Role of Fulvic Acid on Reduction of Cadmium Toxicity on Nile Tilapia *(Oreochromis niloticus*)

Ahmed E. Noor EL Deen , Mona S.Zaki and Hussan A. Osman

Hydrobiology Department, Veterinary Division, National Research Centre, Dokki, Giza, Egypt

dr_ahmednoor2002@yahoo.com

Abstract: Cadmium is one of the most toxic heavy metal, enters the environment from natural sources and as a result of mans activity. Tilapias have the capability of concentrating metals by feeding and metabolic processes, which can lead to accumulation of high concentrations of metals in their tissues. The reduction of toxic elements like cadmium in aquatic environments is needed by any acceptable method. The effect of fulvic acid (FA)on cadmium (Cd) toxicity and the impact on fish immunological, hematological changes in Nile tilapia (*Oreochromius niloticus*) were studied. The fish (100±10g)were exposed to 10 ppm Cd alone or with 0.1, 0.2 and 0.3 ppm for 15 and 45 days. Cd exposure reduced significantly ($P<0.04$) such as erythrocyte count (RBCs), haemoglobin content (Hb), haematocrit value (Hct), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration. These parameters were improved when FA was applied with Cd. The values of RBCs, Hb, Hct, MCH and MCHC were increased significantly to be as in the control fish group. Addition of FA to Cd contaminated medium considerably reduced metal absorption and accumulation in fish tissues, while it was increased metals in water and feces. The present study, is recommended that an optimum dosage of 0.3 g fulvic acid /l can effectively chelate Cd from contaminated fish and water. [Report and Opinion. 2009; 1(5):52-57]. (ISSN: 1553-9873).

Key words: Nile tilapia, Cadmium, immunological, fulvic acid, hematology, liver, gills ,musculature.

1-Introduction

Nile tilapias are considered the most popular widely distributed cheapest and intensively cultured fishes in Egypt. Cadmium is one of the most toxic heavy metal, enters the environment from natural sources and as a result of mans activity e.g. There cycling of scrap metal, electroplating industries manufacturing vinyl plastics, electrical contacts, metallic and plastic pipes. Tilapias have the capability of concentrating metals by feeding and metabolic processes, which can lead to accumulation of high concentrations of metals in their tissues. The reduction of toxic elements like cadmium in aquatic environments is needed by any acceptable method. The most widely used technique for the removal of toxic elements involves the process of neutralization and metal hydroxide precipitation, Hiemesh and Mahadevaswamy (1994). Chemicals can effectively remove certain toxic elements from industrial wastes or polluted media, but is usually costly. However, there are some cheap natural products which are also free from undesirable side effects. In recent years, the remobilization of metals by synthetic anthropogenic chelating agents has received much attention. The literature reported number of chelators that have been used for chelate-induced hyper accumulation, Khangarot and Tripathi (1991). Natural compound like FA is known to be effective chelating agents of heavy metals, Karuppasamy et al,2005. FA is the most commonly used cheater because of its small molecular weight and strong chelating ability for different heavy metals ,Litchfield and Wileoxon (1949) and Donor and William (1993) . Metal bioaccumulation can occur via complication, coordination, chelation, ion exchange and other processes of greater or lesser specificity. Bioaccumulation processes are sometimes due to active (metabolism dependent) metal accumulation by living cells, Aiken et al,1985. In spit of the amount of data published on the effect of water borne exposure of cadmium and FA singly, information on the effects of Cd / FA mixture on aquatic organisms are limited and not uniform. Therefore, FA appears to be promising tool to control cadmium pollution in aquaculture Murry and Linder (1983). The present study, short and long– term bioassays were designed to evaluate the influence of FA on the retention of cadmium in water. It was carried out to investigate the effect of FA on reduction of toxicity of cadmium for enhance the change of blood parameter and enzymes and to assess its impact on some physiological parameters of Nile tilapia *(Oreochromis. Niloticus).*

2-Material and methods

2-1-Collecting sample Tilapia

A healthy 75 fish of Nile tilapia Oreochromis niloticus weighing (100±10g)/ fish were collected from the ponds of Kafr Eel Sheikh governorate fish farms, Egypt. Fish were acclimated in cement fish ponds for 2 weeks. Acclimated fish were exposed to different concentration of cadmium and mortality were observed for 96- h. A static renewable bioassay method, Duncan (1955) was adopted for the determination of 96-h median lethal probity analysis, Santschi (1988) was followed the calculation of 96 hr LC50. A control group was maintained in metal – free tap water. The 96 hr LC50 of cadmium for Oreochromius niloticus was 40 ppm. A stock solution of cadmium was prepared by dissolving 10.686 g 0f annular grad cadmium sulphate ($CdSO_4$– 8/3H_2O) in 1/L of distilled water and the diluted with water to obtain the desired concentration (10 ppm) for this experiment. The fish were distributed randomly in 5 cement pond at a rate of 15 fish / aquarium that containing aerated tap water. These aquaria were divided into five groups with three replicates each per group. Fish were fed frequently a diet containing 25% crude protein (CP) at a rate of 3% of live body weight twice daily for 15 and 45 days. Siphoning three quarters aquariums was done every day for waste removal and replacing it by an equal volume of water containing the same concentration of Cd and FA . Dead fish were removed and recorded daily.

2-2-Sample classification

The first group was free of Cd and FA acid and maintained as a control. The second groups were exposed to 10 ppm of Cd SO_4 only. The third, fourth and fifth group were exposed to 10 mg Cd /L and 0.1, 0.2 and 0.3 g FA /L, respectively. Each aquarium was supplied with compressed air via air-stones from air pumps. Well-aerated water supply was provided from a storage fiberglass tank. The temperature was adjusted at 27□1 oC by means of thermostats Table (1).

2-3-Cd residue :

Cadmium sulphate and fulvic acid was obtained from El- Nasr chemical and a Grotech companies (Egypt) respectively, and prepared in aquatic solution to provide the required concentrations of cadmium and fulvic acid. Cadmium was measured in water, liver , gills, muscle and feces according to method of Norvell(1991).

2-4-Statistical Analysis:

The obtained data were subjected to analysis of variance between means were done at the 5% probability level, using Duncan' s new multiple range test by Spraggue(1973).

3-Results

The present study show that addition of FA to Cd contaminated media, reduced significantly the Cd level in the water and helped to eliminate metal from the fish body(liver, gills, musculature and feces) and and in turn improved the biochemical parameters as compared to fish exposed to Cd alone in Table (2).

3-1-Clinical examination:

The clinical examination of most examined fishes showed asphyxia, some aggregated on the surface, accumulated at the water inlet of the pond and air pump of aquaria. Others appeared dull with loss of escape reflex .

3-2-Haematological parameters:

The results of erythrocyte count (RBCs), haemoglobin content (Hb) and haemetocrit value (Hct) obtained from the fish exposed to sublethal dose of Cd (10 mg/l) alone or with different doses of fulvic acid are given in Table (4). Table 3 shows that the RBCs, HB and HCt were reduce in fish exposed to Cd at both periods and they were less than that of the control ($P<0.05$) The RBCs count decreased significantly in fish exposed to Cd at 15 and 45 days. On the other hand, these parameters were return to the normal values and increased significantly in fish exposed to Cd with 0.2 and 0.3 g of FA /l for 15 and 45 days. These values increased significantly in fish exposed to Cd with 0.3 g FA /l. Blood parameter were improved in fish exposed to Cd with different levels of FA . The blood indices calculated from the mean values of blood parameters for the aforementioned treatments are given in Table(2). Data shows that the MCV increased significantly in fish exposed to Cd alone, while the MCH and MCHC decreased significantly in fish exposed to Cd only when compared with the control. These parameters increased with increasing of exposure time of fish to Cd. Addition of FA to Cd- polluted media maintained the MCV, MCH and MCHC at levels close to those of the control.

Table 1: Field experimental groups and their notation.

S. No.	Groups in field ponds	Nation
1	Control (metal free water)	C
2	Cadmium (10 ppm) alone	Cd
3	Cadmium (10 ppm) +0.1g fulvic acid /l	Cd fulvic acid 1
4	Cadmium (10 ppm) +0.2 fulvic acid /l	Cd fulvic acid 2
5	Cadmium (10 ppm) +0.3g fulvic acid /l	Cd fulvic acid 3

Table 2: Changes in mean cell volume (MCV) , mean celll haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) in the blood of Nile tilapia (O.niloticus) exposed to Cd with or without fulvic acid (FA).

Items	MCV		MCH		MCHC	
Period	15 days	45 days	15 days	45 days	15 days	45 days
Control	95.32 ad	100.02 a	34.35 a	43.21 a	34.77 a	43.32 a
	± 1.86	± 2.243	± 0.342	± 1.432	± 1.121	± 0.928
Cd	106.93 b	106.75 b	33.02 b	36.56 b	31.15 b	37.27b
	± 2.23	± 0.874	± 0.177	± 0.846	± 0.909	± 1.85
Cd+0.1 g	93.45a	95.71 a	34.52 a	32.45 b	37.21a	33..77 b
FA/l	± 2.05	± 4.26	± 1.23	± 1.17	± 1.26	± 1.49
Cd+0.2 g	96.24a	98.77 a	33.23 a	36.76 bc	34.06 ac	37.40 b
FA/l	± 2.64	± 0.909	± 1.72	± 1.49	± 1.76	± 1.20
Cd+0.3 g	101.1db	107.95 b	32.85 a	37.02 ac	33.34 cb	34.87 b
FA	± 2.512	± 2.241	± 1.702	± 1.576	± 0.941	± 1.68

Table 3: Changes in cadmium residue in water (mg Cd/L), liver, gills, muscle and feces (mg Cd/g dry weigh) of Nile tilapia (O. niloticus) exposed to to Cd with or without fulvic acid (FA).

Items	Water	Liver		Gills		Muscle		Feces	
Period/days		15	45	15	45	15	45	15	45
Control	0.041	0.048 a	0.055 a	0.038 a	0.039 a	0.023 a	0.076 a	0.003 ab	0.005 ab
	± 0.02	± 0.02	± 0.004	± 0.02	± 0.04	± 0.002	± 0.005	±0.018	± 0.02
Cd	9.31	2.15 b	5.971 b	1.36 b	2.56 b	0.476 b	1.077 b	0.153 b	0.189 b
	± 0832	± 0.253	± 0.85	± 0.085	± 0.276	± 0.06	± 0.15	± 0.018	± 006
Cd+0.1g	7.15	1.292 c	4.16 b	0.65 c	1.07 c	0.343c b	0.665 c	0.940 c	2.067 c
FA /l	± 0.34	± 0.056	± 0.45	± 0.06	± 0.11	± 0.04	± 0.021	± 0.03	± 0.143
Cd+0.2g	3.78	0.95 d	3.791 b	0.394 d	0.85 c	0.33 cb	0.383 d	2.34 d	5.443 d
FA/l	± 0.01	± 0.054	± 0.29	± 0.052	± 0.06	± 0.08	± 0.034	± 0.069	± 0.345
Cd+0.3g	1.73	0.42 e	2.45 c	0.266 d	0.71 c	0.216 c	0.217 d	5.282 e	7.456 e
FA/l	± 0.02	± 0.034	± 0.23	± 0.073	± 0.42	± 0.03	± 0.025	± 0.32	± 0.528

Table 4: Changes in erythrocyte (count x 106/mm3), hemoglobin content (g/100ml) and haematocrit value (%) in the blood of Nile tilapia (O.niloticus) exposed to Cd with and without fulvic acid(FA) .

Items	Erythrocyte count (RBCs)		Hemoglobin (HB)		Haematocrit value (Hct)	
Period	15 days	45 days	15 days	45 days	15 days	45 days
Control	1.58 a	1.714 a	5.48 a	7.315 a	15..31 a	17..32 a
	± 0.073	± 0.051	± 0.353	± 0.133	± 0.308	± 1.665
Cd	1.268 b	1.06 b	4.21 b	4.12 c	13..5 b	12.01 b
	± 0.064	± 0.073	± 0.235	± 0.354	± 0.47	± 0.576
Cd+0.1g	1.572 a	1.57 d	4.54 ab	5.12 b	14.66 a	15.05 a
FA/l	± 0.064	± 0.023	± 0.395	± 0.136	± 1.454	± 0.76
Cd+0.2g	1.56 a	1.786 ac	5.17 ab	6.605 b	15.02 a	17.65 a
EDTA/l	± 0.086	± 0.032	± 0.458	± 0.305	± 1.72	± 0.916
Cd+0.3g	1.956 c	2.01 c	6.464 c	7.68 a	20.0 c	22.02 c
FA/l	± 0.086	± 0.063	± 0.277	± 0.133	± 0.365	± 1.471

DISCUSSION

The clinical picture in naturally infested and polluted Tilapia sp were revealed some aggregated on the water surface, accumulated at the water inlet of the pond and air pump of aquaria. Almost, appeared dull with loss of escape reflex, Eissa (1994) and Eaton and Stinson (1983).The present study reveals that the fish exposed to Cd alone showed significant reduction in RBCs, Hb and Hct than those exposed to Cd with different level of FA. The reduction of these parameters in Nile tilapia, O niloticus at sublethal levels of cadmium might be due to the destruction of mature RBCs and the inhibition of erythrocyte production due to reduction of haemsynthesis that affected by pollutants, James and Sampath, K (1999) . Also, the decrease in RBCs count may be attributed to haematopathology or acute haemolytic crisis that results in sever anemia in most vertebrates including fish species exposed to different environmental pollutants, Yamawaki (1986) or may be the decrease in the RBCs may be attributed to reduction of growth and other food utilization parameters which results in sever aneamia, Wintrobe (1978). Mousa(1999)found a significant decreased in total erythrocyte count, haemoglobin content, haematocrit value and mean corpuscular haemoglobin concentration in air breathing fish, Channa punctatus after exposure to sublethal dose of Cd (29 mg Cd/L).The addition of FA improves the haematological parameters (RBCs, Hb and Hct) which indicating to the capability of FA to chelate Cd from the media. Subsequently, the Cd toxicity was reduced. These results are in agreement with those of Snedecor and Cochran, W. G. (1982) who observed that Oreochromis mossambicus exposed to copper along with fulvic acid showed a significant improvement in blood parameters over those copper alone. The perturbations in these blood indices (increase MCV, decrease of MCH and MCHC) may be attributed to a defense against Cd toxicity through the stimulation of erythropiosis or may be related to the decrease in RBCs, Hb and Hct due to the exaggerated disturbances that occurred in both metabolic and hemopoietic activities of fish exposed to sublethal concentration of pollutants, Hung et al, 1997. The present results indicate that fulvic acid is effective in removing Cd from water, and reducing Cd bioaccumulation in fish. Particulate organic matter which can scavenge metal from water and help to reduce metal from fish. These results are in agreement with Shalaby (2001) who study that any agent that can remove Cd from water helps to reduce the bioaccumulation of this metal in fish.The present study showed that the addition of fulvic acid to the Cd media reduced significantly ($P<0.05$) the Cd level in water and metal uptake as compared to fish exposed to Cd alone. The Cd concentration in water was 9.31 mg/L and it decreased significantly ($P< 0.05$). The Cd accumulation in liver, gills and muscle of fish exposed to Cd alone was higher than that of FA. These results suggest that FA could chelate Cd ions producing a stable complex, thus reducing the chance for metal uptake by tissues. Besides , the fulvic acid eliminated more amount of Cd from the body through feces. The formation of Cd- FA complex in water and elimination of more amount of Cd in feces evidently reduced the metal burden in tissues and thereby improved the haematological parameters of fish exposed to Cd. Planas-bohne(10) found low level of cadmium in tissues due to increased excretion of metals through feces and urine when rats were administered Cd intravenously along with FA .From the present study, it is recommended that an optimum dosage of 0.3 g FA /l can effectively chelate Cd from contaminated water . Hence, a scientific method detoxification is essential to improve the health of fish in any stressed environmental conditions.

Correspondence to:
Ahmed Ismail Noor El Deen
4 Albhooth St, Doki,Giza,Egypt
Telephone: 002020472751493
Cellular phone: 0020124465620;
0020177891047
Emails: dr_ahmednoor2002@yahoo.com

Reference

1. Aiken,G.R. Mcknight,D.M., and Vac Carthy, P.1985. Humic substances of soil , sediment and water,New York:Wildy Inter science.
2. Donor and William R. (1993).! Humic, Fulvic and Microbial Balance:!Organic Soil Conditioning.!Evergreen Colorado: Jackson Research Center.
3. Duncan,D.B. (1955): Multiple range and multiple (F) test. Biometrics, 11: 1- 42.
4. Eissa, I.A.M and Fatma. M.Fakhry (1994): Clinical picture and residue levels of locally used pesticides (field dose) in some freshwater and marine fishes.J.Egypt Vet.Med.Res.,3,1,96-107.
5. Eaton, D. L and Stinson, M. D. (1983): Concentration of lead, cadmium, mercury and copper in the cray fish (*Pacifasticus leniusculus*) obtained from a lake receiving urban runoff. Arch. Environ. Contam. Toxicol., 12: 693- 700.
6. James, R. and Sampath, K (1999): Effect of the ion-exchanging agent, Zeolite, on reduction of cadmium toxicity: an experimental study on growth and elemental uptake in *Heteropneustes fossilis* (Bloch). J. Aqua. Trop., 14 (1) 65- 74.
7. Hiemesh, S. and Mahadevaswamy, M. (1994): Sorption potential of biosorban: for the removal of copper. Indian. J. Environ. Hlth., 36 :165-169.
8. Huang, J. W.; Chen, J.; Berti, W. R.and Cunningham S. D (1997): Phytoremediation of Pb- contaminated soils: Role of synthetic

chelates in lead phytoextraction. Environ. Sci. Technol., 31: 800- 805.

9. Khangarot, B. S and Tripathi, D. M (1991): Changes in humoral and cell- mediated immune responses and in skin and respiratory surfaces of cat fish *Saccobranchus fossillis*, following copper exposure. Ecot. Envir. Safety., 22 (3): 291- 308.
10. Karuppasamy, R.; Subathra, S and Puvaneswari, S. (2005): Haematological responses to exposure to sublethal concentration of cadmium in air breathing fish, *Channa punctatus* (Bloch). J. Environ Biol., 26(1):123-8.
11. Litchfield, J. T and Wileoxon, F. (1949): A simplified method for evaluatin dose-effect experiments. J. Pharmacol. . Exp. Ther., 96:59-113.
12. Murray,K., and Linder, P.W (1983): Fulvic acid structure and metal binding I.A random molecular model.Journal of soil science, 34, 511-523.
13. Moussa, M. A. (1999): Biological and physiological studies on the effect of the gramoxon and stomp herbicides on Nile tilapia (*Oreochromis niloticus*). Fact. Sci. Zool. Dep. Cairo. Univ., 200p (Ph.D. Thesis)
14. Norvell, W.A (1991): Reactions of metal chelates in soil and nutrient solutions In: Mortvedt, J.J., Cox, F.R., Shuman, L. M and Welch, R. M., Editors, 1991. Micronutrients in Agriculture, 2nd Edition, Soil. Science of America, Madison,Wisconnsin, pp. 187- 227.
15. Planas- Bohne, F and Lehman, M (1983): Influence of chelating agent on the distribution and excretion of cadmium in rats. Toxicol. Appl.Pharmacol., 67:408- 416.
16. Snedecor, G. W. and Cochran, W. G. (1982): Statistical Methods. 6*th* edition. Iowa State Univ. Press., Amer., IA, USA, pp 593.
17. Shalaby, A. M. (2001): Protective effect of ascorbic acid against mercury intoxication in Nile tilapia (*Oreochromius niloticus*). J. Egypt. Acad. Soc. Environ. Develop., (D-Environmental studies)., 2 (3):79-97.
18. Santschi, P. H (1988): Factors controlling the biogeochemical cycle of trace elements in fresh and coastal waters as revealed by artificial radioisotopes. Limnology and oceanography, 33:848- 886.
19. Spraggue, J.B (1973): The ABCs of pollutant bioassay using fish In Biological methods for Assessment of water quality. ASTM 528. Amer. testing. Material., pp.6-30.
20. Wintrobe, M. M (1978): Clinical hematology. Henry Kimpton, London., pp: 448.
21. Yamawaki, K.; Hashimoto, W.; Fujii, K.; Koyama, J.; Ikeda, Y. and Ozaki, H. (1986): Hematological changes in carp exposed to low cadmium concentration. Bull of the Japanese. Soc. Sci. Fish., 59 (3):459- 466.

7/9/2009