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FATE AND EFFECT OF POLLUTANTS IN RIVERS: FROM ANALYSIS TO MODELLING

Mercury, lead, and cadmium in tissues of the Caspian Pond Turtle (*Mauremys caspica*) from the southern basin of Caspian Sea

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Abstract Concentrations of cadmium, lead, and mercury were measured in different tissues (liver, muscle, and shell) of 60 Caspian Pond Turtles collected from Tajan and Shiroud Rivers, southern basin of the Caspian Sea. Based on the results, different tissues showed different capacities for accumulating trace elements. The general trend of metals accumulation was: liver>shell>muscle. Results also showed that accumulation of these elements was not significantly different between sex and river in turtles ($p>0.05$). Based on the results, Hg and Pb concentrations recorded in the present study were higher than some of the maximum concentration permissible. To our knowledge, this is the first report into heavy metal accumulation in tissues and organs of Caspian Pond Turtle from the southern basin of Caspian Sea. Further studies are needed to measure different heavy metals and trace metals in this valuable species.

Keyword Caspian Pond Turtle · *Mauremys caspica* · Heavy metal · Tissues · Sex · Caspian Sea

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Introduction

Heavy metals are accumulated in environment by different ways, e.g., extraction, diffusion, combustion, and industrial activities, and then enter in the aquatic environment by discharge of domestic, industrial, agricultural waste water, accidental leakage, Ballast water discharges by ships and soil erosion (Bahnasawy et al. 2009; Javed 2005). These metals can be strongly accumulated along water and aquatic food chains, thus resulting in sub lethal effects or death in local fish populations and finally in human (Perez-Lopez et al. 2008). Study of some heavy metals, such as cadmium, mercury, and lead are so important due to their toxicity and bio accumulative behavior in aquatic organisms and even in human food chains (Kalantzi et al. 2013). Aquatic species diversity and equilibrium of ecological consumers can be modified by the effects of heavy metals. Aquatic organisms such as fish and turtles cause biomagnification by accumulate metals as Hg to high concentrations than those in water and prey organisms. Selection of biological components such as sea turtles or birds for wide distribution and high position in the food chain can be useful as bioindicator in health monitoring and predict future changes in the environment (Kim et al. 2007).

The Caspian turtle (*Mauremys caspica*) is a species of turtles in the family Geoemydidae, living in the eastern Mediterranean region from northwestern of Saudi Arabia, Iraq, Bahrain, Turkey, Caucasus, Tbilisi to northern, central, and south western part of Iran (Vamberger et al. 2013). This species is widely dispersed in different provinces of Iran, e.g., Mazandaran, Golestan, Guilan, Ardabil, Azerbaijan, Kurdistan, Fars, and Khuzestan (Iverson 1994). There have been many reports of this species in Tajan and Shiroud Rivers. The effects of heavy metals and other contaminants in Caspian Sea fishes have been previously documented (Nasrollahzadeh Saravi et al. 2013), but with the given available data, only one study was done by Yadollahvand et al. (2014) on different tissues of Caspian turtle in Golestan province. On the other hand, Tajan and Shiroud were selected as locations which had agriculture and industrial activity and pesticides such as organophosphates (cinosulfuron, triadimenol, phosphamidon, and tricyclazole). However, despite the importance of biodiversity and high frequency of this valuable species, few studies have been done on it in Mazandaran province. Therefore, this study is not only to measure and compare lead (Pb), mercury (Hg), and cadmium (Cd) in different tissues of both sexes Caspian turtle in Tajan and Shiroud Rivers but also is a complementary study on this species in other rivers of the Caspian Sea basin.

Material and methods

Sixty adult Caspian Pond Turtles were collected from Tajan and Shiroud River in Mazandaran province (Fig. 1), during September and October 2014 in the rainy season. Their feeding habit includes water plants, mollusks, crustaceans, small amphibians, and small fish. Permission to collect of Caspian Pond Turtle was authorized by the Iran Department of Environment (Permission Number: 1184-N/14/2; 2014; 22th September). The Tajan River is one of the most important river, has a watershed of 1.80 million km^2 (36° 43′ 36″ N and 53° 7′ 36″ E). Shiroud River is located in the distance of 8 km from Tonekabon and 15 km from west part of Ramsar at Mazandaran province (44° 50′ 36″ N and 45° 2′ 36″ E).

The turtles were transported alive to the central laboratory of Caspian Sea Ecology Research Center, and their size in curved carapace length (CCL in cm) and weight (g) were measured. They were euthanasia by 200 mg/kg of ketamine hydrochloride (5 %) and 3 mg/kg of diazepam (5 %) before analysis and were dissected with laboratory set, and different tissues of liver, muscle, and shell were quickly removed, washed with distilled water, and refrigerated at −20 °C until chemical analysis (Yadollahvand et al. 2014). All the laboratory materials used were completely acid-washed to prevent contamination of samples (Paez-Osuna et al. 2010).

The procedure used for measuring trace elements concentrations in turtle samples has been described previously (Yadollahvand et al. 2014) with minor modifications. All samples were dried by oven and homogenized using blender. Approximately 0.3 g of the homogenized powder of dried sample was added to 4 ml of concentrated (%65) ultra pure HNO_3 (Merck, Darmstadt, Germany) in a closed cell, polytetrafluoroethylene (Teflon™) lined digestion vessel and incubated for 1 h at 40 °C, followed by heating at 140 °C for 3 h. For mercury digestion, the sample was added to 45 mg V_2O_5. Then, they were diluted to 50 ml with 20 ml distilled water and $K_2Cr_2O_7$ (%2). Samples were filtered through Whatman No. 1 filter paper and then analyzed. Concentrations of Cd and Pb by graphite furnace and Hg using vapor generation were measured using an atomic absorption spectrometer (Thermo M5, Japan). Quality assurance was assessed for each batch of 20 digested samples by inclusion of two blanks and reference materials (RM) TORT-2. Calibration curves were made for the spectrophotometer using SIGMA 3000 (Perkin Elmer). The limit of detection (LOD) for Pb, Cd, and Hg were 0.70, 0.33, and 0.39 ppb, respectively. To rule out possible contamination, weight samples were used with deionized water. To determine the percentage of recovery and evaporation during the digestion process, replicates were used of the reference material (RM) TORT-2. Recovery of RM (TORT-2) and standard repetition added with

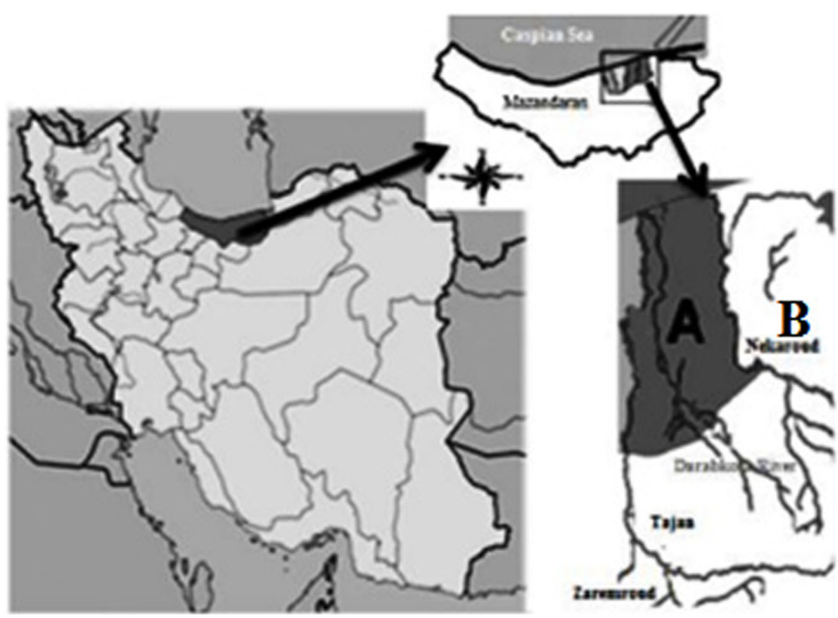

Fig. 1 Sampling sites of Tajan (**a**) and Shiroud (**b**) Rivers (north of Iran), Mazandaran Province (Caspian Sea basin)

Table 1 Biometry data of Caspian pond Turtle from the Tajan and Shiroud Rivers in Mazandaran province Iran

River	Sex	CCL (cm)	Weight (g)	Age (year)
Shiroud	Male	11.3±3.8	277.3±14.2	3±1
	Female	10.84±2.6	271.8±10.6	3±1
	Statistical test	p=0.126	p=0.108	p=0.324
Tajan	Male	11.5±4.1	285.3±13.9	3±1
	Female	10.9±3.5	276.8±10.4	3±1
	Statistical test	p=0.365	p=0.282	p=0.337

The statistical test used is the analysis of variance (ANOVA); statistical test data as mean±SD followed by Duncan's test in parentheses, if significant differences were found. Data are presented as mean±SD. Values in each row with different superscripts show significant difference (p<0.05)

SIGMA 3000, and the percentage of recovery was between 87 and 95 %. The coefficient of variation on replicate, spiked samples ranged up to 10 %. Concentrations of trace elements are expressed as mg kg^{-1} of tissue on dry weight basis.

Reported statistics are arithmetic means and standard deviation (SD). All data obtained from the two sampling sites were used for statistical analyses using SPSS 18.0 Software. One-way analysis of variance (ANOVA) along with Duncan's method was carried out to examine mean differences among the tissues. In this study, p values of ≤0.05 were used to determine significant differences. The correlations among the different heavy metals in tissues were determined using a simple regression model R^2<50 % used as a statistical indicator. Arithmetic means is given to facilitate comparisons with other studies.

Results

The turtles biometric characteristics captured are shown in Table 1. No statistical differences were observed in size or weight by sex or among rivers specimens. The specimens captured can be considered sub-adult by the size.

The heavy metals concentration in various tissues of Caspian Pond Turtle from the Tajan and Shiroud Rivers are presented in Table 2. The results showed that metals concentrations were statistically different among tissues of Caspian Pond Turtles (Pb: $F_{(2, 57)}$=68.1, p=0.001; Cd: $F_{(2, 57)}$=87.3, p=0.001; Hg: $F_{(2, 57)}$=116.4, p=0.001). Pb concentrations were highest among the trace elements examined, while concentrations of Hg were generally lowest in analyzed tissues (Table 2). The trend of metals accumulation was liver>shell>muscle (Table 2). Metal concentrations were generally low in muscle, except for Hg which exhibited the highest mean level than shell. The Duncan's results showed three different groups for all tissues (Table 2). In the present study, mean Pb concentrations in different tissues varied from 21.88±1.27 to 35.46±1.90 mg kg^{-1} dry weight in Caspian Pond Turtle, and the distribution patterns of Pb concentration follows the order: Pb_{liver}>Pb_{shell}>Pb_{muscle} (p value=0.008). Statistical differences were observed in Pb concentration between the three tissues. Mean concentration of Cd and Hg varied from 4.82±2.82 to 2.10±0.10 and 2.79±0.19 to 0.95±0.04 mg kg^{-1} dry weight, respectively. The accumulation patterns of Cd and Hg following these sequences: Cd_{liver}>Cd_{shell}>Cd_{muscle} (p value=0.003) and Hg_{liver}>Hg_{muscle}>Hg_{shell} (p value=0.024) (Table 2). Results showed that accumulation of these elements was not statistically different among sex or river population in turtles (p>0.05). Pearson correlation shows that Pb, Cd, and

Table 2 Mean concentration (±SD) of heavy metals (mg/kg dry weight) in liver, shell, and muscle of Caspian Pond Turtle from the southern basin of Caspian Sea

River	Sex	Tissues	Pb	Cd	Hg
Shiroud	Male	Liver	35.46±1.90 (a)	4.29±0.19 (a)	2.70±0.13 (a)
		Shell	29.32±0.66 (b)	3.3±0.11 (b)	1.04±0.06 (b)
		Muscle	23.82±1.12 (c)	2.10±0.10 (c)	1.63±.0.11 (c)
		Statistical test	p=0.006	p=0.009	p=0.032
	Female	Liver	35.34±2.05 (a)	4.82±2.82 (a)	2.78±0.18 (a)
		Shell	29.7±1.35 (b)	3.4±0.16 (b)	1.05±0.08 (b)
		Muscle	22.12±1.57 (c)	2.29±0.19 (c)	1.66±0.14 (c)
		Statistical test	p=0.005	p=0.003	p=0.021
Tajan	Male	Liver	32.96±1.42 (a)	4.00±0.29 (a)	2.68±0.17 (a)
		Shell	28.8±1.32 (b)	3.43±0.12 (b)	0.95±0.04 (b)
		Muscle	24.28±1.17 (c)	2.13±0.11 (c)	1.57±0.07 (c)
		Statistical test	p=0.008	p=0.024	p=0.018
	Female	Liver	35.34±1.34 (a)	3.87±0.13 (a)	2.79±0.19 (a)
		Shell	28.1±1.02 (b)	3.36±0.13 (b)	1.08±0.09 (b)
		Muscle	21.88±1.27 (c)	2.19±0.15 (c)	1.61±0.08 (c)
		Statistical test	p=0.003	p=0.043	p=0.006

Hg were not significantly correlated with by sex and rivers in length and weight (Table 3) (R^2<0.50; p>0.05). No correlations were found in metals concentrations with size or weight (R^2<0.50; p>0.05).

Discussion

Pb concentrations reported in Caspian Pond Turtle tissues in this study present high concentrations that other metals, particularly in liver and previously reports for fresh water turtles (Overmann and Krajicek 1995; Bishop et al. 2010; Yu et al. 2011; Yadollahvand et al. 2014). Yadollahvand et al. (2014) mention that elevated Pb levels in the Caspian Pond Turtle are worrying, especially considering the fact that this toxic metal could produce serious damage to species health, including infertile eggs, and changes in behavior, growth, and survival of turtles (Zavala-Norzagaray et al. 2014).

The Hg concentrations in liver were 1.4–3.6 times higher than those reported in the Kemp's ridley turtles, *Lepidochelys kempii* (Innis et al. 2008), *Chelonia mydas* (Bezerra et al. 2014) Leatherback sea turtles, *Dermochelys coriacea* (Davenport and Wrench 1990; Perrault et al. 2013) and Loggerhead turtle, *Caretta caretta* (Caurant et al. 1999). High contamination levels of Hg may be related to the high frequency of fish-based diets (Zavala-Norzagaray et al. 2014). Hopkins et al. (2013) observed a negative correlation among Hg levels with reproduction season in the common snapping turtle (*Chelydra serpentina*) and increased the egg infertility and embryonic mortality. Also, Matson et al. (2005) mention that high level of Hg causes chromosomal damage in *Emys orbicularis* and *M. caspica* inhabiting contaminated sites in Azerbaijan (west of Caspian Sea). The Hg concentration found in the muscle tissue of Caspian Pond Turtle (1.65± 0.12) was 1.8–4.2 time higher than those measured in the other sea turtles such as: *Dermochelys coriacea* (Perrault et al. 2013), *Caretta caretta* (Storelli et al. 1998) and *Stenella coeruleoalba* (Storelli et al. 1998). High Hg concentration in muscle found in this study could be accumulated in human brain, liver, and kidney and cause nose irritation, skin burns, irritation of respiratory system, rashes, muscle coordination, and severe diseases such as acrodynia, Hunter-Russell syndrome, and Minamata disease (Tan et al. 2009; Rajeswari and Sailaja 2014).

Table 3 Pearson correlation of heavy metals at different sex and rivers in Caspian Pond Turtle from the southern basin of Caspian Sea

Heavy metals	Rivers/sex	Pb	Cd	Hg
Pb	Male	1	r=0.755[a]	r=0.418[b]
Cd			1	
Hg				1
Pb	Female	1	r=0.783[a]	r=0.385[b]
Cd			1	r=0.442[b]
Hg				1
Pb	Tajan	1	r=0.792[a]	
Cd			1	r=0.526[a]
Hg				1
Pb	Shiroud	1	r=0.721[a]	r=0.431[b]
Cd			1	
Hg				1
Pb	Total	1	r=0.764[a]	r=0.388[a]
Cd			1	r=0.386[a]
Hg				1

[a] Correlation is significant at the 0.01 level (2-tailed)

[b] Correlation is significant at the 0.05 level (2-tailed)

Highest Cd levels were observed in of Caspian Pond Turtle liver; these results are similar than previously observed by Yadollahvand et al. (2014) and higher than those previously reported in liver of green and olive ridley turtles (Gardner et al. 2006) and *Lepidochelys olivacea* (Paez-Osuna et al. 2010) (Table 4). Kitana and Callard (2008) mentioned that high level of Cd reduced proliferation and delay migration of germ cells to genital ridge, finally affect on gonadal developmental processes, and threat the reproductive success of freshwater turtles (*Trachemys scripta*, *Chrysemys picta*).

Certain factors such as body requirements of marine organisms for copper and zinc, excretion of cadmium, lead and mercury, and the other changes could play an important role in heavy metals accumulation in the living organisms (Filazi et al. 2003). Yadollahvand et al. (2014) published that accumulation of these elements was not statistically different among sex, which was confirmed in this study. This shows that the metal accumulation is similar in the whole Caspian Pond Turtle populations in our study area; however, more studies about the relation among heavy metal concentrations and the turtles' age are necessary and have all essential information about whether factors such as age, weight, and size influence on processes of turtle pollution.

Our results indicated that the highest concentration of all metals is found in liver, which is a major tissue of short-term storage in turtles (Thomas et al. 1994; Rie et al. 2001). This is in accordance with previous publications on heavy metals accumulation in Caspian Pond Turtle (Yadollahvand et al. 2014), *Chelonia mydas* (Lam et al. 2004) and *Caretta caretta* (Sakai et al. 2000). Storelli et al. (2008) studied some heavy metals in *Chelonia mydas*; the results showed that Cd concentrations in muscle were about two fold higher than those in the liver. In this study, Cd had the higher concentration in the liver than muscle, suggesting the liver as the center of their accumulation (Torrent et al. 2004; García-Fernández et al. 2009). The difference in accumulation potential between tissues can be justified by the activity of metallothioneins, proteins that are created in liver and present in the muscle and other tissues,

Table 4 Concentrations (μg g^{-1} dry weight) of heavy metals in liver, shell, and muscle of sea turtles

River	Author	Tissues	Pb	Cd	Hg
Mauremys caspica	Current study	Liver	35.46±1.90	4.55±1.32	2.74±0.15
		Shell	29.5±1.82	3.35±0.12	1.04±0.08
		Muscle	23.06±1.06	2.2±0.14	1.65±.0.12
M. caspica	Yadollahvand et al. (2014)	Liver	32.41±6.22	4.29±0.19	NA
		Shell	21.54±7.15	3.54±1.06	NA
		Muscle	27.45±3.69	2.51±0.24	NA
Dermochelys coriacea	Perrault et al. (2013)	Liver	NA	NA	2.11±0.38
		Shell	NA	NA	0.45±0.06
		Muscle	NA	NA	0.83±.0.18
Aspideretes gangeticus	Malik et al. (2013)	Liver	1.93±0.32	1.93±0.32	NA
		Shell	0.98±0.19	0.85±0.03	NA
		Muscle	1.23±1.40	0.23±0.03	NA
Caretta caretta	García-Fernández et al. (2009)	Liver	0.69±0.08	5.85±0.14	NA
		Shell	NA	NA	NA
		Muscle	0.06±0.02	0.04±0.01	NA
Lepidochelys olivacea	Gardner et al. (2006)	Liver	3.32±0.30	3.28±0.22	NA
		Shell	NA	NA	NA
		Muscle	0.01±0.001	3.1±2.4	NA

which have the ability to bind certain trace elements and thus allow the tissue to accumulate them at a high degree (Barbieri 2009; Mashroofeh et al. 2013). Moreover, the differences in levels are the results of polluted water, sediments and foods, age, weight, body width and length, sex, and differences in physiological functions of different tissues and organs and physicochemical characteristics of water (temperature, hardness, and salinity) (Mashroofeh et al. 2013). This result of this study showed that there were no statistical differences in metal levels as a function of gender, rivers, size, and weight (R^2< 0.50; p>0.05). Therefore, gender, rivers, size, and weight do not play an important role to accumulation of these three toxic heavy metals in Caspian Pond Turtle. On the other hand, other aforementioned factors are most important to accumulate of Pb, Cd, and Hg.

In our study, Hg had the lowest concentration among other heavy metals which is in line with findings of Ley-Quinoez et al. (2011) on loggerhead turtles (*Caretta caretta*) and Bezerra et al. (2012) on green turtle (*Chelonia mydas*). Low accumulation of Hg in the tissues is directly related to nourishment and bioaccumulation capacity of this precious specimen (Malik et al. 2013). The Cd emission in rivers are mainly due to the production and use of artificial phosphate fertilizers, mineral phosphate, zinc production processes, and manure manufacturing industries are the most significant emission of cadmium sources (Bonomelli et al. 2003). In the current study, the accumulation of Cd in different tissues followed this pattern: liver>shell>muscle, which was in accordance with findings of Yadollahvand et al. (2014). but which differs from findings of Malik et al. (2013) on soft-shell turtle (*Aspideretes gangeticus*). Some of these activities are carried out in the river of our study area, and these factors could increase the Cd accumulation in Caspian Pond Turtles.

Hg and Pb concentrations recorded in the present study were higher than the acceptable values for designated by standards which is the consequence of Mazandaran wood and paper industry, Antibiotic Company, Municipal and village sewage, Cattle and Poultry Industries, Pasteurized milk factory, MDF Factories, Fisheries Farms, and agricultural activities (Hosseini et al. 2011).

The heavy metal concentrations present in Caspian Pond Turtle could be considered a risk, not only for this species but also for others organisms that develop in the region, including humans. Aguirre and Tabor (2004) established the use "sentinels" species, are species that serve as indicators of their environment. In this case, like the sea turtle, maybe our turtle species can serve as sentinels for the quality of health of aquatics ecosystems, and make contributions to the environmental authorities, who should also monitor the health of human society and make good solutions to reduce the disposal of industrial and toxic waste water to watershed of Caspian Sea and prevent the accumulation of heavy metals in coastal areas.

To our knowledge, this is the first report about heavy metal accumulation in tissues and organs of Caspian Pond Turtle from two rivers of southern basin of Caspian Sea. Further studies are needed to measure different heavy metal concentrations and blood parameter to establish the health risk in the populations of this species.

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