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HISTOPATHOLOGY IS A BIOMONITORING TOOL TO ASSESS PESTICIDE POLLUTION IN THE ENVIRONMENT

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ABSTRACT

Histological alterations are sensitive biomarkers for xenobiotic effects and it is a tool for the diagnosis of direct and indirect toxic effects that affect the animal tissues. Fish gills are multifunctional organs involved in ion transport, gas exchange, acid– base regulation and waste excretion. Furthermore, the gills are the main route of toxicants penetration into the fish organism, thus they are the first organ, which come in contact with environmental pollutants and are sensitive subjects for identifying the effects of water toxicants on the fish tissues. Therefore, it is considered as an excellent method for assessing the environmental quality. Histopathological analysis of gills of *Anabas testudineus* exposed to sublethal concentrations of monocrotophos was studied. Estimation of the maximum allowable toxicant concentration of monocrotophos (MATC) and its application factor (AF) for the *Anabas testudineus*, was derived based on no observable effect concentration (NOEC) and the least observable effect concentration (LOEC). MATC and AF calculated for the pesticide was 13.4164ppm and 0.1308 respectively. The Application Factor, which is worked out for one species can be used for other related species also, provided their 48hr LC_{50} is known. From this the MATC for latter species can be conveniently found out. This will be an important tool for the farmers in deciding the concentrations of pesticides before its application into the paddy fields.

KEY WORDS: Pesticide**,** Monocrotophos, Histopathology, Biomonitoring, MATC, AF.

INTRODUCTION

Agricultural pesticides are nowadays being extensively used for the control of a wide variety of agricultural pests. Most of the pesticides used are highly toxic and are remaining in the environment for a prolonged period, causing pollution. Moreover, due to the repeated applications of the pesticides, their toxic residues in environment and biota have reached an alarming concentration. Unfortunately, many of these toxic chemicals are reported to be mutagenic, carcinogenic or teratogenic to human beings and hydrobionts of the biosphere. Pesticides reach water either through direct application or indirectly from agricultural fields, spray drift, rainwater, sewage and effluents from industries manufacturing pesticides or using them in their processes. It appears that significant proportion of pesticides reach the adjacent water bodies through agricultural run off. Relatively water-soluble pesticides are transported in a dissolved state, while the insoluble forms are transported after being bounded to the particulate matter. Eventhough the pesticides are directly applied against the pests in agricultural fields, it adversely affects the non-target organisms like fishes inhahiting in the paddy fields. 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 wealth due to ubiquitous application of pesticides and thenceforth establishing the necessity for a judicious use of pesticides in agriculture in future.

Histopathology is a biomonitoring tool for the assessment of structural variations of organs due to the pollutants long before its mortality. Gill

histopathology appears to be one of the promising biomarkers for general environmental contamination. Fish gills are multifunctional organs involved in ion transport, gas exchange, acid– base regulation and waste excretion. According to Playle (1998) given the fact that the gills account for over 50% of the surface area of a fish, it is not surprising that one of the major target organs for waterborne toxicants are exactly the gills. Furthermore, according to Rosseland *et al*. (2007) the gills are the main route of toxicants penetration into the fish organism, thus they are the first organs, which come in contact with environmental pollutants and are sensitive subjects for identifying the effects of water toxicants on the fish tissues. Gill structure provides a large surface area for direct and constant contact with water pollutants. Thus, this organ is too sensitive to chemicals in water (Sulekha and Anna Mercy, 2009) and is considered as the primary target organ to the contaminants (Sulekha and Anna Mercy, 2021). It is a metabolically active tissue involved in gaseous exchange, and it accumulates a significant proportion of toxins (Emilia Strzy¿ewska-Worotyñska *et al.*, 2017; Strzy¿ewska *et al*., 2016; Benli and Ozkul, 2008; Evans *et al.,* 2005 and Poleksic and Mitrovic-Tutundzic 1994). Morphological lesions in the gills are easier to detect than functional abnormalities (Fanta *et al*., 2003; Fernandes *et al*., 2007; Velasco-Santamarßa and Cruz-Casallas, 2008), because they may become visible long before behavioural changes in fish can be detected (Yancheva *et al.,* 2015). Sulekha and Mercy, (2021) have been documented several types of gill impairment in fish exposed to phosphamidon. Indeed, the concentration of toxic factors in this organ is identical to the level of xenobiotics found in the water that the fish inhabit.

In the present study, an attempt has been made to evaluatethe histopathological changes in gills, the vital organ of fish exposed to different sublethal concentrations of pesticides would help in assessing the magnitude of pesticide induced pathogenesis in fish. Schwaiger *et al.* (2004) state that the histopathological alterations can be used as indicators for the effects of various anthropogenic pollutants and they are a reflection of the overall health of the entire population in the studied ecosystem. Estimation of maximum allowable toxicant concentration (MATC) of pesticide monocrotophos and the application factor (AF) for the pesticide based on no observable effect concentration (NOEC) and the least observable effect concentration (LOEC) was done based on the histopathology of the gills of *Anabas testudineus*. The application factor (AF), which is worked out for one species can be used for other related species also, provided their 48hr LC_{50} is known. From this the MATC for latter species can be conveniently found out. This will be an important tool for the farmers in deciding the concentrations of pesticides before its application into the paddy fields. Description of sublethal and safe values of pesticides will be helpful in making appropriate recommendations in respect of permissible level of pesticide concentration, which shall not hamper the breeding, development, and survival of fishes in the inundated paddy fields and nearby water bodies so as to safeguard the fishery potential.

MATERIALS AND METHODS

The experiments on the lethal and sublethal toxicity of monocrotophos, an organophosphate pesticide, on the juveniles of *Anabas testudineus* were conducted for 30 days during the period of investigation. The experiments were conducted in the wet lab which has concrete floor with gentle slope, having proper drainage to remove pesticide contaminated water to minimize the hazards. There were provisions for water supply, lighting and adequate ventilation in the shed. The experiment was done in cement cisterns of 35 litres capacity with the following specifications. Clear filtered fresh water drawn from an open well subjected to a fine filtration using nylon bolting cloth, was used for the experiment and the tanks were filled with 32 litres of water.

Juveniles of *Anabas testudineus* were collected from pollution free ponds from the natural habitat. The average size of *Anabas testudineus* with 7.15 ±0.6 cm in total length and 7.50 ± 1.50g in weight were used for monocrotophos exposure. During these periods, they were fed *ad libitum* once a day on fresh clam meat. Monocrotophos is a water soluble organophosphate and is a broad-spectrum systemic and contact insecticide-cum-acaricide with long term residual action. This is effective against sucking, chewing and mining insects on paddy, maize, barley, etc.

Based on the LC_{50} value (102.598 ppm) obtained (Mercy *et al.,* 2000), five nominal concentrations of the pesticides were selected for sublethal toxicity studies. Maximum and minimum sublethal concentrations were chosen based on (Konar, 1969 and Sprague, 1973). The concentrations of pesticides

used for each sublethal exposure were 0.0 ppm, 2.0 ppm, 5.0 ppm, 10.0 ppm, 18.0 ppm and 36.0 ppm of monocrotophos. 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 each 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. Well water was used for the experiment and fresh water was filtered using nylon bolting cloth and aerated to saturation prior to use. The fishes were fed once a day on fresh clam meat *ad libitum*.

Water quality parameters in the experimental tanks were measured by the following methods. Modified standard Wrinkler's method (Strickland and Parsons, 1972) was used for measuring the dissolved oxygen. pH was tested using universal pH indicator solution method. emperature tested using thermometer with an accuracy of 0.1 °C.

After 30 days of the experiments five specimens from each of the treated as well as the control group were sacrificed and gills, the target organ was dissected out and fixed immediately in Bouin's fluid. This fixed organ was washed, dehydrated, cleared and embedded in paraffin wax. Serial sections of the organ was taken at 3 to 5µm thickness and stained with Hematoxylin-eosin staining procedures (Stevens, 1982). Detailed histological observations were carried out with the help of a binocular microscope. Based on the histological lesions observed in the gills of these fishes the index values were calculated. The method followed for the analysis is that of Poleksic and Mitrovic-Tutundzic (1994) who have classified the gill lesions based on two criteria.

First criterion

The first criterion classified gill lesion, based on the type and location of the damaged gill tissue, into five main groups.

(a) hypertrophy and hyperplasia of gill epithelia and related changes
(b) changes in the mucous and/or chloride cells
(c) gill parasites
(d) blood vessel changes
(e) terminal stages

Second criterion

The second criterion - of severity, is based on the scope for repair of the lesions, i.e. the possibility of restoration of normal morphological structure with an improvement in the environmental conditions, or the cessation of pollution.

With regard to this second criterion, gill lesions are classified into three progressive stages:

(a) for the first stage 10^0
(b) for the second stage 10^1
(c) for the third stage 10^2

Of the total of 26 types of gill change, 19 belong to the first stage, five to the second and two to the third (Poleksic and Mitrovic-Tutundzic, 1994).

The sum of the number of lesion types within each of the three stages multiplied by the stage index as above represents the numerical value of the degree of damage in a single fish gill, i.e.

$$I = \sum_{i=1}^{19} a_i + 10 \sum_{i=1}^{5} b_i + 10^2 \sum_{i=1}^{2} c_i$$

Where I is the degree of changes in a single fish gill
a = first stage alterations
b = second stage alterations
c = third stage alterations
The method of calculating a value of I marks it possible to compare the degree of tissue change in a large number of fish from different situation of pollution, and to correlate the intensity of pollution with the intensity of the changes found.

Application of the mathematical equation to derive categories of gill damage

Effects of I values are denoted as follows

I values	Effects
0-10	Functionally normal gills
11-20	Slightly to moderately damaged gills
21-50	Moderately to heavily damaged gills
>100	Irreparably damaged gills.

Based on these I values, obtained for the gills in each experiment no observable effect concentration (NOEC - it is the concentration at which there is no significant change in the histology from that of the control) and least observable effect concentration (LOEC - in which there are significant changes in the histological structure from that of the control) is determined. Concentrations which showed I values less than 10 (functionally normal) were taken as NOEC and concentrations that gave I values between 10 and 20 were taken as LOEC.

Based on these NOEC and LOEC values maximum allowable toxicant concentration (MATC- the concentration in which the animal can tolerate for survival and reproduction without much effect

on its metabolic activities) level is calculated using the formula

$$MATC = (NOEC \times LOEC)^{1/2}$$

From this MATC value application factor was calculated using the formula (Mount and Stephan, 1967)

$$AF = \frac{MATC}{48hr\ LC_{50}}$$

The application factor (AF), which is worked out for one species can be used for other related species also, provided their 48hr LC_{50} is known. From this the MATC for latter species can be conveniently found out. This will be an important tool for the farmers in deciding the concentrations of pesticides before its application into the paddy fields.

An evaluation of histopathological changes in the vital organs of fish exposed to different concentrations of pesticides would help in assessing the magnitude of pesticide induced pathogenesis in fish.

RESULTS

Physico-chemical parameters

Weekly mean temperature, pH and DO values ranged from 27.43 to 28.0 °C, 7.1 to 7.2 and 6.8 to 7.5 $mg.l^{-1}$ respectively.

Histopathological observations

Structure of the gill of Anabas testudineus

A. testudineus is characterized by small number of short gill filaments. The gill filaments are lined externally with the thick stratified primary epitheliumor the gill filament epithelium. It is a fresh water species, so the chloride cells, the characteristic of marine water species is few in number or absent. The secondary lamellae that are the actual sites of gaseous exchange are arranged on the two sides of a filament. This lamella is lined by a double-layered squamous cells epithelium. Internal to the epithelium is the lamellar blood sinuses, lined and spanned by pillar cells of contractile function. A marginal blood channel, lined by endothelium, occurs within the apex of the lamellae. The gills are characterised by equally spaced secondary lamellae, intact cellular layers and no sign of fusion between neighbouring lamellae (Fig. 1).

The details of the number of fishes examined under the monocrotophos exposure and damages noticed in each fish are summarised in the Table 1.

Control

Gills of control fishes in this experiment exhibited only minor alterations such as the hypertrophy and hyperplasia of the respiratory epithelial cells (Fig. 1A). The index value calculated is 1 (Table 1&2).

Concentration 2.0 ppm

Histology of the gills of fishes in this exposure did not vary much from that of the gill structure of control fish except for a few alterations. A few number of lamellae exhibited hypertrophy of the respiratory epithelial cells and certain others exhibited secondary lamellar hyperplasia. Oedematous separation of respiratory epithelium and separation of primary lamellae were also noted rarely (Fig. 1B). The index value calculated is 2 (Table 1&2).

Concentration 5.0 ppm

The changes consisted of hypertrophy and separation of respiratory epithelium in a number of lamellae. Most of the secondary lamellar epithelial cells exhibited hypertrophy, thinning and shortening of secondary lamellae (Fig. 1C). Oedematous separation of respiratory epithelium was frequently observed in two fishes (Fig. 1D). The index value calculated is 3.6 (Table 1 & 2).

Concentration 10.0 ppm

Hypertrophy (Fig. 1E), hyperplasia and fusion of secondary lamellae, oedematous separation of respiratory epithelium (Fig. 1F) were the common and conspicuous alterations that could be observed in this exposure. Fusion of adjacent secondary lamellae either to half of its length or to total length due to secondary lamellar hyperplasia was frequently observed (Fig. 1F). In certain lamellae the hyperplasia and distorted secondary lamellae together obliterated the respiratory surface (Fig. 1F). Telangiectasis (Fig. 1G), thinning of secondary lamellae (Fig. 1E) and shortening of secondary lamellae (Fig.1E) could also be noted in fishes exposed to this concentration. The index value calculated is 6 (Table 1&2).

Concentration 18.0 ppm

In this exposure oedematous separation of respiratory epithelium (Fig. 1H), and filamental blood vessel enlargement (Fig. 1I) were frequently observed in a number of fishes. Severe oedema completely separated the respiratory epithelium, except at its tip and base of the secondary lamellae

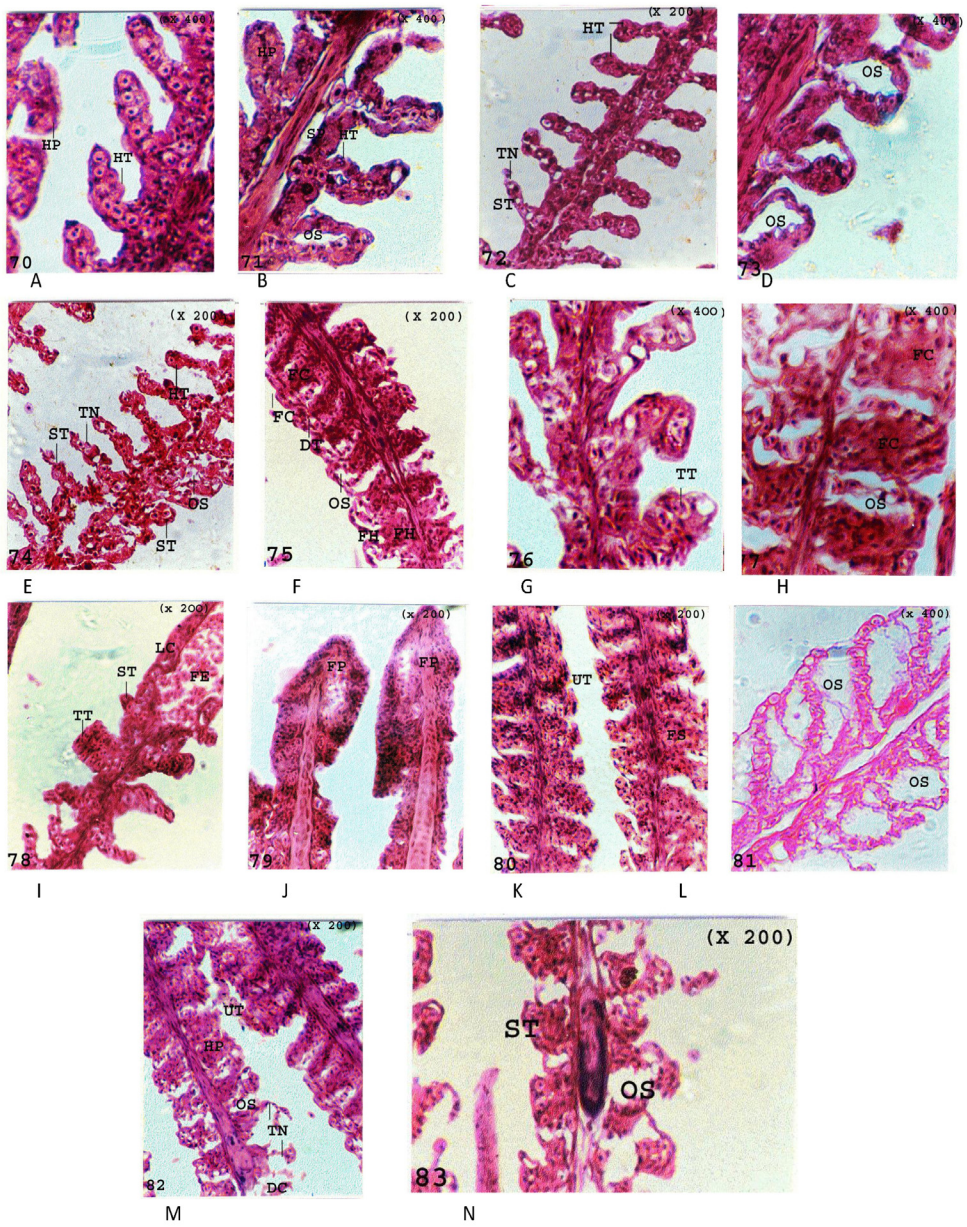

Fig.1 Gill of *A. testudineus* treated with Monocrotophos & Control group. (**A**) Control - hypertrophy (HT) and hyperplasia (HP). H + E x 400. (**B**) 2.0 ppm - hypertrophy (HT), oedematous separation of respiratory epithelium (OS) hyperplasia (HP) and separation of primary lamellae (SP). H + E x 400. (**C**) 5.0 ppm hypertrophy (HT), thinning of secondary lamellae (TN) and shortening of secondary lamellae (ST). H + E x 200. (5.0 ppm oedematous separation of respiratory epithelium (OS). H + E x 400. (**E**)10.0 ppm hypertrophy (HT), oedematous separation of respiratory epithelium (OS), shortening of secondary lamellae ((ST) and thinning of secondary lamellae (TN). H + E x 200. (**F**) 10.0 ppm - complete fusion of secondary lamellae (FC), oedematous separation of respiratory epithelium (OS), distortion of secondary lamellae (DT) and hyperplasia approximately half the length of secondary lamellae (FH). H + E x 200. (**G**) 10.0 ppm telangiectasis (TT). H + E x 400. **(H)** 18.0 ppm complete fusion of secondary lamellae (FC) and oedematous separation of respiratory epithelium (OS). H + E x 400. (**I**) 18.0 ppm - telangiectasis (TT), shortening of secondary lamellae (ST), complete loss of secondary lamellae (LC) and filamental blood vessel enlargement (FE). H + E x 200. (**J**) 18.0 ppm fusion of tip of primary lamellae (FP). H + E x 200. (**K**) 36.0 ppm - uncontrolled thickening (UT) and fusion of secondary lamellae (FS). H + E x 200. (**L**) 36.0 ppm - oedematous separation of respiratory epithelium (OS). H + E x 400. (**M**) 36.0 ppm - uncontrolled thickening (UT), hyperplasia (HP), oedematous separation of respiratory epithelium (OS), thinning of secondary lamellae (TN) and degeneration of epithelial cells (DC). H + E x 200. (**N**) 36.0 ppm - oedematous separation of respiratory epithelium (OS) and shortening of secondary lamellae (ST). H + E x 200

Table 1. The intensity of alterations in each *A. testudineus* treated with monocrotophos

Concentrations (ppm)	C					2.0ppm					5.0ppm					10.0ppm					18.0ppm					36.0ppm				
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	1	2	3	4	5
GILL ALTERATIONS																														
FIRST STAGE																														
1 Hypertrophy of respiratory epithelium	+	+	—	-	-	+	+	-	-	-	+	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
2 Irregular hyperplasia of epithelial cells	+	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-
																					+									
3 Lifting of respiratory epithelium/oedematous separation of respiratory epithelium		-	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	+	-	-	+	+	+	-	-	+	+	+	+	+
																+					+	+				+	+	+	+	+
																					+					+	+			
4 Thinning of respiratory epithelium		-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	+	-	-	-	-	+	-	-	-	+	+	+	+	+
																										+	+	+		
																										+				
5 Hyperplasia from the base to approximately half the length of secondary lamellae		-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	+	+	-	-	+	+	+	+	+
																+										+	+	+	+	+
																										+	+	+	+	
6 Fusion of tips of primary lamella	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+		-	-	-	-	-	-
																					+									
7 Fusion of several secondary lamellae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	+	+	+	+	+
																+										+	+	+		
																										+				
8 Shortening of secondary lamellae	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	+	-	-	+	+	+	+	-
											+					+	+				+	+				+	+			
																										+				
9 Lamellar telangiectasis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+		-	-	-	-	-	-	-	-
																					+									
10 Filamental blood vessel enlargement	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-		+	-	-	-	-	-	-	-	-	-
SECOND STAGE	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-
1 Rupture and peeling of lamellar epithelium																										+	+			
2 Uncontrolled thickening of proliferated tissue	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
																										+	+	+	+	
																										+	+			
3 Complete fusion of all the secondary lamellae	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	+	+	+	+	+
																										+	+	+	+	
																										+				

(+) - mild occurrence; (++) - Moderate occurrence; (+++) - severe occurrence; (-) - no occurrence

(Fig. 1H). Complete fusion of all the secondary lamellae was noted frequently in a few filaments (Fig. 1H). Most of the filaments exhibited the filamental blood vessel enlargement and in those regions the secondary lamellae were either shortened or completely absent (Fig. 1I). Telangiectasis (Fig. 1I) and fusion of the tip of primary lamellae (Fig. 1J) were also depicted in few fishes. The index value calculated is 13 (Table 1&2).

Concentration 36.0 ppm

The histological changes observed in the gills of all the fishes exposed to this highest sublethal concentration were severe hyperplasia (secondary lamellar hyperplasia) (Fig. 1K), fusion of several secondary lamellae and oedematous separation of respiratory epithelium (Fig. 1L).

Severe oedematous separation of respiratory epithelium (Fig. 1L) was frequently noticed. Uncontrolled thickening of secondary lamellar hyperplasia was conspicuous in most of the filaments (Fig. 1K). Secondary lamellar hyperplasia fused the secondary lamellae approximately half of its length or completely Rupture and peeling of lamellar epithelium caused thinning (Fig. 1M) and shortening (Fig. 1N) of secondary lamellae. The index value calculated is 33 (Table 1&2).

From the histopathological studies of the gills of *A. testudineus* exposed to different sublethal concentration of monocrotophos it could be observed that the pathological changes were gradually increasing from the lowest to highest concentrations. Among these concentrations, based on the index values no observable effect concentration (NOEC) is taken as 10.0 ppm (index value <10) and the least observable effect concentration (LOEC) is taken as 18.0 ppm (index value between 10 and 20) (Table 3). The maximum allowable toxicant concentration (MATC) is calculated as $(NOEC \times LOEC)^{1/2}$ is 13.4164 ppm and the application factor (AF) is calculated

$AF = MATC / 48hr\ LC_{50} = 13.4164 / 102.598 = 0.1308.$

The index value calculated for the highest sublethal concentration showed that it can well be placed in the category of moderately to heavily damaged gills. Hence it is evident that *A. testudineus* can be included in the category of fishes which is capable of regaining its original condition if better ecological conditions are provided to its habitat.

Table 2. The index values of the gill alterations (following Poleksic and Mitrovic-Tutundizic, 1994)

A. testudineus treated with monocrotophos	
Treatment(ppm)	Index value
0.0	1.0
2.0	2.0
5.0	3.6
10.0	6.0
18.0	13.0
36.0	33.0

Index values represents average of 5 fishes

DISCUSSION

Biomarkers demonstrate correlations between environmental factors and their outcomes, and they provide insight into both the status of an ecosystem and the status of a given organism (Yancheva *et al.*, 2015). Fish gills have a thin, extensive surface that directly contacts the water, which makes this organ an entry point for multiple environmental factors (Baskar, 2014, Poleksic and Mitrovic-Tutundzic, 1994). In the present study the pathological changes were obviously dose–dependent and maximum severity was noticed in the gills of fishes exposed to the highest sublethal concentration. The overall changes observed due to the exposure to different concentrations of monocrotophos are hypertrophy, hyperplasia, fusion of secondary lamellae, oedematous separation of respiratory epithelium, shortening and thinning of secondary lamellae, lamellar telangiectasis and aneurysm, filamental blood vessel enlargement,uncontrolled thickning and degeneration of lamellae. The observed pathological changes after monocrotophos treatment in the gills of *A. testudineus*are in agreement with reports on other fish species treated with different pollutants (Velmurugan *et al*., 2009; Cengiz and Unlu, 2003 and Sulekha and Mercy, 2021).

Table 3. Maximum allowable toxicant concentration and its corresponding application factor calculated based on the histopathological analysis of *A. testudineus* treated with monocrotophos

Fish species	Pesticides	NOEC (ppm)	LOEC (ppm)	MATC (ppm)	48 hr LC50 (ppm)	AF
A. testudineus	Monocrotophos	10.0	18.0	13.4164	102.598	0.1308

In agreement with Baskar (2014); Poleksic and Mitrovic-Tutundzic (1994) and Sulekha and Mercy (2021), hypertrophy, hyperplasia, oedematous separation of respiratory epithelium, and adhesions of the gill lamellae are defence mechanisms of fishes. These lesions result in an increased distance between the environment and the blood, and thus, they create an additional barrier for environmental factors.

Hypertrophy of the respiratory epithelium found in control and the lower concentrations manifested the possibility that they are the first stage of defense response of the fishas suggested by Morgan and Tovell (1973) and Sulekha and Mercy (2021).

The present investigation revealed mild to acute hyperplasia in agreement with the increasing concentration of pesticides. As the concentration increased, fusion of tips of primary lamellae and uncontrolled thickening and fusion of secondary lamellae were observed in 18 and 36ppm treated fishes. The proliferative changes can increase the water - blood distance and reduce the absorption of xenobiotics, but in turn, hinders the respiratory, secretory, and excretory functions of the gills as proposed by Baskar (2014) Poleksic and Mitrovic-Tutundzic (1994). According to Figueiredo Fernandes *et al.* (2007) cell proliferation with thickening of gill filament epithelium is a histological change, which may lead to the lamellar fusion. Hence, the emergence of fusion and additional significant thickening of the filamentous epithelium could have impact on the respiration and osmoregulation processes in the fish body.

The presence of edema along with the detachment of the lamellar epithelium is a sign of pathology in fish and one of the more frequent lesions observed in gill epithelium of fish exposed to different xenobiotics (Mallatt, 1985). Oedematous separation or lifting of respiratory epithelium from the basement membrane could be observed in *A. testudineus* exposed to all the concentrations of monocrotophos treatment in the current study. The separation of respiratory epithelium due to the lifting was very mild in the lower concentrations but the intensity and frequency was increased towards the exposure to higher concentrations. 36 ppm monocrotophos treated *A. testudineus* exhibited separation of epithelium from both sides of the secondary lamellae and it caused the complete obliteration of respiratory surface. It could serve as a defense mechanism as these alterations increased the distance across which waterborne irritants must diffuse to reach the blood stream (Mallatt, 1985 and Sulekha and Mercy, 2021).

The present study exhibited the blood vessel changes like lamellar telangiectasis or aneurism in *A. testudineus* treated withhigher concentrations of monocrotophos. In the first stage of exposure to pollutants, the marginal channels become dilated, and the blood begins to congest in the gill lamellae results telangiectasis; in the terminal stage, it destruct the pillar cells and develops aneurysms (Camargo and Martinez, 2007). Aneurysms are the most severe alteration in the fish venous system and they are the result of extended vasodilation with the collapse of pillar cells and the breakdown of vascular integrity (Cengiz, 2006). According to Poleksic and Mitrovic-Tutundzic (1994), circulatory changes may be reversible, but they are far more difficult to reverse than epithelial changes. As per the description of Mallatt (1985), vascular damage usually was found only in animals exposed to very high doses of pollutants or to animals that were near death. In the present study also telangiectasis and aneurysm was found in higher concentrations.

Thinning of secondary lamellae appeared in 5, 10, 18 and 36ppm monocrotophos. It was reported previously by Poleksic and Mitrovic-Tutundzic (1994). In the present study, this thinning may be caused by the degeneration of secondary lamellar epithelial cells. It may leads to the weakening of pillar cells and results distortion or curling of secondary lamellae, one of the frequently observed gill alterations, as described by Mallatt (1985) and Sulekha and Mercy (2021). Curling and fusion of the secondary lamellae were also noticed by Cengiz and Unlu (2006) and Velmurugan *et al*. (2007) in their sublethal pesticide toxicity studies.

Similarly shortening of secondary lamellae was observed in the present study except in control and the lowest concentration and the intensity was increased as the concentration increased. In the higher concentrations it has been completely lost. This might be due to the starvation of the fish as the food intake was less in the higher concentration as proposed by Wood and Yasutake (1957) Ramani (2001). In the present study, degeneration of the branchial epithelium observed at the highest concentration reveals the effect of pesticide even in the sublethal chronic exposure. Degenerative changes in the fish gill epithelium were also reported in the work of Butchiram *et al.* (2009) and Hasan *et al.* (2014) after heavy metal and pesticide exposure.

These results suggests that persistant low levels of these pollutants in the environment lead to high levels of bioaccumulation, and to adverse but sublethal effects in these organisms. In general, the gill histopathology appears to be a promising biomarker for general environmental contamination, although tissue preparation for gill histopathological study is time consuming (Oliveira Ribeiro *et al*., 2006).

In the present study, NOEC, LOEC and MATC values of monocrotophos in *A. testudineus* is calculated based on the histopathological studies on the gills. The intensity of pathology is quantified using the index values calculations suggested by Poleksic and Mitrovic-Tutundzic (1994). As stated by them, the index value of gills less than 10 are functionally normal and those between 10 - 20 are slightly to moderately damaged gills. Hence, the concentration which showed index value less than 10 are taken as NOEC and between 10-20 are taken as LOEC. In the present study, the NOEC and LOEC so obtained are given in table 3 and the MATC is thus calculated as 13.4164 ppm. The application factor (0.1308) derived for monocrotophos in *A. testudineus* from the laboratory studies can be directly applied to the field situations. This knowledge of fundamental toxicological and pathological processes is not only important for the regulators of chemicals that are potentially aquatic pollutants, but also for researchers involved in field studies. It also helps in the water quality monitoring and provides an early warning of the conditions of fishes.

Viana *et al.* (2013) suggest that the biomonitoring process should include analyses at different levels of biological organization, from sub-cellular and cellular analysis of tissues and organs, to the population and community levels. Therefore, studies using biomarkers are essential to complement environmental monitoring in order to control pollution effects on the animals that inhabit the water bodies (Camargo and Martinez, 2007).

CONCLUSION

1. Pathological changes were obviously dose–dependent and the maximum severity was noticed in the highest sublethal concentration.
2. The maximum allowable toxicant concentration (MATC) arrived at in the laboratory studies, based on the histopathology of gills, for the pesticides are very sensitive values.
3. The application factors derived from this sublethal study can be directly applied to the field situations.
4. Histopathology can be used as a tool in assessing the intensity of pesticides even at the sublethal levels.
5. Gill histopathology appears to be a promising biomarker for general contamination of aquatic environment.

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