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修士論文

洞爺湖の水生生物における
重金属含有量と健康リスク評価
Heavy metal content in aquatic organisms of Lake Toya and risk assessment for human health

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Contents

Abstract

Heavy metal contamination can be caused by natural and anthropogenic sources. It is recognized that the Japanese archipelago has been affected by volcanic activity emitting heavy metals as a natural source of contamination. The aquatic organisms in the Lake surrounded by or near volcanic areas can contain high amounts of heavy metal as a result of bioaccumulation and biomagnification, and it is considered to cause a potential risk for human beings by being consumed as food. Lake Toya locates in the southwestern part of Hokkaido, which is formed by caldera and surrounded by the active volcano. The purposes of this study are to establish an IC platform for the determination of heavy metals in biological samples and to measure the heavy metal contents in aquatic organisms of Lake Toya for evaluating the human health risks by consumption of the aquatic organisms.

Lake waters, sediment, and three aquatic organisms (*Oncorhynchus nerka, Hypomesus nipponensis, Palaemon paucidens*) of Lake Toya were collected or purchased. Water and sediment samples were stored in the refrigerator and the biological samples were stored in the freezer until analysis. The microwave acid digestion method was applied to the decomposition of sediment and biological samples. Then, ion chromatography (IC) with post-column derivatization method was applied for the separation and detection of heavy metals.

It was confirmed that the IC method proposed in this study can simultaneously separate and measure five heavy metals including Lead (Pb), Copper (Cu), Zinc (Zn), Nickel (Ni), and Cobalt (Co). By applying the microwave acid digestion method, it is possible to determine the content of heavy metals below 1 μg/g in hundreds of micrograms for the biological sample and to confirm the accuracy of the method using the standard reference material. The order for the averaged Cu content in three aquatic organisms was

Palaemon paucidens > *Hypomesus nipponensis* > *Oncorhynchus nerka*, while that for Zn was *Hypomesus nipponensis* > *Palaemon paucidens* > *Oncorhynchus nerka*. Due to the bottom-dwelling habit, *Palaemon paucidens* directly contact with the bottom for feeding, while *Oncorhynchus nerka* and *Hypomesus nipponensis* are benthopelagic fish, which float in the water column. In addition, the order of averaged concentrations of Cu and Zn in *Hypomesus nipponensis* was gill> internal organs > muscle. It can primarily be attributed to the differences in the physiological role of each organ and regulatory ability. Using the upper tolerable daily intakes of Zn and Cu for 40 and 10 mg, respectively, the maximum allowable number of each aquatic organisms were N = 1 of *Oncorhynchus nerka*, 4 of *Hypomesus nipponensis*, and 5 of *Palaemon paucidens* for an adult per day.

1. Introduction

1.1. Overview of heavy metals

Heavy metals are elements with density above 5 g/cm^3, for the 90 naturally occurring elements, which 21 are non-metals, 16 are light metals and the remaining 53 are heavy metals, respectively (Nies, 1999; Duruibe et al., 2007). The majority of heavy metals are extremely toxic to human beings, animals, and plants. Especially it has irreversible harm to human liver, kidney, brain, nervous system and so on (Fu & Wang, 2011). The various harms of heavy metals for the human body are expressed in Fig. 1.

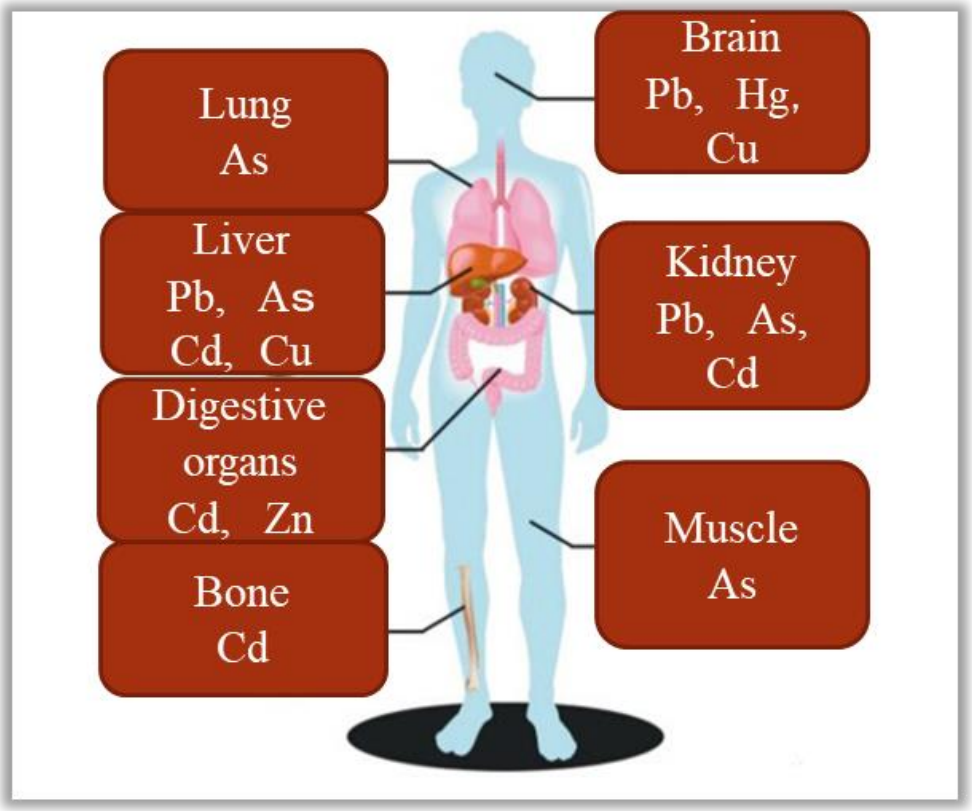


Fig. 1 The harm of heavy metals to the human's body.

The biotoxic effects of heavy metals refer as the harmful effects to the person health when there exceed the bio-recommended limits. The detailed sources and hazards of some heavy metal elements can be summarized as follows:

Zinc (Zn)

Zn is an essential micro-element that is indispensable for human health and it is important for the physiological functions of living tissue. But the excessive ingestion of Zn can cause eminent health problems, such as stomachache, skin irritations, vomiting, and anemia. The main sources responsible for the zinc metal are electroplating industries, pulp industries, and steel making industries (Carolin et al., 2017).

Copper (Cu)

Cu is one of the required microelements for metabolism both in human beings and animals. However, abundant amounts of Cu that goes beyond the level can induce serious toxicological concerns, such as vomiting, cramps, convulsions, or even death (Paulino et al., 2006). The main sources of Cu and its compound pollution are Cu compounds produced by the mining and smelting of Cu-Zn ore, metal processing, machinery manufacturing, steel production.

Nickel (Ni)

In a person's body, Ni often shows the carcinogen that would cause various types of disorders and diseases in human health, when concentrations for Ni expresses more than critical levels. There are many adverse effects, including severe damage to lungs and kidney, skin dermatitis. Ni ions are mainly used in wastewater discharge from electroplating, electronics and metal cleaning industries, which can cause serious water pollution (Borba et al., 2006).

Cadmium (Cd)

Cd also expresses a carcinogenic element and can expose severe risks for people health. Chronic exposure to Cd results in kidney dysfunction and even results in death. The pollution sources are mainly Pb-Zn mines, as well as non-ferrous metal smelting, electroplating, and factories that use cadmium compounds as raw materials or catalysts (Järup, 2003).

Lead (Pb)

Pb is one of the most pervasive contaminants in water environments and soils, even though Pb in low concentration can damage the central nervous system (Naseem & Tahir, 2001). Lead in the environment mainly comes from two aspects. One is natural sources, which refers to Pb released into the environment by natural phenomena such as volcanic eruption smoke, flying ground dust, and forest fire smoke and sea salt aerosols. However, the largest and most frequent source of environmental pollution is man-made activities, including Pb and other heavy metal mining, smelting, battery industry, glass manufacturing, powder metallurgy and the "three wastes" generated by related companies, fuel oil, fuel coal. The production and use of waste gas from combustion, paints, coatings, pigments, glazes, medicines, cosmetics, chemical reagents, and other lead-containing products (Gwiazda & Smith, 2000).

Manganese (Mn)

Excessive exposure or intake of Mn can lead to a condition as manganism which is a neurodegenerative disorder that causes dopaminergic neuronal death and symptoms like Parkinson's disease. The main sources of manganese pollution are wastewater, waste gas and slag discharged from manganese mines, smelters and factories using manganese as raw materials for production (Chillrud et al., 2004).

Cobalt (Co)

Cobalt is an essential micro-element for daily operation of human body. However,

chronic co ingestion has caused serious health problems even injection less than the lethal dose. In 1966, the addition of cobalt compounds to stabilize beer foam in Canada led to a peculiar form for toxin-induced cardiomyopathy, which ultimately regard as beer drinkers cardiomyopathy. In addition, co metal is suspected of causing cancer as per the International Agency for Research on Cancer (IARC) Monographs. The average content of co in the earth's crust is 0.001% (mass), and the total amount of co in the ocean is about 2.3 billion tons. There are nearly one hundred co-containing minerals known in nature, but there are no separate co minerals (Paustenbach et al., 2013).

1.2. Sources of heavy metals pollution

Heavy metal contamination can be caused by the natural and anthropogenic sources. The main anthropogenic sources originate from agriculture, mining, fuel consumption, residual organic matter, and sewage water, etc. The main natural sources of heavy metals generate windblown dust, volcanogenic particles, forest wildfires, and sea salt, etc (Tchounwou et al., 2012; Ali et al., 2019).

The treatment of naturally derived heavy metal-containing soil in Japanese law issued a statement that "soil with natural heavy metals is not soil contamination caused by human activities and doesn’t attribute to pollution". In the Soil Contamination Countermeasures Act in 2003, heavy elements from nature did not restrict from the law. But, in the revision of the Soil Contamination Countermeasures Act in 2009, it is defined that the soil and water of which were naturally introduced the heavy metals should be treated as punitive measure by the law. Thus, the pollution by naturally occurring heavy metals treats in the same way as artificially derived contaminated soil. The reason is about why make the

above punitive measure that the heavy elements by naturally derived can lead to equal risk to the environment and human beings (Uesuna et al., 2013).

Japan is one of the most frequently volcanic areas in the world, including 1,274 times eruptions and 94 recorded volcanoes. Volcanic activity is a major natural origin for heavy metals of which can generate the flooder with heavy metals from the earth's crust to the earth's surface while the heavy metals diffuse into the surrounding soil and atmosphere through the flows of lava, ash, and gases. In addition, after a volcanic eruption, volcanic ash and magma erupt with a large number of heavy metals would infiltrate into water ecosystem, which increase their content and pollution of heavy metal in water environment (Vigneri et al., 2017). Around 2015, other site-specific cancers were found increased in the Sicilian volcanic area but to a lesser extent than thyroid cancer. These findings reflect sparse observations that in the past reported increased cancer in other volcanic areas suggesting the possibility of a wider carcinogenic effect of the volcanic environment (Russo et al., 2015). Major and trace element concentrations were determined in two lichen species from the island of volcanic and around Mt. Etna. Several elements (Br, Pb, Sb, Au, Zn, Cu) resulted enriched with respect to the local substrates (Varrica et al., 2000).

Heavy metals by naturally derived refer as bio-accumulated through the food chain of water system, even if it's concentrations in water are in trace amounts. Ultimately, they will migrate to human beings who are higher consumers at ecosystem and then suffer lots of health effects. Meanwhile, there are many relevant studies that have been researched and published. Bioaccumulation of organic substances is an important component of chemical risk assessment for both scientific and regulatory purposes (Mackay et al., 2018). Bioconcentration factors (BCFs) and bioaccumulation factors (BAFs) are widely used in scientific and regulatory programs to assess chemical hazards (Mackay et al., 2016). For example, the bioaccumulation of heavy metals causes fish deformities and can have

devastating effects on fish populations (Sfakianakis et al., 2015). Through the analysis of food fish off the coast of Malaysia, it is known that bioaccumulation poses a health risk to the population (Agusa et al., 2005). Periodic volcanic eruptions are natural phenomena that can produce global catastrophic effects, due to the emission of large quantities of gas and ash particles into the atmosphere. The physical and chemical properties of the elements expelled in these events, can lead to negative effects on the physiology of plants, arthropods, and fishes, as well as on human respiratory health (Salas-Yanquin et al., 2018).

Therefore, the aquatic organisms in Lake which are affected by volcanic activity possibly express high content of heavy metal and many of them bring to lots of health influence and risk for human beings.

1.3. Lake Toya

Lake Toya locates in the southwestern part of Hokkaido, which is formed by caldera and surrounded by the volcanic. The area of the Lake Toya is the ninth largest in Japan and the third largest caldera lake in Japan, of which this lake is a circular lake that displays the size with 11 km from east to west and 9 km from north to south. Moreover, it also is one of the major tourist areas in Hokkaido adjoined with Mt. Usu, Mt. Showa-shinzan, and Toyako Onsen (Yoshida et al., 2009). There has been little research on heavy metals in the aquatic organisms of Lake Toya, so this study can be used as a preliminary study of heavy metals in Lake Toya's aquatic organisms, which is very meaningful for future heavy metal research. Thus, we systematically research the aquatic systems of the Lake Toya (Fig.2).

Fig. 2 Location and schematic diagram around of Lake Toya.

There are following investigated significance and reasons for the study of Lake Toya. First, the Lake Toya has been repeatedly affected by the volcanic activity of Mt. Usu which erupts every 23 to 50 years (Mori & Ui, 2000). Second, lake water is used for hydroelectric power generation, agricultural irrigation water, and drinking water (Urano, 1987; Urano et al., 2002). Finally, *Oncorhynchus nerka, Hypomesus nipponensis*, and *Palaemon paucidens* in Lake Toya are used in daily diet (Su et al., 2015). Thus, it is an investigation that heavy metals are accumulated in the aquatic organisms of Lake Toya, which is affected by the volcano.

1.4. Determination method for heavy metals in biological samples

In the recent decade, heavy metals are becoming one of the most rigorous environmental problems. Therefore, effective monitoring and management for the heavy metal elements in the aquatic environment are extremely focused on recent decade (Fu &

Wang, 2011). There are a lot of determination methods for measure heavy metals, such as atomic absorption spectrometry (AAS), ion chromatography (IC), inductively coupled plasma atomic emission spectrometry (ICP-AES) and mass spectrometry (ICP-MS) etc.

Atomic absorption spectrometry (AAS) is a traditional determination method for elements including heavy metals applied from the 1950s. It is complementary measure for atomic emission spectroscopy, which is mainly used for the qualitative analysis of inorganic elements and played a major role in quantitative analysis of inorganic compounds (Tüzen, 2003).

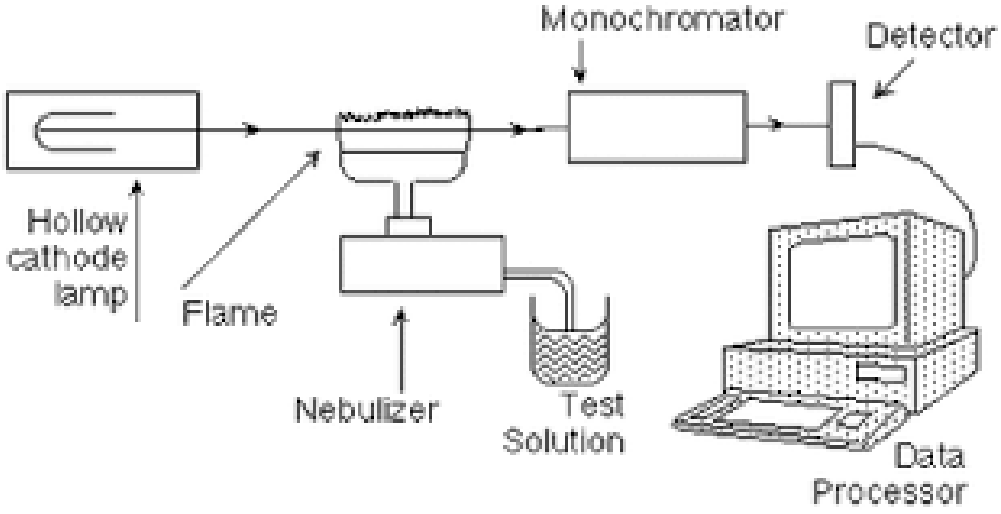


Fig. 3 Schematic diagram of atomic absorption method.

Researchers have revealed several drawbacks in the application period:

First, samples consist of light metals in a high concentration easily suspected by interference of light metals. Secondly, the equipment is difficult to measure multiple heavy metals for a sample in a lower concentration, simultaneously. Finally, require a good amount of measurement.

The first IC was only a suppressor type electrical conductivity detection method, but a

non-suppressor type was later developed, and a non-suppressor type sample ion without light absorption was developed. An indirect absorbance detection method has also been developed that detects the separation elution as a decrease in eluent ions with large light absorption (Nakamura et al., 1989).

In heavy metal measurement, post-column derivatization and absorption detection are relatively high-sensitivity measurement methods. Therefore, the ion chromatography (IC) with post-column derivatization method was fabricated (Thermo Scientific, 2001; Haddad et al., 2008). It exhibits attractive method to survey multiple heavy metals in sample while can alter the conventional AAS measure. Moreover, it also reveals plentiful advantages, such as advanced accuracy and sensitivity, which can measure a sample volume in 1 mL or even less.

In order to match the IC method, appropriate pretreatment methods are required, one of which is the microwave thermal decomposition method. The microwave acid digestion is useful method to pretreat the biological samples for metal analysis. There are some advantages showing as follows (Lamble & Hill, 1998):

(1) This method can effectively decrease the utilization of acid.

(2) Since it is sealed when the sample is decomposed, there is little contamination. It also avoids the sample being polluted by external environment because of sealed during the process of treatment.

(3) This method also efficiently reduces the time to disassemble the sample.

1.5. Purpose of this study

Therefore, the purpose of this study:

(1) Establish an IC platform for measuring heavy metals in biological samples.

(2) Measure content of heavy metal in aquatic organisms of Lake Toya.

(3) Evaluate health risks of heavy metal for human beings by consumption of aquatic organisms in Lake Toya.

2. Materials and methods

2.1. Reagents

Table1 Reagent List.

	Name	Usefulness	Companies
1	Dionex MetPac PAR Postcolumn Reahent Diluent	For post cloumns	Thermometer Fisheries Scientific
2	4-(2-pyridylazo) resorrcinol (PAR)	For post cloumns	FUJIFILM Wako chemicals USA corporation
3	Dionex MetPac Oxalic Acid Eluent Concentrate	For eluent	Thermometer Fisheries Scientific
4	Cu standard solution	Qualitative use	FUJIFILM Wako chemicals USA corporation
5	Zn standard solution	Qualitative use	FUJIFILM Wako chemicals USA corporation
6	Cd standard solution	Qualitative use	FUJIFILM Wako chemicals USA corporation
7	Ni standard solution	Qualitative use	FUJIFILM Wako chemicals USA corporation
8	Pb standard solution	Qualitative use	FUJIFILM Wako chemicals USA corporation
9	Mn standard solution	Qualitative use	FUJIFILM Wako chemicals USA corporation
10	Co standard solution	Qualitative use	FUJIFILM Wako chemicals USA corporation
11	Nitric Acid(1.38)	For microwave decomposition	FUJIFILM Wako chemicals USA corporation
12	Trace Elements in Cod Fish Tissue	Confirmation of analytical methods	National Metrology Institute of Japen

2.2. Biological samples and pretreatment

Biological samples including *Oncorhynchus nerka, Hypomesus nipponensis*, and *Palaemon paucidens* of Lake Toya were chosen in this study. All samples were purchased from the Lake Toya Fisheries Cooperative Shop and stored in the freezer util pretreatment. The detail information of biological samples shows in the Table 2.

Table 2 Detailed information of biological samples used in this study.

Scientific name	*n*	Fresh weight per animal (g)	Body length (cm)	Estimated age (year)
Oncorhynchus nerka	2	115.X±5.X	22.4±1.1	1
Hypomesus nipponensis	10	10.9±1.6	11.4±1.5	1
Palaemon pauciden	15	1.4±0.3	5.5±1.0	1

Fresh biological samples (300-500 mg) including the category in gill, internal organs and muscle and 2 mL of nitric acid were placed in 7 mL PFA internal vessels and close the lid (Fig. 4). Then, the internal vessel was placed in the PTFE container and tightly close the lid with a vise. Place in the microwave with a 100 ml beaker containing 50 mL or more of water. Irradiate microwaves for 3 minutes at an output of 200 W. Take out only the beaker filled with water and irradiate again at 200 W for 3 minutes. Allow to cool for about 3 minutes. Next, irradiate at 200 W for 2 minutes. Finally, filter and quantify. The containers PFA and PTFE used in this study were all purchased from San-ai Science Co., Ltd. I bought a microwave oven from IRIS OHYAMA Inc.

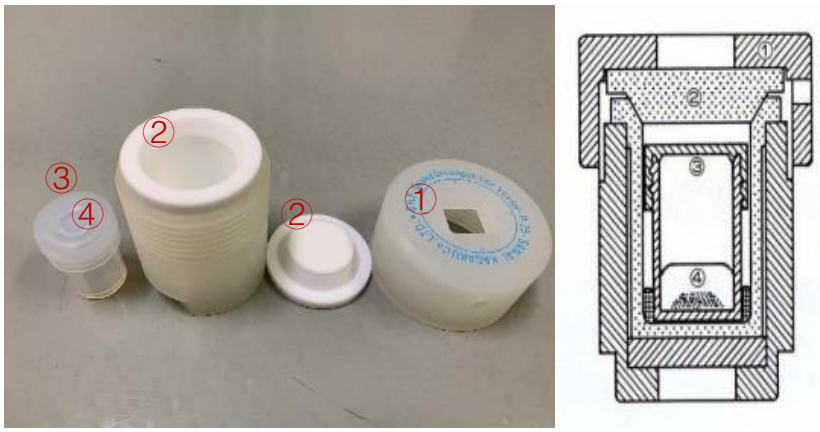


Fig. 4 Reaction vessel used in the microwave digestion method in this study.

① Polypropylene outer cylinder, ② PTFE container,

③ PFA internal small vessel, ④ Coexistence of sample and acid.

2.3. Water and Sediment samples

Lake water samples were collected from different places (Station 1, 2, 3 and 4) at Lake Toya showing in Fig. 5. 490 mL of lake water sample mixing with 10 mL of concentrated nitric acid was gently heated on a hot plate to reduce the volume to 100 mL. This procedure aimed to concentrate the mixing solution for adequately quantifying relevant heavy metals in the sample. Finally, the mixing solutions were filtered and measured.

Sediment samples was obtained in the station A, showing in Fig. 5. For preparing the sediment, dried the sample at room temperature for 24 hours and then passed through a 2 mm sieve. Ultimately, it was digested by the microwave digestion system as mentioned 2.3 for analyzing the qualification of heavy metal elements.

Fig. 5 Sampling points of Lake Toya on Google Maps.

2.4. Ion chromatography (IC) with post-column derivatization method

The schematic diagram of IC method used in this study, displayed in Fig. 6 and Fig. 7, is formed by 4 parts. In first part, the sample and the Dionex MetPac Oxalic Acid Eluent solution are mixed in the auto sampler (2). This mixed solution regularly separated in the Dionex IonPac CS5A Analytical column (3), in the second part. And in next section, separated liquid from column combined in the mixer and efficiently reacted in the length = 6 m, Inner diameter = 0.25 mm, Knitted reaction coil. Finally, each element is detected on a UV detector with a detector wavelength of 530 nm, and we can perform quantitative analysis. Table 3 shows the typical analytical conditions for the IC method. Equipment for ion chromatography analysis method is a device of Thermo Fisher Scientific K.K.

Fig.6 Equipment for ion chromatography analysis method.

1: IC25 Ion Chromatograph 2: AS50 Autosampler 3: LC25 Chromatography Oven 4: UV8020 5: DP8020

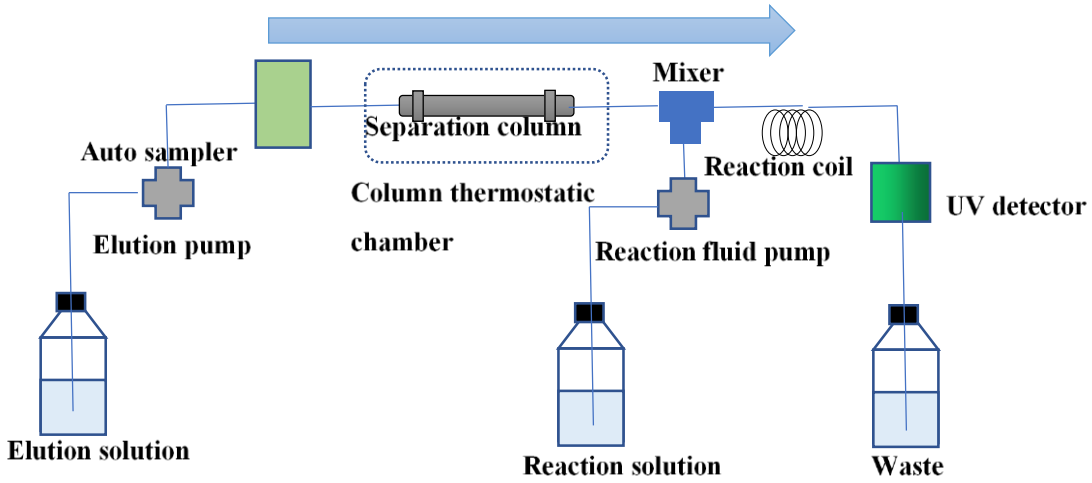


Fig. 7 Schematic diagram of IC with post-column derivatization method.

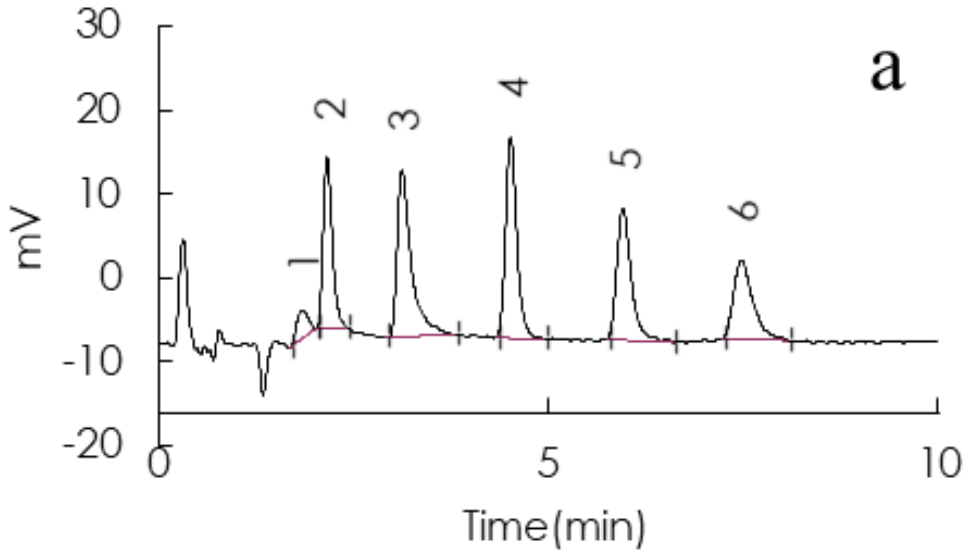
Table 3 IC post column derivatization method conditions.

Columns:	**dionex IonPac CS5A Analytical**
Eluent:	**dionex MetPac Oxalic Acid Eluent**
Flow Rate:	**1.2 mL/min**
Inj.Volume:	**100 µL**
Mixing Device:	**length = 6 m Inner diameter = 0.25 mm Knitted reaction coil**
Postcolumn Reagent :	**0.5 mMPAR in Dionex MetPac PAR Postcolumn Diluent**
Reagent Flow Rate:	**0.2 mL/min**
Detector Wavelength:	**530 nm**

3. Result and discussion

3.1. Validation of IC method for heavy metals in biological samples

3.1.1. Separation, calibration curves and detection limit of IC system for heavy metals



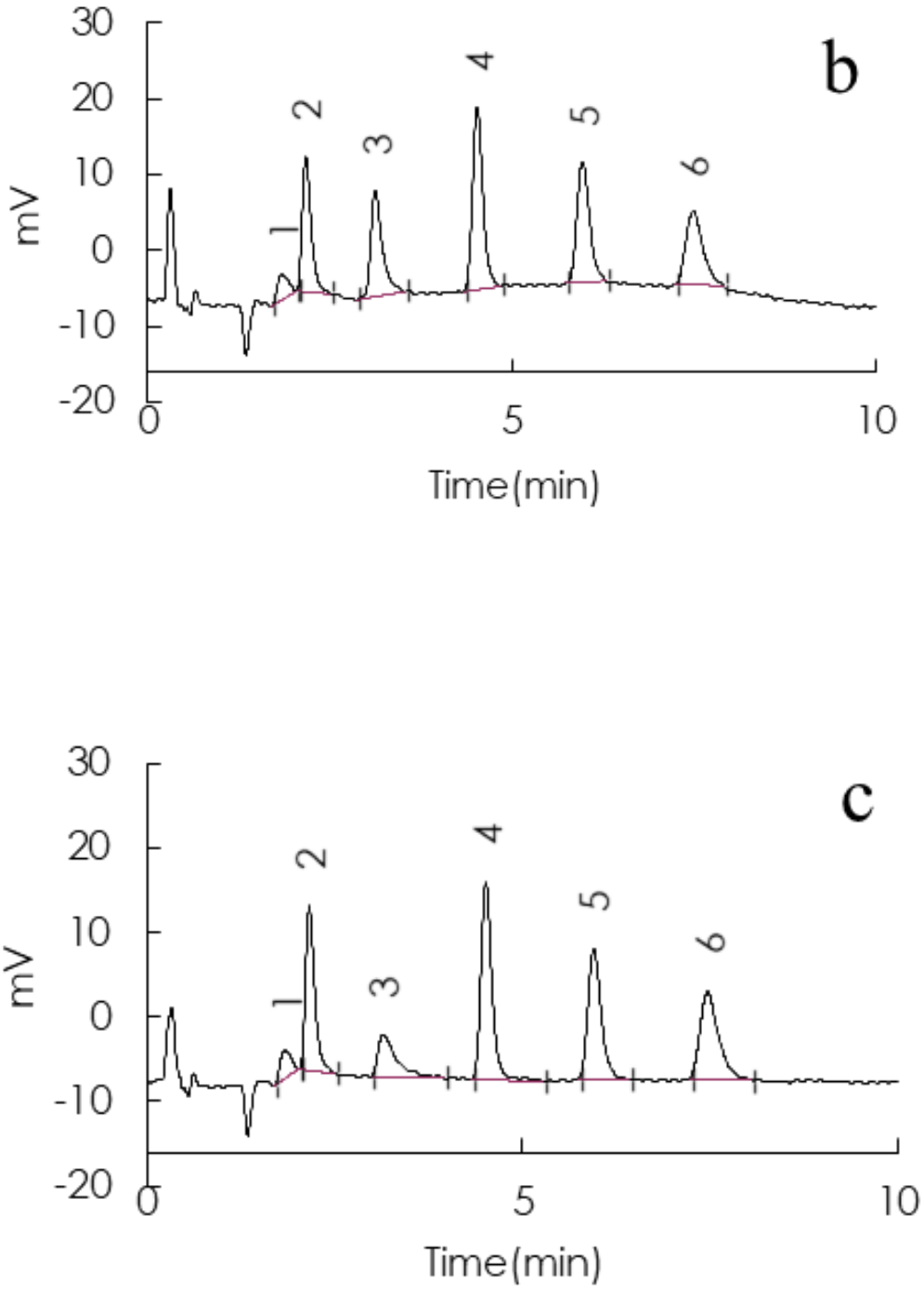


Fig. 8 Chromatograms obtained from the mixture standard solutions containing (a) Cd + Mn, (b) Mn, (c) Cd. Peak identities (concentration, μg/L): 1, Pb (1000); 2, Cu (100); 3, Cd (100) and Mn (100); 4, Co (100); 5, Zn (100); 6, Ni (100).

In the Fig. 8, the IC method employed to measure these heavy metal elements which can be dividing into three groups, separately:

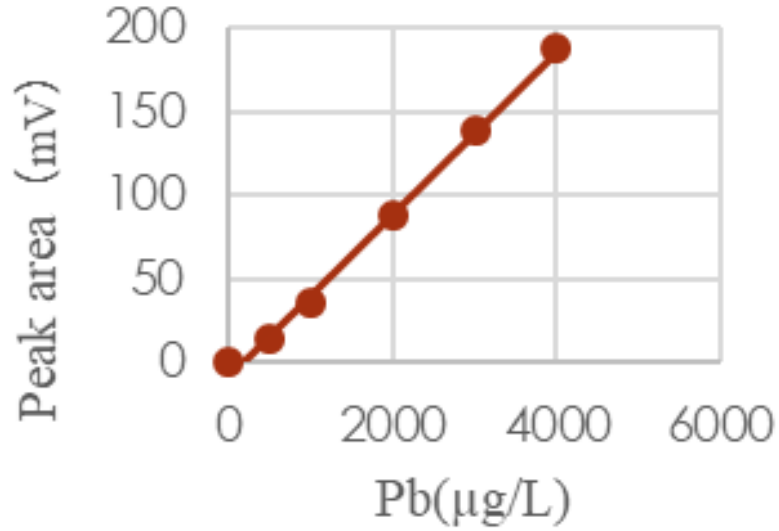
a: Cu, Cd, Mn, Co, Zn, Ni, Pb;

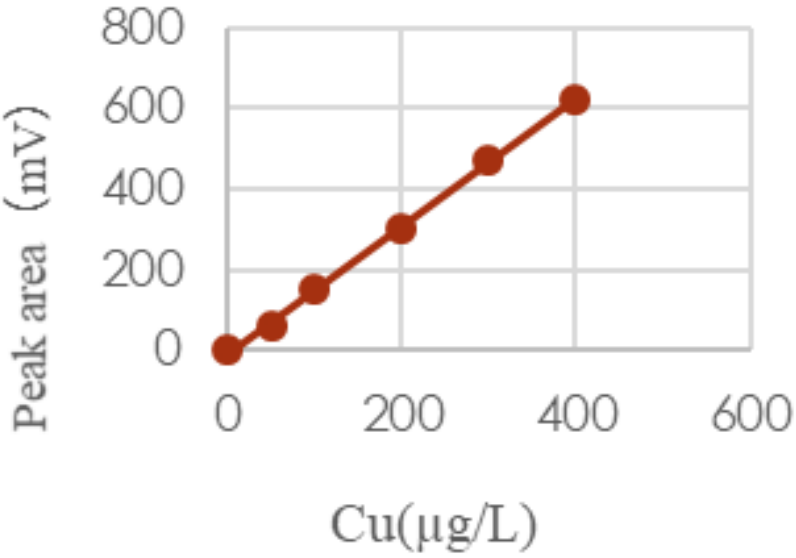
b: Cu, Cd, Co, Zn, Ni, Pb;

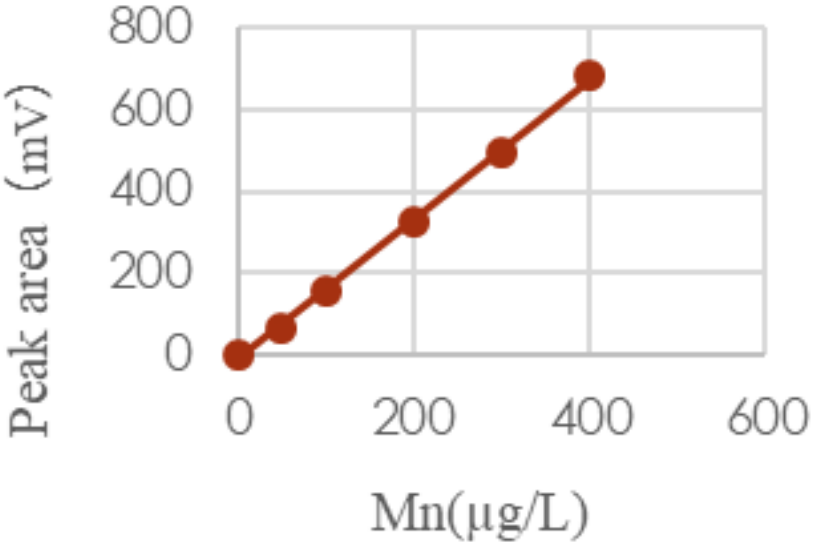
c: Cu, Mn, Co, Zn, Ni, Pb.

Shown in Fig. 8(a), seven heavy metal elements are analyzed, but only six peaks appear, indicating that two peaks overlap. Fig. 8(b) and 8(c) show six peaks after analyzing six elements. From these chromatograms, the peak area for the overlapped Cd with 100 µg/L and Mn with 100 µg/L is 252842 mV s, while those of only Mn with 100 µg/L and of only Mn were 88978 and 155208 mV s, respectively. From these results, it shows that the peaks of Cd and Mn was completely overlapped and could not be separated by IC column under the analytical conditions of this study. In addition, another method can be utilized by changing the retention time of analytical metals to quantify elements. Therefore, this method can only measure five heavy metal elements including Cu, Co, Zn, Ni, and Pb at the same time.

The calibration curve was created by analyzing standard solutions of each elements, including copper (Cu), zinc (Zn), nickel (Ni), cobalt (Co), manganese (Mn), and cadmium (Cd) at concentrations of 0.05, 0.1, 0.2, 0.3, 0.4 mg/L. Due to the standard solution of Pb cannot be measure in micro amount, the samples for the calibration curve were prepared in diverse concentration in 0.5, 1, 2, 3, 4 mg/L, respectively, shown in Fig. 9.


200
150
100
50
0
Peak area (mV)
0
2000
4000
6000
Pb(μg/L)


800
600
400
200
0
Peak area (mV)
0
200
400
600
Cu(μg/L)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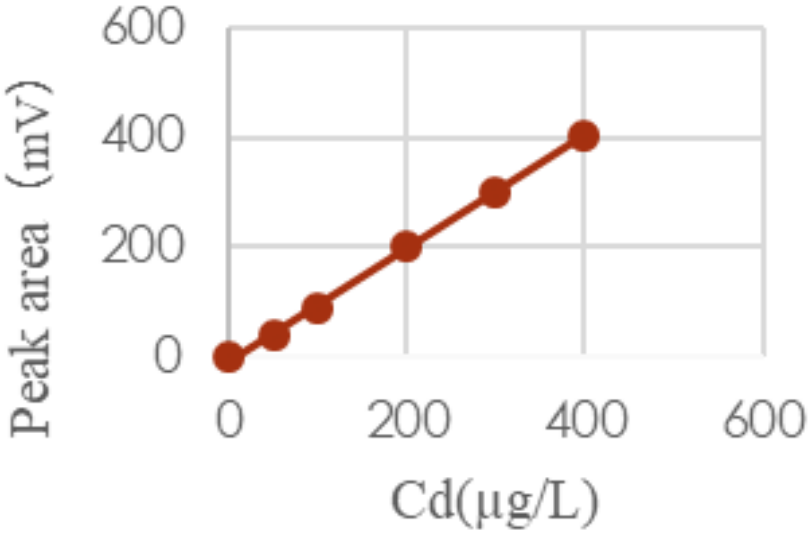
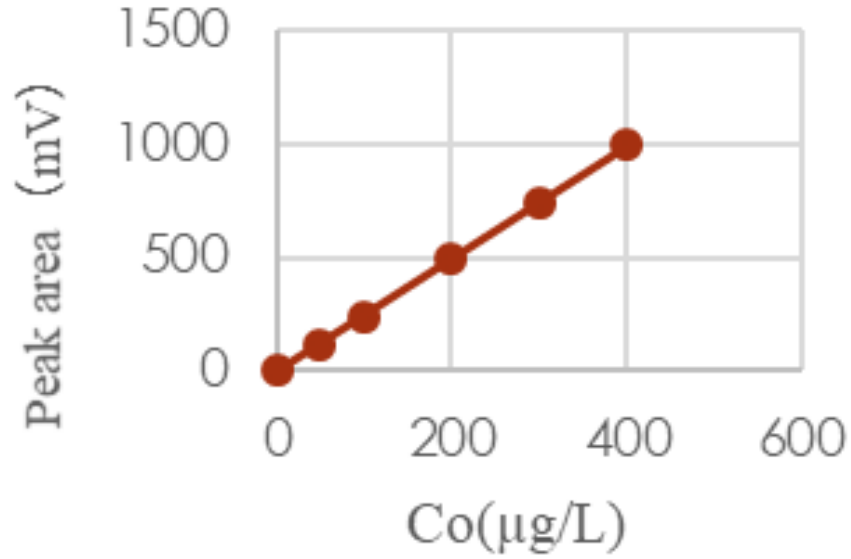
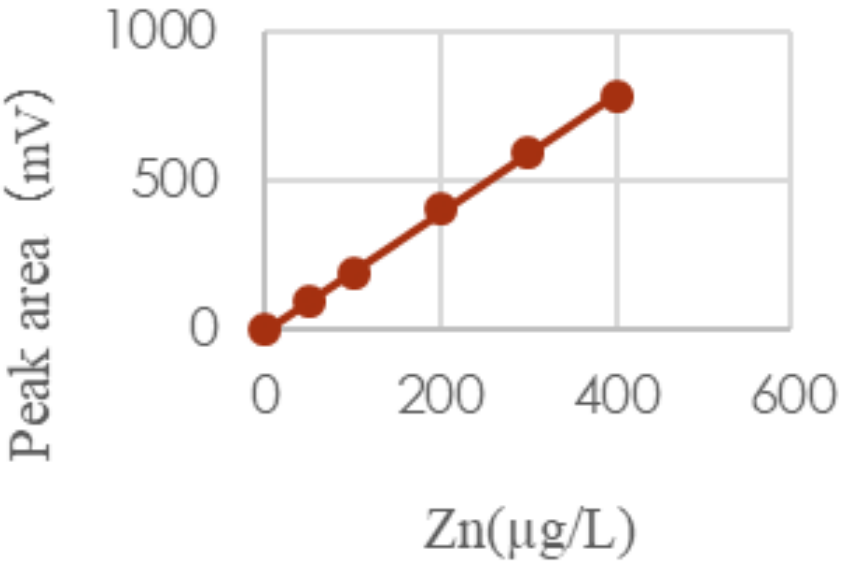
600
400
200
0
Peak area (mV)
0
200
400
600
Mn(μg/L)


600
400
200
0
Peak area (mV)
0
200
400
600
Cd(μg/L)


1500
1000
500
0
Peak area (mV)
0
200
400
600
Co(μg/L)


1000
500
0
Peak area (mV)
0
200
400
600
Zn(μg/L)

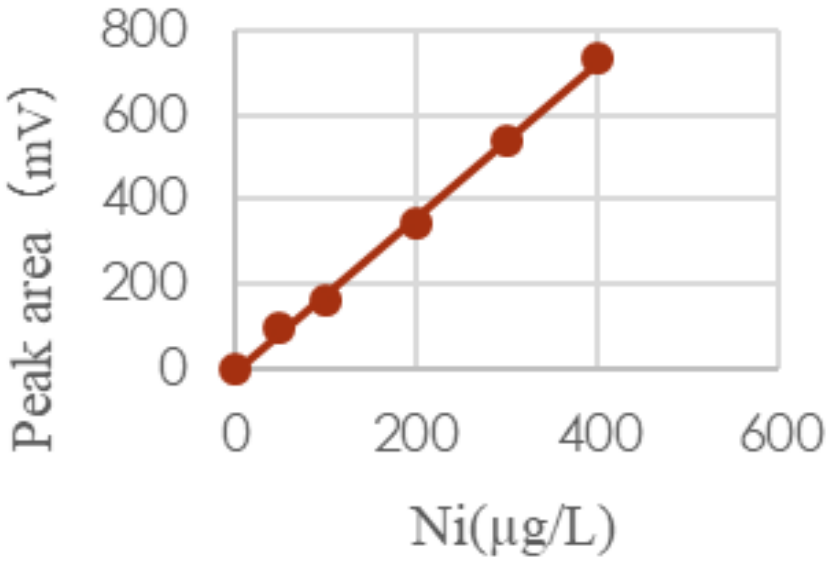


Fig .9 Calibration curves of Cu, Cd, Mn, Co, Zn, Ni and Pb under the optimized analytical conditions.

Table 4 Instrumental detection limits of IC used in this study.

Metals	Range (μg/L)	calibration curve	R^2	device detection limit (μg/L)
Pb	500-4000	y=48.213x-7126	0.9966	250
Cu	50-400	y=1579.5x-7487.4	0.9991	26
Mn	50-400	y=1713.7x-12802	0.9988	28
Cd	50-400	y=1025.9x-7560.1	0.9990	15
Co	50-400	y=2499x-5819.1	0.9997	11
Zn	50-400	y=1983.8x-2278.9	0.9998	16
Ni	50-400	y=1829.5x-8627.4	0.9982	12

The detection limits were summariaed in Table 4 expressing the lowest level that the machine can be measure. Similarly, it cannot be testing the concentration of sample if its elements are lower than this level.

3.1.2. Validation using a certified biological reference material in Cod Fish Tissue.

To inspection this method, certified biological reference material in Cod Fish Tissue that purchased from the Measurement Standards Center was analyzed and compared with the standard solution.

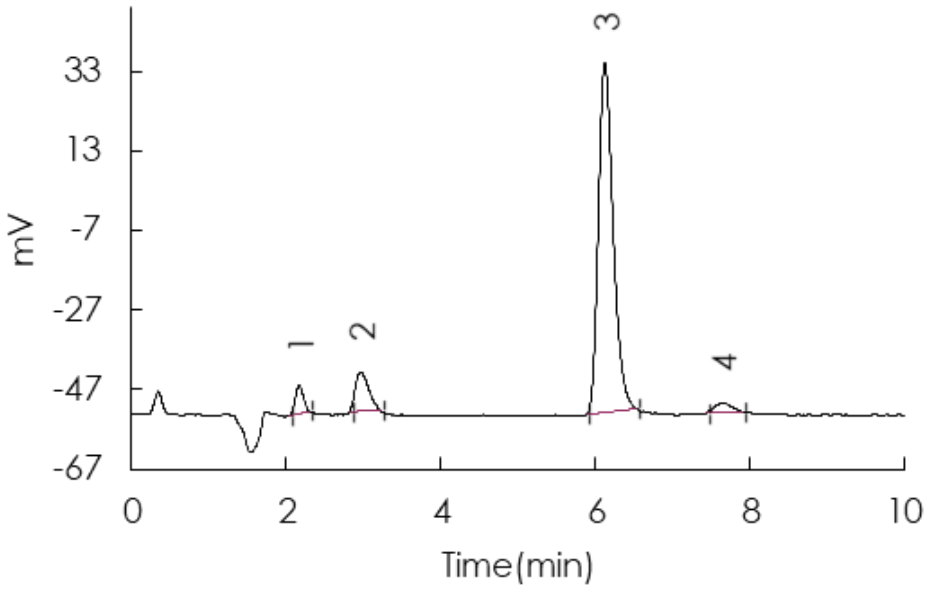


Fig. 10 Chromatogram of Cod Fish Tissue.

Peak identities: 1, Cu; 2, Cd + Mn; 3, Zn; 4, Ni.

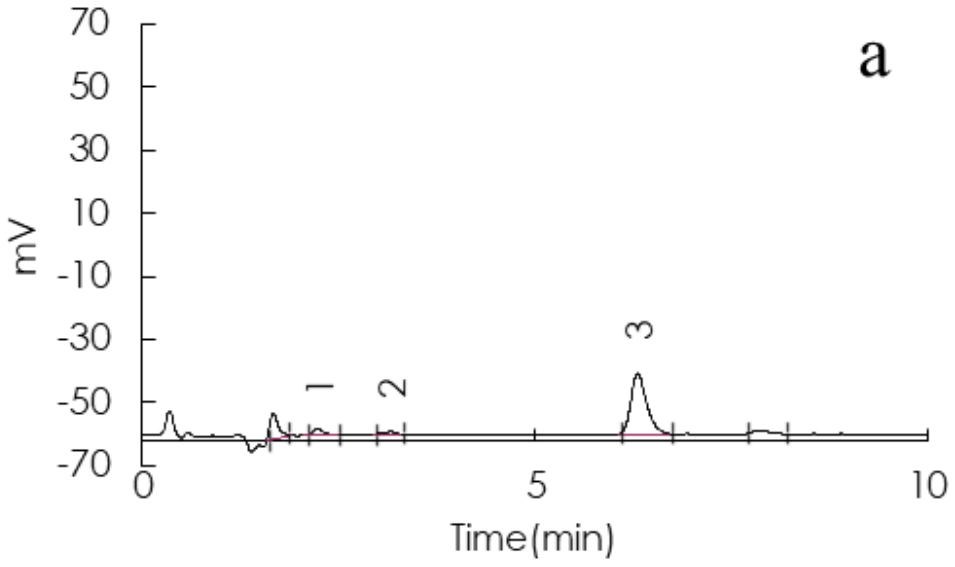
Table 5 Analytical results of certified biological reference materials.

Metals	Certified value (μg/g)	Analytical results (μg/g)	samples detection limit (μg/g)
Pb	0.04	<8.36	8.36
Cu	1.25±0.07	1.23	0.89
Mn	0.41±0.03	<0.95	0.95
Cd	0.009	<0.51	0.51
Co	0.04	<0.38	0.38
Zn	21.3±1.5	22.28	0.56
Ni	0.38±0.05	0.39	0.4

The chromatogram of the standard solution for Cod fish, shown in Fig. 10 and comparing with this figure can qualitatively identify the heavy metal elements. It can be seen from Table 5 that the results of the standard express coherence with the certified value, which shows this method is accurate to measure the heavy elements.

3.2. Analytical results of heavy metals in fish and shrimp in Toya lake

3.2.1. Analytical results of heavy metals in fish and shrimp in Toya lake



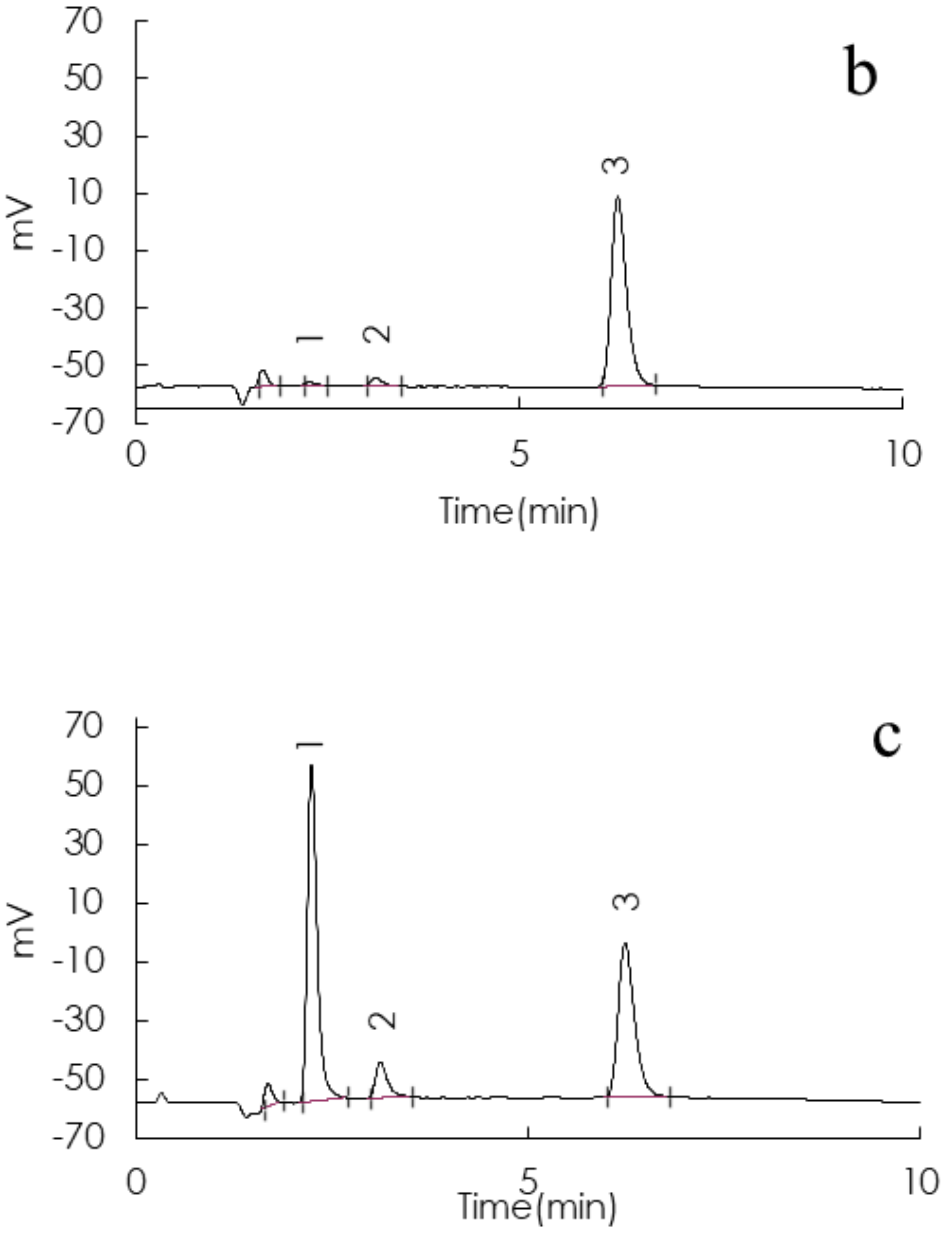


Fig. 11 Typical chromatograms of (a) *Oncorhynchus nerka*, (b) *Hypomesus nipponensis*, (c) *Palaemon paucidens.* Peak identities: 1, Cu; 2, Cd + Mn; 3, Zn.

Comparing the standard solution, the result shows Cu and Zn corresponding to the retention time were confirmed, displayed in Fig. 11. There are not peaks for other heavy

metals because the content of other elements is lower than the detection limit of the sample, showing in Table 6.

Table 6 Heavy metal content of aquatic biological samples.

Scientific name	*N*		Concentration (μg/g wet weight)				
			Cu	Co	Pb	Zn	Ni
Hypomesus nipponensis	10	Gill	6.58±0.21	< 0.38	< 8.36	151±3.10	< 0.4
	10	Internal organs	3.49±0.36	< 0.38	< 8.36	99.4±2.79	< 0.4
	10	Muscle	2.83±0.10	< 0.38	< 8.36	89.8±4.80	< 0.4
Oncorhynchus nerka	2	Muscle	2.89±0.13	< 0.38	< 8.36	30.7±0.50	< 0.4
Palaemon paucidens	15	Whole	126±7.72	< 0.38	< 8.36	70.6±0.70	< 0.4

The results of *Oncorhynchus nerka* show that Cu and Zn shows below the maximum allowable limit of heavy metal concentrations in food (Table 7). The contents of Pb and Ni are <8.36 and <0.4 μg/g, respectively, which exhibit also lower than the maximum allowable limit of heavy metal concentration in food. The content of Co displays <0.38, but the maximum allowable limit of heavy metals in food didn't obtain the value, so it is impossible to analyze (Table 6).

For *Palaemon paucidens*, the Concentration of Cu shows much higher than the maximum allowable limit of heavy metal concentration in food (Table 7). Contrast, the Concentration of Zn expresses lower than the maximum allowable limit for heavy metal concentrations in food. It is the reason why caused this result that possibly due to the Palaemon paucidens frequently activated in the sediment. Deposits often accumulated heavy metals had been published in paper. The contents of Pb and Ni are <8.36 and <0.4 μg/g, respectively, which reveal lower than the maximum allowable limit of heavy metal concentration in food. The content of Co expresses <0.38, but the maximum allowable limit of heavy metals in food don't express value, so it is also impossible to evaluate (Table 6).

For *Hypomesus nipponensis*, the detailed results of Cu and Zn content reveal gill> internal organs> muscle, gill> internal organs> muscle, respectively.

In summary, for human beings, Cu content in all parts exhibits lower than the maximum permissible limit of heavy metal concentration in food (Table 7). However, the Zn content in the gills is higher than the maximum permissible limit of the concentration of heavy metals in food. In human habits that they often eat the whole *Hypomesus nipponensis*, it is bad for person's health even can be toxics. The contents of Pb and Ni show <8.36 and <0.4 μg/g, respectively, which are lower than the maximum allowable

limit for heavy metal concentration of food. The content of Co express <0.38, but the maximum allowable limit of heavy metals in food don't express valuable, so it is impossible to evaluate harmful in the health situation (Table 6).

Table 7 The maximum allowable limit of the concentration of heavy metals in the world's food.

Metals	WAFDR (µg/g)	WHO (µg/g)	FAO (µg/g)	ANHMRC (µg/g)
Cu	-	30	30	30
Pb	-	2	4	2
Zn	-	50-150	30	100
Ni	5.5	5	10	-
Co	-	-	-	-

3.2.2. Comparison with other research results

Table 8 Comparison with other research results

Scientific name	Cu (μg/g)	Zn (μg/g)	Pb (μg/g)	Co (μg/g)	Reference
Brown trout	0.84	8.79	0.65	-	Alibabić et al. 2006
Hypomesus nipponensis	0.44	23.8	-	-	Yamamoto et al. 1992
Pandalus eous	3.88	9.15	0.01	-	Yamamoto et al. 1992
Hypomesus nipponensis	2.83±0.1	89.8±4.8	< 8.36	< 0.38	This study
Palaemon paucidens	126.7±7.72	70.6±0.7	< 8.36	< 0.38	This study
Oncorhynchus nerka	2.89±0.13	30.70±0.52	< 8.36	< 0.38	This study

Comparing with these results, it can be known that the heavy metal content of Lake Toya *Oncorhynchus nerka* illustrates slightly higher than *Brown trout.*

It can be also shown that the elements contents of Lake Toya *Hypomesus nipponensis* and *Palaemon paucidens* are much higher than in Sea (Table 8).

3.2.3. Human health risk assessment of heavy metals via aquatic food in Toya Lake

The daily upper tolerable doses of Zn and Cu for humans are 40 mg and 10 mg for adult males and 35 mg and 10 mg for adult females.

Formula 1 Calculation of the maximum tolerable number of samples.

$$\text{Maximum number of animals to withstand} = \frac{\text{Normal weight} \times \text{heavy metal concentration}}{\text{Dietary Intake Standards}}$$

Table 9 The maximum allowable number of aquatic organisms of Lake Toya for an adults per day.

Scientific name	Averaged weight (g)	Cu (μg/g)	Zn (μg/g)	Maximum capacity (*N*)		Daily allowance (*N*)
				for Cu	for Zn	
Oncorhynchus nerka	120	2.9	30.7	2.8	1.1	1
Hypomesus nipponensis	10.9	3.5	89.8	11.1	4.1	4
Palaemon paucidens	1.4	126.7	70.6	5.6	40	5

The result from Formula 1 can be concluded that the maximum allowable amount for an adult per day, which shows 1 animal *Oncorhynchus nerka*, 4animals *Hypomesus nipponensis* and 5 animals *Palaemon paucidens*, respectively, in Table 9.

3.2.4. Analytical results of heavy metals in water and sediments in Lake Toya.

In the water samples, the contents of Zn, Cu and Ni are reported to be 0.002, 0.003–0.005, 0.0002–0.001, and 0.003–0.004 mg/L, respectively. Co and Ni were not detected because they were lower than the detection limit. Cu has a maximum value in the water at 1 point and a minimum value at 4 points. Zn is only detected in water at 2 points. Ni has a maximum value at 2 points, and a minimum value at 4 points (Table 10). The metal concentrations in the water samples we studied were lower than the allowable standards recommended by the World Organization (Table 11).

Table 10 The concentration of heavy metals in the water sample.

	Sample point heavy metal concentration (mg/L)			
Metals	①	②	③	④
Cu	0.005	<0.003	<0.003	0.003
Co	<0.002	<0.002	<0.002	<0.002
Pb	<0.05	<0.05	<0.05	<0.05
Zn	<0.001	0.002	<0.001	<0.001
Ni	<0.002	0.004	<0.002	0.003

Table 11 Maximum permissible limits for heavy metals concentration in water.

Metals	WHO (mg/L)	PSI (mg/L)	NEQS (mg/L)
Zn	3.0	5.0	5.0
Cu	2.0	1.0-1.5	1.0
Pb	0.01	0.1	0.5
Ni	0.02	-	1.0
Co	-	-	-

In the sediments, the metal contents of Zn, Cu, Ni and Pb in the collected samples were 30.45, 9.29, 5.18 and 488.1μg/g, respectively. Among all heavy metal elements, Pb

was detected as the maximum, while cobalt was not detected. It was found that the lead metal concentration detected in the sediment was higher than the allowable standard (Table 12), which may be caused by heavy metal deposition.

Table 12 Maximum permissible limits for heavy metals concentration in sediments.

Metals	WHO (μg/g)	CEQG (μg/g)	USEPA (μg/g)
Zn	123.0	123.0	-
Cu	25.0	35.7	-
Pb	-	35.0	40.0
Ni	20.00	42.8	-
Co	-	-	-

4. Conclusion

First, a method, ion chromatography, for measuring the content for heavy metal elements in a biological sample was investigated in detail. In addition, the simultaneous separation and measurement of the five elements Pb, Cu, Zn, Ni, and Co express possible in this work. It is necessary to consider the separation of Cd and Mn a little more.

Secondly, the result of Cu and Zn content in *Oncorhynchus nerka*, *Hypomesus nipponensis* and *Palaemon paucidens* shows

Cu: *Palaemon paucidens* > *Hypomesus nipponensis* > *Oncorhynchus nerka*;

Zn: *Hypomesus nipponensis* > *Palaemon paucidens* > *Oncorhynchus nerka*.

It was found that Cu and Zn were lower organisms in long body size (*Oncorhynchus nerka*) and higher organisms in short body size (*Hypomesus nipponensis*). These results also support the viewpoint for biological detoxification. It exhibits that Cu and Zn express micro content in aquatic organisms for floating in the water but show macro content in

organisms which live in sediments. Besides, the abovementioned view is caused by heavy metals sinking to the bottom over time. Cu and Zn play an important role metabolism in human beings. In contrast, if the content shows too high, it will have an adverse effect to the human body. The human being dedicates a huge attention for edibility of this produces in daily life.

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