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RESEARCH ARTICLE

ALTERATIONS IN BIOCHEMICAL PARAMETERS OF A FRESH WATER FISH, *ANABAS TESTUDINEUS* BLOCH ON EXPOSURE TO HEAVY METAL TOXICANT CADMIUM CHLORIDE

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ABSTRACT

The present work aimed to estimate the effect of different concentrations of $CdCl_2$ on biochemical parameters of freshwater fish, *Anabas testudineus*. Electrophoresis of the blood serum, Liver RNA extraction and agarose gel electrophoresis were done. 96-h LC_{50} of $CdCl_2$ was 7ppm. Levels of liver protein, AST and ALT and serum glucose content showed significant change after 14 days of exposure to different concentrations of $CdCl_2$ (2ppm, 3ppm and 4ppm). The electrpherogram showed a reduction in blood protein content on exposure to higher doses of $CdCl_2$ (3ppm and 4ppm) and also a mitigation in liver RNA was observed in the liver of $CdCl_2$ treated fish when compared to the control in the agarosegel electropherogram.

INTRODUCTION

Among all the vertebrate groups, fishes are the most diverse and demonstrate a high degree of heterogeneity in anatomy, physiology, reproductive strategy, behaviour, and ecology (Lagler *et al*., 1977; Janz, 2000). Accordingly, fish have evolved to inhabit a wide variety of environments in brackish, freshwater, and marine systems ranging from approximately 15,000 m above sea level to 10,000 m below (Lagler *et al*., 1977). Pollution of aquatic ecosystems by heavy metals predates all other anthropogenic chemicals by several millennia. Heavy metals exert a broad spectrum of effects on aquatic organisms, especially on the fish species. Heavy metals are soft but highly toxic as they compete for binding with essential metals (Allen, 1995) and consequently they interfere with sulphydryl groups that play an important role for normal function of enzymes and structural proteins. Heavy metals including cadmium (Cd^{2+}) have been shown to act as endocrine disrupting compounds in fish (Mc Master, 2001; Hewitt and Servous, 2001). Endocrine disruptors are environmental chemicals that when absorbed in to the body either mimics or block hormones and disrupts the body's normal physiological functions (Gibson, 2007; Binitha, 2009). Common compounds of cadmium include cadmium chloride [$CdCl_2$], cadmium oxide, cadmium sulphide and cadmium acetate. The two oxidation states of cadmium are the metallic [Cd^0] and divalent [Cd^{2+}].

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Cadmium is a non-essential element with no known biological function, naturally found at low concentrations in natural waters (Martinez *et al*., 1999). The target organs for Cd^{2+} toxicity have been identified as liver, placenta, kidneys, lungs, brain and bones in higher vertebrates (Roberts, 2003). The outbreak of "itai – itai – byo" or "ouch – ouch disease", in Japan was the historical event that drew the world's attention to the environmental hazards of Cd^{2+} poisoning for the first time (Sarunya *et al*., 2006). Bio-enhancement of Cd^{2+} transfer along a food chain was studied by Seebaugh *et al*. (2005) and fish are reported to be used as biological indicators to assess water pollution (Rashed, 2001).

The present work was aimed to determine the biochemical effects of sublethal concentrations of $CdCl_2$ in freshwater Teleost, *Anabas testudineus*. Fish are the group of animals most at threat from aquatic pollution and with their physiological similarity to mammals together with their long term exposure in natural habitats, provides a suitable biomonitor for environmental pollution. Evidences are now accumulating that even low levels of pollutants can disrupt the functioning of the endocrine system of fish (Kime, 1999).

MATERIALS AND METHODS

Prior to experiment the animal model *Anabas testudineus* Bloch collected from local suppliers were acclimatized for two weeks in a glass aquaria filled with dechlorinated tap water

under laboratory conditions [natural photoperiod and temperature 26±2^{0}C]. The fish were fed with protein rich feed on alternate days. Cadmium chloride [$CdCl_2.H_2O$ RM – 469 – 100g purchased from HiMedia, Mumbai] was used as the test chemical and sublethal doses [80, 120 and 160ppm] of cadmium chloride were finalised after determining the LC_{50} of Cd^{2+}. The laboratory acclimatized fish were divided in to ten groups of eight each in separate aquarium tanks. The first group of fish was served as control. Fish in group II, III and IV were exposed to 2mg $CdCl_2$/L of water, groups V, VI and VII to 3mg $CdCl_2$/L and groups of VIII, IX and X 4mg $CdCl_2$/L for a period of 7, 14 and 28 days respectively. After stipulated periods of exposure, fish were sacrificed. Then liver was excised straight away and frozen immediately at $^-80^0$C (NBS, USA) for enzymes assay. The blood was collected from caudal artery and centrifuged at 10,000 r.p.m for 10 minutes in a high speed refrigerated centrifuge [Eppendorf, Germany]. The supernatant was collected and kept in an ultra low freezer at $^-80^0$C until biochemical analysis.

Homogenate preparation and Protein measurement

The chilled liver tissue was blotted, weighed and a 10% homogenate of liver was prepared in cold sucrose (0.25M) using potter Elvehjem type homogeniser with teflon pestle (Arthur Thomas, USA) and centrifuged at 10,000 rpm for 10 minutes. All the operations were carried out at 4^{0}C. The supernatant was collected and used for enzyme's assay. The liver protein concentration for all the enzyme studies was determined using Bradford method (Bradford, 1976).

Measurement of transaminases and serum glucose

Spectrophotometric determination of specific activities of transaminases such as aspartate amino transferase (AST, EC.2.6.1.1) and alanine amino transferase (ALT, EC. 2.6.1.2) were assayed as per standard protocols. Serum glucose values were determined spectrophotometrically using diagnostic kit manufactured by Span diagnostic Ltd. Surat and as per the standard protocol supplied by the manufacturers as implied by the methods of (Tietz, 1976; Wernick, 1985) respectively.

Electrophoresis

For electrophoresis, the blood serum is mixed with sample buffer in the ratio 1:1 analyzed using a mini vertical gel electrophoresis. Each well was loaded with 10µl sample. Electrophoresis was performed under denaturing and discontinuous condition of 10% sodium dodecyl sulfate [SDS] poly acrylamide gel using a mini vertical gel unit [Hoefer, USA] by the method of Laemmli (1970). Known molecular weight protein ranging from 250 to 15 kDa was used as molecular markers [Bio-Rad]. The serum samples loaded in the wells were electrophorised at a constant current of 120V for stacking and running gel in an electrophoresis buffer consisting of 0.1% SDS, 0.05M Tris and 0.384M glycine buffer, pH 8.3 for about 3 hour. The gels were stained with 0.25% Coomassie brilliant blue R 250 in a mélange of 40% methanol and 7% acetic acid and 5% methanol to get the best quality protein bands. Stained gels were then fixed in destain II containing 1% glycerol. The appropriate molecular weights of resolved proteins were determined by comparison with known standards.

RNA Extraction and Agarose gel Electrophoresis

For agarose gel electrophoresis one hundred milligram of the tissue was homogenized in 1ml of Tri Reagent ^{R} (Sigma, USA) using homogenizer (ROTEK, INDIA). Liver RNA was extracted following manufacturer's instructions and the resulting RNA pellet was solubilized and quantified the RNA concentration using spectrophotometry at 260/280nm.1% agarose gel with 0.5µg/mL ethidium bromide was used to fractionate 3µg of liver RNA. Electrophoresis was performed in 1xTBE at 100V for 45 minutes before visualizing under ultraviolet light using transilluminator (Biotech, Chennai).

Chemicals

Acrylamide, Tris, TEMED, ammonium persulphate etc were procured from GE, Amersham chemicals, USA. Glucose kit is purchased from Span diagnostic Ltd. Surat. All other chemicals used were analytical grade and purchased from SRL, Bombay, India.

Statistics

Data were collected from six animals in each group. Statistical analysis was done by SPSS statistical package. Data were analyzed by one – way analysis of variance, which helps to understand whether or not there were differences between groups of means. Groups that were not significantly different in Duncan's (1955) multiple range tests were considered homogenous. Difference was considered significant when $P < 0.05$.

RESULTS

Exposure of fish to different concentrations of $CdCl_2$ revealed significant changes in the biochemical parameters studied at different periods of time in *Anabas testudineus* Bloch. The toxic impact of $CdCl_2$ on hepatic transaminase enzymes were clearly analysed on the basis of the bio assay test, and in comparison of biochemical and haematological examinations with control fish. Cadmium chloride caused effect in the form of hyper hepatic proteinemia (Table 1) and hyperglycemia (Table 2) on exposure to all the three sublethal doses of $CdCl_2$ (2, 3 and 4mg/L).

Table 1. Effect of sublethal doses of $CdCl_2$ (2, 3 and 4mg/L) on total hepatic protein content in *Anabas testudineus*

	Dose of Exposure of $CdCl_2$ (mg/L)		
	2	3	4
Control	0.97±0.005	0.97±0.005	0.97±0.005
7 days	0.97±0.04*	1.01±0.008*	1.097±0.03*
14 days	1.04±0.04*	1.03±0.01*	1.77±0.16**
28 days	1.19±0.04**	1.11±0.05**	2.638±0.19***

Total hepatic protein content expressed as µg/mL.
Each value is mean ± SEM of 6 fish. $^*P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ compared with control.

Table 2. Effect of sublethal doses of $CdCl_2$ (2, 3 and 4mg/L) on blood glucose content in *Anabas testudineus*

	Dose of Exposure of $CdCl_2$ (mg/L)		
	2	3	4
Control	65.52±0.41	65.52±0.41	65.52±0.41
7 days	65.53±0.41	66.08±0.62*	66.81±0.40*
14 days	76.49±0.55**	75.11±0.31**	76.64±0.61**
28 days	82.51±0.51***	82.70±0.73***	84.01±0.41***

Glucose content expressed as mg/dL.
Each value is mean ± SEM of 6 fish. *P< 0.05, **P< 0.01, *** P< 0.001 compared with control.

Table 3. Effect of sublethal doses of $CdCl_2$ (2, 3 and 4mg/L) on hepatic transaminase (AST and ALT) activity in *Anabas testudineus*

		Dose of Exposure of $CdCl_2$ (mg/L)		
		2	3	4
Control	AST	42.98±0.17	42.98±0.17	42.98±0.17
	ALT	17.05±0.79	17.05±0.79	17.05±0.79
7 days	AST	45.24±1.95	54.95±1.45*	55.99±0.5*
	ALT	15.12±1.39*	20.17±0.60*	20.58±0.2*
14 days	AST	61.82±0.90**	63.64±0.94**	63.83±0.92**
	ALT	17.69±0.58*	20.47±0.49**	21.05±0.35***
28 days	AST	65.57±1.12***	66.30±0.89***	67.21±0.63***
	ALT	19.09±1.1*	20.78±1.40**	22.36±0.31***

Transaminase activity expressed as nmoles PO_4 liberated / mg protein / minute.
Each value is mean ± SEM of 6 fish. *P< 0.05, **P< 0.01, *** P< 0.001 compared with control.

Transaminase activity

An increase in transaminase [AST & ALT] activity was observed for all the three periods (7, 14 and 28 days) on exposure to higher doses of $CdCl_2$ (3 and 4mg/L) (Table 3). The activities of AST and ALT are used as a significant stress indicator in fish (Sayed *et al*., 2003).

Electrophoresis

In the electropherogram (Fig 1), M represents the molecular marker, lane-1 represents the control and lanes- 2, 3 & 4 represent blood serum of fish exposed to 2mg/L of $CdCl_2$ for 7, 14 and 28 days respectively. Lanes- 5, 6 & 7 represent blood serum of fish exposed to 3mg/L of $CdCl_2$ for 7, 14 and 28 days respectively, and lanes- 8, 9 & 10 represent blood serum of fish exposed to 4mg/L of $CdCl_2$ for 7, 14 & 28 days respectively. A protein band having molecular weight of 97KDa was intensified when fish was exposed to 2mg/L $CdCl_2$ for 7, 14 and 28 days, and 4mg/L $CdCl_2$ for 14 days but the same band disappeared when fish was exposed to 4mg/L of $CdCl_2$ for 28 days. A new protein band with molecular weight 55KDa appeared on exposure to 2mg/L $CdCl_2$ for 14 days and 4mg/L $CdCl_2$ for 28 days. A protein band with molecular weight 31KDa with less intensity appeared after exposure to 3mg/L of $CdCl_2$ for 28 days. In the present study, it has been confirmed that the exposure to different sub lethal doses of $CdCl_2$ for varied periods led to the appearance and disappearance of some protein bands. Thus the electropherogram showed a decrease in blood protein content.

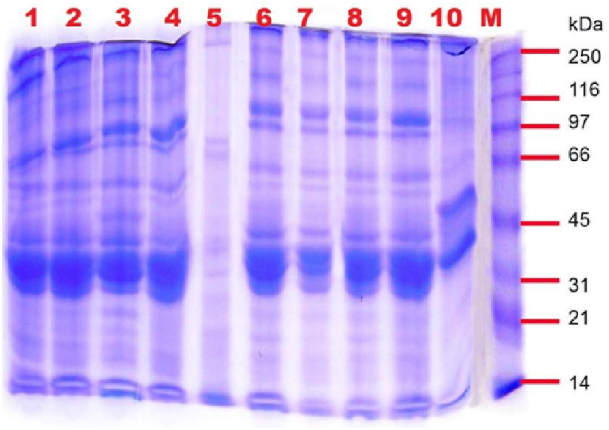

Fig 1. Electropherogram showing serum protein of *Anabas testudineus* exposed to $CdCl_2$

M – Molecular weight marker, 1- Control, 2 to 4 - 2 mg/ L $CdCl_2$ exposure, 5 to 7 - 3mg/ L $CdCl_2$ exposure, 8 to 10- 4mg/ L $CdCl_2$ exposure

RNA Concentration

A variation in RNA concentration was observed in the liver of fish exposed to sublethal doses of $CdCl_2$ when compared to control. Electropherogram showed the presence of three RNA bands in the control fish. Only two RNA bands appeared in the $CdCl_2$ treated fish (Fig 2) and one of the bands showed high intensity. A decrease in total liver RNA was observed in the agarosegel electropherogram.

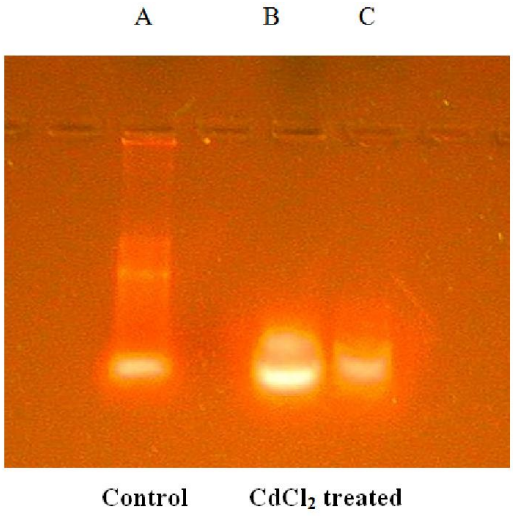

Fig 2. Electropherogram showing liver RNA of *Anabas testudineus* exposed to $CdCl_2$

A-Control B- 4 mg/ L $CdCl_2$ exposure C- 2 mg/ L $CdCl_2$ exposure

DISCUSSION

Energy gain or loss in fish is controlled not only by carbohydrates but also by other macronutrients like proteins. An exposure to the heavy metal toxicant $CdCl_2$ showed an increase in liver protein in *Catla catla* (Sobha *et al*., 2007) as

in the present study. Changes in the protein metabolism caused by the activation of the synthesis or by the protein degradation and the activation or inhibition of certain enzymes such as aspartate amino transferase [AST] and alanine amino transferase [ALT] were reported among the adverse effects caused in the liver by toxic substances (De Smet and Blust, 2001). The data of the present study was well supported by some recent findings where exposure to $CdCl_2$ increased AST and ALT in fish (Zaki *et al*., 2009). In many fish species, the blood plasma glucose level has the tendency to increase due to experimental stress. Hyperglycemic response in Heteroclarias on exposure of Cd^{2+} indicates disrupted carbohydrate metabolism due to enhanced breakdown of liver glycogen mediated by adrenocortical hormones and reduced insulin secretory activity (Kori-Siakpere *et al*., 2006). Increase in serum glucose levels in fish under stress was reported previously by Chowdhary *et al*., 2004 and Bedii and Kenan, 2005. This can be attributed to several factors and one of them is the decrease in the specific activity of some enzymes like phosphofructokinase, lactate dehydrogenase and citrate kinase that decrease the capacity of glycolysis (Al meida *et al*., 2001). The present study illustrated that glucose recorded high values than control group and was explained through gluconeogenesis, which mean formation of glucose from non-carbohydrate source. Hyperglycemia was observed in Grey Mullet on exposure to $CdCl_2$ and in Rainbow truot and *Salmogaidneri* (Vosyliene *et al*., 2006).

Serum proteins are involved in major physiological events and are highly sensitive to heavy metal poisoning (Jacob *et al*., 1977) and the electropherogram showed the decrease in the serum protein may be regarded as an indication of stress. Depletion of protein content has been observed in the muscle, brain and blood of the fish *Catla catla* as a result of mercuric chloride toxicity (Deva Prasanth and Arivoli, 2008). A significant mitigation in liver RNA band in the present study may be due to the stress exerted by the heavy metal toxicant $CdCl_2$. Stressor induced changes in mRNA levels are more transient than the currosponding changes in protein levels (Sanders, 1993). Expression of MT mRNA was decreased significantly in liver and kidney of silver barb, *Puntius gonionotus* during subchronic Cd^{2+} exposure (Alisawangsongsak *et al*., 2007). Therefore the absence of any detectable RNA band in liver of fish exposed to sublethal dose of $CdCl_2$ may be either due to the variations in the ribonuclease activity or changes occurred in cell structure on exposure to the heavy metal toxicant $CdCl_2$. Decrease in RNA, glycogen contents and hepatic enzymes due to mercury have been reported previously (Shakoori *et al*., 1994). On the whole, the results of our study highlight the stress to which freshwater fish are exposed through the uncontrolled discharge of heavy metals in the aquatic environment. It could be concluded that a deleterious effect on the activities of transaminases and haematological parameters was induced by cadmium chloride in this fish and also cadmium can cause an induction or inhibition of a variety of cellular enzymes in fish.

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REFERENCES

Alisa Wangsongsak., Suwimol, U., and Maleeya, K. 2007. Alterations of organ histopathology and metallothionein mRNA expression in silver Barb (*Puntius gonionotus*) during subchronic Cd^{2+} exposure. *J. Environ. Sci.* 19: 1341-1348.

Allen, P. 1995. Accumulation profiles of cadmium and their modification with mercury and lead in the edible tissues of *Oreochromis aureus. Fresh Environ Bull* 2: 745-751.

Almeidaa, J. A., Novelli, E. L., Dal Pai Silva, M., and Junior, R. A. 2001. Environmental Cadmium exposure and metabolic responses of the Nile tilapia (*Oreochromis niloticus*), *Environ Pollut* 114(2): 169-175.

Bedi CiCiK., and Kenan ENGiN. 2005. The effects of Cadmium on levels of glucose in serum and glycogen reserves in the liver and muscle tissues of (*Cyprinus carpio*). *Turk J Vet Anim Sci* 29: 113-117.

Binitha, R. N., and Francis Sunny. 2009. Sewage effluent as a concern for physiological homoeostasis in a freshwater teleost, *Anabas testdineus*. Ph.D Thesis, University of Kerala, Thiruvananthapuram.

Bradford, M. M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle to protein dye binding. *Analyt, Bioche* 72: 248-254.

Chowdhury, M. J., Pane, E. F., and Wood, C. M. 2004. Physiological effects of dietary cadmium acclimation and waterborne cadmium challenge in rainbow trout, respiratory, ionoregulatory, and stress parameters. *Comp Biochem Physiol Part C Toxicol Pharmacol* 139: (1&3): 163-173.

De Smet, H., and Blust, R. 2001. Stress responsed and changes in protein metabolism in carp (*Cyprinus carpio*) during cadmium exposure. *Ecotoxicol Environ Saf* 48(3): 255-262.

Deva Prasath, P. M., and Arivoli, S. 2008. Biochemical study of freshwater Fish *Catla catla* with reference to mercury chloride. *J Environ Health Sci Eng* 5: 109-116.

Duncan, D. B. 1955. Multiple ranges and multiple F test, *Biometrics* 11: 1-42.

Gibson, R. L. 2007. Toxic Baby Bottles: Scientific study finds leaching chemicals in clear plastic baby bottles. *Environ California. Reser. Policy Center.*

Hewitt, M., and Servos, M. 2001. An overview of substances present in the Canadian aquatic environment associated with endocrine disruption. *Wat Qual Res J Canada* 36: 191-213.

Jacob, J. M., Carmicheal, N. and Caranagh, J. B. 1977. Ultra structural changes in the nervous system of rabbit poisoned with methyl mercury. *Toxicol Allo Pharmacol* 39: 249-261.

Janz, D. M. 2000. *In*: Ostrander, G.K. (Eds.), Endocrine System. The Laboratory Fish, San Diego, CA.

Kime, D. E. 1999. Endocrine disrupting chemicals, *Royal Society of chemistry Cambridge* 27.

Kori-Siakpere, O., Ake, J. E. G., and Avworo, U. M. 2006. Sublethal effects of cadmium on some selected haematological parameters of *Hetero clarias. Int J Zool Res* 2(1): 77-83.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature,* 227.

Lagler, K., Bardach, J., Miller, R., and Passino, D. 1977. Ichthyology, 2nd ed. John Wiley and Sons, New York.

Martinez, M., Del Ramo, J., Torreblanca, A., and Diaz-Mayans, J. 1999. Effect of cadmium exposure of zinc levels in the brine shrimp (*Artemia parthenogenetica*). *Aquaculture* 172: 315-325.

McMaster, M. E. 2001. A review of the evidence for endocrine disruption in Canadian aquatic ecosystems. *Water Qual Res J Can* 36: 215-231.

Rashed, M. N. 2001. Cadmium and lead levels in fish (*Tilapia nilotica*) tissues as biological indicator for lake water pollution. *Environ Monit Assess* 68(1): 75-89.

Roberts, M. 2003. Review of risks from metals in the U K. Chemical Stackholder Forum, Fourteenth meeting, 20.

Sanders, B. M. 1993. Stress proteins in aquatic organisms, an environmental perspective. *Crit Rev Toxicol* 23: 49-75.

Sarunya, L., Auratai, A., and Lakana, H. 2006. Effects of Cadmium Level on Chromosome Structure of Snakehead Fish (*Ophiocephalus Striatus*). PhD Thesis, Mahidol University.

Sayeed, I., Parvez, S., Pandey, S., Bin-Hafeez, B., Haque, R., and Raisuddin, S. 2003. Oxidative stress biomarkers of exposure to deltamethrin in freshwater fish, *Channa punctatus* Bloch. *Ecotoxicol Environ Saf* 56: 295-301.

Seebaugh, D. R., Goto, D., and Wallace, W. G. 2005. Bioenhancement of cadmium transfer along a multi level food chain. *Mar Environ Res* 59(5): 473-491.

Shakoori, A. R., Iqbal, M. J., Mughal, A. L., and Ali, S. S. 1994. Biochemical changes induced by inorganic mercury on the blood, liver and muscles of freshwater Chinese grass carp (*Ctenopharingodon idella*). *J Ecotoxicol Environ* 14(2): 81-92.

Sobha, K., Poornima, A., Harini, P., and Veeraiah, K. A. 2007. Study on biochemical changes in the fresh water fish, (*Catla catla*) exposed to the heavy metal toxicant cadmium chloride. *J Sci Eng Tech* 4(1): 4-8.

Tietz, N. W. 1976. In Clinical Guide to Laboratory tests. *W B saunders co Philadelphia* 238.

Vosyliene, M. Z., and Jankaite, A. 2006. Effect of heavy metal model mixture on rainbow trout biological parameters. *Ekologija* 4: 12-17.

Wernick, G. R., Ngayen, T., and Albers, A. A. 1985. Comparison of improved precipitation methods for quantification of high density lipoprotein cholesterol. *Clin Chem* 31: 217-231.

Zaki, M., Suzan, S., Mostafa, O., Olfat, M., Fawzi., Medhat Khafagy., and Faten Bayumi, S. 2009. Clinopathological, Biochemical and Microbiological change on Grey Mullet exposed to cadmium chloride. *Am-Eu.J.Agric. & Environ. Sci* 5(1): 20-23.

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