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# Risk assessment of fish consumption for residents around the international Anzali wetland based on mercury content

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## Abstract

In recent years, the discharge of various pollutants, especially metals, into aquatic environments has exerted negative effects on the biota and health status. Among metals, mercury can induce the highest adverse influence on aquatic ecosystems and humans. Hence, the limited consumption of some fish species including *Sander lucioperca*, *Esox lucius Linnaeus*, *Cyprinus carpio*, and *Carassius auratus gibelio* was determined for the residents around of the Anzali Wetland based on their mercury content. The mercury concentration showed significant differences between the fishes; the highest and lowest levels were detected in the muscle of *Esox lucius Linnaeus* (165.400 ng/g) and *Carassius auratus gibelio* (60.55 ng/g), respectively. The daily consumption rate limit (CRlim) and the allowable number of fish meals per month (CRmm) showed the highest consumption limit for *Esox lucius Linnaeus*, with the highest mercury concentration. *Cyprinus carpio*, and *Carassius auratus gibelio* exhibited the least concern and limited consumption.

**Keywords**: Mercury; CRmm; CRlim; Consumption; Risk.

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## 1. Introduction

Mercury is one of the worst toxic elements due to its accumulative, biomagnification, and persistent properties in the environment and biota (Astani *et al*., 2011; Shahid *et al*., 2020). Its toxicity always depends on the chemical forms. Mercury comes from natural and anthropogenic sources. Geothermal activities and rock mining weathering can release mercury into the air, water and soil, through geothermal activity and the weathering of mercury ore-containing rock. Anthropogenic sources of mercury are abundant around the world, mainly through burning of fossil fuels and urban waste incineration (Zhang *et al*., 2020; Fernández-Martínez *et al*., 2019). Aquatic ecosystems are contaminated by drainage of industrial wastewater and runoff. The direct drainage of industrial wastewater and rainwater runoff leads to mercury contamination in the aquatic ecosystem (ATSDR 1999; Pobi *et al*., 2019).

Despite an ever-decreasing utilization and in turn reducing the release of mercury since 1970, the contamination of mercury has raised concerns because of increasing fossil fuel combustion and hazardous waste discharge in many areas (US EPA, 2000; Singh and Kumar, 2020). Mercury is a trace element in aquatic environments and the toxicity depends on its chemical accumulated forms. Methyl mercury is one of the most toxic substances existing in the terrestrial environments. In aquatic ecosystems, methyl mercury is produced by bacteria in deep organic sediments, such as the sediments of rivers and wetlands. Methyl mercury is biomagnified by predators through food chains and food webs (Hosseini *et al*., 2013). Methyl mercury is a neurotoxin to organisms (Burger and Gochfeld, 1991). A major concern with methyl mercury is that it can pass the mammalian placenta and is also unified into bird eggs during developmental stages, thereby causing abnormalities in young vertebrate during the process of growth (Burger and Gochfeld, 1991; Nogara *et al*., 2019). Thus, organisms can be affected straightly following exposure to mercury contamination and the organisms and their offspring are influenced (Baron *et al*., 1997). It is of high importance to monitor the concentration of mercury in environment. In some cases, fish catch has been forbidden for human consumption due to over-threshold concentration of mercury recommended by the Food and Agriculture/World Health Organization (FAO/WHO, 1972). In many countries, extensive studies were conducted to determine the concentration of methyl mercury in the edible fish. Many countries have triggered a process of detecting mercury contents in natural fish populations by establishing maximum permissible mercury concentrations in fish for human consumption in the range of 0.5–1.0 mg/kg wet weight (Lacerda *et al*., 2000). Hence, in recently years' levels of mercury in fish have been widely reported (Lasorsa and Gill, 1995; Rolfhus and Fitzgerald, 1995; Lacerda *et al*., 2000; Love *et al*., 2003; Golding *et al*., 2017; Manavi and Mazumder, 2018).

The Anzali wetland, located in the southwestern coast of the Caspian Sea. It has special geographical position and is one of the most valuable freshwater aquatic ecosystems in the Iran (Eslaheh Arbani, 1995). Wetland is contaminated by discharging heavy metals and poisonous substances when the rivers water pass through residential and industrial area, paddy fields and farmlands (Eslaheh Arbani, 1995). The Anzali wetland is subdivided into four basins: Eastern, Central, Western and South western. Generally, about 44% of the total inflow water is flowed into eastern basin (complex system of Pirbazar and Pasikhan Rivers), 52% by Siahdarvishan River into central basin and 4% of total inflow water is flowed into western and southwestern basins (JICA, 2004). Groundwater and surface waters enter into the wetland or discharge to the stream flow near mouth of the wetland. The Pirbazare River has a vital role in transition of mercury to the wetland. The Pirbazare River flows into eastern basin; therefore, the discharged tributaries into the Anzali wetland contain elevated mercury and indicate that in the eastern basin of Anzali wetland those tributaries are important source of mercury compounds (Eslaheh Arbani, 1995). Due to the fluctuations in the sea level of the Caspian Sea and entry untreated waste water in to the Anzali wetland, it has begun to deteriorate. Ramsar convection decided to registered the Anzali wetland in the Montro list in 1993 to emphasis that it needs to resuscitation immediate (JICA, 2004). There is little study about risks of consumption fish of the Anzali wetland in terms of mercury level; therefore, this study is aimed to determine risks of consumption of Anzali international wetland fish for the residential around the wetland.

## 2. Material and method

### *2.1. Study area*

The Anzali Wetland, with an area of about 193 km$^2$, is located in Guilan Province at the north of Iran. It lies between 37°22'–37°32'N, and 49°15'–49°36'E. Catchment of the wetland covers an area of about 3610 km$^2$. The waterways to the Anzali Wetland can be divided into four sections of western (Abkenar), eastern (Shijan), central (Hendekhaleh) and south-western (Siahkeshim) (Jamshidi-Zanjani and Saeedi, 2013). The Anzali Wetland is the habitat of unique and invaluable fish species and other flora and fauna, which is internationally known as a route for bird migration and has been registered at Ramsar site since 1975 (Vesali Naseh *et al*., 2012; Zamani Hargalani *et al*., 2014). The Anzali Wetland is the main and largest fresh water coastal wetland in the southern part of the Caspian Sea, which is mainly fed by ten rivers with an average discharge of 76m$^3$/s. PirBazar, Pasikhan and Shijan are among the important rivers in the catchment area of the Anzali Wetland, which play an important role in the transfer of pollutants into the wetland (Jamshidi-Zanjani and Saeedi, 2013). The PirBazar River originates from a forested basin and is formed from the merging of two rivers of Goharrud and Zarjoob. This river, after passing through Rasht City and receiving all kinds of municipal and industrial wastewater, enters the eastern part of the Anzali Wetland (Shijan) with a discharge rate of 9.42 m$^3$/s (Ayati, 2003). The Pasikhan River is one of the independent rivers of the Caspian Sea basin and belongs to the sub-basin of the Anzali Wetland. This river has two main branches, both originated from Latte Berahneh mountain slopes, which are 2667m high. These branches merge and form the Pasikhan River, and then in No-Khaleh region intersect with Shut-Chay River and enter the central part of the Anzali Wetland (Hendekhale) with a discharge rate of 22.8 m$^3$/s (Ayati, 2003; JICA, 2004). In Khomam City, the Khomamrud River intersects with Gurabjir branch, and form the Shijan River. This river, with an average discharge rate of 3.89 m$^3$/s enters the eastern part of the wetland (Ayati, 2003; JICA, 2004). According to JICA studies, more than 40 percent of Gilan Province's population live in the cities located in the catchment area of Anzali Wetland. Different industries such as steel, rubber, ceramic, textile and food industry located in the cities of Rasht, Anzali, Some'e Sara are among the various sources of pollution, and the wastewater of these industries flows into the rivers and finally enters the wetland (JICA, 2004). Farmlands occupy 935.25 $km^2$ of the catchment area of Anzali Wetland. Drains from chemical fertilizers and herbicides and fungicides used in these farmlands are important sources of pollution in the Anzali Wetland. Additionally, water penetration from the Caspian Sea through the shipping channels, and oil leaks from tourist and fishing motor boats are important sources of oil pollution in the Anzali Wetland (Khosheghbal *et al*., 2013; Zamani Hargalani *et al*., 2014).

## *2.2. Analysis procedure*

For measurement of mercury concentration, Muscle of fish samples were freeze-dried at −54°C for 24h to obtain a completely dry sample and then was changed to powder (Malvandi *et al*., 2010). The moisture contents of the tissues were calculated according to weight loss before and after freeze-drying. In mercury analyses of fish, the sum of total mercury in the sample is measured rather than MeHg because the analysis of MeHg is more expensive. Some studies reported that the percentage of MeHg in total mercury ranged from 81 to 95% (Ruggieri *et al*., 2017; Raissy *et al*., 2014). For the purposes of the health risk assessments, 100% of total mercury is assumed to be MeHg thereby erring on the side of caution (Health Canada, 2007). For each aquatic species, at least six duplicate was considered. About 0.03 to 0.05 g dry weight from each sample was weighed and put in the nickel boat of a mercury analyzer. LECO AMA 254 Advanced Mercury Analyzer (USA) according to ASTM, standard no. The LECO AMA 254 is a unique atomic absorption spectrometer specifically designed to determine mercury content in various solids and certain liquids without sample pretreatment. The samples were directly weighed into pre-cleaned combustion boats and automatically inserted into the AMA 254. The sample was dried at 120 °C for 90s and thermally decomposed at 550 °C for 180s under an oxygen flow. Decomposition products were carried by the oxygen flow to an Au-amalgamator. Selectively trapped mercury was released from the amalgamator by a brief heat-up and finally quantified (measuring cycle, 60s) as Hg0 by cold vapor AAS technique at 253.65 nm (Sysalová *et al*., 2013).

## *2.3. Determination of recovery*

Recovery of mercury was determined by adding more amounts of mercury to the samples of two different fish species which were taken through the digestion procedure. The resulting solutions were analyzed for mercury concentration.

## *2.4. Data Analysis*

Three one-way ANOVAs were conducted, two of them to determine whether the differences among concentrations in different fishes could be significant and another to examine whether concentrations of mercury were significantly different in studied fish (Sokal and Rohlf, 1981). Each datum was the mean of metal concentrations in three sub-samples. Duncan's new multiple range test (Zar, 1984) was employed and the results represented that there were statistically significant differences between the group means. All statistical analyses were done in the SPSS software (version 16).

## 3. Results and Discussion

Accumulation of toxins, especially metals, in fish is a potential hazard, especially when they are consumed by humans (Asuquo *et al*., 2004). Fish is a good indicator for assessing water pollution because fish receive pollutants both directly from water and through diet and concentrate in their tissues. Therefore, they are suitable for evaluation through the food network and investigation of the biomagnification (Rumbold *et al*., 2018; Murillo-Cisneros *et al*., 2019). The results of the data obtained from the mean and standard deviation of the mean (SEM) of mercury concentration in the studied fish were analyzed using SPSS software. The results for the mean concentrations of heavy metals (in ng/g fresh weight) in the muscle tissue of the *Sander lucioperca*, *Esox lucius Linnaeus*, *Cyprinus carpio*, and *Carassius auratus gibelio* are presented in Table 1. As the results show, the concentration of mercury in muscle of *Sander lucioperca*, *Esox lucius Linnaeus*, *Cyprinus carpio*, and *Carassius auratus gibelio* were measured 149.8, 165.17, 78.8 and 60.7 ng/g, respectively. Based on the results shown in this study, the highest amount of mercury in *Esox lucius Linnaeus* was determined. Also, based on the FAO/WHO recommended limit in fish, the all the species had values of mercury below the FAO/WHO recommended limit of 500 ng/g wet weight.

One-way ANOVA and Duncan test were used to compare mercury concentrations in the studied fish muscle samples in Anzali wetland. In order to perform one way ANOVA test and then Duncan test, also for normal distribution of data at different stations using Shapiro-Wilk test and homogeneity of variance with levene test. The results of normality and homogeneity of variances test showed that all data follow the normal distribution (*Sig>0.05*) and the variance of the groups is homogeneous (*Sig>0.05*). The results of the one-way analysis of variance are presented in Table 2. As the results show, there is a significant difference between all the fish studied in terms of mercury content.

Table 1. mean and standard deviation and range of mercury accumulation in muscle tissue of *Sander lucioperca*, *Esox lucius Linnaeus*, *Cyprinus carpio* and *Carassius auratus* in Anzali wetland (in ng/g fresh weight)

| Species | SEM ±mean | min –max |
|---|---|---|
| *Sander lucioperca* | 149.93±8.46 | 195.05-122.37 |
| *Esox lucius Linnaeus* | 165.17±4.37 | 271.53–78.2 |
| *Cyprinus carpio* | 78.92±8.83 | 134.48–43.41 |
| *Carassius auratus gibelio* | 60.7±5.51 | 47.06–88.44 |

Table 2. One-way analysis of variance to compare the amount of metals studied in fish muscle

| | SS | df | MS | F | *Sig* |
|---|---|---|---|---|---|
| Between group | 71592.036 | 3 | 23864.012 | 10.901 | .000 |
| Within group | 76617.677 | 35 | 2189.076 | | |
| Total | 148209.713 | 38 | | | |

As the results of one-way analysis of variance showed that there was a significant difference between the concentrations of mercury in fish muscle samples (*Sig<0.05*). According to Duncan test, no significant difference was observed between *Sander lucioperca* and *Esox lucius Linnaeus* (similar letters a), and no significant differences between *Cyprinus carpio*, and *Carassius auratus gibelio* (similar letters b). But there was a significant difference between *Sander lucioperca*, *Esox lucius Linnaeus*, *Cyprinus carpio*, and *Carassius auratus gibelio* (contrasting letters a and b). The highest and lowest amount of this metal was measured in *Esox lucius Linnaeus* and *Carassius auratus gibelio* muscle with 165.14 ng/g and 60.7 ng/g (Figure 1). As the results show, the amount of mercury in carnivorous fish was measured higher than in herbivorous and omnivorous fish. *Sander lucioperca* is a carnivorous species that uses smaller prey than the *Esox lucius Linnaeus* to feed. But the *Cyprinus carpio* diet is mostly vegetarian, it also feeds on worms and benthos, and the *Carassius auratus gibelio* diet is omnivorous (Esmaeili *et al*., 2015; Lu *et al*., 2017).

## *3.1. Determation of CRlim and CRmm*

Due to the importance of determining the amount of mercury entering the human body through the consumption of seafood, the permissible limit for the use of *Sander lucioperca*, *Esox lucius Linnaeus*, *Cyprinus carpio*, and *Carassius*

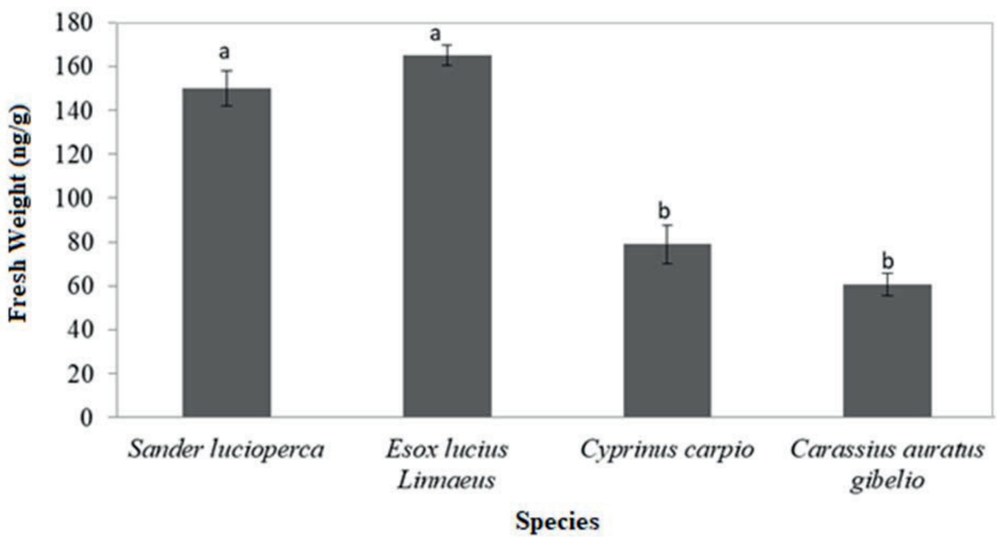


Figure 1. Comparison of mercury concentration in studied fish muscle

Table 3. results of determination of CRlim and CRmm

| Species | CRlim | | | CRmm | | |
|---|---|---|---|---|---|---|
| | children | woman | adult | children | woman | adult |
| *Sander lucioperca* | 0.02 | 0 | 0.05 | 7.3 | 5.6 | 6.17 |
| *Esox lucius Linnaeus* | 0.01 | 0.04 | 0.04 | 6.6 | 5.1 | 5.6 |
| *Cyprinus carpio* | 0.04 | 0.08 | 0.08 | 13.9 | 10.7 | 11.7 |
| *Carassius auratus gibelio* | 0.05 | 0.10 | 0.11 | 18.1 | 13.9 | 15.2 |
| Total of fish | 0.007 | 0.014 | 0.015 | 2.4 | 1.8 | 2.0 |

*auratus gibelio* was obtained by measuring the concentration of mercury in muscle tissue of them in Anzali wetland. The method used by the US Environmental Protection Agency (EPA) was used to calculate the allowable consumption of the fish under study. In fact, the permissible limit of fish consumption has been defined in order to balance the benefits and maintain the general health resulting from its consumption (EPA, 2000). In this method, using a Reference Dose (RfD) formula, it is possible to obtain acceptable levels of fish and fishery products without any carcinogenic effects of mercury consumption over a specific time period.

CRlim = (RfD × BW) / Cm

CRmm = (CRlim × 30) / MS

In the above CRlim equations, the maximum allowable dose per kg, RfD, is the reference dose calculated as 0.1μg/kg BW for mercury. BW; kg body weight, Cm; Hg concentration in micrograms per kg, MS; fish weight per meal (227g for adults and pregnant women and 0.09g for children), and CRmm is the allowable rate of consumption in terms of number of servings per month (EPA 2000). Establishing restrictions on the consumption of fish and other aquatic species in mercury-contaminated waters for the general population or susceptible subgroups of the population, allowing them to reduce mercury exposure while providing nutritional and nutritional benefits. Health benefits of fish consumption. As mentioned earlier, in this study the size of each meal for adults (mean weight 70 kg) and women of reproductive age (mean weight 64 kg) was 0.222 kg and for children aged 8 to 11 years (mean weight 33 kg) was considered 0.09 kg. The results of daily and monthly intake for the studied fish are presented separately and the sum of the four species for adults, women and children is presented in Table 3. As shown in Table 3, the maximum consumption limit is for the *Esox lucius Linnaeus*, which has the highest concentration of mercury. *Cyprinus carpio* and *Carassius auratus gibelio* also have the least concern and limited consumption.

## Conclusion

The purpose of this study is to determination of mercury levels in different species in the Anzali wetland of Iran, and risks of consumption fish of in terms of mercury level. Mercury levels obtained in four species studied between 60.04 to 165.17 ng/g wet weights. All the species had values of mercury below the FAO/WHO recommended limit of 500 ng/g wet weight. The maximum consumption limit is for the *Esox lucius Linnaeus*, which has the highest concentration of mercury. *Cyprinus carpio* and *Carassius auratus gibelio* also have the least concern and limited consumption.

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