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Organochlorine pesticide residues in soil from sugarcane plantations in Kilimanjaro, Tanzania

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ABSTRACT

Soil samples from the Tanganyika Planting Company (TPC) sugarcane plantations in Kilimanjaro, Tanzania, an area of intensive pesticide application, were analysed for historic and current-use pesticide contamination. Twenty eight samples were collected from 7 stations within and outside the plantations during the dry and the rainy seasons. Solid-liquid extraction and gel-permeation chromatography methods were used before analysis of pesticides by GC-ECD and GC-MS. Blank and spiked recovery tests were used to validate the analytical procedure. DDT and its metabolites (*p,p'*-DDD and *p,p'*-DDE), aldrin, dieldrin, heptachlor, heptachlor epoxide, HCHs (-α, -β, -δ and -γ isomers), and chlordane were detected in more than 90% of the samples analysed in concentrations ranging from below detection limits (bdl) to 745.9 ng/g dw. The detection and concentration trends were ∑DDT > ∑HCH > ∑heptachlor > dieldrin > γ-chlordane > aldrin. The highest concentration values were obtained during the dry season and in the application areas. The presence of organochlorine pesticide residues at concentrations reported in this study two decades after cessation of their use emphasizes the need for continuous monitoring and risk assessments.

Keywords: Organochlorine pesticides, soil, sugarcane plantations, environmental contamination.

INTRODUCTION

Organochlorine pesticide residues are regarded as an important class of contaminants, owing to their toxicity, persistence and bioaccumulation (Den Hond et al., 2003). Although the application of these chemicals was banned or restricted in many countries more than two decades ago, their residues and degradation products are still being detected in water, air, soil, sediments and biota at significant levels, not only in the previous application sites, but also in some remote, non-application areas (Guzzella et al., 2005; Concha-Graña et al., 2006; Zhou et al., 2006; Sarkar et al., 2008).

Soil is the primary reservoir of pesticides. This may be through direct application of the chemicals to the soil surface, incorporation in a few inches of the soil surface or off-target drifting during application to crops. Once in the soil, pesticides may undergo a series of transformation and distribution processes. These transformation processes may have a biotic or abiotic origin and cause the degradation of pesticides through several mechanisms such as oxidation, reduction, or

hydrolysis (Berkowitz et al., 2008). The distribution of pesticides can originate from various pathways, including volatilization, leaching, runoff, and absorption by plants. In these processes, the physico–chemical properties of pesticides and the adsorption–desorption equilibrium in soil are the main factors involved (Sánchez-Brunete et al., 2008; Schrecket et al., 2008). Other factors such as land-use practices, type of the soil and the climatic condition of the area determine the fate of pesticides and their degradation products in the soil (Walker et al., 1999). Prolonged pesticide applications in an area may therefore lead to pesticide residues accumulation in soil and other components of the environment (Carabias-Martinez et al., 2003). The major concerns regarding pesticides in soil include the possible carryover of the pesticides and their biologically active degradation products to crops grown in later seasons, biological effects on organisms in terrestrial and aquatic ecosystems, including bioaccumulation and transfer through food chains, surface and groundwater contamination, and effects to soil fertility (Kishimba and Mihale, 2004; Hildebrandt et al., 2008;).

The Tanganyika Planting Company (TPC) sugarcane estate in Kilimanjaro region, northern of Tanzania is one of the oldest large scale pesticide users in the country. TPC was established in 1930 initially as a sisal estate, which was converted into sugar estate in 1932. Pesticides have been intensively applied in the estates since early 1940s, following devastating insect pest outbreaks that caused serious yield losses. Analysis of the yield data from TPC indicated losses of up to 25% during periods of intense pest infestations (TPC, 2002). More than twenty types of pesticides, including organochlorines, organophosphorus, carbamates and pyrethrine insecticides; and different types of herbicides such as oxadiazon were used in the sugarcane fields for more than five decades. The types and amounts of pesticides used kept on increasing over the years. Some of them, like Ethylene Dibromibe (EDB), become non-effective due to development of pest resistance and others like DDT and other organochlorines were banned from use due to environmental concerns.

Despite its over fifty-year history of intensive pesticide application, no study has assessed the soil quality of TPC sugarcane plantations and its environs. This work investigated the status of pesticide contamination in the area by establishing the levels of pesticide residues, their mobility trends, spatial and seasonal distribution trends, and effects of various physicochemical parameters in soil samples. The findings obtained may be useful as baseline information for future monitoring programs.

MATERIALS AND METHODS

Sampling site

The TPC estate is located at about 50 Km South of Mount Kilimanjaro, at Arusha Chini wetlands and Kahe plains. The sugarcane plantation covers an area of about 14,000 ha of cultivated land surrounded by nine villages, namely Chekereni, Kiyungi, Kikavu, Newland, Mtakuja, Msitu wa Tembo, Mserekia, Samanga and Mikocheni, with a shortest distance of about 1 km from the sugarcane farms. Also, there are five camps where workers and their families live. Information about types, duration of use and pesticide application methods at the estate was gathered during field survey.

Seven sampling sites (Figure 1) were selected from which 28 samples (each ~ 100 g) were collected in two sampling campaigns. Sampling sites were selected from the application areas (sugarcane plots) and some locations of increasing distances from the application areas. Sample collection was done using standard methods (Åkerblom, 1995). Samples were packed in cold boxes and transported to the Department of Chemistry, University of Dar es Salaam where they were kept frozen at 4 $^{\circ}$C prior to extraction.

Sample preparation and extraction

Water content in the soil samples was determined by heating 20 g of the samples at

105 °C for 12 h. The samples were then cooled to room temperature and re-weighed to evaluate the loss-on-ignition. The percentage weight losses were equated to the water content in the soil samples. Samples for pesticide residues analysis were homogenized, screened and extracted by the solid-liquid extraction method as described by Åkerblom (1995). Sub-samples of 20 g were put in stoppered flasks, added with 14 ml of 0.2 M ammonium chloride, swirled and left for 15 min to open up the soil structure. 100 ml cyclohexane/acetone (1:1 v/v) solutions were then added to the mixture, shaken vigorously for 1 min and then less vigorously about every 10 min for at least one hour and passed through ultrasonic bath for 5 min. Water was then added cautiously to fill the stoppered flask. The organic phases were transferred using a pipette to E-flasks containing 20 g sodium sulphate, and then decanted through glass wool into evaporation flasks. The sodium sulphate was then rinsed with 30 ml acetone and decanted through the same glass wool plug. The aqueous phases were then discarded. The sample extracts were concentrated in a BÜCHI vacuum rotary evaporator at 30 °C, and the solvent changed to cyclohexane/ethyl acetate (1:1 v/v) for clean-up by gel permeation chromatography (GPC), using the method described by Åkerblom (1995). Cleaned sample extracts were concentrated to 2 ml in cyclohexane/ acetone (9:1 v/v) and kept in teflon-stoppered glass sample vials ready for GC analysis.

Sample analysis

The sample extracts were analyzed by a Varian Star 3400 gas chromatography equipped with ^{63}Ni electron capture detector (ECD) at the Department of Chemistry, University of Dar es Salaam, Tanzania. Non-polar (SE-30) and semi-polar (OV-1701) capillary columns of dimensions 30 m x 0.32 mm x 0.25 μm liquid thickness were used. Nitrogen was used as both a carrier and make up gas, flowing at the rate of 30 ± 1 ml/min. The injector and detector temperatures were set at 250 °C and 300 °C respectively. The column temperature was initially set at 90 °C, held for 1 min and then raised to 180 °C at the rate of 30 °C/min. It was further raised to 260 °C at the rate of 4 °C/min and maintained at this temperature for 12 min.

Pesticide residues were identified and quantified by using external reference standards. Retention times and peak heights of the detected residues were compared to those of the reference pesticides. Parathion was used as an internal reference standard for the determination of compounds' relative retention times. Identified peaks were confirmed by analyzing the same sample extracts by a Hewlett Packard model 5890 GC-MS with CP-Sil 19 CB and CP-Sil 5 CB columns, 20 m x 0.32 mm i.d. x0.25 μm liquid thickness (Chrompack Sverige AB, Nacka Sweden), at the Department of Environmental Assessment, SUAS, Sweden). Helium was used as a carrier gas at 1.9 ml/min. Hydrogen and air were flowing at 120 and 100 ml/min respectively. The injector and detector temperatures were set at 270 °C and 300 °C respectively. The column temperature was initially set at 70 °C, held for 1 min and then raised to 230 °C at the rate of 30 °C/min. It was further raised to 280 °C at the rate of 4 °C/min and maintained at this temperature for 10 min.

Quality control and quality assurance

All reagents used were of analytical grade, and the solvents were of chromatographic grade. Pesticide standards solutions (99% certified purity) were ordered from Dr. Ehrenstorfer (Augsburg, Germany). Sodium chloride and anhydrous sodium sulphate were heated at about 400 °C for two hours and allowed to cool to constant temperature in desiccators to remove interfering substances before use. Glassware used had Teflon caps and were cleaned with detergents and distilled water, thoroughly rinsed with acetone and dried overnight in an oven before use.

The method detection limits (MDLs) of the organochlorines were determined as the concentrations of analytes in a sample that

gave rise to a peak with a signal-to-noise ratio (S/N) of 3. Soil spiked with target compounds were used for evaluation of the detection limits. Blank tests and recovery tests were conducted to check for interference and cross-contamination (Hill, 2000). For every batch of 7 samples analysed, 1 laboratory-grade sand was analysed as a blank sample, and 1 laboratory-grade sand spiked sample were simultaneously analysed. The method validation was done by spiking laboratory-grade sand with mixtures of organochlorine pesticide standard solutions of known concentrations (50–300 ng/l), that were extracted, cleaned-up and analysed as the ordinary samples. The percentage recoveries were calculated as the ratio of the actual concentrations obtained after GC analysis to the added (spiked) concentrations.

Statistical analysis

Mean concentrations of pesticide residues in soil were calculated using a statistical package INSTANT®. Multiple comparison with one way ANOVA were used to test the significant differences of pesticide concentrations among the sampling sites and across the two seasons (α=0.05).

RESULTS

Water content, spiked recoveries and blank tests

Water contents in the soil samples from each sampling site for the two seasons are summarized in Figure 2. The water contents were higher during the rainy season (11.8 – 23.2%) than during the dry season (4.0 – 12.9%). This was expected since during rainfalls the soil is wetter than during the dry season. The mean percentage recoveries and detection limits of the quantified organochlorines are summarized in Table 2. The mean recoveries (68.8 ± 1.3 to 88.7 ± 0.9%) were within the acceptable range of 70 – 120% (Åkerblom, 1995); and the blank reference samples had no traces of the targeted analytes. These data confirmed the practicability of the analytical protocols herein employed in the determination of organochlorine pesticide residues in the soil samples.

Concentrations of organochlorine pesticide residues in the soil samples

The results of the analysis showed the presence of 12 organochlorine pesticide residues and metabolites in the soil samples. The concentration ranges and means of the compounds detected are summarized in Table 3. The mean concentrations ranged from 0.7 ± 1.3 (γ-Chlordane) to 170.4 ± 266.8 ng/g dw (*p,p'*-DDT).

Spatial and seasonal variations of organochlorine pesticide residues in soil

The distribution of various organochlorine pesticide residues in the soil samples from the 7 sampling stations showed a wide range of variation as summarized in Table 4. The concentration trend was ∑DDT>∑HCH>∑heptachlor>dieldrin> γ chlordane>aldrin. The detection frequencies of DDTs and HCHs were 100% of the samples analysed, indicating a wide occurrence of the compounds. Aldrin, dieldrin and chlordane were detected in samples from the sugarcane fields only (S1, S2 and S3). Total organochlorine pesticide levels varied from 25.9 to 1547.8 ng/g dw, with the highest concentrations detected at site S3 and the lowest at S7.

Figure 4 compares the total concentrations of the pesticide residues quantified during the dry season with those of the rainy season. It was observed that in each of the site, concentrations and detection frequencies were higher during the dry season than during the rainy season. This might be attributed to the fact that during the dry season the water content in the soil is low and therefore allows more pesticides to reach bare soil, whereas during rainfall pesticides are washed out from the soil surface and taken away by the water flow.

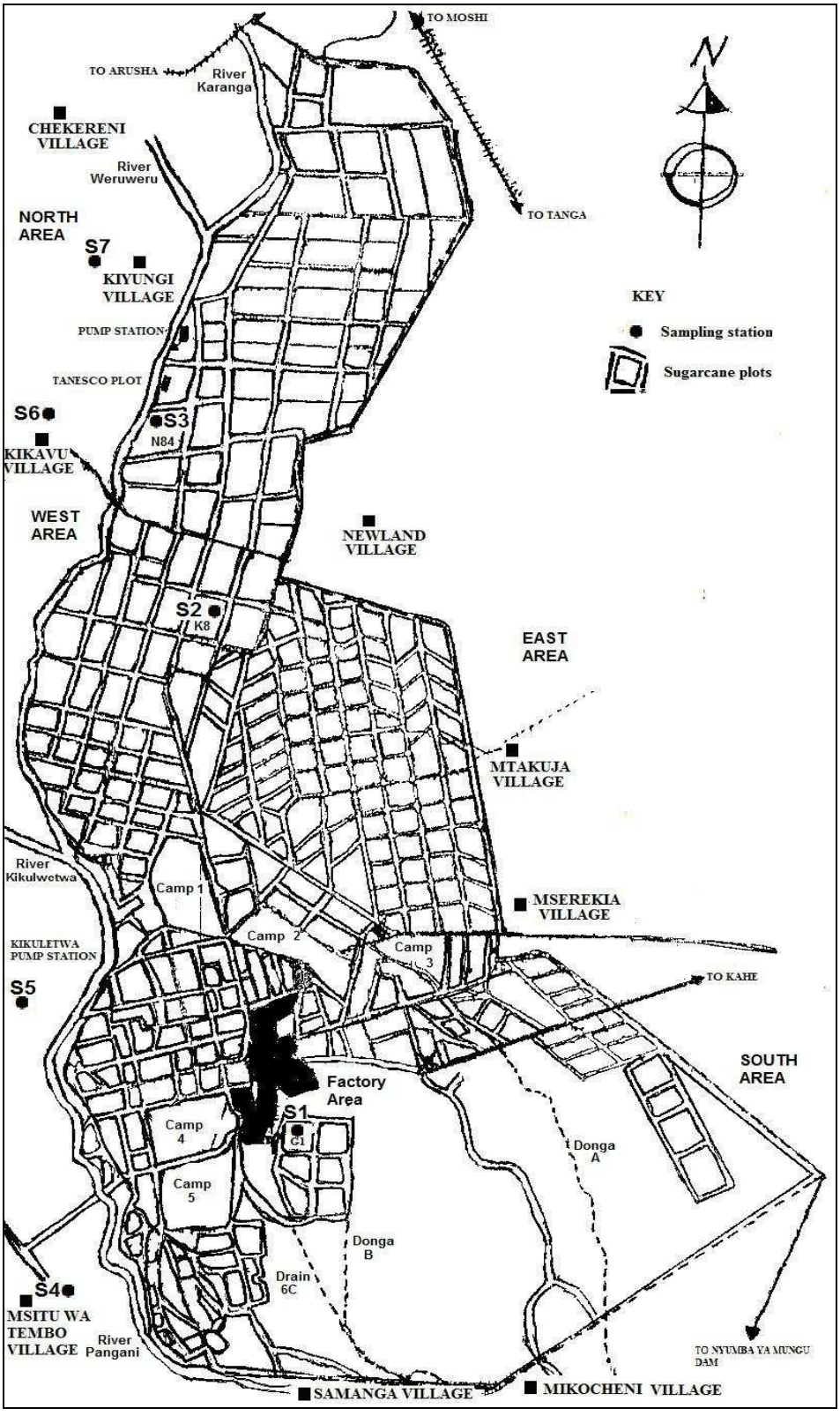

Figure 1: Map of TPC sugarcane plantations showing the sampling stations.

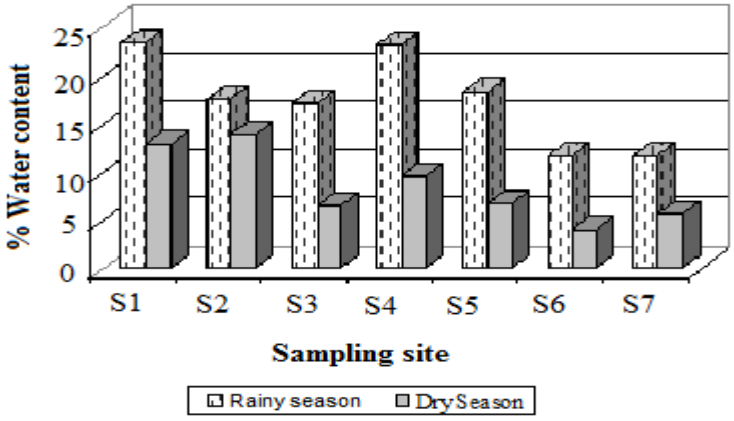

Figure 2: Percentage water contents in soil samples.

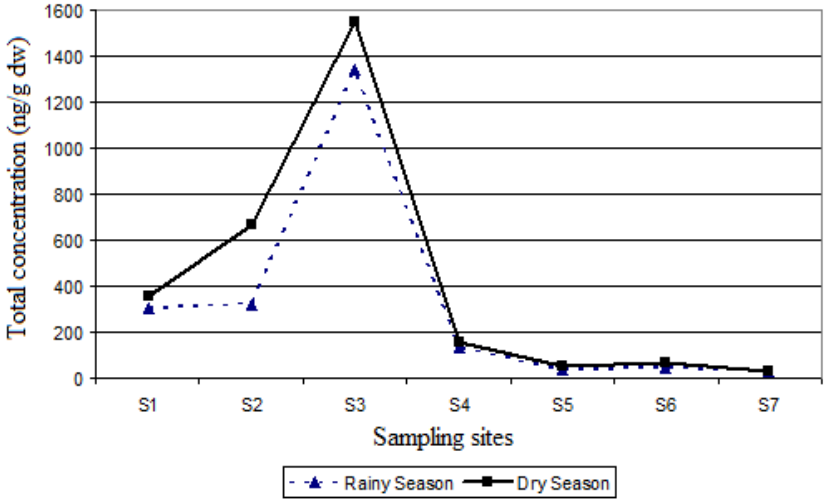

Figure 4: Seasonal variations in pesticide residues' concentrations.

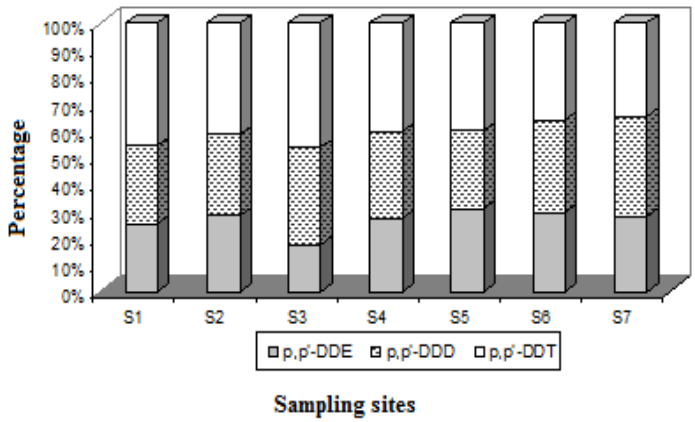

Figure 3: Contribution of individual metabolites to the total DDT concentrations in TPC soil Samples.

Table 1: Sampling sites.

No.	Code	Site name	Site description
1.	S1	G1	Sugarcane plot
2.	S2	K8	Sugarcane plot
3.	S3	N84	Sugarcane plot
4.	S4	Msitu wa Tembo	Non-target area
5.	S5	Kikuletwa	Non-target area
6.	S6	Kikavu	Non-target area
7.	S7	Kiyungi	Non-target area

Table 2: Mean percentage recoveries and detection limits of the analyzed organochlorines obtained from the 4 recovery tests.

Pesticide	% Mean recovery ± SD (n = 4; CL 95%)	Detection limits (ng/g dw)
α-HCH	71.5 ± 0.9	0.1
β-HCH	76.0 ± 0.8	0.3
γ-HCH	81.7 ± 1.7	0.1
δ-HCH	74.0 ± 2.4	0.2
Aldrin	75.2 ± 2.5	0.2
Heptachlor	75.7 ± 1.2	0.4
Heptachlor epoxide	68.8 ± 1.3	0.5
γ-Chlordane	74.5 ± 0.6	0.2
Dieldrin	76.2 ± 2.6	0.2
p,p'-DDE	78.3 ± 0.9	0.3
p,p'-DDD	77.2 ± 1.7	0.4
p,p'-DDT	88.7 ± 0.9	0.5

dw – dry weight

Table 3: Pesticide residues detected in soil samples from TPC plantations and environs (n= 7, CL = 95%).

Pesticide	Rainy season (ng/g dw)		Dry season (ng/g d.w)	
	Range	Mean ± SD	Range	Mean ± SD
α-HCH	<0.1 – 37.3	11.5 ± 12.8	<0.1 – 43.9	12.7 ± 15.1
β-HCH	2.5 – 51.4	16.5 ± 17.2	1.3 – 62.7	17.7 ± 20.9
γ-HCH	<0.1 – 23.9	9.4 ± 9.1	<0.1 – 25.2	9.6 ± 8.2
δ-HCH	<0.2 – 3.5	1.2 ± 1.4	<0.2 – 4.8	1.9 ± 2.2
Aldrin	<0.2 – 2.0	0.6 ± 0.8	<0.2 – 3.4	0.8 ± 0.8
Heptachlor	<0.4 – 5.2	1.8 ± 1.9	<0.4 – 8.4	2.4 ± 4.8
Heptachlor epoxide	<0.5 – 13.5	2.6 ± 4.8	<0.5 – 23.2	3.9 ± 8.5
γ-Chlordane	<0.2 – 3.6	0.7 ± 1.3	<0.2 – 5.9	1.4 ± 2.1
Dieldrin	<0.2 – 13.9	3.6 ± 5.9	<0.2 – 16.8	4.7 ± 7.7
p,p'-DDE	8.4 – 217.2	55.8 ± 74.6	6.5 – 220.4	77.0 ± 90.9
p,p'-DDD	9.0 – 456.7	93.0 ± 162.1	4.9 – 486.3	108.9 ± 172.9
p,p'-DDT	24.6 – 578.4	120.6 ± 205.2	11.2 – 745.9	170.4 ± 266.8

Table 4: Distribution of organochlorine pesticide residues in soil from TPC (n= 7, CL = 95%).

Site	Rainy season (Conc. ng/g dw)							
	*∑HCH	*∑DDT	*∑Heptac.	Aldrin	Dieldrin	Chlord.	Total OCPs	$\frac{DDE + DDD}{\sum DDT}$
S1	110.7±1.3	194.1±0.8	2.7±0.5^{A}	bdl	bdl	bdl	307.5±2.6	0.54
S2	64.3±1.0	237.3±1.6	5.8±0.4	2.0±1.3^{A}	10.5±1.3	bdl	319.9±5.6	0.58
S3	47.9 ±0.7	1252.2±1.0	18.7±1.0	1.5±0.2^{A}	13.9±1.3^{A}	3.6±0.4	1337.8±4.6	0.54
S4	26.5±0.5	106.1±1.1	bdl	bdl	bdl	bdl	132.6±1.6	0.59
S5	11.6±0.5	27.2±1.2	bdl	bdl	bdl	bdl	38.8±1.7	0.59
S6	8.6±0.6	37.5±0.7	bdl	bdl	bdl	bdl	46.1±1.3	0.64
S7	2.5±0.2^{A}	33.1±1.3	bdl	bdl	bdl	bdl	35.6±1.5	0.65
Dry season (Conc. ng/g dw)								
S1	136.6±1.1	214.9±1.9	2.5±1.0^{A}	bdl	bdl	1.3±0.8^{A}	355.3±4.8	0.53
S2	62.1±1.0	583.5±3.4	2.0±0.3	2.1±1.1^{A}	16.8±1.0^{B}	1.9±0.7^{A}	668.4±7.5	0.58
S3	35.9±0.5	1452.6±2.1	36.4±0.8	1.8±0.7^{A}	15.2±0.9^{A,B}	5.9±0.5	1547.8±5.5	0.40
S4	24.1±0.8	129.6±1.0	bdl	bdl	bdl	bdl	153.7±1.8	0.61
S5	17.3±0.5	41.0±0.8	bdl	bdl	bdl	bdl	58.3±1.3	0.55
S6	14.2±0.3	52.7±1.2	bdl	bdl	bdl	bdl	66.9±0.8	0.59
S7	2.3±0.4^{A}	22.6±1.1	bdl	bdl	bdl	bdl	25.9±1.5	0.50

bdl = below detection limit; * For summation bdl was assigned a value of zero; ∑HCH = α-HCH + β-HCH + γ-HCH + δ-HCH; ∑DDT = *p,p'*-DDT + *p,p'*-DDE + *p,p'*-DDT; ∑Heptac. = heptachlor + heptachlor epoxide; Chlord = γ-Chlordane.

DISCUSSION

The detection of organochlorine pesticide residues in soil at this area is associated with the past applications of the chemicals in the sugarcane plantations. From Table 3, it can be observed that the concentrations of DDTs were much higher than the rest of the organochlorines. The ability of pesticide residues to bind to soil particles depends on their lipophilicity, which is estimated by their octanol-water partition coefficient (K_{ow}) (Scheunert, 1992). The high concentrations of DDT and its metabolites may be due to the fact that they have the longest half-life and the greatest partition coefficient, thus they are the most persistent (Hites and Day, 1992). Furthermore, their extremely low solubility in water and heavy applications causes them to be retained and accumulated to a greater degree in the top soil layers (Tao et al., 2005).

Few studies on soil contamination with pesticide residues in agricultural areas are available in Tanzania. One such study was conducted in Lake Victoria basin, an intense cotton growing area with a long-term history of organochlorine pesticides application. The study revealed the presence of *p,p'*-DDT and its metabolite *p,p'*-DDE in concentrations ranging between 2 – 12 ng/g in soil samples, which were lower than in the present study. The different pesticide residues concentrations observed in the two studies might be attributed to a number of factors such as soil properties, climatic conditions and pesticide application history and methods (Henry and Kishimba, 2003). The average composition of DDT compounds in the investigated soil samples had almost similar variations in all the sampling stations, except S3 which had the lowest levels of *p,p'*-DDE (Figure 3). The ratio of the degradation products to the sum DDTs that were greater than 0.5 (Table 4) indicates that the soil samples were dominated by the degradation products from past applications. DDT normally degrades under aerobic condition to DDE and under anaerobic condition to DDD (Tadeo, 2008). The ratio of (DDE + DDD)/ ∑DDT > 0.5 indicate long-term contamination with DDTs (Dimond and Owen, 1996).

Hexachlorohexane (HCH), commonly known as Lindane, was intensively used at TPC since 1946 before it was banned in early 1980s. Table 3 also shows that HCH isomers (α-HCH, β-HCH, γ-HCH and δ-HCH) were detected in the soil samples in concentrations ranging from 1.3 to 62.7 ng/g dw with a general trend of β-HCH>α-HCH>γ-HCH>δ-HCH. The β- isomer was the most abundant isomer, with an average 48% of the total HCHs. These findings are consistent with other studies that reported β-HCH as more resistant to hydrolysis and environmental degradation and thus the dominant isomer in soils (Zhang et al., 2002). δ-HCH is the least persistent of the HCH isomers and seldom found in environmental samples. The low ratio of γ-HCH to α-HCH indicates the past use of technical grade HCH that consists of only about 10 – 12% of γ-HCH and 70 – 80% of α-HCH (Tomlin, 2000).

Heptachlor and heptachlor epoxide were detected in samples from the sugarcane plantations only (Table 3). Concentrations of heptachlor were lower than those of heptachlor epoxide in all of the three sites. It has been established that in the natural environment heptachlor is mostly transformed through both biotic and abiotic processes to heptachlor-epoxide. Consequently, heptachlor is rarely found in environmental samples (Pandit et al., 2001).

Aldrin and dieldrin are two closely related organochlorines due to the fact that when aldrin is applied in the field, it is rapidly broken down to dieldrin (Scheunert, 1992). The overall frequency of detection of aldrin and dieldrin in the analysed soil samples were quite low (Table 3). Both compounds were simultaneously detected in only two of the sites. Concentrations of aldrin were lower than those of dieldrin, indicating the possibility of contribution from long-term degradation of aldrin.

The levels of organochlorine pesticide residues in TPC soils occurred in the order S2>S3>S1>S4>S6>S5>S7 (Table 4). This

shows that pesticide residues concentrations were significantly higher in soil samples from the application areas (S1 to S3) than from the environs (S4 to S7), thus indicating the sugarcane plantations as the source of these pollutants in the area. Similar observation was reported by Henry and Kishimba (2003) in which concentrations of organochlorine pesticide residues were higher in the soil from the cotton fields than the surrounding areas.

Conclusion

Standardized methods were applied for the analysis of organochlorine pesticide residues in soil samples from a sugarcane growing area in Tanzania. Twelve organochlorine pesticide residues and metabolites were detected in the analysed samples. Samples were characterized mainly by DDTs and HCHs which were detected in all of the samples. Heptachlor, chlordane, aldrin and dieldrin were detected in lower concentrations in only three of the sites. Different contamination patterns were observed among sampling sites, where the concentrations of OCPs were higher in soil samples collected from the sugarcane plantation than those from the surrounding areas. The detection of lower levels of parent compounds than the degradation products observed in all sampling stations implies that the contaminants are from past usage of the chemicals. Even though the levels of pesticides residues in the soil reported in this study are generally low, the fact that these chemicals still persist in the environment after a period of nearly two decades after cessation of use emphasizes the need for continuous monitoring and risk assessment. It is therefore recommended that further research should be conducted to determine the amount of pesticide residues in water and sediments, as well as put up mechanisms for monitoring residual levels in the ecosystem and food chain.

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