HPLC analysis of aminoacids in the Mercury and Cadmium treated gill, liver and muscles in *clarias batrachus*

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Abstract: :High performance liquid chromatography (HPLC) based separation of individual amino acids was quite revealing and promising field. It has provided the present study with necessary information on amino acid metabolism in the target tissues of the experimental fish *Clarias batrachus* and also in their relative assimilation, storage and excretion during stress induced response with treated heavy metals like cadmium (Cd).
Key Word: *Clarias batrachus*, Cadmium, HPLC

INTRODUCTION

Cadmium is a metal with no known beneficial properties that support life, there is no evidence that it is either biologically necessary or beneficial (Eisler 1985; Eisler 2000; Nordberg et al. 2007). At low concentrations it is toxic to all life, including plants, fish, birds, mammals (including humans), and microorganisms (Eisler 1985; Eisler 2000; Nordberg et al. 2007, ATSDR 2008). It causes cancer, birth defects and genetic mutations (Eisler 2000, Nordberg et al. 2007). In one study that used comparative acute toxicity testing of 63 heavy metals, cadmium was the most toxic metal (Borgmann et al. 2005). In its elemental form Cadmium does not break down but it can change form into different species and compounds. Some species can bind strongly to soil or sediment particles, depending primarily on the acidity of the surrounding water. Sources of cadmium are coal and other fossil fuels, and shale. It can enter the atmosphere during the burning of coal and household wastes, and can enter water around a sulphide mine as a by-product of acid mine drainage (See e.g. Edmonds and Peplow 2000). In unpolluted waters, natural cadmium concentrations are generally less than 1 ìg/L or 1 part per billion (ppb) (Nordberg et al. 2007). Most cadmium is released from human activities such as mining and smelting of sulphide ores, fuel combustion, and application of phosphate fertilizers or sewage sludge (USPHS 1993). Because it is a non-degradable, cumulative pollutant, continued releases are of global concern (ATSDR 2008; Hutton 1983; Tjell 1983).Animals that accumulate cadmium in their bodies ("body burden") can be eaten by others, and so on, such that cadmium will both accumulate and bio-magnify in the food chain.Fish can accumulate cadmium from the water and eating foods contaminated with cadmium (contaminated food chain). It is important to note that bioaccumulation magnification occur when a substance cannot be easily metabolized or excreted. Cadmium exhibits this persistence. Bio-magnification can happen when the concentration of cadmium increases from one link in the food chain. A bigger fish eats multiple small fish and accumulates the cadmium from those fish in its body tissue. As that fish ages, it keeps accumulating the cadmium from all of the contaminated smaller fish it eats. The cadmium further accumulates – or magnifies - as a bigger fish eats that fish, and so on. Bio-magnification can happen when eagles, bears, or any animals eat prey species that have bio-accumulated cadmium.MATERIALS AND METHODS: The amino acids differential expression in the stress induced experimental fish *Clarias batrachus* with treated heavy metals Mercury (Hg) and cadmium (Cd) were quantified using HPLC technique with pre column. Derivatization by Ophthalaldehyde protein assay and with detection at wave length 338nm as described by Bruckner et al. (1991) the sample tissue was precipitated with equal amount of 10% TCA. Supernatant collected was used for the HPLC analysis. Equal amount of the sample of 10ul, Internal standard and OPA reagent were blended and the resulting mixture was diluted five times (50ul) with orate buffer of pH 10.2. From this mixture 20ul were injected for analysis. RESULTS AND DISCUSSION:

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TABLE 1

Amino acid analysis showing the amino acids level in the Control, Mercury and Cadmium treated Gill in *Clarias batrachus*.

Amino acids	Amino acid level in nm/ml		
	Control	Mercury treated	Cadmium treated
Aspartic acid	26.14	24.18	34.99
Glutamic acid	281.24	485.49	314.89
Serine	31.48	56.37	61.77
Histidine	69.63	190.75	140.34
Glycine	4.11	11.61	17.17
Threonine	0.00	0.00	0.00
Alanine	51.05	119.12	75.78
Arginine	8.26	31.77	13.34
Tyrosine	8.39	22.05	7.45
Valine	6.90	8.98	5.29
Methionine	3.35	12.80	3.66
Phenylalanine	3.06	1.83	2.04
Isoleucine	6.02	9.03	6.02
Leucine	5.25	13.81	7.97
Lysine	253.76	92.48	55.80

The above data in table: 1 confirmed the fact that heavy metals, mercury and cadmium considerably suppressed the bio synthesis of Glutamic acid in the gill of C. batrachus. The level of Glutamic acid, Lycine, Histidine, Alanine Seine, showed a steep decline in mercury treated gill at 485.49 nm/ml and also in cadmium treated gill at 314.89 nm/ml compared to the maximum concentration in control gill 281.24 nm/ml.

TABLE 2

Amino acid analysis showing the amino acids level in the Control, Mercury and Cadmium treated Liver in *Clarias batrachus*.

Amino acids	Amino acid level in nm/ml		
	Control	Mercury treated	Cadmium treated
Aspartic acid	32.24	35.58	24.96
Glutamic acid	304.45	326.11	303.29
Serine	26.69	88.76	27.59
Histidine	46.06	97.91	62.01
Glycine	0.00	19.83	5.08
Threonine	0.00	0.00	0.00
Alanine	10.66	56.04	44.92
Arginine	36.22	19.70	7.94
Tyrosine	9.63	11.18	5.90
Valine	4.60	3.91	2.30
Methionine	7.92	6.70	3.66
Phenylalanine	0.00	2.24	0.00
Isoleucine	0.00	9.36	2.01
Leucine	0.97	5.83	2.14
Lysine	59.87	70.57	61.15

The above data in table: 2 clearly suggested increased biosynthesis of glutamic acid in the liver of C. batrachus exposed to mercury and in cadmium on one hand has diminished the synthesis of glutamic acid. The level of threonine dropped in mercury treated liver of C. batrachus compared with their respective control levels. On the other hands there was a significant increase in the level of glutamic, histidine, lysine, alanine, serine and aspartic acid compared with their respective controls. Cadmium treated liver of *C. batrachus* showed a decreased level of tyrosine, methionine, and Alanine. Glycine Threonine, phenylalanine and isoleucine compared with their respective controls. Only glutamine and histidine levels were upregulated in the liver treated with cadmium.

TABLE 3

Amino acid analysis showing the amino acids level in the Control, Mercury and Cadmium treated Muscle in *Clarias batrachus.*

Amino acids	Amino acid level in nm/ml		
	Control	Mercury treated	Cadmium treated
Aspartic acid	36.17	21.43	24.18
Glutamic acid	916.44	377.18	519.92
Serine	140.33	54.57	82.16
Histidine	223.75	91.39	137.44
Glycine	55.38	14.51	24.18
Threonine	25.16	0.00	0.00
Alanine	166.53	74.19	99.83
Arginine	109.93	16.84	42.26
Tyrosine	50.00	6.83	23.29
Valine	15.42	6.21	10.36
Methionine	26.81	7.92	13.71
Phenylalanine	17.73	6.72	10.60
Isoleucine	27.09	9.36	15.72
Leucine	52.11	13.42	29.75
Lysine	183.18	78.47	115.16

Table 3 summarizes the amino acid levels in control, mercury and cadmium treated muscle of C. batrachus control muscle which exhibited highest concentration of glutamic acid at 916.44 nm/ml and lowest concentration of valine amino acid at 15.42 nm/ml. Mercury treated muscle (Table : 3 and Figure : 8) showed greater concentration of glutamic acid at 377.18 nm/ml while cadmium treated muscle (Table : 3)showed greater concentration of glutamic at 519.92 nm/ml. Threonine amino acid was not detected in both mercury and cadmium treated muscle of C.batrachus. Out of a total of fifteen amino acids in the muscles, fourteen amino acids (glutamic, aspartic acid, serine, histidine, glycine, threonine, alanine, arginine, tyrosine, valine, methionial, phenyulalanine, isolencine, leucine and lysine) showed significantly higher concentration levels due to

mercury treatment compared to control muscle of C. batrachus. Cadmium treated muscle showed higher amino acid levels of twelve amino acids (aspartic, acid, serine, glycine, alanine, arginine, valine, methionine, phenylalanine, isoleucine, leucine and lysine) compared with their respective controls.

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