Research Article

METHOD VALIDATION AND DETERMINATION OF SEVERAL ELEMENTS IN FARMED FISH BY INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (ICP-MS)

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ABSTRACT

In recent years, the fishery sector in Vietnam has remarkably developed. It not only serves the domestic market but also becomes a key export. In 2003, Vietnam was the country with the largest sea products exported to the USA, Japan, China, and Korea. The quality of fish is influenced by various criteria, mainly related to their feeding and living habitat. Among many chemical compositions, the elements could accumulate in many organs and tissues depending on the way of exposure such as through diet or their elevated levels in the environment. Moreover, the accumulation of many metals in the fish body is different due to various affinities of metals to fish tissues, different uptake, deposition, and excretion rates. In this study, microwave-assisted acid digestion and inductively coupled plasma mass spectrometry (ICP-MS) were investigated and validated for the determination of Cr, Mn, Ni, Co, Fe, Cu, Zn, As, Cd, and Pb in fish tissue samples based on AOAC. 2015.01 (2015). The method performed proper linear ranges ($R^2 > 0.995$), favorable repeatability, and reproducibility according to Appendix F. AOAC (2016). The recoveries were from 85.8 to 109.4% for all elements. This method could be applied for routine analysis to determine and control the elements in fish tissue samples. The contents of quantified elements in six fish samples purchased in Ho Chi Minh City were below the permitted level according to 46/2007/QD-BYT (2007). Therefore, the amount of these elements in the fish tissue are safe for human consumption.

Keywords: elements; fish tissue; ICP-MS; microwave

1. Introduction

Today, fisheries and aquaculture play vital roles in reducing hunger and poverty in many countries. In 2016, Global fish production peaked at 171 million tons with 47 percent of the total aquaculture. The total first-sale value of fisheries and aquaculture in 2016 was

362 billion USD, of which 232 billion USD was from aquaculture production and approximately 35 percent of global fish products entered international trade in various forms for human consumption or non-edible purposes. Global aquaculture production in 2016 included 80.0 million tons of food fish and 30.1 million tons of aquatic plants, and 37 900 tons of non-food products (FAO, 2005). In 2016, Vietnam was one of seven countries (including China, India, Indonesia, Vietnam, Bangladesh, Egypt, and Norway) that are the major producers of farmed food fish. Besides, Vietnam was one of the largest exporters of fish and fish products to the European Union (EU), the United States of America, and Japan markets. Vietnam's aquaculture industry has great potential in continuing its current growth. The fish products had significantly increased from 2006 to 2015 (Sidhu, 2003). However, there are many challenges for the sustainable growth of this sector, such as disease management, cumulative impact on the environment, and run out of technology and effective collaboration within the industry. Therefore, it is vital to improve the productivity of fisheries and aquaculture to enhance food and nutrition security, increase income and develop livelihoods, promote economic growth, and protect the environmental resources.

Elements are bioaccumulated at varying levels based on species, different aquatic environments, feeding habits, age, size, and length of the fish and habitats (Kalay, Ay, & Canli, 1999). Larger fish tend to accumulate higher heavy metal contents. It might be explained that their bodies are large, which increases the accumulation of metals through surface action. Besides, the accumulation of elements in fish also relates to the aquatic environment (pH and temperature of the environment or the element concentrations in the surrounding water) (Dhanakumar, Solaraj, & Mohanraj, 2015; Malik & Maurya, 2014; Moiseenko & Kudryavtseva, 2001). For instance, fish living near the smelters have a higher concentration of Cd and Ni than fish living far away with smelters and in water that is not polluted by these metals. The different parts of fish have different bioaccumulation of heavy metals (Batvari et al., 2008). For example, the gill and liver have a higher accumulation of heavy metals compared to fish muscle (Amundsen et al., 1997; Kalay et al., 1999). Elements could be divided into categories, which are essential metals and non-essential metals. Some elements (Co, Cr, Cu, Fe, Mn, Mo, Ni, Se, Zn) are essential and play vital roles in many oxidation-reduction reactions and are constituent of several key enzymes (FAO 764, 1983). Other elements, such as Hg, Pb, Cd, are non-essential elements and potentially toxic only at low concentrations (Nordberg, Nordberg, Fowler, & Friberg, 1986). They are toxic metals causing contact dermatitis, lung fibrosis, cardiovascular, and kidney diseases, as well as nasal cancer (Renieri et al., 2014; Renieri et al., 2019). The effects of these metals in the fish body include decreasing growth and reproductive capacity, swimming imbalance, and inability to capture the prey (Patrick & Loutit, 1978). Fish can accumulate non-essential trace elements through food chain transfer. Hence, the consumption of fish-contaminated

toxic elements can potentially increase the risk of cancer, immune and cognitive dysfunction, and reproductive systems, cardiovascular system (Järup, 2003).

For the measurement of elements, there have been many techniques available, including flame atomic absorption spectrometry (FAAS), graphite furnace atomic absorption spectrometry (GFAAS), inductively coupled plasma optical emission spectrometry (ICP-OES), and inductively coupled plasma mass spectrometry (ICP-MS). Depending on the properties of analyzed elements, a suitable method would be selected. The ICP-MS was developed in the 1970s, and commercial instrumentation was available in the 1980s. It is a coupling of an ICP with mass spectrometric detection (Mester & Sturgeon, 2003). The ICP-MS is the state-of-the-art technique for the trace element analysis available which has many advantages such as the speed of analysis, low detection limits, simultaneous analysis, improved sensitivity, and the ability to do rapid isotopic analysis. Since its commercialization in 1983, approximately 11000 systems have been installed worldwide for many varied and diverse applications including environmental, geological, semiconductor, biomedical, and nuclear application fields (Thomas, 2013). Before the instrumental measurement, the samples need to be digested to obtain the liquids. There has been a variety of digestion techniques for sample preparation procedures such as dry ashing digestion, wet digestion, microwave-assisted digestion, in which microwave-assisted acid digestion was used in this study.

It is important to monitor the amounts of elements in commercial fish to ensure the consumers' health. In Vietnam, the publications on the determination and assessment of elements in the organisms, especially for fish have been limited. This study aimed to (i) optimize the operating parameters on the ICP-MS; (ii) validate the analytical method based on the microwave-assisted acid digestion and ICP-MS for measuring several elements of Cr, Fe, Zn, Mn, Cu, As, Co, Ni, Cd, and Pb; and (iii) determine and assess the variations of these elements in commercial fish samples collected in the markets around Ho Chi Minh City.

2. Materials and methods

2.1. Chemicals and reagents

The deionized water or DI water (Milli-Q, Merck, Germany) was used throughout this experiment. The reagents such as nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) were of analytical reagent grade (Merck, Germany). All glassware and equipment were soaked with 10% (v/v) HNO_3 at least overnight and then rinsed with deionized water before use. Intermediate and working standard solutions (0.10, 1.0, 5.0, 10, 50, 100, 200, 500, 1000, 2000, 5000, and 8000 $\mu g\ L^{-1}$) were prepared by dilutions of a 10 mg L^{-1} multi-elemental stock solution (TraceCERT Sigma-Aldrich, USA) by 2% v/v nitric acid before use.

2.2. *Sample collection, pre-treatment, and storage*

A total number of six samples from four fish types (Figure 1) was purchased at four different markets around Ho Chi Minh City. Then, they were transported to the laboratory in an icebox and washed thoroughly by tap water and DI water. The morphometric data such as total length and body weight measurement were recorded (Table 1).

Table 1. Information of fish samples

No.	Fish type	Sample code	Purchase location (market)	Size (depth x length) (cm)	Weight (g)
1		BS-TD	Tan Dinh	10 x 41.5	900
2	Basa	BS-HB	Hoa Binh	10.5 x 41	900
3		BS-NT	Nhat Tao	9.5 x 36.5	735
4	Red tailed tinfoil	RTT	Tan Dinh	10 x 17.5	245
5	Red Tilapia	RT	Ba Chieu	11 x 40.5	850
6	Snakehead	SH	Tan Dinh	9.5 x 49.5	900

Figure 1. Fish samples

Basa

Red tailed tinfoil

Snakehead

Red Tilapia
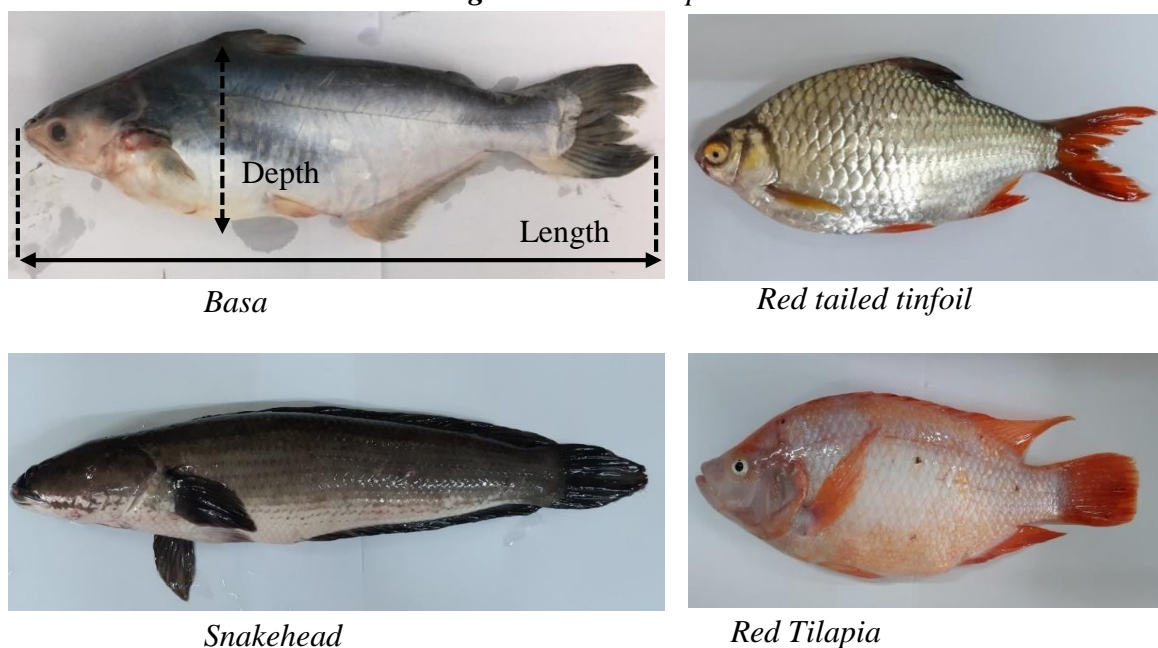
The fish samples were treated in line with the pretreatment procedure as performed in Vietnamese National Standard (TCVN) 11489:2016 (2016). The fish were skinned and shaved to get their tissue part by a plastic knife. The application of a plastic knife was to prevent the potential contamination of elements. The fish tissues were then dried at 60 $^{\circ}$C until their constant mass. Thereafter, these dried fish samples were ground using ceramic pestle and mortar. Finally, the samples were kept in plastic polyethylene bags with zippers and stored in a desiccator at 25 $^{\circ}$C and 70% humidity before further analysis.

2.3. Sample preparation procedure for the determination of elements in fish

The sample preparation for determining the elements in fish was carried out based on AOAC 2015.01 (2015). Briefly, a quantity of 0.2($\pm$0.001) g of dried samples were weighed on an analytical balance and transferred to PTFE vessels. Then, 4($\pm$0.1) mL of 65% (v/v) HNO_3 and 1($\pm$0.1) mL of 30% (v/v) H_2O_2 as the digestion reagents were added to the samples. After that, these vessels were let stand for approximately 30 minutes for the occurrence of preliminary chemical reactions and transferred to the microwave system (Speedwave Xpert, Germany). The microwave pressure and temperature program were shown in Table 2.

Table 2. *Microwave temperature and pressure program for the digestion of the fish sample*

Temperature (^{o}C)	P (bar)	Ramp (min)	Hold (min)	Power (%)
190	35	20	10	90
50	35	20	10	0

The sample solutions were then allowed to cool down to ambient temperature and transferred to 25 mL volumetric flasks. Then, 500 μL of 100 ng L^{-1} internal standard solution was spiked to all samples. The sample solutions were made up to the calibration mark by DI water. The solutions were filtered through 0.22 μm PTFE membranes before being measured with the ICP-MS (iCAP RQ ICP-MS, ThermoFisher Scientific, USA).

2.4. Method evaluation for the determination of elements in fish

2.4.1. Optimization of ICP-MS operation

The ICP-MS instrument was tuned in both STD and KED modes using the iCAP Q/RQ TUNE solution containing seven elements of Ba, Bi, Ce, Co, In, Li, and U. The gas mixture of helium and hydrogen (5 %) was used as collision gas in KED mode. The function of the mass calibration was performed once when setting up the ICP-MS instrument with the aid of iCAP Q/Qnova CALIBRATION solution containing 25 elements.

After tuning in two modes, KED mode was used for all experiments and investigations to minimize polyatomic interferences caused by unknown components and species present in real samples. Qtera and Instrument Control software programs were used to control and monitor the ICP-MS operation.

2.4.2. Estimation of instrument limit of detection (IDL) and quantification (IQL)

The instrument limit of detection (IDL) for each element was estimated by preparing 11 separate 2 % (v/v) nitric acid as the blank solutions. Then, the intensities of each element were recorded, and their concentrations were estimated. The instrument limit of detection (IDL) and quantification (IQL) values was calculated based on their average concentration value ($\overline{X}$) and standard deviation (SD): IDL = $\overline{X}$ + 3SD; IQL = $\overline{X}$ + 10SD (Ellison, Barwick, & Farrant, 2009; Konieczka & Namiesnik, 2016).

2.4.3. Establishment of calibration curves

A range of standard solutions was built from 0.10 to 8000 µg L^{-1} (prepared in 2% v/v nitric acid). The quality control solution of 200 µg L^{-1} was measured immediately after measuring standard solutions and a batch of samples (not exceed 10 samples during a batch analysis and end of the measurement process).

2.4.4. Repeatability and reproducibility of the sample preparation procedure for fish

The repeatability was evaluated based on the relative standard deviation (% RSD, n = 6) and analysis of variance (one way-ANOVA) to check reproducibility within three separate days at the statistical significance level P of 95% (Ellison et al., 2009; Konieczka & Namiesnik, 2016). Repeatability was assessed based on % RSD values for each range of concentration according to Appendix F. AOAC (2016).

2.4.5. Recovery of sample preparation by microwave-assisted acid digestion

The recovery tests were carried out by adding known amounts of standard solution into the sample before transferring the vessels to the microwave system (standard addition method). Because the concentrations of quantified elements were in the different concentration ranges, these elements were divided into two groups (Table 3).

***Table 3.** The spiked standard concentrations*

Group	Elements	Concentration of spiked standard (µg L^{-1})
1	Cr, Fe, Zn	50.0
2	Mn, Cu, As, Co, Pb, Cd	5.00

Each element group was conducted in three replicates (n = 3). The equations to calculate the recovery were as followed: $R\% = \frac{C_{s+c} - C_s}{C_c} \times 100$, whereas C_{s+c}, C_s, and C_c were the concentrations of the spiked sample, non-spiked sample, and theoretical spiked values (µg L^{-1}), respectively.

2.5. Data analysis

All of the analyses were run in triplicate (n = 3) to assure repeatability among the runs. The values of average, standard deviation (SD), and relative standard deviation (RSD) and charts were processed by Microsoft Office Excel 2016 Software.

3. Results and discussion

3.1. Operating parameter optimization for ICP-MS

The parameters obtained after the tuning process (Table 4) were applied to make sure that the instrument operated at its optimal conditions. In the tuning process, gas output flows, torch position, ion lens, and RF power coil were adjusted to maintain the short-term stability of ICP-MS, leading to improve the intensities of interested ions and minimize the effects of polyatomic or double charged interferences. After the calibration mass process, the peak widths of three elements (^{59}Co, ^{115}In, ^{209}Bi) were in the allowed range of 0.650 to 0.850 u.

Table 4. Operating conditions, sensitivity, and mass calibration

Main operating conditions	
▪ Nebulizer	Concentric type
▪ Peristaltic pump speed	40 rpm
▪ Spray chamber	Quartz cyclonic spray chamber with UV shielding cap and Peltier cooling
▪ Sampling cone	Nickel, 1 mm orifice
▪ Skimmer cone	Nickel, 0.75 mm orifice
▪ Plasma power	1200.0 W
Gas output and vacuum	
▪ Plasma gas flow	15 L min^{-1}
▪ Nebulizer gas flow	0.95 L min^{-1}
▪ Auxiliary gas flow	0.80 L min^{-1}
▪ Interface pressure	1.0 mbar
▪ Analyzer pressure	1.55 x 10^{-7} torr
Acquisition parameters	
▪ Mass range	0-300 amu
▪ Dwell time	0.01 s
▪ Number of sweeps	10
▪ Wash time between samples	60 s
▪ Total acquisition time	90 s
Sensitivity	
▪ ^{59}Co	2 134.0 cps
▪ ^{115}In	4 098.0 cps
▪ ^{209}Bi	33 100.0 cps
▪ ^{238}U	61 711.0 cps
▪ ^{140}Ce.^{16}O/^{140}Ce	0.0061
Mass calibration	
▪ ^{59}Co	0.719 u
▪ ^{115}In	0.764 u
▪ ^{209}Bi	0.735 u

The flow of argon nebulizer gas should be thoroughly concerned because it is directly related to the effectiveness of aerosol generation, and then affected the sensitivity of the ICP-MS. If this gas flow rate was too low, the amount of aerosol generated would decrease, and the measured signals would decline. However, polyatomic interferences would appear if the flow of nebulizer gas was too high. The sample had to travel in the hot plasma long enough to vaporize the sample aerosol and convert the analyte elements into ions. Therefore, at a too high level of nebulizer gas, there was insufficient time to convert all of the analytes into elemental ions, the solvent was not evaporated completely. As a result, more oxygen species would enter the plasma and more oxide species would be formed leading to spectral

interferences. This phenomenon could be monitored through the increase of the CeO^+/Ce^+ signal ratios (Olesik & Jiao, 2017). Therefore, the nebulizer gas flow rate should be optimized prior to use, normally between 0.8 L min^{-1} and 1.2 L min^{-1} to provide the highest $^{115}In^+$ ion sensitivity, also to maintain the CeO^+/Ce^+ signal ratios of 0.03 or less for the tuning solution (Wilschefski & Baxter, 2019).

The RF power also influenced the intensities of analytes due to its role as an energy source of plasma where the elemental ions were induced. The higher and the more stable plasma energy was, the higher ionization efficiency of analytes and the better sensitivity could get. However, if the energy of plasma was too high, some elements might be converted to double-charged ions, which caused a decrease in their sensitivity. Therefore, the power of RF should be from 700 to 1 500 W. Moreover, the plasma was also impacted by the plasma gas because this gas played a role as the fuel to generate the plasma. The plasma gas flow rate was monitored at around 12-17 L min^{-1} to make sure that there was enough material to maintain plasma and protect the quartz torch from melting. If the plasma gas flow was too low, the torch would not be cooled enough, causing the increase of the risk of torch melting. Conversely, the temperature of plasma would be declined if the flow of this gas was too high, leading to the recombination of molecules, typically the oxides of alkali and alkali earth metals. Therefore, the plasma gas speed and the RF power should be optimized using the intensities of ^{115}In and Ce^{++}/Ce^+ as the indicators, in which the acceptance value of Ce^{++}/Ce^+ is usually < 2-3% (Pupyshev & Semenova, 2001).

The intensities of ^{115}In were also investigated in the optimization of the auxiliary gas flow rate. In addition to shaping the plasma flame, this gas also introduced the ion lines into the sampler cone, improving the sensitivity of analytes. The optimal auxiliary gas speed was around 1 L min^{-1}. In the tuning process, the torch position (horizontal, vertical, and sampling depth) was automatically optimized to obtain the highest amounts of ions generated by the plasma that could reach the sampler cone.

Polyatomic interferences-the common interferences in ICP-MS could produce unexpected spectral overlaps, which would have negative impacts on the analytical data (Thomas, 2013). Therefore, to minimize their effects, after tuning in both modes, KED mode was used for all experiments.

3.2. *Method validation for the determination of elements in fish samples*

The analytical method for the determination of elements in fish was validated on the iCAP RQ ICP-MS (ThermoFisher Scientific, USA) after the microwave-assisted acid digestion in the microwave digestion system (Speedwave Xpert, Germany) (Table 5).

Table 5. Limit of detection, limit of quantification,
and regression equation (y = ax + b) of elements

No.	Analyte and m/z *	IDL (µg L^{-1})	IQL (µg L^{-1})	Slope (a)	Intercept (b)	R^2
1	^{52}Cr	0.16	0.24	0.00768 ± 0.00014	-0.0004 ± 0.0070	1.0000
2	^{55}Mn	0.23	0.46	0.0557 ± 0.0058	-0.003 ± 0.059	0.9994
3	^{57}Fe	0.56	19	0.002192 ± 0.000065	0.0003 ± 0.0032	1.0000
4	^{59}Co	0.10	17	0.136 ± 0.020	-0.1 ± 1.0	0.9990
5	^{60}Ni	0.16	0.23	0.0364 ± 0.0016	0.003 ± 0.037	0.9999
6	^{63}Cu	0.41	0.93	0.0959 ± 0.0055	0.02 ± 0.13	0.9999
7	^{66}Zn	10	23	0.0204 ± 0.0042	-0.02 ± 0.43	0.9987
8	^{75}As	0.024	0.050	0.0104 ± 0.0012	-0.01 ± 0.12	0.9994
9	^{111}Cd	0.013	0.028	0.0348 ± 0.0012	-0.005 ± 0.026	1.0000
10	^{208}Pb	14	30	0.449 ± 0.048	0.1 ± 1.1	0.9997

(*) m/z used for quantification

For the analysis of elements, it is vital to choose proper isotopes for quantification purposes (Table 5). The isotopes with larger natural abundance are frequently used. However, in certain matrices, the use of alternative isotopes should be necessary to avoid or minimize spectral interferences such as polyatomic, isobaric elements (EPA 6020B, 2014). For example, the argon ions combine with oxygen in the matrix and solvent components to generate ^{40}Ar^{16}O$^+$, which impacts the detection limits of Fe (Thomas, 2013). Therefore, ^{57}Fe was chosen as the quantification isotope although ^{56}Fe is more popular in nature. Another example, ^{111}Cd was analyzed rather than ^{114}Cd because of the isobaric interference of tin (^{114}Sn) (Sandrine Millour, 2011).

The values of IDL and IQL varied for each element. The IDL values of Zn and Pb were higher than those of others because there might be trace element contaminants or impurities in the acid reagents used for preparing these blanks. The solutions containing only DI and IS were also prepared to check if there was any contamination from the DI source in the analytical laboratory. The concentrations of these elements were estimated in DI as below 0.1 µg L^{-1}. Therefore, it could be concluded that the high-intensity values obtained from blank solutions derived from the impurities of the acid reagent used. Sub-boiling acids should be used in this situation; however, because the concentrations of the quantified elements in fish sample solution were higher than the ranges from 0.1 µg L^{-1}, so IDL values estimated based on the current conditions could be accepted. The regression equations were performed in the format of y = ax + b, whereas y stood for the ratio of the intensity of interesting element and the internal standard, and x stood for a concentration of each element. According to Table 5, R^2 values were higher than 0.995, which revealed evidence of the goodness of linearity according to Appendix F. AOAC (2016).

The repeatability was assessed through the calculation of % RSDs for six replicates. These obtained values were lower than the range from 11% to 15% for the concentration between 100 μg Kg^{-1} and 1 mg Kg^{-1}, from 7.3% to 11% for the concentration range of 1-10 mg Kg^{-1}, and from 7.3% to 5.3% for the content between 10 mg Kg^{-1} and 100 mg Kg^{-1}. These % RSD values were acceptable according to Appendix F. AOAC (2016). The analysis of variance (one way-ANOVA) was performed to check reproducibility within three separate days at the statistical significance level P of 95%. The consequences indicated that $p_{calculated}$ (0.07-0.35) > p_{theory} = 0.05, so there was no significant statistical difference between three separate days (P = 95 %) for all elements of interest (Cr, Fe, Zn, Mn, Cu, As, Co, Ni, Cd, and Pb). Therefore, the reproducibility was acceptable (P = 95 %).

The trueness of the analytical method was evaluated by the recoveries of spiked samples. For all elements of interest (Cr, Fe, Zn, Mn, Cu, As, Co, Ni, Cd, and Pb), the recoveries values were in the range from 85.8 to 109.4 %. These obtained recoveries belonged to the range from 80 to 110 percent for the concentration between 100 μg Kg^{-1} and 10 mg Kg^{-1}. These values were acceptable according to Appendix F. AOAC (2016). Therefore, this sample preparation method could be used to determine the concentrations of elements in fish tissues with reliable results.

3.3. *Variations of elements in fish samples*

The validate analytical method by ICP-MS after the microwave-assisted acid digestion was applied for the determination of various elements (Cr, Fe, Zn, Mn, Cu, As, Co, Ni, Cd, and Pb) in fish samples collected in four markets around Ho Chi Minh City (Table 6).

Table 6. *Element concentrations in fish samples calculated in mg Kg^{-1}*

Sample	Value	Cr	Mn	Fe	Cu	Zn	As	Co	Ni	Cd	Pb
SH	Average	6.46	0.702	10.62	0.489	14.48	0.937	-	-	-	-
	± u	0.24	0.017	0.30	0.028	0.41	0.028	-	-	-	-
	% RSD	4.5	2.9	3.5	7.1	3.5	3.6	-	-	-	-
RTT	Average	10.37	0.507	16.05	0.785	11.31	1.325	-	-	-	-
	± u	0.38	0.039	0.37	0.024	0.28	0.060	-	-	-	-
	% RSD	4.4	9.3	2.8	3.7	3.1	5.5	-	-	-	-
RT	Average	12.34	0.789	18.32	0.659	9.25	-	-	-	-	-
	± u	0.21	0.021	0.51	0.026	0.48	-	-	-	-	-
	% RSD	2.1	3.3	3.4	4.9	6.3	-	-	-	-	-
BS-TD	Average	13.84	0.610	24.59	0.828	24.49	1.010	-	-	-	-
	± u	0.21	0.015	0.54	0.025	0.32	0.043	-	-	-	-
	% RSD	1.9	3.0	2.7	3.7	1.6	5.2	-	-	-	-
BS-HB	Average	5.34	0.6625	10.80	0.858	11.66	0.2080	-	-	-	-
	± u	0.26	0.0062	0.19	0.015	0.13	0.0045	-	-	-	-
	% RSD	5.8	1.1	2.1	2.2	1.4	2.7	-	-	-	-
BS-NT	average	6.17	0.653	11.24	1.156	16.44	1.221	-	-	-	-
	± u	0.37	0.038	0.70	0.052	0.51	0.033	-	-	-	-
	% RSD	7.2	7.1	7.6	5.4	3.8	3.3	-	-	-	-

"-" not detected, "u" uncertainty estimated at P = 95% for three replicates

The % RSD values for three replicates of all the fish samples were within the acceptable range according to Appendix F. AOAC (2016). For all fish samples, the elements of Ni, Co, Cd, and Pb were not detected due to their lower concentrations than LODs. In fish tissues, Ni and Co are considered non-essential elements, but trace amounts might be beneficial to activate several enzyme systems (Bird, Mills, & Schwartz, 1999; Grosell, 2012). Besides, the concentration of Ni in the tissues was low, which could support the information that the highest amount of Ni was detected in the gill instead of the tissue due to the result of metallothioneins that trapped heavy metals (Saleh & Marie, 2015). Moreover, Co was not detected in any of the fish muscles, which was in agreement with the study of (Rakocevic, Sukovic, & Maric, 2018).

A one-way ANOVA was applied to test differences among five fish. The consequences showed $p_{calculated} < p_{theory} = 0.05$. Therefore, there was a statistically significant difference among six fish samples regarding several trace element contents. The observed variations in the element contents were probably due to the different affinity of elements to fish tissue, different uptake (through the gills, skin, and the digestive system), deposition and excretion rates (Canli & Atli, 2003). These processes were usually related to environmental factors (e.g. water temperature, pH). Besides, the difference in average concentrations of elements might be caused by the differences of species, sex, age, size, reproductive cycle, etc (Malik, Biswas, Qureshi, Borana, & Virha, 2010; Dvořák, Andreji, Mráz, & Dvořáková-Líšková, 2015; Monikh, Safahieh, Savari, Ronagh, & Doraghi, 2013; Rakocevic et al., 2018).

There were six per ten investigated elements (Cr, Mn, Cu, Fe, Zn, and As) that could be quantified by the method of this study. Zn and Fe are vital for growth, reproduction, and energy metabolism in all organisms. Besides, they are an important part of several enzymes and essential for the synthesis of hemoglobin. In our study, these elements showed higher concentrations in all fish tissues compared to the concentration of other elements, which was in agreement with common findings (Rakocevic et al., 2018). The contents of Zn and Fe in this study were in a range of 9.25-24.49 mg Kg^{-1} and 10.62-24.59 mg Kg^{-1}, respectively. Cu and Mn were also the essential elements (in the permitted contents) for maintaining cellular function and were the integral components of numerous metal-containing enzymes. The concentrations of both elements found in all fish samples were lower than those of Zn and Fe. This phenomenon could be explained that Mn and Cu mainly accumulated in other organs instead of muscles. The highest amounts of Mn and Cu were observed in skin, gills, and gonads; and liver, respectively (Rajkowska & Protasowicki, 2013). Cr was detected in all of the examined samples, and its concentration was dependent on species. Cr is an essential element and has been implicated in carbohydrate, lipid, and protein metabolism (Dallas & Day, 2004). In muscle, Cr concentrations were varied from 5.34 to 13.84 mg Kg^{-1}. The highest content of Cr was found in Basa fish bought at Tan Dinh Market. As

concentrations were quantified in four per five investigated fish samples. As concentrations ranged from 0.2080 to 1.325 mg Kg^{-1}. These values were below the limit of metal in food for consumption, 2 mg Kg^{-1} (Decision No. 46/2007/QD-BYT, 2007). The content of As in Red Tilapia was not found, which might be it was below the LOD of the used method. Several living organisms become acclimated to high levels of arsenic. The concentration of arsenic in the environment can be a result of mining activities and because of the use as herbicides (Dallas & Day, 2004). A significant fraction of this contaminant was presented in fish tissues in the form of organic compounds like arsenobetaine (AsB) which was low toxicity and not metabolized in humans (Oliveira, Ferreira, Oliveira, Nogueira, & Gonzalez, 2017). However, in fish, other species of As could be presented such as methylated arsenic species (dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA)). These species had a moderated toxicity for human health (Oliveira et al., 2017). Hence, the high concentration of As is an environmental concern because As could be accumulated in living organisms and indirectly enters the human body through food sources.

4. Conclusions

The ICP-MS was set up and optimized to obtain proper and reliable working conditions. The microwave was used in the sample preparation procedure. IDL and IQL were estimated for each element. The working ranges for all metal elements were established (from IQL to 8 000 µg L^{-1}) so that they could properly deal with the concentrations of analytes in real samples. The analytical procedure for the determination of the concentrations of Cr, Mn, Ni, Co, Fe, Cu, Zn, As, Cd, and Pb in fish tissue samples was validated based on AOAC. 2015.01 (2015). The results of the recovery, repeatability, and reproducibility were acceptable according to Appendix F. AOAC (2016). Six per ten investigated elements (Cr, Mn, Fe, Cu, Zn, and As) were quantified using this method while the remaining elements were not because their concentrations were below the LODs. Generally, the contents of the quantified elements in the fish samples were lower than the maximum limit of metal in food listed in Decision No. 46/2007/QD-BYT (2007). Hence, the amount of these elements in the fish tissue are safe for human consumption. Nevertheless, it is important to find the potential accumulation of elements in fish such as water environment, food chain, or the elevated concentrations of elements relating to the age of fish so that farmers can carry out proper improvements for their aquaculture production.

REFERENCES

Amundsen, P. A., Staldvik, F. J., Lukin, A. A., Kashulin, N. A., Popova, O. A., & Reshetnikov, Y. S. (1997). Heavy metal contamination in freshwater fish from the border region between Norway and Russia. *Science of the Total Environment*, *201*(3), 211-224. doi: 10.1016/s0048-9697(97)84058-2

AOAC. 2015. 01. Heavy Metals in Food, Inductively Coupled Plasma–Mass Spectrometry (2015).

Appendix F. AOAC. Guidelines for Standard Method Performance Requirements (2016).

Batvari, B. P. D., Kamala-Kannan, S., Shanthi, K., Krishnamoorthy, R., Lee, K. J., & Jayaprakash, M. (2008). Heavy metals in two fish species (Carangoidel malabaricus and Belone stronglurus) from Pulicat Lake, North of Chennai, Southeast Coast of India. *Environmental monitoring and assessment*, *145*(1-3), 167-175. doi: 10.1007/s10661-007-0026-3

Bird, G., Mills, K., & Schwartz, W. (1999). Accumulation of 60Co and 134Cs in lake whitefish in a Canadian shield lake. *Water, Air, and Soil Pollution*, *114*(3-4), 303-322. doi: 10.1023/A:1005146015822

Canli, M., & Atli, G. (2003). The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. *Environmental Pollution*, *121*(1), 129-136. doi: 10.1016/S0269-7491(02)00194-X

Dallas, H. F., & Day, J. A. (2004). The effect of water quality variables on aquatic ecosystems: a review. *Water Research Commission Pretoria*.

Decision No. 46/2007/QD-BYT. Regulation of maximum level of biological and chemical pollution in food (2007).

Dhanakumar, S., Solaraj, G., & Mohanraj, R. (2015). Heavy metal partitioning in sediments and bioaccumulation in commercial fish species of three major reservoirs of river Cauvery delta region, India. *Ecotoxicology and environmental safety*, *113*, 145-151. doi: 10.1016/j.ecoenv.2014.11.032

Dvořák, P., Andreji, J., Mráz, J., & Dvořáková-Líšková, Z. (2015). Concentration of heavy and toxic metals in fish and sediments from the Morava river basin, Czech Republic. *Neuro endocrinology letters*, *36*, 126-132.

Ellison, S. L., Barwick, V. J., & Farrant, T. J. D. (2009). Practical statistics for the analytical scientist: a bench guide: Royal Society of Chemistry.

EPA 6020B: Inductively coupled plasma-mass spectrometry (2014).

FAO (2005). Retrieved from http://www.fao.org/fi/oldsite/FCP/en/VNM/profile.htm (assessed 15/05/2021).

FAO 764. Compilation of legal limits for hazardous substances in fish and fishery products. FAO Fisheries Circular (1983).

Järup, L. (2003). Hazards of heavy metal contamination. *British Medical Bulletin, 68*(1), 167-182. doi: 10.1093/bmb/ldg032

Kalay, M., Ay, Ö., & Canli, M. (1999). Heavy metal concentrations in fish tissues from the Northeast Mediterranean Sea. *Bulletin of environmental contamination and toxicology*, *63*(5), 673-681. doi: 10.1007/s001289901033

Konieczka, P., & Namiesnik, J. (2016). Quality assurance and quality control in the analytical chemical laboratory: A practical approach: CRC Press.

Malik, D., & Maurya, P. K. (2014). Heavy metal concentration in water, sediment, and tissues of fish species (Heteropneustis fossilis and Puntius ticto) from Kali River, India. *Toxicological & Environmental Chemistry*, *96*(8), 1195-1206. doi: 10.1080/02772248.2015.1015296

Malik, N., Biswas, A., Qureshi, T., Borana, K., & Virha, R. (2010). Bioaccumulation of heavy metals in fish tissues of a freshwater lake of Bhopal. *Environmental monitoring and assessment*, *160*(1-4), 267. doi: 10.1007/s10661-008-0693-8

Mester, Z., & Sturgeon, R. (2003). Sample Preparation for Trace Element Analysis: Elsevier.

Moiseenko, T., & Kudryavtseva, L. (2001). Trace metal accumulation and fish pathologies in areas affected by mining and metallurgical enterprises in the Kola Region, Russia. *Environmental Pollution*, *114*(2), 285-297. doi: 10.1016/S0269-7491(00)00197-4

Monikh, F. A., Safahieh, A., Savari, A., Ronagh, M. T., & Doraghi, A. (2013). The relationship between heavy metal (Cd, Co, Cu, Ni and Pb) levels and the size of benthic, benthopelagic and pelagic fish species, Persian Gulf. *Bulletin of environmental contamination and toxicology*, *90*(6), 691-696. doi: 10.1007/s00128-013-0986-7

Nguyen, Q. T., Le, V. T., Nguyen, T. K. P. (2015). Xac dinh ham luong cadimi trong mot so loai ca nuoc ngot bang phuong phap ICP-OES [Determination of cadmium concentrations in freshwater fish by ICP-OES]. *Vietnam Journal of Chemistry, 53*(6), 756-759.

Nordberg, M., Nordberg, G. F., Fowler, B. A., & Friberg, L. (1986). Handbook on the Toxicology of Metals.

Olesik, J. W., & Jiao, S. (2017). Matrix effects using an ICP-MS with a single positive ion lens and grounded stop: analyte mass dependent? *Journal of Analytical Atomic Spectrometry*, *32*(5), 951-966. doi: 10.1039/C7JA00043J

Oliveira, L. H., Ferreira, N. S., Oliveira, A., Nogueira, A. R. A., & Gonzalez, M. H. (2017). Evaluation of distribution and bioaccumulation of arsenic by ICP-MS in tilapia (Oreochromis niloticus) cultivated in different environments. *Journal of the Brazilian chemical society*, *28*(12), 2455-2463. doi: 10.21577/0103-5053.20170101

Patrick, F., & Loutit, M. (1978). Passage of metals to freshwater fish from their food. *Water Research*, *12*(6), 395-398. doi: 10.1016/0043-1354(78)90106-9

Pupyshev, A. A., & Semenova, E. V. (2001). Formation of doubly charged atomic ions in the inductively coupled plasma. *Spectrochimica Acta Part B: Atomic Spectroscopy*, *56*(12), 2397-2418. doi: 10.1016/S0584-8547(01)00301-9

Rajkowska, M., & Protasowicki, M. (2013). Distribution of metals (Fe, Mn, Zn, Cu) in fish tissues in two lakes of different trophy in Northwestern Poland. *Environmental monitoring and assessment*, *185*(4), 3493-3502. doi: 10.1007/s10661-012-2805-8

Rakocevic, J., Sukovic, D., & Maric, D. (2018). Distribution and relationships of eleven trace elements in muscle of six fish species from Skadar Lake (Montenegro). *Turkish Journal of Fisheries and Aquatic Sciences*, *18*(5), 647-657. doi: 10.4194/1303-2712-v18_5_01

Renieri, E. A., Alegakis, A. K., Kiriakakis, M., Vinceti, M., Ozcagli, E., Wilks, M. F., & Tsatsakis, A. M. (2014). Cd, Pb and Hg biomonitoring in fish of the mediterranean region and risk estimations on fish consumption. *Toxics*, *2*(3), 417-442. doi: 10.3390/toxics2030417

Renieri, E. A., Safenkova, I. V., Alegakis, A. K., Slutskaya, E. S., Kokaraki, V., Kentouri, M., Boris, B. D., & Tsatsakis, A. M. (2019). Cadmium, lead and mercury in muscle tissue of gilthead seabream and seabass: Risk evaluation for consumers. *Food and chemical toxicology*, *124*, 439-449. doi: 10.1016/j.fct.2018.12.020

Saleh, Y. S., & Marie, M. A. S. (2015). Assessment of metal contamination in water, sediment, and tissues of Arius thalassinus fish from the Red Sea coast of Yemen and the potential human risk assessment. *Environmental Science and Pollution Research*, *22*(7), 5481-5490. doi: 10.1007/s11356-014-3780-0

Sandrine Millour, L. N. l., Ali Kadar, Rachida Chekri, Christelle Vastel, Thierry Gue´ rin. (2011). Simultaneous analysis of 21 elements in foodstuffs by ICP-MS after closed-vessel microwave digestion: Method validation. *Journal of Food Composition and Analysis*, *24*(1), 111-120. doi: 10.1016/j.jfca.2010.04.002

Sidhu, K. S. (2003). Health benefits and potential risks related to consumption of fish or fish oil. *Regulatory Toxicology and Pharmacology*, *38*(3), 336-344. doi: 10.1016/j.yrtph.2003.07.002

Thomas, R. (2013). Practical guide to ICP-MS: a tutorial for beginners: CRC press.

Vietnamese National Standard (TCVN) 11489:2016. Foodstuffs - Determination of elements and their chemical species – General considerations and specific requirements (2016).

Wilschefski, S. C., & Baxter, M. R. (2019). Inductively Coupled Plasma Mass Spectrometry: Introduction to Analytical Aspects. *The Clinical Biochemist Reviews*, *40*(3), 115. doi: 10.33176/AACB-19-00024

THẨM ĐỊNH PHƯƠNG PHÁP VÀ XÁC ĐỊNH HÀM LƯỢNG NGUYÊN TỐ
TRONG CÁ NUÔI BẰNG PLASMA GHÉP CẶP
CẢM ỨNG CAO TẦN KHỐI PHỔ (ICP-MS)

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TÓM TẮT

Trong những năm gần đây, ngành thủy sản của Việt Nam đã có những bước phát triển vượt bậc, không chỉ phục vụ thị trường trong nước mà còn trở thành mặt hàng xuất khẩu chủ lực. Năm 2003, Việt Nam là quốc gia có lượng hàng thủy sản xuất khẩu lớn nhất sang Mĩ, Nhật Bản, Trung Quốc, Hàn Quốc. Trong đó, chất lượng cá bị ảnh hưởng bởi nhiều yếu tố, chủ yếu phụ thuộc vào thức ăn và môi trường sống. Trong số những thành phần hóa học, các nguyên tố có thể tích luỹ trong nhiều cơ quan và mô cá tùy thuộc vào chế độ ăn uống hoặc nồng độ cao của nguyên tố trong môi trường. Hơn nữa, sự tích luỹ của những kim loại trong cơ thể cá không giống nhau do sự khác biệt về ái lực của mỗi kim loại với mô cá, cũng như sự đa dạng về tốc độ hấp thu, lắng đọng và bài tiết. Trong nghiên cứu này, phương pháp phá mẫu bằng acid có sự hỗ trợ vi sóng và plasma ghép cặp cảm ứng cao tần khối phổ (ICP-MS) đã được khảo sát và thẩm định nhằm xác định hàm lượng của Cr, Mn, Ni, Co, Fe, Cu, Zn, As, Cd và Pb trong mẫu cá dựa trên AOAC. 2015.01 (2015). Phương pháp này cho độ tuyến tính tốt ($R^2 > 0,995$), độ lặp lại và độ tái lặp phù hợp theo Phụ lục F. AOAC (2016). Độ hồi phục từ 85,8 đến 109,4% đối với tất cả các nguyên tố. Phương pháp này có thể áp dụng cho phân tích thường quy nhằm xác định và kiểm soát hàm lượng nguyên tố trong cá. Hàm lượng các nguyên tố định lượng trong 6 mẫu cá mua tại Thành phố Hồ Chí Minh đều dưới ngưỡng cho phép của Quyết định 46/2007/QĐ-BYT (2007). Do đó, lượng nguyên tố này trong mẫu mô cá được xem là an toàn cho người tiêu thụ.

Từ khóa: nguyên tố; mô cá; ICP-MS; vi sóng