Study on sequential extraction of inorganic elements in solid substances

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Contents

Chapter 1

Introduction

1.1. Soil contamination

Soil contamination refers to the destruction of land that making the content of a certain component significantly higher than the original content, causing the existing or potential soil environmental quality deterioration. Soil contamination can occur either because of human activities or because of natural processes. However, mostly it is due to human activities. Heavy metals, organic solvents, pesticides, oils and other substances, which hinder the normal function of the soil, reduce crop yield and quality, often give serious harmful effects for the natural environment, human health and life as soil pollutants [1]. When soil is contaminated by harmful substances, the pollutants leached from soil move to plants, the groundwater, and river water, the contaminated crops and drinking water cause health damage [2-7].

In recent years, with the development of economy, many countries and regions have suffered serious soil pollution. For instance, soils in some Chinese areas surrounding mining, industry activities, and farmland, have been seriously contaminated [8-12]. Moreover, in the 1960s, during its period of faster postwar growth, Japan also had a serious soil pollution problem, Itai-Itai disease, caused by cadmium pollution are well known in worldwide [13-16].

For the past few years, soil contamination due to heavy metals, volatile organic compounds, etc. from old mines or development factories, has become apparent in Japan [17-18]. Therefore, Soil Contamination Countermeasures Law was enacted in 2003 to grasp the situation of soil contamination and to prevent human health damage from soil contamination, as well as survey and measures harmful substances of soil contamination

occurred at factory site etc. [19]. Furthermore, the Soil Contamination Countermeasure Law was revised substantially in 2010. After the enforcement of the new Soil Contamination Countermeasures Law, investigation reports on soil contamination were significantly increased compared to before. 80% of them were the reports for contaminations by heavy metals.

1.2. Heavy metals

Heavy metals generally refer to substances with a specific gravity of 5.0 or higher [20]. The human body needs many essential elements. The lack of these elements affects people's health. However, the excessive heavy metals cause various diseases, such as human immune system is disordered. How serious of it depends on the type of heavy metals [21]. In this study, I described the heavy metals of copper, zinc, cadmium, and lead in sediment samples.

Copper: Copper is commonly used in the manufacture of metallic copper and alloys. At the Ashio copper mine on the upstream catchment of the Watarase river in Japan, copper had been mined until 1973. However, due to the pollution of the river by copper mines, the Ashio copper pollution incident occurred in 1891, and the plants in the downstream farmland also had growth obstacles. Currently, copper concentration of the Watarase River water has been reduced to below 10 ppb in 2010, due to the greening countermeasures, but high concentrations of copper are still unevenly distributed in the sediment [23].

Copper ions are essential elements in living organisms and are the third transition metals contained in the ecosystem that are only less than iron and zinc. Each adult contains about 100 mg of copper. Also, a trace amount of copper ions is an essential element for growth of plants, but high concentrations of copper ions inhibit the growth of plant roots and adversely affect the growth of microorganisms.

Zinc: Zinc content in domestic soils is, on average, 50 to 60 ppm, Zinc in soils is

usually present as an organically bound or insoluble form [24]. Zinc is an essential element in living organisms, and its content is estimated to be 1.4 to 2.3 g in the case of 70 kg adult males. Zinc is also an essential element for plants, but if it is excessive, it can cause growth problems, such as yellowing of new leaves and brown spots.

Cadmium: Cadmium pollution in Japan is known to the Itai-Itai disease occurred in the Jinzu River Basin in Toyama [13-16]. In recent years, cadmium pollution of soil and growing plants is very serious in China. Compared with other heavy metals, cadmium exists as a greater ratio of exchange form in the soil surface, which is easily leached into river or ground waters.

Cadmium content in the blood of a normal person is extremely low ($\leq 4.9~\mu g~L^{-1}$). Cadmium is inherently toxic to both plants and animals, and has non-useful biological effects for any organisms [25-27]. In recent years, cadmium accumulation in the ecosystem as a result of human activities has been discussed as an environmental problem that may endanger the productivity and quality of crops in agricultural ecosystems [28, 29].

Lead: Lead is mainly produced in China and Australia, and is mostly in the form of lead sulfide in nature. In China and other developing countries, because the strict methods for the refining and recycling of lead-acid batteries lack even now, there are many reports on health damage caused by lead pollution [30, 31].

The average intake of lead for adults per day is estimated to be about 2 to 3 mg. Also, lead is an element which is considered unnecessary for plants. It can inhibit the growth of plants when contains large quantities of lead, but is hardly absorbed by plants.

1.3. Arsenic

Arsenic is not in the group of heavy metals. The arsenic (especially arsenite) indicates very high toxicity for human health. Because minerals containing arsenic are widely distributed throughout the world, health damages by arsenic are comparable to

those by heavy metals [32-35].

Inorganic arsenic such as $H_3As(V)O_4$ or $HAs(III)O_2$ in aqueous media cannot be easily destroyed. However, they can be removed by converting to insoluble compounds by binding to elements, such as iron. Artificial arsenic pollutions were caused by spraying of insecticide with arsenic in the agricultures, the production of iron and steel, and/or the inclusion in pollutants generated by coal oxidation. Furthermore, since arsenic exhibits a dynamic behavior similar to that of phosphorus, arsenic compounds coexist and are deposited together with phosphorus. [36, 37].

Most of human health damage by arsenic are caused by ingesting the body via food. Because most arsenic in plants and animals mainly existing as the organic forms, the toxicities are consequently low. The growths of plants are inhibited if plants absorb high concentration of inorganic arsenic in soil, and then the animals ingest the arsenic-contaminated plants and are damaged when the arsenic are significantly accumulated in the body.

1.4. Leaching test

Methods for evaluating the leachabilities of heavy metals in the soil are independently carried out in Japan and abroad. In Japan, standard levels of certain chemicals that contaminate soil are specified by Cabinet Order as being possibly causing human health damage. They are categorized 11 kinds of volatile organic compounds as the first-class specified toxic substances, 9 kinds of heavy metals as the second-class specified toxic substances, and 5 kinds of pesticides as the third-class specified toxic substances [2]. Table 1-1 summarizes the second-class specified toxic substances, which contains the targeted analytes in this study. The elution amounts of their substances from soil are investigated according to test methods summarized in No. 18 Notification from Ministry of the Environment (Table 1-2). The standard method (elution method) is established on the basis of health risks caused by leaching of specified hazardous

substances from contaminated soil into groundwater, and then the groundwater is ingested by humans.

Additionally, the content standard levels in soil are also investigated according to the methods summarized in Table 1-2, which is indicated in Ministry of the Environment Notification No. 19 [38]. The standard method (content method) is established based on the health risk by directly ingesting soil containing specific hazardous substances.

Table 1-1 Standard levels of the second-class specified toxic substances

Substance	Criteria for Designation in soil	
	Elution amount (mg L^{-1})	Content (mg kg^{-1})
Cadmium and its compounds	< 0.01	< 150
Chromium (VI) compounds	< 0.05	< 205
Cyanide	Do not be detected	< 50
Mercury and its compounds	Less than 0.0005 and alkyl mercury do not be detected	< 15
Selenium and its compounds	0.01	< 150
Lead and its compounds	0.01	< 150
Arsenic and its compound	0.01	< 150
Fluorine and its compound	0.8	< 4000
Boron and its compound	1	< 4000

Table 1-2 Sample preparation of soil elution testing method (elution method) and soil content testing method (content method).

Reagent	Elution method	Content method
	Water (pH 5.8 – 6.3)	1 mol L^{-1} HCl
Liquid-solid ratio	10 (more than 50 g / 500 mL)	100 / 3 (more than 6 g of soil)
Extraction method	Shaking (200 rpm, 4-5 cm amplitude), 6h, ordinary temperature pressure	Shaking (200 rpm, 4-5 cm amplitude), 2h, ordinary temperature pressure)
Solid-liquid separation	Centrifugal separation (3000 rpm, 20 min), filter (0.45 μm)	Centrifugal separation (3000 rpm, 20 min), filter (0.45 μm)

1.5. Speciation analysis

Heavy metals present in the environment have different toxicities and absorptions to organisms such as human, or plant, which depending on their chemical forms. At present, as a method of determining elution amount and content of heavy metals, the elution and content testing methods shown in Ministry of the Environment Notification No. 18 and No. 19 are commonly used as mentioned in section 1.3. [38, 39].

However, evaluating the influence of heavy metals on the environment requires not only understanding the total concentration of heavy metals but also their chemical forms concerning to their dissolution properties [40, 41]. Also, it must also be clear that some indistinct points about how the heavy metals move and accumulate in the soil with the environmental conditions such as climate, biological and topography. One of the analysis methods to elucidate these problems is the sequential extraction procedure (SEP) [42-48]. SEP is an extraction method for chemical form of elements, which depends on gradually adding of extractants with the different extraction powers to fractionation of elements associate to carbonate, oxide, organic matter phases, following a stepwise manner.

In this thesis, analysis of chemical form of heavy metals, arsenic and valuable metals contained in soil was carried out by the SEP proposed by Tessier et al [22, 49-51], that is, it is a method that classifies elements contained in soil into five fractions according to the degree of elution and is classified as follows:

Fraction 1 (F1): ease eluted by salts

Fraction 2 (F2): bound to carbonates

Fraction 3 (F3): bound to iron and manganese-oxide

Fraction 4 (F4): bound to organic matter

Fraction 5 (F5): residual

This method can estimate not only the abundance ratios of five fractionations according to the solubilities of targeted metal-ligand complexes in soil, but also the possibility of elution of targeted metal in soil. Currently, the SEP has been applied to farmland soil, sediment, waste residue, sewage sludge, incineration ash, food etc., by many researchers in worldwide [52-58]. It is well-used also for the long-term

environmental impact assessment of soils or sediments contaminated by toxic heavy metals.

1.6. Automatic devise in sequential extraction procedure (SEP)

SEP is an effective extraction method for analyzing the leachability of heavy metals contained in solid samples, by using different extractants.

However, the operation procedures of the conventional SEP operations somewhat complicated because it needs many handling processes such as reagent preparation, centrifugation, filtration and so on. Such batch procedures easily cause sample contaminations, which affects analytical accuracies. To avoid the sample contaminations during the procedures, it is necessary to simplify the complicated treatment processes. Although the several methods to solve such problems are reported [57, 59, 60], we introduced flow analysis method as novel concept to the SEP.

1.6.1. Flow analysis method

Originally, a flow analysis method is also called flow injection analysis system (FIA) [61-64]. The FIA system mixes analyte samples and reagents in a flow path formed by a Teflon tube and connector with an inner diameter of 5 mm or less. The analyte colored by reagent can be measured by visible light or fluorescence detectors as shown in Fig.1-1a.

The FIA can precisely control the solution flow rate, reaction and detection time, so that it can quantify amount of the reaction product, even if the chemical reaction is not achieved to the reaction equilibrium. In addition, the FIA is highly evaluated to promote the concepts of zero emission and green chemistry by contributing to analytical techniques with accuracy, sensitivity, simplification and automation in chemical analysis. The applications of FIA to real samples are spreading over the field of analysis of foods or pharmaceuticals [59]. In 2011, the Japanese Industrial Standard (JIS) "K0170: Water

quality test by flow analysis method" was publicly announced. In addition, several FIA modes according to the properties of analytes were developed, *e.g.*, stopped-in-loop flow analysis [65], multi-syringe flow analysis [66], all injection analysis (AIA) [67].

In this study, AIA system was employed as a novel method in which reagent and sample solutions are introduced into a thin tube flow path, then a flow path was closed with a switching valve and the reaction is performed by circulating and mixing the reagent and the sample in the flow path (Fig. 1-1b). This method has the advantage that the amount of reagent and waste liquid of one analysis can be markedly reduced as compared with the conventional FIA, since it is possible to stop the feeding of the solution except for the channel through which the circulation and mixing are performed.

(a)
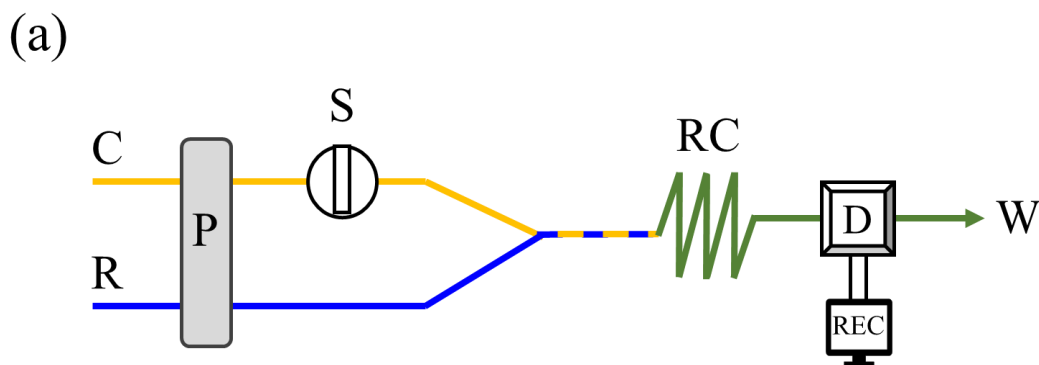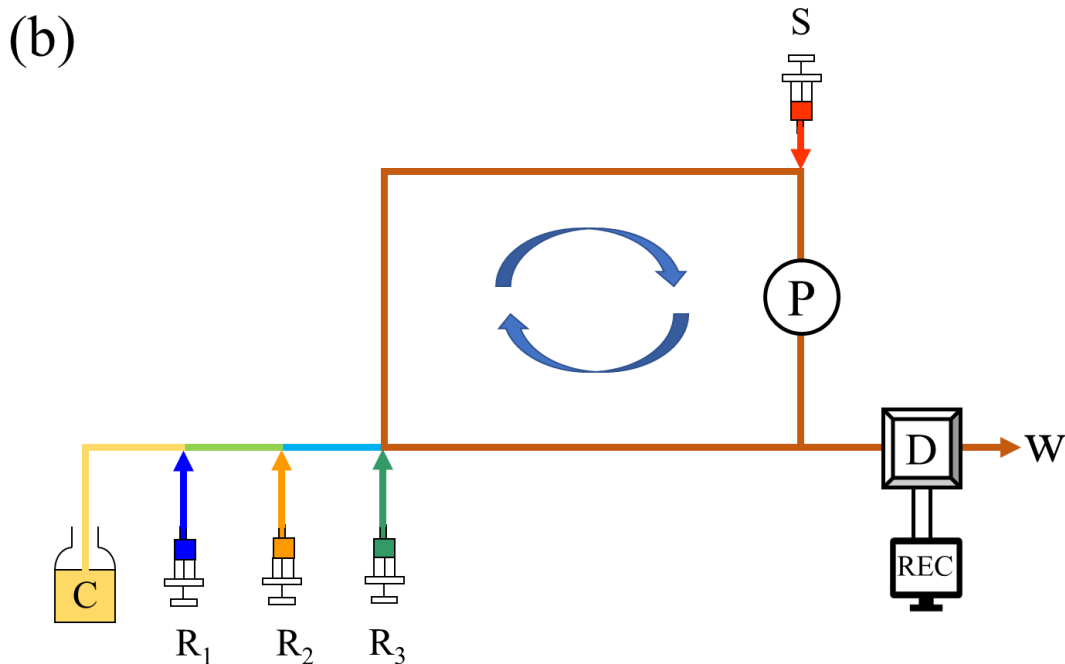
Fig. 1-1 Schematic diagram of flow path of (a) Conventional FIA system and (b) AI analysis system. C: carrier solution; R and R1-R3: reagent; S: Sample solution; P: Pump; RC: Reaction coil; D: Detector; REC: Recorder; and W: Waste.

1.6.2. All injection (AI) heavy metal extraction system

We developed as an automatic inline extraction system that introduced the concept of AIA system (Fig. 1-1b) [68]. This is called "the AI system". As shown in Fig. 1-2, the equipment consists of single rotary pump, single eight-port valve, single four- port valve, and single micro column to pack solid sample (Fig.1-2b). The valve switching or solution loading into the flow channel is controlled by the dedicated control panel installed in the devise. The extraction of heavy metals from soil sample is performed by circulating extractant in closed-flow channel with soil-laden column at certain flow rate.

The extraction system can operate without centrifugation or filtration as compared with the conventional batch system, and effectively suppress amounts of reagents and waste liquid.
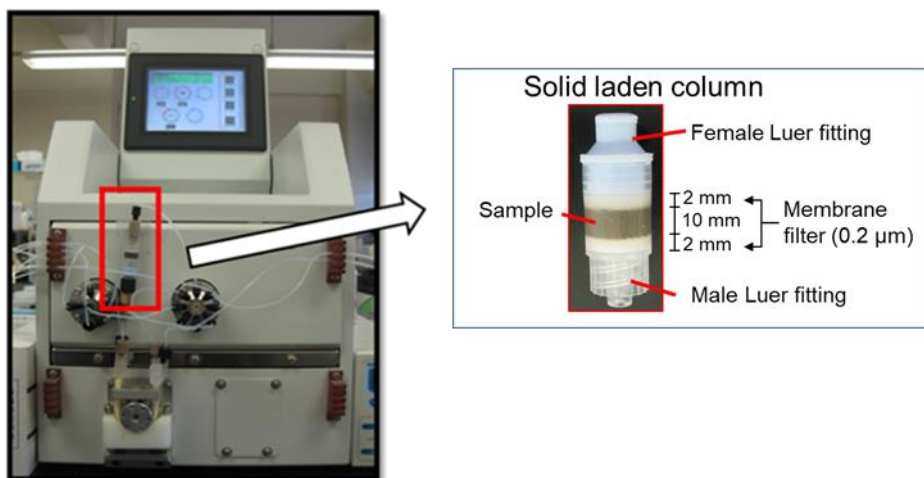
Fig. 1-2 Photos of (a) the AI type-extraction device; and (b) solid laden column

1.6.3. Four-channel circulating flow system

In this study, to increase operation efficiency in the AI system, a four-channel all injection and circulating flow (4-AI) system is developed as the novel extraction platform (as described in chapter 5). The 4-AI system can simultaneously perform the extraction procedures of trace elements from multiple soil samples, by injecting all amount of extractant into multiple flow channels and circulating extractant in closed-channel system by switching the valve. Following the circulation, the solutions including target elements extracted from solid were flushed out by pure water. The 4-AI system could dramatically improve for a four-step SEP, compared to the conventional handling batch procedure and the single channel type AI system as mentioned above. The details are introduced in chapter 5.

1.7. Kusatsu hot spring area

1.7.1. Kusatsu hot spring

In Japan, the large numbers of hot springs containing natural hot and mineral springs are distributed nationwide, especially in Hokkaido, Nagano and Gunma prefectures [69].

Kusatsu hot spring is known as an acidic volcanic hot spring located in the northwest in the Gunma Prefecture. According to the historic record, this hot spring was used as cure from the 1200s, and was promoted as the modern hot-spring therapy by the German doctor, who introduced balneology from German to Japan in the late 1800 [70]. At present, the spa becomes the main tourist sight not only in Japan but also outside of the country.

Kusatsu-town is located in the flatness side of the east foot of Mt. Shirane (2,160m above sea level), which is formed with some pyroclastic flows and lava spouted out with the volcanic activity since about 600,000 years ago [71]. The hot spring water that originates Kusatsu-Shirane volcanism is strong acidic with much sulfate and chlorine, and has strongly affected the water quality of downstream rivers (e.g. Yukawa, Yazawa and Osawa rivers) from the hot spring spa area.

1.7.2. Neutralization of acidic hot spring

Flowing of the strong acidic hot spring into the downstream rivers of Kusatsu hot spring spa area (Yukawa, Yazawa and Osawa rivers), the qualities of the river waters are affected. The hot spring water contains the large amounts of metals, arsenic and trace amounts of valuable metals. Consequently, by flowing of the hot spring water, the rivers contain their metals with acidification of the pHs. For instance, the content of arsenic in Bandaiko hot spring is 9.2 mg L^{-1} [72]. If the acidified rive waters directly flows into dam-lake located in the downstream of the rivers, it will lead to contamination of arsenic and heavy metals in ecosystems living in the lake (Shinaki dam-lake) and depletion of vegetation by the acid in the area surrounding the lake.

Therefore, the neutralizations of the acidified river waters are carried out by adding lime suspensions at Ministry of Land, Infrastructure, Transport and Tourism (MLIT) since 1964 [73]. The corresponding quantities of lime introduced into these rivers are 53, 4.1, and 7.4 tons per day, respectively. As the result that the pH of river water after neutralization rises to 5-7, the harmful and valuable metals are coprecipitated by lime water and deposited in bottoms of the river and Sinaki dam-lake [6].

1.7.3. Shinaki dam-lake

Shinaki dam-lake was constructed in 1965 with the neutralization facility [74]. The neutralizing compounds generated as a result of chemical reaction between calcium hydroxide / calcium carbonate (lime) and acid (sulfuric acid / hydrochloric acid) are accommodated in the dam lake. Also, the stored lake water is used for power generation as clean energy. However, since hot springs supplied from Kusatsu-Shirane volcano at the upstream of the dam contain a large amount of heavy metals and arsenic, these elements are also contained in the sediment in the dam-lake [73].

In this study, we evaluated leachabilities of the heavy metals (Cu, Zn, Cd), arsenic, valuable metal (Pd) and rare earth element (Y) in the sediment of Shinaki dam-lake using the SEP.

1.8. Kusaki dam

In the late Meiji period, the Ashio Copper Mine Incident is known as the starting line of environmental pollution problems in Japan [75]. This pollution incident caused the Ashio Copper Mine happened around the Watarase River in Tochigi and Gunma Prefectures from the 19th century to the 20th century and has drawn great attention. By this incident, high level concentration of poisonous metals including Cu and As were discovered in the Watarase River waters and around of soils.

In 1976 Kusaki dam was built in the Watarase basin. It is currently managed by the Incorporated Administrative Agency Japan Water Agency (JWA), which controls the flood, water supply, industrial water, power generation, etc. However, owing to the influence of typhoon and torrential rain, a large amount of soil and gravel flowed in the dam were accumulated to 52% until 2013 [76]. In addition, removal of the sludge soil was carried out annually by JWA, considering the impact of Ashio copper mine in the upstream area of the Kusaki dam. Therefore, we estimated the leachabilities of heavy

metals in the lake sediments, in order to prevent the secondary pollution by heavy metals in the landfill where damped the sediment removed from the lake using SPE by the AI system (chapters 4 and 5).

1.9. Fly ash

In human life, garbage is necessarily discharged. In Japan, the garbage is incinerated at a garbage incineration plant. At that time, the incineration ashes, which contains main ash and fly ash, are generated. The particle shape of main ash is rough, and the size is very heterogeneous. In contrast, the particle shape of fly ash is fine, and the size is uniform.

Currently, incineration ash is discharged about 17 Mt per year worldwide, most of the incineration ash is not effectively reused, and it is treated with a lot of dumping landfill costs [77, 78].

In Japan, nearly 80% of general municipal waste is incinerated, and the amount of incineration ash has reached 8000,000 t annually. The amounts are expected to continue to increase over the long term in the future. As a result, the lack of landfill land with increase of incineration ash may lead to occurrence of secondary pollution with leaching of heavy metals from the ashes dumped in the landfill [79, 80].

Therefore, we investigated the leaching behaviors of heavy metals of the fly ash collected from the cleaning center of Kiryu city, Gunma Prefecture using SPE by the AI system in chapter 5.

1.10. Purpose of this study

The main purpose of this study is to establish a novel extraction devise that injects and circulates all amount of extractant in the closed-flow channel in order to effectively extract the targeted element and to estimate the leachability. To this end, Kusatsu hot-spring area as the main object field was selected in this study. The monitoring of

environments variation in Kusatsu hot-spring area was investigated detailly, and the leachability of palladium, arsenic and heavy metals contained in the sediment of Kusatsu area was evaluated by using conventional sequential extraction before analyzed by the novel extraction devise.

1.11. Contents of this thesis

This thesis consists of six chapters as follows.

Chapter 1 describes the backgrounds needed to achieve the purposes of this study, subjects to solve problems found from the backgrounds, and purpose of this study.

Chapter 2 describes the environmental variation in Kusatsu hot spring area to seasonal changes by monitoring the annual variations of pH, temperature, and flow rate in river and lake water. Also, this chapter reports that the environment of the river is significantly changed by adding the lime suspension at the neutralization factory, through the monitoring of chemical components (As, Y, Pd and Cd), pH and electrical conductivity (EC) in Yukawa river water before and after neutralization.

Chapter 3 describes the distribution and leachability of Pd, which is a valuable metal, in the river water and sediment samples obtained across the streams of Yukawa and Yazawa rivers, and those of Shinaki dam lake located in the downstream. The leachability of Pd from the sediment samples was estimated using sequential extraction procedure (SEP) by batch system. As the results, the leachabilities of Pd from sediments of the river and lake after adding lime at neutralization were largely different with those before adding lime. This meant that the Pd in river was bound to lime from crystal structure and was then settled on the river and lake beds.

Chapter 4 describes an automatic inline extraction system called the all injection (AI) system, which consists of a rotary pump, an eight-port valve, a micro-column to pack solid sample, a control panel and some reaction coils. This system was developed to aim preventing the sample contaminations during handling operations that were often caused

in the conventional batch system. Trough the SEP, the overall extraction abilities of the AI system for heavy metals and arsenic demonstrated to be comparative and/or higher to those of the batch system. The fact that the AI system could skip the handling procedures, such as centrifugations or filtrations, lead to reducing of waste amounts and experimental times, minimization of sample contamination, and accuracy analytical results.

Chapter 5 describes the SEP of heavy metals and arsenic in lake sediment and fly ash using a novel inline extraction system with four circulation channels "the 4-AI system" developed to carry out the further effective metal extraction than the AI system describes in chapter 4. The results indicate that the SEP by the 4-AI system is a good method for fractionating trace elements in solid samples. The four-channel flow system gave shorter extraction times than can be achieved using conventional batch fractionation methods or the AI system with single circulation channel. The combination of multi-column system and closed-circulation process make the circulation extraction system a facile and reliable method for determining the concentrations of bioavailable metals under steady-state conditions. Furthermore, this chapter explains also for approach to chelating extraction using a chelating reagent.

Chapter 6 summarizes the results obtained in each chapter.

1.12. References

[1] K. M. Scow, and C. R. Johnson, Effect of sorption on biodegradation of soil pollutants, Advances in Agronomy, 58 (1996) 1-56.

[2] The soil contamination counter measures act in Japan
http://www.env.go.jp/water/dojo/law.html <accessed：2017/01/10>

[3] A. A. Meharg, and M. M. Rahman, Arsenic contamination of Bangladesh paddy field soils: implications for rice contribution to arsenic consumption, Environmental Science & Technology, 37 (2003) 229-234.

[4] S. Khan, Q. Cao, Y. M. Zheng, Y. Z. Huang, and Y. G. Zhu, Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China, Environmental Pollution, 152 (2008) 686-692.

[5] M. N. Rashed, Monitoring of contaminated toxic and heavy metals, from mine tailings through age accumulation, in soil and some wild plants at Southeast Egypt, Journal of Hazardous Materials, 178 (2010) 739-746.

[6] M. Arias-Estévez, E. López-Periago, E. Martínez-Carballo, J. Simal-Gándara, J. C. Mejuto, and L. García-Río, The mobility and degradation of pesticides in soils and the pollution of groundwater resources. Agriculture, Ecosystems & Environment, 123 (2008) 247-260.

[7] D. J. Lapworth, N. Baran, M. E. Stuart, and R. S. Ward, Emerging organic contaminants in groundwater: a review of sources, fate and occurrence, Environmental Pollution, 163 (2012) 287-303.

[8] F. J. Zhao, Y. Ma, Y. G. Zhu, Z. Tang, S. P. McGrath, Soil contamination in China: current status and mitigation strategies, Environmental Science & Technology, 49 (2014) 750-759.

[9] L. Wang, X. Cui, H. Cheng, F. Chen, J. Wang, X. Zhao, ... and X. Pu, A review of soil cadmium contamination in China including a health risk assessment, Environmental Science and Pollution Research, 22 (2015) 16441-16452.

[10] X. Tang, C. Shen, D. Shi, S. A. Cheema, M. I. Khan, C. Zhang, and Y. Chen, Heavy metal and persistent organic compound contamination in soil from Wenling: an emerging e-waste recycling city in Taizhou area, China, Journal of Hazardous Materials, 173 (2010) 653-660.

[11] H. Liu, A. Probst, and B. Liao, Metal contamination of soils and crops affected by the Chenzhou lead/zinc mine spill (Hunan, China), Science of the Total Environment, 339 (2005) 153-166.

[12] L. Tang, X. Y. Tang, Y. G. Zhu, M. H. Zheng, and Q. L. Miao, Contamination of polycyclic aromatic hydrocarbons (PAHs) in urban soils in Beijing, China, Environment International, 31 (2005) 822-828.

[13] E. Kobayashi, Y. Suwazono, M. Dochi, R. Honda, and T. Kido, Influence of consumption of cadmium-polluted rice or Jinzu River water on occurrence of renal tubular dysfunction and/or Itai-itai disease, Biological Trace Element Research, 127 (2009) 257-268.

[14] H. Baba, K. Tsuneyama, T. Kumada, K. Aoshima, and J. Imura, Histopathological analysis for osteomalacia and tubulopathy in itai-itai disease, The Journal of Toxicological Sciences, 39 (2014) 91-96.

[15] K. Nogawa, A. Ishizaki, M. Fukushima, I. Shibata, and N. Hagino, Studies on the women with acquired Fanconi syndrome observed in the Ichi River basin polluted by cadmium. Is this Itai-itai disease? Environmental Research, 10 (1975) 280-307.

[16] M. Kasuya, Natural history of Itai-itai disease: a long-term observation on the clinical and laboratory findings in patients with Itai-itai disease, Seventh International Cadmium Conference, London, 1992, Cadmium Association.

[17] T. Arao, S. Ishikawa, M. Murakami, K. Abe, Y. Maejima, and T. Makino, Heavy metal contamination of agricultural soil and countermeasures in Japan, Paddy and Water Environment, 8 (2010) 247-257.

[18] Y. Tashiro, A. Goto, T. Kunisue, T. Kurahashi, and S. Tanabe, Contamination Status of PCBs and Organochlorine Pesticides in the Okinawa Island, Japan: Utilization of

Small Indian Mongoose (Herpestes auropunctatus) as a Bioindicator, Journal of Environmental Chemistry, 26 (2016) 115-122.

[19] Soil Environment Management Division Environmental Management Bureau, Ministry of the Environment Government of Japan, Soil Contamination Countermeasures,

https://www.env.go.jp/en/water/soil/contami_cm.pdf <accessed：2018. 02. 08>

[20] F. L. Fu, and W. Qi, Removal of heavy metal ions from wastewaters: a review, Journal of environmental management, 92 (2011) 407-418.

[21] P. B. Tchounwou, C. G. Yedjou, A. K. Patlolla, and D. J. Sutton, Heavy metal toxicity and the environment, Molecular, Clinical and Environmental Toxicology, 101 (2012) 133-164.

[22] K. Shoji and M. Sugai, The Ashio copper mine pollution case: the origins of environmental destruction. Industrial Pollution in Japan. United Nations University Press, Tokyo, 1992, pp. 18-63.

[23] Y. Iwasaki, M. Soya, M. Takasu, Y. Zushi, T. I. Hayashi, and S, Kashiwada, Spatiotemporal changes in water quality along a historically metal-contaminated river: a retrospective analysis of 50 years of monthly monitoring data, Limnology, 19 (2018) 157-163.

[24] V. S. Saravanan, M. Madhaiyan, and M. Thangaraju, Solubilization of zinc compounds by the diazotrophic, plant growth promoting bacterium Gluconacetobacter diazotrophicus, Chemosphere, 66 (2007) 1794-1798.

[25] S. Khandelwal, J. L. Shukla, and R. Shanker, Modulation of acute cadmium toxicity by Emblica officinalis fruit in rat, Indian Journal of Experimental Biology, 40 (2002) 564-570.

[26] L. Tort and P. Torres, The effects of sublethal concentrations of cadmium on haematological parameters in the dogfish, Scyliorhinus canicular, Journal of Fish Biology, 32 (1988) 277-282.

[27] A. J. Bednarska, M. Opyd, E. Żurawicz, and R. Laskowski, Regulation of body metal

concentrations: toxicokinetics of cadmium and zinc in crickets, Ecotoxicology and Environmental Safety, 119 (2015) 9-14.

[28] L. M. Sandalio, H. C. Dalurzo, M. G. ómez, M. C. Romero-Puertas, L. A. del Río, Cadmium-induced changes in the growth and oxidative metabolism of pea plants, Journal of Experimental Botany, 52 (2001) 2115-2126.

[29] F. Chen, J. Dong, F. Wang, F. Wu, G. Zhang, G. Li, Z. Chen, J. Chen, and K. Wei, Identification of barley genotypes with low grain Cd accumulation and its interaction with four microelements, Chemosphere, 67 (2007) 2082-2088.

[30] H. Zhan, Y. Jiang, J. Yuan, X. Hu, O. D. Nartey, B. Wang, Trace metal pollution in soil and wild plants from lead–zinc smelting areas in Huixian County, Northwest China, Journal of Geochemical Exploration, 147 (2014) 182-188.

[31] H. Sun, Y. Li, Y.Ji, L. Yang, W. Wang, and H. Li, Environmental contamination and health hazard of lead and cadmium around Chatian mercury mining deposit in western Hunan Province, China, Transactions of Nonferrous Metals Society of China, 20 (2010) 308-314.

[32] T. S. Choong, T. G. Chuah, Y. Robiah, F. G.Koay, and I. Azni, Arsenic toxicity, health hazards and removal techniques from water: an overview, Desalination, 217 (2007) 139-166.

[33] K. Jomova, Z. Jenisova, M. Feszterova, S. Baros, J. Liska, D. Hudecova, C.J. Rhodes, and M. Valko, Arsenic: toxicity, oxidative stress and human disease, Journal of Applied Toxicology, 31 (2011) 95-107.

[34] G. M. Panaullah, T. Alam, M. B. Hossain, R. H. Loeppert, J. G. Lauren, C. A. Meisner, Z. U. Ahmed, and J. M. Duxbury, Arsenic toxicity to rice (Oryza sativa L.) in Bangladesh, Plant and Soil, 317 (2009) 31-39.

[35] M. R. Karagas, A. Gossai, B. Pierce, and H. Ahsan, Drinking water arsenic contamination, skin lesions, and malignancies: a systematic review of the global evidence, Current Environmental Health Reports, 2 (2015) 52-68.

[36] A. Hartland, J. R. Larsen, M. S. Andersen, M. Baalousha, and D. O'Carroll,

Association of arsenic and phosphorus with iron nanoparticles between streams and aquifers: implications for arsenic mobility, Environmental Science & Technology, 49 (2015) 14101-14109.

[37] D. O'Carroll, A. Hartland, J. Larsen, and M. Andersen, Association of arsenic and phosphorus with iron nanoparticles in the hyporheic zone, EGU General Assembly Conference Abstracts, 18 (2016) 13815.

[38] Ministry of the Environment Notification No. 19, http://www.env.go.jp/hourei/06/000029.html <accessed：2017. 01. 09>

[39] Ministry of the Environment Notification No. 18, http://www.env.go.jp/hourei/06/000031.html <accessed：2017. 01. 09>

[40] P. Li, C. Lin, H. Cheng, X. Duan, and K. Lei, Contamination and health risks of soil heavy metals around a lead/zinc smelter in southwestern China, Ecotoxicology and Environmental Safety, 113 (2015) 391-399.

[41] B. Keshavarzi, Z. Tazarvi, M. A. Rajabzadeh, and A. Najmeddin, Chemical speciation, human health risk assessment and pollution level of selected heavy metals in urban street dust of Shiraz, Iran, Atmospheric Environment, 119 (2015) 1-10.

[42] M. K. Jamali, T. G. Kazi, M. B. Arain, H. I. Afridi, N. Jalbani, G. A. Kandhro, A. Q. Shah, J. A. Baig, Speciation of heavy metals in untreated sewage sludge by using microwave assisted sequential extraction procedure, Journal of Hazardous Materials, 163 (2009) 1157-1164.

[43] M. B. Arain, T. G. Kazi, M. K. Jamali, N. Jalbani, H. I. Afridi, J. A. Baig, Speciation of heavy metals in sediment by conventional, ultrasound and microwave assisted single extraction methods: A comparison with modified sequential extraction procedure, JournaAl of Hazardous Materials, 154 (2008) 998-1006.

[44] H. Abd El-Azim, K. M. El-Moselhy, Determination and partitioning of metals in sediments along the Suez Canal by sequential extraction, Journal of Marine Systems, 56 (2005) 363-374.

[45] B. P. Cid, A. F. Alborés, E. F. Gómez, E. F. López, Use of microwave single extractions for metal fractionation in sewage sludge samples, Analytica Chimica Acta, 431 (2001) 209-218.

[46] J. Shiowatana, N. Tantidanai, S. Nookabkaew, D. Nacapricha, A flow system for the determination of metal speciation in soil by sequential extraction, Environment International, 26 (2001) 381-387.

[47] A. M. Ure, Ph. Quevauviller, H. Muntau, B. Griepink, Speciation of heavy metals in soils and sediments. An account of the improvement and harmonization of extraction techniques undertaken under the auspices of the BCR of the Commission of the European Communities, International Journal of Environmental Analytical Chemistry, 51 (1993) 135-151.

[48] A. Tessier, P. G. C. Campbell, and M. Bisson, Sequential extraction procedure for the speciation of particulate trace metals, Analytical Chemistry, 51 (1979) 844-851.

[49] J. L. Roulier, S. Belaud, and M. Coquery, Comparison of dynamic mobilization of Co, Cd and Pb in sediments using DGT and metal mobility assessed by sequential extraction, Chemosphere, 79 (2010) 839-843.

[50] A. Giannis, D. Pentari, J. Y. Wang, and E. Gidarakos, Application of sequential extraction analysis to electrokinetic remediation of cadmium, nickel and zinc from contaminated soils, Journal of Hazardous Materials, 184 (2010) 547-554.

[51] V. Jena, S. Gupta, R. S. Dhundhel, N. Matić, S. Frančišković-Bilinski, and N. Dević, Determination of total heavy metal by sequential extraction from soil, International Journal of Research in Environmental Science and Technology, 3 (2013) 35-38.

[52] E. Frossard, P. Demaria, S. Ssinaj, M. Schärer, A flow-through reactor to assess potential phosphate release from agricultural soils, Geoderma, 219-220 (2014) 125-135.

[53] L. Leleyter, C. Rousseau, L. Biree, F. Barayd, Comparison of EDTA, HCl and sequential extraction procedures, for selected metals (Cu, Mn, Pb, Zn), in soils, riverine and marine sediments, Journal of Geochemical Exploration, 116-117 (2012)

51-59.

[54] J. Kim, S. Hyun, Nonequilibrium leaching behavior of metallic elements (Cu, Zn, As, Cd, and Pb) from soils collected from long-term abandoned mine sites, Chemosphere, 134 (2015) 150-158.

[55] L. Dabrowska, A. Rosińska, Change of PCBs and forms of heavy metals in sewage sludge during thermophilic anaerobic digestion, Chemosphere, 88 (2012) 168-173.

[56] H. K. Hansen, A. J. Pedersen, L. M. Ottosen, A. Villumsen, Speciation and mobility of cadmium in straw and wood combustion fly ash, Chemosphere, 45 (2001) 123-128.

[57] W. Boonjob, M. Rosende, M. Miró, V. Cerdá, Critical evaluation of novel dynamic flow-through methods for automatic sequential BCR extraction of trace metals in fly ash, Analytical and Bioanalytical Chemistry, 394 (2009) 337-349.

[58] A. Leufroy, L. Noël, D. Beauchemin, T. Guérin, Use of a continuous leaching method to assess the oral bioaccessibility of trace elements in seafood, Food Chemistry, 135 (2012) 623-633.

[59] M. Rosende, L. M. Magalhães, M. A. Segundo, M. Mirò, Assessing oral bioaccessibility of trace elements in soils under worst-case scenarios by automated in-line dynamic extraction as a front end to inductively coupled plasma atomic emission spectrometry, Analytica Chimica Acta, 842 (2014) 1-10.

[60] M. Rosende, L. Beesley, E. Moreno-Jimenez, M. Mirò, Automatic flow-through dynamic extraction: A fast tool to evaluate char-based remediation of multi-element contaminated mine soils, Talanta, 148 (2016) 686-693.

[61] E. Viollier, P. W. Inglett, K. Hunter, A. N. Roychoudhury, and P. Van Cappellen, The ferrozine method revisited: Fe(II)/Fe(III) determination in natural waters, Applied Geochemistry, 15 (2000) 785-790.

[62] M. Van Son, R. C. Schothorst, and G. Den Boef, Determination of total ammoniacal introgen in water by flow injection analysis and a gas diffusion membrane, Analytica Chimica Acta, 153 (1983) 271-275.

[63] N. Rybkowska, K. Strzelak, and R. Koncki, A comparison of photometric methods for serum iron determination under flow analysis conditions, Sensors and Actuators B: Chemical, 254 (2018) 307-313.

[64] M. Gawin, J. Konefał, B. Trzewik, S. Walas, A. Tobiasz, H. Mrowiec, E. Witek, Preparation of a new Cd (II)-imprinted polymer and its application to determination of cadmium(II) via flow-injection-flame atomic absorption spectrometry, Talanta, 80 (2010) 1305-1310.

[65] A. A. Quezada, K. Ohara, N. Ratanawimarnwong, D. Nacapricha, H. Murakami, N. Teshima, T. Sakai, Stopped-in-loop flow analysis system for successive determination of trace vanadium and iron in drinking water using their catalytic reactions, Talanta, 144 (2015) 844-850.

[66] V. Cerdá, J. M. Estela, R. Forteza, A. Cladera, E. Becerra, P. Altimira, P. Sitjar, Flow techniques in water analysis, Talanta, 50 (1999) 695-705.

[67] H. Itabashi, H. Kawamoto, T. Kawashima, A novel flow injection technique: All injection analysis, Analytical Sciences, 17 (2001) 229-231.

[68] M. Mori, K. Nakano, M. Sasaki, H. Shinozaki, S. Suzuki, C. Okawara, M. Mirò, H. Itabashi, Dynamic leaching and fractionation of trace elements from environmental solids exploiting a novel circulating-flow platform, Talanta, 148 (2016) 617-625.

[69] M. Serbulea and U. Payyappallimana, Onsen (hot springs) in Japan—Transforming terrain into healing landscapes, Health & Place, 18 (2012) 1366-1373.

[70] K. Saito, A general review of latest researches on acid springs, The Journal of Japanese Balneo-Climatological Association, 25 (1961) 287-306.

[71] Y. Hayakawa and M. Yui, Eruptive history of the Kusatsu Shirane volcano, The Quaternary Research (Daiyonki-Kenkyu), 28 (1989) 1-17.

[72] Y. Kikawada, S. Kawai, K. Shimada, and T. Oi, Arsenic Originating in Kusatsu Hot Springs, Gunma, Japan, Journal of Disaster Research, 3 (2008) 261-269.

[73] Y. Kikawada, S. Kawai, T. Oi, Long-term changes in the concentration of dissolved arsenic and its present supply in the Kusatsu hot springs, Gunma, Japan,

Chikyukagaku (Geochemistry), 40 (2006) 125-136.

[74] Shinaki Dam Water Quality Control Office, Ministry of Land, Infrastructure and Transport Kanto Regional Development Bureau, http://www.ktr.mlit.go.jp/sinaki/dam/index.html <accessed：2015. 04. 10>

[75] K. Ohmichi, Y. Seno, A. Takahashi, K. Kojima, H. Miyamoto, M. Ohmichi, and K. Machida, Recent heavy metal concentrations in Watarase Basin around Ashio Mine, Journal of Health Science, 52 (2006) 465-468.

[76] Ministry of Land, Kanto Regional Development Bureau, http://www.ktr.mlit.go.jp/ktr_content/content/000643300.pdf <accessed : 2018. 09. 02>

[77] S. Feng, X. Wang, G. Wei, P. Peng, Y. Yang, and Z. Cao, Leachates of municipal solid waste incineration bottom ash from Macao: Heavy metal concentrations and genotoxicity, Chemosphere, 67 (2007) 1133-1137.

[78] J. M. Chimenos, M. Segarra, M. Fernández, and F. Espiell, Characterization of the bottom ash in municipal solid waste incinerator, Journal of Hazardous Materials, 64 (1999) 211-222.

[79] C. Verma, S. Madan, and A. Hussain, Heavy metal contamination of groundwater due to fly ash disposal of coal-fired thermal power plant, Parichha, Jhansi, India, Cogent Engineering, 3 (2016) 1179243 (*Open Access*).

[80] T. Zhipeng, Z. Bingru, H. Chengjun, T. Rongzhi, Z. Huangpu, and L. Fengting, The physiochemical properties and heavy metal pollution of fly ash from municipal solid waste incineration, Process Safety and Environmental Protection, 98 (2015) 333-341.

Chapter 2

Annual monitoring of environments in Kusatsu hot-spring area

2.1. Introduction

The Kusatsu region, situated northwest in the Gunma Prefecture in Japan, is a famous hot spa area located approximately 1100–1200 m above sea level at the east foot of Mt. Shirane [1]. The hot spring water that originates from the Kusatsu-Shirane volcano indicates a strongly acidic pH owing to the presence of significant quantities of sulfuric and hydrochloric acid, as well as large amounts of arsenic, rare earth elements and metal ions from the hot springs and volcanic eruptions that flow into the rivers surrounding the hot springs [2-4]. To address this issue, acidic river water in the Yukawa, Yazawa, and Osawa tributary rivers has been neutralized by adding a lime suspension since 1964 [5-7]. This process is carried out at a neutralization factory located between the hot spring town and the Shinaki dam-lake (36° 37' 41" N, 138° 38' 04" E), and the corresponding quantities of lime introduced into Yukawa, Yazawa and Osawa rivers are 53, 4.1, and 7.4 tons per day, respectively [8]. Since the addition of lime can shift the pH of the river water from acid to neutral or weak base, many heavy metals present in the water are precipitated as their corresponding hydroxides and/or bound to hydrous ferric oxides (HFOs). This causes sediments containing precipitates with high heavy metal concentrations to flow into the Shinaki dam, thereby necessitating an annual removal by dredging with a high-pressure suction pump [9-10].

In recent years, many researchers have focused on the research of Kusatsu hot-spring. For instance, they reported the speciation of metals and arsenic in hot spring water [11], the relationship between Kusatsu-Shirane volcano and hot spring water [12], and the

effects of hot spring water on human health [13]. However, there were hardly reports on the changes in the conditions of acidic river water, such as pH, river flow rate or water temperature to the seasonal and geographical changes, and the variations of element concentrations caused by those changes.

In addition, although there have been many studies on harmful elements, such as arsenic, in Kusatsu hot springs waters [2], reports of annual variations of valuable metals such as precious metals and rare earth metals in Kusatsu hot spring water were few so far. Moreover, when we conducted a qualitative analysis of the hot spring water in the Kusatsu area, we were surprised to find the existence of Pd and Y. Thus, in this study, we also studied on the annual changes in Pd and Y in river and hot spring water.

Therefore, we here aimed to clarify environmental impacts of the river and lake by adding the lime suspension at neutralization factory.

This chapter first describes the monitoring results that observed the changes in pH, temperature and flow rate of the river water and the lake water (Shinaki dam lake) in 2017. Next, this chapter reports the changes of several elements (As, Y, Pd and Cd) concentrations, and the water qualities (pH and electrical conductivity (EC)) in Yukawa and Yazawa river waters before and after adding lime suspension at neutralization.

2.2. Experimental

2.2.1. Collection of hot spring and river water samples

Water samples annually monitored were collected from before and after neutralization of Yukawa river (B5 and A1 in Yukawa river area in Fig. 2-1) by staffs of the Shinaki dam quality management office of Kanto Regional Bureau of Ministry of land, in each month of 2017. The collections of the hot spring water and the river water samples using water quality monitoring including metal concentrations, pH and EC were undertaken at the Kusatsu hot-spring area on 18 October 2016. Their sampling sites are shown in Fig. 2-1.

The hot spring and river water samples were collected from a spout of hot spring in Sainokawara park, Yubatake, and Bandaiko springs, as well as from different riverbed locations before and after the neutralization points of the Yukawa and Yazawa rivers. A hot spring water in the Sainokawara park (SP) as the comparison with the river water samples was collected from a spout of a hot spring near a stream that flows along Yukawa river in the park, which is in Kusatsu-cho of Gunma Prefecture (36º 37' 27" N, 138º 35' 24" E). Yubatake spring water (YB) was at the facility located in the center of Kusatsu-cho downtown (36º 37' 22" N, 138º 35' 48" E). Bandaiko spring water (BN) was at the hut managed by the Shinaki Dam Water Quality Control Office where poured from the source into the town (36º 37' 23" N, 138º 36' 10" E).
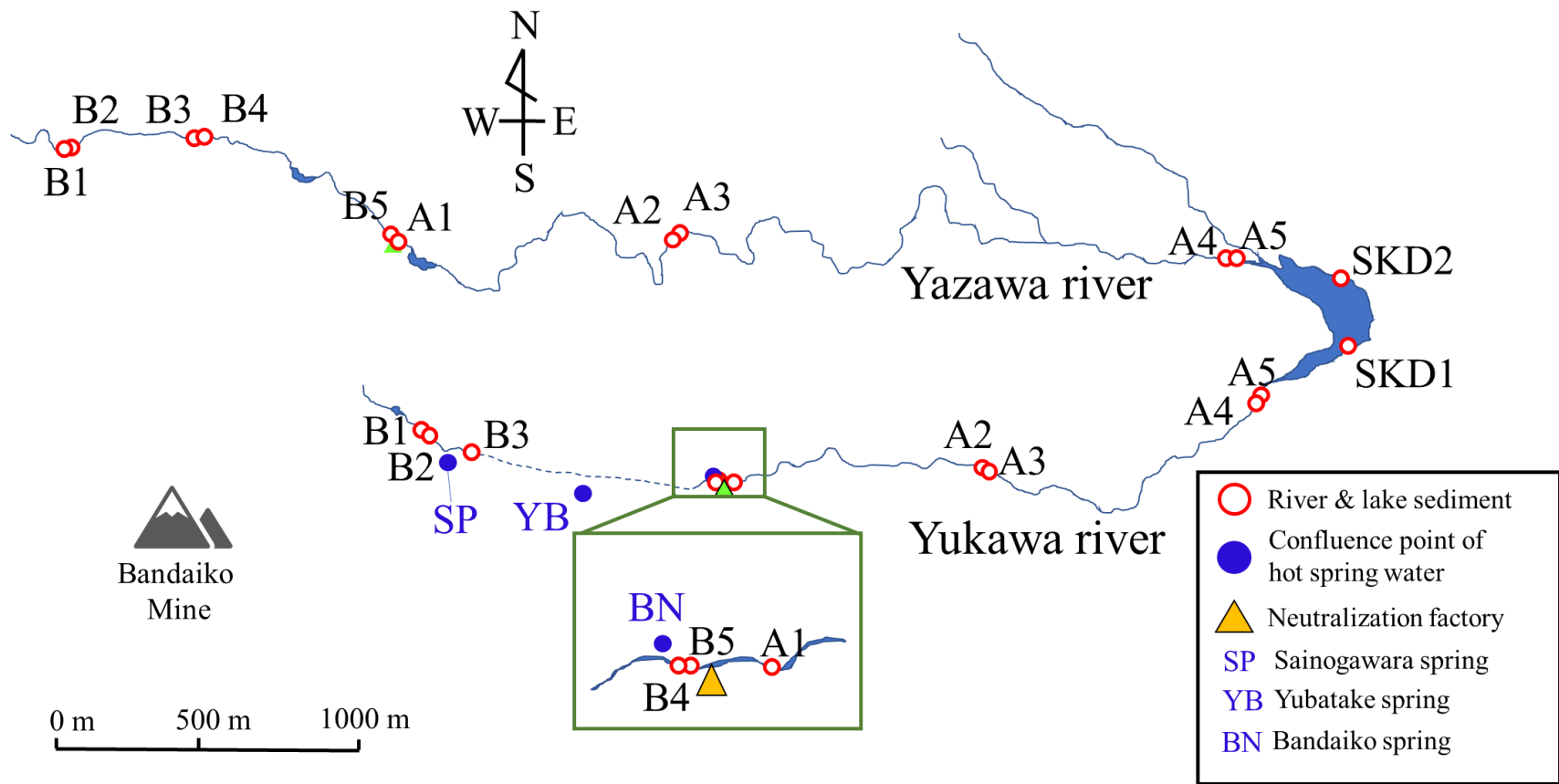
Fig. 2-1 Location map of the sampling points. Red circles identify sampling points for the river water and sediment samples, blue circles represent the hot springs, and green triangles indicate the neutralization points.

2.2.2. Reagents

All reagents used in this study were purchased from Wako Pure Chemical Industries, Co., Ltd. (Osaka, Japan) and were of analytical grade. The standard solutions were prepared using ultrapure water, which was purified using a water distillation system (ASK-2DS, Iwaki Co., Ltd., Tokyo, Japan). The multi-element calibration standards (XSTC-1, XSTC-7 and XSTC-13) of the target elements were purchased from SPEX CertiPrep (Metuchen, NJ, USA).

2.2.3. Apparatuses

A pH meter (HI98129, Hanna Instruments, USA) was used for determining the water samples on-site. Also, another pH meter with a glass-combined electrode (F-22, Horiba, Ltd., Kyoto, Japan) in our laboratory was used during the preparation of the solutions. The concentration of the target element in the water samples were determined using inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7700x, Agilent Technology, Santa Clara, CA, USA).

2.2.4. ICP-MS

Determination of the target elements present in the various samples was carried out by ICP-MS (Agilent 7700x-ICP-MS) analysis suing a collision cell to eliminate any interferences (especially $^{40}Ar^{35}Cl$ in an analysis of ^{75}As) with the target elements [14]. Helium (He), a non-reactive gas, was used in the collision cell. Therefore, the element concentrations indicated herein were the values obtained via the collision mode with He (He-mode).

The operating conditions employed are summarized in Table 2-1. Data acquisition and post analysis were carried out using the G201A Mass Hunter Workstation Software, A.01.02 (Agilent Technology, USA). In order to maintain a linear concentration range,

the samples were diluted with pure water in the range of 1–1000-fold, as necessary. Then, 0.5% nitric acid was added, and the solutions were transferred to 14-mL polypropylene tubes (As-One Corporation, Osaka, Japan). Additionally, a background blank sample without extractant was prepared from water and 0.5% nitric acid. Calibration curves of all measured elements were prepared from multi-element standard solutions with concentrations ranging from 0 (blank) to 1 μg L^{-1} for Pd, from 0 (blank) to 10 μg L^{-1} for As, from 0 (blank) to 20 μg L^{-1} for Cd, from 0 (blank) to 30 μg L^{-1} for Y. The obtained calibration curves exhibited good linearity with correlation coefficients (r^2) > 0.999. In addition, we employed an internal standard method to determine the metal concentration and improve the quantitative accuracy of the ICP-MS analysis. In this approach, germanium (0.5 mg L^{-1} each) was used as an internal standard to determine the ^{75}As, ^{105}Pd, ^{89}Y and ^{111}Cd concentrations.

Table 2-1 Operating conditions of ICP-MS in this study

RF potential	1550 W
Plasma gas	Ar
Gas flow rates	
Main	15 L min^{-1}
Intermediate	0.9 L min^{-1}
Carrier gas	1 L min^{-1}
Sampling cone	Ni
Signal measurement	Scanning
Sweeps/replicate	100
Replicates	3
Dwell time	40 ms
Plasma frequency	27.2 mHz

2.2.5 Monitoring procedures

2.2.5.a Pretreatment of samples

For the water samples, the vessels containing the water samples were rinsed, and 0.45 μm syringe filters were employed to separate the larger particles prior to acidifying with 0.50 vol% nitric acid to prevent metal precipitation or adsorption on the container surface. And then the prepared samples were stored in 100-mL polypropylene bottles.

2.2.5.b Determination of pH, water temperature, daily flowrate and electrical conductivity (EC)

The annual pH, water temperature and water level dates were obtained from Shinaki Dam Water Quality Control Office (Ministry of Land, Infrastructure and Transport Kanto Regional Development Bureau). As well as daily flowrates (Q) of the rivers were estimated by Square dam's formula (JIS K0094) (Eq. 2-1) using the water level data as following;

$$Q = 1.838 \ (w - 0.2h) \ h^{3/2} \times 60 \hspace{3cm} (2\text{-}1)$$

where Q is flow quantity, w is notch width of river and h is overflow level. However, about the samples case, all of them was determined by us using a pH meter (HI98129) on site, to ensure their accuracy.

2.3. Results and discussion

2.3.1. Annual monitoring of pH, water temperature and flowrate of river water and damlake in the Kusatsu area

Initially, the data investigated by Shinaki Dam Water Quality Control Office was summarized as following, especially, the daily flow rates (Q) of the rivers were calculated by Eq. 2-1.

The changes of annual pH in the river water after neutralization and in Shinaki dam-lake are shown in Fig. 2-2a. The pH values of river and lake water samples were risen to 4 - 7, compared to that before the neutralization (pH 2-4) as shown in Fig. 2-3. In addition, although the measurements were carried out at same monitoring site every day, the slight changes in pH values might strongly be affected by changes in the water temperature and flow rate as Fig. 2-2b and Fig. 2-2c, respectively. The water temperatures of the Yazawa river water and the downstream dam-lake water significantly affected by the seasonal changes. The Yukawa river water did not change significantly. This would relatively be stabled by flowing large amount of hot spring water into Yukawa river (Fig. 2-1), which is much higher than that of the other two river waters. Namely, flowing of the high temperature of hot spring water (50-95 $^{\circ}$C) [10] into river water is hardly affected by season changes.

The flow rate of the Yukawa river was significantly larger than that of the Yazawa river as shown in Fig. 2-2c, with a six-fold difference being recorded at the time of samples collection in October 2016. Moreover, we found that the flowrate in September and October reached the highest value. It might be related to influences by typhoon during this period in 2016.
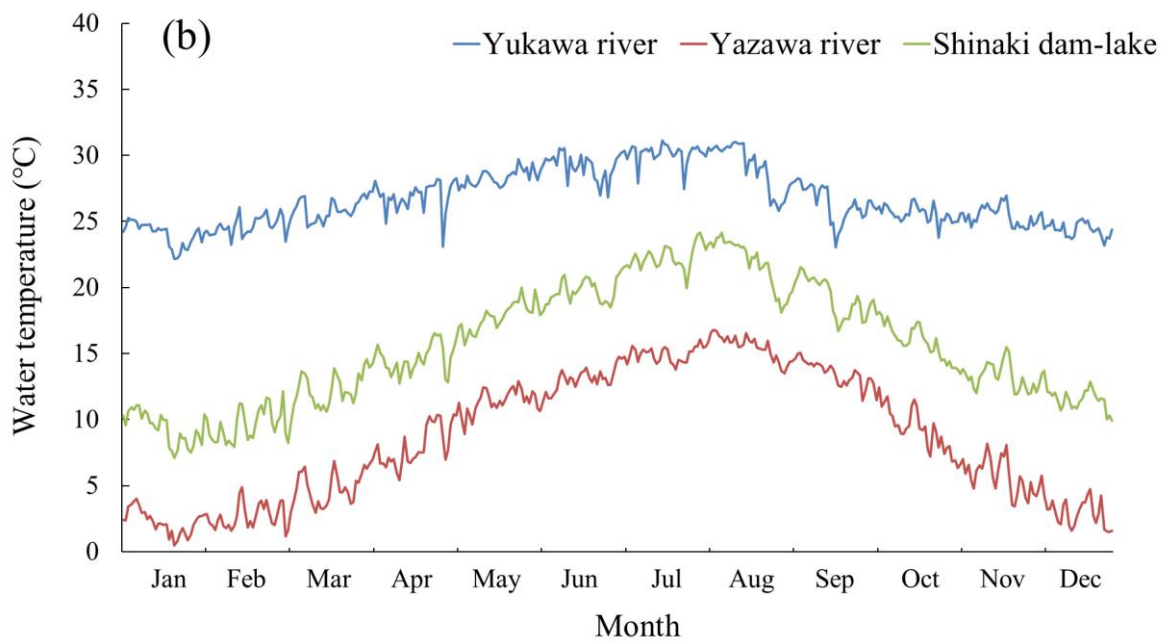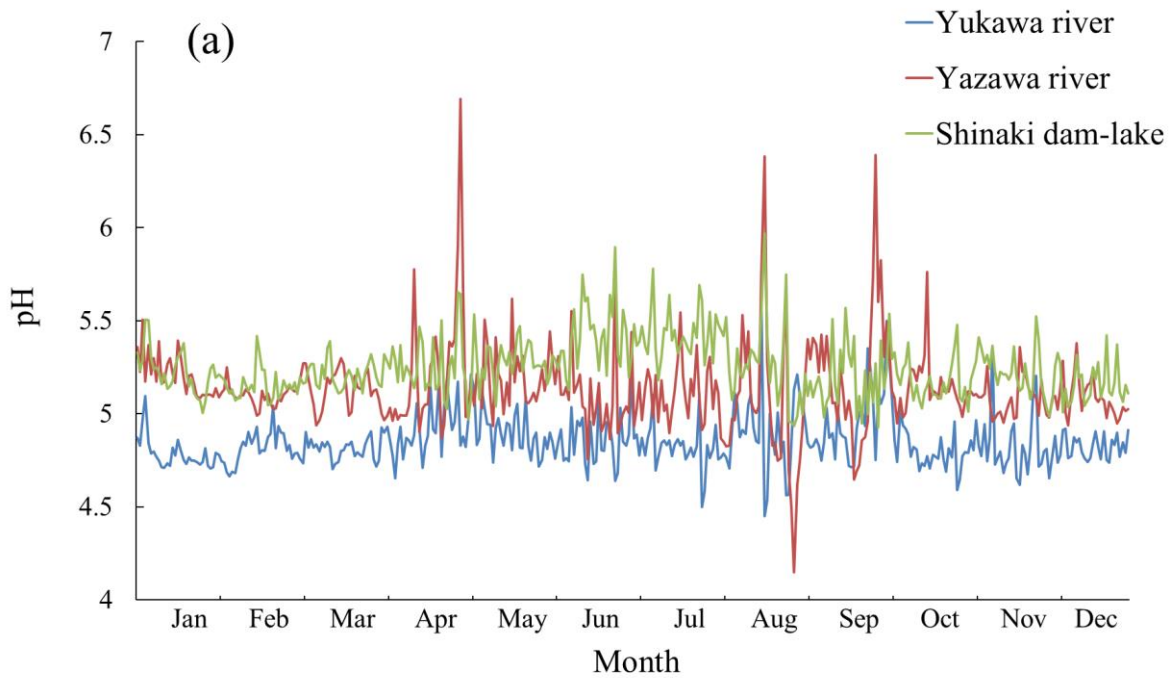
Fig. 2-2 Seasonal variations of pH, water temperature and flow rate measured in the neutralization factories of Yukawa and Yazawa rivers and Shinaki dam-lake. The data was offered from the Shinaki Dam Water Quality Control Office (Kusatsu, Gunma Pref., Japan).
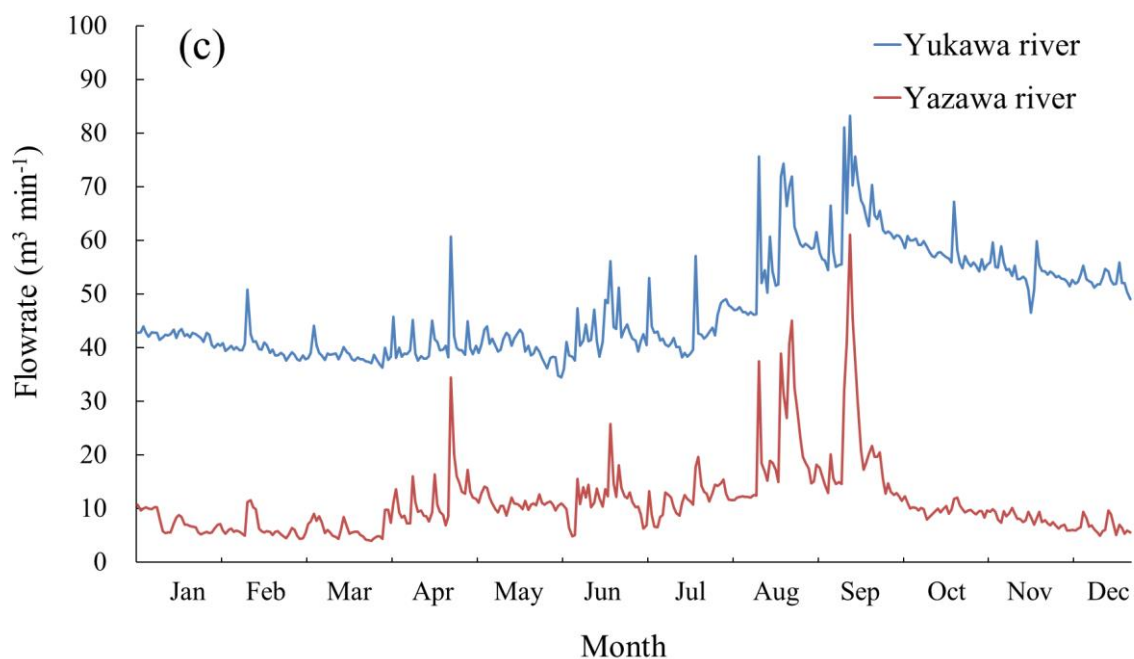
Fig. 2-2 (continued) Seasonal variations of pH, water temperature and flow rate measured in the neutralization factories of Yukawa and Yazawa rivers and Shinaki dam-lake. The data was offered from the Shinaki Dam Water Quality Control Office (Kusatsu, Gunma Pref., Japan).

2.3.2. Changes in pH and electric conductivity of the river water samples

Next, we examined the variations in pH and electric conductivity (EC) of the river water and samples from the Yukawa and Yazawa rivers. As shown in Fig. 2-3, in the case of the Yukawa river, the pH decreased from 4 to 2.4 between collection points B2 and B3 (Fig. 2-3a), while the EC dramatically increased from 380 to 3720 $\mu S\ cm^{-1}$ between points B3 and B4 (Fig. 2-3c). These variations in pH and EC may be affected by the flux of hot spring waters with high acid (mainly H_2SO_4) or electrolyte (Na^+, SO_4^{2-}, Cl^-, etc.) concentrations into the Yukawa river. Water from the Sainogawara hot spring, which has a pH of 1.9 owing to the presence of large quantities of sulfates and chlorides, flows into the river between points B2 and B3, while water from the Yubatake and Bandaiko hot

springs (pH 2.1 and 1.7, respectively) as summarized in Table 2-2 join into the river between points B3 and B4. Interestingly, the EC between points B2 and B3 increased slightly, while that between points B3 and B4 exhibited a significant increase. It therefore appears that the EC may be related to the quantities of electrolytes present in the hot spring water. Indeed, following neutralization by adding lime, the pH increased to 5–6 and the EC decreased from 3500 to ~1500 μS cm^{-1}, likely owing to the precipitation of a number of dissolved cations and anions.

In addition, as shown in Fig. 2-3b, the pH of the Yazawa river increased gradually from 3 to 4 between points B1 to B5, i.e., prior to the neutralization. In this case, the observed increase in pH was smaller than that of the Yukawa river following the addition of lime. The small variation in pH at point A1 in the Yazawa river upon neutralization (an increase from 3-4 to 6) was likely due to the concentration of lime in the Yukawa river being ~3 times greater than that of the Yazawa river. In addition, as shown in Fig. 2-3d, the EC gradually decreased over the full sampling region, and the variation both before and after the neutralization was smaller than that of the Yukawa river.

Indeed, owing to their different inherent lime concentrations, the volumes of lime suspensions required to bring the pH values of the Yukawa and Yazawa rivers to ~6 are 50 and 4.1 t/d, respectively [8]. It should also be noted that the required volume of lime suspension required depends on the flow rate of the river. Upon considering such differences in the river flow rates (Fig. 2-2c), the precipitates deposited on the surface sediment upon neutralization were likely to be distributed further downstream in the faster-flowing Yukawa river.
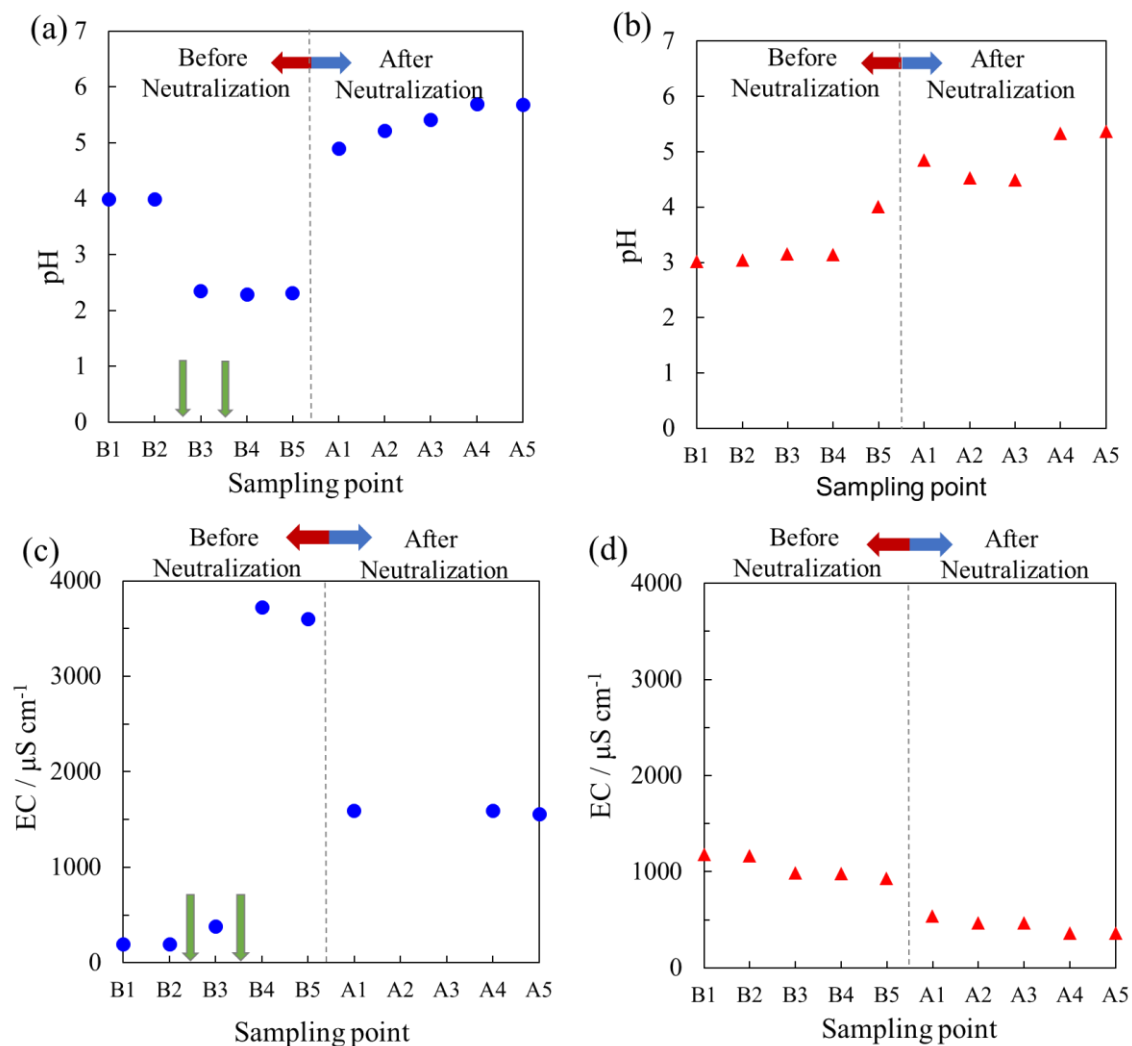
Fig. 2-3 Variations in (a, b) pH and (c, d) EC values for the river water samples collected on 18 October 2016 from the Yukawa river (a, c) and Yazawa rivers (b, d). The green arrows in part (a) indicate the inflow points of hot spring water from the Sainogawara, Yubatake, and Bandaiko springs.

Table 2-2 pH and EC values for hot spring water samples collected on 18 October 2016

Sampling site	pH	EC (μS cm^{-1})
Sainogawara park (SP)	1.93	> 3999*
Yubatake (YB)	2.12	> 3999*
Bandaiko (BN)	1.68	> 3999*

* upper limit of quantitation

2.3.3. Monitoring of annual changes in As, Y, Pd and Cd concentrations in Yukawa river water and Bandaiko hot spring water

Finally, we investigated the changes in As, Y, Pd and Cd annual concentrations in the water samples collected from before neutralization (B5) and after neutralization (A1) of Yukawa river and Bandaiko hot spring (BN). The concentrations of target elements were determined by ICP-MS with a collision mode using He. The sampling data that collected all the water samples were summarized in Table 2-3. The concentrations of four analytes annually changed as shown in Fig. 2-4 and Table 2-4.

In Bandaiko hot spring water, the As indicated extremely high concentration ration compared other targeted metals. The fact that high concentration of As are contained in hot spring water originated from the underground magma of Kusatsu-Shirane volcano was found by Kikawada et al [10]. The concentrations of As were gradually decreased from July to November. The Cd concentrations in Bandaiko hot spring water were trace level (< 20 μg L^{-1}) but the transition to seasonal changes was similar to that of As The variations of Pd concentrations were very large due to the extremely low level to detect by ICP-MS, while the concentrations from July to December were lower than those from January to June like the trends of As and Cd. This might be that the changes in the temperature and pH of the hot spring water during this year caused changes in the solubility of the elements in spring water [15].

In contrast, the Y were constantly contained in the hot spring water as the relative high concentration regardless of seasonal changes. High concentrations of Y might be related to the strong acidity of Bandaiko hot spring water caused higher solubility of these elements than that in river water, though the detailed mechanism could not be clarified in this study.

The As concentration in the Yukawa river before the neutralization indicated the relative constant throughout one year, and that after the neutralization largely reduced in the range from 40-fold to 182-fold by adding the lime (Table 2-4). From these results, the

addition of lime at the neutralization demonstrates that can reduce the concentration of dissolved As to be lower than standard level (< 0.01 mg L^{-1}) [16]. The concentration of Y in the river was lowered after the neutralization but the variations was not correlated to variations before neutralization. Interestingly, the Pd concentration after the neutralization were higher than before the neutralization throughout the one year except October. The concentrations were comparable level to those in Bandaiko hot spring water.

The similar tendency in Pd in which the concentrations after neutralization were higher than those before the neutralization were obtained for the Cd, though the Cd concentration in river waters were extremely lower than that in Bandaiko hot spring water.

This would relate to the concentrations of Pd and Cd in the lime used in the neutralization factory, because the lime contained 10.5 µg kg^{-1} Pd and 622 µg kg^{-1} Cd by our analyses.

Table 2-3 The pH, water temperature and electrical conductivity collected samples

Sample name	Sampling Date	pH / Water temperature(°C) / Electrical conductivity （μs/cm）		
		Bandaiko	Before neutralization of Yukawa river (B5)	After neutralization of Yukawa river (A1)
Jan	2017/1/7	1.21 / 42.7 / ＞3999*	1.86 / 29.1 / ＞3999*	4.30 / 28.9 / 1980
Feb	2017/2/16	1.26 / 42.2 / ＞3999*	1.84 / 31.9 / ＞3999*	4.33 / 31.0 / 2131
Mar	2017/3/16	1.34 / 42.7 / ＞3999*	1.83 / 32.9 / ＞3999*	4.30 / 32.9 / 1753
Apr	2017/4/18	1.36 / 46.8 / ＞3999*	2.00 / 29.8 / 3160	4.21 / 29.8 / 1180
May	2017/5/16	1.33 / 42.5 / ＞3999*	1.92 / 33.1 / 3400	4.42 / 33.1 / 1469
Jun	2017/6/16	1.36 / 42.8 / ＞3999*	1.99 / 34.2 / 3357	4.34 / 34.2 / 1534
Jul	2017/7/18	1.34 / 47.4 / ＞3999*	1.98 / 35.3 /3400	4.28 / 35.3 / 1560
Aug	2017/8/17	1.31 / 42.6 / ＞3999*	2.03 / 30.8 / 3280	4.27 / 30.8 / 1419
Sep	2017/9/19	1.33 / 42.2 / ＞3999*	1.99 / 30.4 / 3146	4.20 / 30.4 / 1383
Oct	2017/10/17	1.38 / 46.8 / ＞3999*	1.92 / 31.6 / 3121	4.30 / 31.6 / 1532
Nov	2017/11/17	1.38 / 4.21 / ＞3999*	1.92 / 28.7 / 2987	4.21 / 28.7 / 1450
Dec	2017/12/18	1.39 / 4.23 / ＞3999*	1.85 / 30.6 / 3270	4.23 / 30.6 / 1538

* upper limit of quantitation
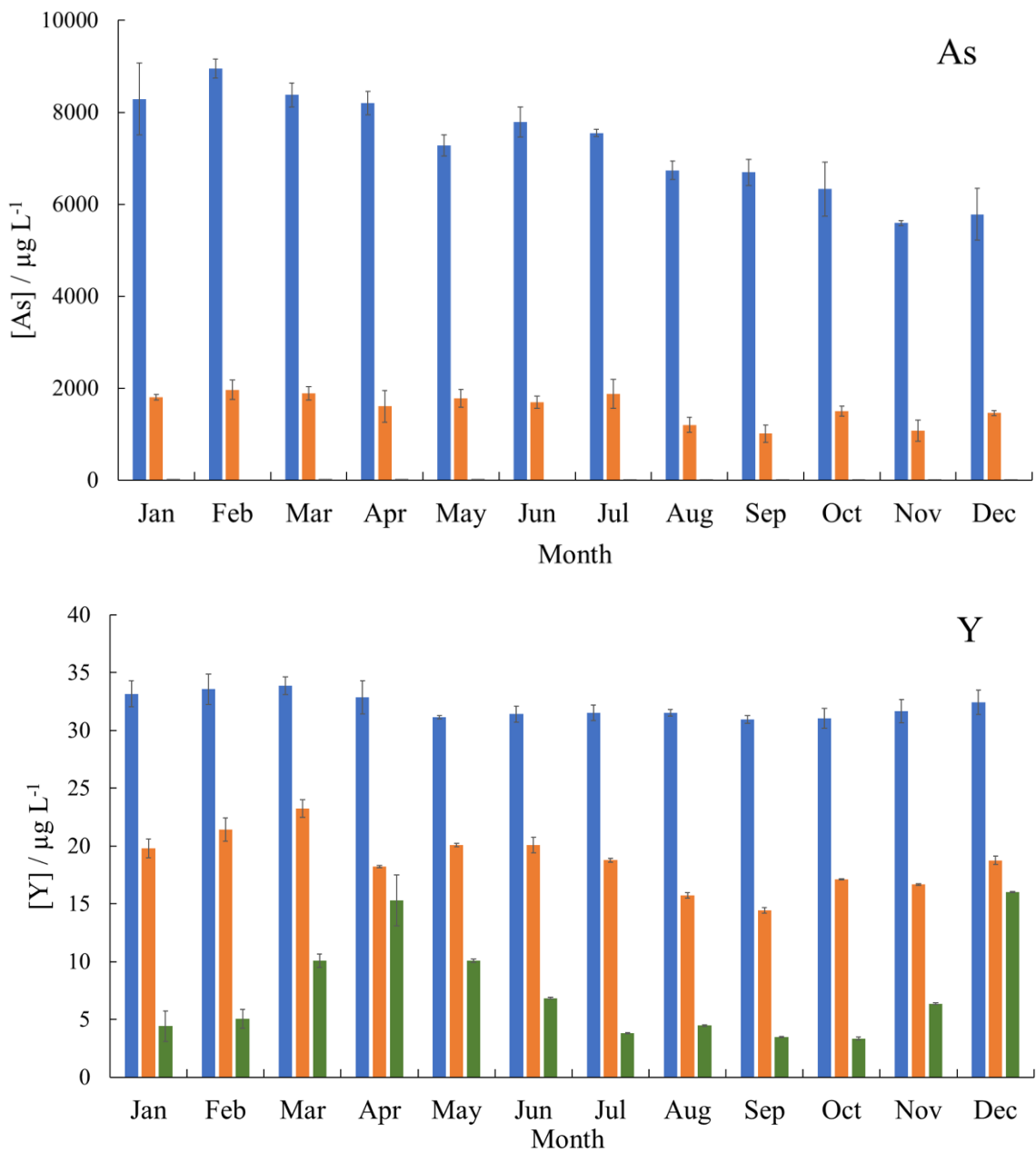
Fig. 2-4 Variations in the concentrations of As, Y, Pb and Cd in the water samples of (■) Bandaiko hot spring, (■) before and (■) after neutralization of Yukawa river collected in each month of 2017.
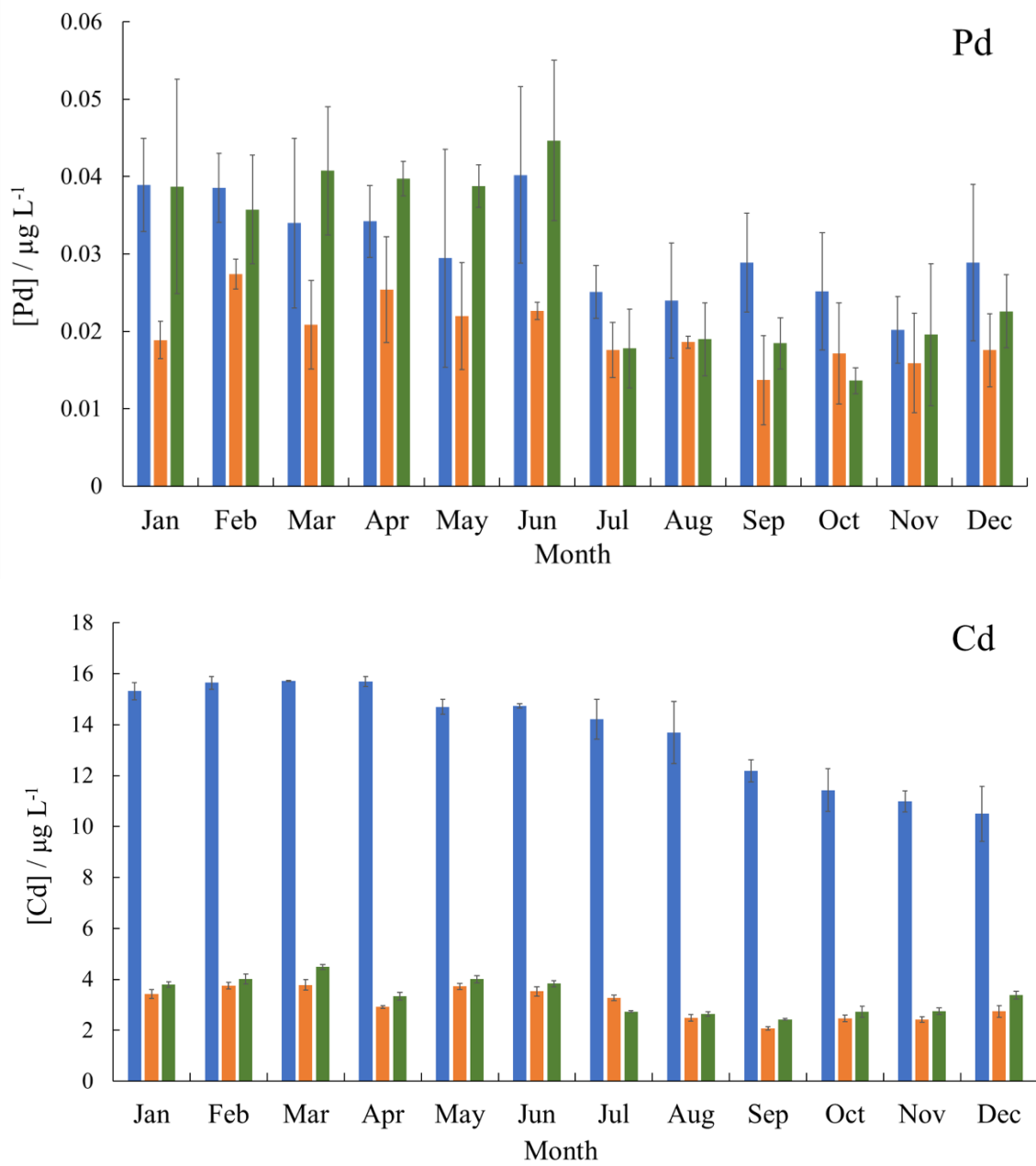
Fig. 2-4 (continued) Variations in the concentrations of As, Y, Pb and Cd in the water samples of (■) Bandaiko hot spring, (■) before and (■) after neutralization of Yukawa river collected in each month of 2017.

Table 2-4 The data on variations in the concentrations of As, Y, Pb and Cd in the water samples of Bandaiko hot spring (BN), before neutralization (B5) and after neutralization (A1) river collected in each month of 2017.

Month	[As] / µg L^{-1}			[Y] / µg L^{-1}			[Pd] / µg L^{-1}			[Cd] / µg L^{-1}		
	BN	B4	A1	BN	B4	A1	BN	B4	A1	BN	B4	A1
Jan	8290 ± 783	1850 ± 58	36.9 ± 1.7	33.2 ± 1.1	19.8 ± 0.8	4.42 ± 1.33	0.04 ± 0.01	0.02 ± 0.00$_2$	0.04 ± 0.01	15.3 ± 0.3	3.44 ± 0.17	3.79 ± 0.11
Feb	8950 ± 204	1970 ± 213	11.1 ± 0.3	33.6 ± 1.3	21.4 ± 1.1	5.05 ± 0.80	0.04 ± 0.00$_4$	0.03 ± 0.00$_2$	0.04 ± 0.01	15.7 ± 0.2	3.75 ± 0.14	4.02 ± 0.20
Mar	8380 ± 258	1890 ± 150	42.2 ± 2.1	33.9 ± 0.8	23.2 ± 0.8	10.1 ± 0.6	0.03 ± 0.01	0.02 ± 0.01	0.04 ± 0.01	15.7 ± 0.0$_3$	3.78 ± 0.21	4.48 ± 0.10
Apr	8200 ± 252	1610 ± 346	30.3 ± 0.5	32.9 ± 1.4	18.2 ± 0.1	15.3 ± 2.2	0.03 ± 0.00$_4$	0.03 ± 0.00$_1$	0.04 ± 0.00$_2$	15.7 ± 0.2	2.92 ± 0.06	3.33 ± 0.15
May	7280 ± 230	1780 ± 197	34.5 ± 0.7	31.1 ± 0.1	20.1 ± 0.1	10.1 ± 0.6	0.03 ± 0.01	0.02 ± 0.00$_3$	0.04 ± 0.00$_3$	14.7 ± 0.3	3.73 ± 0.12	4.01 ± 0.14
Jun	7790 ± 327	1670 ± 132	9.14 ± 0.3	31.4 ± 0.7	20.1 ± 0.7	6.84 ± 0.07	0.04 ± 0.01	0.02 ± 0.00$_1$	0.04 ± 0.01	14.7 ± 0.1	3.53 ± 0.18	3.83 ± 0.12
Jul	7750 ± 75	1880 ± 318	29.6 ± 1.2	31.5 ± 0.7	18.8 ± 0.2	3.81 ± 0.05	0.03 ± 0.00$_3$	0.02 ± 0.00$_4$	0.02 ± 0.01	14.2 ± 0.8	3.27 ± 0.11	2.74 ± 0.04
Aug	6740 ± 200	1200 ± 160	26.3 ± 2.0	31.5 ± 0.3	15.7 ± 0.3	4.47 ± 0.07	0.02 ± 0.01	0.02 ± 0.02	0.02 ± 0.00$_4$	13.7 ± 1.2	2.49 ± 0.12	2.64 ± 0.09
Sep	6690 ± 288	1010 ± 188	25.2 ± 2.3	31.0 ± 0.3	14.4 ± 0.2	3.48 ± 0.05	0.03 ± 0.01	0.01 ± 0.04	0.02 ± 0.00$_3$	12.2 ± 0.4	2.08 ± 0.07	2.43 ± 0.03
Oct	6329 ± 592	1500 ± 110	23.1 ± 2.4	31.0 ± 0.9	17.1 ± 0.1	3.34 ± 0.12	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.00$_2$	11.4 ± 0.8	2.48 ± 0.13	2.73 ± 0.22
Nov	5590 ± 58	1080 ± 231	20.0 ± 1.9	31.7 ± 1.0	16.7 ± 0.1	6.36 ± 0.07	0.02 ± 0.00$_4$	0.02 ± 0.01	0.02 ± 0.01	11.0 ± 0.4	2.42 ± 0.11	2.75 ± 0.12
Dec	5780 ± 565	1460 ± 58	20.0 ± 2.2	32.4 ± 1.1	18.7 ± 0.4	16.0 ± 0.1	0.03 ± 0.01	0.02 ± 0.00$_4$	0.02 ±0.00$_4$	10.5 ± 1.1	2.75 ±0.23	3.38 ± 0.15
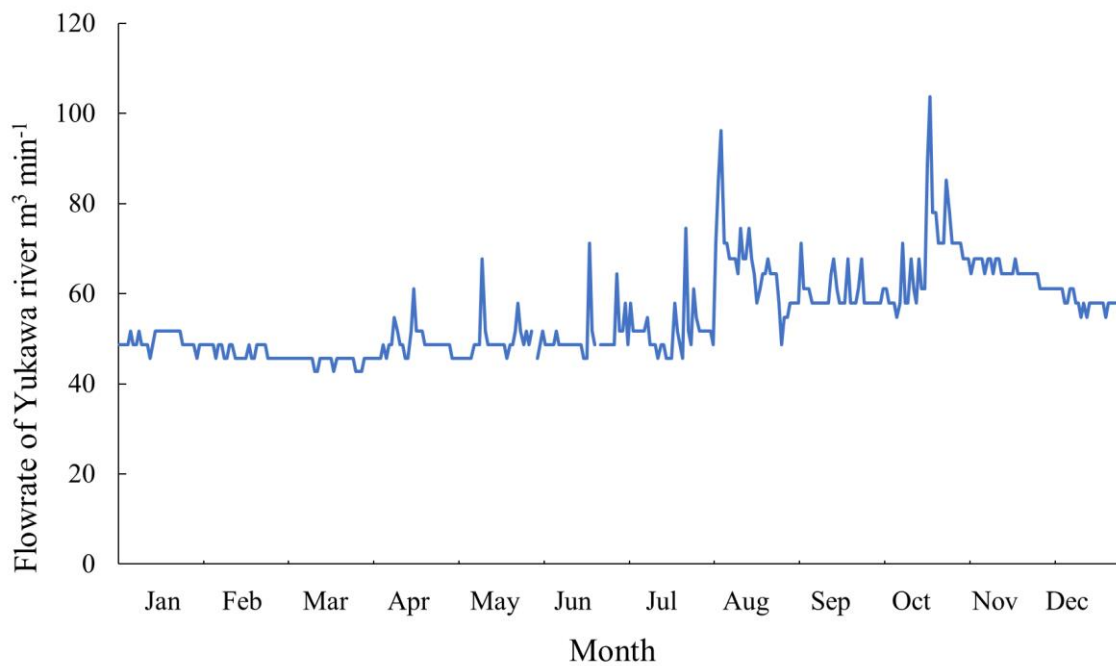
Fig. 2-5 Seasonal variations of flow rate measured in the neutralization factories of Yukawa river in 2017. The data was offered from the Shinaki Dam Water Quality Control Office (Kusatsu, Gunma Prefecture, Japan).

2.4. Conclusions

From these results, the pH, EC, water temperature and flow rate of Yukawa and Yazawa rivers revealed a obvious differences. In both rivers, the pH raised from 2-4 to 4-7 after addition of lime at neutralization factory, while the trends of EC decreased.

Furthermore, while the As concentrations in the Yukawa river was largely reduced after adding the lime, the Pd, which is a valuable metal, in the river water was inversely increased by adding the lime, as well as Cd, which is a toxic metal for human health.

Overall, I considered that the Yukawa/Yazawa river water qualities were dramatically changed by addition of lime in the neutralization. Of course, we expected that the changes in water qualities of the river before and after the neutralization resulted in the changes in the sediment of riverbed.

2.5. References

[1] Nurhasan, Y. Ogawa, N. Ujihara, S. B. Tank, Y. Honkura, S. Onizawa, T. Mori, and M. Makino, Two electrical conductors beneath Kusatsu-Shirane volcano, Japan, imaged by audiomagnetotellurics, and their implications for the hydrothermal system, Earth, Planets and Space, 58 (2006) 1053-1059.

[2] Y. Kikawada, T. Ono, K. Ogawa, M. Fukai, and T. Oi, Chemical geothermometry studies on a geothermal system in Manza hot springs near Kusatsu-Shirane volcano, Japan, Procedia Earth and Planetary Science, 17 (2017) 253-256.

[3] Y. Ogawa, D. Ishiyama, N. Shikazono, K. Iwane, M. Kajiwara, and N. Tsuchiya, Fractionation and Deposition of Indium and Arsenic from the Kusatsu and Tamagawa Acidic Hot Springs, Japan: Possible Man-Made Analogues for Rare Metal Concentrations onto Lake Beds? Economic Geology, 108(2013) 1641-1656.

[4] Y. Kikawada, T. Oi, T. Honda, T. Ossaka, and H. Kakihana, Lanthanoid abundances of acidic hot spring and crater lake waters in the Kusatsu-Shirane volcano region, Japan, Geochemical Journal, 27 (1993) 19-33.

[5] H. Muto, Improvement of Acidic River Water Quality by the Neutralization Method of using Milk of Lime, Proceedings of the Japan Society of Civil Engineers, 275 (1978) 41.

[6] R. E. Villanueva-Estrada, R. M. Prol-Ledesma, A. A. Rodríguez-Díaz, C. Canet, and M. A. Armienta, Arsenic in hot springs of Bahía Concepción, Baja California Peninsula, México Chemical Geology, 348 (2013) 27.

[7] D. Mitsuya, T. Hayashi, Y. Wang, M. Tanaka, M. Okai, M. Ishida, and N. Urano, Isolation of aquatic yeasts with the ability to neutralize acidic media, from an extremely acidic river near Japan's Kusatsu-Shirane Volcano, Journal of Bioscience and Bioengineering, 124 (2017) 43–46.

[8] Shinaki Dam Water Quality Control Office, Ministry of Land, Infrastructure and Transport Kanto Regional Development Bureau,

http://www.ktr.mlit.go.jp/ktr_content/content/000076767.pdf <accessed：2017. 02. 20>

[9] Y. Ogawa, D. Ishiyama, N. Shikazono, K. Iwane, M. Kajiwara, and N. Tsuchiya, The role of hydrous ferric oxide precipitation in the fractionation of arsenic, gallium, and indium during the neutralization of acidic hot spring water by river water in the Tama River watershed, Japan, Geochimica et Cosmochimica Acta, 86 (2012) 367-383.

[10] Y. Kikawada, S. Kawai, K. Shimada, and T. Oi, Arsenic Originating in Kusatsu Hot Springs, Gunma, Japan, Journal of Disaster Research, 3 (2008) 261-269.

[11] A. Sungur, M. Soylak, E. Yilmaz, S. Yilmaz, and Hasan Ozcan, Characterization of heavy metal fractions in agricultural soils by sequential extraction procedure: the relationship between soil properties and heavy metal fractions, Soil and Sediment Contamination, 24 (2015) 1-15.

[12] Y. Ogawa, R. Yamada, K. Shinoda, C. Inoue, and N. Tsuchiya, The fate of arsenic in a river acidified by volcanic activity and an acid thermal water and sedimentation mechanism, Environmental Science: Processes & Impacts, 16 (2014) 2325-2334.

[13] K. Kubota, K. Tamura, H. Kurabayashi, H. Take, T. Shirakura, and J. I. Tamura, Effects of Hot Spring Bathing on Blood Pressure, Heart Rate, Plasma Cortisol and Hematocrit at Kusatsu, The Journal of The Japanese Society of Balneology, Climatology and Physical Medicine, 60 (1997) 61-68.

[14] E. McCurdy, and G. Woods, The application of collision/reaction cell inductively coupled plasma mass spectrometry to multi-element analysis in variable sample matrices, using He as a non-reactive cell gas, Journal of Analytical Atomic Spectrometry Anal, 19 (2004) 607-615.

[15] K. Yutaka, Chemical composition of the spring deposits from the Masutomi spring, Yamanashi Prefecture, The Geochemical Society of Japan, 23(1989) 77-83.

[16] Ministry of the Environment, Environmental Quality Standards for Water Pollution: https://www.env.go.jp/en/water/wq/wp.pdf <accessed：2018. 11. 20>

Chapter 3

Monitoring of palladium concentration in river water and sediment at an acidic hot spring spa area in the Gunma Prefecture

3.1. Introduction

In chapter 2, I characterized the pH, EC, flow rate and metal concentrations in river and lake water samples of Kusatsu hot spring area. From these results, I discussed their values in Yukawa river and Yazawa river, including geographical influences. Also, the metal concentrations in the river water was changed before and after addition of lime in the neutralization. The concentrations of arsenic (As) and yttrium (Y) in the river water after addition of lime at the neutralization factory were reduced compared with those before addition of lime. Namely, As and Y precipitated by adding lime were accumulated in the sediment on the river bed. In contrast, those of cadmium (Cd) and palladium (Pd) were increased after addition of lime. This was considered because Cd and Pd were included in the lime suspension. Therefore, I could understand that the contents of elements in the Yukawa/Yazawa river water was dramatically changed by addition of lime in the neutralization. However, the changes in water qualities of the river before and after the neutralization should result in the changes in the sediment of riverbed.

In this study, I investigated the leachabilities of the As, Cd and Pd, which are the targeted elements in chapter 2, using sequential extraction procedure (SEP) to understand transitions of the elements from sediment to river water, and possibility of collection of their elements, especially Pd, which is an important metal for electrical products and organic synthesis [1-4]. Also, investigating their leachabilities from sediment using SEP is important issue in term of environmental protection, because it relates to risk assessment of river water and lake water ecosystems.

Studies on the leachability of heavy metals from the sediment into ground water or the surrounding areas of the Shinaki dam, using a SEP, are therefore of particular interest

[5]. Kikawada *et al*. determined the arsenic concentration in hot spring water, river water samples collected before and after neutralization, and in precipitates collected from the Shinaki dam [6]. They reported that the largest sources of arsenic were the Bandaiko hot spring and the Yukawa river, with 25 tons of arsenic being deposited in the Shinaki dam annually. In addition, several researchers have focused on the valuable metals present in the hot springs, rivers, and sediments following neutralization. Ogawa *et al*. compared the fractionation and deposition of indium (In) during transport from the Kusatsu acidic hot springs into the Shinaki dam and found that it was removed and adsorbed onto a lime suspension before being transferred to the Shinaki dam [7]. Kikawada *et al*. elucidated the lanthanide patterns of Kusatsu-Yubatake and Tamagawa-Obuki hot spring water samples, in which it was apparent that the lighter lanthanides were more easily leached into water than the heavier ones [8].

Previously, I have also investigated the leachability of harmful elements such cadmium (Cd) and lead (Pb) from the sediments of the Watarase river area, using a SEP to ultimately determine any potential influences on the ecosystem and human health [9]. Recently, I found relatively high concentrations of Pd (40 μg/kg) in the sediments of the Shinaki dam downstream of the Kusatsu hot spa area. The existence of Pd chemical form that can be easily isolated from the sediment or water may potentially lead to a resource creation.

Therefore, in this chapter, I investigated the distribution of Pd by analyzing its concentration in river water samples obtained across the streams of Yukawa and Yazawa rivers, using inductively coupled plasma mass spectroscopy. Sediment samples collected at the same sampling points of the two rivers and Shinaki dam lake samples were characterized by X-ray fluorescence and X-ray diffraction analyses to detect and compare the chemical compositions and crystal structures of the respective components before and after the neutralization process.

In addition, the characterized sediment samples were fractionated by a SEP to estimate the leachability of Pd from the sediment [10]. This is an effective technique for

evaluating the leachability of harmful and valuable metals in soil, sediments, and sludge samples in a stepwise fashion [11-13]. Furthermore, the variations in Pd concentration of the river water and hot spring water samples. The fractionations of the sediment samples were compared with those of other main elements (Cu, Zn, As and Cd) to assist the discussions on the Pd behavior and to know the impact for river and lake environments.

Through this study, I predicted the Pd deposition pathway in the Shinaki dam sediment by examining the Pd distributions and different chemical forms present in the river and dam sediments.

3.2. Experimental

3.2.1. Collection of water and sediment samples

Sampling sites of the hot spring water and river water were mentioned in chapter 2 (see Fig. 2-1 of chapter 2). The sediment samples from the rivers and the Shinaki dam (N36°37′, E138°38′) were collected at the points indicated in Fig. 2-1 (B1–B5 at an upstream location before the neutralization factory, and A1–A5 at a downstream location after the factory). Only water samples were collected at points B4 and A1 of the Yukawa river, as at these points the river bottom was artificially constructed from cement and stone, and no sediment was precipitated owing to the fast river flow. The sediment samples from the rivers were collected at a depth of ~5 cm using a plastic shovel. In contrast, sediment samples (SKD1 and SKD2) from the bottom of the Shinaki dam-lake were obtained from the slurry precipitates, which had been pumped from the dredger to a land facility using a high-pressure pump.

All sediment samples were collected with a soil sampling kit (Daiki Rika Kogyo Co., Ltd., Saitama, Japan) to remove any stone components. After being packed in plastic sachets, the samples were immediately transported to our laboratory. In order to reduce the biological reproduction in the wet sediment samples, the collected samples were air-dried at 25 ± 1 °C after they arrived in the laboratory. The dried samples were pulverized

using a mortar and pestle, and then sieved to obtain particles with a diameter < 2 mm. The exception is that for XRD measurement, it requires the smallest possible sediment samples. Therefore, I also selected some samples less than 0.25 mm for XRD analysis. Finally, all samples were dried at 60 °C for 24 h.

3.2.2. Reagents and apparatuses

All reagents used in this chapter were mentioned in chapter 2, containing the multi-element calibration standards (XSTC-7 and XSTC-13) of the target elements.

The apparatuses used in this chapter were introduced below. Prior to the digestion and speciation analyses, crystal structure analysis of the collected sediment samples was carried out using X-ray diffraction (XRD, RIGAKU RINT-2200V, Rigaku Corp., Tokyo, Japan). In addition, the major elements in the sediment samples were measured by energy-dispersive fluorescent X-ray analysis (XRF, EA1200VX, Hitachi High-Tech Science Corp., Tokyo, Japan).

Furthermore, the separation of solid materials in water sample such as sand or slurry in the collected river water and the residues in the extractants in the SEP were performed by centrifugation (Kokusan H-200, Kokusan Co., Ltd., Tokyo, Japan) at a fixed angle running at 3000 rpm. The extraction solution and sediment samples were mixed using a magnetic stirrer (RS-1DW, As-One Corp., Osaka, Japan) equipped with a rotor. During stirring, a constant temperature (25 °C) was maintained with a thermos regulator (CTR-330, Iwaki, Tokyo, Japan). A pH meter with a glass-combined electrode (F-22, Horiba, Ltd., Kyoto, Japan) was used during the preparation of the solutions. The concentrations of the target elements in the water samples and extractants of the SEP were determined by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7700x, Agilent Technology, Santa Clara, CA, USA).

3.2.3. Contents of the target elements in the sediment samples

To determine the concentrations of the targeted elements which present in the

sediment sample, each sample (1 g) was dried at 60 °C for 24 h and transferred into a polytetrafluoroethylene beaker. And dissolved in a mixture of 36% (w/w) HCl (10 mL) and 60% (w/w) $HClO_4$ (2 mL), then evaporated to dryness at 180 °C using a hot plate equipped with a sand bath. After the addition of a further mixture of 36% (w/w) HCl (10 mL) and 60% (w/w) $HClO_4$ (1 mL), the mixture was again evaporated to dryness at 180 °C for 24 h in a sand bath.

Finally, the dried residue was dissolved in a mixture of 36% (w/w) HCl (3 mL) and 60% (w/w) $HClO_4$ (1 mL), The resulting solution was diluted to 25 mL using ultrapure water and injected into the ICP-MS apparatus after filtering by a 0.45 μm syringe filter. In order to maintain a linear concentration range, the samples were directly injected to ICP-MS without dilution without water, or after diluting with pure water in the range of 10 – 1000-fold as necessary. In fact, according to general method, soil samples should be determined the elements by completely dissolving in a strongly acidic mixture containing HF [14]. However, because use of HF damaged the quartz torch nebulizer of the ICP-MS, we gave up to completely dissolve the final residue.

3.2.4. Sequential extraction procedure (SEP)

SEPs of the target elements present in the sediment samples were carried out according to Tessier's method to evaluate the potential leachability from the sediment to the river water [10]. The specific details for obtaining each fraction are as follows

Fraction 1 (exchangeable form): A portion (1.0 g) of the sediment sample was placed in a 50-mL centrifuge tube, and 1.0 M aqueous $MgCl_2$ (pH 7, 8 mL) was added. After stirring at 600 rpm for 1 h using a magnetic stirrer, the resulting solid-liquid mixture was subjected to centrifugation at 3000 rpm for 30 min. Then, the supernatant was filtered through a 0.45-μm syringe filter and the residues were rinsed using ultrapure water (8.0 mL) by vortexing at 600 rpm for 10 min. The centrifugation and filtration procedures were performed twice. All procedures were carried out at 25 ± 5 °C.

Fraction 2 (bound to carbonates): Fraction 2 was generated by mixing the residue obtained during the preparation of Fraction 1 with a 1.0 M aqueous CH_3COONa solution (pH 5, 8.0 mL) at 25 ± 5 °C for 5 h. The centrifugation, filtration, and rinsing procedures described for Fraction 1 were then performed to obtain Fraction 2.

Fraction 3 (bound to iron and manganese-oxide): Fraction 3 was generated by mixing the residue obtained from the preparation of Fraction 2 with a 0.04 M aqueous solution of $NH_2OH \cdot HCl$ in 25% v/v CH_3COOH (8.0 mL) at 96 ± 3 °C for 6 h. After this time, the resulting mixture was subjected to centrifugation at 3000 rpm for 30 min, and the supernatant was collected. The centrifugation, filtration, and rinsing procedures described for Fraction 1 were then performed to obtain Fraction 3.

Fraction 4 (strong acid elution form): Originally, Fraction 4, which is defined by Tessier *et al* [10]. as the species that is bound to organic matter, was prepared upon treatment of the residue obtained during the preparation of Fraction 3 with a mixture of 8.8 M H_2O_2 (8.0 mL) and 0.02 M HNO_3 (3.0 mL) at 85 ± 2 °C for 5 h to elute the species bound to the organic matter. Importantly, in this process explosive foaming occurred due to the large quantities of calcium carbonate present in the residue. Therefore, I employed an alternative procedure to obtain Fraction 4, in which the residue obtained from the preparation of Fraction 3 was dissolved in a mixture of 36% (w/w) HCl (10 mL) and 60% (w/w) $HClO_4$ (2 mL) and then evaporated the mixture to dryness at 180 °C in a sand bath. After the further addition of a solution of 36% (w/w) HCl (10 mL) and 60% (w/w) $HClO_4$ (1 mL), the mixture was again evaporated to dryness at 180 °C for 24 h in a sand bath.

Finally, the dried residue was dissolved in a mixture of 36% (w/w) HCl (3 mL) and 60% (w/w) $HClO_4$ (1 mL), subjected to centrifugation at 600 rpm for 1 h, and then filtered through a 0.45 μm syringe filter.

Analyte concentrations in all obtained fractions were determined by ICP-MS after dilution to 25 mL using ultrapure water.

According to Tessier's method, Fraction 5 should be determined the elements by completely dissolving the residues obtained during Fraction 4 in a HF/HClO$_4$ mixture. However, because the use of HF damaged the quartz torch nebulizer of the ICP-MS, I must skip this procedure [10].

The resulting weights of the residues per 1 g sediment sample obtained after this four-step sequential extraction are summarized in Table 3-1. As shown in this table, large amounts of residues after Fraction 4 cannot be eluted, and the chemical forms of metal species in these residues were considered by the presence in crystals of silicate rock.

Table 3-1. Weights of the residues obtained after sequential extraction [*]

Sample		Residue / g
Yukawa river	B5	0.807 ± 0.024
	A4	0.270 ± 0.014
Yazawa river	B3	0.835 ± 0.032
	A4	0.756 ± 0.012
Shinaki dam-lake	SKD1	0.470 ± 0.008
	SKD2	0.483 ± 0.024

[*]average ± standard deviation obtained by triplicate measurements

3.3. Results and Discussion

3.3.1. Characterization of the sediment samples

Initially, the collected sediment samples were characterized by elemental analysis using XRF and the respective crystal structures were determined with XRD.

The elemental analysis revealed that the amount of calcium in the sediment sample after neutralization was significantly higher than that before the neutralization (Table 3-2). In the Yukawa river, the calcium amount obtained from collection locations A2, A3, A4 and A5 decreased slightly from 73.1% to 64.8%, respectively, and the calcium amount in SKD1, which was located near the estuary, was 44.4%. In contrast, in the Yazawa river, the calcium levels from collection locations A1 to A3 were ranging from 70.7% to 42.7%, and the calcium amount in the SKD2 sample was lower than that in the A4 sample.

The XRD patterns of the sediment samples, in turn, revealed an obvious difference between the crystal structures of the samples obtained before and after the neutralization process (Fig. 3-1a and 3-1b). Furthermore, the main crystal structure of both sediment samples collected in the Shinaki dam-lake (SKD1 and SKD2) was found be calcite (Fig. 3-1c). A comparison of the XRD patterns with that of lime used in the neutralization (Fig. 3-1d) indicates that the surface of the river sediments after neutralization were covered with precipitated lime.

It was assumed that the variations in the calcium levels in the samples collected from the sampling points after the neutralization were related to the difference in the volumes of lime suspension input in the Yukawa (53-ton day^{-1}) and Yazawa rivers (4.1-ton day^{-1}), and in the flow rates of the Yukawa (33000 L min^{-1}) and Yazawa rivers (8800 L min^{-1}) [15, 6]. Especially for the Yazawa river, since the daily input amount of lime was much less than that of Yukawa river, as well as the low stream flows, the input lime was more likely to precipitates into the riverbed while reacting with the acidic water. Therefore, the content of calcium at A5 is much lower than that at A1.

Moreover, when comparing the calcite intensities in the XRD patterns of SKD1 and SKD2, one can assume that the difference in the river flow rates affected the distribution of the lime precipitates (Fig. 3-1c and Table 3-2)

Table 3-2 Abundance ratios of the elements in the sediment samples from Yukawa and Yazawa rivers, and Shinaki dam-lake, determined by XRF [a]

Yukawa river	Sampling point								
	Before neutralization / %				After neutralization / %				Shinaki dam-lake / %
Element	B1	B2	B3	B5	A2	A3	A4	A5	SKD1
Si	60.2	47.6	58.2	52.9	20.1	24.2	23.5	23.9	16.2
Ca	2.45	4.02	4.23	8.90	73.1	70.4	67.6	64.8	44.4
Fe	20.5	33.2	17.8	17.6	1.02	0.74	2.86	4.59	21.9
S	< 0.01	0.95	0.57	< 0.01	< 0.01	< 0.01	< 0.01	0.150	0.61
Mn	0.26	0.48	0.40	0.28	0.02	0.01	0.07	0.08	0.19
Cu	0.04	< 0.01	0.03	0.04	< 0.01	< 0.01	< 0.01	< 0.01	0.04
Zn	0.02	0.03	0.02	0.04	0.01	< 0.01	0.01	0.01	0.08
Pb	0.29	0.24	0.24	0.59	0.01	0.04	0.03	< 0.01	0.04
As	< 0.01	0.17	< 0.01	0.36	0.08	0.05	0.09	0.12	0.87
Ti	2.90	1.10	1.76	1.71	< 0.01	< 0.01	0.11	0.50	0.71

Yazawa river	Sampling point										
	Before neutralization / %					After neutralization / %					Shinaki dam-lake / %
Element	B1	B2	B3	B4	B5	A1	A2	A3	A4	A5	SKD2
Si	41.0	35.4	35.1	40.6	36.8	25.2	23.8	23.6	37.5	31.8	27.8
Ca	13.0	6.48	9.10	6.26	4.73	70.7	55.7	42.7	12.4	8.75	11.9
Fe	26.8	39.2	36.9	33.0	42.5	0.23	11.7	18.7	37.8	40.2	35.7
Mn	0.46	0.85	0.79	0.47	0.54	0.02	0.35	0.55	0.42	0.93	0.30
Cu	0.03	0.01	0.02	0.02	0.02	< 0.01	0.01	0.03	0.02	0.02	0.07
Zn	0.04	0.05	0.05	0.03	0.04	< 0.01	0.02	0.04	0.03	0.06	0.06
Pb	0.03	0.03	0.04	0.11	0.07	< 0.01	< 0.01	0.02	0.01	0.02	0.42
As	0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.68
Ti	2.03	4.23	3.00	2.09	2.03	< 0.01	0.96	1.64	1.27	3.66	0.94

[a] The weight percentages of each element were estimated from the oxide forms using the fundamental parameter method (Spectroscopy online com. http://www.spectroscopyonline.com/basic-fundamental-parameters-x-ray-fluorescence).
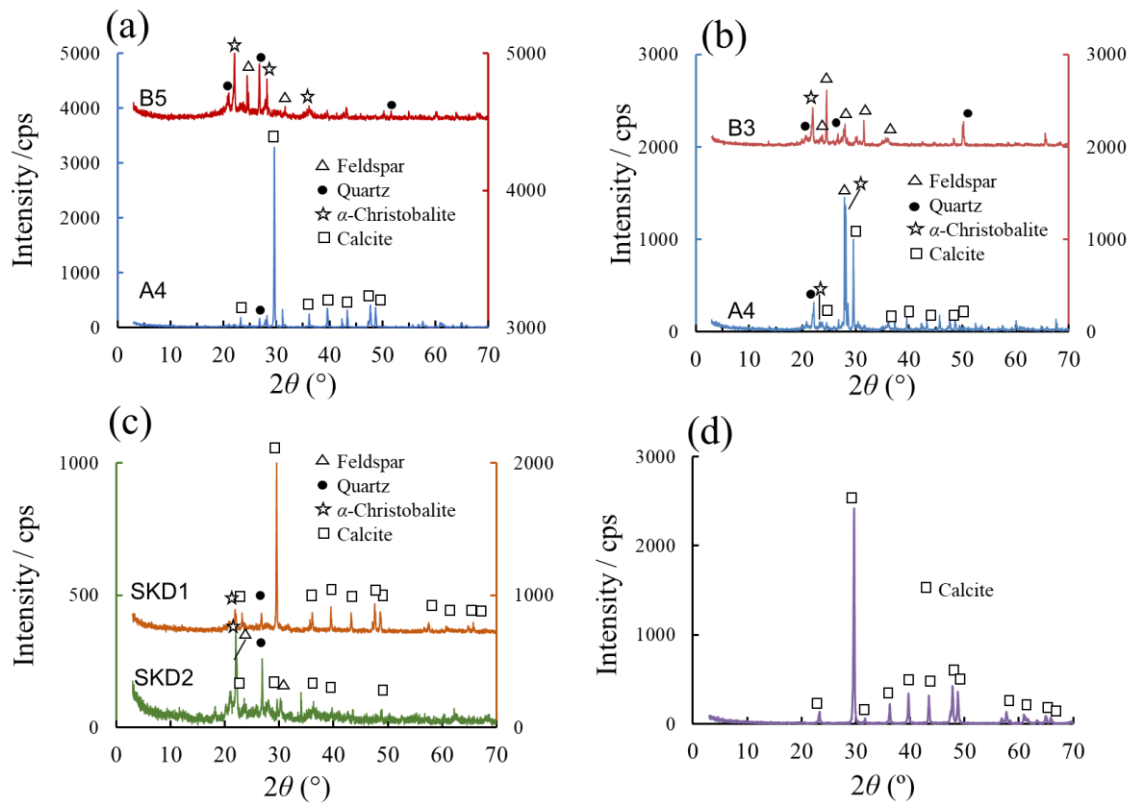
Fig. 3-1 XRD patterns of the river sediments before and after neutralization from the (a) Yukawa (B5, A4) and (b) Yazawa (B3, A4) rivers, (c) sediment from the Shinaki dam lake (SKD1 and SKD2), and (d) lime used in the neutralization factory.

3.3.2. Variations of Pd concentration in the river water and sediments

Next, I investigated the variation in Pd concentration in the river water and sediment samples collected from the Yukawa and Yazawa rivers and compared the obtained values with the Cd and As concentrations. The concentrations of the target elements were determined by ICP-MS in a collision mode using He.

As shown in Fig. 3-2a, the analyte concentrations in water of the Yukawa river exhibited significant increases between sampling points B2 and B3, while the Yazawa river showed a slight decrease in analyte concentrations from upstream to downstream. The standard deviations (SDs) with the analyte concentrations are summarized in Tables 3-3, 3-4, and 3-5. The SDs obtained by ICP-MS were relatively large. This would be related because I must dilute water sample to near the limit of detection (LOD) in order to inject the sample into ICP-MS.

The increase in analyte concentrations between the locations B2 and B3 observed in the Yukawa river (see Fig. 2-1) might be due to the inflow of acidic hot-spring water from Sainogawara park (0.08 μg L^{-1}) inflowed into a stream river, which flows along Yukawa river. In contrast, no highly acidic hot-spring water is introduced into the waters of the Yazawa river and therefore, no such increase was observed.

The Pd concentration in the river water following the neutralization decreased around 0.03–0.04 μg L^{-1} and were almost constant until point A5, which was located near the estuary of the Shinaki Lake-dam. Moreover, the concentrations of Pd and Cd in both rivers were higher than their limitation of quantifications (LOQs: *ca*. 0.01 μg kg^{-1}) following the neutralization, whereas the concentration of As was lower than its LOQ. This may be affected by the input lime, which contains 10.5 μg kg^{-1} Pd and 622 μg kg^{-1} Cd in a lime sample used in the neutralization factory.

By contrast, the concentrations of the various elements in the sediment increased following neutralization. Indeed, Kikawada *et al.*, reported that the high concentrations of As may be related to its interactions with suspended HFO [6]. Thus, from the observed

concentration distributions, it appears that precipitates of Pd with toxic elements such as Cd and As were transported to the Shinaki dam following neutralization (Fig. 3-2a).

Similarly, the variation in Pd concentration in the sediment obtained from the Yukawa river was comparable to that observed for Cd. Indeed, a stronger correlation was observed than for As (Figs. 3-3a and 3-3c), with Student's t-test p-values of 0.0038 and 0.245 being calculated for the correlations between Pd and Cd, and Pd and As, respectively (sampling number: 8, degrees of freedom: 6).

As shown in Fig. 3-2b, in the case of the Yazawa river, the maximum Pd concentration in the river water prior to neutralization was recorded at the most upstream collection point, namely B1. In general, the variations in Pd and Cd concentrations were smaller than for the Yukawa river (as were the variations in pH), although a slight decrease was observed upon moving downstream. This may be related to the fact that no strongly acidic hot spring water is introduced into the waters of the Yazawa river.

Furthermore, prior to the neutralization, little variation in the Pd and Cd concentrations in the sediment samples was observed. However, upon addition of the lime suspension, the concentrations of these elements in the river water decreased, while those of the sediment increased. Moreover, upon moving downstream, the concentrations of Pd and Cd in both the river water and the sediment gradually decreased. The Pd concentrations recorded for the river water and sediment of the Yazawa river were slightly lower than those of the Yukawa river, while the Cd and As concentrations were significantly lower.

I also found that the As concentrations in the river water and sediment were much lower than those recorded for the Yukawa river, and corresponded with the variations in Pd and Cd concentrations, as described above. More specifically, prior to neutralization, the As concentration in the river water decreased from 4.0 μg L^{-1} to 0.70 μg L^{-1} between points B2 and B5, while the As concentration in the sediment increased from 330 μg kg^{-1} to 685 μg kg^{-1}. However, following neutralization, the As concentrations in both the

sediment and the river water were lower. These variations in the Pd (river water and sediment) and As (sediment) concentrations in the Yazawa river will be discussed in the future.

Thus, for the Yazawa river, the variation in Pd concentration in the river water and sediment was similar to that observed for Cd, with a stronger correlation being observed than for As, as was also the case for the Yukawa river (Figs. 3-3b and 3-3d). In this case, the p-values for the correlations between Pd and Cd were 0.0007 for the river water and 0.00006 for the sediment (sampling number: 10, degrees of freedom: 8), while the corresponding values for As were 0.01 and 0.1 respectively.
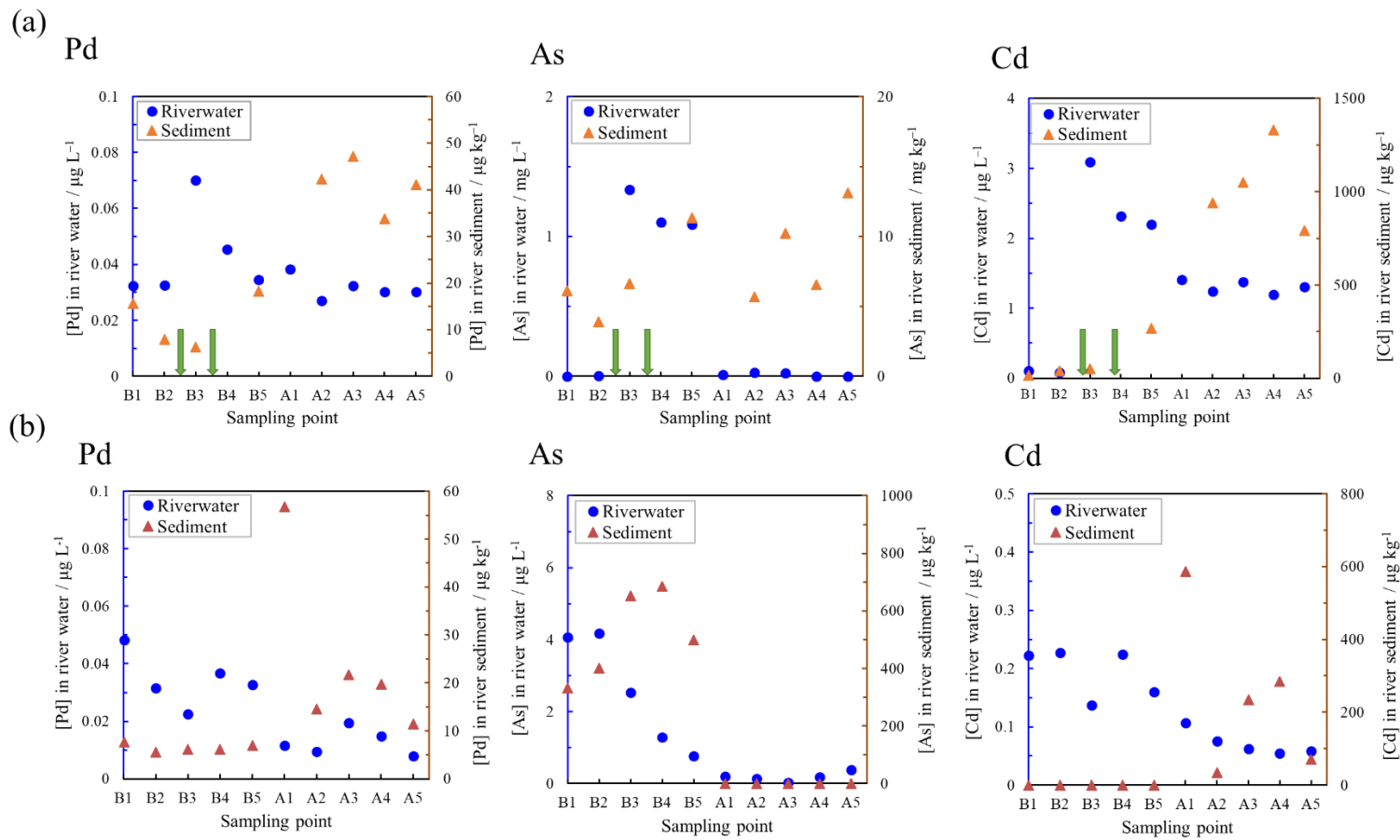
Fig. 3-2 Concentrations of Pd, Cd, and As in the river water and sediment samples collected from (a) the Yukawa river, and (b) the Yazawa river on 18 October 2016. The green arrows in part (a) indicate the inflow points of hot spring water from the Sainogawara, Yubatake, and Bandaiko springs.

Table 3-3 Concentrations of Pd, Cd, and As in Sainokawara park, Yubatake and Bandaiko springs

Sample	Pd / ng L^{-1}	Cd / μg L^{-1}	As / mg L^{-1}
Sainokawara park	80±49 [a]	10.4±0.1	4.07±0.10
Yubatake	53±23	0.554±0.247	0.340±0.026
Bandaiko	48±15	16.3±1.3	7.19±0.46

[a] average ± standard deviation obtained by triplicate measurements.

Table 3-4 Concentrations of Pd, Cd, and As in river water samples [a]

Yukawa river

Collection point	Concentrations /μg L^{-1}									
	B1	B2	B3	B4	B5	A1	A2	A3	A4	A5
Pd	0.03±0.01	0.03±0.01	0.07±0.01	0.05±0.01	0.03±0.01	0.04±0.01	0.03±0.01	0.03±0.01	0.03±0.00	0.03±0.00$_4$
Cd	0.10±0.04	0.08±0.02	3.09±0.04	2.31±0.14	2.20±0.24	1.41±0.13	1.24±0.01	1.37±0.10	1.20±0.04	1.31±0.05
As	1.00±0.61	1.49±0.21	1410±140	1360±74	1540±180	11.4±0.5	55.0±2.5	44.9±3.0	0.93±0.20	0.76±0.12

Yazawa river

	Concentrations /μg L^{-1}									
	B1	B2	B3	B4	B5	A1	A2	A3	A4	A5
Pd	0.05±0.01	0.03±0.00$_2$	0.02±0.02	0.04±0.03	0.03±0.02	0.01±0.00$_2$	0.01±0.01	0.02±0.01	0.02±0.00$_4$	0.01±0.00$_2$
Cd	0.22±0.03	0.23±0.06	0.14±0.07	0.23±0.10	0.16±0.04	0.11±0.01	0.08±0.02	0.06±0.03	0.05±0.03	0.06±0.01
As	4.07±0.38	4.18±0.46	2.54±0.23	1.29±0.17	0.77±0.32	0.19±0.20	0.14±0.16	N.D. [b]	0.18±0.20	0.38±0.10

[a] average ± standard deviation obtained by triplicate measurements.

[b] N.D. = not determined.

Table 3-5 Concentrations of Pd, Cd, and As in sediment samples [a]

Yukawa river

	Concentrations /µg L^{-1}									
	B1	B2	B3	B4[b]	B5	A1[b]	A2	A3	A4	A5
Pd	15.6±8.7	7.89±3.62	6.24±2.20		18.3±6.0		42.3±3.3	47.2±2.9	33.7±6.6	41.1±3.0
Cd	15.8±6.8	39.5±8.0	50.6±3.5		266±5		940±16	1050±58	1330±150	791±36
As	6130±1660	3890±1240	6620±1840		11310±2750		5700±1000	10200±2200	6650±960	13100±6400

Yazawa river

	Concentrations /µg L^{-1}									
	B1	B2	B3	B4	B5	A1	A2	A3	A4	A5
Pd	7.67±0.56	5.61±1.14	6.12±1.66	6.13±0.38	6.93±1.87	56.8±7.2	14.6±3.2	21.8±5.9	19.7±5.1	11.5±3.1
Cd	N.D. [c]	N.D.	N.D.	N.D.	N.D.	587±4	34.8±12.4	234±15	285±27	70.4±5.8
As	333±49	402±58	653±191	685±92	500±106	N.D.	N.D.	N.D.	N.D.	N.D.

[a] average ± standard deviation obtained by triplicate measurements.

[b] In the locations B4 and A1, the sediment samples could not be collected.
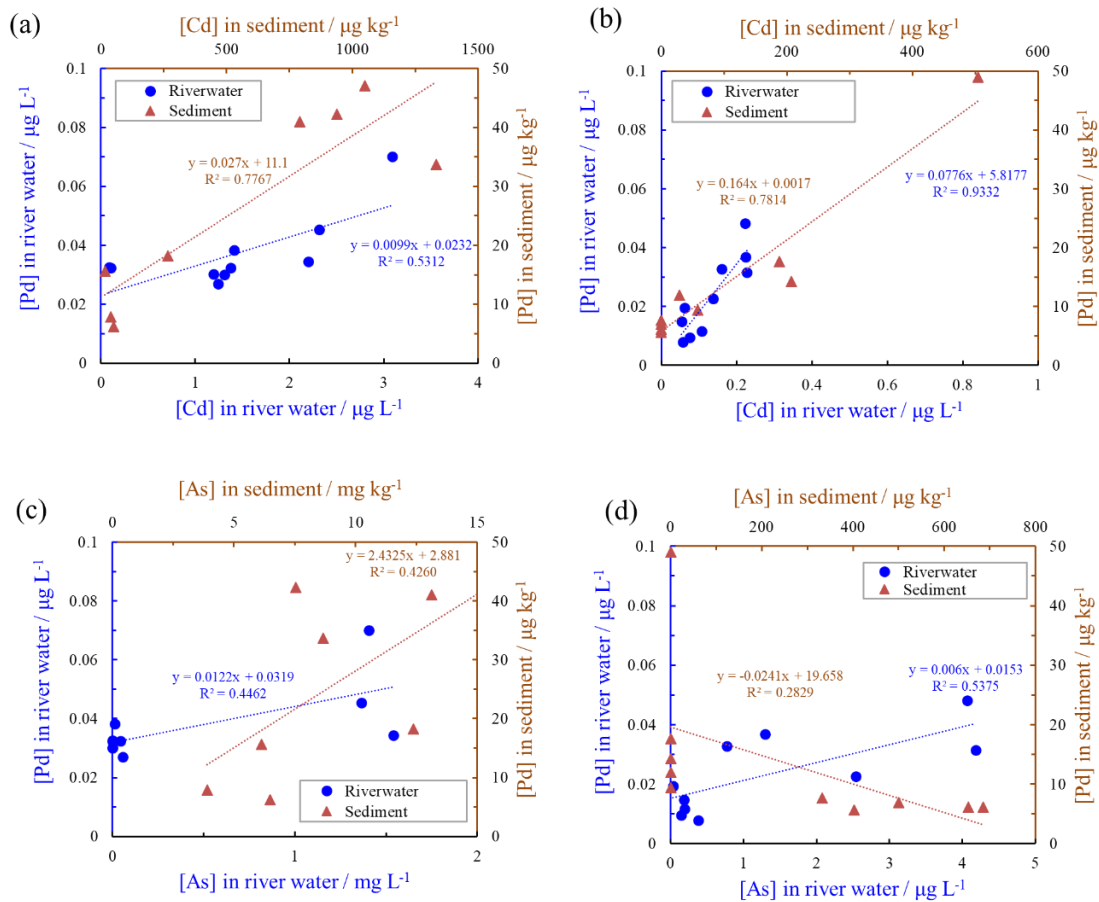
[c] N.D. = not determined.

Fig. 3-3 Relationships (a) between Pd and Cd concentrations in water and sediment of Yukawa river, (b) those of Yazawa river, (c) between Pd and As concentrations in water and sediment of Yukawa river, and (d) those of Yazawa river.

3.3.3. Fractionation of Pd in the sediment samples via a SEP

Next, I investigated the leachability of Pd deposited in the sediment and the relationship between Pd, Cd, and As by fractionation using the SEP, which relies on varying the degrees of solubility. The sediment samples selected for the SEP were from the places where I could collect enough sample amount for repeated experiments.

Prior to the SEP of the sediment samples from the rivers at the Kusatsu hot spring area, I confirmed the precision of the total fractionation of elements in a certified reference material of lake sediment containing trace elements (NMIJ CRM 7303-a). The analyzed elements were Cu, Zn, and Cd, since Pd was not included in the certified value displayed in the specification. The total concentrations of these elements obtained by the sequential extraction procedures for Fractions 1-4, and the recoveries of the certified values as summarized in Table 3-6.

As the results from this experiment, I observed that the total concentrations and recoveries of each element were consequently low compared to the certified values because the sample was not completely dissolved. When comparing these values to the extraction results of Cu, Zn, and Cd from the same sample (NMIJ-CRM 7303-a) in our previous study (Table 3-6), it can be noticed that the experimental t-values ($|t_{exp}|$) of the metals as determined by the paired t-test for every individual metal species at the confidence level of 95% (df = 6) were in all cases below the critical t-value ($|t_{crit}| = 2.77$), i.e., 0.86 for Cu, 2.32 for Zn, and 0.43 for Cd. Therefore, the precisions of the SEP used in this study was maintained [16].

Table 3-6 Concentrations and recoveries of Cu, Zn, and Cd in certified reference materials (NMIJ-CRM 7303-a) [17] extracted via the sequential extraction procedure in the present study and the comparison with those in a previous study [16]

Fraction / mg kg^{-1}	Present			Previous [16]		
	Cu	Zn	Cd	Cu	Zn	Cd
Fraction 1	0.06 ± 0.06 [a]	0.93 ± 0.04	0.147 ± 0.004	0.20 ± 0.06	1.8 ± 0.04	0.17 ± 0.02
Fraction 2	0.85 ± 0.07	0.79 ± 0.05	0.034 ± 0.016	0.30 ± 0.18	0.8 ± 0.05	0.029 ± 0.008
Fraction 3	1.00 ± 0.00	2.69 ± 0.35	0.038 ± 0.014	1.20 ± 0.20	3.5 ± 0.35	0.038 ± 0.007
Fraction 4	7.46 ± 0.11	9.82 ± 0.30	0.036 ± 0.011	8.1 ± 0.4	10.1 ± 0.3	0.024 ± 0.007
Total / mg kg^{-1}	9.36 ± 0.02	14.2 ± 0.6	0.254 ± 0.034	9.8 ± 0.2	16.2 ± 0.2	0.261 ± 0.010
Certified values / mg kg^{-1}	23.1 ± 3.1	107 ± 5	0.34 ± 0.02	23.1 ± 3.1	107 ± 5	0.34 ± 0.02
Recovery / %	40.5	13.3	74.7	42.4	15.1	76.8

[a] average ± standard deviation obtained by triplicate measurements.

Fig. 3-4 shows the fractionation of Pd in the sediment samples via the SEP, and Table 3-7 summarized the analytical values for each fraction and the abundance ratios. The concentrations of other elements are shown in Fig. 3-5 and Table 3-8.

Notably, the highest Pd concentration prior to the neutralization was recorded for Fraction 4. The abundance ratios of Fractions 1 and 2, which are easy elution-forms, suggest that the leachability of Pd from the sediment to the river was lower than 10% (1.0 ± 0.9 μg kg^{-1}). In fact, if the residue obtained after Fraction 4 was completely dissolved in a HF/HClO$_4$ solution, the ratio was expected to be further decreased. The abundance ratios of Pd in the sediment obtained after the neutralization of the Yukawa and Yazawa rivers decreased in the following order: Fraction 3 > Fraction 4 > Fraction 2. However, the fact that the main fractionation of lime was contained in Fraction 3 (84.8%) indicated that the increase in Pd concentration in Fraction 3 after the neutralization was affected by the input of lime suspension (Table 3-7).
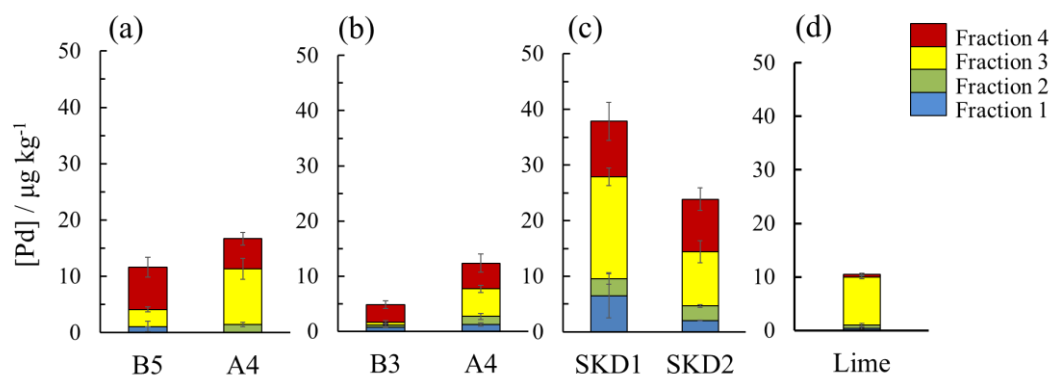

Fig. 3-4 Concentrations and fractionation of Pd in the various sediment samples from the (a) Yukawa river, (b) Yazawa river, and (c) Shinaki dam lake (SKD1 and SKD2), as well as in (d) lime. The error bars on each bar graph were obtained from the standard deviations in triplicate measurements.

Table 3-7 Concentrations of the fractionations and abundance ratios of Pd in the sediment and lime samples used in the neutralization.

Concentration / mg kg^{-1}	Yukawa river		Yazawa river		Shinaki dam		Lime
Sampling point	B5	A4	B3	A4	SKD1	SKD2	Neutralization
Fraction 1	1.09±0.95[a]	N.D.	0.754±0.119	1.22±0.30	6.50±3.99	2.05±0.05	0.365
Fraction 2	N.D.[b]	1.46±0.40	0.420±0.255	1.48±0.56	3.11±1.00	2.62±0.24	0.660
Fraction 3	3.05±0.46	9.86±1.85	0.499±0.288	5.00±0.64	18.3±1.54	9.76±1.96	8.92
Fraction 4	7.48±1.75	5.36±1.12	3.17±0.74	4.67±1.63	10.0±3.4	9.41±2.03	0.570
Abundance ratio / %	Yukawa river		Yazawa river		Shinaki dam		Lime
Sampling point	B5	A4	B3	A4	SKD1	SKD2	Neutralization
Fraction 1	9.4%	–	15.6%	9.9%	17.2%	8.6%	3.5%
Fraction 2	–	8.8%	8.7%	12.0%	8.2%	11.0%	6.3%
Fraction 3	26.2%	59.1%	10.3%	40.4%	48.3%	40.9%	84.8%
Fraction 4	64.4%	32.1%	65.4%	37.8%	26.4%	39.5%	5.4%

[a] average ± standard deviation obtained by triplicate measurements, except for lime.
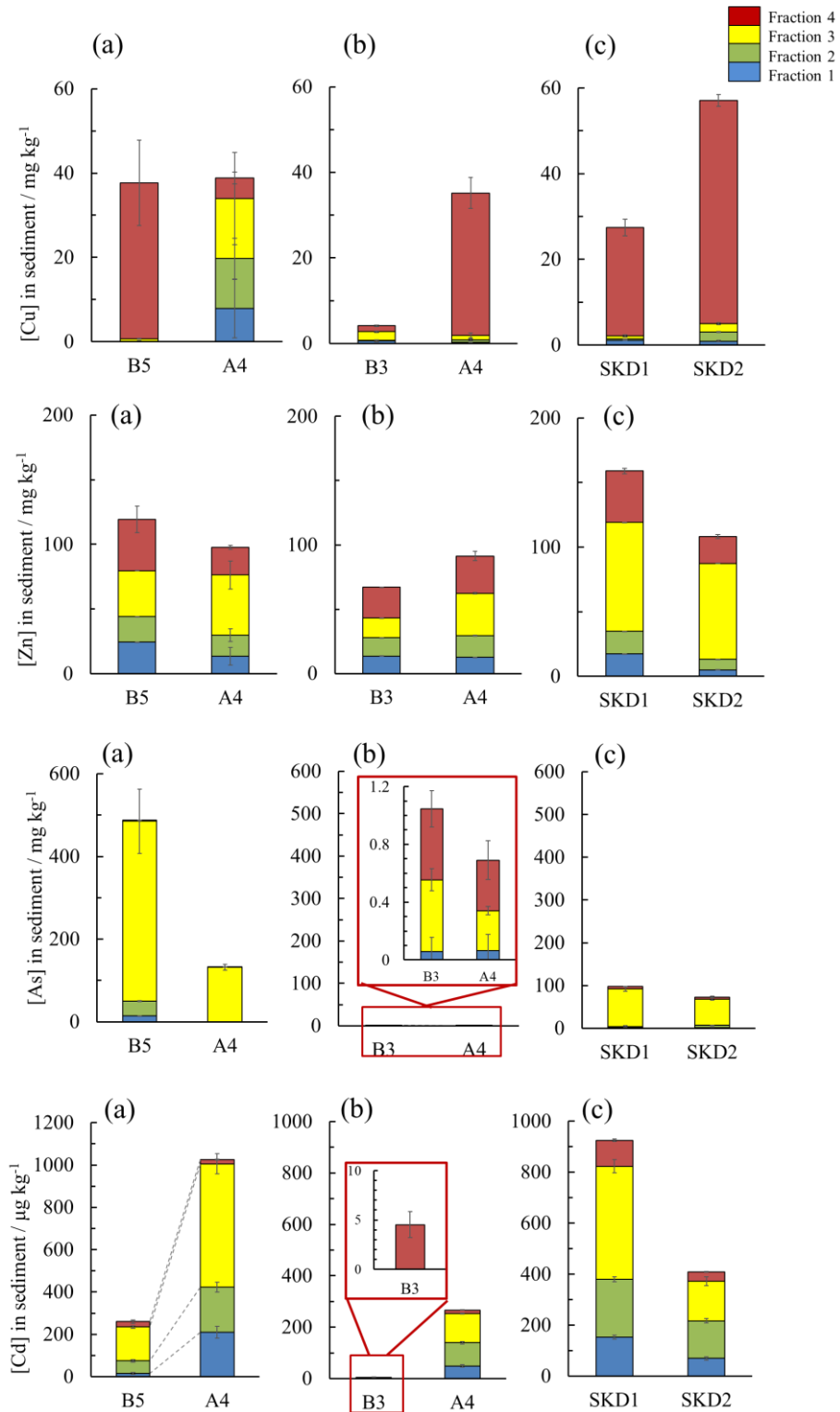
[b] N.D. = not detected.

Fig. 3-5 Concentrations and fractionations of Cu, Zn, As, and Cd from the various sediment samples from (a) Yukawa river, (b) Yazawa river, and (c) Shinaki dam lake. The error bars on each bar graph are obtained from the triplicate measurements.

Table 3-8 Concentrations and abundance ratios in the fractionations of several elements in the sediment samples

Concentrations

		Yukawa river		Yazawa river		Shinaki dam	
Cu	Sampling point	B5 (mg kg^{-1})	A4 (mg kg^{-1})	B3 (mg kg^{-1})	A4 (mg kg^{-1})	SKD1 (mg kg^{-1})	SKD2 (mg kg^{-1})
	Fraction 1	0.090±0.035[a]	7.83±6.93	0.572±0.149	0.239±0.003	1.16±0.09	0.893±0.182
	Fraction 2	0.047±0.020	11.9±4.8	0.210±0.042	0.639±0.250	0.291±0.051	2.14±0.13
	Fraction 3	0.503±0.141	14.2±11.0	1.98±0.19	1.02±0.58	0.714±0.199	2.01±0.22
	Fraction 4	37.0±10.1	4.90±1.42	1.37±0.19	33.3±3.6	25.3±2.0	52.1±1.4
Zn	Sampling point	B5 (mg kg^{-1})	A4 (mg kg^{-1})	B3 (mg kg^{-1})	A4 (mg kg^{-1})	SKD1 (mg kg^{-1})	SKD2 (mg kg^{-1})
	Fraction 1	24.4±14.1	13.5±1.3	13.6±2.8	12.6±2.8	17.5±4.1	4.93±0.73
	Fraction 2	19.8±0.5	16.4±2.4	14.5±5.5	16.8±1.2	17.5±1.1	8.25±0.70
	Fraction 3	35.4±2.7	46.4±1.6	15.1±1.0	32.9±0.8	84.1±2.7	74.2±4.8
	Fraction 4	39.8±14.5	21.4±3.2	24.0±0.4	28.9±6.8	39.8±6.2	20.8±2.6
As	Sampling point	B5 (mg kg^{-1})	A4 (mg kg^{-1})	B3 (mg kg^{-1})	A4 (mg kg^{-1})	SKD1 (mg kg^{-1})	SKD2 (mg kg^{-1})
	Fraction 1	14.8±0.1	0.510±0.129	0.057±0.099	0.064±0.112	1.54±0.09	2.01±0.27
	Fraction 2	34.7±1.2	0.120±0.016	N.D.	N.D.	2.90±1.39	5.10±0.06
	Fraction 3	435±78	132±7	0.497±0.078	0.276±0.029	87.9±5.5	61.1±2.9
	Fraction 4	3.19±0.29	0.498±0.058	0.492±0.126	0.350±0.134	5.42±0.07	5.10±1.47
Cd	Sampling point	B5 (μg kg^{-1})	A4 (μg kg^{-1})	B3 (μg kg^{-1})	A4 (μg kg^{-1})	SKD1 (μg kg^{-1})	SKD2 (μg kg^{-1})
	Fraction 1	15.1±4.7	209±28	N.D.	49.4±5.1	153±8	69.4±6.8
	Fraction 2	59.5±5.9	213±23	N.D.	89.9±4.7	227±10	148±9
	Fraction 3	160±5	584±48	N.D.	113±2	443±26	155±18
	Fraction 4	26.1±7.9	19.4±2.4	4.52±1.32	14.4±0.9	102±5	36.3±2.1

[a] average ± standard deviation obtained by triplicate measurements.

N.D.: not determined.

Table 3-8 (continued)

Abundance ratios of the fractions

		Yukawa river		Yazawa river		Shinaki dam	
Cu	Sampling point	B5	A4	B3	A4	SKD1	SKD2
	Fraction 1	0.2%	20.2%	13.8%	0.7%	4.2%	1.6%
	Fraction 2	0.1%	30.6%	5.1%	1.8%	1.1%	3.7%
	Fraction 3	1.3%	36.6%	47.8%	2.9%	2.6%	3.5%
	Fraction 4	98.3%	12.6%	33.2%	94.6%	92.1%	91.2%
Zn	Sampling point	B5	A4	B3	A4	SKD1	SKD2
	Fraction 1	20.5%	13.8%	20.2%	13.8%	11.0%	4.6%
	Fraction 2	16.6%	16.8%	21.6%	18.4%	11.0%	7.6%
	Fraction 3	29.7%	47.6%	22.5%	36.0%	52.9%	68.6%
	Fraction 4	33.3%	21.9%	35.7%	31.7%	25.1%	19.2%
As	Sampling point	B5	A4	B3	A4	SKD1	SKD2
	Fraction 1	3.0%	0.4%	5.5%	9.3%	1.6%	2.7%
	Fraction 2	7.1%	0.1%	–	–	3.0%	7.0%
	Fraction 3	89.2%	99.2%	47.5%	40.0%	90.0%	83.3%
	Fraction 4	0.7%	0.4%	47.0%	51.0%	5.5%	7.0%
Cd	Sampling point	B5	A4	B3	A4	SKD1	SKD2
	Fraction 1	5.8%	20.4%	–	18.5%	16.6%	17.0%
	Fraction 2	22.9%	20.8%	–	33.7%	24.5%	36.2%
	Fraction 3	61.3%	56.9%	–	42.4%	47.9%	38.0%
	Fraction 4	10.0%	1.9%	100%	5.4%	11.0%	8.9%

The changes in the abundance ratio of fractions before and after neutralization were considered through XRD pattern of the residues obtained following each fractionation. As shown in Fig. 3-6b, the calcite in residue obtained after Fraction 3 in A4, where was collected from place after imputing lime, was not almost observed, compared to those of the Fractions 1 and 2. No changes in XRD patterns of residues in four fractions at B5 (Fig. 3-6a), where was collected from the place before imputing lime, was obtained. The main contents of sediment sample in B5 were expected to be silicate such as feldspar, quartz and α-cristobalite as well as calcite, considering the data in XRF (Table 3-2). Namely, adding lime strongly affects the composition of sediment surface.

Also, Fraction 2 in A4 was enhanced by the neutralization, against Fraction 1 in B5 was reduced. The elemental analysis by XRF (Table 3-2) suggests that the solubility of Pd sediments may be decreased with binding to carbonate such as $CaCO_3$ or HCO_3^-/CO_3^{2-} in Fraction 2 (Fig. 3-4).

Interestingly, the Pd fractionation results from sediment sample of the Shinaki dam lake were comparable to those from the river sediments following the neutralization, thereby indicating that the Pd-containing sediment moves downstream into the Shinaki dam. Moreover, the total Pd, Cd, and As concentrations in SKD1, which is located near the Yukawa estuary, was higher than that in SKD2, situated near the Yazawa estuary.

The leachability of Pd from the sediment was found to be lower than those of homologous elements such as Cd and Zn, because the easily eluted form of Pd exhibited not only low abundance ratios in Fractions 1 and 2 but also a low absolute amount.
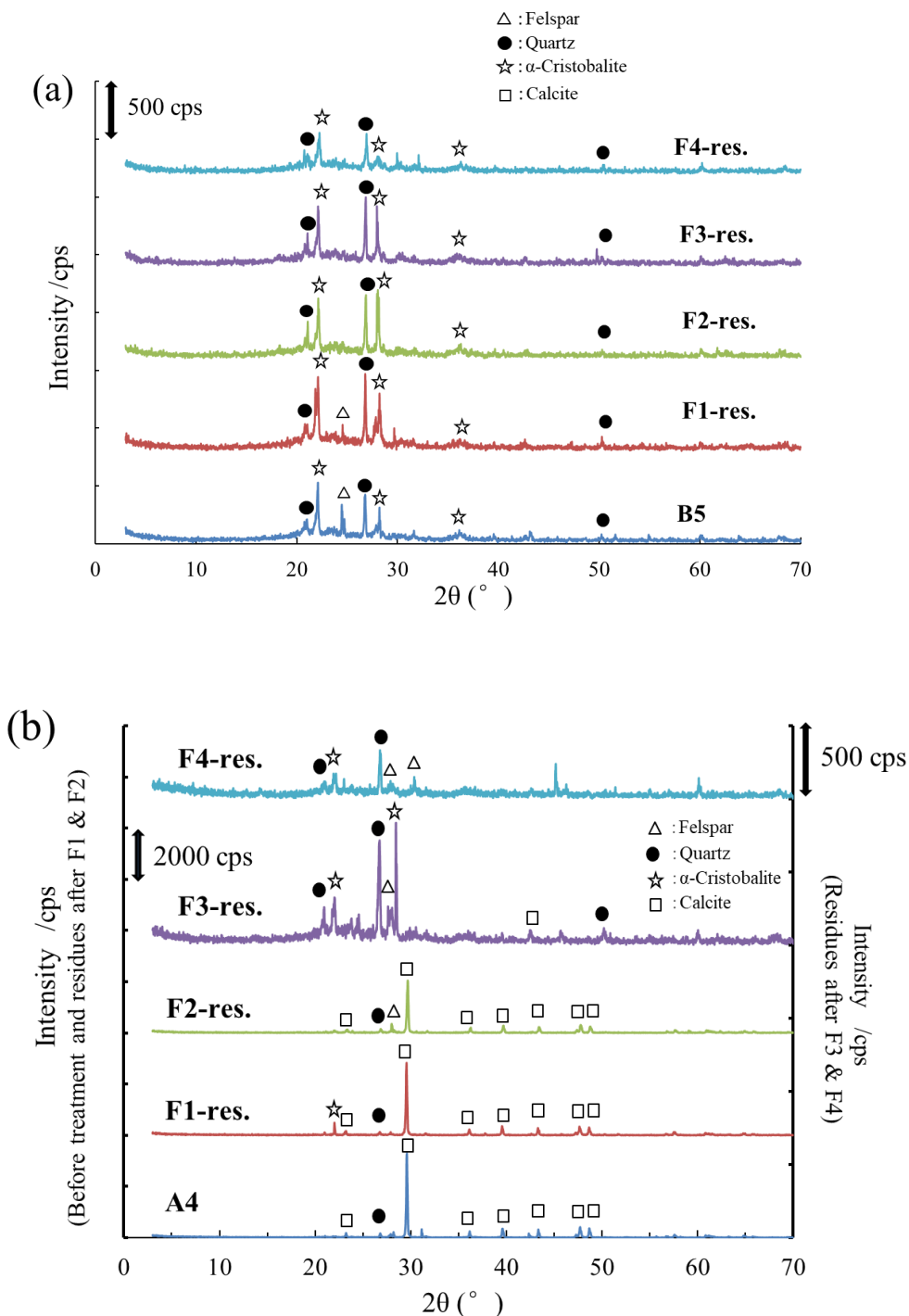
Fig. 3-6 XRD patterns of residues after each fractionation of Yukawa river sediment by sequential extraction procedure (SEP). Sampling point: (a) B5 (before neutralization factory) and (b) A4 (after neutralization factory). The bottom pattern is before SEP. F1-res.: residue of Fraction 1, F2-res.: residue of Fraction 2, F3-res.: residue of Fraction 3

and F4-res.: residue of Fraction 4.

3.3.4. Pathway of Pd precipitation in the sediment of Shinaki dam-lake

In order to discuss the pathway of Pd precipitation in the sediment of Shinaki dam, the Pd amount in each sampling point was calculated.

In the river sediments after the neutralization, the Pd concentrations and the fractionation by the SEP seemed to be affected by lime inputting. However, the estimations of the Pd amounts from the daily inputs of lime in Yukawa and Yazawa rivers were lower than those in their river waters before the lime inputting as summarizing in Table 3-9. Because the variation of the Pd concentration in the river water was smaller than those of As and Cd (Fig. 3-2), the Pd dissolved in the river water before the neutralization may reach the Shinaki dam-lake.

Accordingly, the large amount of Pd precipitated in the lake sediment would mostly originate from Pd dissolved in the Yukawa river, while still being somewhat affected by the lime input. The one of main supply sources of Pd in the river water was considered to be the Bandaiko spring. However, as the Pd amount per daily flow rate in Table 3-9, the sources of Pd in the rivers of Kusatsu hot spring area would also be included from the tributary rivers and hot spring water of several volcanos surrounding Bandaiko.

Table 3-9 Daily transitions of Pd in the Kusatsu hot springs area

Inflow sources	Concentrations of Pd in water sample	Daily flow rate [b]	Pd amount
Yazawa river	0.034 ± 0.009 µg/L [a]	$(16.0 \pm 9.9) \times 10^3$ m^3/day [b]	0.55 g/day
Yukawa river	0.043 ± 0.016 µg/L [a]	$(68.4 \pm 13.5) \times 10^3$ m^3/day [b]	2.93 g/day
Bandaiko spring	0.048 ± 0.015 µg/L	$(12.8 \pm 0.7) \times 10^3$ m^3/day [c]	0.61 g/day
Lime inputted	Total concentration of Pd in Fractions 1–4 by SEP	Daily lime input into river at neutralization [d]	Pd amount
Lime (solid)	10.2 µg/kg	Osawa riv. 7.4 t/day Yazawa riv. 4.1 t/day Yukawa riv. 53.0 t/day	0.075 g/day 0.042 g/day 0.541 g/day
Lake sediments	Total concentration of Fractions 1–4 by SEP	Annual dredging [d]	Pd amount
SKD1	37.9 ± 9.7 µg/kg	15.5 kt/year (42.5 t/day)	588 g/year (1.61 g/day)
SKD2	23.8 ± 2.9 µg/kg		369 g/year (1.01 g/day)

[a] Average Pd concentration in the Yukawa and Yazawa rivers (B1–B5) before the neutralization.

[b] Daily flow rates (Q) of the rivers were estimated by Square dam's formula (JIS K0094) using the water level data investigated by Shinaki Dam Water Quality Control Office (Ministry of Land, Infrastructure and Transport Kanto Regional Development Bureau) as following;

$Q = 1.838 (w - 0.2h) h^{3/2} \times 60,$

where Q is flow quantity, w is notch width of river and h is overflow level.

[c] Daily flow rate of the Bandaiko spring was data provided by Kusatsu-cho office.

[d] The values were the monitoring data obtained by the Shinaki Dam Water Quality Control Office (Ministry of Land, Infrastructure and Transport Kanto Regional Development Bureau).

3.4. Conclusions

I investigated the distribution and fractionation of Pd in the river water and sediments of the Yukawa and Yazawa rivers, and the Shinaki dam. As a result, I found that the concentration of Pd in the river water was slightly affected by the addition of a lime suspension, which contains trace amounts of Pd. Moreover, the leachability of Pd from the sediment following the neutralization was found to be extremely low compared to that of other metals, despite the ease of elution obtained by the sequential extraction procedure.

Consequently, it was expected that the Pd content in the sediment of the Shinaki dam-lake was supplied by the Yukawa river inflowing spring from Bandaiko mine, tributary rivers and hot spring water, rather than the sediment with precipitated Pd from the lime input during the neutralization.

Considering the continuous rising of the market price of Pd (33,030 US$/kg at 29 Nov. 2017 [18], the recovery of Pd from the sediment may be required. However, taking into account the findings of this study, it is likely that this will not be an easy process because of the large amount of acids and reductants needed to extract Pd.

3.5. References

[1] K. Ravindra, L. Bencs, and R. Van Grieken, Platinum group elements in the environment and their health risk, Science of the Total Environment, 318 (2004) 1-43.

[2] I. A. Okorie, J. Enwistle, and J. R. Dean, Platinum group elements in urban road dust, Current Science, 109 (2015) 938-942.

[3] J. Naldrett, and J. M. Duke, Platinum metals magmatic sulfide ores, Science, 208 (1980) 1417-1424.

[4] S. Terashima, N. Mita, S. Nakao, and S. Ishihara, Platinum and palladium abundances in marine sediments and their geochemical behavior in marine environments, Bulletin of the Geological Survey of Japan, 53 (2002) 725-748.

[5] R. E. Price and T. Pichler, Distribution, speciation and bioavailability of arsenic in a shallow-water submarine hydrothermal system, Tutum Bay, Ambitle Island, PNG, Chemical Geology, 224 (2005) 122-135.

[6] Y. Kikawada, S. Kawai, K. Shimada, and T. Oi, Arsenic Originating in Kusatsu Hot Springs, Gunma, Japan, Journal of Disaster Research, 3 (2008) 261.

[7] Y. Ogawa, D. Ishiyama, N. Shikazono, K. Iwane, M. Kajiwara, and N. Tsuchiya, Fractionation and deposition of indium and arsenic from the Kusatsu and Tamagawa acidic hot springs, Japan: Possible man-made analogues for rare metal concentrations onto lake beds? Economic Geology, 108 (2013) 1641-1656.

[8] Y. Kikawada, T. Oi, T. Ossaka, H. Kakihana, and T. Honda, Leaching of lanthanoids from andesitic rocks by acidic aqueous solutions, Geochemical Journal, 29 (1995) 67-84.

[9] K. Nakano, M. Sato, A. Tsuda, H. Sekiya, M. Mori, and H. Itabashi, Speciation Analysis for Heavy Metals in Sediments at Kusaki Dam-Lake with Sequential Extraction Procedure, Bunseki Kagaku, 59 (2010) 829-837.

[10] A. Tessier, P.G.C. Campbell, and M. Bisson, Sequential extraction procedure for the

speciation of particulate trace metals, Analytical Chemistry 51 (1979) 844-851.

[11] B. P. Cid, A. F. Alborés, E. F. Gómez, and E. F. López, Use of microwave single extractions for metal fractionation in sewage sludge samples, Analytica Chimica Acta, 431 (2001), 209-218.

[12] M. K. Jamali, T. G. Kazi, M. B. Arain, H. I. Afridi, N. Jalbani, G. A. Kandhro, A. Q. Shah, and J. A. Baig, Speciation of heavy metals in untreated sewage sludge by using microwave assisted sequential extraction procedure, Journal of Hazardous Materials, 163 (2009) 1157-1164.

[13] A. Sungur, M. Soylak, E. Yilmaz, S. Yilmaz, and Hasan Ozcan, Characterization of heavy metal fractions in agricultural soils by sequential extraction procedure: the relationship between soil properties and heavy metal fractions, Soil and Sediment Contamination: An International Journal, 24 (2015) 1-15.

[14] M. Bettinelli, G. M. Beone, S. Spezia, and C. Baffi, Determination of heavy metals in soils and sediments by microwave-assisted digestion and inductively coupled plasma optical emission spectrometry analysis, Analytica Chimica Acta, 424 (2000) 289-296.

[15] Shinaki Dam Water Quality Control Office, Ministry of Land, Infrastructure and Transport Kanto Regional Development Bureau, http://www.ktr.mlit.go.jp/ktr_content/content/000076767.pdf <accessed：2018. 11. 25>

[16] M. Mori, K. Nakano, M. Sasaki, H. Shinozaki, S. Suzuki, C. Okawara, M. Miró, and H. Itabashi, Dynamic leaching and fractionation of trace elements from environmental solids exploiting a novel circulating-flow platform, Talanta, 148 (2016) 617-625.

[17] NIST/SEMATECH e-Handbook of Statistical Methods, Chapter 1.3.6.7.2., Critical values of the student's-t distribution, https://www.itl.nist.gov/div898/handbook/eda/section3/eda3672.htm <accessed：2018. 04. 05>

[18] Investment Mine HP, 5 Year Palladium Prices and Price Charts, http://www.infomine.com/investment/metal-prices/palladium/5-year/ <accessed : 2018. 03. 25>

Chapter 4

Dynamic sequential extraction procedures of heavy metals and arsenic using a circulating-flow platform

4.1. Introduction

Chapter 3 illustrated the monitoring of Pd concentration in Kusatsu area and concluded that the leachability of Pd from the sediment following the neutralization was found to be extremely low compared to that of other metals by speciation analysis. Indeed, the leaching test and sequential extraction procedures (SEP) [1-5] were established as a method to manage the leachability of the targeted elements from solid substances such as sediment, fly ash, or plants. Also, the results in their tests are used to assess risk for human health with contamination of toxic elements [6,7]. However, the classifications of chemical forms of element species by SEP are usually done by batch procedures with complexed handlings. The extraction resulted in the complexed procedures easily lead to susceptible to sample contamination during the unit operations, such as filtration, centrifugation, and reagent replacement. Therefore, to overcome these problems commonly found in batch procedures, novel automatic flow systems for dynamic extraction procedures that may afford the addition of in-line extraction of metals, filtration of remaining solids, and in-line analysis of extracts has been developed in the recent years. [8-10].

In this chapter, I described an automatic inline-extraction system employing all injection (AI) system that was introduced in chapter 1. The inline-extraction system with AI system consists of a rotary pump, an eight-port valve, a micro-column to pack solid sample, a control panel and some reaction coils [12]. The extraction system can operate

without centrifugation or filtration as compared with the conventional batch system. Also, because of the extractant feed is stopped during the circulation process, the amounts of reagents and waste liquid are significantly decreased as compared with the conventional continuous flow or flow injection manifolds. The overall extraction time was also reduced to one-third of the time spent by batch system.

Furthermore, I judged whether the AI system were suitable for the SEP of heavy metals and arsenic in sediment samples by extractants used according to Tessier's scheme which is a widely used extraction method.

This chapter describes the potentialities of automatic inline-extraction AI system through 1) optimization of the circulation time for extraction abilities of heavy metals (Cu, Zn, Cd and Pb) in a certified reference material (CRM) of lake sediment on the SEP, 2) the abundance ratios of fractionations of metals obtained by this system, and 3) the SEP of arsenic by the AI system. These quantitative results were compared to those in batch system.

4.2. Experimental

4.2.1. Sediment samples

Certified reference material (CRM), NMJ CRM 7303-a, obtained from National Institute of Advanced Industrial Science and Technology (Tsukuba, Japan) was used as reference lake sediment, and certified values were shown in Table 4-1. Furthermore, lake sediment samples were collected from Kusaki dam-lake (N36°33′, E138°22′) in Gunma Prefecture (Pref.). Kusaki dam-lake locates at the upstream of the Watarase River in Midori city of Gunma Pref. The sediment contains relative high concentrations of heavy metals owing to the influence of the Ashio copper mine. The sediment samples of dam lake were collected from surface (0–30 cm) of the lake bottom using an Ekman-Birge bottom sampler (Miyamoto Riken, Co., Ltd., Osaka, Japan) by staffs of Kusaki Dam Operation and Maintenance Office from Japan Water Agency (Numata, Japan) at the sites

of 0.2 km upstream from the weirs of dam reservoir.

Table 4-1　　Certified values of heavy metals and arsenic in CRM, NMIJ CRM 7303-a

Certified value / mg kg^{-1}				
Cu	Zn	Cd	Pb	As
23.1 ± 3.1^{a}	107 ± 5	0.342 ± 0.017	31.3 ± 1.1	8.6 ± 1.0

[a] average $\pm$ standard deviation

The sediment samples collected in Kusaki dam-lake was packed into a plastic sachet and was immediately transported to our laboratory. To reduce the biological reproduction in the wet sediment samples, the collected samples were air-dried at 25 ± 1 °C in our laboratory. The dried samples were pulverized using a mortar and pestle, and sieved to obtain particles with a diameter < 2 mm. Finally, the obtained samples were dried at 60 °C for 24 h.

4.2.2. Reagents

All reagents used in this study were purchased from Wako Pure Chemical Industries, Co., Ltd. (Osaka, Japan) and were of analytical grade. The standard solutions were prepared using ultrapure water, which was purified using a water distillation system (ASK-2DS, Iwaki Co., Ltd., Tokyo, Japan). The extractants of Fraction 1 to Fraction 3 were described in chapter 3. The extractant of Fraction 4 used 1 mol dm^{-1} hydrochloric acid (HCl) instead of the hydrogen peroxide solution (H$_2$O$_2$) indicated by Tessier *et al.* [2]. This was because the gas was produced during the reaction between soil and H$_2$O$_2$ after introducing H$_2$O$_2$ into the flow channel of the AI system, and the pressure in the channel was rapidly increased and damaged.

4.2.3. Apparatuses

The AI system, AIA-IT3, was manufactured by Ogawa, Co. Ltd. (Kobe, Japan) and Kurahashi Giken, Ltd (Kyoto, Japan) in collaboration with our reach group. This system

consists of single rotary pump, an eight-port valve, a four-port valve, a micro column to pack solid sample, and a process control monitor (see details in Fig.1-2b). The valves and column were connected by tubes that made of Teflon (AS ONE, Osaka, Japan) with the diameter of 1mm, and tubes used in pump were made of silicon (2mm i.d., AS ONE).

In the batch procedures, a magnetic stirrer (RS-1 DW, As-One Corp., Osaka, Japan) was used to mix solid samples and the reagents. The pH meter (F-22, Horiba, Ltd., Kyoto, Japan) was used when the pH of reagent was adjusted. Centrifugation equipment (Kokusan H-200, Kokusan Co. Ltd., Tokyo, Japan) was used to separate solids and reagents after the batch-wise extraction.

A targeted element extracted was quantified by a polarized type frame atomic absorption spectrometry Z-5310 (FAAS, Hitachi Z-5310) or inductively coupled plasma mass spectrometry (ICP-MS, HP4500, Agilent) with a collision cell to remove interferences on target elements, according to the concentration of analytes.

4.2.4. Extraction procedures by the AI system

The extraction procedures of heavy metals and arsenic from solid samples the AI system is shown in Fig. 4-1.

First, filling extraction reagent: a 0.25 g of sediment samples were sandwiched between PTFE membrane filters (pore size: 20 nm) and packed in column (C). This column was installed into the circulation channels. The extractant (R) was filled into the flow channel and the solid sample column at a flow rate of 2 mL min^{-1} [12] by using peristaltic pump (P) for 1 minute, because of the column back pressures t were estimated as 53 kg cm^{-2} at 2.1 ml min^{-1} and 46 kg cm^{-2} at 2.0ml min^{-1}, and the flow back pressure in this system should be limited to ca. 50 kg cm^{-2} to prevent mechanical damages. (step 1 in Fig. 4-1)

Second, circulation of extractant: by switching position of 8-port valve, the flow-line was changed into closed-line to circulate extractant to extract metals from solid samples at a given time by a peristaltic pump at a flow rate of 2 mL min^{-1} (step 2 in Fig.

4-1).

Finally, the collection of extractant with analyte extracted: following the extraction operation, the valve was returned to original position, and ultrapure water (W) was brought to flow-line to send out the solution containing an analyte at a flow rate of 2 mL min^{-1} for 5 minutes (step 3 in Fig. 4-1). The solution flushed out was collected into a 20 mL-measuring flask and messed up by ultrapure water.

All fractionations in SEP were carried out at room (ambient) temperature. The analyte was quantified by FAAS or ICP-MS, following the extracting solution dilute with water to 20 mL.

In the SEP on the AI system, after the first fractionation as mentioned above, the extractant for the next fractionation was introduced, and the extraction procedures was performed from step 1 to step 3 in Fig. 4-1. This operation was sequentially performed for F1 to F4. The flow paths were cleaned with 0.1 mol dm^{-1} nitric acid and deionized water for ~25 min to completely remove residues in the flow channels.

(a) Step 1: Filling extraction agent

(b) Step 2: Circulation

(c) Step 3: Collection of extractant
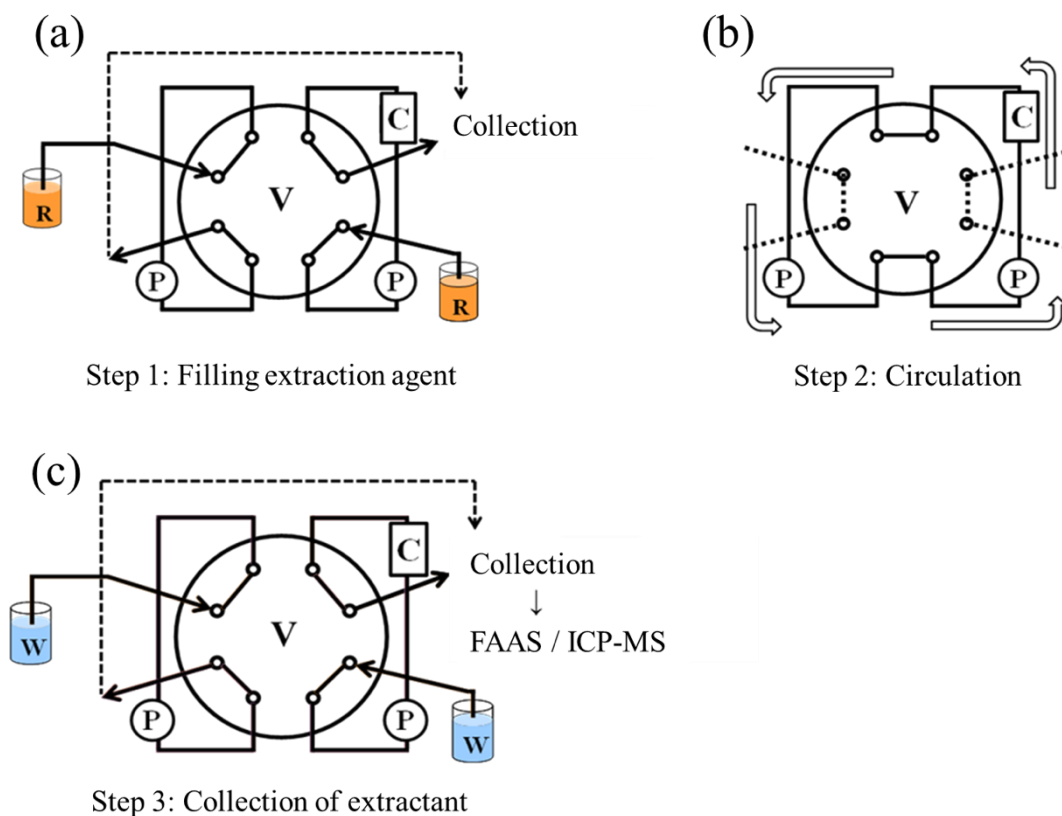
Fig. 4-1 Illustration of the analytical AI system for single extraction and fractionation protocols based on extractants re-circulation.

C: column; P: peristaltic pump; W: water; V: valve; R: extraction reagent

4.2.5. Sequential extraction procedure

4.2.5.a Batch system

The batch SEP was performed according to Tessier *et al* [2]. The operation procedures for each fraction were as follows:

Fraction 1(F1): 8 mL of 1M $MgCl_2$ was added to 1.0 g of the solid sample, and the mixture was stirred at room temperature with a speed of 600 rpm for 1 h by a magnetic stirrer.

Fraction 2 (F2): The residue obtained in the procedures of F1 was mixed with 8.0 mL 1.0 M CH_3COONa (pH=5) with a speed of 600 rpm, at room temperature for 1 h using a magnetic stirrer.

Fraction 3 (F3): The residue obtained in the procedures of F2 was mixed with 8.0 mL 0.04 M $NH_2OH-HCl$ (25% v/v CH_3COOH) with a speed of 600 rpm, at room temperature for 3 h using a magnetic stirrer.

Fraction 4 (F4): The residue obtained in the procedures of F3 was mixed with 8.0 mL 1 M HCl with a speed of 600 rpm, at room temperature for 3 h by a magnetic stirrer.

After the F1–F4 fraction operations, centrifugation of the extraction solution was performed at 3000 rpm for 20 min, and filtered the separated solution through a 0.45 µm syringe filter. the residue after the separation was washed with 8 mL of water, before centrifuged again in the same way.

The obtained extract was dilution to 25 mL, and the concentration of each element was measured by FAAS or ICP-MS.

4.2.5.b The AI system

The same extractants as used in the batch procedures were in the AI system. The

extraction procedures for F1–F4 were successively carried out in the same way as the batch method was performed. The liquid-to-solid ratio of the fractionation was the same as those in the batch method (2 mL extractant and 0.25 g soil or sediment). The extractions were performed at the ambient temperature (25 ± 1 °C). The continuous circulation rate when the extractant passed through the packed column was 2.0 mL min^{-1} [12]. The extracts were diluted to 20 mL with water, then the concentrations of the analytes in the extracts were determined by ICP-MS.

4.3. Results and discussion

4.3.1. Optimization of circulation time

The circulation times for each fractionation were determined to heavy metal concentrations extracted by four types of extractants used in four-step SEP retaining 2.0 mL min^{-1} of circulation rate. The sediment sample of Kusaki dam-lake was used to monitor the dynamic leaching by dividing the circulation time of 0.5, 1, 2, 3 and 5 h.

The circulation time for leaching of trace elements until steady-state extraction conditions was investigated using the extractants used in each fractionation in the four-step SEP. The analyte elements were Cu and Pb, which were commonly employed as analytes on sequential extraction of heavy metals from soil or sediment.

The criteria for determining circulation time for four fractions were judged from relative standard deviations (RSD, $n = 3$) less than 10% for the average times required to reach each maximum concentrations of Cu and Pb. As shown in Fig. 4-2, the well-reproducible extractions of both heavy metals among the four channels were provided in circulation time for 1 h at 1 mol L^{-1} MgCl$_2$ (F1), and 1 h at 1 mol L^{-1} CH$_3$COONa (F2) for both metals. Those with 8.8 mol L^{-1} NH$_2$OH·HCl (F3) and 1 mol L^{-1} HCl (F4) were 3 h for Cu and Pb, respectively. Consequently, the timeframes required to reach steady-state concentrations were 1 h for Fractions 1 and 2, and 3h for Fractions 3 and 4 under circulation rate of 2 mL min^{-1}.
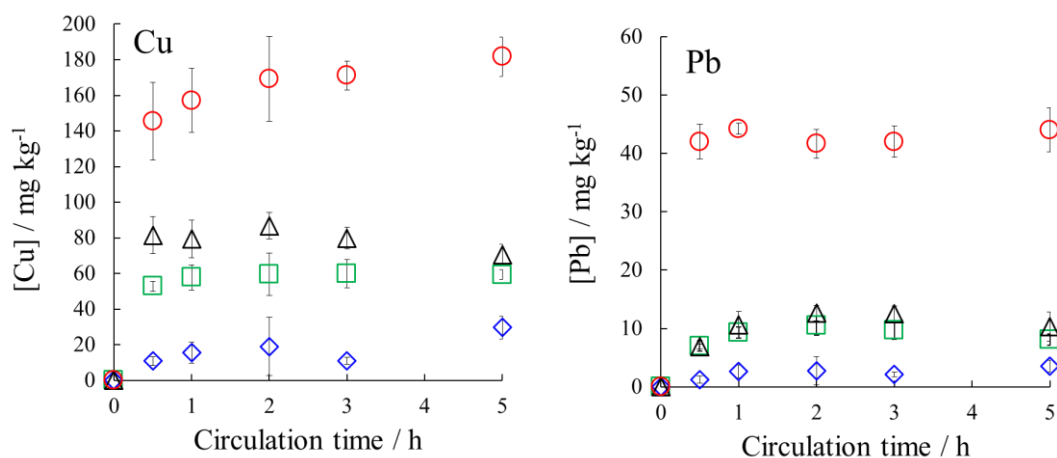
Fig. 4-2 Concentrations of Cu (left) and Pb (right) on each fraction as a function of circulation time. Plot's identities: (◇) Fraction 1 (F1); (□) Fraction 2 (F2); (△) Fraction 3 (F3); and (○) Fraction 4 (F4). Circulation rate: 2 mL min^{-1}.

4.3.2. Comparison between the AI system and batch system on the SEP

SEP of heavy metals (Cu, Zn, Cd and Pb) in lake sediment CRM was carried out using the AI system, and the abundance ratios of the fractionations obtained were compared with the batch system. As shown in Fig. 4-3, in both the AI system and the batch system, the extracted amount tends to increase from F1 to F4 for Cu, Zn and Pb. In the case of Cd, the concentration in F1 was the highest of the fractions. Even in previous study, many fractionations of Cd in sediments tended to contain easy-dissolved form [12], so that Cd is more likely to be dissolved from sediment into environmental water rather than other heavy metals such as Cu and Zn. In addition, except for Cd, the RSD of batch system and AI system were lower than 20%.

The total concentrations of heavy metals in the AI system were slightly larger than those in the batch system. The difference of concentrations of the Cu, Zn, Cd and Pb extracted by the AI system to those by the bath system was +11%, +4.2%, +16% and +8.4%, respectively. This is because the circulation of the extractant in a constant flow path might hardly cause re-adsorption of heavy metal to the sediment sample compared

to stirring or shaking operations in the batch procedure. The recoveries of total metal concentrations in this system to the metal content obtained in CRM ranged from 14% to 87%. The recoveries were comparable to the batch system (13% to 75%).

Generally, the smaller sample loading become easily to be affected to particle size of the sample; the sample amount used in the AI system is 1/4 smaller than that in the batch method. However, the concentrations of heavy metals extracted by the AI system could provide highly accurate analytical results as summarized in Table 4-2.

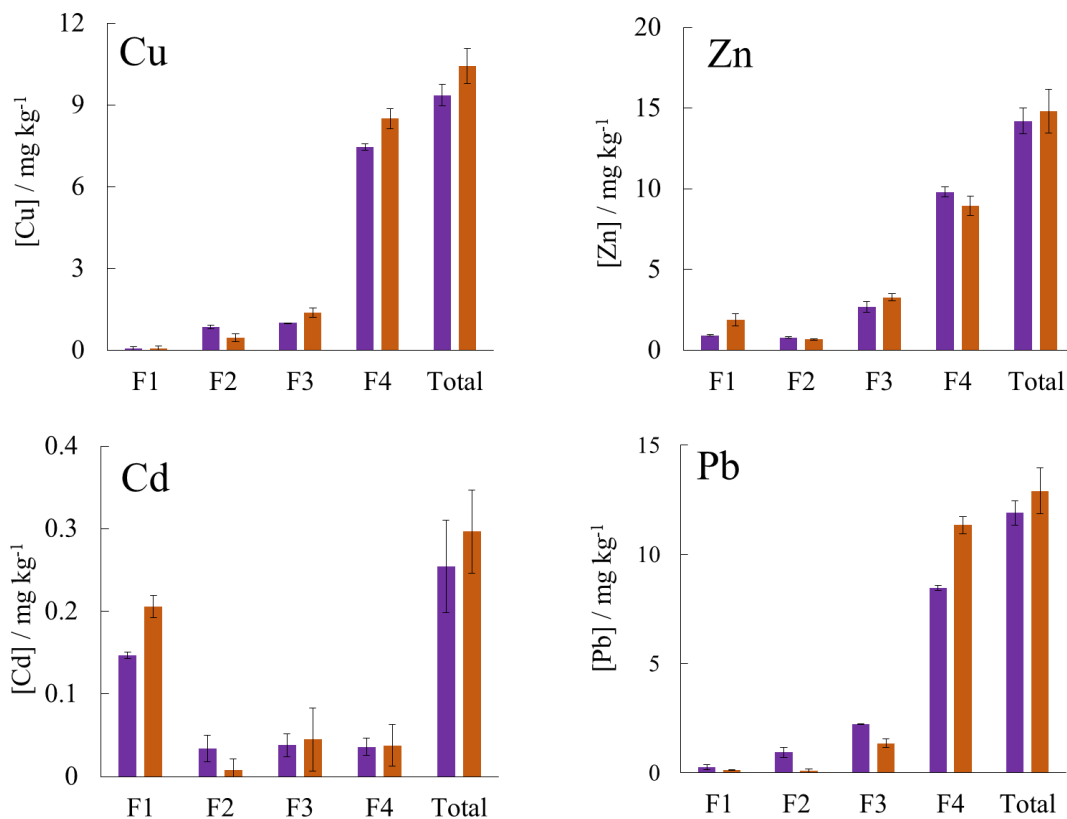Consequently, the AI system can give the heavy metal extraction with high quality performances.

Fig. 4-3 Heavy metals (Cu, Zn, Cd and Pb) concentrations of each fraction by the batch system and the AI system. (■) Batch method and (■) AI system

Table 4-2　Total concentrations of four fractionations (F1-F4) and recovery of heavy metals of certified reference material (CRM) by the AI system and batch system

	Cu		Zn		Cd		Pb	
	Batch	AI	Batch	AI	Batch	AI	Batch	AI
Total concentration / mg kg^{-1}	9.36 ± 0.02	10.4 ± 0.6	14.2 ± 0.6	14.8 ± 1.4	0.255 ± 0.034	0.296 ± 0.050	11.9 ± 0.4	12.9 ± 1.1
Certified value / mg kg^{-1}	23.1 ± 3.1		107 ± 5		0.34 ± 0.02		31.3 ± 1.1	
Recovery / % [a]	41	45	13	14	75	87	38	41

[a] Recovery (%) = [Total concentration] / [Certified values] $\times$ 100

The SEC by the AI system had several advantages compared to that by batch operation as follows;

1) The waste liquid generated by the AI system was 90 mL, while that generated by the operation of batch method was 120 mL.

2) Because the AI system can skip the centrifugation and filtration processes that are indispensable for the batch method and can operate in the closed flow channel, the experimental errors or sample contaminations can be minimized.

3) The whole operation time on the AI system was required for 9 h, against for 16 h in batch operation.

4.3.3. SEP of arsenic by the AI system and batch system

Finally, I evaluated capability of the AI system for the SEP of arsenic. The sample used were the lake sediment CRM and Kusaki dam-lake sediment. The operation method is carried out using the same extractant and circulation time set in section 4.3.1.

As shown in Fig. 4-4, in both the batch system and the AI system, the extracted concentrations of arsenic in sediment samples was confirmed from F1 to F4. The results were similar tendencies to Cu, Zn and Pb. Comparing the recoveries of arsenic in the CRM between the two systems, the recovery obtained by the AI system (58%) was higher than that the batch system (48%), as summarized in Table 4-3. In addition, when the significant difference between the batch method and the AI system was examined by Student's *t*-test ($p = 0.05$), there were no significant differences in the sediment samples of Kusaki dam-lake but were significant differences in the lake sediment CRM. This difference obtained by the lake sediment CRM might be caused because the extracted concentration of F1 and F2 in CRM by the AI system was much larger than that of batch system. Although this cause could not be cleared, the contact efficiency between the sample and the extractant would affect the extraction concentration of arsenic.
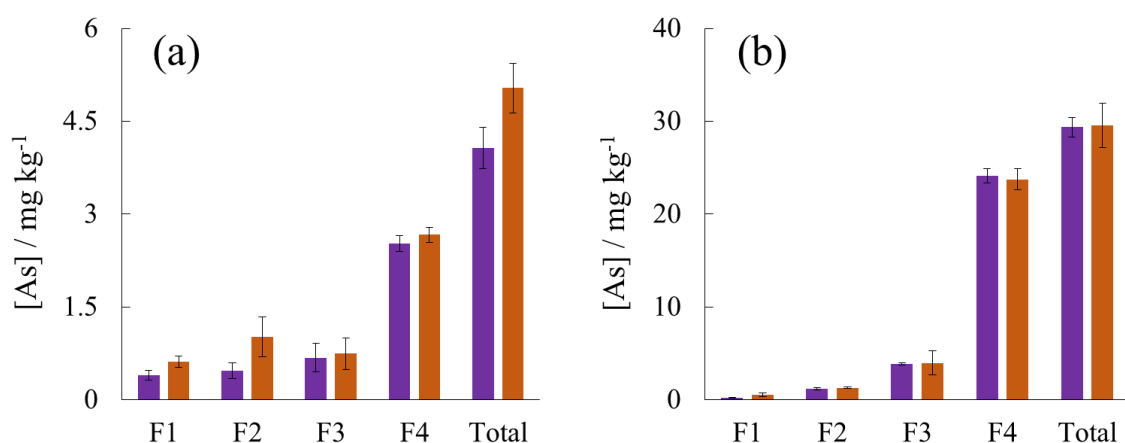
Fig. 4-4 Data comparison of sequential extraction of As from lake sediment of (a) CMR and (b) Kusaki dam-lake by the Batch against the AI system. (■) Batch method and (■) AI system

Table 4-3 Total elution amount (F1-F4) and recovery rate of As in certified reference material (CRM) by the batch system and the AI system.

	Batch	AI
Total concertation / mg kg^{-1}	4.1 ± 0.3	5.0± 0.4
Certified value / mg kg^{-1}	8.6 ± 1.0	
Recovery / % *	48	58

[a] Recovery (%) = [Total concentration] / [Certified values] × 100.

4.4. Conclusions

This chapter described that the AI system that doesn't require a complicated operation (centrifugation, filtration, washing, etc.) was the novel element extraction device in terms of accuracy, amount of waste and time consuming, compared with the conventional batch system. This method could successfully be applied to SEPs of heavy metals (Cu, Zn, Cd and Pb) and arsenic (As). Therefore, because the AI system can down-size, it is expected that can apply to on-site extraction of elements in solids such soil or sediment.

4.5. References

[1] G. Rauret, J. F. Lopez-Sanchez, A. Sahuquillo, R. Rubio, C. Davidson, A. Ure, and P. Quevauviller, Improvement of the BCR three step sequential extraction procedure prior to the certification of new sediment and soil reference materials, Journal of Environmental Monitoring, 1 (1999) 57-61.

[2] A. Tessier, P. G. Campbell, and M. Bisson, Sequential extraction procedure for the speciation of particulate trace metals, Analytical Chemistry, 51 (1979) 844-851.

[3] A. Sahuquillo, J. F. Lopez-Sanchez, R. Rubio, G. Rauret, R. P. Thomas, C. M. Davidson, and A. M. Ure, Use of a certified reference material for extractable trace metals to assess sources of uncertainty in the BCR three-stage sequential extraction procedure, Analytica Chimica Acta, 382 (1999) 317-327.

[4] T. Q. Trang Hoang, and C. Hahn, Arsenic fractionation in agricultural soil in Vietnam using the Sequential extraction procedure, International Conference on Informatics, Environment, Energy and Applications, 2015 (2015), 2184-2199.

[5] K. Leopold, A. Denzel, A. Gruber, and L. Malle, Mobility of traffic-related Pd and Pt species in soils evaluated by sequential extraction, Environmental Pollution, 242 (2018) 1119-1127.

[6] M. Maanan, M. Saddik, M. Maanan, M. Chaibi, O. Assobhei, B. Zourarah, Environmental and ecological risk assessment of heavy metals in sediments of Nador lagoon, Morocco, Ecological Indicators, 48 (2015) 616-626.

[7] X. Ke, S. Gui, H. Huang, H. Zhang, C. Wang, and W. Guo, Ecological risk assessment and source identification for heavy metals in surface sediment from the Liaohe River protected area, China, Chemosphere, 175 (2017) 473-481.

[8] D. Beauchemin, K. Kyser, and D. Chipley, Inductively coupled plasma mass spectrometry with on-line leaching: a method to assess the mobility and fractionation of elements, Analytical chemistry, 74(2002), 3924-3928.

[9] M. Jimoh, W. Frenzel, V. Müller, H. Stephanowitz, and E. Hoffmann, Development

of a hyphenated microanalytical system for the investigation of leaching kinetics of heavy metals in environmental samples, Analytical chemistry, 76(2004) 1197-1203.

[10] D. Nacapricha, P. Sastranurak, T. Mantim, N. Amornthammarong, K. Uraisin, C. Boonpanaid, C. Chuyprasartwattana and P. Wilairat, Cross injection analysis: Concept and operation for simultaneous injection of sample and reagents in flow analysis, Talanta, 110 (2013) 89-95.

[11] M. Mori, K. Nakano, M. Sasaki, H. Shinozaki, S. Suzuki, C. Okawara, M. Mirò, H. Itabashi, Dynamic leaching and fractionation of trace elements from environmental solids exploiting a novel circulating-flow platform, Talanta, 148 (2016) 617-625.

Chapter 5

Dynamic sequential extraction procedures of heavy metals and arsenic in environmental solids using a four-flow channel type circulating-flow platform

5.1. Introduction

In chapter 4, I described an automatic sequential extraction procedure (SEP) of heavy metals and arsenic in the sediment sample by the platform combing the closed circulation system and all injection (AI) system. The extraction system does not involve complicated procedures such as centrifugation, filtration, and unlike conventional batch systems, and can also reduce amounts of reagents and waste liquid. The total extraction time is about two-third of the time taken to perform a batch extraction system. However, the system could not be used to simultaneously process multiple samples because of the apparatus configuration. Namely, if three parallel experiments are required for each sample, the total extraction time is about 24-27 h, because the SEP for each sample takes 8-9 h.

Therefore, in this work I aimed to develop an effective extraction method that can simultaneously process multiple samples. Here, I established a four-channel flow-type extraction device based on the all injection system described in chapter 4. As shown in Fig. 5-1, the system has four flow channels (each containing a column packed with a solid sample), a single-flow peristaltic pump, and eight six-port valves. The extractant is passed into each channel for a specified period, and the valve then is closed. However, in this study, the simultaneous extraction of analytes in the different flow channels were performed using a manual switching valve because this method was before establishing a completely automated system.

This chapter describes the usefulness of the four flow channels on the AI system

through 1) SEP of Cd, Cu, Pb and Zn from a certified reference material (CRM) of lake sediment, a fly ash sample, a soil sample, and a sediment sample, 2) extraction of As, Cd, Cu, Pb and Y from a lake sediment sample of Shinaki dam lake (Gunma Prefecture, Japan) in hot-spring spa area, and 3) the chelating extraction using ethylenediaminetetraacetic acid (EDTA).

5.2. Experimental

5.2.1. Apparatuses

The four-channel flow-type extraction device had eight VH-V200 manual six-port switching valves (FLOM, Tokyo, Japan) and an Eyela MP-2100 Quantitative Liquid Feed Pump double-tube head-peristaltic pump (Tokyo Rikakikai, Tokyo, Japan), on which two tubes could be connected to one tube head. The system is shown in Figs. 5-1 and 5-2. The instrument could extract a sample using two six-port switching valves and one pump. The 1 mm i.d. tubes used to connect the valves, sample-packed column, and reagent reservoirs were made of Teflon (AS ONE, Osaka, Japan), and the 2 mm i.d. tubes used in the pump were made of silicon (AS ONE). The six-port valve is shown in Fig. 5-1. The system could be equipped with four eight-port switching valves, but such valves are more expensive than six-port valves and were not used because of financial constraints.

A solid sample was packed in a Rezorian cartridge (1 cm^3 interior volume, 3.5 cm long; Sigma-Aldrich, St. Louis, MO, USA). A sample (0.25 g soil or sediment, or 0.20 g fly ash) was loosely packed in the column to a depth of 10 mm, then a 20 mm thick polytetrafluoroethylene membrane filter with 0.20 μm pore and 2.0 mm thickness (Advantec, Tokyo, Japan) was placed at each end. Each column was connected to the manifold with Luer lock fittings and nuts. The concentrations of the major elements in the lake sediment samples (described below) were determined by energy-dispersive fluorescent X-ray analysis using an EA1200VX system (Hitachi High-Tech Science, Tokyo, Japan).
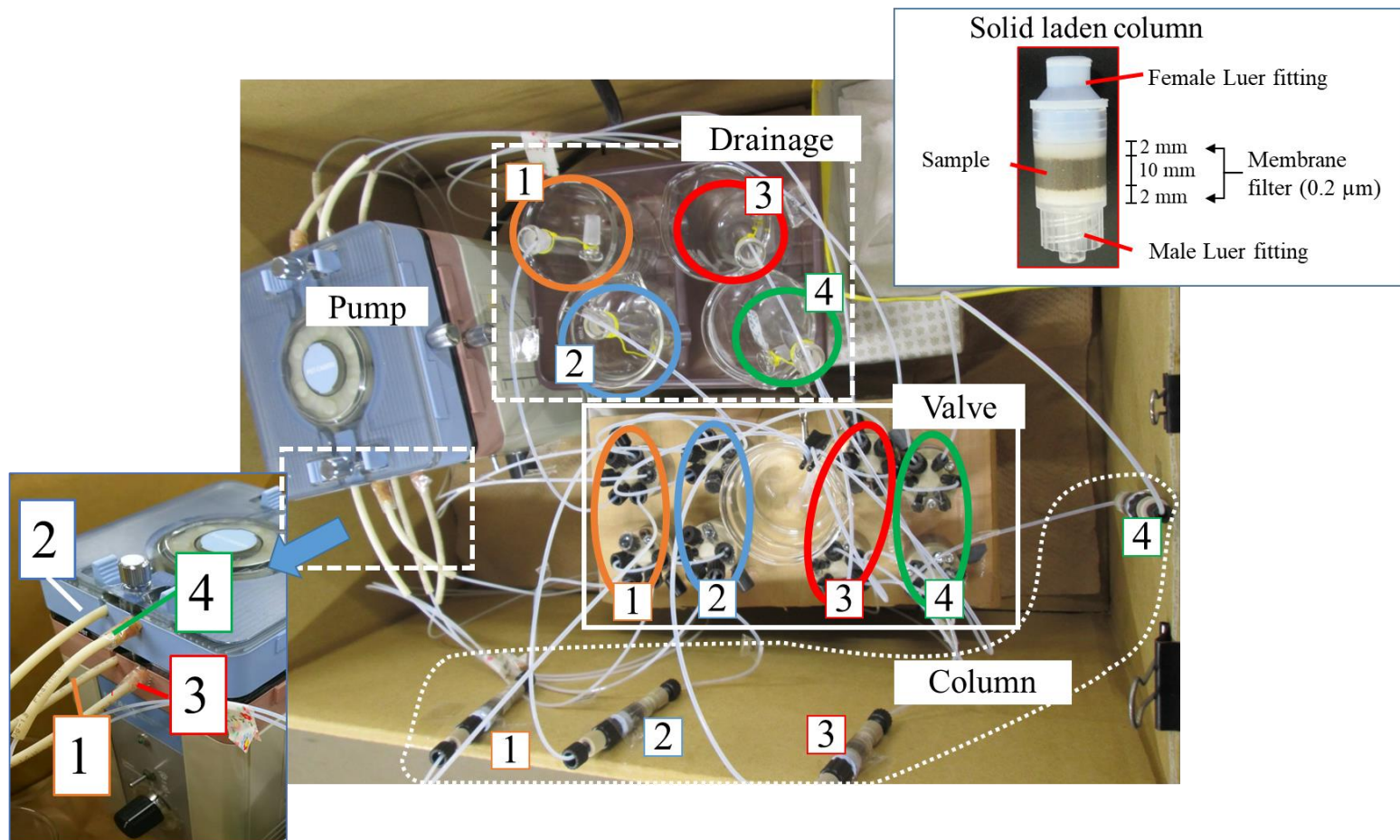
Fig. 5-1 Photo of the proposed extraction system in this study.

The lower left is enlargement of peristaltic pump module, and the upper right is solid laden column.

5.2.2. Extraction procedure

A column containing a sample was connected within a loop between two valves in the six-port switching valves. As shown in Fig. 5-2, the extractant was loaded into the switching valve loop and then circulated through the column at a constant flow rate for a specified time.

The valve switching procedure used to extract each solid sample is shown schematically in Fig. 5-2.

First, loading of extractant: a 0.25 g or 0.2 g of solid samples were packed in the columns (C) and the columns were installed into each circulation channel. The selected extractant was added to the circulation channel at a flow rate of 1 mL min^{-1} by using peristaltic pump (P) for 4 minutes. (Fig. 5-2 a)

Second, changing the position of the six-port valves to switch the flow-line to allow the extractant to circulate through the loop for the specified period, usually 1–5 h at a flow rate of 1mL min^{-1} (Fig. 5-2b).

Finally, collection of sample solution: following the extraction operation, the valves were returned to original position, and ultrapure water (W) was brought to each channel and column to send out the sample solution at a flow rate of 1 mL min^{-1} for 10 minutes (Fig. 5-2c). The solution flushed out was collected into a 20 mL-measuring flask and messed up by ultrapure water.

Switching between valve systems was performed at one-minute intervals because manual switching valves were used. The circulation channel with six-port valves contained about 2.94 mL of liquid (total length of the tubes in one channel is 297 cm), which was flushed from the rotary valve and collected in a 20 mL volumetric flask. At the end of operation, the flow paths were cleaned with 0.1 mol dm^{-1} nitric acid and deionized water to completely remove residues in the flow channels.

The extract was diluted to 20 mL with ultrapure water, then the solution was analyzed by using a polarized type frame atomic absorption spectrometry Z-5310 (FAAS,

Hitachi Z-5310) or an inductively coupled plasma mass spectrometry (ICP-MS), Agilent

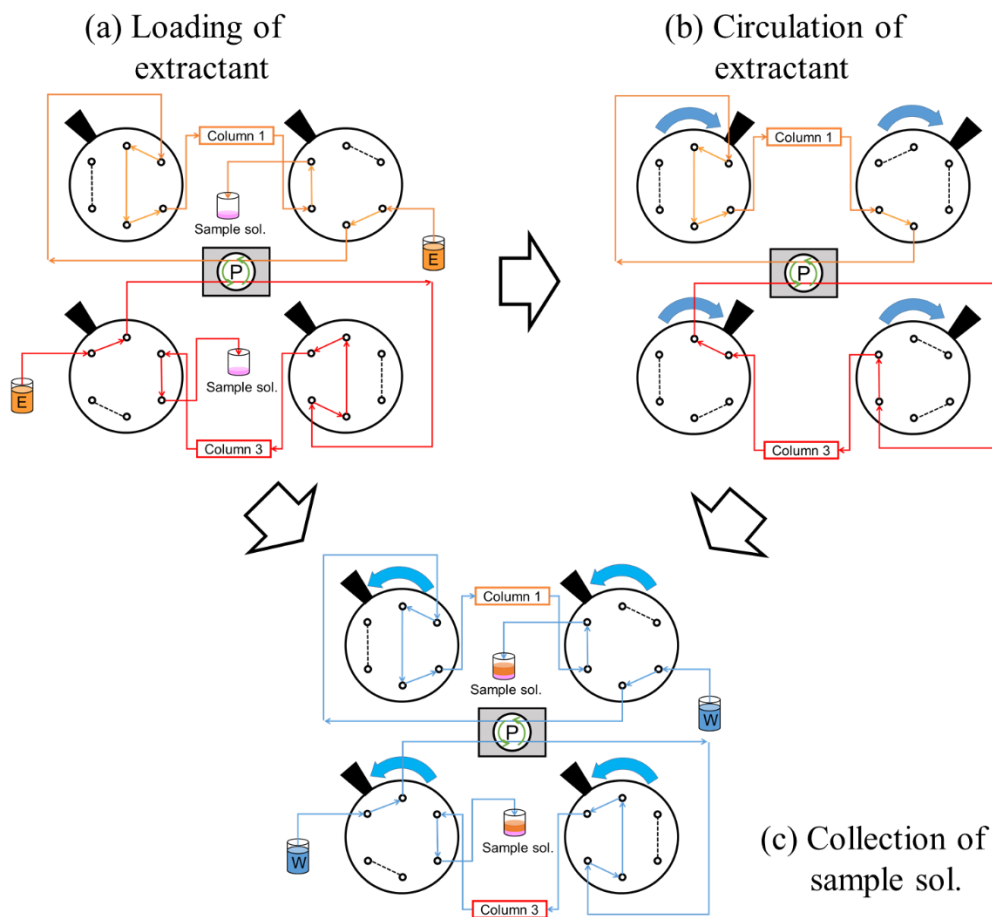7700x-ICP-MS instrument (Agilent Technologies, Santa Clara, CA, USA).

Fig. 5-2 Schematic procedures of value module in the four-channel flow system. This illustrates the 1st and 3rd flow channels set in low stage of the double pump head-peristaltic pump. P: peristaltic pump; E: extractant; W: water

5.2.3. Reagents and sediment samples

The reagents and extractants used in SEP in this chapter were identical to that of chapter 4. The extraction of metals in F4 in the case of sediment in Shinaki Dam lake were performed by batch operation using 60% (w/w) HNO_3-60% (w/w) $HClO_4$ as the extractant, due to large amounts of bubble and gas were caused when the reaction between F3 residue and HCl, and the pressure in the channels were rapidly increased. The EDTA that was used as an extractant in the chelating extraction of metal from a lake sediment sample was added to be 5.0 mmol dm^{-3} in 1.0 mol dm^{-3} sodium acetate, the solution pH was adjusted to 5 with acetic acid.

The solid samples used as following;

1) The lake sediment in certified reference material (CRM) used in chapter 4.

2) The sediments of Kusaki dam-lake that were collected at sampling site located in 4.0 km (K1) and 0.2 km (K2) upstream from the weirs of dam reservoir (Fig. 5-3a).

3) The lake sediment from the Shinaki dam (N36°37′, E138°38) which was pumped from the dredger to a land facility using a high-pressure pump (Fig. 5-3b, S1).

4) A soil sample collected from parking lot (N36°33′, E139°10′) of park surrounding Lake Onuma (Fig. 5-3c, O1) at Mt. Akagi (Maebashi, Gunma Pref., Japan) [1].

5) A fly ash obtained from a domestic waste disposal facility in Kiryu, Gunma Pref., Japan.

As fly ash samples contain hazardous heavy metals at high concentrations, heavy metals can be eluted from fly ash into soil and groundwater after landfill disposal, so it is necessary to clarify these metals' solubility [2,3].

All of the sediment samples were transferred to our laboratory as soon as possible, after being packed in plastic sachet. Before pulverized by a mortar, the sediment samples were air-dried at $25\pm1℃$ to remove biological materials. The grinded samples were sieved to obtain particles with a diameter < 2mm except the sample of Parking lot of Onuma. Finally, they were dried at 60℃ for 24 h with a drying oven.
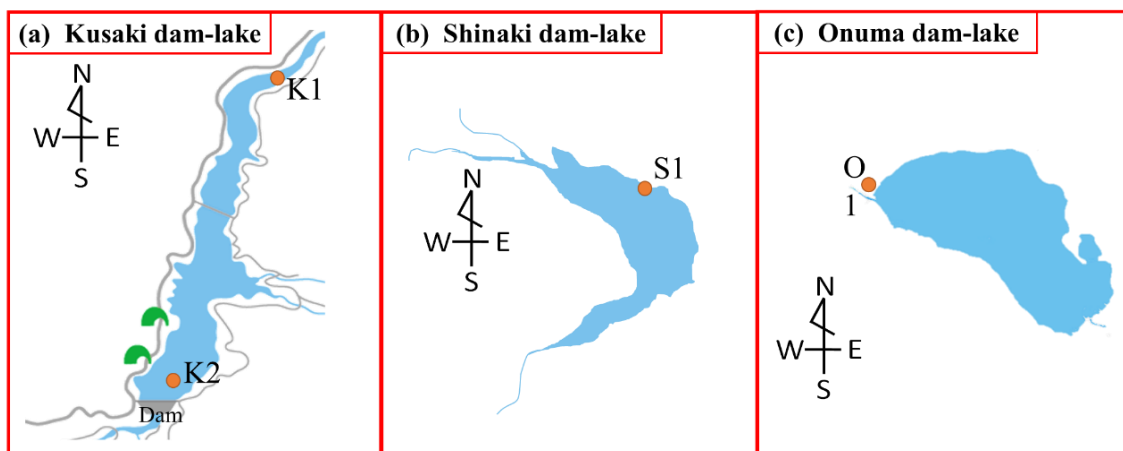
Fig. 5-3 Sediment sampling sites of (a) Kusaki dam-lake, (b) Shinaki dam-lake, and soils from (c) Onuma dam-lake.

5.2.4. SEP

The extractants used in the four-channel circulation extraction system were same as those indicated for the batch procedures (sec. 4.2.5). The extraction procedures for F1-F4 were successively carried out in the same way as the batch method. The liquid-to-solid ratios were twice as much as those in the batch method; specifically, 4 mL of liquid (extractant) and 0.25 g of soil and sediment samples were utilized. All fractionations were performed at ambient temperature (ca. 25 ± 1 °C). The continuous circulation rate passing through the packed column were performed at 1.0 mL min^{-1}. The concentrations of elements extracted were determined by ICP-MS after the volume was made to 20 mL using pure water. The extraction procedure of batch method was identical to the batch procedure of previous chapter.

5.2.5. Chelating extraction using EDTA

Above, I have introduced the application of four channel-hyphenated AI (4-AI) system to extract element by Tessier's sequential extraction scheme. However, the 4-AI

system can not only reliably assess the extracted concentrations, but also selectively extract the targeted elements using chelating reagent. Therefore, EDTA, which is commonly used to extract multivalent metal ions, was used as a model chelating reagent. The chelating extraction was performed according to the procedure of batch method and 4-AI system, with the same liquid-to-solid ratio and the same circulation time of Fraction 2 in the SEP described in chapter 3.

5.3. Results and Discussion

5.3.1. Validation of the circulation extraction system

The abilities of the circulation extraction system and batch system to extract several heavy metals from the lake sediment CRM were compared. The circulation times mentioned above were determined according to previous chapter. The steady-state extractions of Cu and Pb in the lake sediment CRM were achieved in 1 h for F1 and F2 and 3 h for F3 and F4.

The concentrations of Cd, Cu, Pb, and Zn obtained by the circulation time as mentioned above were shown in Fig. 5-4. The Cu, Pb, and Zn concentrations were in the order F4>F3>F2≥F1, and the Cd concentration were in the order F1>F2>F4>F3. The concentrations found using the different channels were not significantly different (Student's t test, P <0.05) [4]. The trends in the concentrations found in the fractions obtained using the circulation extraction system agreed well with the batch system, but the total Cu, Pb, and Zn concentrations obtained by the circulation extraction system were 1.1, 1.2, and 1.4 times higher than that obtained by the batch system, respectively. the circulation extraction system than that by the batch system, as shown in Table 5-1. Extracting with the circulation system was therefore more efficient than extracting by shaking a sample in a bottle (i.e., the batch system method). However, the trend for Cd was somewhat different, because the low Cd concentration caused the difficult effectively

extract. This will be the subject of a future study.

Through the experimental results, I could find out several advantages of the present system against conventional batch SEP. First, in present system, the whole experimental time needed from F1 to F4 was reduced to 9 h because no centrifugation and filtration steps were required, whereas the batch method SEP took at least 14 h. Second, the total amount of effluent consumed in the present system reduced to two-thirds compared to that of in the batch system. Third, the fact that can skip filtration process could minimize the sample contamination or individual operation errors. Comparing to the all injection (AI) system in single channel previously developed (Chapter 4), this system could achieve the simultaneous extractions with small quantitative differences among the four flow channels.
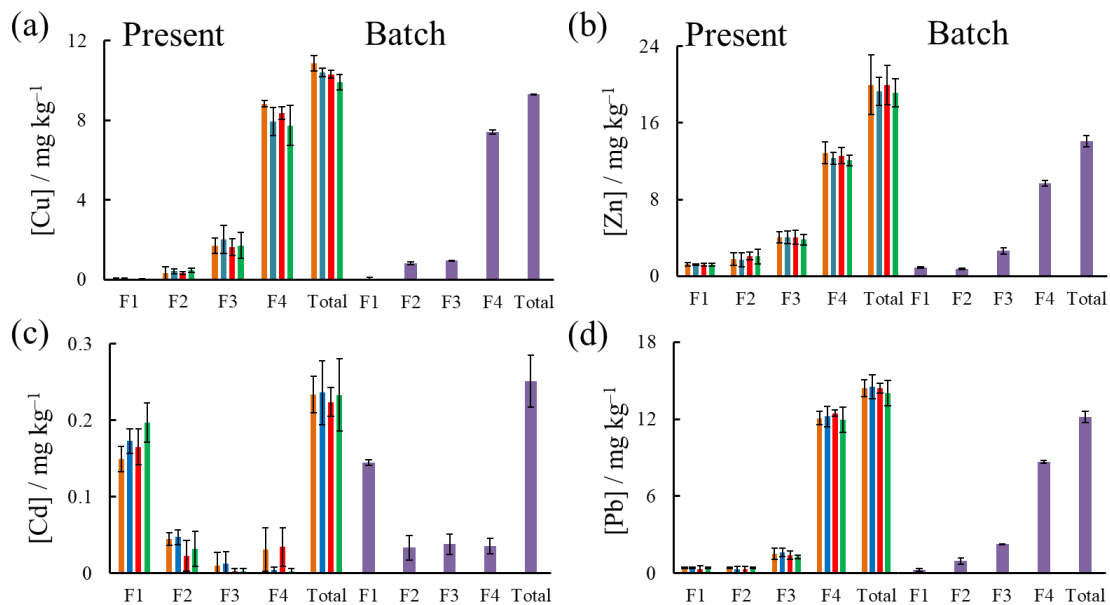
Fig. 5-4 Concentrations of Cu (a), Zn (b), Cd (c) and Pb (d) extracted by this system and batch method. Sample: Certified reference material (lake sediment). Sample volume: this system, 0.25 g; and batch method: 1 g. Circulation time: F1 and F2: 1 h; and F3 and F4: 3 h. Flow channel: ■ 1st channel; ■ 2nd channel; ■ 3rd channel; and ■ 4th channel. "Total" in the graphs means the sums of metal concentrations in Fractions 1–4.

Table 5-1 Concentrations of heavy metals in fractions F1 to F4 and the recoveries for the certified lake sediment reference material determined using the 4 - AI system and the batch system

	Cu		Zn		Cd		Pb	
	This study[a]	Batch	This study	Batch	This study	Batch	This study	Batch
F1 – F4 / mg kg^{-1}	10.37 ± 0.12	9.36 ± 0.02	19.59 ± 0.68	14.21 ± 0.59	0.23 ± 0.19	0.25 ± 0.03	14.35 ± 0.32	11.90 ± 0.42
Certified / mg kg^{-1}	23.1 ± 3.1		107 ± 5		0.34 ± 0.02		31.3 ± 1.1	
Recovery / %	44.9	40.5	18.3	13.3	67.6	73.5	45.8	38.0

[a] Total concentrations determined using the 4-AI system are the means of the total concentrations determined using the four channels.

5.3.2. Effect of the soil sample particle size

The ability of the circulation extraction system to extract analytes was investigated by the soil sample particle size, using the parking lot soil sample surrounding Akagi Onuma, Gunma, Japan. The particle sizes used were <74 μm in channel 1, 74–149 μm in channel 2, 149–2000 μm in channel 3, and <2000 μm in channel 4. Particles <2000 μm (<2 mm) are conventionally analyzed using methods such as the Community Bureau of Reference (BCR) method. Sequential extractions were performed for each sample and the Cd, Cu, and Pb concentrations were determined.

The amounts of the analytes extracted among the four circulation channels were very small differences, as mentioned above and shown in Fig. 5-4, when I assessed the effects of the particle size on the fractionation results.

In contrast, as shown in Fig. 5-5, the highest concentrations were found in the extracts of the smallest soil particles. However, the variations in the concentrations of all the analytes in the fractions were similar for all the particle sizes. The fraction with the highest concentration of an analyte (e.g., F4 for Cu and F1 for Cd) was affected by the total analyte concentration in the F1–F4 extracts. The highest analyte concentrations were found in the smallest particle samples, which had the largest surface areas. Heavy metals tend to sorb efficiently to soil particle surfaces, and silt and clay particle are smaller and have higher surface areas than gravel and sand [5].
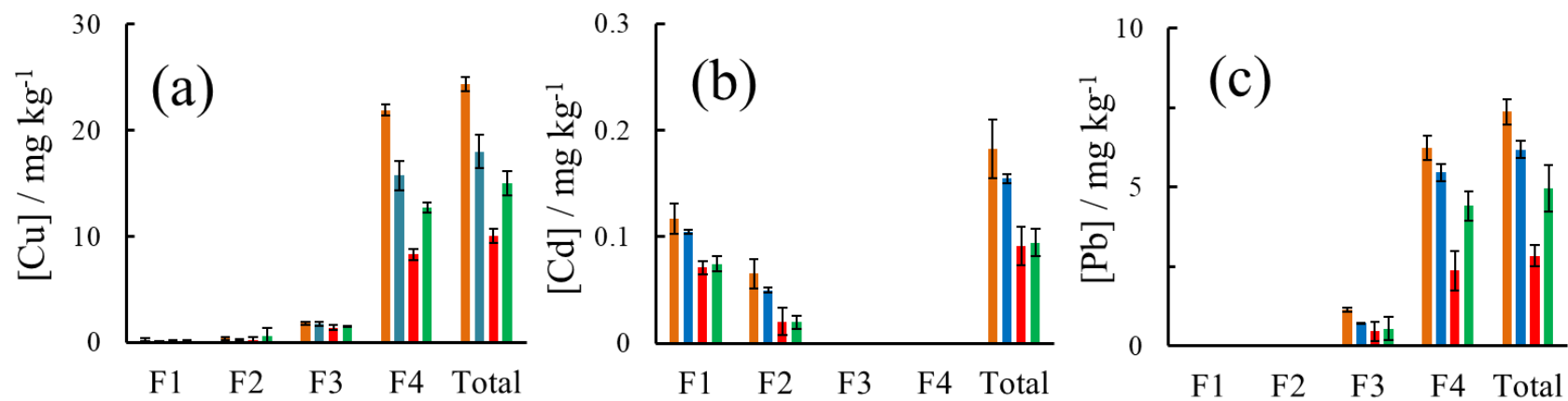
Fig. 5-5 Fractionations of four different elements as function of soil particle size. Sample: soil sample collected in a parking area surrounding Lake Onuma in Mt. Akagi (dryness). Element: (a) Cu, (b) Cd and (c) Pb. Sample amount loaded in the sample column: 0.25 g per sample column. Circulation time: F1 and F2: 1 h, and F 3 and F4: 3 h. Identity of channel: ■ 1st: < 74 μm; ■ 2nd: 74 –149 μm; ■ 3rd: 149 – 2000 μm; and ■ 4th: < 2000 μm.

5.3.3. Extraction of fly ash

Next, the system was used to extract heavy metal in a fly ash sample. As shown in Fig. 5-6, the analyte concentrations in the fly ash were 100–1000 times higher than the concentrations in the CRM. This would have been because heavy metals become concentrated in the small (<74 μm diameter) fly ash particles during combustion. The Cd, Cu, Pb, and Zn concentrations in F3 (66%–83% of the total F1–F4 concentrations) were all higher than in F1, F2, and F4. This would have been related that fly ash from the Gunma Prefecture waste disposal site is produced during the combustion of general waste in air (i.e., in the presence of oxygen) [6].

In the incineration facility, hydrated lime is fluidized in air and injected straight into the exhaust ducting to prevent the ducting becoming oxidized [7]. A proportion of the heavy metals in the ash will have been converted into forms easily dissolved by weak acid, so the contributions of the F2 extracts to the total concentrations were next highest after the contributions of the F3 extracts. Pb is strongly bound to chloride based on the Lewis acids and bases [8], meaning there was a relatively high concentration of Pb in F1.

The differences in the concentrations found using the different channels when fly ash was analyzed were smaller than the differences in the concentrations when the lake sediment CRM was analyzed. The relative standard deviation of heavy metal concentrations found in fly ash were <5%. The fly ash–extractant reaction efficiency would be higher than the sediment–extractant reaction efficiency because the fly ash particles had more uniform physical properties and much smaller diameters than the sediment and soil particles.

The F1 and F2 (easily eluted forms) extraction efficiencies for the fly ash were higher using the circulation extraction system than using the batch system. The Pb concentrations were much higher than the Cd and Cu concentrations in the fly ash F1 and F2 extracts.

Therefore, I will investigate the fly ash collection and disposal methods to determine

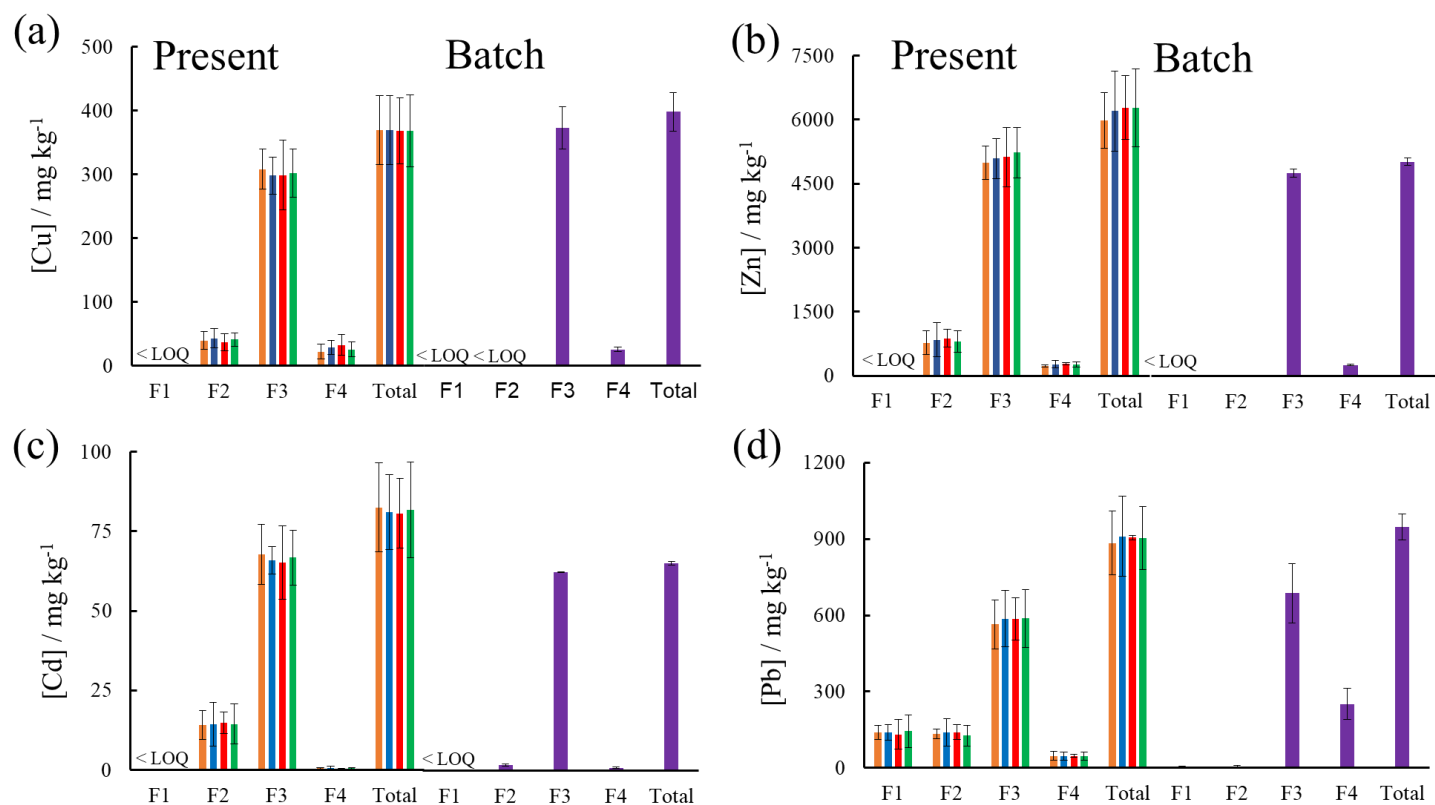how to prevent the ash from releasing Pb into the aquatic environment.

Fig. 5-6 Fractionations of elements in a fly ash sample by the circulation extraction and batch methods. Analyte element: (a) Cu, (b) Zn, (c) Cd and (d) Pb. Volume of fly ash sample loaded: the present system: 0.2 g and batch method: 1 g. The average particle size loaded was less than 74 μm. Circulation time: F1 and F2: 1 h; and F3 and F4: 3 h. Flow channel: ■ 1st channel; ■ 2nd channel; ■ 3rd channel; and ■ 4th channel. "Total" in the graphs means the sums of metal concentrations in Fractions 1–4.

5.3.4. Extraction of heavy metals in sediment

4-AI system was also used to extract heavy metals from sediment of dam-lake. As shown in Fig. 5-7 and Fig. 5-8, the concentrations of heavy metal extracted in Kusaki dam-lake sediments obtained by the AI system provided the similar trends to those by batch system. In addition, the extraction concentration of each target element was higher in the sample at the down-stream (K2) than that collected at the upper-stream side (K1). This might be related to the difference in grain size distribution of Kusaki dam-lake sediment (Fig. 5-9). In the upstream, sediments that particle diameters below 75 µm account for 17% of total sediment samples, while in downstream sediment it up to 95%, that is the smaller particles indicate the larger specific surface area. Also, the extraction concentration between 4-AI system and batch system were examined using the t-test ($P < 0.05$). It was found that there was no significant difference in the sediment of Kusaki dam-lake, except Cd. The relative standard deviation (RSD) of the concentration of the heavy metals in Kusaki dam-lake that were extracted from the four channels ($\leqslant 5\%$) were smaller than the CRM.

The total extracted concentrations of Cu in K1 and K2 were 69.0 and 263 mg kg^{-1}, respectively. The concentration of Cu in down-stream sediment of Kusaki dam-lake was still higher than the environmental standard value of paddy field (125 mg kg^{-1}) [9] now. This seemed to be concerned to the Ashio copper pollution incident that occurred in 1891. The ratio of easy-elution forms (Fraction 1 and 2) contained in Kusaki dam-lake sediments was 31% (i.e. 81.5 mg kg^{-1}). The results indicated that abundance ratio of exchangeable Cu was lower than that of the standard value (125 mg kg^{-1}) [9].
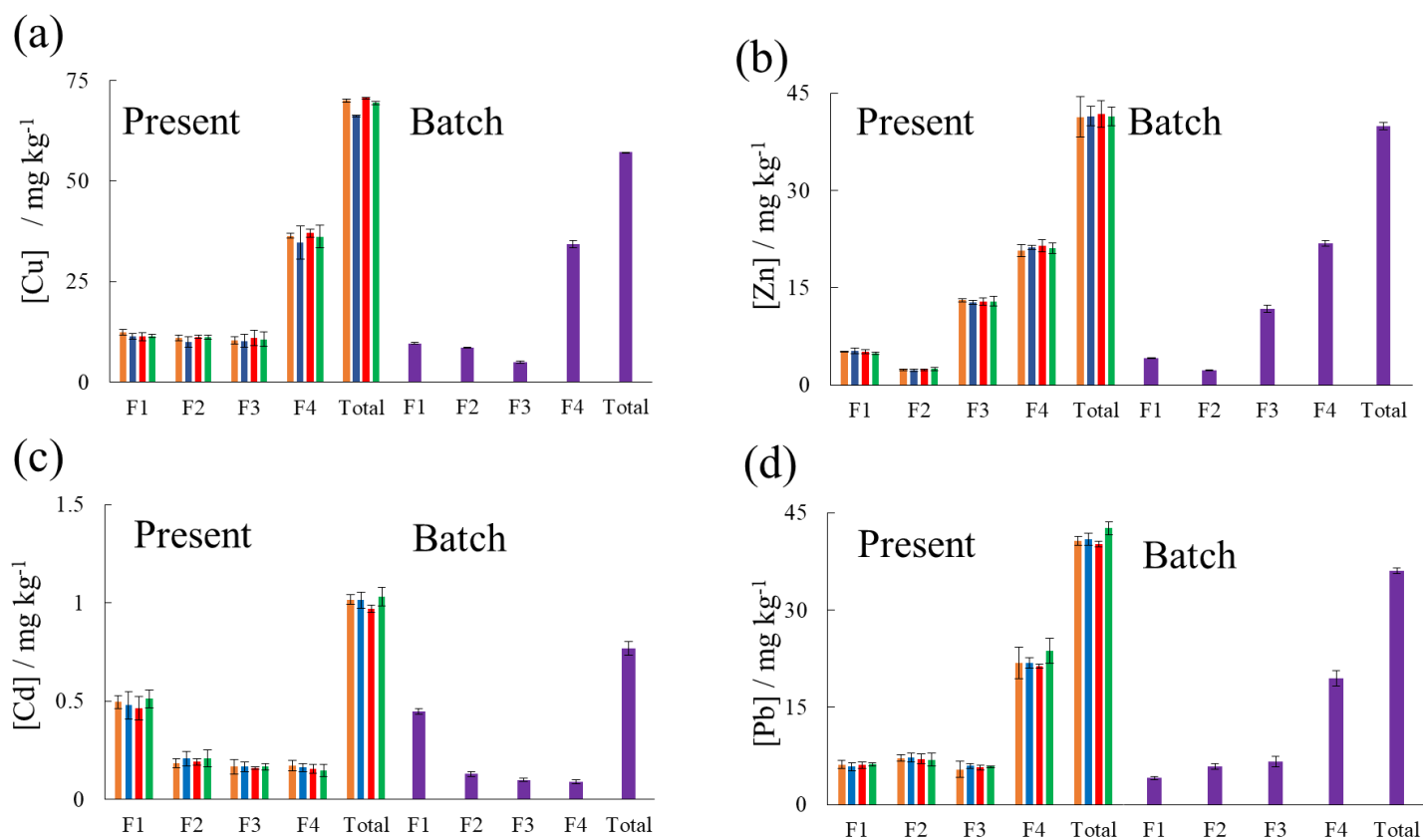
Fig. 5-7 Concentrations of Cu (a), Zn (b), Cd (c) and Pb (d) extracted by this system and batch system. Sample: the upstream sediment of Kusaki dam-lake (K1). Sample volume: this system, 0.25 g; and batch system: 1 g. Circulation time: F1 and F2: 1 h; and F3 and F4: 3 h. Flow channel: ■ 1st channel; ■ 2nd channel; ■ 3rd channel and ■ 4th channel. "Total" in the graphs means the sums of metal concentrations in Fractions 1–4.
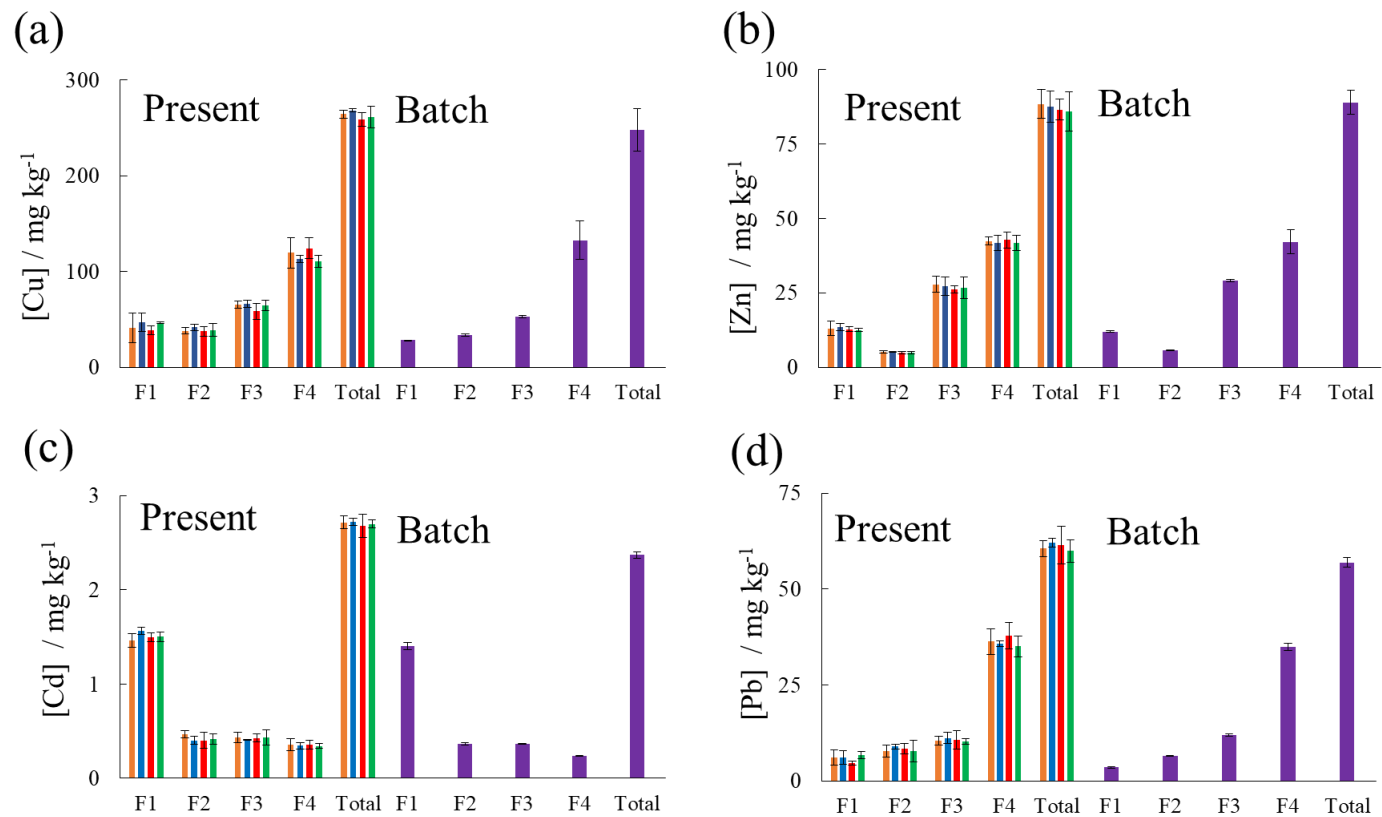
Fig. 5-8 Concentrations of Cu (a), Zn (b), Cd (c) and Pb (d) extracted by this system and batch system. Sample: the downstream sediment of Kusaki dam-lake (K2). Flow channel: ■ 1st channel; ■ 2nd channel; ■ 3rd channel and ■ 4th channel. "Total" in the graphs means the sums of metal concentrations in Fractions 1–4. Other conditions are same as in Fig. 5-7.
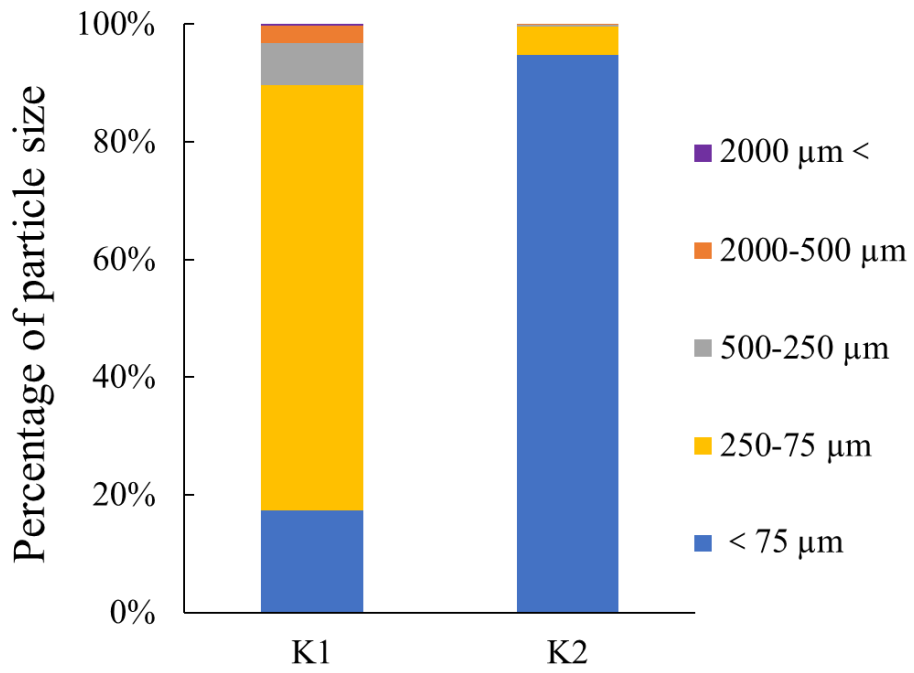
Fig. 5-9 Particle size distributions in sediment of upstream and downstream of Kusaki

dam-lake

5.3.5. Simultaneous speciation analysis

Next, to shorten the operation time in the 4-AI system, the four different extractants used in the SEP were loaded into each four-channel with the column for a lake sediment sample of Kusaki dam-lake, and the simultaneous metal extractions were performed according to the circulation times for each channel as summarized in Table 5-2. The concentrations of analyte metals in four fractionations extracted from sediment samples were calculated from Eqs. 5-1 – 5-4, as following.

F1 = (metal concentration from 1st channel)　　(5-1)

F2 = (metal concentration from 2nd channel) – (metal concentration from 1st channel)　　(5-2)

F3 = (metal concentration from 3rd channel) – (metal concentration from 2nd channel)　　(5-3)

F4 = (metal concentration from 4th channel) – (metal concentration from 3rd channel)　　(5-4)

Table 5-2 Extractant laden in each channel and the circulation time

Channel	Fraction	Extractant	Circulation time
1st	F1	1 mol dm^{-3} MgCl$_2$	1 h
2nd	F1 - F2	1 mol dm^{-3} CH$_3$COONa	1 h
3rd	F1 - F3	0.04 mol dm^{-3} NH$_2$OH-HCl in 25% v/v CH$_3$COOH	3 h
4th	F1 - F4	1 mol dm^{-3} HCl	3 h

Exactly, because the experimental procedures here was different from the originally defined SEP, it was called "imitated-speciation analysis (ISA)". Fig. 5-10 shows the heavy metals concentration in the sediment samples of upstream (K1) of the Kusaki dam-lake by ISA. The fractionations of heavy metals calculated by Eqs. 5-1 – 5-4 were summarized in Table 5-3. Comparing with the batch system, the total amount of heavy metals obtained by ISA agreed with that of the batch method. Moreover, the tendencies for four fractionations of metals were consistent between the ISA and batch system. However, the metal concentrations of F2 obtained by ISA were much smaller than those

by batch system. The extraction efficiency of heavy metals by the extractant corresponding to F2 in the 2nd channel was concluded to be lower than those in other channels.

Although the extraction abilities of ISA had several problems compared to those of the 4-AI system, ISA could shorten the whole extraction time, and extraction operation was completed in 4 h.
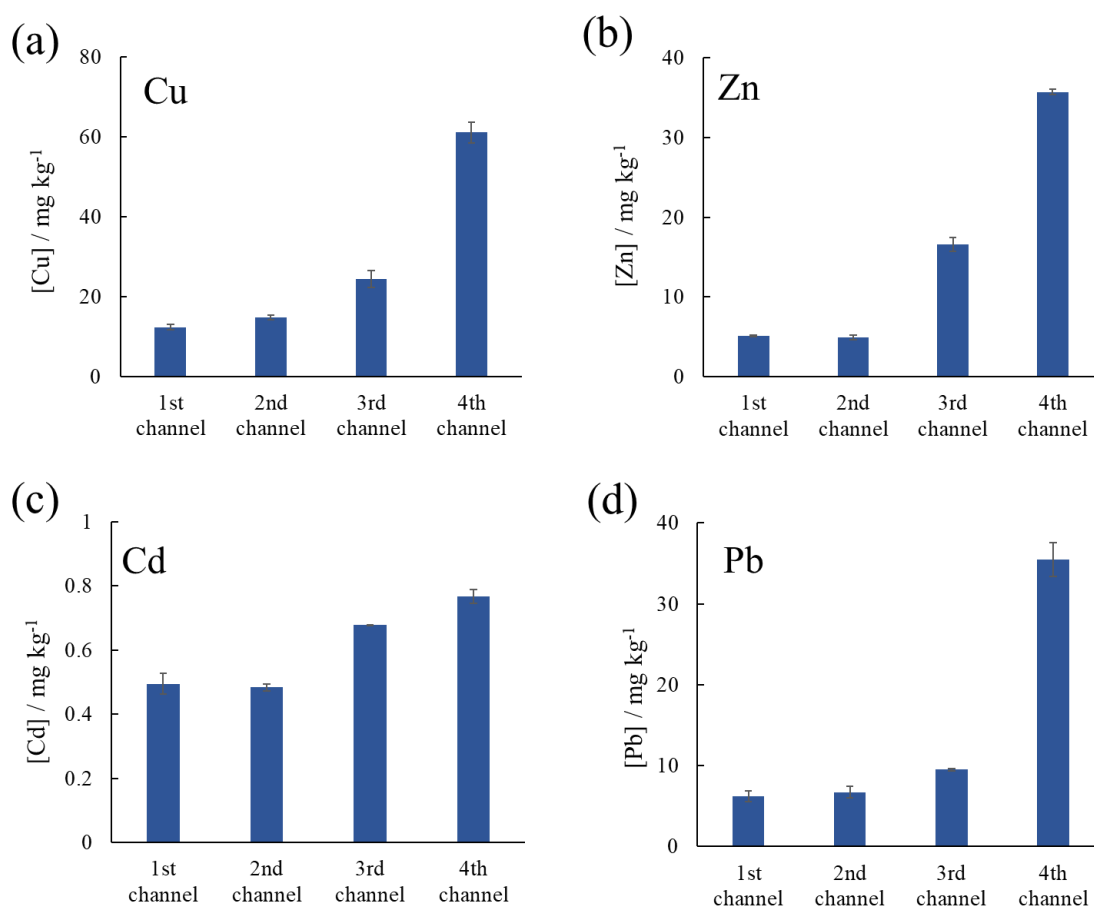

Fig. 5-10 Concentrations of Cu, Zn, Cd and Pb obtained by ISA (n = 3). Sample: upstream sediment (K1) of Kusaki dam-lake; Sample volume: ISA, 0.25 g; Circulation time: 1st and 2nd channels: 1 h; and 3rd and 4th channels: 3 h.

Table 5-3 Concentrations of heavy metals in fractions (F1 to F4) of upstream sediment in Kusaki dam-lake obtained by "imitated"

speciation analysis (ISA) and conventional batch system.

Fraction	Cu		Zn		Cd		Pb	
(mg kg^{-1})	ISA	Batch	ISA	Batch	ISA	Batch	ISA	Batch
F1	12.3 ± 0.7	9.58 ± 0.12	5.13 ± 0.07	4.13 ± 0.09	0.495 ± 0.033	0.448 ± 0.014	6.20 ± 0.64	4.10 ± 0.22
F2	2.43 ± 0.60	8.50 ± 0.07	N.D.[a]	2.24 ± 0.05	N.D.	0.129 ± 0.011	0.493 ±0.701	5.88 ± 0.40
F3	9.66 ± 2.14	4.83 ± 0.29	11.7 ± 0.8	11.7 ± 0.6	0.194 ± 0.002	0.099 ± 0.009	2.80 ± 0.15	6.62 ± 0.81
F4	36.7 ± 2.6	34.3 ± 0.8	19.1 ± 0.3	21.8 ± 0.5	0.090 ±0.021	0.090 ± 0.009	26.0 ± 0.2	19.5 ± 1.2
Total	61.1 ± 5.3	57.2 ± 3.0	35.7 ± 0.9	40.0 ± 3.5	0.767 ±0.027	0.766 ± 0.029	35.5 ± 2.0	36.1 ± 3.1

[a] N.D. = not detected.

5.3.6. As and Y concentrations in lake sediment

Next, the circulation extraction system was used to analyze a lake sediment sample that contained precipitates caused by large amounts of lime that had been added to the river feeding the lake to regulate the pH [10]. I investigated the fractionation of As, which is a very toxic element, and Y, which is a rare earth metal, with heavy metals as above heavy metals. Previously large amounts of As was found in water from the Bandaiko hot springs (Gunma Prefecture) and in river sediment in the Kusatsu hot spring area. At that time, I used SEPs to evaluate the leachability of As from sediment to river water [11]. In contrast, many rare earth metals such as Y enter the environment in areas affected by volcanic eruptions and thermal springs [12,13]. Y was found at a high concentration in the lake sediment, as shown in Fig. 5-11. I expected to find Y because I previously found it in hot spring water and nearby rivers and lakes (see chapter 2).

As is shown in Fig. 5-11, the F4 fraction contained large amounts of As and Y, indicating agreement with resulting in the batch system. These results indicated that As and Y were present in forms that were almost unavailable for exchange into the water. The trends for Cu and Pb are summarized in Table 5-4. Cd was also found at higher concentrations in F3 and F4 than in F1 and F2. This indicates that Cd in the sediment is unavailable for exchange into the water.

I determined the element composition of the lake sediment sample using the energy-dispersive fluorescent X-ray instrument. The sediment sample contained 44% Ca, 21% Fe, 16% Si, 0.6% S (as oxide), and 0.9% As, as shown in chapter 3 (Table 3-2).

Acidic river water upstream of the lake from which the sediment was collected contains high concentrations of metals and As. The river is brought to neutral pH by adding a lime ($Ca(OH)_2$) suspension at a neutralization factory in the middle reaches of the river [10]. This causes sediment to form, and metals and As precipitate to flow downstream into the lake and accumulate in the sediment. The results of this study indicate that Y in the lake sediment will not easily be released into the water in the
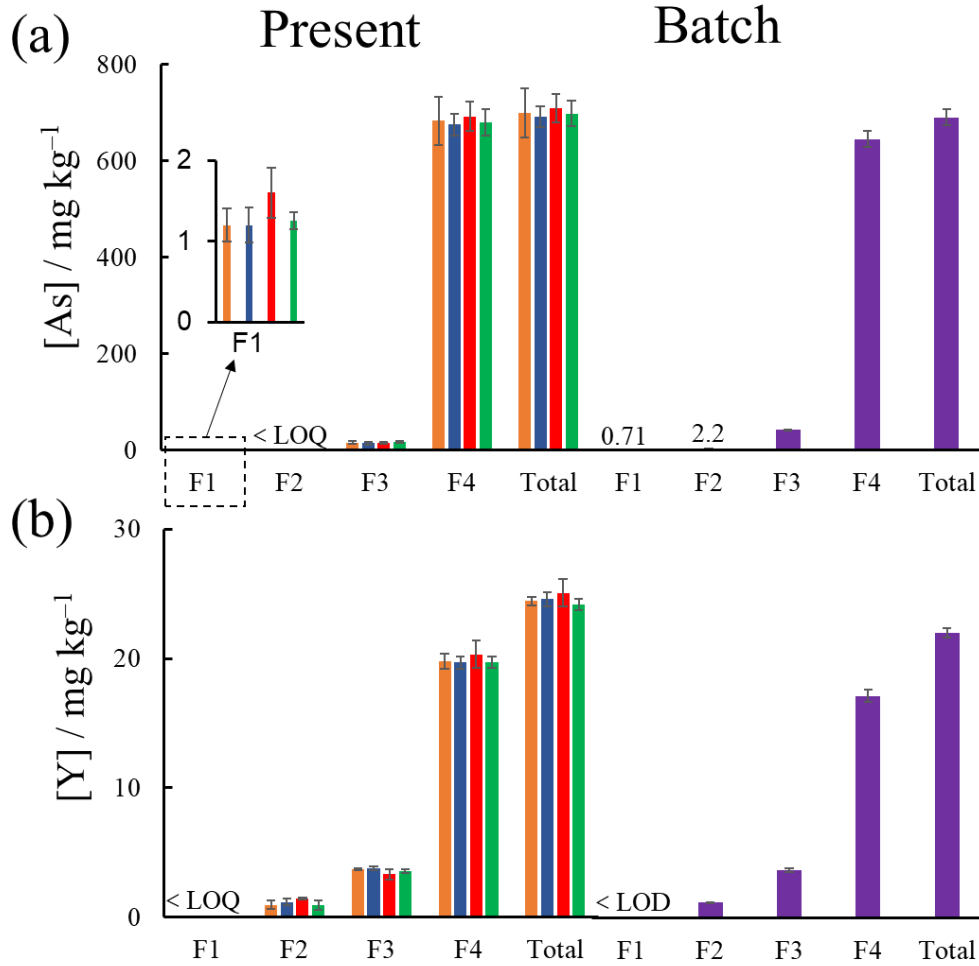
dissolved state.

Fig. 5-11 Fractionations of arsenic and yttrium in a lake sediment located in downstream of acidic hot spring area by the circulation extraction and batch systems. Volume of sediment sample loaded: the present system: 0.25 g and batch system: 1 g. The particle size loaded was within 2 mm. Circulation time: F1 and F2: 1 h; and F3 and F4: 3 h. ■ 1st channel; ■ 2nd channel; ■ 3rd channel and ■ 4th channel.

Table 5-4 Proportions of the analytes found in the F1–F4 extracts of the lake sediment sample determined using the circulation extraction system (Present) and the batch system (Batch)

	Cu		As		Y		Cd		Pb	
	Present	Batch	Present	Batch	Present	Batch	Present	Batch	Present	Batch
F1	0.3%	0.3%	0.2%	0.1%	N.D.	N.D.	8.1%	12.5%	0.4%	0.1%
F2	2.9%	1.9%	N.D.	0.3%	4.5%	5.4%	17.6%	21.2%	2.6%	2.2%
F3	16.3%	16.7%	2.2%	6.1%	14.5%	16.7%	29.9%	20.1%	13.0%	13.4%
F4	80.5%	81.1%	97.6%	93.5%	80.9%	77.9%	44.4%	46.2%	84.3%	84.4%

N.D. = not detected

5.3.7. Correlation between 4-AI systems and batch system

Next, the correlations of heavy metals and arsenic in the lake sediment and fly ash samples obtained between the 4-AI and batch systems are summarized in Table 5-5, and the plots are shown in Fig. 5-12.

In the lake sediment, the concentrations of Cu and Y indicated well-correlations between the 4-AI and batch systems, and those of Zn, Cd and Pb by the 4-AI system revealed higher values than that by batch system. The trends are described also in sections 5.3.1 and 5.3.6. However, the concentration of As in the lake sediment by the 4-AI system was remarkably lower than that by the batch system. As is a nonmetal and the main chemical forms dissolved with water are anions such as arsenite ($HAsO_2$) or arsenate (H_3AsO_4), against many heavy metals are dissolved as cations in water. Because many of As in soil or sediment are bound to metal oxide or metal sulfide [14,15], Fractionations of As to investigate chemical forms in solid substances are often treated by NaOH [16] or NaH_2PO_4 [17]. Accordingly, the SEP of As in the sediment sample are considered to be insufficient on the 4-AI system. Selection of the appropriate extractants of As in SEP will be required as further investigation.

On the other hand, in the fly ash, the concentrations of Zn, Cu and Cd indicated well-correlations between the 4-AI and batch systems, and the concentration of Pb in Fraction 3 and 4 obtained by the 4-AI system revealed lower values than those by batch system. As mentioned in section 5.3.3, many heavy metals in fly ash exists as oxide-forms (Fraction 3). In this study, the concentrations of Pb in Fraction 3 by the AI system were estimated lower than those by the batch system as shown in Fig. 5-6. Accordingly, these results would be related that the total heavy metal concentrations were influenced by the concentration in the fraction having the highest abundance ratio.

Table 5-5 Correlation data on SEPs of heavy metal and arsenic concentrations in lake sediments of the CRM and fly ash obtained by the batch system and the 4-AI system.
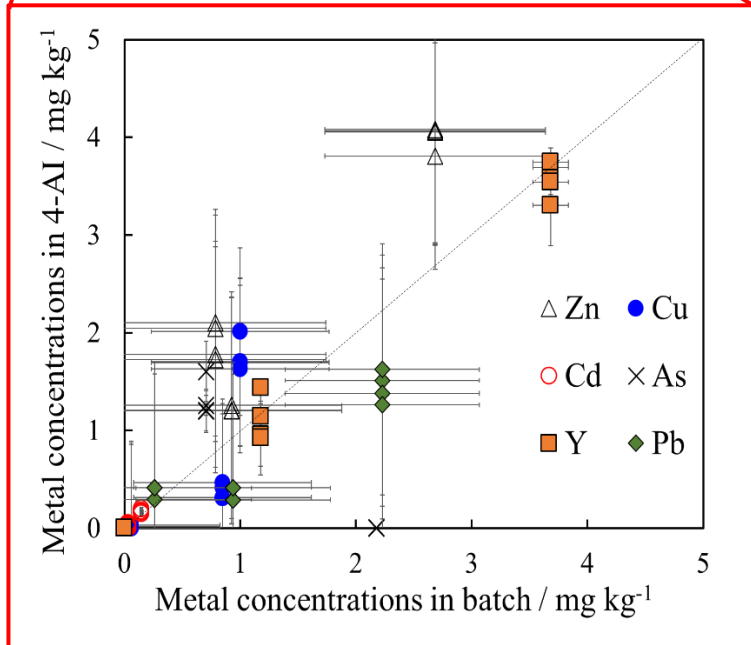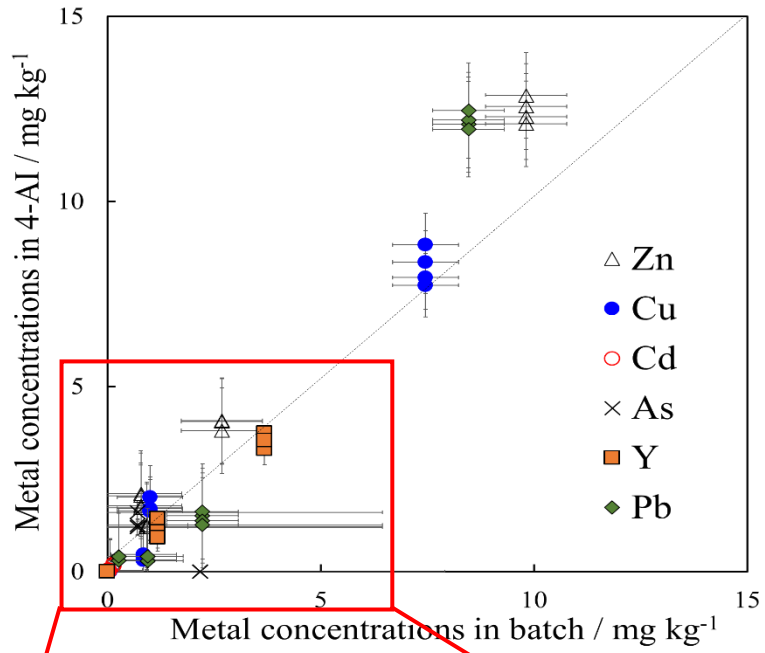
Lake sediment of CRM

Analyte	Approximation formula	Correlation coefficient (r^2)
Cu	$y = 1.100\,x + 0.010$	0.979
Zn	$y = 1.210\,x + 0.594$	0.993
Cd	$y = 1.350\,x - 0.028$	0.929
Pb	$y = 1.520\,x - 0.920$	0.980
Y	$y = 0.971\,x - 0.012$	0.989
As	$y = 0.363\,x + 0.151$	0.982

Fly ash

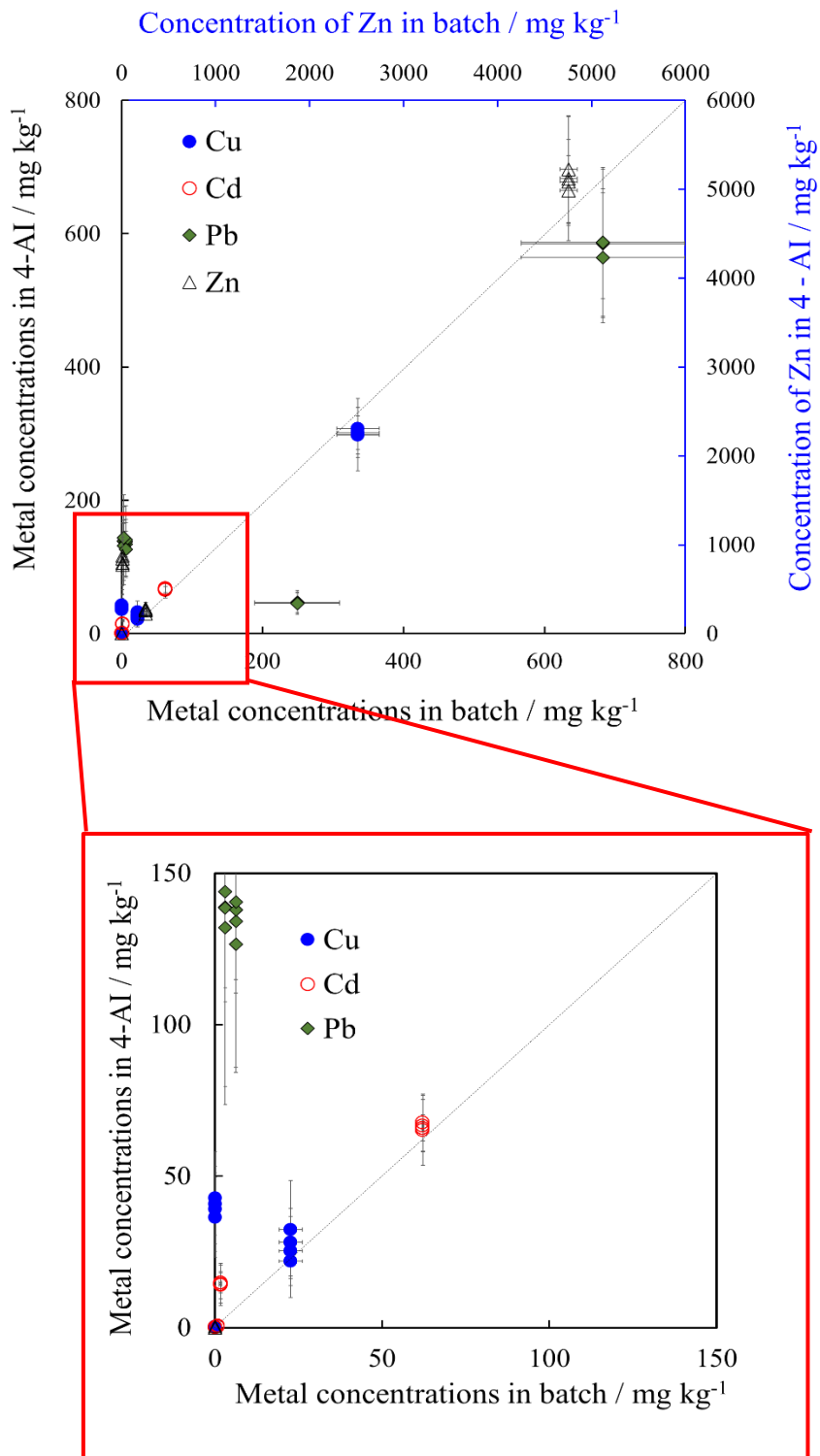| Analyte	Approximation formula	Correlation coefficient (r^2)
Cu	$y = 0.850\,x + 16.211$	0.985
Zn	$y = 1.010\,x + 0.028$	0.974
Cd	$y = 1.010\,x + 4.060$	0.962
Pb	$y = 0.644\,x + 73.700$	0.730

(a)

(b)

Fig. 5-12 Correlation data on SEPs of heavy metal and arsenic concentrations in (a) lake sediments and (b) fly ash obtained by the batch system and the 4-AI system.

5.3.8. Chelating extraction of heavy metal extraction by EDTA

Finally, the 4-AI system based on Tessier's scheme was applied to chelating extraction of heavy metals using ethylenediaminetetraacetic acid (EDTA), which is commonly used to extract polyvalent metal ions and hardness test.

In the chelating extraction, a lake sediment CRM was filled into the extraction column, and then 5.0×10^{-3} mol dm^{-1} EDTA dissolved in acetic acid buffer solution was introduced into the four channels. After the circulation (flow rate: 1.0 mL / min) for 5 h, the extracted solutions were collected, and the heavy metals were analyzed by FAAS.

As shown in Fig. 5-13, when the average concentrations of heavy metals extracted from each channel were compared to the batch system that used the same extractant and performed at the same reaction time, the extraction efficiencies of four heavy metals by the 4-AI system were higher than those by the batch system. Namely, the circulation on the 4-AI system could provide higher extractivity than the stirring or shaking on the batch system.

Furthermore, when the heavy metal concentrations extracted by EDTA were compared to those by the SEP, the concentration level of Cd was equivalent to that of F4 obtained by SPE as shown in Fig. 5-14. Also, the extraction amounts of Cu and Pb by EDTA were higher than that of F3, and the extracted concentration of Zn was equivalent to that of F2. These results were dependent on the leachabilities of four metals from the lake sediment sample while the extractivities might include the degrees of the complexation constants (log K_{ML} at 25 ºC; 18.8 for Cu^{2+}, 16.5 for Zn^{2+}, 16.5 for Cd^{2+}, 18.0 for Pb^{2+}) [18]. Although, the extraction results by EDTA does not relate to those by the SEP, the extractivities of various chelating reagents will effectively be evaluated by using the AI system in the next work.
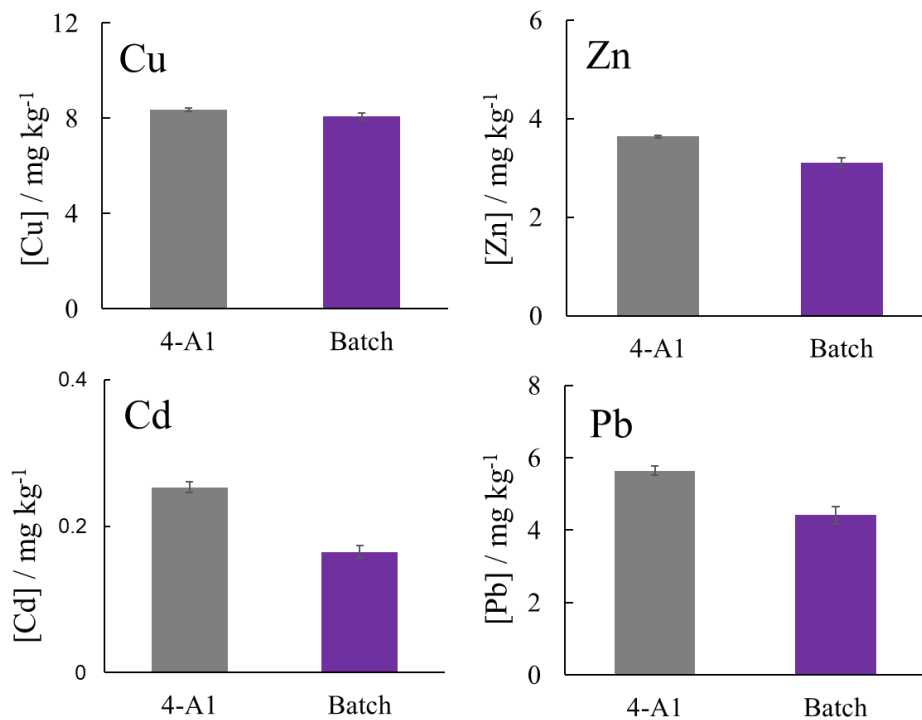
Fig. 5-13 Comparison of extracted amounts by heavy metals the 4-AI system and batch system using EDTA (n = 4). Sample weight: 1 g (Batch system) and 0.25 g (4-AI system). The detailed experimental conditions are described in section 5.2.5.
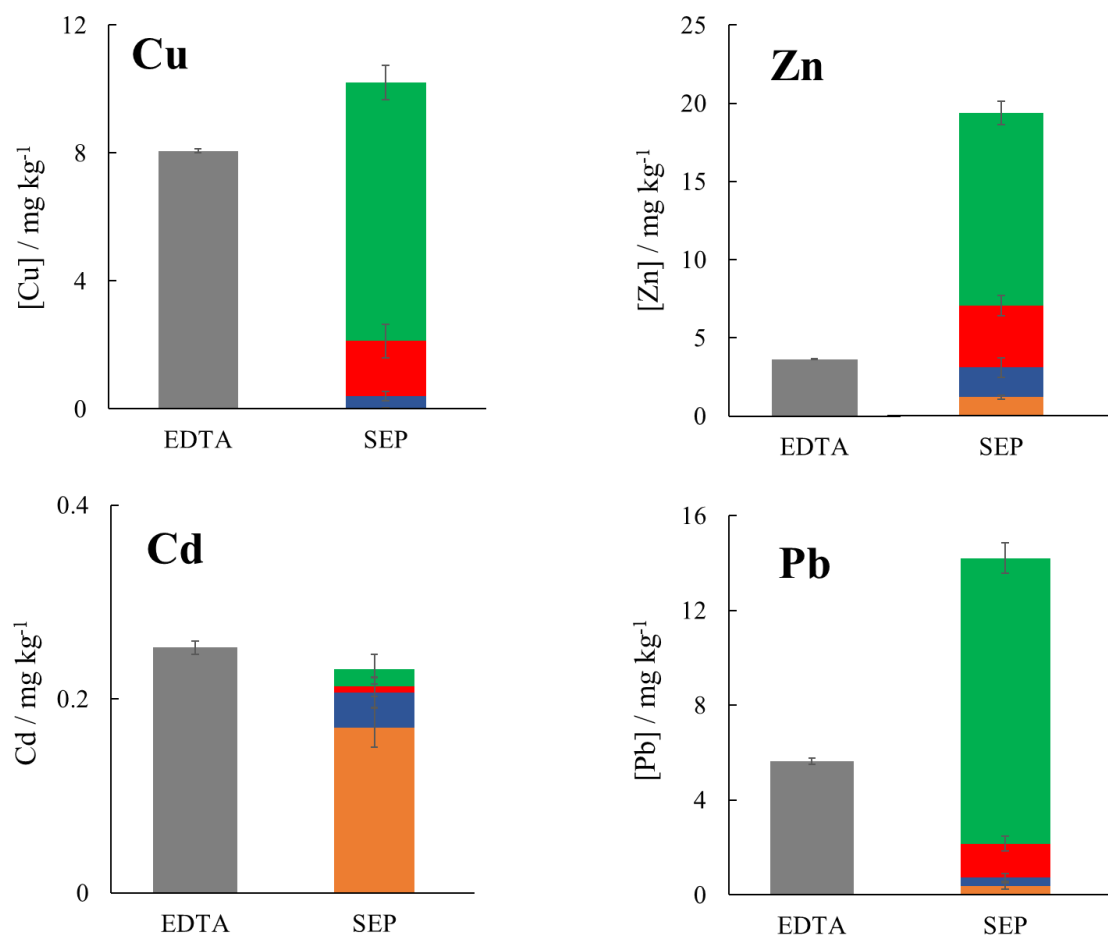
Fig. 5-14 Comparison of metal concentrations extracted with EDTA and the fractionations of heavy metals by SEP using the 4-AI system ($n = 4$). Sample: CRM (lake sediment). Sample volume: this system, 0.25 g; and batch method: 1 g. Fractionation: ■ F1; ■ F2; ■ F3 and ■ F4.

5.4. Conclusions

The results indicate that the circulation extraction system SEP is a good method for fractionating trace elements in solid samples. The four-channel system has a shorter extraction time than a conventional batch fractionation system. The circulation extraction system for one flow channel produced about two-thirds of the waste produced using the batch system. The multi-column system and closed-circulation process were a facile and reliable extraction method for determining the concentrations of bioavailable metals under steady-state conditions. In addition, the system could be applied also to the chelating extraction, as high efficiency and selective extraction of elements.

The 4-AI system is a useful device to simultaneously analyze multiple specimens that have not been reported up to now on the base of AI type. Moreover, if it becomes possible to miniaturize the device, this system will be operated more easily than the batch method. I will investigate the ability of the extraction system to fractionate trace elements in airborne particulate matter and food products.

5.5. References

[1] M. Mori, K. I. Tsunoda, S. Aizawa, Y. Saito, Y. Koike, T. Gonda, S. Abe, K. Suzuki, Y. Yuasa, T. Kuge, H. Tanaka, H. Arai, S. Watanabe, S. Nohara, Y. Minai, Yukiko Okada, S. Nagao, Fractionation of radiocesium in soil, sediments, and aquatic organisms in Lake Onuma of Mt. Akagi, Gunma Prefecture using sequential extraction, Science of The Total Environment 575 (2017) 1247-1254.

[2] F. Jiao, L. Zhang, Z. Dong, T. Namioka, N. Yamada, and Y. Ninomiya, Study on the species of heavy metals in MSW incineration fly ash and their leaching behavior, Fuel Processing Technology 152 (2016) 108-115.

[3] G. Weibel, U. Eggenberger, S. Schlumberger, and U. K. Mäder, Chemical associations and mobilization of heavy metals in fly ash from municipal solid waste incineration, Waste Management 62 (2017) 147-159.

[4] NIST/SEMATECH e-Handbook of Statistical Methods, Chapter 1.3.6.7.2., Critical values of the Student's t-distribution:
http://www.itl.nist.gov/div898/handbook/ ＜accessed：2018. 04. 05＞

[5] C. K. JAIN and D. RAM, Adsorption of metal ions on bed sediments, Hydrological Sciences Journal 42 (1997) 713-723.

[6] M. Mori, Y. Terasawa, and H. Itabashi, Evaluation of heavy metal leaching in incineration ash by sequential extraction, Journal of Industrial Water 645 (2017) 48–57.

[7] British Lime Association, Flue gas treatment:
http://www.britishlime.org/technical/flue_gas_treatment.php ＜accessed：2018. 10. 03＞

[8] J.M.L. Kerbusch, The reaction between solid lead and chlorine gas Author links open overlay panel, Journal of Solid State Chemistry 9 (1974) 197–201.

[9] Contaminated Agricultural Land Countermeasures Act, Ministry of the Environment:
https://www.mhlw.go.jp/shingi/2009/10/dl/s1006-4e.pdf ＜accessed：2018. 11. 10＞

[10] Shinaki Dam Water Quality Control Office, Ministry of Land, Infrastructure and Transport Kanto Regional Development Bureau: http://www.ktr.mlit.go.jp/ktr_content/content/000076767.pdf <accessed：2017. 02. 20>

[11] M. Zhao, N. Wada, H. Shinozaki, N. Seko, M. Mori, and H. Itabashi, Monitoring of palladium concentration in river water and sediment at an acidic hot spring spa area in the Gunma Prefecture, Analytical Sciences Online available.

[12] L. X. Zhao, S. F. Dai, I. T. Graham, X. Li, H. D. Liu, X. L. Song, J. C. Hower, and Y. P. Zhou, Cryptic sediment-hosted critical element mineralization from eastern Yunnan Province, southwestern China: Mineralogy, geochemistry, relationship to Emeishan alkaline magmatism and possible origin, Ore Geology Reviews 80 (2017) 116–140.

[13] P Möller, P Dulski, Y Savascin, M Conrad, Rare earth elements, yttrium and Pb isotope ratios in thermal spring and well waters of West Anatolia, Turkey: a hydrochemical study of their origin, Chemical Geology 206 (2004) 97–118.

[14] D. Ahmann, L.R. Krumholz, H.F. Hemond, D.R. Lovley, F.M.M. Morel, Microbial mobilization of arsenic from sediments of the Aberjona Watershed, Environmental Science & Technology, 31 (1997) 2923–2930.

[15] M. L. Pierce, C. B. Moore, Adsorption of arsenite and arsenate on amorphous iron hydroxide, Water Research, 16 (1982) 1247–1253.

[16] K. Jin, Development of analytical methods for chemical speciation of arsenic and their application to sea algae and bivalve produced in Hokkaido and to some environmental samples, and short review on daily intake of inorganic arsenic and on arsenolipids, Report of the Hokkaido Institute of Public Health, 65 (2015) 1–13.

[17] N. E. Keon, C. H. Swartz, D. J. Brabander, C. Harvey, and H. F. Hemond, Validation of an arsenic sequential extraction method for evaluating mobility in sediments, Environmental Science & Technology, 35 (2001) 2778–2784.

[18] G. Anderegg, Critical survey of stability constants of EDTA complexes, Critical

Survey of Stability Constants of EDTA Complexes, Pergamon Press, Oxford, UK (1977) pp. 1–36.

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Chapter 6

Conclusion and future prospect

6.1. Conclusions

In this thesis, I described study on evaluation of the concentrations of toxic/valuable heavy metals and arsenic contained in environmental solids such as soil or sediment, and the possibility of leaching from solids to environmental water such as river water or ground water by sequential extraction procedure. Also, I established the novel extraction devise that inject all amount of extractant and circulate it in the closed-flow channel to effectively extract the targeted element and to rapidly elucidate their behaviors in the environments. In fact, the extraction devices could estimate the leachability. In this chapter, the achievements in each chapter from chapters 2 - 5 are summarized as described below.

Chapter 2 described the studies on the annual monitoring of Kusatsu hot spring area and the river water from upstream to downstream. The pH, EC, water temperature and flow rate of Yukawa and Yazawa rivers in Kusatsu hot-spring area revealed the obvious differences, depending on the season. In both rivers, the investigation of pH raised from 2-4 to 4-7 after addition of lime at neutralization factory, while the trends of EC decreased. Furthermore, Y and Pd as valuable metals were found in this area. Variations in concentrations of the valuable metals before and after adding lime at the neutralization factory were expected to be related to toxic elements such as Cd and As. This seems due to not only co-precipitation by adding the lime but also the chemical composition.

Chapter 3 described the distribution and fractionation of Pd in the river water and

sediments of the Yukawa and Yazawa rivers, and the Shinaki dam. By using sequential extraction procedure (SEP), the leachability of Pd from the sediment following the neutralization was found to be extremely low compared to that of other metals. Consequently, the recovery of Pd from the sediment may be likely to be very difficult because of the large amounts of reductants and strong acids were needed to extract Pd. Furthermore, considering the data of SEP and the mass balance of Pd, Pd in the sediment of the Shinaki dam-lake was estimated to be supplied from the Yukawa river inflowing spring from volcanos such as Bandaiko mine, tributary rivers and hot spring water, rather than that supplied from the lime inputted at the neutralization.

Chapter 4 described a novel element extraction system (the AI system) from solid substances that does not require a complicated operation (centrifugation, filtration, washing, etc.). As compared with the conventional batch method with handling procedures, the AI system could largely reduce amount of waste and time consuming. Also, this system was applicable to the speciation by SEP of heavy metals such as Cu, Zn, Cd and Pb, but also non-metal element such as As. Because this method can greatly contribute to simplification of experimental operation and reduction of running cost, it may follow the speed up of an onsite environmental monitoring of environmental soil and sediment.

Chapter 5 described utilization of the "4-AI" system developed to extend the performance of the AI-system, which can simultaneously analyze the multiple specimens by simultaneously circulating the extraction reagents in four different channels that insert solid-loaded columns. The 4-AI system for one flow channel produced about two-thirds of the waste produced using the batch system. The multi-column system and closed-circulation process were a facile and reliable extraction method for determining the concentrations of bioavailable metals under steady-state conditions. In addition, the system could be applied also to the chelating extraction to obtain high-efficiency and

selective extraction of an analyte element.

6.2. Future prospect

In field of environmental sciences, the development of automatic extraction method will become very important pretreatment to achieve high-accuracy heavy metal analyses in solid substances. The research areas that require the technique will be expected to be greater in future.

1) Through this study, I am considering that AI system and 4-AI system can be miniaturized to suit for on-site determination and can be applied to fractionate trace elements in polluted farmland and industrial sewage sludge.

2) The AI system and 4-AI system were concluded to be a useful method for trace element extraction in solid samples. However, both systems are still difficult to be applied to on-site measurements because the miniaturization of rotary pump used in circulation the extractant is not progressing. In addition, we do not fully grasp what kind of solid substances can be applied to the systems. Therefore, in future research, our research groups will progress to miniaturize the devices toward on-site extraction.

3) For fractionating trace elements in polluted farmland and industrial sewage sludge, it has been performed by conventional sequential extraction procedures [1,2]. However, those methods have some problems due to the complexed handlings. If I succeed the miniaturization and automation of the AI system, the practical use of the system would be expected as an alternative method to conventional method (batch system).

To achieve the future prospects as mentioned above will be next subjects to design the layout of the flow path and component replacement, as well as to apply AI or 4-AI systems to the pollution farmland and industrial sewage sludge to a serious region of heavy metal-contamination, e.g., Jiangsu province in China where we are researching

now.

6.3. References

[1]   F. Wu, Y. Liu, Y. Xia, Z. Shen, and Y. Chen, Copper contamination of soils and vegetables in the vicinity of Jiuhuashan copper mine, China, Environmental Earth & Sciences, 64 (2011) 761-769.

[2]   C. Wang, X. Hu, M. L. Chen, and Y. H. Wu, Total concentrations and fractions of Cd, Cr, Pb, Cu, Ni and Zn in sewage sludge from municipal and industrial wastewater treatment plants, Journal of Hazardous Materials, 119 (2005), 245-249.

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March 2019, Gumma

Meichao Zhao

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