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 trophic 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 (Messaaoudi 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 al., 2010).

ABSTRACT: Tissue metal accumulations (gills, liver, kidney and muscle) in Persian sturgeon (Acipenser persicus) were compared following 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
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 (Messoudi 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 centrifugation) 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 LC₅₀ of Cd for Acipenser persicus was obtained as 4000 μgL⁻¹ (Mirzaee et al., 2003) and therefore, 400 and 1000 μgL⁻¹ concentrations of Cd were selected for Persian sturgeon exposure on the basis of being 0.1 and 0.25 LC₅₀, 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 filtration) 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
CALIBRATION GRAPH AND Sb DETERMINED, TOO. THEY WERE MEASURED WITH THE SAME CADMIUM CONCENTRATION IN WATER AND FOOD PELLETS WAS CONCENTRATION OF Cd.

DURING EACH DIGESTION RUN, BLANK (HNO3 AND ULTRA PURE WATER) WAS INCLUDED. THE AVERAGE READING OF BLANKS (1.5 µL ml-1) AND 113% VIA SPIKING WEIGHT. RECOVERY PERCENTS WERE CALCULATED BETWEEN 97 AND 113% VIA SPIKING FISH MUSCLE WITH FIVE DIFFERENT CONCENTRATIONS OF Cd.

Cd CONCENTRATION IN WATER AND FOOD PELLETS WAS DETERMINED 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 Cd, METAL, 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 DETRIMENTAL 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 CaCO3; 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 Ci, 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. 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

<table>
<thead>
<tr>
<th>Cd exposure concentration</th>
<th>50 µg L⁻¹</th>
<th>400 µg L⁻¹</th>
<th>1000 µg L⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>y = 70.96 days + 234.2</td>
<td>y = 216.4 days + 273.1</td>
<td>y = 427.8 days + 177.3</td>
</tr>
<tr>
<td>Liver</td>
<td>y = 46.95 days + 473.6</td>
<td>y = 257.6 days + 832</td>
<td>y = 893.3 days + 468.2</td>
</tr>
<tr>
<td>Gill</td>
<td>y = 43.99 days + 259.4</td>
<td>y = 160.4 days + 1175</td>
<td>y = 447 days + 900.2</td>
</tr>
<tr>
<td>Muscle</td>
<td>y = 0.454 days + 4.484</td>
<td>y = 6.26 days + 5.841</td>
<td>y = 21.13 days + 6.384</td>
</tr>
</tbody>
</table>

![Fig. 1: Changes of cadmium concentration in different tissues after 1 day exposure (n=6)](image1)

![Fig. 2: Changes of cadmium concentration in different tissues after 2 days exposure (n=6)](image2)
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 copper 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 anularis, Scorpæna porcus and Scorpæna 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), Urena 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>
<thead>
<tr>
<th>Tissue</th>
<th>50 µg L-1</th>
<th>400 µg L-1</th>
<th>1000 µg L-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>23.88</td>
<td>8.025</td>
<td>6.04</td>
</tr>
<tr>
<td>Liver</td>
<td>17.26</td>
<td>10.92</td>
<td>12.8</td>
</tr>
<tr>
<td>Gill</td>
<td>22.68</td>
<td>8.033</td>
<td>7.077</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.201</td>
<td>0.229</td>
<td>0.297</td>
</tr>
</tbody>
</table>
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 glutathione 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
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 fractionation in gill of Antarctic bivalve Laternula Elliptica that showed Cd in insoluble faction 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.

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Metal bioaccumulation in Persian sturgeon after sublethal exposure


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