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# SUBLETHAL EFFECTS OF HEAVY METALS COPPER, CADMIUM AND ZINC ALONE OR IN COMBINATIONS ON ENZYMES ACTIVITIES OF COMMON CARP *CYPRINUS CARPIO* L.

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## ABSTRACT

Drastic biochemical changes were produced in liver and muscle of common carp; *Cyprinus carpio* L. exposed to sublethal levels (¼ and ½ $LC_{50}$) of either copper, cadmium or zinc alone or a combination of them for 7 and 30 days. The pattern of changes in both treatments was nearly the same except for the intensity of change. The hepatic aspartate amninotransferase (AST) in liver was increased, while acid phosphatase (ACP) activity generally decreased in fish exposed to high level of Cu, Cd, Zn and their mixtures. Also, hepatic alanine aminotransferase (ALT) showed significant increase in fish exposed to all mixture groups, except high level of Cu+Cd+Zn on the 7$^{th}$ day, and then the activity decreased on the 30$^{th}$ day in fish of all treated groups. In muscle, AST showed significant increase in fish exposed to low level of Cu, Zn, Cu+Cd+Zn and high level of Cu and Cd for 7 days. These values decreased significantly after exposure to low level of Zn, Cu+Cd, Cu+Cd+Zn as well as high level of Zn and Cu+Cd+Zn for 30 days. The activity of ALT was increased significantly on the 7$^{th}$ day in fish exposed to low level of mixtures of Cu+Zn, Cd+Zn and Cu+Cd+Zn as well as high levels of Cu, Cd, Cu+Zn and Cu+Cd+Zn treatments, while fish exposed to low level of

Cu+Cd+Zn and high level of Cu+Zn, Cd+Zn and Cu+Cd+Zn treatments showed significant decrease in ALT activity on the 30$^{th}$ day. There was no significant change in muscle ACP activity in all treated fish groups.

## INTRODUCTION

Recent years have witnessed significant attention being paid to the problems of environmental pollution by a wide variety of chemical pollutants including the heavy metals. Industrial processes such as welding, smelting or fibricating of molten metals can produce metals at concentrations often in excess of recommended threshold limit values (Lam *et al.*, 1985). Heavy metals toxicity has been a growing concern in a number of industrial and environmental settings, however, they may reach natural water from mining and/or industrial operation, using as fertilizers and for aquatic weed control.

The progressive use of copper, cadmium, zinc and their compounds in a variety of industrial products and operations caused a marked increase in direct production. Hence, the worldwide production of Cd, Cu and Zn during 1971-80 is estimated to be 1.5 x $10^5$, 82.5 and 63.2 x $10^3$ tons, respectively (Moore and Ramamoorth, 1984).

Moreover, dissolved Cd levels in freshwater generally range from 10 to 500 ng/l and in case of extreme water pollution, concentration of Cd may exceed 17,000 ng/l and Zn concentrations of polluted freshwater in industrial zone rivers may produce total Zn residues of ≥3 mg/l in receiving waters (Moore and Ramamoorth, 1984). Cu concentrations that occurred also in the vicinity of some metal mines are 0.5-2 mg/l (Tyler and Buckney, 1973). Although Cu and Zn are essential elements for some metalloenzymes, high concentrations cause acute and chronic toxicity to fish.

On the other hand, in large amount, the enzymes found in the heart and skeletal muscle, brain, liver and kidney are liberated into the

blood in specific pathological situations and therefore are of clinical importance. Subsequently, there has been increasing interest in using the changes in enzymes activities in fish as an index reflecting metal toxicity (Sastry and Sunita, 1983; Lan *et al.*, 1993). Among the various studied enzymes, much attention has been focused on acid and alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase (Hilmy *et al.*, 1985, 1987; Davolli *et al.*, 1989; Nicholls *et al.*, 1989; Shakoori *et al.*, 1990; Gill *et al.*, 1990, 1991, 1992; Lan *et al.*, 1995).

Therefore, the present study was carried out to investigate the effect of the heavy metals Cu, Cd and Zn either alone or in combinations on the activities of AST, ALT and ACP enzymes in liver and muscle of common carp; *Cyprinus carpio* L. exposed to the toxicants for 7 and 30 days.

## MATERIALS AND METHODS

Healthy fish of common carp (*Cyprinus carpio* L.) weighing 60-90 g/fish were collected from the fish ponds of Central Lab. for Aquaculture Research at Abbassa, Abo-Hammad, Sharkia. Fish were acclimated in indoor tank for 2 weeks and then randomly distributed in glass aquaria of 100-liter capacity at a rate of 10 fish/aquarium that containing aerated water. Air was supplied via air-stones from a central aeration system. The temperature was 25±1 C. Fish were fed at a rate of 3% of live body weight with pelleted fish diet (25% CP) twice daily.

Chemicals used were copper sulfate ($CuSO_4.5H_2O$), cadmium sulfate ($CdSO_4.8H_2O$) and zinc sulfate ($ZnSO_4.7H_2O$); produced by El-Nasr Co. for Chemicals, Egypt as sources for Cu, Cd and Zn, respectively. The 96-hr $LC_{50}$ for each metal was determined according to Behreus and Karber (1953) and it was 3.76, 35.0 and 43.0 mg/l for copper sulfate, cadmium sulfate and zinc sulfate, respectively. Fish was

exposed to ¼ and ½ $LC_{50}$ of copper sulfate (0.94 and 1.88 ppm, respectively), cadmium sulfate (8.75 and 17.50 ppm respectively) and zinc sulfate (10.75 and 12.50 ppm respectively) alone or in combinations of di- or tri- compounds for 7 and 30-day exposure period. Subsequently, the ¼ and ½ $LC_{50}$ for $Cu^{2+}$ was 0.239 & 0.478 ppm, respectively, for $Cd^{2+}$ was 1.278 and 2.556 ppm, respectively and for $Zn^{2+}$ was 2.444 and 4.889 ppm, respectively. The fish were classified into different groups as follows:

Group 1 Control fish

| **Low level** | | **High level** | |
|---|---|---|---|
| **Groups exposed to ¼ $LC_{50}$ :** | | **Groups exposed to ½ $LC_{50}$ :** | |
| Group 2 | Cu (0.239 ppm) | Group 9 | Cu (0.478 ppm) |
| Group 3 | Cd (1.278 ppm) | Group 10 | Cd (2.556 ppm) |
| Group 4 | Zn (2.444 ppm) | Group 11 | Zn (4.889 ppm) |
| Group 5 | Cu + Cd (0.239 +1.278 ppm) | Group 12 | Cu + Cd (0.478 + 2.556 ppm) |
| Group 6 | Cu + Zn (0.239 + 2.444 ppm) | Group 13 | Cu + Zn (0.478 + 4.889 ppm) |
| Group 7 | Cd + Zn (1.278 + 2.444 ppm) | Group 14 | Cd + Zn (2.556 + 4.889 ppm) |
| Group 8 | Cu + Cd + Zn (0.239 + 1.278 + 2.444 ppm) | Group 15 | Cu + Cd + Zn (0.478 + 2.556 + 4.889 ppm) |

Each fish group in two periods consisted of two aquaria. After application of the toxicant, siphoning a portion of water from each aquarium was done every 3 days for excreta removing and an equal volume of water containing the same concentrations of toxicants replaced it.

At the end of each exposure period, the fish were removed and quickly decapitated. Tissue samples were immediately processed after collection for determination of enzyme activities. AST and ALT were determined colorimetrically, using AST and ALT kits supplied by Egyptian American Co. for Laboratory Services, Egypt, according to Reitman and Frankel (1957), and ACP was determined colorimetrically using ACP kit, supplied by Biomereux, France, according to King and

King (1954). Statistical analysis of the data was performed, using Student's 't' test according to Harold and Larson (1982).

## RESULTS AND DISCUSSION

In the present study, the effect of Cu, Cd, Zn and mixtures of them on amino acid metabolism was examined on common carp; *Cyprinus carpio* L. throughout the measurements of aspartate amninotransferase (AST), alanine aminotransferase (ALT) and acid phosphatase (ACP) activities in fish liver and muscle.

The hepatic AST activity of control group was 261.2 ± 24.7 and 266.9 ± 31.8 (IU/g) for 7 and 30 days, respectively. The hepatic AST after 7 days showed a significant increase ($P<0.05$) in fish exposed to low level of Cu and Cu + Zn (462.1± 45.2 and 462.1± 46.6 Iu/g, respectively) and in all treatments of high level except that of Cd + Zn group. After 30 days of exposure, the values of AST were significantly reduced in fish exposed to low level of Cu and Cu + Cd + Zn as well as high level of Cu + Zn and Cu + Cd + Zn compared to control group (Table 1).

The average values of AST in muscle of the control group was 200.9±17.9 and 257.2 ±23.3 (IU/g) after 7 and 30 days. It can be seen from data in Table 2 that, the mean values of AST in muscle were significantly increased in fish raised in low level of Cu, Zn, and Cu + Cd + Zn (276.8 ± 24.2, 261.2±11.1 and 301.4 ± 29.7 IU/g, respectively) as well as high level of Cu and Cd (305.5±39.3 and 307.2± 24.3 IU/g, respectively) after 7 days of exposure. On the other hand, after 30 days the AST activity was significantly decreased in fish exposed to low level of Zn, Cu + Cd and Cu + Cd + Zn (80.4±6.3, 96.4±11.7 and 107.1±9.7 IU/g respectively). Also, it was decreased significantly to 180.7±8.8, 180.8±6.4 and 80.4±6.3 IU/g at high level of Zn, Cu + Zn and Cu + Cd + Zn respectively.

The assessment of hepatic enzyme activities of ALT after treatment with low level of Zn and high level of Cu revealed a significant increase of liver enzyme activity of fish on the 7$^{th}$ day of exposure. Also ALT activity was increased significantly after treatment with low and high level of Cu+Cd and Cu+Zn on the 7$^{th}$ day. Then decreased significantly on the 30$^{th}$ dayafterfish treatment with low level of Cu and all mixture group as well as all treatments of high level except that of Cd+Zn group (Table 3). Similarly, ALT activity in fish muscle that exposed to low level of Cu+Zn, Cd+Zn and Cu+Cd+Zn as well as high level of Cu, Cd, Zn, Cu+Zn and Cu+Cd+Zn increased significantly on the 7$^{th}$ day of exposure. After 30 days of exposing fish to low level, no significant changes were recorded in all treated fish groups except Cu+Cd+Zn group. Whereas, a significant reduction was recorded in fish exposed to mixtures of high level of Cu+Zn, Cd+Zn and Cu+Cd+Zn on the 30$^{th}$ day (Table 4).

The early increase of hepatic AST and ALT activities in some treatments of Cu, Cd, Zn and mixture of them might reflects the early toxic effect of this metal on the hepatic enzyme activity. On the other hand, the opposite effects on hepatic AST and ALT activities at the beginning and at the end of the experiment might be due to liver necrosis induced by toxicants at the end of experiment. These results are in complete agreement with those of El-Zayat *et al.* (1996), who found that chronic lead toxicity caused significant increase in hepatic AST and ALT of albino rat on the 5$^{th}$ day and significant decrease in hepatic AST and ALT on the 20$^{th}$ day of exposure.

Similarly, Mckim *et al.* (1970) found that, sublethal concentration of copper caused significant increase of pALT of *Salvelunus fantinalis* after 6 and 21 days of exposure. Then a significant decrease of pALT was observed after long term exposure (337 days). Mukhopadhyay *et al.* (1982) and Begum and Vijayaragharan (1995) reported that the hepatic

AST and ALT activities were increased significantly in catfish (*Clarias batrachus*) toxicated with carbofuran and dimethoate, respectively. Shakoori *et al.* (1990) found also that the hepatic AST and ALT were increased in freshwater fish *Cirrhinus marigala* toxicated with Cd for 7 days. Gill *et al.* (1991) observed also that cadmium chloride inhibited the activities of AST and ALT in liver, kidney and gills of rosy barb (*Barbus conchonius* Ham.). Moreover, Gill *et al.* (1990) found a marked reduction in hepatic, branchial and renal AST and ALT in rosy barb (*Puntius conchonius*) after toxication with mercuric chloride. They mentioned that, the reduced levels of aminotransferase in various organs may be resulted from tissue damage and consequently the reduction of enzyme turnover causally related to the presence of toxic mercury.

However, it is more plausible to accept that, these metals possess a destructive effect on cell, leading to the relcase of enzymes into blood. In this concern, Koyama *et al.* (1985) and Gill *et al.* (1991) found that Cd caused hepatocellular damage and necrosis in liver of toxicated common carp (*Cyprinus carpio*) and rosy barb (*Barbus conchinius*) and consequent reduction of hepatic AST and ALT activities after exposure for 2 and 6 weeks. This variation in enzymes activities with time of exposure may be due to the relatively slow and constant inhibition of enzymes by these heavy metals since their binding affinity with protein is generally very intense (Mckim *et al.*, 1970).

Concerning the changes in ACP activity in fish liver, data in Table 5 showed a pronounced decrease in fish exposed to low levels of all mixture groups and high levels of Cu, Zn and Cu+Zn group after 7 days of exposure. Similarly, on the $30^{th}$ day, the activity of ACP was significantly decreased in fish exposed to any two levels of Cu, Cd, Cu+Cd+Zn and high levels of Cu+Cd and Cd+Zn fish groups. Therefore, the decrease in ACP activity in the liver of the toxicated fish may be

attributed to the structural damage to cellular machinery concerns with enzyme production. Similar results were obtained by Sastry and Subhadra (1985), who found that, Cd caused reduction of ACP activity in liver and gills of catfish (*Helerapneustes fossilis*). Hilmy *et al.* (1987) reported also that, ACP was decreased in *Clarias lazera* and *Tilapia zillii* toxicated with zinc. Moreover, Gill *et al.* (1991) and (1992) reported that the hepatic, branchial and renal ACP activities were decreased in rosy barb (*Barbus conchonius*) toxicated with Cd and rosy barb (*Puntius conchonius*) toxicated with copper sulfate.

On the other hand, ACP activity in fish muscle exhibited no significant changes in all treated fish groups on the $7^{th}$ day. Likewise, after 30 days of exposure, the ACP activity was increased significantly in fish groups toxicated with low level of Cd and high level of Cu (Table 6). The elevated ACP activity in fish muscle may be due to the alteration in lysosome membrane liability leading to a release of hydrolytic enzymes including ACP. It is known that, lysosomes are rich in ACP and their limiting membrane prevents indiscriminated autolysis by providing a kind of latency. It is likely that, Zn and Cd ions compete for anionic sites, displacing the enzyme and increasing the ease with which they pass through the lysosomes membranes (Chvapil *et al.*, 1972). The incubation of rat liver lysosomes with $Hg^{+}$ was found to increase the lysosomes liability, while Cd, Pb and Zn toxicity decreased it. Gill *et al.* (1991) observed also that ACP activity was elevated in gut and ovary of rosy barb (*Barbus conchonius*) toxicated with Cd. They assumed that these results were due to the induction of Cd-induced enzyme in those organs as part of the biochemical adaptation of these tissues to meet the metabolic needs under toxicant-induced stress and/or an increase in lysosome liability.

It could be concluded from the present results that, the heavy metals; Cu, Cd and Zn showed severe effect on such enzymatic activities

in fish liver and muscle. However, the bi- and tri-mixtures of these metals exhibited antagonistic, synergistic and additive effects among them depending on type of mixture, concentrations of metals, type of enzyme and exposure time.

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Table 1. Changes of aspartate aminotransferase (IU. $g^{-1}$) in liver of common carp; *Cyprinus carpio* L. exposed to low and high levels of Cu, Cd and Zn alone or in combinations for 7 and 30 days.

| Toxicant Conc. | Time of Exposure | |
|---|---|---|
| | 7 days | 30 days |
| **Control** | 261.2 ± 24.7 | 266.9 ± 31.8 |
| **Low levels (¼ $LC_{50}$)** | | |
| **Cu** | 462.1 ± 45.2 ** | 160.2 ± 17.8 * |
| **Cd** | 273.2 ± 18.0 | 320.4 ± 28.4 |
| **Zn** | 305.4 ± 32.2 | 280.0 ± 40.1 |
| **Cu + Cd** | 180.8 ± 14.3 * | 213.1 ± 29.2 |
| **Cu + Zn** | 462.1 ± 46.6 ** | 280.0 ± 33.4 |
| **Cd + Zn** | 321.4 ± 19.2 | 240.3 ± 33.1 |
| **Cu + Cd + Zn** | 251.2 ± 11.9 | 160.1 ± 17.8 * |
| **High levels (½ $LC_{50}$)** | | |
| **Cu** | 401.8 ± 25.4 ** | 320.3 ± 25.4 |
| **Cd** | 381.7 ± 15.6 ** | 320.4 ± 44.1 |
| **Zn** | 401.8 ± 12.9 *** | 373.8 ± 40.9 |
| **Cu + Cd** | 375.0 ± 24.3 * | 380.6 ± 29.9 * |
| **Cu + Zn** | 455.4 ± 38.8 ** | 160.1 ± 17.8 * |
| **Cd + Zn** | 369.3 ± 42.1 | 352.4 ± 54.6 |
| **Cu + Cd + Zn** | 321.4 ± 6.3 * | 160.2 ± 17.8 * |

Data are presented as means of 5 samples ± standard error.

* Significant at P<0.05

** Significant at P<0.01

*** Significant at P<0.001

Table 2. Changes of aspartate aminotransferase (IU. $g^{-1}$) in muscle of common carp; *Cyprinus carpio* L. exposed to low and high levels of Cu, Cd and Zn alone or in combinations for 7 and 30 days.

| **Toxicant Conc.** | **Time of Exposure** | |
|---|---|---|
| | **7 days** | **30 days** |
| Control | 200.9 ± 17.9 | 257.2 ± 23.1 |
| **Low levels (¼ $LC_{50}$)** | | |
| Cu | 276.8 ± 24.2 * | 281.3 ± 9.2 |
| Cd | 241.1 ± 9.6 | 298.6 ± 13.8 |
| Zn | 261.2 ± 11.1 * | 80.4 ± 6.3 *** |
| Cu + Cd | 241.3 ± 24.1 | 96.4 ± 11.7 *** |
| Cu + Zn | 210.9 ± 17.9 | 214.3 ± 14.7 |
| Cd + Zn | 160.7 ± 16.6 | 192.9 ± 16.4 |
| Cu + Cd + Zn | 301.4 ± 29.7* | 107.1 ± 9.7 *** |
| **High levels (½ $LC_{50}$)** | | |
| Cu | 305.5 ± 39.3 * | 192.9 ± 16.4 |
| Cd | 307.2 ± 24.3 ** | 225.1 ± 22.17 |
| Zn | 241.1 ± 6.2 | 180.7 ± 8.8 * |
| Cu + Cd | 208.9 ± 9.8 | 200.9 ± 17.4 |
| Cu + Zn | 200.9 ± 17.9 | 180.8 ± 6.4 * |
| Cd + Zn | 160.7 ± 12.4 | 220.9 ± 22.1 |
| Cu + Cd + Zn | 187.5 ± 14.7 | 80.4 ± 6.3 *** |

Data are presented as means of 5 samples ± standard error.

* Significant at P<0.05

** Significant at P<0.01

*** Significant at P<0.001

Table 3. Changes of alanine aminotransferase (IU. $g^{-1}$) in liver of common carp; *Cyprinus carpio* L. exposed to low and high levels of Cu, Cd and Zn alone or in combinations for 7 and 30 days.

| **Toxicant Conc.** | **Time of Exposure** | |
|---|---|---|
| | **7 days** | **30 days** |
| Control | 148.5 ± 7.7 | 111.4 ± 7.4 |
| **Low levels (¼ $LC_{50}$)** | | |
| Cu | 173.2 ± 15.1 | 74.2 ± 8.8 * |
| Cd | 167.0 ± 15.8 | 83.5 ± 10.9 |
| Zn | 247.5 ± 0.5 ** | 102.1 ± 21.6 |
| Cu + Cd | 197.9 ± 7.7 ** | 51.9 ± 9.1*** |
| Cu + Zn | 197.9 ± 7.7 ** | 49.5 ± 7.5*** |
| Cd + Zn | 185.2 ± 18.2 | 55.7 ± 6.6*** |
| Cu + Cd + Zn | 241.3 ± 25.3 ** | 37.1 ± 3.9 *** |
| **High levels (½ $LC_{50}$)** | | |
| Cu | 341.5 ± 33.9 *** | 83.5 ± 4.5 * |
| Cd | 185.6 ± 16.2 | 74.3 ± 6.7 ** |
| Zn | 159.3 ± 13.1 | 61.9 ± 8.7 ** |
| Cu + Cd | 241.3 ± 25.7** | 141.1 ± 9.2 * |
| Cu + Zn | 259.8 ± 16.6 ** | 49.5 ± 7.5 *** |
| Cd + Zn | 247.5 ± 13.6 *** | 92.8 ± 8.1 |
| Cu + Cd + Zn | 123.7 ± 13.6 | 49.5 ± 7.5 *** |

Data are presented as means of 5 samples ± standard error.

* Significant at P<0.05

** Significant at P<0.01

*** Significant at P<0.001

Table 4. Changes of alanine aminotransferase (IU. $g^{-1}$) in muscle of common carp; *Cyprinus carpio* L. exposed to low and high levels of Cu, Cd and Zn alone or in combinations for 7 and 30 days.

| Toxicant Conc. | Time of Exposure | |
|---|---|---|
| | 7 days | 30 days |
| Control | 204.2 ± 5.5 | 193.1 ± 21.2 |
| **Low levels (¼ $LC_{50}$)** | | |
| Cu | 193.0 ± 18.1 | 182.7 ± 14.8 |
| Cd | 178.2 ± 23.8 | 167.0 ± 14.5 |
| Zn | 178.1 ± 18.2 | 173.2 ± 13.6 |
| Cu + Cd | 222.7 ± 18.2 | 148.5 ± 20.3 |
| Cu + Zn | 196.9 ± 7.7 *** | 163.3 ± 17.3 |
| Cd + Zn | 296.9 ± 7.7 *** | 241.3 ± 27.5 |
| Cu + Cd + Zn | 239.4 ± 11.9 * | 74.2 ± 4.4 *** |
| **High levels (½ $LC_{50}$)** | | |
| Cu | 334.1 ± 14.7 *** | 311.9 ± 27.8 *** |
| Cd | 267.1 ± 16.7 * | 253.4 ± 33.5 |
| Zn | 302.0 ± 12.4 *** | 222.7 ± 31.4 |
| Cu + Cd | 222.7 ± 13.9 | 252.4 ± 42.8 |
| Cu + Zn | 257.9 ± 16.6 *** | 111.4 ± 18.3 * |
| Cd + Zn | 185.6 ± 28.5 | 74.2 ± 6.3 *** |
| Cu + Cd + Zn | 321.7 ± 7.6 *** | 74.2 ± 4.4 *** |

Data are presented as means of 5 samples ± standard error.

* Significant at P<0.05

** Significant at P<0.01

*** Significant at P<0.001

Table 5. Changes of acid phosphatase (IU. $g^{-1}$) in liver of common carp; *Cyprinus carpio* L. exposed to low and high levels of Cu, Cd and Zn alone or in combinations for 7 and 30 days.

| **Toxicant Conc.** | **Time of Exposure** | |
|---|---|---|
| | **7 days** | **30 days** |
| Control | 388.8 ± 27.4 | 215.4 ± 10.7 |
| **Low levels (¼ $LC_{50}$)** | | |
| Cu | 238.4 ± 37.6 | 67.3 ± 7.4 *** |
| Cd | 253.5 ± 29.0 | 72.7 ± 5.1 *** |
| Zn | 335.3 ± 17.6 | 313.1 ± 14.9 *** |
| Cu + Cd | 271.7 ± 18.7 * | 242.4 ± 12.9 |
| Cu + Zn | 222.2 ± 22.5 *** | 212.1 ± 18.6 |
| Cd + Zn | 272.7 ± 28.9 * | 234.5 ± 16.4 |
| Cu + Cd + Zn | 141.1 ± 12.7 *** | 161.6 ± 6.6** |
| **High levels (½ $LC_{50}$)** | | |
| Cu | 149.5 ± 20.6 *** | 136.9 ± 17.2 ** |
| Cd | 339.3 ± 40.3 | 121.2 ± 12.6 *** |
| Zn | 217.6 ± 28.4 ** | 235.6 ± 20.8 |
| Cu + Cd | 379.8 ± 43.9 | 76.8 ± 7.6 *** |
| Cu + Zn | 368.6 ± 7.5 | 132.3 ± 7.8 ** |
| Cd + Zn | 68.7 ± 7.9 *** | 45.5 ± 7.9 *** |
| Cu + Cd + Zn | 222.2 ± 42.7 | 141.6 ± 16.9 ** |

Data are presented as means of 5 samples ± standard error.

* Significant at P<0.05

** Significant at P<0.01

*** Significant at P<0.001

Table 6. Changes of acid phosphatase (IU. $g^{-1}$) in muscle of common carp; *Cyprinus carpio* L. exposed to low and high levels of Cu, Cd and Zn alone or in combinations for 7 and 30 days.

| Toxicant Conc. | Time of Exposure | |
|---|---|---|
| | 7 days | 30 days |
| Control | 56.6 ± 7.6 | 48.4 ± 4.9 |
| **Low levels (¼ $LC_{50}$)** | | |
| Cu | 55.6 ± 7.5 | 68.8 ± 10.4 |
| Cd | 56.6 ± 4.0 | 72.7 ± 4.9 ** |
| Zn | 52.5 ± 8.1 | 50.5 ± 4.5 |
| Cu + Cd | 40.4 ± 3.2 | 32.3 ± 4.9 |
| Cu + Zn | 48.5 ± 8.1 | 56.6 ± 7.6 |
| Cd + Zn | 45.5 ± 7.5 | 56.6 ± 4.0 |
| Cu + Cd + Zn | 55.6 ± 7.5 | 55.6 ± 7.5 |
| **High levels (½ $LC_{50}$)** | | |
| Cu | 72.7 ± 12.1 | 68.7 ± 4.9 * |
| Cd | 40.3 ± 5.6 | 64.6 ± 7.6 |
| Zn | 36.4 ± 7.6 | 48.5 ± 4.9 |
| Cu + Cd | 40.4 ± 6.4 | 60.6 ± 9.0 |
| Cu + Zn | 35.4 ± 7.5 | 60.7 ± 6.5 |
| Cd + Zn | 65.7 ± 9.8 | 50.5 ± 4.5 |
| Cu + Cd + Zn | 52.5 ± 4.9 | 40.4 ± 6.4 |

Data are presented as means of 5 samples ± standard error.

* Significant at $P<0.05$

** Significant at $P<0.01$

*** Significant at $P<0.001$