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Changes in growth and biochemical status of common carp, *Cyprinus carpio* L. exposed to water-born zinc toxicity for different periods

Mohsen Abdel-Tawwab^{1*}, Mohamed NM Mousaad², Khaled M Sharafeldin² and Nahla EM Ismaiel¹

* Correspondence:

mohsentawwab@gmail.com

¹Department of Fish Biology and Ecology, Central Laboratory for Aquaculture Research, Abbassa, Abo-Hammad, Sharqia 44662, Egypt
Full list of author information is available at the end of the article

Abstract

The present study was carried out to investigate the effect of sublethal zinc (Zn) concentrations on the growth, biochemical variables, and Zn residues in common carp, *Cyprinus carpio* L. Fish (18.1 – 19.1 g) were exposed to 0.0 (control), 5.0 and 10.0 mg Zn/L for 7, 14, 28, and 56 days. At each time interval and each treatment, fish were collected, weighed and sampled to measure the growth, biochemical variables, and Zn residues in whole-fish body. Growth performance was significantly reduced with increasing Zn concentrations. However, fish exposed to 10.0 mg Zn/L for 56 days grew lower than that of the control group. Likewise, the optimum feed intake and feed conversion ratio were obtained at control group at 56 days. Furthermore, glucose, AST, ALT, creatinine, and cortisol increased significantly with increasing Zn concentration and exposure time, with maximal values at 56 days. Meanwhile, the highest values of serum protein and lipids of were obtained in the control fish reared for 56 days, whereas the lowest values were observed in fish exposed to 10.0 mg Zn/L for 56 days. The content of whole-body moisture and total ash increased significantly, while crude protein and total lipid contents decreased significantly with increasing Zn concentrations. In addition, Zn exposure increased Zn residues in fish body; however, Zn bioaccumulation in fish body was Zn dose and time dependant. The present study revealed that the growth and health status of common carp were deteriorated by Zn toxicity.

Keywords: Common carp; Zinc toxicity; Biochemical alteration; Zinc residue

Background

With the advent of agricultural and industrial revolution worldwide, most of the water sources are becoming contaminated via discharging toxic and hazardous substances, including heavy metals, into the aquatic ecosystem (Gbem et al. 2001; Khare and Singh 2002; Woodling et al., 2002). Heavy metals have been recognized as strong biological poisons because of their persistent nature and cumulative action (Hoo et al. 2004; Loganathan et al. 2006; Shukla et al. 2007). Zinc (Zn) has been recognized to play a vital role in almost all aspects of living systems either directly or indirectly (Shukla et al. 2007; Srivastava 2007). Fish generally requires Zn in a certain concentration for desirable fish growth (Watanabe et al. 1997) but its overaccumulation is hazardous to exposed organisms (Gupta and Srivastava 2006; Senthil Murugan et al. 2008).

Pollution of the aquatic environment with zinc (Zn) has become a serious health concern in recent years. This metal is introduced into the environment through various routes such as industrial effluents, agriculture pesticide runoff, domestic garbage dumps, and mining activities (Merian 1991). Among aquatic organisms, fish are generally considered to be the most relevant organisms for pollution monitoring in aquatic ecosystems (van der Oost et al. 2003).

Carp species are widely cultivated family in Egypt and worldwide because of their tolerance of wide differences in pond temperature and water quality, their ease of management, and their high growth rates (Tapia and Zambrano 2003). Common carp, *Cyprinus carpio* L. is one of the widely cultured carp species. This fish species may be occurred in the aquatic ecosystem, which may be polluted by Zn. The Zn toxicity may induce changes in blood parameters of fish and affects their growth. Growth performance and blood chemistry analyses often provide vital information aiding the diagnosis for health assessment and management of cultured fish (Cnaani et al. 2004; Abdel-Tawwab et al. 2012). Hence, the present study aims to determine the effect of Zn toxicity on growth performance, feed utilization, biochemical variables, and Zn bioaccumulation in common carp exposed to water-born Zn for different periods.

Methods

Fish and experimental procedures:

Common carp, *C. carpio* L. were obtained from the nursery pond, Central Laboratory for Aquaculture Research, Abbassa, Abo-Hammad, Sharqia, Egypt. Fish were acclimated to laboratory conditions in indoor tanks for 2 weeks. Fish (18.1 – 19.1 g) were randomly distributed at a rate of 10 fish per aquarium, which was filled with aerated tap water and supplied with compressed air via air–stone using air pumps. Fish were fed on a 30% crude protein diet, which was offered up to satiation twice a day for 56 days. Settled fish wastes were siphoned daily together with a half of the water in each aquarium. Aerated tap water containing the same Zn concentration was subsequently added to recover the initial volume of the aquaria. Dead fish were removed and the percentage of fish survival was recorded.

Zn sulfate ($ZnSO_4$, Merck & Co Inc., NJ, USA) was dissolved in distilled water and used in this study. A preliminary study was then conducted to determine the 96-h LC50 of Zn for common carp according to Behrens-Karber's method (Klassen 1991); however, it was 64.0 mg Zn/L. This study was based on a bifactorial design with three Zn sublethal levels (0.0, 5.0, and 10.0 mg/L) and four exposure periods (7, 14, 28, and 56 days). Zinc was added to 24 100-L aquaria to obtain the nominal concentrations of 0.0, 5.0 and 10.0 mg Zn/L and each treatment was represented by 8 aquaria; two aquaria for each period at each Zn concentration. Fish were exposed to the above Zn concentrations for 7, 14, 28, and 56 days.

Growth parameters and feed utilization

Growth performance was determined and feed utilization was calculated as following:

$$\text{Weight gain} = W_2 - W_1;$$

Specific growth rate (SGR) = $100 [\ln W_2 \text{ (g)} - \ln W_1 \text{ (g)}] / T$; where W_2 is final weight, W_1 is initial weight, and T is the experimental period (day);

$$\text{Feed conversion ratio (FCR)} = \text{feed intake} / \text{weight gain}.$$

Biochemical measurements

At 7, 14, 28 and 56 days, five fish from each aquarium were anaesthetized with buffered tricaine methane sulfonate (30 mg/L) and blood was collected from the caudal vein. The collected blood was left to coagulate and centrifuged at 5000 rpm for 15 min at room temperature. The collected serum was stored at -20°C for further assays. Glucose was determined colorimetrically according to Trinder (1969). Total protein in serum was determined colorimetrically according to Henry (1964). Total lipids in serum was determined colorimetrically according to Joseph et al. (1972). Uric acid was measured according to Barham and Trinder (1972) and creatinine was measured colorimetrically as described by Henry (1974). Activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined colorimetrically according to Reitman and Frankel (1957).

Proximate chemical analyses

The proximate chemical analyses of the whole-fish body from each treatment were carried out according to the standard methods (AOAC 1990) for moisture, crude protein, total lipids, and ash. Moisture content was estimated by drying the samples at 85°C in a heat oven (GCA, model 18EM, Precision Scientific group, Chicago, Illinois, USA) for 48 hours. Nitrogen content was measured using a microkjeldahl apparatus (Labconco, Labconco Corporation, Kansas, Missouri, USA) and crude protein was estimated by multiplying nitrogen content by 6.25. Lipid content was determined by ether extraction in multi-unit extraction Soxhlet apparatus (Lab-Line Instruments, Inc., Melrose Park, Illinois, USA) for 16 hours. Total ash was determined by combusting dry samples in a muffle furnace (Thermolyne Corporation, Dubuque, Iowa, USA) at 550°C for 6 hours.

Zinc residue

For measuring Zn residues in the investigated fish body, the whole-fish body was oven dried at 85°C until constant weight and 1.0 g dry weight was ashed in a muffle furnace for 6 hours. Ash was digested with 5 ml conc. H_2SO_4 and gradually kept at 130°C on a hot plate until complete dryness. Then, the digests were diluted with 2 N HCl to a constant volume. The Zn concentration was determined with an atomic absorption spectrophotometer (Thermo 6600, Thermo Electron Corporation, Cambridge, UK), which was calibrated using Zn standard solutions.

Statistical analysis

The obtained data were subjected to two-way ANOVA to test the effect of water-born Zn and exposure periods as two factors simultaneously tested. The differences between means were done by using Duncan's Multiple Range test to compare between means at $P \leq 0.05$. The software SPSS, version 10 (SPSS, Richmond, Virginia, USA) was used as described by (Dytham 1999).

Results and discussion

Growth performance and feed intake, however, were significantly affected by Zn concentrations, exposure periods, and their interaction ($P < 0.05$; Table 1). For instance, fish growth was significantly reduced with increasing Zn concentrations. Fish (26.4 g) exposed to 10.0 mg Zn/L for 56 days grew lower than that of the control group (38.5 g). Likewise,

Table 1 Growth performance and feed utilization (means ± SE) of common carp exposed to different water-born Zn concentrations for different periods

Zn concentrations (mg Zn/L)	Exposure period (days)	Initial weight (g)	Final weight (g)	Weight gain (g)	SGR (% g/day)	Feed intake (g feed/fish)	FCR	Fish survival (%)
Individual treatment means ¹								
0.0	7	18.4	20.5 fgh	2.1 g	0.193 hi	2.0 i	1.18	100.0
5.0		18.7	19.9 gh	1.2 h	0.111 ij	1.5 ij	1.33	96.7
10.0		18.6	19.1 h	0.5 i	0.047 j	0.7 j	1.82	93.3
0.0	14	18.4	24.2 cde	5.8 d	0.490 de	5.8 g	1.01	100.0
5.0		18.7	22.1 efg	3.4 f	0.298 fg	4.5 gh	1.38	96.7
10.0		18.6	21.4 fgh	2.8 g	0.250 gh	3.9 h	1.45	93.3
0.0	28	18.4	28.9 b	10.5 b	0.806 b	13.7 d	1.33	100.0
5.0		18.7	25.6 cd	6.9 cd	0.560 cd	10.7 e	1.62	93.3
10.0		18.6	23.2 def	4.6 e	0.394 ef	8.3 f	1.85	93.3
0.0	56	18.4	38.5 a	20.1 a	1.318 a	28.7 a	1.43	96.7
5.0		18.7	29.8 b	11.1 b	0.832 b	19.7 b	1.82	93.3
10.0		18.3	26.4 c	8.1 c	0.654 c	16.3 c	2.03	93.3
Pooled SE		0.06	0.91	0.92	0.06	1.36	0.10	0.83
Means of main effects ²								
Zn concentration								
0.0		18.4	28.0	9.6	0.702	12.6	1.24 y	99.2
5.0		18.7	24.4	5.7	0.450	9.1	1.54 xy	95.0
10.0		18.5	22.5	4.0	0.336	7.3	1.79 x	93.3
	7	18.6	19.8	1.3	0.117	1.4	1.44 r	96.7
	14	18.6	22.6	4.0	0.346	4.7	1.28 s	96.7
	28	18.6	25.9	7.3	0.587	10.9	1.60 q	95.5
	56	18.5	31.6	13.1	0.935	21.6	1.76 p	94.4
Two way ANOVA		<i>P</i> value						
Zn concentration		0.164	0.001	0.001	0.001	0.001	0.001	0.474
Exposure period (EP)		0.921	0.001	0.001	0.001	0.001	0.039	0.749
Zn conc. x EP		0.984	0.013	0.010	0.025	0.001	0.991	0.978

¹ Treatments means represent the average values of three aquaria per treatment. Duncan multiple range test was conducted for individual means only if there was a significant interaction (ANOVA: $P < 0.05$).

² Main effect means followed by the same letter are not significantly different at $P < 0.05$; x, y, and z for Zn concentration and p, q, r, and s for exposure period.

feed intake decreased, while FCR increased significantly with increasing Zn concentrations ($P < 0.05$; Table 1). The optimum feed intake and FCR were obtained at the control group (28.7 g feed/fish and 1.4, respectively) after 56 days. One hypothesis for these observations is that exposure to elevated Zn concentrations leads to reduced fish appetite, in turn resulting in reduced feed intake and growth. An alternative hypothesis is that due to the reduced feed intake, the energy requirements were met via the decomposition of the

storage-deposited nutrients (Abdel-Tawwab et al. 2006). This hypothesis is supported by a significant decrease in total lipids deposition observed in the current study, and consistent with Shukla and Pandey (1986) who reported significant decreases in growth of *Channa punctatus*, when exposed to 12 mg/L zinc sulfate. Also, (Abdel-Tawwab et al. 2012) found significant decreases in Nile tilapia growth when exposed to 3.5 or 7.0 mg Zn/L for 6 weeks. The water-born Zn exposure regimes employed in the present study were well tolerated by common carp as portrayed by the high fish survival (93.3 – 100.0%).

All the biochemical parameters monitored at 7, 14, 28, and 56 days were significantly affected by the Zn concentrations and exposure periods ($P < 0.05$; Table 2). Glucose

Table 2 Biochemical parameters (means \pm SE) of common carp exposed to different water-born Zn concentrations for different periods

Zn concentrations (mg Zn/L)	Exposure period (days)	Glucose (g/L)	Total protein (g/L)	Total lipids (g/L)	AST (IU/L)	ALT (IU/L)	Uric acid (mg/L)	Creatinine (mg/L)
Individual treatment means ¹								
0.0	7	0.85 g	20.1	10.6 b	12.0 g	11.5 e	18.0	2.8
5.0		0.90 f	17.9	9.2 c	19.0 f	12.2 e	20.0	4.1
10.0		1.00 d	16.4	8.0 de	22.0 ef	13.0 e	24.0	5.3
0.0	14	0.91 ef	21.7	11.9 a	22.0 ef	13.2 e	20.0	3.1
5.0		0.97 d	17.0	8.4 cd	34.0 cde	15.5 d	23.3	5.6
10.0		1.10 c	15.3	7.1 efg	42.0 d	17.2 cd	27.0	7.3
0.0	28	0.98 d	23.5	12.1 a	27.0 e	15.4 d	22.0	3.4
5.0		1.07 c	15.8	7.6 def	57.0 c	18.1 c	27.6	6.6
10.0		1.16 b	13.6	6.6 fg	72.0 b	21.5 b	36.0	8.3
0.0	56	0.96 de	23.7	12.3 a	37.0 d	16.7 cd	25.7	3.6
5.0		1.11 c	14.1	6.9 efg	69.0 b	22.0 b	30.0	7.4
10.0		1.27 a	11.0	6.2 g	82.0 a	27.0 a	39.0	9.4
Pooled SE		0.025	0.86	0.46	4.76	0.93	1.32	0.46
Means of main effects ²								
Zn concentration								
0.0		0.93	22.3 x	11.7	24.5	14.2	21.4 z	3.2 z
5.0		1.01	16.2 y	8.0	44.8	17.0	25.2 y	5.9 y
10.0		1.13	14.1 z	7.0	54.5	19.7	31.5 x	7.6 x
	7	0.92	18.1 p	9.3	17.7	12.2	20.7 s	4.1 s
	14	0.99	18.0 pq	9.1	32.7	15.3	23.4 r	5.3 r
	28	1.07	17.6 q	8.8	52.0	18.3	28.5 q	6.1 q
	56	1.11	16.3 r	8.5	62.7	21.9	31.6 p	6.8 p
Two way ANOVA		P value						
Zn concentration		0.001	0.001	0.001	0.001	0.001	0.001	0.001
Exposure period (EP)		0.001	0.046	0.039	0.001	0.001	0.001	0.001
Zn conc. x EP		0.003	0.164	0.002	0.022	0.002	0.152	0.274

¹ Treatments means represent the average values of three samples per treatment. Duncan multiple range test was conducted for individual means only if there was a significant interaction (ANOVA: $P < 0.05$).

² Main effect means followed by the same letter are not significantly different at $P < 0.05$; x, y, and z for Zn concentration and p, q, r, and s for exposure period.

level increased significantly by increasing Zn concentrations and exposure periods. The highest observation was noticed after 56 days (1.27 g/L) at 10 mg Zn /L, while the lowest value was observed in the control group after 7 days (0.85 g/L). The significant increase of blood glucose during Zn exposure periods indicates to the stressful condition of Zn, which induce chromaffin cells to release catecholamine hormones, adrenaline and non-adrenaline toward blood circulation (Reid et al. 1998). Those stress hormones in conjunction with cortisol mobilize and elevate glucose production in fish through glucogenesis and glucogenolysis pathways (Iwama et al. 1999) to cope with the energy demand produce by stressor for reaction and restoration (Wendelaar Bonga 1997; Barton et al. 2002). Also, this high level was explained through glucogenesis, which mean formation of glucose and glycogen from tissue proteins and amino acids (Almeida et al. 2001). The increase in blood glucose is usually correlated with the mobilization of glycogen and development of a status of hyperglycaemia.

In the present study, serum protein and lipid was significantly decreased with increasing exposure periods ($P < 0.05$; Table 2). Total protein and total lipid in fish serum decreased significantly by increasing Zn concentrations and exposure period. The highest values of protein and lipids were noticed at 56 days at the control group (23.7 and 12.3 g/L, respectively), while the lowest values were observed in fish exposed to 10 mg Zn/L (11.0 and 6.2 g/L, respectively) after the same period. These results might be due to the breakdown of these molecules as energetic substrates to cope with Zn induced stress metabolically (Vijayan et al. 1997), or due to renal excretion, impaired protein synthesis, and/or due to liver disorder (Kori-Siakpere 1995). This decrease may be due to that Zn exposure which causes significant alteration in the protein secondary structure by decreasing the α -helix and increasing the β -sheet content of the gill tissues of rohitia carp, *Labeo rohita* (Palaniappan et al. 2010).

AST and ALT levels increased significantly by increasing Zn concentrations and exposure period ($P < 0.05$; Table 2). The highest values of AST and ALT were obtained at 10 mg Zn /L after 56 days (82.0 and 27.0 IU/L, respectively), while the lowest ones were observed in control group after 7 days (12.0 and 11.5 IU/L, respectively). AST and ALT enzymes are biomarkers of acute hepatic damage, thus their bioassay can serve as a diagnostic tool for assessing liver function (Coles 1989; Coppo et al. 2003). These results agreed with Rajamanickam and Muthuswamy (2008) who studied the effect of cadmium, lead, nickel, and chromium on common carp and found similar results. Firat and Kargin (2010) found increases in ALT and AST activity in Nile tilapia serum caused by the individual and combined effects of exposure to Zn and Cd. Abdel-Tawwab et al. (2012) found significant increases in ALT and AST activity in Nile tilapia when exposed to 3.5 or 7.0 mg Zn/L for 6 weeks.

Uric acid and creatinine levels in fish serum increased significantly by increasing Zn concentrations and exposure periods (Table 2). The highest values were obtained at 10.0 mg Zn /L after 56 days (39.0 and 9.4 mg/L), respectively, while the lowest values were obtained in control group after 7 days (18.0 mg/L and 2.8 mg/L, respectively). Both variables are traditional screening indices for kidney function and renal structural integrity. The increased uric acid and creatinine levels indicated that Zn toxicity had a marked effect on kidney function, perhaps due to the action of water-born Zn on glomeruli filtration rate and/or pathological changes to the kidney resulting in dysfunction. Similar results were obtained by Zaghoul (2001), Ali et al. (2003), and Abdel-Tawwab et al. (2012).

The contents of whole-body moisture increased significantly, while crude protein and total lipid contents decreased significantly with increasing Zn concentrations ($P < 0.05$; Table 3). In this regard, Zaghoul (2001) reported that African catfish, *Clarias gariepinus* exposed to 0.35 mg copper/L individually showed significant ($P < 0.05$) increases in both muscle water and ash contents and significant decreases in either total muscle protein or total lipids percentages. Similarly, Ali et al. (2003) revealed that body moisture and ash contents were the highest, whereas fat content was the lowest for Nile tilapia treated with 0.50 ppm copper as compared with other concentrations (0.15 and 0.30 ppm copper).

The low proteins and lipids in Zn-exposed fish body may be due to the reduction in feed intake. Further, these decreases may be due to the breakdown of those molecules as energetic substrates to cope with Zn-induced stress metabolically (Vijayan et al. 1997).

Table 3 Proximate analysis (means \pm SE; % on dry matter basis) of common carp exposed to different water-born Zn concentrations for different periods

Zn concentrations (mg Zn/L)	Exposure period (days)	Moisture	Crude protein	Total lipids	Total ash	Zn residue (mg/g dry wt)
Individual treatment means ¹						
0.0	7	69.5	58.9	18.9 c	20.4	22.0 g
5.0		73.3	55.6	16.2 d	27.3	40.6 f
10.0		74.9	53.5	14.4 e	28.9	60.9 e
0.0	14	67.8	58.1	19.4 c	19.8	22.5 g
5.0		71.9	57.6	16.3 d	21.6	70.3 d
10.0		76.0	56.1	14.3 e	26.5	97.9 c
0.0	28	66.4	57.3	21.4 b	18.5	23.7 g
5.0		70.3	56.6	19.3 c	23.0	93.5 c
10.0		71.7	55.8	16.2 d	26.1	132.0 b
0.0	56	65.5	59.1	25.2 a	14.6	24.2 g
5.0		68.0	58.5	24.6 a	15.7	101.2 c
10.0		71.0	57.2	21.7 b	15.8	149.8 a
Pooled SE						
Means of main effects ²						
Zn concentration						
0.0		67.3 z	58.4 x	21.2	18.3 z	23.1
5.0		70.9 y	57.1 y	19.1	21.9 xy	76.4
10.0		73.4 x	55.7 z	16.7	24.8 x	110.2
	7	72.6 p	56.0	16.5	25.5 p	41.2
	14	71.9 pq	57.3	16.7	22.6 q	63.6
	28	69.5 q	56.6	19.0	22.5 q	83.1
	56	68.2 r	58.3	23.8	16.0 r	91.7
Two way ANOVA						
		P value				
Zn concentration		0.001	0.039	0.001	0.014	0.001
Exposure period (EP)		0.019	0.055	0.001	0.001	0.001
Zn conc. x EP		0.520	0.202	0.001	0.068	0.001

¹ Treatments means represent the average values of three samples per treatment. Duncan multiple range test was conducted for individual means only if there was a significant interaction (ANOVA: $P < 0.05$).

² Main effect means followed by the same letter are not significantly different at $P < 0.05$; x, y, and z for Zn concentration and p, q, r, and s for exposure period.

Moreover, the loss of protein and lipid levels in the Zn-exposed fish body may be due to increased protein oxidation with Zn exposure (Takahashi et al. 1991). Palaniappan et al. (2010) reported that Zn exposure caused important structural alteration in the existing proteins indicated by a significant reduction in the intensities of the α -helix. They also suggested that Zn exposure causes significant alteration in the protein secondary structure by decreasing the α -helix and increasing the β -sheet content of the gill tissues of rohiya carp. Due to the low feed intake by Zn-exposed fish, the deposited protein and lipid decreased and visa versa. In addition, changes in protein and lipid contents in fish body may be linked with changes in their synthesis and/or deposition rate in fish body (Fauconneau 1985; Abdel-Tawwab et al. 2006), or because fish exerted more energy to challenge the Zn toxicity effect.

The contents of the whole-body ash and Zn residue in the whole-fish body increased significantly by increasing Zn concentrations ($P < 0.05$; Table 3). For instance, Zn residue in the control fish reared for 7 days was the lowest concentration (22.0 mg/g dry weight), while fish exposed to 10.0 mg Zn/L over 56 days accumulated more Zn residue (149.8 mg/g dry weight) than the other treatments. This is consistent with Senthil Murugan et al. (2008) and Palaniappan et al. (2010) who reported similar trends in the *Sole Senegalenis*, *Channa punctatus*, and rohiya carp, respectively. Similar results were obtained by Mohanty et al. (2009) who concluded that Zn accumulation in the whole body of Indian major carp increased with increasing Zn concentrations. Abdel-Tawwab et al. (2012) found that Zn accumulation in the whole body of Nile tilapia is correlated with Zn concentrations.

Conclusion

The present study revealed that Zn exposure had a deteriorate effect on the growth and health status of common carp. However, the biochemical parameters are indicative to Zn toxicity. Also, Zn bioaccumulation in fish body depends on Zn concentrations and exposure periods.

Competing Interests

All authors declare that they have no competing interests.

Authors' contributions

M Abdel-Tawwab: designed this study, did the statistical analysis, write the article and submitted to IAR. MNM Mosaad: designed this study and revised the written article. KM Sharafeldin: did the biochemical analyses. NEM Ismaiel: did the experiment running. All authors read and approved the final manuscript.

Author details

¹Department of Fish Biology and Ecology, Central Laboratory for Aquaculture Research, Abbassa, Abo-Hammad, Sharqia 44662, Egypt. ²Zoology Department, Faculty of Science, Benha University, Benha, Egypt.

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