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ORIGINAL ARTICLE

Cadmium accumulation and biochemical parameters in juvenile Persian sturgeon, *Acipenser persicus*, upon sublethal cadmium exposure

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Abstract The purpose of the present study was to evaluate the effects of exposure to waterborne sublethal cadmium (Cd) concentration on juvenile Persian sturgeon, *Acipenser persicus*. Fish were exposed to 0.68 mg/l of Cd for 1, 7, and 14 days, and metal bioaccumulations, biochemical responses, and gill ions were investigated. There were significant differences ($p<0.05$) in the kidney (1, 7, and 14) and gills (7 and 14) for Cd concentrations between the control and treatment groups. Also, kidney Cd concentrations were significantly higher ($p<0.05$) at metal treatments on day 14 in comparison to day 1. Results showed that there were significant differences ($p<0.05$) in plasma glucose and cortisol concentrations between the experimental and control groups on day 1 only, and at metal treatments, a significant decrease ($p<0.05$) was observed on days 7 and 14 compared to day 1. No significant alterations were observed in plasma and liver protein contents during the course of the study. Neither triiodothyronine or thyroxine levels nor liver catalase or glutathione-*S*-transferase activities changed significantly with sublethal dose and with the time. In contrast, liver superoxide dismutase activities were significantly decreased ($p<0.05$) at Cd treatments both over the control group and during Cd exposure on days 7 and 14. Finally, a comparison between the groups revealed no differences in gill ion levels for 2 weeks. This study demonstrated that sublethal dose of Cd was stressful for Persian sturgeon and resulted in rapid changes in some of the biochemical parameters.

Keywords Cadmium · Cortisol · CAT · SOD · GST · *Acipenser persicus*

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Introduction

Cadmium (Cd) as an important xenobiotic of aquatic ecosystems has detrimental effects on aquatic biota. This heavy metal (HM) usually is found in the aquatic environments at sublethal concentrations. Today, there is a noticeable concern about the contamination of rivers, estuaries, and coastal sediment and waters by Cd (Burger 2008; De Mora et al. 2004a; Moore 1991). Most fishes, especially anadromous species, spend part of their lives in the larval and juvenile stages in such environments where they may probably encounter sublethal doses of Cd.

Cd, as a nonessential HM, has a broad range of deleterious effects on fishes at sublethal concentrations which are manifested in the form of bioaccumulation in vital organs (Asagba et al. 2008), hematological effects (Brucka-Jastrzebska and Protawicki 2005), histological alterations in ion-regulating tissues (Pratap and Wendelaar Bonga 1993; Thophon et al. 2003), plasmatic hormonal changes (Hontela et al. 1996; Pratap and Wendelaar Bonga 1990), osmoregulatory problems (Reid and McDonald 1988), changed behavior (Scott et al. 2003; Sloman et al. 2003), impaired reproduction (Tilton et al. 2003) and growth (Hansen et al. 2002) as well as an inhibition/induction of the activity of some antioxidant defense system enzymes such as catalase (CAT), superoxide dismutase (SOD), and glutathione *S*-transferase (GST) (Almeida et al. 2002; Atli and Canli 2007; Siraj Basha and Usha Rani 2003).

The Persian sturgeon, *Acipenser persicus*, is one of the highest quality sturgeon fishes of the Caspian Sea. Every year, a considerable number of sturgeon fingerlings are produced at different sturgeon proliferation and culture centers in Iran, and they are released into southern rivers and estuaries of the Caspian Sea (Bahmani et al. 2001), but today despite of such attempts, this species is classified as a critically endangered species (IUCN 2010). Generally, water mitigation in migratory rivers, overexploitation, habitat loss, environmental degradation, and water pollution were proposed as possible reasons for sturgeon decline (Barannikova 1995; Billard and Lecointre 2001; De Mora and Turner 2004; Khodorevskaya et al. 1997). Accordingly, some reports have mentioned the accumulation of different contaminants like organochlorines and HMs in the Caspian Sea fish and seal populations (Agusa et al. 2004; Anan et al. 2002, 2005; Kajiwara et al. 2002, 2003; Karpinsky 1992; Kunito et al. 2003; Moore et al. 2003; Pourang et al. 2005; Sadeghi Rad 2002; Watanabe et al. 2002). In addition, contamination of both water and sediments is declared in different parts of the Caspian Sea especially southern parts and rivers (Charkhabi et al. 2005; De Mora et al. 2004a, b; Korshenko and Gul 2005; Parizanganeh et al. 2006, 2008; Saeedi and Karbassi 2006; Saeedi et al. 2006, 2010; Tolosa et al. 2004). On the other hand, a few of these southern rivers are used for anadromous fish stock enhancement program like sturgeons where sometimes HM concentrations in sediments or water are higher than recommended concentrations (Charkhabi et al. 2005; Saeedi et al. 2006, 2010).

Due to the importance of Persian sturgeon for Iranian waters, the aim of this study was to investigate the biochemical responses of Persian sturgeon juveniles after a 14-day exposure to sublethal concentration of Cd in the laboratory condition. Cd seems to be the second most toxic tested HMs for this species to date (Mirzaei et al. 2004). For this purpose, metal bioaccumulations in various tissues, stress-related biochemical responses, and gill ions were examined after 1, 7, and 14 days of exposure.

Materials and methods

Fish

The experiments were carried out on +1-year-old Persian sturgeon, *A. persicus*, juveniles weighing 130±19 g (mean± SD). Fish were supplied by Ecology Faculty of the Caspian Sea, Iranian Fisheries Research Organization (Sari, Mazandaran, Iran) and transferred to the laboratory of Shahid Rajaee Sturgeon Hatchery Center (Sari, Mazandaran, Iran) on mid-June, 2008. The fish were distributed in Veniro tanks for at least 4 weeks before experimental use. Then, fish were transferred from the stock tanks to experimental ones for the sublethal exposure. Fish were fed 3 % of body weight once a day in the morning (at 9:00–9:30 a.m.) for the whole duration of the exposure.

Sublethal exposure

Before commencing the experiments, a stock solution of cadmium chloride (2,000 mg/l of $CdCl_2.2{\cdot}5H_2O$) was prepared, and then, it was diluted to the desired nominal concentration with tank water. The selected nominal Cd concentration (0.68 mg/l) was previously determined as a sublethal concentration for hatchery-reared Persian sturgeon juveniles in our laboratory conditions (i.e., 4.6 % of the calculated 96-h LC_{50}). During sublethal experiments, water parameters were measured daily, including temperature, 22.4±0.5°C; dissolved oxygen, 7.9±0.2 ppm; pH, 7.7± 0.3; hardness, 275±5.5 (milligrams $CaCO_3$ per liter); Cd, 0.6501±0.04 mg/l. Sublethal experiments were conducted in semi-static conditions in Veniro tanks. Each tank contained eight fish at three replicates in 1,000 l of test solution or well water only for control and were sampled after 1, 7, and 14 days of exposure. The Veniros were aerated with air stones attached to an air compressor to saturate with oxygen (Atli and Canli 2007). Fish was starved for 24 h prior to sampling to avoid prandial effects (Siraj Basha and Usha Rani 2003). Cd concentration was monitored daily by inductively coupled plasma optical emission spectrometry (ICP-OES, GBC, Integra XL).

Sampling and analysis

At each sampling time, six fish from each treatment were anesthetized in clove essence solution (at 9:00–9:30 a.m.) and then were weighed, and blood samples were taken from the caudal vein by means of heparinized capillaries and transferred to heparinized tubes held on ice until centrifugation. Immediately after blood collection, the liver, gill, intestine, and kidney tissues were dissected using clean equipment, rinsed by physiological serum, weighed, frozen in liquid nitrogen, and stored at −80 °C until further analysis (Almeida et al. 2002). Blood samples were centrifuged at 10,000 rpm for 3 min at 4 °C to obtain plasma (Atli and Canli 2007) and aliquoted, and stored at -20°C. The livers were homogenized by a homogenizer (TRI-I instrument, England) in 100 mM phosphate buffer (pH 7.4, 1:10, *w*/*v*) containing 2 mM EDTA and 150 KIU/ml aprotinin as a protease inhibitor. The resulting homogenates were centrifuged by a refrigerated centrifuge at 10,000 rpm (Beckman, Avanti^{TM} 30, USA) for 45 min at 4°C, and supernatant was used as an enzyme source. For metal ion determination, we followed the methods described by De Conto Cinier et al.

(1999). In brief, samples were cindered and then dissolved in 1 ml of 65 % super pure nitric acid (Merck, Darmstadt, Germany). The resulting solutions were subsequently diluted to 10 ml with ultra pure water, filtered (0.22 μm cellulose acetate, Sandic, S&S, Germany), and kept in a refrigerator (Kim et al. 2004) until the analysis of metals using ICP-OES.

The glucose levels in the samples were measured with enzymatic colorimetric assay kits (Pars Azmoon, Tehran, Iran) and total protein levels with chemical colorimetric assay kits (Pars Azmoon, Tehran, Iran). Plasma cortisol, triiodothyronine (T_3), and thyroxine (T_4) were assayed with commercial ELISA kits (Diagnostics Biochem Canada Inc, Ontario, Canada). Catalase (CAT, EC.1.11.1.6) and SOD (EC.1.15.1.1) activities were measured using colorimetric assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing City, P.R. China) in microtiter plate format, and ELISA Reader (Sunrise, Tecan, Austria) was used for the optical density recording. The assays were performed according to kit inserts. One unit of enzyme activity is the amount of enzyme that catalyzes the oxidation of 1 μmol substrate per minute. The results are accordingly given as units per milligram of protein. GST (EC.2.5.1.18) activity was measured by monitoring the formation of the product of the reaction between glutathione (GSH) and 1-chloro-2,4-dinitrobenzene (CDNB) at 340 nm (Habig et al. 1974). GST activity was determined by monitoring absorbance changing at 340 nm, which was translated to the rate of CDNB conjugation with GSH. One unit of GST activity was calculated as micromoles CDNB conjugate formed per minute per milligram of protein at 25°C.

Statistical analysis

Statistical analysis of data was carried out using SPSS statistical package programs (ver. 17.0, SPSS Company, Chicago, IL, USA). The values are reported as mean± SEM. Student's *t* test was used to test the difference of the control and treatment groups at each sampling time. Also, data from different days of sampling were compared by one-way analysis of variance (ANOVA), and means were tested by Duncan's multiple range tests. Where data did not meet the assumptions of the ANOVA (normal distribution or equal variances), nonparametric Kruskal–Wallis test was performed (Sokal and Rohlf 1995).

Results

The difference in weights of the fish used in the experiment was not significant ($p>0.05$), and no mortalities occurred during the sublethal Cd exposure. Also, no alterations were observed in water quality parameters with metal contamination. The result of Cd concentrations of gill, intestine, and kidney of *A. persicus* is shown in Fig. 1. There were increases in intestine Cd concentrations in experimental treatments compared to control groups, but these differences were not statistically significant ($p>0.05$). Kidney Cd concentrations in treatment groups were significantly higher compared to the control groups at all days of sampling ($p<0.05$). Moreover, in experimental treatment, kidney Cd concentrations had a significant increase on day 14 compared to day 1 ($p<0.05$), and the maximum average of tissue Cd concentrations was observed in this sampling (3.89 μg Cd/g kidney wet weight). In addition, there were significant increases in fish gill Cd concentrations in comparison to the controls on days 7 and 14 ($p<0.05$).

Plasma glucose levels increased significantly in Cd-exposed fish on the first day of sampling ($p<0.05$), but not on days 7 and 14 ($p>0.05$), and also, a significant decrease in glucose concentrations was observed on days 7 and 14 compared to day 1 ($p<0.05$). No significant alterations were evident in both plasma and liver total protein concentrations with sublethal metal dose or with the time ($p>0.05$). Cd resulted in a significant increase in plasma cortisol concentrations at metal-exposed fish compared to control groups on day 1 ($p<0.05$), and as time elapsed, significant alterations were observed in other stages of sampling ($p<0.05$). Both plasma T_3 and T_4 levels had no significant alterations with the treatment or the time (Fig. 2).

Total liver CAT, SOD, and GST enzyme activities in juvenile Persian sturgeon, *A. persicus*, exposed to sublethal Cd for 14 days are illustrated in Fig. 3. No significant changes were observed in liver CAT and GST activities during experimentation (Fig. 3a, c). Liver SOD activities were significantly decreased at experimental treatments over control groups only on days 7 and 14 ($p<0.05$). In the course of the study and at metal treatments, consistent reductions in liver SOD were evident from the first day of sampling onward, and were significant on days 7 and 14 compared to day 1 ($p<0.05$). Finally, a comparison between groups revealed neither difference in gill ion levels at sodium or potassium nor any significant changes at calcium for 2 weeks ($p>0.05$).

Discussion

In the present study on *A. persicus*, water Cd concentration was actually sublethal for this species, and it caused no mortality during the 14-day exposure. Also, the used nominal Cd concentration (0.68 mg/l) was 13-fold higher than the recent upper limit of Iranian southern coastal water concentration in the Caspian Sea (Varedi et al. 2010).

Results indicated that tissue accumulation of Cd increased significantly over time at both the kidney and gills, and this time dependency in metal bioaccumulation was

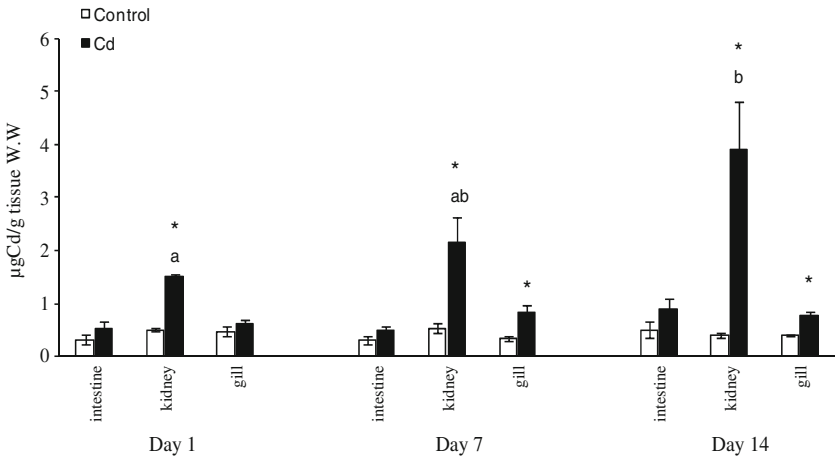

Fig. 1 Changes in tissue metal concentrations (micrograms per gram of tissue wet weight) of *A. persicus* juveniles exposed to 0.68 mg/l of Cd on days 1, 7, and 14 (mean±SE, n=4–6). Significant differences between treatments in each sampling day are denoted by *asterisks* (p< 0.05). Different *letters* indicate significant differences among different days of sampling (p< 0.05)

concomitant with other studies having shown such relationship for waterborne metal accumulation in fish tissues (Isani et al. 2009; McGeer et al. 2000; Wu et al. 2007). In this study, metal accumulations were studied in major osmoregulatory tissues, and it revealed tissue specificity for Cd uptake in *A. persicus*. It should be demonstrated that the distribution of accumulated Cd in fish differs among organs (Asagba et al. 2008; De Conto Cinier et al. 1999). Previous reports have shown that during waterborne metal exposure, high levels of metal accumulations occur in organs like the kidney, liver, gills, and intestine (Cattani et al. 1996; De Conto Cinier et al. 1999; Olsson et al. 1996). During this study and at all stages of sampling, the highest tissue Cd concentrations were detected in juvenile's kidney (Fig. 1). In fresh water fish, Cd uptake occurs mainly through the gills (Williams and Giesy 1978), and Cd entering the fish's body, is distributed, and then is deposited to some other organs (Wu et al. 2007). It is stressed that waterborne Cd ultimately accumulates in the kidney for excretion (Asagba et al. 2008; De Conto Cinier et al. 1999) where it induces the synthesis of metallothioneins (MT). MTs are a class of low molecular weight, sulfur-rich metal-binding proteins with a high affinity for HM ions (Klavercamp et al. 1984; Roesijadi 1996) which control both the kinetics of bioaccumulation and the manifestation of toxic effects, and ultimately determine metal tolerance (De la Torre et al. 2000). Thus, over time, the concentrations of Cd increased in juvenile kidney, and it reached its highest levels on day 14. Moreover, metal accumulation in fish tissues is related to different biotic and abiotic factors (Erickson et al. 2008; Heath 1995; Kim et al. 2004). For instance, water hardness has a peculiar status among them, and here, its high amount (275±5.5 mg $CaCO_3$/l) may affect Cd speciation and mitigate its bioavailability. In brief, exposure of *A. persicus* juveniles to 0.68 mg/l of Cd induced significant but different metal accumulations depending on the length of exposure period and the tissue.

During this study, plasma glucose levels appeared markedly elevated in experimental groups on the first day, but it decreased on other days of sampling (Table 1). Also, a rapid increase was observed in plasma cortisol concentrations in the experimental groups on day 1, but it showed a significant decrease on day 7 (Fig. 2). These results clearly showed that juveniles have experienced a transient elevation in stress parameters that have alleviated over time. Glucose as an energetic substrate is used by fishes during stress condition, and the previous investigations showed that Cd caused hyperglycemia (Cicik and Engin 2005; Ghazaly 1992), but this response usually terminates in a few days (Pratap and Wendelaar Bonga 1990). However, Ricard et al.

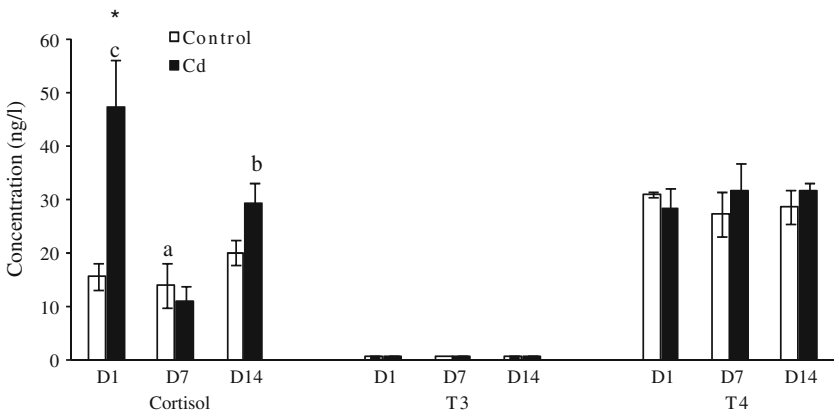

Fig. 2 Plasma hormonal concentrations of *A. persicus* exposed to 0.68 mg/l of Cd on days 1, 7, and 14 (mean±SE, n=5–6). Significant differences between treatments in each sampling day are denoted by *asterisks* (p<0.05). Different *letters* indicate significant differences among different days of sampling (p<0.05)

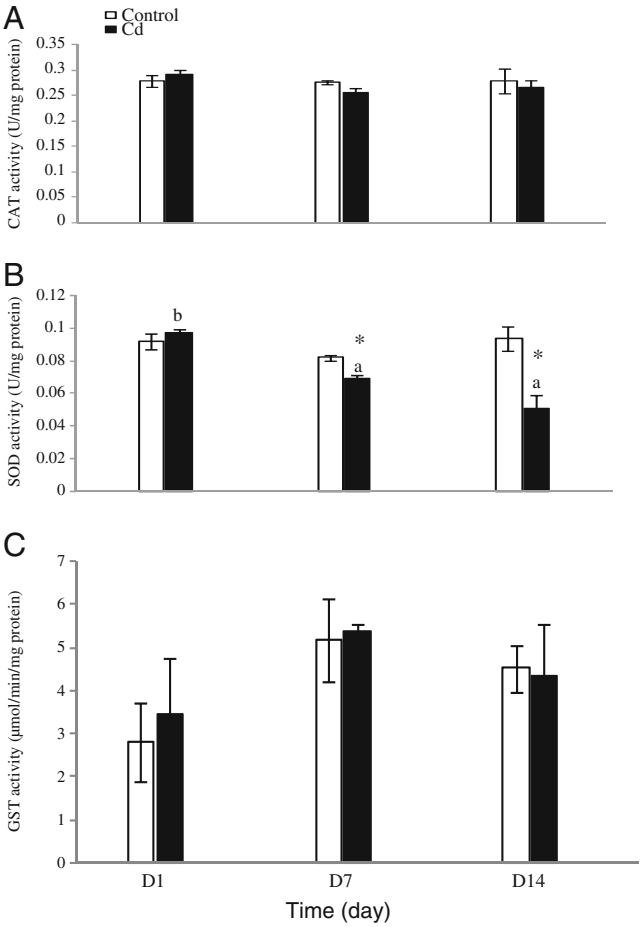

Fig. 3 Liver CAT (**a**), SOD (**b**) (units per milligram of protein) as well as GST (**c**) activities (micromoles per minute per milligram of protein) of *A. persicus* exposed to 0.68 mg/l of Cd on days 1, 7, and 14 (mean± SE, n=5–6). Significant differences between treatments in each sampling day are denoted by *asterisks* (p<0.05). Different *letters* indicate significant differences among different days of sampling (p<0.05)

(1998) illustrated no changes in plasma glucose levels of fish during Cd exposure. Alternatively, Hontela et al. (1996) stressed that Cd may induce liver damage and alter glucose homeostasis. HMs like Cd can induce an osmo-ionic disturbance and activate the hypothalamo–pituitary–interrenal axis that induces cortisol secretion (Fu et al. 1990; Hontela et al. 1995, 1996; Tort et al. 1996) which leads to carbohydrates metabolism and hyperglycemia (Mommsen et al. 1999; Wendelaar Bonga 1997). In contrast, Schreck and Lorz (1978) reported that Cd exposure had no effect on cortisol levels of coho salmon.

No significant changes were observed in both plasma and liver protein contents during three samplings (Table 1). Similarly, other studies showed no alterations in plasma/liver protein contents during Cd exposure (Almeida et al. 2002; De Smet and Blust 2001). It should be stressed that a uniform trend was not yet described in tissue protein changes during waterborne Cd exposure among different studies (Almeida et al. 2001; De la Torre et al. 2000; De Smet and Blust 2001; Ricard et al. 1998). We related obtained responses during this study to both metal concentrations and exposure duration. Cd concentration of 0.68 mg/l was probably lower than the limit which can be effectual on protein metabolism. Protein usually is spared during chronic period of pollutant stress (Garg et al. 2008). We supposed that 14-day Cd exposure did not trigger protein catabolism maybe due to sufficient carbohydrate sources for stress responses.

In this study, there were no significant changes in levels of thyroid gland hormones including T_3 and T_4 during both metal exposure and time, and their trend was opposed to cortisol (Fig. 2). The obtained results from this investigation were inconsistent with results of Hontela et al. (1996) which showed that acute (24 h) exposure to Cd in juvenile rainbow trout increased both plasma cortisol and T_4 not T_3 levels. On the other hand, a 1-week subacute exposure increased the plasma cortisol levels of the exposed fish, but plasma T_4 levels decreased, and plasma T_3 levels remained stable. Generally, thyroid hormones in fishes play important roles in regulation of development, growth, smoltification, reproduction, and toxicant exposure (Hontela et al. 1995; Power et al. 2001). T_4 as a primary secretory product is mostly regarded as a precursor for biological active form of the hormone, T_3, and promotes the secretion of cortisol by the interrenal tissue (Young and Lin 1988). In this study, non-significant T_3 and T_4 changes may be due to the used Cd concentration which was probably below the required threshold which can influence thyroid function. The interactions among these hormones affect some biochemical responses (De Jesus et al. 1990; Hontela et al. 1996). Also, Varghese et al. (2001) pointed out the role of thyroid hormones like T_3 and T_2 in cellular lipid peroxidation and antioxidant enzyme activity for maintaining internal homeostasis.

Exposure of cells to HMs and their compounds can generate excessive reactive oxygen species (ROS) (Filho 1996; Ruas et al. 2008). Although, ROS plays important roles in the cell functions, they cause oxidative damage/stress in amounts which are beyond the cellular antioxidant defense (Almeida et al. 2002). Antioxidant defense system includes both antioxidant enzymes and low molecular weight antioxidants which eliminate oxyradicals (Ahmad et al. 2004; Pandey et al. 2003; Van der Oost et al. 2003). CAT and SOD occur in tandem and make the first line of defense against oxidative stress-inducing xenobiotics (Filho 1996; Halliwell 1994). Our focus on juvenile's liver for antioxidative responses was according to Wilhelm Filho et al. (1993) who introduced the liver as the best organ representing the status of antioxidant defenses. Alternatively, Trenzado et al. (2006) detected the highest CAT and SOD activities in the liver of *Acipenser naccari*.

Table 1 Blood biochemical parameters in *A. persicus* juveniles exposed to 0.68 mg/l of Cd on days 1, 7, and 14

Parameters	Control			Cd		
	Day 1	Day 7	Day 14	Day 1	Day 7	Day 14
Glucose (mg/dl)	53±2.01	41.33±5.93	54±3.21	75.2±5.08*b	52±5.11a	56.75±1.31a
Plasma protein (g/dl)	2.9±.058	2.2±0.4	2.47±.54	2.78±.19	2.68±.22	2.66±.17
Liver protein (mg/mg)	0.126±0.001	0.13±0.003	0.137±0.009	0.13±0.002	0.140±0.007	0.141±0.012

Data were analyzed by Student's *t* test to compare the control and treatment groups at the same treatment time and by one-way ANOVA with Duncan comparisons for different days of sampling. Different letters indicate significant differences among different days of sampling ($p<0.05$). Data are presented as mean±SE, $n=4–6$

*$p<0.05$, significant differences between treatments in each sampling day

After a 14-day Cd exposure, significant alterations were not observed in both liver CAT and GST activities, but SOD activities showed significant changes compared to the control group and over time (Fig. 3a–c). In contrast to these CAT changes, Atli and Canli (2007) reported significant alterations in liver CAT activities during Cd exposure. Data presented here corroborate these authors who have previously mentioned significant hepatic SOD changes during Cd exposure (Almeida et al. 2002; Asagba et al. 2008; Roméo et al. 2000). Observed inhibition in SOD activity during this study can be related to the oxidative stress caused by Cd exposure or direct binding of metal to it as previously reported by Asagba et al. (2008). It is well known that Cd increases ROS production in the tissues and also inhibits the activity of some antioxidative enzymes (Jackim et al. 1970). SOD has the greatest response to oxidative stress (Winston and Di Giulio 1991), and it is the first enzyme which reacts by producing ROS (McCord and Fridovich 1969) and generating H_2O_2 which is catalyzed further by other molecules involved in catabolism like CAT or glutathione peroxidase. Therefore, we observed significant changes in SOD that acts initially. Because of other enzymes which may be involved in catalyzing resulted metabolites, cooperating with CAT, thus they may result in nonsignificant changes of CAT. Also, rapid CAT inactivation at high H_2O_2 levels is reported by Wong and Whitaker (2003) resulting from the converting of active enzyme compounds to inactived ones. Finally, increasing of nonenzymatic mechanisms such as GSH and MTs may be effectual in antioxidant defense system (Price et al. 1990). For instance, we can refer to ascorbic acid as an antioxidant molecule because sturgeon fishes can synthesize it de novo (Dabrowski 2001), and it mitigated the resulted oxidative stress.

GSTs are essential components of the cellular antioxidant defense system and catalyze the conjugation of GSH to various electrophilic compounds, thus providing cellular detoxification (Arrigo 1999). Siraj Basha and Usha Rani (2003) in a study on *Oreochromis mossambicus* exposed to Cd showed significant elevations in liver GST activities from the first day of sampling onward which were then maintained until the 30th day of the experiment. In contrast, Chandrasekera et al. (2008) observed no significant changes of hepatic GST activities of the fish during a 28-day waterborne Cd exposure.

It was observed that gill sodium, potassium, and calcium changes of *A. persicus* were not significant during Cd exposure (Table 2). Plasma/whole body ion changes during Cd exposure have been reported frequently, and Cd can inhibit the ion balances in osmoregulatory organs by binding to Ca^{+2}-ATPase and Na^{+}/K^{+}-APTase (Pelgrom et al. 1995; Pratap and Wendelaar Bonga 1993; Verbost et al. 1988; Wong and Wong 2000), and whereupon inhibits their activities and subsequently the gill Na^{+}/Ca^{2+} transport (Pratap and Wendelaar Bonga 1993; Reid and McDonald 1988). Cd

Table 2 Gill ion concentrations (micromoles per gram of gill wet weight) in *A. persicus* juveniles exposed to 0.68 mg/l of Cd on days 1, 7, and 14

Day	Calcium		Potassium		Sodium	
	Control	Cd	Control	Cd	Control	Cd
1	32.078±1.91	32.724±4.68	31.824±1.57	26.606±1.47	82.985±.79	68.791±6.67
7	48.116±6.83	47.287±22.03	29.867±.89	26.548±5.48	67.631±17.02	80.319±6.24
14	30.693±5.2	29.266±1.59	30.560±8.53	30.821±6.84	78.079±4.43	81.168±9.5

Data were analyzed by Student's *t* test to compare the control and treatment groups at the same treatment time and by one-way ANOVA with Duncan comparisons for different days of sampling. Different letters indicate significant differences among different days of sampling ($p<0.05$). Data are presented as mean±SE, $n=4–5$

*$p<0.05$, significant differences between treatments in each sampling day

affects the Na^+ (Giles 1984) and Ca^{2+} balance and causes hypocalcemia (Fu et al. 1990; Haux and Larsson 1984). Also, there is a scarcity of data about tissue K^+ changes during HM exposure, but its plasmatic changes are usually nonsignificant (McGeer et al. 2000). Due to the lack of measuring the plasmatic ions in *A. persicus* juveniles, making comparison with plasmatic results which are provided by others is impossible. Also, the data about ionic changes of osmoregulatory tissues during HM exposure are sparse. But impaired plasmatic ionic regulation has also been reflected in whole body ion composition (Sayer et al. 1991). During a 14-day exposure in Persian sturgeon juveniles, the Cd concentration increased in gills significantly which might also occur in the chloride cells. In contrast to our expectation based on observation of significant gill ion changes during Cd exposure as a result of impaired branchial ionic pump functions, there were no major ionic disturbances, and this effect can be related to the physicochemical characteristics of experimental media which have probably decreased metal bioavailability.

In conclusion, a 14-day sublethal exposure to 0.68 mg/l of Cd was physiologically stressful for *A. persicus* juveniles as indicated by rapid and transient changes in some of the stress-related parameters. Even though tissue Cd concentration increased, juveniles could, to some extent, adapt themselves to sublethal exposure which is manifested in the form of observed biochemical changes. Because a few significant alterations were observed at the end of the exposure, most of the measured parameters seemed inappropriate biomarkers for monitoring the long-term effects of waterborne sublethal Cd exposure for this species except kidney and gills Cd burdens as well as liver SOD activities which are regarded as suitable biomarkers at least in the laboratory conditions. These findings are helpful in order to standardize the time of exposure to the contaminants and sampling and to provide more robust and authentic results.

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