Impact of anthropogenic stressors with special reference to organochlorine pesticide pollution on *Mugil cephalus* L. in the areas of Kadinamkulam Estuary, South India

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Abstract
Estuaries are the important components of natural environment. To study the impact of anthropogenic stressors with special reference to organochlorine pesticides (OCPs) pollution, the fish Mugil cephalus Linnaeus, 1758 were collected in the summer season from the selected stations (coir retting and non- retting) of Kadinamkulam estuary, South India. The results of the study show decline in total proteins, glycogen and lipid content compared to that of the fishes in no retting station. The organochlorine pesticides residues (α-BHC, δ -BHC, DDT and DDE) are detected in the fish tissues like liver and muscles and the concentrations of OCPs are above the permissible limits of FDA (2001)[7].

Changes in the biomarkers of fishes in the study area may be due to the stress generated by the pollutants released from retting process and other anthropogenic activities near the Kadinamkulam estuary. The pesticides residues detected in fish tissue may be due to the bioaccumulation as large amount pesticides were used in the nearby agricultural fields that can enter the Kadinamkulam lake through runoff water and also from Vamanapuram river. Therefore, the study vividly points out that the fishes in Kadinamkulam estuary were subjected to pollution stress and contamination with organochlorine pesticides.

Keywords: Kadinamakulam estuary, *Mugil cephalus,* Biochemical changes, Organochlorine pesticides.

Introduction

Retting coconut husk in brackish water system is an age old traditional practice carried out for the extraction of coir fiber for which the husk is steeped in water for 6-12 months[4]. To facilitate this, Kerala has long stretches of backwaters and estuaries spread along the coast line from north to south. The total area of this ecosystem was estimated to be around 2,42,600 sq km[3]. Retting is brought about by the pectinolytic activity of micro-organisms, especially bacteria and fungi, liberating large quantities of organic substances like pectin, pentose, fat and tannin into the water. Markedly offensive odour resembling those of hydrogen sulphide is produced from retting zones during the decomposition of pectin[1].

Coconut husk retting is a common practice in most backwaters along the southwest coast of India and is a major source of organic pollution. This retting leads to depletion of dissolved oxygen and production of hydrogen sulphide, destroying the habitat of many species of aquatic organisms. High concentrations of hydrogen sulphide and prolonged periods of anoxia have been noticed in coconut husk retting areas which alter the chemistry and geology of these areas by their chemical composition[2,14].

Fishes are used extensively for environmental monitoring because they uptake contaminants directly from water and diet[13]. Generally the ability of fish to metabolize organochlorine is moderate; therefore, contaminant loading in fish is well reflective of the state of pollution in surrounding environments[8]. Previous studies conducted in the Kadinamkulam estuary showed the presence of Pb, Mn, Fe and Zn in the water and sediments which indicate the metal pollution in the lake[17].

The determination of OC pesticides and biochemical changes in fish may give indication of the extent of aquatic contamination and accumulation characteristics of these compounds in the tropical aquatic biota that will help in understanding the behaviour and fate of these persistent chemicals [10]. This work, therefore, seeks to provide baseline information on levels of pesticide residues in fish and the biochemical changes in *Mugil cephalus* in Kadinamkulam lake with respect to pollutants in the coir retting areas.

Material and Methods

The Kadinamkulam Kayal (Fig. 1) lying in the southern part of Kerala, South India (Lat $8^0$35' - $8^0$40'N; Long 76°44' - 76°51'E) is the largest of its kind in Thiruvananthapuram district connected with the Anchuthengu Kayal in the north and the Veli Kayal in the south. This temporary estuary has no direct connection with the Arabian Sea, but seasonally it becomes connected through the opening of the sand bar at Perumathura.

The fish *Mugil cephalus* Linnaeus, 1758 (commom name: Mullet) was collected from the six selected stations (retting and non-retting stations/control) of the Kadinamkulam estuary. Scientific classification of the fish is given in table 1. All fishes were collected from a single fisherman in order to assure regularity in fishing methods. The fishes with average length between 15 to 17 cm were collected for the study (Fig. 2). The morphological changes of fishes collected from different study stations were noted. All fish samples were immediately stored in an ice-chest at 4^0C and transported to the laboratory for analysis.

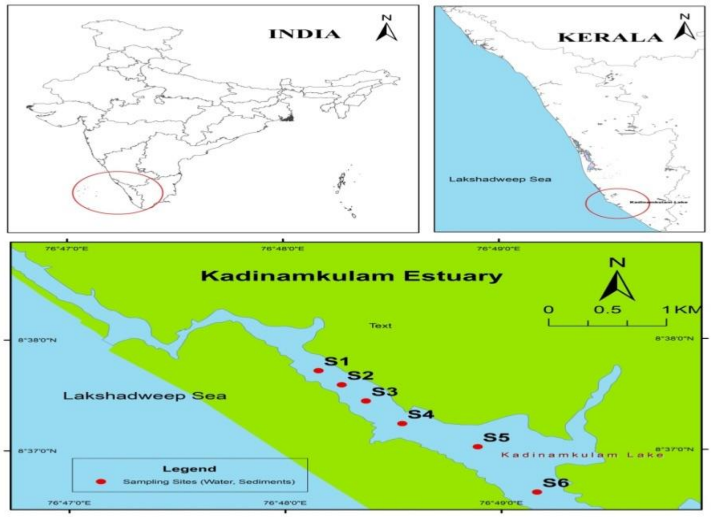

Fig. 1: Location map of the Study area

Fig. 2: *Mugil cephalus* Linnaeus, 1758

Table 1
Scientific Classification of *Mugil cephalus* Linnaeus, 1758

Kingdom	Animalia
Phylum	Chordata
Class	Actinopterygi
Order	Mugil forms
Family	Mugiolidae
Genus	Mugil

For biochemical analysis, the fish were dissected and the gill, liver and muscle tissues were collected in ice cold containers and stored in a freezer for further analysis. The organs were washed with saline to remove impurities. The biochemical parameters like total proteins, total lipids, glycogen and malondialdehyde (MDA) in the gills, liver and muscles were estimated following the standard procedures [9].

Quantitative analysis of organochlorine pesticides was conducted with a Gas chromatograph (Thermo - Trace GC 800) with ECD detector and nitrogen as the carrier gas. The muscle and liver tissue of the fish samples were ground in a blender to obtain a homogenous composite. Accelerated Solvent Extraction System (ASE-Thermo-150) was used for the extraction of organochlorine pesticides (OCP) from the muscle and liver using hexane acetone (9:1 v/v). This extract was poured into glass funnel containing 20 g of anhydrous sodium sulphate and then it is made up to 50 ml using the same solvent. 1µL of purified extract was injected into the injection port of a gas chromatograph with ECD detector. For the analysis, standard mixture of organochlorine pesticide was purchased from Sigma Aldrich, USA (CRM47426).

Results and Discussion

Changes in the biochemical parameters in the tissues of *Mugil cephalus* are given in the table 2. The results show that the protein content in different tissues is in the order: muscle>liver>gill. Fish from the control station showed highest protein content in their tissues. The protein content in muscle tissue ranged from 73.01 mg/gm to 287.24 mg/gm and in liver, it ranged from 35.97mg/g to 142.84 mg/g. In gills, the concentration of protein was highest (124.04mg/g) in fishes of control station and lowest (30.03 mg/g) in fishes collected from station 4. It was found that there is a decrease in protein content in the fishes collected from coir retting stations compared to that of the control station.

The muscles rich in proteins, form mechanical tissues intended for mobility and do not participate in metabolism. Liver being the centre for various metabolisms is also rich in protein. Compared to muscle tissue and liver, the gills showed reduction in proteins in all stations. The decrease in the protein content observed in the most of the fish tissues in retting areas may be due to metabolic utilization of protein for overcoming environmental stress by pollutants in the estuary.

The lipid content in liver ranges from 3.67 mg/gm to 4.94 mg/gm and in muscle, it ranges from 2.09 mg/gm to 2.98 mg/gm. Lipid content is highest in liver compared to that of muscle. Lipid plays a very vital role in physiological activities of the organism. The lipid reserves help the fish in the energy crisis during the periods of toxic stress. The decline in lipid content in fish sample from station 4 may be due to toxic stress by the organic pollutants in the estuary. Decrease in lipid level indicates that hydrolysis of lipids has taken place to derive energy for overcoming the toxic stress[16].

From the results, it is clear that the glycogen contents of liver, muscle and gills of *Mugil cephalus* are reduced in the coir retting stations S1, S2, S3, S4, S5 compared to that in the control station (S6). Glycogen content in fishes from all the stations was found highest in liver and lowest in gills. The glycogen content in liver ranges from 41.46 mg/g. f. wt to 75.81 mg/g. f. wt and in muscle it ranged from 37.42 mg/g f.wt to 59.72 mg/g f.wt. The glycogen content in gills of fishes from station 4 was found lowest (21.96mg/g wt.) and highest (47.58mg/g. f. wt) in control station fishes. The liver contains highest amount of glycogen than in muscles and gills. It is widely accepted that glycogen is an important macromolecule which comes first to rescue fish by providing energy from stress caused by any xenobiotic, since stress imposes an increased energy requirement to the animal[5,12].

In the fishes studied, liver tissue showed highest amount of glycogen and it may be due to the energy required to perform physiological activities and is mostly derived from carbohydrate metabolism. Immediately after intestinal absorption, carbohydrate, especially glucose is carried to the liver, converted and stored as glycogen, to be released for the maintenance of blood glucose level and for meeting the variable energy needs including stressful situations caused by toxicants. Decreased glycogen content in fish tissues expresses high environmental stress in coir retting stations or it may be due to the inhibition of hormone which contributes to glycogen synthesis.

The malondialdehyde (MDA) content (Table 2) in fish liver ranged from 0.219 µM/gm f. wt. (control station) to 4.461 µM/gm f. wt. (in station 4). MDA level was found highest in liver and lowest in muscle tissue. In gills, the malanodialdehyde content varied from 0.096 µM/gm f. wt. to 2.653 µM/gm f. wt. and the MDA content was higher than that in muscle and lower than in liver.

Table 2
Changes in the Biochemical Parameters of *Mugil cephalus*

Station	**Protein (mg/g f.wt)**			**Glycogen (mg/g f.wt)**			**MDA (µ Mol/g f.wt)**			**Lipid (mg/g f.wt)**	
	Liver	**Muscle**	**Gills**	**Liver**	**Muscle**	**Gills**	**Liver**	**Muscle**	**Gills**	**Liver**	**Muscle**
S1	49.73	94.17	30.03	51.34	37.42	28.76	2.653	0.173	1.695	3.70	2.17
S2	107.93	136.50	58.21	59.70	44.50	39.07	1.840	0.076	0.613	3.84	2.71
S3	110.05	217.05	70.29	57.44	40.19	34.03	2.538	0.134	1.53	3.74	2.46
S4	35.97	73.01	30.99	41.46	39.41	21.96	4.461	0.942	2.653	3.67	2.09
S5	110.2	270	94.07	69.02	51.62	47.27	0.942	0.057	0.596	4.28	2.83
S6 (Control)	142.84	287.24	124.04	75.81	59.72	47.58	0.219	0.031	0.096	4.94	2.98

Table 3
Organochlorine Pesticide content in Fish liver and muscle

Stations	**Organochlorine Pesticides (ppm)**							
	DDE		**DDT**		**δ -BHC**		**α- BHC**	
	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver
S1	0.093	BDL	BDL	1.00	BDL	BDL	0.596	BDL
S2	0.546	1.047	0.652	BDL	0.392	0.059	BDL	0.957
S3	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
S4	BDL	BDL	0.719	0.953	0.347	0.257	BDL	BDL
S5	BDL	BDL	BDL	BDL	BDL	0.659	0.103	0.539
S6 (Control)	0.489	1.287	0.974	0.987	BDL	BDL	BDL	0.737

BDL-Below Detectable Limit

The increasing amount of the lipid peroxidation product, MDA indicates the oxidative stress in fish due to the pollutants in the estuary. Due to retting and other anthropogenic activities in the surrounding areas of the estuary, the organic and inorganic pollutants are detected in the estuary water and sediments which degrade the water quality and generate stress in aquatic organisms[17].

The organochlorine content in fish liver and tissue is given in table 3. In the present study, four types of organochlorine pesticide content were detected in muscle tissue and liver (α-BHC, δ -BHC, DDT, DDE). In muscle tissue of fishes from station number 6 (Perumathura), high concentration of DDT (0.974 ppm) was detected. In liver of fishes from station number 6 (Perumathura), high concentration (1.287ppm) of DDE was found. In muscle and liver, the concentrations of pesticides content estimated are above the permissible limits of Food and Drug Administration[7]. Studies reported that only 5% of the sprayed chemicals actually reach the targeted organisms, with the remaining 95% drifting to surrounding areas and eventually becoming an environmental contaminant[15]. It affects the fish directly by being accumulated in the organs of the body. Also it causes serious impairment in metabolic, physiological and structural systems.

Sources of pesticide pollution of estuary water include run-off from the agricultural land, direct discharges of sewage waste water, or direct application as a consequence of treatments for pest control in plantations and paddy fields. Aerially applied pesticides may also be carried by air currents, circulated through the lower troposphere and later deposited by rainfall in distant places[18]. Similar studies were conducted to determine the amount of organochlorine pesticides in freshwater fishes of Punjab showing the predominance of DDT, while other organochlorine pesticides such as lindane, aldrin, dieldrin, chlordane, endosulfan and heptachlor were found at lower levels [11].

Generally, the ability of fish to metabolize organochlorines is moderate; therefore, contaminant loading in fish is reflective of the state of pollution in the surrounding environment[8]. Many of these organochlorine pesticides and their metabolites have also been implicated in a wide range of adverse human and environmental effects including reproduction and birth defects[6].

Conclusion

The present study was conducted to assess the changes in the biochemical constituents of *Mugil cephalus* in the retting areas and non-retting areas of Kadinamkulam estuary. Run off from the land, sewage and coir retting residues were the main source of pollution in the Kadinamkulam estuary. The total proteins, glycogen, total lipids in fish tissues from retting zone show decline in their content than in the fishes from the non retting area (control station). It may due to the increasing metabolic activity of tissues to overcome environmental stress. The continued and intense exploitation of the estuary for retting coconut have a deleterious effect on water quality and lead to adverse changes on biochemical constituents in fishes.

The present study in Kadinamkulam estuary reveals that the fishes are adversely affected by organochlorine pesticides like DDT, DDE, δ–BHC and α- BHC. And these organochlorine pesticides are above the permissible limit of FDA. The biochemical constituents such as total proteins, glycogen, total lipids etc. are decreased in tissues because these energy providing macromolecules help in rescue of the fish from entowering stress caused by xenobiotics. The lipid peroxidation product, malondialdehyde content in the tissue of fishes especially in liver in retting areas was increased due to oxidative stress generated by the organic pollutants.

Malondialdehyde results from lipid peroxidation of polyunsaturated fatty acid and it is a marker of oxidative stress. It may due to overcoming the stress generated by the organic pollutants. Organic pollutants and pesticides have been recognized as serious pollution of aquatic environment. They affect fish directly by being accumulated in their body. They also cause serious impairment in metabolic, physiological and structural systems. The accumulation of pesticides in the tissues of a fish can result in chronic illness and can cause potential damage of population.

This study vividly points out that the coir retting activity causes changes in biochemical constituents of fishes and the *Mugil cephalus* are able to accumulate and retain pesticides from their environment. It is suggested to establish monitoring systems by relevant authorities on handling and disposal of pesticides and pesticide containers for minimizing contamination of surface waters. Determination of levels of organochlorine residues and other pesticides in fish, soil and foodstuff should be carried out regularly in order to evaluate the possible risk to human health. Controlling the release of agriculture effluents into surface water bodies and following proper methods for closed retting can minimize the risk of contamination of estuary and fishes by pollutants.

Acknowledgement

The authors sincerely thank the Registrar, University of Kerala for providing the laboratory and library facilities for conducting this study. We also acknowledge the instrumental facility provided by the Director, CFRD, Konni, Kerala for the determination of pesticide residues in samples.

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(Received 28th August 2025, accepted 07th November 2025)