E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2018; 6(2): 285-289

www.fisheriesjournal.com
Received: 20-01-2018
Accepted: 23-02-2018

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A Preliminary investigation of organochlorine pesticide residue levels in fish and sediment from the Ashaiman Reservoir, Ghana

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Abstract
Fish and sediment samples were used as a case study to determine the levels of organochlorine pesticide residues in the Ashaiman Reservoir, Ghana. The fish species collected from the Reservoir included *Sarotherodon melanotheron*, *Tilapia guineensis*, *Tilapia zilli* and *Clarias gariepinus*. The samples were extracted using solid phase extraction and analyzed using a Gas Chromatography (GC) equipped with Electron Capture Detector (ECD). A total of 15 organochlorines (OCs) namely; β-HCH, δ-HCH, lindane, heptachlor, aldrin, dieldrin, endrin, α-endosulphan, β-endosulphan, endosulphan sulphate, methoxychlor, γ-chlordane, p,p′-DDE, p,p′-DDD and p,p′-DDT were analyzed. In both fish and sediment samples, all the organochlorine pesticides investigated exhibited non-detectable levels at a detection limit of 0.005 mg/kg. The levels of pesticide residues analyzed in the fish samples were below maximum residue limits specified by the European Union and Australia and thus not of appreciable health concern to consumers.

Keywords: Fish, sediment, organochlorine, gas chromatography, maximum residue limit

1. Introduction
Organochlorine pesticides (OCPs) have been used for a very long time in Ghana, both in agriculture and public health, with their residues evident in fish, water, sediments, and in humans among others [1]. In agriculture, pesticides have been used for crop pest control while in public health, they have been used for disease vector control and have significantly contributed to reducing the increase in malaria and livestock disease [2]. They have also been used in termite and tsetse fly control programs [3]. Due to the integral role played by pesticides in agriculture, it has been reported that generally, Ghanaian agriculture, especially vegetable farming is characterized by wrong and excessive use of pesticides [4].

The production and usage of most OCPs have been gradually limited and then banned since the 1970s in most developed nations due to their toxicity and persistence [5]. Ghana has also banned the importation, sales and application of OCPs. In spite of the ban, their usage is still evident in the country [6], and in other developing countries due to their relatively low cost and versatility in controlling various pests [7].

The pathways of OCPs into aquatic environments include runoff, atmospheric deposition, direct dumping of waste into water systems, leaching due to agricultural usage and improper disposal of sewage [5]. Once in the aquatic ecosystem, sediments serve as the major sink for these pesticide residues because they are less soluble in water. Aquatic biota like fish bioaccumulate these organochlorines (OCs) from the food they consume, suspended sediment and organic matter within the water. Fish absorbs pollutants excellently and therefore can be used to give a good indication of the level of pollution within an aquatic environment [8].

The Ashaiman Reservoir is surrounded by residential and agricultural facilities. Artisanal fishing activities do occur in the reservoir. The reservoir was created for the dual purposes of flood control and irrigation of adjoining agricultural fields, hence run-off of agro-chemicals from these fields and domestic waste from surrounding homes into the reservoir cannot be overruled. These materials have the potential to affect the quality of fish and sediment from the reservoir. This study was therefore carried out to provide baseline information on levels of OCP residues in fish and sediment from the Ashaiman Reservoir.

2. Materials and Methods

2.1 Study Area

The Ashaiman Reservoir is located on latitude 5°42'12.04"N and longitude 0°3'14.46"W (Fig. 1).

It forms part of the Ashaiman Irrigation Project. The reservoir stores about 5.6 million m^3 of water and is fed by the Dzorwulu River [9]. Although it was created for the purposes of irrigation and flood control, an important economic activity that has emerged is fishing. Also, the catchment areas of the reservoir have been turned into human settlements resulting in the depletion of the reservoir's vegetative cover. The encroachers also deposit domestic wastes into the reservoir, polluting the water and blocking the normal route of water for irrigation.

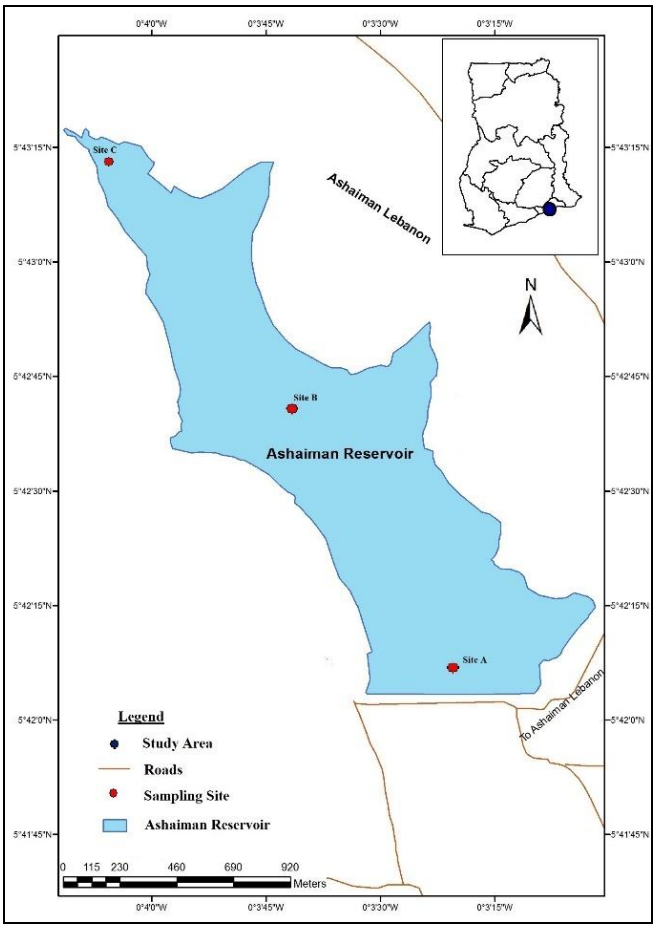

Fig 1: A map of the Ashaiman Reservoir showing sediment sampling sites.

2.2 Sample Collection

Sampling was conducted in January 2017. Sediment samples were grabbed at three locations in the reservoir; sites A, B and C (Fig. 1), using a pre-cleaned Orange Peel grab. At each sampling location, three grab samples were taken across the width and lumped together to form a composite sample. A total of 29 fish samples were obtained from artisanal fishermen. The different fish species were identified and the standard length and body weight of each individual was measured.

All samples were wrapped in aluminium foil, packed in clean zip lock bags and labelled. The samples were then transported on ice to the Pesticide Residue Laboratory of the Ghana Standards Authority (GSA) where they were stored at −20 °C prior to preparation for analysis.

2.3 Sample Preparation

In the laboratory, sediment samples were homogenized, oven dried at 105 °C until constant weight and passed through a 2 mm mesh. Representative sub-samples were then taken for analysis. The fish samples were removed from the freezer, thawed, washed several times with distilled water and descaled. The muscle tissue of the fish was then cut off, minced into smaller pieces and ground using a blender (Panasonic mixer grinder, MX-AC300, India). A homogeneous composite sample was prepared for each of the different fish species.

2.4 Sample Extraction

Extraction of OCPs in sediment was carried out by the EPA method 8081A, with some modifications. A 10 g of the representative sediment sample was transferred into a 250 ml separating flask and mixed with 10 ml of acetonitrile. The flask was corked and sonicated using an Ultrasonic bath (Grant XUB 18UK) for 5 min. Afterwards, a further 10 ml acetonitrile was added. The flask was corked again and placed in a horizontal mechanical shaker set to shake continuously for 30 min. This was followed by allowing the mixture to stand for 10 min to separate into phases. A 10 ml aliquot of the organic phase (top layer) equivalent to 5 g sediment weight was then pipetted into a 50 ml round-bottomed flask. This was then evaporated to 2 ml for extract purification using a Rotary film evaporator (Buchi Ratovapor R-210, USA) in a water bath (Buchi, B-491, USA) at 35 °C, and made ready for the clean-up step.

OCP residues in fish were extracted following the method described by Sun *et al*. [10] with some modifications. 15 ml of acetonitrile was added to 5 g of the sample in a 50 ml centrifuge tube and homogenized for 1 min using a homogenizer (Ultra Turrax, IKA® T25 basic, Germany). Afterwards, a mixture of 1.5 g of NaCl and 4 g of anhydrous $MgSO_4$ (extraction salt) was then added and vortexed for another 1 min. The sample was then centrifugated for 5 min at a speed of 4000 rpm using a centrifuge (Hermle, Z300, Germany). Separation of phases took place and a clear organic layer was formed.

Extracts from sediment samples were cleaned-up using Silica (1000 mg / 6 ml) cartridge with a 1 cm thick layer of anhydrous magnesium sulphate placed on top and conditioned using 10 ml of acetonitrile. The 2 ml concentrated extract was loaded onto the cartridge while a 50 ml pear-shaped flask was placed under the column to collect the eluate. The cartridge was then eluted with 10 ml acetonitrile as the extract was allowed to filter. The filtrate was concentrated below 40 °C just to dryness using a Rotary film evaporator (Buchi Ratovapor R-210, USA) in a water bath (Buchi, B-491, USA). This was followed by re-dissolving the extract in 1 ml ethyl acetate, and then carefully transferring it into a 2 ml standard opening vial prior to GC analysis.

Via a pipette, 6 ml of the organic layer obtained from the fish sample extraction was transferred into a 50 ml centrifuge tube containing 300 mg each of anhydrous $MgSO_4$, Discovery DSC-18 and PSA (Primary Secondary Amide). The content of the centrifuge tube was then vortexed for 1 min. The sample was then centrifugated for 5 min at a speed of 4000 rpm. During centrifugation, purification of the extract took place. 4 ml of the eluate was then transferred into a pear-shaped flask. To the 4 ml eluate, 40 µl of 5% formic acid in acetonitrile was added and concentrated at 38°C just to dryness using a Rotary film evaporator (Buchi Ratovapor R-210, USA) in a water

bath (Buchi, B-491, USA). The eluate was then re-dissolved in 1 ml ethyl acetate, to which 20 µl of ethylene glycol 200 was added using a micro-pipette. The residue was then concentrated to the base of the pair-shaped flask using an ultra-sonic water bath (Clifton, NE2-8D, UK) for effective mixing. A Pasteur pipette was then used to transfer 1 ml of the extract in the pear-shaped flask into an autosampler vial for GC analysis.

2.5 Gas Chromatography Analysis

OCP residues in fish and sediment samples were analyzed using a Gas Chromatograph (Varian, CP-3800, Varian Association Inc., Palo Alto, CA, USA) equipped with ^{63}Ni Electron Capture Detector (GC-ECD) and a CombiPAL autosampler. The analytical column was a 30 m + 10 m EZ Guard × 0.25 mm internal diameter fused silica capillary coated with VF – 5ms (0.25 µm film). The temperature of the injector operating in splitless mode was 270 ˚C, while the detector temperature was 300 ˚C. The temperature of the oven was initially set at 70 °C for 2 min, raised to 180 °C at a rate of 25 °C/min for 1 min and then to 300 °C at 5°C/min. Nitrogen was used as the carrier gas at a constant flow rate of 1 ml/min and the detector make-up gas at 29 ml/min. The injection volume was 1.0 µl.

2.6 Identification and Quantification of OCPs

The pesticides were identified based on comparison of the measured relative retention times to those of known standards. The residue levels of OCPs were quantitatively determined using the peak area. A standard mixture of known concentration of OCPs was run and the response of the detector for each compound ascertained. The peak areas whose retention times coincided with the standards were extrapolated on their corresponding calibration curves to obtain the concentration. The detection limit of the analyzed OC compounds was 0.005 mg/kg.

3. Results

3.1 Species Composition and Size of Fish Samples

Four fish species were obtained from the Ashaiman Reservoir for this study. These belonged to two families namely; Cichlidae and Clariidae. The Blackchin tilapia, *Sarotherodon melanotheron* (Rüppell, 1832), the Guinean tilapia, *Tilapia guineensis* (Bleeker, 1862) and the Redbelly tilapia, *Tilapia zilli* (Gervais, 1848) belonged to the Family Cichlidae whiles the African catfish, *Clarias gariepinus* (Burchell, 1822) was from the Family Clariidae (Table 1).

The mean standard length and mean weight of the fish species were respectively 8.2 ± 1.7 cm and 26.99 ± 14.92 g for *S. melanotheron;* 10.3 ± 2.3 cm and 48.87 ± 33.04 g for *T. guineensis;* 10.4 ± 5.8 cm and 74.24 ± 121.51 g for *T. zilli;* and 27.8 ± 4.1 cm and 246.45 ± 57.73 g for *C. gariepinus*. The clariid was bigger in size than the cichlids. Among the cichlids, however, *S. melanotheron* was smaller than *T. guineensis* and *T. zilli* (Table 1).

Table 1: Species composition, mean standard length (SL) and weight of fish samples.

Species	Family	Mean SL (± SD) (cm)	Mean Weight (± SD) (g)
Sarotherodon melanotheron	Cichlidae	8.2 ± 1.7	26.99 ± 14.92
Tilapia guineensis	Cichlidae	10.3 ± 2.3	48.87 ± 33.04
Tilapia zilli	Cichlidae	10.4 ± 5.8	74.24 ± 12.51
Clarias gariepinus	Clariidae	27.8 ± 4.1	246.45 ± 57.73
SD = Standard Deviation.			

3.2 Organochlorine Pesticide Residues in Fish and Sediment

A total of 15 OCP residues namely; β-HCH, δ-HCH, lindane, heptachlor, aldrin, dieldrin, endrin, α-endosulphan, β-endosulphan, endosulphan sulphate, methoxychlor, γ-chlordane, p,p′-DDE, p,p′-DDD and p,p′-DDT were analyzed in the fish and sediment samples. For all the four fish species, the concentrations of OCP residues were below the detection limit of 0.005 mg/kg (Table 2). Similarly, the concentrations of OCP residues were below the detection limit of 0.005 mg/kg in sediment from the three different sites (Sites A, B and C) in the reservoir (Table 3). From Table 4, OCP residue concentrations of < 0.005 mg/kg in the fish samples were far below the maximum residue limits (MRLs) set by the European Union (EU) and Australia.

4. Discussion

In this study, the Ashaiman Reservoir in Ghana, which was originally created for irrigation purposes and for flood control, has also turned into a habitat for a number of fish species dominated by the cichlids. The non-detection of OCPs in fish and sediment from the reservoir probably suggests that they are either not in use or are scarcely used in the catchment area of the water body. This could be due to the ban on the usage of some OCPs (aldrin, chlordane, DDT, dieldrin, endrin, lindane, heptachlor, beta HCH, and methoxychlor) in Ghana by the Environmental Protection Agency (EPA) [11], although Darko and Akoto [12] reported that management and regulation of pesticides in developing countries like Ghana are often inadequate.

Table 2: Concentrations (mg/kg) of OCP residues in four fish species from the Ashaiman Reservoir.

OCPs	Fish Species			
	***S. melanotheron* (mg/kg)**	***T. guineensis* (mg/kg)**	***T. zilli* (mg/kg)**	***C. gariepinus* (mg/kg)**
Aldrin	< 0.005	< 0.005	< 0.005	< 0.005
γ-chlordane	< 0.005	< 0.005	< 0.005	< 0.005
p,p'-DDE	< 0.005	< 0.005	< 0.005	< 0.005
Dieldrin	< 0.005	< 0.005	< 0.005	< 0.005
Endrin	< 0.005	< 0.005	< 0.005	< 0.005
α- endosulfan	< 0.005	< 0.005	< 0.005	< 0.005
β- endosulfan	< 0.005	< 0.005	< 0.005	< 0.005

Endosulfan sulphate	< 0.005	< 0.005	< 0.005	< 0.005
p,p'-DDT	< 0.005	< 0.005	< 0.005	< 0.005
p,p'-DDD	< 0.005	< 0.005	< 0.005	< 0.005
methoxychlor	< 0.005	< 0.005	< 0.005	< 0.005
β- HCH	< 0.005	< 0.005	< 0.005	< 0.005
δ- HCH	< 0.005	< 0.005	< 0.005	< 0.005
Lindane	< 0.005	< 0.005	< 0.005	< 0.005
Heptachlor	< 0.005	< 0.005	< 0.005	< 0.005

Detection Limit = 0.005 mg/kg

Table 3: Concentrations (mg/kg) of OCP residues in sediment from three different sites in the Ashaiman Reservoir

OCPs	Sediment		
	Site A (mg/kg)	Site B (mg/kg)	Site C (mg/kg)
Aldrin	< 0.005	< 0.005	< 0.005
γ-chlordane	< 0.005	< 0.005	< 0.005
p,p'-DDE	< 0.005	< 0.005	< 0.005
Dieldrin	< 0.005	< 0.005	< 0.005
Endrin	< 0.005	< 0.005	< 0.005
α- endosulfan	< 0.005	< 0.005	< 0.005
β- endosulfan	< 0.005	< 0.005	< 0.005
Endosulfan sulphate	< 0.005	< 0.005	< 0.005
p,p'-DDT	< 0.005	< 0.005	< 0.005
p,p'-DDD	< 0.005	< 0.005	< 0.005
methoxychlor	< 0.005	< 0.005	< 0.005
β- HCH	< 0.005	< 0.005	< 0.005
δ- HCH	< 0.005	< 0.005	< 0.005
Lindane	< 0.005	< 0.005	< 0.005
Heptachlor	< 0.005	< 0.005	< 0.005

Detection Limit = 0.005 mg/kg

Table 4: Comparison of concentrations (mg/kg) of OCP residues in fish species with EU and Australian MRLs.

OCPs	Fish species				EU MRL[a] (mg/kg)	Australian MRL[b] (mg/kg)
	SM (mg/kg)	TG (mg/kg)	TZ (mg/kg)	CG (mg/kg)		
∑DDT	< 0.005	< 0.005	< 0.005	< 0.005	1	1
Dieldrin	< 0.005	< 0.005	< 0.005	< 0.005	0.2	0.1
Lindane	< 0.005	< 0.005	< 0.005	< 0.005	1	1
δ-HCH	< 0.005	< 0.005	< 0.005	< 0.005	-	0.01
β-HCH	< 0.005	< 0.005	< 0.005	< 0.005	0.1	0.01
Endrin	< 0.005	< 0.005	< 0.005	< 0.005	0.05	0.1
Aldrin	< 0.005	< 0.005	< 0.005	< 0.005	-	0.1
∑Endosulfan	< 0.005	< 0.005	< 0.005	< 0.005	0.1	-
∑Chlordane	< 0.005	< 0.005	< 0.005	< 0.005	0.05	-
Methoxychlor	< 0.005	< 0.005	< 0.005	< 0.005	-	-
∑Heptachlor	< 0.005	< 0.005	< 0.005	< 0.005	0.2	0.05

[a] Stefanelli *et al*. [13]; [b] APVMA [14]; Detection Limit = 0.005 mg/kg

SM = *S. melanotheron*; TG = *T. guineensis*; TZ = *T. zilli*, CG = *C. gariepinus*

The non-detection of OCPs such as heptachlor, methoxychlor, endosulfan and chlordane could suggest that pesticides used within the surroundings of the reservoir do not have these compounds as their active ingredient [15]. Furthermore, the non-detection of aldrin and its metabolite dieldrin in the samples could possibly be because aldrin is not in use by farmers.

Although the current work did not detect any OCPs in fish and sediment in the Ashaiman Reservoir, other researchers studying other reservoirs in the country have variously reported the presence of some pesticides, attributing it to the activities of farmers and fishermen in the area [3, 11, 16].

Due to public health risks, DDT, aldrin, dieldrin and heptachlor, among other OCPs have been restricted and banned from agricultural use under the Stockholm convention, in which Ghana is a signatory [17]. Levels of OCP residues in the fish and sediment samples were far below the EU and Australian recommended MRLs, suggesting that the fishes may be safe for human consumption.

The results of the present study are in agreement with Kuranchie-Mensah *et al*. [2] who observed that the levels of OCs in fish from the Densu river were lower than the MRLs set by the EU, US FDA, FAO, Italy and Australia. Also, Akoto *et al*. [16] reported that, detected residue levels of OCPs in fish from the Tono Reservoir were generally low and none were above the WHO/FAO's MRLs. In a similar study, Afful *et al*. [11] observed that all fish species sampled from the Densu river basin had residue levels below the MRLs set by Australia.

5. Conclusion

The study showed that all 15 OCPs analyzed were below the detection limit in fish and sediment samples. They were lower than the EU and Australian MRLs. Fish species in the reservoir are therefore, not contaminated with organochlorine pesticides and are safe for human consumption. Periodic

monitoring is however, recommended to manage OCP levels in fish and sediment from the Ashaiman Reservoir in Ghana.

6. Acknowledgements
The authors wish to express their gratitude to the staff of the Pesticide Residue Laboratory of the Ghana Standards Authority (GSA) for their assistance during sample analysis and also thank the Department of Marine & Fisheries Sciences, University of Ghana, for the use of their facilities.

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