Life Science Informatics Publications
Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences
Journal Home page http://www.rjlbpcs.com/
RJLBPCS
ISSN 2454-6348

**Original Research Article** **DOI: 10.26479/2019.0503.21**

# STUDY ON THE TOXICITY OF COPPER ON BIOCHEMICAL COMPOSITION OF *LITOPENAEUS VANNAMEI* (BOONE 1931)

**S. Arul Prasath[1], V. Valarmathi[2], K. Muthukumaravel[3*]**

1. Department of Zoology, Thiru Vi Ka Government Arts College, Thiruvarur, Tamil Nadu, India.
2. Department of Zoology, A.D.M. College for Women, Nagapattinam, Tamil Nadu, India.
3. P.G. & Research Department of Zoology, Khadir Mohideen College, Adirampattinam, Tamil Nadu, India.

**ABSTRACT:** The effect of sub lethal concentrations of copper on biochemical components such as carbohydrate, protein and lipids were studied in the pacific white shrimp *Litopenaeus vannamei* up to 30 days at an interval of 10 days. The exposed pacific white shrimp are exhibited differential changes in biochemical constituents. The changes were dependent on the period of exposure and concentrations of copper. The effect of 30% sub lethal concentration was to be pronounced than that of 10% sub lethal concentration.

**KEYWORDS:** Toxicity of Copper, *Litopenaeus vannamei*, Biochemical Composition.

**Corresponding Author: Dr. K. Muthukumaravel*** Ph.D.
P.G. & Research Department of Zoology, Khadir Mohideen College, Adirampattinam, Tamil Nadu, India.

## 1. INTRODUCTION

Heavy metal pollution of aquatic ecosystem has long been recognized as a serious environmental concern because of their persistency and tendency to accumulate in organisms undergo food chain amplification. As a result, metal bioaccumulation is a major route through which increased levels of the pollutants are transferred across food chain and creating public health problems wherever man is involved in the food chain. Therefore, it is important to always determine the bioaccumulation capacity for heavy metals by organisms especially the edible ones in order to assess potential risk in human health. Although voluminous data are available on the toxic effect of different pollutants on the aquatic organisms, only few studies are documented on the impact of heavy metals on prawn.


Peer review under responsibility of Life Science Informatics Publications

Hence the present investigation is attempted to elucidate the impact of the heavy metal copper on the prawn *Litopenaeus vannamei*. These studies would ultimately focus the attention on the water criteria, nature and the tolerable extent of the accumulation of heavy metal copper which form the basic steps helpful for conservation of aquatic organisms and to prevent possible disorders caused to human beings by way of biomagnifications of these pollutants.

## 2. MATERIALS AND METHODS

### Prawn Acclimatization

The *Litopenaeus vannamei* of the carapace length of 3.0±0.5 cm and breath of 4.0 ± 0.5 cm were selected for the experiment and were collected from Muthupet mangroves (10° `-20° N, 79° 35`E), Tiruvarur district. Prawns were screened for any pathogenic infections. Plastic troughs were washed with 1% $KMnO_4$ to avoid fungal contamination and then sun dried. Healthy prawns were then transferred to plastic troughs (20" diameter) containing estuarine water (Temperature 29±3°C; DO 4.2 ± 0.5 mg/l; salinity 24.1±0.17 ppt and pH 8.6 ± 0.05). Prawns were acclimated to laboratory conditions for 10 to 15 days prior to experimentation. They were regularly fed with natural food and the medium (estuarine water) was changed daily to remove faeces and food remnants.

### Heavy Metal for Toxicity Studies

Toxicity studies were conducted to obtain reliable data regarding the effects of the toxicant on the test species. Static bioassay tests were conducted as per standards set by the American Public Health Association [4]. The toxicant sample used possessed the following characteristics.

### Heavy metal Copper

Chemical properties of copper

| | | |
|---|---|---|
| Atomic number | : | 29 |
| Atomic mass | : | 63.546 $g.mol^{-1}$ |
| Electro-negativity according to Pauling | : | 1.9 |
| Density | : | 8.9 $g.cm^{-3}$ at 20°C |
| Melting point | : | 1083 °C |
| Boiling point | : | 2595 °C |
| Vanderwaals radius | : | 0.128 nm |
| Ionic radius | : | 0.096 nm (+1) ; 0.069 nm (+3) |

### Acute Toxicity Test

Toxicity tests were conducted in accordance with standard methods [5]. Stock solution of copper with a concentration of 1 g per litre (equivalent to 1 ppt) was prepared in distilled water and different dilutions were prepared by adding required amount of distilled water. Based on the progressive bisection of intervals on a logarithmic scale, log concentrations were fixed after conducting the range finding test. The prawns were starved for 24 hours prior to their use in the experiments as

recommended by storage to avoid any interference in the toxicity of copper by excretory products. After the addition of the toxicant into the test plastic troughs with 5 litres of water having ten prawns, mortality was recorded after 24, 48, 72 and 96 hours. Five replicates were maintained simultaneously. Percent mortality was calculated and the values were transferred into probit scale. Probit analysis was carried out [7]. Regression lines of probit against logarithmic transformations of concentrations were made. Confidential limits (upper and lower) of the regression line with chi-square test were calculated by a computerized programme for Finney's probit analysis [7].

**Chronic Toxicity Test**

Based on acute toxicity test (96h $LC_{50}$) sublethal concentrations (10% and 30%) were derived for copper which were used as the experimental concentration of the copper in the subsequent experiments. Ten prawns were exposed to each concentration for a period of 10, 20 and 30 days. A control batch was maintained simultaneously and six trails were run. The total carbohydrate, protein and lipids were estimated by earlier researchers [8] [14] [21] respectively.

## 3. RESULTS AND DISCUSSION

**Total Carbohydrate**

In the prawn reared as control the carbohydrate content was the highest in hepatopancreas (12.41-12.81 mg/g), followed by muscle (9.22-9.48 mg/g) and low in gill (7.69-7.89 mg/g). The heavy metal copper appeared to cause individually, pronounced dose and time dependent decrease in the carbohydrate content of all tested tissues (Table 1). On day 30, carbohydrate content of *Litopenaeus vannamei* was found to decrease respectively by 44.85 and 65.85% in the hepatopancreas, 47.28 and 63.12 in the gill and 37.55 and 62.45 in the muscle of prawn exposed to 10 and 30% sublethal concentration of copper. In the present study, the highest depletion of carbohydrate content was noted after 30 days of urea exposure in all the tested tissues (Table 8). The percentage depletion was higher on prawn exposed to the 30% sublethal concentration of copper than these exposed to 10% sublethal concentration of copper. Table 8 shows that the change in carbohydrates was dependent significantly on the concentration of copper as well as period of exposure for most of the tissues.

Significant changes in the carbohydrate content in hepatopancreas were also observed in the experiments at different time periods ($F=13426$ and $P<0.01$). Treatment versus duration TXD showed an F value 2861.24 and $P<0.01$(Table 9). Sublethal concentrations of copper have produced significant changes in the carbohydrate content in gill ($F=2930.97$ and $P<0.01$). Similarly the values in the exposure periods showed (TXD) significant changes ($F=607.53$ and $P<0.01$) (Table. 9). Significant changes were also observed in the carbohydrate content of muscle during the time periods ($F=3129.68$ and $P<0.01$). Treatment versus duration (TXD) gave an F value of 770.28 and $P<0.01$ (Table 4).

**Protein**

In *Litopenaeus vannamei* kept as control protein content was the highest in muscle (75.4-76.62mg/g),

followed by hepatopancreas (45.5-46.1 mg/g) and moderate values were observed in gill (31.8-32.61 mg/g). Decrease in protein levels was noted in all the tissues of prawn exposed to the copper (Table 2). The maximum decrease of protein content was observed in the tissues of prawn exposed to 30% sublethal concentration of copper reared for 30 days: 42.3% in hepatopancreas, 44.19 in gill and 44.16 in muscle. Significant changes in the protein content in hepatopancreas were also observed in the experiments at different time periods ($F=988.29$ and $P<0.01$). Treatment versus duration TXD showed an F value 24.66 and $P<0.01$(Table 5). Sublethal concentrations of copper have produced significant changes in the protein content in gill ($F=400.05$ and $P<0.01$). Similarly the values in the exposure periods showed (TXD) significant changes ($F=90.44$ and $P<0.01$) (Table 11). Significant changes were also observed in the protein content of muscle during the time periods ($F=3236.40$ and $P<0.01$). Treatment versus duration (TXD) gave an F value of 562.53 and $P<0.01$ (Table 5).

**Lipid**

The concentration of lipid in the normal prawn was found to be maximum in hepatopancreas (39.81-40.88 mg/g) followed by muscle (20.15-21.31 mg/g), and in gill (17.98-18.31 mg/g). In the prawn exposed to sublethal concentrations of copper, significant decrease were observed in the content of lipid in all the tissues (Table 3). In prawn exposed to the highest sublethal concentration of copper (30%) for 30 days, the concentration of lipid decreased to31.90, 43.37 and 63.88 respectively in the hepatopancreas, gill and muscle. For 10% sublethal concentration of copper the decrease in lipid content was to a lesser extent (Table 12). In general, the decrease in lipid content was maximum in gill and liver compared to hepatopancres, gill and muscle. Significant changes in the lipid content in hepatopancreas were also observed in the experiments at different time periods ($F=2186.12$ and $P<0.01$). Treatment versus duration TXD showed an F value 334.93 and $P<0.01$(Table 6). Sublethal concentrations of copper have produced significant changes in the lipid content in gill ($F=1560.85$ and $P<0.01$). Similarly the values in the exposure periods showed (TXD) significant changes ($F=245.45$ and $P<0.01$) (Table 6). Significant changes were also observed in the lipid content of muscle during the time periods ($F=10990.65$ and $P<0.01$). Treatment versus duration (TXD) gave an F value of 1356.66 and $P<0.01$ (Table 6). The carbohydrate of fishes comprised mainly glycogen and total free sugars and the fluctuations in the carbohydrate content may be due to accumulation and utilization of glycogen and total free sugars at different phases of life like growth, gametogenesis and spawning. In aquatic organisms, generally the carbohydrate reserves may be rapidly utilized under unfavourable conditions and the great variations found in the tissues indicate that the level of mobilizable carbohydrate reserves may fluctuate widely and rapidly in response to fluctuations in the nutritional state of the animal. In the present study the carbohydrate content decreased in the gill tissues of *Litopenaeus vannamei* exposed to sublethal concentrations of copper (Table 1). The fenvalerate exposed *Ctenopharyngodon idellus* showed a decrease in the carbohydrate content in the various tissues [25]. The decrease in total carbohydrate level signifies its utility possibly to meet

the higher energy demands of the fish reeling under metal toxicity. The synthesis and utilization of carbohydrate are therefore, altered in the organism subjected to copper stress. Carbohydrates which supply the major portion of the metabolities for the energy requirements in a normal individual is oxidized for the energy requisites. Carbohydrates may be converted to glycogen or shunted in the metabolic pathway to supply the carbon chain for amino acids or converted in to fat (Priscilla, 1985). At sublethal concentration, when the liver carbohydrate content decreased the haemolyph sugar level increased which suggests the breakdown of hepatopancreas glycogen (glycogenolysis). The mobilization of glucose from the hepatopancreas to the haemolymph and its availability for utilization by the needy tissues for ensuring normal metabolic processes in the body appears inevitable when the prawn is exposed to toxic medium. Many authors have reported decreased carbohydrate level in various tissues of aquatic organisms. The decreased carbohydrate in the brain of *Heteropneustes fossilis* is observed when it is exposed to carboxyl [22]. The decrease in glycogen content of brain in *C. punctatus* after the exposure of endosulfan is reported [17]. In *C. punctatus*, quantative variations in the sugar content of liver and muscle tissues due to pesticidal exposure [28]. In the present study the muscle carbohydrate content of *Litopenaeus vannamei* showed a decrease when it was exposed to sublethal concentrations of copper (Table 1). A fall in muscle carbohydrate level in *L. rohita* when exposed to tannery, electroplating and textile effluents [16]. The high concentration of Nuvacron caused a decline in muscle carbohydrate level in *C. punctatus* [23]. These observations were in conformity with the reports on the fall in muscle glycogen level in *C. punctatus*, when exposed to organophosphate pesticide, Dimethoate [26]. Studies in general have suggested that exposure to metal treatment interferences with the carbohydrate metabolism. A greater decrease of carbohydrate content indicates greater utilization of carbohydrate to cope with enhanced metabolism under stressful situations. Despite a continuous and rapid release of glucose by glycogenolysis in the hepatopancreas, to meet the energy requirement for the increased muscular activity, a fall in the overall of carbohydrate content in prawns subjected to heavy metal treatment is imminent. Proteins are mainly involved in the architecture of the cell. During chronic period of stress, they are also a source of energy [27]. Protein is the most important constituent in living tissues, which is of considerable metabolic and structural value. Therefore, any change in this constituent indicates the stress inflicted on the metabolic functions required for maintaining a healthy physiological state. In this work the protein content of *Litopenaeus vannamei* at different sublethal concentrations decreased in all exposure periods. The depletion in tissue protein of *Litopenaeus vannamei* indicated rapid utilization of energy stores to meet the energy demands warranted by the environment. The observed depletion in tissue protein on treatment with sublethal doses of heavy metal was suggestive of proteolytic activity, possibly to meet the excess energy demands under toxic conditions. The depletion in the protein content in stomach and intestine of *Clarias batrachus* exposed to pesticides endosulfan, malathion and agrofen were reported [12]. The decrease in protein

content of *Lepidocephalichthys thermalis* exposed to sublethal concentrations of fenvalerate is recorded [11]. A significant decrease has been reported in the protein content of the liver and kidney in *Labeo rohita*, when exposed to 20% active ingredient EC. Fenvalerate [2]. A similar decrease in the total and soluble protein content has been observed with fenvalerate in fish [15] [25]. The total protein level of hepatopancreas, gill and muscle were decreased after all the three periods of exposure to the sublethal concentrations of urea. The investigations has revealed a decrease in protein content in *T. mossambica* exposed to different pesticides [13]. The protein content is decreased in liver, muscle, kidney, intestine, brain and gill when *C. punctatus* has been treated with quinaphos [24]. The decreased levels of proteins in gills, testis, ovaries and muscles of marine crab *Uca marionis* exposed to acute and chronic levels of Malathion [30]. The sublethal concentrations of Malathion showed a significant increase in the protein content in kidney of exposed fish during the first week and thereafter, a gradual decrease in protein content has been observed in the later periods of exposure [6]. Lipid is an important constituent of animal tissue, which plays a prime role in energy metabolism. Lipids are also important in cellular and sub-cellular membranes. A gradual decreases in lipid content in various tissues of *Litopenaeus vannamei* after chronic treatments of copper are shown in Table 12. Earlier researchers also suggested that the decrease in lipid content in *C. carpio* may be either due to the uptake of lipid by the tissue for utilization at cellular levels or due to increased lipolysis or mitochondrial injury, which affect the fatty acid oxidation mechanism [3][20][29]. The considerable decrease in total lipid in tissues might be due to drastic decrease in glycogen content in the same tissue which is an intermediate source of energy during toxic stress conditions. After glycogen, lipid content may be used for energy production to overcome toxic stress. Some workers support these results in which lipid content decreased in animals after exposure to pollutants. The significant decrease in lipid of *L. rohita* when exposed to heavy metal cadmium is reported [9]. The effect of dairy effluent on *O. mossambicus* was observed and reported that lipid content was decreased [1]. Similar decrease in lipid content level has also been observed in *C. punctatus* when exposed to mercurial fungicide [19]. Reduction of lipid content of *Litopenaeus vannamei* in this study may have been due to the utilization of lipids for energy demand under stress condition [10].

**Table 1. Levels of total carbohydrate in selected tissues of *Litopenaeus vannamei* exposed to sublethal concentrations of copper**

| Exposure Period | 10 Days | | | 20 Days | | | 30 Days | | |
|---|---|---|---|---|---|---|---|---|---|
| **Tissues** | **HP** | **GL** | **MC** | **HP** | **GL** | **MC** | **HP** | **GL** | **MC** |
| Control | 12.41± 0.56 | 7.71± 0.67 | 9.22± 0.78 | 12.81± 1.02 | 7.69± 0.85 | 9.46± 0.91 | 12.62± 0.69 | 7.89± 0.78 | 9.48± 0.68 |
| 10% SLC | 10.11± 0.81 | 6.92± 0.61 | 8.11± 0.89 | 8.53± 0.72 | 5.52± 0.68 | 7.22± 1.14 | 6.96± 0.73 | 4.16± 0.56 | 5.92± 0.47 |
| % Variation | -18.53 | -10.25 | -12.04 | -33.41 | -28.22 | -23.68 | -44.85 | -47.28 | -37.55 |
| 30% SLC | 9.61± 0.56 | 5.69± 0.38 | 7.32± 0.71 | 6.21± 0.78 | 4.32± 0.59 | 5.64± 0.65 | 4.31± 0.73 | 2.91± 0.61 | 3.56± 0.55 |
| % Variation | -22.56 | -26.2 | -20.61 | -51.52 | -43.82 | -40.38 | -65.85 | -63.12 | -62.45 |

Values are mean ± SD of six observations. – or + indicate percent decrease or increase over control

HP- hepatopancreas; GL – gill; MC – muscle

**Table 2. Levels of total protein in selected tissues of *Litopenaeus vannamei* exposed to sublethal concentrations of copper**

| Exposure Period | 10 Days | | | 20 Days | | | 30 Days | | |
|---|---|---|---|---|---|---|---|---|---|
| **Tissues** | **HP** | **GL** | **MC** | **HP** | **GL** | **MC** | **HP** | **GL** | **MC** |
| Control | 45.5± 0.65 | 32.12± 0.71 | 76.62± 0.69 | 45.9± 0.73 | 31.8± 0.58 | 76.18 ±0.47 | 46.1± 0.39 | 32.61± 0.47 | 75.4± 0.35 |
| 10% SLC | 42.1± 0.51 | 30.5± 0.61 | 70.4± 0.58 | 38.5± 0.67 | 28.2± 0.49 | 62.1± 0.38 | 31.2± 0.46 | 25.6± 0.51 | 51.5± 0.66 |
| % Variation | -7.47 | -5.04 | -8.12 | -29.19 | -11.32 | -18.48 | -32.32 | -21.5 | -31.7 |
| 30% SLC | 37.5± 0.71 | 26.4± 0.65 | 61.6± 0.58 | 32.3± 0.47 | 22.5± 0.51 | 52.2± 0.56 | 26.6± 0.66 | 18.2±0. 72 | 42.1± 0.59 |
| % Variation | -17.58 | -17.8 | -19.6 | -29.63 | -29.25 | -31.48 | -42.3 | -44.19 | -44.16 |

Values are mean ± SD of six observations. – or + indicate percent decrease or increase over control

HP- hepatopancreas; GL – gill; MC – muscle

**Table 3. Levels of total lipid in selected tissues of *Litopenaeus vannamei* exposed to sublethal concentrations of copper**

| Exposure Period | 10 Days | | | 20 Days | | | 30 Days | | |
|---|---|---|---|---|---|---|---|---|---|
| **Tissues** | **HP** | **GL** | **MC** | **HP** | **GL** | **MC** | **HP** | **GL** | **MC** |
| Control | 40.88 ±0.68 | 18.21± 0.57 | 21.31± 0.47 | 40.11 ±0.38 | 17.98 ±0.51 | 20.15 ±0.61 | 39.81 ±0.87 | 18.31 ±0.79 | 21.15± 0.68 |
| 10% SLC | 38.11 ±0.64 | 16.17± 0.51 | 17.21± 0.49 | 34.27 ±0.21 | 14.32 ±0.52 | 13.27 ±0.62 | 31.65 ±0.69 | 12.65 ±0.39 | 10.65± 0.47 |
| % Variation | -6.78 | -11.20 | -19.24 | -14.6 | -20.36 | -34.14 | -20.5 | -30.91 | -49.65 |
| 30% SLC | 35.19 ±0.51 | 14.32± 0.64 | 13.97± 0.38 | 30.61 ±0.46 | 11.22 ±0.51 | 10.12 ±0.38 | 27.11 ±0.51 | 9.27± 0.46 | 7.64±0. 38 |
| % Variation | -13.92 | -21.36 | -34.44 | -23.68 | -37.6 | -49.78 | -31.90 | -43.37 | -63.88 |

Values are mean ± SD of six observations. – or + indicate percent decrease or increase over control

HP- hepatopancreas; GL – gill; MC – muscle

**Table.4. Two way ANOVA table showing the significance of the effect copper on the total Carbohydrates of *Litopenaeus vannamei***

**ANALYSIS OF VARIANCE FOR CARBOHYDRATES – HEPATOPANCREAS**

| SV | DF | SS | MS | F |
|---|---|---|---|---|
| REP (R) | 2 | 0.0242074 | 0.0121037 | 5.87 * |
| TREATMENT | 8 | 221.4956741 | 27.6869593 | 13426.99 ** |
| T (T) | 2 | 162.4090296 | 81.2045148 | 39380.73 ** |
| FACTOR D | 2 | 35.4866741 | 17.7433370 | 8604.76 ** |
| TxD | 4 | 23.5999704 | 5.8999926 | 2861.24 ** |
| ERROR | 16 | 0.0329926 | 0.0020620 | |
| TOTAL | 26 | 221.5528741 | | |

cv = 0.5%

** = significant at 1% level; * = significant at 5% level

**ANALYSIS OF VARIANCE FOR CARBOHYDRATE - GILL**

| SV | DF | SS | MS | F |
|---|---|---|---|---|
| REP (R) | 2 | 0.00227407 | 0.00113704 | <1 |
| TREATMENT | 8 | 78.34367407 | 9.79295926 | 2930.97 ** |
| T (T) | 2 | 55.47754074 | 27.73877037 | 8302.03 ** |
| FACTOR D | 2 | 14.74654074 | 7.37327037 | 2206.77 ** |
| TxD | 4 | 8.11959259 | 2.02989815 | 607.53 ** |
| ERROR | 16 | 0.05345926 | 0.00334120 | |
| TOTAL | 26 | 78.39940741 | | |

cv = 1.0%

** = significant at 1% level

**ANALYSIS OF VARIANCE FOR CARBOHYDRATES - MUSCLE**

| SV | DF | SS | MS | F |
|---|---|---|---|---|
| REP (R) | 2 | 0.00108889 | 0.00054444 | <1 |
| TREATMENT | 8 | 98.44586667 | 12.30573333 | 3129.68 ** |
| T (T) | 2 | 69.27620000 | 34.63810000 | 8809.41 ** |
| FACTOR D | 2 | 17.05482222 | 8.52741111 | 2168.75 ** |
| TxD | 4 | 12.11484444 | 3.02871111 | 770.28 ** |
| ERROR | 16 | 0.06291111 | 0.00393194 | |
| TOTAL | 26 | 98.50986667 | | |

cv = 0.9%

** = significant at 1% level

**Table.5. Two way ANOVA table showing the significance of the effect copper on the total protein of *Litopenaeus vannamei***

**ANALYSIS OF VARIANCE FOR PROTEIN - HEPATOPANCREAS**

| SV | DF | SS | MS | F |
|---|---|---|---|---|
| REP (R) | 2 | 0.046667 | 0.023333 | <1 |
| TREATMENT | 8 | 1278.186667 | 159.773333 | 988.29 ** |
| T (T) | 2 | 878.126667 | 439.063333 | 2715.86 ** |
| FACTOR D | 2 | 241.846667 | 120.923333 | 747.98 ** |
| TxD | 4 | 158.213333 | 39.553333 | 244.66 ** |
| ERROR | 16 | 2.586667 | 0.161667 | |
| TOTAL | 26 | 1280.820000 | | |

cv = 1.0%

** = significant at 1% level

**ANALYSIS OF VARIANCE FOR PROTEIN - GILL**

| **SV** | **DF** | **SS** | **MS** | **F** |
|---|---|---|---|---|
| REP (R) | 2 | 0.0482741 | 0.0241370 | <1 |
| TREATMENT | 8 | 538.4012741 | 67.3001593 | 400.05 ** |
| T (T) | 2 | 396.2960296 | 198.1480148 | 1177.85 ** |
| FACTOR D | 2 | 81.2445630 | 40.6222815 | 241.47 ** |
| TxD | 4 | 60.8606815 | 15.2151704 | 90.44 ** |
| ERROR | 16 | 2.6916593 | 0.1682287 | |
| TOTAL | 26 | 541.1412074 | | |

cv = 1.5%

** = significant at 1% level

**ANALYSIS OF VARIANCE FOR PROTEIN - MUSCLE**

| SV | DF | SS | MS | F |
|---|---|---|---|---|
| REP (R) | 2 | 0.348652 | 0.174326 | 1.21 ns |
| TREATMENT | 8 | 3741.305785 | 467.663223 | 3236.40 ** |
| T (T) | 2 | 2644.214074 | 1322.107037 | 9149.47 ** |
| FACTOR D | 2 | 771.950052 | 385.975026 | 2671.09 ** |
| TxD | 4 | 325.141659 | 81.285415 | 562.53 ** |
| ERROR | 16 | 2.312015 | 0.144501 | |
| TOTAL | 26 | 3743.966452 | | |

cv = 0.6%

** = significant at 1% level; ns = not significant

**Table.6. Two way ANOVA table showing the significance of the effect copper on the total lipids of *Litopenaeus vannamei***

**ANALYSIS OF VARIANCE FOR LIPIDS - HEPATOPANCREAS**

| SV | DF | SS | MS | F |
|---|---|---|---|---|
| REP (R) | 2 | 0.1196741 | 0.0598370 | 1.83 ns |
| TREATMENT | 8 | 571.5171185 | 71.4396398 | 2186.12 ** |
| T (T) | 2 | 403.6912074 | 201.8456037 | 6176.67 ** |
| FACTOR D | 2 | 124.0453630 | 62.0226815 | 1897.95 ** |
| TxD | 4 | 43.7805481 | 10.9451370 | 334.93 ** |
| ERROR | 16 | 0.5228593 | 0.0326787 | |
| TOTAL | 26 | 572.1596519 | | |

cv = 0.5%

** = significant at 1% level; ns = not significant

**ANALYSIS OF VARIANCE FOR LIPIDS - GILL**

| SV | DF | SS | MS | F |
|---|---|---|---|---|
| REP (R) | 2 | 0.0640296 | 0.0320148 | 1.55 ns |
| TREATMENT | 8 | 258.3499185 | 32.2937398 | 1560.85 ** |
| T (T) | 2 | 200.6479407 | 100.3239704 | 4848.95 ** |
| FACTOR D | 2 | 37.3883185 | 18.6941593 | 903.54 ** |
| TxD | 4 | 20.3136593 | 5.0784148 | 245.45 ** |
| ERROR | 16 | 0.3310370 | 0.0206898 | |
| TOTAL | 26 | 258.7449852 | | |

cv = 1.0%

** = significant at 1% level; ns = not significant

**ANALYSIS OF VARIANCE FOR LIPIDS - MUSCLE**

| SV | DF | SS | MS | F |
|---|---|---|---|---|
| REP (R) | 2 | 0.0062519 | 0.0031259 | <1 |
| TREATMENT | 8 | 629.4794519 | 78.6849315 | 10990.65 ** |
| T (T) | 2 | 503.6494519 | 251.8247259 | 35174.69 ** |
| FACTOR D | 2 | 86.9792519 | 43.4896259 | 6074.60 ** |
| TxD | 4 | 38.8507481 | 9.7126870 | 1356.66 ** |
| ERROR | 16 | 0.1145481 | 0.0071593 | |
| TOTAL | 26 | 629.6002519 | | |

cv = 0.6%

** = significant at 1% level

## ACKNOWLEDGEMENT

The authors express profound thanks to the Secretary, Principal, A.D.M. College for Women, Nagapattinam and Head of the Department of Zoology, Thiru Vi Ka Government Arts College, Thiruvarur for providing facilities to carry out this work.

## CONFLICT OF INTEREST

Authors have no conflict of interest.

**REFERENCES**


1. Amutha P, Sangeetha G and Mahalingam S. Diary effluent induced alterations in the protein, carbohydrate and lipid metabolism of a freshwater teleost fish *Oreochromis mossambicus*. Poll. Res. 2002; 21(1): 51-53.
2. Annamani AK. Toxicity of fenvalerate technical grade and 20% Ec to *Labeo rohita* and *Aplocheilus panchox*. M.Phil., Thesis submitted to Nagarjuna University, Nagarjuna Nagar, 1986.
3. Anusha A, Cyril Arunkumar I,Elizabeth Jayanthi F and Selvanayagam M. Quinolphos induced biochemical anomalies in *Cirrhinus mrigala* (Ham.). J. Environ. Biol., 1996; 17(2): 121-124.
4. APHA. Standard methods for the examination of water and waste water (American Public Health Association, Washington, D.C.), 1976; p.1193.
5. APHA. American Public Health Association, American Waterwork Association, Water Pollution Control Federation, standard methods for the examination of water and waste water, 1992; p.1193.
6. Aruna Khare, Sudha Singh and Keerthy Shrivastava. Malathion induced biochemical changes in the kidney of freshwater fish *Clarias batrachus*. J. Ecotoxicol Environ. Monit., 2000; 10(1): 11-14.
7. Finney DJ. Probit Analysis. Cambridge University Press, London, 1971; 333.
8. Floch J, Lees M and Sloane-Stanley SH. A simple method for isolation and purification of total lipids from animal tissues. J. Biol. Chem., 1957; 226: 497-507.
9. Hameed SVS and Muthukumaravel K. Impact of cadmium on the biochemical constituents of fresh water fish *Oreochromis mossambicus*. Indian J. Env. Sci., 2006; 10(1): 63-65.
10. Harpert A, Rodwell NM and Mayer A. A review of physiological chemistry.16, Edition, California Lange, Medical Publication, 1977; 269.
11. Jebakumar SRD, Flora SDJ, Ganesan RM, Jagathesan G and Jayaraman J. Effect of short term sublethal exposure of cypermethrin on the organic constituents of the fresh water fish *Lepidocephalichthys thermalis*. J. Environ. Biol., 1990; 11(2): 203-209.
12. Jha BS and Verma BP. Effect of pesticidal mixture on protein content in the freshwater fish *Clarias batrachus.* J. Ecotoxicol. Environ. Monit., 2002; 12(3): 177-180.
13. Koundinya PR and Ramamurthi R. Haematological studies in *Sarotherodon mossambicus* exposed to lethal ($LC_{50}$/48hrs) concentration of Sumathion and Sevin. Curr. Sci., 1979; 48: 877-879.
14. Lowery OH, Rosebrough NJ, Farr AL and Randall RJ. Protein measurement with folin phenol reagent. J. Biol. Chem., 1951; 193: 265-275.
15. Malla Reddy P and Bashamohideen MD. Toxic impact of fenvalerate on protein metabolism in the branchial tissue of a fish *Cyprinus carpio.* Curr. Sci., 1988; 57: 211-212.

16. Muley DV, Karanjakar DM and Maske SV. Impact of industrial effluents on the biochemical composition of fresh water fish *Labeo rohita*. J. Environ Biol., 2007; 28(2):245-249.
17. Murthy AS and Devi AP. The effect of endosulfan and its isomers on tissue protein, glycogen and lipid in the fish *Channa punctatus*. Pesticidal Biochem. Physiol., 1982; 17: 280-286.
18. Priscilla M. Impact of Bensi hexachloride on carbohydrate metabolism and haematology in freshwater fish *Channa punctatus*. M.Phil. thesis, Annamalai University, India, 1985.
19. Raj Narayan Ram and Sathyanesan AG. Histopathological and biochemical changes of a teleost fish, *Channa punctatus* (Bloch) induced by a mercurial funcide. Environ. Poll., 1987; 47: 135-145.
20. Rao KRS, Sahib IKS, Sailatha D and Rao KVR. Efffect of technical and commercial grade malathion on the oxidative metabolism of the fish *Tilapia mossambica.* Environ. Ecol., 1986; 4: 270-273.
21. Roe JH. The determination of sugar in blood and spinal fluid with anthrone reagent. J. Biol. Chem., 1955; 153: 373-380.
22. Sapna Shrivasatava, Sudha Singh and Keerty Shrivastava. Effect of carbaryl on glucose content in the brain of *Heteropneustes fossilis.* J. Ecotoxicol. Environ. Monit., 2002; 12(3): 205-208.
23. Sastry KV and Dasgupta A. Effect of Nuvacron on the nutritive value of fresh water teleost fish *Channa punctatus*. J. Environ. Biol., 1991; 12(3): 243-248.
24. Sastry KV and Siddiqui A. Some haematological and enzymological parameters of a freshwater teleost fish, *Channa puntatus* exposed to sublethal concentreations of quinalphos. Pestic. Biochem. Physiol., 1984; 22: 8-13.
25. Tilak KS and Yacobu K. Toxicity and effect of fenvalerate on fish *Ctenopharyngodon idellus.* J. Ecotoxicol. Environ. Monit., 2002; 12(1): 09-15.
26. Tripathi PK, Srivastava VK and Singh A. Toxic effects of di methoate (organophosphate) on metabolism and enzyme system of fresh water teleost fish *Channa punctatus*. Asian Fisheries Science, 2003; 16: 349-359.
27. Umminger BL. Physiological studies on super cooled Hillifish *Fundulus heteroditus*, carbohydrate metabolism and survival at sub zero temperature. J. Exp. Zool., 1970; 173: 159-174.
28. Visvanathan P. Maruthanayagam C and Govindaraju M. Effect of malathion and endosulfan on biochemical changes in *Channa punctatus.* J. Ecotoxicol. Environ. Monit., 2009; 19(3): 251-257.
29. Ware GM. Effects of pesticides on non-target organisms. Residue Rev., 1980; 76: 173-301.
30. Yeragi SG, Koli VA and Yeragi S. Effects of pesticide malathion on protein metabolism of the marine crab *Uca merionis*. J. Ecotoxicol. Environ. Monit., 2000; 10(1): 59-62.