ORIGINAL ARTICLE

Effects of sublethal zinc ions and acute crude oil exposure on serum enzymes activity of *Carassius gibelio*

Mohammed Abdulridha Aldoghachi ▪ Faleh Musa Al-Zaidy ▪ Layla Mustafa AlKatrani

MA Aldoghachi (Corresponding author) ▪ **FM Al-Zaidy** ▪ **LM AlKatrani**
Department of Marine Vertebrates, Marine Science Center, University of Basrah, Basrah, Iraq.
email: mohammed.aldoghachi@uobasrah.edu.iq

Received: April 06, 2019 ▪ Revised: May 18, 2019 ▪ Accepted: May 21, 2019

Abstract The current study was intended to investigate the responses of freshwater fish *Carassius gibelio* as an in vivo model by measuring the activity of the enzyme of the liver. Fish exposed to sub-lethal toxicities due to zinc metal and crude oil. Fish were treated to 0.6 and 1.2 mg/l for zinc ions as well as 500 and 2500 mg/l for crude oil during 96h, and the alterations in serum enzyme activities were determined aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Zinc toxicity elevated activity level in hepatic enzymes in a significant way ($P < 0.05$). The present study appeared the slightly augmented levels of AST in the serum of *C. gibelio* when exposed to sub-lethal Zinc concentration while the serum ALT activity increased in response to the high level of Zn exposure when compared to control after (96 h). In addition, acute concentrations of crude oil after 96 h exposure can cause harmful effects on *Carassius gibelio* including the induction of (AST). However, not effecting found (ALT). We are concluded that changes in the activity of the enzyme can be considered as a pointer of the pollution impact in fish.

Keywords: zinc, crude oil, *Carassius gibelio*, AST, ALT, enzymes activity

Introduction

Pollution by heavy elements has become one of the most difficult and important problems facing humans and it has increased in recent decades because of its intensive use in industrial, toxic chemicals and agricultural wastes that have become directly threat to the aquatic system and then indirectly leads to a danger to human life (Tchounwou et al 2012). The heavy metals include zinc that can accumulate in animal tissues and affect several physiological functions. Furthermore, the risk of poisoning largely depends on toxic dose and duration of exposure in addition to the sensitivity of the organism to the toxic substance (Yousafzai and Shakoori 2011; Edori, et al 2013). In other hand, water pollution by crude oil has become an additional danger to the countries of the world in a varied range, and particularly widespread in countries that own the oil industries and most of its economy depends on oil products (Cocarta et al 2017).

Last scientific studies have found that the rise or decline in the serum enzymes activities such as aminotransferases ALT and AST which are intracellular enzymes released by cell damage or death and these activities depend on the toxic mater, fish species, exposure period and water quality (Varol and Sunbul 2017; Rahimikia 2017). Fish are highly sensitive to pollution by toxic metals can disturb many biochemical and histopathological trails in the case of accumulated in fish tissues (Aldoghachi et al 2016). Activities of blood enzymes can be expanded in freshwater fish exposed to toxic chemicals such as heavy metals including the Zinc ions (Cruz et al 2013). Other literature such as Al-Khashali and Alshawi (2013) in changing of enzymes concentration including (ALT) and (AST) in the adult of *Carassius auratus* exposed to diverse salt concentrations. As well as, the study of Edori, et al (2013) in the catfish *Clarias gariepinus* after sub-acute exposure to agricultural pollutant showed a serious biochemical and enzymatic changes, which could be harmful to the fish. A review of Filippov et al (2013) on the activity of fish digestive enzymes, which effects by organic pollutants that induced changes may differ depending on the fish species, toxicant concentrations, and type of hydrolyzed substratum, in addition to experimental conditions.

Most studies on the effect of pollutants are limited to physiological and biochemical changes, while few of these studies have been interested in enzymatic activity in fish blood, especially *Carssius gibelio* which confirmed by Jawad et al (2012) that the existence of *C. gibelio* (Bloch 1782) in a new area in Iraq and this species has similarity to the native crucian carp *Carassius carassius*. Consequently, the main objective of the present research was to investigate the induced effects of Zinc metal and crude oil on serum enzymes (AST, ALT) activities of commercially important freshwater fish *C. gibelio*.

doi.org/10.31893/2318-1265jabb.v7n4p155-159

Materials and Methods

Experimental animals and exposure condition

The study was carried out with *C. gibelio* (with weight mean of 38.03 ± 1.5 g and total length mean of 15 ± 1.3 cm, as mean ± S.E.) was got from commercial fish farms, Medaina, Basrah, Iraq. Acclimatization of fish samples was done in laboratory conditions in aerated glass aquaria for one week at 21±2ºC under a constant photo-period 12:12 light: dark. Acclimatized fish were allowed daily feeding on a dry pellet of commercial food with crude protein content was 25%. Dead organisms were regularly removed by hand net. Experimental aquaria (60× 30× 30 Cm) contained 20 l of dechlorinated tap water and the aerating was continuously available via aerator system, temperature 21±2ºC, pH 8. ± 0.9, dissolved oxygen 7.2 ± 0.2 mg/l. The median lethal concentration (LC50) for the duration of 96 h of exposure ($ZnSo_4.7H_2o$) as described by U.S. EPA (2002) was calculated by the probit transformed concentration-response curves; determined by preliminary test (2.4 mg/l). Fish were distributed in three groups, 12 fish was placed for everyone. The first group was the control samples in which tap water used, and other groups were exposed to 25% and 50% LC50 of Zn which was 0.6 and 1.2 mg\l respectively for 96 hours. The experiment was designed in static renewal conditions; test solutions were completely substituted with new ones of the same concentration in each 24-hour interval until the exposure of 96 hours (Jasim et al 2016). The other side, experimental fish was exposed to different concentrations of crude oil, fish samples were separated into three groups in three replicates each group was containing 30 specimens. Concentrations of crude oil were prepared in glass aquaria one hour prior to fish transfer. First group: Exposed to 10% 96 hr. LC_{50} (500ppm) Group2: Exposed to 50% 96 hr. LC_{50} (2500 ppm) Group 3: used tap water without adding any concentration as a control. Feeding was permissible in control and exposed fish groups per day during the experimental period (96 h) (Yuanyuan et al 2009).

Serum preparation and enzyme activity determination

After the end of the exposure period (96 h), five fish were taken from every aquarium and used as replicates. They were directly anesthetized in ice-cold water and then blood samples were collected from the caudal vein of each fish as mentioned by Zang et al (2015) by using a syringe with a volume of 3 ml. This blood was placed in glass test tubes with volume 5 ml and left for 30 min under room temperature (27 ºC) for clotting, then centrifuged at 3000 rpm for 10 min to separate the serum from the clot using a universal centrifuge model (PLC-036). Serum samples were then kept frozen at -20 ºC in Eppendorf tubes with a volume of 2 ml until the analysis.

The level of aspartate aminotransferase (AST) enzyme and alanine aminotransferase (ALT) enzyme were calculated by UV test technique (Rahimikia 2017) by using commercially reagent kit supplied by Syrbio (Syrian company). Substrate R1 which entering active site of the enzyme for the formation of enzyme products complex and then added color reagent R2. The absorbance of AST and ALT were measured by spectrophotometer model (Humalayzer primus) at 546 nm wavelength. The activity of AST and ALT calculated in (UL) unit.

Data analysis

All data were recorded as mean±standard error, statistically analyzed and carried out to variance analysis (ANOVA) by using the SPSS version 20 statistical software. Differences among treatment means were evaluated at $P < 0.05$ levels.

Results and Discussion

The current research findings found that the activities of AST in Zn exposed fish were either lower or higher than their values in control samples. Convergent values were observed at high or low exposure of zinc (0.6 and 1.2 mg\l) which was (17.26±0.00 and 22.19 IU/L) respectively as against the value of control was 15.45±1.26 IU/L. Moreover, no statistical difference was found in AST concentrations between exposure samples (25 and 50% LC_{50}). While the activity of ALT in the blood serum was elevated over the value of the control sample. The rise in the activity of ALT was dependent on zinc element concentration, with the highest value found at 1.2 mg/L (72.92±5.89 IU/L) as against the least values of control samples (24.51±4.09 IU/L) (Table 1).

Table1 Blood serum AST and ALT in *Carassius gibelio* exposed to Zinc ions for an exposure period (96 h.).

Concentration of Zn (mg/L)	AST (IU/L)	ALT (IU/L)
Control (0)	15.45±1.26	24.70±1.03
0.6	17.26±3.04	25.30±3.02
1.2	22.19±2.96	72.92±5.76

Toxicity of Zinc increased activity levels of hepatic enzymes in a significant level ($P < 0.05$). The current study revealed the levels of AST was a slight increase in the blood serum of *C. gibelio* after treated with sublethal Zinc while the serum ALT activity increased in response to the high level of Zn exposure when compared to control after (96 h).

Many serum enzymes have been reviewed as an appropriate stress indicator. Thus, activities of many serum enzymes, which included AST and ALT, have been frequently applied in the diagnosis of some of the cases of fish diseases,

doi.org/10.31893/2318-1265jabb.v7n4p155-159

in addition, the discovery of impairment in fish tissues induced by environmental pollution. Therefore, the increase in the activity of enzymes in the serum or extracellular fluid is considered a sensible indicator of minor cellular impairment subsequently tissue damage which results in stress (Palanivelu et al 2005). In this study, since there was an elevation of the serum ALT enzyme after sub-acute exposure to zinc metal. This increase attributed to the liver, which appears to be greatest, impacted in the cellular membrane. The toxic materials caused an increase in cell membrane permeability to lead to enzyme leaching or leakage of enzymes from liver to blood or another case the permeability has reduced which directly lead to the accumulation of enzymes in the hepatocytes (Gabriel et al, 2009; Yousafzai and Shakoori 2011).

Generally, the increase in AST and ALT may refer to deterioration changes and dysfunction of liver because the effect of toxicants on the hepatocytes induced in necrosis of the liver and thus, lead to leakage of these cellular enzymes into blood circulation. This is supported by the study of Rahimikia (2017) on gold fish (*Carassius auratus*) underexposure of nickel, he indicated that releasing these transaminases into the bloodstream which attribute to impairment of the hepatic tissue, heart, and kidney under stress affected by metal. In addition, he recommended that serum enzymes could be used in biomarkers to environmental toxicity. Thereby, these enzymes activity in serum of *C. gibelio* is mostly resulted from the release of these enzymes from the aqueous component of the cytoplasm of liver cells into the blood circulation due to liver damage via Zn metal ions.

The current research is in agreement with the finding obtained by Naveed et al (2011) in *Channa punctatus* who noticed that frequent exposure to heavy elements led to augmented activities of both aminotransferases advised greater than before transamination operation. As well as, complemented with the previous research of Li et al (2011) who studied the freshwater rainbow trout (*Oncorhynchus mykiss*) which have been exposed to toxic fungal pesticides, who found that concentration level of AST and ALT in experimental samples were considerably higher than those found in the control samples. Another study by Zikic et al (2001) on *Carassius auratus gibelio* exposed to Cd element ions they proved that high activity in plasma AST and ALT and this rise in activity of enzymes could result from impairment in physiological and anatomical characteristics for tissues. Research of Qiu et al (2009) proved that elevated AST activities in tow fish species silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*), they attribute this increase to mitochondrial disturbance due to severe hepatitis while the increase in ALT activity into the blood prove that the deterioration in hepatocyte membranes. Furthermore, the study of Orbea and Cajaraville (2006) suggested that damage in DNA oxidative resulted in changes in the activity of enzymatic complexes.

Beside that present findings recorded high significant values ($P < 0.05$) of AST in blood serum of specimens exposed to each concentration of crude oil (500 and 2500 ppm) in comparison with control specimens (Figure 1). This increase in the activity of AST indicated the biotransformation pathway affected by the acute concentration of crude oil used, and it is considered a protective response in fish toward oxidative stress.

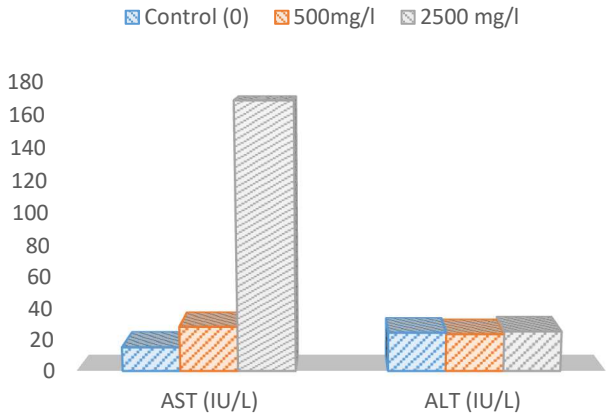

Figure 1 The concentration of serum AST and ALT in *Carassius gibelio* exposed to different concentrations of crude oil for 96h.

The high level in AST recorded in our data corresponds with the finding found in *Oreochromis niloticus* exposed to crude oil by the study of Gad (2011) who observed an increase in enzymatic activity depending on high concentration and exposure time for crude oil. While another study of Zhang et al (2004) on the goldfish *Carassius auratus*, who pointed out antioxidant defenses effected by molecules of diesel oil dissolved in water, and these responses of antioxidant were increased with low concentrations of oil in range (0.05-0.1 mg/l). The present finding concurs with the study of Yuanyuan et al (2009) on the effect of crude oil on the antioxidant defense of liver in *Carassius auratus* who reported that significantly increase in all treated samples, and the uppermost increase has reached more than 6 times in enzyme activity under the 500-ppm exposure.

Our results recorded approximately equal values of ALT at each of the two exposure concentrations of crude oil (500 and 2500 ppm) when compared to the controls (Figure 1). It was indicated that oxidative deamination and inactive transamination have happened. . Rostami and Soltani (2016) reported similar results on sturgeon fish *Acipenser persicus* who demonstrated that crude oil had a devastating effect on erythropoietic cells and prevented all enzymatic activities.

Furthermore, study of Cruz et al (2013) on *Oreochromis niloticus*, who concluded that histopathological changes and dysfunction affected by toxicant on the target

tissue (gill, liver, and kidney) leads to high levels of enzymes activities which was considered as sensitive and rapid bio indicators. In conclusion, of our study demonstrated that zinc ion can effect in enzyme activity for each of AST and ALT.

Conclusions

We are concluded that changes in the activity of the enzyme can be useful as a pointer of the impact of pollution in fish. In addition, crude oil at higher concentration level 2500 ppm after 96 h can cause negative effects on *Carassius gibelio* including the induction of aspartate aminotransferase (AST). However, not effecting found in alanine aminotransferase (ALT) in all exposure concentrations.

Acknowledgments

The authors thank the Marine Science Center, Basrah University, Iraq for the providing of work requirements and materials. This research was reinforced by under research grant 2018, 36ABK and was done at an environmental laboratory in Marine Vertebrates Department.

References

Aldoghachi MA, Azirun MS, Yusoff I and Ashraf M (2016) Ultrastructural effects on gill tissues induced in red tilapia Oreochromis sp. By a waterborne lead. Saudi Journal of Biological Sciences 23:634-640.

Al-khashali M Sh, Al-Shawi S (2013) Effect of salt stress on ALT and AST enzymes activity and cortisol level in adult of *Carassius auratus*. Pakistan Journal of Nutrition 12:97-100.

Cocarta DM, Stoian M, Karademir A (2017) Crude oil contaminated sites: evaluation by using risk assessment approach. Sustainability 9:1365.

Cruz, ZM, de Castro R, Virgens A, Júnior R, Silva A, Ramo, A (2013) Biochemical and cellular changes in *Oreochromis niloticus* related to the water pollution of a degraded river. Acta Scientiarum Biological Sciences 35:419-427.

Edori OS, Dibofori-Orji A, Edori E (2013) Biochemical Changes In Plasma and Liver of Clarias Gariepinus Exposed To Paraquat. Journal of Pharmacy and Biological Sciences 8:35-39.

Filippov AA, Golovanova I, Aminov A (2013) Effects of organic pollutants on fish digestive enzymes: a review. Inland Water Biology 6:155–160.

Fırat O, Cogun H, Yuzereroglu T, Gok G, Kargin F, Kotemen Y (2011) A comparative study on the effects of a pesticide (cypermethrin) and two metals (copper, lead) to serum biochemistry of Nile tilapia, *Oreochromis niloticus*. Fish Physiol Biochem 37:657–666.

Gabriel UU, Obomanu F, Edori O (2010) Biochemical changes in hybrid catfish (*Heterobranchus bidorsalis* and *Clarias gariepinus*) treated with nuracron. Chinese Journal of Applied and Environmental Biology 16:353-357.

Gad NS (2011) Oxidative stress and antioxidant enzymes in *Oreochromis niloticus* as biomarkers of exposure to crude oil pollution. International Journal of Environmental Science and Engineering 1:49-58.

Hedayati A, Jahanbakhshi A (2013) Hematotoxic effects of direct infusion of crude diesel oil on juvenile great sturgeon *Huso huso*. Comparative Clinical Pathology 22:1117-1122.

Jasim MA, Azirun M, Yusoff I, Rahman M (2016) Bioaccumulation and Histopathological Changes induced by Toxicity of Mercury (HgCl2) to Tilapia Fish *Oreochromis niloticus*. Sains Malaysiana 45:119–127.

Jawad L, Al-Faisal A, Mukhtar M (2012) Presence of the Non-Indigenous Fish Species, Gibel Carp, Carassius gibelio (Family: Cyprinidae) in Basrah Province Freshwater Systems, Iraq. Water Research and Management 2:41-44.

Kosti E, Perdikaris C, Athanassopoulou F, Chantzaropoulos A, Vavatsikos M, Ergolavou A, Bithava C, Paschos I (2008) Use of Prussian carp *Carassius gibelio*, pituitaries in the artificial propagation of female common carp, *Cyprinus carpio*. Acta Ichthyologica Et Piscatoria 38:121–125.

Li ZH, Labek V, Velisek J, Grabic R, Machová J, Kolařová J, Li P, Randák T (2011) Antioxidant responses and plasma biochemical characteristics in the freshwater rainbow trout, *Oncorhynchus mykiss*, after acute exposure to the fungicide propiconazole. Czech Journal of Animal Science 56:61–69.

Naveed A, Janaiah C, Venkateshwarlu P (2011) The effects of lihocin toxicity on protein metabolism of the fresh water edible fish, *Channa punctatus* (Bloch). Journal of Toxicology and Environmental Health Sciences 3:18–23.

Orbea A, Cajaraville M (2006) Peroxisome proliferation and antioxidant enzymes in transplanted mussels of four basque estuaries with different levels of polycyclic aromatic hydrocarbon and polychlorinated biphenyl pollution. Environmental Toxicology and Chemistry 25:1616-1626.

Palanivelu V, Vijayavel K, Ezhilar S, Balasubramanian M (2005) Influence of insecticidal derivative (Cartap Hydrochloride) from the marine poly- chaete on certain enzyme systems of the freshwater fish *Oreochromis mossambicus*. Journal of Environmental Biology 26:191–196

Qiu T, Xie P, Guo L, Zhang D (2009) Plasma biochemical responses of the planktivorous filter-feeding silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*) to prolonged toxic cyanobacterial blooms in natural waters. Environmental Toxicology and Pharmacology 27:350–356.

Rahimikia E (2017) Analysis of antioxidants and serum biochemical responses in goldfish under nickel exposure by sub-chronic test. Journal of Applied Animal Research 45:320–325.

Rosa J, Tiago DM, Dias J, Cancela M and Laizé V (2010). Serum-specific stimulation of proliferation and mineralization of fish bone-derived cells. Journal of Applied Ichthyology 26:251-256.

Rostami KH, Soltani M (2016).Effects of acute crude oil exposure on basic physiological functions of Persian sturgeon, *Acipenser persicus*. Caspian Journal of Environmental Science 14:43-53.

Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ (2012) Heavy Metal Toxicity and the Environment. In: Luch A. (eds) Molecular, Clinical and Environmental Toxicology. Experientia Supplementum, vol 101. Springer, Basel. DOI https://doi.org/10.1007/978-3-7643-8340-4_6.

U.S. Environmental Protection Agency (2002) Methods for measuring the acute toxicity of effluents and receiving water to fresh water and marine organisms, 5th ed. EPA -821-R-02-012. Final report, Office of water, Washington, DC.

Varol M, Sunbul M (2017) Organochlorine pesticide, antibiotic and heavy metal residues in mussel, crayfish and fish species from a reservoir on the Euphrates River, Turkey. Environmental Pollution 230:311-319

Yousafzai MA, Shakoori R (2011) Hepatic response of a fresh water fish against aquatic pollution. Pakistan Journal of Zoology 43:209-221.

Yuanyyuan W, Qixing Z, Shengwei P, Lena M, Xiaowei N (2009) Toxic effects of crude-oil-contaminated soil in aquatic environment on *Carassius auratus* and their hepatic antioxidant defense system. Journal of Environmental Sciences 21:612-617.

Zang L, Shimada Y, Nishimura Y, Tanaka T, Nishimura N (2015) Repeated blood collection for blood tests in adult zebrafish. Journal of Visualized Experiments 102:53272.

Zhang JF, Wang X, Guo H, Wu J, Xue Y (2004) Effects of water-soluble fractions of diesel oil on the antioxidant defenses of the goldfish, *Carassius auratus*. Ecotoxicology and Environmental Safety 58:110-116.

Zikic RV, Stajn S, Pavlovic Z, Ognjanovic B, Saicic Z (2001) Activities of superoxide dismutase and catalase in erytrocyte and plasma transaminases of goldfish (Carassius auratus gibelio Bloch.) exposed to cadmium. Physiological Research 50:105–111.

.