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ORIGINAL ARTICLE

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Determination of Pesticide Residues in Freshwater Fish Tissues by Gas Liquid Chromatography

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In this present study, identification and quantification pesticide residues in freshwater fish *Labeo rohita*. Gas Liquid chromatography (GLC) method is successfully developed for the determination of profenofos residues. The fish were exposed to 1/10th sublethal concentration of profenofos 10 µg/L^{-1} for 15 days. After the exposure fish was sacrifice and organs like Gil, Liver, Kidney, and Muscle were separated, the fish samples were extracted with n-Hexane, cleaned up and purified through solid-phase extraction method. The residues are in the following order, Gill > Muscle > Kidney > Liver, these results suggest that prolonged exposure to sublethal concentrations of profenofos in *Labeo rohita* leads to increased accumulation of pesticide residues in tissues.

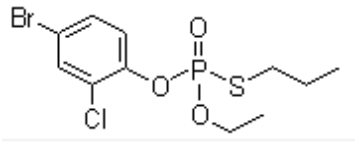
Key words: GLC, Profenofos, Liver, Pesticide, Residue

Aquatic organisms are affected by the pesticides leaching into the waters through agricultural run off, although often the aquatic environment is not the primary site of application of pesticides. Two factors ultimately contribute to the concentration of the pesticides in the aquatic ecosystem: persistence of pesticides in the soils and long-range transport of the pesticides in the atmosphere. As soils receive the major part of the globally used pesticides, and residues are transported eventually to the water bodies, persistence in the soil ultimately constitutes a threat to the aquatic environment. Stringent regulations have been introduced in many countries for the purpose of minimizing the hazards from the use of pesticides, which may leave behind residues or degradation products or metabolites after the desired purpose has been accomplished or when they reach some part of the environment other than intended target. Efforts are also made for development of Chemical pesticides which do not have toxic residue problems. Pesticides of less persistent which are safe to be introduced, Measures for promoting rapid degradation of pesticides including metabolites are being, stepped up.

As an outcome of these studies, selection and measurement of these pesticides is made as an indispensable ingredient of any comprehensive programme taken up to assess or abate their deleterious effects on the biota of various ecosystems.

In the present study profenofos used as toxicant for determination of residue concentration in freshwater fish. Profenofos is widely used to control various mites, lepidopteron pests of cotton, tobacco and on various agricultural crops in India. Due to its wide use in control of insects and mites on many different crops, humans are inevitably exposed to its residues in various ways. Profenofos has been classified as moderately hazardous (toxicity class II) pesticide by WHO and it has a moderate order of acute toxicity following oral and dermal administration (WHO, 1990, US EPA, 2000). Profenofos is extremely toxic to fish and macro-invertebrates (Costa *et al.,,* 2008). The acute toxic action of profenofos is the inhibition of the acetylcholinesterase activity resulting in toxicity also in humans. Biochemical signs of hepatocellular injury and disturbed amino acid metabolism may be of value as markers of exposure to Profenofos (Gomes *et al.,* 1999). Moreover, high doses of the Profenofos induce tissue vacuolization and haemorrhage while swelling of Bowman's capsules and tubular degeneration in the kidney were well reported by (Fawzy *et al*., 2007).

Chemical structure of Profenofos (Organophosphate)



Chemical Abstract name: O-(4-bromeo-2-chlorophenyl) O-ethyl S-propyl phosphorothioate, IUPAC name: O-4-bromo-2chlorophenyl O-ethyl S-propyl Phosphorothioate,

Molecular Formula: C11H15BRCLO3PS,

Molecular Weight: 373.63

CAS No.: 41198-08-7 and

appearance: Pale yellow liquid with garlic-like odour.

Physical-Chemistry

Yellowish liquid. Boiling point: 110°C at 0.001 mm Hg. Flash point: 167°C. Vapor Pressure: 1.24 x 10^{-4} pa at 25°C. Solubility: In water 20 mg/litre at 20°C; 25 mg/litre at 25°C.Readily miscible with most organic solvents. Stability: stable under neutral and slightly acid conditions, unstable under alkaline condition.

Analytical instrument are used in this study to determine, quantify and confirm pesticide residues in fish *Labeo rohita* for both research and regulatory purpose. The pesticides are generally analysed by different types of instruments like spectrophotometry (Bhargavi *et al*., 2006), thin layer chromatography, (TLC), high performance liquid chromatography and gas chromatography (GC), GC-MS (Rathore *et al*., 1993; Thanh *et al*., 2008). The present study describe the method of extraction, clean up and determination of pesticide residue in freshwater fish *Labeo rohita* by using Gas Liquid Chromatography (GLC) for the seperation. The identification of pesticides on fish, vegetables were developed and validated (Radwan *et al*., 2005).

MATERIALS AND METHODS

Experimental Design

The organic solvents hexane and acetone used were HPLC grade and purchased from Loba Chemie. Anhydrous sodium sulphate (AR) from Loba Chemie used for residue extraction was maintained at 200°C overnight and kept in air tight container. The technical and commercial pesticide was purchase from local pesticide market in Guntur of Andhra Pradesh.

The freshwater fish *Labeo rohita* measuring 6 ± 8 cm in length and 6.5 ± 7.5 gm in weight irrespective of the sex were used in the experiment. Fish were washed with 0.1% $KMnO_4$ solution to avoid dermal infection. All the precautions laid down by (APHA, AWWA and WEF, 1998) are followed, for maintaining the fish. The fish were exposed to organophosphorus pesticide profenofos 50% EC, the 96 hours LC_{50} (10 $\mu g/L^{-1}$) sublethal concentrations for 15 days. If mortality occurred during the experimental exposure period, dead fish were removed immediately to avoid depletion of dissolved oxygen level which adversely affects other fish. The water used for acclimatization and conducting experiments was clear unchlorinated ground water. In each test ten fish were introduced in toxicant glass chambers with a capacity of ten litres. The data on the mortality rate of fish was recorded. The dead fish were removed immediately. The acute toxicity tests were conducted to choose the mortality range from ten percent to ninety percent for 24 hrs in static tests. The concentration that produced fifty percent mortality in test species noted. LC_{50} values were calculated by according to Finney's probit analysis (Finney, 1971).

Sample Preparation

The residues from the fish vital organs of liver, kidney, muscle and gill were extracted by the modified Mills FDA procedure (Pesticide Analytical Manual, 1994) method incorporated in the pesticide analytical manual (The Pesticide Manual, 2013). To 1 g of tissue 0.5 g of anhydrous sodium sulfate was added and extracted into 20 ml of Hexane: Acetone, Soft tissues like liver, kidney was homogenized in a tissue homogenizer with minimal quantity of all glass triple distilled water. The homogenized tissue was extracted with 2:1 hexane: acetone. The extract was filtered and evaporated to 1 ml on boiling water bath.

Cleanup and Removal of the co-extractives

After extraction, acetone was washed out and the hexane extract was dried out over Na_2SO_4 (AR grade). The extract was stored in stoppered glass vials and kept in the refrigerator for further processing.

The hexane extract was concentrated to about 1 ml (when the volume of extract was 4 ml or more) and transferred directly on to a florisil column prepared according to the 60-80 mesh PR grade) and was heated overnight at 130 ^{0}C and after cooling, it was deactivated with grades of increasing polarity using hexane and acetone.

Gas-Liquid Chromatography Analysis

The quantitative analysis of test toxicants was carried out with a gas chromatography (Shumatzu) in combination with Flame Ionization Detector (FID). The Flow rate was maintained for carrier gas 1kg/cm^2, Hydrogen gas 1.5 kg/cm^2 ·The temperature of FID detector was maintained at 250 ºC for detector, injection port 220^{0}C, oven 200^{0}C, Oxygen 1.5 kg/cm^2. Nitrogen was used as the carrier gas, 2µl of sample was injected through the injection port by using Hamilton syringe, the column was used in this analysis SE-30 (Packed Column). Quantization of pesticides in different tissues was calculated based on comparison with the standard calibration curve.

Standard preparation

1ml of standard Profenofos was dissolved in 9ml of Hexane makes 100ppm of standard solution and 2µl of this solution was injected. And the retention time of standard was recorded.

Identification and Quantification

The compound was identified by comparing its retention time (RT) with respect to technical grade profenofos standard. The quantitative determination was carried out by calibration curve drawn from chromatographic experiments with standard solution of profenofos.

RESULTS AND DISCUSSION

The results of the gas liquid chromatographic (GLC) analysis in the tissues gill, liver, kidney, and muscle vital organs of the fish *Labeo rohita* are given in Table 1 to 6 and figures 1 to 5, the residue are in the following order: in ppm

Gill > Muscle > Kidney > Liver

Table-1. Method condition:

Parameter		Condition
Carrier gas		Nitrogen
Column		SE-30 (packed column)
Flow	Carrier gas	1kg/cm^2
	Hydrogen	1.5kg/cm^2
	Oxygen	1.5kg/cm^2
Detector		Flame ionized detector (FID)
Temperature	Inject port	220^{0}C
	Oven	200^{0}C
	Detector	250^{0}C
Sample volume		2µl
Runtime		10min
Sample concentration		100ppm
Retention time	Hexane	1.07min
	Profenofos	2.63min

Table-2. Residue level of profenofos pesticide in different tissues of *Labeo rohita* exposed to sublethal concentration for 15 days.

S.No	Fish tissues	Retention time (minutes)	Area	Concentration (ppm) of profenofos
1	Standard	2.63	14982.66	100
2	Gill	2.55	2035.21	13.58
3	Kidney	2.51	608.21	4.06
4	Liver	2.51	206.55	1.38
5	Muscle	2.51	633.70	4.22

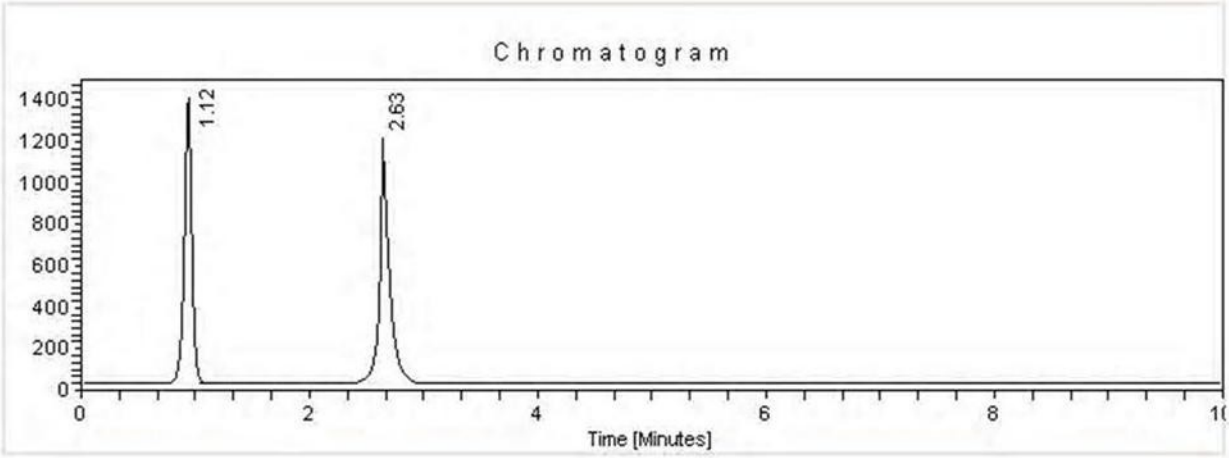


Figure 1. Standard Chromatogram of Profenofos

Table 3. Pesticide Profenofos identified by Gas Chromatography

Table						
Peak No	**Retn.Time**	**Area**	**Height**	**Area %**	**Height %**	**Width@50%**
1	1.12	17325.32	1387.54	53.63	55.07	0.129
2	2.63	14982.66	1132.22	46.37	44.93	0.178
Total		**32307.98**	**2519.76**	**100**	**100**	

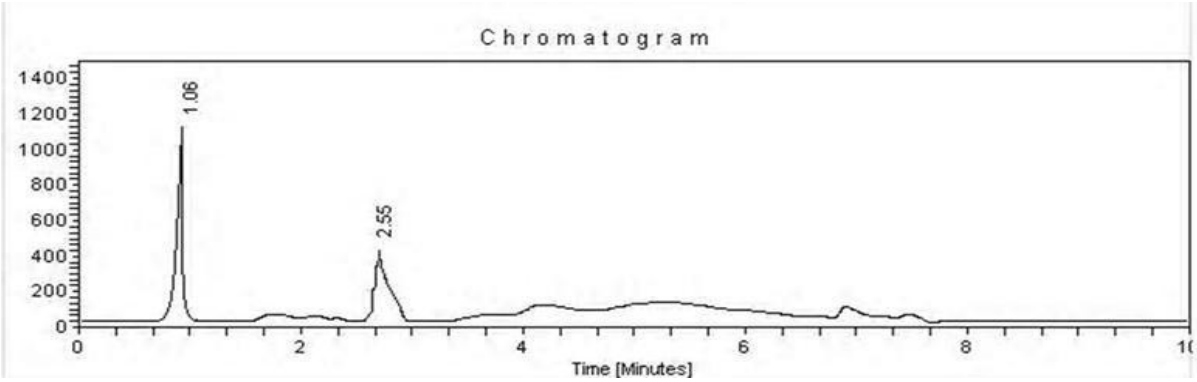


Figure 2. Chromatogram of *Labeo rohita* gill tissue on exposure to profenofos for 15 days

Table 4. Pesticide Profenofos identified in Gill by Gas Chromatography

Table						
Peak No	**Retn.Time**	**Area**	**Height**	**Area %**	**Height %**	**Width@50%**
1	1.06	18126.55	1185.32	89.91	74.63	0.127
2	2.55	2035.21	402.87	10.09	25.37	0.321
Total		**20161.76**	**1588.19**	**100**	**100**	

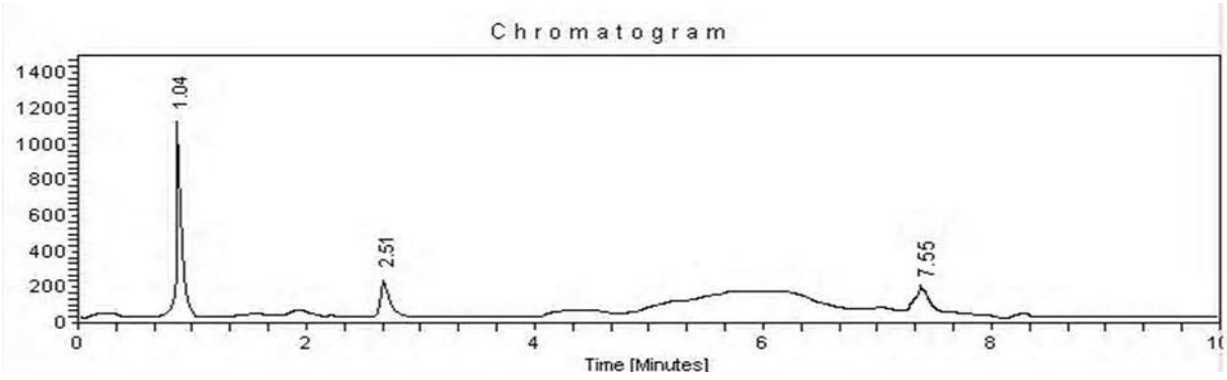


Figure 3. Chromatogram of *Labeo rohita* Kidney tissue on exposure to profenofos for 15 days

Table 5. Pesticide Profenofos identified in Kidney by Gas Chromatography

Table						
Peak No	**Retn.Time**	**Area**	**Height**	**Area %**	**Height %**	**Width@50%**
1	1.04	17563.22	1179.21	93.62	75.49	0.176
2	2.51	608.21	207.22	3.24	13.26	0.125
3	7.55	588.37	175.72	3.14	11.25	0.196
Total		**18759.80**	**1562.15**	**100**	**100**	

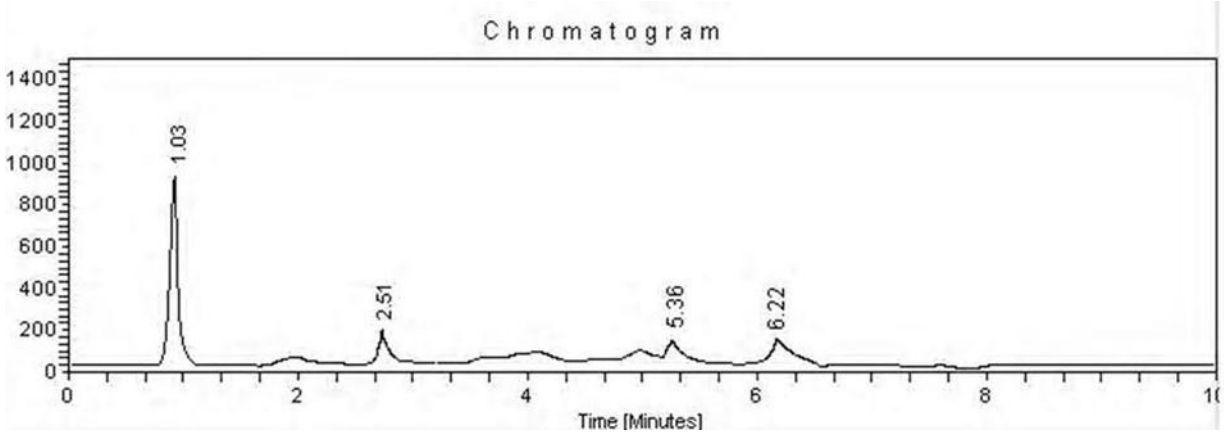


Figure 4. Chromatogram of *Labeo rohita* Liver tissue on exposure to profenofos for 15 days

Table 6. Pesticide Profenofos identified in Liver by Gas Chromatography

Table

Peak No	Retn.Time	Area	Height	Area %	Height %	Width@50%
1	1.04	16289.57	998.56	97.58	69.27	0.175
2	2.51	206.55	207.66	1.24	14.41	0.089
3	5.36	78.90	115.24	0.47	7.99	0.072
4	6.22	118.84	120.03	0.71	8.33	0.189
Total		**16693.86**	**1441.49**	**100**	**100**	

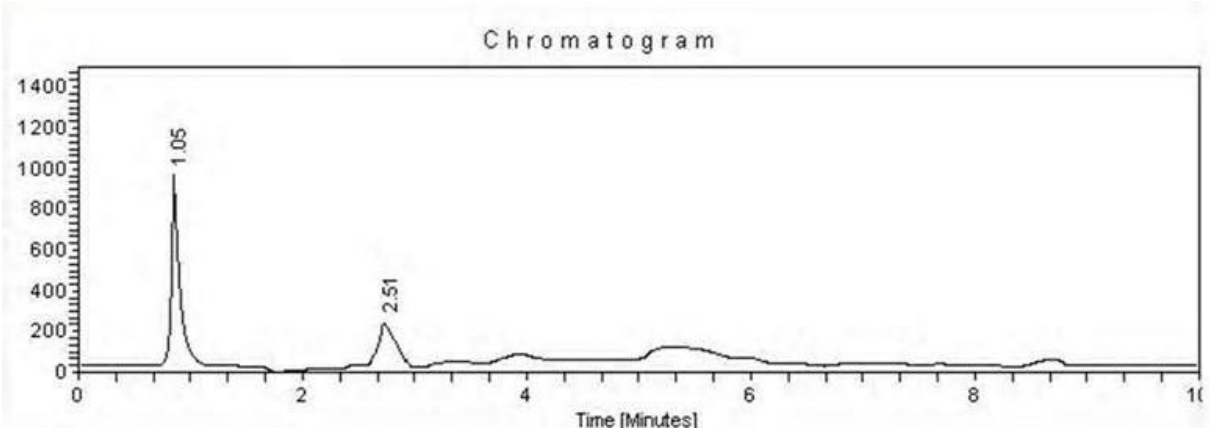


Figure 5. Chromatogram of *Labeo rohita* Liver tissue on exposure to profenofos for 15 days

Table 7. Pesticide Profenofos identified in Muscle by Gas Chromatography

Table

Peak No	Retn.Time	Area	Height	Area %	Height %	Width@50%
1	1.05	16583.97	1037.21	96.32	82.59	0.172
2	2.51	633.70	218.69	3.68	17.41	0.204
Total		**17217.67**	**1255.90**	**100**	**100**	

The variations in the residue analysis are attributed to factors like difference in uptake rate and lipid content of respective animal tissue. The chemical structure, solubility, fish interaction and metabolic pattern are

responsible for pesticide uptake. The results of present study revealed that prolonged exposure to sublethal concentrations led to increase in the accumulation of residue. This is in agreement with the earlier reports by (Tilak *et al*., 2004; Bradbur *et al.,* 1987), the accumulation is a factor responsible for changes in biochemical actions or pathological changes and also disturbance of overall biochemical cyclic reactions which are cumulative causing lethal actions even when the concentrations are sublethal. According to F. Bagheri (2007), residues of OP insecticides in the fish species and the water depend on the physiochemical characteristics of water, time of consumption, pH of water and the ambient temperature.

The residues of the quinalphos in brain were more where the inhibition of AChE activity was also more. The correlation of the residues and the AChE activity by (Coppage *et al*., 1975), also supports the present study (Tilak *et al*., 2004). Also observed that the residues of chlorpyrifos accumulated more in brain than liver in *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala.*

Liver is the main detoxifying tissue containing relatively high levels of detoxifying enzyme. It is also the first organ to face the effect of pesticides being carried through the portal circulation which might have been the cause of the greater accumulation of quinalphos. Mono oxygenase enzymes are found in high concentration in the liver and many tissues such as gonad, kidney intestine, gill and heart (Lindstrom-Seppa *et al*., 1981). The rapid loss of dimethoate from liver was reported by (Begum *et al*., 1994), the lower residue levels in liver are consistent with fish exposed to acute lethal levels of pesticide (Nowak *et al*., 1991 and 1995).

CONCLUSION

The results of the present study according to the above results, it was revealed that prolonged exposure to sublethal concentrations of profenofos in *Labeo rohita* leads to increased accumulation of pesticide residues in tissues. This is in corroboration with the earlier reports of Organophosphorus residues. A thorough literature search revealed that repeated or continuous exposure to low concentrations of pesticides can lead to high residue concentrations without mortalities. Thus the uptake and persistance of profenofos depends not only on a number of physical and chemical properties, but also varies according to the biological factors.

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