Agricultural Journal 7 (5): 316-322, 2012
ISSN: 1816-9155

The Potential Use of Certain Protein Metabolism Parameters as Biomarkers of Heavy Metal (Lead) Stress in the African Catfish, *Clarias gariepinus*

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Abstract: Some protein metabolism parameters were monitored to assess their potential use as bio-indicators of heavy metal stress in the African catfish *Clarias gariepinus*. A total 45 adult fishes were treated to continuous exposure of two sub-lethal concentrations of lead (T_1 0.006 and T_2 0.008 mg L^{-1}) for a period of 42 days. The recovery rate was also assessed over a 7 days period in lead free water. The gill and liver tissues were analyzed every 14 days for examination of their protein biochemistry. The parameters analyzed include Total Crude Protein (TCP), Alanine Aminotransaminase (ALT), Aspartate aminotransaminase (AST), Glucomate Dehydrogenase (GDH), Free Ammo Acids (FAA) and ammonia. There was a reduction in the TCP, ALT and GDH in both gill and liver tissues below the control values while the parameters such as ALT, AST, FAA and ammonia in both tissues showed an increment above the control values. The pattern of variation of the protein metabolism parameters of the gill and liver tissues and their recovery were discussed. The effect of lead on protein metabolism parameters showed their potential use as biomarkers in monitoring the level of lead toxicity in the fish.

Key words: Protein metabolism parameters, potential use, bio-indicators, gill and liver tissues, toxicity

INTRODUCTION

Lead and environmental compounds water quality guidelines for fresh and marine water are; a maximum of 1-5 μg L^{-1} (i.e., 10^{-6} to 5×10^{-6} g L^{-1}) depending on water hardness for fresh water and for the marine water a maximum of 5 μg L^{-1} (i.e., 5×10^{-6} g L^{-1}) (DWAF, 1996).

Studies have been carried out on the effects of lead contamination on freshwater fish species such as rate of uptake and bioaccumulation (Abdelhamid and El-Ayouty, 1991) haematological and immunological effects (Strivastava and Mishra, 1979; Jana *et al*., 1986; O'Brien, 2000). However, information on the effect of lead stress on the protein metabolism parameters is scanty.

Proteins are natural polymers made up of α-amino acid monomers (Schmid, 1996). These monomer units are condensed into a long polypeptide chain. They constitute that class of biochemical compounds most characteristic of protoplasm and life. They are colloidal, hydrophilic and amphoteric (Kachmar *et al*., 1992).

Proteins can be classified according to solubility, constitution and physical nature: those that yield only amino acids upon hydrolysis are simple proteins while those that give other products are conjugated proteins (Schmid, 1996). Amino acids have been described as organic amino substituted acids containing amino (-NH_2) and carboxyl (-COOH) groups (Tewari *et al*., 2004). Simple amino acids (having only one amino group and carboxyl group) can be classified as: α, β, γ and Δ-amino acids depending on the position of the amino group with respect to the carboxyl group.

Of these, the α-amino acids are the most important as they are the final product of hydrolysis of proteins (Tewari *et al*., 2004).

Alanine and aspartate transaminases are involved in the deamination of α-amino acids in which the amino group is transferred to the α-keto-glutarate to yield the α-keto-acid of the original amino acid and glutamate. Glutamine or alanine is responsible for the transport of excess ammonia from the muscles to the liver. The process involves glutamine losing its ammonia to regenerate glutamate which in turn is oxidatively deaminated to release ammonia.

Metals are stored in different places in animals depending on the metal and animal species but bioaccumulation occurs primarily in the bones in the liver

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and in the gills. Fishes can regulate metal concentration over a fairly wide range (Bryan, 1976) after which bioaccumulation occurs (Van Der Putte and Part, 1982). The physiological and biochemical differences in species and the position of each tissue are found to influence bioaccumulation (Kotze, 1997).

The effect of heavy metal pollution has been found to be dependent on the nature of the metal (Kachmar *et al*., 1992), the concentration and duration of exposure (Olojo *et al*., 2005; Olaifa *et al*., 2003). Fish exposed to sub-lethal levels of lead showed significant increase in lead concentration in the liver and muscle which was dose- and time-dependent and that the fishes recovered on transfer of fish to lead free water (Jana *et al*., 1986).

Recovery in fish was faster for those placed under lower concentration of lead than those placed in higher concentration. As such, effects of heavy metal such as lead, on aquatic environment is usually highlighted with respect to their effect on fish (Abdelhamid and El-Ayouty, 1991; Olaifa *et al*., 2003). Using certain proteins as indices, this study is designed to assess lead stress on *C. gariepinus.*

MATERIALS AND METHODS

A to tatal of 36 adult fishes were divided into 6 experimental vats and another 9 fishes were divided into 3 other vats as control all containing fresh water, pH 7.7 for 72 h. Lead (Pb) was introduced directly into the water in form of lead carbonate ($PbCO_3$). Two sublethal concentrations were used, T_1 (0.006 mg L^{-1} of $PbCO_3$ and T_2 (0.008 mg L^{-1} of $PbCO_3$) (Bryan, 1976). Nine vats were used, each treatment having three replicates while the last 3 served as the control. For T_1, 1.2 mg of $PbCO_3$ was weighed using an electronic weighing balance and dissolved in 200 L of water to give a concentration of 0.006 mgL^{-1} $PbCO_3$. For T_2, 1.6 mg of $PbCO_3$ was weighed and dissolved in 5 L of water to give 0.008 mgL^{-1} of $PbCO_3$. Water was changed every 2 weeks with $PbCO_3$ reintroduced to maintain the concentration of the toxicant. Fecal waste was daily removed to maintain the level of dissolved oxygen.

Protein metabolism parameters such as Total Crude Protein (TCP), Alanine Amino Transaminase (ALT), Aspartate Amino Transaminase (AST), Glucomate Dehydrogenase (GDH), Free Amino Acids (FAA) and ammonia were monitored as potential biomarkers of Pb concentration in the liver and gill tissues of *C. gariepinus* every 2 weeks. Crude Protein was determined by the method of AOAC (1990) while determination of free Amino acid was by the method of Barker (1979), ammonia level by Wise (1998) and aminotransaminases by Bates (2001).

RESULTS

Laboratory analysis of the gill and liver tissues of *C. gariepinus* exposed to $PbCO_3$ revealed variations in the protein metabolism parameters, viz., total crude protein. Ammonia, free amino acids, alanine and aspartate aminotransaminases along concentration and time gradients.

Total crude protein: Total crude protein of both gill and liver tissues showed lower values than the control in the two treatments (T_1, T_2). These values showed a steady decline throughout the experimental period (Fig. 1 and 2). Mean maximum TCP values of 32.90±0.35 and 41.18±0.53 both in T_1 were obtained on day 14 while mean minimum values of 14.82±0.53 in T_2 and 21.13±0.55 in T_1 were obtained on day 42 for gill and liver tissues, respectively.

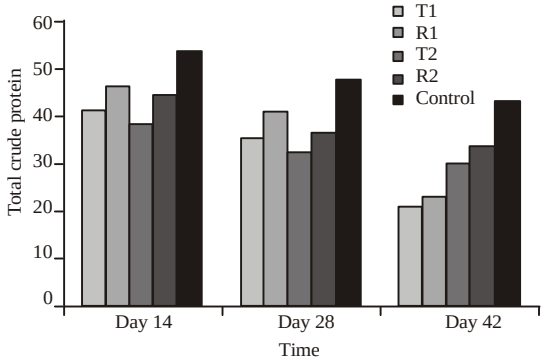

Fig. 1: Changes in total crude protein level in gill of *C. gariepinus* exposed to two concentrations of lead

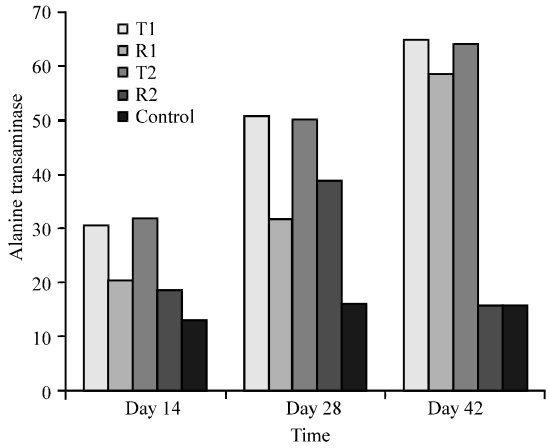

Fig. 2: Changes in alanine transaminase in gill of *C. gariepinus* exposed to two concentrations of lead

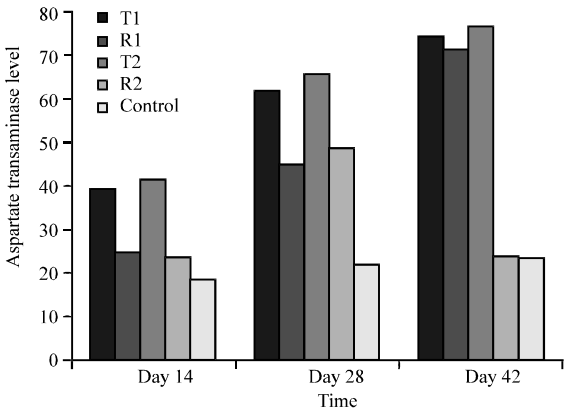

Fig. 3: Changes in aspartate transaminase in gill of *C. gariepinus* exposed to two concentrations of lead

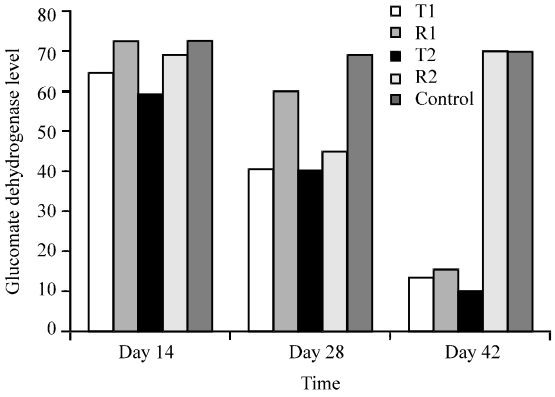

Fig. 4: Changes in glucomate dehydrogenase level in gill of *C. gariepinus* exposed to two concentrations of lead

Alanine aminotransaminase (ALT): In the gill, lower values of ALT than the control were obtained on day 14 in both treatments (T_1 and T_2) after which T_1 showed a steady increase above the control values and T_2 a steady decrease below the control the control values (Fig. 3). However, ALT in liver showed a steady increase above the control values both along concentration gradient and time (Fig. 4).

Aspartate aminotransaminase (AST): AST values in both treatments showed considerable consistent increase above the control values in both gill and liver tissues throughout the experimental period (Fig. 5 and 6) with min-max values of 39.33±1.53 to 76.00±3.61 for gill and 26.33±1.53 to 62.67±2.52 for liver, respectively.

Glucomate Dehydrogenase (GDH): The GDH levels in gill and liver dropped far below the control level but showed

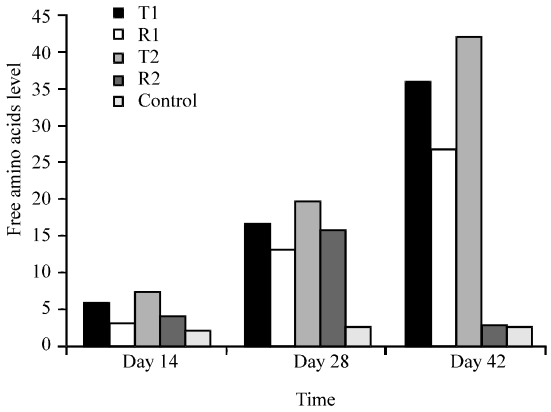

Fig. 5: Changes in free amino acids ileveln gill of *C. gariepinus* exposed to two concentrations oflead

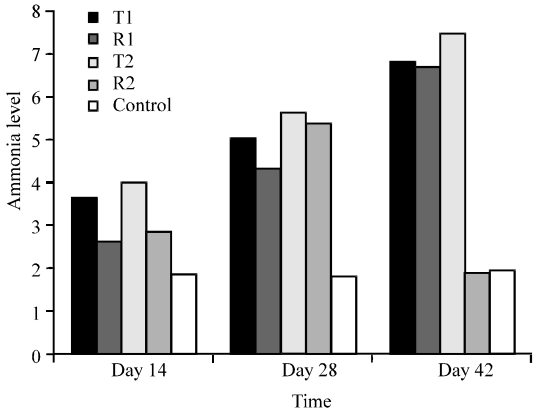

Fig. 6: Changes in ammonia level in gill of *C. gariepinus* exposed to two concentrations of lead

a consistent increase until day 42 when GDH levels in both T_1 and T_2 came closest to the control (Fig. 7 and 8). Maximum value of 64.67 and 78.00 were obtained in both gill and liver tissues, respectively (Fig. 8 and 9).

Free Amino Acids (FAA): Production of free amino acids was higher than in the control on day 14 with minimum values of 6.00 and 7.5 in both T_1 and T_2 for gill and 5.2 and 7.0% in liver, respectively which showed a consistent increase to high values (36.0% in T_1 and 42.0% in T_2) in gill and (34.8% in T_1 and 41.5% in T_2) on day 42 (Fig. 9 and 10).

Ammonia: Ammonia levels in gill and liver tissues of *C. gariepinus* in both treatments T_1 and T_2 recorded higher values than the control on day 14 and showed steady increase throughout the experimental period (Fig. 11 and 12, Table 1 and 2).

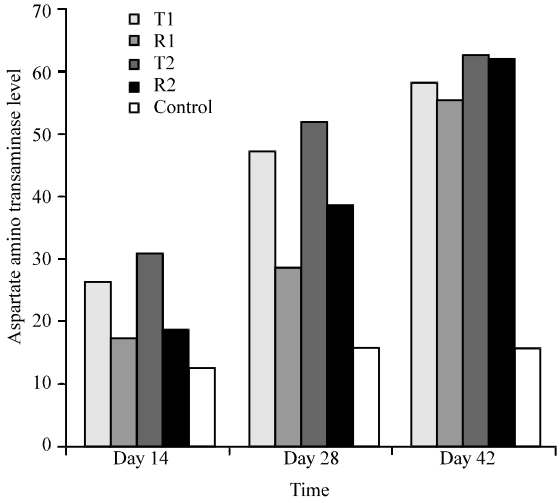

Fig. 7: Changes in aspartate amino transaminase level in liver of *C. gariepinus* exposed to two concentrations of lead

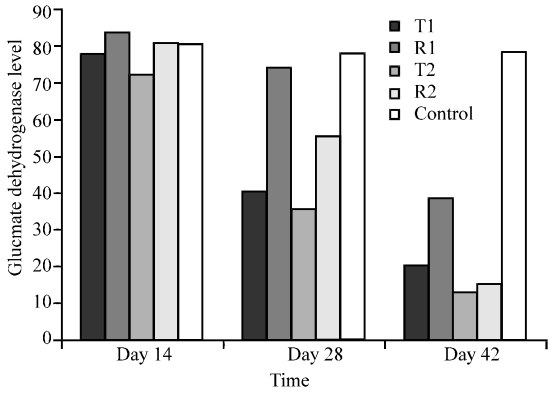

Fig. 8: Changes in glucomate dehydrogenase level in liver of *C. gariepinus* exposed to two concentrations of lead

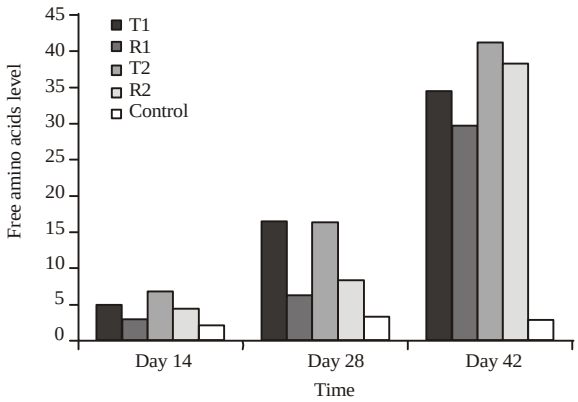

Fig. 9: Changes in free amino acids level in liver of *C. gariepinus* exposed to two concentrations of lead

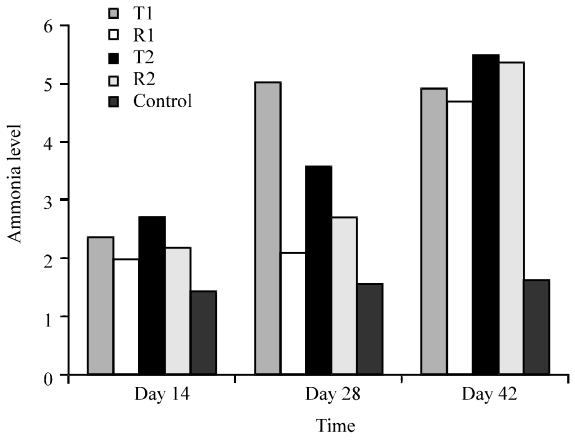

Fig. 10: Changes in ammonia level in liver of *C. gariepinus* exposed to two concentration of lead

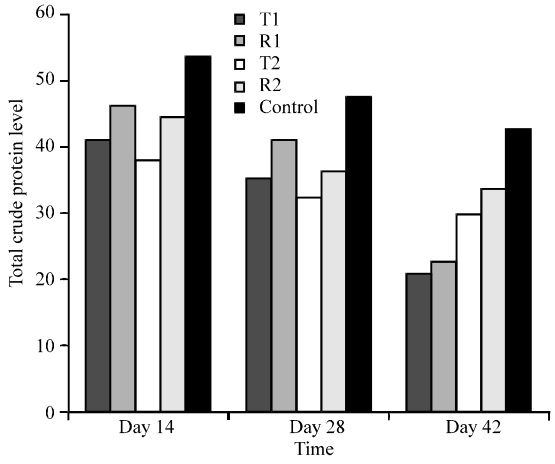

Fig. 11: Changes in total crude protein level in liver of *C. gariepinus* exposed to two concentrations of lead

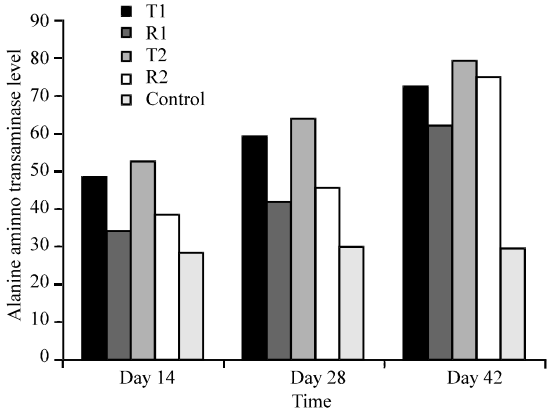

Fig. 12: Changes in alanine aminnotransaminase level in liver of *C. gariepinus* exposed to two concentrations of lead

Table 1: The mean, standard deviation, minimum and maximum values of the gill analysis for day 14

	Gill					
Time (day 14)	TCP	ALT	AST	GLU	FAA	AMM
C (x±SD)	46.00±0.53	13.00±2.00	18.33±1.53	72.33±1.53	1.93±0.10	1.88±0.35
Min-Max	45.50-46.55	11.00-15.00	17.00-20.00	71.00-74.00	1.87-2.05	1.84-1.90
Tl (x±SD)	32.90±0.35	30.33±1.53	39.33±1.53	64.67±1.53	5.98±0.28	3.63±0.17
Min-Max	32.55-33.25	29.00-32.00	38.00-41.00	63.00-66.00	5.79-6.30	3.47-3.81
Rl (x±SD)	38.62±0.53	20.33±2.08	24.33±2.08	72.33±3.06	3.12±0.39	2.63±1:0.10
Min-Max	38.15-39.20	18.00-22.00	22.00-26.00	69.00-75.00	2.72-3.49	2.52-2.72
T2 (x±SD)	29.17±1.13	31.67±2.08	41.67±1.53	59.00±1.00	7.3±0.93	4.01±0.70
Min-Max	28.35-30.45	30.00-34.00	40.00-43.00	58.00-60.00	6.25-7.96	3.94-4.08
R2 (x±SD)	33.72±0.53	18.67±1.53	23.33±0.58	68.67±2.08	4.14±0.48	2.86±0.07
Min-Max	33.25-34.30	17.00-20.00	23.00-24.00	67.00-71.00	3.61-4.53	2.79-2.92

Table 2: The mean, standard deviation, minimum and maximum values of the gill analysis for day 28

	Gill					
Time (day 28)	TCP	ALT	AST	GLU	FAA	AMM
C (x±SD)	43.17±0.53	16.00±2.00	21.67±1.53	68.67±1.53	2.72±0.36	1.83±0.18
Min-Max	42.70-43.75	14.00-18.00	20.00-23.00	60.00-67.00	2.39-3.10	1.63-1.97
Tl (x±SD)	26.02±0.73	50.67±1.53	61.67±1.53	40.33±3.06	16.60±0.82	5.01±0.10
Min-Max	25.20-26.60	49.00-52.00	60.00-63.00	37.00-43.00	15.75-17.38	4.90-5.10
Rl (x±SD)	28.00±0.35	31.67±11.53	44.67±1.53	60.00±2.00	13.14±0.26	4.31±0.21
Min-Max	27.65-28.35	30.00-33.00	43.00-46.00	58.00-62.00	12.92-13.43	4.08-4.49
T2 (x±SD)	24.03±0.53	50.00±l.00	65.33±1.53	24.33±2.08	19.68±0.90	5.64±0.65
Min-Max	23.45-24.50	49.00-51.00	64.00-67.00	22.00-26.00	18.65-20.30	5.58-5.71
R2 (x±SD)	26.25±0.70	38.67±0.58	48.67±1.53	45.00±2.00	15.74±1.16	5.37±0.70
Min-Max	25.55-26.95	38.00-39.00	47.00-50.00	43.00-47.00	14.48-16.77	5.30-5.44

DISCUSSION

Total Crude Protein (TCP): The decline in TCP levels reported in this research could be attributed to protein denaturation which is caused by heavy metals (cations) especially lead. Pb is known to form strong bonds with the negatively charged carboxyl groups on the alkyl groups of proteins, thus disrupting its ionic bonds. This reduces the protein's electrical polarity and thus increases its solubility. This causes the protein to precipitate out of solution (Taylor, 2005). Pb has also been reported to stimulate gluconeogenesis, whereby crude protein is converted into glycogen and that lead-contaminated fishes are initially low on glycogen (Yun, 2004; Davies *et al*., 2003). Pb has been reported to cause inhibition of protein synthesis thereby reducing the TCP over time and with increasing concentration, this is done by preventing the formation of sulphur bridges which hold the amino acids together in a polypeptide chain during translation, thus giving it its primary structure (Mommsen and Walsh, 1992). It has been reported that damage to hepatocyte cells of the liver also stalls the process of protein synthesis as it functions in protein synthesis of proteins such as glucoprotein, serum albumin, glycoprotein, cerruplasmin, lipoprotein and others (Olojo *et al*., 2005). The observed greater percentage drop in TCP in the gills compared to the liver could be attributed to the detoxification of contaminants in the liver by the hepatocyte and the liver being the biochemical hub of the body of the fish while the higher percentage drop in TCP at day 42 in both gill and liver tissues is due to the damage done by the build-up of Pb in the fish. Begun (2004) earlier observed a delayed decrease in the TCP in the liver of *Clarias batrachus* treated with carbofuran insecticide which recovered when placed in lead free water. Also, a significant reduction in TCP has been observed in *C. gariepinus* exposed to PbCl (Abdelhamid and El-Ayouty, 1991) as well as *Oreochromis mossambicus* treated with PbS (O'Brien, 2000). However, this report is in contrast with the report of Jana *et al.* (1986) who observed an increase in TCP in the liver relative to the control values when *C. batrachus* was treated with lead.

Alanine and Aspartate transaminases (ALT and AST): The observed elevated levels of both ALT and AST have been reported to be associated with damage to the gill and liver tissues by heavy metals such as lead (Jiraungkoorskul *et al*., 2003). This trend could also be as a result of conformational changes contributing to the catalytic activity of the enzymes which is enabled by the opening up of an active site that is otherwise closed. Inorganic ions such as Pb^{2+} have been reported to activate enzymes by molding either the enzyme or the substrate into a shape that allows the enzyme or the substrate to be formed again (Taylor, 2005). It is being known that the increase in such enzyme activators stimulates an increase in the enzyme secretion. The earlier

structural damages to the gill and liver tissues due to exposure to lead have been reported to be significantly correlated ($p<0.05$) to the increase in the enzymes secretion resulting in biochemical alteration in the fish (Jiraungkoorskul *et al*., 2003).

Glucomate Dehydrogenase (GDH): The observed elevation of GDH in this study has also been reported by Saha *et al*. (2000) who observed an increase in GDH in the ammonia-forming direction (Begun, 2004) on *C. batrachus* contamination by carbofuran insecticide while Kelly and Stanley (2001) report on the effect of $PbCl_2$ on *O. nilotica* revealed similar trends as a result of lead contamination. According to McKee and McKee (1996), this could be as a result of the increase in Glutamate (GLU) substrate production due to the increased activities of ALT and AST converting alanine and aspartate respectively:

- Alanine + α-keto-glutarate ---→ pyruvate + glutamate
- Aspartate + α-keto-glutarate ←--→ oxaloacetate + glutamate
- Glutamate plays an important role in the body's disposal of excess or waste nitrogen. Glutamate undergoes deamination, an oxidative reaction catalyzed by GDH
- Glutamate + water NAD^- ---→ α-keto-glutarate + $NADH^-$ + ammonia + H^+

Or when glutamate transports NAD from the gill in form of Glutamine (GLU) to the liver where it frees NH^+ from the GLN to regenerate GLU for oxidative deamination to release ammonia.

Free Amino Acids (FAA): The increase in the Free Amino Acids (FAA) level observed in this study has earlier being reported by Jana *et al*. (1986) and Saha *et al*. (2000) who noted a significant increase in the FAA in the liver of *C. batrachus* and *Clarias gariepinus*, respectively as a result of lead exposure, leading to the efflux of FAA into the blood. This elevation in FAA levels could be attributed to the increase in levels of ALT and AST. By transamination, ALT and AST produce more FAA in the fish. GDH is physiologically significant in amino acids metabolism and as such, the increase in GDH would lead to an increase in FAA (Moyes *et al*., 1985). The observed inhibition of protein synthesis by Pb in this research also allows for a build-up of FAA in the fish.

Ammonia: The observed increase in ammonia levels reported in this research could be attributed to the fact that increased activities of the enzymes ALT and AST resulted in increased glutamine and glutamate production as a result of the breakdown of crude protein into amino acids with the generation of nitrogenous wastes. Increased levels of ammonia could also be due to the elevated GDH activity in the fish in terms of amino acids metabolism. Similar report was given by Saha *et al*. (2000) who observed an increase in GDH in the ammonia forming direction. The higher rise in ammonia level in gill compared to liver is as a result of detoxification by the hepatocyte cells of the liver and also due to the increased diffusion distance between blood and water in the gill due to damage (Mary, 2004). This consequently increases the gill ammonia concentration, damaging the gill the more in a positive feedback reaction.

CONCLUSION

Protein metabolism parameters exhibit varying degree of changes when subjected to different factors such as heat and metal toxicity. The changes in six proteins of fish under lead toxicity reported in this study suggests that these protein metabolism parameters such as total crude protein, alanine and aspartate aminotransaminases, glucomate dehydrogenase, ammonia and free amino acids, can be used as potential biomarkers of lead toxicity in *C. gariepinus*.

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