International Journal of Zoology and Applied Biosciences
Volume 3, Issue 2, pp: 184-193, 2018
https://doi.org/10.5281/zenodo.1314225

http://www.ijzab.com

ISSN: 2455-9571

Research Article

EFFECT OF CADMIUM ON ENZYMATIC PARAMETERS OF FRESHWATER CATFISH, *HETEROPNEUSTES FOSSILIS*

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Article History: Received 19th February 2018; Accepted 24th April 2018; Published 27thApril2018

ABSTRACT

Quantitative assessment of enzymes is a reliable indicator of stress imposed on the organism by environmental pollutants such as heavy metals. The activities of Acetylcholinesterase (AChE), alkaline phosphatase (ALP) and acid phoshatase (ACP) enzymes in liver, brain, gill and serum are used as stress indicators. The significant changes in activities of these enzymes in blood plasma indicate tissue impairment caused by stress. In the present study, significant changes were observed in AChE, ALP and ACP activities in liver, brain, gill and serum of *Heteropneustes fossilis* fish exposed to cadmium when compared to the control group. In this study, the AChE activity was inhibited moderately by sub-lethal concentration of cadmium. During 21 days treatment among the tissues tested, the gill AChE was more inhibited than any other tissue AChE. Alkaline phosphatase activities of cadmium treated fish under various sub-lethal concentrations were significantly decreased in liver and increased in brain, gill and serum. Acid phosphatase activities of cadmium treated fish under various sub-lethal concentrations were significantly increased in liver, brain, gills and serum.

Keywords:*Heteropneustes fossilis,* Acetylcholinesterase, Alkaline phosphatase, Acid phoshatase, Cadmium.

INTRODUCTION

Fish are mostly used in the evaluation of aquatic systems quality and some of their physiological changes can be considered as biological markers of environmental pollution (Dautremepuits *et al*.,2004). They have a great potential to serve as sensitive indicators, signaling exposure and understanding the toxic mechanisms of stressors in aquatic ecosystems (Vutukuru *et al*., 2006). The impact of metals as well as other pollutants on aquatic biota can be evaluated by enzymatical assays which are used to detect and evaluate the potential toxicological effects of chemicals on aquatic organisms.

The freshwater catfish, *Heteropneustes fossilis* is an important group of food fishes in India. This stinging catfish (*H. fossilis*) is commercially important and valuable food species also in many Asian countries (Akand*et al*., 1991). *H. fossilis*, commonly known as Shing or Singhi is a popular catfish in India and found naturally in lakes, ponds, swamps and marshes, ditches, floodplains and in muddy rivers. It is characterized by an accessory respiratory organ (air breathing organ) which enables it to exist for hours when out of water or in indefinitely oxygen-poor water and even in moist mud (Akand *et al*., 1991). So, this species is very potential in seasonal water bodies of India.

In recent years, there has been a rapid development of enzymatic biomarkers. This is not only due to advances in biochemistry but also to modern methods of measurement. The measurement of fish cellular enzymes is an indicator of health condition and has been used as diagnostic tool in monitoring programs of aquatic pollution (Fernandes*et al*., 2008). Phosphatases and Acetylcholinesterases are good indicators of stress condition in the biological systems (Verma*et al*., 1980). Acid and alkaline phosphatases are general enzymes present in almost all the tissues. They are hydrolytic enzymes concerned with the process of transphosphorylation and have an important role in the general energetics of an organism. They are associated

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with the transport of metabolites, with metabolism of phospholipids, phosphoproteins, nucleotides and carbohydrate, and with synthesis of proteins (Srivastava*et al*., 1995). Acid phosphatase is a lysosomal enzyme that hydrolyses the ester linkage of phosphate esters and helps in autolysis of cell after its death. Alkaline phosphatase (ALP) is a membrane-bound enzyme related to the transport of various metabolites (Lin*et al*., 1976). It has also been proposed as a good biomarker in ecotoxicology because of its sensitivity to metallic salts (Boge*et al*., 1992). Alterations in acid phosphatase (ACP) and alkaline phosphatase (ALP) activities in tissues and serum have been reported in fish species (Atli & Canli, 2007; Jyothi & Narayan, 2000; Rogers*et al*., 2003).

Acetylcholine is synthesized in neurons soma by combining choline with acetyl (originating from acetyl-CoA) *via* choline acetyl transferase (ChAT). Synthesized acetylcholine is transported to the nerve ends *via* axonal transport and released to the synaptic space (Mesulam *et al*., 2002). Acetylcholinesterase is responsible for hydrolysis of acetylcholine and therefore important for cholinergic neuronal system (Nachmansohn & Wilson, 1951). The inhibition of Acetylcholinesterase causes accumulation of acetylcholine in the neuromuscular synapses and nerve synapses creates abnormal results, the most important one is the over activity of muscle tissues (Roex*et al*., 2003). This over activity in fish leads to changes of behavior such as hyperactivity and anorexia as well as physiological effects such as asphyxia, potentially conducive to death (Beauvais*et al*., 2000).

ALP is a polyfunctional enzyme, present in the plasma membrane of all cells. It hydrolyses a broad class of phosphomonoester substrates, and acts as a transphosphorylase at alkaline pH is 9. It also acts as an early marker of cell differentiation in the osteogenic lineage in bivalve mollusc (Mouri*et al*., 2002). ALP activity has been reported to be sensitive to heavy metal pollutants (Regoli & Principato, 1995). In *Venus gallina* alkaline phosphatase activity is implicated in shell formation (Carpene & Vašák, 1989). ALP in serum and haemocytes of *C. farreri* were more important than any other enzymes in immune defense (Zhang *et al*., 2005).

Acid phosphatase is a major marker enzyme, material to be hydrolyzed is taken into lysosomes by endocytosis and the enzymes catalyze the hydrolysis of most of the major polymeric compounds as well as foreign bodies entered into animal body. Lysosomal enzymes are mainly acid hydrolases and ACP is known to hydrolyse the phosphomonoesters which are produced by hydrolysis of other major phosphates of the cell. Heavy metals accumulate to a relatively high concentration in lysosomes and destabilizing its membrane integrity followed by release of stored lysosomal hydrolases into the haemolymph thereby increasing the activity of the enzyme in haemolymph. The above explanation justifies the hyper activity of ACP as observed at high concentration of metals and the extended period of exposure.

MATERIALS AND METHODS

Collection of fishes

The freshwater catfish, *Heteropneustes fossilis* (Bloch) with a size range of 16-20 cm and, weighing 54 ±4 g irrespective of their sex, have been chosen as the test organism in the present study. The fishes were collected from the domestic fish market located at Guntur city ($16^0$20’ N $80^0$27’ E and 31 m elevation), Guntur district, Andhra Pradesh, India.

Acclimatization

Fishes were acclimatized to the laboratory conditions in large fiber glass tanks with unchlorinated ground water for 3 to 4 weeks at a room temperature of 28 ± 2°C. As these catfishes are benthic in nature, overcrowding was avoided by keeping small numbers of fishes in each tank. Water was changed on alternate days. Tanks were covered with fish netting to prevent the escape of fishes.

Selection of sub-lethal concentrations

In the present study $1/10^{th}$ of the 96h LC_{50} value was taken as sub-lethal concentration (A). The two other doses, B & C, used were a reduction in concentration of the sub-lethal concentration (A) in a graded manner. The half concentration of the sub-lethal concentration A (50 % reduction) was used as the second dose (B) while the third dose (C) was 50 % reduction in concentration of the second dose B (Kayode*et al*., 2016).

Estimation of Enzymatic activities

1. Assay of tissue and serum Acetylcholinesterase activity (AChE): Acetylcholinesterase activity in liver, brain, gill and serum was estimated as per the method of (Ellman*et al*., 1961). The principle underlying in this assay is that the substrate acetylthiocholine when hydrolysed by the enzyme Acetylcholinesterase yields thiocholine. This, on subsequent combination with DTNB forms the yellow anion 5-thio-2nitrobenzoic (TNB) acid which absorbs strongly at 412nm.

Brain, liver and gill tissues of both test and control fish were dissected out and were isolated in ice-cold condition for further studies. The tissues were thoroughly washed in normal saline and homogenized (10%, w/v) for 1 min in sodium phosphate buffer (50 mM, pH 7.5) containing 0.2% Triton X-100 using homogenizer with teflon-coated pestle under ice cold condition. The homogenates were kept in ice for about 10 min and then centrifuged at 10,000 rpm for 30 min in a refrigerated high-speed centrifuge to solubilize

AChE. The clear cell free supernatant of each tissue homogenate was collected and used for determination of AChE activity.

AChE activity was determined using the Ellman's reagent DTNB 5,5-Dithiobis(2-nitrobenzoic acid)and acetylthiocholine iodide as substrate (Ellman *et al.,* 1961). Supernatant/serum of 50µl was taken for assay and 2.3 ml of 0.5 mM DTNB and 100 µl of 2.6mM acetylthiocholine iodide was added. The rate of change of absorbance was measured at 412nm. Blank and samples were taken to make sure that there was no non-specific esterase or other background activity. Protein was estimated as described by Lowry et al,(1951)allowing the calculation of AChE as U (µmol/min) /mg protein.

2. Estimation of tissue and serum Alkaline Phosphatase (ALP): About 10% homogenate of gill, liver and brain were prepared in 0.33 M sucrose solution and centrifuged at 1000 rpm for 15 min. The supernatant obtained was used as the enzyme source. 1.5 ml of carbonate-bicarbonate buffer, 1.0 ml of substrate and 0.1 ml of magnesium chloride and requisite amount of the enzyme source were mixed together. The reaction mixture was incubated at 37°C for 15 minutes. The reaction was terminated by adding 0.1 ml of folin's phenol reagent. Controls were incubated without adding enzyme source and enzyme source were added after the addition of folin's phenol reagent. 1ml of 15% sodium carbonate solution was added and incubated for further 10 minutes at 37°C. The blue colour developed was read at 640 nm against a blank. Standards also were treated similarly. The enzyme activity was expressed as micromoles of phenol liberated per hour/ mg protein.

3. Estimation of tissue and serum Acid Phosphatase (ACP): Both serum and tissue phosphatase activity was determined following the method by Cabrera & Anon Suarez, (1963)

Liver, brain and gill of both control and test fishes were homogenized in isotonic sucrose and were centrifuged at 5000 rpm for 15 minutes. Supernatant obtained was the source of enzymes. 0.5 ml of p-nitro phenyl phosphate was mixed with equal volume of 0.1 M phosphate buffer (pH 4.8). The enzyme was added and incubated for 30 minutes at room temperature. The reaction was arrested by adding 4ml of 0.1 N NaOH. The absorbance of solution was measured spectrophoto-metrically at 410 nm. The amount of p-nitro phenol liberated by the acid phosphatase per hour/ mg protein gives the specific activity. Protein was determined as per the method byLowry *et al.*(1951).

Statistical analysis of the data

The mean and Standard Deviations (SD) were calculated by following the method of (Pillai & Sinha, 1968).

SD was calculated by using the formula:

$$StandardDeviation = \frac{\sqrt{Ex^2 - \frac{(Ex)^2}{n}}}{n-1}$$

Where

'x^{2}' is the sum of square of deviations from the mean

'n' is the number of individual observations.

The significance of the deviations from Normal was calculated by calculating student's t-test by using the formula:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

Where

$\bar{x_1}$ is the mean of first set of observations,

$\bar{x}_2$ is the mean of second set of observations,

$s_1^2 + s_2^2$ are squares of standard deviations of the first and second set of observations, and n_1 and n_2 are number of the first and second sets.

RESULTS AND DISCUSSION

Cadmium interacts with legends in proteins, particularly enzymes and may inhibit their biochemical and physiological activities (Passow*et al.*, 1961). They have strong affinity to bond with the amino acid molecules of protein and may cause changes in enzyme structure. The most obvious consequences of these changes are the inhibition of enzymes.

Acetylcholinesterase (AChE) activity

The calculated values of Acetylcholinesterase activity and percent change over control after exposure to sub-lethal concentrations of cadmium for 7, 14 and 21 days were given in Tables 1 to 4 and Figure 1 to 3.

Acetylcholinesterase activities of the fish exposed to various sub-lethal concentrations of cadmium were significantly decreased. The Acetylcholinesterase activity in the liver, brain, gill and serum showed a continuous decrease as the exposure progressed (Table 4.4.1). Maximum decrease was observed at the highest concentration of cadmium (A) on 21st day exposure. Decrement of the enzyme activity was more intense as the time of exposure increased. In this study, the AChE activity was inhibited moderately by sub-lethal concentration of cadmium. During 21 days treatment among the tissues tested, the gill AChE was more inhibited than any other

tissue AChE. No significant changes were observed in the measured variables of fish maintained in uncontaminated water (control) (Table 4).

The enzyme Acetyl cholinesterase (AChE) which catalyses the hydrolysis of acetylcholine is ubiquitous in the animal kingdom (Walker & Thompson, 1991). It is a well characterized enzyme in vertebrates because of its critical catalytic function at the cholinergic synapses. Acetylcholinesterase is a potential cell membrane marker enzyme (Severson *et al.*, 1972; Steck, 1974; Watts & Pierce, 1978) that hydrolyzes the neurotransmitter acetylcholine to acetic acid and choline (Chuiko, 2000). Several studies have shown that high levels of AChE inhibition are needed to cause significant mortality of aquatic species in both acute and chronic exposures(Ansari & Kumar, 1984).

Acetylcholinesterase (AChE) activity is considered of great interest in evaluating the effects of exposure to neurotoxic compounds in aquatic animals (Cajaraville *et al.*, 2000). It is an enzyme involved in the synaptic transmission of nerve impulses and is inhibited by neurotoxic compounds (Bocquené *et al.*, 1998). However, the responsiveness of AChE to other chemicals including metals has also been reported by Leinio & Lehtonen, (2005). Several studies showed the potential use of this enzyme activity as a useful biomarker for detecting general physiological stress in aquatic organisms caused by exposure to contaminants (Rank *et al.*, 2007). The results of the present study showed significant inhibition of AChE activity after the exposure of *H. fossilis* to cadmium compared to control. The significant responses indicated that the AChE activity decreased to reach a minimum by the end of 21 days treatment. This inhibition may be the result of a neurotoxic effect due to cadmium toxicity. Similar observations have been reported in silver catfish, *Rhamdia quelen* exposed to Cadmium (Pretto *et al.*, 2010). Beldi *et al*,(2006) reported that heavy metal pollution and decreases AChE activity in *Donax trunculus* from industrialized areas and harbour sectors in the gulf of Annaba.

The present study revealed that there was a significant decrease of AChE in the liver, brain, gills and serum when they were exposed to increased concentration of cadmium and extended duration of time. The present results confirm that AChE can be used as a sensitive enzyme marker. Acetylcholinesterase, due to its rate of hydrolysis towards the substrate acetylcholine iodide, confirms its presence in *H. fossilis* is highly sensitive and is recommended as a useful biomarker in bio-monitoring studies.

Alkaline phosphatase activity (ALP)

The calculated values of alkaline phosphatase activity and percent change over control exposed to sub-lethal concentrations of cadmium for 7, 14 and 21 days are given in Tables 5 to 8. The alkaline phosphatase activity in the liver, brain, gill and serum showed a continuous increase as the exposure progressed. Maximum increase was observed at the highest concentration of cadmium (A) at 21 days exposure. Enhancement of the enzyme activity was more intense as the time of exposure increased.

The alkaline phosphatase is composed of several isoenzymes that are present in practically all tissues of the body, especially in cell membranes. It catalyses the hydrolysis of monophosphate esters and has a wide substrate specificity. The functional activity of this enzyme was found to increase during the exposure with heavy metals as an adaptive response in mitigating the metal toxicity (Kopp & Hetesa, 2000).

In the present study, the level of alkaline phosphatase activity increased in the, liver, brain, gill tissue and serum of *H. fossilis* when exposed to cadmium. This result suggests that increased level of alkaline phosphatase might be due to the toxicity effect of cadmium. These increased activities can be attributed to the destruction of cell membrane and lysosomes, which in turn lead to hepatic damage (Thirumavalavan, 2010). The present results are in agreement with (Shalaby & Abbassa, 2007)who obtained a significant increase in ALP in kidney of catfish, *Hetropneustes* sp. after toxication with Cd. In contrast to above results, decreased activity of ALP in liver was obtained by (Sastry & Subhadra, 1985). This decrease may be due to the damage and dysfunction of the liver. Characterization and effect of heavy metals on ALP was made by Mazorra *et al*, (2002) in the clam, *Scrobicularia plana,* and mercury showed highest inhibitory effects on ALP activity in various tissues analysed. ALP, which is sensitive to metals, gives a better picture of the general metabolic condition of the organisms (Regoli & Principato, 1995; Xiao *et al.*, 2002).

Acid phosphatase activity (ACP)

The mean±SD values of acid phosphatase activity and percent change over control after exposed to sub-lethal concentrations of cadmium for 7,14 and 21 days are given in Tables 9 to 12.

Acid phosphataseactivities of cadmium treated fish under various sub-lethal concentrations were significantly increased in liver, brain, gills and serum. Increment of the enzyme activity was more intense as the time of exposure increased. Maximum increase was observed at the highest concentration of cadmium at 21 days exposure.

Table 1. Effect of Cadmium on the Acetylcholinesterase activity (U (μmol/min) /mg protein) in the tissues /serum of *H.fossilis* under exposure to sub-lethal concentrations.

Tissue/ Serum	Treatments											
	Control			A			B			C		
	7th day	14th day	21st day	7th day	14th day	21st day	7th day	14th day	21st day	7th day	14th day	21st day
Liver	0.1506± 0.022	0.1507± 0.028	0.1508± 0.014	0.0954± 0.032	0.0768± 0.044	0.0542± 0.012	0.1122± 0.026	0.0926± 0.018	0.0806± 0.016	0.1276± 0.014	0.1108± 0.012	0.0956± 0.010
Brain	0.4902± 0.041	0.4904± 0.021	0.4900± 0.018	0.2888± 0.046	0.2466± 0.022	0.2056± 0.022	0.4028± 0.012	0.3642± 0.010	0.3022± 0.022	0.4356± 0.066	0.3986± 0.056	0.3576± 0.048
Gill	0.2635± 0.016	0.2636± 0.010	0.2634± 0.016	0.1826± 0.036	0.1245± 0.066	0.0965± 0.024	0.2046± 0.022	0.1722± 0.062	0.1448± 0.026	0.2146± 0.082	0.1964± 0.022	0.1656± 0.066
Serum	0.3274±0.024	0.3272± 0.044	0.3269± 0.042	0.2196± 0.046	0.1654± 0.066	0.1244± 0.076	0.2536± 0.066	0.2122± 0.044	0.1864± 0.028	0.2896± 0.064	0.2525± 0.012	0.2246± 0.042

*Each value is represented as mean ± SD (n=5); Values are significant at p<0.05 (based on t-test).

A = Sub-lethal conc. (2.068 ppm); B = 50% SL of A (1.034 ppm); C = 50% SL of B (0.517 ppm).

Table 2. Changes in specific activity levels of Acetylcholinesterase (U (μmol/min)/ mg protein) and % change over the control in different tissues/serum of *H. fossilis* exposed to sub-lethal concentrations of Cadmium for 7 days.

Tissue/ Serum	Treatments						
	Control	A	% Change	B	% Change	C	% Change
Liver	0.1506±0.022	0.0954±0.032	36.65	0.1122±0.026	25.50	0.1276±0.014	15.27
Brain	0.4902±0.041	0.2888±0.046	41.09	0.4028±0.012	17.82	0.4356±0.066	11.14
Gill	0.2635±0.016	0.1826±0.036	30.70	0.2046±0.022	22.35	0.2146±0.082	18.58
Serum	0.3274±0.024	0.2196±0.046	32.93	0.2536±0.066	31.70	0.2896±0.064	11.54

*Each value is represented as mean ± SD (n=5); Values are significant at p<0.05 (based on t-test).

Table3. Changes in specific activity levels of Acetylcholinesterase (U (μmol/min) /mg protein) and % change over the control in different tissues/serum of *H. fossilis* exposed to sub-lethal concentrations of Cadmium for 14 days.

Tissue/ Serum	Treatments						
	Control	A	% Change	B	% Change	C	% Change
Liver	0.1507±0.028	0.0768±0.044	49.04	0.0926±0.018	38.55	0.1108±0.012	26.48
Brain	0.4904±0.021	0.2466±0.022	49.71	0.3642±0.010	25.73	0.3986±0.056	18.72
Gill	0.2636±0.010	0.1245±0.066	52.77	0.1722±0.062	34.67	0.1964±0.022	25.49
Serum	0.3272±0.044	0.1654±0.066	49.45	0.2122±0.044	35.15	0.2525±0.012	22.83

*Each value is represented as mean ± SD (n=5); Values are significant at p<0.05 (based on t-test).

Table 4. Changes in specific activity levels of Acetylcholinesterase (U(μmol/min) /mg protein) and % change over the control in different tissues/serum of *H. fossilis* exposed to sub-lethal concentrations of Cadmium for 21 days.

Tissue/ Serum	Treatments						
	Control	A	% Change	B	% Change	C	% Change
Liver	0.1508±0.014	0.0542±0.012	64.06	0.0806±0.016	46.55	0.0956±0.010	36.60
Brain	0.4900±0.018	0.2056±0.022	58.05	0.3022±0.022	38.33	0.3576±0.048	27.02
Gill	0.2634±0.016	0.0965±0.024	63.36	0.1448±0.026	45.03	0.1656±0.066	37.13
Serum	0.3269±0.042	0.1244±0.076	61.95	0.1864±0.028	42.98	0.2246±0.042	31.29

*Each value is represented as mean ± SD (n=5); Values are significant at p<0.05 (based on t-test).

Table 5. Effect of Cadmium on the alkaline phosphatase activity (U (µmol/h) /mg protein) in the tissues/serum of *H. fossilis* under exposure to sub-lethal concentrations.

Tissue/ Serum	Treatments											
	Control			A			B			C		
	7th day	14th day	21stday	7th day	14th day	21stday	7th day	14th day	21stday	7th day	14th day	21stday
Liver	1.1608± 0.012	1.1607± 0.018	1.1608± 0.012	1.5554± 0.012	1.7064± 0.024	1.9220± 0.024	1.3022± 0.016	1.4926± 0.018	1.6806± 0.010	1.1976± 0.014	1.2808± 0.022	1.4956± 0.016
Brain	0.9212± 0.041	0.9214± 0.021	0.9212± 0.012	1.8888± 0.016	2.4986± 0.020	2.9056± 0.028	1.2008± 0.012	1.6612± 0.016	2.0022± 0.022	1.0356± 0.016	1.2960± 0.056	1.6570± 0.028
Gill	0.7635± 0.022	0.7638± 0.016	0.7634± 0.016	1.0126± 0.030	1.8745± 0.016	2.3565± 0.020	0.9846± 0.020	1.5422± 0.026	1.8248± 0.026	0.8146± 0.082	0.8264± 0.012	1.2650± 0.018
Serum	5.5374± 0.034	5.5372± 0.014	5.5369± 0.012	6.5116± 0.018	7.1651± 0.026	8.1214± 0.016	6.2236± 0.016	6.7022± 0.014	7.2864± 0.020	5.9896± 0.024	6.3825± 0.010	6.9246± 0.012

**Each value is represented as mean ± SD (n=5); Values are significant at p<0.05 (based on t-test).

A = Sub-lethal conc. (2.068 ppm); B = 50% SL of A (1.034 ppm); C = 50% SL of B (0.517 ppm).

Table 6. Changes in specific activity levels of alkaline phosphatase (U (µmol/h)/ mg protein) and % change over the control in different tissues/serum of *H. fossilis* exposed to sub-lethal concentrations of Cadmium for 7 days.

Tissue/ Serum	Treatments						
	Control	A	% Change	B	% Change	C	% Change
Liver	1.1608±0.012	1.5554±0.012	33.99	1.3022±0.016	12.18	1.1976±0.014	3.17
Brain	0.9212±0.041	1.8888±0.016	105.03	1.2008±0.012	30.35	1.0356±0.016	12.41
Gill	0.7635±0.022	1.0126±0.030	32.62	0.9846±0.020	28.95	0.8146±0.082	6.69
Serum	5.5374±0.034	6.5116±0.018	17.59	6.2236±0.016	12.39	5.9896±0.024	8.16

*Each value is represented as mean ± SD (n=5); Values are significant at p<0.05 (based on t-test).

Table 7. Changes in specific activity levels of alkaline phosphatase (U (µmol/h) /mg protein) and % change over the control in different tissues/serum of *H. fossilis* exposed to sub-lethal concentrations of Cadmium for 14 days.

Tissue/ Serum	Treatments						
	Control	A	% Change	B	% Change	C	% Change
Liver	1.1607±0.018	1.7064±0.024	47.01	1.4926±0.018	28.59	1.2808±0.022	10.34
Brain	0.9214±0.021	2.4986±0.020	171.17	1.6612±0.016	80.29	1.2960±0.056	40.65
Gill	0.7638±0.016	1.8745±0.016	145.41	1.5422±0.026	101.91	0.8264±0.012	8.19
Serum	5.5372±0.014	7.1651±0.026	29.39	6.7022±0.014	21.03	6.3825±0.010	15.26

*Each value is represented as mean ± SD (n=5); Values are significant at p<0.05 (based on t-test).

Table 8. Changes in specific activity levels of alkaline phosphatase (U (µmol/h) /mg protein) and % change over the control in different tissues/serum of *H. fossilis* exposed to sub-lethal concentrations of Cadmium for 21 days.

Tissue/ Serum	Treatments						
	Control	A	% Change	B	% Change	C	% Change
Liver	1.1608±0.012	1.9220±0.024	65.57	1.6806±0.010	44.77	1.4956±0.016	28.84
Brain	0.9212±0.012	2.9056±0.028	215.41	2.0022±0.022	117.34	1.6570±0.028	79.87
Gill	0.7634±0.016	2.3565±0.020	208.68	1.8248±0.026	139.03	1.2650±0.018	65.70
Serum	5.5369±0.01[illegible]	8.1214±0.016	46.67	7.2864±0.020	31.59	6.9246±0.012	25.06

*Each value is represented as mean ± SD (n=5); Values are significant at p<0.05 (based on t-test).

Table 9. Effect of Cadmium on acid phosphatase activity (U (µmol/h) /mg protein) in the tissues/serum of *H. fossilis* under exposure to sub-lethal concentrations.

Tissue/ Serum	Treatments											
	Control			A			B			C		
	7th day	14th day	21st day	7th day	14th day	21st day	7th day	14th day	21st day	7th day	14th day	21st day
Liver	1.2602± 0.032	1.2605± 0.048	1.2602± 0.032	1.3676± 0.044	1.5808± 0.024	1.8956± 0.036	1.3022± 0.046	1.4926± 0.028	1.6806± 0.040	1.2754± 0.032	1.3964± 0.034	1.4520± 0.024
Brain	0.9842± 0.041	0.9844± 0.022	0.9844± 0.042	1.9880± 0.032	2.5960± 0.026	2.9746± 0.040	1.2356± 0.044	1.6416± 0.032	2.1220± 0.032	1.0656± 0.056	1.3260± 0.028	1.8544± 0.040
Gill	0.7950± 0.046	0.7948± 0.046	0.7950± 0.056	1.0526± 0.040	1.8645± 0.034	2.2580± 0.032	0.9960± 0.032	1.6220± 0.052	1.9280± 0.036	0.8240± 0.032	0.8546± 0.032	1.2356± 0.032
Serum	5.3374± 0.044	5.3370± 0.024	5.3372± 0.042	6.3814± 0.038	7.0861± 0.028	7.8714± 0.024	6.0980± 0.042	6.6028± 0.044	7.1984± 0.044	5.8886± 0.022	6.2828± 0.042	6.8256± 0.044

*Each value is represented as mean ± SD (n=5); Values are significant at $p<0.05$ (based on t-test).

A = Sub-lethal conc. (2.068 ppm); B = 50% SL of A (1.034 ppm); C = 50% SL of B (0.517 ppm).

Table 10. Changes in specific activity levels of acid phosphatase (U (µmol/h) /mg protein) and % change over the control in different tissues/serum of *H. fossilis* exposed to sub-lethal concentrations of Cadmium for 7 days.

Tissue/ Serum	Treatments					
	Control	A	% Change	B	% Change	C	% Change
Liver	1.2602±0.032	1.3676±0.044	8.52	1.3022±0.046	3.33	1.2754±0.032	1.21
Brain	0.9842±0.041	1.9880±0.032	101.99	1.2356±0.044	25.54	1.0656±0.056	8.27
Gill	0.7950±0.046	1.0526±0.040	32.40	0.9960±0.032	25.28	0.8240±0.032	3.65
Serum	5.3374±0.044	6.3814±0.038	19.56	6.0980±0.042	14.25	5.8886±0.022	10.33

*Each value is represented as mean ± SD (n=5); Values are significant at $p<0.05$ (based on t-test).

Table 11. Changes in specific activity levels of acid phosphatase (U (µmol/h) /mg protein) and % change over the control in different tissues/serum of *H. fossilis* exposed to sub-lethal concentrations of Cadmium for 14 days.

Tissue/ Serum	Treatments						
	Control	A	% Change	B	% Change	C	% Change
Liver	1.2605±0.048	1.5808±0.024	25.41	1.4926±0.028	18.41	1.3964±0.034	10.78
Brain	0.9844±0.022	2.5960±0.026	163.71	1.6416±0.032	66.76	1.3260±0.028	34.70
Gill	0.7948±0.046	1.8645±0.034	134.59	1.6220±0.052	104.08	0.8546±0.032	7.52
Serum	5.3370±0.024	7.0861±0.028	32.77	6.6028±0.044	23.72	6.2828±0.042	17.72

*Each value is represented as mean ± SD (n=5); Values are significant at $p<0.05$ (based on t-test).

Table 12. Changes in specific activity levels of acid phosphatase (U (µmol/h) /mg protein) and % change over the control in different tissues/serum of *H. fossilis* exposed to sub-lethal concentrations of Cadmium for 21 days.

Tissue/ Serum	Treatments						
	Control	A	% Change	B	% Change	C	% Change
Liver	1.2602±0.032	1.8956±0.036	50.42	1.6806±0.040	33.36	1.4520±0.024	15.22
Brain	0.9844±0.042	2.9746±0.040	202.17	2.1220±0.032	115.56	1.8544±0.040	88.38
Gill	0.7950±0.056	2.2580±0.032	184.03	1.9280±0.036	142.52	1.2356±0.032	55.42
Serum	5.3372±0.042	7.8714±0.024	47.48	7.1984±0.044	34.87	6.8256±0.044	27.89

*Each value is represented as mean ± SD (n=5); Values are significant at $p<0.05$ (based on t-test).

In the present study, the acid phosphatase activity of liver, gill, brain and serum of *H. fossilis*increased in all the three sub-lethal concentrations of cadmium. This increased level of acid phosphatase might be due to the toxic effect of cadmium. The increased level of acid phosphatase activity suggested the involvement of lysosomes in metal toxicity. These results are in agreement with those of (Shalaby & Abbassa, 2007)who found that sub-lethal concentration of Cadmium caused significant increase in ACP of common carp after 7 and 15 days. Sastry & Subhadra, (1985)who also found a significant increased in ACP in kidney of catfish. *Hetropneustes* sp. after toxication with cadmium. The increased concentration of ACP on blood serum indicates impairement of parnchymatous organs. In addition, the increased plasma ACP may be attributed to the hepatocellular damage or cellular degradation by the heavy metals (Yamawaki *et al.*, 1986).

Acid phosphatases are hydrolytic lysosomal enzymes and are released by the lysosomes for the hydrolysis of foreign material; hence it has a role in certain detoxification functions. Increase in acid phosphatase enzyme activity in all the three sub-lethal concentrations might be due to increase in protease activity which causes damage to the lysosomal membrane, thus permitting the leakage of lysosomal enzyme into cytoplasm. Alteration in the enzyme activity is due to adverse effect of xenobiotics on the cell and its organelles. In contrast to above results, decreased acid phosphatase activity in *Sarotherodon mossambicus* exposed to cadmium has earlier been recorded by Rupareliа*et al*, (1992). Gill*et al*,(1991) reported that the hepatic, bronchial and renal acid phosphatase activities were decreased in *Barbus conchonius* toxicated with Cadmium.

CONCLUSION

The heavy metal toxicity seriously impairs various metabolic functions of the fish *H. fossilis*, reflected as alterations in various enzymatical constituents. The activities of acetyl cholinesterase (AChE), alkaline phosphatase (ALP) and acid phoshatase (ACP) enzymes in liver, brain, gill and serum are used as stress indicators. In the present study, there were significant changes in AChE, ALP and ACP activities in liver, brain, gill and serum of fish exposed to cadmium compared to the control group.

The apparent sensitivity of ACP and ALP exhibited through fluctuating activity patterns suggests that analysis of these enzymes at different time periods can be used as biomarkers in metal pollution. In agreement with the observations of other workers, the data obtained once again confirm the reliability of choosing AChE activity pattern as an enzyme marker to assess metal stress.

ACKNOWLEDGEMENT

The authors express sincere thanks to the Head of the Department of Zoology and Aquaculture, Acharya Nagarjuna University for the facilities provided to carry out this research work

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