Effects of aluminum on some tissue enzymes of gills, liver and muscles in common carp (*Cyprinus carpio*)

Miri M.*; Khandan Barani H.

Received: February 2016 Accepted: June 2016

Abstract

The purpose of this study was to evaluate the effects of aluminum on the tissue enzymes of gills, liver and muscles in common carp. In this study, the effects of sub lethal levels of aluminum on metabolic enzymes, Aspartate aminotransferase, Alanine aminotransferase and alkaline phosphatase (AST, ALT and ALP) in organs including gills, liver and muscles of common carp (*Cyprinus carpio*) were evaluated after 24, 48, 72 and 96 h exposure under laboratory conditions. Tissue extracts were prepared from each group. Enzymes were measured using commercial kits and auto-analyzer Selectra-PRO. According to statistical data analysis, it was found that the activity of this enzyme in tissues increased significantly compared to that in the control group. AST enzymes in the liver and muscle increased significantly but were reduced at 96 h. ALP levels in muscle tissue significantly increased at 72 h but were reduced at 96 h. The alterations in the activities of these enzymes indicated that the presence of aluminum interfered with transamination and metabolic process in tissues. As a biomarker in various organs, it can also be useful for toxicology.

Keywords: Aluminum, *Cyprinus carpio*, AST, ALT, ALP

Hamoun International Wetland Research Institute, University of Zabol, Zabol, Iran
* Corresponding author's E-mail: mirimohadeseh@uoz.ac.ir

Introduction

Aluminum is the third most abundant element on earth. About 8% of the Earth's crust is made up of oxygen, silicon, fluorine and other elements in soil, stone, clay and precious stones (Sigel and Sigel, 1988). Exposure to aluminum is almost inevitable and is an active additive substance available in air, water, food and medicines (Li *et al*., 2009). It also has negative effects on human health and animals (Osinska *et al*., 2004). Aluminum has been introduced by the Agency for Toxic Substances and Disease Registry, as a toxic substance to humans and animals (Agency for Toxic Substances and Disease Registry, 2008). This substance can impair the brain, kidney, liver and muscles. Fish have special importance in aqua-toxicology due to economic value and sensitivity to pollutants (Zahedi *et al*., 2014). Enzymatic analysis has been widely used for early detection and metal poisoning (Dutta and Areids, 2003). Aminotransferases catalyze chemical reactions in cells in which the amine group is transferred from a donor molecule to a recipient molecule. These enzymes are essential amino acids and constitute an essential part of the body's tissues. Therefore, they play an important role in the metabolism of carbohydrates and proteins (Burtis and Ashwood, 2001). Aspartate amino transferases (AST) are naturally found in the cytoplasm and mitochondria of cells in different types of tissue such as liver, heart, muscle and brain. The AST level increases, if the tissues are injured. For example, the level of AST increases in myocardial infarction and myotonic dystrophy. The bulk of alanine amino transferase (ALT) in combination with AST is found in the liver where the recipient is most concentrated. Changes in the level of ALT and AST showed tissue damage in the liver, kidney, gills, brain and muscle. Alkaline phosphatases play an important role in the transport of metabolites across the cell membrane and carboxylic acid cycle (TCA) for energy production (Das *et al*., 2004). Changes have been observed in the activity of alkaline phosphatase in tissues, organs and blood of fish exposed to poisons. Studies have shown that enzymes change in the body and blood of fish when exposed to poisons (El-Demerdash and Elagamy, 1999; Das *et al*., 2004). The aim of this study was to evaluate changes in the activity of the enzymes ALT, AST and ALP in liver, gills and muscles of common carp exposed to aluminum.

Materials and methods

In this study aluminum sulfate was used with purity of 99.9% (Merck). Fish were taken from the center of reproduction and nurture Zahak to the Hamoun Wetland International Institute. Fish were distributed after adaptation to laboratory conditions (1,000 liter fiber glass holding tank for three weeks) in 15 aquariums (20 liter) with a density of 10 fish per aquarium. This experiment consists of 4 different aluminum concentrations (1, 2, 4 and 6 (mg/L)) and a control group with three replicates for each concentration. This

dose was selected based on other research (Authman, 2011; Sivakumar *et al.*, 2012; Zahedi *et al.*, 2014). This was done according to the manual test (OECD) and static conditions. Liver, muscle and gill tissue extract were provided after 24, 48, 72 and 96 h of testing, and stored in small glass containers at -20°C (Voegborlo and Akagi, 2007).

Tissue samples were taken to measure the presence of enzymes alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase. These enzymes were washed with saline and then homogenized with phosphate buffer at a ratio of 1 to10 and centrifuged at 12000 g for 15 min (Dai *et al.*, 2009). The enzymes were measured using an autoanalyzer and a commercial kit (Hayan Developers medicine).

Statistical analysis was performed using SPSS software. The Shapiro-Wilk test was used to confirm normality of data. Treatments were compared with the control group in different days using one-way ANOVA and separated in case of significant differences by Duncan's test. The Excel software (Excel 2010) was used for drawing diagrams based on average and standard error.

Results

The results of this study showed that the presence of ALP enzymes in the liver, gills and muscle of fish at different concentrations of aluminum are significantly different when compared with the control group at different times (p<0.05). The lowest and highest amount of ALP observed were 36±0.05 (U/L) in the control group and 80±0.1 (U/L) 96 h after exposure with aluminum (1mg/L) in the liver tissue, respectively,. The highest ALP levels indicated were 103±0.8 (U/L) at 96 h (6mg/L) in the gill tissue and 67±0.6 (U/L) at 48 h (1mg/L) in the muscle tissue. While the lowest values observed were 41±0.4 and 38±0.3 (U/L), in the control group of the gill and muscle tissues, respectively. Also, statistical analysis of ALP values showed significant differences between the times (24, 48, 72 and 96 h) in liver, gills and muscle tissues (p<0.05). The highest level of ALP was reached in the liver and muscle after 96 h. The amount of this enzyme increased in the muscle after 24, 48 and 72 h but significantly decreased at 96 h (p<0.05) (Fig. 1).

The amount of ALT in the liver, gills and muscle of fish showed a significant difference between the different concentrations of aluminum and the control group (p<0.05). The enzyme ALT in the gill tissue of fish exposed to aluminum reduced significantly compared to the control group (p<0.05). The lowest and highest amounts of enzymes found in the gill tissue were 2±0.1 (U/L) at 96 h (concentration 2 mg/L) and 6.6 (U/L) at 24 h (4mg/L), respectively.

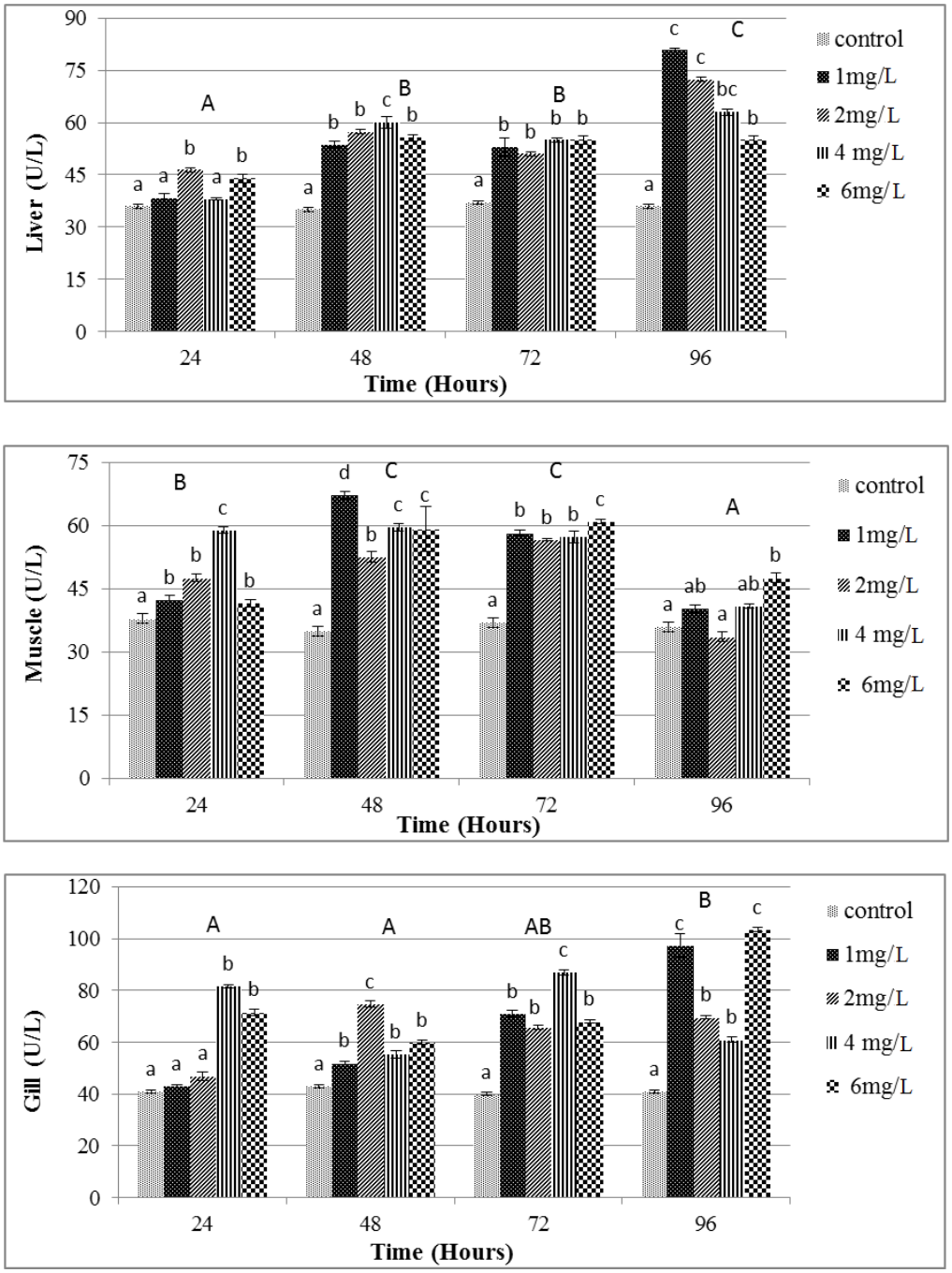


Figure 1: Comparison of average ALP enzymes (U/L) in the tissues of liver, muscle and gills at different times with different concentrations of aluminum (mg/L), different lowercase letters within a time point indicate significant differences between treatments and uppercase letters indicates significant difference between different times, and designate statistically significant values (One-way ANOVA–Duncan test, $p<0.05$).

The ALT levels increased significantly after 48 h ($p<0.05$), but no significant differences were found between the different concentrations and the control group at 96 h in liver tissue ($p>0.05$).

Statistical analysis of ALT values showed that the lowest and highest values observed were 11±0.05 (U/L) in the control group and 48±0.2 (U/L) at 48 h (6mg/L), in the liver tissue,

respectively. The lowest and highest values observed were 3±0.1 (U/L) in the control group and 7.6±0.3 (U/L) at 24 h in the muscle tissue, respectively. The ALT levels were not significantly different between the times in the muscle tissue of fish (p>0.05). However there were significant differences between the times in the liver and gill tissues (p<0.05) (Fig. 2).

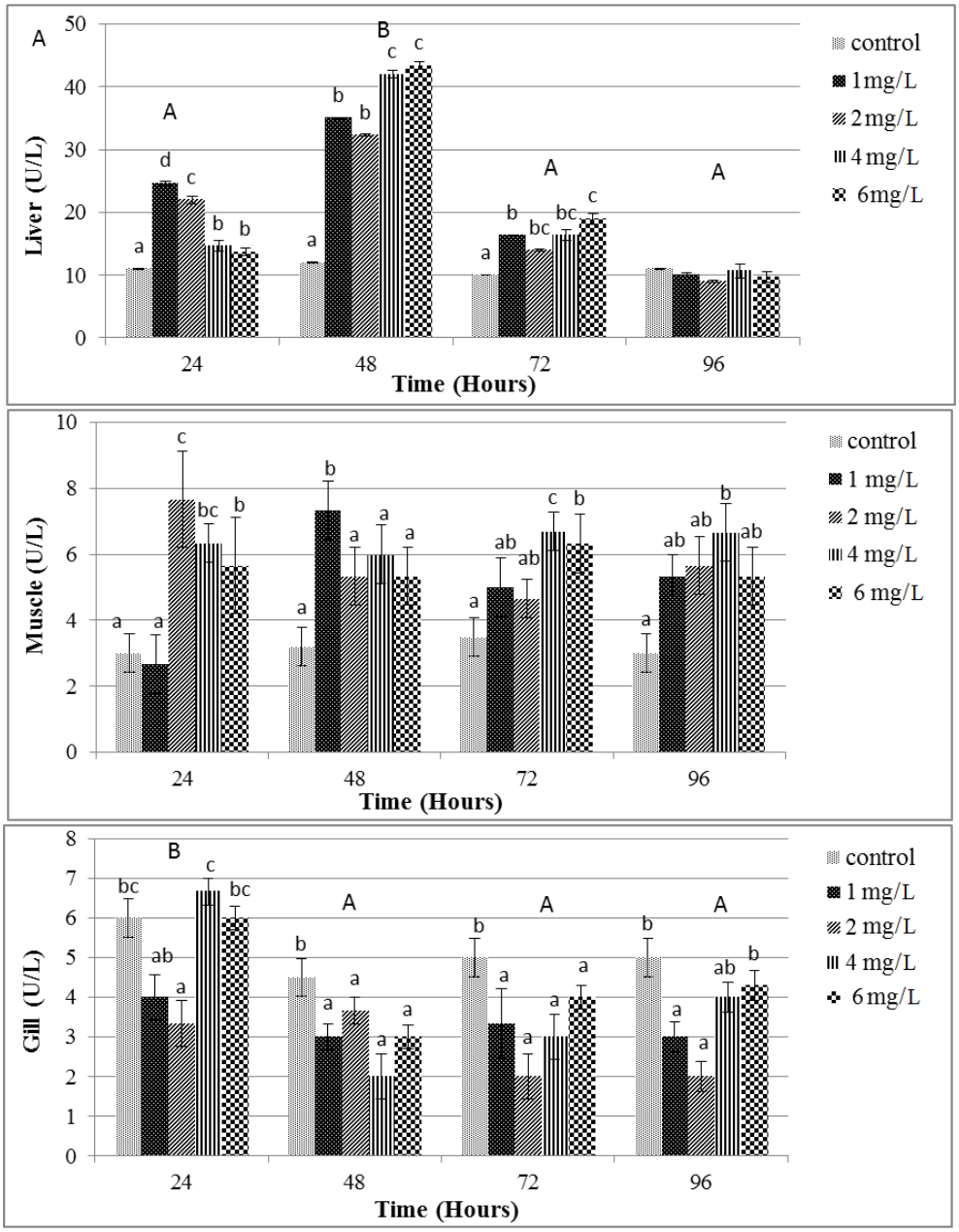
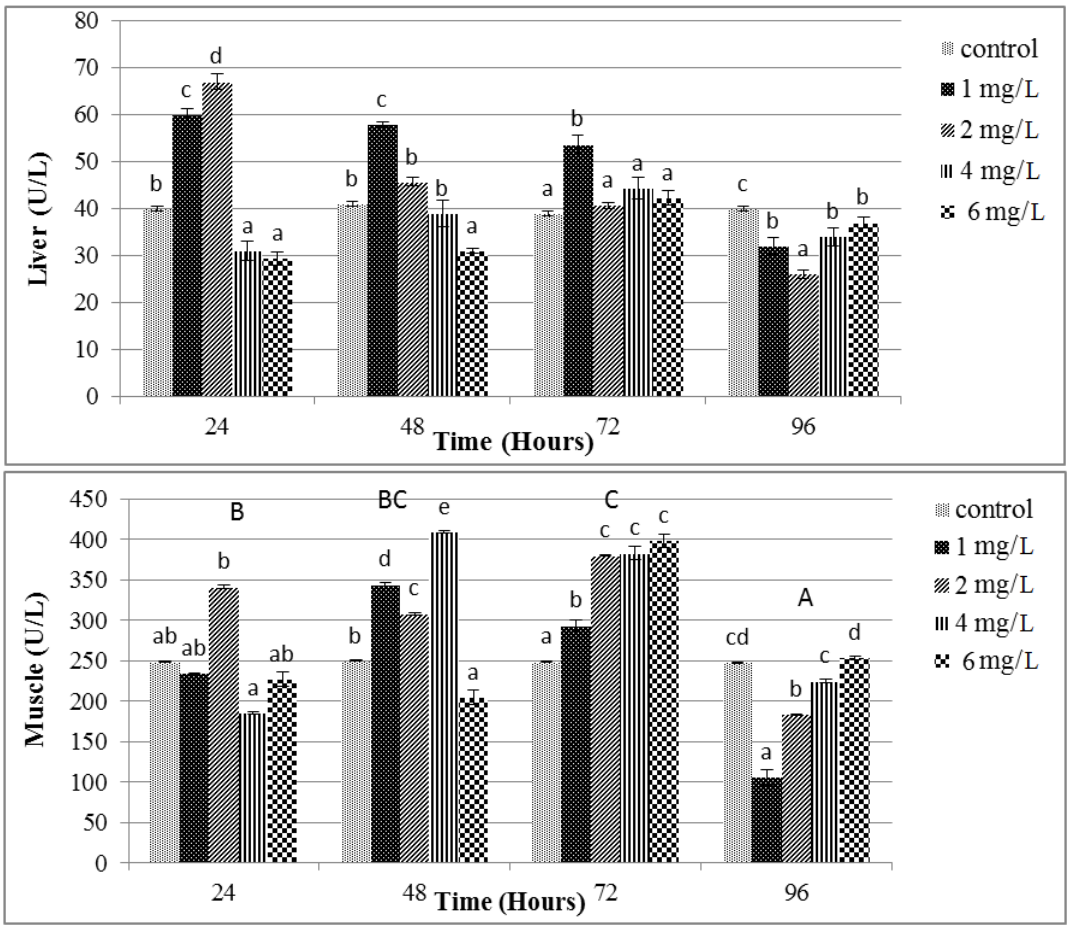


Figure 2: Comparison of the average ALT enzyme (U/L) in the tissues of liver, muscle and gill in different times with different concentrations of aluminum (mg/L), different lowercase letters within a time point indicate significant differences between treatments and uppercase letters indicates significant difference between different times, and designate statistically significant values (One-way ANOVA–Duncan test, p<0.05).

AST activity recorded in different tissues of fish exposed to aluminum is significantly different compared to the control group (p<0.05). The lowest and highest amount of the enzyme observed were 24±0.2 (U/L) at 96 h and 67±0.5 (U/L) at 24 h in the liver tissue, respectively, while the AST values of the gill and muscle tissues increased from 10±0.3 (U/L) in the control group to 19±0.4 (U/L) at 96 h and from 249±0.5 (U/L) in the control group to 383±3.2 (U/L) at 48 h, respectively. Statistical analysis of AST values showed significant differences between the different times for gill tissue (p<0.05). The highest level of enzyme were found in the gills of fish when exposed to aluminum after 96 h. AST level at concentrations of 1 and 2 mg/L increased in the liver after 24 h and reduced at other times, and no significant differences were observed between the different times (p>0.05). The AST significantly increased in muscle tissue after 72 h but reduced at 96 h (p<0.05) (Fig. 3).



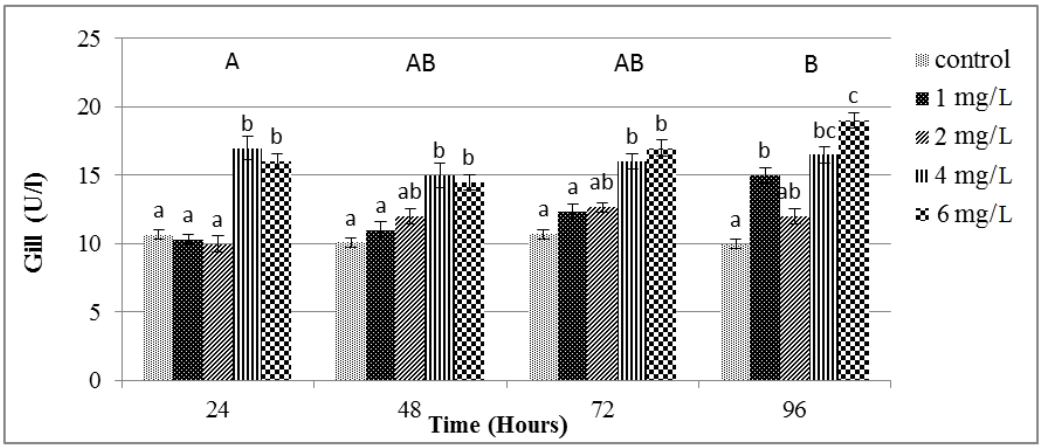


Figure 3: Comparison of the average AST enzyme (U/L) in the tissues of liver, muscle and gills in different times and different concentrations of aluminum (mg/L), different lowercase letters within a time point indicate significant differences between treatments and uppercase letters indicates significant difference between different time, and designate statistically significant values (One-way ANOVA–Duncan test, $p<0.05$).

Discussion

Alkaline phosphates (ALP) are important biomarkers for cellular responses to cell toxicity and genetic toxicity (Lohner *et al.*, 2001). ALP catalyzes the dephosphorization process of organic phosphate (Molina *et al.*, 2005). It also plays a role in bone formation and membrane transport. Karan (1998), studied the impact of copper on ALP activity in the tissues of fish (*Cyprinus carpio*). The study found that enzyme activity increased in the gills of fish. This increase is associated with the changes in tissue and cell damage (Karan *et al.*, 1998; Selamoglu Talas *et al.*, 2012). The study of Alti *et al.* (2007) showed similar results with the present study. The effects metals such as cadmium, lead, zinc and copper on ALP enzyme changes in fish (*Oreochromis niloticus*) were studied for 14 days. This study also found that the metals increased ALP activity in the tissues of fish particularly the liver. Das *et al.* (2004), observed an increase in ALP activity in tissues exposed to nitrite of *Catla catla Labeo rohita*. The study suggested that increase in transferase causes α-amino acid deviation in the tricarboxylic acid cycle (TCA) for energy production. In some studies, the activity of these enzymes has been observed. El-Demerdash and Elagany (1999) observed the inhibition of enzyme activity in some tissues, especially the liver (*Tilapia nilotica*) due to the breakdown of cell membranes balance. Bernet (2001) observed that the activity of these enzymes decreased in fish exposed to wastewater compared to the control group. Also, there were significant decreases in values of ALP compared to the control group in carp (*Cyprinus carpio, L. 1758*) exposed to arsenic ($p<0.05$) (Selamoglu Talas *et al.*, 2012). Selamoglu Talas *et al*. (2014) observed that enzymes activity decreased in the arsenic group compared to the control in *Cyprinus carpio*. This is due to possible disruptions in the

transportation membrane and absorption of nutrients (Bernet, 2001). In the study of Gabriel (2012), the ALP activity was reduced in *Clarias garicpinus* exposed to cypermethrin, because of the reduction in the rate of glycogen synthesis by reducing the metabolic demand (metabolism) and electrolyte imbalance (hyponatremia). Phosphatases play an important role in the transport of metabolites across the cell membrane. Decline in ALP activity in some concentrations may be due to the disruption of membrane transport systems (Gabriel, 2011, 2012). In the present study, the ALP in gills, liver and muscle tissues of common carp exposed to aluminum increased and this increase can be attributed to the change and destruction of cells (Khattab, 2007). ALP increase may also be due to liver damage and bone deformation (Pari and Amali, 2005). Increase of the amount of enzymes in tissues can be seen as a sign of tissue damage for energy production (Atli *et al*., 2006).

Fish need more energy during stress for detoxification and excretion of toxins to reduce the effects of toxins in the body. However, increase of transaminase is effective and efficient against amino acids in different tissues in the fish's metabolic processes and indicates increased stress (Tiwari and Singh, 2004). Increase in ALT and AST yields more energy during stressful conditions, due to high demand and precursors of carbohydrates to preserve the glycolytic pathway (Glycolytic) and stable levels of tolerance in the TCA cycle (Begum, 2005). Alian (2013) studied the effects of cyanide in common carp tissue enzymes (AST, ALT and ALP). They found that the activity of AST in the liver and brain tissues significantly increased compared to the control group. The study reported that the changes of enzyme activity are an indication of metabolic problems caused by chronic poisoning by cyanide in the organ. The results of Begum (2004) are similar to that of the present study. He reported that activity in the face of chemical changes. These changes are due to the turmoil in the biochemical and physiological processes in the body of fish. Sepici-Dincel (2009) observed that ALT and AST increased in the muscles and liver of common carp (*Cyprinus carpio*) when exposed to Cyfluthrin insecticides because of disruption in the Krebs cycle. Yildirim *et al.* (2006) observed that ALT and AST activity increased in the gills and liver of *Oreochromis niloticus* when exposed to deltamethrin for four days. The study reported that increase of protein for energy during the stress of pollution, is due to the increase of these enzymes. Gabriel (2012) observed increase in the activity of these enzymes in the tissues of fish (*C. gariepinus*). Also, Selamoglu Talas *et al*. (2012), observed that ALT and AST values increased when *C. carpio* was exposed to arsenic ($p<0.05$). The study suggested that reducing the activity of the Krebs cycle reduces the intermediates in the cycle. This is therefore compensated by providing α-ketoglutarate which increases this enzyme. AST and ALT are involved in

gluconeogenesis and the amino acid transaminase activity. Increase of transaminase is a safety mechanism that occurs in the early stages of the disease (Chang *et al.*, 2005). On the other hand, decrease of AST and ALT may counteract the effects of poison, maintaining the integrity of liver cell membrane structure and balance the body against toxins in fish (Lohner *et al*., 2001). Changes of enzyme activity are used as a sign of stress. Typically, an increase in liver enzymes ALT and AST represents the disease because of their biological status. Also, an increase of both enzymes AST and ALT may be due to inflammatory disease or liver damage. The results show that aluminum causes significant changes of the enzymes activity in various organs, which are likely to cause tissue damage in fish (Pari and Amali, 2005). The enzymes are used to notice the physiological status of lives and indicators of strees (Lal Shah, 2010).

Studies have observed changes of serum enzyme activity and tissue association due to physiological changes in the fish exposed to metals. Therefore, these enzymes can be used as biomarkers in the science of toxicology.

Reference

Agency for Toxic Substances and Disease Registry, 2008. Toxicological profile for Aluminium. US Department of Health and Human Services, Public Health Service. pp. 1-8.

Alian, S.H., Shahsavani, D. and Baghyshni, H., 2013. Study of tissue enzymes change of cyanide poisoning in common carp. *Iranian Veterinary Journal*, 8(**4**), 44-51.

Atli, G., Alptekin, Ö., Tükel, S. and Canli, M., 2006. Response of catalase activity to Ag^{+}, Cd^{2+}, Cr^{6+}, Cu^{2+} and Zn^{2+} in five tissues of freshwater fish, *Oreochromis niloticus*. *Comparative Biology and Physiology*, 143, 218-24.

Authman, M.M.N. 2011. Environmental and experimental studies of aluminium toxicity on the liver of *Oreochromis niloticus* (Linnaeus, 1758) fish. *Life Science Journal*, 8(**4**), 764-776.

Begum, G., 2004. Carbofuran insecticide induced biochemical alterations in liver and muscle tissues of fish *Clarias batrachus* (Linn) and recovery response. *Aquatic Toxicology*, 66(**1**), 83-92.

Begum, G., 2005. In vivo biochemical changes in liver and gill of *Clarias batrachus* during cypermethrin exposure and following cessation of exposure. *Pesticide Biochemistry and Physiology*, 82, 185-196.

Bernet, D., 2001. Schmidt H, Wahli T, Burkhardt-Holm P. Effluent from a sewage treatment works causes changes in serum chemistry of brown trout (*Salmo trutta L*.). *Ecotoxicology and Environmental Safety*, 48, 140-147.

Burtis, C.A. and Ashwood, E.R., 2001. Tietz fundamentals of clinical chemistry. 5th ed. W.B. Saunders. Philadelphia. pp. 655-657.

Chang, L.M., Yang, W.C., Cheng, J.C., Ho, Y.P., Pan, M.J., Lin,

C,H., and Lin, D.Y., 2005. proportional exaggerated aspartate transaminase is a useful prognostic parameter in late leptospirosis. *World Journal of Gastroenterology*, 11(**35**), 5553-56.

Dai, W., Fu, L., Du, H., Jin, C. and Xu, Z., 2009. Changes in growth performance, metabolic enzyme activities, and content of Fe, Cu, and Zn in liver and kidney of tilapia (*Oreochromis niloticus*) exposed to dietary Pb. *Biological Trace Element Research,* 128, 176-183.

Das, P.C., Ayyappan, S., Das, B.K. and Jena. J.K., 2004. Nitrite toxicity in Indian major carps: sublethal effect on selected enzymes in fingerlings of *Catla catla Labeo rohita*, and *Cirrhinos mrigala*. *Comparative Biology and Physiology,* 138, 3-10.

Dutta, H.M. and Areids, D.A., 2003. Effects of endosulfan on brain acetyl- cholinestarate activity in juvenile blue gill sunfish. *Environmental Research,* 91, 157-162.

El-Demerdash, F.M. and Elagamy, E.I. 1999. Biological effects in *Tilapia nilotica* fish as indicators of pollution by cadmium and mercury. *International Journal of Environmental Research*, 9, 173-186.

Gabriel, U.U., 2011. George ADI. Plasma enzymes in *Clarias gariepinus* exposed to chronic levels of roundup (glyphosate). *Environmental Ecology*, 23, 271-276.

Gabriel, U.U., 2012. Akinrotimi OA, Ariweriokuma VS. Changes in metabolic enzymes activities in selected organs and tissue of *Clarias gariepinus* exposed to cypermethrin. *Chemical Engineering Journal*, 1(**1**), 25-30.

Karan, V., Vitorovic, S., Tutundzic, V. and Poleksic, V., 1998. Functional enzymes activity and gill histology of carp after copper sulfate exposure and recovery. *Ecotoxicology and Environmental Safety*, 40, 49-55.

Khattab, F.K., 2007. Histological and ultrastructural studies on the testis of rat after treatment with aluminium chloride. *Journal of Basic and Applied Scientific,* 1, 63-72.

Lal Shah, S., 2010. Hematological Changes *Tinca tinca* after exposure to letal and subletal doses of Mercury, Cadmium and Lead. Iranian Journal of Fisheries Science, 9(**3**), 434-443.

Li, Z.H., Zlabete, V., Velisek, J., Grabic, R., Machova, J. and Rendak, T., 2009. Physiological condition status and muscle based biomarkers in rainbow trout after long exposure to carbamazepine. *Journal American Toxicology,* 30, 197-203.

Lohner, T.W., Reash, R.J. and Williams, M., 2001. Assessment of tolerant sunfish populations (*Lepomis spp.*) inhabiting selenium-laden coal ash effluent. 2. Tissue biochemistry evaluation. *Ecotoxicology Environmental Safety,* 50, 217-224.

Molina, R., Moreno, I., Pichardo, S., Jos, A., Moyano, R., Monterde, J.G. and Camean, A., 2005. Acid and alkaline phosphatase activities

and pathological changes induced in Tilapia fish (*Oreochromis* sp.) exposed subchronically to microcystins from toxic cyanobacterial blooms under laboratory conditions. *Toxicology,* 46, 725-735.

Osinska, E., Kanoniuk, D. and Kusiak, A., 2004. Aluminium hemotoxicity mechanisms. *Annales Universitatis Mariae Curie-Sklodowska*, . 59(**1**), 411-416.

Pari, L. and Amali, R., 2005. Protective role of tetrahydrocurcumin (THC) and active principle of turmeric on chloroquine-induced hepatoxicity in rats. *Journal of Pharmacology and Pharmaceutics Science*, 8(**1**), 115-23.

Selamoglu Talas, Z., Pinar Dundar, S., Fuat Gulhan, M., Orun, I. and Kakoolaki, S., 2012. Effects of propolis on some blood parameters and enzymes in carp exposed to arsenic. *Iranian Journal of Fisheries Sciences,* 11(**2**), 405- 414.

Selamoglu Talas, Z., Fuat Gulhan, M., Erdogan, K. and Orun, I., 2014. Researching of antioxidant effects of propolis in carp (*Cyprinus carpio*) exposed to arsenic: Biochemical and histopathologic findings. *Diseases of Aquatic Organisms,* 108, 241–249.

Sepici-Dincel, A., Karasu, B.C., Selvi, M., Sarikaya, R., Sahih, D., Ozkul, I.A. and Erkve, F. 2009. Sublethal cyfluthrin to carp *Cyprinus carpio* fingerlings: Biochemical, hematological, histopathological alterations. *Ecotoxicolgy Environmental Safety,* 72, 1433-1439.

Sigel, H. and Sigel, A.E., 1988. Metals ions in dialysis dementia syndrome and aluminiumin toxication. *Nephrology,* 31, 1-10.

Sivakumar, S., Prasad Khatiwada, C,. Sivasubramanian, J., 2012. Bioaccumulations of aluminum and the effects of chelating agents on different organs of Cirrhinus mrigala. *Environmental Toxicology and Pharmacology*, 34, 791-800.

Tiwari, S. and Singh, A., 2004. Piscididal activity of alcoholic extract of *Nerium indicum* leaf and their biochemical stress response on fish metabolism. *Africa Journal Trade CAM,* 1, 15–29.

Voegborlo, R.B. and Akagi, H., 2007. Determination of mercury in fish by cold vapour atomic absorption spectrometry using an automatic mercury analyzer. *Food Chemistry,* 100, 853-858.

Yildirim, M.Z., Benli, K.C., Selvi, M., Ozkul, A., Erko, F. and Kocak, O., 2006. Acute toxicity behavioral changes and histopathological effects of deltamethrin on tissues (gills, liver, brain, spleen, kidney, muscle, skin) of Nile Tilapia (*Oreochromis niloticus*) fingerlings. *Environmental Toxicology*, 21, 614-20.

Zahedi, S., Vaezzade, H., Rafati, M. and Zarei Dangesaraki, M., 2014. Acute toxicity and accumulation of iron, manganese and aluminum in Caspian Kutum fish (*Rutilus kutum*). *Iranian Journal Toxicology,* 8(**24**), 1028-1033.