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Effect of Cd on serum osmolality, ion levels and hematological parameters of tilapia (Oreochromis niloticus) at different salinity levels

Pramita Adi Listiyani, Miftachul Shobirin, Eka Novianti, Bambang Irawan, Sucipto Hariyanto & Agoes Soegianto

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Effect of Cd on serum osmolality, ion levels and hematological parameters of tilapia (*Oreochromis niloticus*) at different salinity levels

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ABSTRACT

Effects of cadmium on serum osmolality, ion levels and hematological parameters of tilapia (*Orechromis niloticus*) were evaluated at different salinities. Serum osmolalities (SOs) in fish unexposed to Cd (0 mg l^{-1}) and exposed to 2.5 mg Cd l^{-1} were not significantly different at salinities of 0, 5 and 10 g l^{-1}, while at 15 g l^{-1}, SO was significantly higher than at 0, 5 and 10 g l^{-1}. Levels of Na^+ and Cl^- in serum at salinities of 5, 10 and 15 g l^{-1} were not significantly different; but were significantly higher than those at 0 g l^{-1} with and without Cd. In media without Cd, the lowest level of K^+ in serum occurred at 15 g l^{-1} salinity, whereas levels of K^+ at 0, 5 and 10 g l^{-1} were not significantly different. The levels of K^+ in Cd-exposed fish at all salinities were not significantly different. At 0 g l^{-1} salinity, hemoglobin, red blood cells, and hematocrit in Cd-exposed fish were significantly lower than controls. At salinities of 5, 10 and 15 g l^{-1}, levels in control and Cd-exposed fish were not significantly different indicating that higher salinity prevented Cd-induced osmotic imbalance and hematological alterations.

Introduction

Cadmium (Cd) is a non-degradable pollutant entering the environment from both anthropogenic activities and natural processes. The concentration of Cd in natural aquatic environment was < 0.1 µg l^{-1}, however in heavily polluted waters, cadmium concentration was up to 2–16.1 mg l^{-1} (Cao et al. 2012). This metal can be accumulated in the aquatic biota including fish. Chronic contamination of freshwater and marine environments with Cd, which is considered to be of severe and pervasive concern (Romeo et al. 2000), is reported frequently. Exposure to sub-lethal concentrations of Cd may cause biochemical and ionic disturbances or adaptive responses in blood and tissues of fish (Pelgrom et al. 1995), and alter the blood composition and immune mechanisms (Wendelaar Bonga and Lock 1992; Witeska 2005; Gabriel, Anyanwu, and Akinrotimi 2007).

Previous observations showed that toxic effects of metals depend on a range of biotic and abiotic factors (Erickson et al. 2008). As an abiotic factor, salinity exerts a significant effect on metal toxicity and accumulation. Toxicity of metals reduces with the increasing medium salinity (Erickson et al. 2008; Loro et al. 2012). Salinity affects metal bioavailability and uptake and its subsequent toxicity by competing with metal ions for binding to biological molecules (Bianchini et al. 2002). On the other hand, bioavailability and toxicity of metals may be also influenced by the physiology and osmoregulatory strategy of an organism (Bielmyer, Brix, and

Grosell 2008; Bielmyer and Grosell 2011). Freshwater teleosts actively combat diffusive losses of ions by taking up Na^+ and Cl^- at the gill, whereas, the hypertonic environment of the saltwater-acclimated fish promotes active excretion of Na^+ and Cl^- at the gill and stimulates ingestion of the surrounding waters, thereby relying on the gills and intestine for ionoregulation, osmoregulation and water balance (Marshall 2002).

Moreover, toxic effects of Cd on fish are persistent and inhibit activity of many enzymes such as Ca^{2+}-ATPase, Na^+/K^+-ATPase, H^+-ATPase, and carbonic anhydrase present in the gills and kidney, which are involved in the uptake of ions and maintenance of ionic balance in freshwater fish (Verbost et al. 1988; Perry et al. 2003).

Tilapias (*Oreochromis niloticus*) tolerate a wide range of salinity. Their ability to thrive in different salinity environments makes them a robust aquaculture species (Sardella et al. 2004; Canonico et al. 2005). Tilapia can be cultured efficiently in freshwater, brackish water and seawater, which is greatly advantageous in the light of global shortage of freshwater representing one of the most severe global challenges of our times (Beuhler 2003). Current projections of global climate change forecast a salinity increase of as much as 9 g l^{-1} in many parts of coastal water systems (Knowles and Cayan 2002). It means that many coastal areas will be inundated by brackish water. This significant salinity increase will have physiological effects on organisms inhabiting these ecosystems. Moreover, in regions, which are impacted by industrial, agricultural and

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domestic activities, fish often encounter both water salinity changes and elevated levels of toxic substances including cadmium, therefore the interaction between salinity acclimation and toxicant becomes important to animals (Adeyemi et al. 2012). Objectives of the present study were to evaluate the effects of cadmium exposures on serum osmolality, ion levels and hematological parameters of tilapia (Oreochromis niloticus) at different salinity levels.

Materials and methods

Fish acclimation and experimental design

Specimens of the fish Oreochromis niloticus (East Java strain, local name: Jatimbulan), approximately 11.3 ± 0.2 cm in length and 15.2 ± 0.5 g in weight, were collected from a commercial farm in Pasuruan, East Java, Indonesia. They were transported to the laboratory and maintained in 250 l holding tanks supplied with a continuous flow of freshwater (FW, 0 g l^{-1}) through a gravel, sand and sponge filter. To avoid osmotic shock, some fish were acclimated to different water salinities (0, 5, 10, and 15 g l^{-1}) gradually, i.e. by increasing salinity at a rate of 5 g l^{-1} per day. The process of acclimation lasted for 14 days. Seawater (SW, 35 g l^{-1}) was obtained from the coast adjacent to the university and fresh water (FW) was obtained from municipal tap water. Salinity was measured using a handheld salinity refractometer (Atago, Japan). The concentrations of Na^+, Cl^- and K^+ in FW and SW were 0.26, 0.19, 0.07 mmol l^{-1} and 459.16, 535.40, 9.80 mmol l^{-1} respectively. Before being used for acclimation and experimentation, tap water was aerated overnight to accelerate dechlorination (Putranto et al. 2014). FW and diluted SW were filtered through a gravel, sand and sponge filter. Dilutions of seawater were prepared by adding adequate volumes of SW to FW until the selected level of salinity was achieved. Throughout the acclimation and experimentation tests, fish were fed twice a day with Takari commercial pellets (30% protein, 3% fat and 4% fiber) at 3% of the fish body weight. The temperature was measured using a mercury in glass thermometer (ºC), pH with a pH meter (Hanna Model HI 981,502, China), and dissolved oxygen (DO) with a DO meter (Lutron DO 5510, Taiwan). The values of temperature, pH, and dissolved oxygen were 28–30℃, 7.56–8.15 and 7.3–7.6 mg l^{-1}, respectively. The fish were maintained in conditions of artificial light-dark cycle 12:12 using cool white fluorescent lamps with a light intensity of 3600–4000 lux for illumination.

Effect on osmoregulation, serum ions and hematological parameters

A short-term test for estimating chronic effects was conducted using a standard semi-static method with test solutions renewed every 48 h. Fish were exposed to nominal Cd concentrations: 0 mg Cd l^{-1} (control, measured-Cd concentration = 0.003 mg l^{-1}) and 2.5 mg Cd l^{-1} for 7 d, at salinities of 0, 5, 10 and 15 g l^{-1} (containing measured-Cd = 2.54, 2.46, 2.49 and 2.42 mg Cd l^{-1}, respectively) in 63 l experimental tanks. The Cd concentrations used in this study were based on the results reported by Nursanti et al. (2017) (the 96 h LC_{50} of Cd was 7.53 mg l^{-1}), and are potentially encountered by fish in the contaminated aquatic environment (Cao et al. 2012). A stock solution of Cd (1000 mg l^{-1}) was prepared by dissolving 2.744 g Cd $(NO_3)_2 \cdot 4H_2O$ (Merck, Darmstadt, Germany) in 1000 ml deionized water. In each medium, the test was run in triplicate, with a total of five fish per replicate. Test media were aerated continuously. Uneaten food and debris were removed daily to maintain the test medium quality. At the end of the exposure period, three groups of fish containing five specimens each were randomly removed from each treatment medium and their serum osmolality, serum ions, and hematological parameters were determined. Prior to blood sampling, fish were anesthetized with 200 mg l^{-1} clove solution (Mohseni et al. 2008). Blood from each fish was obtained by puncturing the heart with a non-heparinized syringe. Then blood samples were collected in heparinized tubes containing ethylenediaminetetraacetic acid as an anti-coagulating agent for the assessment of hematological parameters, and in non-heparinized tubes for the assessment of serum osmolalities and serum ions.

Blood samples from non-heparinized tubes were centrifuged at $4500 \times g$ for 10 minutes to separate blood serum and blood cells (at ambient temperature). Serums were then measured for osmolality, Na^+, Cl^-, and K^+ concentrations. Serum osmolality (SO) was measured using an automated freezing point depression osmometer (Fiske® 210 Micro-Sample Osmometer, USA). The osmolality of the serum sample is expressed as mOsm kg^{-1}. The medium from each treatment was also taken and its osmolality was determined with the same osmometer. Serum ions Na^+, Cl^-, and K^+ were measured with an automated electrolyte analyzer (Jokoh EX-D, Japan) employing the potentiometric (ion-selective electrode) method. Blood samples from heparinized tubes were aspirated directly using the automated hematology analyzer (Sysmex XT-2000i, Japan) to assess the hematological parameters (the red blood cell (RBC) count, hematocrit (Ht), and hemoglobin (Hb) concentration). The Sysmex XT-2000i uses the electric resistance detecting method (impedance technology) with hydrodynamic focusing to measure RBC and Ht. Hb is measured photocolorimetrically using sodium lauryl sulfate-Hb, employing a cyanide-free method. The reagents required for the operation of the Sysmex XT-2000i were obtained from Sysmex Corporation.

Statistical analysis

All data were expressed as the mean ± standard deviation, their normality and homogeneity being verified before the statistical analysis. The comparisons of the effects of different salinities with and without Cd exposure on osmolalities, ion levels and hematological parameters were analyzed using one way analysis of variance. When significant differences were detected ($p < 0.05$), Duncan's multiple range test was used to determine which treatment produced a significant effect on osmolalities, ion levels and hematological parameters at a significance level of 0.05. The comparisons of the effects of different Cd concentrations on osmolalities, ion levels and hematological parameters at the same salinity levels were analyzed using Student's t-test, respectively.

Results

Serum osmolalities (SO) of the Cd-exposed and unexposed fish were not significantly different at 0, 5 and 10 g

l^{-1} ($p > 0.05$, but slightly increase from 0 to 10 g l^{-1}), while at 15 g l^{-1}, SO was significantly higher ($p < 0.05$) than at 0, 5 and 10 g l^{-1}, respectively (Figure 1). At 0 and 5 g l^{-1}, SO levels in control fish (unexposed to Cd) were significantly lower than in Cd-exposed fish ($p < 0.05$). While at 10 and 15 g l^{-1}, SO levels in control and Cd-exposed fish were not significantly different ($p > 0.05$) (Figure 1).

The levels of Na$^+$ in serum at the tested salinity levels (5, 10 and 15 g l^{-1}) were not significantly different; however, they were significantly higher ($p < 0.05$) than those at 0 g l^{-1} with and without Cd exposure, respectively. Na$^+$ levels in serum were significantly higher in Cd-exposed fish than in control fish at 0 and 5 g l^{-1}, respectively ($p < 0.05$). Meanwhile, at salinity of 10 and 15 g l^{-1}, Na$^+$ levels in control and Cd-exposed fish were not significantly different ($p > 0.05$) (Figure 2). The levels of Cl$^-$ in fish serum showed the same tendency as those of Na$^+$. At salinity levels of 5, 10 and 15 g l^{-1}, Cl$^-$ levels were not significantly different ($p > 0.05$); meanwhile, they were significantly higher than those at 0 g l^{-1} with and without Cd exposure ($p < 0.05$), respectively. The

Figure 1. Serum osmolality of *Oreochromis niloticus* exposed to 2.5 mg Cd l^{-1} under different salinities for 7 d. Lowercase letters indicate significant differences under different salinities without Cd ($p < 0.05$, a < b). Capital letters indicate significant differences at different salinities under Cd exposure ($p < 0.05$, A < B). The asterisk above black bars denotes a significant difference between different Cd concentrations under the same salinity ($p < 0.05$). The data presented are the means of five determinations.

Figure 2. Serum Na$^+$ ion of *Oreochromis niloticus* exposed to 2.5 mg Cd l^{-1} under different salinities for 7 d. Lowercase letters indicate significant differences under different salinities without Cd ($p < 0.05$, a < b). Capital letters indicate significant differences at different salinities under Cd exposure ($p < 0.05$, A < B). The asterisk above black bars denotes a significant difference between different Cd concentrations under the same salinity ($p < 0.05$). The data presented are the means of five determinations.

levels of Cl⁻ in serum were significantly higher in Cd-exposed fish than in control fish at 0 and 5 g l^{-1}, respectively ($p < 0.05$). The levels of Cl⁻ in control and Cd-exposed fish were not significantly different at 10 and 15 g l^{-1}, respectively ($p > 0.05$) (Figure 3).

In media without Cd, the lowest level of K$^+$ in serum was noted in fish at salinity of 15 g l^{-1}, meanwhile the levels of K$^+$ at 0, 5 and 10 g l^{-1} salinity levels were not significantly different ($p > 0.05$). The levels of K$^+$ in Cd-exposed fish at all salinities were not significantly different ($p > 0.05$) (Figure 4). K$^+$ levels in Cd-exposed fish were significantly lower ($p < 0.05$) than in control fish at 0 and 5 g l^{-1}, respectively (Figure 4).

The levels of Hb, RBC, and Ht at all salinities were not significantly different in both control and Cd-exposed fish, respectively ($p > 0.05$) (Figures 5, 6 and 7). At salinity 0 g l^{-1}, the levels of Hb, RBC and Ht in Cd-exposed fish were significantly lower ($p < 005$) than in control fish. At

salinity of 5, 10 and 15 g l^{-1}, their levels in both control and Cd-exposed fish were not significantly different ($p > 0.05$) (Figures 5, 6 and 7).

Discussion

The tolerance of different tilapia species and strains to salinity from 0 to 32 g l^{-1} varies considerably (Pullin and McConnell 1982; Suresh and Lin 1992; Avella, Berhaut, and Bornancin 1993). There are species- and strain-specific variations with respect to the effect of salinity on growth performance (Suresh and Lin 1992; Garcia-Ulloa, Villa, and Martinez 2001). Baroiller et al. (2000) reported that *O. niloticus* did not tolerate salinities above 20 g l^{-1} and might not be suitable for culture in full-strength seawater (37 to 40 g l^{-1}). Tilapia (*O. niloticus*) from East Java proved to be hyper-regulators at 0 g l^{-1} (≈ 22 mOsm kg^{-1}) and 5 g l^{-1} (≈ 176 mOsm kg^{-1}) salinities,
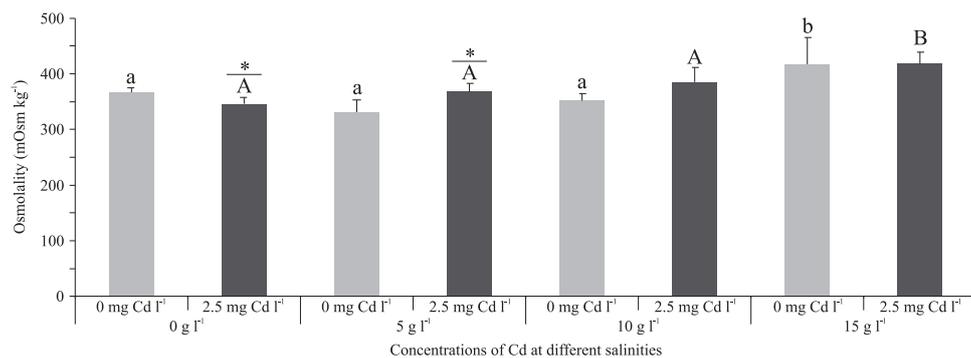

Figure 3. Serum Cl⁻ ion of *Oreochromis niloticus* exposed to 2.5 mg Cd l^{-1} under different salinities for 7 d. Lowercase letters indicate significant differences under different salinities without Cd ($p < 0.05$, a < b). Capital letters indicate significant differences at different salinities under Cd exposure ($p < 0.05$, A < B). The asterisk above black bars denotes a significant difference between different Cd concentrations under the same salinity ($p < 0.05$). The data presented are the means of five determinations.
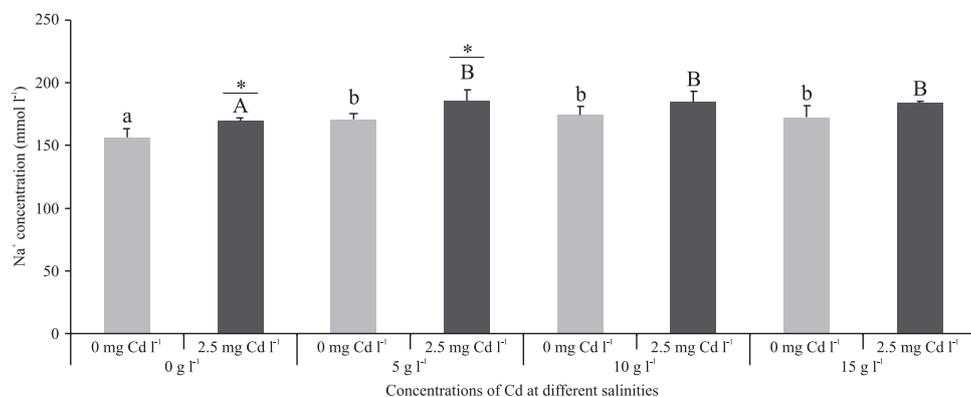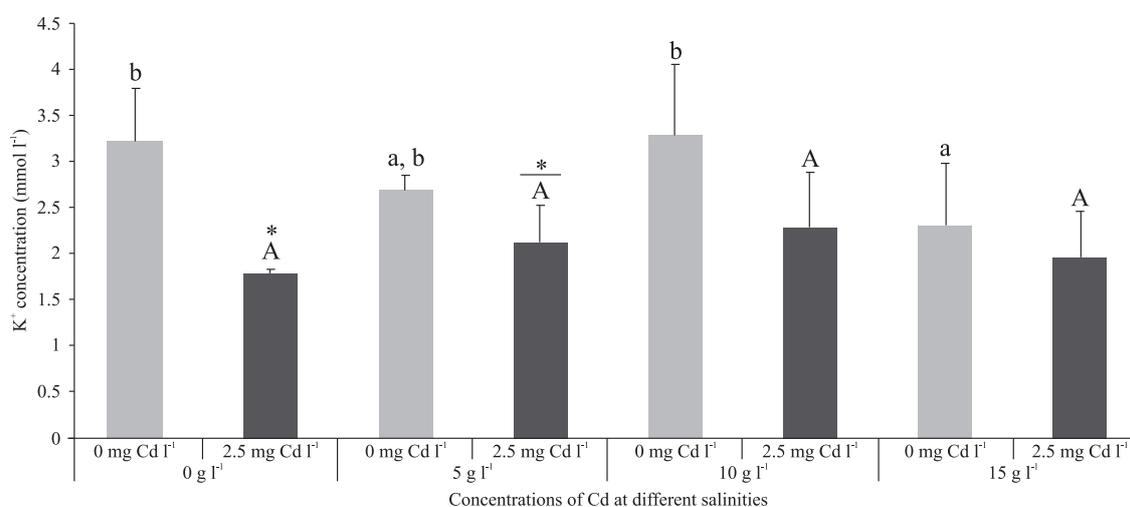

Figure 4. Serum K$^+$ ion of *Oreochromis niloticus* exposed to 2.5 mg Cd l^{-1} under different salinities for 7 d. Lowercase letters indicate significant differences under different salinities without Cd ($p < 0.05$, a < b). Capital letters indicate no significant differences at different salinities under Cd exposure ($p > 0.05$). The asterisk above black bars denotes a significant difference between different Cd concentrations under the same salinity ($p < 0.05$). The data presented are the means of five determinations.
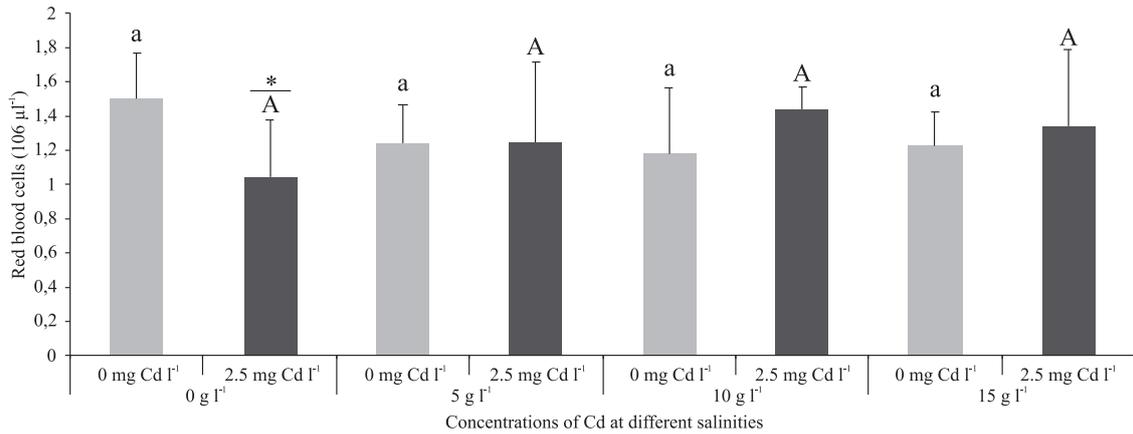
Figure 5. Red blood cells of *Oreochromis niloticus* exposed to 2.5 mg Cd l^{-1} under different salinities for 7 d. Lowercase letters indicate no significant differences under different salinities without Cd ($p > 0.05$). Capital letters indicate no significant differences at different salinities under Cd exposure ($p > 0.05$). The asterisk above black bars denotes a significant difference between different Cd concentrations under the same salinity ($p < 0.05$). The data presented are the means of five determinations.
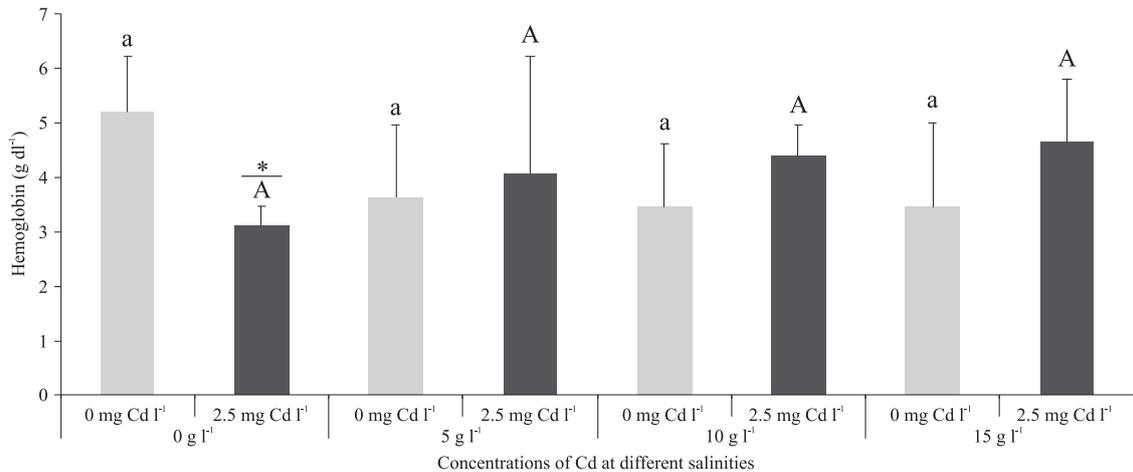
Figure 6. Hemoglobin of *Oreochromis niloticus* exposed to 2.5 mg Cd l^{-1} under different salinities for 7 d. Lowercase letters indicate no significant differences under different salinities without Cd ($p > 0.05$). Capital letters indicate no significant differences at different salinities under Cd exposure ($p > 0.05$). The asterisk above black bars denotes a significant difference between different Cd concentrations under the same salinity ($p < 0.05$). The data presented are the means of five determinations.
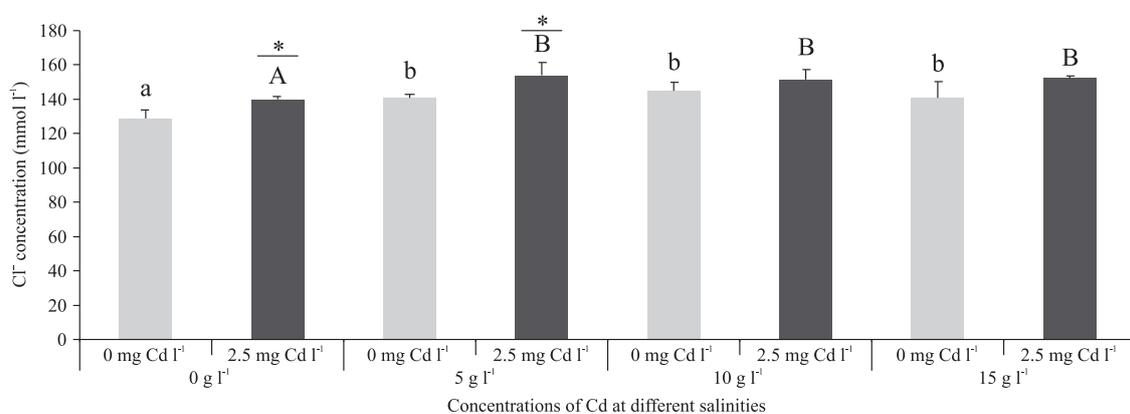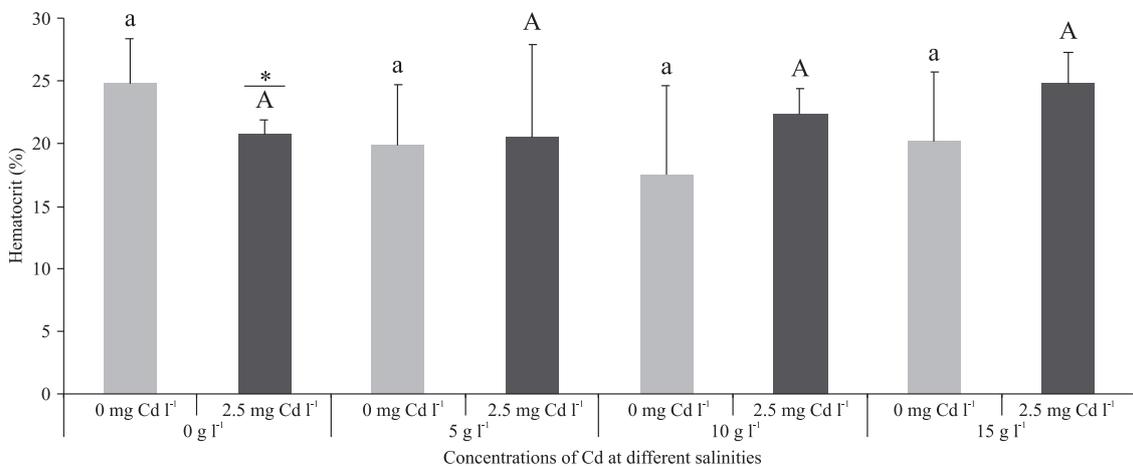

Figure 7. Hematocrit of *Oreochromis niloticus* exposed to 2.5 mg Cd l^{-1} under different salinities for 7 d. Lowercase letters indicate no significant differences under different salinities without Cd ($p > 0.05$). Capital letters indicate no significant differences at different salinities under Cd exposure ($p > 0.05$). The asterisk above black bars denotes a significant difference between different Cd concentrations under the same salinity ($p < 0.05$). The data presented are the means of five determinations.

osmoconformers at 10 g l^{-1} ($\approx$ 335 mOsm kg^{-1}) and hypo-regulators at 15 g l^{-1} ($\approx$ 503 mOsm kg^{-1}) both in the environment with and without Cd at least during the period of the experiment.

In media with and without Cd, the increasing ambient water salinity increased the level of SO with a concomitant increase in Na$^+$ and Cl$^-$ concentrations. In control fish (unexposed to Cd), levels of serum K$^+$ decreased at higher salinities, but in Cd-exposed fish, they were not significantly different. Blood osmolality is determined by the total concentration of solutes, mostly Na$^+$ and Cl$^-$ ions present in the body fluid. In most teleosts, they account for at least 90% of blood osmolality, the rest being made up by other ions such as K$^+$ and Ca^{2+}, proteins and small organic molecules (Gilles and Delpire 1997). Since Na$^+$ and Cl$^-$ are the major ions in the body fluid, regulation of both Na$^+$ and Cl$^-$ is of critical importance for osmoregulation (Kaneko et al. 2006). In the present study, SO, Na$^+$ and Cl$^-$ levels increased at salinities of 5, 10 and 15 g l^{-1} both in control and Cd-exposed fish. Elevated SO, Na$^+$ and Cl$^-$ concentrations in the blood serum of fish at higher salinities might result from the osmotically-induced removal of water from fish and the uptake of ions from the hyperosmotic environment by the fish (Hwang, Sun, and Wu 1989). This fact indicates that this species is adapted to the ambient water salinity ranging from 0 g l^{-1} to 15 g l^{-1} at least for a period of this experiment. The level of serum K$^+$ decreased in control fish, however it did not change in Cd-exposed fish at all salinities. This phenomenon might have occurred to balance the osmotic differences in the intracellular fluid caused by the increase in Na$^+$ and Cl$^-$. Sanders and Kirschner (1983) suggested that in the hyper-osmotic environment, gills of fish are permeable to K$^+$ therefore that efflux is greater than influx. Patridge and Lymbery (2008) suggested that reduced uptake, rather than increased loss of K$^+$, is a more important factor. Meanwhile Nussey, Van Vuren, and Du Preez (1995) suggested that the decrease in serum K$^+$ concentration could be ascribed to osmotic adaptation. Similar findings have been obtained from studies into the Mozambique tilapia and the puffer fish. The concentration of Na$^+$ and Cl$^-$ in the blood serum of the Mozambique tilapia (Vonck, Wendelaar Bonga, and Flik 1998) and the puffer fish (Lin and Lee 2005) in seawater were higher than in fresh water, while concentrations of K$^+$ in the blood serum in seawater were lower than in fresh water.

The present study demonstrated that SO, Na$^+$ and Cl$^-$ levels in Cd-exposed fish were higher than those in control fish at low salinities (0 and 5 g l^{-1}). The mechanism responsible for elevated levels of these serum osmolalities and ions in the Cd-exposed fish most likely involves a greater uptake capacity with the proliferation of chloride cells, a decrease in ions efflux rates due to mucus secretion during waterborne Cd exposure (Wood et al. 1988; Wood 2001), and/or fluid shift from plasma to tissue that may occur during metal induced stress (Wood et al. 1988; Pane, Richards, and Wood 2003). Chowdhury, Pane, and Wood (2004) reported that the greater levels of plasma protein in Cd-exposed fish provide indirect evidence of fluid loss from plasma in Cd-exposed fish. The increase of Na$^+$ level and serum osmolality has been also reported in the ray-finned fish *Prochilodus lineatus* after exposure to water soluble fraction of gasoline (Simonato, Fernandes, and Martinez 2013). These increases were accompanied by an increase in the quantity of chloride cells in the lamellae and of N$^+$/K$^+$-ATPase activity. They suggested that these results reflect the stimulation of the pathway to Na$^+$ uptake, as demonstrated by the activation of Na$^+$/K$^+$-ATPase activity, which resulted in an increase in Na$^+$ concentration and plasma osmolality. Other possible reasons for increased ATPase activities could be related with the period of adaptation processes and/or increased number of enzyme molecules or turnover rates of the enzyme to maintain the ion flux during metal toxicity (Atli et al. 2016).

Cd changed the concentration of serum ions (i.e. Na+, Cl$^-$ and K$^+$) and SO of exposed fish in hypo-osmotic condition (at salinity of 0 and 5 g l^{-1}). The levels of serum Na$^+$, Cl$^-$, K$^+$ ions, and SO of Cd-exposed fish were not significantly different from the controls in the hyperosmotic condition (at salinity 10 and 15 g l^{-1}). The fact that Cd did not affect SO, Na$^+$, Cl$^-$ and K$^+$ levels of *O. niloticus* could indicate that this species can maintain an almost constant osmotic concentration of the serum in media with higher salinities (10 and 15 g l^{-1}). The stable osmotic concentrations in the 10 and 15 g l^{-1} acclimated fish mean stable expenditure of energy (Sampaio and Bianchini 2002), which could increase tolerance of the fish during Cd exposure. Our results suggest that in hypo-osmotic condition (0 and 5 g l^{-1}), Cd might induce reduced growth and/or reproduction rate by increasing osmoregulatory energy expenditure.

The direct effects of metals on blood parameters are usually associated with increased disintegration of erythrocytes or damage to the haemopoietic system (Svobodova, Vykusova, and Machova 1994). We noted that at low salinity (particularly 0 g l^{-1}), the levels of RBC, Hb and Ht in Cd-exposed fish were lower compared to the controls. A significant decrease in the number of RBC suggested that Cd may destroy RBC during circulating erythrocytes. The decrease in RBC coupled with the decrease in Hb and Ht is an indication that *O. niloticus* experienced anaemic conditions or haemodilution. In this condition, the ability of fish to provide sufficient oxygen to the tissues is considerably restricted and will result in decreased physical activity (Wepener, Van Vuren, and Du Preez 1992a, 1992b; Nussey, Van Vuren, and Du Preez 1995). At higher salinities (5, 10 and 15 g l^{-1}), levels of RBC, Hb and Ht in Cd-exposed fish were not significantly

different from the controls. It is likely that salinity plays a significant role in protecting *O. niloticus* under Cd exposure.

Conclusions

In conclusion, our study shows that the Cd-induced osmotic and ionic regulatory impairment is more pronounced in the specimens of tilapia *O. niloticus* acclimated to low salinity than in those acclimated to higher salinities. At salinity 0 g l^{-1}, Cd induced lower levels of RBC, Hb and Ht in Cd-exposed fish compared to the controls. In contrast, at higher salinities (5, 10 and 15 g l^{-1}), levels of RBC, Hb and Ht in control and Cd-exposed fish were not significantly different. This phenomenon indicates that salinity plays an important role in mitigating the toxic effect of Cd on *O. niloticus*.

Acknowledgments

Disclosure statement

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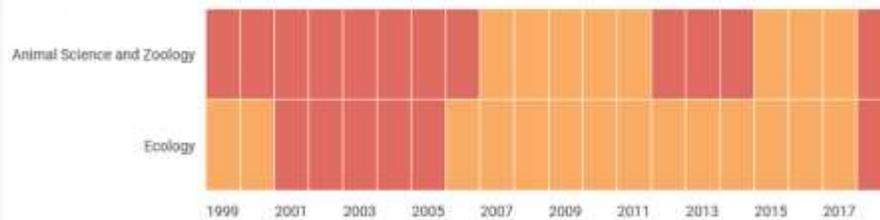|	
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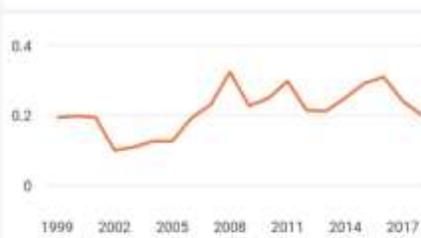
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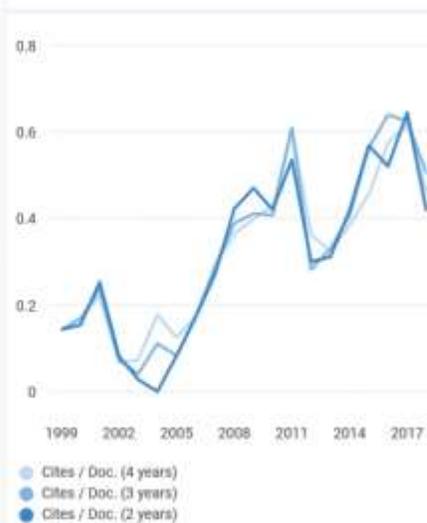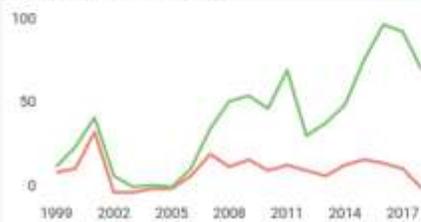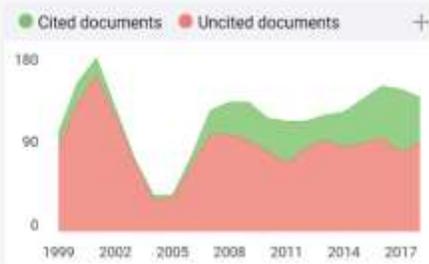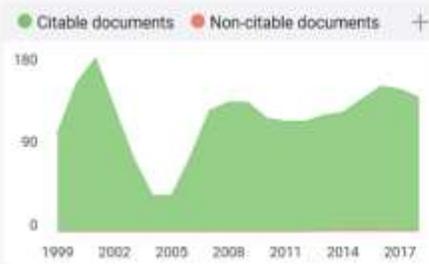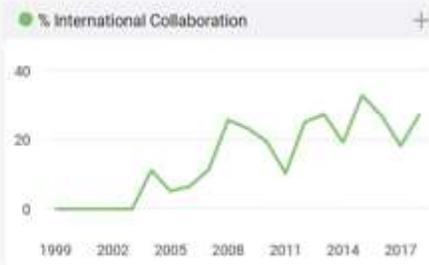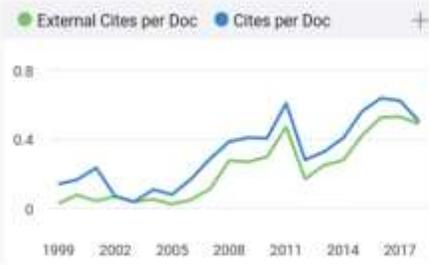
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