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Histological Alternation of the Gills and Muscles organs of Fresh Water Fish *Liza Abu* (Heackl, 1843) after Exposure to sub- lethal concentration of Organophosphates' Chlorpyrifos pesticides

SAPHIA ALI AITTE
Health and Medical Technical College
Community Health Department
Southern Technical University, Basra, Iraq

Abstract:

In the current study, the fresh water fish Liza Abu exposed to sub- lethal concentration of (0,0.02,0.04,0.08) mg /L of Chlorpyrifos pesticides for a period 15 days of exposure to investigate the toxic effect of the pesticide on fish organs included gills and muscles. Observed through the histological sections that there were different levels of degeneration of cells .In the gills hyperplasia , Necrosis and the hemorrhage in gill arches , clubbing shapes and degeneration of secondary lamellae . The muscles changes were degeneration with focal area of necrosis, different size of muscle fiber, splitting of muscle bundles and vacuoles. These hazards became severe with increasing concentrations and exposure time.

Key words: histological, gills , chlorpyrifos , muscle , Liza Abu

INTRODUCTION

Water is vital to the existence of all living organisms, but this valued resource is increasingly being threatened as human populations grow and demand more water of high quality for domestic purposes and economic activities. Water abstraction

for domestic use, agricultural production, mining, industrial production, power generation, and forestry practices can lead to deterioration in water quality and quantity that impact not only the aquatic ecosystem (i.e., the assemblage of organisms living and interacting together within an aquatic environment), but also the availability of safe water for human consumption. It is now generally accepted that aquatic environments cannot be perceived simply as holding tanks that supply water for human activities. Rather, these environments are complex matrices that require careful use to ensure sustainable ecosystem functioning well into the future[1]. Moreover, the management of aquatic environments requires an understanding of the important linkages between ecosystem properties and the way in which human activities can alter the interplay between the physical, chemical and biological processes that drive ecosystem functioning. Water can also contain substances that are harmful to life. These include metals such as mercury, lead and cadmium, pesticides, organic toxins and radioactive contaminants. Water from natural sources almost always contains living organisms that are integral components of the biogeochemical cycles in aquatic ecosystems. However, some of these, particularly bacteria, parasitic worms, fungi, and viruses, can be harmful to humans if present in water used for drinking. [2] There for the Water pollution is one of the main predicaments of the modern world ,It is a cosmopolitan problem that needs urgent attention and prevention . On the one hand we need to conserve and manage natures resources, but on other hand also keep need for further industrial growth in mind , This has resulted in the need for more information regarding the biological effects of water pollutants [3] . also water pollution is mainly due to contamination with hazard chemicals from agricultural runoff and waste water from house hold, One of the major chemicals from agricultural runoff is pesticides which play important part

In increasing agricultural productivity through controlling pest. But on the other hand, they cause hard damage to the non-target organisms both the terrestrial and aquatic environment[4]. Pesticides strong potential health hazard not only livestock and wildlife but also to fish, accumulate pollutants directly from, contaminated water and directly via the food chain [5] [6]. Subsequent to pesticides are the heavy metals from series chemical anthropogenic substance includes insecticides and herbicides [7][8]. Sources are continually released into aquatic system which could aeries threat because of the toxicity, long resistance bio accumulation, bio concentration, and bio magnifications of pesticides and metals in food chain [9] [10], Pollution by persistent chemicals is potentially harmful to the organisms at higher trophic levels in the food chain[11],[12]. It has been reported that the consumption of contaminated fishes is one of the important pathways of human exposure to OCPs Most of aquatic organisms including fish, accumulate the pesticide direct from contaminated water via food chain and indirectly by respiration [13] [14]. So, it is important study the histopathological changes, this can be used as indicator for the effects of various anthropogenic pollutants on organisms and are a reflection of the overall health of entire population in the ecosystem. These histopathological biomarkers are closely related to other biomarker stress since many pollutants have to undergo cellular changes in the affected organisms. Histopathlogical change have been reported in gill of many fish as a result of exposure to different toxicant [15] [16] Several alterations have been reported in the ovary of exposure to pesticide[7] [18]. Histopathological alterations have been reported in the kidney[19] [20] and also reported in the intestine of fish as a result to different toxicants [21] In order to obtain more information about the pathways along which bioaccumulation occurs, it is important to investigate the distribution of

pesticide residues in muscle tissues of fishes. The present study aimed to investigated the impact of the environmental conditions of pesticides on the histopathlogical structure of muscles and gills of Liza Abu.

MATERIALS AND METHODS

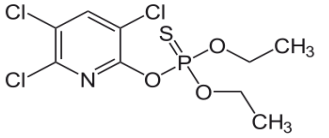
Experimental animals

In experiment, **Liza Abu**, with an average body weight of 12±3 g and length of 11±2 cm, were collected from a freshwater source from north shatt Al -Arab caught by using net with 20 mm mesh size, and acclimated for time period 10-15 days in the laboratory conditions in a plastic aquarium (4200 cm^3)glass aquaria containing decloroinated water .The water of the aquaria was aerated continuously through stone by mechanical compressor at a constant temperature (20 ± 2and pH value tainted 7.6-7.2- Fishes were feed daily with dry algae.

Preparation solution

For the present study, fish were exposed to different concentrations 0.02 .0.04, and 0.08 mg /L of chlorpyrifos pesticide for 15 days continuously. Three replicates of ten fishes for each concentration of the pesticides were used.. Each experiment was accompanied by its respective control, and attended the concentrations of pesticides depending on the mitigation equation (C1V1 = C2V2) and the percentage of the active ingredient installed on a container of pesticides and chemical composition as follows

Effective material of the pesticide chlorpyrifos 48% .



O,O-diethyl O-3,5,6-trichlorpyridin- 2ylphosphorothioate

Preparation of tissue samples for histological analysis

After exposure period, all of the experimental and control fishes were killed for histopathological examination at the end of 15day. Gill, and muscle tissues were fixed in 10% neutral formalin for 24 h. Fixed tissues were washed in running tap water for 24 and dehydrated properly through ascending series of ethanol. Then the tissues were cleared with xylene and embedded in paraffin wax. In order to specify the thickness of serial section for histological purpose, generally sections were cut at 4-5 m thickness and stained with hematoxylin .eosin and methylene blue for light microscopic examination, and sections were examined for investigation of histopathological lesions. Histological preparations were randomly examined three times, and the results from each observation were then combined for the final data[22.23]

RESULTS

Alteration in Gills

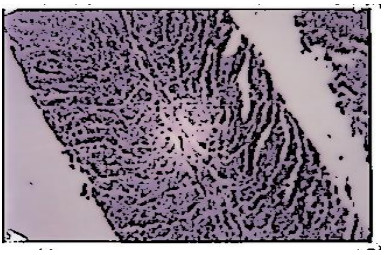


Fig -1- There was no histopathlogical changes in gills tissues of fish control group(Magnification :x400).

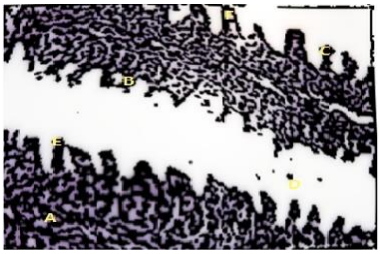


Fig- 2-shows the secondary lamella of gill from Liza Abu a period 15 day of exposure to 0.02mg /L of chlorpyrifos (A) Hyperplasia(B) Necrosis (C) clubbing shape (D) Vaculation of gill (E) Shortening of the secondary lamella (F)) Separation of secondary lamella (Magnification :x400).

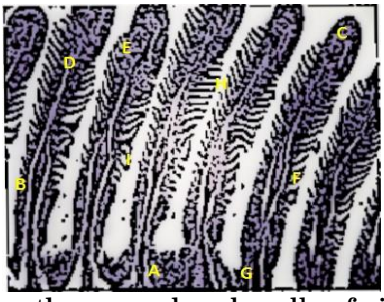


Fig -3 Fig.(3) I shows the secondary lamella of gill from Liza Abu a period 15day of exposure to 0.04mg/L chlorpyrifos A-Hyperplasia B-Hemorrhage C- clubbing Tip D-Higher regeneration of epithelial lamella E-Degeneration of secondary lamella G-Thickness of secondary lamella H- Necrosis (Magnification :x400).

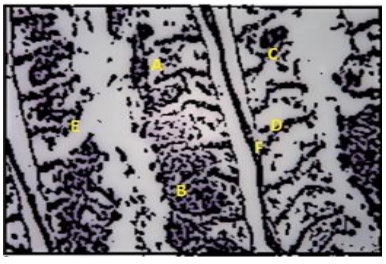


Fig.- 4- shows the secondary lamella of gill from Liza Abu forr a period 16 day of exposure to 0.06mg/L of chlorpyrifos (A) Degeneration (B) Bleeding (C) Necrosis (D) Damage of arches g (E) Vaculation of secondary lamella (F)Splitting (Magnification :x400).

Alteration in Muscle

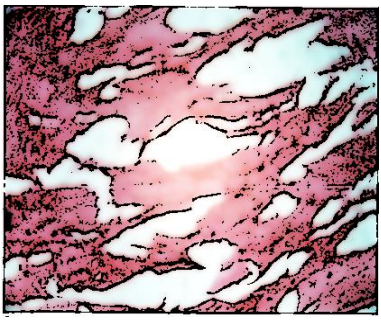


Fig -1-There was no histological changes in the muscle tissues of fish control group(Magnification :x400).

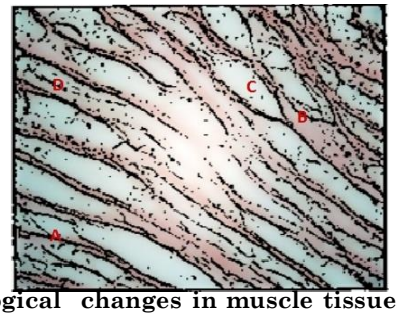


Fig -2-- The histological changes in muscle tissues treated with 0.02 mg/L of chlorpyrifos for a period 15 days exposure showed A- Necrosis in the bundle of muscle B- splitting in the muscle fiber c- vacualation D-nuclear proliferation Magnification : (x400)

Fig -3- The histological changes the muscle tissues of Liza Abu under 0.04mg/l for a period 15 days exposure showed A- edema atrophy B- mild lymphocyte infiltration C- thickness of muscle bundles E- Degeneration of muscle fiber (Magnification :x400)

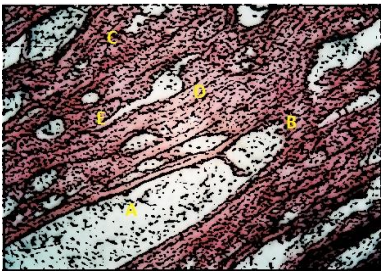


Fig -4- The histological changes in muscle tissues of Liza Abu under 0.08 mg/l of chlorpyrifos for a period 15 days of exposure showed A- vacuoles B- separation of muscle bundle C- degeneration D- lymphocyte infiltration E-focal area necrosis it was leading to vascular degeneration (Magnification :x400)

DISCUSSION

Water pollution contain is a large compound of organic pollutants including organ phosphorous compound chlorpyrifos are insecticides that act by poisoning the aquatic system. The aquatic organisms like fish are able to accumulate several fold higher concentration of pesticide residues than the surrounding water [24].OCPs contribute to many acute and chronic health effect, all these toxic agents are powerful oxidant ,thus the reaction of mechanisms of toxic compound there are have been great important on investigation for antioxidant process, mainly in living organisms[25] The size and diversity of the destruction by water pollution is pretty easy to measure , First ,the human health is at stake . After this , a series of economy activities are affected .Fish and other aquatic biota may be pesticide contaminated water Conversation physiological and behavioral changes or effect on the survival species and this is most dangerous [26] pesticide surface runoff into rivers and stream can be highly lethal to aquatic life , sometimes killing all the fish in a polluted stream [27]. Histopathological changes have been widely used as biomarkers in the polluted environment and accumulation of pollutants evaluation of the

health of fish exposed to contaminants, by examining target organs, including gills, liver, and muscle, that are very important for physiological function like movement ,respiration and excretion [28], furthermore one of the great advantages of the using histopathological biomarkers in environmental monitoring is that category of biomarkers allows examining specific target organs including gills , muscle ,kidney and liver , Histological study of **Liza Abu** gills and muscle tissues exposed to different sub-lethal concentration (0.02,0.04and 0.08 mg/L of chlorpyrifos to 15 days of exposure , histological alterations observed were hyperplasia necrosis ,clubbing shape , and separation of lamella epithelia, degeneration ,hypertrophy), bleeding and fusion of secondary filament ,these noticed are agreement with [28],and [29]observed lamellar oedemas, clumping, cellular degeneration, hyperplasia, and lamellar atrophy in gill of rainbow trout exposed to 2.25, 4.5 and 6.75 µg/L of chlorpyrifos for 24, 48, 72, and 96h. , [30] also reported that sub lethal concentrations of chlorpyrifos (1.46 µl/L and 0.538 µl/L) for 3 and 7 days caused histopathological changes in gill tissues of *Channa punctatus*. The main histopathological changes in gills exposed to the highest concentration were edema, lifting of lamellar epithelia and an intense vasodilatation of the lamellar vascular axis. Lamellar fusion caused by the filamentary epithelium proliferation and some lamellar aneurisms were also found. Hypertrophy; hyperplasia Concentration of. That are responsible for vital function such as respiration ,excretion and the accumulation and any investigated found signs of damage to animal health [31], The muscle that covers the bones and the involuntary movements in the body of the fish, such as swimming. So that organ to be affected by contamination of the water .Results of the present study, showed many alteration in bundles of muscle during exposure to sub lethal concentration of malathion pesticide .fig-1-Muscle group control showed normal structure

.In the treated muscle tissues of **Liza Abu** with sub lethal concentration (0.02,0,04 and 0,08)mg/L for 15days showed degeneration in muscle bundle and increased nuclear see in fig -2-.noticed vascular degeneration in muscle fiber of **Liza Abu**. Fig -3-showed edema atrophy and mild lymphocyte infiltration and degeneration of muscle fiber after exposure to (0.02) mg/L, where fig-4- found splitting of fiber , separation of muscle bundle and focal area necrosis it was leading to vascular degeneration during exposed to (0.04) mg/L .during subjected **Liza Abu** to (0.08) mg/L of the cholorpyrifos where became a seriously damage found an increase in a thick of muscle bundle and filtration of lymphocyte as well as splitting in muscle fiber [32] ,and so in this current study observed vasculation of the muscle fiber according to study by [33] [34]. The histological changes were in agreement with those observed [35] [36] [37].The pathological findings induced degeneration in muscle bundle with aggregation of inflammatory cells between them and focal areas of necrosis[38], and [39] reported the physiological disturbances and morphological changes in the muscle tissues causes pesticides bioaccumulation[40], muscle consistent with what he referred to both [41]. Histopathological changes for aquatic organisms like fishes and the source of chemical contaminant such as pesticides, and these changes does not exceed being morphological and structures only, but extends to the biosynthesis, where are metabolic processes its content and energy products, therefore several studies indicated to biochemical result of damage physiological as result exposed fishes to the different periods and different types of pesticides , so was the biochemical studied good factor helps to see the toxic effects on metabolic action[42]. Biochemical content includes glycogen, protein enzymes, lipid and nucleic acid, the values that are affected by the presence of toxic substances and contaminated compound essential protein enters in the composition and the physiological of the cell and

has an active role in the processes of in cellular metabolism. Pesticides are generally working to decrease in the protein content of the muscles further more; the protein is important for energy needs by fishes and may increase the protein requirement for energy production as it leads to proteolysis protein process. In study made by [43] pointed to decrease muscle protein content. Liver. gills and ovaries because proteolysis protein for energy and do metabolism process [44] , also to lower value of muscle protein content of fishes, in study by [45] Aitte she indicated a decline in fish muscle protein for carp and gold fish exposure to sub lethal concentration of malathion. And the decrease in glycogen content in muscle exposed fish to pesticide observed by [46]. In another study found a significant decrease in the glycogen content in fish muscles exposed to malathion pesticides each acute and sub lethal concentration[47] , and explain the depletion of glycogen content in all the tissues might be due to the utilization of carbohydrates for energy production as a result of toxicant induced hypoxia on Indian major carp *Labeo rohita* fish, therefore the changes that occur in biochemical content in fish as a result of inappropriate for living organisms in the environment aquatic condition[49]. The present study shows that the greater the changes was physiological significant whenever biosynthesis mngerafi natural values so under sub lethal concentration gradient for a period 15 days it shows that concentration of sub lethal contribute subversion it had a little part ,but it is a damage and contribute with time but it is a damage and contribute with time period of exposure where this leads to the accumulation in fish body organs which confirms the toxicity of these pollutants and their negative impact on environment[50]

CONCLUSION

All concentrations of pesticides and especially its long –term effects of toxic fresh water fish *Liza Abu* where physiological changes appeared and both organ gills and muscles damage and sabotages tissues , which in turn reflected on the events vital for fish

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