Heavy Metal Levels in Farmed and Wild Fishes of Aegean Sea and Assessment of Potential Risks to Human Health

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Abstract

The aim of this study was to determine the levels of Ag, Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn in wild seabream (*Sparus aurata*), blotched picarel (*Spicara maena*), pandora (*Pagellus erythrinus*) fished from the Aegean Sea and the farmed seabream (*Sparus aurata*), and to assess the risks depending on their consumption by humans. A total of 40 fishes were collected and the metal levels were determined using ICP-OES. The metal levels were determined to be Ag (Nd), Al (3.20-8.87), Co (0.01-0.01), Cr (0.02-0.51), Cu (0.96-1.28), Fe (0.23-4.30), Mn (0.15-0.39), Ni (0.13-0.22), Pb (0.00-0.10) and Zn (6.18-7.30) (mg kg⁻¹ w.w.) in the study. No Ag or Cd were found in the samples. The heavy metal levels were below the national and international legal limits. The target hazard quotient (THQ) and total target hazard quotient (TTHQ) method was used to determine the possible risks of the heavy metals on human health. THQ and TTHQ values were all less than 1 and it was concluded that they do not pose any risks in terms of human health.

Keywords: Aegean Sea, Heavy metal, Pagellus erythrinus, Sparus aurata, Spicara maena, Risk assessment

Ege Denizi Vahşi ve Çiftlik Balıklarının Ağır Metal Düzeyleri ve İnsan Sağlığı Üzerine Potansiyel Risklerinin Değerlendirilmesi

Özet

Bu çalışmada Ege Denizinden avlanan vahşi çipura (*Sparus aurata*), izmarit (*Spicara maena*), mercan (*Pagellus erythrinus*) ve çiftlik çipura (*Sparus aurata*) türlerinin Ag, Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb ve Zn düzeylerinin belirlenerek tüketimlerine bağlı olarak insan sağlığı risk değerlendirmesi yapılması amaçlandı. Toplam 40 adet balık toplandı ve metal düzeyleri ICP-OES ile belirlendi. Çalışmada balık türlerindeki metal düzeyleri Ag (Nd), Al (3.20-8.87), Co (0.01-0.01), Cr (0.02-0.51), Cu (0.96-1.28), Fe (0.23-4.30), Mn (0.15-0.39), Ni (0.13-0.22), Pb (0.00-0.10) ve Zn (6.18-7.30) (mg kg⁻¹ y.a.) olarak belirlendi. Örneklerde Ag ve Cd'a rastlanmadı. Belirlenen ağır metal düzeyleri ulusal ve uluslararası yasal limitlerin altında bulundu. Ağır metallerin insan sağlığı üzerine olası risklerinin değerlendirilmesinde target hazard quotient (THQ) ve total target hazard quotient (TTHQ) metodu kullanıldı. THQ ve TTHQ değerleride 1'in altında bulundu ve insan sağlığı açısından herhangi bir risk oluşturmadığı sonucuna varıldı.

Anahtar sözcükler: Ege Denizi, Ağır metal, Pagellus erythrinus, Sparus aurata, Spicara maena, Risk değerlendirmesi

INTRODUCTION

Water pollution due to heavy metals is an important issue today. Is not only threatens the aquatic ecosystems, but also human health [1]. The passage of heavy metals into ecological systems is due to human activities mostly [2,3]. Especially during the two centuries since the

industrial revolution, thermal power plants, garbage and waste cremation facilities have been the main sources of heavy metal contamination. Heavy metals create potential hazards for humans because they remain in the environment for a long time, have high levels of toxicity, tend to accumulate in living tissues and affect the top of the food chain more ^[4,5].











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Fish is high quality and valuable food that contains essential amino acids, required for humans, essential fatty acids (omega-3 etc.), carbohydrates, fat soluble vitamins and important macro and micro elements such as Ca, Mg, and Se. Therefore, fish consumption is important to maintain and improve human health [6,7]. The accumulation of heavy metals in fishes is due to the absorption of ions dissolved with suspended particles and foods from the lipophilic membranes in intestines and gills [8,9]. Potentially toxic metals such as Cd, Pb, Ni may harm to human health if they are ingested for long times even in small concentrations. On the other hand, essential minerals such as Cu, Zn, Fe, Mn may become toxic at higher levels [10,11]. Cr causes allergic dermatitis and cancer, short term exposure to Cu causes gastrointestinal problems and long term exposure to it causes liver and kidney disorders and the Wilsons disease. Cd and Pb have toxic effects on kidneys, in addition to chronic toxic effects such as lowering breeding capacity, atherosclerosis, learning difficulties, hypertension, tumors, function disorders in the liver and other organs [5,12].

The southern part of the Aegean Sea is being heavily polluted by discharges from tourism, agriculture, mining and industry. However the literature concerning the levels of metal in the tissues of wild fish that are heavily fished in the fish farms and their environs are scarce ^[7,13]. Determination of metal levels in these fish, and assess them in terms of human health is very important with regard to public health. Metal accumulation in fish is affected by physiological (species, size, sexual maturity and feeding habits) and environmental (seasonal changes, water quality and pollution level) factors ^[7,9,14].

This study aimed to determine and compare the heavy metal levels in muscle tissues of farmed fish and the wild fish caught around them, and to evaluate the health risks that may arise in humans according to consumption scenarios.

MATERIAL and METHODS

Samples, Study Area and Preparation

The muscle tissues excised from the same regions of the fishes were used to determine Ag, Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn levels. 10 each of *Sparus aurata* Linnaeus, 1758 (*SaW*), *Spicara maena* L., 1758 (*Sm*) and *Pagellus erythrinus* Linnaeus, 1758 (*Pe*) were fished around the fish farms in the region between the Göltürkbükü, Torba, Güvercinlik Bay around the Güllük Bay in the city of Muğla at the Aegean shore, and Kuşadası (N 30°30' and E30°30') by hook fishing. Additionally, 10 farmed *Sparus aurata Linnaeus*, 1758 (*SaF*) samples were collected from marine products sales points in the same region to reach a total of 40 fishes. Microwave acid digestion (BERGHOF, Germany) system was used to burn the organic parts to determine the amount of metals in the muscle tissues. Homogenized muscle tissue samples

of 0.5 g each were weighed into vessels. Then 8 mL nitric acid (HNO_3 ; 65%, Merck, Germany) and 2 mL hydrogen peroxide (H_2O_2 ; 30%, Merck, Germany) were added before burning the samples in a 4 step process compliant with the procedure for 15 min at 85% power and 200°C. The samples were filtered through a filter paper and filled up to 25 mL with deionized water and kept in plastic tubes at +4°C prior to analysis.

Analysis

The levels of heavy metal in the filtrates containing Ag, Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn were determined with the Inductively Coupled Plasma Optical Emission Spectrophotometer (ICP-OES) model Optima 3000DV sequential (Perkin-Elmer-Norwalk, USA) device according to USEPA (Method 200.7) [15]. Measurements were triplicated for each sample. Heavy metal concentrations in fish muscles were expressed as mg kg⁻¹ wet weight (ww). The data quality was checked by using standard reference material, the recovery results was satisfactory and ranged from 97.6% to 105.3%. Instrument Limit of Detections (LOD) (mg kg⁻¹) were 0.001 for Cd and Co, Ag, 0.002 for Al, Cr, Cu, Fe and Zn, 0.003 for Mn and Ni, 0.007 for Pb.

Health Risk Assessment

The metal levels of muscle tissues were used to determine the potential health risks arising from *SaW, Sm, Pe* and *SaF* consumption. The target hazard quotient (THQ) method ^[16] with the formula given below, determined by The US Environmental Protection Agency was used in the study to assess the health risks of heavy metal accumulation in fishes.

$$THQ = \frac{E_F \times E_D \times F_{IR} \times C}{R_{fD} \times W_{AB} \times T_A} \times 10^{-3}$$

Where E_F is exposure frequency (365 days/year), E_D is exposure corresponding to average lifespan (70 years), F_{IR} is the consumption rate (g/person/day) varying according to the consumption habits which is 17 g/person/day (6.3 kg/person/year) [17] for Turkey and 52 g/person/day (19.2 kg/person/year) for the world [18], C is the metal concentration in the food (mg kg⁻¹ ww), R_{fD} is the oral reference dose (mg kg⁻¹/day) [19], W_{AB} is the average body weight (bw) (70 kg) and T_A is the average non-carsinogenic exposure duration (E_FxE_D) [7,12,20,21]. THQ values less than 1 shows that the exposed population is not under risk. The Total THQ (TTHQ) with the formula given below is the total of THQ values used in recent studies [12,20,21].

$$\mathsf{TTHQ} = \mathsf{THQ}_{\mathsf{Ag}} + \mathsf{THQ}_{\mathsf{Al}} + \mathsf{THQ}_{\dots} + \mathsf{THQ}_{\mathsf{Zn}}$$

Statistical Analysis

One-way analysis of variance (ANOVA) was performed to determine statistically significant differences. The level of P<0.05 was considered statistically significant.

RESULTS

The heavy metal concentrations determined through ANOVA tests are given as mg kg⁻¹,wet weight \pm sd in *Table 1*. The results of the comparative Tukey multiple test only show a statistically significant difference between the *SaF* for Cr accumulation and *SaW* (P<0.05). The average metal levels in fish muscle tissues were Zn>Al>Fe>Cu> Mn>Cr>Ni>Pb>Co. The metal levels in terms of fish species were ordered *Sm>SaF>Pe>SaW*.

The length (cm) of fish samples ranged from 16.3 to 29.6 for *SaW* with a mean of 22.7 cm, 14.7 to 26.8 for *SaF* with a mean of 20.6 cm, 9.6 to 20.1 for *Sm* with a mean of 15.8 cm and 9.5 to 25.2 *Pe* with a mean of 16.2 cm. The weight (g) of fish samples ranged from 136 to 284 for *SaW* with a mean of 193 g, 92 to 212 for *SaF* with a mean of 156 g, 19.8 to 56.0 for *Sm* with a mean of 33.2 g and 28.9 to 210.2 *Pe* with a mean of 68.8 g.

According to the results, the highest metal levels were (mg kg⁻¹) Al 8.87, Cr 0.51, Cu 1.28, Fe 4.30, Mn 0.39, Ni 0.22, Pb 0.10 and Zn 7.30. The average was 0.01 for Co in all samples, while Pb, Cd and Ag were not detected in *SaW* and *SaF*.

The heavy metal levels for SaF were found to be on average (mg kg⁻¹) Ag (Nd), Al (6.06 ± 5.14), Cd (Nd), Co (0.01 ± 0.01), Cr (0.51 ± 0.25), Cu (0.96 ± 0.79), Fe (3.96 ± 4.90), Mn (0.15 ± 0.10), Ni (0.13 ± 0.13), Pb (Nd) and Zn (6.92 ± 1.95), for SaW they were Ag (Nd), Al (3.20 ± 1.92), Cd (Nd), Co (0.01 ± 0.01), Cr (0.10 ± 0.06), Cu (1.15 ± 2.45), Fe (1.76 ± 2.14), Mn (0.39 ± 0.32), Ni (0.22 ± 0.15), Pb (Nd) and Zn (6.18 ± 3.36). No Ag, Cd and Pb were detected for SaF and SaW. In wild (SaW) and farmed (SaF) S. aurata, there were no statistically significant differences in heavy metal levels except

Cr between the farmed and wild subtypes (P<0.05).

The average metal levels in the muscule tissues of the fish samples in the calculation of the THQ and TTHQ values are given in *Table 2*. THQ values for all fish species were ranged as follows: 0.0E+00 for Ag, 7.8E-04 and 6.6E-03 for Al, 0.0E+00 for Cd, 8.1E-03 and 2.5E-02 for Co, 1.6E-03 and 1.3E-01 for Cr, 5.8-03 and 2.4E-02 for Cu, 8.0E-05 and 4.6E-03 for Fe, 2.6E-04 and 2.1E-03 for Mn, 1.6E-03 and 8.2E-03 for Ni, 0.0E+00 and 1.9E-02 for Pb, 5.0E-03 and 1.8E-02 for Zn. The TTHQ values on the other hand varies between 3.2E-02 and 2.0E-01.

DISCUSSION

Although water products are important for the human diet, they are a source of food that needs to be constantly monitored and assessed for risks in terms of public health due to the risk of heavy metal accumulation [7,21,22]. The absorption of essential metals which are more than the physiological requirements or the absorption of toxic metals which are more than the tolerable limits may cause harmful effects [21]. The aim of this study was to determine the levels of heavy metals in wild and farmed fish in the Aegean Sea, and to compare them to assess the risks caused by metals depending on consumption. The results of the study shows that the accumulation in the muscle tissues of fishes consumed by human were all below the limits determined by national and international authorities (maximum allowed heavy metal amounts in fish muscle tissue, mg kg⁻¹) (Cu:20, Cd:0.05, Pb:0.30 and Zn:50) [23,24].

Dalman et al. [25] have found Pb: <0.02-0.4, Cd: <0.01-0.04, Cu: <0.1, Zn: <0.5-7.2 mg kg $^{-1}$ in their study for the fish (*Dicentrarchus labrax*) obtained from fish farms in the Güllük Bay.

Metals	S. aurata		S. maena	P. erythrinus	Total
	Wild (n:10)	Farmed (n:10)	Wild (n:10)	Wild (n:10)	n:40
Ag	Nd	Nd	Nd	Nd	Nd
Al	3.20±1.92	6.06±5.14	8.87±5.59	5.42±3.05	5.76±4.32
Cd	Nd	Nd	Nd	Nd	Nd
Со	0.01±0.01	0.01±0.1	0.01±0.01	0.01±0.01	0.01±0.01
Cr	0.10±0.06ª	0.51±0.25 ^b	0.24±0.28ab	0.02±0.05ª	0.22±0.26
Cu	1.15±2.45	0.96±0.79	1.28±3.14	1.06±0.74	1.16±1.93
Fe	1.76±2.14	3.96±4.90	4.30±1.78	0.23±0.41	2.60±3.16
Mn	0.39±0.32	0.15±0.10	0.31±0.34	0.37±0.26	0.30±0.27
Ni	0.22±0.15	0.13±0.13	0.19±0.29	0.20±0.17	0.19±0.19
Pb	Nd	Nd	0.10±0.13	0.09±0.11	0.05±0.09
Zn	6.18±3.36	6.92±1.95	6.83±1.64	7.30±0.82	6.81±2.05

Table 2. THQ and TTHQ estimate for individual metals caused by the consumption of wild S. aurata, S. maena, P. erythrinus and farmed S. aurata for inhabitants **Table 2.** Tüketiciler icin vahsi S. aurata, S. maena, P. erythrinus ve ciftlik S. aurata tüketimine bağlı calısılan metaller icin hesaplanan THQ ve TTHQ

Metals	THQs and TTHQs	S. aurata		S. maena	P. erythrinus
		Wild	Farmed	Wild	Wild
Ag	THQ ^f	0.0E+00	0.0E+00	0.0E+00	0.0E+00
	THQ ^t	0.0E+00	0.0E+00	0.0E+00	0.0E+00
Al	THQ ^f	2.4E-03	4.5E-03	6.6E-03	4.0E-03
	THQ ^t	7.8E-04	1.5E-03	2.2E-03	1.3E-03
Cd	THQ ^f	0.0E+00	0.0E+00	0.0E+00	0.0E+00
	THQ ^t	0.0E+00	0.0E+00	0.0E+00	0.0E+00
Со	THQ ^f	2.5E-02	2.5E-02	2.5E-02	2.5E-02
	THQ ^t	8.1E-03	8.1E-03	8.1E-03	8.1E-03
-	THQ ^f	2.5E-02	1.3E-01	5.9E-02	5.0E-03
Cr	THQ ^t	8.1E-03	4.1E-02	1.9E-02	1.6E-03
Cu	THQ ^f	2.1E-02	1.8E-02	2.4E-02	2.0E-02
	THQ ^t	7.0E-03	5.8E-03	7.8E-03	6.4E-03
Fe	THQ ^f	1.9E-03	4.2E-03	4.6E-03	2.4E-04
	THQ ^t	6.1E-04	1.4E-03	1.5E-03	8.0E-05
Mn	THQ ^f	2.1E-03	8.0E-04	1.6E-03	2.0E-03
	THQt	6.8E-04	2.6E-04	5.4E-04	6.4E-04
Ni	THQ ^f	8.2E-03	4.8E-03	7.1E-03	7.4E-03
	THQ ^t	2.7E-03	1.6E-03	2.3E-03	2.4E-03
Pb	THQ ^f	0.0E+00	0.0E+00	1.9E-02	1.7E-02
	THQ ^t	0.0E+00	0.0E+00	6.1E-03	5.5E-03
Zn	THQ ^f	1.5E-02	1.7E-02	1.7E-02	1.8E-02
	THQ ^t	5.0E-03	5.6E-03	5.5E-03	5.9E-03
TTHQ ^f		1.0E-01	2.0E-01	1.6E-01	9.8E-02
TTHQ ^t		3.3E-02	6.5E-02	5.3E-02	3.2E-02

^f Fish ingestion rate by FAO (52 g/person/day) ^[18]; ^t Fish ingestion rate by TURKSTAT (17 g/person/day) ^[17]; **THQ:** Target hazard quotient; **TTHQ:** Total target hazard quotient

Uluturhan and Kucuksezgin ^[26] have determined the levels of Cd: 2.0±0.91, Pb: 59.3±34.7, Zn: 2810±142, Cu: 164±24.1 μ g kg⁻¹ in their study conducted in 2007 on fish from Didim. Tepe et al. ^[27] have determined heavy metal levels of Cd: 0.03±0.01, Co: 0.03±0.00, Cr:0.13±0.05, Cu: 1.56±0.43, Fe: 46.9±12.1, Mn: 0.37±0.07, Ni: 0.31±0.16, Pb: 0.39±0.18, Zn: 5.20±0.81 μ g g⁻¹ for the fish they studied in lzmir.

Comparison of the study performed by Alsaver et al. [28] on the wild and farmed fish from the Aegean Sea in Greece with the present study, the Cr and Al levels were higher while other metal levels were lower. Comparison of the study performed by Yıldız [13] except the Cu, Fe and Zn levels the Co, Cr, Mn, Ni, Pb levels were determines as higher than our study. According to data from Yıldız, Mn, Cd, Cr, Co, Pb and Ni levels were lower, Cu and Zn levels were higher and Fe level was similar [13,28].

The levels detected in different fishes in the present

study are parallel with the levels in the study by Uluturhan and Kucuksezgin $^{[26]}$. In the studies by Dalman et al. $^{[25]}$ and Tepe et al. $^{[27]}$ (mg kg $^{-1}$) the Zn (0.5-7.2), Mn (0.37), Cr (0.13) levels were close to, and the Cd (0.03), Fe (46.9), Ni (0.31), Pb (0.39), Co (0.03) and Cu (1.56) levels were higher than the levels in the present study.

The study of Yabanlı and Alparslan [12] on fish caught on the Muğla shore, reported the heavy metal levels to be (mg kg⁻¹) Cr: 0.27-0.39, Cu: 0.12-0.22 and Pb: 0.10-0.12. Compared to the data from this study, the Cr level is similar, the Cu level is higher and the Pb level is lower than the levels in our study.

In the study performed by Kalantzi et al.^[7] on *SaF* species bred on the Greek shore of the Aegean, they determined the average heavy metal levels to be (mg kg⁻¹) Al: Nd-1.92, Cd: Nd, Co: Nd, Cr: 0.07-0.15, Cu: Nd, Fe: Nd-2.77, Ni: Nd, Pb: Nd and Zn: 4.29-4.99. Compared to the data from this study, the Al, Cr, Fe, Ni and Zn levels are lower, and the Cd,

Co, Cu and Pb levels are similar to ours. Moreover, they analyzed the metal levels of feeds and suggested that the cause of accumulation and the difference of metal levels in fish species may be due to the feed. The analysis results determined the metal levels in the feeds to be (mg kg⁻¹) Al: 29.11, Cd: 0.35, Co: 0.12, Cr: 0.49, Cu: 10.65, Fe: 160.13, Ni: 0.60, Pb: 0.09 and Zn: 149.89. Therefore, in this respect, it is very important to analyze the feeds used in fish farms in terms of the heavy metal levels they possess. However in study that performed by Cheng et al. [29] heavy metal concentrations in all analyzed fish species feed with commercial pellets were below the international legal levels. Basaran et al.[30] who aimed to assess the effects of fish farms on water column and sediment by feed mainly were pointed out that metal levels were tolerable for the marine ecosystem.

According to data from Tepe [31], all studied metal levels were higher on Pe caught on İskenderun Bay, Mersin Bay, Antalya Bay Northern Aegean sea except Cu on Mersin Bay, Antalya Bay Northern Aegean sea and Zn on İskenderun Bay, Mersin Bay, Antalya Bay.

Risk Assessment

R_{fD} is the oral reference dose depending on the fish consumption levels in the world and in Turkey and on the daily exposure levels. The average metal (Ag, Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn) levels in the muscule tissues of the fish samples in the calculation of the THQ values are given in *Table 2*. As for TTHQ, it represents the risk of all metal THQs reveal for all fish species.

All the THQ and TTHQ values in the study were less than 1. According to these results, the consumption of the farm (SaF) and wild (SaW) Sa, Sm and Pe species in the study field does not represent a risk for health in terms of Ag, Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn. When an assessment is made in terms of metals, while Cr (1.3E-01) represents the highest risk, Cd and Ag (0.0E+0.0) represents the lowest. This situation is most probably about the density of the metals in the marine environment and about the bioaccumulation factors. The highest THQ value was Cr in SaF with 1.3-E-01. According to the TTHQ values, the species are ranked as SaF>Sm>SaW>Pe. This situation on the other hand may be about the feeding habits (omnivorous, carnivorous, formulated food etc.), living areas (SaW, SaF and Pm demersal, Sm pelagic), physiologies and environmental conditions which are directly related to metal accumulation [7]. A risk assessment on the consumption of the SaF which is farmed and Saw and Sm which are caught in the coasts of Aegean Sea was not conducted before.

Kalantzi at al.^[7] conducted study on the Al, Cr, Co, Cu, Cd, Fe, Ni, Pb and Zn levels in *SaF* fish collected from the fish farms on the Greek coasts of Aegean Sea, and have found THQ values were less than 1, similar to our study.

Yabanlı and Alparslan [12], in their study on the Cr, Cu, Cd and Pb levels on *Pe* and some other fish caught in Aegean Sea and have found THQ and TTHQ levels values were less than 1, similar to our study.

Tepe [31] conducted study on the Cd, Cr, Co, Cu, Fe, Mn, Ni, Pb and Zn levels in *Pe* fish caught on Mersin Bay, Antalya Bay Northern Aegean sea. And have found compsumption higher than 1.32 kg/week of *Pe* would be reach tolerable weekly intake (TWI) of Cd.

The levels of metals detected in the fish muscle samples analyzed in this study were below the legal and daily consumption limits according to national and international standards and these results show that there is no risk for the public health. Not detecting Ag, Cd and Pb, which are toxic heavy metals, in the wild and farm S. aurata fish muscle tissues and not detecting Ag and Cd in the other wild fish species caught and detecting Pb in very low levels are assessed as a positive result in terms of public health and food safety in the fisheries. Because these results which are found in the fish in this area which contribute to economy to a large extent by their export mainly to EU countries can be accepted as a promising data in terms of food safety and prospective export potential for our country. As waters are exposed to aquatic pollutants and they are important and easily accessed animal protein resources for humans, the heavy metals in the water products should be periodically monitored.

Conclusively, the Ag, Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn levels in the fish muscle tissues of wild *Sparus aurata* Linnaeus, 1758 (*SaW*), *Spicara maena* Linnaeus, 1758 (*Sm*) and *Pagellus erythrinus* Linnaeus, 1758 (*Pe*) caught in Aegean Sea and in farmed *Sparus aurata* Linnaeus, 1758 (*SaF*) were below legal limits, and THQ and TTHQ levels found depending on the feeding habits of consumers were less than 1. Therefore, the consumption of these fish does not represent any risk to human health in terms of the metals detected in the study. It is found that the consumption of *SaF* (260 g/day) being more than 5 times of the world average (52 g/day) may be risky. Therefore, it is important to monitor metal levels of the fish in the area constantly and to make periodic risk assessment on them.

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