African Journal of Environmental Science and Technology Vol. 3 (11), pp. 405-411, November 2009
Available online at http://www.academicjournals.org/ajest
ISSN 1991-637X

Full Length Research Paper

Incidence of organochlorine insecticides (DDT and heptachlor) in Bangladeshi dry fish: Seasonal trends and species variability

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Accepted 22 September, 2009

The concentrations of organochlorine insecticides dichlorodiphenyltrichloroethane (DDT) and heptachlor were investigated to estimate the current status of insecticides used in dry fish in different season and different species. Six most popular species of dry fishes namely Bombay duck (Loittya), Ribbon fish (Chhuri), Shrimp (Chingri), Hilsha shed (Ilish), Chinese pomfret (Rupchanda) and Indian salmon (Lakhua) were collected from Asadgonj (whole sell market for dry fish) of Chittagong, Bangladesh at different seasons, six samples at winter season (December) and six same samples at rainy season (July). The range of DDT concentration at winter was found 5.588 ppb to 250.758 ppb and at rainy season the range of concentration was found 11.054 ppb to 1107.427 ppb. The range of heptachlor concentration at winter was found to range from 0.401 to 2.480 ppb and at rainy season was found to range from 1.087 to 37.780 ppb. The concentrations of DDT and heptachlor were much higher in the samples of rainy season than those of the winter.

Key words: Dry fish, concentration, organochlorine insecticides, DDT, heptachlor, season.

INTRODUCTION

Dry fish (SHUTKI in Bengali), is the most popular food item in Bangladesh. It is the main protein source in many areas including Chittagong, Dhaka, Chandpur, Kuakata, Barisal etc.of this country and recently exported abroad where the main consumers are immigrants and workers of the third world country. The coastal areas, rivers and several haors are famous for producing dry fish, which is dried under the sun from mid October to mid April. Huge amount of fish harvested from fresh and marine water during winter season. During this time the Bay of Bengal, the coastal crisscross channels and other depressions remain calm and quiet and as a result fishing activities are strengthened and more fishes are harvested during this period than the other seasons.

The necessity to cultivate dry fish looms up when huge quantity of fresh fish harvested everyday remains unsold because shortage of customers and cannot be sent to the towns or the metropolis on a daily basis either for shortage of transport or fish traders not willing to pay the right amount of money. As a result winter is considered to be the peak season for drying fish. For long preservation of fish by drying is common practice in Bangladesh. The fisher men are used insecticides to protect the dry fishes from infestation whatever they are getting within their reach.

Studies on the conservation of dry fish showed that a mixture of organochlorine (DDT and heptachlor) is used in dry fish in Bangladesh (Bhuiyan et al., 2008). Some analysis in Bangladesh shows alarming pollutants in fish like DDT and heptachlor (BCAS, 1990). Many people call DDT as white powder. In Kuakata (a fish processing zone in Bangladesh) high level of DDT powder (white powder) is used on dry fish, but DDT is a banned in Bangladesh though as a member of the United Nations, in 1997 (Barua, 2007).

In Bangladesh at present, all Persistent Organic Pollutants (POPs) like DDT and heptachlor import and production have been banned but at least five POPs pes-

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ticides including DDT are still in use under a different name or label (ESDO, 2005). There are no central registers or lists of what in Bangladesh, though some intergovernmental organizations like the Food and Agricultural Organization (FAO) and the World Bank have begun to assemble aggregate use data. Since country-specific data was not found. This did not tell enough about usage to know specifically where and how much of these compounds are being used (ESDO, December 2005). Therefore, to speak documentarily, at present there is no legal use of any POP pesticides in Bangladesh. The Government of Bangladesh signed the Stockholm Convention on POPs on 23 May, 2001. No pesticides and insecticides are registered without reviewing the available toxicological and eco-toxicological information of the candidate pesticide. However, there is no specific legislation for controlling the production and use of hazardous industrial chemicals (UNEP, 2002).

In the last 50 years, people have generated 80,000 types of chemicals. Of them, there are organic chemical pollutants known as 'dirty dozen', which is very harmful to human bodies (Barua, 2007). Among the dirty dozen the chlorinated hydrocarbons (organochlorine compounds), were the first generation of pesticides called wonder drug introduced following the Second World War which comprises DDT, Dieldrin, heptachlor and others. They are designed to kill insects and which, as broad-spectrum poisons. DDT can transfer from generation to generation through breast milk (Solomon and Weiss, 2001).

In areas where it is used for malaria control, infants can be exposed via breast milk in levels that exceed the W. H. O's acceptable daily intake value for DDT (Bouwman et al., 2006; Ntow et al., 2008). It is classified as "moderately toxic" by the US National Toxicological Program and "moderately hazardous" by WHO, based on the rat oral LD50 of 113 mg/kg (WHO, 2005). Farmers exposed to DDT occupationally have an increased incidence of non-allergic asthma (Anthony, 2007). Organochlorine compounds in general have been linked to diabetes (Jones et al., 2008). EPA classified it as a class B2 probable human carcinogen (US EPA, 1987). A study of malaria workers who handled it occupationally found an elevated risk of cancers of the liver and biliary tract (Rogan and Chen, 2005). Exposure to it before puberty increases the risk of breast cancer later in life (Clapp et al., 2008).

On the other hand heptachlor has been shown to bioaccumulates in fish and cattle. Most of the heptachlor that is swallowed is absorbed into blood. It can pass directly from a mother's blood to an unborn baby through the placenta (LDWG, 2007). Animals fed heptachlor throughout their lifetime had more liver tumors than animals that ate food without heptachlor. EPA has classified heptachlor as a probable human carcinogen (B2) and established an oral cancer slope factor of 4.5 per mg/kg-day (LDWG, 2007).

The objectives of this study were to detection and determination of the concentration level of insecticides (DDT and heptachlor) in dry fish at winter and rainy season, used for conservation of dry fish and to elucidate the variation of concentration level of insecticides due to fish species.

MATERIALS AND METHODS

Sample collection

Six most popular dry fish samples namely Bombay duck (Loittya), Ribbon fish (Chhuri), Shrimp (Chingri), Hilsha shed (Ilish), Chinese pomfret (Rupchanda) and Indian salmon (Lakhua) were collected from Asadgonj (whole sell market for dry fish) of Chittagong, Bangladesh at winter season (December) and another six samples of same species from same place were collected at rainy season (July). Total no of samples were twelve.

The control samples of six different fishes were collected from drying yards of Sonadia island (a fish processing zone of Bangladesh) that are known not to be treated with insecticides.

Apparatus

Mincer fish chopper (Weisser No. 81 K), Soxhlet extractor, separatory funnels (500 and 200 ml), Chromatographic tube (20 mm i.d. 50 cm long), sample concentrator (Techne dry block DB.3), round bottomed flask (500 and 100 ml), volumetric flask (50 and 10 ml), Gas Chromatograph (GC-14B, Shimadzu), syringe(10 µl, Hamilton Co.).

Reagents

Acetone, Diethyl ether, Dimethylformamide saturated with petroleum ether, n-hexane, petroleum ether (30 - 60˚C), petroleum ether (30 - 60˚C) saturated with Dimethylformamide, eluting mixture I (petroleum ether + diethyl ether 94:6 v/v), standard solutions , eosin solution (2 mg in 100 ml), sodium sulphate solution (2 g/ 100 ml Na_2SO4 10 H_2O), sodium sulphate anhydrous (heated for at least 2 h at 550˚C temp.), Florisil 60 - 100 mesh (heated for at least 2 h at 550˚C temp., cool and stored in tightly stoppered container, prior to use heated for at least 5 h at 130˚C temp., cool and add 5% w/w water, shake this mixture for at least 20 min and stored in a container for at least 10 h), cotton wool.

All the solvents used for the analysis purchased from MERCK, Germany. DDT and heptachlor standards were obtained from sigma chemicals.

Sample preparation

All the samples are finely comminuted in a mincer; heating of the samples during comminuting is avoided by briefly chopping several times (Peter and Zeumer, 1987).

Extraction

Triturate a sample of 25 gm, with Sodium Sulphate to dry, powdery mixture, with the aid of an extraction thimble; extract the mixture exhaustively with Petroleum Ether in Soxhlet apparatus. Concentrate just to dryness the extract solution by a concentrator and dilute to 25 ml with Petroleum Ether saturated with Dimethylformamide (Peter and Zeumer, 1987).

Clean up

Clean up was done in two steps- (Peter and Zeumer, 1987);

a) ***Dimethylformamide-petrolium ether partition***

Transfer the solution (dissolved in 25 ml Petroleum Ether saturated with Dimethyl Formamide) to 250 ml separatory funnel. Rinse the flask with small portion of a previously measured amount of 75 ml Dimethyl Formamide. Then add the remainder of the Dimethyl Formamide to the separatory funnel and shake vigorously for 1 min. Drain the Dimethyl Formamide phase and again extract the Petroleum Ether phase with 10 ml Dimethyl Formamide. Transfer the combined Dimethyl Formamide phases to a 500 ml separatory funnel and add 200 ml sodium sulphate solution. Add a few drops of eosin solution to achieve better recognition of phase separation in the subsequent partition. Then extract successively with a 40 ml portion and three 25 ml potions of petroleum ether for 1 min each time. Wash the combined petroleum ether phases with 10 ml water, dry on sodium sulphate, filter through a cotton wool plug, add 5 ml n-hexane and concentrate to approx. 5 ml.

b) ***Florisil column chromatography***

About half filled a chromatographic tube with petroleum ether and sprinkle with 30 g Florisil in small portions through a funnel with stopcock open, tapping the column in the process. Cover the Florisil with an approx. 2 cm layer of sodium sulphate. Drain the supernatant solvent to the top of the column packing. Pipette the sample solution on to the column. Let the solution percolate to a level of 1 - 2 mm above the top of the column. Then rinse the flask with small portions of eluting mixture I, add the rinsings to the column and also let them percolate to a level of 1 - 2 mm above the top of the column. Next eluate the column with the remainder of the total 200 ml amount of eluting mixture I, at a flow rate of about 5 ml/min. Concentrate the eluate near to dry and add 5 ml n- hexane to the eluate. Again concentrate the eluate to 1 ml.

Sample analyses

The DDT and heptachlor residues were analyzed by GC-14B, Shimadzu with an Electron Capture Detector (ECD), a manual sampler and GC solution software. A column of 3.1 m x 3.2 mm; I.D glass spiral; stationary phase silicon OV - 17, 5%, aging 300℃, support chromosorb-W-AW-DMCS, mesh 80/100, 1 µm film thickness was used for the chromatographic separation of insecticides. The temperature was fixed for the injector at 250℃, column at 280℃, detector at 280℃. The carrier gas was nitrogen with a 60 ml/min-flow rate. 1.0 µl sample was injected for each run and the running time was 25 min. Standards' peak were identified by injecting high concentration of the standard (0.5 and 0.25 ppm) and the retention time for DDT and heptachlor were determined. Then calibration was done at 3 points (25, 50 and 100 ppb) by composite stock standard solution. GC system was calibrated using external standard technique. Individual standard stock solution (100 mg/l) was prepared by weighing appropriate amounts of active ingredients in a brown bottle with a Teflon-lined screw cap and dissolving the weighed standard in HPLC grade n-hexane. Stock standard solution was used to prepare primary dilution standards. Appropriate volume of each individual stock solution was taken in a volumetric flask and mixed the solutions to obtain composite stock standard solution.

Analytical quality control

Gas chromatograph equipped with ECD was checked for linearity. Instrumental limit of detection for GC-ECD was 1.0 µg/l for organochlorine pesticides. An aliquot of dry fish samples which were collected as blank and treated exactly as a sample including exposure to all glassware, equipments, solvents and reagents used with the sample matrix. No analyte peak was detected in laboratory reagent blank. An aliquot of fortified samples matrix were prepared to which known quantities of the pesticides were added in the laboratory in ppb range. This laboratory fortified matrix was analyzed exactly like the sample. Extraction and clean up were done as mentioned and the recoveries from untreated control samples of dry fish fortified with the analyzed compounds at level of 25 ppb were 96 - 100% for heptachlor and 98 - 100% for DDT. Prior to injection of the first sample solution, a standard solution was injected at least three times to check the operating conditions and the constancy of the detector signals. Further linearity of the ECD signal was checked by injecting serial dilutions of DDT and heptachlor. A standard solution injected after at least every other sample solution so that any alterations of the gas chromatographic system recognized due to column contamination.

RESULTS

Twelve samples of six fish species namely Bombay duck (Loittya), Ribbon fish (Chhuri), Shrimp (Chingri), Hilsha shed (Ilish), Chinese pomfret (Rupchanda) and Indian salmon (Lakhua) were analysed to detect DDT and Heptachlor at winter (December) and at rainy season (July). The results obtained are alarming for Bangladesh. All of the samples contained organochlorine insecticides (except Ribbon fish at winter season, heptachlor was not detected) are shown in Table 1 and the chromatogram of all the samples (1 - 12) are shown in Figures 1 - 12.

The concentration of DDT at winter season for low priced dry fishes (Bombay duck, Ribbon fish and Shrimp) varied from minimum 3.571 ppb (Ribbon fish) to maximum 21.566 ppb (Shrimp) and the concentration for the highly priced dry fishes (Hilsha shed, Chinese pomfret and Indian salmon) varied from 11.054 ppb (Indian salmon) to maximum 250.758 ppb (Chinese pomfret). At rainy season the concentration of DDT for low priced dry fishes varied from 257.210 ppb (Bombay duck) to maximum 556.296 ppb (Ribbon fish) and the concentration for highly priced dry fishes were varied from minimum 233.727 ppb (Indian salmon) to maximum 1107.427 ppb (Chinese pomfret). The concentrations of DDT were generally higher in costly dry fishes in winter season but at rainy season DDT concentrations were much higher than those of winter season for all the dry fish samples.

The concentration of heptachlor at winter season for Ribbon fish, a low priced dry fish, was not detected and the concentration of other two low priced dry fishes were 0.401 ppb (Bombay duck) and 0.915 ppb (Shrimp) and the concentration for the highly priced dry fishes varied from minimum 1.135 ppb (Hilsha shed) to maximum 37.780 ppb (Indian salmon). At rainy season the concentration of heptachlor for low priced dry fishes varied from 1.087 ppb (Bombay duck) to maximum 4.134 ppb (Ribbon fish) and the concentration for highly priced dry fishes were varied from minimum 2.532 ppb (Indian salmon) to maximum 4.011 ppb (Chinese pomfret). The concentrations of heptachlor were generally more or less constant in all samples for both seasons except Indian salmon. Although Indian salmon is highly priced dry fish

Table 1. DDT and heptachlor concentrations in the dry fish samples (the concentrations are in ppb unit).

Name of the samples	Name of insecticides	Winter season	Rainy season
Bombay duck (Loittya)	Heptachlor	0.401	1.087
	DDT	13.104	257.210
Ribbon fish (Chhuri)	Heptachlor	*ND	4.134
	DDT	3.571	556.296
Shrimp (Chingri)	Heptachlor	0.915	2.915
	DDT	21.565	369.567
Hilsha shed (Ilish)	Heptachlor	1.135	2.705
	DDT	152.508	279.102
Chinese pomfret (Rupchanda)	Heptachlor	2.480	4.011
	DDT	250.758	1107.427
Indian salmon (Lakhua)	Heptachlor	37.780	2.532
	DDT	11.054	233.727

*ND - Not Detected, parenthesis indicates the Bengali name.

and it contained lower amount of DDT (11.054 ppb) at winter season but it contained exceptionally higher amount of heptachlor (37.780 ppb) at that season.

DISCUSSION

No studies of OCPs (DDT and heptachlor) in the dry fish of Bangladesh to estimate the current status of insecticides used in dry fish in different season and different species have been carried out. The present study have been undertaken in order to provide the preliminary information on the concentration of DDT and heptachlor in dry fish in different season and in different species of dry fish and to investigate their contamination level. From the study it was found that the fisher men dried fish at winter but they divided the fishes into two categories, some for winter and some for rainy season. At winter the moisture of the weather remain low, the dry fishes do not absorb too much moisture which is suitable for infestation by beetles and mites and they sold that within winter.

Another category of fishes which is dried for selling at rainy season are generally stored in a dump warehouse either at the site or nearby coastal towns. In addition to this, the weather is humid particularly during the rainy season. Due to high moisture content in the weather and dump condition of warehouse, the dry fishes absorbed moisture so rapidly that the fish becomes suitable for infestation by beetles and mites. Most unexpected cause of infestation is that the fishermen do not dry fishes properly due to loss of weight, that is, the fishermen want more profit selling the dry fishes in weight, preparably they do this for high cost fishes such as Hilsha shed, Chinese pomfret and Indian salmon.

The most significant properties of the organochlorine insecticides are their extreme lipophilic nature and resistance to biodegradation, which results in their extreme persistence in environment (Tannenbaum, 1979).

Among the insecticides DDT is a commercial accumulation and concentration in fatty tissues and their organochlorine insecticide that has been widely used on agricultural crops as well as for vector control (ATSDR, 1995). DDT and its by-products can persist in soil and sediments for more than 15 years and are known to bio accumulate in animal tissues. DDT had been banned for all uses in 49 countries and restricted to vector control in 23 (Panna, 1995).

The half-life of DDT in humans is approximately 4 years (Noren and Meironyte, 2000). heptachlor is another organochlorine cyclodiene pesticide that has been used to control termites and as an insecticide on seed grains and food crops. heptachlor epoxide, the main metabolite of heptachlor, is extremely persistent in soil. In some cases, trace amounts of heptachlor epoxide have been found in soil 14 – 16 years after application (Extoxnet, 1996). Plants can draw heptachlor epoxide directly from the soil, and the chemical bioaccumulates in animals. It has been banned or restricted in more than 60 countries (WHO, 1988).

A study showed that countries have restricted and banned heptachlor; levels detected in breast milk have dropped, often by more than 10-fold (Jensen and Slorach, 1991). But another studies showed that in the United States, levels in the Southeast were nearly double the levels in the rest of the country during the period when it was still used (Savage, 1981). However, some of these countries still permit its use for termite and other pest control and many developing nations still use for agricultural purposes (Noronha, 1998). Despite the imposition of a ban on use in the United States in 1988, U.S. customs data showed that it was exported in large quantities through 1994 (Panna, 1997).

As a signatory of the Stockholm Convention, the Government of Bangladesh is required to take action to

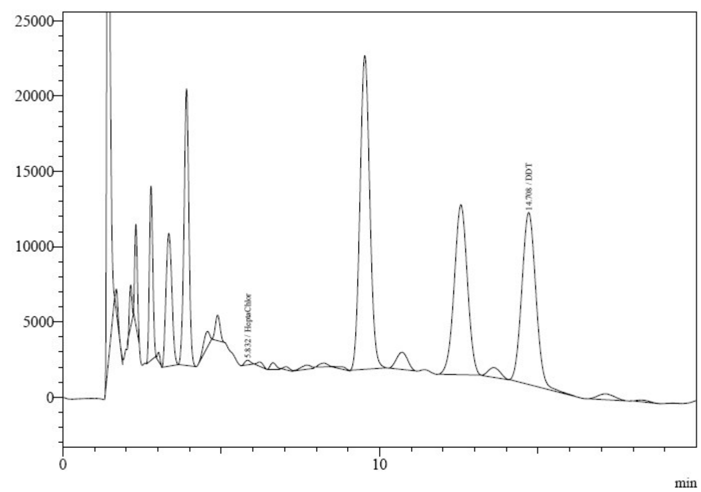

Figure 1. Bombay duck, Winter season.

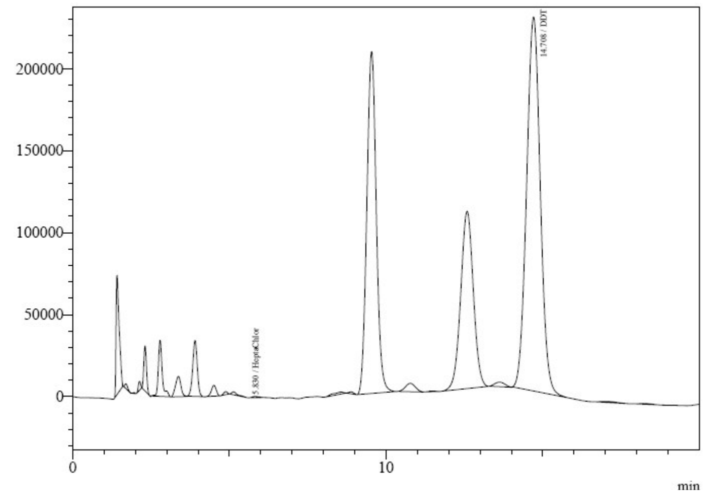

Figure 2. Bombay duck, Rainy season.

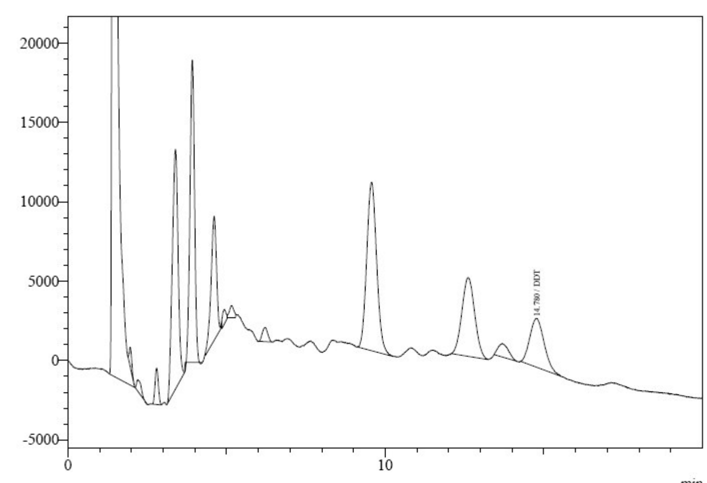

Figure 3. Ribbon fish, Winter season.

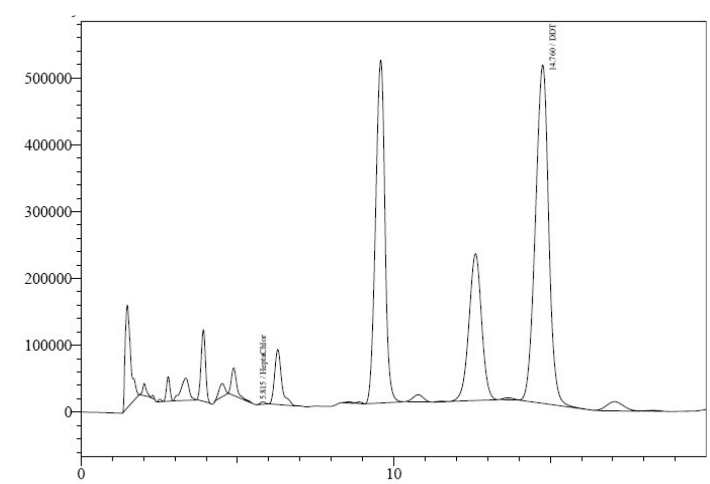

Figure 4. Ribbon fish, Rainy season.

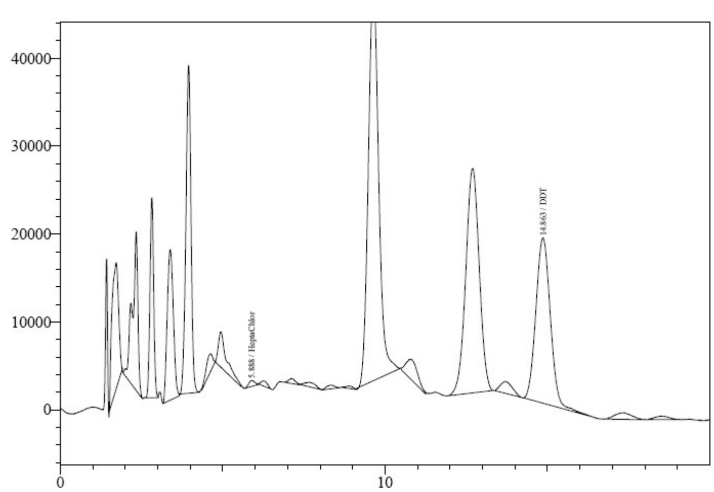

Figure 5. Shrimp, Winter season.

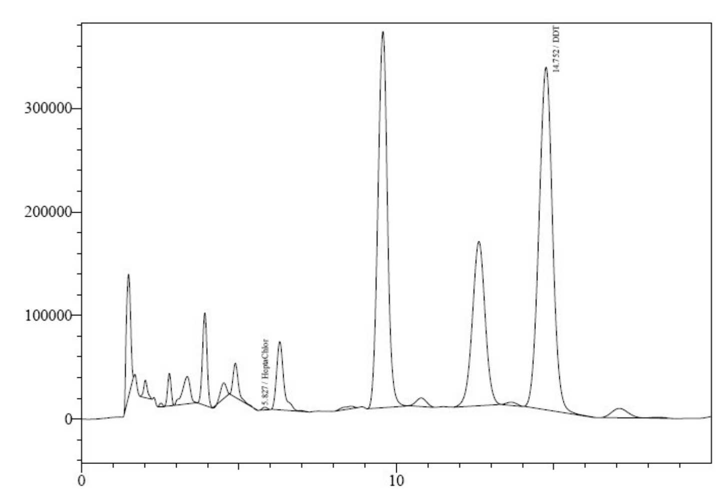

Figure 6. Shrimp, Rainy season.

Figures 1-6. Showed different chromatograms of DDT and heptachlor for 12 samples of dry fishes.

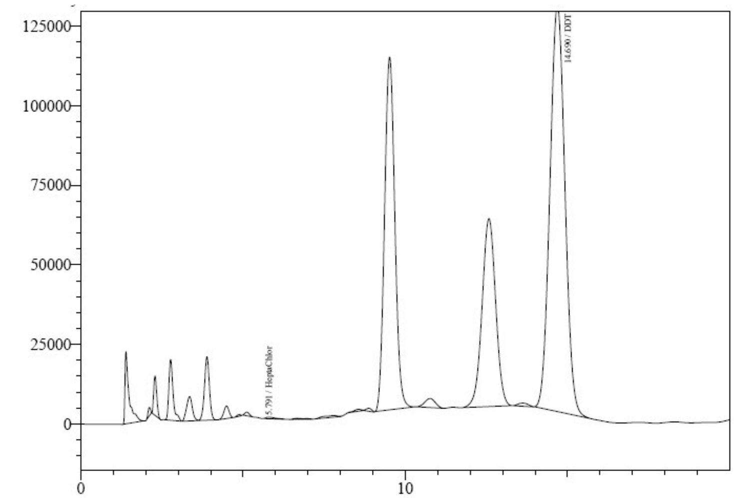

Figure 7. Hilsha shed, Winter season.

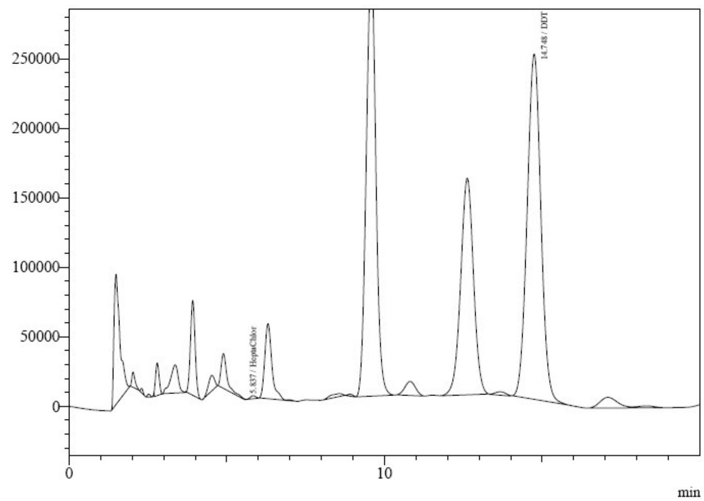

Figure 8. Hilsha shed, Rainy season.

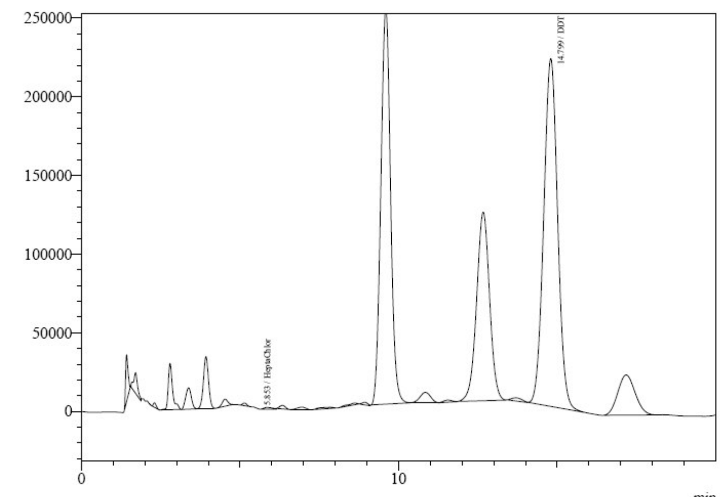

Figure 9. Chinese pomfret season.

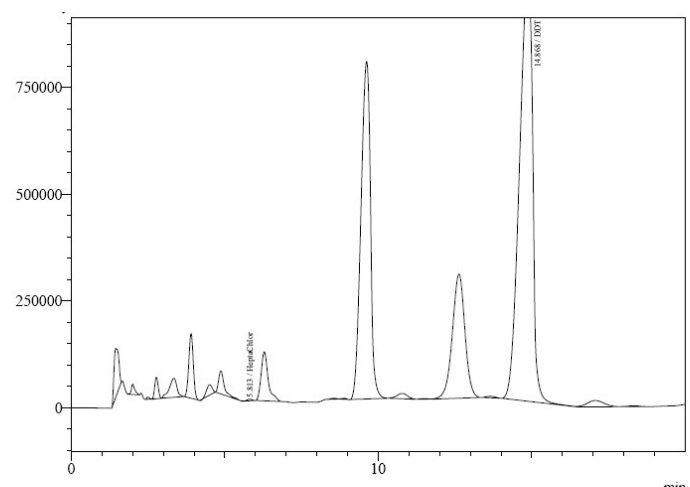

Figure 10. Chinese pomfret, Rainy season.

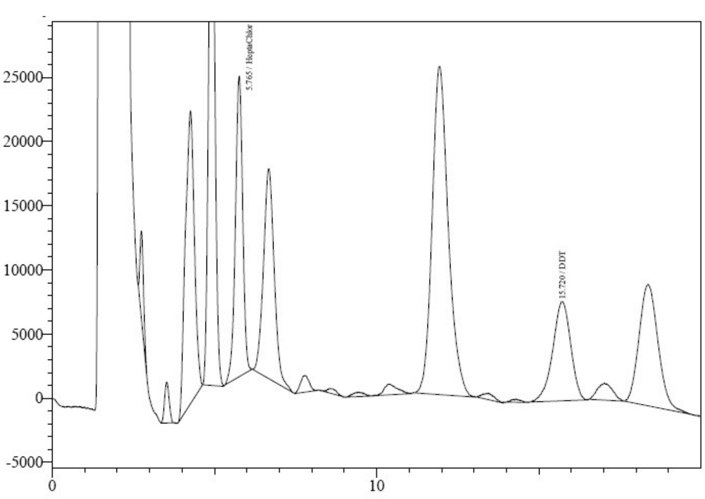

Figure 11. India salmon, Winter season.

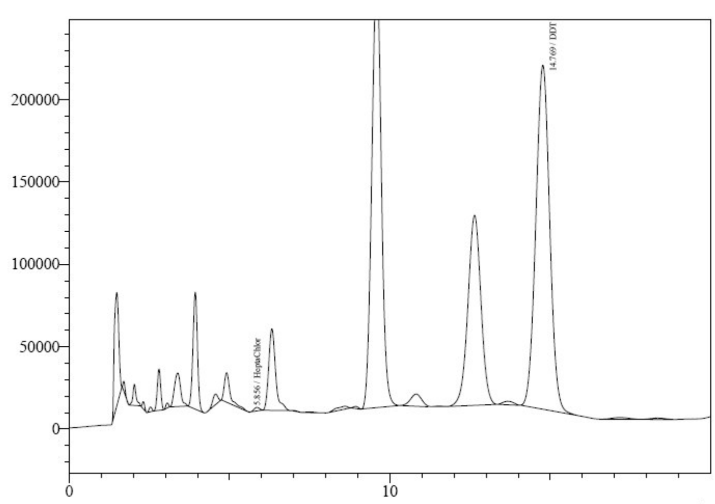

Figure 12. India salmon, Rainy season.

Figures 7-12. Showed different chromatograms of DDT and heptachlor for 12 samples of dry fishes.

generate general awareness of consequences of DDT and heptachlor. Though the government has taken up the initiative to prepare the National Implementation Plan (NIP) (ESDO, December 2005). Other than the inventory on POPs, DOE (Department of Environment) has not taken up any further activities with regard to POPs awareness. There is no government initiated POPs public awareness campaign. To initiate comprehensive mass awareness campaign on POPs need of financial and technical support to encourage action research activities. A variety of chemical and non-chemical alternatives are available for POPs in Bangladesh. Beside the government the stocker should dry correctly and should pack carefully so that the fish can not absorb moisture in monsoon. The other way of preservation can follow such as freezing, canning and curing.

From the study, we can say that, the fisher men and the stocker used DDT and heptachlor in the dry fishes sold in both winter and rainy season but at rainy season the amount is much higher than the winter. It is also higher in the costly fishes.

ACKNOWLEDGEMENTS

This study was done under the Research and Development project (R&D) of Biochemistry Division, BCSIR Laboratories, Chittagong. The authors are indebted to Dr. Mamtaz Dawlatana, CSO, IFST, BCSIR; and Mrs. Sayeda Shahida Begum, PSO, IFST, BCSIR. The authors also wish to thank Aysha Akter, Lecturer, Institute of Marine Sciences, Chittagong University for helping in identification of fish.

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