INVESTIGATION OF SELECTED ORGANIC COMPOUNDS INFLUENCE ON WATER QUALITY ALONG THE OLIFANTS RIVER CATCHMENT

By

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DECLARATION

I, Mulanga Tshimanga Christelle sincerely and solemnly declare that this research work conducted in fulfilment of the requirement of the degree of Master of Science entitled "Investigation of the influence of selected organic compounds on water quality along the Olifants river catchment" is my personal work and that all the sources that I have used or quoted have been specified and acknowledged by means of complete references. The current dissertation has not been submitted or will not be submitted to any university or an institution for the award of a degree.

TC Mulanga

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ABSTRACT

Water is a crucial natural resource, indispensable to food production, life, the environment, power generation, industry, sanitation and hygiene. The presence of Organochlorine Pesticides (OCPs) in the environment is not wanted due to their negative effects on human beings and animals. As a result, there is a need to continuously monitor their presence in the environment. In this study, surface water samples were collected once a month during the dry season and during wet season from the selected five points along the Olifants River and stored at a temperature of < 5^0C before analysis. The OCPs were extracted with dichloromethane (DCM) using the Liquid-Liquid Extraction (LLE) method. After undertaking the sample through the clean- up process, the crude extracts obtained were put into the column chromatography and eluted with hexane, about 1.5 µL of the purified extracts were analysed by the Gas Chromatographic- Mass Spectrophotometer (GC/MS).

The percentage recoveries, varied from 32- 116 % for p,p'-DDT and 4,4'-DDD respectively in triply spiked water samples. The standard deviation for most of the compounds is less than±0.04, with the exclusion of Heptachlor (±0.14). The seasonal variability of OCPs in water samples along Olifants River results show that in dry season, the Olifants River is mostly polluted at the Oxford site with (BHC-beta, Aldrin, Heptachlor-epoxide, Endosulfan-alpha and Endrin), at the Ga- Selati site with (Heptachlor-epoxide and Endrin) and at the Wolvekrans site with (Endosulfan-alpha), with Aldrin up to 834.20 ng/ L indicating the highest hazard toward the aquatic environment while in summer the Olifants River is mostly polluted at the Ga- selati site with BHC-beta and at the Waterval site with (Heptachlor and BHC-gamma) with BHC- gamma up to 560 ng/ L indicating the highest hazard toward the aquatic environment.

The levels reached from the Olifants River catchment were meaningfully above the drinking water quality guidelines for organic chemical recommended by WHO, 2006 i.e. (BHC-gamma, DDT-44, Aldrin, dieldrin and Endrin are (2.0,1.0,0.03,0.03 and 0.6) respectively for the protection of the domestic use, aquatic ecology and agricultural use (irrigation and livestock watering) for compounds with local guideline values; while, the international water quality guidelines to protect the aquatic ecosystems are 0.00083 ng/mℓ (4,4'DDD), 0.00059 ng/mL (4,4'DDE), (4,4'DDT), 0.00021 ng/mL (heptachlor), 0.0092 ng/mℓ (α-HCH), 0.0186 ng/mL (γ-HCH), and chronic values are 0.056 ng/mL (ENDO I and II) and 0.0023 ng/mL endrin) (USEPA, 2002). Levels detected were significantly higher than some research studies conducted up to now in South African aquatic environments. These results confirm the contamination of the Olifants River catchment by the OCPs.

Keywords: OCPs, liquid-liquid extraction, surface water, GC-MS and Olifants River.

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LIST OF ACRONYMS

ANOVA: Analysis of variance

ATSDR: Agency for Toxic Substances and Disease Registry

AVCA SA: Association of veterinary and crop association of South Africa

BHC: gamma-hexachlorocyclohexane

CRM: Certified reference material

DCM: Dichloromethane

DDD: Dichlorodiphenyldichloroethane

DDE: Dichlorodiphenyldichloroethylene

DDT: Dichlorodiphenyltrichloroethane

DO : Dissolved oxygene

DWAF : Department of water affairs and forestry

DWA: Department of water affairs

DWS: Department of Water and Sanitation

E C: Electrical conductivity

EDC: Endocrine disrupting chemical

FAA: Federal aviation administration

GC-ECD: Gas chromatography-electron capture detector

GC/MS: Gas chromatography Mass Spectrometry

GPS: Global positioning system

HS: Head space

HS-SPME: Head space- solid phase micro extraction

HS-LPME: Head space- liquid phase micro extraction

HCB: Hexachlorobenzene

HPCL: High performance liquid chromatograph

IARC: International Agency for Research on Cancer

IUPAC: International union of pure and applied chemistry

IWMI: International water management institute

Kow: Octanol/water partition coeff

LD: Lethal dose

LLE: Liquid liquid extraction

LOD: Limit of Detection

LOQ: Limit of Quantitation

NCMP: National chemical monitoring programme

NTMP: National toxicity monitoring programme

OCs: Organochlorines

OLSPME: On-line solid phase extraction

OCP: Organochlorine pesticides

PAH's: polycyclic aromatic hydrocarbons

PBBs: Polybrominated biphenyls

PBDEs: Polybrominated diphenyls ethers

PCB's: Polychlorinated biphenyls

POPs: Persistent organic pollutants

POPRC: Persistent organic pollutants review committee

RSD% : Relative standard déviation percentage

SADA: South Africa Department of Agriculture

SOCs: Selected organic compounds

S/N: Signal to noise

SPSS: Statistic package social science

S CONV: Stockholm convention

SIM: Selected ion monitoring mode

SPE: Solid Phase Extraction

SPME: Solid-phase micro extraction

UNEP: United Nations Environment Programme

USA: United States of America

US EPA: United States Environmental Protection Agency

WHO: World Health Organisation

WMS: Water management sample

CHAPTER 1: BACKGROUND INFORMATION

1.1 INTRODUCTION

South Africa is one of the World leaders in mining, agriculture and related activities; it is well known for its abundance of mineral resources such as gold, platinum, diamond and coal just to mention a few. According to the department of mineral resources, mining companies and its associated industries in South Africa are key players in the global industry (Kearney, 2012).

Olifants River has been referring to be among the greatest contaminated rivers in the Southern Africa because of the number of anthropogenic stressors that are existent due to different economic activities (Dabrowski, *et. al.,* 2008). These happenings include chrome, chemical and steel manufacturing, intensive coal mining, coal-fired power generation and agriculture. Also, the overall deterioration in the management and operation of waste water treatment infrastructure, particularly sewage treatment complete the list of stressors along the Olifants River.

Often, the pollutants produced from sewage effluent and agriculture activities (nitrogen and phosphorus) and microbiological pollution contain a diversity of potential contaminants. The Olifants River catchment experiences also a general acidification and the mobilisation or input of sulphates plus heavy metal ions; potential acid rain (resulting from air quality being poor) and other pollutants through acid mine drainage.

Hobbs *et. al.,* (2008) noted that the upper Olifants catchment is one of South Africa's most important sources of coal and several reports have documented acidic seepage (acid mine drainage) from both active and abandoned coal mines within this area as shown in Figure 1.1.

Figure 1. 1: Surface coal mining pollution impact in Mpumalanga

Furthermore, Dabrowski *et. al*., (2008) indicated that, there are sets of stressors in the upper Olifants catchment, namely:

- Acidic water, heavy metals and sulphates attributable to mining and industry,
- Excessively high nutrient and microbial inputs from poor sewage treatment practices and, possibly from some agricultural practices are making the greatest contribution to poor water quality.

Over the years, this river is often referred to be one of the extremely polluted systems in the country. Along its length it is being especially impacted by coal mining and industries in the Witbank-Middleburg and Palaborwa areas (Du Preez *et. al.,* 2000). Environmental problem include ground and surface water pollution, in the form of potentially toxic organic compounds and metals taken up by the degradation of the quality of soil, the environment and the damaging of aquatic fauna (Engelbrecht, 2005).

The agricultural pollution arises mainly in the Highveld region of the Olifants catchment where various crops are produced (Van Vuren *et. al.,* 2001). Any pollution contribution from agricultural practices results from substances dissolved in water or else transported by water (Ellis and Coppins, 2007).

1.2 WATER QUALITY BACKGROUND

During recent years, the Olifants River has been systematically impaired because of the increase in industrial development, agricultural, mining and urbanisation activities. This river system is often described as one of the extreme polluted Rivers in South Africa (Van Vuren *et. al.,* 2001). Along the Olifants River there are intensive and subsistence agriculture as well as numerous diffuse sources and point of industrial pollution (Heath and Claassen, 1999). The quality of water in the upper Olifants catchment is under threat from the coal mines (DWAF, 2004b). Anglo Coal reported that fish mortality in the Loskop dam and Wilge River in Mpumalanga, has been related to the cumulative influences of acid mine drainage originating from active, abandoned and old mines, uncontrolled releases from mines and industry and the discharge of raw sewage into the river system (Holman, 2008).

The Groblersdal area is one of South Africa's utmost concentrated agricultural areas and produces deciduous fruit, cereals, citrus, grapes and vegetables. Accusations of pesticide poisoning made by a medical practitioner in Groblersdal during 2007, sent shivers through the region and caused fears amongst the citizenry of health issues connected to the utilisation of pesticides (AVCASA, 2008). The claims of pesticide influences have to date not been validated by scientifically sound toxicological or clinical evidence. The hazard associated with the water resources quality in the Groblersdal area is due to pesticides application and can best be managed by appropriate monitoring programme (DWAF, 2004b).

Knowing that South Africa is one of the World leaders in mining and an agricultural related activity, the research is aimed at probing the concentration of selected persistent organic pollutants (POPs). Predominantly, Organochlorine pesticides in the surface water from a river-reservoir system along the Olifants River catchment. Also, the following water quality parameters Dissolved oxygen (DO), pH, Temperature and Electrical conductivity (EC) were analysised at the site with a hand held YSI instrument.

1.3 PROBLEM STATEMENT

South Africa is among the water-scarce countries of the world (Basson *et. al.,* 2010). There are many water use activities and lands that occur in the Olifants River catchment and are of tactical significance to South Africa. These happenings rely genuinely on a variation of services and goods that they originate from the aquatic ecosystems in the area, to withstand their processes. Nevertheless, the Olifants River has been referred to be among the extremely polluted rivers in Southern Africa, particularly in the upper catchment and the changes to water quality that have

resulted from these activities as illustrated in Figure 1.2 showing the proximity of a coal-fired power plant to the Witbank dam on Olifants River catchment.

The water quality guidelines of the Department of Water Affairs make provision for five water use categories namely: domestic, recreation, industrial, agricultural / aquaculture and the aquatic ecosystem (DWA, 2007).

Figure 1.2: Witbank dam showing the proximity of the coal power station

Recently, Department of Water and Sanitation stated that due to some serious water quality problems in the Olifants River Catchment and the current drought, the following interventions are necessary to overcome the expected water deficit (DWS, 2015).

Interventions that will:

- Reduce the water requirements through water conservation and demand management for the Irrigation, urbanization and mining water use.
- Eliminating unlawful water use.
- Increase the water supply through removal of invasive alien plants
- Increase groundwater development
- Increase treatment of additional decants water from decommissioned and rehabilitated coal mines.

A large section of the rural population in South Africa who do not have access to purified water still rely on surface and groundwater for drinking, domestic and agricultural purposes.

Groundwater equally serves as the major sources of all treated water supplied to the industries and the entire population. If this scarce resource is contaminated with organic waste, the health impacts can be devastating.

It is important for a country like South Africa, to determine the occurrence and levels of these endocrine disrupting chemical (EDCs) in the water systems, in other to take the necessary steps to protect its population.

In light of paucity of studies on the occurrences and levels of Organic contaminant inland water resources such as Olifants River, the present study was undertaken to generate the required data on these over utilised water resources. The concentrations of common physiochemical parameters were also undertaken. It was anticipated that the result of the present study will provide a baseline that can be used to measure the impact of exposure to these compounds within the catchment.

1.4 RATIONALE / JUSTIFICATION OF THE STUDY

Water is a crucial natural resource, essential to life, food production, the environment, industry, power generation, sanitation and hygiene (Blignaut*, et. al.,* 2009). It has been found that a large quantity of the existing water in the Mpumalanga province provisions mines agricultural and industrial sections with their water demands. Nevertheless, rises in water demand, has increased the amount of point and non-point surface pollution resulting in the deterioration of surface water systems (Molale, 2012).

For this reason the Olifants River catchment, as mentioned earlier in Mpumalanga, has been systematically impacted over the past few years due to increasing industrial development, agriculture, mining activities and urbanisation (Grobler *et. al.,* 1994; Hobbs *et.al.,* 2008). The accumulative effect of the use of coal, the negative growing impact on the environment and human health from hypothetically harmful elements and organic compounds has become of concern in this region (Moyo, 2012).

Therefore, information on the concentration and distribution and leaching of these potentially toxic elements from all the economic activities are urgently needed for informed decision making within the catchment.

1.5 HYPOTHESIS

- The presence of organic compounds in surface water at selected sites could impact the water quality of Olifants River over a period of time.
- There is no seasonal variation in water quality along the Olifants River

1.6 GENERAL AIM

The essential aim of this research was to carry out a survey and investigation of concentration of selected organic compounds along the Olifants River catchment in Mpumalanga. In order to provide baseline data required to protect water resources against such chemicals.

1.6.1 Specific objectives

- To determine the presence and levels of selected organic compounds along Olifants River
- To explore the influence of seasonal changes of water quality's properties.
- To identify severely impacted areas that will possibly need further investigation.
- To investigate the movement of organic pollutants along the Olifants River

CHAPTER 2: LITERATURE REVIEW

2.1 INTRODUCTION

The conception of surface water Pollution has been one of the most important environmental problems of the world and many challenges including establishment of public awareness have been introduced over the past few years to deal with this situation (Abel, 1989; Mills, 2001). According to (Oberholster *et. al.,* 2008), they found that anthropogenic activities are a main cause of huge increases in rivers nutrient concentrations. Hence surface water pollution takes place on a worldwide scale; for that reason the fresh water systems of most countries have been negatively affected to some degree (Ellis, 1989; Oberholster *et. al.,* 2008). Other main problems refer to: changes in pollution, global condition and the decrease of natural resources (Miller, 1988; Turk, 1989; Mills, 2001). Thus, environmental problems describe the links between human cultures and the environment in which humans must live on earth (Oberholster *et. al.,* 2008).

2.2 PERSISTENT ORGANIC POLLUTANTS (POPS)

Most of the time Persistent Organic Pollutants are stable, toxic compound that can remain in the environment by resisting chemical, biological and photolytic degradation (Roos, 2011). Numerous POPs can be harmful in high concentrations, but their highest dangerous effects lie in their long-term toxicity, leading to dermal effects, kidney and liver disease, weaknesses of the immune-, reproductive-, nervous-, and endocrine systems, and even cancer (Schecter *et. al.,* 2006). As a result of their lipophilic nature, these contaminants have a tendency to accumulate in matrices rich in organic matter, such as sediment and biota, and can bio-accumulate in food webs (Schecter *et. al.,* 2006).

It has been observed that their chemical and physical characteristics let the compounds to experience long-range transport, letting the pollutants to turn out to be widely disseminated geographically, even to areas where they have never been used or made (Ritter *et. al.,* 2005). As noted earlier, the ecotoxicological effects and environmental behaviour of persistent organic pollutants (POPs) are of universal concern owing to their toxic, bioaccumulative and persistent character both to wildlife and humans (Thomann, 1989; Tanabe et. *al.,* 1998; Guo *et. al.,* 2008; Eqani *et. al.,* 2012).

Usually, the exposure of humans and living organisms to Persistent Organic Pollutants has been related with neurological, immunological, toxicity, a diversity of reproductive, and other side effects (Shaw *et. al.,* 2006; Kalyoncu et. *al*., 2009; Sharma et. *al.,* 2009).

In many instances the production of many organic pollutants from diverse sources such as effluent discharges or runoff within the environment is a subject of big fear in various countries (WHO, 2004). The immediate environmental reservoirs for all kind of organic pollutants are: sea, river and dams (Chee *et. al.,* 1996; Sibali *et.al.,* 2008). These contain organochlorine pesticides (OCPs) such as dichlorodiphenyltrichloroethane (DDT) and its degradation products, DDE and DDD, lindane and endosulfan (Tomkins *et. al*., 1992; Okonkwo *et. al.,* 2007).

Regardless of the restriction and ban on the utilisation of OCPs in western countries during the 1970s and 1980s, some third world countries as well as South Africa are still applying them for public and agricultural objectives due to their efficiency in controlling numerous insects (Tanabe *et.al.,* 1994; Ahmad *et.al.,* 2010).

The following are global studies led on OCPs by some scientists in marine environments: in South Africa (Weaver, 1993; Grobler, 1994; Naude *et. al*., 1998; London *et. al.,* 2000; Fatoki and Awofolu, 2003; Okonkwo *et. al.,* 2007), in Europe (Blair *et. al.,* 1997 and Fernandez-Alba *et. al.,* 1998), in Asia (Iwata *et. al.,* 1994 and Xue *et. al.,* 2006) and America (Dorothea and Muir, 1991; Guillette *et. al.,* 1998) have revealed a well-known existence in marine systems of residues of these pesticides.

However, for the calculation of very small amounts (very low concentrations) of chemicals in the environment Fatoki and Awofolu, 2003; Okonkwo *et. al.,* 2007 have shown that the analytical method procedures must be carried out as follow:

- Separate water sample and organic phase
- Water sample clean-up
- Concentration of the water sample with N_2.
- Quantification by highly selective and sensitive analytical kit, such as gas chromatography / mass spectrometry (GC/MS) (Figure 2.1) or High Performance Liquid Chromatography (HPLC) (Figure 2.2). Both of them playing the same role but having some differences shown below in Table 2. 1.

At present, gas chromatography mass spectrometry (GC/MS) or High performance Liquid Chromatography (HPLC) are the most generally applied analytical methods used for the quantification and detection of POPs. The most important advantage of GC/MS and HPLC techniques is their aptitude to determine the individuality and concentrations of numerous individual chemicals and congeners with realistic precision (Safe, 1995; Hubschmann, 2009).

Figure 2.1: The Gas Chromatography-Mass Spectrometer (GC-MS)

Table 2.1: Differences between HPLC and GC

GC	HPLC
Column (**long**)	Column (**short**)
Temperature programming	High pressure
Stationary phase: **solid**	Stationary phase: **solid**
Mobile phase: **gas**	Mobile phase: **liquid**

Figure 2.2: Figure showing a typical HPLC laboratory set up

2.3 WATER SAMPLE PREPARATION AND CHEMICAL ANALYSIS METHODS

Sample preparation plays a key role in the efficient extraction of components of interest from the sample.

2.3.1 Sampling and sample pre-treatment

Procedure for general environmental sampling necessitates that samples containers and equipment are free from contamination and unpolluted (Olukunle *et. al.,* 2016). Different methods are employed subject to parameters to be tested with the intention that samples are collected in such a way that no impurity is introduced into the sample and no material of interest escapes from the previous sample to analysis (Millar, 1999; USEPA, 2007a; Olukunle *et. al.,* 2016). For volatile organic compounds, methods used according to a review by (Hyotylainen, 2009) include head space (HS), liquid-phase micro extraction (HS-LPME) and solid-phase micro extraction (HS-SPME).

2.3.2 Extraction

The nature of extraction method applied in many instances depends on the medium, such as water, sediment, sewage, dust, air and biological samples. Choosing and optimising a suitable sample preparation method is highly imperative for analytical success (Odusanya *et. al.,* 2006). Numerous illustrations of methods applied for PCBs, OCPs, PBBs and PBDEs and some other POPs in different environmental samples are presented in Table 2.2.

Table 2.2: Application of LLE for extraction of liquids and their respective recoveries

Compounds	**Solvent**	**Sample volume (L)**	**Recovery (%)**	**Reference**
OCP	DCM	0.1L	98.90±7.32-123.7±8.34	(Sibali *et. al.,* 2008)
OCP	DCM	1L	88.22±7.85–109.63±5.10	(Awofolu and Fatoki, 2003)
PBDEs	*n*-Hexane(100 mL)	1L (river water) +20g NaCl	78- 92	(Bacaloni *et. al.*, 2009)
PBDEs	DCM+ 20g NaCl	1L (pure water) +20g NaCl	61- 86	(Odusanya *et. al.,* 2006 and 2008
PBB and PBDEs	DCM(120 mL)	0.5 L	80.5±10.22 – 126.6±1.94	(Olukunle *et. al.,* 2012a)

Some extraction methods have been performed in determining OCPs in water samples (Sibali, *et. al.,* 2008 and Rimayi *et. al.,* 2012). These methods consist of solid-phase micro-extraction

(SPME) and on-line solid phase extraction (OLSPME) (Brossa *et. al.*, 2003) and liquid-liquid extraction (LLE), solid phase extraction (SPE) (Moeder *et. al.,* 2000). Regularly, SPE is in use in various and different parts of analytical chemistry (Sibali *et. al.,* 2008).

2.3.3 Aqueous samples

Methods that are usually used for the extraction of OCPs from liquid samples such as seawater and river, human milk and serum are LLE and SPE (Awofolu, 2003). LLE is an old-style method that utilises large quantity of organic solvents that are of environmental concern, non-selective, time consuming and has a problem of emulsion, it is suitable if only few samples are to be analysed (Olukunle, 2016). In contrast, SPE is highly selective, appropriate for polar and non-polar analytes with availability of large variety of sorbents from non-polar to ion exchange adsorbents, consumes less solvent and saves time (Covaci and Dirtu, 2008). There are however, a number of factors disturbing the recovery of certain analytes from aqueous samples such as sample volume, the ionic strength, pH of the water, type of sorbent and sorbent treatment (Awofolu, 2003).

2.3.4 Liquid-liquid Extraction (LLE)

Solvents that are volatile like, benzene, ethyl acetate, hexane, ether and dichloromethane are frequently applied in the extraction of semi-volatile compounds from water as shown in Table 2.3 (Awofolu and Fatoki, 2003; Sibali *et. al.,* 2008).

Table 2.3: Properties table of common solvents

SOLVENT	CHEMICAL FORMULA	BOILING POINT[9] T	Dielectric constant[10] l	DENSITY	DIPOLE MOMENT
Non-polar solvents					
Pentane	CH_3-CH_2-CH_2-CH_2-CH_3	36^0C	1.84	0.626 g/ml	0.00D
Cyclopentane	C_5H_{10}	40^0C	1.97	0.751g/ml	0.00D
Hexane	CH_3-CH_2-CH_2-CH_2-CH_2-CH_3	69^0C	1.88	0.655g/ml	0.00D
Cyclohexane	C_6H_{12}	81^0C	2.02	0.779g/ml	0.00D
Benzene	C_6H_6	80^0C	2.3	0.879g/ml	0.00D
Toluene	C_6H_5-CH_3	**111^0C**	2.38	0.867g/ml	0.36D
1,4-Dioxane	/-CH_2-CH_2-O-CH_2-CH_2-O/	**101^0C**	2.3	**1.033g/ml**	0.45D
Chloroform	$CHCl_3$	61^0C	4.81	**1.498g/ml**	1.04D
Diethyl ether	CH_3-CH_2-O-CH_2-CH_3	35^0C	4.3	0.713g/ml	1.15D
Dichloromethane (**DCM**)	CH_2Cl_2	40^0C	9.1	**1.3266g/ml**	1.60D
Polar aprotic solvents					
Tetrahydrofuran (**THF**)	/-CH_2-CH_2-O-CH_2-CH_2-/	66^0C	7.5	0.886g/ml	1.75D
Ethyl acetate	CH3-C(=O)-O-CH_2-CH_3	77^0C	6.02	0.894g/ml	1.78D
Acetone	CH_3-C(=O)-CH_3	56^0C	21	0.786g/ml	**2.88D**
Dimethylformamide **(DMF)**	H-C(=O)N(CH_3)$_2$	**153^0C**	38	0.944g/ml	**3.82D**
Acetonitrile **MeCN**	CH3-C≡N	82^0C	37.5	0.786g/ml	**3.92D**
Dimethyl sulfoxide (**DMSO**)	CH_3-S(=O)-CH_3	**189^0C**	46.7	**1.092g/ml**	**3.96D**
Nitromethane	CH_3-NO_2	**100-103^0C**	35.87	**1.1371g/ml**	**3.56D**
Propylenecarbonate	$C_4H_6O_3$	**240^0C**	64.0	**1.205g/ml**	**4.9D**
Polar protic solvents					
Formic acid	H-C(=O)OH	**101^0C**	58	**1.21g/ml**	1.41D
n-Butanol	CH_3-CH_2-CH_2-CH_2-OH	**118^0C**	18	0.810g/ml	1.63D
Isopropanol **(IPA)**	CH_3-CH(-OH)-CH_3	82^0C	18	0.785g/ml	1.66D
n-Propanol	CH_3-CH_2-CH_2-OH0	97^0C	20	0.803g/ml	1.68D
Ethanol	CH_3-CH_2-OH	79^0C	24.55	0.789g/ml	1.69D
Methanol	CH_3-OH	65^0C	33	0.791g/ml	1.70D
Acetic acid	CH_3-C(=O)OH	**118^0C**	6.2	**1.049g/ml**	1.74D
water	H-O-H	100^0C	80	1.000g/ml	1.85D

On the other hand, Dichloromethane has great extraction effectiveness for a wide range of non-polar to polar compounds (Sibali *et. al.,* 2008). Solvent such as dichloromethane is good for immediate analysis because of the next advantages: its boiling point is low and easy to reconcentrate after extraction; it is easy to separate from water because of its superior exact gravity (Awofolu and Fatoki, 2003).

Extraction is ordinarily realised by shaking the solvent and water sample in a separating funnel. Though, huge quantities of emulsion are sometimes formed, it is challenging to isolate the solvent from the aqueous phase. In case this happens, the emulsion is frequently well disseminated (broken down) by adding anhydrous sodium sulfate or by sonicating the mixture in ultrasonic bath, or unceasing liquid-liquid extraction can be executed on samples which form emulsions. Therefore LLE method has great extraction effectiveness; for thermally unstable compounds, it is not appropriate because the extraction period is long (Sibali *et. al.,* 2008).

2.3.5 Clean-up Method of sample extracts

It has been observed that a huge quantity of polar compounds or non-volatile compounds can possibly infect GC injection columns and ports, which in turn causes problems with analysis. As a result, it is very important to remove, or clean up, non-target compounds as much as possible.
According to Labadie *et. al.,* 2010, materials such as silica gel, acidic silica gel (impregnated with H_2SO_4), basic silica gel (impregnated with NaOH), fluorisil, alumina, activated carbon are among the cleaning materials or adsorbents found in literature while anhydrous sodium sulphate is primarily used for drying (removal of water).

2.3.6 Instrumental analysis methods

Simply GC- MS is discussed after this because of the great selectivity, great sensitivity, universality and the big amount of information available on what is frequently considered to be the most suitable analytical instrument for environmental analysis.

2.3.6.1 GC/MS Analysis

It has been documented that to analyse chemicals concentrations proficiently and perfectly, it is still crucial to sensibly choose column temperature conditions, columns, sample injection conditions etc.

2.3.6.2 *Factors Affecting Capillary GC Analysis*

Appropriate separation in the shortest period of time is the ideal (optimum) for good GC analysis. These above factors are linked to the temperature, internal diameter, liquid phase of columns, film thickness and the length.

2.3.6.3 *Mass Spectrometry*

Generally speaking, there are two ways to use mass spectrometers in selected ion monitoring mode (SIM) or scanning mode. For the purpose of selecting the suitable operating mode, one must comprehend some of the key features of these methods.

As noted earlier, the research is aimed at investigating the concentration of selected potentially persistent organic pollutants (POP's) including: Organochlorine pesticides (OCP's) in the surface water from a river-reservoir system along the Olifants River catchment, therefore, the next paragraphs describe them all.

2.4 ORGANOCHLORINE PESTICIDES (OCPS)

Organochlorine pesticides have been used as herbicides, pesticides and insecticides with forms changing from pellet application to sprays for grain and seed storage (Gribble, 2010). They are chlorinated hydrocarbons. Most of them are categorised as Persistent Organic Pollutants (POPs) because they can persist and break down gradually in the environment for long time after application.

Organochlorines (OCs) indicate an essential group of POPs which have created global concern as toxic environmental pollutants (Law *et. al.,* 2003; Covacia, *et. al.,* 2005 and Wurl and Obbard, 2005). Organochlorines (OCPs) are man-made organic chemicals that have been utilised to regulate everything from grasshoppers to fungus. DDT was the first that was applied on a big scale in the United States of America (USA); it was predominantly applied in agricultural regions. Most organochlorine pesticides are no more traded for uses in the U.S.A (Law *et. al.,* 2003; Covacia *et. al.,* 2005 and Wurl and Obbard, 2005).

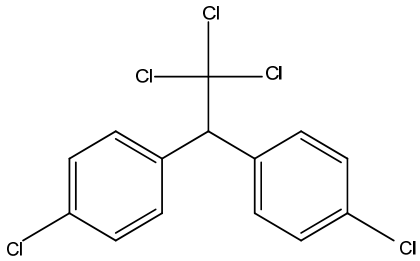
In developing countries like South Africa, DDT is still legitimately used for malaria vector control in some areas within the country. It is mainly documented that some category of OCPs may still be in use secretly under unfamiliar trade names in agriculture for pest control because of their effectiveness and low cost (Fatoki and Awofolu, 2003).

The organochlorine pesticides included in this study are Benzene hexachloride (BHC) alpha, Benzene hexachloride (BHC) gamma, Benzene hexachloride (BHC) beta, Endrin, Aldrin, Dieldrin, Pentachloronitrobenzene, Chlordane trans (gamma), Chlordane cis (alpha), Heptachlor, 4,4' Dichlorodiphenyldichloroethane (DDD), 4,4' Dichlorodiphenyldichloroethylene (DDE), 4,4' Dichlorodiphenyltrichloroethane (DDT),Endosulphan alpha, Endosulphan sulphate, Endosulphan beta, Heptachlor epoxide, Hexachlorobenzene and Mirex.

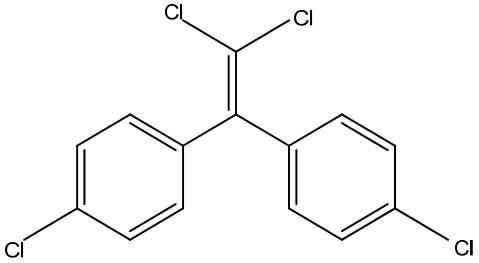
Therefore, the physical and chemical properties, sources, environmental fate and toxicity are discussed briefly below:

2.4.1 Organochlorine pesticides (OCPs)

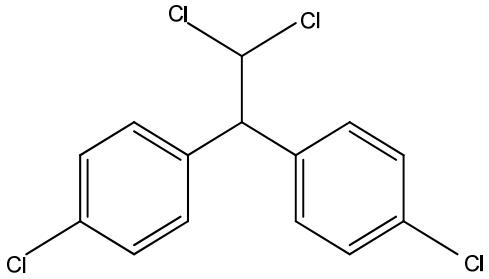
Among the OCPs included in the study, only some of them were a selection of analysis. For this purpose only, these compounds are discussed in the following paragraphs. The chemical structures as shown in Figure 2.3, physical and chemical properties, sources, environmental fate, and toxicity of hexachlorobenzene, heptachlor, mirex(cyclodiene) , 4,4-DDD, 4,4-DDT, 4,4-DDE, Aldrin, Chlorpyrifos-methyl , BHC beta isomer, Endrin, Heptachlor epoxide, BHC delta isomer, BHC alpha isomer, Dieldrin, Endosulfan sulphate, BHC gamma isomer (lindane), Endosulfan- alpha, Endosulfan-beta are discussed in short below.



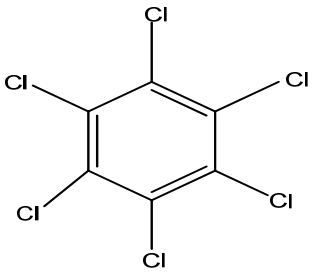
4,4'-DDT



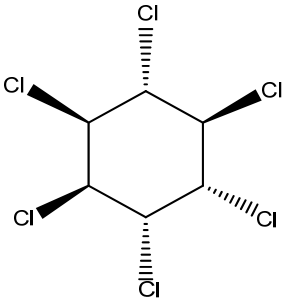
4,4'-DDE



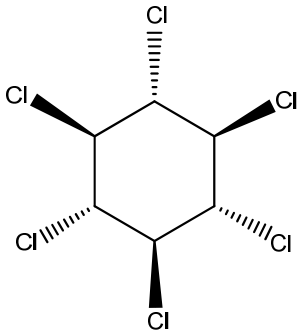
4,4'-DDD



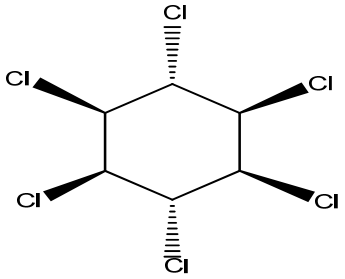
Hexachlorobenzene



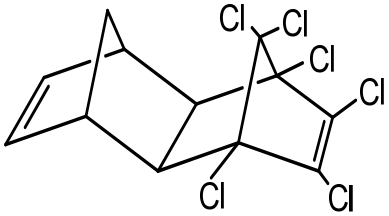
BHC alpha



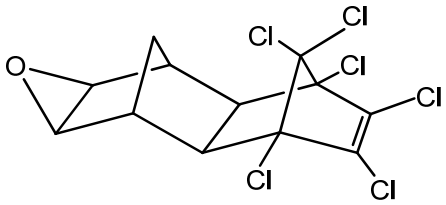
BHC beta



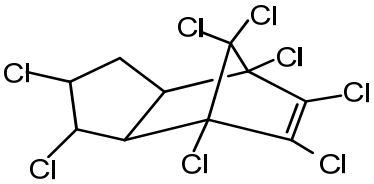
BHC gamma



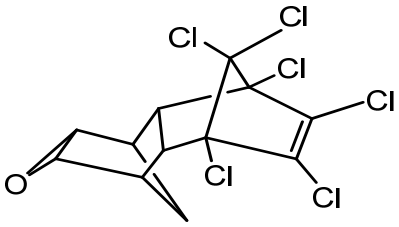
Aldrin



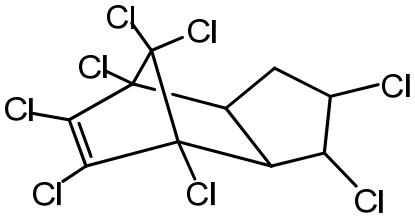
Endrin



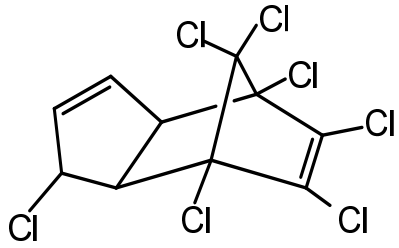
Chlordane alpha



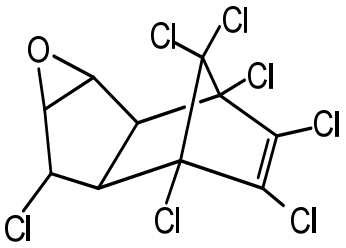
Dieldrin



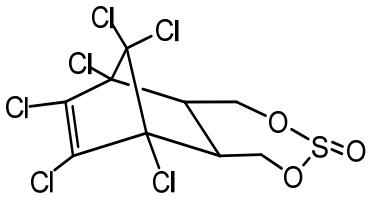
Chlordane trans gamma



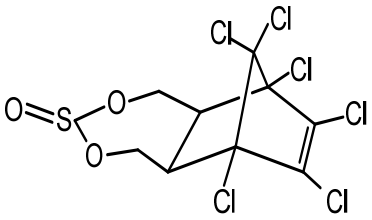
Heptachlor



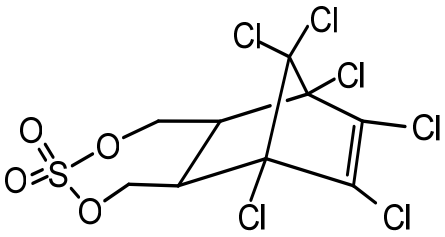
Heptachlor epoxide



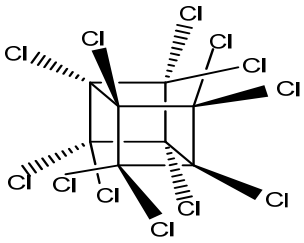
Endosulfan beta



Endosulfan alpha



Endosulfansulphate



Mirex

Figure 2.3: Name and chemical structures of selected organic compounds (SOCs)

2.4.1.1 Hexachlorobenzene (HCB)

For many years until 1965, HCB (C_6Cl_6) was extensively used as a fungicide on seed of onions, wheat, sorghum and other grains. It was also utilised in the making of fireworks, ammunition and synthetic rubber (Sala *et. al.,* 2001). Presently, its production is banned in most countries and it is contained in the Stockholm Convention on persistent organic pollutants stated by the United Nations Environment Programme (UNEP, 2005a).

- ***Physical and Chemical Characteristics***

The application of HCB as an organochlorine compound (chlorinated hydrocarbon) is well-known in the environment, bio-accumulative and lipophilic. According to the Agency for Toxic Substances and Disease Registry (ATSDR), HCB contains 98% HCB of technical agricultural grade, 1.8% pentachlorobenzene and 0.2% tetrachlorobenzene (ATSDR, 2002b). The molecular mass of this compound is 284.76 and is really insoluble in water (0.005 mg/l). The vapour pressure of is 2.3 x 10-3 Pa at 25 °C and a log n-octanol-water coefficient (log K_{OW}) of 3.93 to 6.42 (ATSDR, 2002).

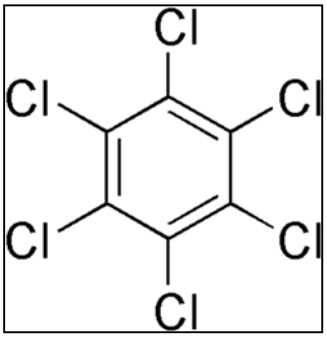


Figure 2.4: Chemical structure of hexachlorobenzene.

HCB is still in application as an industrial chemical even though its use as a fungicide was banned in the 1960's and it is an unintended by-product of several processes, such as during the production of chlorinated solvents (Bailey, 2001). At present, the most important sources of HCB are emissions from combustion processes, metal industries and chemical processes such as perchloroethylene-, chlorobenzene-, and chlorinated organic production (Euro Chlor, 2002). It is also a trace pollutant in some pesticides and may persist in the environment due to historic use as a fungicide (ATSDR, 2002a).

- ***Environmental fate***

Generally speaking, HCB is by nature lipophilic and persistent; the compound is reasonably stable in soil with half-lives ranging from 2.7 to 7.5 years (Augustijn-Beckers *et. al.,* 1994; Roos, 2011). The compound has the potential to degrade aerobically and anaerobically, but its low water solubility causes HCB to have a low mobility in the soil environment. As soon as in the aquatic environment, HCB is broken down quickly. Experimental results on hydro-soil have demonstrated almost complete degradation of HCB to pentachlorophenol and connected compounds in less than 5 days (Augustijn-Beckers *et. al.,* 1994; Roos, 2011).

- ***Toxicity***

The side effects on health produced by HCB are reproductive toxicity. Jarrel and Gocmen (2000) stated on the effects of HCB on a Turkish population unintentionally ingesting HCB-treated seeds. Their most conclusive remark was the absence of children below the age of 5 years in some villages, which would sanction HCB as one of the utmost powerful reproductive toxicants. Whereas some human reproductive health research have shown an affirmative connection between HCB exposure and spontaneous abortion, decreased birth mass, decreased crown-rump length, and reduced gestational period (Jarrel *et. al*., 1998; Schade and Heinzow, 1998; Fenster, *et. al.,* 2006). Others have defined no or non-linear associations (Gladen *et. al.,* 2003; Khanjani and Sim, 2006; Sagiv *et. al.,* 2007).

2.4.2 Heptachlor

Heptachlor ($C_{10}H_5Cl_7$) was largely used in the 1960s and 1970s to exterminate ants, termites and soil insects on seed crops and grains. Meanwhile in South Africa, its registration was withdrawn in 1976 (SADA, 2008) and its use is now banned in most countries (ATSDR, 2007b).

2.4.2.1 Physical and chemical characteristics

Technical heptachlor composition contains of approximately 72% heptachlor and about 28% connected compounds, such as trans chlordane and trans-nonachlor. Its obtainable formulations are contained within wettable powders, emulsifiable concentrates, dusts, and oil solutions (ATSDR, 2007b).

Heptachlor (molecular mass = 373.32) has a water solubility of only 0.056 mg/l, and it is soluble in acetone, alcohol, benzene, cyclohexanone, paraffin and xylene (Kidd and James, 1991). It has a vapour pressure of 3.99 x 10-2 Pa at 25 °C, and a log K_{OW} of between 6.1 and 6.13 (Simpson *et. al.,* 1995; Roos, 2011).

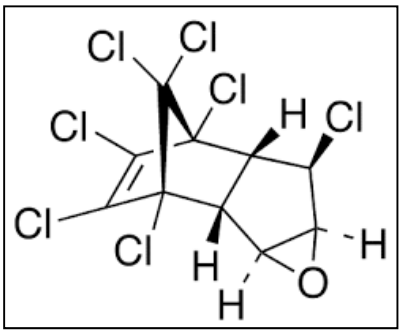


Figure 2.5: Chemical structure of heptachlor.

2.4.2.2 Sources

It appears that there are no natural sources of heptachlor, but heptachlor epoxide is formed by abiotic or biotic transformation of heptachlor in the environment (WHO, 2006). As with the other banned OCPs, heptachlor still be existent in the environment due to remarkable use, unused stockpiles, and in leachates from disposal sites (ATSDR, 2007b). Moreover, heptachlor is also a constituent in plywood glues, and a component of the pesticide chlordane (which, although banned in most parts of the world, is still used for the control of termites) (WHO, 2006).

2.4.2.3 Environmental fate

Naturally, Heptachlor is broken down to heptachlor epoxide in the environment. The metabolite is more expected to be found in the environment than heptachlor (ATSDR, 2007b) and is strong to oxidation, biodegradation, photolysis and hydrolysis (Smith, 1991; Roos, 2011).

In many occasions, Heptachlor and heptachlor epoxide are exposed to long-range environmental and biotic transport, and are detached from the atmosphere by wet and dry deposition (WHO, 2006). Sediment and soil are the major environmental compartments for heptachlor. Both the parent compound and the metabolites are moderately bound to, and persistent in sediments and soils (Augustijn-Beckers *et. al.,* 1994; Roos, 2011). The main route of loss of heptachlor from soil surfaces is via volatilisation. Because heptachlor is nearly insoluble in water, it may enter surface waters mostly via surface runoff. In the aquatic environment, heptachlor is quickly degraded to heptachlor epoxide by hydrolysis and degradation by micro-organisms (Augustijn-Beckers *et. al.,* 1994; Roos, 2011). Adsorption, volatilisation to sediments and photo degradation may also contribute towards the loss or bio-availability of heptachlor and heptachlor epoxide from the water environment (Matsumura, 1985; Smith, 1991; Roos, 2011).

2.4.2.4 Toxicity

Similarly to most OCPs, heptachlor may obstruct with nerve transmission (Ecobichon, 1991; Roos, 2011). In contrast, the negative health effects associated with heptachlor epoxide may be more than the effects associated with heptachlor. However, health effects due to exposure to heptachlor or its metabolites may include hyper excitation of the lethargy, liver damage, convulsions, tremors, coma and stomach cramps (Smith, 1991; ATSDR, 2007b; Roos, 2011). Studies have revealed infertility and wrong development of offspring in mice and rats (Smith, 1991; Roos, 2011). Some experimentation suggests that heptachlor may stimulate the development of tumours in rats (Smith, 1991; Roos, 2011), but evidence is inadequate to evaluate the potential of heptachlor to cause cancer in humans (Roos, 2011).

2.4.3 Mirex

To a larger extent, Mirex ($C_{10}Cl_{12}$) is a chlorinated hydrocarbon that was utilised as an insecticide to control fire ants and leaf cutter ants (typically in South America), mealy bugs (Hawaii), and harvester termites (ATSDR, 1995; Roos, 2011) . Its use was banned in 1976 by the US EPA and it is included as a POP in the Stockholm Convention (UNEP, 2005a).

2.4.3.1 Physical and chemical characteristics

Comparable to the other OCPs, mirex is resistant, persistent and toxic to degradation (ATSDR, 1995; Roos, 2011). Mirex is a white crystalline solid, which is a derivative of cyclopentadiene (C_5H_6).

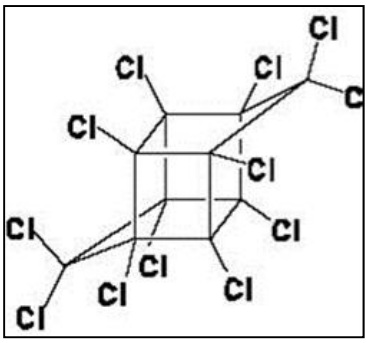


Figure 2.6: Chemical structure of mirex.

2.4.3.2 Sources

Subsequently, it is produced by the dimerization of hexachlorocyclopentadiene in the presence of an aluminium chloride catalyst (Roos, 2011). Even if mirex is commonly known for its insecticidal properties, it was also extensively used as a flame retardant in rubber, paint, plastics, paper and electrical equipment (ATSDR, 1995; Roos, 2011). Its use as an insecticide and fire retardant was banned in the 1970's, but residues of this compound may still stay in the environment due to historical use, disposal, accidental spillages, fires, and volatilisation or leaching from old stockpiles (Roos, 2011).

2.4.3.3 Environmental fate

The presence of Mirex in the environment binds strongly to organic matter in water, soil and sediment. Mirex can be transported for long distances before partitioning into a different phase when bound to particulate matter. Volatilisation and adsorption are the utmost vital environmental fate processes for mirex, while atmospheric transport may also play a role (ATSDR, 1995; Roos, 2011). Owing to its lipophilic nature (high log K_{OW}) and persistence, mirex is bio-magnified and bio-accumulated in food webs.

According to (Roos, 2011) mirex is resistant to biological and chemical degradation in sediment and soil (half-life of >10 years). The major process responsible for the degradation of mirex (to photomirex) is photolysis (Carlson *et. al.,* 1976; Roos, 2011). Through anaerobic degradation mirex in sediment soil are dechlorinated to the monohydro- derivative, whereas aerobic biodegradation plays a negligible role (Carlson *et. al.,* 1976; Roos, 2011).

2.4.3.4 Toxicity

To this date, Statistics on human health effects is missing. Animal studies related mirex exposure to detrimental effects on intestines, liver, the stomach, kidneys, eyes, thyroid, nervous system and reproductive system (ATSDR, 1995; Roos, 2011). In rats, mirex shows toxic effects on foetuses, including cataract formation and liver hypertrophy (UNEP, 2002). It is categorised as a Group 2B possible human carcinogen by the US EPA, but the few experimental results are questionable (Roos, 2011).

2.4.4 Dichlorodiphenyltrichloroethane (DDT)

The South African Department of Agriculture (SADA, 2008) banned the use of DDT in 1993, but its use is still official in certain areas of South Africa to control the disease carrying mosquito, Anopheles sp., the vector of the malaria parasite (Bouwman, 2004).

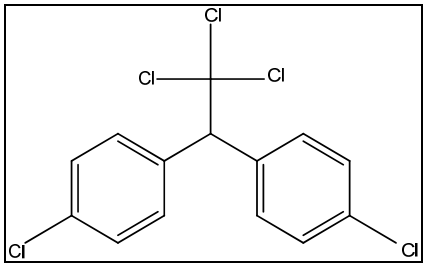
2.4.4.1 Physical and chemical characteristics

DDT ($C_{14}H_9Cl_5$), DDE ($C_{14}H_8Cl_4$) and DDD ($C_{l4}H_{10}Cl_4$) are complex organochlorine elements containing two attached aromatic phenyl rings with chlorine atoms covalently bonded in the ortho- or Para positions. Commercial DDT is a mixture of these closely correlated compounds, with p,p'-DDT being the main component (65 to 80%), and o,p'-DDT and p,p'-DDD present in smaller quantities (15 to 21%, and about 4%, respectively; Beard, 2006). It appears that in its pure form, DDT is a colourless crystalline solid with a weak, chemical odour (ATSDR, 2002b). The pesticide is obtainable in numerous diverse forms including emulsifiable concentrates, granules, aerosols, dustable powders and wettable powders (ATSDR, 2002b).

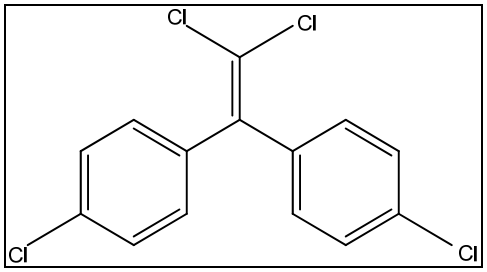
It has been demonstrated that DDT has high log K_{OW} values and a low volatility. Also, DDT and its metabolites are insoluble in water, making these chemicals persistent in aquatic, soils and sediments (ATSDR, 2002b). Volatilisation of DDT, DDE, and DDD is notorious to account for excessive losses of these compounds from water and soil surfaces. Their predisposition to volatilise from water can be estimated by their respective Henry's law constants, which for the respective p,p'- and o,p'- isomers are 8.3x10-6, 2.1x10-5, 4.0x10-6,-5.9x10-7, 1.8x10-5, and 8.2x10-6 atm-m3/mol (Howard and Meylan, 1997; Roos, 2011).

It is soluble and lipophilic in most organic solvents, oils and fats, and hence has the potential to bio-accumulate and bio-concentrate in biota and humans (Zhu *et. al.,* 2005; Beard, 2006). In most environmental conditions, DDT is relatively resistant and stable to degradation. Its less toxic metabolite, DDE, has a stability equal to, or more than, the parent compound. Half-lives

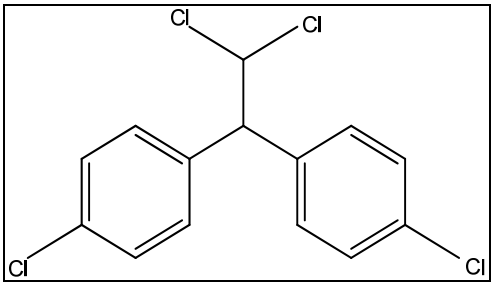
reported for DDT range between 2 and 15 years for soil and as much as 150 years in the aquatic environment (Hooper *et. al.,* 1997; ATSDR, 2002b).



4,4’-DDT



4,4’-DDE



4,4’-DDD

Figure 2.7: Chemical structure of DDT, DDE and DDD.

Table 2.4: International Union of Pure and Applied Chemistry (IUPAC) names and physical and chemical properties of DDT and its metabolites (adapted from ATSDR, 2002b).

Compound	IUPAC name	Molecular mass	Water solubility (mg/l)	Vapour pressure (Pa)	Log K_{OW}
p,p'-DDT	1,1,1-Trichloro-2,2-bis(p-chlorophenyl)-ethane	354.49	0.025	1.6×10^{-7}	6.91
o,p'-DDT	1,1,1-Trichloro-2-(o-chlorophenyl)-2-pchlorophenyl)-ethane	354.49	0.085	1.1	6.79
p,p'-DDE	1,1-Dichloro-2,2-bis(p-chlorophenyl) ethylene	318.03	0.12	6.0×10^{-6}	6.51
o,p'-DDE	1,1-Dichloro-2-(o-chlorophenyl)-2-(pchlorophenyl)ethylene	318.03	0.14		6.00
p,p'-DDD	1,1,-Dichloro-2,2-bis(p-chlorophenyl)-ethane	320.05	0.09	1.35×10^{-6}	6.02
o,p'-DDD	1,1-Dichloro-2-(o-chlorophenyl)-2-(pchlorophenyl)ethane	320.05	0.1		5.87

2.4.4.2 Sources

Traditionally, DDT was released to surface water in case it was used for vector control in the proximity of open waters. This source of release may still be happening in States that depend on DDT in insect pest control close to open waters. DDT also enters surface water as a consequence of wet and dry deposition through gas transfer and from the atmosphere. Atmospheric DDT deposited into Rivers will bring value to the loading in oceans, rivers and lakes. In 1994, the expected stocking of DDT into the Great Lakes region as a consequence of wet and dry deposition was projected at 148 kg, down from 278 kg in 1988 (Howard and Meylan, 1997; Roos, 2011).

For military reasons, DDT was initially instrumental during the second World War for public health purposes to control bubonic plague, malaria and body lice (WHO, 1979; Kumar, *et. al.,*2008). Despite its public health uses, DDT was also functional for a variety of food crops, including soybeans, beans, peanuts, cotton, sweet potatoes, cabbage, tomatoes, cauliflower, corn, and other crops (Casida and Quistad, 1998; Roos, 2011).

Due to the concern over carcinogenicity, adverse health impacts on wildlife and bio-accumulation (Lee *et. al.,* 2001; Kumar *et. al.,* 2008), the utilisation of DDT is banned in most countries, but is still legally manufactured for its use in malaria-endemic areas. Here in South Africa, the common use of DDT was banned in the early 1980's (Kumar *et. al.,* 2008), but it is presently practical for malaria vector control in restricted areas in the northern and eastern parts of Limpopo, the north-eastern parts of Mpumalanga and northern KwaZulu-Natal (Bouwman *et. al.,* 1992; Sharp and Le Sueur, 1996; Coetzee and Hunt, 1998; Roos, 2011). Most of the DDT found in the environment or in areas affected by its use had been prohibited, because of the tenacious nature of the chemical. Traces of DDT measured in areas where the substance has never been applied or produced can be attributed to the compound's potential for long-range transport (Gong *et. al.,* 2007; Hung *et. al.,* 2007).

Due to the fact that the processes used to synthesise DDT and dicofol are similar, dicofol is frequently polluted with DDT (Roos, 2011). Dicofol, a non-systemic acaricide used for the control of mites on crops and orchards, is still listed for use in South Africa and could then be an extra source of DDT contamination (Clark *et. al.,* 1990; Qiu *et. al*., 2005).

2.4.4.3 Environmental fate

By nature DDT is persistent in the environment, and because it tends to associate with organic matter, DDT appears to be relatively immobile in soils. Nonetheless, the loss and degradation in the terrestrial environment include runoff, volatilisation, photolysis, and biodegradation (Beard *et. al*., 2000). However, this will only happen over long periods of time (ATSDR, 2002b). Subsequently, DDE and DDD are breakdown products of DDT and major metabolites in the environment. Overall, the metabolites are also persistent and their physical and chemical characteristics are comparable to that of DDT (ATSDR, 2002b).

As a result, DDT emanates from the atmosphere via emission or volatilisation. Volatilisation of DDT, DDE, and DDD is notorious to account for significant losses of these compounds from water and soil surfaces (Wania and MacKay, 1993; Roos, 2011). Volatilisation loss will rest on the quantity of DDT applied, the quantity of sunlight , proximity to the soil-air interface and proportion of soil organic matter, (depth) (Zhu *et. al*., 2005).

2.4.4.4 Toxicity

In many instances, DDT is gradually altered to DDE and DDD in the human body. While DDD is excreted quickly, DDE and DDT are stored in the fatty tissue, excreted slowly and may bring

about dangerous health effects. DDT and its metabolites are eventually converted into bis (dichlorodiphenyl) acetic acid (DDA) and excreted via the urine (ATSDR, 2002b).

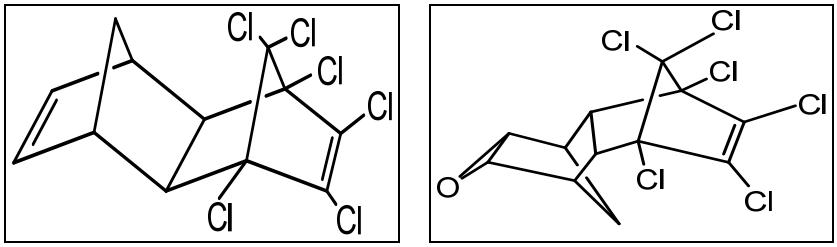
Often, it has been argued that acute effects because of low to moderate exposure to DDT may contain irritation, depression, diarrhoea, increased liver enzyme activity, nausea and excitability. Higher doses may lead to tremors and convulsions (Van Ert and Sullivan, 1992; Beard, 2006). Recent studies on experimental animals have demonstrated that DDT may cause chronic effects on the liver, kidneys, immune system and nervous system (ATSDR, 2002b). There is also clear confirmation that DDT may cause generative effects subject to endocrine disruption (Zeng *et. al.,* 1999; ATSDR, 2002b). A study directed during 2004 to 2006 in the Limpopo Province discovered that women who lived in villages sprayed with DDT gave birth to 33% more boys with urogenital birth defects than women in unsprayed villages (Bornman and Coworkers, 2009). Similarly, blood cell cultures of men occupationally exposed to DDT demonstrated an increase in chromosomal damage (ATSDR, 2002b). To this day, the indication regarding the carcinogenicity of DDT is vague. It has been proven to cause amplified production of tumours of predominantly the liver and lung in test animals. Important association between DDT exposure and pancreatic cancers in chemical workers has been found (ATSDR, 2002b).

In brief, DDT has also demonstrated to have negative influences on animals, particularly birds, where it was directly related to eggshell thinning, and it is toxic to several aquatic invertebrate species (Beard, 2006).

2.4.5 Aldrin and Dieldrin

2.4.5.1 Physical and chemical characteristics

Aldrin ($C_{12}H_8Cl_6$) and dieldrin ($C_{12}H_8Cl_6O$) are insecticides with comparable chemical structures (ATSDR, 2002). They are together debated in this study because Aldrin rapidly changes to dieldrin in the environment and in the body. Usually, Pure Aldrin and dieldrin are white powders with a slight chemical odor (ATRSD, 2002).



Aldrin Dieldrin

Figure 2.8: Chemical structure of Aldrin and Dieldrin.

2.4.5.2 Source

The following compounds (Aldrin and Dieldrin) were first formulated from a waste product of synthetic rubber, cyclopen- tadiene (Jorgenson, 2010). From the 1950s until 1970, Aldrin and Dieldrin were extensively applied insecticides for crops like cotton and corn. Because of apprehensions associated with impairment to the environment and possibly to human health, EPA disqualified all applications of Aldrin and Dieldrin in 1974, except to control termites. In 1987, EPA disqualified all applications (ATSDR, 2002).

2.4.5.3 Environmental fate

Here after is what happen in case Aldrin and Dieldrin penetrate the environment according to (ASTDR, 2002): Aldrin changes to Dieldrin due to bacteria and sunlight and that is the reason why we mostly find Dieldrin in the environment. Most of the time, they stick firmly to soil and gradually vanish to the air. Dieldrin in water and soil breaks down gradually. Plants take in and accumulate Aldrin and dieldrin from the soil. Aldrin speedily changes to dieldrin in animals and plants. Dieldrin is kept in the fat and leaves the body very slowly (ASTDR, 2002).

2.4.5.4 Toxicity

There is no clear indication that Dieldrin or Aldrin cause cancer in humans (ASTDR, 2002). Aldrin and Dieldrin have revealed to cause liver cancer in mice. Pursuant to this, the International Agency for Research on Cancer (IARC) has determined that Aldrin and Dieldrin are not classifiable to human carcinogenicity. However, the EPA has determined that Aldrin and Dieldrin are probable human carcinogens (ASTDR, 2002).

2.4.6 Endosulfan

2.4.6.1 Physical and chemical characteristics

Endosulfan ($C_9H_6C_{l6}O_3S$) is used for controlling a diversity of insects (US EPA, 2006). It is essentially water-insoluble, but freely sticks to clay particles and persists in water and soil for many ages. Its mode of action contains monotonous nerve-discharges definitely connected to increase in temperature. This compound is enormously lethal to most fish (US EPA, 2006).

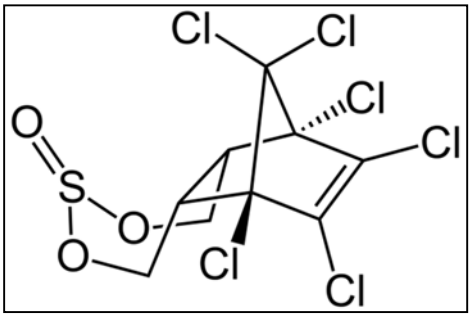


Figure 2.9: Chemical structure of endosulfan.

2.4.6.2 Source

This pesticide is applied to a wide number of crop types including cereals, fruit trees, plantation crops such as coffee and tea and cotton. Nevertheless, due to its relative persistence and semi-volatility, endosulfan is an ever-present environmental pollutant taking place in numerous environmental compartments (Usenko *et. al.,* 2007). Therefore, it has been detected in a variety of environmental media across the world, with the large quantity of reported data on the order of alpha, beta and sulphate (Weber *et. al*., 2006).

2.4.6.3 Environmental fate

Endosulfan is subject to both biotic and abiotic degradation in the environment that may potentially result in oxidation to the corresponding sulphate or hydrolysis in aquatic to endosulfan diol. In turn the diol may degrade further to endosulfan ether, endosulfan alfa-hydroxyether, or endosulfan lactone (Walse *et. al.*, 2003). In the atmosphere, endosulfan is found largely (>95%) in the gas phase (Sofuoglu *et. al.,* 2004), even at the colder temperatures experienced in the Arctic (Hung *et. al*., 2005).

2.4.6.4 Toxicity

This compound (Endosulfan) is severely neurotoxic to mammals and insects, as well as humans (US EPA, 2002). Commonly, symptoms of acute poisoning include tremors, hyperactivity, lack of coordination, convulsions, difficulty breathing, staggering, nausea and vomiting, diarrhoea, and in severe cases, unconsciousness (US EPA, 2002).

2.4.7 Lindane: Gamma-Hexachlorocyclohexane (BHC)

2.4.7.1 Physical and chemical characteristics

Lindane ($C_6H_6Cl_6$) also known as gamma-hexachlorocyclohexane, is an organochlorine chemical variant of hexachlorocyclohexane that has been mainly applied both as a pharmaceutical treatment for scabies and lice and as an agricultural insecticide (US EPA, 2005).

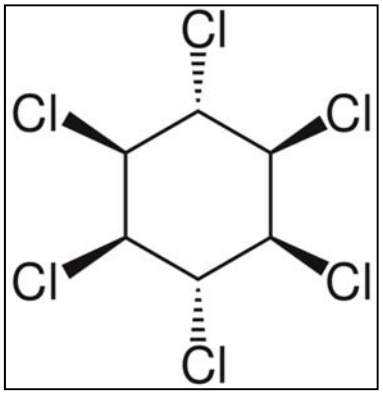


Figure 2.10: Chemical structure of lindane.

2.4.7.2 Environmental fate

Lindane is a persistent organic pollutant: it is reasonably long-lasting in the environment, can be carried a long way by natural processes like global distillation, and it may bioaccumulate in food chains, although it is quickly eliminated when exposure is inaccessible (POPRC, 2007). The production of lindane engenders huge quantities of waste hexachlorocyclohexane isomers, and it is expected that "every ton of lindane produced creates about 9 tons of toxic waste" (POPRC, 2008).

2.4.7.3 Toxicity

At the international level, both the WHO and EPA categorise lindane as "moderately" very poisonous. It has a dermal LD_{50} of 1000 mg/kg and an oral LD_{50} of 88 mg/kg in rats. Most of the adverse human health effects described on lindane have been linked to occupational exposure of seed-treatment workers, chronic and agricultural uses (US EPA, 2006). To a larger extent, exposure to enormous quantities of lindane can damage the nervous system, generating a variety of symptoms from dizziness and headache to seizures, convulsions and, more randomly, death (US EPA, 2006).

2.4.8 Chlorpyrifos-Methyl

2.4.8.1 Physical and chemical characteristics

Chlorpyrifos-methyl ($C_9H_{11}Cl_3NO_3PS$) is an organophosphate pesticide used to control insects on vegetables, fruit and cereal plants. It is beneficial when controlling insects in grain storage areas. This pesticide is a granular crystalline solid with a mercaptan odor. Generally speaking, it is soluble in acetone, acetonitrile, benzene, carbon disulfide, carbon tetrachloride, ethanol,

chloroform, diethyl ether, methanol, *n*-octanol and hexane but also it is insoluble in water (US EPA, 2002).

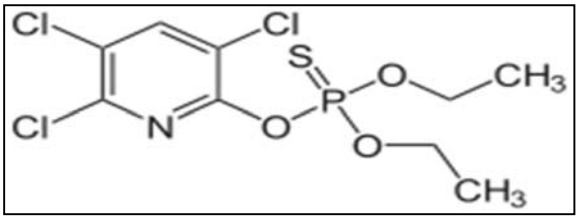


Figure 2.11: Chemical structure of chlorpyrifos .

2.4.8.2 Toxicity

On the medical front, Chlorpyrifos is moderately toxic to humans, and exposure has been related to auto-immune disorders, neurological effects and persistent developmental disorders (US EPA, 2002).

2.4.9 Endrin

2.4.9.1 Physical and chemical characteristics

In the majority of instances, Endrin ($C_{12}H_8Cl_6O$) is water emulsifiable. It is an odorless solid dissolved in liquid carrier and white crystalline. Consequently, it is toxic by skin absorption, inhalation, and/or digestion. When burned or either heated it may release toxic phosgene and hydrogen chloride (US EPA, 2006).

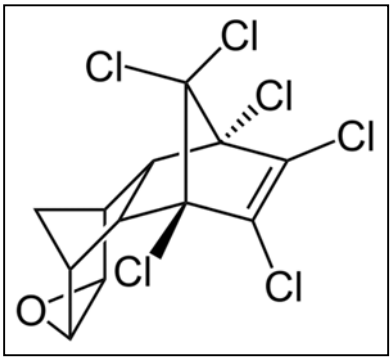


Figure 2.12: Chemical structure of endrin.

2.4.9.2 Source

Practically, pesticides are intended to control insects that appear to be harmful to man. As observed, the insects may be directly harmful, like those acting as disease vectors, or indirectly harmful, as destroyers of crops, food products, or textile fabrics (US EPA, 2006). The 2004

Stockholm Convention (SC) on Persistent Organic Pollutants came into conclusion and listed Endrin as one of the 12 preliminary persistent organic pollutants (POPs) that have been affecting adversely humans and the environment. This above legislation necessitates the participating parties to take measures to restrict or eliminate the production of POPs (SC, 2015).

2.4.9.3 Environmental fate

In the environment Endrin exists as either Endrin ketone or Endrin aldehyde and can be obtained generally in bottom sediments of bodies of water (US EPA, 2015).

2.4.9.4 Toxicity

Exposure to Endrin can occur by ingestion, inhalation of substances containing the compound, or skin contact (US EPA, 2015). Hence, the Federal Aviation Administration (FAA, 2015) stated that it can cause convulsions, seizures, or even death.

CHAPTER 3: MATERIALS AND METHODS

3.1 INTRODUCTION

The objective of this chapter is to outline all specific materials and general procedures used in order to complete the exact aim and objectives of the study. Here the development of avigorous sample preparation and analytical methodology are key to ensure exact measurements. The analysis of the samples collected depended on method validation parameters such as the verification of linearity, accuracy, precision, estimation of linearity, Limit of Detection (LOD), Limit of Quantification (LOQ) and coefficient of regression (R^2).

3.1.1 Materials, reagents and standards

The appropriate choice of materials such as amber glass bottles for sample collection, together with glassware instead of plastic and solvents are essential for a successful and reliable experimental process. With this in mind, care was taken in choosing suitable materials required for the experimental protocols of this research work.

3.1.2 Reagents and their purification

All reagents were of analytical and GC grade (Merck, South Africa) and set aside from impurity. Anhydrous sodium sulphate, 99.5% pure was activated by baking at 450^0C in the muffle heating system for 16 hours before usage. All the solvents used in the analysis included: hexane (69 ºC), dichloromethane (39.8 °C), acetone (56.2ºC) were all of analytical grade and similarly double distilled before use. Anhydrous sodium sulphate, 99 % pure from Rochelle Chemicals (South Africa) was preheated by drying over night before use. Florisil^R 60-100 mesh from Sigma-Aldrich was used for column chromatography. Ten of the organochlorine Pesticides standards were purchased from Supelco (Supelco, Belle-fonte, USA) and the rest of the standards were donated by Dr. David Odusanya of Department of Water and Sanitation, Resource Quality Information Services (Pretoria). Silica gel, Kieselgel Merck Typ 77754, 70 to 230 mesh 100 µm was obtained from Sigma-Aldrich (South Africa). Ultrapure water was distributed from Labostar ultrapure water equipment (Siemens, Germany) provided by Separations Pty (Pretoria, South Africa); Pesticarb and Strata florisil 500 mg x 3mL, provided by Separations Pty (Pretoria, South Africa). Serial dilution of working standards and preparation of standards were done under fume hood.

3.1.3 Cleaning of glassware and apparatus

All glassware were washed water thoroughly and rinsed with distilled water and then with pure acetone. They were then dried in the oven at 110° C over night before use. The glass amber sample bottles were subjected to the same cleaning protocol.

3.2 DESCRIPTION OF STUDY AREA

The Olifants water management area (WMA) falls within three South African provinces (Limpopo, Mpumalanga and Gauteng) it also contains eight District Municipalities and 25 Local Municipalities. It spreads across Phalaborwa to Emalahleni tertiary drainage regions; covering approximately 54 550 km^2 land mass (Figure 3.1).

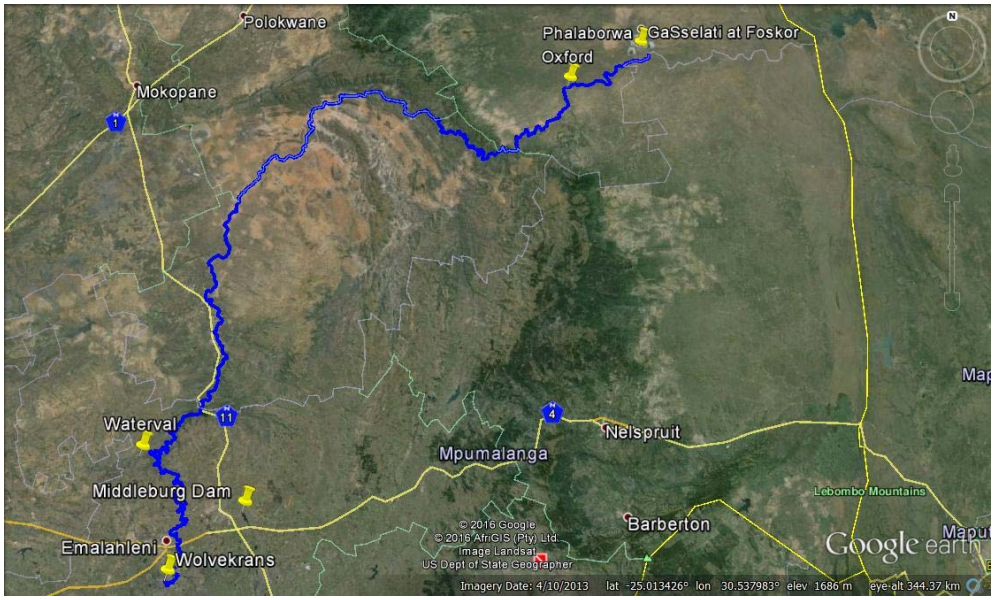


Figure 3. 1:Map of Olifants WMA

The study area is divided into four sub-areas in line with the resource quality objective classification system (Figure 3.1):

Upper Olifants Catchment i.e. catchment of the Olifants River down to Loskop Dam;

Middle Olifants Catchment i.e. the downstream from the Loskop Dam to the confluence with the Steelpoort River;

Steelpoort Catchment corresponds to drainage region of the Steelpoort River; and

Lower Olifants Catchment i.e. between the Steelpoort confluence and the Mozambique border.

According to the International Water Management Institute (IWMI) the Olifants WMA is located on the Southern part of Limpopo and Northern Mpumalanga, conforming to the South African portion of the Olifants River Catchment. Also, originating from the east of Johannesburg (i.e. near Bethal on the Mpumalanga Highveld), the River flows northwards before curving in an

easterly direction via the Kruger National Park, where it joins the Letaba River in Limpopo before flowing into Mozambique and then discharging into the Indian Ocean (IWMI, 2008).

Major tributaries of the Olifants River are the Wilge, Moses, Elands and Ga- Selati on the left bank and Olifants, steelport and Blyde on the right bank, like illustrated in Table3.1.

Table 3.1 Summary Statistics for the major tributaries of the Olifants River (DWA, 2004).

Tributary	**Catchment Area (km2)**	**Mean annual flow (Mm2)**
Wilge	4,356	167
Moses	1,662	39
Elands	6,148	83
Ga-Selati	2,340	80
Klein-Olifants	2,391	81
SteelPort	7,136	396
Blyde	2,842	436

3.2.1 Topography, Geology and Climate of the study area

Topography is wide-ranging, from the reasonably flat and gently sloping Highveld, via mountainous and alpine terrain, and the Drakensberg slope, to the Lowveld. Most of the catchment is very arid with precipitation ranging from 325 mm/a to 750 mm/a. High precipitation of up to 1 000 mm/a takes place only in a thin belt along the slope (DWA, 2004).
On average, the mean annual evaporation for the catchment ranges from 1300mm to 1700mm. Inside the Olifants WMA there is a distinct difference in climatic conditions from cool Highveld in the south-west to sub-tropical in the east of the escarpment. In its report, the Department of Water affairs stated that " primarily the geology in the Olifants River catchment includes hard rock formations, with the happening of the Bushveld igneous complex as the most predominant feature" (DWA, 2004). The eastern limb of this formation stretches through the north part of the water management area. Rich coal deposits take place in the Upper Olifants Sub-area in the surrounding area of Middelburg and Witbank. As observed, a huge dolomitic intrusion spreads along the Blyde River, curving towards the western part along the northern boundary of the water management area (DWA, 2004).

3.2.2 Economic Importance of study area

The headwaters of these rivers are positioned along the Highveld Ridge in the Secunda-Bethal areas and the rivers then flow in a northerly direction towards the Loskop Dam. The Rivers and streams have been widely dammed with the consequence that the stream flow is now highly regulated. The most important impoundments upstream of the Loskop Dam consist of the Witbank Dam, Middleburg Dam, Bronkhorstspruit Dam and Premier Mine Dam (Table 3.2). Middelburg and Witbank are the prevalent urban concentrations (Heath and Claassen, 1999). Economic activity is highly various and ranges from irrigation, mining, metallurgic industries, dry land and subsistence agriculture, to eco–tourism.

Nevertheless the main economic activity is focused in the mining and industrial centres of Witbank/ Middelburg, Phalaborwa and Steelpoort where a variety of minerals are found. To this day, coal mining is the predominant activity, with platinum and other precious metals of growing economic importance.

A number of the largest thermal power stations in the world are located in the Upper Olifants. Wide-ranging irrigation happens in the neighbourhood of Loskop Dam, beside the lower reaches of the Olifants River, near the confluence of the Blyde and Olifants Rivers as well as in the Steelpoort valley and upper Selati catchment.

Loskop Dam was built in 1939, 48 km north of Middelburg and raised in 1977 by 9.1 m. The total catchment area for the dam is estimated to be 12 261 km^2 (SANCOLD, 1978). To a large extent, the total catchment incorporates the most industrialised region of the Olifants River basin and the Loskop Dam is the biggest storage unit in the Olifants River catchment (James and van Wyk, 1993). Rainfall occurs mainly in the summer months; with January experiencing the heaviest rain. The predominant land use is for agriculture purposes (DWAF, 2004b) and 90% of the water available from the Loskop Dam is used for extensive irrigation (James and Van Wyk, 1993).

The majority of the central and north western areas of the WMA are largely undeveloped, with scattered rural settlements. Land use in the WMA is characterised by rain-fed cultivation in the southern and north-western parts, with cotton and grain as main products.

It is well documented that the Olifants River is one of the main rivers which flows through the Kruger National Park and owing to the location of the park at the downstream extremity of the water management area, provision of water for meeting the ecological requirements is one of the controlling factors for managing water resources through the water management area. Water

resources (rivers and wetlands) in the Olifants WMA provide a diversity of ecosystem services such as domestic water use, grazing and livestock watering, cleaning of harvested products tourism, recreation, aesthetic value, education and flood attenuation.

3.2.3 Surface water bodies in the vicinity

The main tributaries are the Wilge, Elands and Ga-Selati Rivers on the left bank and the Steelpoort, Blyde, Klaserie and Timbavati Rivers on the right bank. The Olifants River is one of the highly regulated rivers in the country, with several major dams constructed to supply water for different uses within the WM A see Table 3.2.

Table 3.2: Major rivers and dams in Olifants WMA

Dams in Olifants WMA	**Rivers**
Blyderivierpoort Dam	Blyde River
Bronkhorstspruit Dam	Bronkhorstspruit River
Buffelskloof Dam	Waterval River
Flag Boshielo Dam	Olifants River
Klaserie Dam	Klaserie River
Loskop Dam	Olifants River
Middelburg Dam	Little Olifants River
Ohrigstad Dam	Ohrigstad River
Rhenosterkop Dam	Elands River
Rust de Winter Dam	Elands River
Tonteldoos Dam	Tonteldoos River
Tours Dam	Ngwabitsi River
Vlugkraal Dam	Vlugkraal River
Witbank Dam	Olifants River

3.2.4 Other Developments

Usually, a number of large dams control most surface runoff that is emanating from the mountainous areas and higher precipitation southern (Table 3.3).

Table 3.3: Sampling sites Names, WMS id and GPS coordinates

Sample Point	**WMS Feature ID**	**GPS coordinates**
Oxford	90503	S 24.184 E 30.824
Ga-selati at foskor	90518	S 24.0342 E31.124
Waterval	188223	S 25.579 E 29.128
Middelburg Dam	90414	S 25.773 E 29.544
Wolvekrans	90410	S 26.0064 E 29.254

Source: (NTM, 2014)

The most likely options acknowledged for extra enlargement of surface water resources are raising the flag of Boshielo Dam, the building of a dam on the Steelpoort River and a new dam at Rooipoort on the middle Olifants River. Huge amounts of groundwater are abstracted for rural water supplies throughout most of the WMA as well as for irrigation in the north-western parts of the water management area; increased groundwater utilisation has been acknowledged on the Nebo Plateau.

In addition, extensive water is transferred into the water management area as cooling water for power generation, with smaller transfers to neighbouring WMA.

The case-studies for population growth display a minor rise in population for the rural areas beyond year 2025. Population and economic development are projected to be centred on the industrial and major mining centres of Phalaborwa, Witbank and Middleburg, likewise in the new mining expansions forecast along the eastern limb of the Bushveld Igneous Complex in the Mogoto / Steelpoort area.

In the Mokopane area (Limpopo WMA), water for mining may also be provided from the Olifants River. Moreover, it is also projected that water requirements for power generation in the upper Olifants sub - area will grow (DWS, 2015).

3.3 SELECTION OF SAMPLING SITES

In order, to reduce the cost related to water monitoring, sample collection and analysis, a desk top study was done through the Department of Water and Sanitation, as well as the National Chemical Monitoring Programme (NCMP) sites on the Olifants River. These points were streamed lined according to the National Toxicity Monitoring Programme (NTMP) implementation manual. It was also ensuring that the sites are easily accessible, that the health and safety of the samplers were not at risk. The initial 13 points selected from the NCMP are representative of the land use activities in different zones.

3.3.1 Sampling sites

Water samples were collected from the detected five sampling sites along the Olifants River catchment based on the identification of toxicity hotspots with (*Vibrio fischeri*) field kits under the National toxicity monitoring programme (NTMP). The monitoring point's names, with sampling Id and GPS coordinates were shown earlier in Table 3.3 and Figure 3.1.

3.3.2 Sampling and Sampling techniques

The fundamental ethics of sampling are established upon the extrapolation of part of the sample population to get a representative sample. The population can be described as the whole material whose properties are being inspected and a sample being a fraction of the population carefully chosen for analysis (Bartam and Ballace, 1996). It was vital to find illustrative volumes of water from the sampling points, meanwhile the organic analytical process could not be carried out on the spot; the samples were gathered by a sub-surface grab method which is one of the easiest sampling methods.

3.3.3 Sampling apparatus

A cooler box with ice, a water resistant waders and two litre glass amber bottles with caps were the standard sampling apparatus used.

3.3.4 Sampling procedure

- Grab water samples were collected in 2.5 L pre-cleaned amber Winchester glass containers from 5 detected sampling points to where the water appeared well mixed.
- Prior to sampling, the containers and cap were first cleaned twice with water from stream afterwards absorbed to about 30 centimetres underneath the surface of the tributary at a 45 degree angle to the course of the flow.
- In a cooler box with ice, samples were then conveyed to the laboratory where they were kept in a cold room at 4°C until they were analysed. All samples had to be completely analysed within 30 days of collection.

3.3.5 Sampling frequency

Surface water samples were collected every month during winter season between June and August 2015 and during summer season between September and December 2015 from the selected five points and stored at a temperature of $\leq 5\ ^{o}C$. In situ readings were taken at the spot where water samples were collected with their Global Positioning System (GPS). 60 samples were collected between January and December 2015.

3.4 METHOD DEVELOPMENT

Persistent organic pollutants (POPs) in some instances are present at trace levels in environmental media; as a result, it is necessary to develop optimum extraction and instrumental methods for trace level analytical determinations. The effective determination of environmental

POPs requires the development of a proper analytical method, which should be reliable, fast, and sensitive enough for low levels determinations. In other to detect and quantify this compound in nano and pico-gram levels, the GC instruments need to be optimised. A test of solvents including dichloromethane (DCM), toluene, hexane, acetone and their combinations in different ratios were evaluated.

3.4.1 Preparation of stock solutions

The preparation of stock standard solutions of 100 mg/L were done by evaluating 10 mg of pure standard material into a weighing boat prior to shifting to a 100 mL volumetric container and topping up to the mark with toluene and prudently liquefying by making use of the vortex mixer. Thereafter, 1 mg/L cocktail solution was prepared by adding 1 mL of each of the 18 pure OCP solutions into a 100 mL volumetric container and topping up to the mark with toluene and mixing well by shaking the volumetric container. The calibration standards were made up by serial dilution from the one mg/L cocktail solution to produce nine calibration level standards.

3.4.2 Development of chromatographic conditions (GC-MS)

The mixed Selected Organic Compounds (SOCs) standard was analysed on Shimadzu GC-MS (model 2010). The above model plus gas chromatograph attached with a model QP 2010 ultra-mass spectrometer (Shimadzu, Japan) was injected spontaneously by a Shimadzu AOC-20i auto sampler using electron ionisation. The selected ion-monitoring (SIM) was the operational mode for each compound, the identification of analyte peaks was undertaken using the SIM mode by monitoring the molecular ion (quantifier) and two qualifier ions. The quantification was based on external standard calibration. In the present study the optimisation of the GC-MS was conducted based on the variation of the following:

- Carrier gas and flow rate
- Injector temperature
- Oven temperature
- Column type

3.4.2.1 Carrier gas

Helium was selected and applied as the carrier gas owing to the fact that it shows a flat baseline profile and has also been found to improve the separation of low boiling compounds. It also allows more interaction with the stationary phase. Hence 99.999 % pure helium gas was used as the carrier gas, which was supplied by Afrox gas South Africa. Since there is a relationship

between the average velocities of the carrier gas, its flow rate and the oven temperature programme, several carrier gas flow rates with varied oven programmes were tried so as to achieve better peak resolution and a relatively fast run time as presented in Table 3.4. Carrier gas flow rate of 1.5 ml min^{-1} was found adequate for the GC-MS.

Table 3.4: Ramped oven temperature programmes optimised for GC-MS

Oven Temp Programme	line Temp (°C)	Injector &Transfer Temp (°C)	Ion source flow ($mLmin^{-1}$)	Carrier gas time (min)	Analysis
80°C (2min) to 180°C 10°C,to 300°C (2min)	@200 / 250 @30 °C	300	1	18	
80°C (1min) to 180°C To 250°C (2min)	@ 30°C, @100°C	250/300	250	3	7.03
***100°C (1min) to 160°C $min^{-1}$to 300 °C @ 25°C $min^{-1}$to 325@ 10°C min^{-1}(3min**	**@ 15°C**	**270 / 300**	**250**	**1.5**	**13.17**

*****Optimised instrumental condition

3.4.2.2 Injection temperature

The injector port of the gas chromatograph has to be hot enough so as to ensure adequate and rapid volatilisation of the analytes in the dissolved solvent. Therefore, different temperatures were also tried; hence a temperature of 270 °C was used and found to be hot enough for rapid volatilisation of the Organic compounds investigated.

3.4.2.3 Oven temperature

Some oven temperature programmes were tried and tested in an attempt to obtain good peak resolution of the standards with relatively fast analysis time as indicated in Table 3.4. It was revealed that direct oven heating (isothermal) of the column did not allow satisfactory interaction of the analytes with the stationary phase of the column, leading to poor resolution of the mixture of the Selected Organic Compounds (SOCs) standards within the run time.

3.4.2.4 Column type

Different capillary columns were also tried and tested for their efficiencies of separation. The tested columns included 100% methyl-polysiloxane type (DB1), 30 m x 0.25 mm x 0.25 µm , ZB-5 Capillary column 5% phenyl and 95% dimethylpolysiloxane (30 m x 0.25 mm x 0.25 µm) and DB-5 Capillary column 5% phenyl and 95% dimethylpolysiloxane (30m x 0.25mm, 0.25 µm) was used for separation.

3.5. METHOD VALIDATION

According to ISO 9000 standard series, the method validation can be described as a validation via the provision of objective proof that the requirements for a particular method have been achieved. Validation parameters consist of linearity, linearity verification, Limit of detection (LOD), Limit of Quantification (LOQ), precision, statistical significance testing and trueness. A classic method validation document has to insist on defining the analytical performance requirements, the envisioned use of the method and most essentially deliver unfailing analytical data from validation experiments (Rimayi *et. al*., 2014). In house validations, as contrasting to inter-laboratory analyses have a benefit in that they protect performance parameters such as limits of detection, selectivity, linearity and matrix effects (Rimayi *et. al.,* 2014).

3.5.1 Linearity

Linearity describes the aptitude of the technique to collect test results proportionate to the concentration of the analyte (Rimayi *et. al.,* 2014). Hence, it is advised to produce calibration curves from five upwards calibration points with the utilisation of more than three replicates. The coefficient of regression (*r*) is applied to evaluate the suitability of a calibration curve (Rimayi, *et. al.,* 2014). One of the consequences of applying *r* is its prejudice towards the range of the data. Visual assessment is standard condition to determine whether a calibration curve is non-linear or linear. Statistical test using the null hypothesis is important for the validation of linearity.

3.5.2 Verification of linearity

The competence of calculating the calibration curve linearity as computed by suitable instrumental software regularly needs to be tested. Verification is well-defined as the validation by provision and examination of objective evidence that particular requirements have been satisfied (Cuadros-Rodreguez *et. al.,* 2001).

3.5.3 Precision

In many occasions, precision is a significant parameter of validation and is measured as a function of the true Relative Standard Deviation percentage (RSD %) and is stated as an obligation by most validation guidelines (Stockl *et. al.,* 2009). For a well-defined number of replicates higher than three, a precision of 10 percent higher is considered good. Under the very same operating conditions over a short interval of time, the precision should be conveyed (Rimayi *et. al.,* 2014). Therefore, it is suggested to calculate precision at three different concentrations.

3.5.4 Trueness

Trueness has been described as the difference between a reference quantity value and the average of an infinite number of replicate measured quantity values (Rimayi, *et. al.,* 2014). It is frequently confused by mistake with accuracy which is the difference between the true quantity value of the measured and a measured value. Trueness is best measured via the use of recoveries in GC-MS analysis (Rimayi, *et. al.,* 2014). Evaluating trueness involves estimating separately the proportional bias (in terms of recovery) and the constant bias of the analytical method (Maroto *et. al.,* 2001).

3.5.5 Selectivity

Selectivity is defined as the degree to which an extraction technique can isolate the analyte from intrusions in the original sample (Ferrer and Barcelo, 1999). The sample preparation techniques together with the instrument of analysis were carefully chosen because of the impact on the selectivity of a specific analyte. The method of analysis needs to be enhanced for each specific analyte for efficient selectivity (Cuadros-Rodreguez *et. al.,* 2001).

3.6 CHROMATOGRAPHIC CONDITIONS (GC-MS)

The existence of Selected Organic Compounds (SOCs) in water samples was confirmed using GC-MS. A standard mixture was first injected into the GC-MS to determine the elution times and then the resulting ion peaks. The ion peak profile and the retention time of each standard were then matched to those from the samples. The similar chromatographic conditions that were optimised for the standards were left unchanged for the samples (Cole *et. al.,* 2005), Transfer line temperature was set at 250 ^{o}C, Scan rate: 1 sec^{-1}, Mass defect: 0.0 amu and full scan ion mode (50 - 850).

3.6.1 Peak identification and data assessment

5 ppm well-ordered standards were injected for the determination of the retention time for every single analyte, in order to detect the peaks of interest. Generally in the presence of a matrix, Selective ion monitoring (SIM) mode was configured into the GC-MS, to increase the particularity of the method of analysis.

Meanwhile every single compound has a particular ion spectrum, with allusion to ions from formerly published work, an average of 4 main ion fragments from each analyte were carefully chosen for use in identification of the compounds, using criteria of a balance between the highest mass and abundance (Rimayi *et. al.,* 2014).

Table 3.5: Target and qualifier ions used for SIM analysis

Peak No.	Peak name	(min)	T	Q1	Q2	Q3
1	BHC-alpha	6,631	181	183	217	219
2	Hexachlorobenzene	6,715	284	249	142	214
3	BHC-beta	6,916	181	109	219	217
4	Lindane (BHC gamma)	6,979	181	217	109	219
5	BHC-delta	7,215	183	219	217	109
6	Chloropyriphos-me	7,609				
7	Heptachlor	7,681	272	237	337	135
8	Aldrin	7,992	263	293	66	186
9	Heptachlor-epoxide	8,335	353	237	263	253
10	Endosulfan alpha	8,633	170	241	195	265
11	DDE 4,4'	8,816	246	318	176	316
12	Dieldrin	8,846	263	277	265	108
13	Endrin	9,015	263	245	81	317
14	Endosulfan beta	9,085	195	237	265	159
15	DDD 4,4'	9,141	235	237	165	199
16	Endosulfan-s	9,412	272	229	387	237
17	P,p'-DDT	9,428	235	237	199	165
18	Mirex	10,194	272	274	237	332

T= Target ion; Q= Qualifier ion

3.6.2 Determination of Retention times, standard mixture and limit of detection

3.6.2.1 Retention time determination

The optimal chromatographic conditions were obtained from a series of experiments in Table 3.5. The best instrumental conditions are shown in the same table, of which all selected Organic Compounds (SOCs) and their retention times were determined using those chromatographic conditions. The same oven programme condition was used for the GC-MS.

From the stock standards solution, lower standards were prepared by serial dilution for individual standard as well as the mixture. 1.0 µl of each standard at different concentrations was injected into the GC for essential ideal output.

Lower concentrations (0.3 – 3.0 ng µL-1) of individual and mixtures of standards were prepared from stock solutions of 50 µg /mL by serial dilution. One micro litre of 0.3 – 3.0 ng µL-1 of the individual and standard mixtures was, thereafter, injected into the gas chromatograph using the optimised GC conditions as enumerated and the retention time noted. Retention time was reported as a mean of three determinations (Table 3.5).

3.6.2.2 Determination of instrumental Limits of Detection (LOD)

Scientist like (Rimayi *et. al.,* 2014) stated that the limit of detection can be computed at three times the standard deviation of the blanks or as five percent of the error of identifying the analyte

or low concentration samples when it is not there. The chromatographic LOD can also be determined as the response that gives a signal to noise (S/N) ratio of 3:1 (Rimayi *et. al.,* 2014). The measurement of the LOD using S/N ratio is strongly suggested as it shows the skills of the analytical chemist to optimize the S/N ratio. Lower concentrations (10 to 1) ng µL-1 of each standard was prepared from the working stock solution. 1.0 µL of the diluted standard was injected into the GC to determine the lowest concentration of each Selected Organic Compounds standard that could be detected by the instrument based on the chosen method of analysis. Nine level calibration of mixed standard was carried out with concentration range of (0.006- 1.5) ng µL-1.Limit of detection (LOD) was determined by increasingly lowering the concentration of the mixed standards and taken as 3x signal to noise ratio.

3.6.2.3 Limits of Quantification (LOQ)

The definition of LOQ should be based on principles of trueness, total error or precision. The limit of quantification can be calculated and determine as a function of Relative Standard Deviation (RSD) or as the response that provides a signal to noise (S/N) ratio of 10:1. According to Cuadros-Rodreguez *et. al.,* (2001), most scientists calculate LOQ just as 10 times the standard deviation of the error or as 10% RSD related to detection of the analyte in the blank sample. For this study, the Limit of quantitation (LOQ) was read at 10 x signal to noise ratio. The method was validated by spiking protocol; low concentration of mixed OCP standard was spiked into ultra-pure water and extracted by liquid liquid extraction. The calibration was by external method due to the fact that, it is relatively accurate and reliable when compared to the internal method.

3.6.2.4 External standard calibration method

For the external standard calibration method, samples and the calibration standards were analysed on the developed GC-MS methods without any changes.

3.7 LIQUID -LIQUID EXTRACTION (LLE)

3.7.1 Validation of spiking of deionised water

The validation of the LLE method was carried out by spiking 500mL deionised water with a mixture of OCPs standards and then extracting with 3 times extraction (50 mL for the first time, 30 mL for the second time and 30 mL for the third time) of each of the extracting solvents (dichloromethane, hexane and petroleum ether), before using different combination (1:1) of these solvents. The extracts were mixed, dehydrated with anhydrous sodium sulphate and concentrated to an average of 1.5 mL by means of the rotary evaporator for chromatographic clean-up. The recoveries were thereafter calculated.

The percentage Recoveries of Selected Organic Compounds (SOCs) in spiked deionised water were calculated from the ratio of the amount of (SOCs) recovered from spiked deionised water to the amount added based on the ratio of the peak areas of the standards to that of the spiked solution with the same concentration (Awofolu and Fatoki, 2003).

Figure 3. 2: Liquid-liquid extraction of water samples

Blank extraction of un-spiked deionised water on the other hand was carried out using the DCM extraction and chromatographic clean up method as described before, which gave a clean background. LLE with DCM was chosen for this study because it accomplished good reproducibility and recoveries (Awofolu and Fatoki, 2003; Olukunle *et. al.*, 2012a).

3.7.2 Rotary evaporator

All extracts obtained from LLE were reduced to between 1 and 2 mL in a rotary evaporator (Rota Vapor R-210, BuchiLabortecnik AG, and Switzerland) as seen in Figure 3.3. All extracts were transferred into a round bottom flask and attached to the evaporator then lowered into the water bath. The temperature of the water bath was in tune to about 10 °C below the boiling points of the different solvents used. The extracts were then reduced to about 1mL by switching on the vacuum pump.

Figure 3. 3: Rotary evaporator

3.7.3 Silica Gel Column chromatography

Glass wool was used to separate each layer of materials to enhance the cleaning (Figure 3.4). The Crude sample was introduced into the column before the solvent reached the bed of the sodium sulphate plugged with glass wool. The column was eluted with 4 mL of hexane. The extracts were concentrated to 1.5 µL under the nitrogen using a Reacti-Vap from Thermo Fisher Scientific (Bellefonte P.A, USA) provided by Anatech Pty (Pretoria, South Africa).

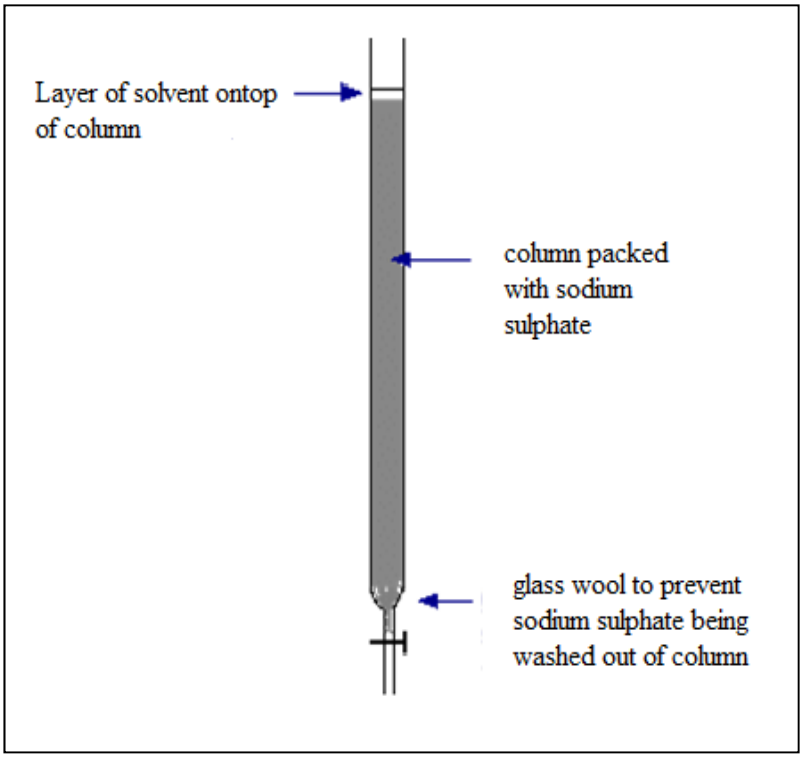


Figure 3. 4: Schematic of clean up by column

3.8 FIELD WATER QUALITY PARAMETER PROFILE

For the purpose of defining the water quality profile along the Olifants River catchment, the following selected water quality parameters: pH, DO, EC and Temperature were measured on the field with a portable YSI instruments. Also field toxicity test was carried out with a toxicity field kit to identify the toxicity hot spots from the list of proposed NTMP points, Figures 3.5 and 3.6 show the YSI and the toxicity field kit used.

Figure 3.5: Toxi-Screening Kit for field Toxicity test

Figure 3. 6 : YSI instrument for onsite water quality measurement

3.9 ANALYSIS OF WATER SAMPLES

500 mL of samples was extracted with DCM as described earlier in LLE method (section 3.7). These extracts were combined, dried and passed through the silica gel column clean up prior to GC-MS.

3.10 STATISTICAL ANALYSIS

Standard deviation and other relevant statistical parameters such as mean, median were calculated using Microsoft Excel while statistical package for social sciences (SPSS) software were used for analysis of variance (ANOVA), correlation and significant difference.

3.11 QUALITY CONTROL/ ASSURANCE

All volumetric containers, pipettes and analytical balance were calibrated before use. Analytical grade reagents were also used for the whole analysis with a purity > 99%. Deionised ultrapure water was obtained from a Millipore Milli-Q system (with organic compound scavenger resin bed). The certified pesticide neat standards had a purity of more or less 98.5% (collected from Chemservice and Dr Ehrenstorfer) and 100 mg/L stock solution and subsequent cocktails were prepared in toluene and kept at $\leq$ -18°C. Spiking solutions were prepared in acetone. Temperatures for the laboratory atmosphere and freezers were scrutinised daily. At the beginning of every sample analysis and after, an initial solvent blank and a laboratory performance standard check were performed using the mixtures of the OCs). This is to ensure stable performance of the GC-MS via; detector sensitivity, peak symmetry and resolution. The spiking method was used in the quality assurance process of analytical method due to unavailability of certified reference material (CRM) for target compounds in water.100 ml of deionised water was spiked with standard mixture (same concentration used for method development) before it was then passed through the same analytical process described in section 3.7 above.

Numerous quality assurance measures were also regularly used in this study, included running check standards and use of surrogate standards during extraction and after clean-up was done to ensure accuracy. Retention times matched those of the standards and quantification was done by monitoring the molecular and reference ions. The limit of detection was taken as 3 times the signal to noise ratio and limit of quantification as 10 times signal to noise ratio for the lowest calibration standard.

To further improve the quality assurance, all analysis was in triplicate, blank extraction of un-spiked and blank sample was run along with each set of analysis to prevent the memory effect on the instrument.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 INTRODUCTION

This chapter presents the results of the analysis of OCPs in water sample collected during winter and summer period in 2015. The pollution of organochlorine pesticides (OCPs) from the selected 5 sites in the Olifants river catchment system was explored to estimate the present-day status of pollution in surface water with a total of 18 OCPs.

The results of the field water quality parameter collected from five selected sites along the Olifants River between January and December, 2015 are shown in Table 4.1 to 4.5.

4.1.1 pH

The monthly pH values along Olifants River in 2015 for the selected five points ranged between 6.87 (at Waterval on Wilge River) in October and 8.6 (at Oxford) in June. The pH values observed at all the points is within the normal range for pH (6.5 to 8.5) in surface water system according to the 1996 South African water quality guidelines, with the exception of the pH (8.51) at Foskor sites in December (Table 4.1). The pH mainly influences corrosivity, solubility and speciation of metals. This effect is of special significance since the toxicity of most compounds is affected by their level of dissociation.

Table 4.1: Monthly pH result for the selected points on Olifants WMA

WMS ID	APRIL	MAY	JUNE	JULY	AUG	SEP	OCT	DEC	Mean
Wolvekrans	7.44	7.63	7.2	7.88	7.66	7.21	7.34	7.27	7.45
Middelburg	7.32	7.41	7.10	7.53	7.83	7.14	7.48	7.83	7.46
Waterval	7.29	7.34	7.1	7.22	7.24	7.63	**6.87**	7.45	7.27
Oxford	7.91	8.31	**8.6**	7.96	8.04	7.78	8.23	8.1	8.12
Foskor	7.1	8.23	7.9	8.1	7.73	7.85	8.37	**8.51**	7.97

4.1.2 Dissolved Oxygen (DO)

Table 4.2 below, shows the monthly dissolved oxygen (mg/l), values along Olifants river in 2015 and these values ranged between 5.99 mg/l in May (at Wolvekrans **)** and 11.67 mg/L in July (at Middelburg Dam). The normal range for DO in surface water is usually from 6 to 14 mg/l. But different aquatic species at various life stages and water temperature need different levels of dissolved oxygen which ranges from 5 to 9.5 mg/l. For warm-water biota the minimum dissolved

oxygen concentration is 5 - 6 mg/ l and 6.5-9.5mg/ l for cold-water biota. More details are given in Alabaster and Lloyd (1982).

Low concentrations of dissolved oxygen, when mixed with the occurrence of poisonous substances may be responsible for stress responses in aquatic ecosystems because the toxicity of certain elements, such as copper, lead and zinc, is raised by low concentrations of dissolved oxygen.

Table 4.2: Monthly DO (mg/l) result for the selected points on Olifants WMA

WMS ID	APRIL	MAY	JUNE	JULY	AUG	SEP	OCT	DEC	Mean
Wolvekrans	7.8	**5.99**	9.2	11.44	11.39	8.5	7.9	6.11	8.54
Middelburg	8.9	10.6	9.65	**11.67**	9.86	10.5	10.2	7.5	9.86
Waterval	10.5	9.6	9.8	12.86	7.9	7	9	9.09	9.47
Oxford	7.8	6.24	6.89	8.65	8.17	8	7.52	6.54	7.48
Foskor	9.68	9.23	9.3	10.08	9.28	8.57	9.89	9.57	9.45

4.1.3 Electrical conductivity (EC)

Electrical conductivity (EC) is a valuable indicator of the mineralisation in a water sample. It associates with the total dissolved solids (TDS) of that sample. Table 4.3, shows the monthly electrical conductivity (mg/l), values along Olifants River in 2015 and these values ranged between 13.8 in May (at downstream Middelburg Dam) and 222.7 mg/l in June (on Ga-Selati river at Foskor). The normal range for EC in surface water according to 1996 South Africa guideline for domestic use is 70 mS/m but health effects of EC happen only at levels beyond 370 mS/m. The effects of high EC may contain disturbances of water balance and salt.

Table 4.3: Monthly EC (mS/m) result for the selected points on Olifants WMA

WMS ID	APRIL	MAY	JUNE	JULY	AUG	SEP	OCT	DEC	Mean
Wolvekrans	45.3	54.8	63.2	73.6	72.2	73.0	44.1	69.0	61.90
Middelburg	35.6	**13.8**	91.57	41.7	46.0	47.0	46.6	49.1	46.42
Waterval	23.6	69.01	42.3	34.7	35.4	35.8	31.4	32.6	38.10
Oxford	27.3	36.8	38.3	47.7	55.7	58.2	56.8	47.0	45.98
Foskor	167.9	186.6	**222.7**	149.9	201.4	101.2	194.2	181.4	175.66

4.1.4 Temperature

The temperatures at which physicochemical measurements are made and at which sample is collected, are key for data interpretation and correlation purposes. For domestic use high temperature may increase the toxicity of many substances such as organic compounds and heavy metals in waters. Table 4.3, shows Monthly temperature between January and December, 2015 for the selected points on Olifants River and the values ranges between 8.9 ^{O}C (Middelburg in June) and 29.45 ^{O}C **(**Ga-Selati River at Foskor in December) for winter and summer respectively.

Table 4.4: Monthly Temperature (ºC) result for the selected points on Olifants WMA

WMS ID	APRIL	MAY	JUNE	JULY	AUG	SEP	OCT	DEC	Mean
Wolvekrans	15.3	14.1	9.0	9.46	13.01	18.5	18.39	20.5	14.78
Middelburg	16.9	15.6	**8.9**	10.31	15.18	15.48	18.28	24.71	15.67
Waterval	18.3	11.3	9.1	9.65	13.45	21.44	20.94	23.24	15.93
Oxford	18.6	19.58	15.2	16.93	20.3	25.32	26.64	28.26	21.35
Foskor	19.8	21.41	14.9	16.17	18.79	23.72	27.52	**29.45**	21.47

4.1.5 Percentage Toxicity

The Table 4.5 below shows the identified monthly toxicity hotspot on Olifants River with the aid of *Vibrio fischeri* Toxicity field kit. All the sites show toxicity response from 3 to 6 months out of the 8 months tested in 2015. The toxicity response observed ranged from 21.3% to 64.7% (Middelburg in October and Oxford in August) respectively. For this period the order of toxicity is Ga-Selati river at Foskor >Oxford>Waterval>Wolvekrans>Middelburg respectively.

Table 4.5: Monthly %Toxicity result for the selected points on Olifants WMA

WMS ID	**APRIL**	**MAY**	**JUNE**	**JULY**	**AUG**	**SEP**	**OCT**	**DEC**	**Mean**
Wolvekrans	4.2	2	4.2	6.4	32.9	1.74	24.7	36.43	14.07
Middelburg	17.4	22.7	17.4	12.1	3	34.4	**21.3**	2.9	16.40
Waterval	21.5	42.5	21.5	0.4	8.9	17.5	45	31.86	23.65
Oxford	24.8	22.5	24.8	27.1	**64.7**	19.4	10.8	21.87	27.00
Foskor	37.7	34.8	37.7	40.5	51.9	36.47	14.4	5.8	32.41

20% toxicity response = limit of detection

4.1.6 Statistical analysis of Field Water Quality Parameter Profile

The profile of water quality parameters from selected sites on Olifants showed that Ga-Selati river at Foskor had the highest EC, %Toxicity and Temperature values and Waterval is the lowest only for EC and Wolvekrans is the lowest for %Toxicity and Temperature values as illustrated in Figures 4.1. This high value is understandable for Ga-Selati River, due to the fact that is located very close to the Foskor mine as well as other mines around Phalaborwa, in Mpumalanga province.

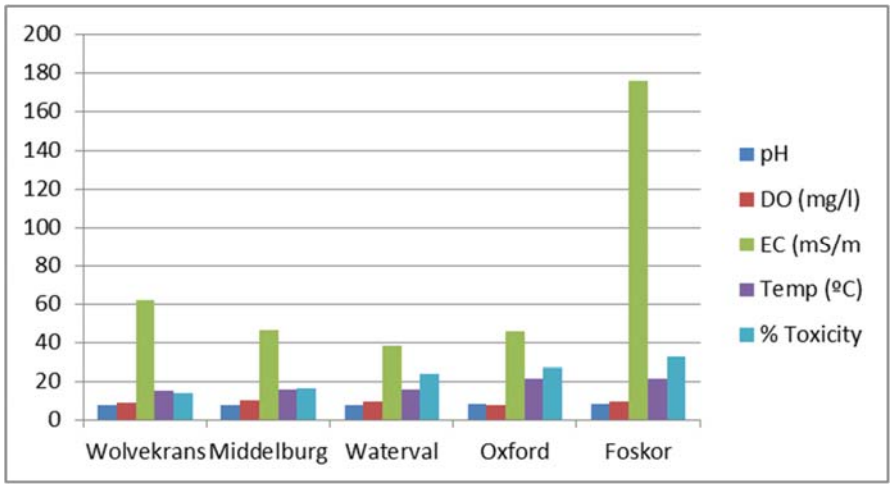


Figure 4. 1: Mean Monthly Water Quality Parameter for Olifants River

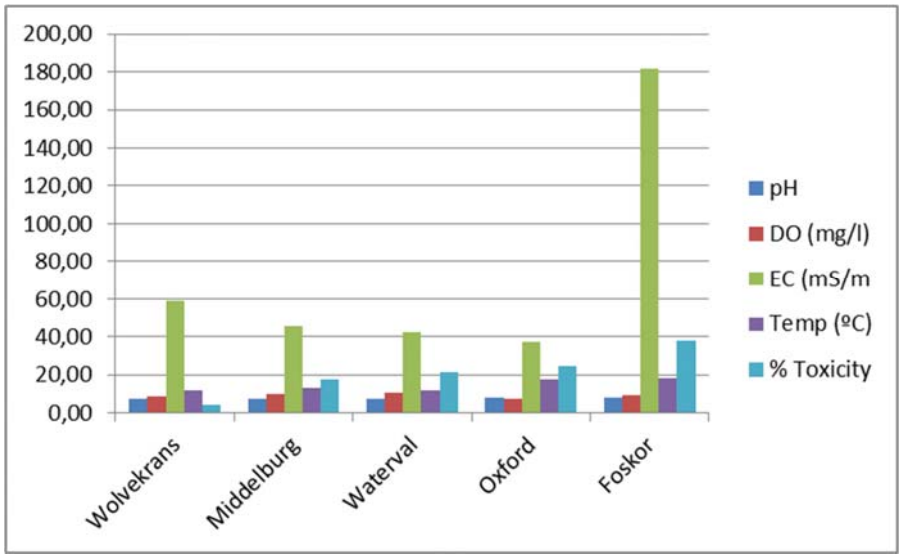


Figure 4. 2: Mean Monthly Water Quality Parameter (Winter 2015)

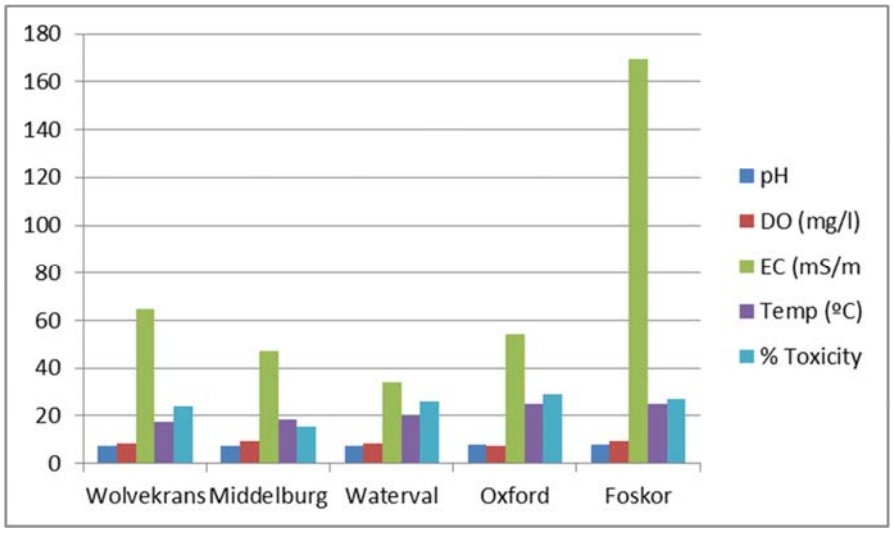


Figure 4.3: Mean Monthly Water Quality Parameters (Summer 2015)

4.2 OPTIMIZATION OF THE GC CONDITIONS

The GC-MS is a technique of choice for routine analysis of environmental samples because it can be used to separate volatile organic compounds and semi volatile organic compounds. Gas chromatography is a split-up method in which the components of a sample partition between two phases: a) the stationary phase and b) the mobile phase (Piatanida and Barron, 2014).The heated and vaporised sample enters the gas stream and is carried out by the gas (N2 or He) mobile phase into the capillary column (stationary phase) where the separation takes place. The detector (e.g. mass spectrometer) measures the quantity of components exiting the column. In this study, GC-MS was applied to the analysis of Organic pesticides in environmental matrices.

The optimisation of the GC conditions is a very important phase in chromatographic analysis. A good optimisation of the GC was reached in terms of relatively high sensitivity, fast analysis and good separations of the Selected Organic Compounds peaks. Helium has been widely used as carrier gas for Organic Compounds (OCs) analysis (Rimayi *et. al.,* 2014); it was set up to be appropriate as a carrier gas in this analysis. Several gas flow rates in conjunction with other instrumental parameters were optimised and a flow rate of 1.5 mL /min was found suitable for the GC-MS system as presented in Table 3.4 (Chapter 3).

Any further attempts to reduce the analysis time proved futile as the resolution of the peaks became poor and this proved problematic for identification purposes. The gas chromatograms of the mixture of the Selected Organic Compounds standard on GC-MS are shown in Figure 4.4.

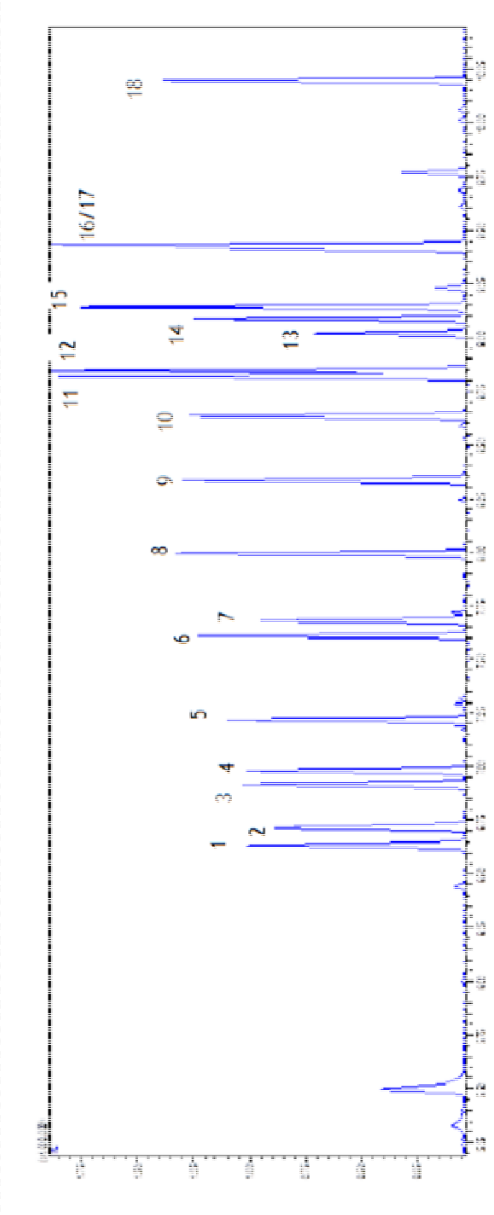


Figure 4. 4: SIM Chromatogram of a (1.5 ng/µL) organochlorine cocktail

(1) BHC-alpha (2) Hexachlorobenzene (3) BHC-beta (4) BHC-gamma (5) BHC-delta (6) Chloropyriphos (7) Heptachlor (8) Aldrin (9)Heptachlor- epox (10) Alpha-Endo (11) pp'-DDE (12) Dieldrin (13) Endrin (14) Endosulfan-b (15) 4,4'-DDD (16) Endosulfan-s (17) pp'-DDT and (18) Mirex

As can be seen from the figure above, the organochlorine compounds were well resolved, the fragmentation and elution patterns observed in both standards and samples are similar to those reported by (Brittain, 2004; Cooper *et. al.*, 2001 and Ackerman *et. al.*, 2005).

4.2.1 Retention times, limits of detection and quantification of standard mixture

The results of retention time (min.) observed from GC-MS for Organic Compounds of interest as presented in Table 4.6, ranged from 6.631 (BHC-alpha) to 10,194 (Mirex) respectively. The BHC-alpha standard eluted first: this might be due to its lower molecular weight (200 – 400) g mol^{-1} and melting point (79 – 82 ^{o}C) compared to other analysed standard. From the two capillary columns that were evaluated, DB-5 Capillary column 5 % phenyl and 95 % dimethylpolysiloxane (30 m x 0.25 mm x 0.25 μm) gave a better result in terms of peak resolution for all the compounds. Related performance by similar capillary column has been reported (Rimayi, *et. al.,* 2014). The instrumental limit of detection (LOD) of the OCs was obtained as the lowest concentration of the analyte that the instrument can identify under the optimised instrumental conditions. The signal to noise (S/N) ratio was utilised to obtain limit of detection (LOD), this was evaluated as three times the blank value for the instrument used. The LOD ranged from (0.006 to 0.2) ng L^{-1} on GC-MS as presented in Table 4.6. The LOD was achieved with analysis time of less than 11 mins for the 18 OCs standards listed in Table 3.5 (Chapter 3).

Table 4.6: Retention time determination, limit of detection and quantification

Name	t_R 1	t_R 2	t_R 3	**LOD**	**LOQ**
BHC-alpha	6,631	6,631	6,631		
Hexachlorobenzene	6,715	6,715	6,715	0.006	0.05
BHC-beta	6,916	6,916	6,916	0.006	0.05
BHC-gamma	6,979	6,979	6,979	0.006	0.19
BHC-delta	7,215	7,215	7,215	0.006	0.05
Chloropyriphos-me	7,609	7,609	7,609	0.006	0.02
Heptachlor	7,681	7,681	7,681	0.006	0.02
Aldrin	7,992	7,992	7,992	0.01	0.09
Heptachlor- e	8,335	8,335	8,335	0.01	0.02
Alpha-Endo	8,633	8,633	8,633	0.02	0.19
P,p'-DDE	8,816	8,816	8,816	0.01	0.05
Dieldrin	8,846	8,846	8,846	0.01	0.05
Endrin	9,015	9,015	9,015	0.01	0.05
Endosulfan-b	9,085	9,085	9,085	0.01	0.19
4,4'-DDD	9,141	9,141	9,141	0.01	0.09
Endosulfan-s	9,412	9,412	9,412	0.006	0.02
P,p'-DDT	9,428	9,428	9,428	0.01	0.05
Mirex	10,194	10,194	10,194	0.006	0.02

4.3 METHOD VALIDATION

4.3.1 Mean percentage recovery of OCPs in water

Generally, it is observed that the yield of any extraction procedure is determined by several factors such as: the solubility of analytes in the extraction mixture, the accessibility of the extraction solvent to the matrix and the extraction time. For this reason, the efficiency of the extraction of the selected OCs was investigated with DCM. Also, about 60 µL of 3 ng/ µL OCPs standard mixture was dissolved in 5mL Dichloromethane (DCM) then spiked into 300 mL ultra-pure water and left for 24 h for equilibration (Olukunle, 2016).

The mixture was, thereafter, extracted by liquid-liquid extraction and final extracts concentrated to 1.5 uL. As indicated before, Dichloromethane gave the best recovery for most of the target analytes in water. Each sample was 3 times extracted with (50 mL for the first time, 30 mL for the second time and 30 mL for the third time with DCM). The mean percentage recoveries are shown in Table 4.7and the chromatogram of the spiked water in Figure 4.5.

Table 4.7: The mean percentage recoveries (Concentration in ng μL-1)

	Recovery experiments						
	Compounds names	C1	C2	C3	Mean	SD	% Recovery
1	BHC-alpha	0.58	0.53	0.5	0.54	0.04	89
2	Hexachlorobenzene	0.48	0.45	0.44	0.46	0.02	76
3	BHC-beta	0.54	0.51	0.47	0.51	0.04	84
4	BHC-gamma	0.54	0.51	0.47	0.51	0.04	84
5	BHC-delta	0.55	0.51	0.48	0.51	0.04	86
6	Chloropyriphos-methyl	0.53	0.48	0.46	0.49	0.04	82
7	Heptachlor	0.57	0.36	0.31	0.41	0.14	69
8	Aldrin	0.55	0.54	0.51	0.53	0.02	89
9	Heptachlor epoxide	0.57	0.54	0.57	0.56	0.02	93
10	.alpha.-Endosulfan	0.53	0.51	0.52	0.52	0.01	87
11	p,p'-DDE	0.45	0.43	0.44	0.44	0.01	73
12	Dieldrin	0.54	0.51	0.52	0.52	0.02	87
13	Endrin	0	0	0	0.00	0.00	0
14	Endosulfan-beta	0.51	0.51	0.51	0.51	0.00	85
15	4,4'-DDD	0.7	0.67	0.71	0.69	0.02	116
16	Endosulfan sulfate	0.5	0.44	0.44	0.46	0.03	77
17	p,p'-DDT	0.23	0.2	0.15	0.19	0.04	32
18	Mirex	0.26	0.25	0.24	0.25	0.01	42

C1,C2 and C3= recovered concentrations, EC= Expected concentration (0.6ppm)

$$\% \text{ Recovery} = \frac{\text{Recovered concentration}}{\text{Expected concentrations}} \mathbf{X}\ 100$$

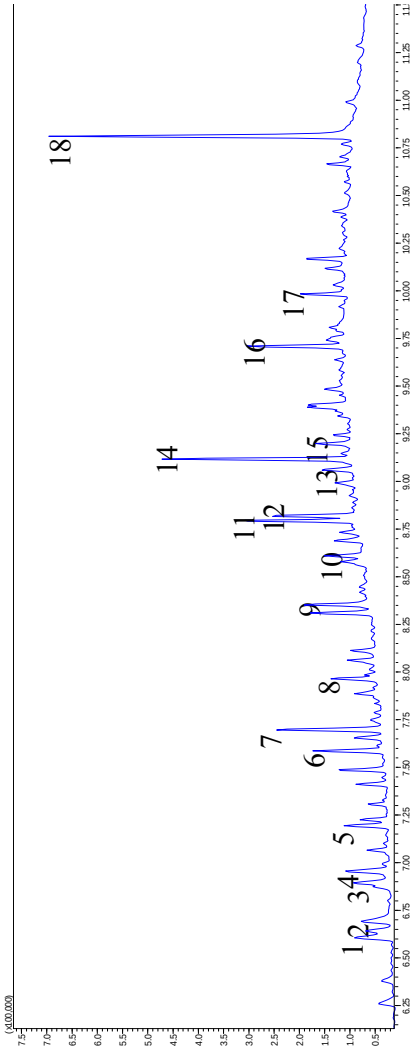


Figure 4. 5: The chromatogram of the spiked ultra-pure water with OCPs

(1) BHC-alpha (2) Hexachlorobenzene (3) BHC-beta (4) BHC-gamma (5) BHC-delta (6) Chloropyriphos (7) Heptachlor (8) Aldrin (9)Heptachlor- epox (10) Alpha-Endo (11) pp'-DDE (12) Dieldrin (13) Endrin (14) Endosulfan-b (15) 4,4'-DDD (16) Endosulfan-s (17) pp'-DDT and (18) Mirex.

4.3.2 GC-MS instrument method validation

In simple terms, Method validation is indispensable as it shows that the method of analysis is accurate in calculating the parameters it is projected to measure. Hence, effective validation of this instrument method validation shows that the methods, protocols and procedures used in the analysis produce effective and reliable data and also make sure that valid assumptions are made as the outcome of the method of validation.

4.3.3 Validation parameters

For the aims of affirmative method validation, the parameters tested were working range, repeatability, linearity verification by Excel, linearity, limits of detection, reproducibility and limits of quantification.

4.3.4 Linearity

Nine independent calibration curves were prepared to validate the linearity of individual analyte analytes (four examples are shown in Figures 4.6 and 4.7; others are in Appendix I). The results are shown below:

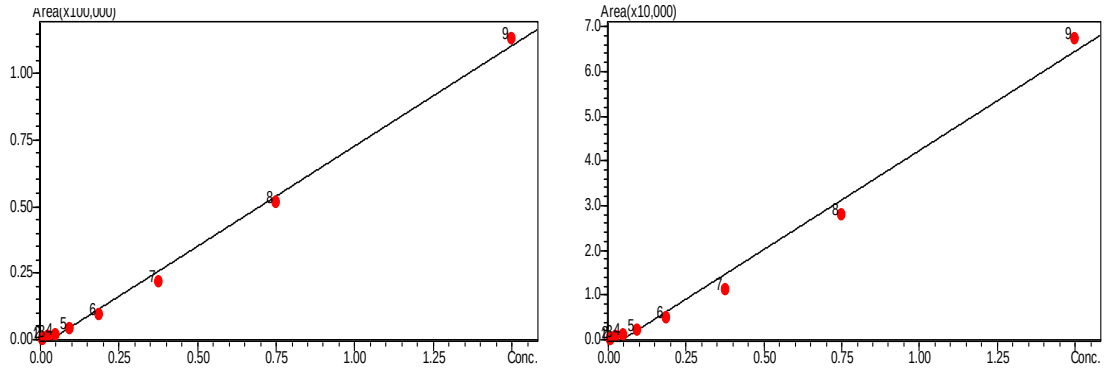


Figure 4. 6: Calibration curve for Chlorpyriphos and Heptachlor

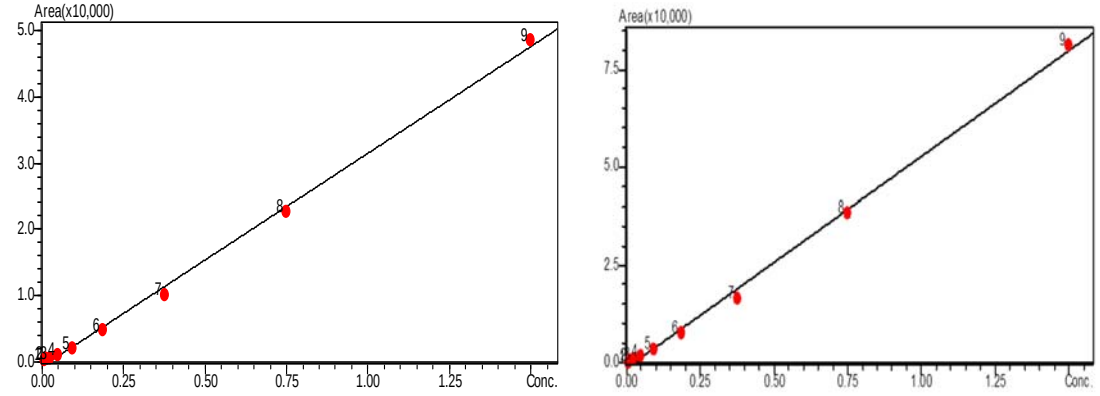


Figure 4. 7: Calibration curve for Hexachlorobenzene and BHC-Alpha

4.3.5 Calibration range

The calibration ranges for the selected organochlorine compounds were verified using 1.5 ppm, 07.5 ppm, 0.375 ppm, 0.1875 ppm, 0.09375 ppm, 0.04688 ppm, 0.02344 ppm, 0.01172 and 0.006 ppm calibration standards. The 0.003 ppm standard was then rejected to distinguish between the analyte peaks and background (noise) peaks for most compounds at this

concentration in order to determine the LOD. Figure 4.7 shows that most of the compounds had good linear ranges with the exception of p,p'-DDT and Heptachlor.

4.3.6 Precision

Precision is defined by the degree of repeatability allowing the measurement of the method of analysis under normal operation. For the purpose of reference, precision was classified into reproducibility and repeatability.

4.3.7 Repeatability

Repeatability was calculated as a fraction of percentage relative standard deviation (%RSD). A 1 ppm standard was analysed 10 times for the determination of the percentage relative standard deviation. Endosulphan beta and Hexachlorobenzene showed the highest degree of repeatability with % RSD values of 2.65% and 2.75% respectively.

$$\% \text{ RSD} = \frac{\text{Standard deviation}}{\text{Mean}} \mathbf{X}\ 100$$

As a quality control procedure, % RSD of less than 10% is thought through to be acceptable. Thus, all analytes investigated revealed a percentage RSD of less than 10%. test

4.4 ANALYSIS OF WATER SAMPLES

4.4.1 Levels of OCPs in water samples

Identification of organic compounds in environmental water samples was completed by comparison of the retention times of the organic compounds in sample extracts with those of the organic compounds individual standards. However, no attempt was made to identify them due to non-availability of individual standard and is out of the scope of this project.

4.4.2 Monthly variability of OCPs in water samples along Olifants River

Tables 4.7 and 4.8, show monthly Aldrin and BHC- gamma between June and December respectively, for the selected points on Olifants River and the values for Aldrin ranged between 5.8 ng/L (Middleburg in September) and 834.20 ng/L (Oxford in July) in winter; while values for BHC-gamma ranged between 8.7 ng/L (Middleburg in September) and 560 ng/L (Waterval in November) in summer.

Table 4.8: Monthly Aldrin in water samples

	Aldrin Concentration (ng/L)						
WMS ID	**June**	**July**	**August**	**September**	**October**	**November**	**December**
Wolvekrans	0	0	10.47	7.2	0	0	20
Middelburg	22.56	0	5.23	**5.8**	9.92	50	20
Waterval	8.52	5.65	6.88	0	0	0	0
Oxford	9.06	**834.20**	7.59	5.76	6.36	0	10
Foskor	17.64	7.25	9.22	0	39.06	0	10

Table 4.9: Monthly BHC-gamma in water samples

	BHC-gamma Concentration (ng/L)						
WMS ID	**June**	**July**	**August**	**September**	**October**	**November**	**December**
Wolvekrans	0	0	14.96	9.6	0	15	0
Middelburg	9.87	10.12	9.41	**8.7**	0	0	0
Waterval	12.78	0	14.61	0	0	**560**	0
Oxford	9.06	48.16	10.62	0	0	0	0
Foskor	33.32	0	0	0	11.16	0	0

4.4.3 Seasonal variability of OCPs in water samples along Olifants River

The mean seasonal concentrations of selected organochlorine found at the five investigated sites along Olifants River are shown in Figures 4.8 and 4.9. The seasonal mean results from this investigation show that in winter the Olifants River is mostly polluted at Oxford (with BHC-beta, Aldrin, Heptachlor-epoxide, Endosulfan-alpha and Endrin) indicating Aldrin up to 834.20 ng/L in July which is the highest hazard toward the aquatic environment, at Ga-Selati with (Heptachlor-epoxide and Endrin) and at Wolvekrans with (Endosulfan-alpha). The concentrations of these compounds are generally found to be above 50 ng/ L for Oxford, Ga-Selati and Wolvekrans. The seasonal mean concentration of Organochlorine compounds found in water samples from Middleburg and Waterval are generally very low, with the exception of Heptachlor-epoxide and Endrin respectively as shown in Figure 4.8.

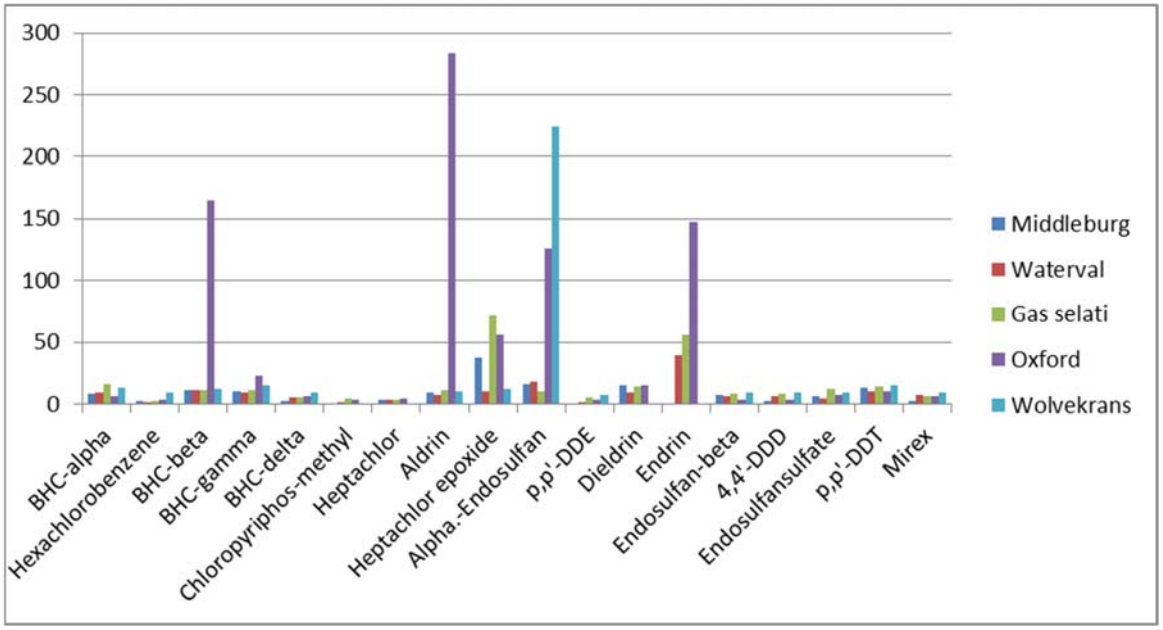


Figure 4.8: Mean concentrations (ng /L) of measured compounds in winter

The results from this investigation show that in summer the Olifants River is mostly polluted at Waterval (with BHC-gamma and heptachlor) indicating BHC- gamma up to 560 ng /L in November which is the highest hazard toward the aquatic environment and at Ga-selati with BHC-beta.

The seasonal mean concentration of Organochlorine compounds found in water samples from Middleburg, Oxford and Wolvekrans are generally very low, with the exception of BHC-beta, BHC-delta and heptachlor at Middleburg, heptachlor at Oxford and Wolvekrans as shown in Figure 4.9.

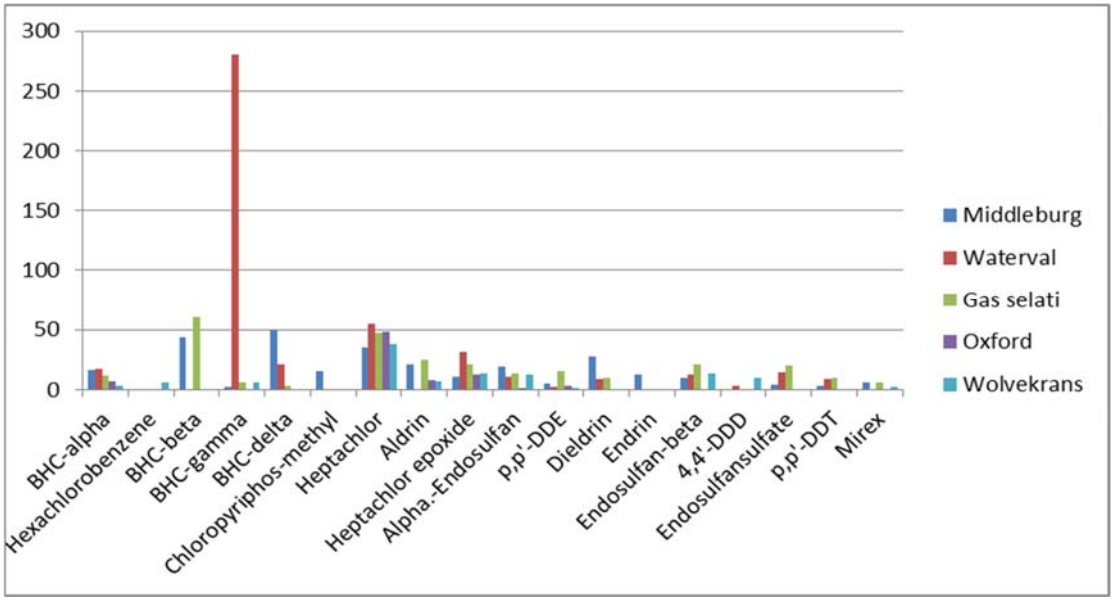


Figure 4.9: Mean concentrations (ng/ L) of measured compounds in summer

4.5 COMPARISON TO OTHER STUDIES

The levels found from the catchment were meaningfully above the water criteria values suggested by US EPA and DWAF for the protection of the aquatic environment (DWS, 2008). Levels obtained were also higher than those of other studies led so far in South African aquatic environments. Therefore, there is an obvious contamination of the Olifants River catchment by the OCPs studied.

Some of the stated levels of OCPs in comparable studies were lower than those obtained in this study. Sibali *et.al*, (2008) studied 13 OCPs in water (filtered and unfiltered) and sediment sample from the Jukskei River catchment. Total levels of OCPs in water studied were described and ranged from 0.631±0.01 to 1540.2±0.19 ng•mℓ-1 (4,4'DDT) and those of sediments ranged from 4.261±0.11 to (γ-HCH) to 22 914±4.85 ng•gdw-1 (2,4'-DDE). Fatoki and Awofolu (2003) and Awofolu and Fatoki (2003) studied water and sediment samples from marine and freshwater sources in the Eastern Cape Province of South Africa that receive runoff from agricultural lands and effluents from industries. The levels of OCPs reported ranged from 5.5 (2,4-DDD) to 450±0.10 ng•ℓ-1 (β-BHC) in water samples and from 0.6 (aldrin and 2,4-DDD) to 184±0.12 ng•g-1 (β-BHC) in sediments for triplicate analyses. Some endocrine disrupting OCPs such as DDT, DDE, heptachlor, ENDO and chlordanes were also detected.

On the other hand, it has been noted in the report on assessment of agricultural pesticides in the upper Olifants river catchment (DWS, 2008) that" various Organochlorines pesticides like

lindane; DDT-4,4'; DDD-4,4' ; DDE-4,4'; dieldrin and endosulfan exceeded the guide line values with mostly higher concentration at the upstream sites within the Olifants catchment" .

Consequently, Organochlorines pesticides are known to be persistent in the environment and have been shown to be capable of undergoing long range atmospheric transport (Ritter *et. al.,* 2005). Moreover, anthropogenic activities like radioactivity (from power generation), acid deposition or discharge (coal and gold mining), manufacturing process, enhanced eutrophication (organic compounds washed from agriculture lands), (Ellis, 2008), may contribute to levels of these pollutants in the environmental media in that way posing great risk to surface and groundwater (Olukunle, 2016). Thus, information on OCPs in different environmental media is very essential to serve as an aid to detecting their sources.

However, this study confirms the existence and levels of OCPs studied in this work from the identified five sampling sites along the Olifants River catchment. This is very essential for data generation as the area has been identified as a source of persistent organic pollutants due to the presence of anthropogenic activities.

CHAPTER 5: CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

From the results of the quality assurance processes carried out for the analysis of selected Organic Compounds in this research, the validation parameter showed a satisfactory recovery for spiked deionised water, field water quality parameters and surface water. Therefore, this study showed LLE with DCM as a good and reliable methodology of extracting Organic Compounds from aqueous samples, this finding is similar to what was reported by Sibali ***et. al.***, (2008). Hence, the GC-MS instrumental conditions were successfully optimised which resulted in chromatographic analysis of 18 selected Organic Compounds with a shorter analysis time (less than 11 min) and the resolution of the peaks was good. Through this research the occurrence of OCPs revealed that the levels reached were also more important than those of other research studies conducted up to now in South African aquatic environments and also showed some seasonal variations. These would show that there is a definite contamination of the OCPs studied in the Olifants River catchment.

Nevertheless, the final constitution of South Africa gives everyone the right to potable water (S 27 (1) (b)), and this implicates that water need to be treated before its distribution to communities (Phaleng, 2009). Poor water resource management thus impact on the cost for treating this water to potable use before distribution. As a result the authorities need to ensure that the industries invest in new technologies rather that opting for the possible way out i.e. discharge (Phaleng, 2009).

As in line with the objective of this study, water samples were collected for winter (April – August) and summer (September – December) 2015; in order to investigate the seasonal variability of the water quality in terms of organic pollution along the River.The results from this investigation show that in winter the Olifants River is mostly polluted at site 90503 (Oxford) with (BHC- beta, Aldrin, Heptachlor-epoxide, Endosulfan- Alfa and Endrin) with Aldrin up to 283.62 ngL-1 indicating the highest hazard toward the aquatic environmental. This winter trend is similar to the trend observed for the annual mean concentration values for oxford, Ga-selati and Wolvekrans sites on Olifants River; indicating that the river is mostly polluted with the selected Organochlorines during the winter period of 2015.

In conclusion, the findings on the effects of selected organic compounds influence on water quality along the Olifants river catchment are unravelled and no South African guideline

values are available for BHC-alpha, BHC-beta, BHC-delta, DDE-4, 4', DDD-4,4 and heptachlor-epoxide (DWAF, 1996).

Hence, the organic contamination of the water quality along the Olifants River have been assessed and found to be of poor water quality with respect to selected organochlorine listed in this study.

5.2 RECOMMENDATIONS

The relatively high levels of analytes like BHC- beta, Aldrin, Heptachlor-epoxide, Endosulfan- alfa and Endrin are a concern.

The study recommends that monitoring of OCPs and POPs should be continual since they are regulated.

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Appendix I: Figures showing calibration curves

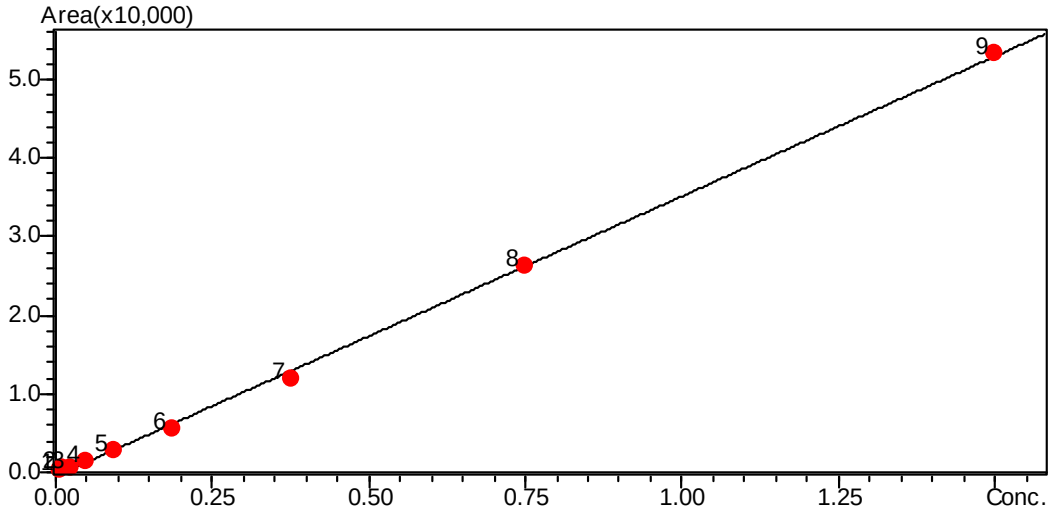

Figure I a: calibration curve for heptachlor-epoxide

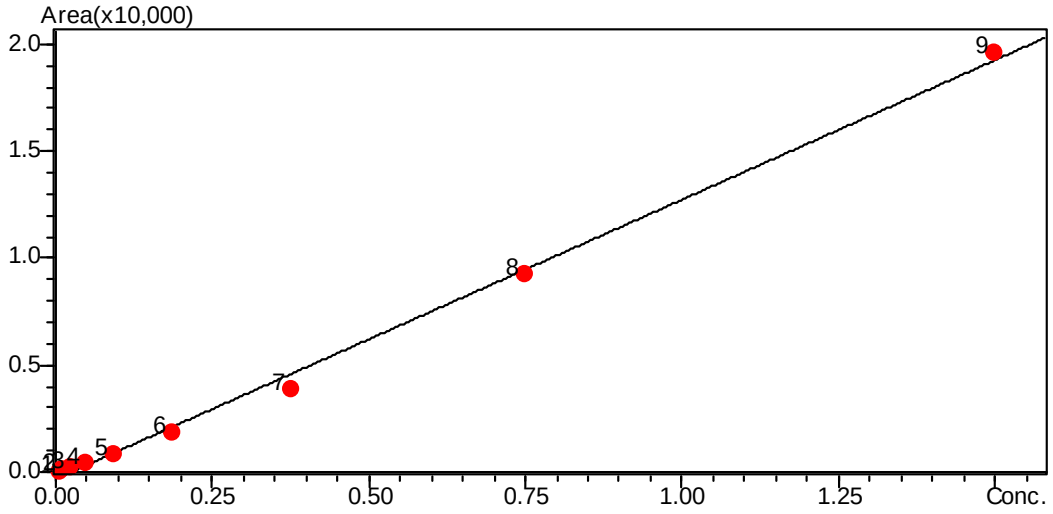

Figure I b: calibration curve for endosulfan-beta

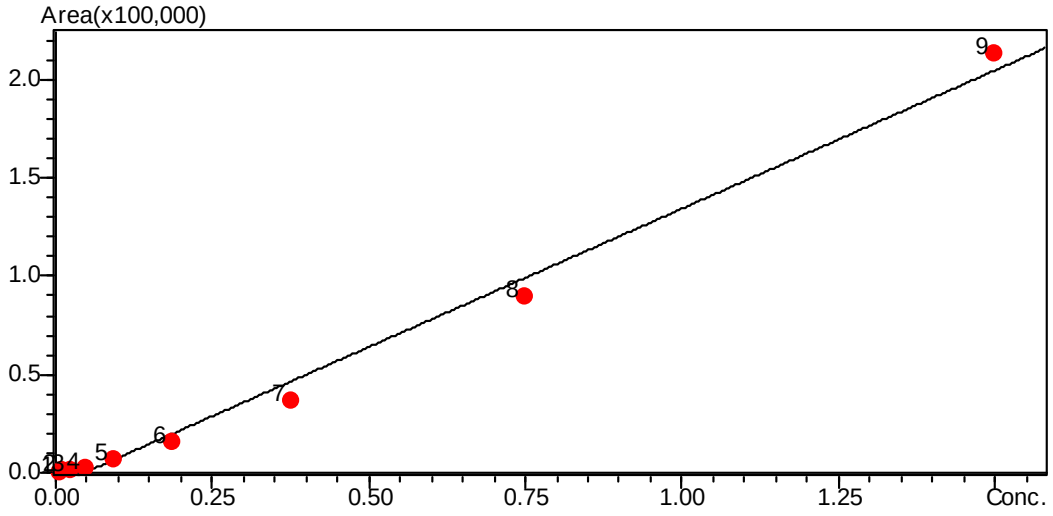

Figure I c: calibration curve for P,p'-DDT

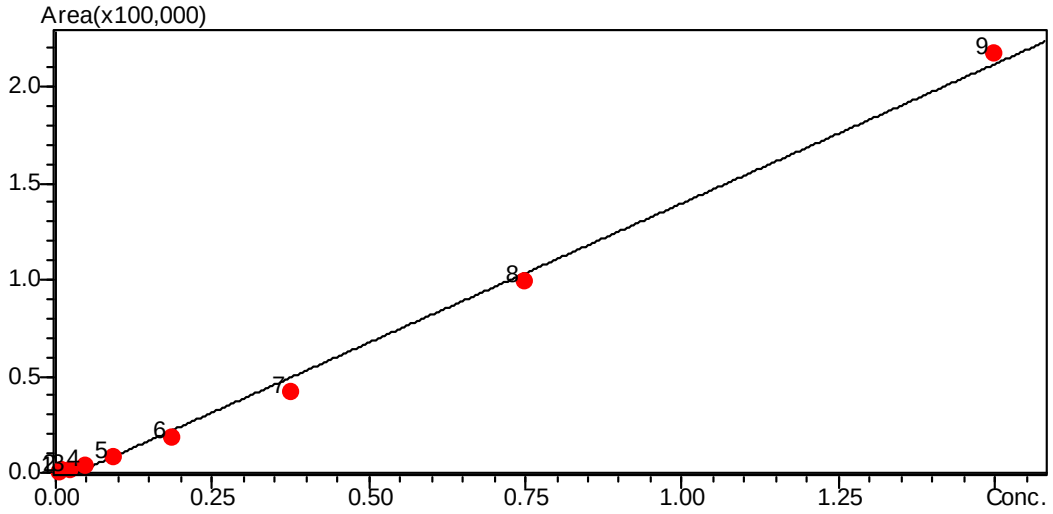

Figure I d: calibration curve for P,p'-DDD

Appendix II: Tables showing levels of OCPs in water

Table II a: Monthly BHC-alpha in water samples

	BHC-alpha Concentration (ng/L)						
WMS ID	**June**	**July**	**August**	**September**	**October**	**November**	**December**
Wolvekrans	0	0	13.46	0	11.16	0	0
Middelburg	8.46	10.12	6.27	8.7	7.44	35	15
Waterval	8.52	8.48	9.46	0	0	35	0
Oxford	9.06	10.32	0	0	**4.77**	0	15
Foskor	**31.36**	7.25	9.22	0	22.32	0	0

Table II b: Monthly Hexachlorobenzene in water samples

	Hexachlorobenzene Concentration (ng/L)						
WMS ID	**June**	**July**	**August**	**September**	**October**	**November**	**December**
Wolvekrans	0	0	13.46	**4.8**	**18.6**	0	0
Middelburg	0	10.12	6.27	0	0	0	0
Waterval	0	8.48	9.46	0	0	0	0
Oxford	0	0	0	0	0	0	0
Foskor	0	0	9.22	0	0	0	0

Table II c: Monthly BHC-beta in water samples

	BHC-beta Concentration (ng/L)						
WMS ID	**June**	**July**	**August**	**September**	**October**	**November**	**December**
Wolvekrans	0	0	11	0	0	0	0
Middelburg	26.79	0	**6.27**	0	0	175	0
Waterval	15.62	0	18.05	0	0	0	0
Oxford	22.65	**460.96**	12.14	0	0	0	0
Foskor	21.56	0	11.52	0	0	0	120

Table II d: Monthly BHC-delta in water samples

	BHC-beta Concentration (ng/L)						
WMS ID	**June**	**July**	**August**	**September**	**October**	**November**	**December**
Wolvekrans	0	0	8.98	0	0	0	0
Middelburg	0	0	6.27	8.7	**3.72**	**185**	0
Waterval	8.52	0	6.88	11.97	0	30	0
Oxford	9.06	0	9.11	0	0	0	0
Foskor	0	7.25	8.07	0	5.58	0	0

Table II e: Monthly Chloropyriphos methyl in water samples

	Chloropyriphos methyl Concentration (ng/L)						
WMS ID	**June**	**July**	**August**	**September**	**October**	**November**	**December**
Wolvekrans	0	0	0	0	0	0	0
Middelburg	0	0	0	0	0	**60**	0
Waterval	0	0	**5.16**	0	0	0	0
Oxford	0	10.32	0	0	0	0	0
Foskor	11.76	0	0	0	0	0	0

Table II f: Monthly Heptachlor in water samples

	Heptachlor Concentration (ng/L)						
WMS ID	**June**	**July**	**August**	**September**	**October**	**November**	**December**
Wolvekrans	0	0	0	46.8	19.84	35	50
Middelburg	0	0	10.45	**37.7**	35.96	15	50
Waterval	0	0	8.60	59.85	0	50	0
Oxford	0	**0**	13.66	40.32	38.16	0	**65**
Foskor	0	0	**9.22**	0	44.64	0	50

Table II g: Monthly heptachlor epoxide in water samples

	Heptachlor epoxide Concentration (ng/L)						
WMS ID	**June**	**July**	**August**	**September**	**October**	**November**	**December**
Wolvekrans	0	11.97	0	0	0	55	0
Middelburg	21.15	85.99	5.23	7.25	0	0	35
Waterval	15.62	3.77	10.32	63.27	0	0	0
Oxford	6.04	129.00	31.87	7.2	4.77	0	25
Foskor	**201.88**	8.46	**3.46**	0	11.16	0	30

Table II h: Monthly Alpha-endosulfan in water samples

	Alpha-endosulphan Concentration (ng/L)						
WMS ID	**June**	**July**	**August**	**September**	**October**	**November**	**December**
Wolvekrans	0	0	**224.38**	12	0	20	20
Middelburg	23.97	18.55	6.27	0	0	75	0
Waterval	31.24	16.96	6.88	20.52	0	0	0
Oxford	27.18	158.24	192.75	0	**4.77**	0	0
Foskor	13.72	6.04	11.52	0	7.44	0	20

Table II i: Monthly p,p'-DDE in water samples

	P,p'-DDE Concentration (ng/L)						
WMS ID	**June**	**July**	**August**	**September**	**October**	**November**	**December**
Wolvekrans	0	0	7.48	**4.8**	0	0	0
Middelburg	0	0	0	0	0	20	0
Waterval	0	0	5.16	5.13	0	0	0
Oxford	0	8.60	0	0	0	0	10
Foskor	**31.36**	0	16.13	0	0	0	30

Table II j: Monthly Dieldrin in water samples

	Dieldrin Concentration (ng/L)						
WMS ID	**June**	**July**	**August**	**September**	**October**	**November**	**December**
Wolvekrans	0	0	0	0	0	0	0
Middelburg	32.43	8.43	**4.18**	0	0	**85**	25
Waterval	17.04	4.71	4.30	17.1	0	0	0
Oxford	18.12	17.20	9.11	0	0	0	0
Foskor	25.48	9.66	6.91	0	0	0	20

Table II k: Monthly Endrin in water samples

	Endrin Concentration (ng/L)						
WMS ID	**June**	**July**	**August**	**September**	**October**	**November**	**December**
Wolvekrans	0	0	0	0	0	0	0
Middelburg	0	0	0	0	0	**50**	0
Waterval	0	0	117.77	0	0	0	0
Oxford	0	**292.40**	148.73	0	0	0	0
Foskor	166.60	0	0	0	0	0	0

Table II l: Monthly Endosulfan-beta in water samples

	Endosulphan- beta Concentration (ng/L)						
WMS ID	**June**	**July**	**August**	**September**	**October**	**November**	**December**
Wolvekrans	0	0	8.98	0	0	25	30
Middelburg	**8.46**	11.80	0	0	0	0	**40**
Waterval	12.78	0	6.02	25.65	0	0	0
Oxford	0	10.32	0	0	**0**	0	0
Foskor	15.68	0	9.22	0	11.16	0	30

Table II m: Monthly 4,4'-DDD in water samples

	4,4'-DDD Concentration (ng/L)						
WMS ID	**June**	**July**	**August**	**September**	**October**	**November**	**December**
Wolvekrans	0	0	8.98	0	0	0	**40**
Middelburg	0	0	8.36	0	0	0	0
Waterval	11.36	0	6.88	**6.84**	0	0	0
Oxford	9.06	0	0	0	0	0	0
Foskor	0	7.25	6.91	0	0	0	0

Table II n: Monthly endosulfan sulfate in water samples

	Endosulfan sulfate Concentration (ng/L)						
WMS ID	**June**	**July**	**August**	**September**	**October**	**November**	**December**
Wolvekrans	0	0	8.98	0	0	0	0
Middelburg	11.28	0	7.32	0	0	0	15
Waterval	8.52	**5.65**	0	29.07	0	0	0
Oxford	0	12.04	9.11	0	0	0	0
Foskor	21.56	0	13.83	0	0	0	**40**

Table II o: Monthly p,p'-DDT in water samples

	P,p-DDT Concentration (ng/L)						
WMS ID	**June**	**July**	**August**	**September**	**October**	**November**	**December**
Wolvekrans	0	0	14.96	0	0	0	0
Middelburg	14.10	16.86	**9.41**	0	12.4	0	0
Waterval	12.78	9.42	8.60	17.1	0	0	0
Oxford	15.10	0	15.18	0	0	0	0
Foskor	**19.60**	12.08	11.52	0	18.6	0	0

Table II p: Monthly Mirex in water samples

	Mirex Concentration (ng/L)						
WMS ID	**June**	**July**	**August**	**September**	**October**	**November**	**December**
Wolvekrans	0	0	8.98	7.2	0	0	0
Middelburg	0	0	6.27	0	7.44	**15**	0
Waterval	9.94	5.65	**5.16**	0	0	0	0
Oxford	9.06	0	9.11	0	0	0	0
Foskor	11.76	0	6.91	0	11.16	0	0

Appendix III: Ethics document

UNISA university of south africa

CAES RESEARCH ETHICS REVIEW COMMITTEE

Date: 02/10/2014

Ref #: **2014/CAES/143**
Name of applicant: **Ms TC Mulanga**
Student #: **55779735**

Dear Ms Mulanga,

Decision: Ethics Approval

Proposal: A review of physico-chemical and biological pollution levels along the Olifants River catchment

Qualification: Postgraduate degree

Thank you for the application for research ethics clearance by the CAES Research Ethics Review Committee for the above mentioned research. Final approval is granted for the duration of the project.

The application was reviewed in compliance with the Unisa Policy on Research Ethics by the CAES Research Ethics Review Committee on 02 October 2014.

The proposed research may now commence with the proviso that:

1) *The researcher/s will ensure that the research project adheres to the values and principles expressed in the UNISA Policy on Research Ethics.*
2) *Any adverse circumstance arising in the undertaking of the research project that is relevant to the ethicality of the study, as well as changes in the methodology, should be communicated in writing to the CAES Research Ethics Review Committee. An amended application could be requested if there are substantial changes from the existing proposal, especially if those changes affect any of the study-related risks for the research participants.*
3) *The researcher will ensure that the research project adheres to any applicable national legislation, professional codes of conduct, institutional guidelines and scientific standards relevant to the specific field of study.*

University of South Africa
Preller Street, Muckleneuk Ridge, City of Tshwane
PO Box 392 UNISA 0003 South Africa
Telephone: +27 12 429 3111 Facsimile: +27 12 429 4150
www.unisa.ac.za

Note:
The reference number [top right corner of this communiqué] should be clearly indicated on all forms of communication [e.g. Webmail, E-mail messages, letters] with the intended research participants, as well as with the CAES RERC.

Kind regards,

Signature
CAES RERC Chair: Prof EL Kempen

PLEASE NOTE THE CONDITIONS

Signature
CAES Executive Dean: Prof MJ Linington

University of South Africa
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Appendix IV: Permission Letter

water & sanitation

Department:
Water and Sanitation
REPUBLIC OF SOUTH AFRICA

Reference: Research Ethics Letter
Enquiries : Mulanga Tshimanga
Tel: 0766780250
Email: Christimulanga82@yahoo.fr

University of South Africa
Collage of Agriculture and Environmental Sciences
P.O.Box 58
UNISA
0002

Dear Madam/Sir,

PERMISSION TO UNDERTAKE SCIENTIFIC RESARCH PROJECT TITLED "A REVIEW OF PHYSICO-CHEMICAL AND BIOLOGICAL POLLUTION LEVELS ALONG THE OLIFANTS RIVER CATCHMENT".

This letter allows Mrs Mulanga Tshimanga, student number 55779735, currently registered for Master in Science (MSc) Environmental Management at UNISA **to undertake the above mentioned project**.

The project entails collection of water and sediment samples, as well as the assessment of the water quality along the river as per the project objectives of the research. **Mrs Tshimanga has also been given permission to use our toxicology laboratories at Roodeplaat for analysing her samples.**

I'm of the view that the information and data from this project will add value to the implementation of the National Toxicity Monitoring Programme (NTMP) to the Olifants Water Management area.

Hope you find this in order.

Yours sincerely,

.....
Dr. David Odusanya
Programme Manager : National Toxicity Monitoring
Tel:0822275415
Date: 23 September 2014

Appendix V: Plagiarism Results

Turnitin Originality Report

MSc Environmental Science dissertation by TC Mulanga

From DES 2016 Submissions (DES 2016 Submissions)

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