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A Quantitative Analysis of Lead, Mercury and Cadmium Intake by Three Commercial Aquatics, *Hypophthalmichthys molitrix*, *Onchorhynchus mykiss* (Walbaum) and *Fenneropenaeus indicus*

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Abstract: At the present study, the distribution of Cd, Hg and Pb in water and their accumulation in fish species (silver carp, rainbow trout and shrimp) in three different farms. Totally 63 samples of three species and the water of their ponds were obtained during summer 2008 from Gilan, Mazandaran and Chabahar-Iran. The concentrations of three heavy metals in fish and shrimp flesh were measured by flameless atomic absorption spectroscopy. Only Lead was found in fish at mean concentrations above the permissible limits proposed by US FDA and EPA. Pb and Hg in the studied species showed the following pattern: silver carp > rainbow trout > shrimp, because Lead and mercury intake by species such as silver carp via phytoplankton consumption seems to be more logical. The accumulation of Cd in rainbow trout found to be more than it in silver carp. Metalloenzyme, an enzyme which found in plankton (the principle food diet of silver carp) that specifically uses cadmium to achieve its biological function, probably is a reason of cadmium increase in water and flesh samples of rainbow trout than that in silver carp. On the other hand, results showed that the significantly lower levels of contamination in shrimp, the main reasons of which might be the short period of feeding of the shrimp and an active mechanism of secretion.

Key words: Mercury · Cadmium · Lead · *Onchorhynchus mykiss* · *Hypophthalmichthys molitrix*

INTRODUCTION

Heavy metals are complex mixtures, which may cause environmental damage [1, 2]. The presence of metals in the environment is partially due to natural processes, such as volcanic activity and erosion [3, 4], but mostly is the result of many different anthropogenic sources, such as industrial, municipal, or agricultural wastes [5, 6]. The concentration of inorganic compounds in many general diet have decreased over the past 25 years. However, there is concern on some elements such as mercury and arsenic, may still be present in the diet at relatively high limits as a result of the consumption of certain food types [7]. The mercury, lead and Cadmium are non-essential toxic metals which are distributed and released into the aquatic environment by industrial sources such as mining, refining of ores, plating process, the use of phosphate fertilizers and gasoline containing Lead that leaks from fishery boats and oil ships [8, 9]. The methyl mercury arguably is the most dramatic and best documented example of high bioaccumulation [10]. The methyl mercury is an organic material that conforms from inorganic mercury. The contamination of mercury in seafood such as fish, shellfish, oyster and other types of seafood is one of great concern in places suffering from pollution humans [11-13].

The cadmium and lead are also common heavy metals with highly toxicity which present in effluents released from industrial activity [14]. The cadmium, lead and mercury injure the kidney and cause symptoms of chronic toxicity, including impaired kidney function, poor reproductive capacity, hypertension, tumors and

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hepatic dysfunction [15]. The effects of long-term exposure to the cadmium can include larval mortality and temporary reduction in growth. Histological effects of near-lethal cadmium concentrations included blackening and damage to gill filaments. Terrestrial invertebrates, on the other hand are relatively insensitive to cadmium exposure, probably due to the presence of effective sequestration mechanisms in specific organs [16]. The cadmium toxicity in fish is characterized by ionic imbalance with reduced plasma Ca (II), Na (II) and Cl-. The probable mechanism underlying this toxicity is inhibition of ion-transporting enzymes by cadmium (II) present in gill membranes. The cadmium has also been shown to interfere with calcium metabolism [17].

The lead usually exists in natural waters as Pb (II). It reacts readily with some major anions (CO_3^{2-}, SO_4^{2-}) at pH values typical of waters. The lead has a high ability and forms stable complexes with S-, P-, O- and N-containing organic ligand which leads to its accumulation in live and dead aquatic biota. At low concentrations of soluble organic ligand, Pb exists primarily in particulate form at pH> 6 [17].

For many cultures, such as Iran, fish and shrimp and their fish consumption are an integral part of daily life. Although fish provides a healthy and nutritious source of protein [18]. The limits of contaminants in fish and shrimp have led to health concerns, particularly for high-risk populations, such as pregnant women and children. [7, 19]. Fish and shrimp are often at the top of the aquatic food chain and may concentrate the large amounts of some metals from the water. Accumulation patterns of contaminants in them depend both on uptake and elimination rates [20, 21].

The present study was undertaken to determine accumulation quantities of some heavy metals (Pb, Hg and Cd) in water and three aquatic species from different sources. Therefore, in the current study the amounts of Pb, Hg and Cd were measured in two fish species include of *Hypophthalmichthys molitrix* (warm water fish) and *Onchorhynchus mykiss* (Walbaum) (cold water fish) and shrimp *Fenneropenaeus indicus*, species that are among the most consumed by the general population.

MATERIALS AND METHODS

Sampling: Totally 63 samples were collected in equal numbers of *Hypophthalmichthys molitrix, Onchorhynchus mykiss* (Walbaum) (350±20 g), which were obtained from different warm and cold fish farms from north of Iran (Guilan and mazandaran) and 21 samples of shrimps, *Fenneropenaeus indicus* (13±32 g) from south of Iran (Chabahar shrimp farm) during summer 2008. At the same time, the water samples were collected from three different farms. After preparation of samples, they were transferred to the toxicology laboratory of veterinary faculty of Tehran University. Abdominal content, gills, head and tail were discarded and flesh samples only were homogenized.

Cadmium and Lead in Flesh (Graphite Furnace Aas. Method): The homogenized tissue samples (2g) were digested with a mixture of Nitric acid (20 mL) and perchloric acid (5 mL) with mild heating until a whitish vapor appeared. After whole digestion, samples were kept in room temperature to become cold. Adding 10 mL of Hydrochloric acid 9% to the samples was the consecutive process. Furnace test then were used as a last implementation. Finally, Cadmium and Lead in flesh was measured by flameless atomic absorption spectroscopy [9, 22].

Mercury in Flesh (Wet digestion): Mercury was analyzed using cold vapor atomic absorption by heating in batch process according to the method [23].

1.0 g of homogenized flesh was added to 5 mL concentrated H_2SO_4 and 2 mL concentrated HNO_3. The final solution then was digested and dissolved at 90°C. 20 mL KMNO4 (5%) was added to sample which was taken from resultant solution and then digestion process was repeated in water bath until the foam disappeared. The sample was taken from the water bath and 5 ml hydroxyl ammonium chloride 10% and 0.5 ml 1-octanol solution were added to it. Finally sample diluted with de-ionized water. Then, 10 g of sample was mineralized by boiling for 10-20 minutes in a mixture of 11 mL of nitric acid and sulphuric acid (ratio 10:1) under a powerful reverse water cooler to prevent leakage of vapors. After 15-20 minutes, the clear yellow solution was cooled and distilled water was added to it. The determination of the amount of mercury in the mineralization product was performed by flameless atomic absorption spectrophotometer.

Mercury, Cadmium and Lead in Water (Graphite Furnace Aas. method): After filtering the water sample, filtered sample directly introduced to Graphite Furnace atomic absorption and read the measurement, ultimately [22].

Statistical method: Data analysis was performed by SPSS software, version 13. One Sample T-Test was used to determine significant difference of burden-heavy metals in compare to international limits. ANOVA test was used to determine the group of selected aquatics (including silver carp and rainbow trout), which has a significant difference in the amount of tissue residual of Pb, Cd, or Hg in compare to other groups.

RESULTS

The mean concentrations of Pb, Cd and Hg in two warm and cold water fish species and *Fenneropenaeus indicus* analyzed in this study are depicted in Tables 1. The maximum mean concentrations of Pb, Cd and Hg in silver carp and rainbow trout fish were 1001.9±237.9, 7.2±1.3, 234.6±107.4, 249.4±88.6, 36±32.2 and 22.1±0.8μg/kg (ppb), respectively (Table 1). The maximum mean concentrations of Pb, Cd and Hg in *Fenneropenaeus indicus* shrimp were 153.9±72.0, 5.6±3.9 and 24±1.4 μg/kg (ppb), respectively (Table 1).

Although, the mean value of lead was higher than lead maximum residual limit (MRL=1000 ppb) in Silver carp, but they didn't show significant difference ($P>0.05$). In the other hand, the mean value of lead was significantly lower than MRL in Rainbow trout and shrimp ($P<0.05$).

The Hg accumulation in silver carp flesh was significantly higher than it in flesh of rainbow trout and shrimp ($P<0.05$) (Table 1). Despite of Hg, there was significant increase of Cd concentration in rainbow trout tissue in comparison with silver carp and shrimp ($P<0.05$) (Table 1). The maximum mean concentrations of Pb, Cd and Hg in silver carp and rainbow trout fish farm water body were 10.6±0.6, 2.2±0.03, 0.53±0.003, 10.2±0.3 2.3±0.03 and 0.52±0.002μg/l (ppb), respectively (Table 1). The maximum mean concentrations of Pb, Cd and Hg in *Fenneropenaeus indicus* shrimp farm water body were 10.3±0.2, 2.3±0.1 and 0.53±0.01 μg/l (ppb), respectively (Table 1).

However, the amount of Hg in both warm and cold fish and shrimp farms water body were less than Hg maximum residual limits in them and there was no significant differences in Hg concentration in different farms water ($P>0.05$) as shown in Table 2. The water body Cd concentration was higher than Cd maximum residual limits in water, although they had shown no significant difference ($P>0.05$) as indicated in Table 2. Also the Pb concentration in cold water farms was higher than its MRL ($P>0.05$) as shown in Table 2.

The results showed that the accumulation of Pb and Hg in silver carp and shrimp flesh were significantly higher than Cd mean value ($P<0.05$) (Fig.1). In contrast, cold water fish Cd concentration showed significant increase in comparison with warm water ($P<0.05$) (Fig. 2).

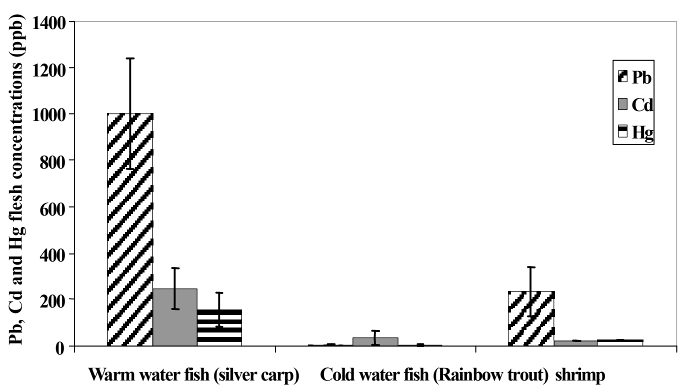

Fig. 1: Concentration of heavy metals in fish and shrimp samples collected from fish and shrimp farms

Table 1: Concentration of heavy metals in fish and shrimp flesh samples collected from fish and shrimp farms with unit Concentration of μg/kg (ppb)

	Silver carp		Rainbow trout		*Fenneropenaeus indicus*	
	Maximum residual limit	Mean in flesh ± SE	Maximum residual limit	Mean in flesh ± SE	Maximum residual limit	Mean in flesh ± SE
Pb	1000	1001.9±237.9	1000	249.4±88.6	1000	153.9±72.0
Cd	1000	7.2±1.3	1000	36±32.2	3000	5.6±3.9
Hg	1000	234.6±107.4	1000	22.1±0.8	1500	24±1.4

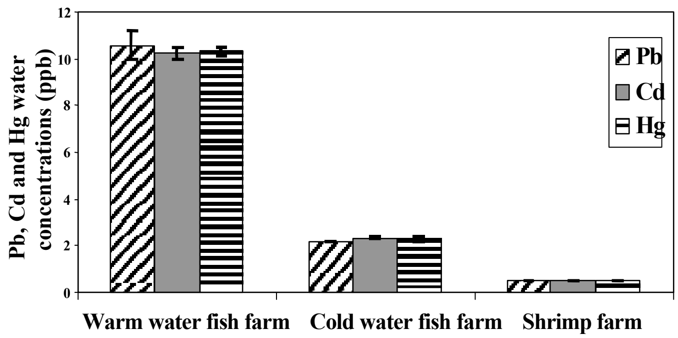

Fig. 2: Concentration of heavy metals in water samples collected from different fish and shrimp farms

Table 2: Concentration of heavy metals in water samples collected from different farms with unit Concentration of μg/l (ppb)

	Warm water fish farms		Cold water fish farms		Shrimp farm	
	Maximum residual limit	Mean in water±SE	Maximum residual limit	Mean in water±SE	Maximum residual limit	Mean in water±SE
Pb	70	10.6±0.6		8	10.2±0.3	100 10.3±0.2
Cd	1	2.2±0.03		0.2	2.3±0.03	2 2.3±0.1
Hg	2	0.53±0.003		1	0.52±0.002	2 0.53±0.01

DISCUSSION

In recent years, a notable numbers of surveys were carried out in different countries have determined the concentrations of metals in various edible aquatic species. However, comparison among studies is not always easy, as fish and seafood species in the different surveys are not generally the same. Fish are often at the top of the aquatic food chain and may concentrate large amounts of some metals, such as lead, cadmium, chromium, copper, mercury, zinc and iron. These metals accumulate differentially in fish organs and cause serious health hazards to humans. For this reason, the problem of fish and other aquatic contamination by toxic metals has received much attention [24]. At the present study, the data of numbers heavy metals were provided for tissues of two fish species, shrimp and water body of three farms. As mean values, such heavy metals were generally found at concentrations not exceeding 10.57, 10.2 and 10.3 ppb for Pb in warm and cold water and shrimp farms, respectively. The highest limits of lead and mercury were both found in water samples which had been taken from individual warm water fish farms. On the base of the present study results, accumulation pattern of the measured heavy metals in the water is according to the following order, Pb>Cd>Hg. Also, a report [25] shown that the lead, cadmium and mercury were the most heavy metals detected in water samples from considered lake and their accumulation pattern was indicated as Pb>Cd>Hg in all seasons. The WHO has recommends that food with mercury levels of$\geq$ 0.5 mg/kg should not be consumed by human [26]. Also in Japan, due to the high consumption of fish, the government has recommended that fish with mercury concentration of $\geq$ 0.3 mg/kg should not be consumed [27]. Between the three species were studied, the silver carp was the most sensitive to heavy metals. As total heavy metal residues, silver carp contained 1001.95 ppb Pb and 234.6 ppb Hg, compared to 249.38 and 22.1 ppb in rainbow trout and 153.9 and 24 ppb in shrimp. Generally, the results f heavy metal analyses in fish indicate that warm water fish (silver carp), had the tendency to accumulate higher limits of Pb and Hg than cold water fish (rainbow trout). A research [28] shown that the heavy metals such as Cd, Cu, Hg, Pb and Zn in the benthivorous fish bream *Abramis brama* of Lake Balaton, Hungary, who was concluded that feeding was the main route for the uptake of heavy metals by adult fish which affected by the heavy metals contents of the sediments and biota rather than the ambient water. Also, other report [29] shown that the Cd, Cr, Cu, Ni, Pb and Zn in various fish species which were raised in secondary treated wastewater, reported that heavy metal accumulation in the fish tissues were more related to the heavy metal concentrations in the food than the water. The absorption of heavy metals from water consumption and through gills exposes fish to the amounts occurring in the ambient water, while feeding on organisms (like phytoplankton) that already concentrated the present heavy metals in the environment, increases amounts of metals ingested by fish [30]. The Phytoplankton were supplied approximately 70% of nutritional requirements of silver carp, a property which contributes notably to the accumulation of heavy metals and the fact that the silver carp culture period relatively long and it takes a year and half in north of Iran, so silver carp can accumulate a considerable amount of such heavy metals [31].

The role of phytoplankton as a natural food to complete food chains so important in carp and shrimp farms, but rainbow trout culture systems are less dependent to natural food availability and more dependent to commercial feeding [32]. The concentration of phytoplankton in current water is lower than warm water farms. Consequently, intake of lead and mercury by species such as silver carp via phytoplankton consumption seems to be more logical [33].

On the other hand, metalloenzyme is an enzyme which found in plankton such as diatom *Thalassiosira weissflogii* that specifically uses cadmium to achieve its biological function. This is the first cadmium enzyme that has been discovered [34]. This finding indicate that, there is direct relation between the amount of cadmium and growth of Plankton, that's why the amount of cadmium is higher in water samples of rainbow trout farms and fish flesh than that in silver carp (Table 1 and 2). Also, a research [17] reported that the cadmium approximately had the highest concentration among the measured heavy metals (Pb and Hg) in freshwater of Arctic area.

In the other hand, results showed significantly lower levels contamination in shrimp. The main reasons for the overall low levels of contamination might be the short period of feeding of the shrimp and an active mechanism of secretion. Besides this, the heavy metals load of the shrimp depended on the fishing season (November or May). The investigation was down by [35] shown that the mercury content in shrimp was 0.033-0.016 µg/g wet weight. It is lower amounts of the investigated elements than the levels of concern of the Federal Institute for Health Protection of Consumers and Veterinary Medicine, i.e. 0.5 µg Hg/g wet weights.

PNUE: Programme des Nations Unies pour l'environnement

FSA: Food Standards Agency Arctic Monitoring and Assessment Programme

EPA: Environmental Protection Agency US Agency for Toxic Substances and Disease Registry

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