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Haematological and biochemical parameters and tissue accumulations of cadmium in *Oreochromis niloticus* exposed to various concentrations of cadmium chloride



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Abstract *Oreochromis niloticus*, weighing 36.45 ± 1.12 g were exposed to 10%, 20% and 30% of the LC₅₀ of CdCl₂ which represents treatments (T1)1.68, (T2)3.36 and (T3)5.03 mg/l, respectively, for a period of 10, 20 and 30 days. It was found that, compared to a control group reading of 0.19 ± 0.03 µg/g dry weight, accumulation of Cd in the gills was significantly ($p < 0.05$) increased in samples ranging between 7.64 ± 0.86 and 61.73 ± 0.82 µg/g dry weight from T1 at 10 days to T3 at 30 days. The accumulation of Cd in the liver, meanwhile, was also observed to significantly increase ($p < 0.05$) with increasing time and concentrations with results ranging between 3.21 ± 0.12 and 181.61 ± 1.32 compared to the control group results of 0.29 ± 0.04 µg/g dry weight. Although muscles exhibited lower levels of accumulation than the gills and liver they still showed the same pattern of increase compared to the control group, with a significant difference ranging between 0.32 ± 0.02 and 2.16 ± 0.08 compared to the control group results of 0.03 ± 0.001 µg/g dry weight. Also, haematological parameters such as red blood cells (RBCs), haemoglobin (Hb) and haematocrit (Hct) were reduced in fish exposed to Cd at all periods, with significant differences ($p < 0.05$). Plasma glucose concentration showed a significant increase. Total protein levels of fish showed a significant reduction ($p > 0.05$) for all exposed treatments. Also,

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the total lipid level increased significantly as fish were exposed to increasing cadmium concentrations, compared to control fish. Finally, the activities of aspartate aminotransferase (AST IU/l) and alanine aminotransferase (ALT IU/l) showed a significant increase ($p < 0.05$) with increasing time and concentrations.

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1. Introduction

Oreochromis niloticus is an important species in commercial fisheries in the world. It is also a species commonly found in brackish water in estuaries around the world (Vijayan et al., 1996) and responds promptly to environmental alterations. Cadmium is widely used in modern industry (Novelli Filho et al., 2000) and, along with other heavy metals; it is known to exert a wide range of pathological effects on fish and other aquatic organisms (Iger et al., 1994). In particular, metal accumulation in fish has been linked to the damage to organ structure (Giari et al., 2007), various changes in the indices of red blood cells (Vosylienė, 1999), changes in the levels of glucose, osmotic regulation (Fu et al., 1990) and alteration in enzyme activities (Lionetto et al., 2000). Furthermore, Cd is known to have severe toxic effects on aquatic organisms when present in excessive amounts (Novelli et al., 1999), including damage to essential biochemical and physiological functions (Heath, 1995).

Chronic sub-lethal exposure to waterborne Cd leads to accumulation of this metal mainly in the fish kidneys, liver and gills (Hollis et al., 1999; McGeer et al., 2000), with the level of accumulation in distinct organs depending on uptake and elimination rates, which are different from one tissue type to other (Lange et al., 2002). Some organs, such as the liver, intestines, gills and kidney are often investigated for metal accumulations. Moreover, these tissues have been identified as the main storage sites for Cd (Kim et al., 2004). Haematological parameters are used as an index to detect physiological changes in a number of fish species and to assess structural and functional health during stress conditions (Adhikari et al., 2004; Suvetha et al., 2010). Fish blood is sensitive to pollution-induced stress, and changes to the haematological parameters, such as haemoglobin content, haematocrit and the number of erythrocytes, can be used to monitor stress caused by pollutants such as heavy metals (Romani et al., 2003; Barcellos et al., 2004).

Biochemical parameters in fish are also sensitive for detecting potential adverse effects of metal accumulations. The activities of various enzymes are considered to be sensitive biochemical indicators before hazardous effects occur in fish and are important parameters for testing water for the presence of toxicants (Gül et al., 2004). Determination of enzymes, such as aspartate alanine aminotransferase (AST, ALT) and lactate dehydrogenase (LDH), is considered a useful biomarker to determine pollution levels during chronic exposure (Basaglia, 2000; Ozman et al., 2006; Younis et al., 2012).

The aims of this study were to investigate the effects of exposure to three concentrations of Cd for varying durations on the Cd accumulation in the gill, liver and muscle tissues of the freshwater fish *O. niloticus*, as well as on some haematological and biochemical parameters.

2. Materials and methods

2.1. Experimental fish

Fingerlings of Nile tilapia, *O. niloticus*, were collected from the fish seed hatchery of King Abdulaziz City for Sciences and Technology Mozahmiya, Riyadh, Saudi Arabia. Fish were acclimatised to laboratory conditions for two weeks prior to experiments.

2.2. Experimental design

One hundred and sixty acclimatised fish, weighing 36.45 ± 1.12 g, were divided into four groups. Three of these groups were exposed to 10%, 20% or 30% of the LC₅₀ of CdCl₂, which represents 1.68(T1), 3.36(T2) and 5.04(T3) mg/l CdCl₂, respectively, for 10, 20 and 30 days. The fourth, unexposed, group served as the control. Glass aquaria with a capacity of 80 l (100 × 50 × 40 cm) were used, with replicates for each concentration. The water was changed once a week to maintain the environmental conditions and CdCl₂ concentrations (Proença and Bittencourt, 1994). Dissolved oxygen was added with diffused air at the top of the biological filter. Fish were fed twice daily with a 32% crude protein diet at a rate of 2% of body weight. Mortalities within each group were recorded.

2.3. Experimental exposure

After acclimatisation, 20 fish were transferred to experimental tanks (80 l) containing dechlorinated tap water. Duplicate cultures were established for each concentration tested, adding calculated amounts of a 1000 mg/l stock solution of CdCl₂ prepared in deionised water, with an unexposed group serving as the control fish. The cadmium treatment level was based on the 96-h LC₅₀ of CdCl₂ in *O. niloticus*, which was previously determined to be 16.8 mg/l by Zirong and Shijun (2007).

2.4. Tissue analysis

After the fish were decapitated, the gills, liver and muscle tissues were collected and freeze-dried. Tissues were washed in fresh water three times and briefly rinsed in double-deionised water; the water left on the tissue surface was blotted dry with filter paper. Tissues were dried at 80 °C overnight, weighed (approx. 0.3 g dry) and then digested. The digested solutions were diluted with double-deionised water and subjected to atomic absorption spectrophotometry. The tissue digestion was conducted according to the method described by Allen (1989). Cd concentration was measured using an Atomic Absorption spectrometer (Varian – Spectra, 220 FS).

2.5. Haematological and biochemical analysis

After each experimental period, samples of the blood, liver, gills and muscle were taken from five fish specimens from each aquarium. Fish were not fed for 24 h before sampling and were anaesthetised with buffered MS222 (50 mg/l). Blood samples were taken from the caudal vein of an anaesthetised fish with a sterile syringe containing heparin solution as an anticoagulant. Plasma was obtained by centrifugation of blood at 3000 rpm for 15 min and non-haemolysed plasma was stored in a deep freezer for further biochemical analyses. These blood samples were used for determining the count of red blood cells (RBCs) following the method of Dacie and Lewis (1984), and the haemoglobin content (Hb) (Van Kampen and Zijlstra, 1961). The haematocrit value (Hct) was calculated according to the formulae mentioned by Britton (1963).

Plasma glucose (mg/l) was determined using assay kits supplied by Human Diagnostics Worldwide according to Trinder (1969). Total protein (g/100 ml) content was determined according to the method of Henry (1964). Total lipid (g/l) content was determined colorimetrically according to Joseph et al. (1972). The activity levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined colorimetrically according to Reitman and Frankel (1957).

2.6. Statistical analysis

The statistical analysis of the data was done using a one way analysis of variance (ANOVA) technique. The means were separated by Fisher's LSD test and compared using Duncan's Multiple Range Test (DMRT) as described by Snedecor and Cochran (1989). Significant differences were defined at $p < 0.05$.

3. Results

3.1. Cd accumulation in gills

Table 1 shows the concentration of Cd in fish exposed to T1, T2 and T3 CdCl₂, with the significantly ($p < 0.05$) increased accumulation of this element in the gills being especially

noteworthy. After 10, 20 and 30 days accumulations in the gills were 7.64, 24.52 and 31.65 µg/g dry weights for the T1 group, 15.83, 32.42 and 42.16 for the T2 group, and 28.96, 40.81 and 61.73 for the T3 group compared to the control group which showed lower accumulations of 0.19, 0.28 and 0.32 mg/l after all the exposed periods, with significant differences ($p < 0.05$) among all groups.

3.2. Cd accumulation in the liver

The highest bioaccumulation of cadmium was observed in the organs mainly implicated in metal intoxication; thus, bioaccumulation was higher in the liver followed by the gills and muscles. The highest amount of Cd residue was found in the liver after 30 days of exposure. Table 1 also shows that the accumulation of Cd in the liver after 10 days was 3.21 ± 0.12 , 28.16 ± 0.61 and 68.41 ± 0.96 respectively for the fish exposed to T1, T2 and T3 CdCl₂, with significant differences ($p < 0.05$) between the treatments and control group (0.29 ± 0.04 µg/g dry weights). The accumulations increased significantly ($p < 0.05$) as the concentration of Cd increased. After 20 days accumulations showed the same trend, being 17.01 ± 0.32 , 36.81 ± 0.72 and 138.12 ± 1.03 respectively with this increase showing a significant difference ($p < 0.05$) between exposed and control fish (0.41 ± 0.02 µg/g dry weights). Also, after 30 days the results illustrated in Table 1 for different concentrations were 32.51 ± 0.51 , 57.67 ± 0.64 and 181.61 ± 1.32 respectively, again with significant differences ($p < 0.05$) between the exposed and control group 0.96 ± 0.05 µg/g dry weights (Table 1).

3.3. Cd accumulation in muscles

Table 1 shows a similar trend with respect to fish muscles exposed to concentrations of Cd for 10 days. This exposure resulted in 0.32 ± 0.02 , 0.68 ± 0.04 and 0.88 ± 0.05 µg/g dry weights respectively, a significant increase ($p < 0.05$) in response to increasing Cd concentrations between the treatments and control groups (0.03 ± 0.001). After a 20 day exposure period the data obtained were 0.64 ± 0.06 , 0.91 ± 0.09 and 1.41 ± 0.04 respectively, again showing a

Table 1 Cadmium accumulation (µg/g dry weight) in some tissues of *O. niloticus* exposed to cadmium chloride for 10, 20 and 30 days.

Tissues	Cd Concentrations	Exposure time (days)		
		10	20	30
Gills	Control*	0.19 ± 0.03 ^a	0.28 ± 0.06 ^a	0.32 ± 0.05 ^a
	T1(10%LC ₅₀)	7.64 ± 0.86 ^b	15.83 ± 0.93 ^b	28.96 ± 0.87 ^b
	T2(20%LC ₅₀)	24.52 ± 0.67 ^c	32.42 ± 0.74 ^c	40.81 ± 0.68 ^c
	T3(30%LC ₅₀)	31.65 ± 0.81 ^d	42.61 ± 0.61 ^d	61.73 ± 0.82 ^d
Liver	Control	0.29 ± 0.04 ^a	0.41 ± 0.02 ^a	0.96 ± 0.05 ^a
	T1(10%LC ₅₀)	3.21 ± 0.12 ^b	17.01 ± 0.32 ^b	32.51 ± 0.51 ^b
	T2(20%LC ₅₀)	28.16 ± 0.61 ^c	36.81 ± 0.72 ^c	57.67 ± 0.64 ^c
	T3(30%LC ₅₀)	68.41 ± 0.96 ^d	138.12 ± 1.03 ^d	181.61 ± 1.32 ^d
Muscles	Control	0.03 ± 0.001 ^a	0.08 ± 0.006 ^a	0.12 ± 0.004 ^a
	T1(10%LC ₅₀)	0.32 ± 0.02 ^b	0.64 ± 0.06 ^b	0.77 ± 0.06 ^b
	T2(20%LC ₅₀)	0.68 ± 0.04 ^{bc}	0.91 ± 0.09 ^c	1.12 ± 0.06 ^{bc}
	T3(30%LC ₅₀)	0.88 ± 0.06 ^{cd}	1.41 ± 0.04 ^d	2.16 ± 0.08 ^d

Values in the same column for each period separately with the same superscript are not significantly different ($p > 0.05$).

* Control (not exposed 0 mg/l CdCl₂).

Table 2 Haematological and biochemical parameters of *O. niloticus* exposed to different concentrations of cadmium chloride for 10 days.

Parameters	Concentrations			
	Control*	T1(10%LC ₅₀)	T2(20%LC ₅₀)	T3(30%LC ₅₀)
RBC (count × 10 ⁶ /mm ³)	1.82 ± 0.06 ^{cd}	1.76 ± 0.07 ^c	1.65 ± 0.03 ^b	1.30 ± 0.05 ^a
Hb (g/100 ml)	6.00 ± 0.30 ^d	5.29 ± 0.18 ^c	5.11 ± 0.41 ^{ab}	4.96 ± 0.22 ^a
Hct (%)	33.26 ± 0.32 ^d	31.70 ± 0.32 ^c	28.41 ± 0.62 ^{ab}	27.58 ± 0.53 ^a
Glucose (mg/l)	60.32 ± 0.57 ^a	66.42 ± 0.68 ^b	72.50 ± 0.71 ^c	76.38 ± 0.92 ^d
TP (g/100 ml)	2.85 ± 0.09 ^d	2.26 ± 0.07 ^c	2.03 ± 0.03 ^{ab}	1.97 ± 0.06 ^a
TL (g/l)	10.96 ± 0.08 ^a	15.31 ± 0.06 ^b	16.06 ± 0.07 ^c	17.11 ± 0.09 ^d
AST (IU/l)	81.65 ± 1.06 ^a	123.04 ± 1.36 ^b	127.40 ± 1.46 ^c	129.60 ± 1.61 ^{cd}
ALT (IU/l)	30.95 ± 0.26 ^a	49.24 ± 0.83 ^{bc}	51.31 ± 1.06 ^c	56.74 ± 1.01 ^d
Survival (%)**	100.00	81.62	73.65	64.86

Values in the same row with the same superscript are not significantly different ($p > 0.05$).

* Control (not exposed 0 mg/l CdCl₂).

** Survival during all experimental periods.

Table 3 Haematological and biochemical parameters of *O. niloticus* exposed to different concentrations of cadmium chloride for 20 days.

Parameters	Concentrates			
	Control*	T1(10%LC ₅₀)	T2(20%LC ₅₀)	T3(30%LC ₅₀)
RBC (count × 10 ⁶ /mm ³)	1.73 ± 0.04 ^d	1.26 ± 0.07 ^c	1.18 ± 0.05 ^b	1.13 ± 0.08 ^a
Hb (g/100 ml)	7.30 ± 0.12 ^d	4.87 ± 0.09 ^c	4.86 ± 0.18 ^b	4.79 ± 0.11 ^a
Hct (%)	31.56 ± 0.23 ^d	26.91 ± 0.41 ^c	25.38 ± 0.24 ^b	24.61 ± 0.39 ^a
Glucose (mg/l)	55.85 ± 0.66 ^a	88.20 ± 0.56 ^b	93.75 ± 0.84 ^c	100.21 ± 0.88 ^d
TP (g/100 ml)	3.05 ± 0.04 ^d	1.69 ± 0.08 ^c	1.53 ± 0.06 ^b	1.42 ± 0.03 ^a
TL (g/l)	11.62 ± 0.02 ^a	17.46 ± 0.05 ^b	18.23 ± 0.08 ^c	20.07 ± 0.06 ^d
AST (IU/l)	72.62 ± 1.03 ^a	132.10 ± 1.05 ^b	138.60 ± 1.11 ^c	143.50 ± 1.64 ^d
ALT (IU/l)	32.65 ± 0.35 ^a	59.11 ± 0.51 ^b	64.30 ± 0.66 ^c	70.24 ± 0.98 ^d

Values in the same row with the same superscript are not significantly different ($p > 0.05$).

* Control (not exposed 0 mg/l CdCl₂).

significant difference ($p < 0.05$) in the increase between the treatment and control groups ($0.08 \pm 0.006 \mu\text{g/g}$ dry weights). A similar trend was evident in the data obtained after the 30 day exposure period, in which the dry weights were 0.77 ± 0.06 , 1.12 ± 0.06 and 2.16 ± 0.08 respectively compared to control group figures of $0.12 \pm 0.004 \mu\text{g/g}$ (Table 1).

3.4. Haematological parameters

Table 2 shows that the RBCs, HB and HCT were reduced in fish exposed to Cd in all periods, as well as being in each case lower than in the control ($p < 0.05$). The RBC count decreased significantly ($p < 0.05$) in T1, T2 and T3 CdCl₂ at 10 days with counts of 1.82 ± 0.06 , 1.76 ± 0.07 , 1.65 ± 0.03 and 1.30 ± 0.05 count × 10⁶ mm³ for the control, T1, T2 and T3 respectively. Also, data obtained for HBs were 5.29 ± 0.18 , 5.11 ± 0.41 and 4.96 ± 0.22 respectively with a significant decrease ($p < 0.05$) evident between the treated and control fish 6.00 ± 0.71 g/100 ml. In the same way, the HCT% showed a significant difference ($p < 0.05$) between exposed fish and the control: 33.26 ± 0.32 , 31.70 ± 0.32 , 28.41 ± 0.62 and $27.58 \pm 0.53\%$ for control, T1, T2 and T3 respectively (Table 1).

3.5. Biochemical parameters

Plasma glucose (mg/l) concentrations, meanwhile, showed significantly ($p < 0.05$) higher values (Tables 2–4) which increased as the concentration and exposure periods increased compared to the control group. The total protein levels of *O. niloticus* for the 10, 20, and 30 day periods are given in Table 2–4. A highly significant reduction ($p < 0.05$) in cadmium is evident for all exposure periods and concentrations compared to the control group. The total lipid levels (g/l) for the fish for the different exposure periods are given in Table 2–4. Increased cadmium concentrations led to a highly significant increase in total lipids ($p < 0.05$) for all periods of exposure.

There are significant differences in AST and ALT activity levels (IU/l) in the serum of fish exposed to cadmium in comparison with the control fish (Tables 2–4). Cadmium appeared to induce a highly significant increase in the levels of these two liver enzymes for all exposure periods as the concentrations of Cd increased. Therefore, survival rates (%) were recorded which decrease by increasing cadmium concentration and time with data ranging between 100 (no mortality) for the control group to 81.62%, 73.65% and 64.86% for T1, T2 and T3 respectively (Table 2).

Table 4 Haematological and biochemical parameters of *O. niloticus* exposed to different concentrations of cadmium chloride for 30 days.

Parameters	Concentrations			
	Control*	T1(10%LC ₅₀)	T2(20%LC ₅₀)	T3(30%LC ₅₀)
RBC (count × 10 ⁶ /mm ³)	1.81 ± 0.04 ^d	1.11 ± 0.07 ^c	1.09 ± 0.06 ^{bc}	1.03 ± 0.03 ^a
Hb (g/100 ml)	7.85 ± 0.11 ^d	4.61 ± 0.09 ^c	4.44 ± 0.07 ^{ab}	4.38 ± 0.08 ^a
Hct (%)	32.21 ± 0.41 ^d	23.73 ± 0.52 ^c	22.41 ± 0.33 ^{ab}	22.51 ± 0.65 ^a
Glucose (mg/l)	54.66 ± 0.48 ^a	107.30 ± 0.67 ^b	115.61 ± 0.56 ^c	125.23 ± 0.44 ^d
TP (g/100 ml)	3.11 ± 0.03 ^d	1.33 ± 0.07 ^c	1.21 ± 0.06 ^b	1.11 ± 0.72 ^a
TL (g/l)	12.05 ± 0.04 ^a	20.96 ± 0.05 ^b	22.11 ± 0.07 ^c	23.01 ± 0.08 ^d
AST (IU/l)	76.52 ± 1.04 ^a	148.7 ± 1.05 ^b	151.20 ± 1.04 ^{bc}	160.13 ± 1.08 ^d
ALT (IU/l)	35.62 ± 0.4 ^a	72.21 ± 0.39 ^b	79.81 ± 0.65 ^c	88.62 ± 0.46 ^{cd}

Values in the same row with the same superscript are not significantly different ($p > 0.05$).

* Control (not exposed 0 mg/l CdCl₂).

4. Discussion

Many studies have investigated Cd accumulation and distribution among organs. The distribution of accumulated Cd in organs differs between these studies, however (Cattani et al., 1996). The inconsistencies in these previous studies may be ascribed to the differences in the cadmium concentrations and exposure times used (Wu et al., 1999). A few papers, however, have compared Cd accumulation among organs in relation to Cd exposure time. Differential responses of Cd exposure and cadmium resistance may be related to oxidative stress biomarkers, increasing the activities of antioxidant enzymes. When the metal concentration increased and overcame the capacity of natural detoxifying systems, various adverse effects occurred and we observed increased mortality. These results were in agreement with data obtained by Nishiyama et al. (1998), who also recommended that oxidative stress, which is defined as the imbalance between oxidant fluxes and antioxidant defences, this might be related with cadmium exposure and mortality. In fish that survived, there were increases in antioxidant defences.

The present data showed that the highest content of Cd was found in the tissues of the liver, gills and muscles, and that these accumulations increased 626, 324, and 72-fold, respectively, after 30 days of exposure, compared to the control group. Results found in carp (*Cyprinus carpio*) indicated that more than 10-fold higher levels of Cd accumulated in the kidneys than in the liver (Kraal et al., 1995). According to the present data, however, in *O. niloticus*, it is the gills that are the temporary target organ of Cd accumulation, with the Cd then being transferred to the digestive tract and organs such as the liver and kidneys via the circulatory system or the enterohepatic circulation. In addition, compared to the organs, the accumulation rate of Cd after 30 days' treatment was the lowest in muscle. In the present study, across all concentrations and periods, the accumulation pattern of cadmium follows the order; the liver > gills > muscles and these results are in full agreement with the data obtained by Cogun et al. (2003), who found a similar distribution pattern for cadmium and copper in tissues of *O. niloticus*. In the marine fish, *Sparus aurata*, however, Souid et al. (2013) reported that the source of Cd-uptake in sea water, metal accumulation in different tissues decreased in the following order: intestine > gills > kidney > liver > muscles for short term exposures of 2, 4 and

24 h. This result might be related to the short term exposure to the metal, which might not have permitted transference of Cd through the portal system from the digestive tract to the liver, which is involved in storage and metal detoxification (Handy, 1993). In bass (*Dicentrarchus labrax*) and flounder (*Scophthalmus maximus*), meanwhile, Cd accumulation followed the order kidneys > liver > gills (Cattani et al., 1996). The finding of the present study is in congruence with (Brown et al., 2006) who suggested that the pollutants may be distributed uniformly within the body tissues of fish but accumulate differently. Overall, fish exposed to Cd leads to accumulate the metal in a concentration-dependent manner (Isani et al., 2009). These results also are in congruence with the finding of the present study. This is in agreement with our data, suggesting that metal accumulation depends largely on the period of exposure and the type of organ. Overall, given long term exposure, the liver accumulates Cd strongly when compared to other studied tissues.

Many studies on fish have demonstrated that the distribution of Cd is tissue specific and depends on the exposure route, as reported by Kraal et al. (1995) and Guinot et al. (2012). Moreover, the results in the present study are in accordance with data obtained by Filipović Marijić and Raspor (2006), who argued that after long term exposure metals, are transferred to storage organs such as the liver or kidney. Studies carried out with different aquatic fish species have shown that the liver is the prime organ for metal accumulation and also plays an important role in storage, redistribution, detoxification or transformation of metals (Erdem and Kargin, 1992; Evans et al., 1993). In accordance with our data Cogun et al. (2003) who observed that muscle in *O. niloticus* generally accumulates the lowest concentrations of metals during exposure of fish for 30 days to cadmium and copper.

The present study reveals that the *O. niloticus* exposed to Cd exhibited a significant reduction in their RBCs, Hb and Hct in comparison with the control. These results are in agreement with Gill and Epple (1993) and Karuppasamy et al. (2005) who each found a significant reduction in the RBCs, Hb and Hct in American eel *Anguilla rostrata* and the air breathing fish, *Channa punctatus*, after exposure to 150 µg and 29 mg Cd/l, respectively. Our results are also in full agreement with data obtained by Mekkawy et al. (2011), who reported that fish exposed to 4.64 mg/l (25% of 96 h LC₅₀) Cd showed a significant decrease in their RBCs Hb and Hct

in *O. niloticus* when exposed to 4.64 mg/l cadmium for 15 and 30 days. Shalaby (2007), found similar results for *O. niloticus* exposed to 10 ppm Cd for 15 and 45 days. The reduction in these parameters at sub-lethal levels of cadmium might be due to the destruction of mature RBCs and the inhibition of erythrocyte production due to a reduction of haemosynthesis that affects haematopathology or causes acute haemolytic crisis which in turn results in severe anaemia in most vertebrates, including fish species, exposed to various environmental contaminants (Khadre, 1988).

Blood glucose is a sensitive and reliable indicator of pollutants causing environmental stress in fish. Our results are in agreement with data obtained by Shalaby (2007) and Mekkawy et al. (2011) who showed that Cd elevated blood glucose levels in Nile tilapia exposed to 4.64 mg/l (25% of 96 h LC₅₀) for 15 and 30 days. Sastry and Subhadra (1985) reported that Cd induced hyperglycaemia with an associated decrease in liver glycogen in catfish, *Heteropneustes fossilis*. Soengas et al. (1996), meanwhile, suggested that hyperglycaemia occurred in Atlantic salmon *Salmo salar* due to cadmium toxicity, possibly associated with changes in liver carbohydrate metabolism which causes activation of liver glycogenolysis and glycolysis as well as increased levels of plasma glucose and lactate.

The decrease in plasma total protein level (hypoproteinaemia) shown in this study was in agreement with the findings of Mekkawy et al. (2011). They recorded that *O. niloticus* exposed to 4.64 mg/l (25% of 96 h LC₅₀) Cd for 15 and 30 days showed a reduction in serum protein levels. This decrease of total protein may be due to the destruction of protein-synthesising subcellular structures and inhibition of the hepatic synthesis of blood protein (Fontana et al., 1998). Loss of protein from damaged kidneys could contribute further to the observed hypoproteinaemia (Gad, 2005). Our data are in accordance with Shalaby (2001), who suggested that Cd-induced increase in serum total lipids in comparison with the control of *O. niloticus* may be due to a disturbance in the metabolism of lipids after fish were exposed to Cd for 15 and 45 days.

The activity of AST and ALT enzymes in blood may also be used as a stress indicator. The significant changes in the activities of these enzymes in blood plasma indicate tissue impairment caused by stress (James et al., 1991; Svoboda, 2001). The increase of AST and ALT values in the fish reveal enzymes exporting from liver into the blood stream (Yang and Chen, 2003; Perez-Rostro et al., 2004). According to Chen et al. (2004) increased levels of these two enzymes in tilapia are associated with hepatic injury. In the present study, there were significant changes in AST and ALT activities in plasma of *O. niloticus* exposed to cadmium for 10, 20 and 30 days compared to the control group. The increased levels of AST and ALT in blood plasma indicate impairment of the liver. In addition, the increase of plasma AST and ALT may be related to hepatocellular damage or cellular degradation caused by cadmium, probably in the liver, heart or muscle (Yamawaki et al., 1986). These results are in agreement with those of Shalaby (2007) who found that sub-lethal concentrations of Cd caused significant increases in AST and ALT of *O. niloticus* after 15 and 45 days of exposure to cadmium. Also, Mekkawy et al. (2011) confirmed similar data for these enzymes in *O. niloticus* exposed to cadmium for 15 and 30 days.

5. Conclusion

The observations of our data showed that metal accumulation depends largely on three factors; the concentration of metal, period of exposure and the type of organ. Overall, given exposure, the liver accumulates Cd strongly when compared to other studied tissues of Nile tilapia (gills and muscles). Haematological and biochemical parameters indicated that cadmium acts as a stressor leading to changes in some blood parameters and accumulation in important tissues.

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