# Journal of
# Fisheries and Aquatic Science

ISSN 1816-4927

Academic
Journals Inc.

www.academicjournals.com

Journal of Fisheries and Aquatic Science 2 (2): 100-109, 2007
ISSN 1816-4927


# Effect of EDTA on Toxicity Reduction of Cadmium in Relation to Growth, Some Haematological and Biochemical Profiles of Nile Tilapia (*Oreochromis niloticus*)

Adel M.E. Shalaby
Department of Fish Physiology, Central Laboratory for Aquaculture Research, Abbassa, Abo-Hammad, Sharkia, Egypt

**Abstract:** The effect of the ion-exchanging (chelating) agent EDTA on cadmium (Cd) toxicity and its impact on fish growth, food utilization, haematological and biochemical changes in Nile tilapia (*Oreochromius niloticus*) were studied. Fish (35-40 g) were exposed to 10 ppm Cd alone or with 0.1, 0.2 and 0.3 g EDTA $L^{-1}$ for 15 and 45 days. Cd exposure reduced significantly ($p<0.05$) the fish growth feed utilizations erythrocyte count (RBCs), haemoglobin content (Hb), haematocrit value (Hct), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration. All these parameters were improved when EDTA was applied with Cd. The values of RBCs, Hb, Hct, MCH and MCHC increased significantly to be as in the control fish group. Significant decreases in alkaline phosphatase activity and total protein (TP) in plasma, muscle and liver also observed in fish exposed to Cd alone. However the plasma glucose concentration, total lipids (LP), aspartate aminotranseferase (AST), alanine aminotransferase (ALT) and acid phosphatase (ACP) increased significantly in fish exposed to Cd alone. Addition of EDTA to Cd contaminated medium enhanced biochemical parameters in fish and the enzyme activities returned to be as the control fish group. Addition of EDTA to Cd-contaminated medium considerably reduced metal absorption and its accumulation in fish tissues, while metal level increased in water and feces. Fish exposed to Cd alone accumulates 2.16 and 5.972 mg Cd $g^{-1}$ dry weight in liver tissue after 15 and 45 days, respectively. Cd concentration was reduced significantly to 1.292 and 4.16; 0.94 and 3.79; 0.42 and 2.45 mg Cd $g^{-1}$ dry weight tissue in fishes exposed to 0.1, 0.2 and 0.3 g EDTA $L^{-1}$ after 15 and 45 days, respectively. Similar trends were observed in gills and muscle.

**Key words:** *Nile tilapia*, cadmium, EDTA, growth performance, feed utilization, haematology, biochemistry, glucose, TP, LP, AST, ALT, ALP, ACP

## INTRODUCTION

The problem of appearance of toxic materials in water ecosystem is presently closely connected with increased concentration of different types of pollutants, which enter water bodies with industrial and communal waste waters or from non point sources. Metals are redistributed naturally in the environment by both geologic and biologic cycles. Many metals, whether organically-complexed or not are known to accumulate in plant and animal tissues to very high level, posing a potential toxic hazard to the organisms themselves, or organisms higher in the food chain including humans, which may consume them (Abel, 1998). Evidence of toxic effect of heavy metals has been reported on fishes and populations eating contaminated food (Chang, 1996).

Cadmium is an extremely toxic heavy metal. It is widely used in mining, metallurgical operation, electroplating industries manufacturing vinyl plastics, used in metallic and plastic

pipes. Effluents from such activities are sources of cadmium into aquatic environments. Most aquatic organisms have the capability of concentrating metals by feeding and metabolic processes, which can lead to accumulation of high concentrations of metals in their tissues. Metals interact with legends in proteins particularlty, enzymes and may inhibit their biochemical and physiological activities (Passow *et al*., 1961).

The reduction of toxic elements like cadmium in aquatic environments is needed by any acceptable method. The most widely used technique for the removal of toxic elements involves the process of neutralization and metal hydroxide precipitation (Hiemesh and Mahadevaswamy, 1994). Chemicals can effectively remove certain toxic elements from industrial wastes or polluted media, but is usually costly. However, there are some cheap chemicals, which are also free from undesirable side effects. In recent years, the remobilization of metals by synthetic anthropogenic chelating agents has received much attention. Literature reported number of chelators that have been used for chelate-induced hyperaccumulation (Huang *et al*., 1997). Synthetic compound like ethylenediamine tetraacetic acid (EDTA) is known to be effective chelating agents of heavy metals (Licop, 1988). EDTA is the most commonly used chelator due to its strong chelating ability for different heavy metals (Norvell, 1991). EDTA has two advantages with respect to -its relative low biodegradability in groundwater systems (Nowack, 1996) and its strong complexing capacity with heavy metals (Kedziorek and Bourg, 2000).

Metal bioaccumulation can occur via complexation, coordination, chelating, ion exchange and other processes of greater or lesser specificity. Bioaccumulation processes are sometimes due to active (metabolism dependent) metal accumulation by living cells. In other cases, bioaccumulation is a strictly aggressive process in which metal ions are sequestered by metal binding site in the interior of the cell. The removal of toxic elements from contaminated water, has potential advantages over the conventional treatment process (ion exchange, precipitation, membranes, etc.) (Kuyack and Volesky, 1990).

In spit of the amount of data published on the effect of waterborne exposure of cadmium and EDTA singly, information on the effects of Cd/EDTA mixture on aquatic organisms are limited and not uniform. Therefore, EDTA appears to be promising tool to control cadmium pollution in aquaculture. In the present study, short and long-term bioassays were designed to evaluate the influence of EDTA on the retention of cadmium in water. It was carried out to investigate the effect of EDTA on reduction of toxicity of cadmium for enhance the change of blood parameter and enzymes also to assess its impact on some physiological parameters of Nile tilapia (*Oreochromis niloticus*).

## MATERIALS AND METHODS

### Fish Culture Management

Healthy fish of Nile tilapia *Oreochromis niloticus* weighing 35-40 g/fish were collected from the ponds of Central Laboratory for Aquaculture Research at Abbassa, Abo-Hammad, Sharkia, Egypt. Fish were acclimated in an indoor tank for 2 weeks to laboratory conditions.

Acclimated fish were exposed to different concentration of cadmium and mortality were observed for 96 h. A static renewable bioassay method (Spraggue, 1973) was adopted for the determination of 96 h $LC_{50}$ (Litchfield and Wileoxon, 1949). A control group was maintained in metal-free tap water. The 96 h $LC_{50}$ of cadmium for *Oreochromius niloticus* was 40 ppm. A stock solution of cadmium was prepared by dissolving 10.686 g of analar grad cadmium sulphate ($CdSO_4$-8/3$H_2O$) in 1 $L^{-1}$ of distilled water and the diluted with water to obtain the desired concentration (10 ppm) for this experiment.

Table 1: Experimental groups and their notation

| Groups | Nation |
|---|---|
| Control (metal free water) | C |
| Cadmium (10 ppm) alone | Cd |
| Cadmium (10 ppm) +0.1 g EDTA $L^{-1}$ | CdEDTA1 |
| Cadmium (10 ppm) +0.2 g EDTA $L^{-1}$ | CdEDTA2 |
| Cadmium (10 ppm) +0.3 g EDTA $L^{-1}$ | CdEDTA3 |

The fish were distributed randomly in 120 L glass aquaria, at a rate of 15 fish/aquarium that containing aerated tap water. These aquaria were divided into five groups with three replicates each per group. The first group was free from Cd and EDTA and maintained as a control. The second groups were exposed to 10 ppm of Cd $SO_4$ only (Equivalent to 1/4 96 h $LC_{50}$). The third, fourth and fifth group were exposed to 10 mg Cd $L^{-1}$ and 0.1, 0.2 and 0.3 g EDTA/L, respectively (Table 1). Each aquarium was supplied with compressed air via air-stones from air pumps. Well-aerated water supply was provided from a storage fiberglass tank. The temperature was adjusted at 27±1°C by means of thermostats.

Cadmium sulphate and EDTA was obtained from El-Nasr Chemical Company (Egypt) and prepared in aquatic solution to provide the required concentrations of cadmium and EDTA.

Fish were fed frequently on a diet containing 30% crude protein (CP) at a rate of 3% of live body weight twice daily for 15 and 45 days. Siphoning three quarters aquariums was done every day for waste removal and replacing it by an equal volume of water containing the same concentration of Cd and EDTA. Dead fish were removed and recorded daily.

**Growth Parameters**

Growth performance was calculated as following:

Weight gain = $W_2$-$W_1$;

Specific Growth Rate (SGR) = 100 (ln $W_2$-ln$W_1$)/T; where $W_1$ and $W_2$ are the initial and final fish weight, respectively and T is the number of days in the feeding period;
Feed Conversion Ratio (FCR) = feed intake/weight gain

**Physiological Analyses**

After 15 and 45 days of the experiment. Samples of blood, liver, gills and muscle were taken from three Fish specimens from each aquarium.

Fish were not fed for 24 h before sampling and were anaesthetized with buffered MS222 (50 mg $L^{-1}$) and blood samples were taken from caudal vein of an anaesthetized fish by sterile syringe using EDTA solution as anticoagulant. These blood samples were used for determining erythrocyte count (Dacie and Lewis, 1984) and hemoglobin content (Van Kampen and Zijlstra, 1961). Heamatocrit value (Hct) were calculated according to the formulae mentioned by Britton (1963).

Plasma was obtained by centrifugation of blood at 3000 rpm for 15 min and nonhaemolyzed plasma was stored in deep freezer for further biochemical analyses. After decapitation of fish, samples of liver and muscle were taken and frozen for further biochemical analyses. Plasma glucose was determined, using glucose kits supplied by Boehring Mannheium kit, according to Trinder (1969). Total protein content was determined colorimetrically according to Henry (1964). Total lipids contents were determined colorimetrically according to Joseph *et al*. (1972). Activities of aspartate amninotransferase (AST) and alanine aminotransferase (ALT) were determined colorimetrically according to Reitman and Frankel (1957), while alkaline phosphatase (ALP) was measured by using Diamond diagnostics kits according to the method of Rec (1972). Also acid phosphatase (ACP) activity was determined according to the method of King and King (1954).

**Cd Residue**

Cadmium rest residues were measured in water, liver, gills, muscle and feces according to method of Eaton and Stinson (1983).

**Statistical Analysis**

The obtained data were subjected to analysis of variance according to Snedecor and Cochran (1982). Differences between means were done at the 5% probability level, using Duncan' s new multiple range test (Duncan, 1955).

## RESULTS

The present study showed that the addition of EDTA to Cd contaminated media, reduced significantly the Cd level in water and helped to eliminate Cd from the fish body, which in turn improved the growth, haematological and biochemical parameters as compared to fish exposed to cadmium alone.

**Growth Perforamance**

Results in Table 2 showed that the final weight, GW, SGR, feed intake and FCR were decreased significantly ($p<0.05$) when Nile tilapia exposed to Cd alone, while fish growth was increased significantly (p0.05) when fish exposed to Cd with 0.2 and 0.3 g EDTA $L^{-1}$ when compared to Cd alone and similar to the control fish group.

**Haematological Parameters**

Table 3 shows that the RBCs, HB and HCt were reduce in fish exposed to Cd at both periods and they were less than that of the control ($p<0.05$) The RBCs count also decreased significantly in fish exposed to Cd at 15 and 45 days. On the other hand, these parameters were return to the normal values and increased significantly in fish exposed to Cd with 0.2 and 0.3 g of EDTA $L^{-1}$ for 15 and 45 days. Blood parameter were improved in fish exposed to Cd with different levels of EDTA.

Data shows (Table 4) that the MCV increased significantly in fish exposed to Cd alone, while the MCH and MCHC decreased significantly in fish exposed to Cd only when compared with the control.

Table 2: Growth performance of Nile tilapia (*O. niloticus*) exposed to Cd with and without EDTA.

| Items | Initial weight (g/fish) | Finial weight (g/fish) | WG (g/fish) | SGR (%) | Feed intake (g feed/fish) | FCR |
|---|---|---|---|---|---|---|
| Control | 38.4±0.04$^{a}$ | 55.6±1.9$^{ab}$ | 17.2±1.3$^{ac}$ | 0.805±0.015$^{a}$ | 47.5±0.22$^{a}$ | 2.76±0.25$^{a}$ |
| Cd | 38.7±0.05$^{a}$ | 42.3±1.8$^{c}$ | 3.6±0.90$^{b}$ | 0.197±0.01$^{b}$ | 44.25±0.06$^{b}$ | 12.29±1.6$^{b}$ |
| Cd+0.1 g EDTA $L^{-1}$ | 38.7±0.05$^{a}$ | 53.31±0.80$^{a}$ | 14.6±1.1$^{c}$ | 0.711±0.021$^{c}$ | 46.91±0.31$^{a}$ | 3.21±0.4$^{a}$ |
| Cd+0.2 g EDTA $L^{-1}$ | 38.6±0.03$^{a}$ | 55.49±1.2$^{ab}$ | 16.9±0.9$^{ac}$ | 0.806±0.03$^{a}$ | 47.75±0.17$^{a}$ | 2.827±0.21$^{a}$ |
| Cd+0.3 g EDTA $L^{-1}$ | 38.6±0.02$^{a}$ | 56.4±1.1$^{b}$ | 17.8±0.8$^{a}$ | 0.842±0.014$^{a}$ | 47.99±0.27$^{a}$ | 2.696±0.52$^{a}$ |

The same letter in the same column is not significantly different at $p<0.05$

Table 3: Changes in erythrocyte (count $\times 10^{6}/mm^{3}$), hemoglobin content (g 100 $mL^{-1}$) and haematocrit value (%) in the blood of Nile tilapia (*O. niloticus*) exposed to Cd with and without EDTA

| | Erythrocyte count (RBCs) | | Hemoglobin (HB) | | Haematocrit value (Hct) | |
|---|---|---|---|---|---|---|
| Items | 15 days | 45 days | 15 days | 45 days | 15 days | 45 days |
| Control | 1.59±0.072$^{a}$ | 1.715±0.051$^{a}$ | 5.49±0.354$^{a}$ | 7.316±0.133$^{a}$ | 15.30±0.308$^{a}$ | 17.33±1.666$^{a}$ |
| Cd | 1.268±0.064$^{b}$ | 1.06±0.073$^{b}$ | 4.21±0.236$^{b}$ | 4.12±0.354$^{c}$ | 13.5±0.47$^{b}$ | 12.02±0.577$^{b}$ |
| Cd+0.1 g EDTA $L^{-1}$ | 1.572±0.062$^{a}$ | 1.57±0.025$^{d}$ | 4.54±0.395$^{ab}$ | 5.12±0.135$^{b}$ | 14.66±1.452$^{a}$ | 15.06±0.76$^{a}$ |
| Cd+0.2 g EDTA $L^{-1}$ | 1.56±0.087$^{a}$ | 1.786±0.032$^{ac}$ | 5.18±0.458$^{ab}$ | 6.606±0.307$^{b}$ | 15.02±1.73$^{a}$ | 17.66±0.918$^{a}$ |
| Cd+0.3 g EDTA $L^{-1}$ | 1.957±0.088$^{c}$ | 2.02±0.061$^{c}$ | 6.466±0.277$^{c}$ | 7.68±0.133$^{a}$ | 20.0±0.365$^{c}$ | 22.01±1.471$^{c}$ |

The same letter in the same column is not significantly different at $p<0.05$

Table 4: Changes in mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) in the blood of Nile tilapia (*O. niloticus*) exposed to Cd with or without EDTA

| | MCV | | MCH | | MCHC | |
|---|---|---|---|---|---|---|
| Items | 15 days | 45 days | 15 days | 45 days | 15 days | 45 days |
| Control | 96.22±1.85$^{ad}$ | 101.04±2.234$^{a}$ | 34.52±0.351$^{a}$ | 42.11±1.456$^{a}$ | 35.88±1.117$^{a}$ | 42.21±0.939$^{a}$ |
| Cd | 106.97±2.23$^{b}$ | 106.76±0.874$^{b}$ | 33.02±0.177$^{b}$ | 36.58±0.846$^{b}$ | 31.18±0.909$^{b}$ | 37.38±1.85$^{b}$ |
| Cd+0.1 g EDTA $L^{-1}$ | 93.25±2.04$^{a}$ | 95.92±4.27$^{a}$ | 34.69±1.32$^{a}$ | 32.61±1.18$^{b}$ | 37.17±1.28$^{a}$ | 33.99±1.51$^{b}$ |
| Cd+0.2 g EDTA $L^{-1}$ | 96.28±2.56$^{a}$ | 98.88±0.914$^{a}$ | 33.20±1.61$^{a}$ | 36.98±1.52$^{bc}$ | 34.07±1.47$^{ac}$ | 37.40±1.16$^{b}$ |
| Cd+0.3 g EDTA $L^{-1}$ | 102.1±2.52$^{db}$ | 108.96±2.243$^{b}$ | 33.84±1.703$^{a}$ | 38.01±1.578$^{a}$ | 32.33±0.943$^{cb}$ | 34.89±1.69$^{b}$ |

The same letter in the same column is not significantly different at $p<0.05$

Table 5: Changes in glucose, total protein and total lipids concentrations in plasma of Nile tilapia (*O. niloticus*) exposed to Cd with and without EDTA

| | Glucose (mg $L^{-1}$) | | Total protein (g 100 $mL^{-1}$) | | Total lipid (g $L^{-1}$) | |
|---|---|---|---|---|---|---|
| Items | 15 days | 45 days | 15 days | 45 days | 15 days | 45 days |
| Control | 60.15±2.89$^{a}$ | 41.32±2.265$^{a}$ | 1.856±0.197$^{a}$ | 3.163±0.193$^{a}$ | 5.706±0.084$^{a}$ | 6.553±0.471$^{ad}$ |
| Cd | 94.86±6.14$^{b}$ | 151.82±7.48$^{b}$ | 1.653±0.207$^{a}$ | 1.530±0.073$^{b}$ | 16.200±0.386$^{c}$ | 13.303±0.307$^{b}$ |
| Cd+0.1 g EDTA $L^{-1}$ | 68.77±1.43$^{c}$ | 76.30±3.95$^{c}$ | 2.173±0.188$^{a}$ | 1.806±0.098$^{b}$ | 13.990±0.571$^{b}$ | 9.391±0.370$^{c}$ |
| Cd+0.2 g EDTA $L^{-1}$ | 49.73±3.76$^{a}$ | 71.50±2.95$^{c}$ | 1.966±0.121$^{a}$ | 1.840±0.158$^{b}$ | 9.533±0.35$^{d}$ | 5.687±0.287$^{d}$ |
| Cd+0.3 g EDTA $L^{-1}$ | 55.05±1.45$^{a}$ | 54.46±3.33$^{d}$ | 2.146±0.143$^{a}$ | 4.536±0.114$^{c}$ | 05.720±0.079$^{a}$ | 7.440±0.325$^{a}$ |

The same letter in the same column is not significantly different at $P<0.05$.

These parameters increased with increasing the exposure time of fish to Cd. Addition of EDTA to Cd-polluted media maintained the MCV, MCH and MCHC at levels close to those of the control.

**Biochemical Parameters**

The present study shows that addition of EDTA to Cd contaminated media reduced significantly the Cd level in the water and helped to eliminate metal from the fish body and in turn improved the biochemical parameters as compared to fish exposed to Cd alone.

The plasma glucose concentration showed higher significant values ($p<0.01$) (94.86±6.14 and 68.77±1.43 mg %) in fish exposed to Cd alone and Cd with 0.1 g of EDTA $L^{-1}$ for 15 days than the control fish group value (60.15±2.89 mg %). The glucose concentration in fish subjected for other groups (Cd+0.2 g EDTA $L^{-1}$ and Cd with 0.3 g EDTA $L^{-1}$) did not significantly be affected. After 45 day of exposure, the plasma glucose concentration increased significantly ($p<0.05$) in all treatments. As shown in Table 5 there was no significant variation in the plasma total protein of nearly all fish under investigation for 15 days. After 45 days, the plasma total protein decreased significantly to be 1.536±0.073, 1.806 ±0.098 and 1.84±0.158 g 100 $mL^{-1}$ in fish exposed to Cd alone and mixture of Cd + 0.1 and Cd + 0.2 g EDTA $L^{-1}$, respectively. This value increased significantly after exposing fish to mixture of Cd with 0.3 g EDTA $L^{-1}$. The plasma total lipids increased significantly in fish exposed to Cd alone and Cd with 0.1 g EDTA $L^{-1}$ for 15 and 45 days when compared to the control group, while it was similar to the control group in fish exposed to mixture of Cd with 0.2 and 0.3 g EDTA $L^{-1}$ for 45 days.

Table 6 shows a significant reduction of total protein in liver and muscle of fish exposed to Cd alone and Cd with EDTA for 15 and 45 days. The addition of EDTA enhanced the total protein in liver and muscle to be better than that of Cd alone, but still lower than that of control group ($p<0.05$). This result indicates that the addition of EDTA failed to recover the total protein in liver and muscle.

Table 7 showed that AST activity increased significantly in plasma of fish exposed to Cd alone. The addition of EDTA decreased significantly the AST activity to be less than that of Cd alone ($p<0.05$). The AST activity in fish exposed to Cd with 0.3 g EDTA $L^{-1}$ became similar to that of control at 15 days and 45 days. The plasma ALT activity increased significantly in fish exposed to Cd alone at 15 and 45 days (53.93 and 83.66 IU $L^{-1}$, respectively. The addition of EDTA enhanced ALT activity to be as in the control especially the groups exposed to Cd with 0.2 and 0.3 g EDTA $L^{-1}$ at both periods.

Table 6: Changes in total protein content (mg/g fresh weight) in liver and muscle of Nile tilapia (*O. niloticus*) exposed to Cd with or without EDTA

| | Liver | | Muscle | |
|---|---|---|---|---|
| Items | 15 days | 45 days | 15 days | 45 days |
| Control | 122.8±4.115[a] | 157.6±2.886[a] | 169.8±2.882[a] | 211.6±4.494[a] |
| Cd | 66.5±2.78[b] | 45.32±1.098[b] | 45.8±1.890[b] | 36.6±1.130[b] |
| Cd+0.1 g EDTA $L^{-1}$ | 58.5±1.739[c] | 101.2±4.094[c] | 58.4±2.788[c] | 92.6±4.168[c] |
| Cd+0.2 g EDTA $L^{-1}$ | 77.92±1.077[d] | 124.6±3.457[d] | 68.1±3.568[d] | 130.5±2.826[d] |
| Cd+0.3 g EDTA $L^{-1}$ | 84.06±2.629[e] | 132.06±4.294[d] | 80.4±2.641[e] | 156.3±4.111[e] |

The same letter in the same column is not significantly different at $p<0.05$

Table 7: Changes in aspartate aminotransferase activity (AST) and alanine aminotransferase (ALT) activity (IU $L^{-1}$) in plasma of Nile tilapia (*O. niloticus*) exposed to Cd with or without EDTA

| | AST | | ALT | |
|---|---|---|---|---|
| Items | 15 days | 45 days | 15 days | 45 days |
| Control | 51.490±1.795[a] | 117.02±2.99[a] | 32.966±2.58[a] | 51.700±2.64[a] |
| Cd | 99.278±1.913[b] | 167.70±3.254[b] | 53.930±0.76[b] | 83.660±2.46[b] |
| Cd+0.1 g EDTA $L^{-1}$ | 98.733±1.543[b] | 127.02±2.27[a] | 52.260±2.86[b] | 61.260±3.21[c] |
| Cd+0.2 g EDTA $L^{-1}$ | 75.866±3.806[c] | 125.43±2.66[a] | 36.510±2.53[a] | 42.050±3.65[a] |
| Cd+0.3 g EDTA $L^{-1}$ | 52.310±1.9854[a] | 130.22±3.28[a] | 44.750±2.45[c] | 51.166±1.981[a] |

The same letter in the same column is not significantly different at $p<0.05$

Table 8: Changes in Alkaline phosphatase (ALP) and Acid phosphatase (ACP) activities (IU $L^{-1}$) in plasma of Nile tilapia (*O. niloticus*) exposed to Cd with or without EDTA

| | ALP | | ACP | |
|---|---|---|---|---|
| Items | 15 days | 45 days | 15 days | 45 days |
| Control | 2.490±0.521[a] | 2.110±0.72[a] | 15.10±2.9[a] | 36.50±27.6[a] |
| Cd | 1.213±0.110[b] | 1.510±0.61[a] | 42.50±4.6[b] | 61.26±3.21[b] |
| Cd+0.1 g EDTA $L^{-1}$ | 1.390±0.323[ab] | 1.532±0.42[a] | 32.60±2.86[bc] | 58.30±4.28[b] |
| Cd+0.2 g EDTA $L^{-1}$ | 1.770±0.326[ab] | 1.690±0.23[a] | 26.51±1.53[c] | 42.05±1.65[ca] |
| Cd+0.3 g EDTA $L^{-1}$ | 1.831±0.234[ab] | 1.980±0.28[a] | 14.75±2.45[a] | 37.16±1.581[a] |

The same letter in the same column is not significantly different at $p<0.05$

The alkaline phosphatase (ALP) in plasma decreased significantly in fish exposed to Cd (1.213±0.110 IU $L^{-1}$, $p<0.05$). On the other hand, addition of EDTA to Cd-polluted media enhanced ALP activity in fish and became similar to that of control fish at 15 and 45 days. After 45 days, the ALP activity showed non significant variation among treatments (Table 8). The acid phosphatase (ACP) increased significantly in fish exposed to Cd alone at 15 and 45 days (42.5 and 61.26 IU $L^{-1}$, $p<0.05$, respectively). Contrarily, the ACP activities of fish exposed to Cd with high dose EDTA became similar to that of control fish group at 15 and 45 days.

**Cd Bioaccumulation**

Addition of EDTA to the Cd polluted media reduced significantly ($p<0.05$) the Cd level in aquarium's water as compared to that of Cd alone (Table 9). The Cd concentration in water exposed Cd alone was 9.32 mg $L^{-1}$ and declined significantly ($p<0.05$) to 7.15, 3.79 and 1.73 mg $L^{-1}$ with 0.1, 0.2 and 0.3 g EDTA $L^{-1}$, respectively. The data showed also a wide variation among the different organs of Nile tilapia subjected to Cd alone or Cd with different doses of EDTA. The highest amount of Cd residue was found in the liver and the lowest amount in the muscle. Table 9 showed that the uptake of Cd in the liver of fish exposed to Cd alone was 2.16 and 5.972 mg $g^{-1}$ dry weight for 15 and 45 days, respectively. it declined significantly to 1.292 and 4.16.; 0.94 and 79; 0.42 and 2.45 mg $g^{-1}$ dry weight in fish group exposed to Cd with 0.1, 0.2 and 0.3 g EDTA $L^{-1}$ for 15 and 45 days, respectively. Similar trends were observed in fish gills and muscles. On the other hand, the Cd residue in fish faces increased significantly with increasing the level of EDTA in aquariums water.

Table 9: Changes in cadmium residue in water (mg Cd $L^{-1}$), liver, gills, muscle and feces (mg Cd $g^{-1}$ dry weigh) of Nile tilapia *(O. niloticus)* exposed to to Cd with or without EDTA

| | | Liver | | Gills | |
|---|---|---|---|---|---|
| Items | Water | 15 | 45 | 15 | 45 |
| Control | 0.042±0.01 | 0.048±0.01$^{a}$ | 0.055±0.004$^{a}$ | 0.038±0.01$^{a}$ | 0.039±0.02$^{a}$ |
| Cd | 9.320±0.83 | 2.160±0.254$^{b}$ | 5.972±0.86$^{b}$ | 1.370±0.086$^{b}$ | 2.550±0.286$^{b}$ |
| Cd+0.1 g EDTA/l | 7.150±0.33 | 1.292±0.054$^{c}$ | 4.160±0.44$^{b}$ | 0.650±0.06$^{c}$ | 1.070±0.11$^{c}$ |
| Cd+0.2 g EDTA/l | 3.790±0.01 | 0.940±0.054$^{d}$ | 3.792±0.29$^{b}$ | 0.394±0.052$^{d}$ | 0.850±0.06$^{c}$ |
| Cd+0.3 g EDTA/l | 1.730±0.01 | 0.420±0.034$^{e}$ | 2.450±0.23$^{c}$ | 0.267±0.076$^{d}$ | 0.710±0.42$^{c}$ |
| | | Muscle | | Feces | |
| Items | Water | 15 | 45 | 15 | 45 |
| Control | 0.042±0.01 | 0.023±0.001$^{a}$ | 0.076±0.005$^{a}$ | 0.003±0.019$^{ab}$ | 0.005±0.01$^{ab}$ |
| Cd | 9.320±0.83 | 0.475±0.06$^{b}$ | 1.078±0.16$^{b}$ | 0.152±0.019$^{b}$ | 0.188±006$^{b}$ |
| Cd+0.1g EDTA/l | 7.150±0.33 | 0.345±0.04$^{ab}$ | 0.667±0.021$^{c}$ | 0.942±0.03$^{c}$ | 2.067±0.142$^{c}$ |
| Cd+0.2g EDTA/l | 3.790±0.01 | 0.330±0.08$^{ab}$ | 0.383±0.034$^{d}$ | 2.340±0.069$^{d}$ | 5.442±0.343$^{d}$ |
| Cd+0.3g EDTA/l | 1.730±0.01 | 0.216±0.03$^{c}$ | 0.217±0.027$^{d}$ | 5.282±0.32$^{e}$ | 7.456±0.526$^{e}$ |

The same letter in the same column is not significantly different at $p<0.05$, Water sample were taken just before siphoning

## DISCUSSION

The results showed that the fish exposed to Cd alone showed a significant lower fish growth, feed intake and feed conversion than those exposed to Cd with different level of EDTA. The reduction of feed conversion rate in *O. niloticus* at sublethal levels of Cd might be due the tissue burden of more Cd, which in turn could reduce food intake, increase in metabolic cost and poor food conversion efficiency. These results are in agreement with those of James *et al*. (1992) who found that *Heeteropenustes fossillis* exposed to mercury along with *Eichhorina crassipes* showed significant improvement in growth of Fish exposed to mercury alone. *Eichhorina crassipes* removed considerable amount of mercury from test medium and thereby indirectly reduced the toxic effects on *H. fossilis* and this observation supports the present study.

The present study reveals that the fish exposed to Cd alone showed significant reduction in RBCs, Hb and Hct than those exposed to Cd with different level of EDTA. The reduction of these parameters in Nile tilapia, *O niloticus* at sublethal levels of cadmium might be due to the destruction of mature RBCs and the inhibition of erythrocyte production due to reduction of haemsynthesis that affected by pollutants (Wintrobe, 1978). Also, the decrease in RBCs count may be attributed to haematopathology or acute haemolytic crisis that results in sever anemia in most vertebrates including fish species exposed to different environmental pollutants (Khangarot and Tripathi, 1991). The decrease in the RBCs may be attributed to reduction of growth and other food utilization parameters which results in sever aneamia (James and Sampath, 1999). Also Gill and Epple (1993) found a significant reduction in the RBCs, Hb and Hct in American eel *(Anguilla rostrata)* after exposure to 150 ug Cd $L^{-1}$. Karuppasamy *et al*. (2005) found a significant decrease in total erythrocyte count, haemoglobin content, haematocrit value and mean corpuscular haemoglobin concentration in air breathing fish, *Channa punctatus* after exposure to sublethal dose of Cd (29 mg Cd $L^{-1}$).

The addition of EDTA improves the haematological parameters (RBCs, Hb and Hct), which indicating to the capability of EDTA to chelate Cd from the media. Subsequently, the Cd toxicity was reduced. These results are in agreement with those of James *et al*. (1998) who observed that *Oreochromis mossambicus* exposed to copper along with EDTA showed a significant improvement in blood parameters over those exposed to copper alone.

The calculated blood indices, MCV, MCH and MCHC have a particular importance in anemia diagnosis in most animals (Coles, 1986). The perturbations in these blood indices (increase MCV, decrease of MCH and MCHC) may be attributed to a defense against Cd toxicity through the stimulation of erythropiosis or may be related to the decrease in RBCs, Hb and Hct which in turn due

to the exaggerated disturbances that occurred in both metabolic and hemopoietic activities of fish exposed to sublethal concentration of pollutants (Moussa, 1999).

The present results indicate that EDTA is effective in removing Cd from water and reducing Cd bioaccumulation in fish. Particulate organic matter can scavenge metal from water and help to reduce metal from fish. These results are in agreement with Santschi (1988) who reported that any agent that can remove Cd from water helps to reduce the bioaccumulation of this metal in fish.

Blood glucose is a sensitive reliable indicator of environmental stress in fish. From the present results, it is clear that Cd which elevated blood glucose level affected as a stress on fish. Cd induced hyperglycemia with decreased in liver glycogen in catfish, *Heteropneustes fossilis* (Sastry and Subhadra 1985). Soengas *et al*. (1996) suggested that hyperglycemia occurred in Atlantic salmon *(Salmo salar*) after toxicity with cadmium, may be due to changes in liver carbohydrate metabolism (activation of liver glycogenolysis and glycolysis) as well as increased levels of plasma glucose and lactate. However, the reduce of glucose concentration in plasma of fish along with EDTA is due to the removal of Cd by EDTA.

Total protein level is a frequently parameter of metal poisoning in fish. However, data available did not allow to assessment of the direction of these changes, since the same metal may cause both an increase or a decrease in total protein. There were no changes in plasma total protein in fish exposed to cadmium at 15 days, while these values were decreased significantly in fish exposed to Cd only or with low levels of EDTA at 45 days. The present study showed that liver protein and muscle total protein was significantly decreased in fish exposed to Cd alone. This result may be attributed to the great demands and cellular damage that occurred in the tissues of Cd-toxicated fish and Cd toxicity may be possible cause protein breakdown. The addition of chelating agent; EDTA to Cd polluted media reduced significantly the retention of Cd in fish body and this indirectly improved the growth and biochemical changes. James and Sampath (1999) found similar results with catfish, *Heteropneustes fossilis*.

Total lipids in plasma increased significantly in fish exposed to Cd alone. On the other hand, addition of EDTA lowered total lipids in fish exposed to cadmium toxicity to be similar to that of the control fish. Shalaby (2001) reported that the absorption of excess heavy metals disturbed the metabolism of lipid.

The activity of AST and ALT enzymes in blood may also be used as a stress indicator. The significant changes in the activities of these enzymes in blood plasma indicates tissue impairment caused by stress (James *et al*., 1991; Svoboda, 2001). In the present study, there were significant changes in AST and ALT activities in plasma of fish exposed to cadmium compared to the control group. The increase in concentration of AST and ALT in blood plasma indicates impairment of parenchymatous organs (namely liver). In addition, the increase of plasma AST and ALT may be attributed to the hepatocellular damage or cellular degradation by these heavy metal, perhaps in liver, heart or muscle (Yamawaki *et al*., 1986). These results are in agreement with those of Shalaby (1997) who found that sublethal concentration of Cd caused significant increases in AST and ALT of common carp after 7 and 15 days.

The decrease of ALP activity in plasma due to Cd toxicity was similar to that obtained by Sastry and Subhadra (1985) who recorded that a significant reduction in ALP in liver and kidney of catfish, *Heteropneustes fossilis* after toxication with Cd. This decrease may be due to the damage and dysfunction of the liver. EDTA reduced the Cd toxicity in water and fish, which in turn enhanced the ALP activity. On other hand, The ACP activity increased significantly in fish exposed to Cd alone more than control. This result is in a agreement with Sastry and Subhadra (1985) who found that a significant increase in ACP in kidney of catfish, *Heteropneustes fossilis*, after toxication with Cd. The obtained results showed that all the tested biochemical parameters were improved due to EDTA application and they were more pronounced in Cd +0.3 g EDTA $L^{-1}$, which is considered as an optimum dose could improve the healthy status of fish.

The present study showed that the addition of EDTA to the Cd media reduced significantly ($p<0.05$) the Cd level in water and metal uptake as compared to fish exposed to Cd alone. The Cd concentration in water was 9.23 mg $L^{-1}$ and it decreased significantly ($p< 0.05$). The Cd accumulation in liver, gills and muscle of fish exposed to Cd alone was higher than that of EDTA treatment. These results suggest that EDTA could chelate Cd ions producing a stable complex, thus reducing the chance for metal uptake by tissues. Besides, the EDTA groups eliminated more amount of Cd from the body through feces. The formation of Cd-EDTA complex in water and elimination of more amount of Cd in feces evidently reduced the metal burden in tissues and thereby improved the haematological and biochemical parameters of fish exposed to Cd. Planas-Bohne and Lehman (1983) found low level of cadmium in tissues due to increased excretion of metals through feces and urine when rats were administered Cd intravenously along with EDTA.

From the present study, it is recommended that an optimum dosage of 0.3 g EDTA $L^{-1}$ could effectively chelate Cd from contaminated water and improve the growth, physiological function and activities of fish.

## REFERENCES

Abel, P.D., 1998. Water Pollution Biology. Taylor and Francis Ltd. London.

Britton, C.J., 1963. Disorders of the Blood. 9th Edn. I.A. Churchill, Ltd. London.

Chang, L.W., 1996.Toxicology of Metals. CRC Press Inc. Lewis Publishers, New York, USA.

Coles, E.H., 1986. Veterinary Clinical Pathology. W.B. Saunders, Philadelphia, pp: 10-42.

Dacie, J.V. and S.M. Lewis, 1984. Practical Haematology. Churchill Living Stone. London.

Duncan, D.B., 1955. Multiple Range and Multiple (F) Test. Biometrics, 11: 1-42.

Eaton, D.L. and M.D. Stinson, 1983. Concentration of lead, cadmium, mercury and copper in the Cray fish (*Pacifasticus leniusculus*) obtained from a lake receiving urban runoff. Arch. Environ. Contam. Toxicol., 12: 693-700.

Gill, T.S. and A. Epple, 1993. Stress-related changes in the hematological profile of the American eel (*Anguilla rostrata*). Ecotoxicol. Environ. Saf., 25: 227-235.

Henry, R.J., 1964. Colorimetric determination of total protein. In: Clinical Chemistry. Harper and Row Publ., New York, pp: 181.

Hiemesh, S. and M. Mahadevaswamy, 1994. Sorption potential of biosorban: For the removal of copper. Indian J. Environ. Hlth., 36:165-169.

Huang, J.W., J. Chen, W.R. Berti and S.D. Cunningham, 1997. Phytoremediation of Pb-contaminated soils: Role of synthetic chelates in lead phytoextraction. Environ. Sci. Technol., 31: 800-805.

James, R., V. Sivakumar, K. Sampath and P. Rajendran, 1991. Individual and combined effects of zinc, cadmium and copper on growth of *Oreochromis mossambicus*. Indian J. Fish, 38: 198-200.

James, R., K. Sampath, V. Jancy Pattu and G. Devakiamma, 1992. Utilization of *Eichhorina crassipes* for the reduction mercury toxicity on food transformation in *Heteropneustes fossilis*. J. Aqua. Trop., 7: 189-196.

James, R., K. Sampath and P. Selvamani, 1998. Effect of EDTA on reduction of copper toxicity in *Oreochromis mossambicus*. Bull. Environ. Contam. Toxicol., 60: 478-493.

James, R. and K. Sampath, 1999. Effect of the ion-exchanging agent, Zeolite, on reduction of cadmium toxicity: An experimental study on growth and elemental uptake in *Heteropneustes fossilis* (Bloch). J. Aqua. Trop., 14: 65-74.

Joseph, A., M. Knight, S. Anderson, M. James and H. Rawie, 1972. Chemical basis of the sulfophospho-vanillin reaction for estimating total serum lipid. Clin. Chem., 18: 198-201.

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

Kedziorek, M.A.M. and A.S.M. Bourg, 2000. Solubilization of lead and cadmium during the percolation of EDTA through a soil polluted by smelting activites. Contam. Hydrol., 40: 381-392.

Khangarot, B.S. and D.M. Tripathi, 1991. Changes in humoral and cell-mediated immune responses and in skin and respiratory surfaces of catfish *Saccobranchus fossillis*, following copper exposure. Ecot. Environ. Safety, 22: 291-308.

King, P.R.N. and J. King, 1954. Estimation of plasma phosphatase by determination of hydrolysed phenol with amino antipyrine. J. Clin. Path., 7: 322-326.

Kuyack, N. and B. Volesky, 1990. Biosorption of Heavy Metal, CRC Press, Boca Raton., pp: 174-198.

Litchfield, J.T. and F. Wileoxon, 1949. A simplified method for evaluatin dose-effect experiments. J. Pharmacol. Exp. Ther., 96: 59-113.

Licop, M.S., 1988. Sodium-EDTA effect on survival and metamorphosis of *Penaeus monodon* larvae. Aquaculture, 74: 239-247.

Moussa, M.A., 1999. Biological and Physiological Studies on the Effect of the Gramoxon and Stomp herbicides on Nile tilapia (*Oreochromis niloticus*). Ph.D Thesis, Cairo. University, pp: 200.

Norvell, W.A., 1991. Reactions of Metal Chelates in Soil and Nutrient Solutions In: Micronutrients in Agriculture, Mortvedt, J.J., F.R. Cox, L.M. Shuman and R.M. Welch (Eds.). 2nd Edn., Soil Science of America, Madison, Wisconnsin, pp: 187-227.

Nowack, B., 1996. Behavior of EDTA in Groundwater: A Study of the Surface Reactions of EDTA-Metal Complexes. Doctoral Thesis, E.T.H.Nr: 11392. Zurich. Switzerlasnd, pp: 75.

Passow, H., A. Rothstein and T.W. Clarkson, 1961. The general pharmacology heavy metals. Pharmacol. Rev., 33: 185-224.

Planas-Bohne, F. and M. Lehman, 1983. Influence of chelating agent on the distribution and excretion of cadmium in rats. Toxicol. Applied Pharmacol., 67: 408-416.

Rec, G.S., 1972. Determination of alkaline phosphatase. J. Clin. Chem. Clin. Biochem., 10: 182.

Reitman, S. and S. Frankel, 1957. Colorimetric determination of glutamic oxaloacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol., 28: 53-56.

Santschi, P.H., 1988. Factors controlling the biogeochemical cycle of trace elements in fresh and coastal waters as revealed by artificial radioisotopes. Limnol. Oceanogr., 33: 848-886.

Sastry, K.V. and K. Subhadra, 1985. *In vivo* effects of cadmium on some enzyme activities in tissues of the freshwater catfish, *Heteropneustes fossilis*. Environ. Res., 36: 32-45.

Shalaby, A.M., 1997. Biochemical and Physiological Studies on Metal Contamination in the Common Carp (*Cyprinus carpio* L). Ph.D. Thesis, Zagazig University, pp: 268.

Shalaby, A.M., 2001. Protective effect of ascorbic acid against mercury intoxication in Nil;e tilapia (*Oreochromius niloticus*). J. Egypt. Acad. Soc. Environ. Develop., (D-Environmental studies)., 2: 79-97.

Snedecor, G.W. and W.G. Cochran, 1982. Statistical Methods. 6th Edn., Iowa State Univ. Press., Am., IA, USA., pp: 593.

Soengas, J.L., M.J. Agra-Lago, B. Carballo, M.D. Andres and J.A.R. Veira, 1996. Effect of an acute exposure to sublethal concentration of cadmium on liver carbohydrate metabolism of Atlantic salmon (*Salmo salar*). Bull. Environ. Contam. Toxicol., 57: 625-631.

Spraggue, J.B., 1973. The ABCs of pollutant bioassay using fish In Biological methods for Assessment of water quality. ASTM 528. Am. Testing. Material., pp: 6-30.

Svoboda, M., 2001. Stress in fish-review. Bul. VURH Vodnany, 37: 69-191.

Trinder, P., 1969. Determination of glucose concentration in the blood. Ann. Clin. Biochem., 6: 24.

Van Kampen, E.J. and N.C. Zijlstra, 1961. Determination of haemoglobin. Clin. Chem. Acta, 5: 719-720.

Wintrobe, M.M., 1978. In: Clinical Hematology. Henry Kimpton, London, pp: 448.

Yamawaki, K., W. Hashimoto, K. Fujii, J. Koyama, Y. Ikeda and H. Ozaki, 1986. Hematological changes in carp exposed to low cadmium concentration. Bull. Japan. Soc. Sci. Fish, 59: 459-466.