ISSN: 2641-1784

Research Article

Advance in Environmental Waste Management & Recycling

Bioaccumulation of Pesticides in the Selected Group of Fishes from Chettuva Backwater, Thrissur, Kerala

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Submitted: 16 Jun 2022; **Accepted**: 05 Jul 2022: **Published**: 15 Jul 2022

Citation: *Sreekala Leelambika Krishnan. (2022). Bioaccumulation of Pesticides in the Selected Group of Fishes from Chettuva Backwater, Thrissur, Kerala. Adn Envi Was Mana Rec, 5(2), 159-165.*

Abstract
In an agricultural country like India, pesticides are a major aquatic chemical pollutant. The pesticides are a broad class of chemical and biological agents that damage or kill pests. There are mainly three groups of synthetic organic pesticides-organochlorines, organophosphates and carbamates. Fishes are the major vulnerable biotic components as they sequester the effects to higher organisms including man through food chain. In this paper an attempt has been made to study the levels of pesticide residues in order to elucidate the status of these chemical contaminants in fish meant for human consumption. Specimens were collected from Chettuva backwater in Thrissur district, Kerala. Samples were cleaned in tap water, muscle tissues dissected, minced into smaller pieces. Quantitative analysis of OCs, OPs and Carbomates were made with Gas Chromatography. The result revealed that among the OPs, Trizophos was detected maximum and it contributed more than 50% to the total OPs, whereas Phorate showed the least contribution. Among organochlorines pesticides, α-endosulfan and endosulfan sulphate were the most frequently detected pesticides. Percentage composition of endosulfan pesticide was also computed and the result revealed that endosulfan sulphate was detected the maximum. The level of total carbamate residue was also recorded. Among carbamate pesticides, Carbofuran were the most frequently detected pesticide. Percentage composition of carbamate pesticides revealed that the Carbofuran contributed more than 65% to the total Carbofuran pesticides.

Keywords: Bioaccumulation, Carbamates, Fish, Organochlorines, Organophosphates, Pesticides

Introduction

In an agricultural country like India, pesticides are the major aquatic pollutants. Water bodies adjoining agricultural fields receive runoff loaded with pesticides [1]. The health status of rivers is generally indicated by the inhabiting floral and faunal components. Among them fishes are well proven to reveal the status better and serve as bio indicators of aquatic ecosystem contamination [2, 3]. To determine the health status of the rivers, fishes are opted owing to their inherent hierarchical position in food chain, capacity to accumulate contaminants even at trace levels so as to allow pollutant detection earlier than would be possible from other ecosystem components. Among many contaminants, pesticides are of much interest owing to their persistence, inherent chemical stability, low biodegradability, high lipid solubility and long term irreversible effects [4]. Fishes are the major vulnerable biotic components as they sequester the effects to higher organisms including man through food chain [5].

There are mainly three groups of synthetic organic pesticides – organochlorines, organophosphates and carbamates. Of these, organochlorines (OCs) were the first group to be invented. They are non-polar, lipophilic, and highly persistent compounds. They reach the aquatic environment through soil percolation, surface runoff, leaching or disposal of empty containers. They remain for years in the environment. Most of them are now banned for agriculture but their residues are still present in many biological and non-biological components [2, 6, 7]. They bio accumulate and bio magnify through food chains and produce harmful effects at every level [5]. But organophosphates are more toxic but less persistent lasting only for days, weeks or months in the environment [8, 9]. They are potent cholinesterase (ChE) inhibitors. They can bind covalently with the serine residues in the active site of acetyl cholinesterase (AChE), and prevent its natural function in the catabolism of neurotransmitters, eventually lead to death. They interfere with all the metabolic processes and they accumulate in vital organs thereby affecting the functional activity of both endocrine and exocrine systems of non-target aquatic organisms including fishes [10]. Carbamates are another group of pesticides. They are esters of N-methyl (or occasionally N, N-dimethyl) carbonic acid (H2N-COOH). The toxicity of these compounds varies according to the phenol or alcohol group. Carbamates are urethanes that affect the nervous system of pests. Like the organophosphate insecticides, the mode of action of carbamates is acetylcholinesterase inhibition with the important difference that the inhibition is more rapidly reversed than with organophosphate compounds [11].

The effectiveness of organophosphorous pesticides, coupled with their relatively cheap cost encourages farmers to use more of these pesticides in their field crops. The pesticides' residues are discharged into the air and water. Through the consumption of foods containing these pesticides, these residues can affect the human body [12]. The widespread use of pesticides may contaminate the environment and freshwater fish which ultimately are consumed by humans [13]. Moreover, moderate to severe respiratory and neurological damage can be caused by many of these compounds, which are genotoxic and carcinogenic [14]. In trace amounts, chlorpyrifos, (OP) has been reported to cause neurological disorders such as attention deficit hyperactivity disorder and a developmental disorder both in foetuses and children [15]. Furthermore, Carbofuran, which is a carbamate, has been reported to cause serious reproductive problems, while occupational exposure to carbaryl has been reported to result in nausea, vomiting, blurred vision, coma and difficulty in breathing [16, 17]. Information on the levels of pesticide residues in the fishes is required to evaluate the health status of Inland ecosystems of Kerala. Hence, the current study documented the levels of a set of OCs (α-Endosulfan, β-endosulfan and Endosulfan sulphate), OPs (Chloropyrifos, Phorate and Triazophos,) and carbamate (Carbaryl and Carbofuran) in two species of fishes (*Etroplus suratensis* and *Mugil cephalus*) collected from Chettuva backwater in Kerala.

Materials and Methods

Two species of fishes, *Etroplus suratensis* and *Mugil cephalus* were collected from Chettuva backwater in Thrissur district, Kerala (Longitude 760 23' E-760 30' E). Three set of samples with six fish each were collected during monsoon season (June – October, 2012). Samples were cleaned in tap water, muscle tissues dissected, minced into smaller pieces. Quantitative analysis of OCs, OPs and Carbamates were made with Gas Chromatography (Agilent Model 7890A Series) equipped with MS (5975 Quadruple) using HP-5ms fused silica capillary column (15m x 0.25mm I.D x 0.25μm film thickness) coated with 5% phenyl and 95% dimethyl polysiloxane. While Helium (IOLAR) was the carrier gas (flow rate: 1.0 ml/min), chromatographic operating conditions were as follows: detector temperature was set at 325 °C; the injector temperature was programmed as 85 °C for 12 sec, increased at 600 °C/min to 325 °C and held for 5 min, then again increased at 10 °C/min to 200 °C (0 min); similarly oven temperature programmed as 70 °C (1min), increased at 25 °C/ min to 150 °C (0 min), again increased at 3 °C/ min to 200 °C (0 min) and then 8°C/ min to 280 °C (10 min). The MS source temperature: 230 °C; MS quadruple: 150 °C; Technique: Electron ionization (EI); total run time: 40.867 min. The calibration standards with a minimum of five calibration points (1.56, 3.26; 6.25, 12.5, 25, 50 and 100 ng/g) spanning the expected concentrations in the samples were used for each analyses. All the samples were analysed for a set of OCs, OPs and Carbamates. An equivalent mixture manufactured by Sigma-Aldrich was used as standard. Concentrations of individual compounds were quantified from the peak area of the sample to that of the corresponding external standard. Recoveries of the compounds from fortified samples (50 μg/kg) ranged from 91 to 102%. Results were not corrected for per cent recovery and expressed in wet weight basis. The limits of detection (LOD) for the method were in the range of 0.25-0.75; 0.29-0.62 and 0.09-0.40 ng/g for the OC, OP and Carbamates respectively. Quantification limits were set at three times the instrumental detection limit for each chemical, and they range from 0.25 ng/g to 0.80 ng/g. Precision for the duplicate samples were between 98-99.5%. Results are presented as mean values and range (min-max). Means were calculated by treating the concentration of the non-detected analyses as zero. Values below the detection limits are reported as below detection limit (BDL).

Result

Bioaccumulation of OP pesticides like chloropyrifos, phorate and triazophos; OC like alpha endosulphan, beta endosulphan and endosulphan sulphate; Carbamate pesticides like carbaryl and carbofuran were analysed in *Etroplus suratensis* and *Mugil cephalus* collected from Chettuva backwater. The total organophosphate pesticide residue level was found to be the maximum in *Etroplus* species (68.9 ± 9.0 ppm) than the *Mugil* cephalus collected from Chettuva backwater (Table 1, 2 and Figure 1).

Table 1: Gas chromatographic quantification of pesticides in Etroplus suratensis (Integrated peak height method)

Pesticide Name	Retention time	% composition with respect to tissue			Quantity in ppm			Mean	SE
		Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3		
Chloropyrifos	21.24	0.0014	0.0011	0.0026	14	11	26	17	4.6
Phorate	24.28	0.0013	0.0007	0.0008	13	7.4	8.4	9.6	1.7
Triazophos	26.36	0.0024	0.0059	0.0044	24	59	44	42.3	10.1
Total OP 68.9									
α endo sulphan	10.41	BDL	0.0056	0.0021	BDL	56	21	25.7	16.3
β-endo sulphan	10.93	BDL	41	0.0003	BDL	41	2.5	14.5	13.3
End. sulphate	11.68	0.002	BDL	0.0062	20	…	62	27.3	18.3
Total OC 67.5									
Carbaryl	30.23	0.0016	0.0023	0.0005	16	23	5	14.7	5.2
Carbofuran	33.2	BDL	0.0066	0.0011	BDL	66	11	25.7	20.4
Total Carbamates 40.4									

Table 2: Gas chromatographic quantification of pesticides in Mugil cephalus (Integrated peak height method)

Pesticide Name	Retention time	% composition with respect to tissue			Quantity in ppm			Mean	SE
		Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3		
Chloropyrifos	21.24	0.0026	0.0009	0.0011	26	9	11	15.3	5.4
Phorate	24.28	0.0029	0.00069	0.0008	29	6.9	7.9	14.6	7.2
Triazophos	26.36	0.0026	0.0052	0.0003	26	52	2.5	26.8	14.3
Total OP 56.7									
α endo sulphan	10.41	0.0031	0.00086	0.0089	31	8.6	89	42.9	24
β endo sulphan	10.93	0.0032	0.00058	0.0014	32	5.8	14	17.3	7.7
End. sulphate	11.68	0.0044	0.0026	0.0045	44	26	45	38.3	6.2
Total OC 98.5									
Carbaryl	30.23	BDL	BDL	0.0005	---		5	1.7	1.7
Carbofuran	33.2	0.0026	0.0044	0.0089	26	44	89	53	18.7
Total Carbamates 54.7									

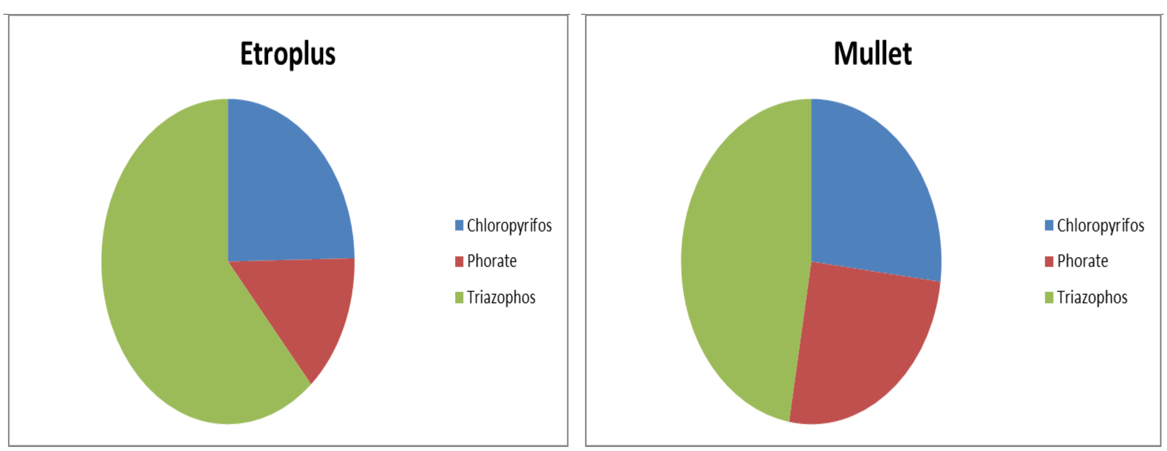
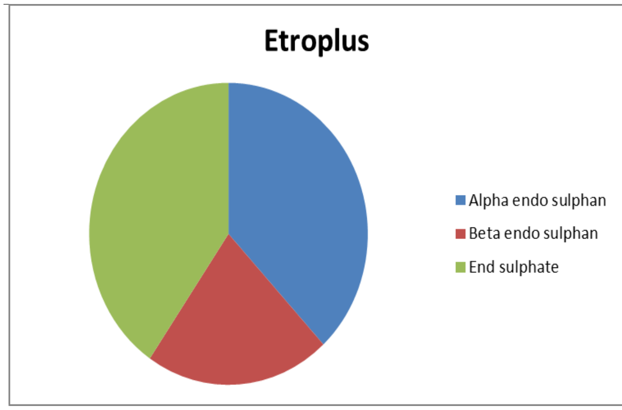


Figure 1: Comparison of OP pesticide concentrations in *Etroplus suratensis* and *Mugil cephalus*

The total OP residue levels did not varied significantly (Kruskal Wallis; X^2=0.048; df=1; P>0.05). Among the organophosphate pesticides, frequency of occurrence of triazophos residues was the maximum (42.3± 10.1 ppm) in *Etroplus* species followed by chloropyrifos in the same species. Percentage composition of OP pesticides was also computed. Among the OPs, triazophos was detected the maximum in all the fishes and it contributed more than 50% to the total OPs, whereas phorate showed the least contribution. The concentration of triazophos was highest in *Etroplus* than in *Mullet* species; like that the concentration of phorate also had least concentration in Etroplus than in *Mullet* species (Figure 2).



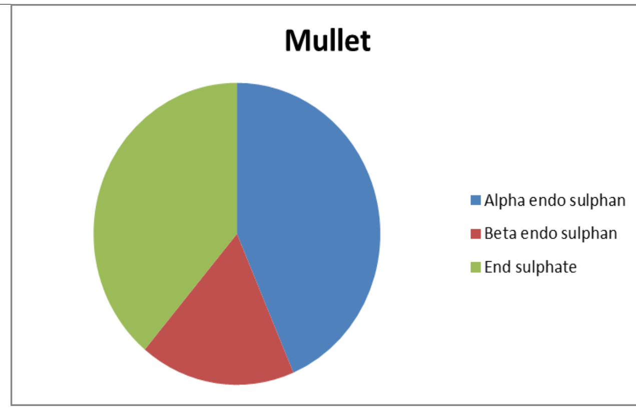
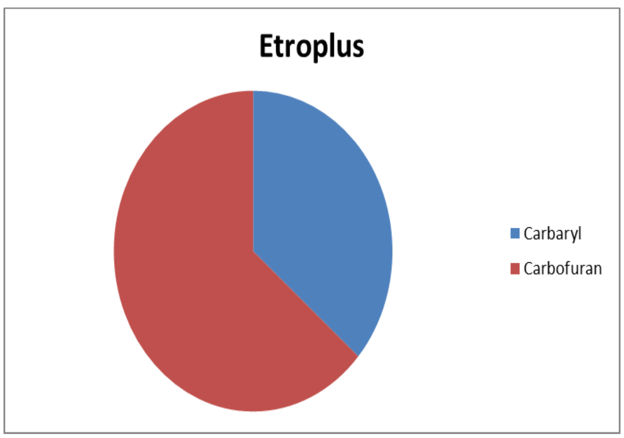


Figure 2: Comparison of OP pesticide concentrations in *Etroplus suratensis* and *Mugil cephalus*



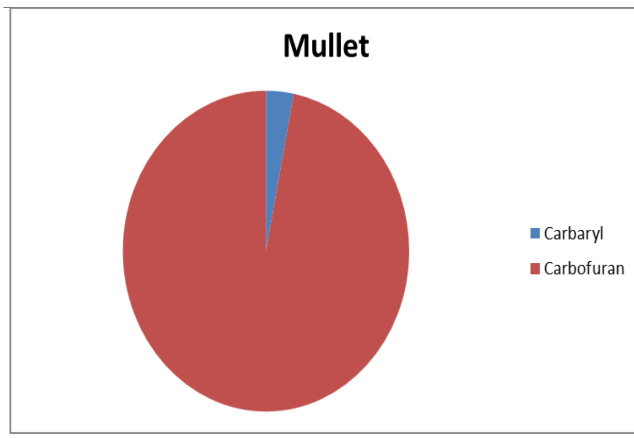
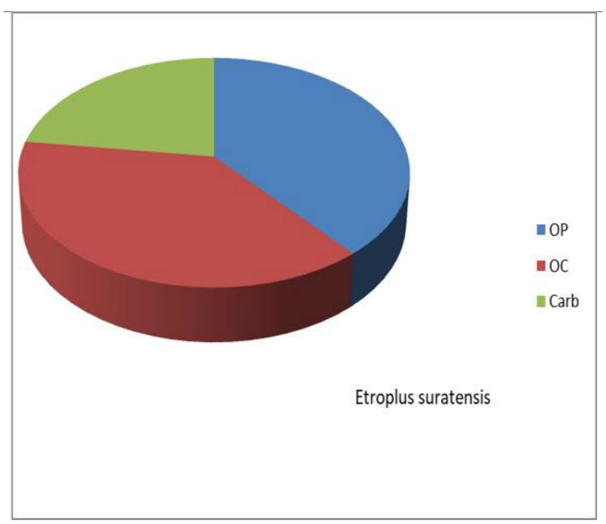


Figure 3: Comparison of OC pesticide concentrations in *Etroplus suratensis* and *Mugil cephalus*



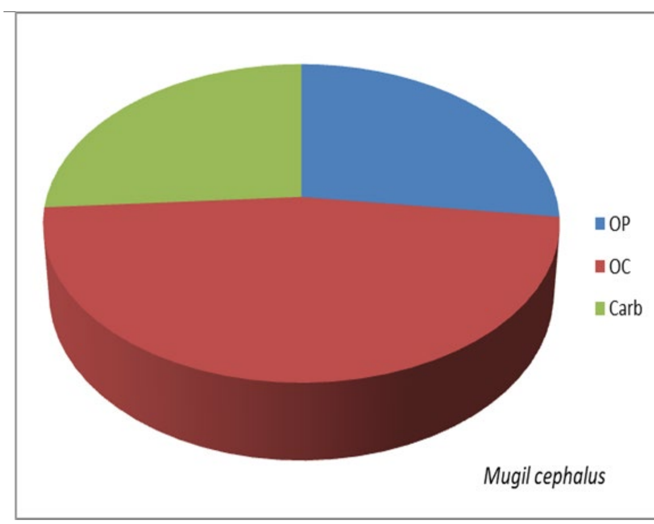


Figure 4: Comparison of Carbamate pesticide concentrations in *Etroplus suratensis* and *Mugil cephalus*

The level of total organochlorines residue was recorded the maximum in *Mugil cephalus* (98.5 ± 24.0 ppm) than the *Etroplus suratensis* (Table 1, 2 and Figure 3). The levels of total endosulfan residue did not varied significantly (Kruskal Wallis; X2=1.19; df=1; P>0.05). Among the endosulfan pesticides, α-endosulfan and endosulfan sulphate were the most frequently detected pesticides in all the fish species analysed. Percentage composition of endosulfan pesticide was also computed and the result revealed that endosulfan sulphate was detected the maximum in *Etroplus suratensis*, and it almost contributed more than 45% to the total endosulfan, but in *Mugil* species α- endosulphan was maximum, whereas β-endosulfan showed the least contribution in both species of fishes (Figure 3).

The level of total carbamate residue was recorded the maximum in Mullet fishes (54.7 ± 24.3 ppm) than in the *Etroplus* fishes collected from Chettuva Backwater (Table 1,2 and Figure 4). The levels of total carbamate residue did not varied significantly (Kruskal Wallis; X^2=1.22; df=1; P>0.05). Between the carbamate pesticides, carbofuran were the most frequently detected pesticides in all the fish species studied. Percentage composition of carbamate pesticides revealed that the carbofuran contributed more than 65% to the total carbofuran pesticides. But the carbaryl showed low concentration in both fish species, among that *Mullet* had least concentration when compare to Etroplus, (Figure 4).

Discussion

Fishes are very sensitive to a wide variety of toxicants in water, various species of fish shows uptake and accumulation of many contaminants or toxicants such as pesticides. Accumulation of pesticides in tissues produces many physiological and biochemical changes in the fishes and freshwater fauna by influencing the activities of several enzymes and metabolites [18]. Fish are used extensively for environmental monitoring because they take contaminants directly from water and diet [19]. Generally the ability of fish to metabolize organochlorines is moderate; therefore, contaminant loading in fish is reflective of the state of pollution in the surrounding environment [20]. Organochlorines pesticides have become ubiquitous contaminants and implicated in a broad range of deleterious health effects in laboratory animals and man. The toxic effect includes reproductive failures and effect on human beings immune system malfunction, endocrine disruption and breast cancers [21-23]. Many of these oranochlorine pesticides and their metabolites have also been implicated in a wide range of adverse human and environmental effects including reproduction and birth defects [24].

Muralidharan *et al.*, (2013) reported that Levels of alpha (α) endosulfan were BDL, trace amounts of beta (β) endosulfan were detected in *Channa striatus* (1.77 ppb) collected from Nilleswaram-Arayi rivers (Kasargod and Kannur Dt). Endosulfan sulphate was the maximum in *Garra sp.* (7.08 ppb) collected from midland areas of Chithar (Kasargod Dt), and minimum in highland areas of Kadalundi, Malappuram Dt (2.03 ppb) in the same species (*Rasbora sp*). Other species those recorded detectable amounts of endosulfan sulphate residues include *Devario sp* from highland (3.61 ppb) and midland (4.98 ppb) areas of Chaliyar river (originates in the Western Ghats range in Elambalari Hills of Tamil Nadu and flows through Malappuram Dt and enters Arabian sea in Kozhikode Dt), *Garra mullya* (4.28 ppb) from Meencolli areas of Kabani river (originates in Wayanad Dt of Kerala and flows eastward to join Kaveri river in Karnataka), *Puntius filamentosus* (3.64 ppb) from low land areas of Mahii, *Devario sp* (2.86 ppb) from Mahii river (near Kannur), *Puntius filamentosus* from Neiyyar (2.59 ppb) (Agasthiyar malai, Trivandrum Dt) and Mamam (2.40 ppb) (originates in Panthalacode hills in Trivandrum Dt). Almost similar observations were obtained in the present study. Ensosulphan suiphate (38.3ppm), alpha endosulphan (42.9 ppm) and beta endosulphan (17.3 ppm) were high in Mullet species. The concentration of beta endosulfan was low in both species of fish when compare to other two OCs.

In a study conducted at Palakkad [25] among OPs analysed, residues of Chlorpyrifos (22.07 ± 4.80 ng/g; 2009-10 Rabi season) and Triazophos (10.47 ± 7.24 ng/g; 2009-10 summer season) were higher in conventional farm sediments. But in the current study among the OPs, triazophos was detected the maximum in all the fishes and it contributed more than 50% to the total OP's. Similarly endosulfan is an off-patent organochlorine insecticide and acaricide that is being phased out globally. Endosulfan became a highly controversial agrochemical due to its acute toxicity, potential for bioaccumulation and role in endocrine disruptions [26]. Because of its threats to human health and the environment, a global ban on the manufacture and use of endosulfan was negotiated under the Stockholm Convention in April 2011. It may be noted that Kerala government banned endosulfan way back in 2002 particularly in Kasargod district, Kerala. The Supreme Court of India has banned Endosulfan in India with effect from May, 2011, with certain exemptions for five additional years [26]. The concentration of sum of endosulfan recorded in the fish species of the present study are higher than the levels (BDL- 4.3 ng/g) reported in nine species of fresh water fishes from Karnataka [2] Da Cuna *et al.*, (2011) studied acute toxicity, histological and physiological changes in fish exposed to endosulfan in laboratory condition. They found decreased blood cells in the fishes. However, the levels of endosulfan detected in the fishes of the current study are lower than the levels reported to cause malformation and mortality.

Pesticides have been recognized as serious pollution of aquatic environment. It affects fish directly by being accumulated in their body. They also cause serious impairment in metabolic, physiological and structural systems. The accumulation of pesticides in the tissues of a fish can result in chronic illness and cause potential damage of population. Fish are able to accumulate and retain pesticides and other pollutants from their environment. Accumulation of pesticides in the tissue of fish is dependent upon exposure concentration as well as other factors such as salinity, temperature, hardness and metabolism of fish. Pesticides effect on specific vital organs such as liver, gill and kidney. Different degree of pesticides accumulation in various tissues depends upon the biochemical characteristic of pesticides. The levels of most of the residues in fish were higher than those found in water.

Conclusion

Organophosphate and carbamate showed higher concentration in fish. But in the case of organochlorines, both species showed considerable differences in its expression. The isomers were the breakdown product of endosulphan. Its breakdown may be depending on the fat content of the fish. So this may be the reason for variation in its concentration in both species. Other group of pesticides like organophosphates and carbamates were directly enters into the body of fish without breakdown. So its accumulation rate is almost similar in both species depend on environmental level. While long-term exposure of fish to low-level pollutants might not show any obvious or visible effects on the fish itself, it could exert deleterious effects on the reproductive organs leading to a decline in number of offspring and eventually to extinction of fish stocks. The data clearly showed that fishes studied from Chettuva backwater have one or the other type of pesticides, although the range of levels varies. Although in most cases the levels are low, it might affect the reproductive system of the species concerned. More importantly, regular consumption of these fishes would lead to health problems. It is therefore suggested that steps may be taken to prevent further pollution of the rivers with pesticides

The pesticides are frequently used in the agricultural fields to yield a higher production of crops, perhaps it acts as a silent killer that have a detrimental effect on environment, damaging and causing that to non-target organism. This type of study can suggest that the use of pesticide in the agricultural field should be in a control rate which does not affect the non-target organism. Government has formulated several action plans but the need of the hour is that the people realize by themselves about the negative effects of the pesticides. The results of the study concluded that even a small amount of pesticide presence in fresh water reservoirs causes harmful effects on fish physiology and subsequently make death of the fish species. So, from the pesticides standpoint, it should take necessary precautions to applied pesticides in natural environment or use of pesticide in minimum quantity to protect the aquatic creatures.

Acknowledgements

The authors acknowledge the University Grant Commission for providing financial support. The authors would also like to thank Department of Zoology, MES Mampad College, Malappuram for providing facilities, the consistent support and interest in this work.

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