ORIGINAL RESEARCH PAPER
Volume - 11 | Issue - 08 | August - 2022 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

HISTOPATHOLOGICAL ALTERATIONS IN MAHASEER FISH EXPOSED TO SUBLETHAL AND ACUTE CONCENTRATIONS OF METHIOCARB

Histopathology

Dr. Brij Mohan Singh Associate Professor, Department of Zoology, S. K. Govt. Girls P.G. College, Sikar, Rajasthan India.

ABSTRACT

Methiocarb is an effective insecticide widely used in agriculture with potential ecotoxicological consequences. The average lethal dose (LD50) and the concentration (LC50) of methiocarb in mahaseer fish were determined. To determine the LD50, a total 50 fishes were assigned to 5 tanks (10 Fish/tank) including one control and 4 treatment group (200, 300, 400, 500 and 550 mg/kg). Fishes were treated with methiocarb for 96 hours. SLD50 was slightly toxic but LC50 was highly toxic to the mahaseer fishes. The degree of tissue change in vital organs from exposed mahaseer fish showed severe damage in gills including necrosis, congestion of blood vessels in gill filament and hyperplasia of epithelial cells. In the liver low number of necrotic hepatocytes and enlarged hepatic perisinusoidal areas containing eosinophilic material, vacuolar dystrophy and hypertrophy of hepatocytes were observed. Trunk kidney showed enlarged sinusoids within an apparently decreased amount of hematopoietic tissue. Some nephrons had occluded glomerular capillaries and separation of the renal tubular epithelium from the surrounding connective tissue. Necrosis was found in hematopoietic tissue, glomerular cells, and tubular cells. Glomeruli had eosinophilic exudation. Melanomacrophage centers (MMC) were scattered throughout the spleen of fish. Exudates and necrosis in the splenic white pulp were observed. This study showed that the rout of exposure to methiocarb not only affects its acute toxicity but also alteration in histopathology of mahaseer fish.

KEYWORDS

Methiocarb, Acute Toxicity, Mahaseer Fish

1. INTRODUCTION

Exposure of insecticide influences some important physiological functions of fish. It greatly affects fish production and human health too through ecological cycling and biological magnification. Accumulated insecticide acts as a prooxidant and enhances oxyradicals generation by reacting with oxygen. Mahseer is the edible fishes from the genus Barbus, and the carp family Cyprinidae. The mahseer fishes are widely found in freshwater rivers and lakes of North and south India. It is an important game and highly valued food fish in India[1][2]. It has thick scales and large powerful jaws. Mahaseer belongs to the bottom feed species as their lips adapted at the bottom for taking its food and they are growing fleshy. The fish can attain a maximum size of about 90 Kgs with a 2-meter length. Some signs and observations indicate to promising possibility of being used for aquaculture in polyculture ponds, since it is herbivorous and detritus feeder. According to [3] mahaseer is one of economically important species and the wild population of mahaseer has been decreasing over the years due to over exploitation, habitat perturbation and river pollution.

The majority of large rivers are also now impounded [4] and rivers are generally used to discharge high quantities of sewage and industrial waste [5]. These stressors have resulted in freshwater fishes being among the most threatened taxa. Approximately 7,588 species of freshwater fish assessed for the IUCN Red List, more than 20% are threatened, with 69 species already 'extinct' or 'extinct in the wild'[6]. [7], [8] and [9] found that mahaseer populations have been declining in most of their natural habitats due to the impacts caused by the industrialization, urbanization, and agricultural developments which are causing ecological alterations and physical changes in the natural environment in lakes and rivers of mid hills.

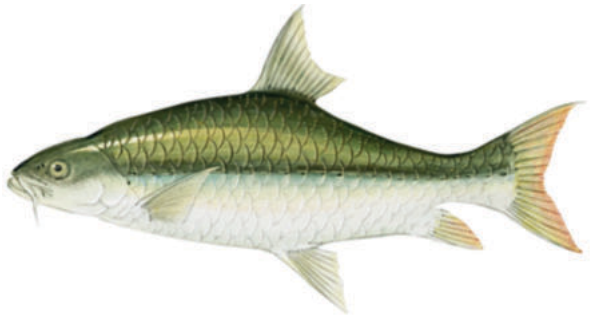


Fig 1: Mahaseer fish (Tor putitora)

Freshwater fishes are facing a challenge due to indiscriminate use of different types of pesticides in agriculture. Annual pesticide consumption has increased by 20 to 25 folds during over three decades in India. They are used to control a wide range of insect pest. Among pesticides, synthetic pyrethroids like methiocarb are broad spectrum photostable organic insecticide. They are used to control a wide range of insect pests. Methiocarb ($C_{11}H_{15}NO_2S$) is a carbamate pesticide (an acetyle cholinesterase inhibitor) which is used as a insecticide, bird repellent, acaricide and molluscicide since the 1960s. Methiocarb has contact and stomach action on mites and neurotoxic effects on molluscs. Seeds treated with methiocarb also adversely affect birds. Other names for methiocarb are mesurol and mercapto-dimethur. About 27 pesticides are to be banned by the Government of India in July, 2021. Due to its acute toxicity, methiocarb is one of the banned pesticides.

Methiocarb exposed fish had necrosis between molecular and granular layer of cerebellum where Purkinje cells present [10]. Other than targeted pests, insecticides can affect nontargeted species such as aquatic animals. In general, methiocarb is moderately toxic to aquatic organisms and the toxic dose depends on species [11]. The toxicity of carbamate substances results primarily from inhibition of acetylcholinesterase (AChE), a key enzyme of the nervous system [12]. The inhibition causes an accumulation of acetylcholine in synapses with disruption of the nerve function, which can result in death [13].

Histopathological studies have been conducted to help establish causal relationships between contaminant exposure and various biological responses. Histopathological investigations have proved to be a sensitive tool to detect direct effects of chemical compounds within target organs of fish in laboratory experiments[14]. This study was aimed to determine the histopathological effects of sublethal concentrations of methiocarb to Mahaseer gills, liver, spleen, brain, and kidney. The present study sought to determine the acute toxicity of commonly used pesticide methiocarb to Mahaseer fish using a static test system. As studies of the lethal times of methiocarb for Mahaseer in the literature are limited, so the objective of this study was to determine the acute toxicity of methiocarb concentration to Mahaseer fish.

Material and methods

(1) Experimental Animals

Mahaseer (Tor putitora) of 2.1±0.1g; 5.0±0.1 cm; Mean ± SD were obtained from the aquarium of department of zoology, Rajasthan university, Jaipur and held in 2 closed recirculating systems (200 L) for at least 15 days to acclimate to laboratory conditions prior to experiments. During the acclimation period, about 50% of the water in each recirculating system was replaced daily. Throughout the acclimation period and subsequent periods of methiocarb exposure, fish were held under a photoperiod of 12 hours of light and 12 hours of darkness. During the acclimation period, the fish were fed 4 times a day with fish food at 5% body weight and once a week with Daphnia sp.

(2) Experimental Design

Experiment was conducted as described by Altinok et al. (2006). After

acclimation, fish from one of the acclimation tanks were randomly transferred to one of 20 glass aquaria containing 25 L of stagnant water. To determine the LD50, a total fifty fishes were assigned to 5 tanks (10 Fish/tank) including one control and 4 treatment group (250, 350, 450, 550 and 600 mg/kg). Fishes were treated with methiocarb for 4 days. Quadruplicate random groups of 10 fish each were subjected to experimentation for 21 days in static fresh water containing 2.4 or 3.65 mg/L methiocarb. Doses selected were based on survivability responses assessed earlier [15][16].

Test solutions of methiocarb were prepared from commercial formulations containing 32.9% and 50% active ingredient (Bayer Crop Science), respectively. The pesticides were dissolved in 100 ml distilled water and then added in aquaria. Control tanks received 100 ml only distilled water. This study was conducted under OECD Guideline No. 203 for static-renewal test conditions (OECD, 1992). 65% of the test solution was renewed each day. During the toxicity experiment, water in each aquarium was aerated. At the end of the 21 days of sublethal toxicity tests, fish were transferred to flow through tanks to observe further effects of methiocarb for during 30 days. At the end of the 30 days of recovery, 20 fish were sampled for histology as described in the histopathology section.

(3) Water quality

The water used for acclimatization and conducting experiment was clear unchlorinated and unpolluted ground water and the hydrographic conditions of water shown in table 1.

Table 1: Characteristics of water used for experiment

SN	Parameter	Value
1	Temperature	15.20 ± 0.4°C
2	pH	7.46 ± 0.24
3	Dissolved oxygen (DO)	8.01± 0.30 mg/L
4	Total hardness as CaCO3	31.0 ± 1.9 mg/L
5	Alkalinity as CaCO3	18.2 ± 0.6 mg/L
6	Un-ionized ammonia	1.3 ± 0.2 mg/L
7	Nitrite	2.3 ± 0.8 µg/L
8	Nitrate	0.9 ± 0.3 mg/L
9	Bicarbonate	427 ± 12 mg/L
10	Total dissolved solids (TDS)	470 ± 20 mg/L

Characteristics of water in each treatment were measured periodically. Various physicochemical characteristics of water were estimated as per standard methods of [17] and [18]. Wrinkler method was used to measure DO in water. Water temperature and pH were determined by thermometer and pH meter respectively. Analysis kits were used to determine other water parameters.

Statistical analysis

Data analysis was done by using Medcalc statistical software. Acute and sublethal toxic effect of methiocarb on mahaseer fish was determined by the use of Finney`s probit analysis. A 95% confidence interval was calculated for the analysis.

Sigma plot ver. 11 software (State software, Inc., CA, USA) was used for other statistical analyses. The Mann-Whitney test was used in comparison of DTC results. The correlation coefficient analysis was done by using SPSS statistical tool. The significance level was set at $P<0.05$.

Histopathology

After 21 days of exposure, 5 fishes from the experimental and 5 fishes from control groups were anesthetized with overdose of MS222 and then the second gill arch, liver, spleen and trunk kidney were carefully removed and preserved in 10% neutral-buffered formalin (NBF) for 48 hours. The same organs were taken from rest of the fish after the 30-day recovery period. Organs were rinsed in 70% ethanol, and stored in 70% ETOH until further processing. They were dehydrated in isopropanol, cleared in xylene, infiltrated in paraffin, and sectioned at a thickness of 4 µm. Sections were stained with hematoxylin and eosin, and examined with a compound microscope.

RESULTS

No fish died during the acclimation period before methiocarb exposure, and three fish died during toxicity tests. Fish exposed to sublethal concentrations of methiocarb did not show any behavioral abnormality compared to control groups (Table 2).

Histological lesions were observed in gills, liver trunk kidney and spleen of fish exposed to Methiocarb.

Effect of methiocarb exposure on fish gills: The lesions observed in the gills of fish exposed to 0.6 or 1.5 µg/L Methiocarb concentration consisted primarily of epithelial lifting which is characterized by a filament cell proliferation (Figure 1A) and lamellar tangencies or clubbed gill filament lifting of the outer layer of the lamellar epithelium with the space under the epithelium filled with eosinophilic material (Figure 1B). Epithelial hyperplasia (5/10) was found with an increased number of epithelial cells at the distal or basal portions (Figure 1B).

Effect of methiocarb exposure on fish liver: The liver shows necrotic of hepatocytes and enlarged hepatic perisinusoidal areas containing eosinophilic material. Sinus dilation and general necrosis of hepatocytes were also observed (Figures 2 A, B, C and D).

Effect of methiocarb exposure on trunk kidney: The trunk kidney of fish exposed to methiocarb had enlarged sinusoids within an apparently decreased amount of hematopoietic tissue (Figure 3b) in comparison to control group (Figure A). Some nephrons had occluded glomerular capillaries and separation of the renal tubular epithelium from the surrounding connective tissue(Figure 3c). Necrosis was present in hematopoietic tissue, glomerular cells, and tubular cells. Glomeruli had eosinophilic exudates(Figures 3 A, B, C and D).

Effect of methiocarb exposure on fish spleen: Melanomacrophage centers (MMC) were scattered throughout spleen. Exudate and necrosis in the splenic white pulp were observed (Figure 4 A-F). Histopathological lesions explained above were not seen in control fish except for MMCs, which were less common and smaller than in the methiocarb exposed fish. A reduction in the number of melanomacrophage centers in the splenic tissue was also observed.

The fish that survived the 21-day methiocarb toxicity tests and were then transferred to other flow-through tanks for observations did not have any abnormal behavior. After histopathological examinations of survivors, fish had no lesions in gills, kidney, spleen, liver, or brain.

Table 2: Histopathological results in different organs of fish after pesticide exposure

Organs	Sublethal concentration of methiocarb	Acute toxicity of Methiocarb (LC50)
Gills	Mild histopathological changes	• Epithelial lifting and lamellar tengiensis • Epithelial hyperplasia found with increased number of epithelial cells at the distal and basal portion.
Liver	Mild histological lesions	• Necrotic of hepatocytes and enlarged hepatic perisinusoidal areas containing eosinophilic material. • Sinus dilation and general necrosis of hepatocytes were also observed.
Kidney	No histopathological changes	• The trunk kidney showed enlarged sinusoids within an apparently decreased amount of hematopoietic tissue. • Some nephrons had occluded glomerular capillaries and separation of the renal tubular epithelium from the surrounding connective tissue. • Necrosis was present in hematopoietic tissue, glomerular cells, and tubular cells. • Glomeruli had eosinophilic exudation
Spleen	Mild histological lesions	• Necrosis and exudates in the splenic white pulp were observed. • A reduction in the number of melanomacrophage centers in the splenic tissue was also observed.

DISCUSSION

Histopathology provides a rapid method to detect effects of irritants in

various organs [19]. The exposure of fish to chemical contaminants is likely to induce a number of lesions in different organs [20]. Gills [21], kidney [22] and liver [23] are suitable organs for histological examination in order to determine the effect of pollution. The exposure of aquatic organisms to very low levels or sublethal concentration of pesticides in their environment may result in various biochemical, physiological, and histological alterations in vital tissues.

Exposure to sublethal and acute concentration of malathion (0.8 and 1.6 mg/L) for 20 days Channa gachua showed degenerative, necrotic changes and congestion of blood vessels in gill filament and severe degenerations in secondary gill lamellae, separation of gill filament from basement membrane, proliferation of mucus cells and atrophy of gill filament [24].

Furthermore, lamellar fusion was only observed in fish exposed to 1.3 µg/L methiocarb. Increasing endosulfan concentrations from sublethal to acute, melanomacrophage centers were scattered throughout the trunk kidney, head kidney, and spleen[16]. Also increasing methiocarb concentrations caused telangiectasis and necrosis between the molecular and granular layers of the cerebellum where Purkinje cells are located [15]. However, correlation of endosulfan and methiocarb concentrations and histopathological changes were weak in fish exposed to sublethal concentrations of these insecticides. Similar findings were also observed by [25].[26]

found the same result when damselfish, Parma microlepis, was exposed to aldrin and dieldrin. Toxic substances can injure gills, thus reducing the oxygen consumption and disrupting the osmoregulatory function of aquatic organisms. Similar findings were also observed by [27].

The results of these studies clearly indicate that sublethal concentration of pesticide has adverse effects on fish gills. Gill lesions (edema with lifting of lamellar epithelium and hyperplasia of lamellar epithelium) suggested that endosulfan causes injury to the gills and increases respiratory diffusion distance [28]. In the present study, gills were found to be the most seriously affected organs compared to liver, spleen, trunk kidney, and brain, perhaps because of the direct contact with the compound.

The liver has the ability to degrade toxic compounds, but its regulating mechanisms can be overwhelmed by elevated concentrations of these compounds, and could subsequently result in structural damage [29]. In the present study methiocarb exposed fish had decreased amount of hematopoietic tissue in the trunk kidney, necrosis in hematopoietic tissue, glomerular cells, and tubular cells. Also the liver had necrosis and hypertrophy. Similar to our findings,[30] found that organochlorines had behavioral disturbances and hepatic and renal injury when fish were exposed to sublethal concentrations of endosulfan.

Similar observations have been made in the whitefish (Coregonus clupeaformis) exposed to nickel and in lake trout (Salvelinus namaycush) exposed to lindane [31]. Therefore the present study is justified with different concentrations of methiocarb test, in which no serious histological damage was found in gill, liver, kidney and spleen of mahaseer fish.

CONCLUSION

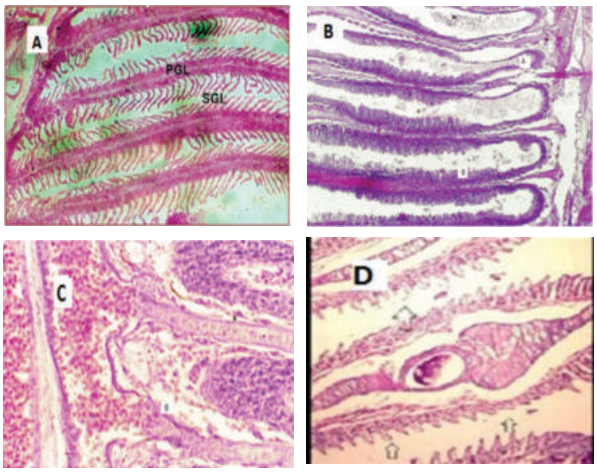
This study showed that methiocarb pesticide is very much detrimental to fish and other aquatic fauna. The exposure of this pesticide can cause serious damage in gill, liver, trunk kidney and spleen. After 30 days of recovery, there was no histological lesion observed in gill, liver, trunk kidney and spleen of fish. Therefore all the changes observed during the exposure were reversible.

Ethical standards: I declare that the experiments were conducted as per the guidelines of the institutional animals ethics committee.

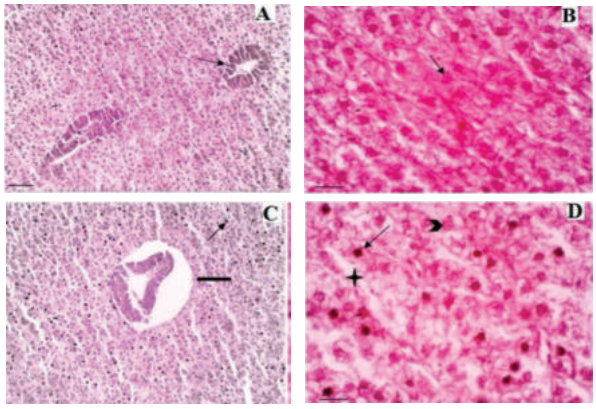
Acknowledgment

The author is highly indebted to the department of zoology and department of chemistry, S. K. Govt. Girls College, Sikar for providing facilities of hydrobiological experimentation. He is very grateful to the pathological laboratory of Saini Hospital, Sikar for Histopathological test of different organs of fish and providing facilities to take photographs. He is also thankful to all those who helped directly or indirectly in the experimentation of the research work.

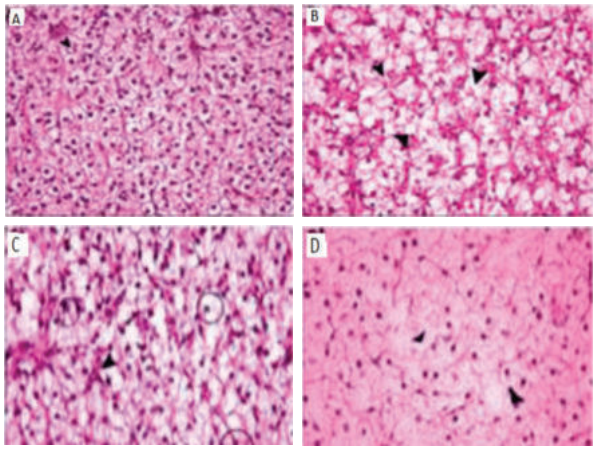
Photographs:



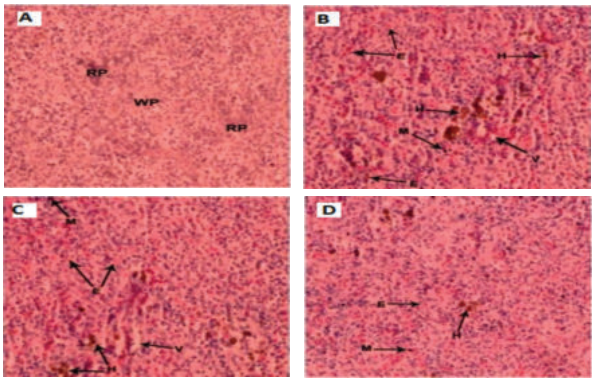
Photograph 1. Effect of methiocarb on gill lamellae of mahaseer fish
A: Gill lamellae of control fish PGL: Primary gill lamellae; SGL: Secondary gill lamellae.
B: Filament cell proliferation and Clubbed gill filament
C: Inner lamellar spaces filled with chloride cells (Mucous metaplasia) and epithelial hyperplasia.
D: Gill treated with methiocarb showing atrophy and necrosis.



Photograph 2. Histological alterations with methiocarb in the liver of mahaseer fish.
A. Normal liver
B. Normal nucleus in hepatocytes
C and D: Structural alterations in exocrine pancreatic duct (thick arrow), pyknosis (narrow arrow), normal nucleus (arrowhead) and sinusoid dilation (star).



Photograph 3. Trunk kidney of control and exposed Mahaseer fish
A. Normal hepatocytes of control group of mahaseer fish
B. Necrosis of hepatocytes (After 7 day exposure)
C. Sinus dilation and general necrosis of hepatocytes and
D. General necrosis of hepatocytes after 7 day exposure of 1.25 mg/l of methiocarb.



Photograph 4. Histological alterations with methiocarb in the Spleen of mahaseer fish.

A. Normal architechture, Red pulp (RP), White pulp (WP)
B. Necrotic eosinophils (E), Melanomacrophage (M), Vacuolation (V), Haemosiderin (H)
C. Necrotic and exudates eosinophil (E), Melanomacrophage (M), Haemosiderin (H), Vacuolation (V);
D. Necrotic and exudates eosinophil (E), Melanomacrophage (M) and Haemosiderin (H).

REFERENCES:

1. Shrestha, T. K., 1990. Behaviour of golden mahseer, T. putitora (Ham) in nature and captivity. J. Fresh w. Biol. 23, 209–2010.
2. Nautiyal, P., 1994. The endangered himalayan mahseer. a decade of retrospection. In: Dehadrai, P.V., Das, P., Verma, S.R. (Eds.), Threatened Fishes of India. Proceedings of The National Seminar on Endangered Fishes of India Held at National Bureau of Fish Genetic Resources, Allahabad, 25–26 April, 1992. NATCON Publication, Muzaffarnagar, pp. 191–196.
3. Ingram B, Sungan S, Gooley G, Sim SY, Tinggi D, De Silva SS (2005) Induced spawning, larval development and rearing of two indigenous Malaysian mahseer, Tor tambroides and T. douronensis. Aquac Res 36:983–995.
4. Poff NL, Schmidt JC (2016). How dams can go with the flow? Science 353: 1099–1100.
5. Keller VD, Williams RJ, Loft house C, Johnson AC (2014) Worldwide estimation of river concentrations of any chemical originating from sewage-treatment plants using dilution factors. Environ Toxicol Chem 33:447–452.
6. Darwall WR, Freyhof J. (2016). Lost fishes, who is counting? The extent of threat to freshwater fish biodiversity. In: Closs GP, Krkosek M, Olden JD (eds) Conservation of freshwater fishes. University Press, Cambridge, pp 1–36.
7. Das, P., Joshi, K.D., 1994. Mahseer conservation: present and future. Mahseer; thegame fish, D3-D9. Nautiyal, P., (Comp. and Ed.), Rachna Publisher, Garhwal, U.P., India.
8. Shrestha, T.K., 1994. Development of mahseer culture towards ranching. Nautiyal,P. (Comp. and Ed.), Mahseer; The Game Fish, D26-D41. Rachna, Srinagar, India.
9. Joshi, C.B., 1988. Mahseer fishery of some hill streams in western Himalayas. Indian J. Fish. 35, 327–329. IlhanAltinok
10. , Erol Capkin, Siyami Karahan, Muhammet Boran (2006). Effects of water quality and fish size on toxicity of methiocarb, a carbamate pesticide, to rainbow trout. Environ toxicol and pharmacol. Vol. 22 (1): 20-26.
11. Johnson, W.W., Finley, M.T.: Handbook of acute toxicity of chemicals to fish and aquatic invertebrates, US Department of the Interior Fish and Wildlife Services, Publication no: 137, 1980.
12. Singh, B.M. (2020) Endosufan mediated some physiological and biochemical changes in a fresh water fish, Int. J. of Life sciences, Vol. 8 (2): 317-326.
13. Tucker, J.W., Thompson, C.Q.: Dangers of using Organophosphorous pesticides and diesel oil in fish ponds. Aquacult. Mag., 1987; 13: 62-63.
14. Schwaiger, J, Fent, K, Stecher, H, Ferling, H, Negele, RD Effects of sublethal concentrations of triphenyl tinacetate on raibow trout (Oncorhynchus mykiss) Arch Environ Contam Toxicol 1996 3032734 Google Scholar | Crossref | ISI.
15. Altinok, I., Capkin, E., Karahan, S., Boran, M.: Effects of water quality and fish size on toxicity of methiocarb, a carbamate pesticide, to rainbow trout. Environ. Toxicol. Pharmacol., 2006; 22: 20-26. Google Scholar | Crossref | Medline | ISI.
16. Capkin, E, Altinok, I, Karahan, S (2006). Water quality and fish size affect toxicity of endosulfan, an organochlorine pesticide, to rainbow trout. Chemosphere 2006 6417931800. Google Scholar | Crossref | Medline | ISI.
17. APHA (American Public Health Association) 2005. Standard methods for the examination of water quality. Newyork, 21st Edition. 2005.
18. Trivedy RK and Goel PK (1986) Chemical and biological methods for water pollution studies. Published by Environmental Publication, Karad, Maharashtra (India).
19. Johnson, LL, Stehr, CM, Olson, OP, Myers, MS, Pierce, SM, Wigren, CA, Mc Cain, BB, Varanasi, U (1993). Chemical contaminants and hepatic lesions in winter flounder (Pleuronectes americanus) from the Northeast Coast of the United States Environ Sci Tech nol 1993 27275971 Google Scholar | Crossref | ISI.
20. Bucke, D, Vethaak, D, Lang, T, Mellergaard, S. (1996). Common Diseases and Parasites of Fish in the North Atlantic: Training Guide for Identification International Council for the Exploration of the Sea Techniques in Marine Environmental Sciences Copenhagen.
21. Poleksic, V, Mitrovic-Tutundzic, V Müller, R, Lloyd, R (1994) Fish gills as a monitor of sublethal and chronic effects of pollution Sublethal and Chronic Effects of Pollutants on Freshwater Fish Oxford, Fishing News Books.
22. Bucher, F, Hofer, R The effects of treated domestic sewage on three organs (gills, kidney, liver) of brown trout (Salmo trutta)Water Res199327255261 Google Scholar | Crossref | ISI.
23. ICES (1997) (International Council for the Exploration of the Sea) Special Meeting on the Use of Liver Pathology of Flatfish for Monitoring Biological Effects of Contaminants. International Council for the Exploration of the Sea Copenhagen Google Scholar.
24. Singh, B.M. (2021). Histopathological study of Channa gachua exposed to sublethal concentration of malathion. Int. J. of Fisheries and Aquatic Studies 2021; 9(3): 174-179. DOI: https://doi.org/10.22271/ fish.2021.v9.i3c.2472
25. Dalela, RC, Bhatnagar, MC, Tyagi, AK, Verma, SR (1979). Histological damage of gills in Channa gachua after acute and subacute exposure to endosulfan and rogor Mikroscopie 1979353017. Google Scholar | Medline
26. Tricklebank, KA (2001) Histological alterations in fish from Sydney reefs: possible biomarkers for environmental effects Biomarker 2001 62632 Google Scholar | Crossref | Medline | ISI.
27. Saravana Bhavan, P, Geraldine, P (2000). Histopathology of the hepatopancreas and gills of the prawn Macrobrachium malcolmsonii exposed to endosulfan. Aquat Toxicol 2000 50331339. Google Scholar | Crossref | Medline | ISI.
28. Nowak, B (1992). Histological changes in gills induced by residues of endosulfan Aquat Toxicol 1992 236583. Google Scholar | Crossref | ISI
29. Bruslé, J, Gonzàlez, I, Anadon, G Munshi, JSD, Dutta, HM The structure and function of fish liver, Fish Morphology 1996 New York Science Publishers Inc Google Scholar
30. Choudhary, N, Sharma, M, Verma, P, Joshi, SC (2003) Hepato and nephrotoxicity in rat exposed to endosulfanJ Env Bio 2003 243058. Google Scholar | Medline | ISI
31. Gill, TS, Pant, JC, Pant, J Gill, (1988) liver and kidney lesions associated with experimental exposures to carbaryl and dimethoate in the fish (Puntiusonchonius am.) Bull Environ Contam Toxicol 1988 41718. Google Scholar | Crossref | Medline | ISI