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EFFECT OF SODIUM CARBONATE ON LEAD AND ZINC TOXICITY AND ITS EFFECT ON GROWTH PERFORMANCE, FEED UTILIZATION, BIOACCUMULATION AND BIOCHEMICAL CONSTITUENTS IN *OREOCHROMIS NILOTICUS*

Mohamed k. Khames[1]; Mohamed M. Zenhom[1] and Mahmoud A. Metwally[2]

Fish Nutrition Department [1] and Physiology Department [2], Central Laboratory for Aquaculture Research, Agriculture Research Center, Egypt.

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ABSTRACT

This study evaluated the effect of sodium carbonate on chronic exposure of water Lead (Pb) and Zinc (Zn) toxicity on *O. niloticus* growth performances, feed utilization bioaccumulation and biochemical constituents. 210 Tilapia niloticus were divided into seven groups each group with three replicates. The group1 (G1) untreated group served as control group, group2 (G2) exposure to concentrations (10 and 20 mmol/L) lead and zinc respectively, group3 (G3) exposure to (15 and 30 mmol/L) lead and zinc respectively, group4 (G4) exposure to (20 and 40mmol/L) lead and zinc respectively, but groups (G5, G6 and G7) exposure to 10 mmol/L of Na_2CO_3 with the same previous concentration of lead and zinc. Each aquarium was containing 10 fish (20.3±0.01g / fish) and the experiments expanded two month. The growth parameters were affected by lead and zinc concentration. High growth performance and feed utilization (WG, SGR, RGR, FCR, PER, PPV, and FI) showed at control group and low concentrations lead and zinc exposure one. Bioaccumulation of lead and zinc increase with increasing concentration exposure dose. Erythrocyte count (RBCs), hemoglobin content (Hb) and hematocrit value (Hct) were significantly affected by high lead and zinc concentrations compared to control one. Blood glucose, Aminotransferase (AST&ALT) activity, total protein, and total lipids were significantly affected by lead and zinc concentration compared to control group. Overall results presented after adding of Na_2CO_3 indicate that improve the growth

performance, feed utilization, biochemical constituents and bioaccumulation decrease in tilapia fish.

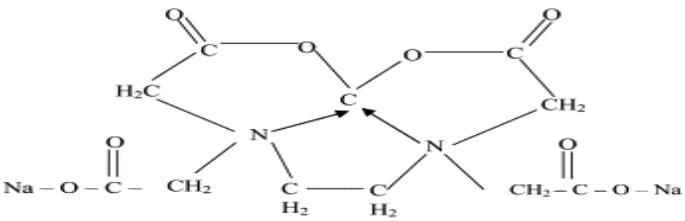
Keywords: *Oreochromis niloticus*, bioaccumulation, growth, lead, zinc and Na_2CO_3.

INTRODUCTION

Given the importance of fish as an inexpensive protein source rich in essential amino acid and an important source for poor third world people, we therefore pay attention to the current study. As a result of the rapid development in industry and modern agriculture, the level of pollution has increased, especially with heavy metals, which in turn affect fish farming as well as humans (Metwally, 2009). Heavy metals are among the pollutants that reach aquatic farms and affect the health and production of fish. The heavy metal has a relatively high density and toxic at low quantity as lead (Pb), mercury (Hg), cadmium (Cd), chromium (Cr), etc. Some 'trace elements' are also known as heavy metals, as, zinc (Zn), copper (Cu), selenium (Se), etc. They are essential to maintaining the body metabolism, but they are toxic at higher concentrations. The heavy metals can enter the bodies via food, drinking water and air (Bradl, 2002). Lead is non- essential element and it is a bone-seeking element, it is processed along with calcium because of its chemical resemblance to calcium. The human body contains 2–3 g zinc, and nearly 90% is found in muscle and bone. Other organs containing negligible concentrations of zinc include prostate, liver, the gastrointestinal tract, kidney, skin, lung, brain, heart, and pancreas (Wastney *et al*., 1986). The fish can take up metals from water, diet and skin (Rauf *et al*., 2009). There is a correlation between the metal content of water and it concentration in fish muscles (Gupta, 2013). Zinc and leads absorbed throughout intestine were distributed via the serum, bound to proteins such as albumin, α-macroglobulin, and transferring to cells and organ (Gupta, 2013). Zinc is a cofactor for the protein splitting enzyme carboxy peptidase; it plays a role in carbohydrate metabolism by convert of pyruvate to lactate in glycolysis (Scott *et al*., 2008). Chronic exposure of Zn and Pb (at 1/3rd of LC50) caused significantly decrease in weight gain, feed intakes and FCE. Also Zn and Pb bioaccumulations varied significantly among fish organs. Fish liver

and kidney accumulated significantly higher Zn and Pb with chronic exposures. However, Zn accumulation was significantly higher than Pb in the fish body. The bioaccumulation of Zn and Pb was dependent upon fish age and concentration of metals (Javed, 2012). Liver and kidney tissues are known to be the target organs for inducing toxic effects by Pb exposure (Patra *et al*., 2001). The highest Pb accumulation in liver and kidney tissues, this may be due to their functions in detoxification (Zhai *et al*., 2017). The spleen of fish is a critical tissue for the removal of blood xenobiotic; it may accumulate a high lead concentration (Kim and Kang, 2004). Heavy metals alter the physiological and biochemical parameters (Basa *et al*., 2003). Acute exposure, water borne Pb can cause Ca2+, chlorides, sodium disruption and spinal deformities, black tail disease, anemia in fish (Dallinger *et al*., 2002).

Calcium disodium complex – Ethylene diamine tetra acetic acid ($CaNa_2$ EDTA) is an efficient chelate of many divalent and trivalent metals (Fatima *et al*., 2000). $CaNa_2$ EDTA is a stable water soluble metal chelated matter, mainly used in food industry, agriculture as micronutrient. Calcium present in a chelated form is penetrates cell membranes and chelates extra cellular metal ions more effectively than intracellular ions *(Ploch et al*., 1999)**.** $CaNa_2$ EDTA is shown in Fig (1).



$CaNa_2$EDTA proved significantly restoring biochemical variables after exposure of nickel chloride in fish (Rengaswamy *et al*., 2009). Tri sodium citrate dihydrate (TCD) and chicken egg shell was introduced as new chelating agents for heavy metal in *Clarius batrachus* species. Where are gave the removal percentage of 90 %, 84 %, 72 % and 100 % for Cu, Cd, Pb and Ni, respectively *(Wan* et al., 2020). The chelating ligands include functional groups such as –OH, –SH and –NH, which can donate electrons for coordination with

the metal (Ellenhorn and Barceloux, 1988). Oxygen deficiency, temperature increase and acidification usually render the fish more susceptible to intoxication, whereas increase in hardness and salinity (mineral content) reduce metal toxicity (Witeska and Jezierska, 2003).The interactions among various metals present in the water may increase or decrease their toxicity effects on fish (Jezierska and Witeska, 2006). The aim of the current research is to reduce the toxicity of lead and zinc by using sodium carbonate through growth performance, bioaccumulation and biochemical component change in *Tilapia niloticus.*

MATERIALS AND METHODS

This research was carried out inside the wet laboratory of the Nutrition Department of the Central Laboratory for Aquaculture Research of the Agricultural Research Center in Egypt.

Chemicals:

Lead acetate and Zinc sulfate and Sodium Carbonate in the present study were obtained from El. Nasr Co., Egypt.

Experimental fish:

Nile tilapias (*O. niloticus*) fish collected from Central Laboratory for Aquaculture farm with average body weight of 20.3g ± 0.01 / fish were used in the present study. The used fish were apparently healthy and free from any abrasions or external parasites. They were acclimatized in glass aquaria for two weeks and supplied with de chlorinated aerated tap water. The aquaria were cleaned and water was changed weekly. Dissolved oxygen was maintained at an acceptable levels (5.5 - 6.6 mg/l), measured by oxygen meter, water temperature was thermostatically controlled at the range of 26 - 28ºC, with thermostatic controlled heater and measured by thermometer, and water pH was adjusted at 7.4, using pH meter.

Experimental design:

A total number of 210 fish *O. niloticus* with body weight (20.3g ± 0.01 /fish) were divided into seven groups (each group has 10 fish with three replicates) all groups fed on normal diet with 30% crud protein, at the rate of 3% of body weight. The group one untreated group served as control group, group two exposure to concentrations (10 and 20 mmol/L) lead and zinc respectively, group three exposure to concentrations (15 and 30 mmol/L) lead and zinc respectively, group four exposure to concentrations (20 and 40mmol/L) lead and zinc respectively, group fife exposure to concentrations (10, 20 and10 mmol/L) lead, zinc and Na_2CO_3 respectively, group sex exposure to concentrations (15, 30 and 10 mmol/L) lead, zinc and Na_2CO_3 respectively, group seven exposure to concentrations (20, 40 and 10 mmol/L) lead, zinc and Na_2CO_3 respectively, The experiment expanded two month, as indicated in Table 1.

Table 1. Experimental design treated by Pb, Zn and Na_2CO_3 concentrations in each group (mmol/L).

Experimental groups	Lead mmol/L	Zinc mmol/L	Na_2CO_3 mmol/L	No of Fish
Group1(control)	0.0	0.0	0.0	30.0
Group 2	10.0	20..0	0.0	30.0
Group 3	15.0	30.0	0.0	30.0
Group4	20.0	40.0	0.0	30.0
Group5	10.0	20..0	10.0	30.0
Group6	15.0	30.0	10.0	30.0
Group7	20.0	40.0	10.0	30.0

Table 2. Chemical analysis of commercial diet used in the experiment (dry wt).

Ingredients	Percent
Dry matter (DM %)	92.5
Crud protein (CP %)	29.65
Ether extract (EE %)	7.1
Crud fiber (CF %)	6.5
Ash	13.2
NFE[1]	43.55
GE (Kcal / 100g)[2]	413.23
P / E ratio[3]	71.75

[1]Nitrogen free extract (NFE)= 100-(CP + EE + CF + Ash)
[2]GE (gross energy) was calculated after NRC (1993) as 5.64, 9.44 and 4.11 Kcal/g for protein, lipid and NFE, respectively.
[3]P/E ratio = Protein to energy ratio in mg protein/kcal of gross energy.

Measurements of fish growth:

At the end of the experimental period, the growth performances and feed utilization parameters were calculated: weight gain (WG), specific growth rate (SGR), food conversion ratio (FCR), and protein efficiency ratio (PER) using the following formulae: according to (Jauncey and Rose, 1982).

WG = Final average weight (g) − initial average weight (g).

SGR (% d –1) = 100 × (ln W_f − ln W_i) / T

where W_f and W_i represent final and initial body weights of fish, respectively, and T represents the duration of the feeding trial.

Relative growth rate (RGR) = weight gain / initial weight.

FCR = dry weight of feed (g) / wet weight gain by fish (g).

PER = weight gain by fish (g) / protein intake (g).

Protein productive value (PPV) = relative protein / protein intake*100.

where protein intake (g) = Protein (%) in feed × total weight (g) of diet consumed /100

Sample collection:

Blood and liver tissue samples were taken from the fish at the end of experimental period, blood samples were taken from the caudal vein of anaesthetized fish by the syringe containing heparin as an anticoagulant. The blood centrifuged at 3000 r.p.m for 15 minutes after blood parameters measurement. The separated Plasma samples were stored at –20°C for Bioaccumulation and biochemical analysis. The fishes were sacrificing freshly after collection of blood samples immediately the liver tissues were taken and homogenate, used for bioaccumulation analysis.

Measured Biochemical parameters:

Erythrocyte count according to Dacie and Lewis, 1984. Hemoglobin content according to Van kampen, 1961 and hematocrit value according to Britton, 1963 were detected. Blood plasma was obtained by centrifugation of the blood was stored in a deep freezer at -20°C till analysis.

Lead and zinc bioaccumulation was measured in both blood plasma (Makino and Takahara, 1981), and tissues (Greig *et al*., 1982) using Perkin - Elmer 2280 Atomic absorption spectrophotometer with slit 0.7. Plasma protein content was determined by Biuret method described by Wootton, 1964. Glucose concentration was measured according to Trinder, 1969 using Boehring Mannheium kits. Total lipids were determined calorimetrically using kits supplied by El Nasr Pharmaceutical Chemical Co. according to Joseph *et al*. 1972. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined calorimetrically using kits supplied by Diamond Diagnostics according to Reitman and Frankel, 1957.

Statistical analysis:

One way analysis of variance (ANOVA) was done using the computer program SPSS and least Significant difference (LSD) post hoc were done to determine significant differences (Tamhane and Dunlop, 2000).

RESULTS

Growth performance and feed utilization:

The results of growth performance of this study after effect of Lead and zinc as chronic toxicity on *Tilapia niloticus* as in Table 3. Results of growth rate and all growth performance were significantly decrease in lead and zinc exposure groups (G2, G3 and G4) Compared to control group (G1), but after treatments the lead &zinc groups with10 mmol/L Na_2CO_3 in groups (G5,G6 andG7)The relative growth rate and specific growth rate (RGR and SGR) were improved and significantly increase in the groups of fish treated with Na_2CO_3 compared to lead and zinc treated groups. Survival rate war improved in all groups of lead & zinc groups after treatment by Na_2CO_3 compared to control as illustrated in Table (3).

Table 3. Growth performance of Nile tilapia exposure to Lead and Zinc after treated by Sodium Carbonate (Mean+SE).

Items	Group 1 control	Group 2 Pb & Zn	Group 3 Pb & Zn	Group 4 Pb & Zn	Group 5 Pb & Zn &Na_2CO_3	Group 6 Pb & Zn &Na_2CO_3	Group7 Pb & Zn &Na_2CO_3
Initial weight (g)	20.31 ±0.32^a	20.32 ±0.01^a	20.28 ±0.01^a	20.28 ±0.02^a	20.31 ±0.02^a	20.29 ±0.02^a	20.30 ±0.01^a
Final weight (g)	45.46 ±0.18^a	40.38 ±0.18^e	38.44 ±0.24^f	37.58 ±0.24^g	44.43 ±0.18^b	43.38 ±0.18^c	41.77 ±0.32^d
Weight gain(g)	25.15 ±0.19^a	20.06 ±0.18^e	18.16 ±0.24^f	17.30 ±0.25^g	24.12 ±0.19^b	23.10 ±0.19^c	21.44 ±0.31^d
RGR	1.2 ±0.011^a	0.99 ±0.01^e	0.90 ±0.012^f	0.85 ±0.013^g	1.19 ±0.01^b	1.14 ±0.01^c	1.05 ±0.02^d
SGR (%/day)	1.34 ±0.008^a	1.15 ±0.007^e	1.07 ±0.01^f	1.03 ±0.012^g	1.31 ±0.008^b	1.27 ±0.008^c	1.20 ±0.012^d
Survival rate (%)	100.0 ±0.00^a	93.3 ±3.33^{ab}	83.0 ±3.33^{cd}	80.0 ±0.00^d	96.68 ±3.33^{ab}	93.3 ±3.33^{ab}	90.0 ±0.00^{bc}

Means with the different letters in the same row for each parameter are significantly different at $P < 0.05$.

The results in Table (4) showed that , feed conversation ratio (FCR) was increased significantly in Tilapia exposed Lead and zinc compared to the control group and significantly different between the all treatments and improved after addition of Na_2CO_3. However, protein intake (PI|), protein efficiency ratio (PER), protein productive value (PPV), showed significantly

decrease all groups exposed to different concentration of lead and zinc compared to control. The same parameters were improved in fish after treatments by Na_2CO_3 compared with lead and zinc groups.

Table 4. Feed intake, feed conversion ratio (FCR), protein intake (PI), protein efficiency ratio (PER) and protein production value (PPV) of Nile tilapia after exposure to Lead and Zinc after treated by Sodium Carbonate (Mean+SE).

Feed utilization	Group 1 control	Group 2 Pb & Zn	Group 3 Pb & Zn	Group 4 Pb & Zn	Group 5 Pb & Zn & Na_2CO_3	Group 6 Pb & Zn & Na_2CO_3	Group 7 Pb & Zn & Na_2CO_3
Feed Intake	41.16 ±0.52^{a}	34.37 ±0.30^{c}	34.98 ±1.05^{c}	34.15 ±0.97^{c}	40.20 ±0.40^{ab}	38.65 ±0.40^{b}	36.17 ±0.47^{c}
FCR	1.64 ±0.009^{b}	1.71 ±0.02^{b}	1.93 ±0.05^{a}	1.97 ±0.03^{a}	1.67 ±0.003^{b}	1.67 ±0.003^{b}	1.69 ±0.003^{b}
PI	12.20 ±0.156^{a}	10.19 ±0.09^{c}	10.37 ±0.31^{c}	10.13 ±0.29^{c}	11.92 ±0.12^{ab}	11.46 ±0.12^{b}	10.72 ±0.14^{c}
PER	2.06 ±0.11^{a}	1.97 ±0.023^{b}	1.75 ±0.048^{c}	1.71 ±0.025^{c}	2.02 ±0.004^{ab}	2.02 ±0.04^{ab}	2.00 ±0.04^{ab}
PPV	13.59 ±1.36^{a}	8.39 ±0.8^{bcd}	6.86 ±0.81^{cd}	6.05 ±0.87^{d}	10.76 ±0.88^{ab}	10.62 ±1.58^{ab}	10.22 ±1.40^{ab}

Means with the different letters in the same row for each parameter are significantly different at $P < 0.05$.

In the present study, the results in Table (5) showed that protein content (CP) and ether extract (EE) in fish body was significantly decreased in fish exposed to lead and zinc, compared to control group. Contrarily, Crud protein (CP) and ether extracts (EE) content in fish body was decreased significantly in fish after treatments by Na_2CO_3 compared to lead and zinc groups. Ash content was higher significantly in fish exposed to lead and zinc compared to the groups after treatment by Na_2CO_3 and the lowest ash content showed in control group.

Table 5 Chemical analysis of the experimental fish at the end of the experimental period of Nile tilapia exposure to Lead and Zinc after treated by Sodium Carbonate (Mean+SE).

Measurements	Zero time	group1 control	group2 Pb & Zn	group3 Pb & Zn	group4 Pb & Zn	Group5 Pb & Zn & Na_2CO_3	Group6 Pb & Zn & Na_2CO_3	Group7 Pb & Zn & Na_2CO_3
DM	23.26 ±0.05^{d}	24.52 ±0.20^{c}	24.97 ±0.19^{abc}	25.14 ±0.11^{ab}	25.42 ±0.16^{a}	24.39 ±0.16^{c}	24.67 ±0.25b^{c}	24.47 ±0.23^{c}
CP	61.23 ±0.06^{d}	64.87 ±0.07^{a}	60.46 ±0.15^{e}	59.49 ±0.14^{f}	58.43 ±0.25^{g}	63.66± 0.13^{b}	62.66 ±1.33^{c}	62.66 ±0.08^{c}
EE	22.76 ±0.05^{b}	23.56 ±0.19^{a}	22.24 ±0.06^{c}	21.36 ±0.93d	21.07 ±0.12^{d}	22.83 ±0.10^{b}	22.59 ±0.17^{bc}	22.49 ±0.20^{bc}
ASH	16.00 ±0.01^{d}	11.61 ±0.26^{g}	17.30 ±0.22^{c}	19.15 ±0.23^{b}	20.50 ±0.26^{a}	13.52 ±0.21^{f}	14.75 ±0.20^{e}	14.85 ±0.29^{e}

Means with the different letters in the same row for each parameter are significantly different at $P < 0.05$.

Heavy metals bioaccumulation:

The results showed significant ($P<0.01$) increase in Pb and Zn bioaccumulation in blood plasma and liver in all treated groups (G 2, G 3, and G 4) of Nile tilapia exposed to different concentration of Lead and Zinc compared to the control group (G 1) and also after treatment the all groups (lead &zinc) with Na_2CO_3 the results showed significant decrease ($P<0.05$) in Pb and Zn bioaccumulation in blood plasma and liver tissues of Nile tilapia after treatments with Na_2CO3 compared to Pb & Zn groups as illustrated in Table (6).

Table 6. Bioaccumulation of Lead and zinc in plasma and liver of Nile tilapia exposure to Lead and Zinc after treated by Sodium Carbonate (Mean + SE).

Items	group1 control	group2 Pb & Zn	group3 Pb & Zn	group4 Pb & Zn	Group5 Pb & Zn &Na_2CO_3	Group6 Pb & Zn &Na_2CO_3	Group7 Pb & Zn &Na_2CO_3
Lead in plasma mg/L	0.55 ±0.006^{f}	1.29 ±0.015^{c}	1.39 ±0.02^{b}	1.50 ±0.02^{a}	0.85 ±0.009^{e}	0.93 ±0.015^{d}	0.94 ±0.023^{d}
Lead in liver mg/kg	1.58 ±0.033^{g}	5.87 ±0.015^{e}	10.22 ±0.013^{b}	15.29 ±0.041^{a}	4.61 ±0.141^{f}	6.80 ±0.159^{d}	8.95 ±0.00^{c}
Zinc in plasma mg/L	5.26 ±0.006^{f}	7.26 ±0.057^{d}	8.92 ±0.034^{c}	10.85 ±0.026^{a}	6.80 ±0.042^{e}	7.83 ±0.195^{d}	9.65 ±0.061^{b}
Zinc in liver mg/kg	14.16 ±0.027^{f}	20.43 ±0.120^{c}	25.20 ±0.054^{b}	27.26 ±0.152^{a}	18.94 ±0.022^{e}	19.74 ±0.035^{d}	20.63 ±0.058^{c}

Means with the different letters in the same row for each parameter are significantly different at $P < 0.05$.

Blood parameters:

The results showed significant (P<0.05) decrease in erythrocyte count, hemoglobin content, hematocrit value, in all treated groups(G 2,G 3, and G 4) of Nile tilapia exposed to different concentration of lead and zinc compared to the control group (G 1). But the results after treatments the all (lead & zinc) groups with Na_2CO_3 were improved significantly (P<0.05) increase in RBCs, Hb, and hematocrit value (P<0.05) in all treated groups (G5, G6, and G7) of Nile tilapia compared to all (lead & zinc) groups. The blood glucose and aminotransferase (AST & ALT) level showed significant increase in treated groups before and after addition of Na_2CO_3 compared to control one but improved results in groups treatments with Na_2CO_3 than untreated (lead & zinc) groups.

Table 7. Biochemical constituents of Nile tilapia exposure to Lead and Zinc after treated by Sodium Carbonate (Mean + SE).

Items	group1 control	group2 Pb & Zn	group3 Pb & Zn	group4 Pb & Zn	Group5 Pb & Zn & Na_2CO_3	Group6 Pb & Zn & Na_2CO_3	Group7 Pb & Zn & Na_2CO_3
Erythrocyte count (RBCs) (count x $10^6/mm^3$)	2.29 ±0.03^a	1.93 ±0.032^d	1.89 ±0.01^{de}	1.83 ±0.017^e	2.16 ±0.01^b	2.14 ±0.018^b	2.03 ±0.015^c
Hemoglobin (Hb) (g/100ml	8.00 ±0.035^a	6.16 ±0.022^e	5.92 ±0.034^f	5.70 ±0.052^g	7.20 ±0.027^b	6.88 ±0.064^c	6.68 ±0.09^d
Hematocrit value (%)	28.29 ±0.04^a	25.94 ±0.032^e	24.36 ±0.03^f	23.16 ±0.03^g	27.16 ±0.01^b	27.05 ±0.03^c	26.80 ±0.04^d
Glucose(mg/dl)	49.65 ±0.94^d	55.03 ±0.93^b	59.10 ±0.89^a	60.75 ±0.24^a	50.69 ±0.32^d	51.30 ±0.5^{cd}	53.10 ±0.4b^c
AST(u/l)	46.52 ±0.22^e	51.98 ±0.245^b	54.03 ±0.41^a	54.68 ±0.29^a	47.26 ±0.078^d	48.0 ±0.075^c	48.30 ±0.03^c
ALT{u/l)	48.01 ±0.33^e	52.28 ±0.32^c	55.24 ±0.14^b	56.45 ±0.22^a	51.32 ±0.11^d	51.51 ±0.25^d	51.67 ±0.14^d
T. Protein(g/dl)	6.66 ±0.064^a	4.92 ±0.037^e	4.76 ±0.062^f	4.58 ±0.052^g	5.87 ±0.069^b	5.61 ±0.02^c	5.39 ±0.03^c
T. Lipid (mg/dl)	4.26 ±0.006^a	3.43 ±0.032^d	3.15 ±0.032^e	2.90 ±0.026^f	3.88 ±0.07^b	3.78 ±0.06b^c	3.65 ±0.061^c

Means with the different letters in the same row for each parameter are significantly different at P<0.05.

Total protein and total lipid level showed significantly decrease in treated groups before and after addition of Na_2CO_3 compared to control one. And also

the improved results in groups treatments with Na_2CO_3 than untreated (lead & zinc) groups. This results illustrated in Table (7).

DISCUSSION

The results of growth performance of this study after effect of lead and zinc as chronic toxicity on *O. niloticus* as in table 3. Results of growth performance (final Wight, Wight gain, RGR, and SGR) in all was treated groups were significantly decrease in treated groups (G2, G3, G4, G5, G6, and G7) Compared to control group (G1). But after treated (lead & zinc) groups with10 mmol/L Na_2CO_3 growth performance were significantly increase compared to (lead & zinc) groups. Survival rate was improved in all groups of (lead & zinc) after treatment by Na_2CO_3 compared to control. As illustrated in Table (3). As similar to (McGeer *et al*., 2000) reported significant interactions growth performance in rainbow trout due to chronic exposures of Cd, Cu and Zn. EDTA reduced significantly Pb and Cd toxicity of fish, and improve the growth performance (Hayam and Abeer, 2012). The reductions in growth rate and food conversion efficiency of fish exposed to lead and zinc are attributed to an alteration in metabolism due to physiological stress (Lupatsch *et al*., 2010). The exposure of fish to metallic toxicity can cause loss of appetite (Javed and Saeed, 2010). Zinc as a trace element is essential for electron transfer in catalytic reactions (Nussey *et al*., 2000). Significant decrease in weights of fish due to manganese exposure has been reported by Hayat *et al*. (2007).

The results showed that feed utilization, feed conversation rate (FCR), protein intake (PI), protein efficiency ratio (PER), and protein productive value (PPV) were decreased significantly in *O. niloticus* exposed to lead and zinc when compared to the control group. FCR decreased with increasing concentrations of heavy metals, FER and PER due to loss of appetite in fish group exposure to Lead and zinc. These results are in agreement with that obtained by Jabeen and Javed. (2011) Trace elements play an important role in metabolism of fish life. Their excessive amounts in water would become toxic and cause deleterious effects on fish. The exposure of fish to different

concentrations of Zn and Pb caused significantly higher feed intakes than the control fish where FCR of fish decreased significantly with increasing metallic ions toxicity (Ali *et al*., 2003). Also the stress of Zn and Pb toxicity caused significant influence on fish appetite (De Boeck *et al*., 1997). Mc Geer *et al*. (2000) showed significant interactions among feed intake, growth, oxygen consumption and ionic regulation in Rainbow trout due to chronic exposures of Cd, Cu and Zn. Heavy metals are toxic and cause reduced growth, behavioral and physiological disorders in the fish (DeBoeck *et al*., 1997).

In the present study, the results showed that protein content in fish body was significantly decrease in fish exposed to 10, 15 and 20 ml mol/L of lead and 20, 30 and 40 ml mol/L of zinc for two compared to control, but the results were improved after addition of Na_2CO_3 compared to non-treatments groups. Also, total lipid content in fish body was decreased significantly in fish that exposed to 10, 15 and 20 ml mol/L of lead and 20, 30 and 40 ml mol/L of zinc respectively when compared to the control group due to the stress and toxic effect of lead and zinc. And these results improved in the group's treatments with Na_2CO_3 fish than that exposed to lead and zinc only may be its make a chemical complex with lead and zinc. These results are in agreement with that obtained by, Rengaswamy *et al*. (2009) reported that the reduction in the protein content in fish after exposure to nickel may be due to its effect on protein metabolism, which considered as the primary biochemical indicator for stress. This synthesizing is influenced by a large number of exogenous substances. May be due to proteolysis, lack of protein biosynthesis.

Heavy metals bioaccumulation:

These results showed significant ($P<0.05$) increase in Pb and Zn bioaccumulation in blood plasma and liver tissues in all treated groups (G2, G3, and G4) of Nile tilapia exposed to different concentration of lead and zinc compared to the control group (G 1) and also, after treatment the all groups (G5, G6, and G7) (lead and zinc) with Na_2CO_3 the results showed significant decrease ($P<0.05$) in Pb and Zn bioaccumulation in blood plasma and liver tissues of Nile tilapia after treatments with Na_2CO3 compared to Pb & Zn

groups as illustrated in Table (6). These results are agreement with the chronic stress caused liver to store significantly higher Zn and Pb, as organs of detoxification, than that of other organs (Nussey *et al*., 2000). The environmental parameters of water, temperature, hardness, salinity, alkalinity and dissolved organic carbon are effect on metals toxicity to fish (Sassi *et al*., 2010). Hardness, also salinity reduces uptake and accumulation of metals by the fish (Stagg and Shuttle worth, 1982). Also dietary intake of $CA_{2}+$ protects the body against Cd and Zn uptakes (McGeer *et al*., 2000). EDTA [ethylene diamine tetra acetic acid), NTA (Nitro tri acetic acid), and DTPA, (Di ethylene tri amine penta acetic acid), are reduce metal accumulation in tissues of aquatic animals (Kargin, 1996).

Hematological and biochemical parameters:

The effect of lead and Zinc toxicity effects on fish erythrocyte number and hemoglobin content, hematocrit value. It's showed significantly decreased compared to the control group, this are indicators for hematopoiesis, Similar results were observed in previous studies by (Güroy *et al*., 2014) reported that the growth and hematological of striped catfish juveniles (*P.hypophthalmus*) and Nile tilapia were greatly inhibited by copper and lead effect. Present study revealed that plasma glucose and AST, ALT values obtained from fish exposed to lead and zinc were significantly increase compared to control group. While, plasma total protein and total lipids value were significantly decreased compared to control one. In this study, plasma glucose and AST, ALT values, which are indicators of stress parameters in *O. niloticus* when exposed to lead and zinc. These results are agreement with the exposure of metals may modify the fish behavior, metabolism, physiology, growth and reproduction (Gohil and Mankodi, 2013). The EDTA treatment reduced significantly Pb and Cd toxicity of fish, also biochemical parameters and growth performance, protein contents, nutrient utilization body carcass were improved (Hayam and Abeer, 2012). The increase of serum AST and ALT may be attributed to the hepatocellular damage and other tissues by heavy

metals, (Yamawaki *et al*., 1986). Sub lethal concentration of Cd had caused significant increases in activities of serum AST and ALT of common carp and *Tilapia zillii* (Shalaby, 2003).

In conclusion, Sodium carbonate effects on lead and zinc toxicity and improved Growth performance, significantly decrease bioaccumulation, physiological and biochemical constituents in treated fish.

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تأثير كربونات الصوديوم على سمية الرصاص والزنك وتأثيرها على أداء النمو والتراكم الحيوي والمكونات البيوكيميائية في البلطي النيلي

[1]محمد خلف خميس، [1]محمد محمد زينهم،
[2]محمود عبدالفتاح متولي

[1]قسم التغذية، [2]قسم الفسيولوجي، بالمعمل المركزي لبحوث الأسماك، مركز البحوث الزراعية، مصر.

الملخص العربي

في هذه الدراسة تم معالجة التسمم بالرصاص والزنك باستخدام كربونات الصوديوم و دراسة مدى تأثير ذلك على أداء النمو والتراكم الحيوي والمكونات البيوكيميائية في البلطي النيلي. تم تجميع 210 سمكة من المزرعة التابعة للمعمل المركزي لبحوث الاسماك وتم تقسيمهم الى سبعة مجموعات وكل مجموعة ممثلة بثلاث مكررات المجموعة الاولى كمجموعة ضابطة لا تحتوى على تركيز من الرصاص أو الزنك. تم استخدام ثلاث تركيزات من الرصاص والزنك . في المجموعة الثانية كان تركيز الرصاص والزنك فيها (10 , 20 mmol) على التوالي . في المجموعة الثالثة كان تركيز الرصاص والزنك فيها (15 , 30 mmol) على التوالي. في المجموعة الرابعة كان تركيز الرصاص والزنك فيها (20 , 40 mmol) على التوالي. في المجموعة الخامسة كان تركيز الرصاص والزنك فيها (10 , 20 mmol) على التوالي بالإضافة الى (10 mmol) من كربونات الصوديوم . في المجموعة السادسة كان تركيز الرصاص والزنك فيها (15 , 30 mmol) على التوالي بالإضافة الى (10 mmol) من كربونات الصوديوم . في المجموعة السابعة والاخيرة كان تركيز الرصاص والزنك فيها (20 , 40 mmol) على التوالي بالإضافة الى (10 mmol) من كربونات الصوديوم . استمرت التجربة لمدة ٦٠ يوما وتم تغذية الاسماك على عليقة تجاريه تحتوى على 30% من البروتين الخام. خلال فترة التجربة تم متابعة معاملات اداء النمو وفي نهاية التجربة تم أخذ العينات من الدم والانسجة لقياس التراكم الحيوى للعناصر الثقيلة في الدم والكبد وكذلك التغيرات الكيميائية الحيوية في الدم. واظهرت النتائج ما يلى: تأثرت سلبيا مؤشرات (WG, RGR, SGR) النمو بزيادة التركيزات المستخدمة من الرصاص والزنك خلال التجربة مقارنة بالمجموعة الضابطة وتحسنت هذه المؤشرات في المجموعات التي تم اضافة كربونات الصوديوم Na_2CO_3 لها وكانت مؤشرات النمو معنوية (RGR WG, SGR) بالنسبة للمجموعات المعاملة Na_2CO_3 مقارنة بالمجموعات المعاملة بالرصاص والزنك فقط.

كذلك تحسنت نتائج التحويل الغذائي FCR و PI, PER, PPV بعد معاملة التسمم بالرصاص والزنك بكربونات الصوديوم مقارنة بالمجموعة الضابطة .

وجد أن محتوى البروتين والدهون في عضلات الاسماك في نهاية التجربة انخفض انخفاضا معنويا في الأسماك المعرضة لتركيزات مختلفة من الرصاص والزنك وقل هذا الانخفاض في المستوى بعد اضافة كربونات الصوديوم الى المجموعات (5,6,7) مقارنة بالمجموعة الضابطة.

بالنسبة لنتائج التراكم الحيوي من الرصاص والزنك في كل من البلازما وأنسجة الكبد تحسنت النتائج بعد معاملة المجموعات المعرضة لتركيزات مختلفة من الرصاص والزنك بكربونات الصوديوم ولاحظنا اعلى تركيزات في المجموعات الغير معاملة بكربونات الصوديوم مقارنة بالمجموعة الضابطة .

اما بالنسبة للمكونات البيو كيميائية فأنخفض مستوى العد الكلى لكرات الدم RBCs ومحتوى الهيموجلوبين Hb ومستوى الهيماتوكريت Hct وكذلك البروتين الكلى والدهون الكلية في الدم وتأثرت تأثرا ملحوظا بزيادة تركيز كل من الرصاص والزنك وتحسنت هذه النتائج في المجموعات المعاملة بكربونات الصوديوم مقارنة بالمجموعة الضابطة .

على عكس ذلك أرتفع مستوى تركيز أنزيمات الكبد ارتفاعا معنويا (ALT, AST) في دم الاسماك المعرضة لتركيزات مختلفة من الرصاص والزنك وانخفض هذا الارتفاع في المجموعات المعالجة بكربونات الصوديوم مقارنة بالمجموعة الضابطة.

في نهاية هذا البحث نؤكد على أن أضافة Na_2CO_3 10mmol/L الى المجموعات المعاملة بالرصاص والزنك الى تخفيض مستوى السمية وتحسين معاملات النمو والتحويل الغذائي والتركيب الحيوي للدم والانسجة في أسماك البلطي النيلي.