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DETERMINATION OF DICHLORODIPHENYLTRICHLOROETHANE (DDT) AND METABOLITES RESIDUES IN GANGETIC MYSTUS (*MYSTUS CAVASIUS*) AND SPOTTED SNAKEHEAD (*CHANNA PUNCTATUS*) OF BANGLADESH

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Abstract: A study was carried out to determine dichlorodiphenyltrichloroethane (DDT) and its metabolites in Gangetic mystus, *Mystus cavasius* and spotted snakehead, *Channa punctatus*. DDTs was extracted by QuEChERS method, cleaned-up by H_2SO_4 treatment and analyzed by GC with Electron Capture Detector. The calibration curves were linear over the range of the tested concentrations as shown by the fact that the correlation coefficients (r^2) for the linearity range were 0.995-0.999. The detection limit was found to be 5ppb for DDTs and the quantification limit was 6.5ppb. Recoveries were found (70-130%); this is acceptable for fish samples according to standard methodology. The DDT and its metabolites were not detected in the liver of *M. cavasius* and C. *punctatus*. In this study, *M. cavasius* was found to contain detectable amount of residual targeted pesticides DDTs at 64.21 ppb in digestive tract, 75.23 ppb in gill, 119.82 ppb in gonad and 45.84 ppb in muscle. The *C. punctatus* residual targeted DDTs were at 8.42 ppb in digestive tract, 4.04 ppb in gill, 56.44 ppb in gonad and 23.15 ppb in muscle. However, this study shows the higher DDT and its metabolites were in the gonad of *M. cavasius* and *C. punctatus*.

Key words: DDTs, Gulsha fish, Taki fish, Bangladesh.

INTRODUCTION

Bangladesh is a small and developing country overloaded with almost unbearable pressure of human population. In the past, people of Bangladesh were mostly dependent upon land-based proteins. But, the continuous process of industrialization and urbanization consumes the land area. Large number of different types of water bodies both inland and marine makes Bangladesh one of the most suitable countries of the world for freshwater aquaculture. Currently, Bangladesh ranked 3rd and 5th in the inland open water capture production and world aquaculture production, respectively (FAO, 2018).

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There are 265 freshwater and 475 marine fish species in the country. About 18 exotic species are being cultured in the country. The total annual fish production is estimated at 30.62 lakh Metric Ton in 2010-11 (Bangladesh fiscal year: 1 July-30 June), of which 14.61 Metric Ton (48%) are obtained from inland aquaculture, 10.54 Metric Ton (34%) from inland capture fisheries, and 5.46 Metric Ton (18%) from marine fisheries (DoF, 2011). Fisheries sector contributes 4.43% to GDP and 22.21% to agricultural GDP and 2.73% to foreign exchange earnings by exporting fish products in 2010-11. Fish supplements to about 60% of our daily animal protein intake. About 10% of the population is dependents directly and indirectly on the fisheries for their livelihood. Average annual growth rate of fish production in last 3 years is 6.11% (DoF, 2011). In the ever-growing trend of environmental-concerned society, it is apparent that many countries are starting to apply strict environmental regulations in almost every aspect associated with human life (Sunarso and Ismadji, 2009). The increasing worldwide need for food demands a higher agricultural productivity, which can only be achieved by an extensive use of pesticides. Unfortunately pesticides contaminate the environment through intensive or inappropriate use. Although organochlorine insecticides like DDT and its metabolites, lindane, aldrin or dieldrin for instance have been banned years ago in many countries based on their mutagenic, carcinogenic and endocrine disrupting properties, they still can be found in environmental samples due to their persistence and lipophilic properties (Lesueur *et al.* 2008). The degree of hazards depends on the amount of pesticides on crops and their toxicity. Since most of the pesticides are toxic in nature, their continuous intake by human even in trace amounts, can result accumulation in body tissues resulting serious adverse effects on health (Handa *et al.* 1999).

Fish is an essential and irreplaceable food in the rural Bangladeshi diet. Paddy-cum fish culture is a common practice in South East Asia. Natural growth of fresh water fish decreased only due to shrinkage of surface water caused by extensive irrigation projects and river siltation but also probably due to extensive use of pesticides including organochlorine compounds (OC) and other pollution. Contamination with toxic residual pesticides is at least partly responsible for fish mortality during hatching of eggs and growing of the post larvae (Hirose, 1975; Park *et al.* 2004; Singh and Singh, 2006).

Aquatic ecosystems are the reservoirs of many contaminants. POPs enter into aquatic ecosystems either due to direct discharge or hydrologic and atmospheric processes. The use of fish for measurement of pollutants is common as fish bioaccumulate many pollutants, especially organochlorine substances thus it's a principal object for monitoring. Furthermore, fish that is

stationary will function as an integrator of the concentrations in water and its feed. Fish and fishery products are generally regarded as a high-risk food commodity in respect to pathogen contents, natural toxins and pesticide residues. Considering the impertinence of food safety, a number of fresh fish was analyzed to determine the Organochlorine pesticide residues.

MATERIALS AND METHODS

Chemicals and reagents: The certified standards (purity, 99%) of *o,p´*-dichlorodiphenyltrichloroethane (DDT) & *p,p´*-dichlorodiphenyltrichloroethane (DDT), *p,p´*-dichlorodiphenyldichloroethylene (DDE) and *p,p´*-dichlorodiphenyldichloroethane (DDD) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Ethyl acetate, acetone, n hexane, dichloromethane (Pesticide-grade quality) and anhydrous magnesium sulphate, sodium chloride (analytical grade), aluminium oxide (alumina) were of Merck, Germany, florisil from ACROS organics, USA, charcoal from Uni-Chem, China. Anhydrous sodium sulfate and concentrated sulfuric acid (H_2SO_4) were purchased from Junsei Chemical Co., Ltd. (Kyoto, Japan) and were used for this analysis.

Standard solution preparation: Primary stock solution 100 mL (100 mg L^{-1}) of *o,p´*-DDT & *p,p´*-DDT, *p,p´*-DDE and *p,p´*-DDD was prepared separately by dissolving 0.0110 g in n-hexane. The primary standard solution was diluted to 20 ppm and 5 ppm middle and working standard solutions, respectively. Calibration curves (0.05, 0.025, 0.01, 0.005 and 0.0025 mgL^{-1}) for each standard were prepared by serially diluting the working standard solutions and limit of detection (LODs) and limit of quantification (LOQs) values were calculated (Table-1). These solutions were stored in amber bottles (100 mL) in the freezer (-24˚C) after labeling their name, concentration and date of preparation.

Samples: Two fresh water fish (5-10 kg) samples such as Gulsha fish (*Mystus cavasius;* Hamilton, 1822), under Bagridae Family and Taki fish (*Channa punctatus*; Bloch, 1794), under Channidae Family were collected from Chandana river, GPS Co-ordinate 23°32'37"N, 89°37'18"E, Modhukhali upazila of Faridpur district and Titas Basin of Brahmanbaria (fish market with GPS Co-ordinate 23°44'19"N, 90°47'23"E, respectively, on 12th May and 23th May, 2012 (Fig 1 & 2) and fishes were identified by Shafi and Quddus (1982). After collection of the fish samples, each of the vital organs such as muscle, gill, liver, digestive tract and gonad etc. were chopped and homogenized in kitchen blender and taking extraction and clean up the samples separately.

All the collected fish samples was wrapped with aluminium foil and kept in a chilled box and transferred immediately to the laboratory. All the collected

fish samples were stored in a freezer at a temperature below -20˚C until dissection.

Fig. 1. Map of Brahmanbaria district showing the River Titas

Extraction (QuEChERS, Anastassiades et al., 2003) and clean-up (Åkerblom, 1995): Each of the collected fish sample was taken out from the freezer. Scales and bones of the fish samples were removed. Then separate vital parts of body i.e. muscle, gill, liver, digestive tract and gonad collected in different petri-dish. The muscle of the fish was chopped into small pieces and homogenized by a kitchen blender. The homogenized samples were divided into several portions of 10g for replicate analysis. Representative homogenized sample (10 g) was taken in a Teflon tube (50ml volume). Then, it was extracted by vortex mixing for 1 minute successively with 10 mL ethyl acetate and adding 6g $MgSO_4$ and NaCl as water remover. The extracts were centrifuged for 5 minutes and the filtrates in a round volumetric flask. The solvent was exchanged from ethyl acetate to n-hexane by evaporation and the volume of the extract was adjusted up to 2mL. The extract (~2 mL; in a graduated test tube) was treated with concentrated sulphuric acid (2 mL) saturated with n-hexane and the test tube was inverted by an inverter carefully ~40 second (not shaked) and vortex for 30 seconds. Then the content was centrifuged for 5 minutes at a rate of 4000 rpm to separate the two layers. The upper clean organic phase was taken into a clean and dried vial for analysis with GC-ECD.

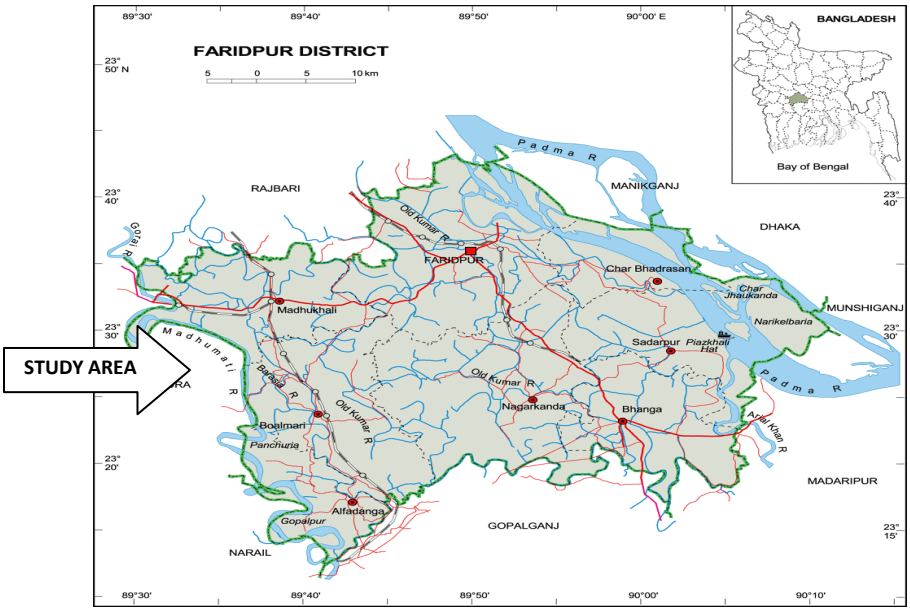
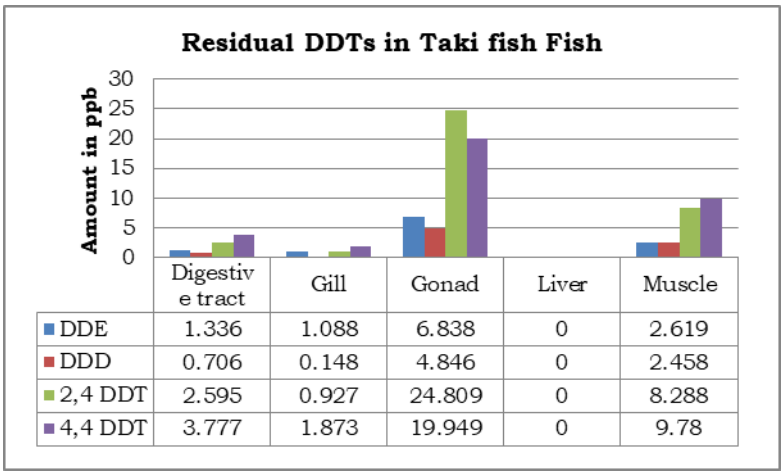


Fig 2. Map of Faridpur district including Modhukhali area

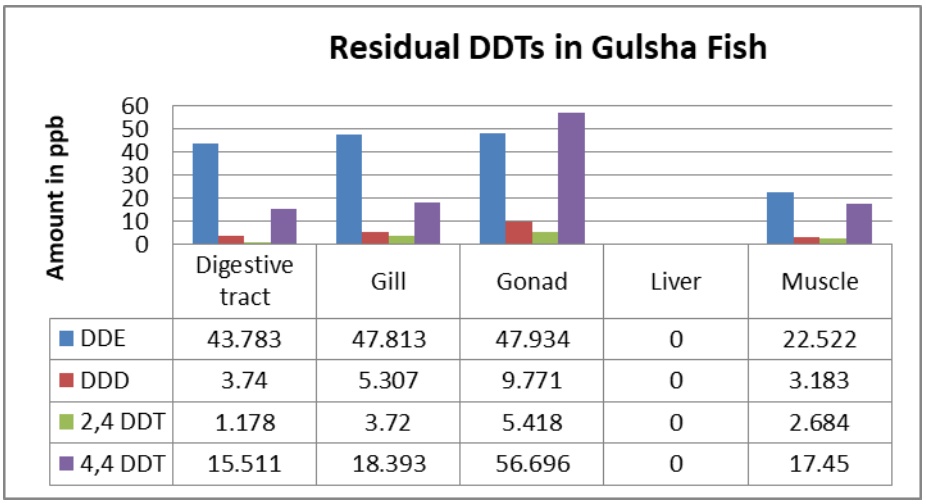
GC-ECD Analysis: A gas chromatograph equipped with an electron capture detector (Shimadzu Corp., Tokyo, Japan) was used for the analysis of all cleaned extracts. The analysis was conducted in a HP-5ms fused silica capillary column (30m×250µm inner diameter and 0.25µm film thickness). Helium was used as the carrier gas (flow rate, 2mL min^{-1}) and makeup gas (flow rate 1mL min min^{-1}). Oven temperature was programmed to 120°C (1-min holding time) and increased upto 285°C, at intervals of 10°C (4min holding time). The injector and detector temperatures were set at 220 and 290°C, respectively. All samples (1-µL volume) were injected in a splitless mode. A Shimadzu 17A gas chromatography (Shimadzu Corp., Tokyo, Japan) adjusted to the same conditions was used for the analysis of pesticide residues in the base laboratory in Bangladesh.

Quality Assurance: A solvent blank was injected into the system prior to the injection of the extracts, in order to get a smooth baseline to ensure the absence of any residual DDTs peaks. To perform recovery experiment, known amount of pesticide standards (0.25 *µ*g) were added drop by drop over to the fish control sample (10 g) and allowed the sample to stand for 30 min to let the pesticides be absorbed into the samples. To determine the LOD, working standard solutions were serially diluted with blank extract to get desired concentrations. The diluted standard solutions were injected one by one, and the limit of detection (LODs) of the tested analytes were determined using signal-



	Digestive tract	Gill	Gonad	Liver	Muscle
DDE	1.336	1.088	6.838	0	2.619
DDD	0.706	0.148	4.846	0	2.458
2,4 DDT	2.595	0.927	24.809	0	8.288
4,4 DDT	3.777	1.873	19.949	0	9.78

Fig 3. Level of residual DDTs in Taki fish.



	Digestive tract	Gill	Gonad	Liver	Muscle
DDE	43.783	47.813	47.934	0	22.522
DDD	3.74	5.307	9.771	0	3.183
2,4 DDT	1.178	3.72	5.418	0	2.684
4,4 DDT	15.511	18.393	56.696	0	17.45

Fig 4. Level of residual DDTs in Gulsha fish.

noise ratio: 3 with reference to the background noise obtained for the blank sample, whereas the limit of quantification (LOQs) were determined with signal-noise ratio: 10.

RESULTS AND DISCUSSION

Method validation: The calibration curves were prepared for *o,p´*-DDT, *p,p´*-DDT, *p,p´*-DDE and *p,p´*-DDD by plotting the peak area against the concentration (0.0025 to 0.05 mg L^{-1}) of each of the standards and the

calibration curves linear to the determination coefficients (r^2) ≥ 0.995 (Table 1). The correlation coefficients (r^2) for the linearity range were 0.995-0.999. The detection limit was found to be 0.39 ppb for both DDE & DDD and for DDT it was 1.56 ppb. The quantification limit was found 1.36 ppb for both DDE & DDD and 4.89 ppb for DDT.

The range of percent recoveries for fish samples in all parts (digestive tract, gill, gonad and muscle) are 72-118%, 73-88%, 75-99% and 80-108% for DDE, DDD, 2,4 DDT and 4,4 DDT, respectively, at the spiking level 0.02 mg L^{-1} and 70-103%, 71-103%, 70-106%, 75-138% for DDE, DDD, 2,4′ DDT and 4,4′ DDT, respectively, at the spiking level 0.1 mg L^{-1} which are acceptable according to standard methodology (Codex, 1993) except gill (133%) and gonad (138%). Precision was expressed as the relative standard deviation (RSD) and it was found to be <16.5 (Table 1).

Table 1. Name, correlation coefficients (r^2), LODs, LOQs, accuracy, and precision of the tested pesticides

Pesticides	Linear range (mg L^{-1})	Linearity (r^2)	LOD (ppm)	LOQ (ppm)	Accuracy (% recovery)± RSD% (Spiking level, ppm)			
					Digestive tract	Gill	Gonad	Muscle
p,p'-DDE	0.0025-0.5	0.998	0.005	0.0165	72 ± 2.15 (0.02) 74± 4.82 (0.10)	118 ± 0.60 (0.02) 91± 7.38 (0.10)	77± 4.42 (0.02) 103± 6.99 (0.10)	85± 3.16 (0.02) 70± 0.84 (0.10)
p,p'-DDD		0.999	0.005	0.0165	79± 14.13 (0.02) 80± 5.53 (0.10)	73± 4.82 (0.02) 103± 2.39 (0.10)	77± 3.83 (0.02) 89± 5.87 (0.10)	88± 4.89 (0.02) 71± 1.58 (0.10)
o,p'-DDT		0.995	0.005	0.0165	75± 5.08 (0.02) 73± 5.12 (0.10)	99 ±7.31 (0.02) 95± 1.38 (0.10)	91± 1.10 (0.02) 106± 3.93 (0.10)	92±3.98 (0.02) 70± 1.80 (0.10)
p,p'-DDT		0.998	0.005	0.0165	94± 16.45 (0.02) 93± 4.36 (0.10)	80± 5.72 (0.02) 133 ± 2.62 (0.10)	108± 6.03 (0.02) 138 ± 1.34 (0.10)	84± 15.03 (0.02) 75± 1.22 (0.10)

DDTs concentrations in fresh fish samples: The total amounts of DDTs were measured to be 8.415, 4.037, 56.442 and 23.146 ppb, respectively, at digestive tract, gill, gonad and muscles in Taki fish and 64.212, 75.234, 119.819 and 45.839 ppb, respectively, at digestive tract, gill, gonad and muscles in Gulsha fish (Table-2). In comparison of two experimental fish analysis *p,p*'-DDE, *p,p*'-DDD, *p,p*'-DDT were found higher amount in Gulsha than that of Taki fish. In

gonad of fishes, *o,p′* -DDT were found higher amount in Gulsha than that of Taki fish (Bar diagram-1,2). The total amounts of DDTs were measured to be 8.42 ppb f.w., 64.21 ppb f.w., at digestive tract of Taki and Gulsha fish, respectively. In gill, 4.04 ppb f.w. and 75.23 ppb f.w. respectively, found in Taki and Gulsha fish. DDTs are found highest amount in gonad than the other organs of fish body, i. e. 56.44 ppb f.w. 119.82 ppb f.w. in gonad of Taki and Gulsha fish respectively. In muscles of Taki and Gulsha fish 23.15 ppb f.w., 45.84 ppb f.w. amounts of DDTs, respectively. No pesticide residues were found to be present in liver of both fishes. The highest amount of DDT and its metabolites (119.82 ppb) were found in the gonad of Gulsha fish which might be due to its high lipid content (Mustafa, 2006) as the whole fish including gonad was taken for analysis. Taki showed small amount of DDT and its metabolites.

Table 2. DDTs in Taki and Gulsha fish

Local name	Organs	*p,p′*-DDE ppb Av. ± SD	*p,p′*-DDD ppb Av. ± SD	*o,p′*-DDT ppb Av. ± SD	*p,p′*-DDT ppb Av. ± SD	ΣDDT ppb	DDT/ DDTs ppb
Taki	Digestive tract	1.33 ±0.003	0.71 ±0.161	2.59 ±0.806	3.78±0.282	8.41	0.44
Gulsha		43.78+2.71	3.74+0.29	1.18+0.75	15.51+0.78	64.21	0.32
Taki	Gill	1.09 ±0.003	0.15 ±0.004	0.93 ±0.006	1.87±0.003	4.037	0.46
Gulsha		47.81+1.95	5.305+0.11	3.72+0.11	18.393+4.78	75.23	0.32
Taki	Gonad	6.84 ± 3.562	4.85 ± 0.069	24.81 ± 1.132	19.95±0.267	56.44	0.35
Gulsha		47.93+0.71	9.77+0.21	5.42+0.27	56.69+0.51	119.82	0.89
Taki	Liver	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Gulsha		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Taki	Muscle	2.62 ± 0.041	2.46 ± 0.002	8.29 ±0.216	9.78±0.18	23.14	0.42
Gulsha		22.52+0.83	3.18+0.32	2.68+0.69	17.45+0.93	45.84	0.61

f.w. = fresh weight; n.d.= not detected

Comparison of DDTs between gulsha and Taki fish in vital organs are found 64.212 & 8.415 ppb in digestive tract, 75.234 & 4.037 ppb in gill, 119.819 & 56.442 ppb in gonad and 45.839 & 23.146 ppb respectively. DDTs are higher in gulsha than that of Taki fish. Both Gulsha and Taki fish are carnivorous but we found more DDTs in Gulsha, because it may be due to the location. Gulsha was collected from Chandana River, Modhukhali, Faridpur District. This area may be more contaminated by DDTs than the location of Taki fish. It might be noted that the amount of 4,4′ DDT is much higher in all four parts compare to its metabolites DDE, DDD and 2,4′ DDT. It is alarming that, Gulsha is susceptible to DDTs. This suggests that the source of fish (different rivers of Bangladesh) contaminated till now. Though, the residual DDTs in all four parts of the fish samples were below maximum residue limit (MRL) of DDTs in fish (5.0 ppm) and

ADI/PTDI 0.01 ppm body weight (FAO, 2000). DDTs were found another study on Boal fish (*Wallago attu*). That means DDTs can be bind with the tissues of catfishes (Anonymous, 2008). Continuous consumption Gulsha will accumulate DDTs in our body which may lead to the concentration enough to cause a threat to our health.

CONCLUSION

Organochlorine pesticides were progressively banned in Bangladesh more than a decade ago and the DDT factory was closed in 1993, the present study showed that organochlorine compounds have persisted in the environment. In the present study DDTs were found in experimental fish samples of Taki and Gulsha fish. Findings of DDT and its metabolites in fish samples indicated that it can be found in other Fresh water fishes also. However, none of the samples was found to contain residual level exceeding the value (5.0 ppm for total DDT in fish) of Maximum Residue Limit (MRL) suggested by FAO/WHO (Codex, 1993). So these fishes are safe for consumption. It should not consume fish during breeding season because DDTs found more in gonad.

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