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Baseline

Potential risk of mercury to human health in three species of fish from the southern Caspian Sea

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ABSTRACT

We aimed to investigate mercury level in three species of fish such as *Sander lucioperca*, *Liza aurata*, and *Rutilus frisii kutum*. Sampling was done in the southern coasts of the Caspian Sea. The ranges of mercury level in *S. lucioperca*, *L. aurata*, and *R. frisii kutum* were 104.67–675.33 ppb, 60.66–175.33 ppb, and 123.33–170.33 ppb, respectively. Results revealed that the mercury level in *S. lucioperca* was more than the allowable limit at several sites, while it was less than the allowable limit in *R. frisii kutum* and *L. aurata* at all sampling sites. Further, the target hazard quotient (THQ) index for *S. lucioperca* was > 1 at some sites and < 1 for other species at all sites. The maximum allowable consumption for each species at the study area was measured daily and monthly.

Nowadays, sea products play a considerable role in food supply for the people worldwide and are increasingly consumed because of their suitability and food priority compared to other proteins (FAO, 2009). Fish meat, which has several advantages, provides many elements required for the body, including phosphorous, calcium, minerals, and vitamins. In addition, fish meat is regarded as a rich source of polyunsaturated fatty acid (PUFA), also called as omega-3, which is beneficial to prevent coronary artery diseases.

Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are the most important omega-3 fatty acids that help in the treatment of atherosclerosis, cancer, arthritis, and aging disease such as Alzheimer's disease. Along with the increasing demand for marine products, serious increase in the rate of pollution in marine environments has intensified probable qualitative problems in these valuable food sources. Industrial development and overgrowth of population in cities and villages in addition to farming land extension and usage of fertilizers and pesticides cause increase in urban and industrial sewage, as well as agricultural runoff containing different chemical compounds such as heavy metals run toward aquatic ecosystems (Kojadinovic et al., 2006).

Mercury is considered as one of the most dangerous heavy metals found in organic and inorganic forms in the natural environment. Studies have indicated that the organic form of mercury (methyl mercury) is much more toxic and bioaccumulate along the food chain. Inorganic mercury is converted into the organic form (i.e., mercury methyl) through methylation and enzymatic process performed by bacteria and other aquatic microorganisms (Porcella, 1994; Adams and McMichael, 1999).

Mercury methyl can be absorbed by aquatic flora, algae, and primary organisms of the food chain, and then it can enter and accumulate in the fish food web. Fish consumption is the most substantial way of introducing mercury into the human body. Owing to the long-term biological half-life of mercury methyl, tremendous and undesirable effects have been observed on human health, particularly on the nervous system resulting in problems including psychopathy, hearing and vision loss, loss of control over the body, general weakness, anxiety attack, and the nervous system of the fetus (Dietz et al., 2000; Ruelas-Inzunza et al., 2008; Horvat et al., 2014).

White fish (*R. frisii kutum*) of the Caspian Sea is distributed in deep waters during autumn while prefers coastal areas (depth of about 30 m) and river estuaries during winter. When inhabiting the sea, they are found in areas with benthic organisms, especially the mollusks. They migrate when thermal changes and water currents occur. This species is found along the coastline of the southern Caspian Sea from the Atrak River to Kora River. In Iranian coasts, many white fish migrate for reproduction to the Shirood River, Havigh River, Lamir River, rivers reaching into Anzali wetland, Sefidrood, Tajan River, Babolrood, and Gorganrood (Shikhshabekov, 1979; Abdoli, 1999).

The Perch fish, *S. lucioperca* from the order Perciformes, inhabits freshwater and saltwater. They are mostly distributed throughout the northern hemisphere, although they are introduced to the southern hemisphere. Their individuals are commonly found in the southwest coast and are regarded as economically valuable fish in the Caspian Sea. In early life, they are zooplanktivorous, and after larval stages, they become predators. The perch feeds seldom on benthos, crustaceans, and

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insect larvae, except for other fish. However, according to the previous studies, its main foods in the Caspian Sea are Caspian roach, Kilka fish, gobies, and shrimp. Unfortunately, the population of this fish has decreased over the past few decades because of various reasons, and its fishing caused a sharp decline (Craig, 2000; Holčik and Oláh, 1992).

The golden mullet (*L. aurata*) was introduced during 1930–1934 into the Caspian Sea and was distributed at different parts. This species is mostly found in the southern coast of the Caspian Sea and can tolerate high temperature and changes in salinity. Often, they feed on mollusk, zooplankton and detritus. White fish, as the most dominant species in catch composition of teleosts throughout the southern Caspian Sea, form > 50% of fishery and > 60% of the fisherman's income. Next to white fish, mullet fish is of great economic importance and their catch provides > 30% of the fisherman's income.

Less information is available on the evaluation of risk associated with the consumption of aquatic organisms; specifically, previous studies are associated with the most edible fish in the Caspian Sea in terms of heavy metals such as mercury, with heavy metal measurements in various tissues of the fish, and the association between metal accumulations with biometric parameters. In the present research, risk evaluation of mercury consumption in three species of the most edible fish in the southern Caspian Sea was studied.

Specimens of white fish (15 fish at each site), mullet (15 fish at each site), and perch (15 fish at each site) were caught from six sampling sites of the Caspian Sea (from Astatara to Gorgan coasts) in winter 2017 by beach seine (Fig. 1) and then transported to the laboratories in ice boxes. The fishes were rinsed with distilled water and weighed using a digital balance. Then, other biometric measurements including standard, fork, and total lengths were analyzed using a digital ruler (with an accuracy of 1 mm). In the laboratory, the mussel between the anterior part of the dorsal fin and lateral line was separated and kept in a freezer at −80 °C until analysis. For mercury analysis, 0.5 g of each homogenized sample from different sites was separately weighed and poured into a 50-ml Teflon-capped container. Then, 5 ml of a mixture of perchloric acid ($HCLO_4$) and nitric acid (HNO) (with the ratio of 1:3) was added and shaken well while the container was capped (AOAC, 1995). For completion of the digestion, the solution was heated from 100 to 150 °C for 45 min in an oven and then sieved through a Whatman paper until a transparent solution was achieved. Afterwards, drying of the solution was carried out at room temperature, and 0.5 ml of 0.1 M SnCl and double distilled water was added to make up the volume to 50 ml. After preparation, samples were measured by the cold vapor technique and using the atomic absorption device. Data were analyzed using statistical package of SPSS (Ver. 16). First, data normality was performed by the Kolmogorov-Smirnov test when $\alpha = 0.05$. Normality test indicated a normal distribution for all data. Thereafter, mean comparisons were done by one-way ANOVA and Duncan's multiple range test. Pearson correlation was applied to assess the association between all measured factors.

Results of biometric data and mercury content in the studied fish are shown in Tables 1 and 2. The range of mercury content in *S. lucioperca* was from 104.67 ppb to 675.33 ppb, with the highest level at site 1 and the lowest level at site 5. Moreover, the highest and lowest content of mercury in *R. frisii kutum* was obtained at sites 4 and 2, respectively. The mean level of mercury in this species was 141.44 ± 28.28 ppb. In *L. aurata*, the highest level of mercury (175.33 ± 6.02 ppb) was detected at site 1, while its lowest level (60.66 ± 7.57 ppb) was observed at site 4.

In the study area, mercury level in *S. lucioperca* showed a decline from west to east. The highest and lowest levels of mercury in *R. frisii kutum* were measured in eastern and western sites, respectively. By contrast, in *L. aurata*, the highest level of mercury was seen in the western site, while the lowest level was obtained in the eastern site.

Difference in heavy metal accumulation in various fish species is pertained to their life, habitat, feeding habit, capability in bioaccumulation, age, and size. In other words, metal bioavailability can be affected by biotic and abiotic factors controlling a specific metal and its bioaccumulation (Spry and Wiener, 1991; Storelli et al., 2005; Kelly et al., 2008). Comparison of mean mercury contents in the three studied species discovered that mercury level in *S. lucioperca* was higher than that in the other two species ($p < 0.05$), while no significant difference was observed in the mercury levels of *R. frisii kutum* and L. *aurata* ($p > 0.05$) (Fig. 2). The higher level of mercury in *S. lucioperca* might be attributed to its carnivorous feeding habit, and this species

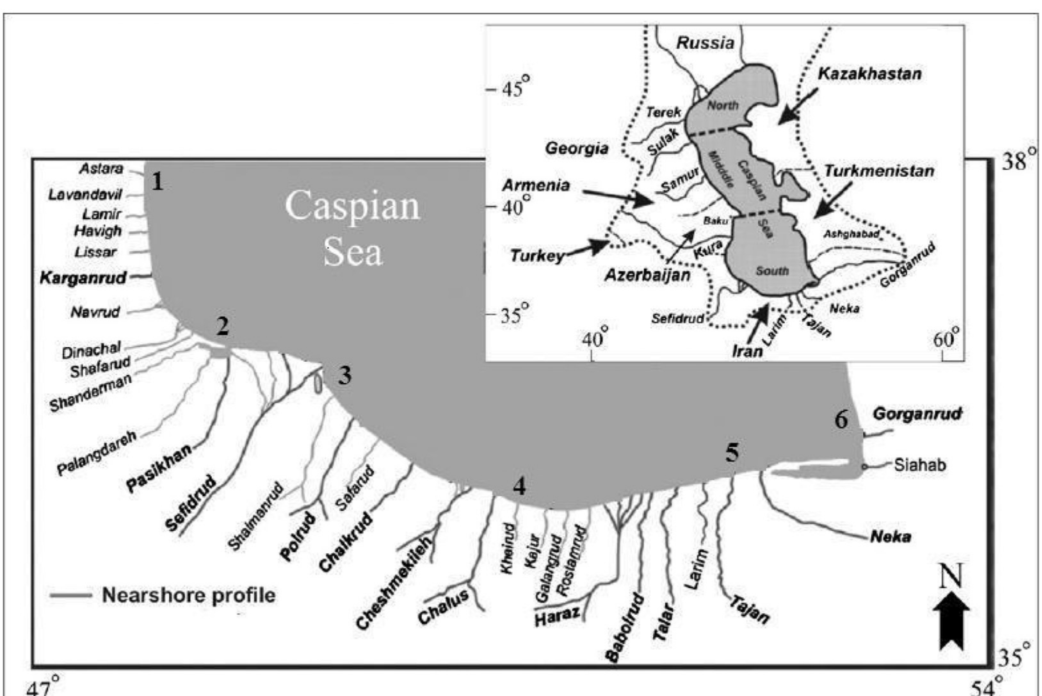

Fig. 1. The locations of the sampling sites at the coastline of the southern Caspian Sea.

Table 1
Biometric data of *S. lucioperca*, *R. frisii kutum*, and *L. aurata* sampled from the southern Caspian Sea.

Parameters	Sampling sites	Geographical location	*R. frisii kutum*	*L. aurata*	*S. lucioperca*
Total length (cm)	1		37.90 ± 1.65	45.66 ± 2.08	38.33 ± 0.28
	2		46.43 ± 2.37	46.83 ± 1.25	42.16 ± 0.76
	3		43.66 ± 6.78	43.33 ± 2.08	39.33 ± 4.07
	4		37.53 ± 2.65	38.66 ± 1.15	47.00 ± 2.00
	5		38.23 ± 2.75	41.33 ± 0.57	47.00 ± 1.32
	6		40.73 ± 2.00	26.80 ± 0.72	–
Fork length (cm)	1		34.60 ± 1.38	39.33 ± 2.30	33.16 ± 0.28
	2		42.50 ± 2.50	39.00 ± 1.00	36.33 ± 0.57
	3		40.33 ± 6.65	36.66 ± 2.30	34.33 ± 3.68
	4		34.16 ± 1.93	34.00 ± 1.00	41.33 ± 2.51
	5		35.43 ± 2.54	33.66 ± 0.288	40.33 ± 3.21
	6		37.86 ± 1.85	22.40 ± 0.69	–
Weight (g)	1		826.17 ± 21.83	901.10 ± 13.13	533.93 ± 1.20
	2		828.00 ± 19.00	868.33 ± 48.56	513.32 ± 40.41
	3		849.93 ± 28.85	851.10 ± 35.26	540.00 ± 153.94
	4		683.37 ± 58.79	403.83 ± 70.78	716.77 ± 10.78
	5		633.67 ± 89.91	433.40 ± 20.70	687.93 ± 33.72
	6		830.00 ± 25.98	124.90 ± 12.91	–

Table 2
Mercury contents (ppb, average ± SD) in the muscle of *S. lucioperca*, *R. frisii kutum*, and *L. aurata* sampled from the southern Caspian Sea. Different letters in each column indicate the significant differences ($P < 0.05$).

Sampling sites	*S. lucioperca*	*R. frisii kutum*	*L. aurata*
1	675.33 ± 36.63[c]	146.67 ± 30.43[a]	175.33 ± 6.02 [d]
2	613.00 ± 134.81[c]	123.33 ± 11.71 [a]	90.00 ± 19.67[bc]
3	244.33 ± 24.00[b]	133.67 ± 19.65[a]	104.33 ± 18.58[c]
4	144.67 ± 76.15[ab]	170.33 ± 13.65[a]	60.66 ± 7.57[a]
5	104.67 ± 15.04[a]	145.33 ± 38.52[a]	62.00 ± 19.67[ab]
6	–	129.33 ± 39.50[a]	75.66 ± 12.89[ab]

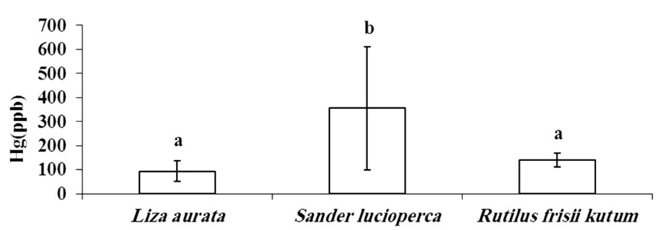

Fig. 2. Comparison of mercury content in *S. lucioperca*, *R. frisii kutum*, and *L. aurata* sampled from the southern Caspian Sea. Different letters indicate the significant differences ($P < 0.05$).

accumulated more mercury through biomagnification than the others. Several previous studies have studied mercury level in different species at various parts of the world (Table 3) (Esmaili-Sari et al. 2006 & b; Hosseini et al., 2013; Coelho et al., 2013; Dadar et al., 2016). On comparison to the present work, some of these studies showed higher or lower level of mercury. This difference might be because of variations in the study area, size, gender, physiological condition of the fish, and environmental traits.

Yet, no single source is proposed to contrast heavy metal concentrations in edible food with standards. Organizations and governments have determined various standards for the levels of these contaminants in food materials. Results of this study were compared with determined allowable standards for mercury in European Union. The comparison showed that mercury content in the tissue of *S. lucioperca* at sites 1 and 2 was more than the allowable limit (0.5 mg/kg), but in the other two species, it was less than the determined standards.

According to the reports of global health organization, mercury is dangerous at any level and there is no particular and healthy level of mercury on human body. Although white fish consumption has no dangerous effect on consumers' health, their consumption by pregnant women and children must be controlled because fetus, infants, and children < 10 years old are more sensitive to mercury toxicity. Hence, determination of the allowable limit for the consumption of these fishes (daily or weekly) is essential (USEPA, 1997).

The association of fish length and weight with heavy metal concentration has been shown in many studies, which was positive or negative in some species (Storelli and Marcotrigiano, 2000; Tabatabaie et al., 2011).). In this research, mercury level had a negative correlation with length and weight of *S. lucioperca* and *R. frisii kutum* (Table 4). This probably implicates that both species have a physiological mechanism, as it prevents tissue accumulation of mercury at higher concentrations. However, there were significant and positive correlations between fork length and weight of L. *aurata* with mercury level ($p < 0.05$).

For calculation of an individual's chances of risk to noncancer diseases, the formula presented by American environmental and conservation organizations was used. Risk index of HQ was the ratio of a pollutant content to its reference level; if < 1, it indicates no detrimental effect of fish consumption on body health and if ≥ 1, it implies a negative effect on human health (USEPA, 2000). HQ index is measured

Table 3
Comparison of Hg content (ppb) in fish, with values taken from the literature.

Sampling sites	*S. lucioperca*	*R. frisii kutum*	*L. aurata*	References
The southern Caspian Sea	356.40 ± 255.95 104.67–675.33	141.44 ± 28.25 123.33–170.33	94.66 ± 42.27 62.00–175.33	This study
Caspian Sea	–	–	432 44–1265	Hosseini et al. (2013)
The Ria de Aveiro lagoon, Portugal	–	–	980 ± 80	Coelho et al. (2013)
the Southern Caspian Sea		894	259	Esmaili-Sari et al. (2006) and (2006)
the Southern Caspian Sea	–	2–4	–	Dadar et al. (2016)
Lake Velenjsko jezero, Slovenia	160 (110–210)	–	–	Zdenka et al. (2010)

Table 4
Correlation between biometric data and mercury content in *R. frisii kutum*, *L. aurata*, and *S. lucioperca*.

R. frisii kutum			
Parameters	Weight	Total length	Forked length
Total length	0.557*		
Forked length	0.536*	0.992**	
Hg	−0.165	−0.137	−0.153
L. aurata			
Total length	0.900**		
Forked length	0.897**	0.987**	
Hg	0.638**	0.430	0.475*
S. lucioperca			
Total length	0.886**		
Forked length	0.878**	0.970**	
Hg	−0.669**	−0.628*	−0.593*

* Correlation is significant at the 0.05 level.
** Correlation is significant at the 0.01 level.

Table 5
Estimated THQ for Hg from the consumption of sampled fish.

Sampling sites	*S. lucioperca*	*R. frisii kutum*	*L. aurata*
1	2.89	0.63	0.75
2	2.63	0.53	0.39
3	1.05	0.57	0.45
4	0.62	0.73	0.26
5	0.45	0.62	0.27
6	–	0.55	0.32

as the following (Chien et al., 2002):

$$THQ = [EFr \times EDtot \times FIR \times C / RfDo \times BWa \times ATn] \times 10^{-3}$$

where THQ is the target hazard quotient; EFr is the exposure frequency (365 days/year); EDtot is the exposure duration (70 years, average lifetime); FIR is the food ingestion rate (30 g/day); C is the heavy metal concentration in fish (l g/g); RfDo is the oral reference dose (Hg = 0.0001 mg/kg/day); Bwa is the average adult body weight (70 kg); and ATn is the average exposure time for noncarcinogens (365 days/year x number of exposure years, assuming 70 years).

Risk index of HQ for *S. lucioperca* was > 1 at sites 1, 2, and 3, and for the other two species, it was < 1 at all stations (Table 5). In risk evaluation, total content of mercury in fish tissue is accounted for methyl mercury because from the viewpoint of human health, methyl mercury is considerably more important than other chemical forms of mercury. Unlike elementary and inorganic forms of mercury, methyl mercury is completely absorbed by the gastrointestinal tract, enters the blood stream, gets and distributed to all tissues. Moreover, it passes through the blood vessels of the brain and damages peripheral and central nervous system. In pregnant women, methyl mercury can reach the fetus through the placenta and cause irrecoverable damage to the growing fetus.

Although seafood products have so many advantages for the consumers, fish consumption is not likely to be right without considering food safety. Therefore, a specific level of fish meat must be consumed at which the mercury level exceeds not more than the RFD. The following equation was applied to calculate the maximum allowable limit (CR) for fish consumption (USEPA, 2000).

$$CR = (RfD \times BW)/C$$

where RfDo is the oral reference dose (Hg = 0.0001 mg/kg/day); Bwa is the average adult body weight (70 kg); and C is the heavy metal concentration in fish.

Accordingly, the allowable limit for consumption of *R. frisii kutum*, *S. lucioperca*, *and L. aurata* was obtained as 0.041–0.057, 0.01–0.067, and 0.04–0.113, respectively (Table 6). Furthermore, the maximum allowable limit for monthly consumption of fish was achieved according to the following formula:

Table 6
Results of risk assessment of mercury in *S. lucioperca*, *R. frisii kutum*, and *L. aurata*.

Sampling sites	*S. lucioperca*		*R. frisii kutum*		*L. aurata*	
	Daily intake	Weekly intake	Daily intake	Weekly intake	Daily intake	Weekly intake
1	1.37	0.010	6.31	0.048	5.28	0.040
2	1.51	0.011	7.50	0.057	10.28	0.078
3	3.79	0.029	6.92	0.052	8.87	0.067
4	6.39	0.048	5.43	0.041	15.25	0.115
5	8.84	0.067	6.37	0.048	14.92	0.113
6	–		7.15	0.054	12.23	0.093

$$CR_m = (CR \times T)/MS$$

where T is the number days in a month; CR is the maximum allowable limit; and MS is the consumption rate of fish. Thus, this limit was measured for *R. frisii kutum* (7 times), *S. lucioperca* (1 time at sites 1 and 2; 3 times at site 3; 6 times at site 4, and 8 times at site 5), and *L. aurata* (10 times at western sites and 15 times at eastern sites) (Table 6).

In this investigation, we focused on mercury concentration and its consumption risk in three important economic species of the southern Caspian Sea. Results exhibited that mercury concentration in *S. lucioperca* captured from western sites was assessed more than the standard limit, whereas in the other two species, it was less than the defined standard limit. Furthermore, significant and positive correlations were found between mercury level in tissues of *L. aurata* with length and weight. So, a comprehensive study on heavy metal concentration of commercial and economical fish in southern Caspian Sea is recommended to determine their toxic metals and allowable consumption limit.

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