HPLC DETERMINATION OF ATRAZINE IN WATER AND SEDIMENT SAMPLES AFTER LIQUID-LIQUID AND SOLID PHASE EXTRACTION: A COMPARATIVE STUDY.

By Margret Pagare

Supervisor: Professor M.F. Zaranyika

FACULTY OF SCIENCE

UNIVERSITY OF ZIMBABWE

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Contents

Acknowledgements ............................................................................................................ 4
Acronyms and Abbreviations ............................................................................................ 5
List of Figures .................................................................................................................... 6
List of Tables ..................................................................................................................... 7

1.0 Introduction ................................................................................................................ 9

1.1 Pesticides .................................................................................................................... 9
  1.1.1 Triazines ............................................................................................................... 9
  1.1.2 Atrazine ............................................................................................................... 10

1.2 Extraction methods for herbicides .............................................................................. 11
  1.2.1 Liquid–liquid extraction ....................................................................................... 12
  1.2.2 Solid Phase Extraction ......................................................................................... 15
  1.2.3 Soxhlet Extraction ............................................................................................... 16
  1.2.4 Accelerated Solvent Extraction (ASE) ................................................................. 17
  1.2.5 Microwave assisted solvent Extraction (MAE) ................................................... 17
  1.2.6 Sonication Assisted Extraction (SAE) ................................................................. 18
  1.2.7 Supercritical fluid Extraction (SFE) .................................................................... 19
  1.2.8 Solid Phase Micro-extraction (SPME) ................................................................. 19
  1.2.9 Matrix solid–phase dispersion (MSPD) ............................................................... 20
  1.2.10 Stir Bar Sorptive Extraction (SBSE) ................................................................. 20

1.3 Chromatographic method of analysis ....................................................................... 21
  1.3.1 Gas Chromatography .......................................................................................... 21
  1.3.2 High Performance Liquid Chromatography ......................................................... 22
  1.3.3 Thin Layer Chromatography .............................................................................. 22
  1.3.4 Ion Exchange ..................................................................................................... 22
  1.3.5 Partition Chromatography .................................................................................. 22
  1.3.6 Size Exclusion .................................................................................................... 23
  1.3.7 Adsorption .......................................................................................................... 23

1.4 Limit of Detection ..................................................................................................... 23

1.5 Methods of analysing results obtained .................................................................... 24

1.6 Objectives .................................................................................................................. 25
  1.6.1 Specific Objectives ............................................................................................... 25

1.7 Justification ................................................................................................................ 26

2.0 Literature Review ...................................................................................................... 27
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Acronyms and Abbreviations

MAC      Maximum admissible Concentration
SPE      Solid Phase Extraction
LLE     Liquid –Liquid extraction
LOD     Limit of detection
LOQ     Limit of Quantitation
WHO     World Health Organisation
USEPA   United States Environmental Protection Agency
HPLC    High Performance liquid Chromatography
EPA     Environmental Protection Agency
SAE     Sonication Assisted Extraction
MSE     Microwave Assisted Extraction
SFE     Supercritical Fluid Extraction
ASE     Accelerated solvent extraction
SPME    Solid phase Micro-extraction
SBSE    Stir Bar Sorptive Extraction
MSPD    Matrix Solid Phase Dispersion
**List of Figures**

Figure 1: Structure of Atrazine ................................................................. 10

Figure 2: Sampling Site Harava Dam in Chitungwiza – 25 km from Harare city centre ........ 30

Figure 3: Rotary Evaporator ....................................................................... 32

Figure 4: Concentration of atrazine standards vs peak area ............................... 36

Figure 5: HPLC-UV Shimadzu LC -10AD VP ............................................. 36

Figure 6: Chromatogram for atrazine of river water extract. (after extraction with 40 ml ethyl acetate) .................................................................................. 40

Figure 7: Chromatogram for atrazine standard of sediment extract. (After extraction with 40 ml ethyl acetate) ................................................................. 41

Figure 8: Chromatogram for atrazine standard of distilled extract. (After SPE with no sample pre-treatment) ................................................................. 43

Figure 9: Chromatogram for atrazine standard of river extract. (SPE with no sample pre-treatment) .................................................................................. 44

Figure 10: Chromatogram for atrazine standard of sediment extract. (After SPE with no sample pre-treatment) ................................................................. 45

Figure 11: Chromatogram for atrazine standard of distilled water extract. (After SPE with sample pre-treatment) ................................................................. 47

Figure 12: Chromatogram for atrazine standard of river water extract. (After SPE with sample pre-treatment) ................................................................. 48

Figure 13: Chromatogram for atrazine standard of sediment extract. (After SPE with sample pre-treatment) ................................................................. 49
List of Tables

Table 1: Calibration results ................................................................. 34
Table 2: Standard curve results for Atrazine Analysis ............................ 35
Table 3: Effect of solvent volume (10ml of ethyl acetate) ...................... 38
Table 4: Effect of solvent volume (20ml of ethyl acetate) ...................... 39
Table 5: Effect of solvent volume (40ml ethyl acetate) ......................... 39
Table 6: Effect of SPE with no sample pre-treatment ............................ 42
Table 7: Effect of SPE with sample pre-treatment ................................ 46
Table 8: Summary of the extraction methods for atrazine on three samples - River water, distilled water and sediment .......................... 51
Abstract

Atrazine is a selective herbicide for control of broadleaf and grassy weeds in corn, sorghum and sugarcane. The extraction efficiency was studied using distilled water, river water and sediment samples. A two-step procedure of extraction and quantification was involved in assessing the recovery of the atrazine residues. Liquid-Liquid and Solid Phase were used for the extraction step. The recoveries for atrazine using the LLE (40ml solvent) were 93.3±2.7%, 94.7±11% and 71.4% for river water, distilled water and sediment sample respectively. The recoveries for atrazine using solid phase with pre-treatment of distilled, river water and sediment sample were 96.6±2.8%, 91.7±3.6% and 113.9±3% respectively. Analysis for atrazine residues was performed by HPLC-UV. The results showed that extraction efficiency is greater with Solid phase extraction than with liquid-liquid extraction.

**Keywords:** Atrazine, Liquid-liquid extraction, Solid phase extraction and pre-treatment
1.0 Introduction

1.1 Pesticides

Pesticides are groups of artificially synthesized substances, toxic and non-biodegradable intended for use in the production, storage and processing of agricultural products in pastures, in the aqueous and industrial environments whose purpose is to change the composition of fauna and flora. This group of substances can be classified according to the purpose for which they are intended, the mode or period of action, or the chemical function. They are extensively used as insecticides, herbicides and nematicides and they are included in the classes of organochlorines, organophosphates and pyrethroids. Pesticides are widely studied as environmental contaminants because of their extensive use in the control of pest affecting agricultural crops, homes and garden.

1.1.1 Triazines

Triazine herbicides are organochlorines which form a wide group of compounds used for weed control. These herbicides are very persistent in water, soil, plant and animals. (Fielding et al., 1992) The water solubility varies over a range of 5-750mg/L. Solubility can be altered by pH (for ionisable groups), presence of dissolved salts, organic materials and temperature. The same parameters can modify the toxicity of these compounds to species. Thus, determination of triazines is very important for environmental control. (Belfroid et al., 1998; Solomon and Chappel, 1998; Rao, 1999). They are inhibitors of the electron transport chain (bind to Quinone binding protein in photosystem II) in photosynthesis. Triazines are some of the oldest herbicides, with research initiated on their weed control properties during the early 1950s. (Fishel, 2006) EPA has classified atrazine as a class c chemical and maximum contaminant level in drinking water is 3.0μg/L and below 0.1μg/L for European Union.
### 1.1.2 Atrazine

Atrazine [2-chloro-4-(ethyl amino) -6-(isopropyl amino) -1, 3, 5- triazine] (figure 1) a member of triazine group is a herbicide that is used to stop pre- and post–emergence broadleaf and grassy weeds in crops such as sorghum, maize, sugarcane and eucalyptus plantations. Atrazine is a product of cyanuric chloride treated sequentially with ethylamine and isopropyl amine. Like other triazine herbicides, atrazine functions by binding to the plastoquinone - binding protein in photosystem II, which animals lack. Plant death results from starvation and oxidative damage caused by breakdown in the electron transport system process. Oxidative damage is accelerated at high light intensity. (Konstantinou et al, 2001)

Atrazine is considered slightly - moderately toxic to humans. Atrazine residues affect the central nervous system, immune system and cardiovascular function. System toxicity is unlikely unless large amounts have been ingested. Atrazine’s effects in humans and animals primarily involve the endocrine system. Studies suggest that atrazine is an endocrine disruptor that can cause hormone imbalance.

![Figure 1: Structure of Atrazine](image)

Studies concerning the environmental fate of atrazine have shown that herbicide transform relatively slowly in the environment with average half-life ranging from 4-57 weeks (Dutta...
and Singh, 2013). Persistence of atrazine has been attributed to the presence of chloride and N alkyl groups. The major route of atrazine transformation observed in soil includes N-dealkylation to products deethylatrazine and deisopropylatrazine. Nitrogen released from atrazine metabolism serves as a nitrogen source for atrazine –degrading bacteria. Atrazine is subject to decomposition by UV irradiation but under normal field conditions this effect is small. It is very stable over several years of shelf life, with only slight sensitivity to natural light. Atrazine is more readily absorbed on muck or clay soil than on soils of low clay and organic matter content. The downward movement or leaching is limited by its adsorption to certain soil constituents. Adsorption is reversible and desorption often occurs readily, depending on factors such as temperature, moisture and pH. (Herbicide -handbook, 1983) Disappearance rate is greater in river water and sediment Concentration of humic substances in sea and river water result in an increase the photo degradation of pesticide residues. A higher content of micro-organism increases bio-degradation. (El-Dib and Abou-Waly, 1998)

1.2 Extraction methods for herbicides

The sample preparation step includes extraction of the analyte of interest from the matrix which leads to sample concentration. There are a number of methods used for extraction such as liquid-liquid, solid phase, solid phase micro-extraction, accelerated solvent extraction, stir bar sorptive extraction, supercritical fluid extraction, sonication assisted extraction, matrix solid phase dispersion and microwave assisted extraction. (Dean, 2009)
1.2.1 Liquid-liquid extraction

The most used extraction method of aqueous samples is liquid-liquid extraction. The principle of LLE is that a sample is distributed or partitioned between two immiscible liquids or phases in which the compound and matrix have different solubilities. Normally, one phase is aqueous (often the denser or heavier phase) and the other phase is an organic solvent (the lighter phase). The basis of the extraction process is that the more polar hydrophilic compounds prefer the aqueous (polar) phase and the more non-polar hydrophobic compounds prefer the organic solvent. (Dean, 2009)

If the method of separation to be used is Reversed phase HPLC then the target organic compounds are the best isolated in aqueous phase so that they are directly injected into the HPLC system or pre-concentrated further using Solid phase extraction. The main advantages of LLE are its wide applicability, availability of high purity organic solvents and the use of low cost apparatus such as separatory funnel.

The distribution coefficient and distribution ratio can be used to explain the solubility differences between solvents. Distribution coefficient is an equilibrium constant that describes the distribution of a compound, X, between two immiscible solvents such as aqueous and organic phase. For example, an equilibrium can be obtained by shaking the aqueous phase containing the compound X, with an organic phase, such as hexane. This process can be written as an equation:

\[ X_{(aq)} \leftrightarrow X_{(org)} \]  \hspace{1cm} (1)

Where (aq) and org are the aqueous and organic phase, respectively. The ratio of the activities of X in the two solvents is constant and can be represented by:
\[ K_d = \frac{[X]_{\text{org}}}{[X]_{\text{aq}}} \] 

Where \( K_d \) is the distribution coefficient, while the numerical value of \( K_d \) provides a useful constant value at a particular temperature, the activity coefficients are neither known nor easily measured. A more useful expression where the fraction of compound (E) is expressed as percentage is (Dean, 2009)

\[ E = \frac{C_o V_o}{C_o V_o + C_{aq} V_o} \] 

(3)

Where:  
- \( C_o \) – Concentration of compound in organic phase,
- \( C_{aq} \) – Concentration of compound in aqueous phase,
- \( V_o \) – Volumes of organic phase,
- \( V_{aq} \) – Volumes of aqueous phase

Or

\[ E = \frac{K_d}{1 + K_d V} \] 

(4)

Where:  
- \( V = \frac{V_o}{V_{aq}} \) which is the phase ratio. (Dean, 2009)

For a one step LLE, \( K_d \) must be large that is greater than 10, for quantitative recovery (>99%) of the compound in one of the phases such as the organic solvent. This is a consequence of the phase ratio \( V \), which must be maintained within a practical range of values: 0.1<\( V \)<10.
Two or three repeat extractions are required with fresh organic solvent to achieve quantitative recoveries. Equation below is used to determine the amount compound extracted after successive multiple extraction.

$$E = 1 - \left[ \frac{1}{1 + K_d V} \right]^n$$  \[5\]

Where: \(n\) is the number of extractions.

It can be a situation that the actual chemical form of the compound in the aqueous and organic phases is not known such as a variation in pH would have a significant effect on a weak acid or base. In this case a distribution ratio is used.

\[D = \frac{\text{Concentration of X in all chemical forms in organic phase}}{\text{Concentration of X in all chemical forms in the aqueous phase.}}\]  \[6\]

Distribution ratio is identical to the distribution coefficient for simple molecules, where no chemical dissociation occurs.

The partition coefficient is dependent on the chemical nature of the analyte and of the two solvents as well as on the temperature. For ionic analytes, the pH of the extraction medium is an essential parameter. Ionic form of the analyte will preferably remain in the aqueous phase, while neutral analytes can be extracted into an organic solvent. The degree of ionization can be controlled by adjusting pH. Extraction of acidic analytes from water into an organic solvent is only practical at pH values more than two units below the pKa value of the acid. Similarly to extract basic analytes into an organic solvent, the pH must be at least two units above the pKb value of the base. (Dean, 2009)
1.2.2 Solid Phase Extraction

Solid phase extraction can be used for both liquid and gaseous samples. It is also often used for clean-up and concentration of liquid extracts. The solid-phase adsorbent is usually packed into small cartridge or column a disk, or a well-plate system. The well-plate system consists usually of 96 small SPE cartridges or disks which are tightly arranged into an array in a standard 8X12 plate. The plate format is compatible with standard microtiter liquid handling technologies and injection systems and it allows parallel sample processing of 96 samples.

Similar stationary phases can be used as in liquid chromatography. The amount of solid absorbent varies from 50mg to 10g. Extraction is performed by forcing the liquid or gaseous sample the sorbent material by means of pressure, vacuum or diffusion. The analytes are partitioned or adsorbed to the adsorbent in a manner similar to the mechanism in chromatography. After the sample has passed through the sorbent, the adsorbed analytes are eluted with a suitable solvent or are thermally desorbed. The analyte can be bound to the solid phase by a number of mechanisms such hydrogen bonding, dipole-dipole interactions, hydrophobic dispersion forces and electrostatic interactions. Normal phase, reverse phase and ion-exchange are the three main modes of SPE clean up. Normal phase, polar adsorbents such as silica or alumina is used. Reversed-phase, non-polar sorbents are used while in ion exchange SPE charged bonded sorbents are applied. For more selective clean up, immunosorbents or molecular imprinted polymers can be applied. SPE clean-up of liquid samples is often carried out in a system consisting of a SPE cartridge or disk and a means of facilitating the flow of both sample and solvent through sorbent. The SPE clean-up can be carried out either manually or automatically with a special robotic system.

The sorbent is conditioned with a suitable solvent before applying the sample. The idea is to wet pores of the sorbent and thus increase the active surface area. Two stage conditioning is usually required. Firstly the sorbent is flushed with an organic solvent such as methanol and
then the excess organic solvent is flushed away with water or buffer. After conditioning, the sample is applied to the sorbent. The amount of sample that can be applied depends on the amount of the sorbent. The sorbent is washed to remove matrix compounds. Analytes are eluted from the cartridge with a suitable solvent. The volume of eluent should be as small as possible to avoid dilution of extract. Flow control in the elution step is important as excessive flow rates might result in induced recoveries of the analytes.

SPE of gaseous samples differ from the system described above. The adsorbent is packed into a tube through which the gaseous sample is either drawn from the pump or simply diffuses. The trapped analytes are often directly thermally desorbed to an analytical instrument. It is also possible to elute with a liquid. Trapping materials include carbon molecular sieves, activated carbon and carbon black or polymeric adsorbents such as Tenax and chromosorb.

1.2.3 Soxhlet Extraction

Soxhlet extraction is continuous liquid extraction that is carried out in a soxhlet extractor. The extractor consists of a solvent flask, a middle chamber with a siphon, a cooled condenser and a heating system. This technique is particularly useful in cases when the pure compound is partially soluble in a solvent and the impurity is not soluble in that solvent and vice versa. The solid sample is placed in an extraction thimble inside the middle chamber. The solvent is placed in the solvent flask below it. The solvent is heated above its boiling point and the vapour from the boiling solvent travels to the condenser where it is condensed: the condensate drips through the sample. The solvent soaks the sample and then drops back into the solvent flask once the solvent level reaches the top of the siphon. Thus, the hot solvent circulates through the samples several times, and in cycle fresh solvent is used as only the clean solvent evaporates while the extracted analytes stay in the solvent flask. Typical extraction times range from 6 to 24 hours and relatively large solvent volumes are required.
1.2.4 Accelerated Solvent Extraction (ASE)

Accelerated solvent extraction (ASE) aim is to enhance liquid extraction by utilising high temperature and pressure. The high temperature and pressure increase the capability of the solvent to penetrate the sample matrix. Typically, the extraction is performed at a temperature above the atmospheric boiling point of the solvent. The solubility and diffusivity of the analytes are increased by the increased temperature, thus making the extraction faster and more efficient. The amount of solvent required is smaller than that required in traditional liquid extraction methods. ASE is used for extraction of organic compounds from solid samples but is not suitable for thermally labile samples. ASE can be performed in either a static or dynamic mode or by a combination of these modes. In dynamic mode, the solvent flows through the sample. Static ASE can be performed manually in a closed vessel. A typical ASE consists of an oven, an extraction cell, a pump and a pressurizing system, several valves and a collection vial. The extraction time ranges from 10-40 minutes and requires 20-50ml of solvent. It is also possible to use water as an extraction solvent in which case the technique is called subcritical water extraction. The properties of water as solvent significantly changes at elevated temperature and pressure. The solubility of non-polar compounds increases strongly with increasing temperature.

1.2.5 Microwave assisted solvent Extraction (MAE)

Microwave Assisted solvent extraction (MAE) is mainly used for solid samples or liquid samples. Microwaves are high frequency electromagnetic waves. In MAE, microwave radiation is utilised to heat up the extraction solvent and also the sample. The microwave energy affects molecules through ionic conduction and dipole rotation. To achieve a successful extraction, the solvent must be able to absorb microwave radiation and pass it on to the sample molecules in the form of heat. Suitable solvents are polar solvents with a high
dipole moment such as water, methanol, and acetone. If non-polar solvents are needed in the extraction, e.g. hexane or toluene, mixing with polar solvents is advisable.

The extraction is typically performed in a closed vessel, such that the pressure increases and the solvent can be heated above its atmospheric boiling point. MAE has two types of systems: open focused and closed vessel. In the open system, sample vessels are irradiated sequentially while in the closed vessel system the vessels are irradiated simultaneously. In a closed vessel, the temperature can be higher than the atmospheric boiling point of a solvent and the extraction is faster due to increased diffusion. It is possible to use dynamic extraction, but this requires special apparatus. The extraction processes typically require 20-50ml of solvent and extraction ranges from 20 to 40 minutes.

1.2.6 Sonication Assisted Extraction (SAE)

Sonication Assisted extraction (SAE) is also known as ultrasound-assisted extraction. SAE can be used for both liquid and solid samples and for extraction of either inorganic or organic compounds. Sonication can aid decomposition and oxidation of organic compounds. Therefore care must be taken to avoid degradation of analytes. Acoustic vibrations with frequencies above 20kHz are applied to a sample. When these vibrations are transmitted through the liquid, cavitation occurs and bubbles with negative pressure are formed. Chemical compounds and particles are mechanically removed from the matrix surface and adsorption sites by the shock waves generated when the cavitation bubbles collapse. The implosion of the cavities creates microenvironments with high temperatures and pressures, which accelerate the extraction. The SAE can either be static open-focused or a closed-vessel system or a dynamic system. The use of dynamic extraction can advantageous, since the analytes are removed as soon as they are transferred from the (solid) matrix to the solvent. In a dynamic system the sample is continuously exposed to fresh solvent. The extraction typically requires 20-200ml of solvent and the extraction time ranges from 2-20 minutes.
1.2.7 Supercritical fluid Extraction (SFE)

Supercritical fluid extraction (SFE), the extraction fluid is in its supercritical state. SFE is performed by dedicated instrument necessary for generating the supercritical and also controlling the pressure, temperature and flow rate of the fluid. A supercritical fluid is defined as an element, substance or mixture that is heated above its critical temperature and pressurized above its critical pressure. The supercritical fluid exists as a single phase and it cannot be liquefied or vaporized by increasing temperature or pressure. The fluid represents an intermediate form of matter between a gas and a liquid, which combines liquid-like high density and dissolving power with gas-like properties such as low viscosity, zero surface tension and high diffusion rate for analytes. The most commonly used SCF is carbon dioxide because it has a low critical temperature and pressure. Modifiers such as methanol and dichloromethane are added to the CO$_2$ to enhance the extraction of more polar analytes.

Usually the extraction is performed in dynamic mode but static extraction or combined static/dynamic extraction can also be applied. The extract is collected either in a suitable solvent or in a solid phase trap. The extraction requires 10-20ml of solvent and the extraction times range from 20-60 minutes. Used for the extraction of organic analytes from solid samples and thermally labile analytes.

1.2.8 Solid Phase Micro-extraction (SPME)

Solid phase micro-extraction (SPME) is another example version of the liquid-solid extraction technique in which the trapping is based on sorption. SPME involves exposing a polymer–coated, fused silica fiber to a sample. The fiber is installed in a syringe like device to facilitate its handling. The analytes are partitioned between the matrix and the stationery phase until an equilibrium is reached, and then the fiber is removed from the sample and the analytes are thermally desorbed from it. It is also possible to remove the analytes from the fiber with a suitable solvent. SPME can be used for both liquid and gaseous samples. The
analytes are not usually quantitatively extracted from the matrix. Advantages of SPME are that it is one of the few solvent free extraction methods and that it does not destroy the sample. SPME sampling can be performed in three ways, namely direct extraction, headspace extraction and extraction with membrane with membrane protection. In the latter, the fiber is shielded from direct contact with the sample by a surrounding membrane, which protects it from high molecular weight compounds.

Instrumental setup of SPME – The fiber is glued to a piece of stainless steel tubing and is mounted in special holder. The holder is equipped with an adjustable depth gauge which makes it possible to control how far the needle is allowed to penetrate into the sample container and injector. During extraction or desorption, the fiber is exposed by depressing the plunger by depressing the plunger. After extraction the fiber is withdrawn into metal needle tube.

1.2.9 Matrix solid–phase dispersion (MSPD)

Matrix solid-phase dispersion (MSPD) is used to extract analytes from semi-solid and viscous samples. The sample is mixed with a solid phase sorbent, and the sample is dispersed over the surface of the sorbent material. The sorbent material is the same as those used in SPE. The sample is place in a mortar with solid phase materials and some solvent. The amount of SP material used is 4-6 times greater than the amount of sample. The mixture is then crushed by a pestle. The Solid phase material acts as both as a grinding aid and adsorbent. After grinding the mixture is placed on an SPE cartridge, which can either be empty or packed with SP material. The analytes can be eluted in the same way described for SPE.

1.2.10 Stir Bar Sorptive Extraction (SBSE)

Stir bar sorptive extraction (SBSE) is carried out with a magnetic rod that is encapsulated in a glass jacket and coated with a relatively thick layer of polydimethylsiloxane. The mechanism
is based on sorption, similar to that in SPME. The coated stir bar is placed in an aqueous sample and spun for a specified time. It is also possible stir bar for headspace sampling of liquid or solid samples. The analytes can be removed from the stir bar either by thermal desorption (for GC) or by liquid extraction (for LC). The use of a stir bar allows a 500-fold increase in enrichment, and thus sensitivity, compared to SPME.

1.3 Chromatographic method of analysis

Chromatography is used for separation of complex mixtures into their individual components and for determining quantitatively the amounts of these components. The most common chromatographic methods are gas chromatography (GC), high performance liquid chromatography (HPLC) and thin layer chromatography (TLC). Chromatographic techniques depend on a particular or a combination of separation functions. The separation functions are a result of the type of interactions between the analyte, the stationery phase and the mobile phase. Separation techniques involved in chromatography include ion exchange, partition, and adsorption and size exclusion. The variety of stationary phases used liquid chromatography (active adsorbent surfaces, polymer loaded glass beads) results in a variety of separation techniques. (Skoog et al, 2004)

1.3.1 Gas Chromatography

The mobile phase in gas chromatography is a carrier gas usually inert or unreactive such as helium or nitrogen respectively. The column is contained in oven temperature of the gas can be controlled. Stationary phase is layer of liquid or polymer on an inert solid support. Gaseous samples being analysed interact with walls of the column which is coated with the stationary phase. This causes each compound to elute at a different time also known as retention time (Harris, 2007).
1.3.2 High Performance Liquid Chromatography

High performance liquid chromatography is a type of chromatography that employs a liquid mobile phase and a finely divided stationary phase. HPLC uses high pressure to force solvent through closed columns containing very fine particles that give high resolution separations. HPLC is important because most compounds are not sufficiently volatile for GC.

1.3.3 Thin Layer Chromatography

Thin layer chromatography is used to separate non-volatile mixtures. It is performed on a sheet of glass which is coated with a thin layer of adsorbent material usually silica gel, cellulose or aluminium oxide also known as the stationary phase. After the sample has been applied on the plate, the solvent mixture is drawn up the plate via capillary action. Plate development is a process in which a sample is carried through the stationary by a mobile phase. Can be used to monitor progress of a reaction, identify compounds present in a given mixture and determine purity of a substance. (Skoog et al, 2004)

1.3.4 Ion Exchange

In ion exchange the stationary phase consists of an insoluble porous resinous material containing fixed charge-carrying groups. Counter ions of the opposite charge are loosely complexed with these groups. The passage of a liquid mobile phase, containing ionised molecules of the same charge as the counter-ions through the system results in the reversible exchange of these ions. The degree of affinity between the stationary phase and feed ions dictates the rate of migration and hence degree of separation between different solute species.

1.3.5 Partition Chromatography

Partition chromatography, the mechanism is absorption into the liquid. The stationary liquid phase is coated onto a solid support such silica gel, cellulose powder, or hydrated silica. The
feed components move through the system at rates determined by their relative solubilities in the stationary and mobile phase.

1.3.6 Size Exclusion

Size exclusion involves molecules of analyte material are separated according to their size or molecular weight. The stationary phase consists of a porous cross-linked polymeric gel. The pores of the gel vary in size and shape such that large molecules tend to be excluded by smaller pores and move preferentially with the mobile phase. The components of a mixture elute in order of decreasing size or molecular weight.

1.3.7 Adsorption

The separation mechanism of adsorption depends upon differences in polarity between the different feed components. The more polar a molecule, the more strongly it will be adsorbed by polar stationary phase. During a surface adsorption process, there is competition for stationary phase adsorption sites between the materials to be separated and the mobile phase. Good separation is achieved by using fairly polar stationary phases and low polarity mobile phases such as hexane. (Harris, 2007)

1.4 Limit of Detection

Limit of detection is the lowest quantity of a substance that can be distinguished from the absence of a substance within a stated confidence limit. The detection limit also known as instrument detection limit (IDL) is estimated from the mean, the standard deviation of the blank and some confidence factor. Most analytical instruments produce a signal even when a blank is analysed. This signal is referred to as noise level. The IDL is the analyte concentration that is required to produce a signal greater than three times the standard deviation of the noise level. This may be practically measured by analysing 8 or more
standards at the estimated IDL then calculating the standard deviation from concentration of these standards. (Skoog, Holler & Crouch, 2007)

1.5 Methods of analysing results obtained

There are two steps involved extraction and analysis of organic substances. Standard solutions of organic substances are prepared and a standard curve is plotted. The extraction efficiency is compared for different methods of extraction. The traditional approach to studying the persistence of organic substances in the environment consists of spiking the appropriate compartment of the environment with the substance, and then collecting samples periodically to determine the amount of the organic substance remaining in the medium at the time the sample was collected. (Zaranyika and Nyandoro, 1993) The concentration of substance remaining at any given time is then plotted as function of time, to give the persistence curve. In most cases the persistence curve resembles a first order decay curve. In the proposed work, the loss in the atrazine herbicide after a given time period will be calculated and plotted as a function of time. The method mentioned above was used in study of the persistence of the herbicide glyphosate and insecticides endosulfan I and II (Zaranyika et al., 2010).

The persistence of pesticides in the environment is often described in terms of half-lives in accordance with the conventional first order kinetics approach to the study of persistence of pesticides and other organic substances (Wania and Mackay, 1999). Konstantinou proposed a first order kinetic model for studying behaviour of pesticides in water. (El-Dib and Abou-Waly, 1998)
1.6 Objectives

Compare the efficiency of liquid-liquid and solid phase extraction of atrazine from water and sediment samples prior to determination by HPLC.

1.6.1 Specific Objectives

1. To determine extraction efficiency of liquid–liquid extraction of atrazine in distilled, river water and sediment.

2. To determine the extraction efficiency of solid phase extraction in distilled, river water and sediment.

3. To find the limit of detection of the HPLC-UV for the analyte Atrazine.
1.7 Justification

Atrazine herbicide is a common herbicide extensively used for weed control in Zimbabwe. It’s more readily absorbed on muck or clay soils than on soils of low clay and organic matter content. These herbicides reach the groundwater by infiltration or by surface runoff, therefore, leading to contamination of water sources. Atrazine is an endocrine disruptor that can cause hormone imbalance. It is considered slightly - moderately toxic to humans. Determination of persistence of triazines is very important for environmental control and use.

The use of atrazine was banned in Italy, the Netherlands, Germany, Sweden, Belgium, France, and Norway. Other European countries where its use is permitted allow only 1/30th the amount allowed in the United States. All representatives of triazines have been reported as contaminants of surface and ground water (Trajkovska, 2001)

While in the Zimbabwe it’s still being used, there is need to monitor environmental effects. Its continued use makes atrazine a significant problem and a critical issue with potentially damaging and unforeseen consequences for the future.

In order to monitor the persistence of herbicides- atrazine effectively, the use of reliable, sensitive methods for analysis is necessary. LLE and SPE were the two methods of extraction available. The organic chemicals for LLE were available as well as the cartridges for SPE. There was need to compare the extraction efficiency for solid phase and liquid-liquid. Extraction methods used depend on the following factors cost chemicals, available equipment and consumables. Liquid-liquid extraction uses a simple separatory funnel and has the ability to extract a wide range of compounds with a wide range of polarities. Solid phase extraction does not require the use of large volumes of solvent, simple, fast and efficient. Therefore, there is need to develop a method which is efficient in extracting the atrazine from the sample matrix.
2.0 Literature Review

Navarro and others studied the s-triazine in river, sea and ground water in Spain using liquid –liquid extraction. 10ml of sample volume were mixed with 40ml of acetonitrile – dichloromethane. The extractant was analysed by GC –NPD and confirmed the identities by GC-MSD. Recoveries varied from 81-101% while the RSD ranged from 1.7-4.3%. No clean up technique was used. (Navarro et al, 2004).

Ghosh and Philip monitored the atrazine degradation in anaerobic environment by mixed microbial consortium in the absence of external carbon and nitrogen source. Liquid–liquid extraction using dichloromethane (10ml) and the extraction efficiency obtained was 95± 0.52% when analysed by UV- Visible Spectrometer. Liquid –liquid extraction was also carried out using ethyl acetate (10ml) as a solvent followed by analysis using GC-ECD, recoveries were 93± 0.55%. (Ghosh and Philips, 2003)

The University of Minnesota monitored the kinetics of hydrolysis in water using liquid partitioning with 30ml of dichloromethane analysed by GC-NPD. Widmer, Olson and Koskinen reported there is very limited loss of atrazine in groundwater samples stored for long periods. The small losses are assumed due to hydrolysis. The rate of hydrolysis of atrazine in water is dependent on environmental factors particularly pH, DOC and temperature. (Widmer, Olson and Koskinen, 1993)

Atrazine pollution in the lower Danube River in Bulgaria was determined at two sampling points. Estimation of seasonal periodic variations in atrazine concentration was carried out. Extraction done by liquid – liquid using 100ml of dichloromethane and analysed by GC-MS (Vitanov, 2003)

Singh et al looked at the sorption behaviour of atrazine, prometon, two thiocarbamates and trialllate. Sorption and degradation are the most important processes affecting the fate and behaviour of pesticides in the environment. Atrazine residues were extracted using liquid-liquid partitioning with n-hexane as the solvent. Herbicide compounds in the extracts were analysed by Gas-Liquid Chromatograph. The recovery of atrazine varied between 97-102%
Rodriguez-Mozaz, Lopez de Alda and Barcelo studied the occurrence of oestrogens, pesticides and bisphenol A in natural waters of Spain and drinking water plants. Solid phase extraction was carried out as a pre-concentration step with an automated Solid Phase from Merck. Analysis was carried by LC-MS. Mean recoveries were between 91-100% for all compounds. Recovery for Atrazine was 94 ± 15%. (Rodriguez-Mozaz, Lopez de Alda and Barcelo, 2004)

Curren and King developed an ethanol modified subcritical water extraction combined with solid phase micro extraction (SPME). (Curren and King, 2001)

Kostik and others developed a simple method to determine chloropyramine active pharmaceutical ingredient in drugs. Liquid–liquid extraction of chloropyramine was done using chloroform. Analysis was done by Shimadzu GC-MS. The average recovery for the tested amount was 99.89 ± 0.15% where n=6.

Zaranyika and Nyandoro studied the kinetics of herbicide glyphosate in distilled water and river water containing sediment. The extraction was done by liquid-liquid extraction. The analysis was done by GC with variable UV detector. The losses due to degradation were 33.5% and 7.9% respectively in the water phase of the experiment and control after 70 days and 60 days respectively. (Zaranyika and Nyandoro, 1993)

Chivinge and Mpofu studied the persistence of atrazine from soil samples obtained from Henderson Research station and its effects of the traces of atrazine in soil on the next crop. Atrazine, prometon and metolachlor plus terbutylazine mixture dissipated rapidly which meant groundnuts could be planted following a maize crop with little danger for injury from herbicide carryover. (Chivinge and Mpofu, 1990)

Manirakiza et al., studied concentration of selected organochlorines pesticides from north end of the end Lake Tanganyika, Burundi, Africa. Pesticides from fish samples were extracted
using hot Soxhlet extraction with a mixture of acetone: hexane followed by a single clean up step. For PCBs and stable pesticides clean-up was done on activated silica gel impregnated with concentrated sulphuric acid while non-acid stable pesticides superposed layers of alumina, silica and florisil impregnated with 15% methanolic solution of potassium hydroxide were successively used. Recoveries of organochlorines ranged from 86% for pp’ DDT to 107% for endrin. (Manirakiza et al., 2002)

Devault et al proposed a fast and reliable analysis of herbicides by employing the accelerated solvent extraction (ASE). They analysed s-triazines in fluvial sediment fractions at 11 sampling sites along mid Garonne River and its tributaries. For sediment samples accelerated solvent extraction was used while for water samples liquid-liquid extraction was used. The analysis of the herbicide residues was done by GC–MSD. Urban and suburban areas were found to the most contaminated sites during the study period compared to the rural areas. Recoveries for the s-triazines were ranging from 82.4-102.2% while the mean was 98.5 % (Devault et al., 2007).

Du Preez characterised the concentrations of atrazine, terbuthylazine and other pesticides in amphibian habitat surface waters of a corn-production area of western Highveld region of South Africa. Extraction of atrazine and terbuthylazine was by liquid-liquid extraction and solid phase extraction using C18 cartridge. The analysis was done by GC-MSD. The maximum atrazine concentrations measured during the study ranged from 1.2 to 9.3μg/L (Du Preez et al, 2005).
3.0 Materials and Methods

3.1 Sampling

A sample is a finite part of a statistical population whose properties are studied to gain information about the whole. Sampling is the process of selecting units from a population of interest so that by studying the sample we may fairly generalize our results back to the population from which they were chosen.

![Figure 2: Sampling Site Harava Dam in Chitungwiza – 25 km from Harare city centre](image)

3.2 Extraction Methods

3.2.1 Liquid-liquid extraction

Atrazine residues from sediment/river water/distilled water samples (10g) were extracted using ethyl acetate (40ml) and anhydrous sodium sulphate (1g) by equilibrating samples on a
rotary shaker for 3hrs. The ethyl acetate was separated and evaporated to dryness at room temperature. The residue thus obtained was re-dissolved in 10ml of acetonitrile (HPLC grade) for analysis. Aqueous samples were injected directly after filtration through 0.45μm filter. (Dutta and Singh, 2013)

Three samples (distilled water, river water and sediment) were taken through the extraction process described above and the results obtained were recorded as controls. Three more samples (distilled water, river water and sediment) were fortified with a 5ppm concentration of atrazine, taken through the extraction process and the volume of ethyl acetate was varied from 10ml, 20ml and 40ml. The concentration obtained recorded and percentage recovery calculated as shown in table 3, 4 and 5.

\[
\text{% recovery} = \left( \frac{\text{concentration obtained}}{\text{actual concentration added}} \right) \times 100
\]

3.2.2 Solid phase extraction

3.2.2.1 Water samples

Passed 1L water through a glass filter and acidified with 2ml of concentrated hydrochloric acid (pH 2.2). Atrazine residues (10ml) were mixed with 20ml of the acidified water. The solution was slowly forced or aspirated through a C\text{18} column preconditioned with 5ml methanol followed by 5ml distilled water. The column was washed with 2ml acetonitrile: water (3:7, v/v). The column was dried for 20mins under vacuum. Elution was carried out with 20ml acetonitrile. Eluate was concentrated on a rotation evaporator.

3.2.2.2 Sediment samples

Sediment (50g) was agitated with methanol: water (8:2, v/v) 100ml for 8hrs and centrifuged for 10min at 3000rpm and the supernatant was removed. The extract was concentrated by
rotary evaporation, diluted in water (100ml) and passed through a C18 solid phase extraction column preconditioned with methanol (4ml) followed by distilled water (4ml) under negative pressure. Atrazine was eluted with methanol (Krutz et al, 2009). Results obtained shown in table 6 and 7.

![Rotary Evaporator](image)

**Figure 3: Rotary Evaporator**

3.3 HPLC-UV Analysis

3.3.1 Materials and Reagents

Methanol HPLC grade from Merck, anhydrous sodium sulphate, acetonitrile HPLC grade, filtered deionised water, filter paper 0.45μm, 97% technical grade Atrazine (Agricura), Concentrated hydrochloric acid. Column type for SPE Sep-Pak Classic C18/0.85ml/360mg
3.3.2 Preparation of solutions

For limit of detection: Technical atrazine (97% active ingredient) was used to prepare the stock solution. A standard solution of 1000ppm of Atrazine was prepared by weighing 1.03g of atrazine and dissolving it 1litre using Acetonitrile: Water (70:30). Prepared standard samples from 0 ; 0.1 ;0.25 ;0.5 ;1.0; 1.5; 2.0 ;3.0; 4.0 and 5ppm. The standard samples were transferred to sample vials. Each sample was analysed three times using HPLC- UV (Skoog et al, 2006). Results shown in figure 5, table 1 and 2 below.

For Percentage recovery: 1000ppm of stock solution was prepared by weighing 0.103g of 97% technical grade atrazine and dissolving CH$_3$CN: filtered deionised water (70; 30) and top to the mark in a 100ml volumetric flask. A 100ppm working standard of atrazine was prepared.

To prepare 5ppm atrazine distilled water 5ml of 100ppm atrazine were placed in 100ml volumetric flask the solution was filled to mark with distilled water.

To prepare 5ppm atrazine river water 5ml of 100ppm atrazine was placed in 100ml volumetric flask the solution was filled to the mark with river water.

To prepare 5ppm atrazine sediment 10ml of 20ppm atrazine were mixed with 30g of sediment.

Calculations for limit of detection

\[
S = mc + S_{bl} / y = mc + b
\]

\[
S_{m} = S_{bl} + K \delta_{bl}
\]

\[
mc + S_{bl} = S_{bl} + K \delta_{bl}
\]

\[
mc = K \delta_{bl} \quad C = K \delta_{bl} /m \quad c – detection \ limit
\]

K - Z = 1.96 at 95% confidence interval

\[
C = 1.96 \times 6064.8/( 2 \times 10^6) = 0.00594 \approx 5.94 \times 10^{-3} \text{ ppm}
\]
Calculations of % recovery and concentration

Concentration (x) ppm= \((y - 250135)/2 \times 10^6\) (equation from figure 4)

% recovery = (concentration obtained/actual concentration added) \times 100

Table 1: Calibration results

<table>
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<th>Retention time</th>
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<th>Mean</th>
<th>Standard Deviation</th>
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Table 2: Standard curve results for Atrazine Analysis

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<th>Conc</th>
<th>Peak area</th>
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<td>8352342</td>
</tr>
<tr>
<td>5.00</td>
<td>9823278</td>
</tr>
</tbody>
</table>
Figure 4: Concentration of atrazine standards vs peak area

Figure 5: HPLC-UV Shimadzu LC -10AD VP
HPLC analysis will be carried out as described by (Dutta and Singh, 2013): C18 column 5µm 90Å, column: 3.9 x 150mm, mobile phase: Acetonitrile: 0.1% O-phosphoric acid (70:30), flow rate 0.5ml/min. Sample injection 20µl, wavelength 222nm, pressure 55 bars.

Vacuum filtration, separatory funnel, Sep-Pak Classic cartridges from Millipore Corporation, Milford LC-18 /0.85ml/360mg: Lot # P2293A1
4.0 Results and Discussion

Table 3: Effect of solvent volume (10ml of ethyl acetate)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ret. Time</th>
<th>Peak Area</th>
<th>Conc ppm</th>
<th>% recovery</th>
<th>% Average</th>
<th>std dev</th>
</tr>
</thead>
<tbody>
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<td>Distilled water sample</td>
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<td>River water spiked</td>
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### Table 4: Effect of solvent volume (20ml of ethyl acetate)

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<th>Sample</th>
<th>Ret. Time</th>
<th>Peak Area</th>
<th>Conc ppm</th>
<th>% recovery</th>
<th>Average</th>
<th>Std dev.</th>
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</thead>
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<tr>
<td>River water</td>
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### Table 5: Effect of solvent volume (40ml ethyl acetate)

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<th>Sample</th>
<th>Ret. Time</th>
<th>Peak Area</th>
<th>Conc ppm</th>
<th>% recovery</th>
<th>Average</th>
<th>Std dev.</th>
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<td>Sample</td>
<td>Ret. Time</td>
<td>Peak Area</td>
<td>Conc ppm</td>
<td>% recovery</td>
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</table>
Figure 6: Chromatogram for atrazine of river water extract. (after extraction with 40 ml ethyl acetate) Atrazine retention time is 4.3
Figure 7: Chromatogram for atrazine standard of sediment extract. (After extraction with 40ml ethyl acetate). Atrazine retention time is 4.2
## Table 6: Effect of SPE with no sample pre-treatment

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ret. Time</th>
<th>Peak Area</th>
<th>Conc ppm</th>
<th>% recovery</th>
<th>Average</th>
<th>Std dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>4.38</td>
<td>8417670</td>
<td>4.0837675</td>
<td>81.67535</td>
<td>82.7</td>
<td>0.9</td>
</tr>
<tr>
<td>10ml sample</td>
<td>4.38</td>
<td>8527024</td>
<td>4.1384445</td>
<td>82.76889</td>
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<td></td>
</tr>
<tr>
<td>washing 2ml Acet/water</td>
<td>4.38</td>
<td>8604450</td>
<td>4.1771575</td>
<td>83.54315</td>
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<td>20ml acetonitrile</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>River Sample</td>
<td>4.35</td>
<td>14190474</td>
<td>6.9701695</td>
<td>139.40339</td>
<td>144.2</td>
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<td>10ml sample</td>
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<td>7.5271805</td>
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<td></td>
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<td>14517348</td>
<td>7.1336065</td>
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<td>20ml acetonitrile</td>
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</tr>
<tr>
<td>River Sample</td>
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<td>4.1428495</td>
<td>82.85699</td>
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<td>0.0</td>
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<tr>
<td>10ml sample</td>
<td>4.37</td>
<td>3372604</td>
<td>1.5612345</td>
<td>31.22469</td>
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</tr>
<tr>
<td>washing 2ml Acet/water</td>
<td>4.38</td>
<td>2763816</td>
<td>1.2568405</td>
<td>25.13681</td>
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<td>20ml acetonitrile</td>
<td>4.37</td>
<td>5695422</td>
<td>2.7226435</td>
<td>54.45287</td>
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<td>Sediment sample</td>
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<td>7394056</td>
<td>3.5719605</td>
<td>71.43921</td>
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<td>5g sample</td>
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<tr>
<td>washing 2ml Acet/water</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 ml acetonitrile</td>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>
Figure 8: Chromatogram for atrazine standard of distilled extract. (After SPE with no sample pre-treatment) Atrazine retention time 4.3
Figure 9: Chromatogram for atrazine standard of river extract. (SPE with no sample pre-treatment)

Retention time for atrazine is 4.3
Figure 10: Chromatogram for atrazine standard of sediment extract. (After SPE with no sample pre-treatment) Atrazine retention time 4.3
Table 7: Effect of SPE with sample pre-treatment

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ret. Time</th>
<th>peak area</th>
<th>conc ppm</th>
<th>% recovery</th>
<th>Average</th>
<th>Std Dev</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water 10ml sample washing acet/water 20 ml acetonitrile</td>
<td>4.25</td>
<td>4273464</td>
<td>2.0116645</td>
<td>40.23329</td>
<td>35.9</td>
<td>15.8</td>
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<td></td>
<td>4.27</td>
<td>2088740</td>
<td>0.9193025</td>
<td>18.38605</td>
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<td></td>
<td>4.27</td>
<td>5148832</td>
<td>2.4493485</td>
<td>48.98697</td>
<td></td>
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</tr>
<tr>
<td>Distilled water 10ml sample washing acet/water 12 ml acetonitrile</td>
<td>4.33</td>
<td>9892779.6</td>
<td>4.8213223</td>
<td>96.42645</td>
<td>96.6</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>4.33</td>
<td>10209192</td>
<td>4.9795285</td>
<td>99.59057</td>
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<tr>
<td></td>
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<td>9639064.8</td>
<td>4.6944649</td>
<td>93.88930</td>
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<td>River water 10ml sample washing acet/water 20ml acetonitrile</td>
<td>4.32</td>
<td>9059538</td>
<td>4.4047015</td>
<td>88.09403</td>
<td>91.7</td>
<td>3.7</td>
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<td>4.30</td>
<td>9790464</td>
<td>4.7701645</td>
<td>95.40329</td>
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<tr>
<td></td>
<td>4.30</td>
<td>9406270</td>
<td>4.5780675</td>
<td>91.56135</td>
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<tr>
<td>River water 10ml sample washing acet/water 20 acetonitrile</td>
<td>4.32</td>
<td>5876140</td>
<td>2.8130025</td>
<td>56.26005</td>
<td>64.9</td>
<td>8.5</td>
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<tr>
<td></td>
<td>4.32</td>
<td>7582228</td>
<td>3.6660465</td>
<td>73.32093</td>
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</tr>
<tr>
<td></td>
<td>4.33</td>
<td>6765936</td>
<td>3.2579005</td>
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<tr>
<td>Sediment 10g sample</td>
<td>4.72</td>
<td>11866021.6</td>
<td>5.8079433</td>
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<td>11756117.6</td>
<td>5.7529913</td>
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<tr>
<td>Sediment 10g sample</td>
<td>4.63</td>
<td>10919820</td>
<td>5.3348425</td>
<td>106.69685</td>
<td>106.7</td>
<td></td>
</tr>
</tbody>
</table>
Figure 11: Chromatogram for atrazine standard of distilled water extract. (After SPE with sample pre-treatment) Atrazine retention time is 4.3
Figure 12: Chromatogram for atrazine standard of river water extract. (After SPE with sample pre-treatment) Atrazine retention time 4.30
Figure 13: Chromatogram for atrazine standard of sediment extract. (After SPE with sample pre-treatment) atrazine retention time 4.2
A two-step procedure is involved in assessing recovery of atrazine residues from distilled water, river water and sediment samples. The two steps include extraction and quantification. The values obtained by linear regression show a high correlation between concentration of the standards and peak area \(r^2=0.9842\). Limit of detection obtained is \(5.94 \times 10^{-3}\) ppm at 95% confidence interval.

The retention time obtained for atrazine was 4.22 min. The concentration and recovery for atrazine was obtained using the equation of the curve on figure 5. The recoveries for atrazine using the LLE (40ml solvent) were 93.3 ± 2.7%, 94.7 ± 11% and 71.4% for river water, distilled water and sediment sample respectively. Results shown in table 5.

LLE for the distilled water and river sample recoveries increased with increase in solvent volume as shown in Table 8. The relationship between solvent increase and recovery is linear. Large amounts of solvent increases solubility of the atrazine residues. Recovery for sediment sample (132%) was higher than the acceptable range (70-120%). Since there was no clean up after LLE, impurities or side reactions may have been present which could have given a signal at the same retention time as atrazine. Recovery for sediment at 40ml of ethyl acetate was lower than expected due to the stronger adsorption bonds which can be broken by sonication, shaking or heating.

Although LLE gave good recoveries, large amounts of solvent need to be used and it becomes expensive to analyse a large number of samples. High purity solvents are expensive to purchase and to dispose of after use. Hence Solid Phase Extraction was carried to compare the recoveries with that of LLE. The recoveries for atrazine using solid phase extraction were 82.7 ± 0.9%; 82.9% and 71.4% for distilled water, river water and sediment sample respectively without pre-treatment shown in table 6. Less solvent was used and the method is saves time. In this case SPE was used as an extraction and clean-up technique.
For the distilled and river water samples, there was direct extraction using a C18 column. For the sediment sample, the sample was mixed with a solvent before passing through the column. SPE with pre-treatment was carried and there was an increase in recovery for all samples. Recoveries for atrazine using solid phase with pre-treatment of distilled, river water and sediment sample were 96.6± 2.8 %, 91.7± 3.6% and 113.9± 3 % respectively shown table 7. Pre-treatment with solid phase extraction increased the recoveries for distilled water, river water and sediment samples.

Table 8: Summary of the extraction methods for atrazine on three samples- River water, distilled water and sediment

<table>
<thead>
<tr>
<th>Sample</th>
<th>Extraction method</th>
<th>Description</th>
<th>% recoveries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled Water</td>
<td>LLE</td>
<td>10ml ethylacetate</td>
<td>44.8 + 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20ml ethylacetate</td>
<td>75.0 + 2.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40ml ethylacetate</td>
<td>94.7 + 11</td>
</tr>
<tr>
<td></td>
<td>SPE</td>
<td>without pretreatment</td>
<td>82.7 + 0.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>With pretreatment</td>
<td>96.6 + 2.9</td>
</tr>
<tr>
<td>River Water</td>
<td>LLE</td>
<td>10ml ethylacetate</td>
<td>53.1 +2.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20ml ethylacetate</td>
<td>20.3 +3.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40ml ethylacetate</td>
<td>93.3 + 2.7</td>
</tr>
<tr>
<td></td>
<td>SPE</td>
<td>without pretreatment</td>
<td>82.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>With pretreatment</td>
<td>91.7 + 3.7</td>
</tr>
<tr>
<td>Sediment</td>
<td>LLE</td>
<td>10ml ethylacetate</td>
<td>132 + 2.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20ml ethylacetate</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>40ml ethylacetate</td>
<td>42.8 + 1.3</td>
</tr>
<tr>
<td></td>
<td>SPE</td>
<td>without pretreatment</td>
<td>71.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>with pretreatment</td>
<td>113.9 + 3.0</td>
</tr>
</tbody>
</table>
5.0 Conclusion

High performance liquid chromatography with a UV-Visible detector was used for the analysis of atrazine residues. The limit of detection for the instrument was 5.94x10^{-3} ppm. The extraction efficiency for atrazine using the LLE (40ml solvent) were 93.3± 2.7%, 94.7± 11% and 71.4% for river water, distilled water and sediment sample respectively. The extraction efficiency for atrazine using solid phase with pre-treatment of distilled, river water and sediment sample were 96.6± 2.8 %, 91.7± 3.6% and 113.9± 3 % respectively. SPE with sample pre-treatment prior to determination gave a higher extraction efficiency compared to LLE.
5.1 Recommendations

- There is need to monitor the effects of atrazine in the environment since it being used extensively at the moment in Zimbabwe.

- To monitor the presence atrazine in drinking water.

- Compare the extraction efficiency of ultrasonic assisted solid phase extraction with that of solid phase and liquid–liquid extraction.
References


WHO Guidelines for drinking water Quality 2011

