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Tracing the effect of pretilachlor on *Channa punctatus* (Bloch)

Angshuman Biswas

Department of Zoology, Sreegopal Banerjee College, Magra, Hooghly, West Bengal. PIN -712148

ABSTRACT:

Pesticides usage is increasing worldwide with the increase in human population. These pesticides are mostly with long half life for better efficiency. Most of the pesticides are drained to the nearby water-bodies by run off or leaching etc. After reaching aquatic system these are accumulated within the aquatic organisms. Bioaccumulation in fish is very common and ultimately threatening to the associated food web especially to the fish eating human community. *Channa punctatus* is a common fish prevalent in different water bodies throughout various parts of India. In this study attempts have been made to understand the possible effect of a common herbicide pretilachlor on the physicochemical characteristics of treated water as well as on the various physiological and anatomical characteristics of *Channa punctatus*. Various alterations have been noted in these parameters which are predominantly related to the pesticide itself. Furthermore data generated through this study could be useful to assess environment risk in terms of pesticide pollution in aquatic system.

KEY WORDS: Pretilachlor, aquatic pollution, *Channa punctatus*, bioindicator, histopathology, haematology, physiology

***Corresponding author**

Angshuman Biswas

Department of Zoology,

Sreegopal Banerjee College, Magra,

Hooghly, West Bengal.

PIN -712148

INTRODUCTION

Since time immemorial earth has been experiencing threats from different kind of pollution. After modern man came into existence and subsequently with the advent of civilization human himself almost singly has caused every kind of insults to the earth. As any deterioration in the character and quality of the environment including air, water or soil, is directly related to the existence of man, therefore, assessment of environmental parameters is not only essential to monitor the community health but also required for management of toxic contaminants. Aquatic pollution has become one of the most frequently discussed topics of recent times because this sort of pollution not only affects the aquatic organisms but also ultimately causes harm to the human community as well. With the ever increasing human population, rapid growth of industrialisation and modern day cultivation, pollutants like heavy metals, detergents and pesticides etc are continuously being thrown out to the water-bodies.

Most of the agriculture based countries, including India are using different kinds of pesticides to increase the production of food, flowers, decorating plants etc. Pesticides are chemical which can kill the pests of a particular organism. The term pesticide is a broad spectrum term which may denote specificallytoall herbicide, insecticides,nematicide, molluscicide, piscicide, avicide, rodenticide, bact ericide, insect repellent,

animal repellent, antimicrobial, fungicide, disinfectant and sanitizer. The most common of these are herbicides which account for approximately 80% of all pesticide use. Pesticides have got detrimental effect on exposed organisms. The major xenobiotic contaminants found in the food chain of different ecosystems are definitely pesticides. In modern agricultural practice use of pesticide is necessary because most of the genetically engineered or hybrid plants are not as resistant to their natural pests as their wild counterparts.

Pretilachlor is a chemical pesticide which is now days widely being used worldwide except in some first world countries. Pretilachlor belongs to the chloroactamide class of herbicides, which inhibits growth and reduces cell division. It is especially designed for early season weed control in wet-sown rice and rice nursery beds. It is a selective pre-emergence broad spectrum herbicide with excellent action against annual grasses and sedges and broadlarved weeds. As this herbicide is used for wet sown rice, therefore, readily goes to the adjacent waterbodies and directly comes in the contact of the aquatic organisms. Lata, *Channa punctatus* (Bloch) is a fresh water fish, which is found in most part of the West Bengal and widely consumed for it's high nutritive value. In the present study aim was to find out the effect of Ptretilachlor on *Channa punctatus* (Bloch) by assessing some physico-chemical parameters of the water concerned along with the evaluation of some of the physiological parameters of the fish itself after exposure.

EXPERIMANTAL SECTION:

Animals and Treatment: *Channa* sp. weighing approximately 70-80 gm were purchased from the local fishermen and were kept in laboratory conditions within aquariums of 15 L capacity. A temperature of 25° C with eight hours of daylight was maintained for two weeks without any artificial aeration. Fish were fed on *Tubifex tubifex* provided *ad libitum* throughout the experimental period. Those fishes were divided into groups each containing five fish. One group was kept as control. 50 % EC Pretilachlor was dissolved in the rest of the aquariums at concentrations of 1.25 ml/L .25 ml/L, 0.5 ml/L, 0.75ml/L, 1 ml/L, 1.25/L and 1.5 ml/l. Those were kept under such conditions for seven days. After LC_{50} determination fish were kept at 0.125ml/L concentration and all subsequent study were done with the fish keeping at this particular concentration of pesticide and later on sacrificed after 120 h.

A. Determination of water parameters: Various water quality parameters were monitored both from the control and the aquaria containing 0.125ml/L concentration which was found to be suitable for survival of the fish, at least for the experimental period.

1. **Determination of dissolved oxygen concentration:** Estimation of dissolved Oxygen was done by Winkler's Method (Winkler 1888).
2. **Determination of free Cabon dioxide:** Free carbon dioxide was measured by titrimetric method with 0.05 N Sodium Hydroxide using phenolpthalein.
3. **pH:** pH was determined by water and soil analysis kit 172 of ESICO.
4. **Temperature:** temperature determined by water and soil analysis kit 172 of ESICO.

B. Estimation of Haemoglobin (Hb%): Haemoglobin percent was estimated by cyanomethhaemoglobin method with colorimeter.

C. Total count of Erythrocytes and Leucocytes: Total RBC count was done with Thoma-Zeiss haemocytometer following standard protocol followed by manual counting under microscope.

D. Determination of plasma glucose: Glucose was measured by GODPOD method with semi auto analyzer Transasia ERBA50 (Trinder 1969).

E. Determination of plasma glucose-6-phosphate dehydrogenase: Plasma glucose-6-phosphate dehydrogenase level was measured by kinetic method (Slein 1965) with semi auto analyzer Transasia ERBA50.

F. Collection of tissue and processing for histology: For histological experiments liver, kidney and gills were fixed in 10% neutral formalin, after that dehydrated and embedded in paraffin before cutting tissue sections of 6μ thickness. Sections were spreaded on slides and stained following standard histological procedure.

G. Regeneration study on fin: Regeneration study was done by equally cutting the caudal fin of the control and treated fin of same size with scissors. In this study 1cm long portion of the fin was cut from both the type of fish.

RESULTS AND DISCUSSION

A. Determination of water quality parameters:

Water quality was estimated after four hours of pesticide administration. pH was found to be a bit alkaline in pesticide treated water. Dissolved O_2 got drastically lowered in pesticide treated water. It was found to be 7.2±0.49 mg/L and 3.75±0.56 mg/L in control and treated water respectively. In reverse free CO_2 was found to be significantly more in pesticide treated water (12.75±1.60 mg/L) compared to the control (3.55±0.36 mg/L).

S No	Parameters	Control	Treated
1	pH	7.31±0.04	7.54±0.04
2	Dissolved O_2 (mg/L)	7.2±0.49	3.75±0.56
3	Free CO_2 (mg/L)	3.55±0.36	12.75±1.60

Table 1: water quality parameters in control and pesticide treated water. Result expressed in Mean ± SD, n=10

B. Estimation of Haemoglobin (Hb%):

A significant decrease in the plasma haemoglobin content in the pesticide treated fish was noted (Fig 1.). In pesticide treated fish Hb% was found to be 6.35 ± 1.37 which was significantly lower than that of the control fish (8.7 ± 0.54).

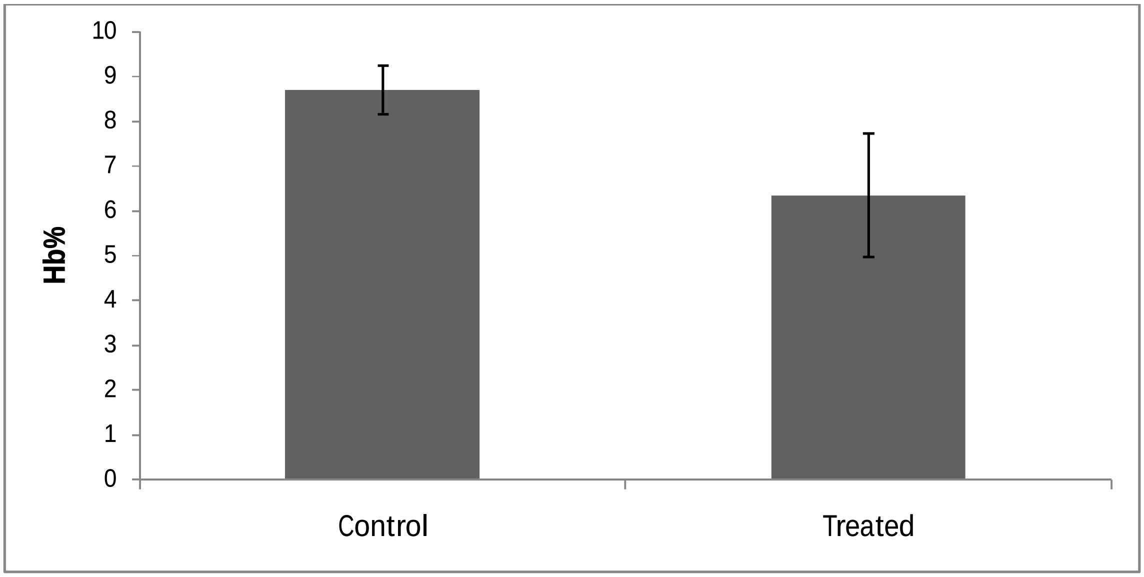

Fig 1. Hemoglobin (Hb%) Content of Normal and Pretilachlor treated Channa sp. Result expressed in Mean ± SD, n=10

C. Total count of Erythrocytes and Leucocytes:

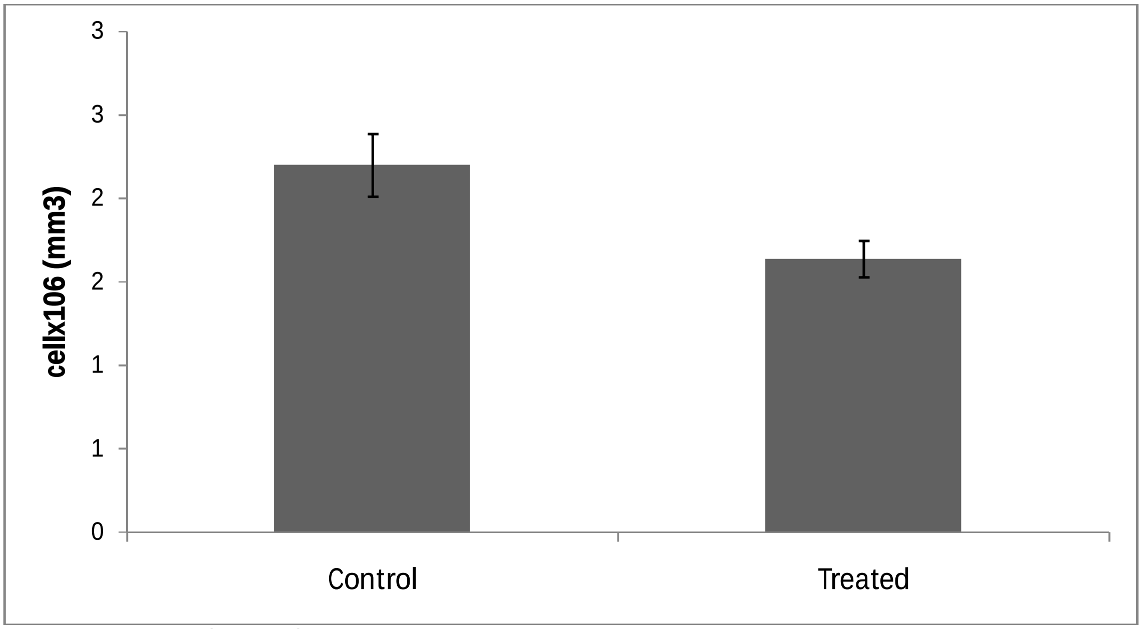

Fig 2. Total Count of RBC of Normal and Pretilachlor treated *Channa* sp. Values are expressed in Mean±SD (n=10)

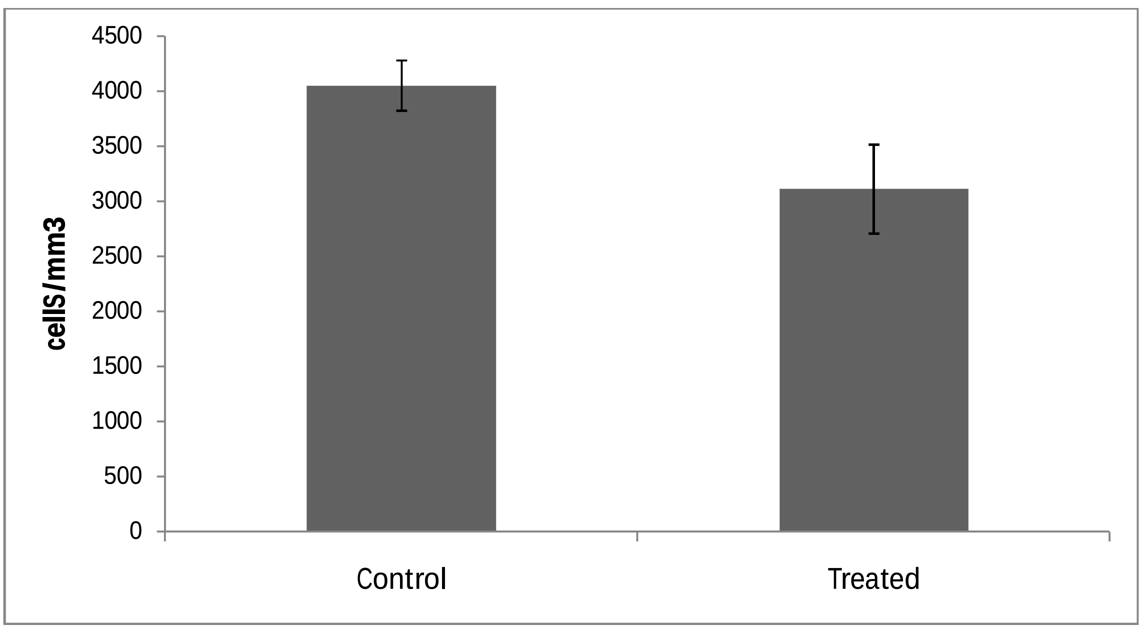

Fig 3. Total Count of WBC of Normal and Pretilachlor treated *Channa* sp. Values are expressed in Mean ± SD (n=10)

Significant decrease was noted in both RBC and WBC counts of the fish kept in pesticide treated water. Normal RBC count was 2.2 ± 0.19 x 10^6/ mm^3 of blood and that of the treated fish was 1.64 ± 0.11 x 10^6/ mm^3 of blood where as WBC count dropped from normal 4050 ± 229 to 3112 ± 403 in the treated.

D. Determination of plasma glucose level:

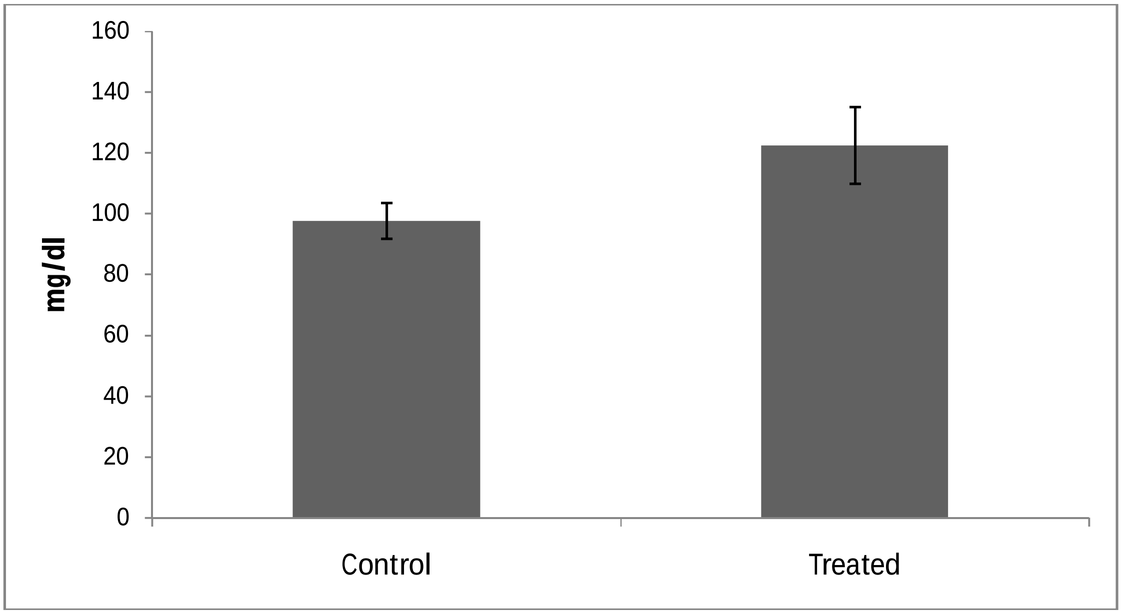

Fig 4. Glucose Level of Normal and Pretilachlor treated *Channa* sp. Values are expressed in Mean ± SD (n=10)

In contrary to other results an increase in the plasma glucose level was noted in the pesticide exposed fish (Fig 4). In control group of fish the plasma glucose level was 97 ± 5.93 mg/dL , whereas in the fish kept in pesticide the level rose to 122.5 ± 12.62 mg/dL.

E. Determination of plasma glucose-6-phosphate dehydrogenase:

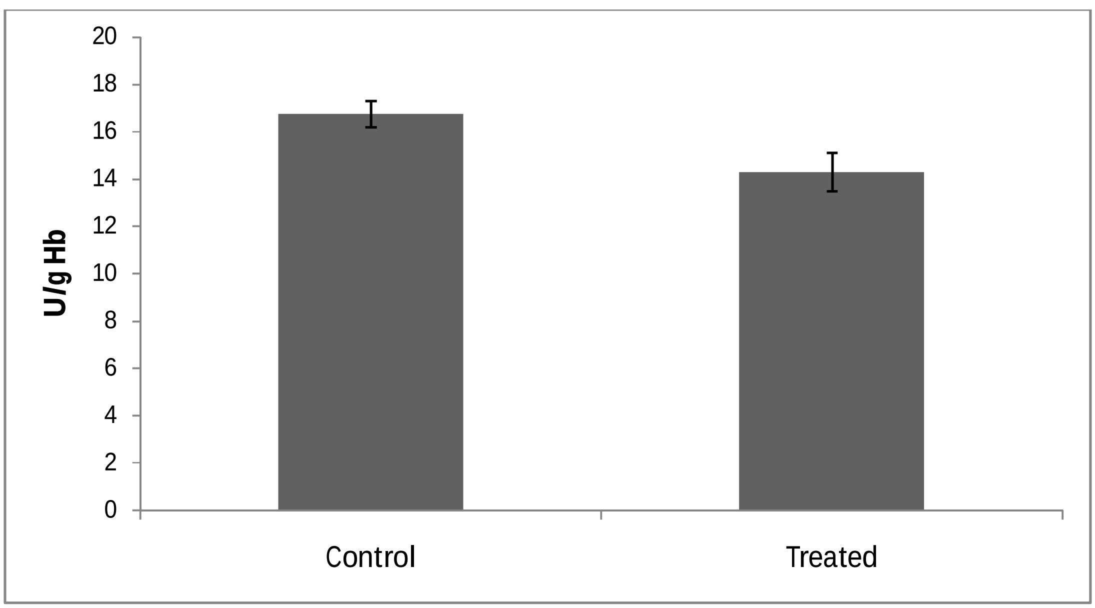

Fig 5. G6PD Level of Normal and Pretilachlor treated *Channa* sp. Values are expressed in Mean ± SD (n=10)

Plasma glucose 6 phosphate dehydrogenase (G-6-PD) level had a significant decrease from normal fish (16.75 ± 0.56 µg/g Hb) to pesticide exposed fish (14.3 ± 0.80 µg/g Hb).

F. Histopathological Study:

Effect of pollution on an organism can directly be assessed by histological study. Histological alterations in fish tissues and organs are direct indications of effects substance under question. Target organs of the agent can be identified by examining the histology. Therefore, histopathology serves as a very important indicator in environment- monitoring. Gills, kidney, and liver carry out vital functions such as respiration, excretion and biotransformation of xenobiotics in the fish respectively. These organs are often found to be the targets of different xenobiotics including pesticides.

a. Histological profile of liver

Liver is the primary organ where biotransformation of different foreign substances takes place. For this reason naturally this organ becomes the primary target of that particular substance as well. Therefore, any alteration in the structure of the liver is the direct reflection of the harmful effect of that particular compound. In this study, histology of the liver of normal fish revealed normal lattice like architecture of parenchymatous cells and sinusoids with a spoke like arrangement towards a large central vein (Fig 1a). Pesticide treated fish liver histology revealed cellular degeneration, hyperplasia, lamellar atrophy etc. Sinusoidal nature was found to be disturbed with necrosis, clumping and intercellular blood clot (Fig 1b). Treated liver also showed hepertrophy of hepatocytes, fibrosis and infiltration of leucocytes.

b. Histological profile of kidney:

In the control fish kidney histology showed normal glomerular and tubular integrity (Fig 1c). Whereas, pesticide treated kidney (Fig 1d) showed irregular diameters of renal tubules, aggregation of RBCs and blood clot. Tubular disruption was also profoundly visible in the affected kidney. Glomerulous was seen to be with clots and aggregations of RBCs and other unidentified entities. Therefore, it can be inferred that marked changes in the histology of the kidney of the affected kidney was the result of direct impact of the pesticide itself.

c. Histological profile of Gill:

In fishes Gill is a multifaceted organ of activity as this organ carry out important functions like respiration, osmoregulation, acid base balance and excretion. Typically in *Channa* on each side of the buccal cavity there are four gill arches which are in turn composed of numerous gill filaments. Gill filaments are with two rows of lamellies and are perpendicularly placed on the filaments. Location-wise gill is also most vulnerable organ in fish because it remains in direct contact with the water and therefore, is in direct contact with the compounds with potential threats. In the pesticide treated gills (Fig 1f) cellular necrosis, epithelial lifting and hypertrophy were noted.

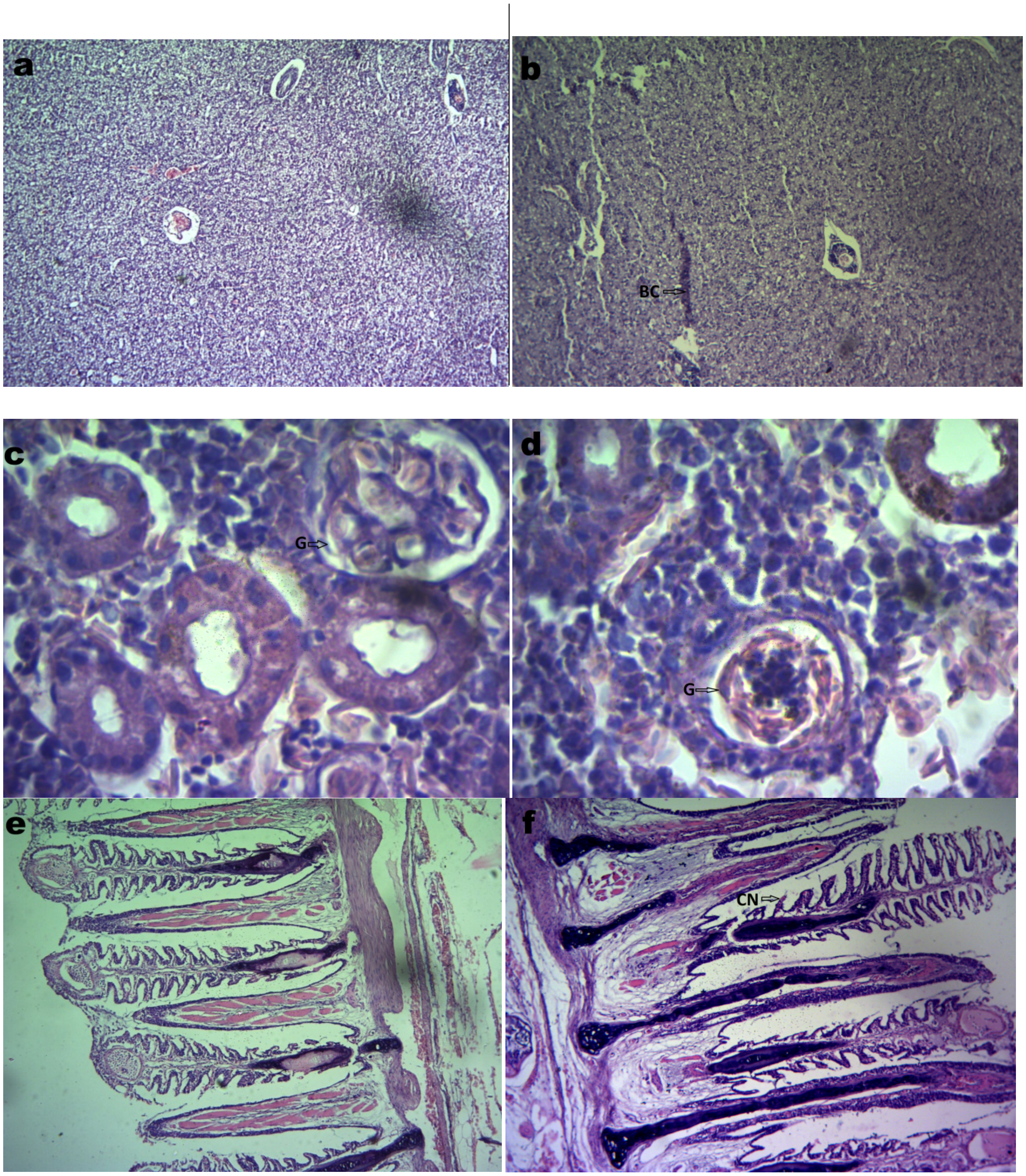

Fig 1: Histopathological study of Liver (a-control & b-treated, 100 X), Kidney (c-control & d –treated, 1000X) & Gill (e-control, f-treated, 1000X): [BC-Blood clot, G-Glomerulous, CN-Cellular necrosis]

G. Study of regeneration of fins:

Regeneration is a process by which damaged or lost organs are either fully perfectly or imperfectly partially reformed. In fishes regeneration is a well marked phenomenon. Caudal fin regeneration study is also a valuable tool in assessing normal physiological state of fish. Regeneration is a process which is influenced by various external chemicals. In our study we have found considerable difference in the pace of regeneration. After one week of surgery, in the control fish lengthwise 0.1 mm of regeneration was noted whereas in the treated fish there was virtually no visible regeneration. After 15 days in the control batch 0.8 ± 0.1 cm increase was noted whereas in treated this was only significantly lower 0.2 ± 0.01 cm.

Ever increasing human population and its ever increasing demand for food have caused our civilization to disturb the ecological balance. Use of pesticide has been increasing worldwide day by day despite several control measures. All pest destroying chemicals are together called pesticides. Most frequently used pesticides are insecticides, herbicides and fungicides. India is the second largest manufacturer of pesticide in Asia after China and globally stands twelfth. In this scenario it is essential to monitor the effect of each chemical compound in air, soil and water so far the ecological health of the community is concerned. Pesticide toxicity to non target aquatic organism like fish is a great concern today. Chemicals like pesticides enter the aquatic environment through atmospheric deposition, surface run off or leaching and accumulate in soft bottom and within the aquatic organisms (Akerblom 2004). Due to bioaccumulation this kind of toxicity even becomes magnified and effects become even more dangerous in the tertiary consumers like man.

This study has been carried out to find out the differences, if any, in physic-chemical properties of water as well as in the physiology of the fish exposed in this water after administration of pesticide. In this study we have assessed amount of dissolved Oxygen and free Carbon-di-Oxide, pH and temperature as the parameters to study the physicochemical changes. We have found a decrease in the DO and an increase in the free CO_2. DO is the only source for oxygen for respiration in aquatic life through gill. Normally different water sources contains DO in the range of 0-18 parts per million (ppm), but major streams, lakes and other natural water bodies require the range of 5-6 ppm of DO to accommodate a diverse life systems (Biswas 2018). Similar result have also been found in earlier studies by Usui and Kasubuchi 2011 where they found decrease in the DO and increase in the free CO_2 after application of herbicide in the paddy field surrounded pond. However, increase in the alkalinity of the water after administration of pesticide cannot be explained right now, however, in many cases, it has been reported that increase in pH may cause degradation of certain pesticides itself.

Pesticides cause serious damage to the physiology of aquatic organisms (Sunanda et. al., 2016). Different hematological, biochemical and histopathological tests, therefore, reveals the extent of damage to the system of the fish. In this study significant decrease in the total WBC and RBC numbers have been found. A decrease in the erythrocyte, leukocyte, hemoglobin and haematocrit mean value was reported earlier in *Channa punctutus* exposed to chlorpyriphos (Ali and Kumar 2012). Changes in the RBC, WBC and Hb percentage were also reported in *Cyprinus carpio* (Ramesh et. al., 2013) and in *Tilapia guineensis* (Chindah et al., 2004) after pesticide treatment. Reduction in the erythrocyte and leukocyte counts were also noted in pesticide treated *Cyprinus* *carpio* (Napit 2013). Decrease in Hb% and RBC count were also observed in Heteropneustes fossilis exposed with Dimecron (Napit 2013).

In this study an increase in the plasma glucose level is being reported. In *Oreochromis niloticus* similar increase in glucose level was reported after pesticide application (Firat et al., 2011). Increase in the plasma glucose level is probably the reflection of impaired metabolic state within the cells or some malfunctioning of glucose uptake mechanism per se. Apart from that stress is also an energy demanding process and glucose is one of the most important indices to study stress of an individual (Firat et al., 2011). G6PD is a very important metabolic enzyme distributed among species from bacteria to human. There are about 100 variants of G6PDs which have been reported from different organisms. Being one of the most important enzymes of the glycolytic pathway this enzyme also takes a pivotal role in the pentose phosphate pathway that supplies reducing energy to cells. Thus this enzyme ultimately controls the level of NADPH. In this study a decrease in the Glucose 6-phosphate dehydrogenase activity was noted. This is also in unison with the result reported by Tripathi and Verma, 2004 after administration of Endosulfan to the freshwater fish *Clarius batrachus*. This observation along with the high plasma glucose level probably points out a lower metabolic activity in the treated fish.

Histopathological effects of pesticides in fishes have been studied extensively. Mainly these pathological changes are prominent in liver, kidney and gills. In parity of the observations found in the pesticide treated organs in this present study, there are ample evidences of similar alteration of the same organs in different other fishes under similar challenges by different pesticides (Murthy et al., 2013). In *Danio rerio* vacuolization and presence of sinusoidal places of liver was reported under chlorpyriphos challenge. Like or reporting shrinkage of glomeruli and wide urinary spaces was reported in fish after Lorsban treatment. Similarly lamellar edema, clumping, cellular atrophy, hyperplasia, lifting of lamellar epithelia were found in the gills of pesticide treated rainbow trout (Sunanda et al., 2016). Significant histological and physiological alterations in gills were observed in *Catla catla, Nandus nandus and Labeo rohita* due to effect of endosulfan, diazinon and chlorpyriphos (Napit 2013).

Regeneration study in organisms like fish reflects the physiological and metabolic status of a fish (Borgave et al., 2016). Glucose itself had a positive influence on the tail fin regeneration in the *Gambusia affinis* as demonstrated by Borgave et al., 2016. It is already demonstrated by Oppedal and Goldsmith 2010, that regeneration of fin in *Danio rerio* is affected by influence of some chemicals. In this study retarded regeneration rate of the caudal fin in the pesticide treated group of fish along with the lower level of glucose might be the reflection of reduced metabolic rate directly influenced by the pesticide under study.

CONCLUSION

Pesticides are one of the most dangerous pollutants of the modern day aquatic system. Direct accumulation in the body causes severe damage to the physiological and anatomical structures of the exposed fishes. Accumulation of pesticide is also dependent on the factors like salinity, temperature, metabolic status of the fish. Vital organs like liver, kidney and gills are the main target regions of the pesticides. *Channa punctatus* is a common teleost fish in India. In this present study we have seen the detrimental effect of the Pretilachlor on the *Channa punctatus* which in turn can serve as the possible bioindicator of pesticide pollution in the waterbodies. Measures to take precaution and restriction in using pesticide is not sufficient to check detrimental use of pesticides. Care and attempts should be taken to stop run off, leaching of pesticide containing water from cultivated lands to nearby water-bodies.

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