Effects of Aluminum on the Biochemical Parameters of Fresh Water Fish, *Tilapia zillii*

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**ABSTRACT**

The effects of aluminum chloride were investigated on the biochemical parameters in adult *Tilapia zillii*. This study was carried out to evaluate the toxicity of three levels of aluminum (25, 50, and 100 µg/L) for 24, 48 and 96 hours in acidic soft water (pH 6.0). Plasma biochemical changes showed significant increase in glucose, total protein, triglycerides, cholesterol, cortisol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatinine, and uric acid values. These results indicate that sublethal levels of aluminum can alter the plasma biochemical contents of tilapia fish in acidified waters. The physiological alterations due to acute short term exposure to aluminum were discussed.

*Keywords*: Aluminum; biochemical parameters; tilapia; fresh water fish.

**INTRODUCTION**

The rapid development of industry and especially chemical industry has created serious problems of water pollution. Human destructive influence on the aquatic environment is in the form of sublethal pollution, which results in chronic stress conditions that have negative effect on aquatic life (Mason, 1991). Heavy metals are considered the most important form of pollution of the aquatic environment because of their toxicity and accumulation by organisms, such as fish (Emami Khansari, Ghazi-Khansari, & Abdollahi, 2005). Besides, the dangers involved from the presence of metals in the environment derive not only from their persistence and toxicity, but also from the remarkable degree of bioaccumulation they undergo through the trophic chain, thus becoming serious danger to man (Bishop, 2000).

Aluminum is the third most abundant element in the earth’s crust and makes up approximately 8% of its rocks and minerals. It occurs naturally in soil, water, air, and many foods (CEPA, 2000). Aluminum enters environmental media naturally through the weathering of rocks and minerals. Anthropogenic releases are in the form of air emissions, waste water effluents, and solid waste primarily associated with industrial processes, such as aluminum production. Furthermore, People are exposed to some aluminum compounds by eating food, drinking water, ingesting medicinal products like certain antacids, buffered aspirin, and intravenous fluids that contain aluminum (ATSDR, 2006).

For many years it has been recognised that aluminum is toxic to aquatic organisms (Muniz & Leivstad, 1980). Examination of the biological effects of aluminum exposure has received significant attention because of the effects of acid precipitation on aluminum bioavailability.
Increasingly, acid environments caused by such acid mine drainage or by acid rain will subsequently cause an increase in the dissolved aluminum content of the surrounding waters (Filipek, Nordstrom, & Ficklin, 1987). Aluminum becomes more soluble and hence, potentially more toxic to aquatic biota at acidic pH (Gensemer & Playle, 1999). In acid waters, Exley (2000) reports that aluminum is the principal toxicant to fish. The mechanism of aluminum toxicity to fish has been attributed to the inability of fish to maintain their osmoregulatory balance, as well as respiratory problems associated with precipitation of aluminum on the gill mucus (IPCS, 1997). Studies carried out with different fish species have revealed that aluminum can produce toxic effects in fish by disturbing physiological activities (Allin & Wilson, 2000), biochemical processes (Poleo & Hytterod, 2003), fertility (Keinanen, Tigerstedt, Kalax, & Vuorinen, 2003), reproduction, growth (Vuorinen, Keinanen, Peuranen, & Tigerstedt, 2003), and mortality (Teien, Salbu, Heier, Kroglund, & Rosseland, 2005).

In recent years, biochemical variables were used more when clinical diagnosis of fish physiology was applied to determine the effects of external stressors and toxic substances. Wepener (1997) suggested that hematology, biochemical changes, growth rate and oxygen consumption of fish be used in determining the toxicity of pollutants. Therefore, biochemical evaluations are gradually becoming a routine practice for determining health status in fish. Some species of fish used as biological indicators to detect the pollution range by metals (Ward & Neumann, 1999). *Tilapia zillii* is distinguished by its adaptation to living in fresh, brackish and nearly saline water, and can survive in partially polluted water (Zyadah, 1997). It is less sensitive to most toxic substances than most other aquatic species. Any toxicant that affects *Tilapia* would most likely be toxic to other aquatic organisms (Murungi & Robinson, 1987). Hence, the present study was designed to explore the sublethal effects of aluminum on the some biochemical parameters of tilapia fish under laboratory condition.

**EXPERIMENTAL**

**Experimental animals:**

Adult freshwater fish *Tilapia zillii*, Gervais, 1848 (Family: Cichlidae), were obtained from the commercial catches of Umhfein Lake (Umalruzam city) on the eastern coast of Libya. On arrival in the laboratory, the fish were placed in large tanks with aerated tap water and were fed with commercially pellets. Fish were acclimatized for 2 weeks under a natural photoperiod and an average temperature of 25°C. The tap water used for the experiment had a pH value of 7±0.1 and a total hardness of 20 mg CaCO₃/L and was replaced every 4 days.

**Experimental Design:**

A total of 60 adult fish of both sexes were used. The average weight of the fish was 101.4±2.9 g. The experiments were conducted in aerated glass aquariums (120 x 40 x 30 cm) each containing 15 fish in 100 L of contaminated test solution and tap water for the control and allowing one hour for acclimation to laboratory conditions. Aluminum was added as a chloride (*AlCl₃*; 6H₂O. Riedel- de Haên). The fish were exposed to dissolved aluminum at varied
concentrations of 25, 50, and 100 µg/L in acidic water (pH 6.0). The last tank was left untreated as control group. Each treated group was divided into 3 subgroups (5 fish each) that exposed to the aluminum for a period of 24, 48 and 96 hours.

**Analytical Techniques:**

At the end of each exposure period, 5 fish were taken from each replicate tank (a total of 20 fish of treated and control groups). One ml of blood samples were collected from the caudal vessels using heparinized syringes. Plasma was obtained by centrifugation immediately at 3000 rpm for 15 min and then placed in sterile tubes for biochemical analysis and any hemolyzed, clotted or insufficient volume samples were discarded. Plasma samples were analyzed for glucose, total protein, triglycerides, cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatinine, and uric acid. Colorimetric determinations were performed using spectrophotometer (Biosystems BTS-302 Spain). The absorbency was detected at an appropriate wavelength ranging from 340 to 546 nm according to the parameter tested. Basis of the tests and procedures steps are outlined by the kits suppliers. Plasma cortisol was determined within two days by using radioimmunoassay (Elecsys 2010 Roche Co. Germany). The leaflet attached with the kit describes steps of analysis.

**Statistical Analyses**

All values from chemical analysis are presented as means ± standard error of the mean. Data obtained were analyzed by one way analysis of variance (ANOVA) test using the Statistical Package for the Social Sciences (SPSS), and means were tested using Least Significant Difference (LSD) test. Statistically significant differences are indicated as follows: * $P<0.05$, **$P<0.01$ and ***$P<0.001$.

**RESULTS AND DISCUSSION**

Changes in the hematologic and blood biochemical values often reflect alteration of physiological state. Although no mortality was observed in the present study, we found physiological effects in the fish after the exposure to aluminum-rich water at pH 6. Result of the quantitative determination of plasma glucose, total protein, triglycerides, and cholesterol in *T. zillii* exposed to aluminum are presented in Fig. 1.

Blood glucose levels were significantly higher in fish exposed to aluminum as compared to the control groups (Fig. 1.A). The increase in glucose values was previously recorded by Kroglund & Finstad (2003) for Atlantic salmon, *Salmo salar*, exposed to aluminum for 3 months at moderately acidic water (pH 5.8). Similar result was also obtained by Royset , Rosseland , Kristensen , Kroglund , Garmo , and Steinnes (2005) for brown trout , *Salmo trutta*, exposed to aluminum in acid fresh waters. Likewise, consistent results have been reported by Poleo & Hytterød (2003) under alkaline conditions (pH 9.5). The depletion of liver glycogen (glycogenolysis) and the rise in blood glucose levels were reported in *T. zillii* as a consequence of water pollution (Abdelmeguid, Kheirallah, Abou-Shabana, Adham, & Abdel-Moneim, 2002). Blood glucose levels have long been used as indicators of stress in fish. It is generally thought
that, under conditions of stress, hyperglycemia may provide additional energy during times of high metabolic need such as a 'fight or flight' response (Goss & Wood, 1988). Consequently, we suggest that aluminum affects glucose dynamics in *T. zillii* in order to obtain more energy to withstand and overcome the existing stress condition.

Fig. 1. Biochemical parameters: (A) glucose, (B) total protein, (C) triglycerides, and (D) cholesterol in blood plasma of *Tilapia zillii* during 24-96 hours of exposure to control, 25 µg Al/L, 50 µg Al/L, and 100 µg Al/L. Data are presented as the mean ± S.E. (*n* = 5). Significant differences with the control groups are indicated with asterisks: *P* < 0.05, **P** < 0.01 and ***P** < 0.001.
In recent work, mean values of total plasma protein were increased significantly (Fig. 1.B). The result is similar to that of Goss & Wood (1988) who found an increase in protein content of plasma in rainbow trout, *Salmo gairdneri*, following aluminum toxicity. Blood serum protein is a fairly labile biochemical system, precisely reflecting the condition of the organism and the changes happening to it under influence of internal and external factors (Shalaby, Khattab, & Abdel-Rahman, 2006). Thus, the influence of toxicants on the total protein concentration of fish has been taken into consideration in evaluating the response to stressors and consequently the increasing demand for energy.

In the present study, triglycerides and cholesterol contents were increased significantly than for the control (Fig. 1.C&D). Triglycerides and cholesterol are known to participate in the rise of total lipid. The rise of these energy reserves in response to pollution could be due to the fact that excess energy reserves (as glucose, triglycerides & cholesterol) are required by organisms to mediate the effects of stress (Lee, Gerking & Jezierska, 1983). Since homeostasis of lipids is one of the principal liver functions, any change in serum triglyceride concentration is used as an indicator of liver dysfunction (Kaplan, Ozabo, & Ophem, 1988.). In addition, the abnormal accumulation of fats in experimental animals could be due to induced imbalance between fat production and utilization (Moore, Pipe & Farrar, 1988).

The changes in plasma cortisol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatinine, and uric acid are shown in Fig. 2.

Data presented in Fig. 2.A indicated that treatment of *T. zillii* with aluminum induced a significant increase in plasma cortisol as compared to the control group. There was a sharp rise in plasma cortisol becoming greatly increased after 48 hour (*P*<0.001) of exposure to aluminum, indicating a severe stress response. Nevertheless, the levels had returned to resting values after 96 hour of exposure. According to Goss & Wood (1988), exposure to acid plus aluminum for rainbow trout produced a much more rapid increase in plasma cortisol which was already significant at the first sample time (1.5 hour). The major corticosteroid produced by teleost fish during activation of the hypothalamo-pituitary–interrenal (HPI) axis, and is considered a principal component of the primary stress response. Increases in plasma cortisol levels and the often-associated rise in blood glucose concentration have been widely employed to assess the extent of stress in fish (Wendelaar Bonga, 1997). Donaldson (1981) suggests that this parameter may be particularly useful for determining stress levels in fish exposed to two or more sublethal stressors. On the basis of the results presented here, the use of cortisol as an indicator of sub-lethal stress under condition such as aluminum plus acid is valid.

In this work, treatment with aluminum resulted in a significant increase in the activities of plasma AST and ALT as compared with control (Fig. 2.B&C). AST and ALT belong to the plasma non-functional enzymes which are normally localized within the cells of liver, heart, gills, kidneys, muscle and other organs. It is also considered to be important in assessing the state of the liver and some other organs (Verma, Rani, & Delela, 1981). Their presence in blood plasma may give information on tissue injury or organ dysfunction (Wells, McIntyre, Morgan, & Davie, 1986). Monitoring of liver enzymes leakage into the blood has proved to be a very useful tool in liver toxic studies (Salah El-Deen & Rogeps, 1993).
As shown in Fig. 2.D, the plasma ALP activity was significantly increased in fish. ALP enzyme is a sensitive biomarker to metallic salts since it is a membrane bound enzyme related to the transport of various metabolites (Lakshmi, Kundu, Thomas, & Mansuri, 1991). Ochmanski and Barabasz (2000) reported that the increase in the activity of ALP in blood might be due to the necrosis of liver, kidney and lung.
Fig. 2. Biochemical parameters: (A) cortisol, (B) AST, (C) ALT, (D) ALP, (E) creatinine, and (F) uric acid in blood plasma of *Tilapia zillii* during 24-96 hours of exposure to control, 25 µg Al/L, 50 µg Al/L, and 100 µg Al/L. Data are presented as the mean ± S.E. (n=5). Significant differences with the control groups are indicated with asterisks: * $P<0.05$, ** $P<0.01$ and *** $P<0.001$.

Herein, we attribute the increase in AST, ALT, and ALP after aluminum exposure to the hepatocellular degeneration and destruction in other tissues. Therefore, the increases of these enzymes in plasma are indicative of liver damage and thus alterations in liver function.

In our study, blood analysis revealed that creatinine and uric acid were significantly greater than the control (Fig. 2.E&F). This rise in creatinine and uric acid might be induced by glomerular insufficiency, increased muscle tissue catabolism or the impairment of carbohydrate metabolism (Murray, Granne, Mayes, & Rodwell, 1990).

**CONCLUSION**

This study provides new evidence, using biochemical parameters, that exposure to levels of aluminium may be an important factor on fish in the acidified soft waters. It is concluded that the fishes can effectively used as monitors of water quality with respect to metals. Also, it can conclude that biochemical parameters could be ranked as possible biomarkers of pollution.

**REFERENCES**


