Cadmium Accumulation in Some Organs of Mosquito Fish *Gambusia holbrooki*

Talib Hussen Ali[1] , Ali Ashkar Abed[2] , Amal abdul Ellah[2]

[1] Department of Biology , college of Education for Girls , University of Mosul , Mosul , Iraq

[2] Department of Biology , college of Education , University of Mosul, Mosul , Iraq

Abstract

The cadmium contents in muscle, brain, gills, liver , intestine and testis from exposed and control animals were determined by atomic absorption spectroscopy. The experiments were planned in three series of a total of 95 fish employing the static test method of acute toxicity. Five fishes were placed in each concentrations of three sublethal concentrations (5, 15 and 25mg/l) for two acute periods (24 and 96h). The experiments were performed as 3 replicates, the Cd accumulation in different fish organs were determined. This showed that the effect of acute Cd on different organs Cd accumulation in the *G. holbrooki* was found to be time dependent fashion. Atomic absorption examination showed that the Cd-content in both testis and intestine increased significantly time dependent by 502%, 167%, 168%, 146%,490%,214% in muscle ,brain, liver, gills, intestine and testis , respectively at short-acute term treatment whereas for long- acute term treatment this increasing were 44%, 82 %,170%,127%,350%,400% in muscle ,brain, liver, gills, intestine and testis respectively.

Keywords: Acute toxicity; LC50; Cadmium chloride; *Gambusia holbrooki*.

Introduction

The major challenge in experimental ecotoxicological studies is to cope with the relative paucity of literature on toxicity induced by environmental pollution may elicit both adaptive and adverse response in animals at different structural levels, i.e, cells, tissues and organs. These reactions depend on a variety of factors, such as the type of contaminant and its concentration, the rate of exposure and the susceptibility, of the organisms (1). Trace metals can be accumulated by fish, both through the food chain and water. Fish living in the polluted water may accumulate toxic trace metals via their food chains (2-3). Among the various heavy metal pollutants, cadmium merits special attention due to its potential hazards to aquatic biota (4).

Cadmium (Cd) is a non-essential metal which could be toxic in traces for aquatic species. Increasing Cd concentrations in fresh water is mainly related to waste waters provided from growing industrial and agricultural activities (5). Cadmium is a heavy metal released into environment as a result of industrial, municipal and agricultural activities. Cadmium appear to be the largest single contributor to many diseases, it is extremely toxic and has toxic biological effect in concentration smaller than any commonly found minerals (6).

Cadmium burden (especially in liver) tends to increase in a linear fashion, consequently. There were differences in animal susceptibility to Cd toxicity regarding concentration level and the reason for this differences is not known and remain to be elucidated (1).

Materials and Methods

Test organisms, fish were collected from north Mosul / Iraq in June 2012 (Mendan Bridge area) . The reference site chosen for this study is where fish from the species *Gambusia holbrooki* have been recorded. Fish were captured using a hand net, kept in maintenance plastic tanks and immediately transported alive to the laboratory. First phase of laboratory maintenance involved a period of quarantine in which the fish were acclimated to the laboratory conditions for at least two weeks (15–30 days) prior to the experiment. The animals were kept in glass aquaria (20×25 ×40) filled with dechlorinated tap water, continuous aeration and temperature of 20 ± 1◦C. The photoperiod was 16:8 (16 h. light/8 h darkness) and fish were fed twice daily with commercially balanced fish food.

Water samples were stabilized at pH2 with 1 M nitric acid prior to direct determination of total metal concentrations. For cadmium analyzing water samples were collected at a depth of 50 cm in clean bottles from the same area. Sampling bottles were previously cleaned by soaking with 10% nitric acid and rinsed with deionizing water.

Ten fish , serving as controls, were placed in clean tap water, and ten fish for each period were placed in plastic aquariums (120x65x60 cm) at depth 3cm of 5, 15 and 25µg/L Cd solution as cadmium chloride ($CdCl_2$) for acute exposure. Each animal represents a replicate, and there were 10 replicates in each treatment. Animals were then sacrificed and brain, liver, gills, muscle, testis and , intestine dissected out for metal quantitative. Fish organ tissues were dried to constant weight for 48 h at 60◦C in Pyrex test tubes. Analysis was carried out according to the procedure described by Karadede and Ünlü (10). Dried tissues were weighed and digested with concentrated nitric acid (Merck, 65%) at 120◦C. When fumes were white and the solution was completely clear, the samples were cooled to room temperature and the tubes were filled to 10 ml with ultra pure water . All samples were analyzed to determine cadmium (Cd) concentrations using a graphite furnace AAS technique (ZE Enit 700). Samples were analyzed in triplicate. The variation coefficient was usually less than 10%. Concentrations of the metal in tissues were calculated on a dry weight basis and expressed as µg/g dry weight.

Statistical analyses

To test statistical significant differences for metal content in test organs between animals from natural sites and Cd exposed , analysis of variance with the Duncan multiple range test was applied to find the significant differences among means of fish organs for cadmium .

Results

Atomic absorption examination showed that the Cd-content in different tested organs increased significantly time dependent by 25, 32, 37%, 71, 256, 390 % in muscle ,brain, liver, gills, intestine and testis respectively at short-acute term treatment ,whereas the increase for long- acute term treatment were 44, 82,170,127, 350,400 % in muscle ,brain, liver, gills, intestine and testis respectively Fig(1)

.

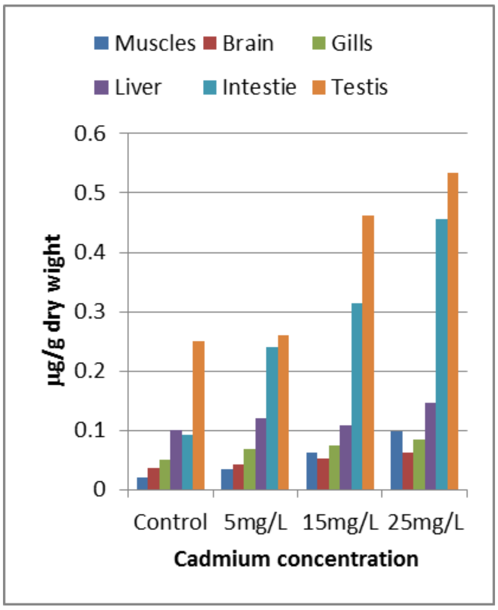

Fig (1) .The relation between the cadmium chloride (24h) exposure and cadmium accumulation in different *G. holbrooki* organs.

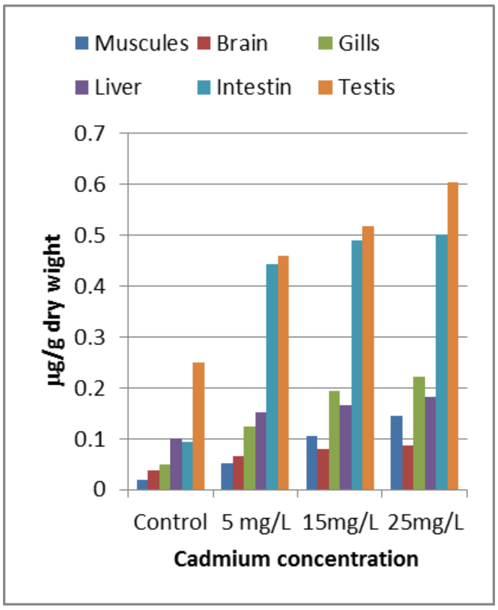

Fig (2): The relation between the cadmium chloride (96h) exposure and cadmium accumulation in different *G. holbrooki* organs.

The SAS system (ANOVA) procedure analysis using Duncan multiple range test of the interaction between concentration ,period, and organism tissues was shown in Table 1 and Table2.

Table1. Concentration of cadmium µg /g dry weight in different *G.holbrooki* organs at 24h exposure period (Duncan multiple range test of the interaction between concentration, period, and organism tissues)

Treatment (Cd / µg/L) / 24h	Organs / µg Cd / g dry weight						
	muscle	Brain	Gills	Liver	Intestine	Testis	Effect of Treatment
Control	0.01970 ± 0.006 M	0.037 ± 0.009 m	0.050 ± 0.011 jki	0.100 ±0.012 fg	0.093 ±0.010 fgh	0.250 ± 0.063 d	0.0922 D
5	0.0349 ± 0.0011 lm	0.042 ± 0.005 klm	0.069 ± 0.008 ghi	0.0121 ± 0.008 f	0.240 ± 0.015 d	0.261 ± 0.010 d	0.131 C
15	0.062 ± 0.0011 hi	0.052 ± 0.004 Jkl	0.074 ± 0.0010 hi	0.109 ± 0.095 f	0.0314 ± 0.0051 c	0.462 ± 0.023 b	0.185 B
25	0.099 ± 0.008 fg	0.062 ± 0.005 i-l	0.084 ± 0.004 ghi	0.146 ± 0.012 e	0.456 ± 0.030 b	0.534 ± 0.037 a	0.235 A
Effect of Organs	0.056 E	0.49 E	0.0.72 D	0.123 C	0.284 B	0.381 A	

Mean with same letter are not significantly different**.**

Table2. Concentration of cadmium µg /g dry weight in different G.holbrooki organs at 96h exposure period. Duncan multiple range test of the interaction between concentration, period, and organism tissues.

	Organs / µg Cd / g dry weight						
Treatment (Cd / µg/L) / 96 h	**Muscle**	**Brain**	**Gills**	**Liver**	**Intestie**	**Testis**	**Effect of Treatment**
Control	0.0197 ±0.006 o	0.037 ± 0.009 no	0.050 ± 0.011 mn	0.100 ±0.012 k	0.093 ± 0.010 k	0.250 ±0.063 k	0.092 D
5	0.0516 ± 0.004 mn	0.067 ± 0.006 kl	0.125 ± 0.003 jk	0.153 ± 0.016 i	0.444 ± 0.013 d	0.460 ±0.049 c	0.223 C
15	0.106 ± 0.010 K	0.081 ± 0.0107 kl	0.194 ± 0.015 g	0.167 ± 0.008 hi	0.490 ± 0.014 c	0.517 ±0.018 b	0.263 **b**
25	0.146 ± 0.008 j	0.087 ± 0.004 kl	0.221 ± 0.001 f	0.182 ± 0.008 gh	0.502 ± 0.022 c	0.603 ± 0.026 a	0.290 **a**
Effect of Organs	0.082 **D**	0.07 **E**	0.151 **C**	0.154 **C**	0.378 **B**	0.46 **A**	

Mean with same letter are not significantly different.

A Indicates the most significant difference compared to controls and other treated groups for that tissue. The statistical significant differences between different treatments and control are reported by different letters a, b, c, d .The values with different letters in the same row are significantly different (Duncan test, $P \leq 0.05$)

Discussion

Atomic absorption examination showed that the Cd-content increased significantly time dependent by 25, 32, 37%, 71, 256, 390 % in muscle ,brain, liver, gills, intestine and testis respectively at short-acute term treatment ,whereas the increase for long- acute term treatment were 44, 82,170,127, 350,400 % in muscle, brain, liver, gills, intestine and testis respectively Fig(1)

It is obvious that the most significant increase observed in the testis and the metal accumulation incrase linearly depened on both concentation and exposure duration as shown in Table. 1 and Table.2.

Cadmium in the environment has the ability to accumulate in the body and that levels of accumulated metals in tissue were related to metal levels in sediment, water and food.(7). This is further supported by Bervoets and Blust saying that it is more likely that tissue levels reflect environmental levels because metal concentrations in tissue follow concentrations in the environment(8). In fresh water fish, cadmium uptake is taking place mainly through three routes namely, gills, skin and also from food via the intestinal wall(9). Different tissues have the ability to accumulate metals differently(7,8, 10,11,12). Loumbourdis and Vogiatzis reported that the liver is one of the main target organs of cadmium accumulation in Rana ridibunda(11). In previuos study Loumbourdis and Wray found that Rana ridibunda liver has higher cadmium concentrations than carcass(10). There are many studies on fish demonstrate that the distribution of Cd in tissue specific and depends largely on certain parameters, such as the exposure route, dose and time. Moreover, it can also vary between species.(13). Our results revealed that Cd accumulation in tissues in the order of muscle > Brain > gills > liver > intestine > testis in both Cd exposure periods in Gambusia. The physiological responses of Gambusia after cadmium exposure and found the highest Cd concentration in the (Testis, intestine and liver wherase the least conentration was in muscle ,brain and gills) after 24h exposure ; this order did not changed by 96h, as the main accumulator organs were then the testis and intestines. Hollis *etal* found Cd accumulation in tissues in the order of kidney > gill > liver 30 days after water borne Cd exposure in rainbow trout(14) . Berntssen *et al* investigated the effect of dietary Cd exposure on Atlantics almonand observed Cd distribution to be Intestine> kidney> liver> gill (15). Chowdhury *et al* found that in Cd-treated rainbow trout both the liver and kidney played an important role in Cd storage: the liver was the most critical organ for detoxification in acute exposure, while the kidney was found to be the eventual storage site in chronic exposure(16). They also demonstrated that nearly three-quarters of intravascular injected Cd were delivered from the plasma to the different tissues within2h, which suggests that the plasma has a fast clearance capacity. In our study a significant increase was detected in the cadmium concentration in the plasma in comparison to the initial control values. Despite this, plasma might have had the ability to eliminate the bulk of the toxicant 3 days after the injection and the elevated metal concentration measured in plasma is due to the presence of Cd-ligand complexes. Zhang and Wang studied the up take of waterborne Cd by black sea bream, and found that the intestine is more important in Cd uptake than the gills in this species(17).

References

1. Wlostowski, T., Krasowska, A. and Bonda, E. (2008). joint effects of dietary cadmium and polychlorinated biphenyls on metallothionein induction, lipid peroxidation and histopathology in the kidney and liver of bank voles. Ecotoxicol . Environ. Saf. 69:403-410 , 2008.

2. Tarrio, J.; Jaffor, M. and Ashraf, M. (1991). Levals of selected heavy metals in commercial fish from 5 fresh water lakes Pakistan. *Toxicol. Environ. Chem*, **33**: 133-140.

3. Hadson, P.V. (1998) .The effect of metabolism on uptake disposition and toxicity in fish. *Aquatic Toxicol,* **11**: 3-18

4. Mayer, W.; Kretschmer, M.; Hoffmann, A. and Harish, G. (1991). Biochemical and histochemical observations on effects of low level heavy metal load (Lead, Cadmium) in different organ systems of the freshwater crayfish, Astacus astacus L. (Crustacea: Decapoda). *Ecotoxicol. Environ. Safe*. **21**, 137-156.

5. Souid ,G.; Souayed,N.; Yaktiti,F. and Maaroufi,K. (2013). Effect of acute cadmium exposure on metal accumulation and oxidative stress biomarkers of Sparus aurata. Ecotoxicology and Environmental Safety 89 :1–7.

6. Ghosh, N.and Bhattacharya, S. (1992) . Thyrotoxicity of the chlorides of cadmium and mercury in rabbit. Biomed.Environ.Sci., 5, 236-240 .

7. Bervoets, L.; Blust, R.; Verheyen, R. (2001). Accumulation of metals in tissues of three-spined stickleback (Gasterousteus aculeatus) from natural fresh waters. Ecotoxicol Environ Saf 48:117–127.

8. Bervoets, L., & Blust, R. (2003). Metal concentrations in water, sediment and gudgeon (Gobio gobio) from a pollution gradient: relationship with fish condition factor. *Environmental Pollution, 126*, 9–19.

9. Karlsson-Norrgren, L. and Runn, P. (1985). Cadmium dynamics in fish: Pulse studies with 109Cd in female Zebrafish, Brachydanio rerio. *J. Fish. Biol.* **27**, 571-581.

10. Loumbourdis, NS. and Wray, D. (1998) .Heavy-metal concentration in the frog Rana Ridibunda from a small river of Macedonia, Northern Greece. Environ Int 24:427–431.

11. Loumbourdis ,NS. and Vogiatzis, A.K. (2002). Impact of cadmium on liver pigmentary system of the frog Rana ridibunda. Ecotoxicol Environ Saf 53:52–58. 30.

12. Burger, J. and Snodgrass, J. (2001) .Metal levels in southern leopard frogs from the Savannah River site: Location and body compartment effects. Environ Res 86:157–166

13. Wu, S.M.; Shih, M.-J. and Ho, Y.-C. (2007). Toxicological stress response and cadmium distribution in hybrid tilapia (Oreochromis sp.) upon cadmium exposure. Comp. Biochem. Physiol. C145, 218–226.

14. Hollis, L.; Hogstrand, C. and Wood, C.M. (2001). Tissue - specificcadmium accumulation, metallothionein induction, and tissue zinc and copper levels during chronic sublethal cadmium exposure in juvenile rainbow trout. Arch. Environ. Contam. Toxicol. 1,468–474.

15. Berntssen, M.H.G; Aspholm, O.O.; Hylland, K.; Baonga,S.E.W and Lundebye, A.K. (2001). Tissue metallothionein, apoptosis and cell proliferation responses in Atlantic salmon (Salmo salar L.) parr fed elevated dietary cadmium. Comp. Biochem. Physiol. C128,299–310.

16. Chowdhury, M.J.; Grosell, M.; McDonald, D.G.; Wood, C.M. (2003). Plasma clearance of cadmium and zinc in non-acclimated and metal-acclimated trout. Aquat. Toxicol. 64,259–275.

17. Zhang, L. and Wang, W.-X. (2007). Gastro intestinal uptake of cadmium and zinc by a marine teleost Acanthopagrus schlegeli. Aquat. Toxicol. 85, 143–153.

التراكم الحيوي للكادميوم في بعض اعضاء اسماك البعوض *Gambusi holbrooki*

طالب حسين علي[1] ، علي اشكر عبد[2] ، امال عبدالاله يونس[2]

[1]*قسم علوم الحياة ، كلية التربية للبنات ، جامعة الموصل ، الموصل ، العراق*

[2] *قسم علوم الحياة ، كلية التربية للعلوم الصرفة ، جامعة الموصل ، الموصل ، العراق*

الملخص

قدر الكادميوم الموجود في العضلات والدماغ والغلاصم و الكبد والامعاء والخصى في الاسماك المعرضة للكادميوم فضلا عن مجموعة السيطرة بواسطة جهاز الامتصاص الذري ،اجريت التجارب بثلاثة مكررات وبعدد كلي للاسماك (95) سمكة. اجري الاختبار الاحصائي لمعرفة السمية الحادة للكادميوم. وضعت خمسة اسماك لكل تركيز من التراكيز تحت القاتلة الثلاثة (5 ، 15 ، 25) ملغرام / لتر،لفترتي التأثير الحاد (24 ، 96) ساعة.اجريت التجارب بثلاثة مكررات.حدد التراكم الحيوي للكادميوم في الاعضاء المختلفة في الاسماك .اشارت النتائج بان التأثير الحاد للكادميوم المتراكم في الاعضاء اعتمد على الوقت .اشارت تجارب الامتصاص الذري ان الكادميوم الموجود في الاعضاء يزداد بصورة معنوية بالاعتماد على الوقت بنسبة 502% و167% و168% و146% و 490% و 214% في العضلات والدماغ و الكبد والغلاصم والامعاء والخصى على التوالي اثناء فترة التعريض الحاد القصيرة .بينما بلغت الزيادة اثناء فترة التعريض الحادة الطويلة 44% و82% و170%و 127% و 350%و400% في العضلات والدماغ والكبد والغلاصم والامعاء والخصى على التوالي.