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# Pesticide Residues in Flesh of *Cirrhinus mrigala* Collected From a Commercial Farm and River Chenab at Trimu Head, Jhang

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**Abstract.-** Farmed and wild samples of two weight Cirrhinus *viz*., 501-900 g and 901-1300 g of *Cirrhinus mrigala* were collected from a Commercial Fish Farm and from river Chenab at Trimu Head Jhang, respectively. The extracted residues were pesticides analyzed through reverse phase high performance liquid chromatography (HPLC) technique. Endosulfan α, p, p'-DDT, methamidophos, carbofuran, diazinon, parathion methyl, dimethoate, malathion, chlorpyriphos, cypermethrin, carbosulfan and isoproturon were detected in farmed fish. All of these pesticide residues except for methomidophos were also identified in the flesh of the wild fish. The level of all pesticide residues was lower than the maximum residue limit but carbofuran exceeded the maximum residue limit (MRL, 0.1 ppm) in farmed (0.11 ppm) and wild fish (0.21 ppm). Total concentration of pesticide residues was found to be highest in farmed (0.06 ppm) as compared with wild fish (0.05 ppm). Higher concentration of pesticide residues was recorded in cultured fish.

**Keywords:** Pesticide residues, *Cirrhinus mrigala*, wild, farmed, HPLC.

## INTRODUCTION

Pesticide losses from areas of application and contamination of non-target sites such as surface and ground water represent threat to environment. Although pesticide residues are present in all foods but fatty foods such as meat, butter, milk, etc are more susceptible for pesticide residues. The animals including fish inhabiting pesticide contaminated or receiving these chemicals, directly or indirectly through food are likely to contains the residues in their tissues.

Solid Phase Extraction (SPE) and Gas Chromatography (GC) were employed by Schenck *et al.* (1994) for detection and estimation of for pesticide residues in the non-fatty fish. Rao *et al.* (1998) investigated the bioaccumulation of pesticide residues of HCH, DDT, aldrin and dieldrin by Thin Layer Chromatography (TLC) in fish. Roche *et al.* (2000) determined the residues of organochlorine (OC) contaminants in muscles of eel, carps and catfish. Shanta *et al.* (2002) found variability of aldrin, dieldrin, BHC and DDT in tissues in *Cyprinus carpio* and *Puntius ticto*. Majority of OCs are banned in Pakistan, however, there are reports of their continuous use in the field (UNEP, 2002; Zhang *et al.*, 2002). Many other recent works have indicated the presence of OC residues in surface waters, sediments, biota and vegetations in Africa (Ntow, 2005) and elsewhere (Hung and Thiemann, 2002; Haozheng *et al*., 2007; Imo *et al*., 2007).

The present study was undertaken to estimate the pesticide residues in flesh of cultured and wild *Cirrhinus mrigala* which is one of the indigenous fish in the Indo-Pak regions. The primary objective of this investigation was to assess the pesticide contamination levels in fish meat being consumed by human.

## MATERIALS AND METHODS

### *Materials*

Total of 28 fish samples of two weight groups $W_1$ (501-900g) and $W_2$ (901-1300g) (7 of each weight group from wild and cultured) *Cirrhinus mrigala* were captured with the help of gill net from Sher Dil Fish Farm and Trimu Head Jhang, Pakistan The fish were transported to laboratory under chilled conditions and stored at -20 °C till processing. Composite sediment samples and water samples were collected from different ponds. All pesticide-residue-grade solvents purchased from Merck (Darmstadt, Germany) were glass distilled

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before use, and they did not yield any interfering chromatographic peak when concentrated from 10 ml to 100 µl. Pesticide residues standards were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Solid phase extraction bond elut C-18 (3 cc/500 mg) were purchased from Varian Inc. (Salt Lake, USA). Glassware was washed with detergent, rinsed with purified water and acetone, and heated at 180°C for 2 h.

*Sample collection and treatment*

Seven samples (fish, sediments and water) were collected randomly from each fish group in the earthen ponds of local fish hatchery. The water samples (1.5 L) were collected into cleaned glass bottles from midstream positions of the pond at approximately 0.4 m depth below the water surface. Then they were pre-filtered through 0.45 µm fiber glass filter (Whatman) to remove debris and suspended materials and preserved by adding 5 ml concentrated sulphuric acid to prevent biological activity (Ntow, 2005). The waterbed sediments were grabbed from the bottom of the pond where fine-textured substrate had accumulated, and they were transferred into glass beakers. The fish samples obtained from hatchery at the various sites around the pond were washed in enough distilled water. All these samples were immediately stored in an ice-chest at 4°C and transported to lab for analysis.

In the lab, the muscle tissues of the fish samples were ground in a blender to form a homogenous composite, while the sediments were air-dried. The pre-filtered water samples were processed using a solid phase extraction technique (Totolin, 2003). After the cartridges (C-18) were conditioned with 5 ml methanol, 5 ml acetone and 5 ml Milli Q water in turn, the water samples (1 L) were passed through cartridges at a flow rate of 5 ml/min. The stationary phase was washed with 5 ml acetone and 5 ml Milli Q water and then centrifuged at 1 000–1 500 rpm for 5 min. The analytes trapped in the cartridges were eluted 3 times with 5 ml portions of hexane. The extracts were concentrated to about 2 ml using a Buchi rotary evaporator operated under 600 Pa at 30°C. The lipids (oils and fats) were extracted from the weighed amount of samples by soxhlet apparatus. A total of 30 g each sample was taken in the extraction thimble.

After the OC residues in sediments and fish samples were extracted using soxhlet extraction[16], 10 g sample was placed into a beaker containing 50 g anhydrous sodium sulfate and mixed thoroughly. The sample mixture was transferred to an extraction thimble and placed in a soxhlet extractor. The mixture was extracted with 150 ml acetone: *n*-hexane (20:80; *V*/*V*) at 50 °C for 4 h, the extracts were filtered and concentrated to 1 ml by vacuum rotary evaporator. Each raw extract was then dissolved in 10 ml hexane and passed through pre-conditioned octadecyl C-18 columns at a rate of 2 ml/min to clean up. The column was washed with 1 ml 30% (*V*/*V*) methanol followed by 1 ml ultrapure water and was allowed to dry. The sample (analyte) which was trapped in the column was eluted 5 times with 0.5 ml aliquots of hexane to recover the pesticide residues. Hexane in the sample was then allowed to evaporate to leave the residue alone in the vial. Dried sample was dissolved in 1 ml portion of hexane, mixed thoroughly with a whirl mixer and then transferred to autosampler vials ready for gas chromatography. Carbamates residues were also extracted from soil by ultrasonic solvent.

*Determination of pesticide residues by HPLC*

The standards, spiked samples, fish, water and sediment samples were analyzed by reverse phase high performance liquid chromatography (HPLC) and UV-Visible detector. Column C-18 (250 × 4.6 mm, 5 µm), oven temperature (30 □), injection loop (20 µl), and flow rate (1 ml/min) were the common parameters for the analysis, whereas, the mobile phase, wavelength and pressure were different for different pesticide analysis (Table I). Different procedures of statistical analysis were used to analyze data. The data were analyzed through computer by using Minitab Package (SAS, 1995).

## RESULTS AND DISCUSSION

Pesticide analysis of fish muscles showed that both wild and farmed *Cirrhinus mrigala* were contaminated with endosulfan α, p, p'-DDT, methamidophos, carbofuran, diazinon, parathion

methyl, dimethoate, malathion, chlorpyriphos, cypermethrin, carbosulfan and isoproturan. Concentration of endosulfan in farmed fish was recorded as 0.02±0.001 and 0.01±ppm and $W_1$ and $W_2$, whereas in wild, the highest concentration was 0.009±0.001ppm under $W_1$ (Tables II). The organochlorine can reach the freshwater bodies through runoff, atmospheric deposition, and leaching due to agricultural applications near water bodies and there sediments work as sink for these pesticides (De Lorenzo *et al.,* 2002).

**Table I.- Mobile phase, wavelength and pressure for pesticide analysis.**

| Pesticide residues | Mobile phase (*V*:*V*) | Wave length (nm) | Pressure (Pa) |
|---|---|---|---|
| Chlorpyrifos, α,β-endosulfan and organophosphatase | acetonitrile: water (4:1) | 208 | 693.6 |
| DDT, DDE and heptachlor | methanol: water (95:05) | 238 | 724.2 |
| Parathion methyl | acetonitrile: water (55:45) | 235 | 499.8 |
| Captan | acetonitrile: water (2:1) | 200 | 622.2 |
| Cypermethrin | acetonitrile: water (3:1) | 225 | 693.6 |
| Carbamates and atrazine | acetonitrile: water (2:1) | 220 | 663.0 |
| Chlorobromuron isoproturan and chlorotoluron | acetonitrile: water (1:1) | 200 | 795.6 |

The concentration of p, p'-DDT in farmed *Cirrhinus mrigala* were detected as 0.01±0.00 and 0.007±0.004ppm under $W_1$ and $W_2$. While in wild *Cirrhinus mrigala* concentration was noted as 0.006±0.0003 and 0.009±0.0004 under $W_1$ and $W_2$ with significant differences (Table II). In the present study, p, p'-DDT was not exceeding the maximum residue limit (MRL) as 7mg/g of fish set by the Codex Alimentarious Commission of FAO–WHO (1997) , which is also in agreement with the findings of Roach and Runcie (1998). They also reported that DDT was not found above MRL in fish at any location of Sydney Harbour. Since our study did not investigate the levels of organochlorines in the entire food web, it cannot be concluded that the accumulation of organochlorines is from the water column rather than another source within the food chain. Therefore, the presence of persistent and hydrophobic organochlorine residues in water, even at low concentrations, poses a risk to health of the biota because such residues have a higher affinity for partitioning into sediment and aquatic organisms. Chemicals with long half-life values and a high solubility in lipids (fats, oils, or waxes) will tend to accumulate in fatty tissue. Such lipophilic chemicals easily move into cells and are sequestered in fat, where they become more persistent.

The concentration of methamidophos noticed in farmed and wild *Cirrhinus mrigala* are given in Table II. Methamidophos was only present in farmed fish. The concentration for carbofuran crossed MRL value (0.1µg/g) in farmed raised fish under $W_1$ (0.15ppm) and wild fish under $W_2$ (0.23ppm). Highest concentration of diazinon was found in wild under $W_2$ was 0.06±0.002ppm, whereas, minimum concentration of parathion methyl was also found in $W_2$ (wild) as 0.05±0.00ppm. Dimethoate had the same value of concentration in both groups of farmed fish (Table II). In farmed *Cirrhinus mrigala* malathion was found to be 0.01±0.0001 and 0.009±0.0001ppm under $W_1$ and $W_2$, whereas, in wild *Cirrhinus mrigala* its concentration was measured as 0.005±0.001 and 0.01±0.0005ppm under $W_1$ and $W_2$ with significant differences (Table II). Chlorpyriphos and carbofuran had maximum concentration in farmed fish under $W_2$, whereas, cypermethrin and isoproturon had maximum concentration in farmed fish under $W_1$ (Table II). As regarded concentration of individual pesticides, the highest concentration of dimethoate (0.16±0.001) and maximum concentration of p, p'-DDT (0.008±0.000ppm) were recorded in farmed *Cirrhinus mrigala.* In wild fish, the highest concentration of carbofuran (0.21±0.004) and lowest concentration of carbosulfan (0.004±0.001) were detected.

The composition of total pesticide concentration in farmed and wild *Cirrhinus mrigala* has shown that the total concentration was highest in farmed fish as compared to wild fish (Table II). This might be due to contaminated supplemented artificial feed ingredients due to spray of various

**Table II.- The concentration (ppm) of pesticide residues in the flesh of farmed and wild *Cirrhinus mrigala*.**

| Pesticides | Fish weight | | | Fish weight | | |
|---|---|---|---|---|---|---|
| | 501-900 g | 901-1300 g | Mean ± S.E | 501-900 g | 901-1300 g | Mean ± S.E |
| Endosulfan α | 0.02±0.001 nopq | 0.01±0.001 opq | 0.01±0.001 LM | 0.009±0.001 opq | 0.001±0.0007 pq | 0.005±0.001 MN |
| P, P'-DDT | 0.01±0.0003 opq | 0.007±0.0004 pq | 0.008±0.0004 MN | 0.006±0.0003 pq | 0.009±0.0004opq | 0.008±0.0003MN |
| Methamidophos | 0.08±0.01 hi | 0.01±0.005 opq | 0.04±0.01 IJ | 0.00±0.00 o | 0.00±0.00o | 0.00±0.00 N |
| Carbofuran | 0.15±0.009 de | 0.08±0.009 hi | 0.11±0.01 E | 0.02±0.003 b | 0.23±0.004 a | 0.21±0.00 4A |
| Diazinon | 0.03±0.001 lm | 0.02±0.001 mn | 0.03±0.001 JK | 0.051±0.001 kl | 0.06±0.002 ijk | 0.05±0.002 GH |
| Parathion methyl | 0.06±0.001 ij | 0.07±0.001 ij | 0.06±0.001 G | 0.06±0.01 jk | 0.05±0.002 jkl | 0.06±0.002 GH |
| Dimethoate | 0.16±0.001 cd | 0.16±0.001 cd | 0.164±0.001 B | 0.15±0.003 cd | 0.13±0.004 f | 0.14±0.004 C |
| Malathion | 0.01±0.0001 opq | 0.009±0.0001 opq | 0.009±0.0001 MN | 0.005±0.001 pq | 0.01±0.0005nopq | 0.008±0.001 MN |
| Chlorpyriphos | 0.01±0.007 nopq | 0.08±0.001 gh | 0.05±0.01 HI | 0.06±0.005 ijk | 0.02±0.005 mno | 0.04±0.006 J |
| Cypermethrin | 0.03±0.001 mn | 0.02±0.001 nop | 0.024±0.001 KL | 0.02±0.001 mn | 0.01±0.001 nopq | 0.02±0.002 L |
| Carbosulfan | 0.05±0.01 jkl | 0.13±0.007 ef | 0.09±0.01 F | 0.008±0.009 opq | 0.001±0.0007 pq | 0.004±0.001 MN |
| Isoproturon | 0.17±0.009 c | 0.12±0.01 f | 0.14±0.009 C | 0.09±0.008 g | 0.16±0.008 cd | 0.13±0.01 D |
| Total mean | 0.06±0.006 | 0.06±0.004 | 0.06±0.005 | 0.05±0.006 | 0.05±0.007 | 0.05±0.005 |

Means with similar letters in a row/column are statistically similar at $P<0.05$.
S.E. = Standard error; ppm = Parts per million
HPLC = High Performance Liquid Chromatography

pesticides and insecticides. The concentration of pesticides in fish might be related with the feed ingredients which were used for the preparation of artificial feed for supplementation to the fish. Isoproturon was detected in rice bran, which was used as one of the ingredient of feed used in the fish hatchery. These findings were in lines with the findings of Cheema (2003) who reported the pathway for the transmission of pesticide residues through feed The highest concentrations of pesticides in farmed fish were probably due to pollution of ground water in the study area and excessive spray of these pesticides on rice, wheat and maize which are basic feed ingredients used in harvesting of fish in ponds.

## CONCLUSIONS

It has been concluded that higher concentration of pesticide residues (0.06ppm) was found in farmed *Cirrhinus mrigala* as compared to wild (0.05ppm). No pesticide residue has crossed maximum residue safe limit except carbofuran which was 0.11 ppm in the farmed and 0.21 ppm in the wild *Cirrhinus mrigala*. The minimum residues found were those of p,p-DDT in farmed fish and endosulfan in the wild fish.

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