ORIGINAL ARTICLE

Distribution pattern of total mercury (T-Hg) in muscle, liver and spleen of Pike (*Esox lucius*) in Anzali wetland, Caspian Sea

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Abstract

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Article history: Accepted 25 December 2023 The present study aimed to investigate the bioaccumulation of total mercury (T-Hg) in dorsal muscle (edible tissue), liver, and spleen of pike (Esox lucius) from Anzali Lagoon, Iran. Acceptable monthly intakes (AMI) were measured based on FDA criteria. Sampling were carried out using gillnet and electro-shocker from July 2020 to July 2021. In addition, 78 specimens (age range 1-5 yr; total length: 10-58^{cm}; weight: 12-1560g.) were collected. Samples were analyzed by LECO AMA254. The measurements were conducted according to the following procedures: drying time 70s, decomposition time 120 s at 600°C in an oxygen atmosphere, and waiting time 50s. The same procedure was used with a blank sample. Each series was preceded by measuring the form of T-Hg in the certified reference material according to ASTM standard NOD-6722 with three standard reference material; SRM 1633b, 2709 SRM and 2711 SRM in three replications. Accuracy degree ranged from 95.5 to 105%. Detection limits was 0.001 mg/kg in dry weight. The min and max of T-Hg in dorsal muscles were ranged between 0.2 and 1.2ppm. The min T-Hg significantly increased with age increased (P<0.05). T-Hg concentration in muscles were significantly higher than liver and spleen (P < 0.05) and higher in females than males (P < 0.05). T-Hg distribution pattern in tissues was as follows: muscle> liver> spleen (P<0.05). AMI estimates for women, men, teenager and children as 270, 320, 125, and 59g, respectively.

Keywords: Heavy Metal, Pollution, Fish, Lagoon.

INTRODUCTION

One of the pollutants at the most relevant to fish assemblage is mercury (Hg), due to its toxicity, biomagnification, high absorption rates, and low excretion (Herring et al. 2017; Seixas et al. 2020). Raising mercury levels in fish was significantly associated with precipitation rates (Rosemary et al. 2017). The total amount of mercury emissions in Europe was reported equal to 342 tonnes per year, while natural activities-based mercury emission into the atmosphere was assessed about 250 to 300 tons per year (Adams & Sonne 2013). Global mercury emissions caused by human activities and natural processes were estimated to be 2700-6000 tons per year respectively (Bosch et al. 2016). Methyl mercury (MeHg) is the main mercury compound accumulated in fishes (Rosemary et al. 2017; Jinadasa & Fowler 2019). Shohreh et al. (2020) studied the heavy metals in some fishes from Caspian Sea and Persian Gulf. The ranges obtained for Hg in fish samples were found as 0.007-0.067mg/kg. Total mercury level in *Rutilus frisii* (0.021), *Parastromateus niger* (0.067), *Pomadasys kaakan* (0.007) and for *Scomberomorus commerson* determined as 0.018mg/kg. Also Ziarati et al. (2017) detected that mercuy level in some frozen fishes in Persian Gulf and reported that total mercury level in *Carcharhinus dussumie* and *Pomadasys furcatus* determined as 0.79 and 0.29, respectively.

In this regard, the consumption of freshwater and marine fishes is recognized as the primary sources of MeHg entering human body (Memet et al. 2017). Some studies have also confirmed that more than 80% of MeHg could penetrate the human body through the consumption of contaminated fish (Ansari et al. 2016; Corrales et al. 2016). In general, different concentrations of mercury increase through accumulation and magnification in the food chain (Lucas et al. 2021; Jinadasa & Fowler 2019), and its highest levels in freshwater sources are found in predatory fish located at the top of the food chain such as pike (Esox lucius) (Ansari et al. 2016; Lucas et al. 2021; Sharma et al. 2008). However, growth rateinduced biodilution is reported in some of fastgrowing fish species (Lajus et al. 2015). Some studies have also found that low levels of mercury in food chains were associated with risks of chemical changes in the nervous system (Weil et al., 2005) and brainheart disorders (Memet et al. 2017; Lajus et al. 2015; Jankovská et al. 2014; Griboff et al. 2017). So far, several reports have been submitted by national and international organizations on the allowable consumption of mercury-contaminated fishes. In this field, the WHO recommended different safe limits of mercury for various species of fishes, such as 0.17 up to 0.5ppm per wet weight for commerciall species, and more than 0.5 up to 1ppm per wet weight for pikes, sharks, and tuna fishes (Scombridae). However, accumulated concentrations more than 1 ppm may be toxic and declared as unallowable levels (USEPA 2004; Lucas et al. 2021). In overall, several studies have been published on changes in the mercury concentration in fish tissues based on biometric parameters and permissible consumptions of mercuryinfected fish. Therefore, the present study aimed to investigate the mercury bioaccumulation in pike (Esox lucius) from Anzali wetland, Guilan province, and Acceptable monthly intake without carcinogenic effects due to mercury.

MATERIAL AND METHODS

The Study area, Anzali International Wetland is located on the southern coast of the Caspian Sea at 37° 28' northern latitudes and 49° 25' eastern longitudes in the Guilan Province (Sadeghi Rad 1997) (37° 28 'N and 49° 25' E), with an area of less than one hundred square kilometers in the southwest of the Caspian Sea. Sampling were conducted using gillnet and electroshocker from July 2020 to July 2021. In addition, 78 specimen of pike (age range 1-5 yr; total length: 10-58cm; weight: 12-1560g.) were collected, then biometric characteristics were recorded. Dorsal muscle, liver, and spleen tissues were immediately frozen at -20°C and transferred to the environmental laboratory of the natural resources and marine

sciences faculty of Tarbiat Modares University. Samples were dried in an oven for 48-72 hours at 60°C and uniformly powdered in a porcelain mortar. Total mercury was measured by the LECO AMA254 D6722 Advanced Mercury Analyzer (Fig 1). The measurements were conducted according to the following procedures: drying time 70s, decomposition time 120s at 600°C in an oxygen atmosphere, and waiting time 50s. The same procedure was used with a blank sample. Each series was preceded by measuring the form of Total mercury in the certified reference material according to American Society for Testing and Materials (ASTM) standard NOD-6722 with three standard reference material; SRM 1633b, 2709 SRM and 2711 SRM in three replications (US EPA 2000) (table 1).

Results indicated different ranges of accuracy (from 95.5 to 105%) for the applied device and tracing accuracy with a standard deviation of RSD <0.05% (in μ g/kg dry weight).

Detection limits was 0.001 mg/kg in dry weight. Accuracy degree ranged from 95.5 to 105%. Statistical analysis was carried out with a standard deviation of reference material (RSD)<0.05%. Acceptable monthly intake without carcinogenic effects due to mercury were calculated according to the criteria of the U.S. Environmental Protection Agency using the following formula (USEPA 2004; Kojadinovic et al. 2006).

Meals/mo =	$RfD \times BW \times 35.2 \times Tap$
	Cm × MS

Meals/mo= Number of meals per month RfD= 0.0001 mg/kg body weight/day BW= Body weight (kg)/ Cm 35.2= Meal size conversion factor (ounces/kg) Tap= 30.44 days/month MS= Meal size (ounces)

According to Pelosi & Sanderer (2003) one-way ANOVA test was used to investigate differences between the mean concentrations of mercury in different fish tissues so that SNK and Dunnett's T3 tests were employed respectively for homogeneity and heterogeneity of variance (Zar 1999). Kruskal–Wallis test was applied for the non-normal distribution of



Fig.1. Diagram presenting the steps of the procedures used in this study.

Table 1. Quality control of THg tracking process accuracy (µg.kg⁻¹ dry weight).

SRM ^a	Ν	Certified value	Obtained mean	SD ^b	R ^c
NIST-1633b	7	0.141	0.135	0.018	95.5
NIST-2709	7	1.4	1.47	0.136	105
NIST-2711	7	6.25	6.34	0.215	101

a: Standard reference material

b: Standard deviation

c: Recovery (%)

Table 2.Statistical values of T-Hg between muscle, liver and spleen VS age, maturity and gender of Esox lucius, Anzali wetland.

Factor		N	Muscle (ppb)		Liver (ppb)		Spleen (ppb)	
		IN	Mean Standard error		Mean	Mean Standard error		Standard error
Maturity I	Immature	53	747.79	99.91	767.91	61.71	355.09	49.35
	Adult	25	245.81	14.05	160.79	4.61	234.01	10.91
Sex	Μ	24	531.84	110.65	388.09	100.3	291.64	75.06
	F	29	926.51	151.78	533.96	75.78	407.6	64.96
Age	1+	25	245.81	14.05	160.79	4.6	234.01	10.91
	2^{+}	5	404.74	112.52	244.88	87.34	165.42	61.9
	3+	12	516.36	139.98	202.96	55.76	151.02	30.29
	4+	22	676.66	99.54	452.14	87.79	354.7	69.53
	5+	14	1180.45	71.3	799.43	143.28	598.28	123.46

data, and the Spearman correlation test was utilized to analyze the correlation between variables. Permissible level of 5% of Type I errors was considered in all stages of statistical analyses, and mean values sharing the same superscript had no statistically significant differences (a>b>c>d>e). Data was analyzed using SPSS software.

RESULTS

The mean and standard error (SE) of T-Hg bioaccumulation in different tissues based on age, maturity and genders are shown in Table 2. Results of the analysis variance related to the T-Hg concentration in different tissues of pike (Table 2) indicated a significant difference in the mean concentrations of T-Hg in muscle, liver, and spleen versus age groups



Fig.2. Comparison of the mean T-Hg between muscle, liver and spleen VS age of Esox lucius, Anzali wetland.

Table 3. Analysis of variance of T-Hg between muscle, liver and spleen VS age, maturity and gender of *Esox lucius*, Anzali wetland.

Sources of variation	Tissue	Test Indices	Р
Age	Muscle	X ² (4)= 21.433 **	0.000
	Liver	X ² (4)= 21.842 **	0.000
	Spleen	F(4, 73)= 5.801 **	0.000
Maturity	Muscle	U=285 **	0.000
	Liver	U=475 *	0.045
	Spleen	U = 616 ns	0.619
Sex	Muscle	T(51)= -2.814 **	0.007
	Liver	U= 217 *	0.019
	Spleen	$U=279^{ns}$	0.218

ns: non-significant; * and **: significant at P<0.05 and P<0.01, respectively

(P<0.01). In muscle tissue, the mean T-Hg concentration had significant positive relationships with the fish age so that the lowest mercury concentration (less than 0.5ppm) was observed for 1⁺ and 2⁺-age group, and the highest level (1.2ppm) was recorded for 5⁺ age class. Regarding the T-Hg bioaccumulation the liver, in significant differentiation between the lowest and highest levels were recognized in 1^+ and 5^+ age class (P<0.05) (Fig. 2). Changes in mercury concentration in the spleen were slightly more complex than in muscle and liver, and T-Hg concentration pattern in spleen dose not fallow age classes (Fig. 2). Maturity and sex parameters had significant effects on the mean concentration of T-Hg in the dorsal muscle and liver (P < 0.05), so that T-Hg concentrations in dorsal muscle and liver tissues of adults were higher than immature individuals (P < 0.05) and its levels in the liver of females was higher than males (P < 0.05).

However, bioaccumulation of T-Hg levels showed no significant differences in the spleen of adults versus immature pike (P < 0.05) and between males and females (P < 0.05) (Table 3, Figs. 3 and 4). The correlation coefficient and significant tests values (Spearman correlation coefficient) for age, body length, and weight of the studied pikes are listed in Table 4. Results of semi partial correlation showed that the mean T-Hg concentrations in dorsal muscle, liver, and spleen were directly related to each other (P < 0.05). The mean T-Hg concentrations in muscle and liver were directly related to the age, total length and weight (P < 0.01). The Results show that mercury bioaccumulation in dorsal muscle, increased significantly whit age increased (P<0.05) (Figs. 5, 6 and 7). However, results indicated (Table 5) that T-Hg concentration in the spleen had no significant correlation with the mentioned parameters (P>0.05)which might mean their nonlinear relationships.



Fig.3. Comparison of the mean T-Hg between muscle, liver and spleen VS maturity of Esox lucius, Anzali wetland.



Fig.4. Comparison of the mean T-Hg between muscle, liver and spleen VS gender of Esox lucius, Anzali wetland.

Table 4. Linear correlation of T-Hg between muscle, liver and spleen VS age, length and weight of *Esox lucius*, Anzali wetland.

	Age	Body length	Weight	Total mercury in muscle	Total mercury in liver	Total mercury in spleen
Age	1.00					
Total L.	0.929 **	1.00				
Weigth	0.929 **	0.995 **	1.00			
T-Hg in muscle	0.525 **	0.481 **	0.491 **	1.00		
T-Hg in liver	0.444 **	0.372 **	0.372 **	0.603 **	1.00	
T-Hg in spleen	0.196 ^{ns}	0.131 ^{ns}	0.141 ^{ns}	0.498 **	0.683 **	1.00

ns: non-significant; * and **: significant at P<0.05 and P<0.01, respectively.

Acceptable monthly intake (AMI) without carcinogenic effects were measured via formula provided by the U.S. Environmental Protection Agency (assuming 100% uptake of mercury into the body). AMI estimated for women, men, juveniles and

children as 270, 320, 125, and 59g. per month, respectively.



Fig.5. The mean T-Hg accumulated between tissue, liver and spleen VS age of Esox lucius, Anzali wetland.



Fig.6. The mean T-Hg accumulated between tissues, liver and spleen VS weight of Esox lucius, Anzali wetland.



Fig.7. The mean T-Hg accumulated between different tissues, liver and spleen VS length of Esox lucius, Anzali wetland.

Appearance traits (X)	Dependent variables (Y)	Statistical model	\mathbb{R}^2	F	Р
	Muscle	Y=331.64-102.33x+52.74x ²	0.969	31.41	0.031
Age	Liver	Y=335.42-201.9x+58.39x ²	0.963	25.93	0.037
	Spleen	Y=446.49-296.19x+60.16x ²	0.988	79.97	0.012
Total Length	Muscle	Y=341.06-15.14x+055x ²	0.997	384.26	0.003
	Liver	Y=300.93-18.73x+0.51x ²	0.952	19.88	0.048
	Spleen	$Y = (197899 - 17462 + 369.36 x^2)^{0.5}$	0.981	50.45	0.019
Weight	Muscle	Y=242.23+0.63x	0.997	1191.25	0.000
	Liver	Y=112.82+0.45x	0.940	46.80	0.006
	Spleen	Y=132.51+0.29x	0.807	12.50	0.038

Table 5. Linear regression analysis between the biometric parameters VS mean T-Hg in muscle, liver, and spleen of *Esox lucius*, Anzali wetland.

DISCUSSION

Based on our findings, the minimum and maximum T-Hg concentrations in dorsal muscle were measured for 1 and 5 years, 0.2 and 1.2ppm respectively. T-Hg concentration in the dorsal muscles of pikes increased significantly with age increased, so that it was exceeded the safe limit (0.17ppm) for all age groups. T-Hg concentrations in dorsal muscles were measured for 1+ and 2+ age group less than 0.5ppm, and for 3^+ and 4^+ age group was more than 0.5ppm, and for 5^+ age group was 1.2ppm, higher than the WHO safe limit 1ppm. Duffy et al. (1999) reported that the maximum concentration of T-Hg accumulated in muscles of pikes from the Andrefski and Sulukna rivers were estimated 1.82 and 1.51ppm, respectively, which exceeded the permissible limit of WHO for pikes. Lockhart et al. (2005) also examined the amount of T-Hg in the edible muscle of fish in northern lakes and found that T-Hg in pike and lake trout exceeded the subsistence consumption guideline (0.17ppm) and often exceeded the higher guideline of 0.5µg.g⁻¹ T-Hg for commercial fish. The researchers also reported the highest levels of T-Hg accumulated in the northern pike at 1.79ppm. In another study, Kojadinovic et al. (2006) reported that the mean value of T-Hg accumulation in 15 commercial tuna fish species of the western Indian ocean were ranged from 0.2 to 3.97ppm (Kojadinovic et al. 2006). Also, Jewett & Duffy (2007) examined 17 fish species from Alaska and found that the mean T-Hg accumulated in the northern pike was higher than other species (>1ppm). Chien et al. (2007) also investigated the permissible consumption of mercury-contaminated fish in Taiwan

and reported the mean T-Hg in swordfish (Xiphias gladius) and sharks (Lamniformes) was 0.77 and 0.73ppm respectively. Shohreh et al. (2020) studied the heavy metals in some fishes from Caspian Sea and Persian Gulf. The ranges obtained for Hg in fish samples were found as 0.007-0.067 mg/kg. Total mercury level in *Rutilusfrisii kutum* (0.021), Parastromateus niger (0.067), Pomadasys kaakan (0.007)and for Scomberomorus commerson determined as 0.018 mg/kg. Also Ziarati et al. detected that mercuy level in some frozen fishes in Persian Gulf and reported that total mercury level in Carcharhinus dussumie and Pomadasys furcatus determined as 0.79 and 0.29 respectively (Ziarati et al. 2017). Davis et al. (2008) examined the levels of mercury in the muscle of 10 species of the Sacramento Delta in California and found that 30% of specimens had more than 0.5 ppm. The present study showed that the mean concentration of T-Hg in muscle and liver increased whit age increased (P < 0.05). This finding agrees with the findings of other researchers (Anan et al., 2005; Davis et al., 2008; Burger, 2009). The mean concentrations of T-Hg in the spleen were not significantly related to the biometric parameter, and T-Hg concentration increased whit age increased in both females and males (P < 0.05) but the gender had significant effect on the mean T-Hg in muscle, liver and spleen (P < 0.05) and was significantly higher in female than meal (P < 0.05).

Differences in T-Hg concentrations between males and females may be due to differences in the metabolic activities of both sexes (Garcia & Carignan 2003; Canli & Atli 2004). The results presented by

Jewett et al. (2003) confirmed that no significant difference was observed between males and females of northern pike whit regard to the mean T-Hg in dorsal muscle (P>0.05).; however, the amount of T-Hg accumulated in the Arctic grayling females was significantly higher than males (P<0.05). In present study the results showed that the mean T-Hg had estimated for muscle more than liver and spleen significantly (P<0.05). Jewett et al. (2003) examined mercury and methyl-mercury levels in the northern pike of western Alaska Rivers and confirmed T-Hg levels in dorsal muscle (1.52ppm) were significantly higher than liver (1.ppm). Hajeb et al. (2009) also evaluated mercury levels in 12 species of commonly consumed marine fishes in Malaysia and indicated that mercury accumulation in muscle is higher than liver. Davis et al. (2008) examined the amount of T-Hg in muscle from 10 species of sport fish captured in the Sacramento-San Joaquin River Delta, California, and found that 30% of pikes had more than 0.5 ppm. The present study showed that the mean concentration of T-Hg in muscle and liver had significantly positive relationship with age, length, and weight, which was consistent with the results founded by other researchers (Canlh & Atli 2003; Black More & Wang 2004; Anan et al. 2005; Davis et al. 2008; Burger 2009). The mean concentrations of T-Hg in the spleen were not significantly related to biometric parameters (P < 0.05).Gender had a significant effect on the man mercury accumulation in muscle and liver (P < 0.05). Our findings showed that T-Hg in females was higher than males (P < 0.05). Evidence suggests that differences in mercury concentrations between males and females may be due to differences in the metabolic activities of both sexes (Garcia & Carignan 2003; Canli & Atli 2004). The results presented by Jewett et al. (2003) showed that no significant differences were recognized between the mean T-Hg concentrations in dorsal muscle of northern pike whit regard to the gender(P > 0.05), however, mercury accumulated in females of grayling was significantly higher than males (P < 0.05). In present study findings revealed that the mean of T-Hg concentration in muscle significantly increased in liver compared to

spleen (P < 0.05). Hajeb et al. (2009) evaluated mercury levels in 12 species of commonly consumed marine fishes in Malaysia and found that overall mercury accumulation in muscle was more than liver (P < 0.05). Similarly, Regine et al. (2006) examined 12 species of fish collected from the French Guiana region (Amazon Basin), and found that the accumulation of mercury in muscle increased significantly compared to other tissues(P < 0.05).

The purification process in the liver can removes different levels of T-Hg (Jewett et al. 2003). Mercury enters the fish body as methyl-mercury through nutrition and binds to the amino acids of the muscle tissue with sulfide bonds, where it accumulates at a higher rate. Since mercury accumulation capacity is directly related to tissue volume (Blakmore & Wang 2004; Chen et al. 2005), it's elimination rate decreases with increasing fish size. (Yamaguchi et al. 2004). On the other hand, low levels of mercury in the environment preferably accumulate in the muscle tissue (Sager, 2004), and methyl-mercury induces the secretion of gonadal hormones from the liver that resulted in reducing mercury accumulation (Yamaguchi et al. 2004). Mercury accumulation in tissues is also affected by several factors, e.g., metabolic and biodilution rates (Anan et al., 2005). Sharma et al., (2008) showed that the rate of mercury biodilution in fish muscle was inversely related to fish length and its accumulation depended on the time of mercury exposure and its accumulation mechanism. Hence, some researchers have reported that mercury accumulation increased with age and length increased (Jewet & Duffy 2007; McIntyre & Beauchamp 2007). Jewet et al. (2003) also showed a significant relationship between mercury accumulation in muscle and body length of northern pike and Arctic grayling collected from rivers in western Alaska (Farkas et al. 2003).

Burger et al. (2009) showed that the body length of bluefish was directly related to the amount of mercury accumulation in muscle. Mercury exposure might be reduced by reducing consumption of larger fish. In the present study, the correlation between pike total length and T-Hg accumulation in muscle was

0.997. calculated equivalent to which is approximately comparable to the values of 0.82 and 0.86, reported by Davis et al. (2008) and Farkas et al. (2003) respectively. Our findings showed that the amount of T-Hg in dorsal muscle of pikes with total length more than 35 cm is exceeded the acceptable limit of 0.5ppm. The acceptable daily intake without carcinogenic effects for T-Hg concentration recognized equal to 0.71µg.day⁻¹.kg⁻¹ body weight by the WHO (Kojadinovic et al. 2006; USEPA 2006) while its rate for all population reported equal to 0.1 and 0.4µg.day⁻¹.kg⁻¹ body weight by the FDA and EPA (Kojadinovic et al. 2006). Jewett et al. (2003) calculated the mean T-Hg concentration in muscle (1.52ppm), estimated that acceptable monthly intake rates of T-Hg was equal to one meal per month for adults and two meals per year for children. Kojadinovic et al. (2006) determined the mean levels of mercury accumulation in 15 commercial tuna fish species of the western Indian ocean between 0.2 and 3.97ppm and ADI for tuna fish were estimated as four meals of 230g per month for adults (except pregnant and lactating mothers). Chien et al. (2007) also studied permissible the consumption of mercurycontaminated fish in Taiwan and reported that mean T-Hg in swordfish and sharks was between 0.77 and 0.73ppm, and the ADI of these fish determined between <50 and <90g. per day respectively. Also Burger (2009) reported that pregnant and lactating mothers, children, and the elderly should avoid eating bluefish with a body length of more than 50cm (average mercury greater than 0.3ppm).

According to the results of the present study, pregnant and lactating mothers, children, and the elderly should avoid eating pike with a body length of more than 35cm (average T-Hg>0.5 ppm). In this study acceptable monthly intake without carcinogenic effects were measured via the formula provided by the U.S. Environmental Protection Agency (assuming 100% uptake of mercury into the body). And acceptable monthly intake without carcinogenic effects for women, men, juveniles and children are 270, 320, 125, and 59 g. respectively.

CONCLUSION

Total mercury distribution in tissue and organs was as follows: muscle> liver> spleen. Pregnant and lactating mothers, children, and the elderly should avoid eating pike with a body length of more than 35 cm (average mercury greater than 0.5ppm). Based on USEPA criteria the acceptable monthly intake of pike from Anzali wetland without carcinogenic effects for women, men, teenager and children are 270, 320, 125, and 59g. per month respectively.

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مقاله كامل

الگوی پراکنش جیوه کل (T-Hg) در ماهیچه، کبد و طحال اردک ماهی (Esox lucius) در تالاب انزلی، دریای خزر

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انستیتو تحقیقات بینالمللی ماهیان خاویاری، موسسه تحقیقات علوم شیلاتی کشور، سازمان تحقیقات، آموزش و ترویج کشاورزی، رشت، ایران.

چکیده: مطالعه حاضر با هدف بررسی تجمع زیستی جیوه کل (T-Hg) در بافت ماهیچه (خوراکی)، کبد و طحال اردک ماهی (Esox lucius) از تالاب انزلی انجام شد. دریافت ماهانه قابل قبول (AMI) براساس معیارهای FDA اندازه گیری شد. نمونه برداری با استفاده از توری گوشگیر و الکترو شوکر از تیر ماه ۱۳۹۹ لغایت تیرماه ۱۴۰۰ انجام شد. علاوه بر این، ۷۸ نمونه (محدوده سنی ۵-۱ سال، طول کل: ۵۸-۱۰ سانتی متر، وزن: ۱۹۶۰-۲۱ گرم) جمع آوری شد. نمونهها توسط IECO AMA254 آنالیز شدند. اندازه گیری ها به این صورت آنالیز شد: زمان خشک شدن ۷۰ ثانیه، زمان تجزیه ۱۲۰ ثانیه در دمای ۲۰۰ درجه سانتی گراد در اتمسفر اکسیژن، و زمان انتظار ۵۰ ثانیه. همین روش برای نمونه شاهد نیز استفاده شد. قبل از هر آزمایش، شکل THg در ماده مرجع گواهی شده مطابق با استاندارد ASTM NOD-6722 با سه ماده ثانیه. همین روش برای نمونه شاهد نیز استفاده شد. قبل از هر آزمایش، شکل THg در ماده مرجع گواهی شده مطابق با استاندارد ASTM NOD-6722 با سه ماده مرجع استاندارد اندازه گیری شد. ASTM 16336 هد. قبل از هر آزمایش، شکل THg در ماده مرجع گواهی شده مطابق با استاندارد یمرجع استاندارد اندازه گیری شد. THg و ۲۷۰۹ مین ترا ۲۹ و ۲۷۱۱ میز مان تجزیه ۲۰ و در ماده مرجع مواهی شده مطابق با ستاندارد اندازه تاب میز است ماده مرجع استاندارد اندازه گیری شد. ASTM 16336 هدار ۲۰۰۹ میلی و ۲۱۷ مین مین T+و در سه تکرار با درجه دقت بین ۵/۵۹ تا ۱۰/۵٪ بود. حد تشخیص ۲۰۰/۰ میلی گرم بر مرجع استاندارد اندازه گیری شد. ASTM 16336 هداره ترا ۲۰۱۰ و ۲۰۱۲ مین ۲۰۸۰ مالی و در جه روزن خشک بود. حد تشخیص ۲۰۰/۰ میلی گرم بر کلوگرم وزن خشک بود. حداقل و حداکثر HH-5 در عضلات پشتی بین ۲/۰ و ۲/۱ و ای در ۲۰۵۰ ۲۰ با فزایش سن بهطور معنی داری افزایش یافت (۲۰/۰۰). کلوگرم وزن خشک جد. حداقل و حداکش میشتر از کبد و طحال بوده (۵/۰۰) و در جنس ماده بیشتر از نر بود (۵/۰۰) ۲۰ در مال