Int. J. Mar. Sci. Eng., 2 (1), 81-90, Winter 2012
ISSN 2251-6743

Metal bioaccumulation in Persian sturgeon after sublethal exposure

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Received 14 February 2011; Revised 1 July 2011; Accepted 16 August 2011

ABSTRACT: Tissue metal accumulations (gills, liver, kidney and muscle) in Persian sturgeon (Acipenser persicus) were compared folowing exposure to sublethal levels of waterborne Cd (50, 400 and 1000 µg•L-1) after periods of 1, 2, 4 and 14 days. The obtained results indicate that at the end of 4 and 14 days of exposure, total tissue cadmium concentration followed the pattern: liver > gill > kidney > muscle. Calculation of bioconcentration factor (BCF) after 14 days exposure showed that at low and high concentrations, highest BCFs were found in kidney and liver, respectively. According to the results, the accumulation capacity of muscle was the lowest at all exposure concentrations. Cd concentration in the cytosol of experimental tissues were measured and the results indicated that Cd levels in the cytosol of liver, kidney and gills increased 240.71, 32.05, and 40.16-fold, respectively 14 days after exposure to 1000 µgL-1 Cd. The accumulation of Cd in cytosol of tissues is in the order of liver> gills> kidney.

Keywords: *Cadmium; Bioaccumulation; Persian sturgeon; Liver; Kidney; Gill*

INTRODUCTION

Metals form an important group of anthropogenic pollutants load in aquatic ecosystems continuously (Campenhout *et al*., 2010). Today, heavy metal pollution in aquatic ecosystems is growing at an alarming rate in many developed and developing countries and becomes a significant worldwide problem. There are different sources of heavy metals due to various anthropogenic activities such as sewage draining, dumping of hospital and other wastes as well as mining and different industrial processes (Malik *et al*., 2010). Also, metals present in small amounts naturally and may enter into aquatic systems through ore-bearing rocks, windblown dust, forest fires and vegetation (Wang and Rainbow, 2008; Fernandez and Olalla, 2000).

As heavy metals can not be degraded, they are deposited, assimilated or incorporated in water, sediments and aquatic animals, so causing heavy metal pollution in water bodies (Malik *et al*., 2010; Mackeviciene, 2002). Metal contamination in water bodies is a critical environmental issue due to metal uptake, accumulation and toxicity in many aquatic organisms, as well as the possibility of their throphic transfer along food chains, eventually reaching humans (Oliveira *et al*., 2010). Cadmium is a biotoxic element and as a widespread environmental pollutant has no biological function which regarded as a priority pollutant (Messaoudi *et al*., 2009; Kalman *et al*., 2010).

It is widely applied in industrial processes and products such as plastics, ceramics, glass, vehicle tires and batteries (Kalman *et al*., 2010; Cao *et al*., 2009). Anthropogenic input considered the main source of Cd contamination in aquatic environments (Kalman et al., 2010). Fish tend to take up Cd from water or food and accumulate it in their tissues (Kalman *et al*., 2010). The rate of uptake depends on some factors, such as route, concentration, and duration of exposure, physiological condition of the organism and environmental parameters like salinity, temperature etc. (Kalman *et*

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al., 2010).

In fish, the gills, skin, and digestive tract are potential sites of absorption of cadmium from water (Ruangsomboon and Wongrat 2006). Fishes have ability to accumulate heavy metals in their tissues by the absorption along the gill surface and gut tract wall to higher levels than toxic concentration in their environment (De Conto Cinier *et al*., 1998; Chevreuil *et al*., 1995; Witeska *et al*., 2006). As fish are often at the top of the aquatic food chain, they may concentrate large amounts of Cd from the water (Cao *et al*., 2009). As pollutants rarely distribute evenly throughout the body of fish, they accumulate in particular organ tissues that for Cd are kidney, liver, and gill (Norey *et al*., 1990).

Due to deleterious effects of Cd on aquatic ecosystems, it is necessary to monitor its bioaccumulation and toxicity in key species, and thus give an indication of the temporal and spatial extent of the process, as well as an assessment of the potential impact on organism health (Messaoudi *et al*., 2009).

When metal analysis in the whole tissue gives the information on total metal content, including metabolically and trophically unaccessible metal forms, metal analysis in the tissue cytosol (including heat sensitive protein (HSP) like enzymes and heat resistant protein such as metallothionein):

– gives the information on trophically and metabolically available metal concentrations; it is especially important if possible harmful effects of toxic metals are considered.

– avoids the use of concentrated acids and indispensable, but rather expensive apparatus for tissue digestion (Podrug *et al*., 2009). Thus, we determined Cd concentration in cytosol (soluble fraction after centrification) in our study.

Nowadays, there is a very serious concern for seafood safety, which also requires knowledge of the bioaccumulated concentrations of metals and their potential risk to humans (Wang and Rainbow, 2008). In this way, with the measurement of metals in water and other parts of aquatic ecosystem, we can estimate accumulated Cd content in desired organism.

In present study, the cadmium bioaccumulation in Cd-exposed Persian sturgeon and bioconcentration factor (BCF) were determined and calculated. Persian sturgeon (Acipenser persicus) is one of the most economically important fishes in the Caspian Sea the stocks of which have decreased during recent years. Formerly omnipresent in the region, illegal catch, poaching and intensive fishing of the sturgeon for caviar have forced this species to be listed as endangered. Since Caspian Sea is an enclosed water body and large amounts of industrial and petroleum effluents as well as municipal wastewater enter to it, it seems necessary to investigate heavy metal bioaccumulation in important aquatic organisms of this region.

Therefore, our main objective was to measure Cd accumulation in liver, kidney, gill and muscle after fish exposure to waterborne sublethal concentrations of cadmium (50, 400 and 1000 μgL^{-1}, 3 replicates for each concentration) during periods of 1, 2, 4 and 14 days. The LC_{50} of Cd for Acipenser persicus was obtained as 4000 $\mu g\ L^{-1}$ (Mirzaee *et al*., 2003) and therefore, 400 and 1000 $\mu g\ L^{-1}$ concentrations of Cd were selected for Persian sturgeon exposure on the basis of being 0.1 and 0.25 LC_{50}, respectively.

MATERIALS AND METHODS

1. Animals and experimental exposure

The Persian sturgeon (1 year old, n=144) weighing 120-200 g was obtained from the Shahid Dr Beheshti Hatchery (Guilan Province-South Caspian Sea) and acclimatized to the experimental conditions for 15 days. Physical and chemical parameters of water including hardness, alkalinity, pH, nitrate, nitrite, phosphate and dissolved oxygen (DO) concentrations were continuously monitored. pH and DO were determined by pH and DO meter, respectively (WTW-Germany). Hardness and alkalinity were determined through titration according to Standard Methods (APHA, 2005). Nitrate, nitrite and phosphate were analyzed through spectrophotometric method (Hach, DR 5000, USA) as Standard Methods (2005).

The stock Cd solution was prepared with CdCl2 from Merck Company)Darmsdat, Germany). Exposure concentrations for experiment were 50, 400 and 1000 µgL-1 Cd with three replications (12 fish in each tank) that performed in static system. During the exposure period, the fish fed daily on commercial fish pellets and the water in bitumen tanks (supplied with 900 liters water from the river after filteration) was changed every two days to minimize metal loss after feeding and thus to reduce contamination of the environment with food remains.

2. Sampling

Six fish from each treatment were sampled in each time for cadmium analysis. The fish were killed by severing the spinal cord behind the head. The tissues (gill, liver, and kidney) were separated from the control and test fishes. The tissues were rinsed with deionized water to remove the adhering blood and maintained in liquid nitrogen (for 24 hours) and then in -50°C freezer until

analysis.

3. Cadmium determination

3.1. Labware

For sample collection and storage, plastic and polyethylene labware were used. To minimize trace metal contamination, they were acid washed prior to use by soaking in 15% nitric acid (for 24 h), followed by rinsing repeatedly with high purity water. After rinsing, the labware was placed to dry under a hood.

3.2. Cytosol and pellet preparation

After addition of sucrose–TRIS buffer (pH=8.6) containing phenylmethylsulfonyl flouride (PMSF) (1.5 µl ml^{-1}) and β-mercaptoethanol (0.1 µl ml^{-1}) to the tissue samples in 1:3 (w/v) ratio, the samples were homogenized using a Polytron PT 3100 (Switzerland) homogenizer in cold room . The homogenates were centrifuged at 30,000 g for 60 min at 0°C using a Tomy Surpema 25 (Japan) centrifuge. Then, the supernatant (cytosol) and pellet were removed and maintained in -20°C for Cd determination.

3.3. Analysis of cadmium in whole tissues

Each gill, liver, and kidney sample was homogenized using a Polytron PT 3100 (Switzerland) homogenizer. Then homogenates were digested with three volume (1:3) concentrated nitric acid (HNO_3, 65%) and heated at 90◦C for 4 hours. When fumes were white and the solution was completely clear, the samples were cooled to room temperature and the tubes were filled to 5 mL with ultra pure water. Cd concentrations were determined using a GBC atomic absorption spectrophotometer (AAS) (model Sens AA, Australia) with a graphite furnace electrothermal atomizer equipped with the auto-sampler. The samples were analyzed in triplicates. The detection limit of method was determined from the standard additions curve. It was based on the usual definition of the concentration of the analyte yielding a signal equivalent to three times the standard deviation of the reagent blank signal (LOD = $3S_b/m$), where m is the slope of the calibration graph and S_b is the standard deviation of five replicates of the blank measurement, n = 5). The LOD of method for Cd was determined as 0.061 µg L^{-1}. During each digestion run, blank (HNO_3 and ultra pure water) was included. The average reading of blanks was subtracted from standards and test samples and then final concentrations were calculated as µg g^{-1} wet weight. Recovery percents were calculated between 97 and 113 % via spiking fish muscle with five different concentrations of Cd.

Cadmium concentration in water and food pellets was determined, too. They were measured with the same method as explained above.

3.4. Cd determination in cytosol and pellet

To one ml of cytosol or weighted amount of resultant pellet, 1 mL concentrated nitric acid was added and heated in water bath 90°C for 4 hours. After that, it was refilled with 5 mL ultra pure water. Then Cd determination in samples was done through above-mentioned method.

4. Statistical analysis

All data were presented as the average of replicate measurements with considering the standard deviation of measurements (Xmean ± S.D). Data related to cadmium and protein concentrations in different sampling times were analyzed for statistical significance by one-way analysis of variance (ANOVA). To identify the significant differences obtained by one-way ANOVA, comparisons were tested with Tukey HSD post hoc method. When the assumptions of the ANOVA and t-test were not satisfied, non-parametric Kruskal–Wallis comparison test was used. The level of significance was set at ≤0.05. Statistical analysis was performed using the Statistical Analysis System (SPSS 17) software.

RESULTS AND DISCUSSION

1. Cadmium

Data found in this investigation showed that the Cd exposure provokes to different extents a significant accumulation in major tissues of A. persicus (liver, gills, and kidney). Cd is a toxic element that has no known biological function and show deleterious effects on aquatic organisms and humans.

The physical and chemical parameters of water were as follows: temperature: 19 ± 2.5°C; pH: 8.1 ± 0.2; dissolved oxygen: 6.4 ± 0.2 mg L^{-1}, total alkalinity: 200.0 ± 10.0 mg L^{-1} $CaCO_3$; total hardness: 400.0 ± 0.1 mg L^{-1} CaCO3; Nitrite: 0.003 ± 0.0002 mg L^{-1}, Nitrate: 3.6 ± 0.3 mgL^{-1} and phosphate: 8.5 ± 0.4 µg L^{-1}.

Mortality was minimal over period of 14 days with 0% for controls and 8% for fish exposed to 1000 µg L^{-1} Cd, respectively. There was no mortality in fish exposed to 50 and 400 µg L^{-1}.

In Table 1, it was shown that after exposure to 50 µg L^{-1} Cd, the order of uptake in the tissues is as the following: kidney > liver > gill > muscle, but after exposure to 400 µg L^{-1} Cd the order was changed to liver > kidney >gill >> muscle and exposure to 1000 µg L^{-1} caused an order as liver > gill > kidney > muscle. It can be observed that in low concentrations, the highest and lowest rate of bioaccumulation were observed in kidney and muscle, respectively. However in maximum

exposure concentration (1000 µg L^{-1}), liver accumulated most of Cd.

In all exposure concentrations, muscle had the lowest accumulation capacity. Linear correlation between Cd accumulation and exposure time in Persian sturgeon as found in present work, has been previously demonstrated in roach liver and carp liver and kidney (De Conto Cinier *et al*., 1998; Bonvick *et al*., 1991).

The trend of Cd accumulation in different tissues after variable exposure times was shown in Figs.1, 2, 3 and 4.

It is obvious that accumulation order after 1 day exposure is as the following: liver > gill > kidney > muscle, but after 2 days, gills had the highest accumulation followed by liver, kidney and muscle. According to Fig. 3 and 4, after 4 and 14 days exposure the order of accumulation was: liver > gill > kidney > muscle. Maybe it is because Cd is initially taken up by

Table 1: The regression equation between Cd concentrations in tissues after exposure to variable waterborne Cd during 1, 2, 4 and 14 days

	50 µg L^{-1}	400 µg L^{-1}	1000 µg L^{-1}
Kidney	y= 70.96 days+234.2	y=216.4 days+273.1	y=427.8 days+177.3
Liver	y= 46.95days+473.6	y= 257.6 days+832	y=893.3 days+468.2
Gill	y= 43.99days+259.4	y=160.4days+1175	y= 447 days+ 900.2
Muscle	y= 0.454 days+4.484	y=6.26 days+5.841	y=21.13days+ 6.384

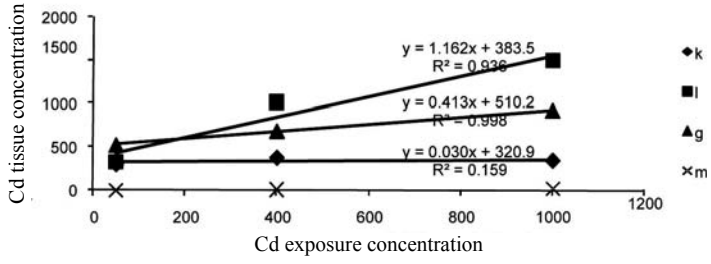

Fig. 1: Changes of cadmium concentration in different tissues after 1 day exposure (n=6)

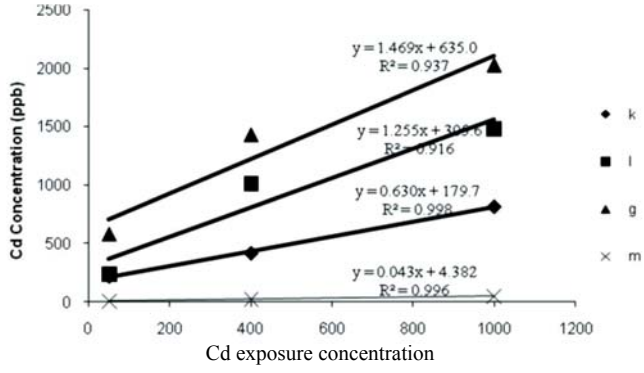

Fig. 2: Changes of cadmium concentration in different tissues after 2 days exposure(n=6)

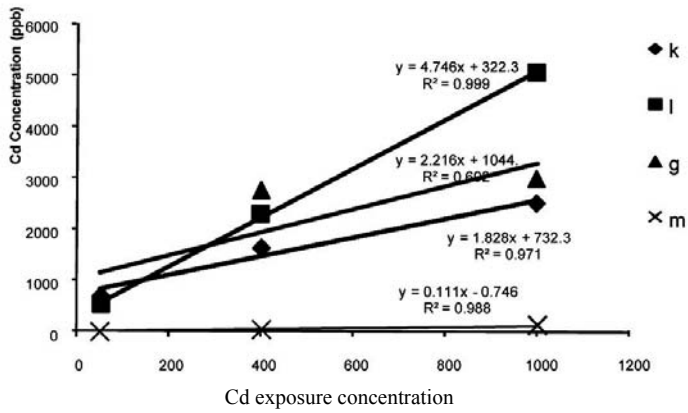

Fig. 3: Changes of cadmium concentration in different tissues after 4 days exposure(n=6)

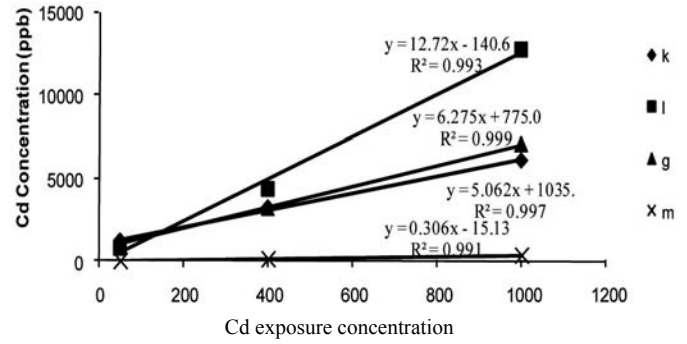

Fig. 4: Changes of cadmium concentration in different tissues after 14 days exposure(n=6)

the liver, where it can bind with reduced glutathione (GSH) and be secreted into the bile. Alternatively, it can bind to metallothionein and subsequently stored (Kim *et al*. 2008). This result was consistent with the work of Ghedira *et al*. (2007) who investigated on the bioaccumulation of cadmium and cupper in liver, gills and kidney of Sparus aurata and concluded that liver had bioaccumulation capacity more than gill and kidney after 48h exposure and Cd was concentrated 20-fold in it. It is in agreement with the data mentioned in the literature such as following: Hamza-Chaffai *et al*. (1995) who reported that Cd and Cu concentrations accumulated in the liver was more than gills in three fish species: Diplodus annularis, Scorpaena porcus and Scorpaena scrofa; Marijic and Raspor (2006) noted a 43-fold increase of Cd level in cytosolic liver and a 5-fold increase in kidney of Mullus barbatus collected from Kastela Bay; Kalman *et al*. (2010) measured Cd concentrations in the liver, gills, intestine and blood of S. aurata at 0, 3 and 6 days after Cd injection and reported that the highest cadmium accumulation was found in the liver, followed by the intestine, gills and blood; Atli and Canli (2008) observed that after 14 days exposure to Cd, the order of accumulation was as liver > gill > muscle; Wong *et al*. (2001) who had reported highest Cd concentration in the liver tissues of Epinephelus areolatus, Loxosceles russelli and Sparus sarba; Creti *et al*. (2008) investigated on Cd bioaccumulation in different tissues of farmed sea bream (Sparus aurata) and they concluded that among tissues, Cd was accumulated more in liver and gut than in other organs analyzed. Some other works done by Woodworth and Pascoe (1983) and Handy (1993) had the same results. Also, in a study was done by Malik *et al*. (2010), it was observed that gills and liver of L. rohita and C. idella accumulated higher levels of

heavy metals than other organs because liver acted as a primary organ for storage and detoxification and gills acted as a depot tissue (Chowdhury *et al*., 2003). The accumulation of metals significantly increased in these organs, as was also observed by Yilmaz (2005) and Nsikak *et al*. (2007).

However, the other authors had contrasting results like Asagba *et al*. (2008) who reported Cd concentrations were also greater in kidney (0.39 ppm) than liver (0.17 ppm) and muscle (0.18 ppm) of fish exposed to different concentrations of Cd during 7 days; Jayakumar and Paul (2006) who reported the patterns of Cd accumulations in the order of gills > kidney > liver > skin > muscle; the result of Wu *et al*. (2007) study on the physiological responses of tilapia (Oreochromis sp.) after waterborne cadmium exposure had been that metal uptake was the highest in the gills followed by the intestine, kidney and liver after 5h; however, this order changed by day 15, as the main accumulator organs were then the intestines, kidney and liver; Hollis *et al*.(2001) found Cd uptake in tissues in the order of kidney > gill > liver 30 days after waterborne Cd exposure in rainbow trout (Onchorhynchus mykiss); Kraal et al. (1995) found that Cd accumulation in kidney of carp (Cyprinus carpio) was 10-fold higher than liver; Kamunde (2009) resulted that after exposure of rainbow trout (Onchorhynchus mykiss) to four concentrations of Cd during 96h, Cd uptake in gills was more than liver.

As shown in to Table 2, bioconcentration factors (BCF) of Cd in different tissues related to water after exposure to four waterborne concentrations of Cd during 14 days were calculated. As observed in lower concentration (50 µg L-1), the order of accumulation was kidney > gill > liver > muscle, but in 400 µg L-1 Cd exposure, the order changed as liver > gill ≈ kidney > muscle and it was liver > gill > kidney > muscle in 1000 µg L-1 Cd exposure. As observed BCF decreased with increase in Cd concentration at all tissues except for muscle, so it was concentration dependent. The above mentioned order was not consistent with the results of Chowdhury *et al*. (2005) that showed after 30 day, the greatest increases in the Cd-acclimated fish were found in the order kidney (656-fold) > gills (400-fold) > liver (122-fold) > carcass (15-fold) in comparison to the control trout.

According to Table 2, bioconcentration of cadmium in the liver, gill, and kidney was higher than muscle. The gill and the liver, along with the kidney, are the main sites of metallothionein (MT) production and metal retention. One of the main reasons for the increased presence of cadmium in these organs is their capacity to accumulate cadmium via induction of the metal binding protein, MT, which is believed to influence the uptake, distribution and toxicity of Cd by binding to it (Asagba *et al*., 2008).

Bioconcentration of Cd was lower in muscles compared to gills, liver, and kidney and it was nearly constant after different concentrations exposure. Galar Burgos and Rainbow (2001) proposed that muscle metal concentration may be regulated and is a poor biomonitor of Cd accumulation in the immediate ambient environment. Also, some other authors such as Canli and Atli (2003), Usero *et al*. (2004), Creti *et al*. (2009), Ureña *et al*. (2007) and Ciardullo *et al*. (2008) had reported the same results obtained in their works. It maybe due to muscle tissues do not come in direct contact with the toxicants. It is also not an active site of detoxification and hence Cd is not transported from other tissues to muscles (Kumar *et al*., 2007). This is particularly important because muscles contribute the greatest mass of the flesh that is consumed as food and based viewpoint of food safety, it is a preferable to us that bioaccumulation in muscles are low (Chowdhury *et al*., 2005).

Many studies have investigated Cd accumulation in total tissues of fish. In this research, Cd concentrations in the cytosol and pellet of experimental tissues were measured, too and their results are shown in Fig. 5-7. Comparison of the results indicated that Cd levels in the cytosol of liver, kidney, and gills increased 240.71-, 32.05-, and 40.16-fold, respectively 14 days after exposure to 1000 µgL-1 Cd in comparison with the control tissue cytosol. Thus, the accumulation of Cd in cytosol of tissues is in the order of liver>gills> kidney and hence Cd concentration

Table 2: Bioconcentration Factors of different tissues after exposure to Cd concentrations after 14 days

	50 µg L-1	400 µg L-1	1000 µg L-1
Kidney	23.88	8.025	6.04
Liver	17.26	10.92	12.8
Gill	22.68	8.033	7.077
Muscle	0.201	0.229	0.297

in the liver elevated faster than kidney and gills that was evident from the slope of the regression lines for different exposure Cd concentrations.

Our findings are in agreement with the results of Marijic and Raspor (2006) who noted a 43-fold increase of Cd level in cytosolic liver and a 5-fold increase in kidney of Mullus barbatus collected from Kastela Bay.

The subcellular distribution pattern differed between three organs. According to Fig. 5a-c, in the kidney, Cd increased in cytosol fraction until four days, but it was decreased in this fraction at the day 14 and increased in insoluble (pellet) fraction.

As shown in Fig 6a-c, Cd increased in soluble form of the liver, but its value increased in pellets 14 days after exposure. Although decrease of Cd in soluble fraction was observed, contribution of soluble part was still more than insoluble fraction. The major part of Cd may be bound to proteins like gluthathione or

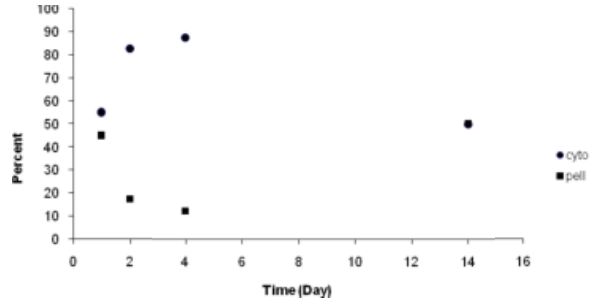

Fig. 5a: Cadmium percent in soluble and insoluble fractions of the kidney after exposure to 50 µg/L Cd , n=6 in every group

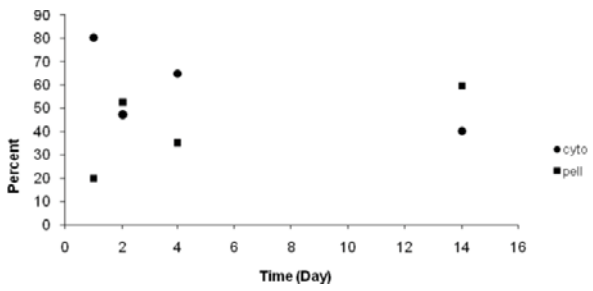

Fig. 5b: Cadmium percent in soluble and insoluble fractions of the kidney after exposure to 400 µg/L Cd , n=6 in every group

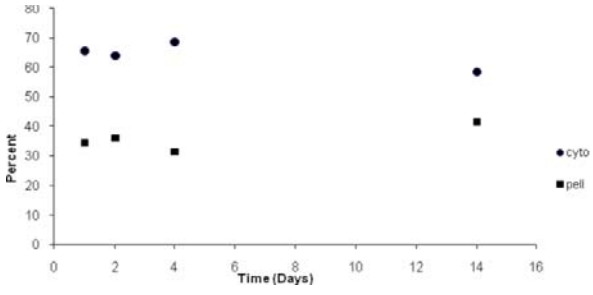

Fig. 5c: Cadmium percent in soluble and insoluble fractions of the kidney after exposure to 1000 µg/L Cd , n=6 in every group

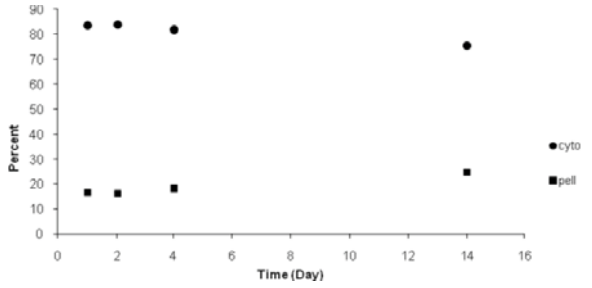

Fig. 6a: Cadmium percent in soluble and insoluble fractions of the liver after exposure to 50 µg/L Cd , n=6 in every group

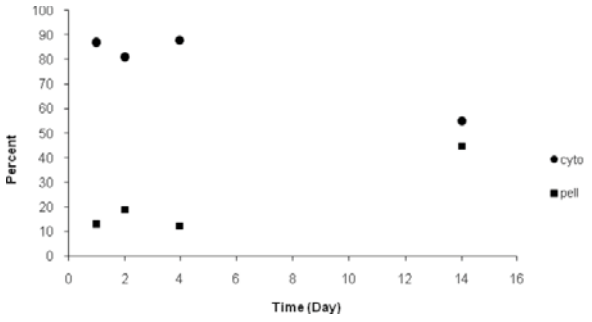

Fig. 6b: Cadmium percent in soluble and insoluble fractions of the liver after exposure to 400 µg/L Cd , n=6 in every group

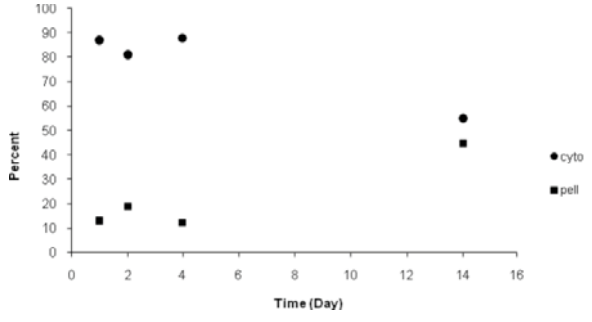

Fig. 6c: Cadmium percent in soluble and insoluble fractions of the liver after exposure to 1000 µg/L Cd , n=6 in every group

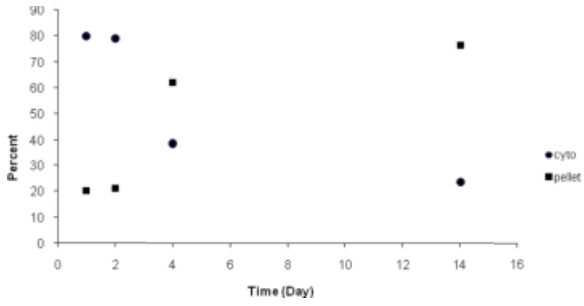

Fig. 7a: Cadmium percent in soluble and insoluble fractions of the gill after exposure to 50 µg/L Cd , n=6 in every group

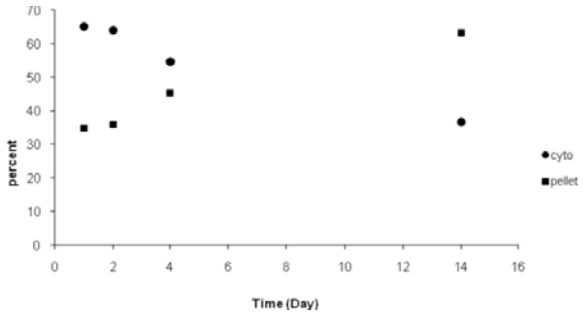

Fig. 7b: Cadmium percent in soluble and insoluble fractions of the gill after exposure to 400 µg/L Cd , n=6 in every group

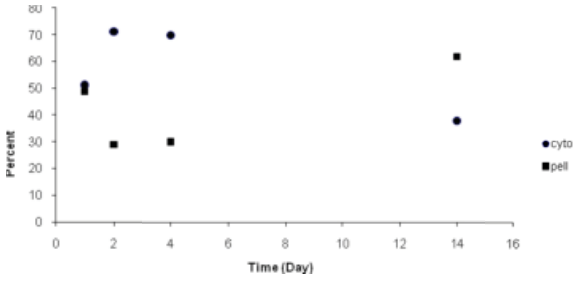

Fig. 7c: Cadmium percent in soluble and insoluble fractions of the gill after exposure to 1000 µg/L Cd , n=6 in every group

enzymes. Campenhout *et al*. (2010) found that metal distribution in soluble and insoluble is different related to tissue and metal type. They concluded that 60-70 percent of metals exist in soluble part of the liver and 50 percent of Cd is in the soluble part of kidney in carassius auratus that is consistent with the findings of this research.

In the gills (Fig. 7a-c), Cd accumulated in both the soluble and insoluble fractions, but mainly in soluble fractions. The relative contribution of insoluble fraction to total Cd accumulation increased with exposure time. Only in 1000 µg L-1 group, Cd concentration was increased till four days and then decreased. Apparently, the major part of Cd is entered to insoluble part that maybe exists in metal rich granules or enters cell organelles and causes deleterious tissue damage. These are in accordance with the results of a study done on Cd farctionation in gill of Antarctic bivalve Laternula Elliptica that showed Cd in insoluble farction increased with the time (Choi et al., 2007).

CONCLUSION

The results of this study indicate that Cd accumulates in liver of Persian sturgeon more than other tissues. The muscle accumulates the lowest amount of Cd that is a benefit for human use as sea foods, because flesh is used by consumer.

ACKNOWLEDGEMENTS

This study is part of a Ph.D thesis. The authors thank Shahid Dr Beheshti Hatchery personnel for their assistance in preparing experimental fish and Agricultural Biotechnology Research Institute of Iran experts for their help. Also, authors gratitude Dr Jamalzad for his assistance in statistical analysis.

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How to cite this article: (Harvard style)
Shariati, F.; Esmaili Sari, A.; Mashinchian Moradi, A.; Pourkazemi, M., (2012). Metal bioaccumulation in Persian sturgeon after sublethal exposure. Int. J. Mar. Sci. Eng., 2 (1), 81-90.