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Organochlorine pesticide residues in sediments from the Uganda side of Lake Victoria

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ABSTRACT

Organochlorine (OC) residues were analysed in 117 sediment samples collected from four bays of the Uganda side of Lake Victoria. The sediments were collected with a corer at a depth of 0–20 cm, and extracted for OC residues using a solid dispersion method. The extracts were cleaned using gel permeation chromatography and analysed for pesticide residues using a gas chromatograph (GC) equipped with an electron capture detector. The results were confirmed using a GC equipped with a mass spectrometer (MS). A total of 16 OC residues, most of them persistent organic pollutants (POPs) were identified and quantified. The OC residue levels were expressed on an oven dry weight (d.w.) basis. Endosulphan sulphate, in the range of 0.82–5.62 µg kg $^{-1}$ d.w., was the most frequently detected residue. Aldrin and dieldrin were in the ranges of 0.22–15.96 and 0.94–7.18 µg kg $^{-1}$ d.w., respectively. DDT and its metabolites lay between 0.11–3.59 for p.p'-DDE, 0.38–4.02 for p.p'-DDD, 0.04–1.46 for p.p'-DDT, 0.07–2.72 for 0.p'-DDE and 0.01–1.63 µg kg $^{-1}$ d.w. for 0.p'-DDT. The levels of γ -HCH varied from 0.05 to 5.48 µg kg $^{-1}$ d.w. Heptachlor was detected only once at a level of 0.81 µg kg $^{-1}$ d.w., while its photo-oxidation product, heptachlor epoxide, ranged between non-detectable (ND) to 3.19 µg kg $^{-1}$ d.w. Chlordane ranged from ND to 0.76 µg kg $^{-1}$ d.w. Based on the threshold effect concentration (TEC) for fresh water ecosystems, aldrin and dieldrin were the only OCs that seemed to be a threat to the lake environment.

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1. Introduction

Organochlorine pesticides in the environment are characterised by high chemical stability, poor water solubility and low vapour pressures (Bouwman, 2004). Consequently they are commonly referred to as persistent organic pollutants (Darko et al., 2008). In East Africa, OC pesticides have been in use since the 1940s and have tended to accumulate in soil (Madadi et al., 2006; Ssebugere, 2010) and sediment (Werimo et al., 2009). Owing to ingestion of sediment, OC pesticides and their residues find their way into edible fish (Licata et al., 2003; Ogwok et al., 2009; Ssebugere et al., 2009). Health risks associated with OC metabolites are well established (Engel et al., 2000; Garry, 2004). As such, out of the 21 POPs that have been ear-marked for phase-out and elimination, fourteen are OC pesticides; dichlorodiphenyltrichloroethane (DDT), aldrin, endrin, dieldrin, chlordane, heptachlor, hexachlorobenzene (HCB), mirex, toxaphene, alpha hexachlorocyclohexane, beta hexachlorocyclohexane, chlordecone, lindane and pentachlorobenzene (Stockholm Convention, 2001, 2009).

In Uganda, the use of pesticides especially in ensuring the sustainability of large quantities of high quality agricultural produce has been steadily increasing over the past half century (Kasozi et al., 2006). Lake Victoria basin has been particularly singled out as the region with the greatest number of activities involving the use of pesticides (NEMA, 2000). The lake, the second largest freshwater body in the world, has an area of 68 800 km², a mean depth of 40 m and an irregular shoreline of about 3440 km in length lying in catchments of about 184 000 km² (Payne, 1986; Wikipedia, 2009). The Victoria Nile at Jinja in Uganda is the lake's only single outlet. The lake basin experiences an equatorial climate, supporting extensive subsistence and commercial farming. In addition to being a source of domestic and industrial water, the lake is believed to contain 350 different fish species (Kwetegyeka et al., 2008) and provides an estimated 220 000 metric tonnes of fish annually for Uganda alone (Ogwok et al., 2009). In the past few decades, however, the lake's productivity has been severely compromised by increased industrial and agricultural activity in its basin, resulting in increased polluted urban runoffs, nutrient and pesticide load, and wetland encroachment (Nyenje et al., 2010). As a result, nearly half of the lake floor currently experiences prolonged anoxia for several months of the year, compared to the 1960s when such anoxia was sporadic and localised (Hecky et al.,

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2010). Concerns over increased pollution as a result of the above activities have led to efforts being made to address the root causes in order to mitigate this environmental impact. Emphasis has been laid on microbiological, eutrophicational, inorganic chemical and suspended solid causes of pollution due to enhanced effluent discharge (Odada et al., 2004). Little, however, has been done to assess the pesticide residue input to the lake's current state of pollution.

The present paper reports and assesses the levels of OC pesticide residues in sediments from Lake Victoria, Uganda. The results might prompt the relevant authorities to put in place appropriate deterrent measures to safeguard the sustainability of the lake.

2. Materials and methods

2.1. Study area

The area of study was the Uganda side of Lake Victoria (Fig. 1). The lake stretches across the equator at 00° 30′N–03° 00′S and 31° 39′W–34° 53′E. This area has undergone rapid ecological changes resulting from agricultural practices that have led to massive deforestation within the vicinity of Lake Victoria. The lake shores, which were historically fringed by extensive papyrus (*Cyperus papyrus*)-dominated wetlands, have been degraded by a rapidly rising human population (Rusongoza, 2003), putting mounting pressure on the lake in terms of sedimentation, water pollution (Mbabazi et al., 2010), algal blooming (Nyakairu et al., 2010) and overfishing. Four

bays of the lake, namely, Murchison, Napoleon, Thurston and Waiya (Fig. 1) were selected as suitable sites for the study because of the large human settlements, industrial and agricultural activities within their immediate vicinities. The sampling points were referenced using a geographical positioning system (GPS).

2.2. Sample collection and preservation

A total of 117 sediment samples were collected at quarterly intervals from 2003 to 2007 inclusive. Sediments were collected in triplicate at 0–20 cm depths per sampling site, because sediments at this level are expected to be the most contaminated, and have the greatest potential for exchange with the water column (Nowell et al., 1999). Sediment composition was not uniform for the four bays. In the Napoleon Gulf and Thurston bay, the sediment was dark and rich in organic matter, whereas in Waiya bay it contained a lot of sand. At Murchison bay, much of the sediment was clay. The samples were taken using a sediment corer, wrapped in aluminium foil and placed in labelled, air-tight bags. They were kept in ice-coolers and transported to the laboratory where they were kept at $-20\,^{\circ}\text{C}$ before extraction.

2.3. Sediment extraction procedure

Sediment samples were extracted for pesticides residues using a dispersion method (Åkerblom, 1995), Gravel was removed from

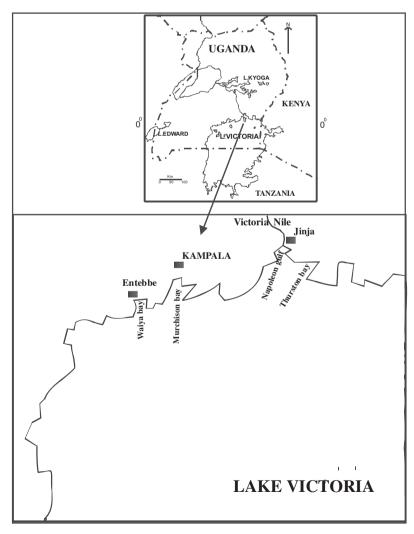


Fig. 1. Schematic map of Uganda showing location of study sites.

each sample prior to extraction. After the sample had been thoroughly homogenised, two portions (10 g each) were separately weighed. The first portion was placed in a pre-weighed petridish and left overnight to dry in an oven kept at 105 °C. After cooling, the portion was reweighed to obtain the dry weight. The second portion was ground with sodium sulphate (40 g), and the powder extracted by successive shaking with 1:1 (v/v) acetone/cyclohexane (50, 30, 20, 20 mL). The combined extract was filtered into a separation funnel and the filtrate shaken with saturated sodium chloride solution (200 mL). The mixture was then left to separate and the aqueous layer further extracted with ethyl acetate/cyclohexane (50 mL; 15:18 v/v). The organic layer obtained from the second extraction was combined with that obtained from the first in an Erlenmeyer flask containing sodium sulphate (20 g). The flask was shaken and the sodium sulphate left to settle. The organic phase was then decanted through glass wool into an evaporating flask and the sodium sulphate washed with ethyl acetate/cyclohexane (20 mL, 15:18 v/v) system which was then passed through the same glass wool. The organic solvents were then evaporated off at 40 °C to dryness using a vacuum rotary evaporator. The extract was dissolved in cyclohexane/ethyl acetate (2 mL; 1:1 v/v), for clean up.

2.4. Clean up of extracts

The sediment extract clean up was performed using gel permeation chromatography (GPC). The GPC consisted of a 50 cm \times 1 cm *i.d.* chromatographic tube, two adapters, Teflon tubing and a sixway valve. Calibration of the GPC was done using ethion and p,p'-DDT as indicator substances. These substances were detected in volumes collected between the 14–25 mL. The extract (1 mL) was loaded on the GPC and eluted at a rate of 1 mL min⁻¹ using ethyl acetate/cyclohexane (1:1 v/v). The final fraction (14–26 mL) collected was carefully concentrated to dryness with nitrogen gas and dissolved in cyclohexane (1 mL) for GC analysis.

2.5. Gas chromatographic analysis

OC residues were analysed using a Varian (CP-3800, Palo Alto. CA, USA) gas chromatograph, equipped with an electron capture detector (ECD) and fitted with a DB-1 non-polar capillary column $50 \text{ m} \times 0.53 \text{ mm}$ i.d. The column was later interchanged with a CP-Sil 19 CB semi-polar one of 30 m \times 0.53 mm i.d. The GC temperature was initially set at 90 °C for 1 min, raised to 180 °C at a rate of 30 °C min⁻¹, then to 260 °C at 4 °C min⁻¹ and finally maintained at that temperature for 10 min. The temperatures of the injector and ECD detector were 230 °C and 300 °C, respectively. The injection volume was 1 μL. Reference pesticide standards were supplied by Dr. Ehrenstorfer GmbH (Augsburg, Germany). Standard solutions for OC pesticides were also obtained from Protocol Analytical and Ultra Scientific Companies (USA). Confirmation of OC residues was done using a GC/MS (Agilent model 6890N) equipped with a quadruple Agilent 5975 inert XL mass selective detector and a fused silica capillary column (HP-5MS). Inter laboratory analysis of the samples was done at the Food and Environmental Toxicology Laboratory of the University of Florida, USA, using a Perkin Elmer Auto System with helium and nitrogen as the carrier and make up gases, respectively. Further confirmatory tests for selected samples were done on GC/MS, at the same laboratory, using an ion trap detector (ITD) in electron ionisation mode (EI). A full scan mode was selected for identification purposes.

2.6. Quality control procedures

Recovery tests were conducted by spiking sediment samples (200 g) with a standard solution equivalent of 10 μ g of aldrin, α -endosulphan, p,p'-DDT and p,p'-DDE. The sediments had been pre-

Table 1Data for computation of lower levels ($\mu g kg^{-1} d.w. \pm SD$) of detection (LLD = $3.29 \times SD$).

<u> </u>		
Component	Mean ^a	LLD
α-Endosulphan	0.0153 ± 0.0025	0.0082
β -Endosulphan	0.0182 ± 0.0018	0.0060
Endosulphan sulphate	0.0172 ± 0.0018	0.0061
Total endosulphan Aldrin Dieldrin Chlordane α -HCH β -HCH γ -HCH	0.0507 ± 0.0061 0.0143 ± 0.0024 0.0148 ± 0.0023 0.0148 ± 0.0016 0.0150 ± 0.0017 0.0163 ± 0.0021 0.0133 ± 0.0016	0.0079 0.0076 0.0052 0.0056 0.0070 0.0053
Total HCH Heptachlor Heptachlor epoxide	0.0446 ± 0.0054 0.0161 ± 0.0018 0.0164 ± 0.0004	0.0060 0.0014
Total heptachlor p,p'-DDT o,p'-DDT o,p'-DDE p,p'-DDE p,p'-DDE p,p'-DDD	0.0325 ± 0.0022 0.0169 ± 0.0014 0.0166 ± 0.0007 0.0141 ± 0.0023 0.0153 ± 0.0015 0.0140 ± 0.0011	0.0046 0.0022 0.0075 0.0051 0.0038
Total DDT	0.0769 ± 0.0070	

a Results for n = 5.

viously analysed and found not to contain these pesticides. The samples were extracted, cleaned up and analysed as described in this section and the amount recovered calculated. Four sets of experiments for each pesticide were performed. Detection limits, the lowest concentration of the analyte that can be detected above the noise from blank chromatograms, were estimated by considering the lower level of detection (LLD) defined as the concentration that produces a signal sufficiently large that 99% of the trials with that amount will produce detectable signals. LLDs for individual components were determined according to Eaton et al. (1995), and are presented in Table 1.

3. Results and discussion

The data was collected every three months throughout the period 2003-2007. This represented the typical equatorial two wet and two dry annual seasonal variations. Fig. 2 shows the mean seasonal variation of the OC levels in sediments over the 4-year period. In general, it was only aldrin and dieldrin that exhibited significant seasonal dependence. A total of 16 different OC residues were identified (cf. Fig. 3), confirmed (cf. Fig. 4), quantified, and compared to freshwater sediment quality assessment criteria as shown in Table 2. All the OC residue levels lay in the range ND-15.96 μg kg⁻¹ d.w. According to these guidelines, a value below the threshold effect concentration (TEC) should not have a harmful effect on sediment dwelling organisms (Burton, 2002). Endosulphan sulphate, the most frequently detected residue, was in the range of $0.82-5.62 \,\mu\mathrm{g \, kg^{-1}}$ d.w., with greatest and least values being recorded in Waiya and Murchison bays, respectively. Endosulphan sulphate is the major degradation product of technical endosulphan which is a mixture of two isomers (α and β) in the ratio of 70–80% α to 30–20% β , w/w (Montgomery, 2000; Leonard et al., 2001). The detection frequencies of the α - and β -isomers in all the samples were 11% and 9%, respectively. Both isomeric endosulphans recorded higher values in the wet seasons than in the dry seasons. No sediment guidelines were available for endosulphan (Pfeuffer, 2008). However, the trend in values of endosulphan isomers suggested increased input of endosulphan into the lake system during wet seasons. Endosulphan was registered for use in

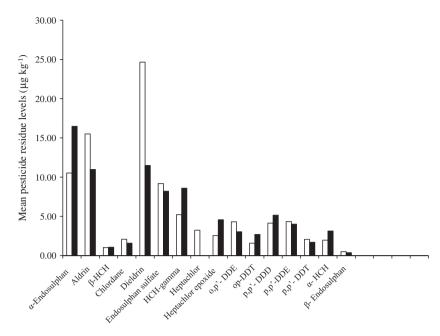
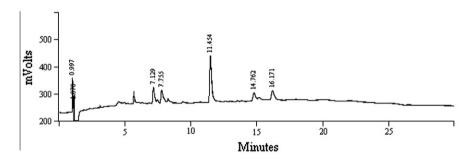


Fig. 2. Mean seasonal variation of OC residue levels in sediments.



Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1		6.4036	0.997	0.000	284298	0.00	BV	1.2		0
2		5.4319	1.078	0.000	241160	0.00	VV	0.6		0
3		12.6913	7.129	0.000	563452	0.00	PV	9.1		0
4	g-HCH	13.2332	7.755	0.025	587509	0.00	PV	10.9		0
5		42.4451	11.454	0.000	1884422	0.00	BB	9.4		0
6	endo. II	8.6756	14.762	0.232	385169	0.00	BV	12.9		0
7	pp-DDT	11.1193	16.171	0.051	493659	0.00	BB	13.8		0
	Totale	100 0000		0.308	4430660					

Fig. 3. Typical OC pesticide residue chromatogram for sediment obtained in the Murchison bay of Lake Victoria.

the country (Kegley et al., 2009) under the trade names thiodan and thionex, but has had an abusive history. In 1998, fish exports from Lake Victoria to the European Union were temporarily banned following observations of tainted fish, which were later proved to have been harvested using endosulphan (European Commission, 1999). The frequencies of detection for aldrin and dieldrin in the samples were, respectively, 8% and 24%. The largest variation in dieldrin levels was found in the Napoleon gulf, with values ranging from 0.22 to 15.96, and an overall mean of 3.80 ± 3.55 SD µg kg⁻¹ d.w. Dieldrin, which is also the major metabolite from the microbial oxidative or epoxidative degradation of aldrin (Montgomery, 2000), was registered for restricted use in Uganda. It was used in the control of tsetse flies, termites and banana weevils within the country until 1989 (NEMA, 2000). Dieldrin was the major pesticide used against Banana weevils. However, its use was stopped when there was a widespread

destruction of Banana plantations in Masaka [central Uganda]. The destruction of banana plantations was attributed to the use of either adulterated Dieldrin or the use of an entirely different chemical imported as dieldrin. Dieldrin was then substituted with furadan and, so it was claimed, the unused dieldrin stocks were subsequently dumped in the environment by farmers. However, a recent inventory on pesticide use within Uganda (Wasswa, 2009) has shown a 6% response use of dieldrin among the commonly used pesticides in the country, suggesting that the pesticide was still being used by farmers to control various pests. On the other hand, the maximum detected aldrin level was 7.18 µg kg⁻¹ d.w. from Thurston bay (Table 2) with 8% detection frequency. Aldrin and dieldrin mean values were greater than the TEC, suggesting that the two residues pose a threat to the lake environment. According to the "Pesticide Action Network" (PAN) registration database for Uganda (Kegley et al., 2009), aldrin was not among

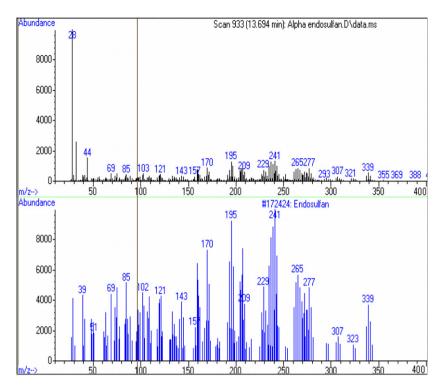


Fig. 4. Typical GC-MS spectrum for α -endosulfan pesticide residue in sediment obtained in the Thurston bay of Lake Victoria [sample (upper) matched against its spectrum from the library].

Table 2 Mean pesticide residue levels (μ g kg⁻¹ d.w. ±SD) in sediments in Lake Victoria, Uganda.

Component	Detection frequency (%)	Range	Median	Mean ^a	TECb
α-Endosulphan	10.63	0.69-9.67	3.40	3.73 ± 2.87	NA ^{d,e}
β-Endosulphan	9.38	0.03-0.22	0.09	0.10 ± 0.06	NA ^{d,e}
Endosulphan sulphate	41.88	0.82-5.62	2.01	2.17 ± 1.43	NA ^{d,e}
Total endosulphan				6.00 ± 4.36	
Aldrin	8.13	0.94-7.18	2.61	3.16 ± 1.85	2.00 ^e
Dieldrin	24.38	0.22-15.96	2.81	3.80 ± 3.55	1.90 ^f
Chlordane	3.13	ND^{c} -0.76	0.37	0.45 ± 0.23	4.50 ^f
α-НСН	3.13	ND ^c -1.27	0.71	0.66 ± 0.41	6.00 ^g
β-НСН	8.13	0.04-0.66	0.23	0.26 ± 0.18	5.00 ^g
γ-НСН	15.63	0.05-5.48	1.83	1.88 ± 1.41	2.37 ^f
Total HCH				2.80 ± 2.00	
Heptachlor	0.63	0.81	0.81	0.81 ± 0.02	1.009
Heptachlor epoxide	14.38	ND^{c} -3.19	0.59	0.86 ± 0.82	2.47 ^f
Total heptachlor				1.67 ± 0.84	
p,p'-DDT	26.25	0.04-1.46	0.39	0.43 ± 0.38	4.20 ^t
o,p'-DDT	3.13	0.01-1.63	0.40	0.62 ± 0.65	4.20
o,p'-DDE	3.75	0.07-2.72	0.65	0.92 ± 0.96	3.20
p,p'-DDE	30.00	0.11-3.59	0.75	1.06 ± 0.94	3.16
p,p'-DDD	26.88	0.38-4.02	0.80	1.21 ± 0.90	3.54
Total DDT				4.24 ± 3.83	

^a Mean values for the 117 samples analysed in triplicate.

the registered pesticides for use in the country. Kasozi (2002) reported that the mean levels of aldrin in surface water from Napoleon gulf were 0.0031 ng mL⁻¹ while the levels of dieldrin were 0.0054 ng mL⁻¹. Earlier studies within Uganda had detected dieldrin in human adipose tissues in the Kampala area, in human

and cow milk, and in the Lake Kyoga water sediment system (Sserunjogi, 1974; Wassermann et al., 1974; Ejobi et al., 1996a,b). The levels of DDT and its metabolites ranged between 0.11–3.59, 0.38–4.02, 0.04–1.46, 0.07–2.72 and 0.01–1.63 µg kg⁻¹ d.w. for p,p'-DDE, p,p'-DDD, p,p'-DDT, o,p'-DDE and o,p'-DDT,

^b Sediment quality guidelines that reflect threshold effect concentration (TEC) below which harmful effects are unlikely to be observed.

c ND = not detected.

d NA = sediment quality assessment guideline had not been developed.

e Pfeuffer (2008).

f MacDonald et al. (2000).

^g Burton (2002).

respectively. Among the metabolites, p,p'-DDE recorded the highest frequency of detection (30%), followed by p,p'-DDD (27%) and p,p'-DDT (26%). Other metabolites were in most cases not detected, with o,p'-DDE being detected with a frequency of 3.75% only in Waiya bay. It is known that p,p'-DDT is the principal isomer of technical DDT, although its structure permits the formation of several different isomeric forms (Montgomery, 2000). Technical DDT contains, w/w, the constituents: p,p'-DDT, 77.1%; o,p'-DDT, 14.9%; p,p'-DDD, 0.3%; o,p'-DDD, 0.1%; p,p'-DDE, 4%; o,p'-DDE, 0.1% and unidentified products, 3.5% (IPCS, 1989). DDE and DDD generally resist further chemical and biological degradation (Montgomery, 2000). This phenomenon is usually used as an indicator for the time lapse of DDT usage. Normally, [p,p'-DDT]/[DDT metabolite] ratios >0.5 indicate recent DDT usage. Therefore, higher detection frequencies together with higher concentration levels of the p,p'-DDT isomer compared to the other isomers would seem to imply a recent exposure of the lake sediment to DDT. However, ratios of p,p'-DDT to p,p'-DDD and p,p'-DDE in this study (0.36 and 0.41, respectively) suggested past inputs of DDT into Lake Victoria sediments. All mean values of the DDT isomers and metabolites detected were lower than the TEC (Table 2). Although it is reported to have been gradually abandoned owing to pest resistance and international concern, DDT seems to have continued to be secretly used by farmers (NEMA, 2000). In a recent interview (Wasswa, 2009), 2% of the farmers confessed to using DDT. Its residues have also been detected in fish samples from Lake Edward (Ssebugere et al., 2009), as well as in soil samples in western Uganda (Ssebugere et al., 2010).

The γ -HCH values were highest in Napoleon gulf (5.48 μ g kg⁻¹ d.w.), while the respective levels in Murchison, Waiya and Thurston bays were 4.22, 3.35 and 2.91 μ g kg⁻¹ d.w. However, the other isomers lay in the range ND-1.27 for α -HCH and 0.04–0.66 µg kg⁻¹ d.w. for β -HCH. The detection frequencies for γ -HCH, α -HCH and β -HCH were 15.6%, 3.1% and 8.1%, respectively, with the mean values being below the TECs. Lindane has been one of the major pesticides used to control cotton-boll worm. However, cases of its abusive use have been reported especially in catching fish and killing bird pests (NEMA, 2000). There have also been reports of aerial deposition onto the lake water of lindane isomers together with α -endosulphan and the DDTs (Wejuli et al., 2002). The other OC pesticide residues detected in Lake Victoria sediments (this work) were heptachlor, heptachlor epoxide and chlordane. Heptachlor was detected only once in Napoleon gulf at a level of 0.81 µg kg⁻¹ d.w., whereas its photo-oxidation product, heptachlor epoxide, ranged from ND to 3.19 μ g kg⁻¹ d.w. (Table 2). Chlordane varied from ND to $0.76 \,\mu\mathrm{g\,kg^{-1}}$ d.w. with a detection frequency of 3%. Heptachlor and chlordane, whose mean concentration values were below the fresh water sediment quality assessment guidelines (TEC), are not registered for use in Uganda. Although there is no history on the use of chlordane in the country, reports suggest that heptachlor has been used to dress beans and maize seeds (NEMA, 2000). As a result, these pesticides find their way into the soil and subsequently into the lake.

4. Conclusions

The results from this study reveal that the levels of the 16 OC pesticide residues/metabolites detected within the lake sediment were in the range ND $-15.96~\mu g~kg^{-1}~d.w$. Endosulphan sulphate was the most frequently detected OC residue. The other OCs identified included dieldrin, aldrin, DDT isomers (p,p'-DDT o,p'-DDT) and its metabolites (p,p'-DDE, p,p'-DDD, o,p'-DDD), lindane isomers (γ -HCH, α -HCH, and β -HCH), heptachlor, heptachlor epoxide and chlordane. However, it should also be noted that Lake Victoria receives drainage from five East African states with the Victoria Nile

at Jinja in Uganda as the lake's only single outlet. Therefore the possibility that the data herein discussed might incorporate residues from these other countries cannot be discounted. Based on the threshold effect concentration (TEC) for fresh water ecosystems, aldrin and dieldrin were the only OCs that seemed to be a threat to the lake environment.

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