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Mercury Levels in the River Otters (*Lutra lutra*) of Iran: Feasibility of Back Calculation for Trace Elements Using Old Stuffed Specimens

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ABSTRACT Otters at the top of the food chain and feeding largely on fish are likely to be especially vulnerable to the effects of bioaccumulating pollutants. This research reports the first data on mercury levels found in hairs of river otters *(Lutra lutra)* from Iran. Between June and December 2007, we collected museum and recent collections of river otter hair. These samples were collected from several provinces of three major regions of Iran. The mean of mercury concentration for all samples (recent and museum) was 5.819±1.557 mg/kg with 95% confidence interval for the mean. The concentrations of Total Hg (THg) in hair ranged from 0.293 to 12.382 mg/kg. No significant variation among the sample regions existed. A comparison of THg concentrations between two decades (1986-1996 and 1996-2006) showed that these differences were significant ($p<0.01$). Also significant difference was found between museum and recent specimens. This study has shown that the river otter has been exposed to THg with increasing trend in two past decades.

Key words: *Back calculation, Hair, Iran, Mercury, River otter*

1 INTRODUCTION

Eurasian otters are distributed through most of Iran's freshwater ecosystems. It had already recorded in many rivers, lakes and pools in various regions at least 13 provinces such as Gilan, Mazandaran, Azarbayejan, Tehran, Kordestan, Kermanshah, Markazi, Isfahan, Khorasan, Chaharmahal-Bakhtiari, Fars, Khozestan, and Lorestan provinces. Since, there are no comprehensive reports on their occurrence (Karami *et al.,* 2006, Rasooli *et al.,* 2007, Mirzaei *et al.,* 2009). It can be found on most rivers and being absent only from the central desert region (Gutleb *et al.*, 1996; Ishunin 1977; Melisch *et al.,* 1996). The species can be found in the Zagros, Elbruz and Koppet-Dagh mountain range and in Iranian Azarbaiejan. It is present in the Hamoon Wetland bordering with Afghanistan and found on the south shores of the Caspian Sea (Ziaie and Gutleb, 1997).

Eurasian otters have been hunted cruelly in Iran because of their economic value, and because they are carnivores. It has been hunted to protect fish farms, and for their pelts for sale, for taxidermy and for decoration in houses and shops. On fish farms, they are hunted with traps, electric fencing and weapons, and this is

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an important factor in reducing numbers. In the north, otters are drowned in fishery nets, where as in the central provinces, habitat destruction and riverbank degradation are the problem. In Iran there was no any information about otter deaths due to pollution (Karami *et al.,* 2006). According to these threats, it classified in NT taxon (near threatened) by the World Conservation Union Mammal Red Data Book (Baillie *et al.,* 2004).

Recently dramatically decline of otter population has been announced by DoE of Iran (Governmental Department of the Environment).

Mercury (Hg) occurs naturally in the environment and as a result of anthropogenic sources. Wildlife species occupying high trophic levels, such as the river otter, are more vulnerable to the toxic effects of Hg (Fortin *et al.,* 2001; Osowski *et al.,* 1995) Wildlife exposure to Hg is primarily due to the consumption of contaminated fish (Eisler, 1987). River otter (*Lutra lutra*) are piscivorous mammals, obtaining up to 90% of their diet as fish (Evans *et al.,* 1998).

Acute and/or chronic exposure to Hg can lead to important health problems and may influence survival and reproduction in top predators that are exposed to the greatest levels of contamination as a consequence of bioamplification along the food chain (Sample and Suter, 1999). Heavy metals like mercury may be partially responsible for the observed decline of the European (*Lutra lutra*) and North American (*Lutra canadensis*) otters in many countries (Dias Fonseca *et al.,* 2005).

Most of the previous studies of mercury in mammals have measured concentrations in tissues like liver and kidneys. Hair has also been used as an indicator of mercury levels in otters in previous studies (Dias Fonseca *et al.,* 2005). Hair possesses advantages as a biopsy material since it can be collected without harming animals and requires no special storage facilities in the field (Cumbie, 1975).

Depending on the stability of Hg in hair over time, it also may be possible to use hair samples from museum collections or other historical deposits to evaluate longer term changes in environmental exposure. From a toxicological viewpoint methyl form of Hg are most significant. However, analytically it is much easier to measure total Hg. Then, it would be best if total Hg concentrations in hair could be used as a surrogate for MeHg in internal tissues such as liver. Total hair concentrations of Hg are strongly correlated with methyl Hg concentrations in liver, indicating that hair is a suitable monitoring tissue (Evans, *et al.,* 1998).

Although aquatic mercury contamination is widespread and high concentrations of mercury have been detected in a variety of piscivorous birds and mammals from several areas of the world. Mercury contamination of Iranian mammals has not been reported yet. Thus this research reports the first data on mercury levels found in hair of river otters (*Lutra lutra*) from Iran.

The aim of this study was two-fold: baseline reporting for concentrations of THg in hairs from river otter in Iran and evaluating the historical and spatial trends of THg by analysis of otters' hair from museum collections.

2 MATERIAL AND METHOD

2.1 Specimen Sources

Twelve museums were contacted for river otter hair specimens. River otter hair were found in ten museums: Zanjan Museum of Natural History, (3 specimens); Kermanshah Museum of Natural History, University of Razi (2 specimens), Arak Museum of Natural History (1specimen), Bushehr Museum of Natural History (1 specimen), Gilan Museum of Natural History (1 specimen), Lorestan Museum of Natural Science (1 specimen), Tehran Museum of Sorkheh-hesar National Park (3 specimen), Babolsar Natural Science Museum (4 specimens). Another Museum of Natural History has several

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specimens but no hair sampling was allowed (Figure 1).

Five recent specimens of river otter were found in refrigerator temperature: -18) of DOE (Department of the Environment, Iran) that had obtained from illegal hunting of the taxon in Golestan province during 2006-2007.

Figure 1 Geographical situation of areas in which the samples were collected.

2.2 Collection Procedures

Otter hair was collected by cutting at the skin surface using a stainless steel scissors by simply pulling out tufts of hair. Samples were placed in envelopes. Each sample was labeled. Museum number, year or decade, location of collection, and other pertinent available information were applied for each tag. Similarly recent samples were collected .Then sent to the analytical laboratory and stored at room temperature.

2.3 Sample preparation

The samples of hair were transferred to the environmental laboratory of Faculty of Natural Resources and Marine Sciences for analysis. At first, the samples were washed with tap water. Then, they were washed with detergent followed by distilled water (3 times) and finally with acetone (Cumbie, 1975). Washing the samples removes all superficial contamination on the hairs. The hair samples were dried at temperature room in a dust-free atmosphere (Mason *et al.,* 1986). Finally they were changed into the powder.

2.4 Mercury Analysis

Mercury was measured by the LECO AMA 254 Advanced Mercury Analyzer (USA) according to ASTM, standard No. D-6722. The LECO AMA254 is a unique Atomic Absorption Spectrometer (AAS) that is specifically designed to determine total mercury content in various solids and certain liquids without sample pre-treatment or sample pre- concentration. Designed with a front-end combustion tube that is ideal for the decomposition of matrices, the instrument's operation may be separated into three phases during any given analysis: Decomposition, Collection, and Detection. In order to assess the analytical capability of the proposed methodology,

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accuracy of total Hg analysis was checked by running three samples of Standard Reference Materials (SRM), National Institute of Standards and Technology (NIST), SRM 1633b, SRM 2709, and SRM 2711 in seven replicates (Zolfaghari *et al.,* 2007). Recovery varied between 94.8% and 105% (Table 1.). As it can be seen, there is a good agreement between obtained mean and certified value. The detection limit of the method used was 0.001 mg/kg in dry weight and repeatability was 0.002 mg/kg or p5% RSD (N =6).

2.5 Statistical analysis

The statistical analysis was done by the SPSS software (Version 11.5). The data were tested for normality using a Kolmogorov–Smirnov test. The data were normally distributed. We used parametric procedures, independent-sample T test to evaluate effects of temporal and spatial trends on hair Hg concentrations. Also, we used one-way ANOVA, Duncan to compare mercury concentration in four provinces.

3 RESULT

3.1 Mercury Concentrations

Twenty-two museum and recent specimens were analyzed for THg .Total mercury was detected in all specimens from the 1986 to the 2007 and from different regions (provinces) in the Iran. The range of mercury concentration in the otters studied was between 0.293 mg/kg (for 1986 from Tehran province) and 12.382 mg/kg (for 2007, recent specimen from Golestan). The mean of mercury concentration for all specimens from the 1986 to the 2007 (recent and museum) was 5.819 ± 1.557 mg/kg with 95% confidence interval for the mean (Table 2).

Table 1 Results of quality assurance procedure for mercury (mg/kg).

SRM[a]	No.	Certified value	Mean	SD[b]	R[c] (%)
NIST-1633b	6	0.141	0.134	0.042	94.8
NIST-2709	6	1.400	1.470	0.131	105
NIST-2711	6	6.250	6.438	0.197	103

[a] Standard reference material, [b]Standard deviation, [c]Recovery.

Table 2 Concentration of Total mercury (mg/kg, dry weight) in hair of rive otter form several provinces of Iran.

Province	Geographical location	THg in hair	n
Golestan	north	8.706	5
Mazandaran	north	4.822	4
Gilan	north	3.204	1
Zanjan	west	4.275	3
Lorestan	west	7.854	1
Hamedan	west	5.67	1
Kermanshah	west	6.21	2
Markazi	Central	7.576	1
Tehran	Central	2.797	3
Bushehr	South	7.264	1
	Total		22

Min	Max	Mean	SD	SE	n
0.293	12.382	5.819 ± 1.557	3.515	0.794	22

%95 confidence interval for the mean.

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In order to examine spatial variation, the mercury concentrations between four provinces including Golestan, Mazandaran, Zanjan and Tehran were compared. We did not find a significant difference in hair Hg concentrations between four provinces. Also in this order every province were compared with another province (for four mentioned provinces). Result showed that only Golestan and Tehran provinces had a significance difference at a confidence level of %95 ($p<0.05$).

The mean THg concentration for north specimens (north province including Mazandaran, Golestan, Gilan) from the period of 1986 to 2007 was 6.602± 2.261 mg/kg; range 0.879 to 12.382 (n = 10). The mean THg concentration in west specimens (west provinces including Zanjan, Kermanshah, Hamedan and Lorestan) from 1986 to 2005 was 5.539 ± 3.381 mg/kg; range 0.975 to 9.931 (n=7). There was no statistically significant difference in the THg of west versus north (Table 3).

To assess temporal trends, according to decade of collection (1986-1996 and 1996-2006) museum samples were grouped (Table 4). The mean THg concentration for hair samples from the period of 1986 to 1996 was 3.14± 2.491 mg/kg; range .293 to 9.43(n = 9). The mean THg concentration in otter hair samples from 1996 to 2006 was 7.029± 1.685 mg/kg; range 3.204 to 9.931 (n = 8). These differences were significant ($P < 0.01$). (Table 5).

A comparison of THg concentrations of museum specimens from the period of 1986-2006[mean= 4.97± 1.708 mg/kg (n = 17)] versus recent samples from 2006-2007 [mean = 8.706± 3.349 mg/kg (n= 5)] showed that recent samples had a significantly higher ($P < 0.05$) concentration of THg than museum specimens (Table 6).

Table 3 Mercury concentration (mg/kg, dry weight) in north versus west.

Geographical location	n	Mean	Min	Max	SD	SE
North	10	6.602± 2.621	0.879	12.382	3.664	1.185
West	7	5.539± 3.381	0.975	9.931	3.664	1.381

Table 4 Grouping of the museum specimens according to decade of collection.

Decade	n	Mean	Min	Max	SD	SE
1986s (1986-1996)	9	3.14± 2.49	0.293	9.43	3.24	1.08
1996s (1996-2006)	8	7.029± 1.685	3.204	9.931	2.014	0.712

Table 5 Results of independent samples t-test for comparing mercury concentrations (mg/kg) between two decades (1986s and 1996s).

Decade1986s		Decade 1996s		t	df	p-value
Mean	SD[a]	Mean	SD[a]			
3.14	3.24	7.029	2.014	-2.9	15	<0.01

[a]Standard deviation

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Table 6 Results of independent samples t-test for comparing mercury concentrations (mg/kg) between recent and museum samples.

1986-2006			2006-2007			t	df	p-value
N[a]	Mean	SD	N	Mean	SD[b]			
17	4.97	3.32	5	8.7	2.697	2.29	20	<0.05

[a]Number, [b] Standard deviation

4 DISCUSSION

Mean mercury detected in river otter hair was 5.819 mg/kg (range from 0.293 to 12.382 mg/kg). Maximum concentration of mercury had been observed for recent samples (samples from Golestan, 2006-2007) (Figure 2).

The levels of Mercury in the hair of river otters from the Iran were higher than Giant otters from the Rio Negro(range, 2.94-3.68; Dias Fonseca *et al.,* 2005) and were approximately similar L. canadensis from Wisconsin (mean, 6.47 mg/kg; max, 63.2 mg/kg) reported by Sheffy and St. Amant (1982) and can be considered low compared to the results reported for L.canadensis from Ontario, Canada (mean, 9.6; range, 4.0 –20.0 mg/kg; Evans *et al.,* 1998), for L. lutra from Finland (mean, 18.5; range, 0.7–61.3 mg/kg; Hyvarinen *et al.,* 2003) and from Britain (mean, 18.7; range, 1.3–85.1 mg/kg; Mason *et al.,* 1986), and for L. canadensis from Maine (mean, 20.3; range, 1.1–33.7 mg/kg; Evers *et al.,* 2002) and from Georgia (mean, 21.2; range, 0.5–54.4 mg/kg; Halbrook *et al.,* 1994). Sheffy and St. Amant (1982) suggest normal background levels of Hg in hair range from 1–5 mg/kg. Similarly, Evers (2005) suggests that adverse sublethal effects in mink and otter are possible when Hg concentrations in hair exceed 20 mg/kg. The mean Hg concentrations in hair of otter from each of the study regions are below this 20 mg/kg benchmark for sublethal effects. Also, individual otters from each region had hair Hg concentrations lower than this benchmark.

4.1 Spatial trends

Although THg was found in museum otters representing different regions of the Iran, no significant geographical variation was observed. This lack of difference may be the result of the small sample size of river otter or show that provinces studied have same pollution.

4.2 Temporal Trends

Based on museum records, the levels of THg in the river otter have increased significantly from the 1986s to 1996s. The ratio of THg in recent samples compared to samples from the 1986 to 2006 was 1.69 and ratio of Thg in decade 1996 compared to decade 1986 was 2.1. One objective of this study was to evaluate the temporal trends of mercury in the river otter. THg was found in all river otter specimens from the 1986s to 2007 and from various regions of the Iran. This study has shown that the river otter has been exposed to THg, at least for the past 20 years, with especially high levels in the 2007. These patterns reflect a long-term and widespread exposure of mercury to this species (Figure 3).

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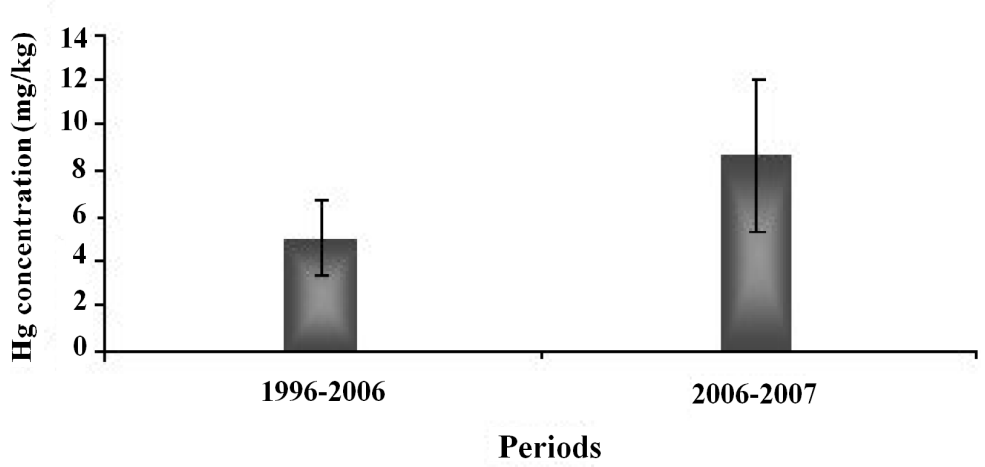

Figure 2 Comparison between museum and recent samples. Values presented are mean ± SE (P<0.01)

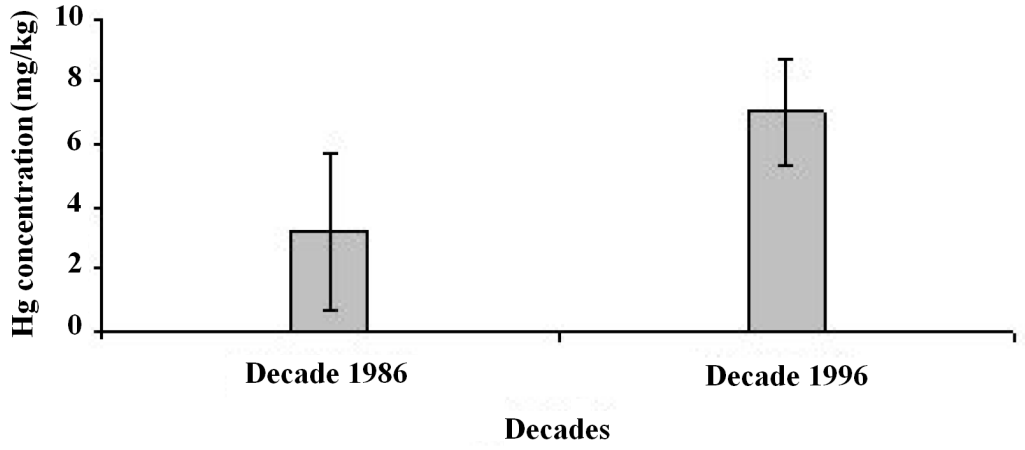

Figure 3 Effect of temporal trend on Hair Hg concentrations. Values presented are mean ± SE (P<0.05)

5 CONCLUSION

Mercury was detected in every hair sample submitted for analysis, which suggests that Hg exposure is widespread throughout Iran particularly in freshwater ecosystem Concentration of mercury in river otters and freshwaters in Iran is increasing. A comparison between museum sample and non- museum sample showed that there was stability of Hg in hair over time for museum samples that had not been threatened by special chemical that use in tanning process and skin preservation. Furthermore, otter hair could have been utilized as an indicator of mercury levels for back calculation. Evans *et al.* (1998) demonstrated a strong relationship between total Hg in hair and MeHg in liver of otter. If such a relationship exists, it facilitates retrospective study of mercury. Moreover provided a large number of specimens exist, we can use museum collections of hair to obtain historical changes in population levels of Hg contamination. Hg accumulation in otters can be a useful tool for prospective studies of Hg patterns and trends. However, as only a few samples were analyzed and at a limited amount of provinces, it is clear that further research is required in order to assess the temporal and spatial trends of mercury more thoroughly.

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سطوح جیوه در شنگ رودخانه‌ای (*Lutra lutra*) در ایران: امکان سنجی پیشینه‌یابی عناصر کمیاب در نمونه‌های موزه‌ای

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چکیده شنگ (سمور آبی) در بالای زنجیره غذایی قرار داشته و تغذیه گسترده‌ای از انواع ماهیان دارد که به ویژه در معرض تجمع زیستی آلاینده‌ها قرار دارند. این تحقیق اولین گزارش داده‌های مقادیر جیوه در خز و موی شنگ رودخانه‌ای (*Lutra lutra*) از ایران است. از تیرماه تا دیماه ۱۳۸۶ نمونه‌های تازه و موزه‌ای را جمع‌آوری نمودیم. این نمونه‌ها از چندین استان و از سه منطقه اصلی جمع‌آوری گردید. میانگین تجمع جیوه برای همه نمونه‌های مو (تازه و موزه‌ای) برابر با mg/kg ۱/۵۵۷±۵/۸۱۹ در سطح اطمینان ۹۵٪ بود. دامنه تغییرات مقادیر جیوه کل (THg) در این نمونه‌ها از ۰/۲۹۳ تا ۱۲/۳۸۲ میلی‌گرم بر کیلوگرم بود. بین نواحی مورد مطالعه تفاوت معنی‌دار آماری دیده نشد. مقایسه جیوه کل بین دو دهه (۱۳۶۵-۱۳۷۵ و ۱۳۷۶- ۱۳۸۵) تفاوت معنی‌دار را نشان داد ($p<0/01$). همچنین بین نمونه‌های موزه‌ای و نمونه‌های تازه تفاوت معنی‌دار یافت شد. این مطالعه نشان داده که در دو دهه اخیر شنگ رودخانه‌ای در معرض روند افزایشی جیوه کل قرار داشته است.

کلمات کلیدی: ایران، پیشینه‌پردازی، جیوه، شنگ رودخانه‌ای، مو

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