Assessment of Aminotransferase Enzymes in Yellowfin Sea Bream (*Acanthopagrus latus*) under Experimental Condition as Biomarkers of Mercury Pollution

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Abstract: The toxic effects of mercury on fish can be measured accurately by studying the relevant enzymes and other physiological and biochemical biomarkers. In this paper, lipase, alanine aminotransaminase (ALT) and aspartat aminotransaminase (AST), Total protein values were assayed. In laboratory, Fish maintained in a seawater re-circulatory system (300-L tanks) and divided to five treatments (0, 10, 20, 40 and 80 µg l). The ALT and AST activity was estimated by commercial chemical Kits. Lipase, Total protein and Glucose activities were determined with Photometric method. Both ALT and AST activities exhibited significant analysis of variance (P<0.01) with lower considerable values than those of the control group. No significant changes occurred in the activities of the Lipase, however it was decrease too. Values recorded for total protein showed significance depletion (P<0.001) with mercury exposed. In conclusion sub acute mercury concentrations may cause several changes in the metabolic and enzymatic parameters of the studied fish.

Key words: Heavy metals · Toxicity · Fish · Ecotoxicology

INTRODUCTION

Human activities may released different type of chemicals to the aquatic ecosystem, either accidentally or by design, may cause adverse effects on the marine environment, including deleterious variation which disrupts metabolic activity at the biochemical level [1]. In recent years, enzymes have been widely used as marine ecosystem biomarkers. These enzymes have an important role in physiological functions determinant for the survival and performance of the marine biota, namely, neutransmission, energy production and detoxification. In spite of seriousness and longevity of heavy metals in the ecosystem, that they are non-degradable with significant oxidizing capacity and substantial affinity for electronegative nucleophilic species in proteins and enzymes.

Changes in serum aspirate aminotransferase (AST), alanine aminotransferase (ALT), enzyme activities in fish have been used frequently as indicators of toxicant and contamination of marine ecosystems [2]. Proteins are involved in major physiological events, so the evaluation of the protein content can be considered as a diagnostic tool to determine the physiological indices of biota. Proteins are highly sensitive to heavy metal poisoning [3]. Blood glucose level in fish is known to be very useful as a criterion for diagnosis of liver and muscle tissue functions [4].

Heavy metals accumulated in the fish tissues may catalyze reactions that generate reactive oxidative species (ROS) which result to environmental oxidative stress. These systems contain different antioxidant defenses. Defensive mechanisms to discomfit the impact of ROS are found in many species including aquatic animals such as fish.

Similar research on fish enzymes have demonstrated that antioxidant systems could provide relevant indices in explaining the sensitivity of some fish species to pollutions [5]. Antioxidants have a very sensitive role in maintaining cell homeostasis and, when these defenses are impaired or surmounted, oxidative stress products, namely reactive oxygen species (ROS), may induce DNA damage, enzymatic inactivation and peroxidation of cell constituents. Fish often increased the levels of protective antioidxants enzymes, as well as non-enzymatic free radical scavengers for prevent and cope again

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abnormality that cause by ROS. Thereupon, one of the suitable biomarker of exposure to heavy metals is the modulation of antioxidants enzymes [6], for example mercury was recognized as a pro oxidant that induces oxidative stress [7].

Changes in serum AST, ALT enzyme activities in fish have been frequently used as indicators of intoxication and water pollution [2].

Induction of oxidative stress causes with mercury make an important contribution to molecular mechanism for liver injury [8]. Recent studies confirmed that mercury causes severe oxidative damages [9] thus mercury is proved to be a potential oxidant in the category of environmental factors.

Proteins are a major constituent in the metabolism of animals and heavy metals may be involved in the normal working of these molecules, therefore, it is important for detection of alterations in protein metabolism induced by metal exposure for further information. Alterations that may occur are the increased synthesis or breakdown of proteins and the inhibition or activation of certain enzymes. These changes can be observed in the total protein content, free amino acid (FAA) concentration and the activity of proteases and transaminases such as ALT and AST [10].

In the present study, enzymatic biomarkers were measured in order to investigate patterns of response in these enzymes and to quantify the extent of alterations caused by the mercury compounds. The enzyme-abundant liver contributes to the aspect of the circulating enzyme pattern in the serum. When damage occurs in enzyme abundant tissue, some enzymes leak from injured cells and the activities of serum enzymes will change. This is because ALT, AST activities in fish serum are known to be very useful as an index for diagnosis of liver function [11]. So the objective of this paper was to characterize the alanine and aspartat transaminase, lipase, glucose and total protein activities and to analyses the *in vitro* effect of mercury on enzyme activity of important economic fish, yellowfin sea bream, to estimate its potential use as a stress biomarker for heavy metals.

MATERIAL AND METHOD

Experimental Design: Forty five yellow fine sea bream (immature males) in same size (120 g final body weight average) were obtained from Mahshahr creeks with hooks in a Upon capture, In laboratory Fish maintained in a seawater re-circulatory system (300-L tanks) equipped with physical/chemical filters and with aeration to the

Mariculture Research Station of the South Iranian Aquaculture Research Center, Mahshahr, Iran. All samples were acclimated for one week. Fish were exposed to nominal mercury concentrations of 0 μg 1 (tank 1), 10 μg 1 (tank 2), 20 μg 1 (tank 3), 40 μg 1 (tank 4), 80 μg 1 (tank 5) and maintained for three weeks with aeration. These sub-lethal doses were chosen on the basis of preliminary toxicity tests and determinations of LC_{50} 96h for this species, suggestive of inducing toxic effects but not lethally so. Conditions within each experimental tank were monitored daily with the temperature 25°C±1, pH 7.8±0.1 and salinity 46±1 ppt under a natural photoperiod (12hL:12hD) in controlled room.

Biochemical Study: Biochemical analyses was done with the automate apparatus Autoanalyser, Metrolab 2300 plus, Argentina (Random Access Clinical Analyzer). Enzyme activity units (IU), defined as micromoles of substrate converted to product at assay temperature per minute, were expressed per liter and mg/dl of serum protein (specific activity). All enzyme assays were performed in duplicate.

Aminotransferases: Both ALT and AST enzymes, were determined with Pars-Azmoon Diagnostics Infinity AST reagent kit (Procedure No. 1 400 018) and Sigma Diagnostics Infinity ALT reagent kit (Procedure No. 1 400 019), respectively by enzymatic methods with a Metrolab 2300 Plus auto-analyze. Results were expressed as units per gram of protein. The AST activity in the serum was estimated by Reitman and Frankel method using Pars Azmoon Kit (1 400 018). The oxaloacetate formed in the reaction is coupled with 2,4-dinitrophenyl hydrazine (DNPH) to give the corresponding hydrazone, which give brown colour in alkaline medium and this is measured colorimetrically at 340 nm and 37 C. A standard curve was obtained using different amounts of pyruvate and enzyme activity was expressed as U/L. The limit of detection (LOD) of the procedure was 2 U/L. Intra-assay and Inter-assay coefficients of variation were of 3.25 and 4.40%, respectively. Intra and Inter-assay Mean±SD were 25.1±0.82 and 25.7±1.13 U/L, respectively.

The ALT activity was also estimated by the method of Reitman and Frankel using Pars Azmoon Kit (1 400 019) with DNPH as colour reagent. Pyruvate formed in the reaction is coupled with 2,4-DNPH to give the corresponding hydrazone, which give brown colour in alkaline medium and this is measured colorimetrically at 340 nm and 37 C. A standard curve was obtained using different amounts of pyruvate and enzyme activity was expressed as U/L. The limit of detection (LOD) of the

procedure was 2 U/L. Intra-assay and Inter-assay coefficients of variation were of 3.25 and 4.40%, respectively. Intra and Inter-assay Mean±SD were 25.1±0.82 and 25.7±1.13 U/L respectively.

Lipase: Lipase activities were determined with Photometric method in Pars-Azmoon Diagnostics Infinity reagent kit (Procedure No. 1 50 24) at 580 nm for detection. The limit of detection (LOD) of the procedure was 3 IU/L.

Protein Analysis: Serum total protein concentrations were determined using Pars Azmoon, Iran (1 500 028) kit, with bovine serum albumin serving as standard by the method of Lowry at 546 nm and 37°C. The limit of detection (LOD) of the procedure was 5 mg/dl. Intra and Inter-assay coefficients of variation were of 0.9 and 1.06% respectively. Intra-assay and Inter-assay Mean±SD were 5.27±0.05 and 5.24±0.06 g/dl respectively.

Statistical Procedures: One-way analysis of variance ANOVA with Tukey or Duncan Post Hoc was used to determine significant differences to evaluate the effect of mercury on parameters. To investigate associations between bioaccumulation and its effects, Pearson correlation coefficients (r) were calculated between mercury concentrations and enzymatic parameters. Multiple regressions were used to determine the relationship between mercury concentration and blood parameters. Regressions follow the model $Y = a \pm bX$; r-correlation coefficient. The differences between means were analyzed at the 5% probability level. Data are reported as means±standard deviation ($\overline{X} \pm SD$).

RESULTS

With respect to raw data, the Kolmogorov-Smirnov normality test was significant at a P<0.05, for all our measured parameters. Results of enzyme and biochemical activity analysis are presented in Table 1. Both ALT and AST activities exhibited significant analysis of variance (P<0.01) with lower considerable values than those of the control group. No significant changes occurred in the activities of the Lipase, however it was decrease too. Values recorded for activity of total protein show significance depletion (P<0.001) with mercury exposed. Although glucose was increase in different treatment, but these changes were not significant.

Fig. 1 presents the *in vitro* enzyme in respect of protein response of yellowfin sea bream exposed to different concentration of mercury chloride. Looking at the differences among blank and treated Fish, it is evident that there is a significant increase (P<0.05) in all the enzymatic activities, However, the increase in AST was not significant.

During *in vitro* results, the correlation between mercury with all pure enzyme and biochemical parameters was statistically tested by analyzing the data obtained during the mercury exposed. Only the Lipase and ALT levels had not statistically significant and other parameter show significant correlation (P<0.05) with mercury exposed, that among all correlation was negative except Glucose (Table 2). Correlation test of same enzyme (Amminotrasaminase) with each other imply that there have not significant correlation, however this correlation was positive.

Table 1: *In vitro* biochemical activities of yellowfin sea bream exposed to mercury chloride

	Control	10 μg l	20 μg l	40 μg l	80 μg l
ALT (U/ L)	6.91±1.2[a]	5.2±0.98[bc]	4.31±0.98[c]	6.88±1.03[a]	6.1±0.73[ab]
AST (U/ L)	135±29.4[a]	111.1±35.9[ab]	97.5±24.7[bc]	67.5±20.48[c]	87.83±20.33[bc]
Lipase (mg/dl)	9.73±1.19[a]	8.93±1.02[a]	9.03±0.96[a]	8.7±1.83[a]	8.45±0.33[a]
Glucose (mg/dl)	66.83±25.89[a]	67.83±3.81[a]	77.33±11.60[a]	92.66±31.72[a]	88.33±29.10[a]
Protein (mg/dl)	7.32±0.90[a]	7.40±1.20[a]	6.29±0.62[a]	5.10±0.78[b]	4.99±1.13[b]

Table 2: *In vitro* correlation of enzyme and biochemical activities of yellowfin sea bream with mercury chloride

	ALT	AST	LIPASE	Protein	Glucose
Pearson correlation (r)	0.12	0.46[*]	0.31	0.67[**]	0.36[*]
sig (p)	0.52	0.01	0.09	0.00	0.04

* Correlation is significant at the 0.05 level, ** Correlation is significant at the 0.01 level

Table 3: *In vitro* curve fit linear regression of enzyme and biochemical activities of yellowfin sea bream with mercury

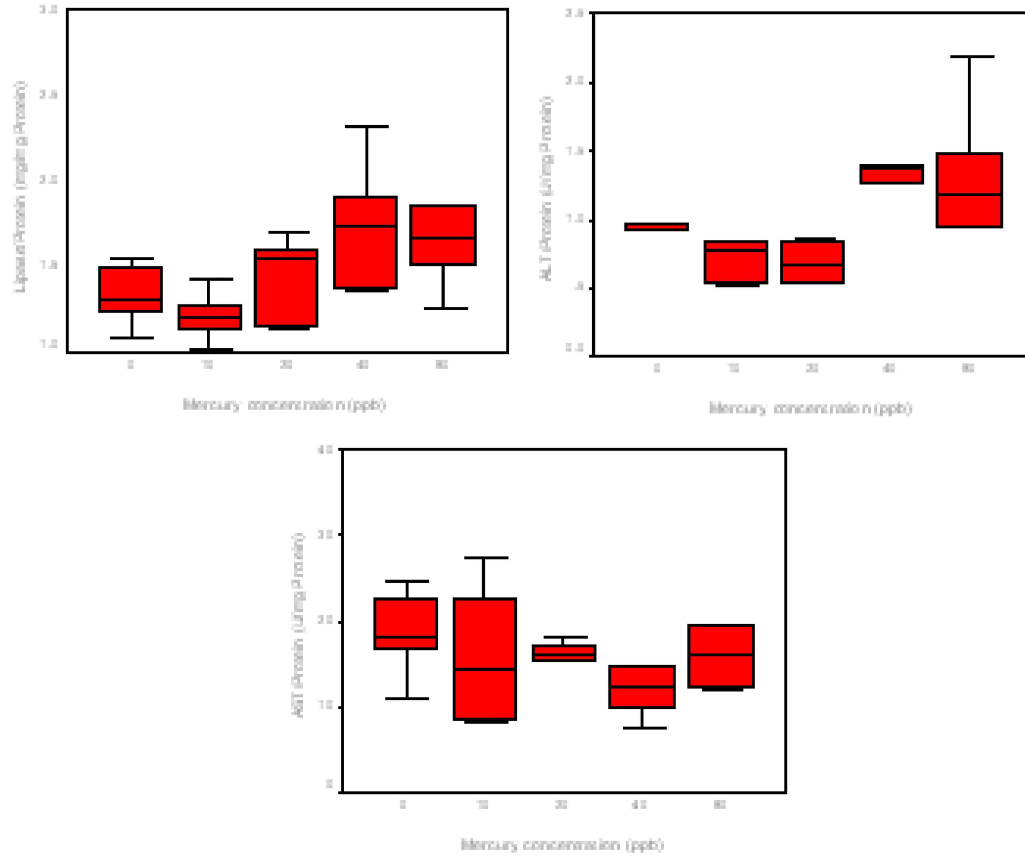|	ALT	AST	Lipase	Protein	Glucose
R square (r^2)	0.011	0.27	0.096	0.45	0.13
F	0.41	7.6	2.99	23.7	4.2
sig (p)	0.52	0.01	0.09	0.00	0.04

Fig. 1: Enzymatic response (ALT, ALT and Lipase) of the yellowfin sea bream during *in vitro* exposed to different concentration of mercury chloride (box plots contain mean and standard deviation). Values of specific enzyme activity are expressed in (U/mg Protein) except Lipase (mg/mg Protein)
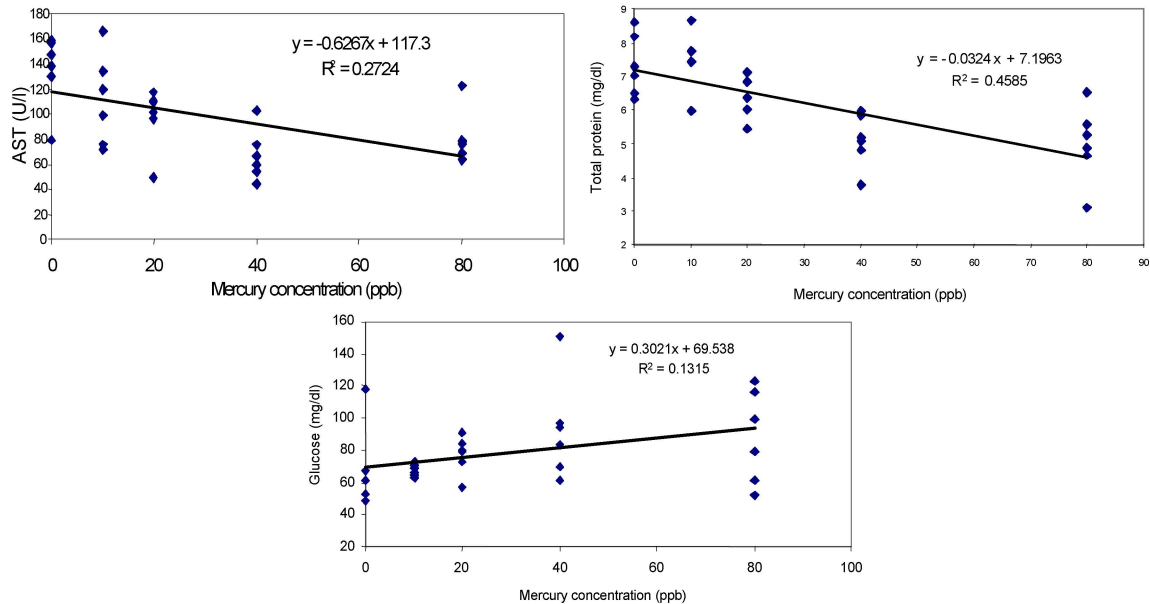

Fig. 2: Regressions model (Y = a±bX) of AST, Total protein and Glucose of the yellowfin sea bream during *in vitro* exposed to different concentration of mercury chloride

Curve estimation regressions data were used to determine the relationship between mercury concentration and pure ALT, AST, Lipase, Protein and Glucose content and enzyme activity. Only the Lipase and ALT levels had not statistically significant and other parameter show significant linear regression (P<0.05) with mercury (Table 3). Regressions model Y = a±bX of significant parameter are in Fig. 2.

DISCUSSION

ALT is an enzymatic stress biomarker and its change identify damages in several tissues and organs of fish [12]. Pacheco Santos [13] reported the ALT activity increase after acute gasoline water-soluble fraction (GWSF) exposure, but He found reduce ALT activity after a longer exposure (6 days) GWSF. Shoba *et al* [14] find serum ALT and AST increase in C. batrachus exposed to trichlorform for 48 h and reducing after 96 h. Serum ALT and AST increased after 96h of clomazone exposure with a little depletion in 192h in silver catfish [15].

Increases in aminotransferase activities were also report after exposure to cadmium for 4 days, however, no elevation in the activity of AST and ALT occurred at the longest exposure concentration [16]. MacInnes *et al.* [17] confirmed that cadmium can inhibit the activity of AST in liver of *autogolabrus adspersus* exposed to Cadmium for 30 days.

As a result, mercury interacts with the biosynthesis of pyridoxal phosphate, a molecule that is an essential requirement for the normal functioning of aminotransferases. With increasing of mercury concentration, the activity of aminotransferases elevated in order to counter the energy crisis during stress, but depletion when high amount of mercury toxicant be accumulated [17]. Therefore based on these studies whereas our study was for 3 weeks exposure, decreased ALT and AST were predicted. ALT and AST depletion constitute a physiological mechanism and may play a role of compensatory mechanism under long time stress in contrary of short time stress.

Due to the fact that proteins are a main part in the metabolism of organisms and mercury may be involved in the normal working of these molecules, it is essential to study the changes in protein metabolism after mercury exposure in further detail. Changes that may occur are the elevate synthesis or breakdown of proteins and the inhibition or activation of certain enzymes. These can be observed as alterations in the total protein content and portion of transaminases such as ALT and AST to protein

[10], so in continuance Portion of ALT and AST to protein were discussed. Our results show a rapid increase after high doses of mercury (20 and 40 ppb).

Exposure of eel to higher dose of deltametrin show elevation in serum AST and ALT [18]. Similar process in transaminase enzymes have been fined in fish exposed to pesticides [19]. Both ALT and AST amounts were elevated by the *Cyprinus carpio* exposure to cadmium [20]. A significant elevation in serum transaminases activities induced by mercury intoxication was reported [21]. Ghorpade *et al.* [22] find that increase of mercury toxicity can leads to elevated AST and ALT activity.

Enhanced ALT and AST activities are indices of initial damage in the liver cells of yellowfin sea bream. The increased levels of aminotransminase enzymes in current study would indicate both injured membrane systems and changes in membrane permeability joint to intracellular metabolism [23]. These disorders seem to be a common effect observed in fish species as consequence of exposure to several contaminants.

Smet and Blust [16] reports elevation of free amino acids (FAAS) accompanied by an elevated activity of AST and ALT in both organs. This indicated that the enhanced protein breakdown is a functional response to deal with the extra energy requirements to cope with mercury stress [24]. The changes in enzyme activities suggest an increased participation of proteins in the energy metabolism in response to an increased energy demand to cope with the stress situation, which is indicative of high protein turnover and amino acid metabolism.

Our finding indicated that the observed proteolysis is intended to increase the role of proteins in the energy production during mercury stress. However, this increased activity of both aminotransferases was not found during exposure to the low dose of mercury concentration, indicating that mercury may also cause an inhibitory effect on the activity of these enzymes above a certain level.

Some researchers report a decrease in total protein content during heavy metal exposure. Such depletions were found in fish *Sarotherodon mossambicus* and the common carp exposed to mercury [10, 24]. Decreased protein content of the *Catla catla* exposed to mercury chloride sub-lethal concentrations were confirmed. Propanil stress in the European eel *Anguilla anguilla* caused protein depletion [25].

The decrease of total protein content may be due to breakdown of protein into free amino acid under the effect of mercury chloride at the lower exposure period [11].

Depletion in protein level in the exposed fish might be due to either arrested metabolism or to use it to build up new cells or enzymes to reduce the stress [26]. The rapid depletion in total protein content was associated with active degradation of proteins under stress. This process is depended to the development of resistance toward to pollutant stress. Proteins being involved in the architecture and physiology of the cell, they seem to occupy a key role in cell metabolism. Catabolism of proteins makes a major contribution to the total energy production in fishes. Under stress situations may constitute a physiological mechanism with an important role in providing energy to cope with the stress situation. So, decrease of total protein content might also be attributed to the destruction or necrosis of cellular function and consequent impairment in protein synthetic machinery [19]. When fish is under pollutant stress, diversification of energy occurs to accomplish the impending energy demands and hence the protein level is decreased [27].

The major findings of this study are that mercury is a toxic substance in yellowfin sea bream, with change enzyme activities in serum of fish exposed to various concentrations. Results of the present study indicated that the sub- acute mercury concentrations tested may cause several changes in the metabolic and enzymatic parameters of the studied fish and we can use these changes as biomarkers of mercury detection. In conclusion, estimation of oxidative stress biomarkers in fish, as in the present study, could provide a useful biomarker of contaminant in marine ecosystems.

ACKNOWLEDGMENTS

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