RESULTS

I.1. Determination of the 96-h LC_{50}:

Fish were exposed to 0, 10, 40, 70, 100, and 130 mg Zn/L for 96 hours. The water physico-chemistry was represented in Table (2). The water temperature range was 23.3 - 23.5 ^{o}C, pH 7.43 - 7.69, dissolved oxygen 5.4 - 5.7 mg/L, total alkalinity 150 - 170 mg/L as $CaCO_3$, and total hardness 220 - 226 mg/L as $CaCO_3$. Moreover, the unionized ammonia increased by increasing Zn concentration, and its values were 0.53, 0.59, 0.64, 0.71, 0.79 and 0.85 mg/L for 40, 70, 100, and 130 mg Zn/L, respectively.

The data obtained herein revealed that the Zn toxicity increased with increasing Zn concentration and/or exposure period. The number of dead fish in relation to the Zn concentrations were assessed and counted during the exposure time at 24, 48, 72 and 96 h then they were removed immediately. No mortality was observed during the 96 h at Zn concentrations of 0.0 and 100% mortality rate was achieved only at 130 mg Zn/L.

Fish survival was adversely affected by water-born Zn concentration. The overall fish mortality increased significantly with increasing Zn concentration (Table 3). For Zn doses 0 and 10 mg/L, the overall mortality after 96-h post-exposure was zero, but for Zn doses 40, 70, 100 and 130 mg/L it was 20, 40, 90, and 100 %, respectively. It was noticed that the 96-h LC_{50} of Zn was 70.0 mg/L (Table 3).

Table 3. The cumulative mortalities and acute 96-h LC_{50} of water-born Zn in Nile tilapia fingerlings according to Behrens-Karber's method (Klassen, 1991).

Zn dose (mg/L)	No. of exposed fish	No of dead fish				Overall deaths within 96 h	A	B	AB
		D1	D2	D3	D4				
0	10	0	0	0	0	0	0	0	0
10	10	0	0	0	0	0	10	0	0
40	10	0	1	1	-	2	30	1.0	30
70	10	2	1	1	-	4	30	3.0	90
100	10	8	1	-	-	9	30	6.5	195
130	10	8	2	-	-	10	30	9.5	285
									∑AB =600

96 h $LC_{50} = LC_{100} - \sum (A \times B)/N = 130 – 600/10 = 70.0$ mg Zn/L.
Where A = differences between the two consecutive doses, B = arithmetic mean of the mortality caused by two consecutive doses and N = Fish number.

I.2. Effect of Zn toxicity and exposure time on the different investigated parameters:

In this experiment, fish fed on 25%-CP diet and exposed to 0.0, 3.5, or 7.0 mg Zn/L for either 1 or 6 weeks. Fish performance, survival, biochemical variables, and Zn accumulation in different fish organs were investigated.

I.2.1. Water physico-chemical parameters:

In the present study, no significant changes were observed in water temperature, dissolved oxygen, pH, water EC, total alkalinity, and total hardness ranges due to Zn concentration, exposure time, and their interaction ($P > 0.05$; Table 4). However, pH was slightly affected by different Zn concentration ($P > 0.05$). After one week, pH value was 7.52 and 7.48 in the treatments of 0.0 and 7.0 mg Zn/L, respectively. After 6 weeks, pH value was 7.52 and 7.41 in the treatments of 0.0 and 7.0 mg Zn/L, respectively. The dissolved O_2 range during the experiment was 4.9 – 5.3 mg/L, water temperature range was 24.4 - 25.0 ^oC, water EC range was 415.2 – 422.3 µS/cm, total alkalinity range was 190.0 – 200.0 mg/L as $CaCO_3$, and total hardness range was 228.0 – 236.3 mg/L as $CaCO_3$.

The unionized ammonia increased significantly by increasing Zn concentration and exposure period (Table 4; Figure 1). The highest ammonia level (0.98 mg/L) was detected when fish exposed to 7.0 mg Zn/L for 6 weeks; meanwhile the lowest value (0.71 mg/L) was obtained in the control

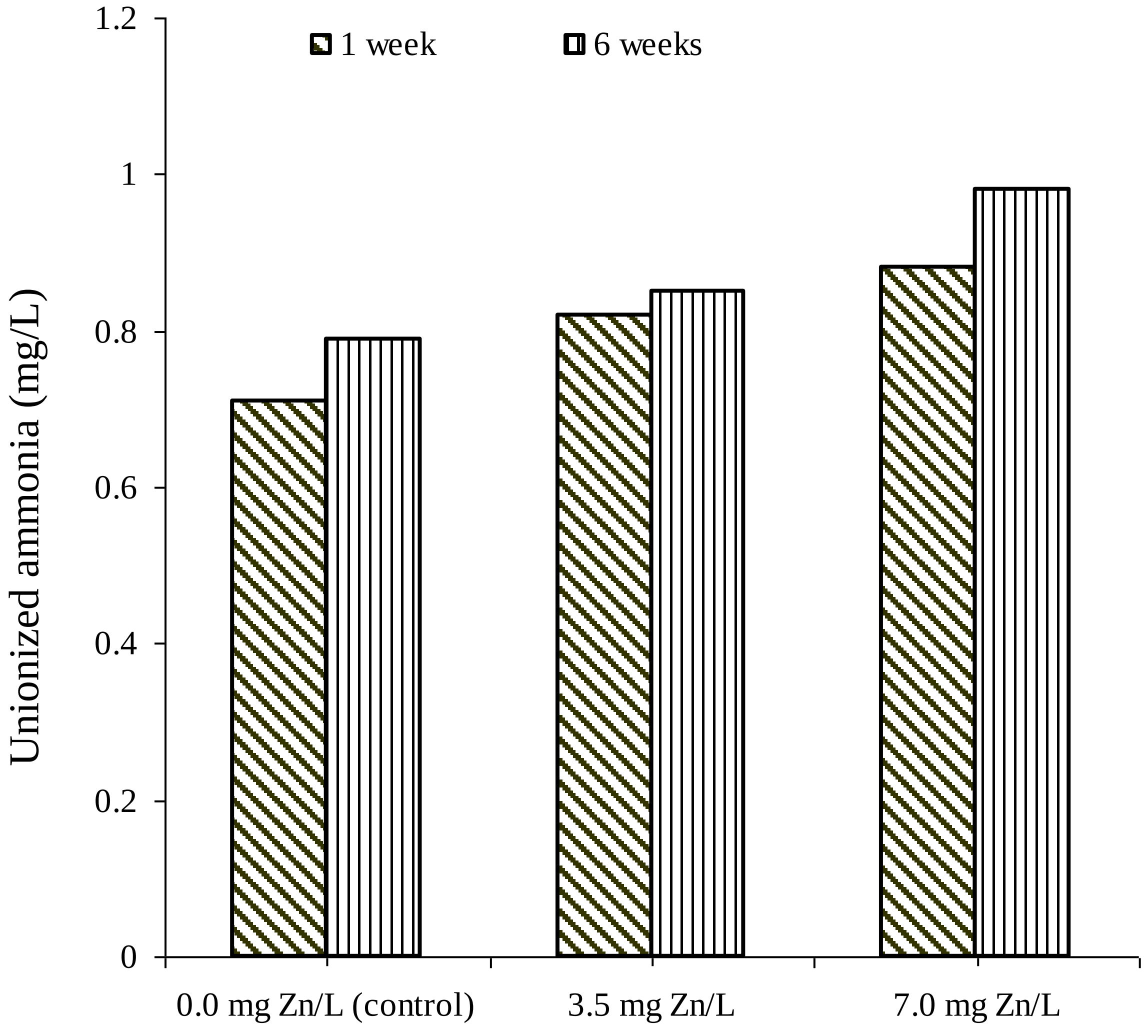

Figure 1. Changes in the unionized ammonia (mg/L) in aquaria's water stocked by Nile tilapia exposed to different Zn concentrations.

group. After one week, NH_3 was 0.71 mg/L and 0.88 mg/L in the treatments of 0.0 mg Zn/L and 7.0 mg Zn/L, respectively. After 6 weeks, NH_3 was 0.79 mg/L and 0.98 mg/L for the same treatments.

I.2.2. Fish performance:

Fish performance and feed utilization were significantly affected by Zn concentration, exposure period, and their interaction ($P < 0.05$; Table 5). Fish growth was reduced significantly by increasing Zn concentration. The growth parameters of fish exposed to 7.0 mg Zn/L for 6 weeks was lower than those of the control group (Figure 2). The final weight, weight gain and SGR were 44.7 g, 19.5 g and 1.365 % g/day for the control group and their values were 32.2 g, 7.1 g and 0.593 % g/day, respectively for 7.0 mg Zn/L at 6 weeks. Moreover, no significant difference in fish survival was observed among the different treatments and its range was 96.7 – 100.0% ($P > 0.05$; Table 5).

Feed intake and FCR were significantly affected by Zn concentration, exposure period, and their interaction ($P < 0.05$; Table 5). Feed intake decreased, while FCR increased significantly with increasing Zn concentration. The best feed intake (32.8 g feed/fish) and FCR (1.68) were obtained at the control group reared for 6 weeks; meanwhile feed intake and FCR were 23.0 g feed/fish and 3.24, respectively for fish exposed to 7.0 mg Zn/L for 6 weeks.

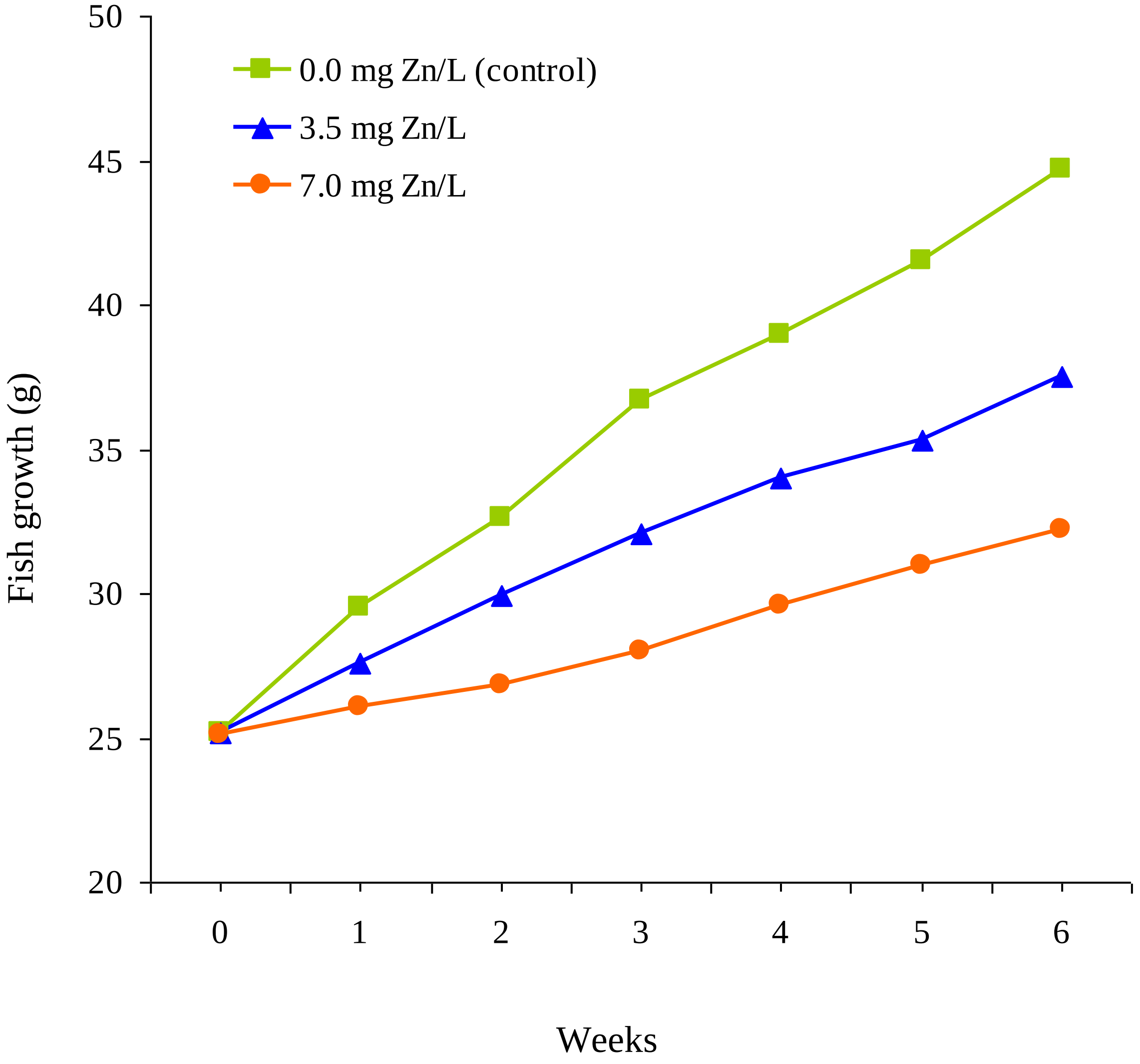

Figure 2. Changes in the weight (g) of Nile tilapia exposed to different Zn levels for 6 weeks in glass aquaria.

I.2.3. Biochemical parameters:

All biochemical parameters of Nile tilapia exposed to different water-born Zn concentrations for 1 or 6 weeks are significantly affected by Zn concentration, exposure period, and their interaction ($P < 0.05$; Table 6). Serum glucose, AST, ALT, creatinine, and cortisol increased significantly with increasing Zn concentration and exposure period. It is noticed that fish exposed to 7.0 mg Zn/L for 6 weeks exhibited the maxima of glucose, AST, ALT, creatinine, and cortisol (143.8 mg/dL, 81.4 IU/L, 59.2 IU/L, 0.79 mg/dL, and 9.39 µg/dL, respectively). The control fish at either 1 or 6 weeks exhibited the lowest values of the previous variables (Table 6). After one week, the values of glucose, AST, ALT, creatinine, and cortisol for control group (0.0 mg Zn/L) were 83.0 mg/dL, 17.1 IU/L, 23.03 IU/L, 0.21 mg/dL and 0.89 µg/dL, respectively. Moreover, their values were 111.3 mg/dL, 41.2 IU/L, 35.0 IU/L, 0.51 mg/dL and 2.56 µg/dL, respectively in fish exposed to 7.0 mg Zn/L. After 6 weeks, these values were 82.8 mg/dL, 38.33 IU/L, 40.8 IU/L, 0.34 mg/dL and 2.21 µg/dL, respectively for control group. They were 143.8 mg/dL, 81.37 IU/L, 59.2 IU/L, 0.79 mg/dL and 9.39 µg/dL, respectively in fish exposed to 7.0 mg Zn/L.

Serum total protein and total lipids decreased significantly with increasing Zn concentration; meanwhile they increased significantly by increasing the exposure period ($P < 0.05$; Table 6).

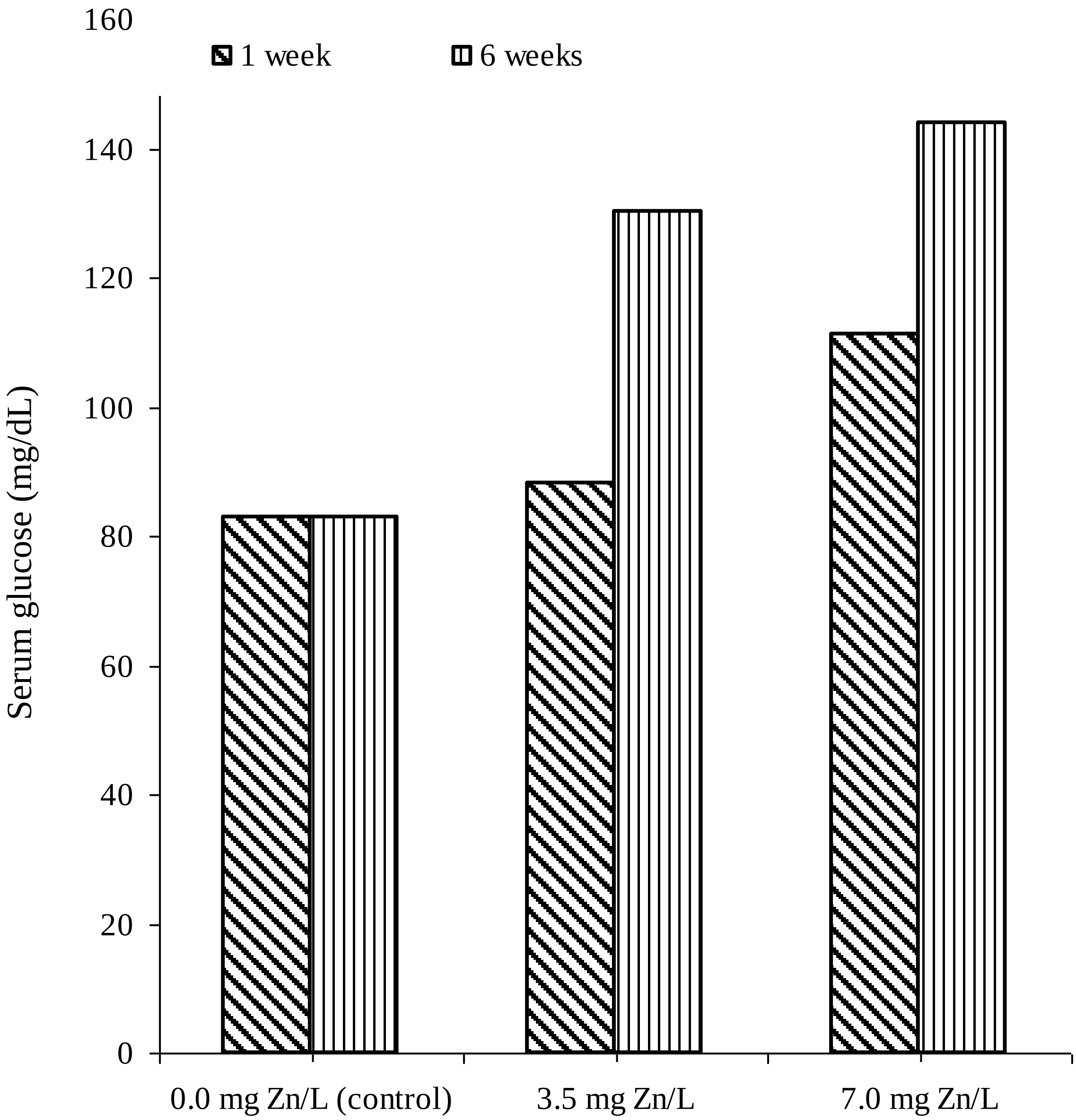

Figure 3. Serum glucose (mg/dL) of Nile tilapia exposed to different Zn concentrations for 1 or 6 weeks in glass aquaria.

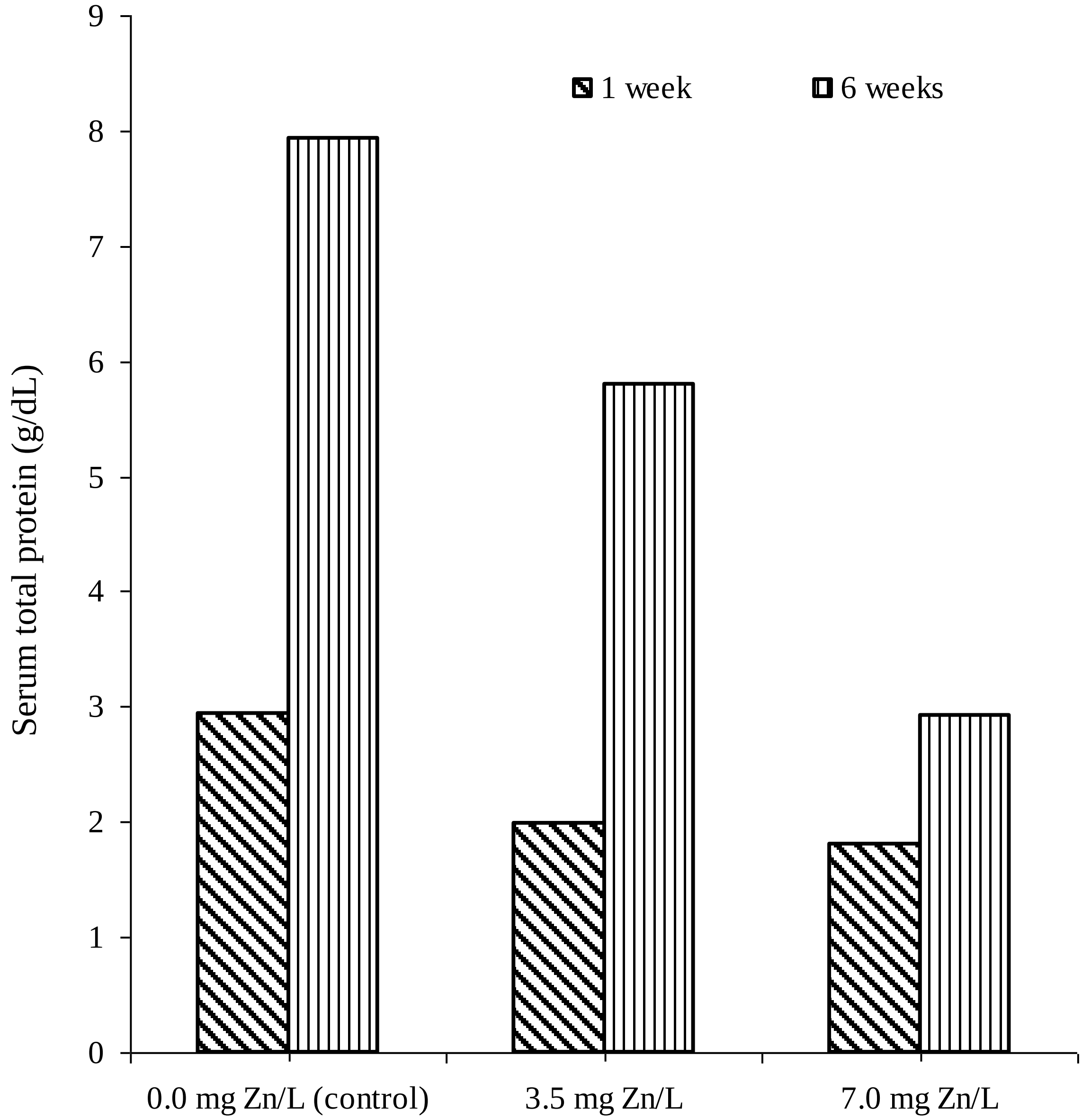

Figure 4. Serum total protein (g/dL) of Nile tilapia exposed to different Zn concentrations for 1 or 6 weeks in glass aquaria.

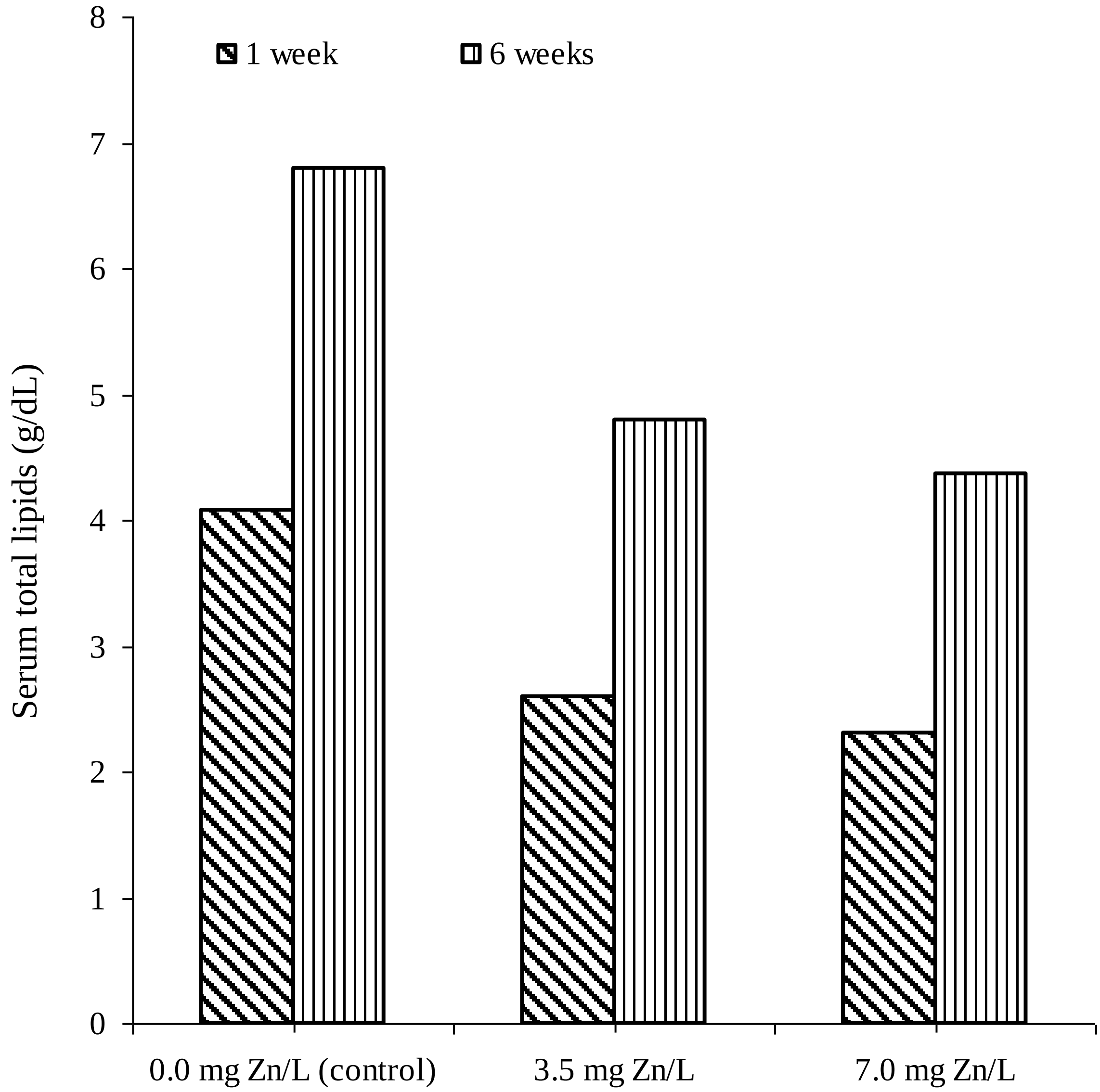

Figure 5. Serum total lipids (g/dL) of Nile tilapia exposed to different Zn concentrations for 1 or 6 weeks in glass aquaria.

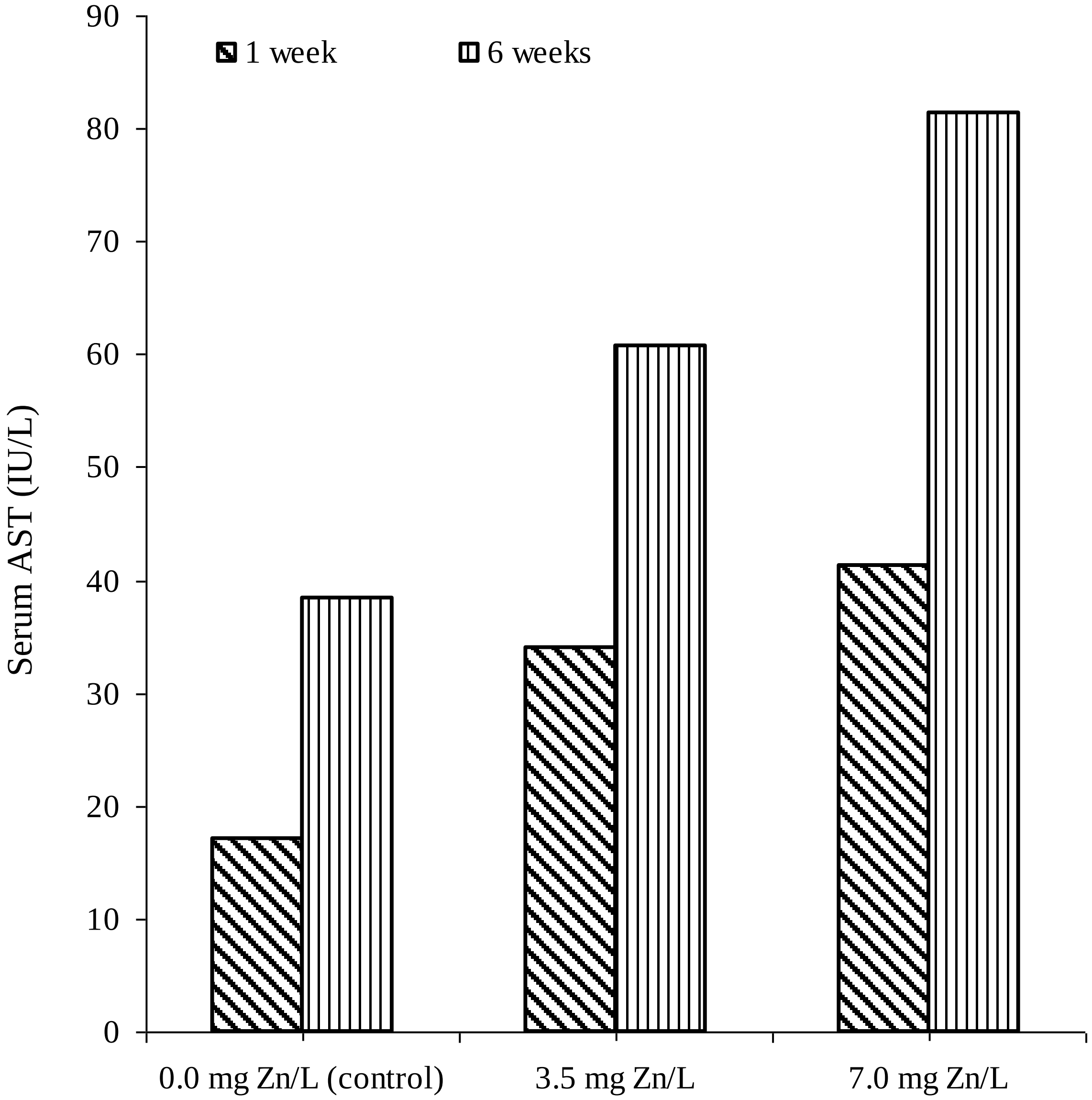

Figure 6. Serum AST (IU/L) of Nile tilapia exposed to different Zn concentrations for 1 or 6 weeks in glass aquaria.

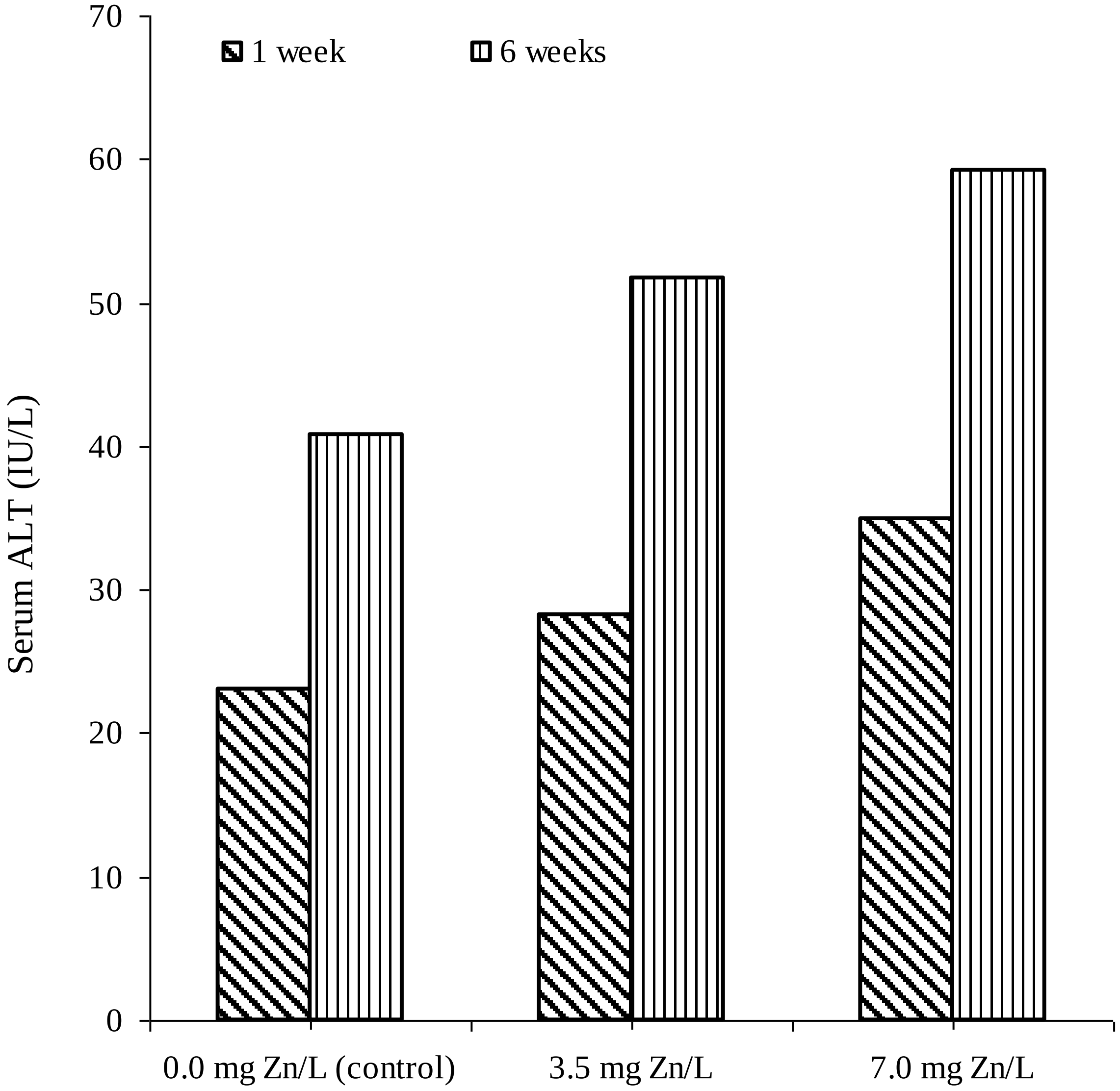

Figure 7. Serum ALT (IU/L) of Nile tilapia exposed to different Zn concentrations for 1 or 6 weeks in glass aquaria.

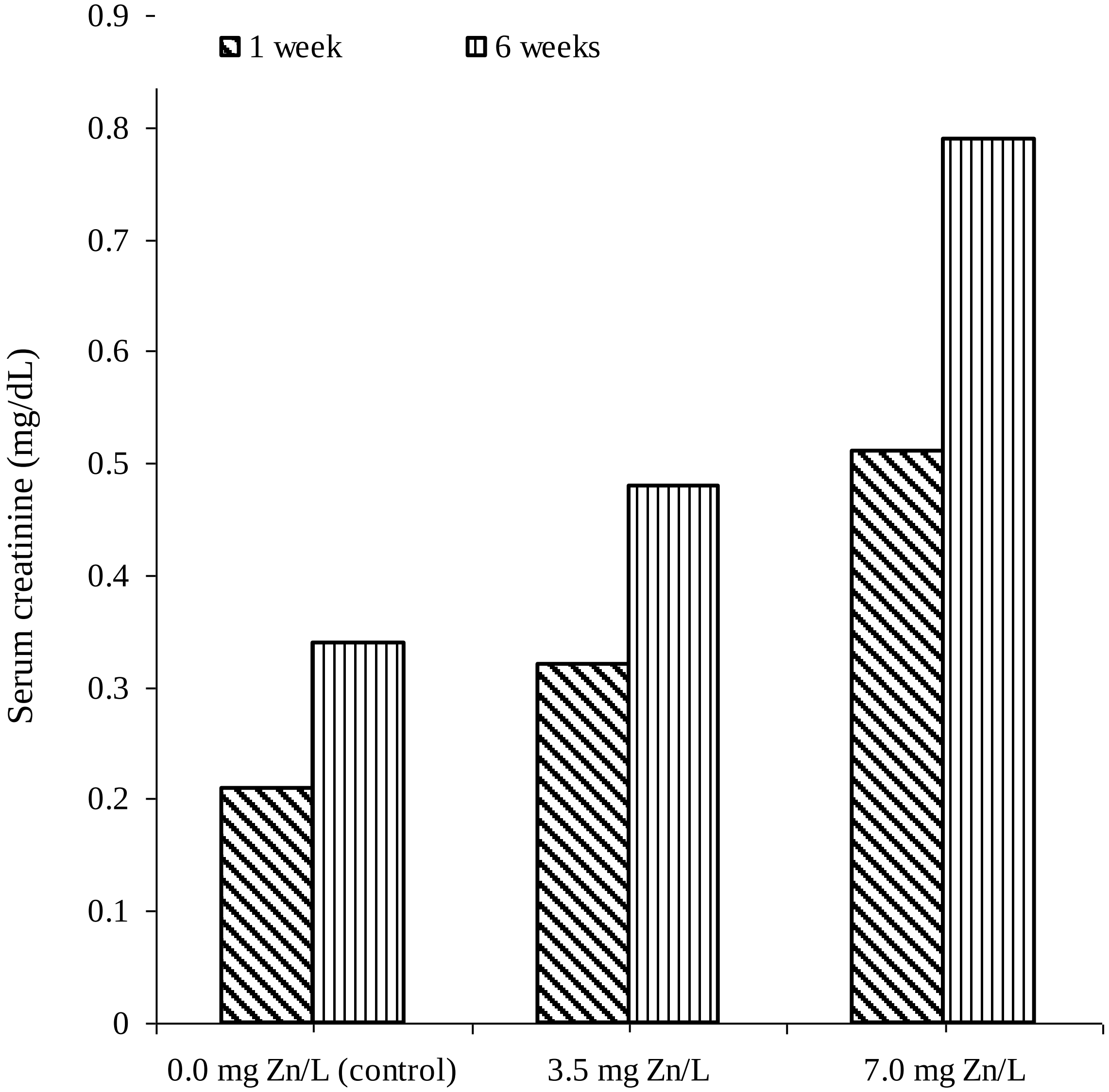

Figure 8. Serum creatinine (mg/dL) of Nile tilapia exposed to different Zn concentrations for 1 or 6 weeks in glass aquaria.

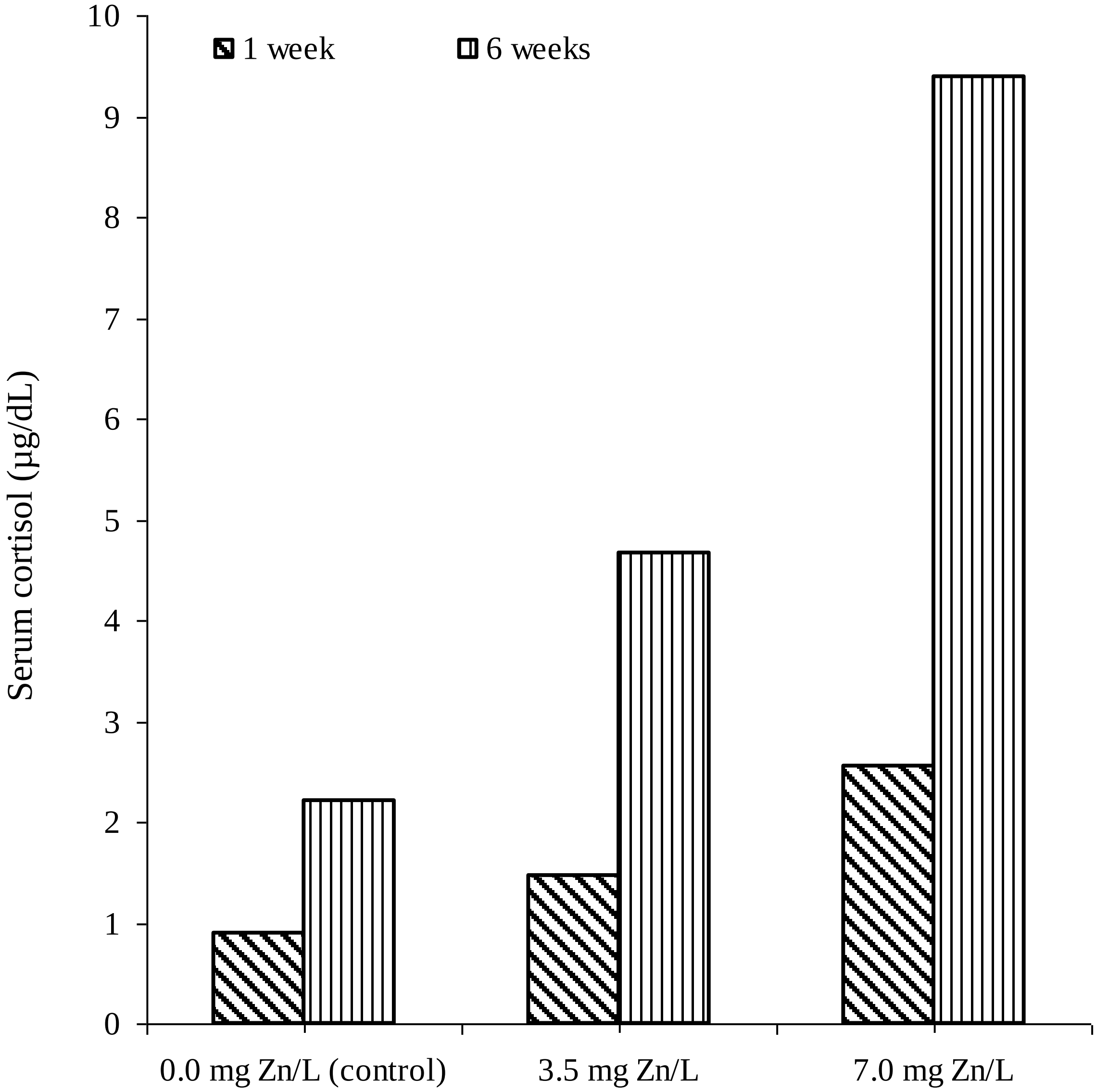

Figure 9. Serum cortisol (µg/dL) of Nile tilapia exposed to different Zn concentrations for 1 or 6 weeks in glass aquaria.

The highest values of serum protein and lipids of (7.93 and 6.85 g/dL, respectively) were obtained in control fish reared for 6 weeks, whereas the lowest values were observed in fish exposed to 7.0 mg Zn/L for 1 week (1.80 and 2.31 g/dL, respectively. Further, total protein and total lipids values after one week were 2.94 g/dL and 4.07 g/dL, respectively for control group and 1.80 g/dL and 2.31 g/dL, respectively in fish exposed to 7.0 mg Zn/L. In control group, they were 7.93 g/dL and 6.85 g/dL, respectively, while they were 2.93 g/dL and 4.37 g/dL, respectively in fish exposed to 7.0 mg Zn/L for 6 weeks.

I.2.4. Proximate analysis of whole-fish body:

All fish body constituents after 6 weeks were significantly affected by Zn concentration ($P < 0.05$; Table 7 and Figure 10). Moisture, crude protein, total lipids, and total ash values in the initial fish samples were 71.3, 66.9, 10.3 and 21.3 %; dry matter basis, respectively. No significant change was observed in moisture content due to Zn toxicity and its range was 71.9 – 73.8%. Crude protein and total lipid contents decreased significantly by increasing Zn toxicity ($P < 0.05$; Table 7). However, the highest protein and lipid contents were observed in the control fish (65.6 and 14.8%, respectively), while the lowest values were observed in fish exposed to 7.0 mg Zn/L (62.7 and 12.4%, respectively).

Table 7. Proximate chemical composition (%; dry matter basis) of whole body of Nile tilapia chronically exposed to water-born zinc for 6 weeks.

Treatments	Moisture	Crude protein	Total lipids	Total ash
0.0 mg Zn/L	71.9±1.81	65.6±1.78 a	14.8±0.51 a	18.4±0.47 c
3.5 mg Zn/L	72.3±1.49	63.8±1.35 b	12.8±0.23 b	21.6±0.56 b
7.0 mg Zn/L	73.8±1.21	62.7±1.01 b	12.4±0.91 b	24.3±0.83 a

Means having the same letter in the same column are not significantly different at $P < 0.05$.

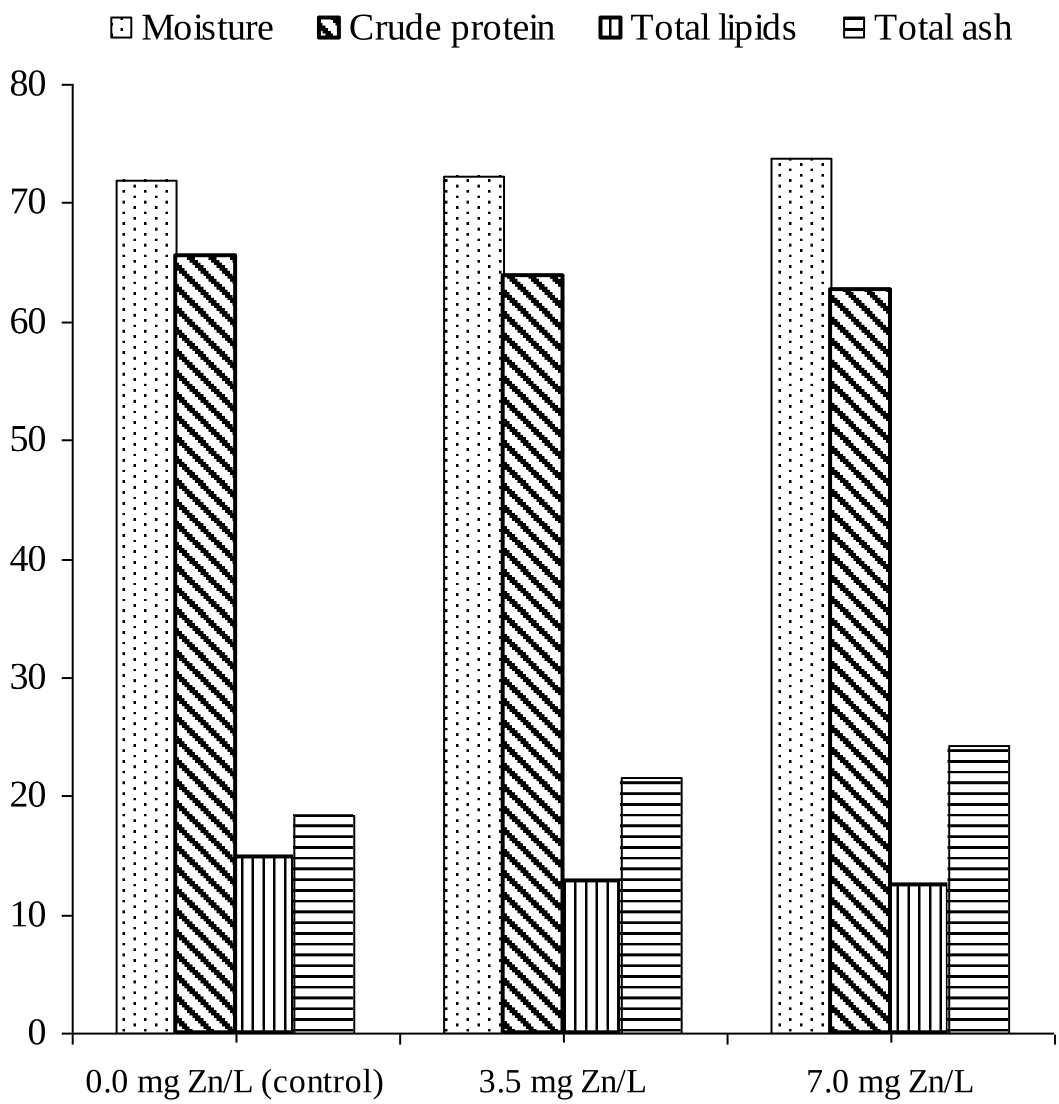

Figure 10. Proximate chemical composition (%; dry matter basis) of whole body of Nile tilapia exposed to different Zn concentrations for 6 weeks in glass aquaria.

No significant changes were observed in protein and lipid contents in fish exposed to 3.5 or 7.0 mg Zn/L (Table 7 and Figure 10). On the other hand, total ash content increased significantly by increasing Zn concentration ($P < 0.05$). The lowest (18.4%) and the highest (24.3%) ash content were observed in control fish and fish exposed to 7.0 mg Zn/L, respectively.

I.2.5. Zinc residue:

The changes in Zn residue in different organs and whole-fish body are significantly affected by Zn concentration, exposure period, and their interaction ($P < 0.05$; Table 8 and Figures 11-15). However, Zn residue increased significantly by increasing Zn concentration and exposure period. The Zn residues in gills, liver, kidney, muscles, and whole body for the initial sample were 22.6, 23.8, 24.2, 9.1, and 91.5 µg/g fresh weight, respectively. The Zn residue in the control fish reared for 1 week showed the lowest values and it was 22.8, 24.9, 24.6, 9.5, and 96.8 µg/g fresh weight for gills, liver, kidney, muscles, and whole-fish body, respectively. The highest Zn residues were observed in fish exposed to 7.0 mg Zn/L for 6 weeks where Zn residues in gills, liver, kidney, muscles, and whole body were 83.2, 109.5, 95.5, 20.2, and 346.7 µg/g fresh weight, respectively. Moreover, in fish exposed to 7.0 mg Zn/L for 1 week, Zn residue in gills, liver, kidney, muscles, and whole body was 59.3, 48.4, 40.9, 12.6, 234.5 µg/g fresh weight, respectively (Table 8 and Figures 11-15).

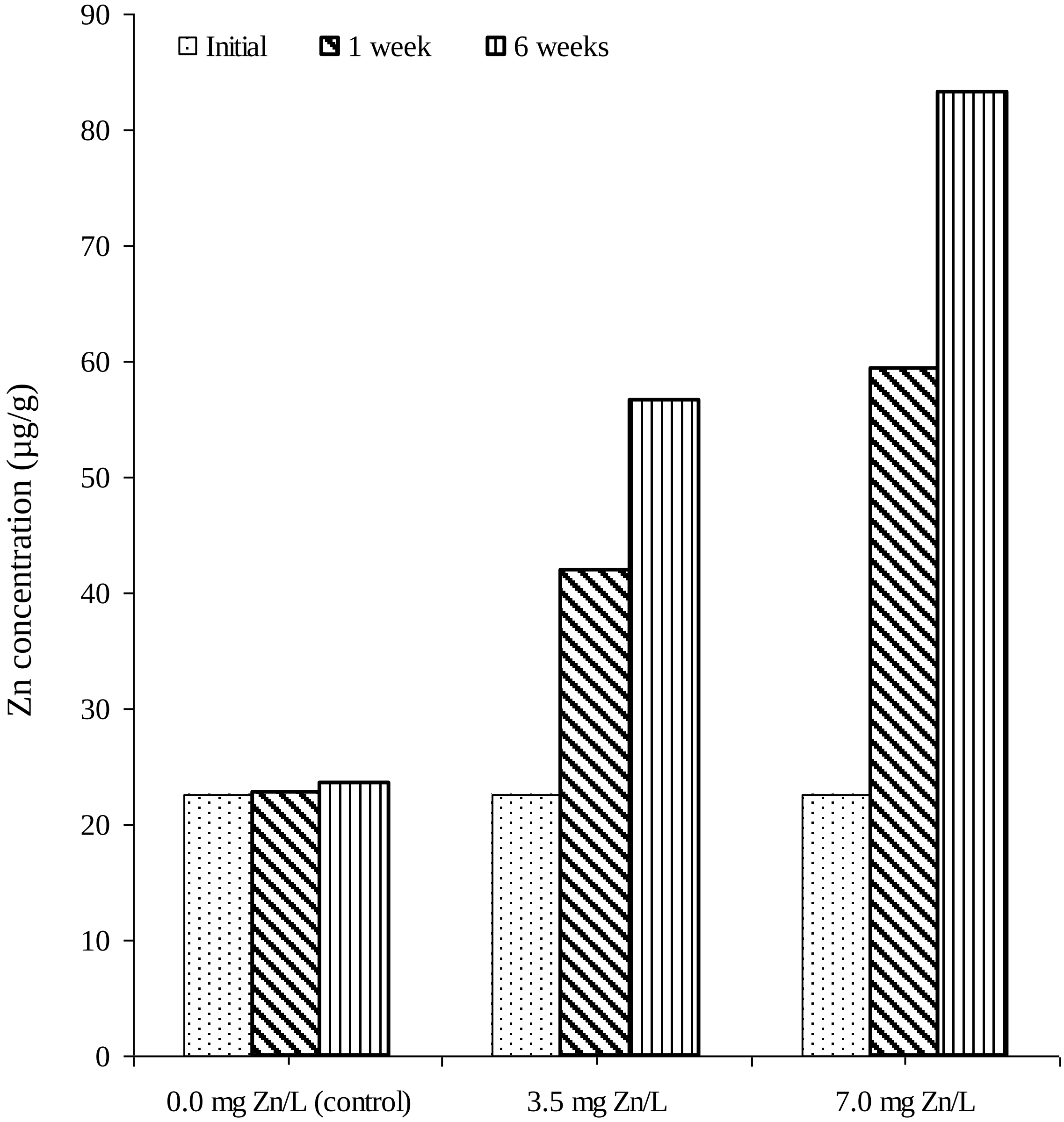

Figure 11. Zinc concentration in gills (µg/g fresh weight) of Nile tilapia exposed to different Zn concentrations for 1 or 6 weeks in glass aquaria.

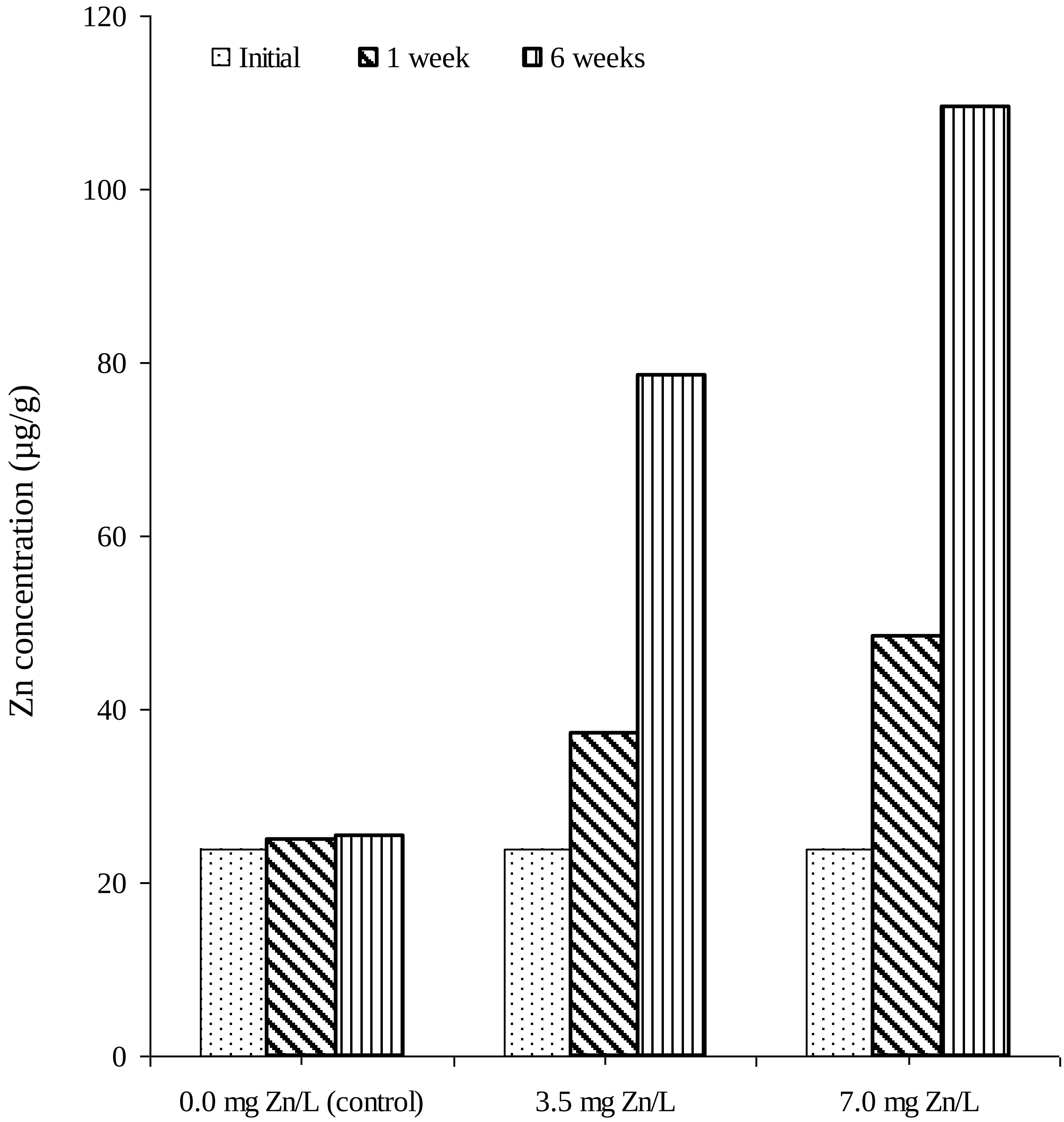

Figure 12. Zinc concentration in liver (µg/g fresh weight) of Nile tilapia exposed to different Zn concentrations for 1 or 6 weeks in glass aquaria.

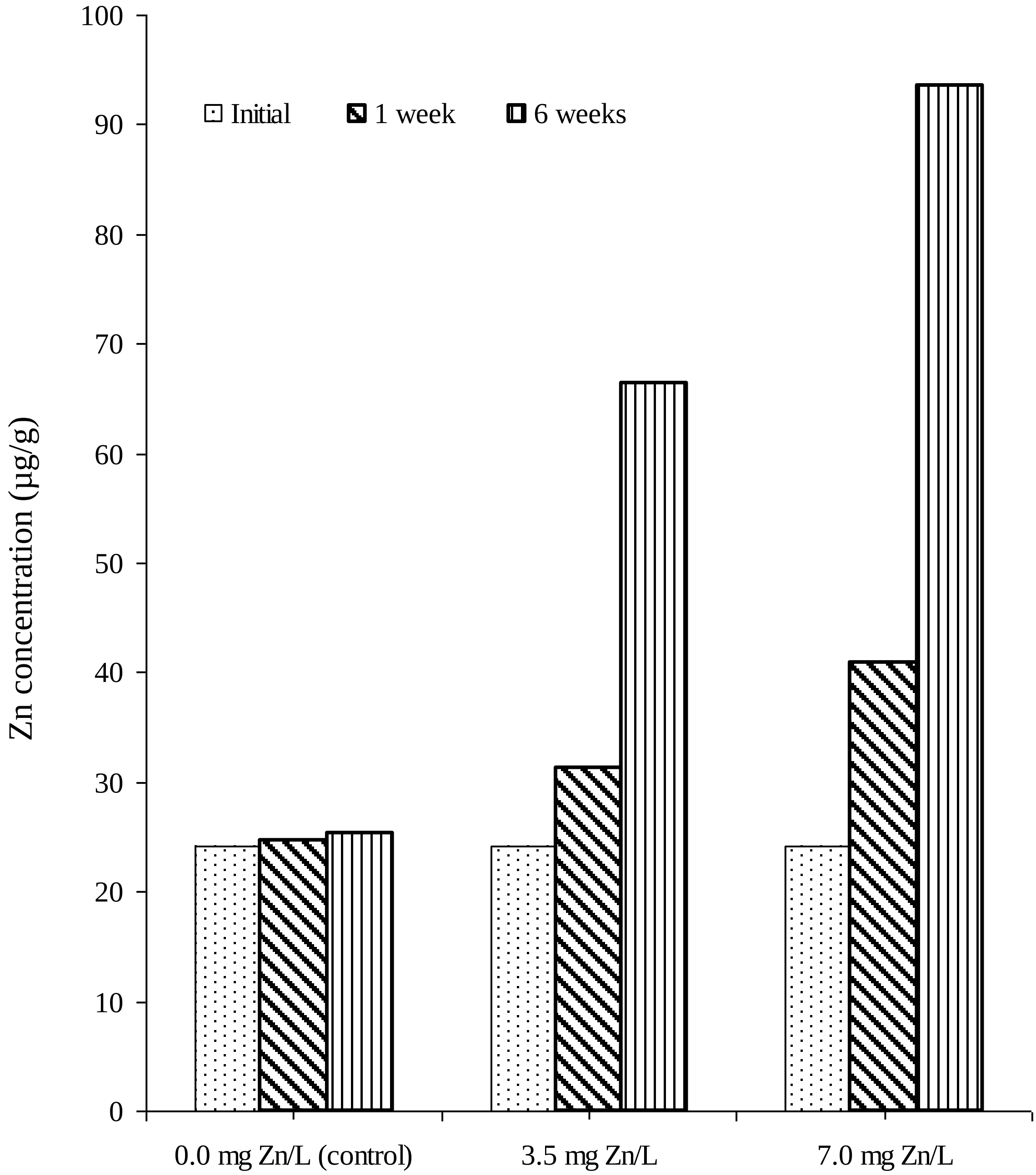

Figure 13. Zinc concentration in kidney (µg/g fresh weight) of Nile tilapia exposed to different Zn concentrations for 1 or 6 weeks in glass aquaria.

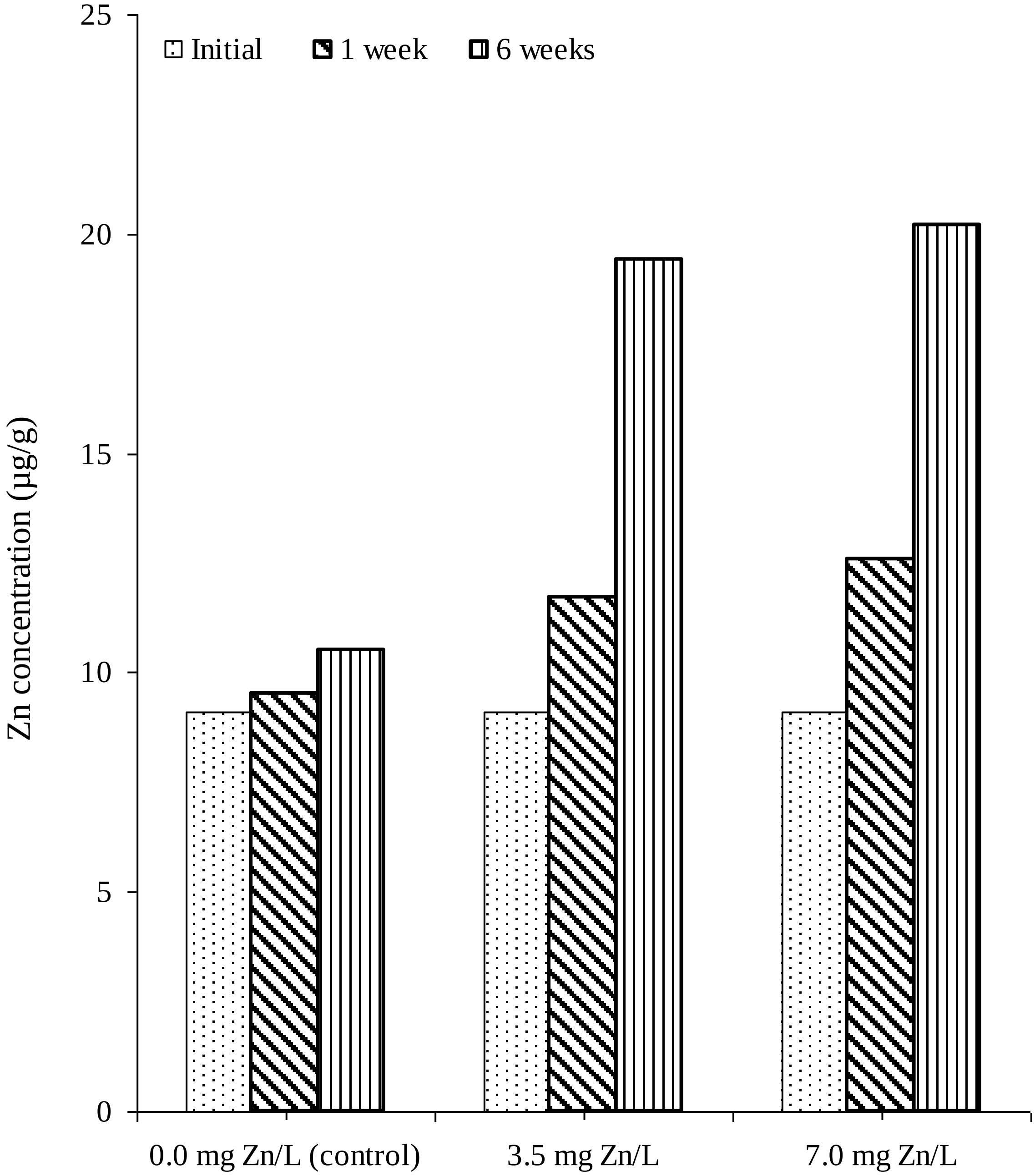

Figure 14. Zinc concentration in muscles (µg/g fresh weight) of Nile tilapia exposed to different Zn concentrations for 1 or 6 weeks in glass aquaria.

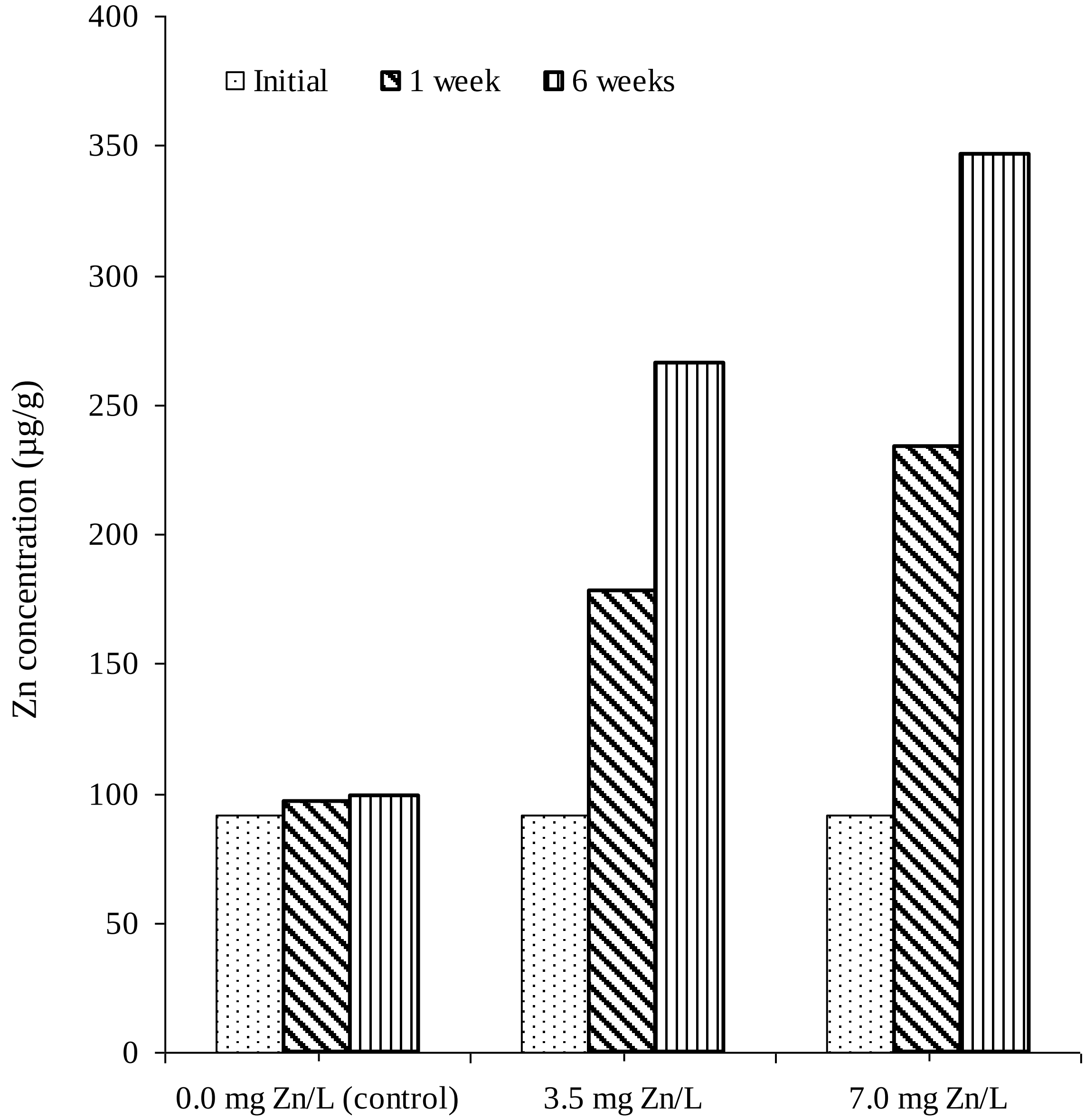

Figure 15. Zinc concentration in whole body (µg/g fresh weight) of Nile tilapia exposed to different Zn concentrations for 1 or 6 weeks in glass aquaria.

It is noticed that the Zn concentrations in all tissues after 6 weeks were higher than those found after 1 week of Zn-exposure. The Zn concentrations after 1 week was found to follow the order of gills > liver > kidney > muscle, meanwhile after 6 weeks it followed the order of liver > kidney > gill > muscle. Moreover, the present findings revealed that liver and kidney are the prime sites of Zn accumulation and Zn load in the muscle was for low as compared to other organs (Table 8 and Figures 11-15).

I.3. Effect of dietary protein level and Zn concentration on the different investigated parameters:

In this experiment, fish were fed on two different diets, which differ in dietary protein level (25% and 45%CP) and exposed to 0.0, 3.5, and 7.0 mg Zn/L for 6 weeks. Fish performance, survival, biochemical variables, and Zn residue in whole-fish body were evaluated.

I.3.1. Water chemistry:

In the present study, no significant changes were observed in water temperature, dissolved O_2, pH value, water EC, total alkalinity, and total hardness due to dietary protein level, Zn concentration, and their interaction ($P > 0.05$; Table 9). However, during the experimental period, water temperature range was 24.2 – 24.6 ^oC, dissolved O_2 orange was 5.2 - 5.7 mg/L, pH range was 7.4 – 7.6, water EC range was 417.5 – 139.0 µS/cm, total alkalinity range was 183.3 - 201.7 mg/L as $CaCO_3$, and total hardness range was 224.7 - 240.0 mg/L as $CaCO_3$.

The unionized ammonia was significantly affected by Zn concentration and dietary protein level (Table 9; Figure 16); however, its level increased by increasing dietary protein level and/or Zn concentration. The highest ammonia level (1.73 mg/L) was detected when fish exposed to 7.0 mg Zn/L and fed on 45%-CP diet; meanwhile the lowest value (0.63 mg/L) was obtained when control fish fed 25%-CP diet. The NH_3 value was 0.63 and 0.81 mg/L for 0.0 and 7.0 mg Zn/L at 25%-CP diet, respectively and it was 0.94 and 1.73 mg/L for 0.0 and 7.0 mg Zn/L at 45%-CP diet, respectively (Table 9 and Figure 16).

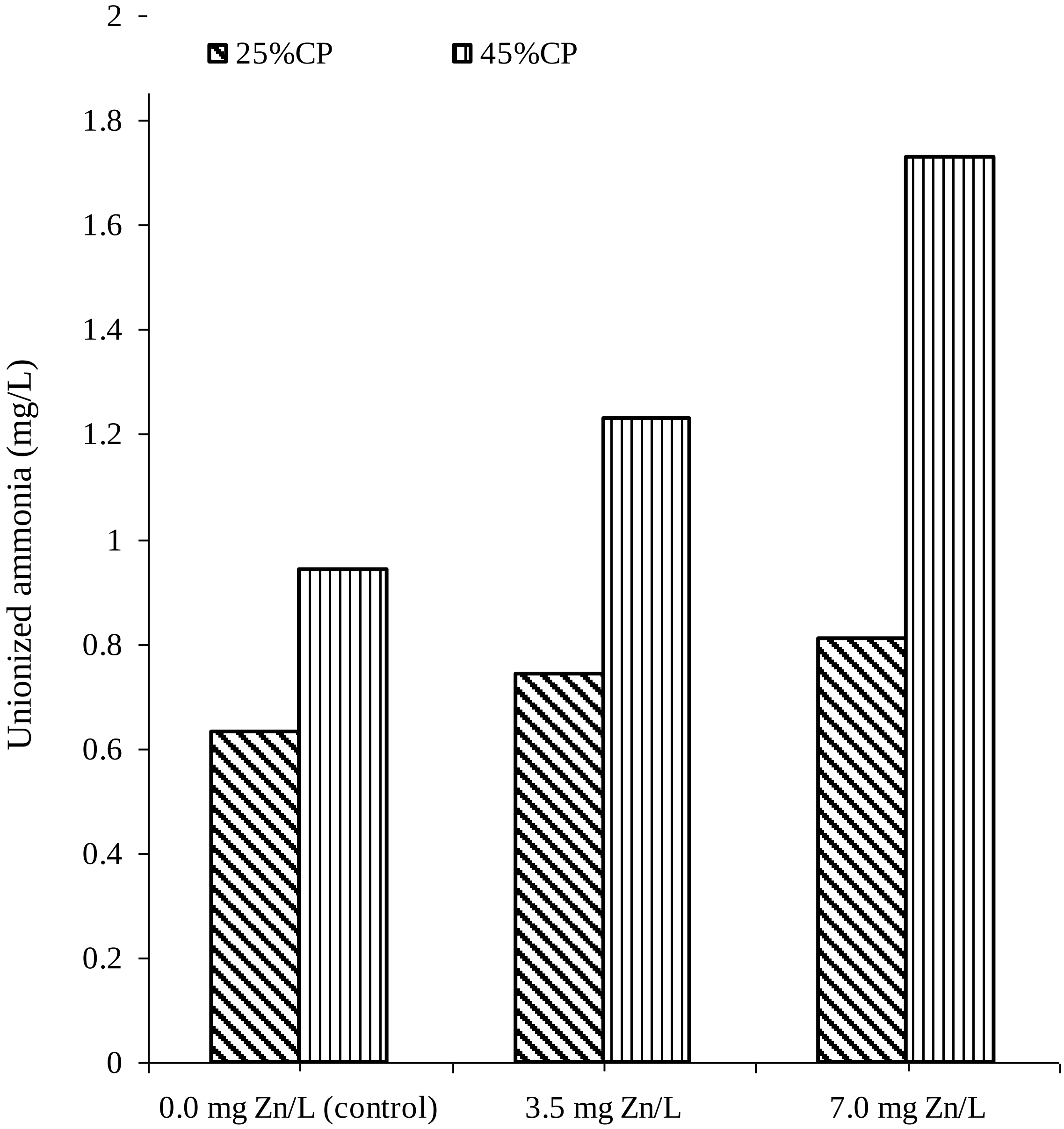

Figure 16. Changes in the unionized ammonia (mg/L) in aquaria's water stocked by Nile tilapia fed different protein levels and exposed to different Zn concentrations for 6 weeks.

I.3.2. Fish performance:

Fish growth decreased significantly by increasing Zn concentration and decreasing dietary protein level (P < 0.05; Table 10 and Figure 17), but their interaction was insignificant (P > 0.05). The growth of fish fed 25% CP diet and exposed to 7.0 mg Zn/L (10.2 g) was the lower as compared to that fed 45% CP diet with no Zn exposure (15.1 g). The final weight, weight gain, and SGR were 13.4 g, 6.0 g and 1.32 % g/day, respectively for 0.0 mg Zn/L-25% CP and they were 10.2 g, 2.7 g and 0.70 % g/day, respectively for 7.0 mg Zn/L-25% CP. In addition, the final weight, weight gain, and SGR were 15.1 g, 7.5 g and 1.64 % g/day, respectively for 0.0 mg Zn/L-45% CP and were 11.8 g, 4.1 g and 1.02 % g/day, respectively for 7.0 mg Zn/L-45% CP.

Feed intake and FCR were significantly affected by Zn concentration and protein level (P < 0.05; Table 10). Feed intake increased, while FCR decreased significantly with reducing Zn concentration, meanwhile feed utilization was improved by increasing the dietary protein level (P < 0.05; Table 10). It is noticed that fish fed 45%-CP diet consumed more feed than that fed 25%-CP diet and FCR was higher in fish fed 25%-CP diet than that fed 45%-CP diet. The best feed intake and FCR were obtained when fish fed 45%-CP diet with no Zn exposure (12.2 g feed/fish and 1.63, respectively). For fish fed 25%-CP diet, feed intake was 10.5 and 7.8 g feed/fish for 0.0 and 7.0 mg Zn/L, respectively, while for fish fed 45%-CP diet, it was 12.2 and 7.9 for 0.0 and 7.0 mg Zn/L, respectively.

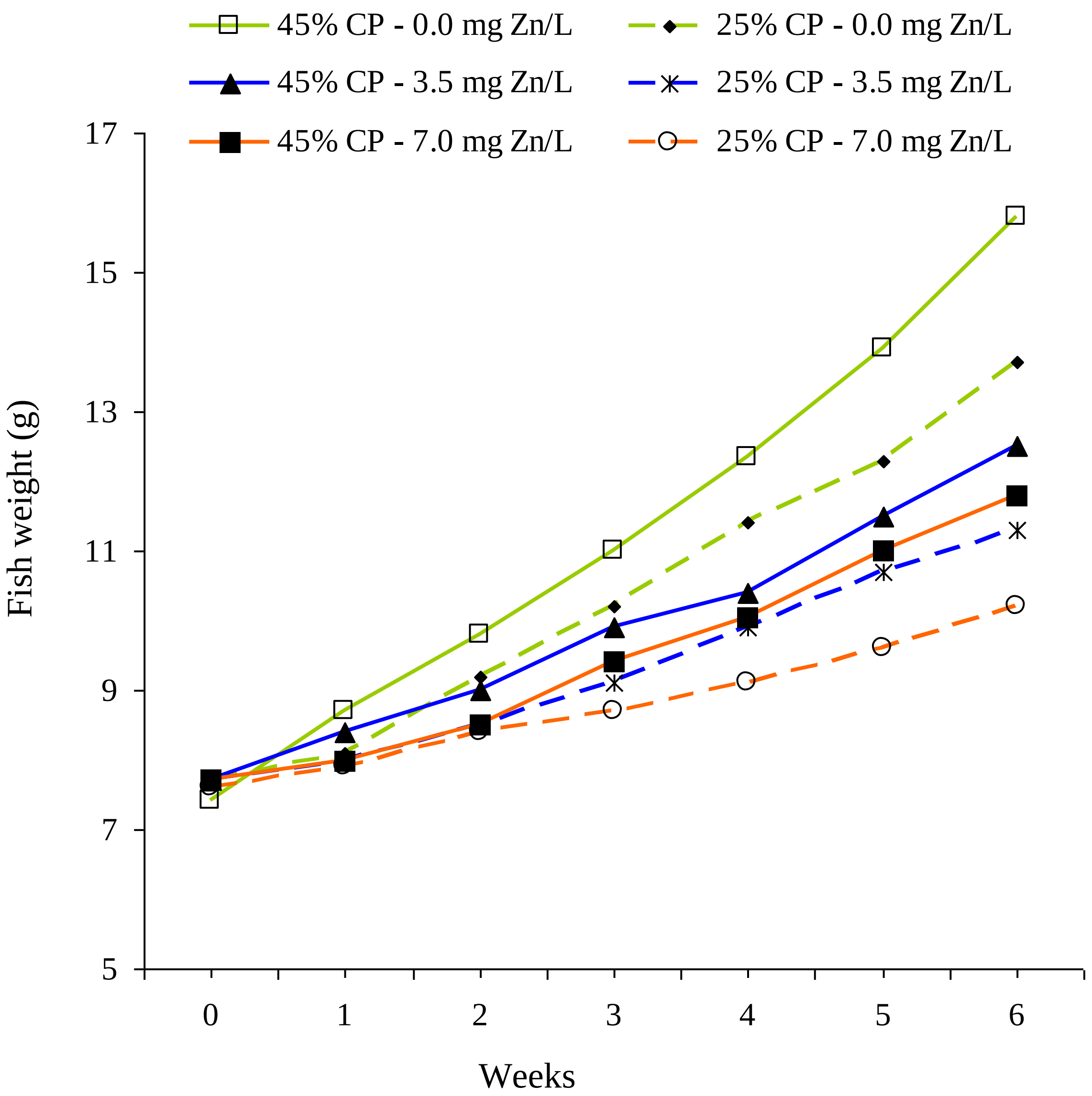

Figure 17. Changes in the weight (g) of Nile tilapia fed diet with different protein levels and exposed to different Zn concentrations for 6 weeks.

FCR, for fish fed 25%-CP diet, was 1.8 and 2.9 for 0.0 and 7.0 mg Zn/L, respectively, while it was 1.6 and 1.9 for 0.0 and 7.0 mg Zn/L, respectively for fish fed 45%-CP diet.

I.2.3. Biochemical parameters:

All biochemical parameters were significantly effected by Zn concentration and dietary protein level ($P < 0.05$; Table 11 and Figures 18-24). However, serum glucose, AST, ALT, and creatinine increased significantly by increasing dietary protein level and/or Zn concentration. The highest values of serum glucose, AST, ALT, and creatinine were obtained in fish fed 45%-CP diet and exposed to 7.0 mg Zn/L (126.0 mg/dL, 81.0 IU/L, 24.7 IU/L, and 0.51 mg/dL, respectively). Serum glucose, AST, ALT, creatinine, and cortisol were increased with increasing Zn concentration. The values of glucose, AST, ALT, creatinine and cortisol in control fish fed 25%-CP diet were 65.7 mg/dL, 54.7 IU/L, 14.0 IU/L, 0.19 mg/dL, and 2.1 µg/dL, respectively. Meanwhile, their values were 106.3 mg/dL, 75.7 IU/L, 21.0 IU/L, 0.31 mg/dL and 9.8 µg/dL, respectively for fish fed 25%-CP diet and exposed to 7.0 mg Zn/L. For control fish fed 45%-CP diet, these values were 64.3 mg/dL, 67.7 IU/L, 14.7 IU/L, 0.22 mg/dL and 3.1 µg/dL, respectively. Meanwhile, they were 126.0 mg/dL, 81.0 IU/L, 24.7 IU/L, 0.51 mg/dL and 10.4 µg/dL, respectively in fish exposed to 7.0 mg Zn/L and fed 45% CP diet.

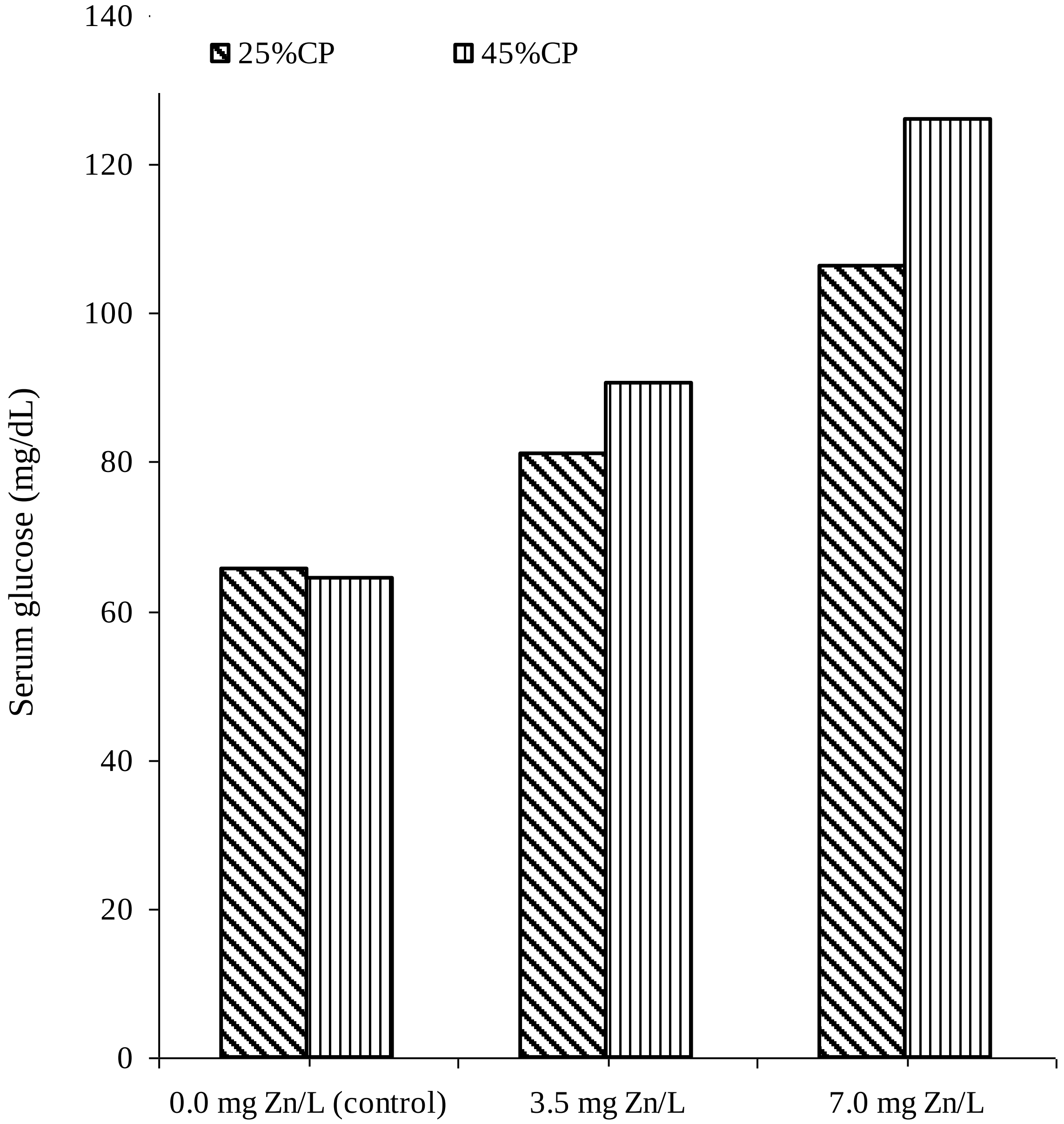

Figure 18. Serum glucose (mg/dL) of Nile tilapia fed diet with different protein levels and exposed to different Zn concentrations for 6 weeks.

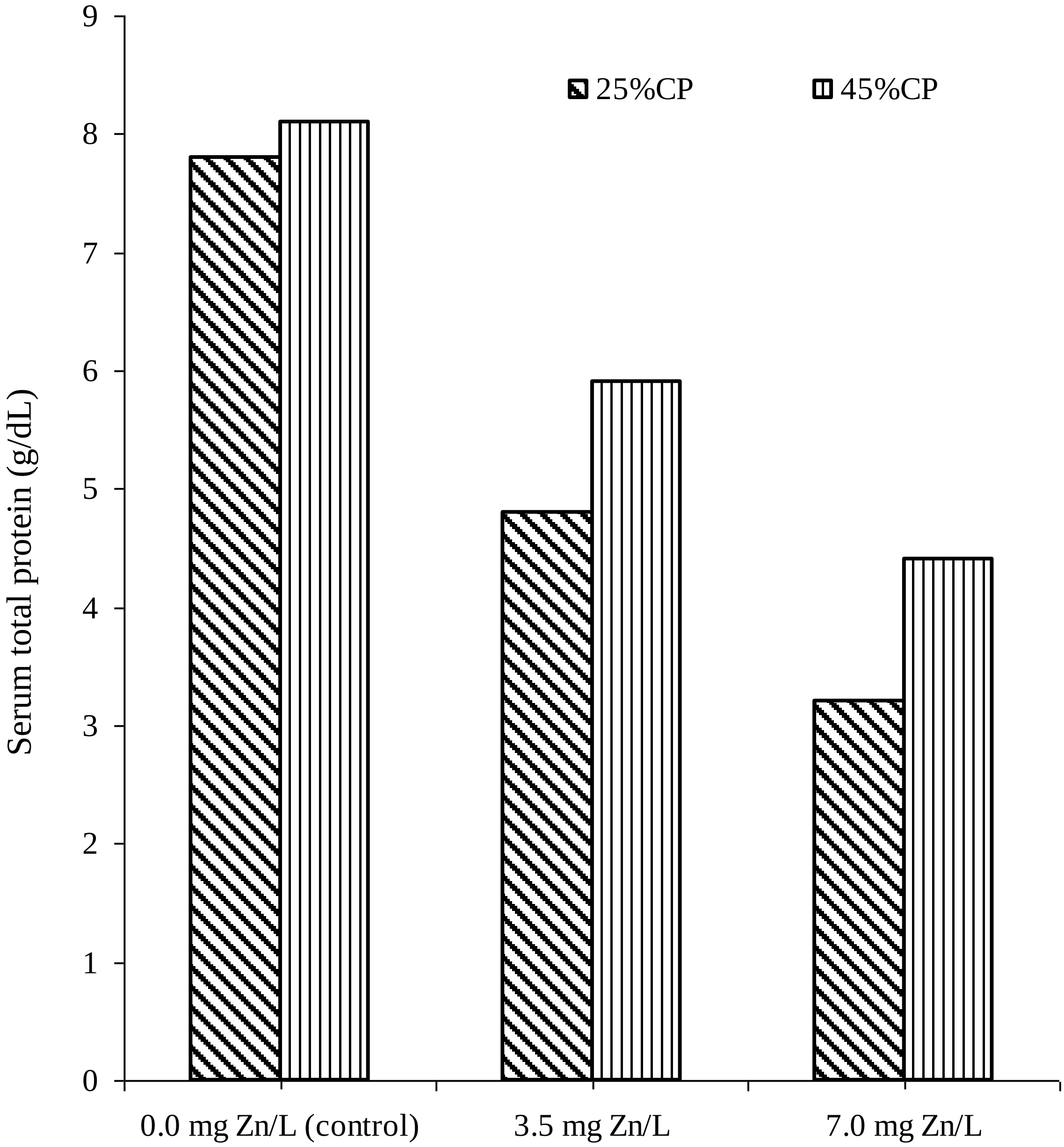

Figure 19. Serum total protein (g/dL) of Nile tilapia fed diet with different protein levels and exposed to different Zn concentrations for 6 weeks.

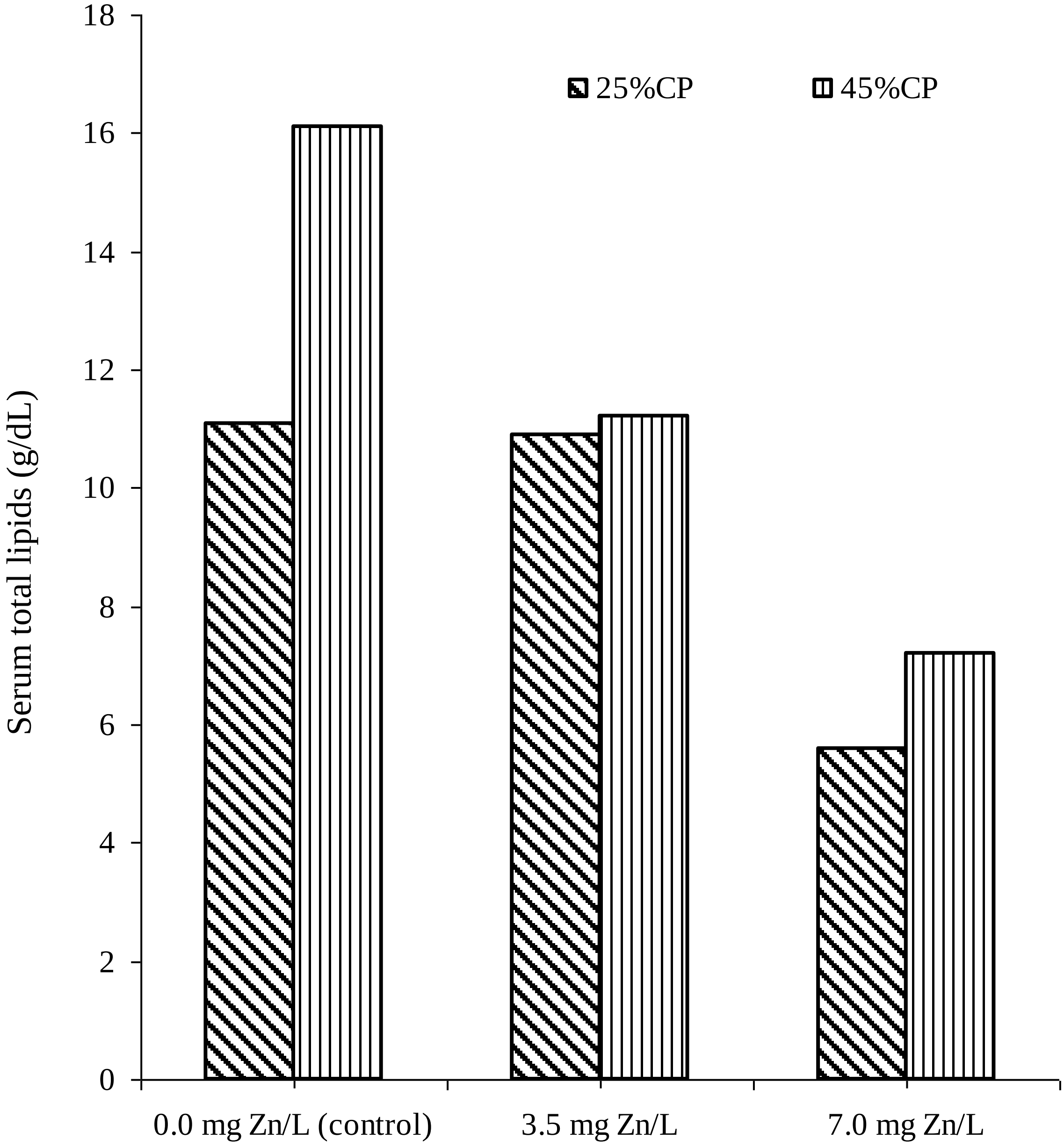

Figure 20. Serum total lipids (g/dL) of Nile tilapia fed diet with different protein levels and exposed to different Zn concentrations for 6 weeks.

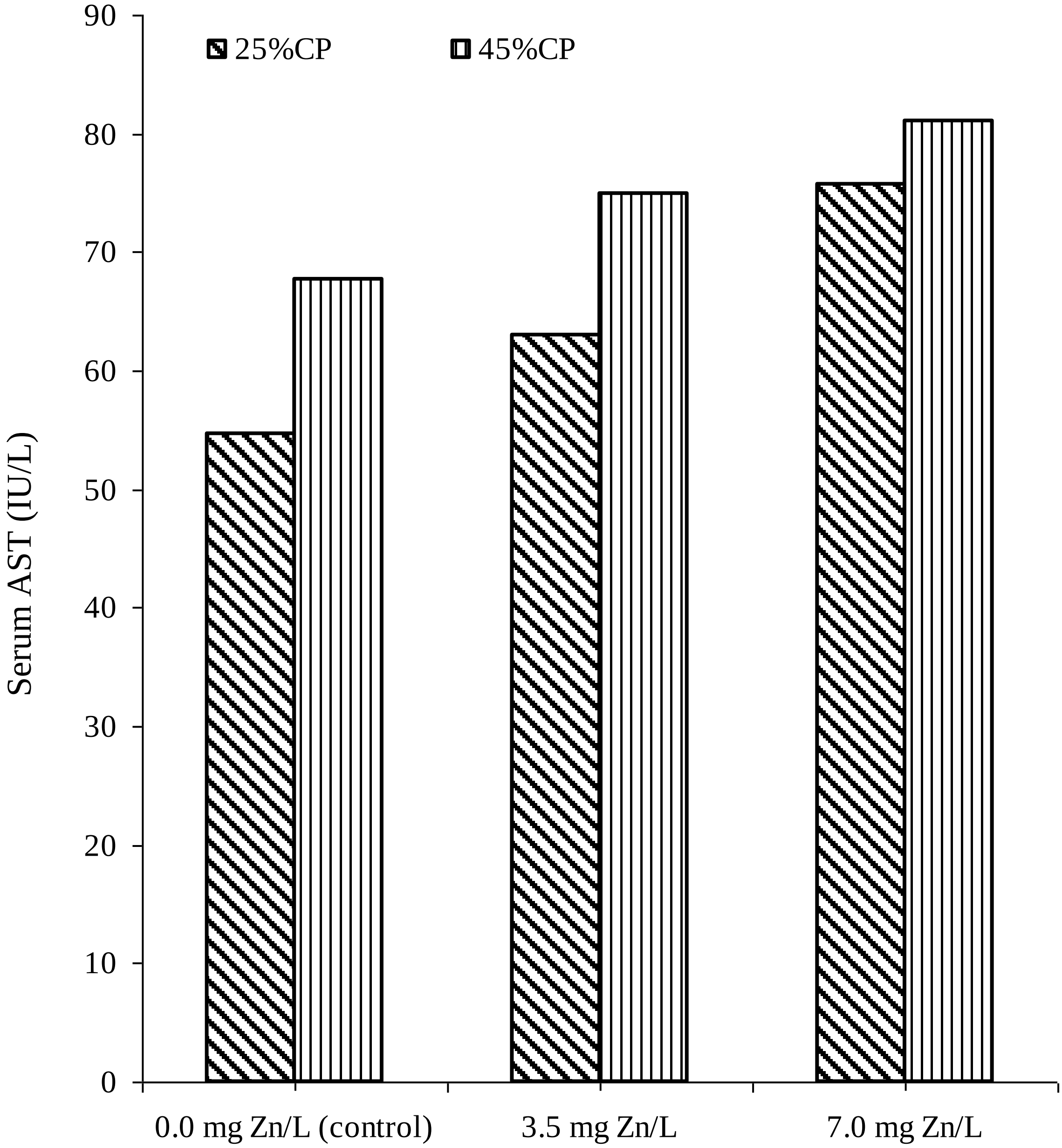

Figure 21. Serum AST (IU/L) of Nile tilapia fed diet with different protein levels and exposed to different Zn concentrations for 6 weeks.

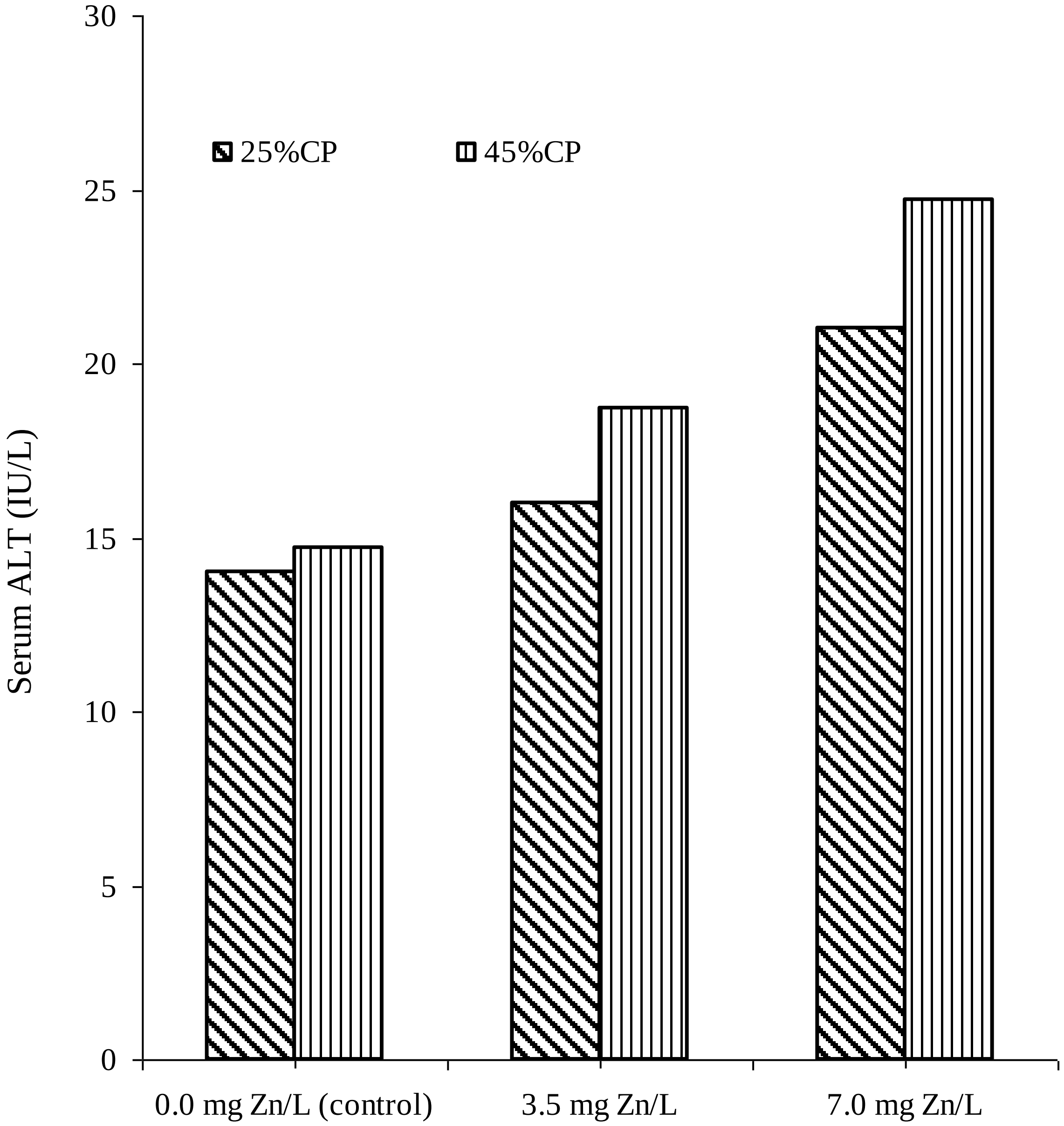

Figure 22. Serum ALT (IU/L) of Nile tilapia fed diet with different protein levels and exposed to different Zn concentrations for 6 weeks.

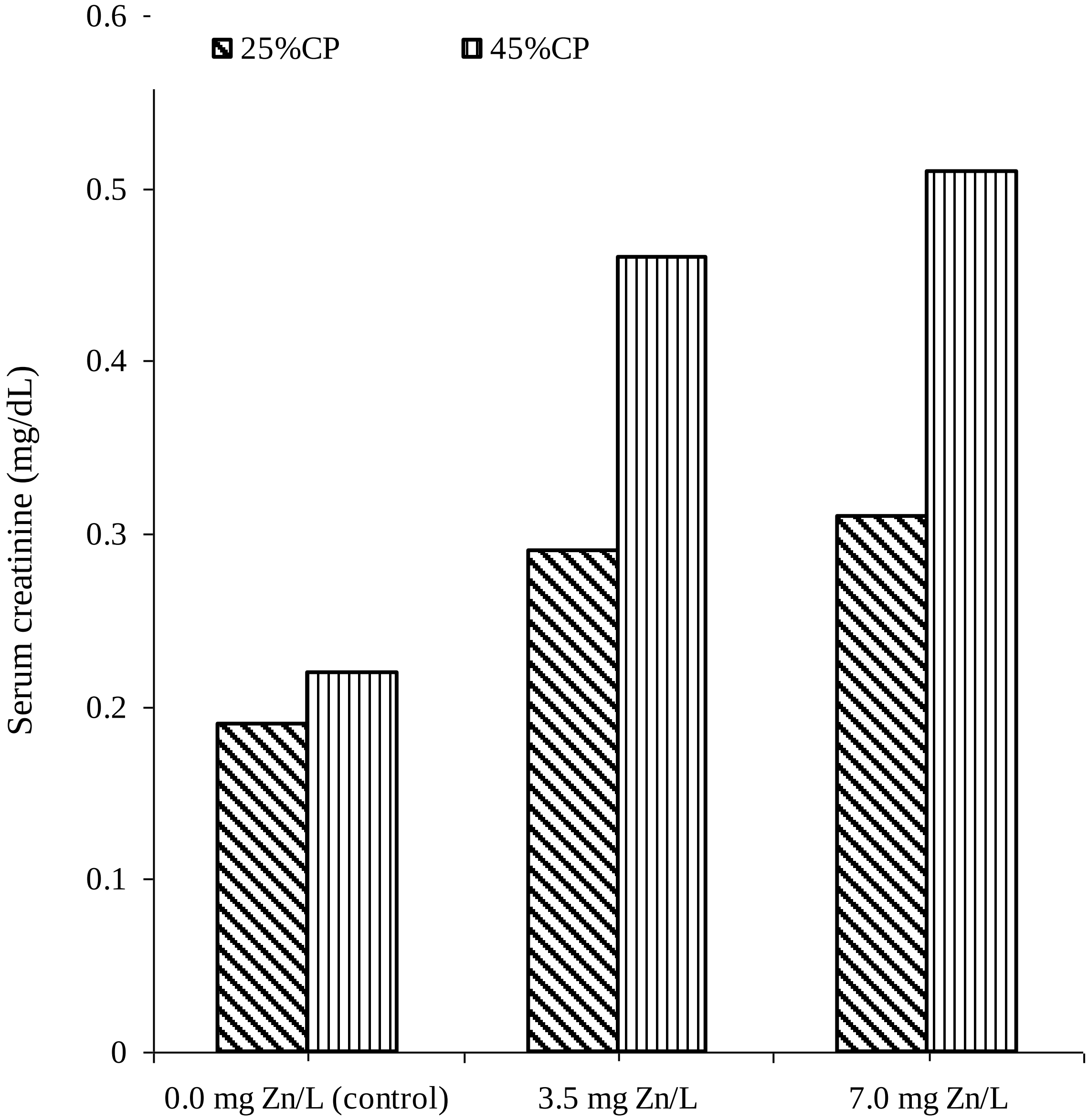

Figure 23. Serum creatinine (mg/dL) of Nile tilapia fed diet with different protein levels and exposed to different Zn concentrations for 6 weeks.

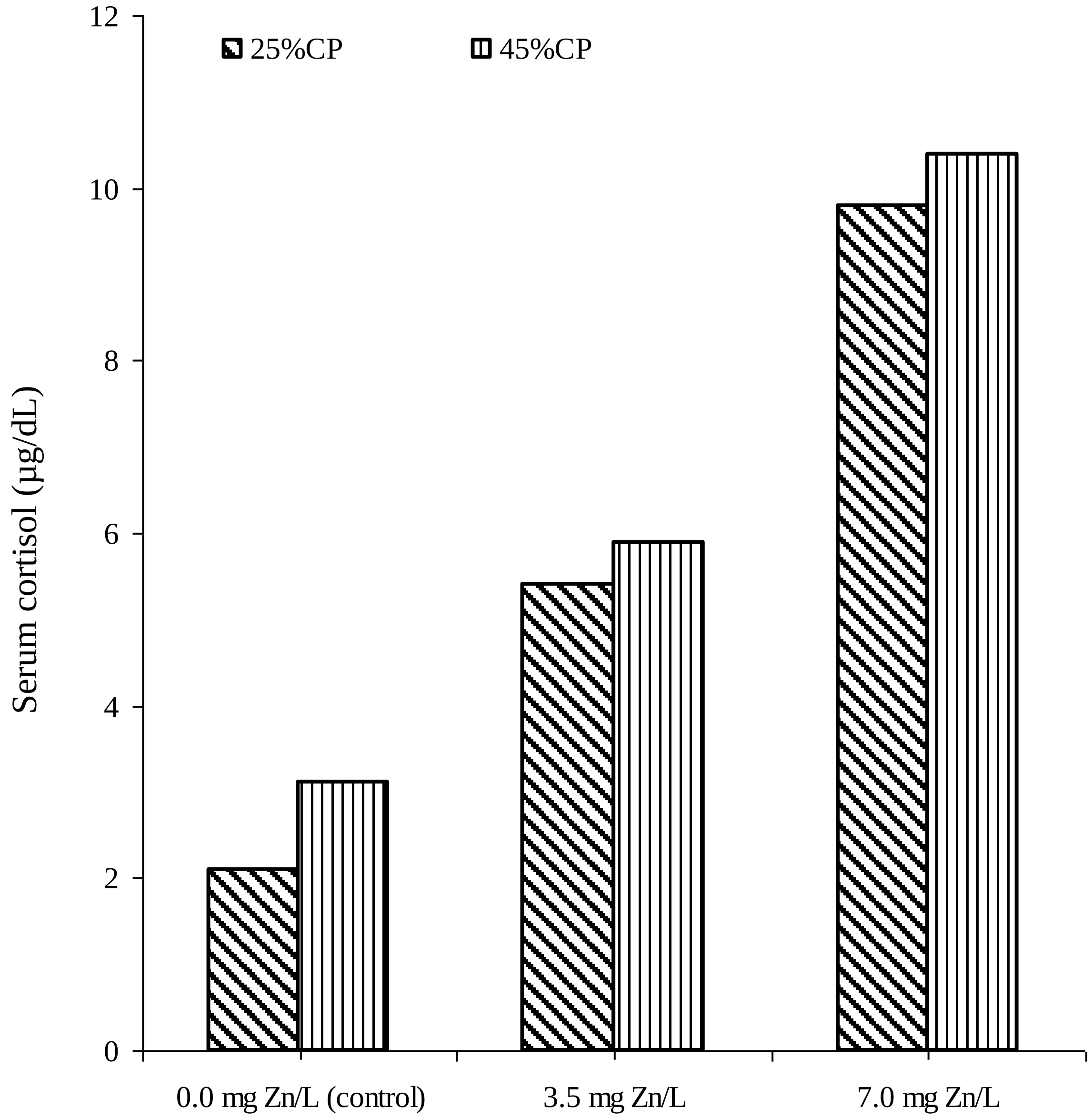

Figure 24. Serum cortisol (µg/dL) of Nile tilapia fed diet with different protein levels and exposed to different Zn concentrations for 6 weeks.

On the other hand, serum total protein and total lipids decreased significantly with increasing Zn concentration and decreasing dietary protein level ($P < 0.05$; Table 11 and Figures 18-24), but their interaction was insignificant ($P > 0.05$). Serum protein and lipids decreased due to Zn exposure and their lowest values (3.8 and 6.1 g/dL, respectively) were obtained when fish fed 25%-CP diet and exposed to 7.0 mg Zn/L. In addition, the increased dietary protein elevated serum protein and lipids and their highest values (8.1 and 16.1 g/dL, respectively) were obtained when fish fed 45%-CP diet with no Zn exposure. Moreover, dietary protein level had no effect on cortisol level, which increased significantly ($P < 0.05$) by increasing Zn concentration. It is noticed that fish fed 25 or 45%-CP diets and exposed to 7.0 mg Zn/L showed the highest cortisol values (9.8 or 10.4 µg/L, respectively; Table 11).

I.3.4. Proximate analysis of the whole-fish body:

All whole-fish body constituents were significantly affected by Zn concentration and protein level (except moisture and ash for protein level) ($P < 0.05$; Table 12 and Figure 25). Moisture, crude protein, total lipids, and total ash contents in the initial fish sample were 71.4, 69.8, 13.3 and 15.5 %, respectively. In addition, moisture content showed no significant response to both tested factors and its range was 68.8 – 70.3%.

Crude protein and total lipids contents increased significantly with reducing Zn concentration (Figure 25). In addition, crude protein increased, while total lipids decreased significantly by increasing dietary protein level. The lowest (64.3%) and highest (69.1%) crude protein contents were obtained in fish fed 25%-CP diet and exposed to 7.0 mg Zn/L and fish fed 45%-CP diet with no Zn exposure, respectively. Crude protein and total lipids decreased significantly with the increase of Zn concentration (Table 12 and Figure 25). Crude protein content was 66.6 and 64.3 % for 0.0 mg Zn/L and 7.0 mg Zn/L-25% CP treatments, respectively and it was 69.1 and 66.3 % for 0.0 and 7.0 mg Zn/L-45%-CP treatments, respectively.

Total lipid decreased with increasing protein level in the diet, where it was 18.6 and 16.2 % for 0.0 and 7.0 mg Zn/L-25%-CP treatments and it was 16.3 and 14.7 % for 0.0 and 7.0 mg Zn/L-45% CP treatments, respectively. In addition, the lowest (14.7%) and highest (18.6%) total lipids contents were obtained in fish fed 45%-CP diet and exposed to 7.0 mg Zn/L and fish fed 25%-CP diet with no Zn exposure, respectively.

Moreover, total ash content increased significantly with increasing Zn concentration only. The highest ash (18.9%) content was detected in fish fed 45-CP diet and exposed to 7.0 mg Zn/L, meanwhile total ash content in control fish fed 25%-CP diet was 14.2%.

I.3.5. Zinc residue:

The Zn residue values were significantly affected by water-born Zn concentration ($P < 0.05$; Table 12 and Figure 26), but the interaction of Zn dose and dietary protein level was insignificant ($P > 0.05$). The Zn residue in whole body increased significantly with increasing Zn concentration (Figure 26). The highest Zn content (0.737 mg/g dry weight) was detected in fish fed 45%-CP diet and exposed to 7.0 mg Zn/L. The value of Zn residue in whole-fish body for the initial samples was 0.30 mg/g dry weight. In fish fed 25%-CP diet, Zn values were 0.32, 0.56 and 0.74 mg/g dry weight for 0.0, 3.5 and 7.0 mg Zn/L treatments, respectively. But in fish fed 45%-CP diet, Zn values were 0.29, 0.55 and 0.65 mg/g dry weight for 0.0, 3.5 and 7.0 mg Zn/L treatments, respectively.

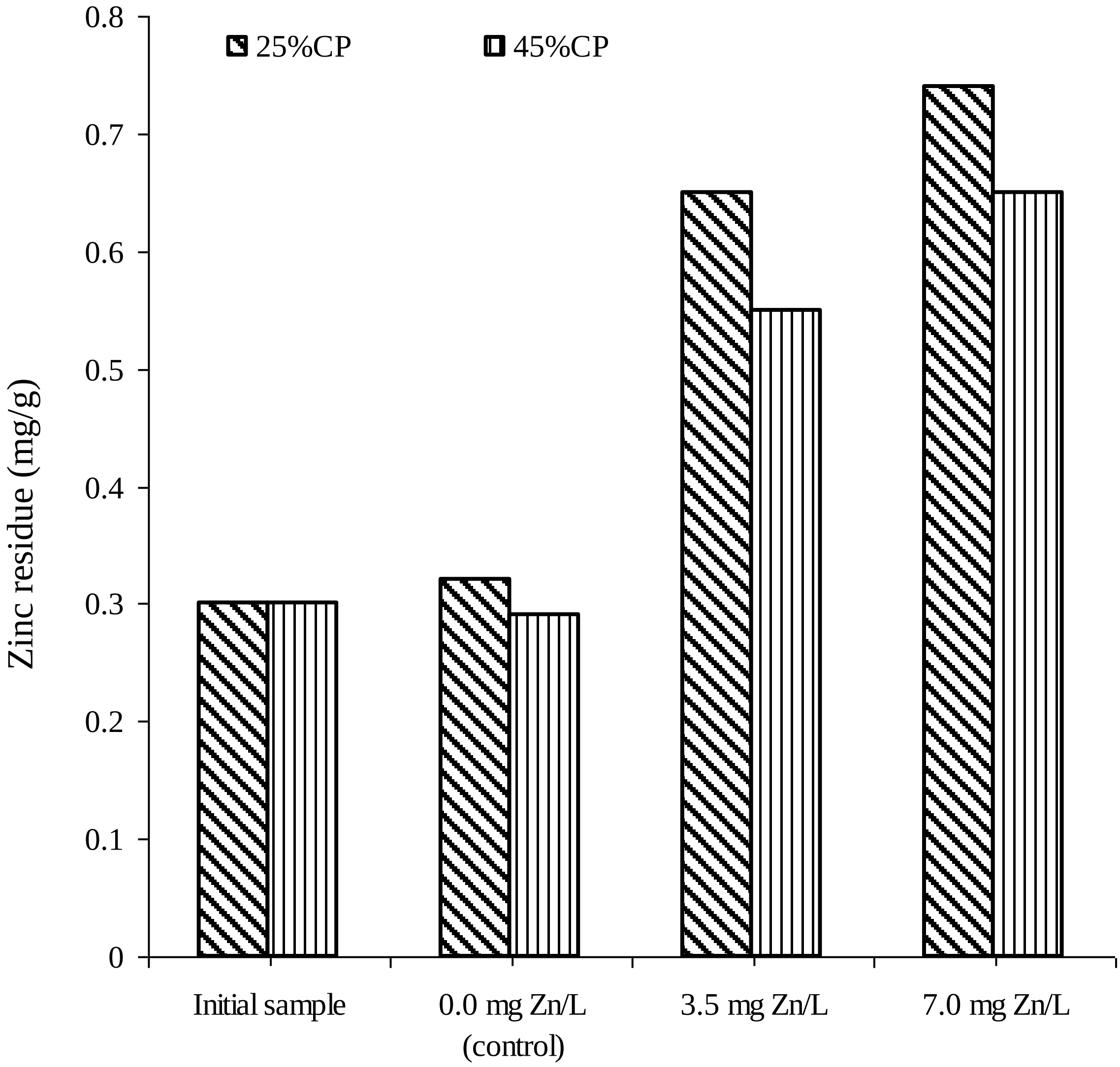

Figure 26. Zinc residue (mg/g dry weight) in whole body of Nile tilapia fed different protein levels and exposed to different Zn concentrations for 6 weeks.