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Bioaccumulation of Some Organochlorine Pesticides Residues in Shrimp/Prawn of South-west Bangladesh and Assessment of Its Probable Risk on Human Health

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Abstract: ***An attempt was made to know the bioaccumulation of some organochlorine pesticides residues in shrimp (Peneus monodon) and prawn (Macrobrachium rosenbergii) by Electron Capture Detector (ECD) of Gas Chromatography during their culture with rice. A Total of six (06) sites viz. Bagerhat Sadar, Kochua, Chitolmari and Rampal Upazila of Bagerhat District as well as Keshobpur and Noapara Upazila of Jessore District were selected for this study from where the shrimp/prawn samples were collected to know the accumulation of some organochlorine pesticides named Heptachlor, DDT, Dieldrin and Endrin. The accumulation of heptachlor residue concentration was 0.016~0.309 ppm; Endrin residue was 0.073 ppm; Dieldrin residue concentration was 0.008~0.017 ppm and DDT residue concentration was not found in the sample Prawn body. In Shrimp sample, accumulation of Heptachlor residue was 0.014~0.031 ppm; Dieldrin concentration was 0.008~0.017 ppm. DDT concentration was 0.005 and Endrin residue was not detected in Prawn body. From the accumulation level of these four pesticide residues concentration in Shrimp and Prawn body, it was noticeable that Heptachlor concentration was in alarming level according to acceptable limit of EU which may cause serious health risk on human. Endrin, Dieldrin and DDT concentration were not in so harmful level in accordance with EU acceptable level. So, proper initiatives should take to inhibit the uses of all classes of hazardous pesticides and chemicals for avoiding any health hazard situation to ensure food safety.***

Keywords: Bioaccumulation, Shrimp and Prawn; Organochlorine Pesticides: DDT, Heptachlor, Dieldrin, Endrin; GC-ECD detector etc.

1. Introduction

In south-west region of Bangladesh around 80% people are practicing Shrimp/Prawn culture with rice as an integrated way for their living. With the growing demand for food, use of chemicals like fertilizer and pesticides in agricultural land has increased since 1954. Constant use of pesticides in crop fields has led to decreased biodiversity of fauna. Pesticides in aquatic ecosystem have become a matter of concern because of their toxicity and tendency to accumulate in food chain. Fishes live in the aquatic column; they are facing challenge for surviving from pollutants, particularly from various chemical fertilizers and pesticides used in or adjacent paddy fields. Pesticides reach aquatic ecosystems by direct application, spray drift, aerial spraying, erosion and run off from agriculture land. These huge amounts of pesticides may get deposited in open water wetlands and rivers of the country [12], [9]. Availability of very limited data [1] indicates that not much has been done in this regards here in Bangladesh. Moreover, there has been no elaborate study on Organochlorine pesticide (OCPs) residues in fishes from Bangladesh except [10].

Organochlorine pesticides (OCPs) including DDT (1,1,1-trichloro-2,2-bis-4-chlorophenyl ethane), aldrin, dieldrin, endrin, lindane, hexachlorobenzene (HCB), chlordane, methoxychlor, toxaphene, heptachlor, heptachlor epoxide and hexachlorocyclohexanes (HCHs) were used in Bangladesh to increase crop production and to eradicate vector diseases from early fifties [13]. However, due to long persistent in the environment, bioaccumulation, and biomagnifications and accumulation to the fatty tissues of human through food chain, the use and production of OCPs became restricted worldwide from nineties [8]. In Bangladesh at present, all Persistent Organic Pollutants (POPs) like DDT and heptachlor import and production have been banned but at least five POPs pesticides including DDT are still in use under a different name or label [3]. In south-west region of Bangladesh like Khulna, Bagerhat and Shatkhira district most of the farmers' culture shrimp and prawn with rice. As a result they have to use various groups of pesticides to control pest of their rice field which ultimate accumulate in sediment and from sediment it transmitted to shrimp and prawn body. Organochlorine (OC) pesticides are among the agrochemicals that have been used extensively for long periods. They have been used widely in agriculture, as well as, in mosquito, termite and tsetse fly control programs [5]. Residues and metabolites of many OC pesticides are very stable, with long half lives in the environment [4]. Thus, present study was aimed to investigate the level of residual organ chlorine pesticides in shrimp and prawn as well as its probable risk on human health.

2. Materials and Methods

2.1 Sample Collection

To determine the bioaccumulation of pesticides residues in shrimp and prawn; samples were collected from Bagerhat Sadar, Kochua, Chitolmari and Rampal Upazila of Bagerhat District as well as Keshobpur and Noapara Upazila of

Jessore District. Three samples were taken from each sampling site. The experiment was conducted during the period of October/2015 to May/2016. The control shrimp samples (without any contact of pesticides) were collected from the internal pond complex of Shrimp Research Station, Bagerhat of Bangladesh Fisheries research Institute, Bangladesh.

2.2 Apparatus

Mincer fish chopper (Weisser No. 81 K), round bottomed flask (500 and 100 mL), volumetric flask (50 and 10 mL), Homogeniger IKAR T25 digital ULTRA-Turrax, Nitrogen evaporator(N-EVAPTM111), SPE Cartidge (C18-REC 300 mg/3 mL) Magnetic Starrier, Gas Chromatograph (GC-2010, Shimadzu).

2.3 Reagents

Methanol, n-Hexane, Ethyl acetate/Acetonitrile, Primary Secondary Amine (all were with high purity 99.99%, HPLC grade) and anhydrous sodium sulphate, anhydrous magnesium sulphate were purchased from Merk Company (Germany). DDT, Dieldrin, Heptachlor and Endrin reference standards were obtained from Sigma Aldrich Chemicals (USA).

2.4 Extraction and cleanup procedure

The samples extractions were done after following the QuEChERS method with few of its modification. At first Ten (10) g of shrimp sample was taken in a Teflon Tube. Then 20 mL Ethyl acetate was added with that sample and the sample was shaken by hand for 1 min. Then the shaken sample was mixed properly with vortex mixture for 2 min. After the vortex, 1.5 g NaCl and 6 g anhydrous $MgSO_4$ was added with the mixture and again shaken by hand for 1 min. Then was shaken with vortex machine for 2 min. After vortex, the mixture was centrifuged at 5000 rpm for 5 min which created two layer *viz.* supernatant (upper aqueous layer) and denatant (lower non-aqueous layer). Then filtration of the supernatant was performed with 20g Na_2SO_4 and 10 mL anhydrous Ethyl acetate. After that ten (10 mL) supernatant was taken in a round bottom flask. Then the filtered solution was evaporated with rotary evaporator at a temperature not more than 40^0C until it was completely dry. Then 5mL n-Hexane was added in that round bottom flask. After that 2mL solution of the previous round bottom flask was taken in a test tube for cleaning-up.

Cleaning-up procedure

Addition of 150 mg PSA (Primary Secondary Amine) and 750 mg anhydrous $MgSO_4$ in that previous test tube was done. Then the solution was mixed with vortex mixture for 1 min. After that the solution mixture was centrifuged at 4000 rpm for 5 min which created two layer *viz.* supernatant (upper aqueous layer) and denatant (lower non-aqueous layer). Then the supernatant was taken in a tube and filtered with a 0.45 µ syringe filter in a 1.5 mL vial. Finally, the vial was kept in injection tray for its real time analysis through GC-ECD or other detector.

2.5 Sample analyses

The target pesticides residues were analyzed by GC-2010, Shimadzu with an Electron Capture Detector (ECD), an auto injector (Shimadzu, AOC 20i) and GC solution software. The capillary column used was Rtx-5MS, length 30.0 m x ID 0.25 mm x film thickness 0.25µm. The GC was run under the following conditions: injector temperature: 200^0C; detector temperature 270^0C; oven temperature programme: 250^0C starting from 100^0C for 1 minute and continued at 5^0C/minute to 250^0C held for 3 minute; injected sample volume: 1µL; mode of injection: Splitless; The carrier gas was N_2 with a 172.0 kPa flow rate. Run time; 16 min. Standards' peak were identified by injecting high concentration of the standard (1 ppm, 0.5ppm and 0.25 ppm) and the retention time for DDT, Heptachlor, Endrin and Dieldrin was determined. Then calibration was done at 3 points (1, 0.50 and 0.25 ppm) 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 methanol. Stock standard solution was used to prepare primary dilution standards. An appropriate volume of each individual stock solution was taken in a volumetric flask and mixed the solutions to obtain stock standard solution.

2.6 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 shrimp samples were collected as blank and treated exactly as a sample including exposure to all glassware, equipment, solvents and reagents used with the sample matrix. No analytic peak was detected in laboratory reagent blank. An aliquot of fortified samples matrix were prepared for known quantities of the pesticides which were added in the laboratory in ppm range. This laboratory fortified matrix was analyzed. Extraction and clean up were done as mentioned and the recoveries from untreated control samples of shrimp fortified with the analyzed compounds at the level of 0.50 ppm were 96 to 100% for heptachlor, 98 to 100% for DDT, 80 to 100% for Dieldrin and 86 to 100% for Endrin. 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, Heptachlor, Endrin and Dieldrin. 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. Any insecticide detected from the tested samples was identified and quantified by the chromatogram of standards. Sample results were quantitated in ppm automatically by the GC-Solution software, which represented the concentration of the final volume injected and from the value, the actual amount of pesticides residues present in the sample was determined by using the following formula:

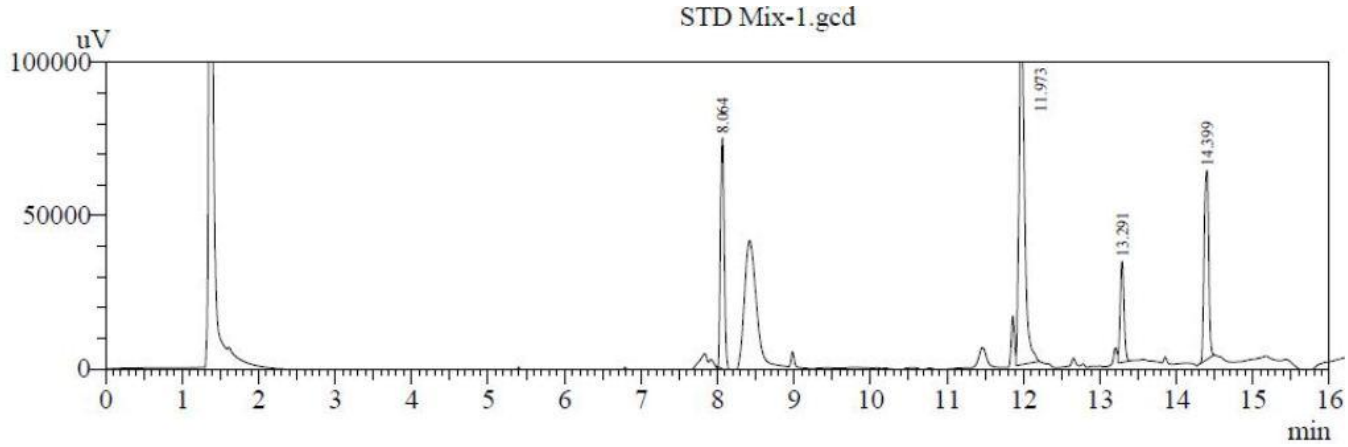
$$= \frac{\text{Concentration of obtained in injected volume (ppm)} \times \text{Quantity of final volume (L)}}{\text{Amount of sample taken (Kg)}}$$

3. Results

Prawn and Shrimp samples were collected from Bagerhat Sadar, Kochua, Chitolmari and Rampal Upazila of Bagerhat district as well as Keshobpur and Noapara Upazila of Jessore district. In these collected samples accumulation of DDT, Dieldrin, Heptachlor and Endrin residue were analyzed. From the analysis, it was found that pesticide residues were in alarming level of few sites. As shown in Table 1, different concentrations of DDT, Dieldrin, Heptachlor and Endrin residues were found.

Table 1: Accumulation of pesticides residues in Shrimp and Prawn body of different sampling sites

Samples Name	Target Pesticides	B'hat Sadar Upazila	Name of sampling location				Control	
			Kochua Upazila	Chitolmari UpazilaUpazila	Keshobpur Upazila	Avaynagar Upazila	Rampal Upazila	SRS Pond Complex
Prawn (*Macrobrachium rosenbergii*)	Heptachlor	0.177 ppm	0.024 ppm	0.309 ppm	0.026 ppm	0.016 ppm	0.020 ppm	ND*
	Dieldrin	0.008 ppm	0.011 ppm	0.008 ppm	0.010 ppm	0.017 ppm	0.009 ppm	ND
	Endrin	0.073 ppm	ND	0.044 ppm	ND	ND	ND	ND
	DDT	ND	ND	ND	ND	ND	ND	ND
Shrimp *(Peneus monodon)*	Heptachlor	0.014 ppm	0.031 ppm	0.024 ppm	0.020 ppm	0.016 ppm	0.026 ppm	ND
	Dieldrin	0.008 ppm	0.008 ppm	0.011 ppm	0.009 ppm	0.017 ppm	0.010 ppm	ND
	Endrin	ND	ND	ND	ND	ND	ND	ND
	DDT	0.005 ppm	ND	ND	ND	ND	ND	ND



*ND= Not Detected

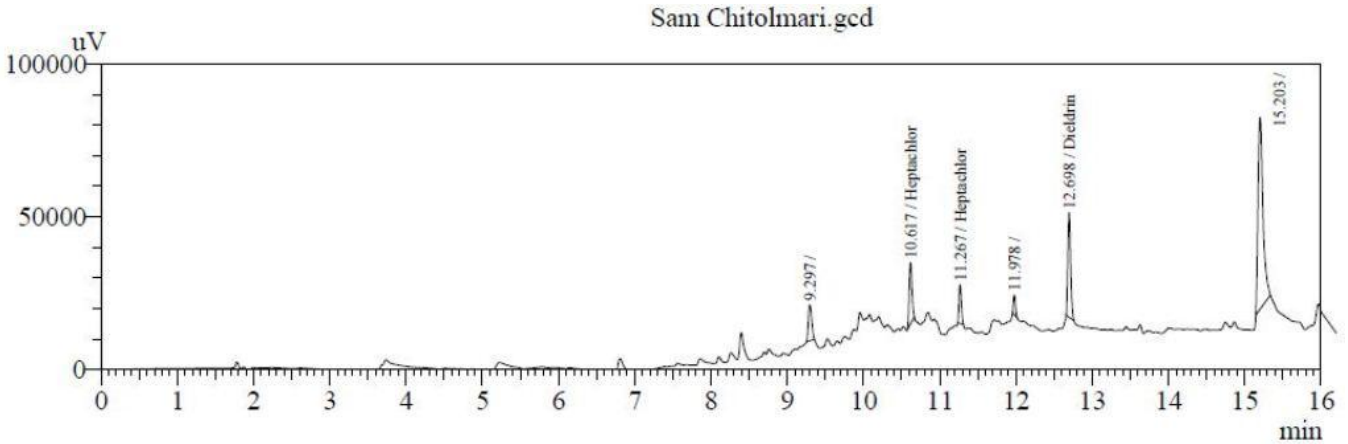


Figure: Chromatogram peaks obtained during real time analysis of standard and sample

Risk assessment of these accumulated pesticides residues on human health:

From these concentrations of pesticides, risk assessment was conducted based on local and country-wide consumption rates for key species [2]. To drive advisory consumption recommendations for the shrimp/prawn analyzed, two following equation were applied which were developed by [15].

Where CR_{lim} is the maximum allowable fish consumption rate (Kg/day), ARL the maximum acceptable individual lifetime risk level (10^{-5}), BW is the consumer body weight (70kg), Cm is the measured concentration of contaminant m

in a given species of fish (mg/kg or ppm), and CSF is the cancer slop factor [mg/kg-day)$^{-1}$]

$$CR_{lim} = \frac{ARL \times BW}{CSF \times Cm}$$

Where CRmm is the maximum allowable fish consumption rate (meals per year). CR_{lim} is the maximum allowable fish consumption rate (kg/d). Tap is the time averaging period (365.25 days per year), and MS is the meal (0.227kg fish/meal)

$$CRmm = \frac{CRlim \times Tap}{Ms}$$

NB: Cancer Slope factor, CSF for DDT=0.34, for others=2.0 [6], Per capita fish consumption in Bangladesh is 51.89 g/day [2]

Table 2: Accumulation of pesticides residue concentration in Prawn (*Macrobrachium rosenbergii*) and its risk based consumption rate for human

Species	Pesticides	ARL	BW (Kg)	CSF	Pesticides Concentration Cm (ppm)	CR_{lim} (Kg/day)	CR_{mm} (Kg/yr)
B'hat Sadar	Heptachlor	0.00001	70	2	0.177	0.002	3.22
Kochua		0.00001	70	2	0.024	0.015	24.14
Chitolmari		0.00001	70	2	0.309	0.001	1.61
Keshobpur		0.00001	70	2	0.026	0.013	20.92
Avaynagar		0.00001	70	2	0.016	0.022	35.39
Rampal		0.00001	70	2	0.020	0.018	28.96
SRS Pond		0.00001	70	2	ND	ND	ND*
B'hat Sadar	Dieldrin	0.00001	70	2	0.008	0.044	70.79
Kochua		0.00001	70	2	0.011	0.032	51.49
Chitolmari		0.00001	70	2	0.008	0.044	70.79
Keshobpur		0.00001	70	2	0.010	0.035	56.32
Avaynagar		0.00001	70	2	0.017	0.021	33.79
Rampal		0.00001	70	2	0.009	0.039	62.75
SRS Pond		0.00001	70	2	ND	ND	ND
B'hat Sadar	Endrin	0.00001	70	2	0.073	0.005	8.05
Kochua		0.00001	70	2	ND	ND	ND
Chitolmari		0.00001	70	2	0.044	0.008	12.87
Keshobpur		0.00001	70	2	ND	ND	ND
Avaynagar		0.00001	70	2	ND	ND	ND
Rampal		0.00001	70	2	ND	ND	ND
SRS Pond		0.00001	70	2	ND	ND	ND
B'hat Sadar	DDT (2,4-Dichloro-diphenyl-trichloro ethane)	0.00001	70	0.34	ND	ND	ND
Kochua		0.00001	70	0.34	ND	ND	ND
Chitolmari		0.00001	70	0.34	ND	ND	ND
Keshobpur		0.00001	70	0.34	ND	ND	ND
Avaynagar		0.00001	70	0.34	ND	ND	ND
Rampal		0.00001	70	0.34	ND	ND	ND
SRS Pond		0.00001	70	0.34	ND	ND	ND

***** ND = Not Detected

Table 3: Accumulation of pesticides residue concentration in Shrimp *(Peneus monodon)* and its risk based consumption rate for human

Species	Pesticides	ARL	BW (Kg)	CSF	Pesticides Concentration Cm (ppm)	CR_{lim} (Kg/day)	CR_{mm} (Kg/yr)
B'hat Sadar	Heptachlor	0.00001	70	2	0.014	0.025	40.23
Kochua		0.00001	70	2	0.031	0.011	17.69
Chitolmari		0.00001	70	2	0.024	0.015	24.14
Keshobpur		0.00001	70	2	0.020	0.018	28.96
Avaynagar		0.00001	70	2	0.016	0.022	35.39
Rampal		0.00001	70	2	0.026	0.013	20.92
SRS Pond		0.00001	70	2	ND	ND	ND*
B'hat Sadar	Dieldrin	0.00001	70	2	0.008	0.044	70.79
Kochua		0.00001	70	2	0.008	0.044	70.79
Chitolmari		0.00001	70	2	0.011	0.032	51.49
Keshobpur		0.00001	70	2	0.009	0.039	62.75
Avaynagar		0.00001	70	2	0.017	0.021	33.79
Rampal		0.00001	70	2	0.010	0.035	56.31
SRS Pond		0.00001	70	2	ND	ND	ND
B'hat Sadar	Endrin	0.00001	70	2	0.073	0.005	8.05
Kochua		0.00001	70	2	ND	ND	ND

Chitolmari		0.00001	70	2	ND	ND	ND
Keshobpur		0.00001	70	2	ND	ND	ND
Avaynagar		0.00001	70	2	ND	ND	ND
Rampal		0.00001	70	2	ND	ND	ND
SRS Pond		0.00001	70	2	ND	ND	ND
B'hat Sadar	DDT (2,4-Dichloro-diphenyl-trichloro ethane)	0.00001	70	0.34	0.005	0.412	662.92
Kochua		0.00001	70	0.34	ND	ND	ND
Chitolmari		0.00001	70	0.34	ND	ND	ND
Keshobpur		0.00001	70	0.34	ND	ND	ND
Avaynagar		0.00001	70	0.34	ND	ND	ND
Rampal		0.00001	70	0.34	ND	ND	ND
SRS Pond		0.00001	70	0.34	ND	ND	ND

***** ND = Not Detected

4. Discussion

Shrimp and prawn samples were analyzed by Gas Chromatography Electron Capture Detector (GC-ECD) for the identification of DDT, Dieldrin, Heptachlor and Endrin residues accumulation in shrimp and prawn as well as its risk based consumption rate for human health. To the best of my knowledge, this is the first time reported information on the bioaccumulation of residual concentration for DDT, Dieldrin, Heptachlor and Endrin in these selected sites *viz*. Bagerhat Sadar, Kochua, Chitolmari and Rampal Upazila of Bagerhat district as well as Keshobpur and Noapara Upazila of Jessore district. Among the obtained results of pesticides residues few are showing alarming concentration level as these are above the acceptable limit of European Union (EU). According to the EU, acceptable limit for Heptachlor and Endrin in shrimp/prawn is 0.01 ppm. Acceptable limit for Dieldrin in shrimp/prawn is 0.02 ppm and DDT in shrimp/prawn is 0.05 ppm. From the above Table-2 and Table-3, it was found that accumulation of Heptachlor residues in Prawn was in alarming level such as 0.177 and 0.309 ppm respectively which were collected from Bagerhat Sadar and Chitolmari Upazila of Bagerhat district. From the final report of [14], it was found that the concentration of heptachlor in bagda was 0 and 0.023 ppm for upstream and downstream waters of the river Karnaphuli, respectively. Using the two equations of [15], it was observed that if anyone consumes 3.22 Kg/year and 1.61 Kg/year of that prawn accumulated with 0.177 and 0.309 ppm Heptachlor residue then after one year he or she may fall into serious health risk like cancer. Accumulation of Endrin residue in Prawn was 0.073 ppm which was collected from Bagerhat Sadar Upazila of Bagerhat district.

From the final report of [14], it was found that the concentration of Endrin in bagda was 0 and 0.013 ppm for upstream and downstream waters of the river Karnaphuli, respectively. Using the two equation of USEPA (2000), it was observed that if anyone consumes 8.05 Kg/year of that prawn accumulated with 0.073 ppm Endrin residue then after one year he or she may fall into serious health risk with cancer. Other pesticides were not so alarming level in Prawn samples. For Shrimp samples, Endrin residue concentration was found 0.073 ppm in the sample collected from Bagerhat Sadar which was at alarming level and the two equation of USEPA (2000) indicate that it may cause for cancer in human health if anyone consumes 8.05 Kg/year of that prawn accumulated with 0.073 ppm Endrin residue concentration. A study [11] on organochlorine pesticides in marine sediments along the coast of Alicante, Spain. The levels of organochlorines in sediments were low. Concentrations ranged as follows: Sum of DDTs -0.3-1 ng/g, HCH 0.01 – 0.12 ng/g, and values of other compounds. Manninen *et al.* (1992) worked on total organochlorine in Finnish water courses. Total organochlorine concentrations measured in waters in a natural condition were below 15 mg/l & in polluted waters from 100-500 mg/l. [7] studied that organochlorine pesticides (OCPs) are synthetic organochlorines which were lipophilic and hydrophobic. Their lipophilicity, hydrophobicity, stability to photo-oxidation, and low vapor pressure, and low chemical and biological degradation rates had led to their accumulation in biological tissues and the subsequent magnification of concentrations in organisms, progressing through to the food chain. Due to our limitation of facilities, we could not study about other organochlorine and organophosphorus but we suspect that there may available other organochlorine pesticides contaminations shrimp and prawn sample. Further work on DDT, Dieldrin, Heptachlor and Endrin residues in blood serum of consumers of the study area is recommended.

5. Conclusion

The aim of this study was to provide information on the bioaccumulation of OCs in the shrimp and prawn sample collected from the selected sampling sites. Heptachlor and Endrin residue were identified as the dominant OC in the sampling sites. In some cases the concentration level of Heptachlor and Endrin cross the maximum acceptable limit recommended by EU which may cause significant health risk for human health. So, we should identify the probable source of this contaminant to reduce such contaminants. In future we will conduct such research to identify the probable source of the above contaminant.

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