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Impact assessment of the fish *Labeo rohita* collected from carbofuran contaminated fresh water of Cuddalore region, Tamilnadu

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Abstract
All life forms require water which is essential for their survival. One of the major ecotoxicological issues that threaten most of the life forms in water bodies is pesticide contamination. The removal of pesticides in aquatic ecosystems has become an important area to reduce the risk to the organisms present. Fish is one of the important sources of protein and other elements to human beings. The fish *Labeo rohita* is taken to study the impact assessment of pesticide contamination in fish which is consumed by most of the people of Tamil Nadu. The samples were collected from fresh water bodies which are nearby to the agricultural field and analyzed for the nutritional value which is required by the human beings. The physical, chemical parameters and microbial analysis of the water samples were done. To assess the pesticide contamination the collected fish samples were then subjected to biochemical analyses. It is noticed that the contamination of inorganic pesticides in water results in the decreased level of prote-in and glycogen levels in fish which will lead to high mortality rate of fishes.

Keywords: pesticides, *Labeo rohita*, protein, carbohydrate, lipids

Introduction
At present throughout the world, it is reported and realized that water quality is deteriorating due to various anthropogenic activities. One of the major issues is usage of pesticides and synthetic fertilizers to safeguard the crop from the insects and pests and to get more yields. The usage of pesticides has become an integral part of today's agriculture. Due to the intensive toxicity towards human, nearly 28 pesticides have been banned. At present Carbofuran is the most commonly used pesticides in cuddalore region. The environmental condition also plays a major role to the increase level of pesticide toxicity. The ecological imbalance of non-target organisms is because of the application of the synthetic pesticides in agriculture. The most commonly used synthetic fertilizers are organophosphates, organochlorines and carbamates which causes toxicity to the soil, fresh water flora and fauna. As a result these compounds damage population dynamics and food web. It is a well-known fact that fishes are good source of protein, water content, minerals, vitamins, calcium and low cholesterol. The biological activity of pesticides directly inhibits the activity of acetylcholine esterase which in turn leads to impeded neurotransmission. Fishes are prone to be sensitive to water contamination. When such contaminants enter into the organ system of fishes it can affect the biochemical process of the organisms. The physico-chemical and the microbiological analysis serve as an indicator of water quality and the changes in the biochemical constituents of the fish shows the effect of pesticides on the metabolic activities. The present study is done to determine the change in the biochemical constituents such as proteins, glycogen and lipid content in fish collected from the carbofuran contaminated water bodies.

Materials and Methods
Sample Preparation
The fish (*Labeo rohita*) was collected from the pond nearby agricultural field where agricultural runoff is observed. Fishes was also collected from a fresh water body of Thookanampakkam in Cuddalore district where there is very meager chance of pesticide contamination. The samples were collected on the 3 rd day of pesticide application. The fish samples were kept inside a container with some amount of tap water for 15 minutes then washed thrice with distilled water. The fins and scales were removed and abdomen was cut open and the organs were collected. Likewise, the edible part of the fish also taken for biochemical analysis. The collected parts were then cut into small pieces and kept in oven for 50-60^{0}C for 6- 8 hours. After drying all the samples were kept in separate air tight glass containers. The samples were ground to fine powder for biochemical analysis. Water samples were also collected and kept below 4^{0}C until physico-chemical analysis was done.

Physico-Chemical Properties of Water
The site selected is as same as where the fish is obtained for analysis. The water for analysis was collected at the middle of the sampling area.
The samples were collected in sterile container and stored under 4^{0}C for further analysis. Temperature, pH and EC were determined on site. Turbidity, total dissolved solvents, Nitrogen, Phosphorus, Dissolved Oxygen, COD, were analyzed later. Each sample was analyzed in triplicates and the reported results are the mean of the values.

Microbial Analysis

The microbial analysis of the collected water sample was done by Nutrient Agar followed by the biochemical test and Gram's staining and the coliform analysis was also done. Both the microbial analysis was done within 24 hours of sample collection.

Determination of Pesticide Concentration

The determination of carbofuran was done by Ghazala *et al*. (2014) by spectrophotometric method. 10 g of carbofuran was taken in a graduated flask and to that 0.3 ml of 1N H2SO4 was added. The standard solution was hydrolyzed in phenol by the addition of 0.5 ml NaOH. To the above solution 1.5 ml of acetonitrile was added and kept it for 5 minutes for shaking. 4M NaOH was prepared and 1 ml was added to the graduation flask and the final volume is made up to 10ml. The extraction of water samples were done with 5ml of chloroform in a separating funnel. Under reduced pressure the extract was subjected to evaporation. Finally the residue was dissolved in 5ml ethanol and finally made it to 50 ml with double-distilled water. The absorbance was measured at 465nm by UV-VIS spectrophotometer for the standards as well as for the water samples.

Biochemical Analysis

Estimation of Protein

The amount of protein present in the fish was estimated by the method followed by Gornell *et al*., (1949). The powdered sample of 0.3 gm was taken and ground into a homogeneous paste in a mortar pestle, and 5 ml of 10% TCA was added. This material was transferred into test tube and centrifuge for 10 minutes at 2000 rpm. The residue was taken and to the residue 1 ml of distilled water and 3 ml of biuret solution was added. Then the residual mixtures were kept in dark place for 30 minutes. The reading was noted on calorimeter at 530 nm after the appearance of lavender color.

Estimation of Glycogen

The glycogen content of the fish was estimated by Kemp *et.al.* (1954) method. 0.1gms of powdered sample was taken and homogenized in a mortar pestle, to which 5ml of 5% TCA was added and transferred in to a centrifuge tube. Then the test tube is further kept in a boiling water bath for 15 minutes at 2000 rpm for digestion. To the 1 ml of supernatant, 3 ml of H2SO4 was added and boiled for 5 minutes. The mixture was shaken well, then immediately cooled and the readings were taken at 530 nm using uv-vis spectrometer.

Lipid Estimation

The method followed by Folch *et al*., (1957) was the method used to estimate the lipid content in fish. 0.05 g of oven dried powdered sample were taken in a centrifuge tube and homogenized with chloroform: methanol (2:1) mixture. To the above mixture 0.2 ml of NaCl (0.9%) was added and the same mixture was centrifuged at 3000 rpm for 5 minutes. After centrifugation the lower phase was separated and evaporated at room temperature for overnight. Then 2 ml of Concentrated H2SO4 was added and boiled for 10 minutes and cooled. 0.1ml of sample was taken in a clean test tube and made it up to 1 ml with concentrated Sulphuric acid.

To the above mixture 2.5 ml of phospho Vanillin reagent was added and kept at incubation for 30 minutes. The reading was noted on calorimeter at 530 nm.

Result and Discussion

Table 1: Physico-chemical properties of water collected (pesticide free site and agricultural runoff) s

Water quality parameter	Control site	Test site
pH	7.2±0.2	7.6±0.1
Odour	Foul smell	Foul smell
Colour	Pale yellow	Pale yellow
TDS mg/l	325±6.4	342± 5.7
EC, µS/cm	0.6±0.01	1.0±0.03
Temperature	28^0C	28^0C
Turbidity NTU	95±2.4	104±2.9
Nitrogen, mg/l	4.0±0.05	3.1±0.04
Phosphorus, mg/l	0.7±0.5	0.9±0.2
Dissolved Oxygen, mg/l	4.2±0.2	4.7±0.2
Chemical Oxygen Demand, mg O2/l	9.5±0.2	16.4±0.3

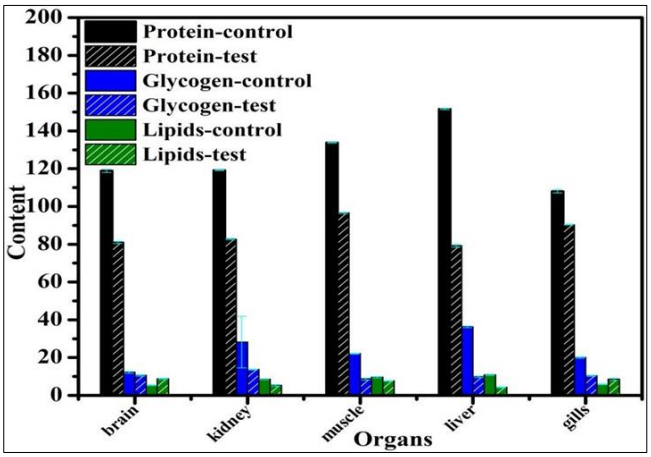

Fig 1: Estimation of Protein, Glycogen and Lipid Content (mg/g)

Results and Discussion

The dissolved synthetic pesticides in water show extensive changes in the physico – chemical properties. Sustainable development depends on the quality and quantity of fresh water which is essential for agriculture. The results of the Physico-chemical properties done by using standard procedures shown in table 1. The maintenance of pH is very important for the survival of the organisms in the water bodies. The changes observed in the pH ranges is because of the presence of the toxic substances present s (Ali J.1991). The total dissolved ions are directly linked with the total dissolved solids present in the water. In the present study it is noted that the conductivity value and the total dissolved solids is less than the recommended value (Bhatt *et.al.*, 1999) [4]. The dissolved oxygen responds to the slight environmental changes which supports the aquatic life. The observed dissolved oxygen fall within the WHO permissible limits (Chapman *et.al.*1992) [5].

The results of the Chemical Oxygen Demand results in the presence of inorganic content because of the acidification of water (Hemant Pathak *et al.*, 2012) [7].

The increased level of nitrate observed is due to the various anthropogenic activities. Nitrate is the important factor to assess the water quality and mostly depends on the presence of nitrifying bacteria (Johnes and Burt., 1993) [8]. In the presence of nitrogen phosphorus regulates the production of phytoplankton. Agricultural runoff tends to increase the phosphate level in water (Shah *et. al.*, 2000) Because of the stress, the aquatic organisms may alter the biochemical contents for their survival. Microbial analysis indicates that the bacterial colonies ranged from 90to 110 CFU/ml. Low level of pH consequently affect the microbial count in the water samples. The fecal coli form contamination will cause severe health issues and no coli form 100 ml of sample is considered as good quality water. The presence of 18-20 CFU/ml of test site indicates that the water is contaminated with fecal matter and it indicates that there is a practice of open defecation (Edema *et. al.*, 2001) [6]. The amount of carbofuran found in the water sample is 0.27±0.01mg/ml.

The biochemical parameters such as protein, glycogen and lipid were estimated in five different tissues. The results indicates the decline in the biochemical composition of the fish which indicates the organism is under stress. The obtained values shows decline in biochemical constituents of the fish which is under stress. The calculated values for protein, glycogen and lipid for the control and test of ishes living in uncontaminated and contaminated sites were given in table 2 are graphical represented in figure 1. The variation seen in the biochemical constituents is because of the differences seen the metabolic activity of the tissues. Fishes are very sensitive towards the contamination in the aquatic environment. The chemical pesticides will reduce the metabolic pathway and in turn generally causes pressure on biologically active molecules (Agrahari *et al.*, 2009) [1]. Proteins are building block of the human beings and during the stress period it will maintain the blood glucose and the energy level (Magar *et al.*, 2012) [9]. The protein content have been decreased gradually in all the tissues of the fish where the site is polluted with pesticide contamination. These study shows that the maximum decline were observed in muscles followed by gills, Kidney, Brain and liver and it was significant over control.

The major storage form of carbohydrates in animals is glycogen which occurs mainly in liver and muscles. Decrease of glycogen content is more in muscles followed by kidney, brain, gills, liver and muscle and. Rapid utilization of glycogen is to meet the energy demands when there is a lack during glycolysis is the major cause for the decline of glycogen in tissues (Anitha Susan *et.al.*,) 2010 [3].The sudden decline of glycogen content indicates the stress response in fish. The present report shows the decrease lipid content of liver, kidney, muscles, gills, and brain has decreased in the present report. Lipids act as a source of energy at the time of demand in carbohydrates to support the physiological functions of the body. Utilization of lipids occurs during the energy demand during pesticide stress (Saravanan *et al*,) 2001 [11].

Conclusion

The present study indicates that the because of the pesticides changes occured in all the bio chemical constituents of the fish *Labeo rohita* when compared to the uncontaminated site of fish tissue. It is found that the alterations found in the protein, glycogen and lipid content of the fresh water fish of contaminated site is because of the toxic substances present in the pesticide. The impact of accumulation of chemical pollutant inhibits the synthesis and leading to an alteration in function which indicates the vulnerability of the tissues. From this study it is concluded that the usage of pesticides to be minimized and to use appropriate level which cause harmful effect to the living organisms.

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