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Alternations of heat shock proteins (*hsp*70) gene expression in liver and gill of Persian sturgeon (*Acipenser persicus* Borodin, 1987) exposed to cadmium chloride

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Abstract

Induction of heat shock proteins (*hsp*$_s$) is considered as an important protective, ecophysiologically adaptive, and genetically conserved response to environmental stress in all organisms. The effects of exposure to sublethal doses of $CdCl_2$ (0.05, 0.1 and 0.2 LC50) during 14 days on mRNA-*hsp*70 expression in liver and gillwere investigated in juveniles of Persian sturgeon (*Acipenser persicus*). First, a fragment of 726 nucleotides coding for *hsp*70 was cloned from the liver. The result showed that in both study tissues, gill and liver, the relative mRNA-*hsp*70 expression level significantly increased ($p \leq 0.05$) on all study days compared to control group. Relative mRNA-*hsp*70 showed a clear time- dependent response in both tissues following the exposure to $CdCl_2$. A significant increase ($p \leq 0.05$) was observed on the second day and then decreased up to day 7 of the exposure and increase level of mRNA-*hsp*70 expression was observed on day 14. The same trends were observed on all the study doses (0.05, 0.1 and 0.2 LC50), in both examined tissues. Although in this study up expression of *hsp*70 was common to both the gill and the liver, it was significantly ($p \leq 0.05$) expressed more in the liver than the gill. At higher dose, the increase level of mRNA expression was found to be more than that of other experimental doses in all study days. The result revealed that these proteins can be used as a promising biomarker for Cd contamination in this species.

Keywords: Heat shock protein, *Acipenser persicus*, Cadmium chloride, Gene expression

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Introduction

The increasing levels of pollutants including heavy metals, pesticides, petroleum carbohydrate in the Caspian Sea during the last decades, have been reported by Anon, 1998; Pourang *et al.*, 2005. Pollution of aquatic systems with heavy metals such as cadmium is a real threat to the environment due to their persistent nature, long distance transport, and toxicity to aquatic organisms (Huang *et al*., 2005; Jayakumar and Paul, 2006; Ling *et al*., 2009). It is generally believed that fresh water fish mainly accumulate cadmium in gills, liver and kidney (Sorenson, 1991; Reynders *et al*., 2006). The gills are considered to be the most important uptake site for waterborne cadmium, whereas the liver and the kidney are the main storage and detoxification organs in fish (Reynders *et al*., 2006). Exposure of fish to even low concentrations of this metal may lead to an increase in body concentration that can result in several effects, including tissue damage, physiological, biochemical and molecular alterations, respiratory changes, and ultimately death which could be used as environmental bio indicators (Sorensen, 1991; Reynders *et al*., 2006; Kim *et al*., 2008; Oner *et al.,* 2008). Some of these existing validated bioassays, mostly based on lethality or reproduction, have been shown to be inadequate in respect to their sensitivity, the duration and expense of the test. In contrast, changes in biochemical level are usually the first detectable responses to environmental perturbation and on the other hand biochemical and molecular endpoints can provide information on the sub lethal cellular effects of stressors in a particular species of interest, and have the potential to be applied as sensitive biomarkers in field studies to monitor fish health. Heat shock proteins have recently been recognized as being one of the primary defense mechanisms that are activated by the occurrences of denatured proteins in the cell (Ritossa, 1962). *Hsp*S are important chaperone molecules maintain cellular processes such as protein folding, repair and transport (Iwama *et al*., 1998; Feder and Hofmann, 1999). Four major stress-proteins have families of 90, 70, 60 and 16-24 kDa, are the most prominent. In terms of ecotoxicological research, these proteins were measured as an important biomarkers for a wide range of stressors including heavy metals (Williams *et al*., 1996; Duffy *et al*., 1999; Singer *et al.,* 2005), industrial effluent (Janz *et al*., 1997; Virjayan *et al*., 1998), pesticides (Sanders, 1993; Hassanin *et al*., 1999), and polycylic aromatic hydrocarbons (Virjayan *et al*., 1998). Nowadays, the actual mechanisms responsible for cadmium induced regulation of gene expression are better understood than in the past. Several possible mechanisms, including the effects on secondary messengers, such as reactive oxygen species (ROS), intracellular Ca^{2+}, transcription factors, cellular signal transition cascade involving kinases, and DNA –cytosine methylation are considered to be responsible for the cadmium induced deregulation of gene expression

(Liveneh and Fishman, 1997; Joseph *et al*., 2001; Waisberg *et al*., 2003). For studies focusing on where and when the *hsp* gene is expressed quantitative real-time PCR can be used that is based on the labeling of primers, probes, or amplicon with fluorogenic molecules and allows detection of the target fragment to be monitored while the amplification is in progress (Pilar, 2005; Mohammadi *et al*., 2012). Persian sturgeon (*A. persicus*) is one of the most economically important fishes in the Caspian Sea, the stocks of which have declined dramatically mainly due to over fishing, illegal catch, pollution and deterioration of habitats and natural spawning grounds (Pourkazemi *et al*., 2000; Yarmohammadi *et al*., 2014). Therefore, like the other sturgeon, it has been listed in the endangered species of IUCN, CITES (Pourkazemi *et al*., 2000; Yarmohammadi *et al*., 2014). Numerous studies have been conducted on physiological and biochemical effects of some types of contamination on fishes (Hedayati *et al*., 2011;Shariati *et al*., 2011; Jahanbakhshi *et al*., 2012; Hedayati and Jahanbakhshi, 2012; Safari, 2013), however studies on molecular level responses of fishes to contaminant (Toxicogenomic) using qPCR is limited (Gharaei *et al*., 2008). Although *hsp* gene expression were studied extensively in other species, most of data about *hsp* in fishes is obtained from proteomic studies (Singer *et al*., 2005; Dowling *et al*., 2006) and it's gene expression information is restricted and including some studies on *A. transmontanus* (Bruno *et al*., 2006); *Huso huso* (Gharaei *et al*., 2008); *Onchorhyncus mykiss* (Atamanalp and Erdogan, 2010; Ceyhun *et al*., 2010);; *Cyprinus carpio* (Jiang *et al*., 2012; Xing *et al*., 2013);; *Tanichthys albonubes* (Jing *et al*., 2013). As no information is available on the induction of *hsp*70 expression due to exposure of *A. persicus* to contaminations, Therefore, present study was done for the first time with the aims of investigating the effects of sub lethal doses of cadmium chloride on the *hsp*70 gene expression in the gill and the liver of Persian sturgeon, compare *hsp*70 expression in the gill and the liver and evaluate the suitability of Persian sturgeon *hsp*70 as a biomarker of cadmium contamination.

Materials and methods

Individuals of *A. persicus* with the average weight of 3-5 g were obtained from Shahid Marjani Breeding and Rearing Center (Golestan, Iran) in May 2012 and maintained in the center of Aquaculture of Gorgan University to acclimate to laboratory conditions (T~ 23°C; pH~ 7.44; DO~ 7.89 mgO_2 L^{-1}) for two weeks before the experiment. During acclimization, fish were fed with live food (*Artemia* biomass) twice in a day. Fish were randomly distributed into 15 tanks of 300 L, and submitted to 0.05, 0.1 and 0.2 LC50 [LC50 was previously determined as 4000µgl^{-1} (Shariati *et al*., 2011)] $CdCl_2$ (Merck-Germany) dose for 14 days, each tank containing 30 fish. Three

replicate tanks were created for each treatment. During the exposure time, the water was continuously monitored for temperature, dissolved oxygen, pH and conductivity. Two- thirds of the water was renewed every 24 h by adding $CdCl_2$ stock solution to minimize metal loss after feeding and thus reduce contamination of tanks with food remains.

Sampling

Nine fish per treatment were rapidly anesthetized with clove powder (0.5 g/l), the livers and gills were taken from samples, immediately deep-frozen in liquid nitrogen and stored at -80°C freezer until RNA extraction.

RNA isolation and cDNA synthesis

Total RNA isolation was done following BIOZOL Reagent protocol (Bioflux-Bioer, China). The concentration of RNA samples was evaluated by Nanophotometer (IMPLEN-P100) reading at 260/280 nm and integrity was verified by ethidium bromide staining of 28 S and 18 S ribosomal RNA (rRNA) bands on non-denaturing agarose gel (1.5%). DNA of samples was removed by treatment with *DNa*se I (Fermentas, France). Then RNA of 3 samples of each replicate were pooled (Roy and Bhattacharya, 2006; Yarmohammadi *et al.*, 2014). cDNA synthesis was carried on according to the Fermentase protocol. Briefly, 1µg RNA was denatured and annealed at 70 °C for 5 min with 0.2 µl of oligo (dT)20. After chilling on ice, 4 µl 5X reaction buffer, 1 µl Ribo Lock Ribonuclease inhibitor (20U/ µl) and 2 µl 10 Mm dNTP were mixed and incubated at 37 °C for 5 min. This mixture was added to 1 µl *M-MuLV* RT (200U/ µl) in final volume of 20 µl, incubated at 37 °C for 10 min, and then at 42 °C for 60 min, finally at 70 °C to stop the reaction (Fermentase, France).

Primer design

To enable species specific qPCR primers, we first designed primer pairs for the *hsp*70 gene from cross-species comparative alignments of other sturgeon sequences available from Gene bank. For the *hsp*70, full or partial length sturgeon specific sequences were available for *A. sinensis* (GenBank no. JF 267328.1), *A. ruthenus* (JN098420.1), *A. transmontans* (AY8802558), *A. Schrenckii* (JQ991596.2) and *A. baerii* (HM348777.1). Universal primers were designed for *hsp*70 within conserved regions in related species using (Primer3: http://frodo.wi.mit.edu/primer3/).

Persian sturgeon specific sequence was PCR amplified in a 25 µL reaction containing 1 x NH4-based reaction buffer, 2.0 mM MgCl2, 200 µM dNTP mix, 0.2 µM of both forward and reverse primers, 1 u/µL of Taq DNA polymerase (Cinagen) and approximately 4 ng of Persian sturgeon liver cDNA. Amplification was performed in a thermal cycler (PEQ STAR, 96 universal gradient) using standard conditions [5 min at 95 °C, 35 x (30 s at 95 °C, 30 s at primer specific annealing temperature, 45 s at 72 °C),

10 min at 72 °C]. Each PCR reaction was visualized for the amplification of a single product on a 1.5 % agarose gel before being cloned into clone-jet vector and sequenced (Bioneer, South Korea) in both directions. Sequence specificity was confirmed via a comparison of homology to other sturgeon *hsp*70 sequence in the blast data base (http://blast.ncbi.nlm.nih.gov/Blast.cgi). To exclude possibility of genomic DNA contamination, (β *2m* with intron) fragment of 1795 bp primer was designed to span an intron. The sequences of primers, melting temperature, GC content and accession No. are listed in Table 1.

Table 1: Name, sequence, and annealing temperature (T) of primers used in the present study to isolate the partial sequence of the *A. persicus Hsp70* gene and to quantify this gene's transcript abundance through Real-time PCR.

Primer name	Gene name Sequence	Tm	Application	Product length
Ap Hsp70q-PCRF	CGCTGGCCTTAATGTTCTCC	56	discovered	249
Ap Hsp70q-PCRR	GCGCTTGAACTCTGCAATGA	56	discovered	
β- actin q-PCRF	TTGCCATCCAGGCTGTGCT	56	Housekeeping gene	215
β- actin q-PCRR	TCTCGGCTGTGGTGAA	56	Housekeeping gene	
β*2m* with intronF	GGGACTTTCCACAAAGGCTA	61	Determining of DNA contamination	1795
β*2m* with intronR	CGACTGATGCTACCGGAACT	61	Determining of DNA contamination	

Real-time PCR

Real-time Quantitative PCR was performed using SYBR Green I technology on iQ5 System (BioRad, USA). The reactions consisted of 1x SYBR Green PCR Master Mix (SYBR biopars, GUASNR, Iran), 300 nm of each specific forward and reverse primers, 10 ng of cDNA template, and nuclease free water to a final volume of 20 μL. PCR reaction mixtures were subjected to the following thermal profile: 94°C during 5 min, 40 cycles at 10s at 94°C followed by 10s at 56°C and 10s at 72°C (Table 1) and each reaction was amplified in triplicate. To validate real-time PCR primers, each gene's specific primer pair was run in duplicate, along a temperature gradient (55-65ºC) in the same plate. The primer specificity assay revealed that at 56ºC the hsp70 and β- actin had low affinity for non-specific product. A melting curve analysis was performed after every amplification program to verify specificity of target and the absence of primer dimmers and a no template control (NTC) was included with each assay to verify that PCR master mixes were free of contamination. To ensure that PCR conditions were optimal, a $\log_{10}$ dilution series was produced from

undiluted cDNA pooled together from randomly selected treatments, used to generate a standard curve. The Standard curve was used to estimate efficiency (*E*) and reproducibility of the assay and was run in triplicate on each PCR. Reproducibility was represented by the R^2 value of standard curve and was greater than 0.95.

Statistical analysis

Relative gene expression was calculated by Pfaffl formula (Pfaffl *et al*., 2002). The ratio between the target (*hsp*70) and housekeeping (β- actin) genes was analyzed by the REST software (Pfaffl *et al*., 2002). A Kolmogorov-Smirnov test was used to assess the normality of distributions. Normalized gene expression data passed Levene's test for homogeneity of variance. Statistics data were Subjected to one way ANOVA with $\alpha=0.05$. Comparisons within each analysis day and within a treatment at different sampling days were performed by Duncan's test. SPSS program ($\alpha=0.05$), version 16 (SPSS, Richmond, Virginia, USA), was used as described by Dytham (1999). Expression difference between study tissues was followed by t- test and $p\leq0.05$ was considered statistically significant.

Results

A fragment of 726 nucleotides coding for *Ap hsp*70 was cloned from liver and deposited into Genebank under accession number KF000408.1. Alignment of nucleotide sequence of *A. persicus* with other representative species can be observed in Fig. 1.

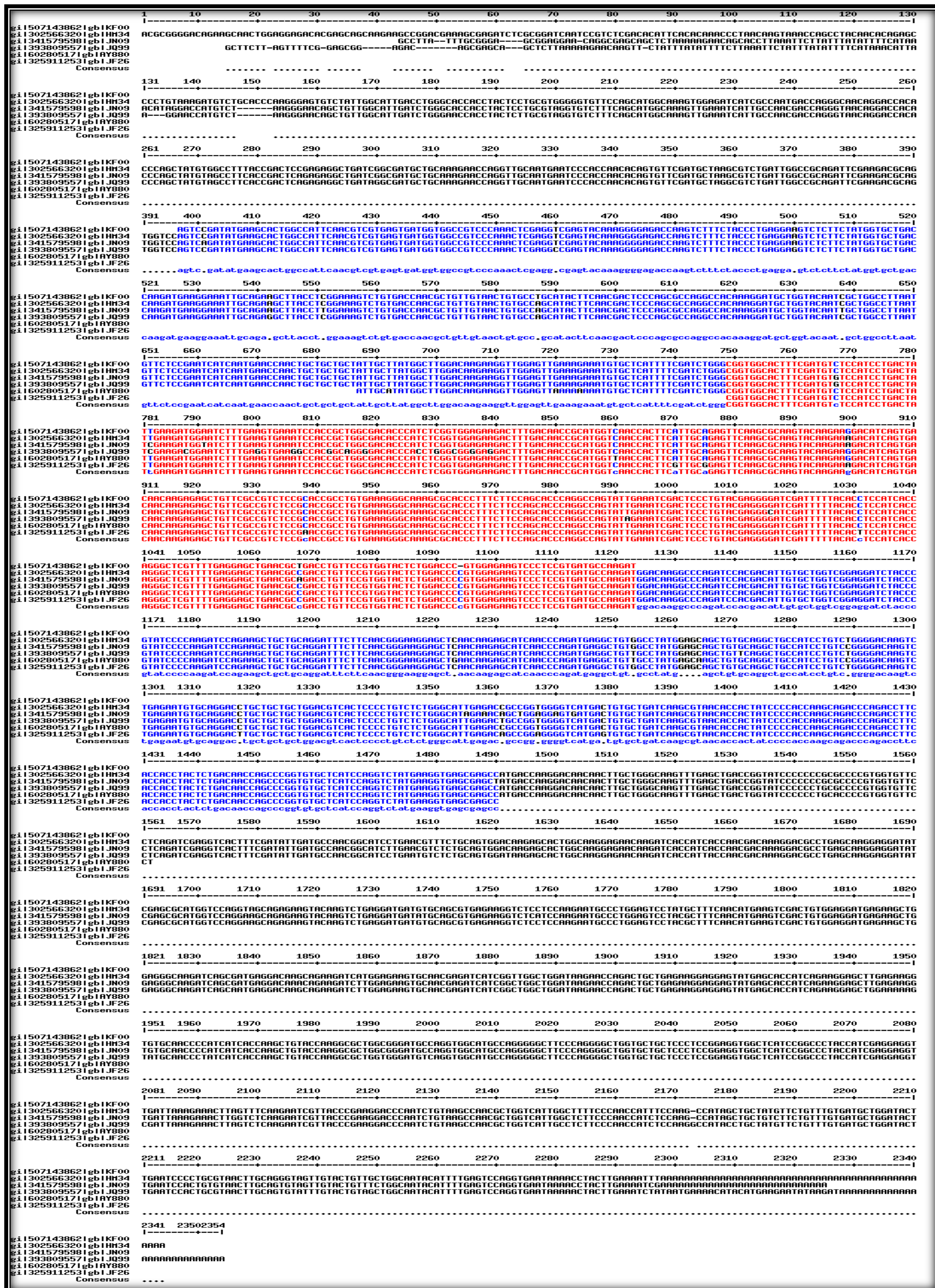

Figure1: Alignment of nucleoetide sequence of *A. persicus*(GenBank no. KF000408.1) with *A. sinensis* (JF 267328.1), *A. ruthenus* (JN098420.1), *A. transmontans* (AY8802558), *A. schrenckii* (JQ991596.2), and *A. baerii* (HM348777.1) using MultAlin software.

Relative up expression of *Aphsp*70 compared to control was observed in both the gill and liver in all the study doses during the $CdCl_2$ exposure time.

Time- dependent profile of Ap hsp70 mRNA expression

In the gill, in 0.05 LC50, the relative expression was the highest on day 2 during exposure time, however, there wasn't significant difference ($p>0.05$) among study days. In 0.1 LC50, the amount of relative expression increased significantly (2.39- fold, $p\leq 0.05$) on day 1 compared to control and reaching the maximum (3.4- fold, $p\leq0.05$) on day 2 of exposure and then decreased significantly (1.9- fold, $p\leq0.05$) on day 7. However the expression increased significantly (2.9- fold, $p\leq0.05$) on day 14 of exposure. The similar trend (4.5- fold, $p\leq0.05$) on day 1, (6.3- fold, $p\leq0.05$) on day 2, (1.7- fold, $p\leq 0.05$) on day 7 and (4.7- fold, $p\leq0.05$) on day 14 was also observed in 0.2 LC50 (Table 2).

In the liver, in 0.05 LC50, the relative expression of *Ap* hsp70 significantly increased (2.9- fold, $p\leq0.05$) on day 1 compare to control and reached the highest (4.4- fold, $p\leq0.05$) on day 2 of exposure which was not significant, and then decreased (3.8- fold, $p>0.05$) on day 7. However, the expression increased (4- fold, $p>0.05$) on day 14 of exposure. In 0.1 LC50, the amount of relative expression significantly increased (5- fold, $p\leq0.05$) on day 1 compared to control and reached the maximum (5.4- fold, $p>0.05$) on day 2 of exposure nonsignificantly and then decreased significantly (4- fold, $p>0.05$) on day 7. However the expression increased nonsignificantly (4.5- fold, $p>0.05$) on day 14 of exposure. The similar trend (5.2- fold, $p\leq0.05$) on day 1, (7.5- fold, $p\leq0.05$) on day 2, (4.1- fold, $p\leq0.05$) on day 7 and (6.36- fold, $p\leq0.05$) on day 14 were also observed in 0.2 LC50 (Table 3).

Table 2: Alternation in the relative mRNA levels to β- actin activity in the liver of *A. persicus* exposed to 0.05, 0.1 and 0.2 LC50 of cadmium chloride ($CdCl_2$) for 14 days.

Concentration/day	Day1	Day2	Day7	Day14
0.05 LC50	$2.9\pm0.34^{b}_{B}$	$4.4\pm0.6^{a}_{B}$	$3.8\pm0.88^{ab}_{A}$	$4\pm0.2^{ab}_{B}$
0.1 LC50	$5\pm1.1^{ab}_{AB}$	$5.4\pm0.6^{a}_{B}$	$4\pm0.2^{b}_{A}$	$4.5\pm0.5^{ab}_{B}$
0.2 LC50	$5.2\pm0.95^{bc}_{A}$	$7.5\pm0.56^{a}_{A}$	$4.1\pm0.76^{c}_{A}$	$6.36\pm1.51^{ab}_{A}$

Results are expressed as means with standard deviation (n=3). Different superscript and subscript letters characterize significant ($p\leq0.05$) difference in each column (A-B) and each row (a-c), respectively

Table 3: Alternation in the Relative mRNA levels to β- actin activity in the Gill of *A. persicus* exposed to 0.05, 0.1 and 0.2 LC50 of cadmium chloride ($CdCl_2$) for 14 days.

Concentration/day	Day1	Day2	Day7	Day14
0.05 LC50	$1.7±0.25^{a}_{B}$	$2.1±0.2^{a}_{C}$	$2±0.22^{a}_{A}$	$1.7±0.35^{a}_{C}$
0.1 LC50	$2.39±0.11^{c}_{B}$	$3.4±0.12^{a}_{B}$	$1.9±0.17^{d}_{AB}$	$2.9±0.24^{b}_{B}$
0.2 LC50	$4.5±0.55^{b}_{A}$	$6.1±0.3^{a}_{A}$	$1.7±0.2^{c}_{B}$	$4.7±0.3^{b}_{A}$

Results are expressed as means with standard deviation (n=3). Different superscript and subscript letters characterize significant ($p≤0.05$) difference in each column (A-C) and each row (a-d), respectively

Dose- dependent profile of Ap hsp70 mRNA expression

In the gill, on the first day of exposure, there wasn't any significant differences in the relative expression of *Aphsp*70 between 0.05 and 0.1 LC50 (1.7- and 2.39- fold, $p>0.05$) respectively, but an increase in 0.2 LC50 (4.5- fold, $p≤0.05$). On the second day the relative expression of *Aphsp*70 increased significantly in different doses (2.1-, 3.4- and 6.1- fold, $p≤ 0.05$). On day 7 of exposure significant difference was observed between 0.05 and 0.2 LC50 ($p≤0.05$) on day 14, the relative expression of *Aphsp*70 increased significantly in different doses in the similar way (1.7-, 2.9- and 4.7- fold, $p≤0.05$). The same trend was observed in the liver, however, the significant increase was observed in the *Aphsp*70 expression between 0.05 and 0.2 LC50 on day 2 (4.4-, 5.4- and 7.5- fold, $p≤0.05$) and the significant difference was found between 0.1 LC50 (4- fold, $p≤0.05$) and the other two (3.8 and 4.1) (Tables 2, 3).

Tissues disturbance of Ap hsp70

*Aphsp*70 expression in the liver was significantly ($p≤0.05$) higher than the gill during the exposure time in all the examined doses except for day 1, 2, 14 in 0.2 LC50 and day 14 in 0.1 LC50 (Fig. 2).

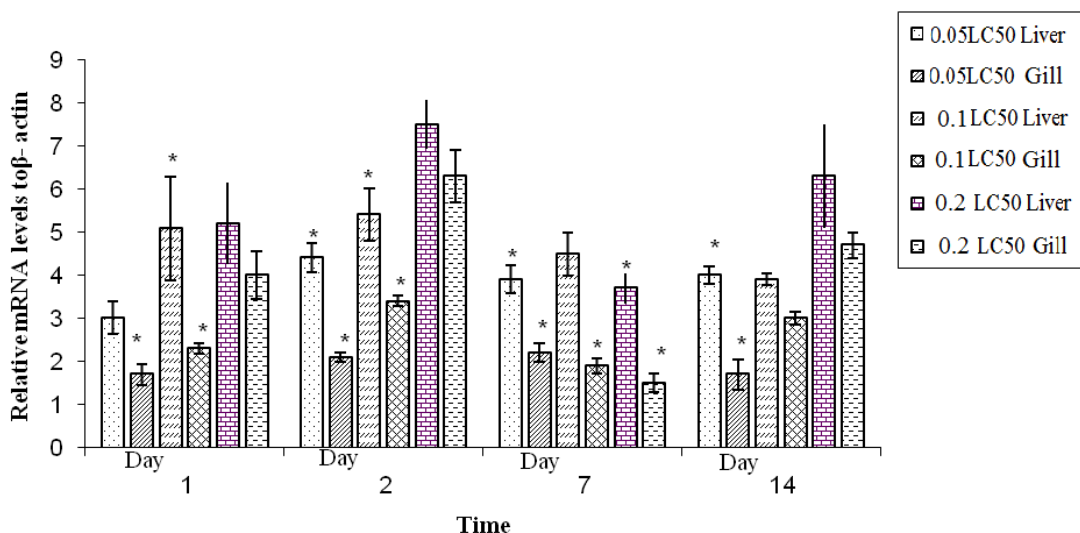

Figure 2: Alternation in the relative mRNA levels to β- actin activity in the gill and liver of *A. persicus* exposed to 0.05, 0.1 and 0.2 LC50 of cadmium chloride (CdCl2) for 14 days (p<0.05).*: sig (p≤0.05).

Discussion

The vast number of chemicals entering the environment either through industrial or other activities has direct or indirect impacts on the environment. Although examinations of the effects of these chemicals on biological system were assessed by behavioural, histopathological and biochemical analysis, evaluating of gene expression can be of predictive value if the target molecule is part of the defense, repair or detoxification machinery of the cell. Given the responsiveness to even minor assauls, the expression of hsp_s may prove useful as a molecular indicator of adverse biological conditions (Hassanein *et al*., 1999; Koehler *et al*., 2001; Varo *et al*., 2002). Among the various *hsp* isoforms, *hsp*70 is often the prominent protein to be expressed following environmental assaults (Iwama *et al*., 1998; Kohler *et al*., 2001). It is demonstrated that the intensity of the *hsp*70 response is not only dependent on the type of pollutant, species, duration, concentration, but also organ-specific (Radlowska and Pempkowiak, 2002; Singer *et al*., 2005; Lee *et al*., 2006; Rhee *et al*., 2009; Ceyhun, 2010; Bao, 2011). In this investigation, changes in *hsp*70 mRNA expression in the liver and gill affected by $CdCl_2$, revealed that this protein can be used as a promising biomarker for Cd contamination in this species. It appears that exposure to cadmium results in increased intracellular Ca^{2+} level (Joseph *et al*., 2001). The cadmium- induced increase in intracellular Ca^{2+} concentration may deregulate the expression of genes directly by allowing excess Ca^{2+} to intract with specific response elements such as the cAMP- response element binding protein (CREB) that are present

in the promoter/ enhancer regions of relevant genes. The effects of cadmium could be indirectly mediated through the activation of protein kinases that cause gene overexpression through phosphorylation (Weisberg *et al*., 2003). Alternations in heat-shock protein expression in response to environmental contaminants were observed in a variety of fish species for evaluating the extent to which an organism is stressed (Geist *et al*., 2007; Rhee *et al*., 2009; Eder *et al*., 2009; Monari *et al*., 2011; Simpkin *et al*., 2013). The studies showed that while heavy metals, invariably caused an up regulation of *hsp*70 expression in broad range of organism, organic chemicals such as Carbon tetrachloride, Fenitrothion and Nonylphenol had differential responses and caused a decrease in *hsp*70 expression (Rhee *et al*., 2009). In the present study the transcriptional expression of *hsp*70 showed a clear time- dependent response in both the gill and liver after the fish were exposed to $CdCl_2$. Although the relative mRNA-*hsp*70 level showed an increase in all the study days compared to control, higher up regulation was observed on the second day. The lower expression on the first day may be attributed to cortisol- *hsp* interaction. The increase in the amount of cortisol on the first day of exposure was observed in simultaneous study on this species (Safari *et al*., 2013). Modulating activity of cortisol on the hsp expression was also noted by Celi *et al*. (2012) in the sea bass head, kidney and isolated leukocytes. A significant increase of *hsp*70 expression ($p\leq0.05$) was observed on the second day that revealed the role of *hsp* as molecular chaperons. By temporarily binding to hydrophobic residues, *hsp*70 prevents these partially denatured proteins from aggregating and allows them to refold. *Hsp*70 may also be one of the early genes that activates late response genes (Weisberg *et al*., 2003). The observed decrease on day 7 may be due to the gene silencing arising from necrosis of the gill and liver tissues. Symptom of necrosis of gills was also reported in simultaneous histopathological study on those samples, on day 7 but the liver wasn’t survived (Safari *et al*, 2013). The decrease in *hsp* expression on day 14 compared to day 2, while it is still higher than the control group might be as a consequent of adaptation to stress. Laboratory studies demonstrate the persistence of increased *hsp* expression long after the removal of the stressor, implying that hsps may be involved in the adaptation of fish to environmental changes (Basu *et al*., 2002; Eder *et al*., 2007). The same trends in *hsp*70 gene expression were observed in all study doses (0.05, 0.1 and 0.2 LC50). Our finding is in agreement with the result of Shariati *et al* (2010) who noted the increase of methallothionein protein in day 4 of exposure to Cd in the similar condition in the liver of *A. persicus*, but a simultaneous study of these two proteins is necessary for better understanding. Similar biphasic

response of the *hsp* induction was also observed in different studies. The moment, rapidity and grade of *hsp* expression is dependent on the xenobiotic (Sander, 1993; Cui *et al*., 2010), tissue (Airaksinen *et al*., 2003; Roy and Bhattacharya, 2006) and species (Piano *et al*., 2004; Fu *et al*.,2011). In the study of Singer *et al* (2005) the *hsp* level in Zebra muscle exposed to palatinum was low on the first 2-3 weeks, but it increased strongly until the sixth week and declined afterwards which can be explained by the adaptation process of muscle to the metal accumulation. In present study, up expression of *hsp* was observed in both the gill and liver from the first day. Since the earlier expression of hsp_s was reported in many studies (Cui *et al*., 2010; Fu *et al*., 2011; Xing *et al*., 2013), for determination of the exact time of alternation in mRNA-*hsp*70 expression, sampling should be done earlier, and in shorter intervals at the beginning of the experiment in future studies. Although, in this study up expression of *hsp* was common to both the gill and liver, it was significantly ($p \leq 0.05$) more expressed in the liver than the gill, which could be attributed to suitable condition in the metabolic tissue of liver for protein kinase that activates *hsp* expression. Geist *et al*. (2007) in a study of striped bass (*Morone saxtalis)* exposed to Esfenvalerate for 24 hours, found the altered *hsp*70 and *hsp*90 transcription in the liver and spleen but not in the muscle, gill or kidney. Eder *et al*. (2009), in their study of *hsp* responses in the liver and muscle of chinook salmon (*O.tshawytscha*) exposed to neurotoxic insecticide observed more sensitivity to chlorpyrifos in the muscle responding and more sensitivity to esfenvalerate in the liver and confirmed that *hsp* expression in these tissues can be useful and a highly sensitive indicator of sub lethal cellular responses to contaminant exposure. Cui *et al*. (2010) in study of swimming carb (*Portunus trituberculatus*) found maximum levels of *hsp* in the gill and referred it to its direct connection to environment. The significant increase in *hsp*70 expression in specific organs such as the liver or gill and not detecting it in the other organs could be attributed to severe sensitivity of this organ to contaminants, leading to impairment of cellular processes including protein biosynthesis and subsequent necrosis (Bradbury *et al*., 1987; Dutta *et al*., 1993, 1998; Rao *et al*., 2003). It seems that the difference between the expression profiles in different tissues is possibly due to inherent difference of various tissues (Fu *et al*., 2009; Rajeshkumar and Munuswamy, 2011). The study presented here demonstrated that the intensity of *hsp* response is dose-dependent. At higher dose, the increase level of mRNA expression was found to be more than that of other experimental doses in all the study days. The up expression of *hsp*70 observed in our research is associated with the protective function of *hsp*70 that is related to its ability to promote folding of nascent polypeptides and to remove

denatured proteins. Our findings are in agreement with Lee *et al*. (2006) who reported cadmium and chromium induced concentration- dependent increases in *hsp* mRNA (6-, 9- and 16-fold expression at 0.2, 2 and 20 mg/L of exposure to Cd and 1.5-, 1.8- and 2.1-fold expression at 0.075, 0.75 and 7.5 mg/L of exposure to Cr). Jiang *et al*. (2012) found up expression of *hsp*70 with the increase of MC-LR concentrations and reached a maximum at 5 µg/L compared to 1 and 0.1 µg/L. Jing *et al*. (2013) also observed a dose-dependent expression pattern in the liver of *T. albonubes* responded to 0.0135mg/L and 0.027mg/L of copper and 2.31 mg/L and 1.15 mg/L of cadmium for 96h and noted an integral role of hsp_s in cross-protection/cross-tolerance, a process that may condition fish to better tolerate a subsequent, more severe stressor in a highly complex environment, and reported that *hsp* has potential to monitor the chronic stressors in culture environments. Conclusions the present findings indicate that the response in Persian sturgeon tissue to uptake and exposure to Cd is manifested by increased *hsp* expression in the liver and gill. But the up expression in the liver was more than the gill. *Hsp*70 may be considered as a sensitive biochemical indicator in ecotoxicological studies.

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References

Airaksinen, S., Raberg, C.M.L., Lahti, A., Kaatrasalo, A., Sistonen, L. and Nikinmaa, M., 2003. Stressor-dependent regulation of the heat shock response in zebra fish, *Danio rerio*. *Comparative Biochemistry and Physiology*, 134, 839-846.

Anon.,1998. Caspian Sea environment national report of I. R. Iran, Department of environment, 129P.

Atamanalp, M. and Erdogan, O., 2010. Alternations of hsp70 gene expression in rainbow trout (*Oncorhyncus mykiss*) exposed to deltamethrin. *Turkish Journal of Veterinary Animal Science*, 34(**4**), 359-363.

Bao, Y., Wang, Q., Liu, H. and Lin, Z., 2011. A small HSP group gene of bloody clam (*Tegillarca granosa*) involved in the immune response against *vibrio parahaemolyticus* and lipopolysaccaride. *Fish and Shellfish Immunology,*30, 729-733.

Basu, N., Todgham, A.E., Ackerman, P.A., Bibeau, M.R., Nakano, K., Schulte, P.M. and Iwama, G.K., 2002. Heat shock protein genes and their functional significance in fish. *Gene*, 295, 173-183.

Bradbury, S.P., McKim, J. M.Coats, J.R., 1987. Physiological responses of rainbow trout (*Salmo gairdneri*) to acute fenvalerate intoxication.

Pesticide Biochemistry Physic, 27, 275-288.

Bruno, J., Osachoff, H. and Skirrow, R., 2004. Gene expression profiles of Columbia River white sturgeon exposed to two industrial effluents. Sturgeon Contaminants Working Group. 21P.

Bustin, S.A., Bens, V., Garson, J.A., Hellemans, J., Huggett, J., Kubista, M.,Mueller, R., Nolan, T.,Pfaffl, M.W., Shipley, G.L., Vandesompele, J. and Wittwer, C. T., 2009. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clinical Chemistry*, 55(**4**), 611-622

Celi, M., Vazzana, M., Sanfratello, M. A. and Parrinello, N., 2012. Elevated cortisol modulate Hsp70 and Hsp90 gene expression and protein in sea bass head kidney and isolated leukocytes. *General and Comparative Endocrinology*, 175(**3**), 424-431.

Ceyhun, S.B., Senturk., M., Ekinci, D., Erdogan, O., Ciltas, A. and Kocaman, E.M., 2010., Deltametrin attenuates antioxidant defense system and induces the expression of heat shock protein 70 in rainbow trout. *Comparetive Biochemistry and Physiology*, 152, 215-223.

Cui, Z., Liu, Y., Luan, W., Li, Q., Wu, D. and Wang, Sh., 2010. Molecular cloning and characterization of a heat shock protein 70 gene in swimming crab (*Portunus trituberculatus*). *Fish and Shellfish Immunology*,28, 56-64.

Dytham, C., 2011. Choosing and Using Statistics: A Biologist's Guide. Third Edition.320P.

Duffy, L.K., Scofiled, T., Patton, M. and Bowyer, R.T., 1999. Comparative baseline levels of mercury, hsp70 and hsp90 in subsistence fish from the Yukon-Kushkokwim delta region of Alaska. Comparative Biochemistry and Physiology. *Pharmacology, Toxicology and Endocrinology*, 124 (**2**), 181-186

Dutta, H.M., Richmonds, C.R. and Zeno, T., 1993. Effects of diazinon on the gills of bluegill sunfish *Lepomis macrochirus*. *Journal of Environmental Pathology and Toxicology*, 12, 219-227.

Dutta, H.M., Roy, P.K., Singh, N.K., Adhikari, S. and Munshim, J.D., 1998. Effect of sublethal levels of malathion on the gills of *Heteropneustes fossils*: Scanning electron microscopic study. *Journal of Environmental Pathology and Toxicology*, 17, 51-63.

Eder, K.J., Kohler, H.R. and Werner, I., 2007. Pesticide and phathogen: Heat shock protein expression and acetylcholinesterase inhibition, in juvenile Chinook salmon in response to multiple stressors. *Environmental Toxicology and Chemistry*, 26, 1233-1242.

Eder, K.J., Leutenegger, C.M., Kohler, H.R. and Werner, I., 2009. Effect of neurotoxic insecticides on heat shoch protein and cytokine transcription in Chinook salmon (*Onchorhyncus tshawytscha*).

Ecotoxicology and Environmental safety, 72, 182-190.

Feder, M.S. and Hofman, G.E., 1999. Heat shock proteins, molecular chaprones, and the stress responses: Evolutionary and ecological physiology. In: Hoffman, J.F. Annual Review of Physiology, 61. Annual Review Inc., Palo Alto, CA, USA, pp. 243-282.

Fu, D., Chen, J., Zhang, Y. and Yu, Z., 2011. Cloning and expression of a heat shock protein 90 gene in the haemocytes of *Crassostrea hongkongensis* under osnotic stress and bacterial challenge. *Fish and Shellfish Immunology*, 31, 118-125.

Geist, J., Werner, I., Eder, K.J. and Leutenegger, C.M., 2007. Comparisons of tissue-specific transcription of stress response genes with whole animal endpoints of adverse effect in striped bass (*Morone saxatilis*) following treatment with copper and esfenvalerate. *Aquatic Toxicology*, 85, 28-39.

Gharaei, A., Esmaili-Sari, A., Jafari Shamoshaki, V. and Ghaffari, M., 2008. Beluga (*Huso huso*, Brandet 1869) bioenergetics under dietary methylmercury. *Fish Physiology and Biochemistry,*34, 473-482.

Hassanein, H.M.A., Banhawy, M.A., Soleiman, F.M., Abdel-Rehim, S.A., Muller, W.E.G. and Schroder, H.C., 1999. Induction of hsp70 by the herbicide oxyfluorfen (Goal) in the Egyptian Nile Fish, *Oreochromisniloticus*. Arch. *Environmental Contamination Toxicology*, 37, 78-84.

Hedayati, A. and Jahanbakhshi, A., 2012. The effect of water-soluble fraction of disel oil on some hematological indices in the great sturgeon *Huso huso*. *Fish Physiology and Biochemistry*, 34, 473-482.

Hedayati, A. and Safahieh, A., 2011. Serum hormone and biochemical activity as biomarkers of mercury pollution in yellowfin seabream *Acanthopagrus latus*. *Toxicology and Industrial Health*, 28, 306-319.

Huang, H.Q., Xiao, Z.Q., Lin, Q.M.., Chen, P., 2005. Characteristics of trapping various organophosphorus pesticides with a ferritin reactor of shark liver (*Sphrna zygaena*). *Animal chemistry*, 77, 1920-1927.

Iwama, G.K.,Thomas, P.T., Forsyth, R.B. and Vijaian, M.M., 1998. Heat shock protein expression in fish. *Reviews in Fish Biology and Fisheries*, 8, 35-56.

Iwama, G.K., Takemura, A. and Takano, K., 1999. Oxygen consumption rates of tilapia in fresh water, sea water, and hypersaline sea water. *Journal of Fish Biology*, 51, 886-894.

Jahanbakhshi, A.R. Shaluei, F. and Baghfalaki, M. 2012. Acute toxicity of cypermethrin on the great sturgeon (*Huso huso*) juvenile. *World journal of fish and marine science*, 4(**2**), 170-174.

Jayakumar, P. and Paul,V.I., 2006. Pattern of cadmium accumulation in selected tissues of the cat fish

Clarias batrachus (Linn.) exposed to sublethal concentration of cadmium chloride. *Veterinarski Arhiv*, 76(**2**), 167-177.

Jiang, J., Shi, Y., Shan, Z., Yang, L., Wang, X. and Shi, L., 2012. Bioaccumulation, oxidative stress and hsp70 expression in *Cyprinus carpio* L. exposed to microcystin-LR under laboratory conditions. *Comparative Biochemistry and Physiology*,155(**3**), 483-90.

Jing, J., Liu, H., Chen, H., Hu, S., Xiao, K. andMa, X., 2013. Acute effect of copper and cadmium exposure on the expression of heat shock protein 70 in the Cyprinidae fish *Tanichthys albonubes*. *Chemosphere*, 91(**8**), 1113-1122.

Joseph, P., Muchnok, T.K., Klishis, M.L., Roberts, J.R., Antonini, J.M., Whong, W.Z. and Ong, T., 2001. Cadmium-induced cell transformation and tumorigenesis are associated with transcriptional activation of c-fos, and c-jun, and c-myc protooncogenes: Role of cellular calcium and reactive oxygen species. *Toxicology Science*, 61, 295-303.

Kim, S.G., Park, D.K. Jange, S.W. Lee, J.S., Kim, S.S. and chung, M.H., 2008. Effects of dietary benzo[a]pyrene on growth and hematological parameters in juvenile rockfish, *Sabates schlegeli* (Hilgendrof). *Bulltin of Environmental Contamination and Toxicology*, 81, 470-474.

Koehler, H.R., Bartussek, C., Eckwert, H., Farian, K., Graenzer, S., Knigge, T. and Kuunz, N., 2001. The hepatic stress protein (hsp70) response to interacting abiotic parameters in fish exposed to various levels of pollution. *Journal of Aquatic Ecosystem Stress Recovery*, 8, 261-279.

Lee, S.M., Lee, S.B., Park, C.H. and Choi, j., 2006. Expression of heat shock protein and hemoglobin genes in *Chironomus tentas* (Diptera, Chironomidae) larvae exposed to various environmental pollutants: A potential biomarker of fresh water monitoring. *Chemosphere*, 65, 1074-1081.

Ling, X.P., Zhu, J.Y., Haung, L. and Huang, H.Q., 2009. Proteomic changes in response to acute cadmium toxicity in gill tissue of *Paralichthys olivaceus. Environmental Toxicology and Pharmacology,* 27, 21-218.

Livneh, E. and Fishman, D.D., 1997. Linking protein kinase Cto cell cycle control. *European Journal of Biochemistry*, 248, 1-9.

Mohammadi, M., Ramezanpour, S.S., Navabpour, S., Soltanloo, H., Kia, SH. and Kalateh Arabi, M., 2012. Study on expression pattern of chalcone synthase and -1,3-glucanase under *Septoria tritici* treatment in wheat by quantitative real time PCR. *American-EurasianJournal ofAgriculture and Environmental Science*, 12(**11**), 1431-1436.

Monari, M., Foschi, J., Rosmini, R., Marin, M.G. and Serrazanetti, G.P., 2011. Heat shock protein 70

response to physical and chemical stress in *Chamelea gallina. Journal of Experimental Marine Biology and Ecology*, 397, 71-78.

Oner, M., Atli, G. and Canli, M., 2008. Changes in serum biochemical parameters of freshwater fish *Oreochromis niloticus* following prolonged metal (Ag, Cd, Cr, Cu, Zn) exposures. *Environmental Toxicology and Chemistry*, 27(**2**), 306-366.

Pfaffl, M.W., Horgan, G.H. and Dempfle, L., 2002. Relative expression software tool (REST) for group wide comparison and statistical analysis of relative expression results in real-time PCR. *Nucleic Acid Research*, 30(**9**), e36.

Piano, A., Valbonesi, P. and Fabbri, E., 2004. Expression of cytoprotective proteins, heat shock protein 70 and metallothioneins, in tissues of *Ostrea edulis* exposed to heat and heavy metals. *Cell Stress Chaperon*. 9, 134-142.

Pilar, H.M., 2005. Hsp70 RNA expression in rainbow trout (*Oncorhynchus mykiss*) clonal lines. M.Sc. thesis in environmental science. Washington State University. 50P.

Pourang, N., Tanabe, S., Rezvani, S. and Dennis, H., 2005. Trace elements accumulation in edible tissues of five sturgeon species from the Caspian Sea. *Environmental Monitoring and Assessment*, 100, 89-108.

Pourkazemi, M., Skibinski, D.O.F., andBeardmore, J.A., 2000. A preliminary study on phylogenetic relationship between five sturgeon species in Iranian coastline of the Caspian Sea. *Iranian Scientific Fisheries Journal*, 2(**1**), 1-12.

Radlowska, M. and Pempkowiak, J., 2002. Stess-70 as indicator of heavy metals accumulation in blue mussel *Mytilus edilus*. *Environment International*, 27, 605-608.

Rajeshkumar, S. and Munuswamy, N., 2011. Impact of metals on histopathology and expression of HSP 70 in different tissues of Milk fish (*Chanos chanos*) of Kaattuppalli Island. *Chemosphere*, 83(**4**), 415-421.

Rao, J.V., Rani, C.H., Kavitha, P., Rao, R.N. and Madhavendra, S.S., 2003. Toxicocity of chlorpyrifos to the fish *Oreochromis mossambicus. Bulletin of Environmental Contamination and Toxicology*, 70, 985-992.

Reynder, H., Campenhout, K.W., Bervoet, L., Coen, W.M.D. and Blust, R., 2006. Dynamics of cadmium accumulation and effects in common carp (*Cyprinus carpio*) during simultaneous exposure to water and food (*Tubifex tubifex*). *Environmental Toxicology and Chemistry*, 25(**6**), 1558-1567.

Rhee, J.S., Raisuddin, S., Lee, K.W., Seo, J.S., Ki, J.S. and Kim, I., 2009. Heat shock protein (Hsp) gene responses of intertidal copepod *Tigriopus japonicus* to

environmental toxicants. *Comparative Biochemistry and Physiology*, 149, 104-112.

Ritossa, F., 1962. A new puffing pattern induced by heat shock and DNP in Drosophila. *Experintia*, 18, 571-573

Roy, S. and Bhattacharya, S., 2006. Arsenic-induced histopatology and synthesis of stress proteins in liver and Kidney of *Channa punctatus*. *Ecotoxicology and Environmental Safety*, 65, 218- 229.

Safari, R., Shabani, A., Ramezanpour, S. and Imanpour, M.R., 2013. Effects of sub lethal doses of $CdCl_2$ on histopathological and biochemical parameters of Persian sturgeon (*Acipenser persicus*). *International Research Journal of Applied and Basic Science*, 5,650-657.

Sanders, B.M., 1993. Stress proteins in aquatic organisms: an environmental perspective. *Critical Reviews in Toxicology*, 23, 49-75.

Sassi, A., Darias, M.J., Said, K., Messaoudi, I. andGisbert, E., 2013. Cadmium exposure affects the expression of genes involved in skeletogenesis and stress response in gilthead sea bream larvae. *Fish Physiology and Biochemistry*, 39(**3**), 649-659.

Shariati, F., Esmaili Sari, A., Mahinchian, A. and Pourkazemi , M. 2011., Metallothionein as potential biomarker of cadmium exposure in Persian Sturgeon (*Acipenser persicus*). *Biological Trace Element Research*, 143, 281-291.

Simpkins, A.M., Tatum, T.E., Cardin, D.L. andWolf, W.C., 2013. Metallothionein and heat-shock protein 70 induction in caged and wild fathead minnows (*Pimephales promelas*) exposed to the Ouachita River, Louisiana. *Journal of Toxicology and Environmental Health*, 76(**2**)**,** 98-106**.**

Singer, C.H., Zimmermann, S. and Sures, B., 2005. Induction of heat shock proteins (hsp70) in the zebra mussel (*Dreissena polymorpha*) following exposure to palatinium group metals (palatinium, palladium and rhodium): Comparision with lead and cadmium exposure. Induction of heat shock proteins (hsp70) in the zebra mussel. *Aquatic Toxicology*, 75, 65-75

Sorenson, E.M., 1991. Metal poisoning in fish. CRC Press, Boca Raton, F.*Journal of Fish Diseases*, 26, 553-561.

Varo, I., Serrano, R., Pitarch, E., Amat, F., Lopez, F.J. and Navarro, J.C., 2009. Bioaccumulation of chlorpyrifos through an experimental food chain: study of protein hsp70 as biomarker of sublethal stress in fish. *Archives of Environmental Contamination and Toxicology*, 42, 229-235.

Vijayan, M.M., Pereria, C., Kruzynski, G. and Iwama, G.K., 1998. Sublethal concentrations of contaminant induce the expression of hepatic heat shock protein 70 in two salmonids. *Aquatic Toxicology*, 40, 101-108.

Vanderpool, A. and Reeves, G., 2001. Cadmium absorption in woman fed processed edible sunflower kernels labeled with a stable isotope of cadmium. ^{113}Cd. *Journal of Environmental Research Science*, A.87, 69-80.

Weisberg, M., Joseph, P., Hale, B. and Beyersmann, D., 2003. Molecular and cellular mechanisms of cadmium carcinogenesis. *Toxicology*, 192, 95-117.

Williams, J.H., Farag, A.M., Stansbury, M.A., Young, P.A., Bergman, H.L. and Petersen, N.S., 1996. Accumulation of hsp70 in juvenile and adult rainbow trout gill exposed to metal contaminated water and or diet. *Environmental Toxicology and Chemistry*, 15, 1324-1328.

Xing, H., Li, S., Wang, X., Gao, X., Xu, S. and Wang, X., 2013. Effects of atrazine and chlorpyrifos on the mRNA levels of HSP70 and HSC70 in the liver, brain, kidney and gill of common carp (*Cyprinus carpio* L.). *Chemosphere*, 90(**3**), 910-916.

Yarmohammadi, M., Pourkazemi, M., Kazemi, R., Hallajian, A., Soltanloo, H., Hassanzadeh Saber, M. and Abbasalizadeh, A., 2014. Persian sturgeon insulin-like growth factor I: Molecular cloning and expression during various nutritional conditions. *Journal of Applied Genetics*, 55(**2**), 239-47.