

Investigation of Selected Organic Compounds Influence on Water Quality Along the Olifants River in South Africa

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Received date: November 08, 2017; Accepted date: November 16, 2017; Published date: November 23, 2017

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Abstract

Water is a crucial natural resource, indispensable to food production, life, the environment, power generation, industry, sanitation and hygiene. Organochlorine Pesticides (OCPs) in the environment are 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 selected five points along the Olifants river in South Africa and stored at a temperature of $\leq 5^{\circ}\text{C}$ 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 and 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 show that during the 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). The summer season data show that most pollution happen at the Ga-selati site with BHC-beta and at the Waterval site with (Heptachlor and BHC-gamma) respectively. The OCPs that reached the river catchment were significantly above the WHO drinking water quality guidelines and its cause for concern for those who are exposed or use it.

Keywords OCPs; Liquid-liquid extraction; Surface water; GC-MS; Olifants river

Introduction

Organochlorine pesticides (OCPs) are an essential group of persistent organic pollutants (POPs) which have created global concern due to their toxicity and negatively affect human beings and the environment [1-4]. POPs in general are priority pollutants due to their mutagenic and carcinogenic effects [5,6]. Therefore, Organochlorines pesticides are known to be persistent in the environment and have been shown to be capable of undergoing long range atmospheric transport [7]. Moreover, the current study area is suspected to be contaminated with persistent organic pollutants through anthropogenic activities such as power generation, coal and gold mining, manufacturing industry and agricultural lands [8].

Common OCPs include dichlorodiphenyltrichloroethane (DDT) and its degradation products, DDE and DDD, HCB, Heptachlor, Lindane and Endosulfan [9,10]. The utilisation of DDT is banned in most countries because of its carcinogenicity, adverse health impacts on wildlife and bio-accumulation [11,12]. Despite its ban, DDT is still legally manufactured for its use in malaria-endemic areas. In South Africa, the common use of DDT was prohibited in the early 1980's

[12], but it is still used strategically to control malaria vector some areas of Limpopo, Mpumalanga and KwaZulu-Natal provinces [1,13-15].

For many years until 1965, HCB (C_6Cl_6) was extensively used as a fungicide on seed of onions, wheat, sorghum and other grains. 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 [16]. It is also a trace pollutant in some pesticides and may persist in the environment due to historic use as a fungicide [17]. Presently, its production is prohibited in most countries and it is contained in the Stockholm Convention on Persistent Organic Pollutants by the United Nations Environment Programme [18].

Heptachlor ($\text{C}_{10}\text{H}_5\text{Cl}_7$) was largely used in the 1960s and 1970s to exterminate ants, termites and soil insects on seed crops and grains [19]. Meanwhile in South Africa, its registration was withdrawn in 1976 and its use is now banned in most countries [9,20]. As with the other banned OCPs, heptachlor still exist in the environment due to widespread previous use, unused stockpiles and in leachates from disposal sites [20].

But, regardless of the restriction and ban on the utilization of OCPs in western countries during the 1970s and 1980s, some third world

countries including South Africa are still using them for public and agricultural safety reasons due to their efficiency in controlling numerous insects [21,22].

Most of the time Persistent Organic Pollutants are stable, toxic compound that can remain in the environment by resisting chemical, biological and photolytic degradation [15,23]. 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 [24]. 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 [13]. POPs chemical and physical characteristics make it possible for long-range transport, allowing them to be widely disseminated geographically, even to areas where they have never been used or made [7].

Extraction is ordinarily realized 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. This study adopted LLE-DCM as a method-solvent combination due to their reliability in extracting organic compounds from aqueous samples [24,25].

Dichloromethane has great extraction efficiencies for a wide range of non-polar to polar compounds, its non-flammable, it has low boiling point and it's easy to re-concentrate after extraction and it is relatively easy to separate from water because of its superior exact gravity [25].

Materials and Methods

Reagents

All reagents used were of analytical and GC grade (Merck, South Africa). Anhydrous sodium sulphate, 99.5% pure was neutralised by dehydrating at 450°C in the muffle heating system for 16 hours before usage. All solvents were subjected to distillation 3 times before utilisation and were ranged from 99.0 to 99.5% pure. Solvents used in the analysis are hexane (69°C), dichloromethane (39.8°C) and acetone (56.2°C). Anhydrous sodium sulphate, 99% pure (Rochelle Chemicals, South Africa) was preheated by drying over night before use. FlorisilR 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). 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 × 3 mL, provided by Separations Pty (Pretoria, South Africa). Double distilled water was used.

Instrumentation

The mixed Selected Organic Compounds (SOCs) standard was analysed on Shimadzu GC-MS. Shimadzu model 2010 gas chromatograph coupled with a model QP 2010 ultra-mass spectrometer (Shimadzu, Japan) using electron ionization and a Shimadzu A0C-20i auto sampling injector. The Operation mode was in the selected ion-monitoring (SIM) 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. Table 1 shows the operating conditions which were used.

Oven Temp Programme	Line Temp (°C)	Injector and Transfer Temp (°C)	Ion source flow (mL/min)	Carrier gas time (min)
80°C (2 min) to 180°C	@200/250°C	300	1	18
10°C to 300°C (2 min)	@30 °C			
80°C (1 min) to 180°C	@30°C,	250/300	250	3
To 250°C (2 min)	@100°C			
100°C (1 min) to 160°C/min to 300°C@25°C/min to 325@10°C/min (3 min)	@15°C	270/300	250	1.5

Table 1: The optimized instrumentation operating conditions.

Preparation of standard stock solutions

OCPs standard stock solutions of 100.0 mg/L were prepared by weighing about 10.0 mg of pure standard material was weighed and quantitatively transferred into a 100.0 mL volumetric flask. Toluene was added up to the mark and the solutions were mixed thoroughly using of the vortex mixer. Intermediate standard stock solutions of 1.0 mg/L were prepared by pipetting 1.0 mL of each the standard stock solutions into a 100.0 mL volumetric flasks and toluene was used to fill up the flasks to the mark. Lower concentrations that ranged from 0.3-3.0 ng/µL of standards mixtures were prepared from the intermediate stock solutions by serial dilution.

Silica gel column chromatography (clean-up)

All crude extracts obtained from liquid-liquid extraction (LLE), were subjected to column clean up before injection into the GC-MS. The clean-up column in use was a solvent-rinsed Pasteur pipette (230.0 mm) plugged with glass wool, packed in layers from bottom with about 0.16 g of silica, 0.06 g Pesticarb, 0.16 g silica and with 0.5 g sodium sulphate.

Glass wool was used to separate each layer of materials and to enhance the cleaning. 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 4.0 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).

Description of the study area and sampling protocols

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.

The 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 325.0 mm/annual to 750.0 mm/annual. High precipitation of up to 1000.0 mm/annual takes place only in a thin belt along the slope [26].

Water samples were collected from the selected 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). Figure 1 shows the location of sampling points around Ga-Selati at Foskor, Oxford, Waterval, Middleburg dam and Wolvekrans using Google Earth.

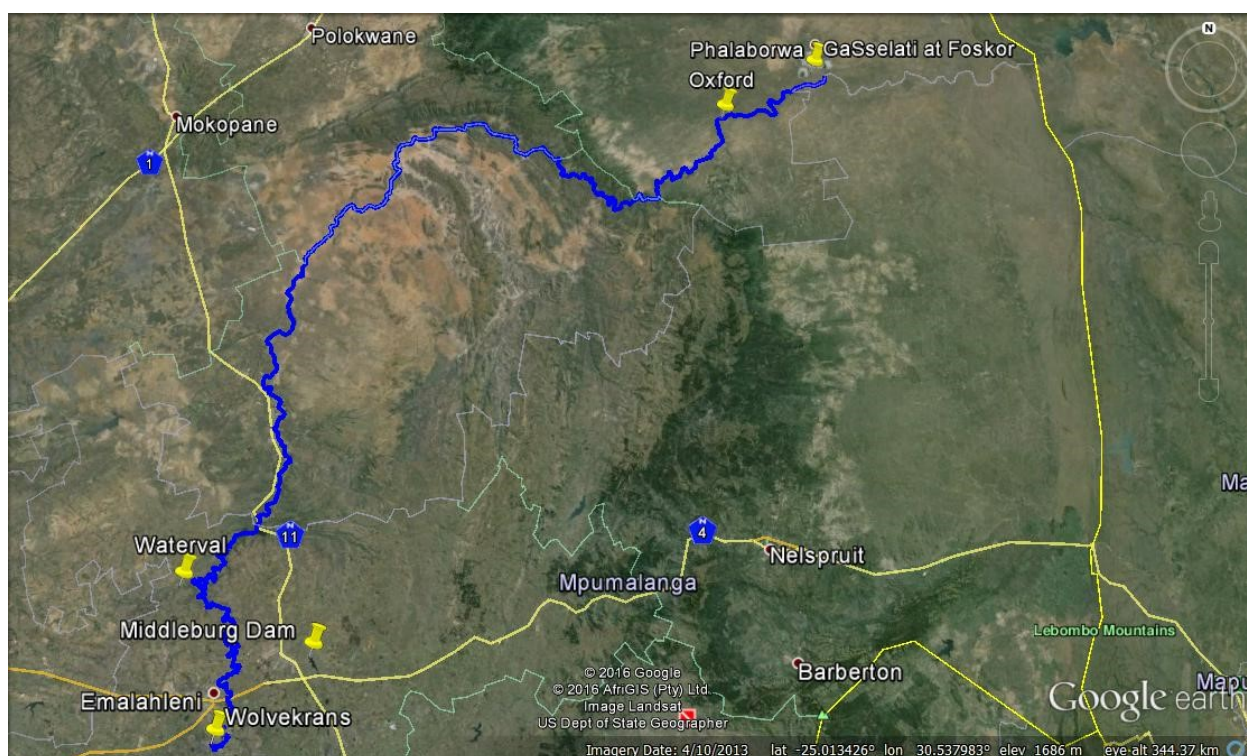


Figure 1: Study area map of the Olifants WMA with monitoring points.

Water sampling

The area of interest is the Olifants river catchment system in the Northern-east province. Surface water samples were collected during the dry season (June and August 2015) and during the wet season (September and December 2015). *In situ* readings were taken at the spot where water samples were collected with their Global Positioning System (GPS).

Prior to sampling, the containers and cap were first cleaned twice with water from stream afterwards absorbed to about 30 centimeters underneath the surface of the tributary at a 45 degree angle to the course of the flow. Grab water samples were collected in 2.5 L pre-cleaned amber Winchester glass containers from 5 selected sampling points to where the water appeared well mixed. Samples were then conveyed in a cooler box with ice to the laboratory where they were kept in a cold room at $<5^{\circ}\text{C}$ until they were analysed. All samples were analysed within 30 days of collection.

Method validation

The mean percentage recovery of OCPs in water

The efficiency of the extraction of the selected OCs was investigated with DCM, with about 60 μL of 3.0 $\text{ng}/\mu\text{L}^{-1}$ OCPs standard mixture was dissolved in 5.0 mL Dichloromethane (DCM) then spiked into 300 mL ultra-pure water and left for 24 h for equilibration (Olukunle).

The mixture was extracted by liquid-liquid extraction and final extracts concentrated to 200 mL. 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).

Results and Discussion

OCP's calibration results

Blank extraction of unspiked doubly distilled water was carried out using the DCM extraction and chromatographic clean up method as described before, which gave a clean background. Water samples did not show any peak that might have had any influence on the results.

Figure 2 shows the chromatogram of spiked ultra-pure water with OCPs standard mixture with individual compounds that are well resolved and are identified as (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. All the individual OCP's were eluted within 11.0 minutes [5,23].

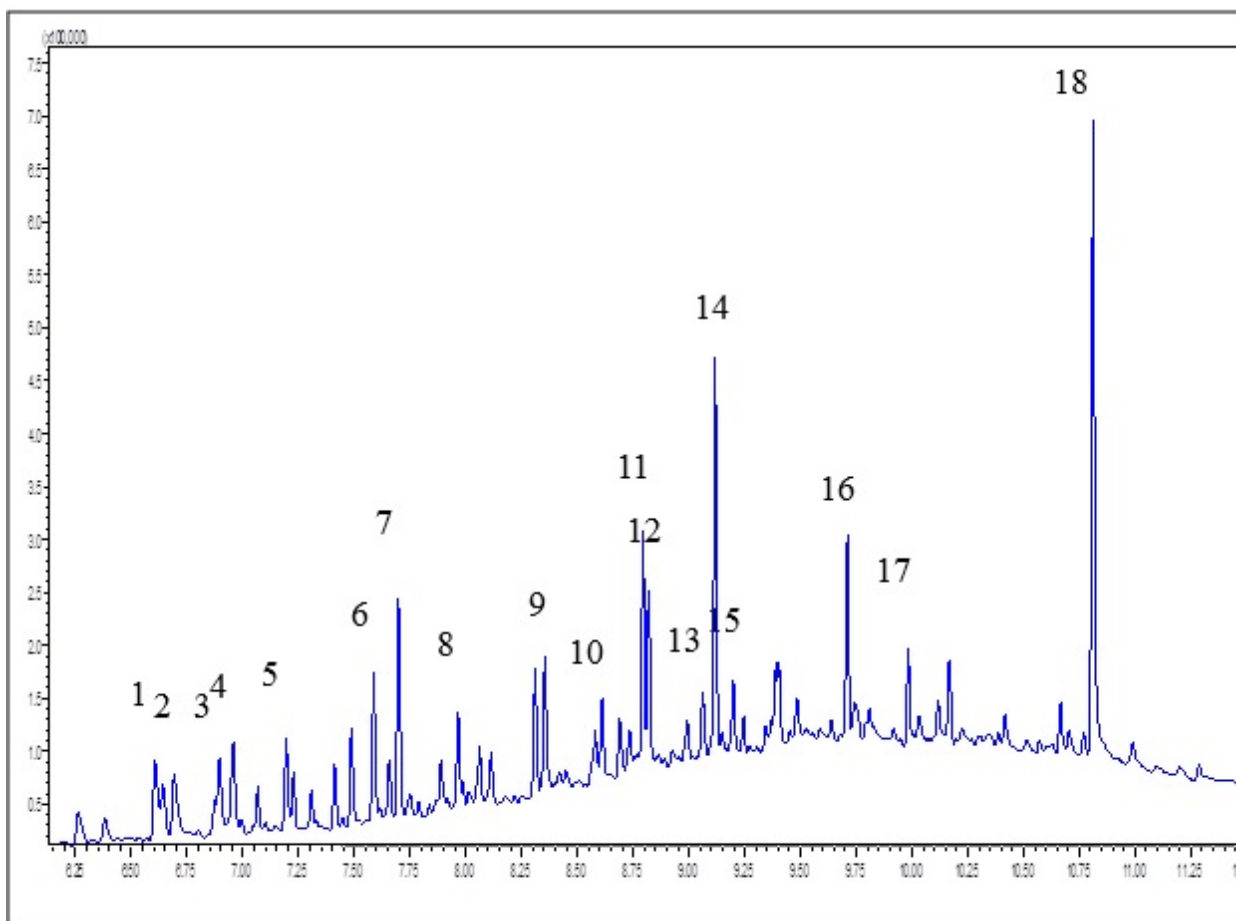


Figure 2: The chromatogram of spiked ultra-pure water with OCPs.

The triplicate retention time (min) results per individual OCPs compound are presented in Table 2. The retention times ranged from 6.631 for BHC-alpha to 10,194 for Mirex respectively. The BHC-alpha standard compound was eluted first: this might be due to its lower molecular weight (200–400) g/mol and boiling point (79–82°C) compared to other analysed standard compound. From the two capillary columns that were evaluated, DB-5 capillary column 5% phenyl and 95% dimethylpolysiloxane (30 m × 0.25 mm × 0.25 μm) produced better results in terms of peak resolution for all the

compounds. Related performance by similar capillary column has been reported [27,28]. The instrumental limit of detection (LOD) of the OCPs was obtained as the lowest concentration of the analyte that the instrument can identify under the optimised instrumental conditions [29]. 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 as shown in the Table below.

Individual OCPs	Triplicate Retention Times Measurements (mins)			LOD	LOQ
	t _R 1	t _R 2	t _R 3		
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

Table 2: Retention times (tR), limits of detection and quantification.

Seasonal variability of OCPs in water samples along Olifants river

The mean seasonal concentrations of selected organo-chlorine found at the five investigated sites along Olifants River are shown in Figures 3 and 4. The seasonal mean results from this investigation show that the Olifants River is mostly polluted in winter at Oxford (with Aldrin, BHC-beta, Endrin, Endosulfan-alpha and Heptachlor-epoxide). Aldrin compound level was the highest with about 834.20

ng/L in July 2015. The highest pollutants observed at other sites are Heptachlor-epoxide (~ 74 ng/L) and Endrin (~ 55 ng/L) at Ga-Selati and Endosulfan-alpha dominated with about 226 ng/L at Wolvekrans. The seasonal mean concentration of Organochlorine compounds found in water samples from Middleburg and Waterval were very low, with the exception of Heptachlor-epoxide and Endrin respectively as shown in Figure 3.

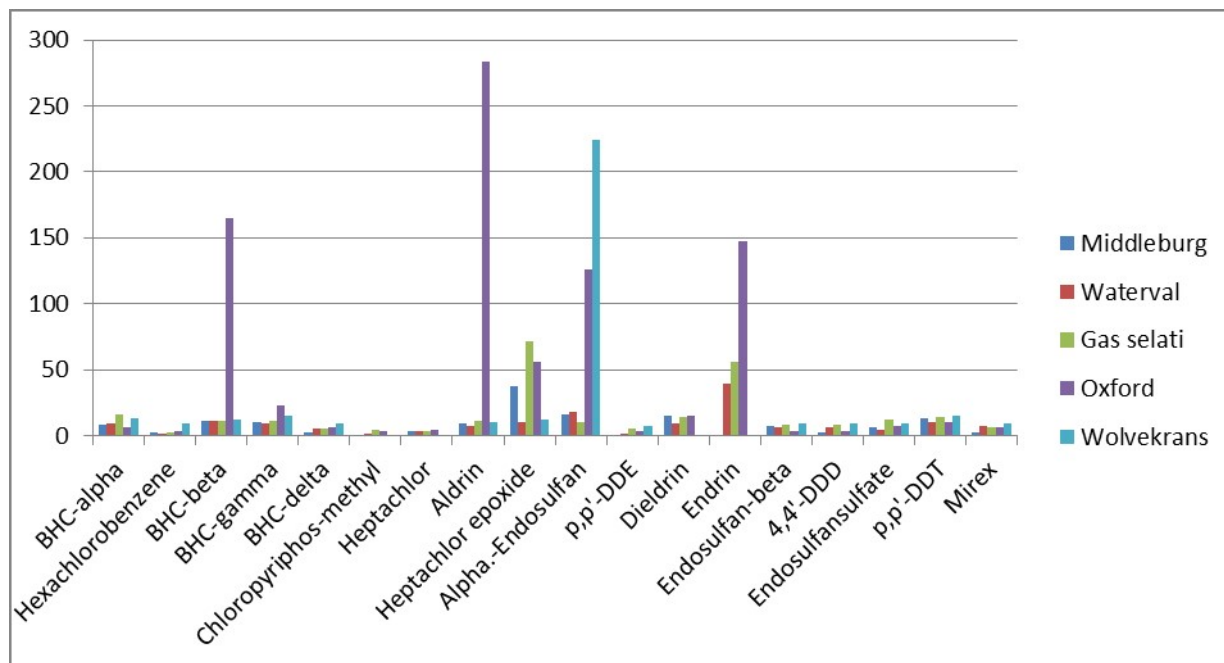


Figure 3: Mean concentrations (ng/L) of measured compounds in winter.

The results from this investigation show that the Olifants River is mostly polluted in summer with about 265 ng/L BHC-gamma and 55 ng/L Heptachlor at Waterval in November, as well as 60 nm/L BHC-beta at Ga-selati.

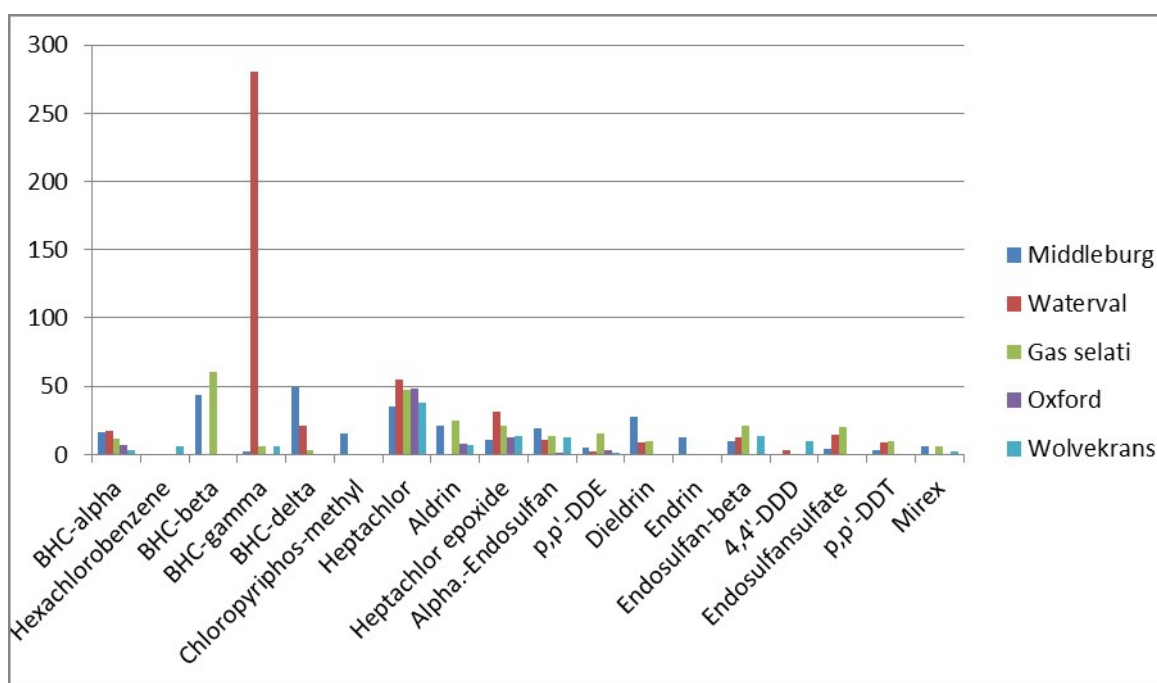


Figure 4: Mean concentrations (ng/L) of measured compounds in summer.

The seasonal mean concentrations of OCP's found in water samples from Middleburg, Oxford and Wolvekrans were generally very low, with the exception of BHC-beta, BHC-delta and heptachlor at Middleburg, heptachlor at Oxford and Wolvekrans. In general, the OCP's levels found from the catchment area were significantly above the local water standard and international water standard [30,31].

DWS reported that the assessment of agricultural pesticides in the upper Olifants river catchment such as lindane; DDT-4,4'; DDD-4,4'; DDE-4,4'; dieldrin and endosulfan exceeded the local guide line values with mostly higher concentration at the upstream sites within the Olifants catchment [32].

In similar studies that took place in the Jukskei River catchment, the highest total levels of 4,4' DDT of OCPs in river water was 1540.2 ± 0.19 ng/mL [25]. Fatoki and Awofolu 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 [24]. The levels of OCPs reported comparable results ranging from 5.5 (2,4-DDD) to 450 ± 0.10 ng/L (β -BHC) in water samples and from 0.6 (aldrin and 2,4-DDD) to 184 ± 0.12 ng/g (β -BHC) in sediments for triplicate analyses. The significant presence of OCPs in river water commonly used for agricultural activities is of major concern. Local authorities should strengthen the monitoring and the penalties against polluters of river water. Some solutions include investing in technologies that effectively treat waste effluents so that the local water bodies remain clean and healthy [33,34].

Acknowledgement

The research work was funded by the University of South Africa. This research work was supervised by staff from the Department of Environmental Affairs and the Council for Geoscience. Post graduate students from the University of South Africa and Tshwane University of Technology are also acknowledged for helping with laboratory work.

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