

Assessment and Monitoring the Fate of Organochlorine Pesticides and Nonyl phenol Ethoxylate, Potential Risks to Human Health and Wildlife along the Kafue River, Zambia.

(Conference ID: CFP/381/2017)

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Abstract

The Kafue River is one of the biggest and important rivers in Zambia in that it that serves as a source of livelihood in terms of fishing and provides drinking water for both humans and wild animals'. It has been termed as the lifeblood of the Kafue national park because of its importance. Nonylphenol ethoxylate and Organochlorine pesticides are potent endocrine disruptors linked to various health problems in both humans and wild animals. NPE is used as a surfactant in the laundry industries whilst OCPs in the agricultural industry for pests elimination..As a result of the vast industries and agricultural activities along the Kafue river, it is imperative to investigate the presence of these two and also determine the extent to which humans and wildlife are at risk.

Soil, sediment, and crops samples were collected from agricultural areas along the Kafue River in Zambia. QuEChERS extraction methods were used for the extraction of organochlorine pesticides and Nonyl phenol ethoxylate.

The separation of the pesticides was performed on a 5975C Gas chromatography mass selective detector (GC/MSD) system with Triple-Axis HED-EM Detector. The Gas chromatography system was equipped with a split-splitless injector and a 7683-auto sampler.

From the data obtained there were no OCPs and NPEs that was detected in the samples that were analysed. These results imply that there's is no potential risk posed by these OCPs and phenol to humans and wildlife, that could possibly bioaccumulate and eventually bio concentrate via the trophic level. QuEChERS can simultaneously be used for analysis of OCPs and NPE. QuEChERS also proved to be a robust method that could be used for the detection of the pollutants and also that it can be used in a laboratory facility that does not have sophisticated equipment.

Key Words: Organochlorine Pesticides, Nonylphenol Ethoxylate, Kafue River, Zambia, GC/MS, QuEChERS

Introduction

Modern pollutants have emerged not only associated with mobile vehicle emissions but with the use of chemicals in both industrial and domestic applications in food, for water treatment, manufacture and processing and also for pest control this has expanded the plethora of chemical pollutants (Jonas, *et al.*, 1999)

The sources of pollution which include industrial emissions, poor sanitation, inadequate waste management, contaminated water supplies and exposures to indoor air pollution from biomass fuels affect large numbers of people. The afore mentioned sources have been found to be prominent in developing countries and Zambia being also one of the developing countries is not spared from such challenges. Though the pollutants may not be in concentrations that are in excess, the effects on human health are usually far from immediate or obvious. As Taubes, (2010). noted, few of the problems of environmental exposure that concern us today imply large relative risks. There is a growing concern of chemicals that affect the endocrine system of humans and wildlife. In order for the endocrine system to be affected it does not need high concentrations of the endocrine disrupting agents. (Allsopp *et al.*, 1997).

Organochlorine pesticides have been a subject of attention from the mid-1950s. OCPs gained attention with a release of a book by Rachel entitled the 'Silent Spring'. OCPs were implicated in causing the quietness of the springs by affecting the bird's eggs. Industrialization and demand for food by the world's growing population brought about increased agricultural activities that lead to the extensive use of pesticides. The pesticides found their way into the environment as a result of run offs from agriculture activities. A worldwide embargo was placed on almost perilous OCPs following the Stockholm Convention held between 2004 and 2009 respectively (Stockholm Convention, 2009).

Extensive research concerning NPEs has been conducted globally, with the developed countries taking the lead. Countries like Mexico, Holland, Japan and Taiwan, United States of America and Israel have reported levels of < 0.1- 100µg/L. This has even lead to legislature being passed on how the two chemicals are used in consumer products and industries (EU, 2000). For instance, in Western Europe all surfactant components of domestic detergents must be biodegradable. The legislature requirement resulted from the fact that the original alkyl benzene sulfonate anionic were based on branched alkenes and these proved resistant to degradation by bacteria at sewage treatment works causing many rivers to suffer from foam. There was also a fear that surfactants could be "recycled" into drinking water (Whitehouse *et al.*,1998a). Similar concerns were expressed with nonylphenol ethoxylates and so in the 1980s the industry moved to linear alkyl benzene sulfonates and alcohol ethoxylates as the major ingredients of their formulations. Effective sewage treatment ensures that detergent components which are part of household effluent water are not discharged untreated into rivers and water courses.

African Perspective

Countries like Nigeria are taking part in the research on the endocrine disruptors with reported surfactants values of 4-NP and 4-t-OP detected in all the water samples collected for the research with concentration ranging between 43.9 ng/L - 79.4 ng/L and 57.1 ng/L - 68.6 ng/L, respectively. However, BPA in sediment samples from both rivers ranged from non-detectable to ≤ 0.4 ng/g, while 4-t-OP and 4-NP were between 2.2 ng/g - 24.5 ng/g and 1.1 ng/g.

Zambian perspective

OCPs are widely used in agricultural practices and have been documented to have both negative effects on humans and the environment. In regards, to the Zambian aquatic environment scanty information is available. The widely researched pesticide which is a potent endocrine disruptor has been 1-1 dichloro-2, 2 bis (p-chlorophenyl) ethane DDT because of its usage in the control of the anopheles vector for malaria.

Norrgren *et al*, (2000) found high concentration of several pesticides, such as DDT, PCBs, dieldrin in Kafue river water. Syakalima, 2006 investigated these OCPS in fish and used a stress marker enzyme Catalase as an indicator of pollution of these pesticides; the levels were found to be high in both the fish and river water. The pesticides/herbicides investigated were heptachlor, dichlorodiphenyltrichloroethane (pp'-DDE), cypermethrin, chlordane, toxaphene, terbufos, kelthane, endosulfan, dieldrin, 1-1 dichloro-2, 2 bis (p-chlorophenyl) ethane (pp'-DDD), DDT, atrazine, disulfoton, d-trans-allevethrin and endrin.

Sichilongo and Torto, 2006 carried out a similar research on the pesticides deltamethrin, heptachlor, aldrin, d-t-allevethrin, endosulfan, pp-DDE, dieldrin, endrin, pp-DDD and DDT in the Lochinvar National Park using GC- MS for the pesticides quantification. Results from the investigation showed that wildlife poised as a potential source of risk to humans living in and on the peripheral of the park through the food chain. Both of these studies by Syakalima ,2006 and Sichilongo,2006 concentrated much of their investigation in the Kafue and Lochinvar National parks, thereby not giving a full representation of the extent of pesticides contamination along the Kafue River. The OCPs covered by Sichilongo,2006 were carried out in the Kafue national park which is in the downstream of this study and Syakalima ,2006 covered one (1) location of the river. Mkandawire,2010 used stress biomarkers to trace the OCPs in fish. Biomarkers of pollution investigated were to, polycyclic aromatic hydrocarbons (PAHs), PCBs, 2, 3, 7, 8 tetrachlorodibenzo-p-dioxin (TCDDs), DDT, organochloride pesticides and heavy metals. The biomarkers investigated included; Cytochrome P450 1A1 (CYP 1A1), Cytochrome P450 3A (CYP 3A) and Metallothionein 1 (MT 1) in Kafue lechwe. This was a good approach since these OCPs tend to bioaccumulate in fatty tissues.

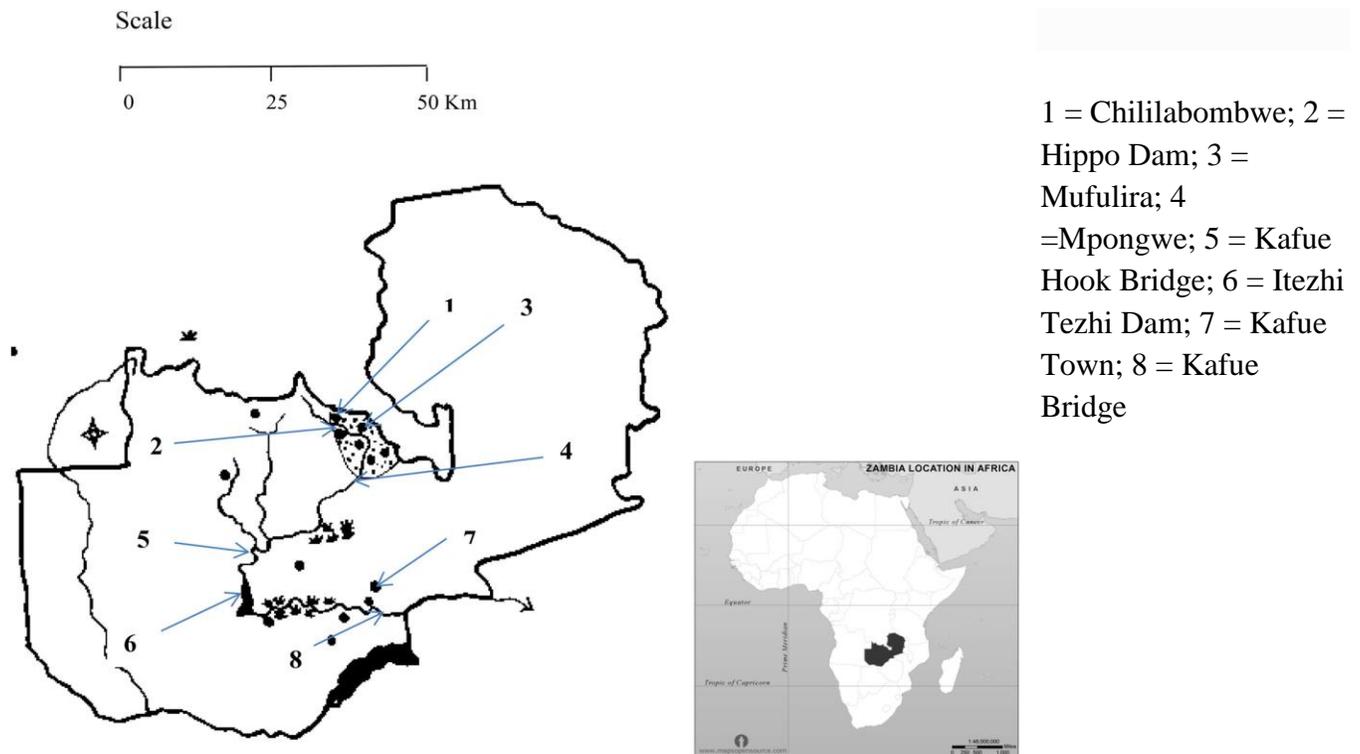
This study however, shows the trend of the OCPs along the Kafue River and will include a plethora of the compounds that were not previously studied and also investigated them in cultivated crops. The study seeks to also to fill a gap in which data concerning endocrine disruptors like Nonylphenol Ethoxylates is scarce. As a result, of their wide usage it has become imperative to investigate both the aquatic and terrestrial environments and ascertain if the Zambian human population and wildlife is at risk of exposure to NPEs and OCPs.

Description of the Study Area

The Kafue River is one of the biggest and important rivers in Zambia that serves as a source of livelihood in terms of fishing and provides drinking water for both human and wild animals'. It has been termed as the lifeblood of the Kafue national park because of the importance's attributed above. It emanates from the Copperbelt region; Also, due to the massive agricultural activities along the river they also concern about pesticide pollution due to agriculture runoffs. The sites were selected as a result of the research carried out by the Environmental Council of Zambia (ECZ) which is now known as the Zambia Environmental Management Agency (ZEMA) which found out that a huge amount of pesticides and herbicides have been used in these areas in the past years. The research also carried out by M'kandawire, 2010 outlines the industries and activities that can potentially leach out NPE to the river and surrounding environment. These areas also carry out a lot of fishing activities. (Syakalima, et al., 2006; ECZ, 2001). The control of this study was carried out on the Luangwa river which has also a lot of fishing activities but does not have active industrial activities that have been implicated in the contamination of the environment Organochlorine pesticides.

MAP OF STUDY AREA

Figure 1: Map of Zambia showing the Kafue River and sampling locations



Source: <http://www.mapsopensource.com> and (Choongo et al.2005)

Sampling

The Kafue river was divided into three sections, the upstream which included sampling sites around Chililabombwe and code named (CHIL) and Hippo dam(HIP), Mufulira code named (MUF)), the middle part of Mpongwe area, and finally the downstream of the Kafue town coded (KFT), Kafue Hook Bridge code named (KB) and Lake Itezhi Itezhi code named (ITT). Samples from Luangwa River were used as controls. These were coded LUA.

Table 1: Showing the sampling sites and GPS coordinates

Sampling Point (Code)	GPS Coordinates	
Hippo Dam (HIP)	12°27'52.40"S	27°51'4.79"E
Chililabombwe (CHIL)	12°28'38.71"S	27°53'54.64"E
Mufulira Bridge (MUF)	12°38'40.45"S	28° 9'52.30"E
Mpongwe (MPO)	13°20'31.08"S	28° 5'0.49"E
Lake Itezhi Tezhi (ITT)	15°46'9.07"S	26° 1'14.33"E
Kafue Hook Bridge (KHB)	14°56'29.60"S	25°54'46.77"E
Kafue Bridge (KB)	15°50'16.49"S	28°14'12.48"E
Luangwa River (LUA)	14°58'36"S	30°12'45"E

OCPS AND NPE EXPERIMENTAL

Materials and Methods

HPLC grade acetone, obtained from Chromasolv, Sigma-Aldrich. HPLC grade Acetonitrile, and distilled water. Pesticides standards:4,4 DDT(1,1,1-trichloro-2,2-bis[p-chlorophenyl]ethane, Methoxychor,β-Endosulfan,α-Endoulfan,4,4 DDE(1,1-dichloro-2,2-bis[p-chlorophenyl]ethylene, Aldrin , 4,4 DDD (Ethane, 1,1-dichloro-2,2-bis(p-chlorophenyl))-, o,p DDT, o,p DDE, hexachlorobenzene, , Naphthalene and Catechol were used as internal standards(IS) (all obtained from Sigma-Aldrich, Germany) QuEChERS extraction tubes (containing 50mg primary and secondary amines (PSA) and 150 mg Magnesium Sulphate, were purchased from Supelco (Bellefonte, PA, USA). Stock standard solutions of 2.00 mg/g of each pesticide were prepared in acetonitrile and stored in -20°C. A pesticide intermediate mixture standard solution (10 mg/kg) was prepared by diluting the stock standard solutions with acetonitrile. The internal standards naphthalene and catechol were prepared in acetonitrile.

Instrumentation

GC-MS

The separation of the pesticides was performed on a 5975C gas chromatography mass selective detector (GC/MSD) system (Agilent technologies, USA), with Triple-Axis HED-EM Detector (Agilent technologies, USA) as seen in Figure 1.0. The gas chromatography system was equipped with a split-splitless injector and a 7683-auto sampler (Agilent Technologies, USA). An HP-5MS (5% phenyl methyl

siloxane), fused silica capillary column 30 m x 320 μm x 0.25 μm (film thickness) (J&W Scientific, Torrance, CA, USA) was employed in the separation of the analytes. Helium was used as the carrier gas at a flow rate of 0.5 mL/min.

Mass Spectrometry

Full scan (m/z 50-600) GC/MS acquisition was performed at 1 scan sec^{-1} . The ionization mode was electron ionization (EI) using a filament operated at 70 eV, with an emission current of 34 μA . A solvent delay of 4 minutes was set to avoid damaging the filament. The mass spectra were compared with those in the NIST/EPA/NIH Mass spectral library, Version 2005 (Newfield NT, USA) using the Automated Mass Spectral Deconvolution and Identifications System (AMDIS) developed by the National Institute for Standards and Technology (NIST) spectral library search. The identities of compounds were also confirmed by comparing their mass spectra and retention times with those obtained for the respective standards.

Sampling

Sampling was done between the periods of August, 2014 and November, 2015. Soil, sediment, and crops samples were collected from agricultural areas along the Kafue River in Zambia. They were dried at room temperature during two days and sieved, obtaining particle sizes <2mm. Finally, the soil was stored at 4 °C. River water samples were also collected since it is used for irrigation of the crops. Crops samples were also collected from the farms, four vegetable samples and a cereal. These were Pumpkin Leaves, Chinese Cabbage Leaves, Rape (Kale) Leaves, Tomato Fruits and Maize Corn.

OCPs Extraction

The commercial kits (Agilent Technology, USA) were used and the manufacturer's instructions were followed. The crops samples were cut and blended (Lab scientific, USA) until a homogenate consistency was reached. 10g of the sample was then weighed and taken for further analysis (Lehotay, 2004)

OCPs GC MS conditions

The injector temperature was held at 230°C, a 1 μL volume was injected in splitless mode, and the carrier gas was helium at a flow rate of 1.7 mL/min. The oven temperature was initially 50°C for 1 min, increased at 20°C/min to 180°C, increased at 10°C/min to 190°C, increased at 3°C/min to 240°C, and finally increased at 10°C/min to 300°C and held for 5 min.

Mass spectrometric parameters: The ion source temperature of the instrument was 200°C, the MS transfer temperature was 280°C, and the solvent delay was 5 min. Ionization was by EI with electron energy of 70 eV. The analysis was done in the SIM (selected ion monitoring) mode based on the use of one quantification and two identification ions. Pesticides were identified according to the retention times, the quantification, and identification ions. The quantification was based on the peak area ratio of the quantification ion divided by the internal standards.

Phenol Extraction

10g sediment was weighed and 10ml water added followed by 10mL Acetonitrile. The mixture was shaken for 1 hour using a shaker. 6g MgSO₄, 1.7g CH₃COONa, 4g NaCl were added to the resulting mixture and this was shaken for 30 sec. The mixture was centrifuged at 5000rpm for 5 min. To 1.5ml aliquot, 0.75mg MgSO₄ was then added then shaken for 30 sec. To 500 μL of the upper layer, 50 μL of acetic anhydride was added for derivatization and this was shaken for 2 min and it was ready for GC injection. The method was adopted from Sanchez *et al*, 2010.

Phenol GC Parameters

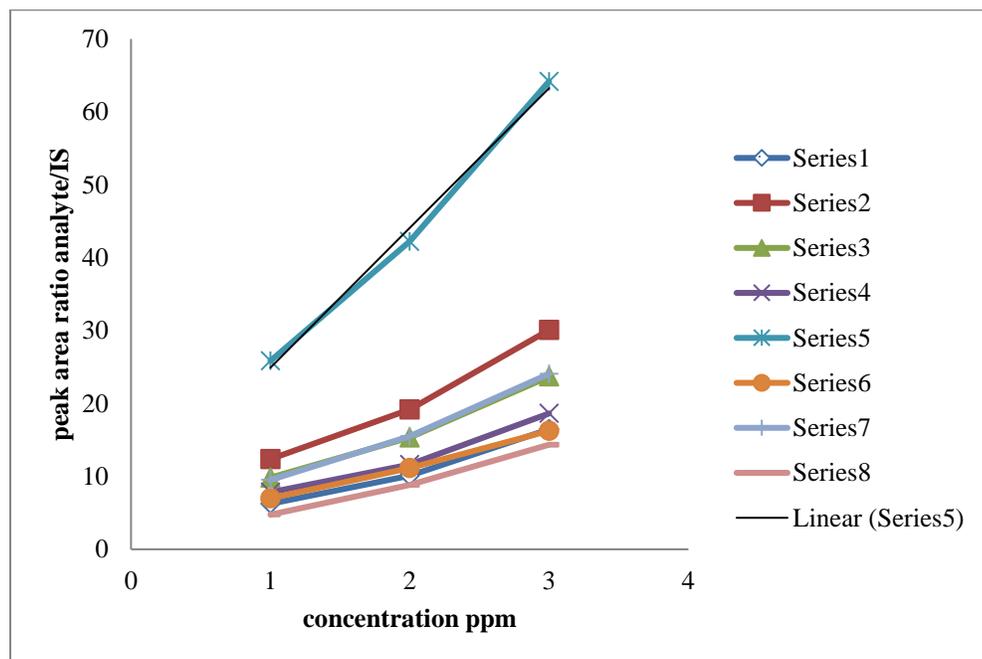
Aliquots of 10μL were injected into the GC system. The injector temperature program was as follows: 70 °C (hold for 0.5 min) to 310 °C (100 °Cmin⁻¹, hold for 10 min). The injector split ratio was initially set at 10:1. Splitless mode was switched on at 0.5 min until 3.5 min. At 3.5 min, the split ratio was 100:1 and at 10 min, 20:1. The column oven program was as follows: 70 °C (hold for 3.5 min) to 300 °C (20 °Cmin⁻¹) to 300 °C (hold 4min).

OCPS AND NPE RESULTS

Table 2: Showing Instrument limits of detection and percentage recoveries for OCP

Figure 3: OCPs Calibration curves

OCPs	regression equation	R ²	instrument LOD(ppm)	percentage recoveries		
				water	Soil	crops
OFN	y = 5.1073x + 0.7228	0.9961	0.45	46	92	65
HCB	y = 8.8541x + 2.8349	0.9827	0.24	22	22	45
Aldrin	y = 7.2779x + 1.8005	0.9897	0.24	77	79	68
Endosulfan I	y = 5.4063x + 1.884	0.972	0.37	76	76	75
DDE	y = 19.171x + 5.7371	0.993	0.05	77	77	77
Endosulfan II	y = 4.6344x + 2.1854	0.9961	0.2	117	117	98
DDD	y = 7.2779x + 1.884	0.9961	0.17	119	119	99
Methoxychor	y = 4.7983x - 0.2862	0.9927	0.42	92	92	90



Serie1=p p, DDE, Series 2=HCB, Series 3=Aldrin, Series 4=Endosulfan I, Series5=Endosulfan II, Series 6=DDD, Series 7=OFN, Series 8=Methoxychor

Table 4: Instrument limits of detection and percentage recoveries and instrument detection limits for phenols

Peak #	alkyl phenol		instrument LOD(ppm)	percentage recoveries	
	regression equation	R ²		Water	soil
Phenol	$y = 588071x + 32842$	R ² = 0.9997	0.17	68	78

Figure 5: OCP standards and retention times

Standard	Retention Time (min)
OFN	4.90
Naphthalene	5.37
HCB	9.84
Aldrin	13.40
Endosulfan I	16.16
DDE	17.25
Endosulfan II	18.57
DDD	19.09
Methoxychor	23.73

Figure 6: Phenol calibration curve

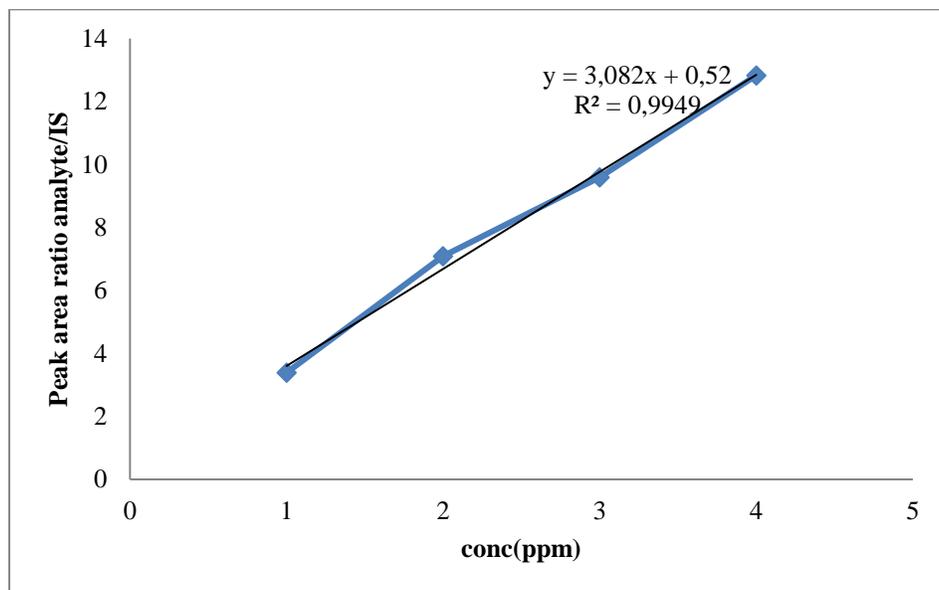
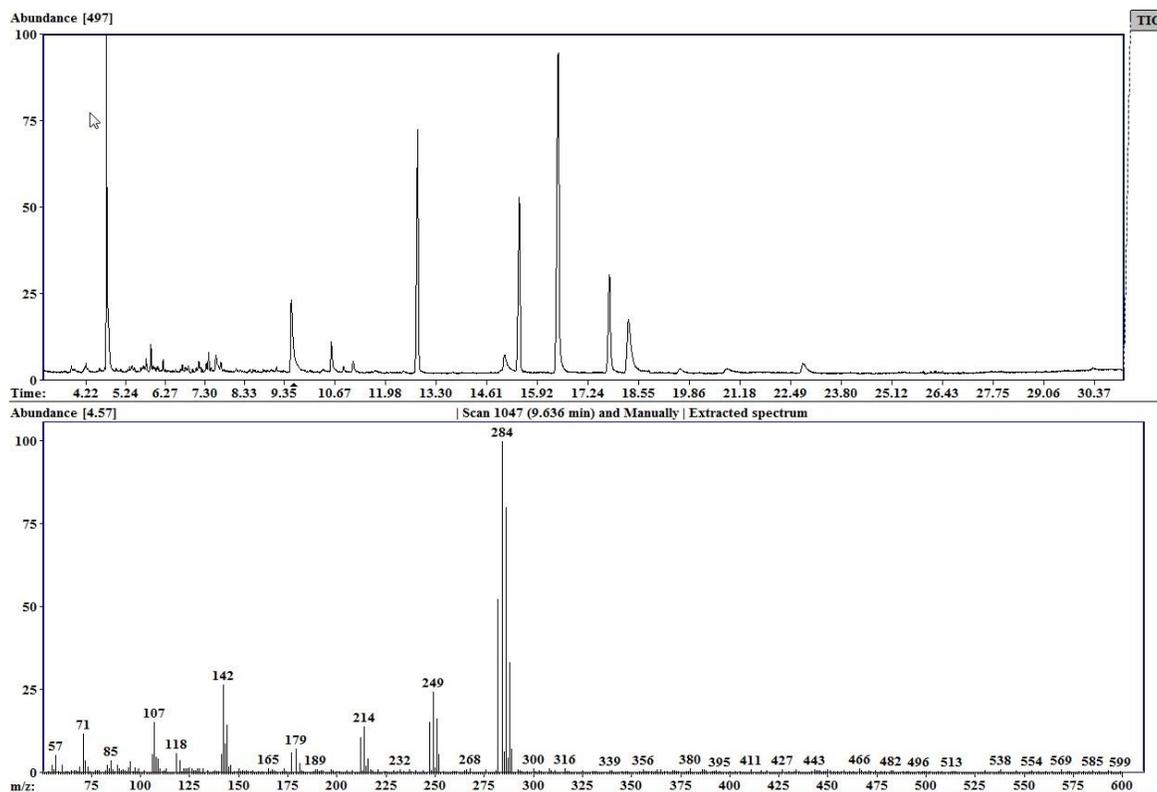


Figure 7: AMDIS chromatogram (Top) and spectrum mass spectrum of HCB extracted at 9.636 min of a crop sample spiked with internal standard and calibration standards



Real Sample Results

Table 8 : Results of water, sediments and crops from the Kafue River

Location	Sample	OCPs	Phenol
HIPO	WATER	ND	ND
	SOIL	ND	ND
	CROPS	ND	ND
MUF	WATER	ND	ND
	SOIL	ND	ND
	CROPS	ND	ND
CHIL	WATER	ND	ND
	SOIL	ND	ND
	CROPS	ND	ND
KB	WATER	ND	ND
	SOIL	ND	ND
	CROPS	ND	ND
KF2	WATER	ND	ND
	SOIL	ND	ND
	CROPS	ND	ND
IT	WATER	ND	ND
	SOIL	ND	ND
MPO	WATER	ND	ND
	SOIL	ND	ND
LUA	WATER	ND	ND

*ND (Not Detected)

Discussion

From the data obtained there were no OCPs and NPEs that was detected in the samples that were analysed. These results imply that there's is no potential risk posed by these OCPs and phenol to humans and wildlife, that could possibly bioaccumulate and eventually bioconcentrate via the trophic level. The greatest danger and exposure of humans is via the food chain through consumption of contaminated crops and other foods. The obtained result could be explained by a possible biodegradation of the NP this was speculated by Ekelund et al. (1993) whose studies of estuaries showed that significant amounts of APEO remain in both sediment and the water column. Biodegradation of APEO and NP was clearly possible, but low, particularly if conditions are anoxic or anaerobic.

When animals ingest NP, the absorption of nonylphenol from the gastrointestinal tract is initially rapid, and probably extensive. The major metabolic pathways are likely to involve glucuronide and Sulphate conjugation. Nonylphenol is distributed widely throughout the body, with the highest concentration in

fat. Available data on bioaccumulation potential from both animal and human studies are inconsistent and do not allow for conclusions on the bioaccumulation potential of NP. The major routes of excretion of NP are via the feces and urine (WHO,2002).

In the environment, the long-chain NPEs biodegrade relatively quickly to short-chain NPEs and NP, which are much more resistant to further degradation. When in water, NPEs can also undergo photo-induced degradation. In the atmosphere, NP will be degraded rapidly by hydroxyl radicals and is not expected to be persistent in air.

Other investigations of NP levels in rivers have found values varying between 2 $\mu\text{g l}^{-1}$ in Delaware River, Philadelphia to 10 $\mu\text{g l}^{-1}$ in the Rhine and 1000 $\mu\text{g l}^{-1}$ in a tributary of the Savannah river, USA, below a wool laundry (BUA, 1991). Levels of NP and NP1EO in the sediments of USA rivers were up to 3 mg kg^{-1} and 0.17 mg kg^{-1} respectively (Naylor et al., 1992).

Whitehouse *et al* (1998a and 1998b) also reported that the species *Mysidopsis Bahia* and fathead minnow *Peepholes promelas* affected by nonylphenol concentrations of 43 $\mu\text{g l}^{-1}$ to 135 $\mu\text{g l}^{-1}$. With regard to bioaccumulation, Whitehouse *et al* (1998a) found bioaccumulation factors for aquatic organisms to be around 300. However, much higher values were found for algae (but this may have been due to adsorption) and when radio-labeled nonylphenol was used. The accumulation of nonylphenol in sediments though bioavailability is considered to be low; nonylphenol has been found to have endocrine disrupting effects in freshwater organisms at concentrations of 20 $\mu\text{g l}^{-1}$.

Organochlorine pesticides are widely used for control of pest and widely used in agricultural applications. These are not easily detected due to their properties such as vapor pressure and partition volumes (Torstermson 1989; Kerle *et al.*, 2007). Most of the studies that have been done for method development and also to improve the detection of the pesticides using various instruments. This study results rules out the possibility of getting contaminated from ingesting or drinking the water from the sampled location has these were not detected. OCPs have been linked to various health implications if ingested or inhaled and endocrine disruption and cancers being some of them (Hernik *et al.*, 2014).

Their health implications depend on the specific pesticide, the level of exposure (dose), the timing of exposure and the susceptibility of the exposed individual (Xavier *et al.*, 2004). Many OCPs have been linked with a broad range of adverse human health and effects, including impaired reproduction, endocrine disruption, immunosuppression and they are also considered as carcinogenic substances (Pardio *et al.*, 2012. Gourounti *et al.* 2008). Studies have showed that DDE may disrupt the action of certain hormones associated with an increased risk of cancer to the human reproductive system, this exposure to DDT has been linked to cancers of the testicles, ovaries, prostate, pancreas and female breasts (ASTDR, 2008; Hernik *et al.*, 2014, Burananatrevedh and Roy, 2001). The mechanism of action by which some OCPs elicit their effects is by disrupting the sodium/potassium equilibrium of the nerve fiber in arthropods like insects and mammals this imbalance leads to hyper excitability and convulsions. A breast cancer research, revealed that the cancer patients blood had DDT and its metabolites (DDD & DDE), dieldrin, heptachlor,

HCH, and its isomer this was is irrespective of age, diet and geographic locality when compared to normal women (Mathur *et al.*, 2002).

Diabetes, neurodegenerative diseases such as Parkinson's, Alzheimer's, mental dysfunction, including memory loss are some of the other health effects. Cox *et al.*, 2007, found that self-reported diabetes was significantly associated with serum levels of β -HCH, p, p DDT and p, p DDE, suggesting that higher serum levels of certain Organochlorine pesticides may be linked with prevalence of diabetes. Studies have also reported the impacts of prenatal exposure to DDT on children's neuro-development (Pardio *et al.*, 2012; Eskenazi *et al.*, 2006).

The highest concentrations of pesticide permissible in food or feed is referred to as the Maximum residue levels (MRLs) these have been used as a means of controlling levels of pesticide levels in food and other consumable product safeguarding the consumers. The standard MRLs in the EU legislation is 0.01 mg/kg for some toxic pesticides (Camino-Sanchez *et al.*, 2011). MRLs are determined by considering the food intake, the average body weight of human beings and the pesticide residue levels under agricultural practices. Different countries have different MRLs making it difficult to have standard international MRLs due to the fact that in different countries the daily intake of particular food may differ. Despite, the legal framework that have established by some countries for regulation of pesticide place (FAO/WHO, 2004), some developing countries still do not have such a framework in place.

Factors such as water solubility, volatility, hydrophobicity, polarity, polarizability, molecular size and their ionizability determine their environmental fate (Torstermson 1989; Gevao *et al.*, 2000). Other factors affecting mobility of pesticides include; vapour pressure, pesticide sorption and other environmental conditions like rainfall, wind, temperature, weather, topography, soil organic matter, texture and structure (Torstermson 1989; Kerle *et al.*, 2007). OCPs are reported to have high affinity for soil due to high values of Log Kow and Log Koc, hence they are retained more in this medium (Noegrohati, 2008). Soluble pesticides are likely to move through water and the degree of plant uptake is determined by pesticide's water solubility (Kerle *et al.*, 2007). Air may be affected as well due to pesticides that volatize or due to air drift and wind erosion. Since, temperature affects vapor pressure, volatilization of pesticides is mainly determined by its vapor pressure has a result of the OCPS volatility (i.e. high vapor pressures), they will move through air at high temperatures. Pesticide persistence is often expressed in terms of their half-life, which is the time required for original concentration to break down to its half. If the pesticide has a longer half-life, it will have greater potential to be transported to long distances in the environment. Many OCPs have been identified as prone to long range transport and they also have long environmental half-lives, in the order of 10-20 years, which makes them to persist for many years (Jones & de Voogt, 1999).

Conclusion

QuEChERS can simultaneously be used for analysis of OCPs and NPE and the in the samples analysed the two groups of pollutants were not detected. QuEChERS also proved to be a robust method that could be used for the detection of the pollutants and also that it can be used in a laboratory facility that does not have sophisticated equipment. It requires simple readily available glassware and the technique is also low cost in that apart from buying commercially available kits, the salts can also be reconstituted as was done when analyzing for phenols.

Acknowledgements

The authors would like to thank the Copperbelt University, the University of Botswana and the Organization for Prohibition of Chemical Weapons (OPCW) through its research fellow exchange program for material support.

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