**	**16**	**40**	**4**	**16**	**40**
***t* (h)**	0-72	0-72	0-72	0-72	0-72	0-72	0-72	0-72	0-72
V_A [µL]	1319	1319	1319	1319	1319	1319	1319	1319	1319
M_O [µg]	0	0	0	0	0	0	0	0	0
C_W [µgL^{-1}]	100	100	100	100	100	100	100	100	100
E_e	17	72	101	42	50	93	69	110	129
***R*s [µL h^{-1}]**	304±1	1319±9	1844±17	764±2	910±4	1711±7	1260±5	2016±15	2362±17
Mean ΔE_a [kJ mol^{-1}]		14			17			12	

The calculation of the activation energy ΔE_a using Eq. (25) was performed on three sets of calibration data, obtained at stagnant water. The activation energies range between 12 and 17 kJ mol^{-1}. The average of all ΔE_a values was 14 kJ mol^{-1}. For a comparison, Vrana et al. (2005) calculated for Chemcatcher average activation energy of 93 kJ mol^{-1}. Thus, the effect of temperature on the MAPS uptake kinetics appears to be less significant than that on Chemcatcher sampling rates.

4.2.2 Effect of Hydrodynamics on Sampler's Uptake Kinetics of Triazines

The sampling rates obtained for individual triazine compounds under stirred and unstirred conditions were compared. A significant increase of sampling rates with change of hydrodynamic conditions from static to turbulent was observed for all compounds under investigation (Figure 4.3). It should be noted that influence of hydrodynamics on the MAPS uptake kinetics was studied previously on acidic compounds (Chimuka et al., 2008). Using the

data obtained previously, sampling rates were calculated and compared under static and under stirred conditions.

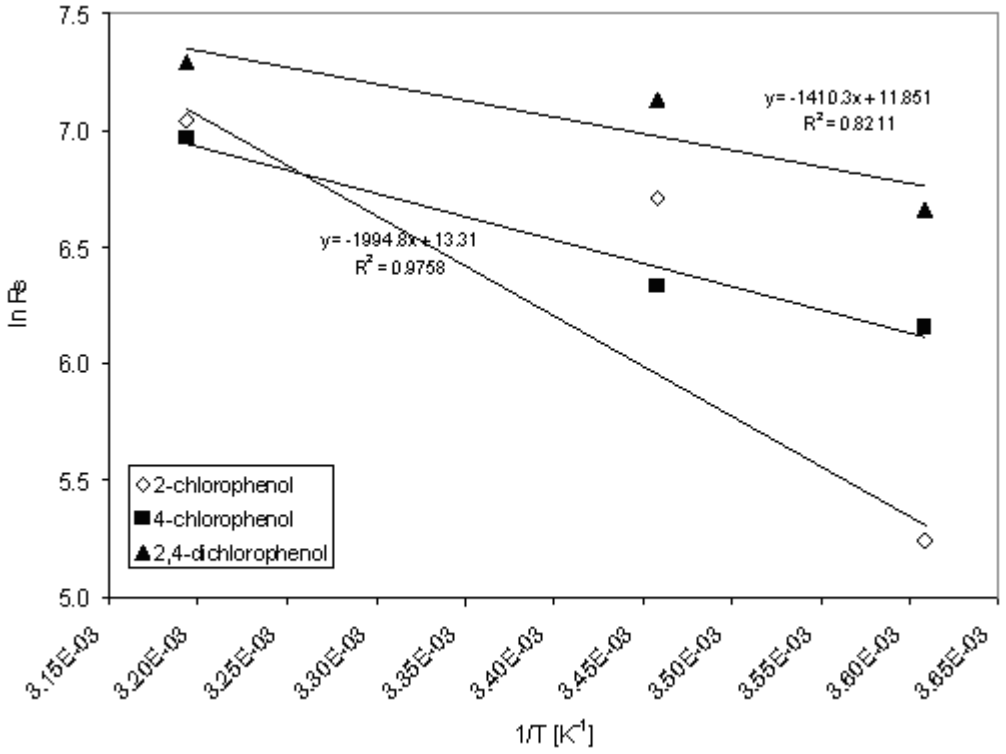


Figure 4.2 A plot of the natural logarithm of R_S against the reciprocal value of absolute temperature ($1/T$). The intercept gives the value of ln A. The activation energy ΔE_a are calculated by multiplying the slope of the regression line ($\Delta E_a/R$) by R.

The results obtained (Figure 4.4) show a significant increase of sampling rates with change of hydrodynamic conditions from static to turbulent. The influence was more on 2,4-dichlorophenol. For 2-chlorophenol with low mass transfer, hydrodynamics had little influence because of incomplete trapping of this compound in the acceptor phase. However, at low turbulence, the diffusion through the aqueous layer limits the mass transfer for more hydrophobic compounds, resulting in higher sampling rates at high turbulence. This trend is consistant for triazine (Figure 4.3). The influence of hydrodynamics on mass transfer in the MAPS is similar to the one observed for the SLM extraction technique (Chimuka et al., 1999; Michel et al., 2009; Chimuka et al., 2009). The extent to which the mass transfer process is

influenced by turbulence is dependent on the polarity of the compound and trapping in the acceptor phase.

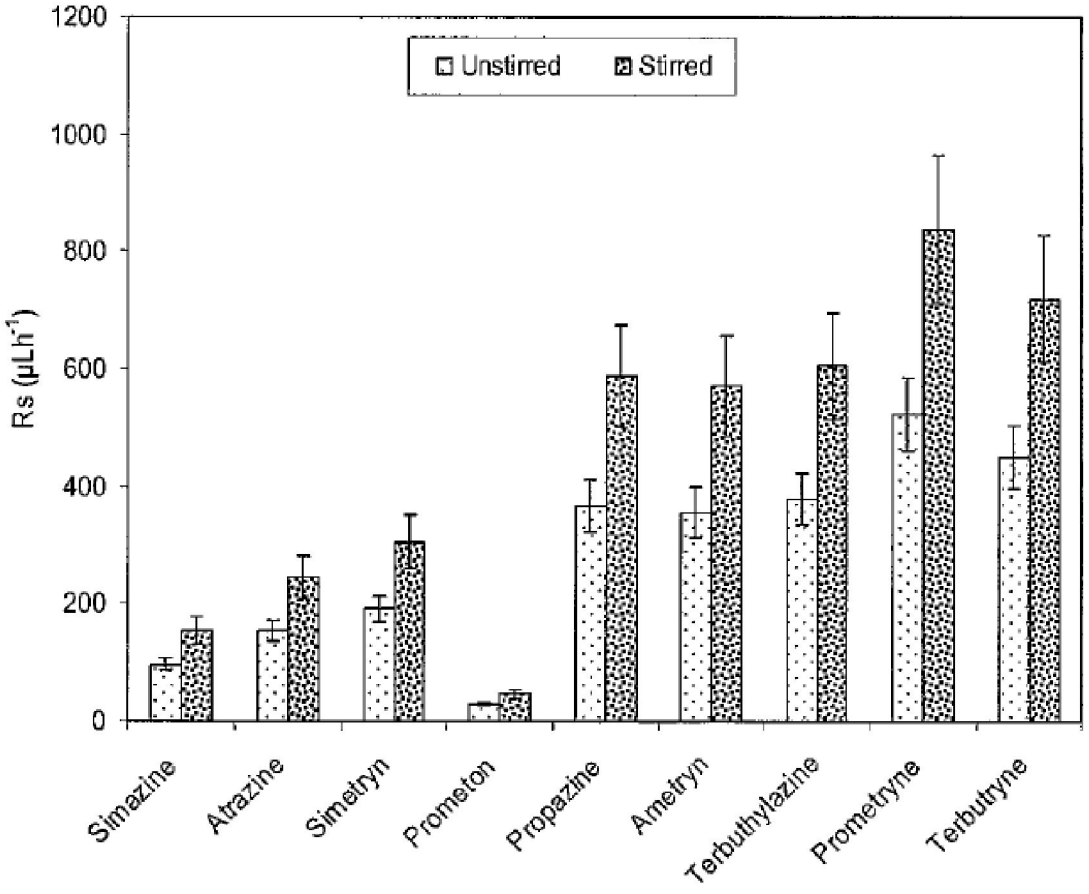


Figure 4.3 Effect of hydrodynamics on the analyte sampling rate values. Data are presented from the exposure experiments conducted at 16 ºC and stirred and unstirred conditions.

For the relatively hydrophobic compound, an increase in turbulence is accompanied by an increase in the sampling rate (Figure 4.3 and Figure 4.4). For most polar compounds, however, not much gain in sampling rate results from an increase in turbulance. The variation of the enrichment factor with turbulence for compounds with varying polarity where the sample is pumped through has been discussed also by Jönsson et al. (1993) in the theoretical treatment of SLM technique. For extraction which is limited by the diffusion of the analyte from the bulk of the donor solution to the membrane surface (donor-controlled extraction), much better concentration enrichment factors are obtained at higher turbulence. This is the case for the more hydrophobic compounds with Log *P* greater than 2, i.e. moderately polar to non-polar

compounds. On the other hand, for the polar compounds with Log *P* less than 2, low dissolution into the membrane limits the mass transfer (membrane controlled extraction). In that case a high turbulence does not result in much gain in sampling rates since the extraction efficiency falls drastically (see equation 26) (Jönsson et al., 1993)

$$E_e = \frac{EV_D}{V_A} = \frac{EF_D t}{V_A} \qquad (26)$$

where F_D is the sample turbulence and t is the extraction time. From equation 26, it is seen that where a sample is pumped through, an increase in donor flow rate increases E_e but E reduces.

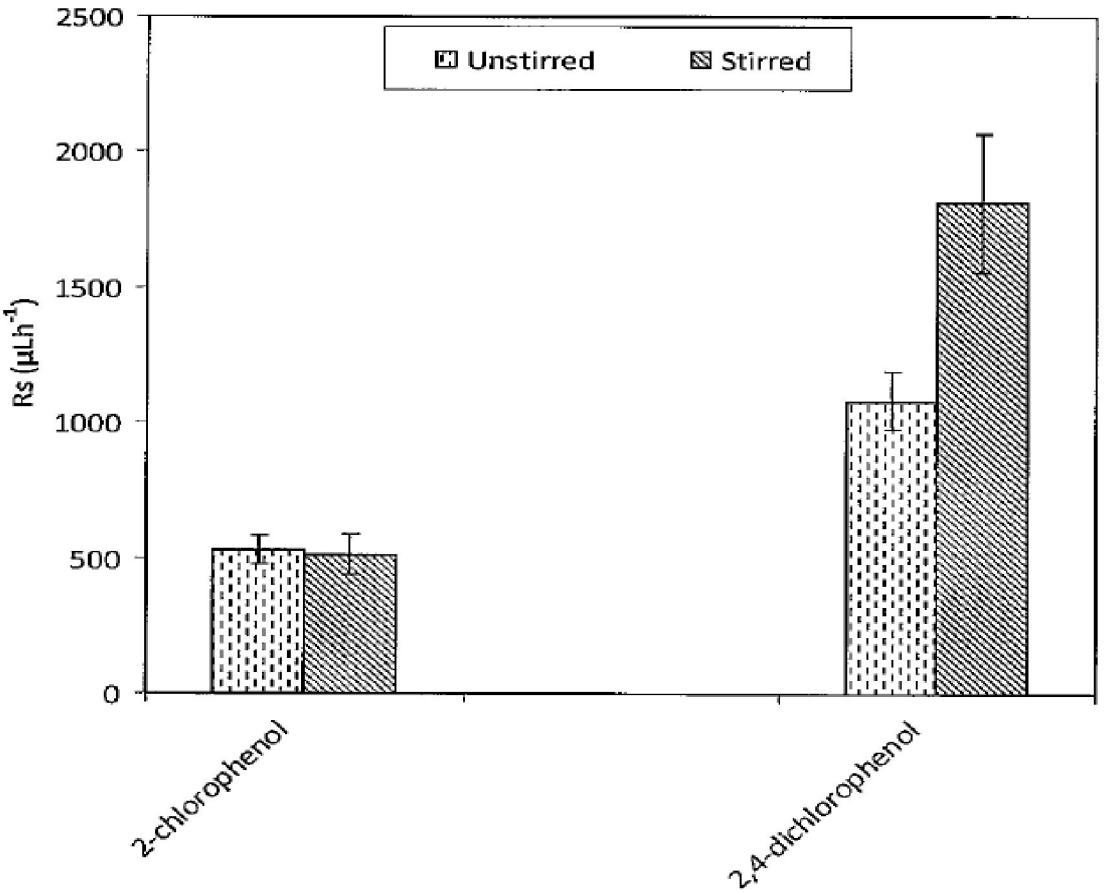


Figure 4.4 Comparison of sampling rates under stirred and unstirred conditions in river water spiked with 10 µgL^{-1} mixtures of chlorophenols and with extraction time of 72 h.

For hydrophobic compounds, *E* does not reduce as much as for more polar compounds whose mass transfer is controlled by dissolution into the membrane (Megersa et al., 2001). In a situation where the sample is stirred as in most applications of passive sampling technique, amount extracted increases with stirring speed both for compounds with low and high Log *P* values. This is because in both cases, the contact time between the sample analytes and the membrane increases. However, at too high a stirring speed, the mass transfer is expected to be limited by the dissolution into the membrane. This is supposed to be more pronounced for compounds with low Log P values. Liu et al. (2007) compared the effect of agitation in a HF-SLM extraction of phenoxy acid herbicides. Static extraction was compared with 100 rpm shaking rate. 90 % extraction efficiency was reached in 8 h under static while this was reduced to 4 h at 100 rpm shaking rate (Liu et al., 2007). The phenoxy acid herbicides studied have Log P values ranging between 2.5 and 2.95 (Chimuka et al., 2000). Zhu et al. (2001) is reported to have varied the stirring speed in the extraction of nitrophenols in a HF-LPME technique. For 3,4-dinitrophenol as a typical example, the extraction efficiency increased with stirring speed until after 1200 rpm where a plateau was reached (Zhu et al., 2001). The log P value of 3,4-dinitrophenol is about 2.17 which is a moderately polar compound. This corresponds well with the theory of diffusion through two films in series (Scheuplein, 1968; Flynn and Yalkowsky, 1972), which predicts a switch in the overall mass transfer to the aqueous phase control for hydrophobic compounds. A similar effect of hydrodynamics has been observed and explained also for Chemcatcher (Vrana et al., 2006a).

4.2.3 Effect of Biofouling Layer

The uptake rates of contaminants in the MAPS in deionised water were compared with those found in river water and wastewater samples collected regularly during the exposure period. The resulting dataset shows that biofouling of the sampler membrane can have an effect on the value of *R*s and this can be difficult to quantify since the development of a biofilm can vary widely at one site at different times of the year and between sites depending on the diversity of fouling organisms present, and the rate of settlement and growth.

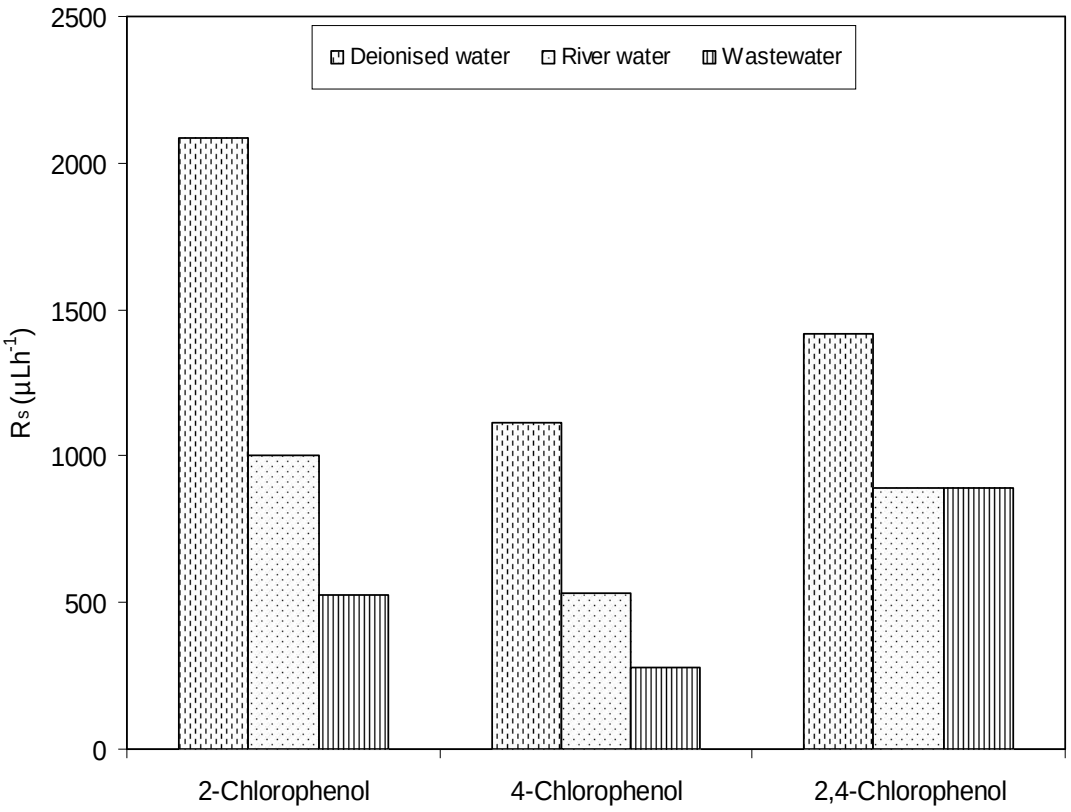


Figure 4.5 Comparison of the MAPS' uptake rates of chlorophenols compounds in deionized water to those found in river water and wastewater samples.

The results (Figure 4.5) show a strong dependency of the sampling rates reductions with sample matrix, with the larger reductions occurring in wastewater samples. The reason for the deterioration of the uptake kinetics on the sampler deployed in river water and wastewater samples could be heavy biofouling observed in all the samplers deployed in those samples (Figure 4.6). This corresponds well with the theory of the effect of biofilm on the concentration profile explained for SPMD devices (Alvarez, 2007) which predicts a deterioration of the contaminant exchange by increased resistance to mass transfer. Figure 4.7 illustrates the basic steps involved in contaminant uptake by the passive samplers. Coming from the surrounding waters, the analytes first have to enter the protective cage where the motion of the water may be reduced relative to the water outside the cage.

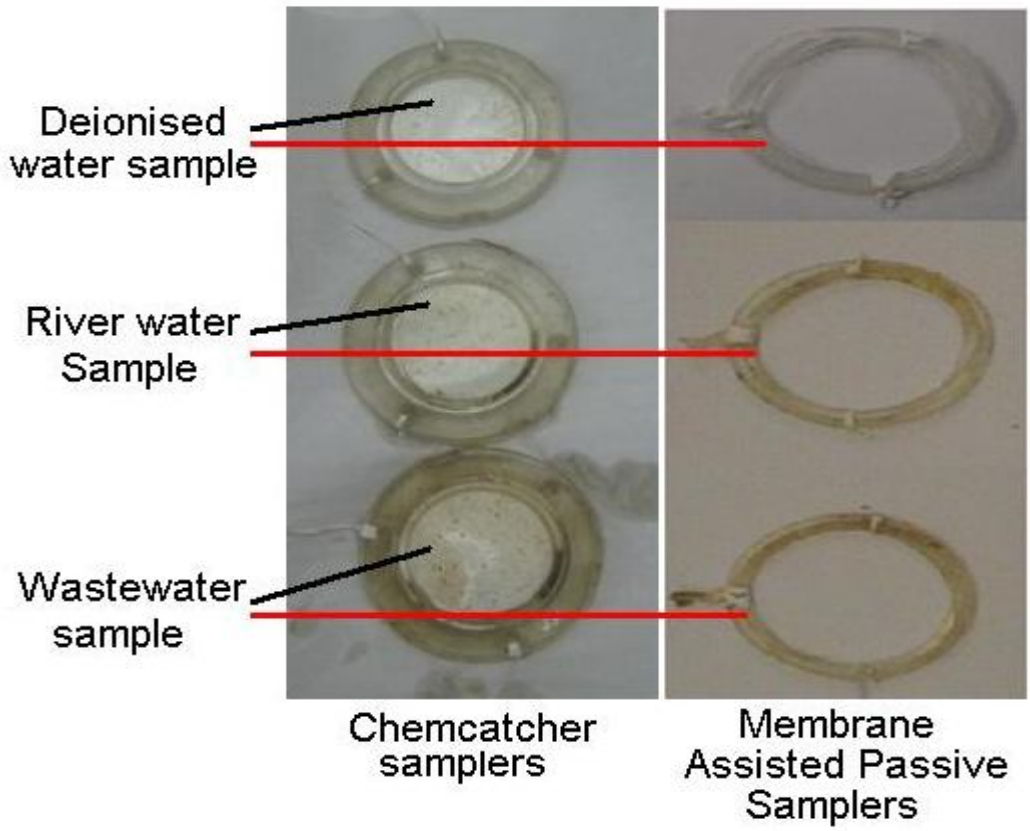


Figure 4.6 Photographs of MAPS and Chemcatcher samplers after 7 days of deployment in deionized water, river water and wastewater samples.

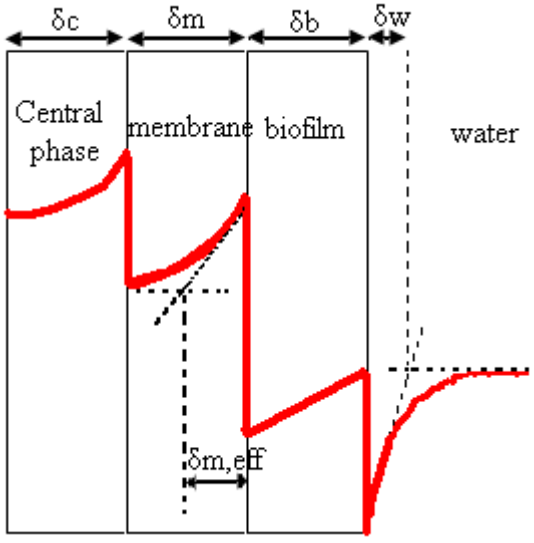


Figure 4.7 Schematic representation of concentration profile in dual-phase PAS with exterior biofilm (i.e. the right half of a symmetrical sampler, or the whole cross section of a sampler with an impermeable boundary located to the left of the central phase). Dashed lines indicate how the effective thickness of the respective phase may be estimated.

Close to the biofouling layer, convective transport of the analyte molecules is reduced more and more, until all transport takes place by molecular diffusion within the water boundary layer (WBL). When ventilating organisms are present, diffusion may be amended with convective currents that are set by organisms. After diffusion through the membrane, analytes are finally sorbed by the central sorption phase. Vrana et al. (2006a) used Confocal Laser Microscopy to obtain semi-quantitative measure (film thickness of the biofilm layer) in polar and non-polar Chemcatcher (Figure 4.8) devices to observe this concentration profile. The biofilm layer is thought to be increased by growth of bacterial mats periphyton and even macrofauna (Flemming et al., 1997).

Richardson et al. (2002) observed that the amounts of organochlorine pesticides and PAHs, absorbed by SPMDs for which the membrane had been absorbed for 1-4 weeks were about 30-40 % lower than the amounts absorbed by unfouled SPMDs. These reductions were higher for OCPs than for PAHs, but did not appear to be related to Log K_{ow}. Similar reductions of phenanthrene uptake by pre-fouled SPMDs (26-39 %) were reported by Ellis et al. (1995). Huckins et al. (2006; 2002; 1997) reported that sampling rates of PAHs by prefouled SPMDs were smaller than for unfoulded SPMDs by 30-70 %. These authors reported weak dependency of the sampling rate reduction with hydrophobicity, with the larger reductions occurring at the higher Log K_{ow} end. Assuming that the biofouling layer can be modelled as a water layer with dispersed organic matter (i.e. similar to a layer of sediments), its conductivity for mass transport is given as (Huckins et al., 2006):

$$k_b K_{bw} = \frac{Ø^2 D_w}{\theta \delta_b} \qquad (27)$$

where $Ø$ is the porosity and θ the tortuosity of the diffusion pathways with the biofilm (i.e. the ratio of the actual diffusion path length and the thickness of the biofilm). Since both $Ø$ and θ are of the order 1, equation 27 states that the biofilm behaves essentially like an immobilized water layer, with a conductivity that is independent of the biofilm-water partition coefficient. As an example, we will apply this model to estimate the thickness of a biofouling layer causes a reduction in the sampling rate of 460 cm^2 SPMDs from 5 to 2.5 L day^{-1}.

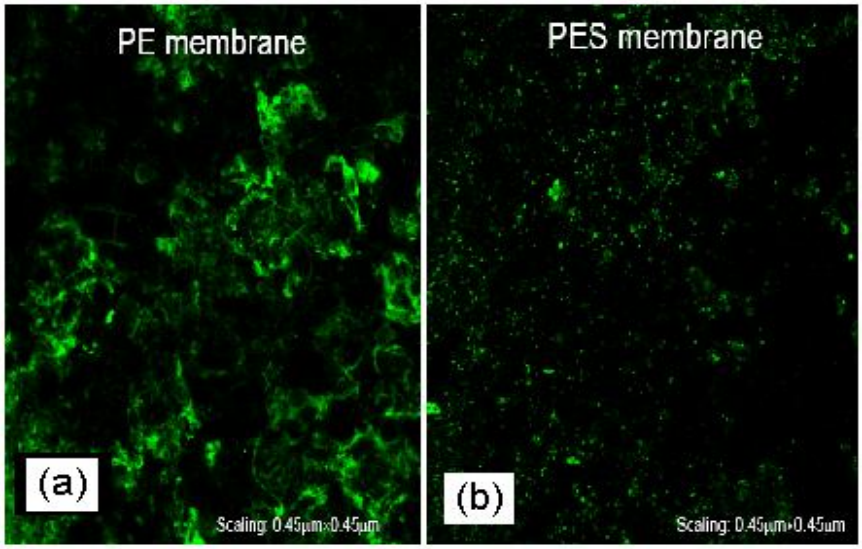


Figure 4.8 A 3D-view of biofouling investigations using confocal laser scanning microscopy of (a) non-polar Chemcatcher PE membrane and (b) polar Chemcatcher PES membrane.

Adopting a porosity of 0.9, a tortuosity of 2 and a D_w value of 5 x 10^{-10} m^2s^{-1}, a biofilm thickness of 160 µm can be calculated, which seems to be a reasonable value. It has been suggested by several authors that the use of PRCs allows to quantify the effect of biofouling on the in situ uptake rates (Huckins et al., 2002; Richardson et al., 2002), but to date the experimental evidence has not yet been presented in the peer-reviewed literature.

4.3 Effects of Humic Substances on Sampler's Performance

4.3.1 Uptake Rates Experiments

A negative influence of humic substances on the MAPS' performance was observed when accumulated concentrations obtained for triazine compounds found in deionised water containing 50 µg L^{-1} of triazines mixture only were compared to those found in deionised water containing 10 mg L^{-1} of humic substances and 50 µg L^{-1} of triazines mixture (Figure 4.9a).

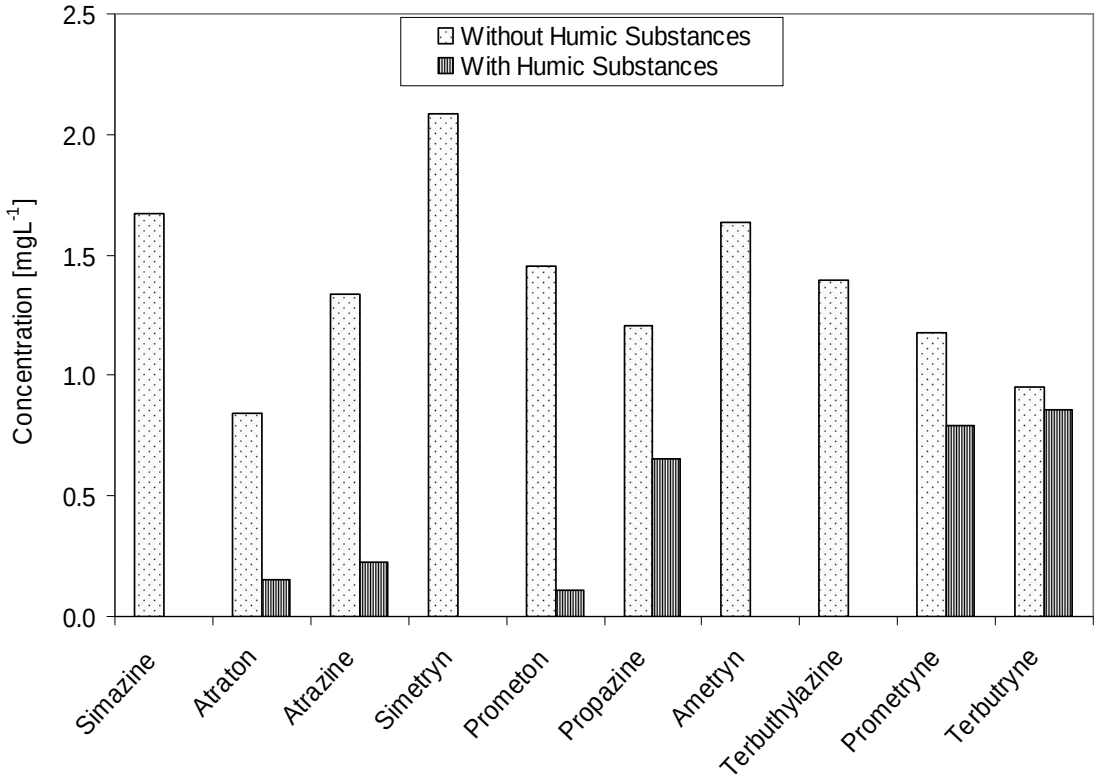


Figure 4.9a Influence of sample matrix (humic substances) on the MAPS' performance for triazines (basic compounds).

A similar effect of humic substances has been observed and explained for triazines by Bjarnason et al., (1999). Humic substances made it difficult to determine low concentrations of triazines, using chromatography and ELISA assays without extensive sample pre-treatment (Baum and Robertshaw, 1995) because of a large amount of interfering compounds. C_{18}-SPE, and C_{18}-SPE in combination with antitriazine molecularly imprinted polymers (MIP) were compared and the results showed triazines are selectively extracted from the highly contaminated sample containing 20 mg L^{-1} humic substances. When C_{18}-SPE column is used, without the combination of the MIP, large amounts of interfering compounds are detected in the chromatogram, making the determination process difficult, if not impossible, as the simazine and propazine peaks are completely hidden by the matrix. Humic substances could be forming some complexes whether weak or strong with some triazines and thus preventing or reduce the dissolution of these basic compounds into the hollow fibre.

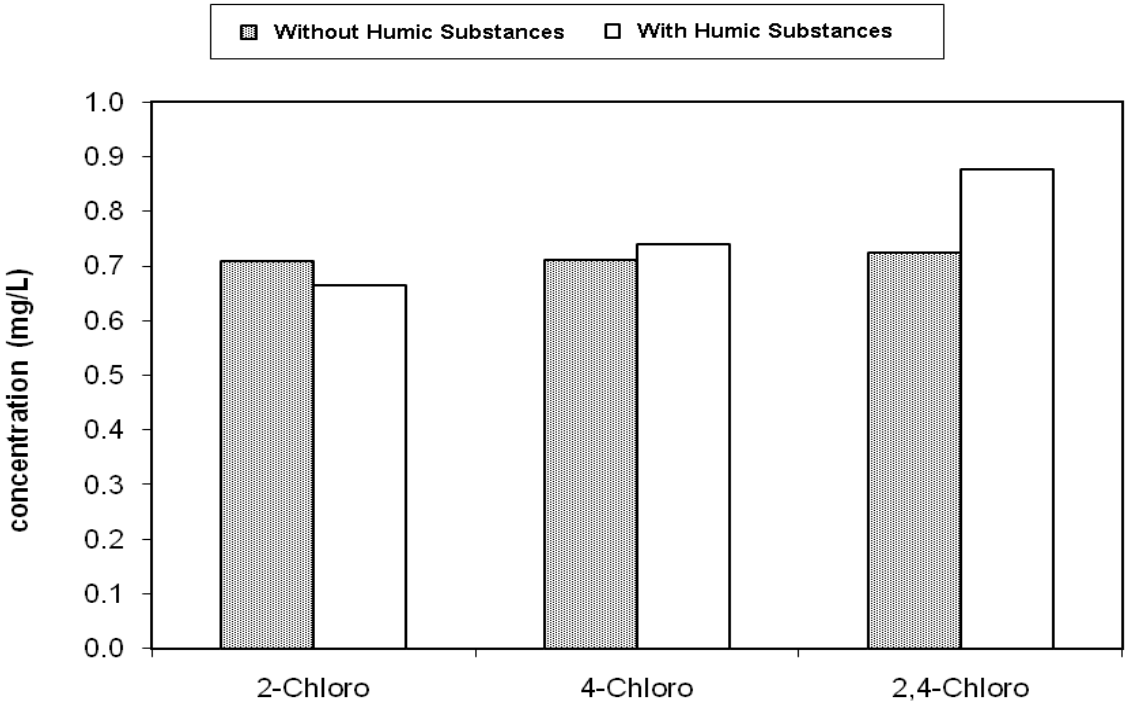


Figure 4.9b Influence of sample matrix (humic substances) on the MAPS' performance for Chlorophenols (acidic compounds)

On the contrary, there was no significant difference noted for acidic compounds under investigation (Figure 4.9b). The concentration accumulated in the acceptor solution of the passive samplers from deionised water samples containing 20 mg L^{-1} humic substances and 100 μg L^{-1} of chlorophenols mixture was similar to that found in deionised water containing 100 μg L^{-1} of chlorophenols mixture. This means that the humic substances in the sampled water do not interfere with the extraction process of the chlorophenols.

4.3.2 Degree of Trapping Experiments

Chlorophenols (acidic compounds)

The degree of trapping or ionisation of the target compounds in the acceptor phase is very important for quantification purposes of the sampler. This also can affect the exposure time. If all analytes in the acceptor phase are completely trapped, there is a linear relationship between

extraction time and amount accumulated in the acceptor phase. A similar linear relationship also exists between the extracted concentration and amount accumulated in the acceptor phase. From theory (Chimuka et al., 1998; Jönsson and Mathiasson, 1999; Jönsson et al., 1993), once the pK_a of the compound is known, it possible to decide the pH of the acceptor solution at which all compounds will be almost or completely trapped. Co-extraction of other matrix compounds especially if these are in high concentration can lead to the acceptor solution reducing its pH value. This is due to neutralisation reactions of the acceptor solution with matrix components.

Figure 4.10 shows the concentration of chlorophenols determined in the acceptor solution of the hollow fibre after exposure to un-spiked deionized water (Acceptor solution 1) and river water (acceptor solution 2). The acceptor solution was spiked with ~ 1.0 mg L^{-1} before being filled in the hollow fibre in each case. The control was the spiked buffer kept in the refrigerator during passive sampler exposure period. The results in Figure 4.10 generally indicate that there was no change in the spiked concentrations of the acceptor solution with time.

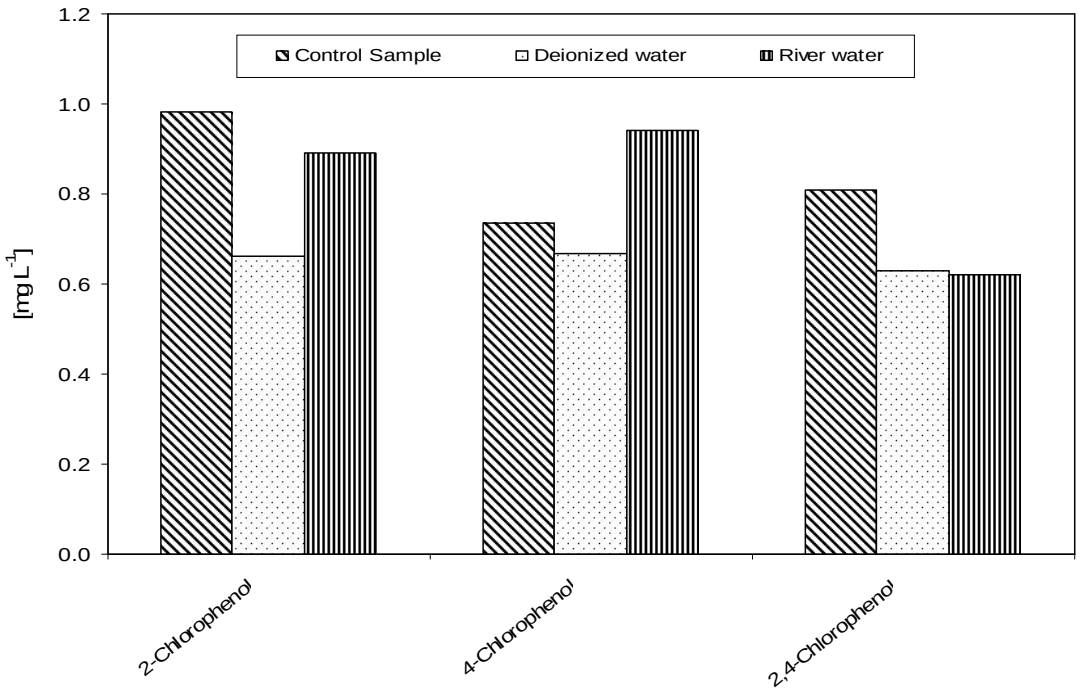


Figure 4.10 Concentrations in the acceptor solutions of the hollow fibres spiked with 1.0 mg L^{-1} after 3 days exposure in deionised water and river water in comparison to a control buffer solution (kept in the refrigerator).

The same results were obtained from deionized water spiked with 20 mg L^{-1} with humic substances (Figure 4.11). The differences seen could be experimental error emanating from pH adjustment before HPLC analysis. This means that compounds were almost completely trapped in the acceptor solution and could not diffuse back and that matrix component in river water or humic substances had no effect on the trapping capacity of the buffer solution.

Triazines (basic compounds)

A similar effect was observed with basic compounds (Figure 4.12). There was no significant change in concentrations for triazine compounds spiked in the acceptor solution with an increase in exposure time. This means that the humic substances in deionised water do not have an effect in the concentration of triazine compounds trapped in the nitric acid acceptor solution. Once these basic compounds are trapped in the acceptor solution they do not diffuse back during the 7 day deployment period.

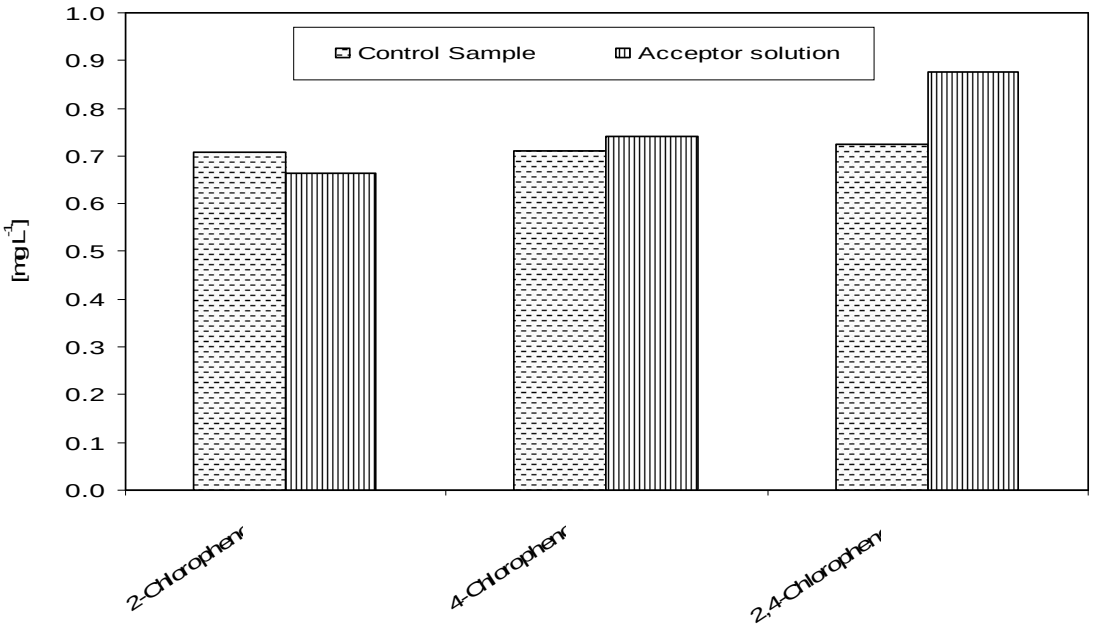


Figure 4.11 Determined concentrations in the acceptor solutions of the hollow fibres previously spiked with about ~ 1.0 mg L^{-1} after passive extraction of deionized water (acceptor solution) spiked with 20 mg L^{-1} humic substances and control buffer solution (kept in the refrigerator).

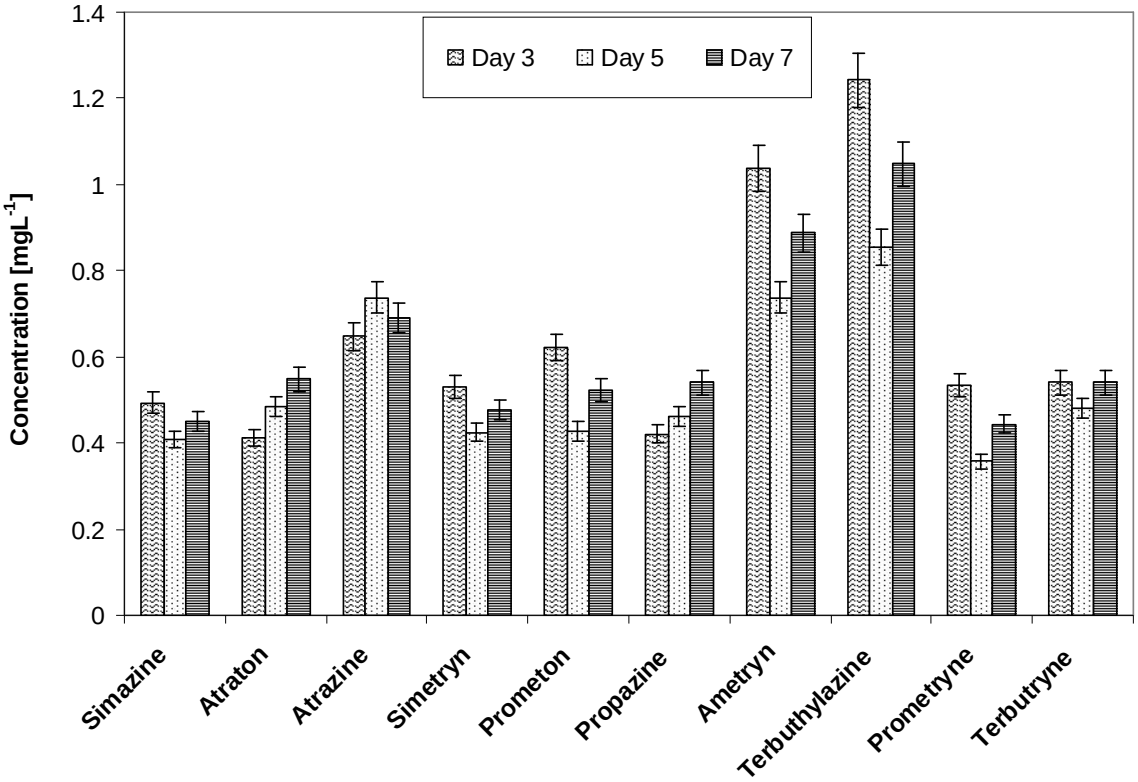


Figure 4.12 Concentration of triazine compounds trapped in the nitric acid acceptor solution during a 7 day exposure period.

4.4 Optimization of MAPS' Extraction Parameters for Triazine Compounds

4.4.1 Extraction Time

The time courses of the amounts of individual test substances in the MAPS are shown in Figure 4.13, Figure 4.14 and Figure 4.15. The chemical uptake of simazine, simetryn, ametryn and terbuthylazine into the passive sampler remains linear and integrative through out the 7 day exposure period. This means that the extraction can go on until all the analytes in the sample are extracted. Such linear relationships have been observed in other passive samplers working in the kinetic regime, such as the Chemcatcher (Kingston et al., 2000) and MESCO (Vrana et al., 2006b) as part of their performance optimization. Prometon and atratone included in the test series could not be detected. A clear relationship between E_e and time in days for atrazine, propazine, prometryne and terbutryne could not be recognised. The amounts

quantified in the MAPS had relative standard deviations mostly between 10 and 20 % (from repeat determinations) and did in no case exceed 30 %. Table 4.2 summarizes the slopes of linear uptake together with correlation coefficients for the MAPS. In comparison with acidic compounds (Chimuka et al., 2008), the slopes for basic compounds are smaller and show delayed effect and suitability for long term exposures.

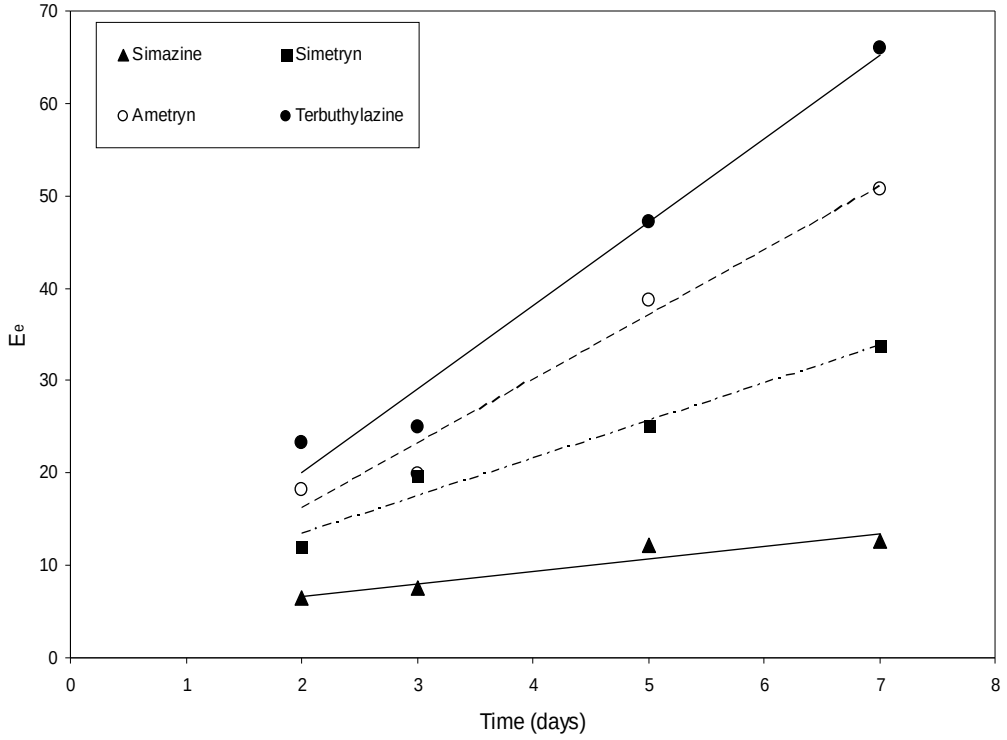


Figure 4.13 Uptake of selected triazine compounds by the MAPS where the receiving phase was 0.5 M nitric acid. The data used represent the 16 ºC exposure at 50 µg L^{-1} nominal water concentration of each analyte.

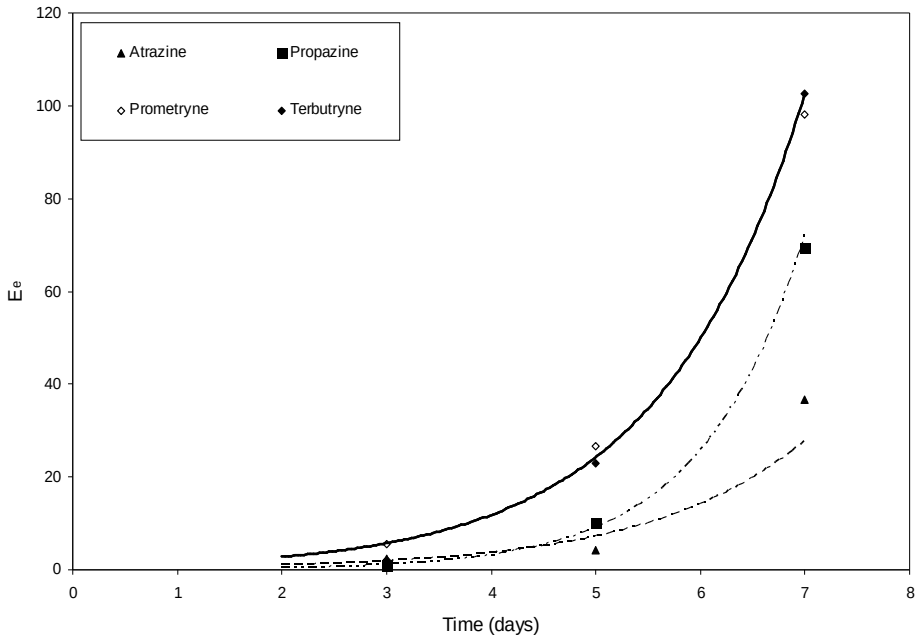


Figure 4.14 Uptake of selected triazine compounds by the MAPS where the receiving phase was 0.5 M nitric acid. The data used represent the 16 ºC exposure at 50 µg L^{-1} nominal water concentration of each analyte.

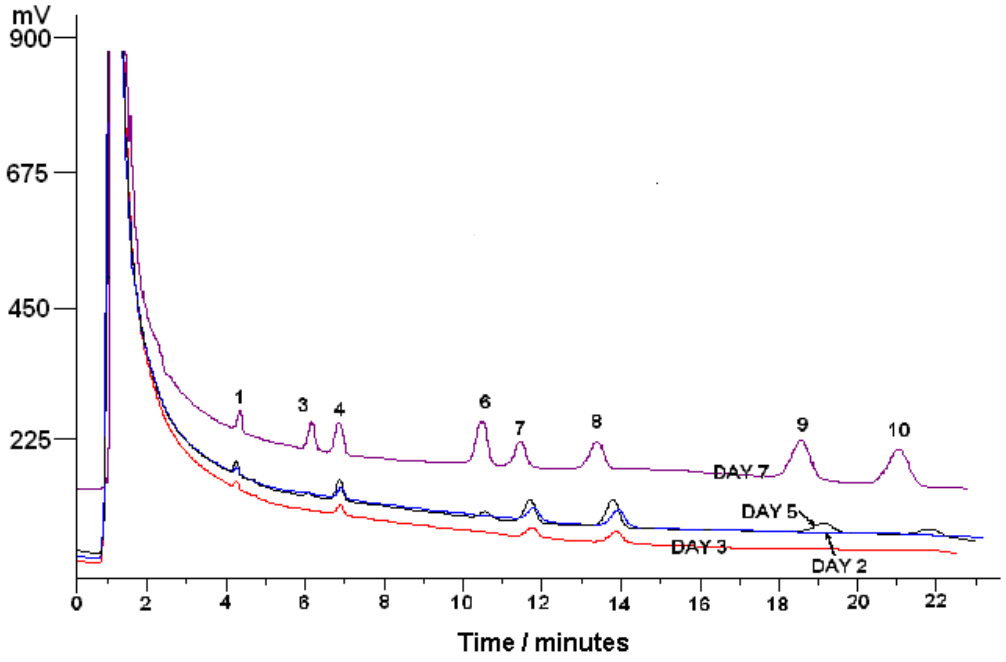


Figure 4.15 Chromatograms (HPLC – UV, with a mobile phase composition of 60 % water and 40 % acetonitrile at a flow rate of 1.0 mL min^{-1}, UV detector was set at 220 nm and a C_{18} column with dimensions 5 µm x 4.6 mm x 25 cm) obtained after passive extraction of deionised water (3 L) spiked with 50 µgL^{-1} mixture of Simazine (1), Atrazine (3), Simetryn (4), Prometon (5), Propazine (6), Ametryn (7), Terbuthylazine (8), Prometryne (9) and Terbutryne (10).

Table 4.2 Slope ($\Delta E_e/\Delta t$) and correlation coefficients (r^2) of linear substance uptake in the MAPS obtained from the serial batch extraction tests at room temperature.

Substance	$\Delta E_e/\Delta t$	r^2
Simazine	1.3369	0.9007
Simetryn	4.0636	0.9716
Ametryn	6.9662	0.9764
Terbuthylazine	9.0361	0.9767

4.4.2 Effect of Protective Cover

Chimuka et al. (2008) observed that the type of cover is paramount when dealing with MAPS as it can affect the mass transfer process of ionizable compounds to the sampler. In their study, they compared iron mesh to nylon mesh. They found that Iron mesh was better than Nylon mesh. Nylon mesh was found to reduce the mass transfer to the sampler, perhaps due to adsorption. However upon inheriting the iron mesh on our experiments, a problem of rusting was encountered (Figure 4.16) which led to a significant reduction of uptake rates of compounds to the sampler.



Figure 4.16 Photo of an iron protective cover showing a problem of rusting encountered.

Thus, a stainless steal mesh was developed. Figure 4.17 shows the mean results of the optimization of the stainless steal protective cover on the sampler performance. The results indicate that the stainless steal protective cover gave better accumulation of triazine to the sampler than iron protective cover. It can also be noted that the type of cover is paramount when MAPS is to be used monitoring basic ionizable compounds.

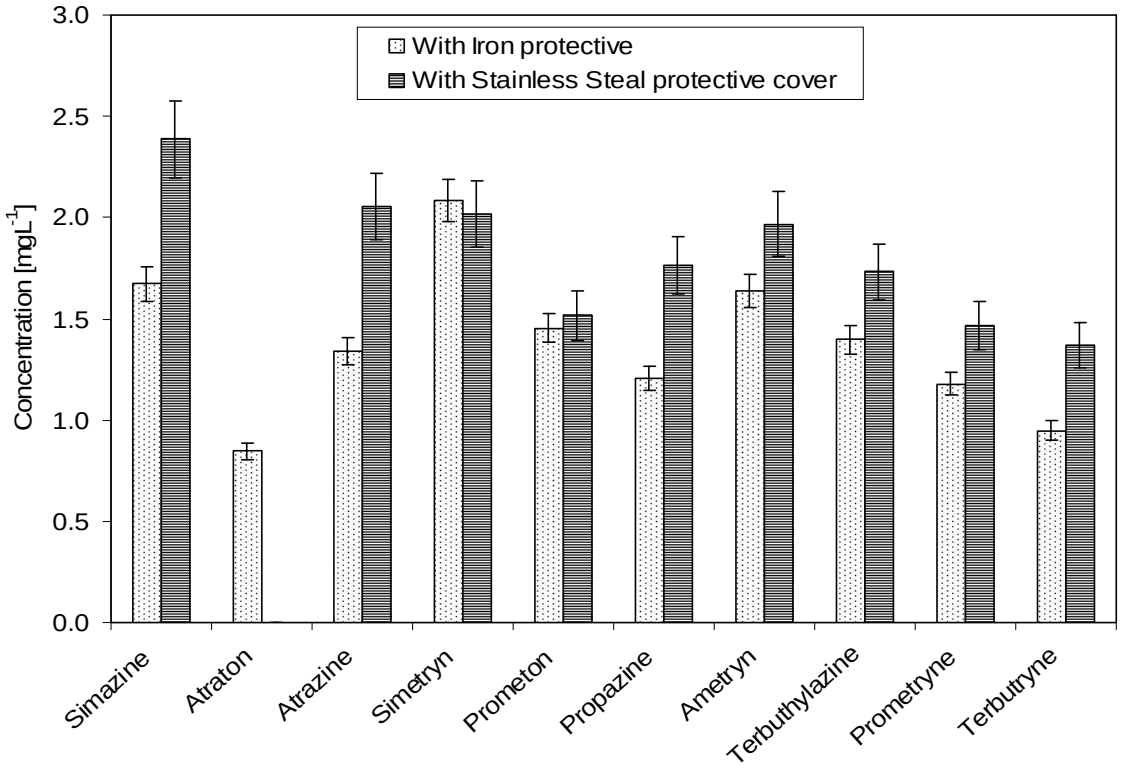


Figure 4.17 Comparison of the effect of different protective covers on the accumulation of triazines in MAPS. Deionised water sample was spiked with 50 µg L^{-1} triazine mixture and extracted for 7 days. All experiments consisted of a hollow fibre filled with 0.5 M nitric acid.

4.5 Field Performance of the MAPS When Used to Monitor Triazines and Chlorophenols.

4.5.1 Triazine Monitoring With SPE, MAPS and Chemcatcher Sampler

There were no detectable triazines in any of the deployed passive samplers in the field after seven days of deployment. Similarly, there were no triazines detected from grab samples extracted by solid phase extraction. This means that concentrations of the target compounds if

present in the dam water are at very trace levels. Figure 5.18 shows typical chromatograms obtained for all the three sampling methods.

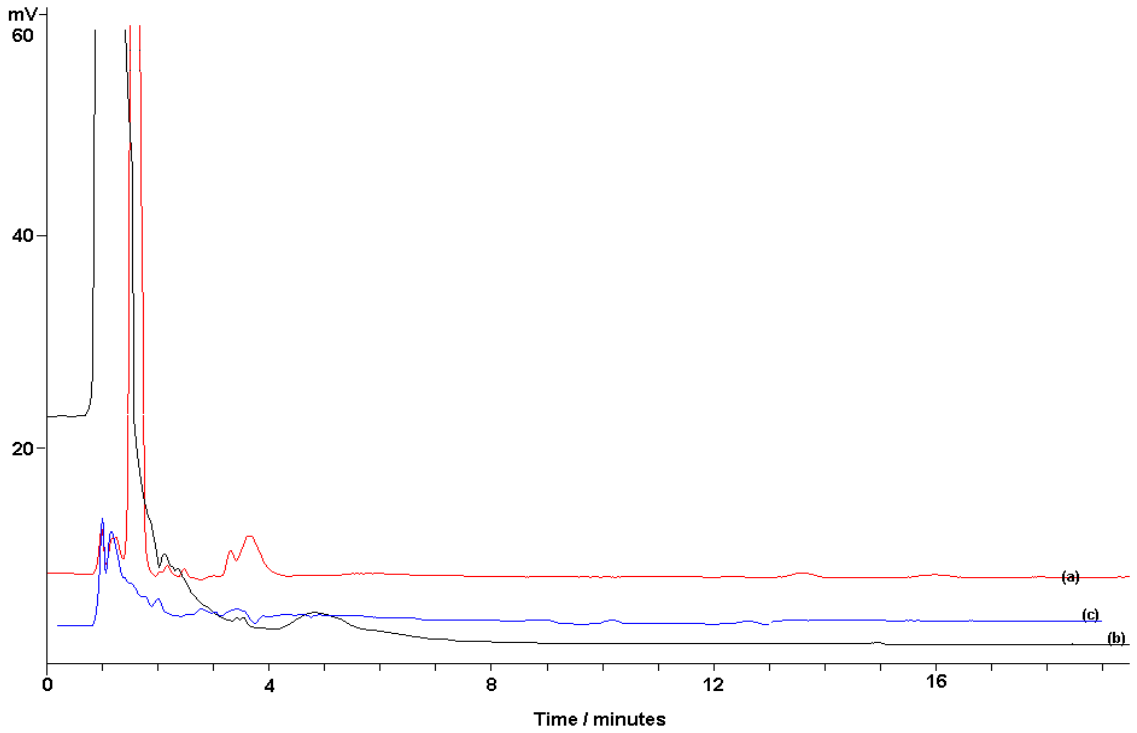


Figure 4.18 Typical chromatogram (HPLC – UV, with a mobile phase composition of 60 % water and 40 % acetonitrile at a flow rate of 1.0 mL min^{-1}, UV detector was set at 220 nm and a C_{18} column with dimensions 5 µm x 4.6 mm x 25 cm) obtained (a) Chemcatcher passive sampling device (b) MAPS device and (c) SPE method.

Nevertheless, for comparison purposes, collected water samples from the Hartebeespoort Dam were spiked with 50 µg L^{-1} triazine mixture and extracted using the three sampling methods. Figure 4.19 shows typical chromatograms obtained for all the three sampling methods. The results showed that MAPS can efficiently sequester basic ionisable triazine compounds in the water environment. All triazine compounds detected in the MAPS were essentially detected in both Chemcatcher and solid-phase extraction techniques.

As expected, the extraction efficiency in the two passive sampling devices was lower than in the solid phase extraction (Figure 4.20). The extraction efficiency in the MAPS ranged from 2 % to 4.3 % while in the Chemcatcher it ranged from 1.1 % to 2.8 % in comparison with that of solid phase extraction which ranged from 23 % to 56 %.

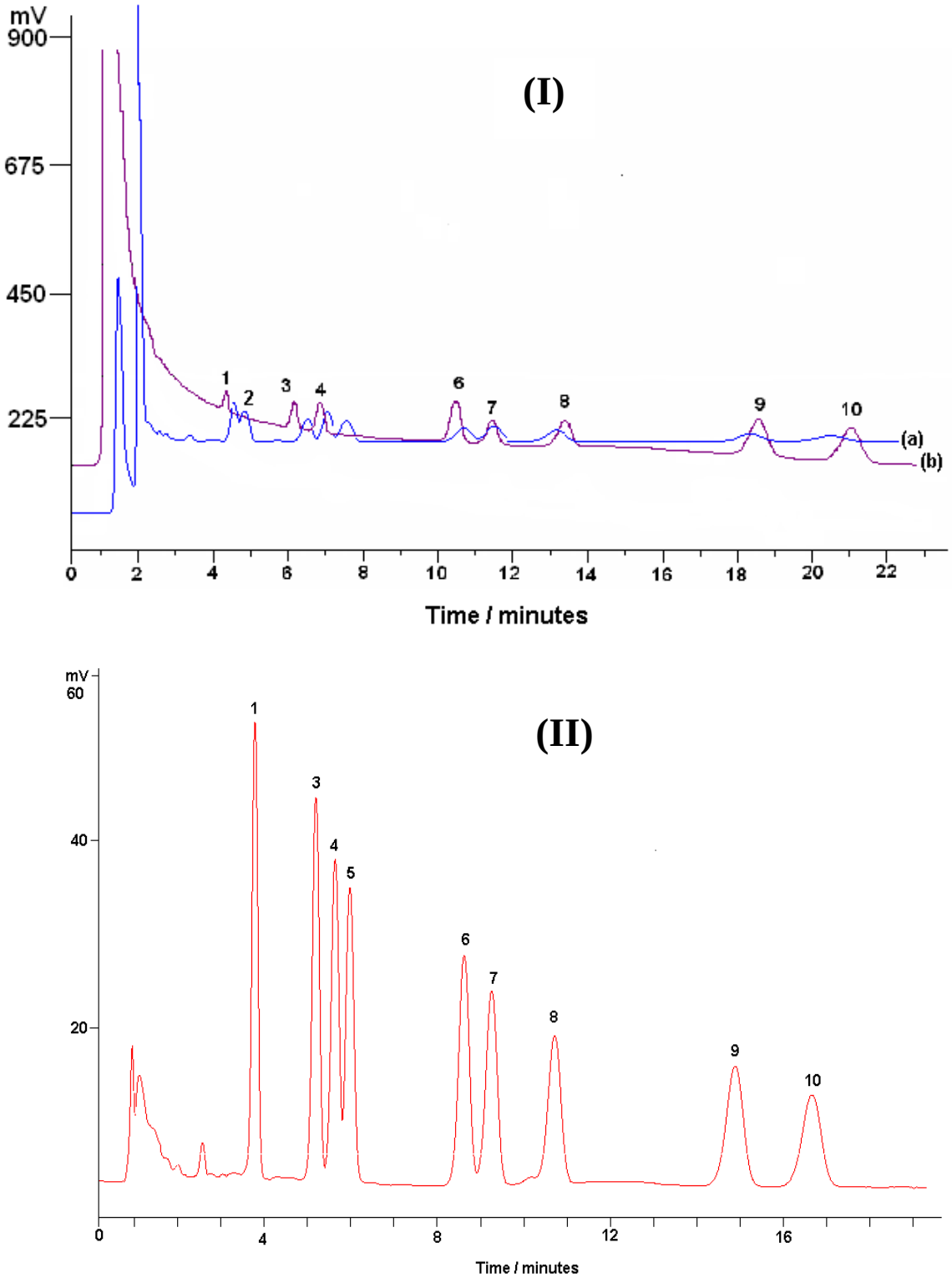


Figure 4.19 Typical chromatogram (HPLC – UV, with a mobile phase composition of 60 % water and 40 % acetonitrile at a flow rate of 1.0 mL min^{-1}, UV detector was set at 220 nm and a C_{18} column with dimensions 5 μm x 4.6 mm x 25 cm) obtained from (**I**) (a) MAPS device (b) Chemcatcher passive sampling device and (**II**) SPE method.

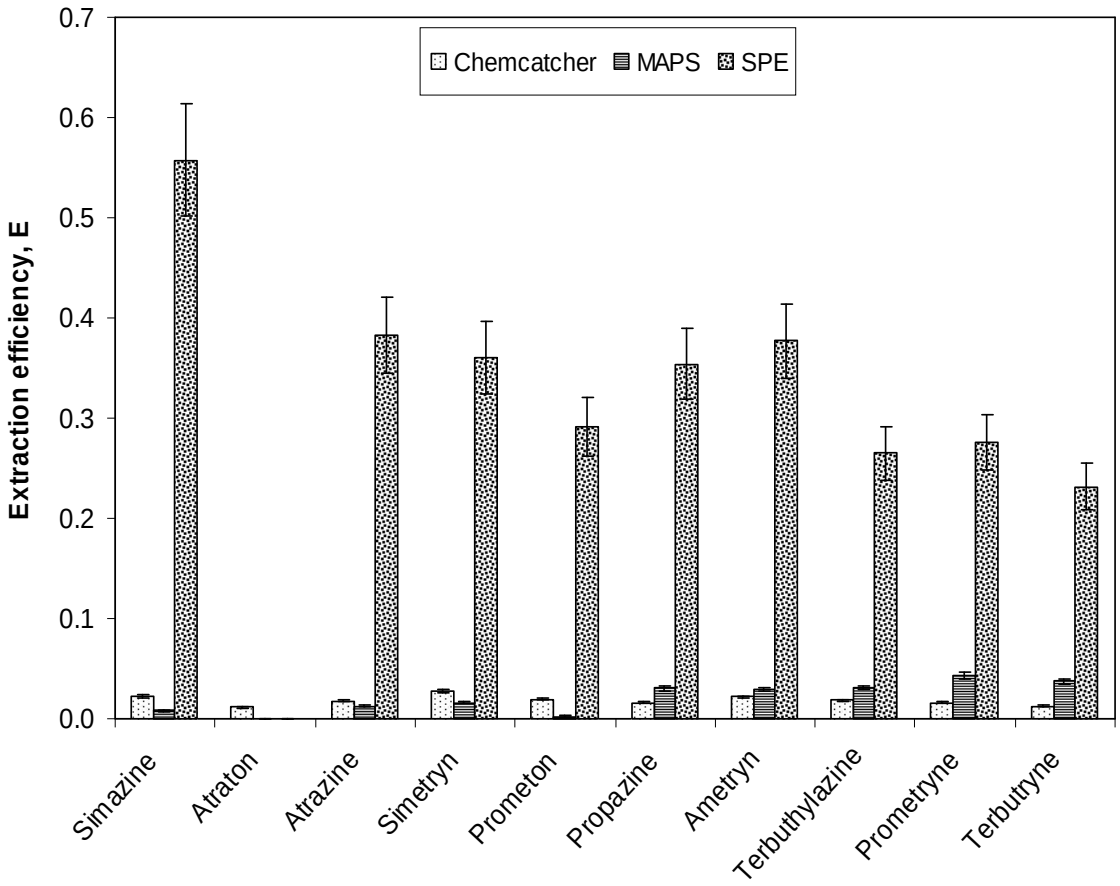


Figure 4.20 Comparison of the extraction efficiencies, in grab water samples from the Hartebeespoort Dam spiked with 50 μgL^{-1} triazine mixture, of the MAPS, Chemcatcher samplers and SPE under laboratory conditions.

The low extraction efficiency in the passive samplers supports the idea that these samplers are not equilibrium samplers but integrative samplers. Many passive samplers have been operated at the equilibrium regime such as semi-permeable membrane devices (SPMDs) (Kingston et al., 2000). The sampler is deployed long enough so that a thermodynamic equilibrium is established between the chemicals in the environmental medium and receiving phase. However, the MAPS just like the Chemcatcher work in the kinetic regime; it is assumed that the rate of mass transfer to the receiving phase is linearly proportional to the difference between the chemical activity of the contaminant in the environmental media and that in the receiving phase (Kingston et al., 2000).

The accumulation of contaminants in the MAPS is not an exhaustive extraction technique. The target analytes are not depleted in the bulk sample solution. This means the passive sampler measures the truly dissolved bioavailable fraction of the chlorophenols. This is important in toxicity studies and ecological assessment of water bodies. Solid phase extraction on the other hand is an exhaustive technique and disturbs the chemical equilibrium of the sample. It therefore does not measure the truly bioavailable fraction of the compounds.

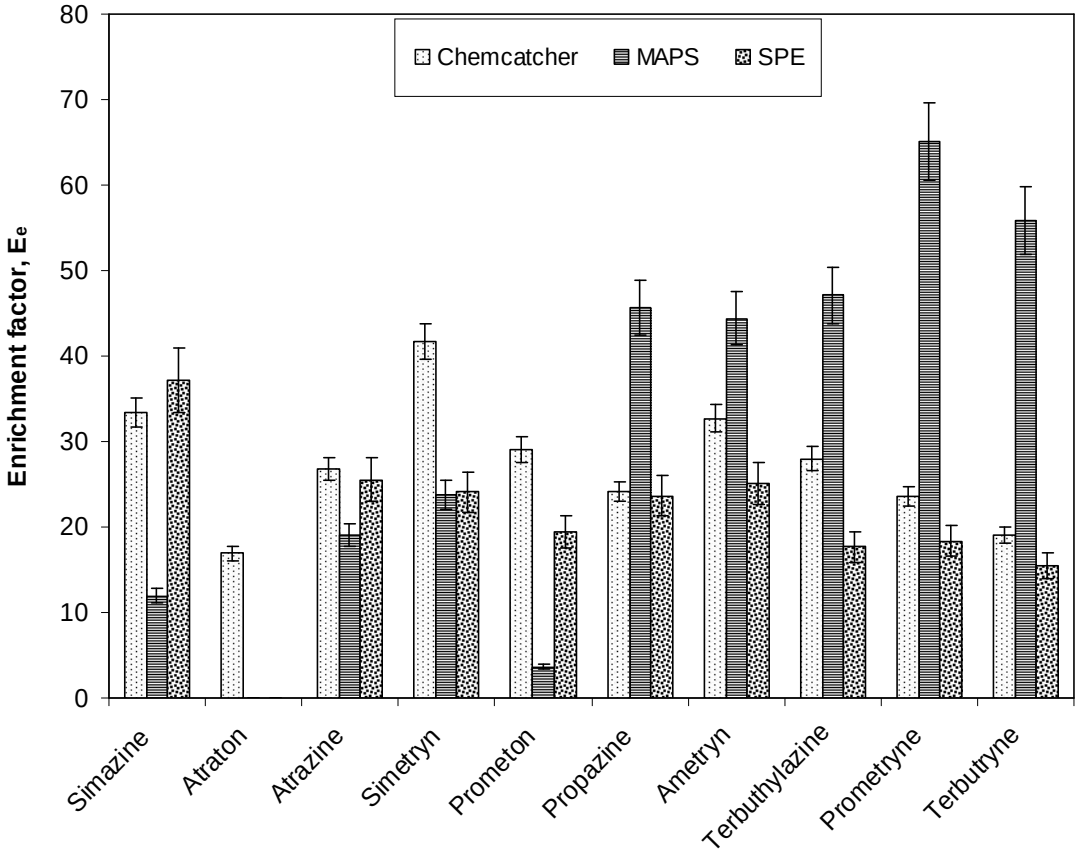


Figure 4.21 Comparison of the enrichment factors, in grab water samples from the Hartebeespoort Dam spiked with 50 µg L^{-1} triazine mixture, of the MAPS, Chemcatcher samplers and SPE under laboratory conditions.

Figure 4.21 compare the obtained enrichment factors of MAPS, Chemcatcher sampling device and solid phase extraction from spiked wastewater samples. The obtained enrichment factors in the passive samplers and SPE are generally comparable with the exception of enrichment factors of propazine, ametryn terbuthylazine, prometryn and terbutryn compounds which are higher for the MAPS ranging from 46 to 65.

4.5.2 Sensitivity of the MAPS and Chemcatcher Over SPE

Using extracts of MAPS, Chemcatcher sampling device and solid phase extraction from Hartebeespoort dam water spiked with 50 µg L^{-1} extracts triazine compounds samples, limits of detection (LOD) of triazine compounds, calculated as three times the concentration of signal to noise, were calculated and these ranged from 11.38 to 61.86 µg L^{-1} for direct injection, 1.082 to 23.077 µg L^{-1} for MAPS, 0.892 to 5.769 µg L^{-1} for Chemcatcher passive sampler and 1.482 to 7.410 µg L^{-1} for SPE (Figure 4.22). The reported LODs in the MAPS and Chemcatcher passive samplers after seven days exposure are comparable to those obtained from solid phase extraction.

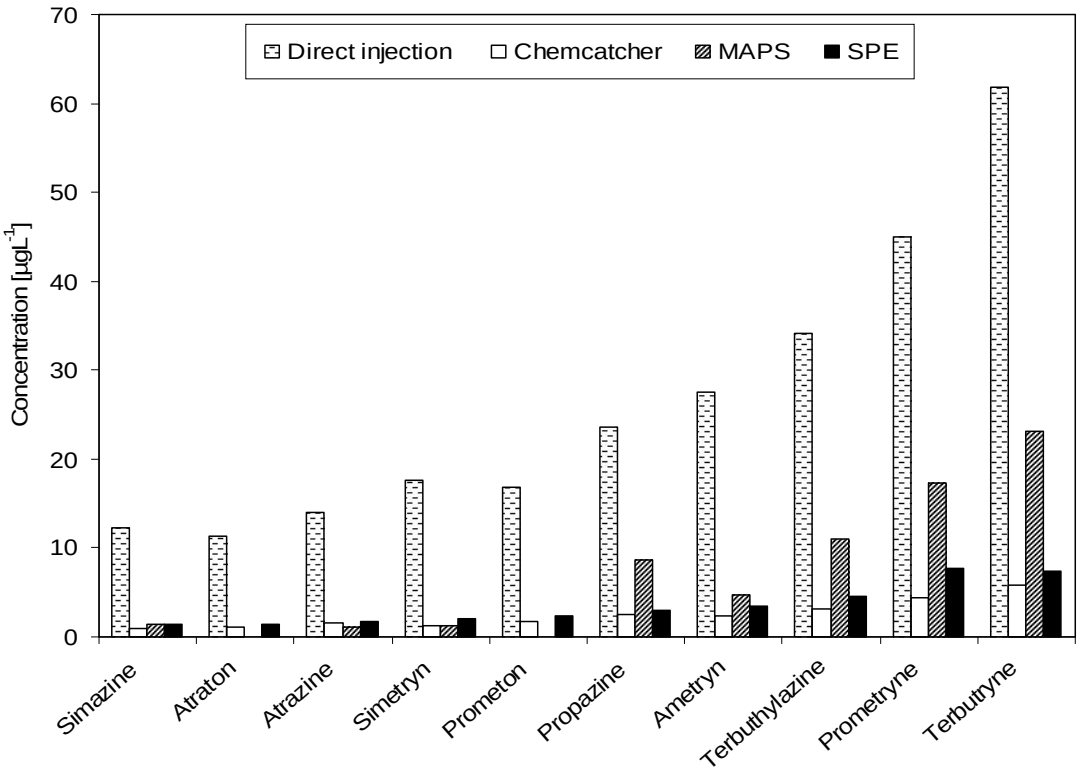


Figure 4.22 Detection limits of triazine compounds in Hartebeespoort dam water samples spiked with 50 µg L^{-1} extracts triazine compounds using MAPS, Chemcatcher sampling device and solid phase extraction.

The laboratory extraction methods, SPE included, commonly used for analysis of spot samples of water are often not sensitive enough to fulfill the required minimum performance criteria

associated with the current environmental quality standards for many priority pollutants. Moreover, several studies have demonstrated a decrease in method reproducibility with decreasing concentrations of organic pollutants in water samples (Coquery et al., 2005). Low volume spot sampling followed by instrumental analysis for the measurement of trace levels of organic contaminants is limited by the LOD and reproducibility that can be achieved. These impacts on the confidence that can be placed in the data obtained with these methods and their fitness for purpose in regulatory environmental decision making. The μg L^{-1} levels of the triazine compounds determined from MAPS and Chemcatcher exposures suggest that the sensitivity of the developed MAPS compares well with the Chemcatcher. Increasing the exposure time in these passive samplers from seven days to ten or more days can further lower the LOD to sub μg L^{-1} levels. The developed MAPS is, therefore, a promising technique for enrichment of trace level of ionizable organic pollutants in environmental samples.

4.5.3 Chlorophenol Monitoring with SPE and MAPS Devices

There were no chlorophenols detected in any of the deployed passive samplers in the field after three days of deployment. Similarly, there were no chlorophenols detected from grab samples extracted by solid phase extraction and from the passive sampler under laboratory conditions. This means that concentration of the target compounds if present in the wastewater are at trace levels. Table 4.3 shows the detection limits which ranged from 1.00 to 100 μg L^{-1} for passive sampler and 1.80 to 2.45 μg L^{-1} for solid phase extraction. The detection limit was taken as the concentration that gives three times signal to noise.

Table 4.3 Comparison of detection limits by direct injection (deionised water) and after hollow fiber passive and SPE in wastewater.

Sample type	**Detection limits (μg L^{-1})**		
	2-chlorophenol	**4-chlorophenol**	**2,4-dichlorophenol**
Direct injection	250	250	300
Passive sampler	2.81	100	1.04
SPE	2.45	2.21	1.80

The detection limits of the passive sampler after three days exposure is comparable to those obtained from solid phase extraction. Increasing the exposure time in the passive sampler from three days to seven or more days can further lower the detection limit to sub μg L^{-1} levels. This is equivalent to increasing the sample volume in solid phase extraction. For 4-chlorophenol, the detection limit was high. The same observation was obtained in the previous application of the passive sampler to river water (Chimuka et al., 2008). In deionised water, 4-chlorophenol was extracted well but poorly in wastewater perhaps due to its interaction with matrix components.

Tables 4.4 and 4.5 show the enrichment factors and extraction efficiency of the chlorophenols in passive sampler and solid phase extraction from spiked wastewater samples. The obtained enrichment factors in the passive sampler are comparable as discussed already on the detection limits. Perhaps what is more interesting is the comparison of the extraction efficiency between the passive sampler and solid phase extraction (Table 4.4). The extraction efficiency in the passive sampler was 3 and 11 % for 2-chlorophenol and 2, 4-dichlorophenol, respectively. In solid phase extraction, the extraction efficiency was 41 % and 67 % for 2-chlorophenol and 2, 4-dichlorophenol, respectively. The low extraction efficiency in the passive sampler supports the belief that it is not an exhaustive extraction technique. The target analytes are not depleted in the bulk sample solution.

Table 4.4 The Enrichment factors obtained after solid phase extraction (SPE) and Passive sampler extraction of 500 mL of waste water spiked with 50 μg L^{-1} of the chlorophenols under laboratory conditions

	PASSIVE SAMPLER			**SPE**		
	2-Chloro	4-Chloro	2,4-Chloro	2-Chloro	4-Chloro	2,4-Chloro
Sample 1	93.7	nd	304	102	113	167
Sample 2	90.0	nd	302	103	113	168
Sample 3	84.4	nd	277	101	113	166
Mean	**89.4**	**nd**	**295**	**102**	**113**	**167**
% RSD	*5.25*	-	*5.23*	*0.759*	*0.010*	*0.415*

Table 4.5 The extraction efficiency, *E*, obtained after solid phase extraction (SPE) and Passive sampler extraction of 500 ml of waste water spiked with 50 µg L^{-1} of the chlorophenols under laboratory conditions

	PASSIVE SAMPLER			**SPE**		
Component	2-Chloro	4-Chloro	2,4-Chloro	2-Chloro	4-Chloro	2,4-Chloro
Sample 1	0.034	Nd	0.11	0.41	0.45	0.67
Sample 2	0.033	Nd	0.11	0.41	0.45	0.67
Sample 3	0.031	Nd	0.10	0.41	0.45	0.67
Mean	**0.033**	**Nd**	**0.11**	**0.41**	**0.45**	**0.67**
% RSD	*5.3*	-	*5.2*	*0.76*	*0.0099*	*0.42*

The passive sampler therefore can be used as an equilibrium sampling and extraction technique. Equilibrium sampling and extraction does not change the chemical equilibrium of the components in the sample. This means the passive sampler measures the truly dissolved bioavailable fraction of the chlorophenols. This is important in toxicity studies and ecological assessment of water bodies. Solid phase extraction on the other hand is an exhaustive technique and disturbs the chemical equilibrium of the sample. It therefore does not measure the truly bioavailable fraction of the compounds. The extraction efficiency in solid phase extraction was generally low perhaps due to matrix components found in wastewater. It is common to have close to 100 % extraction efficiency in solid phase extraction.

Another interesting comparison between the passive sampler and solid phase extraction is the selectivity. This is shown in the resulting chromatograms after passive sampling and solid phase extraction of wastewater (Figure 4.23). From the passive sampler, very clean chromatogram is obtained while some matrix components are seen from solid phase extraction chromatogram. The basis of the selectivity of the passive sampler has been discussed in detail previously (Chimuka et al., 1998). Most of the commercially available passive samplers are also not very selective since they use non-selective solid phase extraction sorbents as trapping media. In solid phase extraction, each extraction consumed about 11 mL of methanol in conditioning, rinsing and elution. This looks small but for routine analysis, it adds up to sizeable amounts of organic solvents. In the developed passive sampler, no consumption of organic solvents is performed. This makes the passive sampler attractive in addition to its selectivity.

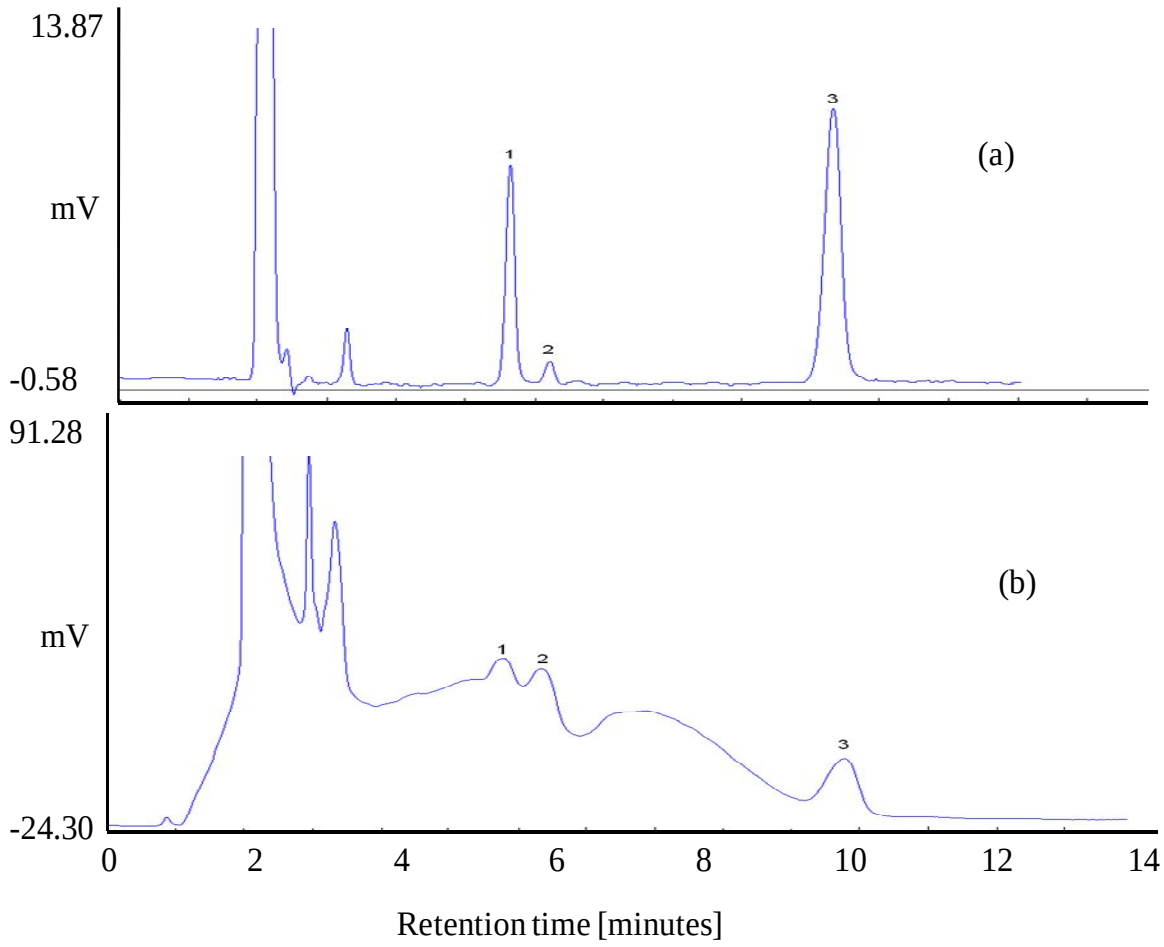


Figure 4.23 Chromatograms (HPLC – UV, with a mobile phase composition of 60 % water and 40 % acetonitrile at a flow rate of 1.0 mL min^{-1}, UV detector was set at 280 nm and a C_{18} column with dimensions 5 µm x 4.6 mm x 25 cm) obtained after passive sampling (a) and after solid phase extraction (b) of wastewater grab samples obtained from Goudkoppies wastewater treatment plant west of Johannesburg. 1 = 2-chlorophenol, 2 = 4-chlorophenol, 3 = 2,4-dichlorophenol.

4.6 Quality Control

In ensuring that the concentration determined using the sampling devices reflect the true picture in the environmental media, quality control procedures to address issues such as contamination and loss of the trapped analytes, accuracy and precision of the results were investigated. Inspection for signs of puncture, discoloring or any malfunctioning upon retrieval to see any possible sources of contamination and/or loss of the trapped analytes (Vrana et al.,

2001; Paschke et al., 2006) was performed. Procedural blanks, Certified Standard Reference Materials, control samples and field blanks were used for identification of any contamination from the process (Vrana et al., 2001; Paschke et al., 2006). Table 4.6 indicates the reproducibility results of the MAPS device in deionized water and wastewater. The percentage relative standard deviations are typically found in other passive samplers under laboratory conditions (Vrana et al., 2005; Stuer-Lauridsen, 2005). For 4-chlorophenol, the percentage standard deviations was high in wastewater. This is due to the low extraction efficiency which was irreproducible. The reproducibility under laboratory conditions also compares well with other active extraction techniques such as solid phase extraction.

Table 4.6 The reproducibility of the passive sampler in spiked deionized water and wastewater. Number of spiked deionized water samples (n) = 9 and number of wastewater samples (n) = 3

	Enrichment factor (E_e)	
Component	**Deionized water**	**Wastewater**
2-chlorophenol	150 (3.8)	89 (8)
4-chlorophenol	95 (3.2)	2.5 (61)
2,4-dichlorophenol	294 (7.6)	295 (5)

~ Chapter Five – Conclusion and Recommendation ~

5.1 Conclusions

The potential of MAPS for passive sampling of acidic and basic ionisable organic compounds has been demonstrated. Variable environmental conditions (e.g. effects of temperature, sample matrix and hydrodynamics) were found to affect the sampler performance. The sampling rate of the MAPS increased with the increasing exposure temperature. The calculated activation energies range between 12 and 17 kJ mol^{-1}. The average of all ΔE_a values was 14 kJ mol^{-1} with a standard deviation of 3.4 kJ mol^{-1}. The effect of temperature on the MAPS uptake kinetics appeared to be less significant than that on Chemcatcher sampling rates. The sampling rates obtained for individual triazine compounds under stirred and unstirred conditions were compared. A significant increase of sampling rates with change of hydrodynamic conditions from static to turbulent was observed for all compounds under investigation. The uptake rates of contaminants in the MAPS in deionised water were compares with those found in river water and wastewater samples collected regularly during the exposure period. The resulting dataset gave a solid basis that when standard MAPS are used without PRCs the potential for biofouling should be available for the period of exposure to compensate for differences in the environmental conditions at each deployment site. The results show a strong dependency of the sampling rates reductions with sample matrix, with the larger reductions occurring in wastewater samples. The reason for the deterioration of the uptake kinetics on the sampler deployed in river water and wastewater samples could be heavy biofouling observed in all the samplers deployed in those samples. A negative influence of humic substances on the MAPS' performance was observed when accumulated concentrations obtained for triazine compounds found in deionised water containing 50 μg L^{-1} of triazines mixture only were compared to those found in deionised water containing 10 mg L^{-1} of humic substances and 50 μg L^{-1} of triazines mixture. Furthermore, the sample matrix e.g. humic substances do not have an effect in the concentration of chlorophenol compounds trapped in the acceptor solution. Once these compounds are trapped in the acceptor solution they do not diffuse back during the deployment period. A strong dependence of the sampling rates reduction on sample matrix and protective

cover used was noted. The chemical uptake of both the acidic chlorophenols and basic triazine compounds into the passive sampler remained linear and integrative through out the exposure periods. The amounts quantified in the MAPS had relative standard deviations mostly between 10 % and 20 % (from repeat determinations) and did in no case exceed 30 %. The behaviour of the MAPS to monitor ionisable triazine compounds in dam water of the Hartebeespoort dam was compared to Chemcatcher and solid phase extraction technique with C_{18} sorbents of spot samples. Similarly, the behaviour of the MAPS to monitor ionisable chlorophenol compounds in wastewater of the Goudkoppies Wastewater Treatment Plant was compared to solid phase extraction technique. There were no triazine and chlorophenol compounds detected in any of the deployed passive samplers in the field applications. However, data from laboratory studies support the feasibility of MAPS to measure the freely dissolved fraction of ionisable organic chemicals in water. Using water from the Hartebeespoort dam spiked with 50 µg L^{-1} triazine compounds, the detection limits of triazine compounds ranged from 11.38 to 61.86 µg L^{-1} for direct injection, 1.082 to 23.077 µg L^{-1} for MAPS, 0.892 to 5.769 µg L^{-1} for Chemcatcher and 1.482 to 7.410 µg L^{-1} for SPE. While using water from Goudkoppies Wastewater Treatment Plant spiked with 100 µg L^{-1} chlorophenols, the detection limits of the passive sampler were comparable with that of solid phase extraction and were around 1.5 µg L^{-1}. Estimation and interpretation of enrichment factors in the passive samplers and SPE were generally comparable ranging from 46 to 295 for chlorophenol compounds. Also, for triazine compounds, the obtained enrichment factors in the passive samplers and SPE are generally comparable with the exception of enrichment factors of propazine, ametryn terbuthylazine, prometryn and terbutryn compounds which are higher for the MAPS ranging from 46 to 65.

5.2 Recommendations for Future Research

More field applications on the same compounds and other ionisable organic compounds to demonstrate the practical application of the laboratory calibration data, obtained in this study, for the measurement of TWA water concentration of ionisable organic contaminants in the field. Empirical and mechanistic models relating the calibration data to physicochemical properties of the sampled compounds will enable to apply the calibration data for measurement of a broader range of pollutants. More research is necessary to provide an understanding of the

effect of biofouling on the sampler performance. Other future research must focus on incorporating the performance reference compounds (PRC) concept into the sampler configurations and incorporation of bioassays in the trapping media.

5.3 Conferences Presentation

1. H. Nyoni, L. Chimuka, T. Nemutandani. E. Cukrowska, H. Tutu. Performance optimisation of a membrane assisted passive sampler for monitoring ionizable organic compounds in water. ChromSAAMS 2008, 12^{th}-17^{th} October. Forever Resorts, Warmbaths, Bela Bela South Africa. *Oral presentation.*

2. H. Nyoni, L. Chimuka, E. Cukrowska. Development of a membrane assisted passive field sampler based on silicone hollow fibre for monitoring ionisable organic compounds in water. ChromSA Student Chromatographer's seminar 2009, 10^{th} September. University of Witwatersrand, South Africa. *Oral presentation.*

3. H. Nyoni, L. Chimuka, B. Vrana, Z. Krascsenits, E. Cukrowska, K. Silharova, P. Tolgyessy. Membrane Assisted Passive Sampler For Aquatic Organic Chemicals – characterization of environmental conditions and field performance. 40^{th} SACI national convention, 30^{th} November – 5^{th} December 2008. Stellenbosch South Africa. *Poster presentations*.

5.4 Publications Emanating from this Project

1. H. Nyoni, L. Chimuka, B. Vrana, E. Cukrowska, H. Tutu. Green passive samplers for water monitoring of organic pollutants. Water SA (Manuscript)

2. H. Nyoni, L. Chimuka, B. Vrana, E. Cukrowska, H. Tutu**.** Comparison of selectivity of silicone based passive sampler with solid phase extraction technique. Water SA (Submitted)

References

Agency for Toxic Substances and Disease Registry (ATSDR) (1999), Toxicological profile for chlorophenols, U.S. Department of Health And Human Services Public Health Service, pp. 1-9

Alvarez, D. A., Petty, J. D., Huckins, J. N., Jones-Lepp, T. L., Goddard, J. P. and Manahan, S. E. (2004), Development of a passive, in situ, integrative sampler for hydrophilic organic contaminants in aquatic environments, *Environ. Toxicol. Chem*., 23, pp. 1640 – 1648

Alvarez, D.A., Huckins, J.N., Petty, J.D., Jones-Lepp, T., Stuer-Lauridsen, F., Getting, D.T., Goddard, J.D., Gravell, A. (2007), R. Greenwood, G. Mills, B. Vrana (Eds.), Passive Sampling Techniques in Environmental Monitoring: Comprehensive Analytical Chemistry, Elsevier, Amsterdam, 48, pp. 141-171.

Alvarez, D.A., Stackelberg, P.E., Petty, J.D., Huckins, J.N., Furlong, E.T., Zaugg, S.D., Meyer, M.T. (2005), Comparison of a novel passive sampler to standard water-column sampling for organic contaminants associated with wastewater effluents entering a New Jersey stream, *Chemosphere*, 61, pp. 610-622.

Arthur C.L., Pawliszyn, J. (1990), Solid phase microextraction with thermal desorption using fused silica optical fibres, *Anal. Chem*., 62, pp. 2145.

Aucamp, P.J., Pieterse, S.A., Vivier, F.S. (1987), Health Problems of the Hartebeespoort Dam. In: Hartebeespoort Dam -Quo Vadis? (Eds. J.A. Thornton and R.D. Wamsley), FRD Ecosys. Prog. Occ. Rep., 25, pp. 83 - 93.

Baltussen, E., Sandra, P., David, F. and Cramels, C. (1999), On the performance and inertness of different materials used for the enrichment of sulfur compounds from air and gaseous samples, *J. Chromatogr. A.*, 864, pp. 345–350.

Barrows, M.E., Petrocelli, S.R., Macek, K.J. (1980), Bioconcentration and elimination of selected water pollutants by bluegill sunfish (Lepomis mncrochirus), In: Haque R, Ed., Dynamics, exposure and hazard assessment of toxic chemicals. Ann Arbor, MI: Ann Arbor Science Publishers Inc, pp. 379-392.

Baum, E. J.; Robertshaw, V. L. (1995), In New Frontiers in Agrochemical Immunoassay; Kurtz, D. A., Skerritt, J. H., Stacker, L., Eds.; *AOAC International*, 17-29.

Beltran, J., Lopez, F.J., Hernandez, F. (2000), Solid-phase microextraction in pesticide residue analysis, *J. Chromatogr. A.,* 885, pp. 389.

Bhandari, A., Novak, J.T., Berry, D.F. (1996), Binding of 4-monochlorophenol to soil. *Environ Sci Technol.,* 30, pp. 2305-2311.

Bjarnason, B., Chimuka, L., Ramstrom, O. (1999), On line solid-phase extraction of triazine herbicides using a molecularly imprinted polymer for selective sample enrichment, *Anal. Chem.,* 71, pp. 2152-2156

Booij K., van Drooge, B.L. (2001), Polychlorinated biphenyls and hexachlorobenzene in atmosphere, sea-surface microlayer, and water measured with semi-permeable membrane devices (SPMDs), *Chemosphere*, 44, pp. 91-98.

Booij, K., Smedes, F., van Weerlee, E.M. (2002), Spiking of performance reference compounds in low density polyethylene and silicone passive samplers, *Chemosphere*, 46, pp. 1157-1161.

Bopp, S., Weiß, H., Schirmer, K., (2005), Time-integrated monitoring of PAHs in groung water using the ceramic dosimeter passive sampling device, *J. Chromatogr. A*, 1072, pp. 137.

Borba da Cunha, A., López de Alda, M., Barceló, D., Pizzolato, T.M., dos Santos, J.H.Z. (2004), Multianalyte determination of different classes of pesticides (acidic, triazines, phenyl

ureas, anilines, organophosphates, molinate and propanil) by liquid chromatography-electrospray-tandem mass spectrometry, *Anal Bioanal Chem*, 378, pp. 940–954.

Buikema, A.L., McGinniss Jr., M.J., Car-ins Jr., J. (1979), Phenolics in aquatic ecosystems: A selected review of recent literature, *Mar Environ Res*, 2, pp.87-181.

Burnside, O.C., Fenster, C.R. Wicks, G.A. (1963), Dissipation and Leaching of Monuron, Simazine and Atrazine in Nebraska Soils, *Weeds (Weed Sci)*, 11, pp. 209-213.

Burnside, O.C., Schmidt, E.L. Behrens, R. (1961), Dissipation of Simazine from the Soil, *Weeds (Weed Sci)*, 9, pp. 477-484.

Carabias-Matinez, R., Rodriguez-Gonxalo, E., Herrero-Hernandez, E. (2005), Determination of triazines and dealkylated and hydroxylated metabolites in river water using a propazine-imprinted polymer, *J. Chromatogr A*, 1085, pp. 199.

Chimuka, L., Mathiasson, L., Jönsson, J. Å., (2000), Role of octanol–water partition coefficients in extraction of ionisable organic compounds in a supported liquid membrane with a stagnant acceptor, *Anal. Chim. Acta*, 416, pp. 77-86.

Chimuka, L., Megersa, N., Norberg, J., Mathiasson, L. and Jonsson, J. A (1998), Incomplete Trapping in Supported Liquid Membrane Extraction with a Stagnant Acceptor for Weak Bases, *Anal. Chem*., 70, pp. 3906-3911.

Chimuka, L., Michel, M., Cukrowska, E., Buszewski, B. (2009), *J. Sep. Sci.*, 32, pp. 1043.

Chimuka, L., Nemutandani, T., Cukrowska, E., Tutu, H. (2008), Performance optimization of a membrane assisted passive sampler for monitoring of ionizable organic compounds in water, *J Environ Monitor*, 10, pp. 129-135.

Chimuka, L., Nindi, M.M., ElNour, M.E.M., Frank, H., Velasco, C. (1999), *J. High Resolut. Chromatogr.*, 22, pp. 417.

Chiou, C.T., Freed, V.H., Peters, L.J. (1980), Evaporation of solutes from water, *Environ Inter.*,3, pp. 23l-236.

Coquery, M., Morin, A., Becue, A., Lepot, B. (2005), Priority substances of the European Water Framework Directive: analytical challenges in monitoring water quality, *TrAC Trends Anal Chem.*, 24, pp. 117-127.

Dawson, J.H., Bruns, V.F. Clore, W.J. (1968), Residual Monuron, Diuron and Simazine in a Vineyard Soil, *Weed Sci*, 16, pp. 63-65.

Dean, J.R. (1998), Extraction Methods for Environmental Analysis, John Wiley and Sons, Chichester.

DiGiano, F.A., Elliot, D., Leith, D. (1989), Application of passive dosimetry to the detection of trace organic contaminants in water, *Environ. Sci. Technol.*, 22, pp. 1365-1367.

Diviš, P., Dočekalová, H., Brulik, L., Pavliš, M., Hakera, P., (2007), Use of the diffusive gradients in thin films technique to evaluate (bio) available trace metal concentrations in river water, *Anal. Bioanal. Chem*., 387, pp. 2239-2244.

Dorsey, J.G., Cooper, W.T. (1994), Retention mechanisms of bonded-phase liquid chromatography, *Anal. Chem*., 66, pp. 857A-867A.

EEC. Drinking Water Guidelines, (1980), 50/779/EEC; EEC.No 1.229/11-29, EEC Brussels.

Ehrig, C., Muller-Wegener, U., Ahlsdorf, B. Schmidt, R. (1991), Entry of pesticides into groundwater, *Mitt Dtsch Entomol Ges*, 66, pp. 291-294.

Eisenreich, S.J., Looney, B.B., Thornton, J.D. (1981), Airborne organic contaminants in the Great Lakes ecosystem, *Environ Sci Technol.*, 15 (1), pp.30-38.

Ellis, G.S., Huckins, J.N., Rostad, C.E., Schmitt, C.J., Petty, J.D., MacCarthy, P. (1995), Evaluation of lipid-containing semipermeable membrane devices for monitoring organochlorine contaminants in the Upper Misissippi River, *Environ. Toxicol. Chem*., 14, pp. 1875.

Environmental Protection Agency (EPA) (1984a), Federal Register, EPA Method 604, Phenols, Part VIII, 40 CFR Part 136, EPA, Washington, DC, USA, pp. 58.

Environmental Protection Agency (EPA) (1984b), EPA Method 625, Base/ Neutral and Acids, Part VIII, 40 CFR Part 136, EPA, Washington, DC, USA, pp. 153.

Environmental Protection Agency (EPA) (1995), EPA Method 8041, Phenols by Gas Chromatography: Capillary Column Technique, EPA, Washington, DC, USA, pp. 1.
Environmental Science Technologies Inc. (USA), http://est-lab.com.

Exon, J.H., Henningsen, G.M., Osborne, C.A. (1984), Toxicologic, pathologic, and immunotoxic effects of 2,4-dichlorophenol in rats, *J Toxicol Environ Health*,14, pp.723-730.

Flemming, H.-C., Schaule, G., Griebe, T., Schmitt, J., Tamachkiarowa, A. (1997), Biofouling-the achilles heel of membrane processes, *Desalination*, 113, pp. 215.

Flynn, G.L., Yalkowsky, S.H. (1972), Correlation and prediction of mass transport across membranes. I. Influence of alkyl chain length on flux-determining properties of barrier and diffusant, *J Pharm Sci*, 61, pp. 838-852.

Fragiadakis, A., Sotiriou, N., Korte, F. (1981), Absorption, balance and metabolism of carbon-14-labeled 2,4,6-trichlorophenol in hydroponic tomato plants, *Chemosphere*, 10, pp. 1315-1320.

Goarlay, C., Miege, C., Noir, A., Ravelet, C., Garric, J., Mouchel, J.M. (2005), How accurately do semi-permeable membrane devices measure the bioavailability of polycyclic aromatic hydrocarbons to *Daphnia magna*? *Chemosphere,* 61, pp. 1734-1739.

Gorecki, T., Namiesnik, J. (2002), Passive sampling , *Trends Anal. Chem*., 21, pp. 276-291.

Haimi, J., Sahninen, J., Huhta, V. (1992), Bioaccumulation of organochlorine compounds in earthworms, *Soil Biol Biochem*., 24 (12), pp.1699-1703.

Heath, R.G.M. and Claassen, M. (1999), An overview of the pesticide and metal levels present in populations of the larger indigenous fish species of selected South African rivers, WRC Report no. 428/1/99, pp. 318

Hedges, J. I., Ertel, J. R., Leopold, E. B. (1982), Lignin geochemistry of a Late Quaternary sediment core from Lake Washington, *Geochim Cosmochim Ac*, 46, pp. 1869-1877

Hely-Hutchinson, J.R., Schumann, E.H. (1997), The Anatomy of a Flash Flood in the Hartebeespoort Dam Catchment, *Water SA*, 23 (4), pp. 345-356.

Heringa, M.B., Hermens, J.L. (2003) , Measurement of free concentration using negligible-depletion-solid phase microextraction (nd-SPME), *Trends Anal. Chem*., 22, pp. 575-587.

Hogenboom, A.C., Niessen, W.M.A., Brinkman, U.A.T. (2001), The role of column liquid chromatography-mass spectrometry in environmental trace-level analysis. Determination and identification of pesticides in water, *J Sep Sci*, 24, pp. 331–354.

http:// Kerouac.pharm.uky.edu, assessed 24 August 2009.

Huckins, J. N., Petty, J. D., Booij, K. (2006), Monitors of Organic Chemical in the Environment: Semipermeable Membrane Devices, *Springer*, New York.

Huckins, J. N., Petty, J. D., Lebo, J. A., Almeida, F. V., Booij, K., Alvarez, D. A., Clark, R. C., Mogensen, B. B. (2002), Development of the permeability/performance reference compound approach for in situ calibration of semi permeable membrane devices, *Environ. Sci. Technol*., 36, pp. 85-91.

Huckins, J. N., Tubergen, M. W. and Manuweera, G. K. (1990), Semi permeable membrane devices containing model lipid: A new approach to monitoring the bioavailability of lipophilic contaminants and estimating their bioconcentration potential, *Chemosphere*, 20, pp. 533-552.

Huckins, J.N., Manuweera, G.K., Petty, J.D., Mackay, D., Lebo, J.A., (1993), Lipid-containing Semipermeable membrane devices for monitoring organic contaminants in water, *Environ. Sci. Technol*., 27, pp. 2489-2496.

Huckins, J.N., Petty, J., Lebo, J.A., Orazio, C.E., Prest, H.F., Tillit, D.E., Ellis, G.S., Johnsson, B.T., Manuweera, G.K. (1996), Semipermeable membrane devices (SPMDs) for the concentration and assessment of bioavailable organic contaminants in aquatic environments. In: Ostrander, G.K. (Ed.), Book Chapter in Techniques in Aquatic Toxicology. CRC-Lewis publishers, *Boca Raton*, FL, pp. 625-655.

Huckins, J.N., Petty, J., Prest, H.F., Lebo, J.A., Orazio, C.E., Gale, R.W., Clark, R.C. (1997), Important considerations in semipermeable membrane devices (SPMDs) design, performance, and data comparability, 18th Annual Meeting of Society of Environmental Toxicology and Chemistry, 16 November 1997, San Francisco, CA, USA.

Huckins, J.N., Petty, J.D., Prest, H.F., Clar, R.C., Alvarez, D.A., Orazio, C.E., Lebo, J.A., Cranor, W.L., Johnson, B.T. (2000), A guide for the use of semipermeable membrane devices (SPMDs) as samplers of waterborne hydrophobic organic contaminants, Report for the American Petroleum Institute (API).

IARC (1999), IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, IARC, Lyon, France, pp. 769.

Isaacson, P.J., Frink, C.R. (1984), Nonreversible sorption of phenolic compounds by sediment fractions: The role of sediment organic matter, *Environ Sci Technol*., 18, pp. 43-46.

Isensee, A.R., Jones, G.E. (1971), Absorption and translocation of root and foliage applied 2,4-dichlorophenol, 2,7-dichlorodibenzo-p-dioxin, and 2,3,7,8-tetrachlorodibenzo-p-dioxin, *J Agr Food Chem.*,19 (6), pp. 1210-1214.

Jönsson, J. Å., Lovkvist, P., Audunsson, G., Nilve´, G. (1993), Mass transfer kinetics for analytical enrichment and sample preparation using supported liquid membranes in a flow system with stagnant acceptor liquid, *Anal. Chim. Acta*, 277, pp. 9-24.

Jönsson, J. Å., Mathiasson, L. (1999), Liquid membrane extraction in analytical sample preparation: II. Applications, *Trends Anal. Chem*., 18, pp. 325-334.

Kilzer, L., Scheunert, I., Geyer, H. (1979), Laboratory screening of the volatilization rates of organic chemicals from water and soil, *Chemosphere*, 8, pp.751l-761.

Kingston, J.K., Greenwood, R., Mills, G.A., Morrison, G. M. and Persson, L. B. (2000), Development of a novel passive sampling system for the time-averaged measurement of a range of organic pollutants in aquatic environments, *J Environ. Monitor.*, 2, pp. 487-495.

Kjeldsen, P., Kjolholt, J., Schultz, B. (1990), Sorption and degradation of chlorophenols, nitrophenols and organophosphorus pesticides in the subsoils under landfills: Laboratory studies, *J Contam. Hydrol.*, 6 (2), pp. 165-184.

Kolpin D. W., Thurman E. M., Linhart S. M. (1998), The environmental occurrence of herbicides: The importance of degradates in groundwater, *Arch. Environ. Contam. Toxicol*., 35, pp. 385.

Kot-Wasik, A., Zabiegała, B., Urbanowicz, M., Dominiak, E., Wasik, A., Namieśnik, J. (2007), Advances in passive sampling in environmental studies, *Anal. Chim. Acta*, 602, pp. 141-163.

Kraaij, R.H., Mayer, P., Busser, F.J.M., van het Bolscher, M., Seinen, W., Tolls, J. (2003), Measured pore-water concentrations make equilibrium partitioning work a data analysis, *Environ. Sci. Technol*., 37, pp. 268-274.

Krijgsheld, K.R., van der Gen, A. (1986), Assessment of the impact of the emission of certain organochloride compounds on the aquatic environment. Part I: Monochlorophenols and 2,4-dichlorophenol, *Chemosphere*, 15 (7), pp. 825-860.

Kuster, M., López de Alda, M., Barceló, D. (2006), Analysis of pesticides in water by liquid chromatography-tandem mass spectrometric techniques, *Mass Spectrom. Rev.*, 25, pp. 900–916.

Leuenberger, C., Ligocki, M.P., Pankow, J.F. (1985), Trace organic compounds in rain. IV. Identities, concentrations, and scavenging mechanisms for phenols in urban air and rain, *Environ Sci Technol*., 19, pp.1053-1058.

Li, W., Zhao, H., Teasdale, P.R., John, R. (2005), Metal speciation measurement by diffusive gradients in thin films technique with different binding phases, *Anal. Chim. Acta*, 533, pp.193-202.

Lindstrom, K., Nordin, J. (1976), Gas chromatography-mass spectrometry of chlorophenols in spent bleach liquors, *J Chromatogr. A*, 128, pp. 13-26.

Liu, J.F., Torang, L., Mayer, P., Jönsson, J. Å., (2007), Passive extraction and clean-up of phenoxy acid herbicides in samples from a groundwater plume using hollow fiber supported liquid membranes, *J. Chromatogr. A*, 1160, pp. 56-63.

Loehr, R.C., Krishnamoorthy, R. (1988), Terrestrial bioaccumulation potential of phenolic compounds, *Hazard Waste Hazard*, 5, pp.109-128.

Lu, Y., Wang, Z. and Huckins, J. (2002), Review of the background and application of triolein-containing semi permeable membrane devices in aquatic environmental study, *Aquat Toxicol.*, 60, pp. 139–153.

MacKenzie, L., Beuzenberg, V., Holland, P., McNabb, P., Selwood, A., (2004), Solid phase adsorption toxin tracking (SPATT): a new monitoring tool that simulates the biotoxin contamination of filter feeding bivalves, *Toxicon*, 44, pp. 901-918.

Mayer, P., Tolls, J., Hermens, J. and Mackay, D. (2003), Equilibrium sampling devices, *Environ. Sci Technolo. A*., 37, pp. 185-191.

Mayer, P., Vaes, W.H.J. Wijnker, F., Legierse, K.C.H.M., Kraaij, R.H., Tolls, J., Hermens, J.L.M. (2000), Sensing dissolved sediment porewater concentrations of persistent and bioaccumulative pollutants using disposable solid-phase microextraction fibers, *Environ. Sci. Technol.*, 34, pp. 5177-5183.

Mergesa, N., Chimuka, L., Solomon T., Jönsson, J.Å. (2001), Automated liquid membrane extraction and trace enrichment of triazine herbicides and their metabolites in environmental and biological samples, *J. Sep. Sci.*, 24, pp. 567.

Michel, M., Chimuka, L., Cukrowska, E., Wieczorek, P., Buszewski, B. (2009), Influence of temperature on mass transfer in an incomplete trapping single hollow fibre supported liquid membrane extraction of triazole fungicides, *Anal. Chim. Acta*, 632, pp. 86-92.

Namiesnik, J., Zabiega1a, B., Kot-Wasik, A., Partyka, M., Wasik, A. (2005), Passive sampling and/or extraction techniques in environmental analysis: a review, *Anal. BioAnal Chem.*, 381, pp. 279-301.

National Institute For Water Research, (1985), The Limnology of Hartebbeespoort Dam. South African National Scientific Programmes Report no., 110, pp. 269.

Olson, C.V., Reifsynder, D.H., Canonva-Davis, E., Ling, V.T., Builder, S.E. (1994), Preparative isolation of recombination human insulin-like growth factor 1 by reversed-phase high –performance liquid chromatography. *J. Chromatogr. A*, 675, pp. 101-112.

Paschke, A., Schwab, K., Brümmer, J., Schüürmann, G., Paschke, H. and Popp, P. (2006), Rapid semi-continuous calibration and field test of membrane enclosed silicone collector as passive water sampler, *J. Chromatogr. A*, 1124, pp. 187–195.

Pawliszyn, J. (1997), Solid-phase Microextraction: Theory and Practice, Wiley, New York, pp. 264.

Peters, A.J., Zhang, H., Davison, W. (2003), Performance of the diffusive gradients in thin films technique for measurement of trace metals in low ionic strength freshwaters, *Anal. Chim. Acta*, 478, pp. 237-244.

Peterson, S.M., Apte, S.C., Batley, G.E., Coade, C. (1995), Passive sampling for chlorinated pesticides in estuarine water, *Chem. Spec. Bioavail.*, 7, pp. 83-88.

Petty, J.D., Huckins, J.N., Alvarez, D.A., Brumbaugh, W.G., Cranor, W.L., Gale, R.W., Rastall, A.C., Jones-Lepp, T.L., Leiker, T.J., Rostad, C.E., Furlong, E.T. (2004), A holistic

passive integrative sampling approach for assessing the presence and potential impacts of waterborne environmental contaminants, *Chemosphere*, 54, pp. 695-705.

Petty, J.D., Orazio, C.E., Huckins, J.N., Gale, R.W., Lebo, J.A., Meadows, J.C., Echols, K.R. and Cranor, W.I. (2000), Considerations involved with the use of semi permeable membrane devices for monitoring environmental contaminants, *J. Chromatogr. A*, 879, pp. 83–95.

Phillips, D.T.H. (1980), Quantitative aquatic biological indicators: Their use to monitor trace metal and organochlorine pollution. Applied Science Publ. London, UK

Piwoni, M.D., Wilson, J.T., Walters, D.M. (1986), Behavior of organic pollutants during rapid-infiltration of wastewater into soil. I. Processes, definition, and characterization using a microcosm, *Hazard Waste Hazard*, 3, pp. 43-55.

Poole, C.F., Wilson, I.D. (2000), Foreword, *J. Chromatogr. A*, 885, pp. 1-2

Reemtsma, T. (2001), The use of liquid chromatography-atmospheric pressure ionization-mass spectrometry in water analysis- Part II: Obstacles, *TrAC, Trends Anal Chem*, 20, pp. 533–542.

Renberg, L., Lindström, K. (1981), C_{18} reversed-phase trace enrichment of chlorinated phenols, guaiacols and catechols in water, *J. Chromatogr. A*, 214, pp. 327-334.

Richardson, B.J., Lam, P.K.S., Zheng, G.J., McClellan K.E., De Luce-Abbott, S.B. (2002), Biofouling confounds the uptake of trace organic contaminants by semi-permeable membrane devices (SPMDs), *Marine Pollut. Bulletin*, 44, pp. 1372.

Richardson, B.J., Zheng, G.J., Tse, E.S.C., Lam, P.K.S. (2001), A comparison of mussels (*Perna viridis*) and semi-permeable membrane devices (SPMDs) for monitoring chlorinated trace organic contaminants in Hong Kong coastal waters *Chemosphere*, 45, pp. 1201-1208.

Rossouw, J.D. (1992), Krokodilrivier (Wes-Transvaal) Opvangsgebiedstudie. Beskrywing van die Fisiese Waterstelsel, , Departement van Waterwese, Verslag No. PA200/00/1292.

Sabaliunas, P. and Södergren, A. (1997), Use of semi-permeable membrane devices to monitor pollutants in water and assess their effects: A laboratory test and field verification, *Environ. Pollut.*, 96, pp. 195-205.

Schellenberg, K., Leuenberger, C., Schwarzenbach, R.P. (1984), Sorption of chlorinated phenols by natural sediments and aquifer materials, *Environ. Sci. Technol*., 18 (9), pp. 652-657.

Scheuplein, R.J. (1968), On the application of rate theory to complex multibarrier flow co-ordinates: membrane permeability, *J Teor. Biol.*, 18, pp. 72-89.

Schwarzenbach, R.P., Westall, J. (1985), Sorption of hydrophobic trace organic compounds in groundwater systems, *Water Sci Technol.*, 17 (9), pp. 39-56.

Scow, K., Goyer, M., Perwak, J. (1982), Exposure and risk assessment for chlorinated phenols (2-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol), Cambridge, MA: Arthur D. Little. EPA 440/4-85-007; NTIS PB85-211951.

Södergren, A. (1987), Solvent-filled dialysis membranes simulate uptake of pollutants by aquatic organisms, *Environ Sci. Technol.*, 21, pp. 855-859.

Solomon, K., Bergman, H., Huggett, R. (1994), A review and assessment of the ecological risks associated with the use of chlorine dioxide for the bleaching of pulp, Canadian Pulp & Paper Association, Pulp Bleaching 1994 International Conference, pp. 145-161.

South African National Committee On Large Dams (SANCOLD) (1978), Loskop Dam. In: Typical Large Dams in South Africa, Published by CIGB ICOLD.

Stover, E.L., Kincannon, D.F. (1983), Biological treatability of specific organic compounds found in chemical industry wastewaters, *J Water Pollut Contr. Fed*, 55, pp. 97-109.

Stuer-Lauridsen, F. (2005), Review of passive accumulation devices for monitoring organic micropollutants in the aquatic environment, *Environ Pollut.*, 136, pp. 503-524.

Sugiura, K., Aoki, M., Kaneki, S., (1984), Fate of 2,4,6-trichlorophenol, pentachlorophenol, p-chlorobiphenyl, and hexachlorobenzene in an outdoor experimental pond: Comparison between observations and predictions based on laboratory data, *Arch Environ Contam. Toxicol.*, 13, pp. 745-758.

Sutton, D.F., Oliveira, M.P. (1987), Hartebeespoort Dam as a receiver of return flows. In: Hartebeespoort Dam - Quo Vadis? (Eds. J.A. Thornton and R.D. Walmsley), FRD Ecosys. Prog. Occ. Rep., 25, pp. 49-61.

Sutton, P.A., Barker, J.F. (1985), Migration and attenuation of selected organics in a sandy aquifer-a natural gradient experiment, *Ground Water*, 23, pp. l0-16.

Thomas, R.G. (1982), Environmental behavior of organic compounds. In: Lyman WJ, Reehl WF, Rosenblatt DH, eds. Handbook of chemical property estimation methods. New York, NY: McGraw-Hill Book Company, pp. 15-9 to 15-31.

Tomlin, C.D.S. (Ed.). (2003), The Pesticide Manual. British Crop Protection Council, Alton, Hampshire, UK, pp. 1344.

Vale, R., Kitunen, V., Salkinjoa-Salonen, M. (1984), Chlorinated phenols as contaminants of soil and water in the vicinity of two Finnish sawmills, *Chemosphere*, 13 (8), pp. 835-844.

van Reit, W.F. (1987), The Hartebeespoort Dam - A magnet to millions? In: Hartebeespoort Dam - Quo Vadis? (Eds. J.A. Thornton and R.D. Walmsley), *FRD Ecosys. Prog. Occ. Rep.*, 25, pp. 83-93.

Veith, G.D., Macek, K.J., Petrocelli, S.R. (1980), An evaluation of using partition coefficients and water solubility to estimate bioconcentration factors for organic chemicals in fish, Aquatic Toxicology ASTM STP707, Eaton JG, Parrish RP, Hendricks AC, eds., Amer Sot Test Mater, pp. 116-129.

Verbruggen, E.M.J., Vaes, W.H.J., Parkerton, T.F., Hermens, J.L.M. (2000), Polyacrylate-coated SPME fibers as a tool to simulate body burdens and target concentrations of complex organic mixtures for estimation of baseline toxicity. *Environ. Sci. Technol*, 34, pp. 324-331.

Verweij, F., Booij, K., Satumalay K., van der Molen N., Van der Oost, R. (2004), Assessment of bioavailable membrane devices (SPMDs), sediments and caged carp, *Chemosphere*, 54, pp. 1675.

Vrana, B., Mills, G.A., Allan, I.J., Dominiak, E., Svensson, K., Knutsson, J., Morrison G. and Greenwood, R. (2005), Passive sampling techniques for monitoring pollutants in water, *Trends Anal. Chem.*, 24, pp. 845-848.

Vrana, B., Mills, G.A., Dominiak, E. and Greenwood, R. (2006a), Calibration of the Chemcatcher passive sampler for the monitoring of priority organic pollutants in water, *Environ Pollut.*, 142, pp. 333-343.

Vrana, B., Paschke, A., Popp, P., (2006b), Calibration and Calibration and field performance of membrane-enclosed sorptive coating for integrative passive sampling of persistent organic pollutants in water, *Environ Pollut.*, 144, pp. 296.

Vrana, B., Popp, P., Paschke, A., Schüürmann, G. (2001), Membrane-enclosed sorptive coating, An integrative passive sampler for monitoring organic contaminants in water, *Anal. Chem.*, 73, pp. 5191-5200.

Wauchope, R.D., Buttler, T.M., Hornsby, A.G., Augustijn-Beckers, P.W.M., Burt, J.P. (1992), The SCS/ARS/CES pesticide properties database for environmental decision- making, *Rev. Environ. Contam. T*, 123, pp. 1

Wilford, B.H., Harner, T., Zhu, J., Shoeib, M., Jones, K.C. (2004), passive sampling survey of polybrominated diphenyl ether flame retardants in indoor and outdoor air in Ottawa, Canada: Implications for sources and exposure, *Environ. Sci. Technol.*, 38, pp. 5312-5318.

Yoshida, K., Shigeoka, T., Yamauchi, F. (1987) Evaluation of aquatic environmental fate of 2,4,6-trichlorophenol with a mathematical model, *Chemosphere*, 16, pp. 253 l-2544.

Yusà, V., Pastor A. and de la Guardia, M. (2005), Microwave-assisted extraction of OCPs, PCBs and PAHs concentrated by semi-permeable membrane devices (SPMDs), *Anal. Chim. Acta*, 540, pp. 355-366.

Zabik, J.M., Aston, L.S., Seiber, J.N. (1992), Rapid characterization of pesticide residues in contaminated soils by passive sampling devices. *Environ. Toxicol. Chem*., 11, pp. 765-770.

Zhang, G.Z., Hardy, J.K. (1989), Determination of phenolic pollutants in water using permeation sampling, *J. Environ. Sci. Health. A*, 24, pp. 279-295.

Zhang, H., Davison, W. (1995), Performance characteristics of diffusion gradients in thin films for the *in situ* measurement of trace metals in aqueous solution, *Anal. Chem.*, 67, pp. 3391.

Zhang, H., Davison, W., Knight, B., McGrath, S. (1998), *In Situ* Measurements of Solution Concentrations and Fluxes of Trace Metals in Soils Using DGT, *Environ. Sci. Technol*., 32, pp. 704-710.

Zhu, L., Zhu, L., Lee, H.K. (2001), Liquid–liquid–liquid microextraction of nitrophenols with a hollow fiber membrane prior to capillary liquid chromatography, *J. Chromatogr. A*, 924, pp. 407- 414.

Appendix

Appendix A1 Description of the design and application of passive samplers for water environment monitoring (Kot-Wasik et al., 2007)

Passive Sampler (full name/description of sampler)	Description of the design	Application		Remarks
		Analytes	Sampling environment	
Semipermeable membrane devices (SPMD)	SPMD is composed of lay flat, low density polyethylene tubing containing a thin film of a pure, high-molecular weight lipid (usually triolein) SPMD Length: (between the welds) 91.4 cm Width: 2.5 cm Wall thickness: 70–95 μm Tubing: lay flat low density polyethylene, additive free Triolein: 99% purity (1.0 mL used for standard 91.4 cm length) Membrane: surface area to total SPMD volume (SA-V) ratio ≈90 cm^2/mL or ≈460 cm2/mL of triolein Lipid-to-membrane: mass ratio ≈ 0.2 Weight of standard SPMD: 4.4–4.6 g	PAHs, PCBs, organotin compounds	Air, water, sediment, wastewater	SPMD was introduced by Huckings and co-workers and till now it attained the greatest importance and widespread application The polymer, often thought to be non-permeable, actually consists of transport corridors of less than 10 Å in diameter. These pores allow for the selective diffusion of hydrophobic organic chemicals, which are then sequestered in the lipid phase
Ceramic dosimeter	The sampler is made of a ceramic membrane (outer layer) and adsorbent material A ceramic tube is the diffusion-limiting barrier, enclosing a receiving phase that consists of solid sorbent beads; the cap material is PTFE (an inner diameter of 1 cm). Filled ceramic tubes are fixed in stainless steel holders of 6 cm length; the receiving phase: Amberlite IRA-743; membrane thickness 0.15 cm; surface area (tube length: 5 cm; tube diameter: 1 cm) is 8.5 cm^2; pore size–5 nm	Polycyclic aromatic hydrocarbons (PAHs) volatile aromatic compounds, volatile chlorinated hydrocarbons, alkylnaphtalenes	Monitoring in groundwater, rivers, lakes, wastewater sewers	The contaminants accumulate by diffusing from the contact water through the membrane into the adsorbent bed. They accumulate with time, depending on the concentration gradient and the effective coefficient of mass transfer across the membrane
Chemcatcher (passive sampler using Empore disk)	The chemcatcher's system uses a diffusion-limiting membrane and a bound, solid-phase receiving phase. A diffusion-limiting membrane and the solid-phase receiving material are supported and sealed in place by inert plastic housing. A number of designs are available with different combinations of receiving phase and a diffusion-limiting membrane	Polar or nonpolar organics, some persistent organic pollutants (POPs): organochlorine pesticides, PCBs, PAHs		Accumulation rates and selectivity are regulated by the choice of both the diffusion-limiting membrane and the solid-phase receiving material. Calibration data available for many chemicals. Deployment: time from 14 days up to 1 month
Diffusive gradients in thin film sampler (DGT)	The whole sampler is made from plastic and consists of three layers. The first one (a filter membrane) consist of a resin-impregnated gel layer (metal-binding layer). A plastic outer-sleeve is placed over the base in order to secure the layers, to maintain an even surface, and to inhibit water ingress into the resin-gel The resin-layer is overlain by a diffusive layer of hydrogen and a filter. Ions have to diffuse through the filter and the diffusive layer to reach the resin layer. It is the establishment of a constant concentration gradient in the diffusive layer that forms the basis for quantitively measuring metal concentration in a solution without the need for separate calibration	The content of trace metals (Cd, Ca, Mg, Zn, Mn)	Natural waters (fresh water, deep sea water, rivers, lakes, estuaries) Application of DGT (1) In situ measurements; (2) monitoring (time averaged concentrations); (3) speciation (labile inorganic and/or organic species); (4) bioavailability (effective concentration); (5) fluxes in sediments and soils; (6) kinetic and thermodynamic constants; (7) high spatial resolution measurements (sub-mm)	Invented by Bill Davison and Hao Zhang in Lancaster. The patent, filed by University of Lancaster in 1993 is granted throughout the world Advantages of the DGT sampler It can be put directly to the sample; low risk of sample contamination or sample preconcentration; simple in use It is held by a rope or string when put in water Problems with measurements when the sample is very diluted (an ionic strength less than 0,2 mmol l^{-1}). The sampler measures a wide range of concentrations. The maximum concentration that can be measured depends on the capacity of the resign (from 30 to 100 mg L^{-1} depending on the metal)

Appendix A1 Description of the design and application of passive samplers for water environment monitoring (*continued*)

Passive Sampler (full name/description of sampler)	Description of the design	Application		Remarks
		Analytes	Sampling environment	
MESCO Membrane-enclosed sorptive coating	The sampler consists of a hydrophobic solid receiving phase enclosed in a water filled hydrophilic semipermeable membrane. Elements of the structure (1) Bar coated with a thin poly (dimethyl siloxane) (PDMS) layer—Gerstel Twister, a commercially available device used for stir bar sorptive extraction used for SBSE (2) a dialysis membrane bag made from regenerated cellulose (3) 3 mL of distilled water (4) Spectra Por enclosures	Pesticides, PCBs, PAHs, selected POPs		The selectivity of the MESCO extraction technique is enhanced in two ways (1) The dissolved molecules become separated from colloids during their diffusion across the dialysis membrane (2) Hydrophobic target analytes are selectively extracted from the inner aqueous solution by the PDMS sorbent coating
Membrane-enclosed sillicone collector	The sampler consists of a hydrophobic solid receiving phase enclosed in a water filled hydrophilic semipreamble membrane. Elements of the structure (1) Bar coated with a thin poly (dimethyl siloxane) (PDMS) layer—Gerstel Twister, a commercially available device used for stir bar sorptive extraction used in SBSE (2) A dialysis membrane bag made from regenerated cellulose (3) 3 mL of distilled water (4) Spectra Por enclosures		Ground waters, surface waters	Laboratory analysis of the passive sampler is faster and less expensive than many conventional analyses of waters, sediments or tissues It combines the advantages of the passive sampling approach with solventless preconcentration organic solutes from aqueous matrices and subsequent desorption of the analytes on-line with capillary gas chromatography It avoids cleanup of extracts required for other samplers and the whole extract is injected Injection of the entire extract makes it quite sensitive despite the small surface area and volume of the sampler Uptake of compounds is linear
POCIS	The POCIS disk is constructed of two 130 μm thick by 47 mm diameter hydrophilic polyethersulfone membranes (0.1 μm pore size). The membranes enclose a resin/adsorbent admix which serves as sequestering medium and consisting of 80 mg of solute ENV+ and 20 mg of Biobeads S-X3 with surface dispersed powdered Ambersorb 1500 carbon or 100 mg of Oasis HLB resin alone.The total surface area corresponding to the 47 mm interior diameter (i.d.) is 18 cm^2 but larger washers (5.1 cm i.d.) can been used in order to increase the sampling rate of the device. When using a larger surface area configuration, the mass of the sorbent must be increased proportionally to maintain a standard surface area - to mass ratio, which is necessary for calibration and quantitation operations	Pesticides, phenoxyacids, pharmaceuticals, sulfa drugs antibiotics, tetracycline—antibiotics, methamphetamine, MDMA (Ecstasy) estradiols and estrone, estriol, alkylphenols benzophenone, caffeine, actibacterial and actifungal agents, organic waster water originated contaminants	Wasterwater effluents, fresh and salt water	The sampler device is manufactured by Environmental Sampling Technology (USA) in a patent agreement with the U.S. Geological Survey. It is marketed under the name of AQUASENSE-P The main driving force in the operation of POCIS is the difference in concentrations. This enables sampling of chemicals by diffusion of the substances through a membrane. Contaminated water penetrates the membrane and infiltrates the sequestration polymers which sorb and immobilize water-soluble compounds. Chemicals are thereby cumulatively collected in the sorption medium and the method is less sensitive to variations in the water since sampling continues over a long period of time The only drawback of POCIS is that for accurate concentrations to be calculated, calibration studies need to be conducted on the analytes of interest

Appendix A1 Description of the design and application of passive samplers for water environment monitoring (*continued*)

Solid-phase adsorption toxin tracking (SPATT)	Passive adsorbtion of biotoxins onto porous syntheticresin filled sachets (SPATT bags) is applied and their subsequent extraction and analysis. The SPATT bags are made from 95 μm polyester mesh (Polymon PES 95/27); 60 mm × 60 mm. Into one seam, a length of polyester string is sewn to fix the bags to the submerged mooring frame. The adsorbtion medium used is DIAION—an aromatic type of adsorbent based on crossed-linked polistyrenic matrix (manufactured in the form of spherical beads and pores)	Toxic micro-algal bloom	Shellfish, seawater	The SPATT techniques is similar to semipermeable membrane devices (SPMDs). The main difference is (1) The transport of solutes through the containment material is not a rate limiting step (2) The adsorption medium in the SPATT bags is less selective The SPATT technique is well suited when shellfish sampling is difficult, dangerous, or where shellfish resources are limited
Passive soil gas and sediment vapor sampling system (PETREX)	PETREX tubes consist of two or three activated carbon adsorption elements, fused to ferromagnetic wire collectors housed in an inert atmosphere in a resealable glass tube	VOCs, SVOCs	Groundwater, soils	A near-surface screening method that can identify a large range of chlorinated and aromatic vapors (such as TCE) and benzene, respectively) migrating to the surface from soil or groundwater. This system provides rapid screening of soils and groundwaters (exposure time: 2 or 3 weeks)
The tubes are usually laid out in a predetermined grid, installed about 16 in. below the surface and left in place for just over 2 weeks.				