Poll Res. 29 (2) : 315-322 (2010)
Copyright © EM International

IMPACT OF WATER POLLUTION ON BIOCHEMICAL CONSTITUENTS OBSERVED IN LIVER, MUSCLES AND GILLS OF *CYPRINUS CARPIO*

G. PRABAKARAN[1], B. SENTHILKUMAR[2], M. RAJASEKARA PANDIAN[3] AND D. SENBAGAM[4]

[1]Department of Botany, Government Arts College, Dharmapuri 636 705. Tamilnadu, India.
[2]Department of Biotechnology, Muthayammal College of Arts & Science, Rasipuram 637 408, Tamilnadu, India.
[3]Department of Zoology, Arignar Anna Arts College, Namakkal 637 001, Tamilnadu, India.
[4]Department of Biotechnology, Vivekananda College of Engineering for Women, Tiruchengode 637 205, Tamil Nadu, India.

ABSTRACT

With continued economic growth population increase, urbanization and technical development more and more waste materials are discharged into water bodies. This results in the pollution of water and affects the aquatic ecosystems. moreover, each type of water pollution affects the biotic and abiotic factors of different aquatic systems to different degrees . In the present study, attempts were made to investigate the impact of pollution on the level of extractable proteins, carbohydrates and total lipids in gills, Liver and muscles of *Cyprinus carpio*.The quantitative and qualitative study of proteins revealed a significant decrease in the different organs of *Cyprinus carpio;* the significant decrease in proteins content is seen in dye factory effluent treatment. This decline has been due to the hydrolysis and oxidation of proteins. The decline in carbohydrate content in the different organs is due to the anaerobic breakdown of glucose available to the cells through enhanced glycogenolysis. The decreased lipid contents is due to the inhibition in the process of lipid biosynthesis or due to greater utilization of the stored lipids to meet the decreased demand for energy due to the stresses following diastic alterations in physio – chemical parameters of waters. The remarakable change has been noted in dye factory effluent followed by agricultural run off sewage and cauvery water. When sewage and cauvery water treatments were compared with agricultural run off the latter shows more effects in the protein, carbohydrate and lipid concentration. However the ground water shows less contamination when compared to all the other water treatments.

KEY WORDS : *Cyprinus carpio,* pollution, cauvery water, Statistical analysis

INTRODUCTION

The term 'water pollution' is referred to the addition of an excess materials to water that is harmful to humans, animals or desirable aquatic life or otherwise causes significant departures from the normal activities of various living communities. Water pollution is one of the major environmental hazards. When toxic substances enter into lakes, streams rivers, oceans and other water bodies, they get dissolved or lie suspended in water or get deposited on the bed. This results in the pollution of water whereby the quality of water deteriorates, affecting aquatic ecosystem. The contamination of water takes place due to the following: (1) a highly enriched, over productive biotic community, such as a river or lake with nutrients from sewage or fertilizer (cultural eutrophication), a body of water poisoned by toxic chemicals which eliminate living organisms or even exclude all forms of life. The major sources of water contamination have been domestic, industrial and agricultural water. Several water borne diseases like cholera, typhoid and infectious hepatitis are directly related to polluted water (Stanley and Alpers, 1975). pH measures the acidity or alkalinity of water. pH of rainwater is about 5.5-6.0. Typically, natural water has pH 6.5-8.5. A pH less than 5 (acidic water) is most damaging to eggs and larvae of aquatic organisms. Most aquatic life (except for some bacteria and

Corresponding author : Department of Botany, Government Arts College, Dharmapuri - 636 705. Tamilnadu, India. Email: gpbiotek@yahoo.co.in Mobile : 09442438280

algae) cannot survive in pH less than 4. Alkalinity is due to carbondioxide (gas) bicarbonates (HCO_3^-); carbonates (CO_3^{2-}) and hydroxyl ions. Carbonate rocks such as limestone and dolomite increase alkalinity. Alkalinity is increased by caustic substances from industry (KOH, NaOH), soil additives in agriculture such as lime $Ca(OH)_2$; superphosphates which is mixture of $Ca(H_2PO_4)_2$ and $CaSO_4$. Natural acidity is due to carbondioxide (gas); HPO_4^{2-}, $H_2PO_4^-$, H_2S, Fe^{3+}, proteins and organic acids. Increases in acidity can be due to acids used in industry and mine drainage.

Each fish species has its preferred range of water hardness. The most commonly used method measures both alkalinity and general hardness as mg/L of calcium carbonate. Water hardness affects fish health because it influences osmoregluation (Morgan *et al*., 1999). As a consequence of osmosis, fresh water fish are subjected to a continuous out flow of water. Against this continuous movement of water into or out of the body, fish have to maintain a constant internal body fluid concentration, osmoregulation. Microorganisms like protozoa, bacteria, viruses, algae and fungi are found in natural water systems. Some of the diseases including typhoid, cholera and amoebic dysentery can result from water-borne pathogens. The excessive growth of algae (algal bloom) can degrade water quality because it lowers dissolved oxygen levels thereby killing other living things.

BOD values are important when they signify that the oxygen supply dissolved in the water will be so greatly reduced that desirable fish no longer can survive or when they signify that conditions for the propagation of dangerous bacteria exist Chemical oxygen demand (COD) may be defined as the amount of oxygen required by organic matter in a sample of water for its oxidation by a strong chemical oxidant and is expressed as ppm of oxygen taken from a solution of potassium dichromate in two hours.

Biochemical cycling of elements is receiving wide attention due to the need for understanding the pathways of pollutants through our present environment. River processes form a major link in the geochemical cycle. The asian rivers are the most polluted in the world with three times as many bacteria (Dauba and Mastrorilla, 2003). Cauvery River runs through one of the most densely populated regions of India. The river system has been impacted by the discharge of untreated sewage, agricultural and industrial effluents. The industrial wastes of mills and industries include metals (Copper, zinc, lead, mercury etc), detergents and acids, many inorganic and organic toxicants.

Pesticides and fertilizers can contaminate groundwater and can present a potential health hazard to nearby groundwater wells or surface water. Ground water, in its percolation through soil and rock, leaches out soluble salts. Of the main nutrients in nitrogen, phosphorus and potassium fertilizers, nitrogen in the form of nitrate is the most common cause of degradation of ground water near agricultural lands.

Most of the Indian rivers and fresh water streams are polluted by industrial wastes or effluents. The industrial wastes or effluents include many inorganic and organic toxicants. All these chemicals have been toxic to animals and may bring about death or sublethal pathology of the liver, kidneys, reproductive systems, respiratory systems or nervous systems in both invertebrates and vertebrate aquatic animals.

A very interesting source of information about water pollution is that it affects aquatic life. Millions of fish are killed each year by a wide variety of different pollutants from many different sources, municipal and industries. Direct acting pollutants create a lethal effect either through their action on the epithelial surfaces of the gills or by absorption of pollutants that enter the body affecting the internal structure and metabolism (Pavlov *et al*., 1999). Keeping in view of the public health and ecology of the water body a critical study in terms of quality and quantity caused due to pollution, is essential. Since quality in terms of biochemical and biotechnological alterations can give better results. The attempt has been made to study the impact of pollution on the level of extractable protein, carbohydrates and total lipids in the gills, liver and muscles of common carp (*Cyprinus carpio*).

MATERIALS AND METHODS

Collection and Acclimatization of Experimental Animal *Cyprinus carpio*.

Freshly caught healthy *Cyprinus carpio* species were brought from Bhavani sagar, Tamilnadu, active specimens of *Cyprinus carpio* (12.08± 0.08 cm in length and 22.32 ± 0.21 gm in weight) of both sexes were used for the experiments. The fishes were maintianted inlarge glass aquaria at 25.7 ± 1.7 º C under diurnal lighting conditions under 12L:12D photo period and acclimatized to the Laboratory

conditions for 28 days. An overhead Philips Fluorescent Lamp (120 cm, 40 W) provided a light intersity of ca 0.65 × 10 quanta cm at the water surface. During the period acclimatization the fish were fed with commercial fish food ad libitum, food and water were changed daily with an intermittent aeration with an aerator to ensure sufficient oxygen supplied. The timing of the reneval was never constand to avoid the possibility of entertainment of any circadian rhythm by a fixed handling schedule feeding was stoped and all fish were fasted for 24th period to experimentation to keep the experimental animal in moe or less similar metabolic state. Test condition were glass aquaria tanks 75 L capacity each can contain a total of 25 litres.

II. Collection of water sample:

Group I: Control water sample was collected in the tap water supply to laboratory.

Group II: Ground water sample collected in the well.

Group III: River water sample collected in the river cavery of Pallipalayam, Erode District, Tamilnadu.

Group IV: Dye-factory (Textile effluent) water sample was collected from Arun dyeing factory in Erode, Tamilnadu.

Group V: Sewage water sample was collected from in place of kaikatty valasu, Erode, Tamilnadu.

Group VI: Agriculture run of off water sample collected from Rasampalayam, Erode District, Tamilnadu.

Experimental Design

In the present investigation, healthy active live fishes were selected and acclimatized. After the short period of acclimatization each group consisting of ten fishes received the following regimen of water treatment for 28 days suitable controls were maintained for each duration of treatment. How ever as there was no obvious difference on any parameter among control group, a common control was employed in the present study.

Group I: Ten fishes were grown in tap water. This was used as control.

Group II: Ten fishes were grown in ground water.

Group III: Ten fishes were grown in cauvery river water.

GROUP IV**:** Ten fishes were grown in dye water (Textile effluent)

Group V: Ten fishes were grown in sewage water.

Group VI: Ten fishes were grown in agricultural run off.

After 28 days of treatment the fishes were sacrificed and the gills. Liver, muscles, were taken to do further biochemical and bio technological studies.

BIO CHEMICAL STUDIES

Estimation of Total Soluble Protein

The total soluble protein concentrations of the tissue (plant and animals) were estimated by the method of Lowery *et al.,* (1951). For the estimation of tissue protein 0.1 ml of the extract were taken and it was made upto a final volume of 1 ml with distilled water. The same procedure was followed as described for the standard. The amount of protein present in the sample was calculated by referring to the standard curve obtained. The protein concentration was expressed in mg/g tissue.

Estimation of Total Carbohydrates

Carbohydrates were estimated by the method described by Dubious *et al*., (1956). To 0.1 ml of the tissue sample in the clean glass test tube, 0.05 ml of the 80 % phenol was added rapidly and 5 ml of stream of acid being directed against the sample surface to obtain good mixing. The tubes were allowed to stand for 10 minutes, shaken and then placed for 20 minutes. Optical density of samples were taken at 490 nm. Glucose was served as the standard.

Estimation of Lipids

The method of Folch *et al.,* (1957) was employed to extract lipids. The total lipid was estimated by the method of Folsch *et al*. (1957). A suitable aliquot of total lipid extract (0.5ml) was taken in a pre weighted petriplate and its dry determined after drying the petriplate in the oven at 16 c over night. This was repeated over 2-3 days interval till a constant weight was obtained. The difference in the weight between the petriplate plus lipid and empty petriplate gives the total lipid content. The total lipid concentration were expressed in mg/g of wet tissue.

Qualitative Assay of Protein

The qualitative estimation of muscle protein was performed by following the method of Laemmli (1970) SDS – PAGE electrophoresis.

Histophathological Studies

A fish liver was taken and washed in 0.9% saline,

from that a small portion of the liver was quickly disserted fixed in 40% formalin. Then the tissues were processed by standard histopathological technique that is dehydration through graded isopropyl alcohol clearing through xylene and impregnated in paraffin wax for 2 hours. then wax blocks were made sections were cut using microtome and stained by hamatoxylin eosin method and photographed under florescent microscope Olympus BH2 – RFCA Magnification (X – 400)

Statistical Analysis

From the results obtained, mean and standard error were calculated.

RESULTS AND DISCUSSION

The results on extractable protein, carbohydrate and total lipid contents in the fishes collected from Bhavani sagar, Tamilnadu. were given in the table (Table 1, 2 and 3).

Quantitative Estimation of Protein (Table 1, Fig. 1)

All the fishes grown in different treatment of waters showed decreased protein content except those fishes grown in ground water. Agricultural runoff water showed the least amount of protein content when compared to all the other water samples.

Fishes grown in sewage water has higher protein content when compared to fishes grown in dye factory effluent and agricultural run off but low protein content when compared with fishes grown in ground water, cauvery water and control.

When compared with different organs of the fish, muscle had more protein content than the gills and liver.

Estimation of Carbohydrates (Table 2, Fig 2)

The muscle, gills and liver carbohydrate contents of all the fishes grown in different water treatments were significantly lower except for those fishes that

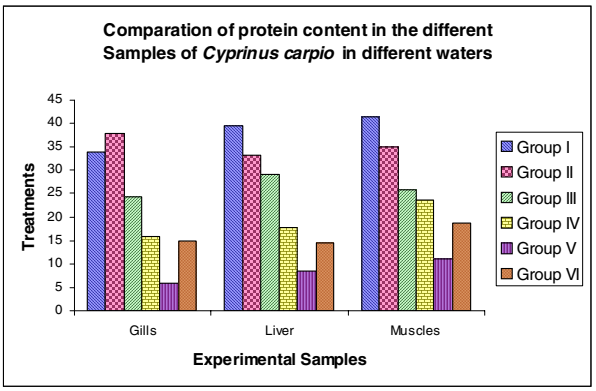


Fig. 1

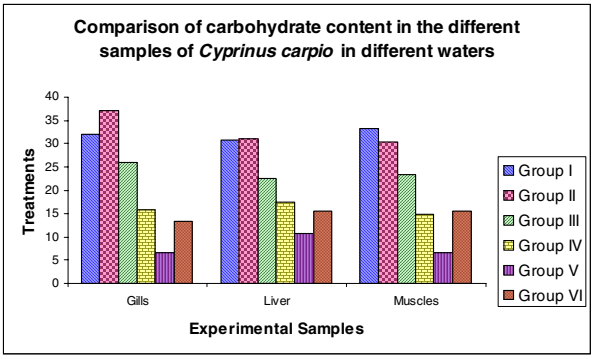


Fig. 2

Table 1. Comparison of protein content in the different samples of *Cyprinus carpio* in different waters

Organs	Treatments					
	Group I	Group II	Group III	Group IV	Group V	Group VI
Gills	33.90±8.56	37.96±9.40	24.32±6.51	15.83±4.62	5.99±2.22	14.90±4.41
Liver	39.55±9.73	33.05±8.38	29.10±7.54	17.80±5.07	8.58±2.89	14.35±4.21
Muscles	41.50±10.13	35.10±8.87	25.80±6.83	23.65±5.20	11.05±3.28	18.75±6.17

Values in artithmetic mean (AM) ± standard error (SE) with 3 replication.

Table 2. Comparison of Carbohydrate content in the different samples of *Cyprinus carpio* in different waters

Organs	Treatments					
	Group I	Group II	Group III	Group IV	Group V	Group VI
Gills	32.06±11.01	37.06±9.22	25.85±6.80	15.68±4.59	6.47±2.33	13.38±4.05
Liver	30.85±7.94	31.05±7.96	22.60±6.14	17.38±4.98	10.70±3.42	15.40±4.52
Muscles	33.10±8.39	30.30±7.80	23.22±6.28	14.75±4.37	6.60±2.38	15.60±4.57

Values in artithmetic mean (AM) ± standard error (SE) with 3 replication.

were grown in groundwater.

Fishes grown in sewage water had slightly greater carbohydrate content than the fishes grown in dye factory effluent and agriculutural run off however, had less carbohydrate content when compared to fishes grown in groundwater, cauvery water and the control.

Estimation of Total Lipids (Table 3, Fig. 3)

The lipid contents of the fishes grown in sewage water, agricultural water and dye factory effluent were low compared to the lipid content of the fishes grown in ground water, cauvery water and the control.

Compared with the different organs of the fish *Cyprinus carpio*, muscle had the highest lipid content than the gills and liver.

Effect of pollution on different concentrations of dye factory effluent (Table 4, 5, and 6), (Fig. 4,5 and 6)

The total protein content, carbohydrate content and lipid content of 1% concentratrion of dye effluent in the different organs of *cyprinus carpio* were high compared to the fishes grown 0.7 %, 0.5% and 0.25% concentration of dye factory effluent.

Compared with the different organs, liver had less protein, carbohydrate and lipid content than the muscles and gills.

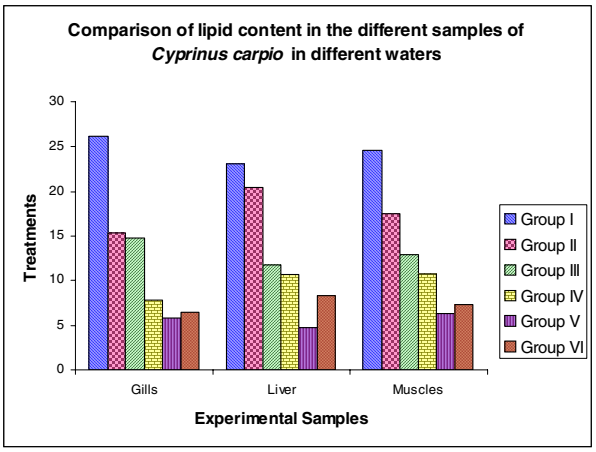


Fig. 3

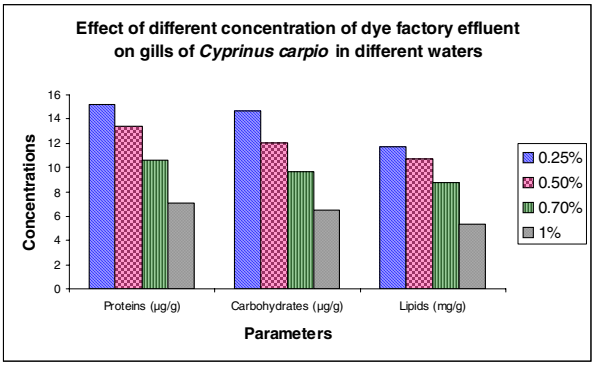


Fig. 4

Histopathology

In the present histopathological, studies the fish liver samples of the fish grown under different water samples observation were made.

The liver of the fish grown under group I (control) and group II (ground water samples) shows normal arrangement of hepatocytes with clearly brought out nuclei The present work was correlated with the result of Zimmerman (1976). The fish grown under

Table 3. Comparison of Lipid content in the different samples of *Cyprinus carpio* in different waters

Organs	Treatments					
	Group I	Group II	Group III	Group IV	Group V	Group VI
Gills	26.10±6.90	15.35±4.85	14.70±4.36	7.87±2.69	5.87±2.17	6.50±2.35
Liver	23.00±6.23	20.45±5.66	11.80±3.68	10.62±3.39	4.72±1.87	8.25±2.81
Muscles	24.50±6.50	17.50±5.00	12.90±3.94	10.83±3.45	6.34±2.31	7.28±2.56

Values in artithmetic mean (AM) ± standard error (SE) with 3 replication.

Table 4. Effect of different concentrations of dye factory effluent on gills of *Cyprinus carpio* in different waters

Parameters	Concentrations			
	0.25 %	0.5%	0.7%	1%
Proteins (µg/g)	15.23±4.56	13.39±4.06	10.65±3.47	7.09±2.51
Carbohydrates (µg/g)	14.68±4.34	12.08±3.75	9.63±3.15	6.52±2.36
Lipids (mg/g)	11.69±3.65	10.72±3.42	8.76±2.93	5.38±2.05

Values in artithmetic mean (AM) ± standard error (SE) with 3 replication.

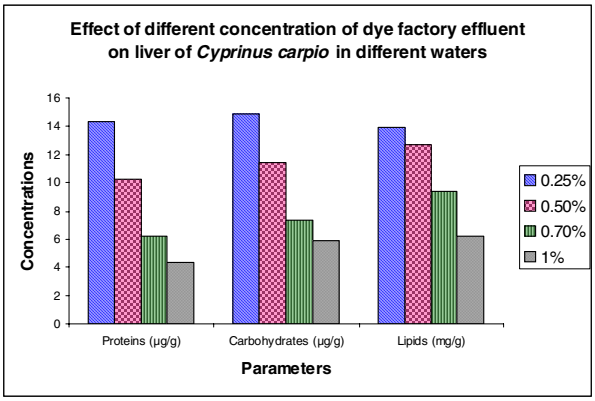


Fig. 5

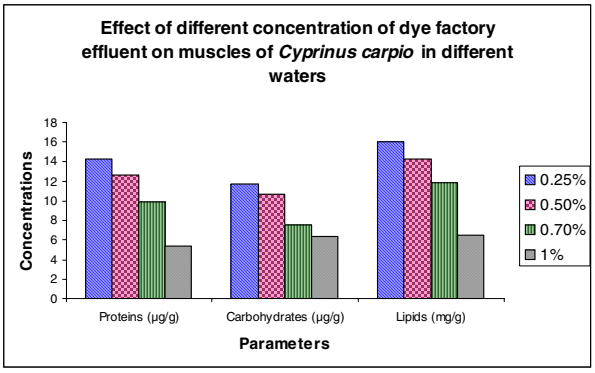


Fig. 6

group III (Cauvery water samples). The cells are slightly affected . group IV grown under dye water samples it indicates the depletion of glycogen group V sewage water samples and the hepatocellular necrosis were observed The fish grown under group VI agricultural run off water samples the cells are highly damaged. The same observation was correlates with the results of Kushal *et al .,* (1999).

Quantitative Protein Assay in Muscles of Dye Treated Fish SDS – PAGE

Intensity of the protein bands gets decreased with an increasing concentration of dye when compared to control. Nonetheless the number of protein bands increased which indicates that some of the house keeping genes or stress protein were expressed.

In our present study, the results revealed that a significant decrease in the extractable proteins, carbohydrates and total lipid contents in the different samples of *Cyprinus carpio* grown in different waters. According to James *et al.,* 1995, and Dave *et al.,* 1979. The decline has been attributed to the hydrolysis and oxidation of proteins, anaerobic breakdown of glucose available to the cells through enhanced glycogenolysis, of inhibition in the process of lipid biosynthesis. The same trend was also observed in our present study.

The observation of the intensity of the protein bands gets decreased with an increasing concentration of dye when compared to control. Nonetheless the number of protein bands increased which indicates that some of the house keeping genes or stress proteins were expressed.

The quality of ground water is getting severely affected because of the widespread pollution of surface water (Crowe and Mutch, 1994). But in our present study the fishes grown in ground water had slight decrease in the lipid content when compared to the control. There was no significant changes in the protein and carbohydrate content. This showed

Table 5. Effect of different concentrations of dye factory effluent on liver of *Cyprinus carpio* in different waters

Parameters	Concentrations			
	0.25 %	0.5%	0.7%	1%
Proteins (µg/g)	14.35±4.28	10.25±3.37	6.25±2.29	4.32±1.73
Carbohydrates (µg/g)	14.92±4.41	11.39±3.42	7.35±2.58	5.92±2.27
Lipids (mg/g)	13.93±4.18	12.67±3.89	9.38±3.09	6.25±2.29

Values in artithmetic mean (AM) ± standard error (SE) with 3 replication.

Table 6. Effect of different concentrations of dye factory effluent on muscles of *Cyprinus carpio* in different waters

Parameters	Concentrations			
	0.25 %	0.5%	0.7%	1%
Proteins (µg/g)	14.26±4.26	12.63±3.88	9.82±3.27	5.38±2.05
Carbohydrates (µg/g)	11.78±3.67	10.67±3.39	7.50±2.61	6.35±2.31
Lipids (mg/g)	15.98±4.66	14.32±4.27	11.87±3.70	6.48±2.35

Values in artithmetic mean (AM) ± standard error (SE) with 3 replication.

that the groundwater was less polluted when compared to the other experimental waters.

Cauvery river runs through one of the most densely populated regions of India. The river system has been impacted by the discharge of untreated sewage, agricultural and industrial effluents. Ramesh *et al.,* 1991). In our present study, the fishes treated in cauvery water showed less amount of protein, carbohydrate and lipid content. The less amount of this different content might be due to action of metabolites. This shows that there is contamination in cauvery water.

Without treatment, crude sewage flows into natural waters and causes pollution. Many waste products produced by animals enter the watercourse and eventually the marine environment. It was observed that fishes grown in sewage water had very low protein, the carbohydrate and lipid – content, when compared with control. Therefore, this proves that the sewage water is highly contaminated.

It is well established that a few heavy metals are essential in trace concentrations for the normal physiological functions in fishes. However, if present in higher concentration than required they can alter the physiological functions of fishes. Industrial waste from dye manufacturing plants contain toxic pollutants like acids, phenolic wastes, ammonium compounds, methyl mercaptans, arsenic, chlorine etc., in the case of acids and phenolic wastes, they affect the mucous in the gills and by direct absorption. It was observed that the fishes grown in dye factory effluent had very low protein, carbohydrate and lipid content

Cyprinus carpio exposed to dye factory effluent, the growth rate and protein conversion efficiency of the fish declined and as a consequence protein contents in the muscle and liver declined. In our present study, fishes grown in dye factory effluent showed significant decreases in the total protein, carbohydrate and lipid content.

A significant decrease in the carbohydrate content of muscle and liver was reported in mystus vittaus exposed to different industrial effluents (Sivakami *et al.,* 1994). Similar results were observed in Cyprinus carpio when it was exposed to dye effluent treatment.

Agricultural water pollution has adverse effects on fishes (Tomlinson, 1970). Pesticides kill or harm many species. It was seen that the protein, carbohydrate and lipid content was decreased to a great extend when compared with the control. This clearly proves that the agricultural runoff water was severely polluted.

The gills are the primarily uptake site of dissolved chemicals in fish. This was in consonance with the present study, which showed that among the various samples of the fish, the gill protein, carbohydrate and lipid content was very low compared to the muscle and liver protein, carbohydrate and lipid content.

Our results proved that the protein, carbohydrate and lipid contents were very much altrered when the fishes treated in different waters. Drastic changes in these contents were observed in dye factory effluent followed by agricultural run off, sewage and cauvery water. Due to less pollution in ground water, there was very less alterations in protein, carbohydrate and lipid contents present in different samples of the *Cyprinus carpio*.

In the present study, the linear samples collects from the fishers treated with different polluted water samples and the observations are coincides with the work of Zimmerman (1976).

REFERENCES

Crowe, A.S. and Mutch, J.P 1994. An expert system approach for assessing the potential for pesticide contamination of ground water. *Ground Water.* 32: 487 – 498.

Dauba, F. and Mastotilla, S. 2003. Long term recovery of microbenthos and fish assemblages after water pollution abatement measures in the river petite basic. *Arch. Environ. Contam. Toxicol.* 33: 277 – 285.

Dave, G., Johansson – Sjobeck. M.L., Larson, A. 1979. *Comp. Biochem. Physiol.* 64: 37-40.

Dubious, M., Gilles, K.A., Hami Ho, J.K., Roberts, P.A. and Smith, F. 1956. *Analt. Chem.* 28: 350-356.

Folch. J., Lees, M and Sleane – Stanely, G.H. 1957. A simple method for isolation and purification of total lipid from animal tissues J. Biol. chem. 226: 497-509. 54. James, R., Sampath, K., Sivakaumar, V ., Babu, S and Shamnugandam, R. 1995. *J. Environ, Biol.* 16: 1-16.

Johannson, D. 1999. The effects of silver on intestinal ion and acid base regulation in the marine teleost fish. *Comp. Biochem. Physiol. Pharmacol. Toxicol. Endocrinol.* 124: 259-270.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assemblage of the head of the bacteriophage T 4. *Nature*. 61: 680-685.

Kushal, Dave, Lennjan, Katysare 1999. Hepatotoxicity and microsomal function. *Environmental Toxicology and Pharmacology* 17: 67-74.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall 1951. *J. Biol. Chem.* 193: 265-275.

Morgan, J., D'cruz, L.M., Dockray, J.J., Linton, T.K., Wood, C.M., 1999. Water hardness and its influence on osmoregulation in fishes. *Ind. J. Fish.* 61: 25-28.

Pavlov, D.F., Chuko, G.M., Govovanova, I.L. 1999. In vitro effects of cadmium on intestinal protease activies in telosts. Comp. *Biochem. Physiol. Toxicol.* 122: 21-25.

Sivakami, R., Premkishore, G. and Chandra, M.R. 1994. *J. Fresh Water. Biol.* 6: 165-175.

Stanley and Alpers 1975. *Man – Made Lakes and Human health,* Academic Press, London.

Tomlinson, T.E. 1970. Trends in nitrate concentration in English rivers in relation to fertilizer use. *J. Soc. At. Treatm. Exam.* 19: 27-235.

Zimmer Man, H.J. 1976. Direct Hepatotoxocins. O. Eichier. *Client Expt. Biol.* 10: 3-15.