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ORIGINAL PAPER

SEASONAL VARIATIONS IN CONCENTRATIONS OF TOXIC TRACE METALS IN DEEP-SEA FISH, IDENTIFIED WITH STAT-AAS AND ICP-AES

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Abstract

Monitoring toxic metal concentrations in fish is very important for human health because fish, which are consumed by humans, can accumulate toxic metals from water up to hazardous levels. The aim of this study has been to monitor the levels of Cd, Pb, Cu, Cr and Ni in three deep-sea fish species as well as to make a risk assessment of their consumption. The samples were digested in a microwave oven and the determinations were made by atomic absorption spectrophotometry (AAS) and inductively coupled plasma-atomic emission spectrophotometry (ICP-AES). The mean lead concentrations in the *Salmo salar* and *Sarda sarda* species were found to be 955 and 948 μg kg^{-1}, respectively, and these levels are three-fold higher than the risk threshold level of 300 μg kg^{-1}. Cd concentrations in only six samples were slightly higher than the maximum allowable concentrations (MACs) of 100 μg kg^{-1}. Mean Cr and Ni concentrations in *Salmo salar* were found to be 866 and 472 μg kg^{-1}, respectively, and these results were higher than in *Sarda sarda* (mean 388 μg Cr kg^{-1} and 356 μg Ni kg^{-1}) and *Merlangius merlangus* (mean 303 μg Cr kg^{-1} and 336 μg Ni kg^{-1}). The measured Pb concentrations in all muscles of *Salmo salar* and *Sarda sarda* were found to be significantly higher than the MAC values, while Cd in all studied samples was around or lower than the MACs. However, there is no carcinogenic risk for humans, and the risk of developing cancer over an average human lifespan is between 2.5 and 13 in 1,000,000.

Keywords: toxic metals, fish, atomic absorption, ICP-AES, risk assessment.

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INTRODUCTION

The pollutants, including toxic metals such as Cd, Pb, Ni, Cr and Cu, are bio-accumulated by aquatic organisms, such as fish, and subsequently transferred to humans through the food chain (Copat et al. 2012, Meredius et al. 2012, Rahman et al. 2012). Those five elements were selected for this study because of their high toxic effects at even low concentrations. 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. Thus, the problem of heavy metal contamination in fish is increasing, attracting attention globally. In addition to toxic effects of Pb on the brain, kidney, the reproductive system, intellectual functioning, fertility, pregnancy and hypertension, the exposure in school-aged children can significantly reduce their intelligence quotient (IQ) and has been associated with aggressive behaviour, delinquency, and attention disorders (Schwartz 1994, Flora 2002, Yaman 2006, IARC 2006). Cadmium injures the kidney and causes the impaired renal function, poor reproductive capacity, hypertension, tumors and hepatic dysfunction (Huff et al. 2007). 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 as "possibly carcinogenic to humans" (Group 2B) (Yaman 2006, IARC 2006). The chromium intake at high concentrations causes severe environmental and public health problems, and Cr(VI) is considered a carcinogenic and mutagenic substance for humans (Rowbotham et al. 2000).

Copper is known to play 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 these enzymes weakens. It is clear that changes in the concentrations of these types of essential elements may cause various metabolic alterations (Ozen et al. 2003, Yaman et al. 2007*a*,). Further, Cu as well as Pb can also accumulate in a human body over time, and then immediately result in chronic poisoning. Nickel can cause a variety of adverse pulmonary health effects, such as lung inflammation, fibrosis, emphysema and tumors (Schaumloffel 2012). Further, Ni compounds have been classified as carcinogenic to humans – Group 1 and metallic nickel as possibly carcinogenic to humans – Group 2B (Silvera, Rohan 2007). Because fish can considerably accumulate those toxic metals from water, the problem of heavy metal contamination in fish is increasing on a global scale.

In terms of risk, children may also differ from adults in their vulnerability to environmental pollutants because of toxicodynamic and other related differences. Toxic chemicals in the environment can cause neurodevelopmental 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 behaviour disorders and reduction of intellectual function, particularly in children. Thus, health risks associated with toxic heavy metals should be assessed based on the target hazard quotients (THQs), which can be derived from concentrations of heavy metals in fish and other consumed food. Current noncancer risk assessment methods are typically based on the target hazard quotient (THQ), which is a ratio of the determined dose of a pollutant to the dose level (a Reference Dose or RfD). If the ratio is less than 1, there will not be any obvious risk. Conversely, an exposed population of concern will experience health risks if the dose is equal to or greater than the RfD. The method for the determination of THQ was provided in the U.S. EPA Region III risk-based concentration table (USEPA 2000, USEPA-IRIS 2007). Islam et al. (2015) evaluated health risks associated with intakes of chromium, nickel, copper, arsenic, cadmium, and lead in fish and vegetables in Bangladesh, in terms of dietary intake and target hazard quotients (THQs). They showed that the inhabitants who consume contaminated fish and vegetables are exposed chronically to metal pollution with carcinogenic and non-carcinogenic consequences. Yaman et al. (2014) reported that although concentrations of Pb in *Mugil cephalus* consumed in Turkey exceed the limits set by the authorities, there is no carcinogenic risk for humans, and the risk of developing cancer over a human lifespan is between 2 and 12 in 1,000,000, depending on children's age, in terms of the estimated non-carcinogenic and carcinogenic health risks by the Target Hazard Quotient and target carcinogenic risk. The same group of researchers found the risk of developing cancer over a human lifespan between 2 and 12 for *Pomatomus saltatrix*, and 2 and 9 for *Scomber scombrus* in 1,000,000, depending on children's age (Yaman, Yaman 2013, 2014). Yaman et al. (2013) found mean metal concentrations: 777 μg Pb kg^{-1}, 501 μg Cr kg^{-1}, 272 μg Ni kg^{-1}, 785 μg Cu kg^{-1} for *Trachurus trachurus* and 439 μg Pb kg^{-1}, 336 μg Cr kg^{-1}, 229 μg Ni kg^{-1}, 394 μg Cu kg^{-1} for *Cyprinus carpio* fish species.

Iqbal and Shah (2014) examined seasonal variations and health risk assessment of heavy metals in Cyprinus carpio from Rawal Lake, Pakistan. They found that Pb, Cd, Cr, Co and Zn levels were higher than the safe limits in relation to the non-carcinogenic risks to human. However, carcinogenic risks related to Cr and Pb clearly exceeded the safe limit depending on the season. Bae and Lim (2012) found that seasonal variations were not detected in lead, but mercury displayed maximal values in winter. Consequently, regular monitoring of concentrations of toxic metals in fish is very important for human health because fish can considerably accumulate toxic metals from water.

The main purpose of this study has been to use a slotted tube atom trap-atomic absorption spectrophotometer (STAT-FAAS) together with an inductively coupled plasma-atomic emission spectrophotometer (ICP-AES) for monitoring the levels of Cd, Pb, Cu, Cr and Ni metals in fish matrices. Three deep-sea fish species, *Salmo salar, Sarda sarda* and *Merlangius merlangus*

were obtained from markets in Elazig, eastern Turkey, during 6 months, from November 2010 to April 2011. A microwave oven was used for digestion of the tissues. Afterwards, direct AAS and ICP-AES determinations were achieved for trace metals concentrations at ppb levels. The risk assessment of fish consumption for children was also performed.

MATERIAL AND METHODS

Apparatus and reagents

Several analytical instruments have been used for the determination of analyte concentrations in different environmental and biological matrices (Yaman, Gucer 1995, Yaman 1999, Yaman et al. 2005, Copat et al. 2013). For instance, flame (FAAS) and electrothermal atomic absorption spectrometry (ET-AAS) are used for determination of As, Pb, Cd, Ni, Cr and Cu by employing chemical vapour generation and in-atomizer trapping techniques (Yaman, Akdeniz 2006, Yaman et al. 2007*b*, Kaya, Yaman 2008*a*, Kaya et al. 2010). Inductively coupled plasma atomic emission spectrometry (ICP-AES), a fast multi-element technique, has also been used for this purpose, since it has the required linear dynamic range (Strugeon 2000).

In this study, an ATI UNICAM 929 model atomic absorption spectrophotometer (AAS) equipped with ATI UNICAM hollow cathode lamps was used together with the STAT for the determinations of Cd, Pb and Cu because high improvment in the sensitivity of detecting these elements was achieved owing to the use of STAT in previous studies (Yaman, Akdeniz 2004, Yaman 2005, Kaya, Yaman 2008*b*). The optimum conditions in FAAS for lead, cadmium and copper were applied, i.e. wavelength: 217.0, 228.8 and 324.8 nm; HCl current: 9.5, 4.0 and 3.0 mA; acetylene flow rate: 0.6, 0.6 and 0.5 L min^{-1}, respectively, and air flow rate: 4.0 L min^{-1} and slit width: 0.5 nm, for three metals. The STAT conditions were as follows - diameter of tube: 6 mm, length of upper slot: 1 cm, length of lower slot: 5 cm and length of tube: 12 cm. Due to the lack of improvement in sensitivity by STAT-AAS for Ni and Cr, a Perkin-Elmer Optima 2100 DV ICP-OES apparatus (Perkin-Elmer California, USA) was used for measurements of the Ni and Cr concentrations. The instrumental conditions for ICP-AES are given in Table 1. Some samples were analyzed using a Perkin-Elmer ELAN 9000 inductively coupled plasma-mass spectrometer (ICP-MS) (Perkin Elmer SCIEX, Concord, Ontario, Canada) to check the accuracy of the measurements. A microwave digestion system (CEM MARSXpress) was used to digest the samples. Unless stated otherwise, all chemicals used throughout the study were of high-purity reagent grade. Doubly distilled water obtained with a water purification system (Millipore® Direct-Q, Millipore Corporation, Bedford, MA, USA) was used for all preparations. When not in use, all Pyrex® glassware was kept permanently full of 1.0 mol L^{-1} nitric acid. Concentrated nitric acid (65%,

Table 1

Operating conditions of ICP-AES for metal determinations

Parameter	ICP-AES
RF power (W)	1450
Plasma gas flow rate (L min^{-1})	15.0
Auxiliary gas flow rate (L min^{-1})	0.2
Nebulizer gas flow rate (L min^{-1})	0.6
Sample flow rate (L min^{-1})	1.5
View mode	axial
Read	peak area
Source equilibration time (s)	15.0
Read delay (s)	45.0
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 (nm)	Ni 231.604 nm Cr 267.716 nm Cu 327.393 nm Cd 228.802 nm Pb 220.353 nm

Merck) was used in the digestion procedure of fish muscle. Proper diluted solutions of the metals were prepared from the stock (1,000 mg L^{-1}) solutions (Merck) by using 1.0 mol L^{-1} nitric acid.

Sampling and digestion of samples

The fish samples for analyses including *Salmo salar, Sarda sarda* and *Merlangius merlangus,* were collected during the months from November 2010 to April 2011, and therefore particular fish samples were obtained from different sources depending on a season. They were randomly purchased from large modern supermarkets and traditional open fish markets in Elazig, a city with a populations of about 500,000, located in the East of Turkey. In each month, at least three different fish samples for each species were collected and their size ranges were between 60-80 cm for *Salmo salar* and 25-30 cm for *Sarda sarda* and 15-25 for *Merlangius merlangus.* Thus, more than 50 fish samples were analyzed in this study. 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 a preferred type of sample because it is the main edible part of fish.

To prevent loss of the studied metals in the dry digestion procedure, the samples were digested in a microwave oven. An amount of 0.5 g of fresh sample was transferred to a digestion vessel and 5 mL of concentrated nitric acid was added. After a few minutes, the vessels were closed and placed in the microwave oven. The samples were digested at 800 W, 180°C for 15 minutes. After complete digestion, the digested samples were allowed to cool to room temperature. Then, the digests were evaporated to 1.0 mL. Each digest was diluted to 5.0 mL with Millipore water. Subsequently, the samples were analyzed for Cd, Pb and Cu using STAT-AAS, and for Cr and Ni using ICP-AES. Blanks were also digested to determine the background contribution of the reagents and treated in the same way as the samples. Consequently, the results are the mean values of at least three different subsamples of the same species.

Analytical performance

The limit of detection (LOD) is defined as 3 X standard deviation of the blank. The limits of detection (LODs) using ICP-AES were found to be (as μg L^{-1}) 4.0 for Ni and 3.0 for Cr. It should be mentioned that sensitivity in ICP -AES is highly dependent on its state including the view mode (axial and radial) and nebulizer type (pneumatic and ultrasonic). The axial view provides better LODs than the radial one. The LODs obtained by STAT-FAAS (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. As a result, each of the samples was analyzed in triplicate, and an average of the three values was used as a mean value on the basis of fresh weight. The lowest concentration in a calibration plot of each element was considered as the limit of quantitation (LOQ) for the measurement step. Taking into consideration the sample's weight of 0.5 g and final volume of 5 mL, LOQ for the sample was calculated as 40 ng g^{-1} for Cd, 260 ng g^{-1} for Pb, 280 ng g^{-1} for Cr, 190 ng g^{-1} for Ni and 400 ng g^{-1} for Cu.

In order to verify the accuracy of the methods, the results obtained were compared with the results achieved by ICP-MS for some of the same samples. The comparison showed 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. Furthermore, the standard additions method was applied in order to establish whether or not any matrix interferences were 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. This shows that there is no matrix interference affecting the determination of the analyzed elements. Hence, the direct calibration method was used for all of the measurements. A table (Table 2) was drawn to compare the results easily for all the data.

Table 2

LOD and LOQ and the linear ranges for STAT-AAS and ICP-AES methods

Element	LOD ($\mu g\ L^{-1}$)		LOQ ($\mu g\ L^{-1}$)		The Linear range	
	STAT-AAS	ICP-AES	STAT-AAS	ICP-AES	STAT-AAS	ICP-AES
Cd	1	2	3	10	3-50	10-300
Pb	3	18	25	100	25-400	100-2000
Cu	5	5	25	25	25-400	25-1000
Ni	NA	4	NA*	20	NA	20-400
Cr	NA	3	NA	20	NA	20-300

* NA – not applicable

The linear ranges for the five elements were determined as follows:

$Y = 2.6X - 0.1$ $R^2 = 0.9999$ for Cd (3-50 $\mu g\ L^{-1}$ by STAT–FAAS),
$Y = 0.3X + 0.5$ $R^2 = 0.9999$ for Pb (25-400 $\mu g\ L^{-1}$ by STAT–FAAS),
$Y = 0.3X - 0.2$ $R^2 = 1$ for Cu (25-400 $\mu g\ L^{-1}$ by STAT–FAAS),
$Y = 294X - 469$ $R^2 = 0.9998$ for Ni (20-400 $\mu g\ L^{-1}$ by ICP-AES),
$Y = 621X - 2369$ $R^2 = 0.9998$ for Cr (20-300 $\mu g\ L^{-1}$ by ICP-AES).

Finally, the fish certified reference material (CRM, IAEA-407) was analyzed. The recommended and obtained metal concentrations were found to be 189 and 183 $\mu g\ kg^{-1}$ for Cd, 120 and 115 $\mu g\ kg^{-1}$ for Pb, 730 and 715 $\mu g\ kg^{-1}$ for Cr, 600 and 584 $\mu g\ kg^{-1}$ for Ni and 3,280 and 3,248 $\mu g\ kg^{-1}$ for Cu, respectively.

To determine the differences between months at a same location and sites in total for metal concentrations in fish samples, one way ANOVA was performed. Possibilities less than 0.05 were considered statistically significant ($p < 0.05$). All statistical calculations were performed with SPSS 15.0.

Estimation of health risk

The methodology for estimating the non-carcinogenic and carcinogenic risks was applied in accordance with that provided in the US EPA Region III Risk-based Concentration table (USEPA-IRIS 2007). The non-carcinogenic risks for each individual metal through fish consumption were assessed by the Hazard Quotient (HQ): "the ratio of a single substance exposure level over a specified time period (subchronic) 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 (HQ=EED/RfD exceeds unity), 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 lifeti-

me, 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 details related with the estimated exposure dose (EED) were given elsewhere (YAMAN et al. 2013, 2014, YAMAN, YAMAN 2014).

The hazard quotient (HQ) for noncancer or systemic effects (such as neurotoxicity) was estimated 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 (USEPA-IRIS, 2007), in the following equation: CR=EEDxSF. RfD is the oral reference dose: 1.0 $x10^{-3}$ mg kg^{-1} day^{-1} for Cd and 4.0 $x10^{-3}$ mg kg^{-1} day^{-1} for Pb (USEPA-IRIS 2007). The RfD represents an estimate of daily oral exposure that is likely to be without an appreciable risk of adverse health effects over a lifetime. The SF is the oral carcinogenic slope factor from the Integrated Risk Information System (USEPA-IRIS 2007) database (8.5$x10^{-3}$ mg kg^{-1} day^{-1} for Pb).

RESULTS AND DISCUSSION

The advantages of microwave digestion compared to the classical dissolution methods are a shorter period of time, less consumption of acid, and keeping 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 an 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 (WHO 2011). Recently, European Food Safety Authority (EFSA) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) have concluded that the existing provisional tolerable weekly intake (PTWI) for lead of 25 μg kg^{-1} body weight is no longer appropriate to protect human health (WHO 2011, EFSA 2010). Concerning cadmium in food, the EFSA Panel on Contaminants in the Food Chain CONTAM Panel establishes a tolerable weekly intake (TWI) for this metal of 2.5 μg kg^{-1} b.w. This value is 2.8 times lower than previously established PTWI for cadmium of 7 μg kg^{-1} b.w. per week (EFSA 2009). JECFA has established a PTWI of 35 μg kg^{-1} per week for Ni, which is equivalent to 2450 μg per week for an adult human weighing 70 kg (WHO 1996). Concerning chromium, in 2001 Dietary Reference Intakes for chromium were established. The research base was insufficient to establish Recommended Dietary Allowances (RDAs), so the Adequate Intakes (AIs) were developed based on average intakes of chromium from food as found in several studies (IMFNB 2001). The US 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 (IMFNB 2001, TRUMBO et al. 2001). It can be concluded

that the food consumption data depend on income, household consumption, age groups and socioeconomic factors (Sutter et al. 2003). In particular, it should be emphasized that diets are significantly different from country to country. Thus, the MAC values established by authorities in developing countries do not reflect the actual intakes in undeveloped countries.

The monthly variations in the Pb concentrations (as μg kg^{-1}) determined for *Salmo salar, Sarda sarda* and *Merlangius merlangus* are shown in Figure 1.

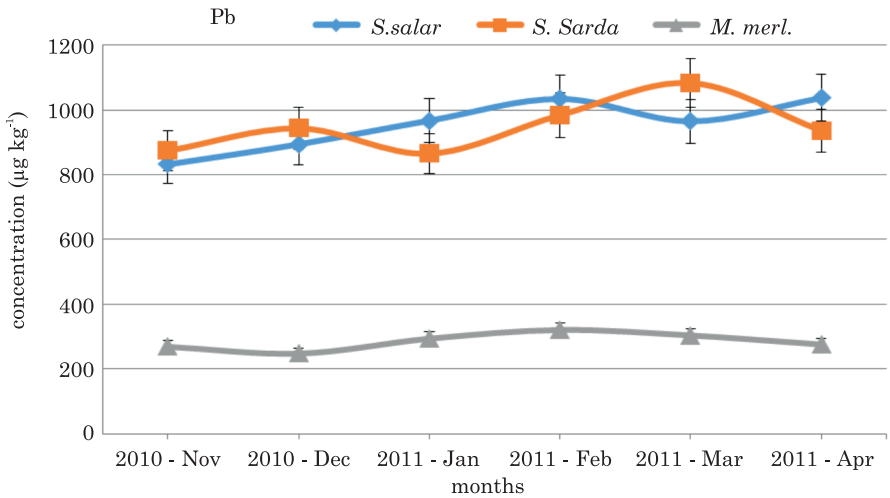

Fig. 1. Seasonal variations in Pb concentrations of *S. salar, S. sarda* and *M. merlangius*

The mean Pb concentrations in *Salmo salar* and *Sarda sarda* were 955 (range 832-1037) μg kg^{-1} and 948 (range 874-1083) μg kg^{-1}. According to the Turkish Food Codex and the European Commission (TFC 2011, EC 2006), maximum allowed concentration (MAC) of Pb in fish tissue is 300 μg kg^{-1}. This limit was exceeded in all studied *Salmo salar* and *Sarda sarda* samples. The mean Pb concentration in *Merlangius merlangus* was found to be 285 (range 248-321) μg kg^{-1}, and those results were lower than the MAC values for Pb in all fish except for two.

The monthly variations in the Cd concentrations determined for *Salmo salar, Sarda sarda* and *Merlangius merlangus* are shown in Figure 2. The mean Cd concentrations in *Salmo salar* and *Sarda sarda* were 95 (range 81-108) μg kg^{-1} and 87 (range 76-97) μg kg^{-1}, respectively. According to the TFC (2011) and the EC (2006), the MAC for Cd in fish tissue is 100 μg kg^{-1} for *Salmo salar* and *Sarda sarda,* and 50 μg kg^{-1} for *Merlangius merlangus*. These limits were not found to be exceeded in any of the studied samples except for two samples of *Salmo salar*.

The monthly variations in the Cu concentrations detected for *Salmo salar, Sarda sarda* and *Merlangius merlangus* are shown in Figure 3. The mean Cu concentration in *Sarda sarda* was 600 (range 543-645 μg kg^{-1}), and these results were higher than in the other two fish species *Salmo salar*

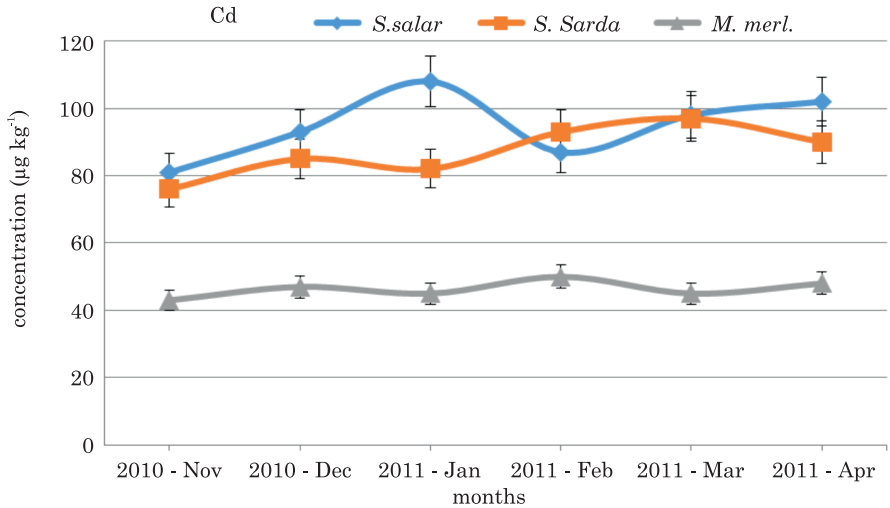

Fig. 2. Seasonal variations in Cd concentrations of *S. salar, S. sarda* and *M. merlangius*

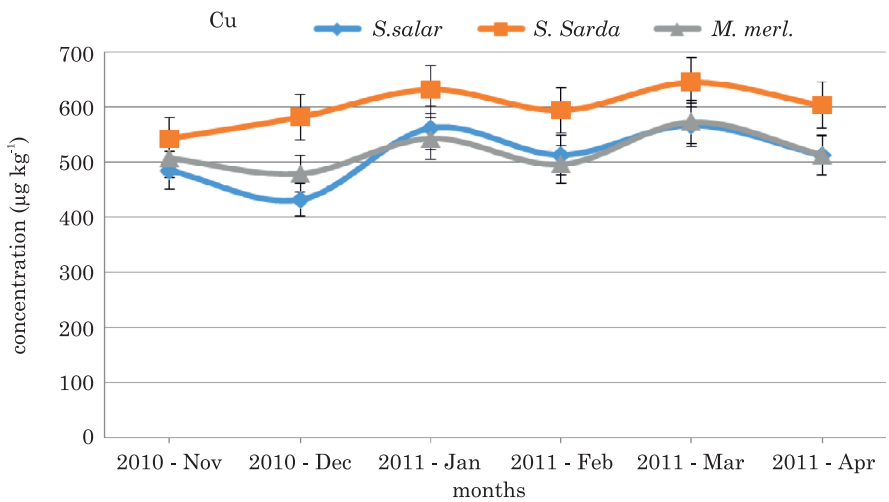

Fig. 3. Seasonal variations in Cu concentrations of *S. salar, S. sarda* and *M. merlangius*

(mean 512, range 432-567 µg kg^{-1}) and *Merlangius merlangus* (mean 519, range 479-573 µg kg^{-1}). According to the TFC (2011), the MAC for Cu in fish tissue is 20,000 µg kg^{-1}. This limit was not found to be exceeded in any of the studied fish samples.

The seasonal variations in the Cr concentrations detected for *Salmo salar, Sarda sarda* and *Merlangius merlangus* are shown in Figure 4. The mean Cr concentration in *Salmo salar,* was 866 (range 756-969), and these values were higher than in *Sarda sarda* (mean 388, range 321-453 µg kg^{-1}) and *Merlangius merlangus* (mean 303, range 269-327 µg kg^{-1}). The TFC

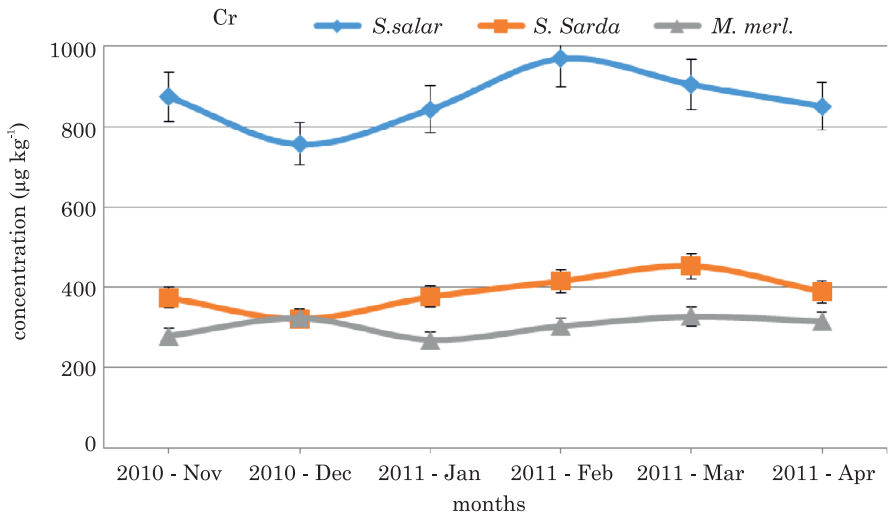

Fig. 4. Seasonal variations in Cr concentrations of *S. salar, S. sarda* and *M. merlangius*

(2011) and other similar authorities (EC 2006) did not establish the MAC for Cr in fish tissue.

The seasonal variations in the Ni concentrations detected for *Salmo salar, Sarda sarda* and *Merlangius merlangus* are shown in Figure 5.

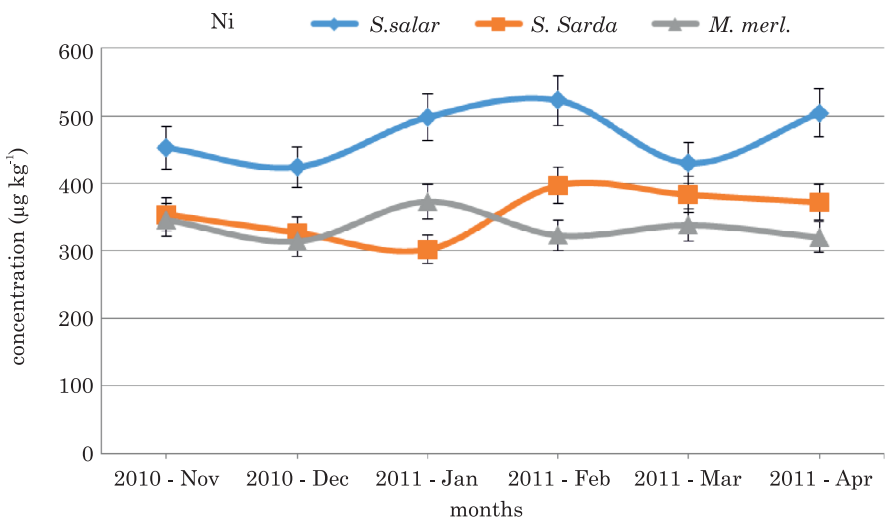

Fig. 5. Seasonal variations in Ni concentrations of *S. salar, S. sarda* and *M. merlangius*

The mean Ni concentrations in *Salmo salar, Sarda sarda* and *Merlangius merlangus* were 472 (range 424-523 µg kg^{-1}), 356 (range 302-397 µg kg^{-1}) and 303 (range 314-373 µg kg^{-1}), respectively. The TFC (2011) and other similar authorities (EC 2006) did not establish the MAC for Ni in fish tissue.

It was found that STAT-AAS can be used directly to determine Cd, Pb and Cu and ICP-AES for Ni and Cr at ppb concentrations. Of more than 50 fish samples analyzed, the highest Pb level was found to be in *Sarda sarda* at 1083 μg kg^{-1} based on fresh weight. Assuming an intake of two *Salmo salar* or *Sarda sarda* portions of 100 g per week, the weekly dose of Pb is calculated to be approximately 200 μg Pb, which accounts for approximately 12% 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. Among possible reasons there could be differences in digestion methods, use of unsufficiently sensitive methods and probable interferences in some methods. 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 μg L^{-1} (even Cd is lower than 3 μg 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. More sensitive analytical techniques such as flameless AAS require expert operators due to a greater proneness to matrix interferences in flameless AAS. Typically, Cd and Pb concentrations in fish were reported from 0.9 and 3.0 μg kg^{-1} to more than 5,000 and 15,000 μg kg^{-1}, respectively.

The potential health risk due to metals in fish tissues was calculated only for Pb in *Salmo salar* and *Sarda sarda*, 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, 2007) for Pb (Table 3). The estimated EED, the HQ and the CR for children were calculated to be an average of 0.95 mg Pb kg^{-1} for both *Salmo salar* and *Sarda sarda*, the ingestion rate (IR) of 0.1135 kg fish per day for children and 0.227 kg for adult, the exposure frequency (EF) of 52 for the people who eat fish once a week, the exposure duration (ED) in years of a child, RfD of $4x10^{-3}$ for Pb and SF of $8.5x10^{-3}$. A CR value higher than the 10^{-6} is an acceptable risk for cancer occurrence. The HQ values were below 1, which indicates that there is no need for concern with regard to developing cancer or other systemic effects such as neurotoxicity. However, it was found that there is the lifetime cancer risks (CR) because the estimated CR values were higher than the $1x10^{-6}$. The estimated non-carcinogenic and carcinogenic health risks by the Target Hazard Quotient and target carcinogenic risk indicate that there is no systemic effect, and the risk of developing cancer over a human lifetime is between 2 to 13 in 1,000,000. This study verifies that fish contamination levels should be carefully and regularly monitored to detect any change in metal contamination which could become hazardous to human safety. Furthermore, in order to

Table 3

Estimated exposure dose (EED), the hazard quotient (HQ) and the lifetime cancer risk (CR) for children according to average 950 μg Pb kg^{-1} for *Salmo salar* and *Sarda sarda*

Child age, years	BW* (kg)	EED	HQ = EED/RfD (0.004 for Pb)	CR = EEDxSF ($8.5x10^{-3}$)
1	10	$1.54x10^{-3}$	0.39	$1.31x10^{-5}$
2	13	$1.18\ x10^{-3}$	0.30	$1.00x10^{-5}$
3	15	$1.02\ x10^{-3}$	0.26	$8.67x10^{-6}$
4	17	$9.04\ x10^{-4}$	0.23	$7.68x10^{-6}$
5	19	$8.08\ x10^{-4}$	0.20	$6.87x10^{-6}$
6	20	$7.68\ x10^{-4}$	0.19	$6.53x10^{-6}$
7	23	$6.68\ x10^{-4}$	0.17	$5.68x10^{-6}$
8	26	$5.91\ x10^{-4}$	0.15	$5.02x10^{-6}$
9	30	$5.12\ x10^{-4}$	0.13	$4.35x10^{-6}$
10	35	$4.39\ x10^{-4}$	0.11	$3.73x10^{-6}$
11	40	$3.84\ x10^{-4}$	0.10	$3.26x10^{-6}$
12	44	$3.49\ x10^{-4}$	0.09	$2.97x10^{-6}$
13	49	$3.14\ x10^{-4}$	0.08	$2.67x10^{-6}$
14	53	$2.90\ x10^{-4}$	0.07	$2.47x10^{-6}$
Adult	70	$4.39\ x10^{-4}$	0.11	$3.73x10^{-6}$

BW* average body weight (kg);
The ingestion rate (IR) of 0.1135 kg fish per day for children and 0.227 kg for adult, the exposure frequency (EF) of 52 for the people who eat fish 1 time a week, the exposure duration (ED) of years of child.

Table 4

The MACs for fish and RFD values for the studied elements

Element	MAC (μg kg^{-1})	RFD (μg kg^{-1} day^{-1} bw)
Cd	50	1
Pb	300	4
Ni	500	20
Cr	1000	1500
Cu	20 000	40

obtain more data on bio-accumulation, this type of investigation should be extended to fish sold in other cities. A table (Table 4) was given including the MACs and RFD values (TFC 2011, EC 2006, USEPA-IRIS 2007, WHO 2011).

CONCLUSIONS

The present study consisted of direct AAS and ICP-AES determinations of trace metals concentrations at ppb levels. Furthermore, evaluation of the food safety of edible fish tissues of *Salmo salar, Merlangius merlangus* and *Sarda sarda* in terms toxic metal contents was performed. In particular, it was verified whether metal levels exceeded the maximum levels set by national and international authorities and a comparison was made with PTWI levels as recommended by those authorities (WHO 1996, IMFNB 2001, USEPA 2007, EFSA 2009, EFSA 2010, WHO 2011). Because many contradictory and unreliable (as can be inferred from the data on weighed samples, final volumes and sensitivity of analytical techniques) concentrations of Cd, Pb and other toxic metals in fish samples have been reported in literature, accurate measurements of these metal concentrations are of high importance. The measured Pb concentrations in all muscles of *Salmo salar* and *Sarda sarda* were found to be significantly higher than the MAC values, while Cd in all studied samples was around or lower than the MAC values. Thus, *Salmo salar and Sarda sarda* species can significantly contribute to human dietary exposure to Pb metal. It was found that the risk of developing cancer over a human lifetime is between 2 to 13 in 1,000,000. Furthermore, it was calculated that there can be systemic effects if 2-year-olds consumed *Salmo salar* and *Sarda sarda* species 3 times in a week. Taking into consideration our results and the ones reported in the literature, more research on toxic metal determination is necessary to monitor fish quality and ensure risk-free human health. We also recommend a survey of the frequency of fish consumption among local inhabitants as an essential input needed for the risk assessment. Finally, our study has demonstrated that STAT-AAS and ICP-AES can be used directly to determine Cd, Pb and Cu, and Ni and Cr at ppb concentrations, respectively.

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REFERENCES

Bae JH., Lim SY. 2012. *Effect of season on heavy metal contents and chemical compositions of chub mackerel (Scomber japonicus) muscle*. J. Food Sci., 77(2): 52-57.

Copat C., Bella F., Castaing M., Fallico R., Sciacca S., Ferrante M. 2012. *Heavy metals concentrations in fish from Sicily (Mediterranean Sea) and evaluation of possible health risks to consumers*. Bull. Environ. Contam. Toxicol., 88: 78-83.

Copat C., Arena G., Fiore M., Ledda C., Fallico R., Sciacca S., Ferrante M. 2013. *Heavy metals concentrations in fish and shellfish from eastern Mediterranean Sea: Consumption advisories*. Food Chem. Toxicol., 53: 33-37.

EFSA 2010. *European Food Safety Authority; Scientific opinion on lead in food*. EFSA J., 8(4):1570 (147 pp.).

EFSA 2009. *European Food Safety Authority; Technical report of EFSA prepared by Assessment Methodology Unit on Meta-analysis of Dose-Effect Relationship of Cadmium for Benchmark Dose Evaluation*. EFSA Sci. Report, 254: 1-62.

EC 2006. *European Commission Regulation N. 1881/2006, 19 Dec. 2006 on setting maximum levels of certain contaminants in foodstuff*. Off. J. Eur. Union:Legis. Ser. 65:5-24.

Flora S.J.S. 2002. *Lead exposure: Health effects, prevention and treatment*. J. Environ. Biol., 23(1): 25-41.

Huff J., Lunn R.M., Waalkes M.P. et al. 2007. *Cadmium-induced cancers in animals and in humans*. Inter. J. Occupat. Environ. Health., 13(2): 202-212.

IMFNB 2001. *Institute of Medicine, Food and Nutrition Board; Dietary reference intakes: vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc*. National Academy Press, Washington, DC, 2001.

Islam M.S, Ahmed M.K., Habibullah-Al-Mamun M. 2015. *Determination of heavy metals in fish and vegetables in Bangladesh and health implications*. Human Ecol. Risk Assess., 21(4): 986-1006.

IARC 2006. *International Agency for Research on Cancer; Working Group on the Evaluation of Carcinogenic Risks to Humans, Inorganic and Organic Lead Compounds*. IARC Monogr. Eval. Carcinog. Risks Hum., 87: 1.

Iqbal J, Shah MH. 2014. *Study of seasonal variations and health risk assessment of heavy metals in Cyprinus carpio from Rawal Lake, Pakistan*. Environ. Monitor. Assess., 186(4): 2025-2037.

Kaya G., Ozcan C., Yaman M. 2010. *Flame Atomic Absorption Spectrometric determination of Pb, Cd, and Cu in Pinus nigra* L. *and Eriobotrya japonica leaves used as biomonitors in environmental pollution*. Bull. Environ. Contam. Toxicol., 84(2): 191-196.

Kaya G., Yaman M. 2008*a*. *Trace metal concentrations in cupressaceae leaves as biomonitors of environmental pollution*. Trace Elem. Electrolytes, 25(3):156-164.

Kaya G., Yaman M. 2008*b*. *Online preconcentration for the determination of lead, cadmium and copper by Slotted Tube Atom Trap (STAT)-Flame Atomic Absorption Spectrometry*. Talanta, 75: 1127-1133.

Medeirus R.J., G. Dos Santos L.M., Freire A.S., Santelli R.E., Braga A.M.C.B., Krauss T.M., Do C. Jacob S. 2012. *Determination of inorganic trace elements in edible marine fish from Rio de Janeiro State, Brazil*. Food Contr., 23: 535-541.

Ozen O.A.**,** Songur A, Sarsilmaz M**.,** Yaman M., Kus I. 2003. *Zinc, copper and iron concentrations in cerebral cortex of male rats exposed to formaldehyde inhalation*. J. Trace Elem. Med. Biol., 17(3): 207-209.

Rahman, M.S., Molla A.H., Saha N., Rahman A. 2012. *Study on heavy metals levels and its risk assessment in some edible fishes from Bangshi River, Savar, Dhaka, Bangladesh*. Food Chem., 134: 1847-1854.

Rowbotham A.L., Levy L.S., Shuker L.K. 2000. *Chromium in the environment: An evaluation of exposure of the UK general population and possible adverse health effects*. J. Toxicol. Environ. Health-Part B-Crit. Rev., 3(3): 145-178.

Schwartz J. 1994. *Low-level lead-exposure and children's IQ-a metaanalysis and search for a threshold*. Environ. Res., 65: 42-55.

Schaumlöffel D. 2012. *Nickel species: Analysis and toxic effects*. J. Trace Elem. Med. Biol., 26: 1-6.

Silvera S.A.N., Rohan T.E. 2007. *Trace elements and cancer risk: A review of the epidemiologic evidence*. Cancer Causes Control, 18: 7-27.

Sturgeon R.E. 2000. *Current practice and recent developments in analytical methodology for trace element analysis of soils, plants, and water*. Commun. Soil. Sci. Plant. Anal., 31(11-14): 1479-1512.

Sutter G.W., Munns W.R. Jr., Sekizawa, J. 2003. *Types of integration in risk assessment and management, and why they are needed*. Human Ecol. Risk Assess., 9(1): 273-279.

Trumbo P., Yates A.A., Schlicker S., Poos M. 2001. *Dietary reference intakes: vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc*. J. Am. Diet. Assoc., 101(3): 294-301.

TFC 2011. Turkish Food Codex, Turk Gida Kodeksi, Ankara: Official Gazette of Republic of Turkey.

USEPA 2000. *United States Environmental Protection Agency. Guidance for assessing chemical contaminant data for use in fish advisories*. Vol. 2. *Risk assessment and fish consumption limits*. 3rd ed. Washington D.C.

USEPA 2007. *Integrated Risk Information System (IRIS) – Database*. Philadelphia; Washington, DC.

WHO 2011. World Health Organization; *Evaluation of certain food additives and contaminants*. Seventy-third report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, No 960, Geneva, Switzerland.

WHO/FAO/IAEA 1996. *Trace elements in human nutrition and health*. World Health Organization, Geneva.

Yaman M. 1999. *Determination of cadmium and lead in human urine by STAT-FAAS after enrichment on activated carbon*. J. Anal. At. Spectrom., 14: 275-278.

Yaman M. 2005. *The improvement of sensitivity in lead and cadmium determinations using flame Atomic Absorption Spectrometry*. Anal. Biochem., 339: 1-8.

Yaman M. 2006. *Comprehensive comparison of trace metal concentrations in cancerous and non-cancerous human tissues*. Curr. Med. Chem., 13(21): 2513-2525.

Yaman M., Akdeniz I. 2004. *Sensitivity enhancement in flame atomic absorption spectrometry for determination of copper in human thyroid tissues*. Anal. Sci., 20(9): 1363-1366.

Yaman M., Akdeniz I. 2006. *Effects of different chemical modifiers on the determination of arsenic by electrothermal atomic absorption spectrometry and application to the poultry and plant samples*. Trace Elem. Electrolytes, 23(4): 237-241.

Yaman M., Atici D., Bakirdere S., Akdeniz I. 2005. *Comparison of trace metal concentrations in malign and benign human prostate*. J. Med. Chem., 48(2): 630-634.

Yaman M., Bal T., Yaman I.H. 2013. *Metal levels in Trachurus trachurus and Cyprinus carpio in Turkey*. Food Add. Contam., Part B. 6(4): 301-306.

Yaman M., Gucer S. 1995. *Determination of cadmium and lead in vegetables after activated-carbon enrichment by Atomic-Absorption Spectrometry*. Analyst, 120: 101-105.

Yaman M., Karaaslan N.M., Yaman I.H. 2014. *Seasonal variations in toxic metal levels of two fish species, Mugil cephalus and Mullus barbatus, and estimation of risk for children*. Bull. Environ. Contam. Toxicol., 93(3): 344-349.

Yaman M., Kaya G., Yekeler H. 2007*a*. *Distribution of trace metal concentrations in paired cancerous and non-cancerous human stomach tissues*. World J. Gastroenterol., 13(4): 612-618.

Yaman M., Kaya G., Simsek M. 2007*b*. *Comparison of trace element concentrations in cancerous and non-cancerous endometrium and ovarian tissues*. Inter. J. Gynecol. Cancer., 17: 220-228.

Yaman M., Yaman I.H. 2013. *Determination of trace metal levels of Cd, Pb, Cr, Ni, and Cu in two fish species by STAT-AAS and ICP-AES*. At. Spectrosc., 34(5): 191-198.

Yaman M., Yaman I.H. 2014. *Variations in toxic metal levels of two fish species, Pomatomus saltatrix and Dicentrarchus labrax, and risk estimation for children*. Spectrosc. Spectral. Anal., 34(2): 300-307.