Sampling and Sample Preparation Techniques for Trace Level Analysis of Pesticides and Their Degradation Products in Water Systems of the Lower Rift Valley of Ethiopia

By

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A thesis submitted to the School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in Chemistry

Feburary 2008

To

My beloved wife, Etenesh Mekonnen, My Lovely kids, Ruhama and Selome and My Late Brother Kebede Berhanu

Acknowledgement

Above all things I would like to Praise my Almighty God for being with me and helping me in all ups and downs, when I was in an uphill battle and down gorges, when no one was insight in the dark and in the light.

I am very much indebted to my supervisors: Dr. Negussie Megersa, Prof. Jan Åke Jönsson and Prof. Theodros Solomon for their unfailing guidance, material support, friendly advice and deep concern about my study. I especially want to cordially thank Dr. Negussie Megersa for accepting me into his project, being friendly during all of my stay with him, for his unfailing material, moral and academic support, and for giving me opportunities to participate in international conferences and workshops.

I am very much grateful to Professor Jan Åke Jönsson, my supervisor at Lund University, for guiding me through the advanced laboratory techniques. What a scientific mind! Thank you very much for giving me right answers to my scientific questions. I am very much indebted for your all time concerns; material support, kindness and close follow up. Thank you very much for inviting me to your home repeatedly for very nice dishes. Thank you very much for the MISTRA meeting in Denmark where I got ideas how to see the ins and outs of things scientifically. My special thanks should go to Dr. Birgitta Fredricksson, the wife of Prof. Jönsson, for her friendly acceptance into their family and very nice dishes at their home. Elna, the bright girl, I appreciate your kept of promise to your grandmother. Thank you very much for your all time concerns.

My sincere thanks should go to Department of Analytical chemistry, Lund University for their hospitality, granting me scholarship to stay for 3 more months to complete my research work and allowing me to use their facilities without limit. I want to extend my sincere thanks to all staffs and colleagues at the Department for their hospitality, help and deep concern. My special thanks should go to Dr. Rikard Westblom, the membrane group: Jing-Fu, Saioa, Roberto, Niklas, Yadolla, Eduardo, Staffan, Barri, Sara, Núria

and all others for the nice scientific discussions we had and for the sharing of valuable experiences.

I am grateful to the Department of Chemistry, Addis Ababa University for allowing me to use all the necessary facilities in the Department to the extent possible during my stay in the Department. I am very much grateful to Dr. Gizachew Alemayehu, the head of the Department, for his unreserved support in all aspects.

My sincere thanks should go to Jimma University for sponsoring me this study and keeping my family in the campus for such a long time.

The time as a PhD student can feel like a very long journey, especially if it is started at late age. Sometimes, it also feels like climbing Mount Everest, when shortage hinders walking, things are scarce, the environment is unfriendly. Thanks God and all of you who have stretched your supporting hands to bring me up here.

This thesis work was done between two universities: Addis Ababa University (AAU), Ethiopia, and Lund University (LU), Sweden. Many people were directly or indirectly involved in the realization of this study. I have received unforgettable material, moral and idea supports and encouragements from in-country and abroad. Therefore, I want to extend my unreserved gratefulness to all of the staffs of the Department of Chemistry, AAU for their material, moral support, encouragements and concerns in all aspects, all of the staffs of Department of Chemistry, Kotebe College of Teacher Education for their all time support, my friends: Drs. Ahmed Hussein, Shimelis Admassie, Ketema Bacha, Diriba Muleta, Mr. Belina Terfassa, Mr. Tadesse Chali, Dejene Tsegaye, Nigussie Lemma, Tesfa Bedhassa, Amenu Assefa, Bizuayehu Tadesse, Denekew, Tesfu, Estifanos Kebede and his family, Menberu Yitbarek and his family, Yitbarek W/Hawaria, Dawit Negassa, Girmaye, Dilnesaw Zerefu, Adamu Belay Tadesse Amera, Nigussie Mecha, Zekarias Asfaw, Eng. Getahun Mekuria, Ms. Askale Mekonnen, Mrs. Senait Negussie, Ms. Israel Habtu and Melat Yadeta for their material, moral support, encouragement, advice, etc.

My deepest and sincere gratitude should go to Mr. Samuel Mekonnen and his family for being my breadbasket during my study. My computer had life to write this thesis because of the help of Mr. Hailegebriel, thanks Haile!

I want to extend my sincere gratitude to Mrs. Trunesh Namara for her hospitality, kindness, her gifts and concerns. Mr. Getachew Darge deserves unreserved thanks for his very nice gift and especially for his unbelievable support on the date of my departure from Lund. I want to extend my sincere thanks to Dr. Gelana Balcha for a very nice gift to my kids. I wish to thank Mr. Negash, Kelemua, Chaltu and others for the nice gifts and the last day dinner party.

Unforgettable time I have spent with Betel Chapel members, thank you very much for all gifts you gave me, heavenly bread you fed me, and for your prayer support. My sincere thanks should go to Mrs. Sehin Kebede and Mr. Sebhat Habtu dedicated sister and brother, God bless you abundantly!!

The International Science Program and the staffs deserve special “thank you’s” for their kind support and allowing me to attend the prestigious Nobel Prize ceremony and for your hospitality at Uppsala. Special thanks should go to Dr. Malin Akerblom, for her motherly concern; Dr. Peter Sundin also for his special concern; Dr. Linnea, for her unreserved help to get us the HPLC system that now we have at the Department.

This work has come to completion because of the help of the Quality and Standards Authority of Ethiopia (QSAE) by allowing me to use their GC-MS which they have got from OPCW by donation; therefore, my sincere appreciation should go to the QSAE. What a staff at QSAE! All are very kind and helpful. I can not exhaustively list them all, to mention just a few, Ato Ameha Bekele, Ato Hirpa Abu, Mrs. Genet G/Medhin, Ato Mulugeta Mekonnen and all the young enthusiastic staffs there for lending me their hands while I was working on GC-MS in their lab.

I am also very much grateful to the Science Faculty Christian Fellowship at AAU, for their continuous prayer, help and concern.

A special note of appreciation is to my wife, Etenesh Mekonnen, who has unselfishly permitted me to pursue my study by continuously shouldering all family burdens for such a long time, whose word of encouragement kept me going through all of the ups and downs of this study. She has been the “rock” in my life. My kids, Ruhama and Selome should also deserve deepest appreciations for their patience, love, understanding, support and concerns through out my study period.

This thesis research work would have been impossible without the financial support of Sida/SAREC.

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Abstract

The main aim of this thesis work is to develop sample preparation and enrichment methods for the determination of various types of pesticide residues in environmental water samples. The other aspect of the study is to determine the levels of pesticide residues and their degradation products in the water systems of lower Rift Valley of Ethiopia. Accordingly, a hollow fiber supported liquid membrane extraction method for the liquid chromatographic determination of dinitrophenolic compounds at parts per trillion levels has been developed. Different variables affecting the extraction process, such as extraction time, shaking speed, acceptor pH, acceptor buffer concentration, salt content and humic acids have been studied. Enrichment factors up to 7000 times were obtained. Validation of the method including the calibration experiments and studies of the linearity of the responses in different matrices has been carried out. Good linearity was obtained in the environmental matrices evaluated. Detection limits ranging from 6.0 to 8.0 ng/L, was obtained showing that the method is suitable for trace analysis and the relative standard deviations do not exceed 7% in terms of repeatability.

In addition, a new design of equilibrium hollow fibre liquid phase microextraction (HF-LPME) method was developed for the determination of freely dissolved organophosphorus pesticides (OPPs) as model compounds. In this new design, a 1.2-1.4 cm length of a hollow fibre, inserted to the end of 20 cm copper wire and impregnated with organic solvent was used to extract the freely dissolved concentration of OPPs in various water systems. This method was applied to the extraction of spiked lake and ground water samples. The ground water sample was spiked at 0.1 μg/L and 0.2 μg/L concentrations of the analytes under study and the average percentage recovery, at the two concentrations, was below 1% showing the non-depletive nature of the extraction. Good linearity (R^2 = 0.991-0.996) was obtained for all of the analytes in both reagent and lake water samples. The method was found to be very simple and inexpensive, with the possibility of running hundreds of samples in parallel with very minimal expenses for the determination of freely dissolved OPPs.

The levels of *s*-triazine herbicides and their degradation products have also been determined using a new design of time-integrating supported liquid membrane (SLM) based portable and on-site sampler. In this work the beauty of SLM technique, i.e., the combination of sampling, extraction, clean-up and enrichment processes, in a single step, has been successfully demonstrated. Besides, the levels of the residues of *s*-triazine herbicides and their degradation products in water samples from the selected sites of Awassa Lake have been determined. Furthermore, the levels of residues of these herbicides and their degradation products in the sediment samples of selected sites of Awassa and Ziway Lakes have been evaluated.

The methods developed in this study are simple, inexpensive and environmentally friendly in terms of use of toxic organic solvents, energy utilization and worker's safety.

Keywords: Pesticides; Awassa Lake; Ziway Lake; Sediment; Organophosphorus pesticides; *s*-Triazine herbicides; Dinitrophenolic compounds; Hollow fibre, Supported liquid membrane extraction; Liquid phase microextraction; Time-integrating portable sampler; *s*-Triazine herbicides degradation products; Tikur Wuha; Water sample; High performance liquid chromatography; Gas chromatography Mass spectrometry

1. Introduction

As W. P. Cunningham and B. W. Saigo said, *humans have always inhabited two worlds. One is the natural world of plants, animals, soils, air and water that preceded us by billions of years and of which we are a part. The other is the world of social institutions and artifacts that we create for ourselves using science, technology and political organization* [1].

Both worlds are essential to our lives, but integrating successfully causes perennial tensions. The key and significant role we play to keep the interconnections between these worlds healthy determines whether we are able to preserve the first world to the coming generation or not.

The interference of human beings in the natural world increases with increasing utilization of materials, energy and space because of the increase in population. This interference of human beings into the first world has come with an increasing flux of anthropogenic chemicals. According to the organization of Economic Cooperation and Development, (OECD), there are presently about 70,000 synthetic chemicals (mostly organic) in daily use and this number increases continuously [2]. As a result, numerous compounds are continuously introduced into the environment in large quantities; examples are solvents, components of detergents, dyes and varnishes, additives in plastics and textiles, chemicals used for construction, antifouling agents, herbicides, insecticides and fungicides. These fluxes of chemicals significantly affect non-target organisms, which are very essential for the well being of the natural world and human beings themselves.

Impacts of these chemicals on the environmental compartments depend, among other parameters, on their concentrations. The environment is not static; materials are constantly transported between the three spheres of the environment: the atmosphere, the hydrosphere and the lithosphere. At each stage of the transportation, the concentration of the pollutants will be altered either by phase transfer, dilution or unexpectedly, reconcentration.

Among the different spheres of the environment, the hydrosphere is given special attention. This is because water is vital for life. We need water not only for drinking and washing (sanitary) but

it is also important for many of the pleasant recreational aspects of life. These may include fishing, navigation, sailing and swimming. Each different use has its own requirements for the composition and purity of water. Water also provides a habitat for a very large number of plants, animals and microbial species. These organisms will directly or indirectly are affected by the composition of the water. Pollution of aquatic environment by different anthropogenic chemicals brings changes in metabolic activities and alters their physiological state by changing the biochemical constituent of the aquatic organisms [3]. One recent study done on acute toxicity test of fenitrothion (an organophosphorus pesticide) on liver esterase of fish showed that this pesticide is toxic to three species of the fish tested [4]. As a result, each body of water to be used needs analysis for its chemical composition, on a regular basis, to confirm its suitability for a particular purpose.

The water systems can be subdivided into natural waters and wastewaters. Natural waters consist of ground, surface and drinking waters. Wastewater, on the other hand, is the water which has had its composition and properties changed by human activities [5].

Among various types of anthropogenic chemicals that contaminate any one of the natural water bodies are various types of pesticides used for agriculture and public health purposes. The cause of contamination of water systems by these pesticides can be attributed to several sources. The pesticide pollution can generally arise from point source contamination; like effluent from pesticide pollution, direct introduction of pesticides, e.g., for weed control in rivers, pest control in fish farms, etc. or non-point source contamination; like spray drift from aerial pesticide application, long distance drift of volatile pesticides, leaching from disposal sites, surface run-off from agricultural areas [5]. On the other hand, pesticides comprise widely varying classes of compounds with varying chemical and physical properties. Some are persistent in the environment and some degrade readily into various types of transformation products, again varying in the degree of toxicity and distribution in a similar manner as their parent compounds. One recent estimate indicate that only 0.1 to 5% of the herbicides applied reach the weeds targeted and that only 0.003% of the insecticides used are consumed by the insects targeted [6]. These estimates suggest that the remaining quantities of pesticides are released to the

environment where they can adversely affect the non-target organism and the ecosystem as a whole.

In addition to their effect on the aquatic ecosystem, they can also induce significant health effect to human beings as a result of their entrance into the water systems. Human beings can be exposed to these pesticides in a variety of ways and to different extents.

Generally exposures are of two types; intentional exposure (suicide and homicide) and unintentional. The unintentional exposure can be through dermal, oral or respiratory contacts and it can be occupational exposure or non-occupational exposure from water, air or food [1]. Usually pesticides can be ingested from food or drinking water, or can be absorbed through skin from clothing or by direct contact.

The broader concern for public health in both developed and developing countries is related to lower levels of exposure that occur on a frequent or long-term basis. Two important sources of long-term exposure for the general population exist. These are the pesticide residues introduced to foods during the production cycle and persistent pesticide contaminants that bioaccumulate in the food chain especially when polluted aquatic organisms consumed as food and/or water itself is directly used for drinking or cooking purposes. The distribution of pesticide residues at lower levels in the environment, not only affect the human health but also have a profound impact on the ecosystem because they can affect entire populations of animals, plants and microorganisms as well as individuals [3, 6, 7-12] on long-term exposure.

Water contamination by pesticides is, therefore, becoming a global environmental concern. Consequently, it is necessary to monitor these pollutants in the aquatic environment to determine the extent to which the water systems have been polluted and to meet the requirements of legislative frameworks and directives.

The difficulty of analyzing for many environmental pollutants, like pesticides, can be awesome. Some of these difficulties in environmental water analysis include [13]:

- Sample matrices are frequently complex containing varieties of other substances, like suspended particles, sediments, dissolved organic and inorganic substances, etc.
- Analytes may have to be measured at very low concentrations, e.g., a few ppb.
- Some analyses can only be made at or very close to the sampling site. Readily portable and robust analytical equipment or remote sensing devices are thus required.

Consequently, analytical methodologies are needed which can detect pesticide residues and their transformation products (TPs) at ultra-trace levels in various types of complex aquatic samples. Therefore, the development of efficient enrichment techniques is vital for environmental aquatic sample analysis.

Ethiopia is a huge tableland, with thousands of meters high altitudes, with slopes or escarpments that form part of the Rift Valley which rise abruptly to the central plateau on the northwest and the eastern plate. Ethiopia hosts the northern extension of the Great African Rift Valley, which is the widest part of the Rift Valley [14]. There are eight major lakes following the descending Ethiopian Rift Valley. These are: Lake Ziway, Lake Abijata, Lake Shala, Lake Langano, Lake Awassa, Lake Abaya, Lake Chamo and Lake Turkana.

There are a number of agricultural farms in these areas of the country. Cotton and maize are harvested in larger quantities each year. There are also state and private farms of horticulture and other cereals in the vicinity of these lakes. Other small-scale farms of cereals and grains that are owned by individual farmers are also very common in the region. As a result, a variety of pesticides are used in this part of the country

According to the Crop Production and Protection Technology and Regulatory Department of Ministry of Agriculture [15], a variety of pesticides that are registered according to the 1990 decree, are being applied during each season to control grain-eating birds, insects, weeds and herbs to promote crop production. Accordingly malathion, fenitrothion, chloropyrifos, diazinon,

fenthion and carbosulfan in different formulations have been used for the control of army worms, grain eating migratory birds and locusts in the country as a whole and mostly in the lake regions.

Besides these, DDT and malathion pesticides are used for public health purpose for the control of mosquitoes, as the lake regions are also malarious. About 75% of the total area of Ethiopia is estimated to be malarious and about 65% of the populations of the country are at risk of malarial infection [16]. As a result, organochlorine and organophosphorus insecticides are widely used against mosquitoes all over the country. During the usage of these pesticides, it is inevitable then that considerable amounts of the applied pesticides can get to water systems by different routes. On the other hand, some of the triazine families, like atrazine, has been used for the control of various weed species in sugarcane, cotton farm, maize and sorghum under different brand names, like Gesapax combi 500 FW, Gesaprim 500 FW, etc [17].

On top of the application of pesticides in agriculture and public health programs, there are indications that stockpiles of obsolete pesticides left dumped in rotting bags and empty drums are contaminating surface and ground water supplies [18].

In spite of the widespread use of these pesticides, there is very little published information on their environmental levels [19]. Particularly the pesticide residue status of these lakes and their tributary rivers has not been determined in an organized manner. Lack of awareness, environmentally friendly analytical methodologies and shortage of environmental research funds etc. may be some of the reasons for the absence of integrated environmental monitoring of the levels of these pesticide residues.

Regarding the advancement of analytical methodologies, in this century, we are witnessing the development of advanced analytical techniques. Chromatography is one of the most widely used separation technique, which is very well advanced in versatility of detection techniques with an excellent separation power. Sensitive detectors have been well developed and are commonly applied in wide fields of studies. Despite these facts, trace and ultra-trace contaminant analysis have been a major challenge for analytical chemists as the current detection limits of chromatographic techniques can not meet all the needs of environmental and toxicological

requirements. Therefore, sample enrichment is frequently required before introduction into the chromatographic systems.

As a result, sample preparation techniques are the bottleneck of environmental analysis, the most time consuming and costly part of the analyses [20]. In a way, the goal of any sample preparation step is to yield the analytes of interest in a form and concentration that can be readily analyzed. Consequently, analytical methodologies are needed which can detect pesticide residues and their transformation products (TPs) at ultra-trace levels in various types of complex water samples. For this reason, the development of efficient enrichment techniques is vital for environmental water analysis globally, in general and this country, in particular.

The other challenge in the environmental samples analysis is the sampling technique itself. Sampling technique varies depending on the type of the analyte and matrices. Therefore, in addition to the requirement of appropriate sample preparation technique, sampling technique is also very essential in collecting representative environmental samples, as it may not be possible to correct any mistake done during sampling process.

A wider overview of the environmental fates of pesticides of our concerns will be presented in the following section. This will then be followed by the discussion of the major classical and modern methods of sample preparation methods from environmental water analysis point of view in a separate chapter.

1.1 Objectives

Scientific awareness and public concerns about the pollution status of our different environmental compartments have led to unprecedented demands on environmental analytical laboratories. Accordingly, there is now much interest in analytical strategies that will help to lower costs, improve efficiency and are environmentally friendly. As briefly discussed above, even after emergence of most advanced instrumental techniques for the final separation, detection, identification and determination of analytes, sample preparation continues to play a

basic role in environmental analysis of complex matrices. In fact, sample preparation steps are often the bottleneck for combined time and efficiency in many overall analytical procedures. In view of this aspect, the main objectives of the present work are:

1. To develop environmentally friendly and cost effective sampling and sample preparation techniques for the analysis of pesticide residues and their likely degradates in environmental water samples. Particularly, development of sample preparation and enrichment methods for the determination of trace levels of phenolic herbicides, organophosphorus pesticides and triazine herbicide residues and their degradation products in environmental matrices, like water was the main focus of this work.
2. To determine the levels of *s*-triazine herbicides in water and sediment samples from the Rift Valley Lakes of Ethiopia.

2 Backgrounds

2.1 Environmental Fates of Pesticides and Their Degradation Products

Pesticides can be defined as any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pest. A pesticide may also be described as any physical, chemical or biological agent that kills an undesirable plant or animal pest [20]. The term *pest* includes harmful, destructive or troublesome animals, plants or microorganisms. Pesticide is a generic name for a variety of agents that are classified more specifically on the basis of the pattern of use and organism killed. In addition to the major agricultural classes that encompass insecticides, herbicides and fungicides, one finds other pest-control agents under different categories such as acaricides, larvacides, miticides, molluscides, pediculicides, rodenticides, scabicides, attractants (pheromones), defoliants, desiccants, plant growth regulators and repellants.

A fundamental contributor to the Green Revolution has been the development and application of pesticides for the control of a wide variety of insects and herbs pests that would otherwise diminish the quantity and quality of food produced. The use of pesticides coincides with the "chemical age" which enabled modern society to have assorted food since the 1950s. Unfortunately, with the benefits of chemistry have also come disbenefits, some so serious that they now threaten the long-term survival of major ecosystems by disruption of predator-prey relationships and loss of biodiversity. Also, pesticides can have significant human health consequences.

The following are the major classes of pesticides according to target pests killed [21]:
–Insecticides - kill insect pests,
–Herbicides - kill weeds and other unwanted plants,
–Fungicides - kills fungi,
–Rodenticides - kill rodents
–Acaricides (miticides) – kill round worm
–Molluscicides - kill mollusces,

–Antimicrobials / biocides – kill microbes/bacteria

–Microbial pesticides, e.g., *Bacillus thuringiensis*

What happens to pesticides once they have been released into the environment is one of the concerns of environmental science. This is because, what happens to them can be beneficial or harmful. Many processes affect what happens to pesticides in the environment. The pesticides once released into the environment can be adsorbed to various solid particles including soil and surface of plants and animals skins, transferred as a result of volatilization, drift, runoff, or leached and degraded into more toxic or less toxic products as the case may be. The general representation of the fate of pesticides in the environment is indicatd in Fig. 2.1

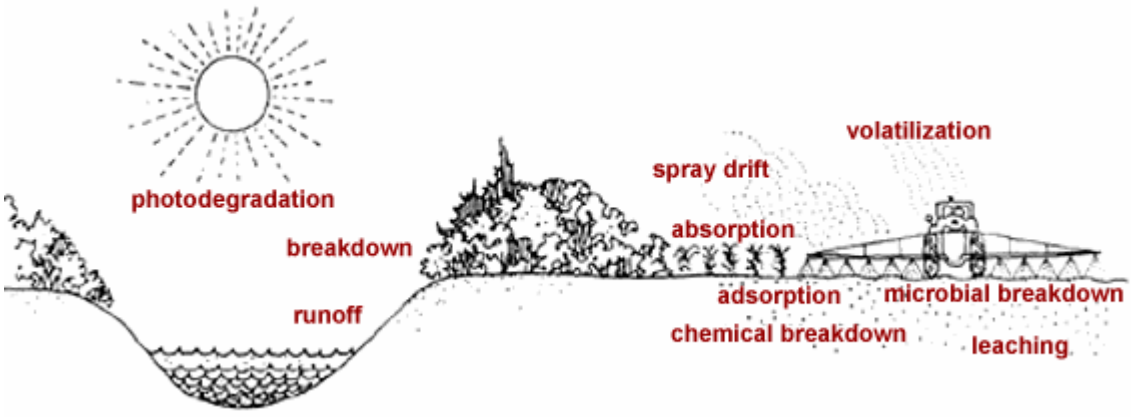


Fig. 2.1 Fate of Pesticides in the Environment [21]

Pesticide characteristics are also important in determining the fate of the chemicals in the environment. These characteristics include solubility in water (water solubility), tendency to adsorb to the soil (soil adsorption), persistence in the environment (half-life), etc [22].

Pesticides with high water solubility, low tendency to adsorb to soil particles and long persistence or half-life have the highest potential to move into water. These three factors: soil adsorption, water solubility and persistence are commonly used to rate pesticides for their potential to leach or move with surface runoff after application.

Brief discussions on some of the major classes of pesticides used in agriculture focusing only on those classes of pesticides covered in this study are given below. Accordingly, the environmental fates of organophosphorus pesticides (OPPs), *s*-triazine herbicides and their degradation products and phenolic herbicides have been presented.

2.1.1 Environmental Fates of Organophosphorus Pesticides

Organophosphorus insecticides have become the most widely used types of pesticides since the removal of more persistent organochlorine insecticides. All of the OPPs run the risk of acute and sub acute toxicity and all share a common mechanism of cholinesterase inhibition and can cause similar symptoms.

These classes of pesticides are efficiently absorbed by inhalation, ingestion and skin penetration. The occurrence of poisoning by these chemicals depends on the rate at which the pesticide is absorbed. Breakdown occurs chiefly by hydrolysis in the liver; rates of hydrolysis vary widely from compound to compound. Those compounds, which degrade slowly, can be stored in body fats. For example diazinon and methyl parathion have significant lipid solubility and are stored in the fat [22].

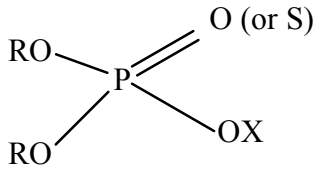
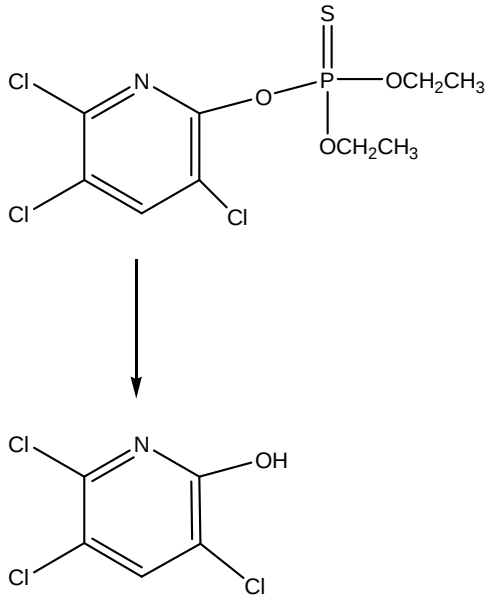


Fig. 2.2 General Formula for OPPs, X represents leaving group (Acyl group)

In the process of degradation, the thions (P=S) readily converted to oxons (P=O) under the influence of oxygen or light in the environment. The oxons are much more toxic than the thions but oxons break down more readily [23]. The general structural formula for these pesticides is depicted in Fig. 2.2.

The degradation pathways of some of these OPPs along with the molecular structure of the parent compounds involved in this thesis work are given below Figs. 2.3-2.5. The main degradation of these compounds is carried out by enzymatic or non-enzymatic hydrolysis of the ester group. Some of these degradates are more toxic than the parent compounds, for example the phosphorothionates are converted into the phosphates, (see Fig. 2.4), which are the actual acetylcholenestrase (AChE) inhibitors. Figs. 2.3 and 2.4 show the enzymatic degradation pathways of chloropyrifos and diazinon, respectively. Fig. 2.5 shows the structural formula of fenthion, one of the OPPs involved in this study.

Chloropyrifos (log P = 4.77 ± 0.40)



3, 4, 6-trichloro-2-pyridinol

Fig. 2.3 Enzymatic degradation of chloropyrifos

Diazinon (log P = 3.44 ± 0.37)

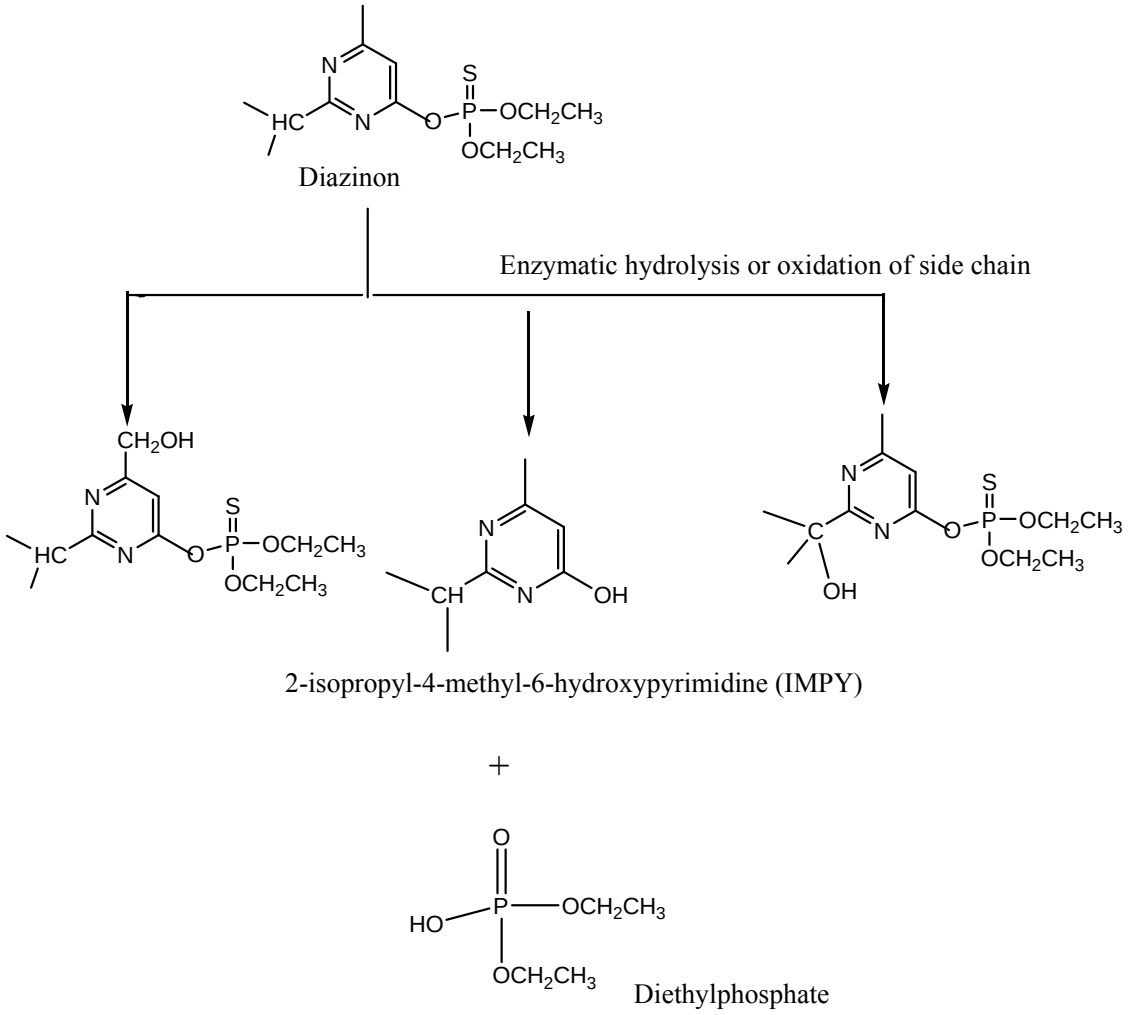


Fig. 2.4. Degradation pathway of diazinon

Fenthion (log P = 3.21± 0.34, pKa = NA)

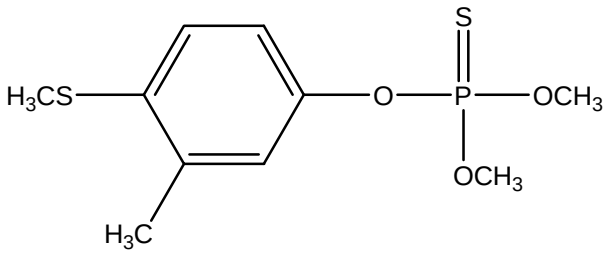


Fig. 2.5 Structural formula for fenthion

The use of relatively less persistent OPPs has brought significant economic benefits to agriculture and public health sectors. Concurrently, the increased use of these pesticides has also caused contamination of surface and ground waters, especially in the agricultural regions. Therefore, there is an increasing interest to monitor the concentrations of these organophosphorus pesticides in surface and ground waters. As these pesticides are very toxic by their nature and less persistent, their freely dissolved concentration in the environmental compartments is far more important from toxicological point of view.

The determination of organophosphorus pesticides in water samples is usually carried out by gas chromatographic (GC) methods [24]. Enzyme inhibition techniques have also been successfully used for the determination of total amount of OPPs in water samples [25, 26]. The enzyme inhibition techniques offer several advantages in terms of short analysis time, selectivity and the inherent ability to provide preconcentration of target analyte. As indicated above, all OPPs inhibit acetylcholenestrase activity and hence, it is not possible to distinguish between individual compounds of the pesticide with this method. As a result, the GC method is still the dominant method in pesticide analysis. The GC method, as indicated in section 2 below, needs complicated sample preparation steps as it cannot directly handle environmental samples. The details of these sample preparation methods have been given in section 2.

From toxicity point of view, the freely dissolved amounts are assumed bioavailable than the bound ones. For this reason, a new kind of sample preparation method based on HF-LPME and compatible with GC analysis, have been developed, (see section 2), for the determination of freely dissolved OPPs in environmental water samples in the current thesis work and is given in section 5.3.

2.1.2 Environmental Fates of *s*-Triazine Herbicides and Their Degradation products

Herbicides are substances or cultured biological organisms used to kill or suppress the growth of unwanted plants and vegetations selectively or non-selectively [27]. Thus they have brought stable crop production and labor saving, they protect crops from undue competition from weeds and enhance the nutritional quality of food. Herbicides are generally used as pre- and post-emergence for the control of weeds in agricultural crops [28]. They constitute the majority of pesticides applied in agriculture. The major classes of herbicides are:

- Bipyridyl - paraquat/diquat which are nonselective herbicides
- *s*-Triazine - most used on monocot crops, selective and most widely used
- Acetamides - used in barnyard grass
- Chlorophenoxy acids - dicot selective

In this thesis work emphasis will be given to the *s*-triazine herbicides as these pesticides are widely used in Ethiopian agricultural sector especially in the Rift Valley Lakes.

Triazine family of herbicides, introduced in 1950s, is one of the largest classes of agrochemicals produced and they are among the most commonly used herbicides. A report based on world market indicated that about 30% of herbicides produced are triazines [29]. The herbicidal properties of the *s*-triazines were discovered in 1952 by a research group of J. R. Geigy in Basel, Switzerland. The first patent application was made in 1954 covering 2-chloro-4, 6- bis (alkyl-amino)-*s*- triazines and their influence on the growth of plants [30]. It is being used as selective

pre-and post- emergence control of leafy and grassy weeds in many agricultural crops like corn, soybeans, wheat, maize, sugar cane and barley [31, 32].

The triazine herbicides contain a six-membered ring holding three nitrogens that give the name to these compounds: the prefix tri- means “three” and azine indicate a nitrogen-containing ring. Triazines are strong inhibitors of photosynthetic electron transport and are very selective herbicides.

Triazine herbicides are slightly basic, with high solubility and moderate persistence in the environment. Attentions have been devoted to the determination of these pesticides, as they are toxic and rather persistent in living systems, soil and aquatic media. These herbicides, particularly simazine and atrazine, are widely used as pre- and postemergent weed control agents to enhance crop yields. Their half-lives vary from few weeks to several months and they are usually transformed into more polar compounds, with more tendencies to stay in aquatic media and the organic matter of soil [33].

The degradation is due to different biochemical processes like dealkylation, dechlorination, hydroxylation, deamination and ring cleavage of the parent compounds.
The formation of these degradation products in several cases can produce compounds not always less phytotoxic than the parent compound; for example, deethylatrazine (DEA) (see Fig. 2.7) has the same toxicity as atrazine [34].

Most of the traizine herbicides have a structure of N-substituted benzene ring with R- groups attached to the carbon atoms (Fig. 2.6). They are the derivatives of symmetrical *s*-triazines [35]. With few exceptions, the *s*-triazines used as selective or general herbicides are substituted diamino *s*-triazines which have commonly chlorine (the common name ending with –azine), methoxy (ending with-tone), methylthio or azido (ending with- etryn) groups attached to the third ring carbon atom [32, 35]. Different substitutions at the 2- , 4- and 6-positions on the triazine ring have produced a wide range of physical, chemical and biological properties of the compounds [36]. The chemical properties of *s*-triazine derivative compounds can be determined

primarily by the constituents in position -2 such as chlorine, methoxy and methylthio groups [32, 35]. Various alkylamino groups [36] usually substitute at position 4 and 6, see Fig. 2.6.

The remarkable stability of *s*-traizine derivatives can be explained by the electronic configuration of the heterocyclic ring, which resembles that of benzene to a certain extent. Both ring systems are stabilized by delocalization of their π-electrons, which are spread over all six-ring atoms. However, essential difference exists in electronic configuration between *s*-triazine and benzene as a consequence of the greater electronegativity of the nitrogen atom compared to that of the carbon atoms. Thus, the π-electrons in the *s*-triazine ring is localized in the vicinity of nitrogen atoms rather than being evenly distributed over the whole ring. The delocalization effect in combination with inductive and mesomeric effects exerted by the substituents at C-2, C-4 and C-6 greatly influences the chemical behavior and physical properties of the *s*-triazine derivatives [32].

Triazines are relatively polar and have a log K_{ow} between 1.6 and 3.7 [27]. Dialkyl amino–*s*-triazines have low solublities in water, the 2-chloro *s*-triazine being less soluble than the 2-methylthio and 2-methoxy analogs. Depending on the substituent in the R-positions, the aqueous solubility of triazines range between 5-750 mg/L [32, 35], practically independent of the pH of the solution. However, a pronounced increase in solubility is observed at pH values where strong protonation occurs, e.g., between pH 5.0 and 3.0 or lower for 2-methoxy and 2-methylthio-*s*-triazines and at pH 2.0 or lower for 2-chloro *s*-triazine [32].

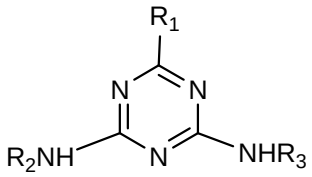


Fig. 2.6 General structure of *s*-triazine

All triazine herbicides are considered somewhat persistent in water and mobile in soil. The physico-chemical properties of triazines make them especially susceptible to leaching into

ground water and runoff from the site of application to surface waters [36] especially during heavy rains. Because of their high water solubility, triazines have a large potential for movement into a solution and only a moderate potential for soil sorption. These properties have resulted in the contamination of surface and ground waters [37]. Therefore, the extent of contamination of surface water should be monitored frequently to study the environmental impacts of these pesticides. That is why in one part of the work of this thesis attention was given to the determination of the levels of these herbicides in lake water system from the Rift Valley Lakes of Ethiopia, where their application is widely observed (see section 4.3).

Triazine herbicides degrade by various routes to a series of degradation products. Scribner *et al*. [38], in their study of the analysis of selected herbicide degradation products in surface and ground water of the United States described the mechanism of degradation of atrazine and other *s*-triazine herbicides as shown in Fig. 2.7. Accordingly, atrazine degrades in soil through both biotic and abiotic reactions to the dealkylated degradation products, DEA and DIA and the hydroxylated metabolite, ATOH (Fig. 2.7) and possibly these degradation products are transported to water systems by different means. DEA may further degrade to the dealkylated hydroxyl degradation products of didealkylatrazine (DDA), hydroxydeethylatrazine (HDEA) and hydroxydeisopropylatrazine (HDIA). DIA may further degrade to the hydroxydegradation products of DDA and HDIA. Hydroxyatrazine (ATOH) may degrade to dealkylated HDIA and HDEA [39]. Field-dissipation studies of the four chlorinated parent triazine herbicides atrazine, cyanazine, simazine and propazine ascertained that they all degrade in soil in similar fashion and form at least one of two common dealkylated degradation products; DIA and/or DEA [40], Fig. 2. 8.

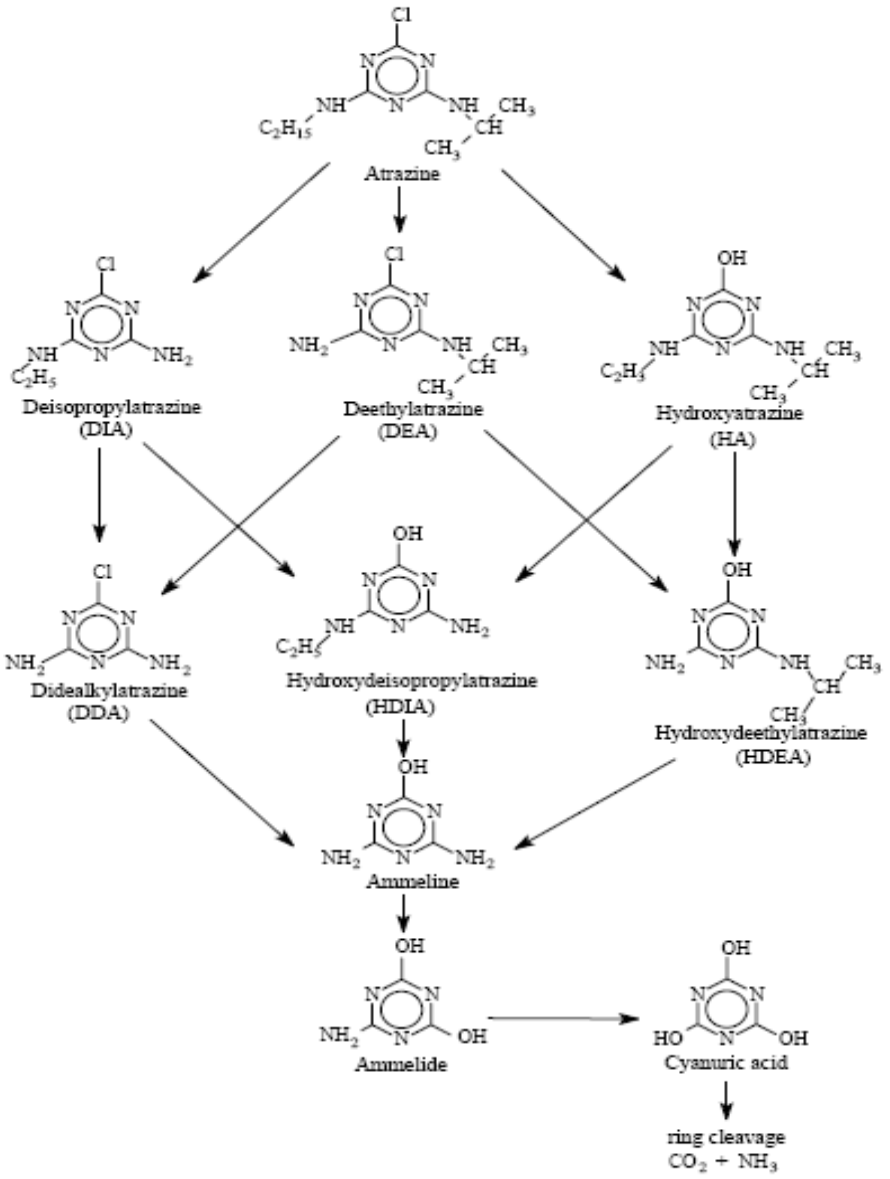


Fig. 2.7 Pathways for degradation of atrazine [38]

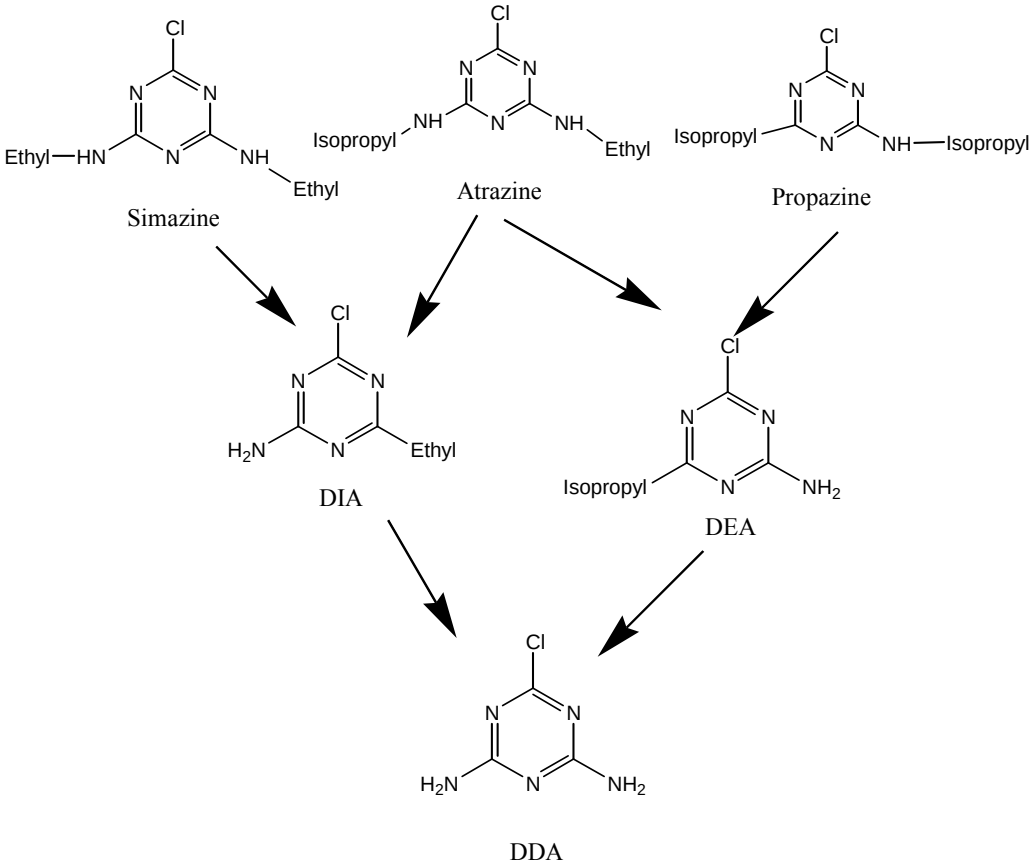


Fig. 2.8 Dealkylatlon reactions of atrazine, simazine and propazine to deethylatrazine (DEA), deisopropylatrazine (DIA) and didealkylatrazine (DDA) [40]

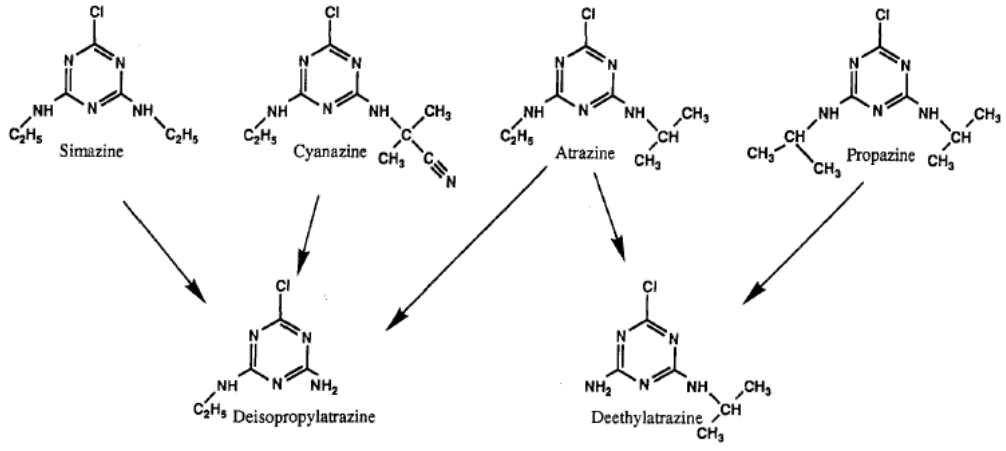


Fig. 2. 9 Pathways for degradation of various triazines to DEA and DIA [41]

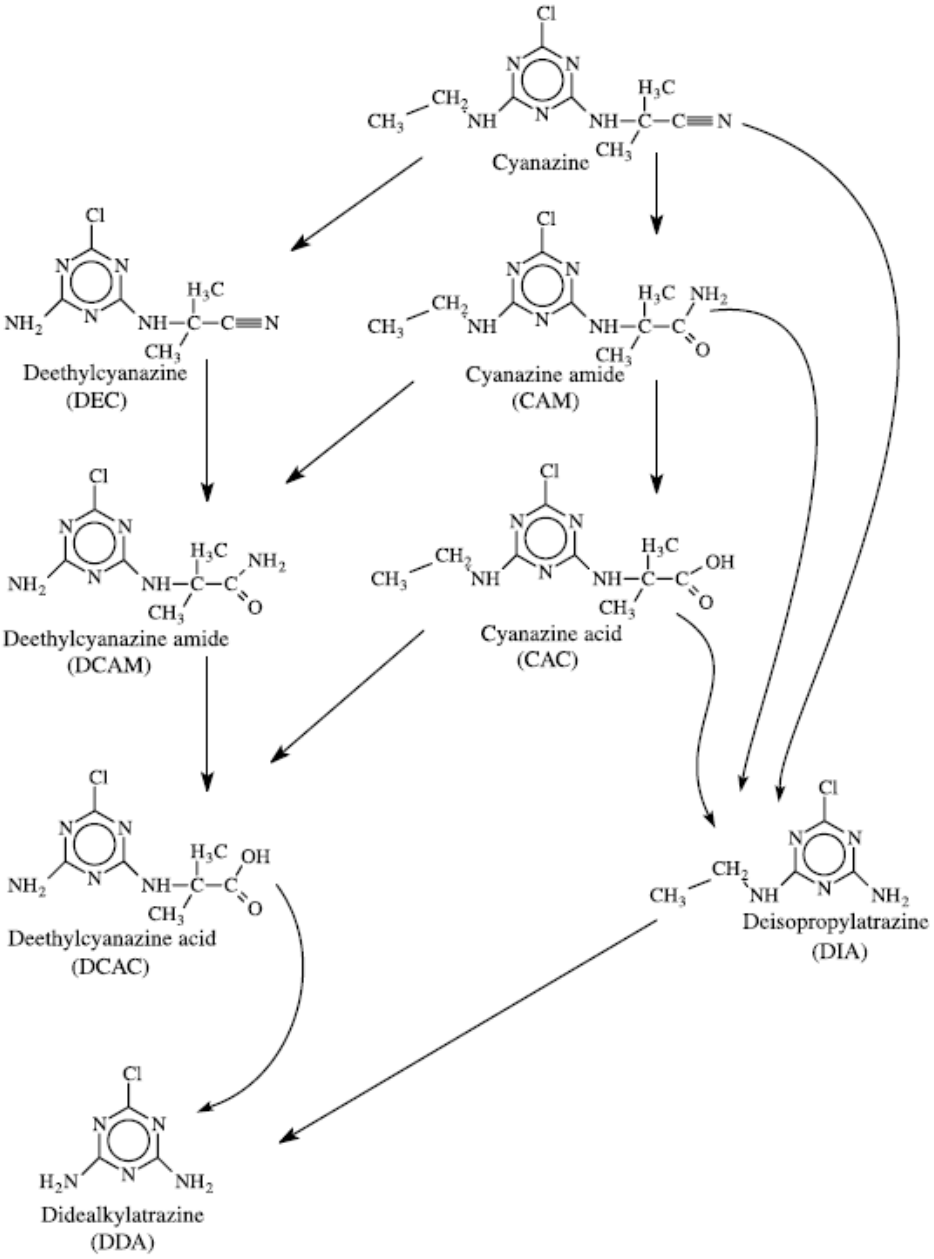


Fig. 2.10 Pathways for degradation of cyanazine [38]

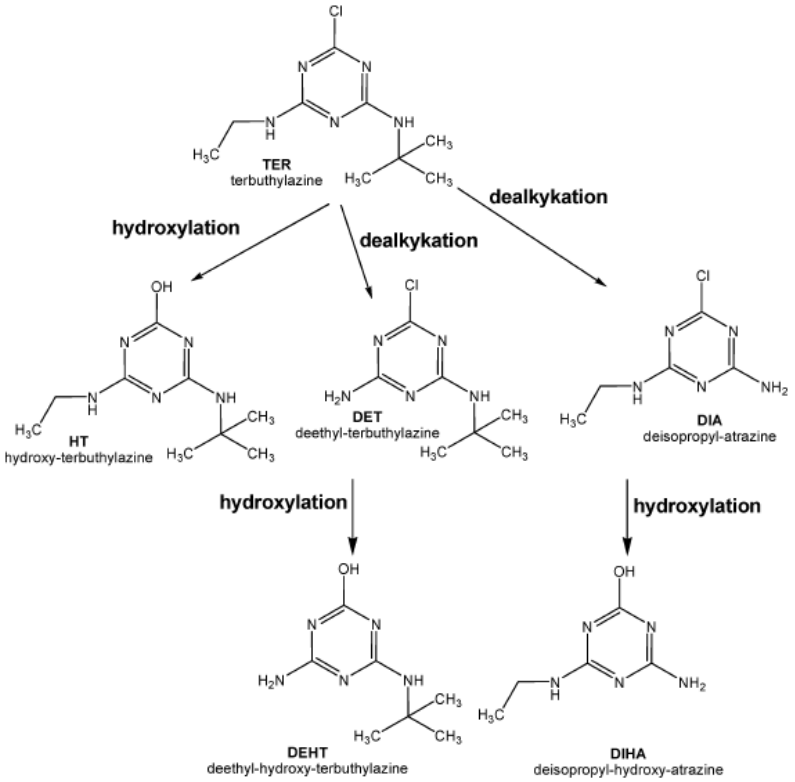


Fig. 2.11 Degradation pathways and respective products of terbuthylazine (TER) [42]

As it can be possible to see from Figs. 2.7 to 2.11, the triazine herbicides degrade into different dealkylated and hydroxylated degradation products. In general, herbicide transformation products (TPs) can be more or less mobile and toxic as their parent herbicides. Battaglin *et al.* [43] in their recent report indicated that of the 89 pesticide degradation products arising from 37 source pesticides, 70 percent were equally toxic as or less toxic than their source compounds and 30 percent were more toxic.

The determination of these transformation products in environmental water systems is, therefore, equally important as their parent compounds. To this effect, the levels of *s*-triazine degradation products along with their parent compounds have been determined in sediment and water samples from selected sites of Awassa and Ziway Lakes in this thesis work (see sections 4.3 and 4.4).

2.1.3 Environmental Fates of Phenolic Herbicides

Nitroaromatic compounds are present in the environment as pesticides and explosives [44]. Nitrophenols found in the environment are often derived from herbicides [45], hydrolytic products of OPPs such as parathion [46], methylparathion [47] or industrial wastes [44, 48]. The toxic effect of nitrophenols on biological systems has led some of them to be classified as priority pollutants in the USA [49].

Phenolic compounds are ubiquitous in the environment as a result of many processes such as industrial [50] and biogeochemical processes and pesticide degradation [51]. Nitrophenols (NPs) are one class of phenols and are present in the environment directly as a result of their use as pesticides or indirectly as decomposed products of carbamate and phosphorus pesticides [50].

Some nitrophenols, especially dinitrophenols (DNPs), are of toxicological concern because of their toxic effects on human beings and other terrestrial and aquatic lives. Certain dinitrophenols like 4,6-*o*-dinitrocresol (DNOC), 2-*sec*-butyl, 4,6-dinitrophenol (dinoseb) and 2-*tert*-butyl-4,6-dinitrophenol (Dinoterb) have been used as herbicides but because of their persistence, high water solubility and toxicity to non-target organisms, both the American Environmental Agency (EPA) [52] and European Union (EU) [53] banned them from being used as pesticides. Another important toxic dinitrophenolic compound is 2, 4-dinitrophenol which is used in the manufacture of dyes, wood preservatives and as a pesticide [54].

These nitrophenols are very toxic to human beings and most aquatic organisms and as a result they are priority pollutants of concern in the environment [55, 56]. Despite the fact that some of these dinitrophenols are banned from use as pesticides, they are being detected in some of environmental matrices because of their formation in the atmosphere and degradation of other chemicals like OPPs. For example, recent screening of pesticides in precipitation done on the selected pesticides and their degradation products and nitrophenols at two stations in Denmark showed the detection of some of these nitrophenols at relatively large concentrations [57]. The EU directive 75/440/EEC states that the maximal concentration of phenolic compounds in surface water for drinking purpose should be 1–10 μg/L [58].

Despite their occurrence in the environment, there is inadequate literature availability on their levels in the tropical water systems. One of the research work done in this thesis work was the development of a method of trace level determination of these pesticides in environmental water samples (section 4.1).

2.1.4 Role of Sediments and the Effect of Contaminated Sediments in Water Ecosystem

Organic pesticides in a water system can be distributed into several compartments, depending on their water solubility.

These compartments include water, aquatic organisms, suspended sediment and bottom sediment [59]. Pesticides, through the processes of erosion and streaming or drifting can come in contact with surface water bodies and contaminate the sediments. The linkage of pesticides to sediment particles delays their migration and increases their persistence with potential risks to ecosystems. This explains, to a large degree, why the toxicity of pesticides to wildlife is predominantly an aquatic problem in comparison with terrestrial ecosystems [60].

Sediment is one of the most important environmental matrices that support the production of aquatic organisms in several ways. For example, hard bottom sediments, which are characteristic of faster-flowing streams and are comprised largely of gravels, cobbles and boulders, provide stable substrates to which algae can attach and grow. Soft sediments, which are common in ponds, lakes and slower-flowing sections of rivers and streams, are comprised largely of sand, silt and clay, i.e., fine sediment. Such sediments provide substrates in which aquatic macrophytes can root and grow. The nutrients that are present in such sediments can also nourish aquatic macrophytes. By providing habitats and nutrients for aquatic plants, sediments support autotrophic production, i.e., the production of green plants in aquatic systems. Sediments play an important role also in supporting primary productivity, both autotrophic and heterotrophic, which

is essential because green plants and bacteria represent the foundation of food webs upon which all other aquatic organisms depend [61].

Studies on pesticide residues in sediment are particularly important because pesticides persist longer in sediment than in other environmental compartments [62]. It was observed that the pesticide concentrations in sediment changed when the physico-chemical properties of sediment and changed with the rate of water flow [63-65] suggesting that the accumulation of pesticides in sediment is probably related with the sedimentation of suspended solids on which pesticides are adsorbed [65, 66].

In addition to their role in supporting primary productivity, sediments also provide essential habitats for many sediment-dwelling invertebrates and benthic fish. Some of these invertebrate species live on the sediments (termed epibenthic species) while others live in the sediments (termed infaunal species). Both epibenthic and infaunal invertebrate species consume the plants, bacteria and other organisms that are associated with the sediments. Invertebrates represent important elements of aquatic ecosystems because a wide range of wildlife species, including amphibians, reptiles, fish, birds and mammals consume them. For example, virtually all fish species consume aquatic invertebrates during all or a portion of their life cycle. In addition, many birds, e.g., dippers, sand pipers and swallows consume aquatic invertebrates. Similarly, aquatic invertebrates represent important food sources for both amphibians (e.g., frogs) and reptiles (e.g., turtles and snakes). Therefore, sediments are of critical importance to many wildlife species due to the role that they play in terms of the production of aquatic invertebrates [61].

More importantly, sediments can also provide habitats for many wildlife species during portions of their life cycle. For example, a variety of fish species utilize sediments for spawning and incubation of their eggs and larvae [61]. In addition, young fish often find refuge from predators in sediments and/or in the aquatic vegetation that is supported by the sediments. Furthermore, many amphibian species hideaway into the sediments in the fall and remain there throughout the winter months, such that sediments provide important over wintering habitats [61].

Sediments also serve as a time-integrated sink of pesticide input from the catchments and therefore many toxic substances that are found at only trace levels in water and can accumulate to elevated levels in sediments. As such, sediments serve both as reservoirs and potential sources of chemicals of potential concerns to the water column [61].

Therefore, contaminated sediments have frequently been demonstrated to be toxic to sediment dwelling organisms and fish. Exposure to contaminated sediments can result in decreased survival, reduced growth or impaired reproduction in benthic invertebrates and fish. As a result of the effects of toxic and bioaccumulative substances, benthic organisms, fish, birds and mammals can be adversely affected by contaminated sediments [61].

Pesticide residues in bottom sediments of streams draining agricultural lands can also be a useful indictor of the entry of pesticides, from crop productions, into aquatic systems [67].

To our knowledge, there has not been any study of any kind of herbicide residue levels in sediment matrices of Ethiopian Rift Valley Lake water systems. Therefore, one of the research works covered in this thesis is the determination of the trace residue levels of *s*-triazine herbicides and the breakdown products in sediment samples from selected sites of Awassa and Ziway Lakes (section 4.4).

2. 2 Sampling and Sample Preparation Methods for the Analysis of Pesticide Residues in Water Samples

2.2.1 Introduction

Major advances have been made during the last few decades in the area of trace analysis in environmental samples. The major work is mainly in the development of analytical instruments. Despite the fact that these instruments are so sophisticated, they cannot handle environmental samples directly. Hence analysis by these instruments should be preceded by appropriate sample preparation steps that concentrate analytes and clean-up interferences [68].

Sample preparation is, in most cases, necessary for one of the following reasons:

- to remove the coexisting interferences since the discriminating power of the analytical instruments between analytes and other sample constituents is limited,
- to prevent deterioration of the analytical system by the components of the matrix under study and,
- to improve the detection limit.

Sample preparation is a key step in pesticide residue analysis in environmental water samples. The pre-treatment may either involve simple procedures, such as filtration, precipitation (e.g. proteins) or it may consist of very laborious multiple extractions followed by evaporation to dryness and constitution in a solvent compatible with the selected analytical technique, etc.

In this regard, liquid–liquid extraction (LLE) is one of the oldest and most widely used sample preparation techniques. It is a versatile sample preparation technique which is prescribed in many standard analytical methods. Despite its widespread use, it has got many limitations. The main limitations of LLE include [69]:

- the use of large amounts of high purity solvents, which are expensive and toxic and result in the production of hazardous laboratory waste,
- its labor and time intensive procedures,

- its tendency to form emulsion,
- its poor potential for automation and
- its multi-step nature which leads to analyte loss.

Further discussions will be made on this sample preparation method as a classical method in subsection 2.2.3

As a result of these limitations of LLE, there is a great need for change in analytical sampling and sample preparation procedures. This need led to the development of new methods which are fast, consume none or negligible quantity of solvents and allow trace level detections. Due to these developments in sample preparation methods, there is increased number of new sample preparation choices in recent years. In many cases, these new protocols provide more accurate results in shorter time and at a lower cost than the older techniques, so deciding which method to use or developing better alternatives is critical to analytical success.

Therefore, the focus of this section is reviewing methods of water sampling and sample preparation processes. Accordingly, the methods of water sampling will be briefly discussed to be followed by the discussion of classical and modern methods of water sample analysis.

2.2.2 Methods of Sampling

In general, sampling methods are classified as either probability or non-probability based methods [70]. In probability samples, each member of the population has a known non-zero probability of being selected. Probability methods include random sampling, systematic sampling and stratified sampling. In non-probability sampling members are selected from the population in some non-random manner. These include convenience sampling, judgment sampling, quota sampling and snowball sampling.

It is difficult to sample water for possible pollution analysis based on probability basis. The main difficulty of water sampling is that natural water is not homogeneous as it looks. Rather natural

water is heterogeneous, both spatially and temporally, making it difficult to obtain representative samples [71].

Therefore, in the monitoring analysis of water, sampling is usually done by collecting discrete grab samples at a given points in the body of water [72]. The samples are commonly obtained by filling a container held just beneath the water surface. This sampling method is cheap and easy to perform. The disadvantage of this method is that the result gives the concentrations for the particular time when the samples were taken and it does not indicate any fluctuations in the concentration in the natural water and episodic pollution events can be missed.

An alternative to grab sampling is the gathering of composite samples using automatic water samplers [72]. Composite samples are made up by pooling a number of high frequency grab samples of a given volume over a given period of time, such that the concentration of a single pooled sample is equivalent to the mean concentration over the sampling period. The frequency of sampling may be constant (time-proportional sampling) or may change with discharge conditions (flow-proportional sampling) in order to weight the pooled sample appropriately. The chief advantage of composite sampling is that it allows obtaining representative water conditions to be characterized with far fewer analyses than would be required using grab sampling [72].

The other commonly used water sampling technique is passive sampling, in which the analytes of interest diffuse through the membrane from the sample medium to the collecting medium or to another material like SPME fiber [73, 74], as a result of difference in chemical potential or concentration gradient of the analyte between the two media. In this sampling method the flow continues until equilibrium is reached or until the user terminates the sampling session. One of the advantages with passive sampling is that it gives a time-weighted average (TWA) of the concentration and, therefore, measures all variations in the sampling media during a fixed time period [75 -77].

The current state-of-the-art of passive sampling and/or extraction methods for long-term monitoring of pollutants in different environmental compartments has recently been reviewed extensively [71].

Supported liquid-membrane (SLM) is another technique useful for sampling of water, which uses minimal amount of organic solvents [78] for extraction of aqueous samples.
This makes it an environmentally friendly technique comprising sampling, sample preparation, enrichment and clean-up procedures in a single step. To this effect, a new portable and time-integrating sampler based on SLM system has been developed and successfully applied to the analysis of *s*-triazine herbicides in lake water samples from the southern agricultural regions of Ethiopia in one of the works covered in this thesis (see section 4.3)

2.2.3 Classical Sample Preparation Methods for Environmental Water Analysis

The sample preparation step in an analytical process typically consists of an extraction procedure that results in isolation and enrichment of components of interest from the sample matrix. LLE or solvent extraction is one of the oldest and most widely used techniques [79] in the preparation of samples been for qualitative and quantitative analysis. It involves the distribution of the sample components between two immiscible liquid phases. As for any sample preparation technique, sample clean-up and/or analyte preconcentration are the most common goals of LLE. The former requires a high selectivity for partitioning of the analyte components over that of potential interference; while the latter is favored by a high distribution ratio, so that the analyte components can be extracted from a large volume of sample solution into a small volume of extractant [79].

In principle, the solution containing the desired constituents must be immiscible with the liquid used to extract the desired constituent. Thus, the extraction of an analyte from one phase into the second phase is dependent upon two main factors: solubility and equilibrium [5]. The principle by which liquid-liquid extraction is successful is that ‘like dissolves like’. To determine the efficiency and success of an extraction, a number of chemical properties can be calculated. Some of these properties are presented in the following paragraphs.

The process of separating a dissolved solute from a liquid, solid or gaseous sample by using suitable solvent can be described by Nernst's distribution law, which is also known as the partition law [5]. This law describes the relationship in which the dissolved substance (solute) is distributed between two immiscible phases depending on the affinity/solubility of the solute for each phase. The ratio of the solute concentration in each phase will be constant. For the extraction of aqueous samples using organic solvents, this ratio is expressed as:

$$S_{aq} \rightleftharpoons S_{org} \quad (1)$$

$$K_d = [S]_{org} / [S]_{aq} \quad (2)$$

where K_d is the distribution coefficient, $[S]_{org}$ represents the concentration of the solute in the organic phases and $[S]_{aq}$ represents the concentration of the solute in the aqueous phase. K_d value is that characteristic for a given solute and solvent systems and is dependent on temperature. The fraction of the analyte extracted is usually the preferred parameter in this kind of extraction and is given by:

$$E = C_o V_o / (C_{org} V_{org} + C_{aq} V_{aq}) \quad (3)$$

or

$$E = K_d V / (1 + K_d V) \quad (4)$$

where C_{org} and C_{aq} are the concentrations of the analyte in the organic and aqueous phases, respectively; V_{org} and V_{aq} are volumes of the organic and aqueous phases, respectively; and V is the phase ratio V_{org}/V_{aq}.

For quantitative recovery from aqueous samples repeated extractions with fresh organic solvent is required. The amount of analyte extracted after successive extractions are given by:

$$E = 1 - [1/(1 + K_d V)]^n \quad (5)$$

where n = number of extractions.

If the actual form of the analyte is not known, then the use of distribution ratio (D) to distribution coefficient is preferred. This is given by the ratio of the concentration of all species of the solute in each phase.

$$D = \frac{\text{Concentration of analyte in all chemical forms in the organic phase}}{\text{Concentration of analyte in all chemical forms in the aqueous phase}} \quad (6)$$

In all of the cases, the selectivity and efficiency of extraction process is governed by the appropriate choice of the two immiscible solvents. With the appropriate choice of the solvent, the extraction of the analyte from one phase to another is an equilibrium process, which depends only on the partition coefficient between the two phases and follows the equation [80]:

$$(C_i^{org}/C_i^{aq})_{eq} = \exp(-\Delta\mu_i^o/RT) = K_d \quad (7)$$

where C_i^{org} and C_i^{aq} are the concentrations of analyte i in the two phases, R is the gas constant, T is the temperature, $\Delta\mu_i^o$ is the difference in the standard chemical potential of the analyte in the two phases and K_d is the distribution coefficient.

As briefly described above, this method of sample preparation technique is labor intensive, costly, environmentally unfriendly and non-automated technique. These limitations initiated the analytical chemists to make all efforts to develop modern types of sample preparation techniques. The following sections, 2.4 to 2.9, deal with these modern sample preparation methods focusing on aqueous sample analysis.

2.2.4 Modern Sample Preparation Methods

Besides the numerous limitations associated with the use of solvent extraction, for sample preparation, the technique is known for its very little selectivity and not lending itself for automations [81]. The analytical community responded to this challenge by trying to develop newer sample preparation techniques which can give higher yields, perform better sample clean-up, cost effective, convenient for workers safety and environmentally friendly. These methods of sample preparation techniques are classified in several ways; one of these classifications is based upon the mode of extraction [82]. Accordingly, fluid–phase partitioning methods, sorptive and membrane based extraction methods are the major ones, which are applied to aqueous analysis. The fluid-phase partitioning methods include: single drop and liquid microextraction, supercritical and pressurized fluid extractions and microwave-assisted extraction. Supercritical fluid extraction (SFE), pressurized liquid extraction and microwave-assisted extractions are more frequently applied to the extraction of solid samples and will not be further treated in this thesis.

Another mode of classification of sample preparation methods is based upon the ways of analytes isolation. Fig. 2.2.1 shows this way of classifying sample preparation techniques as adapted from Kloskowski *et al*. [83] with slight modification.

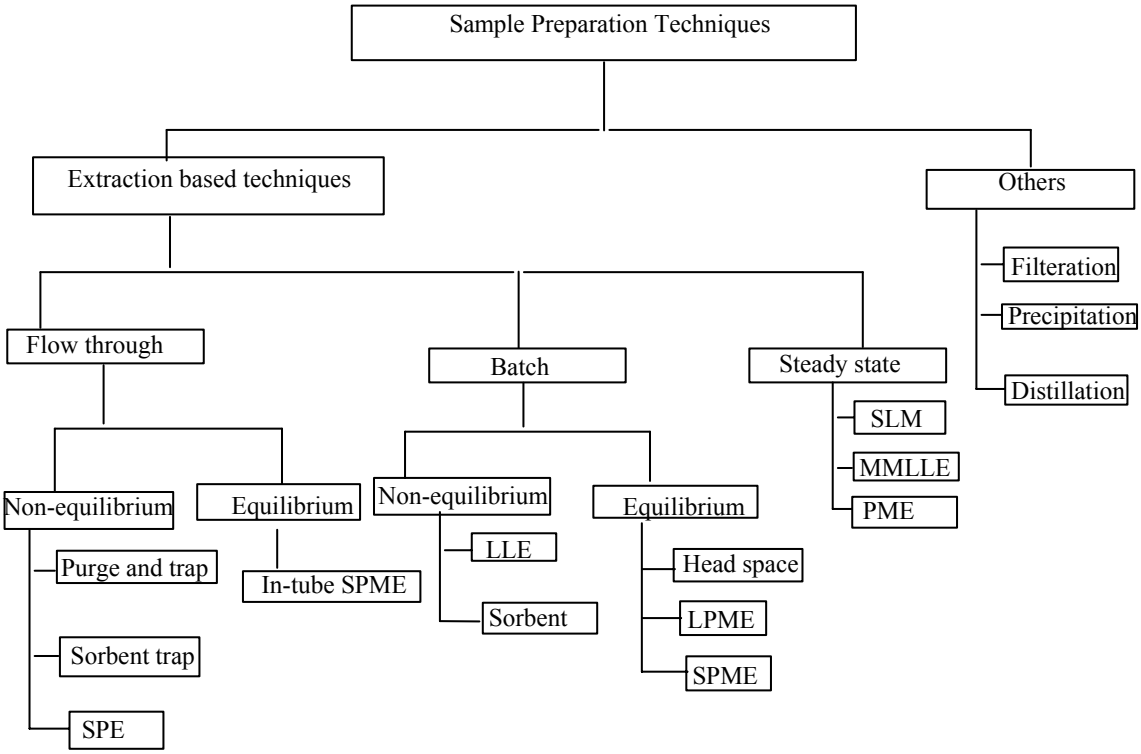


Fig. 2.2.1. Mode of classification of sample preparation techniques based on the ways of analyte isolation [83]

Accordingly, section 2.4 focuses on solid phase extraction and related topics, while various formats of the liquid membrane extraction will be presented in separate section, i.e., sections 2.2.5-2.2.9, for convenience, though they could also be classified under modern sample preparation techniques.

As can be seen from the Fig. 2.2.1, the majority of sample preparation methods are based upon extraction based techniques. These are generally classified into non-equilibrium and equilibrium based extraction methods. Among the extraction based techniques, the sorptive and membrane based extraction techniques have been used for extracting solutes from liquid or gaseous samples. These techniques include: solid phase extraction (SPE), solid phase microextraction (SPME) and stir bar sorptive extraction (SBSE). On the other hand, the membrane based extraction methods are: unsupported bulk liquid membrane (BLM), unsupported emulsion (surfactant) liquid membrane (ELM), supported liquid membrane (SLM) and synthetic

polymeric membranes. As most of these methods are applicable to the extractions of aqueous samples, brief discussion will be presented on each of these techniques.

As Raynie stated [82], extracting solutes from liquid or gaseous samples using sorptive phases has been described as “poor man’s chromatography”. The author has further explained that this is to mean that similar stationary phases are used, but the overall goal is the isolation of compound classes rather than the high resolution typically desired in analytical chromatography. This extraction mechanism likely started with conventional column chromatography, clean-up and fractionation and developed into an extraction mode with what is now considered SPE. That of SPME and SBSE has followed the development of SPE. These techniques will be discussed in the following sections briefly, focusing on their applications to aqueous samples.

2.2.4.1 Solid Phase Extraction (SPE)

SPE has developed as an alternative to liquid–liquid extraction (LLE) for the separation, clean-up, concentration and/or solvent exchange of solutes from solution. It was developed in the early 1970s with the development of small, disposable cartridge systems containing solid absorbents which could speed up the extraction process prior to analysis [79]. It was evolved from the early concept of classical liquid–solid extraction (LSE). SPE uses the principle of modern liquid chromatography, both High Performance Liquid Chromatography (HPLC) and Thin Layer Chromatography (TLC).

Solid phase extraction (SPE) involves bringing a liquid or gaseous sample in contact with a solid phase or sorbent whereby the analyte is selectively adsorbed onto the surface of the solid phase [5]. After washing of the solid phase with appropriate solvent to remove possible adsorbed matrix components the analytes are desorbed by using appropriate eluent. Generally, a SPE procedure consists of four consecutive steps—column conditioning, sample loading, column washing and elution, Fig. 2.2.2. When such a procedure is being developed, a suitable adsorbent material and suitable washing and eluting solvents must be selected, in accordance with the characteristics of the analytes and the matrix and the purpose of the analysis (screening or target

analysis) [20]. The final extract should also be compatible with the final analytical procedure (HPLC or GC).

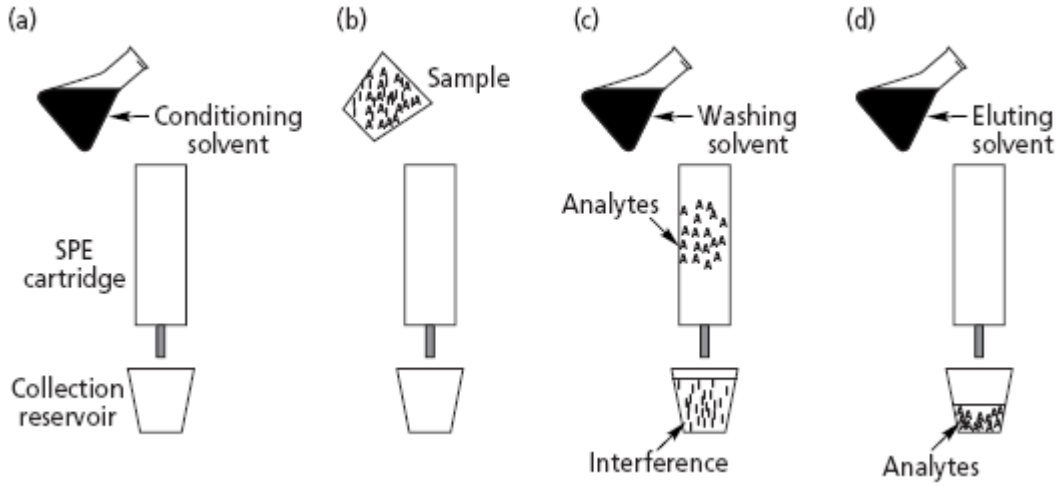


Fig. 2.2.2 Steps in an SPE [84]

SPE is now accepted as an alternative standard sample preparation method to LLE in many US Environmental Protection Agency (EPA) methods for analysis of organic compounds in drinking water and wastewaters [84].

An efficient SPE recovery of analytes from environmental water samples requires selection of a suitable SPE sorbent. This choice of SPE sorbents, techniques and subsequent sample treatment employed for environmental water analysis is dependent upon the analytical instrumentation used for final determination and the type of analytes to be analyzed [85].

Since analytical extractions are generally a preliminary step to the final method that produces the desired information, advantages of time, automation, reproducibility or solvent use must be shown by the type of sorbent chosen for a particular analyte [82]. Hence, SPE sorbents must be able to sorb rapidly and reproducibly, defined quantities of sample components of interest.

According to Sabik *et al*. [20] sorbent-analyte interactions can be categorized into three major types: non-polar, polar and ionic. Other mechanisms are molecular recognition mechanisms [86]

and combination of several interaction mechanisms which come from mixed mode adsorbents [87].

Non-polar sorbents are required for non-polar analytes and polar sorbents are required for polar analytes. Ionic sorbents should be used for ionizable analytes which can be facilitated by adjustment of pHs of the sample solutions. Some of the most commonly used sorbents and adsorbents for the extraction of pesticide residues from aqueous samples are C_8 and C_{18} chemically bonded to silica [88-92], carbon black [93, 94] and polymeric resins [95-98]. These sorbents gives low breakthrough volumes for highly polar pesticides or highly polar degradation products of pesticides [96].

In another mode of classification, the sorbents can be classified as: normal phase, reversed phase and ion exchange [99]. The most common sorbents are based on silica particles to which functional groups are bonded to the surface of silanol groups to alter their retention properties. The bonding of the functional group is not always complete, so unreacted silanol groups remain. These unreacted sites are polar, acidic and can make the interaction with analytes more complex. To reduce the occurrence of these polar sites, some SPE media are 'end-capped', that is, a further reaction is carried out on the residual silanols using a short-chain alkyl group [99]. In addition to silica, some other common sorbents are based on florisil, alumina and macroreticular polymer adsorbents.

Normal phase sorbents have polar functional groups, e.g., cyano, amino and diol (also included in this category is unmodified silica). The polar nature of these sorbents means that it is more likely that polar compounds, e.g., phenolic herbicides, will be retained. In contrast, reversed phase sorbents have non-polar functional groups, e.g., octadecyl, octyl and methyl and conversely are more likely to retain non-polar compounds, e.g., polycyclic aromatic hydrocarbons [99]. Ion exchange sorbents have either cationic or anionic functional groups and when in the ionized form attract compounds of the opposite charge. A cation exchange phase, such as benzene-sulfonic acid, extracts an analytes with a positive charge and vice versa. As such there are no significant differences between the two categories of classifications.

The selection of an appropriate SPE extraction sorbent depends on understanding the mechanism(s) of interaction between the sorbent and the analyte of interest [100]. That understanding in turn depends on the knowledge of the hydrophobic, polar and ionogenic properties of both the solute and the sorbent.

The problem of the extraction of polar analytes in environmental water has been partly solved by the introduction of carbon-based sorbents and highly cross-linked styrene -divinylbenzene (PS–DVB) co-polymers with high specific surface areas in the range of 500 to 1200 m^2/g and high degree of purity [101].

Mixed mode-sorbents containing both non-polar and strong ion (cation and anion) exchange functional groups are another kind of sorbents recently coming up to the extraction of basic compounds in aqueous samples. Restricted access packings which combine size-exclusion and reversed-phase mechanisms are another class of sorbents which allow extraction and clean-up of the samples in one same step. Silica gel is the most polar sorbent available. It is very useful for extract clean-up in the determination of non-polar analytes to remove out the polar interferents.

There are two common designs of SPE device; cartridge and disk formats. The more common of the two formats is the syringe barrel or cartridge. In this format the sorbent material, ranging in mass from 50 mg to 10 g, is positioned between two frits, at the base (exit) of the cartridge which act to both retain the sorbent material and to filter out particulate matter. Typically, the frit is made from polyethylene with a 20 μm pore size.

On the other hand, the disk is a membrane filter. The smaller length-to-diameter ratio of a disk allows greater flow and extraction rates relative to the cartridges [84].

The other new format of disc technology is the 96-well plate format that is used for rapid sample preparation and mainly for sample clean-up in the pharmaceutical and biotechnological industries [102]. The 96-well plates appeared in 1997 and their advantage is that they can be eluted with 100 to 200 μL of solvent.

SPE can be performed off-line; the sample preparation being separated from the subsequent chromatographic analysis, or on-line by direct connection to the chromatographic system [103-105]. On-line techniques do not require further handling of the samples between the trace-enrichment and the separation step and, therefore, are highly suitable for fully automated techniques [106-107].

SPE with different types of sorbents have been used for the determination of a number of pesticide residues in environmental water samples [108-115].

2.2.4.2 Molecular Imprinted Polymer (MIP)

The molecular imprinting technique is one of the most selective separation methods currently in use [116–121]. Various types of molecularly imprinted polymers (MIPs) have been prepared for the selective separation of target compounds from liquid matrices.

Molecular imprinting technologies offer advantages by providing polymers capable of molecular recognition and catalysis which can be used in conjunction with chromatographic separations, chemical sensing or catalytic reactions while at the same time being compatible with different organic solvents. MIPs are polymers produced in a process where functional and cross-linking monomers are copolymerized in the presence of a target analyte molecule, which acts as a molecular template.The concept behind this technique involves molding a material (with the desired chemical recognition properties) around individual molecules (template). Upon removal of the molecular template, the material retains its molded shape to fit and coincide with that of the template molecules. It is well known from both biology and chemistry that molecules tend to stick to receptors or surfaces with complementary shape, i.e., the ‘‘lock and key’’ theory of enzymes [122].

Thus, molecular imprinting results in materials that can selectively bind to molecules of interest. The use of MIPs in chromatographic separations is increasing in the recent years [123, 124]. They have been mostly used as chemical sensing [125]. S. Al-Kindy et al. [126] gave an overview of MIPs and optical sensing applications, an area showing the most important

applications of MIPs within the chemical sensing field. A molecular imprinted polymer (MIP) membrane for atrazine, not containing macropores, was synthesized and implemented in a potentiometric sensor by G. D´Agostino *et al*. [127]. They indicated that it works like a solid ISE, the specific carrier being the imprinted site. The active ion is the protonated atrazine, which was positively charged at lower pH (< 1.8). MIPs have also been employed as sorbents in SPE [128-130].

Molecularly imprinting is a powerful way to achieve three dimensional molecular recognition via the template-directed synthesis of highly cross-linked polymeric matrices. MIPs are attractive materials that enable the selective extraction of small molecule from complex mixtures [131]. MIPs have been extensively used in high performance liquid chromatography (HPLC), CE separation [132–134], thin layer chromatography (TLC) [135], solid phase extraction [136,137], catalysis [138,139], sensors [140–142] and as artificial antibodies in binding assays [143].

2.2.4.3 Solid Phase Microextraction (SPME)

SPE has a number of attractive features compared to traditional solvent extraction. SPE is simple, inexpensiveand uses relatively little solvent. But it is also with some limitations [145]. The limitations for this technique are:

- the interaction between matrix and analytes often results in low recovery. Solid and oily components in a sample matrix may plug the SPE cartridge or block pores in the sorbent causing it to become overloaded.
- sorbent suffer from high carryover values and batch-to-batch variation of sorbents leads to poor reproducibility.
- limited to semi-volatile or non-volatile compounds with boiling temperature substantially above that of the desorption solvent.

Application of sorbing material permanently attached to a fiber addresses these limitations and allows re-use of the same extraction phase. The resulting technique was known as solid phase microextraction (SPME) and was first developed by Pawliszyn in 1990 [146], as a practical

alternative to LLE and SPE. SPME was commercialized by Supelco since 1993 [146] and it is the process whereby an analyte is adsorbed onto the surface of a coated-silica fibre as a method of concentration. This is followed by desorption of the analyte into suitable instrument for separation and quantitation [5, 83]. Its principle is based on the partitioning of analytes between sample matrices and a stationary phase, coated on a fused silica fiber. Figure 2.3 shows the schematic diagram of SPME [147].

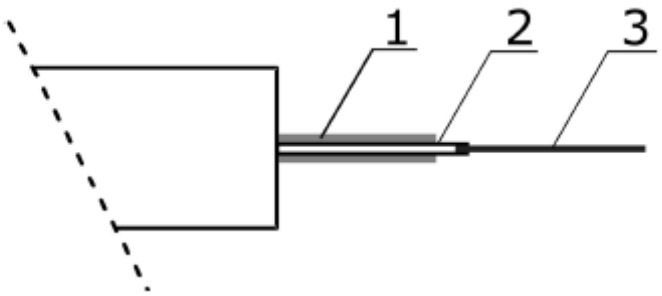


Fig. 2.2.3 Schematic diagram of the SPME device: 1, needle; 2, tube to which the quartz fiber is fixed and 3, the quartz fiber covered with the sorbent film (see text for details) [147]

The superiority of SPME over LLE, SPE and other similar techniques comes from its integration of direct sampling, extraction, enrichment, clean-up and analyte introduction into the analytical instruments in a single device [148]. SPME presents numerous other advantages over LLE, such as simplicity and economy of use; it lends itself well to automation, requires no organic solvents and does not involve dilution of the sample to be analysed [148]. SPME has been widely used in different areas in either headspace or immersion modes for the determination of a variety of compounds [149-153]. The technique is now well established and as a result there are a number of excellent reviews [150, 154 - 163] in the literature. In this dissertation emphasis will be given to its application to aqueous samples for the extraction of pesticide residues and their degradation products. For the details of other applications the reader is kindly referred to other review [160]

At least three modes of SPME applications have been developed: direct-immersion [164], headspace [165] and membrane-protected SPME [166].

In the direct extraction mode the coated fibre is inserted into the sample and the analytes transported directly from the sample matrix to the extracting phase [85]. Agitation is required for rapid extraction under this mode of extraction. In the headspace mode, the analytes are extracted from the gas phase equilibrated with the liquid phase. The primary reason for this modification is to protect the fibre from adverse effects caused by non-volatile, high molecular weight substances present in the sample matrix (e.g., humic acid or proteins). In the third mode of extraction, the fibre is separated from the sample by a selective membrane which allows the analytes pass through the membrane while blocking the interferents. The main purpose of the introduction of the membrane is again to protect the fibre from adverse effects caused by high molecular weight compounds in the matrix when dirty water samples are analyzed [85].

The extraction of a given analyte into the SPME phase mainly depends upon the compounds´ affinity towards the coatings on the fused silica fibre, which can be a liquid polymer, a solid sorbent or a combination of both. Therefore, the choice of appropriate coatings for a particular class of analytes is crucial.

SPME has been successful in its application to the analysis of drugs, food, environmental contaminants and others [99, 167-169]. This success has been achieved through the development of many kinds of new specific coating materials on the fused silica fibre. A large number of fibre coatings based on solid sorbents are commercially available. Most of these are based on poly (dimethylsiloxane) (PDMS) for extracting non-polar analytes and polyacrylate (PA) for extraction of polar analytes. In addition to these coatings there are other innovative designs of copolymers, which further widen the application of SPME. Some of these coatings are PDMS/divinylbenzene (DVB), carbowax/DVB, Carbowax/template resin (TR), carboxen/PDMS and DVB/carboxen/PDMS [170].

The extraction of analytes by these new porous polymer SPME fibers with mixed coating is primarily based on adsorption rather than absorption. Several of these porous polymer SPME fibers with bipolar characteristics are very useful for the simultaneous analysis of pesticides and other contaminants in environmental water samples enlarging the scale of SPME applications [171-174].

Since most of these coatings are applicable to the extraction of non-polar analytes, efforts are undergoing to design coating materials for polar analytes also. Most of these efforts are based upon in-house fibre productions, for polar analytes other than PA. Most of these in house fibre productions are based upon sol-gel technologies. As Chong *et al*. [175] explained, these are tailor-made preparation of fibre coatings that provides efficient incorporation of organic components into inorganic polymeric structures in solution under extraordinarily mild thermal conditions. Dietz *et al.* [176] have presented an overview of the recent developments in new coating procedures and materials, including the respective applications in their recent extensive review.

SPME is attractive for its solvent-free feature, speed of extraction, convenient automation and hyphenation with analytical instruments such as GC, HPLC and capillary electrophoresis (CE). Nearly all of the applications of SPME are based on chromatographic determinations. Most SPME applications have been performed in combination with gas chromatography (GC) where, after the extraction step, the analytes are thermally desorbed into the injector of the chromatograph. This coupling of SPME to GC is generally applied to the analysis of volatile and thermally stables contaminants in aqueous samples. For thermally labile and non-volatile analytes derivatization step is required [177, 178] for GC analysis. Derivatization can be done in the sample directly, on the fibre, after or during extraction process or in the GC injector [179]. The other possible coupling for the application of SPME to the analysis of polar and thermally labile contaminants is its interfacing with high-performance liquid chromatography (HPLC) [180, 181] and with capillary electrophoresis (CE) [182].

SPME–GC and SPME–HPLC interfacings are different from each other in the desorption step of the extraction process (see Fig. 2.2.4). The status of SPME coupling with HPLC and its application to the analysis of different matrices has been the focus of Zamonin's recent review [183]. According to the author, a special type of interfacing is required in the case of SPME-HPLC coupling. Such desorption interface consists of a standard six port HPLC injector with a special fiber-desorption chamber (Supelco, Bellefonte, PA, USA) installed in place of the sample loop. Desorption is performed in the mobile phase or in another solvent mixture. Desorption with

the mobile phase was found to be the best solution and can be performed even in the flowing mobile phase [183].

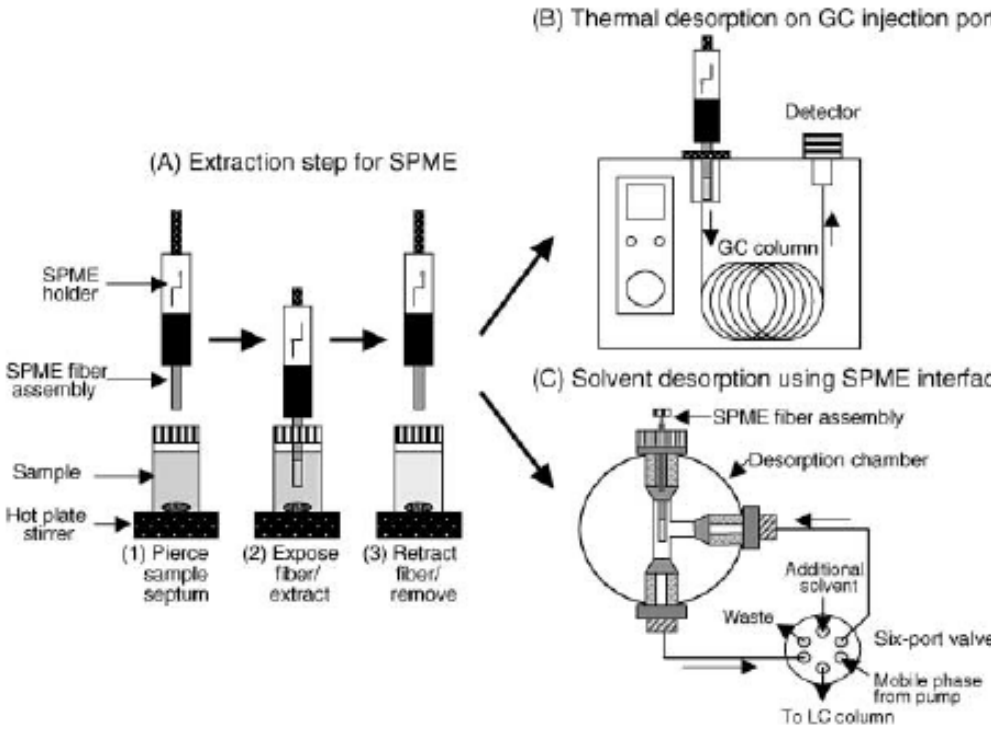


Fig. 2.2.4 Procedure of extraction by fiber SPME and desorption for GC and HPLC analyses [183]

SPME is superior to LLE and SPE sample preparation techniques in that: it is simple and fast to use, solvent free and above all single step sample preparation technique. As a result, SPME has applied to the study of nearly all types of aqueous matrices for pesticide analysis [184]. Among these, the intensively studied pesticides are organochlorine, organophosphorus and triazine. Recently Sánchez-Ortega *et al.* [185] developed a methodology for the analysis of the insecticide fenitrothion and its two main environmental degradation products; fenitrooxon and 3-methyl-4-nitrophenol in water samples and applied to river water samples. They have obtained a satisfactory reproducibility for extractions from water samples at 20 ppb-level with RSD < 12.5% (n = 10). Sakamoto and Tsutsumi [186] have applied an automated headspace SPME (HS-SPME) system for the determination of multi-class pesticides in various water samples and found out that the HS-SPME was effective in pesticide residue identifications. Prosen, *et al.*

[187] have applied SPME to the evaluation of partitioning of certain selected pesticides into dissolved humic acids by measuring the free concentration of these pesticides using SPME. A number of other papers showed the applicability of SPME to the analysis of pesticide residues in environmental water samples in different modes of applications [188-192]. There are also reviews dedicated to the application of SPME to the analysis of pesticide residues in different matrices including water matrices [150, 153].

The basic principles of SPME have been given in detail in reference 119. SPME is an equilibrium extraction device. Hence, the transfer of analytes from the matrix into the coating begins as soon as the coated fiber has been placed in contact with the sample. Then, the process of analyte transfer continues until equilibrium between the sample matrix and the fiber coating is reached. After this time, there would not be any net change in the transfer of analyte from the sample matrix into the fibre coating and the extraction is said to be completed. This equilibrium condition can be expressed as [147]:

$$n = K_{fs}V_fV_sC_o/(K_{fs}V_f + V_s) \quad (8)$$

where n is the amount of analyte extracted by the coating, K_{fs} is a fibre coating/sample matrix distribution constant, V_f is the fibre coating volume, V_s is the sample volume and C_o is the initial concentration of the analyte in the sample. When sample volume is very large ($K_{fs}V_f << V_s$) and equation (7) can be simplified as:

$$n = K_{fs}V_fC_o \quad (9)$$

This case is true in field application where the sample volume is taken to infinitely large. Further details of SPME theory have been given in reference 147.

2.2.4.4 Stir-Bar Sorptive Extraction

Stir-bar sorptive extraction is a new solventless sample preparation method for the extraction and enrichment of organic compounds from aqueous matrices. The method is based on the same principles as solid-phase microextraction (SPME). A stir-bar is coated with a sorbent and immersed in the sample to extract the analyte from solution.

The sample is typically stirred with the coated stir-bar for a specified time depending on the sample volume and the stirring speed to approach equilibrium [193]. The sorbent used for coating the stir-bar is polydimethylsiloxane (PDMS).

Using this sorbent, the primary mechanism of interaction with organic solutes is via absorption or partitioning into the PDMS coating. Extraction of aqueous samples occurs during stirring at a specified speed for a predefined time. After a given stirring time, the bar is removed from the sample and is usually thermally desorbed into a gas chromatograph. This extraction phase is the same as that used on polydimethylsiloxane-coated fibres in SPME, but here the coating uses 50 to 250 times greater amounts of extraction phase [193].

Baltussen and co-workers [194] recently reviewed the principles and applications of sorptive extraction. The main difference between SPME and stir-bar sorptive extraction is the much higher mass of polydimethylsiloxane available in the latter, which results in high recoveries and higher sample capacity.

2.2.5 Membrane-Based Sample Preparation Methods

Membranes can be viewed as a selective barrier between two phases [195]. According to a review by Merbel [195], when a driving force is applied across a membrane, transport of matter from one phase (donor phase) to the other (acceptor phase) occurs, as depicted in Fig. 2.2.5. The most common driving forces are the concentration, pressure, chemical potential and temperature gradients. Separation takes place when one species is transferred from the donor to the acceptor.

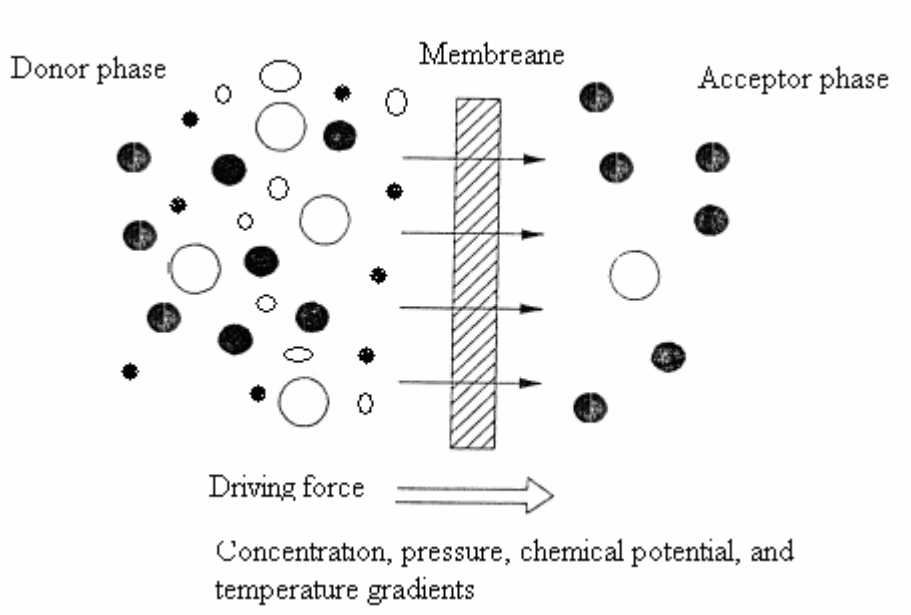


Fig. 2.2.5 Schematic representations of a membrane system [195]

Mass transfer across a given membrane is characterized by the type of the membrane used and the mechanism of mass transfer through the membrane [196]. Jakubowska *et al.* [196] very recently reviewed the membrane extraction methods in determination of various organic compounds in liquid environmental matrices and biological fluids extensively. According to these authors membranes can be classified based upon membrane state, membrane morphology (closely related to their porosity and internal structure) and membrane shape. Fig. 2.2.6 depicts the bases of classification of membranes and the major types of membranes in each classification [196].

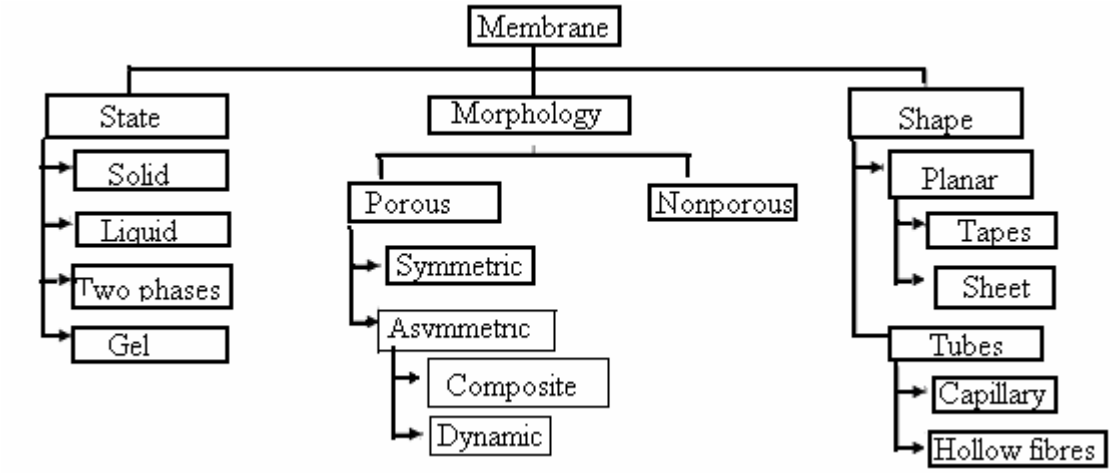


Fig. 2.2.6 Classification of membranes with respect to their state, morphology and shape

The most common mechanisms of membrane transport are [196]: (1) molecular diffusion due to chemical potential and temperature gradients; (2) the electromigration of charged particles in an electric field due to potential difference between the interfaces or under the action of an external current source; and (3) convective transport with a moving medium that contains substances to be separated.

2.2.5.1 Membrane Techniques

There are a number of analytical techniques that make use of different kinds of membranes. Porous membranes separate components of a mixture as a result of the sieving effect (size-exclusion) [195]; molecules with smaller diameter than the pore size of the membrane pass through the membrane. Therefore, its selectivity depends upon its pore size. On the other hand, in non-porous membranes; separation of substances is based upon the solubility and diffusion coefficients of the individual component of the mixture in the membrane material. A porous membrane impregnated with a liquid or a membrane made of a solid material, such as silicone rubber, can be used as a non-porous membrane [195].

Membrane filtration, which includes microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO) [195], is one of the basic membrane techniques used in analytical practices. The driving force of these processes of membrane filtration is the pressure difference across the membrane in a membrane module. Under the pressure gradient applied and due to selective operation of the membrane, some components of a solution penetrate the membrane while others remain in solution.

Dialysis is another membrane technique in which the separation of components of a mixture is based upon concentration gradient. Separation of components is based on differences in diffusion rates of solutes in the membrane material, which in turn results from the differences in the size of molecules [19]. The parameters which control the efficiency of separation by a given membrane in this case are the ratio of the flow rates in the flowing channel, the rate of mass transfer between the two phases, i.e., the donor and acceptor phases, the dimensions of the channels and others. This membrane technique is mainly applied in the field of biomedical and food analyses.

Electrodialysis is another membrane technique in which ionic components of a solution pass across the membrane driven by an external electric field. The membranes used in this process are ion-exchange membranes capable of selective transfer of ions. In this membrane separation, the applied potential across the membrane causes the transfer of the analyte to the acceptor channel and more advantageous over the ordinary dialysis in which the transfer of the analyte is based upon diffusion. As a result, quantitative transfer of analytes can be achieved in electrodialysis as compared to ordinary dialysis.

2.2.5.2 Membrane Extraction

As indicated in Fig. 2.2.6, membranes can morphologically be divided into porous and non-porous membrane systems. In a porous membrane system the two phases on either side of the membrane are physically connected through the pores. These membranes, as briefly discussed above, are used in dialysis and filtration to separate low-molecular-mass analytes from high-molecular-mass matrix components leading to an efficient clean-up but no discrimination

between different small molecules [197]. No enrichment of the small molecules is possible; instead the analytes are diluted since the driving force of the mass transfer process is a simple concentration difference across the membrane.

In most of the cases, membrane extraction techniques utilize non-porous membranes. Non-porous membranes can either be a porous membrane impregnated with organic liquid or entirely solid (such as silicon rubber) [198]. A non-porous membrane may have micropores, as in microporous membrane liquid-liquid extraction technique [198]. The non-porous membrane extraction techniques include supported liquid membrane extraction (SLM), microporous membrane liquid-liquid extraction (MMLLE), hollow fiber based liquid phase microextraction (HF-LPME), polymer membrane extraction, semipermeable membrane devices (SPMDs) and membrane extraction with a sorbent interface (MESI). The last three are briefly described as under and the former three will be dealt with in greater details as these methods were used in this thesis work.

2.2.5.3 Polymeric Membrane Extraction

The polymeric membrane extraction is done using an entirely solid membrane like silicone rubber. The difference in solubility and diffusion of various analytes from the sample towards the polymeric membrane is the basis of separation and selectivity [199]. The trapping of the analytes in the acceptor phase can be enhanced by adjusting the pH of the acceptor phase in such a way that the analytes can be ionized similar to SLM (discussed in section 2.6) [200]. It is possible to trap hydrophobic analytes in organic acceptor phase [201] similar to MMLLE (see also section 2.6.3). The solid nature of the membrane makes the extraction versatile in that it can be applied to aqueous, organic and gaseous samples [195, 202]. In all of the cases, the mass transfer is achieved by the first partitioning of the analytes into the polymer, diffusion through it and then partitioning into the receiving phase [199]. The main disadvantage of the use of solid membrane is that it is not possible to change the chemistry of the membrane as in the case of liquid membrane.

2.2.5.4 Membrane Extraction with Sorbent Interface (MESI)

The membrane extraction with sorbent interface uses gases as acceptor phases and can be applied to the separation of analytes from liquid or gaseous samples.

The most common type of membrane used in this kind of extraction is the non-porous ones like silicon rubber. The extraction is usually carried out using the membrane module made of silicone rubber, into which the analytes extracted from the surrounding gaseous or liquid sample. A stream of inert gas flowing inside the membrane transports the extracted analytes to a cryogenic or sorption trap. The analytes retained in the trap are subsequently released by way of thermal desorption and introduced with a stream of carrier gas onto the inlets of a GC column [203]. The basis of the separation of analytes from the matrices is similar to PME, the differences in solubility and diffusion of the analytes into the non-porous polymer membrane. This technique is only applied to volatile analytes.

2.2.5.5 Semi-Permeable Membrane Devices (SPMDs)

SPMDs are membrane based extraction devices suitable for the determination of freely dissolved contaminants from aqueous samples. In SPMDs, hydrophobic organic contaminants passively diffuse from aqueous donor phase through a polymeric membrane such as polyethylene into the acceptor phase filled with a thin film of high molecular mass neutral lipid like triolein [204]. The dissolution and diffusional transport of the contaminants through the non-porous polymer membrane is the basis of extraction. Therefore, this extraction method provides bioavailable time-weighted average pollutant concentrations over long period of time [205].

2.2.6 Supported Liquid Membrane

Audunsson first introduced SLM in 1986 [206] for the on-line extraction of organic amines from water samples. A number of subsequent applications of supported liquid membrane have been reported since its inception. The extraction principles, mode of application and all other aspects of this technique are discussed below.

SLM consists of a porous membrane support impregnated with a water-immiscible organic solvent [78] trapped by capillary in the membrane pores. It is also possible to use non-porous silicone rubber as a membrane material [195]. This case is not SLM proper, but chemically equivalent. The membrane impregnated with an organic solvent is mounted between two flat blocks made of polytetrafluoro ethylene (PTFE). In these blocks corresponding spiral grooves are machined, forming a flow channel on each side of the membrane. The whole device is connected to pumps which independently pump the donor and acceptor solutions. By proper selection of the solutions pumped through each of the channels on each side of the membrane, compounds can be selectively extracted from one solution (the donor) into the organic membrane liquid and, subsequently, extracted into the other solution (the acceptor).

An SLM system for sample preparation uses minimal amount of organic solvents. This makes it an environmentally friendly technique. The SLM technique and its analytical applications have been reviewed extensively [78, 207] and these reviews showed that SLM offers an advantage of combining selective extraction, enrichment and clean-up processes in a single step over other sample preparation procedures in that it combines selective extraction, enrichment and clean-up in one step.

In SLM the membrane separates two aqueous phases and the pH of the donor channel is adjusted in such a way that the analytes exist in neutral state and the acceptor channel pH would be adjusted in the mode which enables the ionization of the analytes so that they cannot back extracted into the organic phase. Fig. 2.2.7 depicts the principles of SLM extraction [207].

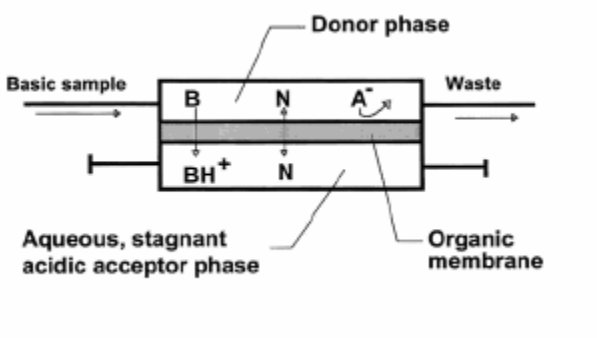


Fig. 2.2.7 Schematic description of the SLM principles

The analytes are partitioned from the donor aqueous sample into the organic membrane and are then re-extracted into the aqueous acceptor phase. The driving force of the mass transfer is the concentration gradient between the donor and acceptor phases. This extraction method is best applied to ionizable contaminants or contaminants which are able to exist in two forms: in neutral non-ionic form on the donor side to be extracted into the organic membrane and in ionized form on the acceptor side in order to be permanently trapped in the acceptor phase. This is accomplished by the adjustment of the pH in both aqueous phases as described above or by adding an ion-pairing or complexing agent in the aqueous sample or in the membrane liquid if the analytes are permanently charged [208]

This technique is superior in its extraction of polar analytes ($2 < \log P < 4$) in which other extraction methods encounter difficulties. Three physical realization of SLM modules have been reported [209] and some are shown in Fig. 2.2.8. These are: the flat, spiral and hollow fibre modules. The ratio between the surface area and its volume of each module plays an important role in analytical applications [210]. This ratio should be high to get high enrichment factors. The flat module and/or the hollow fibre modules are widely applied with channel volumes ranging 10-1000 μL [198]. Some of the hollow fibre modules use less volume, 1.3 μL [211]. According to Audunsson [206], an ideal membrane support is the one that gives high solute permeation and stable membranes. Thin membranes with smaller pores (0.2 μm) with polyethylene backing are generally employed in most of the SLM applications. These kinds of membrane supports were found out to facilitate mass transfer and at the same time be able to retain the impregnated liquid solvents in their pores firmly.

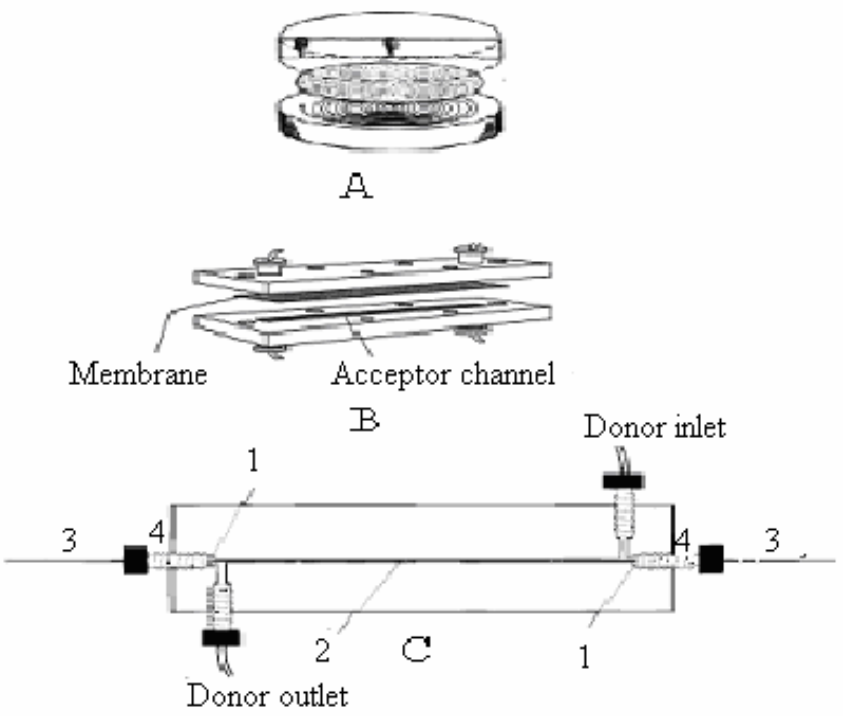


Fig. 2.2.8 Different modules of SLM technical set-ups; (A) Flat (B) membrane unit with 10 µL volume and (C) hollow fibre (1 = O-ring, 2 = polypropylene hollow fibre, 3 = fused silica capillaries, 4 = male nuts)

The liquid membrane solvents most commonly used are long chain hydrocarbons like *n*-undecane or kerosene and more polar compounds like di-*n*-hexyl ether, di-*n*-octyl phosphate and others depending on the type of contaminants analyzed [206].

2.2.6.1 Basic Principles of SLM

The details of the principles of SLM extraction have been given in a number of reviews and research papers [78, 195, 196, 207, 211]. SLM extraction can be made very selective by fine-tuning the three conditions in the three phases as described in Fig. 2.2.9. If all of the conditions are adjusted, as indicated in the figure, analytes belonging to the same family are trapped in the acceptor at a given time. Macromolecules cannot be extracted into the pores of the membrane

because of their large sizes, slower diffusion and usually they are charged. Charged molecules are too polar to dissolve in the organic phase. Neutral molecules may be extracted in the organic phase and diffuse into the acceptor phase but since they will be distributed between the three phases they will not be enriched to any extent.

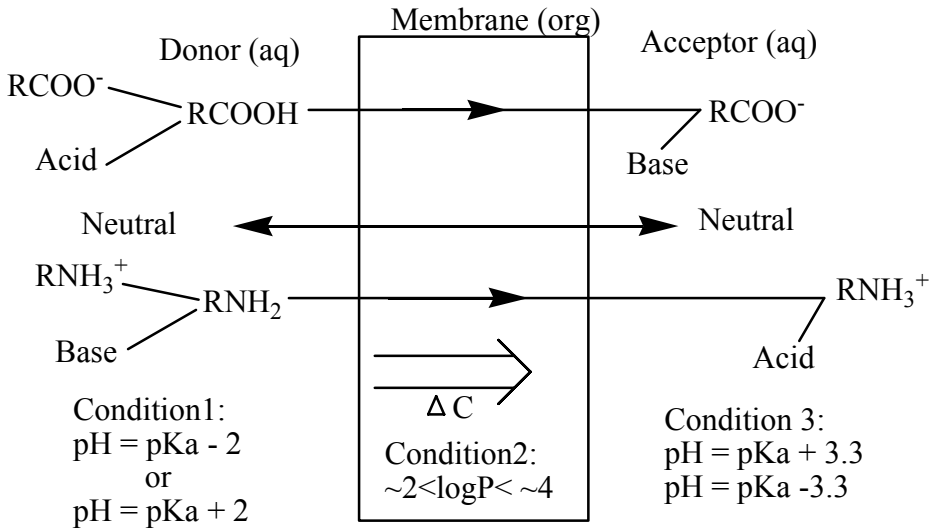


Fig. 2.2.9 Schematic description of the SLM principle for the extraction of basic analytes [206]

Selective transport is not always straightforward without any problem. Sometimes enrichment can be so low because of the low solubility of the analytes in the organic phase despite the fact that the trapping in the acceptor phase can easily be achieved. Under this condition, the use of mobile carrier substance in the membrane that selectively binds the analytes would be a viable solution. The use of carrier molecules makes SLM more versatile and applicable to the extraction of permanently charged analytes like metals [212] and bipyridylnium herbicides [208].

The transport mechanism in liquid membrane has been explained in detail elsewhere [19]. The membrane composition has a significant influence on the selectivity and permeation of the liquid membrane. As Megersa described [19], two transport mechanisms exist across a liquid membrane. These are: passive transport and carrier mediated transport. Passive transport is the one in which mass transfer is achieved by simple diffusion across the membrane.

The carrier-mediated transport is based on the use of a carrier in a liquid membrane. Five carrier mediated transport mechanisms are known: simple carrier mediated transport, simple carrier transport with chemical reaction in the acceptor, carrier mediated coupled co-transport, carrier mediated coupled counter transport and primary active transport [19, 213, 214].

One example of simple carrier transport is the use of Tri-*n*-ocylphophine oxide (TOPO) for the extraction of short chain aliphatic carboxylic acids from acidic donor solution to an alkaline acceptor solution [214]. Other such application is again the use of TOPO for the extraction of *s*-triazine degradation products from environmental water matrices [215].

The enrichment factor, E_e and the extraction efficiency, E, are the two parameters used to evaluate the effectiveness of a particular SLM extraction. The enrichment factor is defined as the ratio of the analyte concentration in the extract to that in the initial donor phase.

$$E_e = C_a/C_d \tag{10}$$

where *C*a and *C*d represent the analyte concentration in the acceptor after extraction and in the initial donor.

The E refers to the fraction of analyte that is extracted into the acceptor such that:

$$E = n_a/n_d = C_aV_a/C_dV_d, = E_eV_a/V_d \tag{11}$$

where n_a and n_d represent the total mass of the analyte in the acceptor and donor, respectively and V_d and V_a are the volumes of the donor and acceptor (sample and extract) channels, respectively.

The extraction efficiency is affected by many factors. Some of the factors which affect the efficiency of extraction in SLM are: the partition coefficient of the uncharged analyte species between the aqueous phase and the organic membrane, the trapping conditions in the acceptor phase, the flow rate of the donor solution and the dimensions, and characteristics of the membrane.

The influence of the partition coefficient, K, is not straightforward. At low values of K, E is small, naturally this is due to the low transfer of analytes from aqueous to the organic membrane. This is the condition where the mass transfer is limited by the diffusion through the membrane (membrane controlled extraction conditions). For intermediate value of K, the mass transfer is limited by the diffusion of analytes in the donor phase (donor controlled condition) and this gives high efficiency of extraction. At very high value of K, for hydrophobic analytes, the rate of analytes transfer from the organic to the acceptor phase would be slow and becomes the limiting factor. This time the extraction efficiency would be reduced, as significant amount of analytes will be left in the membrane phase.

The rate of mass transfer is governed by the concentration difference between the two phases [209, 216].

$$\Delta C = \alpha_D C_D - \alpha_A C_A \qquad (12)$$

where C_D and C_A are the concentrations in the donor (sample) and acceptor phases, respectively; α_D and α_A are the fractions of the analytes that are in the extractable (uncharged) form in the donor phase and acceptor phases, respectively. The extraction condition is adjusted in such a way that the α_D is close to 1 and α_A is very small value. C_A is zero at the beginning of the extraction and increases during extraction process to values greater than C_D.

As long as α_A is kept very small, the second term in equation (11) is negligible and the extraction efficiency will be constant during the extraction process, hence the extracted amount will be directly proportional to the volume of the sample and the concentration of analytes in the sample. This is the exhaustive type of extraction and in most of the cases the one that is required.

SLM system of sample preparation uses minimal amounts of organic solvents [217]. This makes it an environmentally friendly technique. As a result, it has been applied to the analysis of aliphatic amines in urine [218] and blood plasma [219] at low-ppb levels using *n*-undecane as liquid membrane. Short-chain aliphatic amines [220], biogenic amines [221], phenolic

compounds [222], bipyridlium herbicides [208] have also been determined in environmental samples.

This versatility of the technique initiated many researchers to extend its scope of application. To this effect, a time-integrated field sampling has been introduced and applied under laboratory situations by Mathiasson et al. [223] and by Knutsson et al. [224] for long time field sampling of acidic herbicides (six phenoxy acid herbicides) from agricultural lands. More recently, the uses of an entirely automated SLM extraction for carrying out unattended trace enrichment of *s*-triazine herbicides and their breakdown products from various matrices has been demonstrated by Megersa et al. [205].

To further widen the scope of applications of the technique for the environmental real water analysis in its actual settings, we have applied a portable automated time integrating field sampler based on SLM principles to the analysis of s-triazine herbicides and their degradation products in water samples of Lake Awassa, see also section 4.3.

2.2.6.2 Equilibrium Extraction in SLM

If extraction process proceeds for sufficient time, the concentration in the acceptor, C_A increases and eventually the second term in the equation (11) above will become significant and ΔC decreases. This leads to lower rate of mass transfer and a decrease in E. When ΔC has reached zero, all of the three phases are in equilibrium and maximum concentration enrichment factor possible is reached.

$$E_{e(max)} = C_A/C_D = \alpha_D/\alpha_A \qquad (13)$$

If $\alpha_D \cong 1$ and $\alpha_A \leq 0.0005$, signifying complete trapping, $E_{e(max)} \geq 2000$, so it is possible to exhaustively extract the analyte. But if the extraction condition is in such a way that for example $\alpha_A = 0.1$, the maximum enrichment factor is only 10 and this can be achieved very easily and the system would be at equilibrium. So it is possible to use this technique to measure equilibrium concentrations by controlling the extraction conditions.

Indeed SLM extractions have been applied under both equilibrium [225] and exhaustive extraction modes. Therefore, in one of the current thesis works covered, we have applied the principle of this technique to the exhaustive extraction of trace levels of dinitrophenolic compounds in environmental water samples using hollow fibre based SLM (HF-SLM). The use of HF-SLM as a sample preparation methodology has also been termed as liquid phase microextraction (LPME) and will be dealt with in greater details in the following section 2.3.5.

2.2.6.3 Microporous Membrane Liquid-Liquid Extraction (MMLLE)

The microporous membrane liquid-liquid extraction (MMLLE) method of sample preparation is the two phase mode of the SLM technique applied to the extraction of hydrophobic analytes. In this technique the acceptor is an organic solvent and the same solvent forms the liquid membrane by filling the pores in the porous hydrophobic membrane [211]. The hydrophobic membranes are usually PTFE or polypropylene membrane supports. It is best applied to the analysis of uncharged hydrophobic analytes, which cannot be extracted with SLM. As the extracts are organic, the technique is better interfaced with gas chromatography and normal phase HPLC than SLM, which is best compatible with RP-HPLC.

MMLLE was introduced in 1986 by Sahleström and Karlberg [226]. In this technique, there is one extraction step unlike the case of SLM, which involves two extraction steps. However, it allows continuous liquid-liquid extraction to be made in a compact closed system, similar to SLM. The aqueous donor sample is pumped to one side of the microporous membrane while the organic acceptor, on the other side, remains either stagnant or is slowly flowing during the extraction.

Virtually, the principle of MMLLE is similar to that of classical LLE. MMLLE is performing LLE in a continuous and instrumental way. The main differences are: in LLE the system of the two phases are in equilibrium and the only factor affecting the extraction yield is the partition coefficient, K, between the two phases [227]. In non-equilibrium system (for example, high flow

rate in MMLLE), factors such as diffusion and membrane pore size affect the mass transfer and in that way also the extraction efficiency.

If the partition coefficient is very high, it is possible to use a stagnant acceptor phase and achieve considerable enrichment in a small extract volume. The stronger the hydrophobicity of the analyte the higher will be the extraction efficiency. With stagnant acceptor phase the maximum value of the obtainable concentration enrichment factor (E_e) is given by [228]:

$$E_e = K_d \tag{14}$$

In case of smaller partition coefficient, the mass transfer can be enhanced by arranging the acceptor phase to flow slowly during the extraction in order to successively remove the extracted analyte and maintain the diffusion through the membrane. According to Shen et al. [228], the acceptor and donor flow rates influence the extraction efficiency of MMLLE. Assuming that the partition is in equilibrium and that the flows in the channels are parallel, the extraction efficiency is given by the equation:

$$E = 1\text{-}F_D/(F_D + F_A K_d) \tag{15}$$

where F_D and F_A are the donor and acceptor flow rates, respectively.

It is possible to see from the above equation that with increasing acceptor flow rate the extraction efficiency increases. But the concentration of analytes in the acceptor is diluted and the enrichment factor, E_e, decreases:

$$E_e = 1/ (1/K_d + F_A/F_D) \tag{16}$$

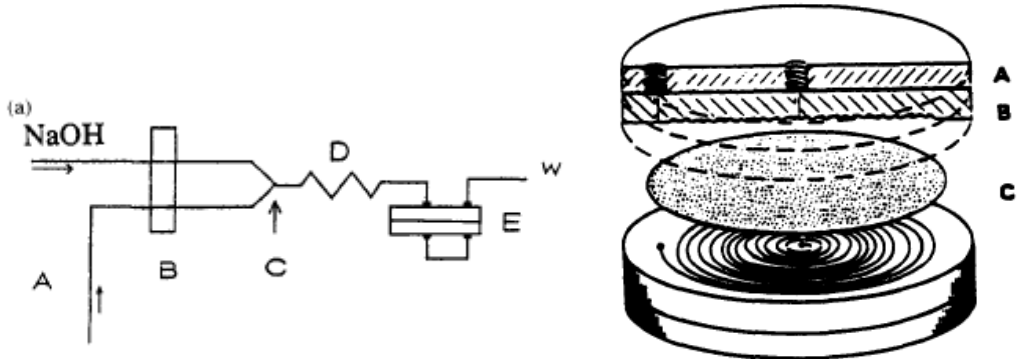
2.2.6.4 Hyphenation and Automation

Automation of sample preparation technique leads to higher sample throughput and less manual operations with obvious economical benefits. Automation significantly increases the efficiency of the general analytical systems as a whole [198]. As described in a recent review of membrane-based techniques for sample enrichment membrane extraction techniques are well suited for automation and automatic connection to chromatographic instruments [198].

2.2.6.5 Flow Systems for Membrane Extraction

A manual off-line membrane extraction flow systems has been built up around peristaltic pumps and membrane units with channel volumes around 1 mL by Knutsson et al. [229], Fig. 2.2.10.

Direct on-line transfer of the acceptor phase from the membrane extraction unit to an HPLC system was originally designed for SLM extraction of chlorinated phenols from natural waters [229] and was further applied to other environmental samples [230] and shown in Fig. 2.2.11.



(a) (b)

Fig. 2.2.10. (a) Set up for membrane enrichment of basic herbicides in water: A, herbicide sample; B, peristaltic pump; C, confluence point of sample and NaOH solution; D, mixing coil; E, membrane separator with stagnant acceptor solution; W, waste. (b) The membrane separator: A, aluminium backup; B, PTFE block with grooves like Archimedes' spiral; C, impregnated liquid membrane [229]

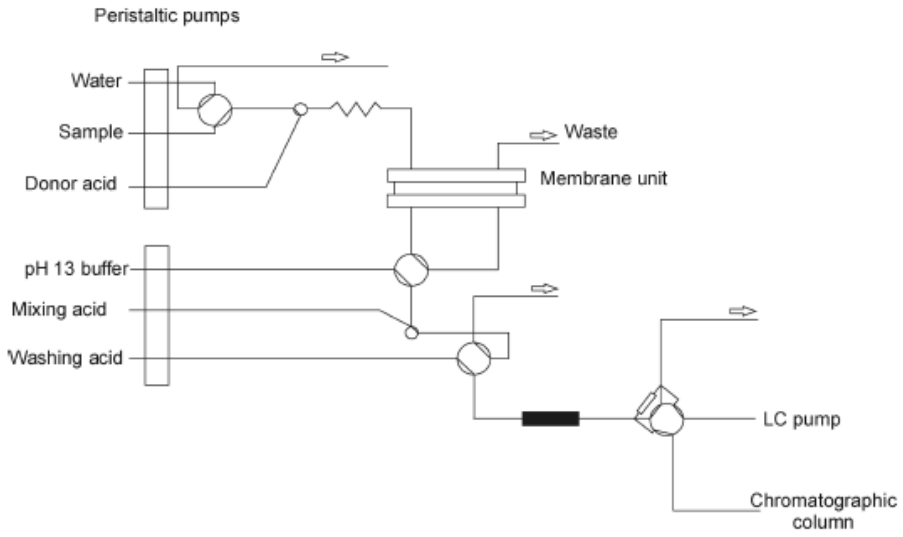


Fig. 2.2.11 SLM systems online connected with HPLC [229]

An MMLLE-GC system has been described by Shen et al [229] showing that it is possible to directly interface the MMLLE system with GC. A newer MMLLE extraction in microscale has recently been developed and applied to the extraction of phthalate esters in aqueous environmental samples [231]. In this particular system the organic extract was directly injected into the GC system by means of an injection needle, directly connected to the extraction cell. Fig. 2.2.12 shows Schematic diagram of the extraction syringe (ESy technique):

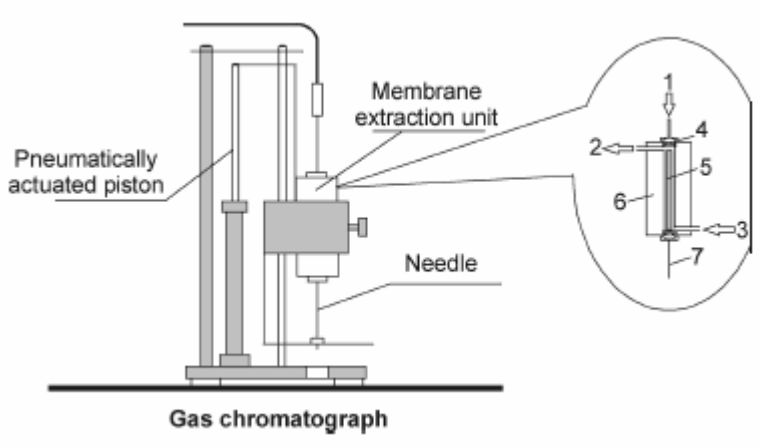


Fig. 2.2.12 Schematic diagram of extraction syringe [232], 1, solvent (acceptor phase) inlet; 2, sample outlet; 3, donor phase inlet; 4, nut; 5, hollow fiber; 6, thermostat (Kel-F block); and 7, needle

2.2.7 Hollow Fibre Based Liquid-Phase Microextraction

Miniaturized LLE or liquid phase microextraction (LPME) was introduced in 1996 simultaneously by Liu and Dasgupta [233] and Jeannot and Cantwell [234]. These novel works were mainly involved the use of hanging droplets in a liquid sample.

The main drawback of the LPME based on hanging droplets is its instability or lack of robustness [235]. The droplets may be lost from the needle tip of the syringe during extraction. This is especially the case when samples are stirred vigorously to speed up the extraction process. In addition, biological samples, such as plasma may emulsify substantial amounts of organic solvents and this may also affect the stability of hanging droplets during extraction.

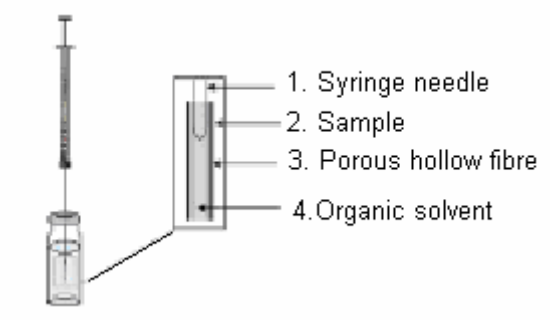
In an attempt to overcome these limitations, keeping their advantage of promising pre-concentration, sample clean-up and solvent saving aspects, Pedersen-Bjergaard and Rasmussen [236] recently introduced an alternative concept for LPME based on the use of single hollow fibre porous polymer made of polypropylene. These hollow fibres are of low-cost, disposable

polypropylene porous polymers [236-250]. Fig. 2.2.13 shows the bundle of such hollow fibre used for extraction.

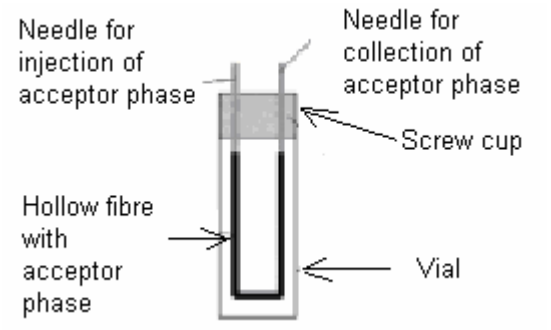
Fig. 2.2.13 Bundles of hollow fibre [251]

Rasmussen and coworkers have reviewed the developments in such hollow fibre based liquid phase microextraction, its basic extraction principles, technical set-up, recovery, enrichment, extraction speed, selectivity, applications and future prospects [252].

The hollow-fiber (HF) membranes can be used either in a rod-like (Fig. 2.2.14 A) or U-shaped (Fig. 2.2.14 B) configuration. The chemical basis of HF-LPME is similar to that of extraction with flat membranes, but the techniques differ significantly in terms of instrumentation and operation. In HF-LPME, both the sample and the extracting phase are stagnant, the membrane (HF) is used only for a single extraction and no instrumentation, such as pumps, is required for sample processing. HF-LPME employs inexpensive membranes, but the automation of the extraction step is difficult [253, 254].



A



B

Fig. 2.2.14 Different hollow fibre modules [255]

2.2.7.1 Extraction Principles

Fig. 2.2.15 illustrates the basic principles of hollow fibre based LPME. The aqueous sample of interest is filled into a small sample vial and a piece of a hollow fibre of porous polypropylene is placed within this sample. The volume of aqueous sample is typically in the range of 0.1-4 mL, depending on the application. The length of the hollow fibre is normally 1.5-10 cm. Prior to extraction, the hollow fibre has been soaked in an organic solvent to immobilize the solvent in the pores of the hollow fibre and other excess solvent has been removed. The solvent is immiscible with water to ensure that it remains within the pores during extraction with no leakage to the aqueous sample.

The organic solvent forms a thin layer within the wall of the hollow fibre, which typically has a thickness of 200 μm and the total volume of organic solvent immobilized is 15-20 μL. For ionizable analytes, similar to SLM, the pH of the sample was adjusted to a value where the analytes were deionised or in a neutral state to reduce their solubility within the aqueous sample and to improve their extractability into the organic phase.

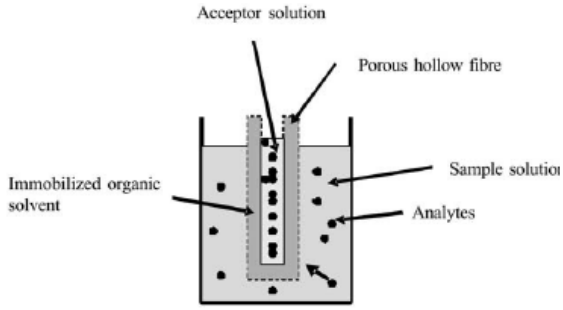


Fig. 2.2.15 Basic principles of extraction in LPME [255]

Thus, analytes are extracted from the aqueous sample, through the organic phase in the pores of the hollow fibre and further into an acceptor solution inside the lumen of the hollow fibre. To speed up this process, extensive agitation or stirring of the sample is applied. The acceptor solution may be the same organic solvent as immobilized in the pores, resulting in extraction of the analyte (A) in a two-phase system, which is similar to the MMLLE, in which the analyte is collected in an organic phase:

$$A_{sample} \rightleftharpoons A_{organic\ acceptor} \quad (17)$$

Two-phase LPME may be applied to most analytes more soluble in an organic solvent immiscible with water than in an aqueous medium. The acceptor solution in this mode is directly compatible with GC, whereas evaporation of solvent and reconstitution in an aqueous medium is required for HPLC or CE. Alternatively, the acceptor solution may be another aqueous phase providing a three-phase system, in which the analytes (A) are extracted from an aqueous sample, through the thin film of organic solvent and into an aqueous acceptor solution:

$$A_{sample} \rightleftharpoons A_{organic\ phase} \rightleftharpoons A_{aqueous\ acceptor} \quad (18)$$

The three phase mode (section 2.7.4) is limited to basic or acidic analytes with ionisable functionalities. After extraction the aqueous acceptor solution can be directly injected into HPLC or CE systems.

These two-phase (section 2.7.3) and three-phase (section 2.7.4) LPME systems are both based on diffusion, in which extraction is promoted by high partition coefficients, as discussed in SLM above. However, the chemical nature of some analytes results in poor partition coefficients that prevent them from being extracted in systems based on diffusion alone. This is especially so for very hydrophilic compounds. In these cases, hollow fibre-based LPME may be accomplished in an active transport mode [227, 228], in which a carrier is added to the sample solution, as illustrated in Fig. 2.2.16. The carrier, which is relatively hydrophobic ion-pair reagent providing acceptable water solubility, forms ion-pairs with the analytes of interest followed by extraction of the ion-pair complexes into the organic phase in the pores of the hollow fibre.

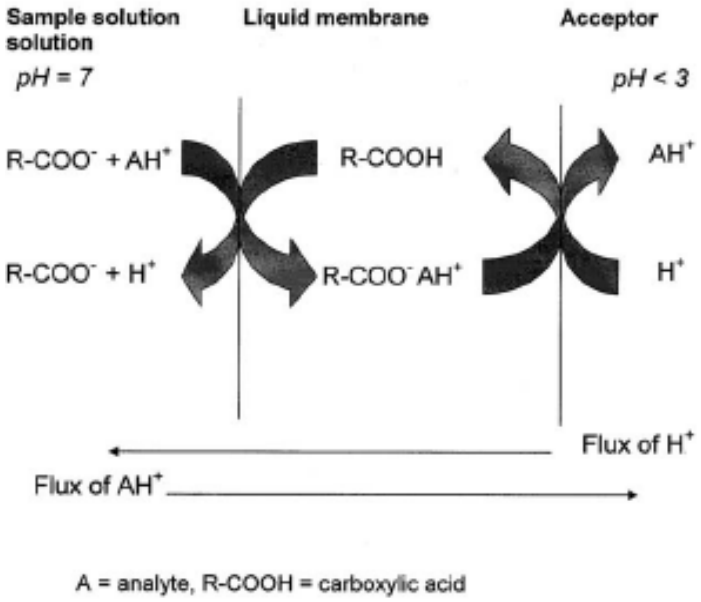


Fig. 2.2.16 Basic principles of active transport in LPME [256]

In the contact region of the organic phase and the acceptor solution, the analytes are released from the ion-pair complex into the acceptor solution, whereas counter ions present in excess in the acceptor solution forms an ion pair with the carrier in the contact area and the new ion-pair

complex is back-extracted into the sample. In the sample again, the carrier releases the transported counter ion, forms an ion-pair with a new analyte molecule and the cycle is repeated. For basic analytes, the carrier may typically be a carboxylic acid with an appropriate hydrophobic moiety (such as octanoic acid), pH in the sample is adjusted to ensure that the analytes are present in their ionised state and pH in the acceptor solution is low to ensure that (1) the carrier is not trapped within this phase and (2) sufficient protons are present to serve as counter ions.

Liu and coworkers [225] applied a slightly different mode of HF-LPME for the determination of freely dissolved chlorophenols in water samples. In this application, hollow fiber pieces of about 15 cm lengths were connected to the needle of the BD Micro-Fine Syringe holding about 0.5 mL of the acceptor solution. Then, the plunger of the syringe was depressed to flush out about 0.1 mL of acceptor to wash and fill the lumen of the hollow fiber. The fiber containing the acceptor solution was dipped into the organic solvent (*n*-undecane) for few seconds to impregnate the pores of the hollow fiber wall, thus forming the organic liquid membrane. After that, the lumen of the fiber was flushed slowly with the rest (about 0.4 mL) of the acceptor solution, at the same time completely filling the lumen with new solution of acceptor phase from the syringe without any air bubbles. To seal the fiber, the two ends were folded three times, enveloped with a strip of aluminum foil and inserted into a piece of small glass tubing. After this preparation, the obtained sampling device has an effective fiber length of about 11 cm with sampling phase volume of about 7 μL. The whole sealed fiber, with filled acceptor and impregnated organic solvent, was immersed into reagent water and shaken for about 1 min to wash out excess organic solvent. After this, the hollow fiber-sampling device was ready for sampling.

For sampling, the hollow fiber-sampling device was immersed into about 1050 mL sample solution, held in a 1000 mL capped volume flask with zero headspace. After static sampling in dark for specified time, the content of the hollow fiber-sampling device was harvested and the water on the fiber surface was absorbed by a paper tissue. One of the sealed ends of the fiber was cut and the fiber was connected to the needle of a BD Micro-Fine Syringe filled with air, then the other sealed end was cut and the acceptor with extracted analytes was flushed into a clean and dried 50 µL glass vial. Normally, 6-7 µL of acceptor could be collected, of which 5 µL was

manually aspirated into a HPLC microsyringe and injected into the HPLC system for analysis. Before aspiration, the HPLC microsyringe was sequentially washed 3 times with water, acetonitrile and water. Samples can also be injected into the HPLC system by an autosampler. Fig. 2.2.17 shows the experimental setup of this mode of hollow fiber application to equilibrium sampling.

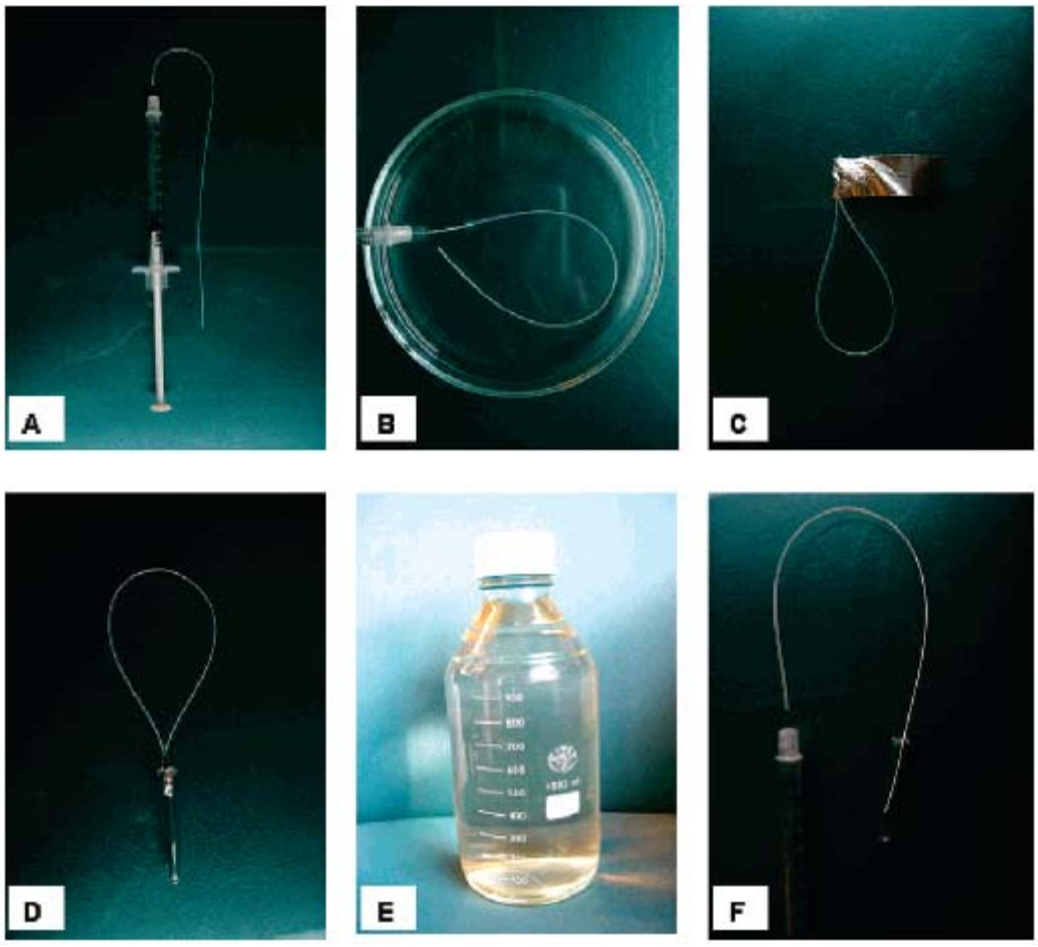


Fig. 2.2.17 Schematic diagram of the equilibrium sampling of chlorophenols in water samples; (A) Filling the lumen of a hollow fiber with acceptor buffer; (B) dipping the hollow fiber into organic solvent for forming the liquid membrane; (C) sealing the two folded ends of hollow fiber with aluminum foil; (D) prepared hollow fiber supported liquid membrane sampling device; (E) sampling water samples and (F) collecting the acceptor buffer solution into a small vial [225]

A similar HF-SLM extraction method has been applied to the determination of dinitrophenolic herbicides in the exhaustive mode and significant enrichment of the analytes were observed in one of the works reported in this thesis (section 4.1).

The three extraction systems described above, i.e., the two phases, three phases and active transport systems are all static. In these modes of extraction the extraction speed is enhanced by extensive agitation of the sample and by careful design of the set-up to minimize diffusion distances of the analytes.

Zhao and Lee [257] and Hou and coworkers [258] showed the dynamic mode of hollow fibre-based LPME applied to the analysis of polyaromatic hydrocarbon (PAH) and pesticides, respectively. Their works were based on the two-phase mode. In this mode, as shown in Fig. 2.2.18, a micro-syringe was filled with a few microliters of organic solvent immiscible with water. A small piece of porous hollow fibre (1-2 cm) is soaked in the same organic solvent to fill the pores, and subsequently, the piece of hollow fibre was connected to the needle of the micro-syringe. The syringe needle and the piece of hollow fibre were placed in an aqueous sample and during extraction; small volumes of the aqueous sample were repeatedly pulled in and out of the hollow fibre using the syringe plunger. During withdrawal of aqueous sample, a thin film of organic solvent is built up in the hollow fibre and vigorously extracts analyte from the sample segment, whereas, during sample expulsion, this thin film recombines with the bulk organic phase in the syringe. During this recombination, the portion of analyte extracted in the current cycle is trapped in the bulk organic solvent. After extraction, which includes many repeated cycles, a portion of the bulk organic solvent is subjected to further chromatographic analysis. The dynamic mode improves the extraction speed as compared to the static one but its limitation is the complication of the instrumentation and adds extra experimental parameters, which have to be optimized and controlled.

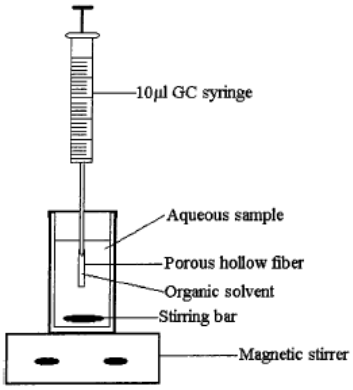


Fig. 2.2.18. Basic apparatus for dynamic LPME [257]

2.2.7.2 Recovery, Enrichment, Extraction Speed and Selectivity

As previously indicated the recovery, enrichment, extraction speed and selectivity parameters have been extensively reviewed by Rassmussen and coworkers [252]. The calculations of these parameters were also given by Ho and coworkers [248]. The detailed mathematical expressions of these parameters are given in the following two sections based on the work of these authors and the reviews cited above.

2.2.7.3 Two-Phase LPME

In two-phase LPME, analytes are extracted by passive diffusion from the aqueous sample (donor solution) directly into the organic acceptor solution. This process is described by equation 17. The extraction process depends on the partition coefficient between the organic acceptor solution and the donor solution ($K_{org/aq}$), defined by equation 19:

$$K_{org/aq} = C_{eq\text{-}org}/C_{eq\text{-}aq} \qquad (19)$$

where $C_{eq,org}$ is the analyte concentration at equilibrium in the acceptor solution and $C_{eq,aq}$ is the analyte concentration at equilibrium in the donor solution. The mass balance, the extraction

recovery, R and the enrichment, E, in the two-phase LPME system may be calculated following equations 19, 23 and 24 as given below.

The initial amount of analyte in the original sample is equal to the sum of the individual amount of analytes in the two phases during the whole extraction process. i.e.,

$$n_i = n_{aq} + n_{org} \tag{20}$$

where n_{aq} is the amount of analytes in the donor aqueous phase and n_{org} is amount of analyte present in the acceptor organic phase.
At equilibrium, equation 4 can be re-written as:

$$C_i V_{aq} = C_{eq,\,aq} V_{aq} + C_{eq,\,org} V_{org} \tag{21}$$

where C_i is the initial analyte concentration in the sample and V_{aq} and V_{org} are the sample volume (donor phase) and acceptor phase volume, respectively.

The amount of analyte extracted into the acceptor phase $n_{eq,org}$ of the system, at equilibrium, can be expressed by [147, 246, 259]:

$$n_{eq,\,org} = \frac{K_{org/aq} V_{org} . C_i V_{aq}}{K_{org\,/\,aq} V_{org} + V_{aq}} \tag{22}$$

The recovery (R) of the analyte is calculated using the equation:

$$R = \frac{100 n_{eq,\,org}}{C_i V_{aq}} = \frac{(K_{org\,/\,aq} V_{org})}{(K_{org\,/\,aq} V_{org}) + V_{aq}} x100 \tag{23}$$

The enrichment factor (E) of the analyte can be calculated using the following formula:

$$E = \frac{C_{org}}{C_i} = \frac{(V_{aq}.R)}{100V_{org}} \quad (24)$$

C_{org} is the concentration of A in the acceptor organic phase at the end of extraction. equtions 19 and 23 may also be used for two-phase LLE. For two-phase LPME, the actual recovery is lower than one calculated by utilizing equation 19 because the fraction of the organic solvent which is immobilized in the pores of the hollow fibre is not available for further analysis; only the fraction present in the lumen may be collected. This is one problem of this technique in its application to non-depletive mode of extraction.

Therefore, in one of the research works covered in this thesis, we have applied the non-depletive mode of HF-LPME to the analysis of freely dissolved organophosphorus pesticides in environmental water samples by using very simple technical set-up and taking out all of the extracts into very small volume of organic solvents. Complete coverage is presented in section 4.2.

2.2.7.4 Three-Phase LPME

In three-phase LPME, the analytes are extracted from the aqueous sample solution (donor phase), through the organic solvent immobilized in the pores of the hollow fibre (organic phase) and further into the acceptor solution (acceptor phase) present inside the lumen of the hollow fibre. This process may be illustrated with the following equation which was given above as equation 18:

$$A_{Sample} \rightleftharpoons A_{organic\ phase} \rightleftharpoons A_{aqueous\ acceptor} \quad (25)$$

In the three-phase system, the initial amount of analyte n_i is equal to the sum of the individual amounts of analyte present in the three phases during the whole extraction process [244]:

$$n_i = n_d + n_{org} + n_a \quad (26)$$

where n_d is the amount of analyte present in the donor (the sample), n_{org} is the amount of analyte present in the organic phase and n_a is the amount of analyte present in the acceptor phase during extraction process. At equilibrium:

$$C_i V_d = C_{eq,d} V_d + C_{eq,org} V_{org} + C_{eq,a} V_a \quad (27)$$

where C_i is the initial concentration in the sample, $C_{eq,d}$, $C_{eq,org}$ and $C_{eq,a}$ are the analyte concentrations in the donor phase, organic phase and acceptor phase at equilibrium, respectively. V_d, V_{org} and V_a are also the volumes of sample (donor phase), organic phase and acceptor phase, respectively.

In the three-phase LPME system, partition coefficients both between the organic phase and the donor phase as well as between the acceptor phase and the organic phase have to be considered:

$$K_{org/d} = \frac{C_{eq,org}}{C_{eq,d}} \quad (28)$$

$$K_{a/org} = \frac{C_{eq,a}}{C_{eq,org}} \quad (29)$$

The partition coefficient between the acceptor phase and the donor phase *K* can be written as:

$$K_{a/d} = \frac{C_{eq,a}}{C_{eq,d}} = K_{org/d} . K_{a/org} \quad (30)$$

The amount of analyte extracted into the acceptor phase of the system can be calculated by substituting $K_{a/d}\, C_{eq,d}$ for $C_{eq,a}$ and by rearrangement of equation 25. At equilibrium the amount of analyte present in the acceptor phase, $n_{eq,a,}$ may be calculated using the following equations [147, 246, 259, 260]

$$n_{eq,a} = \frac{(K_{a/d}.V_a.C_i.V_d)}{(K_{a/d}.V_d + K_{org/d}.V_{org} + V_d} \quad (31)$$

The recovery (R) can be expressed as:

$$R = \frac{100 n_{eq},a}{C_i V_d} = \frac{100 Ka/d.Vd}{(K_{a/d}.V_d + K_{org/d}.V_{org} + V_d)} \quad (32)$$

The enrichment (E) can be calculated by the formula:

$$E = \frac{C_a}{C_i} = \frac{V_d R}{100 V_a} \quad (33)$$

The enrichment factor (E) can expressed interms of extraction efficiency,

$$E_e = E.V_d/V_{org} \quad (34)$$

The extraction efficiency can be obtained from the formula,

$$E = n_A/n_d \quad (35)$$

or alternatively from

$$E' = (n_d - n_A)/n_d \quad (36)$$

Then the recovery would be given by

$$R = E/E'$$

If E = E′, no analyte is lost in the process and the recovery is 100%, but if E< E′ some analyte is either adsorbed in the apparatus or left in themembrane.

On the other hand, equations 31 and 32 may be used to calculate recovery and enrichment for three-phase LPME. In this case, the whole volume of acceptor phase is available for further analysis and the recovery is therefore directly calculated from equation 31.

2.2.8 Equilibrium Sampling Through Membranes for the Determination of Freely Dissolved Environmental Contaminants

Urban and rural aquatic ecosystems are often polluted by a wide variety of contaminants from agricultural, industrial or municipal activities. The usual approach of environmental risk assessment regarding these contaminants is to collect a given quantity of matrix from one of the environmental compartments (i.e., water, sediment, soil and air) and determine the quantity of contaminants or analytes present in the sample. The determined concentrations from the analysis can be compared with objectives set or can be used to approximate the extent to which the given organism is exposed to the contaminant in that particular environmental compartment and deduce certain conclusion [261]. This approach actually does not distinguish between the amount of the contaminant available to the organism and amount, which is bound to different media in that particular element of the environment.

There are considerable behavioral differences between bound and unbound environmental contaminants. In other words, the compounds′ behaviour regarding aqueous mobility, biological uptake, bioaccumulation [262], sediment sorption [263, 264] and potential toxic effect [265] would be changed notably when bound to dissolved organic matter (DOM) or other media in the matrix under investigation.

Therefore, from a toxicological point of view, it is very important to quantitatively determine the freely dissolved fraction of chemical contaminants in the environmental elements in order to evaluate and characterize their bioavailability. Hence, the values of these bioavailable fractions can be used for risk assessment studies and to estimate the maximum achievable degradation levels of these contaminants in different matrices [266-268].

Different methodologies have been developed for estimating the bioavailable fractions. The use of organisms or cells seems to be the preferred option [269], but they are typically slow and imprecise [267]. The application of non-exhaustive extraction techniques [267] is the other alternative to overcome the main limitations of the use of organisms.

For environmental purposes, the most commonly used non-exhaustive methods for sensing the freely dissolved concentrations (C_{free}) of pollutants include diffusion gradients in thin films (DGT) [270] for metals and semipermeable membrane devices (SPMDs) [205] as well as solid-phase microextraction (SPME) [271-273] for organic compounds. Most of the time the DGT and SPMDs are operated in the kinetic uptake regime as they need long time to reach equilibrium, while SPME was successfully used as an equilibrium sampling device due to its relatively short equilibration time [272]. In addition Ramos *et al*. [168], have also found out that negligible depletion SPME was a good technique to determine bioavailable concentrations of hydrophobic chemicals in aquatic environments. The main drawback of SPME is its low extraction efficiency for some polar analytes.

Some of the LPME methods discussed above are non-exhaustive and suitable for equilibrium determination of freely dissolved contaminants in environmental samples. The typical ones are: the miniaturized liquid-liquid extraction (LLE) methods [274, 275].

Recently, the freely dissolved concentrations (C_{free}) of chlorophenols [225] and copper ions [276] were determined by the use of equilibrium sampling through membranes (ESTM), based on HF-SLME in environmental water samples. These works clearly showed the application potential of HF-LPME for the determination of freely dissolved organic and inorganic contaminants in environmental compartments. Moreover, as discussed above in greater details the use of HF-LPME was found to be attractive because of the low-cost, disposable nature of the polypropylene porous polymers and the selectivity of the hollow fiber membrane because of the pores in its wall.

In the majority of applications of HF-LPME discussed above, at the end of extraction the content of the hollow fibre lumen was injected into the separating instruments. In this kind of design it is possible to see two problems: the first one is that the extraction process needs the use of expensive GC or HPLC microsyringes for its applications. Therefore, the analysis of very large number of environmental samples would be expensive as it requires many microsyringes for parallel runs or the sample preparation takes very long time with limited number of microsyringes. The other problem associated with such design is related to the determination of

freely dissolved equilibrium concentrations of the contaminant without depleting the sample. When only the lumen content of the hollow fibre is injected, it is not possible to clearly see whether the equilibrium between the freely dissolved and bound analytes has been disturbed or not. In other words, as the concentration of the analyte in the pores of the hollow fibre is not considered, it is not possible to know whether the extraction is depletive or not.

One of the limiting factors, when it comes to developing countries where there is shortage of funds for environmental researchers, is the cost required to carry out the analysis of the type explained above. To partially overcome such problems, a new design of HF-LPME for the determination of freely dissolved organophosphorus pesticide, as model compounds, has been developed. The design explained in the current study was found to be simpler and inexpensive. We have also demonstrated the possibility of running hundreds of samples in parallel, with very minimal expenses, in each series of experiments.

3. Materials and Methods

Background

This thesis comprises of two categories of research works: developments of sample preparation methods for the analysis of pesticide residues in environmental water samples and determination of levels of *s*-triazne herbicides in water and sediment samples from the rift valley water systems.

In the category of development of sample preparation methods for the analysis of pesticide residues in environmental water samples were included:

- Development of sample preparation methods based upon HF-SLM technique for the trace level determination of dinitrophenolic herbicides in environmental waters.
- Development of a novel sample preparation method for the determination of freely dissolved organophosphorus pesticide residues in environmental waters.

In the second category of this thesis work, the following works were included:

- Designing, construction and application of time-integrated, unattended and automatic portable sampler based upon SLM principles for the analysis of *s*-triazine herbicides from selected sites of Awassa Lake.
- Determination of the levels of *s*-triazine herbicides in selected sites of Awassa Lake; this is the continuation of the application of the portable sampler.
- Determination of the levels of *s*-triazine herbicides in sediment samples from the selected sites of Awassa and Ziway Lakes.

The detailed chemicals and materials used and the methods followed are described below for each part of these topics.

3. 1 Determination of Trace Levels of Dinitrophenolic Compounds in Environmental Water samples

3. 1. 1 Chemicals and Materials

The dinitrophenolic standards used for the method development were: 2, 4-dinitrophenol (DNP), dinitro-ortho-cresol (DNOC), 2-(1-methylpropyl)-4, 6-dinitrophenol (Dinoseb) and 2-tert-butyl-4, 6-dinitrophenol (Dinoterb), all purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). HPLC grade acetonitrile, *n*-undecane and citric acid were obtained from Merck, (Darmstadt, Germany). Humic acid and sulfuric acid (96%) were purchased from Aldrich (Steinheim, Germany). Sodium hydroxide (98.6%) was from EKA Nobel AB (Bohus Sweden). All reagents and solvents used in this study were of analytical grade or HPLC grade.

Twenty milligrams per litre individual standard stock solutions of the standard compounds were prepared by first dissolving in 2–3 mL of acetonitrile and then diluting using appropriate amount of water to the required volume. A mixed solution of the four compounds was prepared by mixing appropriate amount of the stock solution and diluting with water to a given volume. All solutions were stored at 4°C in the refrigerator until use. $NaHCO_3$ (600 mM) acceptor buffer solution was prepared by dissolving appropriate amount of $NaHCO_3$ in certain amount of water and then the pH was adjusted to 10.00 by drop wise addition of 10 M sodium hydroxide then the volume of the solution was filled to the mark of volumetric flask. The donor sample solution was prepared by taking appropriate amount of the mixture solution and diluting to the required volume and then pH was adjusted to 2.00 by 6 M HCl or concentrated sulfuric acid solution. HI 7031 (1413 μS/cm) calibration solution (obtained from HANNA instruments, Melbourne, Australia) was used for calibration in conductivity measurement.

Polypropylene hollow fibre tubing, 50/280 Accurel ® PP (50 μm wall thickness, 280 μm inner diameter, 0.1 μm pore size) was obtained from Membrana GmbH (Wuppertal, Germany). BD MicroFine Syringes (with needle of 0.30 mm outer diameter and 8 mm length, 0.5 mL capacity prepared for U-100 insulin injection) obtained from BD Consumer Healthcare (Franklin Lakes,

USA) were used to fill the acceptor into the lumen of the hollow fiber for extraction and to flush out the acceptor into a small glass vial (200 μL) after extraction.

3. 1. 2 Instruments

The HPLC system used for the analysis included Agilent 1100 series Quaternary Pump, Agilent 1100 series Vacuum Degasser, Agilent 1100 series Autosampler and Agilent 1100 Series Diode Array detector and data acquisition was controlled by the Chemstation 1100 series software (All from Agilent Technologies, Heilbronn, Germany). A RP-Ace 5 C 18 (250 mm × 4.6 mm, 5 μm particle size) was used as analytical column.

The samples were shaken using HT Infors orbital shaker from Infors AG (Bottmingen, Switzerland). A pH 211 microprocesor pH meter (Hanna Instruments, Melbourne, Australia), was used to adjust the sample and acceptor buffer pH and to measure the sample pH. The conductivity of water samples was measured using HI 9033 Multi-range conductivity meter (Hanna instruments, Melbourne, Australia).

3. 1. 3 Extraction Procedures

The hollow fiber was cut manually into approximately 20 cm length and the two ends were looped together, to give appropriate shape, leaving both ends free for subsequent use. Then, the lumen of a single hollow fiber was flushed and filled with the acceptor buffer solution using the BD Micro-Fine syringe. Afterwards, the fiber was dipped in *n*-undecane for few seconds to impregnate the pores of the hollow fiber wall forming the organic liquid membrane. Then, slowly the lumen of the fiber was flushed with more acceptor solution to remove any organic solvent in the lumen and also remove air bubbles from the lumen and filling it completely. The two ends of the fiber were folded and enveloped with a piece of strip of aluminum foil and inserted into a small piece of glass tubing. Subsequently, the filled and sealed fiber was soaked in reagent water for few minutes and this ready hollow fiber supported liquid membrane device was

transferred to 200 mL sample solution. After shaking the whole set-up using an orbital shaker for 5 h, the acceptor solution containing the analyte was collected into 200 μL vials with inserts.

The collection of the sample was as follows: one of the ends of the sealed fiber was cut and connected to a retracted syringe needle and the other end then cut and put in the vial. Next, the syringe plunger was pushed in, to dispense the acceptor solution containing analyte into the vial. After capping, the vial was put on the autosampler of the HPLC for injection. Approximately 10–12 μL solution was collected and 5 μL was injected into the HPLC system.

3. 1. 4 Chromatographic Conditions

Chromatographic separations were carried out using gradient elution with acetonitrile and citric acid buffer in reagent water at pH 2. The flow rate was 0.5 mL/min and the column temperature was maintained at 22^{o}C. Five microlitres of extracted sample was injected and eluted using the following gradient profile: the analysis started with 67% acetonitrile, which was increased linearly up to 90% in 10 min and decreased back to 67% in the next 5 min. The detection was performed at 280 nm wavelengths.

3. 1. 5 Sample Collections

River water was collected from Höje River in the south suburb of Lund, Sweden. The seawater was collected from Öresund, Sweden. The lake water was collected from Lake Awassa, Southern Ethiopia. The samples were filtered with 0.45 μm membrane and acidified to pH 2 using 1 M HCl before each extraction.

3.2 Hollow Fibre Liquid Phase Microextraction Method for Trace Enrichment of Freely Dissolved Organophosphorus Pesticides in Environmental Waters

A sample preparation method for the determination of freely dissolved concentration of organophosphorus pesticide residues was developed based upon the use of hollow fibre liquid phase microextraction with a new kind of technical set up. The materials and methods used in this work are given below.

3. 2. 1 Chemicals and Materials

The pesticide standards, *viz.*, diazinon, fenthion and chlorpyrifos were purchased from Dr. Ehrenstorfer, GmbH, Germany.

Two types of stock solutions of 100 mg/L for each pesticide (diazinon, fenthion, chlorpyrifos) were prepared. One kind was prepared in ethyl acetate (BDH, Laboratory Chemicals Division, England) for calibration and the other in acetone (Analytical reagent, Techno Pharmchem, Bahadurgarh, India) for spiking during the method development. Mixed standard solutions of 10 mg/L, containing each of the pesticides was prepared in ethyl acetate and acetone. The working solutions with concentrations ranging from 0.025 mg/L to 0.25 mg/L, at five points, were prepared every week from the 10 mg/L stock solution for calibration. Another 10 µg/L mixture containing all pesticides was prepared in a mixture of water and acetone for spiking. Then, different concentrations of the OPPs mixture were prepared from the 10 µg/L. Methidathion (Dr. Ehrenstorfer, GmbH, Germany) was used as an internal standard for the GC-MS analysis. *n*-Undecane (Sigma-Aldrich), dihexyl ether (Fluka, Germany) and 1-octanol (Fluka, Chemie GmbH, Buchs, Germany) were tested for use as membrane solvents. All other chemicals, humic acid (ALDRICH, Germany), sodium chloride (Labmerk Chemicals PVT Ltd, India), ethyl acetate (Sigma-Aldrich, Lund, Sweden), acetone (Techno Pharmchem, Bahadurgar, India) and heptane (BDH Poole, England), were of HPLC grade or of analytical grade. The hollow fibre (HF) used was of medium sized Q3/2 type with 0.2 µm pore size, 200 µm wall thickness and 600 µm inner diameter (Membrana GmbH, Germany).

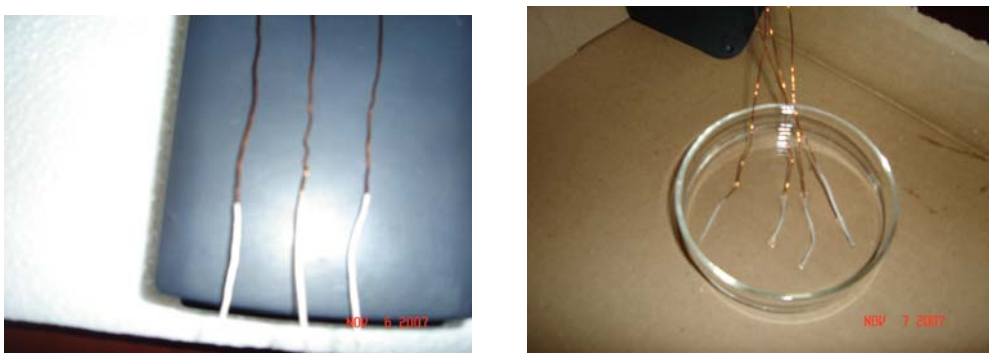
3. 2. 2 Water Sample Collection

Lake water and ground water samples were collected from Awassa Lake and nearby residential houses in Awassa town, respectively. The sampling sites were located in Southern Ethiopia, 275 km away from Addis Ababa. Blank and spiked extraction of the analyte from the water samples were performed as soon as the samples were brought to the Analytical Laboratory at the Department of Chemistry, Addis Ababa University. To investigate the impact of suspended particles, the real water samples were spiked without prior filtrations.

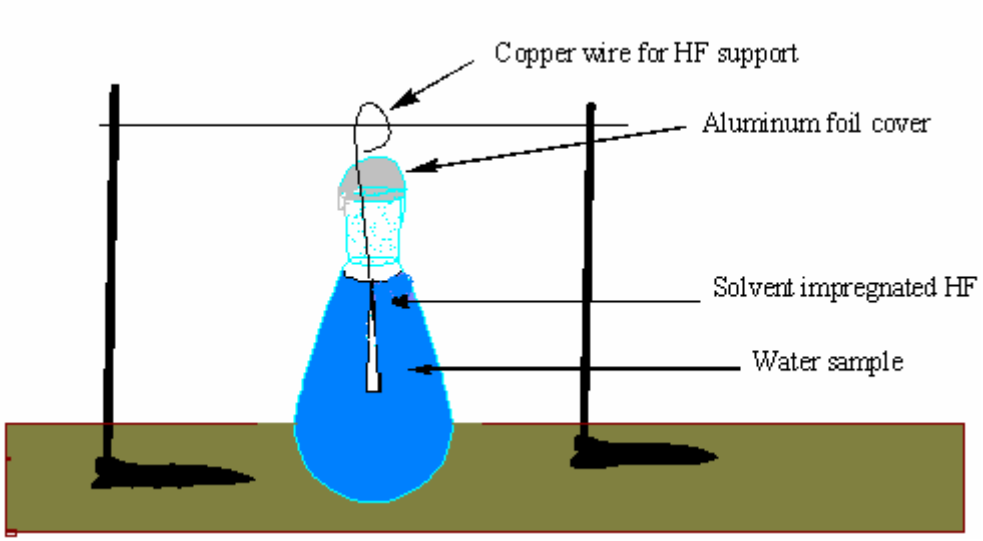
3. 2. 3 Extraction Procedure

Twenty cm copper wire was inserted into one end of 2 cm long hollow fibre to the approximate depth of 0.5 cm. After that, the other end of the hollow fibre was sealed using a cigarette lighter flame. Then, the effective length of hollow fibre was about 1.2-1.4 cm.

The sealed hollow fibre was sonicated in the membrane solvent for about one minute to clean the hollow fibre. Afterwards, it was impregnated with the same extraction solvent for about 4-5 min to fill up the wall as well as the lumen of the hollow fibre (Fig. 3.2.1 A). The soaked extraction unit (Fig. 3.2.1 B) was then rinsed with distilled water in a small beaker to remove excess organic solvent. Subsequently, the copper wire containing the hollow fibre was allowed to suspend in the sample bottle by tying it to a clamp or a rope which was tied to two stands, as shown in Fig. 3.2.1 C. The sample container for all types of extraction was an amber bottle to avoid any possible light interaction with the analytes. The sample bottle was with nearly zero headspace and also covered with aluminum foil. The extraction unit was inserted into the sample bottle through a small orifice, prepared in the middle of the foil covering the top of the bottle. The orifice was then completely covered with additional piece of aluminum foil. Then at the end of the extraction the copper wire was removed from the sample bottle and put into a vial with insert (From Agilent Technologies) containing 40 μL ethyl acetate and internal standard. After that the vial was sonicated to wash out all of the analytes extracted into the organic phase out of the hollow fibre pores and lumen. Afterwards, the vial was capped and put in an autosampler of GC-MS for injection into GC.



A B



C

Fig. 3.2.1 Extraction procedure using HF-LPME: A) Copper wire was inserted into one end of the hollow fibre for support; B) impregnation of the hollow fibre in the extraction solvent and C) hanging of the impregnated hollow fibre in the sample bottle covered with aluminum foil

3. 2. 4 Gas Chromatography–Mass Spectrometric Analysis

The GC–MS analyses were performed using Agilent-6890 Gas Chromatograph (GC) interfaced with Agilent 5975 Mass Selective Detector (MSD), all from Agilent Technologies. The analytes were separated on a HP-5 MS (30 m x 0.25 mm I.D., 0.25 µm film thickness) capillary column from Agilent (S & W., USA). The column was initially maintained for 1 min at 50^{o}C and

subsequently was increased to 193^{o}C at a rate of 50^{o}C/min, then increased to 205^{o}C at a rate of 1^{o}C/min and finally increased to 260^{o}C at a rate of 60^{o}C/min. Helium was used as a carrier gas with a flow rate of 1 mL/min (6 min solvent delay). The inlet temperature was set to 250^{o}C while interface and source temperatures were set to 280^{o}C and 250^{o}C, respectively. A split–splitless injector in the splitless mode was used for the injection of the standards and extracts. The injection volume was 1 µL. All concentrated sample extracts and standard solutions were analyzed by 70 eV electron impact ionization mode. The instrument was operated in the selected ion monitoring mode (SIM) for the quantitative analysis of the analytes. Four fragment ions were monitored for each compound, in order to maximize the detector signal, the most abundant and characteristic ion in the spectrum was selected for quantification and three other peaks for confirmation purposes. Peak identification was based upon the scan mode of the GC-MS. the chromtogram and mass spectra obtained for standard OPPs mixture is given in Fig. 4.2.1, section 4.21. Data acquisition and processing were made using Chemstation, obtained from Agilent Technologies.

3.3 Portable and Time-integrating Field Sampling for the Analysis of s-Triazine Herbicides and Their Degradation products in Awassa Lake, Southern Ethiopia

As indicated in section 2.1 SLM is an environmentally friendly technique comprising sampling, sample preparation, enrichment and clean up procedures into a single step. As a result, a new portable time integrating sampler based on SLM system has been developed and applied to the analysis of *s*-triazine herbicides in lake water samples from the Southern part of the country. First pilot study on the functionality and applicability of the sampler was done and next monitoring of the levels of *s*-triazine herbicides in selected sites of Lake Awassa was carried out.

3. 3. 1 Chemicals

All of the standard *s*-triazine pesticides and their likely degradation products were purchased from Dr Ehrenstorfer GmbH (Augsburg, Germany).

Table 3.3.1 shows all of the *s*-triazine herbicides and their degradation products, which were used in this study. The table also includes some of the physical parameters of interest.

Analytical grade di-*n*-hexyl ether (Sigma Chemicals, St. Louis, MO, USA) was used as a membrane solvent for immobilizing into the membrane. Other chemicals, including sodium dihydrogen phosphate monohydrate, ($NaH_2PO_4 \cdot H_2O$), disodium hydrogen phosphate dihydrate, ($Na_2HPO_4 \cdot 2H_2O$), potassium dihydrogen phosphate, (KH_2PO_4), acetonitrile and hydrochloric acid were from Merck (Darmstadt, Germany). Sodium chloride used in extraction solution was from BDH Laboratory Supplies (Poole, England). 10 % (W/V) tri-*n*-octylphosphine oxide (TOPO) (AlDRICH, Steinheim, Germany) was used as a modifier in the monitoring study of the levels of the *s*-triazne herbicides but it was not used in the pilot study of the application of the sampler. All solutions were prepared from analytical-grade reagents in high purity water obtained

Table 3.3.1 Summary of important physical properties and other relevant information, in SLM context, for the investigated analytes (The general skeletal formula is given in Fig. 2.6)

Analyte Type	**Compound**	**R_1**	**R_2**	**R_3**	**Abbre-viation**	**log K_{ow}***	**pK_a***	**Water solubility (mg/L)**
Dealkylated degradation products	Deethyldesisopropyl atrazine	Cl	H	H	DDA	0	1.5	-
	Desisopropyl atrazine	Cl	H	CH_2CH_3	DIA	1.2	1.3	670
	Deethyl atrazine	Cl	$CH(CH_3)_2$	H	DEA	1.6	1.3	3200
Hydroxylated degradation products	Hydroxy atrazine	OH	$CH(CH_3)_2$	CH_2CH_3	ATOH	1.4	5.15	600
	Hydroxy propazine	OH	$CH(CH_3)_2$	$CH(CH_3)_2$	PROH	-	5.2	-
	Hydroxy terbutylazine	OH	CH_2CH_3	$C(CH_3)_3$	TZOH	-		9
Parent compounds	Cyanazine	Cl	CH_2CH_3	$CCN(CH_3)_2$	CYZN	1.8	1.0	170
	Atrazine	Cl	CH_2CH_3	$CH(CH_3)_2$	ATRZN	2.7	1.68	35
	Prometryn	CH_3S	$C(CH_3)_2$	$CH(CH_3)_2$	PRYN	3.34	4.05	33
	Terbutryn	CH_3S	CH_2CH_3	$CH(CH_3)_2$	TRYN	3.74	4.4	25
	Propazine	Cl	$CH(CH_3)_2$	H $CH(CH_3)_2$	8.6		1.85	

from a Milli Q-RO4 unit (Millipore, Bedford, MA, USA) in Sweden and in Ethiopia doubly distilled water was utilized. One hundred mg/L of *s*-triazine herbicides stock solutions were prepared for the determination of the levels of these compounds in lake water and sediment samples and stored in a refrigerator at 4^{o}C in darkness.

In order to prepare the stock solutions, solid DDA was dissolved first in 5 mL of acetonitrile and then in 5 mL of reagent water. Hydroxylated products were dissolved in 1 mL of 1 M HCl, while all others were dissolved in 1 – 2 mL acetonitrile. All the resulting solutions were diluted to the final volume with acetonitrile.

Mixed working standard solutions of *s*-triazine herbicides and their degradation products at lower concentration levels were made from the aliquots of stock standard solutions by appropriate dilutions with reagent water.

3. 3. 2 Materials and Instruments

A smaller sized membrane module was specially designed so as to fit in a portable plastic box. It is made up of polytetrafluoroethylene (PTFE) blocks with Archimedes spiral grooves of the dimensions of 0.25 mm depth, 1.5 mm width and 438 mm length, yielding a channel volume of about 164.3 µL. Two aluminum blocks, with six screws and an O-ring made the assembly stable and leak tight.

For sample flow, PTFE, fluorinated ethylene propylene (FEP) tubes and flangeless fittings from Genetec (Gothenburg, Sweden) were used. Washers, which were used to prevent leakage, were from Kloehn (Kloehn Ltd., Las Vegas, USA). In order to ensure mixing of sample and donor buffer before extraction, the tube from the donor pump to the membrane was coiled. It was important to minimize the dead volume, especially in the acceptor channel, in order to ensure that no significant amounts of analytes are left in the system after having taken an extract. Therefore, FEP tube with an inner diameter of 0.01" (= 0.25 mm) was used for the acceptor channel between the membrane and the vials. Otherwise,

FEP tube with an inner diameter of 0.03" (= 0.76 mm) was used, except for a larger PTFE tube used for aspiration of water sample during on-site extraction.

The portable SLM sampler system, both for field and laboratory usage is contained in a portable sampler box, without the accompanying power supply and controlling computer. The sampler is built into a plastic box of 42 x 26 x 37 cm dimensions and consists of the membrane holder, two pumps, one valve for handling different extracts, one vial rack, one power transformer, one cooling fan, a sampler probe with filter and various tubes, fittings, wires and support construction in stainless steel. Two syringe pumps, 50300 series model (Kloehn Ltd., Las Vegas, USA) were used to control the flow rates of the donor and acceptor phases, independently. The donor pump is supplied with a 10 mL syringe and the acceptor with a 1 mL syringe. The valve, 50120 series model (Kloehn Ltd., Las Vegas, USA) helps to distribute extracted samples from acceptor phase to the extraction vials through eight different ports, with one port reserved for acceptor waste, so the sampler can handle up to seven extracts for unattended extraction.

In order to make the method more environmentally accustomed, solar cell panels were used to charge the two serially connected 12 V batteries used for field power supply in Ethiopia. The solar cell panels, model NR100G and battery regulator, model Mmini Pro, were from Naps (Skärholmen, Sweden).

It may also be necessary to provide a cooling system for a portable field sampler. The donor pump, which operates almost continuously during the optimised extraction period [215], develops heat. If the constructed portable sampler was kept closed at room temperature, when running the extraction program, the air temperature in the box will approach 45°C after approximately half a day. Increased temperature may influence the extraction. According to the manufacturer, the pumps and the valve shall also be kept at less than 50°C. In addition, the FEP tubes may also have limitations in temperature tolerance (FEP shall be kept below 50°C). To meet these requirements a fan of 6 cm diameter (ELFA, Malmö, Sweden) equipped with a filter was used for cooling purpose in the extraction unit. Care must be taken here so that dusty air does not reach the optical

sensing of the Kloehn pumps, which can be done by using small-mesh air filter or by directing the airflow to the stainless steel support.

The HPLC system used in the first part, i.e., in the pilot study was model 9012, Varian Analytical Instruments, (Sunnyvale, USA) with UV spectrophotometer (Spectroflow 783 model, ABI Analytical Kratos Division, Ramsey, USA) detector. The data was then managed with JCL 6000, for windows, revision 27, chromatographic data system (Jones Chromatography Ltd., Hengoed, Mid-Glamorgan, UK).

In the second part, i.e., in the monitoring of the levels of the herbicides study, HPLC system consisting of Agilent 1100 series Quaternary Pump, Agilent 1100 Series Vacuum Degasser, Agilent 1100 series Autosampler and Agilent 1100 Series Diode Array detector was used. The data acquisition was controlled by the Chemstation 1100 series software (All from Agilent Technologies, Heilbronn, Germany).

Separation of the mixture of triazine herbicides and their degradation products in all of the cases was carried out on C 18 analytical column (Ace 5 C18 Silica; 250 mm x 4. 6 mm, 5 μm film thickness; Advanced Chromatography Technologies, Aberdeen, Scotland). Analytes eluted from the column were detected using an UV detector at 220 nm. The mobile phase flow rate was adjusted at 1 mL/min. The HPLC analysis with gradient elution consisting of the mobile phase: 3.5 mM phosphate buffer (pH = 7.0) and acetonitrile (Merck), was used for sample analysis. The gradient elution was exactly the same as the conditions previously developed for the mixture of the *s*-triazines herbicides and their degradation products [215] and given in Table 3.3.2 below.

Table 3.3.2 Gradient mobile phase composition of acetonitrile and 3.5 mM phosphate buffer at pH 7 wirh varying elution times

Time (min)	Acetonitrile (%)	3.5 mM phosphate buffer at pH 7 (%)
0	10	90
5	15	85
10	15	85
33	70	30
37	70	30
38	10	90
43	10	90

3.3.3 Extraction unit

As in the earlier work [215], a portion of the liquid membrane support (Millipore FG (Millipore, Bedford, MA, USA) with an average pore size of 0.2 μm, a total thickness of 175 μm of which about 115 μm is polyethylene backing and a porosity of 70% was cut and made to fit into the membrane holder described above. It was then immersed into the di-*n*-hexylether for about 30 min and then the soaked membrane was placed between the two PTFE blocks. When the whole construction is clamped, it forms two separate channels, i.e., the donor and the acceptor compartments.

3.3.4 Operation of the Field Sampler

Fig. 3.3.1 shows the schematic diagram of the portable field sampler. Computer connection, the enclosing box and the power system, along with the solar panels are not indicated in the figure. As far as possible, the previously optimised extraction method [215] was followed for field sample extraction. The detail description of the operation is given in the following paragraphs.

Two programs, WinPump® and WinValve® (Kloehn Ltd., Las Vegas, USA), were used to control the two pumps and the valve, respectively. The three devices enabled the SLM extraction either under direct control of PC or by programs pre-hand written and saved in the memories of the devices. The latter is obviously most suitable for field work while the

former for method development in the laboratory or for field trouble-shooting purposes. Each device (the two pumps and the valve) is programmed separately. The steering computer then needs to distinguish between the two pumps and the valves, so for this purpose, there is a number switch on the back of each of these three devices. The donor pump was set in this way to be device 1, the acceptor pump device 2 and the valve device 3. During extraction each device uses its own set of programs. The programs were first written using the WinPump® and WinValve® softwares and then saved into the so-called long time or non-volatile memory of the right device. When operated for field sampling, each device uses one master program, which is activated at power-up.

For extraction and on-site applications, there is a need for synchronization between the pumps and the valve. Firstly, it is important that the valve does not distribute the extract to waste or to a previously collected extract in a vial. Secondly, the acceptor pump must be timed with the donor pump so that it should start pushing out of the extract plug only after the donor pump has pumped the whole sample volume. The operations of the three devices can be synchronized in different ways. Two synchronization methods were used here. Most importantly, the donor pump device can send parallel analog signals to the valve and the acceptor pump devices, which were used for handling ready extracts. Secondly, timed events were used to synchronize more simple operations.

During extraction, the donor pump (P_1), equipped with a 10 mL syringe (Sy1), is used to control the flow of the water sample from the water body, sucked in through the filter (F) (100 μm mesh size) and combined with the buffer from the bottle container. In this process, the water from the lake is first aspirated into Sy1 by the way of port A of valve 1 and the buffer aspirated into the same syringe via port D of the same valve according to the set program. Sy1 pump aspirates 100 μL buffer and 1900 μL sample water five times, giving a total mixture of totally 500 μL buffer and 9500 μL sample in the 10 mL syringe. This mixture is dispensed into the donor channel by the help of the same pump through port B of valve 1, with final mixing in the mixing coil. After passing through the donor channel of the membrane unit, the mixture will be led to the waste. This process is continuously repeated until 3 L of samples has been processed (i.e., 316 times, taking into account the mixing with buffer).

A similar pump, P_2, which is supplied with a 1-mL syringe, (Sy2) controls the flow of the acceptor acid. The acid will be aspirated first into the syringe through port A of valve 2,

then dispensed into the acceptor channel through port B of the same valve and kept stagnant in the membrane until completion of the extraction.

When 3 L sample has been extracted the membrane is washed and the system left for analyte diffusion as previously optimized [215]. Thereafter, an analog signal is transmitted from P1, acting as master device, to the valve device in order to correctly direct valve 3 to the right of eight different ports (labeled A – H) (of which A is left for acid waste from the acceptor channel) to extracts vials, Ex (B – H). Five seconds later, another analog signal is sent from P1 to P2 resulting in aspiration of a fixed volume (1 mL) of acid from the bottle and dispensed into the acceptor channel of the membrane. The acid containing the extract is thus pumped into valve 3 and directed to one of the Ex vials B – H. After extraction, as well as prior to it, both the donor and the acceptor channels were washed with fresh buffer or acid solution (the latter through the A port of valve 3).

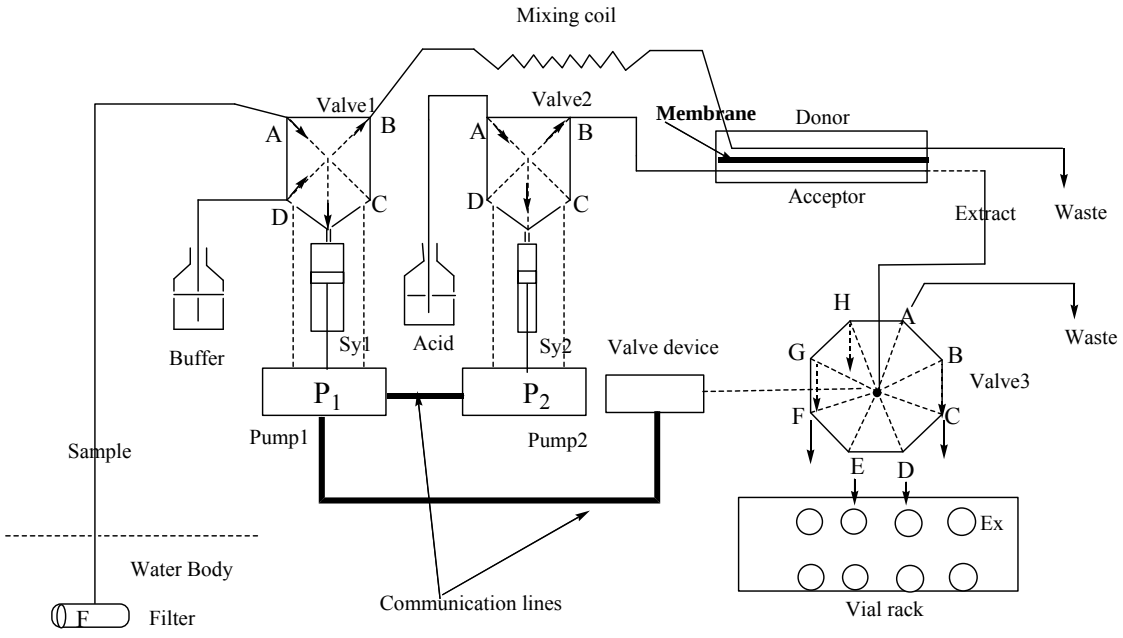


Fig. 3.3.1 Schematic diagram of the portable field sampler (for the description of symbols see the text)

3.3.5 Description of the Sampling Site

Continuous field extraction was carried out at Awassa Lake, 270 km south of Addis Ababa, the capital of Ethiopia. The lake is situated in Ethiopian Great Rift Valley, at an altitude of 1600 – 1700 m above sea level. Its inflow is mainly from a small river, "Tikur Wuha" meaning black water in local language [37] and a number of small streams [14]. It appears that the lake is a closed water basin and its pH is about 8.75. The absence of water outflow from a lake could imply contamination build-up and makes it important to monitor water quality. Farms are situated in close proximity to the lake, using several tonnes of pesticides each year. Thus, residues and degradation products of these different pesticides can easily reach the water bodies of the lake.

Two sampling sites were selected in the northern shore of the lake in the pilot study (March, 2003) and three sites; A, B and C in the monitoring study of levels of *s*-triazne herbicides (December 2003-July 2004), as depicted in Fig. 3.3.2. Site A was included in the second monitoring study. Site A was at the inlet of the tributary river, Tikur Wuha and part of the river, site B was at junction between the river and the lake and site C was in the water mass of the lake. Therefore, during the first sampling time the sample was collected from site B and C. At the time of the first sampling, the water flow at site B was very low and even looked stagnant, at least at the surface. The depth of the water at this point was approximately 2 m and the sampler probe was stabilized at a depth of around 1 m. The second sample site was about one kilometer away from the junction. The water was clearer and shallower than at site B, roughly 1 m deep and the sampler probe was stabilized at a depth of about 0.5 m during all of the extraction periods. During the first sampling time duplicate samplings and thus extractions were carried out at each site, which took *ca* 24 h, for each sampling. However, on site B the first extraction was aborted after having extracted 1260 mL of water. This turned out to be due to the low dispensing pump speed, which was close to the resonance frequency of the pump and vibrations caused the pump to lose its reference point. Such problem was overcome by programming with a quick "go to home" command after extraction of every about 500 mL. Another problem was that the filter probe broke at site 1, thus resulting in some particles reaching the donor pump and the membrane.

For monitoring study, three times of sampling was done mostly at the two sites, A and C and one time at three sites because of the difficulty of accessing site B and mounting the

sampler there as there was an increase in the size of the water and covering the whole vicinity of site B. The sampling times and sites are given in Table 3.3.3

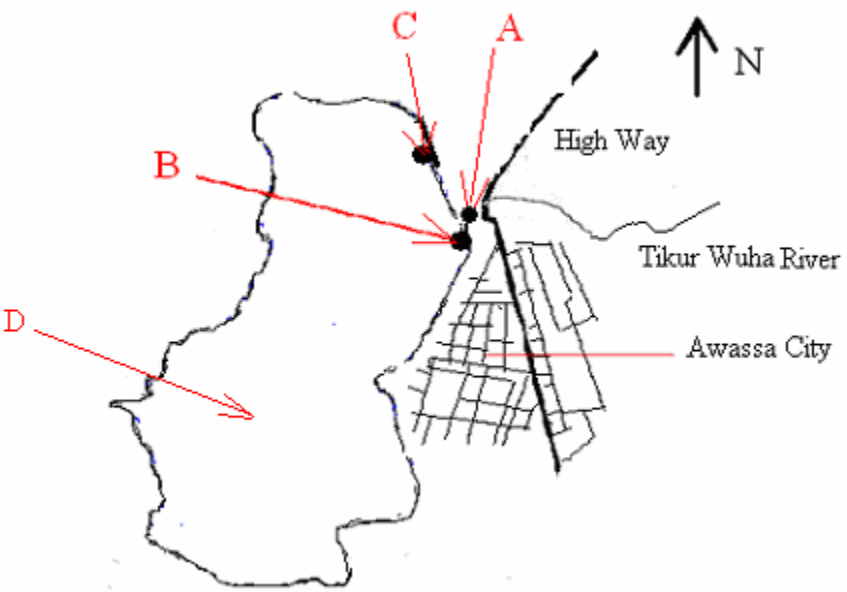


Fig. 3.3.2 Awassa Lake, its surroundings and the sampling sites A, B and C: A = Tikur Wuha river, B = Tikur Wuha river and Awassa lake junction and C = Lake body - 1 km away from the river inlet, D = Body of Awassa Lake, ● represents the sampling site. The map is not drawn to the scale.

Table 3.3.3 Time and site of water sampling from Awassa Lake and its tributary river

Survey	**Time of sampling**	**River inlet**	**Junction (lake/river)**	**Water mass**
1	March 2003		X	X
2	December 2003	X	X	X
3	February 2004	X		X
4	July 2004	X		X

3.4 Pesticide Residue Analysis in Sediment Samples from Selected Sites of Awassa and Ziway Lakes, Southern Ethiopia

The main purpose of this work is to determine the trace residue levels of *s*-triazine herbicides in sediment samples from selected sites of Awassa and Ziway Lakes.

3.4.1 Chemicals

Standards of *s*-triazines herbicides (Atrazine, Cyanazine, Propazine, Ametryn) and their degradation products (Deethylatrazine, DEA, Desisopropyl atrazine, DIA, Deethyldesisopropyl atrazine, DDA, Terbutylazine hydroxide, TrOH and Hydroxy atrazine, ATOH) used in this study were purchased from Dr Ehrenstorfer GmbH (Augsburg, Germany).

Stock standard solutions of 100 mg/L were prepared by weighing 0.5 mg of solid standards in weighing glass and dissolving in 5 mL volumetric flask in acetonitrile. Hydroxy degradation products were first dissolved in small (1-2 mL) volume of 1.0 M HCl, to achieve complete solubility and diluted to the final volume with acetonitrile.

Analytical reagent acetone (99.5% pure, Bahadurgarh, India), 99.5 % pure cyclohexane for residue analysis (Techno pharmachem, Bahadurgarh, India) and double distilled ethyl acetate (BDH, Laboratory Chemicals Division, England) were used as extracting solvents. 99.5 % pure laboratory reagent NaCl (Labmerk chemicals, India) and 98 – 99.9 % pure anhydrous sodium sulphate (Techno pharmachem, Bahadurgarh, India), 37 % extra pure HCl (Riediel-deHaën, Germany), 99.9% pure acetonitrile for HPLC (Sigma Aldrich–GmbH, Germany) were used as a mobile phase solvent.

Mixed working standard of *s*-triazine herbicides and their degradation products at lower concentration levels were made from the aliquots of stock standard solutions by appropriate dilution with reagent water. A series of standard mixtures with individual substance concentrations ranging from 0.0625 to 2 mg/L, at five points, were prepared for external calibration. All solutions were stored at 4^{o}C when not in use.

3. 4. 2 Materials and Instruments

Magnetic stirrer with hot plate (model 04803-02, USA), standard sieve 1.7 mm mesh size (ASTM E- 11, A.S. S.H.O.M-92, USA), Rotary evaporator; (ROTAVAPOR -RE (BUCHI), Switzerland), Analytical Balance (Mettler Toledo, Mettler instruments AG, Switzerland), portable mV/pH meter, (Hana instruments, Portugal), Ultra Sonic heater (England) were used in the process of sample preparation and analysis.

3.4.3 Sediment Sampling

Sampling was done at two lakes in the Ethiopian Rift Valley regions. These were Awassa and Ziway Lakes. There were a total of seven sampling sites, 4 sites at Awassa Lake and 3 sites at Ziway Lake. The locations of the sites are shown in Fig. 3.4.1 and 3.4.2.

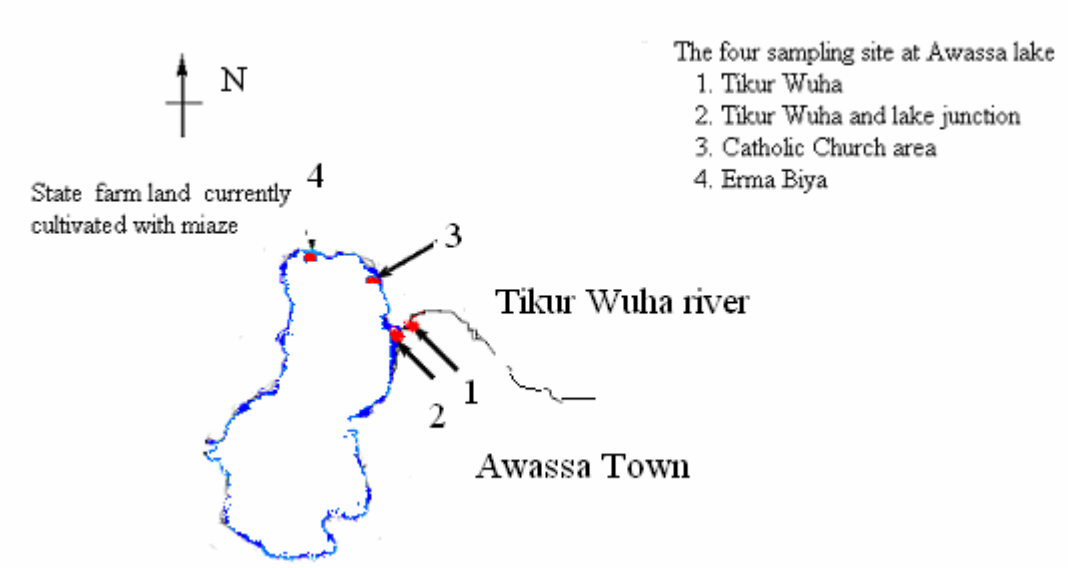


Fig. 3.4.1 Sediment sampling at Awassa Lake, 1= Tikur Wuha, 2 = Tikur Wuha and Awassa Lake junction, 3 = Catholic Church area (Lake water body mass), 4 = Erma Biya. The map is not drawn to the scale.

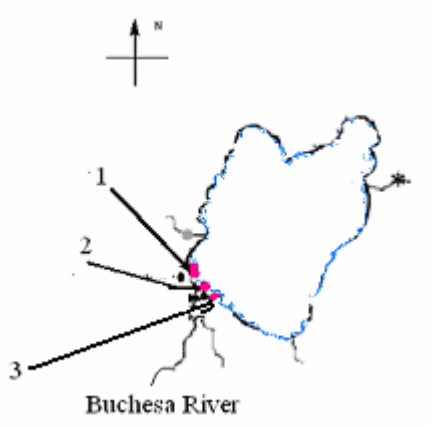


Fig. 3.4.2 Sediment sampling sites at Ziway Lake, 1= Korokonch, 2 = Wafiko, 3 = Buchesa River (outflow of the Lake). The map is not drawn to the scale.

The eastern sides of the sampling sites at Aawassa Lake were Tikur Wuha River and the junction of the river and the lake. The third sampling site was at the northeast side of the lake. The fourth sampling station was at the northwest part of the lake, which is located in proximity to farm areas, where maize crop was cultivated at the first sampling time.

From the sampling stations of Ziway Lake, one was at the out flow of the lake i.e., Buchesa River, located at the southwestern side of the lake. The other two stations were located at the western part of the lake, locally named as korokonch and wafiko areas. Three samples were taken from each sampling sites of the two lakes at approximately 5 m distance from each other above and mixed thoroughly.

As soon as the samples were taken from the lake water, they were rapped up with aluminum foil and put into polyethylene bags followed by labeling with the site number and name on the bags. All samples were stored and kept in ice cooled insulating box until they were transported to the laboratory. Immediately upon arrival at the laboratory, all samples that were taken from the same sampling site were further homogenized on aluminum foil and allowed to air dry at room temperature for 4-5 days until dryness. The sediment samples were stirred and mixed occasionally during drying to break it into smaller pieces. Then, they were sieved through 1.7 mm (USA standard) mesh. This was followed by gentle grinding in mortar before transferring to plastic bags to be kept frozen (-18^{o}C) in a refrigerator until extraction and analysis was done.

3.4.4 Sediment Extraction

The method of extraction was adapted from reference 277. Briefly: sediment sample extraction was carried out by solvent shake method using magnetic stirrer (model 04803-02, USA). Triplicate sub samples of 10 g dry sediment were taken from sediment samples of each sampling site and mixed with 30 g of activated sodium sulphate powder in a mortar. The mixture was then ground in a mortar until the sodium sulphate powder was flown freely. This powder was then extracted four times with 50 mL, 20 mL, 20 mL and 20 mL of acetone / cyclohexane (1:1 v/v) mixture for 15 min, 10 min, 7 min and 6 min, respectively in 500 mL Erlenmeyer flask (E-flask). At the end of each extraction time, the extract was separated from the sediment by decantation into E- flask and then filtered

through filter paper into Buchner flask by using suction filtration. The color of extracts obtained in most of the samples was yellowish.

Two hundred mL of saturated sodium chloride solution was added to the filtrate for salting out of the analytes. The aqueous phase was further extracted with 50 mL of ethyl acetate/cyclohexane (15:85 v/v) mixture. The two organic phases were combined in an E-Flask containing 20 g of sodium sulphate. The extract was then filtered through a plug of glass wool into an E- flask after moisture drying with sodium sulphate. Then, the solvent was reduced by evaporating using rotatory vapor to about 10 mL followed by the clean up procedure.

3.4.5 Sample Clean-up

The extract of the sediment sample was evaporated to dryness using small scale preparative set (AG laboratory glassware, England) at temperatures between 40 and 50^{o}C and the solvent was changed to 4 mL ultra pure water and 1 mL HPLC grade methanol. Then, the reconstituted sample was cleaned up using 50 mg C18 SPE cartridge. The C18 cartridge was pre-washed with 0.7 mL acetonitrile, methanol and ultra pure water, with the flow rate of 0.28 mL/min. Then, the sample was flowed through the cartridge with a flow rate of 1.4 mL/min under vacuum. After extraction of the sample, the cartridge was aspirated for 2 min at the same rate. The cartridge was then eluted twice with 0.3 mL acetonitrile. The extract was pre-concentrated by evaporation under gentle heating using small scale preparative set and was put in the autosampler of HPLC for injection.

3.4.6 Chromatographic Analysis

Analysis was carried out using Agilent 1200 series high performance liquid chromatograph (Agilent Technologies, Germany).

The separation of the ten analytes was performed using ZORBAX Ecllpse XDB-C18 analytical column (4.6 mm x 150 mm, 5 µm particle size) at a flow-rate of 0.5 mL/min

with a gradient of acetonitrile and 5 x10^{-3} M phosphate buffer (pH 7) prepared in ultra pure water purified using EASYpure^{TM} (Barnstead Thermolyne Corporation, USA).

The gradient was 10% of acetonitrile from 0 to 5 min, 15% from 5 min to 10 min, 20% from 10 min to 33 min, 40% upto 37 min and then 50% from 37 to 38 min, followed by 20% at 38 min upto 42 min where it becomes 10%.

4. Results and Discussions

4.1 Determination of Trace Levels of Dinitrophenolic Compounds in Environmental Water samples

Background

Phenolic compounds are ubiquitous in the environment as a result of many processes such as industrial [278] and biogeochemical processes and pesticide degradation [279]. Chloro- and nitrophenols are the main degradation products of many chlorinated phenoxy acid and organophosphorus pesticides, respectively.

Phenolic compounds are considered major environmental risks, either directly, as industrial effluents, or indirectly, as conversion products from natural and synthetic chemicals, including pesticides [280]. Owing to their toxicity both the US Environmental Protection Agency and the European Community (EC) have included some phenols, mainly nitrophenols and chlorophenols, in their lists of priority pollutants. The EC legislation requires that the maximum admissible concentration of phenols in drinking water should be 0.5 μg/L for the total content and 0.1 μg/L for an individual ones [281]. So, detection limits below 0.1μ g/Lare required

Therefore, in this study effort was made to develop a sample preparation method for the trace level determination of these classes of compounds.

To this effect, the main objective of this work was to study the applicability of HFSLM extraction in the kinetic uptake regime followed by HPLC/DAD for the determination of the total concentration of some representative dinitropenols in environmental water samples. In this work, we followed kinetic uptake regime with depletive extraction mode to determine the total concentration of dinitrophenols in environmental water samples. As

these classes of compounds are weak acids and chargeable three phase mode of HFSLM extraction was used.

4.1.1 Extraction Conditions

The pKa values of the analytes varies from 3.42 to 4.42 and the log K_{ow} ranges from 1.74 to 5.55. Since the analytes should be in their neutral state in order to be extracted into the organic phase [282], the pH of the sample solution was kept at pH 2. To trap charged analytes into the acceptor solution the pH of the acceptor phase was adjusted to 10. At this pH most of the analytes exist in their charged anionic form. The pH ranges are sufficiently wide to obtain high enrichment factor and suitable for the determination of trace concentration of the analytes.

Based on previous works [209, 283] and considering its good selectivity, undecane was chosen as the organic solvent for liquid membrane.

4.1.2 Extraction Time

Controlling the extraction time is critical when working in the kinetic uptake regime. HFSLM is three phases extraction system with two liquid-liquid interfaces; as a result the analytes molecules need to have time to diffuse through each phase and cross all interfaces to get into the acceptor phase. Hence, the influence of extraction time on the enrichment factor of the four compounds was studied. Fig. 4.1.1 shows the effect of extraction time on the enrichment factor of the compounds in HFSLM. The amount of analyte extracted increases with longer extraction time before any kind of equilibrium is attained until a maximum is obtained near the equilibrium. For this work 5 h extraction time, which is at the linear position of the curve was chosen for subsequent experiments. The extraction time in this regard is relatively long but it is possible to do many parallel extractions within this time.

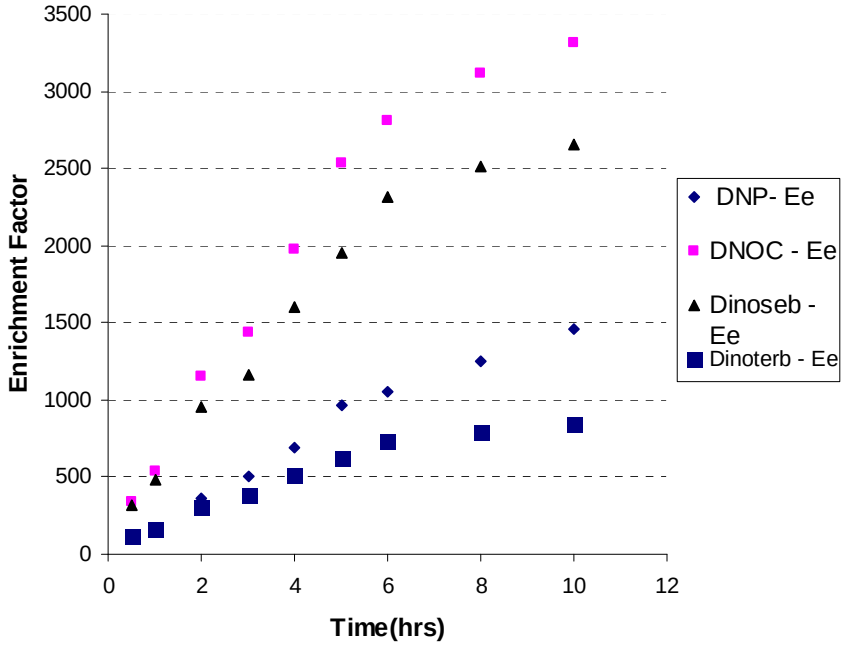


Fig. 4.1.1 Effect of extraction time on the enrichment factor of the four dinitrophenolic compounds. Spiked concentration was 10 µg/L for each compound. Shaking speed was 130 rpm, sample volume was 200 mL and buffer concentration was 400 mM

4.1.3 Effect of acceptor buffer concentration, shaking and stirring speeds

Since the extraction is based on difference in pH values between acceptor and donor phases, the acceptor and donor pH were controlled by appropriate buffer solutions. $NaHCO_3$ was used as acceptor buffer solution. Fixing its pH at 10 to trap the charged analytes in the acceptor solution, the effect of acceptor buffer concentration ranging from 0 to 1000mM was investigated. ANOVA test revealed that there was no statistically significant difference between the mean enrichment factors of each analyte from one level of buffer concentration to another at 95% confidence level. The slight change in the enrichment factor can be attributed to experimental error or to a small change in K_A due to different ionic strengths. Therefore, as a compromise between the need for high acceptor

buffer capacity for real samples [209, 225] to maintain the pH constant and the need for the HPLC mobile phase to acidify the injected sample, 600 mM concentration of the buffer $NaHCO_3$ was chosen as acceptor buffer concentration for the following experiments.

As the extraction process was done in kinetic uptake regime, the system is subjected to the effect of many dynamic processes. One dynamic process that will affect the enrichment factor of the extraction is the agitation of the sample. Agitation can be done either by stirring or shaking. Fig. 4.1.2a shows the comparison of static, stirring and shaking conditions. It is possible to observe that E_e is the highest under shaking condition than in the other two conditions. This may be explained by the fact that during shaking the fiber itself moves and mixes the acceptor solution in the lumen of the fiber and facilitates the transfer of the analytes into the acceptor phase at faster rate. It should be noted that when the static mode is used, very low E_e was obtained (Fig. 4.1.2)

As shaking gives the best enrichment within a given time, the effect of shaking speed was investigated (Fig. 4.1.2b). It can be observed that with increasing shaking speed the enrichment factor also increases up to a certain maximum value. This is so because the diffusion coefficient in the aqueous phase increases with increasing agitation rate, as faster agitation rate decreases the diffusion layer in the aqueous phase around the surface of the membrane. Thus mass transfer increases and also it permits the continuous exposure of the extraction membrane surface to fresh aqueous sample. However, when shaking speed is higher than 200 rpm, E_e decreased, probably due to the formation of air bubbles and disturbance in the liquid membrane. Therefore 200 rpm was used for subsequent experiments.

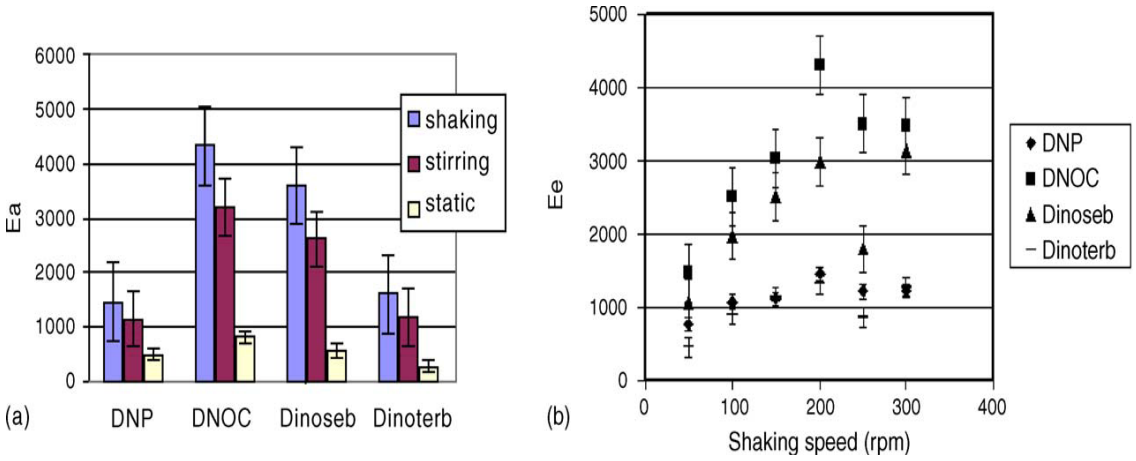


Fig. 4.1.2 The Effect of shaking speed on the enrichment of dinitrophenolic compounds. Concentration 10 µg/L for each compound, extraction time was 3 hr, acceptor buffer concentration was 400 mM and sample volume was 200 mL

4.1.4. Effect of Sample Volume

Effect of sample volume on enrichment factor was studied by taking 25, 50, 100, 200, 500 and 1000 mL of sample solutions adjusted at pH 2.00 using concentrated sulfuric acid. As can be seen from Fig. 4.1.3, the enrichment factor increases with increasing sample volume and levels off after 500 mL. This is reasonable because as sample volume increases the amount of analytes also increases. To keep the volume of the sample at certain constant value, we have chosen 200 mL, even though the enrichment factor was not maximum at this point. This volume was used for the following experiments.

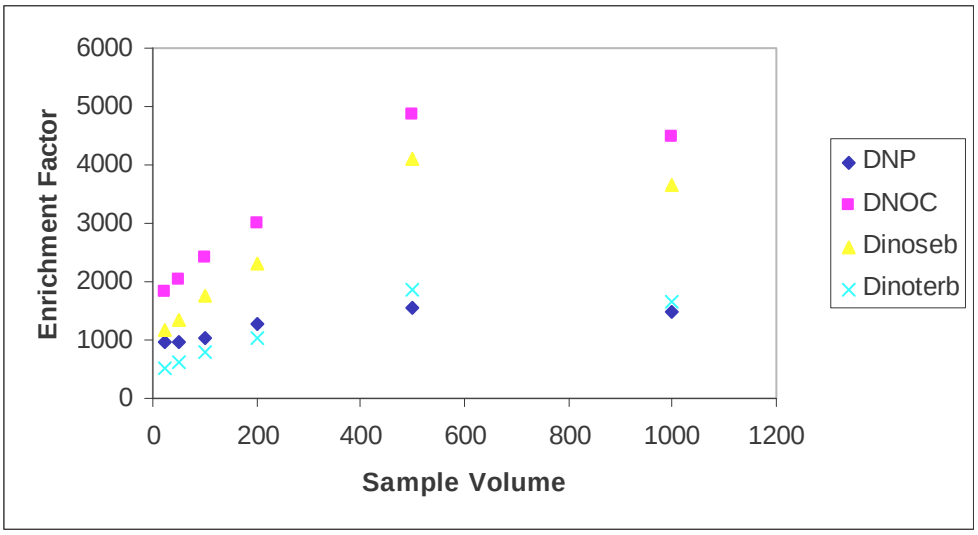


Fig. 4.1.3 The effect of sample volume on the enrichment of dinitrophenolic compounds. Concentration 10 µg/L for each compound, shaking speed was 130 rpm; extraction time was 3 hr, acceptor buffer concentration was 400 mM and sample volume was 200 mL

4.1.5 Effect of Concentration of Analytes

In the above experiments it was found that the enrichment of Dinoterb is lower than theoretically expected and the repeatability was not good. Reducing the concentration of spiked analytes from 10 µg/L to 1µg/L reduced the problem and on further reduction of the concentration to 100 ng/L changes the result significantly. Fig. 4.1.4 shows the effect of enrichment factor time on the extraction factor of the analytes at the reduced concentration of spiked analytes. When this result is compared with that of Fig. 4.1.1 there is significant difference not only with Dinoterb but also with other analytes. The change in the enrichment factor may be due to either the low solubility of Dinoterb at low pH after acidification of the extract or due to low rate of extraction at high concentration of the analyte. The second idea is more plausible as our extraction was done under membrane controlled conditions and hence the extractability of the analytes depends on the activity of the solution. In any case the cause of this change needs further investigation. Since our

target is to extract trace level of analytes we successfully achieved our objective with lower concentration of analytes under current condition. The extraction efficiency under reduced concentration ranges from 12-43%.

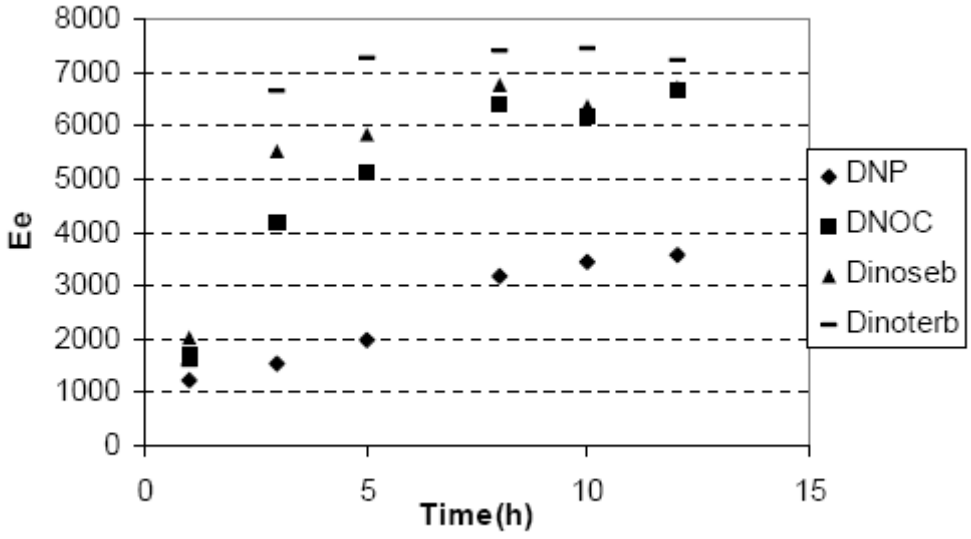


Fig. 4.1.4 Effect of extraction time on the enrichment factor of the four ditrophenolic compounds after reducing the concentration of spiked analytes to 100 ng/L. Extraction conditions: spiked concentration, 100 ng/L for each analyte, shaking speed 200 rpm; sample volume, 200 mL. Acceptor buffer concentration, 600 mM, extraction time, 5 h

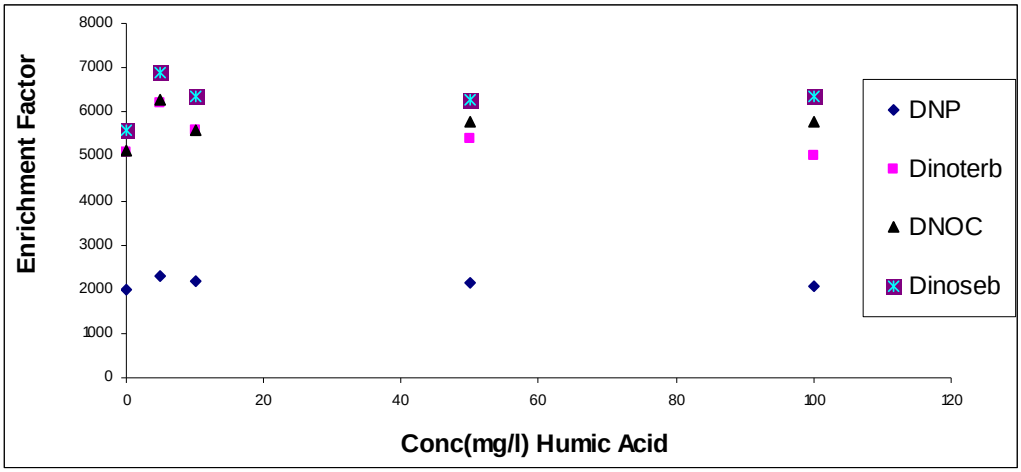
4.1.6 Effect of Humic Acid

It is very common that surface waters contain humic acids. Therefore, the effect of the presence of humic acids on E_e of dinitrophenolic compounds was studied by adding 0, 5, 10, 50 and 100 mg/L of humic acid to the sample solution (two replicates were used for each concentration). Fig. 4.1.5a shows variation of enrichment factor with the concentration of humic acid. The enrichment factor initially increased slightly with increase in the concentration of the humic acid and then dropped down and leveled off quickly. One way ANOVA test revealed that there is no statistically significant difference between the mean enrichment factors of each analyte from one level of humic acid

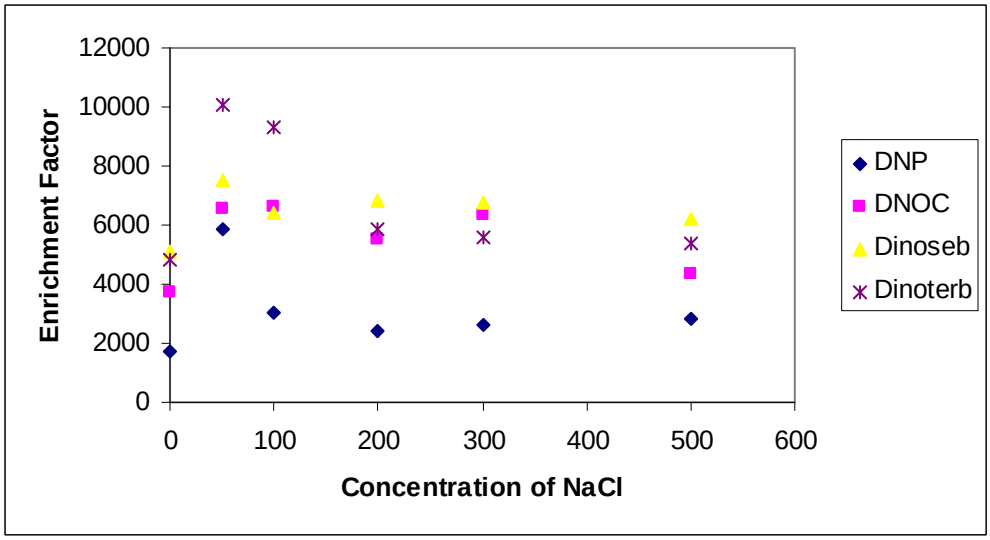
concentration to another ($p > 0.05$, $n = 2$ for each group) in the range tested. There was no interfering peak at the retention times of the analytes with HPLC analysis upto 100 mg/L concentration of humic acid. This shows the selectivity of the HFSLM extraction method. The result also indicates that at the current conditions, the total concentration of analytes is measured. Using conditions for sensing the freely dissolved concentrations, a clear dependence of the results on the concentration of humic acid for similar compound was observed [29].

4.1.7 Effect of Salinity of the Sample on Enrichment Factor

Extraction of analytes can be enhanced or retarded by addition of salts [284] depending on the nature of analytes. As a result, the effect of the salinity of the sample was studied by adding 0, 50, 100, 200, 300 and 500 mM of NaCl to the sample solutions. Fig. 4.1.5b depicts the effect of concentration of NaCl solutions on the enrichment factors of dinitrophenolic compounds in reagent water. The enrichment of more hydrophobic analytes (Dinoterb and Dinoseb) increased significantly initially and levels off later as more salt has been added. This is reasonable because salting out effect increases polarity of the medium and increases extraction rate. Hence, to take care of the effect of salt, 200 mM NaCl was added to the reagent water and real water samples before extraction.



A



B

Fig. 4.1.5 (A) Effect of concentration of humic acid on the enrichment factor, (B) effect of the salinity (conc. NaCl, mM) of the environment on the enrichemnt factor of the four nitrophenolic compounds. Extraction conditions: concentration was 100 ng/L for each analyte, shaking speed was 200 rpm, sample volume was 200mL, acceptor buffer concentration was 600 mM and extraction time was 5 h

4.1.8 Method Validation

Linearity, Limits of Detection, Repeatability and Reproducibility

The linearity of the method is a measure of range within which the results are directly, or by a well defined mathematical transformation, proportional to the concentration of analyte in a sample. For this analysis, the linear relationship was tested by running extractions of solution containing analytes in the concentration range of 25 - 200 ng/L in reagent water sample. All of the analytes exhibited good linearity with squared regression coefficients (r^2) ranging from 0.993 to 0.999 (Table 1).

The MDL was determined according to EPA method 136 [285], i.e., as 3 times the standard deviation of 7 replicate extractions of spiked reagent water at 25 ng/L of each analyte and was found ranging from 6 to 8 ng/L. This shows the analytical capability of HFSLM extraction for trace enrichment of dinitrophenols and other related polar contaminants in environmental water samples. For the comparison of blank and spiked extraction see, Fig. 4.1. 6.

The repeatability was studied by running 6 extractions of reagent water samples spiked at 50 ng/L and 100 ng/L of the analytes concentrations within one day. The relative standard deviations (RSDs) were calculated to be from 2.4 – 6.1% and 3.5 – 6.8 % for 50 ng/L and 100 ng/L concentrations, respectively. The reproducibility of the method was studied by extracting two series of 4 replicate and 6 replicate samples at two different days, which was spiked at 50 ng/L in reagent water. The relative standard deviations were found ranging from 3.1 to 16.6%. The method shows very good repeatability and reasonable reproducibility at such low concentration of analytes.

4.1.9 Application to the Analysis of dinitrophenolic compounds in sea, river and lake water samples

The optimized and validated HFSLM was applied to the extraction of sea, river and lake water samples using previously determined optimum extraction conditions. Only DNP and DNOC were detected in river and seawater samples but they were below quantification limit. None of the analytes were detected in the lake water. As a result, the real samples were spiked with known different concentrations and the linearity of the method was compared with that of the reagent water. The comparison was made by checking the similarity in the slopes of the different matrixes.

The linearity was studied in the analytes concentration range of 25 - 200 ng/L. All of the analytes exhibited good linearity with squared regression coefficients (r^2) ranging from 0.992 – 0.999, 0.994 – 0.999 and 0.997 – 0.999 using peak area as a response variable for river, lake and sea water samples, respectively (Table 4.1.1).

Table 4.1.1 Linearity of HFSLM extraction in reagent, river, lake and seawater samples

Analyte	Matrices	Linear regression	Correlation coefficient (r^2)
DNP	Reagent water	$y = 0.0502x + 0.00$	0.998
	River water	$y = 0.0588x + 0.06$	0.994
	Lake water	$y = 0.064x - 1.09$	0.997
	Sea water	$y = 0.0659x - 0.84$	0.998
DNOC	Reagent water	$y = 0.1706x - 1.30$	0.999
	River water	$y = 0.162x + 0.24$	0.999
	Lake water	$y = 0.1374x - 2.39$	0.994
	Sea water	$y = 0.1664x - 1.56$	0.999
Dinoseb	Reagent water	$y = 0.1791x - 1.82$	0.995
	River water	$y = 0.1148x + 0.08$	0.992
	Lake water	$y = 0.0861x - 1.62$	0.999
	Sea water	$y = 0.1441x - 2.06$	0.997
Dinoterb	Reagent water	$y = 0.1587x - 1.40$	0.993
	River water	$y = 0.069x - 0.12$	0.999
	Lake water	$y = 0.0436x - 0.85$	0.994
	Sea water	$y = 0.0939x - 1.75$	0.997

To study the matrix effect on the extractability of the contaminants, the similarity of the slopes of the linear regression of spiked reagent water and real environmental water samples were tested by using a statistical technique known as the analysis of covariance (ANCOVA) [286]. This test permits the detection of the presence of any matrix effect on the extraction of the analytes. The test results were different for different compounds. There was no significant difference in the slopes of the linear regression of reagent water and real water samples for DNOC and Dinoseb. There was also no significance difference between reagent water and river water for DNP but there was significance difference between reagent water and seawater and reagent water and lake water. For Dinoterb, there was significant difference between the slopes of the linear regression of the reagent water and real water samples. This matrix effect can be attributed to the hydrophobicity of Dinoterb, as it could be strongly bound to dissolved organic compounds in the samples. This idea is substantiated by the DOC measurement, which is given in Table 4.1.2. It is possible to see from this table that the amount of DOC varies significantly between the three matrices. Actually the apparent recovery [287] exactly varies with the amount of DOC in the samples. Thus, the higher is the DOC content, the lower the apparent recovery. Therefore, we recommend that either standard addition method or matrix-matched calibration [288] should be used for the analysis of this analyte in real environmental water samples. Finally, Fig. 4.1.6 shows a chromatogram obtained from the spiked reagent, river, lake and sea environmental water samples in the selected extraction conditions, recorded at a wavelength of 280 nm.

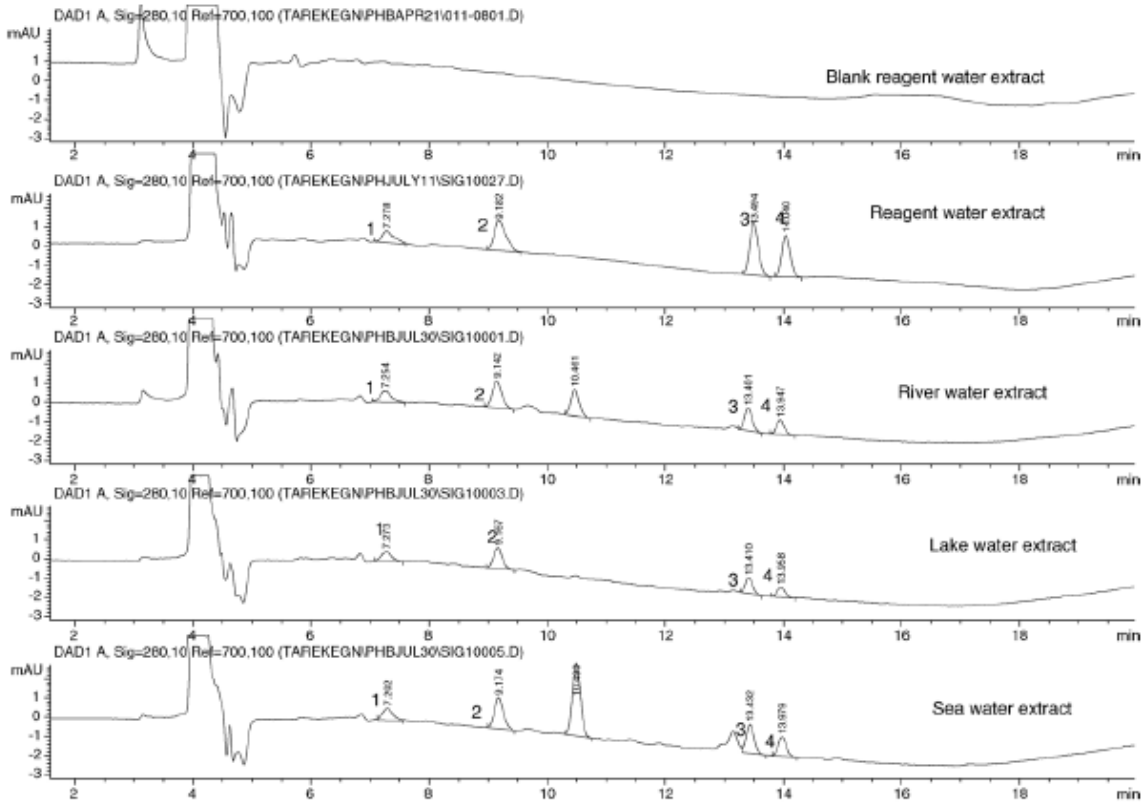


Fig. 4.1.6 Chromatograms (LC-UV at 280 nm) of extracts from 200 mL of reagent water, river water, lake water and seawater samples, respectively, spiked at 100 ng/L of the analytes (1) DNP, (2) DNOC, (3) Dinoseb and (4) Dinoterb

Table 4.1.2 Conductivity dissolved organic carbon (DOC) of the real samples and apparent recovery of the spiked analytes from the environmental water samples.

	River water	**Lake water**	**Sea water**
Conductivity (μS/cm)	507	884	13650
DOC (mg/L)	8	13	4
CO_3 (mg/L)	93	35	19
Apparent recovery (Spiked concentration, 100 ng/L)			
DNP	104	119	130
DNOC	83	73	87
Dinoseb	70	52	76
Dinoterb	50	38	55

4.1.10 Conclusion

The proposed HF-SLM extraction technique appears to be suitable for trace level determination of polar dinitrophenols in environmental water samples by simple means. It is possible to achieve high enrichment factors under optimized extraction conditions and hence suitable for total or depletive extraction of analytes in environmental water samples. As a result of high preconcentrating capacity of HF-SLM, LODs at low parts per trillion levels (6.0-8.0 ng/L) were determined in reagent water samples. This is far below the EU legislations requirement for phenolic compounds and pesticides in surface water for drinking purpose. To our knowledge such low determination levels with very simple extraction system were not reported before and shows the advantage of the developed method over other similar extraction techniques. The other advantage of the method is very clean extracts. As they do not pass the solvent-filled pores, the large molecules like humic acid and fulvic acids cannot be co-extracted with analytes and interferes with the HPLC analysis. No interfering peak was observed from humic acid up to 100 mg/L in the donor phase at the retention times of the analytes. Standard addition or matrix matched calibration is required for the more hydrophobic contaminants. As very small amount of organic solvent is used per sample, the method is also environmentally friendly.

4.2 Hollow Fibre Liquid Phase Microextraction Method for Trace Enrichment of Freely Dissolved Organophosphorus Pesticides in Environmental Waters

Background

As discussed in section 1.1.1, organophosphorus pesticides (OPPs) are esters of phosphoric or thiophosphoric acids and are highly toxic to mammals because of their capacity to phosphorylate the active site of acetylcholinesterase (AChE), leading to accumulation of acetylcholine in synapses [289]. Because of their short persistence in the environment, these compounds have replaced the organochlorine pesticides; however, their toxicity, together with the possibility of bioaccumulation along the trophic chain, may represent a risk for human health [290]. Therefore, determining the bioavailable concentrations of these pesticides in the environmental water samples is vital for fast environmental remediation. To this effect, a new and simple HF-LPME technical set up was developed in this study for the determination of freely dissolved OPPs in environmental water samples. As OPPs are non-ionizable two phase system was found suitable for their extraction unlike those of dinintrophenolic herbicides.

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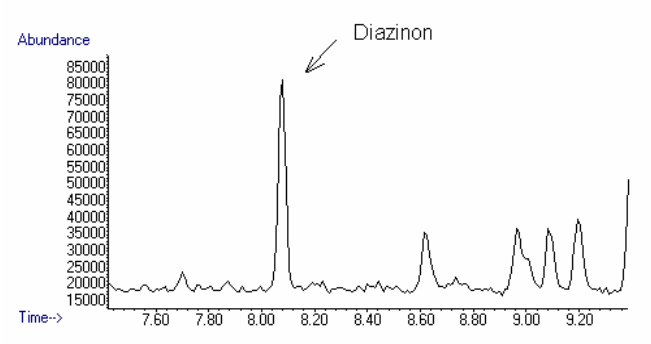
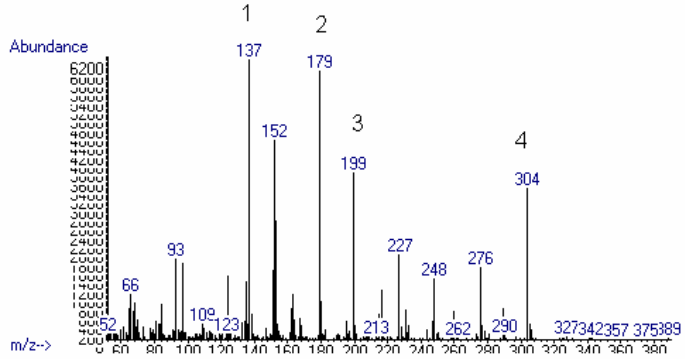
4.2.1 General Information and Evaluation of Extraction Parameters

The compounds studied are listed in Table 4.2.1 along with their log P and water solubility data. It is to be noted that the compounds are reasonably hydrophobic and have low solubility in water. The GC-MS chromatogram obtained from standard sample run with the corresponding mass spectra for the three analytes is given in Fig. 4.2.1, A, B, C, D and E. As indicated in the section 3.2.4, the four ions selected for each analyte quatitation are numbered 1-4 in the spectra.


Abundance
Diazinon
Time-->
7.60 7.80 8.00 8.20 8.40 8.60 8.80 9.00 9.20

A


Abundance
1
2
3
4
137
179
152
199
304
93
227
248
276
66
109
123
213
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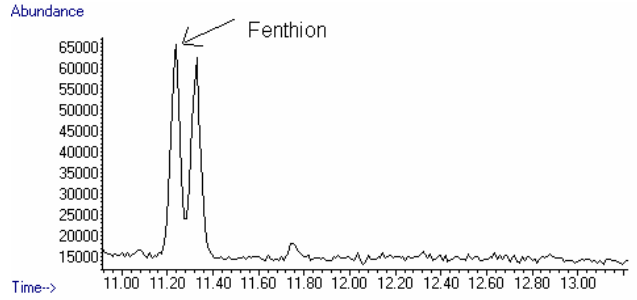
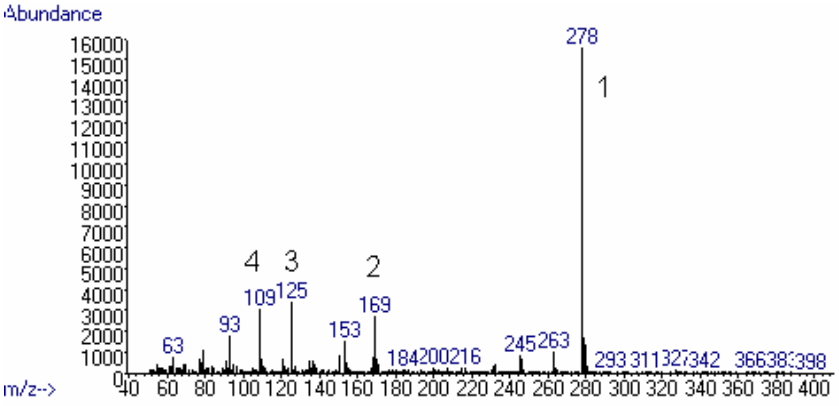
290
327342357 375389
m/z-->

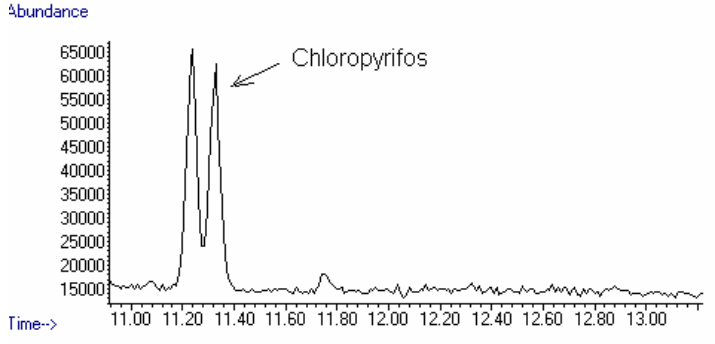
B


Abundance
Fenthion
Time-->
11.00 11.20 11.40 11.60 11.80 12.00 12.20 12.40 12.60 12.80 13.00

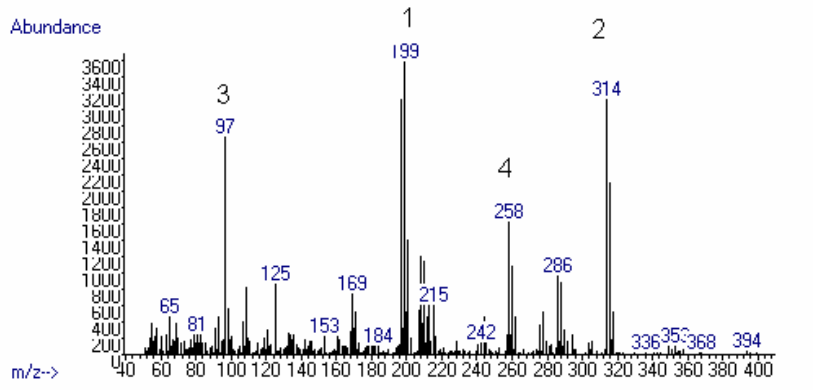
C


Abundance
278
1
4
3
2
109
125
169
93
153
63
245
263
184200216
293 311327342 366383398
m/z-->

D



E



F

Fig. 4.2.1 Chromatogram and Mass spectra for the analytes under study. The numbers show the ions selected for quantitaion in each case

Table 4.2.1 List of studied analytes along with their log P and water solubility data

Compound Name	**log P***	**Water Solubility[a]**
Diazinon	3.44 ± 0.37	60 mg/L
Fenthion	3.21±0.34	4.2 mg/L
Chloropyrifos	4.77 ± 0.40	1.4 mg/L

* Calculated by ACD/Chemsketch (Advanced Chemistry Development Inc. Toronto, Canada). [a] Obtained from reference [291].

During the hollow fibre liquid phase microextraction procedure, a hollow fibre of certain length, containing the membrane liquid phase in its pores and the lumen, was immersed in the aqueous sample solution containing the analyte of concern. Then the analytes partition between the two immiscible solvents. The extraction is said to be completed when the system reaches equilibrium. In this kind of extraction, it is assumed that the dynamics of extraction is a diffusion-limited process [260]. Therefore, parameters related to this equilibrium conditions should be used to optimize the method of extraction. In this regard, the extraction of analyte can be expressed as the enrichment factor, equation 24 or extraction efficiency, as these parameters express the relationships between the amount extracted and amount originally present in the matrix of concern, equation 11. In this study, enrichment factor was used to investigate the parameters governing the effectiveness of the method for the non-depletive extraction of the three OPPs. The enrichment factor (E_e) of the analyte can be evaluated using equation 7

4.2.2 Volume of the Extracting Phase

For method optimization, validation and application studies in non-depletive extraction of the analyte, the volume of extracting phase plays significant role. According to Mayer and

co-workers [261], the non-depletive extracted amount of an analyte on solid phase microextraction fibre should be kept below 5% of the amount dissolved in the sample medium and mathematically expressed as:

$$\frac{(Vsampler.Ksampler/medium)}{Vmedium} \leq 0.05 \qquad (34)$$

where $V_{sampler}$ and V_{medium} are the volume of the sampler and that of the medium from which the analytes are extracted, respectively.

An incomplete trapping, which is similar to the SPME discussed above, was followed in this work. The sampler volume, i.e., the volume of the acceptor in the hollow fibre, therefore, had to be appropriately chosen. The volume of the sampler can be calculated from the length, internal diameter and thickness of the hollow fibre used. Since the internal diameter and thickness of the hollow fibre are constant for a given type of hollow fibre, the sampler volume used for a given sample varies with the length of the hollow fibre used for the extraction. Therefore, in this study, an effective length of 1.2-1.4 cm of the hollow fibre with the above indicated dimensions was used.

4.2.3 Extraction Time

Application of any passive sampler for environmental sampling can be done in any one of the three regimes [261]: kinetic, intermediate and near equilibrium. Passive samplers, which require long time to attain thermodynamic equilibrium, were mostly operated in the kinetic uptake regime [261]. Fig. 4.2.2 shows the generalized uptake profile for a passive sampling device [261]. The kinetic uptake regime is the linear portion of the graph where so amount of the analytes extracted varies with the change in the extrtaction time. Whereas, in the equilibrium uptake regime the amount of analytes extracted will not be basically affected by the time of extraction.

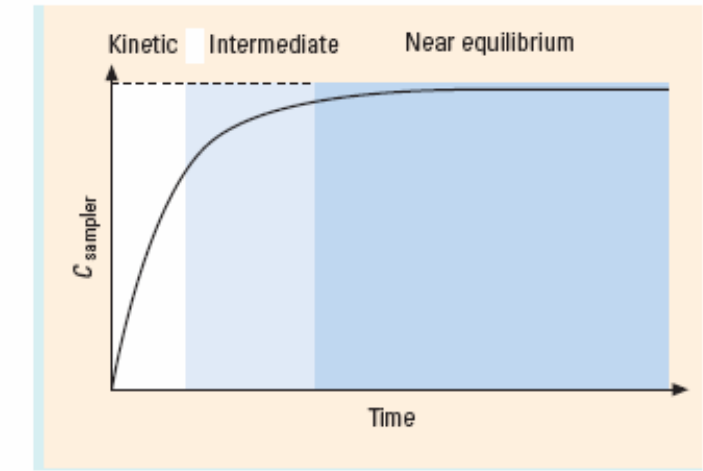


Fig. 4.2.2. Generalized uptake profile for a passive sampling device [261]

Despite the fact that it will take long time, equilibrium uptake regime will be appropriate for biomimetic approaches. Therefore, we have chosen to work at the equilibrium uptake regime so that the measurements were not affected by potential interferents from the matrix on the absorption kinetics [168].

In equilibrium sampling determination of the time at which this thermodynamic equilibrium has reached is very critical. The amounts of mass transfer and hence the enrichment factor increases with increasing time until it reaches the equilibrium. In this study, the determination of equilibrium extraction time was performed by following the enrichment factor of each analyte with increasing extraction time. Fig. 4.2.3 shows the effect of extraction time on the enrichment factors of the three OPPs. The time to reach equilibrium is more or less similar for all of the three analytes. As it could also be seen from Fig. 4.2.3 an equilibrium uptake regime was achieved nearly for all of the analytes under study after 72 h of extraction and, thus, 72 h was chosen for the subsequent experimental works.

It should further be noted that under diffusion controlled conditions, it is possible to reduce equilibration time by stirring or agitating the sample at certain constant speed. Stirring significantly reduced the time to reach the equilibrium, Fig. 4.2. 4. However, if biomimetic approach is required or if application to real environmental situation is targeted, static conditions are preferred to stirring or agitation [225]. Therefore, we have chosen static conditions where there was no disturbance of any kind. This will obviously take very long time, but since the extraction set up developed was so simple and inexpensive, it is possible to run many parallel extractions at the same time, which compensates for the length of time. Hence, it is possible to apply this sample preparation technique to the analysis of very large number of environmental water samples.

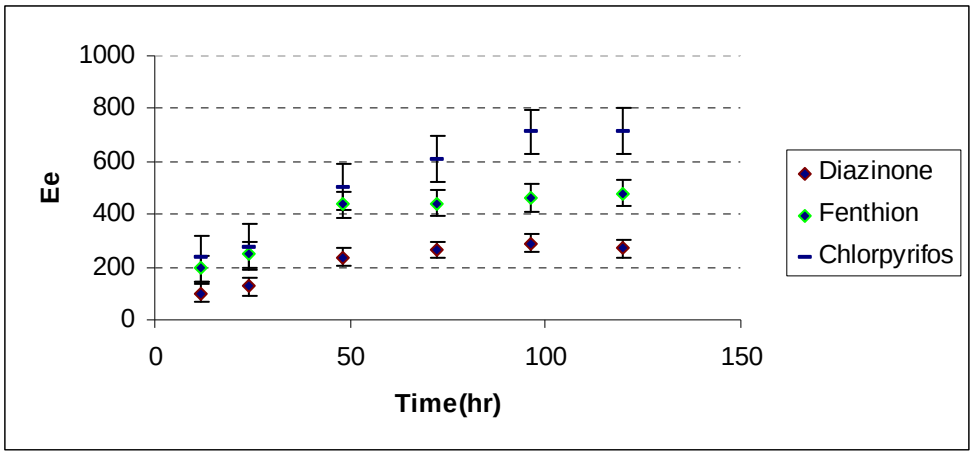


Fig. 4.2.3 Determination of equilibrium uptake time for the analytes studied using HF-LPME. Extraction conditions: 0.4 µg/L of the analytes under study spiked in reagent water. The extraction sample volume was kept at about 80 mL

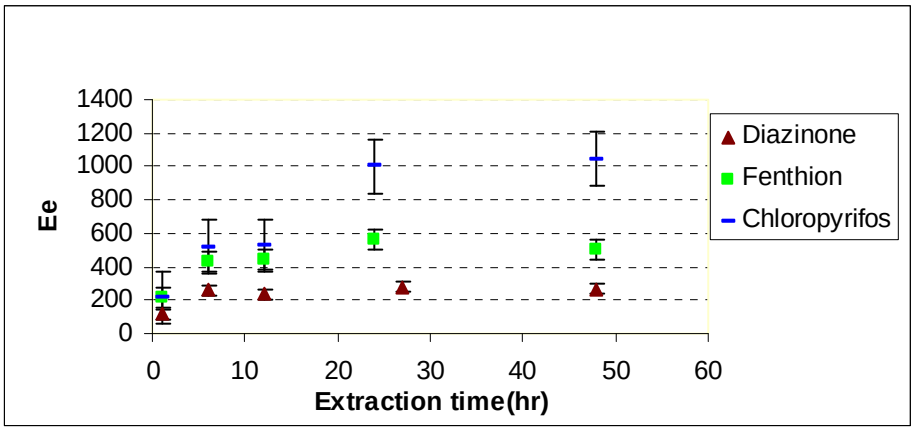


Fig. 4.2.4 Effect of stirring on equilibrium uptake time. Extraction conditions: same as Fig. 4.2.3

4. 2. 4 Choice of the Membrane Solvent

The choice of organic solvent in the HF-LPME technique plays a significant role for efficiently and selectively isolating analytes from various matrices. For attaining maximum permeation, the analytes in question should appreciably be dissolved in the chosen solvent; in a similar manner to common principle of LLE, i.e., "like dissolves like". Accordingly, the particular solvent chosen should be of high solubility for the target analyte, efficiently immobilized in the hollow fiber pores and compatible with the capillary GC column [252]. Based on these considerations, three organic solvents; *viz*. *n*-undecane, di-*n*-hexyl ether and 1-octanol were evaluated for their performances. Fig. 4.2.5 shows the differences in the enrichment factors of the analytes using these organic solvents. The more non-polar analyte, chloropyrifos, was enriched better than the others in the more non-polar solvent (undecane). Furthermore, this solvent exhibited the highest enrichment factor among the solvents investigated. Therefore, *n*-undecane was used as an extraction solvent for the subsequent experimental works.

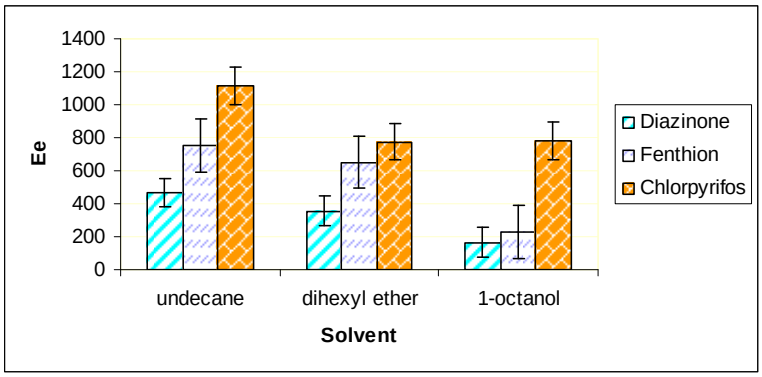


Fig. 4.2.5 Enrichment factor, E_e, of the OPPs for various membrane solvents investigated. Extraction conditions: 0.4 µg/L analyte mixture spiked in reagent water and sample volume was adjusted to 1125 mL

4.2.5 Effects of the Sample Volume

The other important variable, influencing the non-depletive extraction behaviour of the analyte is the sample volume. Evaluation of the extent to which it may affect the enrichment factor is particularly significant when the focus of the research undertaking is aiming towards the real environmental conditions. This is for the reason that the analyte amount extracted into the extracting phase increases with increasing sample volume [292]. One of the conditions used for the non-depletive equilibrium extraction is using very large sample volume so that the extracted amount in small volume of the sampler will be kept to the minimum [260]. Fig. 4.2.6 shows the effect of sample volume on the enrichment factor of the contaminants from reagent water. Equilibrium has been achieved at about 500 mL of the sample volume. But, to entirely avoid any kind of depletion, as indicated by equation (34) above and the convenience of measurement for the amber bottles, 1000 mL of sample volume was chosen for all

subsequent extractions. Furthermore, to work with zero headspace the actual sample volume used during the whole extraction process was adjusted to 1125 mL.

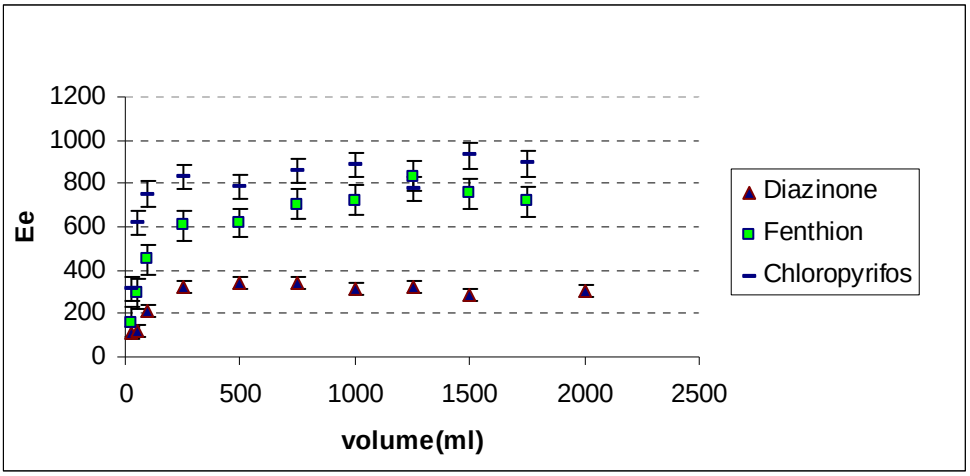


Fig. 4.2.6 Effect of sample volume on the enrichment factor of the pesticides studied. Extraction conditions: 0.4 µg/L of the OPPs mixture was spiked in reagent water

4.2.6 Salting out Effect

Generally, analyte solubility in aqueous solution decreases as ionic strength increases [293]. Therefore, the degree of partitioning of most hydrophobic analytes into the organic phase can be enhanced by addition of certain salts to the sample from which analytes are extracted. In this regard, significant improvement of the enrichment factor has been achieved in a number of studies by the addition of salt solutions to aqueous samples in LLE [294], SLME [215], HF-LPME [295] and SPME [296]. The principal factor responsible for enhanced enrichment is the salting out effect.

In the present study, the effect of addition of a salt on enrichment of the analyte under study has also been carried out by addition of sodium chloride, NaCl, solution varying in concentration from 50-500 mM, keeping the concentration of each analyte at 0.4 µg/L in reagent water. The effects of addition of NaCl addition to HF-LPME is depicted in Fig.

4.2.7. It is evident from the results obtained that the optimum NaCl concentration for the better enrichment of the tested insecticides was 250 mM. In principle, calibrations of the extracting sample with this much amount of salt was required. However, since the principal aim of this study was to extract analytes in an undisturbed natural state, without interfering into their equilibrium system, the extracting samples in subsequent analysis were not calibrated. Therefore, this experiment was done only to evaluate the extent to which the presence of salt, in the natural water environment would affect the enrichment of these analytes by using HF-LPME. Our observation showed that, other things being constant, the determinations of freely dissolved contaminants from salty water systems may be affected more than the non-salty water systems.

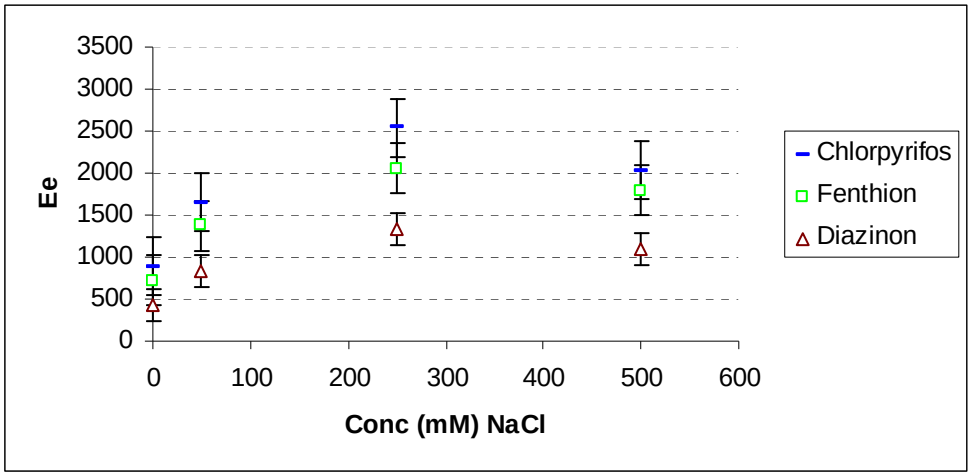


Fig. 4.2.7 Effect of addition of a salt on the enrichment factor of the analytes under investigation. Extraction conditions: 0.4 µg/L of analyte mixture spiked in reagent water and sample volume was adjusted to about 1125 mL

4.2.7 Effect of Humic Acid

Dissolved organic carbon (DOC), which may usually occur in natural waters as humic acid, mainly ranges in concentration from 0.5 to 50 mg/L [168]. These substances can possibly cause interferences when the selective extraction of freely dissolved organic pollutant from environmental water samples is targeted. Consequently, the study of effects of these substances on extraction efficiency and, thus, analyte enrichment requires designing a systematic experimental procedure. This may be needed either to determine the extent to which they will be enriched and thus interfere and/or to minimize their accumulation along the analytes of interest. The other possible effect would be the binding of analytes to these substances and hence their non-availability for extraction. In this context, other workers [296, 297] showed that a certain fraction of the freely dissolved aqueous concentration of organic pollutants can bound to humic acid and hence the freely dissolved amount was decreased in the presence of DOC.

In the current study, interfering potential of the dissolved organic carbon, i.e., HA, in the determinations of freely dissolved OPPs with HF-LPME were carried out by dissolving HA in the concentration range of 0 to 50 mg/L in reagent water. The resulting solution was then allowed to equilibrate for over 3 h under stirring conditions. Subsequently, the extraction was carried out in similar ways to the other spiked reagent water samples discussed above.

Fig. 4.2.8 shows the effect of humic acid on the enrichment factor of the pesticides studied. The free concentration of the most hydrophobic compound decreased more with increasing humic acid concentration than the other two compounds that are relatively less hydrophobic. However, one-way ANOVA test using Statgraphic software has indicated that there was no significant difference between the mean enrichment factors from one level of concentration of HA to another at the 95.0% confidence level for the two compounds studied but there is a decrease for Chloropyrifos, which is the more hydrophobic analyte.

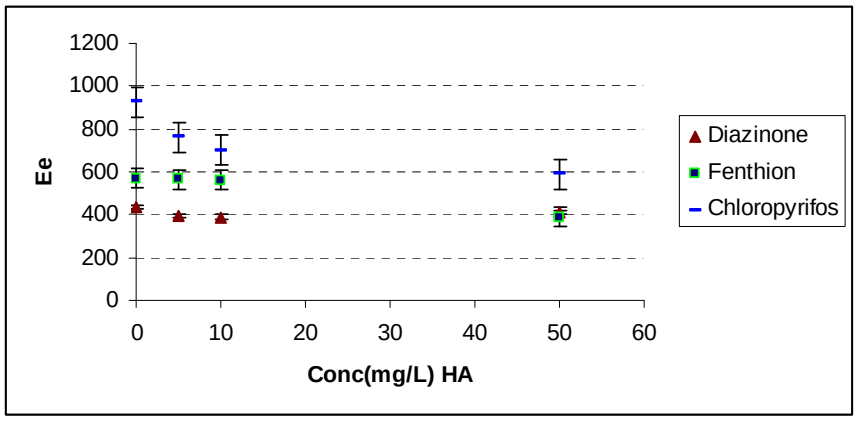


Fig. 4.2.8 Influence of varied concentrations of humic acids on the enrichment factor of the pesticides investigated. Extraction conditions: 0.4 µg/L of OPPs mixture spiked in reagent water and sample volume was adjusted to about 1125 mL

4.2.8 Analytical Performances

Linearity and Limit of Detection

Validation of the method is essential in order to establish that the analytical performance parameters are acceptable for their intended use. The optimized HF-LPME conditions were used to evaluate the linearity of the proposed method over the concentration range of 25–400 ng/L for all of the target analytes in reagent water. Linear regression with proportional weighting was calculated for the plot of peak area versus concentrations of the analytes using Excel® with the DPX plug-in. The evaluated results for regression correlation coefficient and limit of detection (LOD) of the method are given in Table 4.2.2 A linearly proportional relationship between the amount of the extracted analyte and its initial concentration in the sample matrix is critical in developing any sample preparation technique. To this effect, good linearity was obtained for all of the analytes, with correlation coefficients, r^2, ranging from 0.991 to 0.996.

The LOD values were determined from the linear regression line of the calibration curve, based on the principle given in the following equation [298].

$$LOD = Y_b + 3S_b \quad (35)$$

where Y_b is the blank signal and S_b is the standard deviation for the blank signal.
In this case, the standard deviation was taken in place of S_b and the calculated intercept was taken instead of Y_b [298]. The calculated limits of detection (LOD) for all of the target analytes in reagent water were in the range of 26-132 ng/L, Table 4.2.3. This obviates the sensitivity of the method for the determination of freely dissolved OPPs in environmental water samples and its potential application for bioavailability studies.

Table 4.2.2 Linearity and Limit of Detection (LOD) of the method

Analyte	Conc range (ng/L)	R^2	LOD (ng/L)
Diazinone	25-400	0.996	40
Fenthion	25-400	0.991	132
Chlorpyrifos	25-400	0.992	26

4. 2. 9 Repeatability and Reproducibility

Reproducibility and repeatability studies were conducted in order to evaluate the precision of the method. The repeatability of the method (intra-day precision) was studied by running five extractions of spiked reagent water at 200 ng/L concentration for 72 h. The repeatability of the method, expressed as relative standard deviation (RSD), was acceptable for the extraction of all of the analytes, Table 4.2.3.

Reproducibility of the method (inter-day precision), on the other hand, was studied by extracting reagent water samples spiked at 200 ng/L for three different days. It is evident from Table 4.2.3 that the reproducibility of the method, expressed in terms of the relative standard deviation, is different for the three analytes. It is slightly low for chloropyrifos as

compared to the remaining two analytes. This particular observation requires further investigation.

Table 4.2.3 Repeatability and reproducibility of the method expressed in terms of RSD. Concentration of the model analytes for both repeatability and reproducibility was kept at 200 ng/L.

Analyte	Repeatability (RSD, n=5)	Reproducibility (RSD, n=3)
	200 ng/L	200 ng/L
Diazinone	8.7	11.3
Fenthion	13.2	15.6
Chloropyrifos	14.8	30

4.2.10 Applications

The optimized and validated HF-LPME method was applied to the extraction of OPPs in the lake and ground water samples. Diazinone and fenthion were detected in the lake water sample. Since this detection was only based on the retention time corresponding to the SIM mode of the GC-MS analysis and since these are so small that they cannot be confirmed by the scan mode, they were not quantified. None of these pesticides were detected in the sample of the ground water.

The applicability of the method was further investigated by extracting spiked lake and ground water samples. The ground water sample was spiked at 0.1 µg/L and 0.2 µg/L concentrations of the analytes under study. The average percentage recovery at the two concentrations were below 1%, showing that the method is indeed non-depletive [261] and the amount extracted were so small that the equilibrium system of the water was totally undisturbed and the concentration obtained were only those of freely dissolved ones. Hence, our objective was met successfully, as the determination of freely dissolved concentrations are one step closer to the bioavailable amount. Fig. 4.2.9 and Fig. 4.2.10

show the GC-MS chromatograms of the extracts of spiked ground and Lake Awassa water samples.

The linearity of the method in the lake water samples was also compared with that of the reagent water. The comparison was made by considering the similarity between the slopes of the two water matrices. Table 4.2.4 shows the linearity of the method in reagent water compared with that of the lake water samples. All analytes exhibited good linearity with squared regression coefficients (r^2) ranging from 0.991 to 0.996, using peak area as a response variable in all of the water samples.

It is possible to see from Table 4.2.4 that the slopes for the two water samples were significantly different from each other. This further indicates that there is a significant matrix influence on the extraction of freely dissolved concentration of OPPs using HF-LPME. The difference in the slopes for the two water samples may be due to the bound of some of the analytes to the suspended particles in the lake water, as these suspended particles were not filtered before spiking.

Table 4.2.4 Linearity of HF-LPME for the extraction of freely dissolved OPPs in reagent and lake water samples.

Analyte	**Conc range (ng/L)**	**Matrices**	**Linear Regression equation**	**Correlation coefficient (r^2)**
Diazinone	25-400	Reagent water	y = 303165x - 5709.5	0.996
	50-800	Lake water	y = 722.14x - 13023	0.9923
Fenthion	25-400	Reagent water	y = 8688.3x - 120.78	0.9908
	50-800	Lake water	y = 1036x - 14441	0.9947
Chloropyrifos	25-400	Reagent water	y = 4516.2x - 43.391	0.9915
	50-800	Lake water	y = 444.93x - 12729	0.9912

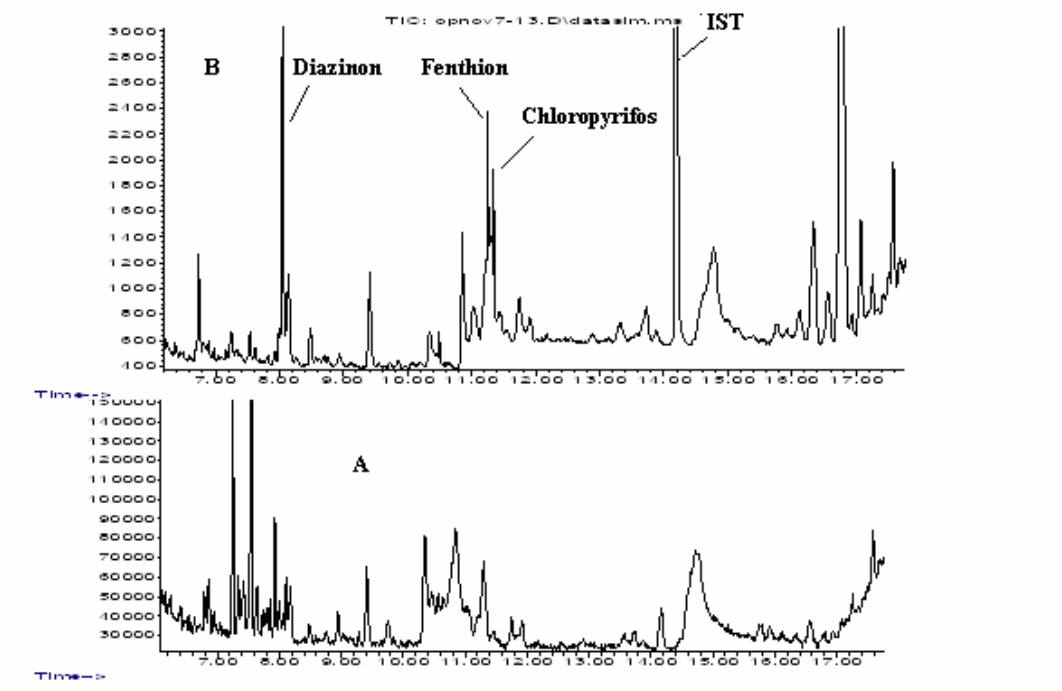


Fig. 4.2.9 Chromatograms of the extracts of the spiked ground water at the concentration level of 200 ng/L of the three analytes A) Scan mode and B) SIM mode

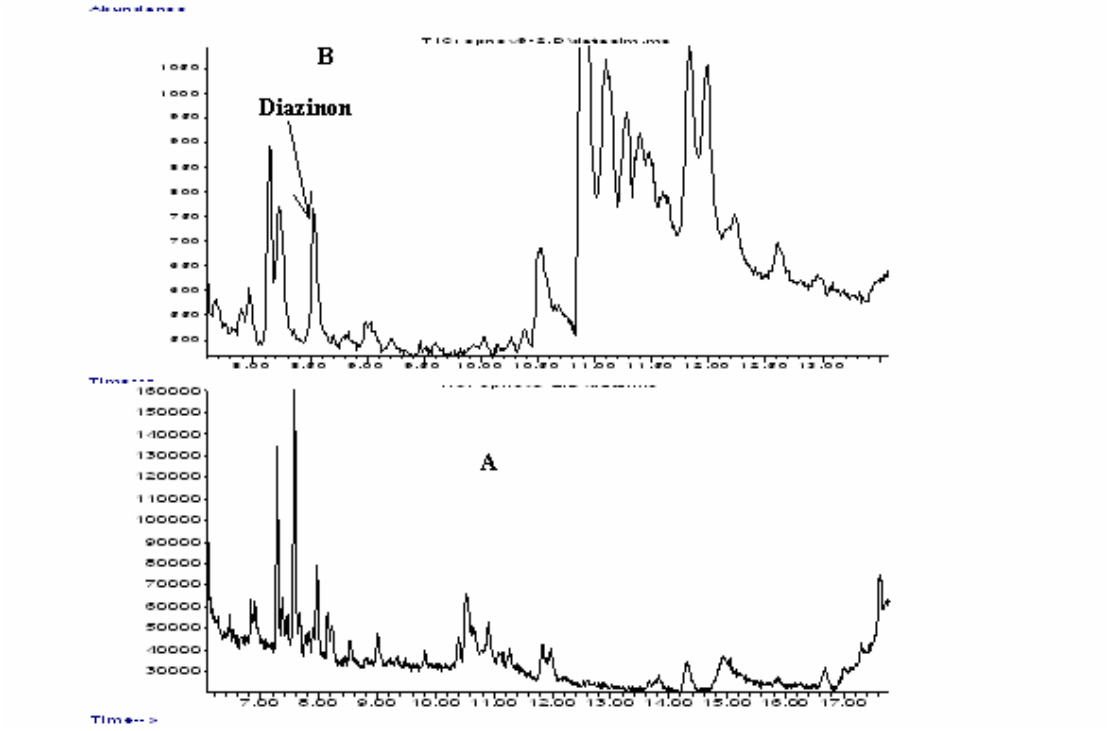


Fig. 4.2.10 GC-MS Chromatogram of extracts from Awassa Lake A) Scan mode B) SIM mode

4. 2. 11 Conclusion

The developed non-depletive equilibrium sampling methodology based on HF-LPME renders an efficient, cost effective and simple sample preparation process for the determination of freely dissolved OPPs in environmental waters. Since the total organic solvent used in this technique per sample was only 51 µl, the technique overcomes the limitations of the conventional methods such as use of expensive and toxic organic solvents and the utilization of tedious and cumbersome procedures. The percent recoveries of the analytes spiked in ground and lake water samples at different concentrations were all below 1% showing that the method is non-depletive. This is, therefore, one alternative to the SPME technique in the determination of bioavailable concentration of organic contaminants in environmental water samples. Overall the method provided satisfactory linearity in the nanogram per liter ranges of contaminant concentrations.

4.3 Portable and Time-integrating Field Sampling for the Analysis of *s*-Triazine Herbicides and Their Degradation products in Lake Awassa, Southern Ethiopia

Background

In most of environmental applications of SLM techniques so far investigated, the usual tradition was collecting grab samples from which composite samples were taken and transported to the laboratories for further treatment followed by analyte extractions [36, 208, 215, 216]. Even under these conditions notable breakthrough have been demonstrated in the application of SLM with regard to the possibility of combining of sampling, sample preparation and clean-up steps, results of which have witnessed improvement in selectivity of the technique for the analyte in question. Efforts have also been made to further upgrade the versatility and flexibility of the SLM techniques. One of these efforts is to carryout a concurrent continuous sampling and extraction processes. To this effect, a number of researchers demonstrated that SLM extraction technique is suitable for time-integrated field sampling [215, 224, 299]. All of these efforts were limited to demonstrations at laboratory levels.

Using the time-integrating field sampler reported in this work, possibility of performing unattended and automated extractions at the actual field sites has been shown with a portable and easily programmed sampler developed. In this application, the usual practice of carrying the water samples from distant areas to laboratories has been solved, which otherwise could have contributed to errors in analytical results [72]. The utilization of the solar energy for recharging of the power supply to the extraction system enabled us to work successfully in remote fields where energy resources are not accessible. In addition it shows that the technique is green with regard to environmental effect and energy utilization.

4.3.1 Performance of the Field Sampler

Performance of the developed time-integrating field sampler has been evaluated by undergoing a pilot study on the extraction of residues of four *s*-triazine herbicides along with six of their likely degradation products as described in section 3.3. The analytes, used in this study, were considered earlier under laboratory conditions for SLM extraction [215], (Table 3.3.1, section 3.3). Some experimental procedures were also followed from the optimized work in order to compare the performances of the newly designed membrane format. However, changes were also made in order to make the sampler flexible and suitable for unattended sampling in the actual field applications.

One difference is, in the previous work, the volume of buffer used to adjust pH, in the donor channel, was as large as the sample itself. In the current work, the relative amount of buffer was significantly reduced, (to 5% of the total volume as has been described in section 3.3.4), for practical reasons. If SLM in the future shall be used for larger-scale, unattended, automatic and time-integrating field sampling in environmental chemical analysis, the volume of buffer should be still smaller.

In the previous work it has been observed that when the membrane solvents, whose composition was modified, by dissolving a carrier, was utilized for high flow rate extractions, the obtained results were less reproducible and sometimes erratic [215]. This could possibly be due to the gradual removal of the carrier by the sample solution in the donor phase. It was then decided that this effect could be worsen when unfiltered lake water samples are extracted at the maximum flow rate under the new sampler conditions in the current work.

Therefore, membrane solvents for the pilot study were used without dissolving any carrier, so as to obtain results that indicate the real field conditions and minimize the variation in the values of the analytical results. This is another difference of point between the current and previous works [215].

On the other hand, this decision was not continued as we have again used the carrier dissolved in the membrane solvent for the monitoring study.

In order to evaluate the performance of the field sampler, 3 litres of spiked reagent water containing the mixture of the analytes at the concentration levels of 10 µg/L were extracted in the laboratory.

The extract volume of 2 mL in combination with the 300 µl each of the phosphate buffer and sodium hydroxide causes dilution of the enriched extract plug in acceptor channel of the membrane. Since the volume of the acceptor channel is about 164.3 µl, this means that an inherent acceptor dilution factor of about 15.8 times has been caused in the method for all extracts. Due to slight difference in amount of sodium hydroxide needed to neutralize the extracts, this factor can vary slightly for different extracts.

In the pilot study, the HPLC system was calibrated by duplicate injections of the solutions with concentration ranges of 10-50 µg/L of all of the analytes. Linear regression with proportional weighting of the analytical signals corresponding to these concentration ranges was determined using Excel™ with the DPX plug-in. Then, peak area signals of spiked extracts and water sample extracts from Awassa Lake were interpolated towards the linear regression lines with a confidence level of 95 %. The obtained concentrations were multiplied with appropriate acceptor dilution factors and thus calculated to C_A-values. For extracts from spiked samples, extraction efficiencies and enrichment factors were calculated according to equations 9 and 10 and presented in Table 4.3.1 below.

A limit of detection (LOD) was calculated as three times the standard deviation of a blank signal, corrected for dilutions and enrichment factors. The calculated LOD-values for this system with a sample volume of 3 L are summarised in Table 4.3.1. However, for DDA the sensitivity was lower than for the other analytes and it was eluted close to the solvent peak. This made it, in general, harder to detect, resulting in no quantification of its LOD.

Table 4.3.1 Extraction efficiency and enrichment factor, with 95 % confidence limits, for extraction of the spiked water samples at the concentration levels of 10 μg/L. Limit of detection (LOD), for all analytes, is given in μg/L.

Analyte	**Extraction Efficiency, E**	**Enrichment Factor, E_e**	**LOD value (μg/*L*)**
DDA	ND*	ND	NQ*
DIA	0.002	2.1 ± 0.2	12
ATOH	0.002	2.1 ± 0.3	12
DEA	0.001	0.76 ± 0.10	11
PROH	0.002	2.2 ± 0.3	15
TZOH	0.006	7.5 ± 0.8	4.2
CYZN	0.001	0.74 ± 0.20	11
ATRZN	0.004	4.8 ± 0.6	3.6
PRYN	0.13	152 ± 26	1.5
TRYN	0.27	314 ± 42	0.9

* LOD for DDA was not quantitated due to lower sensitivity and peak close to solvent peaks.

As depicted in Table 4.3.2, the enrichment factor and extraction efficiency results obtained in this pilot work are much lower than the previous ones [215]. This may be attributed to the absence of a carrier, tri-*n*-octylphosphine oxide, TOPO, in the membrane solvent. It is known that the use of modifiers enhances the extraction of polar analytes [300].

The other possible reason for obtaining lower results from the earlier works could be the reduced size of the membrane. The usual type of membrane used has the following dimensions: 0.25 μm depth, 1.5 mm width and 250 cm length, having a total volume of about 0.95 mL [215]. However, the size of the membrane used in the current study was smaller, as has also been described above, so as to fit into the extraction box. This can obviously make a difference, i.e., as the contact time of the flowing water sample with the membrane is shortened, the mass transfer to the acceptor phase will also be reduced.

Table 4.3.2 Comparison between the extraction efficiency and enrichment factors obtained in the current work and a previous similar work under laboratory conditions.

Analytes	Extraction Efficiency	Enrichment Factor, E_e	LOD value (*µg/L*)	Data from Previous similar work [215]	
				E_e	LOD (µg/L)
DDA	ND	ND	?	-	-
DIA	0.002	0.2	12	4.0	1.2
ATOH	0.002	0.3	12	7.5	0.5
DEA	0.001	0.10	11	9.3	1.2
PROH	0.002	0.3	15	27	0.5
TZOH	0.006	0.8	4.2	33	0.4
CYZN	0.001	0.20	11	1.4	2.0
ATRZN	0.004	0.6	3.6	59	0.04
PRYN	0.13	26	1.5	140	0.02
TRYN	0.27	42	0.9	120	0.01

Based on the values of enrichment factor and extraction efficiency calculated in this case, the amount of analytes in the original sample of Awassa Lake was calculated. Table 4.3.3 shows the concentration levels of *s*-triazine herbicides and their likely degradation products in the lake water sample determined during the pilot study.

Table 4.3.3 Concentration levels of Triazine herbicides and their degradation products in Awasa Lake with 95% confidence intervals (μg/L).

Compound	Sampling sites	
	Junction	**Lake body**
DDA	ND	ND
DIA	19 ± 5	31.4 ± 10
ATOH	25 ± 6	77 ± 44
DEA	NSL	28 ± 15
PROH	ND	ND
TZOH	ND	NSL
CYZN	NSL	ND
ATRZN	NSL	ND
PRYN	ND	ND
TRYN	ND	ND

ND = Not Detected

NSL = Not significantly higher than LOD

4.3.2 Determination of levels of s-Triazine Herbicides and their Degradation products in Selected Sites of Awassa Lake

As indicated above, the determination of the levels of s-triazine herbicides and their degradation products in selected sites of Awassa Lake was done using the time-integrating field sampler continuing from the pilot study. Two portable samplers were constructed at the Department of Analytical Chemistry, Lund University and one was donated to the Department of Chemistry, Addis Ababa University and utilized for this study.

The monitoring study was done in a similar way to the pilot study except that, TOPO was used in the monitoring study unlike that of the pilot study. The other difference was the main sites of sampling. In the monitoring study, sampling was made from the tributary river, Tikur Wuha and the lake water body and only one time sampling was made at the junction where as sampling in the pilot study was from the junction between the river and the lake and the lake body. So the lake water body site was same for both the pilot study and the monitoring study.

Eventhough the same analytical column was used in the cases of pilot and monitoring studies, as indicated in section 3.3.2, the HPLC systems utilized for the analysis of the extracts were different. Hence, determination of new calibration curves and LOD for the instrument was required. Therefore, linear regression with proportional weighting of the signals for concentration ranges indicated in Table 4.3.4 was calculated using Excel™ with the DPX plug-in. The LOD values were determined from the linear regression line of the calibration curve using this program according to the principles described in section 4.2 equation (35). In this case, the standard deviation will be taken in the place of S_b and the calculated intercept will be taken in stead of Y_b [298]. Then, the obtained value of LOD was back calculated for possible values in the donor phase. The correlation coefficient, LOD and the regression equation for these phases are given in Table 4.3.4. As can be seen from the Table, all graphs gave good linear correlation with the correlation coefficients in the range of 0.994 to 0.999.

Evaluation of the precision was based on triplicate injections and measurement of the peak area in all of the cases. The concentration ranges are different for different compounds; this was because it was not possible to detect some of the compounds at the lower concentrations others have been detected.

The peak area signals of Awassa lake water sample extracts were interpolated toward the linear regression results with a confidence level of 95 %. The obtained concentrations were multiplied with appropriate acceptor dilution factors and thus calculated to c_A-values. For extracts from spiked samples, extraction efficiencies and enrichment factors were calculated according to equations 9 and 10 as indicated above. For Awassa lake sample extracts, the E_e-values obtained by extracting 3 L of 10 μg/L-spiked samples in reagent water were used to calculate c_D-values. By using donor dilution factors, the concentrations of analytes in Awassa Lake were determined. The total error for Awassa extracts was estimated in terms of the standard deviation of three extracts from each site. Some of the analytes like CYZN and ATOH were not detected in some of the replicate extracts and, therefore, error estimation was not done for these results. It was observed that in most of the cases the concentration of analytes in the first extract were higher than the second and third extracts (results not shown), as a result the standard deviations for some of the analytes were quite high. This is may be due to the washing away of TOPO through time. The results of this analysis were depicted in Fig. 4.3.1A -B and Fig. 4.3.2 A-C together with data from the pilot study.

Table 4.3.4 Method validation parameters: Concentration range, regression equation, regression coefficient (r^2) and limit of detection (LOD).

Compound	**Conc range (µg/L)**	**Reg. equation[a]**	**r^2**	**LOD (µg/L)**
DDA	50-1000	y = 297.38x + 1.3051	0.999	-
DIA	50-1000	y = 1044.4x + 4.8714	0.999	3.
ATOH	10-1000	y = 293.09x - 2.3625	0.994	3
DEA	10-1000	y = 403.62x + 2.2069	0.999	2
PROH	50-1000	y = 297.24x + 1.3487	0.999	1
TZOH	10-1000	y = 188.71x + 1.579	0.998	1
CYZN	100-1000	y = 322.02x + 8.0421	0.999	8
ATRZN	50-1000	y = 1320.5x + 5.0791	0.999	2
PRYN	50-1000	y = 430.56x - 0.7587	0.999	0.04
TRYN	50-1000	y = 337.88x - 1.6567	0.999	0.02

[a] y peak area, x concentration (µg/L)

* corrected to the original water sample

4.3.2.1 Occurrence of Parent *s*-Triazine Herbicides

Four *s*-triazine parent herbicides were involved in this study. Table 4.3.5 shows the levels of some of the parent s-triazine herbicides. Cyanazine was detected in the tributary river, Tikur Wuha, in higher amount than in the body of the lake. Atrazine was only detected in the first pilot study and it was not detected in any of the following phases. This shows the absence of fresh use of atrazine in recent times. Small amounts of prometryn were detected in two of the sampling times from all of the sampling sites studied. Terbutryn was detected in only one sampling time from the tributary river, Tikur Wuha.

Table 4.3.5 Levels of parent s-triazine herbicides in the selected site of Awassa Lake at the four times of sampling, ND= not detected, Bdl = below detectable level

Parent Compound	Mar.03		Dec. 03			Feb.04		Jul. 04	
	Sampling sites		Sampling sites			Sampling sites		Sampling sites	
	Junction	Lake body	Tik wuha	Junct.	Lake Body	Tik wuha	Lake Body	Tik Wuha	Lake body
CYZN	118±280	Bdl	899.6	Nd	14.1	516.4	Nd	Nd	Nd
ATRZN	4.7±1.4	Bdl	Nd	Nd	Nd	Nd	Nd	Nd	Nd
PRYN	Bdl	Bdl	1.2±64.5	0.2±9.9	0.2±13.4	0.2±21.9	0.1±7.3	Nd	Nd
TRYN	Bdl	Bdl	0.2±27.2	Nd	Nd	Nd	Nd	Nd	Nd

4.3.2.2 Occurrence of Triazine Degradation products

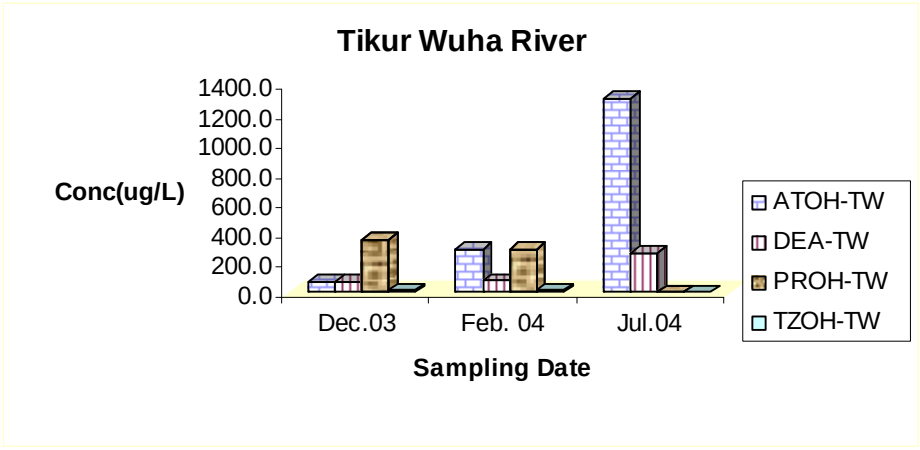
The concentration of different degradation products of *s*-triazine herbicides at different times of sampling is depicted in Figs 4.3.1A and 4.3.1B.

Generally, except for Cyanazine, the parent s-triazine herbicides were found in lower concentrations than their corresponding degradation products. This shows that these pesticides could degrade either in the soil environment around the lake or in the water bodies. Although the presence of these degradation products in higher proportion as compared to their parent compounds is indicative of the herbicides degradation, some of them, especially deethylatrazine (DEA) and deisopropylatrazine (DIA), have similar toxicity, greater water solubility and weaker interaction with soil components than the parent herbicides [301]. Therefore, the presence of these degradation products in the water bodies has an adverse impact on the ecosystem of the aquatic environment. Hence, from the pollution status points of view, the occurrence of these degradation products is alarming.

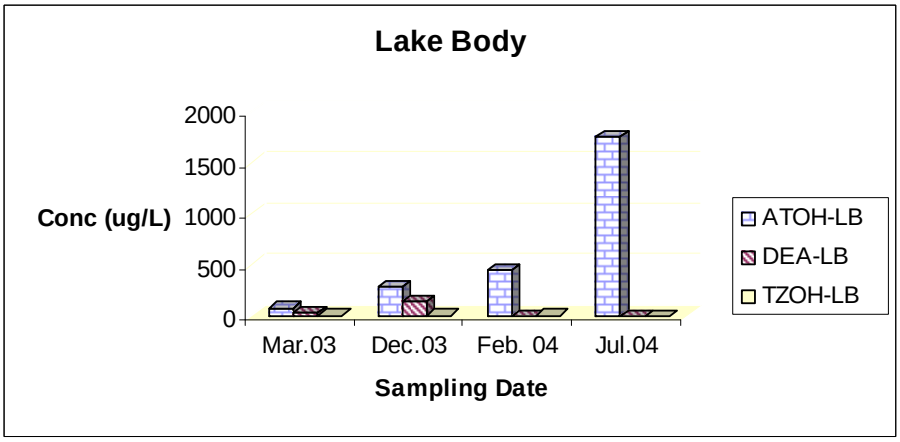
As it is possible to see from Figs 4.3.1A and 4.3.1B, ATOH was the major metabolite found in both the tributary river and the lake body for samples collected in the month of July and it was generally the major degradation products in the body of the Lake. Hydroxyatrazine is the degradation product of atrazine [215] as depicted in Fig.1.7. Research done else where [40] showed that atrazine degradation products accounted for nearly 60% of the atrazine load in northern Missouri streams at preplant, with ATOH the predominant degradation products present. This study showed that the dissolved-phase transport and sediment desorption are the main processes controlling ATOH concentrations in the streams. We also believe that the main cause of this high concentration of ATOH in the tributary river Tikur Wuha and the Lake would be the dissolved- phase transport and sediment desorption as can be evidenced from the low-land position of the river and Lake relative to the farming areas which facilitates run offs to get to the water systems easily. The other evidence comes form the strong turbulences aroused by the movement of Hippopotamus and wave during the extraction time. The strong movement of Hippos and the strong wave enhances desorption of this compound from sediments as it induces the strong stirring of the sediments. Regarding the dissolved-phase transport possibly by run-off, we can raise the question of mechanism of its sorption in soil around the lake. Lerch and coworkers [302] indicated that the majority of bound atrazine residues are sorbed by the mixed-mode mechanisms, i.e., cation exchange and hydrophobic interactions. In this study, we have found significantly high amount of ATOH in the tributary river as well as the lake, Figs 4.3.1A and 4.3.1B. Therefore, the major contribution should have come from desorption of the sediment, but it needs further investigation.

Comparison between the lake and the river, it was detected in higher proportion in the lake than in the river, Fig 4.3.2A. This further substantiate the idea of sediment desorption from sediments as the turbulence was very much significant from the lake than the river.
Terbutylazine hydroxide was another hydroxylated degradation products detected in both the tributary river and the lake in relatively small proportion as compared with ATOH, Fig. 4.3.2B. Comparison between the two sites, it was found in large proportion in the river than the Lake body.

Among the dealkylated degradation products, DDA was detected in nearly all samples studied but not quantitated because of the reason given in section 4.3.1. Among the quantitated degradation products, except for the pilot study, DIA was not detected in any of the following monitoring studies. This is in line with literature in that the deethylation reactions occur much rapidly than deisopropylation reactions [39]. Therefore, DIA can be produced but it is rapidly degraded too, as depicted in Fig. 2.7, preventing accumulation. But on the other hand DEA is resistant to further degradation and accumulates. This shows that the presence of DEA in larger proportion relative to DIA is reasonable. On the other hand the high prevalence of DDA is also reasonable as it can be obtained from two sources, degradation of atrazine and simazine and possibly propazine, Fig. 2.8. As it can be seen from Fig. 4.3.2C, DEA was found in higher proportions in the river than in the body of the lake.



A



B

Fig. 4.3.1 Graph showing the variation of concentration of s-triazine degradation products in A) the tributary river Tikur Wuha and B) the lake water mass. TW= Tikur Wuha, the tributary river, LB= Lake body water mass

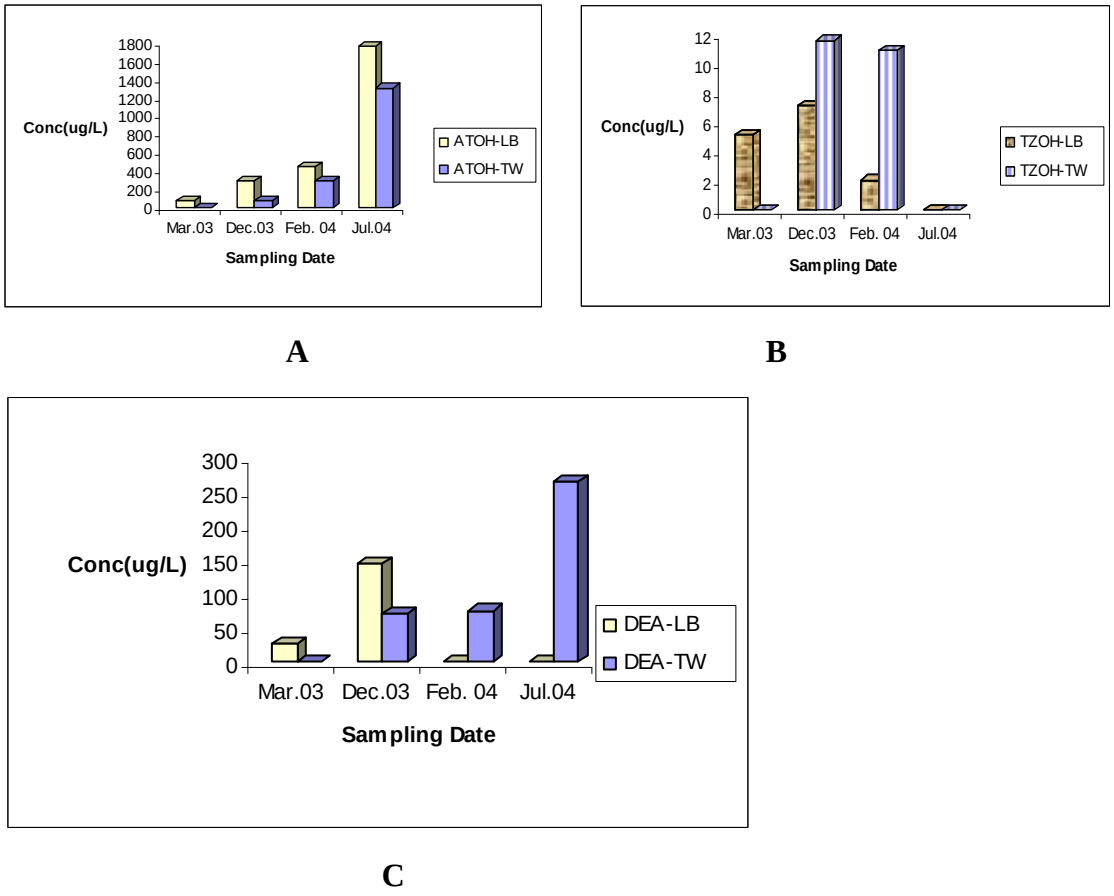


Fig. 4.3.2 Graphs showing the comparison in the variation of concentration of s-triazine degradation products in the tributary river Tikur Wuha and the Lake water mass A) ATOH, B) TZOH and C) DEA. All other abbreviations are as in Fig. 4.3.1

4.3.2.3 Environmental Relevance

Overall, s-triazine pesticides are degraded in Awassa Lake. This is good as this kind of pollutants should not build up in the lake. The residue levels of some of the degradation products are significantly higher than the limit set by the EU directives for drinking water. Even though these values are for drinking water and the lake water is not meant for drinking, the observed values are alarming. The concentrations of parent pesticides are generally below the toxic levels. The exceptions are the relatively high concentration of cyanazine and terbutryn pesticides. For these pesticides, the uncertainty for the

concentration is so high. This is because they were only detected in few of samples extracted and it is impossible to draw definite conclusions.

4.3.3 Conclusion

The time integrating portable SLM sampler has been successfully applied to the onsite field monitoring of environmental water. From this study, it is possible to conclude that most of the s-triazine herbicides reach Awassa Lake and are largely degraded in the lake or in the vicinity of the lake. The concentrations of the investigated s-triazine degradation products are alarmingly high. ATOH was detected in very high concentration in all of the times of sampling and in all of the sites sampled. This is believed to arise from desorption of sediments and dissolved-transport of the degradation products as the lake is found in lower-land compared to the farming areas. The detection of DEA in higher proportion compared to DIA was in line with the available literature [39].

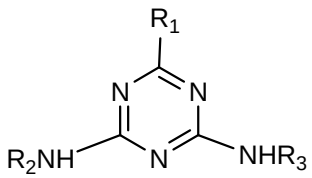
4.4 Pesticide Residue Analysis in Sediment Samples from Selected Sites of Awassa and Ziway Lakes, Southern Ethiopia

Background

As previously pointed out, sediments can serve as a time integrated sink of pesticide input from the catchments and therefore many toxic substances that are found at only trace levels in water can accumulate to elevated levels in sediments. As such, sediments serve both as reservoirs and potential sources of chemicals of potential concerns to the water column [61].

The types and physical properties of the pesticides investigated are given in Table 4.4.1. As it is evident from the table, this class of pesticides are moderately polar and expected to bound to sediment particles. The pH values of the water systems from which the sediment was sampled are given in Table 4.4.2. Except Tikur Wuha River, the Awassa lake water system was slightly basic while those of Ziway Lake are approximately neutral. So, under these conditions the parent s-triazine herbicides preferably exist in their neutral state atleast from chemical point of view.

Table 4.4.1 Some physical properties of the s-Triazine herbicides and their major common degradation products investigated.



Analyte Type	Compound	R_1	R_2	R_3	Abbre-viation	log K_{ow}*	pK_a**	Water solubility (mg/L)
Dealkylated degradation products	Deethyldesisopro - pyl atrazine	Cl	H	H	DDA	0	1.5	-
	Desisopropyl atrazine	Cl	H	CH_2CH_3	DIA	1.2	1.3	670
	Deethyl atrazine	Cl	$CH(CH_3)_2$	H	DEA	1.6	1.3	3200
Hydroxylated degradation products	Hydroxy atrazine	OH	$CH(CH_3)_2$	CH_2CH_3	ATOH	1.4	5.15	600
	Hydroxy propazine	OH	$CH(CH_3)_2$	$CH(CH_3)_2$	PROH	-	5.2	-
	Hydroxy terbutylazine	OH	CH_2CH_3	$C(CH_3)_3$	TZOH	-	-	9
Parent compounds	Cyanazine	Cl	CH_2CH_3	$CCN(CH_3)_2$	CYZN	1.8	1.0	170
	Atrazine	Cl	CH_2CH_3	$CH(CH_3)_2$	ATRZN	2.7	1.68	35
	Ametryn	CH_3S	CH_2CH_3	$CH(CH_3)_2$	Amtn	2.63	4.4	25
	Propazine	Cl	$CH(CH_3)_2$	$CH(CH_3)_2$	PRZN	3.01	1.7	

* Obtained from Ref.19 ** Obtained from Ref. 20

Table 4.4.2 pH of the lake water during sampling, at each station of the sampling site

Lake	Site	pH	
Ziway	Korokonch	7.16	
	Buchesa R.	7.14	
	Wafiko	7.00	
Awassa	Tikur Wuha	6.35	
	catholic church side	8.38	
	Erma biya	8.40	

4.4.1 Occurrence of s-triazine herbicides in Sediment samples from the selected sites of Awassa Lake

Fig. 4.4.1 shows the levels of s-triazine herbicides and their degradation products in selected sites of Awassa Lake. All of the parent herbicides were detected in three of the four sites sampled in Awassa Lake. These parent compounds were detected more frequently than their degradation products. This is reasonable as most of the parent herbicides are more hydrophobic than their degradation products and hence sorbed more strongly to sediment particles than their degradation products, which are more polar. The detection of parent s-triazine herbicides in higher proportions in most of the sites indicates that they are resistant to biodegradation under these environmental conditions.

Among the major degradation products, TROH and ATOH were detected in significant amounts followed by DEA. But DIA was not detected in all of the sites. This may be due its rapid conversion to other degradation products as the ethyl group can be removed easily, as has been discussed in section 4.3.2.2.

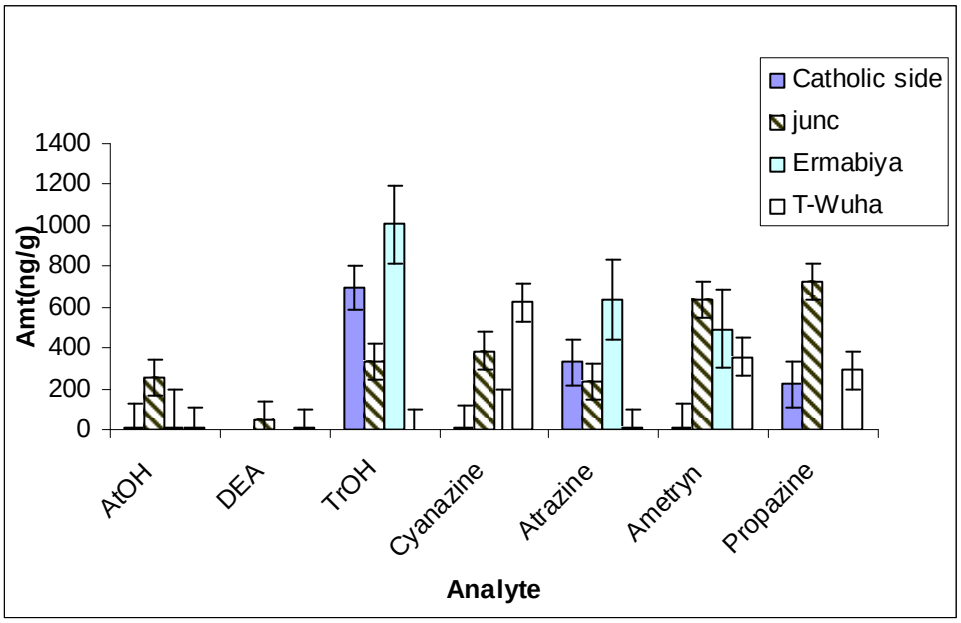


Fig. 4.4.1 Distribution of s-triazine herbicides and their common degradation products

4.4.2 Occurrence of s-triazine herbicides in Sediment samples from the Selected Sites of Ziway Lake

Fig. 4.4.2 shows the levels of s-triazine herbicides in selected sites of Ziway Lake. Similar to the Awassa Lake, the parent herbicides are detected in most of the sites sampled but to the lesser extent than the Awassa Lake. Again, the occurrence of these parent pesticides in sediment samples indicates that these pollutants are indeed resisting degradation under the current environment.

s-Triazine degradation may occur as a result of both chemically and biologically mediated processes in the aquatic environment depending on such factors as microbial population, temperature, pH, redox potential, water content and organic matter content [303]. As it is evident from Table 4.4.2 above, the Awassa Lake is slightly basic than the Ziway Lake and hence the more abundance of these parent compounds may be attributed to this environmental condition, but needs further investigations.

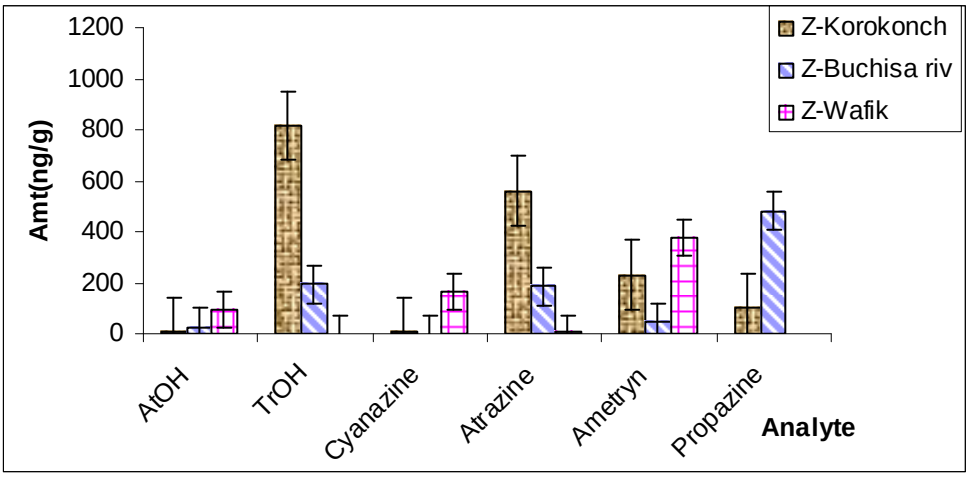


Fig. 4.4.2 Levels of s-triazine herbicides and their degradation products in selected sites of Ziway lake

4.4.3 Comparison between levels of s-triazine herbicides and their Degradation Products in the two lakes

It is evident from Fig. 4.4.3 that on the average, the levels of the parent triazine herbicides in the selected sites of Awassa Lake were more than those in Ziway Lake. This is reasonable as there are more agricultural areas in the vicinity of Awassa Lake than Ziway Lake including the ex-state farm. Among the degradation products, TROH was the most abundant compound in both lakes. This shows the previous use of terbutylazine herbicides in the area.

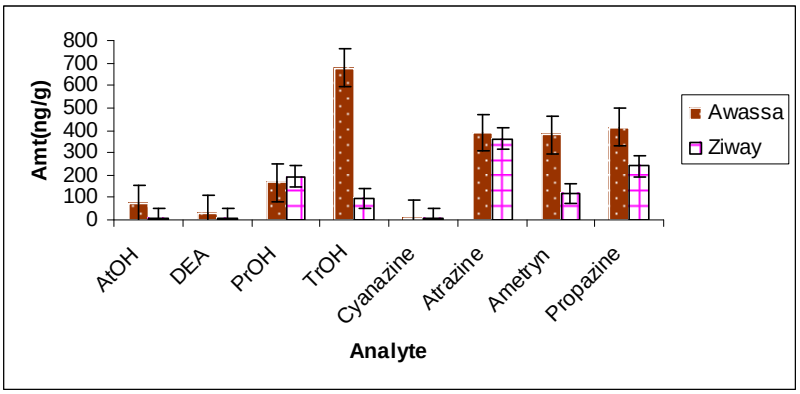


Fig. 4.4.3 Comparison between the levels of s-triazine herbicides and their degradation products in Awassa and Ziway lakes

Generally, the parent herbicides are found in higher proportions in sediment samples as compared to the corresponding amounts in water sample levels as it is possible to see from the results of water analysis discussed in section 4.3. The reverse holds true for degradation products, this is reasonable as degradation products are more polar and hence more soluble in water than in sediment particles. Fig. 4.4.4 and 4.4.5 show the HPLC chromatogram for the extracts of Ziway and Awassa Lakes sediment samples, respectively.

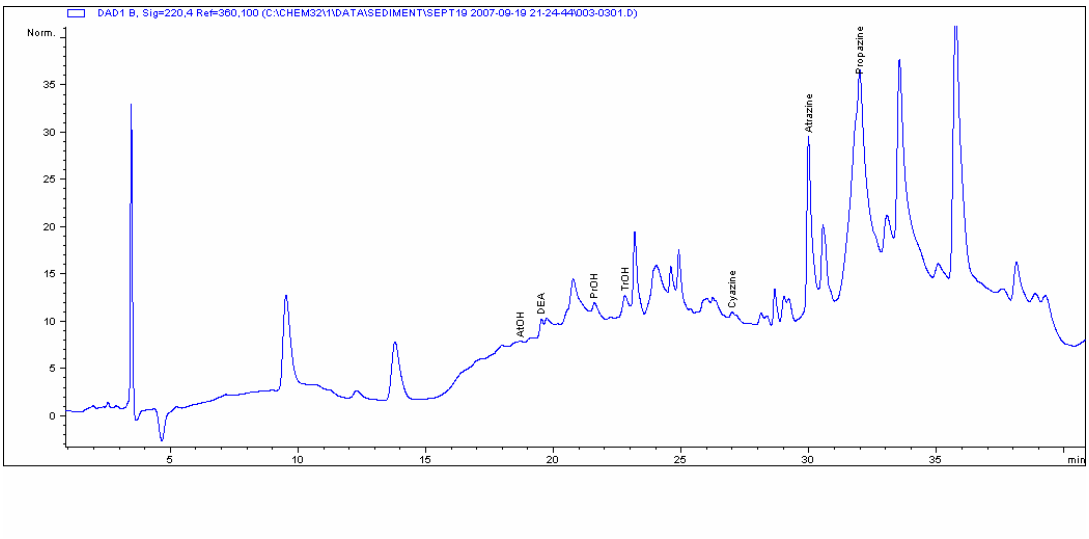


Fig. 4.4.4 Chromatogram of the s-triazine herbicides obtained from the analysis of the sediment extract from Ziway Lake, Buchisa sampling site

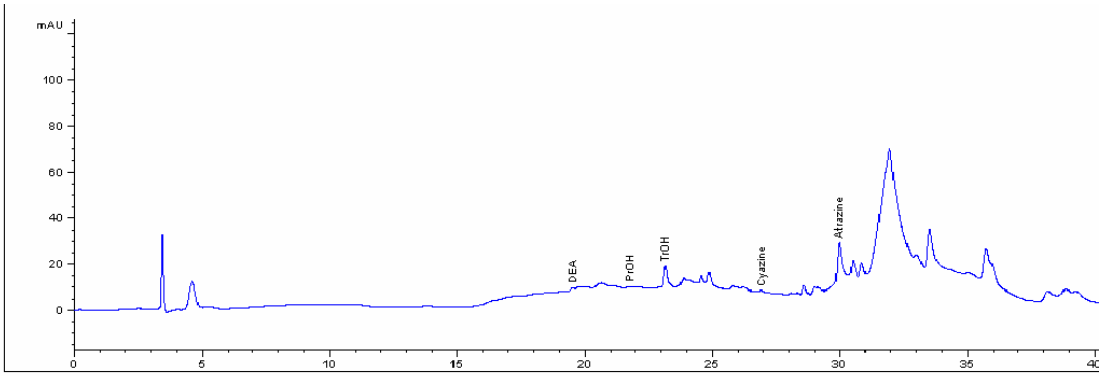


Fig. 4.4.5 Chromatogram of the s-triazine herbicides obtained from the analysis of the sediment extract from Tikur Wuha River sampling site.

4.4.4 Conclusion

As expected from the moderate hydrophobicity of the s-triazine herbicides they were strongly bounded to sediment particles and found in higher proportions in sediment samples as compared to water samples from similar areas. The fact that the parent compounds found in higher proportions indicates that they are resistant to degradation in the sediment column of these environmental compartments.

5. General Conclusion and Recommendations

5.1 Conclusion

In this work an environmentally friendly and cost effective sampling and sample preparation methods for the analysis of dinitrophenolic herbicides, organophosphorus pesticides and s-triazine herbicides as model compounds were developed. These techniques are so simple and cost effective that they could be used in any laboratory especially in developing countries where research funds are scarce and difficult to buy expensive sampling and sample preparation devices. In addition to their simplicity and cost effectiveness the techniques are analytically valid for environmental water trace analysis. The other interesting aspect is the determination of freely dissolved concentration of environmental organic contaminants in a simple way. Since the freely dissolved concentrations of environmental contaminants are one step closer to the bioavailable concentration of these pollutants, this method of equilibrium sampling would be very valuable for environmental remedation studies. The levels of s-triazine degradation products in both water and sediment samples of some of the Rift valley Lakes were significantly high and needs further elaborated study and intervention.

5.2. Recommendations

1. Environmental toxicological assessment should be carried out by including the toxicity of degradation intermediates.
2. Extension of the study to biota and soil is recommended in order to take a strong remedial action towards these pollutants.
3. Collaborative research between chemists, biologists, ecologists and environmental scientists is required to get a meaningful result regarding the environmental fate of pesticides in tropical regions like Ethiopia. We cannot directly associate the results obtained from researches done in temperate zone to our situation as the environmental condition has got huge impact on the degradation, mobility and persistence of these pesticides.

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