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International Journal of Fisheries Science and Research

Research Article

The Relationship between Behavior Responses and Brain Acetylcholinesterase (AChE) Activity of Zebrafish (*Danio rerio*) in Cadmium Stress

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Article Information

Received date: Dec 11, 2017
Accepted date: Dec 20, 2017
Published date: Dec 26, 2017

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Keywords AChE activity; Behavior Strength; Cadmium; Zebrafish; Ecotoxicological Assessment

Abstract

In this research, the toxic effects of Cadmium chloride ($CdCl_2$), which can seriously pollute aquatic environment and threaten human health, are evaluated based on the behavior responses and the brain Acetylcholinesterase (AChE) activity of zebrafish (*Danio rerio*). The results showed that Behavior Strength (BS) of test groups (changed from 0.15 to 0.65), which was recorded using an online behavior monitoring system, was lower than the control groups (changed from 0.65 to 0.85). The behavior responses of zebrafish suggested that both dose and time effect relationships existed between Cd^{2+} stress and zebrafish BS. Meanwhile, the brain Acetylcholinesterase (AChE) activity of zebrafish were strongly inhibited by Cd^{2+}: the AChE activities were lower than 60% after 0.5h Cd^{2+} exposure in both 1 TU (Toxic Unit) and 2 TU. The AChE activities in 0.1 TU Cd^{2+} treatment were about 60% in the first 2h and then increased to about 100% in 4h with a decrease tendency in the following exposure time (8h to 48h), which changed from 100% to 70%-80%. Totally, the brain AChE activities of zebrafish showed similar rules with BS after correlation analysis, which might provide an understanding of the ecotoxicological assessment of heavy metal Cd based on zebrafish.

Introduction

Heavy metals have received more and more attention due to their pollution and the persistence in the aquatic environment, which can affect species at all trophic levels and then endanger the balance of the water ecosystem [1-3]. In all heavy metals, Cadmium (Cd), which is one of the most toxic heavy metals with strong neurotoxicity and can cause great damage to the transduction signal [4], has seriously impacts aquatic environment and threaten human health [5]. As a by-product, Cd might be imported into aquatic environment after mining, forging and other industrial process [6]. Cd pollutions in environment can cause a variety of toxic effects to aquatic organisms, including behavior disturbances, physiological and biochemical changes, reproductive abnormalities, neurological deficits and immunological dysfunctions [7-10].

Acetylcholinesterase (AChE) activity has been widely used as an indicator of environmental stress. It is reported that AChE activity could be inhibited in mouse [11] and zebrafish [12] by heavy metal aluminum. As Cd can affect AChE activity and then induce the loss of nerve conduction ability [13], which can induce strong neurotoxicity on aquatic organisms and seriously damages nervous systems of animals [14], AChE activity analysis is a good approach to realize the understanding of the toxic effects of Cd on the neurotoxicity of aquatic organisms.

Behavior responses of organisms are frequently used as an endpoint to assess environmental stress due to the importance of altered behavior in toxicological studies [8,15,16]. Recent studies have suggested that the swimming behavior associated with low-level chemical toxicants can be observed earlier than changes in physiology [17]. Behavior responses have been widely used in contamination assessment and served as an indicator for environmental stress assessment [18,19].

In the toxic assessment of heavy metals, bioassays only depending on single analytical techniques [20] might be not enough to realize the purpose due to their strong neurotoxicity and bio-accumulation [21]. In fact, some researchers have applied some combined methods to realize the environmental stress assessment. Pereira et al. investigated the effects of endosulfan on the brain AChE activity and the swimming performance in adult zebrafish in 2012 [22], which suggested that that AChE activity inhibition is one of the endosulfan-induced toxicity pathway in fish brain and endosulfan can impairs behavior responses of zebrafish, which can potentially compromises their ecological and interspecific interaction. It is reported by Zhang et al., [23] that Aphanizomenon flos-aquae can affect fish locomotor capacity by damaging the cholinergic system, which suggest that aquatic animals' behavior responses and AChE activity can be used as indicators for investigating environment pollution in nature.

As model species, zebrafish (*Danio rerio*), which are very sensitive to the changes of the environmental stress [24], have been used to realize the water quality assessment due to their large-scale cultivation in water, short growth cycle, simple genomic background, and ability to adapt to a wide range of water conditions, and other characteristics [25]. It is reported that the brain AChE activities of zebrafish could be inhibited by heavy metals [26] and other chemicals [27]. Meanwhile, as the target site of neurotoxicity is brain, the brain AChE activity inhibition could accurately reflect real environmental stress [23]. On the other hand, the behavior responses of zebafish in different environmental stress have been reported [9,22,28], which suggest that biological behavior is the sensitive reactions in response to environmental changes [29]. Therefore, the relationship between behavior responses and brain Acetylcholinesterase (AChE) activity of zebrafish (*Danio rerio*) in Cd^{2+} stress is investigated to: 1) illustrate the difference of zebrafish behavior responses and the brain AChE activity inhibition in different Cd^{2+} treatments; 2) analyze the correlation between zebrafish behavior responses and the brain AChE activity after exposed to Cd^{2+} in different exposure time; 3) discuss the possibility of the ecotoxicological assessment of heavy metal Cd based on the behavior responses and the brain AChE activity of zebrafish. In this study, an online behavior monitoring system was used to automatically sample zebrafish swimming behavior, and then Bradford Protein Assay was used to determine the brain AChE activities in zebrafish in Cd treatments. The relationship between zebrafish swimming behavior and brain AChE activities was analyzed using Detrended Cross-Correlation Analysis (DCCA).

Materials and Methods

Test species and chemicals

The test zebrafish (*Danio rerio*) were obtained from the Institute of Environment and Ecology, Shandong Normal University, China. The stock populations were cultured in our laboratory in circulation system, under temperature of 26 ± 2°C with a photoperiod of 16h light (approximately 4000 lx) and 8h dark conditions. Culture medium was prepared according to the components of the Standard Reference Water. Zebrafish were fed twice a day with flake food (Trea®, Germany) with 8h interval (8:30 am. and 4:30 pm.). Healthy and uninjured adult zebrafish (3 ± 0.2 cm in length, 0.30 ± 0.05 g in weight) were selected randomly from the stock population. Feeding was stopped 24h before the experiment.

As a chemical compound of heavy metal Cd, Cadmium chloride ($CdCl_2$) presents distinctive physicochemical properties, and it can exert the same toxic effects as Cd on aquatic organisms [30]. $CdCl_2$ was purchased from Chinese standard sample center. Acetylthiocholine iodide (ATCh) and 5, 5-Dithio-2, 2-Nitrobenzoic Acid (DTNB) were purchased from Sigma - Aldrich. Coomassie brilliant blue G-250 and Bovine Serum Albumin (BSA) were purchased from SBH - Bio Corporation. All compounds were technical grade (> 95% purity).

Experiment design

In this research, the laboratory conditions were consistent with the culturing room. All determinations were repeated three times. Forty eight hours static exposure experiments were performed using $CdCl_2$ to assess the environmental stress on zebrafish. According to previous research, LC50 - 48h of $CdCl_2$ on zebrafish, which is 42.6 mg/L with 95% confidence interval (41.096 - 43.712 mg/L) [9], were taken as one toxic unit (1.0 TU), and four concentrations (Control, 0.1 TU, 1.0 TU, and 2.0 TU) gradients were set up. Control treatments were used to analyze the normal behavior status of zebrafish during 48h. As LC50 - 48h of $CdCl_2$ on zebrafish, 1.0 TU concentration was always used to assess the acute toxic effects of different chemicals on organisms [18]. As sublethal concentration [31], 0.1 TU Cd^{2+} treatments were used to illustrate behavior responses and the brain AChE acitivity in lower concentration exposure. 2.0 TU Cd^{2+} treatments were used to illustrate behavior responses and the brain AChE acitivity in higher concentration exposure than 1.0 TU.

Forty eight hours exposure test was carried out to assess the effects of Cd on swimming behavior of zebrafish detected by the online behavior monitoring system. Test organisms were divided into four groups. Each group had three fish. The test organisms were placed in a flow-through test chamber (3 cm long, 2 cm in diameter), which was closed off on both sides with nylon nets (250 mm) [8]. No food was fed during the tests. Behavior Strength (BS) of swimming behavior is sampled automatically during the exposure every second, and the average BS every 6 min is used to analyze behavioral changes via control database (Figure 1) [32].

The brain AChE activities in the different treatments were investigated. Fifty test organisms without feeding 24h before the experiment were exposed to Control, 0.1 TU, 1.0 TU, and 2.0 TU, respectively. Sampling time was set up as 0h, 0.5h, 1h, 2h, 4h, 8h, 16h, 32h, and 48h from the beginning of the exposure. The AChE activities in the homogenates were detected as followed: 50 μL enzyme and 50 μL ATCh (5 mm final concentration) were incubated at 30 °C for 15 min in a final volume of 0.1 mL, and then the reaction was stopped by 0.125 mm DTNB-phosphate-ethanol reagent inside 0.9 ml (12.4 mg of DTNB dissolved in 125 mL 95% ethanol, 75 mL distilled water, and 50 mL 0.1 M phosphate buffer, pH 7.5) as the thiol indicator. OD value was measured by UV spectrophotometer at the wavelength of 412nm UV spectrophotometer OD values [33,34]. In this study, the brain AChE activity of zebrafish was detected according to the Bradford Protein Assay in the unit of nmol/min•mg [35,36].

Data analysis

All data are analyzed statistically in MATLAB environment (MATLAB 2010, © 1984-2009 The MathWorks, Inc.). The behavior data were analyzed by a three dimensional surface plotSurf (X, Y, Z). Surf (X, Y, and Z) creates a shaded surface, in which surface height Z stands for zebrafish BS, X stands for exposure time, and Y stands for

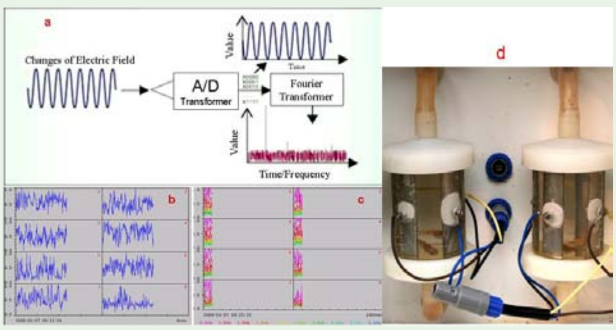

Figure 1: Online behavior monitoring system and test chambers.
(a) The signal acquisition of OMS, (b) The normal signal analysis (BS), (c) The signal analysis after Fast Fourier Transform and (d) test chambers.

Cd^{2+} concentration. In the brain AChE activity analysis, the relative AChE activity (% of each treatment at the beginning of the exposure) was used to analyze the toxic effects of Cd^{2+} on the AChE activity. As Detrended Cross-Correlation Analysis (DCCA) could be applied to quantify the correlation level of time series data [37], the correlation analysis between the brain AChE activity and BS of zebrafish in different Cd^{2+} treatments was analyzed using DCCA in MATLAB environments.

Results and Discussion

Behavior response of zebrafish

The behavior responses of zebrafish under the Cd^{2+} stress were shown in Figure 2. No obvious changes of BS were observed in the control group, which changed from 0.65 to 0.85. In different Cd^{2+} treatments, zebrafish BS changed from 0.15 to 0.70, which suggested an obviously lower result than in the control group. In 1.0 TU and 2.0 TU treatments, the swimming behavior showed evident up-and-down variation tendency. But it showed obviously fluctuations in early period, and then followed by continuous decrease with no adaption ability because of the higher Cd^{2+} concentration in 5.0 TU, which showed similar behavior responses of Daphnia magna in other environmental stress [38].

In 0.1 TU Cd^{2+} treatments, which were regarded as sublethal concentration [31], the behavior responses of zebrafish showed some special characteristics: In the beginning of the exposure, BS was about 0.6 with an increase to about 0.7, a decrease to about 0.55 and then an increase to about 0.65 until the end of the exposure. The swimming behavior of zebrafish in 0.1 TU Cd^{2+} treatment showed evident stepwise behavior responses as described by Zhang et al. in 2013 [23], which included behavior stimulation, acclimation, (re-) adjustment and toxic effects.

Totally, some abnormal phenomena of zebrafish behavior responses in Cd^{2+} treatments could be recorded by the online behavior monitoring system, such as larger magnitude of BS (from 0.15 to 0.70) and the up and down behavior responses, especially the gradual BS increase from the beginning of the exposure, which could be illustrated by the avoidance behavior when aquatic organisms were under threaten of environmental stress [39]. Meanwhile, the behavior responses of zebrafish in Cd^{2+} treatments showed an evident dose- and time- effect relationship in lethal environmental stress, and stepwise behavior responses could be observed in sublethal environmental stress. These results suggested that behavior responses of zebrafish are sensitive to the changes of water quality, and BS could provide a reliable and real-time method for online assessment of aquatic heavy metal pollution [16].

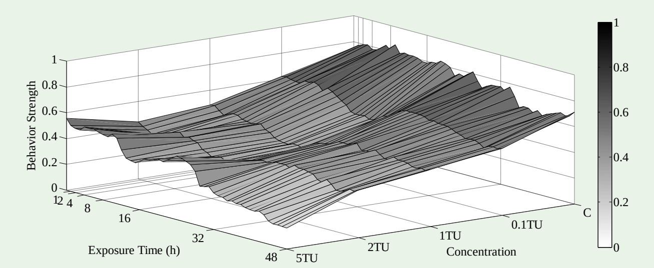

Figure 2: Behavior responses of zebrafish exposed to Cd^{2+}.

AChE activity of zebrafish

Based on the relative values of each treatment at the beginning of the exposure (0h), the brain AChE activities of zebrafishin 48h Cd^{2+} exposure were shown in Figure 3. The overall brain AChE activity showed a general downward trend in 48h exposure. At the beginning, AChE activity decreased due to Cd^{2+} stress, and AChE activity was inhibited evidently in different concentrations after a period of exposure, which suggested that the trend was similar to the behavior responses as shown in Figure 2.

Based on the results shown in Figure 3, the brain AChE activities of zebrafish were strongly inhibited by Cd^{2+}: the AChE activities were lower than 60% after 0.5h Cd^{2+} exposure in both 1 TU and 2 TU. The AChE activities in 0.1 TU Cd^{2+} treatment were about 60% in the first 2h and then increased to about 100% in 4h with a decrease tendency in the following exposure time (8h to 48h), which changed from 100% to 70% - 80%. Therefore, it was advised that the relative brain AChE activities changed with both exposure time and Cd^{2+} concentration, which suggested that the brain AChE activity can be used to evaluate Cd^{2+} stress as previous report [40].

Correlation analysis between BS and brain AChE activity

Previous studies have proved that AChE activity inhibition is correlated with the disorder of swimming behavior [41]. Cd^{2+} could affect AChE activity and Acetylcholine (ACh) synthesis rate; finally induce the collapse of nervous system [31]. In view of this, AChE inhibition could illustrate the intrinsic response mechanism of zebrafish.

In order to analyze correlation between BS and brain AChE activity of zebrafish in Cd^{2+} stress assessment, the DCCA were used based on the correlation coefficient (r) and significance (p). According to Ren et al., [41], $r < 0.3$ means poor correlation, $0.3 < r < 0.5$ means moderate and $r > 0.5$ means high correlation. So the correlation coefficient r was first checked to analyze how much they correlated. Then, p values were checked to see the significance between two variables, in which $p < 0.05$ means significant correlation and $p < 0.01$ means extreme significance. When r is absolutely higher than0.5 with $p < 0.05$, it means a significant correlation.

In Figure 4, the cross correlation results using DCCA showed the relationship between BS values and the relative brain AChE activity of zebrafish in different Cd^{2+} treatments. The correlation could be illustrated by the regression line equation (1) with p = 2.916*e-07 and r = 0.737:

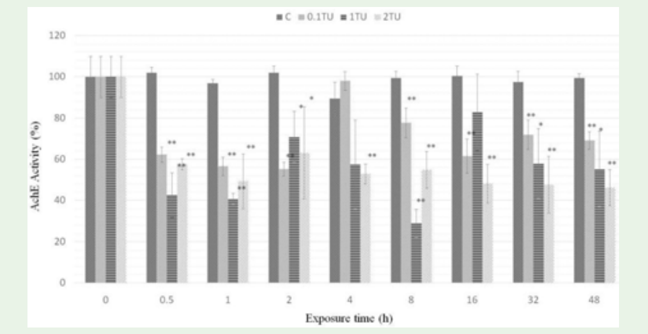

Figure 3: Relative brain AChE activity of zebrafish exposed to Cd^{2+}. Showed as M ± S.D., *$p < 0.5$, ** $p < 0.01$.

$$y = 0.004x + 0.241 \qquad (1)$$

This linear regression with the correlation coefficient ($r > 0.5$) and significance ($p < 0.01$) indicated that BS and the brain AChE activity of zebrafish exposed to Cd^{2+} exposure were highly correlated with extremely significance, which suggested that behavior disorder of zebrafish might be affected mainly by the inhibition of the nerve conduction, in which the AChE is the main neurotransmitter as reported [13]. These results could also be supported by previous researchers [4,14], which illustrated that Cd^{2+} could cause great damage to the transduction signal and then neurotoxicity due to the AChE activity inhibition.

AChE plays a major regulatory role in multiple physiological processes [42]. The activity of the cholinergic system plays an important role in normal behavior and muscle function [43]. In this study, our experimental results show a significant correlation between behavioral changes and brain AChE activity. The present study is consistent with other findings, whicn indicate that Cd^{2+} can affect the AChE activity of zebrafish and lead to change the behavior, which is consistent with previous findings on zebrafish gills [6]. Similar observations were found in the study by Zhang et al. [31], which show that there is a significant dose-effect and time effect at 1.0 TU and 2.0 TU exposure concentrations. It is also found that AChE activity is inhibited almost simultaneously in the brain, gill, muscle and liver under these two higher environmental stresses, whereas it shows a time lag in sublethal treatment (0.1 TU). The inhibition of AChE can also cause behavioral abnormalities. In addition, there are some researchers who have studied organophosphorus pesticides. The results showed that the inhibition of AChE activity may also lead to the loss of nerve conduction ability, eventually causing different types of behavioral changes [8]. Other experiments have also been conducted on rat pups with malathion. The experiment has shown that the AChE activity in the brain of the rat pups is inhibited and the locomotor activity of rat pups exposed to malathion decreased. It demonstrated that the AChE activity in the brain of rat pups exposed to malathion is involved in the change of the behavior [44].

Cd is a neurotoxin metal that has some enrichment in the organism and is not biodegradable. The organisms exposed to Cd have been observed to be barriers to behavioral and biochemical functions [13]. After sub-lethal doses of Cd treatment, AChE in the zebrafish brain and muscle was significantly inhibited and the extent of inhibition varied with the change of time and concentration [45].

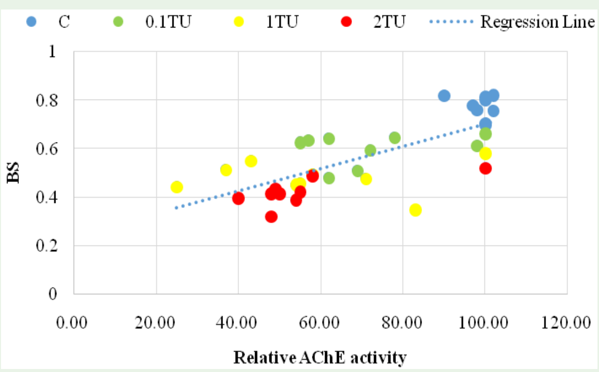

Figure 4: Cross correlation analysis of BS values and the brain AChE activity in different Cd^{2+} treatments.

Conclusion

As it is reported in Almeida et al., [46] Cd^{2+} is a high toxic poison to zebrafish and has a good dose-effect relationship. In this research, the toxic effects of Cd^{2+} were decided by concentrations and exposure time, which suggested that both dose and time effect relationships existed between Cd^{2+} stress and zebrafish BS and brain AChE activity (1.0 TU and 2.0 TU), while a process of adaptation, adjustment and readjustment of zebrafish BS and brain AChE activity was observed under sub-lethal conditions stress (0.1 TU). The correlation analysis using DCCA showed that BS and the brain AChE activity of zebrafish were highly correlated with extremely significance in Cd^{2+} stress, which suggested that behavior disorder of zebrafish might be affected mainly by the inhibition of the nerve conduction.

Cd^{2+} can enter the aquatic environment possibly through sludge and agricultural runoff, or through other industrial processes [45]. The limitation of Cd^{2+}, which is a by-product of mining, forging and other industrial process, is 0.01mg/L in discharged wastewater according to the Environmental Quality Standards for Surface Water in China [47]. However, Cd^{2+} concentration in some discharged wastewater could reach 26 mg/L [48], which induce some heavy Cd^{2+} pollution in some place in China (359.8 g/kg) [49]. Therefore, it is necessary to realize the ecotoxicological assessment of heavy metal Cd^{2+} pollution. As the brain AChE activities of zebrafish showed similar rules with BS after correlation analysis, the results in this study might provide an understanding of the ecotoxicological assessment of heavy metal Cd^{2+} based on zebrafish.

Acknowledgements

This study was financially supported by the Natural Science Foundation of China (21107135), the High-level Talent Project of Shandong Normal University, the Taishan Leader Talent Project of Shandong (tscy20150707) and the Oversea High-level Talent Project of Ji'nan (2013041).

References

1. Vleeschouwer FD, Gérard L, Goormaghtigh C, Mattielli N, Roux GL, Fagel N. Atmospheric lead and heavy metal pollution records from a Belgian peat bog spanning the last two millenia: human impact on a regional to global scale. Science of the Total Environment. 2007; 377: 282-295.

2. Zhang Y, Wang W, Song J, Ren Z, Yuan H, Yan H, et al. Environmental Characteristics of Polybrominated Diphenyl Ethers (PBDEs) in Marine System, with Emphasis on Marine Organisms and Sediments. BioMed Research International. 2016; 20: 1-16.

3. Ren Z, Chon T-S, Xia C, Li F. The monitoring and assessment of aquatic toxicology. BioMed Research International. 2017.

4. Escobar MC, Souza V, Bucio L, Hernández E, Gómez-Quiroz LE, Gutiérrez Ruiz MC. MAPK activation is involved in cadmium-induced Hsp70 expression in HepG2 cells. Toxicology Mechanisms & Methods. 2009; 19: 503-509.

5. Kan H. Environment and health in china: challenges and opportunities. Environmental Health Perspectives. 2009; 117: A530-A531.

6. Pan H, Zhang X, Ren B, Yang H, Ren Z, Wang W. Toxic assessment of cadmium based on online swimming behavior and the continuous ache activity in the gill of zebrafish (Danio rerio). Water Air & Soil Pollution. 2017; 228: 355-363.

7. Adelekan BA, Abegunde KD. Heavy metals contamination of soil and groundwater at automobile mechanic villages in Ibadan, Nigeria. International Journal of Physical Sciences. 2011; 6: 1045-1058.

8. Ren Z, Zhang X, Wang X, Qi P, Zhang B, Zeng Y, et al. AChE inhibition: One dominant factor for swimming behavior changes of Daphnia magna under DDVP exposure. Chemosphere. 2015; 120: 252-257.

9. Qi L, Ma J, Song J, Li S, Cui X, Peng X, et al. The physiological characteristics of zebrafish (Danio rerio) based on metabolism and behavior: a new method for the online assessment of cadmium stress. Chemosphere. 2017; 184: 1150-1156.

10. Xing N, Ji L, Song J, Ma J, Li S, Ren Z, et al. Cadmium stress assessment based on the electrocardiogram characteristics of zebra fish (Danio rerio): qrs complex could play an important role. Aquatic Toxicology. 2017; 191: 236-244.

11. Kaizer RR, Corrêa MC, Spanevello RM, Morsch VM, Mazzanti CM, Gonçalves J, et al. Acetylcholinesterase activation and enhanced lipid peroxidation after long-term exposure to low levels of aluminum on different mouse brain regions. Journal of Inorganic Biochemistry. 2005; 99: 1865-1870.

12. Senger MR, Seibt KJ, Ghisleni GC, Dias RD, Bogo MR, Bonan CD. Aluminum exposure alters behavioral parameters and increases acetylcholinesterase activity in zebrafish (Danio rerio) brain. Cell Biol. Toxicol. 2011; 27: 199-205.

13. Carageorgiou H, Tzotzes V, Pantos C, Mourouzis C, Zarros A, Tsakiris S. In vivo and in vitro Effects of Cadmium on Adult Rat Brain Total Antioxidant Status, Acetylcholinesterase, (Na^+,K^+) - ATPase and Mg^{2+} -ATPase Activities: Protection by L-Cysteine. Basic & Clinical Pharmacology & Toxicology. 2004; 94: 112-118.

14. Rai A, Maurya SK, Khare P, Srivastava A, Bandyopadhyay S. Characterization of Developmental Neurotoxicity of As, Cd, and Pb Mixture: Synergistic Action of Metal Mixture in Glial and Neuronal Functions. Toxicological Sciences. 2010; 118: 586-601.

15. Cazenave J, Nores ML, Miceli M, Díaz MP, Wunderlin DA, Bistoni MA. Changes in the swimming activity and the glutathione S-transferase activity of Jenynsia multidentata fed with microcystin-RR. Water Research. 2008; 42: 1299-1307.

16. Ren Z, Wang Z. Differences in the behavior characteristics between Daphnia magna and Japanese madaka in an on-line biomonitoring system. Journal of Environmental Sciences. 2010; 22: 703-708.

17. Michalec FG, Kâ S, Holzner M, Souissi S, Ianora A, Hwang J. Changes in the swimming behavior of Pseudodiaptomus annandalei (Copepoda, Calanoida) adults exposed to the diatom toxin 2-trans, 4-trans decadienal. Harmful Algae. 2013; 30: 56-64.

18. Ren Z, Zha J, Ma M, Wang Z, Gerhardt A. The early warning of aquatic organophosphorus pesticide contamination by on-line monitoring behavioral changes of Daphnia magna. Environmental Monitoring & Assessment. 2007; 134: 373-383.

19. Wang L, Ren Z, Kim H, Xia C, Fu R, Chon T-S. Characterizing response behavior of medaka (Oryzias latipes) under chemical stress based on self-organizing map and filtering by integration. Ecological Informatics. 2015; 29: 107-118.

20. Farombi EO, Adelowo OA, Ajimoko YR. Biomarkers of Oxidative Stress and Heavy Metal Levels as Indicators of Environmental Pollution in African Cat Fish (Clarias gariepinus) from Nigeria Ogun River. International Journal of Environmental Research & Public Health. 2007; 4: 158.

21. Miao L, Yan W, Zhong L, Xu W. Effect of heavy metals (Cu, Pb, and As) on the ultrastructure of Sargassum pallidum in Daya Bay, China. Environmental Monitoring & Assessment. 2014; 186: 87-95.

22. Pereira VM, Bortolotto JW, Kist LW, Azevedo MB, Fritsch RS, Oliveira RL, et al. Endosulfan exposure inhibits brain ache activity and impairs swimming performance in adult zebrafish (Danio rerio). Neurotoxicology. 2012; 33: 469-475.

23. Zhang DL, Hu CX, Li DH, Liu YD. Zebrafish locomotor capacity and brain acetylcholinesterase activity is altered by aphanizomenon flos-aquae, dc-1 aphantoxins. Aquatic Toxicology. 2013; 138: 139-149.

24. Zhang G, Chen L, Liu Y, Chon T, Ren Z, Wang Z, et al. A new online monitoring and management system for accidental pollution events developed for the regional water basin in Ningbo, China. Water Science & Technology. 2011; 64: 1828-1834.

25. Nemtsas P, Wettwer E, Christ T. Adult zebrafish heart as a model for human heart? An electrophysiological study. Journal of Molecular & Cellular Cardiology. 2010; 48: 161-171.

26. Richetti SK, Rosemberg DB, Ventura-Lima J, Monserrat JM, Bogo MR, Bonan CD. Acetylcholinesterase activity and antioxidant capacity of zebrafish brain is altered by heavy metal exposure. Neurotoxicology. 2011; 32: 116-22.

27. Rico EP, Rosemberg DB, Dias RD, Bogo MR, Bonan CD. Ethanol alters acetylcholinesterase activity and gene expression in zebrafish brain. Toxicology Letters. 2007; 174: 25-30.

28. Yin L, Yang H, Si G, Ren Q, Fu R, Zhang B, et al. Persistence parameter: A reliable measurement for behavioral responses of medaka (Oryzias latipes) to environmental stress. Environmental Modeling and Assessment. 2016; 21: 159-167.

29. Fast DE, Stober QJ. Intragravel behavior of Salmonid alevins in response to environmental changes. Language & Speech. 1984; 15: 270-8.

30. Sunda WG, Engel DW, Thuotte RM. Effect of chemical speciation on toxicity of cadmium to grass shrimp, Palaemonetes pugio: importance of free cadmium ion. Environmental Science & Technology. 1978; 12: 409-413.

31. Zhang T, Yang M, Pan H, Li S, Ren B, Ren Z, et al. Does time difference of the acetylcholinesterase (ache) inhibition in different tissues exist? A case study of zebra fish (Danio rerio) exposed to cadmium chloride and deltamethrin. Chemosphere. 2017; 168: 908-916.

32. Ren Z, Li S, Zhang T, Qi L, Xing N, Yu H, et al. Behavior Persistence in Defining Threshold Switch in Stepwise Response of Aquatic Organisms Exposed to Toxic Chemicals. Chemosphere. 2016; 165: 409-417.

33. Ruisel I, Ruiselová Z, Prokopčáková A. Hepato-protective role of the aqueous and n-hexane extracts of Nigella sativa Linn. In experimental liver damage in rats. Asian Journal of Pharmaceutical & Clinical Research. 2013; 6: 205-209.

34. Zhang X, Yang H, Ren Z, Cui Z. The toxic effects of deltamethrin on Danio rerio: the correlation among behavior response, physiological damage and AChE. RSC Advances. 2016; 6: 109826-109833.

35. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry. 1976; 72: 248-254.

36. Zor T, Selinge Z. Linearization of the Bradford Protein Assay Increases Its Sensitivity: Theoretical and Experimental Studies. Analytical Biochemistry. 1996; 236: 302-308.

37. Zebende GF. DCCA cross-correlation coefficient: Quantifying level of cross-correlation. Physica A Statistical Mechanics & Its Applications. 2011; 390: 614-618.

38. Ren Z, Li Z, Ma M, Wang Z, Fu R. Behavioral responses of Daphnia magna to stresses of chemicals with different toxic characteristics. Bulletin of Environmental Contamination & Toxicology. 2009; 82: 310-316.

39. Zeng Y, Fu XE, Ren Z. The Effects of Residual Chlorine on the Behavioral Responses of Daphnia magna in the Early Warning of Drinking Water Accidental Events. Procedia Environmental Sciences. 2012; 13: 71-79.

40. Carageorgiou H, Tzotzes V, Sideris A, Zarros A, Tsakiris S. Cadmium Effects on Brain Acetylcholinesterase Activity and Antioxidant Status of Adult Rats: Modulation by Zinc, Calcium and L-Cysteine Co-Administration. Basic & Clinical Pharmacology & Toxicology. 2005; 97: 320-324.

41. Ren Q, Zhang T, Li S, Ren Z, Yang M, Pan H, et al. Integrative Characterization of Toxic Response of Zebra Fish (*Danio rerio*) to Deltamethrin Based on AChE Activity and Behavior Strength. BioMed Research International. 2016.

42. Schetinger MRC, Porto NM, Moretto MB, Morsch VM, Rocha JBT, Vieira V, et al. New benzodiazepines alter acetylcholinesterase and atpdase activities. Neurochemical Research. 2000; 25: 949-955.

43. Payne JF, Mathieu A, Melvin W, Fancey LL. Acetylcholinesterase, an old biomarker with a new future? Field trials in association with two urban rivers and a paper mill in newfoundland. Marine Pollution Bulletin. 1996; 32: 225-231.

44. Acker CI, Souza AC, Pinton S, Da RJ, Friggi CA, Zanella R, et al. Repeated malathion exposure induces behavioral impairment and ache activity inhibition in brains of rat pups. Ecotoxicology & Environmental Safety. 2011; 74: 2310-2315.

45. Al-Sawafi AGA, Yan Y. Alterations of Acetylcholinesterase Activity and Antioxidant Capacity of Zebrafish Brain and Muscle Exposed to Sublethal Level of Cadmium. International Journal of Environmental Science & Development. 2013; 4: 327-330.

46. Almeida JA, Barreto RE, Novelli ELB, Castro FJ, Moron SE. Oxidative stress biomarkers and aggressive behavior in fish exposed to aquatic cadmium contamination. Neotropical Ichthyology. 2009; 7: 103-108.

47. GB3838-2002. The surface water environmental quality standard. China Environmental Science Press, 2002 (in Chinese).

48. He H, Zhu T, Liu Z, Chen M, Wang L. Treatment of Cadmium-Containing Wastewater in Zinc Smelting Using Sodium Dimethyl Dithio Carbamate. Environmental Protection of Chemical Industry. 2015; 35: 293-296.

49. Huang Z, Zhou Z, Luo Y. Distribution of Cd in sediments from Xiawan drainage area of the Xiang River. Environment pollution and protection. 2009; 31: 56-58.