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Effects of *In-Situ* Cadmium Exposure on Morphometric Indices of *Anabas testudineus*

Mohd Sham Othman*, Sharifah Nadrah Syed Idrus, Fazlin Hazirah Mohd, Mohd Riduan Abdullah

Center for Toxicology & Health Risk Studies (CORE), Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia

Email: *mso@ukm.edu.my

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Abstract

Anthropogenic activities have greatly affected water resources on a global scale where the world is experiencing water quality and resources issues. Heavy metal is a crucial group of pollutants that is toxic to the environment even at low concentrations due to its bioaccumulation and biomagnification capabilities in living organisms. The detrimental effects of heavy metals on living organisms are due to their bioaccumulation in the aquatic ecosystem. Cadmium may result in adverse health effects due to its high toxicity. The study is conducted to determine the cadmium exposure effects on the morphometric indices of *Anabas testudineus* which are the Scaling Coefficient (SC) and Condition Factor (K) of such species. *Anabas testudineus* is exposed to four different cadmium treatment groups namely the control group, cadmium treatment group of 0.005 mg/L, 0.010 mg/L, and 0.015 mg/L for 16 weeks. The findings of the study have reported inconsistent trends in the values of SC and a decrease in the value of K with increasing cadmium concentration. The trend for the average SC has shown an overall decrease in value while the pattern of the K value is inconsistent in each treatment group with exposure time. Collectively, no significant differences for SC and K of *A. testudineus* in different treatment groups as well as comparison between treatment groups with time exposure.

Keywords

Cadmium, *Anabas testudineus*, Scaling Coefficient, Condition Factor

1. Introduction

The heavy metal pollution scenario is no longer foreign to Malaysia. Excessive urbanization as well as industrial activities are the main factors causing pollution in the Selangor River [1]. This is worrying because the river is the main source of raw water for drinking water in Malaysia. The rapid change of the natural geochemical cycle for metals is due to the disruption of such cycles by humans which causes the accumulation of heavy metals in the soils of urban and rural areas [2].

Cadmium is a type of heavy metal that has been chosen for this study due to its high toxicity properties which in turn can cause negative impacts on living organisms in general. Cadmium has been classified as a Group 1 heavy metal which is carcinogenic to humans [3]. Based on a study conducted by [4], the Agency for Toxic Substances and Disease Registry (ATSDR) has classified cadmium as the seventh most toxic heavy metal. According to [5], cadmium is commercially used in the manufacturing of paint pigments, cosmetics, television screens, and so on. [5], the consumption of food contaminated with cadmium such as leafy vegetables, meat organs, crustaceans, and contaminated water can cause adverse health implications to organisms in the long term. However, human activity is the main source of cadmium exposure to humans such as zinc and lead fusion, battery production processes, plating processes, and also in tobacco use [6].

Cadmium is an element that has a high level of toxicity to mammals as well as fish [7]. Based on the same study, due to the ability of cadmium to concentrate in living organisms, cadmium has become an ecological issue of concern. Short-term exposure to cadmium can cause inflammation which is accompanied by cough, dryness as well as irritation to the nose and throat, chest pain, dizziness, pneumonitis, and pulmonary edema [8]. Nephrotoxic, carcinogenic, teratogenic as well as toxic effects on the endocrine and reproductive systems are due to cadmium [9].

For this study, fish is selected as a biological indicator to identify the effects of cadmium exposure on the organism in terms of changes in the weight and length of fish. According to [10], consumers can experience adverse health problems if fish have been contaminated. A study by [11] has reported that fish can accumulate heavy metals in its body tissues. Hence, the information on the types of fish consumed and the level of heavy metal content contained in them are useful in reducing the danger to public health [11]. *Anabas testudineus* is a fish species selected for the implementation of this study. According to [12], *Anabas testudineus* species can survive and thrive in low dissolved oxygen conditions as well as in low pH and also at a condition with high temperatures.

The study is conducted to identify the effects of cadmium exposure on the Scaling Coefficient (SC) and Condition Factor (K) of *A. testudineus* in different concentrations of cadmium over time exposure of 16 weeks. Fish species of *Anabas testudineus* also known as the climbing perch has been selected as a biological indicator for the effects of cadmium exposure in different cadmium concentration groups with time exposure. According to a study [13] [14] [15] as cited by [16], the climbing perch is a native species which has high endurance against harsh environments which includes hypoxia, high salinity environment, low pH, and contaminants as well.

2. Materials and Methods

The study is an experimental research in which 500 hundred individuals of *A. testudineus* measuring 2 - 3 inches and weighing 5 grams on average are purchased from LKH Aquapets in Pasar Pudu, Kuala Lumpur. Fish rearing has been commenced at the Ecotoxicology Laboratory, Level 3, Block D at the Faculty of Health Science, Universiti Kebangsaan Malaysia. A total of 125 *A. testudineus* individuals are split up and exposed to four different cadmium treatment groups namely the control group, cadmium treatment group of 0.005 mg/L, 0.010 mg/L, and 0.015 mg/L for 16 weeks. The fish are placed in the plastic aquarium throughout the research period. The dechlorinated water is used for fish rearing. A three-decimal point digital scale and caliper are used to measure the length and weight of sampled individuals of *A. testudineus.*

Prior to the fish-rearing process, the tap water is filled in a large container and is left overnight to remove the chlorine content in the water. Each plastic aquarium is filled with dechlorinated water before cadmium treatment is carried out. Cadmium nitrate solution is then placed in each aquarium according to the concentration that has been determined for each treatment group.

According to the [17], the stocking density is 10 - 20 kilograms of fish per 1000 liters of water. Based on the calculation referring to the following study, a total of 20 - 40 individuals are placed in each aquarium for every 10 liters of water with an average weight of 5 grams for each fish. Findings from a study conducted by [18] have shown that water quality is not affected by the stocking density of 4 juveniles per liter within 3 months of fish farming. Before the fish rearing in different cadmium treatment groups, fish are reared in tanks filled with dechlorinated water for one week to allow the fish to adapt to the laboratory setting.

Commercial fish pallets are given once a day to the farmed fish for feeding purposes. The amount of 5% of fish palettes of the average weight of fish is given based on a study conducted by [19]. Ad libitum feeding technique is carried out in which fish are given commercial pallets once a day until satiation is reached [20]. Furthermore, the aquarium cleaning process has been implemented once in every two weeks to ensure the quality of aquarium water is maintained. The optimum dissolved oxygen value is 5 ppm according to [21] as reported by [18], while *A. testudineus* are capable of surviving in water with a pH value of 3.5 to 9.5 [22]. Next, the preferred water temperature for fish farming is between 26.06˚C to 31.97˚C based on [23] as cited by [18].

For every 4 weeks, 20 to 40 A. testudineus individuals are sampled within 16 weeks of the research period. In total, 160 A. testudineus individuals are sampled. A total of 340 excess fish are farmed to ensure that the fish samples for the study are sufficient based on the survival rate of 89% for A. testudineus [24]. The Cold Shock or Ice Water Euthanasia process is implemented before data collection. Next, the sampled fish are dried by using tissue before the weighing process by using a three-decimal point digital scale. The gram unit is used for the fish weight while the fish length is measured by using a caliper. The proper fish dissection technique is practiced ensuring that the kidneys, liver, gonads, and gills of the fish are not damaged which are then used to analyze the morphometric indices of A. testudineus which consist of Scaling Coefficient (SC) and Condition Factor (K) parameters. Scaling Coefficient is the ratio between weight and length of the fins and it is derived empirically from the log-transformed equation of weight versus length graph. The SC value can be determined by calculating the extrapolation logarithm of the weight-length relationship of the fish obtained via linear regression [25] at which the b value is then used to determine the value of K by using the formula following the study conducted by [26]. The Condition Factor (K) is a coefficient that reflects the physical and biological circumstances, hence the general health of a fish.

3. Results and Discussion

3.1. Scaling Coefficient (SC)

Figure 1 shows a comparison of the mean Scaling Coefficient (SC) of *A. testudineus* in four different treatment groups namely control group, 0.005 mg/L group, 0.010 mg/L group and 0.015 mg/L group. The value of SC or also known as the value of b of *A. testudineus* is obtained from the linear regression graph. Next, the value of R^2 is also determined by an empirical formula derived from the linear regression graph.

Based on **Figure 1**, the 0.010 mg/L group is reported with the highest SC value of 3.08 ± 0.10 with R2 = 0.97, followed by the 0.005 mg/L group of 2.99 ± 0.24 with R2 = 0.99. The control group has the lowest SC value of 2.81 ± 0.35 with R2 = 0.96 followed by the 0.015 mg/L group with SC value of 2.95 ± 0.22 and R2 = 0.96. The One-Way ANOVA test is performed as the data distribution for SC value is normal. Based on the findings, there is no significant difference ($p > 0.05$) of SC values in different treatment groups.

Figure 2 shows a comparison of the average SC of *A. testudineus* according to time exposure in four different treatment groups namely control group, 0.005 mg/L

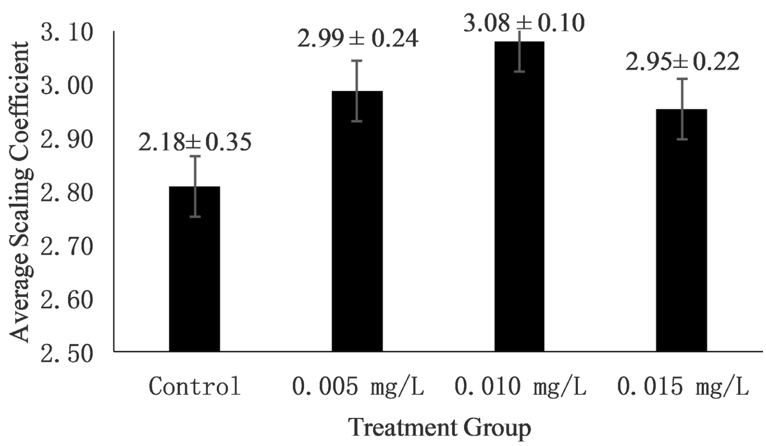

Figure 1. The average Scaling Coefficient (SC) in different treatment groups.

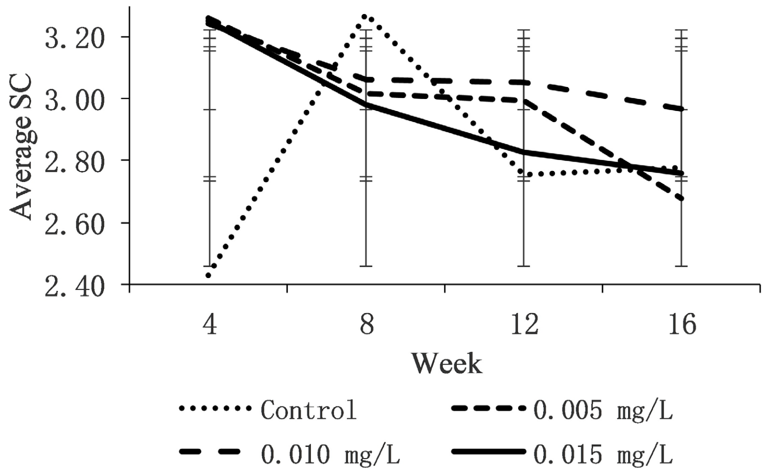

Figure 2. The comparison of average Scaling Coefficient (SC) of each treatment group with time exposure.

group, 0.010 mg/L group and 0.015 mg/L group. Based on the following figure, the trend of the overall average SC for each treatment group has shown a decrease in value over the exposure period.

In the fourth week, the control group has the lowest SC value of 2.43 with a value of $R^2 = 0.90$, followed by the 0.015 mg/L group with an average SC value of 3.25 and a value of $R^2 = 0.96$. Next, the 0.010 mg/L group has the highest mean value where the SC value is 3.29 with the value of $R^2 = 0.98$. The 0.005 mg/L group is reported to have the second-highest SC value of 3.26 with $R^2 = 0.99$. In the eighth week, the control group has shown an increase in SC value of 3.27 with R^2 value = 0.99 which is the highest mean value for SC, followed by 0.010 mg/L group with SC value of 3.06 and R^2 value = 0.98. The 0.015 mg/L group has the lowest SC value of 2.98 with a value of $R^2 = 0.99$, followed by the group of 0.005 mg/L with a total SC value of 3.02 and with a value of $R^2 = 0.99$.

In the twelfth week, the control group has reported a significant decrease in the value of SC of 2.75 with a value of $R^2 = 0.98$ where the mean value is the lowest for SC. Next, the 0.015 mg/L group has the second lowest SC value for Week 12 with an average SC value of 2.83 and an R^2 value = 0.94. The 0.010 mg/L group with SC value of 3.05 and R^2 value = 0.97 is the treatment group with the highest SC value, followed by the 0.005 mg/L group which is 3.00 with R^2 value = 0.99.

In the sixteenth week, the 0.005 mg/L group has the lowest SC value of 2.68 with $R^2 = 0.98$, followed by the 0.015 mg/L group with an average SC value of 2.76 and $R^2 = 0.94$. The 0.010 mg/L group has the highest SC value with an average SC value of 2.97 with a value of $R^2 = 0.95$, followed by the control group with an SC value of 2.78 with a value of $R^2 = 0.97$.

The Two-Way ANOVA test is used for the comparison of SC values for all treatment groups with time exposure. The test is also conducted based on the data distribution of SC which is normally distributed. The test findings have noted that there is no significant difference in the SC values for all treatment groups with time exposure ($p > 0.05$).

Morphometric features are important in understanding the health and reproductive activities of the species concerned in an environment and also in understanding the taxonomy of the studied species [27]. Based on the findings from **Figure 1**, the trend for average SC is inconsistent with increasing cadmium concentrations in different treatment groups. The study of [27] has conceptualized that the increase in fish weight and length is parallel with increased development as well as growth of fish in separate environments.

Based on **Figure 1**, the 0.010 mg/L group has a b value that exceeds the value of 3, where according to [27], a symmetrical or isometric fish growth has a b value that equals to 3 for the relationship of fish weight and length. Next, the 0.015 mg/L group is the second treatment group with the lowest SC value, followed by the control group and also the 0.005 mg/L group where all three treatment groups have a b value lesser than 3. The fish length will increase as well as becoming thinner as a result of negative allometric growth, according to [28]. This is also supported by [29] where a b value of lesser than 3 indicates a negative allometric growth in which the fish will become thinner with increasing body length. The study of [30] has reported that the quality and quantity of food, age and water quality influence fish growth where the increase in biomass of a fish is as a result of changes in food given to fish biomass. Based on **Figure 1**, the group of 0.010 mg/L has the highest mean value of SC which is above the value of 3 where based on the study of [31], higher nutritional efficiency can lead to positive allometric growth [32].

Figure 2 has recorded a mean decrease in the SC pattern for all treatment groups with time exposure. The 0.015 mg/L group has shown a decrease in SC values that are consistent throughout the exposure period, which are also similar to the 0.005 mg/L group and the 0.010 mg/L group. Based on the study of [33], the negative allometric growth is a reference to a smaller b value where the advancement of the fish size produces a lighter fish. Based on the similar study, a fish will be heavier in weight if the value of b is larger with a certain length.

3.2. Condition Factor (K)

Figure 3 shows a comparison of the mean Condition Factor (K) for *A. testudineus* in four different treatment groups namely control group, 0.005 mg/L group, 0.010 mg/L group and 0.015 mg/L group. The 0.005 mg/L group has the highest K value and is followed by the control group which are 5.31% ± 0.36% and 5.19% ± 0.40% respectively. Next, the lowest value for K is the 0.015 mg/L group which is 5.04% ± 0.15%, while the second lowest value of K is 5.13% ± 0.14% which is the 0.10 mg/L group.

The One-Way ANOVA test is applied as the data distribution of K value data is normal. The test results have shown that there is no significant difference ($p > 0.05$) of K values in different treatment groups.

Figure 4 shows a comparison of the mean Condition Factor (K) for *A. testudineus* according to time exposure in four different treatment groups namely

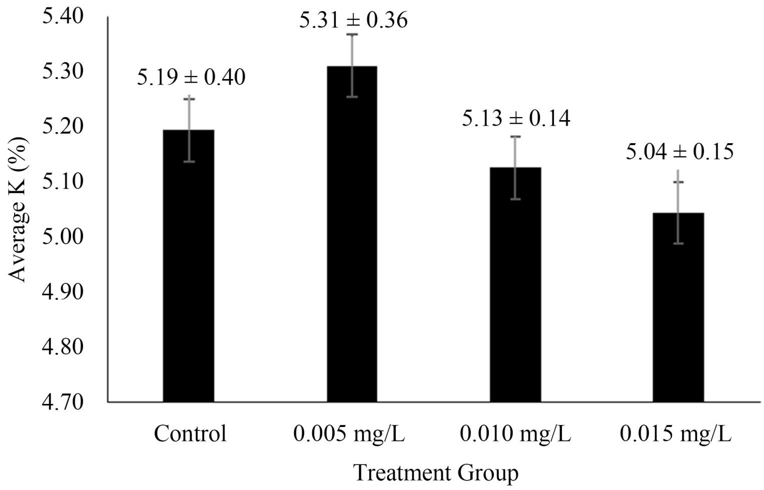

Figure 3. The average Condition Factor (K) in different treatment groups.

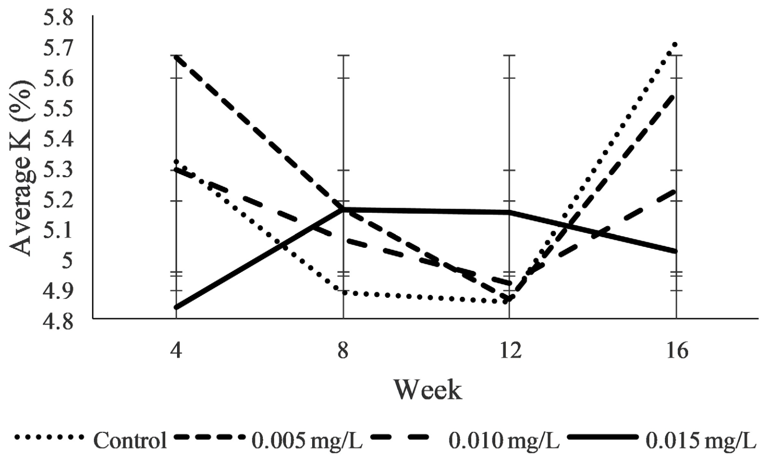

Figure 4. The comparison of average Condition Factor (K) of each treatment group with time exposure.

control group, 0.005 mg/L group, 0.010 mg/L group and 0.015 mg/L group. According to the figure, the overall trend for the treatment groups has shown a decrease in K value from Week 4 to Week 12, but an increase in K value is observed from Week 12 to Week 16. However, the trend for the 0.015 mg/L group is inconsistent throughout the exposure period.

In the fourth week, the 0.015 mg/L group has the lowest mean K value of 4.84 ± 1.16 %, followed by the 0.010 mg/L group with a mean K value of 5.29 ± 0.61 %. Next, the 0.005 mg/L group has recorded the highest mean K value of 5.66 ± 0.62 %, where the control group has the second highest mean K value of 5.32 ± 0.74 %.

In the eighth week, the control group is reported with the lowest mean value of 4.89% ± 0.30% followed by the 0.010 mg/L group with an average K value of 5.06% ± 0.32%. Furthermore, the group with the second highest mean K value is the 0.015 mg/L group with a mean K value of 5.16% ± 0.27%. The 0.005 mg/L group has the highest mean K value of 5.17% ± 0.30%.

In the twelfth week, the 0.015 mg/L group is reported with the highest mean K value of 5.15% ± 0.34%, followed by the 0.010 mg/L group with a mean K value of 4.92% ± 0.37%. Next, the control group has the lowest average K value of 4.86% ± 0.24% and is followed by the 0.005 mg/L group with an average K value of 4.87% ± 0.29%.

In the sixteenth week, the 0.015 mg/L group is the treatment group with the lowest mean K value of 5.02% ± 0.46%, followed by the 0.010 mg/L group with the mean K value of 5.22% ± 0.33%. Next, the control group is the treatment group with the highest mean K value of 5.71% ± 0.39%, while the 0.005 mg/L group is the second highest treatment group with a mean K value of 5.54% ± 0.27%.

The Two-Way ANOVA Test is used for the comparison of Condition Factor values in each treatment group according to time exposure. This test is also performed based on the overall data distribution for the Condition Factor of *A. testudineus* is normal. The results of this test have noted that there is no significant difference for Condition Factor of *A. testudineus* in all treatment groups with time exposure ($p > 0.05$).

Condition Factors for fish are vary based on the interaction between nutritional conditions, parasitic infections and physiological factors as well as the current physical and biological conditions of the fish [26]. The general health condition reflects the Condition Factor of a fish where it is different based on environmental factors, age, sex and breeding period [34]. The understanding of the Condition Factor is important in the identification of different fish body lengths during maturation as well as fish reproduction during the lifespan of the fish [33]. Biotic and non-biotic environmental conditions strongly influence the Condition Factor where it is also used as an index to identify the ecosystem conditions in which the fish live, according to [35].

Figure 3 has noted that the 0.015 mg/L group has the lowest mean value of K and is followed by the 0.010 mg/L group. [34] study has stated that the effects of heavy metals may cause the fish to lose appetite or due to excessive energy consumption which results in a decrease of the K value. Furthermore, the decrease in the value of K also shows that fish are sensitive to ecological and environmental changes [36]. The 0.005 mg/L group has the highest mean K value, followed by the control group. The high K value for the 0.005 mg/L group may be due to the factor of cadmium that can act as an anti-parasite, according to the study of [37]. The study of [33] has reported that the relative fatness of a fish is an expression of the Condition Factor where the better condition of the fish has a greater K value.

Based on **Figure 4**, the average trend of K values for all treatment groups is inconsistent throughout the exposure period. Environmental factors, food supply, ecological conditions of the habitat in question as well as physiological diversity are the factors that influence the diversity in the growth rate of a similar species in different months [26]. While the food supply and environmental factors while rearing the fish could be controlled, the physiological diversity of each fish is seen as the major determinant of the inconsistencies of the K value. The ratio of female to male, along with the number of gravid female individuals in each exposure group would affect the K value of the fish population. If the population has more gravid females, this would increase the K value because the body weight has significantly increased due to the weight of the eggs. If these same females have started laying their eggs, their body weight would decrease significantly, hence decreasing the K value. In this research, decreases in K values for all groups except the 0.015 mg/L group can be seen in Week 12. Based on the same figure, the control group, the 0.005 mg/L group as well as the 0.010 mg/L group have shown an increase in value from Week 12 to Week 16. The subsequent reproductive period as well as the preparatory phase for both sexes that produce high K values may be associated with the increased feeding intensity [33]. The same study also states that the increase may be due to the reconstruction of fish body reserves for the next breeding period for the formation of fish gonads concerned. Based on the findings of the study, the use of Condition Factor parameters to identify the effect of cadmium on *A. testudineus* may be less appropriate, especially starting from Week 12. This is because the total weight of the female fish in the population would include the weight of the eggs as well. Therefore, these extra weights would skewer the weight-length ratio and fish Condition Factor parameter.

4. Conclusions

The study on cadmium exposure to *A. testudineus* has shown effects on the morphometric indices of the species in different treatment groups with an exposure time of 16 weeks. Based on the results of the study findings, the pattern for SC is inconsistent in different treatment groups. The overall trend for the average SC in each treatment group with time exposure has shown a decrease in the value of SC. Next, the pattern for the Condition Factor has shown a decrease in the K value with increasing cadmium concentration. Nevertheless, the inconsistent trend for the average Condition Factor can be observed in each treatment group with time exposure.

Collectively, no significant differences are reported in different treatment groups and for comparison between different treatment groups with time exposure for the Scaling Coefficient and Condition Factor of *A. testudineus*. It can be concluded that cadmium exposure to *A. testudineus* influences the morphometric indices of *A. testudineus* in different treatment groups in a span of 16 weeks. Studies on the effects of cadmium exposure on morphometric indices of *A. testudineus* can be carried out to determine the effect of cadmium on the affected fish population in the short and long term.

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Conflict of Interest

The authors whose names are listed clarify that they have no participation or affiliation in any organizations that involve financial or non-financial interests in the subject or matter discussed in this manuscript.

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