www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 13: 593-602 (2013) DOI: 10.4194/1303-2712-v13_4_04

Effect of Dietborne Cu and Cd on Body Indices of Nile Tilapia (*Oreochromis niloticus*) with Emphasis on Protein Pattern

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Received 25 September 2012
Accepted 23 September 2013

Abstract

The dietborne Cu in minute concentrations are essential for vital functions in fish, whereas Cd is not essential for any vital activities. Fish diets may probably contaminated with some items as metals. Therefore, Nile tilapia (*Oreochromis niloticus*) is used to assess the toxic effects of contaminated diets with Cu, Cd or their mixture. It was recorded that, the condition factor (K), the hepatosomatic index (HSI) and gonadosomatic index (GSI) exhibited marked reduction for fish fed dietborne Cu, Cd, Cu+Cd. The total sarcoplasmic protein showed a mild reduction after 10 days of exposure to dietborne metals. It was increased markedly after 20, 30 days. Similarly, the total sarcoplasmic nitrogen content of *O. niloticus* was increased. The total plasma protein, albumin and globulin contents were increased due to high dietary Cu or Cd or Cu+Cd intake. Fish fed on Cu contaminated diet for 10 days, exhibited disappearance of last four sarcoplasmic protein fractions (sensitive proteins). Also, Cd contaminated diet for 10 days induced disappearance of sarcoplasmic protein 9th fractions. Feeding on Cu+Cd contaminated diet for 10 days, caused disappearance of the last sarcoplasmic protein fraction, bands number 7 and 8 were faintly appeared. In conclusion, the fish diet contaminated with Cu, Cd or their mixture induced deleterious effects of fish body indices as well as sarcoplasmic protein pattern. Thus, it could be mention that high dietborne intake of tested metals induced toxic effects of *O. niloticus*

Keywords: Dietary, Cu, Cd, mixture metals, tilapia, toxicity.

Introduction

In Egypt, tilapia fish represented one of the most common species in the river Nile and numerous lakes. They are extremely recommended as one of successful culturing fish as they primarily exhibit excellent growth rates even on low protein diets. They also tolerate wider ranges of environmental conditions. Moreover, they are highly and widely acceptable as food because of their high delicacy by much more people throughout the world. Consequently, special interest has been given to study the biological and environmental conditions related to improve tilapia production (Balirwa, 1992; Khallaf *et al.*, 2003; Barriga-Sosa *et al.*, 2004).

Copper (Cu) is an essential micronutrient for vertebrate animals as well as fishes and has numerous functions in cellular biochemistry including vital roles in cellular respiration, and as a co-factor for over 30 different enzymes (Linder, 1991). Fish require copper and zinc as micronutrients (Watanabe *et al.*, 1997) and can obtain these metals from either water or their diet (Handy, 1996; Wood, 2001). Teleost fishes have a Cu dietary requirement of about 3-10 mg cu /Kg dry weight (dw) feed, depending on species, feeding regime and life stage (Clearwater *et al.*, 2002). Although, the exact dietary Cu requirements of Nile tilapia, *O. niloticus*, are uncertain, it was 4 mg Cu /Kg feed for tilapia hybrids, *O. niloticus* x *O. aureus* (Shiau and Ning, 2003). Excess dietary Cu starts from 16 to 730 mg Cu/Kg dw feed induce toxicity to freshwater fish (Clearwater *et al.*, 2002). Kamunde and MacPhail (2011) demonstrated chronic toxicity with manifestation of elevated malondialdehyde liver level of rainbow trout (*Oncorhynchus mykiss*) exposed to dietary Cu or Cd at 500 μg/g.

Cadmium (Cd) have unknown biological function but are toxic elements (Baldisserotto *et al.*, 2005). It is one of the most industrial and environmental pollutant. Currently, its risk has rank number 7 in the United States (ATSDR, 2003). Large amounts of Cd have also been released into the environment through the burning of refuse materials that contain Cd and through the use of Cd-contaminated sludge and phosphate salts as fertilizers (Page *et al.*, 1986; ATSDR, 1999). Studies have been

shown that fresh water fish can concentrate Cd to levels 10 to 1000 times higher than the Cd concentration of ambient water (Cao *et al.*, 2012). Berntssen *et al*. (2001) found a significant increase in regulated cell death and proliferation in Atlantic salmon (*Salmo salar* L.) fed elevated dietary Cd (125 mg Cd kg^{-1}) compared to control fish. Prolonged exposures to Cd even at low levels, whether through food and water produces manifestation of chronic toxicity and alterations in the activities of several enzymes and cell injuries (Roméo *et al.*, 2000; Kalman *et al.*, 2010; Shao *et al.*, 2012).

In Egyptian freshwater ponds, Authman et al. (2012) found that the illegal farm water and its inhabiting *O. niloticus* are found to be heavily polluted with metals (Al, Cd, Pb, Hg, Ni) which far exceeded the permissible limits. They also found histopathological lesions in fish tissues as a result of pollution in illegal farm.

Nile tilapia (*O. niloticus*) as one of most common fish species worldwide is posed to the risk of aquatic pollution by metals including Cu and Cd (Rashed, 2001a, b). Besides, wild Nile tilapia is also known to ingest contaminated lake water and mud containing food during feeding with subsequent deleterious effects on their health (Getachew, 1988). Worthy to mention that, knowledge of dietary metals interaction still remains very scanty (Kamunde and MacPhail, 2011).

There is insufficient data concerning the toxic effects of dietary Cu, Cd and their mixture on tilapia (*O. niloticus*), as one of the most important fish species all over the world. Therefore, the present work was intended to investigate some data of the toxicity of dietary Cu or Cd or their combination on some biological responses of Nile tilapia (*O. niloticus*) with special spot on protein prototype.

Materials and Methods

Fish

One hundred and eighty fish of *O. niloticus* were used in the present study which has total length ranged from 19.66 to 22.0 cm and total body weight (158.5-138.96 g). The experimental fishes were obtained during December 2007 from, Islamic company for animal production close to El-Kanater El- Khairia, Qalubia, Egypt, and transported to faculty of science Benha University. They placed into four well aerated experimental aquaria (58 cm length, 97cm width, 85 cm height), (n = 45 fish/ aquarium), the water temperature was kept at 22 ±1 °C by using thermostat.

Fish were apparently healthy and acclimated at laboratory condition for 10 days. They fed on standard fish diet which composed of the following ingredients (Table 1).

Experimental Design

One hundred and eighty healthy fish (*Oreochroomis niloticus* Linnaeus, 1758) were used in the present study. They were divided into four groups in four aquaria, each one contained 45 fish:

- The first group (Control group) fed on standard diet.
- The second group (Cu group) fed on Cu contaminated diet (2 g/Kg dw diet).
- The third group (Cd group) fed on Cd contaminated diet (10 g/Kg dw diet).
- The fourth group (Cu + Cd group) fed on Cu + Cd contaminated diet 2 g Cu + 10 g Cd /Kg dw diet).

Throughout the experimental duration (30 days) the fishes were fed to satiation two times every day. Care was taken to ensure that no uneaten food remained in the tanks after feeding; this was done by siphoning the remained food after 4 hours from feed addition. Fish specimens were sacrificed after 10, 20 and 30 days.

Diet Formulation

The normal diet ingredients were purchased from commercial suppliers at Benha city (about 40 Km North Cairo).

Formulation of Cu – Contaminated Diet

The Cu-contaminated diet was formulated by gelatine coating of the commercial feed with copper sulphate (2g/Kg dw diet). The Copper dose was based according to Shaw and Handy (2006). In order to achieve Cu concentration of 2g/Kg dw diet, 2.358 g of $CuSO_4$ $5H_2O$ was dissolved in 35 ml of distilled water

Table 1. Fish diet ingredients

Ingredients	%
Fish meal	9.1
Soybean flour	52.57
Corn flour	19.25
Starch	7.0
Corn oil	1.80
Cod Liver Oil	1.98
Vitamin premix [a]	2.00
Mineral Premix [b]	2.00
α-Cellulose	3.30
Carboxy- methyl-cellulose	1.00

[a] Viatmin Premix (per kg of Premix), Thiamine, 2.5 g; riboflavin, 2.5 g; Pyridoxine, 2.0 g; inositol, 100 g; biotin, 0.3 g; pantothenic acid, 100.0 g; folic acid,0.75 g; para-aminobenzoic acid, 2.5 g; choline, 200.00gm; nicotinic acid , 10.0 g ; cyanocobalamine, 0.005 g; α-tocopheral acetate, 20.1 g; menadione, 2.0 g; retinol palmitate , 100,000 IU ; Choleclaciferal, 500,000 IU .

[b] Mineral premix (g per Kg of premix: $CaHPO_4.2\ H_2O$, 727.2; $Mg\ CO_3.7H_2O$, 127.5 KCl, 50.0, NaCl, 60.0, Fe $C_6H_5O_7.3H_2O$, 25.0; $ZnCO_3$, 5.5; $MnCl_2.4H_2O$, 2.5 $CuCl_2$,0.785).

with 1.2 g of bovine gelatine to bind the Cu to the food sticks. The gelatine solution was gradually sprayed on to 300 g of the commercial diet, and mixing with food. Then the diet was left 5 minutes to dry and Cu diet was stored into air – tight containers and frozen at -20 °C to prevent lipid peroxidation (Szczerbik *et al.*, 2006; Shaw and Handy, 2006).

Formulation of Cd – Contaminated Diet

The Cd – contaminated diet was formulated by the same way as Cu – contaminated diet. Dose of Cd diet (10 g/Kg dw diet) was determined according to Szczerbik *et al.* (2006). In order to do Cd concentration of 10g/Kg dw diet, 1.476 g of $CdCl_2$ 2½ H2O, (Avondale laboratories, England) was dissolved in 35 ml of distilled water with 1.2 g of bovine gelatine. The gelatine was used to bond the Cd to the food sticks. The gelatine solution was gradually sprayed on to 300 g of the commercial diet. It was mixed carefully. Then, it was left to dry within few minutes and was stored into air – tight containers and frozen at -20 °C to prevent lipid peroxidation (Szczerbik *et al.*, 2006; Shaw and Handy, 2006).

Formulation of (Cu+Cd) – Contaminated Diet

The formulation was done by adding 2.358 g of CuSO4 $5H_2O$ with 1.476 g of $CdCl_2$ 2½ H_2O, and then dissolved in 35 ml of distilled water with 1.2 g of bovine gelatine. The gelatine was used to bind these heavy metals to the food sticks. The gelatine solution was gradually sprayed on to 300 g of the commercial diet, and mixing food. The gelatine coat dried within few minutes and Cu contaminated diet was stored into air – tight containers and frozen at -20 °C to prevent lipid peroxidation (Szczerbik *et al.*, 2006; Shaw and Handy, 2006).

Blood and Tissue Collections

The blood samples were collected by withdrawing blood from caudal vein using heparinized syringe to avoid clotting and then transferred to lithium heparinized tube to avoid clotting, then blood sample left to stand for two hours then centrifuged at 5000 rpm for 10 min. The plasma was pipetted carefully into new eppendorf tubes, then frozen in deep freezer (-20 °C) until analysis.

Muscle samples were isolated from the dorsal epiaxial muscle for protein electrophoresis and heavy metals bioaccumulation.

Body Indices

Body indices measured in the present study are; hepatosomatic index (HSI), gonadosomatic index (GSI) and condition factor (K). They were calculated using the following formulae.

- Hepatosomatic index (HSI) = (Weight of liver /gutted body weight of fish) × 100

- Gonadosomatic index (GSI) = (weight of gonad /gutted body weight of fish) ×100

- $K= (\text{Body Weight of fish} / L^3) \times 100$

Where gutted body weight is the fish weight without the gut and L is the total fish length.

Metals and Nitrogen Analysis

Half g of dorsal epiaxial muscle was digested in 3ml of concentrated. H_2SO_4 and 3ml H_2O using boiling water bath for 1 hour then added 4 ml of perchloric acid for complete digestion and then completed to 25 ml by distilled water. The copper, cadmium, iron and nitrogen were measured using Atomic absorption spectrophotometer (model 2380, U.S.A) at central laboratory, Faculty of Agriculture, University of Benha, Egypt.

Total Protein Determination in Muscle and Plasma

A piece of dorsal epiaxial muscle (0.5 g) was isolated and hoemognized in 3 ml of distilled water, then it was frozen at -20 °C over night. The samples were centrifuged at 4000 rpm for 15 minutes. The supernatant (water soluble muscle proteins) were pipetted into eppendorf tubes. Then it was centrifuged again at 4000 rpm for 15 minutes, the second supernatant was transferred into new eppendorf tube and stored at -20 °C until analysis.

The total protein was determined following the principle of Biuret reaction using Biomerieux reagent Kit No.61 602 (Burtis *et al.*, 1999). The colour intensity developed was measured colorimetrically at wave length 545 nm, using spectrophotometer (model; Jasco 530 V) at wave length 545 nm. Samples total proteins were computed as calibrated using bovine serum albumin.

Determination of Albumin

The determination of albumin in plasma was based on the colorimetric reaction with bromocresol green according to the method recommended by Watson (1965). It was determined Using Stanbio Albumin Liquicolor Kit NO 0285 Using spectrophotometer at wave length 550 nm. The globulin in plasma was computed using the following formula:

Concentration of Plasma Globulin (g/l) = (Concentration of Total plasma protein – plasma albumin).

Protein electrophoresis Technique

Sarcoplasmic proteins fractionation was done using Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) according to Lammeli (1970) using a vertical slab gel unit.

Statistical Analysis

The data of the present work were presented as mean ± standard deviation. Statistical analyses of the data were computed by SPSS (Version 10). Significant differences between every pairwise experimental groups were done using two paired Student's t test (Pipikin 1984).

Results

Body Indices

The data presented in Table 2 exhibited the changes in total length, body weight, Fulton condition factor (K), hepatosomatic index (HSI) and gonadosomatic index (GSI) of Nile tilapia (*O. niloticus*) fed on diet contaminated with heavy metals (Cu or Cd or Cu and Cd). The studied tilapia fish exhibited total length ranged from 19.667: 22.00 cm.

Fishes of the control group recorded average value 1.613 ± 0.090 (g/cm^3) for condition factor (K). The condition factor (K) of fishes fed on Cu-contaminated diet was generally reduced .It was significantly decreased after 10 days and very highly significantly reduced after 20 and 30 days (Table 2).

The cadmium contaminated diet recorded non-significant increase of K value after 10 days, whereas it were very highly significant decreased after 20 and 30 days. The combined effect of Cu and Cd recorded general reduction of K values. It was highly significantly decreased after 10 days, and very highly significantly decreased after 20 and 30 days.

According to the recorded data the HSI (%), the control fish have an average 2.72 ± 0.450 (Table 2). Hepatosomatic index of fishes fed on diet contaminated with Cu was generally reduced. It showed a highly significantly reduction after 20 and 30 days.

The Cd induced a general reduction of HSI. Fish fed with contaminated diet for 20 and 30 days recorded very highly significantly reduction of HSI compared with those of control group. Similarly, the combined effect of Cu and Cd induced the same effect.

The gonadosomatic index (GSI) recorded reduction at all tested experiment. The GSI values when compared between fishes of the control group and fishes fed on diet contaminated with Cu were non-significantly decreased after 10 and 20 days, whereas it was very highly significantly reduced after 30 days (Table 2).

Fishes fed on diet contaminated with Cd exhibited non-significant reduction of GSI after 10 and 20 days, whereas it was highly significantly decreased after 30 days compared with those of the control group. Copper and Cd contaminated diet induced highly significant reduction of GSI after 20 days compared with those of control group.

Muscle Total Proteins and Nitrogen

The data presented in Table 3 exhibited the effect of diet contaminated with heavy metals on the total protein and total nitrogen content (g/g fresh tissue) in muscle of Nile tilapia *O. niloticus* , as well as the significance t-test of the comparison of these data between the control and treated groups.

According to the recorded data, the total protein content in muscle of control fishes recorded average value 0.074 ± 0.005 g/g fresh tissue. The total protein content of fishes fed on diet contaminated with Cu, exhibited highly significant decrease after 10 days, recording percentage difference -13.51% from the value of the control fish. It was increased significantly after 20 days and highly significantly after 30 days, being 13.51 % and 39.19%, respectively, over the control value (Table 3).

Regarding the effect of Cd contaminated diet, the data showed that there was very highly significant reduction of total proteins content after 10 days (-9.46 %), then it was increased significantly after 20 and 30

Table 2. Changes in condition factor (K), hepatosomatic index (HSI) and gonadosomatic index (GSI) of *Oreochromis niloticus* fed on diet contaminated with Cu (2 g/Kg diet) or Cd (10 g/Kg diet) or Cu and Cd (2+10 g/Kg diet)

Duration (Days)	Groups	No	K (g/cm^3)		HSI (%)		GSI (%)	
			Mean±SD	% Diff.	Mean±SD	% Diff.	Mean±SD	% Diff.
10 days	Control	8	1.61±0.09	-	2.72±0.45	-	0.69±0.38	-
	Cu	8	1.48±0.08*	-7.93	2.10±0.45*	-22.79	0.40±0.26	-41.18
	Cd	8	1.63±0.14	1.17	2.11±0.94	-22.43	0.65±0.32	-4.41
	Cu+Cd	8	1.43±0.15**	-11.1	2.46±0.68	-.9.56	0.43±0.17	-36.76
20 days	Control	8	1.69±0.09	-	3.21±0.22	-	0.89±0.24	-
	Cu	8	1.50±0.07***	-10.85	1.89±0.19***	-41.18	0.55±0.38	-38.13
	Cd	8	1.50±0.08***	-10.91	1.69±0.24***	-47.4	0.81±0.28	-8.89
	Cu+Cd	8	1.29±0.11***	-23.24	1.80±0.43***	-43.98	0.49±0.27**	-44.88
30 days	Control	8	2.07±0.08	-	3.89±.34	-	1.34±0.32	-
	Cu	8	1.47±0.15***	-28.86	1.67±0.52***	-57.09	0.48±0.22***	-66.312
	Cd	8	1.60±0.07***	-22.68	1.50±0.09***	-61.46	0.76±0.31**	-43.49
	Cu+Cd	8	1.59±0.07***	-23.36	2.04±1.09***	-47.58	1.07±0.89	-20.45

No= number of fishes, % Diff. = % Difference from the control value, *significant difference (P<0.05) from the control, ** highly significant difference (P<0.01) from the control, ***more highly significant difference (P<0.001) from the control.

days with percentage 13.51 and 37.84 %, respectively.

The combined effect of the Cu and Cd induced non-significant reduction of total proteins content after 10 days, whereas it was very highly significantly increased after 20 and highly significant increased after 30 days, being differed from those reported for control fish with percentage 16.22 % and 64.87 %, respectively, (Table, 2).

The data presented in Table 4 exhibited also the total nitrogen content in muscle of *O. niloticus.* The control fish recorded average value 15.291 ± 2.670 g/g fresh tissue. It is markedly increased due to cu contaminated diet. The recorded data were found statistically very highly significant at all durations, recording percentage increase 76.03, 96.74 and 134.62 over those of control fish.

A drastic rise of total nitrogen content in fish muscle was recorded due to ingestion of Cd contaminated diet. The data exhibited significant increase after 10 days (35.43 %), very highly significant increase after 20 and 30 days (79.03, 110.36 %, respectively).

Similarly the combined Cu and Cd induce obvious increase of total nitrogen content. The data revealed very highly significant increase at all durations, with percentage increase 69.22, 119.35 and 108.73 %) (Table, 2).

Total Plasma Proteins, Albumin and Globulin Content

The data presented in Table 4 showed the effect of diet contaminated with Cu or Cd or Cu and Cd on the total plasma proteins content, albumin and globulin of Nile tilapia (*O. niloticus*), as well as the significant t-values between control and experimental groups. According to the recorded data the total plasma protein content in the control fish recorded average value 46.149 ± 1.569 g/l. Its level was enhanced after dietary Cu after 10 and 20 days. It was found statistically very highly significant differed, recorded percentage difference 33.49 % and 20.29 %. A highly significant decrease was reported after 30 days (-5.96 %).

In case of fishes fed on Cd contaminated diet, very highly significant increase in the plasma total proteins content was recorded after 10 and 20 days, compared to the control value of proteins. On the other hand, the data recorded non-significant decrease

Table 3. Changes in total protein content (g/g fresh tissue) and total nitrogen content (g/g fresh tissue) in muscle of *Oreochromis niloticus* fed on diet contaminated with Cu (2 g/Kg diet) or Cd (10 g/Kg diet) or Cu and Cd (2+10 g/Kg diet)

Groups	No	Total protein content (g/g fresh tissue)		Total nitrogen content (g/g fresh tissue)	
		Mean ±SD	% Diff.	Mean ±SD	% Diff.
Control	8	0.074±0.005	-	15.291±2.67	-
Cu for 10 Days	8	0.064±0.003*	-13.51	26.916±3.878***	76.03
Cu for 20 days	8	0.084±0.002*	13.51	30.083±1.181***	96.74
Cu for 30 days	8	0.0103±0.022**	39.19	35.875±4.725***	134.62
Cd for 10 days	8	0.067±0.001***	-9.46	20.708±6.536*	35.43
Cd for 20 days	8	0.084±0.012*	13.51	27.375±1.65***	79.03
Cd for 30 days	8	01.02±0.007*	37.84	32.167±1.587***	110.36
Cu+Cd for 10 days	8	0.0693±0.004	-6.35	25.875±0.649***	69.22
Cu+Cd for 20 days	8	0.086±0.005***	16.22	33.541±0.85***	119.35
Cu+Cd for 30 days	8	0.122±0.034**	64.87	31.917±6.349***	108.73

No= number of fishes, % Diff. = % Difference from the control value, *significant difference (P<0.05) from the control, ** highly significant difference (P<0.01) from the control, ***more highly significant difference (P<0.001) from the control.

Table 4. Changes in total protein content (g/l), albumin (g/l) and globulin levels (g/l) in plasma of *Oreochromis niloticus* fed on diet contaminated with Cu (2 g/Kg diet) or Cd (10 g/Kg diet) or Cu and Cd (2+10 g/Kg diet)

Groups	No	Total protein (g/l)		albumin (g/l)		Globulin (g/l),	
		Mean ±SD	% Diff.	Mean ±SD	% Diff.	Mean ±SD	% Diff.
Control	8	46.149±1.569	-	16.845±2.033	-	29.304±2.288	-
Cu for 10 Days	8	61.604±2.689***	33.49	15.315±1.935	-9.08	46.289±8.427***	57.96
Cu for 20 days	8	55.514±4.25***	20.29	20.639±5.72**	22.52	34.875±7.224	19.01
Cu for 30 days	8	43.397±2.517**	-5.96	15.726±3.634	-6.64	27.67±6.001	-5.58
Cd for 10 days	8	55.271±1.674***	19.77	18.022±0.653	6.99	37.25±3.702***	27.12
Cd for 20 days	8	57.228±4.352***	24.01	19.683±2.029*	16.85	38.145±3.517***	30.17
Cd for 30 days	8	45.196±2.420	-2.07	18.073±3.61	7.29	27.123±3.641	-7.44
Cu+Cd for 10 days	8	57.242±2.834***	24.04	14.192±2.022*	-15.75	43.25±4.453***	47.59
Cu+Cd for 20 days	8	45.425±2.648	-1.57	22.828±3.003***	35.52	31.598±1.878*	7.83
Cu+Cd for 30 days	8	47.309± 3.261	2.51	17.696±1.201	5.05	29.613±3.755	1.06

No= number of fishes, % Diff. = % Difference from the control value, *significant difference (P<0.05) from the control, ** highly significant difference (P<0.01) from the control, ***more highly significant difference (P<0.001) from the control.

in plasma protein content after 30 days (Table 4).

The diet contaminated with Cu and Cd induced very highly significant increase of plasma proteins after 10 days and non- significant increase after 30 days, being 24.04 % and 2.51 % above the value of control, whereas, there was non- significant decrease in its levels after 20 days (-1.57 %). Concerning the plasma albumin content of fish fed on contaminated diet with Cu, the data exhibited significant decrease after 10 and 30 days, whereas it was increased highly significant after 20 days, compared with those of control group (Table 4).

Regarding, fishes fed on diet contaminated with Cd, the data revealed that the levels of plasma albumin contents were increased at all durations compared to the level of control showing a significant increase after 20 days.

Concerning the combined effect of Cu and Cd on the level values of plasma albumin, the recorded data exhibited significant decrease after 10 days (-15.75) whereas, it was very highly significantly increased after 20 days (35.52 %), compared with those recorded for control fishes.

The total plasma globulin of *O. niloticus* fed on Cu contaminated diet, showed only a very highly significant increase with a percentage of difference (57.96 %) after 10 days. Regarding fish fed on Cd contaminated diet, the data showed very highly significant increase after 10 and 20 days.

The Cu and Cd contaminated diet induced a general rise of total plasma globulins. Statistical analysis revealed a very highly significant difference (47.59 %) from the value of the control after 10 days and after 20 days significant increase was found (7.83%) (Table 4).

Sarcoplasmic Proteins SDS-PAGE

Fractions Appearance

The percentage appearance of muscle protein fractions of different experimental groups are presented in Table 5 and Figure 1. The muscle protein of control fish exhibited 9 fractions. Most of these

Table 5. Changes in percentage appearance of sarcoplasmic protein bands of *Oreochromis niloticus* fed on diet contaminated with Cu (2 g/Kg diet) or Cd (10 g/Kg diet) or Cu and Cd (2+10 g/Kg diet)

Groups	Fraction Number								
	1	2	3	4	5	6	7	8	9
Control	100	100	100	100	100	100	75	25	25
Cu for 10 days	100	100	100	100	100	0	0	0	0
Cu for 20 days	100	100	100	100	100	100	62.5	12.5	0
Cu for 30 days	100	100	100	100	100	100	87.5	62.5	12.5
Cd for 10 days	100	100	100	100	100	87.5	87.5	50	0
Cd for 20 days	100	100	100	100	100	100	62.5	12.5	12.5
Cd for 30 days	100	100	100	100	100	100	100	62.5	0
Cu+Cd for 10 days	100	100	100	100	87.5	62.5	500	12.5	0
Cu+Cd for 20 days	100	100	100	100	87.5	87.5	62.5	12.5	0
Cu+Cd for 30 days	100	100	100	100	100	100	50	25	0

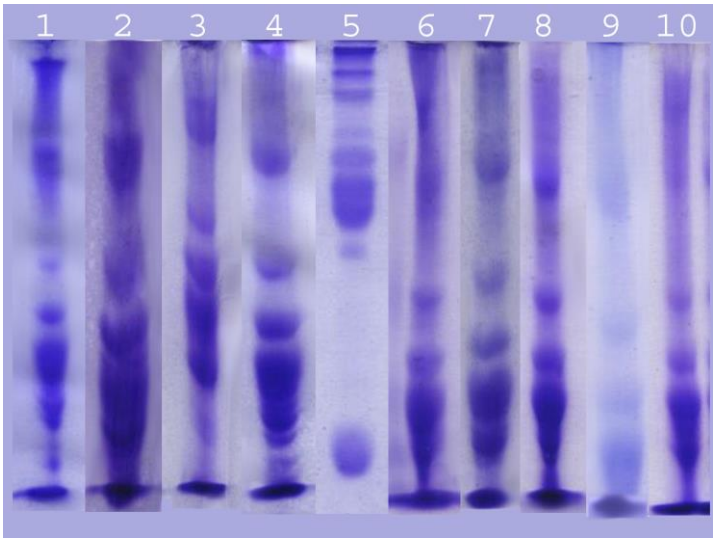

Figure 1.. Photograph of SDS-PAGE of sarcoplasmic proteins of *Oreochromis niloticus*, lane 1: control, lane 2:Cu for 10 days, lane 3: Cu for 20 days, lane 4: Cu for 30 days, lane 5: Cd for 10 days, lane 6: Cd for 20 days, lane 7: Cd for 30 days, lane 8: Cu+Cd for 10 days, lane 9: Cu+Cd for 20 days, lane 10: Cu+Cd for 30 days

Table 6. Changes in relative mobility of sarcoplasmic proteins of *Oreochromis niloticus* fed on diet contaminated with Cu (2 g/Kg diet) or Cd (10 g/Kg diet) or Cu and Cd (2+10 g/Kg diet)

	Fraction numbers								
Groups	1	2	3	4	5	6	7	8	9
Control	0.073±(8) 0.039	0.235±(8) 0.10	0.378±(8) 0.113	0.473±(8) 0.107	0.583±(8) 0.105	0.74±(8) 0.113	0.847±(6)0.021	0.021±(2) 0.0	0.96±(2)0.0
Cu 10 d	0.202±(8)0.035***	0.47±(8)0.05***	0.67±(8)0.07***	0.78±(8)0.07***	0.89±(8) 0.05**	-	-	-	-
Cu 20 d	0.087±(8) 0.03	0.215±(8) 0.086	0.423±(8) 0.041	0.585±(8) 0.042	0.693±(8) 0.053	0.842±(8)0.063	0.933±(5) 0.029	0.97±(1) 0.0	-
Cu 30 d	0.048±(8) 0.041	0.160±(8) 0.05	0.393±(8) 0.10	0.538±(8) 0.106	0.680±(8) 0.086	0.807±(8)0.081	0.898±(7) 0.051	0.945±(5) 0.022	0.980± (1)0.0
Cd 10 d	0.04±(8) 0.01*	0.119±(8)0.056*	0.217±(8)0.052**	0.273±(8)0.049**	0.43±(8)0.238	0.38±(7)0.04***	0.676±(7)0.245	0.960±(4)0.008	-
Cd 20 d	0.063±(8)0.036	0.204±(8)0.120	0.381±(8)0.139	0.574±(8)0.131	0.746±(8)0.128	0.857±(8)0.113	0.903±(5)0.077	0.910±(1)0.0	0.97±(1)0.0
Cd 30 d	0.054±(8)0.034	0.193±(8)0.122	0.304±(8)0.168	0.454±(8)0.182	0.676±(8)0.124	0.802±(8)0.09	0.906±(8)0.058	0.957±(5)0.024	-
Cu+Cd 10 d	0.157±(8)0.141	0.36±(8)0.163	0.473±(8)0.171	0.647±(8)0.201	0.73±(7)0.132	0.808±(5)0.076	0.880±(4)0.036	0.90±(1)0.0	-
Cu+Cd 20 d	0.065±(8)0.07	0.262±(8)0.17	0.367±(8)0.199	0.517±(8)0.202	0.582±(7)0.135	0.768±(7)0.096	0.895±(5)0.061	0.960±(1)0.0	-
Cu+Cd 30 d	0.111±(8)0.058	0.292±(8)0.075	0.461±(8)0.101	0.624±(8)0.109*	0.744±(8)0.102*	0.849±(8)0.10	0.90±(4)0.064	0.920±(2)0.03	-

Number of fishes indicated between brackets, % Diff. = % Difference from the control value, *significant difference (P<0.05) from the control, ** highly significant difference (P<0.01) from the control, ***more highly significant difference (P<0.001) from the control.

fractions were appeared with a high percentage as the 1st, 2nd, 3rd, 4th, 5th and 6th fractions detected with a percentage of 100%. The 7th fraction appeared with a percentage 75%, whereas the 8th and 9th with low percentage (25%).

The 1st, 2nd, 3rd and 4th fractions were appeared with a percentage of 100% in all different contaminated groups (Resistant proteins).

The Nile tilapia fed on diet contaminated with Cu for 10 days exhibited fractions disappearance the last four, (sensitive proteins) whereas first four fractions appeared in all examined fishes (100%).

In fishes fed on diet contaminated with Cu after 10 and 20 days, most of fractions appeared in all examined fishes. Whereas, four protein fractions (6, 7, 8, &9) were missing for fishes subjected to Cu for 10 days. After 30 days no-fractions were missing but last two ones appeared sparsely.

Related to the fishes fed on diet contaminated with Cd for 10 days, the first five fractions were found in all tested individual, whereas fraction number 9 was missing (sensitive protein). Sarcoplasmic protein fractions (6, 7& 8) do not considerably changed from those of control.

Fishes fed Cd contaminated diet for 20 and 30 days have muscle proteinogram fractions (1, 2,3,4,5,6, 7) similar to those of the control (not changeable proteins). The last two fractions was appeared with low percentage (12.5%) (changeable proteins) (Table 5).

Concerning, *O. niloticus* fed on diet contaminated with mixed metals Cu and Cd for 10 days the last fraction was not detected and fraction number 7 and 8 was sharply reduced, four sarcoplasmic protein fractions were found in all tested samples 100% (Resistant proteins), while fraction number 5 was appeared in 87.5% of tested samples. Sarcoplasmic protein fraction number 6 was found in 62.5%.

Regarding the combined effect of contaminated fed with Cu and Cd for 20 days the fraction from 1 to 4 were appeared absolutely 100%, whereas fractions number 5 and 6 were presented in 87.5%. The fraction number 7 was found with percentage 62.5%, but fraction number 8 was found in a low percentage 12.5%. Moreover, fraction number 9 was not detected, so the last two fractions reflected the metals toxicity.

Related to the mixed effect of contaminated diet with Cu and Cd for 30 days, the first six sarcoplasmic protein fractions were not affected (100 % appearance), whereas the 7th and 8th fractions were appeared with a low percentage (50% & 25%). The last fraction was missed.

Relative Mobility

Fish fed with Cu contaminated diet for 10 days induced a drastic rise of protein mobility. Its nobilities of fractions (1, 4) showed significant acceleration. Concerning the fishes fed on diet contaminated with Cu for 20 days, there were non-significant variations for all fractions except the 4th fraction which exhibited significantly accelerated migration on the acrylamide gel. There were no significant variations in the fraction mobility of sarcoplasmic protein between control and those of fishes fed on Cu contaminated diet for 30 days (Table 6).

Regarding tilapia fed on diet contaminated with Cd for 10 days, the relative mobility of the first two fractions changed with significant difference comparing to those of control fish. Whereas, the difference of the mobility of the 3rd, 4th and 6th fractions reduced significantly.

Regarding the effect of diet contaminated with Cd for 20 and 30 days, the relative mobility of sarcoplasmic protein fractions exhibited non-significant differences. Also, the effect of diet contaminated with a mixture of Cu and Cd induced non-significant differences at all tested durations, except in case of bands 4 and 5 where the nobilities were significantly accelerated than those of the control (Table 6).

Discussion

The morphometric indices are of quite interesting indicators for monitoring disturbances of feeding in fish (Riberio *et al.*, 2005). In the present study, there are significant decreases in growth rate and weight of fish fed on metal contaminated diet. Szczerbik *et al.* (2006) reported a significant decrease of appetite and growth rate as well as the decrease of the locomotion activity after exposure to 10 mg Cd g^{-1} of food. The reductions of appetite and growth rate of fish subjected to different stressors have been described earlier (McGeer *et al.*, 2000; Shao *et al.*, 2012).

The growth inhibition could be due to the influence of Cd on fish ability of food intake and assimilation (Szczerbik *et al.*, 2006). It was shown that Cd decreased food intake and assimilation and lead to the decrease of growth rate of fish (McGeer *et al.*, 2000; Shao *et al.*, 2012). The reduction of weight gain may be related to accelerated catabolism (Bryan *et al.*, 1995). The body weight in tilapia (*O. niloticus*) exposed to 2000 mg Cu/ Kg dw feed was significantly lower than in the controls at the first sampling point (day 21), and remained less than the controls throughout the experiment (Shaw and Handy, 2006).

In the present study, there was significant decrease in HSI and K values. These results suggest induced hepatotoxicity with concomitant hepatocellular damage. It is reported earlier that Cd has been shown to induce apoptosis *in vivo* (Pham *et al.*, 2006). The reduction of HSI could be attributed to liver damage induced by pollution (Abdel-Hameid, 2011). The hepatosomatic index (HSI) is useful in ecotoxicological investigations. It is also useful biomarker that reflects the liver function and

physiological status of fish (Riberio *et al.*, 2005). They are indicating a typical weight deficit due to exposure of eels to heavy metals.

The gonadosomatic index (GSI) showed significant decreased due to metal toxicity. This agree with (Szczerbik *et al.*, 2006) and Abdel-Hameid (2011). There is a decrease of ovarian steroid release after Cd exposure (Tilton *et al.*, 2003). The use of the highest cadmium dose caused both stimulation of pituitary function (elevated LH level) and inhibition of ovarian development (Szczerbik *et al.*, 2006). The decrease of the GSI in the group receiving a dose of 10 g Cd Kg−1 could also be due to an effect of elevated cortisol level (Szczerbik *et al.*, 2006). It has been shown that cortisol can inhibit the growth of gonads by decreasing testosterone and estradiol secretion (Carragher and Sumpter, 1990).

In the present study it is found that the total muscle protein content, total plasma protein, albumin and globulin decreased significantly on groups fed on contaminated diet with Cu, Cd, or Cu + Cd than control group. This may be due to that pollutants react with the cell nucleoproteins and nucleic acids and consequently affect the protein synthesis and cellular integrity (Sharf El-deen and Abdel-Hameid, 2002). The increased total proteins and nitrogen contents in the fish flesh observed in the present study induced by dietary metals could possibly explore water turn over from the tissues to the blood (Abdel-Hameid, 1994).

In the present study, the SDS polyacrylamide gel electrophoresis (SDS-PAGE) revealed that total number of sarcoplasmic protein bands in control fish was 9. It was decreased due to Cu intake after 10 days (5 bands) and 8 bands due to Cu feeding for 20 days, Cd for 10 days, Cd for 30 days, and Cu + Cd at all durations. Sharaf-Eldeen and Abdel-Hamid (2002) investigated the exposure of *O. niloticus* to some pollutants and found that six protein fractions were missing due to Cu exposure (high level, 1.0 mg/l).

Kurbanova *et al.* (2004) reported decrease of the intensity of total protein content and albumin concentration, and the increase of gamma globulin and peptidase activity in fish blood plasma exposed to environmental pollution. They considered these changes as adaptive reactions of the fish organism to the change of the environmental conditions directed at the juvenile stage of ontogenesis.

Prolonged feeding of diet contaminated with mixture of Cu+Cd for 30 days induced significant acceleration of relative migration of the 4^{th} and 5^{th} bands over those of the control. This consequence may reflect a genetic damage which concomitantly induced changes in protein quality. Furthermore, the significance change in the relative mobility of muscle protein fractions reported in the present study reflects the genetic damage. This was reported earlier by Sharaf-Eldeen and Abdel-Hamid (2002).

Badawy and El-Serafy (1998) mentioned that in the electrophoretic serum proteinograms of *Clarias gariepinus*, from different polluted water localities, some fractions were completely disappeared and others were polymorphic. The authors found also that the disappearance and polymorphism was dependant on the degree of pollution in water.

Therefore, it be concluded that although Cu is essential element but its elevated dietary level could be toxic for Nile tilapia (*O. niloticus*). On the other hand, Cd is not essential for fish and its contamination to fish diet induced hazardous effects. Furthermore, dietborne Cu, Cd and their mixture induced harmful effects, retarded growth and abnormal protein pattern in Nile tilapia (*O. niloticus*). From the present study, it appears that the toxicity of Cu is higher than that of Cd. Special emphasis as abnormal protein pattern that reflects physiological disturbances and/ or genotoxicity were reported for fish due to dietborne Cu, Cd and Cu +Cd. Therefore, special attention should be given to contamination of the O. niloticus diets with metals during fish farming or in the wild habitat.

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