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Determination of trace metals in fish species of the Ria de Aveiro (Portugal) by electrothermal atomic absorption spectrometry

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Abstract

Cadmium, copper, nickel, lead and zinc were determined in fish samples from seven sampling stations of the Ria de Aveiro (Portugal). The species analyzed were *Anguilla anguilla*, *Mullus surmuletus*, *Trigla lucerna, Mugil cephalus*, *Chelon labrosus*, *Liza aurata* and *Dicentrarchus labrax*, all of which are used for human consumption. For this purpose, procedures for the electrothermal atomic absorption spectrometry determination of cadmium, copper, nickel and lead in these samples were developed, as well as a microwave digestion method for obtaining a fast dissolution of the samples. The concentrations of metals found in the muscle of the fish species were very low. The minor contents corresponded to cadium, lead and nickel with values smaller than 0.043, 0.15 and 0.14 μg/g (wet weight), respectively, except in the case of nickel in the *Anguilla anguilla* species where more elevated concentrations were found (between 0.16 and 0.40 μg/g). The contents of copper in the samples ranged from 0.5 to 1.1 μg/g (wet wt.). Zn is the most abundant element in all fishes, with concentrations around 20 μg/g (wet wt.) in the *Anguilla anguilla* samples and with values oscillating between 4.7 and 12 μg/g in the rest of the species studied. In all cases, the results obtained for all the elements were considerably lower than those recommended by specific legislation for these aquatic organisms. The accuracy of the analytical methodology employed was also evaluated through the analysis of two reference materials (NIST-1577b and IAEA-V10): good agreement was obtained between the experimental results and the certified values.

Keywords: Trace metals; Electrothermal atomic absorption spectrometry; Microwave digestion; Fish

1. Introduction

The Ria de Aveiro is located on the west Portuguese coast, and has an elevated productivity, supporting abundant invertebrates and fish populations, many of which are used as food. It is well known (Boia, 1987; Lucas, Caldeira, Hall, Duarte, & Lima, 1986) that some specific areas of the Ria could be partly contaminated by domestic and industrial wastewater discharges and also by agricultural activities. However, professional fishing in the lagoon is a traditional activity and aquaculture has been developed with greater intensity in recent years. For these reasons, it is important to determine the chemical quality of the marine organisms (Buffle & de Vitre, 1994) particularly the contents of trace metals, in order to evaluate the possible existence of risk to human health of fish consumption.

Food is a constant source of toxic trace metals which accumulate in different parts of the human body (Reilly, 1991; WHO, 1987) and cause damage in many of its basic systems (renal, cardiovascular, gastrointestinal, endocrine, nervous, etc.). Mercury (Hg), lead (Pb) and cadmium (Cd) are considered to be the most dangerous metals (Goyer, Klaassen, & Waalkes, 1995), however, other elements, such as copper (Cu), nickel (Ni), chromium (Cr) and zinc (Zn), although considered as essentials, can also produce toxic effects when the metal intake is excessively elevated. According to the recommendations of various international organizations, such us Codex Alimentarius (Food Chemical Codex, 1996), FAO (Nauen, 1983) and certain regulations (BOE, 1991), the maximum tolerable levels of toxic trace metals in fish and fishery products are very low and habitually lower than 0.1 μg/g (wet wt.) for cadmium,

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lower than 0.5–1 μg/g (wet wt.) for mercury (depending up on the fish species) and lower than 1–2 μg/g (wet wt.) for lead.

In view of these low concentrations, electrothermal atomic absorption spectrometry (ETAAS) is the technique frequently applied for the determination of metals in food samples (Correia, Oliveira, & Oliveira, 2000; González, Gallego, & Valcárcel 1999; Jeng, Lee, & Lin, 1994; Lima, Krug, & Arruda, 1998; Roca, Farré, & Frigola, 1999) and consequently in fish (Blasco, Arias, & Sáenz, 1999; Roméo, Siau, Sidoumou, & Gnassia-Barelli, 1999; Tahvonen & Kumpulainen, 1996). This analytical methodology can be used after dry or wet destruction of the organic matter (Blasco et al., 1999; Correia et al., 2000; Roca et al., 1999; Roméo et al., 1999; Tahvonen et al, 1996) or can be applied directly, using the slurry-sampling technique (González et al., 1999; Jeng et al., 1994; Meeravali & Kumar, 2000).

The main aim of this work was to determine the total contents of Cd, Cu, Ni, Pb and Zn in different fish species collected from various sampling points of the Ria de Aveiro, since they are an important component of the human diet in this zone. For this purpose the samples were dissolved using a microwave digestion method, proposed in this work, and the determination of metals was carried out by atomic absorption spectrometry (AAS) using flame (for Zn) and graphite furnace (for Cd, Cu, Ni and Pb) as atomization systems. The furnace operating conditions and the influences of chemical modifiers were evaluated when the use of graphite furnace was required. Certified reference materials were used to check the analytical methods employed.

2. Materials and methods

2.1. Sampling

The samples studied were collected in seven sampling stations distributed along the Ria de Aveiro (Fig. 1) from July 1999 to March 2000. They were fished using a traditional method with the supervision of professional fishermen. Immediately after collection, the fish were brought to the laboratory and only those species eaten by humans were separated and stored frozen until they were analyzed. *Anguilla anguilla*, *Mullus surmuletus*, *Trigla lucerna, Mugil cephalus*, *Chelon labrosus*, *Liza aurata* and *Dicentrarchus labrax* were the species found in all samplings, the three first being the most abundant.

2.2. Apparatus

A Varian Atomic Absorption Spectrophotometer (SpectrAA-300 Plus) equipped with a deuterium lamp as background correction and a GTA-96 graphite tube atomizator were used for Cd, Cu, Ni and Pb determinations; varian graphite partition tubes (63-100012-00) were used throughout. A GBC, 904 AA model, atomic absorption spectrophotometer was used for Zn measurements using an air/acetylene flame with a flow rate of 11-1 l/ min. Hollow cathode lamps (Cathodeon) were used as the energy sources. Lamp intensity and band-pass width were used according to the manufacturer's recommendations. The resonance line used for Zn was 213.9 nm and the slit width was 0.5 nm. The instrumental parameters for the other elements studied are shown in Table 1.

A 45-ml capacity Parr reactor (model 4782) together with a 650 W power domestic microwave oven (Crown, model NW 070) was used for acid-digestion of fish samples. A porcelain mortar was employed to grind and homogenize the muscle of the wet fish samples.

2.3. Reagents

All reagents were of analytical reagent grade. High purity water (Millipore Milli-Q System) was used throughout. The stock solutions of metals (1000 μg/g)

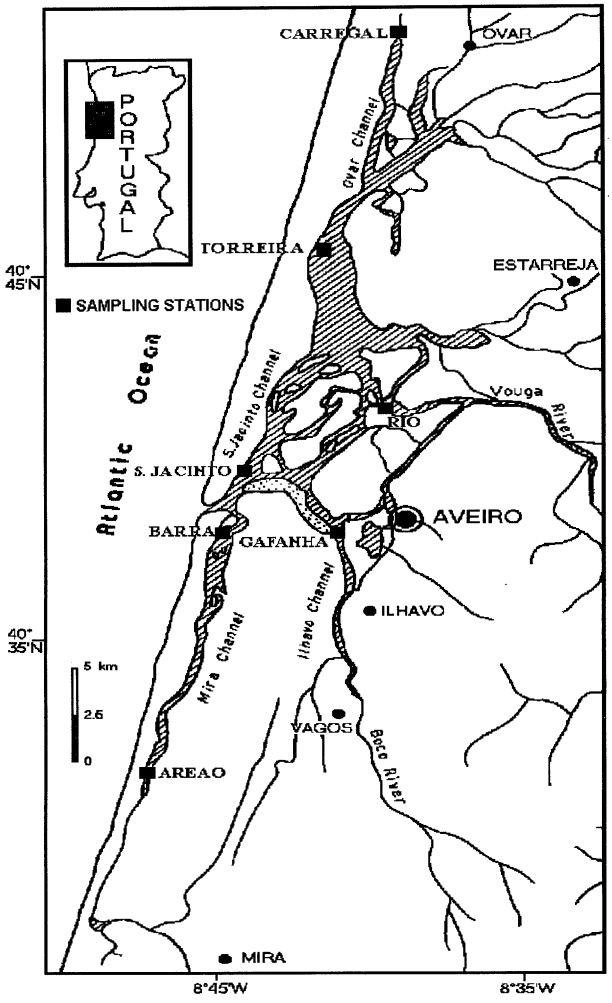

Fig. 1. Map of the Ria de Aveiro including the sampling stations.

were obtained by dissolving the appropriate salts or the corresponding metals. $Mg(NO_3)_2$ and $NH_4H_2PO_4$ (Merck) were used as chemical modifiers for Cu–Ni and for Cd–Pb, respectively. Concentrated nitric acid (65%) was used for the digestion of the samples. Certified reference materials, such as bovine liver (No. 1577b) from the National Institute of Standard Technology (NIST), and hay (V-10) from the International Atomic Energy Agency (IAEA), were used to validate the method. High purity Argon was used as inert gas.

2.4. Sample pretreatment and microwave digestion

The muscle of the wet fish samples was homogenized using a porcelain mortar and after this it was dissolved by microwave digestion using the following simplified procedure, proposed in this work: approximately 800 mg of wet sample, 4 ml of nitric acid (65%) and 1 ml of ultrapure water were placed into the PTFE vessel of the Parr reactor. The vessel was closed and heated for 120 s at 325 W in the microwave oven. The reactor was then cooled by means of an ice bath before it was opened. Then, the resultant solution was quantitatively transferred into a 10 ml volumetric flask and made up to volume with ultrapure water. The solution was decanted and stored at 4°C in stoppered polyethylene bottles until it was analyzed.

Table 1
Instrumental parameters and optimized graphite furnace conditions for the determination of Cd, Cu, Ni and Pb in fish samples (injection volume 20 μl)

	Cd	Cu	Ni	Pb
Instrumental conditions				
Wavelength (nm)	228.8	324.8	232.0	283.3
Bandpass (nm)	0.5	0.5	0.2	0.5
Lamp current (mA)	3	3	4	6
Furnace programs				
Drying 1				
Temperature	90	90	90	90
Ramp rate (s)	20	20	20	20
Hold time (s)	30	30	30	30
Flow rate (ml/min)	Ar 300	Ar 300	Ar 300	Ar 300
Drying 2				
Temperature	120	300	300	120
Ramp rate (s)	10	10	10	10
Hold time (s)	5	15	20	5
Flow rate (ml/min)	Ar 300	Ar 300	Ar 300	Ar 300
Pyrolysis				
Temperature	800	1000	1200	900
Ramp rate (s)	15	15	10	15
Hold time (s)	10	15	15	10
Flow rate (ml/min)	Ar 300	Ar 300	Ar 300	Ar 300
Atomization				
Temperature	2100	2000	2500	1500
Ramp rate (s)	1	1	1	1
Hold time (s)	2	2	2	2
Flow rate (ml/ min)	0 (read)	0 (read)	0 (read)	0 (read)
Cleaning				
Temperature	2200	2700	2600	2500
Ramp rate (s)	1	1	1	1
Hold time (s)	3	3	3	3
Flow rate (ml/ min)	Ar 300	Ar 300	Ar 300	Ar 300

3. Results and discussion

3.1. Pyrolysis–atomization curves

Fig. 2 shows the pyrolysis and atomization curves obtained for Cd, Cu, Ni and Pb in a dissolved fish sample. The influence of chemical modifiers (5 μl) was also evaluated for all of them. According to the literature recommendations, 0.1% (w/v) and 0.5% (w/v) $NH_4H_2PO_4$ solutions were used as chemical modifiers for Cd (Campillo, Viñas, Löpez García & Hernández Cordoba, 1999) and Pb (Parsons & Slavin, 1999; Spevácková & Smíd 1999), respectively. Similarly, $Mg(NO_3)_2$ at 1.6 and 10 mg/ml was used for Cu (Capelo, Filgueiras, Lavilla, & Bendicho, 1999) and Ni (González et al., 1999), respectively.

Fig. 2a and b show that the influence of chemical modifier was obvious, on the pyrolysis and atomization temperatures of Cd and Pb. In the case of Cd (Fig. 2a), although the absorbance signal hardly changed when the chemical modifier was used, the pyrolysis and atomization temperatures were increased by around 300 and 800°C, respectively. This effect was also noted for this element in other studies (Blust, Van der Linden, Verheyen, & Decleir, 1988; Silva, Goreti, Vale, & Caramão, 1999) using different modifiers. Thus, for Cd determinations, the addition of chemical modifier to the sample was needed and the most favourable pyrolysis and atomization temperatures were 800 and 2100°C, respectively.

Pb shows a different behaviour (Fig. 2b), given that the absorbance signal was considerably increased when the $NH_4H_2PO_4$ was introduced as a matrix modifier. Moreover, the pyrolysis and atomization temperatures were also both improved. The influence of the chemical modifier for Pb determination was also observed in other biological samples (Correia et al., 2000; Spevácková et al., 1999); however, in the study of slurries, some authors proved that the slurry particle itself acts as a modifier, stabilizing the analyte during the pyrolysis (Silva et al., 1999). Based on these considerations the pyrolysis and atomization temperatures were fixed at 900 and 1500°C, when the chemical modifier was employed. Similar temperatures were also selected for lead determinations in urine and blood samples using $NH_4H_2PO_4$ as chemical modifier (Campillo et al., 1999).

In the case of Ni, the pyrolysis and atomization temperatures were tested in the presence and absence of $Mg(NO_3)_2$ as chemical modifier, in the ranges of 400–1400°C, and 1700–2700°C, respectively. By taking into account the results of Fig. 2c it was possible to ensure that the presence of the chemical modifier was favourable in the pyrolysis temperature, because it could be increased by around 100°C and, moreover, the absorbance signal was also improved. In contrast, the atomization temperature and the absorbance signal varied little when the modifier was added. The best graphite furnace conditions for this element were achieved using temperatures of 1200 and 2500°C for pyrolysis and atomization, respectively. These results were not in agreement with other studies (González et al., 1999) where this element was determined in wheat four samples without the addition of chemical modifier.

In relation to Cu, the addition of the $Mg(NO_3)_2$ to the sample did not allow higher pyrolysis and atomization temperatures and even slightly reduced the Cu atomic absorption signal (Fig. 2d). Thus, sample modification did not provide practical advantages for the determination of copper in fish samples as has also been proved by other authors for biological samples (Blust et al., 1988; Silva et al., 1999). The optimized temperatures for this element were 1000 and 2000°C for pyrolysis and atomization, respectively.

Other parameters of the graphite furnace temperature, such as ramp and hold times for the pyrolysis, atomization stages, drying and cleaning steps, were also investigated and the optimum values are summarized in Table 1.

3.2. Matrix effect

The effect of chemical interferences, caused by different components of the fish sample on the determination of Cd, Cu, Ni, Pb and Zn, was evaluated by comparing the slopes of the aqueous calibration and the sample standard addition graphs. The results of this study are shown in Table 2. According to these results the slopes of the aqueous calibration and standard addition graphs appear to be very different for Cd, Cu, Ni and Pb, where the matrix composition provides depressive effects with negative changes of the slope between 7.59 and 41.44%. Moreover, significant differences ($P = 95\%$) were found between the Cd, Cu, Ni and Pb slopes when they are statistically compared (Miller & Miller, 1993). Therefore, it was only possible to work with aqueous calibration for Zn and the other elements had to be measured using the standard addition graphs. This calibration method was also employed by other authors for determination of Cd and Pb in other biological samples (Campillo et al., 1999). In contrast, no matrix interferences were found for Cd and Pb in infant cereal

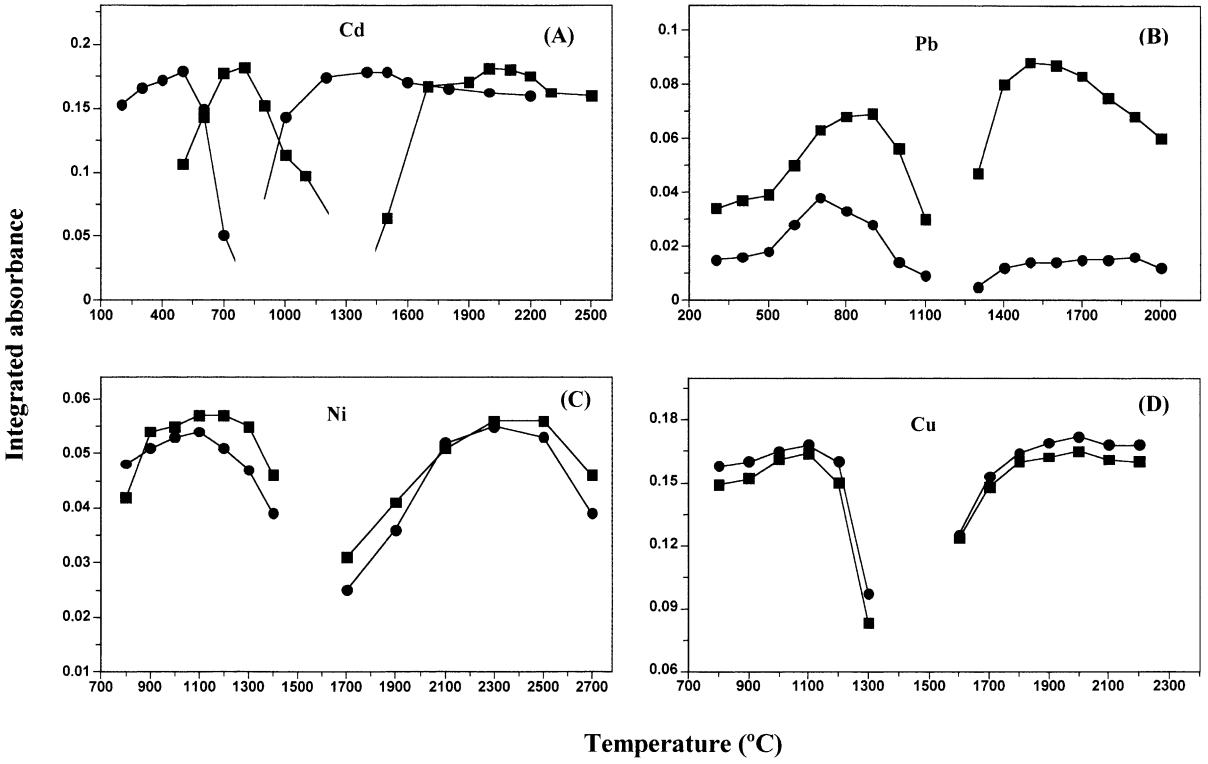

Fig. 2. Pyrolysis and atomization curves for Cd, Pb, Ni and Cu in a dissolved fish sample. With chemical modifier (■), without chemical modifier (●).

samples (Roca et al., 1999) or for Cu and Ni in algae matrix (Meeravali & Kumar, 2000).

3.3. Validation of the analytical method

The accuracy of the analytical methodology employed was evaluated through the analysis of two biological reference materials (NIST-1577b and IAEA-V10) with certified contents of all the metals studied, except IAEA-V10 which did not have quantifiable levels of Ni. The experimental results obtained for both samples, expressed as mean value (mg/kg) ± standard deviation of three determinations, are shown in Table 3. As can be seen, good agreement with the certified contents was obtained for all them, reaching percentages of recovery ranging from 96.89 to 105%. In fact, no significant differences were obtained when the experimental and certified results were statistically compared at confidence level of 95%.

The values of the limits of detection (LOD) and quantification (LQD) for all the elements studied are given in Table 4. The LOD and LQD were defined as 3 and 10 σ/m, respectively, where σ is the standard deviation of 10 measurements of a blank and m is the slope of the corresponding calibration graph (aqueous calibration or standard addition).

The sensitivity, i.e. the characteristic mass (m_o) defined as the mass of analyte corresponding to 0.0044 absorbance units, was also calculated for those elements measured by ETAAS and the values obtained are included in Table 4.

Table 2
Comparison of slopes corresponding to aqueous calibration (AC) and standard addition (SA) graphs for metal determination in fish samples[a]

	Aqueous calibration slope (l/μg)	Standard addition slope (l/μg)	% Change in slope SA–AC[b]
Cd	0.1204 (0.9997)	0.0705 (0.9993)	−41.44
Cu	4.934×10^{-3} (0.9991)	3.996×10^{-3} (0.9975)	−19.01
Ni	1.442×10^{-3} (0.9999)	1.073×10^{-3} (0.9997)	−25.61
Pb	3.613×10^{-3} (0.9996)	3.3387×10^{-3} (0.9998)	−7.59
Zn[c]	0.2299 (0.9998)	0.2274 (0.9989)	−1.08

[a] The correlation coefficient (r) is given in brackets.
[b] The slope change was calculated using the following ratio: [(slope of standard addition (SA) − slope of aqueous calibration (AC))/slope of aqueous calibration (AC)]×100.
[c] These values are expressed as ml/μg.

Table 3
Analytical results obtained in two biological certified reference materials

	Certified values[a] (mg/kg)	Found values[b] (mg/kg)	Recovery (%)
NIST-1577b			
Cd	0.03 (0.02–0.05)	0.031 ± 0.0045	103.3
Cu	9.40 (8.80–9.70)	9.46 ± 0.48	100.6
Ni	4.20 (3.80–4.90)	4.42 ± 0.76	105.2
Pb	1.60 (0.8–1.90)	1.68 ± 0.20	105.0
Zn	24 (23–25)	24.48 ± 3.15	102.0
IAEA-V10			
Cd	0.50 ± 0.03	0.49 ± 0.02	98.4
Cu	160.0 ± 8.0	155.3 ± 0.03	97.1
Ni	–	–	–
Pb	0.129 ± 0.004	0.126 ± 0.002	97.67
Zn	127.0 ± 16	123.03 ± 5.43	96.87

[a] Certified values and their confidence intervals.
[b] Mean values of three determinations ± standard deviation ($n = 3$).

Table 4
Limits of detection (LOD), limits of quantification (LOQ) and characteristic mass (m_o) obtained for all metals studied in fish samples

	LOD (μg/l)	LOQ (μg/l)	m_o (pg)
Cd	0.0788	0.260	0.624
Cu	0.344	1.147	11.01
Ni	1.825	6.083	41.00
Pb	0.968	3.250	13.17
Zn	0.0127[a]	0.0423[a]	–

[a] These values are expressed as μg/ml.

3.4. Analysis of fish samples

The *Anguilla anguilla*, *Mullus surmuletus*, *Trigla lucerna, Mugil cephalus*, *Chelon labrosus*, *Liza aurata* and *Dicentrarchus labrax* species caught in the sampling stations of the Ria de Aveiro (Fig. 1) were analyzed following the above methodology. The results obtained for all the studied elements, expressed as mean value (μg/g wet wt.)±standard deviation of three determinations, are shown in Table 5. The profiles of metal concentrations for all the species studied are shown in Fig. 3.

According to the results (Table 5) the metal contents in the samples studied depends on the analyzed species, the sampling station and the period of sampling. In general, the lower concentrations correspond to Cd, Pb and Ni with values smaller than 0.043, 0.15 and 0.14 μg/g, respectively, except in the case of Ni in the *Anguilla anguilla* samples, where the values obtained ranged from 0.169 to 0.394 μg/g. The contents of Cu in the samples oscillated between 0.516 and 1.04 μg/g; the lowest values of this element (about 0.5 μg/g) correspond to the *Mugil cephalus* and the *Trigla lucerna* samples proceeding from Barra sampling station in October of 1999 and February of 2000; the rest of the samples present values of Cu between 0.637 and 1.04 μg/g. As expected, the most abundant element in all the species studied is Zn; the greatest concentration of this element was found in *Anguilla anguilla*, with values close to 20 μg/g in most of the analyzed samples; the lowest Zn concentrations correspond to *Mullus surmuletus*, *Trigla lucerna* and *Mugil cephalus*, with values ranged from 4.71 to 7.79 μg/g, in all samples. The rest of the analyzed fish species (*Chelon labrosus*, *Liza aurata* and *Dicentrarchus labrax*) present a similar content of Zn, close to 11 μg/g for all of them.

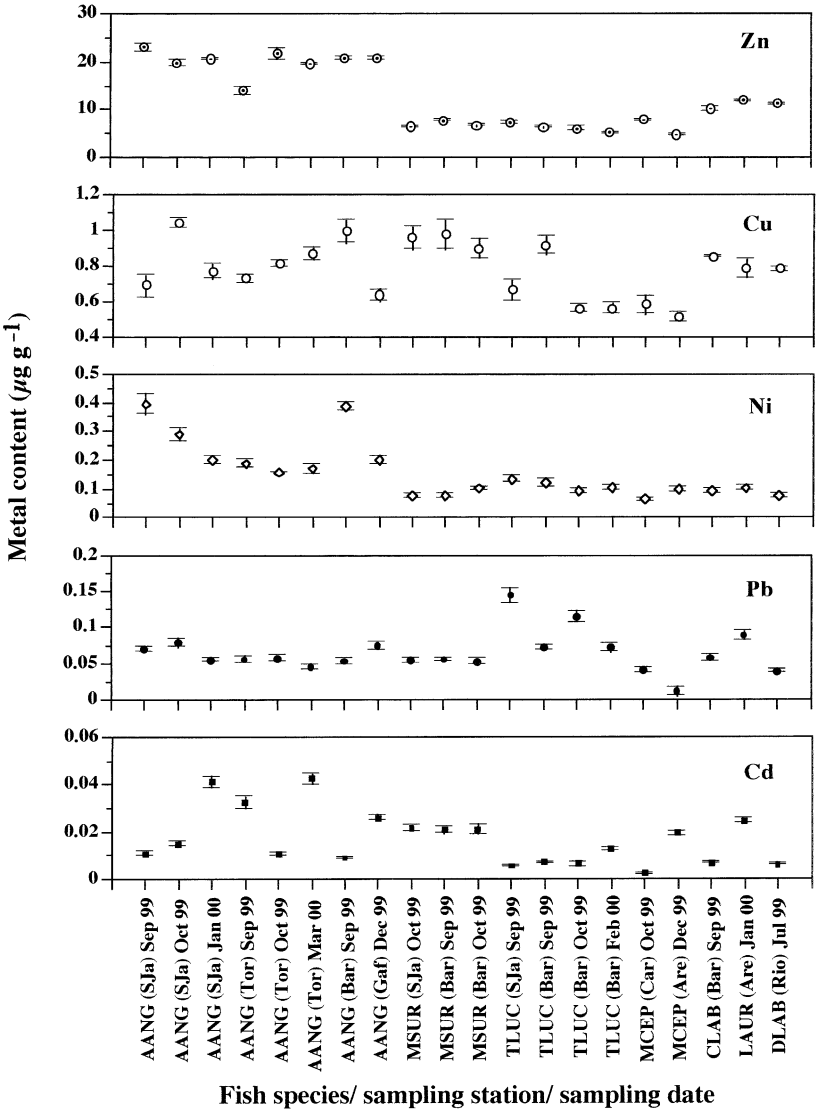

Fig. 3. Profiles of metal concentrations in the fish samples. Error bars indicate the standard deviation.

Table 5
Metal concentrations in muscle tissues of fish species from the Ria de Aveiro[a]

Species/sampling station	Sampling date	Cd[b]	Cu[b]	Ni[b]	Pb[b]	Zn[b]
Anguilla anguilla (AANG)						
São Jacinto (SJa)	September 1999	$0.0109\pm1.03\times10^{-3}$	0.693 ± 0.066	0.394 ± 0.035	$0.0699\pm2.87\times10^{-3}$	23.1 ± 0.83
São Jacinto (SJa)	October 1999	$0.0150\pm1.16\times10^{-3}$	1.04 ± 0.026	0.288 ± 0.022	$0.0783\pm6.24\times10^{-3}$	19.7 ± 0.70
São Jacinto (SJa)	January 2000	$0.0411\pm2.31\times10^{-3}$	0.771 ± 0.041	0.200 ± 0.015	$0.0541\pm2.72\times10^{-3}$	20.7 ± 0.28
Torreira (Tor)	September 1999	$0.0324\pm2.65\times10^{-3}$	0.728 ± 0.021	0.187 ± 0.013	$0.0551\pm4.65\times10^{-3}$	14.0 ± 0.89
Torreira (Tor)	October 1999	$0.0109\pm7.02\times10^{-4}$	0.812 ± 0.021	$0.157\pm1.00\times10^{-3}$	$0.0564\pm4.40\times10^{-3}$	21.7 ± 1.15
Torreira (Tor)	March 2000	$0.0423\pm2.20\times10^{-3}$	0.868 ± 0.032	0.169 ± 0.015	$0.0440\pm2.94\times10^{-3}$	19.6 ± 0.14
Barra (Bar)	September 1999	$8.98\times10^{-3}\pm4.90\times10^{-4}$	0.991 ± 0.064	0.387 ± 0.016	$0.0526\pm4.21\times10^{-3}$	20.8 ± 0.21
Gafanha (Gaf)	December 1999	$0.0258\pm1.17\times10^{-3}$	0.637 ± 0.035	0.200 ± 0.016	$0.0744\pm5.14\times10^{-3}$	20.7 ± 0.29
Mullus surmuletus (MSUR)						
São Jacinto (SJa)	October 1999	$0.0215\pm1.27\times10^{-3}$	0.955 ± 0.062	$0.0751\pm8.00\times10^{-3}$	$0.0536\pm3.25\times10^{-3}$	6.42 ± 0.19
Barra (Bar)	September 1999	$0.0206\pm1.35\times10^{-3}$	0.974 ± 0.081	$0.0754\pm6.34\times10^{-3}$	$0.0543\pm2.57\times10^{-3}$	7.64 ± 0.15
Barra (Bar)	October 1999	$0.0211\pm1.98\times10^{-3}$	0.897 ± 0.053	$0.102\pm4.62\times10^{-3}$	$0.0519\pm4.07\times10^{-3}$	6.67 ± 0.11
Trigla lucerna ((TLUC)						
São Jacinto (SJa)	September 1999	$5.59\times10^{-3}\pm2.38\times10^{-4}$	0.665 ± 0.056	0.133 ± 0.011	0.145 ± 0.010	7.21 ± 0.25
Barra (Bar)	September 1999	$7.08\times10^{-3}\pm3.20\times10^{-4}$	0.917 ± 0.050	0.122 ± 0.013	$0.0715\pm2.72\times10^{-3}$	6.35 ± 0.21
Barra (Bar)	October 1999	$6.31\times10^{-3}\pm9.75\times10^{-4}$	0.564 ± 0.022	$0.0918\pm7.98\times10^{-3}$	$0.115\pm7.81\times10^{-3}$	5.94 ± 0.49
Barra (Bar)	February 2000	$0.0127\pm4.51\times10^{-4}$	0.564 ± 0.028	$0.103\pm8.83\times10^{-3}$	$0.0711\pm6.15\times10^{-3}$	5.09 ± 0.14
Mugil cephalus (MCEP)						
Carregal (Car)	October 1999	$2.27\times10^{-3}\pm4.39\times10^{-4}$	0.583 ± 0.054	$0.0635\pm6.21\times10^{-3}$	$0.0404\pm3.65\times10^{-3}$	7.79 ± 0.16
Areão (Are)	December 1999	$0.0192\pm8.50\times10^{-4}$	0.516 ± 0.026	$0.0976\pm9.04\times10^{-3}$	$0.0103\pm6.25\times10^{-3}$	4.71 ± 0.08
Chelon labrosus (CLAB)						
Barra (Bar)	September 1999	$6.81\times10^{-3}\pm4.90\times10^{-4}$	$0.852\pm4.62\times10^{-3}$	$0.0918\pm6.68\times10^{-3}$	$0.0571\pm4.10\times10^{-3}$	10.14 ± 0.67
Liza aurata (LAUR)						
Areão (Are)	January 2000	$0.0245\pm8.88\times10^{-4}$	0.787 ± 0.051	$0.102\pm8.79\times10^{-3}$	$0.0885\pm6.21\times10^{-3}$	11.89 ± 0.28
Dicentrarchus labrax (DLAB)						
Rio (Rio)	July 1999	$6.19\times10^{-3}\pm2.59\times10^{-4}$	0.785 ± 0.013	$0.0761\pm7.16\times10^{-3}$	$0.0380\pm2.25\times10^{-3}$	11.24 ± 0.19

[a] Abbreviated names are given in brackets.
[b] All results are given as mean value (μg/g wet weight)±standard deviation of three determinations.

Fig. 3 shows that it is possible to conclude that the concentrations of Ni and Zn in the *Anguilla anguilla* samples were higher than those found in the other analyzed fish species. In contrast, in the case of Pb, the lowest concentration values correspond to *Anguilla anguilla*, *Mullus surmuletus* and *Mugil cephalus*. Due to the wide variability of the Cd and Cu results, no clear tendency was observed for these elements in the species studied.

The results obtained from the analyzed fish samples in this work were in good agreement with some values found in muscle tissues of other fish species from Mauritanian (Roméo et al., 1999) and Spanish (Blasco et al., 1999) waters. In all cases, the values obtained in this study were much below the maximum limits recommended by international organizations (Food Chemical Codex, 1996; Nauen, 1983) and specific regulations (BOE, 1991) for these aquatic organisms, and consequently, in what concerns possible effects of the studied metals, the consumption of these fish by humans should be perfectly safe.

4. Conclusions

The results obtained in this work allow us to conclude that the employed microwave digestion method could be considered as a fast procedure for dissolving biological materials, since only 2 min are required for a complete dissolution of the sample. The accuracy of the method was evaluated when two biological reference materials were analyzed and the experimental results were not significantly different from the certified values, for all metals.

On the other hand, the results of this study supply valuable information about the metal contents in different fish species from different sampling stations of the Ria de Aveiro. This could be considered as a bioindicator of the environmental contamination in this zone by estimating the bioavailability of metals to the marine biota. Moreover, these results can also be used to test the chemical quality of the marine food, in order to evaluate the possible risk associated with their consumption by humans. In relation to this, the low metal

contents found in all the studied fish samples are insufficient to cause toxicological effects on human health when these fish are included in the diet.

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