



HUMAN HEALTH RISK ASSESSMENT FROM ARSENIC EXPOSURE  
AFTER SEA BREEM (*SPARUS AURATA*) CONSUMPTION IN  
AEGEAN REGION, TURKEY

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**Summary**

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The aim of the present study was to determine the amount of arsenic in the muscular tissues of wild sea breams, sea breams cultured in soil ponds and sea breams cultured in off-shore marine cage systems in the Aegean Region of Turkey. Then an estimated daily intake (EDI) and target hazard quotient (THQ) based risk assessment by sea bream consumption in terms of arsenic was performed for both children and adults. The arsenic concentrations in the muscular tissues of fish were detected by inductively coupled plasma-mass spectrometry method after digestion by microwave wet burning. According to the results obtained, the highest average arsenic concentration was found in sea bream cultured in soil pond while the lowest was determined in off shore marine cultured sea bream. The arsenic concentrations determined in all fish groups were found out to be below 1 mg.kg<sup>-1</sup> wet weight which is the international legal limit. Also the EDI values determined for children and adults were lower than 0.30 µg/kg/day, the oral reference doses (RfDo) value for arsenic and THQ value was found out to be lower than the value assessed as potentially carcinogenic. Summarising, the results of this study do not underline any potential risk in term of arsenic deriving from consumption of sea bream cultured and caught in Aegean Sea.

**Key words:** arsenic, public health, risk assessment, *Sparus aurata*

**INTRODUCTION**

Arsenic is a carcinogenic element known for its poisonous characteristics which is regarded as one of the most critical environmental threats of the world for millions of people (Celik *et al.*, 2008; Ravenscroft *et al.*, 2009). Arsenic is a naturally exist-

ing element and its presence in food proves that it is usually accumulated from the environment (Roychowdhury *et al.*, 2002).

People can be exposed to inorganic and organic arsenic in various ways (envi-

ronmental, dietary, medicinal and work). The researches conducted have shown that 90% of nutritional exposure to arsenic occurs via fish and other seafood and the remaining 10% – via other food (Han *et al.*, 1998).

Arsenic levels in aquatic environments are usually higher than in inland (Rose *et al.*, 2007). Moreover, the arsenic concentrations increase going up at the top of the food chain (Turkmen *et al.*, 2005). A considerable amount of the arsenic present in fish and crustaceans is organic (methylated arsenic compounds) and a very little amount is inorganic arsenic (Schoof *et al.*, 1999). The organic forms of arsenic (arsenobetaine, arsenocholine, dimethylarsinic acid, monomethylarsonic acid, trimethylarsine oxide, and tetramethylarsonium ion) found in fish are practically accepted as nontoxic (Muñoz *et al.*, 2000). Inorganic arsenic (arsenite and arsenate) is quite toxic and carcinogenic for humans (Neff, 1997). Norin *et al.* (1985) reported that about 5–12% of total arsenic in the muscular tissue of fish is inorganic. Besides, according to Roychowdhury *et al.* (2002), 10% of total arsenic in marine products is inorganic arsenic. Starting from this study at least 10% of the total arsenic concentration in the analysed samples is presumed to be inorganic arsenic.

Nutritionists recommend regular fish intake (Cirillo *et al.*, 2010). Fish consumption is beneficial for health because of its proteins, polyunsaturated essential fatty acids (eicosapentaenoic acid, docosahexaenoic acid), minerals (calcium, iron, selenium, zinc, etc.) and vitamins (mainly A, B<sub>3</sub>, B<sub>6</sub>, B<sub>12</sub>, E, and D) (Steffens, 1997; Sidhu, 2003). Fish fatty acids are of great importance especially in preventing coronary artery disease in humans.

In Turkey, Mugla is the most productive area for aquaculture and the greatest

amount of the national production of sea bream (35.701 tonnes only in 2013) is carried out in this region. In addition to the farmed production, about 50% of the sea bream gained through hunting are caught in the Aegean Sea (483.0 tonnes of 943.5 tonnes in 2013) (TURKSTAT, 2014). Together with sea bass (*Dicentrarchus labrax*), sea bream (*Sparus aurata*) is among the most consumed and well-appreciated marine aquaculture species in Turkey and in all Southern European countries (European Commission, 2008).

Currently there are no maximum levels established for arsenic in food at EU level and also in USA, although these maximum levels are laid down in national legislation of some European Members States (EFSA, 2014). According to the European Food Safety Authority (EFSA, 2014), currently the assessments of the exposure to arsenic is uncertain and hence, more analytical data on arsenic, in particular in fish, seafood and in food groups that provide a significant contribution to the dietary exposure to inorganic arsenic (e.g. rice and wheat-based products) are strongly requested in order to reduce this lack.

In this context, the purpose of this research was to detect the arsenic concentration in sea breams bred in different culture environments (soil ponds and marine cage systems) and wild specimens caught from Mugla Region and to determine the potential health risks for children and adults consuming this fish species.

## MATERIALS AND METHODS

### *Fish samples*

Fish samples were collected seasonally in 2013 for one year. Three different groups of sea breams were considered during the

**Table 1.** The biometric properties of the three studied groups of sea bream

	Total length (cm)		Weight (g)	
	Mean $\pm$ SD	Min – Max	Mean $\pm$ SD	Min – Max
I (n=12)	29.25 $\pm$ 2.09	(25.00 – 31.00)	493.33 $\pm$ 104.91	(300.00 – 580.00)
II (n=12)	25.42 $\pm$ 3.68	(19.00 – 31.00)	328.17 $\pm$ 96.49	(200.00 – 550.00)
III (n=12)	27.00 $\pm$ 1.86	(24.00 – 30.00)	366.25 $\pm$ 28.05	(330.00 – 420.00)

SD: standard deviation, Min: minimum, Max: maximum

research: group I consisted of wild sea bream caught in the Gulf of Gulluk (Eastern Aegean Sea), group II – sea bream cultured in off-shore system in the Gulf of Gulluk and group III – fish cultured in soil ponds around Milas (Mugla, Western Anatolia). Every season a total of 3 fish per each group were randomly caught and brought under cold conditions to the Laboratory Department of Toxicology of Veterinary Control Institute (Izmir). During the research a total of 36 (12 specimens for each group) of sea breams were analysed. The main biometric properties of the studied fish are reported in Table 1.

#### *Analysis of arsenic content*

Ashoka *et al.* (2009) indicated that the method of dissolution with nitric acid and hydrogen peroxide in high-pressure closed microwave systems bore fruitful results in fish tissue. Following this method, in this study 250 mg of muscular tissue was put in a polytetrafluoroethylene (PTFE) microwave container (DAP 60). Then 3 mL of nitric acid (65%, suprapur Merck) and 2 mL of hydrogen peroxide (30%, suprapur Merck) was added on it. The container was left to pre-dissolution at ambient temperature. Later, 3 mL of ultra-pure water was added in the container, the lid was closed and it was placed in the microwave unit (Berghof speedwave MWS-3) and dissolution process was carried out

by the digestion programme reported in Table 2.

The liquefied dissolved samples were put in ICP-MS (Agilent 7700x) device under operating conditions reported in Table 3. The calibration curve was prepared in 5 different concentrations (5-10-50-100-200  $\mu\text{g.L}^{-1}$ ) using the mixed element standard (AccuTrace Mes-21-1).

**Table 2.** Microwave burning programme

Step	1	2	3	4	5
Temperature, (°C)	150	175	220	50	50
Pressure, psi	35	35	35	35	35
Rump time, (min)	5	5	5	1	1
Hold time, (min)	5	5	17	1	1
Power (%)	90	90	90	10	10

**Table 3.** ICP-MS instrument operating conditions

Radio frequency power	1550 W
RF matching	2.1 V
Sampling depth	8 mm
Carrier gas	0.95 l/min
S/C temperature	2°C
Nebulizer type	MicroMist

TORT-2 lobster hepatopancreas trace element certificated reference matter (21.6  $\pm$  1.8  $\text{mg.kg}^{-1}$ ) was used in the study to prove the reliability of analysis data. By

triplicate analyses of the certified reference matter, the resulting arsenic concentration was  $20.67 \pm 0.22$  mg.kg<sup>-1</sup>. The recovery ratio was 95.69%.

The results of the analyses are reported as mg.kg<sup>-1</sup> wet weight (w.w.).

#### Estimation of daily intake of arsenic

The estimated daily intake (EDI) is suggested as an alternative approach in hazards assessment in food (Sofuoglu & Kavcar, 2008; Petroczi & Naughton, 2009). It was assessed by the formula suggested by Islam *et al.* (2014) and reported below:

$$\text{EDI } (\mu\text{g As/kg body weight/day}) = \frac{\text{FIR} \times \text{C}}{\text{BWa}}$$

where: C=the average arsenic concentration in fish species (μg/kg), FIR=daily fish ingestion rate (kg/person), BWa=average body weight.

A body weight of 10 kg was applied for 1 year-old children, 15 kg for 3 years-old children, 20 kg for 6 years-old children, 44 kg for 12 years-old children (Yaman *et al.*, 2014). In this risk assessment, 70 kg of body weight is usually applied for adults (Brodberg & Klasing, 2003). The fish ingestion rate in Turkey in 2013 was 6.307 kg/person/year (TURK-STAT, 2014).

By comparing the daily intake with the chronic oral reference dose (RfDo), it is possible to determine whether a person is exceeding acceptable health guidance levels (Patrick *et al.*, 2008). The RfDo value for arsenic is  $3 \times 10^{-4}$  mg.kg<sup>-1</sup>.day<sup>-1</sup> (USEPA, 2000).

#### Determination of target hazard quotient

The target hazard quotient (THQ) is a ratio of determined dose of a pollutant to the dose level (RfDo). It is used at health risk assessment in order to determine the carcinogenicity of the samples (Islam *et*

*al.*, 2014). The formula below suggested by Chien *et al.* (2002) was used to calculate the THQ. If the THQ value obtained is <1 (safe level), there are no adverse effects for the exposed population.

$$\text{THQ} = \frac{\text{Efr} \times \text{EDtot} \times \text{FIR} \times \text{C}}{\text{RfDo} \times \text{BWa} \times \text{ATn}} \times 10^{-3}$$

In the formula, Efr stands for frequency of exposure (365 days/year), EDtot: period of exposure (average life expectancy: 70 years), FIR: food intake rate (g/day), C: mean arsenic concentration in fish muscular tissue (mg/kg), RfDo: oral reference dose of arsenic (mg/kg/day), BWa: average body weight, ATn: period of average exposure for non-carcinogens (365 days/year×number of exposure years i.e. 70 years).

#### Statistical analyses

The statistical evaluation of data was carried on by IBM SPSS Statistics V.20 program. The non-parametric Kruskal Wallis and Mann-Whitney U tests were used to assess differences in arsenic content among the groups. The statistical significance was determined through 0.05 alpha level. If P<0.05, it was evaluated as there was a statistically significant difference between the groups. Spearman correlation analysis was applied to evaluate bivariate correlations.

## RESULTS

The arsenic concentrations in fish muscular tissues are given in Table 4. Higher average arsenic concentration was encountered in the sea bream cultured in soil ponds whereas the lower one in marine cultured sea breams. Statistically significant differences were determined between soil pond-marine cage sea bream and soil pond-wild sea bream (P<0.05) in terms of

**Table 4.** Arsenic concentrations ( $\text{mg.kg}^{-1}$ ) in the muscular tissues of the three groups of fish studied

	Total arsenic, $\text{mg.kg}^{-1}$			Inorganic arsenic
	Min	Max	Mean $\pm$ SD	
Group I	0.31	0.45	$0.38 \pm 0.04$	0.038 <sup>b</sup>
Group II	0.32	0.41	$0.35 \pm 0.03$	0.035 <sup>a</sup>
Group III	0.54	0.67	$0.60 \pm 0.03$	0.060 <sup>a,b</sup>

Values with the same superscripts letter were found significantly different ( $P < 0.05$ ) in Kruskal Wallis and Mann-Whitney U tests.

**Table 5.** Estimated daily inorganic arsenic amounts (EDI) ( $\mu\text{g/kg/day}$ ) that can be ingested by fish consumption in children and adults

		Group I	Group II	Group III
Children	1 year of age	0.066	0.060	0.104
	3 years of age	0.044	0.040	0.069
	6 years of age	0.033	0.030	0.052
	12 years of age	0.015	0.014	0.024
Adults		0.0094	0.0086	0.015
Chronic oral reference dose (RfDo)		0.30		

arsenic concentrations detected in the muscular tissue, whereas no statistically significant differences were detected between marine cage and wild sea bream (Table 4).

EDI values calculated for children and adults with connection to consumption of the three groups of fish examined in this study are given in Table 5. According to it, the highest EDI value ( $0.104 \mu\text{g/kg/day}$ ) was calculated for sea breams farmed in soil ponds and the lowest EDI value – in marine cultured sea breams ( $0.0086 \mu\text{g/kg/day}$ ).

The values of THQ obtained for the inorganic arsenic derived by sea bream consumption were found lower than the critical value of 1 (Table 6).

## DISCUSSION

EFSA (2014) reported the As contents for different fish species. These values repre-

sent the most updated references for Europe but the values differ considerably among different fish species (i.e.  $0.005 \text{ mg.kg}^{-1}$  for halibut and  $0.049 \text{ mg.kg}^{-1}$  for sardine) underlining the importance of *ad hoc* studies for each fish species. Nevertheless, although the sea bream is one of the preferred fish consumed in Europe, currently no analytical data on this species are available by EFSA. The data presented in our study could be useful to

**Table 6.** Estimated inorganic arsenic target hazard quotients (THQs) for children and adults from the consumption of sea bream from the three studied groups

	Group I	Group II	Group III
1 year	0.219	0.202	0.346
3 years	0.146	0.134	0.230
6 years	0.109	0.101	0.173
12 years	0.050	0.046	0.079
Adults	0.031	0.029	0.049

cover this lack and will represent a reference for future studies.

In our study, a lower average arsenic value was found in marine cultured sea breams compared with those obtained in cultures soil ponds. It could be a result of the controlled farming conditions of the fish growing in a more isolated environment than natural sea breams. According to Brazilian, Singaporean, Malaysian and Australian legislations, the maximum total arsenic level that could be found in fish and fish products is  $1 \text{ mg.kg}^{-1}$  w.w. (Muñoz *et al.*, 2000; Medeiros *et al.*, 2012). In our study, the samples did not exceed the critical level of  $1 \text{ mg.kg}^{-1}$  in any of the three fish groups.

A total arsenic concentration similar to the one obtained in the present study was reported by Tuzen (2009) for ten different sea fish species caught in the Black Sea. Their reported values resulted between the range of  $0.11\text{--}0.32 \text{ mg.kg}^{-1}$  w.w. Total average arsenic lower than the limiting value ( $0.19\text{--}0.21 \text{ mg.kg}^{-1}$  w.w.) was determined in the sea breams bought from local markets in Nantes (France) (Cardinal *et al.*, 2011). Total average arsenic concentration ( $0.39 \text{ mg.kg}^{-1}$  w.w.) in the sea breams bought from fish markets in Trabzon (Turkey) did not exceed the limiting value (Aydın & Tokaloğlu, 2014).

An average of  $3.135\text{--}11.024 \text{ mg.kg}^{-1}$  w.w. total arsenic was detected in five different fish species caught in the Mediterranean Sea in a study conducted in Catania (Italy) (Copat *et al.*, 2013). These values exceeded the international limiting value and the average values in this study. Average total arsenic concentrations determined in striped marlin (*Tetrapturus audax*) and sailfish (*Istiophorus platypterus*) caught in the Gulf of California were  $4.0$  and  $5.1 \text{ mg.kg}^{-1}$  respectively and this results exceeds the limiting value

(Soto-Jiménez *et al.*, 2010). Total average arsenic levels detected in four fish species caught in the Adriatic Sea were  $0.43\text{--}5.91 \text{ mg.kg}^{-1}$  w.w. and the concentration obtained for *Mullus surmelatus* exceeded the limiting value (Bilandžić *et al.*, 2011).

Total average arsenic amounts ( $1.54\text{--}4.48$  and  $0.88\text{--}2.94 \text{ mg.kg}^{-1}$  w.w. respectively) determined in Japanese sea basses (*Lateolabrax japonicus*) cultured in marine fish cages and red sea breams (*Pagrus major*) in Fujian (China) exceeded the limiting value (Onsanit *et al.*, 2010). A range of  $0.98\text{--}1.74 \text{ mg.kg}^{-1}$  w.w. of total average arsenic was determined in three fish species caught in the Gulf of Iskenderun (Turkey) with concentrations exceeded the limiting value in the species of *Triglia lucerna* and *Solea lascaris* (Yılmaz *et al.*, 2010). Total average arsenic concentrations detected in culture and wild sea breams on the coasts of Croatia were higher than the limiting value being  $3.01\text{--}3.90 \text{ mg.kg}^{-1}$  and  $4.70\text{--}14.90 \text{ mg.kg}^{-1}$  respectively (Rožič *et al.*, 2014). Total arsenic amounts were determined ( $0.1\text{--}6.1 \text{ mg.kg}^{-1}$  w.w.) in 11 sea fishes in Rio de Janeiro (Brazil) and the limiting value was determined to be exceeded (Medeiros *et al.*, 2012).

In a study conducted in Canada, it was determined that cooking had an effect on arsenic concentrations in fish. According to the study, while the total arsenic amount in raw fish meat is  $2466 \text{ }\mu\text{g.kg}^{-1}$  w.w., a value of  $3048 \text{ }\mu\text{g.kg}^{-1}$  w.w. was detected in cooked fish meat. The researchers attributed this rise to loss of weight by cooking (Dabeka *et al.*, 1993).

The possible reasons for the differences in arsenic concentrations reported in the different studies, could be due to seasonal and biological differences in the fish (species and their physiology) but also, to the nutritional source and region

where the fish were caught and environmental conditions (Alasalvar *et al.*, 2002; Yildiz, 2008; Bhourri *et al.*, 2010).

Dietary intake studies showed that the greater share in people's arsenic intake via diet belongs to marine products (Schoff *et al.*, 1999). Almost 90% of daily arsenic intake in the United States occurs with the consumption of sea food (Gunderson, 1995). An inorganic arsenic amount that can be ingested with the consumption of sea fish similar to this study was determined to be 0.003–0.096  $\mu\text{g}/\text{kg}/\text{day}$  in China (Zhang & Wang, 2012). The inorganic arsenic EDI value (0.014) calculated for marine cultured fish consumption was found to be lower than RfDo (Onsanit *et al.*, 2010). Daily inorganic arsenic amount ingested with fish in Italy was 0.670–2.357  $\mu\text{g}/\text{kg}/\text{day}$  in children and 0.305–1.073  $\mu\text{g}/\text{kg}/\text{day}$  in adults (Copat *et al.*, 2013). These values were higher than those found in our study and exceeded the RfDo value of 0.30  $\mu\text{g}/\text{kg}/\text{day}$ .

THQ values similar to this study were obtained in a research analysing the arsenic concentration in fish bought from markets in Bangladesh (Saha & Zaman 2013). Moreover, the inorganic arsenic THQ value from consumption of marine cultured fish in China (0.05) was found to be the critical level (Onsanit *et al.*, 2010). With regard to Portugal, the inorganic arsenic THQ values determined for children and adults from consumption of marine fish in NW were  $<0.01$  and this posed no risk from the consumption of fish in term of arsenic (Vieira *et al.*, 2011).

The THQs determined by Copat *et al.* (2013) in Italy from fish consumption once a week ranged between 0.3–1.1 in children and 0.1–0.5 in adults. Those values were higher than the results of this study. A carcinogenic risk was not assessed for children who consume *Mullus*

*barbatus* once a week (THQ=1.1) in the study at issue. The inorganic arsenic THQs calculated derived from consumption of billfish caught in the Gulf of California were within the ranges of 0.4–2.5 for children and 0.2–1.7 for adults. These values underline a potential carcinogenic risk for human (Soto-Jiménez *et al.*, 2010). The THQ arsenic risk assessment derived from consumption of tilapia (*Oreochromis mossambicus*) cultured in Southwest Taiwan (Kar *et al.*, 2011), equaled 1.22, a value higher than the critical level of 1 and indicating a potential carcinogenic effect.

The risk assessments in terms of human health revealed that none of the three analysed groups, exceeded the value of 1  $\text{mg}\cdot\text{kg}^{-1}$  w.w. which is the international limit of arsenic level. EDI ratio in children and adults resulted less than the arsenic RfDo value of 0.30  $\mu\text{g}/\text{kg}/\text{day}$  and the determined THQ values were  $<1$ , a level assessed as potentially carcinogenic for children and adults.

In conclusion, the results of this study revealed that there is no risk from consumption of sea bream cultured and caught in Aegean Region with respect to the intake of arsenic. The results also underline the importance to assess risk for public health in terms of heavy metals such as arsenic and other contaminants in food and especially in marine products.

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