Determination of Trace Metal Levels of Cd, Pb, Cr, Ni, and Cu in Two Fish Species by STAT-AAS and ICP-AES

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INTRODUCTION

The pollutants, including toxic metals such as Cd, Pb, Ni, and Cr, are bio-accumulated by aquatic inhabitants, including fish, and subsequently transferred to humans through the food chain. All trace elements, including the essential ones, may be toxic when taken in excessive amounts or may lead to deficiencies when taken in insufficient amounts (1-2). For example, lead (Pb) is toxic for the brain, kidney, and the reproductive system, and can also cause impairment in intellectual functioning, fertility, miscarriage, and hypertension. In addition, Pb exposure in school-age children can significantly reduce their IQ and has been associated with aggressive behavior, delinquency, and attention disorders. The general assumption is that Pb interacts with the function of enzymes, signal systems, and membranes, probably by binding to certain sites of proteins (3-6).

Cadmium (Cd) injures the kidney and causes symptoms of chronic toxicity, including impaired kidney function, poor reproductive capacity, hypertension, tumors, and hepatic dysfunction (7). The U.S. environmental protection agency (EPA) has classified Cd and Pb as probable human carcinogens (Group B2) (8). The International Agency Research on Cancer (IARC) has placed Cd and Cd compounds in Group 1 (Carcinogenic to humans), and classified inorganic Pb compounds as "probably carcinogenic to humans" (Group 2A), while Pb

ABSTRACT

The concentrations of five toxic metals (Cd, Pb, Cr, Ni, and Cu) were determined in two fish species, *Scomber scombrus* and *Sparus auratus*, obtained from fish markets in Turkish cities. For the determinations, slotted tube atom trap-atomic absorption spectrometry (STAT-AAS) and inductively coupled plasma atomic emission spectrometry (ICP-AES) were used. The obtained lead concentrations (mean $630 \mu\text{g kg}^{-1}$) for all *Scomber scombrus* samples were found to be higher than the maximum allowance concentrations (MAC) of $300 \mu\text{g kg}^{-1}$. Cadmium concentrations in both fish species (mean $90 \mu\text{g kg}^{-1}$ for *Scomber scombrus* and $54 \mu\text{g kg}^{-1}$ for *Sparus auratus*) were found to be around the MAC values. The mean Cr, Ni, and Cu concentrations in *Scomber scombrus* were $313 \mu\text{g kg}^{-1}$, $280 \mu\text{g kg}^{-1}$, and $420 \mu\text{g kg}^{-1}$, respectively. The same metal levels in *Sparus auratus* were $324 \mu\text{g kg}^{-1}$ Pb, $268 \mu\text{g kg}^{-1}$ Cr, $323 \mu\text{g kg}^{-1}$ Ni, and $382 \mu\text{g kg}^{-1}$ Cu. Although some metal concentrations exceed the limits set by the authorities, the estimated non-carcinogenic and carcinogenic health risks by the Target Hazard Quotient and target carcinogenic risk indicate that there are no systemic effects, and the risk of developing cancer over a human lifetime is between 2-9 in 1,000,000.

as "possibly carcinogenic to humans" (Group 2B) (9).

Chromium (Cr) intake at high concentrations causes severe environmental and public health problems, and is considered a carcinogenic and mutagenic substance for humans (10). Copper is known to take a role in the activation of one of the most important enzymes, namely zinc-containing superoxide dismutase (Cu-ZnSOD). If the concentrations of copper increase or decrease with time, the activity of this enzyme gets worse. It is clear that changes in the concentrations of these types of essential elements may cause various metabolic alterations (11-12). Further, Cu as well as Pb, can also accumulate in the human body over time and then result in chronic poisoning.

Nickel (Ni) normally occurs at very low levels in the environment and can cause a variety of pulmonary adverse health effects such as lung inflammation, fibrosis, emphysema, and tumors (13). In 1996, the World Health Organization (WHO) classified Ni as a 'probable' essential trace element, given its role in Ni-containing enzymes found in plants and microorganisms (14). However, similar functions of Ni in humans are not evident at present. In contrast, Ni compounds can display a tumor-promoting capability via a number of mechanisms including inhibition of intercellular communication, immortalization of fibroblasts and epithelial cells. Based on the evaluation of epidemiological studies, the International Agency for Research on Cancer (IARC) concluded that "...There is sufficient evidence in

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humans for the carcinogenicity of nickel sulphate and of the combinations of nickel sulphides and oxides encountered in the nickel refining industry." Thus, Ni compounds have been classified as carcinogenic to humans (Group 1) and metallic nickel as possibly also carcinogenic to humans (Group 2B) (15).

Among nutrition sources, the consumption of fish has increased in importance due to the high protein supply, low saturated fat, and omega fatty acids. Because fish can considerably accumulate toxic metals from water, the problem of heavy metals contamination in fish is increasing on a global basis. Thus, heavy metal pollution in fish has become an important worldwide concern, not only because of the threat to fish, but also due to the health risks associated with fish consumption. Hence, regular monitoring of the concentrations of toxic metals in fishes is very important for human health.

In terms of risk, children may also differ from adults in their vulnerability to environmental pollutants because of toxicodynamic and similar differences. Toxic chemicals in the environment can cause neuro-developmental disabilities, and the developing brain can be particularly sensitive to environmental contaminants. For example, elevated blood lead levels and prenatal exposure to even relatively low levels of lead can result in behavior disorders and reduction of intellectual function particularly in children.

Several analytical instruments have been used in the determination of analyte concentrations in different environmental and biological matrices (16-19). For instance, flame (FAAS) and electrothermal atomic absorption spectrometry (ET-AAS) are used for the determination of As, Pb, Cd, Ni, Cr, and Cu by employing chemical vapor gen-

eration and atomizer trapping techniques (20-22). Inductively coupled plasma atomic emission spectrometry (ICP-AES), a fast multi-element technique, was also used since it has the required linear dynamic range (23).

The main purpose of this study was to determine Cd, Pb, Cu, Cr, and Ni in two fish species, *Scomber scombrus* and *Sparus auratus*, obtained from markets in Elazig and Malatya cities, Eastern Turkey. The samples were collected over an eight-month period (October 2010 to May 2011). A microwave oven was used for digestion of the tissues. Cadmium, Pb, and Cu determinations were carried out by slotted tube atom trap STAT-FAAS, and Cr and Ni were determined by ICP-AES. The risk assessment from fish consumption for children was also calculated.

EXPERIMENTAL

Instrumentation

A model ATI UNICAM 929 atomic absorption spectrometer,

equipped with ATI UNICAM hollow cathode lamps, was used together with the STAT for the determination of Cd, Pb, and Cu. The operating parameters used in the AAS measurements were chosen as described in the manufacturer's handbook. In the literature, STAT was used to improve the sensitivity of some elements (24-27). The concentrations as low as 3 µg L⁻¹ for Cd, 25 µg L⁻¹ for Pb, and 25 µg L⁻¹ for Cu were determined by using STAT-FAAS.

A PerkinElmer® Optima™ 2100 DV ICP-OES (PerkinElmer, Inc., Shelton, CT, USA) was used for measurement of the Ni and Cr concentrations. The instrumental conditions for ICP-OES are given in Table I.

Some samples were analyzed using the PerkinElmer ELAN® 9000 inductively coupled plasma mass spectrometer (ICP-MS) to check the accuracy of the measurements.

A microwave digestion system (CEM MARSXpress) was used to digest the samples.

TABLE I
Operating Conditions of ICP-OES for Ni, Cr, and Cu Determination

Parameter	ICP-OES
RF power	1450 W
Plasma gas flow rate	15.0 L min ⁻¹
Auxiliary gas flow rate	0.2 L min ⁻¹
Nebulizer gas flow rate	0.6 L min ⁻¹
Sample flow rate	1.5 L min ⁻¹
Viewing mode	Axial
Readout	Peak area
Source equilibration time	15.0 s
Read delay	45.0 s
Replicates	3
Background correction	2-point (manual point correction)
Spray chamber	Scott type spray chamber
Detector	CCD
Purge gas	Nitrogen
Shear gas	Air
Gas	Argon
Analytical wavelengths	Ni 231.604 nm, Cr 267.716 nm, Cu 327.393 nm

Reagents and Standard Solutions

Unless stated otherwise, all chemicals used throughout the study were of high-purity reagent grade. Doubly distilled water, obtained with a Model Direct-Q water purification system (Millipore Corporation, Bedford, MA, USA), was used for all preparations. When not in use, all Pyrex® glassware was kept permanently full of 1 mol L⁻¹ nitric acid. Concentrated nitric acid (65%, E. Merck, Darmstadt, Germany) was used in the digestion procedure of fish muscle. Proper diluted solutions of the metals were prepared from the stock solutions (1000 mg L⁻¹) (Merck) by using 1.0 mol L⁻¹ nitric acid.

Sampling and Digestion of Samples

Before carrying out the experimental work, all glassware was washed with chromic acid, then rinsed with distilled water. All glass containers were kept clean in order to prevent elemental contamination when not in use.

The fish samples were collected during the months of October 2010 and May 2011. In each month from each city, at least one fish sample from each fish species was taken. They were randomly purchased from large modern supermarkets and traditional open fish markets in two neighboring Turkish cities, Elazığ and Malatya, with populations of at least 500,000. After collection, the fish samples were skinned, cut, and the internal organs removed. The samples were cleaned, transported to the laboratory immediately, and stored in a refrigerator prior to analysis. The muscle is preferred because it is a major target tissue for metals storage and is the main edible part of the fish.

To prevent loss of the studied metals in the dry digestion procedure, the samples were digested

using a microwave. An amount of 0.5 g of fresh sample was transferred to a digestion vessel and 5 mL of concentrated nitric acid added. After a few minutes, the vessels were closed and the bombs placed in the microwave oven. The samples were digested at 800 W, 180 °C, for 15 minutes. After complete digestion, the samples were allowed to cool to room temperature. Then, the digests were evaporated to 1 mL. This digest was diluted to 5.0 mL with doubly distilled water. The digestion vessels were cleaned after each run using the same reagents as well as the program as applied for the sample decomposition, followed by rinsing with doubly distilled water. Subsequently, the samples were analyzed for Cd, Pb, and Cu using STAT-AAS, and Cr and Ni using ICP-AES. Blanks were also digested to determine the background contribution of the reagents and treated the same way as the samples.

Analytical Performance

The limits of detection (LODs) using ICP-AES, calculated as three times the standard deviation of the blank, were found to be (as µg L⁻¹) 4.0 for Ni, 3.0 for Cr, and 4.0 for Cu. The LODs obtained by STAT-FAAS, calculated as three times the standard deviation of the blank, (as µg L⁻¹) were 0.5 for Cd, 2.5 for Pb, and 3.0 for Cu. The blank digests were prepared and analyzed in the same way. All of the experiments were performed three times.

In order to verify the accuracy of the methods, the results obtained were compared with the results obtained by ICP-MS. From these results, it was observed that there was no significant difference between the data obtained in this study and by ICP-MS using the Student's *t*-test at the 90% confidence level. Finally, the standard addition method was applied in order to establish whether or not any

matrix interferences are caused by the matrix. It was observed that the slopes of the calibration plots obtained with the standards (given in the following equations) were very close to those obtained using the standard additions method for each element: 2.6 for Cd, 0.29 for Pb, 0.3 for Cu, 291 for Ni, and 625 for Cr. This shows that there is no matrix interference affecting the determination of the studied elements. Hence, the direct calibration method was used for all of the measurements. The linear ranges for the five elements were determined as follows:

$$Y = 2.6 X - 0.1 \quad R^2 = 0.9999$$

for Cd (3–50 µg L⁻¹ by STAT-FAAS)

$$Y = 0.3 X + 0.5 \quad R^2 = 0.9999$$

for Pb (25–400 µg L⁻¹ by STAT-FAAS)

$$Y = 0.3 X - 0.2 \quad R^2 = 1$$

for Cu (25–400 µg L⁻¹ by STAT-FAAS)

$$Y = 294X - 469 \quad R^2 = 0.9998$$

for Ni (20–400 µg L⁻¹ by ICP-AES)

$$Y = 621X - 2369 \quad R^2 = 0.9998$$

for Cr (20–300 µg L⁻¹ by ICP-AES)

$$Y = 819X - 2618 \quad R^2 = 0.9999$$

for Cu (20–300 µg L⁻¹ by ICP-AES)

$$Y = 802X - 1605 \quad R^2 = 0.9999$$

for Cd (20–300 µg L⁻¹ by ICP-AES)

The lowest concentration in the calibration plot for each element was considered as the limit of quantitation (LOQ) for the measurement step. Taking into consideration the sample weight of 0.5 g and a final volume of 5 mL, the LOQ for each sample was calculated using 40 µg kg⁻¹ for Cd, 260 µg kg⁻¹ for Pb, 280 µg kg⁻¹ for Cr, 190 µg kg⁻¹ for Ni, and 400 µg kg⁻¹ for Cu. As a result, each sample was analyzed in triplicate, and an average of the three values was used as the mean value on the basis of the original fresh weight.

Estimation of Health Risk

The methodology for estimating the non-carcinogenic and carcino-

genic risks was applied in accordance with that provided in the U.S. EPA Region III Risk-based Concentration table (8). The non-carcinogenic risks for each individual metal through fish consumption were assessed by the HQ=hazard quotient which means "...the ratio of a single substance exposure level over a specified time period (sub-chronic) to a reference dose (RfD) for that substance derived from a similar exposure period." The HQ assumes a level of exposure (RfD) below which it is unlikely for even sensitive populations to experience adverse health effects. If the exposure level exceeds this threshold value $HQ = EED / RfD$ (estimated exposure dose)/RfD, there may be concern for potential non-carcinogenic effects. Higher HQ values mean a higher probability of experiencing long-term non-carcinogenic effects. For carcinogens, risks are estimated as the incremental probability of an individual to develop cancer over a lifetime as a result of exposure to that potential carcinogen. Acceptable risk levels for carcinogens range from 10^{-4} (risk of developing cancer over a human lifetime is 1 in 10,000) to 10^{-6} (risk of developing cancer over a human lifetime is 1 in 1,000,000).

The estimated exposure dose (EED) was calculated using the following equation (8) to estimate the human health risk from consuming metal-contaminated fish:

$$EED = \frac{\text{Conc.} \times IR \times EF \times ED}{BW \times AT}$$

- where EED is the estimated metal exposure dose (mg/kg/day);
- Conc. is the metal concentration in fish (mg/kg, wet weight);
- IR is the ingestion rate (adult: 0.227 kg fish per day; child: 0.1135 kg fish per day);
- EF is the exposure frequency, or number of exposure events per year of exposure (52 days/year for people who eat fish 1 time a week);

- ED is the exposure duration (adult: 70 years; child: his/her age years);
- BW is the body weight (adult: 70 kg; child: his/her body weight);
- AT is the average time or the period over which cumulative exposures are averaged ($AT = ED \times 365$ days/year for non-cancer risk and $AT = 70 \text{ years} \times 365$ days/year for lifetime cancer risk).

The hazard quotient (HQ) for noncancer or systemic effects (such as neurotoxicity) was estimated by dividing the calculated EED by the metal reference dose (RfD), while the lifetime cancer risk (CR) was obtained by using the chemical slope factor (SF) (8) in the following equation: $CR = EED \times SF$. The RfD is the oral reference dose: 1.0×10^{-3} mg/kg/day for Cd and 4.0×10^{-3} mg/kg/day for Pb (9). The RfD represents an estimate of daily oral exposure that is likely to be without appreciable risk of adverse health effects over a lifetime. The SF is the oral carcinogenic slope factor from the Integrated Risk Information System (8) database (8.5×10^{-3} mg/kg/day for Pb).

RESULTS AND DISCUSSION

The advantages of microwave digestion compared to the classical dissolution methods are a shorter period of analysis time, less consumption of acid, and being able to keep volatile compounds in the solution. The Provisional Tolerable Weekly Intake (PTWI) is the maximum amount of a contaminant to which a person can be exposed per week over a lifetime without unacceptable risk of health effects. The tolerable intake of heavy metals as PTWI is set by the Food and Agriculture Organization/World Health Organization (FAO/WHO) Joint Expert Committee on Food Additives (JECFA) (14). Consequently, WHO established the PTWI for Cd and Pb at 5 and 25 $\mu\text{g kg}^{-1}$ body

weight, respectively, for all human groups, which are equivalent to 350 and 1750 $\mu\text{g/week}$, respectively, for an adult human weighing 70 kg (14). JECFA has established a PTWI of 35 $\mu\text{g/kg/week}$ for Ni, which is equivalent to 2450 $\mu\text{g/week}$ for an adult human weighing 70 kg (28). With regard to Cr and Cu, the authorities have established the Recommended Dietary Allowance (RDA) of 50–200 $\mu\text{g/day}$ for Cr and Acceptable Daily Intake (ADI) of 1.3 mg/day for Cu for an adult weighing 70 kg (14,29). The U.S. dietary guidelines for adequate daily chromium intake were lowered in 2001 from 50–200 μg for an adult to 30–35 μg (adult male) and to 20–25 μg (adult female) (30). The maximum allowance concentrations (MAC) for each food are based on domestic food consumption data. The total amount of the metal of interest in those food species should not exceed the amounts above: weekly, 350 μg Cd, 1750 μg Pb, and 2450 μg Ni, and daily, 50–200 μg Cr and 1.3 mg Cu, for an adult human weighing 70 kg. It is clear that the food consumption data are dependent on income, household consumption, age groups, and socioeconomic factors. In particular it should be emphasized that the diet is significantly different from country to country. Thus, the MAC values established by authorities in undeveloped countries do not reflect the actual intake in developed countries.

Variations in Metals in *Scomber scombrus*

Pb and Cr

The seasonal variations in the lead and chromium concentrations found in *Scomber scombrus* are shown in Figure 1. The mean Pb concentration in *Scomber scombrus* was $574 \pm 259 \mu\text{g kg}^{-1}$ (range 215–889 $\mu\text{g kg}^{-1}$) for Elazig city and $685 \pm 282 \mu\text{g kg}^{-1}$ (range 240–1015 $\mu\text{g kg}^{-1}$) for Malatya city. According to the Turkish Food

Codex (31) and the European Commission (32), the maximum allowance concentration (MAC) of Pb in fish tissue is $300 \mu\text{g kg}^{-1}$. This limit was exceeded in all but three of the studied *Scomber scombrus* samples. The mean Cr concentration in *Scomber scombrus* was $286 \pm 29 \mu\text{g kg}^{-1}$ (range 240–316 $\mu\text{g kg}^{-1}$) for Elazig city and $340 \pm 14 \mu\text{g kg}^{-1}$ (range 320–358 $\mu\text{g kg}^{-1}$) for Malatya city. Up to now, the Turkish Food Codex and other similar authorities did not establish the MAC for either Cr or Ni in fish tissue.

Cu and Ni

The seasonal variations in the Cu and Ni concentrations detected for *Scomber scombrus* are shown in Figure 2. The mean Cu concentration in *Scomber scombrus* was $404 \pm 45 \mu\text{g kg}^{-1}$ (range 343–462 $\mu\text{g kg}^{-1}$) for Elazig city and $436 \pm 28 \mu\text{g kg}^{-1}$ (range 409–474 $\mu\text{g kg}^{-1}$) for Malatya city. According to the Turkish Food Codex (31), the maximum allowance concentration (MAC) of Cu in fish tissue is 20,000 $\mu\text{g kg}^{-1}$. This limit was not exceeded in all studied *Scomber scombrus*. The mean Ni concentration in *Scomber scombrus* was $267 \pm 40 \mu\text{g kg}^{-1}$ (range 217–322 $\mu\text{g kg}^{-1}$) for Elazig city and $292 \pm 34 \mu\text{g kg}^{-1}$ (range 226–327 $\mu\text{g kg}^{-1}$) for Malatya city.

Variations on Metals in *Sparus auratus*

Pb and Cr

The seasonal variations in the lead and chromium concentrations determined for *Sparus auratus* are shown in Figure 3. The mean Pb concentration in *Sparus auratus* was $251 \pm 59 \mu\text{g kg}^{-1}$ (range 186–328 $\mu\text{g kg}^{-1}$) for Elazig city and $396 \pm 167 \mu\text{g kg}^{-1}$ (range 165–617 $\mu\text{g kg}^{-1}$) for Malatya city. As can be seen, the MAC limit was exceeded only in two *Sparus auratus* samples obtained in Elazig city and five samples from Malatya city. The mean Cr concentration in *Sparus auratus* was $201 \pm 9 \mu\text{g kg}^{-1}$ (range 190–214 $\mu\text{g kg}^{-1}$) for Elazig city and $335 \pm 86 \mu\text{g kg}^{-1}$ (range 203–417 $\mu\text{g kg}^{-1}$) for Malatya city.

Cu and Ni

The seasonal variations in the Cu and Ni concentrations determined for *Sparus auratus* are shown in Figure 4. The mean Cu concentration in *Sparus auratus* was $379 \pm 12 \mu\text{g kg}^{-1}$ (range 365–397 $\mu\text{g kg}^{-1}$) for Elazig city and $384 \pm 33 \mu\text{g kg}^{-1}$ (range 319–416 $\mu\text{g kg}^{-1}$) for Malatya city. The MAC for Cu in all *Sparus auratus* samples studied was not exceeded. The mean Ni concentration in *Sparus auratus* was $320 \pm 45 \mu\text{g kg}^{-1}$ (range 267–375 $\mu\text{g kg}^{-1}$)

for Elazig city and $325 \pm 21 \mu\text{g kg}^{-1}$ (range 299–353 $\mu\text{g kg}^{-1}$) for Malatya city.

MAC of Cd for *Scomber scombrus* vs. *Sparus auratus*

Table II lists the mean Cd concentration found in *Scomber scombrus* as $82 \pm 14 \mu\text{g kg}^{-1}$ (range 60–98 $\mu\text{g kg}^{-1}$) for Elazig city and $98 \pm 10 \mu\text{g kg}^{-1}$ (range 82–109 $\mu\text{g kg}^{-1}$) for Malatya city. According to the Turkish Food Codex, the MAC of Cd in fish tissue depends on the fish species. The MAC for *Scomber scombrus* was 100 $\mu\text{g kg}^{-1}$ Cd, while MAC for *Sparus auratus* was 50 $\mu\text{g kg}^{-1}$ Cd based on fresh weight (31). The MAC limit was exceeded in only four *Scomber scombrus* samples from Malatya city. The mean Cd concentration in *Sparus auratus* was found to be $48 \pm 5 \mu\text{g kg}^{-1}$ (range 42–55 $\mu\text{g kg}^{-1}$) for Elazig city and $59 \pm 3 \mu\text{g kg}^{-1}$ (range 55–64 $\mu\text{g kg}^{-1}$) for Malatya city. The MAC of Cd was exceeded in all *Sparus auratus*, except for six samples from Elazig.

Of the more than 100 fish samples analyzed (taking into consideration that some of the data points in Figures 2–4 are the mean value of five sub-samples), the highest metal levels were found in the

Fig. 1. Seasonal variations in Pb and Cr concentrations of *Scomber scombrus*. El: Elazig city, Ml: Malatya city.

Fig. 2. Seasonal variations in Cu and Ni concentrations of *Scomber scombrus*. El: Elazig city, Ml: Malatya city.

Scomber scombrus: 109 $\mu\text{g kg}^{-1}$ Cd, 1015 $\mu\text{g kg}^{-1}$ Pb, 358 $\mu\text{g kg}^{-1}$ Cr, 474 $\mu\text{g kg}^{-1}$ Cu, and 327 $\mu\text{g kg}^{-1}$ Ni based on fresh weight.

Assuming an intake of two fish portions of 100 g *Scomber scombrus* per week, the weekly doses of Cd and Pb are calculated to be approximately 22 μg Cd and 203 μg Pb, which accounts for 7% of PTWI for Cd (350 μg Cd) and 11% of PTWI for Pb (1750 μg Pb).

In the literature, there are a number of contradictory results particularly with regard to Cd and Pb concentrations in fish samples. With a sample weight of less than 0.5 g in a microwave digestion procedure, considering the MAC and unpolluted fish, the Cd, Pb, Cr, and Ni concentrations are generally lower than 20 $\mu\text{g L}^{-1}$ (even Cd is lower than 3 $\mu\text{g L}^{-1}$) depending on the final dilution volume. It is known that these concentrations are too low for the sensitivity of direct flame AAS. In this study, successful results were obtained using STAT-AAS because it improved the sensitivity of flame AAS. However, it should be emphasized that unreliable results for Cd, Pb, Ni, and Cr concentrations are inevitable when the final solution was diluted to

a volume of more than 10 mL for 0.5 g fish sample and using direct flame AAS.

The potential health risk due to metals in fish tissues was calculated only for Pb in *Scomber scombrus*, since this element surpassed or was close to international and national criteria used for human health protection (Figure 1). We determined the EED, HQ, and CR from the RfD and SF as set by the USEPA (2000) for Pb, and the results are listed in

Table III. The estimated exposure dose (EED), the hazard quotient (HQ), and the lifetime cancer risk (CR) for children was calculated to be an average of 0.63 mg kg^{-1} Pb for *Scomber scombrus*, the ingestion rate (IR) of 0.1135 kg fish per day for children and 0.227 kg for adults, the exposure frequency (EF) of 52 for people who eat fish once a week, the exposure duration (ED) in years of a child, RfD of 4×10^{-3} for Pb and SF of 8.5×10^{-3} . A CR value

TABLE II
Seasonal Variations in Cd Concentrations
Depending on Fish Species and Cities (n=3)

Period	<i>Scomber scombrus</i> Cd (ng/g)		<i>Sparus auratus</i> Cd (ng/g)	
	Elazig	Malatya	Elazig	Malatya
2010-Oct.	80±7	106±10	47±6	56±7
2010-Nov.	60±6	82±7	43±5	58±7
2010-Dec.	96±9	109±10	53±6	64±6
2011-Jan.	83±8	89±9	48±5	61±6
2011-Feb.	67±8	96±10	42±4	59±7
2011-March	98±11	105±11	55±6	55±7
2011-April	75±9	91±11	50±5	62±6
2011-May	92±9	103±11	47±6	56±7
Mean	82±14	98±10	48±5	59±3.2

Fig. 3. Seasonal variations in Pb and Cr concentrations of *Sparus auratus*. El: Elazig city, Ml: Malatya city.

Fig. 4. Seasonal variations in Cu and Ni concentrations of *Sparus auratus*. El: Elazig city, Ml: Malatya city.

TABLE III
Risk of Developing Cancer

(Estimated exposure dose (EED), hazard quotient (HQ), and lifetime cancer risk (CR) for children according to an average of 0.63 mg kg⁻¹ Pb for *Scomber scombrus*, the ingestion rate (IR) of 0.1135 kg fish per day for children and 0.227 kg for adults, the exposure frequency (EF) of 52 for people who eat fish once a week, the exposure duration (ED) of age of child.)

Child Age (years)	BW ^a (kg)	EED	HQ=EED/RfD (0.004 for Pb)	CR=EEDxSF (8.5x10 ⁻³)
1	10	1.01x10 ⁻³	0.25	8.59x10 ⁻⁶
2	13	7.70 x10 ⁻⁴	0.19	6.55x10 ⁻⁶
3	15	6.57 x10 ⁻⁴	0.16	5.58x10 ⁻⁶
4	17	5.88 x10 ⁻⁴	0.15	5.00x10 ⁻⁶
5	19	5.26 x10 ⁻⁴	0.13	4.47x10 ⁻⁶
6	20	5.00 x10 ⁻⁴	0.13	4.25x10 ⁻⁶
7	23	4.35 x10 ⁻⁴	0.11	3.70x10 ⁻⁶
8	26	3.85 x10 ⁻⁴	0.10	3.27x10 ⁻⁶
9	30	3.33 x10 ⁻⁴	0.08	2.83x10 ⁻⁶
10	35	2.86 x10 ⁻⁴	0.07	2.43x10 ⁻⁶
11	40	2.50 x10 ⁻⁴	0.06	2.13x10 ⁻⁶
12	44	2.27 x10 ⁻⁴	0.06	1.93x10 ⁻⁶
13	49	2.04 x10 ⁻⁴	0.05	1.73x10 ⁻⁶
14	53	1.89 x10 ⁻⁴	0.05	1.61x10 ⁻⁶
adult	70	2.91 x10 ⁻⁴	0.07	2.47x10 ⁻⁶

^a BW: Average body weight (kg).

higher than the 10⁻⁶ is an acceptable risk for cancer to be occurring. The HQ values were below 1.0 which indicates that there is no need for concern with regard to causing cancer or other systemic effects such as neurotoxicity. From Table III it can be seen that the risk of developing cancer over a human lifetime is between 2-9 in 1,000,000. This study verifies that fish contamination levels should be carefully and regularly monitored to detect any change in metals contamination which could become hazardous to human safety. Furthermore, this type of investigation should be expanded to the fish sold in other cities in order to obtain more data on bio-accumulation.

CONCLUSION

Because of the very contradictory and unreliable concentrations of Cd, Pb, and other toxic metals in fish samples reported in the literature, the results obtained in this study are of great importance because the authors are aware of all possible error sources which are mentioned in the results and discussion sections. It was also verified whether metal levels exceeded the maximum levels set by national and international authorities and a comparison was made with Provisional Tolerable Weekly Intake (PTWI) levels as recommended by the Joint Expert committee on Food Additives (JECFA). The measured Pb concentrations in all *Scomber*

scombrus tissues were significantly higher than the Maximum Allowance Concentration (MAC) values, while the Cd levels were higher than the MAC values in only four of these samples. Thus, it can be stated that *Scomber scombrus* can significantly contribute to dietary lead exposure, thus, the risk of developing cancer over a human lifetime is between 2-9 in 1,000,000. The Pb concentrations (mean 324 µg kg⁻¹) in the studied *Sparus auratus* samples were slightly higher than the MAC values (300 µg kg⁻¹). However, this excess may be considered as unimportant because the overage value is in the range of the standard deviation. Based on the results of this study and in published literature, certainly more research on toxic metals determination is necessary to monitor fish quality and to ensure safe human health. Our final recommendation for health risk assessment purposes is the implementation of a standard survey to continuously monitor the frequency of fish consumption in different localities.

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