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Histopathology – a Biomonitoring Tool to Characterize the Health Status of Fish in the Paddy Fields of Kuttanad, Kerala, India

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ABSTRACT

High population growth have led to a tremendous intensification of rice production, which in turn has significantly increased the amount of pesticides applied in rice cropping systems. Since pesticides are toxic by design, there is a natural concern on the impact of their presence in the environment on human health and environmental quality. The present study was conducted to assess the nature and extent of pesticide induced pathogenesis in the tissues of gills, liver and kidney of *Etroplus maculatus* inhabiting the paddy fields of Kuttanad, Kerala, India, which is subjected to long term exposure to sublethal concentrations of a common pesticide, phosphamidon, used in these area. The organ index calculated based on various reaction patterns of the different organs. The study showed that the gills are severely affected, liver is moderately affected and the kidney is the mildly affected organ. As an indicator of pollution, histology represents a useful tool to assess the degree of pollution, particularly for sub lethal and chronic effects.

Key words : *Pesticides, Phosphamidon, Histopathology, Organ index, Gill, Liver, Kidney*

Introduction

Since pesticides are toxic by design, there is a natural concern on the impact of their presence in the environment on human health and environmental quality. Pesticides have become an indispensable part of modern agricultural practices and act as one of the vital factors in increasing food production. Over spray and run off of pesticides from agricultural fields may easily find their way into the natural water sources and adversely affect the quality of water and creates hazards for aquatic life resulting in serious damage to non-target species, including fishes (Magar and Bias, 2013). Many scientists reported the presence of pesticide residues in soil, water bodies, air, food materials and the bodies of living beings (Brahmaprakash and Sethunathan, 1987; Gangamma and Satyanarayana, 1991; Mencher, 1991; Ganeshwade, 2012). Water pollution induces histological changes in organisms. In fish, water pollution can lead to different changes ranging from biochemical alterations in single cell up to changes in whole populations. In the present study, gills, liver and kidney are selected for histological examination in order to determine the effect of pesticide pollution. These organs are primary markers for aquatic pollution as gills exhibit large surfaces, which are in direct and permanent contact with po-

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tential irritants. The liver plays a key role in metabolism and subsequent excretion of xenobiotics and is also the site of vitellogenin production. The kidney is important for the maintenance of a stable internal environment with respect to water and salt excretion and partially, for the metabolism of xenobiotics. Bernet *et al*.(1999) suggested an organ index for the calculation of the histopathological lesions of gill, liver and kidney.

The present study, conducted to assess the nature and extent of pesticide induced pathogenesis in the tissues of gills, liver and kidney of *Etroplus maculatus* inhabiting the paddy fields of Kuttanad, Kerala, India, which is subjected to long term exposure to sub lethal concentrations of a common pesticide, phosphamidon used in these area. In addition to that a comparative analysis of the lab and field observation of the same has been done.

Materials and Methods

The experiments on the lethal and sublethal toxicity of phosphamidon on the juveniles of *Etroplus maculatus* was conducted for 48 hours and 30 days respectively during the period of investigation. *Etroplus maculatus* (6.70 ± 0.30 cm in total length and 5.00 ± 1.00g in weight) collected from non-polluted natural ponds. During the period of exposure, they were fed *adlibitum* once a day on fresh clam meat. Based on the LC50 values, five nominal concentrations (Table 1) of the pesticides were selected for sublethal toxicity studies. Maximum and minimum sublethal concentrations were chosen based on Konar (1969) and Sprague (1973). This sublethal exposure was done in a static system where water and pesticide medium were renewed every 24 hr to maintain the desired pesticide concentration. A control, free of pesticide, was also maintained in this experiment. All the treatments and the controls were made in triplicates. Ten healthy fishes chosen at random from the acclimated stock were reared in 32 litres of water in seasoned cement cisterns.

After 30 d of the experiments five specimens from each of the treated as well as the control group were sacrificed and the target organs were dissected out and fixed immediately in Bouin's fluid. Theses organs were washed, dehydrated, cleared and embedded in paraffin wax. Serial sections of each organ was taken at 3 to 5µm thickness and stained with Hematoxylin-eosine staining procedures (Stevens, 1982). Detailed histological observations were carried out with the help of a binocular microscope. Simultaneously same species of fishes were collected from paddy fields of Kuttanad and analyzed in the same way. In the present study, histopahtological conditions of different organs were assessed based on Bernet *et al*. (1999) who classified the histopathological changes of each organ in to 5 reaction patterns. Each pattern includes several alterations in respect of either functional unit of the organ or as entire organ.

Calculation of the index values were 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 importance 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 are also considered.

Mathematical calculation of lesion indices.

- Reaction index of an organ (I org rp)

Table 1. 48 hr LC50 and Sublethal concentrations of phosphamidon

Fish Species	Pesticide	48 hr LC50 (ppm)	Sublethal concentrations $mg.l^{-1}$					
E. maculatus	Phosphamidon	2.97	0.0	0.06	0.1	0.3	0.5	1.0

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)*

Reaction pattern value	Functional unit of the tissue	Alteration	Importance	Score factor	Index
Gills		Haemorrhage/hyperaemia/aneurysm	WGC1=1	aGC1	IGC
Circulatory		Intercellular oedema	WGC2=1	aGC2	
disturbances	Epithelium	Architectural and structural alterations	WGR1=1	aGR1	IGR
Regressive		Plasma alterations	WGR2=1	aGR2	
changes		Deposits	WGR3=1	aGR3	
		Nuclear alterations	WGR4=2	aGR4	
		Atrophy	WGR5=2	aGR5	
		Necrosis	WGR6=3	aGR6	
		Rupture of the pillar cells			
	Supporting	Architectural and structural alterations	WGR7=1	aGR7	
	tissue	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	Hypertrophy	WGP3=1	aGP3	
	tissue	Hyperplasia	WGP4=2	aGP4	
Inflammation		**Exudate**	**WGI1=1**	**aGI1**	**IG1**
		Activation of RES	WGI2=1	aGI2	
		Infilteration	WGI3=2	aGI3	
Tumour		**Benign tumour**	**WGT1=2**	**aGT1**	**IGT**
		Malignant tumour	WGT2=3	aGT2	
					IG
Liver		**Haemorrhage/hyperaemia/aneurysm**	**WLC1=1**	**aLC1**	**ILC**
Circulatory		**Intercellular oedema**	**WLC2=1**	**aLC2**	
disturbances	**Liver tissue**	**Architectural and structural alterations**	**WLR1=1**	**aLR1**	**ILR**
Regressive		Plasma alterations	WLR2=1	aLR2	
changes		Deposits	WLR3=1	aLR3	
		Nuclear alterations	WLR4=2	aLR4	
		Atrophy	WLR5=2	aLR5	
		Necrosis	WLR6=3	aLR6	
		Vacuolar degeneration			
	Interstitial	Architectural and structural alterations	WLR7=1	aLR7	
	tissue	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	

Table 2#. *Continued ...*

Reaction pattern value	Functional unit of the tissue	Alteration	Importance	Score factor	Index
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	
Tumour		**Benign tumour**	**WLT1=2**	**aLT1**	**ILT**
		Malignant tumour	WLT2=3	aLT2	
					IL
Kidney		**Haemorrhage/hyperaemia/aneurysm**	**WKC1=1**	**aKC1**	**IKC**
Circulatory		**Intercellular oedema**	**WKC2=1**	**aKC2**	
disturbances	**Tubule**	**Architectural and structural alterations**	**WKR1=1**	**aKR1**	**IKR**
Regressive changes		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	
Tumour		**Benign tumour**	**WKT1=2**	**aKT1**	**IKT**
		Malignant tumour	WKT2=3	aKT2	
					IK

Extracted from Bernet *et al*. (1999)

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

Concentrations	0.0 ppm					0.06ppm					0.1 ppm					0.3 ppm					0.5 ppm					1.0 ppm				
No.offishes	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	-	-	-	-	-	-	-	-	-	-	2/2	4/4	-	-	4/4	-	2/2	4/4	2/2	4/4	4/4	6/6	4/4	6/6	2/2	6/6	4/4	4/4	6/6	6/6
WGC2=1	2/2	-	-	2/2	-	2/2	2/2	2/2	-	2/2	-	2/2	2/2	2/2	-	4/4	4/4	2/2	2/2	4/4	6/6	6/6	4/4	4/4	4/4	6/6	6/6	6/6	6/6	6/6
WGR2=1	-	-	-	-	-	2/2	2/2	2/2	-	2/2	2/2	2/2	6/6	4/4	2/2	2/2	2/2	2/2	2/2	2/2	-	2/2	2/2	2/2	2/2	2/2	-	-	2/2	4/4
WGR5=2	-	-	-	-	-	-	-	-	-	-	4/2	8/4	4/2	8/4	4/2	4/2	4/2	4/2	8/4	4/2	12/6	8/4	4/2	8/4	4/2	12/6	8/4	8/4	8/4	12/6
WGR6=3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6/2	12/4	12/4	-	6/2	18/6	12/4	6/2	12/4	-
WGR7=1	-	-	-	-	-	-	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	2/2	4/4	6/6	2/2
WGR11=2	-	-	-	-	-	4/2	4/2	4/2	-	-	12/6	8/4	4/2	4/2	8/4	8/4	4/2	4/2	8/4	4/2	8/4	8/4	12/6	12/6	4/2	12/6	12/6	12/6	8/4	12/6
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	4/2	4/2	4/2	4/2	4/2	12/6	12/6	4/2	4/2	8/4	12/6	8/4	12/6	8/4	12/6	12/6	12/6	12/6	8/4	8/4
WGP4=2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8/4	4/2	8/4	-	4/2	8/4	8/4	8/4	4/2	8/4	12/6	8/4	8/4	12/6	12/6
WGTI=2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4/2	8/4	-	-	4/2
Index value of each fish	4	2	2	2	-	14	12	10	6	10	28	30	24	24	24	40	34	28	26	32	58	62	60	46	44	86	72	60	68	66
Meanorgan Index of 5 fishes			2					10.4					26.0					32.0					54.0					70.4		

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 phosphamidon (following Bernet *et al.*, 1999)

Concentrations	0.0 ppm					0.6 ppm					0.1 ppm					0.3 ppm					0.5ppm					1.0 ppm				
No. offishes	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																														
WLC1=1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	2	-	-	2	-	-	-	-	-
WLR1=1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
WLR2=1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	2	2	6	4	6	6	4	6	6	4	6	6
WLR4=2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8/4	4/2	8/4	8/4	4/2	12/6	8/4	8/4	8/4	8/4
WLR5=2	-	-	-	-	-	-	-	-	-	-	4/2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4/2	-	4/2	4/2	-
WLR6=3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6/2	6/2	-
WLT1=2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6/2	-	-	-	-
Organ Index	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	2	-	2	2	16	10	14	14	10	28	14	22	24	14
Mean of organ index of 5 fishes			0					0					0.8					1.2					12.8					20.4		

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

The lesions within one organ only 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.

- 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

- Organ index (I org)

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

(abbreviations same as in reaction index formula)

This index represents a measure of the overall health status based on the histological lesions.

Results and Discussion

The organ index was calculated based on various reaction patterns of the different organs of the fish showed that gills are severely affected organ (Table 3&9), whereas the liver is moderately affected and kidney is the mildly affected organ (Table 4 & 5). Fishes of same species collected from Kuttanad also showed that gill is the severely affected organ followed by liver and kidney (Table 6-8). The total index indicated the overall health status of the fishes in each concentration and collected from the paddy fields of Kuttanad (Table 9). There was a gradual decrease in the health status of fish according to the increase in the concentration of pesticides.

The organ index is used for comparing the severity of lesions in different organs. The organ indices are used for calculating the total index, which gives the health status of an organism in particular, under altered environmental condition. The organ Index was calculated by Bernet *et al*., 1999. Sulekha and Anna Mercy, (2009); (2022) calculated the same in different fishes. 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 concen-

Table 5. Organ index values of the kidney of *E. maculatus* exposed to phosphamidon (following Bernet *et al*., 1999)

Concentrations	0.0 ppm					0.06ppm					0.1 ppm					0.3 ppm					0.5 ppm					1.0 ppm				
No.offishes	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	2/2	4/4	4/4	2/2	4/4	4/4	4/4	4/4	6/6	2/2
WKR1=1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6/6	2/2	4/4	-	6/6	6/6	4/4	6/6	2/2	2/2
WKR2=1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2/2	4/4	-	-	4/4	2/2	4/4	-	-
WKR5=2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4/4	8/4	4/2	-	-
WKP2=2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4/2	-	-	-	-	-	-	-	-	-	4/2	8/4	8/4	4/2	4/2
Organ index of each fish	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	2	-	4	2	8	8	12	4	8	22	26	26	12	8
Mean organ index of 5 fishes			0					0					0					3.2				8.0					18.8		

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

Table 6. Organ index values of the gills of *E. maculatus* collected from the paddy fields of Kuttanad (following Bernet *et al.*, 1999)

Fishspecies	*E.maculatus*									
No. offishes	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
WGC2=1	4/4	4/4	2/2	2/2	4/4	4/4	2/2	4/4	2/2	4/4
WGR5=2	8/4	8/4	4/2	4/2	12/6	8/4	8/4	4/2	12/6	12/6
WGR6=3	12/4	18/6	12/4	6/2	6/2	12/4	12/4	6/2	12/4	12/4
WGR7=1	6/6	4/4	4/4	2/2	4/4	4/4	4/4	2/2	6/6	4/4
WGR11=2	12/6	4/2	8/4	4/2	4/2	8/4	12/6	12/6	8/4	4/2
WGP2=2	12/6	8/4	8/4	12/6	8/4	12/6	12/6	12/6	8/4	4/2
Organ index of each fish	58	48	42	36	40	50	54	44	54	44
Mean organ index of 10 Fishes							47.0			

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

Table 7. Organ index values of the liver of *E. maculatus* collected from the paddy fields of Kuttanad (following Bernet *et al*., 1999)

Fish Species	*E.maculatus*									
No offishes	1	2	3	4	5	6	7	8	9	10
Alterations										
WLC1=1	2/2	-	2/2	-	-	-	2/2	2/2	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	-
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	-	-	-	-	-	-	-	-	-	-
Organ Index of 26 each fish	8	10	6	6	8	26	26	22	6	
Mean organ Index of 10 fishes	13.6									

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

Table 8. Organ index values of the Kidney of *E. maculatus* collected from the paddy fields of Kuttanad (following Bernet *et al*., 1999)

Fish Species	*E.maculatus*									
No offishes	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
WKR5=2	8/4	-	-	4/2	4/2	-	-	4/2	4/2	4/2
WKP2=2	4/2	4/2	8/4	4/2	4/2	4/2	4/2	4/2	4/2	-
Organ Index of each fish	16	6	8	12	12	6	8	10	10	6
Meanorgan index of 10 fishes	9.4									

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

Table 9. Total index of *E. maculatu*s exposed to different sub lethal concentrations of phosphamidon Collected from Kuttanad based on the organ index.

Treatment (ppm)	Organ Index			Total Index
	Gill	Liver	Kidney	
0.0	2.0	0	0	2.0
0.06	10.4	0	0	10.4
0.1	26.0	0.8	0	26.8
0.3	32.0	1.2	3.2	36.4
0.5	54.0	12.8	8.0	74.8
1.0	70.4	20.4	18.8	109.6
Collected from Kuttanad	47.0	13.6	9.4	70.0

trations of phosphamidon. The total index value of *E. maculatus* collected from Kuttanad was 70, which is comparable to the total index value of *E. maculatus* exposed to sublethal concentrations of phosphamidon between 0.3 ppm and 0.5 ppm (total index 36.4 and 74.8 respectively). The result showed that the histological conditions of the fishes from the paddy fields of Kuttanad, were worse. That means the concentration of pesticide in the study area is very high. It will leads to functional disturbance or dysfunction of the organs. This gradually leads to mortality and in turn affects the population of the ecosystem. So we also agreed with Yancheva *et al*., 2016, histopathology should be more often included in monitoring programs on contaminated aquatic systems, along with other biomarkers and chemical analyses of waters and sediments.

Conclusion

Chronic exposure of gills, liver and kidney to phosphamidon will lead to severe histopathological conditions. Based on the organ index value arrived at from the lab and the field study, gill is found as the most sensitive organ. The histopathological changes in fish should become one of the methods used for water Quality assessment in sublethal and chronic situations as lower levels of biological organizations occur prior to the organismic changes. It is therefore, provides a rapid "early warning system".

References

Bernet, D., Schmidt, H., Meir, W., Burkhardt- Holn, P. and Wahli, T. 1999. Histopathology in fish: Proposal for a protocol to assess aquatic pollution. *Journal of Fish Diseases.* 22 : 25-34.

Brahmaprakash, G. P. and Sethunathan, N. 1987. Relative Persistence of Hexachloro cyclohexane, Methyl Parathion and Carbofuran in an Alluvial Soil under Flooded and Non-flooded Conditions. *Agricultural Economics.* 30 : 71- 80.

Ganeshwade, R. M. 2012. Histopathological Changes in the Gills of Puntiusticto (HAM) Under Dimethoate Toxicity. *The Bioscan*. 7(3): 423-426.

Gangamma, G. N. and Satyanarayana, B. S. 1991. Danger Posed by Pesticides. *Spice India*. 32(9): 7-9.

Konar, S. K. 1969. Two organophosphorus insecticides DDVP and phosphamidon as selective toxicants. *Transactions of the American Fisheries Society.* 98 : 430-437.

Magar, R. S. and Bias, U. E. 2013. Histopathological Impact of Malathion on the Ovary of the Fresh Water fish *Channa punctatus*. *International Research Journal of Environment Sciences*. 2(3): 59- 61.

Mencher, J. P. 1991. Agricultural Labor and Pesticides in Rice Regions of India: Some Health Considerations. *Economic and Political Weekly.* 26(39): 2263-2268.

Sprague, J. B. 1973. The ABC's pollutant bioassay using fish. In: J. Cairns, Jr. and K.L. Dickson (eds.). *Biological Methods for the Assessment of Water Quality*. ASTM STP 528, American Society for testing and Materials, Philadelphia. 6-30.

Stevens, A. 1982. The Haematoxylins. In Bancroft, John; Stevens, Alan (eds.). *The Theory and Practice of Histological Techniques (2nd ed.)*. Longman Group Limited. 109.

Sulekha B. T. and Anna Mercy T. V. 2009. Pesticide induced histopathological changes in the freshwater fishes of Kuttanad, Kerala – A tool to assess water quality and the health status of fishes. *Asian Fisheries Science*. 22: 729-749.

Sulekha, B.T. and Anna Mercy, T. V. 2022. Sublethal Effects of Monocrotophos on Histological Architecture of Liver and Kidney of a Freshwater Fish, *Anabas testudineus*. *Ecology, Environment and Conservation.* 28 (3) : 528-537.

Yancheva, V., Velcheva, I., Stoyanova, S. and Georgieva, E. 2016. Histological Biomarkers in Fish As A Tool in Ecological Risk Assessment and Monitoring Programs: A Review. *Applied Ecology and Environmental Research.* 14 (1):47-75.