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Sublethal Effects of Monocrotophos on Histological Architecture of Liver and Kidney of a Freshwater Fish, *Anabas testudineus*

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ABSTRACT

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. Pesticides usage in agricultural fields to control pests is extremely toxic to non-target organisms like fish and affects fish health through impairment of histological structure, sometimes leading to mortality. The present histopathological study reveals the potential adverse effects of monocrotophos, an organophosphate pesticide, on a fresh water fish, *Anabas testudineus* and it document a dose- dependent reaction of liver and kidney histology. The histological changes observed in liver included fatty-vacuolation and the displacement of nuclei to the periphery of the hepatocytes, congested and constricted liver sinusoids, condensed hepatocytes, destructed hepatocytes and pyknotic nuclei. The major histological alterations identified in the kidney were melano-macrophage centres, shrunken renal tubules and degenerated renal tubule. Calculation of organ index is used for comparing the severity of lesions in different organs. These organ indices are used for calculating total index. In the present study the total index showed the health status of fishes in each sublethal concentration "Calculation of organ index is used for comparing the severity of lesions in different organs. These organ indices are used for calculating total index. In the present study the total index showed the health status of fishes in each sublethal concentration and the status became worse in the higher sublethal concentrations. " and the status became worse in the higher sublethal concentrations. Histopathology can be used as a tool for assessing the sublethal conditions of water quality and it gives a "rapid early warning system".

Key words : *Histopathology, Liver, Kidney, Pesticide, Organ index*

Introduction

All pesticides are toxic but they are essential for maintaining or increasing agricultural production. According to WHO, about 76,000 people die each year in India from pesticide poisoning. Many of the deaths are suicides made easy by the wide availability of toxic pesticides. Kuttanad, the rice bowl of Kerala, is a region where there is overdose application of pesticide during the Punja cultivation periods. According to the data compiled byKuttanad 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

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alone (KWBSP, 1990). Punja crop, the traditional crop of Kuttanad, is sown in November to December and the harvesting takes place by the end of March. The punja period is invariably prolonged and may lasts up to May. The prolongation of the crop season has a direct bearing in the increase in pest problem. There is no systematic crop surveillance and therefore, farmers arbitrarily apply pesticides at regular intervals. These means of treatments are ineffective as well as wasteful and can cause severe damage to Kuttanad ecosystem. Monocrotophos, Henosan, Dimecron, Thymet, Fernoxan and Nuvacron are the major components of the pesticides being used in Kuttanad.

Other than targeted pests, pesticides affect a wide range of non-target organisms, such as invertebrates and fin fish inhabiting aquatic environment (Burkepile *et al*., 2000). Pesticide pollution severely affects aquatic organisms and, in turn, the entire food chain including human beings (Svensson, 1994). Hence water pollution can lead to different changes, ranging from biochemical alternations in single cell into changes in whole populations. In 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 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.

A number of studies revealing the changes in functions are initiated by changes in cellular level (Sulekha and Mercy, 2021a; Bilal, 2019; Cuevas, 2016; Tabassum, 2016). In the case of aquatic animals such as fish, pollution leads to morphological and cytological changes in the kidney and liver (Amin *et al*., 2013). The present study, conducted in the laboratory to assess the nature and extent of pesticide induced pathogenesis in the tissues of liver and kidney of *Anabas testudineus,* a true denizen of Kuttanad paddy feids, which is subjected to long term exposure to sub lethal concentrations of monocrotophos, a widely used pesticides in Kuttanad. According to WHO, swallowing 1,200 mg of monocrotophos can be fatal to humans. In 2009, it called for India to ban the product because of its extreme toxicity.

The liver and kidney are important for the maintenance of a stable internal environment with respect to water and salt excretion and partially for the metabolism of xenobiotics (Hinton, 1993). The histopathological changes give an early warning of the damage caused in the fish at the histological level before their mortality. Furthermore, the data generated could be useful in the environmental risk assessment of freshwater and marine organisms. Histopathology can be used as a tool for assessing the sublethal conditions of water quality and it gives a "rapid early warning system".

Materials and Methods

The study on the sublethal toxicity of monocrotophos on the juveniles of *Anabas testudineus* were conducted for a period of 30 days. 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 risk of hazards. There were provisions for water supply, lighting and adequate ventilation in the shed. The sublethal bioassay was done in cement cisterns of 35 l capacity. 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 l of water. Juveniles of *A. testudineus* were collected from pollution free ponds from the natural habitat. The average size of *A. testudineus* was 7.15 ± 0.6cm in total length and 7.50 ± 1.50g in weight was 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 LC50 value (102.59ppm) 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 pesticide used for each sublethal exposure were 2.0ppm, 5.0ppm, 10.0ppm, 18.0ppm and 36ppm. This sublethal exposure was done in a static system where water and pesticide medium were renewed every 24 hr to maintain the desired pesti-

cide 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 l of water in seasoned cement cisterns. The filtered fresh well water was aerated to saturation prior to use. 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. Temperature tested using thermometer with an accuracy of 0.1°C. 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.

Organ index based on histopathological conditions

In the present study, histopahtological conditions of different organs were assessed based on the method of Bernet (1999) who classified the histopathological changes of each organ into 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.

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

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.

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.

Table 1. Physico-chemical parameters in the experimental tanks during the exposure of *A.testudineus* to monocrotophos

Physico-chemical Parameters	Weeks			
	1	2	3	4
Temperature (°C)	28.0 ± 0.0	27.43 ± 0.49	27.71 ± 0.25	27.71 0.45
pH	7.16 ± 0.5	7.1 ± 0.7	7.2 ± 0.6	7.2 ± 0.6
Dissolved Oxygen $mg.l^{-1}$	6.9 ± 1.1	6.8 ± 1.1	7.07 ± 1.1	7.5 ± 0.7

Results

Physico-chemical parameters

Weekly mean temperature, pH and DO values were reported in Table 1.

Histopathology of Liver

Control: This liver consisted of parenchymatous, homogeneous, polygonal cells formed from double layers of liver cells separated from each other by capillary blood spaces called liver sinusoids. The hepatocytes were compact and carried centrally placed nucleus. This hepatocytes and the liver sinusoids were observed as intact (Fig. 1A). The organ index is zero (Table 2).

2.0 ppm: The structure of hepatic parenchyma was almost similar to that of control fish. The hepatocytes were compact and carried large roundish nuclei with regular outline and located approximately in the centre of the cells. A few hepatocytes of one fish exhibited slight constriction of liver sinusoids (Fig. 1B). Calculated organ index is 0.4 (Table 2).

5.0 ppm: The parenchymatous, polygonal hepatocytes in the liver in this exposure were similar to that of the hepatocytes of control fishes. Slightly congested liver sinusoids could be manifested in this exposure (Fig. 1C). Calculated organ index is 0.8(Table 2).

10.0 ppm*:* The hepatocytes in this concentration were also seen as more or less similar to that of con-

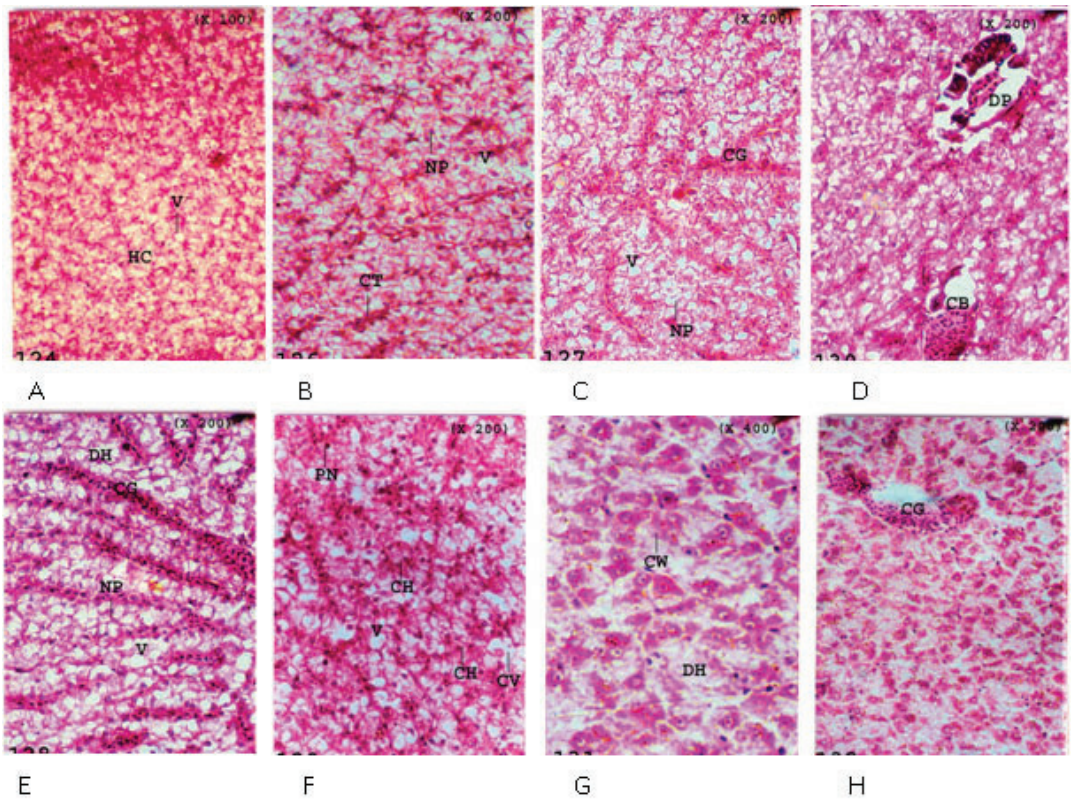

Fig. 1. Liver of *A. testudineus* **(A)** control - hepatocytes (HC), Vacuoles (V). H+E x 200. **(B)**treated with 2.0ppm monocrotophos - constriction of the liver sinusoids (CT), peripherally located nucleus (NP), vacuoles (V). H+Ex200.**(C)**treated with 5.0ppm monocrotophos - congestion of liver sinusoids (CG), peripherally located nucleus (NP), vacuoles (V). H+Ex200. **(D)**treated with 10 ppm monocrotophos - coagulated blood (CB) and destructed hepatocytes (DH). H+Ex200.**(E)**treated with 18 ppm monocrotophos - vacuoles (V), peripherally located nucleus (NP), congested liver sinusoids (CG) and coalesence of vacuoles (CV) and destructed hepatocytes (DH). H+Ex200. **(F)** treated with 36 ppm monocrotophos - vacuoles (V), coalescence of vacuoles (CV), condensed hepatocytes (CH) and pyknotic nuclei (PN). H+Ex200.**(G)**treated with 36ppm monocrotophos - destructed cell wall (CW) and destructed hepatocytes (DH). H+Ex400.**(H)**treated with 36ppm monocrotophos - coagulated blood cell (CB). H+Ex200.

Table 2. Organ index values of liver of *A. testudinues* exposed to monocrotophos (following Bernet *et al.*, 1999)

Concentrations	Control					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																														
WLCl=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 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). eg. WLC1 = 1 means importance factor = 1.

Table 3. Organ index values of 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 fishes	0					0					0					3.2					7.2					17.6				

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

trol fish. But the blood cells inside the liver sinusoids were slightly coagulated. Wall of the blood vessel was also seen damaged in one fish (Fig. 1D). Calculated organ index is 1.2 (Table 2).

18.0 ppm: The liver of fishes exposed to this concentration showed severe hepatocellular vacuolation. The vacuoles varied in size and shape. This extensive intra cellular vacuolization resulted in the displacement of the nucleus to the cell margin. As the number of vacuoles increased they were coalesced. Number of liver sinusoids could be seen as severely congested (Fig. 1E). Calculated organ index is 9.6 (Table 2).

36.0 ppm: In this highest sublethal concentration, the liver cells lost their normal architecture in some fishes. Most of the fishes exhibited vacuolated hepatocytes and at certain regions the vacuoles were coalesced. Pyknotic nuclei and condensed hepatocytes were noticed in a few fishes (Fig. 1F). The walls of hepatocytes were destructed completely in one fish but the nuclei were prominent. The degenerated or destructed hepatocytes were also noticed in few fishes (Fig. 1G). Coagulated blood cells in the liver sinusoids could also be observed in this exposure (Fig. 1H). Calculated organ index is 13.2 (Table 2).

Histopathology of Kidney

Control: The kidney of fishes maintained in the control consisted of renal corpuscles containing vascularised glomeruli, renal tubules and hematopoietic cells. All these structures

were seen as intact (Fig. 2A). The organ index value is zero (Table 3).

2.0 ppm: The glomeruli, renal tubules and the hematopoietic cells were similar to that the structure of the kidney of control fishes (Fig. 2B). No alterations could be observed. The organ index value is zero (Table 3). The organ index value is zero (Table 3).

5.0 ppm: The kidney structure of the fishes in this concentration also did not exhibit any pathological conditions. The glomeruli, renal tubules and the hematopoietic cells were normal in appearance. (Fig. 2C). The organ index value is zero (Table 3).

10.0 ppm: Shrinkage of renal tubules and its degeneration were observed in some fishes exposed to this concentration. (Fig. 2D). The glomeruli and the hematopoietic cells were normal in structure. The organ index value is 3.2 (Table 3).

18.0 ppm: The renal tubules of these fishes were affected by shrinkage. Melano-macrophage centres were also detected frequently in all the fishes (Fig. 2E). The organ index value is 7.2 (Table 3).

36.0 ppm: The renal tubules of kidney in this highest exposure exhibited severe shrinkage (Fig. 2F) and severe accumulation of melano-macrophage centre (Fig. 2G). The degeneration of renal tubules could also be observed in this exposure (Fig. 2G).The organ index value is 17.6 (Table 3).

Table 4. Total index of *A. testudineus* exposed to different sublethal concentrations of monocrotophos based on the organ index.

Treatment (ppm)	Organ Index		
	Liver	Kidney	Total Index
0.0	0	0	0.0
2.0	0.4	0	0.4
5.0	0.8	0	0.8
10.0	1.2	3.2	4.4
18.0	9.6	7.2	16.8
36.0	13.2	17.6	30.8

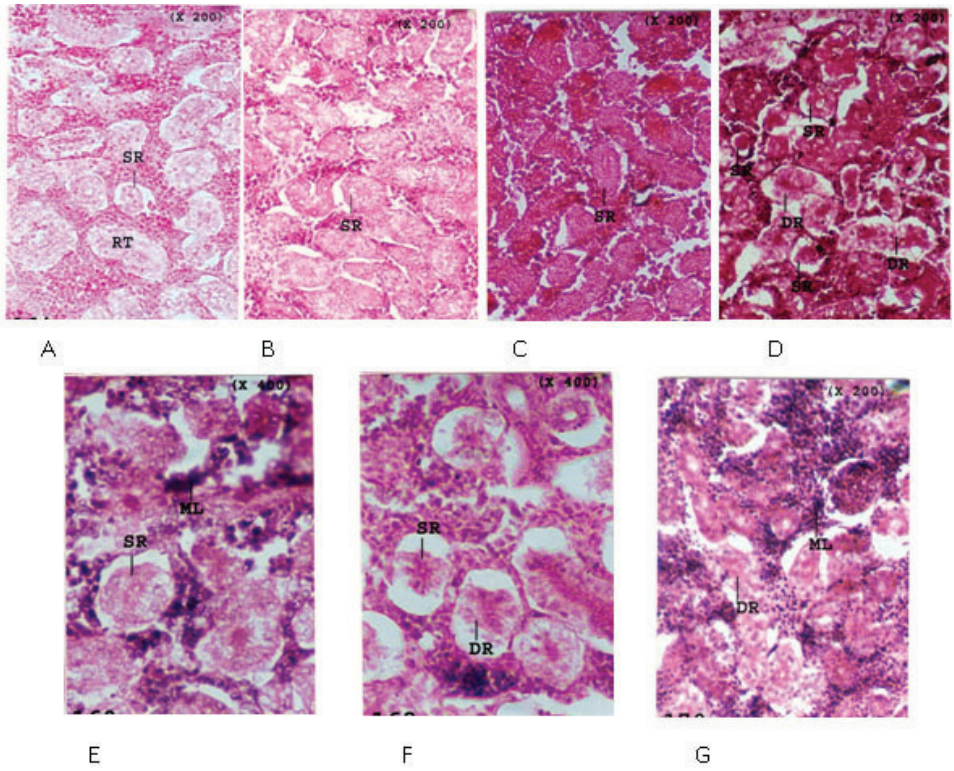

Fig. 2. Kidney of *A. testudineus*(A) control - renal tubules (RT) and slight shrinkage of renal tubules (SR). H + E x 200.**(B)**treated with 2.0ppm monocrotophos - renal tubules (RT). H + E x 200. **(C)**treated with 5.0ppm monocrotophos - shrunken renal tubules (SR). H + E x 200. **(D)**treated with 10.0ppm monocrotophos. - shrunken renal tubules (SR) and degenerated renal tubules (DR). H + E x 200.**(E)**treated with 18.0ppm monocrotophos - shrunken renal tubules (SR) and melano-macrophage centers (ML). H + E x 400.**(F)**treated with 36.0ppm monocrotophos - shrunken renal tubules (SR) and degenerated renal tubules (DR). H + E x 400. **(G)**treated with 36.0ppm monocrotophos - melano-macrophage centers (ML) and degenerated renal tubules (DR). H + E x 200.

The total index of control, 2, 5, 10,18 and 36ppm treated fishes were 0.0, 0.4, 0.8, 4.4, 16.8 and 30.8 respectively (Table 4). The organ index calculated based on various reaction patterns of the organs showed that liver is moderately affected and kidney is the mildly affected organ. The total index indicated the overall health status of the fishes in each concentration (Table 2 - 4). There was a gradual decrease in the health status of fish according to the increase in concentration of pesticides.

Discussion

In the present study, the histopatholgical effects produced by the pesticide, monocrotophos in the liver and kidney of *A. testudineus* document a dose-dependent reaction of liver and kidney histology. The pathological changes included fatty-vacuolation and the displacement of nuclei to the periphery of the hepatocytes, congested and constricted liver sinusoids, condensed hepatocytes, destructed cell membrane and pyknotic nuclei.

The liver of *A. testudineus* treated with 18.0 and 36.0ppm monocrotophos exhibited hepatocellular vacuolation. As the concentration increased, the size of the vacuoles also increased due to coalescence of small vacuoles. This extensive intra-cellular vacuolization resulted in the displacement of the nucleus to the cell margin. Most of the scientists considered this vacuolation as the accumulation of fat in the hepatocytes (Sulekha and Mercy, 2021b; 2022; Banik, 2016; Cengiz and Unlu, 2006). In the present study, the diet and other conditions were normal, the fishes were juveniles and therefore, the observed fatty livers must be considered as pathological due to pesticides. According to Biagianti-Risbourg and. Bastide (1995), accumulation of fat gave the fish modicum of protection from its toxic effects. Liver lipoid sequestration of synthetic molecules may therefore, be one of the more common and effective adaptive mechanism (i.e. non-genetic resistance) as defined by Wedemeyer and Goodyear (1984). However, accumulation and sequestration of contaminants can only be effective as long as the capacities of the organ involved are not overloaded (Moore, 1985). Such pathology indicated the transition to the third step of the stress process (exhaustion) as revealed by the increase in mortality rates. Similarly, a transition stage to third step of the stress was noted in higher concentrations of the present sublethal toxicity study.

In the present study, the condensed hepatocytes were noticeable in 36.0 ppm treated fishes. We could identify that the food intake was very less in higher concentrations and this starved condition might have resulted in the use of stored glycogen in the liver, which might have led to the shrinkage of liver cells. Such a condition was reported during starvation by Storch and Jucaria (1983), Segner and Moller (1984).

Constriction of liver sinusoids have shown in 2ppm treated fishes which might be due to the pesticide reaction in the wall of the blood vessel during the detoxification process of the pesticides (Sulekha and Mercy, 2021b; 2022; Kranz and Peters, 1985). Congested liver sinusoids were noticed in 5.0 and 18.0 ppm monocrotophos treated *A. testudineus*. The liver is a detoxifying organ and the congestion in the liver sinusoid might be due to the increased blood flow towards the liver for the detoxification process. Sahoo *et al*. (2001) suggested that the marked congestion in most of the organ during acute trials is clear indication of toxic effect. Naidu *et al*. (1983) and Peters *et al*. (1987) found the local blood congestion in the liver sinusoids in fishes from the Lower Elbe as a consequence of pollution.

Blood coagulation inside the liver sinusoids could be noticed in *A. testudineus* treated with 10.0 and 36.0 ppm monocrotophos. It might be due to the pesticide reaction during the detoxification process of liver. In the pollutant - induced hepato-pathology, a gradual increase in damages is noticed with larger duration of test, showing significant damages in hepatocytes and coagulation of blood in sinusoids (Sulekha and Mercy, 2021b; 2022; Sultan and Khan, 1983; Bhattacharya, 1985).

A. testudineus in 36.0 ppm monocrotophos showed a destruction of hepatocytes. This might be due to the adverse reaction of the pesticide on the hepatocytes. Since this lesion was found only in the higher concentrations, it might be assumed that it is a severe damage that occurs when the fish is exposed to higher concentrations. Such a structural impairment in liver leading to the destruction of hepatocytes was reported in *Puntius conchonius* (Kumar and Pant, 1981). Rupture of outer membrane of the liver was observed in *Oryzias melastigma* and *Colisa fasciatus* exposed to lindane (Verma, 1975) and *Channa punctatus* exposed to dimecron (Sastry and Malik, 1979).

As in the case of liver, lesions are also non-specific in kidney and hence it is not possible to at-

tribute the occurrence of a given type of lesion to a particular pollutant but an attempt is made here to discuss the pathological changes in relation to concentrations between chemical pollution and specific kidney conditions in the fishes under study. The alterations observed in the histology of kidney are the presence of melano-macrophage centres, shrunken glomerulus and renal tubules, degenerated renal tubules and tubular vacuolation.

Melano-macrophage centres have been considered as a potentially sensitive indication of fish health or of stressful environmental conditions (Sulekha and Mercy, 2021b; Bucke, 1991). In higher teleosts, macrophages are organised into discrete centres, which occur not only in the hemopoietic tissues but also in other sites. These centres are called melano-macrophage centres or macrophage centres or pigment modules or macrophage aggregates or macrophage accumulations. They have been recognized as an integral part of the fishes' reticulo-endothelial system (Agius, 1985).

Many studies have suggested that the general functions of these aggregates are the centralization of destruction, detoxification or recycling of endogenous and exogenous materials (Vogelbein *et al.,* 1987; Ferguson, 1976; Wolke, 1992). Thus the activity of macrophages is one of the first indicators for the presence of a stressor (Anderson, 1990).

In the present study, the melano-macrophage centres were observed in 36.0 ppm treated fishes and it is the clear indication of stressful environmental conditions due to pesticides, as reported by (Bucke, 1991; Agius, 1985; Wolke, 1985b; Blazer, 1987). There are data of changes in number and the capacity of melano-macrophage centres caused by environmental changes (Weeks and Warinner, 1986; Weeks, 1986; Secombes, 1991). Degenerated renal tubules were observed in *A. testudineus* treated with 36.0 ppm monocrotophos. This degeneration of renal tubules probably occurred because of the lack of oxygen as a result of acute anemia as suggested by Taveekijakran *et al.* (1996). It may be noted that in fish exposed to the above concentrations, the gill was also severely damaged. They may retard the oxygen intake through the gill lamellae and cause anaemia in the fish.

The shrinkage of renal tubules were noticed in higher concentrations of monocrotophos (10.0, 18.0 and 36.0; ppm) exhibited a dose related shrinkage in renal tubules. It may be due to the reaction of pesticides on the wall of the renal tubules or due to the osmotic imbalance and it gradually diminishes the excretory surface area. Later it causes the reduction of excretion and gradually leads to the death of the fish itself. Pandey *et al.* (1997) reported this condition as oedema.

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. In the present study the total index showed the health status of fishes in each sublethal concentration and the status became worse in the higher sublethal concentrations.

Conclusion

Liver and kidney exhibited a dose dependent degeneration in histology when it was exposed to various sublethal concentrations. Since the liver has a regenerating capacity, it is not much affected by the exposure to toxicant medium. If better environmental conditions are provided the liver can regain its original functions. But chronic exposure to the toxicant medium will definitely damage the organ. Based on the organ index value, kidney is less affected than liver. As the concentration of pesticides increased, the damages also increased in both liver and the kidney. Histopathology can be used as a tool for assessing the sublethal conditions of water quality and it gives a "rapid early warning system".

Conflict of Interest: 'The Authors declare that there is no conflict of interest'.

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