Asian Journal of Water, Environment and Pollution, Vol. 17, No. 3 (2020), pp. 49-53.
DOI 10.3233/AJW200035

Effects of Cd, Zn and Cd+Zn Combination on Osmoregulation of Tilapia (*Oreochromis niloticus*)

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Received February 2, 2020; revised and accepted June 5, 2020

Abstract: The objectives of this study were to evaluate the effects of cadmium (Cd), zinc (Zn) and Cd+Zn combinations on serum osmolality and ions in *Oreochromis niloticus.* A total of 60 *O. niloticus* with five fish per tank and two tanks per group were used during this experiment. Group I was held in media without metal (as control) and other groups were exposed to 7.5 mg/L Zn, 15 mg/L Zn, 2.5 mg/L Cd, 7.5 mg/L Zn + 2.5 mg/L Cd and 15 mg/L Zn + 2.5 mg/L Cd for 7 days. The osmolalities of fish exposed to Cd, Zn and Cd+Zn combinations were higher than the control. Fish exposed to 2.5 mg/L Cd presented the highest level of Na^+ and Cl^-, and the lowest level of K^+. This study shows that the osmotic and ionic regulatory impairment is more pronounced in fish exposed to Cd than in Zn and Cd+Zn combinations. This phenomenon indicates that Zn plays a prominent role in suppressing the toxic effect of Cd on *O. niloticus*.

Key words: Fish, cadmium, zinc, osmolality, ions, serum.

Introduction

Anthropogenic activities, such as agriculture, mining and industry, have exponentially increased the amount of metals in aquatic ecosystems (Guimaraes-Soares et al., 2006). Exposure of aquatic animals to metals can lead to several toxic effects, such as alterations of biochemical and physiological mechanisms, and can ultimately cause mortality (Heath, 1995).

Change in physiological mechanisms such as osmoregulation and plasma ion levels can be used to identify environmental contamination before the health of aquatic organism is seriously affected (Mayer et al., 1992). Shifts in the hydro-mineral balance may be a consequence of the action of pollutants on organs involved in osmoregulation, on the endocrine system, on metabolism or on active transport processes (Martinez and Colus, 2002).

Tilapia *Oreochromis niloticus* is a widely distributed freshwater fish that can persist in a highly polluted habitat and has the potential to develop as a biological monitor of environmental pollution (Firat and Kargin, 2010). Aquatic environments are generally contaminated with mixtures of heavy metals; however, many studies examining the metal toxicity to the serum biochemistry of fish have evaluated the effects caused by exposure

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to a single metal (Adhim et al., 2017; Listiyani et al., 2018). Metal interaction in natural waters can be seen as synergism, antagonism or additive, and can affect the absorption, distribution, excretion and also biological functions of metals (Brzoska and Moniuszko-Jakoniuk, 2001).

Cadmium and Zn are chemically similar metals and can be taken up through similar transport pathways (Rainbow and Blackmore, 2001). Cadmium is a nonessential and highly toxic metal, whereas Zn is an essential metal that can be toxic at high concentrations (Chen et al. 2008; Soegianto et al. 2013). Cadmium uptake was suppressed in the presence of Zn in a number of marine phytoplankton or cell models (Sunda and Huntsman 2000; Noel et al. 2006). Thus, the aim of this study was to determine the effects of individual Cd, Zn, and combined Cd and Zn exposures on osmolality and ions level of *O. niloticus*.

Materials and Methods

Fish Acclimation and Experimental Design

Oreochromis niloticus (55.6 ± 2.4 g, 14.6 ± 0.5 cm; mean ± SD) obtained from Pasuruan, East Java, Indonesia were transferred to the laboratory. Fish were acclimatised to laboratory conditions for 14 days in 250 L holding tanks supplied with a continuous flow of dechlorinated tap water through gravel, sand and sponge filter before exposure (Soegianto et al., 2017). The laboratory was illuminated with artificial light-dark cycle 12:12 using cool white fluorescent lamps with a light intensity of 3600–4000 lux. Fish were fed twice a day with Takari commercial pellets (30% protein, 3% fat and 4% fiber) for every 3% of the fish body weight. Uneaten food and debris were removed daily to maintain good water quality. Throughout the acclimation and experimentation tests, the values of temperature, pH and dissolved oxygen were measured to be 28-30ºC, 7.60 – 8.10 and 7.1 – 7.5 mg/L, respectively. The experiments in the present study were conducted in accordance with the principles and procedures that were approved by the Institutional Animal Care of Universitas Airlangga.

We used a total of 60 *O. niloticus* with five fish per tank and two tanks per group. Experimental tanks contained 50 L of experimental media. Group I was held in tap water as control and other groups were exposed to 7.5 mg/L Zn, 15 mg/L Zn, 2.5 mg/L Cd, 7.5 mg/L Zn + 2.5 mg/L Cd and 15 mg/L Zn + 2.5 mg/L Cd for 7 days. Zn and Cd concentrations were selected based on 96-h LC_{50} values (36.5 mg/L for Zn and 7.5 mg/L for Cd) of *O. niloticus*. The stock solutions of Cd (1000 mg/L) and Zn (1000 mg/L) were prepared by dissolving 2.744 g Cd $(NO_3)_2.4H_2O$ (Merck, Darmstadt, Germany) and 4.332 g $ZnSO_4.7H_2O$ (Merck, Darmstadt, Germany) in 1000 ml deionised water, respectively. The test media were changed every 2 days to minimise decreases in the metal concentrations. During the test, fish were fed twice a day with Takari commercial pellets at 3% of the fish body weight.

Analysis of Serum Osmolality and Ions Level

At the end of exposure period, five fish were randomly removed from each treatment. Prior to blood sampling, fish were anesthetised with 200 mg/L clove solution (Adhim et al., 2017). Blood samples of each fish were taken by puncturing the heart using a non-heparinised syringe. Then, blood samples were placed in anti-coagulant-free tubes for the assessment of serum osmolalities and serum ions.

Serum was obtained by centrifugation of the blood at 4500 × g for 10 minutes and measured for osmolality, Na^+, Cl^- and K^+ concentrations. Serum osmolality was measured using an automated osmometer (Fiske® 210 Micro-Sample Osmometer, USA). The osmolality of the serum sample is expressed as mOsm/kg. Serum ions Na^+, Cl^- and K^+ were measured by potentiometic (ion-selective electrode) method using an automated electrolyte analyzer (Jokoh EX-D, Japan).

Statistical Analysis

All data were expressed as mean ± standard deviation and their normality and homogeneity verified before statistical analysis. The comparisons of the effects of metals on osmolalities and ion levels were analysed using one way analysis of variance, respectively. When significant differences were detected ($p<0.05$), Duncan's multiple range test was used to determine which treatment is resulting in significant effect on osmolalities and ion levels at a significance level of 0.05.

Results and Discussion

The levels of osmolality and ion of tilapia serum after being exposed to different levels of metals (Cd, Zn and Cd+Zn) are presented in Figures 1 to 4. The osmolalities of all exposed fish to Cd, Zn and Cd+Zn combinations were higher than the control (Figure 1). Only fish exposed to 2.5 mg/L Cd presented the level of Na^+ higher than the control (Figure 2). Similarly, only fish exposed to 2.5 mg/L Cd demonstrated the level of Cl^- higher than the control (Figure 3). Only fish exposed to 2.5 mg/L Cd presented the lowest level of K^+ (Figure 4).

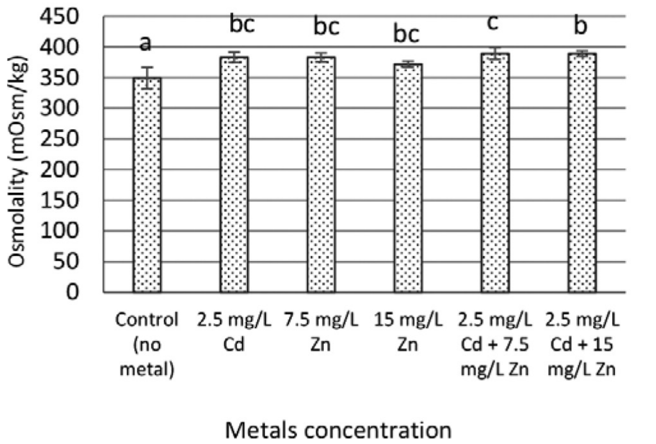

Figure 1: Osmolality of serum (mOsm/kg) of *O. niloticus* after being exposed to different levels of metals. Different lowercase letter indicates significance difference according to Duncan test (p< 0.05).

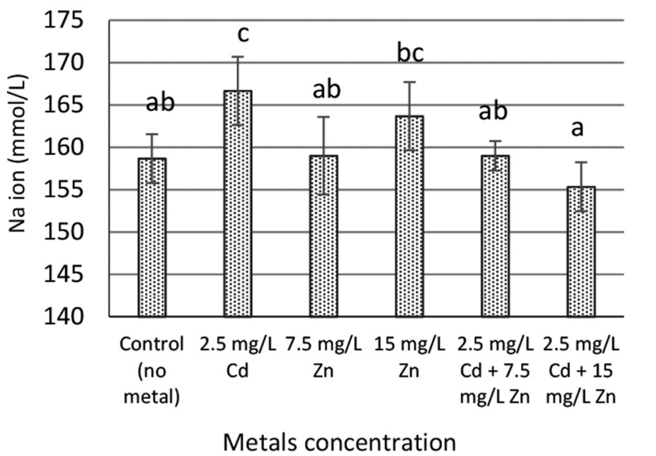

Figure 2: Na^+ level (mmol/L) in serum of *O. niloticus* after being exposed to different levels of metals. Different lowercase letter indicates significance difference according to Duncan test (p< 0.05).

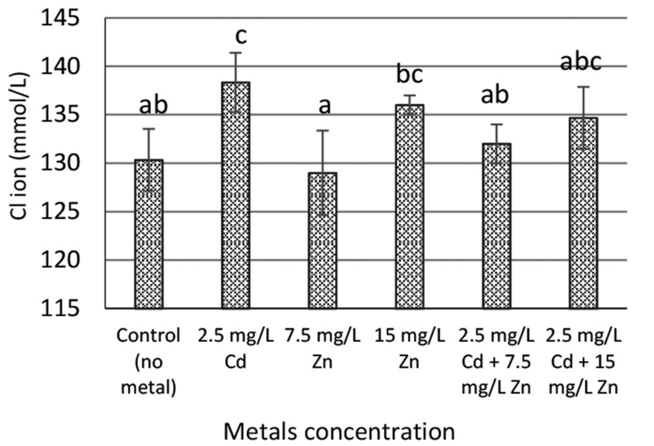

Figure 3: Cl^- level (mmol/L) in serum of *O. niloticus* after being exposed to different levels of metals. Different lowercase letter indicates significance difference according to Duncan test (p< 0.05).

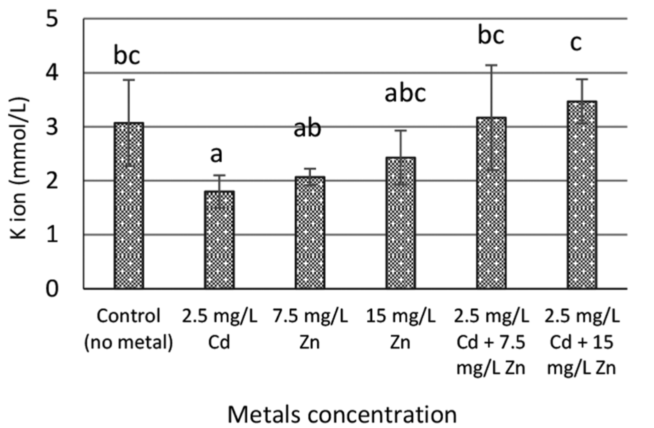

Figure 4: K^+ level (mmol/L) in serum of *O. niloticus* after being exposed to different levels of metals. Different lowercase letter indicates significance difference according to Duncan test (p< 0.05).

The higher levels of Na^+ and Cl^- in the Cd-exposed fish than in other metal-exposed fish suggest that it is a temporary state of ion imbalance as has been reported in tilapia exposed to Pb (Adhim et al., 2017). Since there were no significant difference of osmalities among metals exposed fish (Cd, Zn and Cd+Zn combinations), the increases of Na^+ and $Cl^−$ in fish following exposure to Cd might indicate that ion uptake mechanisms were not yet down-regulated, resulting in greater net uptake under conditions of greater NaCl availability (Kolbadinezhad et al., 2012). Gilles and Delpire (1997) reported that significantly increased levels of Na^+ after being exposed to Cd are in line with increasing the serum osmolality. This is because osmotic concentration is influenced by the amount of inorganic ions, especially Na^+ and Cl^- ions (Gilles and Delpire, 1997).

Eroglu and Canli (2013) showed that there was a significant decrease in Na^+/K^+-ATPase in the gill *O. niloticus* when exposed to Cd, but it increased significantly when exposed to Cd+Zn. This shows that Cd uptake was suppressed in the presence of Zn. Attar and Maly (1982) reported that the toxicity of Cd to *Daphnia magna* was higher than the toxicity to the Cd+Zn combination for 96 hours. Chen et al. (2008) demonstrated that the absorption of Cd was suppressed by Zn to some extent in the *Bacillus firmus* bacterium.

The level of serum K^+ decreased only in Cd-exposed fish, however, it did not change in other metal-exposed fish. This phenomenon might have occurred to balance the osmotic differences in the intracellular fluid caused by the increase in Na^+ and Cl^-. Listiyani et al. (2018) suggested that in metals-exposed fish, gills are permeable to K^+ , therefore, that efflux is greater than influx. Patridge and Lymbery (2008) suggested that a reduced uptake rather than increased loss of K^+ is a more important factor. Meanwhile, Nussey et al. (1995) suggested that the decrease in serum K^+ concentration could be ascribed to osmotic adaptation.

Conclusion

In conclusion, our study shows that the osmotic and ionic regulatory impairment is more pronounced in the specimens of tilapia *O. niloticus* exposed to Cd than in Zn and Cd+Zn combinations. This phenomenon indicates that Zn plays an important role in suppressing the toxic effect of Cd on *O. niloticus*.

Acknowledgements

This research was supported by the grant from Faculty of Science and Technology, Universitas Airlangga (PUF, 1408/UN3/2019). The authors would like to thank Prof. Bambang Irawan for constructive comments and Mrs. Sulaiman Budi Santoso and Alif Firmansyah Maidi for technical assistance during the experiment.

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Asian Journal of Water, Environment and Pollution

Volume 17 Number 2 **April – June, 2020**

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