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Pesticide-induced Histopathological Changes in the Freshwater Fishes of Kuttanand, Kerala-A Tool to Assess Water Quality and the Health Status of Fishes

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Abstract

Kuttanad, the rice bowl of Kerala, is a region where overdose application of pesticide is prevalent during the punja cultivation periods. According to the data compiled by Kuttanad Water Balance Study Project, 485 tonnes of pesticides were applied in Kuttanad on an annual basis of which 370 tonnes were used for the punja crop alone (KWBSP, 1990). In such a degraded aquatic environment, particularly where pollutants occur at chronic sublethal concentrations, changes in the structure and functions of aquatic organisms occur more frequently than their mass mortality. Therefore, one of the possible methods of assessing the effects of pollutants on fresh water fish inhabiting this ecosystem is to examine their organs for morphological changes. In fishes, apart from lethal effects of pesticides and the consequent mortality of eggs, larvae, and adults, their prolonged exposure in sublethal concentration may also result in reproductive abnormality, stock recruitment, deformities of eggs and larvae, retardation of hatchling percentage and body abnormalities. In the present study, a tool developed by Bernet et al. (1999) is used to assess the histopathological conditions; hence, histopathology is used as a tool to assess the health status of two freshwater fishes of Kuttanad, *viz*., *Etroplus suratensis* and *Anabas testudineus*. The organ index calculated based on various reaction patterns of the different organs of fishes exposed to sublethal concentrations of monocrotophos for a period of 30 days showed that gills were severely affected, liver was moderately affected, and kidney was the mildly affected organ, irrespective of fish species. Histopathology provides evidences of adaptation to degeneration, and histopathological alterations can be used as biomarkers of environmental pollution by organic chemicals. Histological changes in fish gill should become a rapid "early warning system" for water quality assessment in sublethal and chronic situations, as the toxicants induce changes at lower levels of biological organization prior to organismic changes.

Introduction

Kuttanad, the rice bowl of Kerala, India, is a region where overdose application of pesticide is prevalent during the punja cultivation periods. Traditionally, there are three cropping seasons for paddy in Kerala, the virippu, mundakan, and punja seasons. Punja crop, the traditional crop of Kuttanad, is sown in November to December and the

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harvest takes place by the end of March. The peak period of the pest damage, particularly by the brown plant hopper, is from February to March, which not only reduces the yield but also entails additional expenses for pesticides. There is no systematic crop surveillance and therefore, farmers arbitrarily apply pesticides at regular intervals. These ways of treatments are ineffective as well as unwanted and can cause severe damage to Kuttanad ecosystem. (KWBSP 1990).

According to the data compiled by Kuttanad Water Balance Study Project, 485 tonnes of pesticides were applied in Kuttanad on an annual basis of which 370 tonnes were used for the puncha crop alone (KWBSP 1990). Dimecron, Monocrotophos, Henosan, Thymet, Fernoxan, and Nuvacron are the major components of the pesticides being used in Kuttanad. In such a degraded aquatic environment, particularly where pollutants occur at chronic sublethal concentrations, changes in the structure and functions of aquatic organisms occur more frequently than their mass mortality. Therefore, one of the possible methods of assessing the effects of pollutants on fresh water fish inhabiting this ecosystem is to examine their organs for morphological changes.

In fishes, apart from lethal effects of pesticides and the consequent mortality of eggs, larvae, and adults, their prolonged exposure in sublethal concentration may also result in reproductive abnormality, stock recruitment, deformities of eggs and larvae, retardation of hatchling percentage, and body abnormalities. Evidence of retardation of natural propagation of fishes is already discernible in Kuttanad due to very low yield registered from these regions (Kurup et al. 1990). Hence, water pollution can lead to different changes, ranging from biochemical alterations in single cell into changes in whole populations. In general, the end points used in toxicity studies are mortality, survival, and growth with acute toxicity tests. These parameters are quite appropriate, but for long-term sublethal concentrations, these relevant parameters are difficult to ascertain. In the past, there were no tools to measure the magnitude of histopathological conditions in the affected organs. However, at present, many tools are available. Hence, in the present study, histopathological parameters are used to assess the nature and magnitude of toxic effects of pesticides that are being widely applied in the paddy fields of Kuttnad. This analysis is "user friendly" for the field investigator (Hinton 1993).

The advantage of histopathology as a biomarker lies in its intermediate location with regard to the level of biological organization (Adams et al. 1989). Histological changes appear as a medium-term response to sublethal stressors, and histology provides a rapid method to detect the effects of irritants, especially chronic ones in various tissues and organs (John et al. 1993). Histopathological analysis yields data on a number of organs and permits localization of lesions within specific cell types. With a thorough prior knowledge of normal anatomy, the investigator can use histological analysis to detect alterations in tissues and organs caused by exposure to toxicants. When concentration of a toxicant is sufficient to result only in cellular injury and not death, sublethal (adaptive) changes can be observed in affected cells.

The exposure of fish to chemical contaminants is likely to induce a number of lesions in body organs like gills, liver, and kidney. These organs are suitable for histological examination to determine the effect of extent of pollution (Hinton 1993). Gills exhibit large surfaces, which are subjected to direct and permanent contact with potential irritants. Liver plays a key role in metabolism and subsequent excretion of xenobiotics and is also the site of vitellogenin production. Kidney is important for the maintenance of a stable internal environment and partially involved in the metabolism of xenobiotics (Hinton 1993).

In the above-mentioned conditions, it is felt that a study on the pesticide-induced histopathological changes in selected fishes would be helpful in bringing out the lethal effect caused to fish health due to ubiquitous application of pesticides and henceforth establishing the necessity for a judicious use of pesticides in agriculture in future.

Materials and methods

Juveniles of *Etroplus maculatus* (*E. maculatus*) and *Anabas testudineus* (*A. testudineus*) were collected from pollution-free ponds from the natural habitat. These fishes (size 47.5 ± 9.0 mm and 71.5 ± 6.0 mm in total length and 330 ± 80 mg and 750 ± 150 mg in weight, respectively) were acclimatized to the laboratory conditions for 14 days prior to the bioassay. During these periods, they were fed *ad libitum* once a day on fresh clam meat. The experiments on the lethal and sublethal toxicity of monocrotophos- an organophosphate pesticide-on the juveniles of *E. maculatus* and *A. testudineus*, the true denizens of Kuttanad paddy fields, were conducted for 48 hours and 30 days, respectively, during the period of investigation.

Based on the LC_{50} values (Mercy et al. 2000), five nominal concentrations of the pesticide were selected for sublethal toxicity studies. Maximum and minimum sublethal concentrations were chosen based on Konar (1969) and Sprague (1973). The experimental fishes were exposed to such sublethal concentrations for a period of 30 days. The concentrations of pesticides used for each sublethal exposure are given in Table 1.

Table1. Forty-eight-hour LC_{50} values and sublethal concentrations chosen for the experiment

Fish species	Pesticide	48-hr.LC_{50} (ppm)	Sublethal concentrations ($mg.L^{-1}$)					
Etroplus maculatus	Monocrotophos	3.36	0.0	0.1	0.3	0.6	1.0	1.5
Anabas testudineus		102.59	0.0	2.0	5.0	10.0	18.0	36.0

Sublethal exposure was carried out in a static system where water and pesticide medium were renewed every 24 hours to obtain the desired pesticide concentration. A control free of pesticide was also maintained in each experiment. All the treatments were made in triplicates. Ten healthy fishes, each of the target species, chosen at random from the acclimated stock were reared in 32 litres of water in seasoned cement cisterns. The ratio of the animal wet-weight to water volume ranged from 0.4899 to 2.7875 gm.L^{-1}. The tanks were covered with plastic mesh nets to prevent the escape of the fishes by jumping. All the experiments were conducted in ambient temperature (28 ± 2°C). The dissolved oxygen, pH, and temperature in the different treatments were measured immediately before and after the pesticide inoculation. After 30 days, i.e. after the termination of the experiments, five specimens from each of the treated as well as the control group were killed and the target organs such as the gills, liver, and kidney were dissected out and fixed immediately in Bouin's fluid. Histological sections were prepared based on standard procedures and stained using hematoxylin and eosin. Each organ is observed for its detailed histology. Same species of fishes were collected from the paddy fields of Kuttanad during the months of February and March, and histological preparations were carried out for the target organs and were observed for their histopathological lesions.

In the present study, histopathological conditions of different organs were assessed based on Bernet et al. (1999) who classified the histopathological changes of each organ into five reaction patterns (Table 2). Each pattern includes several alterations with respect to either functional unit of the organ or entire organ. Calculation of the index values was based on an importance factor (*w*) and score value (*a*).

Importance factor (w)

The relevance of a lesion depends on its pathological importance, i.e. how it affects organ function and the ability of the fish to survive. This is taken into account by an importance factor assigned to every alteration listed in the histological description.

The alterations are classified into three important factors:

1) Minimal pathological importance, the lesion is easily reversible as exposure to irritants ends; 2) Moderate pathological importance, the lesion is reversible in most cases if the stressor is neutralized; and 3) Marked pathological importance, the lesion is generally irreversible, leading to partial or total loss of the organ function.

Score value (*a*) Every alteration is assessed using a score ranging from 0 to 6, depending on the degree and extent of alteration: (0) unchanged; (2) mild occurrence; (4) moderate occurrence; and (6) severe occurrence (diffuse lesion). Intermediate values were also considered.

Table 2. Histopathological assessment tools for 3 fish organs (i.e. gills, liver, and kidney). An importance factor ($W_{org\ rp\ alt}$) ranging from 1 to 3 is assigned to every alteration: it is composed of the respective organ (org), the reaction pattern (rp), and the alteration (alt)*. # Extracted from Bernet et al. (1999)

Reaction pattern	Functional unit of the tissue	Alteration #	Importance factor	Score value	Index
Gills		Hemorrhage/hyperemia			
Circulatory disturbances		/aneurysm	WGC1 = 1	aGC1	IGC
		Intercellular edema	WGC2 = 1	aGC2	
Regressive changes	Epithelium	Architectural and structural alterations	WGR1 = 1	aGR1	IGR
		Plasma alterations	WGR2 = 1	aGR2	
		Deposits	WGR3 = 1	aGR3	
		Nuclear alterations	WGR4 = 2	aGR4	
		Atrophy	WGR5 = 2	aGR5	
		Necrosis	WGR6 = 3	aGR6	
		Rupture of the pillar cells			
	Supporting tissue	Architectural and structural alterations	WGR7 = 1	aGR7	
		Plasma alterations	WGR8 = 1	aGR8	
		Deposits	WGR9 = 1	aGR9	
		Nuclear alterations	WGR10 = 2	aGR10	
		Atrophy	WGR11 = 2	aGR11	
		Necrosis	WGR12 = 3	aGR12	
Progressive changes	Epithelium	Hypertrophy	WGP1 = 1	aGP1	IGP
		Hyperplasia	WGP2 = 2	aGP2	
	Supporting tissue	Hypertrophy	WGP3 = 1	aGP3	
		Hyperplasia	WGP4 = 2	aGP4	
Inflammation		Exudate	WGI1 = 1	aGI1	IG1
		Activation of RES	WGI2 = 1	aGI2	
		Infilteration	WGI3 = 2	aGI3	
Tumor		Benign tumor	WGT1 = 2	aGT1	IGT
		Malignant tumor	WGT2 = 3	aGT2	
					IG
Liver		Hemorrhage/hyperemia	WLC1 = 1	aLC1	ILC
Circulatory disturbances		/aneurysm			
		Intercellular edema	WLC2 = 1	aLC2	
Regressive changes	Liver tissue	Architectural and structural alterations	WLR1 = 1	aLR1	ILR
		Plasma alterations	WLR2 = 1	aLR2	
		Deposits	WLR3 = 1	aLR3	

Table 2. Continued..

		Nuclear alterations	WLR4 = 2	aLR4	
		Atrophy	WLR5 = 2	aLR5	
		Necrosis	WLR6 = 3	aLR6	
		Vacuolar degeneration			
	Interstitial tissue	Architectural and	WLR7 = 1	aLR7	
		structural alterations			
		Plasma alterations	WLR8 = 1	aLR8	
		Deposits	WLR9 = 1	aLR9	
		Nuclear alterations	WLR10 = 2	aLR10	
		Atrophy	WLR11 = 2	aLR11	
		Necrosis	WLR12 = 3	aLR12	
	Bile duct	Architectural and			
		structural alterations	WLR13 = 1	aLR13	
		Plasma alterations	WLR14 = 1	aLR14	
		Deposits	WLR15 = 1	aLR15	
		Nuclear alterations	WLR16 = 2	aLR16	
		Atrophy	WLR17 = 2	aLR17	
		Necrosis	WLR18 = 3	aLR18	
Progressive					
changes	Liver tissue	Hypertrophy	WLP1 = 1	aLP1	ILP
		Hyperplasia	WLP2 = 2	aLP2	
	Interstitial tissue	Hypertrophy	WLP3 = 1	aLP3	
		Hyperplasia	WLP4 = 2	aLP4	
	Bile dudct	Hypertrophy	WLP5 = 1	aLP5	
		Hyperplasia	WLP6 = 2	aLP6	
		Wall proliferation of bile ducts or ductules			
Inflammation		Exudate	WLI1 = 1	aLI1	IL1
		Activation of RES	WLI2 = 1	aLI2	
		Infilteration	WLI3 = 2	aLI3	
Tumor		Benign tumor	WLT1 = 2	aLT1	ILT
		Malignant tumor	WLT2 = 3	aLT2	
					IL
Kidney					
Circulatory		Hemorrhage/hyperemia			
disturbances		/aneurysm	WKC1 = 1	aKC1	IKC
		Intercellular edema	WKC2 = 1	aKC2	

Table 2. Continued..

Regressive changes	Tubule	Architectural and structural alterations	WKR1 = 1	aKR1	IKR
		Plasma alterations	WKR2 = 1	aKR2	
		Deposits	WKR3 = 1	aKR3	
		Nuclear alterations	WKR4 = 2	aKR4	
		Atrophy	WKR5 = 2	aKR5	
		Necrosis	WKR6 = 3	aKR6	
	Glomerulus	Architectural and structural alterations	WKR7 = 1	aKR7	
		Plasma alterations	WKR8 = 1	aKR8	
		Deposits	WKR9 = 1	aKR9	
		Nuclear alterations	WKR10 = 2	aKR10	
		Atrophy	WKR11 = 2	aKR11	
		Necrosis	WKR12 = 3	aKR12	
	Interstitial tissue	Architectural and structural alterations	WKR13 = 1	aKR13	
		Plasma alterations	WKR14 = 1	aKR14	
		Deposits	WKR15 = 1	aKR15	
		Nuclear alterations	WKR16 = 2	aKR16	
		Atrophy	WKR17 = 2	aKR17	
		Necrosis	WKR18 = 3	aKR18	
Progressive changes	Tubule	Hypertrophy	WKP1 = 1	aKP1	IKP
		Hyperplasia	WKP2 = 2	aKP2	
	Glomerulus	Hypertrophy	WKP3 = 1	aKP3	
		Hyperplasia	WKP4 = 2	aKP4	
		Thickening of Bowman's capsular membrane			
	Interstitial tissue	Hypertrophy	WKP5 = 1	aKP5	
		Hyperplasia	WKP6 = 2	aKP6	
Inflammation		Exudate	WKI1 = 1	aKI1	IK1
		Activation of RES	WKI2 = 1	aKI2	
		Infilteration	WKI3 = 2	aKI3	
Tumor		Benign tumor	WKT1 = 2	aKT1	IKT
		Malignant tumor	WKT2 = 3	aKT2	

Mathematical calculation of lesion indices:

1. Reaction index of an organ ($I_{org\ rp}$)

Only the lesions within one organ are studied, the following indices are applicable.

$$I_{org\ rp} = \sum_{alt} (a_{org\ rp\ alt} \times w_{org\ rp\ alt}),$$

where org = organ; rp = reaction pattern (constant); alt = alteration; a = score value; w = importance factor. The quality of the lesion in an organ is expressed by the reaction index.

2. Organ index (I_{org})

$$I_{org} = \sum_{rp} \sum_{alt} (a_{org\ rp\ alt} \times w_{org\ rp\ alt})$$

(Abbreviations same as in reaction index formula). This index represents the degree of damage to an organ

3. Total index (Tot-I)

$$Tot\text{-}I = \sum_{org} \sum_{rp} \sum_{alt} (a_{org\ rp\ alt} \times w_{org\ rp\ alt})$$

(Abbreviations same as in reaction index formula). This index represents a measure of the overall health status based on the histological lesions.

Results

The organ index calculated based on various reaction patterns of the different organs showed that gills were severely affected, liver was moderately affected, and kidney was the mildly affected organ, irrespective of fish species (Table 3–13).

Fishes of same species collected from Kuttanad also showed similar pattern except for *A. testudineus* in which liver was less damaged than kidney (Table 10–11). The total index indicated the overall health status of the fishes in each concentration and in Kuttanad. There was a gradual decrease in the health status of fish according to the increase in concentration of pesticide

Table 3. Organ index values of the gills of *E. maculatus* exposed to monocrotophos (following Bernet et al. 1999)

Concentrations	0.0 ppm					0.1 ppm					0.3 ppm					0.6 ppm					1.0 ppm					1.5 ppm				
No. of fishes	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Alterations																														
WGC1=1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4/4	-	-	4/4	2/2	2/2	4/4	4/4	2/2	4/4	6/6	6/6	4/4	4/4	4/4
WGC2=1	-	-	-	-	-	6/6	4/4	2/2	2/2	-	4/4	2/2	4/4	4/4	2/2	2/2	4/4	4/4	2/2	4/4	4/4	2/2	4/4	4/4	2/2	4/4	4/4	4/4	4/4	4/4
WGR2=1	-	-	-	-	-	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	-	-	-	-	-	4/4	2/2	2/2	2/2	2/2	4/4	2/2	2/2	2/2	2/2
WGR5=2	-	-	-	-	-	-	8/4	4/2	8/4	-	8/4	4/2	4/2	4/2	-	8/4	8/4	4/2	4/2	-	-	-	-	-	-	12/6	8/4	8/4	8/4	4/2
WGR6=3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12/4	6/2	6/2	12/4	-	12/4	12/4	12/4	12/4	12/4
WGR7=1	-	-	-	-	-	-	2/2	-	-	-	2/2	-	-	-	-	4/4	-	2/2	2/2	2/2	6/6	4/4	4/4	6/6	4/4	6/6	4/4	6/6	4/4	6/6
WGR11=2	-	-	-	-	-	8/4	4/2	4/2	-	-	4/2	8/4	4/2	-	8/4	12/6	8/4	4/2	8/4	4/2	12/6	12/6	8/4	8/4	12/6	8/4	12/6	12/6	8/4	8/4
WGP1=1	2/2	2/2	-	-	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	-	-	2/2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
WGP2=2	4/2	-	4/2	-	-	4/2	8/4	4/2	4/2	8/4	8/4	12/6	12/6	4/2	4/2	4/2	4/2	8/4	8/4	4/2	12/6	8/4	8/4	12/6	8/4	4/2	8/4	8/4	4/2	4/2
WGP4=2	-	-	-	-	-	-	-	-	-	-	12/6	8/4	4/2	4/2	8/4	12/6	8/4	4/2	4/2	8/4	8/4	8/4	4/2	4/2	4/2	8/4	12/6	8/4	12/6	12/6
WGT1=2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4/2	-	-	-	-
Organ index of each fish	6	2	4	-	2	22	30	18	18	12	42	38	30	18	26	46	32	26	32	24	60	46	40	50	36	68	68	64	58	56
Mean organ index of 5 fishes	2.8					20.0					30.8					32.0					46.4					62.8				

Denominator value denotes the score value: Numerator value = (score value x importance factor) WGC1 = 1 means importance factor = 1.

Table 4. Organ index values of the liver of *E. maculatus* exposed to monocrotophos (following Bernet et al. 1999)

Concentra-tions	0.0 ppm					0.1 ppm					0.3 ppm					0.6 ppm					1.0 ppm					1.5 ppm				
No. of fishes	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Alterations																														
WLR1=1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2/2	2/2	-	-	-	-	-	-	2/2	-	-	-	-	-
WLR2=1	-	-	-	-	-	-	-	-	-	-	2/2	-	2/2	2/2	-	4/4	-	4/4	2/2	2/2	4/4	6/6	4/4	6/6	4/4	6/6	6/6	4/4	6/6	4/4
WLR4=2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4/2	-	4/2	-	-	4/2	8/4	4/2	8/4	8/4	8/4	8/4	4/2	8/4	4/2
WLR5=2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4/2	4/2	-	8/4	-
Organ index of each fish	-	-	-	-	-	-	-	-	-	-	2	-	2	2	-	8	2	10	2	2	8	14	8	14	16	18	18	8	22	8
Mean organ index of 5 fishes			0					0					1.2					4.8					12.0					14.8		

Denominator value denotes the score value: Numerator value = (score value x importance factor). WLC1 = 1 means importance factor = 1.

Table 5. Organ index values of the liver of *A. testudinues* exposed to monocrotophos (following Bernet et al., 1999)

Concentrations	Control					2.0 ppm					5.0 ppm					10.0 ppm					18.0ppm					36.0 ppm				
No. of fishes	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Alterations																														
WLCI=1	-	-	-	-	-	2/2	-	-	-	-	-	2/2	-	2/2	-	-	2/2	2/2	-	2/2	2/2	4/4	-	4/4	-	2/2	2/2	2/2	4/4	2/2
WLR1=1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2/2	-	2/2	-	-
WLR2=1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4/4	4/4	2/2	6/6	2/2	4/4	2/2	6/6	4/4	2/2
WLR4=2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4/2	4/2	-	8/4	-	-	4/2	4/2	-	-
WLR5=2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4/2	-	8/4	-	4/2	12/6	-
Organ Index of each fish	-	-	-	-	-	2	-	-	-	-	-	2	-	2	-	-	2	2	-	2	10	12	2	22	2	16	8	18	20	4
Mean organ index of 5 fishes			0				0.4					0.8					1.2					9.6					13.2			

Denominator value denotes the score value: Numerator value = (score value x importance factor). WLC1 = 1 means importance factor = 1

Table 6. Organ index values of the kidney of *E. maculatus* exposed to monocrotophos (following Bernet et al., 1999)

Concentrations	0.0 ppm					0.1 ppm					0.3 ppm					0.6 ppm					1.0 ppm					1.5 ppm				
No. of fishes	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Alterations																														
WKC2=1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4/4	2/2	-	4/4	2/2	4/4	6/6	4/4	2/2	2/2
WKR1=1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2/2	-	2/2	-	2/2	-	-	-	-	-	-	-	-	-	-
WKR5=2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4/2	-	4/2	8/4	-	8/4	12/6	8/4	2/2	2/2
Organ index of each fish	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	2	-	2	8	2	4	12	2	12	18	12	4	4
Mean organ index of 5 fishes			0					0					0					1.2					5.6					10.0		

Denominator value denotes the score value: Numerator value = (score value x importance factor). WKC1 = 1 means importance factor = 1.

Table 7. Organ index values of the kidney of *A. testudineus* exposed to monocrotophos (following Bernet et al., 1999)

Concentrations	0.0 ppm					2.0 ppm					5.0 ppm					10.0 ppm					18.0 ppm					36.0 ppm				
No. of fishes	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Alterations																														
WKC2=1	-	-	-	-	-	-		-	-	-	-	-	-	-	-	2/2	-	4/4	-	2/2	2/2	4/4	2/2	4/4	4/4	6/6	4/4	6/6	6/6	2/2
WKR5=2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4/2	-	4/2	-	-	-	-	-	-	-	8/4	4/2	-	4/2	-
WKP2=2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4/2	4/2	4/2	4/2	4/2	12/6	8/4	8/4	8/4	12/6
Organ index of each fish	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	-	8	-	2	6	8	6	8	8	26	16	14	18	14
Mean organ index of 5 fishes			0					0					0					3.2					7.2					17.6		

Denominator value denotes the score value: Numerator value = (score value x importance factor). WKC2 = 1 means importance factor = 1.

Table 8. Organ index values of the gills of *A. testudineus* exposed to monocrotophos (following Bernet et al. 1999)

Concentrations	0.0 ppm					2.0 ppm					5.0 ppm					10.0 ppm					18.0ppm				
No. of fishes	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Alterations																									
WGC2=1	-	-	-	-	-	2/2	2/2	2/2	-	2/2	2/2	2/2	4/4	2/2	4/4	4/4	2/2	4/4	2/2	4/4	6/6	4/4	2/2	6/6	4/4
WGR1=1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
WGR5=2	-	-	-	-	-	-	-	-	-	-	4/2	4/2	-	4/2	4/2	4/2	4/2	4/2	4/2	4/2	4/2	4/2	8/4	4/2	4/2
WGR7=1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2/2	2/2	-	4/4	2/2	4/4	2/2	-	2/2	-
WGR11=2	-	-	-	-	-	-	-	-	-	-	8/4	4/2	-	4/2	4/2	8/4	8/4	-	4/2	4/2	8/4	8/4	12/6	4/2	4/2
WGP1=1	2/2	2/2	-	-	-	2/2	-	2/2	2/2	-	6/6	2/2	2/2	4/4	2/2	2/2	4/4	2/2	2/2	4/4	-	-	-	-	-
WGP2=2	4/2	-	-	4/2	4/2	4/2	4/2	-	-	4/2	-	-	-	-	-	8/4	4/2	4/2	4/2	-	8/4	4/2	4/2	8/4	4/2
WGP4=2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2/2	-	4/4	2/2	2/2	4/4	2/2	2/2	-	2/2
Organ index of each fish	6	2	-	4	4	8	6	4	2	6	20	12	6	14	14	30	24	18	22	20	34	24	28	24	18
Mean organ Index of 5 fishes			3.2					5.2					13.2					22.8					25.6		

Denominator value denotes the score value: Numerator value = (score value x importance factor). WGC1 = 1 means importance factor = 1.

Table 9. Organ index values of the gills of *E. maculatus* and *A. testudineus* collected from the paddy fields of Kuttanad (following Bernet et al., 1999)

Fish species	*E. maculatus*										*A. testudineus*									
No. of fishes	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Alterations																				
WGC1=1	4/4	2/2	4/4	6/6	2/2	2/2	4/4	4/4	6/6	4/4	4/4	2/2	-	2/2	4/4	-	4/4	4/4	2/2	-
WGC2=1	4/4	4/4	2/2	2/2	4/4	4/4	2/2	4/4	2/2	4/4	6/6	4/4	6/6	2/2	6/6	4/4	4/4	6/6	4/4	2/2
WGR5=2	8/4	8/4	4/2	4/2	12/6	8/4	8/4	4/2	12/6	12/6	8/4	12/6	4/2	12/6	-	4/2	8/4	8/4	12/6	-
WGR6=3	12/4	18/6	12/4	6/2	6/2	12/4	12/4	6/2	12/4	12/4	12/4	6/2	6/2	-	12/4	12/4	12/4	6/2	6/2	6/2
WGR7=1	6/6	4/4	4/4	2/2	4/4	4/4	4/4	2/2	6/6	4/4	6/6	4/4	6/6	4/4	4/4	4/4	2/2	6/6	2/2	6/6
WGR11=2	12/6	4/2	8/4	4/2	4/2	8/4	12/6	12/6	8/4	4/2	8/4	-	4/2	4/2	8/4	8/4	4/2	8/4	-	8/4
WGP2=2	12/6	8/4	8/4	12/6	8/4	12/6	12/6	12/6	8/4	4/2	8/4	12/6	-	4/2	12/6	12/6	8/4	4/2	12/6	8/4
Organ index of each fish	58	48	42	36	40	50	54	44	54	44	52	40	26	28	46	44	42	42	38	30
Mean organ index of 10 fishes	47.0										38.8									

Denominator value denotes the score value: Numerator value = (score value x importance factor). WGC1 = 1 means importance factor = 1.

Table 10. Organ index values of the liver of *E. maculatus* and *A. testudineus* collected from the paddy fields of Kuttanad (following Bernet et al., 1999)

Fish species	*E. maculatus*										*A. testudeneus*									
No of fishes Alterations	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
WLC1=1	2/2	-	2/2	-	-	-	2/2	2/2	2/2	-	-	-	2/2	2/2	2/2	4/4	4/4	-	2/2	-
WLR2=1	6/6	4/4	4/4	2/2	2/2	4/4	6/6	6/6	6/6	2/2	-	-	-	-	-	-	-	-	-	-
WLR4=2	8/4	4/2	4/2	-	-	4/2	8/4	8/4	8/4	-	4/2	8/4	4/2	4/2	4/2	8/4	8/4	4/2	4/2	8/4
WLR5=2	4/2	-	-	4/2	4/2	-	4/2	4/2	-	4/2	-	-	-	-	-	-	-	-	-	-
WLR6=3	6/2	-	-	-	-	-	6/2	6/2	6/2	-	-	-	-	-	-	-	-	-	-	-
WLT1=2	-	-	-	-	-	-	-	-	-	-	4/2	-	4/2	-	-	8/4	-	8/4	-	-
Organ Index of each fish	26	8	10	6	6	8	26	26	22	6	8	8	10	6	6	20	12	12	6	8
Mean organ Index of 10 fishes	13.6										9.6									

Denominator value denotes the score value: Numerator value = (score value x importance factor). WLC1 = 1 means importance factor = 1.

Table 11. Organ index values of the Kidney of *E. maculatus* and *A. testudineus* collected from the paddy fields of Kuttanad (following Bernet et al., 1999)

Fish species	*E. maculatus*										*A. testudeneus*									
No of fishes	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Alterations																				
WKC2=1	4/4	2/2	-	4/4	4/4	2/2	4/4	2/2	2/2	2/2	2/2	4/4	-	6/6	4/4	2/2	-	-	-	2/2
WKR5=2	8/4	-	-	4/2	4/2	-	-	4/2	4/2	4/2	4/2	12/6	4/2	8/4	-	-	4/2	-	4/2	-
WKP2=2	4/2	4/2	8/4	4/2	4/2	4/2	4/2	4/2	4/2	-	12/6	12/6	8/4	12/6	4/2	-	-	4/2	8/4	-
Organ Index of each fish	16	6	8	12	12	6	8	10	10	6	18	28	12	26	8	2	4	4	12	2
Mean organ index of 10 fishes	9.4										11.6									

Denominator value denotes the score value: Numerator value = (score value x importance factor). W_{KC2}= 1 means importance factor = 1.

Table 12. Total index of *E. maculatus* and *A. testudineus* exposed to different sublethal concentrations of monocrotophos based on the organ index.

Fish	Treatment (ppm)	Organ Index			Total Index
		Gill	Liver	Kidney	
E. maculatus	0.0	2.8	0	0	2.8
	0.1	20.0	0	0	20.0
	0.3	30.8	1.2	0	32.0
	0.6	32.0	4.8	1.2	38.0
	1.0	46.4	12.0	5.6	64.0
	1.5	62.8	14.8	10.0	87.6
A. testudineus	0.0	3.2	0	0	3.2
	2.0	5.2	0.4	0	5.6
	5.0	13.2	0.8	0	14.0
	10.0	22.8	1.2	3.2	27.2
	18.0	25.6	9.6	7.2	42.4
	36.0	33.2	13.2	17.6	64

Table 13. Total index of *E. maculatus* and *A. testudineus* collected from Kuttanad based on the organ index

Fish Species	Organ Index			Total Index
	Gill	Liver	Kidney	
E. maculatus	47.0	13.6	9.4	70.0
A. testudineus	38.8	9.6	11.6	60.0

Discussion

The organ indices are used for calculating the total index, which gives the health status of an organism in particular, under altered environmental condition. In the present study, the total index showed the health status of fishes in each sublethal concentration and in the field conditions. The health status became worse in the higher sublethal concentrations of both the fishes treated with monocrotophos. The total index of *E. maculatus* collected from Kuttanad is 70, which is comparable to the total index value of *E. maculatus* exposed to sublethal concentrations of 1.0 ppm monocrotophos (total index 64). The total index value of *A. testudineus* collected from Kuttanad is 60, which is comparable to the total index value of *A. testudineus* exposed to sublethal concentrations of 36 ppm monocrotophos (total index 63.4).

It is evident from the index value that the gills are in the irreversibly damaged condition as per Poleksic & Mitrovic-Tutundzic (1994). This irreversible condition in the gills of *E. maculatus* and *A. testudineus* may be due to the chronic microtoxicosis (sublethal effects) as a result of toxicants in the medium. Thus, gill degeneration can be considered as a factor, which seriously impairs the viability of organisms, while this may not represent a hazard to the life of the individual. It has great importance as far as the survival of the population is concerned. Szakolczai et al. (1994) have also reported that structural changes in gills can be considered suitable to monitor the level of environmental contamination, especially the sublethal and chronic effects of pollutants, particularly in those cases where other methods of assessments are not satisfactory, and to compliment the assessment of the average level of pollution. It should be emphasized that fish gill can maintain their vital functions even when some lamellae are heavily damaged. However, chronic exposure of these gill lamellae to pesticides will lead to the histological degeneration of the irreversible condition that will lead to functional disturbance or dysfunction of the organ. This gradually leads to mortality and in turn affects the population of the ecosystem. Hence, the present study carried out on the natural population supports the view of Poleksic & Mitrovic-Tutundzic (1994) that histological changes in fish gills should become one of the methods used for assessment of water quality in sublethal and chronic situations.

The histopathological changes are one of the most sensitive parameters for the evaluation of chronic toxicity test effects and thus also for the derivation of Maximum Allowable Toxicant Concentration as reported by Poleksic & Mitrovic-Tutundzic (1994). Moreover, the sublethal concentrations may become lethal for populations confronted

with additional stresses. This should be taken into serious consideration when evaluating the effects of mixtures of toxicants in fresh water fish under natural conditions.

In the natural ecosystem, fishes are exposed simultaneously to more than one biocide or contaminant because some chemicals are applied continuously and are highly persistent or others are applied as combinations to increase the efficiency or reduce costs (Marking 1977). Kuttanad is an area where the pesticides and weedicides are applied simultaneously or intermittently. The problems of toxicity of mixtures of pesticides on fish have been recognized only recently and notable among the studies are those of Macek (1977); Fabacher et al. (1976); Nair et al. (2000). While studying the individual and combined acute lethal toxicity of monocrotophos and 2,4-D on the juveniles of *E. suratensis*, a highly favored food fish of Kuttanad region, Nair et al. (2000) reported that a strictly additive nature of their combined toxicity and the sequential and even simultaneous use in the ecosystem increases the potential for pollution. Significant increase in sensitivity could be achieved from histological studies when compared with routine parameters like survival and mortality. Histopathology provides evidences of adaptation to degeneration, and this certainly represents the major advantage of the use of histopathological alterations as biomarkers of environmental pollution by organic chemicals.

Therefore, it is proposed that the histological changes in fish gill should become one of the methods used for assessment of water quality in sublethal and chronic situations as the toxicants induce changes at lower levels of biological organization occurring prior to organismic changes. It should therefore provide a rapid "early warning system" as suggested by Moore (1985).

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