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# TOXICITY OF CADMIUM AND COPPER AND THEIR EFFECT ON SOME BIOCHEMICAL PARAMETERS OF MARINE FISH *MUGIL SEHELI*

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## ABSTRACT

The present work aimed to estimate the toxicity of cadmium and copper to fingerlings of marine fish *Mugil seheli*, as well as the effect of different concentratios of Cd and Cu on some biochemical parameters (Aspartate and alanine transaminase enzymes, glucose, glycogen, lipid and protein) in the fish organs. 96-h $LC_{50s}$ of Cd and Cu were 5.36 and 1.64 ppm, respectively. Levels of aspartate and alanine transaminase were reduced within 2 days of exposure to different concentrations of Cd (2.0 and 0.5 ppm) and Cu (0.5 and 0.2 ppm) in both plasma and muscle tissue. Glucose was increased reaching the highest values after 4 days of the treatment with Cd and Cu, while muscle glycogen was increased at first days then dropped after 14 days below control level. Generally, total lipids and proteins in plasma and muscle recorded higher values than the control one. The present study showed high toxicity of copper to fish *Mugil seheli* comparing to cadmium.

## INTRODUCTION

The toxicity tests are necessary in water pollution evaluation because chemical and physical measurements alone are not sufficient to assess potential effects on aquatic biota (Tarzwell, 1971). In addition, it is an important step to detect the levels of toxicants to be used in the experimental studies of the accumulation and effect of these toxicants to the marine organisms. There are many studies concern with the toxicity of cadmium on vertebrates and invertebrates (Rasmussen and Andersen, 2000, Adami *et al,* 2002 and Filiovic and Raspor, 2003). Fish exposed to high concentration of cadmium quickly develop lack of calcium and low blood hemoglobin. Microorganisms may suffer growth inhibition at cadmium concentration of 0.25 mg/l (Roberts, 2003).

Biochemical and physiological biomarkers are frequently used for detecting or diagnosing sublethal effects in fish exposed to different toxic substances (Theodorakis *et al.,* 1992). Sublethal effects are biochemical in origin as the most toxicants exert their effects at basic level of the organism by reacting with enzymes or metabolites and other functional components of the cell. Such effects might lead to irreversible and detrimental disturbances of integrated functions such as behavior, growth, reproduction and survival (EIFAC, 1975 and Waldichuk, 1979).

Transaminase enzymes play vital role in carbohydrate-protein metabolism in

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fish and other organisms' tissues (Eze 1983). Changes in enzymes activity and other biomarkers have been studied as possible tools for aquatic toxicological research (Moore and Simpson 1992; Arellano *et al,* 2000 and Abou El-Naga *et al.,* 2001).

The present study aimed to determine the toxicity ($LC_{50}$) of Cd and Cu to the fingerlings of marine fish *Mugil seheli*. Also to evaluate the effect of different concentrations of Cd and Cu on transaminases in plasma and muscle tissue and other energetic metabolites.

## MATERIAL AND METHODS

**(1) Toxicity tests:**

Live fingerlings of *Mugil seheli* (mean weight of 3.6 g and mean length of 5.2 cm) were collected from Dersa Pond in the Suez Canal and transported to the wet lab in National Institute of Oceanography and Fisheries, Suez, in aerated tank. Fish were kept in a holding tank filled with seawater as stock. Acclimatization took place for 7 days.

Stock solutions of 1000 mg Cd/1 and 1000 mg Cu/1 were prepared freshly using Cd $SO_2$ and Cu $SO_2.5H_2O$ of pure grade. The pH of the stock solutions was adjusted to about 7 to prevent adsorption of the metals on the wall of the container.

Several experimental glass aquaria of dimensions 40 x 20 x 30 cm were prepared (cleaned and washed by running tap water, then by the same sea water used in the experiment). The aquaria were filled with a known volume of sea water before adding the test solutions. Different concentrations of cadmiun (from1.0 to 15.0 ppm) and copper (from 1.0 to 3.0 ppm) were used. Thirty minutes after adding the toxicants, ten fish were randomly picked from the stock tank and were introduced to each of the experimental aquarium in addition to the control aquarium. Observations for mortality were made and dead fish were removed and recorded. The test was terminated after 96 h, and repeated three times to confirm the data.

The percentage mortality in each concentration was corrected for control mortality using Abbott's formula (Finney, 1971); however, mortalities in controls were never greater than 10 %.

Abbott's formula was as follow:

$C = O – X / 100 - X$

Where,

C is the corrected mortality.

O is the percentage of observed mortality.

X is the percentage of the control fish that have died at the relevant observation time.

96 h $LC_{50}$ for each metal was estimated by probit methods (Finney, 1971) using graphical analysis. To construct the graph, plot percentage mortality as the ordinate against log concentration as the abscissa. The concentration causing 50 % mortality from the fitted line; is the estimated $LC_{50}$ for the exposure time.

**(2) Biochemical studies:**

Live adult fish of *Mugil seheli* were collected and handled by the same procedure described for fingerlings. The fish were distributed in glass aquaria filled with seawater containing 0.5 and 2.0 ppm cadmium and 0.2 and 0.5 ppm copper in addition to control group. Samples of fish were taken after 2, 4, 7 and 14 days.

Aspartate and alanine transaminases were investigated using kits (Pasteur Lab, Egypt, Lot No. 213). Total protein in plasma and muscle was determined according to Gronall *et al.* (1949). Total lipids were determined by the method of Knight *et al.* (1972) and glycogen was detected according to Seifter *et al.* (1950).

Statistical analysis (mean ± S.E., student *t*- test) was carried out using computer program, STATISTICA Package.

## RESULTS

**1- Toxicity of Cd and Cu ($LC_{50}$):**

The experiments were conducted during winter season. The conditions of the experiments were as follow: temperature ranged from 19.5 to 21.5 °C, pH ranged from

8.22 to 8.31, salinity ranged from 40.90 to 41.03 ‰ and dissolved oxygen varied from 4.38 to 4.97 mg $l^{-1}$.

Acute toxicity tests of cadmium and copper to *Mugil seheli* are shown in (Fig. 1). The present study recorded that 96 h-$LC_{50}$ values of Cd and Cu were 5.36 and 1.64 ppm, respectively.

**2- Transaminases:**

**a- Aspartate transaminase (AST) enzyme (u/l):**

Aspartate transaminase (AST) enzyme activity in plasma was decreased after 2 days from the exposure to different concentrations of Cd (0.5 and 2.0 ppm) and Cu (0.2 and 0.5 ppm) recording 47.61±6.94, 34.93±4.23, 30.02±8.57 and 34.93±4.23 u/l, respectively (Fig. 2). The enzyme activity still lower than control value (72.26±5.96 u/l) until the 7$^{th}$ day except for 2.0 ppm Cd. While after 14 days from the exposure to Cd and Cu the activity of enzyme elevated to reach around control value. Significant differences ($p<0.05$) in AST levels were observed for fish groups exposed to 0.5 ppm Cd (at 4 and 7 days) and 2.0 ppm Cd (at 4 days) and 0.5 ppm Cu (at 2 days).

Muscle AST activity recorded low values in groups exposed to 0.5 and 2.0 ppm Cd and 0.2 and 0.5 ppm Cu (3.40±0.41, 3.20±0.15, 3.04±0.25 and 2.36±0.26 u/g, respectively) comparing to the control group level (4.52±0.53 u/g) after 2 days from the exposure time. After 14 days, the activity of enzyme reached to the control value for fish exposed to 0.5 ppm Cd only, while the others still below the control level. Variation in AST activity showed significant differences ($p<0.05$) for fish groups exposed to 0.5 ppm Cd ( at 2, 4 and 7 days) and 2.0 ppm Cd (at 4 days) and 0.2 ppm Cu (at 4 days).

**b- Alanine transaminase (ALT) enzyme (u/l):**

Plasma ALT activity decreased after 2 days of exposure to different concentrations of Cd and Cu recording 5.38±1.32, 3.76±1.04, and 2.64±0.52, 2.45±0.69 u/l, respectively (Fig. 2) in comparison to control group level (10.01±2.49 u/l). The enzyme activity increased gradually and reached to control value after 14 days except for group subjected to 0.5 ppm Cu which still below the control value (3.92±0.08 u/l). Insignificant differences ($p>0.05$) in ALT level were recorded through all the time intervals of experiment except for group exposed to 2.0 ppm Cd at 2 days.

Muscle ALT enzyme showed decrease in its activity after 2 and 4 days. After 14 days from exposure to different concentrations of Cd and Cu, enzyme activity increased recording values of 0.49±0.01, 0.50±0.01, 0.56±0.03 and 0.49±0.04 u/g higher than control value (0.44±0.08 u/g). Insignificant differences ($p>0.05$) of muscle ALT were recorded through all the experiments except for group exposed to 0.5 ppm Cd at 14 days.

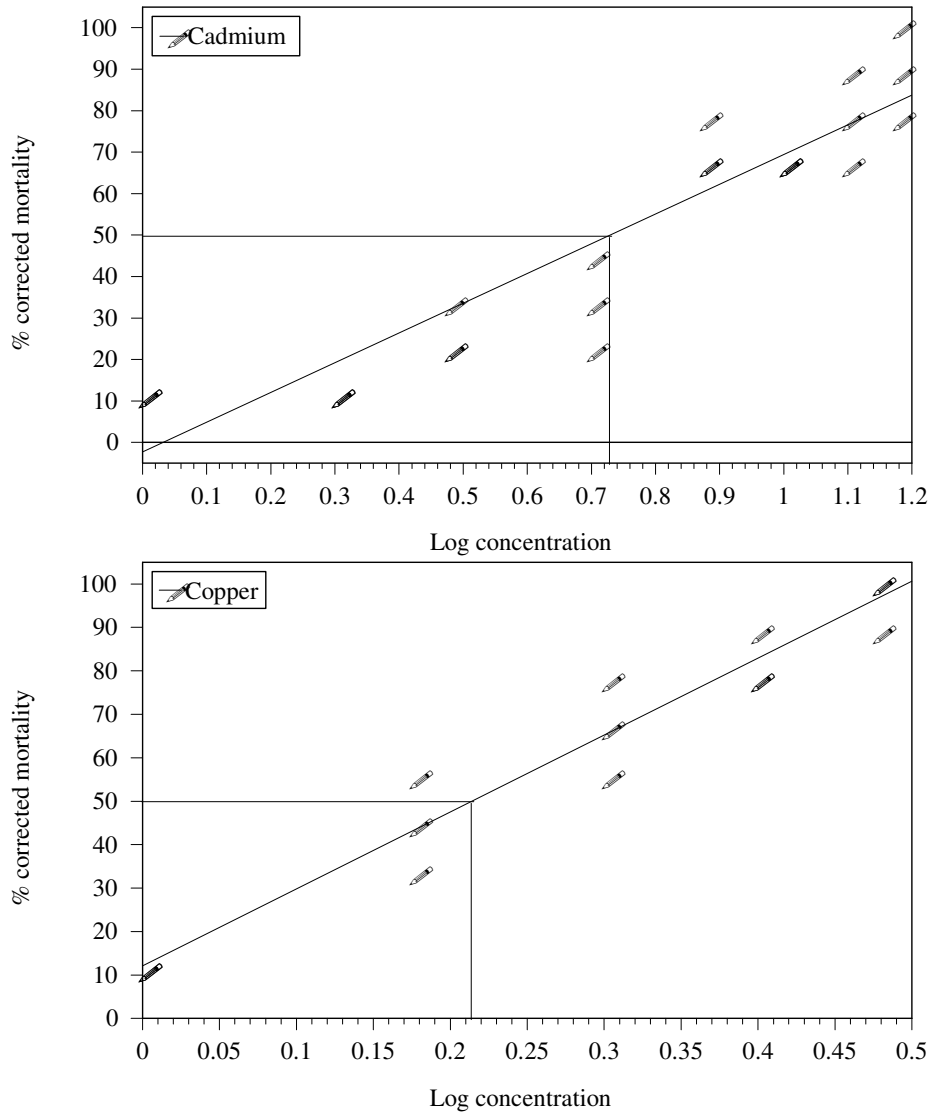


**Fig. (1): Toxicity of cadmium and copper (96h $LC_{50}$) to the fingerlings of fish *Mugil seheli***

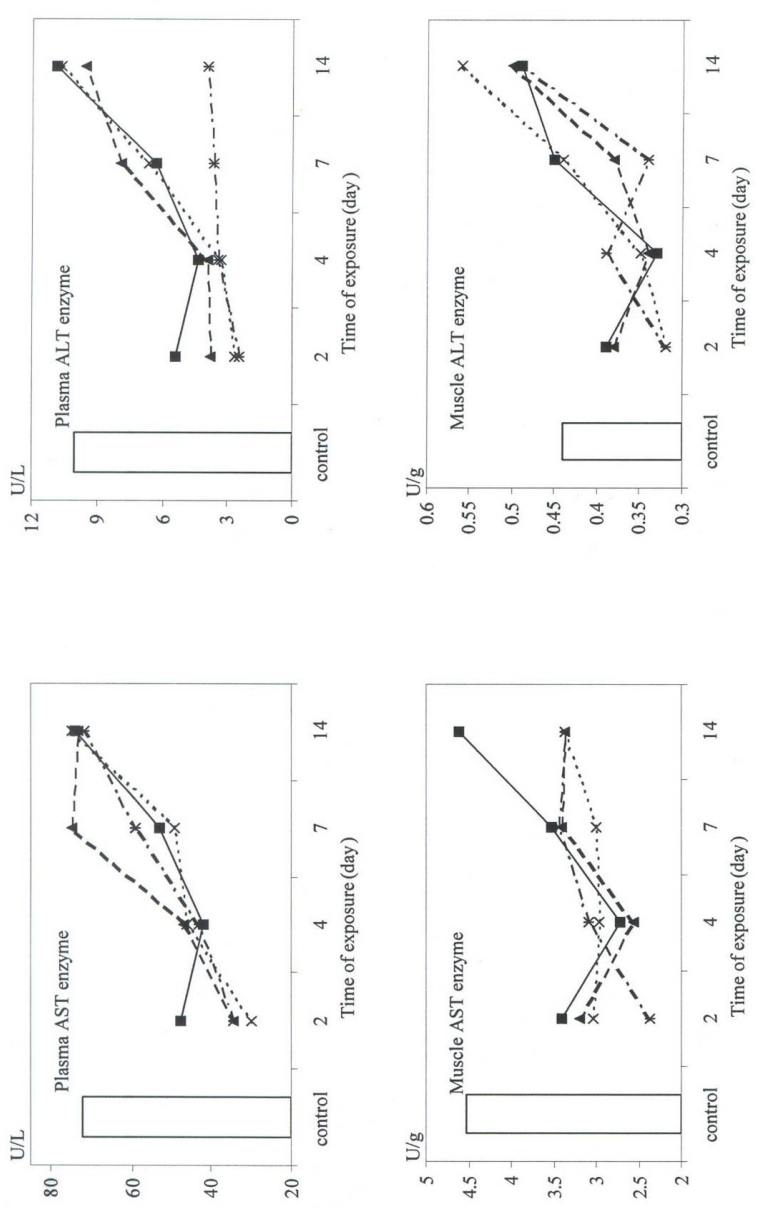


**Fig. (2): Effect of different concentrations of cadmium and copper on transaminases in plasma and muscle of *Mugil seheli***

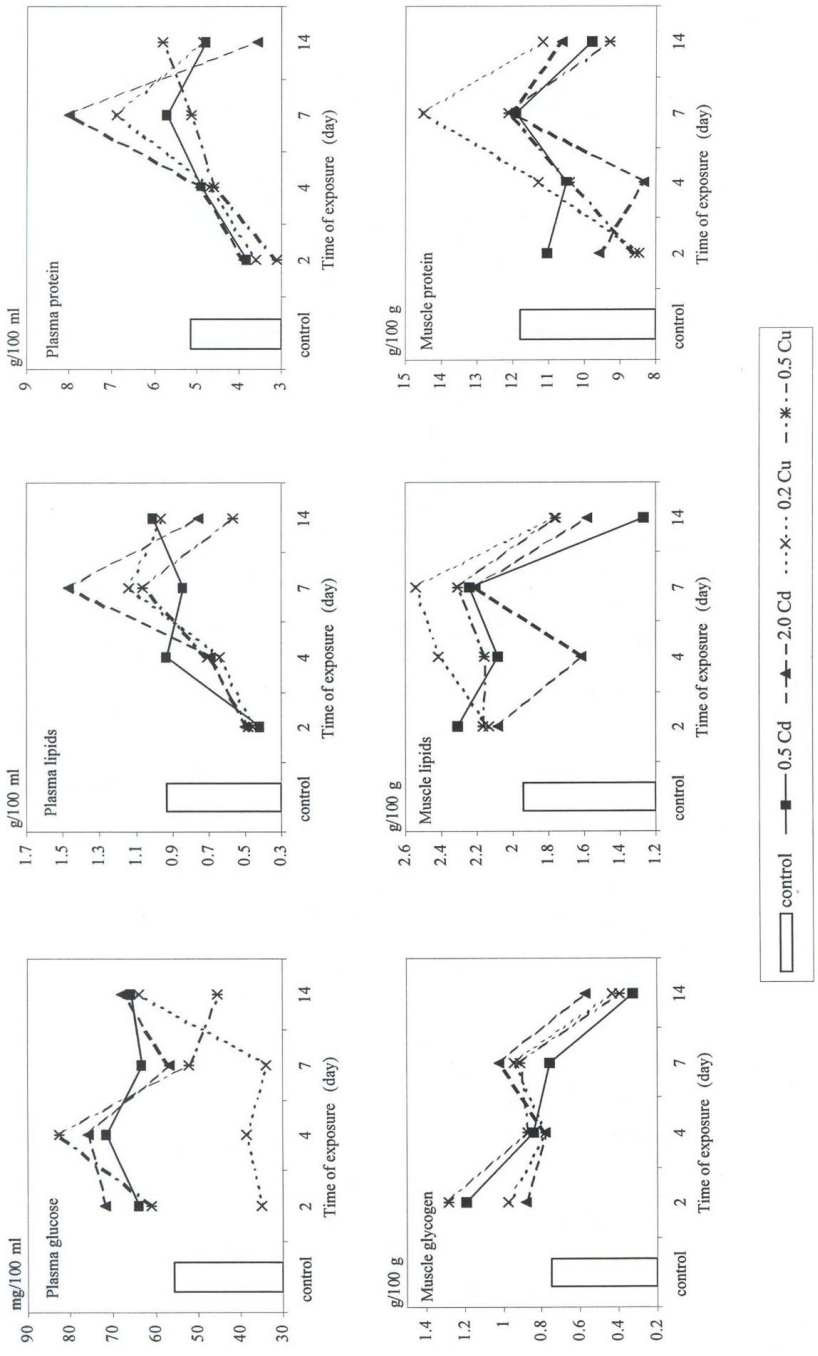


**Fig. (3): Effect of different concentrations of cadmium and copper on biochemical parameters in plasma and muscle of *Mugil seheli***

**3- Plasma glucose (mg%) and muscle glycogen (g/100 g tissue)**

**Plasma glucose:**

Figure (3) shows that concentration of glucose increased reaching the maximum level at 4 days of exposure to 0.5 and 2.0 ppm Cd and 0.5 ppm Cu recording 71.58±13.69, 75.81±5.14 and 82.50±6.91 mg %, respectively. While fish group exposed to 0.2 ppm Cu showed a marked depression of glucose level (33.77±4.89 mg%) at the 7$^{th}$ day. After 14 days exposure to Cd and Cu, glucose level was still higher than control (55.56±9.53 mg %) except for 0.5 ppm Cu. Insignificant differences ($p>0.05$) in plasma glucose value were recorded in all groups exposed to different concentrations of Cd and Cu at different time intervals.

**Glycogen in muscle:**

Glycogen content in fish muscle recorded high values (1.19±0.13, 0.88±0.07, 0.97±0.12 and 1.28±0.04 g/100 g tissue) and (0.76±0.07, 1.02±0.12, 0.94±0.09 and 0.91±0.16 g/100 g tissue) comparing to the control group (0.75±0.06 g/100 g tissue) after 2 and 7 days of exposure to Cd (0.5 and 2.0 ppm) and Cu (0.2 and 0.5 ppm), respectively. After 14 days of exposure to different concentrations of Cd and Cu, the stored glycogen were depleted to levels lower than control group recording 0.32±0.02, 0.57±0.11, 0.43±0.07 and 0.39±0.03 g/100 g tissue (Fig., 3). Significant difference ($p<0.05$) in glycogen content existed after 2 days from exposure to 0.5 ppm Cd and 0.5 ppm Cu and after 14 days for 0.5 ppm Cd.

**4- Plasma and muscle total lipids:**

Figure (3) shows that plasma total lipid of control group recorded value of 0.93±0.33 g %. After 2 days of exposure to different concentrations of Cd and Cu, the level of total lipid were decreased to its minimum values (0.42±0.07, 0.50±0.08, 0.46±0.19 and 0.48±0.09 g %, respectively). While after 7 days the total lipid was elevated to 0.84±0.29, 1.47±0.21, 1.14±0.16 and 1.05±0.16 g % from exposure to 0.5 and 2.0 ppm Cd and 0.2 and 0.5 ppm Cu, respectively.

Generally, total lipid in muscle recorded high values for different groups exposed to different concentrations of Cd and Cu after 2, 4, 7 days. While after 14 days, level of total lipid decreased to 1.27±0.26, 1.58±0.27, 1.77±0.19 and 1.76±0.24 g/100 g tissue in comparison to control group value (1.94±0.31 g/100 g tissue).

Insignificant differences ($p>0.05$) were recorded in lipid values for both plasma and muscle total lipids at all time intervals for all groups except 2.0 ppm Cd for muscle after 7 days.

**5- Plasma and muscle total proteins:**

Total protein in plasma exhibited the lowest values after 2 days of exposure to 0.5 and 2.0 ppm Cd and 0.2 and 0.5 ppm Cu recording 3.83±0.74, 3.89±0.32, 3.62±0.57 and 3.09±0.25 g % in comparison to control value (5.13±0.32 g %). At the 7 days, total protein increased to level higher than the control. While at the 14 days, protein values returned around and below the control value (Fig. 3).

Total protein in muscle was decreased to 11.5±1.12, 9.60±1.11, 8.44±0.96 and 8.60±1.30 g/100 g tissue in comparison to control value 11.80±1.13 g/100 g tissue after 2 days of exposure to different concentrations of Cd an Cu. At 4-14 days, level of protein was elevated around control value (Fig. 3).

Insignificant differences ($p>0.05$) were recorded in plasma protein at all time intervals for different concentrations of Cd and Cu except at 7 days for 0.5 ppm Cu ($p<0.05$). Also insignificant differences ($p>0.05$) were recorded between control and exposed groups at different time interval except at 2 days in case of muscle protein for 0.2 ppm.

**6- Comparison between the effect of cadmium and copper at same concentration (0.5 ppm) on biochemical constiututes:**

Table (1) shows the percent of change in biochemical constitutes in plasma and muscle after exposure to 0.5 ppm of cadmium and copper for 2, 4, 7 and 14 days in comparison to control value (plamsa and muscle AST and ALT, Plasma glucose and muscle glycogen and plasma and muscle lipid and protein). Significat difference ($p<0.05$) found in plasma AST activity at 2 and 4 days of exposure to Cd and at 2 days of expoure to Cu. Also significant difference ($p<0.05$) found in ALT activity of exposure to Cu. Muscle AST activity recorded significant difference ($p<0.05$) at 2, 4 and 7 days of expoure to Cd. Significant difference ($p<0.05$) was exist at 2 and 14 days of expoure to Cd and at 2 day of expoure to Cu.

## DISCUSSION

The results of toxicity test in the present study indicated that the ionic form of Cu is more toxic than the ionic form of Cd to *Mugil seheli,* and the fingerlings are more sensitive to copper toxicity than that of cadmium. Denton and Burdon-Jones (1986); Cui-Keduo *et al.* (1987) and Wu-Yulin *et al.* (1990) reported that copper is more toxic than cadmium for the larvae and adult fish species.

Spehar (1976) reported that the 96 h $LC_{50}$ of Cd for flag fish, *Jordanella floridae*, was 2.5mg /l. Hamed (1992) found that the 72 h $LC_{50}$ of Cd for *Mugil seheli* was 4.87 mg/1. El-Moselhy (2001) stated that toxicity of Cd to *Mugil seheli* decreased with increasing the exposure time and the recording $LC_{50}$ values were 12.34, 8.92, 6.01 and 3.45 mg/l for 24, 48, 72 and 96 hours, respectively. The 96 h $LC_{50}$ values of copper was 1.83 ppm for fish *Etroplus maculaus* reported by Gaikwad (1989). The variation in the $LC_{50}$ values for the same metal may be due to; species type, chemical structure of metal compound, and the conditions of the experiment (water temperature, salinity, oxygen content and pH).

Taylor (1981) reported $LC_{50}$ values of about 0.3 to 50 mg Cd/1. While 96 h $LC_{50}$ of Cu ranged from 0.2 to 3 mg/1 for various marine fish and crustaceans (Bryan 1976). Pagenkopf (1986) studied the toxicity of copper, cadmium, lead and zinc to fishes. He derived at least four general conclusions from the analysis of literature: 1) some metals are more toxic than other, 2) an increase in water hardness tends to reduce the heavy metals toxicity, 3) naturally occurring materials often reduce the concentrations of toxic species. These parameters are not the only ones affecting toxicity, but they can be utilized to rationalize many observed metal toxicity results.

Fish are responding to various stressors by a series of biochemical and physiological stress reactions, so called secondary stress responses comparable to those of higher vertebrates (Mazeaud and Mazeaud, 1981).

In the present work, protein value in plasma and muscle was decline (exhausted) at first 7 days from the exposure to different concentrations of Cd and Cu, this due to supplying fish with energy to do vital activities. Brett and Groves (1979) reported that protein is the major source of energy. Also this explanation was enhanced through decreasing lipid levels in plasma and muscle in the same period.

The present study illustrated that glucose recorded high values than control group level; also muscle glycogen content was increased at the same time intervals. This high level was explained through glucogensis, which mean formation of glucose and glycogen from non-carbohydrate source.

Heath (1987) reported that muscle glycogen was increased in the same fish organ without other organs. The reduction in the availability of carbohydrates for energy was partially compensated by increasing the activity of glutamate dehydrogenase and α-amino oxidase; which are the enzymes of

controlling the utilization of amino acids for energy.

On the other hand, the responsible enzymes of protein-carbohydrate metabolism (aspartate and alanine transaminases) showed low levels than control group value due to its sharing in transforming proteins to glycogen. Christensen (1971) attributed the reduction in enzyme activity into inhibitory effect of metal. De la Torre *et al.* (1998) reported that the activity of ATPase was decreased while AST and ALT in muscle of *Cyprinus carpio* was not affected.

Cadmium can cause an induction or inhibition of variety of cellular enzymes in marine fish, when rainbow and red trouts exposed to sublethal concentration of cadmium for 2 or 3 months (Reberts *et al.* 1979).

The present study showed that plasma enzymes (AST and ALT) were greatly affected of expoure to Cu than Cd. Glucose recorded high values of exposure to Cd rather than Cu. Plasma lipids and protein were affected by Cu more than Cd. Muscle glycogn, lipids and protein were affected by Cu more than Cd.

**Table (1): Percent of change in plasma and muscle constitutes of *Mugil seheli* after exposure to 0.5 ppm cadmium and copper in comparison to control mean values at different time intervals.**

| Parameters | Control mean values | Cadmium (0.5 ppm) | | | | Copper (0.5 ppm) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 2 days | 4 days | 7 days | 14 days | 2 days | 4 days | 7 days | 14 days |
| Plasma | | | | | | | | | |
| AST | 72.62±5.96 | -34.43 | -42.19* | -26.39* | 2.3 | -52.55 | -41.37 | -19.61 | -2.59 |
| ALT | 10.01±2.49 | -46.25 | -56.34 | -36.56 | 8.39 | -75.52* | -65.63 | -63.23 | -60.83 |
| Glucose | 55.56±9.53 | 14.97 | 28.83 | 13.89 | 18.43 | 9.89 | 48.48 | -6.44 | -18.3 |
| Lipids | 0.93±0.33 | -54.83 | 0 | -9.67 | 8.6 | -48.38 | -23.65 | 12.9 | -38.7 |
| Protein | 5.13±1.32 | -25.34 | -4.67 | 11.69 | -7.01 | -39.76 | -9.94 | 0.58 | 13.45 |
| Muscle | | | | | | | | | |
| AST | 4.52±0.33 | -66.03* | -72.82* | -64.83* | -65.84 | -47.87 | -31.85 | -23.89 | -25.66 |
| ALT | 0.44±0.08 | -11.36 | -25 | 2.27 | 11.36 | -27.27 | -11.36 | -22.77 | 11.36 |
| Glycogen | 0.75±0.06 | 58.66* | 12 | 1.33 | -57.33* | 72.00* | 16.00 | 21.33 | -48 |
| Lipids | 1.53±0.51 | 50.98 | 35.94 | 46.4 | 3.26 | 41.83 | 41.17 | 50.98 | 36.6 |
| Protein | 11.80±1.31 | -6.35 | -11.18 | -5.67 | -16.84 | -27.11 | -11.18 | 2.62 | -21.18 |

* = significant difference (P<0.05)

## CONCLUSION

From the results stated above, it can be concluded that, although copper is an essential metal for various physiological processes, it showed a toxic effect to fingerlings of *Mugil seheli* higher than those of cadmium, which is known as toxic metal and not required for physiological reactions. This result should be impelled us to make a focusing on the copper and other essential elements that can produce a hazardous influences on the marine organisms, and in general on the marine life and environment.

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