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Spectrum of Organochlorine Pesticide Residues in Fish Samples from the Densu Basin

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Abstract: In this study, the levels of organochlorine pesticide residues in the Densu basin have been investigated using fish samples as a case study. Six fish species namely *Heterotis niloticus*, *Channa obscura*, *Hepsetus odoe*, *Tilapia zilli*, *Clarias gariepinus* and *Chrysichthys nigrodigitatus* were collected from the sampling towns, Weija and Nsawam along the Densu river basin in the Greater Accra Region of Ghana. Composite samples were homogenized, freeze dried and ground to obtain powdered samples. The powdered samples were extracted by soxhlet extraction procedure using (3:1) hexane: acetone mixture. The extracts were cleaned-up using florisil adsorbent and characterized for organochlorine content using Gas Chromatography (GC) equipped with Electron Capture Detector (ECD). The GC recoveries of spiked samples were between 80 to 96%. Fourteen organochlorines (OCs) namely gamma-HCH, delta-HCH, heptachlor, aldrin, gamma-chlordane, p,p'-DDE, alpha-endosulfan, dieldrin, endrin, endrin-aldehyde, endosulfan-sufate, p,p'-DDT, endrin-ketone and methoxychlor were identified and quantified. A 100% incidence was recorded for gamma-HCH, delta-HCH, heptachlor, aldrin, gamma-chlordane, alpha-endosulfan, dieldrin and p,p'-DDT, while 75% incidence was recorded for the metabolites, p,p'-DDE and endosulfan-sulfate. The concentrations of OCs ranged from 0.3 to 71.3 µg/kg and were however, below the Australian Maximum Residue Limits (MRL) of 50 to 1000 µg/kg for fresh water fish.

Key words: Baseline data, chromatographic, levels, organohalogen, persistency, pollution

INTRODUCTION

Pesticides can broadly be classified as insecticides, fungicides and herbicides. Insecticides are mainly organochlorines, organophosphorus, carbamates and pyrethroids. Organochlorine compounds are synthetic organic insecticides that contain carbon, hydrogen, chlorine and sometimes oxygen. The essential structural feature about organochlorine insecticides is the present of carbon-chlorine bond or bonds (Stimman *et al*., 1985). They are therefore also called chlorinated hydrocarbons. Notably among organochlorine pesticides is the dichlorodiphenyltrichloroethane (DDT)

Organochlorine insecticides had been the most used pesticide but they have now been replaced with organophosphorus insecticides because of their environmental persistency. The environmental persistency of organochlorine insecticides has led to the ban of most of them not only as agrochemicals to control pest attack in agriculture but also for the formulation of other pesticide products such as mosquito coils (Bouwman, 2004). The recent Stockholm Convention on Persistent Organic Pollutants (POPs) has banned the use of most organochlorines. These banned organochlorines referred to as "dirty dozen" by the convention include aldrin, chlordane, dichlorodiphenyltrichloroethane (DDT), dieldrin, endrin, heptachlor, hexachloro-benzene (HCB). The convention also curtails inadvent production of dioxins and furans

Organochlorines pesticides are characterized by high persistence, low polarity, low aqueous solubility and high lipid solubility (lipophilicity) and as a result they have a potential to bioaccumulate in the food chain posing a great threat to human health and the environment globally (Lars, 2000). Organochlorines have been implicated in a broad range of adverse human health effects including reproductive failures and birth defects, immune system malfunction, endocrine disruptions, and cancers (Garabrant *et al*., 1992). Studies have shown that dichlorodiphenyltrichloroethane (DDT), dieldrin and polychlorinated biphenyls (PCBs) have endocrine disrupting capacities (Mckinnney and Waller, 1994). Similarly, epidemiological studies have suggested an etiological relationship between exposure to organochlorines and Parkinson's diseases (Fleming *et al*., 1994).

The organochlorine contamination pathways to water bodies are likely to be nonpoint sources via runoff, atmospheric deposition, and leaching due to agricultural applications, vector pest control and improper waste

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disposal methods. Sediments act as a sink for the persistent contaminants, which increases organochlorine bioavailability and accumulation in the food chain through re-suspension. This allows for fish contamination through ingestion, dermal absorption and respiration

Despite the adverse side effect of pesticides, including organochlorines, they form an integral component of modern agriculture. They are used to control pests and thus improve crop yield. The benefits are increased supply of food, but problem arise when significant amount of the chemicals are left on the field as residues which tend to affect non-target organisms and river bodies are one of the main recipient of pesticide residues generated on farming fields.

The pollution of water bodies in the country including the Densu river basin via runoff, direct deposition and leaching due to agricultural activities is alarming. Farmers along the bank of the Densu river basin are engaged in farming activities and in the process use pesticides to control pests attack. Residues of these pesticides generated on the field, are washed into the Densu river when it rains. These chemicals being hydrophobic can potentially bio-concentrate in the fatty tissues of the fishes. As a result of feeding habits of the fishes, there is bioaccumulation, which may lead to biomagnifications in humans on the top of the food chain.

The Weija dam which supplies water to greater part of Greater Accra is built on the Densu river. The Densu river basin is also one of the main rivers used for fishing industry in the Greater Accra Region of Ghana. In Ghana fish is consumed by large population as a major source of protein due to its availability and relative cheaper cost compared to other alternative sources of protein. The possible contamination of fish species with organochlorine pesticides need to be investigated, considering the adverse health effects organochlorine pesticides pose to humans. At the moment, there is no baseline data regarding the pesticide residues content in fish samples in the Densu River. It is therefore important to determine the levels of organchlorine pesticide residues in fish samples from the Densu to help establish a baseline data for organochlorine pesticide residues content in the Densu river basin.

In the present study the spectrum of organochlorine pesticide residues in six fish species, sampled from Weija and Nsawam, which form part of the Densu basin were analyzed using gas chromatographic technique to determine the level of organochlorine pesticide contamination in the fish samples.

MATERIALS AND METHODS

The study was conducted at the Chemistry Department of National Nuclear Research Institute, Ghana Atomic Energy Commission from August 2008 to July 2009.

Chemicals and reagents: All chemicals and reagents used in this study were of high purity quality and were of analytical grade. Hexane (95+%), ethyl acetate (99.8%), nitric acid and anhydrous sodium sulphate were purchased from Sigma-Aldrich, Germany. Acetone (99.5%) was purchased from BDH, England. Florisil adsorbent was purchased from Hopkin and William Ltd, England. The individual reference pesticide standards (ISO 9001 Certified) used for GC analysis of the organochlorines were purchased from Dr. Ehrenstorfer GmbH of Augsburg in Germany

Study area: The Densu basin lies between latitudes 5º30′ N to 6º20′ N and longitudes 0º10′ W to 0º35′ W as shown in Fig. 1. The basin area is about 2488.41 km^2 with an average length of 225.6 Km (Kusimi, 2008). Its main tributaries are the Kuia, Adaiso, Nsaki and Aprapon. The Densu Basin passes through three major regions in Ghana namely; Eastern, Greater Accra and Central Regions and falls under ten district administrations. The Greater Accra region is the most industrialized region in Ghana consisting of the two major industrialized cities in Ghana, Accra and Tema harbor city. The basin plays a critical role in the socio-economic development of these two cities and many towns, satellites villages dotted within it. Most of the urban centers such as Accra, Koforidua, Nsawam, Suhum among others get treated water from the Densu River. Other small settlements also depend on untreated water from the Densu River and its tributaries. The Densu Basin is also intensively used for the cultivation of both cash and food crops. Principal food crops cultivated within the basin are cassava, maize, yam, plantain, banana and cocoyam. Cash crops include cocoa, oil palm, papaya, pineapple, mangoes and citrus. Vegetables cultivation also takes place along the banks of the Densu River. Other land use activities include housing, sand winning, animal rearing, salt mining etc. These activities have seriously depleted the vegetative cover of the basin with hydrological and geomorphologic implications such as flooding, soil erosion, siltation of the river channel and evaporation. Another economic activity in the Densu basin is fishing. Most of the towns and villages including city dwellers in Accra get their fish supply from the Densu basin. Commercial fishing sites in the basin include Nsawam and Weija. Human waste from public and private places of convenience was disposed directly into the Adeeso River, a tributary of the Densu at Adeeso in the Eastern Region (ADRA International, 2008).

Sample collection: Six fish species from Weija and Nsawam were collected for the study. Fish samples were bought alive directly from commercial fishermen at the landing sites at Weija and Nsawam. The fish samples

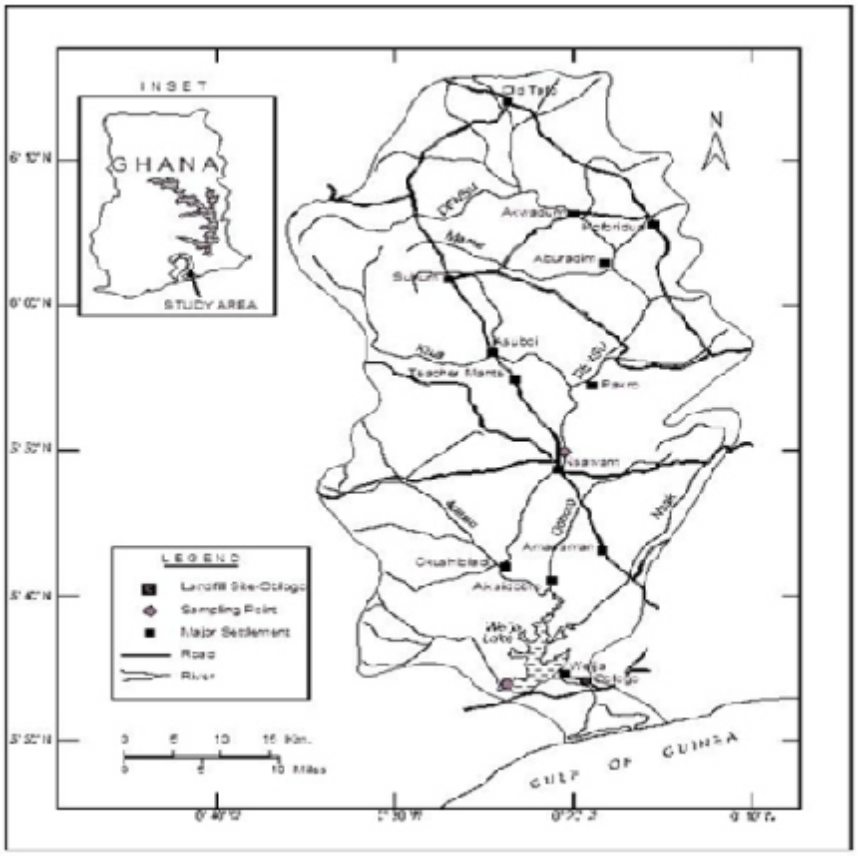


Fig. 1: Map of the Densu Basin showing the sampling point

were kept in thermo insulator box and transported to the laboratory. At the laboratory, the fish samples were washed several times with deionized water to clean them from sediments and other entangled materials. The fish samples were identified and given unique identification codes as shown in Table 1.

The fish samples were wrapped in aluminium foil and the wrapped samples were each placed in a polyethylene bag and kept in the freezer prior to sample preparation and analysis.

Preparation of powdered fish samples: Fish samples were removed from the freezer and rinsed several times with deionised water. Prior to preparation, each sample was weighed using a calibrated weighing balance. Each fish sample was gutted to remove intestines. The scales, head, tails, fins and bones were also removed using a stainless steel knife. A composite sample was prepared for each different species for the five different kinds of fishes. The muscle tissues of the fish samples were ground in a warring blender to obtain a homogenous composite sample. A 100 g of each composite sample were weighed and wrapped in aluminium foil. Samples were kept in the freezer for one hour prior to freeze-drying. The samples were freez dried for 72 h. The dried samples were ground using mortar and pestle and stored in pre-cleaned glass bottles for analysis.

Table 1: Fish species and sampling sites

Species	Sampling site	Identification codes
Clarias gariepinus	Weija	CGW
Chrysichthys nigrodigitatus	Weija	CNW
Tilapia zilli	Weija	TZW
Heterotis niloticus	Nsawam	HNN
Channa obscura	Nsawam	CON
Hepsetus odoe	Nsawam	HON
Tilapia zilli	Nsawam	TZN
Clarias gariepinus	Nsawam	CGN

Soxhlet extraction: About 10 g of dried powdered fish samples were weighed and wrapped in a filter paper. The wrapped fish samples were placed in a cellulose extraction thimble. The fish samples were extracted with 160 mL of 3:1 hexane: acetone mixture for 5 h by soxhlet extraction. After extraction the extract was concentrated to dryness on a rotary evaporator fitted to a vacuum pump. The residues were re-dissolved in 10 mL of hexane. A virgin cellulose extraction thimble together with a filter

Table 2: Mean concentrations of organochlorines in the fish samples (µg/kg)

Organochlorines	CGW	CNW	TZW	HNN	CON	HON	TZN	CGN
γ-HCH	1.4±0.02	1.9±0.2	1.3±0.02	9.3±0.11	21.4±0.03	17.7±0.20	6.5±0.10	6.0±0.21
δ-HCH	5.0± 0.01	0.9±0.07	0.4±0.01	0.4±0.10	35.2±0.04	14.6±0.20	0.7±0.01	0. 8±0.10
Heptachlor	1.1±0.10	0. 9±0.03	3.2±0.02	6.3±0.10	16.1±0.20	21.5±0.24	5.1±0.01	1.6±0.02
Aldrin	0. 8±0.02	0. 5±0.10	0. 7±0.20	0. 6±0.02	0. 8±0.01	2.9±0.60	1.4±0.40	0. 3±0.10
γ-Chlordane	0. 5±0.10	2.3± 1.00	0. 7±0.10	5.1±0.20	5.2±0.01	10.2±1.20	2.9±0.01	0. 6±0.20
α-Endosulfan	0. 8±0.21	1.2±0.01	0. 6±0.01	16.4±2.20	71.3±0.60	44.6±0.10	10.4±0.03	8.7±0.20
p.p'-DDE	8.5±0.02	1.3±0.61	nd	6.8±1.20	nd	34.6±0.94	12.0±0.03	7.2±0.10
Dieldrin	0.11±0.01	0. 4±0.01	0. 2±0.03	19.2±0.10	17.7±0.01	9.3±0.02	6.7±0.12	4.8±2.00
Endrin	0.5±0.20	nd	nd	10.3±1.10	2.4±0.80	13.9±0.30	5.3±1.20	0.3±0.04
p.p'-DDT	0. 6±0.13	0. 8±0.01	1.0±0.02	7.5±0.60	8.9±0.44	12.5±1.23	5.0±0.60	4.4±0.01
Endrin-aldehyde	nd	nd	nd	2.3±0.10	0. 3±0.10	1.3±0.02	0. 4±0.01	0. 9±0.01
Endosulfan-Sulfate	nd	2.1±0.20	nd	18.1±2.16	8.5±0.65	1.7±0.01	9.5±0.20	2.4±0.10
Endrin ketone	nd	nd	1.8±0.40	2.1±0.02	0. 9±0.01	13.4±0.10	2.6±0.02	7.4±0.10
Methoxychlor	2.5±0.70	nd	1.6±0.12	1.8±0.05	0. 9±0.30	1.7±0.010	1.0±0.02	2.2±0.20

nd = not detected

CGW=*Clarias gariepinus* (Weija), CNW= *Chrysichthys nogrodigitatus* (Weija), TZW= *Tilapia zilli* (Weija)
HNN= *Heterotis niloticus* (Nsawam), CON= *Channa obscura* (Nsawam), HON= *Hepsetus odoe* (Nsawam),
TZN= *Tilapia zilli* (Nsawam), CGN= *Clarias gariepinus* (Nsawam)

paper was extracted in the same manner as the samples to obtain the blank. One sample each in a batch was spiked with 1 mL of 0.01 mg/kg mixed standard of organochlorines for the recovery analysis.

Florisil clean-up of extracts: Florisil Solid Phase Extraction (SPE) columns were prepared by packing 6 mL extraction columns with 1 g pre-activated florisil adsorbent with 0.5 g anhydrous Na_2SO_4 packed on top of the florisil. The columns were each conditioned with 10 mL n-hexane prior to clean-up. The extracts of the fish samples, blanks and spiked samples were eluted through the columns and the eluate collected into a 50 ml conical flask. The column was further eluted with 10 mL n-hexane. The eluate was concentrated to dryness on the rotary evaporator and recovered into 1ml ethyl acetate. The 1ml extracts were transferred quantitatively into 2 mL glass vials using pasteur pipette for GC analysis. Recovery of the column was determined using 0.01 mg/kg mixed standard solution of organochlorine pesticides

GC analysis: A Gas Chromatograph model CP-3800 (Varian) equipped with ^{63}Ni Electron Capture Detector (ECD) of activity 15 mCi with an auto sampler was used for the analysis. A volume of 1.0 µL of the extracts was injected. The operating conditions were capillary column: VF-5ms, 30m x 0.25mm x 0.25µm, temperature programme: 70ºC (2 min) to 180ºC (I min) 25ºC/min to 300ºC 5ºC/min, Injection temperature: 270ºC, Detector temperature: 300ºC, Carrier gas: Nitrogen at 1.0 mL/min, Make up gas: Nitrogen at 29 mL/min. A 0.1 ppm mixed standard solution of organochlorine pesticides were analyzed in a similar manner for identification. Peak identifications were conducted by comparing the retention time of authentic standards and those obtained from the extracts. Concentrations were calculated using a four point calibration curve.

RESULTS AND DISCUSSION

Table 2 shows individual organochlorine pesticide and their respective mean concentrations when samples were analysed. Margins of errors are standard deviation based on triplicate determination of each pesticide. In all, 14 organochlorines namely γ-HCH, δ-HCH, heptachlor, aldrin, γ-chlordane, α-endosulfan, p,p'-DDE, dieldrin, endrin, p,p'-DDT, endrin-aldehyde, endosulfan-sulfate, endrin-ketone and methoxychlor were detected in the samples. The detection of 14 organochlorines in the fish samples indicates the wide use of these chemicals. As at December 2008, the organochlorine pesticides; aldrin, chlordane, DDT, dieldrin, endrin, lindane (δ-HCH), heptachlor were among banned pesticides by Environmental Protection Agency of Ghana. However, the concentrations of the 14 organochlorines residues detected in this study fell below the Australian Maximum Residue Limits (MRL) of 50 to 1000 µg/kg for fresh water fish (Australian MRL, 2009). The Maximum Residue Level (MRL), is the maximum amount of the pesticide residue which if found in food substances will not cause any health effect or hazard (Gerken *et al*., 2001). The concentrations of the pesticide residues range from 0.30 to 71.3 µg/kg. The highest concentration of 71.3 µg/kg was α-endosulfan measured in *Channa obscura* and sampled from Nsawam. The lowest concentration of 0.30 µg/kg was measured for aldrin, endrin and endrin-aldehyde in *Clarias gariepinus*, *Clarias gariepinus* and *Channa obscura* respectively. All these species were sampled from Nsawam. The results further show that the organchlorines, γ-HCH, δ-HCH, heptachlor, aldrin, γ-chlordane, α -endosulfan, dieldrin and p,p'-DDT were present in all the fish samples. Concentrations of δ-HCH were generally lower than for γ-HCH except for *Channa obscura*. A mean δ-HCH concentration of 0.126 µg/kg in fish from the Lake

Bosomtwi in Ghana had been reported (Darko *et al*., 2008), Ntow also reported mean δ-HCH level of 0.008 µg/L in water and 2.30 µg/kg in sediments sampled from the Volta Lake in Ghana (Ntow, 2005). These concentrations recorded in Ghana previously are comparable to results obtained from this work. However, levels of 559000 and 118200 µg/kg of δ-HCH in *Tilapia zilli* and *Chrysichthys nigrodigitatus* respectively were reported in Lagos lagoon, Nigeria (Adeyemi *et al*., 2008). These levels reported in Nigeria were extremely high compared to levels recorded in this work.

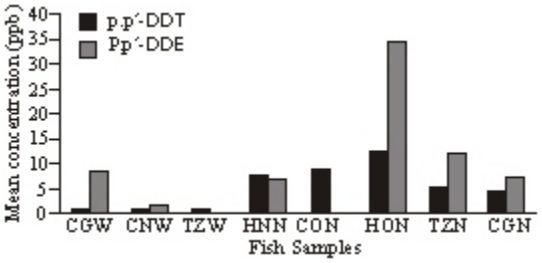
Among the seven banned organochlorine pesticides (aldrin, chlordane, DDT, dieldrin, endrin, lindane (δ-HCH), heptachlor) in Ghana detected, δ-HCH recorded the highest residue concentration of 35.2 µg/kg in *Channa obscura* (CON). This can be explained by the wide use of δ-HCH (lindane) in Ghana previously as a pesticide with the trade name gamalin-20.

Figure 2 shows the distribution of p,p'-DDT and its metabolite, p,p'-DDE while Fig. 3 show the distribution of α-endosulfan and its metabolite endosulfan sulfate in the fish Samples. The result in Fig. 2 generally shows higher concentrations of p,p-DDE than the parent p,p-DDT in almost all the samples. The low concentrations of p,p-DDT compare to its metabolite, p,p-DDE is an indication that there might not be recent input of DDT in the Densu basin. The detection of p,p-DDE is an indication of photochemical degradation of p,p-DDT (Wandiga, 1995).

It is obvious from Fig. 3 that concentrations of α-endosulfan were generally higher than its metabolite, endosulfan-sulfate. This comes as no surprise as it was only in December, 2008 that the use of endosulfan in Ghana was restricted to the cotton industry by EPA. The detection of endosulfan sulphate is an indication that metabolism of the parent endosulfan occurred via oxidation (Wandiga, 1995). The highest concentration of endosulfan-sulfate, 18.1 µg/kg was measured in *heterotis niloticus* (HNN). The metabolite was however, not detected in *Clarias gariepinus* (CGW) and *tilapia zilli* (TZW).

CONCLUSION

The results of this work indicates that organchlorines pesticide residues are present in the Densu basin however, the concentrations of the organochlorines residues determined in the fish samples were below the stipulated Australian Maximum Residue Limit (MRL) of 50-1000 µg/kg for fresh water fish. In all fourteen oranochlorine pesticides were identified of which seven are among the banned pesticides of the Environmental Protection Agency (EPA) of Ghana. These banned organochlorines detected are; aldrin, chlordane, DDT, dieldrin, endrin,



Fig, 2: Distribution of p, p-DDT and p,p-DDE in the fish samples

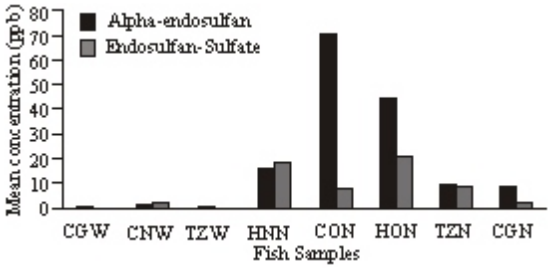


Fig. 3: Distribution of α-endosulfan and endosulfan-sulfate in the fish samples

lindane (δ-HCH), and hepthachlor. The mean concentrations of the banned organochlorines detected range from 0.30-35.2 µg/kg with the highest concentration of 35.2 µg/kg recorded for δ-HCH in the *Channa obscura* sp. sampled from Nsawam. The lowest mean concentration of 0.30 µg/kg was recorded for endrin, aldrin and endrin-aldehyde in the species *Clarias gariepinus*, *Clarias gariepinus* and *Channa obscura* respectively. The metabolites namely p.p-DDE and endosulfan sulfate were also detected in six of the eight fish species used for the investigation. From the results of this work, organochlorine pesticide residues in the Densu river basin are likely to have originated from various sources such as the use of pesticides products for agricultural purposes by farmers along the Densu river basin.

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