Evaluation of pesticide residues in fish tissue samples collected from different markets of Jorhat district of Assam, India

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Abstract-Five sample of each fish in each location per month was collected from three markets of Jorhat districts for three months. Three markets are Jorhat fish market, Lichubari fish market and Teok fish market of Jorhat district. The fish species are Puthi(*Puntius sophore*),Muwa (*Amblypharyngodon mola*) Indian major carp (*Cirrhinus mrigala*), Catla(*Catla catla*), Rohu(*Labeo rohita*), Kuhi(*Labeo goniuf*), Common Carp(*Cyprinus carpio*), Calbasu (*Labeo calbasu*). The samples were homogenized and grind with the help of an electrical grinder. The samples were analyzed by multi residues method using GC-ECD. The residues of various pesticides in fish tissues were investigated and the main group of contaminant was the OCs followed by SPs. The contamination by OC group *viz.*, α-HCH ranged from 0.001 to 0.0019 µg g^{-1} **,** β-HCH ranged from 0.001 to 0.0016 µg g^{-1} **,** δ-HCH ranged from 0.0012 to 0.0053 µg g^{-1} **,** alachlor ranged from 0.001 2 to 0.0013 µg g^{-1}**,** aldrin ranged from 0.001 to 0.0015 µg g^{-1} , endosulfan-I ranged from 0.001 2 to 0.0015 µg g^{-1}, butachlor ranged from 0.0012 to 0.0014 µg g^{-1}, P,P'-DDE ranged from 0.001 to 0.0014 µg g^{-1}**,** endosulfan-II ranged from 0.0012 to 0.0013 µg g^{-1} **,** P,P'-DDD ranged from 0.001-0.0014µg g^{-1} and P,P'-DDT ranged from 0.0012-0.0014µg g^{-1} . The contamination by SP group *viz.* fenpropathrin ranged from 0.001 to 0.0012µg g^{-1} , permethrin-I ranged from 0.001 to 0.0012µg g^{-1} ,λ-cyhalothrin ranged from 0.001 to 0.002µg g^{-1} , α-cypermethrin ranged from 0.001 to 0.0021µg g^{-1} and deltamethrin ranged from 0.001 to 0.0013µg g^{-1} . Among the OC compound the major contaminants were α-HCH followed by β-HCH, δ-HCH, P,P'-DDT and P,P'-DDE

Key words: Fish, Organochlorine pesticides. Synthetic pyrethroids , India

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1 INTRODUCTION

The pollution of soils and water resources by pesticides, detergents, solvents and a variety of industrial organics is pressing a worldwide problem. The indiscriminate use of pesticides in agriculture can cause environmental problems especially to aquatic system by altering the quality of water and so affecting the physiology and biochemistry of non target organism such as fish, (Shakoori, et al, 1996). Several publications revealed the existence of pesticide residues mainly organochlorine compounds in various aquatic ecosystems components, (Badawy, 1998; El-Kabbany, et al, 2000 ; Gupta, et al, 2002 ; Radwan & Atalla, 2005). The aquatic environment is the ultimate sink for pollutants and any compounds produced on an industrial scale are likely to reach this environment sooner or later (Murty, 1986). Pesticides reach the aquatic environment through rainfall run-off from agricultural fields, direct entry from spray operations, industrial effluents, spraying of cattle and dust storms. Once in the aquatic environment, the pesticides are absorbed by aquatic organisms and concentrated in the trophic food chain thus endangering the life of fish and other organisms.

Organochlorine pesticides are still in use in developing countries despite their ban in many countries due to their associated problems of indiscriminate potency and persistency. The chemical stability of these compounds, their high lipid solubility and toxicity to human and animals (Aulakh et al, 2006), has led government and researchers to be concerned with their presence in the environment. Organochlorines continue to be the potential group of chemicals used in control of agricultural pests and vectors of diseases like malaria (David, et al, 2003), even though many new broad spectrum pesticides have been developed in recent years. The pesticides applied on land eventually find their way to the aquatic environment, thus contaminating soil and water for several years and subsequently get accumulated in aquatic organisms (Singh, et al, 2005). The aquatic organisms like fish are able to accumulate several fold higher concentration of pesticide residues than the surrounding water (Siddiqui, et al, 2005). It has been found that greater than 80% of the total intake of pesticide residues in human beings is through the food chain (Martinez, et al, 1997) and considerable amount of residues find their way into humans through consumption of contaminated fishes (Mwevura,et al, 2002).

Studies have also related the presence of organochlorine residues in breast milk and consumption of contaminated fish, and their role in alteration of thyroid function (Hagmar, et al, 2001). India is now both the largest manufacturer and consumer of pesticides in South Asia. Despite the proliferation of different types of pesticides, organochlorines such as hexachlorocyclohexane (HCH) and DDT still account for two thirds of the total consumption in the country (Kumari, et al, 2001) for agriculture and public health purposes respectively. In India as most of the rivers pass through agricultural fields, they are subjected to contamination with different pesticides used for crop protection. India is the third largest producer of fish and second in inland fish production (Feroz and Panikkar, 2006) with annual production of 7.75 million tonnes. In India there are many studies on the presence of organochlorine residues in aquatic system; water and fishes (Sarkar, et al, 2003) freshwater fish (Kaur, et al, 2008) and marine fish (Muralidharan, et al, 2009). Studies done by Kole et al. (2001) reported the presence of endosulfan and HCH residues in fishes sold at Calcutta market.

Pyrethroids are synthetic derivatives of pyrethrins, the natural insecticides that are produced by certain species of *Chrysanthemum*. Pyrethroids of greatest interest to water quality include cypermethrin and fenvalerate. Pyrethroids are extremely toxic to aquatic organisms, with lethal concentration (LC50) values less than 1.0 ppb. They are applied in urban areas primarily for structural pest control, in agricultural areas and in the home as pet sprays and shampoos. Some of the new pyrethroids such as cypermethrin, which is used in much lower amounts, could be up to 20 times more toxic than permethrin (Amweg, et al, 2005). The primary transport pathway for pyrethroids is receding waters from agricultural and urban applications through runoff. Pyrethroids are persistent compounds and feebly soluble in water (Laskowaski, 2002). Several recent monitoring studies in California have reported synthetic pyrethroid contamination of both surface waters and sediments (Hengel, et al, 1997; Weston, et al, 2004; Gan, et al, 2005).Assam is North Eastern state of India which has great potential for integration of inland wetland fishes. The people of North Eastern state mainly Assam make fish an integral part of their dish. There is huge domestic production of inland water fish of Assam which is consume by the people of Assam along with their daily dishes. So fish become an integral part of the dishes of Assamese people. So the present study was aimed at assessment of OPs and SPs in selected species of fishes in inland wetlands of Assam.

2 MATERIAL AND METHODS

Five sample of each fish in each location per month was collected from three markets of Jorhat districts for three months. Three markets are Jorhat fish market, Lichubari fish market and Teok fish market of Jorhat district. Individual species of fish samples were mixed and analyzed in triplicate. The fish species are Puthi(*Puntius sophore*),Muwa (*Amblypharyngodon mola*) Indian major carp (*Cirrhinus mrigala*), Catla(*Catla catla*), Rohu(*Labeo rohita*), Kuhi(*Labeo goniuf*), Common Carp(*Cyprinus carpio*), Calbasu (*Labeo calbasu*).The samples were kept in deep freeze. The allotted samples were taken out from the deep freezer and put the sample one by one in polythene bag and were kept in running water for thawing without direct contact with running water. After thawing each samples were peeled and take edible portion only for sample analysis. Thoroughly homogenized, the whole sample in a homogenioser. From this weigh 25 g of the meet were taken for analysis and balance were sample kept in deep freezer**.** This thoroughly grounded sample were taken in a mortar add 100 gm anhydrous sodium sulphate were added to combine with water present and to disintegrate sample. Mixed with spatula until sample and sodium sulphate were well mixed. These mixtures were taken in a 500 ml conical flask and add 150 ml petroleum ether and shake well for 15 minutes with a shaker. Decanted petroleum ether through anhydrous sodium sulphate taken in a glass funnel which is plugged with glass wool and collect in another beaker/conical flask and re-extracted residue with two, 100 ml portions of petroleum ether by shaking 15 minutes each time with a shaker. Combined extracts together and concentrated to 5 ml in vacuum flash evaporator. The extract quantitatively transferred to a 100 ml separating funnel, 15 ml acetonitrile saturated with petroleum ether 60-80^0 C was added and shaken well and layers allowed to separate. The bottom layer containing the pesticide was transferred in to 1 litre-separating funnel containing 600 ml water, 100 ml petroleum ether and 40 ml of saturated sodium chloride solution, extraction with acetonitrile was

repeated for 2 more time and the bottom layer poured to the 1 litre separating funnel, shaken well and allowed to separate.The bottom aqueous layer was transferred to another 1 litre-separating funnel containing 100 ml petroleum ether. It was also shaken well and allowed to separate. The aqueous layers were discarded and the petroleum ether layers from the two were pooled, dried with anhydrous sodium sulphate and vacuum flash evaporator.

The samples were then cleaned by column chromatography. The column of 22mm inner diameter was packed by placing solvent washed cotton plug at bottom of the column above which 4 g of cleaned dried anhydrous sodium sulfate was placed. Then 25 g activated florosil was placed slowly on the uniformly leveled clean anhydrous sodium sulphate. The florosil was leveled properly by shaking and above it again 10g clean anhydrous sodium sulphate was placed and leveled properly. The above was filled with petroleum ether and allowed to drain. When the petroleum ether to down to the surface of column acetonitrile clean up sample were taken in minimum quantity of petroleum ether quantitatively without the disturbing the column package. Then elute first with petroleum ether containing 6% diethyl ether, 200 ml was collected. Column was then eluted with petroleum ether containing 15% diethyl ether and again 200 ml were collected. Both these solutions were pooled together and evaporated to dryness in a vacuum flash evaporator. Then 100ml n-haxen was added to vacuum flash evaporator and final volume was made up to 5 ml (Sharma,2007).

GC analysis: Samples were analyzed by using Shimadzu Gas Chromatograph (GC-2010) equipped with "Ni electron capture detector (ECD) and capillary column (DB-1,30mx ID-0.25mm)with AOC-20i auto injector. Operating conditions were: Injector temperature:280^{0}C and Detector Temperature:300^{0}C oven temperature programmed at 170^{0}C (hold for 5 minute) -220^{0}C (hold for 10 minute and increased @1.5^{0}C)-280^{0}C (hold for 7.0 minute and increased

@4.0^{0}C).The nitrogen gas was used as carrier gas with flow rate 7.7 ml per minute with split less ratio.

Reference standard of 21 pesticide mixture was obtained from M/S sigma Aldrich, USA. The list of pesticide along with retention time (Rt) are given in the table1.

Table 1: List of Organochlorine and Synthetic Pyrethroids pesticide along with retention time (Rt)

Peaks	Pesticide	Rt(Minute)	Recovery (%)	LOQ (ng/L)
Organochlorine				
1	α-HCH	9.02	83.08	0.02
2	β-HCH	10.08	84.00	0.02
3	δ-HCH	11.28	82.23	0.05
4	Alachlor	15.89	90.45	0.01
5	Aldrin	18.48	86.25	0.05
6	Endosulfan-I	24.69	88.03	0.05
7	Butchlor	25.77	92.00	0.01
8	P,P'-DDE	27.38	91.08	0.03
9	Endosulfan-II	27.85	93.09	0.05
10	P,P'-DDD	31.21	87.05	0.02
11	P,P'-DDT	35.41	94.04	0.01
12	Dicofol	41.93	87.30	0.03
Synthetic pyrethroids				
13	Fenpropathrin	43.14	88.09	0.02
14	λ-Cyhalothrin	51.41	94.40	0.01
15	Permethrin-I	55.20	87.32	0.06
16	Permethrin-II	55.91	88.90	0.03
17	β-Cyfluthrin	58.99	96.03	0.01
18	α-Cypermethrin	60.18	89.09	0.04
19	Fenvalerate-I	62.85	90.01	0.03
20	Fenvalerate-II	63.63	84.04	0.02
21	Delamethrin	65.64	92.08	0.03

3 RESULTS AND DISCUSSION

Recovery experiments were carried out with the entire representatives group. The recoveries from fish samples fortified at the level of 0.1 and 0.01 mg/kg varied from 82.23-96.03 per cent for all Organochlorine and Synthetic pyrethroids pesticides for electron captured detector.

Table2: Pesticide residues (μg g^{-1}) in tissues of fish sample collected from different markets of Jorhat districts of Assam during the month of November,2010.

S. No.	Pesticides	F-1	F-2	F-3	F-4	F-5	F-6	F-7	F-8
1	α-HCH	0.0019	BDL	BDL	BDL	BDL	BDL	0.001	BDL
2	β-HCH	BDL	BDL	0.0013	BDL	BDL	BDL	BDL	BDL
3	δ-HCH	BDL	0.0053	BDL	BDL	BDL	0.0018	BDL	BDL
4	Alachlor	BDL	BDL	BDL	BDL	BDL	BDL	0.0013	BDL
5	Aldrin	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
6	Endosulfan-I	0.0012	BDL	BDL	BDL	BDL	BDL	BDL	BDL
7	Butchlor	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
8	P,P'-DDE	BDL	BDL	BDL	BDL	BDL	0.0013	BDL	BDL
9	Endosulfan-II	BDL	BDL	BDL	0.002	BDL	BDL	BDL	0.0012
10	P,P'-DDD	0.0013	BDL	BDL	0.001	BDL	BDL	BDL	BDL
11	P,P'-DDT	BDL	BDL	0.0014	BDL	BDL	BDL	BDL	BDL
12	Dicofol	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
13	Fenpropathrin	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
14	λ-cyhalothrin	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
15	Permethrin-I	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.0012
16	Permethrin-II	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
17	β-cyfluthrin	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL

18	α-cypermethrin	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
19	Fenvalerate-I	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
20	Fenvalerate-II	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
21	Delamethrin	BDL	BDL	BDL	BDL	0.001	BDL	BDL	BDL
Total		**0.0044**	**0.0053**	**0.0027**	**0.003**	**0.001**	**0.0031**	**0.0023**	**0.0024**

Table 3: Pesticide residues (μg g^{-1}) in tissues of fish sample collected from different markets of Jorhat districts of Assam during the month of December,2010.

S. No.	Pesticides	F-1	F-2	F-3	F-4	F-5	F-6	F-7	F-8
1	α-HCH	BDL	BDL	0.0015	BDL	BDL	BDL	0.0013	BDL
2	β-HCH	0.0016	BDL	BDL	0.0012	BDL	BDL	BDL	BDL
3	δ-HCH	BDL	0.0014	BDL	BDL	0.0012	BDL	BDL	BDL
4	Alachlor	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
5	Aldrin	BDL	BDL	BDL	BDL	BDL	BDL	0.001	BDL
6	Endosulfan-I	BDL	BDL	0.0012	BDL	BDL	BDL	BDL	0.0015
7	Butchlor	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
8	P,P'-DDE	BDL	BDL	BDL	BDL	0.0014	BDL	BDL	BDL
9	Endosulfan-II	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
10	P,P'-DDD	BDL	BDL	BDL	BDL	BDL	0.0014	BDL	BDL
11	P,P'-DDT	BDL	0.0012	BDL	BDL	BDL	BDL	BDL	BDL
12	Dicofol	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
13	Fenpropathrin	BDL	BDL	BDL	BDL	0.001	BDL	BDL	BDL
14	λ-cyhalothrin	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
15	Permethrin-I	0.001	BDL	BDL	BDL	BDL	BDL	BDL	BDL

16	Permethrin-II	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
17	β-cyfluthrin	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
18	α-cypermethrin	BDL	BDL	BDL	0.001	BDL	BDL	BDL	0.001
19	Fenvalerate-I	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
20	Fenvalerate-II	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
21	Delamethrin	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Total		**0.0026**	**0.0026**	**0.0027**	**0.0022**	**0.0036**	**0.0014**	**0.0023**	**0.0025**

Table4: Pesticide residues (μg g^{-1}) in tissues of fish sample collected from different markets of Jorhat districts of Assam during the month of January,2011.

S. No.	Pesticides	F-1	F-2	F-3	F-4	F-5	F-6	F-7	F-8
1	α-HCH	0.0013	BDL	BDL	BDL	0.001	BDL	BDL	BDL
2	β-HCH	BDL	BDL	0.0012	BDL	BDL	BDL	0.001	BDL
3	δ-HCH	BDL	BDL	BDL	0.0014	BDL	0.0016	BDL	BDL
4	Alachlor	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.0012
5	Aldrin	BDL	BDL	BDL	BDL	0.0015	BDL	BDL	BDL
6	Endosulfan-I	0.0014	BDL	BDL	BDL	BDL	BDL	BDL	BDL
7	Butchlor	BDL	BDL	BDL	0.0012	BDL	BDL	0.0014	BDL
8	P,P'-DDE	BDL	BDL	0.001	BDL	BDL	BDL	BDL	BDL
9	Endosulfan-II	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.0013
10	P,P'-DDD	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
11	P,P'-DDT	BDL	0.0012	BDL	BDL	BDL	BDL	BDL	BDL
12	Dicofol	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
13	Fenpropathrin	BDL	BDL	BDL	BDL	0.0012	BDL	BDL	BDL

14	λ-cyhalothrin	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
15	Permethrin-I	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
16	Permethrin-II	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
17	β-cyfluthrin	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
18	α-cypermethrin	BDL	0.0021	BDL	BDL	BDL	BDL	BDL	BDL
19	Fenvalerate-I	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
20	Fenvalerate-II	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
21	Delamethrin	BDL	BDL	0.0013	BDL	BDL	BDL	BDL	BDL
Total		**0.0027**	**0.0033**	**0.0035**	**0.0026**	**0.0037**	**0.0016**	**0.0025**	**0.0025**

Average of three replicates, BDL: Below Determination Level

F-1 = *Puntius sophore* , F-2 = *Amblypharyngodon mola* , F-3 = *Cirrhinus mrigala* , F-4 = *Catla catla* , F-5 = *Labeo rohita* , F-6 = *Labeo goniuf* , F-7 = *Cyprinus carpio* , F-8 = *Labeo calbasu*

Table 5: Month wise number of contamination of samples by pesticides

Samples	Nevemver,2010	December,2010	January,2011	Total
Puntius sophore	3	2	2	7
Amblypharyngodon mola	1	2	2	5
Cirrhinus mrigala	2	2	3	7
Catla catla	2	2	2	6
Labeo rohita	1	3	3	7
Labeo goniuf	2	1	1	4
Cyprinus carpio	2	2	2	6
Labeo calbasu	2	2	2	6

The residues of various pesticides in fish tissues are presented in table 2, 3 and 4.The results revealed that the main groups of contaminants are the OCs followed by SPs. However,

the degree of contamination of later group is negligible. The contamination by OC group *viz.*, α-HCH ranged from 0.001 to 0.0019 µg g^{-1} **,** β-HCH ranged from 0.001 to 0.0016 µg g^{-1} **,** δ-HCH ranged from 0.0012 to 0.0053 µg g^{-1} **,** alachlor ranged from 0.001 2 to 0.0013 µg g^{-1}**,** aldrin ranged from 0.001 to 0.0015 µg g^{-1} , endosulfan-I ranged from 0.001 2 to 0.0015 µg g^{-1}, butachlor ranged from 0.0012 to 0.0014 µg g^{-1}, P,P'-DDE ranged from 0.001 to 0.0014 µg g^{-1}**,** endosulfan-II ranged from 0.0012 to 0.0013 µg g^{-1} **,** P,P'-DDD ranged from 0.001-0.0014µg g^{-1} and P,P'-DDT ranged from 0.0012-0.0014µg g^{-1} . The contamination by SP group *viz.* fenpropathrin ranged from 0.001 to 0.0012µg g^{-1} , permethrin-I ranged from 0.001 to 0.0012µg g^{-1} ,λ-cyhalothrin ranged from 0.001 to 0.002µg g^{-1} , α-cypermethrin ranged from 0.001 to 0.0021µg g^{-1} and deltamethrin ranged from 0.001 to 0.0013µg g^{-1} . The degree of contamination during the three months are almost same. The highest contamination seen in *Puntius sophore, Cirrhinus mrigala* , *Labeo rohita* followed by *Catla catla, Cyprinus carpio* and *Labeo calbasu* . The results shows that among the OC compound the major ones were α-HCH followed by β-HCH, δ-HCH P',P'-DDT and P,P'-DDE. Almost all the samples were contaminated by one or more pesticides. These may be due to the fact that the isomers of α-HCH is relatively stable. This is stable to light, high temperature, hot water and acid, although it is dechlorinated in alkali (Matsumura, 1985).

The concentrations of β and δ -HCH were the most dominated among isomers of HCHs. The predominance of occurrence of β -HCH, the most persistent form is due to the isomerisation of α - and δ -HCH into β -HCH and also due to its high stability. Presence of high concentrations of β -HCH has been reported in various biological components (Kumari, et al, 2001; Kole, et al, 2001). Earlier study conducted by Kumari et al (2001) also reported high concentration of HCH in fish and food products and possibilities of higher dietary intake of pesticides through fishes. Although no major ill effects in man have been correlated with the levels of HCH, altered thyroid function was reported in women (Hagmar, et al, 2001). Among the metabolites p,p-DDT, p,p-DDE had significantly higher burden in fishes. The high frequency of occurrence of p,p-DDE can be due to its past use and the residues brought from surrounding areas. India banned DDT for agricultural purposes in 1989, but continues to use for malaria control. The presence of p,p-DDT and its metabolites in fish species indicates its recent exposure at the minimum levels. Moreover, the conversion of p,p - DDT to p,p-DDE could be due to the activity of the mixed

function oxygenase enzyme through metabolic functions. The presence of SPs pesticide in fish is partly due to their past use.

4 CONCLUSION

The investigation reveals that the consumption of fish is safe from consumer's point of view as residues of all the pesticides were far below their MRLs, though the fish eater is not so safe for which safety measures have to be adopted to protect the future generation.

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