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DICHLORODIPHENYLTRICHLOROETHANE (DDT) RESIDUES STATUS IN FISHES AND PRAWNS OF CHITTAGONG CHEMICAL COMPLEX AREA, BANGLADESH

Farzana Khalil*, Mohammad Shoeb, M. I. R. Mamun, Tonima Mustafa, and Nilufar Nahar

Department of Zoology, Jagannath University, Dhaka-1100, Bangladesh

Abstract: Fourteen various fish samples *i.e.*, rui *Labeo rohita*, catla *Gibelion catla*, striped dwarf catfish *Mystus vittatus*, stinging catfish *Heteropneustes fossilis*, walking catfish *Clarias batrachus*, Ganges river sprat *Corica soborna*, mola carplet *Amblypharyngodon mola*, common carp *Cyprinus carpio*, Nile tilapia *Oreochromis niloticus*, climbing perch *Anabas testudineus*, spotted snakehead *Channa punctatus*, striped snakehead *Channa striata*, bronze featherback *Notopterus notopterus*, wallago catfish *Wallago attu* and mixed juvenile prawns were collected from a pond of closed down DDT (Dichlorodiphenyltrichloroethane) factory area of Chittagong Chemical Complex (CCC) of Bangladesh to assess the concentrations of DDT and its metabolites (4,4'-DDD and 4,4'-DDE). The fish flesh and shell-free prawn muscle samples were extracted by QuEChERS method and these were cleaned up using H_2SO_4. The cleaned sample extracts were analyzed using a Gas Chromatograph coupled with Electron Capture Detector (GC-ECD). The cultured *L. rohita* was used as a control which was found to contain no detectable amount of DDTs. Recovery experiments with control samples were spiked with certified DDTs standard at three different concentrations (0.01 mg kg^{-1}, 0.02 mg kg^{-1} and 0.05 mg kg^{-1}) levels. The percent recoveries were 72-114 % (0.05 mg kg^{-1}), 70-101 % (0.01 mg kg^{-1}) and 87-105 % (0.02 mg kg^{-1}). LOD and LOQ of the standards were found to be 0.0625 µg kg^{-1} and 0.2063 µg kg^{-1}, respectively and R^2 of 4'-DDE, 4,4'-DDD, 2,4'-DDT and 4,4'-DDT were found to be 0.999, 0.999, 0.999 and 0.998, respectively. The concentrations of total DDTs residue in fish and prawn samples ranged between 0.66 ng g^{-1} in *W. attu* to 8.90 ng g^{-1} in *H. fossilis*. The ratios of 4,4'-DDT/∑DDTs in the studied fish samples indicated the historical use of DDTs in the adjacent region around the DDT factory of the CCC area. The concentrations of total DDTs in all the samples were within the permissible Maximum Residue Level (MRL) recommended by FAO-WHO. To assess human health risks, Health risk Indexes (HI) of fish and prawn samples were calculated. HIs<1 in all fish and prawn samples indicated that the fish samples are safe to consume but the daily consumption of the fish together with other contaminated food may cause human health hazards.

Key words: DDT, DDD, DDE, Fish, Health hazard and DDT factory

*Author for corresponding: <farzana.khalil@aiub.edu>
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INTRODUCTION

Organochlorine pesticides (OCPs) suggests a significant group of persistent organic pollutants (POPs), which are hypothetical to be imaginable carcinogens or mutagens as well as endocrine disruptors (Haddaoui *et al.* 2016). The question of Persistent Organic Pollutants (POPs) has given rise to a global campaign created by the United Nations Environmental Program (UNEP) to abolish or diminish these health and environment threatening chemicals world-wide. The UNEP has listed 12 such chemicals that are chlorine containing organic compounds of which nine are pesticides (UNEP 2001). The production and usage of OCPs have been banned or restricted in developed countries. Whereas these bans and restrictions were legislated during the 1970s and 1980s, some developing countries are still using OCPs for agricultural and public health purposes because of their low cost and versatility in controlling various pests (Sabljic 2001). The Stockholm Convention identified 12 POPs and recently included 13 more and DDT is one of them. DDT which is an OCPs metabolite to 4,4'-DDE, 4,4'-DDD and 2,4'-DDT. DDTs can transport to a long distance and accumulate in fatty tissues where it has never been used. Food is the primary route of exposure to DDTs. In animals, DDT causes adverse health effects such as reproductive and developmental failure and may cause immune system defects (Qua *et al.* 2019).

Considering the harmful effect of DDTs on human and environment, a treaty was formulated in 2001 to stop production, application and elimination of OCPs. Bangladesh is a member of it and has been expending the fees regularly to the secretariat and actively participating in biannual conference (Conference of the Parties). However, report says that DDT is still being used illegally in the country (Takada *et al.* 2003).

Pesticide was first introduced in Bangladesh in 1951 (Rahman and Alam 1997). In early sixties, DDT used to be imported to Bangladesh to increase crop production and to eradicate vector diseases. After independence, organochlorine pesticides including DDTs was available for the farmers in local market to ensure food production. Soon after people around the world became concern about POPs for their chronic toxicity. In 1993 the use of DDT had been banned in Bangladesh (Matin *et al.* 1998). So, Bangladesh closed the DDT factory in 1995 without deciding, what should happen to the stored DDT in the factory of the Chittagong Chemical Complex (CCC) area. It was unclear what happened to the stockpile DDT in the godown of the CCC area. DDTs can spread to far away from the CCC factory areas by air, water day by day. For this reason, the human and environment near the DDT factory are at risk.

High amount of DDT and its metabolites were randomly distributed in the soil samples from CCC area (Nahar 2006). According to Al Mahmud *et al.* (2015), soil, pond sediment and pond water from different sites of the godown contained very high amount of DDT and its metabolites. The DDTs were approximately equally distributed in all directions of the water bodies (0.59–3.01 μg L^{-1}). From these research findings it can be happened that DDTs was dumped randomly to the factory surroundings after its closing. Thus, DDTs may be a risk factor for many components of the ecosystem there. Former employees of CCC are at high risk of accumulation of DDTs as they bath, drink and cultivate fishes in that pond.

The main objective of the present study is to determine the concentrations of DDT and its metabolites in fish samples in the pond of Chittagong Chemical Complex area and estimate the health risk of that fishes to human.

MATERIAL AND METHODS

Chemicals and Reagents: Certified standards (purity, 99%) of 4,4'-DDE, 4,4'-DDD, 2,4'-DDT and 4,4'-DDT were purchased from Dr. Erhenstrofer GmbH (Augsburg, Germany). Silica gel 60 (70-230 mesh, Merck, Darmstadt, Germany) was activated by heating over-night at 300 °C and allowing it to cool to room temperature before use. Anhydrous sodium sulphate (Na_2SO_4) (Merck, Darmstadt, Germany) of analytical grade and n-Hexane was of pesticide grade (Merck, Darmstadt, Germany). Sulfuric acid (98%, w/w, RCI Labscan, Ltd.) and water were of HPLC grade. All required glass wares like volumetric flasks and pipettes were calibrated by BSTI (Bangladesh Standard Testing Institute). All glassware were cleaned by detergent, rinsed with distilled water followed by acetone, dried in oven at 105°C and then finally were rinsed with solvent. The analytical balance (Adventure, AR 1140 by Ohaus Corp, USA) was calibrated by BSTI. The other instruments used include centrifuge machine, rotary evaporator for reducing solvent, ultrasonic bath and oven for drying.

Sample collection: Fourteen different fish and one prawn samples were collected on June 2016 from a pond, south of the storage region of Chittagong Chemical Complex (CCC) area which is situated near by Barabkunda Bazar in Chittagong district (22.584000° N, 91.685005° E). All samples were brought to Dhaka in a chilled box and immediately transferred to the Organic Research Laboratory, Chemistry Department of Dhaka University for further analysis. Samples were identified first by morphological characteristics analysis following Fishbase (2014), Rahman (2005) and Shafi and Quddus (1982) then stored at −20 °C in freezer.

Extraction and Clean-up: Before extraction each sample were thawed well in normal temperature. Only the edible portion of the samples were chopped into small pieces and homogenized by a kitchen blender. The grinded sample (10 g) were extracted by QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method (Mastoaska and Matrices, 2006) and cleaned-up with sulphuric acid treatment (Akerblom 1995). The cleaned extracts were analyzed by Electron Captured Detector (GC-ECD). Three replicates were used for analysis of each sample.

Table 1. Retention times (RT), determination coefficients (R^2), LOD and LOQ of fish

Pesticides	Linear range	RT (min)	Linearity (R^2)	LOD	LOQ
4,4'-DDE	0.00025– 1	10.39	0.999	0.0625	0.2063
4,4'-DDD		11.04	0.999		
2,4'-DDT		11.14	0.999		
4,4'-DDT		11. 68	0.998		

Preparation of standard solution, LOD, LOQ and Recovery experiment: Primary stock solutions (100 mg/kg) of 4,4'- DDE, 4,4'-DDD, 2,4'- DDT and 4,4'-DDT were prepared separately by dissolving 0.0101 g of each analyte in n-hexane (100 mL). The primary stock solution was diluted to obtain a 20 mg/kg medium standard and the medium standard was diluted to 5 mg/kg in order to obtain the working standard solutions. Calibration curves (0.5, 0.25, 0.125, 0.05, 0.01 and 0.005) for each standard were prepared by serially diluted the working standard solution and the limits of detection (LODs) and limit of quantification (LOQs) values were determined (Table 1). These solutions were stored in amber bottles (100 mL) at -24°C for pending analysis.

Recovery: Recovery experiment was done on culture *Labeo rohita* fish and known amount of certified four standards at 3 different concentration levels (0.05, 0.10, 0.20 µg/mL or mg/kg) were spiked separately then calculate the percentage recoveries (Table 2).

Table 2. Data of the recovery experiments for fish

Pesticides	% Recovery ±RSD %		
	Spiking level (0.05 mg kg^{-1})	Spiking level (0.1 mg kg^{-1})	Spiking level (0.2 mg kg^{-1})
4, 4' -DDE	92±2.76	93±7.13	87±3.02
4, 4' -DDd	114±1.54	101±6.17	102±16.34
2, 4' -DDT	76±2.05	88±8.30	105±9.29
4, 4' -DDT	72±3.64	70±0.78	91±15.05

GC-ECD: A Gas Chromatograph having Electron Captured Detector, (GC-ECD) (Shimadzu-2010 Japan) was used for identification and quantification of organochlorine compounds. A capillary column (HP-5) of 30-meter length, 0.25 mm inner diameter (ID) and 0.25 µm film thickness was used for analysis. Nitrogen was used as carrier and make-up gas (flow rate, 1 mL min^{-1}). Oven temperature was programmed from 120°C (1 min holding time), chronological increasing of temperature from 20 °C min^{-1} to 260 °C (hold 0 min) and finally 5 °C min^{-1} increased up to 280 °C (hold 4 min). The injector and detector temperatures were fixed at 220 °C and 290 °C, respectively. All samples (1 µL volume) were injected in a split less−split mode.

Quality Assurance: The concentration of DDT and its metabolites were quantitatively determined by external standard method using peak area. Linear calibration curves for DDTs were done by ECD over eight calibration levels (0.5, 0.25, 0.125, 0.05, 0.025, 0.01, 0.005 and 0.001 µg L^{-1}) (Fig. 1) The instrumental limit of detection (LOD) of DDTs was determined using signal-to-noise ratio(S/N) of 3 with reference to the background noise obtained for the solvent blank sample, whereas the limit of quantification was determined with S/N of 10.

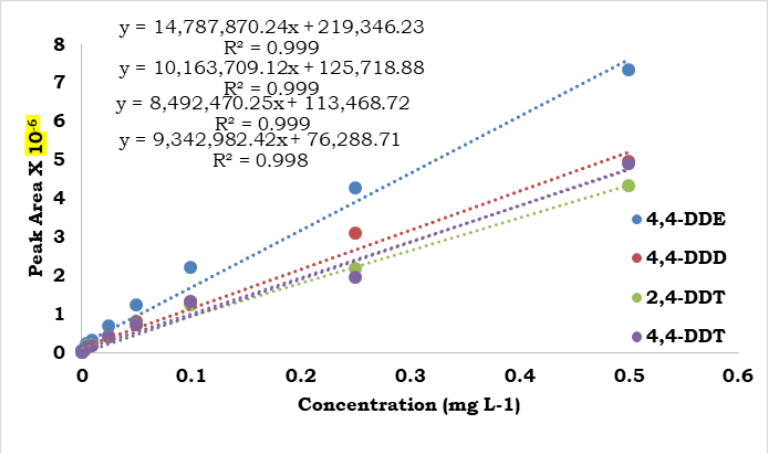

Fig. 1. Calibration curve of standard DDTs solutions

Lipid determination: Amount of lipid in the fish and prawn samples (n=15) were determined. The n-hexane extract was evaporated to dryness with gentle flow of nitrogen until constant weight was obtained. The dry materials determined the amount of lipid present in the fish samples

Human health risk estimation: Human health risk for consuming contaminated fish is estimated through Health risk Index (HI) following the equations referred by Darko *et al.* 2008 and Akoto *et al.* 2015. The concerned

food is acceptable if the value of HI is below 1 while the value above 1 considered the food as health risk to consumers.

The HIs were estimated by using the following equation.

$$HI = EDI / ADI$$

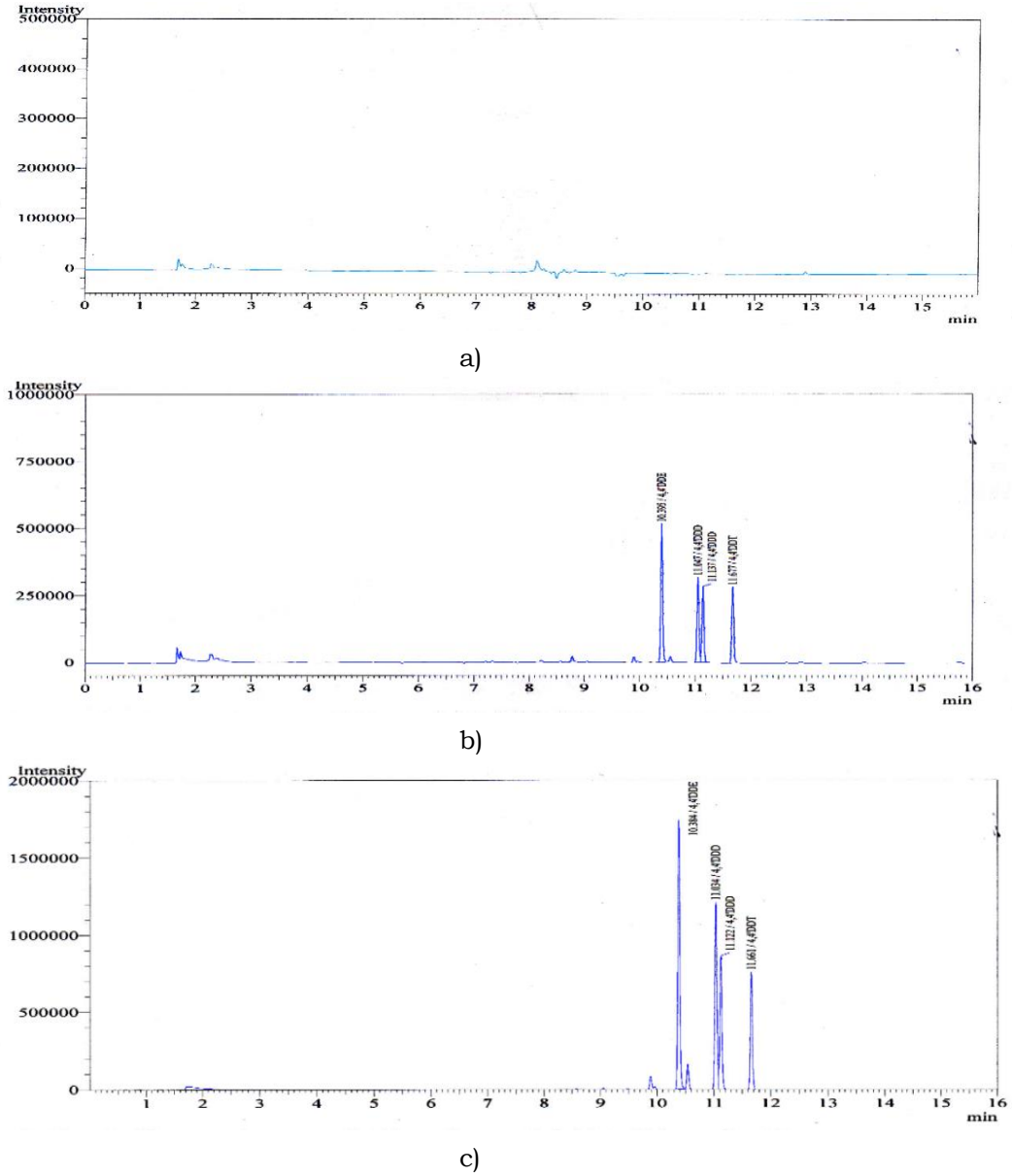

Fig. 2. Gas chromatography electron capture detection of a) blank sample, b) DDTs standards at 0.05 mg L^{-1}, and c) fish sample (sequence of peak: 4, 4'-DDE, 10.39; 4, 4' -DDD, 11.04; 2, 4' -DDT, 11.14; and 4, 4' -DDT, 11.68)

Here EDI is Estimated Daily Intake that was calculated for every fish sample and ADI is Acceptable Daily Intakes referred by WHO/FAO (FAO/WHO 2010). The EDIs of DDTs were calculated as follows equation where the unit is nanogram per kilogram body weight per day (ng/kg bw/d).

$$EDI = (C \times DR) / BW$$

Here C = Estimated concentration of DDTs (ng g^{-1}), DR = The average daily intake rate of fish (g day^{-1}) and BW = body weight (kg).

RESULTS AND DISCUSSION

Lipid contents: The lipid contents (%) of different fish and prawn species ranged from 0.19 % in *C. punctuatus* to 2. 70 % in *H. fossilis* (Table 3). However, similar values of the lipid contents were also found in seven fresh water fish species from Meghna river of Bangladesh ranged between 0.32 to 2.99 % (Mustafa *et al.* 2019) and in nine fresh water fish species of China ranged between 0.59 to 0.22 % (Zhang *et al.* 2014). while the lipid contents in three fresh water fish species of Bangladesh range between 3.9 to 8.7 % (Mustafa *et al.* 2015), in fifty-five fresh water, marine and culture fish and prawn species ranged between 0,03 to 18.3 % (Bogard *et al.* 2015) and in four fresh water fish species of Kenya ranged between 1.40 to 4.77 % (Keriko *et al.* 2010) are more or less similar to range of the present study despite the upper limit being higher.

DDT and its metabolites: The mean concentrations of 4,4´-DDE, 4,4´-DDD, 2,4´-DDT and 4,4´-DDT residues in the fishes and prawn species are presented in the Table 3. The concentrations of 4,4´-DDE residue ranged from 0.10 ± 0.01 ng g^{-1} in *O. niloticus* to 4.75 ± 0.60 ng g^{-1} in *H. fossilis*. The concentrations of 4,4´-DDD residue ranged from 0.06 ± 0.01 ng g^{-1} in *C. batrachus* to 2.68 ± 0.34 ng g^{-1} in *H. fossilis*. The mean concentrations of 2,4´-DDT residue ranged from 0.02 ± 0.01 in *C. soborna* to 0.82 ± 0.13ng g^{-1} in *H. fossilis*. The concentrations of 4,4´-DDT residue ranged from 0.05 ± 0.01 ng g^{-1} in *C. punctatus* to 3.02 ± 0.05 ng g^{-1} in *C. soborna*.

Total DDTs (∑DDTs) residues: The mean concentrations of total DDTs (∑DDTs) residue (ng g^{-1} ww) in fish and prawn are shown in Table. 3. ∑DDTs ranged from 0.66 ng g^{-1}in *W. att*u to 8.90 ng g^{-1} in *H. fossilis*. DDT and its metabolites are present in all the samples in different concentrations. The wide distribution of the residues indicates the aquatic habitat is polluted with DDTs. The variations of concentration in fish may be due to their different habitat, feeding habit, age and seasons etc. (Kidd *et al.* 2000). As the environment of the CCC is contaminated with DDT and its metabolites (Al Mahmud *et al.* 2015) which could be wash out with rain water into the pond and fish uptake these residues from their surrounding environment. Hossain *et al.* 2016; Mustafa et

al., 2019; Nahar et al., 2008 showed that fish from flood plains of Sonargaon region, the Kangsha and Titas river, culture pond and common fish markets of Bangladesh also contained higher level of DDT residues.

Recent or historical use of DDT: The ratio of 4,4'-DDT/∑DDTs indicates the time of exposure of DDTs in environment. If the ratio exceeds 0.5, DDT can be used as fresh input instead of degraded as historical resides (Zamir *et al.* 2008) These ratios in all samples of the present study were in the range of 0.01-0.46 indicated the past input of DDTs in the adjacent region around DDT factory of the Chittagong Chemical Complex (CCC) (Table 3, Fig. 2.). This could be due to the damping of DDTs in the surrounding area of the DDT factory in CCC during its close down time, 1995 (Al-Mahmud 2015).

Table 3. Lipid contents, different DDTs residues, Total DDTs, 4,4'DDT/∑DDT and HI of different fish and prawn species of CCC (Values of lipid is expressed as mean in % and of different DDTs and Total DDTs is expressed as mean ± SD in ng g^{-1} in f.w., n= 3 replicates)

Local name	Scientific name	Lipid (g%)	4,4'-DDE (µg kg^{-1})	4,4'-DDD (µg kg^{-1})	2,4'-DDT (µg kg^{-1})	4,4'-DDT (µg kg^{-1})	∑DDTs (µg kg^{-1})	4,4'DDT /∑DDT	HI
Rui	*Labeo rohita*	0.40	0.38±0.03	0.36±0.01	0.09±0.36	0.31±0.04	1.14	0.27	0.0001
Katla	*Gibelion catla*	0.21	0.37±0.01	0.26±0.04	0.14±0.02	0.08±2.05	0.85	0.09	0.0001
Tengra	*Mystus vittatus*	0.46	1.05±0.04	1.32±0.03	0.05±0.02	0.06±.12	2.48	0.02	0.0002
Shing	*Heteropneustes fossilis*	2.70	4.75±0.60	2.68±0.34	0.82±0.13	0.65±1.08	8.90	0.07	0.0008
Magur	*Clarias batrachus*	1.98	0.22±0.03	0.06±0.01	0.42±0.07	0.38±0.07	1.08	0.35	0.0001
Kachki	*Corica soborna*	0.76	2.71±1.68	0.80±0.12	0.02±0.01	3.02±0.93	6.54	0.46	0.0006
Mola	*Amblypharyngodon mola*	0.91	3.63±0.43	0.43±0.05	0.37±0.05	0.12±0.02	4.55	0.03	0.0004
Karfo	*Cyprinus carpio*	0.92	0.94±0.09	1.14±0.03	0.18±0.6	0.29±0.9	2.55	0.11	0.0002
Telapia	*Oreochromis niloticus*	0.43	0.10±0.01	0.39±0.01	0.45±0.05	0.15±0.05	1.09	0.14	0.0001
Koi	*Anabas testudineus*	1.13	1.09±0.05	1.14±0.14	0.65±0.04	1.42±0.07	2.90	0.01	0.0003
Taki	*Channa punctatus*	0.19	0.56±0.04	0.22±0.02	0.36±0.04	0.05±0.1	1.19	0.04	0.0001
Shol	*Channa striata*	0.24	1.78±0.31	0.17±0.01	0.16±0.01	0.07±0.65	2.18	0.03	0.0002
Chitiol	*Notopterus notopterus*	0.20	0.54±0.08	0.76±0.03	0.08±0.01	0.08±3.02	1.46	0.05	0.0001
Boal	*Wallago attu*	0.87	0.27±0.01	0.21±0.02	0.09±0.01	0.09±0.03	0.66	0.14	0.0001
Gura Chingri	*Unidentifiedi*	0.43	0.37±0.04	0.63±0.09	0.16±0.04	0.26±0.05	1.42	0.18	0.0001

*f.w.= fresh weight, SD= Standard deviation

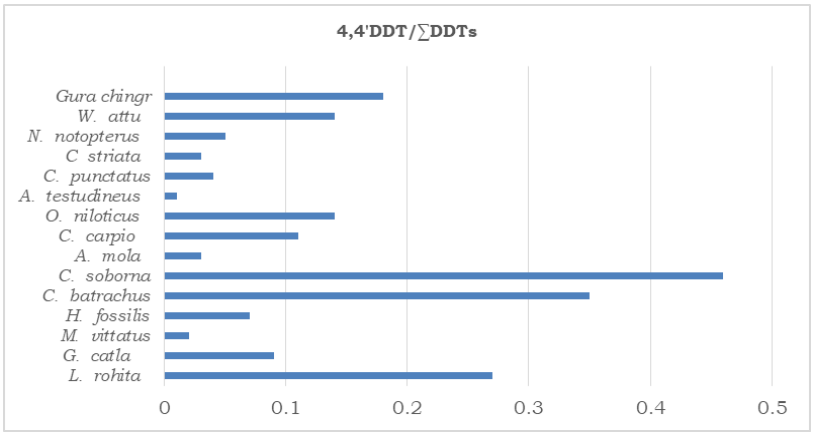

Fig. 3 Ratios of 4,4'-DDT/∑DDTs in different fish and prawn species of CCC

Human health risk assessment: Health Risk Index (HI) for the DDTs residues of the samples recorded less than 1 (HI<1) (Table 3). Moreover, the values of DDTs residues, found in fish tissue are below the Maximum Residue Level (MRL) value of 5000 ng g^{-1} w.w. for consumption set by Codex Alimentarius Commission of FAO/WHO (2012). So, all the fish are safe for consumption.

CONCLUSION

The DDT residues identified in all fish and prawn samples of the pond of Chittagong Chemical Complex (CCC) area indicating wide distribution of that pollutant in that region. The variation of the residual concentration in fish may due to their different feeding habit, lipid content and metabolic rates etc. Ratios of 4,4'-DDT/∑DDTs in the studied fish reflect the past expose of DDT in that area. The concentrations of total DDTs residues were below the limit of Maximum Residue Level (MRL) for consumption set by Codex Alimentarius Commission of FAO/WHO (2012). From the estimated Hazard Index (HI) of studied fish, it can be said that the fish together with other contaminations in food further cause complex hazard issues.

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