

Acute Toxicity of Cadmium on Silver Barb, *Barbonymus gonionotus* (Bleeker, 1850); Nile Tilapia, *Oreochromis niloticus* (Linnaeus, 1758); and Climbing Perch, *Anabas testudineus* (Bloch, 1792)

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Received: May 24, 2023; Revised: July 4, 2023; Accepted: October 4, 2023

Abstract

The use of fish species as environmental biomarkers was proposed. A bioindicator and monitor of their effect on the health of creatures and aquatic ecosystems may be found by investigating the toxicity of heavy metals in fishes. The acute toxicity of cadmium and their toxicological effects on median lethal concentration (LC₅₀) of widely consumed three freshwater fishes included Silver barb, *Barbonymus gonionotus* (Bleeker, 1850), Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) and Climbing perch, *Anabas testudineus* (Bloch, 1792) were observed for 96 hrs to determine suitable ranges to be employed in the lethality test's final trials, preliminary trials were done. In the final trials, mortalities were assessed and LC₅₀ values were calculated values by Probit analysis. The results showed that LC₅₀ values increased with a decrease in mean exposure times for Cd. The LC₅₀ values of Cd on *B. gonionotus* for 24, 48, 72 and 96 hrs were 26.9946, 17.7884, 12.1246, and 5.1713 mgCd/L respectively. The values of LC₅₀ on *O. niloticus* were 57.6966, 46.2676, 42.4668, and 41.7624 mgCd/L, respectively; and the LC₅₀ values of Cd on *A. testudineus* were 134.5610, 108.5223, 73.2544, and 47.3254 mgCd/L, respectively. In the control group, no mortality and normal behavioral response of fishes were occurred during the experimental period. These results revealed higher tolerance among *A. testudineus* than *O. niloticus* and *B. gonionotus*. The outcome of this study provides index for management, monitoring, and surveillance of Cd accumulation and consumption of these fish species.

Keywords: Climbing perch; Nile tilapia; Silver barb; Heavy metals; LC₅₀; Toxicity

1. Introduction

Freshwater are the most impacted habitats by human activities because they are exposed to xenobiotics and natural contaminants (Cruz-Esquivel *et al.*, 2023). Through a variety of environmental processes, pollutants are moved from their sources to rivers and streams (Thanigaivel *et al.*, 2023).

Heavy metal contamination of these ecosystems from mining, metallurgy, battery production, sewage treatment discharge, agricultural runoff, and domestic stormwater runoff has been extensively studied, primarily because of its detrimental effects on public health and high toxicity for aquatic organisms

(Alengebawy et al., 2021; Das and Poater, 2021; Elgarahy et al., 2021; Islam and Mahdi, 2022).

Cadmium, mercury, lead, chromium, and copper are the most poisonous heavy metals, which can be fatal to many fish species at a low concentration $\mu\text{g/L}$ (Balali-Mood et al., 2021). It has been thoroughly studied how poisonous cadmium is to fish, and it is widely acknowledged that the metal's free cadmium ion form is the most easily absorbed form (Liu et al., 2022). Freshwater fishes mainly take up waterborne Cd through their gills, and it is transported to other organs and tissues via the circulatory system (Liu et al., 2022). Bioaccumulation of Cd can lead to various toxic effects, the main targets being ion regulation and oxidant-antioxidant balance (Khan et al., 2022; Liu et al., 2022). Given that fish is one of the most important protein sources, it is quite possible that Cd can be enhanced in fish and transported into humans through the food chain (Liu et al., 2022). Cd accumulation in the human body plays havoc with the cardiovascular, immune, reproductive, and nervous systems (Mielcarek et al., 2022). Simultaneously, it interrupts cell cycle, growth, DNA biosynthesis, and repair, and then impacts the apoptosis pathway (Liao et al., 2021). Fish are a crucial group of aquatic creatures that make up food chain apex and are extremely vulnerable to Cd pollution (Kakade et al., 2023). Long-term exposure to Cd contamination causes numerous tissues in fish to absorb and store Cd, which alters the gonad, liver, and gill structure and function (Rani et al., 2022; Zhang et al., 2023). Additionally, exposure to Cd impairs their physiological metabolism, immunological, and reproductive systems, ultimately resulting in metabolic problems, physical disorders, or even death (Noor et al., 2020; Ferro et al., 2021; Zheng et al., 2021). Recent research has demonstrated that Cd can cause various epigenetic changes, which affect the chemical modification of DNA, histones, and chromatin but not the sequence of DNA nucleotides. These changes affect DNA methyltransferase, histone acetyltransferase, histone deacetylase, histone methyltransferase, and micro RNA, among other epigenetic processes. (Aguilera et al., 2023). Consequently, consuming these

fish may cause disease and development of several cancer types (Cai et al., 2020; Ibrahim et al., 2021; Zhu et al., 2021). As a result, using these indicators to show how Cd contaminants affect the environment is a valuable technique to assess the ecosystem's safety.

In order to address the impact of toxicants on species physiology and survival, numerous environmental agencies and researchers have implemented regulatory guidelines for polluted environments, made suggestions for improving them, and established prevention criteria based on maximum permissible pollution levels (Manisalidis et al., 2020; Ferro et al., 2021). The concentration needed to kill 50% of the population at a specific time after exposure is known as the lethal concentration (LC_{50}) (Zhao and Newman, 2004). It is generally employed as an endpoint indicator because reduction in survival affects population dynamics (Stark et al., 2015). The LC_{50} value is currently incorporated into most guidelines for evaluating environmental status and sanitation improvement since it offers clear, repeatable results and benefits like quick assay times and cheap operating costs (Jager and Ashauer, 2018). Therefore, this study was conducted to determine the acute toxicity (median lethal concentration: LC_{50}) of cadmium on silver barb, Nile tilapia, and climbing perch.

2. Methodology

2.1 Ethics statement

All procedures and experiments on fish were certified by the Institutional Animal Care and Use Committee of Khon Kaen University (License number, IACUC-KKU-38/66), based on the Thai Government under the authority of the Institute Animals for Scientific Purpose Development, the National Research Council of Thailand (License number, U1-08815-2563).

2.2 Experimental fishes

Healthy silver barb, *Barbonymus gonionotus* (Bleeker, 1850), Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758), and climbing perch, *Anabas testudineus* (Bloch,

1792) fingerlings with average body weights (2.39 ± 0.43 , 2.95 ± 0.52 , and 3.80 ± 0.67 g, respectively); average total lengths (6.46 ± 0.34 , 5.32 ± 0.29 , and 5.67 ± 0.36 cm, respectively); and age on 48, 38, and 48 days, respectively were obtained from a hatchery at the Nam Pong District, Khon Kaen, Thailand and transported to the laboratory of Department of Fisheries, Faculty of Agriculture, Khon Kaen University, Thailand. Fingerlings of each species were acclimatized in 3 circular fiberglass tanks (300 fingerlings per tank) containing 200 L of dechlorinated tap water and equipped with aeration for 8 days under laboratory conditions. During the acclimatization, the fish were fed once daily. Each tank was monitored daily to check fish mortality, and dead fish were removed from the tank daily.

2.3 Experimental chemicals

The analytical 99% AR grade cadmium (II) nitrate ($\text{Cd}(\text{NO}_3)_2$) was obtained from Loba Chemie Pvt. Ltd. (Mumbai, Maharashtra, India) to prepare the stock solution (10,000 mgCd/L), which modified from Palanippan and Karthikeyan (2009) by dissolving 27.4430 g of $\text{Cd}(\text{NO}_3)_2$ in 1,000 mL distilled water.

2.4 Acute toxicity tests

Before conducting the experiments, tap water was kept in four 200 L plastic tanks equipped with aeration for 7 days to remove chlorine.

Acute toxicity bioassay was aimed to determine median lethal concentration (LC_{50}) for 96 hrs of Cd on *B. gonionotus*, *O. niloticus* and *A. testudineus*. Based on the results of preliminary trials, which were conducted to establish a narrower concentration range in the definitive toxicity test. In the definitive toxicity test, it consisted of seven Cd concentrations as 0 (control), 5, 10, 15, 30, 50, and 80 mgCd/L for *B. gonionotus*; 0 (control), 10, 20, 30, 60, 90, and 120 mgCd/L for *O. niloticus*; and 0 (control), 30, 60, 90, 120, 160, and 200 mgCd/L for *A. testudineus*, with three replications per treatment per species of fish. The test solutions for each treatment were prepared by diluting the stock solution with 10 L

dechlorinated tap water in a glass tank (48 x 23 x 28 cm) as a diluent. Ten fingerlings were distributed into each glass tank. Sufficient aeration was provided during the experiment. Mortality rates of fish (%) were recorded at 24, 48, 72, and 96 hrs after the start of exposure. Behavioral alterations of fishes were studied during Cd acute toxicity tests. Physiological observations were recorded for startle responses after exposure to Cd, such as swimming, equilibrium, and general activity of fish during the experiments. The periods were 1, 2, 4, 6, 12, 24, 48, 72, and 96 hrs. Dead fishes were immediately removed from the glass tank immediately (Nekoubin et al., 2012).

2.5 Water quality analysis

The water quality parameters before and after experiments, i.e., dissolved oxygen (DO), potential of hydrogen ions (pH), and water temperature, were measured by a dissolved oxygen meter (Horiba model LAQUAact-DO110, Horiba Scientific Inc., Kyoto, Japan); a pH meter (Thermo Scientific™ Eutech™ 150 & 450 Series, Thermo Fisher Scientific Inc., Singapore); and a thermometer, respectively.

2.6 Statistical analysis

The DO, pH, and water temperature values were expressed as mean \pm standard deviation (SD). The LC_{50} value of Cd on *B. gonionotus*, *O. niloticus*, and *A. testudineus* were determined through "Probit Analysis" (Finney, 1971). Statistical analysis was executed using SPSS version 28.0.1.0 for Windows at level of significance $p < 0.05$ (95% confidence level).

3. Results and Discussion

3.1 Acute toxicity of Cd

Mortality rate (%) and the median lethal concentration (LC_{50}) of Cd on *B. gonionotus* (Bleeker, 1850), *O. niloticus* (Linnaeus, 1758), and *A. testudineus* (Bloch, 1792) against duration exposure, i.e., 24, 48, 72, and 96 hrs, are shown in Tables 1 and 2.

The three species of fish that indicated the adverse consequences of increased Cd exposure had the fastest mortality rates: *B. gonionotus* (50 and 80 mgCd/L), *O. niloticus* (120 mgCd/L), and *A. testudineus* (120, 160, and 200 mgCd/L), but no mortality of fishes in the control groups (0 mgCd/L) was observed during the experiments. The LC₅₀ values for 24, 48, 72, and 96 hrs of Cd exposures on *B. gonionotus*, and *O. niloticus* were 26.9946, 17.7884, 12.1246, and 5.1713 mgCd/L, respectively; and 57.6966, 46.2676, 42.4668, and 41.7624 mgCd/L, respectively. Including the LC₅₀ values of Cd on *A. testudineus* were 134.5610, 108.5223, 73.2544, and 47.3254 mgCd/L, respectively. Figure 1 shows regression line between the probit mortality value and log concentration of Cd at 96 hrs in *B. gonionotus*, *O. niloticus*, and *A. testudineus*, where the linear regression equation were $y = 3.8066x + 2.2839$, $R^2 = 0.8279$; $y = 3.3415x - 0.4158$, $R^2 = 0.8877$; and $y = 3.6449x - 1.1056$, $R^2 = 0.6900$, respectively.

When exposed to Cd concentrations, the species *B. gonionotus*, *O. niloticus*, and *A. testudineus* were observed to demonstrate a range of behavioral alterations. Throughout the study period, the control groups showed no mortality and normal behavior. The Cd exposure groups in three fish species, showed behavioral alterations on Cd higher concentrations (Table 3). The behavioral alterations difference after exposure Cd were rapid opening and closing of the operculum with the mouth open on *B. gonionotus*; pectoral and anal fin hemorrhage on *O. niloticus*; and the expulsion of a large amount of mucus on *A. testudineus*.

In general, fish are more likely to absorb and accumulate heavy metals the higher the concentration of those metals in the environment (Adams et al., 2011). These data unequivocally demonstrate that fish mortality increased with concentration increase and that shorter exposure times were required to bring about 50 percent fish mortality. The control groups showed no mortality and normal behavioral changes simultaneously. When formulating ideas for the management and protection of the environment, a toxicity test on aquatic creatures is crucial

(Johnson and Radhakrishnan, 2015). The LC₅₀ tests can measure animal's susceptibility and survival potential to particularly toxic substances such as heavy metals. High LC₅₀ values are less toxic because greater concentrations are required to produce 50% mortality in animals (El-Agri et al., 2021). At extreme low quantities of heavy metals like arsenic, cadmium, mercury, and chromium are harmful to aquatic species and are never suitable for living things (Shuhaimi-Othman et al., 2010). The acute toxicity test is used to establish the amount of a test substance or agent that, when exposed to a group of test organisms for a brief period under controlled circumstances, has a harmful effect on organisms (Rani et al., 2011). If Cd is poorly regulated by organisms, thereby increasing the likelihood that whole-body residues will increase with increasing exposure concentration, affecting experimental organisms' survival. Larval mortality and transient growth inhibition can be side consequences of long-term exposure (Rahman et al., 2018).

Several studies reported the assessment of the acute toxicity of Cd in various fish species. In the present study, 96 hrs LC₅₀ values of Cd on *B. gonionotus*, *O. niloticus*, and *A. testudineus* were 5.1713, 41.7624, and 47.3254 mgCd/L, respectively. In earlier studies 96 hrs LC₅₀ value of Cd for *Oreochromis niloticus* were 20.10 mgCd/L (average weight of 10.00 g) (Silva et al., 2017); 3.751 mgCd/L (mean total length 3.30 ± 0.30 cm; mean weight 1.50 ± 0.20 g) (Taweel et al., 2013); and for *A. testudineus* was 40.898 mgCd/L (Rita and Abhik, 2014). The toxicity level of any chemical depends on the physiological situations of the fish exposed, their habitat and the chemicals' purity (Abdelzاهر et al., 2022). Following are the similar observations recorded by different investigators experimented with Cd and different fish species - the LC₅₀ value 96 hrs of Cd on *Phallogeros caudimaculatus* (average weight of 0.14 ± 0.15 g; average total length of 1.90 ± 0.71 cm) was 13.99 mgCd/L (Sanches Filho et al., 2017); and *Silurus meridionalis* (weighed nearly 40 g) was 6.85 mgCd/L (Liu et al., 2021).

Table 1. Correlation between the concentrations of cadmium and mortality rate (%) on time (24 - 96 hrs.) for *B. gonionotus* (Bleeker, 1850), *O. niloticus* (Linnaeus, 1758), and *A. testudineus* (Bloch, 1792)

Species of Fish	Concentrations of Cd (mgCd/L)	Mortality Rate (%)				
		N	24 hrs.	48 hrs.	72 hrs.	96 hrs.
<i>B. gonionotus</i> (Bleeker, 1850)	0	30	0.00	0.00	0.00	0.00
	5	30	0.00	13.33	23.33	50.00
	10	30	13.33	23.33	40.00	83.33
	15	30	10.00	23.33	56.67	100.00
	30	30	50.00	66.67	70.00	100.00
	50	30	83.33	100.00	100.00	100.00
	80	30	100.00	100.00	100.00	100.00
<i>O. niloticus</i> (Linnaeus, 1758)	0	30	0.00	0.00	0.00	0.00
	10	30	0.00	6.67	13.33	13.33
	20	30	0.00	6.67	6.67	6.67
	30	30	3.33	3.33	3.33	3.33
	60	30	46.67	60.00	66.67	70.00
	90	30	96.67	96.67	96.67	96.67
	120	30	100.00	100.00	100.00	100.00
<i>A. testudineus</i> (Bloch, 1792)	0	30	0.00	0.00	0.00	0.00
	30	30	0.00	0.00	0.00	0.00
	60	30	3.33	16.67	53.33	93.33
	90	30	3.33	23.33	60.00	96.67
	120	30	10.00	30.00	66.67	100.00
	160	30	90.00	100.00	100.00	100.00
	200	30	96.67	100.00	100.00	100.00

Table 2. Median lethal concentration (LC₅₀) of Cd on *B. gonionotus* (Bleeker, 1850), *O. niloticus* (Linnaeus, 1758), and *A. testudineus* (Bloch, 1792) by Probit analysis

Species of Fish	Period of Exposure (hrs.)	LC ₅₀ of Cd (mgCd/L)
<i>B. gonionotus</i> (Bleeker, 1850)	24	26.9946
	48	17.7884
	72	12.1246
	96	5.1713
<i>O. niloticus</i> (Linnaeus, 1758)	24	57.6966
	48	46.2676
	72	42.4668
	96	41.7624
<i>A. testudineus</i> (Bloch, 1792)	24	134.5610
	48	108.5223
	72	73.2544
	96	47.3254

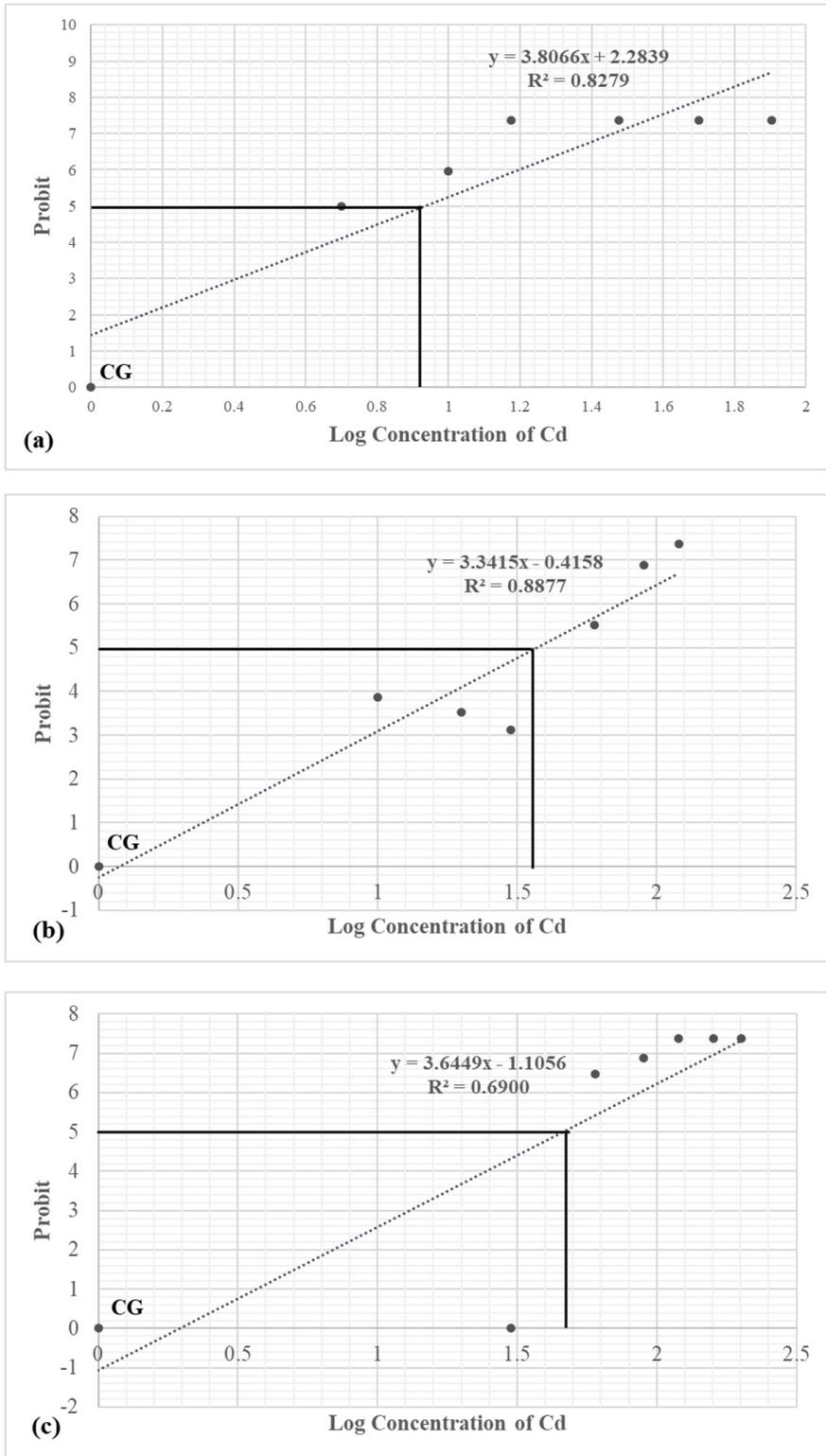


Figure 1. Regression line between the probit mortality value and log concentration of Cd at 96 hrs in (a) *B. gonionotus* (Bleeker, 1850), (b) *O. niloticus* (Linnaeus, 1758), and (c) *A. testudineus* (Bloch, 1792), CG = Control Group

Table 3. Behavioral alterations of *B. gonionotus* (Bleeker, 1850), *O. niloticus* (Linnaeus, 1758), and *A. testudineus* (Bloch, 1792) in control groups and exposure to Cd groups

Species of Fish	Period of Exposure (hrs.)	Behavioral alterations
Control groups (0 mgCd/L)	24 - 96	-Not behavior changes
<i>B. gonionotus</i> (Bleeker, 1850)	24 - 96	-being unresponsive to stimulation, -losing their balance, -lying on their sides, -moving in spiral fashion with sudden jerky movements, -contracting of muscles and fins, -rapid opening and closing of the operculum with the mouth open, -and death.
<i>O. niloticus</i> (Linnaeus, 1758)	24 - 96	-pectoral and anal fin hemorrhage, -being unresponsive to stimulation, -losing balance, -lying on their sides, -moving in a spiral fashion with sudden jerky movements, -contracting muscles and fins, -and eventually dying
<i>A. testudineus</i> (Bloch, 1792)	24 - 96	-the expulsion of a large amount of mucus, -being unresponsive to stimulation, -losing balance, -lying on their sides, -contracting muscles and fins, -and eventually dying

The LC₅₀ value at 96 hrs of Cd for certain fishes were- *Poecilia reticulata* 30.40 mgCd/L (Yilmaz et al., 2003); *Channa punctatus* (length range 12 - 16 cm; weight range 50 - 65 g) 80.62 mgCd/L (Singh and Saxena, 2020); *Ctenopharyngodon idella* (average weight of 40 ± 4 g) and *Hypophthalmichthys nobilis* (average weight of 12 ± 2.6 g) were 4.164 and 5.590 mgCd/L, respectively (Vajargah and Hedayati, 2017). Different LC₅₀ values of Cd for the different fish species were found. This may be due to the differences in age, feeding, habitat, sex, accessory breathing organ (labyrinth organ of *A. testudineus*), and feed behavior (i.e., herbivorous, omnivorous, and carnivorous fish) as well as experimental conditions (Kim et al., 2020; Abdelzاهر et al., 2022). As a result, the susceptibility and tolerance of *B. gonionotus*, *O. niloticus*,

and *A. testudineus* in this investigation varied. Fish are the most important bio monitors in aquatic ecosystems for assessing metal pollution levels because they provide a variety of unique benefits in defining natural features of aquatic ecosystems and monitoring habitat changes (Abdel-Aziz et al., 2022). In addition, they are also at the top of the aquatic food chain and may accumulate heavy metals and pass them to humans through food, causing chronic or acute diseases (Sauliutė and Svecevičius, 2015). Studies from the field and laboratory work showed that heavy metals accumulation in a tissue primarily depends on metal concentrations and exposure period. Although other environmental factors like water temperature, oxygen concentration, pH, hardness, salinity, alkalinity, and dissolved organic carbon may affect and play significant

roles in metals accumulation and toxicity to fish (Jitar *et al.*, 2015; Abdel-Aziz *et al.*, 2022). Fish can accumulate heavy metals in their tissues to levels higher than the harmful quantity in their environment by absorption along the gill surface and gastrointestinal tract wall (Rajeshkumar and Li, 2018). Heavy metal bioaccumulations may result in a high mortality rate or biochemical and histological changes in the fish that survive (Abalaka *et al.*, 2020; Vajargah, 2021).

3.2 Water quality parameters

The water quality parameters measured before and after the experiments including DO, pH, and water temperature are shown in Table 4. The values of the measured parameters (DO, pH, and water temperature) were compared with standards recommended for aquaculture of *B. gonionotus*, *O. niloticus*, and *A. testudineus* (not less than 4.00 mg/L (DO); 6.50 - 8.00 (pH); and 24.00 - 30.00 °C (water temperature), respectively). The values revealed that the water quality parameters are suitable for the aquaculture (National Bureau of Agricultural Commodity and Food Standards, 2010; Department of Fisheries, 2019).

The average DO measure before and after the experiments of Cd on *B. gonionotus*, *O. niloticus*, and *A. testudineus* were 7.11 ± 0.33 , 8.55 ± 0.46 , 7.25 ± 0.39 mg/L (before); and 6.57 ± 0.35 , 8.11 ± 0.51 , 7.16 ± 0.16 mg/L (after), respectively. DO is the most important factor for the lives of aquatic animals available for breathing and growing. The DO values appropriate for aquaculture are not less than 3.00 mg/L (Thailand Pollution Control Department: TPCD, 2022). DO in the water is depends on various factors such as temperature, salinity, turbidity, species of plants and phytoplankton in the water (Khamlerd, 2019).

The average pH measure before and after the experiments of Cd on *B. gonionotus*, *O. niloticus*, and *A. testudineus* were 8.35 ± 0.32 , 8.04 ± 0.19 , 7.74 ± 0.07 (before); and 8.30 ± 0.14 , 8.26 ± 0.37 , 8.01 ± 0.31 (after), respectively. The pH range appropriate in the aquaculture is 6.50 - 9.00 (Thailand Pollution Control Department: TPCD, 2022) which depends on physical water and pollution activities occurring the water source. pH is important for aquatic animals and plants (Khammanichanh and Neeratanaphan, 2015). If the pH of the water changes from its optimal range, it can impact fish performance and survival (White *et al.*, 2014).

Table 4. Values of water quality parameters measured before and after the acute toxicity experiments of cadmium

Species of Fish	Water quality parameters					
	Before			After		
	DO (mg/L)	pH	Water temperature (°C)	DO (mg/L)	pH	Water temperature (°C)
<i>B. gonionotus</i> (Bleeker, 1850)	7.11 ± 0.33	8.35 ± 0.32	25.38 ± 0.07	6.57 ± 0.35	8.30 ± 0.14	25.38 ± 0.07
Control group (0 mgCd/L)	7.19 ± 0.46	8.60 ± 0.06	25.57 ± 0.06	6.77 ± 0.25	8.28 ± 0.05	25.53 ± 0.06
<i>O. niloticus</i> (Linnaeus, 1758)	8.55 ± 0.46	8.04 ± 0.19	25.13 ± 0.09	8.11 ± 0.51	8.26 ± 0.37	25.23 ± 0.09
Control group (0 mgCd/L)	8.54 ± 0.55	8.11 ± 0.95	25.13 ± 0.15	8.16 ± 0.52	8.58 ± 0.07	25.43 ± 0.12
<i>A. testudineus</i> (Bloch, 1792)	7.25 ± 0.39	7.74 ± 0.07	25.38 ± 0.07	7.16 ± 0.16	8.01 ± 0.31	25.38 ± 0.07
Control group (0 mgCd/L)	7.15 ± 0.04	8.43 ± 0.07	25.53 ± 0.06	7.12 ± 0.04	8.50 ± 0.12	25.53 ± 0.06
Standard *	> 3.00	6.50 - 9.00	23.00 - 32.00	> 3.00	6.50 - 9.00	23.00 - 32.00

* Water quality index for freshwater aquaculture, Thailand Pollution Control Department (TPCD), Ministry of Natural Resources and Environment, Thailand

The average water temperature measure before and after the experiments of Cd on *B. gonionotus*, *O. niloticus*, and *A. testudineus* were 25.38 ± 0.07 , 25.13 ± 0.09 , 25.38 ± 0.07 °C (before); and 25.38 ± 0.07 , 25.23 ± 0.09 , 25.38 ± 0.07 °C (after), respectively. Water temperature is an important factor that influences the lives of aquatic animals and other physical changes in water, such as density, viscosity, dissolved oxygen, water stratification, water circulation, and mineral circulation (USEPA, 1999). The range of water temperature appropriate in the aquaculture is 23 - 32 °C (Thailand Pollution Control Department: TPCD, 2022). Fish metabolic rates rise as a result of increasing ambient temperatures, which increases their appetites. Furthermore, energy expenditure and metabolic needs were be impacted by the water temperature as well (Shackleton, 2012).

4. Conclusion

The LC₅₀ values for 24, 48, 72, and 96 hrs of Cd exposures on *B. gonionotus*, *O. niloticus* and *A. testudineus*, were 26.9946, 17.7884, 12.1246, and 5.1713 mgCd/L; 57.6966, 46.2676, 42.4668, and 41.7624 mgCd/L, and 134.5610, 108.5223, 73.2544, and 47.3254 mgCd/L, respectively. LC₅₀ values of Cd decreased with increase in exposure period and increase in exposure duration of the Cd become toxic even at lower concentrations. These results indicated that *B. gonionotus* was more sensitive to Cd than *O. niloticus* and *A. testudineus*. Also, *A. testudineus* had a higher tolerance to Cd than *O. niloticus* and *B. gonionotus*. The behavioral alterations in three species of fish after being exposed to Cd were being unresponsive to stimulation, losing balance, lying on their sides, moving in a spiral fashion with sudden jerky movements, contracting muscles and fins, and eventually dying. The results of this study provide guidelines for managing, monitoring, and supervising of Cd accumulation and consumption by these species of fish.

Acknowledgement

The authors thank Research Fund for Supporting Lecturer to Admit High Potent Student to Study and Research on His Expert Program Year 2021 (Research fund code, 641T216). Thanks, are also due to Department of Fisheries, Faculty of Agriculture, Khon Kaen University for facilities.

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