Available online at www.journalijmrr.com

INTERNATIONAL JOURNAL OF MODERN RESEARCH AND REVIEWS

Int. J. Modn. Res. Revs.

Volume 3, Issue 1, pp 28-35, January, 2015

ISSN: 2347-8314

ORIGINAL ARTICLE

EFFECTS OF INDUSTRIAL EFFLUENTS ON THE BIOCHEMICAL COMPONENTS OF THE FISH *Arius maculatus* FROM UPPANAR ESTUARY, CUDDALORE DISTRICT, TAMILNADU

T. Kumaresan

Department of Zoology,Annamalai University,Chidambaram,Cuddalore Dist-608002,Tamilnadu

Article History: *Received 5th January,2015, Accepted 30th Jan,2015, Published 31st January,2015*

ABSTRACT

The present study is aimed to analyse the total protein, total carbohydrates, total protein and total lipids in gill, muslce liver and kidney tissues of *Arius maculatus* exposed to sub lethal concentration industrial effluents collected from Uppanar estuary, Cuddalore, District,Tamilnadu. The level of total carbohydrates, total proteins and total lipids were decreseaed in gill, liver, muslce and kidney tissues of *Arius* maculatus exposed to sub lethal concentration industrial effluents. The present study concludes that the effect of industrial effluents alter the biochemical parameters in *Arius* maculatus

Keywords: Industrial effluents, Biochemical study, *Arius maculatus*

1.INTRODUCTION

Aquatic toxicology addresses not only all the facets related to the toxicity of chemicals to aquatic organisms, but also the chemical interaction with the life process of an ecosystem. The toxicity of chemicals in the aquatic environment is determined by interplay of inter-organismic, intra-organismic and environmental factors. On the organismic level, species, age, sex, health status, trophic level, ecological niche, toxicant level, inductive status, and physiology are determinant factors in the assessment of risk. Similarly on the environmental front, water quality, distribution, temperature, light, absorption, and solubility are the major attributes. The play of environmental factors upon the physico-chemical nature of toxicants, availability of toxicants and physiology of aquatic organisms provides a sliding scale with countless variations adding to the complexities of real hazard assessment.

Despite, the publicity spotlight on global warming due to the "green house effect", the power plants release halogenated hydrocarbons into the air which affects the ozone layer of the earth's atmosphere. Few people realize the impact of manmade chemicals on fish that live in the abyssal depths of the ocean. Continual pollution of our ocean could result in greater damage to the ecosystem and may soon present just as serious a threat to the global ecosystem like "green house effect". Not only these chemicals and their toxic metabolites can have an impact on aquatic organisms, but as members of the food web they inturn affect the consumers; and if left unchecked it would turn into the case of the murdered takes his revenge

Effluent and run off from fields comprising chemicals of versatile nature, exert their toxic effects on fish population by depleting the dissolved oxygen, altering the pH, salinity and changing the carbondioxide content (Soundarapandian *et al.*, 2009; Sankaran *et al.*, 2011) thereby directly or indirectly affecting the life cycle as well as the metabolic pathways of the fish at the biochemical level (Puvaneswari *et al.*, 2009). Man- made pollution is perhaps the biggest threat to the estuaries, in many instances; estuaries are being used as “sewers and sinks” for untreated waste water. Pollution of estuaries is difficult to assess because of the special qualities of this ecosystem; estuarine pollution is different from river pollution as the pollutants remain trapped in the ecosystem for a long period due to tidal isolation; and pollution damage of estuaries is the product of man's as well as nature's activities.

Contrary to the opinion that the seas are bottomless pits, man is now realizing that they have very specific ocean floors and a limit to the quantum of solute they can hold. Water pollution is now proving to be one of the main causes for public health hazards. Most of the Indian rivers are

Corresponding author: *Dr.* ***T. Kumaresan*** *Assistant Professor, Department of Zoology,Annamalai University, Annamalainagar-608002, Tamilnadu*

seriously polluted by industrial effluents carrying toxic chemicals which bring about death or sublethal pathological changes particularly in liver, kidney, respiratory, reproductive and nervous tissues of the aquatic animals (Singh *et al.,* 1997; Purushothaman and Chakrapani, 2007; Pandey *et al.,* 2005).

Fish have survived millions of years in the most diverse, adverse and advanced environments. Many organisms have been used as bioindicators in polluted environment, including fish. Fish are known to accumulate metals from waste water and therefore, can act as an environmental indicator and also biomonitor for any pollutant (Widianarko *et al.,* 2000). It is expected that all known mechanisms of chromosomal changes could have occurred in the evolution resulting in a characteristic karyotype. Fish karyotypes are not identical, as in humans or other animal species, so we do not have a standard karyotype for fish species (Karuppasamy *et al.,* 2010).

Metals released through effluents are accumulated in organic form in fish and other marine organisms. Such heavy metal toxicity is believed to have impact on the metabolism (Karuppasamy, 2001), physiology (Karuppasamy *et al.,* 2005) and alterations in fish larvae development (Puvaneswari *et al.,* 2009). Local people who consume fish from the polluted water have to face grave consequences. Birds and mammals, living on fish meal also cycle mercury. Ramana *et al.* (2001) noted that sewage sludge is known to contain high concentration of metals and its use in agriculture has resulted in the accumulation within vegetable and edible tissues of plants. Similarly many aquatic organisms like crab, fish and molluscs also bioaccumulate with various metals from the surrounding waste water (Faronibi *et al.,* 2007; Puvaneswari and Karuppasamy, 2008; Subathra and Karuppasamy, 2008).

2.MATERIALS AND METHODS

Fish *Arius maculatus* was collected from three different stationsat Uppanar estuary and they were immediately transferred to ice box.From the experimental station of Uppanar estuary, fish were caught either using cast net or hand lines. Cast net are made of nylon. The mesh varies from 10 to 15 mm. Hand lines with mono filament and hooks were used with prawn or mussel as bait for the capture of *Arius maculatus*. The fish were transported to the laboratory and maintained in the same way as the control fish collected from Perumal Lake. The water was renewed once in two days.

Procurement of fish

Live specimens of *Arius maculatus* with an average length of 8.5 ± 0.50 cm and weight of 15.0 ± 0.5 g were collected from Uppanar brackish water by operating cast net. The fish were acclimatised in the aquaria of 120 litres capacity containing well aerated sea water (salinity 28 ‰; pH 7.69; oxygen content 4.32 mg/l) and water temperature (32.6°C) for a period of one week prior to experiment. During acclimatization, the fish were fed withchopped prawn and clams. Food was withheld one day before the' commencement of the experiment. The water was changed along with waste feed and faecal matter every 24 hours. Fish collected from Perumal lake (Plate 1A) were used as control and Uppanar brackishwater area was selected as experimental site

Acute Toxicity Test

The raw and partially treated effluent was collected from the discharging point of industries surrounding the Uppanar eaturry for acute toxicity test. In the acute toxicity bioassay, mortality could be observed within a short period. LC_{50} was calculated by the following method of Finney (1978) to observe mortality and behavioural response of the test fish, *Arius maculatus* on exposure to effluents of different concentrations.

Static acute toxicity was employed to evaluate the adverse effects of industrial effluents surrounding the Uppanar estuary on the fish, *Arius maculatus* under standardised laboratory conditions.

Food was withheld one day before the toxicity test with a view to avoid the possible change in the toxicity of the pollutants after addition of the effluent into the test tank with 100 litres of sea water having 10 fishes. Mortality was recorded after 24, 48, 72 and 96 hr and five replicates were maintained simultaneously for the purpose. Fishes showing respiratory and lack of response to tactile stimuli were considered, nearing dead and removed immediately. Percentage mortality was calculated and the values were subjected to Probit analysis (Reddy *et al.,* 1992). Confidential limits (upper and lower) of the Regression coefficient with Chi - square test were calculated.

Design of sublethal toxic study

Sublethal studies are helpful to assess the response of the test organism to stress caused by the effluents. Based on acute toxicity test two sublethal concentrations (2% and 6%) on *Arius maculatus* were derived and used as the experimental concentrations. Sublethal of safe level concentration were derived from 96 hr LC_{50} value.

In the present study 2% (1/50) and 6% (1/30) dilution (in *Arius maculatus*) of the 96 hr LC_{50} were selected as sublethal concentrations. The experimental fish were exposed in each concentration for a period of 7, 15 and 30 days. A control batch corresponding to each test group was maintained simultaneously.

Estimation of Total Carbohydrates

The amount of total carbohydrates present in the extracts was estimated by Anthrone method (Roe, 1955). 100 mg of the different tissues like gill, muscle, liver and kidney were weighed and homogenized by a glass homogenizer using 5 ml of freshly prepared 5% trichloro acetic acid (TCA). The content was centrifuged for 10 minutes at 3000 rpm and the supernatant was collected and mixed with 10 ml of anthrone reagent (500 mg of anthrone and 10 g of thiourea dissolved in one litre of 66% sulphuric acid by volume) and then incubated for 15 minutes in a boiling waterbath. Finally the colour intensity was measured at 620 nm in Bausch and Lomb Spectronic 21 Spectrophotometer. Glucose was taken as standard and the values were expressed in µ/g wet tissue.

Estimation of Total Proteins

The total proteins in the above tissues were determined by following the procedure of Lowry *et al.* (1951) with Folin-phenol reagent.

100 mg of wet tissue of gill, muscle, liver and kidney was weighed and homogenised in glass homogeniser using 1 ml of

1 N sodium hydroxide solution and centrifuged for 10 minutes at 3000 rpm. 1 ml of the supernatant was taken and mixed with 5 ml of alkaline copper reagent (prepared by mixing 1 volume of 0.5% copper sulphate in 1% sodium tartarate with 50 volumes of 2% sodium carbonate in 0.1 N sodium hydroxide). The contents was mixed well and allowed to stand for 10 minutes at room temperature and then 0.5 ml of 1 N Folin - Ciocalteau reagent was added quickly and mixed well for a second or two and read in Bausch and Lomb Spectronic 21 Spectrophotometer at 550 nm. The unknown protein concentration of the tissue samples was calculated by comparing with the standard curve obtained by using Bovine serum albumin. The results were expressed in µg/g wet tissue.

Estimation of Total Lipids

Lipid in tissues was extracted as per the method of Folch *et al.* (1957) and the estimation as described by Barnes and Blackstock (1973). 50 mg of wet tissue of gill, muscle, liver and kidney were homogenized with 5 ml of chloroform methanol (2:1 v/v) and 0.2 ml of 0.9% sodium chloride solution was added to the homogenized contents, which was mixed well and centrifuged at 3000 rpm for about 10 minutes. Using a syringe the lower phase was separated free from fluff. The volume was then made to the original quantity of 5 ml with chloroform. This method is based on the sulphophosphovanillin reaction, which depends on the reaction of lipids with sulphuric acid, phosphoric acid and vanillin to give a red coloured complex. 0.5 ml of extract was measured into a clean test tube and left to dry overnight. The dried lipid was dissolved in 0.5 ml of concentrated sulphuric acid and mixed well. The tubes were plugged with non - absorbent cotton wool and they were placed in a boiling water bath for 10 minutes. The test tubes were then cooled to room temperature. From this acid digest, 0.5 ml of aliquots was taken and 2.5 ml of vanillin reagent (2 mg of vanillin powder in 800 ml of 88% phosphoric acid and 200 ml of distilled water) was added. The tubes were shaken well and allowed to stand for 30 minutes. The colour developed was read at 520 nm using Spectronic 21 Spectrophotometer. The results were expressed in µg/g wet tissue. Standard and blank were run simultaneously using standard cholesterol (E Merck). Standard solution of cholesterol was prepared by dissolving 80 mg cholesterol in 100 ml of 2% chloroform and methanol. This was diluted to 1: 10 in the solvent prior to the experiment.

Statistical Analysis

Data from the present studies were subjected to Standard deviation and the significance of difference obtained was assessed by two way ANOVA for the study between the various periods of exposure as well as concentrations. The significant difference within the groups of exposure periods and within the concentrations were separately assessed by one way ANOVA and by the Turkey - HSD test (Multiple range test). The 't' test was assessed to study the significant difference between the control group and the test samples of field study using SPSS package (Statistical package for social science). Acute toxicity study was analysed using Probit analysis and Chi - square test.

3.RESULTS

Level of total carbohydrates in tissues

Gill

The total carbohydrate content in the control groups during the exposure period of 7, 15 and 30 days were estimated to be 8.173, 8.018 and 7.188 µg/g respectively (Table 1). In the lower sublethal concentration (2%) at the different periods of exposure showed a decreased value of 8.098 (for 7 days), 7.183 (for 15 days) and 7.095 µg/g (for 30 days). At 6% sublethal concentration, there was a decline in the total carbohydrates during different exposure periods (7, 15 and 30 days) and indicated the values as 7.813, 7.100 and 6.690 µg/g respectively (Table 1).

Muscle

The total carbohydrate content in the control groups during the exposure period of 7, 15 and 30 days were estimated to be 8.303, 6.890 and 6.780 µg/g respectively (Table 1). In the lower sublethal concentration (2%) at the different periods of the exposure, showed a lower value of 7.698 (for 7 days), 6.795 (for 15 days) and 6.468 µg/g (for 30 days). Similarly at 6% sublethal concentration there was a decline in the total carbohydrates during the different exposure periods and indicated the values as 7.490, 6.600 and 6.115 µg/g on 7, 15 and 30 days respectively (Table 1).

Liver

Total carbohydrate content in the control groups during the exposure period of 7, 15 and 30 days were estimated to be 8.198, 8.175 and 7.190 µg/g respectively (Table 1)At the lower sublethal concentration (2%) during the different periods of exposure showed a value of 7.720 (for 7 days), 7.698 (for 15 days) and 7.183 µg/g (for 30 days) respectively. At 6% sublethal concentration, there was a decline in the total carbohydrates during different exposure periods (7, 15 and 30 days) and indicated the values as 7.495, 7.292 and 7.098 µg/g respectively (Table 1).

Kidney

Total carbohydrate content in the control groups during the exposure period of 7, 15 and 30 days were estimated to be 7.023, 6.875 and 6.598 µg/g respectively (Table 3.35 and Graph 14). In the same way the lower sublethal concentration (2%) at the different periods of exposure showed a declining value of 6.885 (for 7 days), 6.292 (for 15 days) and 5.703 µg/g (for 30 days). Similarly at 6% sublethal concentration, there was a fall in the total carbohydrates during different exposure periods (7, 15 and 30 days) and indicated the values as 6.685, 6.098 and 5.097 µg/g respectively (Table 1).

The total carbohydrate content in the tissues of field specimen showed a decreased value in the carbohydrates as 6.150, 5.393, 6.403 and 5.217 µg/g with respect to the control group as 7.118, 6.232, 7.318 and 6.098 µg/g respectively (Table 3.39 and Graph 15).

The Two way ANOVA for quantification of carbohydrates in all the tissues showed a significant value at 1% level between the exposure days and the two sublethal concentrations (Table 3.36). One way analysis of variance on the muscle showed an insignificant value between the control and experimental concentrations. The Multiple range test for gill and liver showed a significant value at 5% level particularly between

control and 6% concentration. Similarly, the kidney showed a significant value at 5% level between control and the two sublethal concentrations Generally all the tissues showed a significant value at 1% level irrespective of the periods of exposure. Besides, multiple range tests for liver showed a significant value at 5% level. An analysis on the total carbohydrate content in the field tissue sample showed a significant attribute at 1% level

Table 1 Quantification of carbohydrate (µg/g wet wt) in the tissues of *Arius maculatus* exposed to sublethal concentrations (% 96 hr LC_{50}) of the effluent

Period of exposure	Tissues	Concentration level (% 96 hr LC_{50})		
		Control	2%	6%
7 Days	Gill	8.173 ± 0.816	8.098 ± 0.894	7.813 ± 0.543
	Muscle	8.303 ± 0.624	7.698 ± 0.157	7.490 ± 0.265
	Liver	8.198 ± 0.457	7.720 ± 0.548	7.495 ± 0.254
	Kidney	7.023 ± 0.218	6.885 ± 0.248	6.685 ± 0.259
15 Days	Gill	8.018 ± 0.125	7.183 ± 0.549	7.100 ± 0.354
	Muscle	6.890 ± 0.247	6.795 ± 0.324	6.600 ± 0.359
	Liver	8.175 ± 0.654	7.698 ± 0.548	7.292 ± 0.541
	Kidney	6.875 ± 0.549	6.292 ± 0.862	6.098 ± 0.264
30 Days	Gill	7.188 ± 0.324	7.095 ± 0.548	6.690 ± 0.856
	Muscle	6.780 ± 0.652	6.468 ± 0.248	6.115 ± 0.642
	Liver	7.190 ± 0.459	7.183 ± 0.324	7.098 ± 0.365
	Kidney	6.598 ± 0.687	5.703 ± 0.284	5.097 ± 0.851

Values are mean of five replications ± SD

Level of of total proteins in tissues

Standard known weight of major tissues such as gill, muscle, liver and kidney were isolated from control and experimental fish to determine the total amount of proteins. The total amount of proteins was estimated following the protocol of toxicity including the exposure periods of 7, 15 and 30 days under treated condition of the effluent for the two sublethal concentrations at 2% and 6% (Table 2).

Gill

The total amount of proteins estimated in the gill of control sample revealed a mean value of 78.791, 70.690 and 68.682 µg/g for an exposure periods of 7, 15 and 30 days respectively (Table 2). But experimental sample exposed to lower sublethal concentration of 2% at different exposure periods however, showed a lower value of 66.200 (for 7 days), 59.035 (for 15 days) and 54.215 µg/g (for 30 days) of proteins. Similarly at higher sublethal concentration of 6% the values declined in the total proteins during different exposure periods (7, 15 and 30 days) and the values were 62.390, 49.603 and 45.890 µg/g respectively (Table 2).

Muscle

The total amount of proteins in the muscle of the control sample were estimated to be 99.592, 96.595 and 91.693 µg/g for an exposure period of 7, 15 and 30 days respectively (Table 3.40 and Graph 17). Similarly at lower sublethal concentration of 2% for the different exposure periods showed a value of 85.390 (for 7 days), 81.305 (for 15 days) and 77.293 µg/g (for 30 days). Contrastingly at higher sublethal concentration of 6% showed a decline in the total proteins during different exposure periods (7, 15 and 30 days) and indicated the values as 80.298, 70.403 and 65.190 µg/g (Table 2).

Liver

The total amount of proteins estimated in the liver of the control samples were 85.495, 81.480 and 78.574 µg/g towards the exposure periods of 7, is and 30 days respectively. In the lower sublethal concentration of 2% for the different exposure periods showed a value of 73.463 (for 7 days), 67.278 (for 15 days) and 64.397 µg/g (for 30 days). Similarly at higher sublethal concentration of 6% showed a decline in the total proteins for the same exposure periods (7, 15 and 30 days) and indicated the values as 66.393, 57.293 and 57.103 µg/g wet wt (Table 2).

Kidney

The total proteins estimated in the kidney of the control sample revealed a mean value of 73.973, 70.791 and 66.875 µg/g for the exposure periods of 7, 15 and 30 days respectively (Table 3.40 and Graph 19). The lower sublethal concentration of 2% at different exposure periods showed a value of 61.598 (for 7 days), 57.393 (for 15 days) and 55.193 (for 30 days) µg/g. Similarly at higher sublethal concentration of 6% the total protein content was declined during different exposure periods (7, 15 and 30 days) and indicated the values as 53.298, 47.620 and 43.578 µg/g (Table 2).

The total proteins estimated in the tissues of field specimen such as gill, muscle, liver and kidney of *Arius maculatus* showed a decline in the protein level as 54.595, 61.078, 75.598 and 60.298 µg/g with respect to control group 75.170, 83.270, 96.428 and 72.253 µg/g respectively (Table 3.44 and Graph 20).

The Two way ANOVA for quantification of proteins in the tissues showed a significant difference at 1% level between the exposure days and the two sublethal concentrations (Table 3.41) The F value confirmed significance of the above values between the two concentrations in all the tissues. Furthermore, it indicated that there was a significant variation within the given concentrations at 5% level. The multiple range test showed a significant value in kidney between 7 and 30 days of exposure. The F value showed an insignificant attribute between the periods of exposure in all the tissues. In the field tissue samples of *A. maculatus* a significant value in the total protein at 1% level was observed between the control and test samples.

Table 2 Quantification of protein (µg/g wet wt) in the tissues of *Arius maculatus* exposed to sublethal concentrations (% 96 hr LC_{50}) of the effluent

Period of exposure	Tissues	Concentration level (% 96 hr LC_{50})		
		Control	2%	6%
7 Days	Gill	78.791 ± 0.325	66.200 ± 0.365	62.390 ± 0.653
	Muscle	99.592 ± 0.321	85.390 ± 0.695	80.298 ± 0.251
	Liver	85.495 ± 0.365	73.463 ± 0.369	66.393 ± 0.325
	Kidney	73.973 ± 0.213	61.598 ± 0.345	53.298 ± 0.263
15 Days	Gill	70.690 ± 0.362	59.035 ± 0.632	49.603 ± 0.348
	Muscle	96.595 ± 0.324	81.305 ± 0.895	70.403 ± 0.287
	Liver	81.480 ± 0.365	67.278 ± 0.365	57.293 ± 0.152
	Kidney	70.791 ± 0.254	57.393 ± 0.562	47.620 ± 0.127
30 Days	Gill	68.682 ± 0.265	54.215 ± 0.354	45.890 ± 0.214
	Muscle	91.693 ± 0.689	77.293 ± 0.265	65.190 ± 0.264
	Liver	78.574 ± 0.378	64.397 ± 0.365	57.103 ± 0.450
	Kidney	66.875 ± 0.365	55.193 ± 0.452	43.578 ± 0.261

Level of total lipidsin tissues

Standard known weight of major tissues such as gill, muscle, liver and kidney were isolated from control and experimental samples to determine the total amount of lipids. The total amount of lipids was estimated in accordance to the exposure period of 7, 15 and 30 days for the two sublethal concentrations of 2% and 6% (Table 3).

Gill

Analysis of total lipids in the gill of the control sample indicated a mean value of 7.393, 6.388 and 6.300 µg/g for the exposure periods 7, 15 and 30 days respectively. In the lower sublethal concentrations of 2% for the different exposure periods showed a decline in the lipid level as 6.093 (for 7 days), 5.915 (for 15 days) and 5.698 µg/g (for 30 days). Similar trend was repeated at higher sublethal concentration of 6% and the corresponding values were 5.493, 5.413 and 4.293 µg/g for the different exposure periods (Table 3.45 and Graph 21). The F value confirmed significance of the above values between the two concentrations in all the tissues. Furthermore, it indicated that there was a significant variation within the given concentrations at 5% level (Table 3.46). The multiple range test showed a significant value in kidney between 7 and 30 days of exposure (Table 3.45). The F value showed an insignificant attribute between the periods of exposure in all the tissues. In the field tissue samples of *Arius maculatus* a significant value in the total lipid at 1% level was observed between the control and test samples (Table 3).

Muscle

Analysis of total lipids in the muscle of the control sample indicated a mean value of 6.890, 6.710 and 6.598 µg/g for the exposure periods of 7, 15 and 30 days respectively (Table 3). At lower sublethal concentrations of 2% for the different exposure periods showed a lower lipid level as 6.393 (for 7 days), 6.190 (for 15 days) and 5.368 µg/g (for 30 days). Similar trend was shown at higher sublethal concentration of 6% and the values were 5.855, 5.535 and 4.598 µg/g for the different exposure periods (7, 15 and 30 days) respectively (Table 3).

Liver

Analysis of total lipids in the liver of the control sample indicated a mean value of 10.218, 9.895 and 9.823 µg/g for the exposure periods of 7, 15 and 30 days respectively (Table 3). Similarly at the lower sublethal concentrations of 2% for different exposure periods showed a decline in the lipid level as 9.195 (for 7 days), 9.190 (for 15 days) and 9.103 µg/g (for 30 days). Similar trend was shown at higher sublethal concentration of 6% and the values were 8.798, 8.383 and 8.20511µg/g for the different exposure periods (7, 15 and 30 days) respectively (Table 3).

Kidney

Analysis of total lipids in the kidney of the control sample indicated a mean value of 9.805, 9.618 and 9.358 µg/g for the exposure periods of 7, 15 and 30 days respectively (Table 3). But in the lower sublethal concentrations of 2% for the different exposure periods showed a decline in the lipid level as 9.215 (for 7 days), 9.180 (for 15 days) and 9.100 µg/g (for 30 days). Similar trend was shown at higher sublethal concentration of 6% and the values were recorded as 9.127, 8.498 and 8.238 µg/g for the different exposure periods (7, 15 and 30 days) respectively (Table 3).

Analysis of the total lipids in the tissues of field specimen (gill, muscle, liver and kidney) of *Arius maculatus* showed a decline in the lipid level as 5.310, 5.553, 8.218 and 8.380 µg/g with respect to the control group (6.842, 6.840, 10.403 and 9.692 µg/g) respectively.The Two way ANOVA on the quantification of total lipids in the tissues showed a significant value at 1% level, between the exposure periods and the two sublethal concentrations (Table 3.46). Furthermore the F value confirmed a significant relation between concentration at 1% level in all the tissue (Table 3.47). Except liver and kidney, all the tissues showed a significant value for the periods of exposure. Similarly the Multiple range test showed a significant attribute at 5% level in the gill, muscle and kidney between 7 and 30 days of exposure. The interaction between the pollutant and the organism can be understood properly if the various biochemical changes that take place inside the body of the organism are known. Begum (2004), Luskova *et al.* (2002) stated that health of the organisms is directly related to the chemical composition of the organism concerned. However, a clear picture of the metabolic changes that could take place under stressed conditions is not clearly understood. Hence, the present investigation is pursued to understand the biochemical interaction that occurs in the tissues of *A. maculatus* exposed to different concentration of the effluent.

Table 3 Quantification of lipid (µg/g wet wt) in the tissues of *Arius maculatus* exposed to sublethal concentrations (% 96 hr LC_{50}) of the effluent

Period of exposure	Tissues	Concentration level (% 96 hr LC_{50})		
		Control	2%	6%
7 Days	Gill	7.393 ± 0.362	6.093 ± 0.124	5.493 ± 0.314
	Muscle	6.890 ± 0.215	6.393 ± 0.167	5.855 ± 0.245
	Liver	10.218 ± 0.324	9.195 ± 0.157	8.798 ± 0.365
	Kidney	9.805 ± 0.324	9.215 ± 0.124	9.127 ± 0.284
15 Days	Gill	6.388 ± 0.324	5.915 ± 0.164	5.413 ± 0.340
	Muscle	6.710 ± 0.245	6.190 ± 0.127	5.535 ± 0.284
	Liver	9.895 ± 0.325	9.190 ± 0.135	8.383 ± 0.654
	Kidney	9.618 ± 0.240	9.180 ± 0.176	8.498 ± 0.324
30 Days	Gill	6.300 ± 0.365	5.698 ± 0.143	4.293 ± 0.327
	Muscle	6.598 ± 0.247	5.368 ± 0.245	4.598 ± 0.482
	Liver	9.823 ± 0.249	9.103 ± 0.249	8.205 ± 0.159
	Kidney	9.358 ± 0.365	9.100 ± 0.365	8.238 ± 0.178

Values are mean of five replications ± SD

4. DISCUSSION

Total carbohydrates

It is established fact that tissue level carbohydrate plays a major role as energy precursors for fish exposed to stress conditions (Umminger, 1970;Karuppasamy, 2000d. In the present study a significant depletion of carbohydrate in the tissue of the fish was observed. The effects on the carbohydrate metabolism observed during exposure are in general agreement with alterations noted in chronically exposed rainbow trout (Larsson and Hause, 1982) and catfish, *Heteropneustes fossilis* (Sastry and Subhadra, 1982).

Decrease in carbohydrate may be attributed to the utilization of energy in view of increase in acitivity under stress condition. Excessive carbohydrate depletion was reported by Berry and Symthe (1959) in mice heavily poisoned with

sodium arsenite. Carbohydrate depletion was reported by Szinciz and Forth (1988) in view of toxicity caused by trivalent arsenicals. A similar decrease was observed in the midgut gland and muscle of *Metapenaeus monoceros* exposed to methyl parathion, carbaryl and aldrin. Jayaprada *et al.* (1991) reported inhibition of glycolysis with the onset of gluconeogenesis in *Penaeus indicus* treated with phosphamidon. Besides, Soundarapandian *et al.* (1997) reported maximum reduction in total carbohydrates in the muscle, hepatopancreas and in the body.

The decrease in the carbohydrate may be due to the decrease in the glycogenesis or gluconeogenesis (Szinciz and Forth, 1988). Increase in the breakdown of glycogen would suggest a higher energy demand in the animals living under extreme stress conditions including stress by toxicants. This is confirmed with the work done by Shoba Rani *et al.* (2000).

It is obvious that decline of carbohydrate content in the gill, muscle and liver was more pronounced than the kidney exposed to sublethal concentration of the effluent. Such depletion of carbohydrate could be a response to withstand the toxicants which imposed stress conditions in the fish (Keller and Andrew, 1973).

Venkata Reddy (1993) observed a decline in the glycogen content in the gill, muscle, liver and kidney of the fish exposed to the raw effluent for 30 days in the field also showed depletion in the total carbohydrates in different proportions. This confirms the findings of Begum (2004). It may also be due to the prevalence of hypoxic and anoxic conditions which normally increase carbohydrate utilization. Similarly, a decrease in the whole animal consumption, tissue respiratory potentials and a condition similar to hypoxia was already noticed in the fish *Oreochromis mossambicus* (Janardana Reddy *et al.*, 1991), *Labeo rohita* (Janardana Reddy *et al.*, 1998) and fresh water snail, *Lumnaea acuminata* (Tripathi and Singh, 2002) after exposure to phosalone.

A decline in the glycogen content in the of fish, *Clarias batrachus* exposed to fluoride (Anand kumar *et al.*, 2007) and crab *Oziotelphusa senex* exposed to phosalone (Venkata Reddy, 1993) suggested that it may be due to either a reduction in glycogenesis or increased glycogen utilization through the glycolytic pathway.

Thus the influence of pollutants of the estuary has induced modulations in carbohydrate metabolic profile of the experimental fish and it can be considered as an index for determining the environmental pollution. The exposures of the fish to the effluent have resulted in the depletion of carbohydrates which may be attributed to the decrease in the synthesis of carbohydrates.

Total proteins

Proteins are one of the most important group of biological materials comprising of nitrogenous constituent performing distinct biological functions. Therefore an assessment of protein content may be considered as a diagnostic tool to determine the physiological status of an organism (Manoj and Ragothaman, 1999). Proteins are highly sensitive to heavy metals and hence indicators of heavy metal poisoning (Jacobs *et al.*, 1977). The impairment of protein synthesis due to heavy metal stress has been reported by many investigators (Yoshino *et al.*, 1966; Kuznetsov *et al.*, 1984). Any alteration that takes place in the protein turnover, may have an adverse impact on the synthesis of organic molecules. Jana and Choudhari (1984) reported moderate depletion of protein content in the liver and intestine of fish due to the invasion of heavy metals and similar observations were also made by Shakoori *et al.* (1976), Rath and Misra (1980), Ramalingam (1990) and Vega *et al.* (2002).

Incidentally the current pursuit disclosed a significant decrease in the total protein of gill, muscle, liver and kidney at all intervals of study. The decline was constant and deviated from that of control after 7, 15 and 30 days of exposures to 2% and 6% concentrations of the effluent and along with the exposure to raw effluent in the field for 30 days.

The decline in the protein suggests an intestinal proteolysis in the respective tissues which in turn could contribute to the hike of free amino acids to be fed into the tricarboxylic acid cycle as ketoacid, and it support the hypothesis of Kabeer (1979). Begum (2004) who stated that qualitative and quantitative variation in the aminoacids of tissues exposed to toxicants. Anand Kumar *et al.* (2007) observed that the protein content was decreased in fresh water male catfish (*Clarias batrachus*, Linn.) exposed to toxicity of fluoride. Nevertheless, the fish may eventually succumb due to its inability to cope up with such environment. The decreased protein content in the fish is also in general agreement with Karuppasamy (2000b) who reported the conversion of protein into amino acid residues so as to increase aminoacid pool. Inhibition in the protein content was reported to be possible due to non - selective blocking of phosphorylation process in the central nervous system and tissues (Kuznetsov *et al.*, 1984 and Kawamata *et al.*, 1987). Umminger (1970) attributes protein as an energy source to spare during the chronic period of stress.

Total lipids

Lipids are the primary source of essential fatty acids necessary for anabolism. Currently a significant decrease in the total lipids in view of 30 days of exposure in the field as well as 2% and 6% sublethal concentrations for the different exposure periods was observed in relation to the respective control groups and it may be attributed to the severity of stress which demands more energy.

Similar decrease in the levels of total lipids in the tissues of the freshwater prawn, *Macrobrachium lamarrei* treated with tannery effluent was reported by Maruthanayagam *et al.* (1996). Depletion in the lipid reserves in the present study might be attributed to the utilization of lipids for the energy demand as warranted by the situation of stress (Roe and Rao, 1981; Begum and Vijayaragavan, 2001). Anand Kumar *et al.* (2007) observed that the lipid content was decresead in fresh water male catfish (*Clarias batrachus*, Linn.) exposed to toxicity of fluoride. Thus the biochemical perturbation is an indicator of altered metabolic activities due to the stress of pollutants of the estuary.

Anandkumar, S., Tripathi, N., and M. Tripathi, 2007. Fluoride-induced biochemical changes in fresh water catfish (*Clarias batrachus*, Linn.) *Res. Report Fluor.*, **40(1)**: 37-41.

Barnes, H., and J. Blackstoch, 1973. Estimation of lipid in marine animals and tissues. Detailed investigations of the sulphophosphovanillin method for total lipids.

J. Expt. Mar. Biol. Ecol., **12**: 103-118.

Begum, G., 2004. Carbofuran insecticide induced biochemical alterations in liver and muscle tissues of the fish *Clarias batrachus* (linn) and recovery response. *Aquat. Toxicol.,* **66**: 83–92

Begum, G., and S. Vijayaraghavan, 2001. Carbofuran toxicity on total lipids and free fatty acids in air breathing fish during exposure and cessation of exposure *in vivo*. *Environ. Monit. Assess.,* **70**: 233–239

Berry, L. J., and D. S. Symthe, 1959. Carbohydrate metabolism in normal and altitude exposed mice following arsenite poisoning. *Am. J. Physiol.,* **97**: 37-40.

Farombi, E.O., Adelowo, O.A., and Ajimoko, Y.R. 2007biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African cat fish (*clarias gariepinus*) from Nigeria Ogun river. Int. J. environ. Res. Public health 4: 158 –165.

Folch, J., Lees, M., and G. S. H. Stanley, 1957. A simple method or the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.,* **226**: 497-508.

Jacobs, J.M. N. Carmichael and J.B. Cavanagh. 1977. Ultra structural changes in the nervous system of rabbits poisoned with methyl mercury. *Toxicol. Appl. Pharmacol.*, 39: 249-261.

Jana, B., and M. A. Choudhari, 1984. Synergistic effect of heavy metal pollutants on senescence in submerged aquatic plants. *Water Air Soil Poll.*, **21**: 351-357.

Janardana Reddy, S., Kalaivani, V., Tharakanatha, B., Reddy., and R. Ramamurthi, 1998. Changes in energy metabolism of the fish *Labeo rohita* in relation to prolonged lead exposure and recovery. *J. Ecotoxicol. Environ. Monit*., **8(1)**: 43-53.

Janardana Reddy, S., Venkata Reddy, B., and R. Ramamurthi, 1991. Impact of chronic phosalone toxicity on erythropoietic activity of fish, *Oreochromis mosambicus. Biochem. Inf.,* **23(3)**: 547-552.

Jayaprada, P., Reddy, M.S., and K. V. Rao, 1991. Subacute physiological stress induced by phosphamidon on carbohydrate metabolism in midgut gland of prawn, *Penaeus indicus. Biochem. Inf.,* **23(3)**: 507-514.

Kabeer A. S.J. 1979. Studies on some aspects of protein metabolism and associated enzyme systems, in the freshwater teleost *Tilapia mossambica* to malathion exposure. Ph.D. Thesis, *Sri Venkateswara University*, Tirupati, pp. 136-184.

Karuppasamy, R. 2000. Effect of phenyl mercuric acetate on carbohydrate content of *Channa punctatus* Uttar Pradesh *J. Zool.* 20(3): 219-225.

Karuppasamy, R., 2001. Effect of phenyl mercuric acetate on the succinic and lactic dehydrogenase activities in the tissue of fish *C. punctatus* (Bloch). *J. Exp. Zool. Ind.,* **4**: 81-91.

Karuppasamy, R., Subathra, S., and S. Puvaneswari, 2005. Haematological responses to exposure to sublethal concentration of cadmium in air breathing fish *Channa punctatus* (Bloch.). *J. Environ. Biol.,* **26(1)**: 123-128.

Karuppasamy, R., Zutshi, B., and K. Bhavani, 2010. Karyotype of a bagrid catfish, *Mystus vittatus* from the fresh water system of Chidambaram, Tamil Nadu, India. *Sci. Acta.*, **36**: 157-160.

Kawamata, O., Kasoma, H., Omata, S., and H. Sugano, 1987. Decrease in protein phosphorylation in control and peripheral nervous tissue of methylmercury treated rats. *Arch. Toxcicol.,* **59(5)**: 345-352.

Keller, R., and E. M. Andrew, 1973. The site of action of the crustacean hyperglycemic hormone. *Gen. Camp. Endocrinol.,* **20**: 572-578.

Kuznetsov, D. A., Zivijalov, A., Govorkov, V., and M. T. Sibileba, 1984. Methyl mercury induced non - selective blocking phosphorylation process as a possible causes of protein synthesis inhibition *in vitro* and *in vivo. Toxicol. Lett.,* **36(2)**: 153-160.

Larsson, A., Haux, C., Sjobeck, M. L. and Lithner, G. 1985. Physiological effects of an additional stressor on fish exposed to a simulated heavy-metal-containing effluent from a sulfide ore smeltery. *Ecotoxicol. Environ. Saf.* Vol. 8. P. 118–128.

Lowry, O.H., Rosenbrough, N.J., Farr. A and Randall. R.J 1951. Protein measurement with folin phenol reagent. *J. Biol Chem.* 193: 265 - 273.

Luskova, V., Svoboda, M. and J. Kolarova, 2002. The effect of Diazinon and blood plasma biochemistry in carp (*Cyprinus carpio*). *Acta. Vet. Biol.,* **71**: 117-123.

Manoj, K., and G. Ragothaman, 1999. Mercury, copper and cadmium induced changes in the total protein level in muscle tissue of an edible estuarine fish *Boleophthalmus dussumieri* (Cuv). *J. Environ. Biol.,* **20(3)**: 231-234.

Maruthanayagam, C., Krishnamoorthy, P., and P. Subramanian, 1996. Effect of tannery effluent on the biochemical constituents of the freshwater prawn *Macrobrachium lamerrei* (H. Milne Edwards). *J. Environ. Biol.,* **17(4)**: 285-294.

Pandey, S., R.Kumar., N.S.Sharma., S.K.Sirivastava and M.S.Verma, 2005. Acute toxicity bioassays of mercuric chloride and malathion on air breathing fish *Channa punctatus* (bloch). *Ecotoxicol. Environ. Saf*., 61:114-120.

Purushothaman, P., and G. J. Chakrapani, 2007. Heavy metals fractionation in Ganga river dediments, India. *Environ. Monit. Assess.*, **132**: 475–489.

Puvaneswari, S., Marimuthu, K., Karuppasamy, R., and M. A. Haniffa, 2009. Early embryonic and larval development of Indian cat fish *Heteropneustes fossilis*. *Eur. Asian J. Biosci.*, **3**: 84-96.

Ramana, A., Biswas, A. K., Kundu, S., Saha, S., and R. B. R. Yadav, 2001. Efficacy of distellary effluent on seed germination and seedling growth in mustard, cauliflower and radish. *Proc. Na. Acad. Sci. India,* **71**: 29-135.

Rath, S., and B. N. Misra, 1980. Changes in nucleic acid and protein content of *Tilapia mossambiea* exposed to dichlores (DDVP). *Indian J. Fish,* **27**: 76-81.

Roe, J. R., 1955. The determination of sugar in blood and spinal fluid with anthros reagents. *J. Biol. Chem.,* **20**: 335-343

Sankaran, S., Sonkamble, S., and K. Krishnakumar, 2011. Integrated approach for demarcating subsurface pollution and saline water intrusion zones in SIPCOT area: a case study from Cuddalore in Southern India. *Environ. Monit. Assess.,* doi.10.1007/s10661-011-2327-9.

Sastry K.V and Subhadra K. 1982. Effects of cadmium on some aspects of carbohydrate metabolism in a fresh water cat fish *Heteropnenskes fossilis*. *Toxicol. Lett*., 14 (1-2): 45-55.

Shakoori, A. R., Saleem, A. Z., and S. A. Mohammed, 1976. Effect of malathion, dieldrin and endrin on blood serum proteins and free amino acid pool in *Channa punctatus* (Bloch). *Pak J. Zool.,* **8**: 124-134.

Shoba Rani, A., Sudharsan, R., Reddy, T. N., Reddy, P. U. M., and T. N. Raju, 2000. Effect of sodium arsenite on glucose and glycogen levels in freshwater teleost fish, *Tilapia mossambica. Poll. Res.*, **19(1)**: 129-131.

Singh, M., Ansari, A. A., Mueller, G., and I. B. Singh, 1997. Heavy metals in freshly deposited sediments of the Gomati river (a tributary of the Ganga river): effects of human activities. *Environ. Geol.,* **29**: 246–252.

Soundarapandian, P., Kannupandi, T. K., and M. John Samuel, 1997. Effect of starvation on biochemical composition of freshwater prawn juveniles of *Macrobrachium malcolmsonii* (H. Milne Edwards). *Indian J. Expt. Biol.,* **35(5)**: 502-505.

Szinciz, L., and W. Forth, 1988. Effect of As_20_3 on gluconeogenesis. *Arch. Toxicol.,* **61**: 444-449.

Tripathi, P. K., and A. Singh, 2002. Toxic effect of dimethoate and carbaryl pesticides on carbohydrate metabolism of fresh water snail *Lumnaea acuminata*. *Bull. Environ. Contam. Toxicol.* **68**: 606–611.

Umminger, B. L., 1970. Physiological studies on super cooled hillfish *Pundulus heteroclitus.* III. Carbohydrate metabolism and survival at subzero temperature. *J. Expt. Zool.,* **173**: 159-174.

Venkata Reddy, B., 1993. Effect of organo phosphorus insecticide phosalone on aspects of hydro mineral and energy metabolism in the field crab, *Oziotelphusa sensenece* (Fabricus) Ph.D. Thesis, *Sri Venkateswara University,* Tirupati, India, pp. 110-145.

Yoshino, V., Mozai T., and K. Makoda, 1966. Biochemical changes in the brain in rats poisoned with an alkyl - mercury compound with special reference to inhibitions of protein synthesis in brain cortex slices: *J. Neurochem.,* **13**: 1223-1230.